Pergamon

0031-9422(95)00943-4

Phytochemistry, Vol. 42, No. 3, pp. 789–794, 1996

0031-9422/96 $15.00 + 0.00

20,29-EPOXYSALACIANONE AND 6β-HYDROXYSALACIANONE, TWO LUPANE TRITEREPENES FROM *SALACIA BEDDOMEI*

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(*Received* 5 *October* 1995)

Key Word Index—*Salacia beddomei*; Celastraceae; new triterpenoids; 20,29-epoxysalacianone; 6β-hydroxysalacianone.

Abstract—Two new lupane triterpenoids, 20,29-epoxysalacianone (20,29-epoxylupane-3,21-dione) and 6β-hydroxysalacianone [6β-hydroxylup-20(29)-ene-3,21-dione] have been isolated from the hexane and ethyl acetate extracts of the stem bark of *Salacia beddomei*, together with the known compounds 3β,28-dihydroxylup-20(29)-ene (betulin) and 2α,3β-dihydroxylup-20(29)-ene. Their structures have been elucidated with the help of NMR and mass spectroscopic techniques.

INTRODUCTION

Our previous report of the phytochemical studies on the stem bark of *Salacia beddomei* (Gamble, ref. [1]) deals with the isolation and characterization of six known compounds lup-20(29)-en-3-one, friedelan-3-one, 15α-hydroxyfriedelan-3-one, sitosterol, 15α-hydroxyfriedelane-1,3-dione and pristimerin, together with two new lupane compounds named salacianone [lup-20(29)-ene-3,21-dione] and salacianol [21β-hydroxylup-20(29)-en-3-one] [**2**]. Further studies of the hexane–ethylacetate extracts of the title plant yielded four more lupane triterpenes (**1–4**) out of which two new compounds (**1** and **2**) have been characterized as derivatives of salacianone (**5**) while the other two (**3** and **4**) are identified as known compounds.

RESULTS AND DISCUSSION

Salacia beddomei stem bark powder was extracted first with hexane and subsequently with ethyl acetate. The concentrated hexane extract was chromatographed on silica gel columns according to the procedure described previously [2]. Compound **1** was obtained from chloroform fractions. The concentrated ethyl acetate extract was separately chromatographed over a silica gel column which was eluted with solvents of increasing polarity. Compound **2** was isolated from benzene fractions and compounds **3** and **4** from chloroform–ethyl acetate fractions.

The ^{1}H and ^{13}C NMR data for **1–4** revealed the presence of structurally similar lupane triterpenes. Compound **3** exhibited the NMR spectral characteristics of a dihydroxylupane which has been identified as betulin [3β,28-dihydroxylup-20(29)-ene] from a comparison of its ^{1}H and ^{13}C NMR spectral data with those reported in the literature [3]. The ^{1}H and ^{13}C NMR shifts of **4** also showed the characteristics of a lupane compound with two secondary hydroxyl groups. The ^{1}H NMR spectrum of **4** showed two carbinol methine protons at δ 3.68 (1H, *ddd*, J = 10.5, 9.6 and 4.5 Hz) and 2.97 (1H, *d*, J = 9.6 Hz) and the decoupling studies revealed the connectivity between these carbinol methine protons, indicating a vicinal diol group in the molecule. This compound has been identified as 2α, 3β-dihydroxylup-20(29)-ene, a lupane 2,3-diol, first reported from *Pterocarpus santalinus* (Leguminosae) [4] and later from *Leucas nutans* (Labiatae) [5] from their identical ^{1}H NMR spectral data and physical constants.

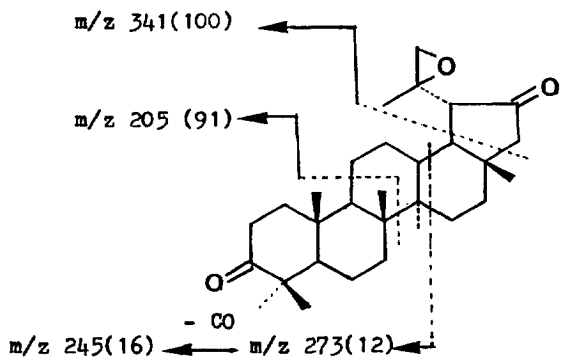
20,29-Epoxysalacianone (**1**), a new lupane triterpenoid, obtained as crystals in small amounts, was also positive to the Liebermann–Burchard colour reaction. Its ^{1}H NMR spectrum (500 MHz, $CDCl_3$) showed seven tertiary methyl groups as singlets at δ 0.87, 0.96, 1.04 (6H) 1.09, 1.12 and 1.36, two one-proton doublets at δ 2.53 (J = 4.8 Hz) and 2.42 (J = 4.8 Hz), a pair of one-proton multiplets at δ 2.49 and 2.40, two AB methylene doublets at δ 2.15 (J = 16.7 Hz) and 1.93 (J = 16.7 Hz), a one-proton doublet at δ 1.91 (J = 11.6 Hz) and a one-proton multiplet at δ 1.89. The ^{13}C and $\{^1H\}$–^{13}C INEPT (75 MHz, $CDCl_3$) spectra showed 30 carbons including seven CH_3 carbons, 10 CH_2 carbons, five CH carbons and eight quaternary carbons. The EI mass spectrum showed a $[M]^+$ ion at m/z 454 (65%), which is in agreement with a molecular formula $C_{30}H_{46}O_3$ in corroboration with the NMR data.

Two of the oxygens were keto functions as was evident in the ^{13}C NMR spectrum from the appearance

of two carbonyl carbon signals at δ 217.0 and 218.0 and no signals due to hydroxylated or vinylic carbons. The ^{1}H and ^{13}C NMR spectral features of **1** were very similar to lupane triterpenoids except that it lacked the signals of the exomethylene double bond at the Δ20(29)-position. The absence of an exomethylene double bond, as well as the hydroxyl group in **1**, therefore indicated the presence of the third oxygen as a C-20, C-29 terminal epoxide. The ^{13}C NMR chemical shifts of a quaternary carbon at δ 56.34 and a CH_2 carbon at 55.52, as well as the two one-proton doublets, attributable to epoxymethylene protons, at δ 2.53 and 2.42 in the ^{1}H NMR spectrum supported this assignment. Therefore, **1** was assigned a 20,29-epoxylupane skeleton and the positions of the carbonyl groups were determined on the basis of its EI mass spectral fragmentation, analysis of the ^{1}H and ^{13}C NMR chemical shifts, multiplicity of the signals and comparison of the spectral data with those for salacianone (**5**), a 3,21-diketolupane isolated from the title plant [2].

The EI mass spectrum showed prominent ions in the high mass region at *m/z* 436 $[M-H_2O]^+$ (68%), 426 $[M-CO]^+$ (92%) 424 $[M-HCHO]^+$ (51%) and 394 $[M-C_3H_8O]^+$ (38%). The ions at *m/z* 424 and 394 may be rationalized in terms of the cleavages in the epoxy side chain. The EI mass spectrum showed an abundant ion at *m/z* 205 (90%), formed by the C-ring cleavages, which indicated a fragment ion constituted by rings A and B with a C-3 keto function [2]. Similarly, the ion at *m/z* 341 (100%) constituted by the rings A, B, C and D with a 3-keto function, formed by the E-ring cleavage as shown in Scheme 1, indicated the location of the second carbonyl function in the E-ring. A comparison of the ^{13}C NMR chemical shifts in both **1** and **5** revealed the identical shifts for the carbon atoms up to the D-ring, thus suggesting that both molecules shared the same structural features. Therefore, the positions remaining for the second keto group substitution were in the E-ring at either C-21 or C-22. The evidence for the C-21 keto substitution was provided by the H-19 proton signal, which appeared as a doublet at δ 1.91 with a coupling constant of 11.6 Hz due to the diaxial interaction with the H-18 proton. The multiplicity of this signal as a doublet clearly indicated the absence of H-21 protons due to the keto group substitution at this site. The methylene AB doublets at δ 2.10 (J = 16.7 Hz) and 1.93 (J = 16.7 Hz), assignable to the isolated H-22 protons adjacent to the carbonyl group, are also consistent with C-21 keto substitution, thereby characterizing **1** as a 20,29-epoxy derivative of salacianone. The NMR chemical shift pattern, as well as the multiplicities of the signals in **1** and **5**, were found to be comparable (Tables 1 and 2) and were verified with decoupling studies, NOE difference spectroscopy and a ^{1}H–^{1}H COSY experiment. The one proton multiplets at δ 2.49 and 2.40 were assignable to H-2 protons, and the ^{1}H–^{1}H COSY spectrum revealed their coupling with H-1 proton multiplets at δ 1.89 and 1.41. Similarly, the connectivity between the epoxymethylene proton doublets at δ 2.53 and 2.42 and also between the H-22 proton doublets at δ 2.15 and 1.93 were established by decoupling studies and a ^{1}H–^{1}H COSY experiment. The assignment of the H-19 proton at δ 1.91 was verified by NOE difference spectroscopy. The irradiation of the H-17 methyl resonance at δ 0.87 showed enhancements in the proton doublets at δ 1.91 (H-19) and 2.10 (H-22a), confirming their β-orientation.

6β-Hydroxysalacianone (**2**), a new lupane triterpenoid, was obtained as crystals, mp 243° and was positive to the Libermann–Burchard colour reaction. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) showed six tertiary methyl singlets at δ 0.93, 1.01, 1.15, 1.42, 1.45 and 1.47 and a vinyl methyl singlet at δ 1.65. The absorption of two exomethylene protons at δ 4.82 (*br s*) and 4.95 (*br s*) as well as the vinyl methyl resonance at δ 1.65 suggested a lup-20, 29-ene skeleton for **2**. The ^{1}H NMR spectrum showed the presence of a methylene AB system at δ 2.19 (1H, *d*, J = 17.1 Hz) and 1.98 (1H, *d*, J = 17.1 Hz), a pair of diastereotopic protons adjacent to a carbonyl group at δ 2.81 (1H, *td*, J = 6.1, 14.4 Hz) and 2.24 (1H, *m*), a one-proton doublet at δ 2.69 (J = 11.9 Hz) and a carbinol methine proton at δ 4.50 (*br s*). The ^{13}C and $\{^1H\}$–^{13}C INEPT (75 MHz, $CDCl_3$) spectra showed 30 distinct carbon signals including seven CH_3 carbons, nine CH_2 carbons, six CH carbons and eight quaternary carbons. The EI mass spectrum showed a $[M]^+$ ion at *m/z* 454 (91%), which was in agreement with a molecular formula $C_{30}H_{46}O_3$, also supported by the NMR data. The appearance in the



Scheme 1. EI-mass spectral fragmentation of **1**. Relative intensities are given in parentheses.

Table 1. Diagnostic ^{1}H NMR shifts of compounds **1**, **2** and **5** (in $CDCl_3$, TMS = 0, 500, 300 and 270 MHz, respectively)

H	1	2	5
1	1.91 *m*,	1.91 *m*,	1.90 *m*,
	1.40 *m*	1.40 *m*	1.40 *m*
2	2.49 *m*,	2.81 *td* (*J* = 6.1,	
		14.4 Hz)	2.49 *m*,
	2.40 *m*	2.25 *m*	2.40 *m*
6	—	4.50 *br s*	—
19	1.91 *d*(*J* = 11.6 Hz)	2.69 *d*(*J* = 10.8 Hz)	2.68 *d*(*J* = 10.8 Hz)
22	2.10 *d*(*J* = 16.7 Hz),	2.19 *d*(*J* = 16.2 Hz)	2.19 *d*(*J* = 16.2 Hz)
	1.93 *d*(*J* = 16.7 Hz)	1.96 *d* (*J* = 16.2 Hz)	1.98 *d*(*J* = 16.2 Hz)
23	1.08 *s*,	1.15 *s*	1.07 *s*
24	1.04 *s*	1.45 *s*	1.05 *s*
25	0.96 *s*	1.42 *s*	0.95 *s*
26	1.12 *s*	1.47 *s*	1.12 *s*
27	1.04 *s*	1.01 *s*	1.04 *s*
28	0.87 *s*	0.93 *s*	0.92 *s*
29	2.53 *d* (*J* = 4.8 Hz),	4.82 *br s*,	4.80 *br s*
	2.42 *d* (*J* = 4.8 Hz)	4.95 *br s*	4.87 *br s*
30	1.34 *s*	1.65 *s*	1.65 *s*

^{13}C NMR spectrum of two carbonyl carbon signals at δ 217.87 and 216.58 and a carbinol methine carbon signal at δ 69.45 suggested that **2** was a lup-20(29)-ene with two carbonyl groups and a secondary hydroxyl group. The position of the carbonyl groups and the secondary hydroxyl group in the basic skeleton was determined on the basis of its EI mass spectral fragmentation, analysis of the ^{1}H and ^{13}C NMR chemical shifts, multiplicity of the signals and comparison with model compounds.

The diagnostic ions in the EI mass spectral frag-

Table 2. ^{13}C NMR data for compounds **1**, **2** (75 MHz, $CDCl_3$) and **5** (69.5 MHz, $CDCl_3$, TMS) and **6** (25.2 MHz, $CDCl_3$) [7]

C	1	2	5	6
1	39.54	42.07	39.53	42.2
2	34.12	34.38	34.05	34.5
3	217.00	216.58	217.68	216.7
4	47.31	48.93	47.28	49.0
5	54.83	56.55	54.90	56.6
6	19.59	69.45	19.57	69.7
7	33.26	41.75	33.23	42.2
8	40.94	40.13	40.85	40.0
9	49.51	50.48	49.56	50.7
10	36.85	36.77	36.84	36.8
11	21.47	21.01	21.22	21.3
12	26.99	25.32	25.30	25.2
13	36.78	36.25	37.25	37.2
14	42.83	42.93	42.72	43.0
15	26.98	26.97	26.90	27.5
16	34.63	34.73	34.81	35.5
17	37.96	37.81	37.83	43.2
18	46.55	47.03	46.96	48.3
19	54.69	59.00	59.01	48.0
20	56.34	143.38	143.43	150.8
21	218.02	217.87	217.81	29.8
22	51.31	55.33	55.37	39.9
23	24.65	24.96	26.60	25.0
24	21.04	23.70	20.99	23.7
25	15.79	16.97	15.89	17.0
26	15.97	16.89	15.73	17.1
27	14.54	14.79	14.49	14.8
28	18.93	18.67	18.69	18.0
29	55.52	115.10	114.95	109.4
30	21.93	20.83	20.81	19.3

mentation is shown in Scheme 2. The location of one of the carbonyl groups in ring E and other groups in rings A and B is evident from the appropriate fragment ions as shown in Scheme 2. A keto group substitution at the C-21 position in **2** was evident from the identical NMR shifts observed for the atoms in rings D and E and the isopropenyl group in comparison with those shifts in salacianone (**5**) [2]. The one-proton doublet at δ 2.69 (J = 11.9 Hz) in the ^{1}H NMR spectrum of **2** was due to the allylic H-19 proton as in the case of compound **5**. The multiplicity of this signal as a doublet as well as the higher J value can be explained in terms of the diaxial interaction of H-19 with H-18, indicating the absence of H-21 protons due to the carbonyl group substitution. Further evidence for this assignment came from the chemical shift and multiplicity of the H-22 protons, which appear as AB doublets at δ 2.19 (J = 17.1 Hz) and 1.98 (J = 17.1 Hz) as in the case of **5** (Table 1). Moreover, the ^{13}C chemical shifts of the carbon atoms in rings D and E and the isopropenyl group were in perfect agreement with the corresponding shifts in **5** (Table 2), thus confirming the C-21 keto substitution in **2**.

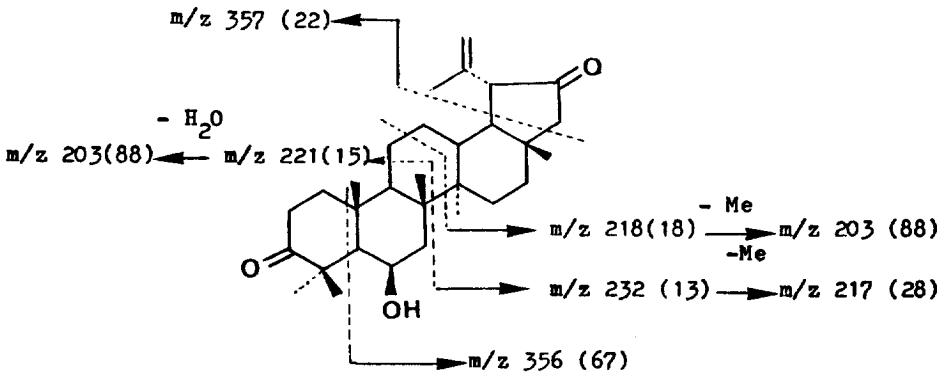
The positions of the remaining keto group and the hydroxyl group in **2** were determined from the following observations. A C-3 oxygenation in the form of a hydroxyl or keto group is often proposed in most of the triterpenoids due to biogenetic reasons. The ^{1}H and ^{13}C NMR data for **2** seem to be well in agreement with shifts of a C-3 keto triterpenoid rather than the corresponding C-3 hydroxyl derivative, irrespective of its α- or β-stereochemistry. This observation is based on the obvious changes noticed in the ^{13}C NMR chemical shifts of C-2, C-4 and C-23 carbons in C-3 keto lupane compounds in comparison with their C-3 hydroxyl derivatives. Therefore, the ^{1}H NMR signals at δ 2.81 (1H, *td*, J = 6.3 and 9.1 Hz) and 2.24 (1H, *m*) can be assigned for the diastereotopic H-2 protons, and their mutual connectivity has been established by decoupling studies. The multiplicity of these signals ruled out the possibility of hydroxylation at the C-1 position. Therefore, the positions remaining for the hydroxyl group substitution now appeared only at C-6 or C-7.

An analysis of the ^{1}H and ^{13}C NMR spectral data for 6β-hydroxylupane compounds such as 6β-hydroxylup-20(29)-en-3-one, 6β,20-dihydroxylupan-3-one, and 6β,28-dihydroxylup-20(29)-ene, all of which were isolated from *Pleurostylia opposita* (Celastraceae) [6, 7], indicate that a 6β-hydroxyl substitution in all these compounds is characterized by a carbinol methine proton shift at δ 4.50 and the corresponding carbinol methine carbon shift at δ 69.7. Another interesting observation to support this assignment is the unique and noticeable deshielding observed in the ^{1}H NMR shifts of three tertiary methyl groups at C-24, C-25 and C-26. This effect is explained in terms of 1,3-diaxial interaction of the above methyl groups with the axially oriented 6β-hydroxyl group [7]. The ^{1}H and ^{13}C NMR shifts of the carbinol methine proton and carbon in **2** appeared at δ 4.50 and 69.45, respectively, indicating a 6β-hydroxyl substitution. This assignment was further validated from the considerable deshielding observed for the ^{1}H NMR shifts of three methyl groups at C-24, C-25 and C-26 in **2** which appeared at δ 1.42, 1.45 and 1.47, i.e. 0.4–0.5 ppm greater than the corresponding shifts in our previously isolated lupane compounds without 6β-hydroxylation [2]. Moreover, the ^{1}H and ^{13}C NMR chemical shifts of the atoms in rings A, B and C in **2** are in perfect matching with those signals in 6β-hydroxylup-20(29)-en-3-one (**6**), thus confirming a 6β-hydroxy substitution and thereby characterizing **2** as 6β-hydroxysalacianone.

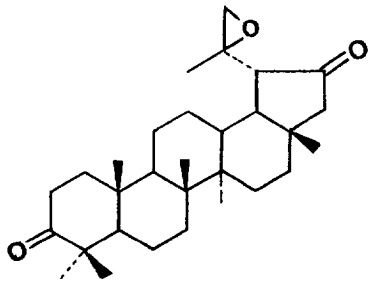
EXPERIMENTAL

^{1}H NMR spectra were recorded at 300 and 500 MHz in $CDCl_3$ using TMS as int. standard. The ^{13}C NMR spectra were recorded at 75 MHz using TMS as int. standard and multiplicities were determined by INEPT techniques. TLC was performed on 0.2 mm silica gel plates and the spots were detected by spraying with vanilline–H_2SO_4 followed by heating at 110° until the visualization of characteristic colour.

Plant material. The stem bark of *S. beddomei* was collected from Palode, Kerala, and identified by Dr A. Nazarudeen, Tropical Botanic Garden & Research



Scheme 2. EI-mass spectral fragmentation of **2**. Relative intensities are given in parentheses.



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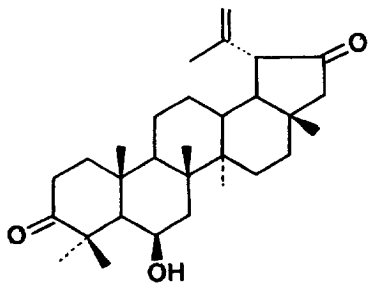

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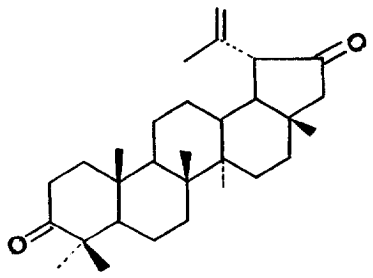

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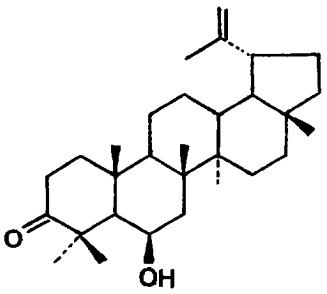

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Institute, Palode, Kerala, where a voucher specimen has been deposited.

Extraction and isolation. The shade-dried stem bark powder (500 g) was repeatedly extracted with hexane and subsequently with EtOAc. The conc hexane extract was chromatographed over silica gel as described previously [2] and **1** was isolated from $CHCl_3$ frs and eluted after 15α-hydroxyfriedelane-1,3-dione. The concd EtOAc extract (5 g) was separately chromatographed over silica gel and the column was eluted with hexane, hexane–C_6H_6, C_6H_6, C_6H_6–$CHCl_3$, $CHCl_3$ and $CHCl_3$–EtOAc. Compound **2** was isolated from C_6H_6 frs and **3** and **4** from $CHCl_3$–EtOAc frs.

20,29-*Epoxysalacianone* [20(29)-*epoxylupane*-3,21-*dione*] (**1**). Crystals ($CHCl_3$–MeOH) (3 mg) mp 205–208°. ^{1}H NMR (500 MHz, $CDCl_3$): Table 1; ^{13}C NMR (75 MHz, $CDCl_3$): Table 2: EI MS (70 eV) *m/z* (rel. int): 454 $[M]^+$ (62), 436 $[M-H_2O]^+$ (68), 426 (92), 424 (50) 394 (39), 341 (100), 340 (43), 245 (16), 235 (20), 219 (27), 217 (53), 205 (91), 203 (37), 190 (38), 188 (40).

6β-*Hydroxysalacianone* [6β-*hydroxylup*-20(29)-*ene*-3 3,1*dione*] (**2**). Crystals ($CHCl_3$–MeOH) (15 mg), mp 243°. ^{1}H NMR (300 MHz, $CDCl_3$): Table 1; ^{13}C NMR (75 MHz, $CDCl_3$): Table 2: EI MS (70 eV) *m/z* (rel. int): 454 $[M]^+$ (91), 436 $[M-H_2O]^+$ (25), 421 $[M-H_2O-Me]^+$ (22), 412 (7), 393 (9), 356 (67), 331 (19), 271 (9), 245 (20), 243 (18), 232 (13), 221 (15), 219 (32), 203 (88), 189 (37), 175 (51) 135 (75), 108 (89), 105 (95), 96 (100).

Betulin [3β-28-*dihydroxylup*-20(29)-*ene*] (**3**). Crystals ($CHCl_3$–MeOH) (10 mg), mp 234–36° (lit. ref. [3], mp 236–38°). ^{1}H NMR (300 MHz, $CDCl_3$): 0.76 (3H, *s*, H-24), 0.82 (3H, *s*, H-25), 0.97 (3H, *s*, H-23), 0.98 (3H, *s*, H-27), 1.02 (3H, *s*, H-26), 1.68 (3H, *s*, H-30), 2.38 (1H, *td*, J = 5.4, 11.3 Hz, H-19), 3.18 (1H, *m*, H-3), 3.33 (1H, *d*, J = 10.8 Hz, H-28b). 3.79 (1H, *d*, J = 10.8 Hz, H-28a), 4.58 (1H, *br s*, H-29b), 4.68 (1H, *br s*, H-29a), 0.65–2.00 (*m*, rest of the protons). ^{13}C NMR (75 MHz, $CDCl_3$) (C1-C30): 38.67, 27.04, 78.97, 38.87, 55.27, 18.27, 34.2, 40.89, 50.37, 37.13, 20.79, 25.15, 37.29, 42.69, 27.35, 29.16, 47.77, 48.72, 47.77, 150.46, 29.76, 33.93, 27.95, 15.35, 16.09, 15.94, 14.75, 60.58, 109.69, 19.05.

2α, 3β - *Dihydroxylup* - 20(29) - *ene* (**4**). Crystals ($CHCl_3$–MeOH) (3 mg), mp 232–34° (lit. ref. [4], mp 234°). ^{1}H NMR (300 MHz, $CDCl_3$): 0.79 (3H, *s*, H-24), 0.81 (3H, *s*, H-28), 0.90 (3H, *s*, H-23), 0.94 (3H, *s*, H-25), 1.01 (3H, *s*, H-27), 1.03 (3H, *s*, H-26), 1.68 (3H, *s*, H-30), 1.92 (2H, *m*, H-22a, H-1b) 2.37 (1H, *dt*, J = 5.4, 11.3 Hz), 2.04 (1H, *dd*, J = 5.4, 11.7 Hz, H-1a), 2.97 (1H, *d*, J = 9.6 Hz, H-3), 3.68 (1H, *ddd*, J = 10.5, 9.6, 4.5 Hz, H-2), 4.57 (1H, *br s*, H-29b), 4.69 (1H, *br s*, H-29a); ^{13}C NMR (75 MHz, $CDCl_3$ + CD_3OD) C-1–C-30): 42.68, 68.68, 83.27, 38.21, 55.20, 17.77, 33.96, 40.68, 49.6, 39.07, 20.83, 24.84, 37.81,

42.70, 27.21, 35.37, 42.81, 48.3, 46.33, 150.80, 29.63, 39.79, 28.23, 16.34, 17.11, 15.80, 14.29, 18.15, 19.05, 109.17.

Acknowledgement—This research was supported by the Science, Technology and Environment Department of Kerala through a research grant.

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