

Constituents of the Leaves of *Thevetia neriifolia*

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CONSTITUENTS OF THE LEAVES OF *THEVETIA NERIIFOLIA*

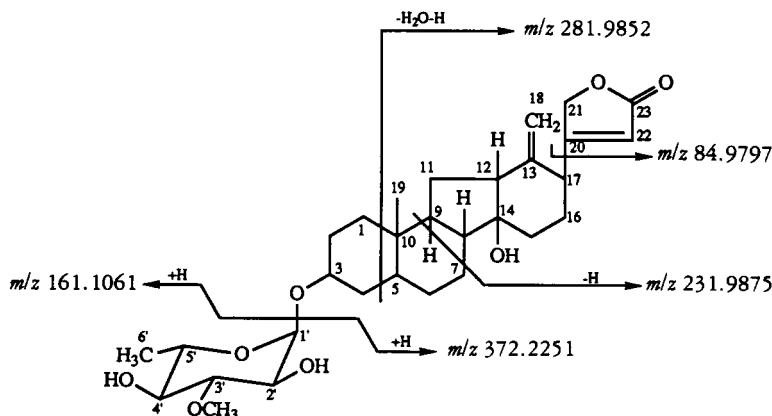
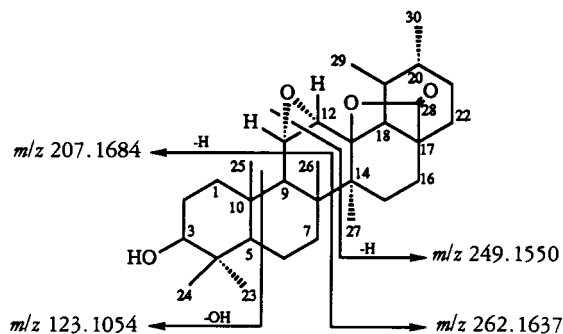
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ABSTRACT.—A new pentacyclic triterpene **1** has been isolated from the fresh, uncrushed leaves of *Thevetia neriifolia* along with a cardenolide, 3 β -O-(α -L-thevetose)-3 β ,14 β -dihydroxy-14(13 \rightarrow 12)-abeo-5 β ,12 β ,14 β -carda-13(18),20(22)-dienolide [**2**]. The structures of **1** and **2** have been elucidated through detailed 1D and 2D nmr studies.

Thevetia neriifolia Juss. (Apocynaceae) is distributed in tropical America and the West Indies. The tree has been used in folk medicine as a purgative, an emetic, and a remedy for intermittent fever (1). It possesses cardiotoxic activity (2) as well as insecticidal properties (3) and also affects alkaline phosphatase activity (4). Studies undertaken by various groups on *T. neriifolia* in view of its medicinal significance have led to the isolation of several cardiac glycosides

(5), sterols (6), iridoid-glucosides (7), and triterpenes (8). We report the isolation and characterization of a new pentacyclic triterpene 3 β -hydroxy-11 α ,12 α -epoxy-urs-13 β ,28-olide [**1**] and a cardenolide, 3 β -O-(α -L-thevetose)-3 β ,14 β -dihydroxy-14(13 \rightarrow 12)-abeo-5 β ,12 β ,14 β -carda-13(18),20(22)-dienolide [**2**] along with seven known compounds from the fresh uncrushed leaves. [Compound **2** was presented in the Fifth International Symposium on Natural Product Chemis-



try held in Karachi, Pakistan, Jan. 5–9, 1992. However, at the time of submission of this manuscript we came across a reference in which the same compound has been communicated from the same source (9).] The known compounds were identified as neolupenyl acetate, 11-oxo-urs-12-en-28-oic acid, lupeol acetate, oleanolic acid, ursolic acid, stigmast-5-en-7-one, and β -sitosterol, based on comparison with the reported data (10–18). The structures of **1** and **2** have been

determined on the basis of detailed ^1H - and ^{13}C -nmr studies including 2D experiments (COSY-45, *J*-resolved and hetero-COSY).

RESULTS AND DISCUSSION

The eims of **1** showed the molecular ion peak at *m/z* 470, an exact measurement of which gave 470.3379 corresponding to a formula $\text{C}_{30}\text{H}_{46}\text{O}_4$. The ^1H -nmr spectrum (Table 1) showed a doublet at δ 1.55 (*J* = 11.23 Hz, H-18)

TABLE 1. ^1H - ^{13}C -Hetero-COSY of **1** and **2**.

Position	1		Position	2	
	δ C	δ H ^a		δ C	δ H ^a
1	37.87	1.31 m	1	30.31	1.83 m
2	21.35	1.15 m	2	25.16	1.74 m
3	78.84	3.24 dd(10.95, 5.34)	3	73.41	1.62 m
4	38.91	—	4	31.25	1.59 m
5	56.35	0.69 dd(11.34, 3.03)	5	36.55	3.94 m
6	17.66	1.12 m	6	26.63	1.36 m
7	31.43	1.17 m	7	20.13	1.33 m
8	41.40	—	8	49.73	1.63 m
9	51.55	1.53 m	9	38.38	1.29 m
10	36.58	—	10	35.03	1.32 m
11	54.71	3.11 dd(3.95, 2.21)	11	21.08	2.05 m
12	54.67	2.94 d(3.95)	12	50.00	2.33 m
13	89.04	—	13	145.93	—
14	40.65	—	14	79.95	—
15	26.79	1.09 m	15	32.04	1.83 m
16	22.82	1.28 m	16	27.59	1.74 m
		1.06 m	17	44.11	1.29 m
17	43.90	—	18	111.90	1.23 m
18	60.75	1.55 d(11.23)	19	22.73	3.40 m
19	40.30	1.85 m	20	173.77	5.16 br s
20	37.58	1.35 m	21	72.66	4.99 br s
21	30.63	1.31 m	22	116.30	0.92 s
22	31.20	1.23 m	23	171.30	—
23	27.64	1.09 s	C-1'	97.00	4.84 d(4.44)
24	17.21	1.12 s	C-2'	73.18	3.58 dd(9.09, 4.44)
25	16.42	0.99 s	C-3'	84.50	3.24 t(9.09)
26	15.11	0.78 s	C-4'	74.72	3.13 t(9.09)
27	18.88	1.02 s	C-5'	67.47	3.75 dq(9.09, 6.30)
28	178.90	—	C-6'	17.51	1.23 d(6.30)
29	17.27	1.08 d(7.40)	O-Me	60.55	3.67 s
30	20.30	1.04 d(6.30)			

^aCoupling constants, in Hz, were calculated from ^1H -nmr and 2D *J*-resolved spectra.

a double doublet at δ 3.24 ($J = 10.95$, 5.34 Hz, H-3), five methyl singlets at δ 1.12, 1.09, 1.02, 0.99, and 0.78, and two methyl doublets at δ 1.08 and 1.04. These data indicated that compound **1** is a triterpene of the α -amyrin type. The molecular formula showed eight double bond equivalents, five of which were accounted for by the ring nucleus. The ir absorption at 1755 cm^{-1} and a carbon signal at δ 178.9 suggested the presence of a lactone which possessed two double bond equivalents. No double bond was indicated by the nmr, yet two coupled proton signals at δ 3.11 (dd, $J = 3.95$, 2.21 Hz) and 2.94 (d, $J = 3.95$ Hz) indicated an epoxy ring and accounted for the remaining double bond equivalent. These protons were related to two C-H carbons at δ 54.71 and 54.67 in the hetero-COSY spectrum, which supported the epoxide moiety. The splitting pattern of these protons and mass fragments at m/z 207.1684 and 262.1637 (see structure) suggested location of the epoxide ring at C-11, C-12; and the doublet of H-12 showed that C-12 is adjacent to a quaternary carbon (C-13). Hence a lactone ring was presumed between C-13 and C-17 and this conclusion was supported by quaternary carbons at δ 89.04 (C-13) and 43.90 (C-17) in the ^{13}C -nmr spectrum (BB). The C-17 shift and shifts of other carbons of ring D/E are consistent with a carboxyl or lactone function at C-17 as in ursolic acid (19). The multiplicities and coupling constants of the epoxide protons further showed α disposition of the epoxide ring. Thus the structure of **1** was proposed as 3β -hydroxy- 11α , 12α -epoxy-urs- 13β , 28 -olide, which was confirmed through comparison of the spectral data of the acetyl derivative of **1** with those of an authentic sample obtained earlier synthetically (19,20). However, this is the first instance of the isolation of **1** as a natural product.

The hrms of **2** showed an ion at m/z 372.2251 ($\text{C}_{23}\text{H}_{32}\text{O}_4$) while positive fab showed an $[\text{M} + 1]^+$ peak at m/z

533, suggesting a sugar moiety with 178 amu. The ir spectrum showed peaks at 3500 (OH), 1780, 1740 (α,β -unsaturated five-membered lactone), and 1610 cm^{-1} ($>\text{C}=\text{C}$). One anomeric carbon signal (δ 97.0) suggested that **2** has only one sugar moiety. The chemical shift and coupling constant of H-1' showed an α -glycosidic linkage with the aglycone. The protons of the sugar were assigned using their coupling constants in the ^1H nmr (Table 1), 2D J -resolved, and COSY-45 as well as double resonance experiments. Thus, the anomeric proton (H-1') at δ 4.84 (d, $J = 4.44$ Hz) was coupled to H-2' at δ 3.58 (dd, $J = 9.09$, 4.44 Hz). Using same techniques H-3' was assigned at δ 3.24 (t, $J = 9.09$ Hz), H-4' at δ 3.13 (t, $J = 9.09$ Hz), H-5' at δ 3.75 (dq, $J = 9.09$, 6.30 Hz), and H-6' at δ 1.23 (d, $J = 6.30$ Hz). The coupling constants of H-2'-H-5' indicated that these protons are axially oriented. The methoxy group (δ_{H} 3.67, δ_{C} 60.55) was placed at C-3' due to the downfield chemical shift of C-3' (δ_{C} 85.4). These observations led to characterization of the sugar as 6-deoxy-3-*O*-methyl- α -L-glucose (α -L-thevetose) (21). The carbons of the sugar moiety were assigned on the basis of hetero-COSY. The ^1H -nmr spectrum of compound **2** further showed two double doublets of one proton each at δ 4.69 and δ 4.56 for H_a-21 ($J = 17.53$, 3.77 Hz), and H_b-21 ($J = 17.53$, 1.85 Hz), and a one-proton double doublet at δ 5.94 ($J = 3.77$, 1.85 Hz) for H-22 of the butenolide ring. Characterization of the sugar as α -L-thevetose led to assignment of the ion at m/z 372.2251 ($\text{C}_{33}\text{H}_{32}\text{O}_4$) to the aglycone and demonstrated that it had one oxygen in addition to the hydroxyl functions at C-3 and of the butenolide ring. This was attributed to a hydroxyl function at C-14 due to the presence of a quaternary carbon at δ 79.95 in the ^{13}C -nmr spectra (BB, DEPT). An exomethylene was observed at δ 111.90, which indicated a terminal double bond.

The corresponding olefinic protons (hetero-COSY) were observed at δ 5.16 (br s) and 4.99 (br s) in the ^1H -nmr spectrum. Furthermore, a quaternary carbon signal at δ 145.93 and only one angular methyl signal ($\delta_{\text{C}} = 22.73$, $\delta_{\text{H}} = 0.92$) in the nmr indicated that the second angular methyl group has been oxidized to an exocyclic double bond. The ^{13}C -nmr shifts of ring A and B carbons were comparable to those of digitoxigenin (22), indicating that compound **2** has 5β hydrogen and further that the Me-19 was intact and Me-18 had been oxidized to form an exocyclic methylene group accompanied by cleavage of the C-13—C-14 bond and formation of a new bond between C-12 and C-14. This conclusion was supported by the presence of six methine and eight methylene carbons in **2** instead of five methines and nine methylenes in the digitoxigenin steroidal nucleus. Assignment of the carbons of the aglycone part was also based on hetero-COSY as well as comparison with the chemical shifts of digitoxigenin (22). Thus, the structure of compound **2** is 3β -O-(α -L-thevetose)- 3β , 14β -dihydroxy- $14(13\rightarrow 12)$ -abeo- 5β , 12β , 14β -carda- $13(18)$, $20(22)$ -dienolide.

Characterization of the known compounds rests on comparison of spectral data of these compounds with those reported earlier (10–18). Neolupenylacetate, 11-oxo-urs-12-en-28-oic acid, and stigmast-5-en-7-one are previously unreported from this source, whereas lupeol acetate, ursolic acid, and β -sitos-terol were reported earlier (14–16, 18).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Ms: Finnigan MAT 112 and 312 double focusing mass spectrometers connected to a PDP 11/34 computer system; nmr spectra (CDCl_3): 500 MHz for ^1H and 75 MHz for ^{13}C . Chemical shifts are reported in δ (ppm) and coupling constants are in Hz. In the ^{13}C -nmr, carbon signals were identified through BB, DEPT, and hetero-COSY spectra and comparison with the chemical shifts of carbons of related compounds (22–27). Si gel PF₂₅₄ and Si gel 9385 have been used for tlc and flash cc (Model Eyla), respectively. The plant

was identified as *T. neriifolia* by Prof. S.I. Ali (Department of Botany, University of Karachi), and a voucher specimen (No. 33010 KUH) has been deposited in the Herbarium.

Fresh undried and uncrushed leaves (22 kg) of *T. neriifolia* were collected from the Karachi region in the month of December and percolated with MeOH at room temperature. The syrupy concentrate obtained on removal of solvent was partitioned between EtOAc and H₂O. The EtOAc layer was partitioned with 4% aqueous Na₂CO₃ to separate acidic and neutral fractions. The EtOAc layer (neutral fraction) was washed with H₂O and dried (Na₂SO₄). The residue obtained on removal of the solvent from this layer was divided into hexane-soluble and hexane-insoluble portions. The hexane-insoluble fraction (25 g) was subjected to vacuum liquid chromatography (vlc) (0–10% MeOH in CHCl₃), which gave nine fractions. Fraction 1, which eluted with CHCl₃, was again subjected to vlc (0–10% EtOAc in hexane), which furnished eight fractions (I–VIII). Fraction II (390 mg) was further separated by flash cc (29) (0–10% EtOAc in hexane), ultimately furnishing three compounds. The first two that were eluted with hexane, in the order of polarity, were identified as neolupenyl acetate (24 mg) and lupeol acetate (30 mg), while the third, obtained on elution with hexane-EtOAc (99:1), was identified as β -sitos-terol (26 mg). Fraction IV (1.94 g) was subjected to flash cc (0–10% MeOH in CHCl₃), ultimately yielding five compounds. The CHCl₃ eluate furnished stigmast-5-en-7-one (35 mg), oleanolic acid (210 mg), ursolic acid (530 mg), and 3β -hydroxy- 11α , 12α -epoxy-urs- 13β , 28 -olide [**1**] (29 mg), in order of polarity, while CHCl₃-MeOH (9.6:0.4) eluate gave 3β -hydroxy- 11 -oxo-urs- 12 -en- 28 -oic acid (19 mg). Fraction V was characterized as ursolic acid (853 mg), while fraction VI furnished 3β -O-(α -L-thevetose)- 3β , 14β -dihydroxy- $14(13\rightarrow 12)$ -abeo- 5β , 12β , 14β -carda- $13(18)$, $20(22)$ -dienolide [**2**] (52 mg) on flash cc and elution with CHCl₃-MeOH (98:2).

3β -Hydroxy- 11α , 12α -epoxy-urs- 13β , 28 -olide [**1**].—White plates (29 mg) (MeOH): $[\alpha]_{\text{D}} +49.4^\circ$ (CHCl₃); mp 283 – 284° ; uv (MeOH) λ max 200 nm; ir (CHCl₃) ν max 3400, 2900–2850, 1755, 1020 cm^{-1} ; ^1H and ^{13}C nmr see Table 1; hrms m/z (rel. int. %) [$\text{M}]^+$ 470.3379 (C₃₀H₄₆O₄) (29), [$\text{M} - \text{H}_2\text{O}]^+$ 452.3272 (C₃₀H₄₄O₃) (11), [$\text{M} - \text{COOH}]^+$ 425.3387 (C₂₉H₄₅O₂) (9), 262.1637 (C₁₆H₂₂O₃) (31), 207.1684 (C₁₄H₂₃O) (55), 189.1605 (C₁₄H₂₁) (100), 81.0713 (C₆H₉) (70).

3β -O-(α -L-thevetose)- 3β , 14β -dihydroxy- $14(13\rightarrow 12)$ -abeo- 5β , 12β , 14β -carda- $13(18)$, $20(22)$ -dienolide [**2**].—Colorless needles (52 mg) (EtOH): $[\alpha]_{\text{D}} +20.6^\circ$ (MeOH); mp 152 – 153° ; uv (MeOH) λ max 217 nm; ir (CHCl₃) ν max 3450,

2900–2850, 1780, 1740, 1610, 1120, 1040 cm^{-1} ; ^1H and ^{13}C nmr see Table 1; hrms m/z (rel. int. %) 372.2251 ($\text{C}_{23}\text{H}_{32}\text{O}_4$) (19), 354.2176 ($\text{C}_{23}\text{H}_{30}\text{O}_3$, aglycone - H_2O) (33), 336.2109 ($\text{C}_{23}\text{H}_{28}\text{O}_2$, aglycone - $2 \times \text{H}_2\text{O}$) (45), 321.1840 ($\text{C}_{22}\text{H}_{25}\text{O}_2$, aglycone - $2 \times \text{H}_2\text{O}$ - Me) (3), 241.1261 ($\text{C}_{18}\text{H}_{17}\text{O}_2$) (25), 161.1061 ($\text{C}_7\text{H}_{13}\text{O}_4$) (20), 121.0903 ($\text{C}_8\text{H}_9\text{O}$) (19), 74.0370 ($\text{C}_3\text{H}_6\text{O}_2$) (100).

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