STUDIES IN THE SUGAR ALCOHOL, STEROID AND HETEROCYCLIC SERIES.

T H E S I S

Submitted by

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for the degree

of

Doctor of Philosophy

in the

Faculty of Science

of the

University of Glasgow.

May, 1948.

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Preface.

The author desires to express his deep appreciation of the valuable advice and encouragement, so freely given throughout this work, by Professor J.W. Cook, F.R.S.

Grateful acknowledgment is made to Mr. J. Robertson, B.Sc., for helpful discussion in the course of the work dealing with mannitol; and to Dr. R.B. Moffett (Kansas, U.S.A.) for gifts of microphotostat copies of certain Japanese publications, and of an authentic specimen of "α"-hyodeoxycholic acid.

He is further indebted to Professor C.H. Browning, F.R.S., and to Dr. F.H.S. Curd for providing results of biological investigation of certain compounds which are described in the third part; and to Mr. J.M.L. Cameron, who carried out all of the micro analyses.

The work described in the first part was financially aided by a Research Grant from Cefoil Ltd., and that in the third part by a Studentship from the Medical Research Council, to which bodies, acknowledgment is also made.

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Part I.

Some Chemical Transformations of D-mannitol.

(i) The Hydrogenolysis of D-mannitol.

(ii) The Conversion of D-mannitol into 2:3:4:5-tetra acetyl-aldehydo-D-arabinose.

Introduction.

The sweetish exudate, manna, which is obtained by incision of the trunks of certain kinds of trees such as the manna ash (Fraxinus ornus), has been known since early biblical times. In 1806, Proust[1] isolated in crystalline form, the sweet principle which is now known as D-mannitol. It was early recognised as possessing properties similar to those of other naturally occurring, sweet tasting substances such as cane sugar (sucrose), dextrose (glucose), and fruit sugar (fructose), and was shown to be of widespread occurrence in nature, being isolated from the tissues of materials as diverse as certain land plants, fungi and seaweed. The last, owing to its abundance, appears to be a potentially important raw material for the production of mannitol on the large scale, for example, it has been recently reported[2] that the dried fronds of Laminaria Claustoni, harvested in waters round the coast of Scotland during the month of August, contain as much as 36.7% of mannitol.

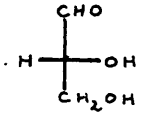
In the latter part of the nineteenth century, Berthelot[3] studied the action of various organic acids on mannitol and thereby established that it is a polyhydric alcohol, similar to glycerol and erythritol in its chemical behaviour. Several years later, Schützenberger[4] prepared and analysed its

hexaacetate and thus showed that mannitol is a hexahydric alcohol, $C_6H_{14}O_6$. The chemical relationship between mannitol and glucose, which had been previously shown to be a straight chain pentahydric aldehyde (aldohexose)[5], was demonstrated by work, notably of E. Fischer and his school[6]. In 1887, Fischer showed[7] that oxidation of mannitol with dilute nitric acid gives, as well as the previously observed oxidation product, fructose, a new sugar, mannose $C_6H_{12}O_6$, whose phenylhydrazone is distinct from that of glucose, but which yields on further treatment with phenylhydrazine, an osazone which is identical with glucosazone. He later (1889)[8] obtained n-heptoic acid from mannose by means of the series of reactions previously used by Kiliani[5] in the case of glucose, and thus showed that the new sugar is an aldohexose, isomeric with glucose. He correctly interpreted the fact that mannose can be converted into glucosazone, on the basis of the newly propounded theory of van't Hoff - Le Bel, by asserting that mannose differs from glucose only in respect to the steric configuration of the groups attached to the carbon atom which is adjacent to the aldehyde group. Fischer inferred from the evidence that mannose is readily reduced to mannitol and that glucose is only slowly reduced by sodium amalgam under alkaline conditions to the same hexitol, that mannitol is not the primary reduction product of glucose, and he showed

later[9] that under the influence of dilute alkali glucose does in fact undergo rearrangement to give mannose.

It is not proposed to give a description here of the methods used and arguments advanced by Fischer[6] which led ultimately to the elucidation of the configurational relationships which exist between the various members of the series of isomeric aldohexoses and aldopentoses, and of the relationship between each member and d-glyceraldehyde, which was adopted as the ultimate reference compound, since these topics have been previously adequately reviewed. (See e.g. [10]).

The configurational structures (written in abbreviated forms of the conventional projection formulae) of d-glyceraldehyde, D-glucose, D-mannose (open chain forms), and D-mannitol are represented respectively by (I), (II), (III) and (IV).



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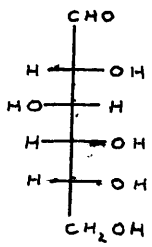

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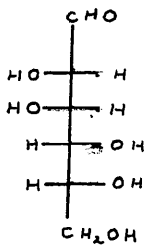

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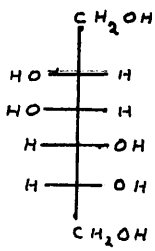


(IV)

It is to be noted that according to convention, the prefix D- when applied to a member of the sugar series, implies that the sugar is theoretically derived from d-glyceraldehyde

in respect to its configuration[11], and does not necessarily define its sign of optical rotation. The reference compound, d-glyceraldehyde, is arbitrarily assigned the absolute configuration shown in (I) and hence all members of the D-series of sugars are represented by projection formulae in which the hydroxyl group attached to the lowest asymmetric carbon atom is written on the right hand side..

The Hydrogenolysis of D-Mannitol.

The reaction of simple sugars with hydrogen, under pressure in the presence of a catalyst, was first studied by Ipatieff[12], who obtained a nearly quantitative yield of D-sorbitol by hydrogenation of D-glucose in alcohol in presence of a nickel catalyst, under 100 atmospheres, at 130°. Cake[13] was able to reduce D-glucose at ordinary pressures in presence of a platinum catalyst. Connor, Covert and Adkins[14], using a nickel-kieselguhr catalyst, hydrogenated D-glucose and D-fructose in aqueous solution at 150° under pressure and obtained D-sorbitol and D-mannitol respectively in nearly quantitative yields. Yoshikawa[15], on the other hand, reported that hydrogenation of D-fructose in presence of a nickel catalyst, under pressure at 130° yields a mixture of equal quantities of D-sorbitol and D-mannitol.

All of these hydrogenation experiments were carried out at temperatures of less than 200°, and hydrogenation ceased at the polyhydroxy alcohol stage. The first observations on the hydrogenation of sugars and polyhydroxy alcohols at higher temperatures appear to be contained in an I.G. Farbenindustrie patent application (1926)[16] which was followed by other patents to the same firm,[17), 18), 19), 20), 21] to Du Pont de Nemours[22), 23] and others[24), 25]. The general

theme of these publications (e.g., [20]) which are couched in the general terms usually employed in the patent literature, is that hydrogenation of hexitols such as D-sorbitol and D-mannitol in aqueous solution, under high pressures at 200-300°, in presence of various catalysts, causes fission of the C_6 chains into C_3 fragments, with the production of glycerol and 1:2-propylene glycol. Aldohexoses such as D-glucose and D-galactose give the same products, hydrogenation being best carried out in two stages, first at about 150° in order to produce the corresponding hexitols, and then at about 250°. More complex carbohydrates such as sucrose, starch and cellulose, preferably after preliminary hydrolysis[21], behave similarly. It was claimed[20] that good yields of glycerol can be obtained by interrupting the course of hydrogenation and by the use of a nickel catalyst, whereas the use of a catalyst which contains copper gives improved yields of propylene glycol.

Schmidt[26], from an examination of the nature of the products obtained by hydrogenation of glycerol, erythritol, 1-methyl glycerol, xylitol, 1:4-dimethylerythritol, sorbitol, mannitol, dulcitol and other polyhydroxy alcohols, in presence of a cobalt-zinc-barium catalyst at 300 atmospheres and 200-250°, concluded that whether a polyhydroxy alcohol possesses a chain of six, five or four carbon atoms, fission

occurs mainly between C_3 and C_4. Thus propylene glycol is the main product obtained from the three hexitols. Glycerol was not found among the reaction products. Schmidt put forward the view that the first stage in the reaction of a hexitol consists in dehydrogenation of the latter by the action of the catalyst at temperatures above 200°, to give the corresponding aldose or ketose. He postulated that these intermediates, owing to their capacity for undergoing enolisation, should be more susceptible to chain fission than the original starting materials. This view is directly opposed to that which may be inferred from results set forth by the patent literature, for according to these publications (e.g., [20]), hexitols are formed as intermediates during the destructive hydrogenation of sugars. Weidenhagen and Wegner[27] studied the hydrogenation of sucrose in aqueous solution under pressure, in presence of a highly active nickel catalyst at 170-180° and found that large quantities of acetol (2-ketopropan-1-ol) can be isolated from the reaction mixture if hydrogenation is arrested immediately after the initially rapid hydrogen uptake, which begins at a temperature of 170°, has ceased. Hexitols were not found in the products of the reaction and were found to be unaffected by these reaction conditions. Since the acetol which is produced cannot be converted by means of further hydrogenation into

propylene glycol, unless an alkali such as calcium hydroxide is added to the reaction mixture, these authors concluded that in hydrogenation of sugars there are at least two courses which the reaction may take, viz: (a) conversion of hexoses, possibly with trioses as intermediates, into methyl glyoxal which then undergoes hydrogenation (in neutral solution) to give acetol which is resistant to further hydrogenation, or (b) reduction of hexoses to hexitols which then undergo fission at temperatures above 200° into mainly C_3 fragments. Zartman and Adkins[28], examined the products of hydrogenation of D-glucose, D-sorbitol, D-mannitol, sucrose, lactose and maltose in ethanol suspension in presence of a copper-chromium oxide catalyst. The same reaction conditions were used in each case, namely an initial hydrogen pressure of 300 atmospheres and a temperature of 250°, and it was found that under such conditions, all of these compounds undergo rapid degradation to methanol, ethanol, propylene glycol and higher boiling products. Glucose, and the two hexitols give similar yields of propylene glycol and of higher boiling material, for example D-mannitol gives propylene glycol and higher boiling material in yields corresponding to 50% and 14% respectively of the weight of starting material used. The three disaccharides give comparable yields of products in each case, but the yields of propylene glycol are much lower than those obtained from the

C_6 compounds and the yields of higher boiling materials are correspondingly higher. Glycerol was not found in any of the higher boiling fractions.

It is evident from these examples which have been discussed, that in the hydrogenation of hexitols and sugars, experimental conditions, especially temperature and the nature of the catalyst used, play an important part in determining the course of reaction and the nature of the products formed. The experiments which are described in the sequel were carried out in order to study the products of reaction of D-mannitol with hydrogen under various conditions of temperature and pressure, in presence of each of the two catalysts, copper-chromium oxide[29] and Raney nickel[30], with the object of ascertaining, if possible, experimental conditions which might lead to maximum yields of propylene glycol and glycerol. The conditions used in each experiment and the products formed, are described in the table (page 15).

In presence of copper-chromium oxide, a suspension of mannitol in ethanol rapidly absorbed hydrogen at an initial pressure of 200 atmospheres when the reaction mixture was heated to 230°. The reaction products contained propylene glycol and higher boiling material (from which no glycerol could be isolated) in quantities corresponding to 50% and 9% respectively, of the starting material. These results are in close agreement with those previously reported by

other workers[28]. The reaction proceeded only very slowly at an initial hydrogen pressure of 200 atmospheres, and a temperature of 200°. After a prolonged period under these conditions, much starting material remained undhanged and the yield of propylene glycol was correspondingly lower. In presence of copper-chromium oxide, the nature of the solvent used was found to be of importance, for in one experiment under conditions which had previously produced rapid conversion of mannitol, except that water was substituted for ethanol as solvent, the starting material was recovered unchanged even after a prolonged period under these conditions. Conditions of temperature and also pressure were found to be of even more importance in presence of Raney nickel. Over the latter catalyst, and in ethanolic suspension, reaction proceeded slowly at 200°, under a pressure of about 150 atmospheres. On interruption of the hydrogenation after several hours under these conditions, about one third of the starting material was recovered, together with about 12% of propylene glycol and 9% of higher boiling material, which was found to contain about one half of its weight of glycerol. This was the only experiment (No.5) in which glycerol could be isolated from the reaction products. In order to attempt to obtain complete conversion of mannitol, the conditions were altered by increasing the reaction temperature to 230°.

This necessitated the use of water as solvent instead of ethanol, since the latter is reported[31] to be unstable in the presence of Raney nickel at temperatures greater than 225°. However, at the increased temperature a very vigorous reaction set in. The formation of gaseous hydrocarbons presumably methane, ethane, etc. was indicated by a sudden, great increase in pressure and the only products which could be isolated consisted of a mixture of lower alcohols, mainly ethanol, and a very small amount of propylene glycol.

It may be inferred from the results so far discussed, that the main reaction (neglecting side reactions) probably proceeds in at least four consecutive stages, viz:-

a) fission of the C_6 chain with the formation of glycerol,

b) hydrogenation of the latter to propylene glycol,

c) destructive hydrogenation of the glycol to ethanol and methane and, d) complete reduction of ethanol to ethane.

a) $CH_2OH.CHOH.CHOH.CHOH.CHOH.CH_2OH \longrightarrow 2CH_2OH.CHOH.CH_2OH$

b) $CH_2OH.CHOH.CH_2OH \longrightarrow CH_3.CHOH.CH_2OH$

c) $CH_3.CHOH.CH_2OH \longrightarrow CH_3CH_2OH + CH_4 + H_2O$

d) $CH_3CH_2OH \longrightarrow C_2H_4 + H_2O$

The conversion of glycerol into propylene glycol by hydrogenation has been previously reported[20),32)]. In order to test the validity of the assumption made in regard to stage c),

hydrogenation of propylene glycol in presence of Raney nickel was studied. At a temperature of about 230°, a sudden increase in pressure, caused by the formation of gaseous hydrocarbons occurred, and the only products which could be isolated from the reaction mixture were ethanol (in approximately 35% of the theoretical yield) and a corresponding quantity of water. It is obvious that the reactions represented by c) and d) proceed at slower rates than do those represented by a) and b), but if the conditions used are sufficiently vigorous, degradation of propylene glycol takes place almost as soon as it is formed. In water, in presence of Raney nickel, mannitol was almost completely unaffected by hydrogen under a pressure of 170 atmospheres, at temperatures of 190° or less. On reduction of the pressure to about 100 atmospheres and elevation of the temperature to 230°, it was possible to effect complete conversion, which resulted in the production of somewhat higher yields of propylene glycol (16%), and of higher boiling material (13%), than were obtained when an alcoholic medium was used. When the temperature was maintained at 230°, and the pressure reduced still further to approximately 70 atmospheres, a lower yield of propylene glycol (11%) and higher yield of higher boiling products (25%), were obtained.

To sum up, it may be stated that under certain specified conditions, mannitol can be readily converted by means of

hydrogenolysis under high pressures and at high temperatures in presence of copper-chromium oxide into fair yields of propylene glycol, but in presence of the more active catalyst, Raney nickel, conditions of temperature and pressure are too critical to make the method of any practical value for the production of reasonable quantities of either propylene glycol or glycerol. It is realised that in the use of the latter catalyst, the large number of possible combinations of variables in respect to temperature and pressure, were not thoroughly explored, but it is considered that the experiments performed were sufficiently representative to justify the conclusions stated above.

Hydrogenolysis of mannitol

Expt. No	mannitol (g.mols.)	solvent	catalyst	P (atmos)	Maximum T	Duration at Max. T.	unchanged mannitol	propylene glycol Yield	Higher boiling products Yield
1	1	EtOH	CC	200	200°	12 hrs.	40 %	14 %	b.p. 174 - 182° / 12 mm. 9 %
2	1	EtOH	CC	170	230°	3·5 hrs.	—	50 %	b.p. 110 - 190° / 12 m.m. 8 %
3	1	H_2O	CC	180	230°	10 hrs.	50 %	—	—
4	1	EtOH	RN	150†	200°	6 hrs.	30 %	12 %	b.p. 160 - 180° / 22 mm. 9 %
5	1	H_2O	RN	> 200†	230°	4·5 hrs.	-	3 %	< 2 %
6	0·5	H_2O	RN	160	170°	5 hrs.	ca 100 %	—	—
7	0·5	H_2O	RN	168	190°	5 hrs.	ca 100 %	—	—
8	0·5	H_2O	RN	96	230°	2·5 hrs.	—	16 %	b.p. 100 - 195° / 12 mm 13 %
9	0·5	H_2O	RN	70	230°	2·5 hrs.	—	11 %	b.p. 120 - 260° / 12 mm 25 %

CC = copper chromium oxide
RN = Raney nickel

In each experiment, the amount of catalyst used was 10 % of the weight of mannitol

Approximately equal weights of mannitol and solvent were used.

% yields are calculated on the weight of starting material.

† some pressure had to be released owing to its sudden rapid increase.

Experimental.

The apparatus in which all of the high-pressure hydrogenation experiments were carried out consisted of a Baskerville and Lindsay stainless steel autoclave of total capacity 1.5 litres, fitted with a stainless steel, reciprocating stirrer which was actuated by an external solenoid coil. The apparatus was electrically heated, and the internal temperature, which was measured by means of a thermocouple located in a stainless steel pocket dipping into the reaction mixture, could be regulated by means of a rheostat. Hydrogen pressure was measured by means of a standard gauge.

Copper-chromium oxide and Raney nickel catalysts were prepared according to the procedures of Folkers, Connor and Adkins[29] and of Covert and Adkins[32] respectively. For the sake of uniformity, all of the hydrogenation experiments were carried out using samples from a single batch of each catalyst.

The conditions of each hydrogenation experiment and the results therefrom are given in the table (page 15), and therefore, only certain salient experimental features will be described in the sequel. The general procedure used in each experiment is illustrated by a description of experiment No.2:-

A mixture of D-mannitol (182 g.), absolute ethanol (200 c.c.) and copper chromium oxide (18 g.) was subjected to

a hydrogen pressure of 170 atmospheres and heated to 220-230°. Rapid absorption of hydrogen occurred and ceased at the end of 3.5 hours. On cooling, the reaction mixture was filtered from catalyst and the filtrate freed from solvent and volatile products by distillation from an oil bath until the temperature of the distillate rose above 100°. The residue was fractionally distilled (20 cm. column) under reduced pressure, and the following fractions collected: (a) b.p. 89-91°/12 mm. (90.4 g.), (b) b.p. 110-190°/12 mm. (14.8 g.). Fraction (a) gave a positive reaction with Denigès reagent[33], and on treatment with 3:5-dinitrobenzoyl chloride in pyridine it gave propylene glycol di(3:5-dinitrobenzoate), which formed rosettes of colourless needles (from ethanol), m.p. 174-175°. (Found: C, 44.2; H, 2.8; N, 12.1. $C_{17}H_{12}O_{12}N_4$ requires C, 44.0; H, 2.6; N, 12.1%). Fraction (b) was redistilled into four fractions: (b 1) b.p. 70-80°/1.2 mm. (2.8 g.), (b 2) b.p. 85-112°/1.2 mm. (2.3 g.), (b 3) b.p. 112-125°/1.2 mm. (4.6 g.) and (b 4) b.p. 125-132°/1.2 mm. (2.4 g.). Treatment of fractions (b 4) and (b 3) with 3:5-dinitrobenzoyl chloride yielded uncrystallisable gums. The same result was obtained on benzoylation of a fraction b.p. 155-160°/11 mm. which was obtained by refractionation of fraction (b 4).

Experiment No.4.

After removal of unchanged mannitol by filtration of the cold reaction mixture, and of solvent and volatile products by distillation of the filtrate until the temperature reached 100°, the residue was fractionally distilled (20 cm. column) into the two fractions: (a) b.p. 100-110°/22 mm. (21 g.), (b) b.p. 160-180°/22 mm. (16.5 g.). Fraction (a) was identified as propylene glycol by the methods described above. Fraction (b) was refractionated and a fraction b.p. 166-170°/11 mm. (8.1 g.) collected. A small portion of the latter, on treatment with benzoyl chloride in pyridine, yielded glycerol tribenzoate, m.p. 71-72°, alone or on admixture with an authentic sample[34].

Experiment No.5.

When the reaction temperature reached 210-230°, the pressure within the bomb rapidly increased from about 140 to over 200 atmospheres. The apparatus was allowed to cool and the pressure slowly released, the escaping gas being led through a train of traps cooled in solid CO_2-acetone. No condensate collected in the traps. Hydrogenation was continued at 220-230° under 140-160 atmospheres for 4 hours, there being no further increase in pressure. Fractional distillation of the reaction mixture (after removal of catalyst) gave the following fractions: (a) b.p. 70-97°

(25 g.), (b) b.p. 99-100° (285 g.), (c) b.p. 185-189° (6.2 g.). A small amount (1.8 g.) of non-volatile residue remained. Fraction (c) consisted of propylene glycol. Fraction (a) was dried (CaO) and redistilled. Treatment of a small sample of the distillate (b.p. 77-83°; 18 g.) with 3:5-dinitrobenzoyl chloride in a mixture of benzene and pyridine gave a solid ester which on repeated recrystallisation from light petroleum (b.p. 60-80°) yielded ethyl 3:5-dinitrobenzoate, m.p. 86-90°, alone or on admixture with an authentic sample[35] of m.p. 93°. Fraction (b) consisted mainly of water.

Experiments Nos. 8 and 9.

The higher boiling fractions obtained in each experiment were refractionated under reduced pressure. Benzoylation of each of the several fractions collected, yielded no crystalline product.

Hydrogenation of propylene glycol.

A solution of the glycol (45 g.) in water (100 c.c.) was hydrogenated over Raney nickel (approximately 15 g.), under 190 atmospheres at 220-230° for 3 hours. When the temperature reached 225°, the pressure began to increase rapidly and had to be reduced. After being filtered, the reaction mixture was fractionally distilled. The first

fraction consisted of aqueous ethanol, which after being dried (K_2CO_3) and redistilled, yielded 8.3 g., b.p. 78°. A small sample, on treatment with 3:5-dinitrobenzoyl chloride yielded pure ethyl 3:5-dinitrobenzoate. The second fraction consisted entirely of water (117 g.), and there was no higher boiling residue.

Addendum.

The experimental studies on the hydrogenation of D-mannitol which are described in this dissertation were completed in 1942, and since that time, some results obtained by other authors working on similar topics have been published. These results are briefly discussed below.

In an attempt to develop a method for the production of glycerol on the large scale, alternative to the usual method which involves saponification of fats, Lenth and du Puis[36] studied the hydrogenation of sugars in presence of various catalysts, under varied conditions of temperature and pressure and extended their experiments with both glucose and sucrose to the pilot-plant stage. It was found that maximum conversion occurs in an alcoholic medium at pressures of 100 to 140 atmospheres and temperatures of 225° to 250°, in presence of copper-chromium oxide or a similar catalyst consisting of copper-aluminium oxide. The products of reaction are propylene glycol (in 36% yield) and smaller quantities (25% yield) of a higher boiling mixture, of which glycerol forms a small proportion but cannot be isolated from it by means of fractional distillation. These results are in qualitative agreement with those obtained in the present work.

By means of studies involving several hundred experiments

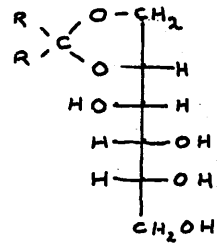
on the hydrogenation of glucose and other carbohydrates in aqueous and alcoholic media in presence of various catalysts, Natta, Rigamonti and Beati[37] arrived at similar conclusions, namely that optimum conditions for producing high yields of propylene glycol involve the use of copper catalysts in alcoholic media at temperatures of 220° to 230°. It was claimed that yields of glycol as great as 80% may be obtained by submitting the higher boiling products to rehydrogenation. It was also claimed that hydrogenation of glucose in aqueous solution in presence of various nickel catalysts, e.g., nickel-kieselguhr, nickel-cobalt, nickel-copper and nickel-chromium, leads to lower yields (e.g., 30-35%) of glycol and higher yields of glycerol. These authors apparently use the term "glycerol" to denote the mixtures of higher boiling substances which usually accompany propylene glycol in the reaction products. Raney nickel was found to be an unsuitable catalyst, yields of glycol as low as 6% being obtained in its presence, but its action under varied conditions of temperature and pressure was apparently not examined.

(ii) Conversion of D-mannitol into 2:3:4:5-tetraacetyl-aldehydo-D-arabinose.

It was early recognised that certain properties displayed by simple aldoses, such as their ability to form two isomeric series of semi-acetals and of fully esterified derivatives, the phenomena of mutarotation, and the inability of many of them to give a positive aldehyde reaction with fuchsin-sulphurous acid, indicated that the constitutions of these substances are better expressed by ring structures in which the carbon atom of the terminal reducing group forms one member of an oxide ring, rather than by acyclic, polyhydroxy aldehyde structures. It is now generally accepted[38] that the normal form of glucose and of most of the other aldoses and pentoses, contains a six-membered oxide (pyranose) ring. Other forms of many of these sugars and their derivatives are known to possess five-membered (furanose) ring structures, but these forms are not encountered so frequently. However, derivatives of acyclic sugars have been prepared, mainly by a method which involves first, the formation of the sugar diethyl acetal, then protection of the free hydroxyl groups by esterification or etherification, followed by removal of the thioacetal groups by means of selective hydrolysis. Wolfrom and co-workers were able to prepare thus a number of crystalline, acyclic, sugar acetates. Other, less general, methods include catalytic reduction of fully acetylated aldonic acid chlorides[39], and selective hydrolysis

of acetylated, sugar oximes and semicarbazones, followed by treatment with nitrous acid[40]. These acyclic (aldehydo) sugar derivatives are of interest since they display the properties of true aldehydes. For example, they are capable of undergoing reaction with methyl magnesium bromide to give the corresponding methyl carbinols[41], and with diazomethane to give 1-deoxy ketose derivatives[42].

The experiments which are described in the sequel were initiated with the objects of exploring a novel route to derivatives of aldehydo D-arabinose starting from D-mannitol, and if possible, of determining an improved procedure for the preparation of the somewhat difficultly accessible D-form of arabinose, of which the L-form is that occurring in nature. It is obvious, from a consideration of the structure of D-mannitol, that a derivative (V) in which the 1:2-hydroxyl groups are engaged by acetal formation, corresponds to a derivative in which the 5:6-hydroxyl groups are similarly engaged, and that esterification of (V) to give (VI), followed by selective hydrolysis of the acetal group, would give the partially esterified derivative (VII) in which both 1:2-hydroxyl groups are free. This derivative (VII) should be capable of undergoing reaction with lead tetra acetate, a reagent which has been shown by Criegee[43] to be capable of effecting fission of 1:2-glycols with the formation of carbonyl compounds, and should thus give a molecule of



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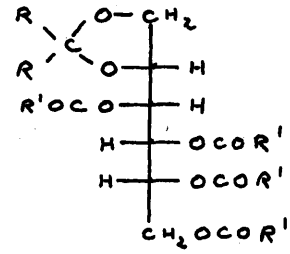

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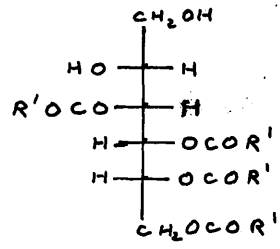
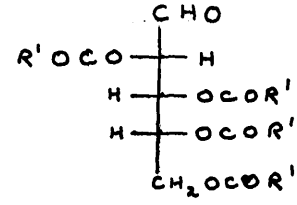


(VII)



(VIII)

formaldehyde and the esterified aldehydo D-arabinose derivative (VIII). Irvine and Patterson[44] obtained by graded hydrolysis of triacetone mannitol, a monoacetone mannitol, m.p. 85°, to which they assigned the structure (V; R = $-CH_3$), since on methylation followed by selective hydrolysis of the acetone residue, and oxidation of the resulting tetramethyl derivative, it gives what was believed to be a tetramethyl mannonic acid. However, Müller[45] showed that the monoacetone mannitol gives a dityrityl derivative and therefore probably contains two free primary hydroxyl groups, and also that it is obtained by hydrolysis of the benzoate groups of 1:6-dibenzoyl monoacetone mannitol. He suggested that the monoacetone mannitol is in fact, the

3:4-derivative and this view was later confirmed by Brigl and Grüner[46] who obtained dibenzoyl D-glyceraldehyde by lead tetra acetate oxidation of the tetrabenzoyl mannitol which is derived from it by means of benzoylation followed by selective hydrolysis of the acetone residue[47]. Vargha[48] obtained a monoacetone mannitol, m.p. 167°, by acetonisation of mannitol in presence of boric acid, and formulated it as the 1:2-derivative (V; R = CH_3) for the following reasons:- (a) It differs from the monoacetone derivative (shown to be 3:4-) of Irvine and Patterson. (b) It gives a monotrityl derivative and therefore probably possesses only one free primary hydroxyl group. (c) On further acetonisation it is converted into a diacetone mannitol which is identical with that previously isolated from the products of acetonisation of mannitol in presence of hydrochloric acid, and formulated as the 1:2-5:6-diacetone derivative[49]. The 1:2-monoacetone mannitol of Vargha[48], on acetylation gives a nearly quantitative yield of (VI; R = R^1 = $-CH_3$), which on graded hydrolysis yields a crystalline 3:4:5:6-tetraacetyl-D-mannitol (VII; R^1 = $-CH_3$). In the present work, the latter was prepared and submitted to oxidation by lead tetraacetate. The crystalline 2:3:4:5-tetraacetyl-aldehydo-D-arabinose (VIII; R^1 = CH_3) which was obtained, corresponded in its properties to the tetraacetyl aldehydo-D-arabinose which

was prepared by Wolfrom and co-workers[42] from D-arabinose diethyl mercaptal. Further proof of their identity was provided by comparison of the properties of their semicarbazones.

This conversion of the monoacetone mannitol of Vargha into (VIII; $R' = CH_3$), obviously affords further confirmation of the structure previously assigned to the former compound. One serious disadvantage of the process, as a means of convenient synthesis of D-arabinose, lay in the fact that low and inconsistent yields were obtained in the first stage, namely the acetonisation in presence of boric acid. Vargha[48] claimed a yield of monoacetone derivative of 14.8%. In our hands, this was approached only once out of several preparations, and other workers have evidently experienced similar difficulty. In a paper which was published after the present work was completed, Baer[50] states that "... the outcome of the synthesis was unpredictable. With the chemicals then (1934) available (in Basel) we succeeded only twice out of many trials in obtaining any yield of diacetone mannitol."

Since any 1:2-monoacetal derivative of D-mannitol would serve theoretically as a starting point for the process, attention was next directed to the study of the constitution of a monofurfurylidene derivative which was

obtained, apparently in good yield, by Bredereck and Papademetriu[51] by the direct action of furfuraldehyde in presence of dilute nitric acid, on D-mannitol. These authors did not assign a constitution to this derivative. It was considered that it might possess the required 1:2-constitution, in view of the fact that whereas the monoacetone mannitol which is produced on graded hydrolysis of triacetone mannitol is the 3:4-derivative, that which is obtained by the direct action of acetone (in presence of boric and sulphuric acids) on mannitol is the 1:2-isomer.

In an attempt to determine the number of free primary hydroxyl groups present, the compound was treated with triphenyl methyl chloride in pyridine, but a crystalline product could not be obtained. Treatment of the monofurfurylidene derivative with acetic anhydride and benzoyl chloride resulted in the formation of uncrystallisable gums in each case. The syrupy benzoylation product however, on graded hydrolysis, yielded a gum from which two crystalline products were isolated. Of these, one m.p. 118-119° had the composition of a tetrabenzoyl mannitol and its identity with 1:2:5:6-tetrabenzoyl mannitol[47),46)] was proved by means of a mixed melting point determination made with an authentic sample of the latter. The other, m.p. 163°, had the

composition of a tribenzoyl mannitol, and is probably identical with the 1:2:6-derivative which has been previously described[46].

These results show therefore, that in the original monofurfurylidene D-mannitol, the acetal group is attached to C_3 and C_4. The production of a certain amount of tribenzoyl mannitol by hydrolysis of its benzoylation product may be explained on the grounds that either incomplete reaction occurred at the benzoylation stage, or partial hydrolysis of the benzoyl groups of the fully benzoylated acetal took place to some extent, under the mild conditions used for removal of the acetal group.

Further work, with the object of obtaining more readily accessible mannitol derivatives containing free hydroxyl groups in the 1:2-positions, could not be carried out owing to the departure of the author to take up a war-time appointment with May and Baker Ltd. However, since the experiments described above were completed (in 1943), one of their initial objects, namely the determination of a process whereby D-mannitol might become a convenient source for the preparation of D-arabinose, has been achieved by another author. Wiggins[52] improved the procedure of Irvine and Patterson[44] for the preparation of 1:2-3:4-diacetone D-mannitol and showed that lead tetraacetate oxidation of the latter yields diacetone aldehydo-D-arabinose, which on hydrolysis gives D-arabinose.

Experimental.

1:2-Monoacetone D-mannitol.

The procedure of Vargha[48] was followed. Yields obtained in a series of runs were:- 13.2, 10.1, 6.2, 7.1, 0, and 4.6%. The use of A.R. acetone was found to be necessary.

New synthesis of 2:3:4:5-tetraacetyl-aldehydo-D-arabinose.

Lead tetraacetate[53] (1.09 g.) was added in small portions with shaking after each addition, to 3:4:5:6-tetra-acetyl-D-mannitol[48] (0.82 g.) suspended in anhydrous, thiophene-free benzene (30 c.c.). The mixture was shaken for 3 hours and then filtered. The residual lead salts were washed with benzene and the filtrate and washings evaporated under reduced pressure. The residue was extracted with acetone and the extracts evaporated under reduced pressure. Crystallisation of the residue from ether-petroleum ether (b.p. 60-80°) gave colourless needles (0.42 g.), m.p. 112-114° $[\alpha]_{5460}^{27^\circ}$ +67.6° (c = 4.02 in chloroform). (Found: C, 49.5; H, 5.5. Calc. for $C_{13}H_{18}O_9$: C, 49.0; H, 5.7%). The substance restored the colour to Schiff's reagent. Wolfrom et al.[42] record for the same compound prepared from D-arabinose:- m.p. 113-115°, $[\alpha]_D^{23^\circ}$ +65°. (c = 4.1 in chloroform).

2:3:4:5-Tetraacetyl-aldehydo-D-arabinose semicarbazone.

A solution of the above tetraacetyl aldehyde (0.2 g.) in warm water (2 c.c.) was treated with a mixture of semicarbazide hydrochloride (0.1 g.) and potassium acetate (0.13 g.). The product, on recrystallisation from methanol, gave glittering plates m.p. 183-185°. (Found: N, 11.0. Calc. for $C_{14}H_{21}O_9N_3$: N, 11.2%). Wolfrom et al[40] give m.p. 183-185°.

Tritylation of monofurfurylidene D-mannitol.

A solution of the monoacetal[51] (0.5 g.), in anhydrous pyridine (5 c.c.), was treated with triphenyl methyl chloride (1.07 g.; 2 mols.) and then set aside for 7 days. It was poured into ice-cold water, and the gum which separated extracted by means of ether. The ethereal extract was washed several times with water, dried (Na_2SO_4), and then evaporated. The residual syrup (1.2 g.), could not be rendered crystalline by treatment with solvents or by cooling to 0° for several days.

Acetylation of monofurfurylidene mannitol.

The monoacetal (0.3 g.), dissolved in anhydrous pyridine (2 c.c.), was treated with acetic anhydride (1 c.c.). After being kept at room temperature for 24 hours, the mixture was treated with ice-cold water. The resulting yellow gum

could not be rendered crystalline.

Benzoylation of monofurfurylidene mannitol.

A solution of the monoacetal (0.51 g.) in anhydrous pyridine (5 c.c.) was cooled to 0° and treated dropwise with redistilled benzoyl chloride (1.2 g.; 4.1 mols.). After being set aside for 48 hours, the mixture was poured into water and extracted with ether. The ethereal extract was washed with dilute sodium bicarbonate solution and water, dried (Na_2SO_4), and then evaporated under reduced pressure. The residual yellow syrup (1.4 g.) could not be rendered crystalline by solvent treatment.

Hydrolysis of benzoylated monofurfurylidene mannitol.

A solution of the above syrup in glacial acetic acid (12 c.c.) was treated gradually with hydrochloric acid (d 1.19; 3 c.c.), whereupon a small quantity of oil separated. An homogeneous solution was obtained by shaking for 1 hour, and was kept at room temperature for 3 hours. During this time the initially light-yellow solution turned deep claret. It was poured into water and extracted with ether. The ethereal extract was washed successively with sodium bicarbonate solution and water, dried (Na_2SO_4), and then evaporated under reduced pressure. The residual brown syrup was dissolved in warm benzene (5 c.c.), an equal volume of petroleum

ether (b.p. 80-100°), added and the solution allowed to cool. The solid which separated (0.45 g.), on fractional crystallisation from benzene yielded, as more soluble product, long, colourless needles (0.2 g.), m.p. 118-119°, of 1:2:5:6-tetrabenzoyl D-mannitol. (Found: C, 68.6; H, 5.2. Calc. for $C_{34}H_{30}O_{10}$: C, 68.2; H, 5.1%). Its m.p. was not depressed on admixture with an authentic sample (m.p. 119-121°), which was prepared from 3:4-monoacetone D-mannitol by the method of Fischer[47]. The less soluble product (0.18 g.), formed short needles, m.p. 163°, of tribenzoyl mannitol. (Found: C, 66.1; H, 5.2. Calc. for $C_{27}H_{26}O_9$: C, 65.6; H, 5.3%). (Brigl and Grüner[46] reported that 1:2:6-tribenzoyl D-mannitol has m.p. 163°.)

Bibliography.

1). Proust, Ann.Chim., 1806, 57, 143.

2). Black, Nature, 1948, 161, 174.

3). Berthelot, Compt.rend., 1855, 41, 452.

4). Schützenberger, Ann.Chim., 1870, 21, 256,
Annalen, 1871, 160, 92.

5). Kiliani, Ber., 1886, 19, 767.

6). E. Fischer, "Untersuchen über Kohlenhydrate und Fermente",
Vol.I, 1884-1908, Springer, Berlin, 1909.

7). E. Fischer, Ber., 1887, 20, 821.

8). E. Fischer and Hirschberger, Ber., 1889, 22, 365.

9). E. Fischer, Ber., 1890, 23, 2133.

10). Wolfrom, in Gilman's "Organic Chemistry", 2nd Edn.,
Wiley, New York, 1942, p.1532.

11). Rosanoff, J.Amer.Chem.Soc., 1906, 28, 114.

12). Ipatieff, Ber., 1912, 45, 3225.

13). Cake, J.Amer.Chem.Soc., 1922, 44, 859.

14). Connor, Covert and Adkins, J.Amer.Chem.Soc., 1932, 54,
1651.

15). Yoshikawa, Ch.Abs., 1935, 29, 2434.

16). DR - P 524 101 (Ch.Abs., 1931, 25, 3665).

17). DR - P 541 362 (Ch.Abs., 1932, 26, 1939).

18). DR - P 544 666 (Ch.Abs., 1932, 26, 3514).

19). Brit. - P 299 373 (Ch.Abs., 1929, 23, 3232).

20). French - P 662 874

21). French - P 739 951 (Ch.Abs., 1933, 27, 2158).

22). U.S. - P 1963 997 (Ch.Abs., 1934, 28, 5077).

23). U.S. - P 2004 135 (Ch.Abs., 1935, 29, 4772).

24). French - P 816 952 (Ch.Abs., 1938, 32, 2255).

25). Brit. - P 490 211 (Ch.Abs., 1939, 33, 787).

26). Schmidt, Z.physikal.Chem., 1932, (A), 159, 337.

27). Weidenhagen and Wegner, Ber., 1938, 71, 2712.

28). Zartman and Adkins, J.Amer.Chem.Soc., 1933, 55,4559.

29). Folkers, Connor and Adkins, J.Amer.Chem.Soc., 1932, 54, 1138.

30). Covert and Adkins, J.Amer.Chem.Soc., 1932, 54, 4116.

31). Adkins, "Reactions of Hydrogen with Organic Compounds", University of Wisconsin, Wisconsin, 1937, p.26.

32). Connor and Adkins, J.Amer.Chem.Soc., 1932, 54, 4678.

33). Denigès, Ann.Chim., 1879, 18, 174.

34). Einhorn and Hollandt, Annalen, 1898, 301, 95.

35). Reichstein, Helv.Chim.Acta, 1926, 9, 799.

36). Lenth and du Puis, Ind.Eng.Chem., 1945, 37, 152.

37). Natta, Rigamonti and Beati, Ber., 1943, 76, 641.

38). W.N. Haworth, "The Constitution of Sugars", Arnold, London, 1929.

39). Cook and Major, J.Amer.Chem.Soc., 1936, 58, 2410.

40). Wolfrom and Georges, J.Amer.Chem.Soc., 1934, 56, 1794.

41). Gätzi and Reichstein, Helv.Chim.Acta, 1938, 21, 914.

42). Wolfrom, Weisblat, Zophy and Waisbrot, J.Amer.Chem.Soc., 1941, 63, 201.

43). Criegee, Ber., 1931, 64, 260.

44). Irvine and Patterson, J., 1914, 105, 898.

45). Müller, Ber., 1932, 65, 1055.

46). Brigl and Grüner, Ber., 1933, 66, 931.

47). E. Fischer, Ber., 1915, 48, 266.

48). Vargha, Ber., 1933, 66, 1394.

49). E. Fischer and Rund, Ber., 1916, 49, 91.

50). Baer, J.Amer.Chem.Soc., 1945, 67, 338.

51). Bredereck and Papademetriu, Ber., 1937, 70, 797.

52). Wiggins, J., 1946, 13.

53). Oesper and Deasy, J.Amer.Chem.Soc., 1939, 61, 972.

Part II.

The Configuration of the C_6 Hydroxyl Groups in "α"- and "β"-Hyodeoxycholic Acids and the Partial Synthesis of these Bile Acids from Cholesterol.

Introduction.

Extensive investigations directed towards the elucidation of the constitutions of the complex organic acids which can be isolated from the bile of mammals and fishes, were initiated near the beginning of this century and were carried out in the first place, notably by Wieland. These studies proceeded apace with investigations on the nature of sterols, particularly the readily accessible cholesterol, the structural investigation of which was undertaken by Windaus in 1903, and it was soon recognised that the chemical structures of bile acids and sterols are closely related. The isolation, reported in 1929 by both Doisy and Butenandt, of oestrone was followed soon after by the isolation of a series of biologically active sex hormones of similar character, and recognition that these substances are steroid, led to intensified activity in the study of the group as a whole. When in 1932, Rosenheim and King suggested that the nuclear skeleton of cholesterol and the bile acids, is essentially that of chrysene, and this formulation was shortly afterwards modified by Wieland and Dane, who suggested that the carbon skeleton is that of 1:2-cyclopentenophenanthrene, it became possible to accommodate the vast amount of experimental facts then known about the sterols and bile acids by means of a single carbon framework common to both groups.

Work in the steroid field now in progress is concerned mainly with the elucidation of fine points of structure, particularly stereochemical, the confirmation or modification of older structures, the examination of more recently isolated natural steroids such as those of the adrenal cortex and finally with model experiments directed towards total synthesis. In connection with the last mentioned aim, it has been stated recently[1] that the way to a total synthesis of cholesterol and its derivatives is now open.

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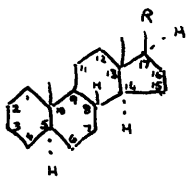
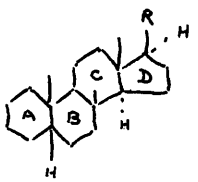
Modern views, which have been admirably summarised by Shoppee[2], on the stereochemical configuration of the steroid nucleus, are based on a combination of both chemical and physical studies, and in particular, recent X-ray crystallographic studies of cholesteryl iodide[3] have confirmed the configurations (I) and (II) assigned to the nucleii of the commoner sterols and bile acids respectively.



(I)



(II)

In both (I) and (II), rings B/C and C/D are trans-fused. In (I), rings A/B are trans- and in (II) cis-fused. The

angular methyl groups attached to C_{10} and C_{13} are by convention regarded as projecting forward from the plane of the paper. Since the absolute configuration of no steroid centre has yet been determined, the actual configurations of (I) and (II) may be their respective mirror-images.

The convention used for the description of substituted steroids in the sequel, is that proposed by Fieser,[4] and is the one in common use at the present time. A dotted line attached to a substituent indicates that the latter is trans- in respect to the methyl group attached to C_{10}, i.e., it has the (α) configuration. An unbroken line attached to the nucleus indicates a cis- relationship to the C_{10} angular methyl group, i.e, a (β) orientation. The trivial indices "α" and "β" are not used in order to indicate a definite stereochemical orientation, but only to distinguish between closely related isomers, the configurations of which are not known with certainty.

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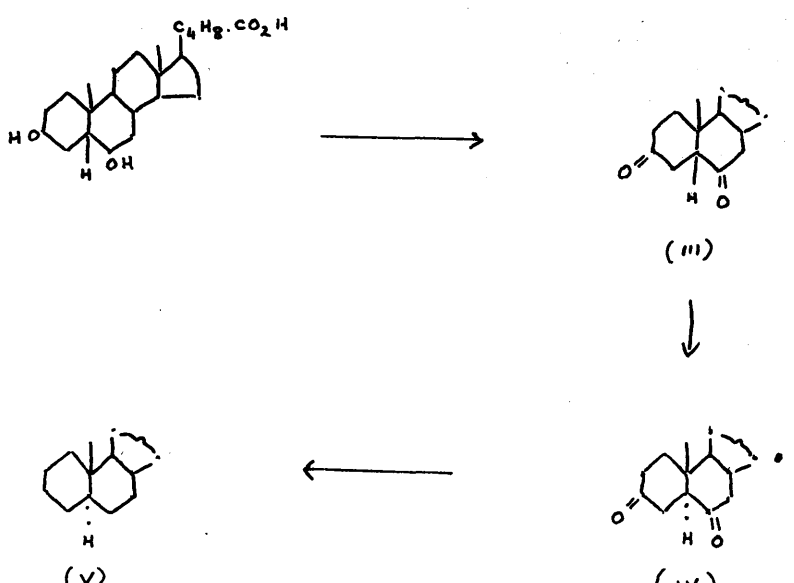
The structural investigation of hyodeoxycholic acid which was first discovered in pig bile in 1847 by Gundelach and Strecker[5] was commenced by Windaus, and in 1923 he described[6),7] some properties of the free bile acid and its isolation from pig bile. Three years later he announced the results of further work,[8] which settled the essential

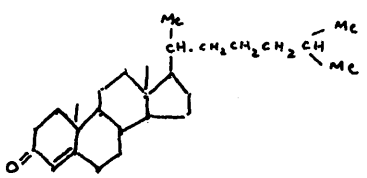
features of its constitution, and showed that (in terms of the modern, cyclopentenophenanthrene structure) hyodeoxycholic acid is a 3:6-dihydroxycholanic acid. Since dehydrohyodeoxy cholic acid (III), the diketo acid obtained by chromic acid oxidation of hyodeoxycholic acid, on Clemmenson reduction yields allo-cholanic acid, it was believed, prior to 1926, that the bile acid belonged to the cholestane series, and was therefore exceptional among all of the other bile acids then known. However in that year it was shown by Windaus,[8] that the primary oxidation product, namely the "α"-dehydro acid (III), in presence of hot dilute acid or alkali, readily undergoes allomerisation, and gives the more stable isomer 3:6-diketoallocholanic acid ("β"-dehydrohyodeoxycholic acid) (IV), which undergoes Clemmensen reduction in the normal way, to give allocholanic acid (V).



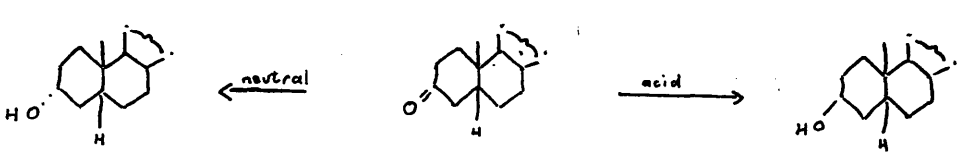
This observation together with other chemical and physical[9] evidence provided strong indication that in the bile acid (coprostane) series, rings A and B are fused as in cis-decalin while in the cholestane series, the mode of fusion is that of the more stable trans-decalin.

Evidence that hyodeoxycholic acid, in common with most of the other naturally occurring bile acids, belongs to the epicoprosterol series, i.e., the C_3 - OH, is in the trans position with respect to the C_{10} angular methyl group follows from the results of work which is briefly summarised as follows. It was shown by Ruzicka and co-workers,[10] that catalytic hydrogenation with a platinum catalyst of coprostanone (VII), which in turn is obtained on reduction of coprostenone (VI) over a palladium catalyst[11], in acid solution gives coprostanol (VIII), whereas in neutral solution the epimeride, epicoprostanol (IX) is formed.

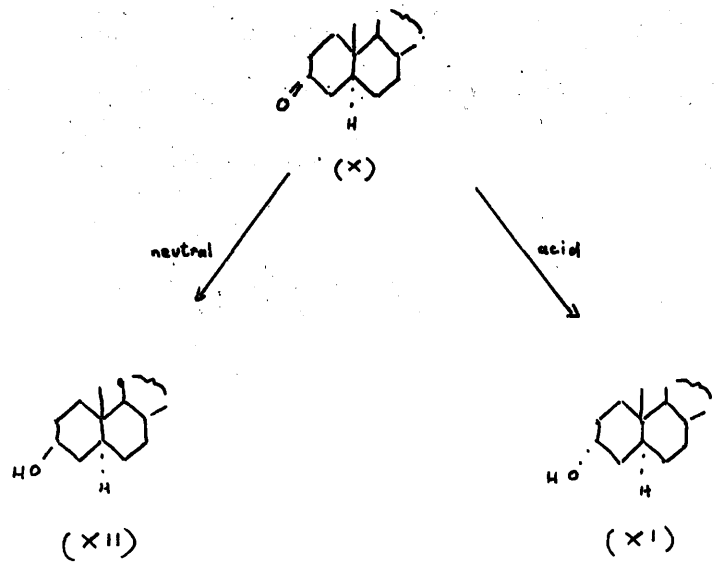
In the same way, cholestanone (X) gives in an acid medium, epi-cholestanol (XI), and in neutral solution, cholestanol (XII).



(VI)



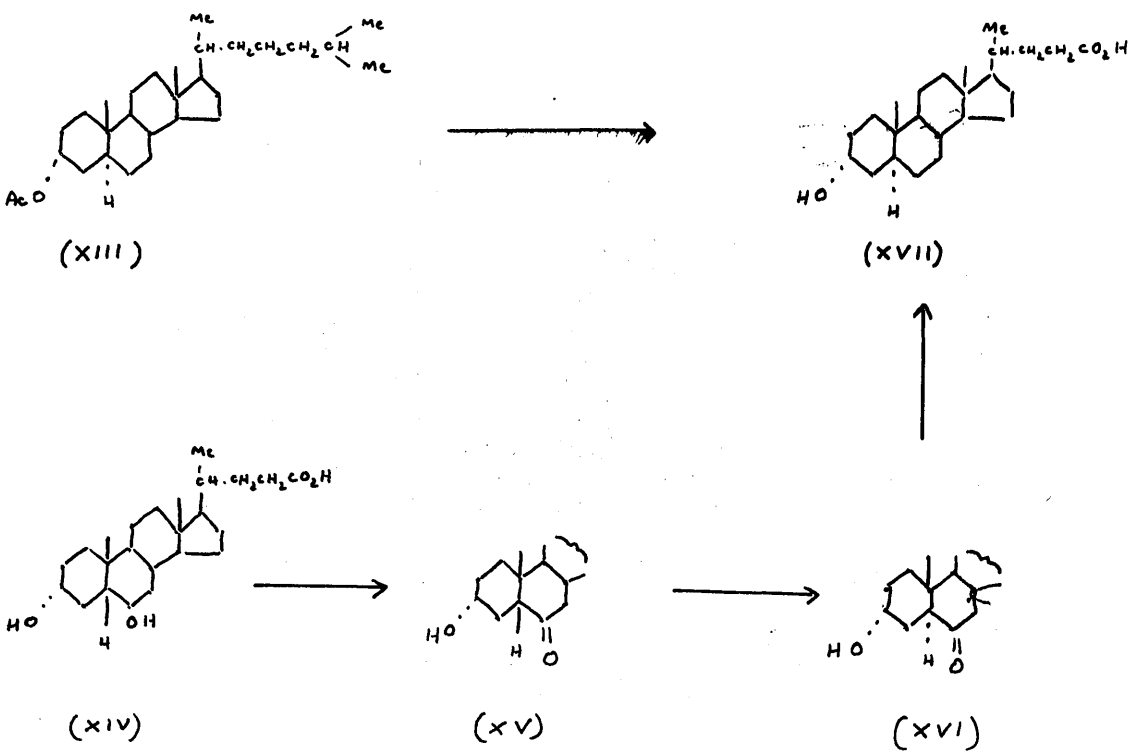
(IX) (VII) (VIII)



By application of the Auwers-Skita hydrogenation rule[12)13] that neutral media favour the formation of trans-modifications, and that acid media lead to cis-structures, it was inferred that the configurations of the C_3 - OH with respect to the C_5 hydrogen atom in each of the hydrogenation products, are as shown. Ruzicka recognised that application of the rule can be made with reference to the configuration of the bond extending from C_5 into ring B, instead of to that of the C_5 hydrogen atom, in which case the configurations of the C_3 - OH groups, as shown, would be reversed. His choice of the latter was later shown to be justified through Lettré's[14)] studies on the behaviour towards lactonisation, of certain acids derived from the rupture of ring B.

Dalmer and co-workers[15)] isolated from the products of chromic acid oxidation of epicholestanol acetate (XIII),

an acid, identical with 3-hydroxyallocholanic acid (XVII), which had been previously obtained by Wieland and Dane[16] from hyodeoxycholic acid (XIV), by means of the series of reactions (XIV), to (XVII).

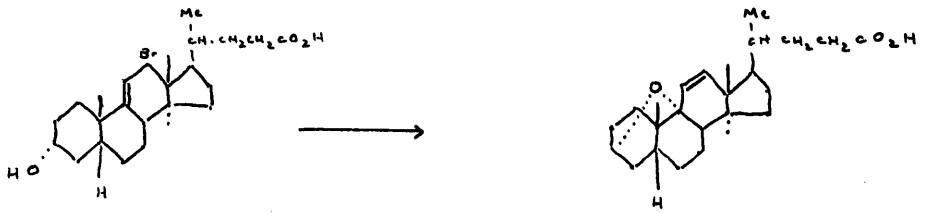


(XIII) (XVII)

(XIV) (XV) (XVI)

Thus the configuration of the C_3 - OH group in hyodeoxycholic acid was shown to be the same as in epicholestanol (XI). Recently, results which furnish good confirmation of the previous assumptions in regard to the configuration of bile acids have been obtained by Kendall and co-workers[17]. Treatment of methyl 3(α)-hydroxy-12-bromo-chol-9(11)-enate

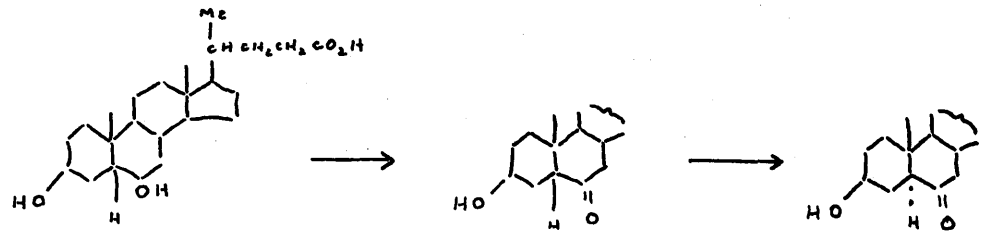
(XVIII) (which is derived from deoxycholic acid), with pyridine or sodium bicarbonate solution, gives an epoxy derivative (XIX). Evidence was adduced that the compound is a 3:9-epoxy derivative.



(XVIII) (XIX)

It is possible to construct Stuart models[18] of (XIX), only if rings A/B are cis-fused, and if the configuration of the original C_3 - OH in (XVIII) is (α).

During the course of a close examination of pig bile, Kimura[19] isolated from it, as well as hyodeoxycholic acid, smaller quantities of a new bile acid which is isomeric with the latter, and yields "α"-dehydrohyodeoxycholic acid on chromic acid oxidation. The new acid, named "β"-hyodeoxycholic acid, must therefore differ from the "α"-acid only in respect to the configurations of one or both hydroxyl groups. Applying a reaction sequence, formulae (XX) to (XXII), analogous to that employed by Wieland and co-workers[20],[16] in the case of the "α"-isomer (p. 44), Kimura proved that the new acid has its C_3 - OH in the (β)-configuration.



(xx) (xxi) (xxii)

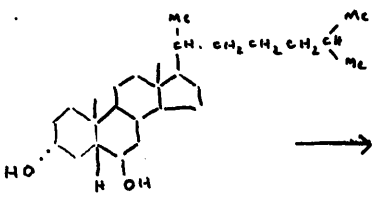
He put forward the view that the two natural acids are C_3 epimers, but it is obvious that in the absence of further evidence, the possibility of the acids being epimeric about C_6 also, cannot be excluded. Kimura followed the example of Sugiyama,[22] who assigned the (β)-configuration to the C_6 - OH in "α"-hyodeoxycholic acid, without advancing any adequate proof, and formulated "α"- and "β"-hyodeoxycholic acids as 3(α):6(β)- and 3(β):6(β)-dihydroxycholanic acids respectively. Other workers,[22),23] also have employed these configurations, although it would be more accurate to formulate the acids as 3(α):6- and 3(β):6-dihydroxycholanic acids until more definite evidence may be obtained.

The experiments which are described in the sequel were initiated in order to examine this problem of the configurations of the C_6 - OH groups in both natural bile acids. A subsidiary project which was envisaged at the outset, was the conversion of cholesterol into "α"- or "β"-hyodeoxycholic acid. Although the former bile acid

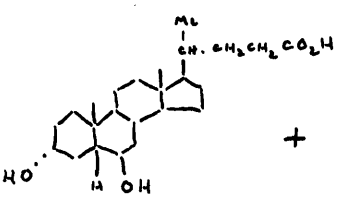
has been stepwise degraded to each of the sex hormones, androsterone,[15] and progesterone[24], its partial synthesis from a sterol has not yet been achieved. It was considered that these objects could be accomplished by the following means. Several examples of oxidative degradation of sterol derivatives to the corresponding bile acid derivatives are extant in the literature. For example, the isolation of allo^{5}cholanic and cholanic acids from the products of oxidation of cholestane and pseudocholestane (coprostane) respectively, afforded early evidence of the similarity of the nuclei of sterols and bile acids[25], and the degradation of epicholestanol (XI) to 3-hydroxyallocholanic (XVII) acid has been already mentioned. Chromic acid oxidation of cholesterol acetate dibromide gives small quantities of both 3(β)-hydroxyallo-chol-5-enic acid and 3(β)-hydroxyandrost-5-en-17-one[26]. Windaus and Hossfeld[27] found that oxidation of 3(β):6(α)-diacetoxycholestane (for discussion of configuration see p.52) leads to opening of ring A, but Marker and co-workers[28] were able to isolate low yields of both 3(β):6(β)-dihydroxyallo-cholanic acid and 6(β)-hydroxyisoandrosterone from the products of chromic acid oxidation of 3(β):6(β)-diacetoxy-cholestane (for discussion of configuration, see p. 52).

In view of these experiments, it was considered that if means for converting cholesterol into 3(α):6(β)- and 3(β):6(β)-dihydroxycoprostane, (XXIII) and (XXIV), could

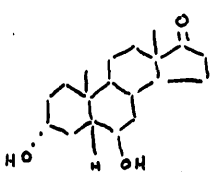
be determined, then oxidation of these sterol derivatives (after protection of the hydroxyl groups), might give the corresponding cholanic acid derivatives (XXV) and (XXVI), which should be accompanied by the C_{17}-ketone derivatives, (XXVII) and (XXVIII).



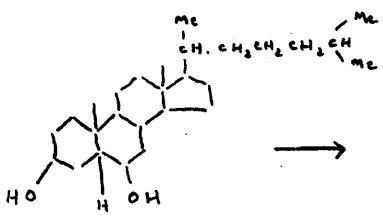
(XXIII)



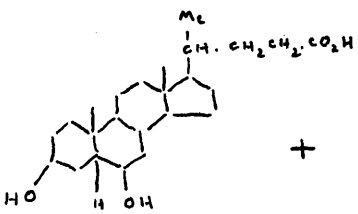
(XXV)



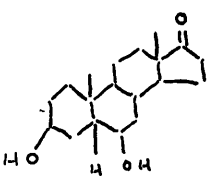
(XXVII)



(XXIV)



(XXVI)



(XXVIII)

The identities or otherwise of (XXV) and (XXVI) with "α"- and "β"-hyodeoxycholic acids respectively, would then indicate the configurations of the C_6 - OH groups in the natural bile acids.

In the first place, therefore, the problem consisted in the determination of means whereby cholesterol might be converted into the 3:6-dihydroxycoprostane derivatives

(XXIII) and (XXIV) in such ways that the configurations of the hydroxyl groups in the products might be known with certainty. It should be mentioned that Paige[29] had previously attempted to attain these ends, but his attention was diverted to the study of some interesting examples of acyl migrations, which were encountered in the course of his experiments in the sterol series.

Recently, Prelog and Tagmann[30] described the preparation of coprostan-3(β):6(β)-diol (XXIV), by means of catalytic hydrogenation of Δ^4-cholesten-3(β):6(β)-diol (XXXIX), in alcoholic suspension, and therefore a method of obtaining the starting material for the proposed degradation, (XXIV) to (XXVI) was available at the outset. It is evident that the saturated diol (XXIV), obtained by Prelog and Tagmann, corresponds to its unsaturated precursor (XXXIX), in respect to the configurations of its 3:6-hydroxyl groups, and since an exact knowledge of the configuration of the former is essential for the work now described, a brief summary of the available evidence as to the configuration of the unsaturated diol, is given below.

Hydrogen peroxide[31] oxidation of cholesterol (XXIX), gives a 3(β):5:6-triol ("triol I") (XXX), whereas oxidation with permanganate[32], or with osmium tetroxide and hydrogen peroxide[33] yields an isomeric 3(β):5:6-triol ("triol II")

(XXXI). It was shown by Criegee[34] that triol-I and triol-II are trans- and cis-α-glycols (C_5 - C_6) respectively*. Ellis and Petrow[36] studied the behaviour towards bromination of the acetoxy ketone (XXXIII), prepared from the diacetyl derivative of triol-I, by partial hydrolysis to give (XXXII), followed by oxidation. It was shown that (XXXIII) gives a 2-bromo derivative (XXXIV), which on treatment with alkali yields a trans-annular oxide (XXXV). The alternative mechanism whereby bromination occurs at C_4 to give ultimately a C_4 - C_5 oxide was excluded because of the properties of the oxide obtained. Oxidation of the 6-monoacetate (XXXII) of triol-I gives a lactonic acid (XXXVI), which on hydrolysis gives a dilactone (XXXVII). Thus bromination of the 3-ketone (XXXIII) gives a 2-bromo derivative, and oxidation leads to rupture at $C_2 \| C_3$, a behaviour which is known to be characteristic of C_3-keto steroids in which rings A and B are trans-fused[37]. On the assumption that this behaviour is maintained when C_5 carries a hydroxyl group instead of a hydrogen atom, Ellis and Petrow concluded that triol-I is a cholestane derivative, and therefore formulated it as cholestan-3(β):5:6(β)-triol (XXX).

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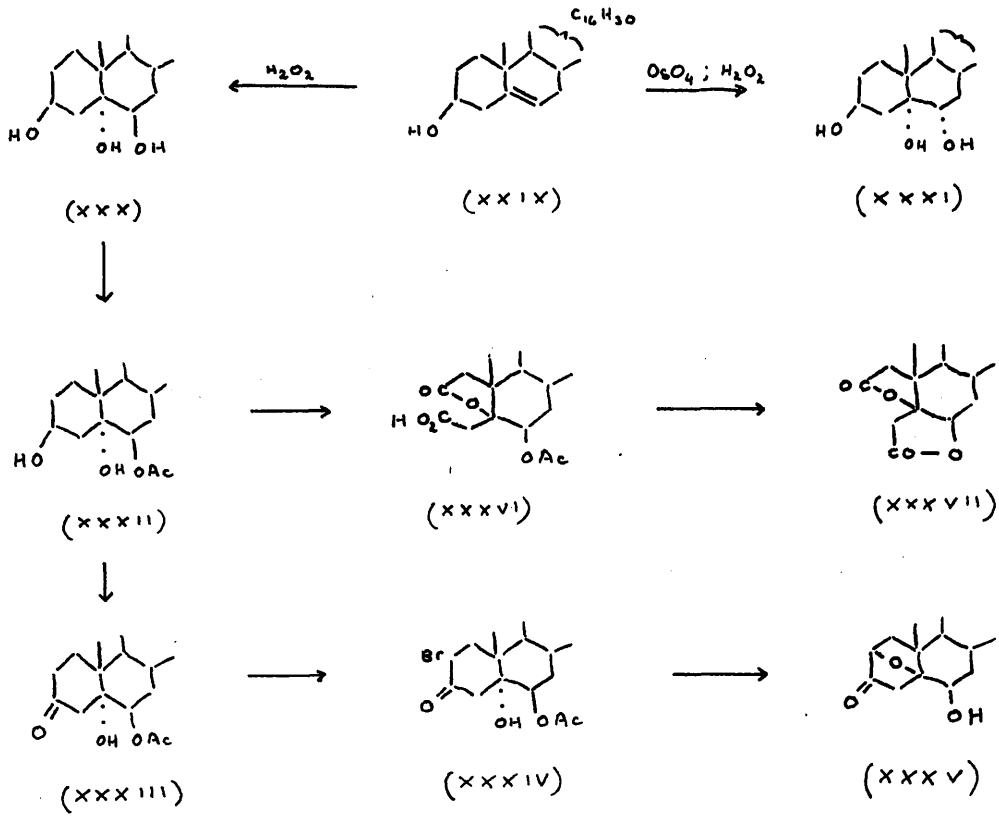
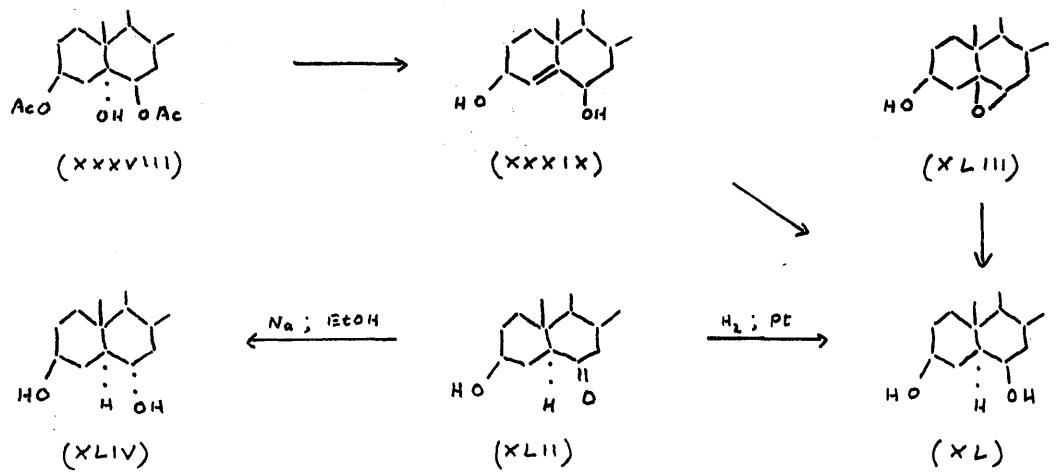
* It has been shown recently[35], that contrary to earlier statements[32),31)] both triols, on Oppenauer oxidation yield cholestan-5-ol-3:6-dione, and therefore differ only in respect to the configurations of the C_6 - OH groups.

Spring[38] pointed out that "the configuration adopted for triol-I appears to be independent of the assumption made by Ellis and Petrow. First, the immediate lactonisation of the (not isolated) C_2 // C_3 dicarboxylic acid to the lactonic acid (XXXVI) and of the (not isolated) hydroxy-lactone-acid to the dilactone (XXXVII) requires that rings A and B in triol-I be trans-fused."



Dehydration of the diacetate (XXXVIII) of triol I with thionyl chloride and pyridine[39] takes place, presumably with retention of configuration at C_3 and C_6, and gives the diacetate of Δ^4-cholesten-3(β):6(β)-diol (XXXIX). Still further evidence which confirms the configuration

assigned to the unsaturated diol is provided by results obtained by other workers. Catalytic hydrogenation of the unsaturated diol (XXXIX), in presence of acetic acid,[39] gives a cholestan-3(β):6-diol, m.p. 192° (XL), which is also obtained by catalytic hydrogenation of 6-ketocholestan-3(β)-ol (XLII),[24] and by hydrogenation of the acetate of cholesterol-β-oxide (XLIII)[40]. An epimeric cholestan-3(β):6-diol, m.p. 217° (XLIV), is obtained by sodium and alcohol reduction of 6-ketocholestan-3(β)-ol[41]. Plattner and Lang[42] studied the behaviour of the diacetates of each of the C_6 epimeric diols towards mild conditions of alkaline hydrolysis, and found that whereas the diacetate of the higher melting diol (XLIV), undergoes hydrolysis readily, both acetoxyl groups being hydrolised at similar rates, the diacetate of the lower melting diol (XL), possesses one acetoxyl group which is relatively difficult to hydrolyse. These authors pointed out that comparison of Stuart models[18] of the two diols (XL) and (XLIV) shows that the C_6 - OH group in (XL) should be sterically hindered (by the C_{10}-methyl group) to a greater extent than the C_6 - OH group in (XLIV), and therefore the higher melting diol is the 3(β):6(α)-diol and its epimeride is the 3(β):6(β)-diol.



(XXXVIII) (XXXIX) (XLIII)

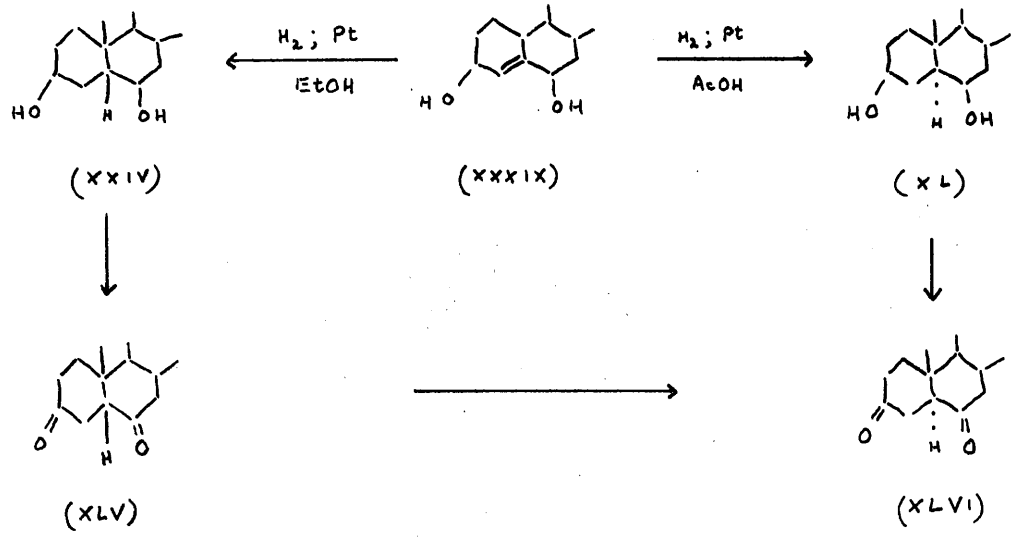
(XLIV) (XLII) (XL)

Although it has been pointed out[43),18)] that results obtained by representation of molecules by means of Stuart models should be interpreted with caution, their application by Plattner and Lang would appear to be justified since the diacetates of both epimeric diols, (XL) and (XLIV), were available for comparative experiments.

Thus evidence for assigning the configuration 3(β):-6(β)- to the hydroxyl groups in the unsaturated diol (XXXIX) derived from triol-I, rests on a firm basis.

It seemed to be remarkable that, as already mentioned, hydrogenation with a platinum catalyst of (XXXIX), in acetic acid, should give cholestan-3(β):6(β)-diol[39)], whereas hydrogenation with a similar catalyst, in ethanol, gives coprostan-3(β):6(β)-diol[30)]. Evidence for the coprostane configuration of the latter diol was presented by Prelog and Tagmann[30)] as follows. Their diol (XXIV), is different from the known cholestan-3(β):6(β)-diol[39),42)] (XL), and is oxidised by chromic acid to give the hitherto

unknown coprostan-3:6-dione (XLV). This dione is distinct from the known cholestan-3:6-dione (XLVI),[45] but is transformed into the latter on treatment with hot dilute acid. This behaviour is exactly analogous to that of "α"-dehydrohyodeoxycholic acid, which undergoes rearrangement to the C_5-allomer on treatment with acids or alkalis (p. 41).

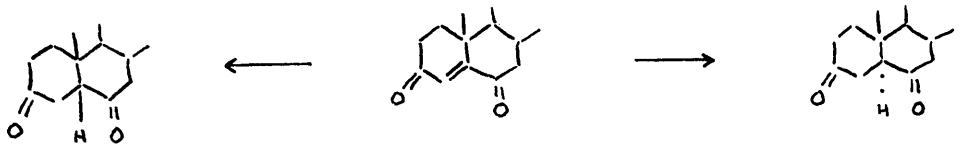


(XXIV) (XXXIX) (XL)

(XLV) (XLVI)

Bretschneider[44] showed that hydrogenation, with a palladium catalyst, in acetic acid, of Δ^4-cholesten-3:6-dione (XLVII), which is obtained by chromic acid oxidation of cholesterol[45),46], gives cholestan-3:6-dione (XLVI). It was considered, in the present work, that by analogy with the behaviour of (XXXIX) towards hydrogenation, discussed above, hydrogenation of the unsaturated dione in ethanol solution, over palladium, might be expected to yield

coprostan-3:6-dione. The production of the latter would afford confirmation of the coprostane configuration assigned to the diol (XXIV) of Prelog and Tagmann and further, if the dione (XLV) were obtained in good yield, it would provide a convenient starting material for further hydrogenation experiments using a platinum catalyst, with the object of converting it into one or more of the four theoretically possible, epimeric, coprostan-3:6-diols. In connection with the last mentioned project, it was realised that in the course of hydrogenation of (XLV), allomerisation at C_5 might take place, as was observed previously in the cases of catalytic hydrogenation (in acetic acid) of 6-keto-Δ^3-cholenic acid,[16] and "α"-dehydrohyodeoxycholic acid[8].



(XLV) (XLVII) (XLVI)

In fact, hydrogenation of the unsaturated dione (XLVII), in ethanol, over palladium black, gave a mixture from which both (XLVI) and (XLV) were isolated in yields of about 40% and 20% respectively. The latter product was identical with the dione obtained by chromic acid oxidation of coprostan-3(β):6(β)-diol[30], and thus the coprostane configuration of the diol is confirmed.

Chromic acid oxidation of the diacetate of the latter diol (XXIV), under vigorous conditions, gave a mixture of products. From the acidic constituents of the mixture there was isolated a low yield (3%) of a crystalline acid, m.p. 250°, which had the composition of a dihydroxycholanic acid, and therefore was considered to be 3(β):6(β)-dihydroxycholanic acid (XXVI). From the neutral constituents, there was isolated a crystalline semicarbazone, m.p. 218-220°, which had the composition of the semicarbazone of a diacetoxyaetiocholanone, and therefore was considered to be 3(β):6(β)-diacetoxyaetiocholan-17-one semicarbazone. Hydrolysis of the latter yielded 3(β):6(β)-dihydroxyaetiocholan-17-one, (XXVIII), m.p. 209-210°.

The nature of the dihydroxy acid, m.p. 250°, was further investigated as follows. On chromic acid oxidation it yielded a diketo acid, m.p. 161-162°, which crystallised with one molecule of water of crystallisation, from aqueous ethanol. Treatment of the hydrated, diketo acid with hot dilute hydrochloric acid gave an isomeric (anhydrous) diketo acid, m.p. 205°. Oxidation of the dihydroxy acid with a limited amount of chromic acid yielded an hydroxy keto acid, m.p. 189°, which on treatment with hot dilute sodium hydroxide solution gave an acid, m.p. 236-237°. The melting points of the new dihydroxy acid, and of its oxidation

products are compared with those previously reported in the literature for the two naturally occurring 3:6-dihydroxy cholanic acids, "α"- and "β"-hyodeoxycholic acids and their corresponding oxidation products[6),8),16),20),19)], in the table below.

	"α"-hyo acid 3(α):6-	"β"-hyo acid 3(β):6-	New acid 3(β):6(β)-
Free dihydroxy acid	197°	190°	250°
"α"-dehydro acid	162°	166°	162°
"β"-dehydro acid	209°	209°	205°
3-hydroxy-6-keto acid	173°	154°	189°
3-hydroxy-6-keto allo acid	194°	238°	237°

(Melting ranges are not shown).

Thus it is evident that the new dihydroxy acid, m.p. 250°, gives "α"-dehydrohyodeoxycholic acid on oxidation, and therefore retains the cholane (coprostane) configuration at C_5. The identity of the diketo acid, m.p. 161-162°, was subsequently confirmed by means of a mixed m.p. determination carried out with a sample of "α"-dehydro acid

derived from a sample of "α"-hyodeoxycholic acid which had been isolated from pig bile.

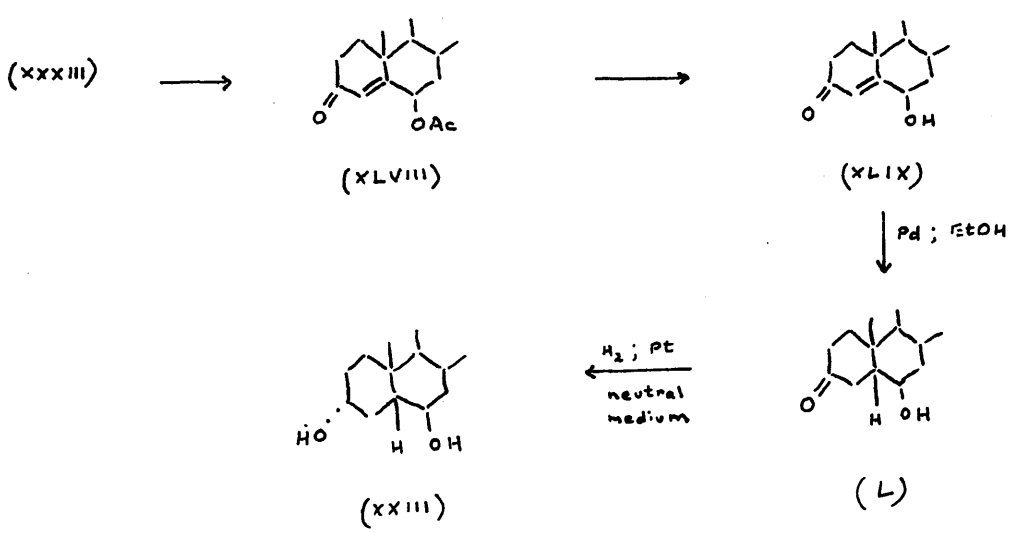
The slight discrepancy in melting points between the "α"-dehydro acids obtained by Windaus and Kimura from "α"- and "β"-hyodeoxycholic acids respectively, is probably explained as follows. Windaus[6] obtained the acid in the form of a monohydrate, m.p. 161.5-162°, whereas Kimura[19] apparently obtained an anhydrous form, m.p. 166°, as is indicated by the combustion results reported in his paper. In the present work, the acid was obtained in the form of a monohydrate, m.p. 161-162°, but in a second preparation, the acid after being dried at 140°, had m.p. 166-167°, although its combustion results still indicated the presence of a certain amount of water of crystallisation. The more serious discrepancy in melting points of the hydroxy-keto acids obtained on partial oxidation of "β"-hyodeoxycholic acid[19] and the new acid, was ascribed to either error, or the existence of polymorphic forms.

Since the properties of 3(β):6(β)-dihydroxycholanic acid (m.p. 250°) do not correspond to those of "β"-hyodeoxycholic acid (m.p. 189-190°)[19], it follows that the latter must be 3(β):6(α)-dihydroxycholanic acid.

The preparation of 3(α):6(β)-dihydroxycholanic acid (XXV), and its comparison with "α"-hyodeoxycholic acid,

would serve to settle the question of the configuration of the C_6 - OH group in the latter. Therefore, the possibility of converting cholesterol into coprostan-3(α):6(β)-diol (XXIII), for the purpose of carrying out oxidative degradation of the latter to the corresponding cholanic acid derivative, was next examined.

The following considerations appeared to indicate a feasible route to the desired diol (XXIII). Ellis and Petrow[36] showed that partial hydrolysis of the diacetate of triol-I gives in good yield 3(β):5-dihydroxy-6-acetoxy cholestane (XXXII; p. 51) which on oxidation gives 6(β)-acetoxycholestan-5-ol-3-one (XXXIII; p. 51). Dehydration of the hydroxy acetoxy ketone (XXXIII) with thionyl chloride in pyridine, or acetic anhydride, gives 6(β)-acetoxy-Δ^4-cholesten-3-one (XLVIII) which, on saponification, gives the free unsaturated hydroxy ketone (XLIX).

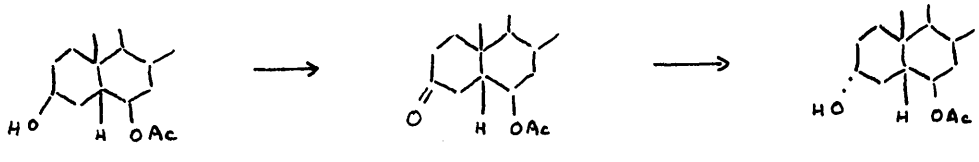


Since (XLIX) is ultimately derived from triol-I, its C_6 - OH must possess the (β)-configuration. Moreover, since (XLIX) is structurally very similar to coprostenone, which on hydrogenation over palladium yields coprostanone[10),11)] (p. 42), it was considered that (XLIX) might behave similarly and thus give coprostan-6(β)-ol-3-one (L). By analogy with the previously discussed (p. 42) case of coprostanone, which on hydrogenation (platinum catalyst) in neutral media yields mainly epicoprosterol[10)], the hydroxy ketone (L) might be expected to give similarly, coprostan-3(α):6(β)-diol (XXIII). It should be mentioned that a somewhat similar process of reasoning led Paige[29)] to study the behaviour of 6(β)-acetoxy- Δ^4 -cholesten-3-one (XLVIII) towards hydrogenation over a palladium catalyst. It was found by that author, however, that "although hydrogen was rapidly absorbed, a crystalline product could not be isolated." In the present work, this result was considered to be not inconsistent with observations previously made by Petrow, Rosenheim and Starling[47),39)] in connection with the hydrogenation of Δ^4-cholesten-3(β):6(β)-diol. It was shown that whereas hydrogenation of the unsaturated diol (in acetic acid) leads smoothly to cholestan-3(β):6(β)-diol (p. 53), hydrogenation of the diol diacetate yields a mixture of mainly cholestane, cholestan-3-ol and cholestan-3(β):6(β)-diol.

Accordingly, the behaviour of the free unsaturated hydroxy ketone (XLIX) towards hydrogenation in ethanol, over palladium, was then investigated. Absorption of hydrogen occurred rapidly and ceased after about one molecule had been taken up. The resulting gum could not be rendered crystalline, but on treatment with semicarbazide it gave, in good yield, an homogeneous crystalline product which had the composition of coprostan-6(β)-ol-3-one semicarbazone. The product obtained by hydrolysis of the semicarbazone was also a gum, which would appear to consist essentially of coprostan-6(β)-ol-3-one (L), since on chromic acid oxidation it gave, in good yield, coprostan-3:6-dione, which was identical with the dione obtained by oxidation of coprostan-3(β):6(β)-diol.

Concurrently with these preliminary experiments, a possibly more convenient route to coprostan-6(β)-ol-3-one (L) was investigated. There are in the literature, several examples which show that in certain cases, preferential hydrolysis of C_3-acetoxy groups in polyacetoxy steroids can be effected by the use of sufficiently mild experimental conditions, presumably because in these cases, the C_3-grouping is subject to less "steric hindrance" than corresponding groupings in, e.g., the C_5-, C_6-, or C_{12}- positions in the steroid molecule. The partial hydrolyses of 3(β):6(β)-diacetoxycholestane which gives 6(β)-acetoxy-cholestan-3(β)-

ol, and of the diacetate of triol-I which gives 3(β):5-dihydroxy-6-acetoxy cholestane, have been already discussed (pp. 52, 50). It was considered that it might prove possible to effect partial hydrolysis of the diacetate of coprostan-3(β):6(β)-diol. The resulting 3(β)-hydroxy-6(β)-acetoxycoprostane (LI) should then be capable of undergoing oxidation to give the acetoxy ketone (LII), which on catalytic hydrogenation (after saponification, if necessary), over a platinum catalyst, in neutral solution, would be expected to give 3(α)-hydroxy-6(β)-acetoxy-coprostane (LIII).



(LI) (LII) (LIII)

Experimental conditions for the conversion of the diacetate of coprostan-3(β):6(β)-diol[30] into a crystalline hydroxyacetoxy coprostane, which was presumed to be (LI), were found, but further work along the lines indicated above was not carried out owing to the circumstances, first, that at this stage, the author came into receipt of a Studentship, awarded by the Medical Research Council, which required that his attention be diverted to studies in another field, and second, that shortly before these experiments were

completed, the author learned through private communication with Dr. R.B. Moffett that the latter had obtained evidence which, when considered together with evidence accruing from the present work, shows that "α"-hyodeoxycholic acid is, in fact, 3(α):6(α)-dihydroxycholanic acid.

This evidence, and other related topics, are discussed in the "Addendum".

Addendum.

(i) In the first place, through the kindness of Dr. Moffett, it became possible for the author to gain access to information contained in certain war-time, Japanese publications of which the author had not been hitherto aware, owing to the fact that they are not available in this country, and were not noticed by the Abstracting Journals. Some results of work therein described are relevant to the present discussion, and are briefly summaried as follows. Tukamoto[48] succeeded in isolating the four, theoretically possible 3:6-dihydroxycholanic acids from the products of aluminium isopropoxide reduction of "α"-dehydrohyodeoxycholic acid, which was prepared from "α"-hyodeoxycholic acid. Two of these acids, m.p. 197° and 190°, were found to be identical with "α"- and "β"-hyodeoxycholic acids respectively, as might be expected. Of the other two, one m.p. 258°, was found to give on oxidation with a limited amount of chromic acid, an hydroxyketocholanic acid, m.p. 189°, which was found tobe identical with the product similarly obtained from "β"-hyodeoxycholic acid, and therefore the dihydroxy acid, m.p. 258°, differs from the latter only in respect to the configuration of its C_6 - OH group. By the same method of partial oxidation, the remaining dihydroxycholanic acid, m.p. 208°, was shown to be related to "α"-hyodeoxycholic acid in the same way. Tukamoto arbitrarily assigned the configuration 3(α):6(α)- to the hydroxyl groups in "α"-hyodeoxycholic acid ("Diese Säure ist konstitutionell höchst wahrscheinlich 3(α):6(α)-Dioxycholansäure") and accordingly, the configuration 3(α):6(β)- to those in the acid, m.p. 208°. His evidence for assigning the 3(β):6(β)- configuration to the dihydroxycholanic acid, m.p. 258°, is apparently based on the following. In a later paper[49] he showed that aluminium isopropoxide reduction of 3(α):6-ketocholanic acid gives a mixture of "α"-hyodeoxycholic acid and its C_6-epimer (m.p. 208°), the former being isolated in higher yield. Similar reduction of 3(β):6-ketocholanic acid gives a mixture of "β"-hyodeoxycholic acid and the acid, m.p. 258°, the latter being isolated in higher yield. On the assumption that "α"-hyodeoxycholic acid is the 3(α):6(α)- isomer, and therefore, that in aluminium isopropoxide reduction of a 3-hydroxy-6-ketocholanic acid, the main course of reaction leads to the formation of that isomer which bears the same configuration at C_6 as at C_3, he assigned the configuration 3(β):6(β)- to the acid, m.p. 258°. It is clear that Tukamoto's evidence does not furnish adequate proof of the

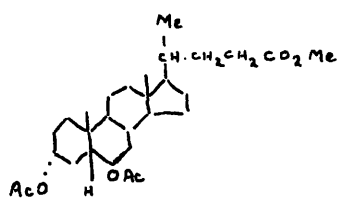
configurations of the hydroxyl groups at C_6 in the four dihydroxycholanic acids; however, it is satisfactory that his acid, m.p. 258°, coincides in its properties with 3(β):6(β)-dihydroxy cholanic acid, m.p. 250°, which is described in the present work. For example, both acids have melting points in fair agreement, give sparingly soluble sodium salts and evidently give the same hydroxy keto acid, (m.p. 188° and 189°) on partial oxidation (c.f., Kimura[19]). Together with these reduction experiments of Tukamoto, the degradation which is described in the present work, of cholesterol to "α"-dehydrohyodeoxycholic acid constitute partial syntheses of both natural hyodeoxycholic acids and their C_6 epimers, from the sterol.

(ii) After the experiments described in this dissertation were completed, Moffett[50] adduced the following evidence. Partial hydrolysis of the diacetate of methyl "α"-hyodeoxycholate, followed by successive oxidation with chromic acid and saponification, yields 6-hydroxy-3-ketocholanic acid, in which the configuration of the C_6 - OH group presumably persists. Catalytic hydrogenation of the hydroxy keto acid, over platinum, in an acidic medium, yields 3(β):6-dihydroxycholanic acid, m.p. 191-192°, the methyl ester of which is precipitable by digitonin. The acid is evidently identical with "β"-hyodeoxycholic acid, and therefore the C_6 - OH groups in both natural "α" and "β" acids must possess the same configuration, which is shown to be (α) as a consequence of the present work.

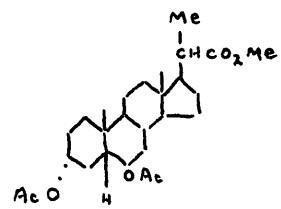
(iii) In a recent review article, which was published shortly before the results of Moffett became available, Shoppee[2], in dealing with "α"-hyodeoxycholic acid, puts forward the view that "the configuration at C_6 appears to be (β) because various workers have been able to achieve partial hydrolysis of 3:6-diacetoxy- to 6-monoacetoxy-compounds[24)51)22)23]." This view is based on the interpretation by Plattner and Lang[42] of the behaviour of each diacetate of the two epimeric cholestan-3(β):6-diols towards mild hydrolytic conditions, on the basis of Stuart models (see p. 52).

However, it is considered by the present author that Shoppee's suggestion in regard to "α"-hyodeoxycholic acid is not justified by the available evidence. Previous workers[51)22)] encountered great difficulty in effecting partial hydrolysis of methyl diacetoxy "α"-hyodeoxycholate (LIV), the resulting hydroxy acetoxy compound being formed in such low yield that it could not be isolated as such from the hydrolysis products. Further, it was found not

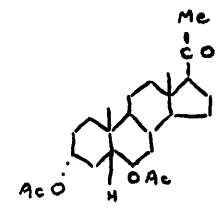
possible[23] (c.f., [24]) to effect partial hydrolysis of either of the diacetates of bis-nor-hyodeoxycholic acid methyl ester (LV) and 3:6-dihydroxypregnan-20-one (LVI), which are derived from (LIV) by means of processes of degradation of the side chain.*



(LIV)



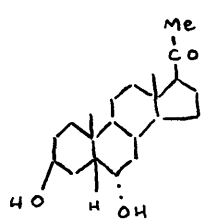
(LV)



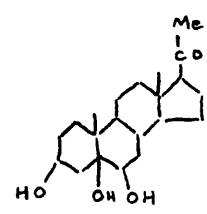
(LVI)

The difficulty encountered in effecting partial hydrolysis of (LIV) is in contrast to the relative ease with which 3(β):6(β)-diacetoxycoprostane is converted into a monoacetate (see Experimental, p. 79), and it is possible that the diacetate of the C_6 epimer of "α"-hyodeoxycholic acid may similarly undergo partial hydrolysis with ease. In view of Shoppee's suggestion, it would be of interest to prepare from "α"-hyodeoxycholic acid this compound, which has been previously described[22] and examine its behaviour towards mild hydrolytic conditions.

It is worthy of note, that Ehrenstein and Stevens[53] found that of the diacetates of the two C_6 epimeric 20-keto-pregnan-3(β):5:6-triols (LVII) and (LVIII), only that of the trans-triol (LVII), in which the C_6 - OH group presumably has the (α)-configuration, could be transformed into the 6-monoacetate by means of hydrolysis with very dilute alkali.



(LVII)



(LVIII)

* From these negative results, it might be inferred that the side chain in (LIV) exerts some slight "steric hindering" effect on the C_6-acetoxy group. However, examination of Stuart models of (LIV) shows that the carbomethoxy group of the side chain can be brought into close proximity to the C_6-acetoxy group only by profound distortion of the model. The model was constructed in such a way that the bile acid side chain at C_{17} was in the (β) configuration, which is in accordance with modern views[52].

However, it was realised by these authors[54] that conclusive evidence as to the pregnane rather than the allopregnane configuration of the triols was not obtained. If the latter configuration obtains, then the configuration at C_6 in each triol would be the reverse of that shown above, and then the results of these partial hydrolysis experiments would be in line with other, more recent findings[42].

Experimental.

Melting points greater than 220° are uncorrected.

Adams PtO_2 catalyst was prepared by the method of Bruce[55], and Pd black by the method of Heilbron, Sexton and Spring[56].

Δ^4-Cholesten-3:6-dione.

This was prepared by the methods of Windaus[45] and Ross[46]. Owing to the troublesome emulsions formed in the latter procedure, the former was found to be preferable for the preparation of small quantities.

Hydrogenation of Δ^4-cholesten-3:6-dione.

A solution of the dione (1.0 g.) in warm, absolute ethanol (25 c.c.) was cooled quickly to room temperature, and the resulting suspension of small crystals shaken with palladium black (0.1 g.) in an atmosphere of hydrogen. Uptake reached a maximum (ca 1.3 mols.) after 1 hour, and shaking was continued for a further hour. The mixture was warmed and filtered from catalyst, and the crystalline material which separated on cooling, combined with a further amount obtained by evaporation of the mother-liquors. Fractional crystallisation of the material from ethanol yielded, as less soluble product, needles (0.4 g.), m.p. 168-170°, $[\alpha]_D^{19°}$ +10°. (c = 0.902 in chloroform). The latter product was identified as cholestan-3:6-dione, by

comparison with an authentic sample, prepared by reducing the unsaturated dione with zinc dust in acetic acid[57]. The more soluble product formed long needles (from ethanol), (0.2 g.), m.p. 175-179°, $[\alpha]_D^{19°}$ -82° (c = 0.268 in chloroform) of coprostan-3:6-dione. A sample of the latter dione prepared according to Prelog and Tagmann[30] had m.p. 175-179°, $[\alpha]_D^{19°}$ -79° (c = 0.33 in chloroform). (cf. these authors who give m.p. 170-174°, $[\alpha]_D^{18°}$ -57° ±8° (c = 0.262 in chloroform)), and showed no depression in m.p. on admixture with the more soluble product. It showed a marked depression in m.p. on admixture with cholestan-3:6-dione.

Δ^4-Cholesten-3(β):6(β)-diol.

This was prepared by means of the series of reactions:- cholesterol ⟶ cholesteryl acetate[58] ⟶ cholestan-3(β):5:6(β)-triol diacetate[31] ⟶ Δ^4-cholesten-3(β):6(β)-diol diacetate ⟶ free unsaturated diol[39]. An alternative method, whereby the unsaturated diol was reported to be obtained in 25% yield on selenium dioxide oxidation of cholesterol in acetic anhydride[59], was not employed, since it was considered that complete removal of selenium from the product (essential for subsequent catalytic hydrogenation) might have proved difficult.

Coprostan-3(β):6(β)-diol.

The procedure of Prelog and Tagmann[30] was slightly modified, in order to provide a more convenient means of manipulation of larger quantities.

A suspension of finely powdered Δ^4-cholesten-3:6-diol (10 g.) in absolute ethanol (500 c.c.), was shaken with platinic oxide catalyst (0.5 g.) in an atmosphere of hydrogen. Uptake reached a maximum after about 3 hours, and shaking was continued for 2 hours longer. The catalyst was removed by filtration and the filtrate evaporated under reduced pressure on the water-bath. Crystallisation of the residue from ethyl acetate gave needles, m.p. 194-195° (8.75 g.). The diacetate, prepared essentially according to the procedure of Prelog and Tagmann, formed large elongated plates (from methanol) (yield 99%), m.p. 140-141°.

Oxidation of coprostan-3(β):6(β)-diol diacetate.

A preliminary experiment carried out with 4 g. of material provided information which led to the adoption of the following procedure.

A mechanically stirred solution of the diol acetate (22.9 g.) in glacial acetic acid (900 c.c.), was heated on the water-bath and treated dropwise during 6 hours with a solution of chromium trioxide (50 g.) in glacial acetic acid (150 c.c.), and water (28 c.c.). Heating on the

water-bath was continued for 4 hours longer. After being kept overnight at room temperature, the mixture was treated with ethanol (25 c.c.) and then evaporated under reduced pressure on the water-bath, to small bulk. The residue was treated with 2N-sulphuric acid (750 c.c.) and extracted with ether. The ethereal extract was washed thrice with water, and then extracted with 2N-sodium hydroxide solution (110 c.c.). The alkaline extract was saponified by heating on the water-bath for 2 hours. The sparingly soluble sodium salt thus obtained was separated by centrifuging the mixture, washed with a little water, partly dissolved in hot water, and the free acid precipitated by the addition of concentrated hydrochloric acid. The crude material was filtered off and purified by extraction with boiling ethyl acetate (Soxhlet), followed by crystallisation from dilute ethanol, giving colourless plates (870 mg.), m.p. 250°, of 3(β):6(β)-dihydroxycholanic acid. (Found: C, 73.4; H, 10.1. $C_{24}H_{40}O_4$ requires C, 73.5; H, 10.3%).

The ethereal solution, from which acidic substances had been removed, was washed with water and evaporated. The residual oil was dissolved in warm methanol (45 c.c.), the solution allowed to cool, and seeded with a crystal of starting material. The unchanged diol diacetate (1.6 g.)

which crystallised was filtered off and the filtrate evaporated. The residue was freed from volatile, pleasant-smelling substances by distilling in steam. The residue was extracted with ether, the ethereal solution washed with water and evaporated. The residual gum was dissolved in warm ethanol (30 c.c.), the solution treated with semicarbazide hydrochloride (0.85 g.) and sodium acetate (1.02 g.), and the mixture boiled under reflux on the water-bath for 2 hours. After cooling, ether and water were added. The ethereal layer was separated and washed repeatedly with small portions of water, until solid material started to separate. When separation was complete, the material was filtered off and washed with a little ether. Crystallisation from methanol gave microscopic, white needles (170 mg.), m.p. 218-220° (decomp.), of 3(β):6(β)-diacetoxyaetiocholan-17-one semicarbazone.

(Found: C, 64.3; H, 8.0; N, 9.3. $C_{24}H_{37}O_5N_3$ requires C, 64.4; H, 8.3; N, 9.4%). This semicarbazone (149 mg.) was dissolved in warm ethanol (7.5 c.c.), the solution treated with a mixture of concentrated sulphuric acid (0.75 c.c.) and water (1.5 c.c.), and boiled under reflux for 30 minutes. After cooling, the mixture was treated with water and ether, and the ethereal layer washed with water and then evaporated. The residue was boiled under reflux with 2% methanolic potassium hydroxide (4.5 c.c.) for 1 hour. The solution

was treated with water and extracted with ether. The ethereal layer was washed with water and then treated with light petroleum (b.p. 40-60°). Recrystallisation of the precipitate from ether-light petroleum (b.p. 40-60°) gave colourless rhombs, m.p. 209-210°, of 3(β):6(β)-dihydroxy-aetiocholan-17-one. (Found: C, 74.8; H, 9.5. $C_{19}H_{30}O_3$ requires C, 74.5; H, 9.8%).

Investigation of the dihydroxycholanic acid, m.p. 250°.

Attempted acetylation: A solution of the acid (100 mg.) in anhydrous pyridine (1.5 c.c.) was treated with freshly redistilled acetic anhydride (1c.c.). The solution was kept at room temperature for 48 hours, poured into water and extracted with ether. The ethereal extract was washed with dilute hydrochloric acid, water, and then evaporated. The residue was a yellow gum which could not be rendered crystalline either by solvent treatment or by filtration of its solution in benzene through a column of activated alumina, followed by elution of the column with chloroform-methanol.

Oxidation with excess of chromic acid.

A solution of the acid (200 mg.) in warm acetic acid (7 c.c.) was cooled to room temperature (solution supercooled) and treated gradually, with shaking, with a solution of 80% acetic acid (3 c.c.), which contained chromium trioxide

(128 mg.; 2.5 mols.). The solution was set aside for 3-5 hours and then treated with 0.1 N-sulphuric acid. The crystalline product was washed with water and then recrystallised from acetic acid. Further repeated recrystallisation from aqueous ethanol gave rectangular leaflets, m.p. 161-162°, of "α"-dehydrohyodeoxycholic acid monohydrate. (Found: C, 71.2; H, 9.1. Calc. for $C_{24}H_{36}O_4 \cdot H_2O$: C, 70.9; H, 9.4%). This sample had been dried at 90° for analysis. The diketo acid obtained in a second oxidation experiment was dried at 140°/12 mm., and then had m.p. 166-167°. (Found: C, 71.6, 71.6; H, 9.3, 9.2. Calc. for $C_{24}H_{36}O_4$: C, 74.2; H, 9.3%. Calc. for $C_{24}H_{36}O_4 \cdot \frac{3}{4}H_2O$: C, 71.7; H, 9.4%). The m.p. of the diketo acid (m.p. 161-162°) was not depressed on admixture with an authentic sample of "α"-dehydrohyodeoxycholic acid, prepared from natural "α"-hyodeoxycholic acid.

"β"-Dehydrohyodeoxycholic acid.

The above diketo acid (m.p. 161-162°; 90 mg.) was boiled under reflux with acetic acid (1.8 c.c.) containing one drop of concentrated hydrochloric acid for 1.5 hours. The hot solution was treated dropwise with water until it became turbid, and the material which crystallised on cooling was repeatedly recrystallised from ethanol, giving minute, colourless leaflets, m.p. 205°.

(Found: C, 73.9; H, 9.4. Calc. for $C_{24}H_{36}O_4$: C, 74.2;

H, 9.3%).

Oxidation with a limited amount of chromic acid.

A solution of the dihydroxy acid (200 mg.) in warm acetic acid (8 c.c.) was mechanically stirred and quickly cooled to 10-12°, whereupon a small proportion separated in the form of very small crystals. At that temperature, and with vigorous stirring, the suspension was treated dropwise during 45 minutes, with a solution of 80% acetic acid (5 c.c.) which contained chromium trioxide (35 mg. = 0.685 mols.). The resulting solution was kept at 0° for 1.5 hours and then at room temperature for 1.5 hours. It was treated with a few drops of ethanol, concentrated to small bulk under reduced pressure (bath temperature 25-30°) and the residue diluted with water. The gum which separated was extracted into ether, and the ethereal extract washed with water until the washings were neutral to litmus. Stout needles separated from the ethereal solution on standing and these were filtered off and combined with a further crop obtained by concentrating the mother-liquor. The material (98 mg.) was repeatedly recrystallised from ether-petroleum ether (b.p. 40-60°) and gave colourless leaflets, m.p. 189°, of 3(β)-hydroxy-6-ketocholanic acid. (Found: C, 74.2; H, 9.4. Calc. for $C_{24}H_{38}O_4$: C, 73.8; H, 9.7%). For the same compound, Kimura[19] gives m.p. 154°,

and Tukamoto[48] m.p. 188°.

3(β)-Hydroxy-6-ketoallocholanic acid.

The above hydroxy keto acid (39 mg.) dissolved in acetic acid (0.5 c.c.) containing a drop of concentrated hydrochloric acid, was heated at 100° for 2 hours in a sealed tube. On being cooled and treated with water, the reaction mixture gave a yellow gum containing some crystalline material. It was concluded that allomerisation had not proceeded to completion. The gum was isolated by means of ether, and heated on the water-bath with N-sodium hydroxide (4 c.c.) for 3 hours. The sparingly soluble sodium salt thus obtained was separated (centrifuge), dissolved in boiling water and acidified with dilute hydrochloric acid. The free acid was recrystallised from ethyl acetate and gave short needles, m.p. 236-237°. The same acid, obtained from different sources, has been previously described by Wieland et al.[20] and by Kimura[19], who give melting points, 235° and 237-238°, respectively. The m.p. of the acid was strongly depressed on admixture with 3(β):6(β)-dihydroxycholanic acid.

Hydrogenation of Δ^4-cholesten-6(β)-ol-3-one.

A solution of the unsaturated hydroxy ketone[36] (400 mg.) in absolute ethanol (20 c.c.) was shaken with palladium black (40 mg.) in an atmosphere of hydrogen.

Absorption of hydrogen ceased after 20 minutes when approximately 1.1 mols. had been taken up. After being shaken for 1 hour longer, the solution was filtered and the filtrate evaporated under reduced pressure. The residue was a brittle gum which could not be obtained crystalline by solvent treatment. It was dissolved in light petroleum (b.p. 60-80°; 20 c.c.) and the solution poured on to a column (1.5 x 21 cm.) of activated alumina. The column was successively washed with light petroleum (b.p. 60-80°) (20 c.c.), 2% - (30 c.c.), 5% - (30 c.c.) and 10% - (30 c.c.) methanol-light petroleum. Portions (10 c.c.) of the filtrate were collected and evaporated, but no residue was obtained until the column was finally washed with 10% methanol-benzene, whereupon a gum (330 mg.) was rapidly eluted, apparently without any separation having taken place.

Coprostan-6(β)-ol-3-one semicarbazone.

A solution of the above gum in ethanol (10 c.c.) was treated with semicarbazide hydrochloride (137 mg.) and sodium acetate (167 mg.) and then boiled under reflux on the water-bath for 2 hours. After being cooled, the mixture was filtered and the residue washed with water. Crystallisation from ethanol gave minute leaflets (240 mg.), m.p. 202-203° (decomp.). (Found: C, 72.7; H, 10.2; N, 9.3. $C_{29}H_{49}O_2N_3$ requires C, 73.1; H, 10.7; N, 9.2%).

The m.p. of the product was unchanged after repeated recrystallisation, and a search of the various mother-liquors did not reveal the presence of any appreciable quantity of an isomeric compound.

Hydrolysis of the semicarbazone: The compound (147 mg.) was dissolved in a mixture of ethanol (7.5 c.c.), concentrated sulphuric acid (0.75 c.c.) and water (1.5 c.c.), and the solution boiled under reflux on the water-bath for 30 minutes. It was poured into water and extracted with ether. The ethereal extract was washed with several portions of water and then evaporated under reduced pressure. The residue consisted of a yellow, brittle gum which could not be rendered crystalline by solvent treatment.

Oxidation of the hydrolysis product: The above gum (76 mg.) was dissolved in acetic acid (4 c.c.), and the solution treated dropwise, with shaking, with 90% acetic acid (3.71 c.c.) which contained chromium trioxide (23.6 mg.). After being kept at room temperature for 15 hours, the solution was treated with a few drops of methanol, poured into water, and extracted with ether. The ethereal extract, after being washed with dilute sodium carbonate solution, then with water, and evaporated, left a crystalline residue which, on recrystallisation from methanol, gave long needles (60 mg.), m.p. 175-179°, $[\alpha]_D^{19°}$ -76° (c = 0.332 in chloroform), of coprostan-3:6-dione, the m.p. of which was not

depressed on admixture with an authentic sample, prepared by oxidation of coprostan-3(β):6(β)-diol.

Partial hydrolysis of coprostan-3(β):6(β)-diol diacetate.

(i) A mechanically stirred solution, at room temperature, of the diol diacetate (1.55 g.) in absolute ethanol (100 c.c.) was treated dropwise during 1.5 hours, with 0.22 N-ethanolic potassium hydroxide (14.4 c.c.; 1 mol.). The mixture, from which a small quantity of crystalline material separated, was stirred for 1 hour longer, and then set aside for 70 hours. After being acidified with a few drops of glacial acetic acid, the mixture was filtered from a trace of unchanged starting material and concentrated under reduced pressure to small bulk. The solid which separated (1.2 g.) was filtered off and washed with water. Fractional crystallisation of the product from ethanol showed that it was a mixture consisting mainly of unchanged starting material, and no other constituent could be isolated in a pure condition.

(ii) A solution of the diol diacetate (488 mg.) in methanol (13.5 c.c.) was boiled under reflux on the water-bath and treated dropwise during 30 minutes with 0.198 N-methanolic potassium hydroxide solution (5.56 c.c.; 1.05 mols.). The mixture was boiled for 10 minutes longer and set aside for 12 hours. After being acidified with a few drops of

glacial acetic acid, the solution was evaporated on the water-bath to small bulk. The solid which separated was filtered off and triturated with water, giving a crystalline substance (300 mg.; m.p. 125-132°) which on repeated recrystallisation from methanol gave long, colourless leaflets, m.p. 143°, of 6(β)-acetoxycoprostan-3(β)-ol, (Found: C, 78.2; H, 11.2. $C_{29}H_{50}O_3$ requires C, 78.0; H, 11.2%). It showed a marked depression in m.p. (mixed m.p. 118-122°) on admixture with starting material.

Bibliography.

1). Cornforth and Robinson, Nature, 1947, 160, 737.

2). Shoppee, Ann.Repts.Chem.Soc., 1946, 43, 200.

3). Carlisle and Crowfoot, Proc.Roy.Soc., 1945, A. 184, 64.

4). Fieser, "The Chemistry of Natural Products Related to Phenanthrene", 2nd Edn., New York, 1937, p.398.

5). Gundelach and Strecker, Annalen, 1847, 62, 205.

6). Windaus and Bohne, Annalen, 1923, 433, 278.

7). Windaus, Z.angew.Chem., 1923, 36, 309.

8). Windaus, Annalen, 1926, 447, 233.

9). Ruzicka, Furter and Thomann, Helv.Chim.Acta, 1933, 16, 327

10). Ruzicka et al., Helv.Chim.Acta, 1934, 17, 1407.

11). Grasshof, Z.physiol.Chem., 1934, 223, 249; 225, 197.

12). von Auwers, Annalen, 1920, 420, 84.

13). Skita, Ber., 1920, 53, 1792.

14). Lettré, Ber., 1935, 68, 766.

15). Dalmer et al., Ber., 1935, 68, 1814.

16). Wieland and Dane, Z.physiol.Chem., 1932, 212, 41.

17). Kendall et al., J.Biol.Chem., 1946, 164, 569.

18). Maitland, Ann.Repts.Chem.Soc., 1939, 36, 236.

19). Kimura, Z.physiol.Chem., 1937, 248, 280.

20). Wieland, Dane and Martius, Z.physiol.Chem., 1933, 215, 18.

21). Sugiyama, J. of Biochem. (Japan), 1937, 25, 157.

22). Hoehn, Linsk and Moffett, J.Amer.Chem.Soc., 1946, 68,1855

23). Moffett, Stafford, Linsk and Hoehn, J.Amer.Chem.Soc., 1946, 68, 1857.

24). Marker and Krueger, J.Amer.Chem.Soc., 1940, 62, 79.

25). Windaus and Neukirchen, Ber., 1919, 52, 1915.

26). Wallis and Fernholz, J.Amer.Chem.Soc., 1935, 57, 1504.

27). Windaus and Hossfeld, Z.physiol.Chem., 1925, 145, 177.

28). Marker, Krueger, Adams and Jones, J.Amer.Chem.Soc., 1940, 62, 645.

29). Paige, J., 1943, 437.

30). Prelog and Tagmann, Helv.Chim.Acta, 1944, 27, 1880.

31). Pickard and Yates, J., 1908, 93, 1678.

32). Windaus, Ber., 1907, 40, 257.

33). Ushakow and Lieutenberg, Nature, 1937, 140, 466.
J.Gen.Chem.Russia, 1939, 9, 69.

34). Criegee, Ber., 1932, 65, 1770.

35). Prelog and Tagmann, Helv.Chim.Acta, 1944, 27, 1867.

36). Ellis and Petrow, J., 1939, 1078.

37). Butenandt and Wolff, Ber., 1935, 68, 2091.

38). Spring, Ann.Repts.Chem.Soc., 1940, 37, 358.

39). Petrow, Rosenheim and Starling, J., 1938, 677.

40). Plattner, Petrzilka and Lang, Helv.Chim.Acta, 1944, 27, 513.

41). Windaus, Ber., 1917, 50, 133.

42). Plattner and Lang, Helv.Chim.Acta, 1944, 27, 1872.

43). Marvel and Glass, J.Amer.Chem.Soc., 1938, 60, 1051.

44). Bretschneider, Ber., 1941, 74, 1361.

45). Windaus, Ber., 1906, 39, 2249.

46). Ross, J., 1946, 737.

47). Petrow, Rosenheim and Starling, J., 1937, 383.

48). Tukamoto, J. of Biochem.(Japan), 1941, 32, 451.

49). Tukamoto, J. of Biochem.(Japan), 1941, 32, 467.

50). Moffett, Private communication;
Moffett and Hoehn, J.Amer.Chem.Soc., 1947, 69, 1995.

51). Gallagher and Xenos, J.Biol.Chem., 1946, 165, 365.

52). Shoppee, Chem. and Ind., 1947, 109.

53). Ehrenstein and Stevens, J.Org.Chem., 1940, 5, 318.

54). Ehrenstein, J.Org.Chem., 1939, 4, 506.

55). Bruce, J.Amer.Chem.Soc., 1936, 58, 687.

56). Heilbron, Sexton and Spring, J., 1929, 929.

57). Windaus, Ber., 1903, 36, 3755.

58). Rayman, Bull.Soc.Chim., 1887, 47, 899.

59). Butenandt and Hausmann, Ber., 1937, 70, 1157.

Part III.

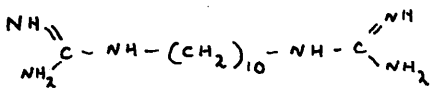
Attempts to Find New Trypanocides.

The Synthesis of Certain Compounds Containing Condensed Polycyclic Heterocyclic Ring Systems.

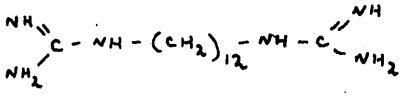
Introduction.

It is generally admitted that at the present time considerations employed in the initiation of research directed to the synthesis of chemical compounds having specific, antagonistic activities towards given pathogenic micro-organisms largely rest on empirical bases. This is necessarily the case, since no clear correlation between chemical structure and biological activity has been ascertained, in spite of the fact that various theories which accommodate a limited number of experimental facts have been put forward from time to time. Nevertheless, although the complex problems of mechanism of drug action and of the delicate relationships existing between drug, parasite and host are as yet imperfectly understood, many very valuable results in the whole field of chemotherapy have been obtained during the course of its relatively short history. As knowledge of the biological activities displayed by various classes of chemical compounds accumulates, it may be expected that evidence, which will serve to indicate further, fruitful lines of work, may be forthcoming. Some of the processes of reasoning previously used in the evolution of valuable new drugs are well illustrated in the cases of two classes of compounds, namely, the aromatic diamidines, and the phenanthridinium compounds.

Studies which eventually led to the discovery of the therapeutic properties of certain aromatic diamidines may be traced back to an early observation by Koch that urine from animals with parathyroid tetany contained methyl guanidine. Administration of the latter compound to healthy animals was found to cause a fall in the blood sugar content. Similarly constituted compounds were investigated, and two which displayed minimum toxicity associated with maximum activity in respect to this property were found, namely, decamethylene diguanidine (synthalin) (I), and dodecamethylene diguanidine (synthalin B) (II).



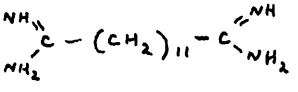
(I)



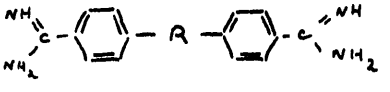
(II)

When it became known that trypanosomes require a large amount of glucose for their metabolic processes, synthalin was tested in experimental trypanosomiasis, and, in fact, was found to exert marked trypanocidal activity. However, the fact that synthalin owes its activity, not to its effect on blood sugar concentration, but to its direct lethal action on the parasites, was demonstrated in 1937 by <u>in vitro</u> experiments carried out by Lourie and Yorke[1]. Following this observation, the trypanocidal action of a number of chemically similar compounds, e.g., many polymethylene

diguanidines, monoguanidines, cyclic guanidines, polymethylene isothioureas, polymethylene monoamines and diamines, aliphatic monoamidines and polymethylene diamidines, was studied[2),3)]. It emerged from this work that the alkylene diamidines show an even greater activity than the corresponding diguanidines, the most active against T.rhodesiense infections in mice being undecane diamidine (III).



(III)

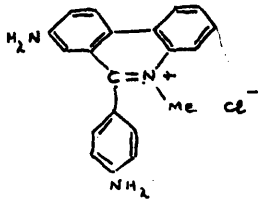


(IV)

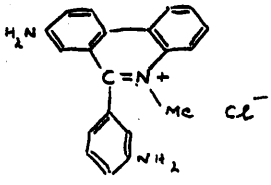
This work was still further extended by Ewins and coworkers[4)] who synthesised a series of aromatic diamidines, represented by (IV), where the two aromatic nuclei are linked by various chains, containing oxygen, carbon, nitrogen and sulphur atoms. Of these compounds, the most active were found to be 4:4'-diamidinodiphenyl ether ("phenamidine"; R = O), 4:4'-diamidinodiphenoxy-propane ("Propamidine"; R = $O(CH_2)_3O$) - pentane ("Pentamidine"; R = $O(CH_2)_5O$) and 4:4'-diamidinostilbene ("Stilbamidine"; R = -CH=CH-). These, and in particular the last named and variants[5)], form an important, recent contribution to the field of chemotherapy in tropical disease[6),7)].

The discovery of pronounced trypanocidal properties of certain members of the phenanthridinium series of compounds resulted from an extended series of investigations initiated by Morgan and Walls in 1931[8] on the chemistry of the heterocyclic base, phenanthridine and its derivatives. Biological investigations carried out by Browning and co-workers showed that several of these derivatives possess marked antiseptic properties. Doubtless by analogy with observations previously made in the acridine (e.g., acriflavine), styryl quinoline[9], and 4-aminoquinoline[10] series, in which it was found that trypanocidal activity is greatly enhanced by quaternisation of cyclic nitrogen atoms, the phenanthridinium compounds corresponding to the more powerfully antiseptic phenanthridine compounds were investigated. Two of them were found to exert therapeutic effects on both T. brucei and T. congolense infections in mice[11]. These results led to further investigations of a large number of basically substituted derivatives of 9-phenyl and 9-methyl phenanthridine quaternary ammonium salts[12], many of which were found to possess marked trypanocidal activity in experimental animals. The constitutional features which contribute towards maximum activity have been recently discussed by Walls[13]. The most active products were found to be phenidium chloride (V),

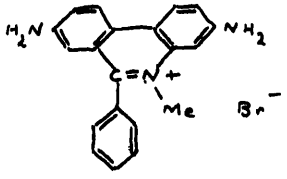
metidium chloride (VI), and dimidium bromide (VII).



(V)



(VI)

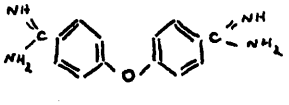


(VII)

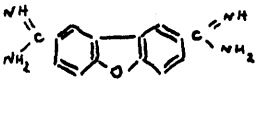
Both (V) and (VII) showed results of promise in the treatment of African bovine trypanosomiasis in the field, and were found to be superior to all of the drugs previously used in T. congolense infections of experimental animals[14].

The experiments which are described in the sequel were initiated with the object of synthesising certain new heterocyclic, condensed-ring compounds, which by their general structural similarity to some of the more active, known compounds, might be expected to display therapeutic properties, possibly to a greater degree, in trypanosome infections in experimental animals.

In the first place, it was considered that modification of the molecule of one of the more active aromatic diamidine compounds, "Phenamidine" (VIII), by the formation of a bond between the carbon atoms in the 2:2'-positions, to give 3:6-diamidinodibenzfuran (IX) might give rise to



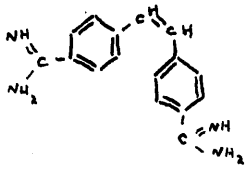
(VIII)



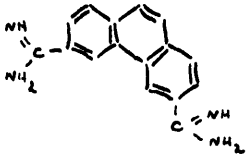
(IX)

results of interest, especially since the dibenzfuran ring system forms a part of the molecules of other biologically active compounds[15], such as morphine. The effects of such modification of the aromatic diamidines do not appear to have been extensively studied, although in a recent publication devoted to a description of the synthesis of 3:6-diamidinophenanthrene (X), Barber and Slack[16] indicated that the latter (which may be considered to be related to

"Stilbamidine" (XI) in the same way as (IX) is to (VIII)) possessed only slight trypanocidal activity.



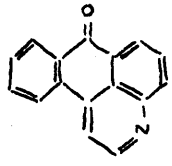
(XI)



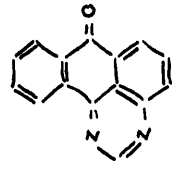
(X)

The dibromo derivative which is obtained by bromination of dibenzfuran in carbon disulphide solution[17], was shown, by means of its synthesis by an unambiguous route[18], to be 3:6-dibromodibenzfuran, and this compound provided a convenient starting point for the synthesis of (IX). The dibromo compound reacted with cuprous cyanide in boiling quinoline to give 3:6-dicyanodibenzfuran. The latter, on treatment with anhydrous ethanolic hydrogen chloride, according to modifications[4] of Pinner's general method for the preparation of imino ethers[19] gave the corresponding diimino ether hydrochloride, which was converted into 3:6-diamidinodibenzfuran dihydrochloride dihydrate (IX) (M.1), on treatment with ethanolic ammonia. The product showed some activity against both T. brucei and T. congolense infections in mice, (see p. 109), but the order of activity was not high and accordingly, it was considered that the results did not warrant the preparation of further compounds of this series.

Attention was next directed to an examination of the possibility of preparing derivatives of two hitherto unknown heterocyclic systems, analogous to the known, so-called anthrapyridine and anthrapyrimidine ring systems, the parent compounds of which are shown in (XII) and (XIII) respectively.

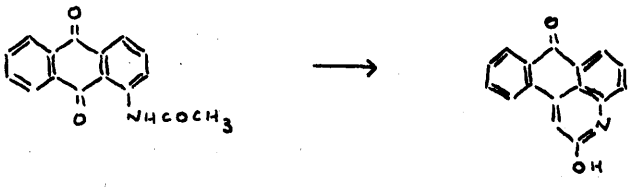


(XII)



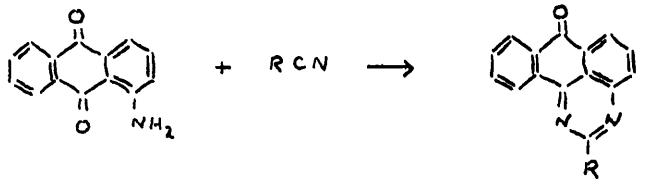
(XIII)

In a series of patents (summarised in [20]) granted to I.G. Farbenindustrie, there are outlined methods of preparation of derivatives of (XII) and (XIII), some of which are stated to possess dyestuff properties. It would appear from these publications, that 1-acetamidoanthraquinone (XIV) and derivatives, readily undergo cyclisation to give anthrapyridone (XV) (and derivatives) under the influence of aqueous alkali[21], or of sodium hydroxide suspended in nitrobenzene[22]. The use of other reaction conditions such as the action of boiling acetic anhydride[21], alkali metal acetates[23], tertiary bases[24], or merely heating above the melting point[25], is mentioned in the usual broad language of the patent literature.



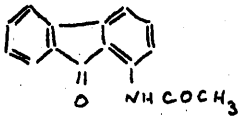
(XIV) (XV)

Anthrapyrimidines are prepared by condensation of 1-amino-anthraquinones with amides[26], N-alkyl benzimino chlorides[27], or aryl cyanides at high temperatures in presence of condensing agents[28], or by the reaction of 1-acylaminoanthraquinones with ammonia under pressure[29].

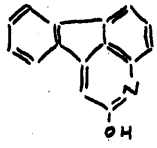
e.g., 

It was considered in the present work that 1-acetamido-fluorenone (XVI) might similarly undergo cyclisation, under the influence of aqueous alkali to give 3-hydroxy-4-azafluoranthene (XVII) which then might be converted into 3-chloro-4-azafluoranthene (XVIII). Condensation of the latter with various amines would be expected to yield 4-azafluoranthenes containing basic substituents in the 3-position (XIX), and the investigation of such derivatives in experimental protozoal infections would then follow. It was considered that these 4-azafluoranthene derivatives would be more suited for biological examination than the corresponding anthrapyridine derivatives owing to the

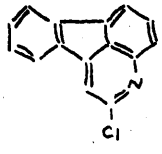
presence of a quinone carbonyl group in the latter compounds.



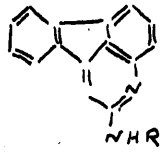
(XVI)



(XVII)

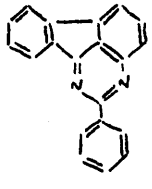


(XVIII)

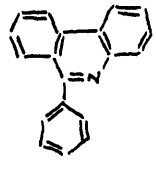


(XIX)

By analogy with the anthrapyrimidine compounds, it was also considered that means of converting 1-aminofluorenone into derivatives of 3-phenyl-2:4-diazafluoranthene (XX) could be ascertained.



(XX)



(XXI)

The structure of (XX) bears some resemblance to that of 9-phenylphenanthridine (XXI), which, as has already been mentioned, is the parent compound of certain phenanthridinium compounds which are powerful trypanocides.

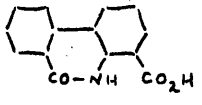
In the first place, it was necessary to determine suitable means of preparing 1-aminofluorenone, the intermediate required for these projected transformations. Chromic acid oxidation of fluoranthene gives fluorenone-1-carboxylic acid in reasonable yield[30]. The conversion of the latter, by means of Hofmann hypobromite degradation of its amide into 1-aminofluorenone has been previously reported on two occasions. Goldschmidt[31] obtained the amino ketone by this method in unspecified yield, and reported its m.p. to be 110°. On the other hand, Huntress, Pfister and Pfister[32], who prepared it in 56% yield, described its m.p. as 118-118.5°. In the present work, however, although the melting point for the product given by the latter authors was confirmed, yields of only approximately 25-30% were obtained in experiments carried out on a larger scale. It was considered that application of the conditions of the Schmidt reaction[33] to the case of fluorenone-1-carboxylic acid might afford a convenient route to the amino ketone, although it was realised at the outset that since hydrazine in presence of sulphuric acid reacts not only with carboxylic acids to give amines, but also with ketones to give amides, the presence of the cyclic keto group might give rise to complications. Apparently the only keto acid whose behaviour under the conditions of

the reaction has been previously studied, is laevulinic acid (XXII), which was found to give mainly acetyl β-alanine (XXIII), together with a smaller quantity of N-methyl succinic monoamide (XXIV)[33].

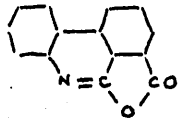
$$CH_3COCH_2CH_2CO_2H \longrightarrow CH_3CONHCH_2CH_2CO_2H + CH_3NHCOCH_2CH_2CO_2H$$

(XXII) (XXIII) (XXIV)

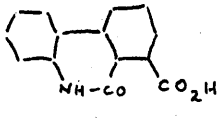
In the present work it was found that treatment of fluorenone-1-carboxylic acid with rather more than one molecule of hydrazoic acid in presence of sulphuric acid gave a mixture, from which two products, one of which was acidic and the other neutral, were isolated. From their combustion results, these products were considered to be (XXV) and (XXVI) respectively, although the less probable,



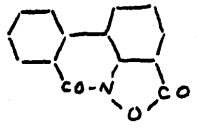
(XXV)



(XXVI)



(XXVII)



(XXVIII)

alternative structures (XXVII) and (XXVIII) (which contains a β-lactam ring) cannot be excluded. The reaction was not further investigated since it was evident that preferential reaction at the ketonic group had occurred.

Application of the conditions of the Curtius reaction[34] to fluorenone-1-carboxylic acid eventually gave the desired amino ketone in approximately 50% overall yield. Treatment of a solution of the acid chloride[31] in acetone, with an aqueous solution of sodium azide, gave in nearly theoretical yield the corresponding acid azide which on being heated with concentrated hydrochloric acid was converted into 1-aminofluorenone. Fluorenonyl-1-isocyanate, which is presumably an intermediate in the degradation, could be isolated from the solution obtained by warming the azide under anhydrous conditions, in toluene.

All attempts to effect cyclisation of 1-acetamidofluorenone were unsuccessful. Heating the acetamido ketone with methanolic sodium methoxide on the water-bath, or with sodium mexthoxide suspended in nitrobenzene at 140°, resulted in hydrolysis to the free amine, although under the latter conditions a proportion of the starting material was recovered unchanged. Heating with sodium methoxide in boiling xylene yielded mainly an intractable gum, and the starting material was recovered unchanged after prolonged boiling of its solutions in acetic anhydride or in ethanol containing piperidine. It was considered that these negative results, which are in contrast with previously made observations of the facile cyclisation of 1-acetamidoanthraquinone

derivatives under the influence of dilute alkali (see, e.g., [35]), might be connected with the stereochemical structure of the fluorenone molecule*. In the case of anthraquinone, the three rings are evidently co-planar but the problem of the configuration of the rings in fluorene is not yet settled (for discussion, see Rieveschl and Ray[36]). From a consideration of evidence obtained from X-ray studies, Cook and Iball[37] concluded that in fluorene, each benzene ring is inclined at 20° to the 5-membered ring and at 40° to each other, and some support for this non-planar configuration is provided by recently reported results from resolution experiments with 2-substituted 9-fluorenylamines[38].

It was then considered that cyclisation experiments with derivatives of 1-acetamidofluorenone, in which the methylene group of the side chain is activated by the presence of substituents possessing powerful electron-attractive properties, might offer better prospects of success, and preliminary experiments carried out with 1-acetoacetamidofluorenone (XXIX; R = $-COCH_3$) which was prepared by condensation of 1-aminofluorenone with ethyl acetoacetate at 165°, justified this reasoning.

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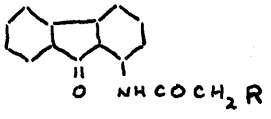
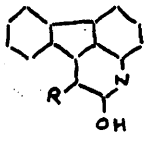
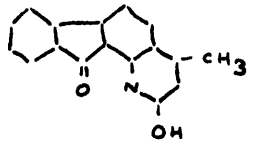
* In this connection it is of interest to note that Goldschmidt[31] observed that fluorenone-1-carboxylic acid, on treatment with hydroxylamine, yields an oxime and not an oxime anhydride which might be expected to be produced by analogy with the behaviour of other keto carboxylic acid systems, e.g., 3-benzoyl picolinic acid.



(XXIX)



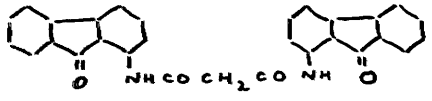
(XXX)



(XXXI)

The acetoacetamido compound (XXIX; R = $-COCH_3$) on treatment with sodium methoxide in nitrobenzene at 140°, gave a product which is believed to be 3-hydroxy-2-acetyl-4-azafluoranthene (XXX; R = $-COCH_3$). However it is conceivable that cyclisation may have taken place in an alternative direction to give the isomeric 2-hydroxy lepidine derivative (XXXI), and in order to examine this point, an attempt was made to prepare (XXXI) by heating the acetoacetamido compound (XXIX; R = $-COCH_3$) with concentrated sulphuric acid at 65°, these being the conditions usually employed for cyclodehydration of acetoacetanilide derivatives to lepidine derivatives (see, e.g., Misani and Bogert[39]). However, this treatment resulted in hydrolysis of (XXIX; R = $-COCH_3$) to the free amine, and the matter was not further investigated. In order to attempt to prepare derivatives of (XXX) where R represents groups which might be subsequently readily eliminated, possible means of preparing 1-cyanoacetamido- (XXIX; R = -CN) and 1-carbethoxyacetamidofluorenone (XXIX; R = $-CO_2Et$) were examined.

Condensation of 1-aminofluorenone with ethyl cyanoacetate at 190° gave a low yield of (XXIX; R = -CN), together with a compound, m.p. 233°, which was subsequently identified as NN'-di(1-fluorenonyl)-malonamide (XXXII). The production of the latter compound was unexpected, but it

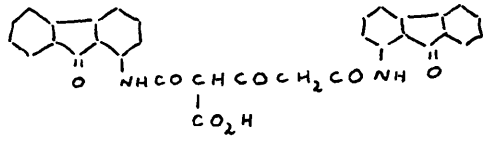


(XXXII)

was later found that analogy exists in the literature which shows[40] that a proportion of NN'-diphenylmalonamide is formed on heating aniline with ethyl cyanoacetate. Some observations made by Naegeli and Tyabji appeared to offer a more favourable route to (XXIX; R = -CN). These authors showed[41] that aromatic isocyanates react with carboxylic acids to give mixture of the corresponding acylamine and sym-urea derivatives. The latter are predominantly formed in reactions with acids such as benzoic and acetic, but the former are formed almost exclusively in the case of strong acids, such as cyanoacetic and trichloracetic. In the present work, a more improved yield of (XXIX; R =-CN) was obtained by treatment of fluorenone-1-isocyanate with cyanoacetic acid, than by heating the amine with ethyl cyanoacetate, but attempts to cyclise the product were not

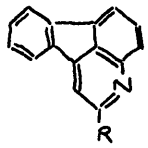
made, owing to the fact that concurrently performed experiments which are described below, indicated a probably more convenient route to 3-hydroxy-4-azafluoranthene.

Condensation of 1-aminofluorenone with a large excess of diethyl malonate at 190° yielded mainly, 1-carbethoxyacetamidofluorenone (XXIX; R = $-CO_2Et$) together with a small quantity of the malonamide derivative (XXXII). Somewhat similar overall yields of the former product were also obtained by means of reaction between fluorenone-1-isocyanate and carbethoxy acetic acid. Preliminary attempts to effect cyclisation of (XXIX; R = $-CO_2Et$) were unsuccessful. Heating the substance above its melting point, or in boiling nitrobenzene gave the malonamide derivative (XXXII). Prolonged boiling with a mixture of acetic anhydride and sodium acetate yielded gummy products from which only 1-acetamidofluorenone could be isolated. Treatment with approximately one equivalent of dilute sodium hydroxide solution gave mainly 1-aminofluorenone, together with a low yield of an acidic substance, whose behaviour on heating and analytical results, indicated for it the structure of the β-ketonic acid derivative (XXXIII)



(XXXIII)

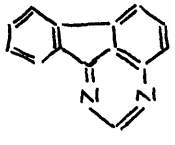
However, under those conditions which had led to successful cyclisation of 1-acetoacetamidofluorenone (XXIX; R = $COCH_3$), namely the action of sodium methoxide in nitrobenzene at 140°, the carbethoxyacetamido compound (XXIX; R = $-CO_2Et$) was converted into 3-hydroxy-4-azafluoranthene-2-carboxylic acid (XXX; R = $-CO_2H$) in good yield. The latter was smoothly decarboxylated on heating with quinoline in the presence of copper carbonate, to 3-hydroxy-4-aza-fluoranthene (XXX; R = H), which on treatment with phosphoryl chloride gave 3-chloro-4-azafluoranthene (XXXIV; R = Cl). The latter chloro compound was condensed with ammonia and a



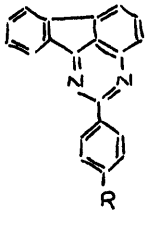
(XXXIV)

variety of amines to give the following 4-azafluoranthenes, (XXXIV), 3-amino- (R = NH_2) (M.5), 3-acetamido- (R = $-NHCOCH_3$) (M.8) (prepared by acetylation of M.5), 3-diethylamino- (R = $-NEt_2$) (M.4), 3-β-diethylaminoethylamino- (R = $-NHCH_2CH_2NEt_2$) (M.7), 3-γ-diethylaminopropylamino- (R = $-NHCH_2CH_2CH_2NEt_2$) (M.6) and 3-δ-diethylamino-α-methylbutylamino-4-azafluoranthene (R = $-NH.CH(CH_3).CH_2CH_2CH_2NEt_2$) (M.9). Results of biological investigation of (M.8) and the hydrochlorides of (M.5), (M.4), (M.7), (M.6) and (M.9) are described on p. 109 - 112.

Under conditions somewhat similar to those previously described in the patent literature for the preparation of derivatives of anthrapyrimidine (XIII), condensation of 1-aminofluorenone with formamide occurred to give a low yield of 2:4-diazafluoranthene (XXXV). Condensation of 1-aminofluorenone with benzonitrile, p-chlorobenzonitrile and p-nitrobenzonitrile at temperatures of ca. 190° and in presence of hydrogen chloride, gave the following 2:4-diazafluoranthenes (XXXVI), 3-phenyl-(R=H) (M.2), 3-p-chlorophenyl- (R=Cl) (M.3) and 3-p-nitrophenyl-2:4-diazafluoranthene (R=NO_2).



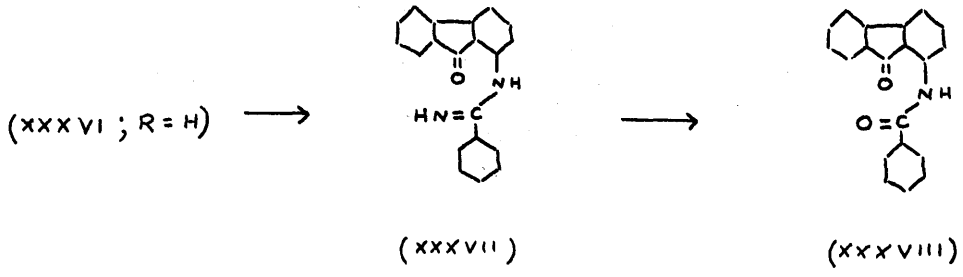
(xxxv)



(xxxvi)

Reduction of the nitro compound (XXXVI; R = $-NO_2$) with iron and dilute hydrochloric acid gave the amine (XXXVI; R=NH_2) (M.10) from which the acetyl derivative (XXXVI; R=$-NHCOCH_3$) (M.11) was prepared. The last named, and the hydrochlorides of the bases (M.2), (M.3) and (M.10) were found to have no therapeutic effect on T.brucei, T.congolense or T.cruzi infections in mice (see p. 110). That the compounds (XXXVI; R=H) and (XXXVI; R=Cl) are only very weakly basic, was demonstrated by the fact that their hydrochlorides on heating with water, decomposed to give the corresponding

free bases. With the object of preparing more strongly basic derivatives, which would be more closely analogous to the active phenanthridinium compounds, attempts were made to prepare quaternary ammonium salts of (XXXVI; R=H). The base was recovered unchanged after being boiled with methyl iodide in benzene. Heating with dimethyl sulphate in anhydrous nitrobenzene, i.e., under the usual conditions of quaternisation of cyclic nitrogen compounds, gave a gum from which a neutral, crystalline product was isolated in some 30% yield. The compound had the composition, $C_{20}H_{13}O_2N$, which indicates the loss of one atom of nitrogen (as ammonia) from the starting material (XXXVI; R=H) with addition of two molecules of water, and corresponds to the composition of a benzamidofluorenone. A similar result was obtained on prolonged boiling of (XXXVI; R=-NH_2) with strong ethanolic hydrogen chloride, which gave a product having the composition of the hydrochloride of a p-amino-benzamidofluorenone, $C_{20}H_{14}O_2N_2$. It seemed to be most reasonable to interpret the formation of the neutral product of the former reaction in terms of the following sequence of reactions. First, hydrolysis of the starting material accompanied by ring fission occurs, to give the substituted amidine (XXXVII), which then undergoes further hydrolysis, with loss of ammonia, to give 1-benzamidofluorenone (XXXVIII).



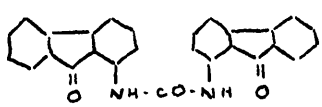
(XXXVII) (XXXVIII)

However, this interpretation was later found to be wrong owing to the fact that the melting point of an authentic sample of 1-benzamidofluorenone, prepared by benzoylation of 1-aminofluorenone[32] was strongly depressed on admixture with the product, $C_{20}H_{13}O_2N$. The melting point of the latter was strongly depressed also on admixture with 1-benzamidofluorenol ($C_{20}H_{15}O_2N$), which was prepared by means of aluminium isopropoxide reduction of 1-benzamidofluorenone. The quantities of ring-fission products available were too small to permit their further investigation.

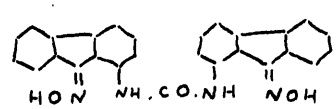
Addendum.

Some Reactions of NN'-di(1-fluorenonyl)-urea.

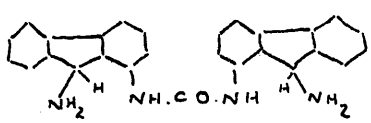
This compound (XXXIX) was obtained as a by-product in the course of several experiments devised for the purpose of ascertaining optimum conditions for the conversion of fluorenone-1-carboxylic acid azide into 1-aminofluorenone. Since it contains the symmetrical urea configuration which is also a feature of the molecule of the powerful trypanocide, "Antrypol" (Bayer 205), it was considered worthy of biological examination after suitable modification in order to render it more basic and water soluble. It was considered that a product possessing these properties could be obtained by preparation of the dioxime (XL), followed by reduction of the latter to give the di(fluorenylamine) derivative (XLI)



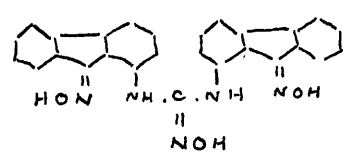
(XXXIX)



(XL)



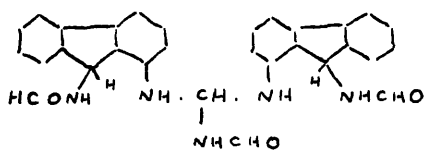
(XLI)



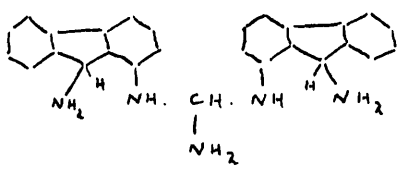
(XLII)

However, treatment of (XXXIX) with hydroxylamine hydrochloride in boiling pyridine yielded a product whose analytical data clearly excluded the dioxime structure (XL) and indicated that it had the composition of the trioxime (XLII), containing one half of a molecule of water of crystallisation. This result was unexpected in view of the well known lack of reactivity displayed by amides in general towards carbonyl reagents, and so the matter was further investigated. Treatment of the oximation product with zinc and acetic acid, i.e.,

reagents previously employed for conversion of fluorenone oxime into fluorenylamine[42], gave a non-basic product which could not be rendered crystalline. However, the sym-urea derivative (XXXIX), on boiling with formamide according to the conditions of the Ingersoll modification[43] of the Leukart reaction, yielded a product which had the composition of the triformyl pentamine (XLIII), and which on boiling with concentrated hydrochloric acid gave a hydrolysis product which had the composition of the corresponding pentamine (XLIV) in the form of its trihydrochloride monohydrate.



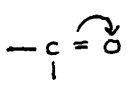
(XLIII)



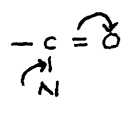
(XLIV)

The latter was further characterised by its conversion into the free pentamine (XLIV) which gave a well-crystalline dipicrate. Nitrogen values found for the trihydrochloride monohydrate, dipicrate, and free base were low, and in the last case, inconsistent, evidently owing to the formation of nitrogenous charcoals during combustion.

As far as has been ascertained by the author, the phenomenon of a urea carbonyl group displaying properties of a ketonic carbonyl group has not been previously recorded in the literature, although the opposite case, in which reactivity of a ketonic carbonyl group is suppressed by the influence of other groups present in the molecule is known, and is well exemplified by the case of Michler's ketone (XLVII), whose carbonyl group is only feebly reactive towards nucleophilic reagents. According to current theory, reactivity of ketones towards such reagents is occasioned by a displacement of electrons taking place, represented by (XLV), whereby the carbon atom becomes cationoid in character. In the case of carboxylic acid amides this character of the carbon atom is neutralised, owing to the occurrence of electromeric displacements, shown in (XLVI).

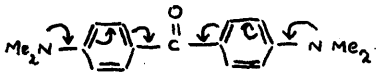


(XLV)

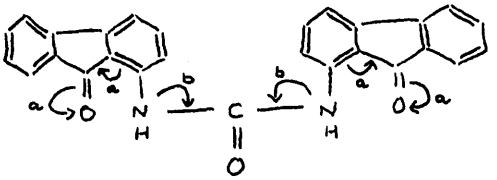


(XLVI)

Similarly, the ketonic character of the carbonyl group in Michler's ketone is masked, owing to the occurrence of the electromeric displacements shown in (XLVII)[44].



(XLVII)



(XXXIX)

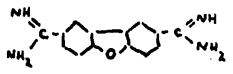
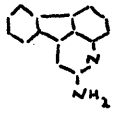
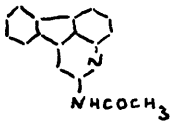
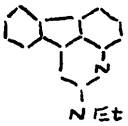
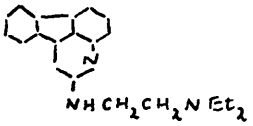
It seems reasonable to suppose that the observed reactivity of the urea carbonyl group in (XXXIX) arises from the fact that the mesomeric effects (a) exerted by both cyclic keto groups (situated in ortho positions) inhibit, either wholly or partly, the neutralising effects (b) exerted by the nitrogen atoms on the cationoid character of the carbon atom to which they are linked. In this connection, it would be of interest to examine the behaviour towards nucleophilic reagents, of derivatives of carbanilide (NN'-diphenyl urea) containing various electron-attractive substituents located in the ortho and para positions of the benzene rings, and it is intended to do this when opportunity permits.

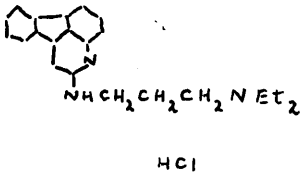
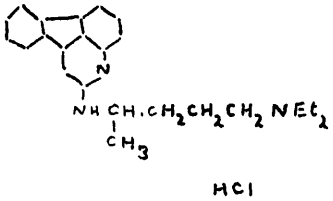
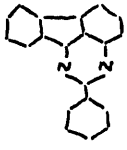
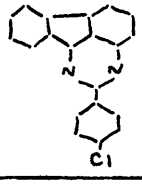
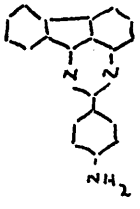
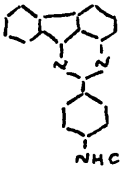
The trihydrochloride monohydrate of the pentamine (XLIV) is a well-crystalline, freely water-soluble substance. It had no therapeutic effect on the course of T.brucei or T.congolense infections in mice.

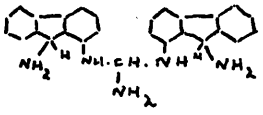
Biological Results.

(i) Examination for Trypanocidal Activities.

(Note:- These investigations were carried out in the Department of Bacteriology by other workers under the supervision of Professor C.H.Browning, F.R.S., to whom the author is indebted for the results which are summarised below).

No.	Formula	M.T.D. mg./kg.	Organ-ism	Dose mg./kg.	Activity
M.1	 $2HCl \cdot 2H_2O$	50	a b	33 5 3.3 33 10 1	Cure (Cure) (Cure) Cure (Cure) 0
M.5	 $HCl \cdot H_2O$	10	a b c	6.7 6.7 6.7	0 0 0
M.8		500*	a	200	0
M.4	 $HCl \cdot 3H_2O$	500	a b c	200 200 100	0 0 0
M.7	 HCl	100	a b c	67 67 67	0 0 0

No.	Formula	M.T.D. mg./kg.	Organ-ism	Dose mg./kg.	Activity
M.6		200	a b	100 100	0 0
M.9		100	a	67	0
M.2		50	a b	10 20	0 0
M.3		100	a b	100 100	0 0
M.10		200	a b	200 200	0 0
M.11		1000*	a b	200 500	0 0

No.	Formula	M.T.D. mg./kg.	Organ-ism	Dose mg./kg.	Activity
M.12	 3 HCl. H_2O	200	a b	167 167	0 0

Administration (to mice) was by the subcutaneous route.

M.T.D. = approximate maximum tolerated dose.

* = the compound was administered in the form of a suspension.

a = T. brucei.

b = T. congolense (strain I - see Calver[14]).

c = T. cruzi.

Toxicities and doses are expressed in terms of mg. per kg. of body weight (mice).

cure = complete sterilisation of infection.

(cure) = only a proportion of animals cured.

0 = no effect.

Antimalarial activities.

Dr. F.H.S. Curd reported that M.7, M.6, and M.9 were inactive against P. gallinaceum in chicks.

Amoebicidal activities.

According to Dr. Curd, each of the compounds M.7, M.6 and M.9 displayed slight activity on examination according to the method of Jones[45]. He states:-

"We would regard their activities merely as a possible useful lead. There does not seem to be any doubt about the activity since all three compounds of the type show it, although it is not very high."

Experimental.

3:6-Dicyanodibenzfuran.

An intimate mixture of 3:6-dibromodibenzfuran[17] (5.48 g.) and cuprous cyanide (6.5 g.) was added in small portions during 15 minutes to boiling, purified quinoline (20 cc.), and the resulting dark mixture boiled under reflux for 30 minutes. After being cooled somewhat, it was added to concentrated hydrochloric acid (200 cc.). The mixture was boiled for a few minutes, cooled and then filtered. The residue was washed with dilute hydrochloric acid and water, and then sublimed in a high vacuum. The yellow product (3.4 g.), which sublimed at 220-230°/0.5 mm., was recrystallised from acetic acid, giving colourless needles (2.9 g.; 80%), m.p. 299°. (Found: C, 76.9; H, 2.8; N, 12.8. $C_{14}H_6ON_2$ requires C, 77.1; H, 2.8; N, 12.8%).

3:6-Diamidinodibenzfuran.

A suspension of the above dicyano compound (2.79 g.) in anhydrous ethanol (45 cc.) was cooled at 0° and then treated with a stream of dry hydrogen chloride until it was saturated. The suspension was kept at room temperature for 14 days. Reaction appeared to be very slow, and at the end of that time it was evident that a proportion of the starting material was still unchanged. The mixture was filtered, the residue washed with anhydrous ether and then kept in a vacuum

desiccator containing sodium hydroxide, for several hours. The crystalline mixture (4.5 g.) was cooled to 0° and treated with anhydrous ethanol (45 cc.) which had been previously saturated with ammonia. The mixture was heated at 45-50° for 6 hours, allowed to cool and then filtered. The residual solid (3.67 g.) was separated by means of extraction with boiling 2N-hydrochloric acid into unchanged dicyano compound (0.9 g.: 32%), and the diamidine dihydrochloride dihydrate, which crystallised from 2N-hydrochloric acid in long, colourless needles (2.8 g.: 60%), m.p. greater than 320°. (Found: C, 46.7; H, 4.9; N, 15.2. $C_{14}H_{12}ON_4 \cdot 2HCl \cdot 2H_2O$ requires C, 46.5; H, 5.0; N, 15.5%).

The Schmidt reaction applied to fluorenone-1-carboxylic acid.

Sodium azide (0.72 g.) was added in small portions to a mechanically stirred solution, heated at 45°, of the keto acid[30] (2.24 g.) in a mixture of concentrated sulphuric acid (5 cc.) and chloroform (30 cc.). A steady evolution of gas occurred while the mixture was stirred at 45° for 20 minutes. After being kept at room temperature for 2 hours, the mixture was cooled to 0°, diluted with chloroform (120 cc.), treated with crushed ice, and then basified by the addition of strong sodium hydroxide solution. The chloroform layer was separated, washed with water and evaporated. The residue was crystallised from benzene, giving light-orange needles (0.8 g.), m.p. 223-

224°, of an anhydro phenanthridone carboxylic acid, (XXVI or XXVIII). (Found: C, 76.2; H, 2.8; N, 6.4. $C_{14}H_7O_2N$ requires C, 76.0; H, 3.2; N, 6.3%). The orange-red solid (1.2 g.; m.p. 200-205°), obtained on acidification of the aqueous-alkaline extract, was separated by means of a process involving fractional crystallisation from ethanol followed by extraction with benzene, into unchanged starting material (0.2 g.) as more soluble product, and as less soluble product, a phenanthridone carboxylic acid (XXV or XXVII), which crystallised from acetic acid in short, yellow needles (0.3 g.), m.p. 229° (decomp.). (Found: C, 70.5; H, 3.7; N, 5.9. $C_{14}H_9O_3N$ requires C, 70.3; H, 3.8; N, 5.9%).

1-Aminofluorenone.

(i) By Hofmann reaction on fluorenone-1-carboxylic acid amide.

Repetition of the procedure of Huntress, Pfister and Pfister[32], using 10 g. of the amide, gave the amino ketone, m.p. 114-118°, in 26% yield.

(ii) By Curtius Reaction.

Fluorenone-1-carboxylic acid azide.

Fluorenone-1-carboxylic acid[30] (20 g.) was boiled under reflux with thionyl chloride (40 cc.) for 30 minutes. Excess thionyl chloride was removed by distillation under reduced pressure, from a water-bath, and the residual solid freed from last traces by means of repeated evaporation under reduced

pressure with anhydrous benzene. The residue of acid chloride was dissolved in dry acetone (440 cc.). The solution was cooled at 0-5° and treated dropwise during 5-10 minutes, with mechanical stirring, with a solution of sodium azide (8.5 g.) in water (25 cc.). After being stirred at 0-5° for 30 minutes longer, the mixture was treated with water (960 cc.). The precipitated azide was filtered off and washed with water. (It was best dried at room temperature, spread thinly on a clock glass, followed by drying over phosphorus pentoxide in a vacuum desiccator. On one occasion, when the azide was warmed at 40-42° under reduced pressure for a few hours, decomposition occurred with evolution of heat and gas). It was in the form of a yellow crystalline solid (20.8 g.; 94%), m.p. 90-91° (with effervescence). (0.206 g. heated in toluene evolved 18.5 cc. of nitrogen measured at N.T.P. Theory for $(C_{13}H_7O)CON_3$ requires 18.6 cc.).

Conversion to the amino ketone.

The partially dry azide obtained from such an experiment was divided into two equal portions. Each portion was cautiously heated under reflux with concentrated hydrochloric acid (250 cc.), with occasional shaking, until evolution of gas was complete. The mixture was then boiled under reflux for 5 hours, diluted with water (200 cc.), boiled for 1 hour longer, and then filtered while hot. The combined yields of

hydrochloride which separated from the filtrates on cooling were treated with aqueous ammonia, and gave a crude product (9.7 g.; m.p. 116-117°) which on recrystallisation from dilute ethanol gave long, orange-yellow leaflets (8.8 g.; 50% overall yield from the acid), m.p. 118-119°.

In one experiment, in which the solution obtained by heating the dry azide (25 g.), under reflux with anhydrous toluene (250 cc.), was boiled with concentrated hydrochloric acid (250 cc.), the yield of 1-aminofluorenone was only 4% (4 g.). The main product was a neutral solid (8.5 g.), which on crystallisation from pyridine gave yellow needles, m.p. 264°, of NN'-di(1-fluorenonyl) urea (XXXIX). (Found: C, 77.6; H, 3.8; N, 6.5. $C_{27}H_{16}O_3N_2$ requires C, 77.9; H, 3.9; N, 6.7%). The latter was unaffected by prolonged boiling with concentrated hydrochloric acid.

Fluorenone-1-isocyanate.

The dry azide (2.8 g.) was heated with anhydrous toluene (15 cc.) on the water-bath for 30 minutes. The smooth evolution of gas was complete in 5-10 minutes. The mixture was filtered while hot from a small amount of tar, and the product which crystallised from the filtrate was recrystallised from toluene, giving clusters of orange rhombs (2 g.; 80%), m.p. 144-146°. (Found: N, 6.2. $C_{14}H_7O_2N$ requires N, 6.3%).

1-Acetamidofluorenone.

The procedure of Huntress et.al.[32] was slightly modified as follows:-

A solution of the amino ketone (3 g.) in benzene (45 cc.) was boiled under reflux with acetic anhydride (3 cc.) for 1.5 hours. The product, isolated from the benzene solution, formed long yellow needles (from ethanol) (3.24 g.; 89%), m.p. 138-139°. (Found: N, 5.9. Calc. for $C_{15}H_{11}O_2N$: N, 5.9%). Huntress et al.[32] obtained the compound, m.p. 138-138.3° in 75% yield.

Attempts to cyclise 1-acetamidofluorenone.

(i) The compound (200 mg.) was boiled under reflux on the water-bath with 2.2N-methanolic sodium methoxide (10 cc.) for 5 hours. The product (160 mg.: m.p. 117-118°), was identified as 1-aminofluorenone by m.p. and mixed m.p.

(ii) A solution of the compound (1.0 g.) in warm, anhydrous nitrobenzene (10 cc.) was treated with powdered sodium methoxide (0.251 g.; 1.1 mols.) and the mixture heated at 140° for 6 hours. After being cooled, acidified with dilute hydrochloric acid and steam distilled, the mixture gave a gum from which were obtained by means of treatment with methanol, followed by chromatography (Al_2O_3) of the more soluble products, starting material (30 mg.), and 1-aminofluorenone (700 mg.).

(iii) A solution of the compound (456 mg.), in warm, anhydrous

xylene (15 cc.), was treated with sodium methoxide (0.126 g.; 1.1 mols.) and then boiled under reflux for 1.5 hours. After being cooled, the dark-coloured mixture was washed with water. The xylene layer, on evaporation under reduced pressure, gave a dark-red gum which on trituration with methanol gave a trace of crystalline material. The latter, on recrystallisation from benzene gave light-brown crystals, m.p. 230-237° (micro m.p.). There was insufficient for further investigation and no other crystalline product could be isolated from the methanol mother liquors.

(iv) The acetamido compound (200 mg.) was boiled under reflux with acetic anhydride (3 cc.) for 6 hours. On working up, only starting material (120 mg.) identified by m.p. and mixed m.p. was obtained.

(v) A solution of the acetamido compound (200 mg.) in warm anhydrous ethanol (3 cc.) was treated with purified piperidine (0.1 cc.) and then boiled under reflux for 6 hours. The starting material (190 mg.) was recovered unchanged.

1-Acetoacetamidofluorenone (XXIX; R = $-CO_2Et$).

1-Aminofluorenone (5 g.) was added in small portions, during 30 minutes, to ethyl acetoacetate (25 cc.), heated to 160-165°. The mixture was heated at that temperature for 20 minutes longer. After being cooled, the mixture was distilled under reduced pressure from a water-bath in order

to remove excess ethyl acetoacetate. The residual, red, viscous gum was triturated with petroleum ether (45 cc.; b.p. 60-80°). The resulting solid was extracted with boiling ethanol (175 cc.), and the ethanol extract concentrated on the water-bath, to small bulk. The material which crystallised on cooling (3.3 g.; m.p. 134-136°) was recrystallised from ethanol giving orange needles (3.0 g.), m.p. 139-140°. (Found: C, 73.3; H, 4.6; N, 5.2. $C_{17}H_{13}O_3N$ requires C, 73.1; H, 4.7; N, 5.0%). The insoluble residue from the ethanol extraction was a yellow solid (0.15 g.), m.p. 225-230°. This was submitted to hydrolysis by boiling under reflux with dilute acetic acid containing a few drops of concentrated hydrochloric acid. The product was a gum which was dissolved in benzene (10 cc.), and the solution poured on to a column (1.5 x 6 cm.) of activated alumina. Elution of the resulting, lower orange band with 1% methanol-benzene gave 1-amino-fluorenone (20 mg.). Elution of the more strongly adsorbed yellow band with 10% methanol-benzene gave a few crystals, m.p. 264-267°, identified by m.p. and mixed m.p. as the compound which is probably 3-hydroxy-2-acetyl-4-azafluoranthene (XXX; R = $-COCH_3$), and which is described below.

Cyclisation of 1-acetoacetamidofluorenone.

A solution of the compound (1.0 g.) in anhydrous nitrobenzene (10 cc.) was heated to 100° and then treated

with powdered sodium methoxide (0.21 g.). The temperature was raised gradually to 135-140°, and heating continued for 4 hours longer. After being cooled, the mixture was treated with 2N-hydrochloric acid (10 cc.), and then distilled in steam. The residual tarry gum, on trituration with chloroform, gave a solid, which on crystallisation from acetic acid (charcoal), yielded long, yellow needles (0.45 g.), m.p. 265-268°, of cyclisation product, which is probably 3-hydroxy-2-acetyl-4-azafluoranthene (XXX; R = $-COCH_3$).
(Found: C, 78.2; H, 4.2; N, 5.4. $C_{17}H_{11}O_2N$ requires C, 78.2; H, 4.2; N, 5.4%). A small quantity (0.15 g.) of 1-aminofluorenone was recovered by chromatography of the gum, which was obtained on evaporation of the chloroform mother liquors.

Treatment of 1-acetoacetamidofluorenone with sulphuric acid.

A solution of the compound (0.5 g.) in concentrated sulphuric acid (1 cc.) was kept at room temperature for 15 minutes, and then heated at 65° for 3 minutes. After being cooled and poured into water, the reaction mixture yielded 1-aminofluorenone (0.32 g.) identified by m.p. and mixed m.p.

1-Cyanoacetamidofluorenone (XXIX; R = -CN).

(i) By condensation of 1-aminofluorenone with ethyl cyanoacetate.

The amino ketone (5 g.) was added portionwise to ethyl cyanoacetate (25 cc.), heated to 170°. The temperature was raised gradually to 190° and maintained at 190-195° for 30 minutes. The reaction mixture was distilled under reduced pressure from an oil-bath at 100-120°. The residual gum was treated with warm acetone (40 cc.), and the mixture allowed to stand for several hours. The insoluble solid (0.66 g.), formed minute, yellow prisms (from acetic acid), m.p. 233°, of NN'-di(1-fluorenonyl)-malonamide (XXXII).
(Found: C, 75.8; H, 3.9; N, 6.0. $C_{29}H_{18}O_4N_2$ requires C, 76.0; H, 3.9; N, 6.1%). Evaporation of the acetone solution yielded a red gum, from which were obtained by means of treatment with ethanol followed by fractional crystallisation from the same solvent, unchanged 1-aminofluorenone (1.8 g.), and a product (0.4 g.), which on crystallisation from acetic acid followed by repeated recrystallisation from ethanol gave yellow needles, m.p. 203°, of 1-cyanoacetamidofluorenone. (Found: C, 73.2; H, 3.7; N, 10.9. $C_{16}H_{10}O_2N_2$ requires C, 73.3; H, 3.8; N, 10.7%).

(ii) By interaction of fluorenone-1-isocyanate and cyanoacetic acid.

A mixture of the isocyanate (0.5 g.), anhydrous cyanoacetic acid[46] (0.4 g.) and anhydrous toluene (5 cc.) was

boiled under reflux for 16 hours. After being cooled, the mixture was treated with water and then filtered. The residue was extracted with boiling ethanol, and the extracts concentrated to small bulk. The product, on recrystallisation from ethanol, gave yellow needles (0.32 g.; 54%), m.p. 198-202°, of 1-cyanoacetamidofluorenone, which showed no mixed m.p. depression on admixture with the compound described above.

1-Carbethoxyacetamidofluorenone (XXIX; R = $-CO_2Et$).

(i) By condensation of 1-aminofluorenone with diethyl malonate.

A mixture of the amino ketone (6.0 g.) and diethyl malonate (30 cc.) was heated at 190-200° (air condenser) for 17 hours. After being cooled somewhat, the mixture was distilled under reduced pressure, from an oil-bath at 110-130°. The residual gum was washed with petroleum ether (b.p. 60-80°) and then extracted with warm acetone. The insoluble residue (0.6 g.) on recrystallisation from ethanol gave yellow prisms, m.p. 233°, of NN'-di(1-fluorenonyl)-malonamide (XXXII). Evaporation of the acetone solution, followed by recrystallisation of the residue from ethanol, gave orange, prismatic needles (7.8 g.; 82%), m.p. 126°, of the carbethoxyacetamido compound. (Found: C, 70.2; H, 4.6; N, 4.4. $C_{18}H_{15}O_4N$ requires C, 69.9; H, 4.9; N, 4.5%).

(ii) Interaction of fluorenone-1-isocyanate and carbethoxy

acetic acid[47], in boiling toluene gave the carbethoxy-acetamido compound in 27% yield, together with NN'-di-(1-fluorenonyl)-urea in 22% yield.

Unsuccessful attempts to cyclise 1-carbethoxyacetamido-fluorenone.

(i) A solution of the compound (439 mg.) in anhydrous nitrobenzene (4.4 cc.) was boiled under reflux for 6 hours. After removal of solvent by steam distillation, the residue was separated by means of fractional crystallisation from ethanol into unchanged starting material (100 mg.) and NN'-di(1-fluorenonyl)-malonamide (120 mg.), which was identified by m.p. and mixed m.p.

(ii) The compound (200 mg.) was boiled under reflux with acetic anhydride (3 cc.), and anhydrous sodium acetate (200 mg.) for 6 hours. Treatment of the reaction mixture with water yielded a dark gum, from which was isolated a small quantity of yellow needles, m.p. 135°, identified by m.p. and mixed m.p. as 1-acetamidofluorenone.

(iii) The finely powdered compound (390 mg.) was boiled under reflux with 1.02 N-sodium hydroxide solution (1.86 cc.; 1.5 mols.) for 2.5 hours. After being cooled, the mixture was extracted with chloroform. Evaporation of the chloroform extract gave a crystalline residue (180 mg.) which on re-crystallisation from ethanol gave 1-aminofluorenone, identified

by m.p. and mixed m.p. Acidification of the aqueous-alkaline layer with dilute hydrochloric acid gave a crystalline solid (80 mg.), which on crystallisation from acetic acid and then from ethanol gave yellow needles, m.p. 155-156° (with effervescence) of an acid, which is probably the saponified product of Claisen condensation of two molecules of starting material (XXXIII). (Found: C, 70.1; H, 3.7; N, 5.6. $C_{32}H_{20}O_7N_2$ requires C, 70.6; H, 3.7; N, 5.2%).

3-Hydroxy-4-azafluoranthene-2-carboxylic acid

(XXX; R = CO_2H).

A mechanically stirred solution of 1-carbethoxy-acetamidofluorenone (5 g.) in anhydrous nitrobenzene (50 cc.) was treated with powdered sodium methoxide (0.96 g.), and then heated at 135-140° for 11 hours. After being cooled, the mixture was treated with concentrated hydrochloric acid (2 cc.), set aside for several hours, and then filtered. The orange, crystalline residue was washed with benzene and boiled under reflux with N-sodium hydroxide solution (50 cc.) for 3 hours. The resulting solution was filtered while hot. Acidification of the hot filtrate with concentrated hydrochloric acid gave a precipitate of practically pure product (3.4 g.; 78%), m.p. 308-310°, which on recrystallisation from acetic acid gave orange needles, m.p. 310° (decomp.).

(Found: C, 72.9; H, 3.5; N, 5.3. $C_{16}H_9O_3N$ requires C, 73.0; H, 3.4; N, 5.3%).

3-Hydroxy-4-azafluoranthene (XXXIV; R = -OH).

A solution of the above carboxylic acid (3.96 g.) in quinoline (15 cc.) was heated to 230° and treated portionwise with basic copper carbonate (0.3 g.), with occasional stirring. The mixture was boiled for 15 minutes longer. After being allowed to cool somewhat, it was poured into 2N-hydrochloric acid (200 cc.). Recrystallisation of the product from acetic acid gave yellow needles (3.0 g.; 90%), m.p. 288-290°. (Found: C, 82.1; H, 4.2; N, 6.4. $C_{15}H_9ON$ requires C, 82.2; H, 4.1; N, 6.4%).

3-Chloro-4-azafluoranthene (XXXIV; R = -Cl).

The above hydroxy compound (3.3 g.) was boiled under reflux with phosphoryl chloride (33 cc.) for 3.5 hours. The resulting solution was cooled and then distilled under reduced pressure from a bath at 30-50°. The residue was treated with crushed ice and dilute ammonia solution and extracted with chloroform. The chloroform extract was washed with water, treated with charcoal, filtered and evaporated. Recrystallisation of the residue from ethanol gave yellow needles (3.2 g.; 90%), m.p. 120-121°. (Found: Cl, 15.1. $C_{15}H_8NCl$ requires Cl, 15.0%).

3-Amino-4-azafluoranthene.

The above chloro compound (0.5 g.) was heated with ethanolic ammonia (5 cc.) at 185-190° for 17 hours in a sealed tube. The reaction mixture was evaporated on the water-bath. The residue was treated with dilute sodium hydroxide solution and extracted with chloroform. The chloroform solution was well washed with water, dried (Na_2SO_4) and then percolated through a column (2 x 10 cm.) of activated alumina, which was then washed with pure chloroform. The main yellow band was eluted by means of 1% methanol-chloroform. Evaporation of the filtrate followed by recrystallisation of the residue from benzene gave short, yellow, prismatic needles (0.3 g.), m.p. 209-211°. (Found: C, 82.7; H, 4.6; N, 12.8. $C_{15}H_{10}N_2$ requires C, 82.6; H, 4.6;, N, 12.8%). The monopicrate formed yellow needles (from acetic acid), m.p. 268-270° (decomp.). (Found: C, 56.4; H, 3.0. $C_{15}H_{10}N_2 \cdot C_6H_3O_7N_2$ requires C, 56.4; H, 2.9%). The monohydrochloride monohydrate was prepared by passing dry hydrogen chloride into a solution of the base in anhydrous chloroform. It formed stout yellow rhombs which were reconverted into the free base on heating to 120-150°. (Found: C, 66.0; H, 4.7. $C_{15}H_{10}N_2 \cdot HCl \cdot H_2O$ requires C, 66.1; H, 4.8%).

3-Acetamido-4-azafluoranthene.

Prepared by boiling the above amino compound with acetic anhydride, it formed pale-buff needles (from methanol), m.p. 233°. (Found: C, 78.3; H, 4.6. $C_{17}H_{12}ON_2$ requires C, 78.5; H, 4.6%).

3-Diethylamino-4-azafluoranthene.

The corresponding chloro compound (0.5 g.) was heated with anhydrous diethylamine (5 cc.) at 190-200° for 16 hours in a sealed tube. The product was a gum which gave a monopicrate, forming yellow needles (from acetone-ethanol), m.p. 210-212° (decomp.). (Found: C, 59.8; H, 4.3; N, 13.8. $C_{19}H_{18}N_2 \cdot C_6H_3O_7N_3$ requires C, 59.6; H, 4.2; N, 13.9%). The free base, on regeneration from the purified picrate by means of treatment with dilute sodium hydroxide solution, was a light-yellow gum, which on treatment of its (moist) ethereal solution with hydrogen chloride gave yellow crystals, m.p. 65-68° (decomp.) of the monohydrochloride trihydrate. (Found: C, 62.3; H, 6.6; Cl, 9.9. $C_{19}H_{18}N_2 \cdot HCl \cdot 3H_2O$ requires C, 62.5; H, 6.9; Cl, 9.7%).

3-β-Diethylaminoethylamino-4-azafluoranthene.

The corresponding chloro compound (0.5 g.) was heated with β-diethylaminoethylamine (0.98 g.) and benzene (1.5 cc.) at 185-190° for 12 hours in a sealed tube. The reaction

mixture was treated with dilute sodium hydroxide solution and extracted with ether. The benzene-ethereal layer, after being washed with water, was extracted with 0.5 N-hydrochloric acid. The extract was basified with dilute sodium hydroxide solution and extracted with ether. The ethereal extract was washed several times with water and evaporated. The residual oil on treatment with a solution of picric acid in ethanol gave a dipicrate which formed yellow prisms (1.0 g.) (from 2-methoxy ethanol), m.p. 235-237° (decomp.). (Found: C, 51.1; H, 3.7; N, 16.3. $C_{21}H_{23}N_3 \cdot 2C_6H_3O_7N_3$ requires C, 51.1; H, 3.7; N, 16.3%). The free base on regeneration from the purified picrate was a light-brown viscous oil, which could not be rendered crystalline. (Found: C, 79.1; H, 7.0. $C_{21}H_{23}N_3$ requires C, 79.5; H, 7.2). Attempted distillation under 1 mm. of a small portion resulted in decomposition. The base was readily soluble in one equivalent of 0.1 N-hydrochloric acid, to give a solution which was neutral to litmus.

3-γ-Diethylaminopropylamino-4-azafluoranthene.

This was prepared in a similar manner by condensation of the chloro compound with γ-diethylaminopropylamine. It was purified through its dipicrate, which formed yellow needles (from 2-methoxy ethanol), m.p. 204-205°. (Found: C, 51.8; H, 3.9; N, 16.1. $C_{22}H_{25}N_3 \cdot 2C_6H_3O_7N_3$ requires C, 51.7; H, 3.9; N, 16.0%). The free base, regenerated from the

purified dipicrate, formed a yellow-brown, fluorescent oil, (Found: C, 79.7; H, 7.7. $C_{22}H_{25}N_3$ requires C, 79.8; H, 7.6%), which dissolved in one equivalent of 0.1 N-hydrochloric acid to give a neutral solution.

3-δ-Diethylamino-α-methylbutylamino-4-azafluoranthene

Prepared by condensation of the chloro compound with δ-diethylamino-α-methylbutylamine. It was purified through its dipicrate, which formed stout, golden rhombs (from acetone-ethanol), m.p. 184-185°, (Found: C, 53.0; H, 4.2; N, 15.4. $C_{24}H_{29}N_3 \cdot 2C_6H_3O_7N_3$ requires C, 52.9; H, 4.3; N, 15.4%) and formed a golden, viscous oil (Found: C, 80.1; H, 8.1. $C_{24}H_{29}N_3$ requires C, 80.2; H, 8.1%) which was soluble in one equivalent of 0.1 N-hydrochloric acid.

2:4-Diazafluoranthene (XXXV).

1-Aminofluorenone (0.5 g.) and purified formamide (5 cc.) were heated under an air condenser to 180-185° for 18 hours. Trituration of the reaction mixture with water, followed by extraction of the resulting brittle, brown gum with warm acetone gave a small quantity (50 mg.) of a sparingly-soluble solid, which could not be satisfactorily recrystallised from solvents. It was purified by means of sublimation at 200-220°/0.2 mm., and formed soft, white needles which charred on heating to 230-240°. (Found: N, 13.7. $C_{14}H_8N_2$ requires N, 13.7%).

3-Phenyl-2:4-diazafluoranthene.

A stream of dry hydrogen chloride was passed into a mixture of 1-aminofluorenone (1 g.) and benzonitrile (5 cc.), heated at 180-185°, during 11 hours. The reaction mixture was distilled in steam. The residue was treated with dilute sodium hydroxide solution and extracted with chloroform. The chloroform solution was washed with water, treated with charcoal, filtered and then evaporated. The residual, brown gum was dissolved in anhydrous benzene (30 cc.), and the solution percolated through a column (3 x 9 cm.) of activated alumina. The column was repeatedly washed with benzene until the main, faintly-yellow band had passed into the filtrate. Evaporation of the latter gave a crystalline residue, which on recrystallisation from ethanol, gave lemon-yellow needles (0.9 g.), m.p. 130-131°. (Found: C, 85.8; H, 4.2; N, 9.9. $C_{20}H_{12}N_2$ requires C, 85.7; H, 4.3; N, 10.0%). The monohydrochloride was prepared by passing dry hydrogen chloride into a solution of the base in benzene. It formed orange crystals, m.p. 151-152° (Found: C, 76.0; H, 3.9. $C_{20}H_{12}N_2 \cdot HCl$ requires C, 75.8; H, 4.1%), which on heating with water were transformed into the free base.

3-p-Chlorophenyl-2:4-diazafluoranthene.

This was prepared by an essentially similar process, using p-chlorobenzonitrile[48] (2.2 g.), dissolved in

anhydrous nitrobenzene (3 cc.), in place of benzonitrile. Purified by means of chromatography, the base formed yellow needles (from ethyl acetate) (yield 60%), m.p. 189°. (Found: C, 76.6; H, 3.6; N, 8.9. $C_{20}H_{11}N_2Cl$ requires C, 76.3; H, 3.5; N, 8.9%). It gave a hemihydrochloride, orange crystals (from benzene-hydrogen chloride), which decomposed on heating to 160-170°, to give the free base. (Found: C, 72.0; H, 3.6; N, 8.5. $C_{20}H_{11}N_2Cl.\frac{1}{2}HCl$ requires C, 72.1; H, 3.5; N, 8.4%).

3-*p*-Nitrophenyl-2:4-diazafluoranthene.

Prepared by passing hydrogen chloride into a mixture of 1-aminofluorenone (2 g.), *p*-nitrobenzonitrile[49] (1.7 g.) and nitrobenzene (5 cc.), heated at 180-190°, during 10 hours. The tarry product could not be purified by means of chromatography owing to its insolubility in the common organic solvents. It formed light-tan needles (from xylene) (yield 27%), m.p. 275°. (Found: C, 74.0; H, 3.4; N, 12.8. $C_{20}H_{11}O_2N_3$ requires C, 73.8; H, 3.4; N, 12.9%).

3-*p*-Aminophenyl-2:4-diazafluoranthene.

A suspension of the finely powdered nitro compound (0.89 g.) in ethanol (20 cc.) containing 1 N-hydrochloric acid (1.25 cc.) was boiled under reflux and treated with small portions of iron powder (0.9 g.). The mixture was boiled under reflux on the water-bath for 3 hours, diluted

with ethanol (25 cc.), treated with 1 N-sodium hydroxide solution (1.3 cc.), heated to the b.p. and then filtered. The residue was extracted with hot ethanol and the combined filtrate and extracts evaporated on the water bath. The residual red syrup was dissolved in chloroform (50 cc.) and the solution washed with water, dried (Na_2SO_4) and then percolated through a column (3 x 4 cm.) of activated alumina. The column was thoroughly washed with alcohol-free chloroform. The combined filtrates were evaporated on the water-bath and the residual crystalline solid recrystallised from ethanol to give orange-red needles (0.7 g.), m.p. 211°.
(Found: C, 81.3; H, 4.4; N, 14.1. $C_{20}H_{13}N_3$ requires C, 81.4; H, 4.4; N, 14.2%).

Treatment of a dilute solution of the analytically pure amino-compound, in ethanol, with 2 N-ethanolic hydrogen chloride, gave a precipitate of minute, purple needles, m.p.258-260°, of the hydrochloride hydrate (Found: C, 66.9; H, 4.3; N, 12.1; Cl, 12.0. $C_{20}H_{13}N_3 \cdot 1\frac{1}{4}HCl \cdot H_2O$ requires C, 66.9; H, 4.5; N, 11.7; Cl, 12.4%), which could not be satisfactorily recrystallised owing to its insoluble nature. On treatment with dilute ammonia solution it gave back the free base. On prolonged boiling of a very dilute solution of the hydrochloride in saturated ethanolic hydrogen chloride solution, the original deep-purple colour of the solution was discharged. The product, which was obtained by concentrating the resulting

light-yellow solution, formed minute yellow crystals (from ethanol), m.p. 231-232°, which had the composition of the hydrochloride of a p-aminobenzamidofluorenone (Found: C, 68.6; H, 4.4; N, 8.4. $C_{20}H_{14}O_2N_2 \cdot HCl$ requires C, 68.5; H, 4.3; N, 8.0%), but whose nature remains obscure.

3-p-Acetamidophenyl-2:4-diazafluoranthene.

Prepared by boiling under reflux a suspension of the amine (430 mg.) in benzene (13 cc.) containing acetic anhydride (0.3 cc.). It formed orange needles (450 mg.) (from ethanol), m.p. 244°. (Found: C, 78.2; H, 4.4; N, 12.6. $C_{22}H_{15}ON_3$ requires C, 78.3; H, 4.5; N, 12.5%).

Attempts to prepare quaternary ammonium salts of 3-phenyl-2:4-diazafluoranthene.

(i) The compound was recovered unchanged after prolonged boiling of its solution in a mixture of benzene and methyl iodide.

(ii) A solution of the compound (300 mg.) in anhydrous xylene (3 cc.) was heated to 140° (bath temperature) and then treated with purified dimethyl sulphate (1.62 g.). The solution became pink and after a few minutes a red oil separated. The mixture was distilled in steam. The aqueous phase was filtered while hot from an insoluble gum, and on evaporation under reduced pressure left no appreciable residue.

The gum, on treatment with ethanol gave a crystalline product (100 mg.; m.p. 150-152°) which on repeated recrystallisation from ethanol gave long yellow needles, m.p. 153-154°, which had the composition of a benzamidofluorenone. (Found: C, 80.6; H, 4.6; N, 4.6. $C_{20}H_{13}O_2N$ requires C, 80.3; H, 4.4; N, 4.7%). It contained no sulphur. The same compound was obtained by treatment of the starting material in nitrobenzene at 165-170° with dimethyl sulphate and was the only product which could be isolated in a crystalline state. It showed a marked mixed m.p. depression, a) on admixture with a sample, m.p. 150-152°, of 1-benzamidofluorenone (mixed m.p. 115-120°) which was obtained by benzoylation of 1-aminofluorenone[32], and b) on admixture with a sample, m.p. 163-165°, of 1-benzamidofluorenol (mixed m.p. 125-128°) prepared as described below.

1-Benzamidofluorenol.

1-Benzamidofluorenone[32] (200 mg.) was boiled under reflux with a solution of aluminium isopropoxide (500 mg.) in anhydrous toluene (5 cc.) for 4 hours, and the solution concentrated by slow distillation during 2 hours longer. The mixture was diluted with benzene and washed with dilute hydrochloric acid. The organic phase was then washed with water and evaporated under reduced pressure. Crystallisation of the residue from benzene gave faintly yellow, elongated

prisms, m.p. 163-165° (Found: C, 79.6; H, 5.1; N, 5.0. $C_{20}H_{15}O_2N$ requires C, 79.7; H, 5.0; N, 4.7%), which showed a marked mixed m.p. depression on admixture with a sample of starting material.

Reaction of NN'-di(1-fluorenonyl) urea (XXXIX) with hydroxylamine.

The sym-urea derivative (1 g.), suspended in purified pyridine (50 cc.), was boiled under reflux with hydroxylamine hydrochloride (0.67 g.) for 2 hours. The resulting solution was concentrated to small bulk by distillation under reduced pressure and then poured into water. The product (0.95 g.; m.p. 220-222°) on repeated recrystallisation from aqueous ethanol, gave yellow rhombs, m.p. 226-228°, of the trioxime hemihydrate. (Found: C, 68.7; H, 4.2; N, 14.5. $C_{27}H_{19}O_3N_5 \cdot \frac{1}{2}H_2O$ requires C, 68.9; H, 4.3; N, 14.9%).

Attempted reduction of the trioxime hemihydrate.

Zinc dust (0.65 g.) was added portionwise to a mechanically stirred mixture, heated under reflux to its b.p., of the above product (0.45 g.), acetic acid (10 cc.), and water (1 cc.). The mixture was boiled under reflux for 3 hours longer, and then filtered while it was hot. The filtrate (from which a quantity of zinc acetate trihydrate separated

on cooling) was evaporated under reduced pressure. The residue, on trituration with N-sodium hydroxide solution, gave a non-basic, pale-buff, amorphous solid (0.39 g.), m.p. 170-185°, which could not be rendered crystalline by solvent treatment.

Action of formamide on NN'-di(1-fluorenonyl)-urea.

The sym-urea derivative (3 g.) was boiled under reflux with purified formamide (21 cc.) for 90 minutes. Vigorous frothing occurred at first. The material which crystallised on cooling (1.36 g.) was washed with methanol and recrystallised (charcoal) from ethanol, giving white leaflets (1.2 g.), m.p. 230-233°, of di(9-formamido-1-fluorenylamino)-formamido-methane (XLIII). (Found: C, 71.6; H, 4.6; N, 14.3; $C_{30}H_{25}O_3N_5$ requires C, 71.6; H, 5.0; N, 13.9%).
A further quantity (80 mg.) of the compound, and also some 1-aminofluorenone (0.4 g.), were obtained by working up the formamide mother liquors.

Hydrolysis of the triformyl derivative (XLIII).

The above compound (600 mg.) was boiled under reflux with concentrated hydrochloric acid (18 cc.) for 10 minutes. The resulting solution, on cooling, deposited large, colourless rhombs. These were filtered off, recrystallised from 5N-hydrochloric acid, and then from ethanol-ether, giving

glittering rhombs (0.61 g.), which decomposed on heating to 210-220°, of di(9-amino-1-fluorenylamino)-methylamine trihydrochloride monohydrate (XLIV). (Found: C, 58.9; H, 5.2; N, 12.1; Cl, 19.2. $C_{27}H_{25}N_5 \cdot 3HCl \cdot H_2O$ requires C, 59.3; H, 5.4; N, 12.8; Cl, 19.5%). The compound was readily soluble in water. Basification of its aqueous solution with dilute ammonia solution gave a solid which on recrystallisation from ether-petroleum ether (b.p. 60-80°), gave minute, yellow rhombs, m.p. 97-99°, with previous sintering, of the corresponding base. (Found: C, 77.5; H, 5.7; N, 13.9, 15.2. $C_{27}H_{25}N_5$ requires C, 77.3; H, 6.0; N, 16.7%). The base gave a dipicrate which formed yellow needles (from ethanol), m.p. 215-220° (decomp.). (Found: C, 53.4; H, 3.6; N, 15.9. $C_{27}H_{25}N_5 \cdot 2C_6H_3O_7N_3$ requires C, 53.4; H, 3.5; N, 17.5%).

(The formation of charcoals which resisted combustion was observed during the course of Dumas nitrogen estimations of the base and its dipicrate).

Bibliography.

1). Lourie and Yorke, Ann.Trop.Med.Parasit., 1937, 31, 435.

2). King, Lourie and Yorke, Lancet, 1937, (2), 1360.

3). King, Lourie and Yorke, Ann.Trop.Med.Parasit., 1938, 32, 177.

4). Ewins et al., J., 1942, 103.

5). Fulton and Yorke, Ann.Trop.Med.Parasit., 1942, 36, 131.

6). Yorke, Brit.Med.Bull., 1944, 2, 60.

7). Heathcote, J.Trop.Med. and Hyg., 1946, 49, 1.

8). Morgan and Walls, J., 1931, 2447.

9). Browning et al., Proc.Roy.Soc., 1933, 113 B, 300,293 ibid, 1932, 110 B, 249, 372 and previous papers.

10). Jensch, Angew.Chem., 1937, 50, 891.

11). Browning et al., J.Path.Bact., 1938, 46, 203.

12). Browning, Walls et al., J., 1938, 389; 1945, 294; 1946, 1031; 1947, 67; 1948, 188.

13). Walls, J.S.C.I., 1947, 66, 182.

14). Calver, Trop.Dis.Bul., 1945, 42, 704.

15). Kirkpatrick and Parker, J.Amer.Chem.Soc., 1935, 57, 1123.

16). Barber and Slack, J., 1945, 167.

17). Hoffmeister, Annalen, 1871, 159, 215.

18). McCombie, MacMillan and Scarborough, J., 1931, 536.

19). Pinner, Ber., 1877, 10, 1889; "Die Imido Äther".

20). Houben and Fischer, "Das Anthracen und die Anthrachinone", Thieme, Leipzig, 1929, p.643, 650.

21). DR - P 192 201

22). DR - P 203 752

23). DR - P 209 033

24). DR - P 290 984

25). DR - P 212 204

26). Brit. - P 385 295

27). Brit. - P 386 861

28). Brit. - P 415 069

29). Brit. - P 412 005

30). Fieser and Seligman, J.Amer.Chem.Soc., 1935, 57, 2174.

31). Goldschmidt, Monatsh., 1902, 23, 886.

32). Huntress, Pfister and Pfister, J.Amer.Chem.Soc., 1942, 64, 2845.

33). Wolff, in "Organic Reactions", Vol.III, Wiley, New York, 1946.

34). Smith, ibid., p.337.

35). Dupont, Bull.soc.chim.Belge., 1943, 52, 7. Chem.Abs., 1944, 38, 5215.

36). Rieveschl and Ray, Chem.Rev., 1938, 23, 378.

37). Cook and Iball, Chem. and Ind., 1936, 55, 467.

38). Ray and Krieser, J.Amer.Chem.Soc., 1947, 69, 3068.

39). Misani and Bogert, J.Org.Chem., 1945, 10, 347, 351.

40). Quenda, Ber., 1892, 25, Ref.326.

41). Naegeli and Tyabji, Helv.Chim.Acta, 1933, 16, 349: ibid., 1934, 17, 931.

42). Ingold and Wilson, J., 1933, 1499.

43). Ingersoll et al., J.Amer.Chem.Soc., 1936, 58, 1808.

44). Robinson, "Outline of an Electronic Theory of the Course of Organic Reactions", Institute of Chem.Lectures, 1932.

45). Jones, Ann.Trop.Med.Parasit., 1946, 40, 130.

46). Phelps and Tillotson, Ch.Abs., 1909, 3, 1533.

47). Marguery, Bull.soc.chim., 1905, [3], 33, 544.

48). von Scherpenzeel, Rec.trav.chim., 1897, 16, 106.

49). Bogert and Kohnstamm, J.Amer.Chem.Soc., 1903, 25, 479.