

**409.** *The Chemistry of the Triterpenes and Related Compounds.*  
*Part XXXVI.\* Some Constituents of Trametes odorata (Wulf.) Fr.*

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3 $\beta$ -Hydroxylanosta-8 : 24-dien-21-oic acid has been isolated as its methyl ester from the sporophores of the fungus *Trametes odorata*. Three other acids have also been isolated as their methyl esters.

IN continuation of our investigations of the wood-rotting fungi we have made a preliminary examination of the constituents of *Trametes odorata* (Wulf.) Fr. found on fir and pine trees in Norway and Austria. The constituents were first examined by Gruber and Proske<sup>1</sup> in 1950. From a cold ether extract of the dry pulverised sporophores of fungus they isolated "trametenolic acid," m. p. 259—261°,  $[\alpha]_D +47^\circ$  in methanol. They suggested a formula of C<sub>30</sub>H<sub>48</sub>O<sub>3</sub> or C<sub>31</sub>H<sub>50</sub>O<sub>3</sub>. The acid was characterised as a secondary hydroxy-acid with two double bonds and was converted<sup>2</sup> by standard procedures into the parent hydrocarbon "trametene." The chief products of dehydrogenation were reported<sup>3</sup> to be 1 : 2 : 5 : 6-tetramethylnaphthalene and 1 : 2 : 7 : 8-tetramethylphenanthrene. These results suggest that trametenolic acid is a tetracyclic triterpene although the dehydrogenation products are unusual.

For our investigation a small amount (500 g.) of *Trametes odorata* was available. It was extracted first with ether and then with alcohol, approximately by the procedure of Gruber and Proske<sup>1</sup> in the hope of isolating trametenolic acid. The extracts were separated into acidic and neutral fractions. The latter were very small and not investigated further. The two acidic fractions were methylated with diazomethane, and the resulting esters separated by alumina chromatography.

The main component of the esters resulting from the ether extract was methyl 3 $\beta$ -hydroxylanosta-8 : 24-dien-21-oate (I) previously obtained by reduction of the corresponding keto-compound, methyl pinicolate<sup>4</sup> (II), with sodium borohydride. From a comparison of the constants of the hydroxy-ester (I) and its derivatives it does not appear to be methyl trametenolate (cf. Table, p. 2040). For comparison with the trametenolic acid

\* Part XXXV, preceding paper.

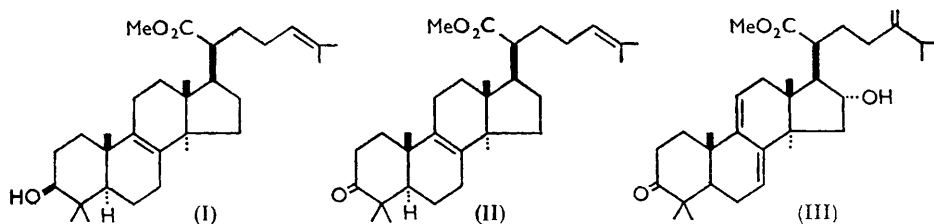
<sup>1</sup> Gruber and Proske, *Monatsh.*, 1950, **81**, 837.

<sup>2</sup> *Idem, ibid.*, p. 1024.

<sup>3</sup> *Idem, ibid.*, 1951, **82**, 255.

<sup>4</sup> Guider, Halsall, and Jones, *J.*, 1954, 4471.

of Gruber and Proske the acid from ester (I) had to be prepared. However, the methyl ester, like methyl pinicolate A,<sup>4</sup> was not hydrolysed by boiling methanolic potassium hydroxide in 24 hours. To bring about hydrolysis of the esters (I) and (II) it was necessary to use boiling diethylene glycol as solvent. In contrast, methyl trametenolate is hydrolysed<sup>1</sup> under the milder conditions, as is also methyl polyporenate C (III).<sup>5</sup> The greater ease with which the ester group of methyl polyporenate C is hydrolysed may be due to the presence of the 16 $\alpha$ -hydroxyl group. Adjacent hydroxyl groups can, if suitably placed, facilitate ester hydrolysis.<sup>6</sup>



In addition to methyl 3 $\beta$ -hydroxy-24-dien-21-oate three other esters (ester A; m. p. 159–65°,  $[\alpha]_D +49^\circ$ ; ester B, m. p. 152–153°,  $[\alpha]_D +66^\circ$ ; ester C, m. p. 197–199°) have so far been isolated, but owing to the small amounts available they have not yet been investigated in detail. Most is known about ester B which has the formula C<sub>32</sub>H<sub>48</sub>O<sub>6</sub> or C<sub>33</sub>H<sub>50</sub>O<sub>6</sub> and appears to be the dimethyl ester of a C<sub>30</sub> or C<sub>31</sub> hydroxyketo-dicarboxylic acid. Its ultraviolet spectrum has a broad band at 2130 Å ( $\epsilon$  16,600), which suggests the presence of an  $\alpha\beta$ -unsaturated ester group (the true maximum due to such a group will be at a longer wavelength after allowance for end-absorption of isolated double bonds), and a low-intensity band at 2750–2800 Å suggestive of an isolated carbonyl group although the intensity ( $\epsilon$  120) is rather higher than that usually associated with ketones. The infrared spectrum (in Nujol) had a band due to a hydroxyl group and a very broad band in the carbonyl region with two peaks (1709 and 1722 cm.<sup>-1</sup>; the former slightly more intense). There was also a band of moderate intensity at 1650 cm.<sup>-1</sup>. These bands are consistent with a keto-group in a six-membered ring, an isolated ester group, and an  $\alpha\beta$ -unsaturated ester group. The keto-group is unreactive since ester B does not react with Girard's reagent or with sodium borohydride. Oxidation of ester B gave a compound, whose analysis indicated that it had two hydrogen atoms less than the starting material and hence that one hydroxyl group had been oxidised. The oxidation product had no band due to a hydroxyl group but bands at 1735 and 1710 (very broad) cm.<sup>-1</sup> (in carbon disulphide).

Alcoholic extracts of the fungus were intensely coloured by a pigment which was deposited from concentrated solutions as an amorphous reddish-brown powder and for which the name "trametin" is provisionally proposed. It does not contain nitrogen and hence differs from cinnabarin (polystictin) from *Trametes cinnabarina* and *Coriolus sanguineus*.<sup>7</sup>

#### EXPERIMENTAL

The general experimental conditions described in Part XXXV apply also to this paper.

*Extraction of T. odorata (Wulf.) Fr.*—The chopped air-dried fungus (500 g.) was suspended in boiling ether for 24 hr. The ether was decanted and the fungus re-extracted thrice more in the same manner. From each extract a reddish-yellow solid (5.24; 2.12, 2.87, and 1.17 g.) was obtained on evaporation.

The residual fungus was extracted with cold alcohol for 1 month. The red-black extract

<sup>5</sup> Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2548.

<sup>6</sup> Cf. Henbest and Lovell, *J.*, 1957, 1965.

<sup>7</sup> Cf. Cavill and Tetaz, *Chem. and Ind.*, 1956, 986.

was decanted, filtered, and evaporated under reduced pressure to 500 c.c. The extract was then kept at 20° for a few days. During this period *ca.* 2 g. of an amorphous orange-brown pigment were deposited. It was filtered off and washed with light petroleum. It was then suspended successively in boiling light petroleum for 15 min. and in boiling ether for 15 min., filtered off, and dried. The substance provisionally called "trametin" darkened but did not melt when heated to 300° (Found: C, 54.75; H, 5.4%; N, 0). It was insoluble in most solvents except alcohol and acetone. The red colour of dilute solutions was intensified by addition of hydrochloric acid, but changed to a dark green in cold alcoholic alkali. It had  $\lambda_{\max}$  (in 95% ethanol) 2300, 2760, and 4310 Å, shoulders at 4600 and 4950 Å. The minimum  $E_{1\%}^{1\text{cm}}$  values at 2670 and 4310 Å are 204 and 186.  $\nu_{\max}$  (in Nujol) were at 3310 (broad) (OH), 1630 and 1580  $\text{cm}^{-1}$ .

*Examination of the Ether Extract.*—The residue from the first extract was dissolved in 1:1 methanol-ether (1 l.) and extracted with 10% potassium hydroxide solution. The neutral fraction (420 mg.) was obtained from the ethereal layer, and the acidic fraction was isolated from the aqueous phase by acidification with acetic acid and ether-extraction.

The acidic fraction was methylated with an excess of ethereal diazomethane, and the resulting mixed methyl esters (4.8 g.) were adsorbed from benzene (60 c.c.) on alumina (activity I—II) (300 g.) and eluted with the following solvents: benzene (0.5 l.), fraction 1 (0.51 g.), yellow viscous oil; benzene-ether (9:1) (4 l.), fraction 2 (1.05 g.), solid; benzene-ether (3:1) (3 l.), fraction 3 (0.33 g.), gum; 1:1 benzene-ether (1 l.), fraction 4 (0.68 g.) gum; ether (2 l.), fraction 5 (0.14 g.), yellow gum.

Fractions 1 and 5 did not crystallise and were not investigated further.

Fraction 2 was acetylated in pyridine (20 c.c.) with acetic anhydride (10 c.c.) at 20° for 24 hr. Isolation with ether yielded a product which crystallised from methanol as stout needles (920 mg.), m. p. 133—137°. These were adsorbed from light petroleum (30 c.c.) on alumina (85 g.). Elution with light petroleum-benzene (3:1; 400 c.c.) gave methyl 3 $\beta$ -acetoxyylanosta-8:24-dien-21-oate as needles (from methanol), m. p. 147—149°,  $[\alpha]_{\text{D}} + 63^\circ$  (*c.* 0.98) (Found: C, 77.2; H, 10.3. Calc. for  $\text{C}_{33}\text{H}_{52}\text{O}_4$ : C, 77.3; H, 10.2%). The compound showed no significant ultraviolet absorption. The methyl ester acetate (820 mg.) in methanol (100 c.c.) was hydrolysed overnight with 10% methanolic potassium hydroxide (100 c.c.) at 20°. Isolation with ether afforded a gum which gelatinised in contact with methanol. It was adsorbed from benzene (30 c.c.) on alumina (80 g.) and eluted with benzene (300 c.c.) to give methyl 3 $\beta$ -hydroxyylanosta-8:24-dien-21-oate as needles (from propan-2-ol), m. p. 132—135°,  $[\alpha]_{\text{D}} + 52^\circ$  (*c.* 0.99) (Found: C, 78.85; H, 10.8. Calc. for  $\text{C}_{31}\text{H}_{50}\text{O}_3$ : C, 79.1; H, 10.7%). Oxidation of the hydroxy-ester (470 m.g.) in acetone (100 c.c.) in the usual manner<sup>8</sup> gave methyl picolate, m. p. 119—120°.

*Hydrolysis of Methyl 3 $\beta$ -Hydroxyylanosta-8:24-dien-21-oate.*—(a) The hydroxy-ester (500 mg.) was heated under reflux with 10% methanolic potassium hydroxide (100 c.c.) for 24 hr. Dilution with water and extraction with ether yielded unchanged ester (465 mg.).

(b) The hydroxy-ester (500 m.g.) in diethylene glycol (25 c.c.) and potassium hydroxide (0.4 g.) were heated under reflux for 2 hr. After acidification with acetic acid and dilution with water ether-extraction afforded a brown gum which was adsorbed from benzene (20 c.c.) on alumina (50 g.; 10% deactivated). Elution with ether (500 c.c.) gave 3 $\beta$ -hydroxyylanosta-8:24-dien-21-oic acid as needles (from acetone and propan-2-ol), m. p. 253—258°,  $[\alpha]_{\text{D}} + 43^\circ$  (*c.* 0.94 in pyridine) (Found: C, 79.0; H, 10.45.  $\text{C}_{30}\text{H}_{48}\text{O}_3$  requires C, 78.9; H, 10.6%). Hydrolysis of methyl picolate A under the same conditions afforded picinic acid A.

The hydroxy-acid (200 mg.) in pyridine (5 c.c.) was treated with acetic anhydride (2 c.c.) for 24 hr. at 20°. Isolation with ether gave 3 $\beta$ -acetoxyylanosta-8:24-dien-21-oic acid as needles (from dilute acetone), m. p. 240—244° (decomp., sublimes 225°),  $[\alpha]_{\text{D}} + 48^\circ$  (*c.* 1.3 in pyridine) (Found: C, 76.6; H, 10.2.  $\text{C}_{32}\text{H}_{50}\text{O}_4$  requires C, 77.05; H, 10.1%).

*Fractions 3 and 4 from the Ether Extract.*—These fractions (1.0 g.) were combined and adsorbed from benzene (25 c.c.) on undecivated alumina (80 g.). Elution with benzene-ether (1:1; 900 c.c.) gave ester A (probably a mixture of hydroxy-esters) as a gum (650 mg.) which crystallised from dilute ethanol and methanol as needles, m. p. 159—165°,  $[\alpha]_{\text{D}} + 49^\circ$  (*c.* 0.73),  $\lambda_{\max}$  2350, 2430, and 2570 Å [intensity indicating the presence of *ca.* 9% of 7:9(11)-diene],  $\nu_{\max}$  (in Nujol) 3440, 1720, 1675, 885, 845, and 820  $\text{cm}^{-1}$ .

*Examination of the Alcohol Extract of the Fungus.*—The concentrated alcohol extract was diluted with ether (500 c.c.) and extracted with potassium hydroxide solution. The colour of the extract immediately changed from red-black to green-black. The alkaline layer was

separated and extracted further with ether (500 c.c.). From the combined ethereal extracts a neutral fraction (0.34 g.) was obtained. Acidification of the alkaline phase with acetic acid produced a gelatinous suspension from which the acidic fraction (6.3 g.) was extracted (with difficulty) by three extractions with ether (1½ l.).

The acidic fraction was suspended in acetone (150 c.c.) and methylated with an excess of ethereal diazomethane. The resulting mixed methyl esters (4 g.) were adsorbed from benzene (200 c.c.) on alumina (250 g.; 10% deactivated). Elution with benzene (2 l.) gave a yellowish solid (2.3 g.) (fraction 1). Elution with benzene-ether (1 : 1; 2 l.) gave a yellow gum (1.3 g.) (fraction 2).

*Examination of Fraction 1 from the Alcohol Extract.*—This fraction was adsorbed from benzene (150 c.c.) on alumina (150 g.) and eluted as follows: benzene (600 c.c.), oil (200 mg.) (fraction 1); benzene-ether (9 : 1) (400 c.c.), yellow oil (540 mg.) (fraction 2); benzene-ether (9 : 1) (3.5 l.), solid (1.15 g.), m. p. 143—151° (fraction 3); benzene-ether (3 : 1) (100 c.c.), solid (30 mg.), m. p. 130—138° (fraction 4); benzene-ether (3 : 1) (100 c.c.), solid (20 mg.), 137—148° (fraction 5); benzene-ether (3 : 1) (100 c.c.), solid (20 mg.), m. p. 133—160° (fraction 6); benzene-ether (1 : 1) (100 c.c.), solid (30 mg.), m. p. 176—178° (fraction 7); and benzene-ether (1 : 1) (100 c.c.), solid (40 mg.), m. p. 182—187° (fraction 8).

Fractions 1 and 2 (740 mg.) were combined and adsorbed from light petroleum (50 c.c.) on alumina (60 g.). Elution with light petroleum-benzene (3 : 2; 200 c.c.) and benzene (200 c.c.) afforded small quantities of impure crystalline material, m. p. 135—155° and 129—139° respectively.

Fraction 3 (1.15 g.) was recrystallised several times from methanol to yield *ester B* as needles, m. p. 149—152°,  $[\alpha]_D +66^\circ$  (*c* 1.13) (Found: C, 72.45; H, 9.55.  $C_{32}H_{48}O_6$  requires C, 72.7; H, 9.15.  $C_{33}H_{50}O_6$  requires C, 73.05; H, 9.3%).  $\lambda_{max}$  (broad) at 2130 and 2750—2800 Å ( $\epsilon$  16,600 and 120),  $\nu_{max}$  (in Nujol) 3450, 1720, and 1708 (broad band with two maxima of approx. equal intensity), 1650, 860, and 830  $cm^{-1}$ .

Fraction 8 (40 mg.) crystallised from methanol to give *ester C* as needles, m. p. 197—199°. This substance showed no significant ultraviolet absorption.  $\nu_{max}$  (in Nujol) were at 3300, 1720, 1708, 1650, 900, 860, and 830  $cm^{-1}$ .

*Treatment of Ester B with Girard's Reagent T.*—The ester (770 mg.) in ethanol (50 c.c.) containing acetic acid (0.96 c.c.) was heated under reflux with Girard's reagent T (720 mg.) for 1 hr. The cooled mixture was poured into a solution of sodium carbonate (770 mg.) in ice-water. Extraction with ether yielded a solid which was adsorbed from benzene (50 c.c.) on alumina (80 g.). Elution with benzene-ether (3 : 1; 800 c.c.) gave *ester B* as needles, m. p. 147—151°. The aqueous solution was acidified with concentrated hydrochloric acid and kept at 20° for 1 hr. Ethereal extraction did not afford a ketonic fraction.

*Attempted Reduction of Ester B with Sodium Borohydride.*—*Ester B* (450 m.g.) in pure dioxan (30 c.c.) was treated with sodium borohydride (85 m.g.) in aqueous dioxan (1 : 1; 30 c.c.) at 20° for 1 hr. Dilution with water and isolation with ether gave a product which was adsorbed from benzene (30 c.c.) on alumina (45 g.). Elution with benzene-ether (9 : 1; 900 c.c.) yielded starting material, m. p. 147—148° (from methanol or acetone).

*Oxidation of Ester B.*—*Ester B* (130 mg.) in acetone (25 c.c.) was oxidised with chromic acid in the usual manner.<sup>8</sup> Isolation with ether gave a gum which was adsorbed from light petroleum (20 c.c.) on alumina (10 g.). Elution with light petroleum-benzene (1 : 1; 500 c.c.) yielded a gum (90 mg.) which crystallised from dilute methanol or dilute alcohol as needles, m. p. 158—159°,  $[\alpha]_D +88.5^\circ$  (*c* 1.09) (Found: C, 73.4; H, 8.85.  $C_{33}H_{48}O_6$  requires C, 73.3; H, 8.95. Calc. for  $C_{33}H_{48}O_6$ : C, 72.95; H, 8.8%). This compound had  $\lambda_{max}$  (broad) at 2130 Å ( $\epsilon$  18,000) and  $\nu_{max}$ . 1735 and 1708 (very broad and intense band with two maxima), 1650, 860, and 820  $cm^{-1}$ .

*Examination of Fraction 2 from the Alcohol Extract.*—This fraction (1.3 g.) was adsorbed from benzene (120 c.c.) on alumina (100 g.) and eluted as follows: benzene-ether (9 : 1; 300 c.c.), solid (170 mg.), m. p. 143—146° (fraction 1); benzene-ether (1 : 1; 700 c.c.), sticky solid (550 mg.) (fraction 2); ether (400 c.c.), yellow gum (380 mg.) (fraction 3).

Fraction 1 crystallised from methanol, to give *ester B* as needles, m. p. 146—149°.

Fraction 2 was adsorbed from benzene (20 c.c.) on alumina (35 g.). Elution with benzene-ether (1 : 1; 300 c.c.) yielded a small amount of crystalline material, m. p. 190—193° (mainly *ester C*).

<sup>8</sup> Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2555.

Fraction 3 did not crystallise.

Comparison of constants.

Name of compound	M. p.	[ $\alpha$ ] <sub>D</sub>	Constants of the corresponding trametenolic acid derivative	
			M. p.	[ $\alpha$ ] <sub>D</sub>
3 $\beta$ -Hydroxylanosta-8 : 24-dien-21-oic acid...	253—258°	+43° *	259—261°	+47° †
3 $\beta$ -Acetoxylanosta-8 : 24-dien-21-oic acid ...	240—244	+48 *	209—211	—
Me 3 $\beta$ -hydroxylanosta-8 : 24-dien-21-oate ...	132—135	+52	127—128	+59
Me 3 $\beta$ -acetoxylanosta-8 : 24-dien-21-oate ...	147—149	+63	104—106	—
Me 3-oxolanosta-8 : 24-dien-21-oate <sup>a</sup> .....	121—123	+69	105—107	—
3-Oxolanosta-8 : 24-dien-21-oic acid <sup>a</sup> .....	197—202	+68	204—206	—
Lanosta-8 : 24-diene-3 $\beta$ : 21-diol <sup>b</sup> .....	189—192	+57 *	199—200	—
Lanosta-8 : 24-diene <sup>c</sup> .....	79.5—80	+65	85—87	+66

\* In pyridine. † In methanol.

<sup>a</sup> Ref. 4. <sup>b</sup> Halsall and Sayer, preceding paper. <sup>c</sup> McGhie, Pradhan, and Cavalla, *J.*, 1952, 3176.

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