1028. *Avenaciolide, an Antifungal Lactone from* Aspergillus avenaceus.

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Avenaciolide, $C_{15}H_{22}O_4$, a neutral antifungal metabolite of *Aspergillus avenaceus*, has structure (I) with absolute configuration as shown. The acid fraction from *A. avenaceus* contains β-nitropropionic acid.

When grown on aqueous media, *Aspergillus avenaceus* G. Smith yields culture filtrates which possess antifungal properties. The active principle has been isolated as a neutral crystalline solid, for which we propose the name avenaciolide. This has a double m. p. 49—50° and 54—56°, although on one occasion the higher-melting form was obtained. The acid fraction contained β-nitropropionic acid, previously isolated from *Penicillium atrovenetum* and several *Aspergillus* species (for refs. see 1).

Avenaciolide, $C_{15}H_{22}O_4$, shown below to have structure (I), contains one *C*-methyl group (Kuhn–Roth) and no methoxyl or acetoxyl groups. The infrared spectrum in bromoform shows no band attributable to a hydroxyl group, a single band (at 1786 cm.$^{-1}$) in the carbonyl region, and a band at 1667 cm.$^{-1}$ attributable to an ethylenic double bond.

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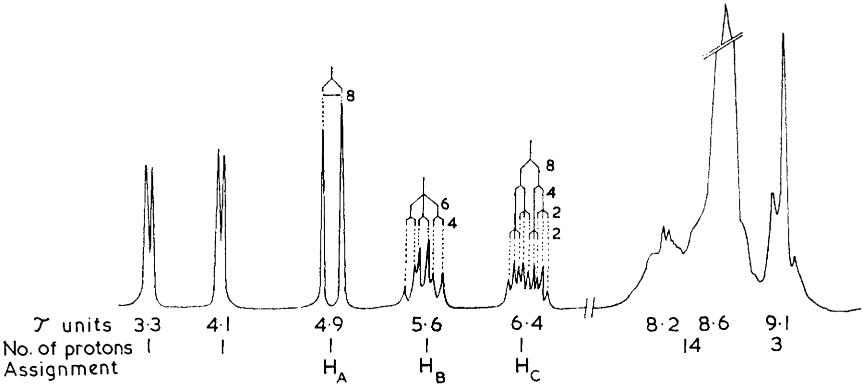
[1] Raistrick and Stössl, *Biochem. J.*, 1958, **68**, 647.

On alkaline hydrolysis, avenaciolide consumed two equivalents of alkali. Isolation of the product yielded a hydroxy-acid (II; R,R′ = CH_2, R″ = H), $C_{15}H_{24}O_5$, which shows an infrared band at 1777 cm.$^{-1}$ in addition to the expected bands due to carboxyl and hydroxyl groups and to an ethylenic double bond. Relactonisation of the hydroxy-acid regenerated avenaciolide. Methanolysis of avenaciolide gave the hydroxy-ester (II; R,R′ = CH_2, R″ = Me), $C_{16}H_{26}O_5$, $\nu_{max.}$ 1782 cm.$^{-1}$. These facts indicate that avenaciolide contains two γ-lactone groups, accounting for all the oxygen atoms in the molecule.

Hydrogenation of avenaciolide, $\lambda_{max.}$ 210 mμ (ε 10,000), yielded the dihydro-compound (III; R = Me, R′ = H) which contains two *C*-methyl groups, and which shows no selective ultraviolet absorption or infrared double-bond absorption. Avenaciolide therefore possesses one double bond which is present as a methylene group conjugated to one of the lactone-carbonyl groups. This is supported by the ready addition of methanol to avenaciolide under mild basic conditions,[2] to give the dilactone (III; R = CH_2·OMe, R′ = H). The lactone rings and the double bond account for all the unsaturation of avenaciolide.



Nuclear magnetic resonance spectrum of avenaciolide. Numerals on peaks are *J*, in c./sec.

The hydroxy-acid (II; R,R′ = CH_2, R″ = H) produced by alkaline hydrolysis of avenaciolide shows only end-absorption in the ultraviolet region (ε_{200} 8850), suggesting that in the hydroxy-acid it is the αβ-unsaturated lactone which has opened. This conclusion is supported by hydroxylation of the hydroxy-acid with osmium tetroxide to give the triol (II; R = CH_2·OH, R′ = OH, R″ = H), having $\nu_{max.}$ 1735 cm.$^{-1}$ in bromoform, compared with 1700 cm.$^{-1}$ for the starting material.

In accordance with the presence of an α-methylene-γ-lactone group, hydroxylation of avenaciolide with osmium tetroxide yielded a diol which with lead tetra-acetate gave formaldehyde, carbon dioxide, and the hydroxy-acid (IV; R = R′ = H), $C_{13}H_{22}O_5$, which formed an oily methyl ester monoacetate showing no infrared hydroxyl absorption. The hydroxy-acid (IV; R = R′ = H), $\nu_{max.}$ 1790 and 1745 cm.$^{-1}$ (in acetonitrile), titrated as a monobasic acid monolactone and was recovered unchanged from the lactone titration. The hydroxy-acid (IV; R = R′ = H) was also obtained by ozonolysis of avenaciolide, followed by decomposition of the ozonide with dilute hydrochloric acid. The formation of the hydroxy-acid involves the rupture of two carbon–carbon bonds, a feature which is characteristic of the " abnormal " ozonolysis[3] of compounds containing a conjugated carbonyl group.

The nuclear magnetic resonance spectrum (see Figure) of avenaciolide confirms the presence of a conjugated terminal methylene group (two doublets, at τ 3·3 and 4·1), and one

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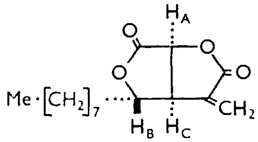
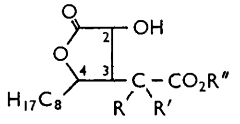
[2] Fukushima and Gallagher, *J. Amer. Chem. Soc.*, 1951, **73**, 196; Kulkarai, Paul, Rao, Kelkar, and Bhattacheryya, *Tetrahedron*, 1961, **12**, 178.

[3] Bailey, *Chem. Rev.*, 1958, **58**, 925; Sutherland, *Chem. and Ind.*, 1961, 1607.

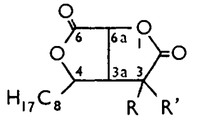
methyl group which must be attached to a CH_2 group (triplet at τ 9·1). In addition there are three one-proton multiplets between τ 4·8 and 6·5. The multiplet at lowest field, H_A (τ 4·9) shows by its splitting and intensity ratio [4] that it is coupled to a proton, H_C (τ 6·4), approximately 1·7 τ units to the high-field side. Similar approximate treatment of the two high-field peaks in the multiplet centred at τ 5·6 (H_B) shows H_B to be coupled to a proton (H_C) approximately 1 τ unit to the high-field side. Since the splitting pattern of H_B shows that it must also have a CH_2 group adjacent to it, avenaciolide must contain partial structure (VII). H_B, H_C, and the CH_2 protons form an ABX_2 system with the A proton (H_C), further split by H_A, giving in the general case a twelve-line spectrum as shown in the Figure; the splitting of H_C into triplets with J 2 c./sec. is as expected for an ABX_2 spectrum. This " virtual " coupling [5] is the first reported example for a saturated system where $J_{AX} = 0$.



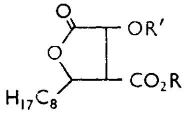
(I)



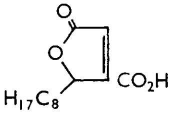
(II)



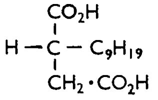
(III)



(IV)

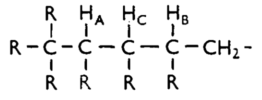


(V)



$$\mathrm{H}-\underset{\mathrm{CH_2\cdot CO_2H}}{\overset{\mathrm{CO_2H}}{\mathrm{C}}}-\mathrm{C_9H_{19}}$$

(VI)



$$\mathrm{R}-\underset{\mathrm{R}}{\overset{\mathrm{R}}{\mathrm{C}}}-\underset{\mathrm{R}}{\overset{\mathrm{H_A}}{\mathrm{C}}}-\underset{\mathrm{R}}{\overset{\mathrm{H_C}}{\mathrm{C}}}-\underset{\mathrm{R}}{\overset{\mathrm{H_B}}{\mathrm{C}}}-\mathrm{CH_2}-$$

(VII)

With the added knowledge that avenaciolide is a di-γ-lactone, $C_{15}H_{22}O_4$, consideration of the nuclear magnetic resonance spectrum leads to structure (I) which accounts satisfactorily for all the evidence so far presented.

Confirmation of structure (I) for avenaciolide was obtained as follows. Ozonolysis of avenaciolide in glacial acetic acid followed by decomposition of the ozonide with zinc dust and water gave, in addition to the hydroxy-acid (IV; R = R′ = H), a 13% yield of the cross-conjugated acid (V), $\nu_{\max.}$ 1760, 1725, and 1633 cm.$^{-1}$, $\lambda_{\max.}$ (in $CHCl_3$) 218 mμ (ε 11,640). The hydroxy-acid (IV; R = R′ = H) does not react with lead tetra-acetate, suggesting that the hydroxyl group is not α to the carboxyl group. In spite of this, dehydration to the unsaturated compound (V) was difficult, the only effective reagent being a refluxing solution of phosphorus pentoxide in pyridine. This resistance to dehydration is reminiscent of malic acid,[6] and is due to destabilisation of the incipient carbonium ion by the carbonyl group of the γ-lactone.

On hydrogenation in glacial acetic acid with Adams catalyst the unsaturated acid (V) absorbed 2 mol. of hydrogen, giving (*R*)-(+)-nonylsuccinic acid (VI), $[\alpha]_D^{23}$ +11·7°, whose solution infrared spectrum was identical with that of a racemic specimen synthesised according to Barry and Twomey's method.[7] Distillation of the diacid (VI) gave (+)-nonylsuccinic anhydride, $\lambda_{\max.}$ 1865 and 1790 cm.$^{-1}$, $[\alpha]_D^{22}$ +4·2°.

The relative stereochemistry of avenaciolide is readily obtained by consideration of the nuclear magnetic resonance spectrum of the methyl ester (IV; R = Me, R′ = H). The resonances corresponding to H_A and H_B are superimposed at τ 5·15, whilst that corresponding to H_C consists of a double doublet at τ 6·77 (J = 8, 4 c/sec.): the " virtual " coupling has vanished as a result of the increase in the relative chemical shift between H_B and H_C. The fall in the τ value of H_B on conversion of avenaciolide into the hydroxy-

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[4] Pople, Schneider, and Bernstein, " High Resolution Nuclear Magnetic Resonance," McGraw-Hill, New York, 1959, p. 121.

[5] Musher and Corey, *Tetrahedron*, 1962, **18**, 791.

[6] Kashichi, Ono, and Shin-ichi Hirayama, *J. Chem. Soc. Japan*, 1937, **58**, 1089.

[7] Barry and Twomey, *Proc. Roy. Irish Acad.*, 1947, **51**, *B*, 137.

ester (IV; R = Me, R′ = H) is readily explained in terms of the diamagnetic anisotropy of the methoxycarbonyl group,[8] provided that this group is *cis* to H_B. This would result in a dihedral angle between H_B and H_C of about 130° and a coupling constant (calculated by the Karplus equation[9]) of about 3 c./sec., close to that observed (4 c./sec.). The coupling constant between H_A and H_C of 8 c./sec. indicates a dihedral angle of about 0°, and thus a *cis*-relation of the carboxylic acid and the hydroxyl group, a conclusion which is supported by the ready relactonisation of the hydroxy-acid (II; R,R′ = CH_2, R″ = H). The relative stereochemistry is therefore as shown in (I).

The naturally derived nonylsuccinic acid has a weakly positive optical rotatory dispersion curve, confirming[10] the absolute configuration (VI). Hydrogenation of the acid (V) would take place predominantly from the less hindered side of the molecule; therefore, since all optically active centres have been related to the configuration of the side-chain by the nuclear magnetic resonance evidence above, avenaciolide must have the absolute stereochemistry of (I), with the 3a*S*,4*S*,6a*S* absolute configuration.

At pH 3·5, avenaciolide inhibits the germination of spores of a wide range of fungi at concentrations of 1—10 μg./ml., *e.g.*, germination of *Botrytis allii* and *Penicillium gladioli* is inhibited at 3 μg./ml. Some strains, *e.g.*, *Mucor erectus*, are notably resistant, requiring concentrations up to 100 μg./ml. to inhibit germination. Avenaciolide is weakly antibacterial, solutions containing 200 μg./ml. giving zones of inhibition of 13·5 cm. in cylinder plate assays against *B. subtilis*.

EXPERIMENTAL

Unless otherwise stated, infrared spectra were determined for Nujol mulls, optical rotations and ultraviolet spectra were measured for ethanol solutions, light petroleum had b. p. 40—60°, and silica gel used for chromatography was either B.D.H. chromatographic grade or Hopkin and Williams M.F.C. Nuclear magnetic resonance spectra were determined for deuterochloroform solutions on a Varian A.60 spectrometer, with tetramethylsilane as internal standard.

Isolation of Avenaciolide.—*Aspergillus avenaceus*, G. Smith (CMI 16140; No. 1349 in our collection), was grown at 25° as surface culture in ceramic vessels each containing 1 l. of Czapek–Dox medium. The fungus was harvested 15 days after inoculation and the culture filtrate (43 l.) was acidified and extracted with ethyl acetate (1 × 8 l. and 3 × 4 l.). The ethyl acetate solution was concentrated to *ca.* 1 l. and extracted with sodium hydrogen carbonate, to give neutral and acid fractions.

The neutral fraction (brown semi-solid; 13·0 g.) was percolated through a column of silica gel (100 g.), and the effluent was evaporated to dryness, giving a solid (9·9 g.) which was recrystallised twice from ether–light petroleum to give *avenaciolide* (8·0 g.) as elongated plates, double m. p. 49—50° and 54—56°, $[\alpha]_D^{26\cdot5}$ −41·6° (*c* 1·2) [Found: C, 67·7, 67·7; H, 8·4, 8·5; *C*-Me, 4·9, 6·7%; *M* (*X*-ray), 271 ± 3; Equiv. (lactone), 130. $C_{15}H_{22}O_4$ requires C, 67·6; H, 8·3; *C*-Me, 5·6% (for 1); *M*, 266; Equiv., 133 (dibasic)], $\lambda_{max.}$ 210 mμ (ε 10,000), $\nu_{max.}$ 1775 and 1667 or, in bromoform, 1786 and 1667 cm.$^{-1}$. On one occasion avenaciolide was obtained as needles, m. p. 54—56°, $\nu_{max.}$ 1795m, 1765s, and 1668w cm.$^{-1}$.

The crude acid fraction (11·3 g.) was chromatographed on silica gel (100 g.), to give the following fractions: (*a*) (eluted with benzene) avenaciolide (260 mg.); (*b*) [eluted with chloroform–benzene (1 : 9 and 1 : 1)] a solid (2·5 g.), which was purified by crystallisation from benzene and sublimation at 65°/10^{-4} mm., to give β-nitropropionic acid (1·5 g.), m. p. and mixed m. p. 67—69°, with infrared spectrum identical with that of an authentic sample kindly provided by Professor J. H. Birkinshaw, London School of Hygiene and Tropical Medicine; (*c*) [eluted with chloroform–benzene (1 : 1) and chloroform] oily crystals shown to be impure hydroxy-acid (II; R,R′ = CH_2, R″ = H) by infrared spectrum, paper chromatography, and formation of avenaciolide on treatment with acid.

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[8] Jackman, " Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, p. 124.

[9] Karplus, *J. Chem. Phys.*, 1959, **30**, 11; *J. Phys. Chem.*, 1960, **64**, 1793.

[10] Fredga, *Tetrahedron*, 1960, **8**, 126.

Hydrogenation of Avenaciolide.—Hydrogenation of avenaciolide (97 mg.) in glacial acetic acid (4 ml.) over palladium (22 mg.) gave an uptake of 1·05 mol. hydrogen. Isolation of the product gave a solid which was recrystallised from ether–light petroleum to give (3a*S*,6a*S*)-3-*methyl*-4(*S*)-*octylfuro*[3,4-b]*furan*-2,6-*dione* (III; R = Me, R′ = H) (90 mg.) as plates, m. p. 72—73·5°, $[\alpha]_D^{22}$ −5·32° (*c* 0·19) (Found: C, 67·3, 67·1; H, 9·1, 9·1; *C*-Me, 9·7. $C_{15}H_{24}O_4$ requires C, 67·1; H, 9·0; 2*C*-Me, 11·2%), $\varepsilon_{200} = 300$, $\nu_{max.}$ 1780 cm.$^{-1}$ or, in bromoform, 1787 cm.$^{-1}$.

Hydrolysis of Avenaciolide.—A suspension of avenaciolide (205 mg.) in 0·1N-sodium hydroxide (25 ml.) was heated at 75° for 90 min., to give a clear solution which was allowed to cool, washed with ether, acidified, and immediately extracted with ethyl acetate. This led to a solid (229 mg.), m. p. 83—93°, that was purified by chromatography on silia gel (3 g.) in chloroform–benzene (1 : 1). Recrystallisation from ether–light petroleum gave (2*S*,3*S*,4*S*)-3-1′-*carboxyvinyl*-2-*hydroxydodecan*-4-*olide* (II; R,R′ = CH_2, R″ = H) as small rocks (127 mg.), m. p. 91—93°, $[\alpha]_D^{24}$ +100·6° (*c* 0·5) (Found: C, 63·3; H, 8·5. $C_{15}H_{24}O_5$ requires C, 63·4; H, 8·5%), end absorption only (ε_{200} 8850), $\nu_{max.}$ 3430, 3335s, 3150br, 1786vs, 1771vs, 1702, and 1635 cm.$^{-1}$ or, in bromoform, 3320m, 1779vs, 1700s, and 1628w cm.$^{-1}$.

Relactonisation of the hydroxy-acid (II; R,R′ = CH_2,R″ = H) (15 mg.) with a boiling mixture of ethyl acetate (5 ml.) and concentrated hydrochloric acid (1 drop) for 5 hr. gave avenaciolide, m. p. 48—50°, identified by infrared spectrum.

Methanolysis of Avenaciolide.—A solution of avenaciolide (111 mg.) in methanol (10 ml.) containing concentrated sulphuric acid (0·3 ml.) was set aside at room temperature for 7 days. The solution was diluted with water and extracted with ethyl acetate, to give an oily solid (119 mg.) which crystallised from ether–light petroleum (four times), affording the hydroxy-ester (II; R,R′ = CH_2, R″ = Me) as plates (57 mg.), m. p. 86—87°, $[\alpha]_D^{26}$ +98·3° (*c* 0·2) (Found: C, 64·6; H, 8·8. $C_{16}H_{26}O_5$ requires C, 64·4; H, 8·8%), ultraviolet end absorption only ($\varepsilon_{200} = 8000$), $\nu_{max.}$ 3595s, 3550w,br, 2950s, 1885m, 1782vs, 1717vs, and 1635w cm.$^{-1}$ or, in bromoform, 3495, 1782, 1717, and 1635 cm.$^{-1}$.

Reaction of Avenaciolide with Methanolic Potassium Hydroxide.—A solution of avenaciolide (110 mg.) in 5% methanolic potassium hydroxide solution (25 ml.) was set aside at room temperature for 4 hr. Recovery in ether afforded gummy crystals (118 mg.) which were chromatographed on silica gel in benzene, to give a colourless oil (110 mg.) which crystallised from aqueous ethanol as needles (21 mg.), m. p. 66—67°, $[\alpha]_D^{26}$ +11·6° (*c* 0·26), of (3a*S*,4*S*,6a*S*)-3-*methoxymethyl*-4-*octylfuro*[3,4-b]*furan*-2,6-*dione* (III; R = CH_2·OMe, R′ = H) (Found: C, 64·1; H, 8·85. $C_{16}H_{26}O_5$ requires C, 64·4; H, 8·8), $\nu_{max.}$ 1791vs or, in bromoform, 1790vs cm.$^{-1}$.

Oxidation of Avenaciolide with Osmium Tetroxide.—A solution of avenaciolide (91 mg.) and osmium tetroxide (100 mg.) in dioxan (12 ml.) was set aside overnight at room temperature. The resulting black solution was treated with hydrogen sulphide, filtered, and evaporated to dryness. The crystalline residue (90 mg.) was recrystallised three times from ether–light petroleum, to give (3a*S*,4*S*,6a*S*)-3-*hydroxy*-3-*hydroxymethyl*-4-*octylfuro*[3,4-b]*furan*-2,6-*dione* (III; R = CH_2·OH, R′ = OH) as needles, m. p. 84—94°, $[\alpha]_D^{26·5}$ +4·97° (*c* 0·2) (Found: C, 60·0; H, 8·1. $C_{15}H_{24}O_6$ requires C, 60·0; H, 8·05%), $\nu_{max.}$ 3380s, 390sh, 1816s, and 1774vs or, in acetonitrile, 3440s and 1790vs cm.$^{-1}$.

Hydroxylation of the Hydroxy-acid (II; R,R′ = CH_2, R″ = H).—A solution of the hydroxy-acid (II; R,R′ = CH_2, R″ = H) and osmium tetroxide (197 mg.) in dry dioxan (3 ml.) was kept at room temperature for 24 hr. and the product was isolated as above. The crystalline residue (73 mg.) was recrystallised from benzene–methanol (50 : 1), giving (2*S*,3*S*,4*S*)-3-(1-*carboxy*-1,2-*dihydroxyethyl*)-2-*hydroxydodecan*-4-*olide* (II; R = CH_2·OH, R′ = OH, R″ = H) as prisms (32 mg.), m. p. 107—110°, $[\alpha]_D^{26}$ +26·8° (*c* 0·4) (Found: C, 56·5; H, 8·3%; equiv., 315. $C_{15}H_{26}O_7$ requires C, 56·6; H, 8·2%; equiv., 318), $\nu_{max.}$ 3510vs, 3300m, 3150m, 1803w, 1775vs, 1730, 1700w or, in bromoform, 3500s, 3300s, 1785vs, 1735m cm.$^{-1}$.

Cleavage of the Hydroxy-lactone (III; R = CH_2·OH, R′ = OH).—A stream of nitrogen was passed successively through a flask containing lead tetra-acetate (425 mg., 3·5 mol.) and traps containing (*a*) saturated aqueous dimedone, (*b*) empty (at −80°), and (*c*) 0·1N-barium hydroxide. The hydroxy-lactone (III; R = CH_2·OH, R′ = OH) in 60% acetic acid (5—10 ml.) was introduced into the flask. Barium carbonate was formed almost immediately in trap (*c*) and, after 3 hr., titration with 0·1N-hydrochloric acid showed that 0·84 mol. of carbon dioxide had been liberated.

The contents of the reaction flask were distilled at 0·1 mm. into trap (*b*), and the distillate was added to trap (*a*) and set aside overnight to give formaldehyde dimethone (7·5 mg.), m. p. 182—189°.

The residue in the flask was treated with 2N-sulphuric acid and extracted with ethyl acetate, to give a colourless solid (60 mg.) which was recrystallised from ether–light petroleum, to give (2*S*,3*S*,4*S*)-3-*carboxy*-2-*hydroxydodecan*-4-*olide* (IV; R = R′ = H) as plates, m. p. 114—118°, $[\alpha]_D^{26}$ +84·2° (*c* 0·2) [Found: C, 60·55; H, 8·6; Equiv., 231 (lactone 127). $C_{13}H_{22}O_5$ requires C, 60·4; H, 8·6%; Equiv., 258; Equiv. (lactone), 129], $\nu_{max.}$ 3320, 3200br, 1760vs, 1738s, and 1726s, or, in acetonitrile, 1785 and 1735 cm.$^{-1}$.

No carbon dioxide was evolved (barium hydroxide trap) during 30 min. at room temperature when the acid (IV; R = R′ = H) was treated with lead tetra-acetate.

Methylation with diazomethane gave the *hydroxy-ester* (IV; R = Me, R′ = H), m. p. 75—76°, $[\alpha]_D^{22}$ +82·2° (*c* 0·5) (Found: C, 61·5; H, 8·8. $C_{14}H_{24}O_5$ requires C, 61·7; H, 8·9%), $\nu_{max.}$ 3475, 1788vs, 1765s, 1746, or, in bromoform, 3550m, 3450sh, 1785vs, 1745vs cm.$^{-1}$. Nuclear magnetic resonance spectrum (with number of protons in parentheses): τ = 5·15 (2, multiplet), 6·23 (3, singlet), 6·77 (1, double doublet) (*J* 8,4 c./sec.), 8·71 (14), and 9·11 (3, triplet).

The *acetyl derivative* (IV; R = Me, R′ = OAc), prepared in pyridine at room temperature, of the methyl ester (IV; R = Me, R′ = H) was an oil, b. p. 120°/0·05 mm., which crystallised from ether–light petroleum at −80° (Found: C, 6·1; H, 8·3. $C_{16}H_{26}O_6$ requires C, 61·1; H, 8·3%) and had $\nu_{max.}$ 1790 and 1747 cm.$^{-1}$.

Ozonolysis of Avenaciolide.—(*a*) A stream of ozonised oxygen (2 equiv. during 5 min.) was passed through a solution of avenaciolide (203 mg.) in glacial acetic acid (10 ml.). The solution was set aside for 90 min., diluted with ether (10 ml.) and water (3 ml.), treated with zinc dust (100 mg.), and set aside overnight at room temperature. The mixture was diluted with water (50 ml.) and extracted with ether. The aqueous layer was distilled into saturated aqueous dimedone, to give formaldehyde dimethone (40 mg.). Evaporation of the ether solution yielded gummy crystals (198 mg.) which were chromatographed on silica gel (6 g.). Elution with ether–light petroleum (1 : 9) yielded (S)-(+)-3-*carboxydodec*-2-*en*-4-*olide* (V) as plates (28 mg.), m. p. 131—133°, $[\alpha]_D^{22}$ +23·4° (*c* 0·4) (Found: C, 64·8; H, 8·4. $C_{13}H_{20}O_4$ requires C, 65·0; H, 8·4%), $\nu_{max.}$ 1745, 1716, 1035, and 700 cm.$^{-1}$ together with a broad carboxylic OH band, or in chloroform, 1760vs, 1725s, and 1635w, $\lambda_{max.}$ 218 mμ (ε 11,640). Nuclear magnetic resonance spectrum (with number of protons in parentheses): τ = 3·18 (1) (H_2) (*J* = 2 c./sec.), 4·75 (1, H_4), 8·71 (14), and 9·13 (3).

Continued elution with increasing percentages of ether in light petroleum gave (with 15% of ether) avenaciolide (42 mg.) and (with 20% of ether) the hydroxy-acid (IV; R = R′ = H).

(*b*) Avenaciolide (1 g.) in acetic acid (15 ml.) was ozonised for 50 min. (5 equiv.), and the solution was left at room temperature for 2 hr. After treatment with 2N-hydrochloric acid (15 ml.), followed by acetic acid (35 ml.), to give a clear solution, the mixture was left at room temperature overnight. Recovery in ether gave a solid (546 mg.) which on crystallisation from ether–light petroleum gave the hydroxy-acid (IV; R = R′ = H) (491 mg.), m. p. 114—116°.

Extraction of the aqueous layer with chloroform gave a semi-solid (409 mg.) which was chromatographed on silica gel to (*a*) further hydroxy-acid (IV; R = R′ = H) (96 mg.) and (*b*) a solid (53 mg.), recrystallised from ether–light petroleum as plates (14 mg.), m. p. 60—62°, which was not investigated further.

Dehydration of the Hydroxy-acid (IV; R = R′ = H).—Phosphorus pentoxide (2 g.) was added to a refluxing solution of the hydroxy-acid (IV; R = R′ = H) (1·14 g.) in pyridine (20 ml.). After 15 min. the mixture was diluted with water (75 ml.) and extracted with ether, giving a brown gum (894 mg.) which was chromatographed on silica gel (30 g.). Elution with benzene–ether (9 : 1) gave gummy crystals (450 mg.) shown by the infrared spectrum to be a mixture of the unsaturated acid (V) and the expected β-lactone. Extraction with sodium hydrogen carbonate solution gave (*a*) a solid acid (328 mg.) which, on recrystallisation from acetone–light petroleum, yielded crystals (214 mg.) of the unsaturated lactone (V), and (*b*) a neutral intractable gum.

Continued elution of the column with benzene–ether (17 : 3) gave starting material (190 mg.).

Hydrogenation of the Acid (V).—The acid (V) (11·5 mg.) in acetc acid (10 ml.) was hydrogenated over platinum [from Adams catalyst (21·5 mg.)]. After 8 min. (uptake 1·85 mol) the solution was filtered and evaporated to dryness, to give a semicrystalline solid (12·8 mg.) which recrystallised from aqueous ethanol or light petroleum (b. p. 60—80°) to give (+)-(*R*)-nonylsuccinic acid (VI) (5 mg.), m. p. 92—93°, $[\alpha]_D^{23}$ +11·7° (*c* 0·9) (Found: C, 63·8; H, 9·7.

$C_{13}H_{24}O_4$ requires C, 63·9; H, 9·9%), $\nu_{max.}$ (broad, OH), 3100—2500, 1740sh, 1685vs, or, in acetonitrile, 3250br and 1740vs cm.$^{-1}$, mixed melting point with a specimen of (±)-nonylsuccinic acid,[10] 90—95°.

Distillation of nonylsuccinic acid (119 mg.) at 160°/15 mm. yielded an oil (80 mg.) which rapidly crystallised. Recrystallisation from acetone–light petroleum gave nonylsuccinic anhydride (20 mg.), m. p. 54—55°, $[\alpha]_D^{22}$ +4·2° (*c* 0·5), $\nu_{max.}$ 1846vs, 1825w, 1779vs, or, in acetonitrile, 1865s and 1795vs.

We are indebted to Mr. J. F. Grove for helpful discussion and advice, to Professor W. Klyne for measuring the optical rotatory dispersion curve of (+)-nonylsuccinic acid, to Dr. A. Melera for the nuclear magnetic resonance spectrum of avenaciolide, to Miss E. M. Gregory for technical assistance, to Mr. G. L. F. Norris for the fermentation, and to Mr. B. W. F. Fitzpatrick for measurement of biological activity.

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[*Received, May* 10*th*, 1963.]