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DAMMARANE TRITERPENES FROM THE RESIN OF *BOSWELLIA FREERANA*

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Key Word Index—*Boswellia freerana*, Burseraceae, triterpenes, 3β-acetoxy-16(*S*),20(*R*)-dihydroxydammar-24-ene

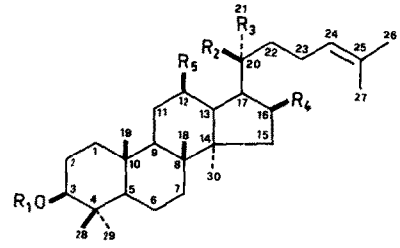
Abstract—The resin of *Boswellia freerana* afforded in addition to the known 3β,20(*S*)-dihydroxydammar-24-ene, its 3-acetyl derivative and (20*S*)-protopanaxadiol, a new triterpene that was characterized as 3β-acetoxy-16(*S*),20(*R*)-dihydroxydammar-24-ene on the basis of chemical and physico-chemical evidence

INTRODUCTION

Some species of *Boswellia* (Burseraceae) growing mainly in the tropical and subtropical regions are a source of 'incense', a resin which contains a rich mixture of terpenoids

Recently a chemical investigation on the exudate of *B freerana*, distributed in the northern regions of Somalia, led to the isolation of epilupeol and lupeol [1], in addition to 10 monoterpenes, among which the most abundant component was *p*-cymene [2]

In the present study we have examined the more polar fraction of this resin This has resulted in the isolation of a new triterpene which was shown to be 3β-acetoxy-16(*S*),20(*R*)-dihydroxydammar-24-ene (**1**) As detailed in the Experimental section, four compounds have been isolated by chromatography of concentrates of the chloroform extracts from the exudate of *B freerana* From physical and spectroscopic data, and also by comparison with authentic samples, three of these compounds were found to be known products, namely 3β,20(*S*)-dihydroxydammar-24-ene (**3**), its 3-acetylderivative (**4**) and (20*S*)-protopanaxadiol (**5**) The structure determination of the remaining compound (**1**) is described



	R_1	R_2	R_3	R_4	R_5
1	Ac	Me	OH	OH	H
2	H	Me	OH	OH	H
3	H	OH	Me	H	H
4	Ac	OH	Me	H	H
5	H	OH	Me	H	OH
6	Ac	Me	OH	H	H
7	Ac	Me	OH	O-tosyl	H

RESULTS AND DISCUSSION

Compound **1** ($C_{32}H_{54}O_4$, from analytical data) crystallized from MeOH, mp 183–185° An intense fragment ion at *m/z* 442 $[M-60]^+$ indicated the presence of an acetoxy group which was corroborated by IR (ν_{max} 1735 cm^{-1}), and ^1H NMR spectral data [$CDCl_3$, δ2 03 (3H, *s*, CH_3CO–) and 4 50 (1H, *dd*, *J* = 10 and 5 Hz, =CHOAc)] showed it to be secondary The remaining two oxygen atoms must be present as hydroxy groups, as indicated by the mass spectrum (*m/z* 484 $[M-H_2O]^+$ and 466 $[M-2H_2O]^+$), one of which was secondary (δ4 47, 1H, *dt*, *J* = 5 and 5 5 Hz)

In the ^1H NMR spectrum eight methyl signals appeared as singlets at δ1 70 and 1 63 (3H each, vinyl methyls), 1 28 (3H, methyl on an oxygen-bearing carbon atom), 1 03 (3H) and 0 86 (12H) A vinyl proton on a trisubstituted double bond (δ5 15, 1H, *bt*, *J* = 7 Hz) was shown to be allylically coupled with the methyls by homonuclear decoupling experiments, thus establishing the presence of a terminal $-CH_2-CH=C(CH_3)_2$ group

Alkaline hydrolysis of **1** afforded the triol **2**, $C_{30}H_{52}O_3$ (from analytical data), ^1H NMR δ4 50 (1H, *dt*, *J* = 5 and 5 5 Hz), 3 18 (1H, *dd*, *J* = 10 and 5 Hz), 1 67, 1 61, 1 28, 1 00, 0 95, 0 84, 0 83 and 0 76 (3H each, singlets)

The above data strongly suggested that **1** could be a monoacetylderivative of a trihydroxytriterpene having a tetracyclic skeleton Chemical support for this hypothesis was obtained by treatment of **1** with *p*-toluensulphonyl chloride and sodium borohydride reduction of the resulting ester which afforded in good yields 3β-acetoxy-20(*R*)-hydroxydammar-24-ene (**6**) identified by comparison of its physical properties with those reported in the literature [3] From this result structure **1** was proved except for the location of the secondary alcoholic function at C-16, which was assigned as follows

In the ^1H NMR spectrum of **2** the H-16 signal appeared at δ4 50 as a double triplet (*J* = 5 and 5 5 Hz) and by

difference double-resonance experiments was shown to be coupled with three protons resonating as double doublets at δ1 79 (J = 10 and 5 5 Hz), 1 65 (J = 11 and 5.5 Hz) and 1 50 (J = 11 and 5 Hz), which by irradiation collapsed into three doublets, thus establishing the presence in **2** of the following partial structure –CH–CH–CH(OH)–CH_2–C– Incorporation of this structure in the dammarane skeleton is compatible only by locating the hydroxy group at C-16 Assignment of the *S*-configuration to this carbon atom was made by application of the GC modification of the Horeau method according to Brooks and Gilbert [4] on the compound **1**

The co-occurrence in the resin of *B freerana* of **1** and **3–5**, having opposite configurations at C-20 is quite surprising However, the possibility of an epimerization during the isolation procedure cannot be excluded, taking into account that the hydroxy group at C-20 of dammarane-type triterpenes with a double bond at C-24 were shown to epimerize easily [5]

EXPERIMENTAL

Mps are uncorr Specific rotations and IR spectra were measured in $CHCl_3$ ^{1}H NMR spectra at 250 MHz were obtained in $CDCl_3$ soln using TMS as int standard MS were determined at 70 eV

Extraction and purification of compounds **1**, **3**, **4** *and* **5** The exudate of *B freerana* (50 g), kindly supplied by Incense National Agency (Somalia), was Soxhlet-extracted with $CHCl_3$ for 36 hr and the extract concd The residue (43 g) was chromatographed on a silica gel column using as eluent solvent mixtures in increasing polarities from C_6H_6 to C_6H_6–Et_2O (7 3) Elution with C_6H_6–Et_2O mixtures in 9 1, 8 2 and 7 3 ratios afforded fractions A (1 g), B (0 16 g) and C (0 48 g), respectively, which were used for the isolation of compounds **1**, **3**, **4** and **5** as described below

Fraction A was further fractionated by prep TLC (silica gel, C_6H_6–Et_2O, 9 1) to give **3** (0 150 g) and **4** (0 390 g) identified by comparison of their properties with those of authentic samples

Fraction B, by HPLC (RP-18, CH_3CN), afforded compound **1** (0 030 g)

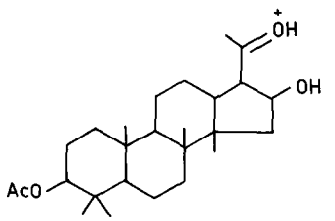
Fraction C was rechromatographed by prep TLC (silica gel, C_6H_6–Et_2O, 2·3) to give **5** (0 180 g) identified by comparison with an authentic sample

3β-Acetoxy-16(S),20(R)-*dihydroxydammar*-24-*ene* (**1**) Colourless crystalline compound, mp 183–185° (MeOH), $[\alpha]_D$ +25 (*c* 0 9) EIMS, *m/z* (rel int) 484 $[M-H_2O]^+$ (6), 466 $[M-2H_2O]^+$ (35), 451 $[M-2H_2O-CH_3]^+$ (1), 442 $[M-CH_3COOH]^+$ (12), 423 $[M-H_2O-CH_3COOH]^+$ (4), 419 [**a**]$^+$ (12), 402 [**a** – OH]$^+$ (7), 357 [**b**]$^+$ (10), 341 $[\mathbf{a}-H_2O-CH_3COOH]^+$ (25), 297 $[\mathbf{b}-CH_3COOH]^+$ (14), 249 [c – H]$^+$ (7), 189 $[\mathbf{c}-CH_3COOH-H]^+$ (75), 109 [side chain – H_2O]$^+$ (100) IR and NMR data are reported in the Results and Discussion (Found C, 76 28, H, 10 71% $C_{32}H_{54}O_4$ requires C, 76 45, H, 10 83%)

3β,16(S),20(R)-*Trihydroxydammar*-24-*ene* (**2**) Treatment of compound **1** (15 mg) with 10% KOH in 80% EtOH for 2 hr under reflux yielded compound **2** (12 mg), mp 212–214°, $[\alpha]_D$ +17 8 (*c* 1 0) EIMS *m/z* 442 $[M-H_2O]^+$ The ^{1}H NMR spectrum is reported in the Results and Discussion (Found C, 78 42, H, 11 40% $C_{30}H_{52}O_3$ requires C, 78 21, H, 11 38%)

Reduction of compound **1** A soln of **1** (15 mg) and *p*-toluensulphonyl chloride (8 mg) in dry pyridine (2 ml) was kept at room temp for 16 hr Following the usual work-up crude compound **7** (18 mg) was isolated and, without further purification, dissolved in $CHCl_3$ (0 5 ml) After addition of $NaBH_4$ (4 mg) in H_2O (0 2 ml) and Adogen 464 (Ega Chemie, 2 mg), the mixture was stirred at room temp for 4 hr The residue obtained after evaporation of the organic phase, purified by TLC (silica gel, C_6H_6–Et_2O 8 2), afforded 6 mg of 3β-acetoxy-20(*R*)-hydroxydammar-24-ene (**6**), identified by comparison of its properties (mp, $[\alpha]_D$, NMR and IR) with those reported in ref [3]

Application of the GC modification of the Horeau method to compound **1** Compound **1** (2 mg) in dry pyridine (4 μl) was treated with an excess of (±)-α-phenylbutyric anhydride and was kept at 40° for 1 5 hr Conventional work-up [4] led to the identification by GC of a preponderance of (*R*)-α-phenylbutyric acid



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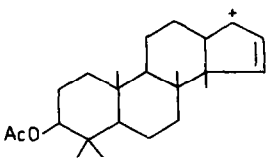

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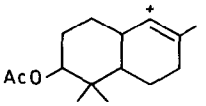

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REFERENCES

1 Proietti, G, Strappaghetti, G and Corsano, S (1981) *Planta Med* **4**, 417
2 Strappaghetti, G, Corsano, S, Craniero, A and Proietti, G (1982) *Phytochemistry* **21**, 214
3 Baker, P M, Barreiro, E J L and Gilbert, B (1976) *Phytochemistry* **15**, 785
4 Brooks, C J W and Gilbert, J D (1973) *J Chem Soc Chem Commun* 194
5 Nagari, Y, Tanaka, O and Shibata, S (1971) *Tetrahedron* **27**, 881