

Isolation and Identification of Eburicoic Acid from *Fomes pinicola*

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The petroleum ether extract of *Fomes pinicola*, upon chromatography on an alumina column, yielded ergosterol and a crystalline compound which was identified as eburicoic acid by means of IR and NMR spectroscopy. Ultraviolet spectrum analysis indicated that eburicoic acid was contaminated with trace impurities of dehydro-eburicoic acid.

BASIDIOMYCETES belonging to family *Polyporaceae* have been found to be rich sources of triterpene acids. The genera *Polyporus* and *Fomes* are noteworthy members among this group. Kariyone and Kurono (1), in 1940, isolated eburicoic acid, a triterpene acid, from *Fomes officinalis*. Since then it has been isolated from a number of other species such as *P. sulphureus*, *P. anthracophilus*, *P. eucalyptorum*, etc., when these species were grown on a synthetic medium (2). Besides eburicoic acid, its hydroxylated derivatives such as sulphurenic acid and tumulosic acid have also been encountered (3, 4). When grown on a synthetic medium, the mycelia of *P. sulphureus* produced eburicoic acid in huge yields (as much as 50% or more of the dried mycelial weight). This ready availability of eburicoic acid has led to extensive investigation of its biogenesis on the one hand (5) and degradation to steroids of various structures for potential medicinal usage on the other (6).

Polyporus pinicola is now recognized as the same species as *Fomes pinicola* (7). From this species, Halsall and co-workers (8-11) have isolated, among other steroidal derivatives, five closely related triterpenes. They are 21-hydroxylanosta-7,9(11)-24-triene-3-one (Ia), lanosta-7,9(11)-24-triene-3 β ,21-diol (Ib), 3 α -oxylanosta-8,24-diene-21-oic acid (IIa), picnicolic acid A (IIb), and polyporenic acid C (III). Eburicoic acid (IVa), which can be viewed as a reduced form of polyporenic acid C (III), has not been reported to occur in *F. pinicola*. This fact, coupled with the widespread occurrence of eburicoic acid in species of *Fomes* and *Polyporus*, led to the investigation of this species. This paper reports that eburicoic acid indeed occurs in *F. pinicola*.

RESULTS AND DISCUSSION

The petroleum ether extract of *F. pinicola*, upon thin-layer chromatography, revealed a complex mixture of sterols-triterpenes. Hence the mixture was chromatographed over a column of alumina. Elution with chloroform removed a sterol which after several crystallizations from diluted ethanol gave a homogeneous compound melting at 159-161°. It was identified as ergosterol from its characteristic UV spectrum with $\lambda_{max}^{CHCl_3}$ at 264, 273, 283, and 294 μ . Undepressed mixed melting point with an authentic specimen of ergosterol and their superimposable IR spectra further verified the identity of the isolate as ergosterol.

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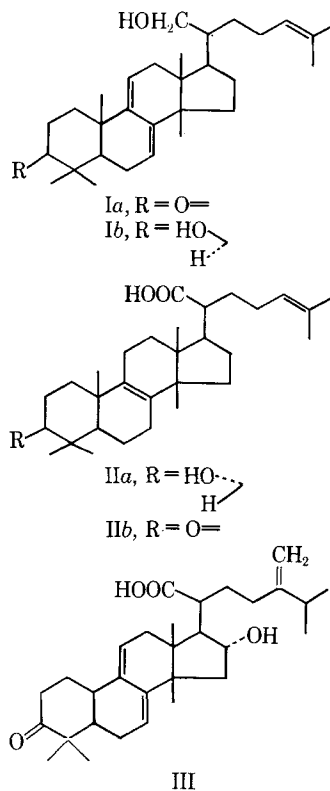
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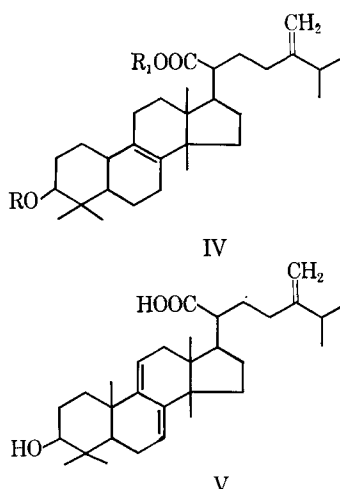
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Continued elution of the column with methanol yielded a highly polar compound which after repeated crystallizations from dilute ethanol gave fine needles of a compound, m.p. 280-283°. It gave a positive Liebermann-Burchard color reaction and it was identified as eburicoic acid (IVa) from the following evidence.

The IR spectrum (KBr pellet) of the isolate (IVa) showed strong absorption bands at 3430 ($-\text{OH}$), a group of small bands at 2700, a strong absorption band at 1700 ($-\text{COOH}$), and bands at 1640 and 887 cm^{-1} ($>=\text{CH}_2$). In the IR spectrum of the acetyl derivative (IVb), the absorption band in the 3500 cm^{-1} region was eliminated while the bands due to free carboxyl and terminal methylene groups were maintained. An additional band in the carbonyl region at 1725 cm^{-1} indicated the esterification of the hydroxyl group in IVb. On the other hand, the IR spectrum of the methyl ester (IVc) showed the absorption bands due to free hydroxyl (3420 cm^{-1}) and terminal methylene (1640 and 885 cm^{-1}) groups but it lacked absorptions due to a free carboxyl group. Instead, a single strong absorption band at 1730 cm^{-1} indicated the esterification of the free



IVa, eburicoic acid; R = R₁ = H
 IVb, acetyl eburicoic acid; R = CH₃CO, R₁ = H
 IVc, methyl eburicoate; R = H, R₁ = CH₃

carboxyl group of IVa. The NMR spectra of methyl eburicoate (IVc) and acetyl eburicoic acid (IVb) were in complete agreement with those recorded by Fried *et al.* (3).

Finally, the IR spectra of the isolate (IVa) and its methyl ester (IVc) were superimposable over those of eburicoic acid and its methyl ester,¹ thus confirming the identity of the isolate as eburicoic acid.

The UV spectrum of the isolate (IVa) showed a trio of absorption bands of low intensity but characteristic $\lambda_{\text{max}}^{\text{MeOH}}$ at 234, 243, and 251 m μ . These absorption bands are ascribed to the presence of multicyclic terpenes with a $\Delta^{7,9(11)}$ conjugated diene system. These dienes, often called dehydro compounds, occur frequently as adjuncts to the more common terpenes with a $\Delta^{8,9}$ double bond. Holker *et al.* (12) have commented upon the co-occurrence of dehydroeburicoic acid (V) along with eburicoic acid (IVa) and also on the very great difficulty of separating these compounds from each other. Hence, the characteristic but low intensity UV absorption bands in the sample of the isolate must be ascribed to the traces of dehydroeburicoic acid in it.

The melting points of eburicoic acid isolated from different sources and its derivatives prepared by different workers vary considerably (*cf.* under *Experimental*). These variations can be ascribed possibly to the differing amounts of dehydroeburicoic acid which may be present in the samples of eburicoic acid.

EXPERIMENTAL

Melting points were determined using a Kofler micromelting point apparatus. Ultraviolet spectra were recorded on a Beckman spectrophotometer, model DB. Infrared spectra were recorded on a Beckman IR-8 spectrophotometer. NMR spectra were determined in CDCl₃ on a Varian Associates A-60 instrument through the courtesy of Dr. F. T. Bond, Oregon State University.

¹ Eburicoic acid and methyl eburicoate were obtained through the courtesy of the Squibb Institute for Medical Research, New Brunswick, N. J., and Professor Josef Fried, Ben May Laboratory for Cancer Research, University of Chicago, Chicago, Ill., respectively.

Material—Naturally occurring carpophores of *Fomes pinicola*, growing on larch trees (*Larix occidentalis*) in Linn County, Oreg., were collected during the summer of 1966. The collection was identified by Dr. Lewis F. Roth, Oregon State University.

Extraction—Approximately 250 Gm. of fungus was cut up in small bits and pulverized in an Abbé mill. The powdered material was exhaustively extracted with petroleum ether in a Soxhlet extractor for 48 hr. The extract was concentrated under reduced pressure at 40° to a small volume of syrupy residue.

Isolation of Ergosterol—About one-half the quantity of the concentrated petroleum ether extract was dissolved in 75 ml. of benzene and the solution was chromatographed on an alumina² column (500 Gm., 35 × 5 cm.). Careful elution with chloroform yielded a fraction which deposited crystals upon evaporation. The crystals were collected and crystallized once from ethyl acetate and then thrice from dilute ethanol. Colorless plates were obtained m.p. 159–161°, [α]_D = –128° (CHCl₃). [Reported m.p. 163°, [α]_D = –133° (CHCl₃) (13).]

Isolation of Eburicoic acid—After ergosterol was eluted, the column was developed with solvents of increasing polarity. Methanol eluted a chalky component which was found to contain inorganic impurities. These were removed by treating the compound with boiling water for 10 min. and then collecting it on a Büchner funnel. The compound thus collected was crystallized four times from dilute ethanol. Colorless needles of eburicoic acid (IVa) were obtained, m.p. 280–283°, [α]_D = +31° (pyridine). [Reported m.p. 283° (1); m.p. 280° (14); m.p. 292–293°, [α]_D = +34° (pyridine) (15).] Mixed melting point with an authentic sample of eburicoic acid (m.p. 275–277°) did not show any depression.

Preparation of Acetyl Eburicoic Acid—Eburicoic acid (48 mg.) was dissolved in 1.0 ml. of pyridine, and 1.0 ml. of acetic anhydride was added to the solution. After refluxing the mixture for 30 min., it was poured onto crushed ice. The precipitate was collected, washed thoroughly with water, and crystallized thrice from ethanol. Acetyl eburicoic acid was obtained, m.p. 248–253°, [α]_D = +40° (CHCl₃). [Reported m.p. 240° (1); m.p. 259° [α]_D = +42° (CHCl₃) (15).]

Preparation of Methyl Eburicoate—A chilled solution of diazomethane (prepared from 5 Gm. of *N*-nitrosomethylurea) in ether was added to a chilled solution of eburicoic acid (150 mg.) in methanol (100 ml.). The mixture was allowed to evaporate spontaneously and the residue was crystallized repeatedly from *n*-hexane. Fine needles of methyl eburicoate were obtained, m.p. 138–141°, [α]_D = +42° (CHCl₃). [Reported m.p. 141° (1); m.p. 146–147°, [α]_D = +45° (CHCl₃) (15).] An admixture with an authentic sample of methyl eburicoate (m.p. 132–137°) did not show depression in melting point.

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² Alumina activated, chromatographic grade, Matheson, Coleman, and Bell, East Rutherford, N. J.

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Opium Alkaloids V. Structure of Porphyroxine

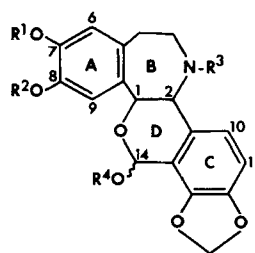
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Porphyroxine is a monophenolic alkaloid isolated from opium. It contains a methyl acetal group and has three asymmetric centers. The position of the phenolic hydroxyl group has been determined by NMR spectroscopy. Evidence has been presented for the configuration at position-14.

IN 1837, MERCK (1) discovered a minor opium alkaloid which he named porphyroxine because it produced a red color when heated with dilute mineral acids. He suggested that this reaction might be used as a test for opium in forensic cases (2). The concentration of the alkaloid varies with the source of opium, and the color reaction is useful for determining the geographic origin of opium seized in illicit traffic (3, 4). Although there has been a great deal of interest in porphyroxine for more than 100 years and many investigators claim to have isolated it, it is only recently that it has been obtained in a form sufficiently pure for structure studies (5-7). In 1962 the empirical formula was determined by Pfeifer and Teige (6) as $C_{20}H_{21}NO_6$. In a series of investigations of the alkaloids of the genus *Papaver*, Pfeifer *et al.* (8, 9) described a total of six alkaloids which gave the characteristic red color with mineral acids. They coined the name "papaverrubines" for these compounds. All papaverrubines are secondary amines. The *N*-methyl derivatives, several of which have been found in the *Papaveraceae* family, do not give the red color reaction with acids. The gross structures of some of these alkaloids are illustrated in I-V.

Papaverrubine A (I), glaudine (III), isorhoeadine (IV), and glaucamine (V) have a *trans*-configuration at the junction of ring B and ring D, whereas papaverrubine E (I), oreodine (III), and rhoeadine (IV) have the *cis*-configuration (10-12).

Porphyroxine (II) has been established as a member of the B/D *trans*-series by methylation to glaudine (13). The only structural features of porphyroxine which have not yet been reported are the exact position of the hydroxyl group in ring A and the stereochemistry at position-14. During studies



- I, $R^1 + R^2 = CH_2$, $R^3 = H$, $R^4 = CH_3$; papaverrubine A and E
 II, $R^1 = H$ or CH_3 , $R^2 = CH_3$ or H , $R^3 = H$, $R^4 = CH_3$; papaverrubine D (porphyroxine)
 III, $R^1 = R^2 = R^3 = R^4 = CH_3$; glaudine and oreodine
 IV, $R^1 + R^2 = CH_2$, $R^3 = R^4 = CH_3$; isorhoeadine, rhoeadine
 V, $R^1 = R^2 = R^3 = CH_3$, $R^4 = H$; glaucamine

of the minor alkaloids of opium, this alkaloid was isolated. The isolation and subsequent studies of the structure of porphyroxine are described.

EXPERIMENTAL

Isolation—Four pounds of powdered opium of Indian origin were extracted, and a preliminary separation of alkaloid groups was carried out as described in a previous communication (14). The phenolic alkaloid fraction was subjected to preparative thin-layer chromatography on silica gel with chloroform-methanol (9:1). Bands were obtained of reticuline, isoboldine, and scoulerine. One band with a high R_f value was a mixture of several alkaloids which were separated on an alumina column (Merck, neutral, activity IV) with chloroform. The first fractions contained an alkaloid which, when crystallized from methanol, melted at 190° (micro m.p. K.). [Lit. m.p. 192° (6).] It gave a single peak by gas chromatography on silicone rubber SE-30 (3.8%, 4 ft., 200°) and single spots by thin-layer chromatography on silica gel in two different solvent systems (15). The spots produced a red color when

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