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# MINOR TRITERPENOIDS OF *FOMES OFFICINALIS*

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**Key Word Index**—*Fomes officinalis*; Polyporaceae; fungi; triterpenoids; 3β,6β-dihydroxy-4,4,14α-trimethyl-Δ⁸-5α-pregnene-20-one; 3-keto-dehydrosulfurenic acid.

**Abstract**—The degraded triterpenoid, 3β,6β-dihydroxy-4,4,14α-trimethyl-Δ⁸-5α-pregnene-20-one (II) and 3-keto-dehydrosulfurenic acid (III), have been isolated from the extracts of *Fomes officinalis* and their structures determined.

## INTRODUCTION

THE WOOD rotting fungus *Fomes officinalis* (Polyporaceae) has proven to be a rich source of new and unusual triterpenoids related to lanosterol (see Table 1). We have recently isolated from *F. officinalis* two new triterpenoids. The first, a degraded triterpenoid related to I, has been identified on the basis of chemical and spectral evidence as 3β,6β-dihydroxy-4-4,14α-trimethyl-Δ⁸-5α-pregnene-20-one (II). The second compound, a triterpenoic acid (characterized as the methyl ester), proved to be 3-keto-dehydrosulfurenic acid (III). The structure of this molecule was confirmed by converting known methyl sulfurenate (IV) to the methyl tetrahydro-derivative of III.

## RESULTS AND DISCUSSION

The light petroleum soluble material obtained by extraction of the whole mycelium was subjected to an involved separational scheme previously described by Epstein and Van Lear.[5] After removal of squalene, ergosterol, eburicol, and 3α-hydroxy-4,4,14α-trimethyl-Δ⁸-5α-pregnene-20-one, a new compound, 3β,6β-dihydroxy-4,4,14α-trimethyl-Δ⁸-5α-pregnene-20-one (II), was isolated by chromatography on Florisil.

The mass spectrum molecular weight of 374 combined with analytical data indicated a formula of $C_{24}H_{38}O_3$ for II. The presence of a tetra-substituted double bond was indicated by a positive tetranitromethane test combined with a lack of any olefinic protons in the NMR spectrum of II. A methyl ketone was suggested by a strong 1685 $cm^{-1}$ band and further substantiated by a single methyl resonance in the NMR spectrum of II at τ7·8. The existence of two secondary hydroxyl groups was indicated by the presence of an intense 3350 $cm^{-1}$ band in the IR spectrum and two down-field doublets in the 100 mc. NMR spectrum of II (τ5·50, $J = 5$ Hz τ5·98, $J = 5$ Hz, $d_6$-DMSO), which disappeared upon addition of $D_2O$. Further evidence for the presence of two hydroxyls was the formation of a diacetate (IIa). The mass spectrum of IIa possessed no molecular ion, but had an intense M-60 (M–$CH_3COOH$) peak, a situation not uncommon among many sterol acetates.[7]

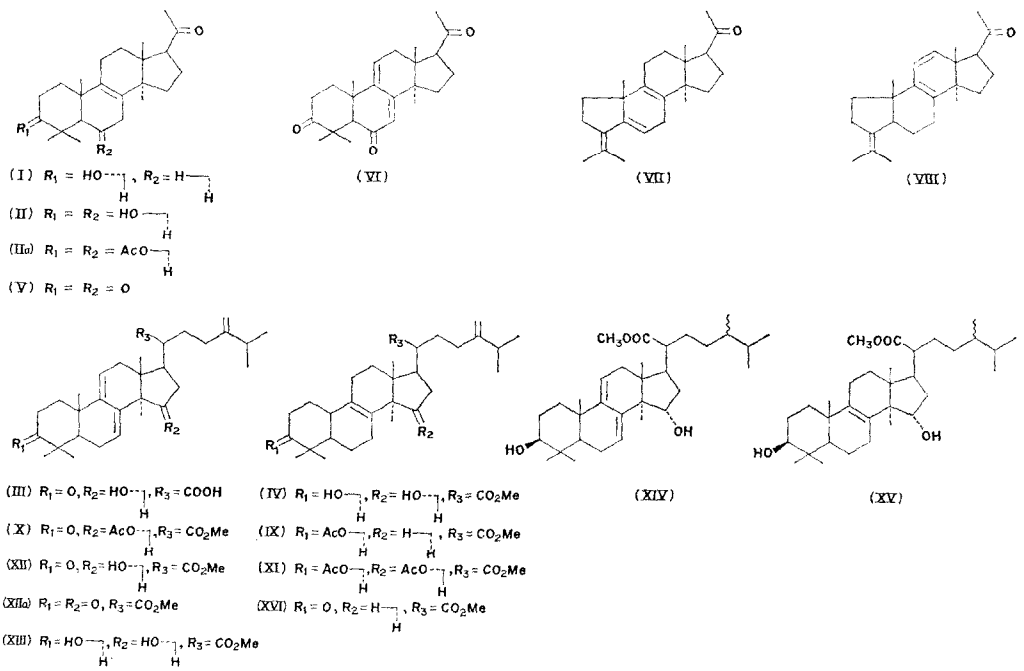
The presence of two acetate moieties in IIa was established by the existence of a new 6-H singlet at $\tau$7·99 in the NMR spectrum of the molecule.

TABLE 1. TRITERPENOIDS OF *F. officinalis*

| Compound | Ref. |
|---|---|
| Eburicoic acid (3$\beta$-Hydroxy-24-methylenelanost-8-en-21-oic acid) | 1 |
| Dehydroeburicoic acid (3$\beta$-Hydroxy-24-methylenelanosta-7,9(11)-dien-21-oic acid) | 2 |
| Sulfurenic acid (3$\beta$-15$\alpha$-Dihydroxy-24-methylenelanost-8-en-21-oic acid) | 3 |
| Officinalic acid* | 4 |
| Eburicol (3$\beta$-Hydroxy-24-methylenelanost-8-ene) | 5 |
| Eburicodiol (3$\beta$-21-Dihydroxy-24-methylenelanost-8-ene) | 3 |
| Eburical (3$\beta$-Hydroxy-24-methylenelanost-8-en-21-al) | 3 |
| 3$\alpha$-Hydroxy-4,4,14$\alpha$-trimethyl-$\Delta^8$-5$\alpha$-pregnene-20-one | 5 |

* Officinalic acid is a triterpenoic acid with a molecular formula of $C_{30}H_{44}O_6$.

The similarities in the NMR spectra of I and II, coupled with the above data, suggested that II could be a dihydroxy tetracyclic system possessing an 8,9 double bond and an acetyl side chain. Substantial evidence for this suggestion was obtained when it was found that




the optical rotary dispersion spectrum of II was again similar to that of I and consistent with a *trans*-C-D ring juncture possessing two angular methyl groups and a 17$\beta$-acetyl function.[6]

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Oxidation of II in benzene using aqueous $CrO_3$–acetic acid afforded two products, V and VI. The major product (V) had a MS molecular weight of 370 ($C_{24}H_{34}O_3$), and notably absent from the IR and NMR spectra were peaks associated with a hydroxyl function. In addition to the carbonyl adsorption at 1685 $cm^{-1}$ in II, a second adsorption appeared at 1715 $cm^{-1}$ in V. The above data are consistent with the oxidation of the secondary hydroxyl groups to ketones. The minor component (VI) had a MS molecular weight of 368 ($C_{24}H_{32}O_3$) and a UV maximum of 290 nm (log $\epsilon$ 4·0). The significance of this compound in the reaction mixture will be discussed later. Upon reaction with $POCl_3$ at 0°, II smoothly underwent a retropinacol rearrangement characteristic of 3$\beta$-hydroxy-4,4-dimethyl steroids to yield the isopropylidine derivative VII, evidenced by loss of NMR methyl signals at $\tau$9·28 and 9·14 and subsequent gain at $\tau$8·10 and 8·15, as well as dehydration of the second hydroxyl. The isopropylidine derivative also showed adsorption at 247 nm (log $\epsilon$ 4·1), characteristic of a heteroannular diene, indicating the second hydroxyl must be in the vicinity of either the first hydroxyl or the original double bond.

The assignment of the second hydroxyl to the 6-position becomes apparent when one considers the following line of reasoning: Tertiary positions 5, 17 and 21 were eliminated on the basis that the remaining hydroxyl must be secondary. The triketone V did not possess the properties of a 1,2 or a 1,3 diketone, thereby ruling out secondary positions 1,2, and 16; nor did V show adsorption above 200 nm, excluding enone positions 7 and 11 as possibilities. Moreover, position 15 was eliminated because a ketone derived from oxidation of a hydroxyl group in a five-membered ring should have an intense adsorption near 1745 $cm^{-1}$ in the infrared.[8] This leaves positions 6 and 12 as possible sites for the second hydroxyl function.

That the second hydroxyl group is located at the 6-position was substantiated by the results of the dehydration experiment using $POCl_3$. If the hydroxyl group was originally at C-12, then the triene obtained upon dehydration of II would have had the spectral properties of a homoannular conjugated diene as in VIII ($\lambda^{max}_{calc.}$ 273 nm). Since the UV spectral properties of the dehydration product VII were those of a heteroannular diene ($\lambda^{max}_{obs.}$ 247 nm, log $\epsilon$ 4·1, $\lambda^{max}_{calc.}$ 252 nm), position 6 was established as the likely position bearing the second hydroxyl function. This conclusion was further substantiated by the presence of VI ($\lambda^{max}_{obs.}$ 290 nm, log $\epsilon$ 4·0) in the reaction mixture obtained upon $CrO_3$-oxidation of II. The spectral properties of VI are characteristic of the highly conjugated 6-keto-$\Delta^{7,9(11)}$-steroid system ($\lambda^{max}_{reported}$ 292 nm, log $\epsilon$ 4·1),[9] which can be envisaged as having arisen from V by further oxidation of the 8,9 double bond to the more stable 7,9(11) system. The 8,9 double bond is known to be very susceptible to oxidative attack by a variety of reagents to give the 7,9(11) system.[10] In the presence of a 6-keto group, an 8,9 double bond would be expected to be more vulnerable to oxidative attack, since the 7,9(11) system formed would be further stabilized by conjugation with the 6-keto group.

The assignment of the 6-hydroxyl to the $\beta$ or axial configuration was based primarily on molecular rotation differences.[11] The 3$\beta$,6$\alpha$-dihydroxy system had a predicted molecular rotation of +184°, whereas the 3$\beta$,6$\beta$-dihydroxy system had a predicted value of +79°.

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Since II had an observed molecular rotation of +74°, the 6-hydroxyl was assigned the β-orientation. The same argument was applied successfully to the diacetate of II to yield a predicted value of +29° for the 3β,6β-diacetoxy derivative and an observed value of +40°, further substantiating the above conclusion. Easy elimination of a 6-axial hydroxyl under mild conditions is again consistent with our assignment. Thus, II was confirmed to be 3β,-6β-dihydroxy-4,4,14α-trimethyl-$\Delta^8$-5α-pregnene-20-one.

The second compound, a triterpenoic acid characterized as 3-keto-dehydrosulfurenic acid (III), was isolated in small quantities from the chloroform extracts of the whole fungus (isolation procedure is described in detail in experimental section). Chromatography on Florisil of the methyl acetyl derivatives of the weak-acid fraction afforded methyl acetyleburicoate (IX) followed by the new compound, methyl-3-keto-acetyldehydrosulfurenate (X) and finally methyl diacetylsulfurenate (XI). X would not crystallize but when allowed to stand overnight in 0·1 N ethanolic-KOH, long slender needles of methyl-3-ketodehydrosulfurenate (XII) crystallized from the solution.

Methyl-3-keto-dehydrosulfurenate (XII) had a mass spectrum molecular weight of 496, consistent with a formula of $C_{32}H_{48}O_4$. Like methyl eburicoate and methyl sulfurenate, XII possessed a terminal methylene, as evidenced by bands in the IR at 1650 and 890 $cm^{-1}$ and NMR resonances at τ5·25 (1 H) and 5·32 (1 H). The presence of a $\Delta^{7,9(11)}$ diene system in XII was inferred from a trio of bands in the UV spectrum at 236, 243 and 252 nm (log ε = 4·10, 4·23 and 4·0, respectively) and NMR signals at τ4·10 (1H) and 4·48 (1 H). An intense 1725 $cm^{-1}$ band in the IR spectrum of XII, which disappeared upon reduction with $NaBH_4$ was indicative of an aliphatic ketone. A 3450 $cm^{-1}$ band in the IR spectrum coupled with the fact that XII underwent $CrO_3$-oxidation to yield a diketone (XIIa) showed the existence of a secondary hydroxyl group.

The above data taken in conjunction with the fact that XII resisted hydrolysis by strong bases at elevated temperatures (characteristic of a C-21 carbomethoxy group) led to the speculation that XII could be a $\Delta^{7,9(11)}$-sulfurenic acid derivative. Furthermore, the fact that XII possessed no band characteristic of a 3β-hydroxyl (C–O stretch at 1035 $cm^{-1}$)* led to the possibility that XII was methyl-3-keto-dehydrosulfurenate.

The structure of XII was unambiguously established by the following sequence of reactions: $NaBH_4$ reduction of XII yielded the dihydro-derivative XIII, which possessed a 3β-hydroxyl, suggested by a band at 1033 $cm^{-1}$ in the IR spectrum.† Catalytic reduction of XIII in the presence of Adam's Catalyst afforded the tetrahydro-derivative XIV.

When known methyl sulfurenate (IV) was catalytically reduced over Adam's Catalyst and the product (XV) subsequently oxidized with *m*-chloroperbenzoic acid,[10] a compound resulted which was identical in every respect to XIV (m.p. and m.m.p., comparison of IR, NMR and MS). Accordingly, the structure of XII was established to be methyl-3-keto-dehydrosulfurenate.

The most unusual compounds found in *Fomes officinalis* are the degraded triterpenoids I and II. Their unusual nature is derived from the fact that they do not possess the normal eight or nine-carbon side chain found in most lanosterol related compounds. Furthermore, after a thorough survey of the literature, we have not been able to find another example of a naturally occurring 4,4,14-trimethyl-pregnene derivative. Whether 4,4,14-trimethyl-

* This conclusion is based upon a comparison of infrared spectra of a large number of compounds possessing the 4,4-dimethyl-3β-hydroxyl system.

† This is further supported by the results of model $NaBH_4$ reductions of methyl-3-keto-dihydroeburicoate (XVI) and other 3-keto-4,4-dimethyl steroids.

pregnene compounds have any hormonal effect in fungi similar to the unmethylated pregnene systems in animals is conjecture at this point.

## EXPERIMENTAL

Unless otherwise specified, m.ps (uncorrected) were determined on a Fisher–Johns m.p. Apparatus; optical rotations were measured in $CHCl_3$ solution on a Perkin–Elmer Model 141 Polarimeter; IR spectra recorded as KBr pellets on a Beckman IR-5a; 60 mc NMRs were recorded in $CDCl_3$ solution on a Varian A-60 spectrometer; 100 mc NMR recorded on a Varian XL-100 instrument; MS determined on a Perkin–Elmer Model 270 using PFK as an internal standard; UV spectra were run in 95% EtOH on a Cary Model 15 apparatus and ORD spectra were obtained on a Cary 60 instrument. All TLC was performed using silica gel G according to Stahl.

*Extraction of* F. officinalis *and treatment of extract.* Previously described by Epstein and Van Lear.[5]

*Isolation of* 3β,6β-*dihydroxy*-4,4,14α-*trimethyl*-$\Delta^8$-5α-*pregnene*-20-*one* (II). The neutral material (21 g) obtained by the above method was chromatographed on 900 g Florisil. Materials eluted from the column by less polar eluents than benzene were combined and rechromatographed on Florisil. The only identified component of this mixture was 30 mg of squalene, previously reported as a constituent of *Fomes officinalis*.[5] The substance eluted by solvent systems from benzene to 1:19 ether in benzene were rechromatographed on Florisil. Eburicol (150 mg) was eluted by benzene and 3α-hydroxy-4,4,14α-trimethyl-$\Delta^8$-5α-pregnene-20-one by 1:99 ether in benzene. The title compound (II) was eluted by 1:99 MeOH in ether and after five crystallizations from isopropyl ether yielded 220 mg of prisms, m.p. 188–189°; $[\alpha]_D^{24}$ + 20° ($c$ = 1·0); IR bands: 3350 and 1685 $cm^{-1}$; NMR methyl resonances at $\tau$ 9·28, 9·14, 9·05, 9·01 and 7·80 in ratio of 1:1:2:1:1; ORD: $[\alpha]_{600}$ + 54°, $[\alpha]_{589}$ + 54°, $[\alpha]_{313}$ + 895°, $[\alpha]_{268}$ − 574°, $[\alpha]_{247}$ 0° (c = 0·14 in dioxane); $M^+$ 374; TLC; ethyl acetate–petrol (3:2). Anal: Calc. for $C_{24}H_{38}O_3$; C, 76·96%; H, 10·23%. Found: C, 76·66%; H, 10·24%.

3β,6β-*Diacetoxy*-4,4,14α-*trimethyl*-$\Delta^8$-5α-*pregnene*-20-*one* (IIa). The acetate of II was prepared by treating II with acetic anhydride and pyridine. Recrystallization from MeOH gave the diacetate IIa, m.p. 258–260°; $[\alpha]_D$ +24° ($c$ = 0·24 in dioxane); IR bands: 1739, 1718 and 1247 $cm^{-1}$ ($CCl_4$); NMR methyl resonances at at $\tau$ 7·99 (6 H); $M^+$ − 60 at 398; TLC: acetone–$CHCl_3$ (1:9).

4,4,14α-*Trimethyl*-$\Delta^8$-5α-*pregnene*-3,6,20-*trione* (V) *and* 4,4,14α-*trimethyl*-$\Delta^{7,9(11)}$-5α-*pregnadiene*-3,6,20-*trione* (VI). To a solution of 20 mg of II in 5 ml of benzene was added 45 mg of $CrO_3$ in 3 ml of $H_2O$ and 1 ml HOAc. After stirring for 24 hr, benzene (25 ml) was added and the aqueous layer removed. The benzene layer was washed with portions of sat. $NaHCO_3$ solution and $H_2O$ and dried over $Na_2SO_4$. Removal of solvent *in vacuo* afforded a two component mixture which was separated by chromatography on 20 g of Florisil. Benzene–ether (1:3) eluted 12 mg of V, m.p. 165–168°; IR bands: 1685 and 1715 $cm^{-1}$; $M^+$ 370; only end adsorption in the UV. Ether eluted 4 mg of a light yellow compound (VII) which had a UV maximum at 290 nm (log $\epsilon$ = 4·0); $M^+$ 368; TLC: ethyl acetate–petrol (2:3).

14α-*Methyl*-3-*isopropylidene*-$\Delta^{5,8}$-*A-nor-pregnadiene*-20-*one* (VII). To a solution of 10 mg of II in 1 ml of pyridine at 0° was added 2 drops of $POCl_3$. The mixture was allowed to stand at refrigerator temp. for 2·8 hr, poured over ice and extracted with ether. Drying with $Na_2SO_4$ followed by removal of solvent yielded VII (6 mg) as the sole product. NMR methyl resonances at $\tau$ 8·10 (3 H) and 8·15 (3 H); UV maximum at 247 nm (log $\epsilon$ = 4·1); TLC: ethyl acetate–petrol (2:3).

*Isolation of methyl*-3-*keto-dehydrosulfurenate* (XII). The $CHCl_3$ extracts of the fungus were separated into neutrals, strong and weak acids by the usual methods. The weak acid fraction (8·5 g) after removal of $CHCl_3$ *in vacuo* was taken up in MeOH and allowed to stand for several days. The precipitate which formed was characterized as eburicoic acid (2·1 g). MeOH was removed from the mother liquor *in vacuo* and the residue (5·6 g) acetylated using acetic anhydride-pyridine. The resulting weak acid acetates were converted to methyl esters with $CH_2N_2$. Chromatography on Florisil afforded methyl acetyleburicoate (IX, 150 mg, benzene–ether 3:1) followed by a new compound, methyl-3-keto-acetyldehydrosulfurenate (X, 110 mg, benzene–ether 1:1) and finally methyl diacetylsulfurenate (XI, 140 mg, benzene–ether 1:3). All attempts to crystallize X failed, but when X was allowed to stand overnight in 0·1 N ethanolic-KOH, long slender needles of methyl-3-keto-dehydrosulfurenate (XII, 88 mg) crystallized from solution: m.p. 173–175°; $[\alpha]_D^{25}$ +67·5° ($c$ = 2·0); IR bands: 3450, 1725, 1700, 1650, 1060 and 890 $cm^{-1}$; several NMR methyl resonances in the region $\tau$ 8·8–9·25 (21 H); 6·32 (3H), 5·32 (1 H), 5·25 (1 H), 4·48 (1 H) and 4·10 (1 H); UV maxima at 236, 243 and 252 nm (log $\epsilon$ = 4·10, 4·23 and 4·00, respectively); $M^+$ 496; TLC: ethyl acetate–petrol (1:4).

*Methyl dehydrosulfurenate* (XIII). To a solution of 60 mg of XII in 5 ml of 95% EtOH was added 100 mg of $NaBH_4$ and the mixture stirred for 2·5 hr at room temperature. The product was worked up by diluting with a large volume of 0·1 N $H_2SO_4$ and extracting with ether. Removal of solvent *in vacuo* followed by crystallization of the residue from aqueous MeOH afforded 52 mg of feathers, m.p. 194–196°; $[\alpha]_D^{25}$ +62·5° ($c$ = 0·78); IR bands: 3350, 3400, 1710, 1650, 1060, 1033 and 890 $cm^{-1}$; $M^+$ 498; TLC: ethyl acetate–petrol (1:4).

*Methyl-3,15-diketo-dehydrosulfurenate* (XIIa). The title compound (XIIa) was prepared by oxidizing 40 mg of XII in 20 ml of benzene with 80 mg of $CrO_3$ in 5 ml of $H_2O$ and 3 ml HOAc. Crystallization from aq. MeOH after work-up yielded needles (28 mg), m.p. 178–180°; $[\alpha]_D^{24}$ +55° ($c$ = 0·39); IR bands: 1725 and 1710 $cm^{-1}$; $M^+$ 494; TLC; ethyl acetate–petrol (1:4).

*Attempted hydrolysis of methyl-3-keto-dehydrosulfurenate.* A solution of 11 mg of XII in 0·1 N ethanolic–KOH was refluxed for 4 hr and worked-up by diluting with a large vol. of $H_2O$ and extracting with ether. Removal of the ether *in vacuo* and crystallization of the residue from MeOH yielded starting material, m.p. and m.m.p. 173–175°.

*Methyl-24,28-dihydro-dehydrosulfurenate* (XIV). To a suspension of 5 mg of $PtO_2$ in 5 ml HOAc was added a solution of 22 mg of methyl dehydrosulfurenate (XIII) in 25 ml HOAc and the resulting mixture agitated in the presence of $H_2$ until uptake was complete (2 hr). The catalyst was removed by filtration and the solution diluted with a large volume of $H_2O$ and extracted with ether. After washing the ether layer with sat. $NaHCO_3$ solution and $H_2O$, the solvent was removed *in vacuo* and the residue crystallized from MeOH to yield XIV (15 mg), m.p. 205–208°; $[\alpha]_D^{24}$ +65° ($c$ = 0·62); $M^+$ 500. No bands attributable to a terminal methylene could be found in the NMR or IR spectra of XIV.

*Methyl-24,28-dihydrosulfurenate* (XV). Methyl sulfurenate (IV, 60 mg) was hydrogenated according to the above procedure and crystallized from MeOH to yield 55 mg of XV, m.p. 204–205° (reported m.p. 202–203°).[12]

*Oxidation of methyl-24,28-dihydrosulfurenate with* m-*chloroperbenzoic acid.* To 55 mg of methyl dihydrosulfurenate (XV) in 20 ml of $CHCl_3$ was added 200 mg of technical grade *m*-chloroperbenzoic acid and the reaction set aside at room temp. for 5 hr. After the addition of 10 ml HOAc, the solution was refluxed 1 hr. The $CHCl_3$ was washed with sat. $NaHCO_3$ solution followed by $H_2O$ and the solvent removed *in vacuo*. Two crystallizations of the resulting residue from MeOH afforded 34 mg of a compound which was identical in every respect to XIV (m.p. and m.m.p., comparison of IR NMR and MS spectra).

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