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TETRACYCLIC TRITERPENOIDS FROM *GLYCOSMIS ARBOREA*

AJIT K. CHAKRAVARTY, BINAYAK DAS, KAZUO MASUDA* and HIROYUKI AGETA*

Indian Institute of Chemical Biology, Calcutta-700 032, India; *Showa College of Pharmaceutical Sciences, Machida, Tokyo 194, Japan

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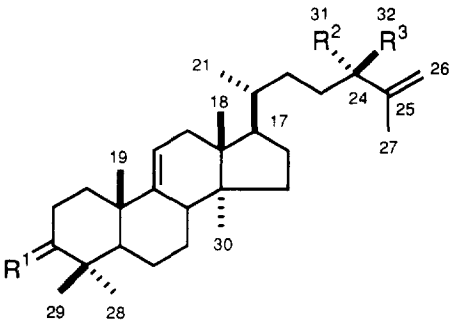
Key Word Index—*Glycosmis arborea*; Rutaceae; triterpene alcohols.

Abstract—Two new tetracyclic triterpene alcohols, along with (24*S*)-24-methyl-5α-lanosta-9(11),25-dien-3β-ol and 24,24-dimethyl-5α-lanosta-9(11),25-dien-3β-ol, were isolated from the petrol extract of the overground part of *Glycosmis arborea* and their structures were elucidated to be (24*S*)-24-methyl-5α-lanosta-9(11),25-dien-3α-ol and 24,24-dimethyl-5α-lanosta-9(11),25-dien-3α-ol on the basis of 2D NMR and mass spectral analyses.

INTRODUCTION

Glycosmis arborea (Roxb.) DC, an Indian medicinal plant popularly known as Ashshoura, Bon-nimbu, etc., is locally used for the treatment of fever, liver complaints and certain other diseases [1]. It has been shown to contain several alkaloids [2–5] and pentacyclic triterpenoids [6–8]. In a re-investigation of the plant, we have isolated four minor tetracyclic triterpenoids (**1a–4a**) in the petrol extract of the overground part of the plant. Their structures were elucidated as (24*S*)-24-methyl-5α-lanosta-9(11),25-dien-3α-ol (**1a**), (24*S*)-24-methyl-5α-lanosta-9(11),25-dien-3β-ol (**2a**), 24,24-dimethyl-5α-lanosta-9(11),25-dien-3α-ol (**3a**) and 24,24-dimethyl-5α-lanosta-9(11),25-dien-3β-ol (**4a**) on the basis of 2D NMR and mass spectral studies on their acetates (**1b–4b**). Of the four compounds, **1a** and **3a**, were new and the other two compounds were recently reported from *Neolitsea aciculata* [9]. We report herein on the isolation and structural elucidation of the compounds.



1a R^1 = α OH, β H; R^2 = Me; R^3 = H
1b R^1 = α OAc, β H; R^2 = Me; R^3 = H
2a R^1 = α H, β OH; R^2 = Me; R^3 = H
2b R^1 = α H, β OAc; R^2 = Me; R^3 = H
3a R^1 = α OH, β H; R^2 = R^3 = Me
3b R^1 = α OAc, β H; R^2 = R^3 = Me
4a R^1 = α H, β OH; R^2 = R^3 = Me
4b R^1 = α H, β OAc; R^2 = R^3 = Me

RESULTS AND DISCUSSION

Repeated chromatographic purification of the petrol extract of the plant over silica gel and neutral alumina yielded a triterpene fraction which was further purified through its acetate resulting in the isolation of, besides the previously reported arborinyl acetate and isoarborinyl acetate, a sticky substance containing a number of triterpenoid acetates and ketones. Repeated preparative HPLC (C_{18} column) yielded pure triterpenoid acetates **1b–4b**, besides arborinyl acetate, isoarborinyl acetate and arborinone.

The high resolution mass spectra of **1b** and **2b** showed the molecular formula for both compounds to be $C_{33}H_{54}O_2$. Both compounds showed identical mass spectral fragmentation patterns with slight differences in the intensities of the peaks. It was therefore presumed that the compounds must be stereoisomeric. The 500 MHz ^{1}H NMR spectra of both **1b** and **2b** (Table 1) displayed very close signals for eight methyl groups of which two are secondary and six tertiary including one vinylic, one trisubstituted vinylic methine proton and two disubstituted vinylic methylene protons. Moreover, while the spectrum of **1b** showed a carbinyl proton signal as a broad singlet overlapped with the broad singlet for the vinylic methylene protons, the carbinyl proton signal of **2b** appeared as a double doublet with J = 11.7 and 4.3 Hz, clearly demonstrating that the two compounds were epimeric at the carbinyl carbon.

A comparison of the ^{13}C NMR spectra of **1b** and **2b**

Table 1. ^{1}H NMR data (500 MHz, $CDCl_3$) for compounds **1b–4b**

H	1b	2b	3b	4b
H_3-18	0.649	0.638	0.644	0.631
H_3-19	1.070	1.065	1.068	1.063
H_3-21	0.878 (*d*, 6.1)*	0.869 (*d*, 6.1)	0.875 (*d*, 6.4)	0.865 (*d*, 6.5)
H_3-27	1.641	1.638	1.689	1.684 (*d*, 0.5)
H_3-28	0.852	0.863	0.852	0.863
H_3-29	0.927	0.888	0.927	0.887
H_3-30	0.776	0.729	0.775	0.728
H_3-31	1.000 (*d*, 7.0)	0.997 (*d*, 7.0)	1.014†	1.014
H_3-32	—	—	1.020†	1.014
H-3	4.665 (*br s*)	4.482 (*dd*, 11.7, 4.3)	4.658 (*br s*)	4.481 (*dd*, 11.0, 4.3)
H-11	5.235 (*d*, 6.1)	5.223 (*d*, 6.1)	5.233 (*d*, 6.1)	5.220 (*d*, 5.8)
H_2-26	4.665 (*br s*)	4.664 (*br s*)	4.661 (*d*, 1.0)	4.657 (*d*, 1.8)
			4.724 (*dd*, 1.5, 1.5)	4.721 (*m*)
OAc	2.066	2.051	2.065	2.053

*Figures in parentheses denote the coupling constants in Hz.
†May be interchanged.

(Table 2) revealed that the spectra were different with respect to the signals for C-1 to C-5 and C-29. The signals for C-1 and C-5 were shielded by *ca* 5 ppm, the signals for C-2 and C-4 were shifted upfield by *ca* 1 ppm and those of C-3 and C-29 were deshielded, respectively, by *ca* 2.5 and *ca* 5 ppm in **1b**. This is consistent with 3α- and 3β-acetoxytriterpenoid structures for **1b** and **2b**, respectively.

The skeleton of the compounds was determined by detailed analyses of the 2D NMR spectra of **1b** as detailed below. For unambiguous assignment of ^{13}C and ^{1}H chemical shifts of **1b**, PND, DEPT, ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY, HSQC and HMBC spectra were recorded. The two- and three-bond correlations of various protons with carbons obtained from the HMBC spectrum are summarized in Table 3. The correlations observed for eight methyl proton signals clearly led to the formulation of three partial structures **A**, **B** and **C**. Further correlations of the vinylic methine proton (H-11) signal joined the structures **A** and **B** to form structure **D**. Connectivities shown by dotted lines in the partial structure **D** were then established from the correlations observed for the methine protons H-5 and H-8, and the methylene protons H_2-15, demonstrating that the compound has a tetracyclic triterpenoid skeleton. Although no definite evidence was obtained for the connectivity between the methylene carbons C-22 (of **D**) and C-23 (of **C**), probably due to the complex low intensity multiplets expected for both the methylene groups, joining the two carbons resulted in a 24-methyllanosta-9(11),25-dien-3α-yl acetate structure for **1b**.

The assigned structure (**1b**) was also corroborated by the mass spectral fragmentation pattern. Thus, the spectrum showed a prominent peak at *m/z* 355 due to the elimination of the side chain and two hydrogen radicals from the molecular ion. Moreover, the fragment ions at *m/z* 255, 241 and 229 are diagnostic [10]

Table 2. ^{13}C NMR data (125 MHz, $CDCl_3$) of **1b–4b***

C	1b	2b	3b	4b	C	1b	2b	3b	4b
1	31.25	35.80	31.26	35.81	18	14.40	14.14	14.38	14.37
2	23.23	24.17	23.24	24.17	19	22.03	22.30	22.05	22.30
3	78.36	80.89	78.36	80.88	20	36.05	36.05	36.63	36.62
4	37.11	38.01	36.99	38.01	21	18.41	18.41	18.55	18.53
5	47.74	52.58	47.76	52.58	22	34.00	33.98	30.81	30.79
6	21.16	21.24	21.16	21.25	23	31.47	31.47	37.34	37.33
7	27.96†	28.01†	27.99	28.01	24	41.61	41.62	38.72	38.71
8	41.90	41.76	41.90	41.76	25	150.19	150.22	152.40	152.40
9	148.56	148.13	148.58	148.14	26	109.38	109.38	109.29	109.29
10	39.30	39.25	39.32	39.25	27	18.62	18.64	19.42	19.41
11	114.65	115.21	114.66	115.20	28	27.89	28.18	27.90	28.19
12	36.98	37.14	37.08	37.11	29	22.18	16.80	22.18	16.81
13	44.25	44.27	44.25	44.25	30	18.62	18.50	18.62	18.50
14	47.09	47.00	47.11	47.00	31	20.19	20.18	27.27†	27.25†
15	33.91	33.91	33.91	33.91	32	—	—	27.52†	27.52†
16	27.98†	27.97†	27.99	27.99	OAc	21.37	21.34	21.38	21.35
17	50.91	50.88	50.81	50.76		170.88	170.99	170.88	170.98

*Assignments were made on the basis of ^{1}H–^{1}H COSY, ^{1}H–^{13}C COSY and HMBC spectra.
†Values in a vertical column may be interchanged.

Table 3. Two- and three-bond ^1H–^{13}C correlation data for **1b**

^1H signals (δ_H ppm)	Multiple bond correlation cross peaks (δ_C ppm)			
0.852 (H_3-28)	22.18 (C-29)	37.11 (C-4)	47.74 (C-5)	78.36 (C-3)
0.927 (H_3-29)	27.89 (C-28)	37.11 (C-4)	47.74 (C-5)	78.36 (C-3)
1.070 (H_3-19)	31.25 (C-1)	39.30 (C-10)	47.74 (C-5)	148.56 (C-9)
0.776 (H_3-30)	33.91 (C-15)	41.90 (C-8)	44.25 (C-13)	47.09 (C-14)
0.649 (H_3-18)	36.98 (C-12)	44.25 (C-13)	47.09 (C-14)	50.91 (C-17)
0.878 (H_3-21)	34.00 (C-22)	36.05 (C-20)	50.91 (C-17)	
1.000 (H_3-31)	31.47 (C-23)	41.61 (C-24)	150.19 (C-25)	
4.665 (H_2-26)	18.62 (C-27)	41.61 (C-24)		
1.641 (H_3-27)	41.61 (C-24)	109.38 (C-26)	150.19 (C-25)	
5.235 (H-11)	36.98 (C-12)	39.30 (C-10)	41.90 (C-8)	44.25 (C-13)
4.665 (H-3)	170.88 (OAc)			
1.30 (H-5)	22.03 (C-19)	22.18 (C-29)	27.89 (C-28)	27.98 (C-7)
	39.30 (C-10)			37.11 (C-4)
2.18 (H-8)	21.16 (C-6)			
1.35 (H_2-15)	18.62 (C-30)	27.98 (C-16)	47.09 (C-14)	50.91 (C-17)

of $\Delta^{9(11)}$, Δ^8 or Δ^7 triterpenoids. In addition, the spectrum exhibited intense peaks at *m/z* 467 $[M - CH_3]^+$, 422 $[M - AcOH]^+$, 407 $[M - CH_3 - AcOH]^+$ as expected.

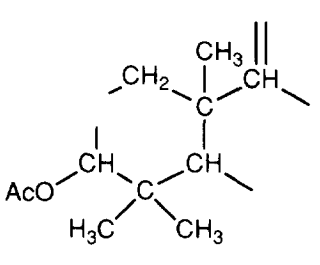
A comparison of the ^1H chemical shifts (Table 1) of the vinylic methyl protons (H_3-27) of **1b** and **2b** (δ 1.641 and 1.638, respectively) with those [11–13] of the 24-epimeric sterols, codisterol (24*S*, δ 1.635) and epicodisterol (24*R*, δ 1.650), clearly demonstrated that both **1b** and **2b** possess 24*S* stereochemistry.

The relative stereochemistry of the ring chiral centres of **1b** was determined from the NOE interactions observed in its NOESY spectrum, as depicted in Fig. 1.

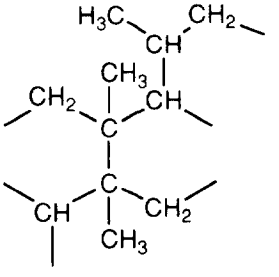
On the basis of the above evidence, **1b** and **2b** could be represented by (24*S*)-24-methyl-5α-lanosta-9(11),25-dien-3α-yl acetate and (24*S*)-24-methyl-5α-lanosta-9(11),25-dien-3β-yl acetate, respectively.

The high resolution mass spectra of **3b** and **4b** showed the molecular formula for both compounds to be $C_{34}H_{56}O_2$ (M^+ at *m/z* 496). The ^1H (Table 1) and ^{13}C (Table 2) NMR spectra of the compounds showed them to be stereoisomeric at the carbinyl carbon, C-3, with the OH group axially oriented in **3b** and equatorially oriented in **4b**. The spectra also revealed that the compounds contained one more methyl group compared to **1b** and **2b**.

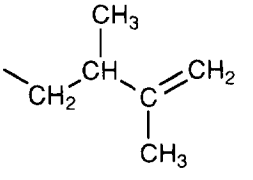
The HMBC spectrum of **3b** (Table 4) clearly demonstrated the presence of the partial structure shown by heavy lines in **5**, in which C-24 is substituted by a *gem* dimethyl group. The mass spectral fragmentation pat-



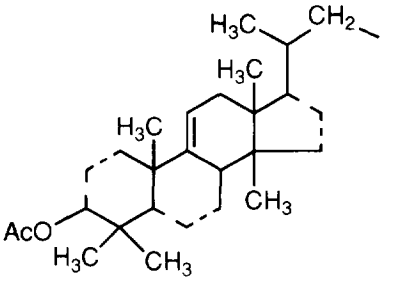
A



B



C



D

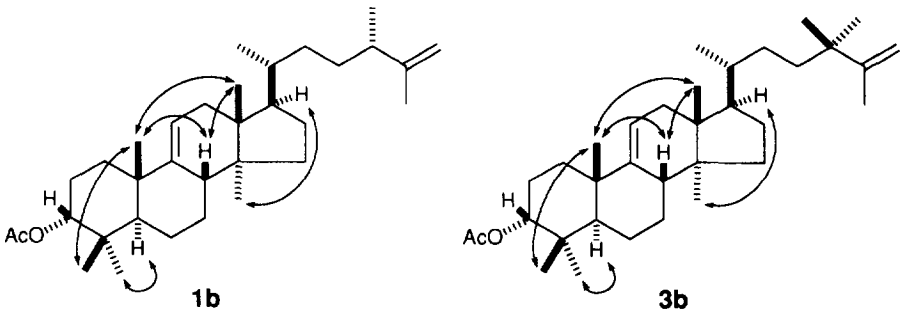


Fig. 1. NOE interactions observed in the NOESY spectra of compounds **1b** and **3b**.

Table 4. Two- and three-bond $^{1}H-^{13}C$ correlation data for **3b**

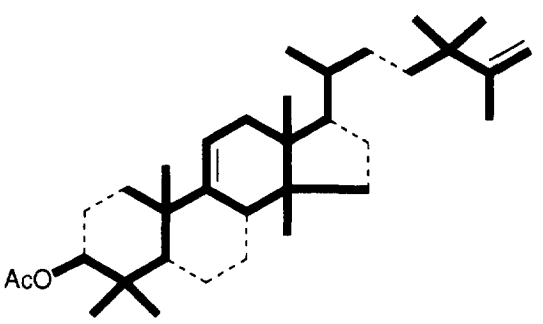
^{1}H signals (δ_H ppm)	Multiple bond correlation cross peaks (δ_C ppm)			
0.852 (H_3-28)	22.18 (C-29)	36.99 (C-4)	47.76 (C-5)	78.36 (C-3)
0.927 (H_3-29)	27.90 (C-28)	36.99 (C-4)	47.76 (C-5)	78.36 (C-3)
1.068 (H_3-19)	31.26 (C-1)	39.32 (C-10)	47.76 (C-5)	148.58 (C-9)
0.775 (H_3-30)	33.91 (C-15)	41.90 (C-8)	44.25 (C-13)	47.11 (C-14)
0.644 (H_3-18)	37.08 (C-12)	44.25 (C-13)	47.11 (C-14)	50.81 (C-17)
0.875 (H_3-21)	30.81 (C-22)	36.63 (C-20)	50.31 (C-17)	
1.014 (H_3-31)	27.52 (C-32)	37.34 (C-23)	38.72 (C-24)	
1.020 (H_3-32)	27.27 (C-31)	37.34 (C-23)	38.72 (C-24)	
4.661 (H_a-26)	19.42 (C-27)	38.72 (C-24)		
4.724 (H_b-26)	19.42 (C-27)	38.72 (C-24)		
5.233 (H-11)	41.90 (C-8)	44.25 (C-13)		

tern (see Experimental) was also found to be in excellent agreement with a 24,24-dimethyl-lanosta-9(11),25-dien-3α-yl acetate structure for **3b**. The relative configuration of all the ring chiral centres could be established from the NOE interactions observed in its NOESY spectrum, as shown in Fig. 1.

Compounds **3b** and **4b** were, therefore, represented as 24,24-dimethyl-5α-lanosta-9(11),25-dien-3α-yl acetate and 24,24-dimethyl-5α-lanosta-9(11),25-dien-3β-yl acetate, respectively.

EXPERIMENTAL

Mps: uncorr.; crystallization: Me_2CO; prep. HPLC: Senshu PAK, ODS-3251-D column (25 cm × 8 mm i.d., 5 μm), MeCN–$CHCl_3$ (9:1) as mobile phase (flow rate, 4 ml min^{-1}) using a RI detector. GC: 1.4% SE-30 on Chromosorb HP glass column (1 m × 4 mm i.d.) at 260°; cholestane was used as int. standard and its R_t was set at 3 min. HR-EIMS: 30 eV, direct inlet; ^{1}H NMR (500 MHz): $CDCl_3$, with TMS as int. standard.



5

Isolation of **1a–4a**. The overground part (10 kg) of *G. arborea* (supplied by M/s United Chemical and Allied Products, Calcutta; voucher specimen deposited in the company's herbarium) was milled and extracted with petrol in a Soxhlet apparatus for 24 hr. The extract was concd and subjected to CC on silica gel (1 kg) to obtain a triterpenoid fr. (2 g) which was acetylated with Ac_2O–pyridine at room temp. for 24 hr. The acetate mixt. on repeated CC over silica gel and neutral alumina yielded, besides arborinyl acetate and iso-arborinyl acetate, a sticky mass (0.24 g) which on repeated prep. HPLC furnished **1b–4b** in pure form.

(24S)-24-*Methyl*-5α-*lanosta*-9(11),25-*dien*-3α-*yl acetate* (**1b**). Mp 149–150°, $[\alpha]_D$ +42 (*c* 0.3, $CHCl_3$), GC *RR*$_t$ 3.72, HPLC R_t 24.6; IR ν_{max}^{KBr} cm^{-1}: 1735, 1240 (OAc), 1641, 978 (=CH_2); EIMS *m/z* (rel. int.): 482.4138 $[M]^+$ (32), 467 (48), 422 (7), 407 (100), 355 (65), 316 (8), 295 (10), 255 (9), 241 (8), 229 (10).

(24S)-24-*Methyl*-5α-*lanosta*-9(11),25-*dien*-3β-*yl acetate* (**2b**). Mp 204–205° (lit. [9] mp 178–180°), $[\alpha]_D$ +78 (*c* 0.4, $CHCl_3$), GC *RR*$_t$ 4.32, HPLC R_t 26.8; IR ν_{max}^{KBr} cm^{-1} 1728, 1240 (OAc), 1641, 978 (=CH_2); EIMS *m/z* (rel. int.): 482.4133 $[M]^+$ (51), 467 (67), 422 (9), 407 (54), 355 (100), 316 (12), 255 (7), 241 (6), 229 (9).

24,24-Dimethyl-5α-lanosta-9(11),25-dien-3α-yl acetate (**3b**). Mp 194–195°, $[\alpha]_D$ +46 (*c* 0.1, $CHCl_3$), GC *RR*$_t$ 4.57, HPLC R_t 28.3; IR ν_{max}^{KBr} cm^{-1}: 1731, 1240 (OAc), 1639, 979 (=CH_2); EIMS *m/z* (rel. int.): 496.4308 $[M]^+$ (50), 481 (54), 436 (8), 421 (100), 355 (90), 316 (24), 295 (18), 255 (15), 241 (11), 229 (11).

24,24-Dimethyl-5α-lanosta-9(11),25-dien-3β-yl acetate (**4b**). Mp 234–235° (lit. [9] mp 231–234°), $[\alpha]_D$ +87 (*c* 0.4, $CHCl_3$), GC *RR*$_t$ 5.44, HPLC R_t 30.6; IR ν_{max}^{KBr} cm^{-1} 1740, 1250 (OAc), 1641, 982 (=CH_2): EIMS *m/z* (rel. int.): 496.4328 $[M^+]$ (42), 481 (64), 439 (10), 421 (56), 355 (100), 316 (28), 255 (11), 241 (10), 229 (12).

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