683. *The Chemistry of the Triterpenes and Related Compounds. Part XXXI.* The Isolation of Further Triterpene Acids from* Polyporus pinicola *Fr.*

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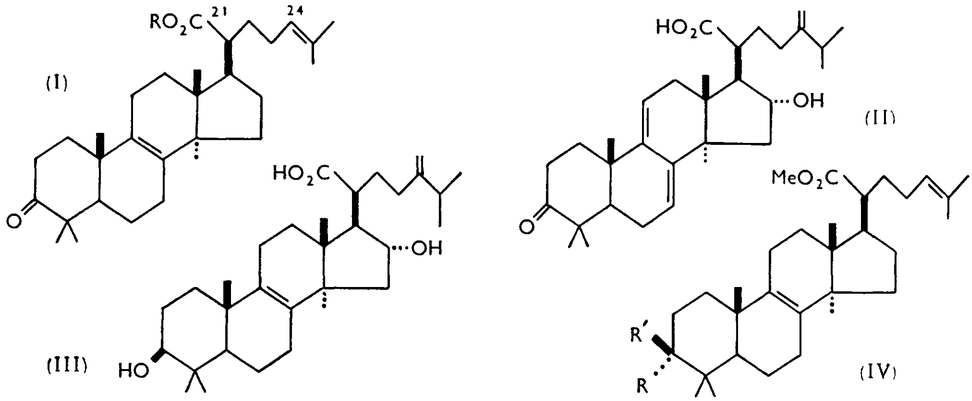
The examination of the triterpene constituents of *Polyporus pinicola* Fr. has been continued and two new acids have been isolated. The methyl ester monoacetate of one has been identified as methyl 3α-acetoxylanosta-8 : 24-dien-21-oate (IV; R = OAc, R′ = H). The other, which occurs along with its dehydro-derivative, is a dioxo-acid.

In Part XXVII[1] the isolation of methyl pinicolate A (methyl-3-oxolanosta-8 : 24-dien-21-oate) (I; R = Me) from triterpene constituents of *Polyporus pinicola* Fr. was described. Further investigation of the mixture of triterpenes present in the fungus has shown that it is very complex. Recent results of other workers support this view.

Shibamoto, Minami, and Tajima[2] have isolated from *P. pinicola* two acids, both described as $C_{30}H_{46}O_4$. One is a hydroxy-oxo-acid and the other a dihydroxy-acid. It is possible that the first is polyporenic acid C (II)[3-5] and the second tumulosic acid (III)[3, 6, 7] although the agreement between the constants of the Japanese acids and those of polyporenic acid C and tumulosic acid is not good (see Experimental section). Polyporenic acid C is certainly present in *Polyporus pinicola* Fr.[8]



(I)

(II)

(III)

(IV)

Schmid and Czerny[9, 10] have isolated an oxo-alcohol and three acids from *Polyporus pinicola*. The contents of the oxo-alcohol[9] (m. p. 116°; $[\alpha]_D$ +71°; $\lambda_{max.}$ 2360, 2450, and 2520 Å, ε 5400, 6000, and 4200) are in good agreement with those (m. p. 114—117°; $[\alpha]_D$ +68·5°; $\lambda_{max.}$ 2360 and 2430 Å, ε 4000 and 4500; inflexion : 2510 Å, ε 3000) of a mixture, as yet unresolved, of 21-hydroxylanosta-8 : 24-dien-3-one (75%) and the corresponding dehydro-derivative (25%) which has been isolated from the neutral portion of an extract of *Polyporus pinicola* Fr. by Guider, Halsall, and Jones.[11] The formulæ proposed by Schmid and Czerny[9, 10] for their three acids, I, II, and III are $C_{30}H_{48}O_3$, $C_{30}H_{48}O_3$, and

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* Part XXX, *J.*, 1957, 753.

[1] Guider, Halsall, and Jones, *J.*, 1954, 4471.

[2] Shibamoto, Minami, and Tajima, *J. Japan. Forest Soc.*, 1953, **35**, 56.

[3] Cross, Eliot, Heilbron, and Jones, *J.*, 1940, 632.

[4] Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2548.

[5] Bowers, Halsall, and Sayers, *J.*, 1954, 3070.

[6] Cort, Gascoigne, Holker, Ralph, Robertson, and Simes, *J.*, 1954, 3713.

[7] Guider, Halsall, Hodges, and Jones, *J.*, 1954, 3234.

[8] Fazakerley, Halsall, Jones, and Whitham, unpublished work.

[9] Schmid and Czerny, *Scientia Pharm.*, 1953, **21**, 258.

[10] *Idem, Monatsh.*, 1954, **85**, 1307.

[11] Guider, Halsall, and Jones, unpublished work.

$C_{30}H_{48}O_4$. The constants are: I, m. p. 215—216°, $[\alpha]_D$ +50°; II, m. p. 196—198°, $[\alpha]_D$ +62°; and III, m. p. 265—267°. The constants of acid II suggested that it is pinicolic acid A (I; R = H) (m. p. 197—202°, $[\alpha]_D$ +68°); a specimen, kindly provided by Professor L. Schmid did not depress the m. p. of pinicolic acid A.

To simplify the separation of the triterpene constituents the fungus was first extracted with light petroleum. The extract was hydrolysed and the acidic and neutral fractions were separated. This paper is concerned with some of the constituents of the acidic fraction. The methyl esters from this fraction were separated by use of Girard's reagent T into two fractions called "light petroleum ketonic esters" and "light petroleum non-ketonic esters." This treatment does not necessarily separate all ketonic from non-ketonic esters as there may be present esters with oxo-groups (e.g., at $C_{(16)}$ in trimethyl-steroids) which do not react with Girard reagent T.

From the "light petroleum ketonic esters" methyl pinicolate A (I; R = Me) was isolated by chromatography on alumina along with a mixture of the methyl esters of a dioxo-acid (80%) and its dehydro-derivative (20%). The isolation of this type of mixture from wood-rotting fungi is quite common.[cf. 7, 12] The mixed esters had infrared bands at 1700 cm.$^{-1}$ indicative of a keto-group in a six-membered ring, and at 1723 cm.$^{-1}$ (very strong), indicative of both an ester group and a keto-group in a five-membered ring. A trimethyl-steroid skeleton being assumed for the dioxo-acid, the six-membered ring keto-group can be placed at $C_{(3)}$ since oxygen has, so far, been found in all trimethyl-steroids at $C_{(3)}$. The most likely site for the other carbonyl group is $C_{(16)}$. The molecular-rotation contribution [13, 4] of a $C_{(16)}$ carbonyl group is approximately −500°, whilst the molecular rotation of the methyl ester mixture ($[M]_D$ −217°, a molecular weight of 482 [$C_{31}H_{46}O_4$] being assumed) is 541° less than that of methyl pinicolate A. The nature of the side-chain of the dioxo-acid (cf. V) has not yet been elucidated but the infrared spectrum of the methyl ester mixture indicates that the vinylidene group is absent.

Chromatography of the "light petroleum non-ketonic esters" on alumina gave a number of fractions, including two main fractions containing hydroxy-esters. The first of these has been examined in detail. It was acetylated and the product chromatographed on alumina. The first compound obtained was a methyl ester monoacetate, $C_{33}H_{52}O_4$, containing no significant amount of diene impurity; its infrared spectrum indicated absence of a vinylidene group. Its constants were not identical with those of the known methyl 3β-acetoxylanosta-8 : 24-dien-21-oate (IV; R = H, R′ = OAc) ($[\alpha]_D$ +67°).[1] Instead, its low rotation ($[\alpha]_D$ +7°) suggested the presence of a 3α-acetoxy-group which is known to have a negative molecular-rotation contribution.[14] Proof that it was methyl 3α-acetoxylanosta-8 : 24-dien-21-oate (IV; R = OAc, R′ = H) was obtained by hydrolysing the acetoxy-group with methanolic potassium hydroxide at room temperature and then oxidising the hydrolysis product with chromic acid, methyl pinicolate A (methyl 3-oxolanosta-8 : 24-dien-21-oate) (I; R = Me) being obtained.

A second fraction (m. p. 142—144·5°; $[\alpha]_D$ +50·5°; showing no diene absorption) was shown by careful chromatography to be a mixture of two closely related methyl ester monoacetates which it was not possible to separate completely.

The preparation of methyl 3β-acetoxylanosta-7 : 9(11)-dien-21-oate, needed as a reference compound, is described.

EXPERIMENTAL

M. p.s were determined on a Kofler block and are corrected. Rotations were determined in chloroform at room temperature. The alumina used for chromatography, unless otherwise stated, was Peter Spence Grade "H" which had been deactivated with 5% of 10% acetic acid. Light petroleum refers to the fraction with b. p. 60—80°. Ultraviolet spectra were determined in ethanol.

[12] Holker, Powell, Robertson, Simes, Wright, and Gascoigne, *J.*, 1953, 2422.
[13] Klyne, *J.*, 1952, 2916.
[14] Klyne and Stokes, *J.*, 1954, 1979.

Extraction of Polyporus pinicola *Fr. with Light Petroleum.*—The air-dried fungus (1 kg.) was extracted five times with cold light petroleum (4 l. each) for 16 hr., and the extracts were combined and concentrated to a dark brown paste (81 g.). Further extraction gave negligible quantities of gum. The extract (80 g.) was heated under reflux with ethanol (90 c.c.), water (275 c.c.), and sodium hydroxide (50 g.) for 20 hr. The cooled solution was diluted with water and extracted with ether (10 × 200 c.c.) to yield the " light petroleum neutral fraction " (18·6 g.). The aqueous phase was acidified with hydrochloric acid and extracted with ether (5 × 250 c.c.) to yield the " light petroleum acidic fraction " (43 g.).

Methylation of the " Light Petroleum Acidic Fraction."—The acidic fraction (43 g.) in ether (400 c.c.) and acetone (10 c.c.) was treated with an excess of diazomethane in ether and kept overnight. The excess of diazomethane was then decomposed with acetic acid. The ethereal solution was decanted from a small amount of polymeric material, washed with 2N-sodium hydroxide solution and water, and dried. Evaporation gave a brown gum (42 g.). These esters (42 g.) in ethanol (400 c.c.), water (20 c.c.), and acetic acid (40 c.c.) were heated under reflux with Girard's reagent T (25 g.) for 1 hr. The cooled mixture was poured into ice-cold aqueous sodium carbonate (28·4 g.) and extracted with ether to yield the " light petroleum non-ketonic esters " (28 g.). The aqueous phase was acidified (Congo red) with concentrated hydrochloric acid and kept at 20° overnight. Extraction with ether then afforded the " light petroleum ketonic esters " (13 g.) as a pale yellow gum which deposited feathery needles.

Chromatography of the " Light Petroleum Ketonic Esters."—Chromatography of the " light petroleum ketonic esters " was carried out on Whatman No. 1 paper with a reversed phase system. The stationary phase was odourless kerosene [from a 20% (v/v) solution in ether], and the eluant was the lower phase of the system, propan-2-ol–water–odourless kerosene (7 : 3 : 1) by vol.).[15] The triterpenes were detected by treatment of the paper with antimony trichloride in acetic acid at 90° for 2 min. and then with the Noller reagent,[16] or by treatment with the Noller reagent alone. In this case two spots, R_f 0·39 and 0·73, were obtained. Methyl pinicolate A[1] gave a spot with R_f 0·39.

The esters (10·8 g.) were adsorbed from light petroleum–benzene (9 : 1; 200 c.c.) on alumina (1 kg.), deactivated with 10% of 10% aqueous acetic acid. Elution with light petroleum–benzene (1 : 1; 2500 c.c.) gave methyl pinicolate A[1] (methyl 3-oxolanost-8 : 24-dien-21-oate) (7·5 g.) which crystallised from methanol as needles, m. p. and mixed m. p. 119—121°, $[\alpha]_D$ +71° (Found: C, 79·4; H, 10·2. Calc. for $C_{31}H_{48}O_3$: C, 79·4; H, 10·3%). The ultraviolet absorption spectrum of the methyl pinicolate A indicated that it contained a small amount (3—4%) of methyl dehydropinicolate A [methyl 3-oxolanosta-7 : 9(11) : 24-trien-21-oate]. Light absorption: Max. 2430 and 2740 (broad) Å, ε 670 and 40. Inflexions, 2360 and 2520 Å, ε 690 and 450.

Further elution with light petroleum–benzene (2 : 3, 400 c.c.; 3 : 7, 800 c.c.) yielded a gum (1·8 g.) which from methanol gave the dimorphic *methyl ester* of a dioxo-acid, m. p. 128—130° (plates) and 133—136·5° (needles), $[\alpha]_D$ −45° (*c* 1·46) (Found: C, 77·4; H, 9·35. $C_{31}H_{46}O_4$ requires C, 77·15; H, 9·6. $C_{31}H_{44}O_4$ requires C, 77·45; H, 9·25. $C_{32}H_{48}O_4$ requires C, 77·35; H, 9·75. $C_{32}H_{46}O_4$ requires C, 77·7; H, 9·35%). The ultraviolet absorption spectrum indicated that the dioxo-ester contained about 20% of a 7 : 9(11)-dehydro-component. Light absorption: Max. 2350, 2430, and 2520 Å; ε 3400, 3600, and 2600 ($M = 482 \equiv C_{31}H_{46}O_4$ being assumed). Infrared absorption in Nujol; bands at 1723 (vs), 1700 (s), 1660 (w) (sh), and 810 (w) cm.$^{-1}$. Further elution with benzene and ether afforded a gum (1·5 g.) from which it was not possible to obtain any crystalline derivative.

Chromatography of the " Light Petroleum Non-ketonic Esters."—Paper chromatography of the esters (see above) gave a chromatogram having two spots with R_f values of 0·37 and 0·67. The former is probably due to traces of methyl pinicolate A not removed by separation with reagent.

The esters (9·4 g.) were adsorbed from light petroleum (100 c.c.) on alumina (1000 g.) deactivated with 10% of 10% aqueous acetic acid. Elution with the solvents indicated gave the following six fractions: (i) light petroleum–benzene (9 : 1, 1200 c.c.) (1·5 g.); (ii) light petroleum–benzene (8 : 2, 1200 c.c.) (0·3 g.); (iii) light petroleum–benzene (7 : 3, 750 c.c.; and 1 : 1, 750 c.c.) (1·1 g.); (iv) light petroleum–benzene (4 : 6, 600 c.c.) (0·5 g.); (v) light petroleum–benzene (3 : 7, 900 c.c.; and 1 : 9, 900 c.c.) (3·3 g.); (vi) benzene–ether and ether–methanol (1800 c.c.) (2·95 g.).

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[15] Mills and Werner, *J.*, 1955, 3132.

[16] Noller, Smith, Harris, and Walker, *J. Amer. Chem. Soc.*, 1942, **64**, 3047.

Further chromatography and infrared examination showed fraction (i) to be very probably a mixture of fatty acid esters. Fraction (iii) gave only gums on rechromatography.

The infrared spectra of fractions (iv)—(vi) had bands due to hydroxyl groups. Fraction (v) was adsorbed from light petroleum–benzene (4 : 1, 50 c.c.) on alumina (350 g.). Elution with light petroleum–benzene (1 : 1, 300 c.c.; and 4 : 6, 800 c.c.) gave a gum (0·5 g.) which was discarded since its infrared spectrum showed no hydroxyl band. Elution with light petroleum–benzene, benzene, and ether gave a series of gums (*ca.* 3 g.) which were combined and acetylated at 20° for 24 hr. to give a gum. This was adsorbed from light petroleum (60 c.c.) on alumina (350 g.). Elution with light petroleum (200 c.c.) and light petroleum–benzene (9 : 1, 400 c.c.) gave an oil (0·1 g.) which was discarded. Further elution with the solvents indicated gave the following fractions: (*a*) light petroleum–benzene (8 : 2, 200 c.c.) (70 mg., solid); (*b*) light petroleum–benzene (7 : 3, 700 c.c.) (1·2 g., gum); (*c*) light petroleum–benzene (6 : 4, 500 c.c.) (1 g., solid); (*d*) light petroleum–benzene (1 : 1, 400 c.c.; 4 : 6, 400 c.c.) (0·1 g.), and (*e*) benzene (200 c.c.) (90 mg., solid).

Fraction (*a*) gave plates (from methanol), m. p. 149—161°. Fraction (*b*) gave methyl 3α-acetoxylanosta-8 : 24-dien-21-oate as needles (50 mg.) (from aqueous acetone), m. p. and mixed m. p., 132—136°, $[\alpha]_D$ +9° (*c* 0·93). Light absorption: inflexions at 2430 and 2510 Å; ε 230 and 160. The mother liquors from these crystallisations yielded needles, m. p. 136—140·5° $[\alpha]_D$ +17° (*c* 0·18); no selective light absorption above 2200 Å.

Fraction (*c*) gave *methyl* 3α-*acetoxylanosta*-8 : 24-*dien*-21-*oate* as needles (from aqueous acetone), m. p. 135·5—137°, $[\alpha]_D$ +7° (*c* 1·5) (Found: C, 77·2; H, 10·15. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·2%). Light absorption: Inflexions 2430 and 2510 Å; ε 125 and 85.

Fraction (*d*) gave needles (30 mg.), m. p. 142—144·5°, $[\alpha]_D$ +50·5° (*c* 0·68) (Found: C, 77·7; H, 10·05. $C_{34}H_{54}O_4$ requires C, 77·5; H, 10·3. $C_{35}H_{56}O_4$ requires C, 77·7; H, 10·45%); no selective light absorption above 2200 Å. Further chromatography of corresponding material indicated that it was a mixture of two closely related compounds. The first fraction from the chromatogram had m. p. 139—148°, $[\alpha]_D$ +54° (*c* 1·14), whilst the last fraction had m. p. 144—146·5°, $[\alpha]_D$ +54° (*c* 1·13).

From fraction (*e*) needles (from methanol), m. p. 141—160°, were obtained. Light absorption: Max., 2360 and 2430 Å; ε 7000 and 8000. Inflexion ε *ca.* 5000.

Examination of fraction (vi) from the initial chromatogram of the "light petroleum non-ketonic esters" will be detailed in a future publication.

Conversion of Methyl 3α-*Acetoxylanosta*-8 : 24-*dien*-21-*oate into Methyl* 3-*Oxolanosta*-8 : 24-*dien*-21-*oate* (*Methyl Pinicolate A*).—The acetoxy-ester (150 mg.) was treated with methanolic potassium hydroxide (15 c.c.; 10%) at 60° and then kept for 24 hr. at 20°. After dilution with water extraction with ether afforded a gum which was dissolved in acetone (20 c.c.) and oxidised with chromic acid solution (8N) according to the method of Bowers *et al.*[4] The product (98 mg.) was adsorbed from light petroleum–benzene (9 : 1; 10 c.c.) on alumina (10 g.). Elution with light petroleum–benzene (1 : 1; 100 c.c.) gave a solid (76 mg.) which yielded methyl 3-oxolanosta-8 : 24-dien-21-oate (methyl pinicolate A) (from aqueous acetone), m. p. and mixed m. p. 118—120°, $[\alpha]_D$ +70° (*c* 0·62). The infrared spectrum was identical with that of an authentic sample.

Preparation of Methyl 3β-*Acetoxylanosta*-7 : 9(11)-*dien*-21-*oate.*—Methyl 3β-acetoxylanost-8-en-21-oate[1] (220 mg.) was oxidised with perbenzoic acid (equiv. to 1·9 mol.) in benzene (10 c.c.) at 20° for 7 days. The mixture was then poured into sodium carbonate solution (5%).

Comparison of the Constants of Derivatives of the Japanese Acids and of Polyporenic Acid C and Tumulosic Acid.

	Japanese acid A series[2]		Polyporenic acid C series[4]	
Derivative	m. p.	$[\alpha]_D$	m. p.	$[\alpha]_D$
Free acid	260—263°	+ 8·6° (Py)	273—276°	+ 8° (Py)
Methyl ester	181—182·5°	+20·8°	198—199°	+10°
Monoacetate	177—180°	—	206—210°	—

	Japanese acid B series[2]		Tumulosic acid series[6]	
Derivative	m. p.	$[\alpha]_D$	m. p.	$[\alpha]_D$
Free acid	305—308°	+43°	306°	+ 8·1° (Py)
Methyl ester	178—178·5°	+44°	164—164·5°	+27°
Acetate	160—166°		214° (228°)	

Extraction with ether afforded a gum which was treated in acetic acid (10 c.c.) with concentrated sulphuric acid (3 drops) at 100° for 5 min. and at 20° for 18 hr. The product (215 mg.), which contained an αβ-unsaturated ketone (band at 1665 cm.$^{-1}$), was adsorbed from light petroleum–benzene (2 : 1; 15 c.c.) on alumina (13 g.). Elution with light petroleum–benzene (3 : 2; 60 c.c.) afforded a solid (80 mg.) which gave *methyl* 3β-*acetoxylanosta*-7 : 9(11)-*dien*-21-*oate* as needles (from aqueous methanol), m. p. 146—148°, $[\alpha]_D$ +65° (*c* 0·8) (Found : C, 77·1; H, 10·2. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·2%). Light absorption : Max. 2360, 2430, 2520 Å; ε 14,950, 17,500, and 11,700.

The authors thank Professor E. R. H. Jones, F.R.S., for his interest and advice, and Professor N. A. Sörensen, of the Institutt for Organisk Kjemi, Norges Tekniske Høgskole, Trondheim, for his generous help in collecting and supplying *Polyporus pinicola* Fr. One of them (J. B.) also thanks the United States Public Health Service for a Fellowship and another (H. F.) the Department of Scientific and Industrial Research for a maintenance grant.

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[*Received, February* 15*th*, 1957.]