

TRITERPENOIDS AND THEIR GLYCOSIDES FROM *TERMINALIA CHEBULA*

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Abstract—Two new triterpenoid glycosides, chebuloside I and II were isolated from the stem bark of *Terminalia chebula* and shown to be β -D-galactopyranosyl $2\alpha,3\beta,23$ -trihydroxyolean-12-en-28-oate and β -D-glucopyranosyl $2\alpha,3\beta,6\beta,23$ -tetrahydroxyolean-12-en-28-oate, respectively, based on their spectral data and some chemical transformations.

INTRODUCTION

Terminalia chebula L., an Indian plant used in Indian folk medicine [1, 2], has been examined previously for its chemical constituents [3–5]. In the most recent publication, Singh [5], has reported on the isolation of a new triterpene, 2α -hydroxymicromeric acid, from the leaves of this plant. This paper reports the isolation and structure elucidation of two new triterpene glycosides, chebuloside I and II, in addition to the isolation and identification of arjunglucoside I and bellericoside from the stem bark of the plant.

RESULTS AND DISCUSSION

The neutral fraction obtained as indicated in the Experimental afforded besides arjunglucoside I [6] and bellericoside [7], two new triterpenoid glycosides as indicated by Liebermann–Burchard and Molisch tests.

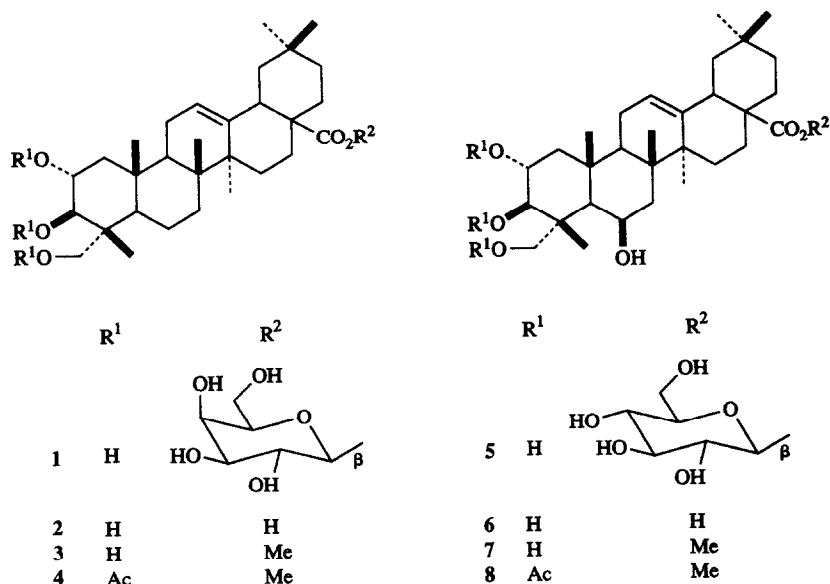
The first glycoside was designated chebuloside I (1), $C_{36}H_{58}O_{10}$ (elemental analysis and MS). The M_r of the glycoside was determined to be 650 by liquid-secondary-ion mass spectrometry (LSI-MS) [8]. This spectrum displayed ion peaks at m/z 673 and 689 formed by cationization of the molecule with Na^+ and K^+ , respectively. Chebuloside I (1) was suggested to be an ester glycoside as its IR spectrum showed a band at 1730 cm^{-1} . The glycoside 1 on alkaline hydrolysis with 5% methanolic KOH (aq.) afforded an aglycone and a sugar constituent. The aglycone (2), $C_{30}H_{48}O_5$, on treatment with an ethereal solution of diazomethane yielded a methyl ester (3) (δ 3.62, 3H, s, CO_2Me) which on acetylation, furnished a methyl ester acetate (4). The mass, 1H and ^{13}C NMR spectral data of the aglycone 2 as well as the 1H NMR data of the methyl ester acetate 4 suggested

the identity of the aglycone to be that of $2\alpha,3\beta,23$ -trihydroxyolean-12-en-28-oic acid (arjunolic acid) (2) previously isolated from *Terminalia arjuna* [9]. The physical and spectroscopic data of 2 compared well with those reported for arjunolic acid.

The sugar constituent obtained by hydrolysis of the glycoside 1 was identified by PC as D-galactose by comparison with an authentic sample. The presence of D-galactose was ascertained by GC analysis after preparation of its alditol acetates. The attachment of D-galactose to the CO_2H -28 group of arjunolic acid (2) was shown by the ^{13}C NMR spectrum of the glycoside, chebuloside I (1). The spectrum showed a signal at δ 96.0 assignable to C-1 of the galactose unit. Thus, the structure of chebuloside I was established as β -D-galactopyranosyl $2\alpha,3\beta,23$ -trihydroxyolean-12-en-28-oate. The ^{13}C NMR data (Table 1) of chebuloside I were in good agreement with the proposed structure. Complete assignment of the ^{13}C NMR data of 1 was accomplished by comparison with those of the aglycone, 2 and methyl β -D-galactopyranoside using known chemical shift rules [10, 11] and glycosylation shifts [12, 13].

The M_r of chebuloside II (5) was determined to be 666 by LSI-mass spectroscopy. The spectrum showed ion peaks at m/z 689 and 705 attributable to $[M+Na]^+$ and $[M+K]^+$, respectively. Alkaline hydrolysis furnished a genin (6) and a sugar constituent, the latter being identified as D-glucose by PC and GC. The genin 6, $C_{30}H_{48}O_6$ yielded a methyl ester (7) on treatment with an ethereal solution of diazomethane. The ester 7 on acetylation furnished a methyl ester triacetate (8) as was evident from its 1H NMR spectrum. Thus the nature of five out of six oxygen atoms in the genin was determined. The ^{13}C NMR spectrum of 6 indicated that the remaining oxygen function in the genin is also hydroxy in nature. The hindered nature of the fourth hydroxy group was evident by the formation of the triacetate (8) from 7 by

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acetylation at ambient temperature. Moreover, the mass spectral fragmentation pattern of the methyl ester triacetate was typical of Δ^{12} -oleanene or Δ^{12} -ursene triterpenes [14] and the retro-Diels-Alder fragments indicated that all the four hydroxy in **6** are present in the part containing rings A/B. That the hindered hydroxy group is present at the 6β position was evident by the downfield shifts of *ca* 0.3 ppm for the C-25 methyl singlet in the ^1H NMR spectrum of the triacetate (**8**) compared to an oleanene triterpene not containing a 6β hydroxyl group, e.g. arjunolic acid (**2**). Thus the genin was identified as $2\alpha,3\beta,6\beta,23$ -tetrahydroxyolean-12-en-28-oic acid (terminolic acid). The identity was confirmed by comparison of its physical and spectral data with those reported for terminolic acid [15]. The attachment of D-glucose to the carboxylic group of terminolic acid was determined from the ^{13}C NMR spectrum of chebuloside II (**5**). All the carbon chemical shifts of the glycoside was assigned by comparison with those of terminolic acid and methyl β -D-glucopyranosyl using known chemical shift rules [10, 11], glycosylation shifts [12, 13], off resonance and DEPT studies [16]. Consequently the structure of chebuloside II was established as β -D-glucopyranosyl $2\alpha,3\beta,6\beta,23$ -tetrahydroxyolean-12-en-28-oate.

The acidic fraction obtained from the *n*-butanol soluble fraction on purification by repeated CC and preparative TLC led to the isolation of **2** and **6** besides arjungenin and belleric acid reported previously from *T. bellerica* [7].

EXPERIMENTAL

The plant material was collected from Bankura, West Bengal and was identified in the Indian Botanical Garden, Howrah. A voucher specimen is deposited in the herbarium of IICB. Mps: uncorr; ^1H NMR: 99.6 and 399.8 MHz, CDCl_3 ; ^{13}C NMR: 100 MHz, pyridine- d_5 ,

$\text{DMSO}-d_6$, TMS as int. standard; IR: KBr; MS: direct inlet, 70 eV; GC: ECNSS-M, 3% on Gas Chrom. Q at 190° for alditol acetates; TLC: silica gel G (BDH) using the solvent system (A) CHCl_3 -MeOH- H_2O (35:13:2), (B) CHCl_3 -MeOH- H_2O (80:19:1) and (C) C_6H_6 - CHCl_3 -EtOAc (5:3:2). TLC plates were developed by spraying with L.B. reagent. PC: Whatman paper No. 1 using the solvent system *n*-BuOH-pyridine- H_2O (6:4:3). A satd soln of aniline oxalate in H_2O was used as staining agent. Liquid secondary-ion mass spectra (LSI-MS) were obtained in a nitrobenzyl alcohol (NBA) matrix operating at 5-kV accelerating voltage, equipped with a 20-kV conversion dynode. Cesium ion was used as a bombarding particle.

The air-dried and powdered stem-bark of *T. chebula* (2 kg) was first defatted with petrol (60–80°) and then exhaustively extracted with MeOH. The methanolic extract on removal of the solvent under red. pres. yielded a viscous dark greenish brown mass (250 g). A part of the MeOH extract (125 g) was partitioned between *n*-BuOH and H_2O . The *n*-BuOH soluble part was separated into acidic and neutral fractions by treatment with a sat soln of NaHCO_3 .

Isolation of chebuloside I (1) and chebuloside II (5). The neutral fraction (7.5 g) was chromatographed on silica gel (150 g) and eluted with petrol, petrol- CHCl_3 (1:1), CHCl_3 , and CHCl_3 -MeOH (19:1, 9:1, 87:13, 43:7, 17:3 and 4:1). Frs (250 ml each) were monitored by TLC. Fractions eluted with CHCl_3 -MeOH (9:1, 87:13 and 43:7) were combined (2.8 g) and subjected to prep. TLC with solvent system (A) to give 4 chromatographically pure fractions, A (190 mg), B (400 mg), C (180 mg) and D (195 mg) according to the increasing order of polarity. Fractions B and C were found to be identical with arjunglucoside I and bellericoside respectively [7].

Chebuloside I (1). Crystallized from MeOH as a powder, mp 238–240°, $[\alpha]_D^{+42^\circ}$ (MeOH; *c* 0.25): IR

Table 1. ^{13}C NMR spectral data ($\delta_{\text{C}} \pm 0.1$) of compounds 1, 2, 5 (in pyridine- d_5) and 6

C	2	1	6 (DMSO- d_6)	5
1	47.1	47.3	48.9	50.0
2	68.9	68.8	67.4	69.0
3	78.7	78.7	75.6	78.3
4	43.5	43.5	43.1	42.8
5	48.4 ^a	48.5 ^a	47.4 ^a	48.8
6	18.6	18.7	66.0	67.6
7	33.1	33.1	39.6	41.0
8	40.1	40.2	38.1	41.0
9	48.5 ^a	48.4 ^a	46.7 ^a	48.8
10	38.5	38.6	36.9	38.1
11	23.8 ^b	23.8 ^b	22.6 ^b	22.6 ^a
12	123.5	123.4	121.7	123.5
13	144.1	144.1	143.3	143.5
14	42.4	42.0	41.8	42.8
15	28.3	28.2	27.1	28.2
16	23.9 ^b	23.5 ^b	22.9 ^b	24.0 ^a
17	47.0	47.1	45.5	47.0
18	43.5	41.8	40.9	41.8
19	46.3	46.3	45.7	47.0
20	30.7	30.6	30.4	30.7
21	34.2	34.1	33.3	34.0
22	33.0	33.3	32.1	32.5
23	67.2	67.2	63.7	66.1
24	14.0	14.1	14.9	15.9
25	17.6 ^c	17.4 ^c	17.8 ^c	18.8 ^b
26	17.2 ^c	17.1 ^c	18.1 ^c	19.0 ^b
27	26.1	26.2	25.7	26.1
28	178.6	176.4	178.6	176.4
29	29.1	29.2	32.9	33.1
30	23.7	23.9	23.3	23.6
Gal-1		96.0		
Gal-2		71.7		
Gal-3		75.3		
Gal-4		69.1		
Gal-5		76.4		
Gal-6		61.6		
Glc-1				95.6
Glc-2				74.0
Glc-3				79.1 ^c
Glc-4				71.1
Glc-5				78.6 ^c
Glc-6				62.1

^{a-c}Assignments within a column may be interchanged.

$\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3200–3500 (OH), 1730, 1635, 1450, 1060;
 ^{13}C NMR: Table 1 (found: C, 66.50; H, 8.92; $\text{C}_{36}\text{H}_{58}\text{O}_{10}$
 requires: C, 66.43; H, 8.98%).

Chebuloide II (5). Crystallized from MeOH as a powder mp 215°, $[\alpha]_{\text{D}} + 25^\circ$ (MeOH; c 0.75); IR $\nu_{\text{max}}^{\text{KBr}}$ cm^{-1} : 3200–3500 (OH), 1730, 1635, 1450, 1060;
 ^{13}C NMR: Table 1 (found: C, 64.58; H, 8.69; $\text{C}_{36}\text{H}_{58}\text{O}_{11}$
 requires C 64.84; H, 8.77%).

Alkaline hydrolysis of chebuloide I (1). Chebuloide I (1, 110 mg) was hydrolysed with 5% methanolic KOH (aq.) under reflux for 3 hr and worked-up in the usual way. The residue on chromatographic purification fol-

lowed by crystallization from MeOH, yielded arjunolic acid (2, 65 mg); methyl ester mp 250–252°; $[\alpha]_{\text{D}} + 62^\circ$ (MeOH; c 0.52); (lit. [17] mp 248–250°; $[\alpha]_{\text{D}} + 68^\circ$); MS of the methyl ester and ^1H NMR of the methyl ester triacetate were comparable with those reported in the literature [18]. The aq. layer was passed through a column of Dowex 1- \times 2 (OH^-) and $-50\text{W} \times 8$ (H^+), respectively, and then concd under red. pres. The residue obtained was tested for sugar by PC; D-galactose was identified by comparison with an authentic sample. The presence of D-galactose was also confirmed by GC after prepn of the alditol acetate.

Alkaline hydrolysis of chebuloide II (5). Chebuloide II (5, 120 mg) was hydrolysed with 5% methanolic KOH (aq.) and worked-up as described above for chebuloide I to yield D-glucose (identified by PC and GC) and terminolic acid (6); methyl ester mp 166–167°; $[\alpha]_{\text{D}} + 38^\circ$ (CHCl_3 c 0.5); [lit.[15]; Me ester 165–168°; $[\alpha]_{\text{D}} + 40^\circ$ (CHCl_3 ; c 0.78)]. 2 α , 3 β , 23-Triacetoxy 6 β -hydroxy methyl terminolate as leaflets (MeOH), mp 165–166°; $[\alpha]_{\text{D}} + 17^\circ$ (CHCl_3 ; c 1.2); [lit.[15]]. Me ester 165–168°, $[\alpha]_{\text{D}} + 40^\circ$ (CHCl_3 ; c 0.78) methyl triacetyl terminolate, mp 160–162°; $[\alpha]_{\text{D}} + 19^\circ$ (CHCl_3 ; c 1.53). EIMS m/z (rel. intensity): 644 $[\text{M}]^+$ (3), 626 $[\text{M}-\text{H}_2\text{O}]^+$ (1), 585 $[\text{M}-\text{CO}_2\text{Me}]^+$ (2), 584 $[\text{M}-\text{HOAc}]^+$ (5), 524 $[\text{M}-2\text{HOAc}]^+$ (3), 262 $[\text{a}]$ (91), 203 $[\text{a}-\text{CO}_2\text{Me}]^+$ (100), 202 $[\text{a}-\text{OHAc}]^+$ (25), 189 (20), ^1H NMR (399.8 MHz): δ 0.91 (3H, s), 0.94 (3H, s), 1.02 (3H, s), 1.09 (3H, s), 1.28 (3H, s), 1.46 (3H, s) (together 6 \times Me), 1.99 (3H, s, OCOMe), 2.03 (3H, s, OCOMe), 2.07 (3H, s, OCOMe), 3.63 (3H, s, CO_2Me), 3.72 (1H, d , $J=12$ Hz, H-23a), 3.95 (1H, d , $J=12$ Hz, H-23b), 4.35 (1H, br s, H-6 α), 5.01 (1H, d , $J=11$ Hz, H-3 α), 5.23 (1H, td , $J=11$, 6 Hz, H-2 β) and 5.34 (1H, t -like. H-12).

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