The Chemistry of the Triterpenes and Related Compounds. Part XXVII. Pinicolic Acid A.*

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A trimethyl-steroid acid, pinicolic acid A, has been isolated from *Polyporus pinicola* Fr. It possesses the lanostane carbon skeleton and has been shown to have the structure (XI).

In 1939, Cross, Eliot, Heilbron, and Jones (*J.*, 1940, 632; cf. also Cross and Jones, *J.*, 1940, 1491) commenced a systematic investigation of the constituents of the higher fungi by examining the birch tree fungus, *Polyporus betulinus*, Fr. (cf. Parts XIII, XIV, XV, XVIII, XIX, XXIV, XXV, and XXVI; *J.*, 1953, 457, 464, 468, 2548, 3019; 1954, 2385, 3070, 3234). Attention has now been turned to the pine-rotting fungus, *Polyporus pinicola* Fr. In 1928 and 1929 Froschl, Hartmann, and Zellner (*Monatsh.*, 1928, **50**, 193; 1929, **53—54**, 186) investigated the alcohol-soluble constituents of this fungus and isolated two acids, termed α- and β-pinicolic acid, m. p. 198—208·5°, $[\alpha]_D$ +35·7°, and m. p. 265—271°, $[\alpha]_D$ +23·4° respectively. From the analyses of these compounds Zellner and his co-workers suggested the common formula, $C_{19}H_{30}O_2$, but Cross *et al.* (*loc. cit.*) indicated that the analytical data were consistent with a formula $C_{30}H_{50}O_3$, which suggests the possibility of the acids being trimethyl-steroids.

For the current investigation of *Polyporus pinicola* Fr. we have been very generously helped by Professor N. A. Sörensen of the Institutt for Organisk Kjemi, Norges Tekniske Høgskole, Trondheim, who has obtained supplies of this fungus for us from Norway.

Extraction of the fungus with cold ethanol, followed by separation and methylation of the acidic fraction of the extract, gave a mixture of methyl esters corresponding to about 5% of the total weight of air-dried fungus. Chromatographic separation of the mixed esters yielded a fraction (20%) which gave the crystalline methyl ester of a new acid, pinicolic acid A. The present communication is concerned with the structure of this acid, the companion substances will be discussed later.

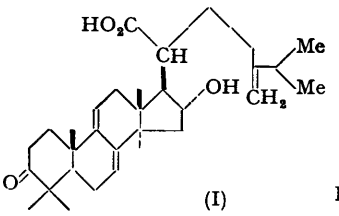
Methyl pinicolate A, m. p. 120—123°, was found by analysis to be either $C_{31}H_{48}O_3$ or $C_{32}H_{50}O_3$. Typical samples had low-intensity absorption at 2360, 2430, and 2520 Å (*e.g.*, ε 635, 610, and 410), suggesting the presence of a small amount of an impurity containing a conjugated diene system. Absorption at these wave-lengths arises from the heteroannular diene system found in polyporenic acid C (I) (Bowers, Halsall, Jones, and Lemin, *J.*, 1953, 2548; Bowers, Halsall, and Sayer, *J.*, 1954, 3070), dehydroburicoic acid (II) (Gascoigne, Robertson, and Simes, *J.*, 1953, 1830), and dehydrolanosterol (III) (cf. Dawson, Halsall, and Swayne, *J.*, 1953, 590). In view of the co-existence of eburicoic and dehydroburicoic acids in a number of fungi (Gascoigne, Holker, Ralph, and Robertson, *J.*, 1951, 2346) and of (IV) and its dehydro-derivative in *Polyporus betulinus* Fr. (Guider, Halsall, Hodges, and Jones, *J.*, 1954, 3234), it may reasonably be concluded that the contaminant is methyl dehydropinicolate A, and it could be inferred that pinicolic acid A has a ring system similar to that found in eburicoic acid and lanosterol (V). In addition to the maxima due to diene absorption a broad maximum was also observed at 2740 Å (ε 77), indicative of a carbonyl group, the presence of which in methyl pinicolate A was confirmed by the formation of a 2 : 4-dinitrophenylhydrazone. The infra-red spectrum of the methyl ester (in CS_2) had bands at 1709 cm.$^{-1}$ (keto-group in a six-membered ring) and at 1736 cm.$^{-1}$ (methoxycarbonyl group). Hydrogenation of methyl pinicolate A gave a dihydro-derivative. Pinicolic acid A is therefore a monoketo-acid with at least one double bond. The formula $C_{31}H_{48}O_3$ or $C_{32}H_{50}O_3$ for methyl pinicolate A corresponds to a tetracyclic structure with two double bonds or a pentacyclic structure with one double bond.

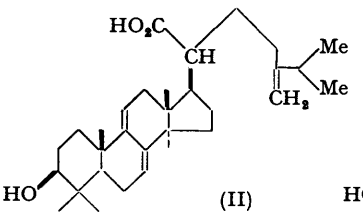
Ozonolysis of methyl pinicolate A gave acetone, isolated as its 2 : 4-dinitrophenylhydrazone in good yield, and the non-volatile fragment was acidic. This conforms with

* Part XXVI, *J.*, 1954, 3234.

a side chain terminating in the grouping $-CH{:}CMe_2$, which might be of the C_8 lanosterol type (cf. V) rather than the C_9 eburicoic acid type terminating in the grouping $-C(:CH_2)\cdot CHMe_2$.

Methyl pinicolate A was not easily hydrolysed and the free acid melted without decarboxylation. These properties are similar to those shown by methyl polyporenate C and the corresponding acid, and suggest corresponding locations of the carboxyl group.


(I)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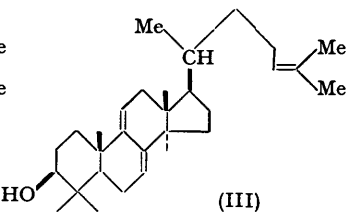
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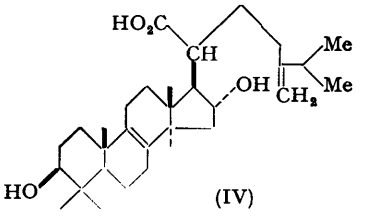
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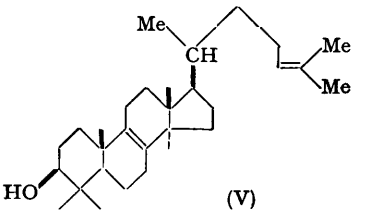
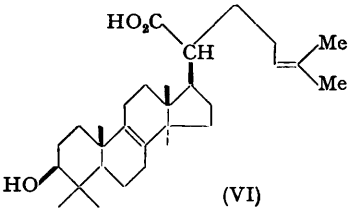

(V)

Reduction of methyl pinicolate A with sodium borohydride gave the hydroxy-ester, the acid (VI; see below for proof of structure) corresponding to which has recently been isolated in these laboratories from the fungus *Trametes odorata* (Wulf.) Fr. (Halsall, Hodges, Jones, and Sayer, unpublished work). It is not possible to decide from the published constants whether this acid is identical with the so-called trametenolic acid isolated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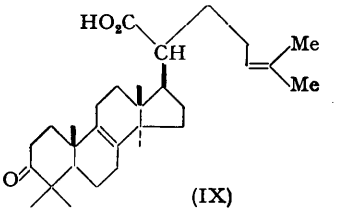
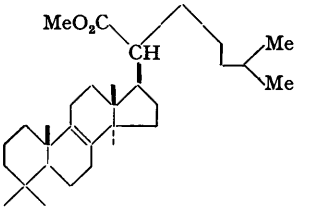

(IX)

CH_2N_2; H_2–Pt; Wolff–Kishner; CH_2N_2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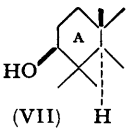
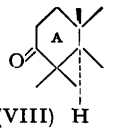

(VII)


(VIII)

from *Trametes odorata* by Gruber and Proske (*Monatsh.*, 1950, **81**, 877, 1024; 1952, **82**, 255); the properties of their acid are consistent with the C_{30} formulation (VI).

The hydroxy-ester was hydrogenated to the dihydro-derivative and this was smoothly dehydrated with phosphorus pentachloride in light petroleum to a product which on

ozonolysis yielded acetone, isolated in good yield as its 2 : 4-dinitrophenylhydrazone. This dehydration is characteristic of the grouping (VII) (cf. Christen, Dünnenberger, Heusser, and Jeger, *Helv. Chim. Acta*, 1952, **35**, 1757) and indicates that pinicolic acid A possesses a typical triterpene ring A, but containing a keto-group as in (VIII).

On the basis of the evidence so far presented (IX) appeared to be a possible structure for pinicolic acid A. This being so, hydrogenation of the methyl ester, followed by Wolff–Kishner reduction of the dihydro-ester, and methylation of the product should give a known compound, methyl 28-noreburic-8-en-21-oate (X) (Holker, Powell, Robertson, Simes, Wright, and Gascoigne, *J.*, 1953, 2422). Methyl dihydropinicolate A was reduced (Wolff–Kishner), and methylation then gave an ester identical with an authentic sample of (X) very kindly provided by Professor A. Robertson, F.R.S., and Dr. J. S. E. Holker. No isomerisation occurs at $C_{(20)}$ during the alkaline hydrolysis of the methyl esters of pinicolic acid A derivatives, which indicates that the carboxyl group is in the more stable (lanostane) configuration (cf. Bowers, Halsall, and Sayer, *loc. cit.*). Since the positions of the carbonyl group and of the reducible double bond are known, respectively, from the phosphorus pentachloride dehydration reaction and the ozonolysis of methyl pinicolate A described above, pinicolic acid A must be formulated as (IX) (3-oxolanosta-8 : 24-dien-21-oic acid). This is the first example of an acid of the trimethylsteroid series possessing the lanostane skeleton.

Fieser and Fieser (" Natural Products related to Phenanthrene," 3rd Edn., 1949, Reinhold Publ. Corp., p. 207) present a table which shows that the compounds of the two 24-methylcholestane series, *i.e.*, of the ergostane and campestane series, have molecular rotations which are, respectively, more negative and more positive than those of the corresponding cholestane compounds. Comparison of the molecular rotations (cf. Table) of derivatives of lanost-8-ene with those of eburic-8-ene indicate that the latter have the same configuration at $C_{(24)}$ as ergostane, dihydroeburicoic acid, for instance, being 4 : 4 : 14-trimethylergost-8-en-21-oic acid.

Compound	$[M]_D$	Compound	$[M]_D$	Δ
Cholestanol [1]	+ 93°	Ergostanol [1]	+ 64°	−29°
Cholestanol [1]	+ 93	Campestanol [1]	+125	+32
Methyl 3β-hydroxylanost-8-en-21-oate [2]	+228	Methyl 3β-hydroxyeburic-8-en-21-oate [3]	+214	−14
Cholestanyl acetate [1]	+ 60	Ergostanyl acetate [1]	+ 27	−33
Cholestanyl acetate [1]	+ 60	Campestanyl acetate [1]	+ 80	+20
Methyl 3β-acetoxylanost-8-en-21-oate [2]	+528	Methyl 3β-acetoxyeburic-8-en-21-oate [3]	+294	−34
Lanost-8-ene [4]	+273	Eburic-8-ene [5]	+234	−39

[1] Fieser and Fieser, " Natural Products related to Phenanthrene," 3rd edn. 1949, Reinhold Publ. Corp., p. 206. [2] This paper. [3] Lahey and Strasser, *J.*, 1951, 873. [4] Ruzicka, Rey, and Muhr, *Helv. Chim. Acta*, 1944, **27**, 472; Ruzicka, Denss, and Jeger, *ibid.*, 1945, **28**, 759; Dorée, McGhie, and Kurzer, *J.*, 1947, 1467. [5] Gascoigne, Holker, Ralph, and Robertson, *J.*, 1951, 2346.

Hydrogenation of polyporenic acid A derivatives gives two series of dihydro-compounds (Jones and Woods, *J.*, 1953, 464). Since ergostane derivatives have lower rotations than those of campestane it is probable that methyl dihydro-(I)-polyporenate A and its derivatives, with lower rotations than those of methyl dihydro-(II)-polyporenate A (cf. the Table given by Jones and Woods, *J.*, 1953, 466), have the same configuration at $C_{(24)}$ as ergostane.

Experimental

Rotations were determined in $CHCl_3$ at room temperature. M. p.s were determined on a Kofler block and are corrected. The alumina used for chromatography had an activity of II. Light petroleum refers to the fraction with b. p. 60—80°.

Extraction of Pinicolic Acid A (IX) *from* Polyporus pinicola *Fr. and Isolation of the Methyl Ester.*—The air-dried fungus (800 g.) was extracted with cold ethanol (7 l.) for 16 hr. The extract was evaporated to a volume of 200 c.c., diluted with ether (2 l.), and extracted with sodium hydroxide solution (10%). The acidic fraction obtained was methylated with an excess of ethereal diazomethane, and the resulting mixed esters (38 g.) were adsorbed from light

petroleum–benzene (4 : 1; 500 c.c.) on alumina (2·5 kg.) deactivated with 10% of 10% aqueous acetic acid. Elution with light petroleum–benzene (4 : 1; 1500 c.c.) gave a gum (700 mg.) which was discarded. Further elution with light petroleum–benzene (1 : 1; 3000 c.c.) afforded a yellow gum (7·6 g.) which was readsorbed from benzene on alumina (500 g.). Elution of this alumina with benzene–ether (9 : 1; 750 c.c.) gave *methyl pinicolate A* (*methyl 3-oxolanosta-8 : 24-dien-21-oate*) (3·5 g.) which crystallised from methanol as needles, m. p. 117—119°, raised by further crystallisations to 121—123°, $[\alpha]_D$ +69° (*c*, 1·38) (Found: C, 79·5; H, 10·25. $C_{31}H_{48}O_3$ requires C, 79·45; H, 10·3%). The ultra-violet absorption spectrum of the methyl pinicolate A indicated that it contained a small amount of methyl dehydropinicolate A. Light absorption in ethanol: Max. 2430 and 2740 (broad) Å; ε 610 and 77. Inflexions, 2360 and 2520 Å; ε 635 and 410.

Isolation of Pinicolic Acid A.—The acidic extract from the fungus (2 g.) was adsorbed from benzene (20 c.c.) on alumina (100 g.) deactivated with 10% of 10% aqueous acetic acid. Elution with benzene–ether (1 : 1; 300 c.c.) gave a gum (300 mg.) which was discarded. Further elution with ether gave a solid fraction (400 mg.) which was crystallised from methanol to give *pinicolic acid A* (*3-oxolanosta-8 : 24-dien-21-oate*) as needles, m. p. 182—190°, raised by several crystallisations from acetone to 197—202°, $[\alpha]_D$ +68° (*c*, 0·83) (Found: C, 79·0; H, 10·6. $C_{30}H_{46}O_3$ requires C, 79·25; H, 10·2%). Light absorption in ethanol: Max. 2430 Å; ε 930. Inflexions, 2350 and 2520 Å; ε 950 and 625.

Methylation of this acid with ethereal diazomethane, followed by chromatographic purification, gave methyl pinicolate A identical with an authentic sample.

Attempted Hydrolysis of Methyl Pinicolate A.—Methyl pinicolate A (200 mg.) in methanol (10 c.c.) was treated with methanolic potassium hydroxide (30 c.c.; 10%) for 16 hr. at 20°. Dilution with water followed by ether-extraction yielded unchanged methyl pinicolate A (180 mg.), m. p. 117—120°.

Methyl Pinicolate A 2 : 4-Dinitrophenylhydrazone.—Methyl pinicolate A (150 mg.) in methanol (25 c.c.) was heated under reflux for ½ hr. with 2 : 4-dinitrophenylhydrazine (200 mg.) in the presence of a few drops of concentrated sulphuric acid. The mixture was diluted with water and extracted with benzene. The product was adsorbed from benzene (50 c.c.) on alumina (15 g.) and eluted with benzene (400 c.c.), to give *methyl pinicolate A 2 : 4-dinitrophenylhydrazone* (165 mg.) which crystallised as plates, m. p. 197—198°, from ethyl acetate–methanol (Found: C, 68·7; H, 7·95; N, 8·45. $C_{37}H_{52}O_6N_4$ requires C, 68·5; H, 8·1; N, 8·65%).

Ozonolysis of Methyl Pinicolate A.—Methyl pinicolate A (420 mg.) in acetic acid (30 c.c.) was treated with ozonised oxygen (6%) for 30 min. at 20°. Water was added and the mixture steam-distilled, the distillate being passed into saturated aqueous dimedone. No precipitate resulted. The dimedone solution was steam-distilled, the distillate being passed into a solution of 2 : 4-dinitrophenylhydrazine (400 mg.) in methanol containing a few drops of concentrated sulphuric acid. Dilution with water followed by extraction with benzene gave a product which, after purification by chromatography was crystallised from methanol, to give acetone 2 : 4-dinitrophenylhydrazone (150 mg.) as plates, m. p. 126—127°, undepressed on admixture with an authentic specimen.

Hydrogenation of Methyl Pinicolate A.—Methyl pinicolate A (200 mg.) in acetic acid (7 c.c.) was hydrogenated at 20° in the presence of Adams's catalyst (10 mg.) (uptake of H_2, 1·06 mol.). After filtration and evaporation the product was adsorbed from benzene (20 c.c.) on alumina (10 g.). Elution with benzene–ether (9 : 1; 200 c.c.) gave *methyl dihydropinicolate A* (*methyl 3-oxolanost-8-en-21-oate*) as needles (150 mg.), m. p. 114—116° (from methanol) raised by further crystallisations from methanol to 119—121°, $[\alpha]_D$ +59° (*c*, 1·025) (Found: C, 79·5; H, 10·8. $C_{31}H_{50}O_3$ requires C, 79·1; H, 10·7%).

Wolff–Kishner Reduction of Methyl Dihydropinicolate A.—Methyl dihydropinicolate A (300 mg.) in diethylene glycol (50 c.c.) was heated at 100° for 1 hr. with hydrazine hydrate (1 c.c.; 60%); the excess of water and hydrazine were then removed by distillation. Potassium hydroxide (0·5 g.) was added and the solution heated under reflux for 5 hr. After acidification with acetic acid the cooled mixture was diluted with water, and the product was isolated with ether and remethylated with an excess of ethereal diazomethane. The crude methyl ester (300 mg.) was adsorbed from light petroleum (20 c.c.) on alumina (20 g.). Elution with light petroleum–benzene (3 : 1; 100 c.c.) afforded methyl 28-noreburic-8-en-21-oate (methyl lanost-8-en-21-oate) (200 mg.) which crystallised from methanol as needles, m. p. 100—103°, raised by further crystallisations from methanol to 102—105°, undepressed on admixture with an authentic sample of m. p. 105—107°; the reduction product had $[\alpha]_D$ +47° (*c*, 1·16) (Found: C, 81·45; H, 11·5. Calc. for $C_{31}H_{52}O_2$: C, 81·5; H, 11·5%). Light absorption in ethanol:

Max. 2430 Å; ε 620. Inflexion 2360 and 2520 Å; ε 610 and 430. The infra-red spectrum determined in " Nujol " was identical with that of an authentic sample of methyl 28-noreburic-8-en-21-oate.

Reduction of Methyl Pinicolate A with Sodium Borohydride.—To a solution of methyl pinicolate A (400 mg.) in dioxan (10 c.c.) sodium borohydride (100 mg.) in aqueous dioxan (10 c.c.) was added, and the solution was kept for 1 hr. at 20°. Dilution with water and isolation with ether gave a product which did not crystallise readily. It was treated with acetic anhydride (5 c.c.) and pyridine (5 c.c.) for 16 hr. at 20°. The acetic anhydride and pyridine were then removed under reduced pressure and the residue was crystallised from methanol, to give *methyl* 3β-*acetoxylanosta*-8 : 24-*dien*-21-*oate* as needles (300 mg.), m. p. 137—140°, raised by further crystallisations from methanol to 145—147°, $[\alpha]_D$ +67° (*c*, 0·965) (Found : C, 77·15; H, 9·95. $C_{33}H_{52}O_4$ requires C, 77·3; H, 10·2%).

Hydrolysis of Methyl 3β-*Acetoxylanosta*-8 : 24-*dien*-21-*oate.*—The acetate (130 mg.) in methanol (15 c.c.) was treated with methanolic potassium hydroxide (15 c.c.; 10%) for 16 hr. at 20°. Dilution with water and extraction with ether yielded *methyl* 3β-*hydroxylanosta*-8 : 24-*dien*-21-*oate* which was crystallised several times from aqueous *iso*propanol to give needles (90 mg.), m. p. 131—134°, $[\alpha]_D$ +50° (*c*, 0·92) (Found : C, 78·75; H, 10·6. $C_{31}H_{50}O_3$ requires C, 79·1; H, 10·7%). Light absorption in ethanol : Max. 2430 and 2520 Å; ε 280 and 195. Inflexion 2360 Å; ε 305.

Hydrogenation of Methyl 3β-*Hydroxylanosta*-8 : 24-*dien*-21-*oate.*—Methyl 3β-hydroxylanosta-9 : 24-dien-21-oate (1·5 g.) in acetic acid (60 c.c.) was hydrogenated at 20° in the presence of Adams's catalyst (100 mg.) until absorption of hydrogen ceased. The product obtained after filtration and evaporation was crystallised from *iso*propanol, to give *methyl* 3β-*hydroxylanost*-8-*en*-21-*oate* as fine needles (1·3 g.), m. p. 149—151° raised by further crystallisations from methanol to 151—153°, $[\alpha]_D$ +48·5 (*c*, 0·955) (Found : C, 79·0; H, 11·45. $C_{31}H_{52}O_3$ requires C, 78·75; H, 11·1%). Light absorption in ethanol : Max. 2430 Å; ε 400. Inflexions 2360 and 2510 Å; ε 420 and 270. The hydroxy-ester (700 mg.) was acetylated in pyridine (10 c.c.) with acetic anhydride (10 c.c.) at 20° for 16 hr., to give *methyl* 3β-*acetoxylanost*-8-*en*-21-*oate* which crystallised from methanol as needles (500 mg.), m. p. 144—146°, $[\alpha]_D$ +64° (*c*, 0·95) (Found : C, 77·0; H, 10·6. $C_{33}H_{54}O_4$ requires C, 77·0; H, 10·6%).

Dehydration of Methyl 3β-*Hydroxylanost*-8-*en*-21-*oate.*—Methyl 3β-hydroxylanost-8-en-21-oate (300 mg.) in light petroleum (30 c.c.) was shaken with phosphorus pentachloride (160 mg.) for 20 min. at 20°. After washing with sodium hydrogen carbonate solution and water, evaporation of the petroleum solution gave the dehydration *product* which crystallised from methanol–acetone as needles (250 mg.), m. p. 139—144°, raised by further crystallisations from acetone to 143—146°, $[\alpha]_D$ +46° (*c*, 0·915) (Found : C, 82·1; H, 11·05. $C_{31}H_{50}O_2$ requires C, 81·9; H, 11·1%). Light absorption in ethanol : Inflexions 2360, 2430, and 2520 Å; ε 230, 130, and 120.

Ozonolysis of Dehydration Product.—A stream of ozonised oxygen (6%) was passed into a solution of the dehydration product (320 mg.) in acetic acid (120 c.c.) for 30 min. at 20°. Water was added and the mixture steam-distilled, the distillate being passed into a saturated aqueous solution of dimedone. No precipitate was formed. The dimedone solution was in turn steam-distilled and the distillate passed into methanolic 2 : 4-dinitrophenylhydrazine (300 mg.) containing a few drops of concentrated sulphuric acid. Extraction of this solution with benzene gave a product which, after purification by chromatography, was crystallised from methanol to give acetone 2 : 4-dinitrophenylhydrazone (160 mg.) as plates, m. p. and mixed m. p. 125—127°.

The authors thank Professor N. A. Sörensen for collecting and supplying the *Polyporus pinicola*, and Professor A. Robertson, F.R.S., and Dr. J. S. E. Holker for the authentic sample of methyl 28-noreburic-8-en-21-oate. One of them (J. M. G.) thanks the Department of Scientific and Industrial Research for a maintenance grant. The authors thank Mr. E. S. Morton and Mr. H. Swift for the microanalyses.

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