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ISOLATION OF LANOSTA-8,25-DIEN-3β-OL FROM THE FUNGUS *FOMES FASTUOSUS*

AMOLAK C JAIN and SUSHIL K GUPTA

Department of Chemistry, University of Delhi, Delhi-110007, India

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Key Word Index—*Fomes fastuosus*, Polyporaceae, wood-rotting fungus on *Emblica officinalis*, lanosta-8,25-dien-3β-ol

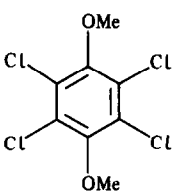
Abstract—A new lanostane triterpene has been isolated from the fungus *Fomes fastuosus* which causes wood-rotting of *Emblica officinalis* The compound is assigned the structure lanosta-8,25-dien-3β-ol on the basis of spectral data and correlation with lanosterol

INTRODUCTION

The medicinally important tree *Emblica officinalis* is sometimes observed locally to be infected with wood-rotting fungi The one affecting mid-stem is *Ganoderma australe* which has recently been found [1] to produce ergosterol palmitate, ergosta-7,22-dien-3-one, ergosterol and lanosta-7,9(11),24-trien-3β,21-diol The other fungus growing at the base is *Fomes fastuosus* (specimen No 8447, incorporated in Forest Research Institute Herbarium, Dehradun) The latter fungus was examined earlier by Singh and Rangaswami [2] who reported the isolation of ergosterol and 2,3,5,6-tetrachloro-1,4-dimethoxybenzene (**1**)

RESULTS AND DISCUSSION

The petrol extract of *F fastuosus* was found to be a mixture on TLC The residue on column chromatography yielded besides the chlorobenzene derivative (**1**) a new triterpene compound It is assigned the structure lanosta-8,25-dien-3β-ol (**2**) on the following grounds The positive Liebermann–Burchard test showed it to be triterpenoid in nature and the elemental analysis coupled with a molecular ion peak at *m/z* 426 in the mass spectrum gave its molecular formula as $C_{30}H_{50}O$ That it belonged to the lanostane group, which is usually the case with fungal triterpenes, was shown by its mass spectrum Thus, it exhibited two important mass fragments, one at *m/z* 315 due to loss of the side chain alone (see formula **3**) and another at *m/z* 273 which represents further loss of 42 amu due to loss of carbons at positions C-15, C-16 and C-17 [3] (see formula **4**) The only oxygen atom present in the molecule was found to be in the form of a hydroxyl group as it formed a monoacetate with MS *m/z* 468 $[M]^+$, 408 $[M - AcOH]^+$ and a ^1H NMR singlet at δ 2 01 due to one acetyl group Moreover, the hydroxyl group was present in the position C-3β as shown by a



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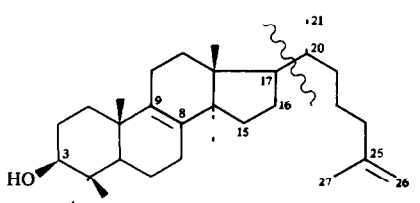

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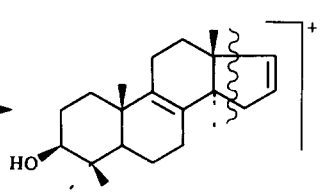
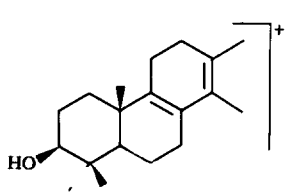


3 *m/z* 315



4 *m/z* 273

characteristic ^{1}H NMR multiplet at δ 3 22 due to the 3α-hydrogen [4] The UV spectrum did not show any chromophore but the presence of two double bonds is indicated by the molecular formula Obviously, these two double bonds are isolated Since in the formation of mass fragments of *m/z* 315 and 273 (see above), the side chain loss of 111 amu was smaller by two hydrogen atoms than a saturated C_8 side chain, it follows that one double bond was present in the side chain Consequently, the other double bond was in the ring system The presence of an olefinic methyl group at δ 1 69 in the ^{1}H NMR spectrum and IR bands at 1630, 1610 and 878 cm^{-1} due to an unsymmetrically disubstituted ethylenic system indicated that the double bond in the side chain occupied a terminal position at C-25 This was further supported by the appearance in the ^{1}H NMR spectrum of a doublet of one olefinic proton at δ 4 68 and a quartet of one olefinic proton at 4 55 (the smaller δ values of the olefinic protons as compared to the usual olefinic signals at 5 3 is significant and points to the same environment) Since the ^{1}H NMR spectrum showed only two olefinic protons in the side chain, the second double bond in the ring should have no proton, and that is possible only if there is a double bond at the C-8 position Finally the ^{1}H NMR spectrum showed five tertiary methyl groups as singlets at δ 0 75, 0 78, 0 82, 0 95, 1 03 and one secondary methyl group as a doublet at 0 93 (J = 3 Hz) as required by the lanostane skeleton Hence the new triterpene can be assigned the structure **2** which was supported by correlation with lanosterol (lanosta-8,24-dien-3β-ol) as follows Catalytic hydrogenation of compound **2** acetate gave a product which was identical by mmp, TLC and its IR spectrum with 3-acetoxylanost-8-ene obtained by reduction of lanosteryl acetate Thus, compound **2** represents a new naturally occurring triterpene isomeric to lanosterol The presence of a double bond in the C-25 position is rather rare and has been noted only recently in the case of (24*S*)-3-methoxy-5-lanosta-9(11),25-dien-24-ol [5]

The identification of the chlorocompound **1** was made from its mass spectrum which showed four mass ions (*m/z* 282, 280, 278 and 276) above the molecular ion (*m/z* 274) and many mass fragments differing in 2 amu

EXPERIMENTAL

Unless stated otherwise, mps are uncorr, petrol used had a boiling range 60–80°, silica gel was used for column chromatography and TLC, R_f values refer to TLC for which the solvent systems were (A) petrol, (B) C_6H_6, UV spectra were recorded in MeOH, ^{1}H NMR spectra (90 MHz) chemical shifts are expressed in the δ scale downfield from TMS as internal standard

Extraction of Fomes fastuosus Air-dried fungus (200 g) was powdered and extracted exhaustively with petrol This extract on evaporation gave a light yellow oil (1 3 g) which when left overnight in petrol (10 ml) in the refrigerator yielded 2,3,5,6-tetrachloro-1,4-dimethoxybenzene (**1**) as colourless needles (100 mg) The remaining soln was found to be a complex mixture on TLC and hence subjected to column chromatography when the following fractions were obtained

2,3,5,6-*Tetrachloro*-1,4-*dimethoxy benzene* (**1**) This compound was obtained in the initial fractions on elution from the column by petrol It crystallized from petrol as colourless needles (250 mg), mp and mmp with a synthetic sample [6] 166–167° (lit [2] mp 164–165°), R_f 0 95 (solvent A) The IR and ^{1}H NMR spectral data agreed with that reported previously [2], MS *m/z* 282 $[M+8]^+$, 280 $[M+6]^+$, 278 $[M+4]^+$, 276 $[M+2]^+$ (which is the most intense peak in the molecular ion region), 274 $[M]^+$, 265, 263, 261, 259, 218, 213, 211, 209, 207, 203, 189, 174, 164, 162, 149, 135, 121, 119, 109, 107, 105, 95, 93, 91, 87, 85, 83, 81, 78, 69, 57 and 55

Lanosta-8,25-*dien*-3β-*ol* (**2**) This compound was eluted from the column by petrol–C_6H_6 (3 1) followed by petrol–C_6H_6 (1 1) It crystallized from MeOH as colourless needles (120 mg), mp 204°, positive LB test, R_f 0 4 (solvent B) (red spot on H_2SO_4 spray and heating at 120° for 10 min), $[\alpha]_D$ +23 8° (*c* 1 4, $CHCl_3$), IR ν_{max}^{KBr} cm^{-1} 3380, 2920, 1630, 1610, 1445, 1376, 1183, 1032, 878, UV λ_{max}^{MeOH} nm 205 5, ^{1}H NMR ($CDCl_3$) δ 0 75, 0 78, 0 82, 0 95, 1 03 (3H each, 5*s*, 5 × tert Me), 0 93 (3H, *d*, J = 3 Hz, sec Me), 1 70 (3H, *d*, J = 1 Hz, olefinic Me), 3 22 (1H, *br m*, H-3α), 4 55 (1H, *q*, J = 1 5 Hz, olefinic H), 4 68 (1H, *d*, J = 3 Hz, olefinic H), MS *m/z* 426 $[M]^+$, 411 $[M-Me]^+$, 408 $[M-H_2O]^+$, 393 $[M-Me-H_2O]^+$, 316, 315 $[M-$side chain$]^+$, 273 $[M-$side chain $-42]^+$, 272, 257, 247, 234, 220, 218, 207, 189, 175, 161, 147, 135, 121, 109, 107, 95, 93, 81, 69 and 55 [base ion]$^+$ (Found C, 84 3, H, 11 8 $C_{30}H_{50}O$ requires C, 84 5, H, 11 7%)

Compound **2** (30 mg) was acetylated at room temp with Ac_2O–pyridine to yield a monoacetate which crystallized from MeOH as colourless needles, mp 185–187°, $[\alpha]_D$ +25 4° (*c* 1 35, $CHCl_3$), R_f 0 8 (solvent B), ^{1}H NMR ($CDCl_3$) δ 0 80, 0 85, 0 94, 1 02 (18H, 4*s*, 6 × Me), 1 68 (3H, *s*, 1 olefinic Me), 2 01 (3H, *s*, OAc), 4 45 (1H, *br m*, H-3α), 4 5, 4 62 (*m*, 2H, olefinic Hs), MS *m/z* 468 $[M]^+$, 408 $[M-AcOH]^+$, 365, 297 $[M-AcOH-$side chain$]^+$, 257, 249, 229, 218, 217, 216, 215, 203, 189, 187, 185, 161, 147, 146, 135, 133, 121, 119, 109, 107, 105, 95, 83, 81, 69, 57, 55, 43 and 41

3β-*Acetoxylanost*-8-*ene* Commercial 'pure lanosterol' (purchased from ICN Pharmaceuticals, New York, U S A) (250 mg) was treated with Ac_2O (5 ml) and pyridine (1 ml) and kept at room temp overnight The product was chromatographed and eluted with petrol–C_6H_6 (3 1) The eluate crystallized from $CHCl_3$–MeOH mixture as colourless crystals (120 mg), mp 105–108°, R_f 0 85 (solvent B) Its ^{1}H NMR spectrum and lower mp (lit mp 113–114° [4, 7], 129–130° [8]) suggested that it was still a mixture of 3β-acetoxylanosta-8,24-diene and 3β-acetoxylanost-8-ene as also mentioned by McGhie *et al* [8] Since the latter impurity did not interfere in our desired reaction, it was directly hydrogenated as such A soln of the above acetate (80 mg) in EtOAc was stirred with Pd–charcoal (40 mg), in contact with H_2 at atmospheric pressure for 3 hr The catalyst was filtered and the soln evaporated to dryness The residue crystallized from $CHCl_3$–MeOH mixture to give 3β-acetoxylanost-8-ene as colourless crystals (40 mg), mp 120–121° (lit mp 120–121° [7], 117–118° [9]), R_f 0 85 (solvent B) IR ν_{max}^{KBr} cm^{-1} 2955, 2875 (sh), 2840 (sh), 1728 (ester carbonyl), 1640, 1620, 1450, 1380, 1268 and 1035, ^{1}H NMR ($CDCl_3$) δ 0 62, 0 76, 0 83, 0 93 (24H, 4*s*, 8 × Me), 1 95 (3H, *s*, OAc), 4 41 (1H, *br m*, H-3α)

A soln of compound **2** acetate (40 mg) obtained from natural **2** was dissolved in EtOAc (10 ml) and reduced with H_2 in the presence of Pd–charcoal (20 mg) and the product worked up as described above The product crystallized from $CHCl_3$–MeOH mixture as colourless crystals (20 mg), mp and mmp identical with the above sample of 3β-acetoxylanost-8-ene (120–121°) superimposable IR spectrum, and co-TLC with those of the authentic sample

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A BUTENOLIDE ATYPICAL OF THE RANUNCULACEAE: AQUILEGIOLIDE FROM *AQUILEGIA ATRATA* (VAR. *ATROVIOLACEA*)

ANTONIO GUERRIERO and FRANCESCO PIETRA

Istituto di Chimica, Università di Trento, 38050 Povo-Trento, Italy

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Key Word Index—*Aquilegia atrata*, Ranunculaceae, butenolides

Abstract—Roots of *Aquilegia atrata* have afforded 6β-hydroxy-2(6,7-dihydro-7aβH)-benzofuranone (aquilegiolide) and its 7aαH-isomer, or their enantiomers, two butenolides atypical of the Ranunculaceae Hot aqueous 20% sulphuric acid rapidly equilibrates the two isomers in a 1 4 ratio

INTRODUCTION

Ranunculaceae have afforded some C_5 α,β-unsaturated lactones [1, 2] among which, however, only protoanemonin (**1**) [3, 4] is not an artifact of the extraction process [5]

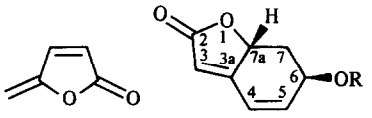
It is interesting, therefore, to have now found in *Aquilegia atrata*, var *atroviolacea*, of the Trentino area, two ring-fused butenolides (**2** and **4** or their enantiomers), which are atypical of the Ranunculaceae

One of these butenolides (**4**) has already been described (but this needs some correction here) as the only butenolide from acid hydrolysis of menisdaurin, a nitrile glucoside isolated from *Menispermum dauricum* (Menispermaceae) [6]

RESULTS AND DISCUSSION

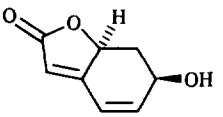
Roots were lyophilized and extracted first with ethyl ether and then with methanol at reflux The residue from solvent evaporation was column chromatographed at medium pressure on silica gel with ethyl ether, whereby little pure **2** and **4** were obtained from the first and the last fraction, respectively Efficient separation of **2** and **4** was then completed by reverse phase HPLC

Compound **2** (0 01% in fresh roots) was obtained as colourless needles The conjugated chromophore is suggested by the 253 nm absorption, while the IR spectrum clearly shows hydroxyl, conjugated lactone carbonyl, and olefine absorptions The high-resolution mass spectrum revealed the elemental composition of the molecular ion, whilst linked scans showed the loss, from the molecular ion, of water, formyl, ketene and carbon dioxide and, from $[M-CHO]^+$, of carbon monoxide The fragmentation pattern is clearly in accordance with structure **2**, which is further supported by MS deuteriation experiments with deuteriated methanol These showed incorporation of one deuterium in all ions except *m/z* 134, clearly in accordance with alcoholic hydrogen exchange for an hydroxyl group at either C-6 or C-7 In fact, loss of either water or of DHO from the molecular ion introduces a $\Delta^{6,7}$ double bond Moreover, all carbon resonances, with the expected multiplicities, could be observed for **2** Finally, the



1

2 R = H
3 R = Ac



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