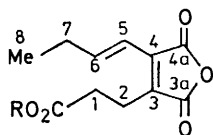


## A New Tricarboxylic Acid Anhydride from *Paecilomyces variotii*

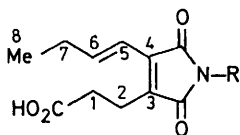
By David C. Aldridge,\* Raymond. M. Carman, and Richard B. Moore, Imperial Chemical Industries Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire SK10 4TG

The metabolite (3*Z*,5*E*)-octa-3,5-diene-1,3,4-tricarboxylic acid 3,4-anhydride (1) has been isolated in high yield from *Paecilomyces variotii*.

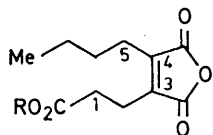
THE mould *Paecilomyces variotii*, when grown on aqueous media, yields culture filtrates containing large quantities of an acidic crystalline solid  $C_{11}H_{12}O_5$ . The  $^1H$  n.m.r. spectrum indicated a *trans*-coupled but-1-enyl group, an isolated allylic dimethylene group, and one exchangeable proton. I.r. stretching vibrations showed a conjugated maleic anhydride and an aliphatic carboxylic acid. These factors led to structure (1) which was confirmed by carbon magnetic resonance and by mass spectrometry.



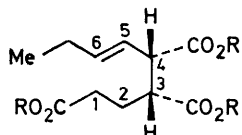
(1) R = H  
(2) R = Me



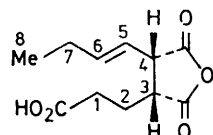
(3) R = H  
(4) R = Ph



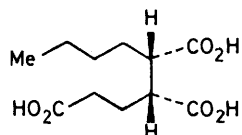
(5) R = H  
(6) R = Me



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



(9)



(10)

Compound (1) was stable to a range of conditions (see Experimental section). Methylation gave the liquid ester (2) while ammonia and aniline gave the crystalline imines (3) and (4). Catalytic hydrogenation removed one double bond to give the acid (5) which was methylated to the ester (6). Reduction of compound (1) with zinc in acetic acid gave predominantly the acid (7) which could be purified *via* the anhydride (9). The relative stereochemistry of the acid (7), its ester (8), and anhydride (9) follow from the spectrum of compound (9). In this compound 4-H is coupled to both 5-H and 3-H by 8 Hz. The 8 Hz coupling between 3-H and 4-H is of a magnitude normal for a *cis*-coupling in substituted succinic anhydrides, and larger than the normal *trans*-coupling. The anhydride (9) readily reverts to the acid (7) in moist air.

The acid (7) gives an n.m.r. spectrum showing 4-H as a

triplet with  $J_{3,4} = J_{4,5} = 9$  Hz. This large coupling is again characteristic of succinic acids with an n.m.r. doublet of triplets coupled to 4-H by 9.5 Hz and to the two C-2 methylene protons, which it must see differently, by 9.5 and 3.5 Hz. These couplings suggest that the 3-H is arranged in predominantly an antiperiplanar and a synclinal fashion respectively to the two 2-H protons.

Hydrogenation of the acid (7) gave the previously unknown octane-1,3,4-tricarboxylic acid (10).

All compounds showed consistent n.m.r., i.r., u.v., and mass spectra.

Biosynthetically, acid (1) can be formally derived<sup>2</sup> by a condensation between hexanoic acid and 2-oxoglutaric acid.

### EXPERIMENTAL

N.m.r. spectra are for  $CDCl_3$  solutions with internal  $SiMe_4$ . G.l.c. analyses were run at 210 °C over 5% SE 30. U.v. spectra are for methanol solutions. *J* Values are in Hz.

*Isolation and Characterisation of the Anhydride (1).*—*Paecilomyces variotii* Bain (ICI isolate, No. 3 142 in our collection) was grown by stirred, aerated fermentation in a vessel containing 5-l of Czapek-Dox medium with 5% sugar. After 90 h the filtrate was extracted at pH 2 with ethyl acetate ( $1 \times 1\frac{1}{2}$  l,  $1 \times 1$  l) to give a light brown solid (16.0 g). The extract in ether was passed down a short silica column to give (3*Z*,5*E*)-octa-3,5-diene-1,3,4-tricarboxylic acid 3,4-anhydride (1) (8.45 g) as prisms from diethyl ether-light petroleum, m.p. 101–102 °C (Found: C, 58.9; H, 5.3.  $C_{11}H_{12}O_5$  requires C, 58.9; H, 5.4%),  $\nu_{max}$  (Nujol) 3 100—2 600, 1 825, 1 770, 1 750, 1 695, 1 650, 1 610, 1 285, 1 275, 1 225, 980, 940, and 915  $cm^{-1}$ ;  $\delta$  H 7.27 (d of t, *J* 16, 7.5, 7.5, H-6), 6.31 (d, of t, *J* 16, 1.5, 1.5, H-5), 2.79 ( $A_2B_2m$ , H-1 and H-2), 2.35 (pentuplet, *J* 7, H-7) and 1.11 (t, *J* 7, H-8)  $\delta_c$ : 178.2 (s, C-1a), 165.9 (s, C-3a or C-4a), 164.3 (s, C-4a or C-3a), 150.2 (d, C-5), 138.4 (s, C-3 or C-4), 136.3 (s, C-4 or C-3), 116.4 (d, C-6), 31.3 (t, C-1 or C-2), 27.5 (t, C-2 or C-1), 19.2 (t, C-7), and 12.5 (q, C-8);  $\lambda_{max}$  211 and 268 nm ( $\log \epsilon$  4.03 and 4.23); *m/e* 224.069 8 (*M*,  $C_{11}H_{12}O_5$  requires 224.068 4), 196, 178, 164, 146, 118, 105, 91, 79, and 77.

Compound (1) could also be recrystallized from aqueous methanol, aqueous acetic acid, and aqueous sulphuric acid. It was recovered unchanged from concentrated sulphuric acid upon addition of water, from dry hydrogen chloride in glacial acetic acid, from aqueous perchloric acid after 4 h at 100 °C, from refluxing pyridine in methanol, and from furan. The compound was unchanged after 4 days with iodine and sodium hydrogen carbonate in ethyl acetate, after overnight treatment with hydrogen peroxide and sodium carbonate, and after treatment with maleic anhydride in toluene at 100 °C for 24 h.

*The Methyl Ester (2).*—The acid (1) in concentrated sulphuric acid was poured onto methanol. Addition of water

and extraction with ethyl acetate gave, after a washing with hydrogen carbonate solution and flash distillation, the ester (2) as a colourless mobile oil (Found: C, 60.5; H, 6.0.  $C_{12}H_{14}O_5$  requires C, 60.5; H, 5.9%),  $\nu_{\max}$  (neat) 1 830, 1 760, 1 730, 1 645, 972, and 912  $cm^{-1}$ ;  $\delta_H$  7.30 (d, t, *J* 16, 7.5, 7.5, H-6), 6.35 (d t, *J* 16, 1.5, 1.5, H-5), 3.68 (s, OMe), 2.78 ( $A_2B_2m$ , H-1 and H-2), 2.36 (pent., *J* 7, H-7), and 1.11 (t, *J* 7, H-8); *m/e* 238 (*M*), 206, 166, 135, 124, 109, 105, 91, 79, and 77. The compound gave one peak on g.l.c.

Treatment of the acid (1) with ethyl chloroformate followed by methanol using the mixed anhydride method also gave the ester (2). Reaction of diazomethane in ether with the acid (1) gave the ester (2) contaminated by a higher molecular weight impurity, *m/e* 252 ( $C_{13}H_{16}O_5$ ).

**The Imide (3).**—The acid (1) was recovered unchanged from ammonia (0.880) at 20 °C after 1 h. After 3 h at 100 °C the solution was taken to dryness, the residue taken into aqueous methanol and treated with IR 120(H) resin. Filtration followed by removal of the methanol gave the imide (3), m.p. 119–120 °C (from water) (Found: C, 59.0; H, 5.9; N, 6.3.  $C_{11}H_{13}NO_4$  requires C, 59.2; H, 5.9; N, 6.3%),  $\nu_{\max}$  (Nujol): 3 250, 1 775, 1 725, 1 710, and 1 650  $cm^{-1}$ ;  $\delta_H$  (Me<sub>2</sub>SO) 6.94 (d t, *J* 16, 6.5, 6.5, H-6), 6.34 (d, t, *J* 16, 1, 1, H-5), 2.52 ( $A_2B_2m$ , H-1 and H-2), 2.22br (pent., *J* ca. 6.5, H-7), and 1.02 (t, *J* 7, H-8);  $\lambda_{\max}$  (MeOH) 230 and 335 nm (log  $\epsilon$  4.09 and 3.54); *m/e*: 223(*M*), 205, 177, and 164.

**The Phenylimide (4).**—The acid (1) in toluene with an excess of aniline at 20 °C for 2 h gave, on addition of hexane, the phenylammonium salt of an anilide, m.p. 90–91 °C (from ether) (Found: C, 67.2; H, 6.3; N, 6.5.  $C_{23}H_{26}N_2O_5$  requires C, 67.3; H, 6.4; N, 6.8%),  $\nu$  (Nujol): 1 770, 1 700, 1 640, 1 625, 1 595, 1 540, and 1 500  $cm^{-1}$ ;  $\delta_H$  7.7–6.2 (m, phenyl and vinyl), 2.9–2.5 ( $A_2B_2m$ , H-1 and H-2), 2.30br (pent., *J* 7, H-7), and 1.10 (t, *J* 7, H-8);  $\lambda_{\max}$  (MeOH): 232 and 268 nm (log  $\epsilon$  4.33 and 4.09).

This salt on crystallization from acetic acid–water gave the phenylimide (4), m.p. 90–91 °C (Found: C, 68.0; H, 5.7; N, 4.7.  $C_{17}H_{17}NO_4$  requires C, 68.2; H, 5.7; N, 4.7%),  $\nu_{\max}$  (Nujol): 3 120, 1 770, 1 720, 1 640, 1 595, and 1 500  $cm^{-1}$ ;  $\delta_H$  9.8br (COOH), 7.36 (m, 5 H), 7.22 (dt, *J* 16, 7, 7, H-6), 6.36 (dt, *J* 16, 2, 2, H-5), 2.80 ( $A_2B_2m$ , H-1 and H-2), 2.30br (pent., *J* 7, H-7) and 1.10 (t, *J* 7, H-8);  $\lambda_{\max}$  (MeOH) 228, 280, and 350 nm (log  $\epsilon$  4.34, 3.83, and 3.40); *m/e* 299 (*M*), 281, 253, and 240 (*M* – CH<sub>2</sub> – COOH).

**The Dihydro-derivative (5).**—The acid (1) in glacial acetic acid was hydrogenated at atmospheric pressure at 20 °C over Adam's catalyst, to give (3*Z*)-oct-3-ene-1,3,4-tricarboxylic acid 3,4-anhydride (5), m.p. 64–65 °C (from aqueous acetic acid) (Found: C, 58.5; H, 6.2.  $C_{11}H_{14}O_5$  requires C, 58.4; H, 6.2%),  $\delta_H$  10.7 (s, COOH), 2.80 (4 H, sharp s, H-1 and H-2 coincidental), 2.53 (t, *J* 7, H-5), 1.5 (m, H-6 and H-7), and 0.98 (distorted t, *J* 7, H-8);  $\nu_{\max}$  (Nujol): 3 000–2 500, 1 830, 1 775, 1 710, 1 670, 908, 756, and 738  $cm^{-1}$ ; *m/e* 208 (*M* – H<sub>2</sub>O), 180, 166, and 138.

**The Ester (6).**—The acid (5) in concentrated sulphuric acid was poured onto methanol. Work-up as above for compound (2) gave a colourless mobile oil, b.p. 120 °C/0.1 mmHg (Found: C, 60.3; H, 6.8.  $C_{12}H_{16}O_5$  requires C, 60.0; H, 6.7%),  $\delta_H$  3.67 (s, OMe), 2.72 ( $A_2B_2m$ ), 2.50 (t, *J* 7.5, H-5), 1.5 (m, H-6 and H-7), and 0.95 (distorted t, *J* 7, H-8);  $\nu_{\max}$

(neat) (No OH), 1 840, 1 775, 1 745, 1 670, and 910  $cm^{-1}$ ; *m/e* 208 (*M* – MeOH), 198 (*M* – CO<sub>2</sub>), 180, and 166.

**The Dihydro-acid (7).**—The acid (1) in glacial acetic acid was reduced at 100 °C for 3 h with zinc dust which was added portionwise in excess during the reaction. The solution was filtered and taken to dryness. The residue was extracted into ethyl acetate and the product recrystallized from ethyl acetate–hexane to constant m.p. to give the acid (7) (ca. 70%) contaminated with another isomer, m.p. 145 °C (Found: C, 54.0; H, 6.6.  $C_{11}H_{16}O_6$  requires C, 54.1; H, 6.6%). Further recrystallization or chromatography did not effect a purification.

Flash distillation (180 °C, 0.02 mmHg) gave the anhydride (9) as a mobile oil (Found: C, 58.4; H, 6.4.  $C_{11}H_{14}O_5$  requires C, 58.4; H, 6.2%),  $\nu_{\max}$  (neat) 3 250–2 650, 1 850, 1 780, 1 710, 1 675infl, 1 220, 970, and 925  $cm^{-1}$ ;  $\delta_H$  10.0 (s, CO<sub>2</sub>OH), 5.87 (dt, *J* 16, 6, 6, H-6), 5.44br (dd, *J* 16, 8, 1, 1, H-5), 3.44br (t, *J* 8, H-4), 3.07 (d, t, *J* 7, 7, 8, H-3), 2.67 (t, *J* 7.5, H-1), 2.14 (m, H-2 and H-7), and 1.01 (t, *J* 7, H-8); *m/e* 226(*M*), 208, 180, 166, and 154.

The anhydride (9) crystallised within 48 h upon exposure to the atmosphere to give pure (3*RS*,4*SR*,5*E*)-oct-5-ene-1,3,4-tricarboxylic acid (7), m.p. 154–157 °C (from ethyl acetate–hexane) (Found: C, 54.0; H, 6.6.  $C_{11}H_{16}O_6$  requires C, 54.09; H, 6.60%),  $\nu_{\max}$  3 100–2 700, 1 705br, 1 660infl, 1 420, 1 410, 1 250, 975, and 925  $cm^{-1}$ ;  $\delta_H$  (Me<sub>2</sub>SO) 12.2 (three COOH), 5.66 (dt, *J* 16, 6, 6, H-6), 5.26br (q, *J* 16, 9.5, 1, H-5), 3.04 (t, *J* 9.5, H-4), 2.3–1.4 (m, H-1, H-2, H-3, H-7), and 0.92 (t, *J* 7, H-8); *m/e* 226 (*M* – H<sub>2</sub>O); 208.073 7 ( $C_{11}H_{12}O_4$  requires 208.073 5), 180 ( $C_{10}H_{12}O_3$ ), 162, and 154, with metastables at 191.4 (226→208) and 145.8 (180→162).

Methylation of the acid (7) in methanol with ethereal diazomethane gave the triester (8), b.p. 120 °C/0.01 mmHg, as a colourless mobile oil (Found: C, 58.7; H, 7.8.  $C_{14}H_{22}O_6$  requires C, 58.7; H, 7.8%),  $\nu_{\max}$  (neat) (No OH), 1 730, 1 260–1 160s, br and 970  $cm^{-1}$ ;  $\delta_H$  5.70 (dt, *J* 16, 6, 6, H-6), 5.30 (dd with each line further split into a fine t, *J* 16, 9.5, 1.5, 1.5, H-5), 3.63 (s, three OMe), 3.28 (t, *J* 9.5, H-4), 2.71 (dt, *J* 9.5, 9.5, 3.5, H-3), 2.5–1.4 (m, H-1, H-2, H-7), and 0.96 (t, *J* 7, H-8); *m/e*: 286(*M*), 255 (*M* – OMe), 254, 226, 222, and 194 with a metastable at 201.1 (254→226).

**The Triacid (10).**—The triacid (7) in methanol was hydrogenated over Adam's catalyst to give (3*RS*,4*SR*)-octane-1,3,4-tricarboxylic acid (10), m.p. 122 °C (from ethyl acetate–hexane). The compound was hygroscopic [Found (for a sample freshly dried at 50 °C/0.02 mm): C, 53.7; H, 7.4.  $C_{11}H_{18}O_6$  requires C, 53.65; H, 7.4%),  $\nu_{\max}$  (Nujol) 3 000–2 600, 1 695, 1 408, and 935  $cm^{-1}$ ;  $\delta_H$  (Me<sub>2</sub>SO): 12 (three COOH), 2.2 (2 H, m), 1.75 (2 H, m), 1.6–1.25 (8 H, m), 0.86 (3 H, distorted t); *m/e*: 210 (*M* – 2H<sub>2</sub>O), 182, 165, 154, and 138.

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