Tetrahedron. Vol. 28, pp. 5105 to 5111. Pergamon Press 1972. Printed in Great Britain.

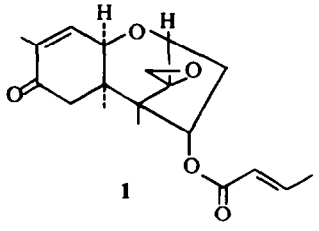
THE STRUCTURES OF TRICHODIOL AND TRICHODIENE

S. NOZOE and Y. MACHIDA
Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo, Japan

(*Received in Japan* 14 *December* 1971; *Received in the UK for publication* 9 *February* 1972)

Abstract—Two new metabolites, trichodiol and trichodiene, have been isolated from *Trichothecium roseum*. Spectral evidence was used in deriving their structures.

MANY NATURALLY occurring, physiologically active compounds have been found that are the esters of a group of sesquiterpene alcohols possessing the trichothecane nucleus. For example, the antifungal and cytotoxic substances, trichothecin (**1**)[1] isolated from *Trichothecium roseum*, verrucarins and roridins[2] isolated from *Myrothecium* species, and diacetoxyscirpenol[3] and the acetates of nivalenol[4] isolated from *Fusarium* species belong to the above esters.



1

All the sesquiterpene alcohols are assumed to be derived biogenetically from the hydrocarbon having structure **16**, described in the literature[5] as a hypothetical precursor of trichothecin. Experimental verification that trichothecin is actually formed from farnesyl pyrophosphate by a cyclization process including a 1,2-methyl double migration has been reported.[6, 7]

In the course of investigations on the biosynthesis of fungal isoprenoids, two new sesquiterpenes have been isolated from a strain of *Trichothecium roseum* for which the names trichodiol* and trichodiene[9] are proposed. Trichodiene was found to be the hydrocarbon actually possessing structure **16**.

In the present paper we describe the isolation and structural elucidation of these substances.

Trichodiol-A*

Trichodiol-A (**2**) was obtained from the nonsaponifiable fraction of the fermentation broth from *Trichothecium roseum* as colourless crystals, m.p. 81–83° (from ether–hexane), $[\alpha]_D$ + 52° ($CHCl_3$). Although the highest value in the mass spectrum of trichodiol-A was 234·162 corresponding to $C_{15}H_{22}O_2$ (calc. 234·162), elemental analysis and the data from various derivatives confirmed the molecular formula of this

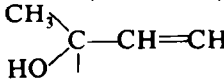
* Trichodiol previously reported[8] has been found to be an artefact produced from compound **15** by alkali saponification. therefore we assigned the name trichodiol-A to compound **2** and trichodiol to compound **15**.

compound to be $C_{15}H_{24}O_3$. The IR spectrum of trichodiol-A showed an absorption at 3460 cm^{-1} indicating the presence of OH group(s) but had no absorption in the carbonyl region. The UV spectrum showed only the end absorption (210 nm, $\varepsilon = 360$) suggesting the presence of a non-conjugated double bond. On acetylation, trichodiol-A gave a monoacetate which still had an abosrption band at 3600 cm^{-1} due to a tertiary OH group.

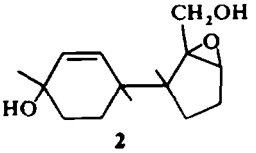
The NMR spectrum of trichodiol-A revealed the presence of three tertiary methyl groups (singlets at 0·86, 0·96, 1·15, 3H each), $C\underline{H}_2OH$ (3·48, 4·06, 2H, ABq, $J = 12$ Hz) attached to non-hydrogen bearing carbon atom, —$C\underline{H}$=$C\underline{H}$— (5·46, 5·73, 2H, ABq, $J = 10$ Hz) both carbon atoms which are attached to quaternary carbon atoms, and a methine proton (3·17, 1H, bs) probably of an oxirane ring. The signals at 3·48 and 4·06 shifted to 4·33 and 4·54 upon acetylation.

The mass spectrum of trichodiol-A showed it to be very readily cleaved into two parts on electron impact; (a) $C_8H_{13}O$ (obs 125·099, calc. 125·097) and (b) $C_7H_{11}O_2$ (obs 127·074, calc. 127·076). Other prominent fragments were C_8H_{11} (a-H_2O), C_8H_{12} (a-OH), C_7H_9O (b-H_2O), C_6H_8O (b-CH_2OH). In the mass spectrum of trichodiol-A acetate the peak at 127 disappeared and a new peak at 169 (b + CH_2CO) appeared, whereas the peak at 125 (a) remained. This shows that the CH_2OH is in the b portion.

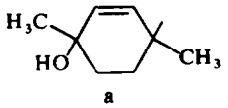
LAH reduction of trichodiol-A gave a triol (**3**), the NMR spectrum of which had no absorption at 3·17. When treated with $Pb(OAc)_4$ in benzene, triol **3** afforded a norketone (**4**) which showed an IR band at 1732 cm^{-1} due to a five membered ring ketone. The NMR and mass spectra revealed the loss of CH_2OH. These data indicate that part b of trichodiol-A contains 1-hydroxymethylcyclopentene oxide moiety. Jones oxidation of **4** gave a diketone (**5**). The IR spectrum of this compound showed an absorption due to an α,β-unsaturated ketone at 1665 cm^{-1} and a band due to five membered ring ketone (1735 cm^{-1}). The UV spectrum had an absorption at 237 nm ($\varepsilon = 12{,}600$). In the NMR spectrum of **5**, one of the three tertiary methyl groups and the AB system due to double bond protons that appeared in the spectrum of norketone **4** disappeared, instead, a broad singlet corresponding to three hydrogens and a multiplet corresponding to one hydrogen appeared at 1·89 and 5·58, respectively.

These data show the presence of a β-methyl-α,β-unsaturated ketone in diketone **5** and the allylic carbinol system,  , in compound **4**, and hence in trichodiol-A. As the molecular formula requires the presence of one more carbocyclic ring or double bond and as there was no indication of a double bond, it is concluded that there is a carbocyclic ring and that this ring, which must be in part a, should be six membered as suggested by the position of the IR band of compound **5**.

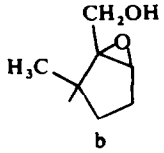
Since six carbon atoms have been shown to be present in part a and other six carbon atoms in part b, it can be concluded that two tertiary methyl groups exist in part a and one in part b, affording structure **2** to trichodiol-A except the position of substitution of part a and the methyl group on the cyclopentane ring of part b.



2



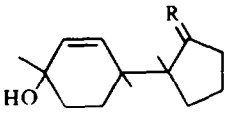
a



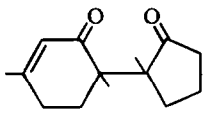
b

In order to determine this position, the following transformations were carried out. Trichodiol-A was hydrogenated over Pd/C with one mole of hydrogen, affording a mixture of compound **6** and the saturated derivative **7**. LAH reduction of this mixture gave glycols **8** and **9**, which on periodic oxidation yielded a mixture of norketones (**10** and **11**). The unsaturated and saturated norketones could be separated by means of $AgNO_3$ impregnated silica gel column cnromatography. It was found that norketone **10** was a mixture of two double bond isomers which might be formed by isomerization during hydrogenation. The saturated derivative (**11**) showed in its NMR spectrum only two protons at a lower field than 1·90 indicating the carbon atom next to carbonyl group is quaternary.

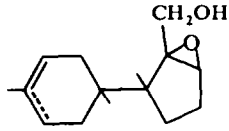
These observations established the structure of trichodiol-A as **2**.



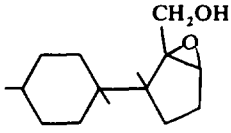
3, R=CH_2OH, OH
4, R=O



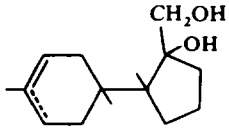
5



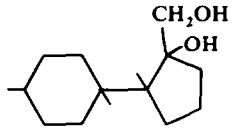
6



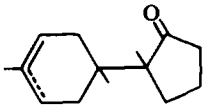
7



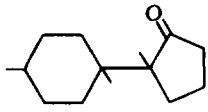
8



9

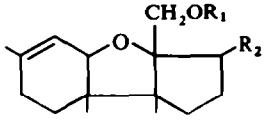


10



11

Trichodiol-A is very sensitive to acid. Thus treatment of **2** with HCl afforded a chlorohydrin (**12**). When dilute HCl was used during work-up in the preparations of the monoacetate of trichodiol-A and the triol **3**, similar reactions took place, giving compounds **13** and **14**, respectively.

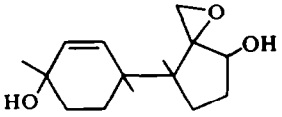


12, R_1=H, R_2=Cl
13, R_1=Ac, R_2=Cl
14, R_1=R_2=H

Inspection of the structure of trichodiol-A suggests the possibility that it is derived from a compound possessing the isomeric structure **15** during the alkali saponification. And in fact the broth extract, which had not been treated with alkali, was separated to afford compound **15**, which showed mass fragmentation peaks including 234 (M—H_2O), 219 (M—H_2O—CH_3), 125 (a), 109 (b-H_2O), 108, and 107 (a-H_2O). This compound is very difficult to separate from concomitant impurities but when acetylated

it is easily separable by column chromatography. Thus acetylation of the crude fraction containing compound **15** gave the monoacetate. It showed NMR signals due to three tertiary methyl groups (singlets at 0·92, 1·00, 1·27, 3H each), $C\underline{H}_3COO—C\underline{H}<$ (2·01, 3H, s; 4·63, 1H, bs), oxirane methylene group (2·74, 3·45, 2H, ABq, $J = 4·5$ Hz), and —C<u>H</u>=C<u>H</u>— (5·46, 5·64, ABq, $J = 10$ Hz). The mass spectrum showed intense peaks at 169 (b + CH_2CO), 125 (a), 109 (b-AcOH), 108, and 107 (a-H_2O). Alkali treatment of this compound gave trichodiol-A, establishing the structure of the compound.

As the broth extract before saponifaction does not contain trichodiol-A (**2**), it was shown that this compound is indeed an artefact derived from the isomeric, trichodiol (**15**).



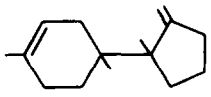
15

Trichodiene

Trichodiene (**16**) is a colourless, oily material isolated from the extract of the mycelium of *T. roseum*. The molecular formula was determined as $C_{15}H_{24}$ by mass spectrum which showed a molecular ion peak at 204 and intense peaks at 189, 161, 133, 121, 119, 109 (base peak, C_8H_{13}), 95 (C_7H_{11}), 93, and 67. The NMR spectrum of trichodiene showed singlets at 0·85 and 1·04 due to two tertiary methyl groups, a broad singlet at 1·63 due to an olefinic methyl group, a multiplet at 5·23 due to an olefinic proton, and two broad singlets at 4·71 and 4·92 due to olefinic, possibly exomethylene, protons. The IR spectrum of trichodiene included bands at 3065 cm^{-1} ($\nu_{as=CH}$), 1645 cm^{-1} ($\nu_{C=C}$), and 890 cm^{-1} (ν_{C-H} of $C=CH_2$).

Treatment of trichodiene with *m*-chloroperbenzoic acid afforded a monoepoxide (**17**) which on ozonization followed by treatment with Zn/AcOH yielded a norketone (**18**) showing an IR band (1732 cm^{-1}) due to a five membered ring ketone and a small amount of a diepoxide (**19**).

Since the data described above are compatible with structure **16**, chemical correlation of this compound with trichodiol was undertaken.

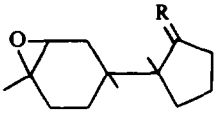


16

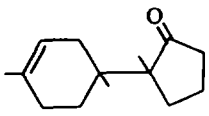
The norketone **18** was treated with NaI, NaOAc, and Zn/AcOH to yield compound **20**. The mass and IR spectra as well as TLC behaviour of compound **20** were the same as those of compound **10** derived from trichodiol, but the NMR spectra were slightly different in intensities suggesting that compound **20** is identical with one of the isomers of compound **10**. Reduction of norketone **18** with LAH afforded an epimeric mixture of diols (**21**), which on Jones oxidation gave ketoalcohol **22**. Treatment of **10** with *m*-chloroperbenzoic acid afforded a mixture of two isomeric epoxides whose NMR

spectrum again suggested that one of the isomers would be identical with the corresponding epoxide **18**. LAH reduction of the product followed by Jones oxidation led to a single isomer of ketoalcohol, which was identical in all respects (mass, NMR, and IR spectra and TLC and VPC behaviour) with ketoalcohol **22**. That the single isomer of ketoalcohol **22** was formed from **10** would be explained by assuming that the epoxidation had occurred stereoselectively, probably from the less hindered side of the cyclohexene ring in ketone **10**. This agrees with the observation that the epoxide **17** derived from trichodiene was also a single isomer.

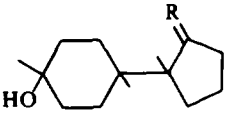
From these results, the structure of trichodiene was established as **16**.



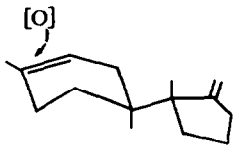
17, R=CH_2
18, R=O
19, R=

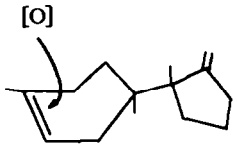


20



21, R=H, OH
22, R=O





Preparation and feeding experiments of isotopically labelled trichodiene are under investigation.

EXPERIMENTAL

UV spectra were determined in EtOH and IR spectra were measured in $CHCl_3$ soln. Mass spectra were recorded on a Hitachi RMU-6D spectrometer unless otherwise stated. NMR spectra were recorded on a Japan Electron JNM-4H-100 spectrometer and chemical shifts are expressed in ppm downfield from TMS as internal standard. Silica gel (Wakogel C-200) was used for column chromatography.

Isolation of trichodiol-A (**2**). The EtOAc extract of the fermentation broth from *T. roseum* was saponified with 10% ethanolic KOH at room temp. The mixture was concentrated to *ca.* $\frac{1}{3}$ volume below 40° under reduced pressure. This was extracted with ether and the extract washed with water, dried and the solvent evaporated. Repeated column chromatography (60% ether–benzene) of the nonsaponifiable material gave **2** which crystallized from ether–hexane to give a pure sample, m.p. 81–83°; $[\alpha]_D$ +52°; mass spectrum (Japan Electron JMS-01SG mass spectrometer); 234·162 ($C_{15}H_{22}O_2$, M—H_2O, calc. 234·162), 127·074 ($C_7H_{11}O_2$, b, calc. 127·076), 125·099 ($C_8H_{13}O$, a, calc. 127·097), 108·095 (C_8H_{12}, a-OH, calc. 108·094), 107·088 (C_8H_{11}, a-H_2O, calc. 107·086), 109·065 (C_7H_9O, b-H_2O, calc. 109·065), 96·056 (C_6H_8O, b-CH_2OH, calc. 96·058). (Found: O, 18·84. $C_{15}H_{24}O_3$ requires: O, 19·02%).

Trichodiol-A *monoacetate*. A soln of **2** in Ac_2O-pyridine (1 : 1) was allowed to stand overnight at room temp and then poured into cold water and ether extracted. The ether layer was washed with aq. $NaHCO_3$ and water, dried, and the solvent removed. The residue was evaporated *in vacuo* until all the pyridine was removed giving quantitative yield of the crude acetate. Column chromatography (5% ether–benzene) gave a pure sample, $C_{17}H_{26}O_4$; mass spectrum: 294 (M^+), 169, 125, 119, 117, 110, 109, 107; NMR: 0·95 (3H, s), 0·99 (3H, s), 1·21 (3H, s), 1·97 (3H, s), 3·15 (1H, bs), 4·33, 4·54 (2H, ABq, J = 12 Hz), 5·51, 5·67 (2H, ABq, J = 10 Hz); IR: 3600, 1745, 1245 cm^{-1}.

Triol **3**. To a soln of **2** (40 mg) in THF (3 ml) was added LAH (60 mg) with stirring and the mixture refluxed for 3 hr. After cooling, sufficient wet ether was added and the mixture washed with water. The ether layer was dried and the solvent removed. Column chromatography (30% ether–benzene) afforded **3** (25

mg), $C_{15}H_{26}O_3$; mass spectrum: 236 (M—H_2O), 125, 107; NMR: 0·88 (6H, s), 1·17 (3H, s), 3·35, 3·79 (2H, ABq, J = 12 Hz), 5·48, 6·07 (2H, ABq, J = 10 Hz).

Norketone **4**. A mixture of **3** (50 mg), $Pb(OAc)_4$ (100 mg), and benzene (3 ml) was stirred for 4 hr at room temp. Inorganic materials were filtered off and the filtrate was evaporated. The desired product was obtained by column chromatography (20% ether–benzene). $C_{14}H_{22}O_2$; mass spectrum: 222 (M^+), 125, 107; NMR: 0·92 (3H, s), 1·02 (3H, s), 1·17 (3H, s), 5·53, 5·68 (2H, ABq, J = 10 Hz); IR: 3600, 1732 cm^{-1}.

Diketone **5**. To a soln of **4** (10 mg) in acetone (0·5 ml) was added Jones reagent with stirring at room temp. After stirring for 10 min the mixture was diluted with water and extracted with ether. The ether layer was washed with water, dried, and the solvent evaporated to give a residue (7 mg) which was chromatographed to afford **5** (2·6 mg), $C_{14}H_{20}O_2$; mass spectrum: 220 (M^+), 124, 123, 82; NMR: 0·97 (3H, s), 1·09 (3H, s), 1·89 (3H, bs), 5·58 (1H, bs).

Ketones **10** *and* **11**. **2** (130 mg) was shaken in H_2 with 5% Pd/C (50 mg) in EtOH (14 ml) until one equivalent of H_2 was absorbed. Separation by column chromatography (20% ether–benzene) gave a mixture of **6** and **7** (72 mg). This was treated with LAH as described in preparation of triol **3**. The crude product was dissolved in MeOH (6 ml), to which was added 0·54 M aq. HIO_4 with stirring. After 5 min most of the MeOH was removed under reduced pressure and the residue was extracted with ether, washed with aq. $NaHCO_3$ and water, dried, and the ether evaporated. Separation using 10% $AgNO_3$ impregnated silica gel column (2% ether–benzene) gave **10** (10 mg) and **11** (8 mg). **10**, $C_{14}H_{22}O$; mass spectrum: 138 (M-68), 110, 109, 98, 68, 67; NMR: 0·88 (3H, s), 0·97 (3H, s), 1·61 (3H, s), 5·20 (1H, b); IR: 1735 cm^{-1}. **11**, $C_{14}H_{24}O$; mass spectrum: 208 (M^+), 111, 98; NMR: 0·90 (3H, s), 0·935 (3H, s), 0·905 (3H, d, J = 6 Hz); IR: 1735 cm^{-1}.

Chlorohydrin **12**. A soln of **2** (16 mg) in ether (5 ml) was shaken with 15% HCl (2 ml) for 3 min. The ether layer was separated, washed with aq. $NaHCO_3$ and water, dried, and the solvent removed to leave crude **12** (15 mg), purification by column chromatography (20% ether–benzene). $C_{15}H_{23}O_2Cl$; mass spectrum: 270 (M^+), 255, 239; NMR: 0·81 (3H, s), 1·03 (3H, s), 1·71 (3H, bd, J = 5 Hz), 3·64, 3·79 (2H, ABq, J = 12 Hz), 3·67 (1H, bd, J = 5 Hz), 4·20 (1H, t, J = 4 Hz), 5·44 (1H, bd, J = 5 Hz); IR: 3600 cm^{-1}.

Isolation of trichodiol (**15**). The EtOAc extract of the fermentation broth from *T. roseum* was chromatographed (60% ether–benzene) to give crude **15**, which after separation by prep TLC, gave a pure sample. $C_{15}H_{24}O_3$. IR: 3600 cm^{-1}.

Trichodiol monoacetate. The drude fraction of **15** was acetylated in the usual manner and the product isolated by column chormatography (5% ether–benzene), $C_{17}H_{26}O_4$. IR: 3600, 1730, 1250 cm^{-1}.

Isolation of trichodiene (**16**). The mycelium from the fermentation of *T. roseum* was suspended in warm acetone for 3 hr. After filtration, the mycelium was resuspended in acetone and allowed to stand overnight at room temp and then filtered. The combined acetone extracts were concentrated to *ca.* ¼ volume, to which water was added. Extraction with EtOAc followed by successive washing with water, drying, and removal of solvent afforded a brown residue, which was saponified as described in isolation of **2** to obtain the nonsaponifiable material. Column chromatographic separation was first effected using ether–benzene. The first fraction (100% benzene) was further chromatographed using a $AgNO_3$ impregnated silica gel column (hexane elution) to obtain **16**, $[\alpha]_D$ + 21°.

Epoxide **17**. To a soln of the crude trichodiene fraction (204 mg) in CH_2Cl_2 (4 ml) was added a soln of *m*-chloroperbenzoic acid (200 mg, 85% pure) in CH_2Cl_2 (6 ml) dropwise with stirring. After stirring for 10 min at room temp, the mixture was washed with aq. Na_2SO_3 and water, dried, and concentrated. The residue was chromatographed to afford **17** (108 mg), $C_{15}H_{24}O$; mass spectrum: 220 (M^+), 125, 107, 96, 95; NMR: 0·85 (3H, s), 1·02 (3H, s), 1·26 (3H, s), 2·76 (1H, d, J = 5 Hz), 4·69 (1H, bs), 4·93 (1H, bs).

Norketone **18** *and diepoxide* **19**. Ozone was passed through a soln of **17** (54 mg) in CH_2Cl_2 (3 ml) until a blue colour persisted. The mixture was diluted with ether (10 ml), to which Zn (50 mg) and AcOH (2 ml) were added, then stirred for 10 min at room temp, inorganic materials were filtered and the filtrate washed with aq. $NaHCO_3$ and water, dried and the solvent removed to leave a residue (50 mg). Column chromatography (benzene) afforded **19** (3 mg), $C_{15}H_{24}O_2$; mass spectrum: 236 (M^+); NMR: 0·83 (6H, s), 1·24 (3H, s), 2·75 (1H, bd, J = 5 Hz), 2·62, 3·17 (2H, ABq, J = 4·5 Hz) and **18** (17 mg), $C_{14}H_{22}O_2$; mass spectrum: 125 (M-97), 107, 98; NMR: 0·89 (3H, s), 0·96 (3H, s), 1·23 (3H, s), 2·74 (1H, t, J = 2·5 Hz); IR: 1732 cm^{-1}.

Ketone **20**. A mixture of **18** (17 mg), NaI (75 mg), NaOAc (20 mg), Zn (14 mg), AcOH (1 ml), and water (0·1 ml) was stirred at room temp for 2 hr. Sufficient ether was added and the inorganic materials were

filtered. The filtrate was washed thoroughly with aq. $NaHCO_3$ and water, dried, and evaporated. The residue was chromatographed to give **20** (7 mg), $C_{14}H_{22}O$; mass spectrum: 108 (M-98), 98, 67; NMR: 0·88 (3H, s), 0·98 (3H, s), 1·62 (3H, bs), 5·20 (1H, m); IR: 1735 cm^{-1}.

Ketoalcohol **22**. To a soln of **18** (9 mg) in THF (3 ml) was added LAH (15 mg) and the mixture heated under reflux for 1 hr. The mixture was worked up as usual and the product chromatographed (50% ether–benzene) to afford **21** (8 mg). Jones oxidation was performed as described in the preparation of **5** to give the desired **22**, $C_{14}H_{24}O_2$; mass spectrum: 224 (M^+), 206, 126, 125, 109, 98; NMR: 0·92 (3H, s), 0·97 (3H, s), 1·12 (3H, s); IR: 3600, 1734 cm^{-1}.

Acknowledgement—We are grateful to Professor S. Okuda for providing us the strain of *Trichothecium roseum* which was used for the present investigation, to Dr. Y. Nakayama, Nippon Kayaku Co. Ltd., for kindly supplying the fermentation materials, and to Mr. T. Kinoshita, Central Research Laboratories, Sankyo Co. Ltd., for the measurement of the high resolution mass spectrum of trichodiol-A. We also thank the Hoansha for its financial support to this work.

REFERENCES

[1] G. G. Freeman, J. E. Gill and W. S. Waring, *J. Chem. Soc.* 1105 (1959); J. Fishman, E. R. H. Jones, G. Lowe and M. C. Whiting, *Ibid.* 3948 (1960); W. O. Godtfredsen and S. Vangedal, *Acta Chem. Scand.* **19**, 1088 (1965)

[2] R. Traxler, W. Zürcher and Ch. Tamm, *Helv. Chim. Acta* **53**, 2071 (1970)

[3] A. W. Dawkins, J. F. Grove and B. K. Tidd, *Chem. Comm.* 27 (1965); A. W. Dawkins, *J. Chem. Soc* (*C*) 116 (1966); E. Flury, R. Mauli and H. P. Sigg, *Chem. Comm.* 26 (1965); H. P. Sigg, R. Mauli, E. Flury and D. Hauser, *Helv. Chim. Acta* **48**, 962 (1965)

[4] B. K. Tidd, *J. Chem. Soc.* (*C*) 218 (1967); T. Tatsuno, Y. Fujimoto and Y. Morita, *Tetrahedron Letters* 2823 (1969); J. F. Grove, *J. Chem. Soc.* (*C*) 375 (1970)

[5] J. D. Bu'Lock, *The Biosynthesis of Natural Products* (McGraw-Hill, 1965); R. Bentley and I. M. Campbell, in *Comprehensive Biochemistry*, Vol 20, p. 452, ed. M. Florkin and E. H. Stotz; Elsevier (1968)

[6] J. R. Hanson and B. Achilladelis, *Chem. and Ind.* 1643 (1967); B. Achilladelis, P. M. Adams and J. R. Hanson, *Chem. Comm.* 511 (1970); P. M. Adams and J. R. Hanson, *Ibid.* 1569 (1970)

[7] E. R. H. Jones and G. Lowe, *J. Chem. Soc.* 3959 (1960)

[8] S. Nozoe and Y. Machida, *Tetrahedron Letters* 1177 (1970)

[9] S. Nozoe and Y. Machida, *Ibid.* 2671 (1970)