Metabolites of *Fomes officinalis*[1a]

WILLIAM W. EPSTEIN AND GEORGE VAN LEAR[1b]

Department of Chemistry, University of Utah, Salt Lake City, Utah 84112

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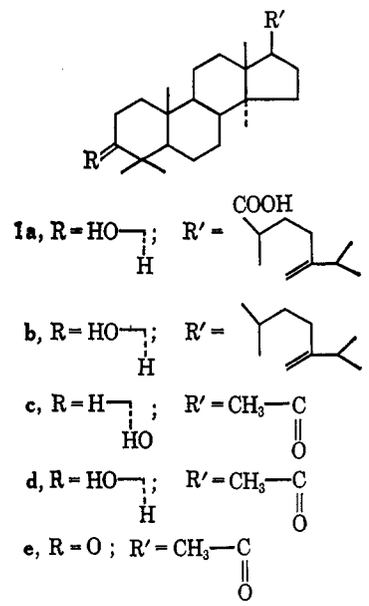
Our interest in finding new lanostane derivatives has led to a phytochemical survey of the *Polyporaceae* family (Agaricales order) genus *Fomes*. The present study has been concerned with some of the petroleum ether soluble metabolites of *Fomes officinalis*. Although previous work on this species showed ergosterol,[2] agaricinic acid[2] (an unknown acid which was not found in the present study), agaric acid,[3] and eburicoic acid (**1a**),[4] to be present, our work indicates that this species is still a good source of new and unusual compounds.

The petroleum ether soluble material obtained by extraction of *F. officinalis* was subjected to an involved separational scheme described in detail in the Experimental Section. After ergosterol was removed from the mixture by crystallization, squalene, eburicol (**1b**), a degradation product of eburicoic acid previously unreported as naturally occurring, and a new compound, 3α-hydroxy-4,4,14α-trimethyl-Δ^2-5α-pregnen-20-one (**1c**), were isolated after chromatography on Florisil.[5] Squalene and eburicyl acetate were identified by comparison of their physical characteristics with those of authentic samples.

The structure of **1c** was established from a consideration of the following data. The mass spectrum molecular weight[6] of 358 combined with analytical data indicated a molecular formula of $C_{24}H_{38}O_2$. The 3550-cm^{-1} band in the infrared spectrum coupled with the formation of a monoacetate showed the existence of a hydroxyl group. A methyl ketone was deduced from a strong 1695-cm^{-1} carbonyl band with a 1366-cm^{-1} band stronger than the 1475-cm^{-1} band[7] and further substantiated by a singlet methyl resonance in the nuclear magnetic resonance (nmr) spectrum at τ 7.92.

Notably absent from the infrared and nmr spectra were peaks which might be related to some type of carbon–carbon double bond. The ultraviolet spectra possessed a trio of bands characteristic of a $\Delta^{7,9(11)}$-diene system which is present in many triterpenes related to eburicoic acid. The very low intensity of these bands indicates that the compound possessing this chromophore must be an impurity, a situation often encountered with multicyclic terpenes containing an 8,9 double bond.

The above data coupled with the fact that the nmr spectrum showed a large number of singlet methyl resonances led to speculation that **1c** could be a 3-hydroxy tetracyclic system similar to lanosterol with an acetyl group in place of the side chain. Support for this supposition was gained when it was found that the optical rotatory dispersion (ORD) spectrum of **1c** showed a positive Cotton effect with an amplitude of +164° consistent with a *trans*-C–D ring juncture possessing two angular methyl groups and a 17β-acetyl function.[8] Compound **1d**, 3β-hydroxy-4,4,14α-trimethyl-Δ^8-5α-pregnen-20-one, has been prepared[9] from eburicoic acid; the physical properties reported for **1d** were similar to but not identical with those of **1c**. An authentic sample of **1d** was obtained[10] and found to have a different R_f on silica gel thin layer chromatography (tlc) from that of **1c**. As the two compounds were obviously different, the possibility of **1c** and **1d** being epimeric at position 3 was considered because of the difficulty noted in the preparation of the acetate of **1c**. Compound **1d** contains an equatorial hydroxyl whereas **1c** would possess the hindered axial hydroxyl. Oxidation of **1c** or **1d** using Jones reagent[11] gave rise to the same diketone (**1e**) establishing **1c** as the 3 epimer of **1d**.



Recent work[12] has shown that tritium-labeled farnesyl pyrophosphate was incorporated into eburicoic acid grown in *Polyporus sulphureus*. The above fact taken in conjunction with the known biosynthetic pathway of two farnesyl pyrophosphates coupling to give squalene followed by cyclization to lanosterol provides a rather complete early picture of the biosynthesis of eburicoic acid. The stepwise conversion of lanosterol to eburicoic acid, is quite obscure. In our study the presence of lanosterol was indicated from tlc but could not be confirmed by isolation of the pure compound. The occurrence of squalene, eburicol, and perhaps lanosterol

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suggests that eburicol may be a key intermediate in the conversion of lanosterol into eburicoic acid.

The isolation of **1c** is the first instance of the occurrence in a microorganism of a compound related to lanosterol which does not have the normal eight- or nine-carbon side chain.

Experimental Section

All melting points are uncorrected. Analyses were performed by Galbraith Laboratories, Knoxville, Tenn. Infrared spectra were determined on a Beckman IR 5. Ultraviolet spectra were run in 95% EtOH on a Beckman DK2. Nmr spectra were run on a Varian A-60 instrument. ORD spectra were obtained on a Cary 60 apparatus. All tlc was performed using silica gel G according to Stahl.

Extraction of *F. Officinalis*.—Finely ground specimens of *F. officinalis* (9 kg) were placed in a Lloyd extractor and extracted with petroleum ether (bp 30–60°) for a period of 3 weeks. Solvent was removed each day and the solution was concentrated. The extracted material was combined and stored at 10° until the extraction was completed.

Isolation of Ergosterol.—The petroleum ether was removed and the mixture was dissolved in methanol and allowed to stand for several days. The precipitate which formed was separated into strong and weak acids and neutral compounds by extraction of an ether solution with sodium bicarbonate and sodium hydroxide. The neutral materials were recrystallized from methanol to give 3.5 g of ergosterol, mp 160–162° (lit.[13] mp 162°). The acetate of ergosterol was prepared by use of acetic anhydride and pyridine to give material: mp 170–172° (lit.[13] mp 181°); ultraviolet 263, 271, 282, and 294 mμ. The methanol was removed from the mother liquors and the mixture was treated with urea under complexing conditions[14] to remove most of the fatty substances. The isolated fatty materials were not investigated further. The strong and weak acids were removed as before from the material which did not complex with the urea.

The neutral material was saponified in 0.75 *N* potassium hydroxide in methanol and worked up in the usual fashion,[15] and the respective weak and strong acids were combined with those from above. The unsaponifiable portion was treated with urea as before to give 21 g of material which did not form a complex with urea. The mixture was chromatographed on 900 g of Florisil.[5]

Materials eluted from the column by less polar eluents than benzene were combined and rechromatographed on Florisil.[5] The only identified component of this mixture was 30 mg of squalene which was established by comparison of the vapor phase chromatography retention time, tlc, and infrared spectrum with those of known squalene.

Eburicol (1b).—The substances eluted by solvent systems from benzene to (5:95) ether in benzene were rechromatographed on Florisil.[5] Benzene eluted 150 mg of eburicol: mp 159–162° from methanol, $[\alpha]_D$ +62.5° (*c* 0.12, chloroform) (lit.[16] mp 158–159°, $[\alpha]_D$ +66°). Eburicyl acetate was prepared by use of acetic anhydride and pyridine to give material: mp 142–143°, $[\alpha]_D$ +68.5° (*c* 0.07, chloroform) (lit.[16] mp 138–139°, $[\alpha]_D$ +66°). Eburicyl acetate synthesized from eburicoic acid was found to be identical in all of its characteristics with those of the naturally occurring material.

3α-Hydroxy-4,4,14α-trimethyl-Δ⁸-5α-pregnen-20-one (Compound 1d).—Compound 1c was eluted from the column with (1:99) ether in benzene. Two recrystallizations from methanol gave 80 mg of compound: mp 230–232°; $[\alpha]_D$ +109° (*c* 0.18, dioxane); infrared bands at 3550 and 1695 cm^{-1}; nmr methyl peaks at τ 9.27, 9.12, 9.02, and 7.92 and in a 1:1:3:1 ratio; ultraviolet λ_{max} 235 mμ (log ε 3.08), 243 (3.11), 250 (2.90); ORD $[\alpha]_{625}$ +90°, $[\alpha]_{589}$ +109°, $[\alpha]_{313}$ +2370°, $[\alpha]_{267}$ −2170°, $[\alpha]_{260}$ −1950° (*c* 0.18, dioxane); mass spectrum mol wt 358; tlc 1:9 acetone in chloroform.

3α-Acetoxy-4,4,14α-trimethyl-Δ⁸-5α-pregnen-20-one.—To a solution of 1 ml of acetic anhydride was added 15 mg of compound **1c**. The mixture was heated at reflux for 4 hr and worked up as before. One recrystallization from methanol gave acetate, mp 250–252°, $[\alpha]_D$ +48° (*c* 0.05, chloroform), tlc 100% chloroform.

4,4,14α-Trimethyl-Δ⁸-5α-pregnen-3,20-dione (Compound 1e).—To 0.36 ml of a solution containing 20 mg of chromium trioxide and 32 mg of sulfuric acid per milliliter of 90% aqueous acetone was added with stirring a solution containing 24 mg of **1c** in 10 ml of reagent grade acetone. The reaction was allowed to proceed for 20 min and excess reagent was destroyed by adding 1 ml of 95% ethanol. Water was added and the diketone was extracted with chloroform. The chloroform extract was dried over sodium sulfate and evaporated to dryness *in vacuo*. Recrystallization from acetone furnished 20 mg of the 3,20-diketone **1e**: mp 207–210°, $[\alpha]_D$ +138° (*c* 0.10, chloroform), and infrared at 1709 cm^{-1}. This compound had the same R_f on tlc (100% $CHCl_3$), superimposable infrared, and no depression of mixture melting point when compared with the diketone prepared from an authentic sample of the β isomer.

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Mesoionic Oxazolones. A New Synthesis and Electrophilic Substitution Reaction

W. DICKINSON BURROWS[1]

Clothing and Organic Materials Division, U. S. Army Natick Laboratories, Natick, Massachusetts

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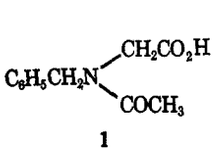
Attempting to prepare the acid chloride of N-acetyl-N-benzylglycine (**1**) by treatment with oxalyl chloride, we isolated instead a bright yellow, crystalline material of empirical formula $C_{13}H_{10}ClNO_4$. The product was an acid chloride, but not the expected one. The identity of this material was suggested by the work of Huisgen and co-workers, who prepared a new class of mesoionic oxazolones (*e.g.*, **2**) from substituted glycines by a modified Dakin–West procedure.[2]



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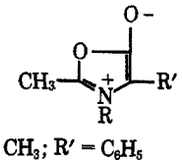

2, R = CH_3; R′ = C_6H_5
3, R = $CH_2C_6H_5$; R′ = COCOCl
4, R = $CH_2C_6H_3(OCH_3)_2$; R′ = COCOCl
5, R = $CH_2C_6H_5$; R′ = H

That our product was the 4-chloroglyoxyloyl derivative (**3**) was established by the infrared spectrum (potassium bromide disk) which showed strong bands at 1800 and 1785 cm^{-1} due to the acid chloride and α-carbon carbonyl groups, respectively, and a strong, broad band at about 1610 cm^{-1} probably resulting from the ring carbonyl and iminium groups, and by the nmr spectrum (hexadeuterioacetone) which displayed a five-proton band at δ 7.43 due to the phenyl group, a two-proton singlet at 5.71 due to the α-benzyl hydrogens, and a three-proton methyl singlet at 2.75.

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