Polyolefinic 18-Methyl-19-oxoicosenoic Acid Pigments from the Fungus *Piptoporus australiensis* (Wakefield) Cunningham

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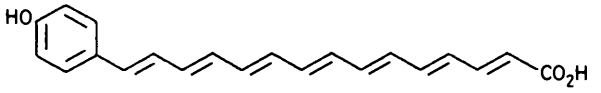
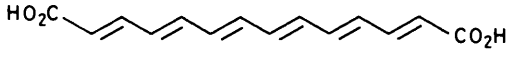
Three pigments present in the bright orange fruiting body of the basidiomycete *Piptoporus australiensis* are identified as (all-*E*)-18-methyl-19-oxoicosa-2,5,7,9,11,13,15,17-octaenoic acid (piptoporic acid) (4), its methyl ester (3), and (all-*E*)-(3*R*)-3-acetoxy-18-methyl-19-oxoicosa-5,7,9,11,13,15,17-heptaenoic acid (5). The absolute configuration of the last named metabolite is established by chemical correlation with methyl (3*R*)-3-acetoxy-4-carboxybutanoate.

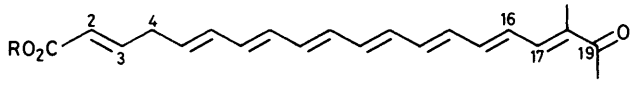
REPORTS of the occurrence of non-isoprenoid polyene pigments in the basidiomycetes are rare. Only two such metabolites have hitherto been isolated, namely cortisalin (1) and corticrocin (2) which were obtained from the fruiting bodies of *Corticium salicinum* [1] and *C. sulfureum*,[2] respectively. On the other hand, carotenoid pigments [3] and (generally) colourless poly-yne and poly-yne-ene metabolites [4] enjoy a wider distribution among the higher fungi. We describe here the isolation and characterisation of three novel polyolefinic fatty acid derivatives, (3), (4), and (5), which are responsible for the bright orange colour of the fresh fruiting body of the basidiomycete *Piptoporus australiensis* (Wakefield) Cunningham.



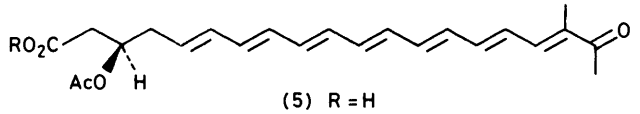
(1)



(2)



(3) R = Me
(4) R = H



(5) R = H
(6) R = Me

Sporophores of *P. australiensis*, a 'bracket' fungus native to Australia and New Zealand, possess moist flesh which is coloured intensely orange when fresh but which fades rapidly on drying in air, becoming almost colourless. Acetone extraction of fresh sporophores gave a deep-red gum containing three major yellow pigments. Preliminary purification of this mixture by trituration with light petroleum removed a colourless metabolite, 3-[(*Z*)-hexadec-7-enyl]-4-methylfuran-2,5-dione,[5] together with linoleic acid, some ergosterol, and a proportion of the pigment content. Further purification of the compounds remaining was facilitated by 'flash' column chromatography [6] which allowed rapid separation and efficient recovery of the unstable polyene metabolites (3), (4), and (5) in a combined yield of *ca.* 11% of the total acetone extractives from the fungus.

Piptoporic acid methyl ester (3) † is the least polar of those pigments isolated. It was obtained after 'flash' chromatography in 2.1% yield as an orange-red oil. In common with the other polyenes described here the ester (3) proved extremely unstable when free from solvent. However, it can be stored in the absence of light at low temperature for several weeks without significant spectroscopic or chromatographic deterioration if maintained in chloroform solution under argon. High resolution mass spectrometry established the molecular formula $C_{22}H_{26}O_3$ for the metabolite (3). The presence of a conjugated heptenone chromophore was indicated by long wavelength u.v. absorption at 422.5, 398.5, and 378.5 nm (log ε 4.82, 4.89, and 4.74, respectively ‡) in hexane solution [7] and by less well resolved absorption bands at 415sh and 402.5 nm (log ε 4.82 and 4.83) in methanol,[8] and was confirmed when addition of sodium borohydride to a methanolic solution of compound (3) [8] produced u.v. maxima at 400.5, 378.5, 359, and 344sh (log ε 4.93, 4.95, 4.75, and 4.46, respectively) typical of an all-*trans* conjugated heptene.[7,8] The absence of a diagnostic '*cis*-peak' in the near u.v. region [8] is consistent with the assignment of all-*trans* stereochemistry to the conjugated polyene chain. The i.r. spectrum of the pigment (3) shows no absorption due to hydroxy-groups and contains, in addition to a conjugated carbonyl band at 1 660 cm^{-1}, a second carbonyl absorption at 1 730

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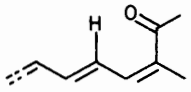
† The trivial name piptoporic acid is suggested for (all-*E*)-18-methyl-19-oxoicosa-2,5,7,9,11,13,15,17-octaenoic acid (4); the pigments (3) and (5) are named accordingly as derivatives of piptoporic acid.

‡ The acute instability in the dry state of this and the other polyenes made the reproduction of extinction coefficients difficult. The figures quoted are probably low.

cm^{-1} consistent with the presence of an ester group. A strong band at 970 cm^{-1} and the absence of absorption near to 780 cm^{-1} further supports the assignment of all-*trans* double bond stereochemistry. The ^{13}C n.m.r. spectrum of compound (3) shows resonances at δ 199.2 and 166.9 and sixteen olefinic carbon signals appear between δ 146.3 and δ 121.9, indicative of the presence of one ketone carbonyl, one ester carbonyl, and eight double bonds. The pigment (3) must therefore be acyclic and must contain one double bond which is not conjugated with the principal all-*trans* heptenone chromophore.

The ^1H n.m.r. spectrum of compound (3) reveals methyl proton singlets at δ 3.72, 2.33, and 1.91 identifying, respectively, a methyl ester, a methyl ketone (*cf.* ref. 9), and a vinyl methyl group adjacent to the ketone carbonyl.[10] The ketone and vinyl methyl signals are broadened somewhat by incompletely resolved long-range coupling with 17-H; irradiation at frequencies towards the low-field end of the olefinic proton envelope (*ca.* δ 7.05, see below) sharpened and increased the intensity of these signals. The remainder of the ^1H n.m.r. spectrum consists of a two-proton triplet (*J* 6 Hz) at δ 3.04 and an envelope between δ 5.60 and 7.19 containing fifteen olefinic proton resonances. These data are accommodated fully in the icosaoctenoate structure (3). The *trans*-stereochemistry of the isolated double-bond was assigned upon examination of the olefinic proton n.m.r. pattern. Thus, double triplets at δ 5.86 (*J* 15.0, 1.5 Hz) and δ 6.98 (*J* 15.0, 6.5 Hz) which collapse to mutually coupled doublets when the sample is irradiated at the frequency of the doubly allylic methylene group are assigned to the olefinic protons 2-H and 3-H, respectively. The 15 Hz coupling constant between these protons defines the stereochemistry between C-2 and C-3 as *trans*. The stereochemistry at the ketonic terminus of the molecule (3) is clear from the absence of any olefinic proton resonance below δ 7.19. This observation precludes the possible alternative configuration at C-18 depicted in partial structure (A) since the proton 16-H in such a molecule would experience strong deshielding by the carbonyl group and should resonate near to δ 8.0.[11]



(A)

Confirmation of the position at C-18 of the vinyl methyl group in compound (3) was obtained by catalytic hydrogenation of the pigment. The product (7), $C_{22}H_{42}O_3$, m.p. 35—36 °C, has not hitherto been reported but its identity as methyl 18-methyl-19-oxoicosanoate is readily established from its mass spectrum. The molecular ion at *m/z* 354 fragments by cleavage β to the ketone carbonyl to give both the base peak at *m/z* 72 ($C_4H_8O^+$) by a McLafferty rearrangement and the second most abundant ion at *m/z* 283 ($C_{18}H_{35}O_2^+$) which is also observed in the spectrum of methyl 19-oxoicosanoate itself.[12] The ^1H and ^{13}C n.m.r. spectra are also in full accord with structure (7).

A second pigment, $C_{21}H_{24}O_3$ (mass spec.), isolated in 5.5% yield was identified spectroscopically as piptoporic acid (4), the free acid corresponding to the methyl ester (3). Methylation of the metabolite (4) with diazomethane afforded material indistinguishable from the ester (3). Hydrogenation of piptoporic acid gave 18-methyl-19-oxoicosanoic acid (8), m.p. 53—55 °C, the structure of which followed by spectroscopic comparison with, and methylation to, the ester (7).

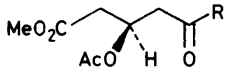
$$RO_2C\text{-}(CH_2)_{15}CH_2CH(Me)COMe$$

(7) R = Me
(8) R = H

A third and the most polar pigment characterised from *P. australiensis* was shown to be (3*R*)-3-acetoxy-2,3-dihydropiptoporic acid (5). It was obtained still slightly impure after chromatography and proved difficult to separate completely from piptoporic acid (4) and from two other minor pigments. However, methylation of the pigment and further chromatography afforded the pure ester (6) (3.1%), $C_{24}H_{30}O_5$, $[\alpha]_D$ +195° (MeOH), which was subsequently characterised. In the mass spectrum, the ester (6) exhibits a molecular ion at *m/z* 398 which eliminates the elements of acetic acid to give the base peak at *m/z* 338 coincident with the molecular ion of piptoporic acid methyl ester (3). Similar loss of acetic acid in the mass spectrum of the pigment (5) itself suggested that this metabolite is an acetoxylated dihydropiptoporic acid derivative. The u.v. spectrum of the ester (6) in methanol and in methanol containing sodium borohydride established the presence of the same conjugated all-*trans* heptenone chromophore common to the pigments (3) and (4) and thereby restricts the possible site of the acetoxy-substituent to C-2, C-3, or C-4. Its position at C-3 is established clearly by the ^1H n.m.r. spectrum, which lacks the resonances characteristic of the 2-H and 3-H olefinic protons of piptoporic acid methyl ester (3) (see above). Instead, there is a two-proton doublet at δ 2.58 and a one-proton quintet at δ 5.26, mutually coupled by 6.0 Hz, which identify the β-acetoxybutyrate end group in the ester (6).[13] Other significant proton resonances include a triplet at δ 2.48 assigned to the C-4 methylene group and an acetate methyl singlet at δ 2.00. The remainder of the ^1H n.m.r. spectrum closely resembles that of the ester (3) and together with the ^{13}C spectrum (Experimental section) fully supports the structure (6) for the methyl ester of pigment (5).

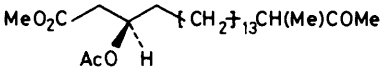
Finally, the absolute configuration at C-3 in the metabolite (5) was established as *R* by chemical correlation with methyl (3*R*)-3-acetoxy-4-carboxybutanoate (9).[14] Ozonolysis of the ester (6) followed by oxidative decomposition of the ozonide with Jones reagent gave the crude half-ester (9) which was isolated in pure form as the crystalline ureide derivative (10), m.p. 138—

139 °C (lit.,[14] 142 °C), $[\alpha]_D$ −16.1° ($CHCl_3$) [lit.,[14] −15.7° ($CHCl_3$)]. The half-ester (9) itself exhibited a plain *positive* o.r.d. spectrum[14] but could not be obtained chemically pure even after repeated bulb-to-bulb (Kugelröhr) distillation (*cf.* ref. 14). Similarly, plain positive o.r.d. spectra were obtained for the (3*R*)-3-acetoxyicosanoate (11), the product of catalytic hydrogenation of the ester (6), and for methyl (3*R*)-3,5-diacetoxypentanoate (12). The diacetoxypentanoate (12) was obtained from the ester (6) by ozonolysis followed by sodium borohydride work-up and subsequent acetylation of the intermediate alcohol (13).

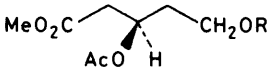


(9) R = OH

(10) R = −N(CONH−(*p*-$Me_2NC_6H_4$))−(*p*-$Me_2NC_6H_4$)



(11)



(12) R = Ac

(13) R = H

Unlike the methyl ester (3) which co-occurs with piptoporic acid (4) in sporophores of *P. australiensis*, no trace of (3*R*)-3-acetoxy-2,3-dihydropiptoporic acid methyl ester (6) could be detected by t.l.c. in the total extract of the fungus. This fact, together with the observation that the ester (6), when pure, shows no propensity whatsoever to β-elimination, provides strong evidence that the pigments (3) and (4) are true metabolites.

While the polyene metabolites (3), (4), and (5) are the most abundant pigments in *P. australiensis*, three minor yellow compounds are visible in chromatograms of the crude extracts, and there is the suggestion from the mass spectra of the pigments (3), (4), and (5) themselves that vinylogues of these pigments may be present in trace amounts in the fungus. The polyolefinic compounds (3), (4), and (5) represent, therefore, the first examples of what may be a new class of pigments present in members of the Polyporaceae.

EXPERIMENTAL

I.r. spectra were measured as solutions in carbon tetrachloride on a Perkin-Elmer 683 spectrometer unless stated otherwise. N.m.r. spectra were recorded on Varian HA-100 (100 MHz, ^1H) and JEOL JNM-FX60 (15.04 MHz, ^{13}C) spectrometers for solutions in deuteriochloroform with tetramethylsilane as internal standard. Optical rotations at the sodium D-line (589 nm) and o.r.d. spectra were measured using a JASCO ORD/UV-5 spectrometer. U.v. spectra were obtained on a Varian DMS 90 spectrophotometer. Mass spectra were run on GEC-AEI MS 902 and VG-Micromass 7070 instruments operating at 70 eV. Melting points were determined on a Kofler hot stage and are uncorrected. Boiling points cited refer to Kugelröhr air-bath temperatures.

Materials.—Light petroleum of boiling range 60—80 °C was used. Throughout, ether refers to diethyl ether. Merck Kieselgel 60 (230—400 mesh) was used for 'flash' chromatography.[6] Pigments when pure were transferred using spectroscopic grade chloroform and were stored in the same solvent under an argon atmosphere at −20 °C in the absence of light. Thin layer chromatograms were analysed visibly and then with molybdophosphoric acid (5% in ethanol).

A voucher specimen of *Piptoporus australiensis* (Wakefield) Cunningham is on deposit at the Herbarium of the Royal Botanic Gardens, Edinburgh, under collection number WAT. HERB. 13878-E.

Isolation and Purification of Pigments from P. australiensis.—Whole fresh sporophores (three specimens; 15—18 cm in diameter, *ca.* 6 cm in thickness), collected at Marker Point, N.S.W., Australia, were chopped and immersed overnight in acetone (1 l) at room temperature. After filtration and re-extraction (2×) the combined extracts were evaporated to leave a red gum (50 g). Trituration with light petroleum (3 × 500 ml) at room temperature,[5] followed by re-extraction of the light petroleum-washed residue (34 g) with acetone (250 ml), gave a red solution; this was filtered to remove colourless materials (*ca.* 8 g) which included ergosterol. Evaporation of the filtrate afforded a red gum (*ca.* 26 g) which was chromatographed in portions (0.5 g) on a 4 cm (diameter) column of silica gel which had been packed in and was subsequently eluted with methylene dichloride–acetone–methanol (12 : 8 : 1), precisely as advocated by Still.[6] The first yellow zone to be eluted (60 mg) contained the pigment (3) together with a second yellow metabolite (R_F 0.54 and 0.34, respectively, in methylene dichloride–5% ethyl acetate) and was re-chromatographed[6] (4 cm column, methylene dichloride–5% ethyl acetate) to afford *methyl* (*all*-E)-18-*methyl*-19-*oxoicosa*-2,5,7,9,11,13,15,17-*octaenoate* (*piptoporic acid methyl ester*) (3) (20 mg, 2.1%) as an unstable orange-red oil, R_F 0.95 [ethyl acetate–light petroleum–methanol (40 : 10 : 1)] (Found: M^+, 338.1884. $C_{22}H_{26}O_3$ requires *M*, 338.1882); $\lambda_{max.}$ (hexane) 422.5 (log ε 4.82), 398.5 (4.89), 378.5 (4.74), 302 (3.99), 290 (3.87), and 245 nm (4.02); $\lambda_{max.}$ (MeOH) 415sh (4.82), 402.5 (4.83), 306 (4.02), and 247 nm (4.00); $\lambda_{max.}$ (MeOH + $NaBH_4$) 400.5 (4.93), 378.5 (4.95), 359 (4.75), 344sh (4.46), 286 (3.83), 277 (3.70), and 233 nm (4.13); $\nu_{max.}$ 1 730 and 1 660 (C=O), 970 cm^{-1}; δ_H 1.91 (s, 18-Me), 2.33 (s, MeCO), 3.04 (t, *J* 6 Hz, 4-H_2), 3.72 (s, CO_2Me), 5.74 (dt, *J* 15.0, 7.0 Hz, 5-H), 5.86 (dt, *J* 15.0, 1.5 Hz, 2-H), 5.96—7.19 (12 H, m, 6-H to 17-H), and 6.98 (dt, *J* 15.0, 6.5 Hz, 3-H); δ_C 199.2 (s, C-19), 166.9 (s, C-1), 146.3 (d, C-3), 139.8, 139.0, 136.6, 136.3, 135.8, 134.9, 133.3, 131.8, 131.0, 130.3, 129.9, 129.7, 128.8, and 128.1 (C-5 to C-18), 121.9 (d, C-2), 51.5 (q, CO_2Me), 35.2 (t, C-4), 25.6 (q, C-20), and 11.6 (q, 18-Me).

The second yellow band to elute consisted of (*all*-E)-18-*methyl*-19-*oxoicosa*-2,5,7,9,11,13,15,17-*octaenoic acid* (*pipto-*

poric acid) (4) (53 mg, 5.5%), R_F 0.53 [ethyl acetate–light petroleum–methanol (40 : 10 : 1)] (Found: M^+, 324.1750. $C_{21}H_{24}O_3$ requires M, 324.1725); $\lambda_{max.}$ (MeOH) 415sh (log ε 4.67), 404 (4.68), 305 (3.90), and 249 nm (3.94); $\lambda_{max.}$ (MeOH + $NaBH_4$) 400.5 (4.82), 378.5 (4.84), 358.5 (4.66), 344sh (4.39), 287.5 (3.94), 276 (3.90), and 235 nm (4.12); $\nu_{max.}$ 3 700—2 400br (OH), 1 700 and 1 660 (C=O), 970 cm^{-1}; δ_H 1.92 (s, 18-Me), 2.06 (s, OH), 2.35 (s, MeCO), 3.09 (t, J 6 Hz, 4-H_2), 5.75 (dt, J 15.0, 7.0 Hz, 5-H), 5.87 (br d, J 15 Hz, 2-H), 5.98—7.20 (12 H, m, H-6 to H-17), and 7.10 (dt, J 15.0, 6.0 Hz, H-3); δ_C 199.5 (s, C-19), 171.4 (s, C-1), 148.7 (d, C-3), 140.0, 139.2, 136.6, 136.3, 135.9, 134.9, 133.3, 131.8, 130.6, 129.9, 128.9, and 128.4 (C-5 to C-18, two resonances obscured), 121.8 (d, C-2), 35.3 (t, C-4), 25.6 (q, C-20), and 11.6 (q, 18-Me).

The third intensely yellow zone (86 mg) contained the pigment (5), R_F 0.35 [ethyl acetate–light petroleum–methanol (40 : 10 : 1)] (Found: M^+, 384.1932. $C_{23}H_{28}O_5$ requires M, 384.1936) contaminated with piptoporic acid (4) and two minor yellow metabolites [R_F 0.44 and 0.24 in ethyl acetate–light petroleum–methanol (40 : 10 : 1)]. Treatment of the impure pigment (5) with an excess of diazomethane in ether followed by further chromatography [6] (4 cm column, methylene dichloride–5% ethyl acetate) gave *methyl* (3R)-(*all*-E)-3-*acetoxy*-18-*methyl*-19-*oxoicosa*-5,7,9,11,13,15,17-*heptaenoate* [(3R)-3-*acetoxy*-2,3-*dihydropiptoporic acid methyl ester*] (6) (30 mg, 3.1%) an unstable orange-yellow oil, $[\alpha]_D$ +195° (*c* 0.7 in MeOH), R_F 0.41 (methylene dichloride–5% ethyl acetate) (Found: M^+, 398.2093. $C_{24}H_{30}O_5$ requires M, 398.2093); $\lambda_{max.}$ (MeOH) 415sh (log ε 4.94), 403 (4.95), 305 (4.23), and 248 nm (3.16); $\lambda_{max.}$ (MeOH + $NaBH_4$) 420sh (4.34), 401 (5.07), 378.5 (5.08), 359 (4.89), 345sh (4.59), 287 (4.04), 276 (3.93), and 234 nm (4.20); $\nu_{max.}$ 1 748 and 1 660 (C=O), 970 cm^{-1}; δ_H 1.90 (s, 18-Me), 2.00 (s, $MeCO_2$), 2.32 (s, MeCO), 2.48 (t, J *ca.* 6 Hz, 4-H_2), 2.58 (d, J 6.0 Hz, 2-H_2), 3.65 (s, CO_2Me), 5.26 (quintet, J 6.0 Hz, 3-H), 5.68 (dt, J 15.0, 7.0 Hz, 5-H), and 5.94—7.20 (12 H, m, 6-H to 17-H); δ_C 199.1 (s, C-19), 170.5 (s, C-1 and $MeCO_2$), 139.7, 138.9, 136.7, 136.5, 136.2, 135.7, 134.8, 133.4, 131.7, 130.3, 129.9, 129.2, 129.0, and 128.7 (C-5 to C-18), 69.6 (d, C-3), 51.7 (q, CO_2-*Me*), 38.3 and 37.4 (both t, C-2 and C-4), 25.6 (q, C-20), 21.0 (q, *Me*CO_2), and 11.6 (q, 18-Me); m/z 398 (M^+, 71%), 338 ($M^+ - C_2H_4O_2$, 100), 129 (25), 128 (19), 109 (30), 105 (22), 91 (28), and 43 ($MeCO^+$, 56).

Hydrogenation of Piptoporic Acid Methyl Ester (3).—The ester (3) (20 mg) in ethanol (25 ml) was hydrogenated in the presence of 5% Pd–carbon until the consumption of hydrogen ceased. The catalyst was filtered off and washed with ethanol, and the filtrate was evaporated. Crystallisation of the residue from pentane (−20 °C) gave *methyl* 18-*methyl*-19-*oxoicosanoate* (7) (20 mg) as fronds, m.p. 35—36 °C (Found: C, 74.8; H, 12.05. $C_{22}H_{42}O_3$ requires C, 74.5; H, 11.95%); $\nu_{max.}$ 1 745 and 1 715 cm^{-1} (C=O); δ_H 1.07 (d, J 7.0 Hz, 18-Me), 1.16—1.80br (30 H, CH_2), 2.12 (s, MeCO), 2.30 (t, J 7.5 Hz, 2-H_2), 2.49 (sextet,* J 7 Hz, 18-H), and 3.65 (s, CO_2Me); δ_C 212.8 (s, C-19), 174.3 (s, C-1), 51.4 (q, CO_2*Me*), 47.3 (d, C-18), 34.2 (t, C-2), 33.0 (t, C-16), 29.7 (t, C-4 to C-15), 27.9 (q, C-20), 27.3 (t, C-17), 25.1 (t, C-3), and 16.2 (q, 18-Me); m/z 354 (M^+, 8%), 323 ($M^+ - CH_3O$, 7), 283.2629 ($C_{18}H_{35}O_2^+$, 22), 74 (10), 72.0572 ($C_4H_8O^+$, 100), 69 (10), 55 (15), and 43 (21); *2,4-dinitrophenylhydrazone*, yellow prisms, m.p. 93—96 °C (from ethanol) (Found: C, 63.05; H, 8.7; N, 10.45. $C_{28}H_{46}N_4O_6$ requires C, 62.9; H, 8.65; N, 10.5%).

* Signal partially obscured by 2-H triplet; multiplicity confirmed by shift experiment.

Hydrogenation of piptoporic acid (4) (90 mg) in an analogous fashion gave 18-*methyl*-19-*oxoicosanoic acid* (8) (104 mg) as fronds, m.p. 53—55 °C (from light petroleum) (Found: C, 73.7; H, 12.0. $C_{21}H_{40}O_3$ requires C, 74.1; H, 11.85%); $\nu_{max.}$ 3 500—2 400br (OH) and 1 715 cm^{-1} (C=O); δ_H 1.07 (d, J 7.0 Hz, 18-Me), 1.14—1.80 (30 H, $-CH_2-$), 2.12 (s, MeCO), 2.34 (t, J 7 Hz, 2-H), and 2.50 (sextet, J 7 Hz, 18-H); δ_C 213.1 (s, C-19), 179.5 (s, C-1), 47.3 (d, C-18), 34.0 (t, C-2), 33.0 (t, C-16), 29.7 (t, C-4 to C-15), 28.0 (q, C-20), 27.3 (t, C-17), 24.8 (t, C-3), and 16.2 (q, 18-Me); m/z 340 (M^+, 4%), 98 (10), 85 (13), 73 (17), 72 (100), 69 (13), 57 (14), 55 (22), and 43 (30). Methylation of the acid (8) with diazomethane gave the ester (7).

Ozonolysis of (3R)-3-*Acetoxy*-2,3-*dihydropiptoporic Acid Methyl Ester* (6).—Ozone was passed through a solution of the ester (6) (64 mg) in chloroform (20 ml) at −78 °C until the yellow colour was discharged and an excess of oxidant was detected at the outlet. The excess of ozone was removed with nitrogen, after which the solution was warmed to room temperature and the solvent removed under reduced pressure. The ozonide in acetone (5 ml) was exposed to an excess of Jones reagent at 0 °C for 0.5 h after which propan-2-ol, then water, were added and the products were isolated with ether. The residue (63 mg) was distilled twice (Kugelröhr, b.p. 85 °C/0.02 mmHg) to give methyl (3*R*)-3-acetoxy-4-carboxybutanoate (9) (14 mg) as an oil, $[\alpha]_D$ +5° (*c* 0.5 in $CHCl_3$); δ 2.03 (s, $MeCO_2$), 2.74 (d, J 6.0 Hz, 2-H_2), 2.80 (d, J 6.0 Hz, 4-H_2), 3.68 (s, CO_2-Me), 5.52 (quintet, J 6.0 Hz, 3-H), and 8.34br (OH).

The half ester (9) (10 mg) and bis(*p*-dimethylaminophenyl)carbodi-imide [15] (14 mg) were heated under reflux in ether (3 ml) for 3 h. Removal of the solvent and purification of the residue by p.l.c. in methylene dichloride–10% ethyl acetate gave 1-[(3*R*)-3-acetoxy-4-methoxycarbonylbutanoyl]-1,3-bis(*p*-dimethylaminophenyl)urea (10) (12 mg) as tan plates, m.p. 138—139 °C (lit.,[14] 142 °C) (Found: C, 61.5; H, 6.65; N, 11.5. Calc. for $C_{25}H_{32}N_4O_6$: C, 61.95; H, 6.65; N, 11.55%); $[\alpha]_D$ −16.1°, $[\alpha]_{400}$ −46.0° (*c* 0.326 in $CHCl_3$) {lit.,[14] $[\alpha]_D$ −15.7° ($CHCl_3$)}; δ 2.02 (s, $MeCO_2$), 2.64 (4 H, d, J 6 Hz, 2-H_2 and 4-H_2), 2.88 (s, Me_2N), 2.98 (s, Me_2N), 3.66 (s, CO_2Me), and 6.62—7.44 (8 H, ArH).

Methyl (3R)-3,5-*Diacetoxypentanoate* (12).—The ester (6) (35 mg) in methanol (25 ml) at −70 °C was ozonised until the yellow colour was discharged. To this solution at −10 °C was added sodium borohydride (23 mg) and the mixture was maintained at −10 °C for 1.5 h. Addition of 20% aqueous sodium hydrogenphosphate and extraction with ether gave the alcohol (13) which was acetylated in pyridine (2 ml) with acetic anhydride (5 drops). Extractive work-up and distillation gave the *diacetate* (13) (10 mg) as an oil, b.p. 70 °C/13 mmHg (Found: C, 51.65; H, 7.0. $C_{10}H_{16}O_6$ requires C, 51.7; H, 6.95%); $[\alpha]_D$ +5.5°, $[\alpha]_{400}$ +16.7°, $[\alpha]_{300}$ +36.1° (*c* 0.36 in MeOH); $\nu_{max.}$ (film) 1 740br cm^{-1} (C=O); δ 2.04 (6 H, s, $MeCO_2$), 2.08 (m, obscured by signal due to acetate protons, 4-H_2), 2.63 (d, J 6 Hz, 2-H_2), 3.69 (s, CO_2Me), 4.14 (t, J 6 Hz, 5-H_2), and 5.32 (quintet, J 6 Hz, 3-H).

Methyl (3R, 18RS)-3-*Acetoxy*-18-*methyl*-19-*oxoicosanoate* (11).—Hydrogenation of the ester (6) (68 mg), as described above for pigment (3), gave after distillation the *icosanoate ester* (11) (30 mg) as an oil, b.p. 125 °C/0.3 mmHg (Found: C, 70.4; H, 10.4. $C_{24}H_{44}O_5$ requires C, 69.85; H, 10.75%); $[\alpha]_D$ +3.0°, $[\alpha]_{400}$ +5.7° (*c* 0.835 in $CHCl_3$); $\nu_{max.}$ (film)

1 745br and 1 715 cm^{-1} (C=O); δ_H 1.08 (d, J 7 Hz, 18-Me), 1.14—1.80 (28 H, CH_2), 2.02 (s, $MeCO_2$), 2.12 (s, MeCO), 2.56 (d, J 6 Hz, 2-H_2), 3.68 (s, CO_2Me), and 5.22 (quintet, J 6 Hz, 3-H); δ_C 213.0 (s, C-19), 171.0 and 170.5 (s, C-1 and $MeCO_2$), 70.7 (d, C-3), 51.8 (q, CO_2Me), 47.3 (d, C-18), 39.1 (t, C-2), 34.2 (t, C-4), 33.0 (t, C-16), 29.8 (t, C-6 to C-15), 28.1 (q, C-20), 27.3 (t, C-17), 25.2 (t, C-5), 21.2 (q, $MeCO_2$), and 16.2 (q, 18-Me).

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