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ZIZYBERANALIC ACID, A PENTACYCLIC TRITERPENOID OF *ZIZYPHUS JUJUBA*

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Key Word Index—*Zizyphus jujuba*; Rhamnaceae; 3α-hydroxy-2β-aldehydo-A(1)-norlup-20(29)-en-28-oic acid; zizyberanalic acid.

Abstract—A novel pentacyclic triterpenoid, zizyberanalic acid, has been isolated from both bark and roots of *Zizyphus jujuba*. The structure and stereochemistry of the compound have been unambiguously settled as 3α-hydroxy-2β-aldehydo-A(1)-norlup-20(29)-en-28-oic acid from detailed spectroscopic evidence and by its conversion to a compound of established structure and stereochemistry.

INTRODUCTION

Zizyphus jujuba Lam. has been reported to possess a wide variety of chemical constituents including peptides and triterpenoids [1, 2]. The roots of the plant are used in the treatment of fever and of wounds/ulcers while the bark is used as a remedy in diarrhoaes [3]. A preliminary pharmacological investigation (unpublished observations: performed in collaboration with Pharmacological Research Unit, CCRAS, University College of Medicine, Calcutta-20) of the different extracts of the plant gave encouraging results. This paper reports the isolation of an unique compound, zizyberanalic acid (**1**), which is the first naturally ocurring pentacyclic triterpenoid having a formyl and a hydroxyl group in vicinal arrangement on a five-membered ring. It belongs to the hitherto unknown epiceanothic acid series, and is a potential biosynthetic intermediate of ceanothic acid derivatives and their isomers. The triterpenoids lupeol (**2**), betulinic acid (**3**) and ceanothic acid (**4**) were also isolated during this investigation.

RESULTS AND DISCUSSION

Zizyberanalic acid (**1**), $C_{30}H_{46}O_4$ (M^+ 470), mp 263–265°, $[\alpha]_D^{20}$ +3° (py) gave a Liebermann–Burchardt colour reaction for triterpenes. It exhibited in its IR spectrum bands at 3380 (OH), 1698, 1717 (COOH/CHO) and at 1644 and 828 cm^{-1} (=CH_2). The 300 MHz ^{1}H NMR spectrum of the compound in d_5-pyridine was highly informative and contained signals at δ 0.65 (6H, *s*), 0.70 (6H, *s*), 1.10 (3H, *s*, C-10 Me), 1.63 (=C–Me), 2.52 (1H, C-2H, *dd*, J = 4.6 and 8.7 Hz), 3.36 (1H, *m*, allylic proton), 4.57 (1H, *d*, –CHOH, J = 8.7 Hz), 4.63 and 4.78 (1H, *s*, each =CH_2) and at 10.01 (1H, *d*, –CHCHO, J = 4.6 Hz). The presence of a secondary hydroxyl group was confirmed by the formation of a mono-acetate (**1a**), $C_{32}H_{48}O_5$ ([M]$^+$ 512), mp 127–131°, ν_{max} 3450 and 1695 (COOH), 1725 and 1238 (OAc), 1638 and 880 cm^{-1} (=CH_2), ^{1}H NMR (300 MHz, $CDCl_3$): δ 0.83, 0.86, 0.98, 1.03, 1.03 (3H, *s* each, C-5 Me); 1.66 (=C–Me), 2.00 (–OCOMe), 2.33 (C-2H, *dd*, J = 4.2 and 8.9 Hz), 4.60 and 4.70 (1H, *s* each, =CH_2), 5.36 (1H, *d*, J = 8.9 Hz, –CH–OAc) and at δ 9.81 (–CHO, 1H, *d*, J = 4.2 Hz). Comparison (Table 1) of ^{13}C NMR spectral (75.5 MHz, $CDCl_3$) data of **1** and **1a** with those of ceanothic acid dimethyl ester (**4a**) and betulinic acid acetate (**3a**) isolated by us showed a very close resemblance in the carbon resonances in rings B–E in these compounds and showed significant differences in the resonance signals for ring-A carbons. This suggested that zizyberanalic acid was a pentacyclic triterpene of the lupeol series where ring-A was five-membered and contained a formyl at C-2. The proton at C-2 appeared as a double doublet in both **1** and **1a** due to coupling with a proton at C-3 and the proton of the C-2 formyl, the proton of which gave a doublet in **1** and **1a**. Furthermore, the signals for the ring-junction carbons (C-5 and C-10) in the ^{13}C NMR spectra of **1** and **1a** were shifted accordingly from the values of these carbons in betulinic acid acetate where ring-A is six-membered.

Thus, zizyberanalic acid appeared to be a triterpene of the lupeol series containing a carboxylic group, an aldehyde, a double bond in an isopropenyl group and a secondary hydroxyl on a five-membered ring in a β-position to the formyl. Compelling evidence for the vicinal arrangement of the –OH and –CHO groups in ring-A was obtained from the isolation of a conjugated aldehyde, zizyberenalic acid (**5**), when **1** was heated at its melting point. Compound **5**, $C_{30}H_{44}O_3$ ([M]$^+$ 452), mp 218–220°, λ_{max}^{EtOH} 237 nm (log ε 3.91, conjugated aldehyde), ν_{max} 3400, 1687 (COOH/CHO), 1638, 877 (=CH_2) cm^{-1} showed in its ^{1}H NMR (300 MHz, $CDCl_3$) spectrum signals at δ 0.95, 0.96, 0.98, 1.11, 1.26 (3H, *s* each, C-5 Me), 1.66 (3H, *s*, vinylic methyl), 2.98 (1H, *m*, allylic proton), 4.59, 4.73 (1H, *s* each =CH_2), 6.53 (1H, *s*, C-3H), 9.70 (1H, *s*, –CHO). The ^{1}H NMR spectrum of **5**, as expected, lacked the proton signals for –CHOH and H-2. Instead, there appeared two sharp singlets at δ 6.53 and 9.70 attributable to H-3 and an aldehydic proton, respectively, thereby confirming the vicinal arrangement of the –OH and –CHO groups in ring A. The above experiment, however, could not establish the orientation of these two groups with respect to each other. Thus, four different

Table 1. ^{13}C NMR speetral data of compounds **1**, **1a**, **3a** and **4** (75.5 MHz, $CDCl_3$)

C		1*	1a	3a	4a
1	*s*	206.18	203.80	38.28 *t*	176.55[a]
2	*d*	80.93	81.89	18.07 *t*	84.63
3	*d*	73.66	77.20	80.90	65.33
4	*s*	48.26	48.54	37.71	49.33
5	*d*	63.02	62.49	55.31	56.49
6	*t*	18.59	17.94	20.76	18.38
7	*t*	34.70	34.07	34.14	33.85
8	*s*	43.11	42.90	40.62	43.17
9	*d*	49.78	49.36	50.29	49.36
10	*s*	41.25	41.97	37.05	41.54
11	*t*	24.94	22.95	23.59	23.45
12	*t*	25.68	24.58	25.34	25.33
13	*d*	38.55	38.24	38.35	38.51
14	*s*	42.32	42.75	42.34	42.72
15	*t*	31.26	29.76	30.50	30.55
16	*t*	32.95	32.20	32.08	32.12
17	*s*	56.58	56.25	56.35	56.43
18	*d*	50.56	50.13	49.19	46.81
19	*d*	47.84	46.93	46.87	44.50
20	*s*	151.20	149.99	150.23	150.18
21	*t*	30.42	30.52	29.60	29.74
22	*t*	37.63	37.07	36.97	36.82
23	*q*	26.58	25.21	27.86	30.69
24	*q*	17.07	16.59	16.36	19.05
25	*q*	25.68	24.58	16.07	18.32
26	*q*	15.08	16.59	15.95	16.38
27	*q*	14.90	14.77	14.56	14.62
28	*s*	178.82	178.99	182.54	175.20[a]
29	*t*	110.07	109.92	109.62	109.44
30	*q*	19.50	19.30	19.26	19.34
			–O–CO–Me	–O–CO–Me	2–COOMe
			170.71 *s*	170.95 *s*	51.12 *q*
			20.76 *q*	21.20 *q*	51.08 *q*

*Measured in pyridine-d_5.

[a]Assignments may be interchanged.

configurations (i–iv) of ring-A substituents in **1** are possible (Scheme 1). The splitting pattern of H-3 as a doublet in both the parent compound ($J = 8.6$ Hz) and its acetate ($J = 8.9$ Hz) clearly indicated that the two groups (–OH and –CHO) were arranged as shown in i, ii or iv and eliminated the *trans*-configuration (iii) where the dihedral angle (from Dreiding models) is $\sim 100°$ which means that J would have to be 0–1 Hz [4]. Comparison of the coupling constants of H-2, H-3 in **1** and **1a** with those reported [5] for ceanothic acid dimethyl ester acetate (**4b**) and its corresponding epimers and isomers strongly indicated that zizyberanalic acid has the A-ring configuration as shown in iv, where H-2–H-3 dihedral angle is $\sim 140°$. This led to structure **1** for zizyberanalic acid. In order to confirm the assigned structure, **1** was acetylated with acetic anhydride–pyridine to form a monoacetate (**1a**) which on Jones' oxidation and subsequent methylation with diazomethane in ether (Scheme 1) afforded a dimethyl ester acetate (**6**), $C_{34}H_{52}O_6$ (M^+ 556), mp 150–152°, $[\alpha]_D^{20°} + 9°$ ($CHCl_3$; *c* 0.1), ν_{max} 1722, 1240 (OAc), 1740 (COOMe) and 880 ($=CH_2$) cm^{-1}. The ^{1}H NMR spectrum (300 MHz, $CDCl_3$) of the compound contained signals at δ 1.61 (3H, *s*, vinylic methyl), 2.02 (3H, *s*, –OCOMe), 2.38 (1H, *d*, $J = 9.5$ Hz, C-2H), 3.64 and 3.65 (3H, *s*, 2 –COOMe), 4.60 and 4.72 (1H, *s* each $=CH_2$), 5.26 (1H, *d*, $J = 9.5$ Hz, C-3H).

Table 2 lists the coupling constants between the protons on C-2 and C-3 for four possible configurations of ring-A substituents in the dimethyl acetoxy ceanothate series [5] and compound **6**.

These values unequivocally established the structure and stereochemistry of the derived dimethyl ester acetate as methyl 3α-acetoxy-2β-methoxy-carbonyl-A(1)-norlup-20(29)-en-28-oate (**6**), thereby confirming the structure and stereochemistry of zizyberanalic acid as represented by **1**. The detailed ^{13}C NMR analysis of triterpenoids of the lupane series with a modified A-ring is reported for the first time. The structure (**1**) for zizyberanalic acid received further support from its mass spectral fragmentation pattern. The genesis of the ion-fragments at *m/z* 452, 424, 248, 234 and 203 corresponding to species, **a–e** respectively could be explained in terms of the structure (**1**) for zizyberanalic acid.

Thus **1** is the first member to the epiceanothic acid series to be isolated from nature. Biogenetically, it could be derived from 2-hydroxybetulinic acid by oxidative cleavage across the C-2–C-3 bond to form an intermediary dialdehyde which, through an aldol-type condensation, could generate **1**. Studies are in progress to isolate other possible bio-intermediates.

EXPERIMENTAL

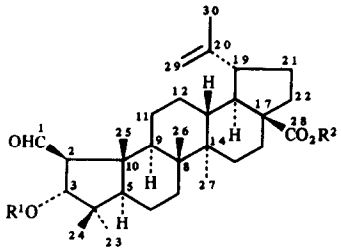
Plant material was collected locally and identified by Dr S. R. Das, Survey Officer, Regional Research Institute (Ay.), Calcutta-700 009. A voucher specimen has been deposited at the Department of Pure Chemistry, Calcutta University. Mps: uncorr; UV: 95% aldehyde-free EtOH; ^{1}H NMR (300 MHz) and ^{13}C NMR (75.5 MHz): $CDCl_3$ (except were otherwise stated) with TMS as int. standard. Silica gel (BDH, 60–120 mesh) and silica gel G (Merck) were used for CC and TLC respectively. Samples were routinely dried over P_2O_5 for 24 hr.

Extraction and separation of the compounds. The dried and powdered bark (5 kg) of *Zizyphus jujuba*, collected locally in November 1986, was extracted with hot petrol (60–80°). The crude extract after evapn of the solvent was chromatographed over silica gel. Elution with C_6H_6–EtOAc furnished lupeol (**2**) (20 mg), betulinic acid (**3**) (300 mg) and zizyberanalic acid (**1**) (150 mg). The EtOAc extract of the defatted roots of the same plant on chromatographic resolution over silica gel yielded ceanothic acid (**3**) (350 mg) in the C_6H_6–EtOAc (1 : 1) eluates.

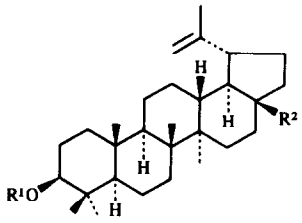
Lupeol (**2**). Colourless needles; mp 222–224° (Et_2O), identified as lupeol (co-IR).

Betulinic acid (**3**). Isolated as a powder which was directly acetylated with Ac_2O–pyridine. Usual work-up afforded betulinic acid acetate (**3a**). Colourless needles; mp 258° (dec.) (petrol); ^{1}H NMR ($CDCl_3$): δ 0.81, 0.82, 0.83, 0.91, 0.95 (3H, *s* each, 5 × Me), 2.02 (3H, *s*, OAc), 4.46 (1H, *m*, 3α-H), 4.60 and 4.72 (1H, *s*, each, $=CH_2$); ^{13}C NMR ($CDCl_3$): Table 1; identified as betulinic acid acetate (co-IR).

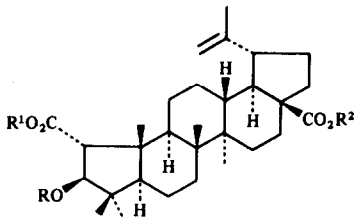
Zizyberanalic acid (**1**). Colourless granules; mp 263–265° (EtOAc); $[\alpha]_D^{20} + 3°$ (pyridine; *c* 1.15); $C_{30}H_{46}O_4$; MS *m/z*: 470 $[M]^+$, 452, 424, 248, 234, 203; IR ν_{max}^{KBr} cm^{-1}: 3380, 1717, 1698, 1644, 828; ^{1}H NMR (pyridine-d_5): δ 0.65 (6H, *s*, 2 × Me), 0.70 (6H, *s*, 2 × Me), 1.10 (3H, *s*, C-10 Me), 1.63 (3H, *s*, vinylic Me), 2.52 (1H, *dd*, $J = 4.6$, 8.7 Hz, H-2α), 3.36 (1H, *m*, H-19), 4.57 (1H, *d*, $J = 8.7$ Hz, H-3β), 4.63 and 4.78 (1H, *s* each, $=CH_2$), 10.01 (1H, *d*, $J = 4.6$ Hz, CHO); ^{13}C NMR (pyridine-d_5): Table 1.



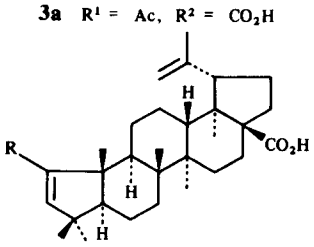
1 $R^1 = R^2 = H$
1a $R^1 = Ac$, $R^2 = H$



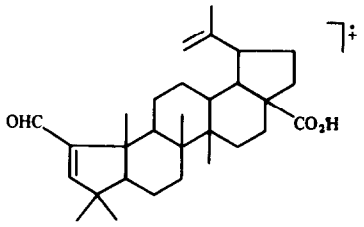
2 $R^1 = H$, $R^2 = Me$
3 $R^1 = H$, $R^2 = CO_2H$
3a $R^1 = Ac$, $R^2 = CO_2H$



4 $R^1 = R^2 = R^3 = H$
4a $R^1 = H$, $R^2 = R^3 = Me$
4b $R^1 = Ac$, $R^2 = R^3 = Me$



5 R = CHO
5a R = H



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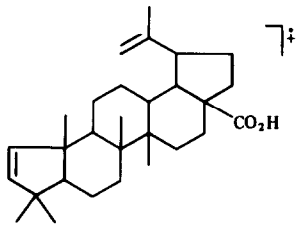

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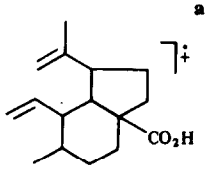

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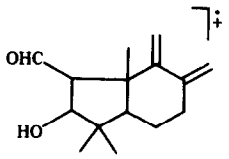

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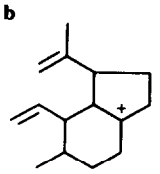

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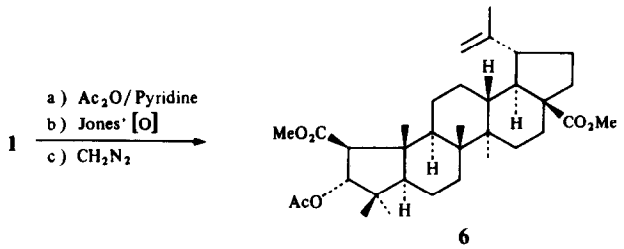

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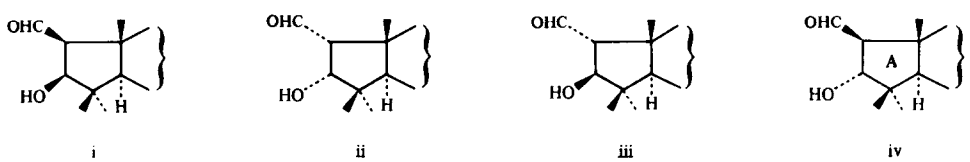

i ii iii iv

Scheme 1.

Table 2. Coupling constants of C-2 and C-3 protons

		Configuration of ring-A substituents			
		2α, 3β;	2β, 3β;	2α, 3α;	2β, 3α
		Observed $J_{2,3}$ (Hz)			
Dimethyl ceanothate series:	(3–OAc)	0.2	7.6	7.6	9.5
Compound **6**	(3–OAc)	—	—	—	9.5

Acetylation of zizyberanalic acid. Zizyberanalic acid (**1**) (75 mg) in pyridine (1 ml) and Ac_2O (5 ml) was heated at 80° for 1 hr and kept overnight at room temp. After usual work-up, it was purified by prep. TLC (C_6H_6–EtOAc 19:1) to give the acetate **1a** (60 mg). Amorphous solid; mp 127–131°; $C_{32}H_{48}O_5$: MS *m/z*: 512 $[M]^+$; ^{13}C NMR ($CDCl_3$) (Table 1).

Zizyberenalic acid (**5**). Zizyberanalic acid (**1**) (10 mg) was heated at its mp (265°) and the product after extraction with $CHCl_3$ was chromatographed over silica gel. Elution with a mixture of C_6H_6–EtOAc (4:1) yielded **5** (7 mg). Colourless needles; mp 218–220° (Et_2O); $C_{30}H_{44}O_3$; MS *m/z*: 452 $[M]^+$.

Ceanothic acid (**4**). Light colourless needles; mp 328–331° (C_6H_6–EtOAc); IR ν_{max}^{KBr} cm^{-1}: 3420, 1692, 1640, 882; ^1H NMR (pyridine-d_5): δ 1.04, 1.12, 1.20, 1.34, 1.36 (3H, *s* each, 5 × Me), 1.60 (3H, *s*, vinylic Me), 3.12 (1H, *s*, H-2β), 4.82 (1H, *s*, H-3α), 4.72 and 4.86 (1H, *s* each $=CH_2$).

Dimethylceanothate (**4a**). Ceanothic acid (**4**) (200 mg) was methylated with ethereal CH_2N_2 to afford dimethylceanothate (**4a**) (180 mg). Colourless granules; mp 216–218° (light petrol), lit. [6] 220–222°; ^{13}C NMR ($CDCl_3$): Table 1.

Dimethylceanothate acetate (**4b**). Acetylation of **4a** (50 mg) gave **4b** (40 mg). Colourless needles; mp 164–166° (MeOH), lit. [6] 168–169°; ^1H NMR ($CDCl_3$): δ 0.90, 0.96, 0.98, 1.06, 1.23 (3H, *s* each, 5 × Me), 1.70 (3H, *s*, vinylic Me), 2.08 (3H, *s*, OAc), 2.61 (1H, *s*, H-2β), 3.75 and 3.76 (3H, *s* each, 2 × CO_2Me), 4.63 and 4.76 (1H, *s* each, $=CH_2$), 5.13 (1H, *s*, H-3α).

Decarboxylative dehydration of ceanothic acid (**4**). Ceanothic acid (**4**) (20 mg) was heated at its mp (331°) and the product after usual work-up and purification (silica gel, C_6H_6) afforded **5a** (10 mg). Colourless needles; mp 228–230° (*n*-pentane); lit. [6] 230–232°; ^1H NMR ($CDCl_3$): δ 0.91, 0.93, 0.94, 0.99, 1.01 (3H, *s* each, 5 × Me), 1.68 (3H, *s*, vinylic Me), 4.60 and 4.73 (1H, *s* each, $=CH_2$), 5.43 (1H, *d*, $J \simeq 5.1$ Hz, H-2), 5.91 (1H, *d*, $J = 5.1$ Hz, H-3).

Methyl 3α-acetoxy-2β-methoxycarbonyl-A(1)-norlup-20(29)-en-28-oate (**6**). Zizyberanalic acid acetate (**1a**) (30 mg) in Me_2CO (15 ml) on Jones' oxidation and usual work-up afforded a solid which was methylated with ethereal CH_2N_2. The product on chromatography (neutral Al_2O_3, petrol–benzene 49:1) furnished **6** (25 mg). Colourless needles; mp 150–152° (MeOH); $[\alpha]_D^{20}$ +9° ($CHCl_3$; *c* 0.1), lit. [5] 176–178°, $[\alpha]_D^{20}$ +9° ($CHCl_3$).

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