STUDIES

OF

FUSICOCCIN CHEMISTRY

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A B S T R A C T

A new metabolite has been isolated from the culture filtrates of the fungus *Fusicoccum amygdali* Del. The constitution and stereochemistry of this new compound has been established by its synthesis from fusicoccin A.

This new fusicoccin has been labelled with tritium at C-8 and fed to the fungus *Fusicoccum amygdali* Del. It was specifically incorporated into fusicoccin A with efficiencies of up to 20.6%.

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To Dominiko Ssalongo and Bitoria Nnalongo

and

Cosmas Ssalongo and Puddy Nnalongo

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R E V I E W

INTRODUCTION

Many natural products contain a repeated isopentane skeleton. These compounds include the mono-, sesqui-, di-, ses- and tri- terpenoids containing respectively, 10, 15, 20, 25, and 30 carbon atoms, as well as the sterols, carotenoids, the natural rubbers, the polyprenols, and any substances containing terpenoid elements in their molecules.

Several theories[1-5] on terpenoids have been put forward. Of these, the "Isoprene Rule" has proved a very useful guide in the elucidation of structures of terpenoids. However, there have been exceptions[1,2] to the "Isoprene Rule."

Biosynthetic studies[6-16] using isotopic labelling, showed that a number of substances e.g. leucine, isovaleric acid, acetone, acetate, acetoacetate and 3-hydroxy-3-methylglutaric acid, could be incorporated into terpenoids. However, later work[16-21] suggested there might be a redistribution of the label, since 3-hydroxy-3-methylglutaric acid was in rapid equilibrium, in various biological systems, with 3-methylcrotonic, 3-hydroxy-isovaleric and 3-methylglutaconic acids, as well as with acetyl- and acetoacetylcoenzyme A.

The key substance in the biosynthesis of terpenoids, was discovered by Skeggs et al.[22-24] in distillers' soluble residues. This compound, 3-hydroxy-3-methylpentano-5-lactone (mevalonic acid lactone) has been investigated extensively in the biosynthetic studies of terpenoids.

Tavermina et al[25] observed that synthetic [2 - C^{14}]- DL - mevalonic acid was incorporated by liver preparations, into cholesterol with an efficiency of 43%. The carboxyl carbon (C - 1) was eliminated as CO_2, in converting mevalonic acid to cholesterol.[26,27] These observations indicated that mevalonic acid gives rise to a branched chain C_5 intermediate which is used as such, in the biosynthesis of squalene or sterols.

Gould et al.[28] reported that after administration of DL — [2 - ^{14}C]- mevalonic acid to mice, 40% of the radioactive dose could be recovered in the body cholesterol, 10% in the expired CO_2, and 50% in urine.

Cornforth et al[29] showed [2 - ^{14}C]-mevalonic acid to be a precursor of squalene. Chemical degradation of the resulting [^{14}C] squalene[29,30] showed that squalene contained only six labelled positions in the chain of the molecule The branched methylcarbons of squalene were not labelled. This indicated that all the carbon atoms in mevalonic acid retained their individuality during the biosynthesis. It was also established that isoprene is not an intermediate in going from mevalonic acid to squalene or sterol[29].

The ^{14}C/^{3}H ratio of squalene biosynthesized from [2-^{14}C:5-^{3}H] mevalonic acid by particle-free extract of baker's yeast,[31] was the same as in the mevalonic acid. This excluded the possibility that C-5 of the mevalonic acid was oxidised to carboxyl before condensation.

It was observed[32-34] that C-2 of mevalonic acid is not incorporated into the angular methyl groups of squalene, but is located entirely in the main chain of squalene. It was also shown[35] that the "biological isoprene unit" is not derived from mevalonic acid by oxidative reactions.

The overall conversion of mevalonic acid to squalene requires ATP, Mn^{++} and reduced pyridine nucleotide as cofactors.[36,37] Previously, it had been observed that fractionation[38] of a crude extract of yeast, gave fractions A and B, both of which were required for the conversion of mevalonic acid to squalene (in presence of ATP, Mn^{++} and reduced pyridine nucleo tide). Fraction A was shown[39] to catalyse the phosphorylation of mevalonic acid by ATP. The enzyme in fraction A was shown[40] to be mevalonic kinase, which catalyses the formation of phosphomevalonic acid:

mevalonic acid + ATP ⟶ phosphomevalonic acid + ADP.

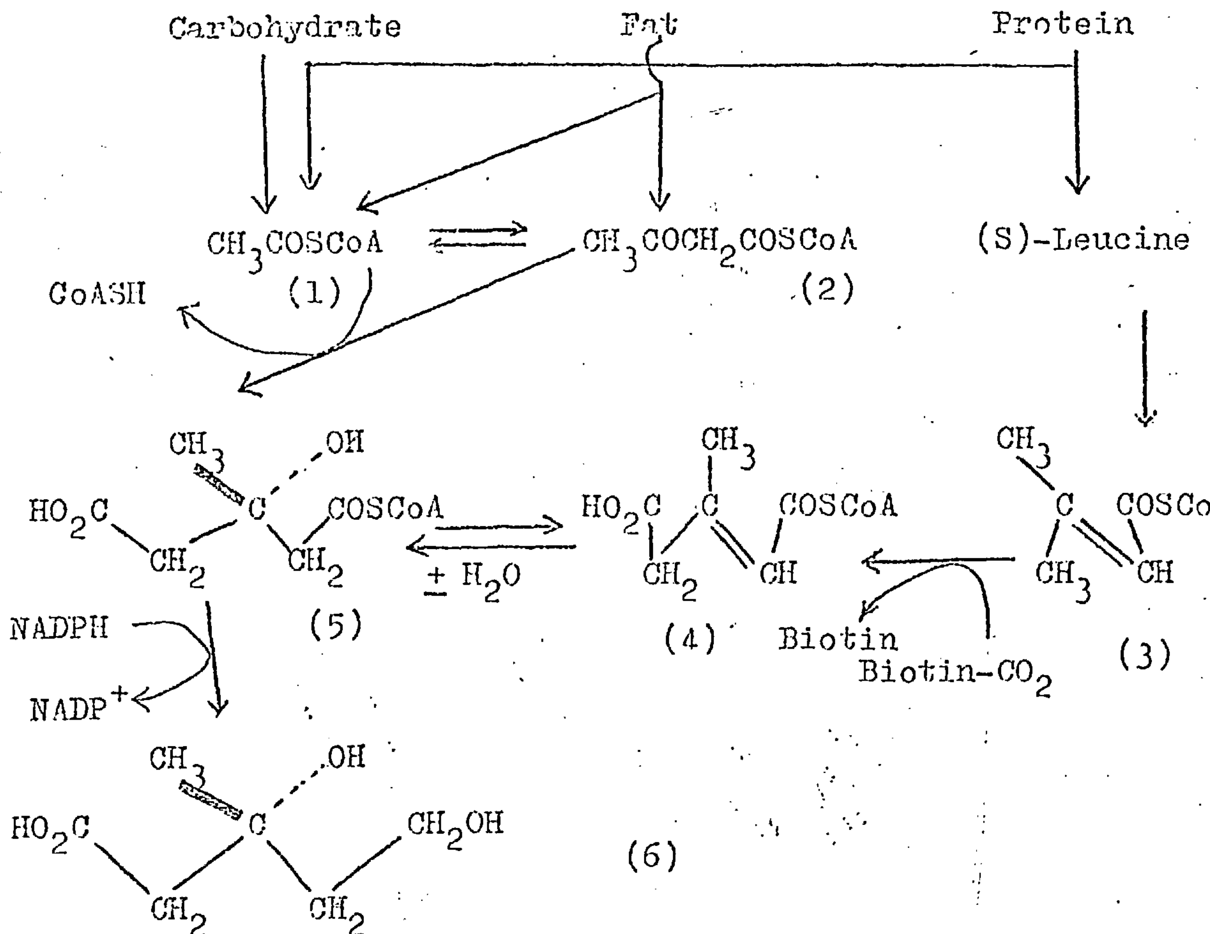
In the presence of ATP, phosphomevalonic acid was shown to be converted to another mevalonic acid derivative[41] which gave rise to squalene in the absence of ATP. Tchen[40] therefore suggested that phosphomevalonic acid is the first intermediate in the transformation of mevalonic acid to squalene.

It was reported[42] that the "biological isoprene unit" which undergoes condensation, possesses terminal methylene groups which arise by elimination of water and phosphate, from mevalonic acid or its phosphorylated derivatives. Tchen[40] suggested that the purpose of phosphorylation of mevalonic acid is to facilitate the elimination of the hydroxyl group, since phosphate esters are better leaving groups than hydroxyls.

It has been shown[43] that a protein fraction of yeast extract catalyses the synthesis from mevalonate, of farnesyl pyrophosphate which was converted to squalene on addition of yeast cell particles and reduced pyridine nucleotide. Lynen et al[44] reported that 3, 3-dimethylallyl pyrophosphate and geranyl pyrophosphate were intermediates preceding the formation of farnesyl, pyrophosphate. 5-Phosphomevalonate and 5-diphosphomevalonate were isolated from liver-enzyme preparations[45,46]. Both substances were shown to be more

effective precursors of cholesterol than DL-mevalonic acid itself. These observations were followed by the discovery[47,48] of Δ^3-isopentenyl-pyrophosphate, geranylpyrophosphate and farnesylpyrophosphate in the biosynthesis of squalene. Geraniol and farnesol were synthesized[49] from mevalonate-2-^{14}C and from (R)5-phosphomevalonate-2-^{14}C, with soluble liver-enzymes.

Today it is believed that terpenoid biosynthesis is initiated by the enzymic reduction of (S)-3-hydroxy-3-methylglutaryl coenzyme A(5), as shown in scheme 3. Two hydrogens are transferred from reduced nicotinamide-adenine dinucleotide phosphate (NADPH). This reduction producing (R)-mevalonic acid (6), is not easily reversible. So far as is known, mevalonic acid is produced by no other pathway and is used solely for terpenoid synthesis. In contrast, (S)-3-hydroxy-3-methyl glutaryl coenzyme A (5) is produced by several biosynthetic pathways.



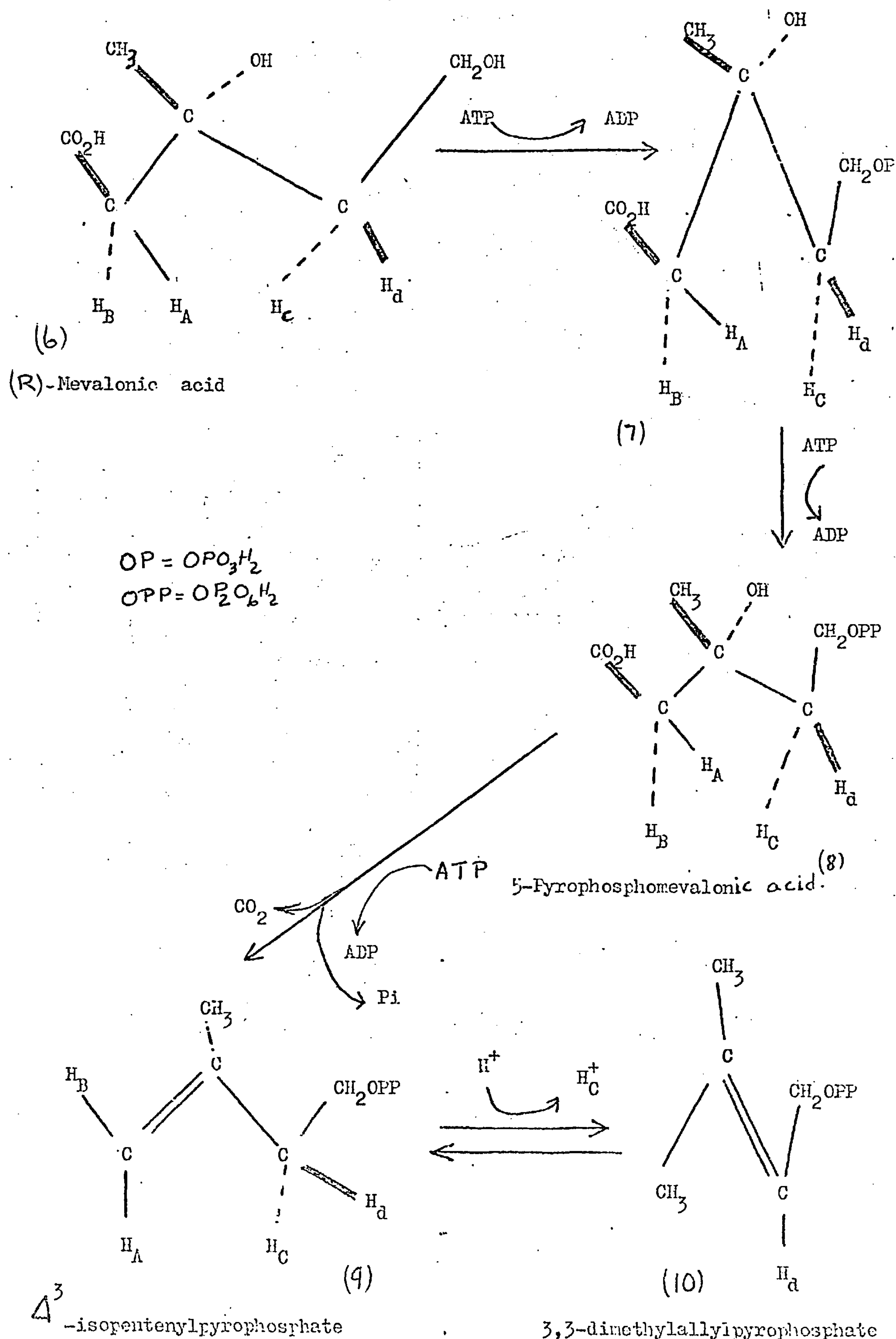
Scheme 3.

In the next two enzymic processes, (R)-mevalonic acid[40] is phosphorylated to produce successively, 5-phosphomevalonic acid (7) and 5-pyrophosphomevalonic acid (8). In each case the donor is the terminal phosphoryl group in adenosine triphosphate (ATP).

5-Pyrophosphomevalonic acid (8) reacts on the enzyme with adenosine triphosphate to give adenosine diphosphate (ADP), inorganic phosphate, CO_2, and Δ^3-isopentenylpyrophosphate (9).[40a] The oxygen of the tertiary hydroxyl group was found in the inorganic phosphate after the reaction.[51] This suggests the phosphorylation of this hydroxyl group by ATP before elimination, but no intermediate phosphate has been detected. The new double bond is formed by a concerted elimination rather than by dehydration followed by decarboxylation; for no hydrogen from the aqueous medium appears in the product. This process is a trans-elimination,[54] as shown in scheme 4.

In the next step Δ^3-isopentenylpyrophosphate (9) is converted, by a prototropic shift, into 3,3-dimethylallylpyrophosphate (10). The equilibrium in this reversible reaction, leans heavily towards (10). The elimination of a proton in the change (9)→ (10) is stereospecific, the hydrogen atom H_c being the one eliminated. Thus, (9) with a relatively unreactive pyrophosphoryl group and a nucleophilic double bond, is converted into a highly reactive electrophilic allylpyrophosphate (10).

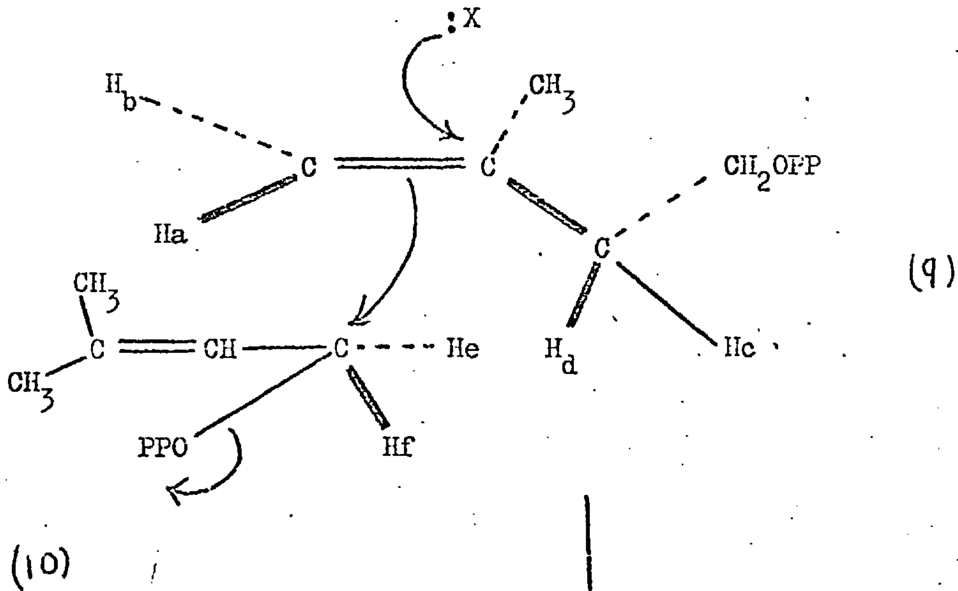
Scheme 4.



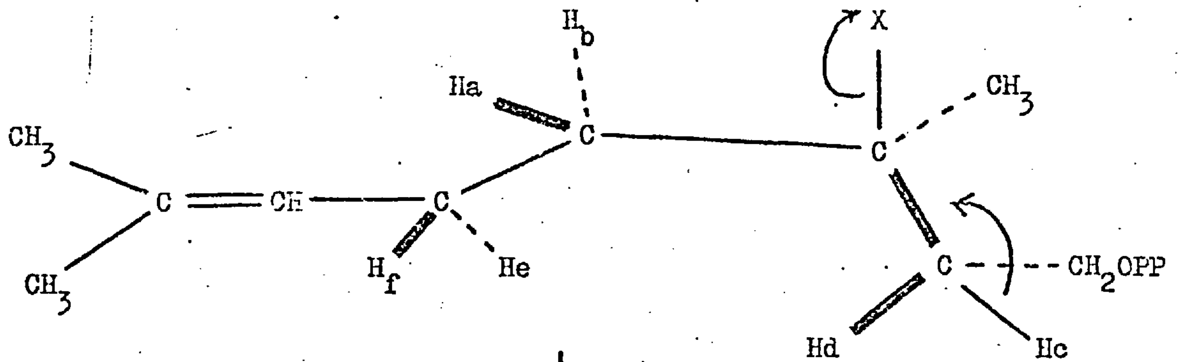
Both (9) and (10) are joint substrates for the enzyme catalysing the next stages. The process is like polymerization in some respects, (10) being the initiating and (9) the propagating, species. The combination of these two molecules, with loss of a pyrophosphate ion from one of them and a hydrogen ion from the other, produces geranylpyrophosphate (11) (scheme 5a). This product then replaces (10) in a further, exactly similar, reaction with (9) to form farnesylpyrophosphate, the precursor of sesquiterpenes.

Experiments using asymmetric labelling with hydrogen isotopes[55] have shown that establishment of the new carbon-to-carbon bond above, is accompanied by complete inversion of configuration at the allylic carbon atom, indicating a bimolecular nucleophilic substitution reaction. Further, the fixed stereochemical relation between addition of the allylic C_5 unit and elimination of the hydrogen ion suggests that the enzymic process proceeds in two distinct steps: (i) trans-addition of the allylic unit and of an electron donating group X, and (ii) trans-elimination of X and of the hydrogen ion, as shown in scheme 5a.

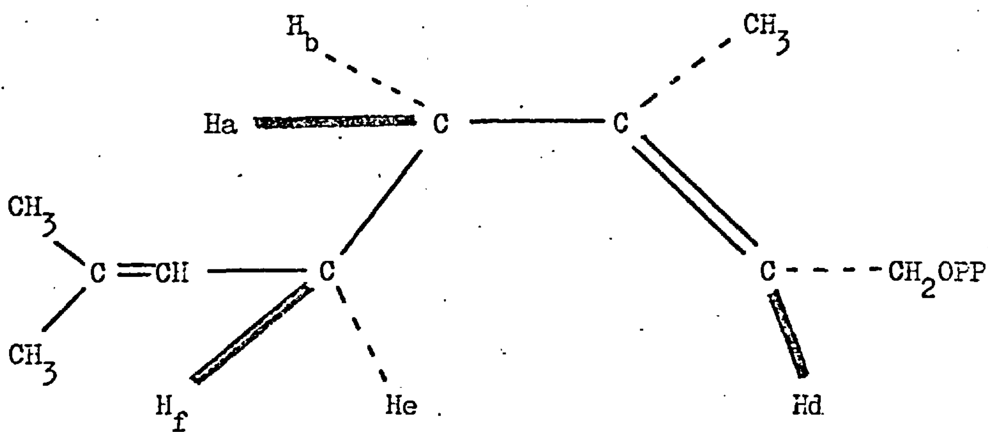
In the biosynthesis of rubber, the elimination of hydrogen ion produces a cis double bond and the hydrogen atom eliminated is the prochiral hydrogen[87] H_d in scheme 5b. This result could be produced by a simple change in orientation of the substrate (9) on the enzyme without any alteration in the mechanism of reaction, as shown in scheme 5b.



(10)

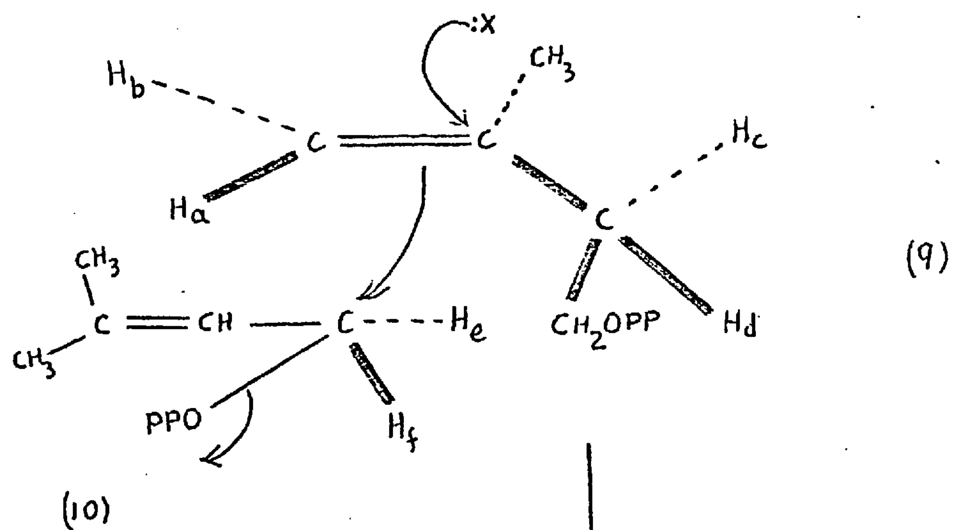


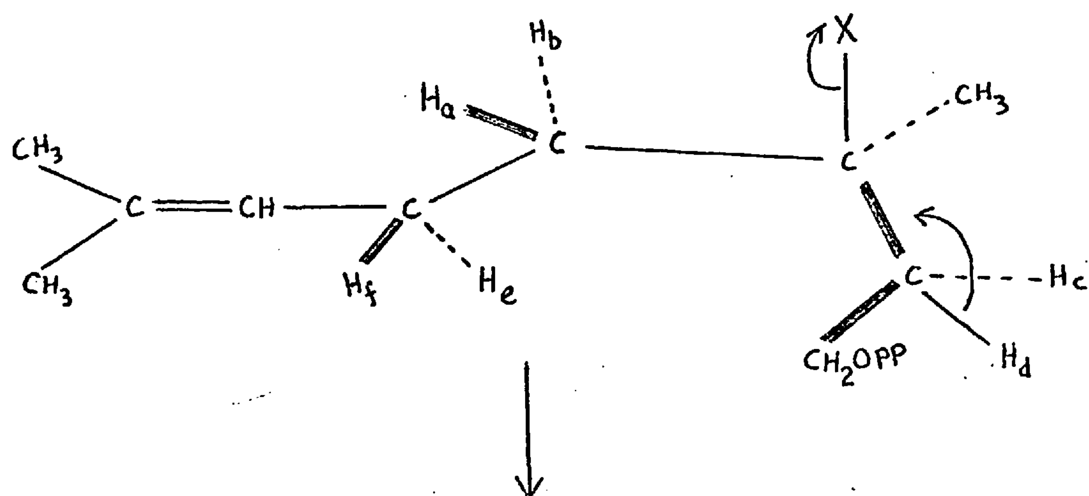
OPP = $OP_2O_6H_2$

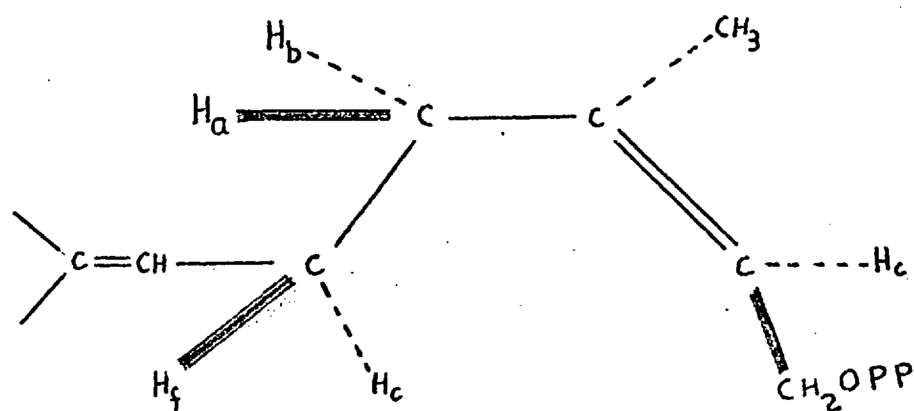


(11) geranylpyrophosphate

Scheme 5a







Scheme 5b

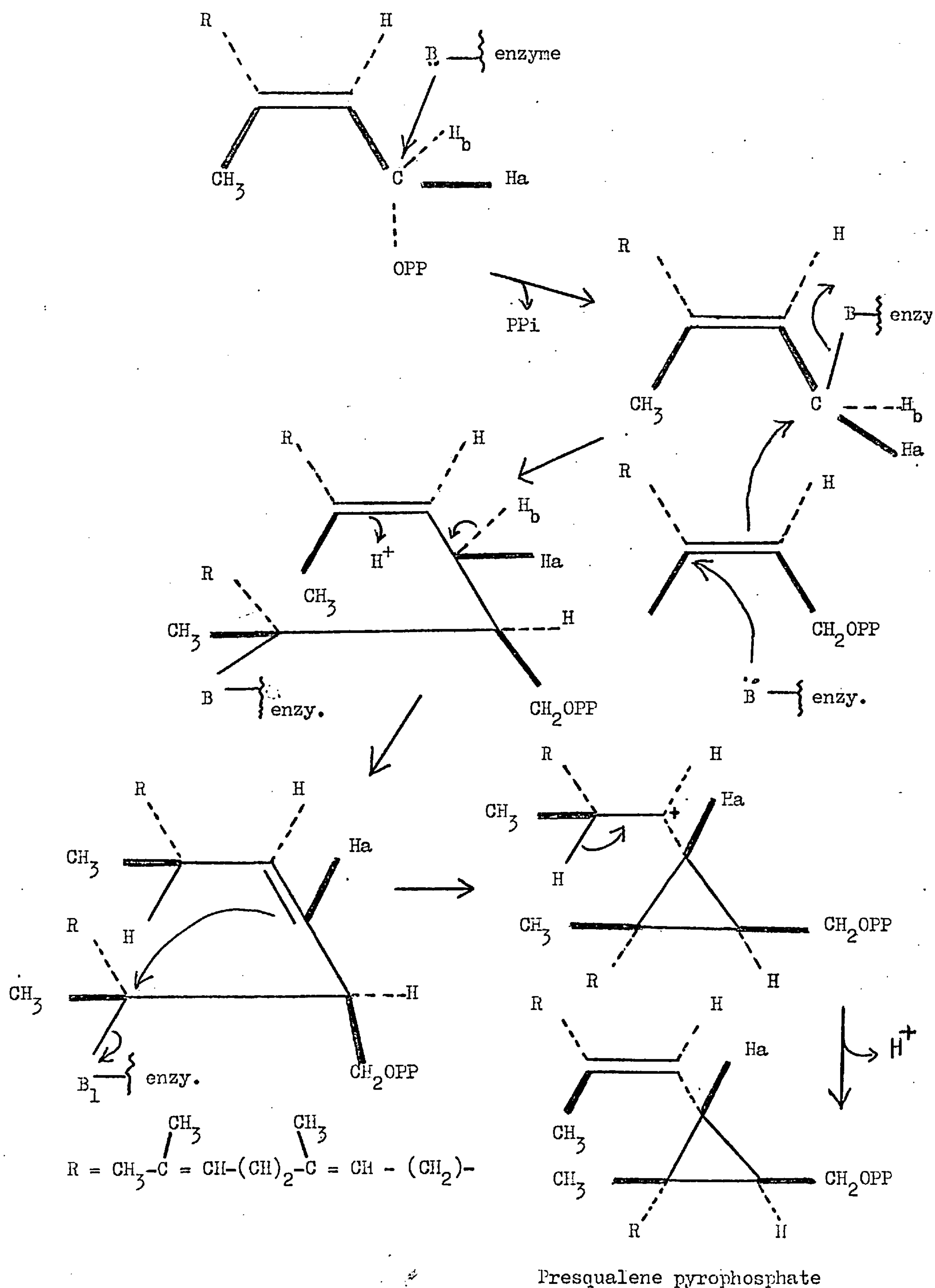
Two molecules of farnesyl pyrophosphate can condense to yield presqualene pyrophosphate[88] as shown in Scheme 6. This substance gives rise to triterpenes.

Coupling of farnesyl pyrophosphate with one molecule of Δ^3-isopentenyl pyrophosphate yields geranylgeranyl pyrophosphate. This substance can cyclise with elimination of the pyrophosphate group to give diterpenes.

Two molecules of geranylgeranyl pyrophosphate can condense to give prelycopersene pyrophosphate[89] as shown in Scheme 7. This substance gives rise to carotenes.

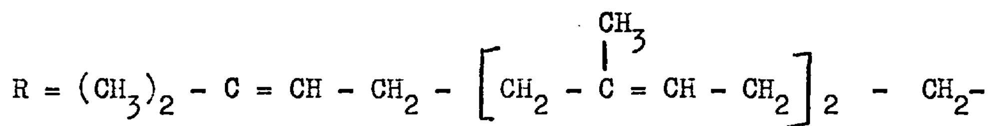
Condensation of geranylgeranyl pyrophosphate with Δ^3-isopentenyl pyrophosphate gives rise to geranylfarnesyl pyrophosphate. This compound yields sesterterpenes. Scheme 8 is a summary of the biosynthesis of terpenes.

Scheme 6.



prelycopersene pyrophosphate

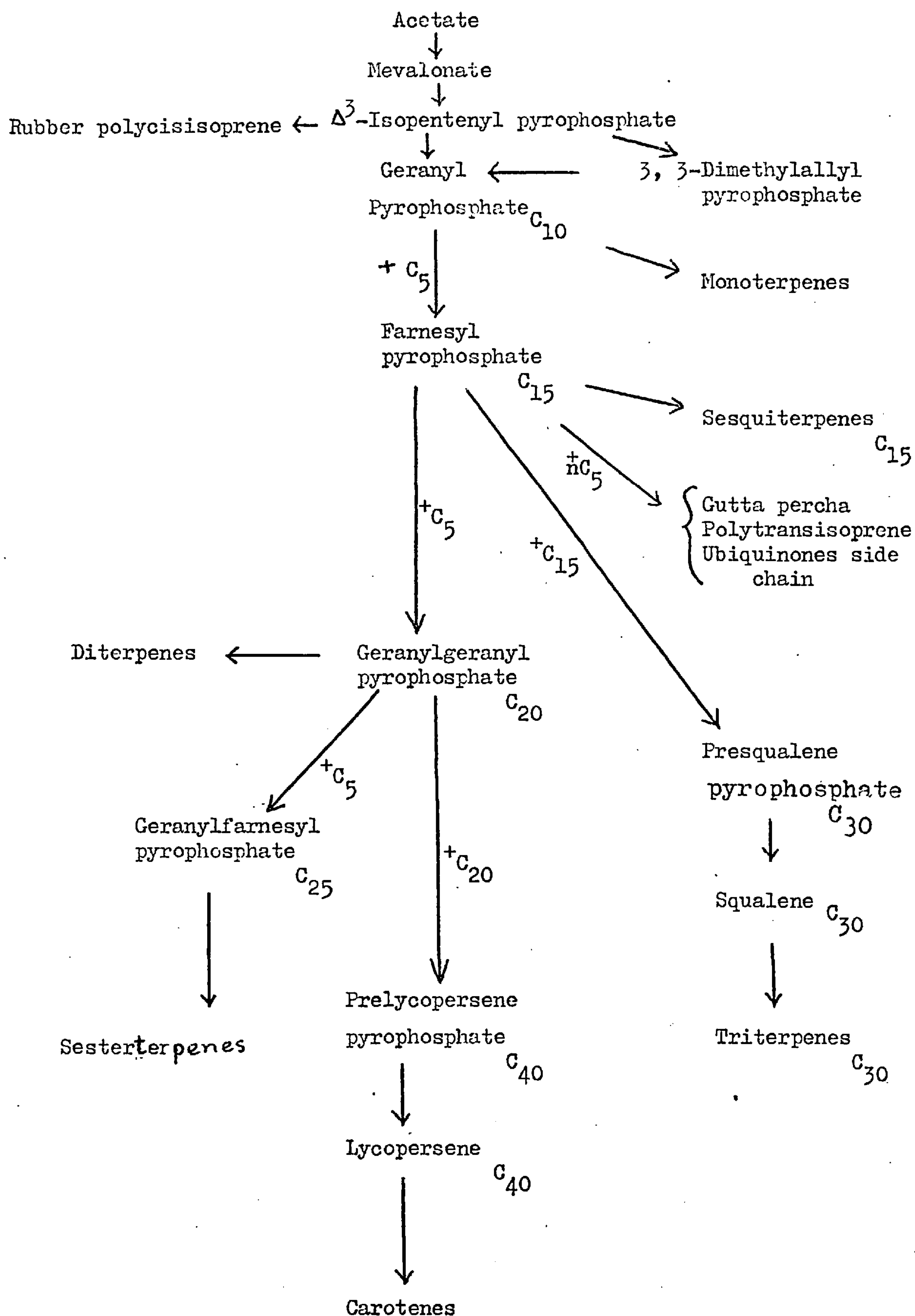
Lycopersene



$$R = (CH_3)_2 - C = CH - CH_2 - \left[CH_2 - \overset{\displaystyle CH_3}{\overset{|}{C}} = CH - CH_2 \right]_2 - CH_2 -$$

H* = Hydrideion from N A D P H

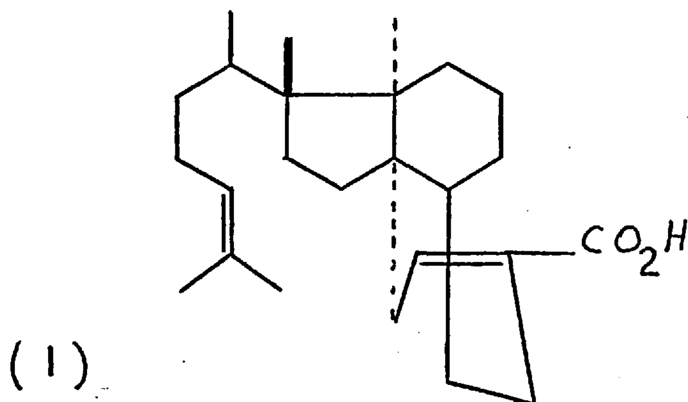
Scheme 7.



Scheme 8.

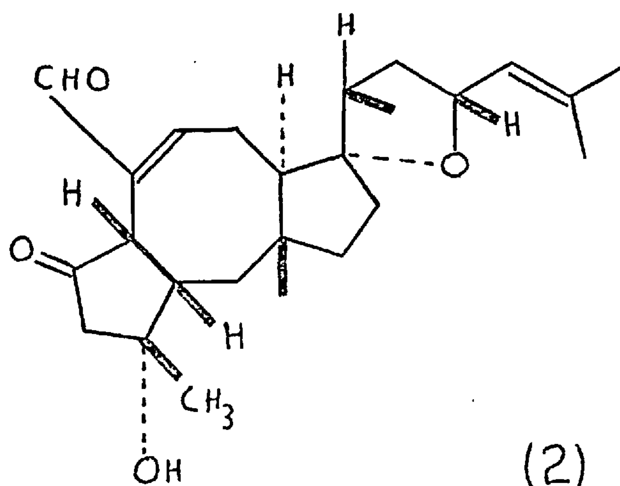
SESTERTERPENES

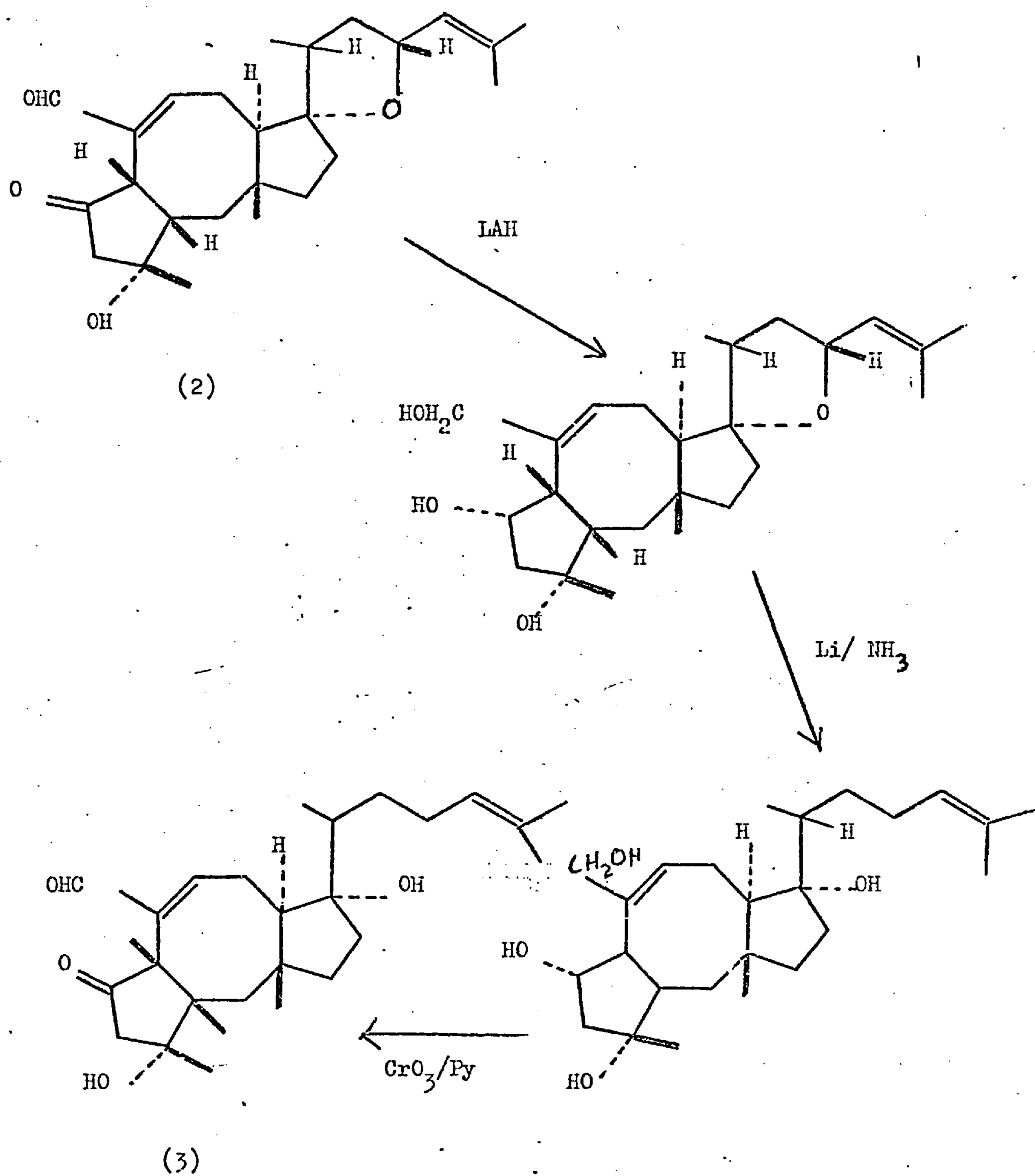
Sesterterpenes are believed to originate from geranylfarnesyl pyrophosphate. Gascardic acid (1) below was isolated from the secretion of the



insect Gascardia madagascariensis[90]. The structure of this compound was established by degradative studies and biogenetic reasoning.

Compounds called ophiobolins have been isolated from the fungus Ophiobolus (cochliobolus) miyabeanus and Helminthosporium species of fungi. Ophiobolin A (2) was first isolated by Ishibashi and Nakamura[91].





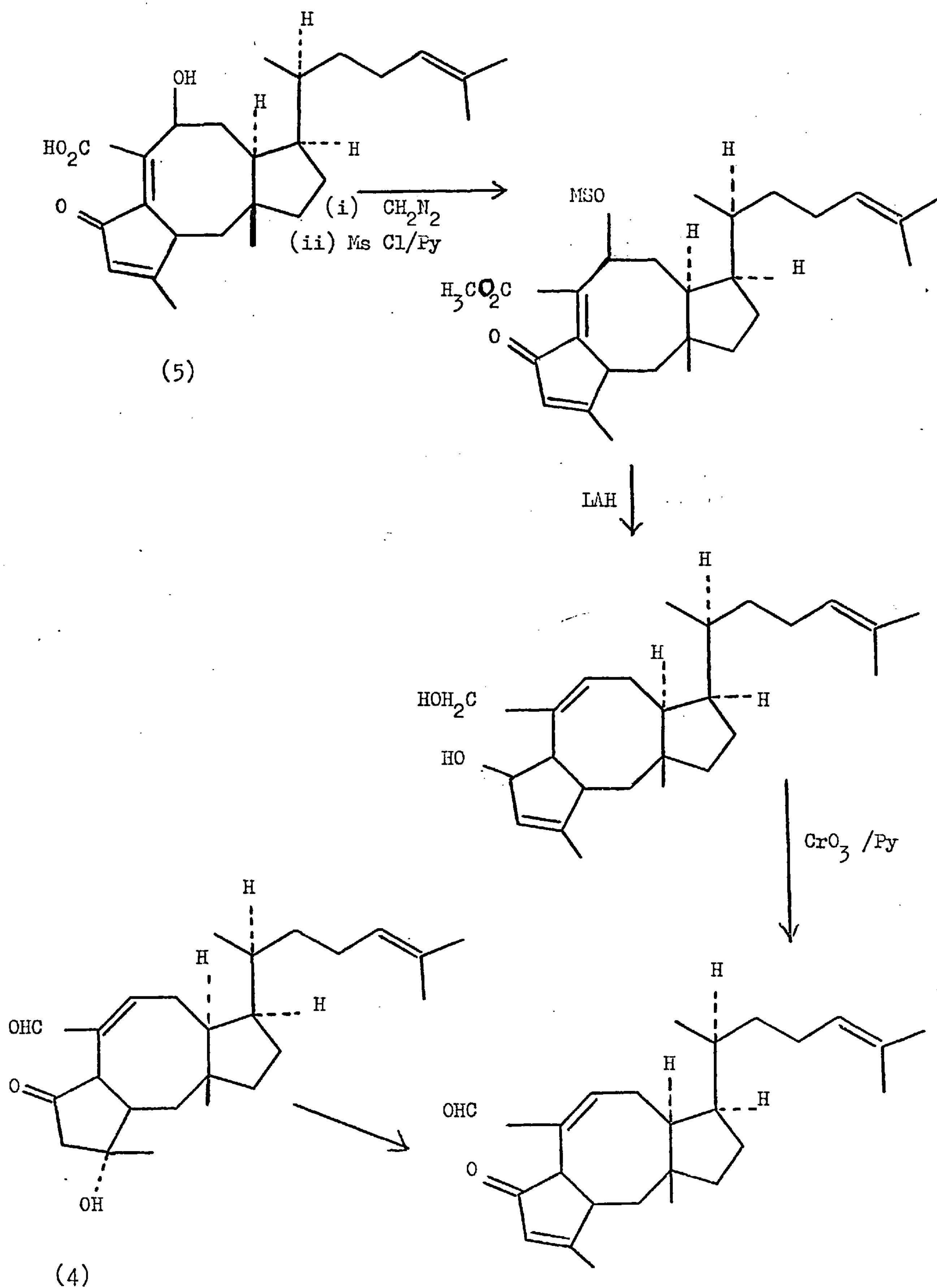
Scheme 9.

The structure and absolute configuration of this compound was established by Nozoe et al,[91] from the X-ray crystallographic data of its bromomethoxy derivative.

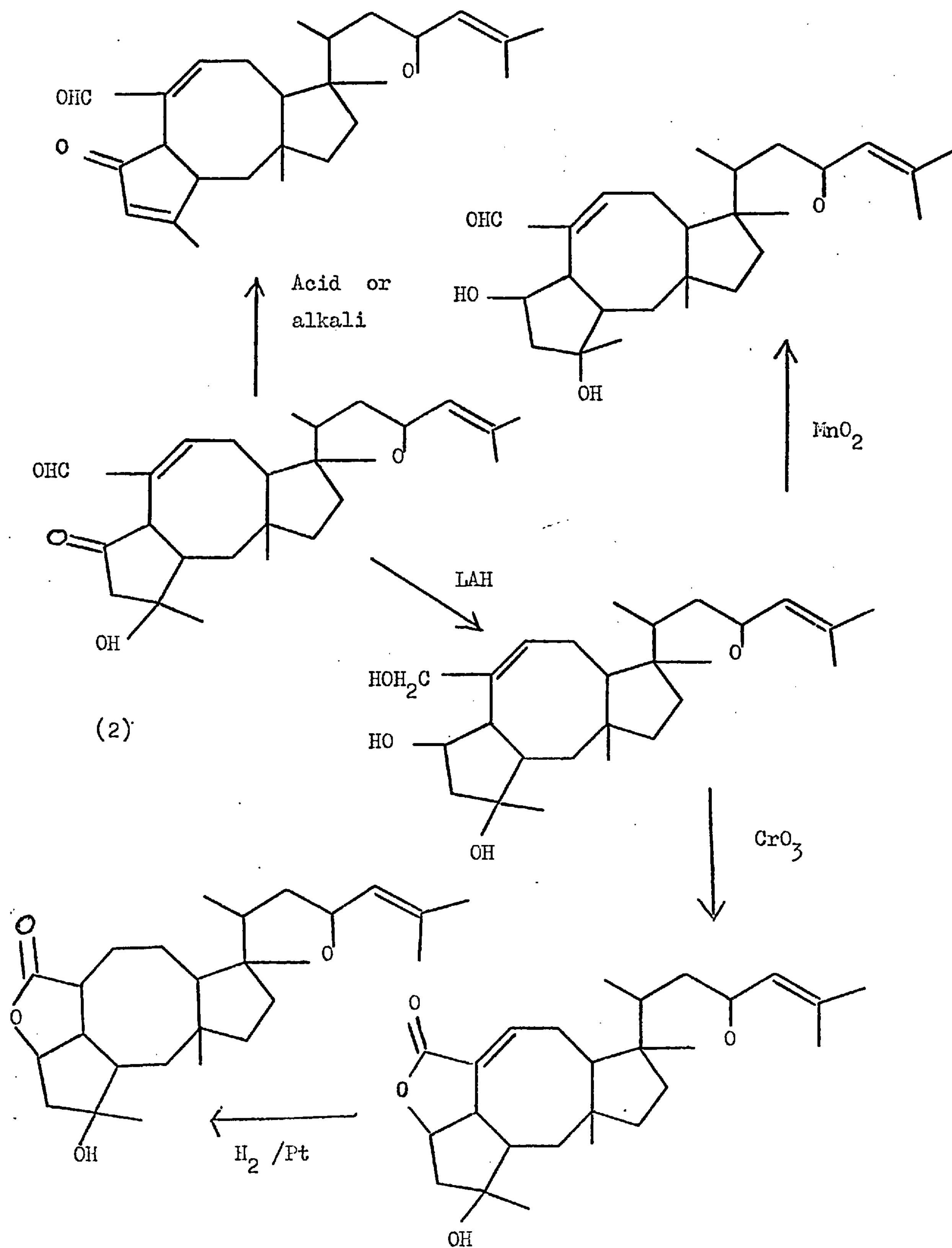
Nozoe et al[91c] isolated ophiobolin B (3) and established its structure by chemical and spectroscopic correlation with Ophiobolin A (2) (Scheme 9). These workers also reported the isolation of Ophiobolin C (4), Scheme 10.

Itai et al[91d] isolated Ophiobolin D (5) from <u>Cephalosporium caerulens</u>. The structure and absolute configuration of this compound was established by X-ray crystallographic analysis of its bromoacetate. Ophiobolin D has been correlated[91e] with Ophiobolin C, Scheme 10.

Canonica et al[91f] reported the chemical degradation of Ophiobolin A and Ophiobolin B, isolated from <u>Helminthosporium Orizae</u> (Scheme 11).

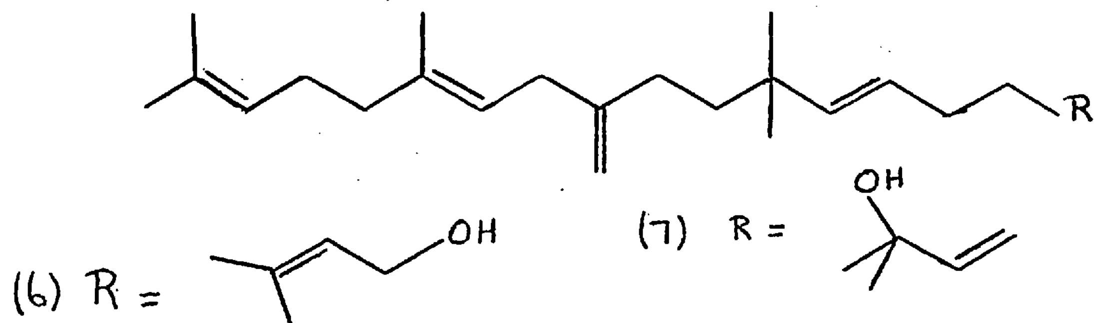


Scheme 10

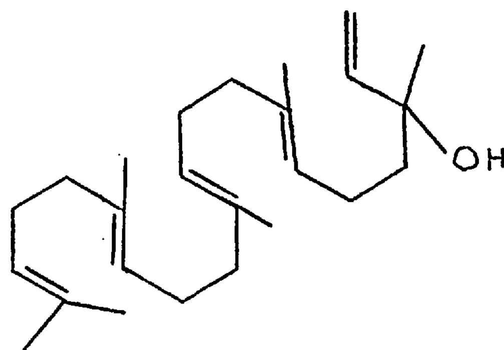


Scheme 11

Tschesche et al[92] isolated meonocinol (6) and its isomer (7) from the antibiotic moenomycin.



Geranylnerolidol (8) and Ophiobolin F (9) were isolated from the fungus which causes the leaf spot disease in maize. Nozoe et al[93] demonstrated that Ophiobolin F is produced when synthetic all-trans geranylfarnesyl pyrophosphate is incubated with a fungal enzyme system. The structure and stereochemistry of Ophiobolin F was established by biogenetic reasoning[93] and chemical correlation with Ophiobolin C (4), Scheme 12.



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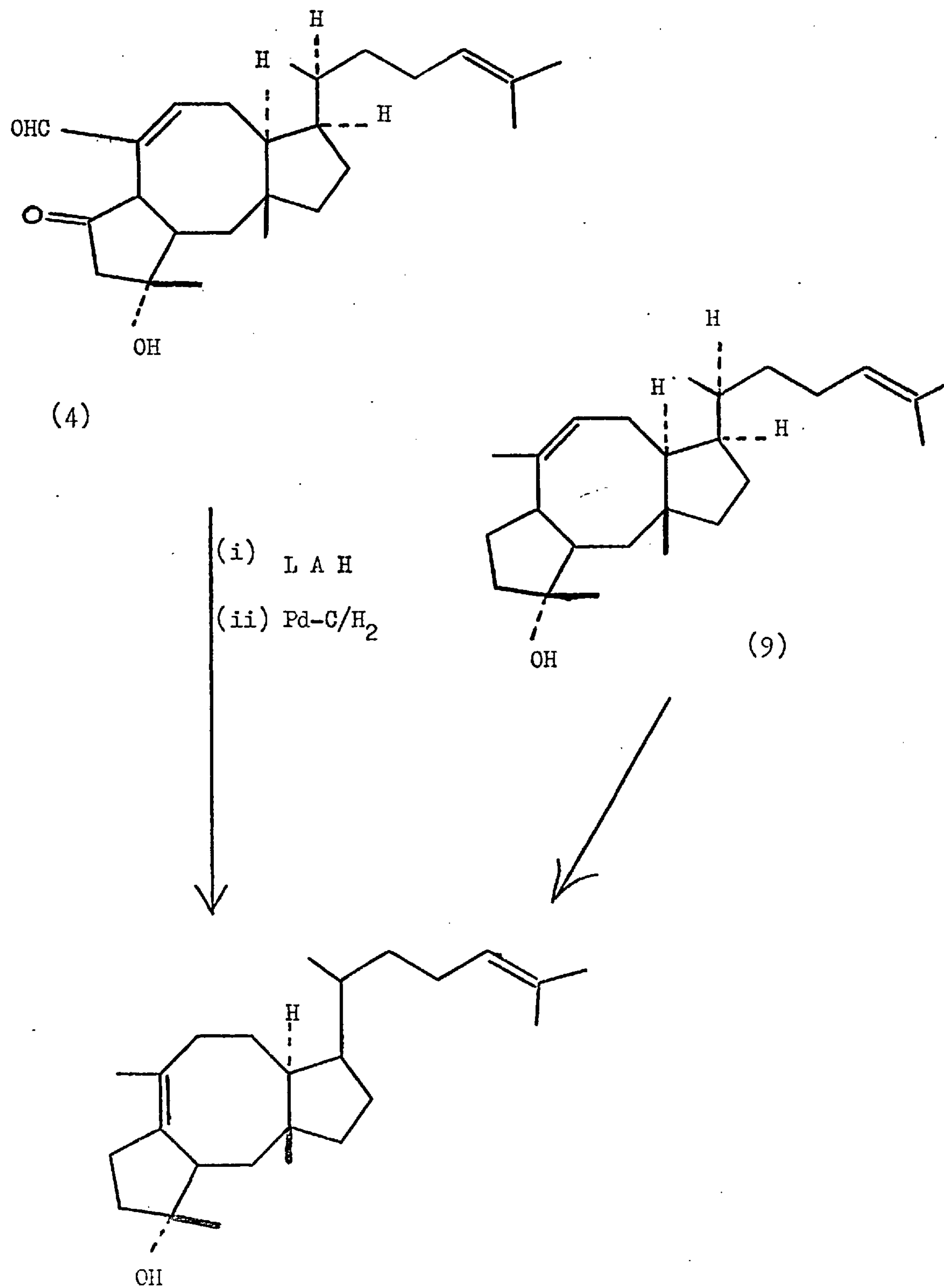
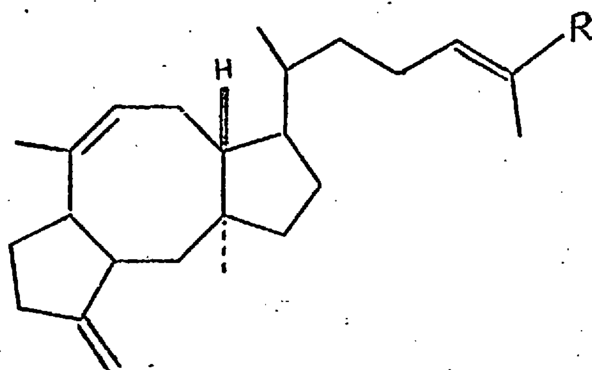
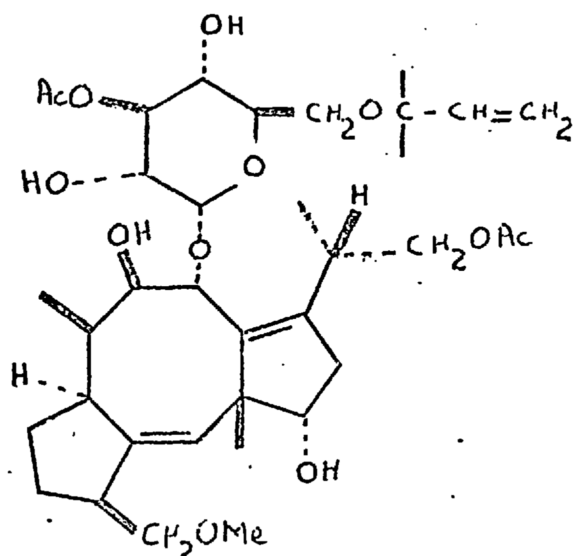


Scheme 12.

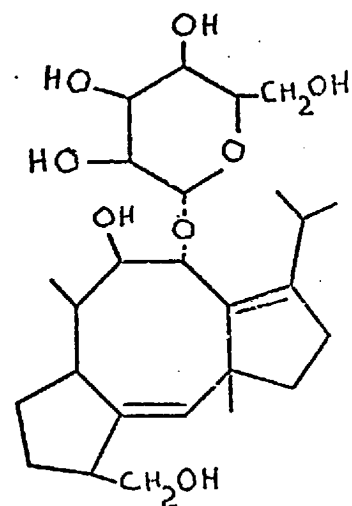
Iitaka et al[94] isolated ceroplasteric acid (10a) from the wax secreted by the insect Ceroplastes albolineatus. The structure of this compound was established by X-ray analysis of the 4-bromobenzoate of ceroplastol (10b)



Chain et al[95] isolated fusicoccin A (11a) from the fungus Fusicoccum amygdali Del. This compound has the ophiobolin ring system and was thought to be derived from a sesterterpene



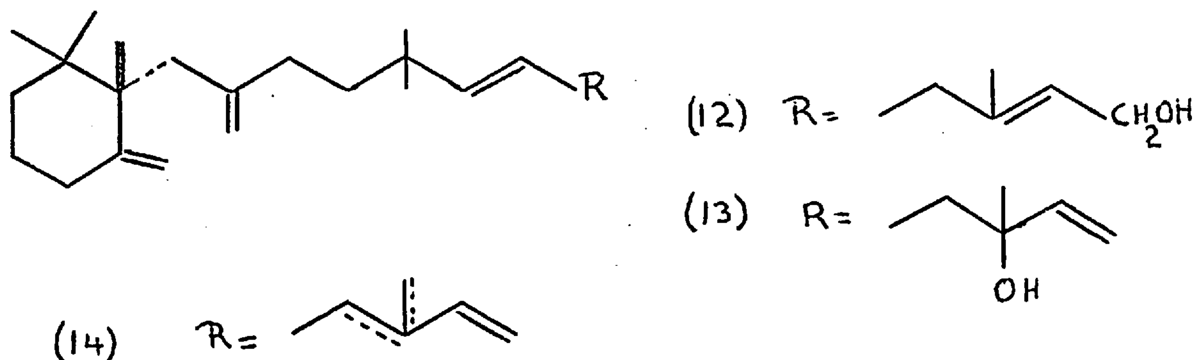
(11a)



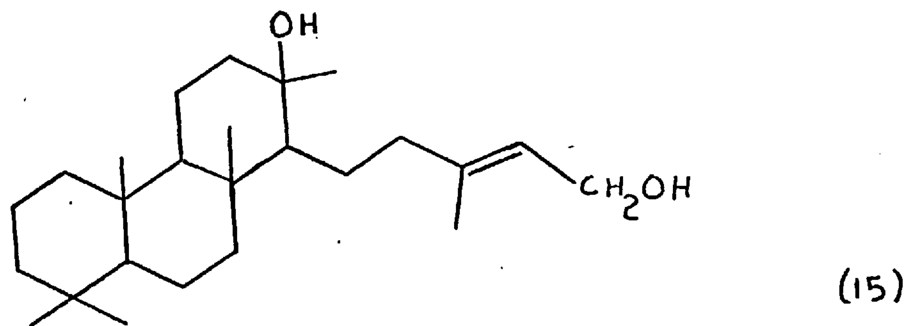
(11b)

precursor. However, the isolation of fusicoccin H (11b) is strong evidence that fusicoccin A may be a diterpene.

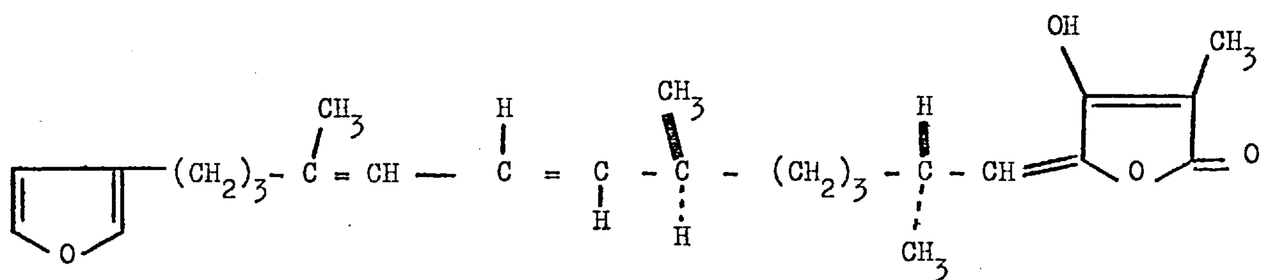
Slusarchyk et al[96] have isolated diumycinol (12), isodiumycinol (13) and diumycene (14), from the phosphorus containing antibiotic diumycins.



The isolation of cheilanthatriol (15) has been reported by Sukh Dev et al[97].



Fasciculatin (16), Furospongin-I (17), Ircinin-I (18), and Nitenin (20) were isolated from Marine Sponge[98]. Faulkner[98] has recently isolated variabilin (19) from <u>Ircinia Variabilis</u>.



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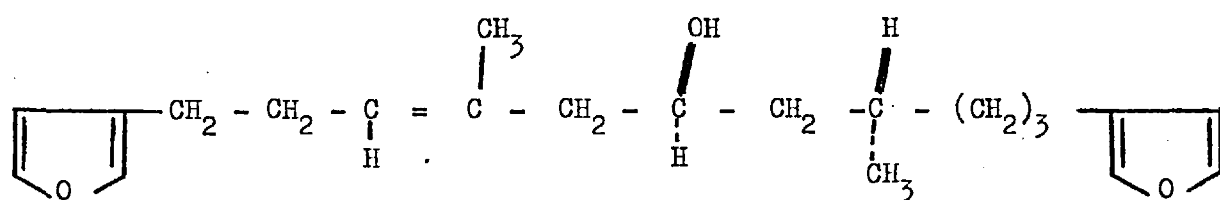

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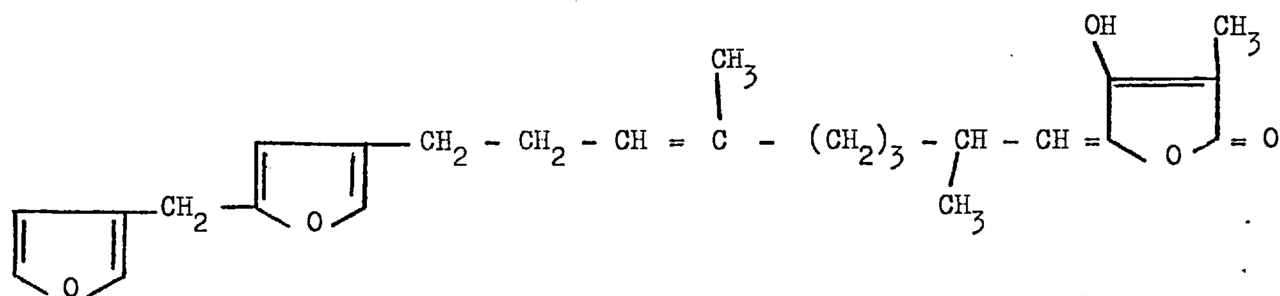

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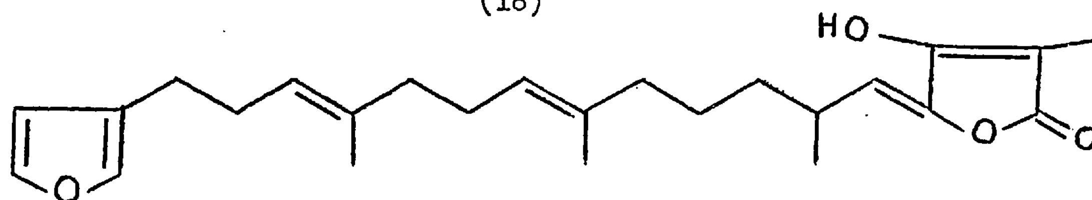

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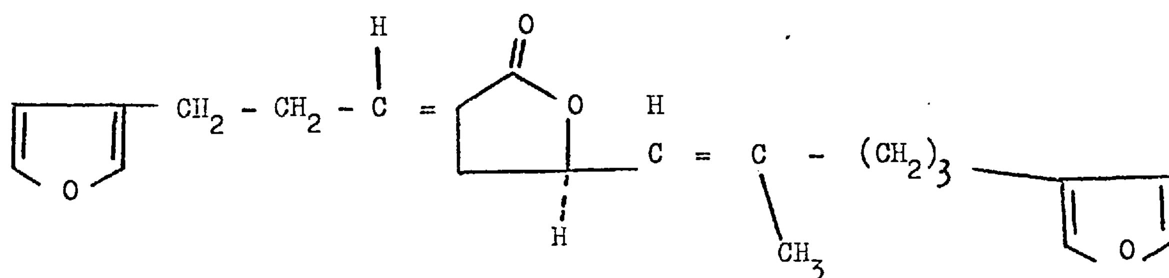

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RESULTS AND DISCUSSION

Fusicoccin A (I) was isolated by Chain et al[1] from culture filtrates of the fungus Fusicoccum amygdali Del., which causes a wilting disease of the almond tree (Prunus amygdalus St.). They observed that fusicoccin A showed phytotoxic activity in the tomato plant test in concentrations of 0.1 - 0.2 ug/ml. The elementary analysis of fusicoccin A (from ethylacetate) indicated the formular $C_{38}H_{58}O_{13}$. Molecular weight determination by X-rays gave a value of 733.5($\pm$ 2%); isothermal distillation gave a value of 697.3($\pm$ 4%). The mass spectrum showed the highest mass peak at m/e 704. This peak was thought to be due to loss of one molecule of water from $C_{38}H_{58}O_{13}$(mol. wt. 722). Fusicoccin A yielded one molecule of glucose on acid hydrolysis. Elementary analysis and n.m.r. indicated one methoxy- and two acetoxy- groups. When hydrogenated in presence of a catalyst, fusicoccin A absorbed one mole of hydrogen to give dihydrofusicoccin A, which exhibited the highest mass peak at m/e 706. Dihydofusicoccin A had about half the biological activity of fusicoccin A.

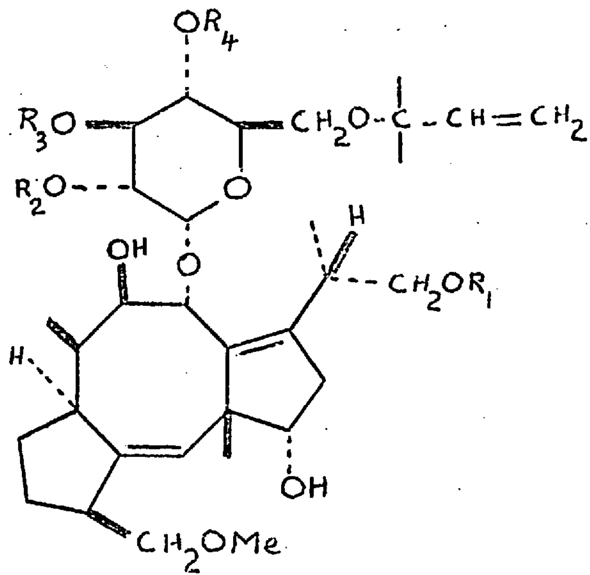
Barton[2] and Chain reported that the mass spectrum of fusicoccin A showed a minor peak at m/e 722 which fragmented into the aglycone $C_{23}H_{36}O_6$ (m/e 408), and a diacetylglucosyl fragment $C_{10}H_{15}O_7$ (m/e 247) which gave rise to peaks at m/e 205 (loss of CH_2CO), 145 (205 - CH_3COOH), and the base peak at m/e 69 (C_5H_4). Although they observed only two acetyl groups in the n.m.r. (100 MHz) spectrum of fusicoccin A, the mass spectrum of deacetylfusicoccin $C_{32}H_{52}O_{10}$ (M^+596), aglycone ion m/e 366, indicated the loss of three acetyl groups. Acetylation of fusicoccin A with acetic anhydride in pyridine gave a product $C_{42}H_{62}O_{15}$ (M^+ 806) whose n.m.r. spectrum showed five acetoxy-groups. Acetylation of fusicoccin A with $^2H_6$ -acetic anhydride gave a product (M^+ 815), requiring the addition of three acetoxy-groups. In addition to the

very small peak at m/e 722, fusicoccin A showed a more intense ion at m/e 680. Barton[2] et al suggested that this peak at m/e 680 was due to the molecular ion (M^+). The minor m/e 722 ion requiring three acetoxy-groups, and the diacetylglucosyl ion at m/e 247, in the mass spectrum of fusicoccin A were thought to arise through a thermal transacetylation[2,3] prior to ionisation of the parent compound (M = 680). Barton[2] et al. suggested the revised molecular formular of fusicoccin $C_{36}H_{56}O_{12}$, containing two acetoxy-groups shared between the glucosyl unit and the aglycone.

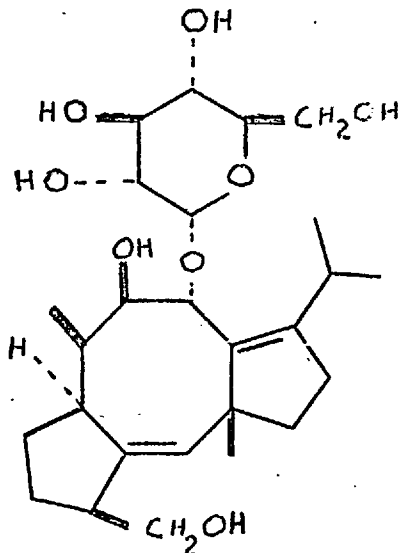
The structure and streochemistry of fusicoccin A was established by degradative and spectroscopic studies[4,5] and by an X-ray crystallographic analysis[6] of a mercuribromide of its aglycone. The same structure and stereochemistry of fusicoccin A was independently suggested by Ballio et al[3] on its degradative and spectroscopic data, and X-ray crystallography of its p-iodobenzenesulphonyl derivative.

Derivatives of fusicoccin A, (I a - g) have been observed in culture filtrates of Fusicoccum amygdali Deb., and also when fusicoccin A is incubated at room temperature at the pH of the culture filtrate. The structures of these compounds have been established by mainly n.m.r. spectral data[7].

Barton et al[8] have isolated fusicoccin H (II) from culture filtrates of Fusicoccum amygdali Del. The structure of fusicoccin H was established by degradative studies and by chemical correlation with the known fusicoccin series. Feeding experiments showed that fusicoccin H can act as a precursor of fusicoccin A, which strongly suggests that fusicoccin A is a diterpenoid and not a degraded sestertepenoid.



I	R_1, R_3 = $COCH_3$; R_2, R_4 = H
(a)	R_1 = $COCH_3$; R_2, R_3, R_4 = H
(b)	R_1, R_2, R_3, R_4 = H
(c)	R_1, R_2 = $COCH_3$; R_3, R_4 = H
(d)	R_1, R_4 = $COCH_3$; R_2, R_3 = H
(e)	R_2 = $COCH_3$; R_1, R_3, R_4 = H
(f)	R_4 = $COCH_3$; R_1, R_2, R_3 = H
(g)	R_3 = $COCH_3$; R_1, R_2, R_4 = H



II

In the following work a new metabolite has been isolated from culture filtrates of Fusicoccum amygdali Del. The structure and stereochemistry of this minor metabolite have been established as (28) (Scheme 9) by its synthesis from fusicoccin A. Feeding experiments have shown that compound (28) can act as a precursor of fusicoccin A.

A chloroform extract of a culture filtrate of Fusicoccum amygdali Del. was fractionated on a silica gel M.F.C. column, eluting with isopropanol and chloroform (5:95). Fractions containing fusicoccin A (I) were combined, concentrated, and the fusicoccin A (I) crystallised. A t.l.c. examination of the mother liquor showed the presence of several new minor metabolites of mobilities between fusicoccin A (I) and deacetylfusicoccin (Ib). The mother liquor was evaporated to dryness to give a yellow gum. Attempts to purify the new metabolites by p.l.c. failed. The crude material was acetylated with acetic anhydride and pyridine at 80°. The reaction mixture went almost black but p.l.c. separation gave a small amount of compound (I) (Scheme 1).

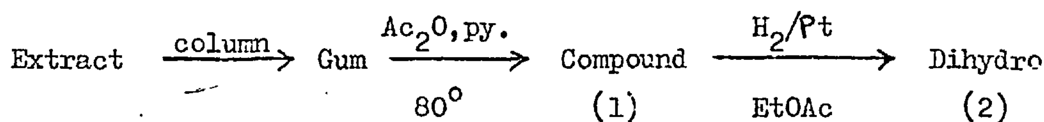
The mass spectrum of compound (I) showed the highest mass peak at m/e 732 (16%), with other peaks at m/e 672 (4%) (732 - $MeCO_2H$), 663 (1.3%) (732 - C_5H_9), 603 (1%) (663 - $MeCO_2H$), 442 (8.4%), 417 (5%), 374 (18.5%) (732 - $C_{17}H_{26}O_8$), 373 (18.5%) (732 - $C_{23}H_{35}O_3$), 357 (21%) (732 - $C_{23}H_{35}O_4$), 314 (33.3%) (374 - $MeCO_2H$), 313 (33.2%) (373 - $MeCO_2H$), 289 (99.4%), 229 (80.2%), 169 (74%), 109 (53%) and 69 (100%).

Compound (I) was hydrogenated in presence of a catalyst to give compound (2). The mass spectrum of compound (2) showed the highest mass peak at m/e 734, with other peaks at m/e 674, 663, 603, 444, 417, 374, 373, 357, 314, 289, 229, 169, 109, and the base peak at m/e 71.

From the mass spectra, compounds (I) and (2) appeared to have 4 acetoxy-groups. Three acetoxy-groups were on the glucopyranose unit as indicated by the presence of the characteristic glucopyranose triacetate ion series m/e 289, 229, 169, and 109. The fourth acetoxy-group was on the ion m/e 374, fragmenting into the ion m/e 314 (374 - $MeCO_2H$). The base peaks at m/e 69 and 71 in the mass spectra of (1) and (2) respectively, indicated the presence of an isopentenyl unit[4] (C_5H_9) in (1). This isopentenyl unit was catalytically hydrogenated to give compound (2).

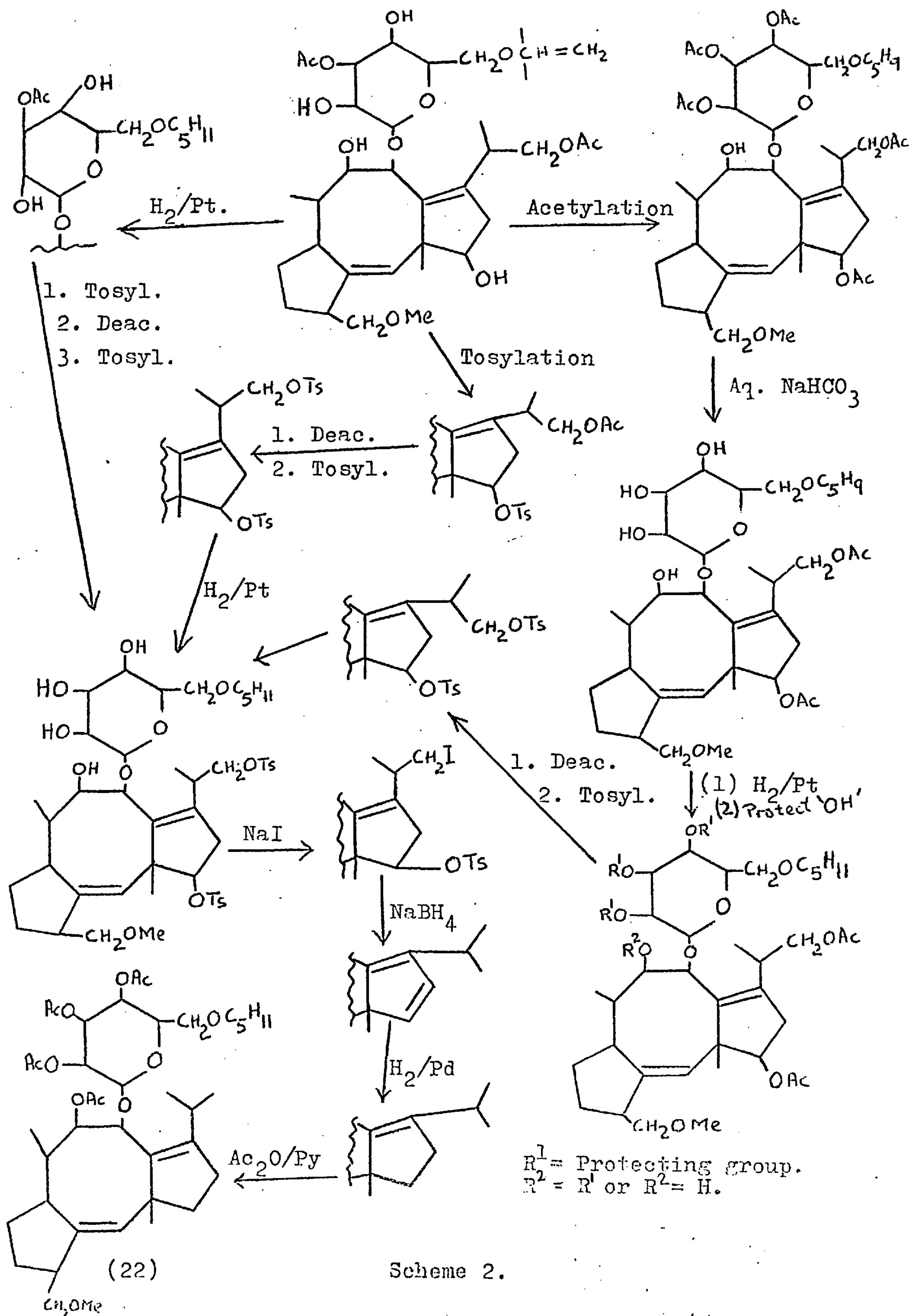
From the mass spectrum of compound (8) (Scheme 4), it appeared that the mass spectrum of the derivative of (2), corresponding to (8), would give valuable information. Compound (2) was deacetylated and treated according to Scheme 4, but the mass spectrum of the product corresponding to (8), was not helpful.

The samples of compounds (1) and (2) were insufficient for further characterisation. It was assumed that (1) and (2) had the same carbon skeleton as the known fusicoccins and structure (22) (Schemes 2 and 7) was suggested for compound (2), based on the mass spectral data.



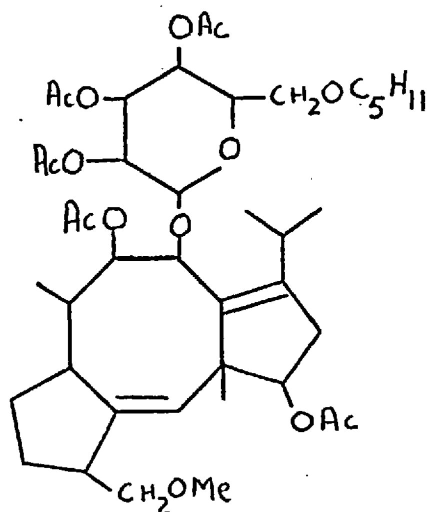
Scheme 1.

Attempts were made to synthesise structure (22) from fusicoccin A, according to Scheme 2.

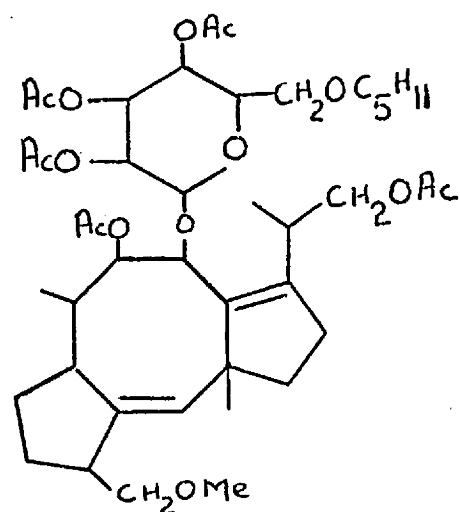


Scheme 2.

However, when the compound (22) was synthesised it differed from compound (2) in the mass spectrum. On t.l.c. the synthetic compound (22) was less polar than compound (2), indicating that (2) might be of a higher oxygenation than (22). Structures (a) and (b) below looked equally possible for compound (2). The molecular weight of (a) or (b) is 794. It was thought that the absence of a peak at m/e 794 in the mass spectrum of (2) might be due to elimination of CH_3COOH from the parent molecule (a) or (b) prior to ionisation.



(a)



(b)

Compound (27) of structure (a) was synthesised from fusicoccin A, according to Scheme 8 and was found to be identical with (2). Both compounds (2) and (27) were non-crystalline.

A chloroform extract of a culture filtrate of Fusicoccum Amygdali Del., was treated with acetic anhydride and pyridine under nitrogen at 80°, to give compound (1). This product was deacetylated to give a crystalline compound (28) (Scheme 9), which was later observed in the culture filtrate of Fusicoccum amygdali Del. The constitution and stereochemistry of (28) was

established by its synthesis from fusicoccin A.

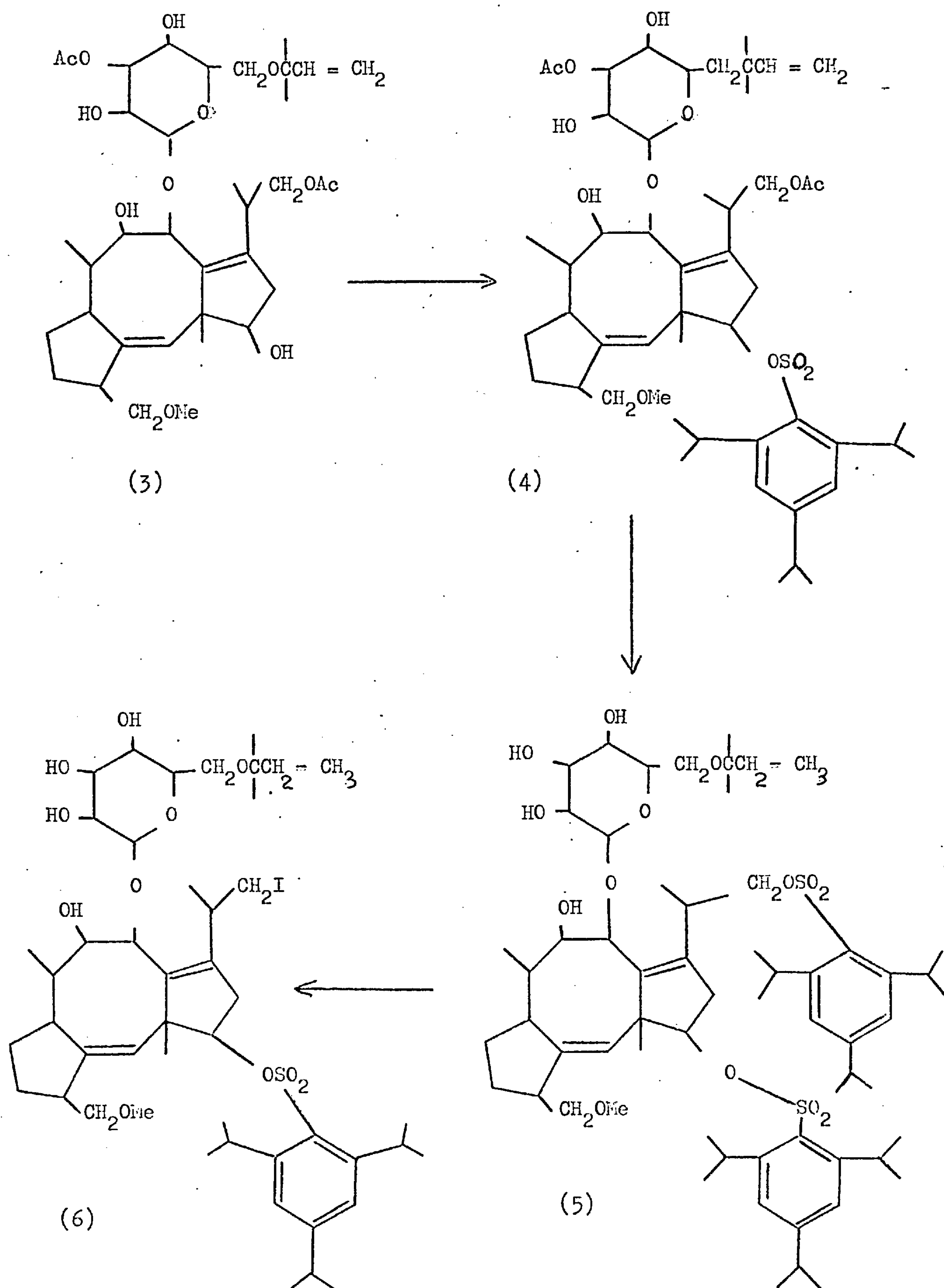
Synthesis of Compound (22).

Treatment of triacetylfusicoccin[4] A (9) with 0.2M aqueous $NaHCO_3$ for 1½ hours afforded a mixture of products. From this mixture compound (10) (Scheme 5) was isolated by p.l.c. and crystallised from ether to give needles, m.p. 99-100°, M^+ 680, diacetylaglycone ion m/e 450, isopentenyl unit m/e 69; n.m.r. τ 7.93 (3H, s, CH_3CO) and τ 8.05 (3H, s, CH_3CO). When treated with H_2/Pt in ethylacetate, compound (10) took up one mole of hydrogen to give the di-hydro derivative (14). Compound (14) crystallised from ether as needles, m.p. 75°, M^+ 682 and the C_5 side chain m/e 71; n.m.r. τ 7.93 τ(3H, s, CH_3CO) and τ 8.05(3H, s, CH_3CO).

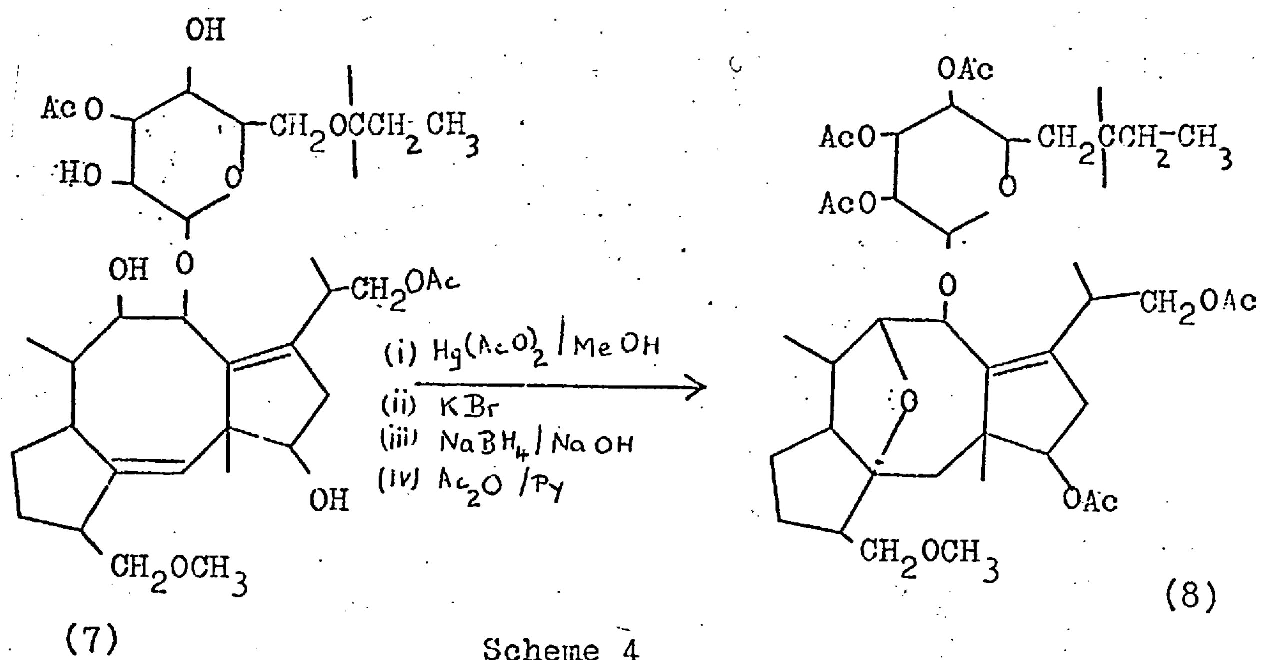
To synthesise di-(2,4,6-triisopropylbenzenesulphonyl)-deacetyldihydrofusicoccin (18) (Scheme 7), it appeared necessary to protect the sugar hydroxyls in compound (10) or (14), with groups stable to conditions under which both the acetoxy-groups can be removed.

It was established that all the hydroxyls in deacetylfusicoccin[4] (16) (Scheme 6) could be benzylated by treating deacetylfusicoccin with NaH and benzylbromide, to give hexabenzyldeacetylfusicoccin. The protection of the free hydroxyls in compounds (10) and (14) by benzylation was attempted by treating compound (10) or (14) with methyllithium, tetramethylpiperidine and benzylbromide. However, it appeared deacetylation occurred and the experiment failed.

Treatment of compound (10) with orthonitrobenzylchloride in pyridine at room temperature, yielded the diorthonitrobenzoyl derivative (11) (Scheme 5b).



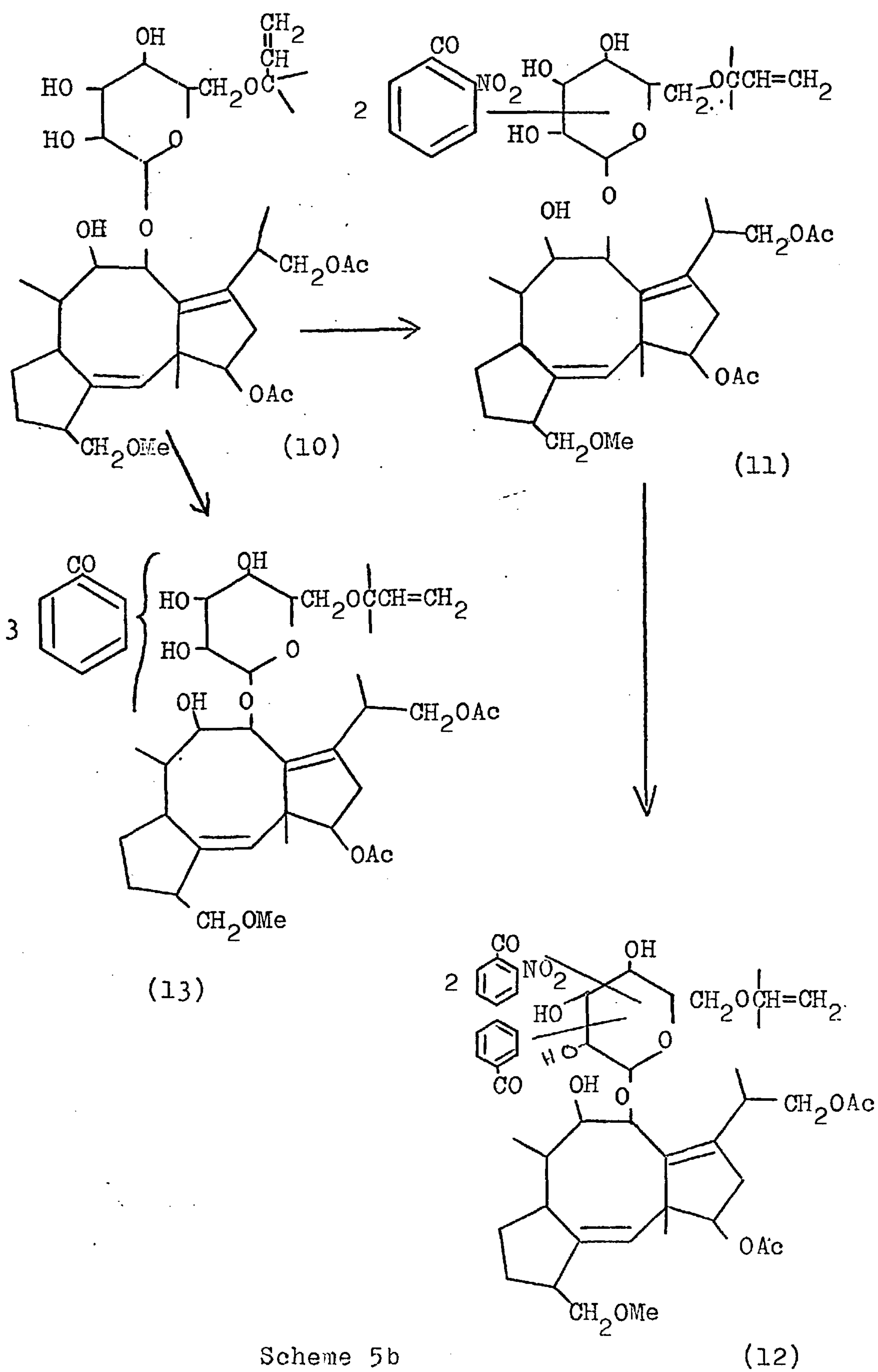
Scheme 3



Scheme 4

(9) (10) (14) (15)

Scheme 5a



Scheme 5b

Treatment of compound (11) with more orthonitrobenzylchloride in pyridine at 55° had no effect. When compound (11) was treated with benzoylchloride in pyridine at room temperature, it yielded the monobenzoyl derivative (12).

Treatment of compound (12) with 0.2M aqueous $NaHCO_3$ at 35° overnight or 0.2M Na_2CO_3 at 5° for 30 minutes afforded a mixture of products, in addition to unchanged starting material. T.l.c. showed that the reaction mixtures included deacetylfusicoccin (16) (Scheme 6) and compound (10) (Scheme 5). In the case of the 0.2M aqueous Na_2CO_3 reaction mixture a benzoate without nitrogen (elementary analysis) was isolated. This indicated that the benzoyl group was more stable than the O-nitrobenzoyl groups, under these conditions. It appeared that a tribenzoyl derivative might undergo deacetylation without debenzoylating, under the conditions above.

Treatment of compound (14) with benzoylchloride in pyridine at room temperature, yielded the tribenzoyl derivative (15). Treatment of compound (15) with 0.2M aqueous Na_2CO_3 gave a mixture of products, including deacetyldihydrofusicoccin, compound (14) and unchanged (15). It was hoped that a bigger base would remove the acetoxy-groups without affecting the benzoyl groups. However, treatment of (15) with piperidine in dry toluene gave mainly compound (14) and a small amount of deacetyldihydrofusicoccin. There was a small amount of unchanged (15).

Treatment of compound (14) with a mixture of dihydropyran, toluene and concentrated hydrochloric acid at 80°, gave a yellow oil on evaporation (Scheme 7). This product was deacetylated and treated with 2,4,6-triisopropylbenzenesulphonyl chloride in pyridine, at room temperature for several days. The reaction mixture was worked up and the tetrahydropyranyl ethers cleaved by treating the product with dilute hydrochloric acid in

ethanol, to give a mixture of products. Separation of this mixture by p.l.c. gave compound (18), λmax 281 mμ (ϵ 3700), n.m.r. τ 2.86(2H, s) (aromatic, 1ry group) and τ 2.92 (2H, s) (aromatic, 2ry group).

Treatment of (18) with NaI in acetone, gave mainly compound (19), λmax 281mμ (ϵ 1900), n.m.r. τ 2.90 (2H, s)(aromatic, 2ry group). Compound (19) was identical with compound (6) in Scheme 3.

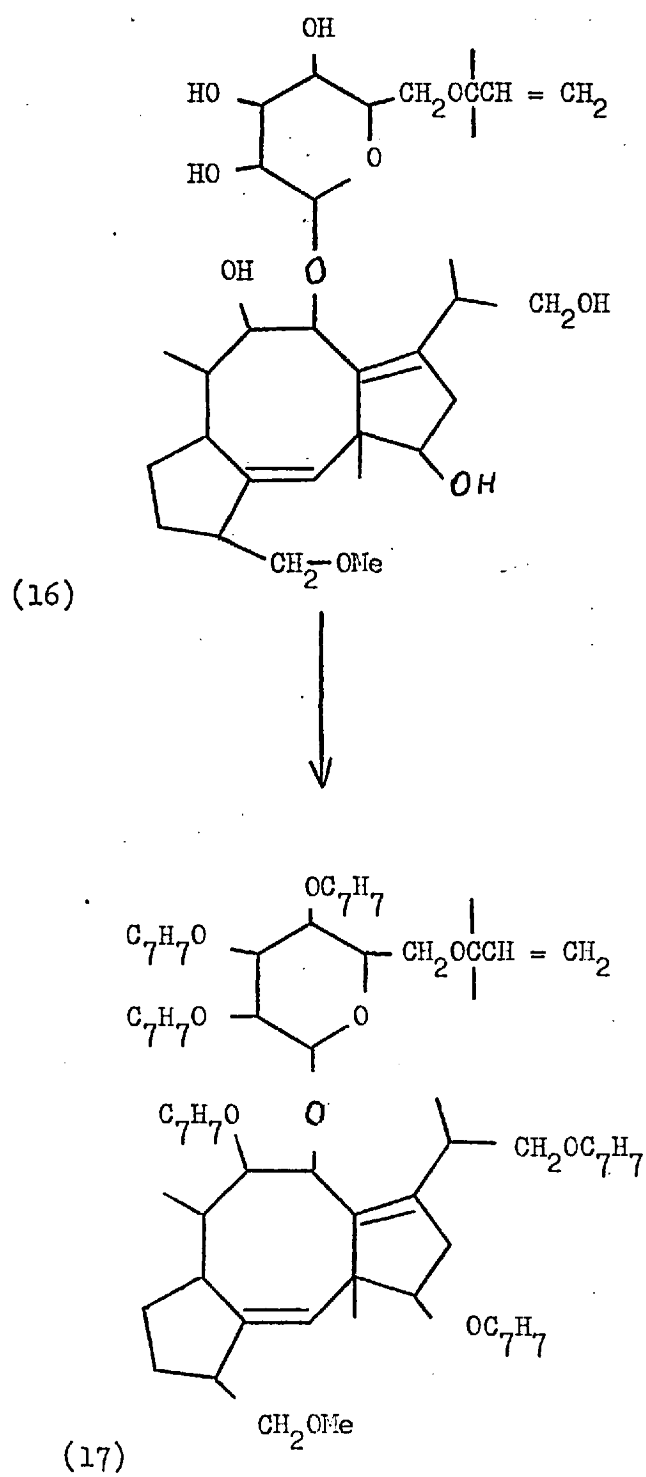
Compound (19) reacted with $NaBH_4$ in dimethylsulphoxide, giving the cyclopentadiene (20) M^+ 564 ($C_{32}H_{52}O_8$ m. wt. 564), λmax 270 mμ (ϵ 1200), n.m.r. τ 3.86 (2H, s) assigned to the cyclopentadiene protons.

Compound (20) was hydrogenated in the presence of 10% Pd/Strontium carbonate, to give compound (21), without the cyclopentadiene absorption in its U.V. spectrum. The n.m.r. spectrum of (21) did not show the cyclopentadiene protons at τ 3.86.

Treatment of (21) with acetic anhydride and pyridine at room temperature for 4 days, gave compound (22), M^+ 734 ($C_{40}H_{62}O_{12}$ m. wt. 734). This product was different from compound (2) in Scheme 1. T.l.c. examination of acetylation mixtures of extracts of cultures of Fusicoccum amygdali Del. showed no signs of a compound identical with (22).

Synthesis of Compound (27).

Treatment of dihydrofusicoccin[4] (23) with a mixture of dihydropyran, toluene and hydrochloric acid, gave a light yellow oil on evaporation. This product was deacetylated and treated with 2,4,6-triisopropylbenzene-sulphonyl chloride at room temperature. The reaction mixture was worked up



Scheme 6

and the tetrahydropyranyl ethers cleaved by treating the product with dilute hydrochloric acid, to give a mixture of products. Separation of this mixture by p.l.c. gave compound (24), λmax 281 mμ (ε 2400), n.m.r. τ 2.86 (2H, s) (aromatic, 1ry group).

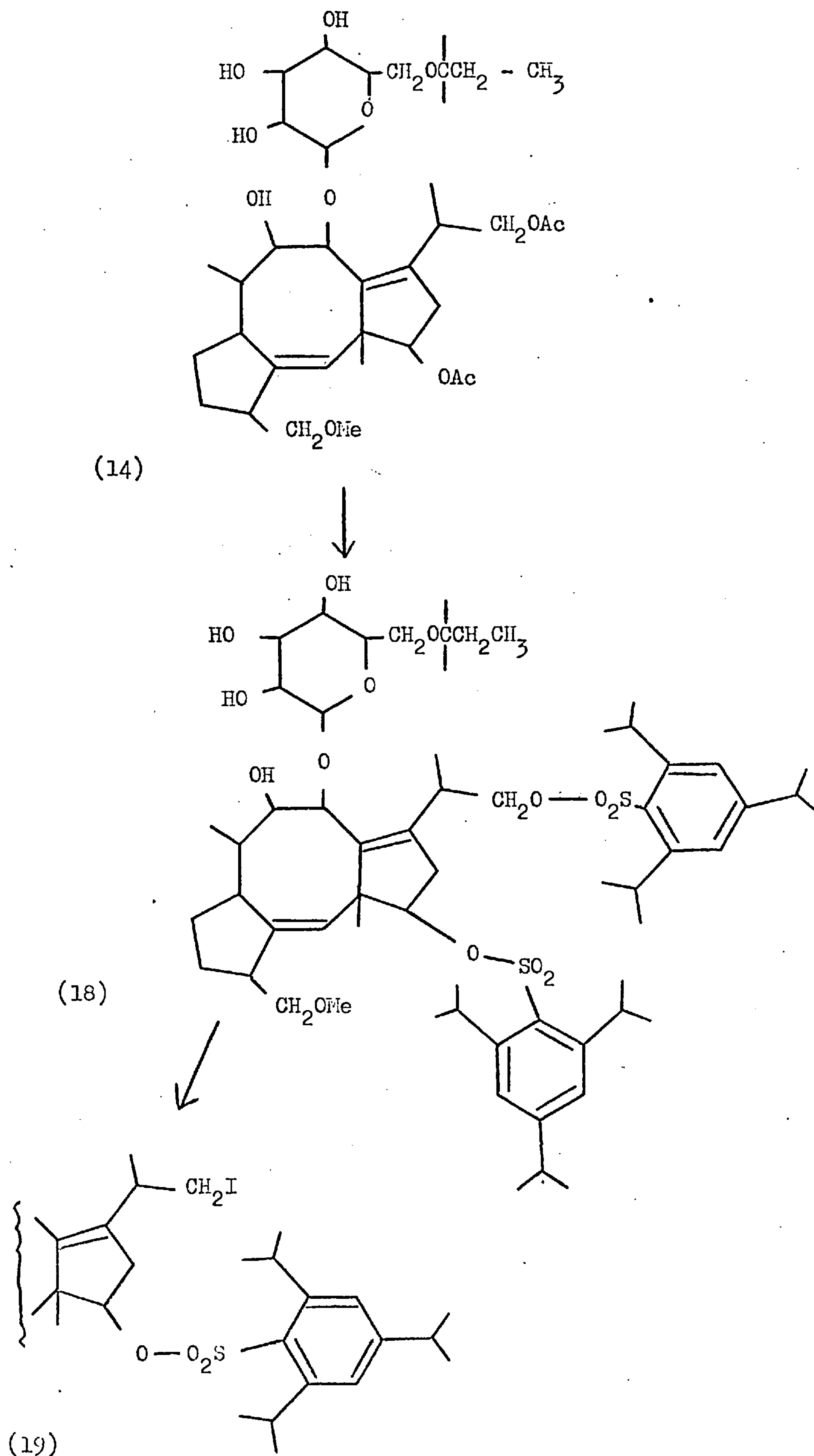
Treatment of compound (24) with excess sodium iodide in acetone, yielded the iodide (25), λmax 224 mμ (ε 7500), and 259mμ (ε 1600). This product analysed for $C_{32}H_{51}IO_9$ and gave a positive Beilstein test.

Compound (25) reacted with $NaBH_4$ in DMSO to give compound (26) identified by its mass spectrum only, m/e 582 (M^+, $C_{32}H_{54}O_9$ m. wt. 582), 350 (aglycone ion), 332, 314, 259, 207, 151, 109 and the base peak m/e 71.

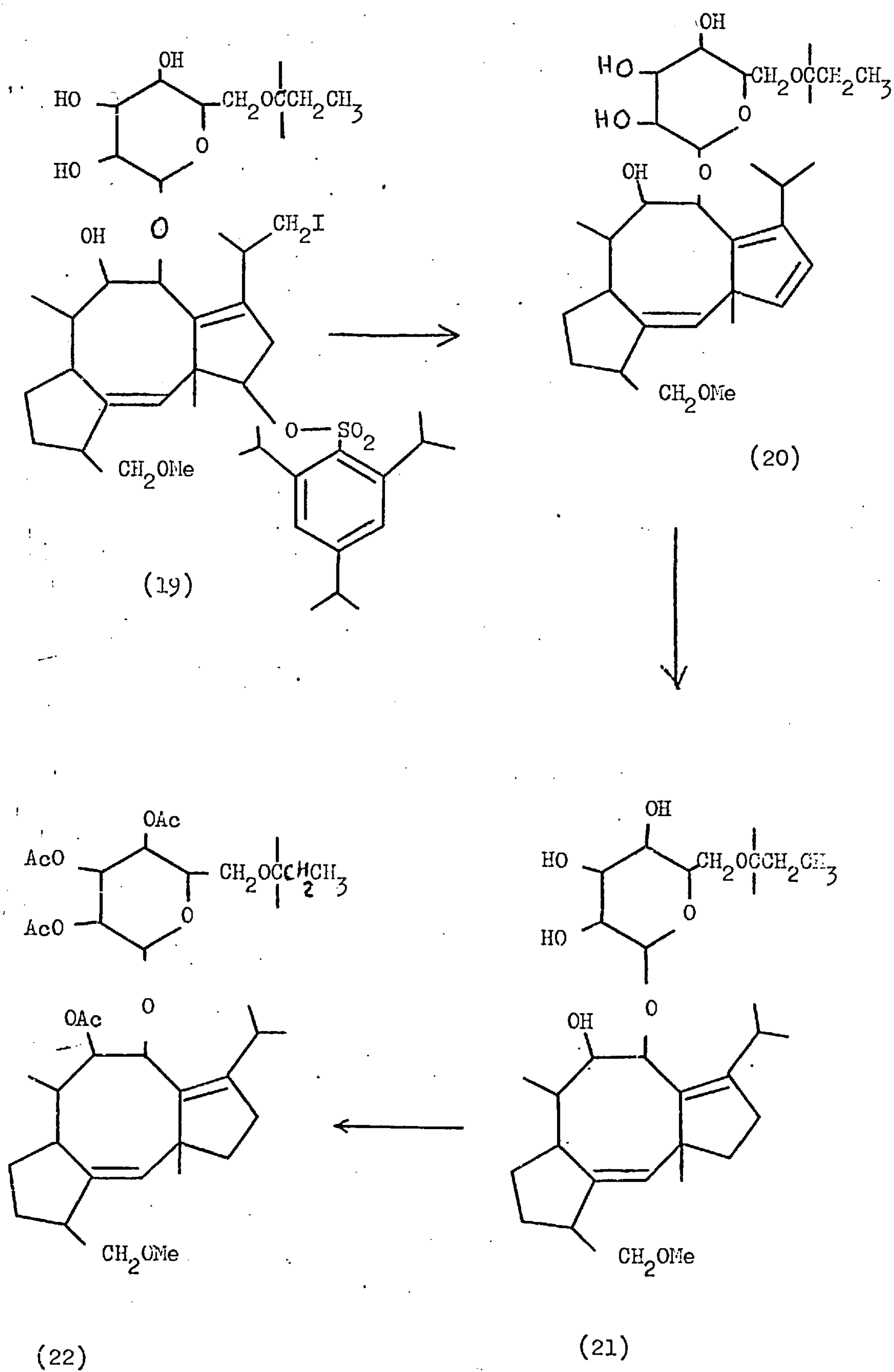
Treatment of compound (26) with acetic anhydride in pyridine at room temperature overnight, yielded two products, the minor one being fully acetylated. However, acetylation of (26) at room temperature for 7 days or at 80° under nitrogen overnight, gave mainly the fully acetylated product (27). The mass spectrum of (27) showed a small peak at m/e 794 (M^+, $C_{42}H_{64}O_{14}$ m. wt. 794), a large peak m/e 734 (M^+ - CH_3COOH), and the rest of the spectrum was identical with the mass spectrum of compound (2) in scheme 1. Compounds (2) and (27) were also identical on t.l.c.

Compound 28.

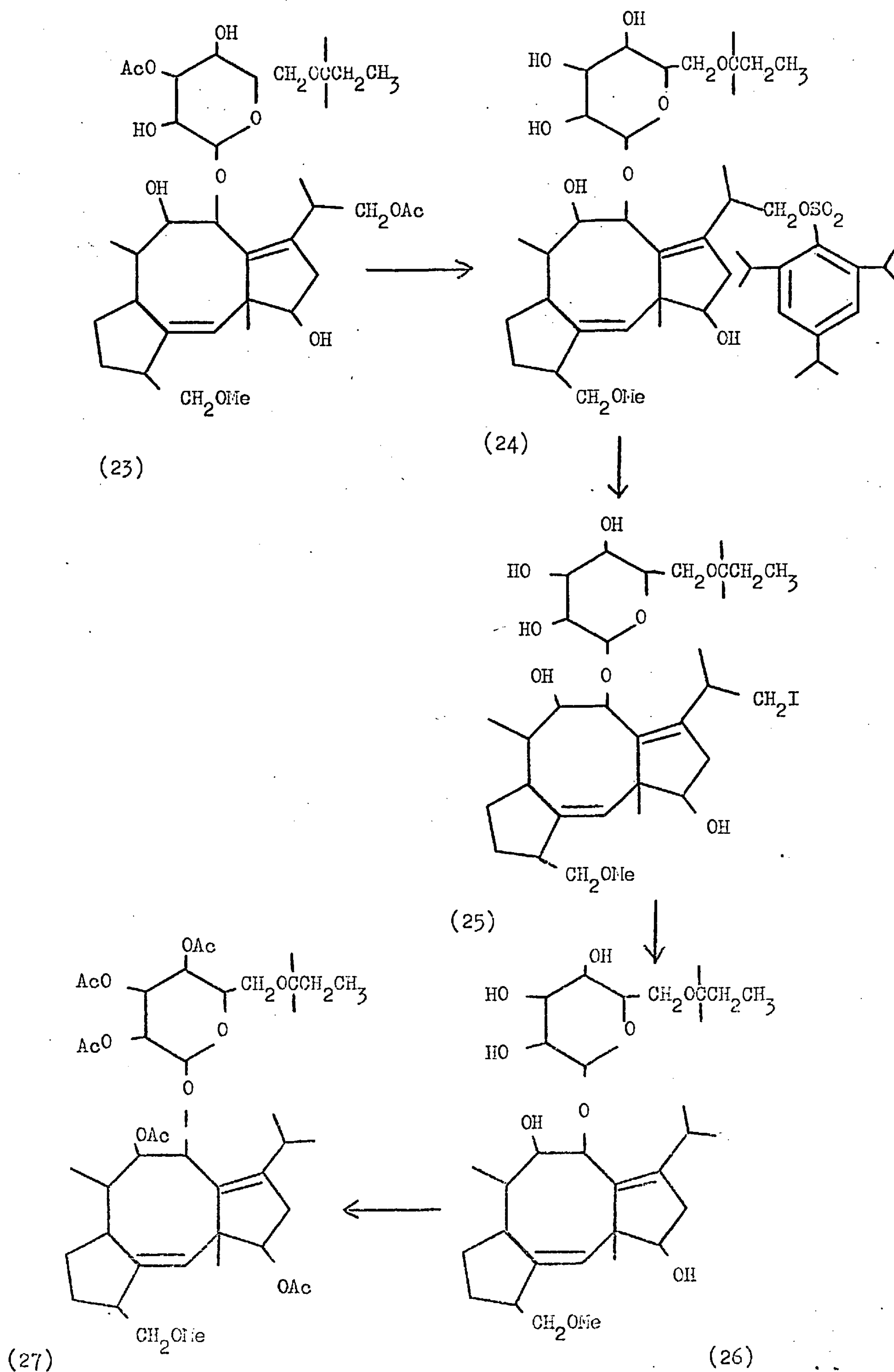
A chloroform extract of a culture filtrate of *Fusicoccum amygdali* Del. was treated with acetic anhydride and pyridine at 80° under nitrogen (Scheme 9). The reaction mixture was worked up and fractioned on a silica gel column, to give a fraction containing compound (1). This fraction was fractionated by p.l.c. to give a crude sample of (1). Deacetylation of this product and separation by p.l.c. on silica gel did not yield a pure



Scheme 7a



Scheme 7b



Scheme 8

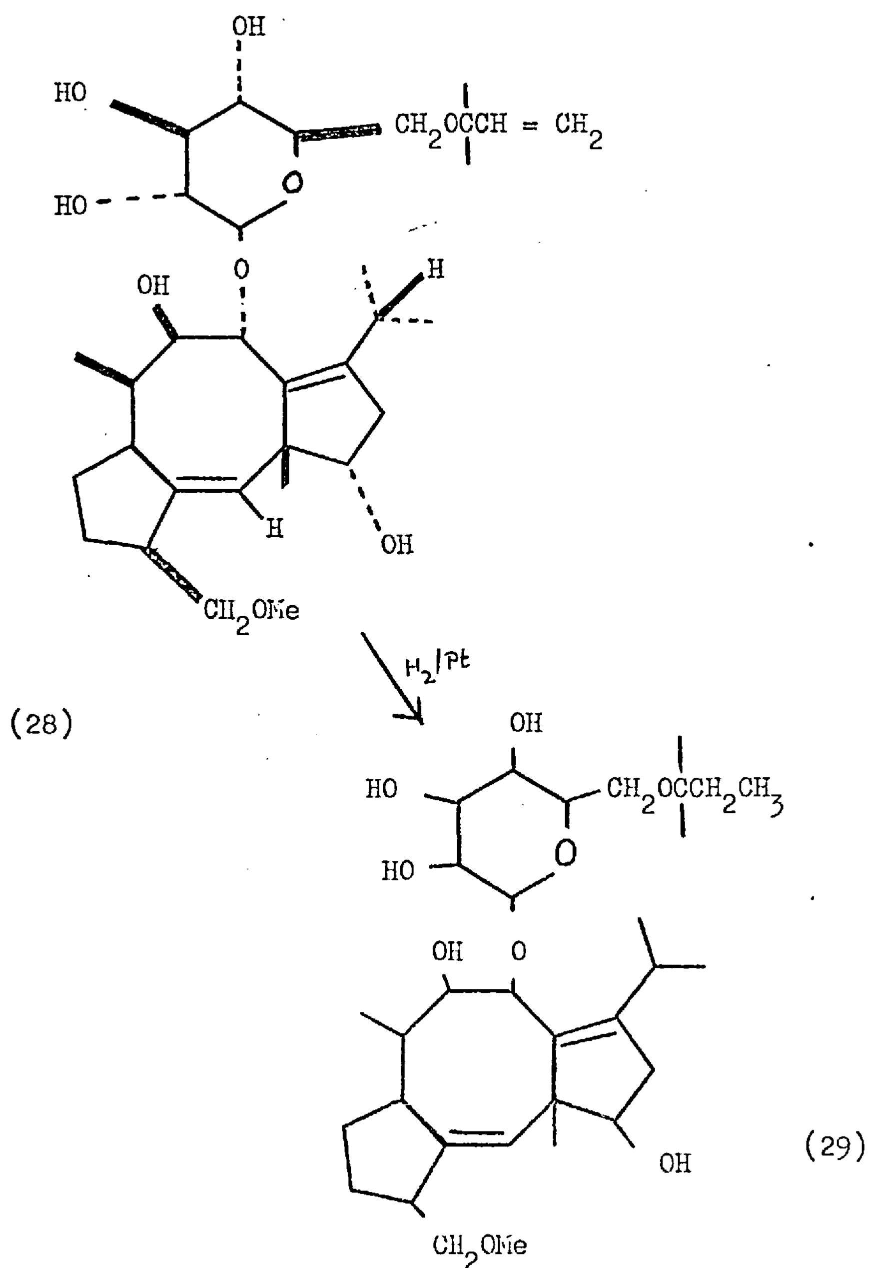
Fusicoccum amygdali

Culture Extract

(1) Acetylation

(3) Partial Purification

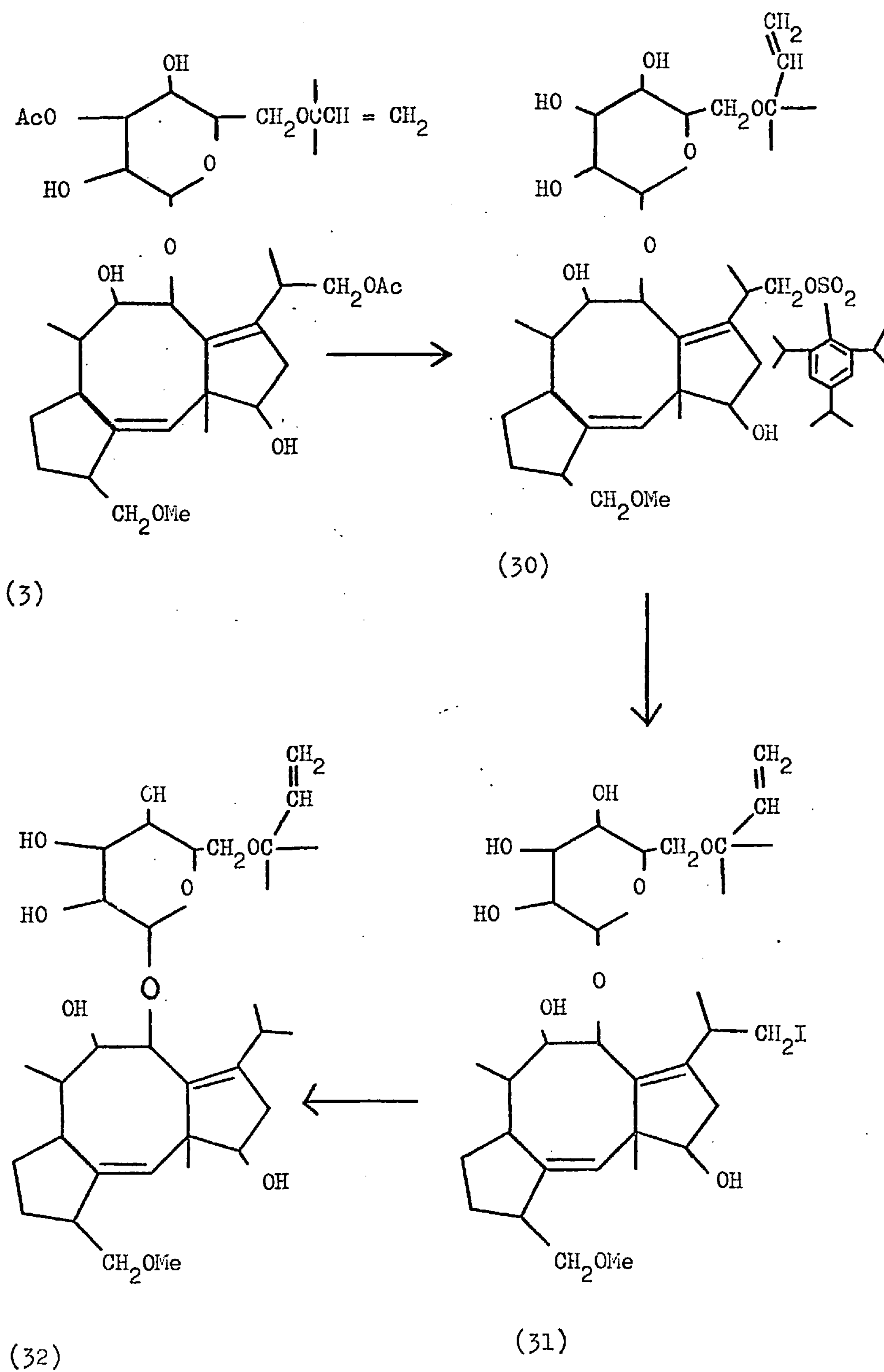
(4) Deacetylation



Scheme 9

compound. The crude compound from p.l.c. was purified by p.l.c. on 5% $AgNO_3$ impregnated silica gel plates, to give compound (28), which crystallised from ether. In the crystalline form, compound (28) is only slightly soluble in ether but it can be brought into ether solution by dissolving it first in ethanol and evaporating the solution to dryness in vacuo. The residue, which may be a gum or a solid foam, readily dissolves in ether. Compound (28) obtained above had to be recrystallised from ether several times, to obtain a pure sample. The pure sample of (28) melted at 194 - 203° with decomposition, on a Kofler block. In a sealed tube under nitrogen (28) melted at 204-6° without any sign of decomposition.

The mass spectrum of compound (28) showed the highest mass peak at m/e 580 (M^+, $C_{32}H_{52}O_9$ m. wt. 580), with significant peaks at m/e 350 (aglycone ion, $M^+ - C_{11}H_{18}O_5$), 332 (350 - H_2O), 151 (332 - $C_{11}H_{17}O_2$), and the base peak m/e 69. When hydrogenated in presence of Adam's catalyst (28) absorbed one mole of hydrogen to give the dihydro derivative (29), which was identical with compound (26). The mass spectrum of (29) showed the highest mass peak at m/e 582 (M^+, $C_{32}H_{54}O_9$ m. wt. 582) with significant peaks at m/e 350 (aglycone ion, $M^+ - C_{11}H_{20}O_5$), 332 (350 - H_2O), 151 (332 - $C_{11}H_{17}O_2$), and the base peak m/e 71. The base peaks at m/e 69 and 71 in the mass spectra of (28) and (29) respectively, indicated the presence of an isopentenyl unit[4] (C_5H_9) in (28). The i.r. spectrum of (28) displayed absorption at 3420(OH) and 1645 ($RCH = CH_2$) cm^{-1}. The n.m.r. spectrum of (28) run in deuteriochloroform showed a six-proton singlet at τ 8.77 due to the gem dimethyl substituent, and a one-proton double doublet centred at τ 4.27 due to the X proton of the vinyl ABX system present in the isopentenyl unit[4]. Further, the n.m.r. spectrum showed one methoxy-group (τ 6.70), one tertiary (τ 8.83) and three secondary methyl groups, τ 8.96 (3H, d, J6Hz), τ 9.04 (3H, d, J6Hz),



Scheme 10

and τ 9.17 (3H, d, J7.5Hz).

The dihydroderivative (29) was crystallised with difficulty from a mixture of diethylether and petroleum ether (b.p. 40-60°). All attempts to crystallise the corresponding compound (26) in the synthetic series, failed. Thus, a good comparison of (26) and (29) could not be made. Compound (28) was easily crystallised from diethylether. Therefore, an attempt was made to synthesise (28) from fusicoccin A, despite the risk of losing the isopentenyl unit during acid treatment.

Treatment of fusicoccin A (3) (Scheme 10) successively with a mixture of dihydropyran, toluene and hydrochloric acid, 2N sodium hydroxide, 2,4,6-triisopropylbenzenesulphonyl chloride, and finally hydrochloric acid, gave compound (30), λmax 281 mμ (ε 1900), n.m.r. τ 2.85 (2H, s)(aromatic, 1^{ry} group) , τ 4.21 (1H, dd, J10, 18Hz) isopentenyl unit. Compound (30) in acetone was heated with NaI in presence of triethylamine to give the iodide (31), νmax (KBr) 3450 (OH) and 1645 (RCH = CH_2)cm^{-1}; λmax 224 mμ (ε 7400) and 259mμ(ε 1800); n.m.r. τ 4.25 (1H, dd, J10, 18Hz) isopentenyl unit. Treatment of compound (31) with $NaBH_4$ in DMSO at 100° overnight, yielded a 50:50 mixture of two products and a small amount of unchanged (31). When this reaction was followed by t.l.c. it was found that only one product was formed within the first ½hour of reaction. After 30 minutes the second product appeared and increased with time. In later preparations the reaction was run for 20 to 25 minutes to give compound (32) along with unchanged (31). P.l.c. separation of this mixture gave (32), which was crystallised from ether. Compound (32) melted at 194-201° with decomposition, on a Kofler block. In a sealed tube under nitrogen (32) melted at 202-4° without any sign of decomposition. A mixed m.p. determination of compounds (28) and (32) showed no depression, Compounds (28) and (32) were identical in all other

respects. This synthesis established the structure and stereochemistry of (28) as shown in scheme (9).

Compound (32) was labelled with tritium and fed to Fusicoccum amygdali Del. It was incorporated into fusicoccin A with a high efficiency, indicating that (28) can act as a precursor of fusicoccin A.

Labelling of (32).

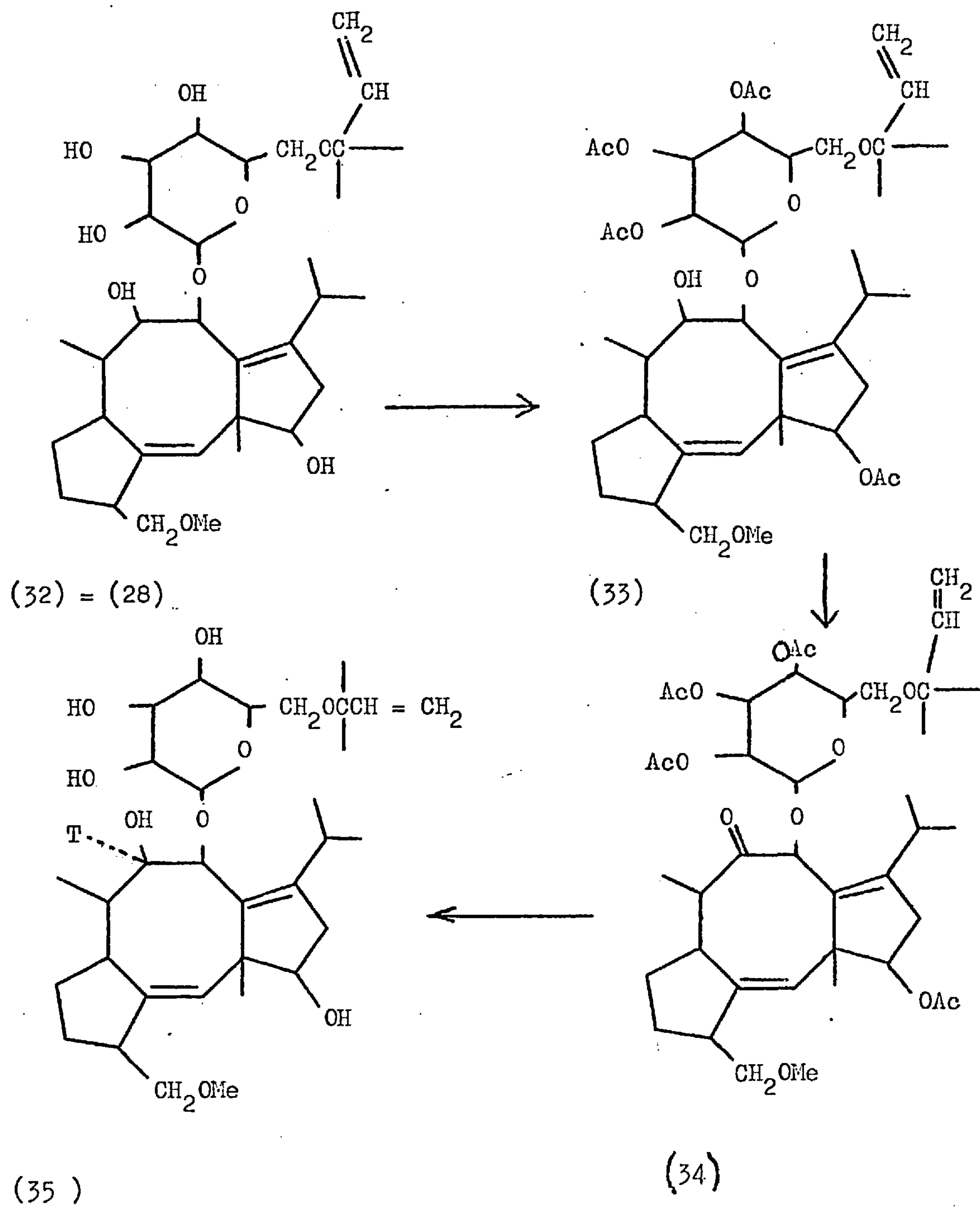
Treatment of (32) with pyridine and acetic anhydride at 2° overnight, gave the tetraacetate (33), scheme 11. This product, identified by its i.r. and n.m.r. spectra, failed to crystallise. Oxidation of (33) with Jones reagent[8,9] gave a crude product, presumably containing the ketone (34). This crude product was treated with $LiBT_4$ to give compound (35), which was identical with (32). Compound (35) was recrystallised to constant specific activity, 9.69×10^5 d/m/mg.

Feeding Experiments.

Two feeding experiments were carried out.

(a) Compound (35) (1.07mg: total activity fed 10.37×10^5 d/m was fed to one flask of 100ml culture of Fusicoccum amygdali Del. and worked up after 5 days.

(b) Compound (35) (3.61mg: total activity fed 34.98×10^5 d/m was fed to 2 flasks each of 100ml culture of Fusicoccum amygdali Del., and worked up after 7 days. In both cases the crude extracts of the culture filtrates were deacetylated and fusicoccin D (36) isolated by p.l.c. and recrystallised to constant specific activity. In these two experiments (35) was incorporated with efficiencies of 20.1 and 20.6%, respectively. These incorporation efficiencies are higher than those



Scheme 11

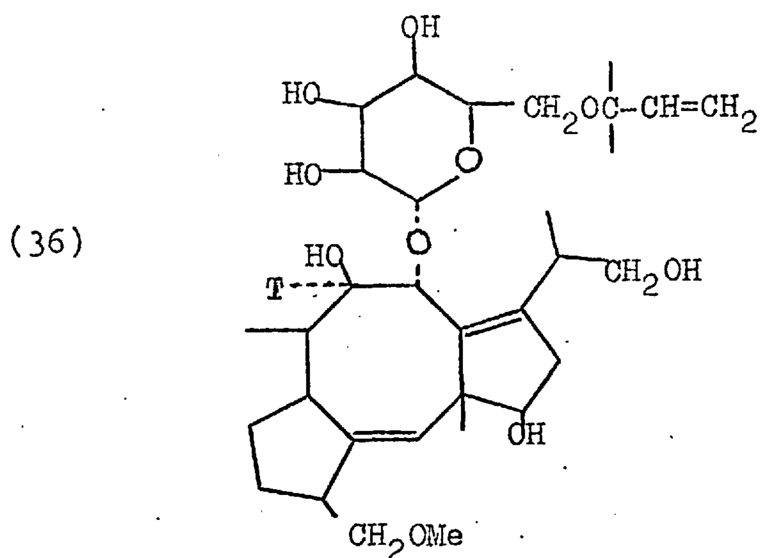
recorded for fusicoccin H (II)[8] (1.25 and 2.3%), showing that (32) is a more advanced precursor than fusicoccin H.

Specificity of Incorporation.

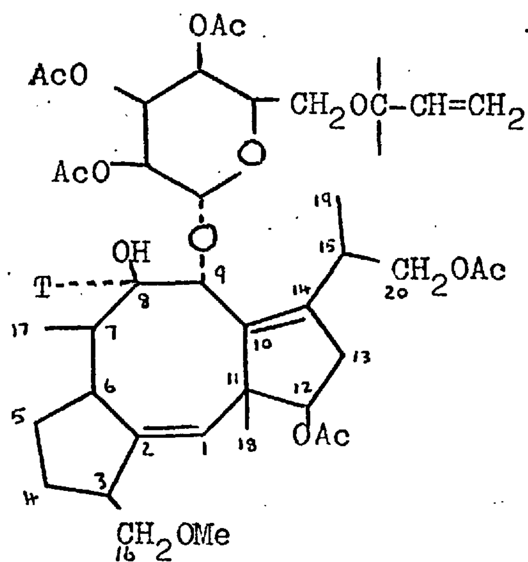
Treatment of compound (36) with acetic anhydride and pyridine at 2° overnight, gave triacetylfusicoccin (37). Jones oxidation of (37) at 5° for 1/2 hour, gave (38), with a loss of 89.0% of the label.

It was presumed that the residual tritium (11%) in (38), might be at C-7 or C-14 as a result of scrumbling during Jones oxidation. Therefore, (38) would be expected to lose its tritium when treated with a base. Treatment of (38) with $K^{+}\bar{O}Bu^{t}/HOBu^{t}$ gave a single non-crystalline product. On t.l.c. this product was much more polar than (38) and probably did not have any acetates. The specific activity of this product corresponded to 94.5% loss of the tritium originally present in (37).

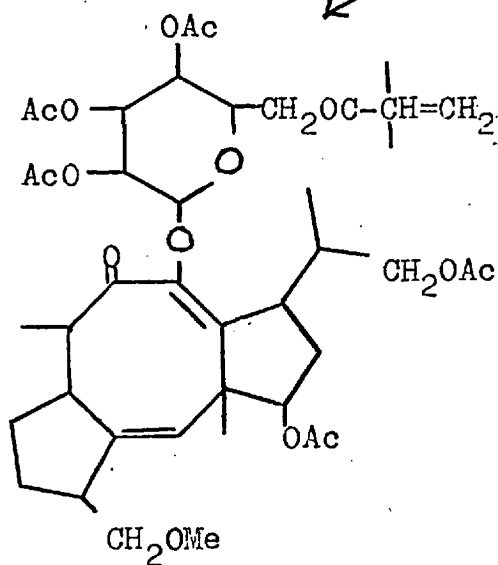
(36)



(37)



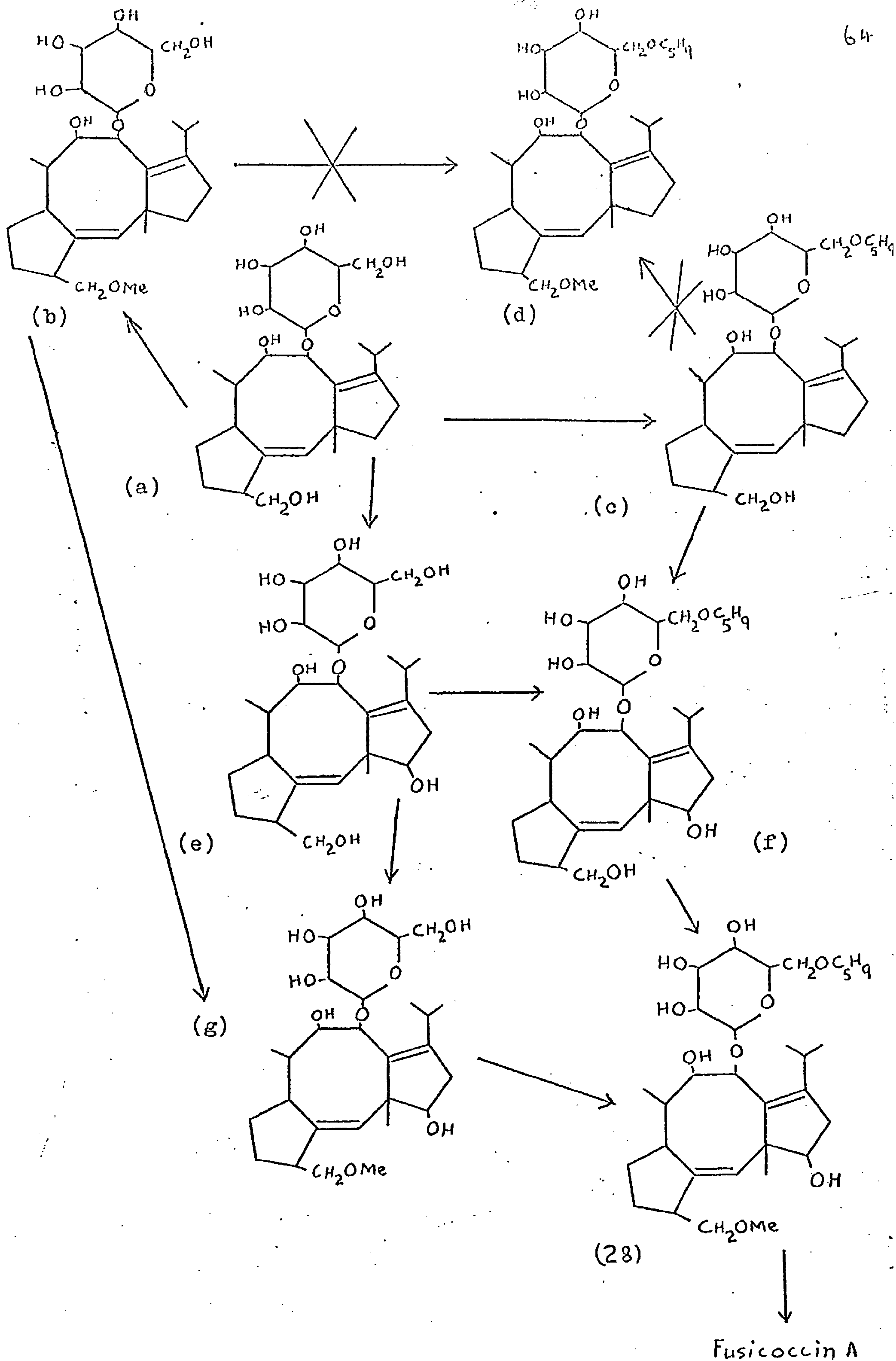
(38)



Scheme 12

Acetylation of extracts of culture filtrates of Fusicoccum amygdali Del. failed to yield compound (22) on hydrogenation. Therefore, it is unlikely that compound (d) (scheme 13), is an intermediate in the conversion of fusicoccin H (a) to fusicoccin A. Scheme 13 shows the possible pathways for the conversion of fusicoccin H (a) to the new metabolite (28). None of the compounds (b) - (g) have yet been isolated from the fungus.

The final stages in the biosynthesis of fusicoccin A, must therefore involve the specific hydroxylation of one of the geminal methyl groups of the isopropyl residue in (28), followed by the enzymic acetylation of this primary hydroxyl group and the sugar hydroxyl group.



Scheme 13

EXPERIMENTAL

Unless otherwise stated, the following data applies to this section:

Melting points were determined on a Kofler block and are uncorrected. Infra-red spectra were recorded on a Unicam SP200 spectrometer, as solutions in carbontetrachloride. Ultraviolet spectra were recorded on a Unicam SP800 spectrometer. N.m.r. spectra were recorded in deuterochloroform on a T-60 or a varian H.A.100 spectrometer. Mass spectra were run on a Perkin Elmer 270 low resolution or an A.E.I. MS9 high resolution spectrometer. Rotations were measured on a Perkin Elmer 141 polarimeter, in chloroform solutions.

Radiocounting was carried out with a Beckmann Liquid Scintillator Counter. The efficiency of ^{3}H-counting was ca. 50% with a background of ca. 50 dpm.

Thin layer (t.l.c.) and preparative layer chromatography (p.l.c.) were performed on Kieselgel GF_{254} activated at 110°C for 1½ hours.

Solvents were removed in vacuo on a rotary film evaporator in water baths at 50 - 60°. Products were freed from water or pyridine by repeated addition and evaporation of ethanol-toluene, in vacuo on a rotary film evaporator, in water baths at 50 - 60°.

Isolation of Compound (1)

A chloroform extract of a culture filtrate of Fusicoccum amygdali Del. was concentrated and fractionated on a silica gel M.F.C. column, eluting with 5% isopropanol in chloroform. Fractions containing fusicoccin A^2 were combined, concentrated and the fusicoccin A^2 crystallised. A portion of the mother liquor concentrate (92mg) was treated with acetic anhydride in pyridine at. 85° for 24 hours. The mixture was poured into ice water and extracted with ether. The ether extracts were evaporated to dryness and the residue dried. Separation of the residue by t.l.c., eluting with 30% ethylacetate in petroleum ether (b.p. 40-60°), gave (1) R_f(ca. 0.3) (1 mg) as a gum. The mass spectrum of (1) showed the highest recorded peak at m/e 732 (16%) (M^+ - CH_3COOH), with significant peaks at m/e 672 (4%), 663 (1.3%), 603 (1%), 442 (8.4%), 417 (5%), 374 (18.5%), 373 (18.5%), 357 (21%), 314 (33.3%), 313 (33.2%), 289 (99.4%), 229 (80.2%), 169 (74%), 109 (53%), and 69 (100%).

Dihydro derivative of (1).

Hydrogenation of (1) in ethylacetate in presence of Adam's catalyst, gave (2). The mass spectrum of (2) showed a small peak at m/e 794 (M^+), and a larger peak at m/e 734 (M^+ - CH_3COOH), with other peaks at m/e 674, 663, 603, 444, 417, 374, 373, 357, 314, 289, 229, 169, 109 and 71.

2,4,6-triisopropylbenzenesulphonylfusicoccin A(4).

Fusicoccin A (3) (1g) in pyridine was treated with 2,4,6-triisopropylbenzene-sulphonyl chloride (1.5g) at room temperature for 3 months. The mixture was treated with excess ethanol and evaporated to dryness. Separation by p.l.c. eluting with 5% isopropanol in chloroform, gave (4) R_F(ca 0.5), which crystallised

from methanol, m.p. 92-3° (891 mg). $[\alpha]_D^{22°}$ + 8° (c, 0.93); ν max 3500 (OH), 1725 (C = O); 1650(RCH = CH_2), 1605 (aromatic) cm^{-1}; λ max 281 mμ (ε 2500). NMR, τ 2.88 (2H, S) aromatic, τ 4.22(IH, dd, J10, 18Hz), τ 6.73(3H,S), τ 7.90(3H,S), τ 7.96(3H,S), τ 8.73 - 8.80 (27H, 2s), τ 8.98(3H, d, J6.5Hz), τ 9.21 (3H, d, J7Hz).
(Found: C, 64.8; H, 8.3; S, 3.3. $C_{51}H_{78}O_{14}S$ requires C, 64.5; H, 8.2; S, 3.4%).

Di-(2,4,6-triisopropylbenzenesulphonyl)-deacetyldihydro-fusicoccin (5).

2,4,6-triisopropylbenzenesulphonylfusicoccin (4) (450mg) was deacetylated by treatment with 2N NaOH and methanol (1:4), at room temperature overnight. Dilution with water, extraction with ether and evaporation of the ether extracts, gave the deacetyl product. This substance was dried and treated with 2,4,6-triisopropylbenzenesulphonyl chloride (lg) in pyridine at room temperature for 6 days. The reaction mixture was treated with excess ethanol and purified as in the above experiment, to give a t.l.c. pure non-crystalline product (326mg). Hydrogenation of this substance in ethanol in presence of Adam's catalyst or 10% Pd/$CaCO_3$, gave 2 products. Hydrogenation of the substance (250mg) in ethylacetate in presence of Adam's catalyst gave the non-crystalline di-(2,4,6-triisopropylbenzenesulphonyl)-deacetyldihydrofusicoccin(5) as the sole product (243mg). $[\alpha]_D^{20°}$ + 21° (c,0.17); λ max 281 (ε 4800). NMR, τ 2.77 - 2.90 (4H,2s) aromatic, 6.70(3H, s).

Reaction of di-(2,4,6-triisopropylbenzenesulphonyl)-deacetyldihydrofusicoccin(5) with sodium iodide.

Di-(2,4,6-triisopropylbenzenesulphonyl)-deacetyldihydrofusicoccin(5) (124mg) was treated with sodium iodide (lg) in acetone (19ml), under reflux overnight.

The reaction mixture was extracted into ether. Removal of solvent and crystallisation from petroleum ether (b.p. 40-60°) and diethyl ether, gave iodo-(2,4,6-triisopropyl-benzenesulphonyl)-deacetyldihydrofusicoccin (6)(87mg) as needles, m.p. 101-103°; a mixed m.p. determination with iodide (19) showed no depression. $[\alpha]_D^{20°}$ -48° (c, 1.2); λ max 281mμ(ε 2100); NMR, τ2.88(2H,s); τ6.18 (6H,S, broad), τ6.74(3H,s), τ8.74 - 8.86 (28H, 3s), τ 8.94(3H, d, J6Hz), τ9.11(3H, d J6Hz), τ9.21(3H, t, J3.5Hz). (Found: C, 58.0; H, 7.8; I, 12.9. $C_{47}H_{73}IO_{11}S$ requires C,58.1; H, 7.7; I, 13.1%).

Triacetyldihydrofusicoccin cyclic ether (8).

Dihydrofusicoccin[1] (7) (500mg) was treated with mercuric acetate (1.6g) in methanol (30ml) at room temperature for 4 days. The reaction mixture was poured into aqueous KBr solution (3.2g in 100ml of water) and kept at room temperature for 20 minutes. The mixture was extracted with ether and then with chloroform. The ether and chloroform extracts were combined and evaporated to dryness, to give a five component residue, (all the components quenched U.V. light). This residue was dissolved in methanol (40ml) and treated with 2N NaOH(8ml), followed by $NaBH_4$(400mg) and more 2N NaOH(8ml). The mixture was stirred at room temperature overnight. The reaction mixture was poured into water and extracted with ether. The ether extracts were evaporated to dryness and the residue dried. This residue was treated with excess acetic anhydride in pyridine at room temperature, for 3 days. The reaction mixture was poured into ice water and extracted with ether. The ether extracts were evaporated to dryness, to give (8) (200mg) as needles m.p. 141-144° (from ether). $[\alpha]_D^{20°}$ + 16°(c,0.6); ν max 2975, 1745 (C = O), 1375, 1245 and 1050cm^{-1}. NMR, τ6.73(3H,s), τ7.96(3H s), τ7.98(6H,s), τ8.06(6H,s) τ8.95(9H, s), τ 9.02(3H, d, J4Hz) τ9.16(3H,t,J7.5Hz).
M.S. m/e 808 (5%). M^+, 626 (8%), 556 (16%), 497 (8%), 432 (41%), 390 (50%),

372 (47%), 289 (100%), 229 (97%), 169 (85%), 109 (43%), 71 (67%), (Mass measurement at M(626), found 626.2965: $C_{31}H_{46}O_{13}$ requires 626.2937). (Found: C, 62.5; H, 7.9. $C_{42}H_{62}O_{15}$ requires C, 62.5; H, 7.8%).

Diacetyldeacetylfusicoccin A (10).

Triacetylfusicoccin A[1] (9) (602mg) was dissolved in methanol 75ml and treated with 0.2M aqueous sodium bicarbonate (15ml) at 35° for 1½ hours. The reaction mixture was diluted with water and extracted with ether and the ethereal extracts evaporated to dryness. Separation of the residue by p.l.c., eluting with 5% isopropanol in chloroform, gave (10) (R_F Ca 0.3) (210mg) as needles, m.p. 99-100° (from ether).
$[\alpha]_D^{20°}$ + 31° (c, 0.3), ν max 3475 (OH), 2970, 1730 (C = O), 1380, 1245 and 1050 cm^{-1}. N M R, τ 4.24 (IH,dd,J10, 18Hz), τ6.70 (3H,s), τ7.93 (3H,s) τ8.05 (3H,s), τ 8.77 (6H,s), τ 8.80(3H s), τ8.92(3H,d, J6.5Hz), τ 9.15(3H,d,J7H_2).
MS m/e 680 (M^+, $C_{36}H_{56}O_{12}$ mol. wt 680), 450 (diacetylaglycone), 390, 330 and 69. (Found: C,63.3; H,8.2. $C_{36}H_{56}O_{12}$ requires C,63.5; H,8.3%).

The di-O-nitrobenzoate (11).(P. 47)

O-nitrobenzoyl chloride (2g) was added to pyridine (15ml), dropwise with stirring. A precipitate was formed. The diacetate (10) (217mg) in pyridine (5ml) was added and the mixture stirred at room temperature for 22 hours. Ice was added and the mixture diluted with aqueous sodium chloride and extracted with ether. The ether extracts were evaporated to dryness. Separation of the residue by p.l.c., eluting with 5% isopropanol in chloroform, gave (11) (band R_FCa 0.5) as a gum (257mg). $[\alpha]_D^{22°}$ + 83° (C,1.94), ν max 3515(OH), 2995, 1735(C = O), 1610, 1555 and 1355 cm.$^{-1}$; λ_{max} 262.5 mμ (ε 9800).
NMR, τ 2.40(8H m) aromatic, τ 6.70(3H,s), τ 8.06(3H,s), τ8.08(3H,s),

τ 8.78(9H, s, overlapping), τ 8.95(3H,d,J6.5Hz), τ 9.18(3H,d,J7Hz). (Found: C, 61.2; H, 6.2; N, 3.0. $C_{50}H_{62}N_2O_{18}$ requires C, 61.3; H, 6.4; N, 2.9%).

Attempted O-nitrobenzoylation of (11).

Compound (11) (50mg) in pyridine (1ml) was added as above, to a mixture of O-nitrobenzoyl chloride (1g) and pyridine (10ml). The mixture was stirred at 55° for 3 days and worked up as above. The product was unchanged (11), which analysed correctly for $C_{50}H_{62}N_2O_{18}$.

The mono-benzoyl derivative of (11).

Compound (11) (133mg) in pyridine (3ml) was treated with benzoyl chloride (600mg) at room temperature overnight. The mixture was poured into ice water and extracted with ether. The ether extracts were evaporated to dryness. Separation of the residue by p.l.c., eluting with 5% isopropanol in chloroform, gave (12) (band R_F ca 0.6) as a gum (95mg).
$[\alpha]_D^{22°}$ + 86° (C, 0.6), ν max 3510 (OH), 1735(C = O) cm.$^{-1}$; λ max 255 mμ (ε 15900), 262 mμ (ε14500). NMR, τ2.38(13H,m), τ 6.70(3H,s), τ 8.05(3H,s), τ 8.07(3H,s), τ 8.76(9H,s, overlapping), τ8.93(3H,d,J6.5Hz), 9.16(3H,d,7Hz). (Found: C, 63.4; H, 6.4; N, 2.6. $C_{57}H_{66}N_2O_{19}$ requires C, 63.2; H, 6.1; N, 2.6%).

Attempted deacetylation of compound (12).

Compound (12) (5mg) was treated with methanol (5ml) and 0.2M aqueous $NaHCO_3$ (1ml) at 35° overnight. The reaction mixture was diluted with water and extracted with ether. Evaporation of the ether extract to dryness gave a residue which was a mixture of compounds including (12), (10) and deacetyl-

fusicoccin[2] (16). Similar results were obtained when the experiment was repeated with 0.2M aqueous sodium carbonate at 5° overnight.

Tribenzoates (13) and (15).

The diacetate (10) (68mg) in pyridine (3ml) was treated with benzoyl chloride (500mg) at room temperature overnight. The reaction mixture was worked up as in the preparation of (12). The product was separated by t.l.c., eluting with 2½% isopropanol in chloroform, to give (13) (band R_F Ca 0.6), as a gum (45.5mg). $[\alpha]_D^{22^o}$ + 36 (C, 0.5), ν max 3510 (OH), 1730 (C = 0) cm.$^{-1}$; λ max 231 mμ (ε54400), 275mμ (ε5700), 282mμ (ε4500). NMR, τ2.16 (6Hm), τ2.72 (9H,m), τ4.20 (1H,dd,J10, 18Hz), τ6.71(3H,s), τ7.93(3H,s), τ8.04(3H,s), τ9.03(6H s), τ9.20(3H,d,J3.5Hz), τ9.25(3H,s,J 3.5Hz).

When the experiment was repeated with the dihydro (14), compound (15) (P.46) was obtained as a gum. (Found: C, 68.7; H, 7.0. $C_{57}H_{68}O_{15}$ requires C, 68.9; H, 6.9%).

Attempted deacetylation of compound (15) using Na_2CO_3.

Compound (15) (5mg) was treated with methanol (6ml) and 0.2M aqueous sodium carbonate (1ml) at room temperature for 35 minutes. The reaction mixture was worked up as in the preparation of (10). The product was a mixture of compounds including (15), (14), and the deacetylation product of (14).

Attempted deacetylation of (15) using piperidine.

Compound (15) (27mg) in dry toluene (1ml) was treated with dry piperidine (0.2ml) at room temperature for 20 hours. T.l.c. indicated one product in addition to unchanged (15). The reaction mixture was then kept at 35° for 20

hours. T.l.c. indicated two products in addition to unchanged (15). The reaction mixture was then put under nitrogen and heated at 100° for 3 hours. T.l.c. indicated that a considerable amount of unchanged (15) remained. More piperidine (0.8ml) was added and the mixture kept at 100°, under nitrogen, overnight. T.l.c. indicated the presence of unchanged (15). The two products formed earlier, had decreased, and (14) had been formed.

The dihydroderivative of (10).

Compound (10) (575mg) was dissolved in ethylacetate and hydrogenated in presence of Adam's catalyst, at room temperature and atmospheric pressure, to give (14) (480mg) as needles (from ether), m.p. 75°.
$[\alpha]_D^{22^\circ}$ + 23° (c, 0.6), ν max 3450 (OH), 1730 (C = O) cm.$^{-1}$, NMR, τ 6.71 (3H,s), τ 7.94(3H,s), τ 8.06(3H,s), τ 8.91(9H s), τ 8.93(3H, d, J5Hz), τ 9.14(3H, d, J7Hz), τ 9.18(3H J7Hz). M.S. m/e 682(M^+, $C_{36}H_{58}O_{12}$ mol. wt. 682), 450 (diacetylaglycone), 390, 330, 71.
(Found: C, 63.4; H, 8.3. $C_{36}H_{58}O_{12}$ requires C, 63.5; H, 8.3%).

Hexabenzyldeacetylfusicoccin (17).

Deacetylfusicoccin[1] (16) (200mg) in N,N-dimethylacetamide (5ml) was stirred with NaH (300mg) under nitrogen at room temperature until no more hydrogen was given off. Benzyl bromide (1.5ml) was added and the mixture was stirred at 50°, under nitrogen, overnight. The mixture was poured into ice water and extracted with ether. The ether extracts were evaporated to dryness. Separation of the residue by p.l.c., eluting with ethylacetate-petroleum ether (b.p. 40-60°). (10:100), gave (17) (band R_F Ca 0.4) as a gum (216mg). $[\alpha]_D^{20^\circ}$ + 14° (C, 1.65); ν max 2900-2950, 1505, 1465 and 1370 cm^{-1}; λ max 210 mμ (ε46,000), 259mμ (ε1740). M.S. m/e 1136(M^+, $C_{74}H_{88}O_{10}$ mol. wt. 1136).

(Found: C, 78.6; H, 7.9. $C_{74}H_{88}O_{10}$ requires C, 78.3; H, 7.8%).

Attempted benzylation of compound (14).

Dry ether (50ml) was placed in a 2-neck flask provided with a water condenser and nitrogen. 5.3% methyllithium in ether (0.8ml) was injected below the surface of ether and the mixture was stirred at room temperature. Tetramethylpiperidine (465mg) was added and the mixture stirred for several hours, to allow the formation of tetramethylpiperidinelithium. The diacetate (14) (99.6mg) in dry ether, was added and the mixture stirred at room temperature overnight. The mixture became cloudy. Dry N,N-dimethylacetamide was added until a clear solution was formed. Benzylbromide (1ml) was added and the mixture stirred at room temperature overnight. The mixture was poured into ice water and extracted with ether. The ether extracts were evaporated to dryness. T.l.c. indicated the residue was a mixture of at least 7 compounds, including the deacetylation product of (14). Separation by p.l.c., eluting with 5% isopropanol in chloroform, gave the major product (band R_F Ca 0.3) as a gum (13mg). λ max 218 mμ (ε7443) and 259 mμ. (ε345), $[\alpha]_D^{20^\circ}$ 0° (C, 0.7). NMR, τ 6.70 (3H,s), τ 8.03(3H,s), τ 8.57(3H,s), τ 8.73(6H,s) τ 8.99(3H,s), τ 9.23(3H,d); there was a broad signal centred at τ 2.60.

Di-(2,4,6-triisopropylbenzenesulphonyl)-dihydrodeacetylfusicoccin (18).

Compound (14) (297mg) in dihydropyran (10ml) and toluene (5ml), was stirred with concentrated hydrochloric acid (1 drop), under nitrogen, at 80° overnight. The mixture was evaporated to dryness, to give a yellowish oil. This product was deacetylated with 2NNaOH (2ml) in methanol (10ml) at room temperature overnight. The reaction mixture was diluted with water and extracted with ether. The ether extracts were evaporated to dryness and the residue dried. The

residue was dissolved in pyridine (5.4g) and treated with 2,4,6-triisopropylbenzenesulphonyl chloride (2.1g), at room temperature for 104 hours. The reaction mixture was treated with excess ethanol, evaporated to dryness, and the residue freed from pyridine. The residue was dissolved in ethanol (75ml), and treated with hydrochloric acid (1ml) in water (4ml) at 30° overnight, to cleave the tetrahydropyranylethers. The reaction mixture was then diluted with water and extracted with ether. The ether extracts were washed with aqueous sodium bicarbonate and evaporated to dryness. The residue contained both the mono- and di- 2,4,6-triisopropylbenzenesulphonyl products. Separation by p.l.c. eluting with ethylacetate-petroleum ether (b.p. 40-60°) (1:5) and then with 5% isopropanol in chloroform afforded (18) (band R_FCa 0.4, the mono-product band R_FCa 0.0.39) as a gum (115mg). $[\alpha]_D^{21^{\circ}}$ + 12°(C,0.9); ν max 3530(OH), 3000, 1605. 1475 and 1440 $cm.^{-1}$; λ max 281 (ε 3700). NMR, τ 2.86(2H,s)(aromatic, 1^{ry} group), τ 2.92(2H,s) (aromatic, 2^{ry} group), τ 6.75(3H,s), τ 8.72-8.97 (45H,5s), τ 9.02(3H, d, J6.5Hz), τ 9.10(3H, d, J7Hz), τ 9.14(3H, t, J7Hz).

Iodo-2,4,6-triisopropylbenzenesulphonyl compound (19).

Compound (18) (87mg) in acetone (15ml) was heated under reflux with sodium iodide (1.5g) overnight. The reaction mixture was diluted with water and extracted with ether. The ether extracts were evaporated to dryness. Separation of the product by p.l.c., eluting with 5% isopropanol in chloroform, gave (19) (band R_F Ca 0.35) (44mg) as needles (from diethylether - petroleum ether b.p. 40-60°), m.p. 102-103°. A mixed m.p. determination with the iodide (6) showed no depression. $[\alpha]_D^{18^{\circ}}$ - 49° (c, 0.92); ν max 3450(OH), 1605, 1475, 1440$cm.^{-1}$; λ max 281 mμ (ε 1900). NMR, τ 2.90(2H,s) (aromatic, 2^{ry} group), τ 6.74(3H,s), τ 8.74-8.85(27H,4s) τ 8.96(3H, d, J6Hz), τ 9.10(3H, d, J6Hz) τ 9.20(3H, t, J3.5Hz).

Cyclopentadiene (20).

The iodide (19) (140mg) in dimethyl-sulphoxide (20ml) was stirred with $NaBH_4$ (1g) at 140° for 3 days. The reaction mixture was poured into water and extracted with ether. The ether extracts were washed with aqueous $NaHCO_3$ to remove dimethylsulphoxide, and evaporated to dryness. Elution of the product on p.l.c. using 5% isopropanol in chloroform, afforded the cyclopentadiene (20) (band R_F Ca 0.2) as a gum (15mg). $[\alpha]_D^{20^0}$ + 53° (C, 0.12); ν max 3420(OH), 1705, 1470 cm.$^{-1}$; λmax 270 mμ (ε1200). NMR, τ 3.86(2H,s) (cyclopentadiene), τ 6.70(3H,s), τ 8.76(6H,s), τ 8.82-8.96 (≈15H, q, overlapping) τ 9.14(3H, t, J7Hz).

M.S. m/e 564 (M^+ $C_{32}H_{52}O_8$ mol. wt. 564). (Found: C, 67.9; H, 9.5% $C_{32}H_{52}O_8$ requires C, 68.0; H, 9.3%).

Hydrogenation of the cyclopentadiene (20).

The cyclopentadiene (20) (14mg) in ethylacetate was hydrogenated in presence of 10% Pd on strontium carbonate, at room temperature and atmospheric pressure.

1 mol. of hydrogen was absorbed, giving a single product (14mg). The product had no U.V. absorption at λ270 mu. and did not show the cyclopentadiene signal at τ 3.86 in its n.m.r. spectrum. This product was identified as compound (21) without further characterisation.

Acetylation of (21) to compound (22).

Compound (21) (14 mg) in dry pyridine (0.3ml) was treated with acetic anhydride (0.5ml) at room temperature for 4 days. The reaction mixture was diluted with water and extracted with ether. The ether extracts were evaporated to dryness. Purification by p.l.c., eluting with 30% ethylacetate in petroleum ether (b.p. 40 - 60°), gave (22) (band R_F ca 0.7) as a gum (15mg). M.S. m/e 734(3%) (M^+ $C_{40}H_{62}O_{12}$ mol. wt. 734), 378(12%), 360(8.5%), 359(11%), 358(11%), 315(17%), 300(28%), 289(44%), 256(22%), 229(28%), 169(28%), 151(28%), 109(26%), and 71(100%). This product was not characterised further.

2,4,6-triisopropylbenzenesulphonyldeacetyldihydrofusicoccin (24).

Dihydrofusicoccin[2] (23) (1g) in dihydropyran (30ml) and toluene (10ml) was stirred with concentrated hydrochloric acid (2 drops) under nitrogen at 70° overnight. The mixture was evaporated to dryness, to give a yellow oil. This product was deacetylated with 2N NaOH(5ml) in methanol (25ml), at room temperature overnight. The reaction mixture was worked up and dried as in the preparation of (18). The product was dissolved in pyridine and treated with 2,4,6-triisopropylbenzenesulphonyl chloride (6g), at room temperature for

69 hours. The reaction mixture was treated with ethanol and work up as for (18). The product in ethanol (150ml) was treated with concentrated hydrochloric acid (2ml) in water (8ml), at 30° overnight. The product was worked up and purified as for (18), to give (24) (band R_F ca 0.5) as a gum (786mg). $[\alpha]_D^{21°}$ + 49° (c, 1.8); ν max 3500 (OH), 3000, 1605, 1475, 1440, and 1355cm^{-1}; λ max 281 mμ (ε 2400). NMR, τ 2.86 (2H, s)(aromatic 1^{ry}group), τ 6.71 (3H, s), τ 8.73(9H, s) τ 8.79(12H, s), τ 8.90 (6H, s) τ 8.97 (3H, d, J6.5Hz), τ 9.12(3H, d, J6.5Hz), τ 9.17 (3H, t, J7Hz).
(Found: C, 65.1; H, 8.5; S, 3.6. $C_{47}H_{76}SO_{12}$ requires C, 65.4, H, 8.7, S, 3.7%).

The iodide(25)

Compound (24) (415mg) in acetone (15ml) and sodium iodide (1.5g) were stirred and heated under reflux overnight. The reaction mixture was diluted with water, worked up and purified as for (19), to give the iodide (25) (band R_F ca 0.45), as needles (from ether) m.p. 137 -140°, (276mg). The product gave a positive Beilstein test. $[\alpha]_D^{20°}$ + 4° (c, 0.7); λ max 224 mμ (ε 7500), and 259mμ (ε 1600).
(Found: C, 54.3; H, 7.4. $C_{32}H_{51}IO_9$ requires C, 54.4; H, 7.2%).

Reduction of the iodide (25).

The iodide (25) (61mg) in dimethylsulphoxide (23ml) was stirred with $NaBH_4$ (200mg) at 100° for 30 minutes. The reaction mixture was poured into water and worked up as for (20). Separation of the product by p.l.c., on triple elution with 3% isopropanol in chloroform, afforded compound (26) (band R_F ca 0.43) as a gum 30mg. The product gave a negative Beilstein test. M.S. m/e 582 (1%) (M^+, $C_{32}H_{54}O_9$ mol. wt. 582), 350 (29%) (aglycone),

332 (34%), 314 (10%), 259 (13%), 207 (20%), 151 (27%), 109 (31%) 71 (100%).
This compound was not characterised further.

Acetylation of (26).

Compound (26) (30mg) in pyridine (0.5ml) was treated with acetic anhydride (1ml) under nitrogen at 70° overnight. The reaction mixture was poured into ice water, worked up and purified as for (22), to give (27) (band R_F Ca 0.4) as a gum (32mg). $[\alpha]_D^{22°}$ + 15 (c, 2.8), ν max 1740 (C = O), 1370, 1240, and 1045cm.$^{-1}$ NMR, τ 6.78 (3H, s), τ 8.03 - 8.11 (15H, 3s), τ 8.78 (6H, d, J7Hz), τ 8.96 (9H s), τ 9.14 (3H, d, J7Hz), τ 9.18 (3H, t, J7Hz). M.S. m/e 794 (M^+ $C_{42}H_{66}O_{14}$ mol. wt. 794), 734, 373, 331, 289, 229, 169, 109 and 71. This product (27) was identical with compound (2) on t.l.c. and the i.r. and mass spectra were identical. There was no further characterisation.

Isolation of Compound (28)

A chloroform extract of a culture filtrate of Fusicoccin amygdali Del. was concentrated and fractionated on a florisil column, eluting with isopropanol-chloroform mixtures. The fractions containing fusicoccin A[2] (3) were concentrated and the fusicoccin A crystallised. The mother liquor was evaporated to dryness. The product (26g) in pyridine (40ml) was treated with acetic anhydride (160ml) under nitrogen at 70° for 2 days. The mixture was worked up and dried as for compound (22). T.l.c. indicated the product contained compound (1). Fractionation of this product on a column of silica gel G.F.C. (61 x 6.5cm, 1150g) eluting with 30% ethylacetate in petroleum ether (b.p. 40-60°), gave impure compound (1). This product was further purified by p.l.c. eluting with 30% ethylacetate in petroleum ether (b.p. 40 - 60°). The product was still impure and it was deacetylated with 2N NaOH (4ml) in methanol (20ml)

at room temperature overnight. The reaction mixture was worked up as usual. Separation of the product by p.l.c. on 5% silver nitrate/silica gel G F_{254}, eluting with 10% isopropanol in chloroform, gave (28) (band R_F Ca 0.4) (300mg) as white crystals (from ether), m.p. 194 - 203° (with decomposition), 204 - 6° under nitrogen in a sealed tube (no decomposition). $[\alpha]_D^{18°}$ + 24° (c, 0.54); ν max.(KBr) 3420 (OH), 1645 ($RCH = CH_2$), 1460, 1385, 1350, 1275, 1240 and 1150 $cm.^{-1}$ N.M.R., τ 4.27(1H, dd, J10, 18Hz), τ 6.70 (3H, s) τ8.76 (6H, s), τ8.82 (3H, s), τ8.96 (3H, d, J6Hz), τ9.04 (3H, d, J6Hz), τ 9.17 (3H, d, J7Hz).
MS., m/e 580 (0.5%), (M^+ $C_{32}H_{52}O_9$ mol. wt. 580), 350 (15%), 332 (26%0, 259 (20%), 207 (15%), 151 (23%), 109 (38%) 69 (100%).
(Found: C, 65.9; H, 9.1. $C_{32}H_{52}O_9$ requires C, 66.2; H, 9.0%).

The dihydro derivative of (28).

Compound (28) (50mg) in ethylacetate was hydrogenated in presence of Adam's catalyst. 1 mol. of hydrogen was absorbed to give the dihydro (29) (50mg). This product crystallised from diethyl ether - petroleum ether (b.p. 40 - 60°) (with difficulty), as needles m.p. 106 - 8°. It was identical with product (26) on t.l.c. $[\alpha]_D^{21°}$ + 22° (c, 0.89); ν max 3500 (OH), 1475, 1400, 1380 and 1055$cm.^{-1}$ M.S. 582 (1%) (M^+ $C_{32}H_{54}O_9$ mol. wt. 582), 350 (23%) (aglycone), 332 (29%), 314 (9%), 259 (10%), 207 (19%) 151 (28%), 109 (31%) and 71 (100%). The product was identified without further characterisation.

2,4,6-triisopropylbenzenesulphonyldeacetylfusicoccin A (30).

Fusicoccin A[2] (3) (500mg) in dihydropyran (10ml) and toluene (5ml) was stirred with concentrated hydrochloric acid (2 drops) under nitrogen at room temperature for 5 days, and then at 65° for 7 hours. The mixture was evaporated to dryness to give a light yellow oil. The product was deacetylated

with 2N NaOH (2ml) in methanol (13ml), at room temperature overnight. The reaction mixture was worked up and dried as for (24). The product was dissolved in pyridine and treated with 2,4,6-triisopropylbenzenesulphonyl chloride (800mg), at room temperature for 19 days. The reaction mixture was treated with excess ethanol and worked up as for (24). The pyridine free product was dissolved in ethanol (75ml) and treated with concentrated hydrochloric acid (1ml) in water (4ml) at 30° for 17 hours. The product was worked up and purified as for (24), to give (30) (band R_F Ca 0.4) as a gum (197mg). $[\alpha]_D^{20°}$ + 57° (c, 1.2); ν max 3500 (OH), 1605, 1475, 1435, 1385, 1355 and 1270 cm.$^{-1}$, λ max 281 mμ (ε 1900). NMR, τ 2.85 (2H, s)(aromatic,1^{ry} group), τ 4.20 (1H, dd, J10, 18Hz), τ 6.69 (3H, s), τ 8.72 - 8.78 (27H, 3s), τ 8.96 (3H, d, J6.5Hz), τ 9.12 (3H, d, J7Hz). (Found: C,65.3, H, 8.5, S, 3.6. $C_{47}H_{74}SO_{12}$ requires C, 65.4; H, 8.7; S, 3.7%)

<u>The iodide (31)</u>.

Compound (30) (306mg) in acetone (15ml) was stirred with sodium iodide (1.5g) in presence of triethylamine (1ml), and heated under reflux for 24 hours. The product was diluted with water, worked up and purified as for (25), to give (31) (band R_F Ca 0.38) as needles (from ether) (211mg), m.p. 157°. $[\alpha]_D^{20°}$ + 5° (c, 0.8); ν max (KBr) 3450 (OH), 1645(RCH = CH_2), 1465, 1385, 1370 and 1260 cm.$^{-1}$; λ max 224 (ε7400), and 259 (ε 1800). NMR, τ 4.25(1H, dd, J10, 18Hz), τ 6.70 (3H, s), τ 8.74 (6H, s), τ 8.82 (3H, s), τ8.85 (3H, d, J5Hz), τ9.14 (3H, d, J7Hz). (Found: C, 54.6; H, 7.2; I, 17.7. $C_{32}H_{49}IO_9$ requires C, 54.4; H, 7.2; I, 18.0%).

Reduction of the iodide (31).

The iodide (31) (782mg) in dimethylsulphoxide (23ml) was stirred with $NaBH_4$ (1g) at 100° for 23 minutes. The mixture was poured into aqueous $NaHCO_3$ solution (200ml) and extracted with ether (2 x 50ml). The ether extracts were washed with fresh aqueous $NaHCO_3$ (2 x 30ml) and evaporated to dryness. Separation of the product by p.l.c., on triple elution with 5% iso-propanol in chloroform, gave (32) (band R_F Ca 0.36) (391mg) and unchanged (31) (166mg). The product (32) crystallised from diethylether m.p. 194 - 201° (with dec.), 202 - 4° under nitrogen in a sealed tube. A mixed m.p. determination with (28) showed no depression. Compounds (28) and (32) were identical on t.l.c. and the i.r., n.m.r. and mass spectra were identical. $[\alpha]_D^{20}$ + 24° (c, 0.6); ν max (KBr) 3420 (OH), 1645 (RCH = CH_2), 1460, 1385, 1350, 1275, 1240 and 1150 cm^{-1}.

M.S. 580 (0.5%), (M^+) 350 (15.6%), 332 (28%), 259 (20.6%) 207 (17%), 151 (23%), 109 (35%), 69 (100%). NMR, τ 4.26 (H, dd, J10, 18Hz), τ 6.70 (3H, s), τ 8.75 (6H, s), τ 8,85 (3H, s), τ 8.96 (3H, d, J6Hz), τ 9.03 (3H, d, J6Hz), τ 9.16 (3H, d, J7Hz).

(Found: C, 66.1; H, 9.0. $C_{32}H_{52}O_9$ requires C, 66.2; H, 9.0%).

Tetraacetyl- derivative of (32).

Compound (32) (187mg) in dry pyridine (800mg) was treated with acetic anhydride (2.5g), at 2° overnight. The reaction mixture was poured into ice-water and the product extracted with ether. Separation by p.l.c. eluting with 30% ethyl-acetate in petroleum ether (b.p. 40-60°), yielded compound (33) (band R_F ca 0.27), as a gum (218mg), νmax 3570 (OH), 1750 (C=O) and 1645 ($RCH=CH_2$) cm^{-1} N.n.r. τ4.32 (1H, dd, J10,17.5Hz), τ6.70 (3H, s), τ7.94 (3H, s), τ8.03 (3H,s), τ8.06 (3H, s), τ8.07 (3H, s), τ8.80 (9H, s), τ8.87 (3H, d, J6.5 Hz), τ8.97 (3H, d, J6.5 Hz), and τ9.19 (3H, d, J6.5 Hz). This product was identified without further characterisation.

Jones Oxidation of Compound (33).

Compound (33) (76mg) in acetone (5ml) was cooled to -5° in a salt-ice bath.[8] Jones reagent[9] (0.5ml) was added. The flask was shaken by hand at -5° for 2 minutes. Excess isopropanol (3ml) was added. The mixture was poured into ice water and extracted with ether. The ether extracts were washed with water and evaporated to dryness. The residue, presumably containing (34), was dried in vacuo at room temperature for 2 days and used without purification.

Compound (35)

To $LiBH_4$ (170mg) in THF (freshly distilled over $LiAlH_4$) (10ml) was added ^3H_2O (6 Ci/ml) (75 ul). The mixture was stirred and refluxed for $1^1/2$ hours. The crude product from Jones oxidation above, was dissolved in dry THF (7ml) and added. The mixture was refluxed for 15 hours and cooled to room temperature. 2N NaOH (5ml) and methanol (5ml) were added and the mixture kept at room temperature for 7 hours. The reaction mixture was then diluted with a saturated aqueous solution of NaCl (50ml) and extracted with ether (2x20ml). The ether extracts were washed with more brine (20ml) and evaporated to dryness in vacuo. The residue was taken up in ethanol and the solution evaporated to dryness, repeating the process several times, to free the product from labile activity. The product was recrystallised to constant specific activity from diethylether, to give (35) scheme 11, (17.6mg), 9.69×10^5 d/m/mg. This product was identical with (32) on t.l.c.

Feeding Experiments.

Fusicoccum amygdali Del. was grown in shaken flasks [500 ml capacity, 100ml medium of the composition: glucose (3.0%), $NaNO_3$ (0.33%), KH_2PO_4 (2.0%), KCl (0.05%), $MgSO_4.7H_2O$ (0.05%), $FeSO_4.7H_2O$ (0.001%), soya bean meal(0.2%), and tap water to volume.] The flasks were incubated at 24° for 27 hours. Compound (35) was dissolved in a few drops of warm ethanol, diluted with distilled water and fed to the cultures,rinsing the vessels with aqueous ethanol (7:3). The cultures were shaken again at 24° for 5 or 7 days. The cultures were then filtered through muslin, washing the mycelium with distilled water (4×25ml). The filtrate and washings were combined and extracted with isobutylmethyl ketone (3×100ml) and diethylether (100ml). The extracts were combined and evaporated to dryness in vacuo at 45°. The residue was dissolved in methanol (25ml) and treated with 2N NaOH (5ml) at room temperature overnight. The mixture was diluted with water and extracted with IBMK (3×30ml) and diethylether (30ml). The extracts were combined and evaporated to dryness in vacuo. Fusicoccin D (36) was isolated from the residue by p.l.c.,on elution with 10% isopropanol in chloroform. Compound (36) was recrystallised to constant specific activity, from a (1:1) mixture of acetone and petroleum ether (b.p. 40-60°). The results are given in table I below.

TABLE I

Compound (35): Specific activity 9.69×10^5 d/m/mg.						
Wt. fed. in mg	Total activ. fed. $\times 10^5$ d/m	Volume of culture in ml	Period of incubaion in days.	Yield of Fus.D (36) in mg	Total activ. rec'd $\times 10^5$ d/m	% Incorp. Eff.
1.07	10.37	100	5	11	2.079	20.1
3.61	34.98	2x100	7	17.6	7.216	20.6

Specificity of Incorporation.

Compound (37).

^{3}H-Fusicoccin D (36) (22.4mg) in pyridine 500mg was treated with acetic anhydride (1.5g) at 2° overnight. The mixture was poured into ice-water and extracted with ether. The ether extracts were evaporated to dryness and the product recrystallised to constant specific activity, from petroleum ether (b.p. 60-80°) to give triacetylfusicoccin A (37) (28mg), m.p. 116° (Lit.m.p. 116-7°)[4], 3.068×10^6 d/m/m mole.

Compound (38).

Compound (37) (28mg) in acetone (10ml) was stirred, cooled to 5° and treated with Jones reagent[9] (1.2ml) dropwise. The mixture was stirred at 5° for 1/2 hour. Excess isopropanol (5ml) was added. The mixture was diluted with water (50ml) and extracted with ether (2×20ml). The ether extracts were combined, washed with water (2×20ml) and evaporated to dryness in vacuo. The product was recrystallised to constant specific activity, from petroleum ether (b.p. 60-80°), to give the α,β-unsaturated ketone (38) (21mg), m.p. 136-9°; $[\alpha]_D^{20^o}$ + 80° (c, 1.6), λ_{max} 235 mu (ε 2400), [Lit. m.p. 137-8°; $[\alpha]_D$ + 80°; λ_{max} 230 mu (ε 9500)][5]. This product had a specific activity of 3.366×10^{5} d/m/m mole, corresponding to 89.0% loss of the label. Treatment of (38) with $K^{+}\bar{O}Bu^{t}$/ $HOBu^{t}$ at 30° overnight, gave an uncharacterised non-crystalline product. This product had a specific activity of 1.70×10^{5} d/m/m mole, corresponding to 94.5% loss of the tritium originally present in (37).

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