Metabolites of the Higher Fungi. Part 22.[1] 2-Butyl-3-methylsuccinic Acid and 2-Hexylidene-3-methylsuccinic Acid from Xylariaceous Fungi

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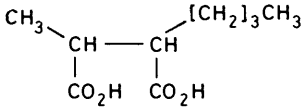
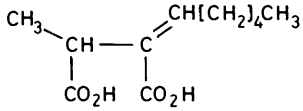
2-Butyl-3-methylsuccinic acid has been isolated from the culture medium of *Hypoxylon illitum*. 2-Hexylidene-3-methylsuccinic acid is the major metabolite produced by *Poronia piliformis*, *H. deustum*, and four *Xylaria* species. Methods for the synthesis of the diacids are examined.

In the previous paper in this series we described the isolation and identification of a number of substituted mellein derivatives from members of the xylariaceous genera *Hypoxylon* and *Nummularia*.[1] During this study it became clear that the largest variation in metabolite production among members of the *Hypoxylon* genus occurred in the sub-section primo cinerea. Since members of the primo cinerea can be related morphologically to other xylariaceous genera such as *Rosellinia*, *Xylaria*, and *Poronia* it was of interest to examine the metabolites produced by members of these genera.



(1)



(2)

One member of the *Hypoxylon* sub-section primo cinerea, *viz.* *H. illitum*, in addition to producing 5-methyl-, 5-carboxy-, and 5-hydroxymethyl-mellein as already described[1] also produced a substantial quantity of a colourless optically active metabolite which we have identified as compound (**1**). A second species of the same sub-section *H. deustum*, produced small quantities of a second diacid (**2**). Diacid (**2**) is also produced in substantial quantities as the major metabolite by the dung fungus *Poronia piliformis* and by four members of the morphologically related genus *Xylaria* (Table). Members of the genus *Xylaria* bear a close affinity to *Poronia*, differing only in their habitat of dead and decaying wood. In the cases of *P. piliformis*, *X. hypoxylon*, *X. polymorpha*, and *X. mali* it was possible to crystallise the diacids directly from the crude metabolic extracts but for *H. deustum* and *X. longipes* chromatography was required prior to crystallisation. The diacid from *X. mali* and *X. hypoxylon* had the same m.p. (155 °C) as the synthetic acid (see below) and had zero rotation. However, the diacid from *P. piliformis*, *X. polymorpha*, *X. longipes*, and *H. deustum* had a m.p. 23 °C lower than that of the synthetic acid. The rotations of the diacid from two of the *Xylaria* species and *P. piliformis* were the same and strongly negative, whereas the diacid from *H. deustum* showed a positive rotation.

Six *Rosellinia* species were also examined; none of these produced mellein derivatives or either of the diacids (**1**) or (**2**). *Rhophalostoma gracile*, a new and as yet undescribed species from Thailand, is another xylariaceous fungus considered to be closely related to *Xylaria*; metabolic liquors of this fungus yielded succinic acid as the major metabolite.

Diacid (**1**), $C_9H_{16}O_4$, m.p. 142 °C, $[\alpha]_D^{23°}$ +22° from the metabolic liquors of *H. illitum*, was detected on t.l.c. plates as a colourless opaque area which developed as the plates which had been sprayed with diazotised *p*-nitroaniline began to dry; the acid was crystallised directly from the crude metabolic extract. The mass spectrum does not show a molecular ion, but a weak ion (1.1%) occurs at *m/z* 170 corresponding to the loss of water from the hypothetical molecular ion M^+ 188; the first major ion (5.3%) occurs at *m/z* 142 ($M - H_2O - CO$). A further loss of CO gives rise to an ion at *m/z* 114 (35.7%). The loss of a butyl side-chain is indicated by an ion at *m/z* 132 (7.2%) and this is confirmed by the occurrence of the base peak at *m/z* 56. In the ^{1}H n.m.r. spectrum a triplet (3 H) at δ 0.90 and a doublet (3 H) at δ 1.2 indicated the presence of CH_3CH_2– and CH_3CH– entities in the molecule.

Table. Optical rotation of diacid (**2**) from various fungal sources

Source	$[\alpha]_D^{23}$ (°) (*c* 1.0 in MeOH)
H. deustum	+31
X. polymorpha	−89
X. longipes	−89
P. piliformis	−89
X. mali	0
X. hypoxylon	0

Rohr[2] and Arigoni[3] have reported the isolation of two isomers of this diacid from the fungus *Anthostomella avocetta*; the (+)-*erythro*, m.p. 142 °C $[\alpha]_D$ +24.4° and the (+)-*threo*, m.p. 98 °C, $[\alpha]_D$ + 23.3°, and accordingly the diacid from *H. illitum* is ascribed the (+)-*erythro* structure. A synthetic acid described by Marvel and Fuller[4] is reported to have m.p. 85—105 °C, and repetition of their synthesis confirmed this observation. However, we were easily able to separate this synthetic acid into the component *erythro* and *threo* isomers by recrystallisation from nitromethane; complete purification of each component was possible by chromatography. The least soluble, high m.p., *erythro* isomer (m.p. 142 °C) proved to be identical with the natural product.

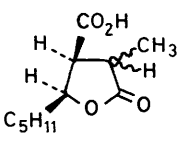
Diacid (**2**), m.p. 155 °C or 132 °C, $C_{11}H_{18}O_4$ was similarly detected as a colourless opaque spot on dried plates or by a yellow stain with iodine. In this case a weak molecular ion (0.2%) is observed in the mass spectrum but the ion at *m/z* 196 ($M - 18$) is the first major ion. In the i.r. spectrum (KBr) a broad absorption occurs at 3 450—2 500 cm^{-1} and two separate acid carbonyl absorptions occur at 1 711 and 1 678 cm^{-1}. In the ^{1}H n.m.r. spectrum the olefinic proton occurs as a triplet at δ 6.88 (*J* 7 Hz) and is coupled to a multiplet methylene group at δ 2.24. An intense signal at δ 1.34 (6 H) and a triplet at δ 0.87 (3 H) show the presence of a C_4H_9 group constituting part of the hexylidene side-chain. The remaining CH–CH_3 entity absorbs at δ 3.7 (1 H, q), and δ 1.28 (3 H, d). Calculations based on the Pascual formula[5] lead to chemical-shift values of δ 6.78 and δ 6.20 for the olefinic proton of the *E*- and *Z*-form respectively and allows assignment of the *E* configuration to the new acid.

This is supported by a comparison with quoted values for the olefinic protons of several *E* and *Z* substituted crotonic acids where the *E* isomers show the lower field signals.[6] The final confirmation of the structure was sought by synthesis.

Although many methods are described in the literature for the synthesis of alkylidenesuccinic acids derived from succinic acid itself, there are few reports of the successful synthesis of the alkyl-substituted alkylidene compounds. We have examined a number of possible routes for the synthesis of diacid (**2**) and have been able to confirm the structure of the diacid but we have been unable to find a high-yield synthesis for this compound.

The Stobbe reaction between ketones and succinate esters is usually a high yielding route to unsaturated succinic acids. However, with aldehydes, the strongly basic conditions of the reaction often lead to self-condensation of the aldehyde, and there are few reports of its successful use with alkyl-substituted succinate esters. In our hands all attempts to get hexanal to react with diethyl methylsuccinate in the presence of either potassium t-butoxide or sodium hydride failed to yield any of the required product. The simplest alternative route to the acid appeared to be *via* the appropriately substituted paraconic (tetrahydro-5-oxo-3-furoic) acid, the esters of which are reported to undergo ring opening and dehydration in the presence of sodium ethoxide.[7,8] The simple paraconic acids derived from succinic acid are readily available by the condensation of aldehydes with sodium succinate in the presence of acetic anhydride.[8–13] The products from the similar reaction with alkylated succinic acids are less well known but heptanal is reported to yield a difficultly separable mixture of two paraconic acids when treated with methylsuccinic acid,[14] these arising from the reaction of the aldehyde at the two alternative sites of the acid.

For the preparation of the required paraconic acid, hexanal was condensed with sodium methylsuccinate in the presence of acetic anhydride at 125 °C. The product comprised a mixture of the required acid lactone (**3**) and two stereoisomers of the acid lactone (**5**); these isomers were readily separated by chromatography and are distinguishable by differences in m.p. and ^{1}H n.m.r. chemical shifts. The required lactone (**3**) showed absorption of the 5-methine proton as a multiplet at δ 4.4 and the 3- and 4-methine protons an overlapping multiplet between δ 2.56—3.12. By comparison the 3-methylene proton resonances of compound (**5a**) occur as well separated doublets at δ 3.1 and 2.4 (*J* 16 Hz) and those in isomer (**5b**) at δ 2.95 and 2.36. Lactones (**5a**) and (**5b**) can also be distinguished by differences in the chemical shift of the 5-methine proton; in (**5a**) this occurs as a triplet at lower field (δ 4.62) than that of (**5b**) (δ 4.21). Similar downfield shifts have been observed in *trans–cis*-lactone isomers of related structure.[15]



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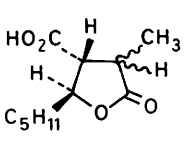
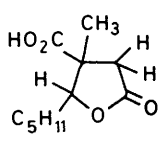


(4a and b)



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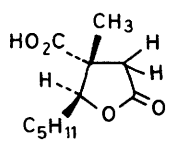
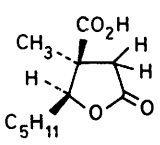


(5a)



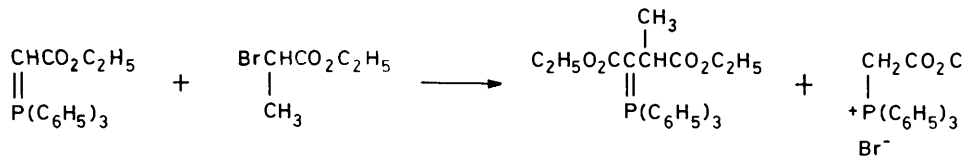
(5b)

An alternative synthesis of compound (**3**) was achieved by treating ethyl 3-oxo-octanoate with methyl 2-bromopropionate in the presence of sodium, and reduction of the resulting keto diester with sodium borohydride. The paraconic ethyl ester on hydrolysis gave a mixture of isomeric paraconic acids which were separated by chromatography. The major component (75%) of the mixture was identical with lactone (**3**). The remaining 25% of the product consisted of two additional isomers (**4a**) and (**4b**). These were distinguishable in their ^{1}H n.m.r. spectrum from (**3**) by the occurrence of their 5-methine proton at lower field and the narrower range of absorption of the 3- and 4-methine proton multiplet. The lower field 5-methine proton absorption in these compounds suggests a *cis* arrangement of this proton with the carboxy group in both these compounds. The i.r. spectra of the lactones show interesting differences which may be reconciled with differences in association within the molecules. In the solid state (KBr) isomers (**3**) and (**4a**) show a lactone carbonyl absorption at 1 750 cm^{-1} and an acid absorption at 1 730 cm^{-1}. However, isomer (**4b**) is quite different; the lactone absorption occurs at higher frequency (1 770 cm^{-1}) and two acid absorptions occur at 1 725 and 1 698 cm^{-1}. The former band is relatively weak compared with the latter which is of similar intensity to the lactone absorption. In chloroform solution these three compounds show similar absorptions at 1 775 cm^{-1} (lactone) and 1 735—1 720 cm^{-1} (acid); the latter absorption appears to comprise more than one absorption band. The spectra of the two isomers (**5a**) and (**5b**) in the solid state are also different. In (**5a**) two lactone absorptions are observed at 1 785 (shoulder) and 1 758 cm^{-1} with the acid band at 1 715 cm^{-1}. In (**5b**) lactone and acid absorptions coalesce to give a single broad absorption between 1 755—1 730 cm^{-1}. In $CHCl_3$ solution both compounds show a lactone band at 1 785 cm^{-1} and carboxyl absorption at 1 735—1 710 cm^{-1} (broad).

Treatment of the ethyl ester of compound (**3**) with sodium ethoxide over varying periods of time gave only a low yield of the required acid (**2**); also produced was a small quantity of methylsuccinic acid, the latter presumably arising from a reversed aldol reaction on the intermediate hydroxy acid.

The low yields encountered in the final hydrolysis and dehydration stage of the paraconic ester led us to examine a possible alternative Wittig route to this acid. Wittig reactions between aldehydes and phosphonium ylides of succinic acid or its esters proceed readily with the formation of alkylidenesuccinic acids.[16] However, there are no reports of the similar reaction between aldehydes and phosphonium ylides of alkylated succinic acids. Two methods for the preparation of the phosphonium ylide of methylsuccinic acid were investigated: (a) a modification of the reaction used to prepare deuterio ylide derivatives of succinic acid whereby the triphenylphosphonium salts of acetic acid are treated with deuteriobromoacetic ester[17] (Scheme). In our hands substitution of the bromoacetic ester by ethyl 2-bromopropionate in this reaction gave only a low and very variable yield of the ethyl triphenylphosphoranediyl-(methyl)succinate. (b) Addition of triphenylphosphine hydrobromide to diethyl citraconate (methylmaleate), a reaction which proceeds readily with diethyl fumarate,[18] gave low yields of the required product which were not improved by the addition of benzoyl peroxide. The bromide was readily converted into the stable phosphorane with dilute alkali. However, this product could not be induced to react with aldehydes and even after 7 days reflux a substantial quantity of unchanged starting material was recovered and no trace of the alkylidene ester could be detected; instead a low yield of diethyl methylfumarate was obtained resulting from an elimination reaction.

After this work had been completed a new method for the preparation of alkylated 1,2-bis(alkoxycarbonyl)ethylidene-(triphenyl)phosphoranes appeared; in this reaction 1,2-bis(ethoxycarbonyl)ethylidenetriphenylphosphorane is lithiated and the anion is alkylated.[19] The author reports the



Scheme.

elimination of triphenylphosphine and the formation of alkylated fumaric esters when these compound are heated in an inert solvent in the presence of benzoic acid. Significantly, even the anion fails to react with aldehydes.

The formation of methylsuccinic acid by a retro-aldol reaction on the paraconic ester suggested a method for determination of the stereochemistry of diacid (**2**) by relating the rotation of the degradation product to those of the known methylsuccinic acids. However, all attempts to convert diacid (**2**) into the lactone, either by hydration or hydrohalogenation, failed. The acid did not react with either sulphuric acid, HCl, or HBr over extended periods and the ester failed to oxymercuriate with mercury(II) acetate during 7 days. Methyl crotonate by contrast reacted with mercury(II) acetate in 45 min.

The occurrence of butylmethylsuccinic acid as a metabolite of *Hypoxylon illitum* closely links this species with *H. serpens* which produces a butyrolactone bearing the same alkyl substituents.[20] Similarly the two acids described in this paper closely link *Poronia*, *Xylaria*, and members of the *Hypoxylon* sub-section primo cinerea. Presumably both butyl(methyl)-succinic acid and the alkylidenesuccinic acid arise from a similar metabolic pathway possibly involving condensations between a keto acid of the glyoxylic acid cycle and a fatty acid.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus, i.r. spectra on a Perkin-Elmer 681 spectrophotometer, u.v. spectra on a Unicam SP 800 spectrophotometer, ^{1}H n.m.r. spectra on a JEOL JNM-MH-100 spectrometer (with $SiMe_4$ as internal standard), mass spectra on an AEI MS 902 spectrometer, and optical rotations on a Perkin-Elmer 141 polarimeter. All t.l.c., preparative layer (p.l.c.), and column chromatography was done on Merck Kieselgel PF 256 + 366; p.l.c. was performed on silica gel (16 g) on 20 × 20 cm glass plates. Unless otherwise stated, light petroleum refers to the fraction boiling in the range 60—80 °C.

Isolation of 2-*Butyl*-3-*methylsuccinic Acid* (**1**) *from* Hypoxylon illitum.—*Hypoxylon illitum* was cultured on malt solution (4% Boots) in 21 Thomson bottles for 8 weeks. The colourless mycelium was filtered off and the pale brown medium was extracted with ethyl acetate (3 × 1 l). The extract was dried (Na_2SO_4) and evaporated. The semi-solid residue (4.3 g) was dissolved in nitromethane; on being cooled the solution produced needles which were recrystallised from nitromethane to yield *erythro*-2-butyl-3-methylsuccinic acid (**1**), m.p. 142 °C (lit.,[2] 142 °C); $[\alpha]_D^{20}$ + 22° (*c* 1.0 in CH_3OH) (Found: C, 57.3; H, 8.7. Calc. for $C_9H_{16}O_4$: C, 57.4; H, 8.6%); $\nu_{max.}$(KBr) 3 000br and 1 710 cm^{-1}. The strain of *H. illitum* used was the same as that used for the isolation of the mellein derivatives[1] but had been sub-cultured several times on malt agar. The mellein derivatives from the above isolation remained in the nitromethane mother liquor.

Isolation of 2-*Hexylidene*-3-*methylsuccinic Acid* (**2**) *from* Xylaria mali *and* X. longipes.—The fungus was cultured as described above. The fungus grew rapidly, forming a hard black crusty mycelium with a yellow underside. Fruiting occurred in several of the bottles. The medium (**9e**) was extracted with ethyl acetate (3 × 1 l) and the extract was dried (Na_2SO_4) and evaporated to yield a gummy yellow solid (140 mg). Recrystallisation from nitromethane gave needles of racemic (E)-2-*hexylidene*-3-*methylsuccinic acid* (**2**) (110 mg), m.p., 155 °C (Found: C, 61.7; H, 8.6. $C_{11}H_{18}O_4$ requires C, 61.7; H, 8.5%); $\nu_{max.}$(KBr) 3 450, 1 711, 1 678, and 1 635 cm^{-1}. In the case of *X. longipes* the crude gummy extract was chromatographed on silica gel in the solvent system toluene–ethyl formate–formic acid (50:49:1). Crystallisation of the gummy product from nitromethane gave needles of (*E*)-2-hexylidene-3-methylsuccinic acid (**2**) (135 mg), m.p. 132 °C; $[\alpha]_D^{23}$ −89° (*c* 1.0 in CH_3OH) (Found: C, 61.7; H, 8.4%). Yields from the other *Xylaria* species were similar but were substantially higher (1.59 g) from 10 l in the case of *P. piliformis*.

Separation of Synthetic erythro- *and* threo-2-*Butyl*-3-*methylsuccinic Acids*.—The synthetic acid prepared by the method of Marvel and Fuller[4] (2.5 g) was dissolved in the minimum of boiling nitromethane and the solution was set aside. The colourless solid which separated (1.04 g), m.p. 140 °C comprised the *erythro* isomer contaminated with a little of the *threo* isomer. Purification by column chromatography with chloroform–acetic acid (95:5) as eluant yielded the pure *erythro* isomer (R_F 0.47) which recrystallised from nitromethane as needles (0.9 g), m.p. 142 °C. The nitromethane mother liquor from the first crystallisation was evaporated to dryness and the residue (1.0 g) which comprised mainly the *threo* isomer was chromatographed as described above. The *threo* isomer (R_F 0.4) recrystallised from water as needles, m.p. 90 °C (0.8 g).

4-*Carboxy*-4,5-*dihydro*-3-*methyl*-5-*pentylfuran*-2(3H)-*one* (**3**) *and* 4-*Carboxy*-4,5-*dihydro*-4-*methyl*-5-*pentylfuran*-2(3H)-*one* (**5a**) *and* (**5b**).—A mixture of the sodium salt of methylsuccinic acid (17.6 g, 0.1 mol), hexanal (10 g, 0.1 mol), and acetic anhydride (0.1 mol) was heated at 128 °C for 24 h. The cooled mixture was dissolved in water (50 ml) and the solution was washed with ether. The aqueous layer was acidified and extracted with ether. The ether extract was extracted with M-aqueous sodium carbonate, the aqueous layer was acidified, and the resulting oil was again extracted into ether. Evaporation of the dried (Na_2SO_4) extract gave a pale brown viscous oil (7 g) which was applied to a column of silica gel and eluted with light petroleum–ether–acetic acid (70:30:3) to yield, after evaporation of the solvent, three fractions in the order (i) a solid (1.27 g) which gave lustrous needles of 4-*carboxy*-4,5-*dihydro*-3-*methyl*-5-*pentylfuran*-2(3H)-*one* (**3**), m.p. 89 °C (from light petroleum) (Found: C, 61.9; H, 8.3. $C_{11}H_{18}O_4$ requires C, 61.7; H, 8.5%), (ii) a solid (1.92 g) which gave needles of 4-*carboxy*-4,5-*dihydro*-4-*methyl*-5-*pentylfuran*-2(3H)-*one* (**5a**), m.p. 78—79 °C (from light petroleum) Found: C, 61.9; H, 8.4%), and (iii) a solid (1.2 g) which gave leaflets of the C-4 *epimeric* 4-*carboxy*-4,5-*dihydro*-4-*methyl*-5-*pentylfuran*-2(3H)-*one* (**5b**), m.p. 98 °C (from light petroleum) (Found: C, 61.6; H, 8.2%).

Ethyl 3-*Oxo-octanoate*.—A solution of hexanoyl chloride (40 g) in dichloromethane (100 ml) was added dropwise to a solution of Meldrum's acid (2,2-*dimethyl*-1,3-*dimethyl*-1,3-*dioxane*-4,6-*dione*) (39.7 g in pyridine (50 ml) at 0 °C. The red solution was set aside overnight at room temperature and was then

poured into 2M-HCl (300 ml). The lower layer was separated, washed with water, dried (Na_2SO_4), and evaporated to yield a brown oil (61 g). A solution of the oil in absolute ethanol (200 ml) was refluxed for 5 h and then evaporated, and the residue was distilled to yield ethyl 3-oxo-octanoate (35.9 g), b.p. 90—96 °C at 4 mmHg.

Diethyl 2-Hexanoyl-3-methylsuccinate.—Ethyl 3-oxo-octanoate (46.5 g) was added dropwise during 3 h to a suspension of powdered sodium (5.75 g) in toluene (500 ml). Ethyl 2-bromopropionate (18.1 g) was added and the mixture was heated on a water-bath (100 °C) for 16 h. After being cooled, the solution was washed with water and the toluene layer was dried (Na_2SO_4) and evaporated. Distillation of the residue gave diethyl *2-hexanoyl-3-methylsuccinate* (35 g), b.p. 140—150 °C at 4 mmHg (Found: C, 64.6; H, 8.6. $C_{16}H_{26}O_5$ requires C, 64.4; H, 8.7%); $\nu_{max.}$ 1 735, 1 717, and 1 710 cm^{-1}; $\delta(CDCl_3)$ 4.08—4.2 (4 H, m), 3.88 (1 H, d, *J* 11 Hz), 3.24 (1 H, m), 2.60 (2 H, t, *J* 7 Hz), 1.12—1.7 (15 H, m), and 0.92 (3 H, t).

4-Ethoxycarbonyl-4,5-dihydro-3-methyl-5-pentylfuran-2(3H)-one.—Sodium borohydride (3.7 g) was added to a solution of the above keto ester (28.6 g) in ethanol (100 ml) and water (40 ml). After 3 h the solution was acidified and extracted twice with ether. Evaporation of the dried (Na_2SO_4) extract and distillation of the residue (× 2) gave the furanone as an oil (7.2 g), b.p. 120—124 °C at 0.1 mmHg (Found: C, 64.7; H, 9.3. $C_{13}H_{22}O_4$ requires C, 64.4; H, 9.15%); $\nu_{max.}$ 1 775 and 1 732 cm^{-1}.

Hydrolysis of 4-Ethoxycarbonyl-4,5-dihydro-3-methyl-5-pentylfuran-2(3H)-one.—A mixture of the above lactone ester (2.5 g) and 2M-aqueous sodium hydroxide (25 ml) was refluxed for 3 h. The cooled solution was extracted twice with ether and the aqueous solution was then acidified and extracted three times with ether. Evaporation of the latter dried (Na_2SO_4) extracts gave an oil (1.97 g) which rapidly solidified. T.l.c. in the solvent system light petroleum–ether–formic acid (70:30:3) showed the presence of 3 components (bromocresol green indicator). The mixture was applied to a column of silica gel and eluted with the above solvent system to yield two fractions after evaporation of the solvent: (i) a solid which recrystallised from light petroleum (b.p. 80—100 °C) as lustrous needles, m.p. 89 °C, identical by mixed m.p. and ^1H n.m.r. spectrometry with isomer (**3**) described above. (ii) A solid (0.12 g) comprising two components which were separated by p.l.c. in the solvent system light petroleum–chloroform–acetic acid (50:50:3) to yield (a) a solid (25 mg), the acid (**4a**), which recrystallised from light petroleum (b.p. 80—100 °C) as needles, m.p. 153 °C (sublimes 120—150 °C) (Found: C, 61.5; H, 8.3. $C_{11}H_{18}O_4$ requires C, 61.7; H 8.4%); $\delta(CDCl_3)$ 0.86 (3 H, t), 1.24—1.66 (11 H, m), 1.84—3.22 (2 H, m), and 4.66 (1 H, q); (b) a solid (65 mg), the *acid* (**4b**), which recrystallised from light petroleum (b.p. 80—100 °C) as small needles, m.p. 112 °C (Found: C, 61.9; H, 8.5. $C_{11}H_{18}O_4$ requires C, 61.7; H, 8.4%); $\delta(CDCl_3)$ 0.84 (3 H, t), 1.24—1.66 (11 H, m), 2.82—3.22 (2 H, m), and 4.66 (1 H, q).

2-Hexylidene-3-methylsuccinic Acid by Hydrolysis of the Lactone Ethyl Esters.—The isomeric mixture of lactone ethyl esters (1.16 g), prepared by the borohydride reduction described above, was refluxed in sodium ethoxide solution [from ethanol (15 ml) and sodium (0.23 g)] for 24 h. The solvent was evaporated off and the solid residue was dissolved in 2M-sodium hydroxide (10 ml) and the mixture was refluxed for 3 h. The mixture was extracted with ether and the aqueous layer was acidified. Re-extraction with ether gave an oil which was dissolved in nitromethane (1.5 ml). On being cooled the solution deposited needles of 2-hexylidene-3-methylsuccinic acid (175 mg), m.p. 155 °C (Found: C, 61.8; H, 8.5. $C_{11}H_{18}O_4$ requires C, 61.7; H, 8.5%), identical by mixed m.p. and ^1H n.m.r. spectrometry with the natural product from *X. mali.*

The mother liquor from the above recrystallisation was evaporated to dryness. P.l.c. separation of the residue in the solvent system chloroform–acetic acid (95:5) gave a second acid (7 mg), m.p. 110—112 °C, identified as methylsuccinic acid by its ^1H n.m.r. spectrum and mixed m.p. with an authentic sample.

Preparation of Ethoxycarbonylmethylene(triphenyl)phosphorane.—Ethyl 2-bromoacetate (83.5 g) was added dropwise during 30 min to a stirred solution of triphenylphosphine (131 g) in benzene (600 ml) at room temperature. The temperature rose to 35—40 °C during the addition. The mixture was stirred for a further 5 h and was then set aside overnight. The precipitate was filtered off, washed successively with benzene and light petroleum, and dried *in vacuo* at water-pump pressure at 50 °C to yield the bromide (195 g).

The bromide (190 g) was dissolved in cold water (2 l) and 2M-aqueous sodium hydroxide was added dropwise until the mixture was alkaline to phenolphthaleine. The precipitate was filtered off and air-dried to give the phosphorane (140 g), m.p. 117 °C (lit.,[21] 116—117 °C).

Preparation of 1,2-Bis(ethoxycarbonyl)propylidene(triphenyl)phosphorane.—(i) A solution of ethoxycarbonylmethylene(triphenyl)phosphorane (14 g), and ethyl 2-bromopropionate (3.62 g) in dry ethyl acetate (100 ml) was refluxed for 72 h. The mixture was filtered hot and evaporated under reduced pressure. Recrystallisation of the residue from ethyl acetate gave the title phosphorane as cubes (2.3 g), m.p. 168—171 °C (lit.,[19] 168—170 °C) (Found: C, 72.4; H, 6.4; P, 7.1. Calc. for $C_{27}H_{29}O_4P$: C, 72.3; H, 6.4; P, 6.9%).

(ii) A solution of diethyl citraconate (diethyl methylmaleate) (7.4 g) and triphenylphosphine hydrobromide (14 g) in dry acetonitrile (20 ml) was refluxed for 24 h. The mixture was poured into water (100 ml) and the solution was extracted with ether (3 × 50 ml). The aqueous solution was rendered alkaline with 2M-aqueous sodium hydroxide and the opaque mixture was again extracted with ether (3 × 50 ml). The yellow gummy solid obtained by evaporation of the dried latter extract was triturated with benzene (5 ml) and the mixture was filtered after 1 h. Recrystallisation of the solid from ethyl acetate yielded the phosphorane as plates, m.p. and mixed m.p. with the sample prepared above 168—171 °C.

Reaction of 1,2-Bis(ethoxycarbonyl)propylidene(triphenyl)phosphorane with Hexanal.—A solution of the phosphorane (4.5 g) in dry benzene (60 ml) was added dropwise during 15 min to a solution of hexanal (1.0 g) in dry benzene (10 ml) under nitrogen. The mixture was refluxed for 24 h and the yellow solution was evaporated to yield an oily yellow solid which was washed with light petroleum. The residue (2.2 g) comprised unchanged phosphorane. The washings were evaporated to dryness and the residue was refluxed (2 h) with a mixture of 2M-aqueous sodium hydroxide (25 ml) and ethanol (15 ml). The alcohol was evaporated off and the aqueous solution was extracted with ether (3 × 15 ml). The aqueous layer was acidified with 2M-HCl and re-extracted with ether (3 × 15 ml). Evaporation of the dried (Na_2SO_4) latter extracts and crystallisation of the residue from nitromethane gave methylfumaric acid (340 mg), m.p. and mixed m.p. with an authentic sample 202 °C.

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