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LOW-MOLECULAR-WEIGHT MUSHROOM METABOLITES. V. EBURICOIC ACID FROM *Polyporus ailanthus*

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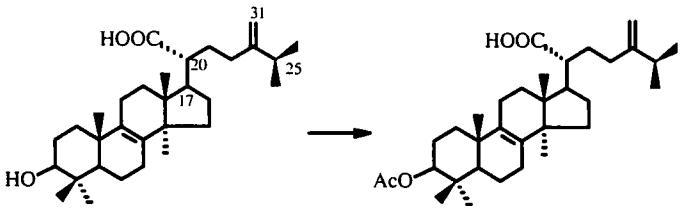
The triterpene acid isolated from Polyporus ailanthus *(Aphyllophorales) is identified as eburicoic acid on the basis of ^1H and ^{13}C NMR, electron-impact mass, and IR spectra.*

Key words: mushrooms, triterpene acid.

In continuation of studies of secondary metabolites of mushrooms [1], we studied the wood-destroying fungus *Polyporus ailanthus* (Aphyllophorales) growing on apple trees.

A crystalline compound $C_{31}H_{50}O_3$ (**1**) was isolated from the methanol extract of the mushrooms. The ^1H NMR of this compound contains signals of five tertiary and two secondary methyls at high field and two exomethylene protons at 4.90 and 4.95 ppm (see Experimental). An absorption band at 890 cm^{-1} in the IR spectrum also is consistent with an exomethylene in **1**. Carbonyl (1720 cm^{-1}) and hydroxyl (3332 cm^{-1}) bands are also observed.

The appearance of a signal at 178.77 ppm (Table 1) in the ^{13}C NMR spectrum indicates that the carbonyl absorption in the IR spectrum is due to a carboxyl. Therefore, the third O atom is a secondary hydroxyl. This is consistent with a doublet of doublets at 3.46 ppm with spin—spin coupling constant (SSCC) $^3J_1$ = 8.7 Hz and $^3J_2$ = 7.2 Hz in the ^{1} H NMR spectrum that belongs to a proton geminal to the aforementioned hydroxyl. A signal for a secondary-alcohol C atom at 78.02 ppm in the ^{13}C NMR spectrum confirms this. Acetylation of **1** with acetic anhydride in pyridine produces the monoacetate **2**.



1 **2**

The ^{13}C NMR spectrum of hydroxyacid **1** contains signals at 135.50 and 135.26 ppm, which belong to C atoms of the tetrasubstituted double bond, in addition to signals at 107.02 and 155.96 ppm, which belong to the C atom of the exomethylene and the C atom bonded to it, respectively.

The molecular formula of **1**, $C_{31}H_{50}O_3$, indicates that the degree of unsaturation is 7. Therefore, it should include a system from four rings, taking into account the carboxyl and the two double bonds. This suggests to us that **1** is a lanostane derivative [2].

As a rule, the "extra" C atom in the C_{31} derivatives of lanostan is situated on C-24. The methylene doublets with identical SSCC in the ^{1} H NMR of **1** and **2** should be assigned to gem-dimethyls on C-25. The chemical shift of C-25 (33.80 ppm) in the ^{13}C NMR spectrum of **2** is practically identical to that of cycloeucalenol and its derivatives [3] and identifies the position of an exomethylene on C-24.

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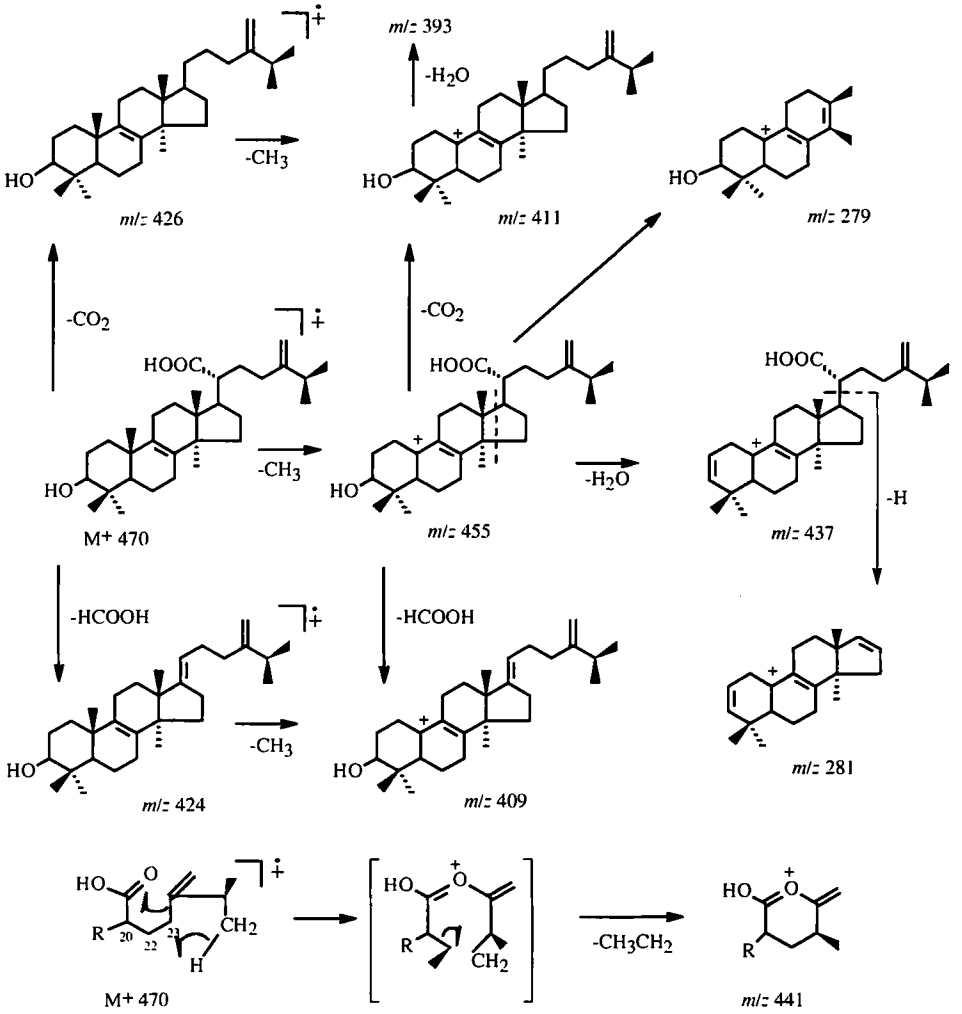

R-polycyclic part of the molecule

Fig. 1. Mass spectrometric fragmentation of eburicoic acid (**1**).

The mass spectrum of **1** contains peaks of ions resulting from successive elimination of methyl radical, water molecule, and side chain with *m/z* 455, 437, and 281 (Fig. 1). The corresponding ions with *m/z* 497, 437, and 281 also appear in the mass spectrum of **2**. The appearance of an ion with *m/z* 281 suggests that the carboxyl is located in the side chain and that it can only be located on C-20. This is also consistent with the appearance of a rearranged ion with *m/z* 441 (M^+ - 29) that results from loss of an ethyl radical including C-22 and C-23. This is characteristic of carboxylic acids with an alkyl chain [4].

Only one position, -Δ^8, is possible for the tetrasubstituted double bond in the polycyclic part of the lanostan backbone. Thus, the studied compound is based on the 24-methylenelanost-8-ene backbone.

The magnitude of the chemical shifts of the protons geminal to the hydroxyl and acetoxyl in the ^{1}H NMR spectrum of **1** and **2** and their SSCC in addition to the size of the chemical shifts of the corresponding C atoms in the ^{13}C NMR spectra of these compounds define the position and configuration of the O groups as 3β.

Thus, **1** has the structure of 24-methylenelanost-8-en-3β-hydroxy-21-oic acid. This is the same structure as eburicoic acid, which was isolated previously from *Polyporus arhracophilus* Cooke, *P. eucalyptorum* Fr., *P. sulfureus* (Bull) Fr., *Fomes officinalis* Fr., and *Lentinus dactyloides* Clel. [5, 6].

TABLE 1. Chemical Shifts of C Atoms in Eburicoic Acid (**1**) and Its Acetate (**2**)

C atom	DEPT	Compound	
		1	**2**
1	CH_2	36.14	35.43
2	CH_2	26.84	24.18
3	CH	78.02	80.70
4	C	37.41	36.94
5	CH	50.91	50.48
6	CH_2	21.28	20.89
7	CH_2	27.50	27.08
8	C	135.50	134.30
9	C	135.26	134.13
10	C	39.54	37.83
11	CH_2	18.73	18.08
12	CH_2	30.90	30.35
13	C	49.88	49.51
14	C	44.93	44.29
15	CH_2	32.78	31.97
16	CH_2	28.71	26.34
17	CH	49.28	47.16
18	CH_3	19.42	19.18
19	CH_3	16.39	16.52
20	CH	47.74	47.74
21	C	178.77	181.97
22	CH_2	29.39	28.84
23	CH_2	31.86	30.92
24	C	155.96	155.21
25	CH	34.23	33.80
26	CH_3	21.90	21.76
27	CH_3	22.02	21.85
28	CH_3	24.52	24.33
29	CH_3	28.64	27.89
30	CH_3	16.35	15.98
31	CH_2	107.02	106.81
COO	C	-	170.99
CH_3	CH_3	-	21.32

Spectra were obtained in deuteropyridine (eburicoic acid, **1**) and deuterochloroform (its acetate, **2**).

It is interesting that peaks of the (M^+ - H_2O) and (M^+ - AcOH) ions do not appear in the mass spectra of **1** and **2**, respectively. This suggests that elimination of the methyl radical from the molecular ions precedes loss of water or acetic acid. However, elimination of the methyl radical and CO_2 occurs in parallel and passes through ions with *m/z* 455 and 426 to the same ion with *m/z* 411. The same can be said about the elimination of the methyl radical and formic acid, which produce the ion with *m/z* 409. Loss of a methyl radical from the three allylic positions C-10, C-14, and C-25 would seem to be equally probable. However, the observation of ions with *m/z* 281 and 279 confirms that elimination of the C-19 methyl predominates.

The ^{13}C NMR spectra were interpreted using DEPT experiments and ^{13}C NMR spectra of isomultiflorenol [7] and bryonolic acid, which have rings *A*, *B*, and *C* identical to those in eburicoic acid, and of lanosterol [8], dihydrolanosterol [8, 9], and cycloeucalenol and its derivatives [3].

EXPERIMENTAL

General Observations. TLC was performed on Silufol plates. Spots were detected visually by spraying with 25% phosphotungstic acid in ethanol followed by heating for 5 min at 100-110°C. Silica gel (L grade) with particle size 50-100 μm was used for column chromatography.

The following solvent systems were used: 1) $CHCl_3$—CH_3OH (10:1), 2) $CHCl_3$—CH_3OH (50:1).

^1H and ^{13}C NMR spectra were recorded on a UNITYplus 400 instrument in deuteropyridine and deuterochloroform. ^{13}C NMR spectra were recorded with full C–H decoupling and under DEPT conditions (δ, ppm, 0 = TMS). Mass spectra and empirical formulas were measured on an MX-1310 spectrometer at 70 eV ionization potential and 100-170°C. IR spectra were obtained on a Perkin—Elmer System 2000 FT-IR spectrophotometer in KBr.

Isolation of Eburicoic acid (1). Air-dried ground *Polyporus ailanthus* (213.6 g) were extracted with methanol (3×300 ml). The methanol extracts were evaporated to a small volume and diluted with an equal amount of water. The aqueous solution was treated with $CHCl_3$. The $CHCl_3$ extract was evaporated. The solid was chromatographed on a column using system 1 for elution. Yield: crystalline eburicoic acid (**1**), 10 mg, $C_{31}H_{50}O_3$.

IR spectrum (KBr, ν, cm^{-1}): 3332 (OH), 1720 (C=O carboxyl), 1648 (C=C), 890 (=CH_2).

Mass spectrum, *m/z* (%): M^+ 470 (100), 455 (88.1), 441 (20.9), 437 (95.5), 426 (25.4), 424 (26.9), 423 (28.4), 411 (40.3), 409 (26.9), 397 (11.9), 393 (14.9), 341 (16.4), 315 (16.4), 313 (16.4), 311 (14.9), 301 (14.9), 299 (19.4), 297 (13.4), 295 (10.4), 281 (25.4), 279 (13.4), 256 (46.3), 213 (34.3).

^1H NMR (δ, ppm, J, Hz, C_5D_5N): 1.02 (CH_3, s), 1.0375 (CH_3, d, ^3J = 2.8 Hz), 1.0405 (CH_3, d, ^3J = 2.8 Hz), 1.05 (CH_3, s), 1.09 (CH_3, s), 1.11 (CH_3, s), 1.26 (CH_3, s), 3.46 (H-3, dd, $^3J_1$ = 8.7 Hz, $^3J_2$ = 7.2 Hz), 4.90 (H-31A, t, $^2J = {}^4J$ = 1.3 Hz), 4.95 (H-31B, q, $^2J = {}^4J_1 = {}^4J_2$ = 1.37 Hz). For the ^{13}C NMR spectrum, see Table 1.

Eburicoic Acid Acetate (2) from 1. Eburicoic acid (8 mg) was acetylated with acetic anhydride (0.25 ml) in pyridine (0.5 ml) at room temperature for 24 h. Then solvent was evaporated. The solid was chromatographed on a column using system 2. Yield: crystalline eburicoic acid acetate (**2**), 6 mg, $C_{33}H_{52}O_4$.

IR spectrum (KBr, ν, cm^{-1}): 1735, 1251 (ester), 1717 (C=O of carboxyl), 1644 (C=C), 890 (=CH_2).

Mass spectrum, *m/z* (%): M^+ 512 (30.4), 497 (43.5), 483 (8.1), 437 (100), 423 (21.7), 419 (7.5), 353 (11.8), 341 (18.6), 315 (15.5), 301 (11.2), 295 (11.8), 281 (34.8).

^1H NMR spectrum (δ, ppm, J, Hz, $CDCl_3$): 0.76 (CH_3, s), 0.87 (CH_3, s), 0.88 (CH_3, s), 0.90 (CH_3, s), 0.99 (CH_3, s), 1.003 (CH_3, d, ^3J = 4.8 Hz), 1.02 (CH_3, d, ^3J = 4.8 Hz), 4.49 (H-3, dd, $^3J_1$ = 11.1 Hz, $^3J_2$ = 4.9 Hz), 4.68 (H-31B, q, $^2J = {}^4J_1 = {}^4J_2$ = 1.3 Hz), 4.76 (H-31A, t, $^2J = {}^4J$ = 1.25 Hz). For the ^{13}C NMR spectrum, see Table 1.

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