New Triterpenoids from *Corchorus trilocularis*

Amir AHMED, Muhammad ASIM, Muhammad ZAHID, Akbar ALI, and Viqar Uddin AHMAD*

HEJ Research Institute of Chemistry, International Center for Chemical Sciences, University of Karachi; Karachi 75270, Pakistan. Received December 18, 2002; accepted March 11, 2003

Two new tetracyclic triterpenoid trilocularol A and trilocularol A 3-glucoside and one pentacyclic triterpenoid tirlocularoside A were isolated from *Corchorus trilocularis* L., their structure were elucidated as 3β,6α,16α,20(*S*),27-pentahydroxydammar-24(*Z*)-ene (1), 3β-D-glucopyranosyloxy-6α,16α,20(*S*),27-tetrahydroxydammar-24(*Z*)-ene (2) and 2α,3β,19α,30-tetrahydroxyurs-12-en-24,28-dioic acid 28-*O*-β-D-glucopyranosyl ester (3). respectively, on the basis of detailed spectroscopic studies.

Key words *Corchorus trilocularis*; Tiliaceae; triterpenoid

Corchorus trilocularis L. (Tiliaceae) is commonly found throughout Pakistan in plains and low hills at moist shady places. The bitter seed of the plant have been used by natives in fevers and abdominal obstruction.[1)] Previous phytochemical investigation of this genus have led to the isolation of flavonoids, triterpenoids and cardiac glycoside from the various species.[2—6)] The previously isolated corchoionoside 'B' shows inhibitory activity on the histamine released from rat peritoneal exudate cells induced by antigen-antibody reaction.[7)] The plant *Corchorus trilocularis* has, however, not yet been investigated and this paper describes the structure elucidation of three new triterpenoids isolated from this plant.

Results and Discussion

Trilocularol A (**1**) was isolated as an amorphous powder. The high-resolution electron impact (HR-EI) MS of **1** showed the $[M]^+$ at *m/z* 492.3814 in agreement with the molecular formula $C_{30}H_{52}O_5$, indicating five degrees of unsaturation. The IR spectrum showed absorption peaks at 3413 (OH) and 1602 (C=C) cm^{-1}. The ^{1}H-NMR spectrum exhibited seven methyl signals at δ 0.90 (s, H_3-19), 0.94 (s, H_3-29), 1.02 (s, H_3-18), 1.15 (s, H_3-30, 21), 1.27 (s, H_3-28), 1.75 (s, H_3-26), an olefinic proton at δ 5.29 (t, J=7.1 Hz, H-24) and three oxygenated methines at δ 3.11 (dd, J=4.9, 11.9 Hz, H-3), 4.01 (dt, J=3.2, 10.4, 13.7 Hz, H-6) and 4.18 (m, H-16), suggesting that the molecule is a tetracyclic triterpenoid. The ^{13}C-NMR spectrum shows 30 signals identified as: seven methyls, nine methylenes, eight methines and six quaternary carbons on the basis of DEPT experiment. The detailed analysis of ^{1}H–^{1}H correlation spectroscopy (COSY), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC), and heteronuclear multiple bond connectivity (HMBC) experiment permitted us to determine the structure. The olefinic proton was correlated to the carbon at δ 129.3 (C-24) in the HMQC and showed long range correlations with the vinyl methyl carbon at δ 21.5 (C-26) and oxymethylene carbon at δ 61.4 (C-27) in the HMBC spectrum (Fig. 2). This showed that both methyl and oxymethylene are attached to the olefinic carbon and are located at the terminal of the side chain. The stereochemistry of $\Delta^{24,25}$ was arrived as *Z* based on chemical shift value of C-26 and C-27 in ^{13}C-NMR,[8)] furthermore no interaction was observed in nuclear Overhauser enhancement spectroscopy (NOESY) experiment between vinylic proton at C-24 and oyxmethylene proton. The chemical shift value at δ 79.6 (CH) was attributed to hydroxyl

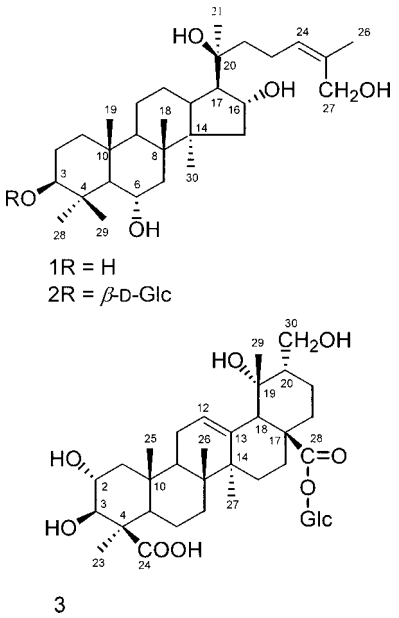
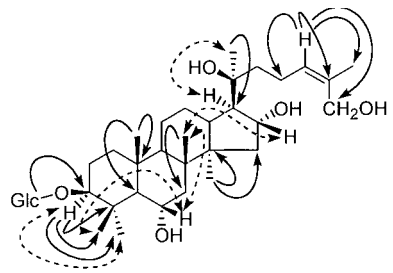
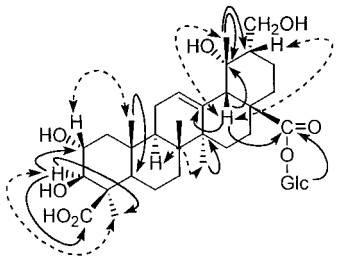


Fig. 1



Significant correlation observed in HMBC (→) and NOESY (↔) spectra of **2**.



Significant correlation observed in HMBC (→) and NOESY (↔) spectra of **3**.

Fig. 2

* To whom correspondence should be addressed. e-mail: vuahmad@cyber.net.pk

group present at C-3. The β orientation of the hydroxyl group was inferred from the chemical shift and coupling pattern of C-3 proton. The downfield signal at δ 68.9 (CH) was assigned to C-6 bearing an α-hydroxyl group.[9] The latter also causes a downfield shift of the C-28 methyl group to δ 31.4. On the other hand the signal at δ 74.8 is due to C-16 and the corresponding H-16 absorbs at δ 4.18. This assignment was based on the multiplicity of the signals at H-15 and H-17 and the pronounces downfield shift of the C-17 signal to δ 59.4 as compared to the usual chemical shifts of C-17 in compounds which do not have the C-16 hydroxyl group.[10,11]

The complete stereochemistry was established by NOESY. The cross peaks observed between H_3-29/H-6, H_3-18/H-6 and between H_3-18/H-16 confirm the α disposition of hydroxyl groups at C-6 and C-16. The C-20 configuration of **1** was established to be *S* on the basis of comparison of the ^{13}C-NMR chemical shifts of C-20, 21 and 22 with those of dammarenediol I (20*R*), dammarenediol II (20*S*)[12] and cucurbitacin **8**.[17] Consequently, the structure of triloculatrol A **1** was elucidated as 3β,6α,16α,20(*S*) and 27-pentahydroxydammar-24(*Z*)-ene.

Triloculatrol A 3-glucoside (**2**) was isolated as a gummy solid. The ^{13}C-NMR spectrum showed 36 signals, of these 30 signals suggested that the agylcone of **2** was **1** (Discussed earlier, Table 1). Negative FAB-MS (*m*/*z* 653 for [M$-$H$^-$]) deduced the molecular formula as $C_{36}H_{62}O_{10}$.

Six additional signals were from a hexose, which was characterized as a β-glucopyranose on the basis of ^{1}H–^{1}H coupling constant observed in ^{1}H-NMR spectrum. The ^{13}C-NMR glycosidation shift at C-3 and the HMBC correlation observed between at δ 4.31 (H-1′) of the glucose and at δ 90.8 (C-3) of the aglycone confirmed that the sugar moiety is attached to C-3 of the aglycone. Thus triloculatrol A 3-glucoside (**2**) was identified as 3β-D-glucopyranosyloxy-6α,16α,20(*S*),27-tetrahydroxydammar-24(*Z*)-ene.

Triloculatroside A (**3**) was obtained as an amorphous powder. Its IR spectrum showed the absorption at 3386 (OH), 1733 (br. CO_2R and CO_2H) and 1567 (C=C) cm^{-1}. The negative FAB-MS showed the molecular ion peak at *m*/*z* 695 for [M$-$H]$^-$ which suggests the molecular formula as $C_{36}H_{56}O_{13}$, which was further confirmed by ^{13}C-NMR and distortionless enhancement by polarization transfer (DEPT) spectra. The ^{1}H-NMR spectrum showed five methyl signals at δ 1.05 (s, H_3-26), 1.15 (s, H_3-25), 1.47 (s, H_3-29), 1.51 (s, H_3-27), 1.65 (s, H_3-23), a sharp singlet at δ 2.76 (H-18) which is the characteristic signal for the ursane type triterpenoid with 19 hydroxy substitution,[13] a broad singlet at δ 5.34 (H-12) of olefinic proton and two oxygenated methines at δ 3.35 (d, *J*=8 Hz, H-3) and at δ 4.86 (br m, H-2). The signal of H-2 (δ 4.86) was found interacting with H_3-25 in NOESY experiment, showing its β stereochemistry while that of H-3 (δ 3.35) interact with H_3-23 showing its α disposition.

The ^{13}C-NMR spectrum of **3** showed a total 36-carbon signals, out of which 30 were attributed to the triterpenoid moiety and 6 to the saccharide part. The ^{13}C-NMR and DEPT spectra suggested that the 30 signals of the aglycone moiety comprised of five methyls, nine methylenes, seven methines and nine quaternary carbons. The six peaks in the range of δ 61—95 corresponding to the presence of glucose moiety and the anomeric carbon signals at δ 95.0 (CH) showed an ester linkage with the aglycone.[14] The H-3 also showed *trans* coupling (8.7 Hz) with H-2. The ^{1}H-NMR also contains one anomeric proton signal at δ 6.00 (d, *J*=8.0 Hz) suggesting one β-linked sugar in the molecule.

Table 1. ^{13}C-NMR (100 MHz) Spectral Data of Compounds **1**, **2** and **3**

Carbon	**1**[a]	**2**[a]	**3**[b]
1	40.1	40.0	47.6
2	27.8	27.7	68.6
3	79.6	90.8	83.7
4	40.5	41.0	49.6
5	62.8	62.5	57.0
6	68.9	68.8	20.5
7	47.8	48.0	33.4
8	42.4	42.4	41.5
9	50.8	51.0	46.4
10	40.3	40.1	38.2
11	22.4*	22.4*	23.7
12	27.7	27.0	128.5
13	42.9	43.0	137.8
14	50.0	50.1	42.1
15	42.3**	42.3**	28.4
16	74.8	75.0	25.6
17	59.4	59.7	48.0
18	17.8	17.8	53.6
19	17.6	17.9	73.0
20	75.2	75.2	47.7
21	25.3	25.3	21.2
22	43.0**	43.0**	36.7
23	22.9*	23.0*	25.2
24	129.3	129.2	180.0
25	135.5	135.5	15.1
26	21.5	21.6	16.7
27	61.4	61.4	23.5
28	31.4	31.3	177.2
29	16.1	16.8	26.6
30	18.1	18.1	63.1
1′		106.7	95.0
2′		75.7	72.8
3′		77.6	77.0
4′		71.7	70.2
5′		78.3	78.0
6′		62.8	61.4

*, ** Assignment may be interchangeable in the same column. *a*) CD_3OD, *b*) pyridine d_5+D_2O.

Comparison of ^{13}C-NMR with reported compound trachelosperoside A-1[15] show good general agreement except for the carbons of ring E (C-20, 21, 30). The absence of C-30 methyl signal in ^{1}H- and ^{13}C-NMR spectrum and the downfield shifts of C-20 and C-30 helped us in assigning hydroxymethylene residue at C-30.[16] The NOESY interactions between H-18/H_3-29 and H-18/H-20 confirmed the α orientation of hydroxmethyl at C-30 and of hydroxyl group at C-19.

The ^{13}C-NMR glycosidation shift at C-28 and the HMBC correlations observed between at δ 6.00 (H-1′) of the glucose and δ 177.2 (C-28) of the aglycone confirmed that the sugar moiety is attached to C-28 of the aglycone. Thus the triloculatroside A (**3**) was identified as 2α,3β,19α,30-tetrahydroxyurs-12-en-24,28-dioic acid 28-*O*-β-D-glucopyranosyl ester.

Experimental

General Experimental Procedure Melting points were determined on a Buchi-535 melting point apparatus and are uncorrected. Optical rotations were taken with a JASCO DIP-360 automatic digital polarimeter. The IR spectra were recorded on a Bruker FTIR Vector 22 spectrophotometer. The

^{1}H- and ^{13}C-NMR spectra were measured with Bruker AM-400 and 500-AM spectrometer using tetramethylsilane as an internal standard. The FAB-MS were recorded on Jeol JMS HX-110 mass spectrometer, EI-MS was recorded with a Finnigan MAT-312 double focusing mass spectrometer. Kieselgel 60 (35—70 mesh, Merck) and sephadex LH-20 were used for column chromatography, silica gel 60 F_{254} (Merck) was used for the TLC. Flash chromatography was carried out with an Eyela EF-10 flash chromatograph.

Plant Material The plant material was collected from Karachi region in October 2001. A voucher specimen of the plant is deposited at the herbarium of department of Botany, University of Karachi.

Extraction and Isolation Air-dried plant (6 kg) of *Corchorus triloculares* were cut into small pieces and were extracted with 95% methanol at room temperature. The solvent was removed by rotary evaporator and the dark brown residue (275 gm) was dissolved in H_2O and extracted successively with hexane, EtOAc and *n*-butanol. The EtOAc exctract was concentrated *in vacuo* to give 125 g of the solid residue. Which was subjected to silica gel vacuum liquid chromatography (VLC). Elution was carried out with the gradient of $CHCl_3$ in MeOH. The fraction obtained from VLC (95 : 5) $CHCl_3$–MeOH were again subjected to column chromatography, eluting with $CHCl_3$–MeOH to give compound **1** (15 mg). Similarly the *n*-butanol extract was concentrated *in vacuo* (85 g) and subjected to VLC (gradient $CHCl_3$–MeOH). The VLC fraction eluted with $CHCl_3$–MeOH (90 : 10) gave a mixture of crude saponins that was subjected to sephadex LH-20 column chromatography using of water–MeOH (1 : 1) as an eluent. The fraction obtained were subjected to reversed phase column flash chromatography using Lichrospher RP-18 silica (20 g, cat no. 16105, Em Science) using water–MeOH (1 : 1) as an eluent to afford **2** (50 mg).

The VLC fraction eluted with $CHCl_3$–MeOH (2 : 1) was subjected to sephadex LH-20 column chromatography using water–methanol (1 : 1) as an eluent to give a semi pure compound. Re-chromatography of the fraction on a saphadex LH-20 column using water–MeOH (3 : 7) afforded **3** (18 mg). The purity of the compounds was checked on the HP-TLC plates and the spot is visualized by spraying with ceric ammonium sulfate reagent followed by heating.

Triloculerol A (**1**): Amorphous solid, mp 90—92°C (decomp.) $[\alpha]_D^{25}$ +17.39° (c=0.138 MeOH). IR (KBr) cm^{-1}: 3413 (OH) and 1602 (C=C). EI-MS *m*/*z* (rel. int., %): 492 (10) [M$^+$], 474 (11), 456 (32), 376 (18) 207 (41), 125 (100), 55 (89). HR-EI-MS *m*/*z*: 492.3874 (Calcd for $C_{30}H_{52}O_5$, 492.3814). ^{1}H-NMR (400 MHz, $CDOD_3$) δ: 0.90 (3H, s, H-19), 0.94 (3H, s, H-29), 1.02 (3H, s, H-18), 1.15 (6H, s, H-30, 21), 1.27 (3H, s, H-28), 1.75 (3H, s, H-26), 1.78 (1H, m, H-17), 3.11 (1H, dd, J=4.9, 11.9 Hz, H-3), 4.01 (1H, dt, J=3.2, 10.4, 13.7 Hz, H-6), 4.07 (2H, s, H-27), 4.18 (1H, m, H-16), 5.29 (1H, t, J=7.1 Hz, H-24). ^{13}C-NMR (100 MHz, CD_3OD): Table 1.

Triloculerol A 3-Glucoside (**2**): Gummy material. $[\alpha]_D^{25}$ +10.0° (c=0.15 MeOH). IR (KBr) cm^{-1}: 3417 (OH) and 1600 (C=C). FAB-MS (negative mode) *m*/*z*: 653 [M−H]$^-$, 491 [M−H−162]$^-$, 455 [M−H−162−2×H_2O]$^-$. HR-FAB-MS *m*/*z*: 653.4434 (Calad for $C_{36}H_{61}O_{10}$ [M−H]$^-$, 653.4448). ^{1}H-NMR (500 MHz, $CDOD_3$) δ: 0.90 (3H, s, H-19), 1.02 (6H, s, H-18, 29), 1.15 (6H, s, H-30, 21), 1.36 (3H, s, H-28), 1.75 (3H, s, H-26), 1.79 (1H, m, H-17), 3.13 (1H, dd, J=4.5, 11.6 Hz, H-3), 3.16 (1H, m, H-2′), 3.25 (1H, m, H-4′), 3.36 (1H, m, H-5′), 3.38 (1H, m, H-3′), 3.62 (1H, dd, J=5.3, 11.7 Hz, H-6′A), 3.82 (1H, dd, J=2.2, 11.7 Hz, H-6′B), 4.00 (1H, dt, J=3.5, 10.6, 14.2 Hz, H-6), 4.07 (2H, s, H-27), 4.18 (1H, m, H-16), 4.31 (1H, d, J=7.7 Hz, H-1′) 5.28 (1H, t, J=7.0 Hz, H-24). ^{13}C-NMR (100 MHz, CD_3OD): Table 1.

Triloculoroside A (**3**): Amorphous solid, mp 187—189 °C (decomp). $[\alpha]_D^{25}$ +12.12° (c=0.198 MeOH). IR (KBr) cm^{-1}: 3386 (OH), 1733 (br CO_2R and CO_2H), and 1567 (C=C). FAB-MS (negative mode) *m*/*z*: 695 [M−H]$^-$, 533 [M−H−162]$^-$. HR-FAB-MS *m*/*z*: 695.4075 (Calcd for $C_{36}H_{55}O_{13}$ [M−H]$^-$, 695.4095). ^{1}H-NMR (400 MHz, C_5D_5N+D_2O) δ: 1.05 (3H, s, H-26), 1.15 (3H, s, H-25), 1.37 (1H, m, H-20), 1.47 (3H, s, H-29), 1.51 (3H, s, H-27), 1.65 (3H, s, H-23), 1.75 (1H, m, H-9), 2.76 (1H, s, H-18), 3.35 (1H, d, J=8.7 Hz, H-3), 4.06 (1H, m, H-5′), 4.22 (1H, m, H-2′), 4.31 (1H, m, H-3′), 4.37 (1H, m, H-4′), 4.41 (1H, dd, J=4.0, 12.0 Hz, H-6′A), 4.48 (1H, dd, J=2.5, 12.0 Hz, H-6′B), 4.86 (1H, m, H-2), 5.34 (1H, br s, H-12), 6.00 (1H, d, J=8.0 Hz, H-1′). ^{13}C-NMR (100 MHz, C_5D_5N+D_2O): Table 1.

Acid Hydrolysis of 2 and 3 A sample of **2** (10 mg) in MeOH was refluxed for 4 h with 1 M HCl. After cooling the reaction mixture, MeOH was evaporated *in vacuo*. Distilled water (3 ml) was added and the solution was extracted with EtOAc. The aqueous layer was neutralized with Ag_2CO_3, filtered and evaporated *in vacuo*. The residue obtained was compared with authentic sugar sample on the silica gel card using the solvent system EtOAc–MeOH–HOAc–H_2O (11 : 2 : 2 : 2). The spots were visualized with aniline phthalate reagent, which indicated that the only sugar unit present was D-glucose. Similarly, D-glucose was obtained when **3** was hydrolyzed.

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