SYNTHETIC AND BIOSYNTHETIC STUDIES ON NITROGEN HETEROCYCLES

A Thesis Presented to the University of Glasgow for the Degree of Doctor of Philosophy

by

John W.M. Mackinnon

Chemistry Department

August, 1976.

CONTENTS

PAGE

ACKNOWLEDGEMENTS

I am extremely grateful to Professor Gordon W. Kirby for his friendly advice and discussions during the course of this work and in preparation of this thesis.

I also wish to thank the following for their kind advice and assistance:-

My parents, my wife, Elisabeth Breslin, Janet Christie, John Corrie, Jim Gall, Bob Hill, Helen Prentice, David Robins, Fred Rowell, Ram Sharma, Jim Sween y, Ratnakar Talekar and Ivor Williams.

Finally, I would like to thank the Science Research Council for financial assistance and the staff of the Chemistry Department for technical assistance.

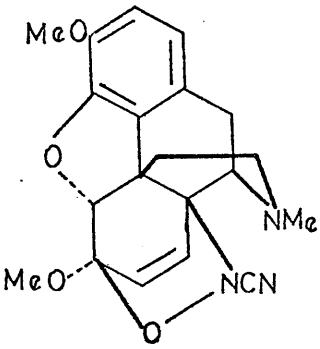
SUMMARY

The reaction of nitrosocarbonyl-compounds with a series of simple dienes to form Diels-Alder adducts has been investigated. The modification of such adducts in further synthesis has been studied and their limitations assessed. A series of adducts, designed for de-acylation under a variety of mild conditions, have been formed from the novel alkyl nitrosoformate reactive intermediates and C-nitroso-formamide adducts have also been briefly examined. The chemistry of adduct formation between ergosteryl acetate and various nitrosocarbonyl-compounds has been examined in detail and a novel rearrangement discovered in this field.

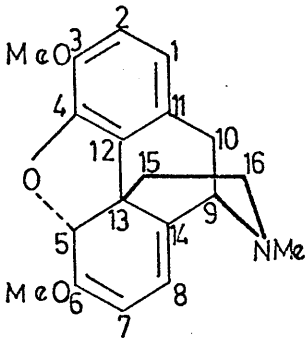
The synthesis of a postulated biphenyl intermediate in the biosynthesis of the Amaryllidaceae alkaloids norpluviine and lycorine has been investigated by several different routes.

Tracer experiments with ^{3}H- and ^{14}C-tyrosine have confirmed that D- and L-tyrosine are incorporated with equal efficiency into these alkaloids and that D-tyrosine is converted into L-tyrosine before incorporation. The later stages in the biosynthesis of norpluviine and lycorine have been re-examined. A partial retention at position 2 in lycorine of tritium from [3,5-$^{3}H_2$]tyrosine is noted in Clivia miniata Regel and 'Twink' daffodils. Degradation studies suggest that this partial retention results from scrambling of the 2-methylene protons of norpluviine and a mechanism for this is postulated.

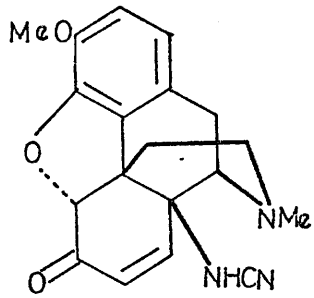
SECTION 1



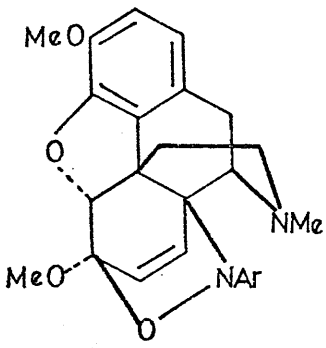
(1)



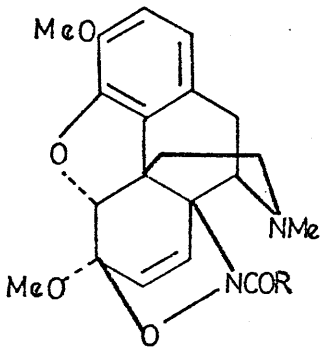
(2)



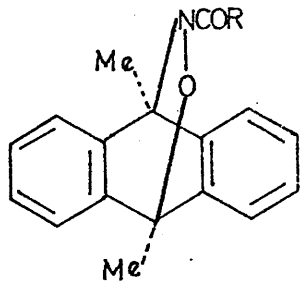
(3)



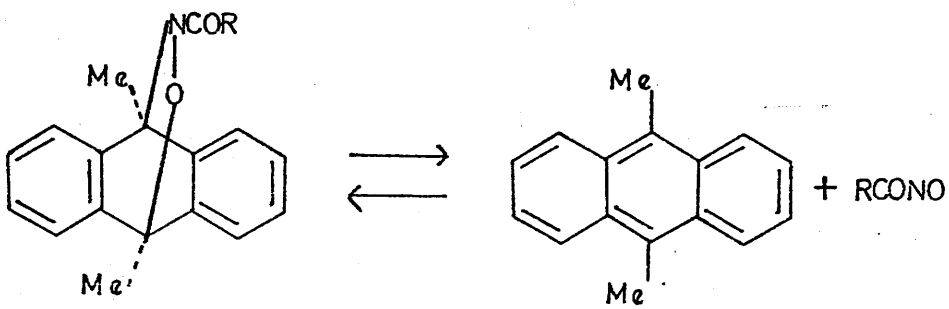
(4)



(5) a;R=Ph
b;R=Me



(6) a;R=Ph
b;R=Me

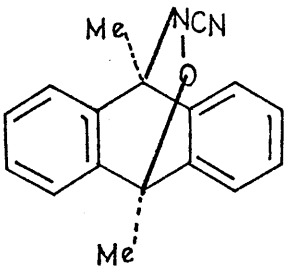


(fig.1)

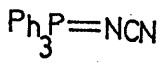
INTRODUCTION

It is well-known that nitrosoarenes and nitrosoalkenes with electron-withdrawing substituents in the α- position form 1,4-cyclo-adducts with conjugated dienes.[1] It was not surprising therefore that good evidence[2] for the existence of activated C-nitroso-compounds first came from the trapping of nitrosyl cyanide as its cyclo-adduct (1) with thebaine (2). Evidence for the cyclic nature of this adduct came from its hydrogenation to the cyanamide (3) and from its spectral similarities with the known[3,4] thebaine/nitrosoarene adducts (4). Unlike the adducts (4) however, the adduct (1) did not dissociate detectably in solution at room temperature. Final evidence for the existence of nitrosyl cyanide came later when its structure was established by microwave spectroscopy.[5]

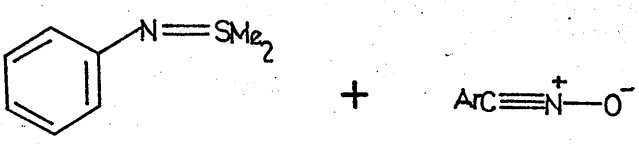
Replacement of the cyano group in nitrosyl cyanide with acyl groups gives nitrosocarbonyl—compounds, and indeed such compounds had first been postulated by Beckwith and Evans[6] to explain the occurrence of benzoic acid and its derivatives when alkyl nitrites were pyrolysed in the presence of benzaldehyde. Later, other groups[7,8,9] proposed nitroso-carbonylalkanes and nitrosocarbonylarenes as transient intermediates in the oxidation of aliphatic and aromatic hydroxamic acids to explain the occurrence of NO-diacylhydroxylamines and other acyl derivatives. The first direct evidence[10] for these transient species came when benzohydroxamic acid and acetohydroxamic acid were oxidised by tetraethylammonium periodate[11] in the presence of thebaine to

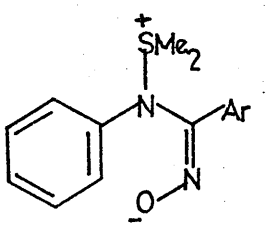


(7)

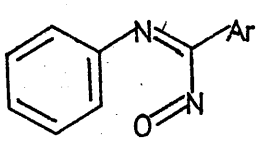


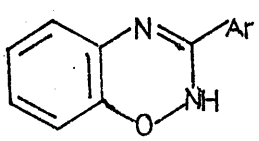
(8)





(fig.2)

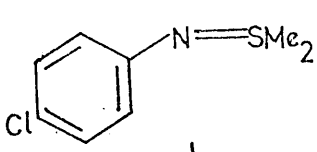




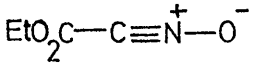
(9)

yield the Diels-Alder adducts (5a) and (5b) respectively. Strong evidence for the free existence of nitrosocarbonylbenzene and nitrosocarbonylmethane came from the nature of their crystalline 9,10-dimethylanthracene adducts (6a) and (6b). Both these adducts were stable in solution at room temperature; however, in hot benzene in the presence of thebaine, a rapid intermolecular transfer of RCONO took place to give the corresponding thebaine adducts. Further evidence[12] for the suggested equilibrium (fig. 1) comes from the rapid reaction of the nitrosocarbonylarene/9,10-dimethylanthracene adducts with triphenylphosphine to form aryl isocyanates. For a range of aryl adducts (6) this reaction was found to follow first-order kinetics with a rate constant similar in magnitude to the first-order rate constant measured for the transfer of the nitrosocarbonylbenzene moiety from 9,10-dimethylanthracene (D.M.A.) to thebaine. This suggested a slow rate-determining dissociation step followed by either a fast association with thebaine or a fast reaction with triphenylphosphine. Analogously, the D.M.A./nitrosyl cyanide adduct (7) reacted with triphenylphosphine at a rate similar to that with thebaine, to give the phosphinimine derivative (8).

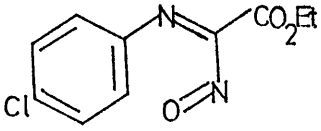
Another class of activated C-nitroso-compounds are the C-nitroso-imines. These were first proposed by Gilchrist, Harris and Rees[13] as intermediates (fig. 2) in the preparation of 1,2,4-benzoxadiazines (9) from N-arylsulphimides and nitrile oxides. In a later communication[14] it was shown that, in the



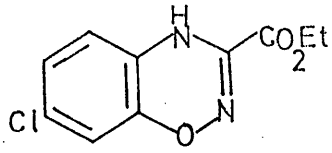
(10)



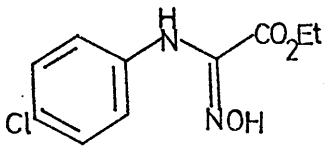
(11)



(13)



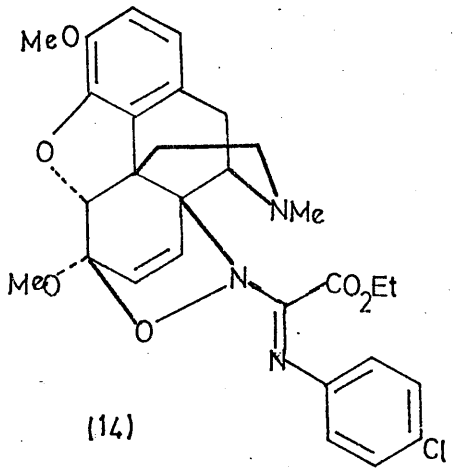
(12)



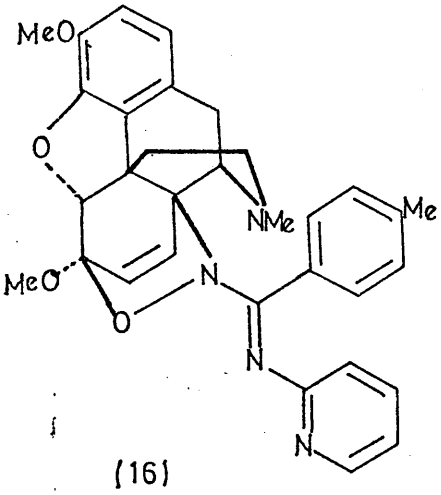
(15)

Pb(OAc)$_4$

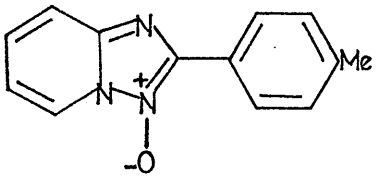
(2)



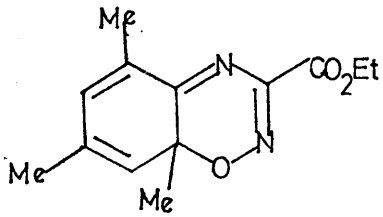
(14)



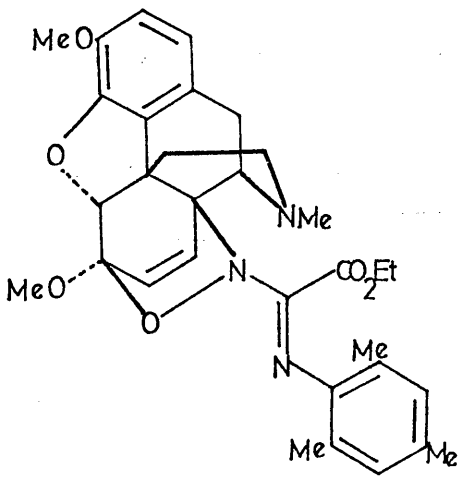
(16)



(17)

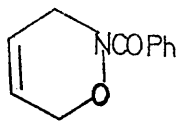


(18)

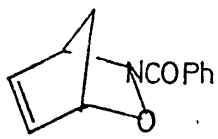


(19)

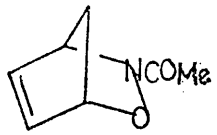
reaction of the sulphimide (10) with the nitrile oxide (11) to give the 1,2,4-benzoxadiazine (12), the C-nitroso-imine intermediate (13) could be trapped as its thebaine adduct (14). The same outcome was found when the nitroso-imine was generated by the oxidation of (15). Unlike the nitrosocarbonylarene and nitrosocarbonylalkane adducts with thebaine, the nitroso-imine adducts dissociate at moderate temperatures. Thus heating the adducts (14) and (16) in benzene yields the 1,2,4-benzoxadiazine (12) and the N-oxide (17) respectively. When aromatisation of the oxadiazines was blocked[15] by a substituent in the ortho position as in the case of the oxadiazine (18), the corresponding thebaine adduct (19) could be formed merely by stirring a mixture of the oxadiazine and thebaine in methylene chloride for 24 hrs at room temperature.



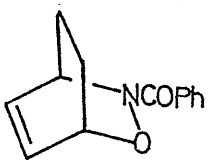
(20)



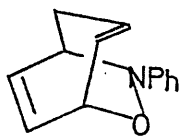
(21)



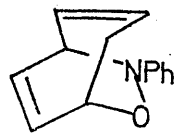
(22)



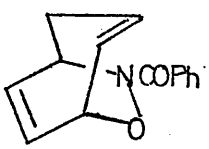
(23)



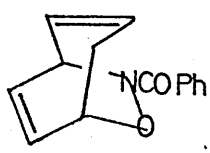
(24)



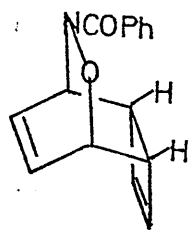
(25)



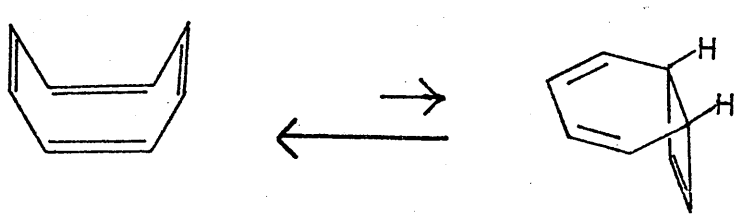
(26)



(27)



(28)



(fig. 3)

DISCUSSION

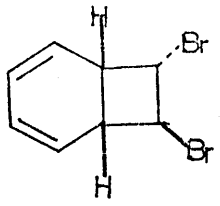
1.1. Nitrosocarbonyl/Diene Adducts

Initially it was planned to test the generality of diene/ RCONO adduct formation. The adducts (20), (5) and (6) were already known.[10] The cyclopentadiene/nitrosocarbonylbenzene adduct (21) was formed by the oxidation of benzohydroxamic acid in the presence of cyclopentadiene and was obtained as a crystalline solid; the acetyl analogue (22) prepared in the same fashion, was however, obtained as a colourless oil. The structures (21) and (22) were consistent with the n.m.r. spectra of the adducts of cyclopentadiene with nitrosocarbonylbenzene and nitrosocarbonylmethane respectively and both adducts showed a strong i.r. absorption characteristic of an amide carbonyl. Preparation of the 1,3--cyclohexadiene/nitrosocarbonylbenzene adduct (23) was equally successful and again a crystalline solid was produced.

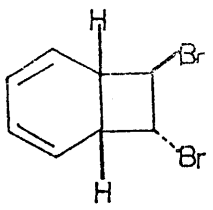
Nitrosoarene adducts[1] with both cyclopentadiene and 1,3-cyclohexadiene are well known, but 1,3-cycloheptadiene[1] does not form an adduct on prolonged treatment with nitrosobenzene. However, an adduct of nitrosobenzene and 1,3,5--cycloheptatriene is reported in the literature[16] and assigned the structure (24) or (25) on the basis of its n.m.r. spectrum. An attempt to prepare the corresponding cycloheptatriene/ nitrosocarbonylbenzene adduct (26) or (27) by the direct method (viz. oxidation of a hydroxamic acid in the presence of the appropriate 'diene') gave an unstable compound which

decomposed at room temperature. However the n.m.r. spectrum of the decomposing compound was concordant with either structure (26) or (27). To ensure that the reason for the low yield of this compound was not merely due to a slow reaction of PhCONO with 1,3,5-cycloheptatriene competing inefficiently with self-decomposition of PhCONO or reaction of PhCONO with benzohydroxamic acid, an attempt was made to transfer PhCONO from the 9,10-dimethylanthracene/nitroso-carbonylbenzene adduct (D.M.A./PhCONO) to cycloheptatriene (the indirect method). However, when the transfer reaction was carried out in hot benzene no evidence for the formation of the unstable adducts (26) or (27) was found and only a slow decomposition of D.M.A./PhCONO to D.M.A. and benzoic anhydride was noted. It was felt therefore, that in this case although the nitrosocarbonylbenzene reacted much faster with cyclo-heptatriene than does nitrosobenzene, the adduct formed is much less stable than the corresponding cycloheptatriene/nitrosobenzene adduct.

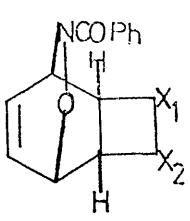
Some reaction of cyclo-octatetraene with nitrosobenzene has been observed.[1] However the authors of this report were unable to isolate or characterise any product. It was not entirely surprising therefore, that an attempt to react nitrosocarbonylbenzene with cyclo-octatetraene by the 'direct' route produced no adduct. In contrast to the corresponding cycloheptatriene experiment, no t.l.c. or n.m.r. evidence for the formation of any adduct was found.



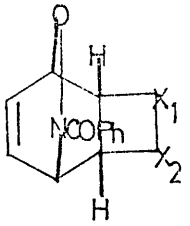
(29a)



(29b)



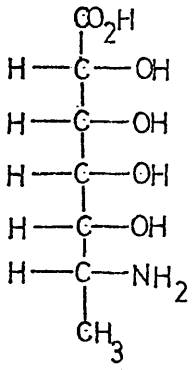
(30a)



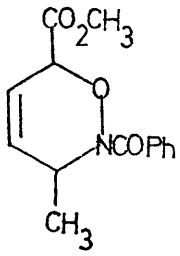
(30b)

$X_1 = \alpha$-Br, $X_2 = \beta$-Br

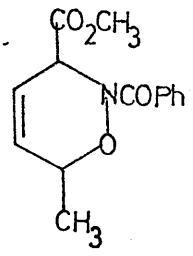
$X_1 = \beta$-Br, $X_2 = \alpha$-Br



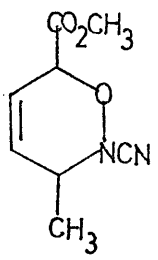
(31)



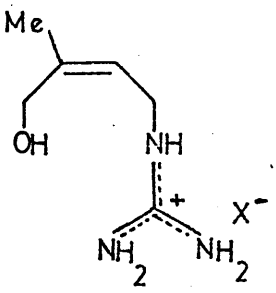
(32a)



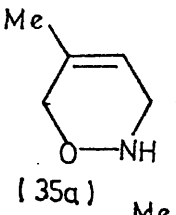
(32b)



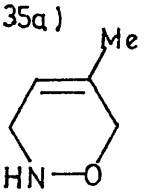
(33)



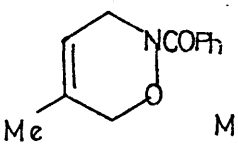
(34)



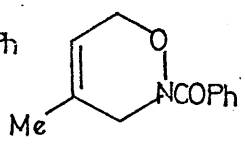
(35a)



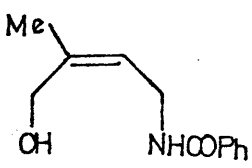
(35b)



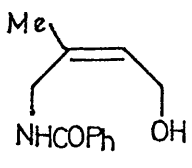
(36a)



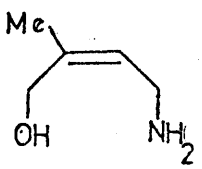
(36b)



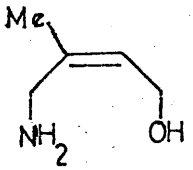
(37a)



(37b)



(38a)



(38b)

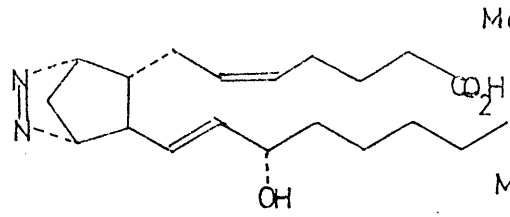
Cyclo-octatetraene is well known to react as a 'sluggish' diene because its major valence tautomer (fig. 3) does not contain a planar, conjugated diene system.[17] It was thought that although a reaction between cyclo-octatetraene and nitrosocarbonylbenzene might occur, its rate could be slow relative to the rates of decomposition of PhCONO by other routes. This idea was put to the test and found to be correct when a preparation of the crystalline cyclo--octatetraene/nitrosocarbonylbenzene adduct (28) was achieved by the 'indirect' reaction of nitrosocarbonylbenzene with cyclo-octatetraene. Under the conditions of the 'indirect' generation of RCONO, a low steady-state concentration of free RCONO is maintained by the equilibrium (fig. 1) and allows the transfer of RCONO from D.M.A. to the appropriate 'diene'. However self-decomposition of RCONO is discouraged as the free species is at low concentration and may always reform the adduct with D.M.A. rather than undergoing a slow, bimolecular[18] self-decomposition. This method of generating the cyclo-octatetraene/nitroso-carbonylbenzene adduct was unsuitable for the preparation of the adduct in quantity and an alternative route was sought. Bromination of cyclo-octatetraene with 1 molecular equivalent of bromine gives trans-dibromocyclo-octatetraene[19] (29) which, unlike cyclo-octatetraene, is known[20] to be a reactive diene towards Diels-Alder addition. The nitroso-carbonylbenzene dieneophile was no exception to the rule and the isomeric mixture of adducts (30) was formed readily and

in good yield by the direct method. No attempt was made to separate the diasterioisomeric pairs of isomers and the non--crystalline mixture was carried through to the next stage of this preparation which involved the reductive elimination of bromine by the action of zinc dust in dimethylsulphoxide.[20] This reaction also took place in good yield to yield the adduct (28), identical in all respects to the sample prepared from cyclo-octatetraene itself.

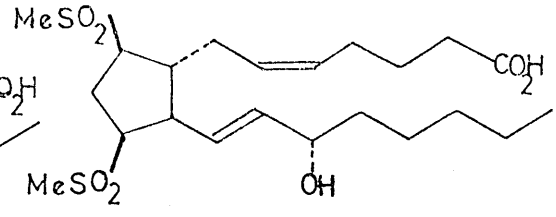
A further purpose in the investigations of the chemistry of nitrosocarbonyl-compounds was to explore the synthetic utility of their Diels-Alder adducts. In general, nitroso-alkanes and nitrosoarenes react slowly with dienes and the rapid reaction of nitrosocarbonyl-compounds with dienes was thought to be capable of exploitation. The amino-sugar (31) was synthesised by Belleau and co-workers[21] by osmylation and hydrolysis of the dihydro-oxazine (32a). The dihydro-oxazine was itself prepared by the benzoylation of the product formed by *in situ* ethanolysis of the adduct of methyl *trans*,*trans*--sorbate and 1-chloro-1-nitroso-cyclohexane.[22] It was hoped that a more efficient single step preparation of the key compound (32a) could be achieved by formation of the adduct between PhCONO and methyl *trans*,*trans*-sorbate. However, attempts to prepare this intermediate by both the 'direct' and 'indirect' methods gave neither of the possible adducts (32a) or (32b). This was a surprising shortcoming of nitroso-carbonyl-dieneophiles as the methyl *trans*,*trans*-sorbate/ nitrosyl cyanide adduct (33) is known.[23] It is not known

whether RCONO generally does not react with electron-deficient dienes.

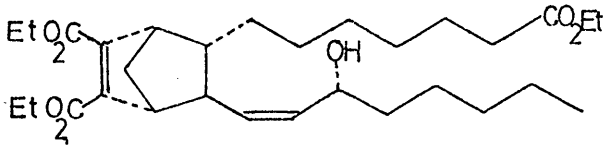
The naturally occurring hydroxygalegine (34) has been synthesised by Leonard and Playtis[24] by zinc reduction of the dihydro-oxazine (35a). The oxazine itself was obtained by the in situ ethanolysis of the cyclo-addition product formed from isoprene and 1-chloro-1-nitrosocyclohexane. The authors claim that only one of the two possible geometrical isomers (35) is formed but confess that the yield of this isomer is very low due to the predomination of the 'ene reaction at the allylic methyl group. The 'ene reaction of the RCONO species has been studied[18] and is known to proceed at a rate which is much slower than the reaction of RCONO with a diene. With this fact in mind an efficient synthesis of (34) was forecast. When PhCONO reacted in the 'direct' manner with isoprene a chromatographically homogeneous product was obtained in good yield. However, the CH_3-resonance in the n.m.r. of the oil consisted of two singlets of different intensities. The product was thought to be a mixture of two isomers (36a) and (36b). Despite this, the zinc/acetic acid reduction of the mixture was carried out. This reduction took place to give the mixture of amido alcohols (37a) and (37b), but the reduction itself took several days to reach completion and the yield was not high. The sluggishness of this reaction is not witnessed in the zinc reduction of diene/nitrosoarene adducts[1] and was attributed to the presence of the carbonyl group in the



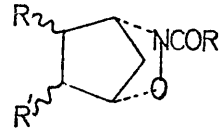
(39)



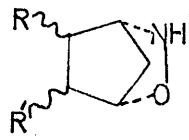
(40)



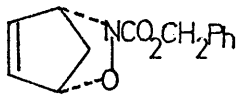
(41)



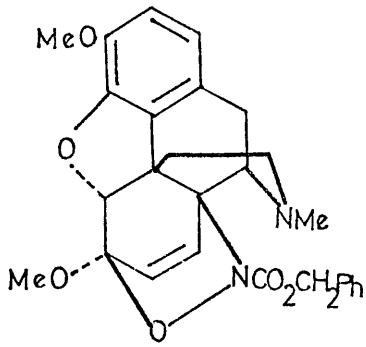
(42)



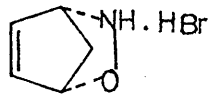
(43)



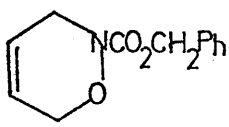
(44)



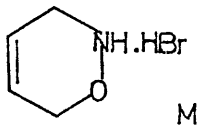
(45)



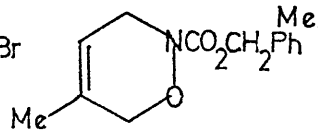
(46)



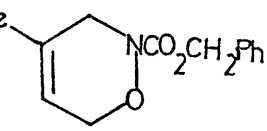
(47)



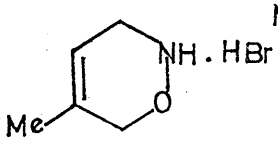
(48)



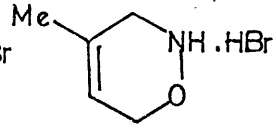
(49a)



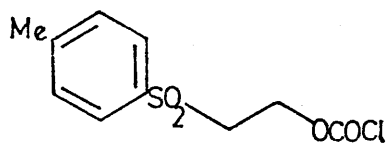
(49b)



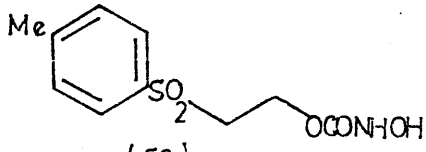
(50a)



(50b)



(51)



(52)

RCONO adducts. This was the first major disadvantage encountered in the attempts at synthesis with RCONO cyclo-adducts.

Attempts to hydrolyse the amido alcohols (37) in strong aqueous base gave only a poor recovery of starting material and it was suspected that the products, the amino alcohols (38a) and (38b) were more soluble in water than in organic solvents.

Another field of interest was the synthesis of prostaglandin endoperoxide aza-analogues. Corey and co-workers[25] have synthesised the azo-prostanoid (39) by simultaneous displacement of the methanesulphonyl groups in (40) with hydrazine and dehydrogenation of the resulting derivative. Of more general applicability is the synthesis of the analogue (41) by Abatjoglou and Portoghese,[26] the primary starting materials in this synthesis being diethyl acetylenedicarboxylate and cyclopentadiene. A similar synthetic sequence with RCONO would yield an analogue of general structure (42) but, as the ultimate target was a hydroxylamine derivative (43), the crucial step was the de-acylation of (42). Hydrolysis of the amide linkage in RCONO cyclo-adducts proved to be the second major stumbling block in attempts at synthesis with these compounds. Attempted enthanolysis of the cyclopentadiene/nitroso-carbonylmethane adduct under mild conditions was unsuccessful and O-methylation of the cyclopentadiene/nitrosocarbonyl-benzene adduct followed by mild acid hydrolysis gave only

methyl benzoate. An alternative approach to de-acylation was clearly desirable and is described later.

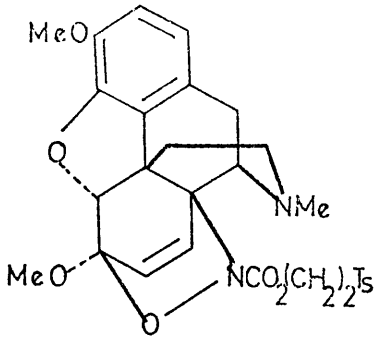
9,10-Dimethylanthracene is expensive and difficult to make,[12] it was hoped, therefore, that the cyclopentadiene/RCONO adducts might be used in place of D.M.A./RCONO adducts in the thermal transfer of RCONO to other dienes. In order to test whether cyclopentadiene/RCONO adducts were subject to the same dissociative thermal equilibrium as the D.M.A./RCONO adducts, a solution of the cyclopentadiene/PhCONO adduct in hot benzene was treated with triphenylphosphine and the reaction monitored by i.r. spectroscopy. It was already known[12] that PhCONO, liberated thermally from its adduct with D.M.A., is converted efficiently by triphenylphosphine into phenyl isocyanate. The hoped-for dissociation was revealed by presence of a phenyl isocyanate -N=C=O absorption of increasing intensity. Having shown that the dissociation did occur, a successful transfer of PhCONO from cyclopentadiene to thebaine was carried out in good yield.

1.2. Nitrosoformate and Nitrosoformamide/Diene Adducts

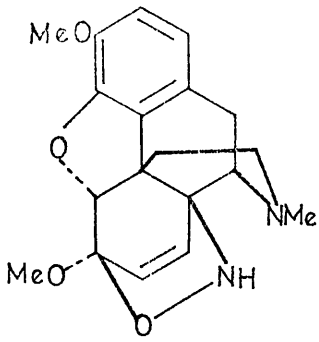
The two outstanding obstacles to the use of the RCONO adducts in further synthesis was the resistance of the N-O bond to reductive cleavage in the presence of the amide carbonyl group, and the lack of a sufficiently mild method of de-acylation of the adducts. In an attempt to overcome these problems, it was thought that nitrosoformate species

(ROCONO), analogous to RCONO, may well exist transiently and form more easily de-acylated cyclo-adducts. The oxidation of N-hydroxy-urethanes had been studied by Boyland and Nery[27] and products of the general formula ROCONHOCOR obtained but the authors did not postulate ROCONO as a possible intermediate in the oxidation. Breslow and co-workers[28] had however postulated such an intermediate when azidoformates were thermally decomposed in dimethyl sulphoxide. Benzyl-N-hydroxycarbamate[27] was oxidised with tetraethylammonium periodate in the presence of cyclopentadiene, and the adduct (44) obtained as an oil. The corresponding thebaine/benzyl nitrosoformate adduct (45) however was obtained, in a similar fashion, as a crystalline solid in good yield. In an attempt to convert the adduct (44) to the salt (46), the adduct was treated with HBr in acetic acid[29] at room temperature. Although carbon dioxide was evolved and benzyl bromide isolated, none of the desired salt was obtained. This was possibly due to a retro-Diels-Alder formation of cyclopentadiene under the reaction conditions.

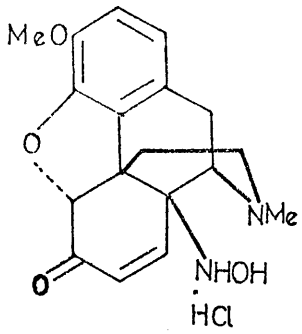
To test this theory, and to ensure that the conditions for de-acylation employed were correct, it was decided to form and de-acylate a simpler adduct. The adduct of choice was the butadiene/benzyl nitrosoformate adduct (47) because the salt (48) would have little ring strain unlike the strained, bicyclic hydrobromide (46). The adduct (47) was obtained by the 'direct' method. Treatment of (47) with



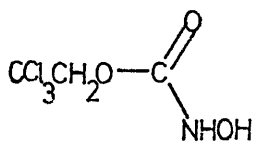
(53)



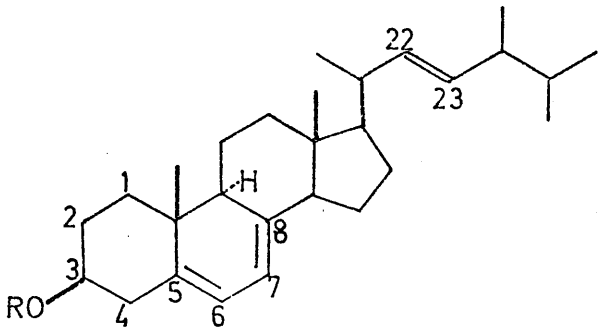
(54)



(55)

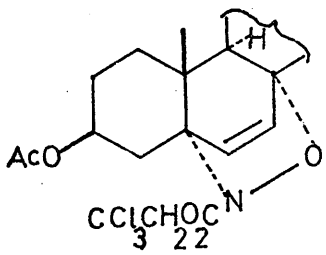


(56)

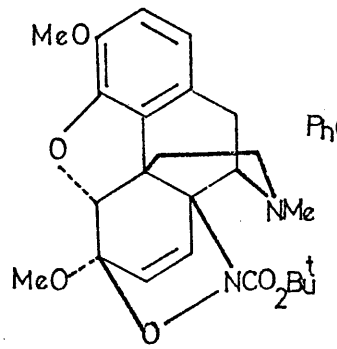


(57) a; R=Ac

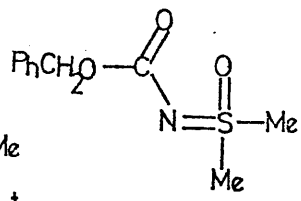
b; R=H



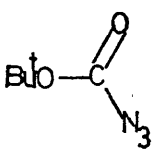
(58)



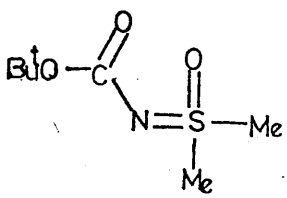
(59)



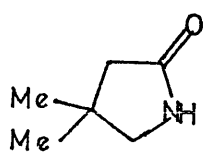
(60)



(61)



(62)



(63)

HBr in acetic acid, under the same conditions which failed to give (46) afforded a good yield of the crystalline hydrobromide (48).

Having determined that the de-acylation of benzyl nitrosoformate cyclo-adducts could be performed efficiently it was decided to apply this system to the problem of synthesising the amino alcohols (38). The mixture of isoprene adducts (49) was obtained by the 'direct' method, and converted with HBr in acetic acid into a mixture of the isomeric salts (50). This mixture was treated with zinc dust in acetic acid but none of the desired mixture of amino alcohol could be extracted from the aqueous phase after the neutralisation of the acetic acid. Since the alcohols (38) were not obtained in two different reactions, one a hydrolysis and the other a reduction; it was assumed that this was due to their high solubility in water.

Having had a limited success with the de-acylation of benzyl nitrosoformate adducts we decided to investigate other nitrosoformate adducts which might de-acylate under a range of mild conditions. One amino protecting group which is labile under mild basic conditions[30,31] is the β-(4-toluene-sulphonyl)-urethane derivative of amines. The chloroformate (51) was prepared by the literature method[31] and treated with hydroxylamine to give the new N-hydroxy-carbamate (52). This was converted by periodate oxidation into the corresponding nitrosoformate which was trapped as its thebaine adduct (53). Treatment of this adduct with 1,5-diazabicylo-

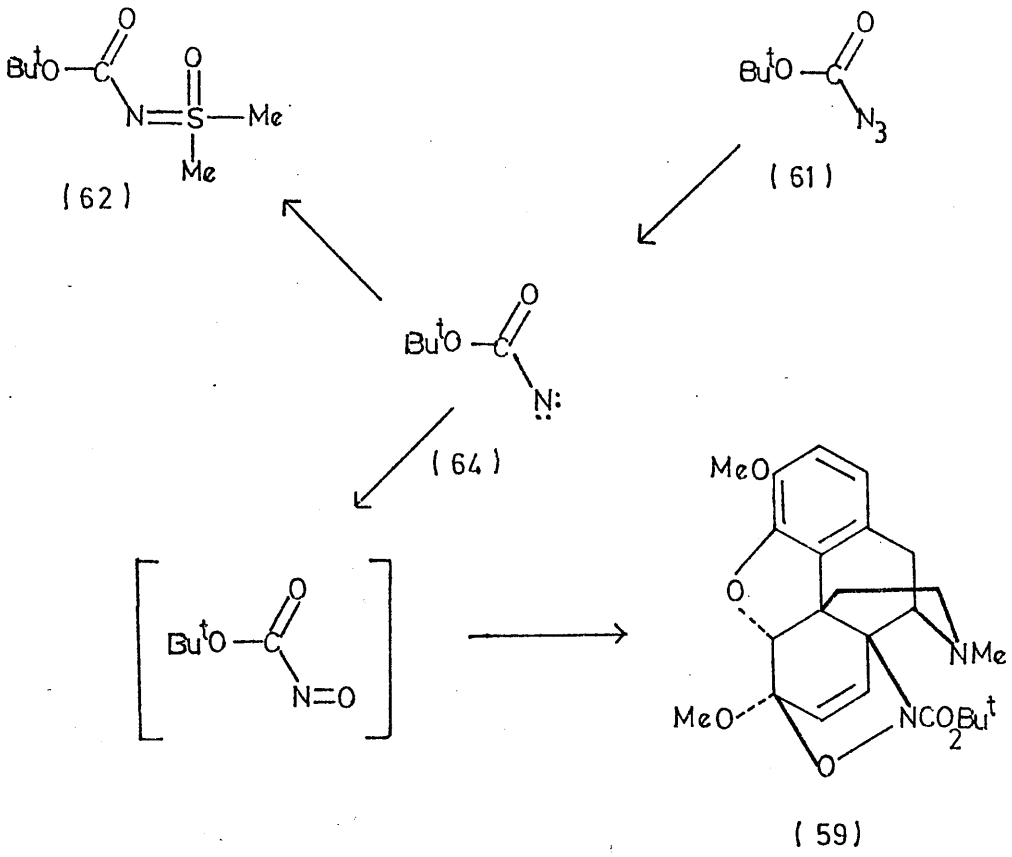
[4.3.0]non-5-ene[30] in benzene gave an unstable compound whose n.m.r. spectrum was consistent with the structure (54). This 'HNO adduct' (54) could not be crystallised, and decomposed in a few hours to thebaine. However, when the de-acylation was repeated and an *in situ* acid hydrolysis of (54) carried out, 14β-hydroxyaminocodeinone was obtained in good yield as its hydrochloride (55).

In the hope of obtaining a reductively labile nitrosoformate adduct, trichloroethyl chloroformate[32] was converted to 2,2,2-trichloroethyl *N*-hydroxycarbamate (56). This was oxidised in the presence of ergosteryl acetate (57a) to yield a single crystalline adduct (58). However, when this adduct was treated with zinc in acetic acid[32] the major product of the reaction was ergosteryl acetate. The adduct (58) itself was stable in acetic acid and it was concluded that, although de-acylation had occurred, the bicyclic dihydro-oxazine produced had undergone a retro-Diels-Alder reaction producing the parent diene. The orientation of addition of 2,2,2-trichloroethyl nitrosoformate to ergosteryl acetate was assigned as shown for reasons which will be discussed below.

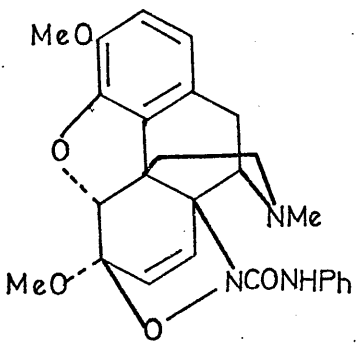
Another amine protecting group is the t-butoxycarbonyl group which is labile in mild acid.[33,34] Oxidation of known[35] t-butyl *N*-hydroxycarbamate in the presence of thebaine gave the nitrosoformate adduct (59). It was hoped that cleavage of this adduct with dry HCl might give the

hydrochloride of 'HNO adduct' (54). However when a dry methanolic solution of (59) was treated with dry HCl and diluted with ether only the hydrochloride (55) of the hydrolysis product was obtained. The deprotection had again been successful but the strained ketal system in the product was obviously labile to hydrolysis by traces of atmospheric moisture.

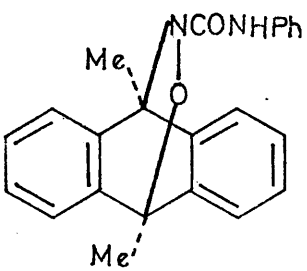
Noting the reference of Breslow[28] to nitrosoformate transients, Kirby and Sharma[36] had studied the thermal decomposition of benzyl azidoformate in dimethyl sulphoxide. In the absence of thebaine the principal product was the sulphoximine derivative (60) and in the presence of thebaine both the sulphoximine (60) and the adduct (45) were obtained. It was desirable to test the generality of these findings by repeating the experiments with another azidoformate. When t-butyl azidoformate (61) was heated alone in dimethyl sulphoxide at 115°C the sulphoximine (62) was obtained. However the n.m.r. spectrum of the crude sulphoximine indicated the presence of smaller amounts of other compounds having quaternary methyl groups. These by-products are thought to be a result of radical decomposition of the azide. A radical mechanism had been proposed by Breslow[28] and radical decomposition products had been detected in the case of benzyl azidoformate.[36] Another possible by-product was the oxazolidone (63) which is the major product of the thermal decomposition of t-butyl azidoformate in inert solvents.[37] However no spectral evidence for (63) as a by--product could be found. When five molecular equivalents



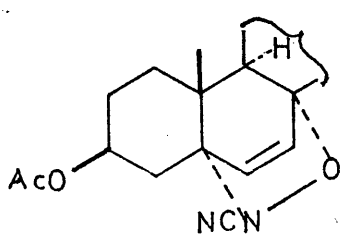
(fig. 4)



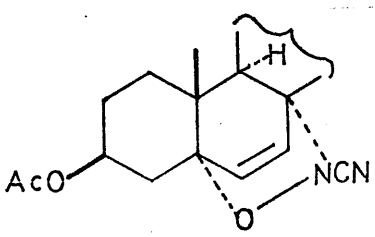
(65)



(66)



(67a)



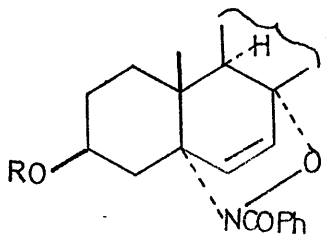
(67b)

of the azide were decomposed in dimethyl sulphoxide containing one molecular equivalent of thebaine both the sulphoximine (62) and the adduct (59) were obtained but no thebaine. However when thebaine was in excess the sulphoximine (62) was still obtained together with the adduct (59) and unreacted thebaine. This indicates a partitioning of the nitrene (64) between attack at sulphur and attack at oxygen in dimethyl sulphoxide (fig. 4) and is in accordance with the behaviour of benzyl azidoformate.

Having established that ROCONO species exist long enough to form cyclo-adducts with dienes, we wondered whether the corresponding nitrosoformamides, RR'NCONO, might behave in a similar fashion. Accordingly oxidation of N-hydroxy-N'-phenylurea in the presence of thebaine yielded the thebaine/N-phenylnitrosoformamide adduct (65). An attempt to make the D.M.A. adduct (66) by the same route gave an unstable product which could not be crystallised. An n.m.r. spectrum of the crude product showed two sharp singlets in the region expected for the methyl groups of adduct (66) but also the methyl resonance for D.M.A. itself which became comparatively stronger as the adduct decomposed at room temperature.

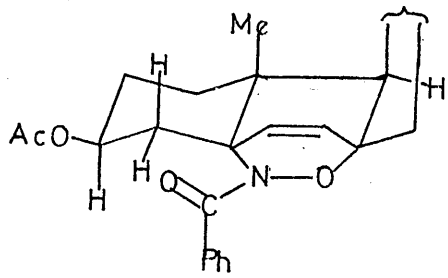
1.3. Adducts of Ergosteryl Acetate with Activated C-Nitroso--compounds.

Nitrosyl cyanide generated by the 'indirect'method had been shown to react with ergosteryl acetate (57a)[23] to yield

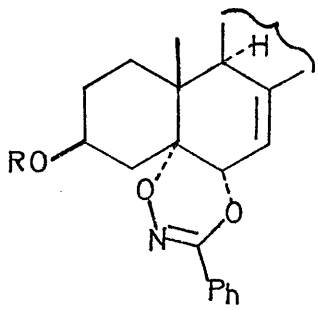


(68) ; R=Ac

(72) ; R= H

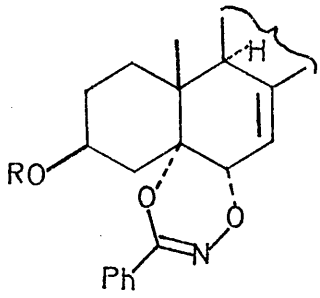


(69)



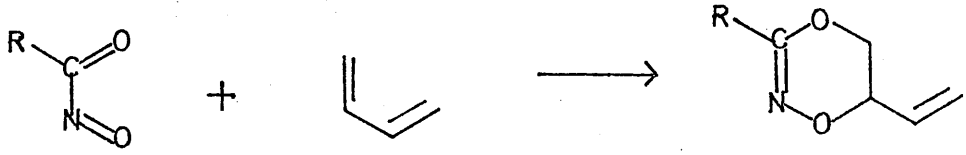
(70a);R=Ac

(71a);R=H

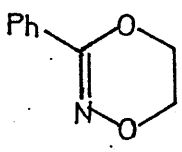


(70b);R=Ac

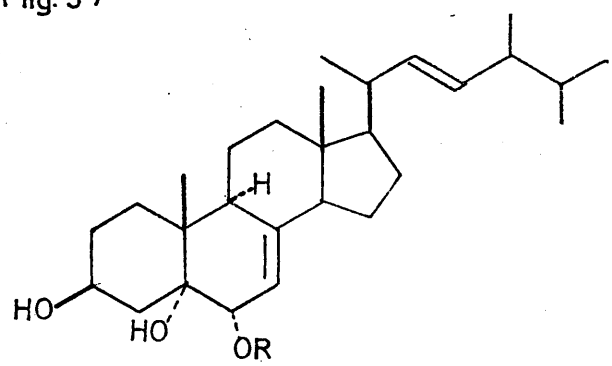
(71b);R=H



(fig. 5)



(73)



(74) ; R=COPh

(75) ; R=H

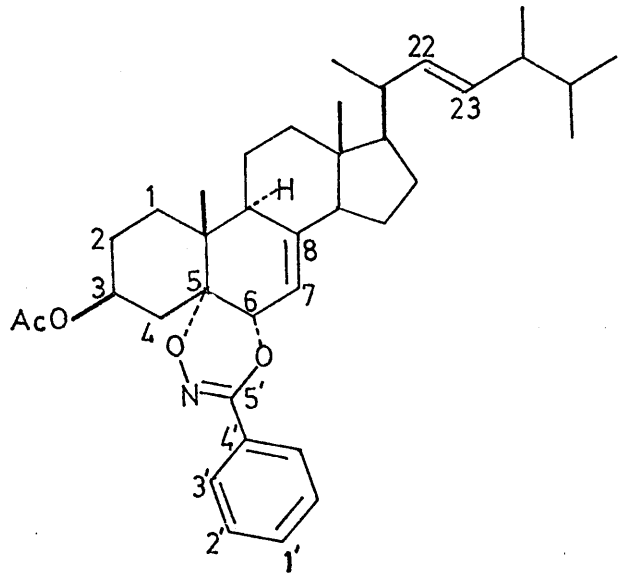
two adducts. These adducts were assigned the structures (67a) and (67b), both showing two doublets, J 9Hz, in the region τ 3 - 4 which were attributed to the olefinic protons at C-6 and C-7. It was of interest then to investigate the reaction of nitrosocarbonylarenes and nitrosocarbonylalkanes with this large, unsymmetrically substituted diene. Reaction of ergosteryl acetate with PhCONO in the 'direct' fashion gave a mixture of two adducts. The adduct of higher Rf, referred to as the type I adduct, did not have an n.m.r. spectrum resembling those of either of the adducts (67) and no amide carbonyl absorption was present in its i.r. spectrum. The lower Rf adduct (type II), by contrast, showed a characteristic pair of doublets in the region τ 3 - 4 and a strong i.r. amide carbonyl absorption; also the n.m.r. spectrum of this adduct had a one-proton doublet of doublets, J 13Hz and 5Hz, at τ 6.38, well separated from the rest of the saturated steroid signals. Examination of models showed that this resonance could only be reasonably attributed to the 4α-H of the structure (68). This is seen more clearly in the diagram (69). The 4α-H is deshielded due to the magnetic anisotropy of the amide carbonyl. The 13Hz coupling is due to vicinal coupling with the 4β-H and coupling with the 3-H, by the Karplus rule, would be ca. 5Hz. Decoupling experiments established that the 5Hz coupling was indeed with the proton at position 3.

The n.m.r. spectrum of the type I adduct displayed two broadened singlets at τ 4.95 and 5.37. Because no other reasonable assignment could be made, these resonances were attributed to the protons at C-6 and C-7. It was further hypothesised that this assignment was consistent with a structure (70a) or (70b), the broadening of the two singlets being due to residual coupling between the 6-H and the 7-H. These structures would also explain the absence of the amide carbonyl in the i.r. spectrum of the type I adduct. Furthermore, analysis and mass spectroscopy were consistent with the molecular formula of an adduct of PhCONO with ergosteryl acetate and it was credible that a 1,2-cyclo-addition with respect to the diene component (fig. 5) had occurred. Ergosterol (57b) formed corresponding type I and type II adducts with PhCONO. These were assigned the structures (71) and (72) respectively, on the basis of their spectral similarity with their corresponding ergosteryl acetate adducts. The type I adducts (70) and (71) were interconvertible by hydrolysis and acetylation suggesting that substituents at the C-3 position were not involved in the formation of the unusual type I adducts (70) and (71).

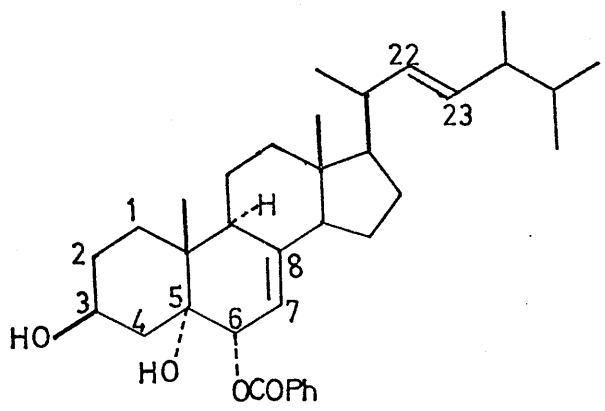
In order to confirm its structure, a degradation of the type I adduct (70) was sought. However, the adduct was resistant to strong base and when hydrolysed in an acidic medium the necessary conditions were so forcing that the major products were ones which had lost any hydroxyl

groups by elimination of water. Hydride reduction was also unsatisfactory because a host of low-yield products was produced. Since degradation was difficult, it was decided to seek information from the ^{13}C n.m.r. spectrum of the type I adduct. For comparison the known compounds (73) and (74) were prepared. The former[38] was prepared by the action of potassium benzohydroxamate and potassium carbonate on 1,2-dibromoethane; the latter[39] by the action of perbenzoic acid[40] on ergosterol. It was gratifying to note that the C-6 and C-7 protons absorbed as broadened singlets in the benzoate (74). The ^{13}C n.m.r. spectra of the type I adduct and the two model compounds were recorded and an assignment of the low field nuclei made. This assignment is summarised in Table 1 and was made with the help of off-resonance decoupling experiments and with reference to the published[41] assignments for the spectrum of ergosterol. A good correlation was found between the steroid carbons of the type I adduct (70a) or (70b) and the benzoate (74). The correlation between the aromatic ^{13}C resonances of (73) and the type I adduct were also good, and a fair correlation between the 'imine' carbons, in both compounds, existed.

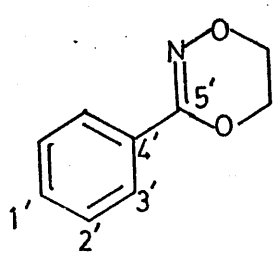
The benzoate (74) was converted by base hydrolysis, to the known steroidal triol[39] (75). The triol (75) was then employed to identify (chromatrographically) its presence among the hydride reduction products of the type I adduct. This was accomplished and the triol (75) was isolated from


22
23
H
1
2
3
8
5
6
7
AcO
4
O
O
N
5'
4'
3'
2'
1'

(70a)


22
23
H
1
2
3
8
5
6
7
HO
4
HO
OCOPh

(74)

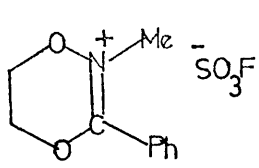

N
O
5'
O
4'
1'
3'
2'

(73)

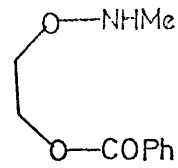
Table 1

^{13}C n.m.r. Chemical Shifts - δ ($CDCl_3$)

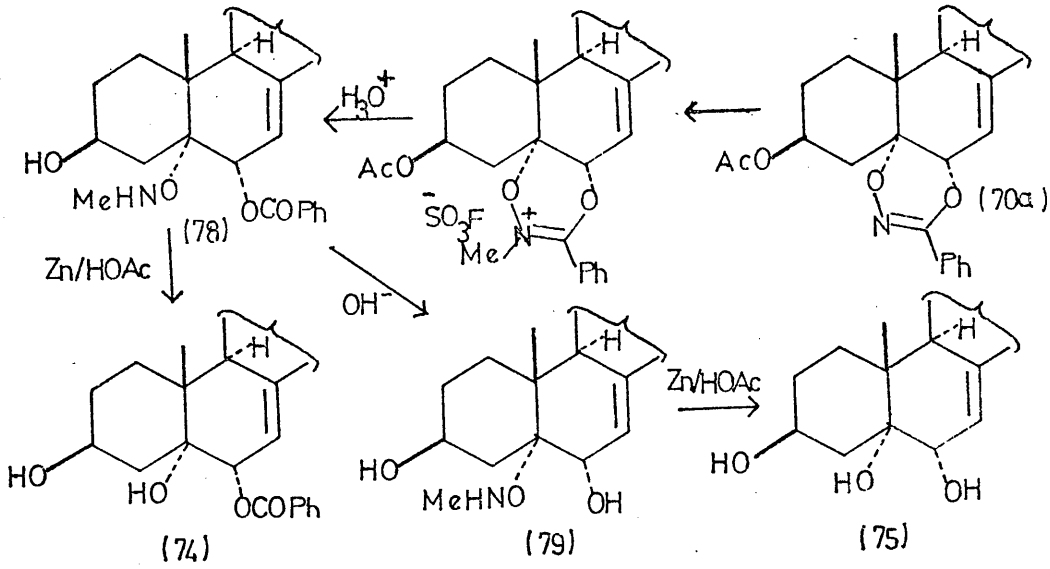
Carbon Atoms	(70a)	(74)	(73)
8	145.3	144.5	-
23	135.4	135.5	-
22	132.1	132.3	-
7	114.5	115.3	-
5	75.5	75.6	-
6	72.6	74.7	-
3	69.9	67.4	-
5'	150.8	-	153.9
4'	130.9	-	130.9
1'	130.1	-	130.3
3'	128.1	-	128.2
2'	125.6	-	125.6



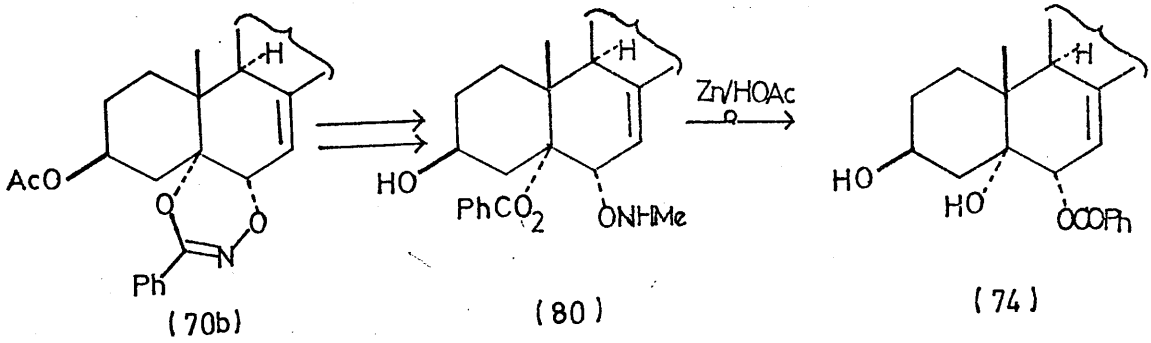
(76)



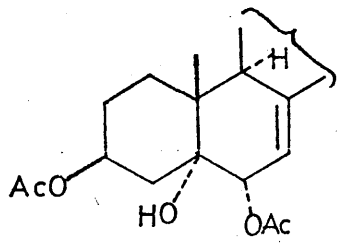
(77)



(fig. 6)



(fig. 7)



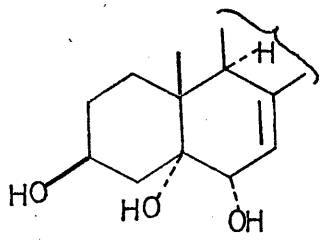
(81)

the reduction mixture in ca. 10% yield and was identical in all respects with the authentic material.

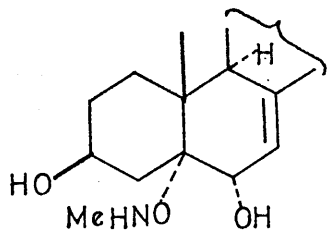
Isolation of (75) was strong supporting evidence for one of the structures (70a) or (70b) but not until N--alkylation of the type I adduct was achieved could the structure be unequivocally assigned. Attempts to alkylate the model compound (73) with methyl iodide and benzyl bromide were unsuccessful but 'magic methyl' (methyl fluorosulphonate)[42] proved effective. The resulting fluorosulphonate salt (76) was hydrolysed in dilute acid, at room temperature, to give a benzoate which was assigned structure (77) on spectral evidence. Having carried through the proposed degradation for the model compound, we applied the same sequence of reactions to the type I adduct and obtained the benzoate (78). The structure of this compound was defined when zinc/acetic acid reduction of (78) produced the known compound (74). Base hydrolysis of the benzoate (78) gave the substituted hydroxylamine (79) which, in turn, gave the previously prepared triol (75). These transformations (fig. 6) confirm that structure of type I adduct is (70a) and not (70b). The basis of this assignment rests on the identification of (74) as a known compound thereby showing that the hydroxylamino group is attached to position 5 rather than 6 in the sterol (79). It might be argued however, that, during the zinc reduction of (80) [hypothetically derived from (70b)], trans-esterification had occurred (fig. 7) to yield (74).

This would mean that the assignment of (70a) as the structure of the type I adduct was in error. To confirm that the benzoyl group was at position 6 in (78) it was decided to compare the 6-H chemical shifts of all the compounds in the degradation scheme and to complete the picture the triol (75) was acetylated to give its diacetate[39] (81). Table 2 lists the chemical shifts of the 6- and 7-protons in the compounds of interest. It is easily seen that for the steroids not acylated at position 6 the 6-H has a chemical shift higher than τ 5.7. In the type I adduct itself this proton's chemical shift occurs lower down-field at τ 5.37, whereas in the acetate (81) and the benzoate (74) the shift of this proton is below τ 5.0. The benzoate in question (78) has a 6-H chemical shift of τ 4.56 which agrees so well with corresponding resonance at τ 4.50 in the benzoate (74) that there is no doubt that the benzoyl group is at the 6-position in (78).

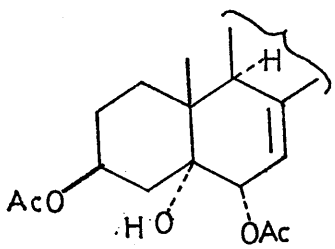
Having established that there were present, in this system, two types of adduct we wished to test their relative stabilities. Both the type I adduct (68) and the type II adduct (70a) were stable in benzene at 80°C. The type I adduct was stable in toluene at 111°C even in the presence of triphenylphosphine. However the type II adduct decomposed to give ergosteryl acetate when heated with triphenylphosphine in toluene at 111°C. This indicated a reversal of adduct formation at this temperature and, when the adduct was heated without triphenylphosphine at 111°C, a slow conversion



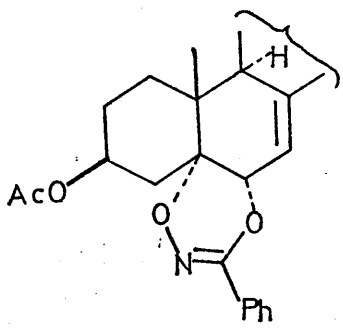
(75)



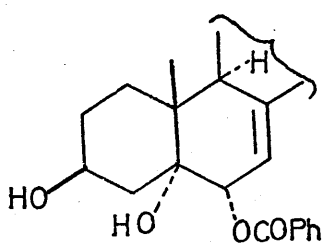
(79)



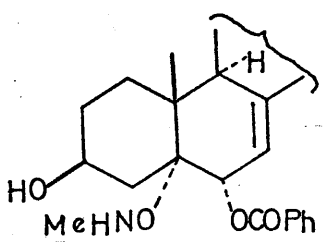
(81)



(70a)



(74)

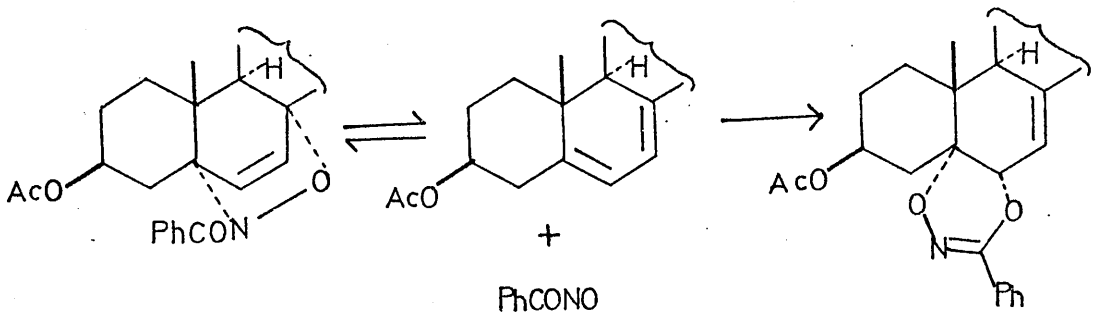


(78)

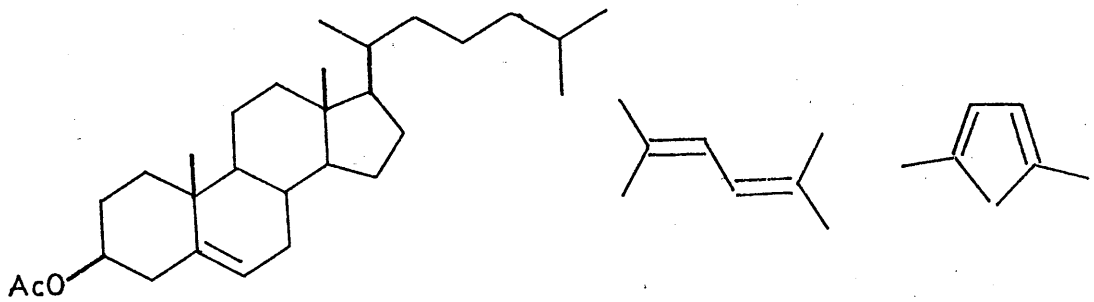
Table 2

^{1}H n.m.r. Chemical Shifts

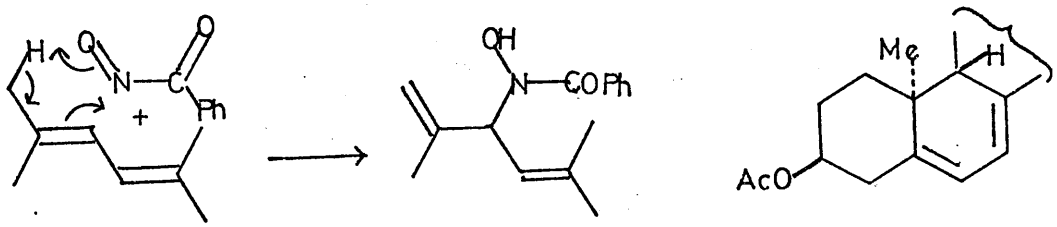
Compound	Solvent	τ (7-H)	τ (6-H)
(75)	D_6-D.M.S.O.	5.16	5.71
(79)	$CDCl_3$	4.83	5.90
(81)	$CDCl_3$	5.09	4.95
(70a)	$CDCl_3$	4.95	5.37
(74)	$CDCl_3$	4.96	4.50
(78)	$CDCl_3$	5.11	4.56



(fig. 8)



(82) (83) (84)



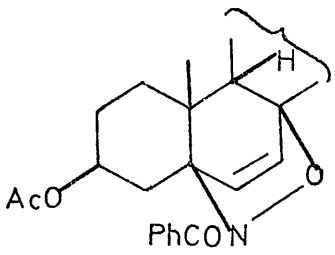
(85) (86)

(87) (88)

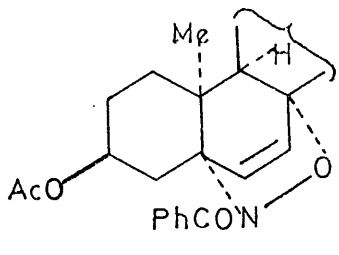
to adduct type I was noted. These observations show that the type II, but not the type I adduct dissociates at 111°C and the type I adduct is the thermodynamically more stable (fig. 8).

A search for other type I adducts was made starting with an attempt to prepare the type I adduct of cholesteryl acetate (82) and PhCONO. However both the 'direct' and 'indirect' methods of generating PhCONO were unsuccessful and it was apparent that the extra double bond was necessary, perhaps to 'activate' the 5,6 double bond. In order to test this theory a diene which was unable to react as a Diels-Alder 4π electron system in a cyclo-addition reaction was selected. 2,5-Dimethyl-2,4-hexadiene (83), because of the steric interaction of the methyl groups in the conformation (84) is predominantly in the form (83) under normal conditions. An attempt to react (83) with PhCONO in the 'direct' manner was totally unsuccessful and when the 'indirect' method was employed only a slow 'ene reaction[18] to produce the substituted hydroxamic acid (85) occurred. In a move nearer to ergosteryl acetate, the reaction of PhCONO (generated by the 'direct' method) with lumisteryl acetate† (86), pyrocalciferyl acetate[43] (87), and isopyrocalciferyl acetate[43] (88), was studied. Lumisteryl acetate formed no adduct at all; but lumisteryl acetate[44] is known to be a less reactive diene than ergosteryl acetate; whilst pyrocalciferyl and isopyrocalciferyl acetates each formed single 1,4 (type II) adducts [(89) and (90) respectively].

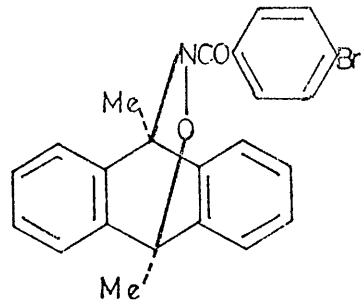
† courtesy of Professor Sir D.H.R. Barton



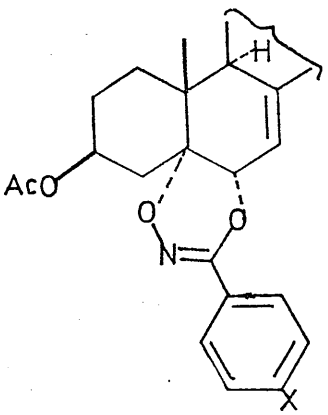
(89)



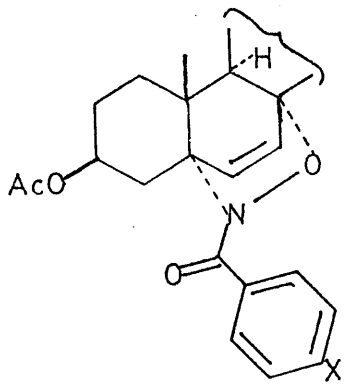
(90)



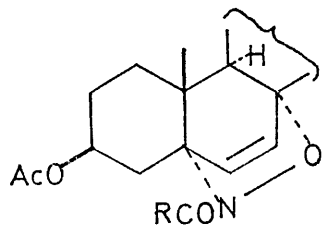
(91)



(92) a; X=Br
b; X=NO_2
c; X=OMe

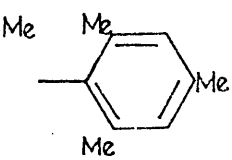


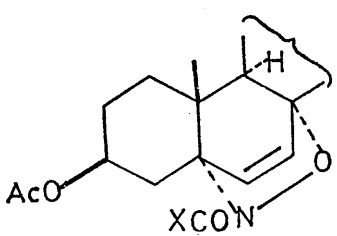
(93) a; X=Br
b; X=NO_2
c; X=OMe



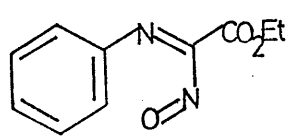
(94); R=Me

(95); R=

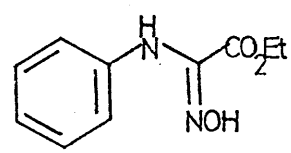




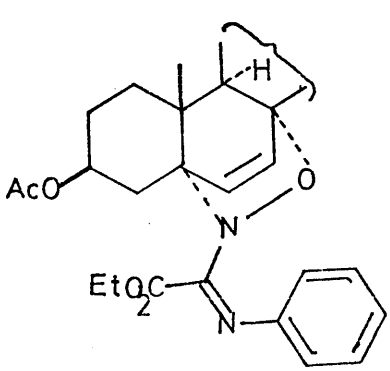
(96) a; X= OCH_2Ph
b; X= NHPh
(58); X= OCH_2CCl_3



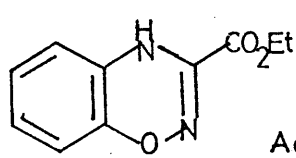
(97)



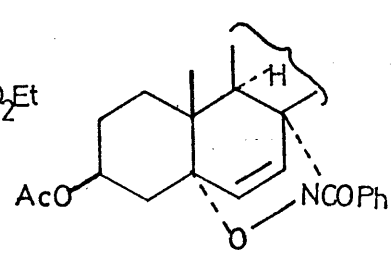
(98)



(99)



(100)



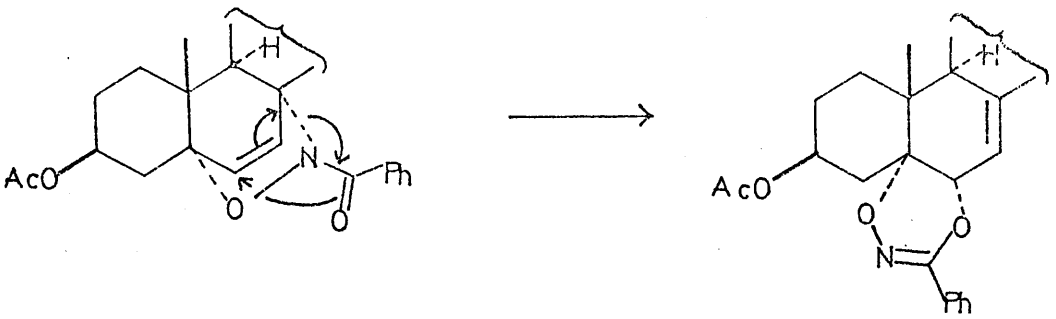
(101)

A range of nitrosocarbonyl-compounds were next added to ergosteryl acetate in order to find out how general formation of the type I adducts was with respect to variation in the structure of the nitrosocarbonyl moiety. 4-Bromonitrosocarbonylbenzene, when generated by both 'direct' and 'indirect'[from (91)] methods, gave adducts with ergosteryl acetate which were spectrally similar to the type I and type II PhCONO adducts [(92a) and (93a) respectively]. 4-Nitronitrosocarbonylbenzene gave two adducts (92b) and (93b) and 4-methoxynitrosocarbonylbenzene likewise [(92c) and (93c)]. However, nitrosocarbonylmethane reacted with ergosteryl acetate in the 'direct' manner to give a single type II adduct (94). This led to the idea that only nitrosocarbonylarenes would form type I adducts, due to the electronic effect of the conjugated aromatic ring. 2,4,6-Trimethylnitrosocarbonylbenzene, generated by oxidation of mesitohydroxamic acid, like nitrosocarbonylmethane, gave only a type II adduct (95). This gave support to the argument that conjugation of the aromatic ring was important because, as is well known, due to the steric effects of the ortho-methyl substituents in mesitoic acid derivatives, the aromatic ring is twisted out of conjugation with the carbonyl group. To test the generality even further benzyl nitrosoformate, 2,2,2-trichloroethyl nitrosoformate, and N-phenyl nitroso-formamide were all generated oxidatively in the presence of ergosteryl acetate and a single type II adduct [(96a) (58) and (96b) respectively] obtained in each case.

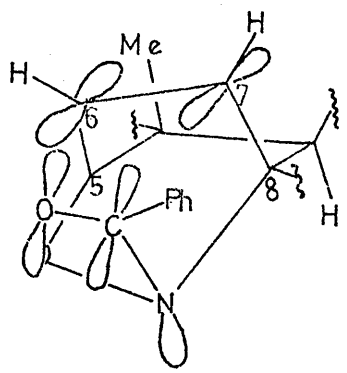
Finally, when Rees's C-nitroso-imine (97) was generated[14] by the lead tetra-acetate oxidation of the amidoxime (98) in the presence of ergosteryl acetate, only the type II adduct (99) and the 1,2,4-benzoxadiazine (100) were obtained.

In an attempt to determine whether the occurrence of only type II adducts in the case of non-aromatic nitroso-carbonyl-compounds was due to kinetic control, the nitroso-carbonylmethane adduct (94) was heated in toluene at 111°C but no trace of any other adduct was detected.

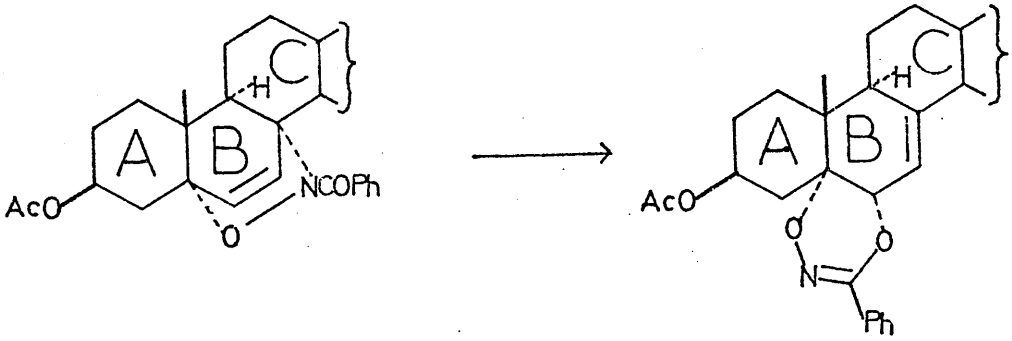
On reflection, the fact that only one 1,4 and one 1,2 adduct were formed between nitrosocarbonylbenzene and ergosteryl acetate seemed strange, especially in light of the two known 1,4 ergosteryl acetate/nitrosyl cyanide adducts (67a) and (67b). The 'directly' generated PhCONO was allowed to react with ergosteryl acetate, as before, and after a careful work-up, at low temperature, the crude reaction mixture was examined by n.m.r. spectroscopy. The expected resonances for the type II adduct were present, but none of the signals associated with the type I adduct were noted and, in their stead, were signals (notably a second pair of doublets in the region τ 3 - 4) which could only be attributed to a type III adduct (101). This mixture of adducts was heated in benzene at 60°C and the n.m.r. spectrum of the mixture taken periodically. A gradual conversion of the type III adduct (101) into the type I adduct (70a) and no change in the type II adduct (68) was noted. It was at last clear that



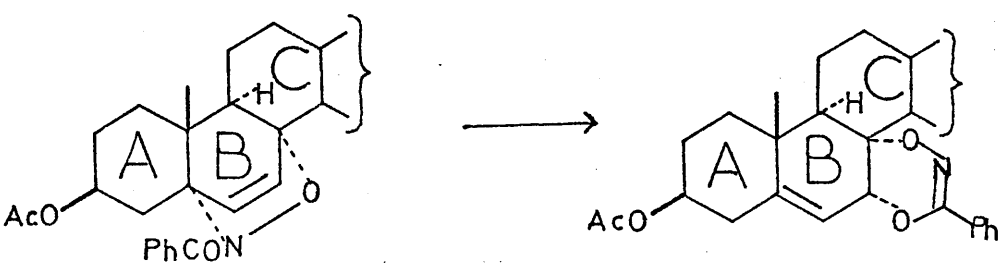
(fig. 9)



(fig.10)



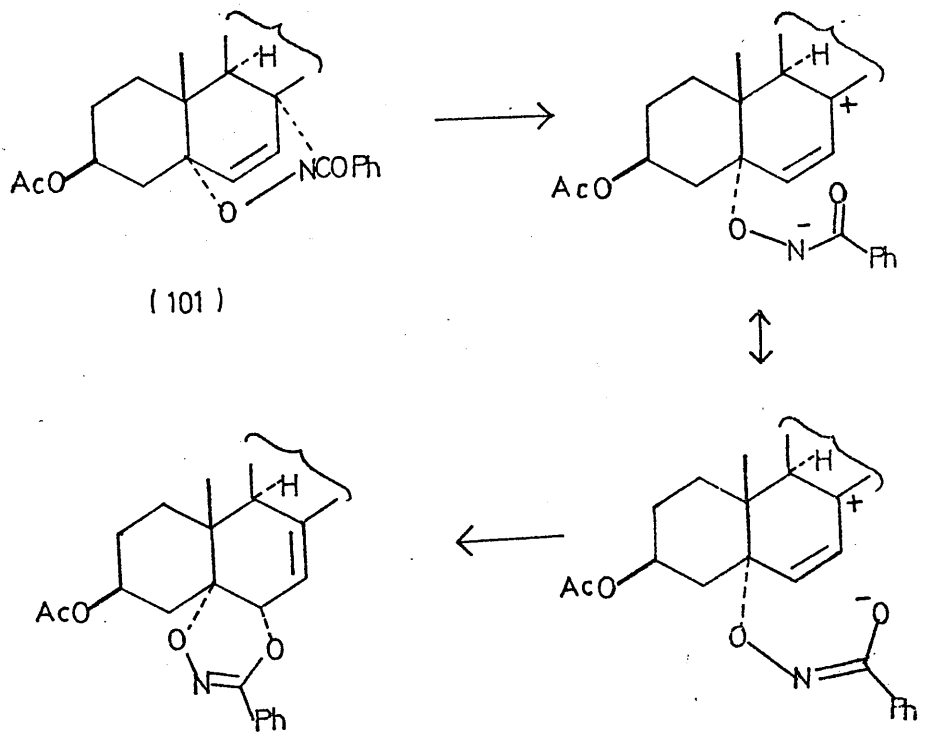
(101) (70a)



(68) (102)

the type I adduct was an 'adduct' only in the formal sense of the word and that, in truth, it was a rearrangement (fig. 9) product of the type III adduct. Inspection of models shows that if the amide nitrogen is briefly pyramidal the p-orbitals of the olefinic bond and the carbonyl are in close proximity (fig. 10). The same is true for a hypothetical rearrangement of the type II adduct but no corresponding rearrangement occurs in this case. This is probably due to the fact that in the rearrangement (101) to (70a) the strain in (101) due to a cis-B/C ring fusion is relieved. However, although (68) also possesses a cis-B/C ring juncture, a corresponding rearrangement would result in another cis-B/C fused system (102) and there would be no equivalent driving force for the rearrangement to take place. The rearrangement as written (fig. 9) implies a thermally allowed 6π electron concerted process and this may well be the case. An ionic mechanism (fig. 11), through the tertiary carbenium ion, cannot however be ruled out. The rate and outcome of the rearrangement are unaffected by the addition of triphenylphosphine which scavenges[12] free RCONO and so it is almost certain that the rearrangement does not follow a dissociative pathway.

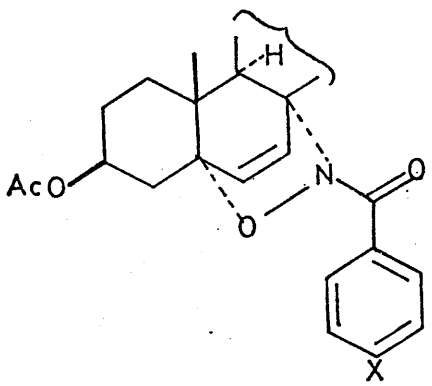
To investigate the possibility of this rearrangement in a simpler system the cyclopentadiene/nitrosocarbonylbenzene adduct (21) was heated at 111°C in toluene. However only a slow decomposition to an intractable mixture was noted.



(101)

(70a)

(fig.11)



(103) ; X=NO_2

(104) ; X=OMe

The corresponding type III adducts were also observed in the 4-nitro- and 4-methoxynitrosocarbonylbenzene cases [(103) and (104) respectively] and a rough order for the rate of their rearrangement to the corresponding type I adducts assessed. This order may be summarised:-

$$\underline{p}\text{-}CH_3O\text{-}C_6H_4 > C_6H_5 > \underline{p}\text{-}NO_2\text{-}C_6H_4$$

Since the relative amounts of the type I and type III adducts formed in any particular case is subject to kinetic control and since both adducts are stable under the conditions required for 'indirect' generation of RCONO (80°C), further information on the nature of the RCONO intermediate became available. For both nitrosocarbonylbenzene and 4-bromo-nitrosocarbonylbenzene the relative amounts of type I and type II adducts obtained with ergosteryl acetate was independent of the method ('direct' or 'indirect') of generation of RCONO. This points to a common intermediate, which is unlikely to be anything other than the free nitroso-carbonylarene and lends strength to previous arguments for the independent existence of RCONO.

EXPERIMENTAL

General Methods

The following instruments were used in acquisition of physical data:-

m.p. (uncorrected)	-	Kofler block
I.R. spectra	-	Perkin Elmer 257
	-	Pye Unicam SP100
^{1}H n.m.r.	-	Varian T-60
	-	Varian HA-100
^{13}C n.m.r.	-	Varian XL-100
	-	Varian CFT-20
Mass spectra	-	A.E.I. MS9
	-	A.E.I. MS12

1.1 EXPERIMENTAL

Cyclopentadiene/Nitrosocarbonylbenzene Adduct (21)

To an ice-cooled solution of freshly distilled cyclopentadiene (660 mg, 10 mmol) and tetraethylammonium periodate[11] (2.50 g, 8 mmol) in redistilled methylene chloride (125 ml), was added, with stirring and in portions over 15 minutes, benzohydroxamic acid (2.0 g, 15 mmol). The reaction mixture was stirred for a further 45 minutes and then washed with saturated aqueous sodium thiosulphate (2 x 50 ml), 10% aqueous caustic soda (2 x 50 ml) and brine (2 x 50 ml). The organic layer was dried ($MgSO_4$) and evaporated to yield the crude cyclopentadiene/nitrosocarbonylbenzene adduct as an oil which crystallised [benzene/light petroleum (b.p. 60 - 80°C)] as colourless prisms, m.p. 78 - 79°C (1.50 g, 74% based on cyclopentadiene) (Found: C, 71.4; H, 5.46; N, 6.91. $C_{12}H_{11}NO_2$ requires C, 71.6; H, 5.51; N, 6.96%), m/e 201, ν_{max} (KBr) 1 630 cm^{-1} (C=O), τ ($CDCl_3$) 2.1-2.9 (5H, m, Ph), 3.78 (2H, bs, olefinic H), 4.74 (2H, bs, O-C-H and N-C-H), 7.90 (1H, dm, J 9Hz, methylene H), 8.22 (1H, d, J 9Hz, methylene H).

1,3-Cyclohexadiene/Nitrosocarbonylbenzene Adduct (23)

This adduct was prepared according to the procedure detailed for the cyclopentadiene/nitrosocarbonylbenzene adduct (21) by the oxidation of benzohydroxamic acid (200 mg, 1.5 mmol) with tetraethylammonium periodate (250 mg, 0.8 mmol) in the presence of 1,3-cyclohexadiene (80 mg, 1 mmol).

The adduct (23) was obtained (181 mg, 84% based on diene) as colourless needles m.p. 108 - 110°C from benzene/light petroleum (b.p. 40 - 60°C) (Found: C, 72.4; H, 6.04; N, 6.30. $C_{13}H_{13}NO_2$ requires C, 72.6; H, 6.05; N, 6.51%), m/e 215, ν_{max} (KBr) 1 640 cm^{-1} (C=O), τ ($CDCl_3$) 2.2-2.9 (5H, m, Ph), 3.48 (2H,m,olefinic H), 4.90 (2H, m, O-C-H), 5.26 (2H, m, N-C-H), 7.95 (2H, bd, J 9Hz, methylene H), 8.52 (2H, bd, J 9Hz, methylene H).

Attempted Preparation of Cycloheptatriene/Nitrosocarbonylbenzene Adducts (26) and (27)

An attempt to prepare these adducts following the procedure described for the cyclopentadiene/nitrosocarbonylbenzene adduct (21) yielded an oil (71% based on cycloheptatriene) which was inhomogeneous by t.l.c. However, n.m.r. indicated the probable presence of the desired adducts:- τ ($CDCl_3$) 3.97 (4H, bm, olefinic H), 4.64 (1H, bm, O-C-H), 4.98 (1H, bm, N-C-H), 8.50 (2H, bm, methylene H). Isolation of the principal band from p.l.c. (silica GF_{254}, eluent chloroform) gave a mixture of products similar to the original mixture and attempts at direct crystallisation from the crude mixture were unsuccessful.

9,10-Dimethylanthracene/Nitrosocarbonylbenzene Adduct or D.M.A./PhCONO (6a)

This was prepared as for the cyclopentadiene/nitrosocarbonylbenzene adduct (21) by oxidation of benzohydroxamic acid (2.0 g, 15 mmol) with tetraethylammonium periodate (2.5 g, 8 mmol) in the presence of D.M.A. (2.0 g, 10 mmol).

Crystallisation of the crude product from benzene/light petroleum (b.p. 60 - 80°C) gave colourless prisms of D.M.A./PhCONO (1.36 g, 40% based on D.M.A.) m.p. 127 - 128°C, identical in all respects with samples prepared by previous workers.[10]

Cyclo-octatetraene/Nitrosocarbonylbenzene Adduct (28) Method A

An attempt to prepare this adduct was made in accordance with the preparation of the cyclopentadiene/nitrosocarbonylbenzene adduct (21). However this method yielded none of the desired adduct but a mixture of cyclo-octatetraene and benzoic anhydride.

Method B

A stirred solution of D.M.A./PhCONO (34 mg, 0.1 mmol) and cyclo-octatetraene (0.1 ml, excess) in sodium-dried benzene (5 ml) was heated at 80°C, under nitrogen, for 6 hr. T.l.c. indicated the presence of D.M.A. and one other spot of lower Rf. than D.M.A./PhCONO. P.l.c. (silica GF_{254}, eluent chloroform) afforded pure cyclo-octatetraene/nitrosocarbonylbenzene adduct (28) which crystallised from benzene/light petroleum (b.p. 40 - 60°C) as colourless needles (14.5 mg, 61% based on D.M.A./PhCONO) m.p. 114 - 116°C (Found: C, 75.4; H, 5.42; N, 5.50. $C_{25}H_{13}NO_2$ requires C, 75.3; H, 5.48; N, 5.85%), m/e 239, ν_{max} 1 645 cm^{-1} (C=O), τ ($CDCl_3$) 2.1-2.8 (5H, m, Ph), 3.75 (2H, m, cyclohexene olefinic H), 3.99 (2H, s, cyclobutene olefinic H), 4.83 (1H, m, O-C-H), 5.10 (1H, m, N-C-H), 6.68 (2H, m, bridgehead H).

Method C

According to the published method,[19] a solution of bromine (16.0 g, 0.1 mmol) in chloroform (50 ml) was added dropwise, over 30 minutes to a stirred solution of redistilled cyclo-octatetraene (10.4 g, 0.1 mmol) in chloroform (250 ml), cooled in a sodium chloride-ice freezing mixture. After a further hour stirring at room temperature the pale orange reaction mixture was evaporated free of chloroform on a rotary evaporator and then distilled under reduced pressure (b.p. 86 - 90°C/0.12 mm) to yield racemic trans-dibromocyclo-octatetraene (21.0 g, 81%).

Oxidation of benzohydroxamic acid (200 mg, 1.5 mmol) with tetraethylammonium periodate (250 mg, 0.8 mmol) in the presence of this dibromide (264 mg, 1 mmol) by the method described for the cyclopentadiene/nitrosocarbonylbenzene adduct (21) gave the diastereoisomeric mixture of dibromocyclo-octatetraene/nitrosocarbonylbenzene adducts (30) (381 mg, 92% based on dibromide), ν_{max}^{oil} 1 640 cm^{-1} (C=O), m/e 399, τ ($CDCl_3$) 2.2-2.7 (5H, m, Ph), 2.7-3.5 (2H,m, olefinic H), 4.70 (1H, m, O-C-H), 5.18 (2H, m, Br-C-H), 5.80 (1H, m, N-C-H), 6.40 (2H, m, C-H). No attempt was made to separate the diastereoisomers and the mixture, as an oil, was used directly at the next stage.

To a solution of dibromocyclo-octatetraene/nitrosocarbonylbenzene adducts (30) (80 mg, 0.2 mmol) in dry, redistilled dimethyl sulphoxide[20] (2 ml) was added powdered zinc (100 mg). The reaction mixture was stirred under

nitrogen at room temperature for 20 hr and then for a further 14 hr after the addition of more zinc (100 mg). The resulting solution was diluted with water (100 ml), filtered free of zinc, and extracted with methylene chloride (3 x 25 ml). The methylene chloride solution was dried ($MgSO_4$) and evaporated *in vacuo* to yield an oil, which after p.l.c. (silica GF_{254}, eluent chloroform), and crystallisation gave cyclo-octatetraene/nitrosocarbonylbenzene adduct (34 mg, 71%); mixed m.p. with sample from method B undepressed and spectrally identical with sample from method B.

Attempted Preparation of Methyl Sorbate/Nitrosocarbonylbenzene Adducts (32)

Following the method described in the case of the cyclopentadiene/nitrosocarbonylbenzene adduct (21), benzohydroxamic acid was oxidised in the presence of freshly distilled methyl sorbate. The reaction mixture was washed with saturated aqueous sodium thiosulphate, saturated aqueous sodium hydrogen carbonate, and brine. The dried (Na_2SO_4) organic phase was evaporated to an oil which on tituration with light petroleum (b.p. 40 - 60°C) gave *O*-benzoylbenzohydroxamic acid (5%, based on benzohydroxamic acid) m.p. 162 - 165°C (lit.[8] 166°C). The supernatant petroleum was removed with a pasteur pipette and evaporated to yield methyl sorbate (85% recovery).

A further attempt to prepare the desired adduct (32a) by thermal transfer of the nitrosocarbonylbenzene moiety from D.M.A./PhCONO to methyl sorbate was made as described for

cyclo-octatetraene/nitrosocarbonylbenzene adduct (28). The reaction was followed by t.l.c. (silica GF_{254}, eluent benzene/chloroform 1:1) and after 8 hr all the D.M.A./PhCONO was spent. N.m.r. of the crude reaction mixture showed no evidence of the adducts(32), and p.l.c. (same system as above) gave back methyl sorbate (80% recovery).

Cyclopentadiene/Nitrosocarbonylmethane Adduct (22)

This was prepared as detailed in the case of the cyclopentadiene/nitrosocarbonylbenzene adduct (21). The adduct (22) was obtained as an oil which repeatedly failed to crystallise and was purified by column chromatography (grade III alumina, eluent chloroform) and evaporated free of solvent to oil-pump vacuum (70% based on cyclopentadiene); m/e 139, ν_{max} (thin film) 1 662 cm^{-1}, τ ($CDCl_3$) 3.55 (2H, dm, olefinic H), 4.71 (2H, bs, O-C-H and N-C-H), 7.80 (2H, m, $-CH_2-$), 8.00 (3H, s, CH_3-).

Attempted Ethanolysis of Cyclopentadiene/Nitrosocarbonylmethane Adduct (22)

Cyclopentadiene/nitrosocarbonylmethane adduct (139 mg, 1.0 mmol) was dissolved in a solution of sodium ethoxide (340 mg, 5.0 mmol) in ethanol (15 ml). This solution was stirred in a flask fitted with a drying tube ($CaCl_2$), at room temperature, for 14 hr. T.l.c. (silica GF_{254}, eluent chloroform) indicated complete exhaustion of adduct (22). The reaction mixture was diluted with water (100 ml) and the aqueous phase extracted with ether (3 x 10 ml). The dried ($MgSO_4$) ethereal extracts were saturated with dry hydrogen

chloride to no effect. Evaporation of the solvent gave no product.

Methylation and Subsequent Hydrolysis of Cyclopentadiene/Nitrosocarbonylbenzene Adduct (21)

To a stirred, ice-cooled solution of the adduct (21) (101 mg, 0.5 mmol) in sodium-dried benzene (5ml) was added, in one portion, methyl fluorosulphonate (72 mg, 0.5 mmol). The reaction was monitored by t.l.c. (silica GF_{254}, eluent chloroform), and little change was noted after 2 hr. More methyl fluorosulphonate (288 mg, 2.0 mmol) was added and the mixture stirred at room temperature. After 1 hr the adduct (21) was no longer detectable by t.l.c. and the reaction mixture was diluted with methanol (10 ml) and 10% aqueous HCl (2.0 ml). This solution was stirred for a further 2 hr. The resulting mixture was basified with saturated sodium hydrogen carbonate solution (100 ml) and extracted with ether (2 x 20 ml). The combined ether extracts were dried (Na_2SO_4) and evaporated to a pale brown oil. I.r., t.l.c., and n.m.r. of this oil indicated the presence of methyl benzoate among numerous other minor products. The oily mixture was taken-up in anhydrous ether (10 ml) and the ethereal solution saturated with dry hydrogen chloride. A sparse, flocculent precipitate resulted which on attempted filtration became an oil (2 mg). The filtrate was re-examined by t.l.c., and appeared to be largely the original mixture of products.

Isoprene/Nitrosocarbonylbenzene Adducts (36a) and (36b)

These adducts were prepared, by oxidation of benzohydroxamic acid (2.0 g, 115 mmol) with tetraethylammonium periodate (2.5 g, 8 mmol) in the presence of isoprene (680 mg, 10 mmol), and purified according to the method described for the cyclopentadiene/nitrosocarbonylmethane adduct (22). The adducts (36a) and (36b) (1.56 g, 77% based on isoprene) were obtained as an oil; m/e 203, ν_{max} (thin film) 1 643 cm^{-1} (N-C=O), τ ($CDCl_3$) 2.2-2.9 (5H, m, Ph), 4.59 (1H, bs, olefinic H), 5.90 (4H, bs, CH_2-N and CH_2-O), 8.32 and 8.43 (sum equivalent to 3H, singlets, CH_3 in (36a) and CH_3 in (36b)). The presence of two CH_3 resonances in the n.mr. spectrum indicated the presence of both (36a) and (36b) in a mixture which was homogeneous by t.l.c.

Zinc-Acetic Acid Reduction of Isoprene/Nitrosocarbonylbenzene Adducts (36a) and (36b)

The isomeric mixture (36a) and (36b) of adducts (203 mg, 1 mmol) was dissolved in glacial acetic acid and treated with zinc dust (100 mg). After stirring for 3 days at room temperature a further portion of zinc dust (100 mg) was added and stirring continued for 4 days longer. The reaction mixture was diluted with water (100 ml) and neutralised by the addition of solid sodium hydrogen carbonate. The aqueous solution was extracted with chloroform (4 x 20 ml), the combined chloroform extracts dried ($MgSO_4$), and evaporated to yield an oil which showed a single major spot on t.l.c. (silica GF_{254}, 5% methanol in chloroform). Column chromato-

graphy (grade III neutral alumina, eluent chloroform/methanol) gave the pure isomeric mixture of amides (37a) and (37b) as an oil (79 mg, 39%); m/e 205, ν_{max} 1 647 cm^{-1}, τ ($CDCl_3$) 2.2-2.8 (5H, m, Ph), 3.40 and 3.72 (sum equivalent to 1H, two overlapping triplets, J_1 and J_2 7Hz, olefinic H in (37a) and (37b)), 5.8-4.2 (4H, m, CH_2-O and CH_2-N), 4.0 (2H, bm, NH and OH, exchangeable with DCl), 8.27 (3H, s, CH_3-).

Attempted Base Hydrolysis of Hydroxy-amides (37a) and (37b)

An isomeric mixture of the hydroxy-amides (100 mg) was dissolved in a hot 6N solution of potassium hydroxide in aqueous dioxan and the resulting solution refluxed, with stirring, for 2 hr. The reaction mixture was diluted with water (50 ml) and extracted with chloroform (4 x 10 ml). The combined chloroform extracts were washed with brine (2 x 10 ml), dried ($MgSO_4$), and evaporated carefully to yield only starting material (15% recovery), which was pure by t.l.c. (silica GF_{254}, 10% methanol in chloroform).

Reaction of Cyclopentadiene/Nitrosocarbonylbenzene Adduct (21) with Triphenylphosphine

Triphenylphosphine (52.4 mg, 0.2 mmol) was added in one portion to a solution of the adduct (21) (40.6 mg, 0.2 mmol) in sodium-dried benzene. The resulting solution was heated, with stirring under nitrogen, at 80°C and i.r. spectra of the reaction mixture (reference benzene) run periodically. The intensity of the amide carbonyl (1 650 cm^{-1}) fell off with a concomitant increase in intensity of the phenyl isocyanate band (2 250 cm^{-1}). After 3 hr the adduct (21) was

completely decomposed and the intensity of the absorption at 2 250 cm^{-1} had reached a maximum.

Attempted Thermal Transfer of Nitrosocarbonylbenzene from Cyclopentadiene/Nitrosocarbonylbenzene Adduct to D.M.A.

The cyclopentadiene/nitrosocarbonylbenzene adduct (20 mg, 0.1 mmol) and D.M.A. (21 mg, 0.1 mmol) were dissolved in sodium-dried benzene (3 ml) and stirred at 60°C under nitrogen.

The reaction mixture was monitored by t.l.c. (silica GF_{254}, eluent chloroform) and after 6 hr no change was apparent. The temperature was raised to 80°C but after 2 hr only starting materials were present in the reaction mixture.

Thermal Transfer of Nitrosocarbonylbenzene from Cyclopentadiene/Nitrosocarbonylbenzene Adduct (21) to Thebaine

A solution of cyclopentadiene/nitrosocarbonylbenzene adduct (20 mg, 0.1 mmol) and thebaine (2) (31 mg, 0.1 mmol) in sodium-dried benzene (5 ml) was stirred at 60°C under nitrogen. The reaction was followed by t.l.c. (silica GF_{254}, eluent chloroform) and judged complete after $6\frac{1}{2}$ hr. P.l.c. of the resulting mixture (same system as above) yielded thebaine/nitrosocarbonylbenzene adduct as colourless needles from benzene/light petroleum (b.p. 60 - 80°C) (31 mg, 69%). A mixed m.p. with an authentic sample[10] of the adduct (5a) was undepressed (170 - 172°C) and the sample from this experiment was spectrally identical with (5a).

1.2

Cyclopentadiene/Benzyl Nitrosoformate Adduct (44)

This compound was prepared by the method described for the adduct (21) by oxidation of benzyl N-hydroxycarbamate[27] (2.5 g, 15 mmol) with tetraethylammonium periodate (2.5 g, 8 mmol) in the presence of excess cyclopentadiene (1 ml). The adduct (44) was obtained as an oil (2.46 g, 71%, based on $PhCH_2OCONHOH$) after column chromatography (grade III neutral alumina, eluent chloroform/benzene); m/e 231, ν_{max} (KBr) 1 705 cm^{-1} (C=O), τ ($CDCl_3$) 2.74 (5H, s, Ph), 3.73 (2H, m, olefinic H), 4.91 (2H, s, Ph-CH_2-O), 4.8-5.2 (2H, m, N-C-H and O-C-H), 8.13 (1H, dm, J 9Hz, methylene H), 8.20 (1H, d, J 9Hz, methylene H), (Found: M^+ 231.0897. $C_{13}H_{13}NO_3$ requires 231.0895).

Thebaine/Benzyl Nitrosoformate Adduct (45)

This compound was prepared after the method described for the preparation of the cyclopentadiene/nitrosocarbonylbenzene adduct (21) by the oxidation of $PhCH_2OCONHOH$ (250 mg, 1.5 mmol) with tetraethylammonium periodate (250 mg, 0.8 mmol) in the presence of thebaine (311 mg, 1 mmol). The adduct (45) (366 mg, 76% based on thebaine) was obtained as colourless prisms from benzene/light petroleum (b.p. 60 - 80°C), m.p. 104 - 106°C (Found: C, 67.9; H, 5.93; N, 5.81. $C_{27}H_{28}N_2O_6$ requires C, 68.1; H, 5.88; N, 5.88%, m/e 476, ν_{max} (KBr) 1 716 cm^{-1} (C=O), τ ($CDCl_3$) 2.69 (5H, s, Ph), 3.37 (2H, m, 1-H and 2-H), 3.98 (2H, s, 7-H and 8-H), 4.96 (2H, s, Ph-CH_2-O), 5.43 (1H, s, 5-H), 6.20 (3H, s, O-CH_3), 6.51 (3H, s, O-CH_3), 7.56 (3H, s, N-CH_3).

De-acylation of Cyclopentadiene/Benzyl Nitrosoformate Adduct (44)

The adduct (460 mg, 2.0 mmol) was shaken in a solution of HBr in acetic acid (0.66 ml of a 48% w/v solution; equivalent to 4.0 mmol HBr) at room temperature. The solution rapidly became black in colour and after 1 hr no more carbon dioxide was evolved. The solution was diluted with anhydrous ether (50 ml) and the residual black oil re-extracted with anhydrous ether (2 x 50 ml). Attempts to crystallise the residual oil were unsuccessful and n.m.r. ($CDCl_3$) of the oil showed it to be largely benzyl bromide in acetic acid. The ethereal extracts gave only traces of benzyl bromide on evaporation. The reaction was repeated on half the above scale.

The reaction mixture went rapidly black once more and carbon dioxide evolution ceased after 45 minutes. The reaction mixture was diluted first with methanol (5 ml) and then anhydrous ether (150 ml), a flocculent brown precipitate (1 mg) was filtered off and the solution allowed to stand for a prolonged period at 0°C. However no crystallisation occurred.

Butadiene/Benzyl Nitrosoformate Adduct (47)

This was prepared as for the cyclopentadiene/nitroso-carbonylbenzene adduct (21) by the oxidation of $PhCH_2OCONHOH$ (600 mg, 3 mmol) with tetraethylammonium periodate (600 mg, 2.4 mmol) in a solution of butadiene (2 ml, excess) in nitromethane (25 ml) at -10°C. The crude reaction product

was purified by column chromatography (grade III neutral alumina, eluent benzene/chloroform) to give the adduct (47) as a colourless oil (570 mg, 72% based on $PhCH_2OCONHOH$); m/e 221, ν_{max} (thin film) 1 710 cm^{-1} (C=O), τ ($CDCl_3$) 2.69 (5H, s, Ph), 4.25 (2H, s, olefinic H), 4.82 (2H, s, Ph-CH_2-O), 5.68 (2H, m, CH_2-O), 5.92 (2H, m, CH_2-N).

De-acylation of Butadiene/Benzyl Nitrosoformate Adduct (47)

Butadiene/benzyl nitrosoformate adduct (47) (221 mg, 1.0 mmol) was shaken in a solution of HBr in acetic acid (0.33 ml of a 48% w/v solution; equivalent to 2.0 mmol HBr) at room temperature. After 45 minutes evolution of carbon dioxide had ceased and the solution was diluted first with methanol (5 ml) and then anhydrous ether (100 ml). From this solution colourless needles of 3,6-dihydro-2H-1,2-oxazine hydrobromide (48) (107 mg, 65%) gradually crystallised, m.p. 132 - 134°C (Found: C, 28.7; H, 4.88; N, 8.84; Br, 48.6 C_4H_8BrNO requires C, 28.9; H, 4.82; N, 8.44; Br 48.2%).

Isoprene/Benzyl Nitrosoformate Adducts (49a) and (49b)

Following the method outlined for the cyclopentadiene/nitrosocarbonylbenzene adduct (21) oxidation of $PhCH_2OCONHOH$ (1.2 g, 7.2 mmol) with tetraethylammonium periodate (1.5 g, 0.64 mmol) in the presence of isoprene (4 ml, excess) gave the crude adducts (49a) and (49b). Purification by column chromatography (grade III neutral alumina, eluent chloroform) yielded the pure product mixture (1.3 g, 77% based on $PhCH_2OCONHOH$) which was homogeneous by t.l.c. (silica GF_{254}, numerous solvent mixtures); m/e 235, ν_{max} (thin film) 1 715 cm^{-1}

(C=O), τ ($CDCl_3$) 2.73 (5H, s, Ph), 4.66 (1H, m, olefinic H), 4.86 (2H, s, Ph-CH_2-O), 5.71 (2H, m, CH_2-O), 6.03 (2H, m, CH_2-N), 2.34 (3H, bs, CH_3).

De-acylation of Isoprene/Benzyl Nitrosoformate Adducts (49a) and (49b)

The adducts (49a) and (49b) (233 mg, 1.00 mmol) were shaken in a solution of HBr in acetic acid (0.33 ml of a 48% w/v solution; equivalent to 2.0 mmol HBr). After 45 minutes evolution of carbon dioxide had ceased and the solution was diluted with methanol (5 ml) followed by anhydrous ether (100 ml). None of the desired salts (50a) and (50b) precipitated but a substantial amount of rubber was formed. The ethereal solution was decanted free of rubber and evaporated free of ether, and finally dried *in vacuo* to give the crude mixture of salts (50a) and (50b) (60 mg, 33%). The salt mixture was taken up in saturated sodium hydrogen carbonate (15 ml) and the resulting solution extracted with methylene chloride (3 x 10 ml). The combined methylene chloride solutions were dried (Na_2SO_4) and carefully evaporated to yield a mixture of the free bases (35a) and (35b); ν_{max} (thin film) 3 450 cm^{-1} (N-H), τ ($CDCl_3$) 4.44 (1H, m, olefinic H), 4.80 (2H, s, Ph-CH_2-O), 5.78 (2H, m, CH_2-O), 6.59 (2H, m, CH_2-N), 8.29 (3H, bs, CH_3).

Reduction of Oxazines (35a) and (35b)

The crude mixture of oxazines (35a) and (35b) (32 mg) from the above experiment were dissolved in glacial acetic acid (1.0 ml) and stirred with powdered zinc (50 mg), at room

temperature for 1 hr. The reaction mixture was diluted with water (50 ml), decanted from residual zinc and extracted with ether (4 x 20 ml). The dried (Na_2SO_4) ether extracts were carefully evaporated but no product was obtained. No more success was met with when the aqueous solution was extracted with methylene chloride (4 x 20 ml) after saturation with sodium chloride.

2-Toluene-p-sulphonylethyl N-hydroxycarbamate (52)

A solution of 2-hydroxy-1-toluene-p-sulphonylethane [31] (14.0 g, 0.07 mol) in sodium-dried benzene (100 ml) was added over 30 minutes and with vigorous stirring to an ice-cooled solution of phosgene (1.17 mol) in toluene (100 ml). After complete addition the resulting mixture was stirred at room temperature for 16 hr. Unreacted phosgene and solvents were removed on a rotary evaporator (fume hood) to leave the crude chloroformate [31] (51) as an oil, ν_{max} (thin film) 1 790 cm^{-1} (C=O).

The crude chloroformate (0.07 mol) was quickly dissolved in dry methanol (30 ml) and added over 5 minutes to an ice-cooled, strongly stirred solution of hydroxylamine (0.1 mol) (prepared from the hydrochloride and sodium methoxide) in dry methanol (150 ml). The resulting solution was allowed to stand at room temperature for 16 hr after which it was treated with acetone (25 ml) and evaporated to give a dry crystalline residue which crystallised (ethanol) as colourless needles of 2-toluene-p-sulphonylethyl N-hydroxycarbamate (52), m.p. 123 - 126°C (6.1 g, 60%), which gave an

intense blue colouration with ethanolic ferric chloride solution (Found: C, 46.1; H, 5.10; N, 5.65; S, 12.04. $C_{10}H_{13}NO_5S$ requires C, 46.3; H, 5.02; N, 5.40; S, 12.36%), m/e 259, ν_{max} (KBr) 3 405 and 3 295 (O-H and N-H), 1 715 cm^{-1} (C=O), τ ($(CD_3)_2CO$) 0.28 (2H, m, exchangeable with D_2O, NH and OH), 2.17 (2H, d, J 8Hz, aromatic H ortho to $-SO_2$), 2.53 (2H, d, J 8Hz, aromatic H meta to $-SO_2$), 5.64 (2H, t, J 7Hz, $-CH_2-O$), 6.47 (2H, t, J 7Hz, $-CH_2-SO_2$), 7.56 (3H, s, CH_3-).

Thebaine/2-(Toluene-p-sulphonyl)ethyl Nitrosoformate Adduct (53)

This was prepared by oxidation of (52) (129 mg, 0.5 mmol) with tetraethylammonium periodate (83 mg, 0.33 mmol) in the presence of thebaine (104 mg, 0.33 mmol) after the method described for the adduct (21). The purified adduct (53) was obtained as a glass (135 mg, 72% based on thebaine) after chromatography (grade III neutral alumina, eluents methanol and chloroform). Repeated attempts at crystallisation were unsuccessful; m/e 311 (no molecular ion), ν_{max} ($CHCl_3$) 1 713 cm^{-1} (C=O), τ ($CDCl_3$) 2.24 (2H, d, J 8Hz, aromatic H ortho to $-SO_2$), 2.66 (2H, d, J 8Hz, aromatic H meta to $-SO_2$), 3.34 (1H, d, J 8Hz, 1-H or 2-H), 3.40 (1H, d, J 8Hz, 1-H or 2-H), 3.92 (2H, s, 7-H and 8-H), 5.47 (1H, s, 5-H), 5.59 (2H, t, J 6.5Hz, CH_2-O-CO), 6.23 (3H, s, $O-CH_3$), 6.46 (3H, s, $O-CH_3$), 6.61 (2H, t, J 6.5Hz, CH_2-SO_2), 7.54 (3H, s, $N-CH_3$, or $Ar-CH_3$), 7.55 (3H, s, $N-CH_3$ or $Ar-CH_3$).

De-acylation of Thebaine/2-(Toluene-p-sulphonyl)ethyl Nitrosoformate Adduct (53)

A solution of the adduct (53) (57 mg, 0.1 mmol) in sodium-dried benzene (1.0 ml) was treated with a solution of 1,5-diazobicyclo[4.3.0]non-5-ene (D.B.N.)[30] (12.4 mg, 0.1 mmol) in dry benzene (0.10 ml). After 1 hr, t.l.c. (silica GF_{254}, 5% methanol in chloroform) indicated the presence of starting material and a further addition of D.B.N. (0.2 mmol) was made. After 30 minutes more, t.l.c. indicated complete reaction and the reaction mixture was evaporated free of solvent and separated by p.l.c. (above system) to give recovered thebaine (12 mg, 39%) and the HNO adduct (54) as a colourless oil (19 mg, 56%); τ ($CDCl_3$) 3.37 (2H, m, 1-H and 2-H), 3.94 (1H, d, J 9Hz, 7-H or 8-H), 4.10 (1H, d, J 9Hz, 7-H or 8-H), 5.40 (1H, s, 5-H), 6.19 (3H, s, O-CH_3), 6.46 (3H, s, O-CH_3), 3.59 (3H, s, N-CH_3). All attempts to crystallise the thebaine/HNO adduct (54) were unsuccessful as the adduct quickly reverted to thebaine.

The above reaction was repeated exactly and when all the starting material was used (t.l.c.) the reaction mixture was vigorously stirred for 15 minutes with 10% aqueous HCl (2.0 ml). The resulting mixture was treated with solid sodium hydrogen carbonate (excess), diluted with water (15 ml) and extracted with methylene chloride. The combined organic extracts were dried (Na_2SO_4) and evaporated to give 14-β--hydroxyamino-codeinone. This product was isolated as its hydrochloride salt from methanol/ether (26 mg, 71%), m.p.

240 - 244°C, ν_{max} (KBr) 3 400 and 3 220 (N-H and O-H), 1 679 cm^{-1} (C=O); spectrally identical with an authentic[10] sample and mixed melting point with authentic sample undepressed.

N-(2,2,2-Trichloroethoxycarbonyl)hydroxylamine (56)

A stirred solution of hydroxylamine (20 mmol) in methanol (50 ml), at 0°C, was treated with 2,2,2-trichloroethylchloroformate (2.12 g, 10 mmol), dropwise over 10 minutes. The resulting solution was allowed to stir at room temperature for 12 hr, treated with acetone (10 ml), and evaporated to give a crude crystalline mass which was recrystallised [benzene/light petroleum (b.p. 60 - 80°C-] to a constant m.p. of 87 - 88.5°C (440 mg, 21%) (Found: C, 17.5; H, 1.98; N, 7.00; Cl 50.6. $C_3H_4Cl_3NO_3$ requires C, 17.3; H, 1.92; N, 6.72; Cl 51.1%), m/e 211, 209, 207, ν_{max} (KBr) 3 360 and 3 260 (N-H and O-H), 1 715 cm^{-1} (C=O), τ ($CDCl_3$) 0.66 (1H, bm exchangeable with D_2O, N-H or O-H), 1.51 (1H, bm, exchangeable with D_2O, N-H or O-H), 5.17 (2H, s, $-CH_2-$).

Ergosteryl Acetate/2,2,2-Trichloroethyl Nitrosoformate Adduct (58)

This was prepared according to the method outlined for the cyclopentadiene/nitrosocarbonylbenzene adduct (21) by the oxidation of 2,2,2-trichloro-urethane (0.75 mmol) in the presence of ergosteryl acetate (0.5 mmol). The adduct (58) was obtained as colourless needles (86% based on ergosteryl

acetate) from methanol, m.p. 157 - 159°C (Found: C, 61.6; H, 7.59; N, 2.10; Cl, 16.3. $C_{33}H_{48}Cl_3NO_5$ requires C, 61.5; H, 7.45; N, 2.17; Cl, 16.5%), m/e 438 no molecular ion, ν_{max} (KBr) 1 728 cm^{-1} ($CH_3C{=}O$ and $N(C{=}O)O$), τ ($CDCl_3$) 3.75 (2H, s, 6-H and 7-H), 4.73 (1H, bm, 3-H), 4.88 (2H, m, side-chain olefinic H), 5.03 and 5.55 (2 x 1H, doublets, J 13 Hz, CH_2-O), 6.65 (1H, bdd, J 14Hz, 5Hz, 4α-H), 2.02 (3H, s, CH_3-CO).

De-acylation of Ergosteryl Acetate/2,2,2-Trichloroethyl Nitrosoformate Adduct (58)

The adduct (58) (161 mg, 0.25 mmol) was stirred in glacial acetic acid (5 ml) with powdered zinc (130 mg) for 2 hr. The reaction mixture was diluted with water (50 ml), filtered free of unreacted zinc, and basified to pH 10 with 10% aqueous sodium hydroxide. The aqueous solution was extracted with methylene chloride (3 x 20 ml), washed with brine (2 x 10 ml), dried (Na_2SO_4), and evaporated to an oil. Separation of this oil by p.l.c. (silica GF_{254}, eluent 10% methanol in chloroform) gave ergosteryl acetate (89 mg, 81%) and two minor products (<5 mg each) neither of which showed an N-H or O-H absorption in the i.r.

Thebaine/t-Butyl Nitrosoformate Adduct (59)

Following the procedure detailed for the preparation of cyclopentadiene/nitrosocarbonylbenzene adduct (21), N-hydroxy-t-butyl carbamate (0.5 mmol) was oxidised in the presence of thebaine (0.33 mmol) to yield thebaine/t-butyl

nitrosoformate adduct (59) as an oil (74% based on thebaine). Despite purification by column chromatography (grade III neutral alumina, eluents chloroform and methanol) the adduct could not be persuaded to crystallise. [Found (as a foam): C, 65.1; H, 6.89; N, 6.07. $C_{24}H_{30}N_2O_6$ requires C, 65.1; H, 6.83; N, 6.33%], m/e 442, ν_{max} (thin film) 1 710 cm^{-1} (C=O), τ ($CDCl_3$) 3.30 (1H, d, J 8Hz, 1-H or 2-H), 3.44 (1H, d, J 8Hz, 1-H or 2-H), 3.89 (1H, d, J 8.5Hz, 7-H or 8-H), 4.03 (2H, d, J 8.5Hz, 7-H or 8-H), 5.48 (1H, s, 5-H), 6.20 (3H, s, O-CH_3), 6.41 (3H, s, O-CH_3), 7.54 (3H, s, N-CH_3), 8.57 (9H, s, $(CH_3)_3C$).

De-acylation of Thebaine/t-Butyl Nitrosoformate Adduct (59)

The adduct (59) (44 mg, 1.0 mmol) was dissolved in dry methanol (2 ml) and the resulting solution saturated with gaseous HCl. This solution was diluted with anhydrous ether to give a white precipitate which crystallised on scratching with a glass rod. This precipitate was identified by spectral techniques as 14β-hydroxyamino-codeinone hydrochloride (33 mg, 90%). Mixed melting point with an authentic sample[10] of the hydrochloride was undepressed.

Thermal Decomposition of t-Butyl Azidoformate in Dimethyl Sulphoxide

A solution of t-butyl azidoformate (500 mg, 3.5 mmol) in dry D.M.S.O. (2.0 ml) was heated with stirring at 115°C for 2 hr. The solution turned dark-red and nitrogen was evolved. The resulting solution was diluted with water (200 ml) and extracted with methylene chloride (4 x 25 ml). The combined methylene chloride extracts were washed with water

(5 x 200 ml), dried (Na_2SO_4) and evaporated *in vacuo* to yield a crude oil (467 mg). This oil was purified by p.l.c. (silica GF_{254}, 10% methanol in chloroform) to yield *N*-t--butoxycarbonyl-2,2-dimethylsulphoximine as an oil (384 mg, 58%). This oil recrystallised from benzene/light petroleum (b.p. 60 - 80°C) as colourless needles, m.p. 72 - 74°C (308 mg, 47%) (Found: C, 43.7; H, 7.92; N, 7.20; S, 16.32. $C_7H_{15}NO_3S$ requires C, 43.5; H, 7.78; N, 7.25; S, 16.68%), *m/e* 193 ν_{max} (KBr) 1 660 cm^{-1} (C=O), τ ($CDCl_3$) 6.76 (6H, s, $(CH_3)_2S$), 8.56 (9H, s, $(CH_3)_3C$).

The above experiment was repeated using *t*-butyl azidoformate (42 mg, 0.25 mmol) and D.M.S.O. (2 ml) containing thebaine (156 mg, 0.5 mmol). After p.l.c. (above system) thebaine (114 mg, 73%) was recovered along with the sulphoximine (62) (18 mg, 40% based on *t*-butyl azidoformate) and thebaine/*t*-butyl nitrosoformate adduct (59) (27 mg, 25% based on *t*-butyl azidoformate) identical with the sample prepared by oxidation of *N*-hydroxy-t-butyl carbamate in the presence of thebaine.

The reaction was again repeated using t-butyl azidoformate (360 mg, 2.5 mmol) and D.M.S.O. (2 ml) containing thebaine (156 mg, 0.5 mmol). After p.l.c. (above system) no thebaine was recovered, only the sulphoximine (62) (243 mg, 51% based on *t*-butyl azidoformate) and the thebaine adduct (59) (186 mg, 17% based on *t*-butyl azidoformate or 84% based on thebaine).

The yields quoted in the above experiments refer to materials purified by chromatography but not crystallisation, unless otherwise stated.

Thebaine/N-Phenylnitrosoformamide Adduct (65)

This adduct was prepared by oxidation of N-hydroxy-N'--phenylurea[45] (0.75 mmol) in the presence of thebaine (0.5 mmol) following the procedure described for the cyclopentadiene/nitrosocarbonylbenzene adduct (21) and gave thebaine/N-phenylnitrosoformamide adduct (65) as colourless needles (59% based on thebaine) from ethanol, m.p. 185 - 188°C (dec.) (Found: C, 67.7; H, 5.80; N, 9.19. $C_{26}H_{27}N_3O_5$ requires C, 68.0; H, 5.90; N, 9.10%), m/e 461, ν_{max} (KBr) 3 420 (N-H), 1 695 (C=O), τ ($CDCl_3$) 2.26 (1H, m, exchanges with DCl, NH), 2.5-2.9 (5H, m, Ph), 3.34 (2H, m, 1-H and 2-H), 3.88 (2H, s, 7-H and 8-H), 5.15 (1H, d, J 7Hz, 9-H), 5.30 (1H, s, 5-H), 6.17 (3H, s, O-CH_3), 6.30 (3H, s, O-CH_3), 7.46 (3H, s, N-CH_3).

Attempted Preparation of 9,10-Dimethylanthracene/N-Phenylnitrosoformamide Adduct (66)

Following the procedure described for the formation of the cyclopentadiene/nitrosocarbonylbenzene adduct an attempt was made to form the adduct (66). T.l.c. of the crude reaction mixture (silica GF_{254}, eluent chloroform) showed D.M.A. and one other intense spot and n.m.r. ($CDCl_3$) showed three sharp singlets above 6τ:- 6.94, 7.31 and 7.73 in the ratio 1:2:2 respectively. This was taken to represent a 1:2 mixture of D.M.A. and D.M.A./PhNHCONO (66) respectively.

P.l.c. (above system) of the mixture returned a similar mixture when the major band was isolated and all attempts at direct crystallisation were unsuccessful.

1.3

Ergosteryl Acetate/Nitrosocarbonylbenzene Adducts (70a) and (68) Method A

When PhCONHOH (1.5 mmol) was oxidised in the presence of ergosteryl acetate (1 mmol) as described for adduct (21) t.l.c. (silica GF_{254}, eluent chloroform) indicated the presence of two products which were separated by p.l.c. (above system). The higher Rf material ergosteryl acetate/ nitrosocarbonylbenzene adduct type I (70a) was initially obtained as an oil (56% based on ergosteryl acetate) which gave colourless needles from methanol, m.p. 190 - 191°C (50%) (Found: C, 77.3; H, 9.02; N, 2.72. $C_{37}H_{51}NO_4$ requires C, 77.5; H, 8.96; N, 2.44%), m/e 573, ν_{max} (KBr) 1 728 cm^{-1} (CH_3-C=O), τ ($CDCl_3$) 2.1-2.3 (2H, m, aromatic H ortho to C=N), 2.5-2.8 (3H, m, aromatic H not ortho to C=N), 4.6-5.2 (1H, bm, 3-H), 4.7-4.9 (2H, m, 22-H and 23-H), 4.95 (1H, bs, 7-H), 5.37 (1H, bs, 6-H), 8.04 (3H, s, CH_3CO), ^{13}C δ(TMS) 170.0 (C=O), 150.8 (O-C=N), 145.3 (8-C), 135.4 (23-C), 132.1 (22-C), 130.9 (substituted aromatic C), 130.1 (aromatic C para to substituent), 128.1 (aromatic C ortho to substituent), 125.6 (aromatic C meta to substituent), 114.5 (7-C), 75.5 (5-C), 72.6 (6-C), 69.9 (3-C).

The material of lower Rf, ergosteryl acetate/nitroso-carbonylbenzene adduct type II (68), was obtained as an oil which, so far, has not crystallised (33%), m/e 438 no molecular ion, ν_{max} (thin film) 1 733 cm^{-1} (CH_3-C=O), 1 654 cm^{-1} (N-C=O), τ ($CDCl_3$) 2.3-2.8 (5H, m, Ph), 3.43 (1H, d, J 9Hz, 6-H or 7-H), 3.77 (1H, d, J 9Hz, 6-H or 7-H), 4.5-5.0

(1H, bm, 3-H), 4.84 (2H, m, 22-H and 23-H), 6.33 (1H, bdd, J 14Hz, 5Hz, 4α-H), 7.95 (3H, s, CH_3-C=O).

Method B

Following the method described for cyclo-octatetraene/nitrosocarbonylbenzene adduct (28), thermal transfer of nitrosocarbonylbenzene from D.M.A./PhCONO (0.1 mmol) to ergosteryl acetate (0.1 mmol) was carried out yielding the type I adduct (70a) (44% before crystallisation and based on D.M.A./PhCONO), and the type II adduct (68) (27%).

Ergosterol/Nitrosocarbonylbenzene adducts (71a) and (72)

Following the procedure described for the cyclopentadiene/nitrosocarbonylbenzene adduct (21), benzohydroxamic acid (0.4 mmol) was oxidised in the presence of ergosterol (0.25 mmol) to yield the crude mixture of type I adduct (71a) and type II adduct (72).

Separation of the mixture by p.l.c. (silica GF_{254}, eluent 10% methanol in chloroform) gave as the higher Rf. fraction type I adduct (71a) as an oil (56% based on ergosterol) which later crystallised from methanol as colourless needles (52%) m.p. 170 - 172°C (Found: C, 79.12; H, 9.09; N, 2.85. $C_{35}H_{49}NO_3$ requires C, 79.05; H, 9.29; N, 2.63%), m/e 531, ν_{max} (KBr) 3 240 cm^{-1} (O-H), τ ($CDCl_3$) 2.20 (2H, m, aromatic H ortho to C=O), 2.65 (3H, m, aromatic H, not ortho to C=O), 4.83 (2H, m, 22-H and 23-H), 4.97 (1H, bs, 7-H), 5.38 (1H, bs, 6-H), 5.98 (1H, bm, 3-H).

The second fraction, adduct type II (72) was obtained as an oil (38%), m/e 396 (no molecular ion), ν_{max} (thin film) 3 390 (O-H), 1 662 cm^{-1} (N-C=O), τ ($CDCl_3$) 2.20 (2H, m, aromatic H ortho to C=O), 2.68 (3H, m, aromatic H not ortho to C=O), 3.48 (1H, d J,9Hz, 6-H or 7-H), 3.77 (1H, d,J 9Hz 6-H or 7-H), 4.81 (2H, m, 22-H and 23-H), 5.84 (1H, bm, 3-H), 6.43 (1H, dd,J 14Hz, 5Hz, 4α-H).

Interconversion of Ergosteryl Acetate/Nitrosocarbonylbenzene Adduct Type I (70a) and Ergosterol/Nitrosocarbonylbenzene Adduct Type I (71a)

The ergosterol adduct type I (71a) (53 mg, 0.1 mmol) was stirred in a solution of acetic anhydride (1 ml) and pyridine (0.25 ml) at room temperature for 4 hr. The resulting solution was diluted with water (25 ml) and treated with excess, solid sodium hydrogen carbonate. The neutralised solution was extracted with chloroform (3 x 10 ml) and the combined chloroform extracts washed with brine (2 x 10 ml), 10% aqueous HCl (2 x 10 ml) and again with brine (10 ml). The organic layer was dried ($MgSO_4$) and evaporated to an oil which crystallised from methanol (38 mg, 67%) to give the ergosteryl acetate type I adduct (70a), identical in all respects with a previously prepared sample.

The ergosteryl acetate type I adduct (70a) (57 mg, 0.1 mmol) prepared by the oxidation of benzohydroxamic acid in the presence of ergosteryl acetate, was stirred at room temperature in a mixture of methanol (30 ml) and 20% aqueous

sodium hydroxide (0.5 ml) for 12 hr. The reaction mixture was evaporated to ca. 0.5 ml, diluted with water (30 ml) and extracted with chloroform (3 x 15 ml). The combined chloroform extracts were dried ($MgSO_4$) and evaporated to an oil which crystallised from methanol (41 mg, 77%) to yield the ergosterol type I adduct (71a), identical in all respects with an authentic sample.

Attempted Base Hydrolysis of Ergosteryl acetate/Nitrosocarbonylbenzene Adduct Type I (70a)

The adduct (70a) (53 mg, 0.01 mmol) was dissolved in a solution of sodium hydroxide (200 mg, 5 mmol) in methanol (25 ml) and water (0.5 ml). After stirring at room temperature for 6 hr no change beyond (71a) could be detected by t.l.c. (silica GF_{254}, eluent 5% methanol in chloroform). The solution was heated under reflux for 6 hr to give only recovered ergosterol adduct (71a) (41 mg, 86%).

Attempted Acid Hydrolysis of Ergosteryl Acetate/Nitrosocarbonylbenzene Adduct Type I (70a)

The adduct (70a) (110 mg, 0.21 mmol) was heated under reflux in a solution of 12N HCl (10 ml) in methanol (50 ml). After 5 hr t.l.c. (silica GF_{254}, eluent chloroform) indicated the exhaustion of starting material and the reaction mixture was evaporated free of methanol. The resulting solution was basified to pH 11 with 20% aqueous sodium hydroxide, diluted with water (150 ml) and extracted with chloroform (3 x 30 ml) to give a mixture showing several spots on t.l.c;

the major component was isolated after p.l.c. (above system) (68 mg), but showed only simple aliphatic and olefinic infra-red absorption. The n.m.r. spectrum of this component showed no aromatic absorption.

Hydride Reduction of Ergosteryl acetate/Nitrosocarbonylbenzene Adduct Type I (70a)

The adduct (70a) (100 mg) in sodium-dried benzene was treated with sodium dihydro-bis(2-methoxyethoxy)aluminate (1.0 ml of a 1.0M solution in benzene). After 30 minutes, t.l.c. (silica GF_{254}, eluent 10% methanol in chloroform) indicated complete reduction of the 3-O-acetyl group, and after 3 hr no further change. The solution was then heated at 80°C, under nitrogen, for 10 hr. P.l.c. (above system) gave 13 narrow, well-resolved bands one of which yielded 5α, 6α-dihydroxy-5α,6α-dihydroergosterol (75) which crystallised from ethyl acetate as colourless needles m.p. 241 - 242°C (12 mg 16%). A sample[39] prepared by the cis-hydroxylation of ergosterol had identical spectral properties and an undepressed mixed m.p. with this sample.

3-Phenyl-5,6-dihydro-1,4,2-dioxazine (73)

After the method of Johnson[38] and co-workers, the dioxazine (73) was prepared by the action of potassium benzohydroxamate (4.9 g) and anhydrous potassium carbonate (7.7 g) on redistilled 1,2-dibromoethane (5.8 g) in a solution of methanol (21 ml) and water (14 ml).

The product distilled (b.p. 112 - 116°C/0.1 mm Hg) as a colourless oil (1.95 g, 45%), ν_{max} (thin film) 1 605 cm^{-1} (O-C=N), τ ($CDCl_3$) 2.1-2.3 (2H, m, aromatic H ortho to C=O), 2.5-2.7 (3H, m, aromatic H not ortho to C=O), 5.45-5.62 (2H, half of A_2B_2 system J,6Hz, 5Hz, $-CH_2-$), 5.80-5.95 (2H, half of A_2B_2 system, $-CH_2-$), ^{13}C δ(TMS) 153.9 (O-C=N), 130.9 (substituted aromatic C), 130.3 (aromatic C para to substituent), 128.2 (aromatic C ortho to substituent), 125.6 (aromatic C meta to substituent), 64.6 and 63.8 (5-C and 6-C).

Alkylation and Subsequent Hydrolysis of 3-Phenyl-5,6-dihydro-1,4,2-dioxazine (73)

A solution of the dioxazine (73) (324 mg, 2.0 mmol) in anhydrous ether (10 ml) was heated under reflux with methyl iodide (284 mg, 2.0 mmol) for 14 hr. On cooling no precipitation occurred and the reaction mixture was evaporated free of ether and methyl iodide. The recovered dioxazine was taken up in sodium-dried benzene (10 ml) and heated under reflux with redistilled benzyl bromide (342 mg,2.0 mmol) for 14 hr. On cooling no precipitation occurred.

A fresh solution of the dioxazine (324 mg, 2.0 mmol) in dry benzene was treated with methyl fluorosulphonate (228 mg, 2.0 mmol). After stirring for 10 minutes at room temperature the solution became cloudy, and after 2 hr a substantial precipitate of colourless needles had formed. N-Methyl-3-phenyl-5,6-dihydro-1,4,2-dioxazine fluorosulphonate(76) was recovered by filtration, m.p. 135 - 137°C (dec.) (505 mg, 91%)

(Found: C, 43.5; H, 4.60; N, 5.08. $C_{10}H_{12}FNO_5S$ requires C, 43.4; H, 4.34; N, 5.05%).

The salt (76) (276 mg, 1.0 mmol) was dissolved in 10% aqueous HCl (5.0 ml) and stirred at room temperature for 14 hr. The resulting solution was diluted with water, and neutralised with solid sodium hydrogen carbonate (excess). The resulting solution was extracted with chloroform (3 x 25 ml). The combined chloroform extracts were dried (Na_2SO_4) and evaporated to yield N-methyl-O-(2-benzoylethyl)hydroxylamine as a colourless oil (181 mg, 93%), m/e 195, ν_{max} (thin film) 3 420 (N-H), 1 727 cm^{-1} (C=O), τ ($CDCl_3$) 1.9-2.1 (2H, m, aromatic H ortho to C=O), 2.4-2.7 (3H, m, aromatic H not ortho to C=O), 5.08 (1H, bm exchangeable with D_2O, N-H), 5.50 (2H, m, PhCO-O-CH_2), 6.06 (2H, m, N-O-CH_2), 7.33 (3H, s, $-CH_3$).

Degradation of Ergosteryl Acetate/Nitrosocarbonylbenzene Adduct Type I (70a)

Following the procedure used in the case of the dioxazine (73), the adduct (70a) (200 mg, 0.35 mmol) was stirred at room temperature with methyl fluorosulphonate (40 mg, 0.35 mmol) in sodium-dried benzene (10 ml). T.l.c. (silica GF_{254}, eluent 5% methanol in chloroform) indicated complete salt formation after 45 minutes but as no precipitate had formed, in situ hydrolysis was effected by addition of 12N HCl (0.1 ml) in methanol (10 ml) and water (0.1 ml) and stirring the resulting mixture at room temperature. After 12 hr the mixture was diluted with water (100 ml), basified to pH 11

with 20% aqueous sodium hydroxide, and extracted with ether (3 x 25 ml). The combined ethereal extracts were dried ($MgSO_4$), and evaporated to yield the disubstituted hydroxylamine (78) which remained as an oil (110 mg, 56%) even after p.lc. (above system); m/e 563, ν_{max} (thin film) 3 400 (N-H and O-H), 1 721 cm^{-1} (Ph-C=O), τ ($CDCl_3$) 1.9-2.1 (2H, m, aromatic H ortho to C=O), 2.4-2.8 (3H, m, aromatic H not ortho to C=O), 4.56 (1H, bs, 6-H), 4.84 (2H, m, 22-H and 23-H), 5.11 (1H, bs, 7-H), 6.37 (1H, bm, 3-H), 7.35 (3H, s, $N-CH_3$).

Base Hydrolysis of the Disubstituted Hydroxylamine (78)

The disubstituted hydroxylamine (50 mg, 0.09 mmol) was stirred at room temperature in methanol (5 ml) containing 20% aqueous sodium hydroxide (0.5 ml). After 2 hr the reaction was complete (t.l.c. silica GF_{254}, eluent 10% methanol in chloroform) and the solution was diluted with water (50 ml) and extracted with ether (3 x 20 ml). The combined ethereal extracts were dried ($MgSO_4$), and evaporated to give (79) as an oil, which after p.l.c. (above system), crystallised as colourless needles from methanol (28 mg, 69%), m.p. 179 - 181°C (Found: C, 76.0; H, 10.77; N, 2.80. $C_{29}H_{49}NO_3$ requires C, 75.9; H, 10.68; N, 3.05%), m/e 459, ν_{max} (thin film) 3 400 cm^{-1}(NH,OH), τ ($CDCl_3$) 4.83 (3H, m, 7-H, 22-H and 23-H), 5.90 (1H, bs, 6-H), 6.15 (1H, bm, 3-H), 7.40 (3H, s, $N-CH_3$).

Zinc Reduction of the Disubstituted Hydroxylamine (78)

The disubstituted hydroxylamine (78) (50 mg, 0.09 mmol) was dissolved in glacial acetic acid (5 ml) and stirred, at room temperature, with powdered zinc (195 mg) for 14 hr. The resulting mixture was diluted with water (50 ml), treated with excess sodium hydrogen carbonate and extracted with chloroform (3 x 25 ml). The combined chloroform extracts were dried ($MgSO_4$), and evaporated to give (74) as an oil which crystallised as colourless needles from methanol (35 mg, 74%), m.p. 194 - 195°C. A sample prepared[39] by the action of perbenzoic acid on ergosterol was spectrally identical and had an undepressed mixed m.p. with this sample.

Zinc Reduction of (79)

Following the method outlined in the case of (78) the substituted hydroxylamine (79) was reduced by the action of zinc in glacial acetic acid. The triol (75) crystallised from methanol as colourless needles, m.p. 241 - 242°C (59%). An authentic sample prepared by the peroxidation of ergosterol[39] was spectrally identical and had an undepressed mixed m.p. with this material.

5α,6α-Dihydroxy-5α,6α-dihydroergosteryl 6-Benzoate (74)

According to the method of Luttringhaus,[39] an ice-cooled solution of ergosterol (396 mg, 1.0 mmol) in chloroform (100 ml) was treated, in one portion, with a solution of perbenzoic acid[40] (138 mg, 1.0 mmol) in ice-cold chloroform (1.0 ml). The resulting solution was allowed to stand at 0°C until a starch iodide test was negative (3 days). The reaction mixture was extracted with saturated aqueous sodium

hydrogen carbonate (2 x 20 ml), dried (Na_2SO_4), and evaporated to small bulk. Column chromatography (grade III neutral alumina, eluent 10% methanol in chloroform) yielded the desired product which crystallised from methanol as colourless needles (168 mg, 32%), m.p. 194 - 195°C (literature[39] 194 - 195°C), ν_{max} (KBr) 3 430 (O-H), 1 728 cm^{-1} (C=O), τ ($CDCl_3$) 1.8-2.1 (2H, m, aromatic H ortho to C=O), 2.4-2.7(3H, m, aromatic H not ortho to C=O), 4.50 (1H, bs, 6-H), 4.82 (2H, m, 22-H and 23-H), 4.96 (1H, bs, 7-H), 4.01 (1H, bm, 3-H), ^{13}C δ(TMS) 166.2 (C=O), 144.5 (8-C), 135.5 (23-C), 133.1 (aromatic C para to C=O), 132.3 (22-C), 130.4 (substituted aromatic C), 129.8 (aromatic C ortho to C=O), 128.4 (aromatic C meta to C=O), 75.6 (5-C), 74.7 (6-C), 67.4 (3-C).

5α,6α-Dihydroxy-5α,6α-dihydroergosterol (75)

The benzoate (74) (134 mg, 0.25 mmol) was heated under reflux in a 5% solution of potassium hydroxide in methanol (50 ml). After 3½ hr the solution was evaporated to dryness, diluted with water (50 ml), and extracted with chloroform (3 x 20 ml). After drying ($MgSO_4$) and evaporating the combined chloroform extracts 5α,6α-dihydroxy-5α,6α-dihydroergosterol crystallised from methanol as colourless needles (94 mg, 70%), m.p. 241 - 242°C (literature[39] 241 - 242°C), ν_{max} (KBr) 3 410 cm^{-1} (O-H), τ (D_6-DMSO) 4.82 (2H, m, 22-H and 23-H), 5.16 (1H, bs, 7-H), 5.71 (1H, bs, 6-H), 4.26 (1H, bm, 3-H).

5α,6α-Dihydroxy-5α,6α-dihydroergosteryl 3,6-Diacetate (81)

A suspension of the ergosterol diol (75) (70 mg, 0.16 mmol) in analar acetic anhydride (2.0 ml) and pyridine (0.5 ml) was stirred for 14 hr at room temperature. The resulting clear solution was diluted with water (150 ml), treated with excess sodium hydrogen carbonate and extracted with chloroform (3 x 30 ml). The total chloroform extract was washed with 2N aqueous HCl (2 x 20 ml) and brine ((2 x 50 ml). After drying ($MgSO_4$) and evaporating the chloroform extract the residual diacetate (81) crystallised from methanol (66 mg, 79%) as colourless needles, m.p. 171 - 173°C (literature [39] 172 - 173°C) ν_{max} (thin film) 1 738 cm^{-1} (C=O), τ ($CDCl_3$) 4.75 (2H, m, 22-H and 23-H), 4.8-5.1 (1H, bm, 3-H), 4.95 (1H, bs, 6-H), 5.09 (1H, bs, 7-H), 7.89 (3H, s, CH_3C=O), 7.98 (3H, s, CH_3C=O).

Attempted Reaction of Cholesteryl Acetate with Nitrosocarbonylbenzene

Method A

Following the procedure outline for the preparation of cyclopentadiene/nitrosocarbonylbenzene adduct (21), benzohydroxamic acid (0.75 mmol) was oxidised in the presence of cholesteryl acetate (0.5 mmol). T.l.c. (silica GF_{254}, eluent chloroform) gave no indication of a new adduct and p.l.c. (above system) gave recovered cholesteryl acetate (85%).

Method B

An attempt to thermally transfer nitrosocarbonylbenzene to cholesteryl acetate using the method outlined in the

preparation of cyclo-octatetraene/nitrosocarbonylbenzene adduct (28) was unsuccessful. The DMA/PhCONO adduct slowly decomposed but no new adduct was formed and after p.l.c. (above system) cholesteryl acetate (83%) was recovered.

Reaction of 2,5-Dimethyl-2,4-hexadiene with Nitrosocarbonylbenzene

Method A

Benzohydroxamic acid (1.5 mmol) was oxidised in the presence of 2,5-dimethyl-2,4-hexadiene (1 mmol) by the method detailed for the cyclopentadiene/nitrosocarbonylbenzene adduct (21). The reaction mixture showed no product by t.l.c. (silica GF_{254}, eluent chloroform) and n.m.r. and i.r. indicated only starting material and benzoic anhydride recovered.

Method B

Following the procedure detailed for the preparation of cyclo-octatetraene/nitrosocarbonylbenzene (28), D.M.A./PhCONO (0.1 mmol) slowly decomposed in the presence of 2,5-dimethyl-2,4-hexadiene (0.2 mmol).to yield after p.l.c. (above system) N-hydroxy-3-benzamido-2,5-dimethyl-1,4-hexadiene (85) as an oil (23 mg, 94% based on D.M.A./PhCONO). This oil later crystallised as pinkish prisms from benzene/light petroleum (b.p. 60 - 80°C), m.p. 58 - 60°C, which gave a violet colour with enthanolic ferric chloride; m/e 245, ν_{max} (KBr) 3 420 (O-H), 1 616 cm^{-1} (C=O), τ ($CDCl_3$) 2.53 (5H, s, Ph), 4.41 (1H, bd, J 10Hz, N-C-H), 4.91 (1H, bd, J

10Hz, =C-H), 5.05 (2H, s, $=CH_2$), 8.23 and 8.24 (6H, singlets, $=C(CH_3)_2$), 8.69 (3H, s, $=C(CH_3)-$) (Found: $\underline{M}^+$, 245.1416 $C_{15}H_{19}NO_2$ requires $\underline{M}$, 245.1416).

Pyrocalciferyl Acetate[43] /Nitrosocarbonylbenzene Adduct (89)

After the method described for (21), benzohydroxamic acid (0.15 mmol) was oxidised in the presence of pyrocalciferyl acetate (0.05 mmol). T.l.c. (silica GF_{254}, eluent chloroform) and n.m.r. of the crude reaction mixture indicated only one product. After p.l.c. (above system) pure pyrocalciferyl acetate/nitrosocarbonylbenzene adduct (89) was obtained which crystallised from methanol as colourless needles (67% based on pyrocalciferyl acetate), m.p. 95 - 98°C (Found: C, 77.3; H, 8.83; N, 2.80. $C_{37}H_{51}NO_4$ requires C, 77.5; H, 8.96; N, 2.44%), $\underline{m/e}$ 438 (no molecular ion), ν_{max} (KBr) 1 738 (CH_3-C=O), 1 655 cm^{-1} (N-C=O), τ ($CDCl_3$) 2.4-2.9 (5H, m, Ph), 3.04 (1H, d, $\underline{J}$ 8Hz, 6-H or 7-H), 3.75 (1H, d, $\underline{J}$ 8Hz, 6-H or 7-H), 4.77 (1H, bm, 3-H), 4.89 (2H, m, 22-H and 23-H), 6.75 (1H, bd $\underline{J}$, 15Hz, 4α-H), 7.52 (1H, dd, $\underline{J}$, 15Hz, 4Hz, 4β-H), 7.96 (3H, s, CH_3-C=O).

Isopyrocalciferyl Acetate[43] /Nitrosocarbonylbenzene Adduct (90)

Following the method used for cyclopentadiene/nitrosocarbonylbenzene adduct (21), this adduct was prepared by the oxidation of benzohydroxamic acid (0.15 mmol) in a solution containing isopyrocalciferyl acetate (88) (0.05 mmol). T.l.c. (silica GF_{254}, eluent chloroform) and n.m.r.

of the crude reaction mixture indicated one adduct which after p.l.c. (above system) gave colourless prisms of isopyrocalciferyl acetate/nitrosocarbonylbenzene adduct (90) from methanol, m.p. 124 - 126°C (63% based on diene) (Found: C, 77.7; H, 8.93; N, 2.36. $C_{37}H_{51}NO_4$ requires C, 77.5; H, 8.96; N, 2.44%), m/e 438 (no molecular ion), ν_{max} (KBr) 1 735 (CH_3-C=O), 1 628 cm^{-1} (N-C=O), τ ($CDCl_3$) 2.3-2.6 (2H, m, aromatic H ortho to C=O), 2.6-2.8 (3H, m, aromatic H not ortho to C=O), 3.47 (1H, d, J 8Hz, 6-H or 7-H), 3.81 (1H, d, J 8Hz, 6-H or 7-H), 4.89 (2H, m, 22-H and 23-H), 4.97 (1H, bm, 3-H), 6.8-7.4 (2H, m, 4-H), 7.93 (3H, s, CH_3-C=O).

Attempted Formation of Lumisteryl Acetate/Nitrosocarbonylbenzene Adduct

Following the method described for the preparation of cyclopentadiene/nitrosocarbonylbenzene adduct (21), benzohydroxamic acid (0.25 mmol) was oxidised in the presence of lumisteryl acetate* (86) (0.1 mmol). However only benzoic anhydride and lumisteryl acetate (92% recovery) were present in the resultant mixture.

Effect of Heat on Ergosteryl Acetate/Nitrosocarbonylbenzene Adduct Type I (70a)

A solution of the adduct (70a) in sodium-dried toluene was heated under nitrogen at 111°C for 20 hr. After this time only starting material was detectable by t.l.c. (silica GF_{254}, eluent chloroform).

* Sample from Professor D.H.R. Barton's collection

Attempted Reaction of Ergosteryl Acetate/Nitrosocarbonylbenzene Adduct Type I (70a) with Triphenylphosphine

A solution of the type I adduct (70a) (57 mg, 0.1 mmol) and triphenylphosphine (52 mg, 0.2 mmol) was heated at 111°C under nitrogen for 6 hr. T.l.c. (silica GF_{254}, eluent chloroform) showed only the presence of both starting materials in the reaction mixture.

Reaction of Ergosteryl Acetate/Nitrosocarbonylbenzene Adduct Type II (68) with Triphenylphosphine

A solution of the type II adduct (57 mg, 0.1 mmol) and triphenylphosphine (52 mg, 0.2 mmol) was heated, under nitrogen, at 111°C for 4 hr. T.l.c. of the reaction mixture (silica GF_{254}, eluent chloroform) showed that the adduct (68) was no longer present and p.l.c. (same system) gave ergosteryl acetate (34 mg, 78%).

Effect of Heat on Ergosteryl Acetate/Nitrosocarbonylbenzene Adduct Type II (68)

A solution of the type II adduct (68) (57 mg, 0.1 mmol) in sodium-dried benzene (5 ml) was heated at 80°C for 2 hr with no change apparent by t.l.c. (silica GF_{254}, eluent chloroform). The resulting solution was evaporated free of benzene and taken up in sodium-dried toluene (5 ml). The toluene solution was heated under nitrogen and a slow appearance of the type I adduct (70a) with concomitant disappearance of the type II adduct (68) noted (t.l.c.). After 16 hr the transformation was complete and p.l.c. (above system) gave the type I adduct (70a) (31 mg, 55%) identical in all respects with previously prepared samples.

Ergosteryl Acetate/Nitrosocarbonylbenzene Adduct Type III (101)

The previously described preparation (Method A) of the ergosteryl acetate/nitrosocarbonylbenzene adducts type I (70a) and type II (68) was repeated exactly. The crude ice-cooled reaction mixture in methylene chloride (50 ml) was washed with ice-cooled solutions of saturated aqueous sodium thiosulphate (2 x 25 ml), 10% aqueous sodium hydroxide (2 x 25 ml), and brine (2 x 25 ml). The resulting solution was dried ($MgSO_4$) and evaporated at 0°C to yield the crude product as an oil. This oil was quickly dissolved in deuterochloroform and its n.m.r. spectrum recorded immediately. The n.m.r. spectrum showed none of the resonances associated with the type I adduct (70a) and, as well as the resonances of the type II adduct, additional peaks consistent with an adduct of structure type III (101) were present:- τ ($CDCl_3$) 3.51 (d, J 8Hz, 6-H or 7-H), 3.71 (d, J 8Hz, 6-H or 7-H) and 8.09 (s, $CH_3C{=}O$).

This mixture of adducts type II (68) and type III (101) was heated in sodium-dried benzene at 60°C and the n.m.r. spectrum of the mixture examined periodically. After 7 hr the mixture contained only adducts type I (70a) and type II (68) whilst at intermediate times a mixture of all three types of adduct was noted.

The mixture of adducts type II (68) and type III (101) was again prepared and heated with triphenyl phosphine (2 molar excess based on total adduct concentration), in dry benzene at 60°C. Periodic n.m.r. spectra of this mixture

showed again the gradual rearrangement of adduct type III (101) to adduct type I (70a), at the same rate as observed in the absence of triphenyl phosphine.

Effect of Heat on Cyclopentadiene/Nitrosocarbonylbenzene Adduct (21)

A solution of the adduct (21) in sodium-dried benzene was heated at 80°C under nitrogen for 3 hr. No change in the t.l.c. (silica GF_{254}, eluent 5% methanol in chloroform) of the solution was evident after this time.

The solution was evaporated free of benzene and taken up in sodium-dried toluene. The toluene solution was heated at 111°C for 48 hr, during this time the t.l.c. of the mixture slowly changed from that of the starting material to a mixture of numerous minor spots and the solution itself became dark brown in colour.

9,10 Dimethylanthracene/4-Bromonitrosocarbonylbenzene Adduct (91)

This was prepared as for the cyclopentadiene/nitrosocarbonylbenzene adduct (21) from D.M.A. (1.0 g, 5 mmol) and 4-bromobenzohydroxamic acid[46] (1.6 g, 7 mmol). Crystallisation of the crude product from benzene/light petroleum (b.p. 60 - 80°C) gave D.M.A./p-BrC_6H_4CONO (1.0 g, 41% based on D.M.A.) as colourless plates m.p. 125 - 128°C (Found: C, 66.0; H, 4.48; N, 3.20. $C_{23}H_{18}BrNO_2$ requires C, 65.7; H, 4.28; N, 3.34%), m/e 206 (no molecular ion), ν_{max} (KBr) 1 680 cm^{-1} (C=O), τ ($CDCl_3$) 2.3-2.8 (12H, m, aromatic H), 7.25 (3H, s, O-C-CH_3), 7.96 (3H, s, N-C-CH_3).

Ergosteryl Acetate/4-Bromonitrosocarbonylbenzene Adducts (92a) and (93a) Method A

Following the method outlined for the cyclopentadiene/nitrosocarbonylbenzene adduct (21), 4-bromobenzohydroxamic acid (0.9 mmol) was oxidised in a solution of ergosteryl acetate (0.5 mmol). P.l.c. (silica GF_{254}, eluent chloroform) gave as the first fraction (65% based on ergosteryl acetate) ergosteryl acetate/4-bromonitrosocarbonylbenzene adduct type I (92a) which crystallised from methanol as colourless needles (57%), m.p. 224 - 226°C (dec.) (Found: C, 66.9; H, 7.75; N, 2.43. $C_{37}H_{50}BrNO_2$ requires C, 67.0; H, 7.93; N, 2.22%), m/e 651, ν_{max} (KBr) 1 735 cm^{-1} (C=O), τ ($CDCl_3$) 2.37 (2H, d, J 8Hz, aromatic H meta to Br), 2.58 (2H, d, J 8Hz, aromatic H ortho to Br), 4.85 (2H, m, 22-H and 23-H), 4.92 (1H, bm, 3-H), 5.02 (1H, bs, 6-H), 5.40 (1H, bs, 7-H), 8.03 (3H, s, CH_3-C=O).

The second fraction, which was type II adduct (93a) (33%), crystallised from methanol as colourless prisms, m.p. 143 - 146°C (29%) (Found: C, 67.2; H, 7.65; N, 1.84. $C_{37}H_{50}BrNO_2$ requires C, 67.0; H, 7.93; N, 2.22%), m/e 438 (no molecular ion), ν_{max} (KBr) 1 735 (CH_3-C=C), 1 653 cm^{-1} (N-C=O), τ ($CDCl_3$) 2.60 (4H, s, aromatic H), 3.48 (1H, d J, 8Hz, 6-H or 7-H), 3.79 (1H, d, J 8Hz, 6-H or 7-H), 4.6-5.1 (1H, m, 3-H), 4.82 (2H, m, 22-H and 23-H), 6.35 (1H, dd, J 14Hz, 5Hz, 4α-H), 7.93 (3H, s, CH_3-C=O) Rf. irradiation at τ 4.85 caused 4α-H doublet of doublets to collapse to a simple doublet, J 14Hz.

Method B

Thermal transfer of 4-bromonitrosocarbonylbenzene from D.M.A./p-BrC_6H_4CONO (0.25 mmol) to ergosteryl acetate (0.25 mmol) was carried out as for the preparation of cyclo--octatetraene/nitrosocarbonylbenzene adduct (28). Separation of the product mixture by p.l.c. (above system) gave adduct type I (92a) (53% based on ergosteryl acetate) and adduct type II (93a) (25%).

Ergosteryl Acetate/4-Methoxynitrosocarbonylbenzene Adducts

Following the method described for cyclopentadiene/nitrosocarbonylbenzene adduct (21), 4-methoxybenzohydroxamic[47] acid (1.5 mmol) was oxidised in the presence of ergosteryl acetate (1 mmol). P.l.c. (silica GF_{254}, eluent chloroform) gave type I adduct (92c) (47%, based on ergosteryl acetate), which crystallised (methanol) as colourless needles (34%), m.p. 216 - 218°C (Found: C, 75.9; H, 8.84; N, 2.61. $C_{38}H_{53}NO_5$ requires C, 75.6; H, 8.79; N, 2.32%), m/e 603, ν_{max} (KBr) 1 740 cm^{-1} (CH_3-C=O), τ ($CDCl_3$) 2.24 (2H, d, J 9Hz, aromatic H meta to CH_3-O-), 3.13 (2H, d J, 9Hz, aromatic H ortho to CH_3-O-), 4.80 (2H, m, 22-H and 23-H), 4.89 (1H, bm, 3-H), 4.96 (1H, bs, 6-H), 5.40 (1H, bs, 7-H), 6.20 (3H, s, CH_3-O), 8.03 (3H, s, CH_3-C=O).

The lower Rf. fraction from p.l.c. gave ergosteryl acetate/4-methoxynitrosocarbonylbenzene adduct type II (93c) as an oil (23%) which slowly crystallised from benzene/light petroleum (b.p. 60 - 80°C) as colourless needles (16%), m.p. 153 - 156°C (Found: C, 75.4; H, 8.87; N, 2.60. $C_{38}H_{53}NO_5$

requires C, 75.6; H, 8.79; N, 2.32%), m/e 438 (no molecular ion), ν_{max} (KBr) 1 737 (CH_3-C=O), 1 655 cm^{-1} (N-C=O), τ ($CDCl_3$) 2.41 (2H, d, J 9Hz, aromatic H meta to CH_3-O-), 3.18 (2H, d, J 9Hz, aromatic H ortho to CH_3-O-), 3.45 (1H, d, J 9Hz, 6-H or 7-H), 3.83 (1H, d, J 9Hz, 6-H or 7-H), 4.74 (1H, bm, 3-H), 4.81 (2H, m, 22-H and 23-H), 6.18 (3H, s, CH_3-O), 6.33 (1H, dd, J 14Hz, 5Hz, 4α-H), 7.99 (3H, s, CH_3-C=O).

The preparation was repeated with a work-up at 0°C and the products separated on p.l.c. (above system) and their n.mr. spectra quickly taken. The n.m.r. of the type II adduct (93c) fraction was unchanged but the n.m.r. of the fraction corresponding to type I adduct (92c) showed as well as the signals attributed to type I adduct additional resonances consistent with the presence of the type III adduct (104):- τ ($CDCl_3$) 2.25 (2H, d, J 9Hz, aromatic H meta to CH_3-O-), 3.06 (2H, d, J 9Hz, aromatic H ortho to CH_3-O-), 3.54 (1H, d, J 9Hz, 6-H or 7-H), 3.72 (1H, d, J 9Hz, 6-H or 7-H), 6.16 (3H, s, CH_3-O), 8.05 (3H, s, CH_3-C=O).

This mixture was taken up in sodium-dried benzene and heated at 60°C. N.m.r. spectra of the mixture were taken periodically and in 6 hr all the type III adduct (104) had been converted into type I adduct (92c).

Ergosteryl Acetate/4-Nitronitrosocarbonylbenzene Adducts

4-Nitrobenzohydroxamic acid[48] (0.8 mmol) was oxidised in a solution of ergosteryl acetate (0.25 mmol) in methylene chloride as described for the preparation of cyclopentadiene/nitrosocarbonylbenzene adduct (21). P.l.c. (silica GF_{254},

eluent chloroform) gave two fractions. The fraction of higher Rf. (58% based on diene) slowly crystallised from methanol (50%) as fibrous needles of ergosteryl acetate/4--nitronitrosocarbonylbenzene adduct type I (92b), m.p. 201 - 203°C (Found: C, 72.1; H, 8.00; N, 4.30. $C_{37}H_{50}N_2O_6$ requires C, 71.9; H, 8.09; N, 4.53%), m/e 618, ν_{max} (KBr) 1 740 cm^{-1} (C=O), τ ($CDCl_3$) 1.79 (2H, d,J 9Hz, aromatic H ortho to $-NO_2$), 2.02 (2H, d,J 9Hz, aromatic H meta to $-NO_2$), 4.80 (2H, m, 22-H and 23-H), 4.89 (1H, bm, 3-H), 4.95 (1H, bs, 6-H), 5.29 (1H, bm, 7-H), 2.03 (3H, s, CH_3-C=O). The lower Rf. fraction (25%) gave colourless needles of adduct type II (93b) (17%) from methanol/water, m.p. 147 - 149°C (Found: C, 71.8; H, 7.89; N, 4.33. $C_{37}H_{50}N_2O_6$ requires C, 71.9; H, 8.09; N, 4.53%), m/e 438 (no molecular ion), ν_{max} (KBr) 1 738 (CH_3-C=O), 1 660 cm^{-1} (N-C=O), τ ($CDCl_3$) 1.85 (2H, d,J 9Hz, aromatic H ortho to $-NO_2$), 2.44 (2H, d,J 9Hz, aromatic H meta to $-NO_2$), 3.49 (1H, d,J 9Hz, 6-H or 7-H), 3.75 (1H, d,J 9Hz, 6-H or 7-H), 4.77 (1H, bm, 3-H), 4.82 (2H, m, 22-H and 23-H), 6.24 (1H, dd,J 14Hz, 5Hz, 4α-H), 7.98 (3H, s, CH_3-C=O).

The above preparation was repeated with a work-up at 0°C. After p.l.c. (same system) the two fractions were examined by n.m.r. spectroscopy. The second fraction was unchanged but the first fraction showed none of the resonances associated with adduct type I (92b) but an entirely new spectrum attributed to ergosteryl acetate/4-nitronitroso-carbonylbenzene adduct type III (103):- τ ($CDCl_3$) 1.86 (2H, d

J 9Hz, aromatic H ortho to $-NO_2$), 2.27 (2H, d, J 9Hz, aromatic H meta to $-NO_2$), 3.52 (2H, d, J 9Hz, 6-H or 7-H), 3.69 (2H, d, J 9Hz, 6-H or 7-H), 4.79 (2H, m, 22-H and 23-H), 4.89 (1H, bm, 3-H), 8.10 (3H, s, CH_3-C=O). An i.r. spectrum of this compound also showed two carbonyl absorptions:- ν_{max} (thin film) 1 745 (CH_3-C=O), 1 660 cm^{-1} (N-C=O). All attempts to crystallise adduct type III (103) yielded only adduct type I (92b).

A solution of adduct type III (103) in dry benzene was heated at 60°C. Periodic n.m.r. spectra showed a gradual rearrangement to adduct type I (92b) which was complete in 8 hr.

Ergosteryl Acetate/Nitrosocarbonylmethane Adduct (94)

This was prepared as for the adduct (21) from ergosteryl acetate (0.5 mmol) and acetohydroxamic acid (1.3 mmol). Type II adduct (94), the only adduct produced, crystallised (methanol) as shining needles (84% based on diene) m.p. 142 - 145°C (Found: C, 75.1; H, 9.62; N, 3.00. $C_{32}H_{49}NO_4$ requires C, 75.2; H, 9.59; N, 2.74%), m/e 511, ν_{max} (KBr) 1 743 (O-C=O), 1 675 cm^{-1} (N-C=O), τ ($CDCl_3$) 3.64 (1-H, d J 9Hz, 6-H or 7-H), 3.78 (1-H, d J 9Hz, 6-H or 7-H), 4.74 (1H, bm, 3-H), 4.80 (2H, m, 22-H and 23-H), 6.53 (1H, dd, J ,14Hz, 5Hz, 4α-H), 7.99 (3H, s, CH_3-(C=O)-O), 2.15 (3H, s, CH_3-(C=O)-N).

Effect of Heat on Ergosteryl Acetate/Nitrosocarbonylmethane Adduct (94)

A solution of the adduct (94) in sodium-dried toluene was heated at 111°C under nitrogen and the reaction mixture

periodically examined by t.l.c. (silica GF_{254}, eluent 5% methanol in chloroform). After 6 hr no change was noted but eventually (4 days) the reaction mixture decomposed to give a brown solution which streaked on t.l.c.

Ergosteryl Acetate/2,4,6-Trimethylnitrosocarbonylbenzene Adduct (95)

This was prepared as described for the adduct (21) from mesitohydroxamic acid[49] (1.5 mmol) and ergosteryl acetate (0.5 mmol), the single adduct product, type II adduct (95) was obtained as a glass (80% based on diene) which could not be crystallised. (Found (glass): C, 78.3; H, 9.35; N, 2.40%. $C_{48}H_{57}NO_4$ requires C, 78.1; H, 9.27; N, 2.28%), m/e 438 (no molecular ion), ν_{max} 1 736 (CH_3-C=O), 1 658 cm^{-1} (-N-C=O), τ ($CDCl_3$) 3.22 and 3.33 (2 x 1H, singlets, aromatic H), 3.54 (1H, d, J 9Hz, 6-H or 7-H), 3.75 (1H, d, J 9Hz, 6-H or 7-H), 4.77 (1H, bm, 3-H), 4.76 (2H, m, 22-H and 23-H), 6.27 (1H, dd, J 14Hz, 5Hz, 4α-H), 7.68 (3H, s, $C\underline{H}_3$-Ar, para to C=O), 7.76 and 7.96 (2 x 3H, singlets, $C\underline{H}_3$-Ar, meta to C=O), 8.04 (3H, s, CH_3-C=O), τ (C_6D_5-CD_3) 3.38 (2H, s, aromatic H), 3.85 (1H, d, J 9Hz, 6-H or 7-H), 4.07 (1H, d, J 9Hz, 6-H or 7-H), 4.30 (1H, bm, 3-H), 4.78 (2H, m, 22-H and 23-H), 5.96 (1H, dd, J 14Hz, 5Hz, 4α-H), 7.47 (3H, s, $C\underline{H}_3$-Ar para to C=O), 7.88 (6H, s, $C\underline{H}_3$-Ar meta to C=O), 8.21 (3H, s, CH_3-C=O). The multiplicity of the aromatic protons and the $C\underline{H}_3$-Ar protons in the $CDCl_3$ spectrum was attributed to hindered rotation.

Ergosteryl Acetate/Benzyl Nitrosoformate Adduct (96a)

After the method described for the cyclopentadiene/benzyl nitrosoformate adduct (44), benzyl N-hydroxycarbamate (0.4 mmol) was oxidised in the presence of ergosteryl acetate (0.25 mmol) to give only the type II adduct (96a) which crystallised as colourless prisms from methanol (80% based on ergosteryl acetate), m.p. 141 - 143°C (Found: C, 75.3; H, 8.94; N, 2.53. $C_{38}H_{53}NO_5$ requires C, 75.6; H, 8.79; N, 2.32%), m/e 438 (no molecular ion), ν_{max} (KBr) 1 738 (CH_3-C=O), 1 712 cm^{-1} (O-(C=O)-N), τ ($CDCl_3$) 2.75 (5H, s, Ph), 3.78 (2H, s, 6-H and 7-H), 4.80 (1H, bm, 3-H), 4.83 (2H, m, 22-H and 23-H), 4.84 and 5.04 (2 x 1H, doublets, J 13Hz, CH_2-O), 6.68 (1H, dd, J 14Hz, 5Hz, 4α-H), 7.99 (3H, s, CH_3-C=O).

Ergosteryl Acetate/N-Phenylnitrosoformamide Adduct (96b)

This was prepared by oxidation of N-hydroxy-N-phenylurea (0.4 mmol) in the presence of ergosteryl acetate (0.25 mmol) by the procedure described for the preparation of cyclopentadiene/nitrosocarbonylbenzene adduct (21). The adduct (96b) gave colourless prisms from methanol, m.p. 146 - 148°C (20% based on ergosteryl acetate) (Found: C, 74.9; H, 8.97; N, 4.8. $C_{37}H_{52}N_2O_4$ requires C, 74.6; H, 9.16; N, 4.92%), m/e 438 (no molecular ion), ν_{max} (KBr) 3 390 (N-H), 1 735 (CH_3-C=O), 1 695 cm^{-1} (N-C=O), τ($CDCl_3$) 2.08 (1H, bs, exchangeable with DCl , N-H), 2.5-3.1 (5H, m, Ph), 3.72 (2H, s, 6-H and 7-H), 4.63 (1H, bm, 3-H), 4.76 (3H, m, 22-H and 23-H), 6.50 (1H, dd, J 14Hz, 5Hz, 4α-H), 7.98 (3H, s, CH_3-C=O).

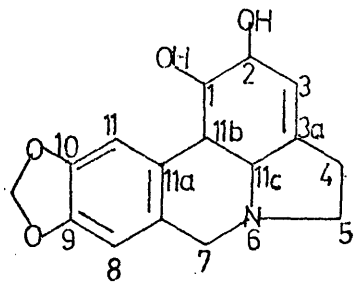
Ergosteryl Acetate/Ethyl Nitrosoglyoxylate Anil Adduct (99)

Following the general procedure of Rees and co-workers[14] lead tetra-acetate (96 mg, 0.25 mol) was added in portions to a stirred, ice-cooled, solution of ergosteryl acetate (110 mg, 0.25 mmol) and ethyl nitrosoglyoxylate anil (69 mg, 0.33 mmol) in methylene chloride (25 ml). After complete addition (10 minutes) the reaction mixture was allowed to stir for 50 minutes longer. The crude reaction mixture was washed with 5% aqueous sodium hydroxide (1 x 25 ml) and brine (2 x 25 ml). The methylene chloride solution was dried (Na_2SO_4), and evaporated to yield an oil which on separation by p.l.c. (silica GF_{254}, eluent chloroform) gave the adduct (99) as colourless needles from methanol, m.p. 153 - 154.5°C (105 mg, 65%) (Found: C, 74.6; H, 8.74; N, 4.17. $C_{40}H_{56}N_2O_5$ requires C, 74.5; H, 8.69; N, 4.34%), m/e 438 (no molecular ion), ν_{max} (KBr) 1 738 (CH_3-C=O), 1 725 (O-C=O), 1 643 cm^{-1} (C=N), τ ($CDCl_3$) 2.6-3.3 (5H, m, Ph), 3.50 (1H, d, J 9Hz, 6-H or 7-H), 3.68 (1H, d, J 9Hz, 6-H or 7-H), 4.58 (1H, bm, 3-H), 4.79 (2H, m, 22-H and 23-H), 6.09 (2H, q, J 7Hz, CH_2-O), 6.30 (1H, dd, J 14Hz, 5Hz, 4α-H), 8.02 (3H, s, CH_3-C=O).

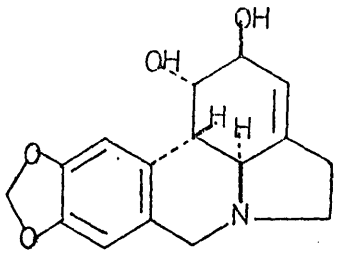
A minor by-product also isolated by p.l.c. was 3-ethoxycarbonyl-4H-1,2,4-benzoxadiazine (100) (10 mg, 15%) as yellow needles (chloroform/light petroleum (b.p. 40 - 60°C)), m/e 206, m.p. 84 - 86 °C, τ ($CDCl_3$) 2.9-4.6 (4H, m, aromatic H), 5.57 (2H, q, J 7Hz, CH_2-O), 8.64 (3H, t, J 7Hz, CH_3-CH_2).

An n.m.r. spectrum of the crude reaction mixture indicated only the presence of both isolated products.

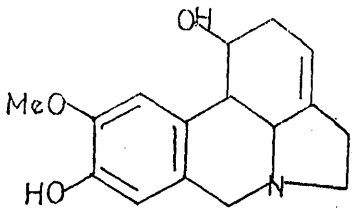
SECTION 2



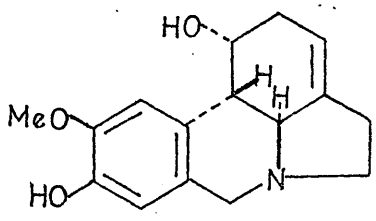
(105)



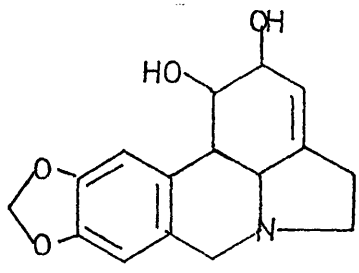
(106)



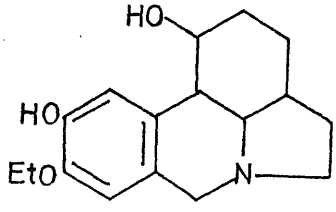
(107)

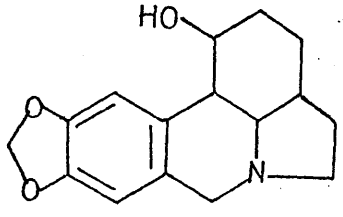


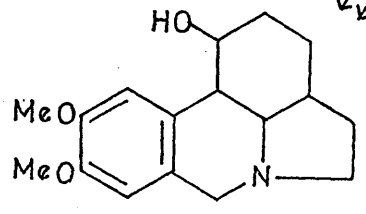
(108)

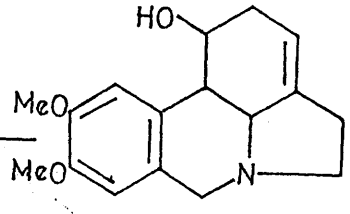


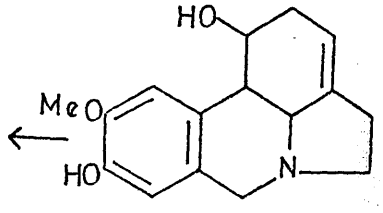
(105)











(107)

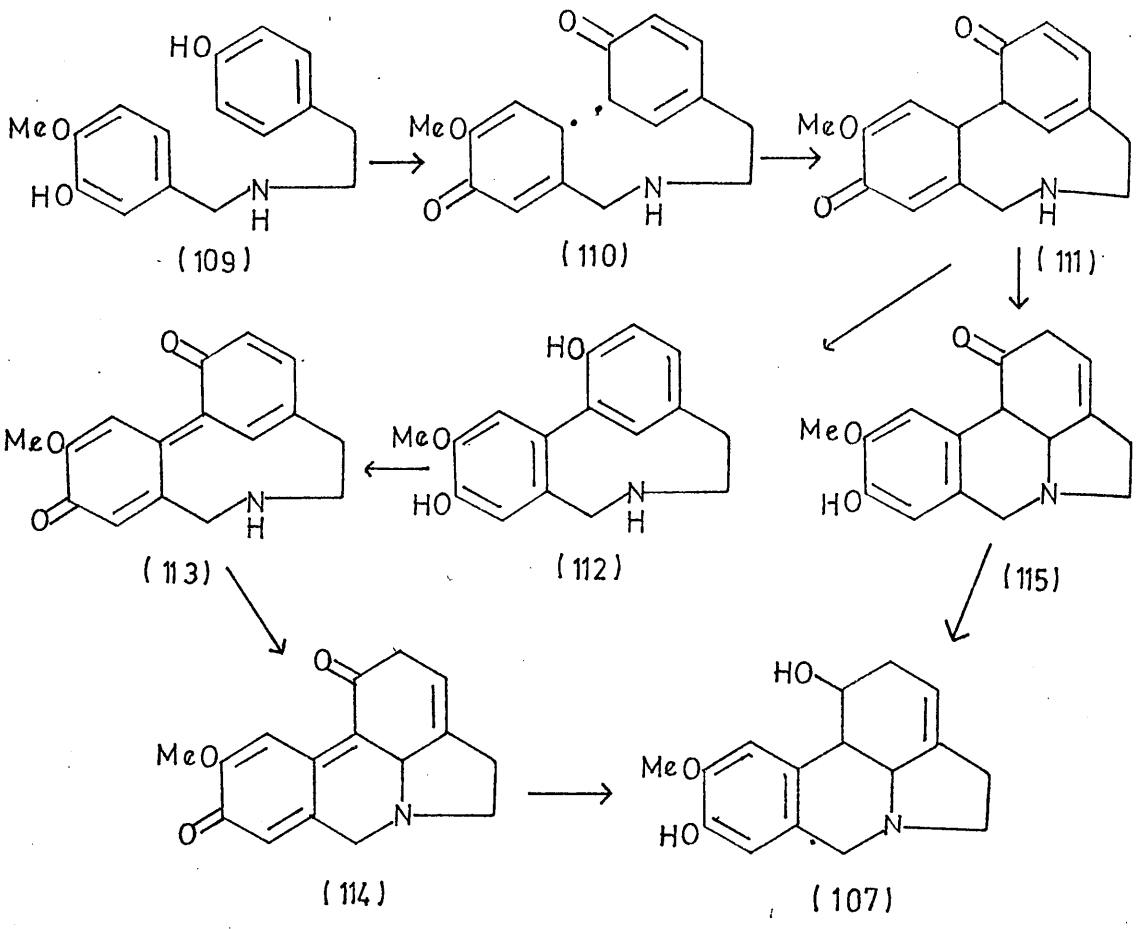
(fig.12)

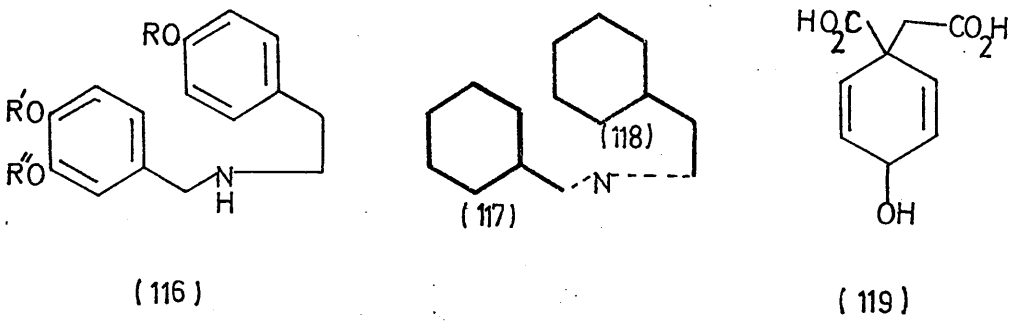
INTRODUCTION

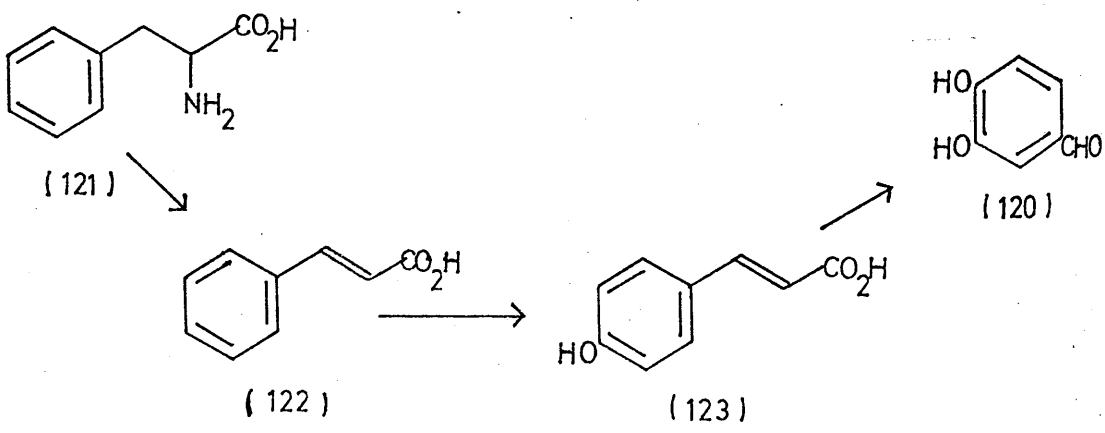
The Amaryllidaceae alkaloid lycorine (105) was first isolated by Morishima[50] from Lycoris radiata Herb. in 1897. Its structure (105) was proposed by Uyeo and Yanaihara[51] in 1958 but not until 1966, with the advent of X-ray structure analysis, was the absolute stereochemistry[52] (106) assigned.

Norpluviine (107) was originally isolated in 1959 by Uyeo[53] from the same plant and was assigned the structure[51] (107) at the same time as the structure of lycorine was assigned. The absolute stereochemistry of norpluviine (108) followed by the chemical inter-relation (fig. 12) of lycorine and norpluviine.[53,54,55]

In 1957, Barton and Cohen[56] proposed that many naturally occurring compounds might be formed by phenol oxidative coupling. The norbelladine derivative (109) was proposed as a precursor for Amaryllidaceae alkaloids. Oxidation of (109) to the di-radical (110) followed by ortho-para coupling would give the bis-dienone (111). Re-aromatisation of the dienone would give the biphenyl (112) which, after further oxidation, would yield the diphenoquinone (113). The diphenoquinone could then cyclise by attack of nitrogen at the C-ring giving the quinonemethine (114) which could be converted into norpluviine (107) by reduction and aromatisation. A shorter scheme was later proposed by Barton.[57] By this scheme cyclisation of the intermediate (111) to give the ketone (115) may occur if attack by nitrogen on ring-C of (111) proceeds faster than aromatisation to (107). Finally reduction of the


HO
MeO
HO
N
H
(109)
O
MeO
O
N
H
(110)
O
MeO
O
N
H
(111)
O
MeO
O
N
H
(113)
HO
MeO
HO
N
H
(112)
O
MeO
HO
N
(115)
O
MeO
O
N
(114)
HO
MeO
HO
N
(107)


RO
R'O
R''O
N
H
(116)
(118)
N
(117)
HO2C
CO2H
OH
(119)

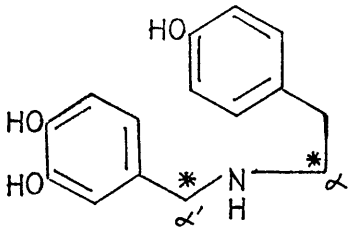

CO2H
NH2
(121)
CO2H
(122)
HO
HO
CHO
(120)
CO2H
HO
(123)

ketone (115) would give norpluviine (107).

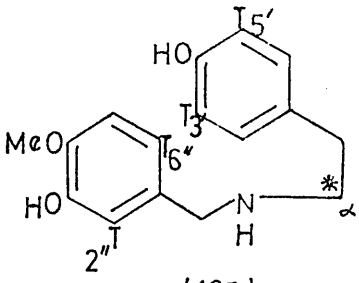
The norbelladine-type precursor (116) may be divided into two parts; a C_6-C_1 hydro-aromatic unit (117) and a C_6-C_2 hydro-aromatic unit (118).

Tyrosine[58,60,61] and tyramine[62] have been shown to be precursors for the C_6-C_2 unit in lycorine and the carboxyl group of tyrosine[63] is not incorporated to a significant extent. [β-^{14}C]Phenylalanine[62] but not [α-^{14}C]phenylalanine[62] was incorporated into lycorine in Narcissus incomparibilis. Degradation showed that the activity of lycorine resided only in the C_6-C_1 portion of the molecule. In higher plants, phenylalanine and tyrosine are independently derived from prephenic acid (119).[64,65] Thus tyrosine is a specific precursor for the C_6-C_2 portion and phenylalanine must be a specific precursor for the C_6-C_1 unit of lycorine. Protocatechuic aldehyde (120)[66] has been shown to be a precursor for the C_6-C_1 unit in lycorine and it has been shown that it must arise from the sequence[67]:- phenylalanine (121) ⟶ trans-cinnamic acid (122) ⟶ p-coumaric acid (123) ⟶ protocatechuic aldehyde (120).

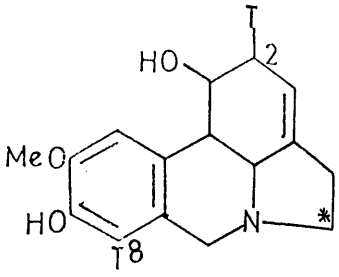
In 1964 Battersby and co-workers[63] fed [α,α'-^{14}C]norbelladine (124) to Nerine bowdenii daffodils and showed that radioactive lycorine (105) was obtained and that the ratio of the activities in carbons 5 and 7 was the same as the ratio of activities in carbons α and α' respectively of the precursor. This showed that norbelladine was incorporated



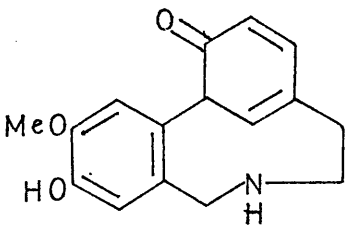
(124)



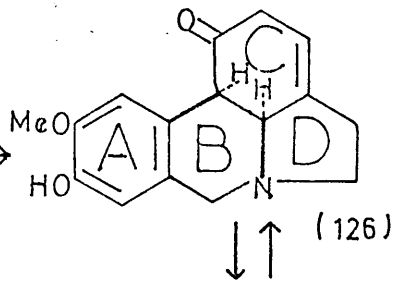
(125)



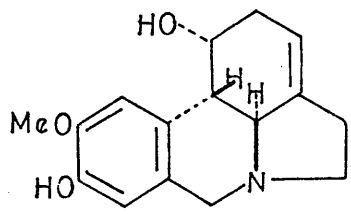
(107)



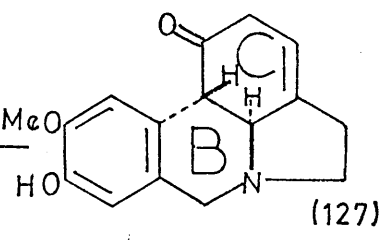
(111)



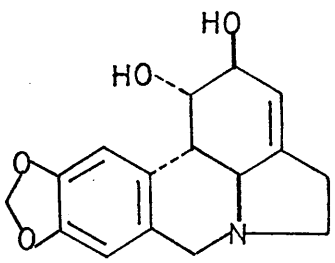
(126)



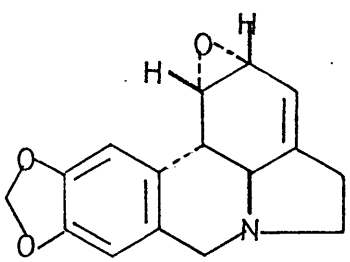
(108)



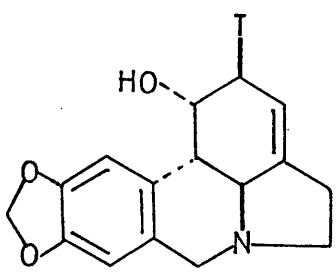
(127)



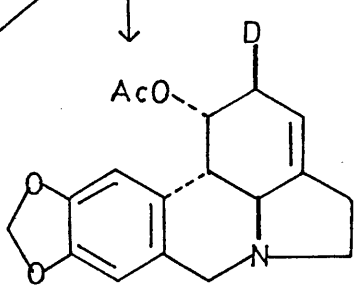
(106)



(129)



(128)



(130)

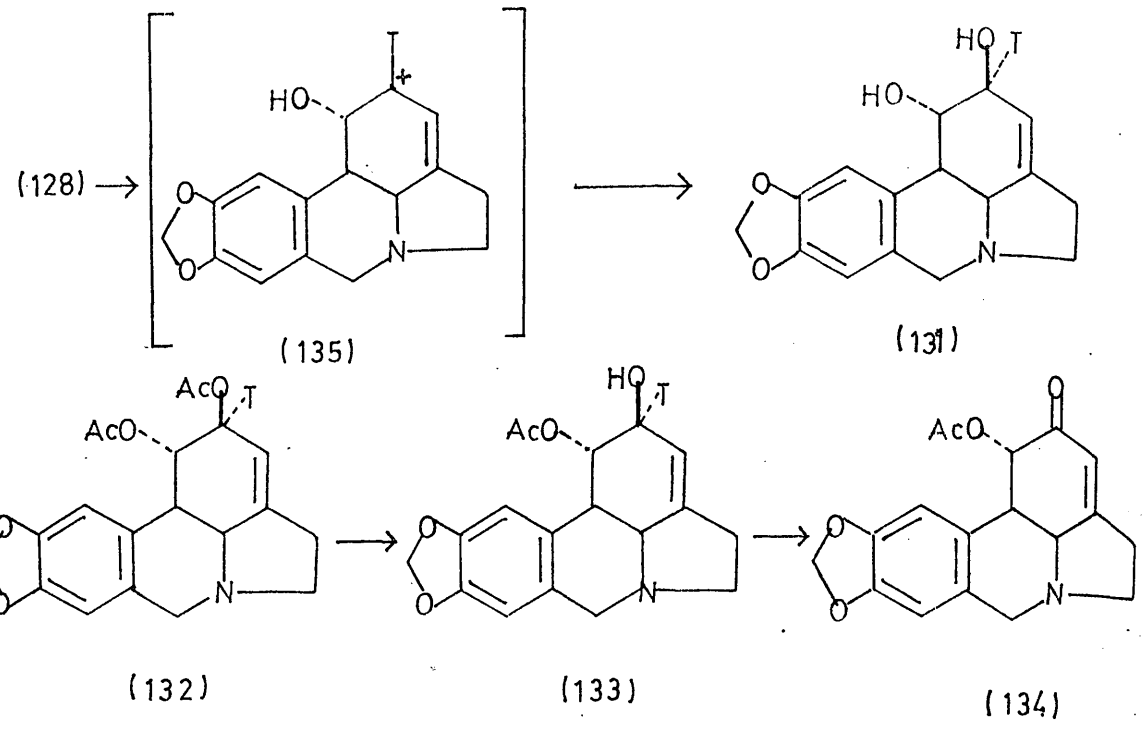
intact into lycorine and was the first strong supporting evidence for Barton and Cohen's theory.

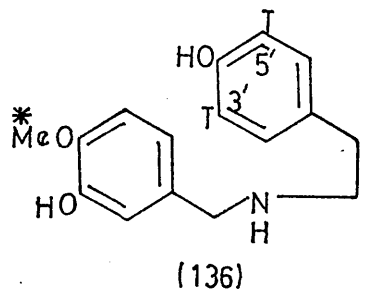
Kirby and Tiwari[68] showed that [3',5',2'',6''-$^{3}H_{4}$,α-^{14}C] O-methylnorbelladine (125) was incorporated into norpluviine in 'Texas' daffodils. Furthermore, half the tritium of the precursor had been lost in norpluviine and the residual tritium was shown by degradation to be divided equally between the 2 and 8 positions of norpluviine (107). This was in complete accord with Barton and Cohen's early biosynthetic proposals but Barton's second proposal (see p. 81) did not predict the loss of the 11b proton observed by Kirby and Tiwari. However Kirby and Tiwari proposed a modification of Barton's shortened route which could account for loss of the 11b proton by exchange. Inspection of models suggests that in Barton's proposed attack of nitrogen on ring C of (111) the cis-B/C fused compound (126) would result. However norpluviine itself is known[69] to have a trans-B/C ring fusion and it is possible that the 11b proton could be lost by an enolisation which epimerised (126) to the natural epimer (127).

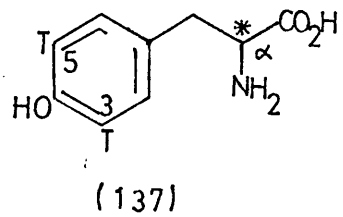
Battersby and colleagues[63] demonstrated that [methyl-^{14}C]methionine fed to 'Twink' daffodils gave incorporation into the methoxyl group of norpluviine and the methylenedioxy group of lycorine. Later Bruce and Kirby[70] showed that [methyl-^{14}C]O-methylnorbelladine gave norpluviine labelled in the methoxyl group and lycorine with a radioactively labelled methylenedioxy group, when fed to 'Twink' daffodils.

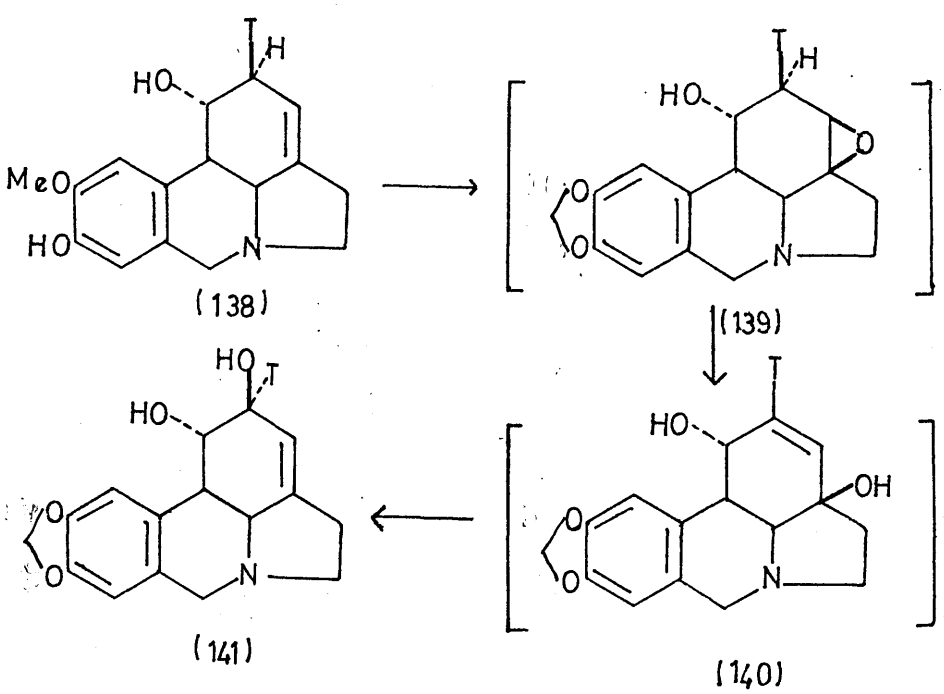
Earlier feeding experiments had not established whether both D- and L-tyrosine were incorporated into lycorine and norpluviine. Bruce and Kirby[70] decided to settle the issue by feeding 'Twink' daffodils with mixtures of D-[3,5-$^{3}H_{2}$] and DL-[α-^{14}C]-tyrosine ($^{3}H/^{14}C$=14.9), and L-[3,5-$^{3}H_{2}$] and L-[α-^{14}C]-tyrosine ($^{3}H/^{14}C$=15.4). Both experiments gave similar incorporations into lycorine (0.1%) and norpluviine (0.16%) and the $^{3}H/^{14}C$ ratios were ca. 6.2 in all cases. Thus, although the loss of tritium was substantially greater than the expected 50% (see later), the similar ratios indicated an equal incorporation of D- and L-tyrosine. In order to determine whether incorporation of both D- and L-tyrosine was due to interconversion by α-racemisation or transamination, Bruce and Kirby[71] fed DL-[α-^{3}H,α-^{14}C]tyrosine to 'Twink' daffodils and isolated radioactive norpluviine and lycorine, both with total retention of tritium label. It was concluded that neither transamination nor racemisation could have been operative as both these processes demand a loss of α-tritium.

Wildman and Heimer[72] in 1967 partially synthesised [2β-^{3}H]caranine (128) by reduction of lycorine-1,2α-epoxide (129) with lithium aluminium [^{3}H]hydride. The epoxide (129) was prepared by treatment of lycorine with phosphorus oxychloride and was assigned the β- configuration principally on the grounds of n.m.r. spectroscopic evidence. Reduction of the epoxide (129) with lithium aluminium [^{2}H]hydride, followed by acetylation, gave [2β-^{2}H]caranine acetate (130)


(128)
HO
T
+
O
O
N
(135)
HO
T
HO
O
O
N
(131)
AcO
T
AcO
O
O
N
(132)
HO
T
AcO
O
O
N
(133)
AcO
O
O
O
N
(134)


T
HO
5'
3'
T
*
MeO
HO
N
H
(136)


T
5
HO
3
T
*
CO2H
α
NH2
(137)

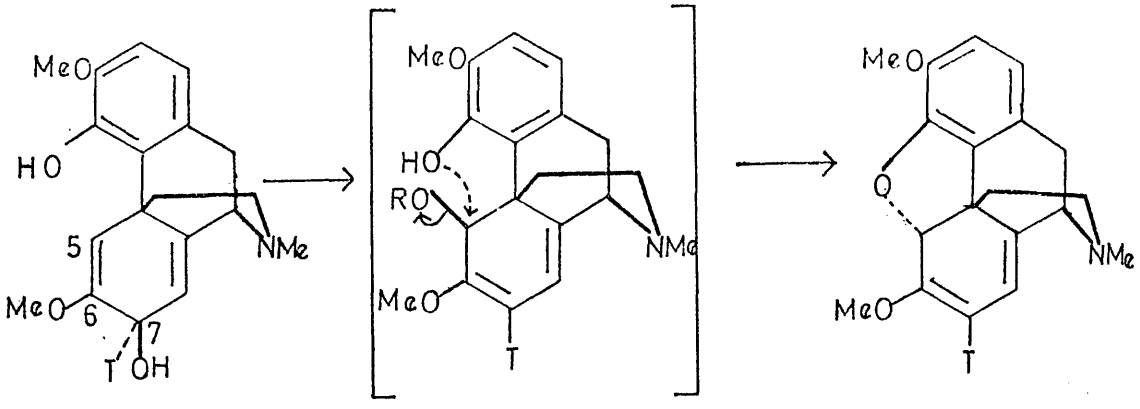

H
HO
MeO
HO
N
(138)
H
HO
O
O
O
N
(139)
HO
T
HO
O
O
N
(141)
HO
T
HO
OH
O
O
N
(140)

which, by n.m.r. spectroscopy, had 100% of the deuterium at C-2. If attack of deuteride ($^2H^-$) had occurred on the epoxide (129) with inversion then the ^2H atom would be in the β-configuration. Evidence of this came from the mass spectrum of (130) which showed a significant increase in M-61 (loss of CH_3CO_2D) over the mass spectrum of natural acetylcaranine, no such increase being expected had the ^2H been in the α-configuration. The [2β-^{3}H]caranine had its configuration assigned by analogy.

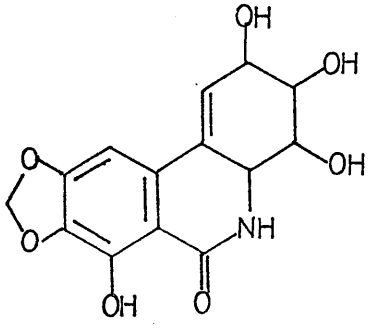
When the [2β-^{3}H]caranine was fed to Zephyranthes candida radioactive lycorine (131) (7% incorporation) was isolated. This was converted into diacetyl lycorine (132) and 1-O--acetyl-lycorine (133) without loss of tritium but, when (133) was oxidised to 1-O-acetyl-lycorin-2-one (134), a 96% loss of tritium ensued. Thus hydroxylation must occur, to some extent, with inversion of configuration at C-2 and lycorine cannot be formed from a 2-oxo-derivative.

Hydroxylation at saturated carbon generally[73-80] occurs with retention of configuration and to explain this exceptional result Heimer and Wildman suggested that hydroxylation might proceed through the allylic cation (135) which would be preferentially hydroxylated from the less hindered α-face to give lycorine (131).

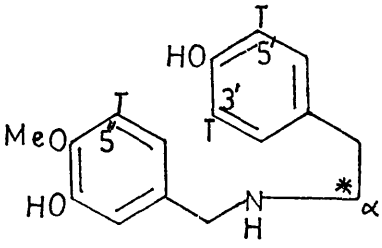
In 1968 Bruce and Kirby showed that [70,81] in 'Twink' daffodils norpluviine (138) and lycorine (141) derived from [3',5'-$^3H_2$,methyl-^{14}C]O-methylnorbelladine (136) or [3,5-$^3H_2$, α-^{14}C]tyrosine (137) both retained 50% of the precursors'



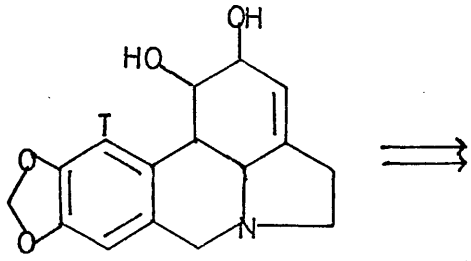
(142) (143) (144)

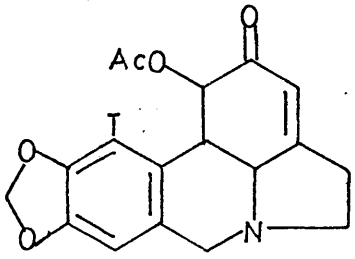


(145)

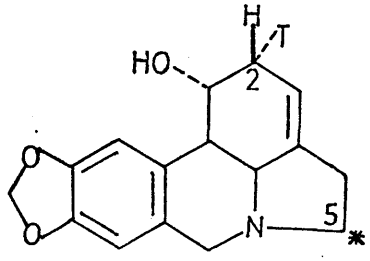


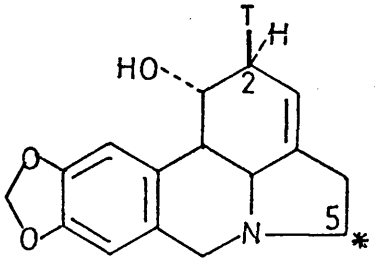
(146)





(147) (148)





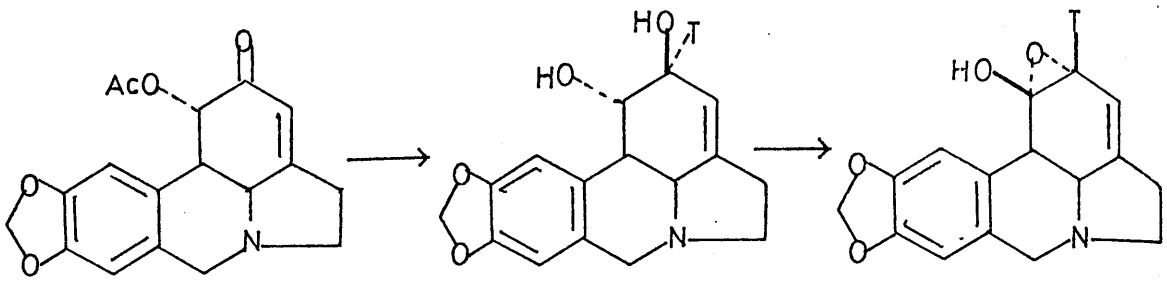
(149) (150)

tritium. The configuration of the tritium in norpluviine was known[68] to be β-, the same as the 2-hydroxyl configuration in lycorine. Thus the hydroxylation at C-2 in norpluviine had occurred with a stereospecific inversion of configuration. Bruce and Kirby explained this unusual hydroxylation with retention by suggesting that the first oxidation product might be the epoxide (139) formed by attack at the less hindered face of norpluviine. Stereospecific ring-opening would give the allylic alcohol (140) which could rearrange to lycorine (141).

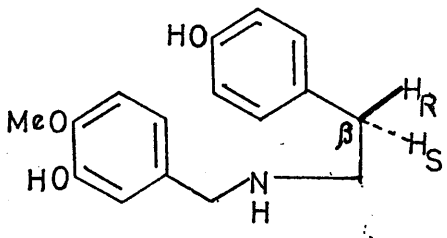
A similar allylic rearrangement with retention of configuration has been postulated by Barton and co-workers[82] in morphine alkaloid biosynthesis. Cyclisation of [7α-^{3}H]-salutaridinol (142), but not the 7-epimer, to thebaine (144) was shown to occur with retention of tritium. The postulated allylic rearrangement to the 5β-hydroxy derivative (143), followed by S_N2 displacement as shown would then avoid the apparent requirement for an anti S_N2' process.

Fuganti, Staunton and Battersby,[83] whilst studying the biosynthesis of narciclasine (145) in 'Twink' and 'Deanna Durban' daffodils from [3',5'-$^{3}H_2$,methyl-^{14}C]O-methylnorbelladine (136), isolated norpluviine and lycorine both with a 49% retention of tritium. This was in effect a repetition of the work of Bruce and Kirby[70] which confirmed their findings.

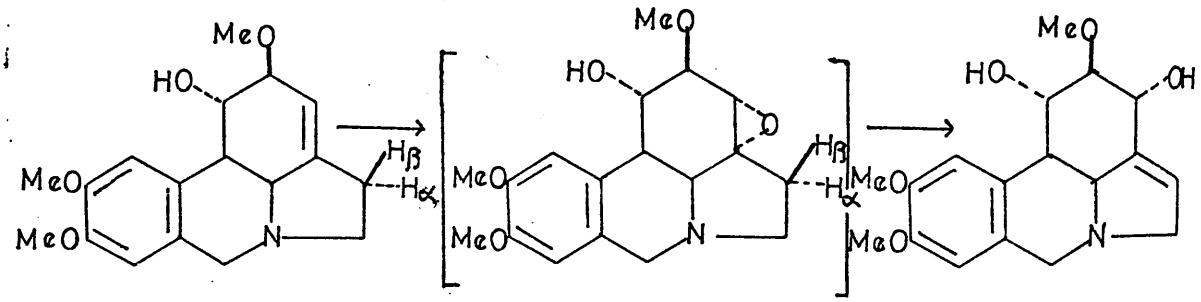
Fuganti and Mazza,[84] however, made a chance observation that, in Clivia miniata Regel plants, [3',5'-$^{3}H_2$,methyl-^{14}C] O-methylnorbelladine was incorporated into lycorine with



(134) (151) (152)



(153)

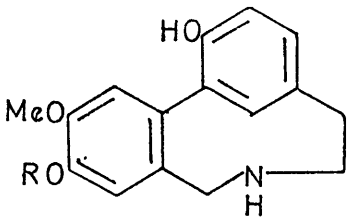


(154) (155) (156)

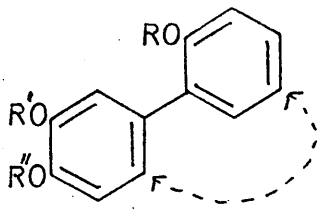
total loss of tritium. In confirmation of these results [3',5',5''-$^{3}H_{3}$,α-^{14}C]O-methylnorbelladine (146) fed to Clivia miniata was found to give lycorine (147) with a 37% retention of tritium. Conversion of this lycorine to 1-O--acetyl-lycorin-2-one (148) occurred with almost total retention of tritium. Although neither norpluviine nor caranine has been isolated from this plant, it seemed that hydroxylation of caranine to lycorine had occurred by a different steric course from that established for 'Twink' daffodils. To establish this supposition, Fuganti and Mazza prepared [2α-^{3}H,5-^{14}C]caranine (149) and [2β-^{3}H, 5-^{14}C]norpluviine (150). The latter precursor was obtained by mixing norpluviine derived in 'Twink' from [α-^{14}C]O--methylnorbelladine and from [3',5'-$^{3}H_{2}$]O-methylnorbelladine. [2α-^{3}H]Caranine was prepared by reduction of 1-O-acetyl--lycorin-2-one (134) with sodium boro-[^{3}H]-hydride to [2-^{3}H]lycorine(151), conversion of [2-^{3}H]lycorine to [2-^{3}H]-1, 2α-lycorine epoxide (152) by Heimer and Wildman's procedure, and lithium aluminium hydride reduction of this epoxide. [2α-^{3}H]Caranine, thus obtained, was mixed with caranine biosynthesised from [α-^{14}C]O-methylnorbelladine in 'Twink' daffodils as before, to give the former precursor. Clivia miniata incorporated [2α-^{3}H,5-^{14}C]caranine into lycorine with 92% tritium retention whilst [2β-^{3}H,5-^{14}C]norpluviine gave incorporation with a 20% ^{3}H retention. Fuganti and Mazza concluded that (a) conversion of O-methylnorbelladine into lycorine in Clivia miniata occurred via norpluviine

and caranine; (b) hydroxylation at C-2 took place with removal of the β-H (retention of configuration); and (c) norpluviine and caranine intermediates in the biosynthesis of lycorine in Clivia miniata retain the 5'-H of O-methyl-norbelladine in the 2β-position.

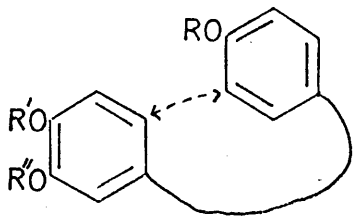
Fuganti and co-workers[85] considered that, in the biosynthesis of narcissidine (156), an epoxide analogous to that proposed by Bruce and Kirby (155) might open, in the manner shown to yield narcissidine. [β-^{3}H,α-^{14}C]O-Methyl-norbelladine (153), fed to Sempre avanti daffodils gave galanthine (154) with 98% tritium retention and narcissidine (155) with 46% tritium retention. This indicated that the hydroxylation was proceeding in a stereospecific manner and subsequent experiments with [2R-^{3}H,α-^{14}C] and [2S-^{3}H,α-^{14}C] O-methylnorbelladine showed that the 4α-H of galanthine (154) was stereospecifically removed in oxidation to narcissidine (156); the 4β-H being completely retained in (156). The stereochemistry observed was taken by the authors as evidence against intermediates of the type (155) in general.



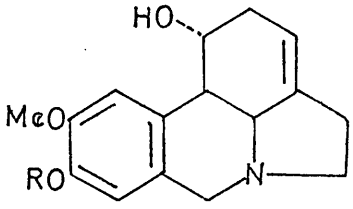
(112)



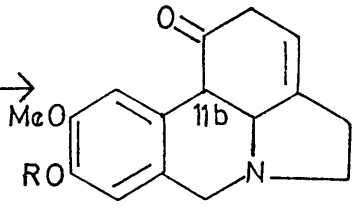
(157)



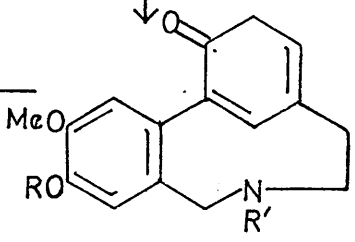
(158)



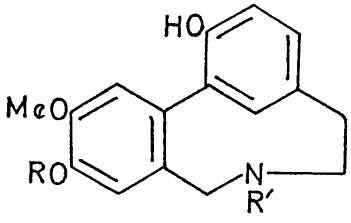
(159)



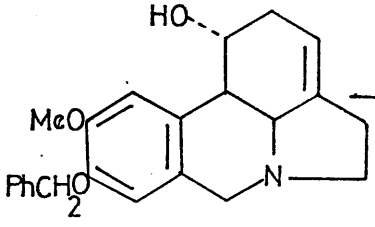
(160)



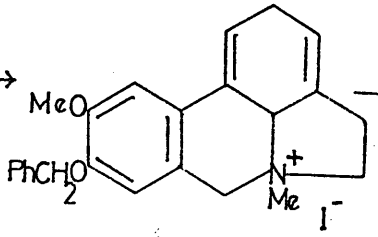
(161)



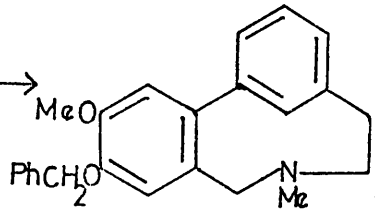
(162)



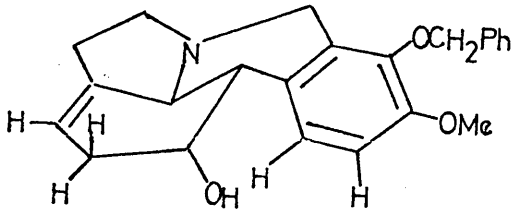
(163)



(164)



(165)

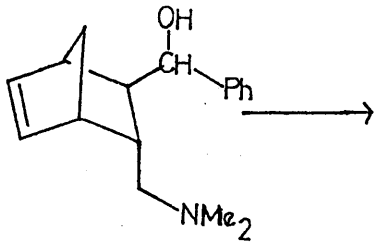


(166)

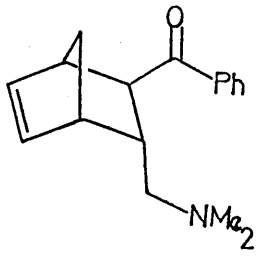
DISCUSSION

2.1. Attempted Syntheses of a Postulated Biosynthetic Intermediate

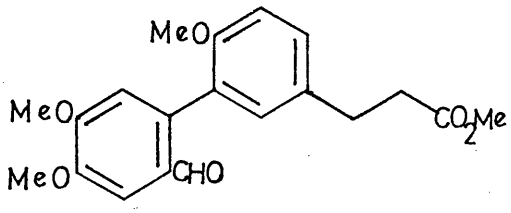
One question about the biosynthesis of the lycorine type of Amaryllidaceae alkaloids which remains to be answered is do these alkaloids originate from a cyclisation as postulated by Barton and Cohen, or from the shorter route later suggested by Barton alone? One feature which distinguishes these two routes (see p. 81) is the presence of the biphenyl intermediate (112) in the former route. If this biphenyl could be synthesised, tritium could easily be exchanged into positions ortho- to the phenol groups and the resulting radioactively labelled biphenyl could be fed to an appropriate plant. Incorporation into norpluviine or lycorine would suggest that Barton and Cohen's scheme was the one operating in Nature whereas non-incorporation (especially in a cell-free system) would be strong evidence for Barton's modified scheme. Moreover, chemical or enzymic oxidation of (112) might provide an interesting synthetic route to the alkaloids' ring system. With these aims in mind it was hoped to synthesise the biphenyl (112). This biphenyl poses a difficult synthetic problem as, not only is the molecule a biphenyl contained in a nine-membered heterocyclic ring, but the biphenyl portion of the molecule is ortho- meta bridged, a situation which creates additional strain in an already severely strained ring system. This problem was approached from three directions. Firstly a partial synthesis was



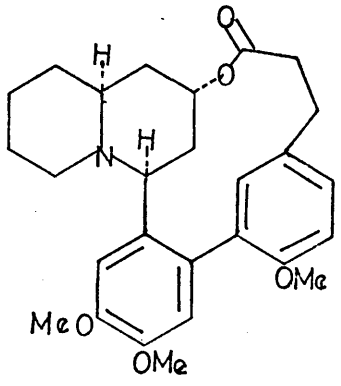
(167)



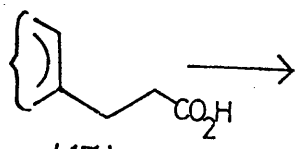
(168)



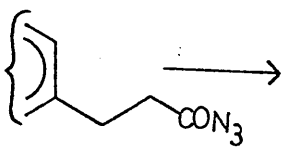
(169)



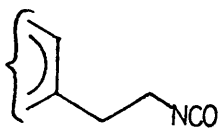
(170)



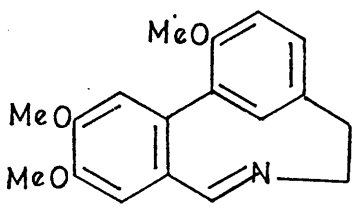
(171)



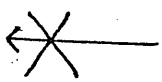
(172)

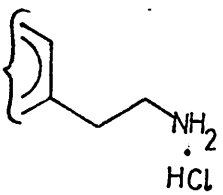


(173)

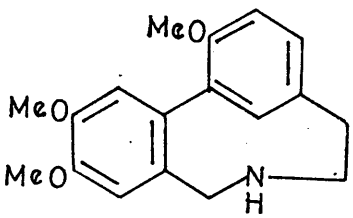


(175)





(174)



(176)

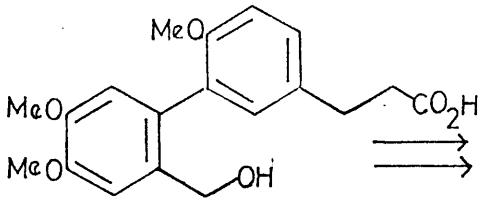
attempted; secondly the biphenyl section was synthesised first and an attempt to close the heterocyclic ring made latterly (157); and thirdly a ring closure to form the biphenyl link in the final step was attempted (158).

The proposed partial synthesis involved oxidation of the protected norpluviine (159) to a protected norpluviin--one (160). N-Alkylation, or perhaps even N-protonation, followed by a base-catalysed Hoffman elimination of the activated 11b proton would yield the dienone (161) which would readily rearrange to give the doubly protected biphenyl (162). There was good precedent for such an elimination as Kirby and Bruce[81] had shown in the Hoffman elimination of 1,11b-anhydro-9-O-benzylnorpluviine methiodide (164) to 9-O-benzylnorpluviine anhydromethine (165). The literature preparation of 9-O-benzylnorpluviine (163) was repeated but a successful oxidation of 9-O-benzylnorpluviine was not achieved despite attempts with a series of mild oxidising agents. This was thought to be due to two reasons. Firstly, the secondary alcohol is in a sterically hindered position (166) and secondly part of the molecule consists of a substituted benzylamine which is susceptible to oxidative cleavage. Poos and Lehman[86] state that oxidation of a secondary alcohol in the presence of a tertiary nitrogen is generally difficult and in their particular case chromic anhydride/pyridine succeeded in oxidising (167) to (168) where numerous other oxidants had failed. However, chromic anhydride/pyridine was no more successful than any of the

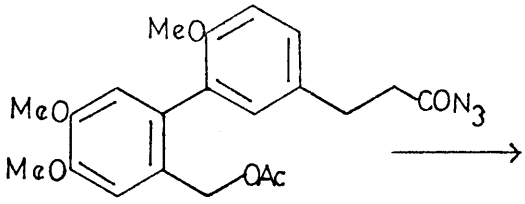
other systems which were applied to the oxidation of 9-O-benzylnorpluviine. Eventually the attempt at partial synthesis had to be abandoned due to insufficient quantities of norpluviine which was obtained by extraction of 'Twink' daffodils.

The second approach (synthesis and cyclisation of a biphenyl) was initiated by the reported synthesis of the biphenyl (169) by Loev, Lantos, and Van Hoeven[87] in the total synthesis of the Lythraceae alkaloid derivative (±)-methyl-decinine (170). Although (169) was fully methylated this seemed a good model system to start with and a protected biphenyl could be used when a successful synthesis had been achieved with the model system.

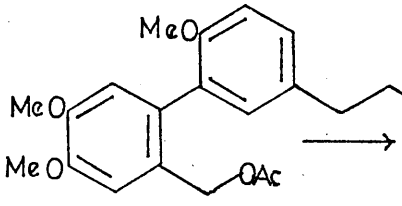
The literature preparation of (169) was repeated and after hydrolysis the acid (171) was converted into the azide (172) which was transformed to the isocyanate (173) by a Curtius rearrangement. Acid hydrolysis of the isocyanate gave the amine hydrochloride (174). When this hydrochloride was basified in very dilute solution, the expected Schiff's base (175) was not obtained but a white solid was formed which was insoluble in all common solvents with the exception of trifluoro-acetic acid. Acid catalysed hydrolysis of this material gave a partial recovery of the amine hydrochloride (174) and its mass spectrum showed two intense peaks of 2M-31 and M-31 where M is the molecular weight of (175). Accurate mass measurements showed that these intense peaks



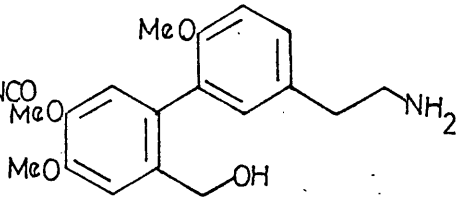
(177)



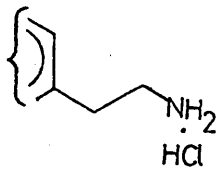
(178)



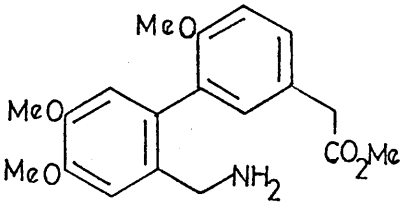
(179)



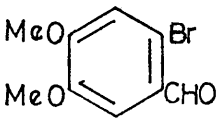
(180)



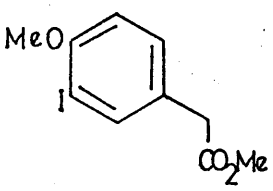
(181)



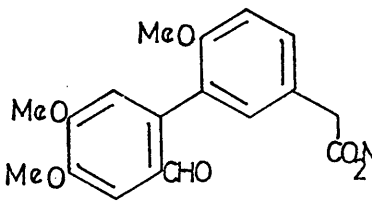
(182)



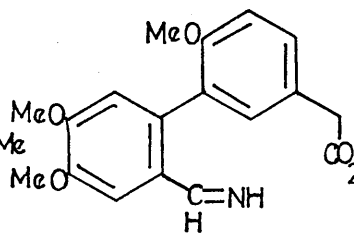
(183)



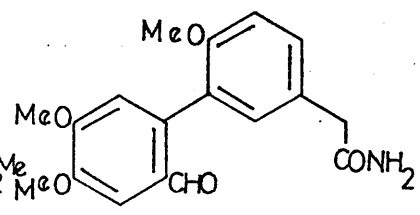
(184)



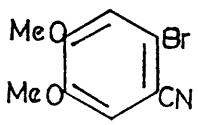
(185)



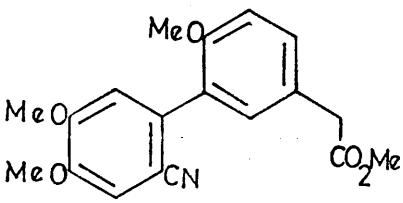
(186)



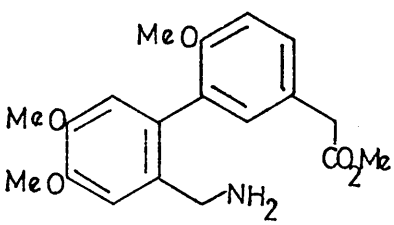
(187)



(188)



(189)



(190)

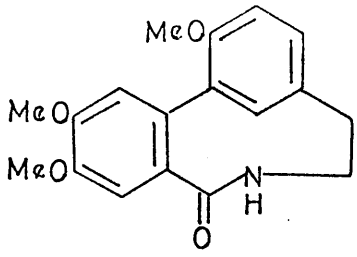
corresponded exactly to loss of CH_3O- from a dimer of (175) and loss of CH_3O- from (175) respectively. It was concluded that this white solid was polymeric or oligomeric in nature. Repeated attempts at formation of the Schiff's base (175) by this method consistently gave the same polymeric product.

An attempt to synthesise the amine (176) by sodium cyanoborohydride reduction of the hydrochloride (174) at pH 3-4 in methanol/water was also unsuccessful, a large number of compounds of low yield being obtained.

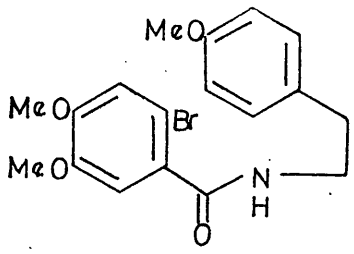
It was thought that the imine bond of (175) may have been introducing more strain into an already strained system and so an attempt at cyclisation of fully saturated side-chains was made. The aldehyde (171) was reduced with sodium borohydride to the alcohol (177) which was converted into the azide (178) and then into the isocyanate (179). Acid hydrolysis of the isocyanate with a basic work-up gave the desired amine (180) which was isolated as its hydrochloride (181). It was hoped that chlorination of the alcohol, followed by *in situ* basification would yield the desired amine (176) by nucleophilic displacement. However, although a variety of chlorinating agents were used and the basification was carried out with aqueous and non-aqueous bases, only recovered starting material (180) and a mixture of numerous minor products could be obtained in each case. It was thought that the by products were a result of some *N*-chlorination and intermolecular nucleophilic displacement.

Hoping that cyclisation might be more facile if the nitrogen atom were in the benzylic position of an acyclic precursor we attempted to synthesise the amine (182). The ester (185) was synthesised by a mixed Ullman coupling of (183) and (184) in the same fashion as the synthesis of (169). Treatment of the ester (185) with ammonia in methanol showed no evidence for the formation of the desired imine (186), but only a slow formation of the amide (187). In an attempt to trap the imine (186) the reaction mixture was quenched by the addition of excess lithium aluminium hydride, before formation of the amide (187) was detectable, but this procedure yielded no basic product.

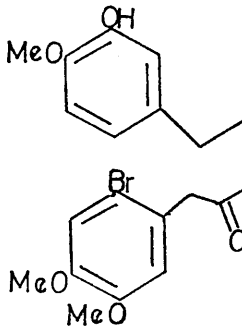
In a further attempt to produce an acyclic precursor of (176) with nitrogen in a benzylic position the cyano-ester (189) was synthesised by a mixed Ullman coupling of the nitrile (188) and the ester (184). According to reports[88] an ester function is reduced more slowly than a nitrile function by diborane in tetrahydrofuran. However when the cyano-ester (189) was treated with diborane in tetrahydrofuran the ester function reduced much faster (adjudged by t.l.c. and i.r. spectra) than the nitrile and it was not possible to obtain any of the desired amine (190). An attempted reduction of (189) with a sodium borohydride/cobaltous chloride mixture was also unsuccessful in that an intractable mixture was obtained as product.



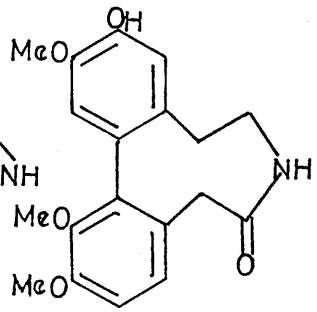
(202)



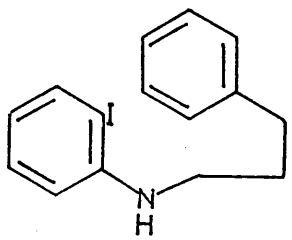
(203)



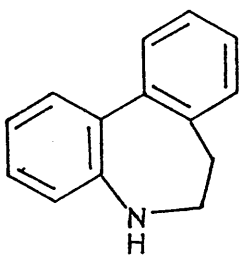
(204)



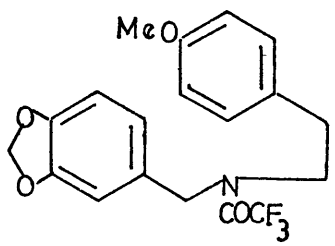
(205)



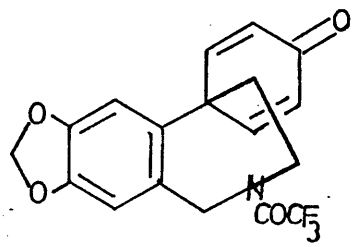
(206)



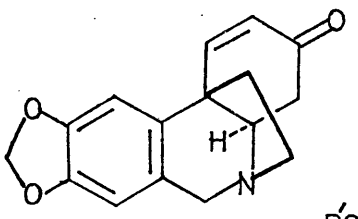
(207)



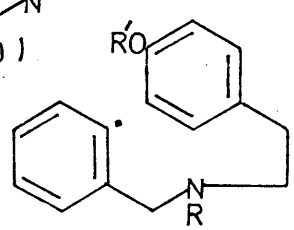
(208)



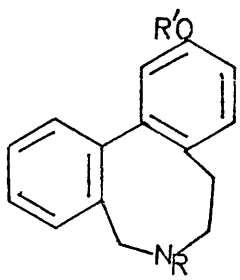
(209)



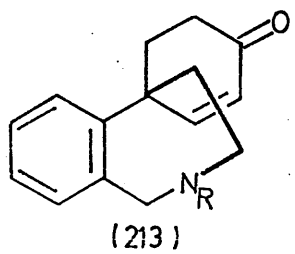
(210)



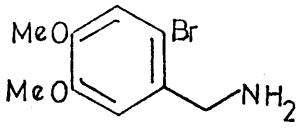
(211)



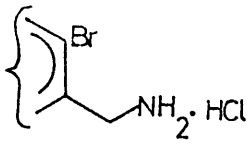
(212)



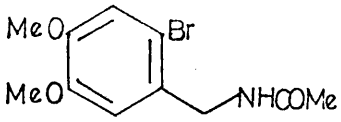
(213)



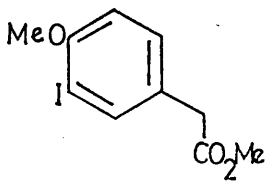
(191)



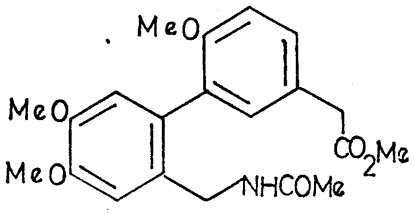
(192)



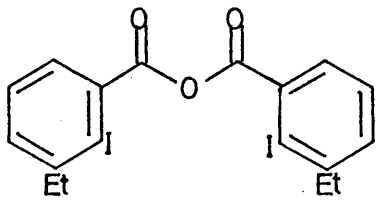
(193)



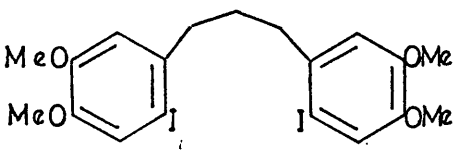
(184)



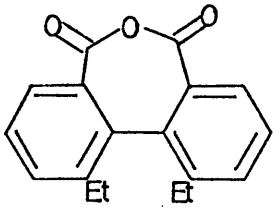
(194)



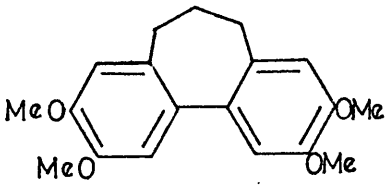
(195)



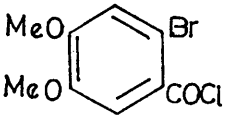
(196)



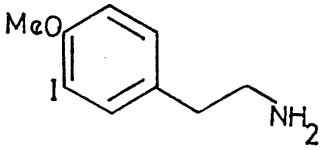
(197)



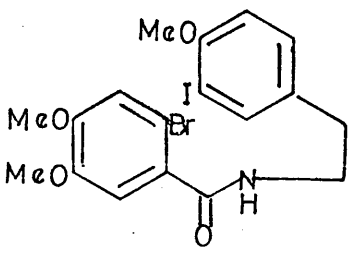
(198)



(199)



(200)

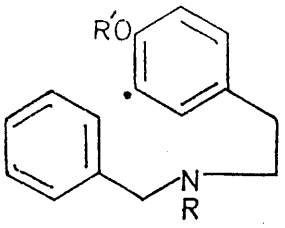


(201)

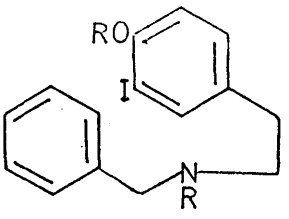
The last attempt at synthesising a substituted benzylamine precursor for (176) was an attempted Ullman coupling of the amide (193) and the ester (184). The product (194), although detectable by mass spectroscopy, was present only in minute quantities and could not be isolated in the pure state.

The third approach (158) to the synthesis of the biphenyl (112) was begun with an attempted Ullman cyclisation. The biphenyls (197) and (198) had been synthesised[89] by an Ullman coupling of their respective acyclic precursors (195) and (196). As these biphenyls are linked by seven-membered rings there seemed a reasonable chance of success with the system under study. The amide (201) was prepared by the reaction of (199) and (200). When this amide was subjected to the same 'Ullman conditions' which had been successful in the preparation of acyclic biphenyls the hoped-for biphenyl (202) was not detectable and the major product isolated was the reduction product (203).

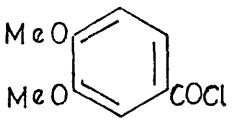
The synthesis[90] of the Erythrina alkaloid erybidine (205) by the photolysis of the acyclic amide (204) is one example of many photochemical syntheses of cyclic biphenyls. The close similarity of the alkaloid (205) to the biphenyl (112) was an encouragement for investigation of a possible photochemically induced radical coupling (158). Jeffs and Hansen[91] had shown that photolysis of the amine (206) produced the biphenyl (207) as the only cyclic product and oxidative radical coupling of the amide



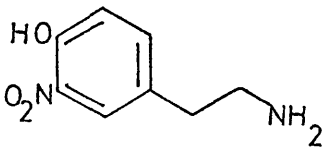
(214)



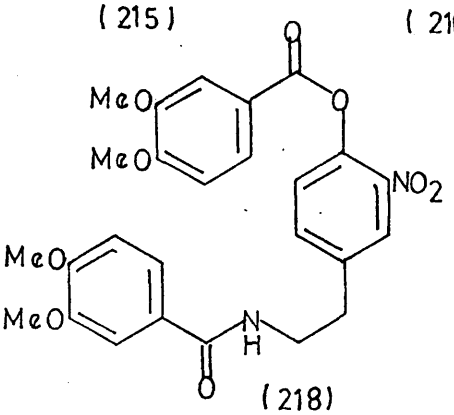
(215)



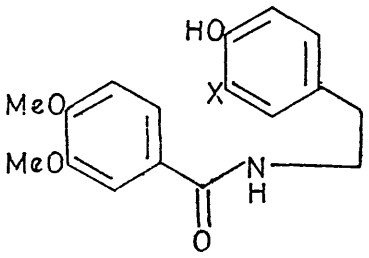
(216)



(217)



(218)

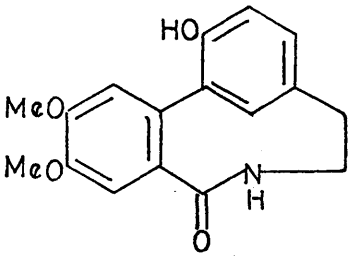


(219); X= NO_2

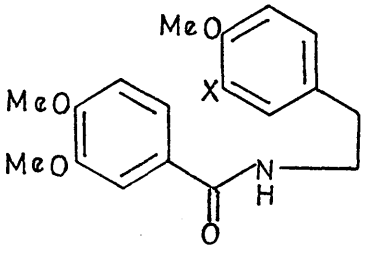
(220); X= NH_2

(221); X= $N_2^+Cl^-$

(222); X= I



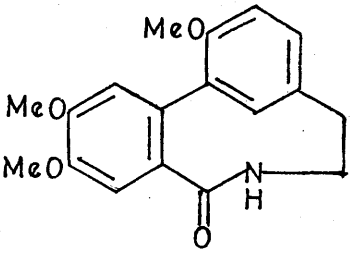
(223)



(224); X= I

(225); X= H

(226); X= Ph

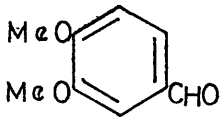


(227)

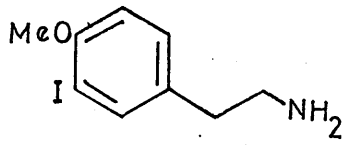
(208) gave (209) as an intermediate in the synthesis of (±) oxocrinine[92] (210). It was therefore obvious that if the radical initially formed by photolysis had the general structure (211) the least strained, and therefore the most probable products would be (212) and (213). To avoid this, the projected synthesis must involve a radical of the type (214) which could be generated from the corresponding iodo compound (215).

The amide (220) was obtained from veratroyl chloride (216) and 3-nitrotyramine (217) by hydrogenation of the intermediate amide (219). However decomposition of the diazonium salt (221) derived from this amine in the presence of potassium iodide yielded an intractable brown mixture instead of the iodo-phenol (222). Although this route to iodo arenes had to be abandoned a brief attempt at Pschorr cyclisation of the diazonium salt (221) to yield the biphenyl (223) was made, but again an intractable mixture resulted.

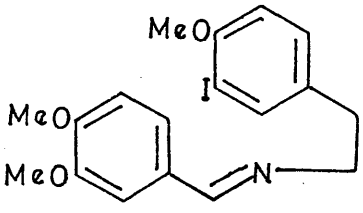
Condensation of veratroyl chloride (216) and the amine (200) gave the amide (224). Irradiation of a methanolic solution of this amide with a low pressure mercury lamp gave (225) as the major product. To eliminate the possibility of reduction by participation of an alcoholic solvent the photolysis was repeated in benzene but again solvent capture of the photolytically produced radical gave the biphenyl (226).



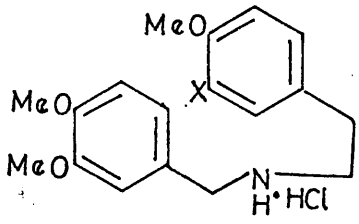
(228)



(200)

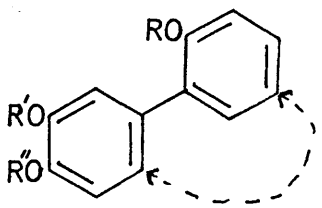


(229)

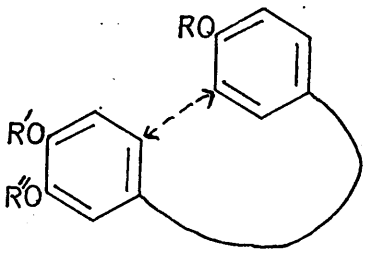


(230); X=I

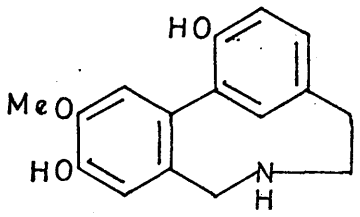
(231); X=H



(157)



(158)



(112)

Apparently the photolysis was producing a radical but in each case the desired cyclisation to (227) would not occur. In case the amide linkage was introducing an extra strain in the heterocyclic ring of (227) which prevented the cyclisation occurring, photolysis of the amine hydrochloride (230) was investigated. This compound was prepared by sodium borohydride reduction of the Schiff's base (229) formed from veratraldehyde (228) and the amine (200). The hydrochloride was photolysed in a methanol/water mixture and the photolysis, which was much slower than the photolysis of the corresponding amide (224), again produced only the reduction product (231).

Thus it seems that, in the second approach (157), when a desired precursor was attained inter-molecular coupling predominated, and in the third approach (158), although the desired radical generally formed, any escape route was preferable to intra-molecular cyclisation. All hope for the future synthesis of (112) must therefore lie in the partial synthesis already outlined or, more generally in the ring--opening of a fully-synthetic tetracyclic ring system.

2.2. Biosynthetic Studies

As Leistner, Gupta, and Spenser[93] make abundantly clear one cannot be too careful when assigning precursor configuration in biosynthetic precursor-product relationships. With this thought in mind it seemed desirable to confirm the findings of Bruce and Kirby which indicated an equal

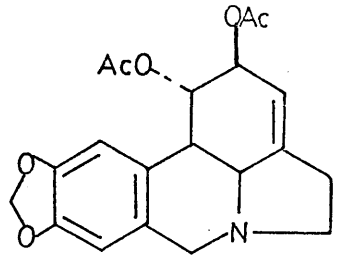
incorporation of D- and L-tyrosine into lycorine and norpluviine with loss of the α-H of tyrosine in each case.

In experiments complementary to those of Bruce,[71] mixtures of DL-[3,5-$^{3}H_2$]- and L-[α-^{14}C]-tyrosine and of DL-[3,5-$^{3}H_2$]- and L-[α-^{14}C]-tyrosine were prepared and fed to 'Twink' daffodils. In addition, the fates of the α-H of D- and L-tyrosine were re-examined by feeding 'Twink' daffodils separately with L-[α-^{3}H,α-^{14}C]- and D-[α-^{3}H,α-^{14}C]--tyrosine. The results of these experiments are summarised in Table 3. Both DL-[3,5-$^{3}H_2$]/L-[α-^{14}C]- and DL-[3,5-$^{3}H_2$]/D-[α-^{14}C]-tyrosine mixtures were incorporated with similar efficiences and similar ^{3}H/^{14}C ratios. This showed clearly that D- and L-tyrosine are utilised equally well by the plant for alkaloid synthesis and that Bruce's earlier findings were correct. However, although L-[α-^{3}H,α-^{14}C]- and D-[α-^{3}H,α-^{14}C]-tyrosine were incorporated with comparable efficiencies the L-isomer retained most of its α-tritium whereas the D-isomer lost nearly all α-tritium. This result was in conflict with earlier results of Bruce and to resolve the anomaly DL-[α-^{3}H,α-^{14}C]-tyrosine was fed to 'Twink' in repetition of Bruce's experiment. Norpluviine showed approximately 50% loss of α-^{3}H (Table 3) in agreement with the foregoing experiments. These results can be explained in terms of a fast utilisation of L-tyrosine and a slow transformation of D-tyrosine to L-tyrosine either by a racemase or a transaminase. Conversion of D-tyrosine into

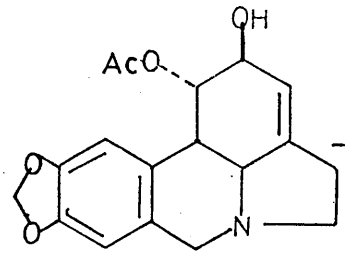
Table 3

Feeding Experiments with 'Twink' Daffodils Relating to Precursor Configuration for Norpluviine and Lycorine

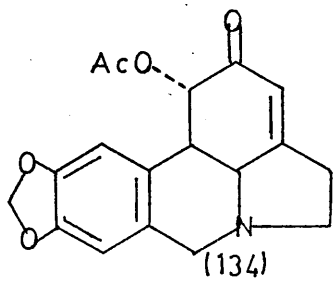
Year	Tyrosine Precursor	Norpluviine		Lycorine	
		Incorporation (%)	Tritium Retention (%)	Incorporation (%)	Tritium Retention (%)
1974	DL-[3,5-$^{3}H_2$]/L-[α-^{14}C]	0.99	49.6	0	-
1974	DL-[3,5-$^{3}H_2$]/D-[α-^{14}C]	0.95	51.6	0	-
1974	L-[α-^{3}H,α-^{14}C]	0.48	98.6	0.01	100
1974	D-[α-^{3}H,α-^{14}C]	0.56	0.9	0.01	0
1975	DL-[α-^{3}H,α-^{14}C]	0.15	45.4	0	-



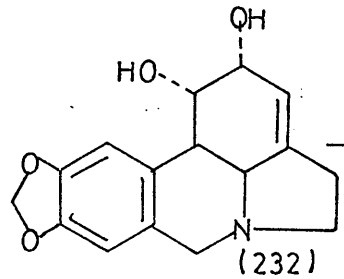
(233)



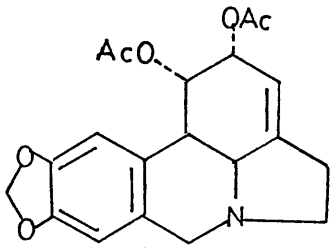
(234)



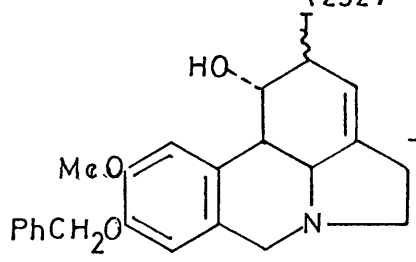
(134)



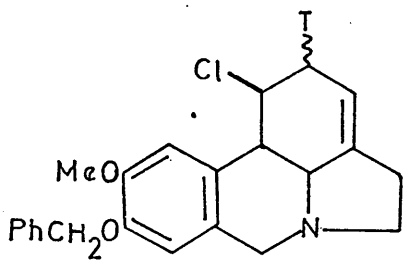
(232)



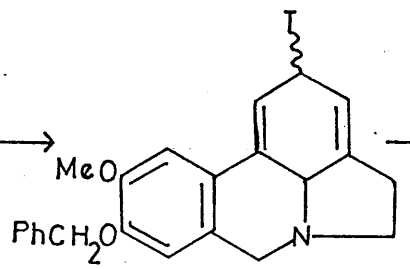
(235)



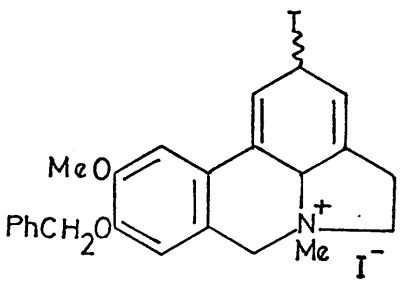
(236)



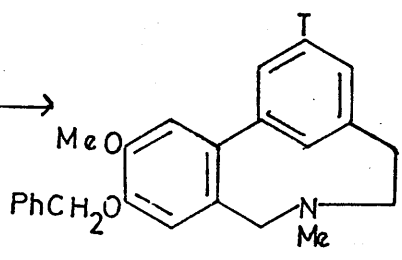
(237)



(238)



(239)



(240)

tyramine (with retention of tritium), a known intermediate in the pathway, by oxidative decarboxylation can however be safely ruled out. The difference between these results and those of Bruce may be due to variations in the conditions of the plants fed in each series of experiments. It is noteworthy that only 45% of the precursor's tritium was retained when DL-[α-^{3}H,α-^{14}C] was fed to 'Twink' plants. The loss of 5% may have been due to a partial reversible epimerisation of L-tyrosine before incorporation.

Fuganti and Mazza's paper on the stereochemistry of hydroxylation in the biosynthesis of lycorine in Clivia miniata, reported that this hydroxylation followed the opposite stereospecific course to that found in daffodils. This implied that plants of the same family had evolved different enzymes to perform essentially the same operation. Such a situation appeared so unlikely that further investigation was necessary.

These anomalies might be explained if lycorine, isolated from either daffodils or Clivia plants had been contaminated with 2-epilycorine (232). The optical rotation of lycorine freshly isolated from 'Twink' daffodils was taken and compared with the rotation of an authentic sample isolated by J.D. Loudon[94] and co-workers. The optical rotations were the same within experimental error and so it was decided to seek chromatographic evidence for 2-epilycorine in 'Twink' plants. After the method of Nakagawa and Uyeo,[95] diacetyl--lycorine (233) was selectively hydrolysed to 1-O-acetyl--lycorine (234) which was oxidised to 1-O-acetyl-lycorin-2-

-one (134). Reduction of the ketone (134) with lithium aluminium hydride gave 2-epilycorine which was converted into its diacetate (235). Lycorine diacetate (233) and 2-epilycorine diacetate (235) were widely separated on t.l.c. but none of the latter could be detected in the crude mixture obtained by acetylation of 'Twink', chloroform-insoluble alkaloids.

An opportunity to check the results of Kirby and Bruce was provided by the set of related feeding experiments (reported above) with DL-[3,5-$^{3}H_{2}$,α-^{14}C]-, DL-[3,5-$^{3}H_{2}$]/D-[α-^{14}C]-, and DL-[3,5-$^{3}H_{2}$]/L-[α-^{14}C]-tyrosine in 'Twink' daffodils in 1974. However (Table 4), although all these experiments gave incorporation into norpluviine with the expected 50% retention of tritium, only the first experiment gave a significant incorporation into lycorine. Only 30% (rather than 50%) of the precursor's tritium was present in this sample but, as the incorporation of radiolabelled tyrosine into lycorine was not high, repetition of the experiment was necessary. In Spring 1975, DL-[3,5-$^{3}H_{2}$,α-^{14}C]tyrosine was fed to 'Twink' on two occasions but a high incorporation into lycorine could not be achieved. Again, norpluviine retained half of the tyrosine tritium and again lycorine had a tritium retention of ca. 30%.

In order to establish the position of the tritium label in the biogenetic lycorine the radioactive lycorine diacetate was converted, as before, into the mono-acetate (133) and then to the ketone (134). Little change in tritium

Table 4

Feeding Experiments Relating to the 2-Hydroxylation of Norpluviine

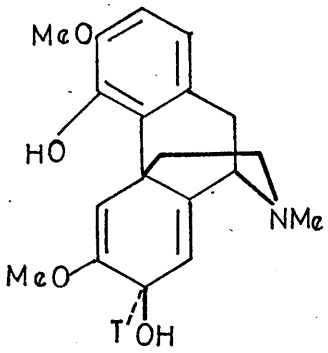
Plant and Year	Tyrosine Precursor	Compound*	Incorporation (%)	Tritium Retention (%)
'Twink' 1974	DL-[3,5-$^{3}H_2$]/ L-[α-^{14}C]	Norpluviine	0.99	49.6
		Lycorine	0	-
'Twink' 1974	DL-[3,5-$^{3}H_2$]/ D-[α-^{14}C]	Norpluviine	0.95	51.6
		Lycorine	0	-
'Twink' 1974	DL-[3,5-$^{3}H_2$, α-^{14}C]	Norpluviine	1.75	47.7
		Lycorine	0.02	30.3
'Twink' 1975	DL-[3,5-$^{3}H_2$, α-^{14}C]	Norpluviine	0.44	48.1
		Lycorine	0.04	32.8
'Twink' 1975	DL-[3,5,-$^{3}H_2$, α-^{14}C]	Norpluviine	1.40	47.7
		Lycorine	0.04	29.9
		(133)	-	26.4
		(134)	-	1.2**
'Twink' 1976	DL-[3,5-$^{3}H_2$, α-^{14}C]	Norpluviine	1.95	47.9
		Lycorine	0.06	28.8
		(238)	-	44.2**
		(240)	-	38.4
Clivia Miniata 1976	DL-[3,5-$^{3}H_2$, α-^{14}C]	Lycorine	0.08	19.6
		(133)	-	17.5
		(134)	-	0.4

* Norpluviine and Lycorine counted as diacetate derivatives

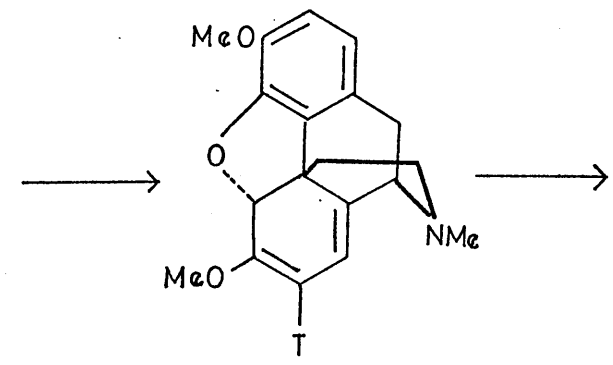
** Not crystallised to constant activity

retention was noted in (133) but when this alcohol was oxidised the ketone obtained had lost 99% of the precursor's tritium activity. This established that almost all the tritium in lycorine (131) was present at the 2 position.

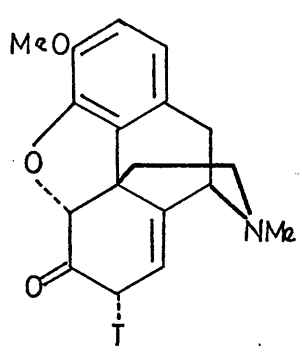
When DL-[3,5-$^{3}H_{2}$,α-^{14}C]tyrosine was yet again fed to 'Twink' in 1976 a slightly better incorporation into lycorine was noted and again norpluviine and lycorine retained, respectively, ca. 50% and 30% of the precursor's tritium. If partial epimerisation of the tritium label at C-2 of norpluviine had occurred this could explain the apparent partial loss of tritium in hydroxylation to lycorine. To establish whether such were the case, the method of Bruce and Kirby[81] for determination of the stereochemistry of the tritium at C-2 in norpluviine was followed. Biogenetically--derived norpluviine from the 1976 feeding experiment was diluted with inactive norpluviine and converted to the 9-O-benzyl derivative (236). Treatment of this derivative with phosphorus oxychloride and pyridine gave 1,11b-anhydro--9-O-benzylnorpluviine (238) via the chloride (237). The anhydro-derivative (238) was converted into its methiodide salt (239) which upon treatment with sodium ethoxide underwent rapid elimination to yield the anhydromethine (240). A small loss in tritium was noted in conversion of norpluviine (107) into the anhydro-derivative (238) and the anhydromethine (240) retained only 38% of the tritium present in the precursor. When Bruce carried out the same reaction sequence with norpluviine biogenetically derived



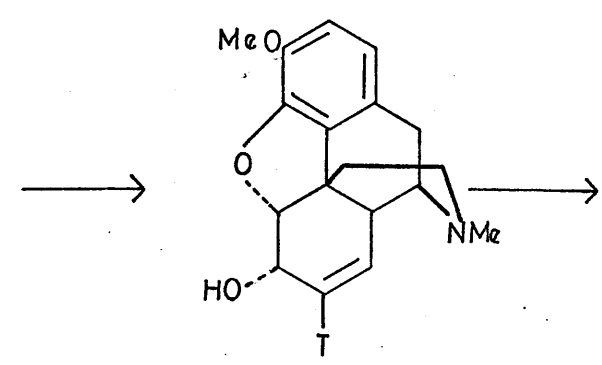
(142)



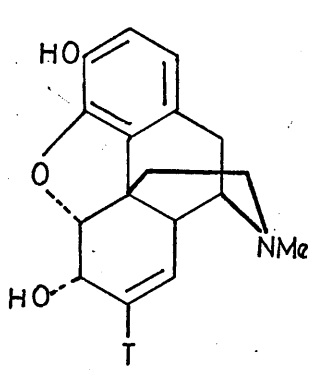
(144)



(241)



(242)



(243)

from the same precursor and in the same plant the anhydromethine (240) lost 96% tritium. Bruce and Kirby had been careful to establish that the elimination of hydrogen iodide to form the anhydromethine followed the second order kinetics expected of a 1,4-conjugate E2 elimination which are believed, admittedly on limited evidence, to take place in the cis[96] fashion. It was therefore unlikely that in our case the tritium retention after elimination was the result of a tritium isotope effect in a non-stereospecific reaction.

It seemed likely then that tritium resident at C-2 in our sample of norpluviine was present in both the α and β configurations. This situation could arise by a reversible enolisation, without enzymic catalysis, of either the intermediate (114), proposed by Barton and Cohen, or the intermediate (115) later proposed by Barton. Such an enolisation could take place with little loss of tritium, due to a tritium isotope effect, and with variable amounts of scrambling of a 2β-tritium, which would not be evident until stereospecific hydroxylation at C-2 took place in the formation of lycorine.

It is worth noting here that, in feeding [2β-^{3}H,5-^{14}C]-norpluviine biogenetically derived from 'Twink' to Clivia miniata, Fuganti and Mazza obtained lycorine with 20% retention of starting tritium activity. This result at least would be consistent with a scrambling of tritium configuration in 'Twink' norpluviine. Barton and co-workers,[97] in studying the incorporation of 7-^{3}H salutaridinol (142)

into thebaine (144), codeine (242), and morphine (243), found that significant amounts of the tritium present in thebaine were lost in conversion into codeine and morphine. Barton hinted that the reason for such a loss may have been enolisation of the intermediate neopinone (241) with attendant exchange of tritium. There is therefore precedent for enolisation at natural pH.

Having repeated the experiments of Bruce and Kirby and found a variation from their results, we decided to repeat the work of Fuganti and Mazza with Clivia miniata Regel. Initially, however, lycorine was isolated from this plant and found to have an optical rotation close to that of lycorine samples isolated recently from 'Twink' and earlier by J.D. Loudon.[94] Furthermore, no chromatographic evidence for the presence of 2-epilycorine in this plant could be found. Young Clivia miniata were fed a sample of DL-[3, 5-$^{3}H_{2}$,α-^{14}C]tyrosine identical to that fed previously to 'Twink' daffodils. Radioactive lycorine (Table 4) was isolated with a retention of 20% of precursor tritium whereas on the basis of Fuganti's experiments, complete loss of tritium was expected. This result suggests that in Clivia miniata a partial scrambling of tritium label may have occurred. Although norpluviine and caranine have not been isolated from this plant, Fuganti and Mazza recently demonstrated that they were the progenitors of lycorine. This being so it is possible that scrambling occurred before the norpluviine stage or by reversal of norpluviine to the

ketones (114) or (115). The scrambling of tritium configuration at C-2 of norpluviine explains the variability of results obtained in this field. However the findings of Wildman are still a glaring contrast to the results obtained by Fuganti using radioactively labelled caranine synthesised by Wildman's procedure. Although reversal of caranine to a ketone analogous to (115) or (114) and resulting scrambling of label cannot be ruled out it would be more satisfying to see a double-labelled feeding experiment with [2β-^{3}H]caranine repeated for Zephyranthes candida and Clivia miniata.

EXPERIMENTAL

General Methods

In addition to the instruments detailed in the previous experimental section the following were used:-

Optical activity - Hilger and Watts M511

Radioactivity - Phillips PW4510

Unless otherwise stated, all activities quoted (dpm= disintegrations per minute; 1 μCi = 2.22 x 10^6 dpm) refer to samples which have been chromatographed and crystallised to constant activity. Scintillation fluid was a solution of P.P.O. (5 g) and P.O.P.O.P. (0.1 g) in toluene (1 l). Samples of tyrosine were dissolved in ethanolic HCl, diluted with dimethylformamide, and added to scintillation fluid. Other samples were dissolved in dimethylformamide and added. Feeding of labelled tyrosine precursors was carried out by injection of aqueous solutions into the leaves and hollow stems of the plants. After all the precursor had been administered the plants were further injected with distilled water for a few days and allowed to grow for another five days before extraction.

DL-[α-^{14}C]Tyrosine was supplied in 50 μ Ci ampoules by the Radiochemical Centre, Amersham.

2.1 EXPERIMENTAL

9-O-Benzylnorpluviine (163)

After the method of Bruce,[71] norpluviine (200 mg, 0.73 mmol) was added to a stirred suspension of sodium hydride in dry dimethylformamide (10 ml) at room temperature, under nitrogen. After 30 minutes the solution became clear and benzyl chloride (93 mg, 0.73 mmol in dry dimethylformamide (1 ml) was added. The solution was heated at 80° for 4 hr. and the resulting suspension cooled and diluted with water (50 ml). The aqueous solution was extracted with chloroform (4 x 15 ml) and the combined chloroform extracts were washed with water (2 x 15 ml), dried ($MgSO_4$), and evaporated to an oil which after p.l.c. (alumina GF_{254}, eluent 20% ethyl acetate in benzene), crystallised from ethyl acetate/light petroleum (b.p. 60-80°C) as colourless plates (120 mg, 45%), m.p. 168-171°C (lit.,[71] 166-170°C), ν_{max}(KBr) 3540 cm^{-1} (O-H), τ ($CDCl_3$) 2.5-2.8 (5H, m, Ph), 3.05 (1H, s, 11-H), 3.36 (1H, s, 8-H), 4.64 (1H, m, 3-H), 4.92 (2H, s, Ph-CH_2-O), 5.27 (1H, m, 1-H), 5.90 (1H, d, J 14Hz, 7-methylene H), 6.13 (3H, s, CH_3-O), 6.52 (1H, d J, 14Hz, 7-methylene H).

Attempted Dimethyl Sulphoxide/N,N'-Dicyclohexylcarbodiimide/H_3PO_4 Oxidation of 9-O-Benzylnorpluviine

9-O-Benzylnorpluviine (30 mg, 0.08 mmol) was dissolved in a solution of N,N'-dicyclohexylcarbodiimide (52 mg, 0.25 mmol) in dimethyl sulphoxide (1 ml) and ethyl acetate (1 ml). The mixture was stirred until homogeneous (5 minutes) when

H_3PO_4 (5 mg, 0.05 mmol) was added. The reaction was followed by t.l.c. (alumina GF_{254}, eluent 20% ethyl acetate in benzene), each base line spot being basified with ammonia (d 0.88), but after 1 week starting material was largely unchanged.

Attempted Dimethyl Sulphoxide/Acetic Anhydride Oxidation of 9-O-Benzylnorpluviine

9-O-Benzylnorpluviine (30 mg) was dissolved in a mixture of acetic anhydride (0.3 ml) and dimethyl sulphoxide (0.6 ml) and the resulting solution stirred at room temperature for 16 hr. The solution was then diluted with water (40 ml) and extracted with chloroform (3 x 30 ml). The combined chloroform extracts were dried ($MgSO_4$) and evaporated to small bulk and separated on p.l.c. (alumina GF_{254}, eluent 20% ethyl acetate in toluene) to yield as the major fraction (10 mg) 1-O-acetyl-O-benzylnorpluviine. ν_{max} (thin film) 1735 cm^{-1} (CH_3-C=O).

Attempted Aqueous Chromic Acid Oxidation of 9-O-Benzylnorpluviine

9-O-Benzylnorpluviine (37 mg, 0.1 mmol) was stirred in and ice-cooled solution of 2.5M aqueous sulphuric acid (5 ml) and chromium trioxide (10 mg, 0.1 mmol) added in one portion. The resulting solution was stirred for 45 minutes before being diluted with water (25 ml) and treated with excess sodium hydrogen carbonate. The neutralised solution was extracted with chloroform (3 x 30 ml) and the combined

chloroform extracts dried ($MgSO_4$) and evaporated to a brown oil. This oil gave only one p.l.c. (alumina GF_{254}, eluent 20% ethyl acetate in benzene) fraction of weight greater than 4 mg. This was recovered 9-O-benzylnorpluviine (16 mg, 42%). A small portion of the reaction mixture gave no precipitate with acidic ethanolic 2,4-dinitrophenylhydrazine.

Attempted Ruthenium Tetroxide Oxidation of 9-O-Benzylnorpluviine

9-O-Benzylnorpluviine (40 mg, 0.11 mmol) was stirred in ethanol-free chloroform (5 ml) and a suspension of ruthenium dioxide (5 mg, 0.04 mmol) in water (5.0 ml) added. The heterogeneous mixture was stirred vigorously and saturated aqueous potassium periodate solution added dropwise until the yellow colour of ruthenium tetroxide persisted. The yellow mixture was stirred for 30 minutes, diluted with water (20 ml), and extracted with chloroform (3 x 15 ml). The combined chloroform extracts were dried ($MgSO_4$) and evaporated to yield a brown intractable oil.

Attempted Oxidation of 9-O-Benzylnorpluviine with Chromic Anhydride/Pyridine Complex

Following the method of Ratcliff and Rodehurst[98] the reagent was prepared by the addition of chromium trioxide (600 mg, 6 mmol) to a stirred solution of dry pyridine (950 mg, 12 mmol) in dry methylene chloride (15 ml). This solution (1.5 ml), was added to 9-O-benzylnorpluviine (30 mg, 0.08 mmol) in methylene chloride (5 ml). After 2 hr no change was detectable by t.l.c. (alumina GF_{254}, eluent

20% ethyl acetate in benzene). After 6 hr the reaction mixture was diluted with water (50 ml) and extracted with chloroform (3 x 30 ml).

The chloroform extracts were dried ($MgSO_4$) and evaporated to dryness (final traces of pyridine removed by azeotropic distillation with benzene). The resultant oil yielded only one major p.l.c. (above system) fraction viz. recovered 9-O-benzylnorpluviine (12 mg, 40%).

Attempted Manganese Dioxide Oxidation of 9-O-Benzylnorpluviine

Freshly prepared[99] active, basic manganese dioxide (100 mg, 1.2 mmol) in dry benzene (1 ml) was added to a solution of 9-O-benzylnorpluviine (35 mg, 0.1 mmol) and the resulting suspension stirred at room temperature. T.l.c. (alumina GF_{254}, eluent 20% ethyl acetate in benzene) of the reaction mixture showed a slow decomposition of starting material to numerous, equally intense spots. Starting material was still detectable after 6 days.

2-Formyl-4,5,2'-trimethoxy-5'-(2-hydroxycarbonylethyl)-biphenyl (171)

According to the method of Loev, Lantos and Van Hoeven[87] an intimate mixture of methyl β-(3-iodo-4 methoxyphenyl)-propionate[87] (320 mg, 1 mmol), 6-bromoveratraldehyde (245 mg, 1 mmol) and copper bronze (635 mg, 10 mmol) were heated at 200° ± 5°C, in a sealed tube, for 30 minutes. The tube was opened and its contents extracted with hot acetone (100 ml). The acetone solution was evaporated to an oil which

was separated by p.l.c. [alumina GF_{254} eluted twice with 20% ethyl acetate in light petroleum (b.p. 60-80°C)] to yield the desired biphenyl (169) (149 mg, 42%), m/e 358, ν_{max} (CCl_4) 1 738 (O-C=O), 1 675 cm^{-1} (H-C=O), τ ($CDCl_3$) 0.38 (1H, s, H-C=O), 2.3-3.3 (5H, m, aromatic H), 6.04 (6H, s, CH_3-O meta and para to H-C=O), 6.28 (3H, s, CH_3-O-Ar), 7.19 (4H, A_2B_2 m, J 6Hz, $(CH_2)_2$).

A solution of this ester (260 mg, 0.72 mmol) in methanol (50 ml) containing 5% aqueous sodium hydroxide (10 ml) was stirred at room temperature for 1 hr. The resulting solution was evaporated to ca. 10 ml diluted with 5% aqueous HCl (100 ml), and extracted with chloroform (3 x 30 ml). The combined chloroform extracts were washed with brine (2 x 10 ml), dried ($MgSO_4$) and evaporated to give the crude acid (171) as a creamy solid which crystallised (ether) as colourless prisms m.p. 132 - 134°C (lit.,[100] 132 - 135°C) (172 mg, 75%), ν_{max}($CHCl_3$) 3 240 (O-H), 1 710 (O-C=O), 1 675 cm^{-1} (H-C=O).

Amine Hydrochloride (174)

The foregoing acid (171) (69 mg, 0.20 mmol) was stirred at 0°C in dry acetone (10 ml) and freshly distilled triethylamine (22 mg, 0.22 mmol) in dry acetone (5 ml) added in one portion followed by redistilled ethyl chloroformate (25 mg, 0.22 mmol) in dry acetone (5 ml), dropwise over 10 minutes. After stirring the resulting solution for 1 hr sodium azide (15 mg, 0.23 mmol) in water (0.5 ml) was added

slowly and this solution was stirred at 0°C for 1½hr. The reaction mixture was diluted with water (100 ml) and extracted with ether (3 x 50 ml). The dried ($MgSO_4$) ethereal extracts were evaporated to give the acid azide (172) as an oil (66 mg), ν_{max}(thin film) 2 125 (N_3), 1 710 (N-C=O), 1 670 cm^{-1} (H-C=O).

The azide was dissolved in sodium-dried benzene 5 ml and heated at 80°C, under nitrogen, for 2 hr. The resulting solution was evaporated free of solvent to give the isocyanate (173) as an oil, ν_{max}(thin film) 2 260 (N=C=O), 1 670 cm^{-1} (H-C=O).

The isocyanate was stirred in acetone (5 ml) with 20% aqueous HCl (5 ml). After 14 hr the solvents were removed *in vacuo* to yield an oily mixture which solidified on titration with ether. Recrystallisation from ethanol/ether gave needles (33 mg, 30%) of the amine hydrochloride (174), m.p. 207 - 210°C (decomp. with weeping from 194°C) (Found: C, 61.4; H, 6.30; N, 3.72. $C_{18}H_{22}ClNO_4$ requires C, 61.4; H, 6.24; N, 3.96%), ν_{max}(KBr) 3 400 (N-H), 1 670 cm^{-1} (N-H), τ (D_2O) 0.65 (1H, s, H-C=O), 2.61 (1H, dd, *J* 8Hz, 2Hz, aromatic H *meta* to $-CH_2-$), 2.68 (1H, s, aromatic H *ortho* to H-C=O), 2.8-3.0 (3H, m, aromatic H *ortho* to $-CH_2-$), 3.19 (1H, s, aromatic H, *meta* to H-C=O) 6.07 and 6.10 (2 x 3H, singlets, CH_3O- *meta* and *para* to H-C=O), 6.32 (3H, s, CH_3-O *para* to $-CH_2-$), 6.66 (2H, t, *J* 6Hz, $C\underline{H}_2-NH_3^+$), 6.99 (2H, t, *J* 6Hz, $C\underline{H}_2-Ar$).

Basification of the Amine Hydrochloride (174)

The hydrochloride (30 mg), in water (150 ml) was basified to pH 11 by dropwise addition of 5% aqueous sodium hydroxide, with stirring, at 0°C. A white suspension quickly formed and filtration gave a white solid (26 mg), m/e 563 and 266, ν_{max}(KBr) 3 400 w (N-H), 1 636 cm^{-1} (C=N), τ (trifluoroacetic acid) 2.2-3.3 (5H, m, aromatic H), 5.94 and 5.96 (2 x 3H, broad singlets, CH_3-O *meta* and *para* to H-C=O), 6.33 (3H, bs, CH_3-O *para* to $-CH_2-$), 6.15 (2H, bm, CH_2-N), 6.82 (2H, bm, CH_2-Ar). (Found M^+, 563.2555. $C_{35}H_{35}N_2O_5$ requires 563.2546; M^+ 266.1181. $C_{17}H_{16}NO_2$ requires 266.1181). This solid material was insoluble in every common solvent except trifluoroacetic acid and was thought to be a mixture of polymeric imines.

A suspension of this solid (20 mg) was refluxed in 20% aqueous HCl (20 ml) for 2 hr. The resulting solution was evaporated to dryness *in vacuo* to give complete recovery of crude, pale-red amine hydrochloride (174) ν_{max}(KBr) 3 400 (N-H), 1 670 cm^{-1} (H-C=O).

Treatment of the Amine Hydrochloride (174) with Sodium Cyanoborohydride

The hydrochloride (75 mg) in dry methanol (20 ml) was stirred at 0°C and sodium cyanoborohydride (20 mg) added over 30 minutes. The resulting mixture was allowed to stir at room temperature for 12 hr whilst maintaining pH 3-4 (methyl orange) by the addition of 1 M HCl, dropwise as required. The reaction mixture was diluted with water (100

ml), acidified to pH 1 with 20% aqueous HCl and extracted with chloroform (2 x 10 ml).

The chloroform extracts were discarded and the aqueous layer basified to pH 11 with 20% aqueous sodium hydroxide and re-extracted with chloroform (3 x 20 ml). The chloroform extracts were dried ($MgSO_4$) and evaporated to a yellow oil (40 mg) which showed ca. twelve spots of equal intensity on t.l.c. (alumina GF_{254}, eluent 30% ethyl acetate in benzene).

Hydroxy-acid (177)

The foregoing ester (169) (480 mg, 1.34 mmol) in methanol (30 ml) was treated with sodium borohydride (26 mg, 0.80 mmol) in portions over 1 hr. The resulting solution was stirred at room temperature for 1 hr and then for 14 hr longer after the addition of 20% aqueous sodium hydroxide (5 ml). The solution was evaporated to dryness in vacuo, taken up in water (20 ml) and acidified to pH 1 by the dropwise addition of 20% aqueous HCl. The acidified solution was extracted with chloroform (3 x 25 ml) and the combined extracts dried ($MgSO_4$) and evaporated free of solvent to give the hydroxy-acid (177) which crystallised from ether/light petroleum (b.p. 60 - 80°C) (318 mg, 69%) as colourless needles, m.p. 120 - 122°C (Found: C, 65.7; H, 6.34. $C_{19}H_{22}O_6$ requires C, 65.9; H, 6.40%), m/e 346, ν_{max} (thin film) 3 400 (O-H), 1 705 (C=O) cm^{-1}.

Acetylation of Hydroxy-acid (177)

The hydroxy-acid (173 mg, 0.5 mmol) was stirred for 14 hr at room temperature in a solution of pyridine (0.5 ml) and acetic anhydride (2.0 ml). The resulting mixture was poured onto an ice-water slurry (100 g) and acidified by the dropwise addition of 20% aqueous HCl. The acidic solution was extracted with chloroform (4 x 20 ml) and the combined chloroform extracts washed with brine (2 x 10 ml), dried ($MgSO_4$) and evaporated to an oil. This oil was freed from traces of acetic acid by evaporation *in vacuo* (oil pump). The crude acetate (178) gave colourless plates (132 mg, 68%), m.p. 90 - 92°C from ethanol/ether, m/e 388, ν_{max} (thin film) 3 300 (O-H), 1 720 cm^{-1} (HO-C=O and CH_3-C=O), τ ($CDCl_3$) 2.7-3.3 (5H, m, aromatic H), 4.89 (2H, s, Ar-CH_2-O), 6.07 and 6.13 (2 x 3H, singlets, CH_3-O *meta* and *para* to -CH_2-O), 6.27 (3H, s, CH_3-O *para* to -$(CH_2)_2$-, 7.10 (4H, m, $(CH_2)_2$), 8.00 (3H, s, CH_3-C=O).

Amino-alcohol (180)

Following the method described for the preparation of the amine hydrochloride (174) the acetate (178) (580 mg, 1.5 mmol) was converted to the isocyanate (179) ν_{max} (thin film) 2 280 (N=C=O), 1 720 cm^{-1} (CH_3-C=O). Both the isocyanate and acetate functions were hydrolysed by heating a solution of (179) in tetrahydrofuran (15 ml) and 20% aqueous HCl (5 ml), under reflux for 6 hr. The resulting solution was evaporated to *ca.* 5 ml, diluted with water (50 ml) and

basified to pH 11 by the addition of 20% aqueous sodium hydroxide. The alkaline solution was extracted with chloroform (3 x 20 ml) and the combined chloroform extracts dried ($MgSO_4$) and evaporated to an oil which after p.l.c. (alumina GF_{254}, eluent 40% light petroleum (b.p. 40 - 60°C) in ethyl acetate) yielded the amino-alcohol (180) as a glass (268 mg, 57%), m/e 317, ν_{max} (thin film) 3 400 cm^{-1} (N-H and O-H), τ [$(CD_3)_2C=O$] 2.0-3.4 (5H, m, aromatic H), 6.19 and 6.26 (2 x 3H, singlets CH_3-O meta and para to $-CH_2$-O), 6.34 (3H, s, CH_3-O para to $-(CH_2)_2$), 6.61 (2H, t, J 6Hz, N-CH_2), 7.23 (2H, t, J 6Hz, Ar-CH_2-CH_2).

The free base was converted to its hydrochloride by saturation of an ethereal solution with dry HCl to give the salt as a precipitate of fine white needles, m.p. 222 - 225°C (decomp.) (Found: C, 61.2; H, 6.96; N, 4.20. $C_{18}H_{24}ClNO_4$ requires C, 61.2; H, 6.80; N, 3.96%).

Attempted Cyclisation of Amino-alcohol (180)

The hydrochloride (181) (50 mg) was stirred for 12 hr in thionyl chloride (1 ml). The resulting solution was poured, with vigorous stirring, on to 2N sodium hydroxide solution (100 ml). A brown suspension was produced which was extracted with chloroform (3 x 30 ml). The combined chloroform extracts were dried ($MgSO_4$), and evaporated to a dark oil which gave a continuous streak on t.l.c. (alumina GF_{254}, several eluents).

A similar intractable mixture was obtained when the reaction was carried out in dioxan using 1 mol equivalent of thionyl chloride, or when phosphorus oxychloride in acetonitrile was used, or when the reaction mixture was worked-up with triethylamine instead of aqueous sodium hydroxide.

Methyl 3-Iodo-4-methoxyphenylacetate (184)

This was prepared by the method noted[87] for methyl β-(3-iodo-4 methoxyphenyl)propionate and gave the ester (184) as an oil in 58% overall yield from 4-hydroxyphenylacetic acid, m/e 306, ν_{max} (thin film) 1 735 cm^{-1} (C=O), τ ($CDCl_3$) 2.36 (1H, d, J 2Hz, aromatic H ortho to I), 2.69 (1H, dd, J 8Hz, 2Hz, aromatic H para to I), 3.15 (1H, d, J 8Hz, aromatic H meta to I), 6.17 (3H, s, CH_3-O-Ar), 6.35 (3H, s, CH_3-O-C=O), 4.52 (2H, s, $-CH_2-$).

2-Formyl-4,5,2'-trimethoxy-5'-methoxycarbonylmethylbiphenyl (185)

This biphenyl (185) was prepared exactly by the method described in the case of the homologous ester (169) and was obtained as an oil (44%), m/e 344, ν_{max} (thin film) 1 740 (O-C=O), 1 675 cm^{-1} (H-C=O), τ ($CDCl_3$) 0.36 (1H, s, H-C=O), 2.5-3.3 (5H, m, aromatic H), 6.07 (6H, s, CH_3-O meta and para to H-C=O), 6.28 (3H, s, CH_3-O para to $-CH_2-$), 6.30 (3H, s, CH_3-O-C=O), 6.40 (2H, s, $-CH_2-$).

Amination of Ester (185)

The ester (185) (30 mg) was stirred in dry methanol (1 ml) at room temperature and excess ammonia solution (d 0.88; 0.1 ml) added in one portion. After 3 hr, t.l.c. [alumina GF_{254}, eluent 40% light petroleum (b.p. 40 - 60°C)] indicated one major product and the reaction mixture was diluted with water (100 ml) and extracted with chloroform (3 x 20 ml). The combined chloroform extracts were dried and evaporated to yield the amide (187) as a glass (19 mg, 65%), m/e 329, ν_{max} (thin film) 3 340 (N-H), 1 680 (H-C=O) and 1 660 cm^{-1} (N-C=O).

The reaction was repeated on the same scale and after 10 minutes when no amide was detectable by t.l.c. the reaction mixture was quickly quenched by the addition of lithium aluminium hydride (100 mg). This mixture was allowed to stir for 10 minutes and then after the addition of anhydrous tetrahydrofuran (10 ml) for a further period of 2hr. Unreacted lithium aluminium hydride was decomposed by the dropwise addition of ethyl acetate and the resulting suspension evaporated to dryness under a stream of nitrogen. The residue was acidified to pH 1 with 20% aqueous HCl, diluted with water (50 ml) and extracted with ether (3 x 10 ml). The ethereal extracts were discarded and the acidic layer treated with excess solid sodium hydrogen carbonate. The resulting aqueous solution was extracted with ether (3 x 20 ml) and the total ethereal extract dried ($MgSO_4$). However, evaporation of this ethereal extract yielded no product.

2-Cyano-4,5,2'-trimethoxy-5'-methoxycarbonylmethylbiphenyl (189)

According to the method used in the case of the ester (169) methyl 3-iodo-4-methoxyphenylacetate was coupled with 2-bromo-4,5-dimethoxybenzonitrile[101] to give the cyano-ester (189) as an oil (20%), m/e 341, ν_{max} (thin film) 2 230 (C≡N), 1 735 cm^{-1} (O-C=O), τ ($CDCl_3$) 2.5-3.2 (5H, m, aromatic H), 6.09 (6H, s, CH_3-O *meta* and *para* to -C≡N), 6.18 (3H, s, CH_3-O *para* to $-CH_2-$), 6.31 (3H, s, CH_3-O-C=O), 6.40 (2H, s, $-CH_2-$).

Hydrolysis of Ester (189)

The foregoing ester (189) (20 mg 0.06 mmol) was dissolved in methanol (5 ml) and stirred at 0°C with aqueous sodium hydroxide (5N; 1.0 ml). After 40 minutes the reaction mixture was diluted with water (50 ml), acidified to pH 1 by the addition of 20% aqueous HCl, and extracted with chloroform (5 x 5 ml). The combined chloroform extracts were dried ($MgSO_4$) and evaporated to dryness to give the corresponding crude acid which crystallised (14 mg, 71%) from ethyl acetate/light petroleum (b.p. 60 - 80°C) as colourless rhombs, m.p. 162 - 165°C (Found: C, 65.8; H, 5.46; N, 4.27. $C_{18}H_{17}NO_5$ requires C, 66.1; H, 5.23; N, 4.28%), m/e 327, ν_{max} (thin film) 3 100 (O-H), 2 220 (C≡N), 1 715 cm^{-1} (O-C=O).

Diborane Reduction of Ester (189)

A stirred solution of (189) in anhydrous tetrahydrofuran (5 ml) was treated with a solution of diborane in tetrahydrofuran (1.0M in BH_3, 1.0 ml) in one portion at room temperature

and the reaction mixture monitored by t.l.c. (alumina GF_{254}, eluent 50% light petroleum (b.p. 40 - 60°C) in ethyl acetate) and i.r. spectroscopy. After 30 minutes t.l.c. showed the presence of starting material and three other compounds of lower Rf. I.r. spectra of the reaction mixture showed the continued presence of the nitrile absorption long after the disappearance of the ester absorption band.

2-Bromo-4,5-dimethoxybenzylamine Hydrochloride (191)

2-Bromo-4,5-dimethoxybenzonitrile (242 mg, 1 mmol) in dry tetrahydrofuran (5 ml) was stirred at 0°C and a solution of diborane in tetrahydrofuran added over 10 minutes. The resulting mixture was stirred at room temperature for 6 hr and then evaporated free of solvent under a stream of nitrogen. The residue was carefully diluted with water (100 ml) and basified to pH 11 with 20% aqueous sodium hydroxide. The alkaline solution was extracted with ether and the combined ethereal extracts dried ($MgSO_4$) and saturated with dry HCl to give 2-bromo-4,5-dimethoxybenzylamine hydrochloride (191) as colourless granules (200 mg 85%), m.p. 254 - 255°C (Found: C, 38.5; H, 4.70; N, 4.70. $C_9H_{13}BrClNO_2$ requires C, 38.3; H, 4.60; N, 4.96%).

The free amine of (191) was obtained by basification of an aqueous solution of the hydrochloride and extraction with chloroform; ν_{max} (thin film) 3 475 cm^{-1} (N-H), τ ($CDCl_3$) 3.03 (1H, s, aromatic H ortho to Br), 3.12 (1H, s, aromatic H meta to Br), 6.16 (8H, bs, 2 x CH_3-O and CH_2-N), 8.15 (2H, bm exchangeable with D_2O, N-H).

N-Acetyl-2-bromo-4,5-dimethoxybenzylamine (193)

A solution of 2-bromo-4,5-dimethoxybenzylamine (1.105 g) in acetic anhydride (5 ml) and dry pyridine (2 ml) was stirred at room temperature for 10 hr. The resulting solution was poured on to an ice-water slurry (250 g) and acidified to pH 1 with 5M aqueous HCl. The acidic solution was extracted with chloroform (4 x 100 ml) and the combined chloroform extracts carefully washed with saturated aqueous sodium hydrogen carbonate (3 x 50 ml). The chloroform extract was then dried ($MgSO_4$) and evaporated to give the acetamide (193) which crystallised as colourless rhombs (895 mg, 69%) from ethanol/ether, m.p. 142 - 144°C m/e 288, ν_{max} (KBr) 3 278 (N-H), 1 645 cm^{-1} (C=O), τ ($CDCl_3$) 3.00 (1H, s, aromatic H meta to Br), 3.05 (1H, s, aromatic H para to Br), 3.90 (1H, bm exchangeable with DCl, N-H), 5.57 (2H, d, J 6Hz, collapsed to a singlet on addition of DCl, $-CH_2-$), 6.13 (6H, s, 2 x CH_3-O), 8.00 (3H, s, $CH_3-C=O$).

Attempted Preparation of 2-Acetylaminomethyl-4,5,2'-trimethoxy--5'-methoxycarbonylmethylbiphenyl (194)

Following the procedure which was successful in the case of the aldehydo-ester (169) an attempt was made to couple methyl 3-iodo-4-methoxyphenylacetate and N-acetyl-2--bromo-4,5-dimethoxybenzylamine. The product was detectable in the crude reaction mixture (m/e 388) but no substantial amounts of (194) could be isolated after chromatography and examination of numerous fractions.

Attempted Cobaltous Chloride/Sodium Borohydride Reduction of the Cyano-ester (189)

The cyano-ester (189) (34 mg, 0.1 mmol) and cobaltous chloride hexahydrate (48 mg, 0.2 mmol) were stirred in methanol (5 ml) at room temperature and sodium borohydride (38 mg, 1 mmol) added in one portion. After stirring for 2 hr the mixture was diluted with water (50 ml) and extracted with chloroform (3 x 10 ml). The combined chloroform extracts were dried ($MgSO_4$) and evaporated to give an intractable brown residue.

NO-Diveratroyl-3-nitrotyramine (218)

3-Nitrotyramine[102] (182 mg, 1 mmol) in dry pyridine (5 ml) was treated with veratroyl chloride[103] (401 mg, 2 mmol) in portions over 15 minutes and with vigorous stirring. After complete addition, the solution was stirred for 1 hr before being poured on to crushed ice (5 g). The resulting solid was filtered and recrystallised to give cream coloured needles of NO-diveratroyl-3-nitrotyramine (218) (367 mg, 73%), m.p. 160 - 162°C (Found: C, 60.9; H, 5.32; N, 5.15. $C_{26}H_{26}N_2O_9$ requires C, 61.2; H, 5.13; N, 5.49%), m/e 510, ν_{max} (KBr) 3 290 (N-H), 1 735 (O-C=O), 1 637 (N-C=O), τ [$(CD_3)_2C=O$] 1.9-3.1 (7H, m, aromatic H), 6.05, 6.09, 6.15 and 6.17 (4 x 3H, singlets, 4 x CH_3-O), 6.31 (2H, t, J 7Hz, CH_2-N), 6.89 (2H, t, J 7Hz, Ar-CH_2-), 7.12 (1H, bs exchangeable with D_2O, N-H).

N-Veratroyl-3-nitrotyramine (219)

NO-Diveratroyl-3-nitrotyramine (255 mg, 0.5 mmol) was stirred at room temperature in a solution of 20% aqueous sodium hydroxide (1 ml) in ethanol (10 ml) for 12 hr. The red solution was then acidified to pH 1 with 20% aqueous HCl and the resulting pale yellow suspension evaporated free of ethanol, diluted with water (100 ml) and extracted with ethyl acetate (3 x 20 ml). The combined ethyl acetate extracts were dried ($MgSO_4$) and evaporated to a yellow solid which gave N-veratroyl-3-nitrotyramine (219) as yellow needles (230 mg, 90%) from ethanol, m.p. 129 - 131°C (Found: C, 58.7; H, 5.40; N, 8.10. $C_{17}H_{18}N_2O_6$ requires C, 59.0; H, 5.24; N, 8.10%), m/e 246, ν_{max} (KBr) 3 450 and 3 250 (N-H and O-H), 1 655 cm^{-1} (N-C=O), τ ($CDCl_3$) 1.67 (2H, bm, O-H and N-H), 2.1-3.3 (6H, m, aromatic H), 6.16 and 6.20 (2 x 3H, singlets, 2 x CH_3-O), 6.38 (2H, t, J 6Hz, N-CH_2-), 7.12 (2H, t, J 6Hz, O-CH_2-).

N-Veratroyl-3-aminotyramine (220)

N-Veratroyl-3-nitrotyramine (1.38 g, 4 mmol) was allowed to take up hydrogen (271 ml, theoretical amount 267 ml) while stirring in ethanol (250 ml) containing 10% Pd/C (140 mg).

The resulting solution was filtered through celite, and the filtered Pd/C washed with ethanol (2 x 100 ml). The combined filtrates were evaporated to give N-veratroyl--3-aminotyramine (220) as a colourless oil (1.20 g, 95%),

m/e 316, ν_{max} (thin film) 3 350 (N-H and O-H), 1 625 cm^{-1} (N-C=O), τ ($CDCl_3$) 2.6-3.8 (6H, m, aromatic H), 6.17 and 6.18 (2 x 3H, singlets, 2 x CH_3-O), 6.41 (2H, t, J 7Hz, CH_2-N), 7.29 (2H, t, J 7Hz, CH_2-Ar).

The free base was converted to its hydrochloride salt by saturation of an ethereal solution of (220) with dry HCl. The hydrochloride recrystallised from ethanol/ether as colourless prisms, m.p. 223 - 224°C (Found: C, 57.6; H, 6.19; N, 7.79. $C_{17}H_{21}ClN_2O_4$ requires C, 57.3; H, 5.95; N, 7.95%).

Attempted Sandmeyer Reaction with N-Veratroyl-3-aminotyramine (220)

An ice-cooled solution of N-veratroyl-3-aminotyramine hydrochloride (352 mg, 1.0 mmol) in water (4 ml) was slowly treated with a freshly prepared 1M solution of nitrous acid (1 ml) with stirring. The resulting solution was slowly added to a stirred solution of potassium iodide (166 mg, 1 mmol) in ice-cold water (2 ml). Gradual evolution of nitrogen and formation of a flocculent brown precipitate was observed. When evolution of nitrogen had ceased the reaction mixture was diluted with water (25 ml) and extracted with ether (3 x 25 ml). The combined ethereal extracts were washed with saturated aqueous sodium thiosulphate (2 x 20 ml), dried ($MgSO_4$), and evaporated to a brown oil which gave a continuous streak on t.l.c. (alumina GF_{254}, eluent 30% ethyl acetate in benzene).

Attempted Pschorr Cyclisation of N-Veratroyl-3-aminotyramine (220)

A solution of N-veratroyl-3-aminotyramine hydrochloride (117 mg, 0.33 mmol) in water (20 ml) was diazotised by the slow addition of a 0.1M nitrous acid solution (10 ml) with stirring at 0°C. The solution was then heated on a steam-bath for 4 hr and the resulting solution cooled and extracted with chloroform (3 x 10 ml). The combined chloroform extracts were dried ($MgSO_4$) and evaporated to an oil which was intractable.

3-Iodo-O-methyltyramine (200)

This was prepared as for the amine hydrochloride (174) to give the amine (200) as an oil (67%, based on β-(3-iodo--4-methoxyphenyl)propionic acid), m/e 277, ν_{max} (thin film) 3 360 cm^{-1} (N-H), τ ($CDCl_3$) 2.35 (1H, d, J 2Hz, aromatic H ortho to I), 2.80 (1H, dd, J 8Hz, 2Hz, aromatic H para to I), 3.20 (1H, d, J 8Hz, aromatic H meta to I), 7.07 (3H, s, CH_3-O), 7.23 (4H, m, $-(CH_2)_2-$), 8.66 (2H, bm exchangeable with D_2O, N-H).

The amine hydrochloride precipitated when an ethereal solution of the free base was saturated with dry HCl. The hydrochloride gave colourless needles from methanol-ether, m.p. 230 - 234°C (Found: C, 34.2; H, 4.26; N, 4.80. $C_9H_{13}ClINO$ requires C, 34.5; H, 4.15; N, 4.46%).

N-(6-Bromoveratroyl)-3-iodo-O-methyltyramine (201)

A solution of 3-iodo-O-methyltyramine (50 mg, 0.18 mmol) was stirred at 0°C in dry pyridine (4 ml) and 6-bromoveratroyl chloride (56 mg, 0.2 mmol) added in portions over 30 minutes. After complete addition, the reaction mixture was stirred for a further 1 hr and poured on to crushed ice (40 g). The resulting solution was acidified to pH1 with 20% aqueous HCl and extracted with chloroform (3 x 20 ml). The combined chloroform extracts were dried ($MgSO_4$) and evaporated to give the crude amide (201) which gave colourless needles from ethanol/ether (71 mg, 77%), m.p. 131 - 133°C (Found: C, 41.4; H, 3.90; N, 2.90. $C_{18}H_{19}BrINO_4$ requires C, 41.6; H, 3.66; N, 2.69%), m/e 518, 520, ν_{max} (KBr) 3 290 (N-H), 1 632 cm^{-1} (N-C=O), τ [$(CD_3)_2C=O$] 2.3-3.3 (5H, m, aromatic H), 3.85 (1H, bm exchangeable with DCl, NH), 6.14 (9H, bs, 3 x CH_3-O), 6.38 (2H, t, J 6Hz CH_2-N), 7.17 (2H, t, J 6Hz, Ar-CH_2).

Attempted Cyclisation of N-(6-Bromoveratroyl)-3-iodo-O--methyltyramine (201)

The amide (201) (26 mg, 0.05 mmol) was ground to an intimate mixture with copper bronze (32 mg, 0.5 mmol) and the mixture heated in a sealed tube at 210°±5°C for 30 minutes. The tube was broken and the contents extracted with acetone (25 ml). The acetone extract was evaporated to an oil which showed one major spot by t.l.c. (alumina GF_{254}, eluent 50% light petroleum (b.p. 40 - 60°C) in

chloroform). Crystallisation (ethanol/ether) gave N-(6--bromoveratroyl)-O-methyltyramine (10 mg, 51%), m/e 392, 394, ν_{max} (thin film) 3 400 (N-H), 1 640 cm^{-1} (N-C=O), τ ($CDCl_3$) 2.5-3.3 (6H, m, aromatic H), 3.85 (1H, bm exchangeable with DCl, N-H), 6.08 (6H, s, 2 x CH_3-O), 6.12 (3H, s, CH_3-O), 6.22 (2H, t, J 6Hz, N-CH_2), 7.14 (2H, t, J 6Hz, CH_2-Ar).

N-Veratroyl-3-iodo-O-methyltyramine (224)

This amide (224) was prepared exactly according to the method described for the preparation of N-(6-bromoveratroyl)-3-iodo-O-methyltyramine and gave colourless needles (67%) from ethanol, m.p. 126-129°C (Found: C, 48.7; H, 4.40; N, 3.35. $C_{18}H_{20}INO_4$ requires C, 48.9; H, 4.54; N, 3.35%), m/e 441, ν_{max} (KBr) 3 290 (N-H), 1 636 cm^{-1} (N-C=O), τ ($CDCl_3$) 2.3-3.3 (6H, m, aromatic H), 3.85 (1H, bm exchangeable with DCl, N-H), 6.09 (6H, s, 2 x CH_3-O), 6.14 (3H, s, CH_3-O), 6.87 (2H, t, J 6Hz, N-CH_2), 7.16 (2H, t, J 6Hz, Ar-CH_2).

Attempted Photocyclisation of N-Veratroyl-3-iodo-O-methyltyramine (224)

A solution of the amide (224) (22 ml, 0.05 mmol) in benzene (89 ml) was stirred with 5% aqueous sodium thiosulphate (1 ml) and irradiated with a low-pressure mercury lamp at room temperature. After 4 hr t.l.c. [alumina GF_{254}, eluted twice with 40% chloroform in light petroleum (b.p. 40 - 60°C)] indicated almost complete conversion to a product which could not be fully resolved from starting material. However, after p.l.c. and isolation of the appropriate part of the main fraction pure N-veratroyl-3-phenyl-O-methyltyramine (226)

was isolated as an oil (9 mg) which gave colourless needles from ethanol/ether (7 mg), m.p. 108 - 110°C, m/e 391, ν_{max} ($CHCl_3$) 3 480 (N-H), 1 640 cm^{-1} (N-C=O), τ[$(CD_3)_2C=O$] 2.25 (1H, m exchangeable with DCl, N-H), 2.5-3.2 (11H, m, aromatic H), 6.17 (3H, s, CH_3-O), 6.21 (3H, s, CH_3-O), 6.24 (3H, s, CH_3-O-), 6.42 (2H, t, J 6Hz, CH_2-N), 7.12 (2H, t, J 6Hz, Ar-CH_2), (Found: M^+, 391.1783. $C_{24}H_{25}NO_4$ requires M 391.1784).

The above irradiation was repeated with the amide (45 mg, 0.1 mmol) in methanol (89 ml) and 5% aqueous sodium thiosulphate (1 ml). After irradiating for 36 hr the principal product was isolated by p.l.c. (above system). This was N-veratroyl-O-methyltyramine (225) and gave colourless needles from ethanol/ether (20 mg, 62%), m.p. 158 - 160°C, m/e 315, ν_{max} ($CHCl_3$) 3 450 (N-H) 1 648 cm^{-1} (N-C=O), τ [$(CD_3)_2C=O$] 2.5-3.3 (7H, m, aromatic H), 6.12 (6H, s, 2 x CH_3-O), 6.23 (3H, s, CH_3-O), 6.37 (2H, t, J 6Hz, CH_2-N), 4.10 (2H, t, J 6Hz, Ar-CH_2), (Found: M^+ 315.1469. $C_{18}H_{21}NO_2$ requires M 315.1470).

N-(3,4-Dimethyoxybenzyl)-3-iodo-O-methyltyramine Hydrochloride (230)

3-Iodo-O-methyltyramine (245 mg 0.89 mmol) in methanol (10 ml) was added dropwise and over 30 minutes to a stirred solution of veratraldehyde (145 mg, 0.089 mmol) in methanol (20 ml) at room temperature. The mixture was allowed to stir for 24 hr and sodium borohydride (500 mg) was added in one portion to the solution cooled at 0°C. After a further

3 hr stirring the solution was evaporated free of methanol and extracted with anhydrous ether (3 x 20 ml). The combined ethereal extracts were washed with water (2 x 10 ml), dried ($MgSO_4$), and acidified with saturated ethanolic HCl to yield the hydrochloride (230) as colourless granules (352 mg, 82%), m.p. 197 - 199°C. (Found: C, 46.8; H, 5.15; N, 3.16. $C_{18}H_{23}ClINO_3$ requires C, 46.6; H, 4.96; N, 3.02%), ν_{max} (KBr) 3 500 - 2 600 (N-H), τ (CD_3OD), 2.2-3.2 (6H, m, aromatic H), 5.83 (2H, s, Ar-$C\underline{H}_2$-N), 6.10 (3H, s, CH_3-O), 6.12 (6H, s, 2 x CH_3-O).

Attempted Photocyclisation of N-(3,4-Dimethoxybenzyl)-3-iodo--O-methyltyramine Hydrochloride (230)

A solution of the hydrochloride (30 mg) in methanol (5 0 ml), water (39 ml), and 5% aqueous sodium thiosulphate (1 ml) was irradiated with a low-pressure mercury lamp and the reaction monitored by t.l.c. [silica GF_{254}, eluent 20% methanol in chloroform, after basification of base line spots with ammonia (d 0.88)]. After 5 days the reaction mixture was evaporated free of methanol and treated with excess solid sodium hydrogen carbonate. The neutralised solution was extracted with chloroform (3 x 20 ml) and evaporated to an oil which was separated by p.l.c. (above system) to give N-(3,4-methoxybenzyl)-O-methyltyramine (231) as an oil which was taken up in ether and treated with saturated ethanolic HCl to give the corresponding hydrochloride which was recrystallised from methanol/ether (14 mg, 64%) as small colourless needles, m.p. 195 - 198°C (Found: C, 63.9; H,

7.21; N, 4.40. $C_{18}H_{24}ClNO_3$ requires C, 64.1; H, 7.13; N, 4.16%).

The free amine when regenerated had the following spectral characteristics:- ν_{max}(thin film) 3 420 cm^{-1} (N-H), τ ($CDCl_3$) 2.8-3.4 (7H, m, aromatic H), 6.15 (6H, m, 2 x CH_3-O), 6.21 (3H, s, CH_3-O), 6.25 (2H, m, CH_2-N), 7.15 (4H, m, Ar-$C\underline{H}_2$).

2.2

Extraction of Norpluviine (105) and Lycorine (107)

Following the general procedure of Wildman and co-workers[104] whole "Twink" daffodils (typically 500 g wet weight) were macerated in a 1% ethanolic tartaric acid solution (400 ml) and allowed to stand for 1 week. The suspension was filtered and the plant material re-extracted (400 ml, 3 days). The combined filtrates were evaporated to ca. 50 ml and diluted with water (150 ml) and 2N hydrochloric acid (15 ml). The acidified solution was washed with chloroform (3 x 30 ml) and the washings back extracted with 2N hydrochloric acid (3 x 20 ml). The combined acid extracts were treated with excess solid sodium hydrogen carbonate and extracted with chloroform (6 x 200 ml). The combined chloroform extracts were dried ($MgSO_4$) and evaporated to ca. 5 ml. The resulting suspension was chilled to 0°C, filtered and washed with cold chloroform (2 x 5 ml). The solid mixture of lycorine and norpluviine was stirred in 20% aqueous sodium hydroxide, at room temperature for 12 hr and the resulting suspension filtered and washed with water (3 x 10 ml) to give crude lycorine (typically 100 mg, 0.02%). The combined filtrates were acidified to pH 9 by the dropwise addition of 20% aqueous HCl and the resulting suspension filtered to yield crude norpluviine (typically 150 mg, 0.03%).

The same procedure was employed in the extraction of Clivia miniata Regel for lycorine, the only 'chloroform insoluble' alkaloid present in this plant (typically 0.03%).

'Twink lycorine' $[\alpha]_D^{25}$-184° (c 1.06 in 50% pyridine in ethanol)
'Clivia miniata lycorine' $[\alpha]_D^{23}$-180° (c 1.21 in 50% pyridine in ethanol)
Authentic lycorine[94] $[\alpha]_D^{25}$-184° (c 1.39 in 50% pyridine in ethanol)

Recovery of lycorine and norpluviine from crystallisation was poor and the alkaloids were usually purified as their diacetates by chromatography and crystallisation. Crystallised samples of 'Twink' and Clivia miniata lycorine had i.r. spectra (KBr disc) identical with an authentic sample from Professor J.D. Loudon's collection.

Diacetyl-lycorine (233)

Lycorine was acetylated in a pyridine-acetic anhydride mixture according to the literature method[68] and gave colourless plates (90%) from ethyl acetate/light petroleum (b.p. 60 - 80°C), m.p. 217 - 219°C (dec.) (lit.,[68] 221 - 222°C ν_{max} ($CHCl_3$) 1 730 cm^{-1} (C=O).

Diacetylnorpluviine

Norpluviine was acetylated in a pyridine-acetic anhydride mixture by the literature procedure[68] and gave colourless prisms (85%) from ethyl acetate/light petroleum (b.p. 60 - 80°C), m.p. 150 - 152°C (lit.,[105] 151 - 152°C), ν_{max} ($CHCl_3$) 1 760 and 1 725 cm^{-1} (C=O).

1-O-Acetyl-lycorine (234)

A solution of diacetyl-lycorine (232 mg, 0.63 mmol) in ethanol (100 ml) and 35% aqueous HCl was stirred at room temperature and the hydrolysis followed by t.l.c. (alumina GF_{254}, eluent 40% ethyl acetate in benzene; baseline spots neutralised with a drop of ammonia (d 0.88); Rf. 0.05 lycorine, 0.25 1-O-acetyl-lycorine, 0.83 diacetyl-lycorine). When the spot corresponding to 1-O-acetyl-lycorine was at its most intense (7 hr) the reaction mixture was evaporated to dryness. The residue was dissolved in water (10 ml), treated with excess sodium hydrogen carbonate, and extracted with chloroform (3 x 20 ml). The combined chloroform extracts were dried ($MgSO_4$) and evaporated to an oil which was separated by p.l.c. (above system) to yield 1-O-acetyl-lycorine which gave colourless needles (90 mg, 44%) from ethanol/water, m.p. 214 - 216°C (lit.,[95] 215 - 216°C), ν_{max} (KBr) 3 400 (O-H), 1 738 cm^{-1} (C=O), τ ($CDCl_3$) 3.34 (1H, s, 11-H), 3.42 (1H,s, 8-H), 4.08 (2H, s, O-CH_2-O), 4.3-4.5 (2H, m, 1-H and 3-H), 5.86 (1H, d, J 14Hz, 7-CH_2), 5.82 (1H, m, 2-H), 6.46 (1H, d, J 14Hz, 7-CH_2), 7.16 (1H, bm exchangeable with D_2O, O-H), 8.03 (3H, s, CH_3-C=O).

1-O-Acetyl-lycorin-2-one (233)

1-O-Acetyl-lycorine (89 mg, 0.27 mmol) in ethanol-free chloroform (5 ml) was stirred with active[99] manganese dioxide (600 mg) in dry benzene (2 ml) at room temperature. After 5 hr the suspension was filtered and the manganese dioxide

washed with chloroform (2 x 10 ml). The combined filtrates were evaporated to dryness and separated on p.lc. (alumina GF_{254}, eluent 20% ethyl acetate in benzene) to give 1-O--acetyl-lycorin-2-one (31 mg, 35%) as colourless prisms from ethanol-water, m.p. 186 - 188°C (decomp.), [lit.[95] 191°C (decomp.)] ν_{max} (thin film) 1 740 (O-C=O), 1 675 cm^{-1} (C=O), τ ($CDCl_3$) 3.27 (1H, s, 11-H), 3.43 (1H, s, 8-H), 4.00 and 4.03 (2 x 1H, singlets, 1-H and 3-H), 4.09 (2H, s, O-CH_2-O), 5.84 (1H, d, J 14Hz, 7-CH_2), 6.40 (1H, d, J 14Hz, 7-CH_2), 8.03 (3H, s, CH_3-C=O).

Diacetyl-2-epilycorine (235)

1-O-Acetyl-lycorin-2-one (31 mg, 0.01 mmol) was stirred in anhydrous tetrahydrofuran (10 ml) with lithium aluminium hydride (20 mg) for 1 hr at room temperature. Excess lithium aluminium hydride was destroyed by the addition of a few drops of water and the solution evaporated to dryness under a current of nitrogen. The residue was extracted with chloroform (50 ml) and the chloroform extract evaporated free of solvent and stirred in a mixture of pyridine (0.25 ml) and acetic anhydride (1 ml) for 6 hr. The resulting solution was poured on to ice (25 g), treated with excess solid sodium hydrogen carbonate, and extracted with chloroform (3 x 15 ml). The combined chloroform extracts were dried ($MgSO_4$) and evaporated to dryness (final traces of pyridine removed as an azeotropic mixture with benzene). The residue was separated by p.l.c. (alumina GF_{254}, eluent 30% ethyl acetate in benzene) to yield diacetyl-2-epilycorine (16 mg, 45%) as colourless

needles from ethanol/water, m.p. 192 - 194°C (lit.,[95] 192 - 194.5°C), ν_{max} ($CDCl_3$) 1 743 cm^{-1} (O-C=O), τ ($CDCl_3$) 3.29 (1H, s, 11-H), 3.45 (1H, s, 8-H), 3.90 and 3.94 (2 x 1H, singlets, 1-H and 3-H), 4.10 (2H, s, O-CH_2-O), 4.65 (1H, bs, 2-H), 5.48 (1H, d, J 14Hz, 6-CH_2-), 6.48 (1H, d, J 14Hz, 7-CH_2-) 7.94 and 8.00 (2 x 3H, singlets, CH_3-O). $[\alpha]_D^{23}$ - 152° (c 0.71 in $CHCl_3$) [lit.,[95] $[\alpha]_D$ -158° (c 1.04 in $CHCl_3$)].

1,11b-Anhydro-O-benzylnorpluviine

According to the method of Bruce,[71] freshly distilled phosphorus oxychloride (0.24 ml) was added slowly to a stirred solution of 9-O-benzylnorpluviine (163) (103 mg, 0.28 mmol) in dry pyridine (1 ml) at 0°C. After 3½ hr stirring at room temperature the resulting suspension was poured on to an ice/saturated aqueous sodium hydrogen carbonate slurry and extracted with chloroform (5 x 20 ml). The combined chloroform extracts were washed with water (2 x 20 ml), dried (Na_2SO_4), and evaporated to give crude 1,11b-anhydro-O-benzylnorpluviine as a yellow oil which gave colourless needles (85 mg, 89%) from methanol/ether m.p. 198 - 201°C (decomp.) [lit.,[71] 197 - 202°C (decomp.)].

1,11b-Anhydro-O-benzylnorpluviine Methiodide (164)

A solution of 1,11b-anhydro-O-benzylnorpluviine (74 mg) and methyl iodide (1 ml) was allowed to stand at room temperature, in the dark, for 16 hr. The resulting solution was evaporated to yield cream crystals of the methiodide (164) which crystallised as colourless needles from methanol/

ether (86 mg, 82%), m.p. 185 - 187°C (decomp.) [lit.,[71] 185 - 187°C (decomp.)].

O-Benzylnorpluviine Anhydromethine (165)

A stirred suspension of the methiodide (164) (65 mg) in ethanol (10 ml) was treated at room temperature, and in one portion, with 4N aqueous sodium hydroxide (0.1 ml). The suspension dissolved in 5 minutes and the pale-yellow solution was evaporated to dryness. The residue was taken up in water (10 ml) and extracted with chloroform (3 x 10 ml). The combined chloroform extracts were dried (Na_2SO_4) and evaporated to a yellow oil which after p.l.c. (alumina GF_{254}, eluent 50% light petroleum (b.p. 40 - 60°C) in benzene) gave anhydromethine (165). This slowly crystallised from methanol as colourless plates (32 mg, 74%), m.p. 80 - 81°C (Found: C, 80.3; H, 7.05; N, 4.20. $C_{24}H_{25}NO_2$ requires C, 80.2; H, 7.01; N, 3.90%), τ ($CDCl_3$) 2.2-3.3 (6H, m, aromatic H), 4.84 (2H, s, $PhCH_2$-O), 6.02 (3H, s, CH_3-O, 7.61 (3H, s, CH_3-N).

Racemisation of L-Tyrosine

According to the method of Bergmann and Zervas[106] L-tyrosine (2.92 g, 20 mmol) was dissolved in 1N aqueous sodium hydroxide (40 ml) and mixed with acetic anhydride (50 ml). The resulting solution was heated at 100°C for 4 hr, cooled, and acidified to neutral pH with 1N aqueous HCl (40 ml). The neutral solution was evaporated to dryness and then heated under reflux in 4N aqueous HCl (100 ml) for 3 hr. The hot acidic solution was treated twice with charcoal cooled, and adjusted to pH 6 with 4N aqueous sodium hydroxide. The

precipitated tyrosine was filtered off and dried (2.07 g, 70%), $[\alpha]_D^{23}$ -0.08° (c 4.0 in 6N HCl) [lit.,[107] L-tyrosine $[\alpha]_D^{22}$ -7.98 (c 4.0 in 6N HCl)].

DL-[3,5-$^3H_2$]Tyrosine

DL-Tyrosine (100 mg) was dissolved in 4N DCl (0.5 ml) and heated in a sealed tube at 100°C for 24 hr and the n.m.r. spectrum of the resulting tyrosine taken:-

proton	integral
3,5	0.00
2,6	1.97
α	1.02
β	2.00

Integration indicated 100% exchange at the 3,5 positions.

Tritiated water (0.5 ml, ca. 12.5 mCi/mmol) was treated with thionyl chloride (0.08 ml) to give 4N tritiated aqueous hydrogen chloride. This was added to a Carius tube containing DL-tyrosine (37 mg). The tube was sealed and heated at 100°C for 24 hr. The tube was carefully opened and the contents brought to pH 6 by the dropwise addition of ammonia (d 0.88). The precipitated DL-[3,5-$^3H_2$]tyrosine was filtered off, washed with water (2 x 10 ml), and dried *in vacuo* (36 mg, 97%; 8.39 mCi/mmol, 67% exchange).

DL-[α-^3H]Tyrosine

According to the method of Kirby and Michael[108] N-acetyl--O-methyltyrosine (237 mg, 1 mmol) was dissolved in anhydrous dioxan (5 ml) and acetic anhydride (306 mg, 3 mmol). The

resulting solution was heated under reflux for 45 minutes and the solvents removed *in vacuo*. The residue was taken up in dry dioxan (2 ml), deuterium oxide (0.5 ml, 25 mmol) and dry pyridine (0.2 ml). After standing at room temperature for 15 minutes the solution was heated under reflux for 15 minutes longer before again removing the solvents *in vacuo*. The pale yellow oil gave needles of [α-^{2}H]-*N*-acetyl-*O*--methyltyrosine (193 mg, 82%), m.p. 149 - 151°C (lit.,[109] 147 - 148°C).

[α-$^2H_2$]-*N*-Acetyl-*O*-methyltyrosine (40 mg, 0.16 mmol) was dissolved in 48% aqueous HBr (5 ml) and heated under reflux for 1 hr. The solution was then evaporated to dryness *in vacuo* (aqueous sodium hydroxide trap) and the residue dissolved in water (2 ml) and again evaporated to dryness. The residual tyrosine hydrobromide was taken up in 5% aqueous sodium hydroxide (1 ml) and adjusted to pH 6 by dropwise addition of glacial acetic acid. The resulting suspension was chilled to 0°C and filtered to give pale brown plates of DL-[α-$^2H_2$]tyrosine (22 mg, 74%), n.m.r. (DCl) showed the absence of α-H (100% exchange).

The above sequence of reactions was repeated on half the scale and using tritiated water (0.5 ml; *ca*.12.5 mCi/mmol, 31% exchange).

DL-N-Chloroacetyltyrosine

DL-Tyrosine (100 mg) was dissolved in one-third of a solution of sodium hydroxide (200 mg) in water (3 ml). The

remaining aqueous sodium hydroxide was added to the stirred tyrosine solution in small alternate portions with chloroacetic anhydride (300 mg) over one hour. The solution was stirred until clear (2 hr more) and concentrated hydrochloric acid added dropwise to pH 2. Unreacted tyrosine and chloroacetic acid (total 10 mg) precipitated and were filtered off. The filtrate was evaporated to dryness *in vacuo* and extracted with acetone (25 ml). The acetone extract was evaporated to an oil which gave crystals of DL-*N*-chloroacetyltyrosine (40 mg, 29%) from cold chloroform, m.p. 160 - 162°C (lit.,[110] 160 - 162°C).

Resolution of DL-*N*-Chloroacetyltyrosine

According to the literature method[110] DL-*N*-chloroacetyltyrosine (40 mg) was suspended in water (1 ml) and dissolved by the dropwise addition of 1*N* aqueous lithium hydroxide (final pH 7.5). Bovine carboxypeptidase A (1 mg) was dissolved in a few drops of saturated aqueous lithium chloride and added to the solution of DL-*N*-chloroacetyltyrosine. After 18 hr at 23°C the crystalline L-tyrosine which had separated was filtered off and dried (10 mg, 72%). The filtrate was diluted with water (5 ml), acidified to pH 1 with concentrated hydrochloric acid and extracted with ethyl acetate (4 x 5 ml). The ethyl acetate was evaporated to a clear oil which was heated under reflux in 20% aqueous HCl (5 ml), evaporated to dryness and taken up in the minimum of 10% aqueous sodium hydroxide. The basic solution

was adjusted to pH 6 with glacial acetic acid, chilled to 0°C and the precipitated D-tyrosine filtered off and dried (11 mg, 79%).

L-[α-^{3}H,α-^{14}C]Tyrosine and D-[α-^{3}H,α-^{14}C]Tyrosine

These were prepared by mixing 10% aqueous hydrochloric acid solutions of inactive DL-tyrosine, (80 mg) DL-[α-^{3}H]tyrosine (20 mg) and commercial DL-[α-^{14}C]tyrosine (0.16 mg). The resulting solution was adjusted to pH 6 with ammonia (d 0.88) and the precipitated DL-[α-^{3}H,α-^{14}C]tyrosine collected. The DL-[α-^{3}H,α-^{14}C]tyrosine was converted into its N-chloroacetyl derivative and resolved, as previously described (p. 138), to give L-[α-^{3}H,α-^{14}C]tyrosine and D-[α-^{3}H,α-^{14}C]tyrosine.

L-[α-^{14}C]Tyrosine and D-[α-^{14}C]Tyrosine

These were prepared by mixing 10% aqueous hydrochloric acid solutions of inactive DL-tyrosine (200 mg) and DL-[α-^{14}C] tyrosine (0.16 mg). The resulting solution was adjusted to pH 6 with ammonia (d 0.88) and the precipitated, diluted DL-[α-^{14}C]tyrosine filtered off. This was converted into its N-chloroacetyl derivative and resolved as previously described (p. 138), to give L-[α-^{14}C]tyrosine and D-[α-^{14}C]tyrosine.

DL-[3,5-^{3}H$_2$]/L-[α-^{14}C]Tyrosine and DL-[3,5-^{3}H$_2$]/D-[α-^{14}C]-Tyrosine

DL-[3,5-^{3}H$_2$]/L-[α-^{14}C]Tyrosine was prepared by mixing 10% aqueous hydrochloric acid solutions of DL-[3,5-^{3}H$_2$]tyrosine (0.06 mg) and L-[α-^{14}C]tyrosine (15 mg). The resulting solution was brought to pH 6 and DL-[3,5-^{3}H$_2$]tyrosine collected by filtration.

DL-[3,5-$^{3}H_2$]/D-[α-^{14}C]Tyrosine was prepared in a similar fashion from DL-[3,5-$^{3}H_2$]tyrosine (0.14 mg) and D-[α-^{14}C]tyrosine (22 mg).

DL-[3,5-$^{3}H_2$,α-^{14}C]Tyrosine and DL-[α-^{3}H,α-^{14}C]Tyrosine

DL-[3,5 -$^{3}H_2$,α-^{14}C]Tyrosine was prepared by mixing typically 10% aqueous hydrochloric acid solutions of inactive DL-tyrosine (15 mg), DL-[3,5-$^{3}H_2$]tyrosine (1.2 mg) and DL-[α-^{14}C]tyrosine (0.04 mg). The pH of the resulting solution was adjusted to pH 6 with ammonia (d 0.88) and the precipitated DL-[3,5-$^{3}H_2$,α-^{14}C]tyrosine collected.

DL-[α-^{3}H,α-^{14}C]tyrosine was prepared in a similar way from inactive DL-tyrosine (12 mg), DL-[α-^{3}H]tyrosine (7 mg) and DL-[α-^{14}C]tyrosine (0.06 mg).

L-[α-^{3}H,α-^{14}C]Tyrosine Fed to 'Twink', Spring 1974

Wet weight of plant	= 230 g
Precursor	
Total weight	= 18 mg
^{14}C specific activity	= 7.81×10^{6} dpm/mg
Total ^{14}C activity	= 1.41×10^{7} dpm
^{3}H activity/^{14}C activity	= 5.47
Norpluviine	
Total weight isolated	= 67 mg (0.029%)
^{14}C specific activity (as diacetate)	= 1.30×10^{3} dpm/mg
^{14}C total activity	= 6.70×10^{4} dpm
Incorporation of precursor	= 0.48%
^{3}H activity/^{14}C activity	= 5.39
Tritium retention	= 98.6%
Lycorine	
Total weight isolated	= 43 mg (0.019%)
^{14}C specific activity (as diacetate)	= 49 dpm/mg
^{14}C total activity	= 161 dpm
Incorporation of precursor	= 0.01%
^{3}H activity/^{14}C activity	= 5.48
Tritium retention	= 100.0%

D-[α-^{3}H,α-^{14}C]Tyrosine Fed to 'Twink', Spring 1974

Wet weight of plant	= 175 g
Precursor	
Total weight	= 13 mg
^{14}C specific activity	= 7.75×10^{6} dpm/mg
Total ^{14}C activity	= 1.01×10^{7} dpm
^{3}H activity/^{14}C activity	= 5.15
Norpluviine	
Total weight isolated	= 43 mg (0.025%)
^{14}C specific activity (as diacetate)	= 1.74×10^{3} dpm/mg
Total ^{14}C activity	= 5.69×10^{4} dpm
Incorporation of precursor	= 0.56%
^{3}H activity/^{14}C activity	= 0.05
Tritium retention	= 0.9%
Lycorine	
Total weight	= 34 mg (0.019%)
^{14}C specific activity (as diacetate)	= 42 dpm/mg
Total ^{14}C activity	= 1.09×10^{3} dpm
Incorporation of precursor	= 0.01%
^{3}H activity/^{14}C activity	= 0
Tritium retention	= 0.0%

DL-[3,5-$^{3}H_{2}$]/D-[α-^{14}C]Tyrosine Fed to 'Twink', Spring 1974

Wet weight of plant = 170 g

Precursor

Total weight = 21 mg

^{14}C specific activity = 3.42×10^{5} dpm/mg

Total ^{14}C activity = 7.29×10^{6} dpm

^{3}H activity/^{14}C activity = 1.76

Norpluviine

Total weight isolated = 59 mg

^{14}C specific activity (as diacetate) = 1.51×10^{3} dpm/mg

Total ^{14}C activity = 6.84×10^{4} dpm

Incorporation of precursor = 0.95%

^{3}H activity/^{14}C activity = 0.91

Tritium retention = 51.6%

Lycorine

Total weight isolated = 27 mg (0.016%)

^{14}C specific activity (as diacetate) = 0 dpm

Incorporation of precursor = 0%

DL-[3,5-$^{3}H_2$]/L-[α-^{14}C]Tyrosine Fed to 'Twink', Spring 1974

Wet weight of plant = 120 g

Precursor

Total weight = 14 mg

^{14}C specific activity = 2.01×10^{5} dpm/mg

Total ^{14}C activity = 2.83×10^{6} dpm

^{3}H activity/^{14}C activity = 1.64

Norpluviine

Total weight isolated = 20 mg (0.017%)

^{14}C specific activity (as diacetate) = 1.84×10^{3} dpm/mg

Total ^{14}C activity = 2.81×10^{4} dpm

Incorporation of precursor = 0.99%

^{3}H activity/^{14}C activity = 0.82

Tritium retention = 49.6%

Lycorine

Total weight isolated = 29 mg (0.024%)

^{14}C specific activity (as diacetate) = 0 dpm

Incorporation of precursor = 0%

DL-[3,5-$^{3}H_{2}$,α-^{14}C]Tyrosine fed to 'Twink', Spring 1974.

Wet weight of plant = 150 g

Precursor

Total weight = 14.5 mg

^{14}C specific activity = 8.29×10^{6} dpm/mg

Total ^{14}C activity = 1.21×10^{7} dpm

^{3}H activity/^{14}C activity = 5.44

Norpluviine

Total weight isolated = 47 mg (0.032%)

^{14}C specific activity (as diacetate) = 5.94×10^{3} dpm/mg

Total ^{14}C activity = 2.12×10^{5} dpm

Incorporation of precursor = 1.75%

^{3}H activity/^{14}C activity = 2.59

Tritium retention = 47.7%

Lycorine

Total weight isolated = 31 mg (0.021%)

^{14}C specific activity (as diacetate) = 1.12×10^{2} dpm/mg

Total ^{14}C activity = 2.68×10^{3} dpm

Incorporation of precursor = 0.02%

^{3}H activity/^{14}C activity = 1.65

Tritium retention = 30.3%

DL-[3,5,-$^{3}H_{2}$,α-^{14}C]Tyrosine fed to 'Twink', Spring 1975

Wet weight of plant	= 500 g
Precursor	
Total weight	= 15.5 mg
^{14}C specific activity	= 1.89×10^{6} dpm/mg
Total ^{14}C activity	= 2.93×10^{7} dpm
^{3}H activity/^{14}C activity	= 5.48
Norpluviine	
Total weight isolated	= 101 mg (0.020%)
^{14}C specific activity (as diacetate)	= 1.71×10^{3} dpm/mg
Total ^{14}C activity	= 1.30×10^{5} dpm
Incorporation of precursor	= 0.44%
^{3}H activity/^{14}C activity	= 2.63
Tritium retention	= 48.1%
Lycorine	
Total weight isolated	= 80 mg (0.016%)
^{14}C specific activity (as diacetate)	= 1.82×10^{2} dpm/mg
Total ^{14}C activity	= 1.12×10^{4} dpm
Incorporation of precursor	= 0.04%
^{3}H activity/^{14}C activity	= 1.79
Tritium retention	= 32.8%

DL-[3,5-$^{3}H_2$,α-^{14}C]Tyrosine Fed to 'Twink', Spring 1975 (Repeat)

Wet weight of plant = 490 g

Precursor

Total weight = 15.5 mg

^{14}C specific activity = 1.89×10^{6} dpm/mg

Total ^{14}C activity = 2.93×10^{7} dpm/mg

^{3}H activity/^{14}C activity = 5.48

Norpluviine

Total weight isolated = 55 mg (0.011%)

^{14}C specific activity (as diacetate) = 3.79×10^{3} dpm/mg

Total ^{14}C activity = 4.59×10^{5} dpm

Incorporation of precursor = 1.40%

^{3}H activity/^{14}C activity = 2.56

Tritium retention = 47.7%

Lycorine

Total weight isolated = 76 mg (0.016%)

^{14}C specific activity (as diacetate) = 1.33×10^{2} dpm/mg (2.14 μCi/mmol)

Total ^{14}C activity = 1.26×10^{4} dpm

Incorporation of precursor = 0.04%

^{3}H activity/^{14}C activity = 1.64

Tritium retention = 29.9%

1-O-Acetyl-lycorine (133)

^{14}C specific activity = 1.30×10^{2} dpm/mg (1.85×10^{-2} μCi/mmol)

^{3}H activity/^{14}C activity = 1.45

Tritium retention = 26.4%

1-O-Acetyl-lycorin-2-one* (134)

^{14}C specific activity = 1.31×10^{2} dpm/mg (1.85×10^{-2} μCi/mmol)

^{3}H activity/^{14}C activity = 0.07

Tritium retention = 1.2%

* Crystallised only once.

DL-[α-^{3}H,α-^{14}C]Tyrosine fed to 'Twink', Spring 1975

Wet weight of plant = 510 g

Precursor

Total weight = 13 mg

^{14}C specific activity = 1.71×10^{6} dpm/mg

Total ^{14}C activity = 2.22×10^{7} dpm

^{3}H activity/^{14}C activity = 3.53

Norpluviine

Total weight isolated = 26 mg (0.005%)

^{14}C specific activity (as diacetate) = 1.71×10^{2} dpm/mg

Total ^{14}C activity = 3.38×10^{4} dpm

Incorporation of precursor = 0.15%

^{3}H activity/^{14}C activity = 1.60

Tritium retention = 45.4%

Lycorine

Total weight isolated = 50 mg (0.010%)

^{14}C specific activity (as diacetate) = 0 dpm

Incorporation of precursor = 0%

DL-[3,5-$^{3}H_2$,α-^{14}C]-Tyrosine Fed to 'Twink', Spring 1976

Wet weight of plant = 360 g

Precursor

Total weight = 10 mg

^{14}C specific activity = 3.49×10^{6} dpm/mg

Total ^{14}C activity = 3.49×10^{7} dpm

^{3}H activity/^{14}C activity = 4.12

Norpluviine

Total weight isolated = 45 mg (0.013%)

^{14}C specific activity (as diacetate) = 1.99×10^{4} dpm/mg

Total ^{14}C activity = 6.83×10^{5} dpm

Incorporation of precursor = 1.95%

^{3}H activity/^{14}C activity = 1.93

Tritium retention = 47.9%

Diluted norpluviine ^{14}C specific activity = 2.8×10^{-1} μCi/mmol

1-11b, Anhydro-O-benzylnorpluviine* (238)

^{14}C specific activity = 1.79×10^{3} dpm/mg (2.65×10^{-1} μCi/mmol)

^{3}H activity/^{14}C activity = 1.82

Tritium retention = 44.2%

O-Benzylnorpluviine anhydromethine (240)

^{14}C specific activity = 1.80×10^{3} dpm/mg (2.87 μCi/mmol)

^{3}H activity/^{14}C activity = 1.58

Tritium retention = 38.4%

* Crystallised only once

Lycorine

Total weight isolated	= 71 mg (0.020%)
^{14}C specific activity (as diacetate)	= 3.94×10^2 dpm/mg
Total ^{14}C activity	= 2.15×10^4 dpm
Incorporation of precursor	= 0.06%
^{3}H activity/^{14}C activity	= 1.18
Tritium retention	= 28.8%

DL-[3,5-$^{3}H_{2}$]Tyrosine Fed to Clivia miniata Regel, Summer 1976

Wet weight of plant = 140 g

Precursor

Total weight = 10 mg

^{14}C specific activity = 3.49×10^{6} dpm/mg

Total ^{14}C activity = 3.49×10^{7} dpm

^{3}H activity/^{14}C activity = 4.12

Lycorine

Total weight isolated = 80 mg (0.57%)

^{14}C specific activity (as diacetate) = 4.25×10^{2} dpm/mg (6.84×10^{-1} μCi/mmol)

Total ^{14}C activity = 2.62×10^{4} dpm

Incorporation of precursor = 0.08%

^{3}H activity/^{14}C activity = 0.81

Tritium retention = 19.6%

1-O-Acetyl-lycorine (133)

^{14}C specific activity = 4.74×10^{2} dpm/mg (6.76×10^{-1} μCi/mmol)

^{3}H activity/^{14}C activity = 0.72

Tritium retention = 17.5%

1-O-Acetyl-lycorin-2-one (134)

^{14}C specific activity = 4.76×10^{2} dpm/mg (6.73×10^{-1} μCi/mmol)

^{3}H activity/^{14}C activity = 0.02

Tritium retention = 0.4%

REFERENCES

1. J. Hamer and M. Ahmad, '1,4-Cycloaddition Reactions' ed. J. Hamer, Academic Press, New York, 1967, ch. 12.

2. P. Horsewood and G.W. Kirby, J.C.S. Chem. Comm., 1971, 1139.

3. P. Horsewood and G.W. Kirby, Loughborough University of Technology, Dep. Chem. Science Final Year Stud. Proj. Thesis, 1969, 10, 147.

4. K.W. Bentley, P. Horsewood, G.W. Kirby and S. Singh, J.C.S. Chem. Comm., 1969, 411.

5. R. Dickinson, G.W. Kirby, J.G. Sweeney and J.K. Tyler, J.C.S. Chem. Comm., 1973, 241.

6. A.L.J. Beckwith and G.W. Evans, J. Chem. Soc., 1962, 130.

7. B. Sklarz and A.F. Al-Sayyab, J. Chem. Soc., 1964, 1318.

8. J.E. Rowe and A.D. Ward, Austral. J. Chem., 1968, 21, 2761.

9. U. Lerch and J.G. Moffatt, J. Org. Chem., 1971, 36, 3391.

10. G.W. Kirby and J.G. Sweeney, J.C.S. Chem. Comm., 1973, 704.

11. A.K. Qureshi and B. Sklarz, J. Chem. Soc. (C), 1966, 412.

12. J.E.T. Corrie, G.W. Kirby and R.P. Sharma, J.C.S. Chem. Comm., 1975, 915.

13. T.L. Gilchrist, C.J. Harris and C.W. Rees, J.C.S. Chem. Comm., 1974, 485.

14. T.L. Gilchrist, M.E. Peek and C.W. Rees, J.C.S. Chem. Comm., 1975, 913.

15. T.L. Gilchrist, M.E. Peek and C.W. Rees, J.C.S. Chem. Comm., 1975, 914.

16. G. Kresze and G. Schulz, Tetrahedron, 1961, 12, 7.

17. L.A. Paquette, Tetrahedron, 1975, 31, 2855.

18. J.E.T. Corrie and G.W. Kirby, Unpublished work.

19. K. Klager, W. Reppe, O. Schlichting and T. Taepel, Annalen, 1948, 560, 1.

20. M. Oda, Y. Kayama and Y. Katahara, Tetrahedron Letters, 1974, 23, 2019.

21. B. Belleau and Yum-Kin Au-Young, J. Amer. Chem. Soc., 1963, 85, 64.

22. E. Muller, H. Metzgir and D. Fries, Chem. Ber., 1954, 87, 1449.

23. G.W. Kirby and J.G. Sweeney, Unpublish work.

24. N.J. Leonard and A.J. Playtis, J.C.S. Chem. Comm., 1972, 133.

25. E.J. Corey, K.C. Nicolaou, Y. Machida, C.L. Malmsten, and B. Samuelsson, Proc. Nat. Acad. Sci. U.S.A., 1975, 72, 3355.

26. A.G. Abatjoglou and P.S. Portoghese, Tetrahedron Letters, 1976, 1457.

27. E. Boyland and R. Nery, J. Chem. Soc. (C), 1966, 354,

28. D.S. Breslow, A.F. Marcantonio and T.J. Prosser, Tetrahedron Letters, 1964, 2479.

29. D. Ben-Ishai and A. Berger, J. Org. Chem., 1952, 17, 1564.

30. E.W. Colvin, T.A. Purcell and R.A. Raphael, J.C.S. Chem. Comm., 1972, 1031.

31. A.T. Kader and C.J.M. Stirling, J. Chem.Soc., 1964, 258.

32. D.B.R. Johnston and T.B. Windholz, Tetrahedron Letters, 1967, 2555.

33. N.F. Albertson and F.C. McKay, J. Amer. Chem. Soc., 1957, 79, 4686.

34. K. Hofmann, W. Haas, M.J. Smithers, R.D. Wells, Y. Wolman, N. Yanaihara and G. Zanetti, J. Amer. Chem. Soc., 1965, 87, 620.

35. B.A. Carpino, L.A. Carpino and C.A. Giza, J. Amer. Chem. Soc., 1959, 81, 955.

36. G.W. Kirby and R.P. Sharma, Unpublished work.

37. G.H. Bockhorn and R. Kreher, Angew. Chem., 1964, 76, 681.

38. W.C. Cunningham, L.J. Hayes, Jui Shung Hwong, J.E. Johnson, D.L. McClaugherty and J.R.Springfield, J. Org. Chem., 1971, 36, 284.

39. A. Luttringhaus and A. Windaus, Annalen, 1930, 481, 119.

40. G. Braun, Org. Synth. Coll. Vol. 1, 431.

41. M. Jantelat, M.T. Messe, H.J. Reich, J.D. Roberts and F.J. Weigert, J. Amer. Chem. Soc., 1969, 91, 7445.

42. M.G. Ahmed, R.W. Alder, G.H. James, M.L. Sinnott and M.C. Whiting, J.C.S. Chem. Comm., 1968, 1533.

43. F.A. Askew, R.B. Bourdillon, H.M. Bruce, R.K. Callow and J.S.L. Philpot, Proc. Roy. Soc. (London), 1932, B109, 488.

44. D.H.R. Barton, G. Leclerc, P.D. Magnus and I.D. Menzies J.C.S. Chem. Comm., 1972, 448.

45. C.D. Hurd, J. Amer. Chem. Soc., 1923, 45, 1472.

46. G.R. Gale, J.B. Hynes and A.B. Smith, J. Medicin. Chem., 1970, 13, 571.

47. J. Hase, K. Kobashi, N. Kawaguchi and K. Sakamoto, Chem. and Pharm. Bull. (Japan), 1971, 19, 363.

48. A. Werner and W. Skiba, Chem. Ber., 1899, 32, 1654

49. C. Grundmann and H. Frommeld, J. Org. Chem., 1966, 31, 157.

50. K. Morishima, Arch. Exptl. Path. Pharmakol., 1897, 40, 221.

51. S. Uyeo and N. Yanaihara, J. Chem. Soc., 1958, 172.

52. M. Shiro, T. Sato and H. Koyama, Chem. Ind. (London), 1966, 1229.

53. W.C. Wildman, "The Alkaloids", ed. R.H.F. Manske, Academic Press Inc., London, 1959, vol. VI, p.328.

54. L.D. Quin, Selecta Chim., 1962, 20, 37.

55. W.C. Wildman, "The Alkaloids", ed. R.H.F. Manske, Academic Press, Inc., London, 1959, vol. VI, p.319, 324.

56. D.H.R. Barton and T. Cohen, "Festchrift Arthur Stoll", Birkhauser, Basle, 1957, p.117.

57. D.H.R. Barton, Proc. Chem.Soc. (London), 1963, 293.

58. D.H.R. Barton, G.W. Kirby, Proc. Chem. Soc. (London), 1960, 392.

59. D.H.R. Barton, G.W. Kirby, J.B. Taylor and G.M. Thomas, J. Chem. Soc., 1963, 4545.

60. A.R. Battersby, R. Binks and W.C. Wildman, Proc. Chem. Soc., 1960, 410.

61. A.R. Battersby, H.M. Fales and W.C. Wildman, J. Amer. Chem. Soc., 1961, 83, 4098.

62. R.J. Suhadolnik, A.G. Fischer and J. Zulalian, J. Amer. Chem. Soc., 1962, 84, 4348.

63. A.R. Battersby, R. Binks, S.W. Breur, H.M. Fales, W.C. Wildman and R.J. Highet, J. Chem. Soc., 1964, 1595.

64. E. Ramstad and S. Agurell, Ann. Rev. Plant. Physiol., 1964, 15, 143.

65. S.A. Brown, D. Wright and A.C. Neish, Canad. J. Biochem. and Physiol.,1959, 37, 25.

66. R.J. Suhadolnik, A.G. Fischer and J. Zulalian, Biochem. Biophys. Res. Comm., 1963, 11, 208.

67. D.R. McCalla and A.C. Neish, Canad. J. Biochem. and Physiol., 1959, 37, 537.

68. G.W. Kirby and H.P. Tiwari, J. Chem. Soc. (C), 1966, 676.

69. S. Mizukami, Tetrahedron, 1960, 11, 89.

70. I.T. Bruce and G.W. Kirby, J.C.S. Chem. Comm., 1968, 207.

71. I.T. Bruce, Ph.D. Thesis, University of London, 1968.

72. N.E. Heimer and W.C.Wildman, J. Amer. Chem. Soc., 1967, 89, 5625.

73. M. Hayno, M. Gut, R.I. Dorfman, O.K. Sefek and D.H. Peterson, J. Amer. Chem. Soc., 1958, 80, 2336.

74. E.J. Corey, G.A. Gregoriou and D.H. Peterson, J. Amer. Chem. Soc., 1958, 80, 2338.

75. S. Bergstrom, S. Lindstredt, B. Samuelson, E.J. Corey, and G.A. Gregoriou, J. Amer. Chem. Soc., 1958, 80, 2337.

76. E.J. Corey and G.A. Gregoriou, J. Amer. Chem. Soc., 1959, 81, 3127.

77. M. Hayno, M. Gut, A. Schubert, R.I. Dorfman and R. Siebert, Biochim. Biophys. Acta, 1959, 32, 269.

78. L.J. Morris, Biochem. Biophys. Res. Comm., 1967, 29, 311.

79. R. Howe and D.F. Jones, 5th International Symposium on the Chemistry of Natural Products, 1968, (Abstracts), p.174.

80. A.R. Battersby, J.E. Kesley and J. Staunton, J. Chem. Soc. (D), 1971, 183.

81. I.T. Bruce and G.W. Kirby, Chimica (Switz.), 1968, 22, 314.

82. D.H.R. Barton, D.S. Bhakuni, R. James and G.W. Kirby, J. Chem. Soc. (C), 1967, 128.

83. A.R. Battersby, C. Fuganti and J. Staunton, J.C.S. Chem. Comm., 1971, 1154.

84. C. Fuganti and M. Mazza, J.C.S. Chem. Comm., 1972, 936.

85. C. Fuganti, D. Ghiringhelli and P. Grasseli, J.C.S. Chem. Comm., 1974, 350.

86. G.I. Poos and M.M. Lehman, J. Org. Chem., 1961, 26, 2576.

87. B. Loev, I. Lantos and H. Van Hoeven, Tetrahedron Letters, 1974, 1101.

88. H.C. Brown, P. Heim and N.M. Yoon, J. Amer. Chem. Soc., 1970, 92, 1637.

89. P.E. Fanta, Synthesis, 1974, 9.

90. K. Ito and H. Tanaka, Chem. and Pharm. Bull (Japan), 1974, 22, 2108.

91. P.W. Jeffs and J.F. Hansen, J. Amer. Chem. Soc., 1967, 89, 2798.

92. E. Kotani, N. Takeuchi and S. Tobinaga, J.C.S. Chem. Comm. 1973, 551.

93. E. Leistner, R.N. Gupta and I.D. Spenser, J. Amer. Chem. Soc., 1972, 95, 4040.

94. J.W. Cook, J.D. Loudon and P. McCloskey, J. Chem.Soc., 1954, 4176.

95. Y. Nakagawa and S. Uyeo, J. Chem. Soc., 1959, 3736.

96. S.J. Cristol, W. Barasch and C.H. Tieman, J. Amer. Chem. Soc., 1958, 80, 2562.

97. D.H.R. Barton, A.R. Battersby, T.A.Dobson, G.W. Kirby, H. Ramuz, W. Steglich and G.M. Thomas, J. Chem. Soc., 1965, 2423.

98. P. Ratcliff and R. Rodehorst, J. Org. Chem., 1970, 35, 4000.

99. J. Attenburrow, A.F.B. Cameron, J.H. Chapman, R.M. Evans, B.A. Hems, A.B.A. Jansen, and T. Walker, J. Chem. Soc., 1952, 1094.

100. B. Loev, I. Lantos and H. Van Hoeven, Personal Communication.

101. R. Pschorr, Annalen, 1912, 391, 23.

102. E. Waser and H. Sommer, Helv. Chim. Acta, 1923, 6, 54.

103. H. Meyer, Monatsh., 1901, 22, 428.

104. W.C. Wildman, "The Alkaloids", ed. R.H.F. Manske, Academic Press, New York, 1960, Vol. VI, p. 293.

105. R.E. Hale, E.A. Kieler, J.R. Crowder and W.C. Wildman, J. Amer. Chem. Soc., 1934, 56, 930.

106. M. Bergmann and L. Zervas, Biochem. Z., 1928, 203, 280.

107. M.S. Dunn and L.B. Rockland, "Advances in Protein Chemistry, 1947, 3, 296.

108. G.W. Kirby, and J.D. Michael, Unpublished work.

109. P. Karrer, M. Gisher, E. Horlacher, F. Locher, W. Mader and H. Thomann, Helv. Chim. Acta, 1922, 5, 469.

110. J.B. Gilbert, J.P. Greenstein and V.E. Price, J. Biol. Chem., 1949, 180, 473.