Fungal Products. Part IV.[1] The Structure of Heveadride, a New Nonadride from *Helminthosporium heveae*

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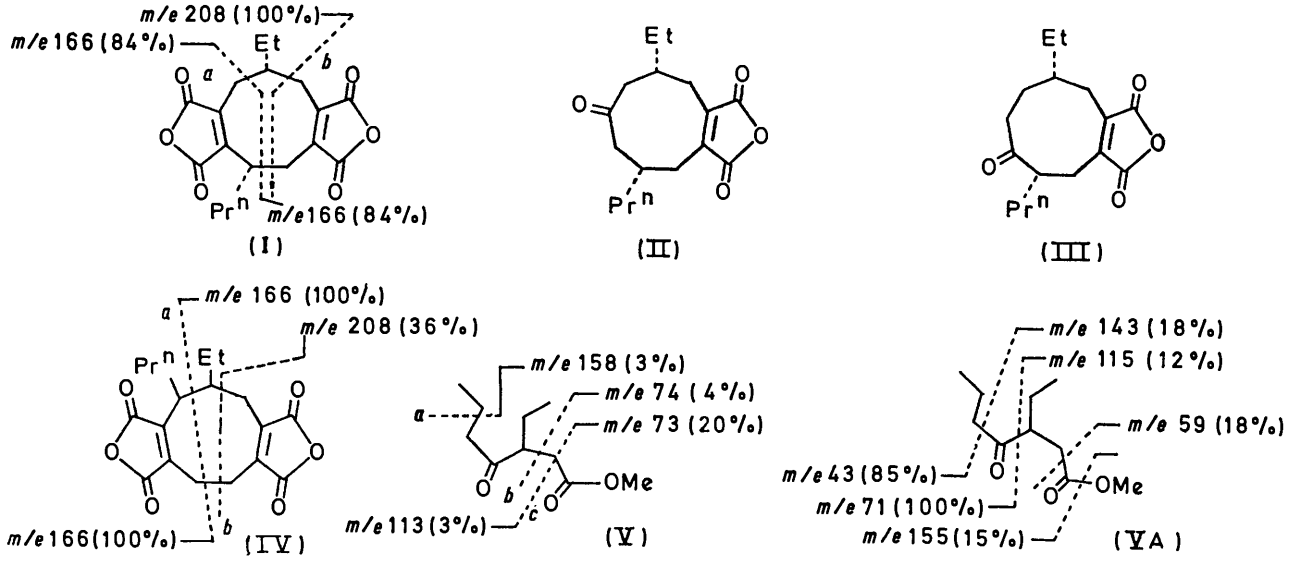
Heveadride, a fungal product from *Helminthosporium heveae*, has been shown to be the nonadride (IV) (8-ethyl-7-propylcyclonona-1,5-diene-1,2,5,6-tetracarboxylic 1,2:5,6-dianhydride) from its spectroscopic properties and its oxidation to succinic acid and to a series of acids [corresponding to the methyl esters (V), (VI), (VII), (X), (XII) or (XIII), and (XIV)].

HEVEADRIDE, isolated from the mycelium of *Helminthosporium heveae*, was characterised as an isomer of the nonadride,[2] byssochlamic acid (I),[3] as follows. The molecular formula, $C_{18}H_{20}O_6$, was established by high resolution mass spectrometry, and the i.r. spectrum of a chloroform solution contained five-membered ring anhydride absorption at 1830 and 1775 cm.$^{-1}$ The u.v. absorption [$\lambda_{max.}$ (cyclohexane) 244 nm; (ε 9500)] was identical to that of byssochlamic acid and indicated the presence of two maleic anhydride chromophores; this was supported by the formation of a tetramethyl ester and a bis-*p*-bromophenylhydrazide. The room temperature 100 MHz n.m.r. spectra of solutions in chloroform, chloroform–benzene, and pyridine, were unhelpful even in the τ 9 region. However, the presence of two methyl triplets was clearly observed in 220 MHz spectra for solutions in *o*-dichlorobenzene at 150° [Figure 1(f)] and in chloroform at −20° [Figure 1(a)]. The effect of temperature on the 220 MHz spectra (Figure 1) indicated at least two readily interconvertible conformations and made further interpretation of the spectra difficult.

Like byssochlamic acid,[3] heveadride formed a single mono-*N*-hydroxyimide which was converted, *via* the toluene-*p*-sulphonate, into two ketones, (A) and (B). These ketones did not appear to be analogous to the ketones (II) and (III), obtained[3] from byssochlamic acid, since the minor ketone (B), m.p. 116—117°, was converted by boiling 5N-sodium hydroxide into the major ketone (A), m.p. 127—128°, as judged by g.l.c. and optical rotation. The major ketone (A) was virtually unchanged under the same conditions. Thus, although their relationship was not studied in detail, ketones (A) and (B) appear to be epimers. At this point degradation of heveadride in a manner analogous to byssochlamic acid[3] was abandoned in favour of the following oxidative degradation which showed that heveadride was not a diastereoisomer of byssochlamic acid.

Heveadride was oxidised with potassium perman-



[1] Part III, J. MacMillan, A. E. Vanstone, and S. K. Yeboah, *J.C.S. Perkin I*, 1972, 2898.

[2] J. E. Baldwin, D. H. R. Barton, J. L. Bloomer, L. M. Jackson, L. Rodriguez-Hahn, and J. K. Sutherland, *Experientia*, 1962, **18**, 345.

[3] J. E. Baldwin, D. H. R. Barton, and J. K. Sutherland, *J. Chem. Soc.*, 1965, 1769.

ganate and the methylated crude product was examined by combined gas chromatography–mass spectrometry (g.l.c.–m.s.). The total ion current traces are shown in Figure 2; peaks A—O were scanned from 0 to 500

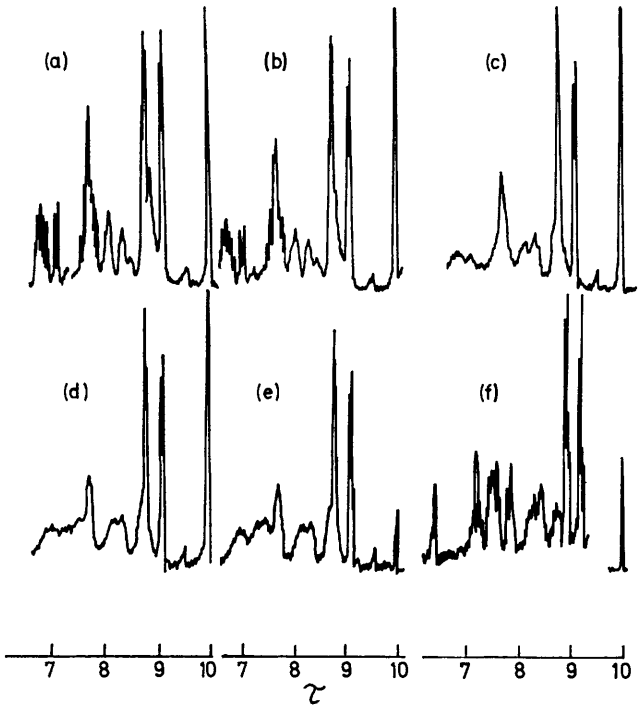


FIGURE 1 220 MHz N.m.r. spectra of heveadride in $CDCl_3$ at −20° (a), 20° (b), 30° (c), 50° (d), and 60° (e), and in *o*-dichlorobenzene at 150° (f)

mass units at 4 s per mass decade. The mass spectrum from scan A was identical with that of dimethyl succinate. Although structures could be deduced from most of the other scans, the compounds in peaks B—F, J, L, and M were isolated for further investigation from the methylated crude oxidation product by successive column, preparative gas–liquid, and thin-layer chromatography.

Compound B was assigned structure (V) from the following data. The molecular formula, $C_{10}H_{18}O_3$, was established by accurate mass measurement on the molecular ion. The i.r. spectrum showed carbonyl absorption at 1735 and 1715 $cm.^{-1}$ The n.m.r. spectrum showed methyl triplets (J 8 Hz) at τ 9·16 and 9·12, a methoxy-singlet at τ 6·38, a multiplet at τ 7·0—8·0 assigned to five protons adjacent to carbonyl, and a multiplet due to four methylene protons centred at 8·5. The mass spectrum showed the McLafferty rearrangements *a* and *b* and the branched chain cleavage *c* illustrated in structure (V), together with the α-carbonyl cleavages in structure (VA). Further fragmentation of the *m/e* 115 ion by losses of 74, 60, and 32 mass units accounts, respectively, for the observed ions at *m/e* 41(35%), 55(40), and 83(17).

Compound C (VI) showed carbonyl absorption at 1730 cm^{-1}. The n.m.r. spectrum contained signals assigned to two coincident methyl triplets (J 8 Hz) at τ 9·16 and to two coincident methoxy-singlets at τ 6·34; a complex AB multiplet centred at τ 7·3 was assigned to two protons adjacent to carbonyl groups and multiplets centred at τ 8·4 (4H) and 8·8 (2H) to the remaining methylene protons. The mass spectrum showed no molecular ion but the ion *m/e* 185 had the composition $C_{10}H_{17}O_3$ corresponding to the loss of methoxyl from a molecular ion $C_{11}H_{20}O_4^+$. McLafferty rearrangements *a* and *b* and the branched chain cleavage *c* shown in structure (VI), and the α-carbonyl cleavages shown in structure (VIA), were observed. Other intense ions can be derived by further fragmentation of these ions; for example, the base peak at *m/e* 55 from the ion *m/e* 115 by loss of 60 mass units, and the ion *m/e* 41(51%) from the ion *m/e* 101 by the same loss. The ion *m/e* 97(44%) corresponds to the successive loss of 59 and 60 mass units from the molecular ion. The foregoing spectroscopic data define structure (VI) for compound C.

Compound D (VII) showed carbonyl absorption at 1735 cm^{-1}. The n.m.r. spectrum contained two coincident methyl triplets (J 7 Hz) at τ 9·10 and two coincident methoxy-singlets at τ 6·35, together with

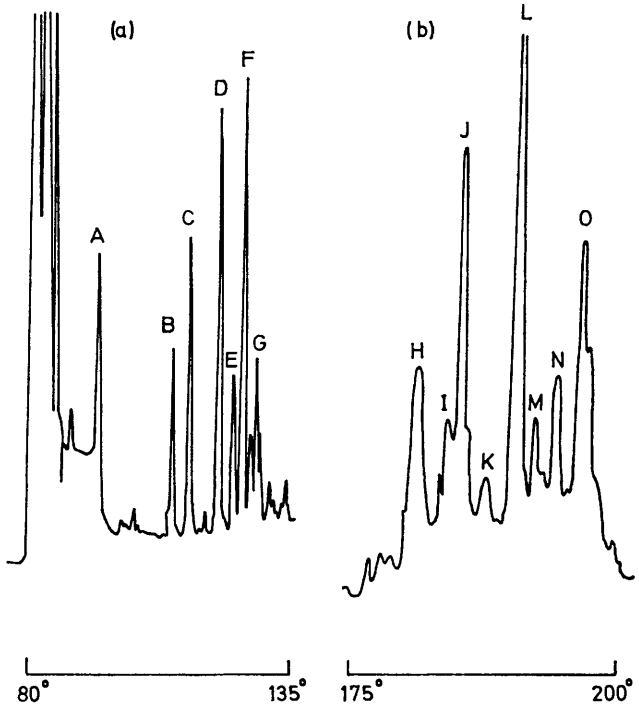
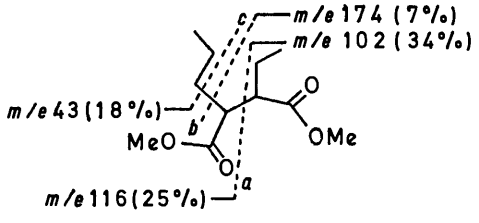


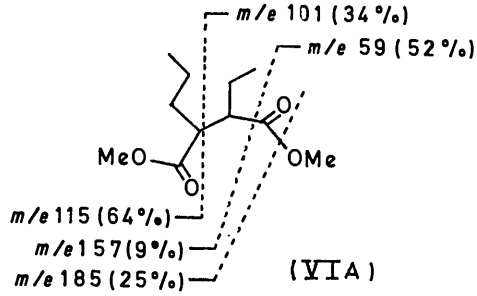
FIGURE 2 Total ion current traces of methylated oxidation product of heveadride; temperature programmed (a) from 80° at 5° min^{-1}, and (b) from 160° at 3° min^{-1}

four- and six-proton multiplets centred at τ 7·7 and 8·7, respectively. In the mass spectrum the highest mass peak (*m/e* 199) had the composition $C_{11}H_{19}O_3$ corresponding to the loss of methoxyl from an absent molecular ion $C_{12}H_{22}O_4^+$. The McLafferty rearrangements

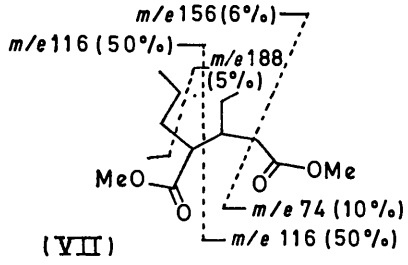
shown in structure (VII), and the branched chain fragmentations shown in structure (VIIA), were observed together with ions at *m/e* 199(14%) and 171(9) from α-carbonyl cleavage. Further fragmentation of the ion *m/e* 115 can occur by loss of 74, 60, 32, and 28 mass units to give the observed ions at *m/e* 41(50%), 55(100), 83(25), and 87(95).



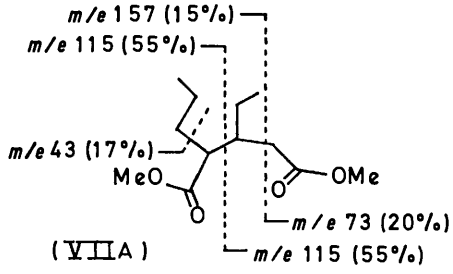
(VI)



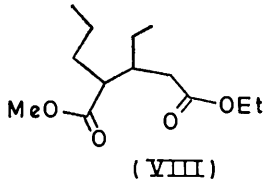
(VIA)



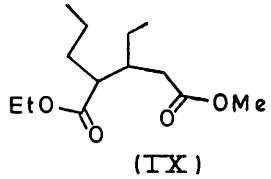
(VII)



(VIIA)



(VIII)



(IX)

Peak E probably contained a mixture of the mono-ethyl mono-methyl esters (VIII) and (IX). The i.r. spectrum contained broad carbonyl absorption at 1730 cm^{-1} and the n.m.r. spectrum showed a methoxy-singlet at τ 6·34, an ethoxy-quartet and triplet at τ 5·87 and 8·75, respectively, and two coincident methyl triplets at τ 9·10. In the mass spectrum, the highest mass ion (*m/e* 213) had the composition $C_{12}H_{21}O_3$ derivable from an absent molecular ion $C_{13}H_{24}O_4^+$ by loss of methoxyl. The fragmentation pattern was similar to that of compound D (VII); the McLafferty rearrangement ions *m/e* 116 and 88, diagnostic of (VIII), and those at *m/e* 130 and 74, diagnostic of (IX), were all present, indicating a mixture. The source of the ethyl group, observed also in peak M (see later) is unknown.

Compound F (X) showed γ-lactone (1785) and ester (1740 cm^{-1}) carbonyl absorption. The n.m.r. spectrum contained two methyl triplets (*J* 7·5 Hz) at τ 9·08 and 9·06, one methoxy-singlet at τ 6·22, a three-proton multiplet at τ 7·0—8·0, and a six-proton multiplet at τ 8·0—9·0. In the mass spectrum the ions at *m/e* 172 ($C_8H_{12}O_4$) and 155 ($C_9H_{15}O_2$) corresponded to the respective losses of C_3H_6 and $CH_3{\cdot}CO_2$ from an absent molecular ion $C_{11}H_{18}O_4^+$. The principal fragmentations are shown in structure (X), which is defined by the spectroscopic data.

Compound G was not isolated but structure (XI) is proposed from the g.l.c.–m.s. scan. The McLafferty rearrangement *a*, the α-carbonyl fragmentations, and the branched chain cleavages shown for structure (XI) are consistent with the proposed structure. Further fragmentation of the illustrated ions can account for the other observed ions. For example, the loss of 60 mass units from the ion *m/e* 115 gives the base peak *m/e* 55; the loss of 60, 74 and (73 + 29) mass units gives, respectively, the ions *m/e* 111(30%), 97(60), and 69(66); and the loss of 59 + 60 mass units from M^+ gives the ion *m/e* 139(46%).

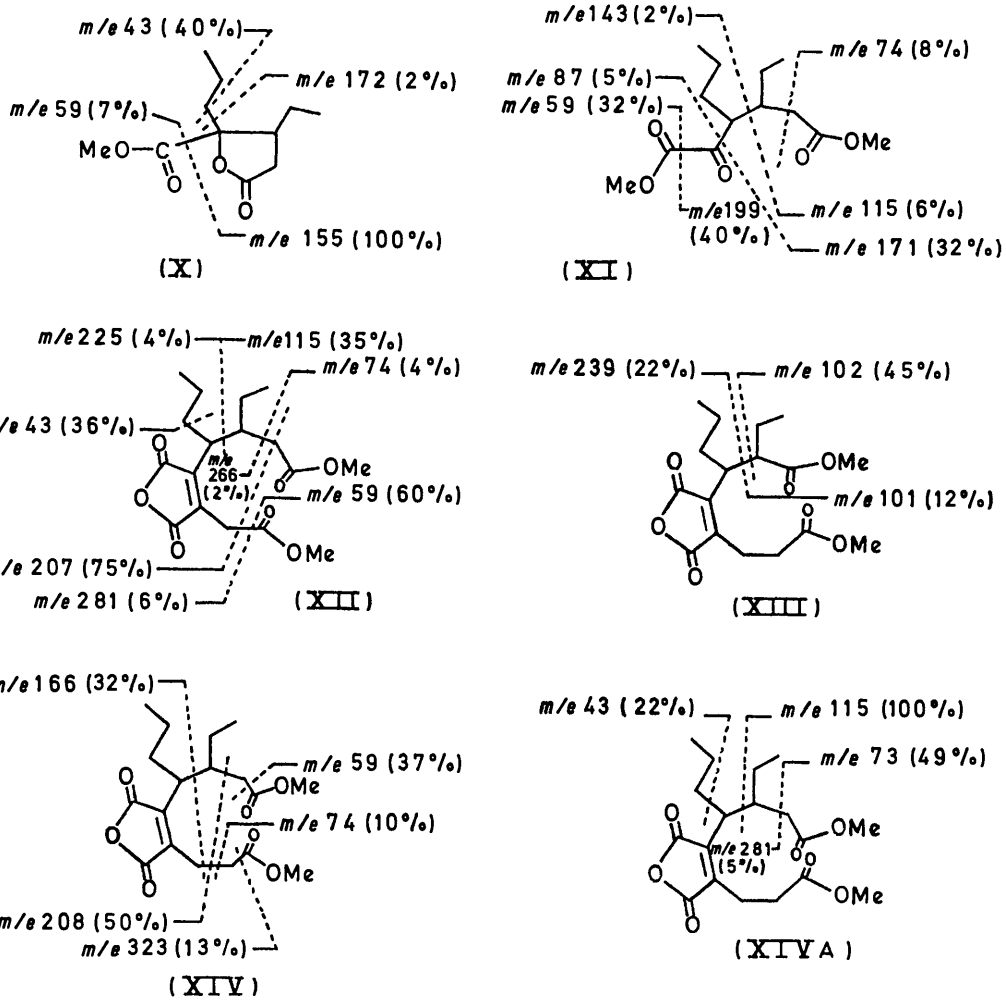
Compound J, $C_{16}H_{20}O_6$, from mass-matching of the $M^+ - 32$ ion, showed anhydride (1835 and 1770) and ester (1735 cm^{-1}) carbonyl absorption. The n.m.r. spectrum contained two coincident methyl triplets at τ 9·12 and two methoxy-singlets at τ 6·34 and 6·46. Together with the foregoing data, the mass spectrum was consistent with either structure (XII) or (XIII) except for the ions at *m/e* 115 and 102. The former ion is diagnostic of branched chain cleavage of structure (XII) and the latter ion is most readily derived by a McLafferty rearrangement of structure (XIII). Since both ions were observed, both compounds may be present.

Compound L (XIV) showed five-membered ring anhydride (1835 and 1765) and ester (1735 cm^{-1}) carbonyl absorption. The n.m.r. spectrum showed two methyl groups at *ca.* τ 9·0 and two methoxy-singlets at

τ 6·3 and 6·4. In the mass spectrum the highest mass ion (*m/e* 323, $C_{17}H_{23}O_6$) corresponds to the loss of methoxyl from an absent molecular ion $C_{18}H_{26}O_7^+$. The observed McLafferty and α-carbonyl cleavages shown in structure (XIV), together with the branch chain fragmentations shown in (XIVA), are in accord with the proposed structure. Other intense ions can be rationalised by further fragmentation of these ions.

Peak M appeared to be composed of a mixture of the methyl ethyl diesters (XV) and (XVI). The molecular ion had the composition $C_{19}H_{28}O_7$ and the i.r. spectrum showed anhydride (1835 and 1775) and ester (1730 cm^{-1}) carbonyl absorption. The n.m.r. spectrum indicated a mixture, the main component showing the following signals: two methyl triplets at τ 9·10 and 9·08, one methoxy-singlet at τ 6·38, and an ethoxy-quartet (τ 5·83) and triplet (τ 8·7). The minor component showed a methoxy-singlet at τ 6·32 and ethoxy-signals at *ca.* τ 5·85 and 8·74. The fragmentation of the mixture was similar to that described for compound L (XIV). The relative intensities of the ions at *m/e* 115(90%) and *m/e* 129(13), derived by branched chain cleavage as indicated in structures (XV) and (XVI), indicated that the ester (XV) was the main component. This conclusion was supported by the presence of the McLafferty rearrangement ion at *m/e* 74(7%) for structure (XV) and the absence of the corresponding ion at *m/e* 88 from structure (XVI).

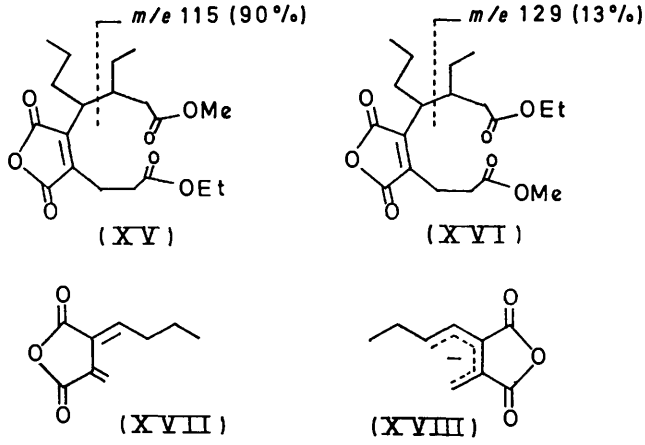
Compounds from peaks K, N, and O were not isolated and no structure could be assigned on the basis of the g.l.c.–m.s. scans.



From the structures of the foregoing oxidation products, structure (IV) is deduced for heveadride. This structure may be derived formally by head-to-head concerted cycloaddition of the two C_9 units (XVII) and (XVIII), analogous to the suggested [4] biogenesis of byssochlamic acid (I) by head-to-tail linkage of these two units. The differences in the mass spectra of heveadride and byssochlamic acid can be explained by their structures. In byssochlamic acid (I), the allylic

[4] C. E. Moppett and J. K. Sutherland, *Chem. Comm.*, 1966, 772.

fragmentations *a* and *b* are of comparable probability whereas in heveadride (IV), fragmentation *a* is the more probable since it is both allylic and branched chain. The oxidation products (VI), (VII), (X), and (XIV)



(XV) (XVI)

(XVII) (XVIII)

retain optical activity and their stereochemistry is under investigation.

EXPERIMENTAL

Optical rotations (Perkin-Elmer 141 polarimeter) and i.r. spectra (Perkin-Elmer 257 spectrometer) were determined for chloroform solutions unless stated otherwise. U.v. spectra were determined on a Unicam SP 800 instrument. G.l.c.–M.s. was performed on an LKB 9000 instrument and preparative g.l.c. on a Varian Aerograph 712. For other general procedures see Part II.[5]

Isolation of Heveadride.—Helminthosporium heveae (C.M.I. 80,137, No. 1971 in our collection) was grown as surface culture in Glaxo vessels each containing 250 ml of Raulin-Thom medium. After 13 days, the mycelium (dry weight 60·3 g) from 20 flasks was air-dried at 40° and extracted with chloroform (Soxhlet). The chloroform was evaporated off and the residue (9·5 g) was washed with hot light petroleum and crystallised from ethyl acetate–light petroleum to give needles (2·52 g). Recrystallisation from ethyl acetate–light petroleum and from methanol gave *heveadride* (*8-ethyl-7-propylcyclonona-1,5-diene-1,2,5,6-tetracarboxylic 1,2:5,6-dianhydride*) (IV) as rosettes, m.p. 161—162°, $[\alpha]_{589}$ +63°, $[\alpha]_{365}$ +351° (*c* 1·19 in CH_2Cl_2) (Found: C, 65·2; H, 6·0%; *m/e* 332·125. $C_{18}H_{20}O_6$ requires C, 65·05; H, 6·1%; *M*, 332·126); $\lambda_{max.}$ (cyclohexane) 244 nm (ε 9500); $\nu_{max.}$ 1830, 1775, and 1650 cm^{-1}; *m/e* 332(10%, $C_{16}H_{20}O_6$), 260(75%, $C_{16}H_{12}O_3$), 217(20%, $C_{13}H_{13}O_3$), 208(100%, $C_{12}H_{16}O_3$), and 166(84%, $C_9H_{10}O_3$).

The *tetramethyl ester*, obtained directly from ethereal diazomethane and a methanolic solution of heveadride, was purified by preparative t.l.c. (silica gel HF and 2% formic acid–benzene) to give an oil (Found: M^+, 424·209. $C_{22}H_{32}O_8$ requires *M*, 424·210); $\nu_{max.}$ 1730 cm^{-1}; τ 6·32 (12H, 4 × OMe); *m/e* 424(0·5%) and 180(100%, $C_{10}H_{12}O_2$).

The *bis*-p-*bromophenylhydrazide*, prepared as for bysso-chlamic acid,[3] was purified by preparative t.l.c. (silica gel and 2% formic acid–benzene) to give plates, m.p. 176—178° (from methanol–benzene) [Found: M^+, 668, 670, and 672 (rel. int. 1 : 2 : 1). $C_{30}H_{30}N_4O_4{}^{79}Br_2$, $C_{30}H_{30}N_4O_4$, $^{79}Br^{81}Br$, and $C_{30}H_{30}N_4O_4{}^{81}Br_2$ require *M*, 668, 670, and 672 (rel. int. 1 : 2 : 1)].

Degradation of Heveadride to the Ketones A and B.—Heveadride (810 mg) was dissolved in warm 0·2N-sodium hydroxide (100 ml) and the solution was cooled to room temperature, titrated with 3N-hydrochloric acid to pH 10, and treated with hydroxylamine hydrochloride (400 mg) for 20 h at room temperature. Acidification with 3N-hydrochloric acid to pH 3, extraction into chloroform, and re-extraction with N-sodium hydrogen carbonate (4 × 50 ml) gave a red solution of the anion of the *N*-hydroxy-imide. Acidification, and recovery in chloroform, gave the *N*-hydroxy-imide as a foam (770 mg) (Found: M^+ 347·138. Calc. for $C_{18}H_{21}NO_6$: *M*, 347·137); $\nu_{max.}$ 3600—2300, 1830, 1770, and 1720 cm^{-1}. The imide (1·9 g) containing traces of heveadride (detected by t.l.c. on silica gel in 1% formic acid–benzene), was dissolved in dry pyridine (9 ml) and toluene-*p*-sulphonyl chloride (3·0 g) was added. After 4 days at 0° the solution was poured into ice–water (200 ml). After 1 h acidification with 3N-hydrochloric acid and extraction with chloroform gave a foam (2·4 g) which was purified by column chromatography on silica gel. Elution with light petroleum removed traces of toluene-*p*-sulphonyl chloride; elution with 10—100% benzene–light petroleum eluted traces of heveadride, and elution with 5—20% chloroform–benzene eluted the toluene-*p*-sulphonate (1·3 g). A sample further purified by preparative t.l.c. on silica gel with 1% formic acid–benzene had $[\alpha]_{589}$ +7°, $[\alpha]_{365}$ +175° (*c* 0·2 in CH_2Cl_2); $\nu_{max.}$ 1830, 1770, and 1750 cm^{-1}.

The toluene-*p*-sulphonate (470 mg) in ethanol (4·4 ml) and 1·4N-potassium hydroxide (20 ml) was heated under reflux for 7 h. The solution was washed with chloroform, acidified, and extracted with chloroform to give a solid (230 mg), which was chromatographed on a column of silica gel. Elution with 20—50% benzene–light petroleum gave the ketone (A) (45%), which crystallised from acetone–light petroleum in needles, m.p. 128°, $[\alpha]_{589}$ +10°, $[\alpha]_{365}$ +64° (*c* 1·23) (Found: C, 69·3; H, 8·0%; M^+, 278·150. Calc. for $C_{16}H_{22}O_4$: C, 69·0; H, 8·0%; *M*, 278·151); $\lambda_{max.}$ (MeOH) 256 and 216 nm (ε 5100 and 5800); $\nu_{max.}$ 1830, 1775, 1715, and 1675 cm^{-1}; *m/e* 278(6%, $C_{16}H_{22}O_4$), 236(45%, $C_{13}H_{16}O_4$), 180(35%), and 152(100%, $C_8H_8O_3$). Further elution of the column with 50—100% benzene yielded a gum which, after preparative t.l.c. on silica gel HF with 2% formic acid–benzene, yielded the ketone (B), prisms, m.p. 116—117° (from acetone–light petroleum), $[\alpha]_{589}$ −112°, $[\alpha]_{365}$ −572° (*c* 0·46) (Found: C, 69·3; H, 7·7. Calc. for $C_{16}H_{22}O_4$: C, 69·0; H, 8·0%); $\nu_{max.}$ 1830, 1775, 1715, and 1635 cm^{-1}, *m/e* 278(35%), 236(55), 180(53), and 152(100).

Treatment of the Ketones (A) and (B) with 5N-Sodium Hydroxide.—(*a*) A solution of ketone (B) (2·6 mg) in 5N-sodium hydroxide (1 ml) was heated under reflux for 5 h, acidified, and centrifuged. The precipitate was washed with water and dissolved in acetone, and the filtered solution was evaporated. The dried residue (1·9 mg), $[\alpha]_{589}$ +2·9°, $[\alpha]_{365}$ +23·6 (*c* 0·19), showed only one peak on g.l.c. at 190° on 2% QF-1, 2% SE-33, and 2% XE-60 columns, with the same retention time as the ketone (A).

(*b*) The ketone (A) was virtually unchanged under the

[5] Part II, J. MacMillan, T. J. Simpson, A. E. Vanstone, and S. K. Yeboah, *J.C.S. Perkin I*, 1972, 2892.

foregoing conditions. G.l.c. on the three columns, as in (*a*), indicated the presence of traces of ketone (B).

Oxidation of Heveadride.—A solution of heveadride (2·0 g) and potassium permanganate (6·93 g) in acetone (900 ml) was set aside for 1 h at room temperature. 2N-Hydrochloric acid (50 ml) and sodium hydrogen sulphite (20 g) in water (200 ml) were added, the acetone was evaporated off, and the aqueous residue was extracted continuously with ether for 45 h to give a gum (1·95 g), which was methylated in methanol with ethereal diazomethane.

G.l.c.–M.s. of the Methylated Oxidation Products.—A column (1·7 m × 3 mm) of 2% SE-33 on Gaschrom Q was used with a helium flow rate of 30 ml min^{-1}. Two temperature programmes were used: (*a*) from 80° at 5° min^{-1} and (*b*) from 160° at 3° min^{-1}. The peaks A—O (Figure 1) were scanned from 0 to 500 mass units at 4 s per mass decade at 70 eV.

Peak A. The scan was identical to that obtained from dimethyl succinate under the same conditions.

Peak B. *m/e* 41(35%), 42(8), 43(85), 45(6), 55(46), 56(19), 57(6), 59(18), 69(12), 70(11), 71(100), 72(6), 73(20), 74(4·5), 83(17), 87(8), 98(8), 115(12), 143(18), and 186(3).

Peak C. *m/e* 41(51%), 42(6), 43(18), 45(16), 53(9), 55(100), 56(16), 57(7), 59(52), 67(8), 69(23), 70(9), 71(9), 73(24), 74(4), 81(6), 83(35), 84(6), 85(7), 87(72), 88(7), 95(5), 97(44), 98(7), 99(8), 101(34), 113(29), 114(19), 115(64), 116(25), 125(7), 127(8), 129(7), 142(15), 143(5), 145(27), 146(7), 155(5), 156(9), 147(9), 174(7), 185(25), and 216(0).

Peak D. *m/e* 41(50%), 42(6), 43(17), 45(8), 53(10), 55(100), 56(11), 54(45), 67(10), 68(6), 69(25), 73(20), 74(11), 81(15), 83(25), 84(9), 85(6), 87(95), 95(8), 96(17), 97(25), 99(8), 109(6), 110(6), 111(8), 114(6), 115(55), 116(50), 127(15), 128(12), 138(8), 139(9), 141(11), 156(9), 157(15), 169(7), 170(18), 171(9), 188(5), 199(14), and 230(0).

Peak E. *m/e* 41(34%), 43(13), 45(12), 53(5), 55(100), 56(9), 57(6), 59(46), 69(14), 73(35), 74(3), 81(6), 83(13), 87(24), 97(84), 98(7), 101(8), 115(7), 125(16), 129(5), 157(32), 185(58), 199(2), 213(2), and 244(0).

Peak F. *m/e* 41(11%), 43(40), 55(19), 57(16), 59(7), 69(9), 71(42), 83(8), 109(8), 143(7), 155(100), 156(9), 172(2), and 214(0).

Peak G. *m/e* 41(42%), 42(5), 43(36), 45(7), 53(7), 55(100), 56(10), 57(13), 59(32), 67(8), 69(66), 71(13), 73(11), 74(8), 81(10), 83(24), 85(9), 87(5), 95(7), 97(60), 98(6), 111(30), 115(6), 139(46), 140(6), 155(17), 171(32), 199(40), 227(3), and 258(0).

Peak J. *m/e* 41(48%), 42(10), 43(36), 45(13), 53(15), 55(100), 57(14), 59(60), 65(14), 67(14), 69(24), 70(31), 71(11), 73(6), 74(4), 77(26), 79(28), 81(12), 81(17), 87(45), 91(28), 93(17), 95(12), 97(15), 101(13), 102(45), 103(7), 105(18), 107(13), 109(10), 111(8), 113(5), 115(35), 125(11), 133(12), 135(10), 137(10), 139(11), 151(10), 161(10), 165(17), 166(12), 167(11), 179(20), 205(11), 206(24), 207(75), 208(15), 220(10), 239(22), 248(22), 249(22), 266(2), 281(6), 308(34), 309(17), and 340(1).

Peak L. *m/e* 41(29%), 43(22), 53(10), 55(90), 57(51), 59(37), 65(11), 73(49), 74(10), 77(18), 79(20), 83(65), 91(21), 93(12), 105(13), 115(100), 166(32), 208(50), 240(15), 281(5), 295(1), 323(13), and 354(1).

Peak M. *m/e* 41(42%), 43(48), 45(13), 53(10), 55(100), 57(15), 59(44), 67(11), 69(16), 71(10), 73(50), 74(7), 77(14), 79(18), 81(10), 83(66), 91(16), 93(12), 97(11), 101(16), 105(11), 115(90), 129(13), 166(30), 207(12), 208(46), 249(10), 281(6), 295(4), 323(8), 337(5), and 368(2%).

Peak N. *m/e* 41(38%), 43(32), 45(12), 55(100), 57(10), 59(60), 67(13), 69(22), 73(24), 79(10), 81(12), 83(34), 95(14), 97(13), 109(11), 113(14), 115(92), 129(15), 183(26), 197(12), 209(24), 237(10), 241(13), 257(11), 269(24), 301(9), 325(9), 357(8), 385(3), and 416(1).

Peak O. *m/e* 41(41%), 43(24), 55(100), 56(14), 59(48), 69(26), 74(3), 83(70), 97(12), 115(38), 139(15), 199(11), 227(19), 228(15), 230(10), 279(11), 309(9), 311(46), 451(6), and 371(0·4).

Isolation of the Oxidation Products.—The crude methylated oxidation product (1·8 g) was adsorbed on a column of silica gel (90 g) and eluted with light petroleum (b.p. 30—40°; 350 ml). Successive 100 ml fractions were then collected by elution with light petroleum containing 0·25, 0·5, 1, 2, and 5% ether, which was thereafter increased in 5% steps to 75%.

Fraction 10 (70 mg), eluted with 20% ether, was dissolved in ethyl acetate (140 μl) and subjected to preparative g.l.c. on a column (4·6 m × 0·95 cm) of 5% SE-30 on Gaschrom Q (60—80 mesh). The temperature was programmed from 125 to 140° at 1° min^{-1}. The column was baked at 240° after each injection. The carrier gas was nitrogen at a flow-rate of 200 ml min^{-1}. From seven injections (20 μl), peaks B, C, D, and E were collected.

Fraction 11 (138 mg), eluted with 25% ether, was dissolved in ethyl acetate (140 μl) and subjected to preparative g.l.c. as described for fraction 10. From seven injections (20 μl) peaks B, C, and D were collected.

Fraction 14 (169 mg), eluted with 35% ether, was dissolved in ethyl acetate (150 μl) and six injections (25 μl) were made under the same conditions as above except that the column was kept at 170° until peak F was collected. The temperature was then raised to 220° at 1° min^{-1} and peaks J, L, and M were collected.

Fraction 15 (132 mg), eluted with 40% ether, was treated as for fraction 14 to give peaks F and L.

Methyl 3-ethyl-4-oxoheptanoate (V), obtained by preparative t.l.c. of combined peaks B on silica gel with 10% ether–light petroleum (b.p. 30—40°) containing 2% methanol, was obtained as an oil (2·0 mg) (Found: M^+, 186·125. $C_{10}H_{18}O_3$ requires M, 186·126).

Dimethyl 2-ethyl-3-propylsuccinate (VI), was purified by preparative t.l.c. of the combined peaks C on silica gel with 20% ether–light petroleum (b.p. 30—40°) containing 2% methanol; it was obtained as an oil (9·3 mg); $[\alpha]_{589}^{20}$ −17·5° (*c* 0·8) [Found: *m/e*, 185·118. ($C_{11}H_{20}O_4$ − CH_3O) requires M, 185·118].

Dimethyl 3-ethyl-2-propylglutarate (VII), obtained in the same way from combined peaks D, was an oil (30·0 mg); $[\alpha]_{589}^{20}$ +11·0 (*c* 2·7) [Found: *m/e*, 199·134. ($C_{12}H_{22}O_4$ − CH_3O) requires M, 199·133].

3-Ethyl-4-methoxycarbonylheptan-4-olide (X), purified as above from combined peaks F, was obtained as an oil (21·0 mg); $[\alpha]_{589}^{20}$ −9° (*c* 1·6) [Found: *m/e*, 172·074 and 155·107. ($C_{11}H_{18}O_4$ − C_3H_6) requires M, 172·074; ($C_{11}H_{18}O_4$ − $C_2H_3O_2$) requires M, 155·107].

Compound [(XII) and/or (XIII)] was isolated by preparative t.l.c. of combined peaks J as described for compound (V); it was obtained as an oil (2·6 mg) [Found: *m/e*, 308·125. ($C_{17}H_{24}O_7$ − CH_4O) requires M, 308·126].

6-Ethyl-1,7-bismethoxycarbonyl-5-propylhept-3-ene-3,4-dicarboxylic anhydride (XIV), purified from combined peaks L as described for compound (VI), was obtained as an oil (34·0 mg); $[\alpha]_{589}^{20}$ +6·6° (*c* 2·72) [Found: *m/e*, 323·149. ($C_{18}H_{26}O_7$ − CH_3O) requires M, 323·149].

The mixture of ethyl esters (VIII) and (IX) was isolated as an oil (4·9 mg) [*m/e*, 213·149. Calc. for ($C_{13}H_{24}O_4$ – CH_3O): *M*, 213·149] from combined peaks E as for compound (V). Similarly the mixture of ethyl esters (XV) and (XVI) was obtained by preparative t.l.c. of combined peaks M as for compound (VI) as an oil (7·5 mg) (Found: M^+, 368·184. Calc. for: $C_{19}H_{28}O_7$: *M*, 368·183).

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