The Nonadrides. Part VI.[1] Dimerisation of the C_9 Unit *in vivo* and *in vitro*

By R. K. Huff, C. E. Moppett, and J. K. Sutherland,*† Chemistry Department, Imperial College, London SW7

But-1-enyl(methyl)maleic anhydride (4) has been synthesised and shown to act as an efficient (55% incorporation) precursor of the cyclononadienes, glauconic and glaucanic acids, in *Penicillium purpurogenum*. Base-catalysed dimerisation of compound (4) yields 5% of an epimer of glaucanic acid.

In Part V [1] evidence, based on feeding of labelled acetate, succinate, glucose, and n-butyl(methyl)maleic anhydride, was presented supporting the hypothesis that a key step in the biosynthesis of glaucanic acid (1) was the dimerisation of a C_9 unit and that glaucanic acid was further oxidised to glauconic acid [2] (2). The anhydride (4) appeared to be the most likely C_9 unit which dimerises to (1) since it is of the correct oxidation level and functionality.

The synthesis of the anhydride (4) was based on our findings [3] that α-keto-esters condense with α-phosphonato-ester anions in a Wadsworth–Emmons reaction [4] to give high yields of maleic esters with little or none of the corresponding fumarates. The required keto-ester (5) was synthesised from ethyl 2-oxohexanoate, which, was first converted into the enol acetate (6) by the action of acetic anhydride and toluene-*p*-sulphonic acid. Treatment of compound (6) with *N*-bromosuccinimide gave a high yield of the bromide (7), $\nu_{max.}$ 1770, 1720, and 1660 cm^{-1}, τ ($CDCl_3$) 3·45 (1H, d, J 11 Hz), 5·40 (1H, dt, J_d 11 Hz, J_t 8 Hz), and 8·0 (3H, s). When we were unable to convert the bromide (7) directly into the keto-ester (5) another route was developed; [5] reduction of (7) with zinc in acetic acid gave the βγ-unsaturated ester (8), $\nu_{max.}$ 1730 and 1670 cm^{-1}, τ ($CDCl_3$) 3·15—3·50 (3H, m) and 8·0 (3H, s) which, on acid-catalysed ethanolysis, was converted into the enol (9), $\nu_{max.}$ 3500, 1740, and 1670 cm^{-1}, τ ($CDCl_3$) 4—4·5 (2H, m) and 5·55 (1H, m). Manganese dioxide oxidation then yielded the keto-ester (5), $\lambda_{max.}$ (EtOH) 237 nm (ε 4700), $\nu_{max.}$ 1730, 1700, 1680, and 1630 cm^{-1}, τ ($CDCl_3$) 2·90 (1H, dt, J_d 16, J_t 6 Hz) and 3·50 (1H, d, J 16 Hz). The *trans* stereo-

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[1] Part V, J. L. Bloomer, C. E. Moppett, and J. K. Sutherland, *J. Chem. Soc.* (*C*), 1968, 588.

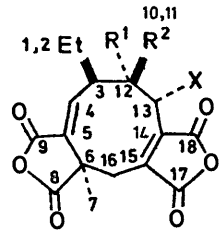
[2] D. H. R. Barton, L. M. Jackman, L. Rodriguez-Hahn, and J. K. Sutherland, *J. Chem. Soc.*, 1965, 1772.

[3] R. K. Huff, C. E. Moppett, and J. K. Sutherland, *J. Chem. Soc.* (*C*), 1968, 2725.

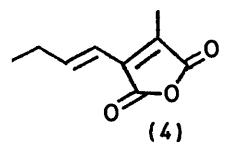
[4] W. D. Emmons and W. S. Wadsworth, *J. Amer. Chem. Soc.*, 1961, **83**, 1733.

[5] C. E. Moppett and J. K. Sutherland, *J. Chem. Soc.* (*C*), 1968, 3040.

chemistry of (5) follows from the J values, and its reduction to the enol (9) with sodium borohydride indicates similar stereochemistry for (8). An alternative synthesis of the keto-ester (5) in fewer steps is



(1) $R^1 = X = H$, $R^2 = Et$
(2) $R^1 = H$, $R^2 = Et$, $X = OH$
(3) $R^1 = Et$, $R^2 = X = H$



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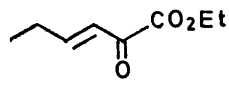

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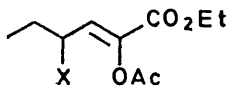
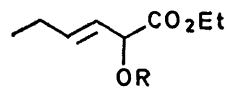
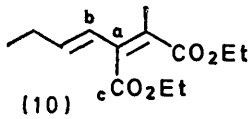


(6) X = H
(7) X = Br



(8) R = Ac
(9) R = H



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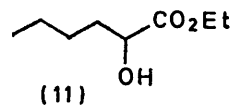

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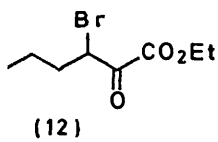

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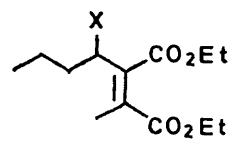
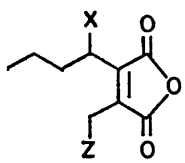
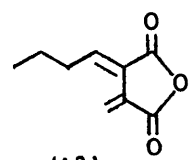


(13) X = H
(14) X = Br



(15) X = Z = H
(16) X = Br, Z = H
(17) X = Z = Br



(18)

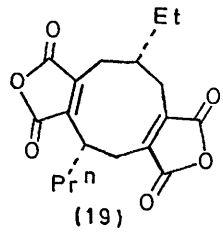
based on the formation of α-bromopyruvate [6] from lactate by reaction with N-bromosuccinimide; reaction of the hydroxy-ester (11) with N-bromosuccinimide (2 mol. equiv.) gave the bromide (12), $\nu_{max.}$ 1730 cm^{-1}, τ ($CDCl_3$) 4·97 (1H, t, J 8 Hz), which on dehydrobromination with lithium bromide and lithium carbonate in dimethylformamide [7] yielded the keto-ester (5); because of the low overall yield and the difficulty in obtaining a pure product on a large scale this is not the method of choice.

Condensation of compound (5) with the anion of ethyl 2-diethoxyphosphorylpropionate generated the maleic ester (10), $\nu_{max.}$ 1740, 1650, and 1620 cm^{-1}, $\lambda_{max.}$ (EtOH) 266 nm (ε 17,800), τ ($CDCl_3$) 3·65 (1H, d, J 16 Hz), 4·10 (1H, dt, J_d 16, J_t 6 Hz), and 8·0 (3H, s), which on hydrolysis with aqueous ethanolic sodium hydroxide followed by chromatography gave the anhydride (4), $\nu_{max.}$ 1860, 1810, 1765, and 1650 cm^{-1}, $\lambda_{max.}$ (hexane) 303 nm (ε 12,100), τ ($CDCl_3$) 2·90 (1H, dt, J_d 16, J_t 6 Hz), 3·90 (1H, d, J 16 Hz), and 7·95 (3H, s). The most efficient (31%) way of converting (5) into (4) is to hydrolyse the crude reaction mixture from the condensation and chromatograph the product on silica gel impregnated with formic acid. The inversion of the chemical shift of the vinyl protons in going from structure (10) to structure (4) deserves comment; the shifts for (4) are those expected for the planar transoid conformation depicted. Those for (10) fit neither the transoid nor the cisoid conformation (the latter is unlikely on steric and electronic grounds). Torsional twisting of bonds a–b or a–c in (10) could account for the anomalous shifts by placing the β-vinyl proton in the shielding region of the carbonyl group. The high extinction of compound (10) in the u.v. region makes the latter the more likely mechanism.

The anhydride (4) has been prepared by three other less efficient routes. In the first the crude bromo-ester (14) [prepared by reaction of the ester (13) [1] with N-bromosuccinimide] was dehydrobrominated with lithium carbonate in dimethylformamide [7] and the crude product was hydrolysed with alkali to give, after chromatography, a small yield of (4). The next synthesis of compound (4) was inadvertent insofar as it was designed to generate compound (18), the fulgenic anhydride postulated as the C_9 precursor of byssochlamic acid [8] (19). Treatment of anhydride [1] (15) with N-bromosuccinimide gave the monobromide (16), τ (CCl_4) 5·23 (1H, t, J 8 Hz) and 7·85 (3H, s), which on further reaction with N-bromosuccinimide gave a 1 : 1 mixture of compounds (16) and (17), τ (CCl_4) 5·15 (1H, t, J 8 Hz) and 5·72 (2H, s). Since efforts to maximise the yield of (17) without increasing the complexity of the reaction mixture were unsuccessful, the 1 : 1 mixture was debrominated with sodium iodide in acetone and, after chromatography, yielded 9% of the anhydride (4). That this isomerisation [(18) ⟶ (4)] was due to the conditions of reaction and/or isolation rather than a 1,5-sigmatropic shift [9] was established by the successful synthesis of compound (18). Again a phosphonate condensation was chosen for the generation of the second double bond, and the required phosphonate (20) was synthesised starting from diethyl citraconate, which on photocatalysed bromination [10] gave a 4 : 1

[6] P. F. Kruse, N. Geurkink, and K. L. Grist, *J. Amer. Chem. Soc.*, 1954, **76**, 5796.
[7] R. Joly, J. Warrant, G. Nominé, and D. Bertin, *Bull. Soc. chim. France*, 1958, 366.
[8] J. E. Baldwin, D. H. R. Barton, and J. K. Sutherland, *J. Chem. Soc.*, 1965, 1787.
[9] R. B. Woodward and R. Hoffmann, *Angew. Chem. Internat. Edn.*, 1969, **8**, 781.
[10] W. R. Vaughan and K. M. Milton, *J. Amer. Chem. Soc.*, 1951, **73**, 5497.

mixture of *erythro*-[τ ($CDCl_3$) 4·95 (1H, s) and 7·82 (3H, s)] and *threo*-[τ ($CDCl_3$) 5·06 (1H, s) and 7·84 (3H, s)] dibromides (21). Dehydrobromination in refluxing pyridine yielded a 4 : 1 mixture of compounds (22), τ ($CDCl_3$) 7·90 (3H, s), and (23) τ ($CDCl_3$) 7·82 (3H, s), which reacted at 150° with triethyl phosphite to form a mixture of (24) and (25), τ ($CDCl_3$) 7·73 (d, J 3·5 Hz) and 7·94



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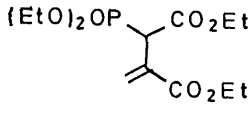

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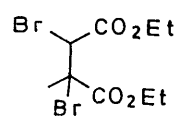

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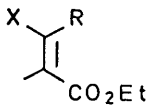
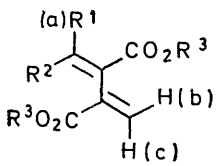
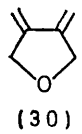


(22) X = CO_2Et, R = Br
(23) X = Br, R = CO_2Et
(24) X = $PO(OEt)_2$, R = CO_2Et
(25) X = CO_2Et, R = $PO(OEt)_2$



(26) R^1 = Pr^n, R^2 = H, R^3 = Et
(27) R^1 = H, R^2 = Pr^n, R^3 = Et
(28) R^1 = H, R^2 = Pr^n, R^3 = H
(29) R^1 = H, R^2 = Pr^n, R^3 = Me



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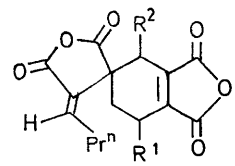


(31) R^1 = H or Pr^n
R^2 = Pr^n or H

(d, J 2 Hz) (35%), and (20), τ ($CDCl_3$) 3·67 (1H, d, J 4·5 Hz) and 3·53 (1H, d, J 4·5 Hz). Though unnecessary for the furtherance of the synthesis it was possible to convert the mixture solely into compound (20) by anion formation followed by protonation. Indeed α-hydrogen exchange in (20) occurs in deuterium oxide, and in this way we were able to confirm (by its retention) that the 4·5 Hz coupling constant is due to P–H allylic coupling. The disposition of functional groups and the carbon skeleton in the compounds of the mixture was confirmed by zinc dust–acetic acid reduction [components (24) and (25) reduced] followed by catalytic (Pd–C) hydrogenation [(20) reduced] to a product identical with that obtained by alkylating the anion of ethyl 2-diethoxyphosphorylpropionate with ethyl α-bromopropionate.

Reaction of the anion(s) formed from the mixture of (20), (24), and (25) with butanal gave a mixture of the fulgenic esters (26), τ (CCl_4) 3·88 (1H, t, J 7 Hz), 3·94 (1H, s), 4·42 (1H, s), and (27) τ (CCl_4) 3·15 (1H, t, J 7 Hz), 3·64 (1H, s), and 4·54 (1H, s). The ratios of (26) and (27) obtained varied from 1 : 1 to 3 : 7, and an extensive series of control experiments forced us to the conclusion that the only factor causing this variation was the concentration of the reagents; the corollary that the isomers are formed in reactions of different kinetic order is difficult to understand. Saponification of the ester mixture converted compound (27) into the crystalline acid (28), τ ($CDCl_3$), 3·08 (1H, t, J 8 Hz), 3·49 (1H, d, J 1·5 Hz), and 4·40 (1H, d, J 1·5 Hz), whereas the ester (26) was transformed into a crude γ-lactone (i.r.), which was not investigated further. Reaction of the acid (28) with diazomethane yielded the ester (29); the virtual identity of the vinyl proton n.m.r. spectrum of (29) with that of (27) established that compounds (27) and (28) were of the same stereochemical series. The anhydride (18) was prepared from the acid (28) by reaction with oxalyl chloride–triethylamine in dichloromethane; it was not possible to isolate pure samples of this material, owing to its instability ($t_{\frac{1}{2}}$ in CCl_4 at 0°, 30 min), but it was characterised spectroscopically: $\nu_{max.}$ 1825, 1770, and 1650 cm^{-1}, $\lambda_{max.}$ (hexane) 260 nm, τ (CCl_4) 2·90 (1H, t, J 7 Hz), 3·50 (1H, s), 3·90 (1H, s), and 7·48 (2H, q, J 7·5 Hz). It is now necessary to consider the stereochemical assignments made primarily on the basis of the chemical shifts of the vinyl protons (see Table 1). The chemical shifts for (26) are those

TABLE 1

Vinyl proton chemical shifts of fulgenates (τ values)

Compound	H(a)	H(b)	H(c)
(26)	3·88	3·94	4·42
(27)	3·15	3·64	4·54
(29)	3·08	3·49	4·40
(18)	2·90	3·50	3·90

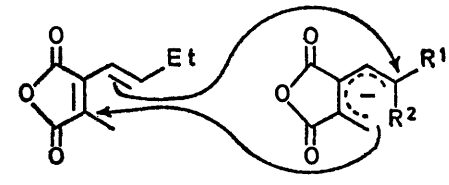
expected for the αβ-unsaturated ester units[11] in the absence of any perturbing effect from the neighbouring double bond. However in structures (27) and (29) H(b) is deshielded; this might be due to differing degrees of deviation of the diene systems from planarity. A reversal of these assignments would require H(c) to be shielded by a neighbouring double bond (in a transoid conformation) or by an ethoxycarbonyl group in the less likely cisoid arrangement; either possibility seems unlikely. Again it seems difficult to meet these conditions by twisting a carbonyl group out of the plane of the diene system. In the anhydride (18) where the diene systems must be cisoid the chemical shifts are in accord with our previous results[2] on anhydrides, and presumably the alterations are due in part to the geometrical changes consequent on five-membered ring formation

[11] See L. M. Jackman and S. Sternhell, 'Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry,' 2nd edn., Pergamon, Oxford, 1969.

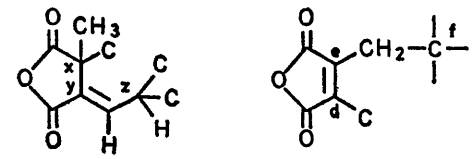
altering the positions of the carbonyl groups relative to the vinyl protons. It is notable that the vinyl protons of (30) differ by 0·45 p.p.m. in chemical shift,[11] and it might have been expected that the deshielding effect of the carbonyl groups would have significantly altered this difference.

One piece of chemical evidence which tends to support the assignments comes from the boron-trifluoride-catalysed dimerisation of the acid (28) [to (31), $\nu_{max.}$ 1825, 1815, 1775, and 1650 cm^{-1}, $\lambda_{max.}$ (hexane) 226 (ε 10,000) and 250infl nm, τ ($CDCl_3$) 2·90 (1H, t, *J* 7 Hz)]. The characteristic u.v. and i.r. spectra of the product indicate the presence of maleic and itaconic anhydride chromophores,[12] and the chemical shift of the vinyl proton unambiguously indicates the stereochemistry. If we accept that stereochemistry is retained in this reaction, this assignment agrees with that for the monomer. The anhydride (18) was isomerised to (4) by treatment with triethylamine in benzene in 33% yield, so completing another formal synthesis of (4).

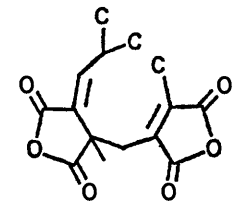
With the postulated C_9 precursor of glaucanic acid available we first investigated the feeding of labelled anhydride (4) to *Penicillium purpurogenum*. Treatment of the anion of ethyl diethoxyphosphorylacetate with [^{14}C]methyl iodide gave ethyl [3-^{14}C]-2-diethoxyphosphorylpropionate, which was utilised in the first synthesis described to give compound (4) labelled in the vinyl methyl group. When a culture was grown in the presence of labelled (4) (introduced as an aqueous solution of the disodium salt), isolation of glaucanic (1) and glauconic (2) acids showed 4·3 and 50·8% incorporation of activity. Degradation established that 97·5% of the activity in the acid (2) was located at C-7 and C-16. This result, in conjunction with previous results indicating a dimerisation, leaves little doubt that the acid (1) arises from two molecules of the anhydride (4). There are a number of possible mechanisms for this condensation but an attractive one is a [6 + 4] cycloaddition of the anion (33) with the cisoid anhydride (32) to give (1) (X = —); reaction *via* an *endo* transition state would give the correct relative stereochemistry. In the hope of achieving this reaction *in vitro* we treated the anhydride (4) with sodium hydride in dimethylformamide and obtained a 2% yield of a crystalline compound (3), the properties of which were similar to but not identical with those of glaucanic acid. Using triethylamine as base improved the yield to 4%. The spectroscopic properties are summarised in Table 2; the i.r. and u.v. data show the presence of itaconic and maleic anhydride units. The close correspondence between the n.m.r. data for the synthetic product and for compound (1) allows deduction of the part structures (35). Analytical and spectroscopic properties show that compound (3) is monocarbocyclic and that there are seven chain-terminating groups (methyl or oxidised equivalent) present; thus there must be seven branches from the carbocyclic system, but the part structures (35) contain eight branch points so that one or more must be common to the two main units. By examining the possible identity of x, y, z, d, e, and f in (35) it becomes clear



(32) (33) R^1 = Et, R^2 = H (34) R^1 = H, R^2 = Et



2 x C—Et (35)



2 x C—Et (36)

that only x and f can be common, so allowing extension of the part structure to (36).

The structure can now be completed in three distinctive ways by ring formation to give (*a*) a three-membered ring, (*b*) an eight-membered ring, or (*c*) a nine-membered one. These different modes of ring closure can be distinguished by oxidative degradation since the fragment of unknown structure would yield respectively (*a*)

TABLE 2

Spectral properties

	Isoglaucanic acid (3)	Glaucanic acid (1)
$\lambda_{max.}$ (cyclohexane)/nm	220 (ε 10,700), 250infl	220 (ε 10,700), 250infl
$\nu_{max.}$ (Nujol)/cm^{-1}	1850, 1830, 1765, 1670, 1655	1850, 1830, 1765, 1670, 1655
Mass spectrum: *M*+	332	332
base peak (*m/e*)	166	166
τ($CDCl_3$; 100 MHz); *J* in Hz		
H on: C-1, C-10	9·22 (t, *J* 6), 8·83 (t, *J* 5)	9·21 (t, *J* 6), 8·92 (t, *J* ?)
C-4	3·13 (d, *J* 11)	3·08 (d, *J* 12)
C-7	8·51 (s)	8·53 (s)
C-10	7·48, 6·82 (AB, *J* 13)	7·37, 6·80 (AB, *J* 13)

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

two monocarboxylic acids, (*b*) a substituted succinic acid, or (*c*) a substituted glutaric acid. Prolonged ozonolysis of compound (3), followed by oxidation of the products with alkaline hydrogen peroxide, gave propane-1,3,3-tricarboxylic acid [confirming part structure (36)] and a liquid glutaric acid, $C_9H_{16}O_4$ (anhydride, $\nu_{max.}$ 1810 and 1760 cm^{-1}), establishing the presence of the C_9 ring. *erythro*-2,3-Diethylglutaric acid has already been synthesised [13] from *meso*-diethylsuccinic acid; repetition of this procedure with (±)-diethylsuccinic acid gave (±)-*threo*-2,3-diethylglutaric acid, which was identical (i.r., n.m.r., and mass spectra) with the degradation product. By the same criteria the dimethyl esters were identical; however the properties of the *erythro*-derivatives were so similar that it was not possible to distinguish the isomers with certainty. As we were unable to separate compounds of the two series by t.l.c. or g.l.c. we turned to the only crystalline derivatives we could prepare readily on a small scale, the *p*-phenylphenacyl esters; again the spectral properties of the *threo*- and *erythro*-derivatives were virtually indistinguishable but the m.p.s (*erythro*, 104·5°; *threo*, 86—88°) were different. However the derivative of the degradation product, although identical spectrally, had m.p. 81—82°; the most likely explanation is that some epimerisation occurred during degradation and that it is a mixture of the two isomers. Thus there is little doubt that the degradation product is a 2,3-diethylglutaric acid, but it is not possible to assign stereochemistry unambiguously. The stereochemistry of compound (3) can be deduced in this way: the magnitude (11 Hz) of the coupling between the protons on C-3 and C-4 implies an antiperiplanar relationship which is possible only when C-7 and the C-3 ethyl group are *trans*, so that the relative stereochemistry at these two positions is the same as that of glaucanic acid (1). Thus isoglaucanic acid (3) must have the two ethyl groups *trans*. If it is accepted that the *in vivo* and *in vitro* dimerisations are mechanistically similar, then there are three possible explanations for the difference in stereochemistry.

$$\underbrace{(32) + (33)}_{endo} \longrightarrow (1) \longleftarrow \underbrace{(32) + (34)}_{exo}$$

$$\overbrace{(32) + (34)} \longrightarrow (2) \longleftarrow \overbrace{(32) + (33)}$$

If the same anion (33) or (34) is used to produce both products then the transition state geometry must be *exo* in one case and *endo* in the other, which seems unlikely. There remain the possibilities that isomerisation of the anion (33) to (34) occurs in either the biochemical or the chemical reaction; at present there is no way of distinguishing these.

Attempts to dimerise the anhydride (18) or mixtures of it with (4), so generating the byssochlamic acid (19) type of compound, were unsuccessful. As already noted, reaction of the anhydride (18) with base in non-polar solvents causes isomerisation to (4) and thence dimerisation to isoglaucanic acid. In polar aprotic solvents only polymeric material was obtained from (18).

EXPERIMENTAL

Reaction mixtures, unless otherwise stated, were worked up by dilution with water, extraction with ether, and washing the extract, unless inappropriate, with 2N-hydrochloric acid, saturated sodium hydrogen carbonate solution, and brine. Extracts were dried ($MgSO_4$) and concentrated *in vacuo*. T.l.c. and p.l.c. were carried out on Kieselgel GF_{254}.

Ethyl 2-Acetoxyhex-2-enoate (6).—Ethyl 2-oxohexanoate [14] (16·3 g) was refluxed with acetic anhydride (80 ml) and toluene-*p*-sulphonic acid (100 mg). Acetic acid was distilled off continuously (spinning-band column). When no more acetic acid distilled over, the mixture was added to saturated aqueous sodium hydrogen carbonate and worked up in the usual way to give the *enol acetate* (6) (16·4 g), b.p. 83—85° at 1 mmHg (Found: C, 59·9; H, 7·5. $C_{10}H_{16}O_4$ requires C, 60·0; H, 8·0%).

Ethyl 2-Acetoxy-4-bromohex-2-enoate (7).—The enol acetate (6) (17·8 g) in carbon tetrachloride (80 ml) was refluxed with *N*-bromosuccinimide [16·3 g; freshly crystallised from benzene and mixed with benzoyl peroxide (40 mg)] for 45 min. After cooling the mixture and filtering off the succinimide, the solvent was removed and the product was distilled (b.p. 100—103° at 0·1 mmHg) to give the *bromide* (7) (27 g) (Found: C, 43·4; H, 5·4; Br, 28·0. $C_{10}H_{15}BrO_4$ requires C, 43·0; H, 5·4; Br, 28·6%).

Ethyl 2-Acetoxyhex-3-enoate (8).—Zinc dust (38 g) was added during 30 min to a cooled and stirred solution of the bromide (7) in acetic acid (150 ml). After filtration, work-up in the usual way yielded the *ester* (8) (17 g), b.p. 68—70° at 0·5 mmHg (Found: C, 60·2; H, 8·4. $C_{10}H_{16}O_4$ requires C, 60·0; H, 8·1%).

Ethyl 2-Hydroxyhex-3-enoate (9).—The ester (8) (17·1 g) was refluxed with ethanol (130 ml) containing 4% (w/w) hydrogen chloride for 1·5 h. Work-up in the usual way and distillation of the product gave the *alcohol* (9) (10·6 g), b.p. 62—64° at 1 mmHg (Found: C, 60·3; H, 8·4. $C_8H_{14}O_3$ requires C, 60·7; H, 8·9%).

Ethyl 2-Oxohex-3-enoate (5).—(*a*) The alcohol (9) (20 g) was stirred for 20 min with a suspension of manganese dioxide (160 g) in chloroform (1 l). Evaporation of the filtered solution *in vacuo* afforded the *ketone* (17 g), b.p. 63—64° at 0·5 mmHg, characterised as its 2,4-dinitrophenylhydrazone, m.p. 139° (from ethanol–ether) (Found: C, 49·7; H, 4·5; N, 16·7. $C_{14}H_{16}N_4O_6$ requires C, 50·0; H, 4·8; N, 16·7%).

(*b*) Ethyl 2-hydroxyhexanoate (10 g) and *N*-bromosuccinimide (23·8 g) were refluxed in dry carbon tetrachloride (120 ml) under irradiation from a 500 W tungsten lamp until the solution was colourless. Filtration, evaporation of solvent, and distillation gave ethyl 3-bromo-2-oxohexanoate (10·4 g), b.p. 74—78° at 0·6 mmHg.

The α-bromo-ketone (9·48 g), lithium bromide (5·2 g), and lithium carbonate (5·92 g) in dry dimethylformamide (60 ml) were stirred under nitrogen for 20 min at 100°. After dilution with ether (400 ml), aqueous 2·5% sulphuric acid (230 ml) was added and the system was stirred vigorously for

[13] D. H. R. Barton, L. D. S. Godhino, and J. K. Sutherland, *J. Chem. Soc.*, 1965, 1779.
[14] K. Dietzch and W. Pritzkow, *Chem. Ber.*, 1960, **93**, 1733.

4 h. After work-up of the ethereal layer in the usual way the residue was distilled to give the ketone (2·8 g).

Sodium borohydride reduction of the ketone (5) regenerated the alcohol (9).

Diethyl 2-(*But*-1-*enyl*)-3-*methylmaleate* (10).—Ethyl 2-diethoxyphosphorylpropionate (42·2 g) in dimethoxyethane (120 ml) was added dropwise to a stirred slurry of sodium hydride (9·61 g of a 53·3% dispersion in oil) in dimethoxyethane (100 ml). After cessation of hydrogen evolution, the ketone (5) (28·5 g) in dimethoxyethane (100 ml) was added dropwise and the solution stirred for 24 h. Work-up in the usual way gave an oil (35 g). A portion (1·1 g) was purified by chromatography on silica gel; elution with benzene–chloroform (1 : 1) gave the ester (10) (408 mg).

2-(*But*-1-*enyl*)-3-*methylmaleic Anhydride* (4).—(*a*) The crude product from the previous experiment (34 g) in ethanol (80 ml) and 2N-sodium hydroxide (80 ml) was set aside under nitrogen for 6 h. Work-up in the usual way gave an oil which was chromatographed on silica gel (1 kg) impregnated with formic acid (110 ml). Elution with benzene gave the anhydride (8·7 g).

The 3-[^{14}C]methyl compound was prepared by the same route (by use of ethyl[3-^{14}C]-2-diethoxyphosphorylpropionate prepared from [^{14}C]methyl iodide and the anion of ethyl diethoxyphosphorylacetate.

(*b*) Diethyl 2-butyl-3-methylmaleate (13) (400 mg), *N*-bromosuccinimide (292 mg), and benzoyl peroxide (40 mg) in carbon tetrachloride (4 ml) were refluxed for 10 min. Filtration and removal of solvent gave the unstable bromo-ester (14), which was dissolved in dimethylformamide (3 ml) containing lithium carbonate (75 mg) and a few crystals of hydroquinone and heated for 15 min at 130°. Work-up in the usual way gave an oil which was purified by preparative t.l.c. on silica gel [benzene–formic acid (38 : 1)] to give the anhydride (4) (60 mg).

(*c*) 2-Butyl-3-methylmaleic anhydride (168 mg) in carbon tetrachloride (2 ml) was refluxed with *N*-bromosuccinimide (196 mg) and benzoyl peroxide (2 mg). After 2 h the succinimide was filtered off and further *N*-bromosuccinimide (600 mg) was added; the mixture was then refluxed for a further 3 h. Filtration and evaporation gave a 1 : 1 mixture of compounds (16) and (17) which was dissolved in acetone (5 ml) and stirred with sodium iodide (150 mg) for 15 min at room temperature. After addition of solid sodium disulphite work-up in the usual way followed by preparative t.l.c. gave the anhydride (4) (15 mg).

Preparation of the Allyl- and Vinyl-phosphonates (20), (24), *and* (25).—The mixture of bromo-esters (22) and (23) (11·8 g) was heated in a sealed tube with triethyl phosphite (14·8 g) for 18 h at 150°. Distillation gave the mixture of isomeric phosphonates (20), (24), and (25) (7·0 g), b.p. 108° at 0·03 mmHg (Found: C, 48·2; H, 7·0. Calc. for $C_{13}H_{23}O_7P$: C, 48·4; H, 7·2%).

The phosphonate mixture (97 mg) was stirred for 2 h at room temperature with sodium hydride (15 mg of a 53·3% dispersion in oil) in dimethylformamide (2 ml). Work-up in the usual way yielded only the allylphosphonate (20) (78 mg).

Diethyl 2-*Diethyoxyphosphoryl*-3-*methylsuccinate*.—(*a*) Ethyl diethoxyphosphorylacetate (6·89 g) in 1,2-dimethoxyethane (50 ml) was stirred with sodium hydride (1·43 g of 53·3% dispersion in oil) for 1 h, and for a further 60 h after addition of ethyl 2-bromopropionate (5·66 g). After filtration and removal of solvent the residue was chromatographed on grade 3 alumina (300 g). Elution with 1,2-dichloroethane gave the *succinate* (5·1 g), b.p. 121—122° at 0·1 mmHg (Found: C, 48·7; H, 8·2. $C_{13}H_{25}O_7P$ requires C, 48·2; H, 7·7%).

(*b*) The mixture of allyl- and vinyl-phosphonates (20), (24), and (25) (97 mg) was stirred with zinc dust (250 mg) in acetic acid (2 ml) for 24 h at room temperature. The zinc was filtered off and the filtrate worked up in the usual way. The residue in ethanol (10 ml) was hydrogenated over 10% palladium–charcoal. Filtration and removal of solvent gave the phosphonate, identical (spectrally) with the sample prepared in (*a*).

Diethyl (E)- *and* (Z)-2-*Butylidene*-3-*methylenesuccinates*.—The phosphonate mixture [(20), (24), and (25)] (644 mg) in tetrahydrofuran (20 ml) was added dropwise to a stirred slurry of sodium hydride (90 mg of 53·3% dispersion in oil). After a clear solution had been obtained butyraldehyde (650 mg) in tetrahydrofuran (20 ml) was added. The mixture was stirred at 50° for 24 h and then worked up in the usual way. The product was purified by p.l.c. (chloroform–benzene, 1 : 1) and distillation to give the mixture of esters (26) and (27) (287 mg), b.p. 90—95° (bath) at 0·05 mmHg (Found: C, 65·0; H, 8·5. Calc. for $C_{13}H_{20}O_4$: C, 65·0; H, 8·4%).

(E)-2-*Butylidene*-3-*methylenesuccinic Acid* (28).—The ester mixture [(26) and (27)] (157 mg) was stirred with 2N-sodium hydroxide (3 ml) and ethanol (3 ml) under nitrogen for 18 h. After acidification of the mixture with 2N-sulphuric acid, the usual work-up gave an oil which was dissolved in acetonitrile. On cooling to 0° a solid separated, which, after sublimation at 90—100° and 10^{-5} mmHg followed by crystallisation from ether–light petroleum (b.p. 40—60°) gave the *acid* (60 mg), m.p. 149—151° (Found: C, 58·6; H, 6·6. $C_9H_{12}O_4$ requires C, 58·7; H, 6·6%).

Methylation of the acid with diazomethane in ether gave the *dimethyl ester*, b.p. 80° (bath) at 0·3 mmHg (Found: C, 62·0; H, 7·7. $C_{11}H_{16}O_4$ requires C, 62·3; H, 7·6%).

Reaction of the Acid (28) *with Boron Trifluoride–Ether*.—The acid (100 mg) was refluxed with boron trifluoride–ether (200 mg) in dioxan (10 ml) for 60 h. The solvent was removed under reduced pressure and the residue refluxed with saturated aqueous sodium hydrogen carbonate for 30 min. After acidification with 6N-sulphuric acid, extraction with chloroform, followed by concentration of the extract, gave a gum which solidified on trituration with ether. Crystallisation from benzene–ether gave the *dimer* (31), m.p. 160—161° (Found: C, 64·8; H, 6·0. $C_{18}H_{20}O_6$ requires C, 65·0; H, 6·1%).

(E)-2-*Butylidene*-3-*methylenesuccinic Anhydride* (18).—Triethylamine (100 mg) was added to the acid (28) (185 mg) in dichloromethane (100 ml). The mixture was cooled to 0° and oxalyl chloride (0·5 ml) was added. After 24 h at 0° the solvent was removed *in vacuo* and the residue extracted with carbon tetrachloride at 0°. The unstable anhydride could be obtained by evaporation under reduced pressure at 0°. Its n.m.r. spectrum was measured at −5°.

When the anhydride (30 mg) in benzene (9 ml) containing triethylamine (100 mg) was set aside at 25° for 24 h it was converted into the maleic anhydride (4) (10 mg), isolated by t.l.c. [benzene–formic acid (90 : 5) as eluant].

Isoglaucanic Acid (3).—The anhydride (4) (6 g) in dimethylformamide (45 ml) was stirred at 25° with sodium hydride (6 g of 53·3% dispersion in oil) for 18 h. After acidification with 4N-hydrochloric acid the mixture was continuously extracted with ether. Chromatography of the

residue obtained from the ether extract on Woelm grade 1 silica gel (300 g) impregnated with formic acid (33 ml) gave on elution with benzene–ether (9 : 1) a yellow oil which was further purified by p.l.c. [benzene–ether–formic acid (18 : 4 : 1) as eluant]. The solid obtained was sublimed at 135° and 10^{-5} mmHg and crystallised from benzene–ether to give *isoglaucanic acid*, m.p. 169—169·5° (Found: C, 65·0; H, 6·1. $C_{18}H_{20}O_6$ requires C, 65·1; H, 6·1%).

When the anhydride (4) (160 mg) in dimethylformamide (5 ml) was stirred at 25° with triethylamine (85 mg) for 24 h, work-up as before gave isoglaucanic acid (10 mg).

Ozonolysis of Isoglaucanic Acid.—Isoglaucanic acid (111 mg) in acetic acid (20 ml) was ozonised at 25° for 19 h. Excess of ozone was removed by flushing with nitrogen and the acetic acid was evaporated off *in vacuo* at 25°. The residue was treated with 1N-sodium hydroxide (4 ml) and 30% hydrogen peroxide (2·8 ml) for 30 min at 25°. After addition of platinum residues to destroy peroxides the solution was heated at 95° for 15 min. The cooled mixture was acidified with 2N-sulphuric acid and continuously extracted with ether for 20 h. After concentration the extract was triturated with chloroform to give a white solid which was recrystallised from ethyl acetate [m.p. 166—171° (decomp.)] and identified by comparison with an authentic sample as propane-1,2,2-tricarboxylic acid.

The residue from the chloroform filtrate was purified by p.l.c. [4% acetic acid in chloroform as eluant]. Removal of the band with the same R_F value as the synthetic 2,3-diethylglutaric acids gave an oil (20 mg), identical (i.r.) with the authentic 2,3-diethylglutaric acids. The methyl esters (made with diazomethane) and the anhydrides (made with acetyl chloride) were also identical (g.l.c.).

A bis-*p*-phenylphenacyl ester, m.p. 81—82°, was prepared in the usual way.

(±)-2,3-threo-*Diethylglutaric Acid.*—This acid was prepared by the method described for the synthesis of the *erythro*-isomer but from (±)-diethylsuccinic acid. It was obtained as an *oil*, b.p. 105° at 10^{-5} mmHg (Found: C, 57·5; H, 8·7. $C_9H_{16}O_4$ requires C, 57·4; H, 8·6%) and gave a *bis*-p-*phenylphenacyl ester*, m.p. 86—88° (from methanol) (Found: C, 77·0; H, 6·4. $C_{37}H_{36}O_6$ requires C, 77·1; H, 6·3%).

Feeding Experiments.—These were carried out as described previously,[1] as were the degradations and radioactive counting. Compound (4) was fed as an aqueous solution of its disodium salt.

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