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24-METHYLENE TETRACYCLIC TRITERPENES FROM *POLYALTHIA LANCILIMBA*

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Key Word Index—*Polyalthia lancilimba*; Annonaceae; 24-methylene tetracyclic triterpene; lanostane.

Abstract—Two new 24-methylene tetracyclic triterpenes that possess the lanostane skeleton have been isolated from the stem barks of *Polyalthia lamcilimba*. Their structures were determined by spectral analysis and comparisons with other lanostane type triterpenes described in the literature. The two new components (**1** and **3**) were elucidated as 24-methylenelanosta-7,9(11)-dien-3β,15α-diol and 24-methylenelanosta-8-en-2β,3β,21-triol.

INTRODUCTION

The genus *Polyalthia* has 120 species. Earlier work on 14 species of this genus has yielded diterpenes, triterpenes, nitrogen heterocycles, (zincpolyenamine), indolosesquiterpenes, benzylisoquinolines, protoberberines, bisbenzylisoquinolines and aporphines. About 14 diterpenes have been isolated from 6 *Polyalthia* species [1–3], but only one new triterpene, polycarpol, together with some sterols (sitosterol, stimasterol, campesterol and cholesternol) have so far been reported [4, 5]. Thus few triterpene derivatives have been reported from the Annonaceae family.

For *Polyalthia lancilimba* collected from Yunnan, China, no chemical studies have been reported to date. In this paper, we wish to report the isolation and structural elucidation of two new 24-methylene tetracyclic triterpenes.

RESULTS AND DISCUSSION

We have isolated two main components, **1** and **3**, from the ethanolic extract of the stem barks of *Polyalthia lancilimba*.

Compound **1**, represented 0.8% of the plant material. The ^{1}H NMR spectrum indicated the presence of two olefinic protons, an exomethylene, two oxymethines, three secondary and five tertiary methyls. The ^{13}C NMR spectrum showed 31 carbons including 8 methyls, 8 methylenes, 8 methines and 7 quaternary carbons. The HR-EI mass spectrum showed the molecular ion peak at m/z 454.3812 (calc. mass 454.3811) corresponding to the molecular formula $C_{31}H_{50}O_2$. These data suggested that **1** was a triterpene which had one additional sp^2-methylene [6]. The fragment ion peak in the EI-mass spectrum at m/z 327 represented the loss of 9 carbon atoms, which unit (C_9H_9+2H) was due to the cleavage of the bond between C-17 and C-20. Thus compound **1** is a tetracyclic triterpene bearing a 9 carbon side chain. The ^{13}C NMR data corresponding to these 9 carbons of the side chain are almost identical upon comparison with those of 24-methylenelanosta-7,9(11)dien-3β-ol (**2**) [7]. This enabled the 9 carbon skeleton of the side chain of **1** to be identified and assigned.

The co-incidence of ^{13}C NMR and ^{1}H NMR spectral data with those reported in the literature suggested that there were striking resemblances between **1** and **2**, such as two *endo*-double bonds, a 3β-OH and other groups in the ring A, B and C besides the side chain. The ^{13}C NMR signals at δ 121.3, 140.9, 146.2, 116.0 and the ^{1}H NMR signals at δ 5.82, 5.28 indicated that the two double bonds were positioned between C-7 and C-8 and between C-9 and C-11. In the COLOC spectrum, two important correlation spots between one olefinic

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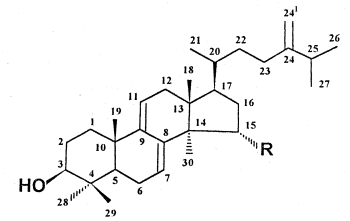

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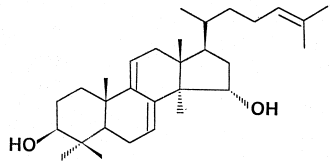

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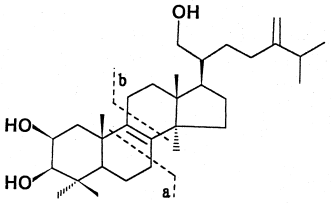

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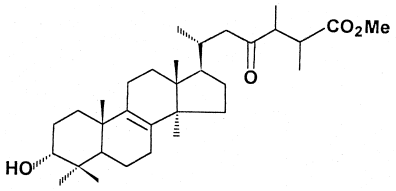

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carbon and H-19, another olefinic carbon and H-30 also supported this suggestion. The peaks of one hydrogen at δ 3.22 (*dd*) correspond to H-3. Indeed, a NOE effect between H-3 and H-28 indicated that H-3 was α-oriented and therefore the 3-OH was a β-OH as in **2**. These facts established that **1** was a 24-methylene-7,9(11)-diene of the lanostane type of triterpene.

The differences between **1** and **2** were in the presence of an extra hydroxyl in the ring system (besides 3β-OH) and the spectral data of the vicinal protons under the influence of this extra OH. The additional hydroxyl was positioned on ring D (possible position: 15-OH or 16-OH), because in the EI-mass spectrum, the peak at *m*/*z* 273 which included only one hydroxyl (3-OH) was due to the cleavage of ring D. A close observation of the chemical shifts in the ^{13}C NMR spectra of **1** and **2** of C-14, C-15, C-16 and C-17 showed differences. However the chemical shifts of C-20, C-18 and C-30 were identical (see Table 2).

In the ^{1}H–^{1}H COSY spectrum, the correlation between H-20 and a methine proton (H-17 at δ 1.68) was determined, then the corresponding carbon (C-17) was assigned at δ 48.9 in the ^{1}H–^{13}C COSY spectrum. The upfield chemical shift of C-17 (2.0 ppm) is negligible by comparison with **2**. Thus, we inferred the hydroxyl was not positioned at C-16, but at C-15. This suggestion was supported by the ^{1}H–^{1}H COSY spectrum: H-17 also correlated with the protons of a methylene (at δ 1.72 and 1.95). Based on the above analysis, the other hydroxyl has been placed at C-15 and the NOE effect between H-15 and H-18 indicated that the 15-OH was α-oriented.

After determination of 15α-OH, the structure of **1** was established as 24-methylenelanosta-7,9(11)dien-3β,15α-diol. The EI-mass spectral fragmentation pattern also supported the structure.

Compound **3** is a component with 31 carbons, three oxy-groups (δ 71.3, 78.3 and 62.5 in the ^{13}C NMR spectrum, δ 4.04, 3.13 and 3.65 in the ^{1}H NMR spectrum), together with a molecular ion peak at *m*/*z* 472.3925 (calc. mass 472.3916) in the HR-EI-mass spectrum which revealed the formula of **3** as $C_{31}H_{52}O_3$. It had 6 unsaturated positions, including two double bonds (δ 133.8, 135.3, 106.2, 156.6 in the ^{13}C NMR spectrum) and 4 rings. These facts suggested that **3** possesses a 9 carbon side chain. The data in the ^{13}C NMR and ^{1}H NMR spectra demonstrated a terminal olefinic bond, 2 methyls and a methine which were almost identical by comparison with C-24 to C-27 and C-24 of **1**. This indicated the 24-methylene 9 carbon skeleton of the side chain of **3**.

The chemical shifts at δ 133.8 and 135.3 of two quaternary carbons in ^{13}C NMR spectrum indicated that **3** had only an *endo*-olefinic bond. By comparing with the ^{13}C NMR spectral data of both the related compound **4** [8] and lanosterol [9–12], the chemical shifts of the carbons of rings B, C, D (from C-5 to C-18 and C-30) were similar (see Table 2). These facts showed that compound **3** was a lanostane type triterpene having a double bond between C-8 and C-9. On the other hand, the cleavage of ring B or C in the EI-mass spectrum supported the position of this double bond [8]. The fragment ion peak at *m*/*z* 271 $[M–\mathbf{a}–H_2O]^+$ represented successive losses of fraction **a** and water from the molecular ion at *m*/*z* 472, together with an

ion at m/z 133 [M–**a**–side chain–Me]$^+$, m/z 161[M–**b**–H_2O–$(Me)_2$]$^+$.

The protons of a methylene substituted by a hydroxyl did not show a single or doublet peak in the ^1H NMR spectrum, but two *dd* were observed. This indicated that the hydroxyl is not located at one of the methyls of the carbon rings, but rather at one of the methyls in the side chain (the possible positions were C-26, C-27 and C-21). Also in the ^{13}C NMR spectrum the chemical shift of C-25 (δ 33.8, the same for **1** and **2**) was assigned. In the ^1H–^1H COSY spectrum, H-25 (δ 2.21) showed two correlation spots with protons of two methyls whose chemical shifts were at higher field (H-26 at δ 0.97, H-27 at δ 0.98). Thus the first hydroxyl was not positioned at C-26 or C-27, but was located at C-21.

For the two other hydroxyls, a β-OH positioned at C-3 was determined by comparison with the spectral data of **2** and **4**. After H-3 (δ 3.13) and C-3 (δ 78.3) were assigned, the ^1H–^1H COSY spectrum showed that H-3 was only correlated with one H-2 (δ 4.04). Therefore the third hydroxyl was positioned at C-2. The NOE effect between H-3 and H-28 supported the β-orientation of the 3-OH. Another NOE effect between H-2 and H-3 indicated that the 2-OH was also β-oriented. The analysis therefore confirmed the structure of compound **3** as 24-methylenelanosta-8-en-2β,3β,21-triol.

Few triterpenoids have been reported from the Annonaceae and only a new triterpene, polycarpol (**5**), has been reported which was isolated from *P. oliveri* Engl. and *P. suaveolens* Engl. et Diels [5, 6] and was regarded as a useful chemotaxonomic marker of *Polyalthia*. The isolation and identification of **1** and **3**, which possess a 24-methylenelanostane structure may be an addition chemotaxonomic matter.

EXPERIMENTAL

M.p. uncorr. UV was obtained on a Shimatsu UV-210A. IR was recorded on a Perkin-Elmer 577. EI-MS was measured on a Finnigan-4510. NMR were recorded with a Bruker AM-400 in $CDCl_3$, with TMS as int. standard. The stems of *Polyalthia lancilimba* were collected from Pingbian of the Yunnan province and its specimen was determined by Mrs Huang Suhua in Yunnan University.

Extraction and isolation

Stem barks of *Polyalthia lancilimba* (2700 g) were extracted with 95% EtOH and the extract concentrated *in vacuo*. It then was suspended in H_2O and extracted with $CHCl_3$. The $CHCl_3$ fraction (50 g) was subjected to CC on silica gel eluting with a gradient of petroleum–EtOAc (100:0 to 0:100) and afforded **1** (2130 mg) and **3** (60 mg).

24-Methylenelanosta-7,9(11)-dien-3β,15α-diol (**1**): Yield: 0.8‰, white powder m.p. 144–146°, $[\alpha]_D^{29} = +63.8°$ (c 0.48, C_5H_5N). IR ν_{max}^{KBr} cm^{-1}: 3300, 2930, 1640, 1460, 1370, 1035, 990, 890. UV λ_{max}^{MeOH} nm (log ε): 196.5 (3.13), 234 (3.73), 243.5 (3.84), 251.5 (3.67). EIMS, 70 eV, m/z (rel. int.): 454 (100), 439 (9), 439 (9), 436 (6), 421 (31), 403 (5), 355 (6), 327 (45), 311 (6), 273 (26), 255 (19), 239 (16), 228

Table 1. ^1H NMR spectral data of compounds **1**, **2** and **3**† (δ ppm; J, Hz; in $CDCl_3$)

H	**1**	**2**	**3**
H-1	2.01 *br*		1.38 *d*, *J* = 2.8; 2.15 *d*, *J* = 2.8
H-2	1.68 *m*		4.04 *m*
H-3	3.22 *dd*, *J* = 10.9, 5.0	3.24 *dd*	3.13 *d*, *J* = 3.7
H-5	1.07 *m*		1.80 *br t*
H-6	2.11 *br*		1.71 *m*
H-7	5.82 *d*, *J* = 6.9	5.30 *d*	2.03 *br*
H-11	5.28 *d*, *J* = 6.0	5.46 *br*	2.06 *m*
H-12	2.05 *br*; 2.24 *br*		1.19 *m*; 1.57 *m*
H-15	4.25 *dd*, *J* = 9.6, 5.8		1.95 *m*; 2.10 *m*
H-16	1.72 *m*; 1.95 *m*		1.43 *m*; 1.56 *m*
H-17	1.68 *m*		1.12 *m*
H-18	0.58 *s*	0.56 *s*	0.68 *s*
H-19	0.91 *s*	1.00 *s*	1.33 *s*
H-20	1.32 *m*		1.44 *s*
H-21	0.86 *d*, *J* = 6.5	0.96 *s*	3.69 *dd*, *J* = 9.4, 3.3; 3.61 *dd*, *J* = 11.0, 3.8
H-22	1.11 *m*; 1.50 *m*		1.63 *m*; 1.77 *m*
H-23	1.85 *m*; 2.10 *m*		1.26 *br*; 1.95 *br*
H-25	2.19 *hept*, *J* = 6.4		2.21 *hept*, *J* = 6.6
H-26	1.00 *d*, *J* = 6.9‡	1.01 *d*	0.97 *d*, *J* = 6.9‡
H-27	0.99 *d*, *J* = 6.8‡	1.02 *d*	0.98 *d*, *J* = 6.9‡
H-28	0.97 *s*	0.97 *s*	0.97 *s*
H-29	0.85 *s*	0.87 *s*	0.97 *s*
H-30	0.95 *s*	0.87 *s*	0.84 *s*
H-24[1]	4.62 *s*; 4.69 *s*	4.65 *s*; 4.71 *s*	4.64 *s*; 4.69 *s*

†Compounds **1** and **3**: Bruker AM-400 MHz; compound **2**: 360 MHz [7].
‡Assignments of H-26 and H-27 may be interchanged.

Table 2. ^{13}C NMR spectral data of the compounds **1**, **2**, **3** and **4** (δ ppm, in $CDCl_3$)

C	1	2	3	4
C-1	35.8 *t*	35.7 *t*	41.3 *t*	30.2 *t*
C-2	27.8 *t*	27.8 *t*	71.3 *t*	25.9 *t*
C-3	78.9 *d*	78.9 *d*	78.3 *d*	76.7 *d*
C-4	38.7 *s*	38.6 *s*	38.3 *s*	37.1 *s*
C-5	49.1 *d*	50.3 *d*	44.6 *d*	44.4 *d*
C-6	23.0 *t*	22.9 *t*	17.9 *t*	18.3 *t*
C-7	121.3 *d*	120.1 *d*	26.4 *t*	26.2 *t*
C-8	140.9 *s*	142.7 *s*	133.8 *s*	134.1 *s*
C-9	146.2 *s*	145.9 *s*	135.3 *s*	134.9 *s*
C-10	37.5 *s*	37.3 *s*	36.5 *s*	37.7 *s*
C-11	116.0 *d*	116.2 *d*	21.1 *s*	21.0 *t*
C-12	38.6 *t*	38.7 *t*	30.7 *t*	30.9 *t*
C-13	44.4 *s*	43.7 *s*	44.3 *s*	44.7 *s*
C-14	52.0 *s*	49.1 *s*	50.0 *s*	50.1 *s*
C-15	74.9 *d*	27.8 *t*	31.4 *t*	31.1 *t*
C-16	40.1 *t*	31.4 *t*	28.5 *t*	28.4 *t*
C-17	48.9 *d*	50.9 *d*	50.5 *d*	50.4 *d*
C-18	15.8 *q*	15.6 *q*	16.0 *q*	15.9 *q*
C-19	22.8 *q*	22.6 *q*	20.8 *q*	19.0 *q*
C-20	36.0 *d*	36.2 *d*	43.1 *d*	32.6 *d*
C-21	18.5 *q*	18.4 *q*	62.5 *t*	19.9 *q*
C-22	35.0 *t*	34.9 *t*	30.6 *t*	48.9 *t*
C-23	31.3 *t*	31.3 *t*	27.6 *t*	213.2 *s*
C-24	156.5 *s*	156.8 *s*	156.6 *s*	48.5 *d*
C-25	33.8 *d*	33.8 *d*	33.8 *d*	40.9 *d*
C-26	21.9 *q**	21.8 *q*	21.8 *q*	176.4 *s*
C-27	22.0 *q**	21.9 *q*	21.8 *q*	14.3 *q*
C-28	28.2 *q*	28.1 *q*	29.5 *q*	28.0 *q*
C-29	16.0 *q*	15.7 *q*	16.9 *q*	22.3 *q*
C-30	17.2 *q*	25.5 *q*	24.3 *q*	24.3 *q*
C-24[1]	106.2 *t*	105.9 *t*	106.2 *t*	13.4 *q*

Compounds **1** and **3**: Bruker AM-400,100 MHz; compound **2**: 90.56 MHz [7]; compound **4**: JEOL.GX-400, 100 MHz [8].
*Interchangeable.

(5), 211 (12), 199 (15), 187 (22), 159 (36), 145 (29), 133 (33), 119 (42). ^1H NMR, ^{13}C NMR spectra see Tables 1 and 2.

24-Methylene-lanosta-8-en-2β,3β,21-triol (**3**): Yield: 0.022‰, white needles (MeOH), m.p. 152–154°, $[\alpha]_D^{29} = +70.9°$(*c* 0.41, MeOH). IR ν_{max}^{KBr} cm^{-1}: 3400, 2910, 1450, 1365, 1020. UV λ_{max}^{MeOH} nm (log ε): 203.5(3.85); EIMS, 70 eV, *m*/*z* (rel. int.): 472 $[M]^+$ (73), 457 $[M–Me]^+$ (57), 439 $(M–Me–H_2O]^+$ (34), 421 $[M–Me–2H_2O]^+$ (25), 315 $[M–C_9H_{17}O–Me–H]^+$ (17), 297 $[315\text{-}H_2O]^+$ (11), 282 $[297–Me]^+$ (12), 271 (11), 161 (31), 133 (39), 121 (51), 109 (61), 95 (69), 69 (79). ^1H NMR, ^{13}C NMR spectra see Tables 1 and 2.

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