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TRITERPENOIDS AND THEIR GLUCOSIDES FROM *TERMINALIA BELLERICA*

ASHOKE K. NANDY, GURUDAS PODDER, NIRANJAN P. SAHU* and SHASHI B. MAHATO*†

Department of Chemistry, School of Tropical Medicine, Calcutta 700 073, India; *Indian Institute of Chemical Biology, 4, Raja S. C Mullick Road, Jadavpur, Calcutta 700 032, India

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Key Word Index—*Terminalia bellerica*; Combretaceae; belleric acid; bellericoside; triterpene; triterpene glucoside

Abstract—In addition to the isolation and identification of arjungenin and its glucoside, the structures of a new triterpene, belleric acid and its glucoside, bellericoside have been defined as 2α,3β,23,24-tetrahydroxyolean-12-en-28-oic acid and its β-D-glucopyranosyl ester.

INTRODUCTION

Terminalia bellerica Roxb. is a handsome tree which occurs widely in the moist valleys of India. It has been used for the treatment of various ailments [1], and has been reported to contain β-sitosterol, gallic acid, ellagic acid, ethyl gallate, galloyl glucose, mannitol, glucose, galactose, fructose and rhamnose [2, 3]. This paper reports the isolation and structure elucidation of a new triterpene, belleric acid and its glucoside, bellericoside, in addition to the isolation and identification of arjungenin and its glucoside.

RESULTS AND DISCUSSION

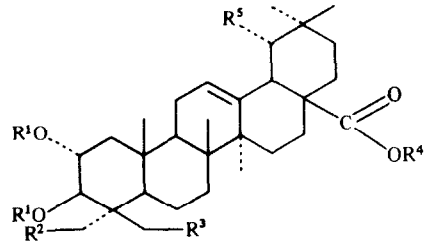
The *n*-butanol-soluble fraction of the methanol extract of the stembark of *T. bellerica* was separated into acidic and neutral fractions. The acidic fraction yielded two acids, both of which gave a positive Liebermann–Burchard test for triterpenes. The first acid was characterized as arjungenin (**1**) by comparison of its physical and spectroscopic data with those reported for an authentic sample [4]. The second acid, designated belleric acid (**2**), $C_{30}H_{48}O_6$ (elemental analysis and MS), yielded a methyl ester (3H, δ3.62, COOMe) (**3**) on treatment with diazomethane. The ester (**3**), on acetylation, afforded a methyl ester tetraacetate (**4**) (four acetoxy methyls at δ1.9, 2.0, 2.08 and 2.21). Thus the nature of six oxygen atoms in belleric acid was defined. The presence of an α-glycol system was indicated by the consumption of 1 mol periodate/mol of the acid (**2**) and, in the ^{1}H NMR spectrum of the methyl ester acetate (**4**), by signals at δ5.14 (*d*, $J = 10$ Hz) and 5.18 (*m*) attributable to H-3α and H-2β. This spectrum also demonstrated the presence of two acetoxy methylene groups showing that the acid (**2**) contains two primary hydroxyls.

The mass spectrum of methyl ester **3** showed retro-Diels–Alder fragments typical of Δ^{12}-oleanene or Δ^{12}-ursene triterpenes [5]. The appearance of the fragment ions at *m/z* 255 (**a**) and 262 (**b**) suggested that the four hydroxyl groups of acid (**2**) are present in the part containing rings A/B, and that the carboxyl group is located in the part containing rings D/E. The formation of other significant fragment ions could also be rationalized. As the ^{13}C NMR spectrum (Table 1) of the acid (**2**) exhibited six quaternary carbons within the range 0–60 ppm, its skeleton proved to be that of oleanane. The spectrum further disclosed the presence of nine methylenes, three methines, two secondary alcohols, two primary alcohols, an acid carbonyl, five quaternary methyls and a trisubstituted double bond. Assignment of all the carbon chemical shifts of belleric acid (**2**) was accomplished by comparison with those of trachelosperoside E-1 isolated by Abe and Yamauchi [6] and the monodesmoside of oleanolic acid [7]. Compelling evidence for the presence of 23,24-hydroxyls was obtained as follows: The methyl ester (**3**) on treatment with trityl chloride [8] yielded the trityl derivative **5**, which on acetylation afforded compound **6**. On removal of the trityl groups, compound **6** furnished the partially acetylated product **7** which on treatment with acetone and concentrated sulphuric acid [9] yielded the acetonide **8**, suggesting the presence of primary hydroxyls at C-23 and C-24. The evidence relating to the presence of the 2α,3β-glycol system has already been described. Thus, the structure of the acid was established as 2α,3β,23,24-tetrahydroxyolean-12-en-28-oic acid (**2**).

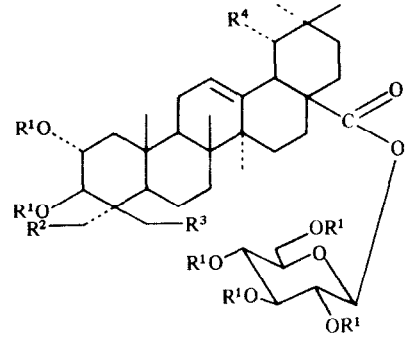
The neutral fraction of the *n*-butanol-soluble fraction yielded two triterpenoid glucosides as indicated by Liebermann–Burchard and Molisch tests. The first glucoside (**9**) on hydrolysis (5% MeOH–KOH aq. solution) liberated arjungenin (**1**) and D-glucose, the latter being identified by PC and GLC. The glucoside was identified as β-D-glucopyranosyl 2α,3β,19α,23-tetrahydroxyolean-12-en-28-oate (arjunglucoside I) (**9**) by comparison of its physical data with the literature values [4]. The availability of arjungenin (**1**) and its glucoside (**9**) enabled complete elucidation of the ^{13}C NMR spectra (Table 1), taking into consideration the ^{13}C NMR data of arjunetin and arjunglucoside II [10].

Bellericoside (**10**), $C_{36}H_{58}O_{11}$, on alkaline hydrolysis, afforded belleric acid (**2**) and D-glucose. The attachment of D-glucose to the 28-COOH group was evident from the ^{13}C NMR spectrum of the glucoside (**10**) (signal at δ95.6

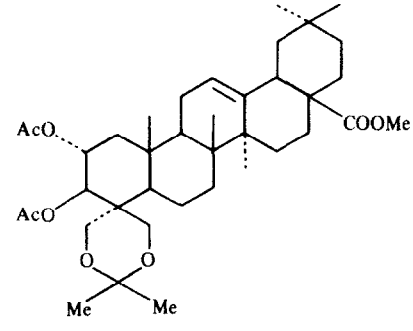
†Author to whom correspondence should be addressed



1 $R^1 = R^3 = R^4 = H$, $R^2 = R^5 = OH$
2 $R^1 = R^4 = R^5 = H$, $R^2 = R^3 = OH$
3 $R^1 = R^5 = H$, $R^2 = R^3 = OH$, $R^4 = Me$
4 $R^1 = Ac$, $R^2 = R^3 = OAc$, $R^4 = Me$, $R^5 = H$
12 $R^1 = Me$, $R^2 = R^3 = OMe$, $R^4 = R^5 = H$



9 $R^1 = R^3 = H$, $R^2 = R^4 = OH$
10 $R^1 = R^4 = H$, $R^2 = R^3 = OH$
11 $R^1 = Me$, $R^2 = R^3 = OMe$, $R^4 = H$



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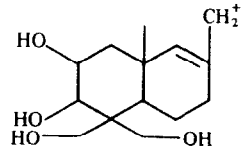
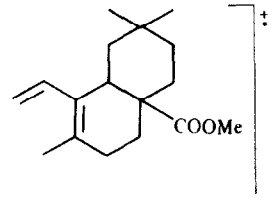


(a) *m/z* 255



(b) *m/z* 262

assigned to C-1 of glucose) Moreover, the ^{1}H NMR spectrum of the permethylate (**11**) prepared by treatment of the glucoside (**10**) with sodium hydride–methyl iodide in hexamethyl phosphoramide [11] displayed signals ascribable to eight methoxy groups This permethylate, on hydrolysis, yielded tetra-*O*-methyl bellenic acid (**12**) and 2,3,4,6-tetra-*O*-methyl-D-glucose, identified by GC Thus the structure of bellericoside (**10**) was established as *β*-D-glucopyranosyl 2*α*,3*β*,23,24-tetrahydroxyolean-12-en-28-oate

Although a number of new triterpenes have been isolated from the genus *Terminalia* [12 14], belleric acid is the first triterpene isolated from this genus containing 23,24-hydroxyl functions. In fact, to our knowledge, trachelosperoside E-1 [6], a triterpene glucoside, and clethric acid [15] are the only two 23,24-hydroxyl-containing triterpenes so far isolated from a natural source

EXPERIMENTAL

The plant material was collected from Burdwan, West Bengal A voucher specimen has been deposited in the herbarium of IICB Mps uncorr, ^{1}H NMR 99 6 and 300 133 MHz, $CDCl_3$, ^{13}C NMR 25 05 MHz, pyridine-d_5. TMS as int standard, IR KBr, MS direct inlet, 70 eV, GC (i) ECNSS-M, 3% on Gas Chrom Q at 190 for alditol acetates and (ii) OV-225 on Gas Chrom Q at 195 for partially methylated alditol acetates, TLC silica gel G (BDH) using the solvent systems (A) $CHCl_3$–MeOH–H_2O (35 13 2), (B) $CHCl_3$–MeOH–H_2O (78 19 1), and (C) C_6H_6–$CHCl_3$–EtOAc (5 3 2) TLC plates were developed by spraying 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

The air-dried powdered stem-bark of *T bellerica* (1 5 kg) was extracted successively in a Soxhlet apparatus with petrol (60–80) and MeOH The MeOH extract on removal of the solvent under red pres yielded a viscous dark greenish brown mass (200 g) A part of the MeOH extract (100 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 satd soln of $NaHCO_3$

Isolation of arjungenin (**1**) *and belleric acid* (**2**) The acidic fraction (2 5 g) was chromatographed on silica gel (40 g) and eluted with petrol petrol–$CHCl_3$ (1 1), $CHCl_3$, $CHCl_3$–MeOH

Table 1 ^{13}C NMR data ($\delta_c \pm 0.1$) of arjungenin (**1**), arjunglucoside-I (**9**), bellenic acid (**2**) and bellericoside (**10**) (pyridine-d_5)

C	1	9	2	10
1	47 4	47 3	47 9	46.9
2	68 9	68 8	69 0	69.0
3	78 4	78.6	80 0	79 9
4	43 6	43 3	47 7	47 0
5	48 2	48.2	48 4	48 3
6	18 8	18 9	19 3	19.2
7	33 6[a]	32 9	33 2	33.0[a]
8	40 1	40 2	40 0	40.0
9	48 5	49 6	48 9	48 8
10	38 6	38 9	38 3	38 2
11	28 8	24 2	24 3	24 2
12	123 5	123 7	122.4	123 5
13	144.9	143 9	144 9	144.1
14	42 2	42 1	42 4	42 2
15	29 2[b]	28 0[b]	28 4	28 2
16	24 3	28 4	23 9	23 5
17	46 0	46 3	46 7	47 0
18	44 8	44 4	42 1	41.8
19	81 3	81.3	46 7	46.3
20	35 7	35 3	31 0	30.7
21	28.4[b]	28 8[b]	34.4	34 1
22	33 1[a]	32 9	33 2	33 3[a]
23	66 8	67 1	64 1	64.0
24	14 2	13 8	63.8	63 6
25	17 7[c]	17 7[c]	17.4[b]	17 4[b]
26	17.3[c]	17 1[c]	17 2[b]	17 2[b]
27	24 9	24 8	26 2	26 0
28	180 8	177 8	180 0	176 3
29	29 2	29 0	33 5	33 3
30	24 9	24 8	23 9	23.8
g-1		95 5		95.6
g-2		73 8		73 9
g-3		78 6		78 8
g-4		71 2		71 3
g-5		78 6		78 7
g-6		62 3		62 5

g, Glucose, [a, b, c] may be interchanged in each vertical column.

(19.1 and 9 1). The $CHCl_3$–MeOH (19 1) eluate (1 7 g) was subjected to prep TLC using solvent system (B) to give two chromatographically pure fractions. Fractions having a high R_f value crystallized from MeOH to give **1**, mp 293–295° (dec), $[\alpha]_D$ +33° (MeOH, *c*, 0 51), methyl ester, mp 179–180°, $[\alpha]_D$ +31° ($CHCl_3$; *c*, 0 2), methyl ester triacetate, mp 138–140°, $[\alpha]_D$ +8° ($CHCl_3$, *c*, 0 18) (lit [4] mp 137–138°, $[\alpha]_D$ +8° (EtOH, *c* 1 8); ^{1}H NMR (300 MHz) δ0 66 (*s*, 3H), 0.88 (*s*, 3H), 0.95 (*s*, 3H), 0.96 (*s*, 3H), 1 06 (*s*, 3H), 1.22 (*s*, 3H) (together 6 × Me), 1 98 (3H, *s*,–OAc), 2 01 (3H, *s*,–OAc), 2 06 (3H, *s*,-OAc), 3.13 (*br s*, H-18), 3 33 (*br s*, H-19), 3 6 (*s*,–COOMe), 3 75 and 3 55 (2H, ABq, CH_2OAc, J = 12 Hz), 5 06 (*d*, H-3α, J = 12 Hz), 5 15 (*td*, H-2β, J = 12, 4 Hz) and 5 42 (*t*-like, H-12)

The fraction having a low R_f value furnished crystals of bellenic acid (**2**) from MeOH, mp > 300°, $[\alpha]_D$ +77° (MeOH; *c* 0 33) IR ν_{max}^{KBr} cm^{-1} 3300–3500, 1675, 1040 (Found C, 71.35, H, 9 60, $C_{30}H_{48}O_6$ requires C, 71 39; H, 9 59)

Bellenic acid methyl ester (**3**) Bellenic acid (**2**, 100 mg) in MeOH (20 ml) was treated with an ethereal soln of CH_2N_2. The reaction mixture was worked up as usual to give a residue which was crystallized from MeOH to yield bellenic acid methyl ester (**3**), mp 234–235°, $[\alpha]_D$ + 69° ($CHCl_3$, *c* 0 33), IR ν_{max}^{KBr} cm^{-1} 3300–3500, 1725, 1260, 1160, 1040; MS *m/z* (rel. int.): 518 $[M]^+$ (17), 503 $[M-Me]^+$ (3), 500 $[M-H_2O]^+$ (6), 482 $[M-2H_2O]^+$ (7), 470 $[M-H_2O-CH_2O]^+$ (3), 467 $[M-Me-2H_2O]^+$ (4), 459 $[M-COOMe]^+$ (7), 458 $[M-COOMe-H]^+$ (9), 452 (5), 451 (3), 441 $[M-COOMe-H_2O]^+$ (7), 434 (5), 423 (7), 393 (5), 296 (8), 262 (**b**, 98), 255 (**a**, 12), 237 (**a** $-H_2O$, 24), 219 (**a** $-2H_2O$, 20), 203 (**b** – COOMe, 100), 202 (**b** – COOMe – H, 96) and 189 (94) ^{1}H NMR: δ0 70 (*s*, 3H), 0 92 (*s*, 9H), 1.12 (*s*, 3H) (together 6 × Me), 3 62 (3H, *s*, –COOMe) and 5 35 (*t*-like, H-12) (Found. C, 71 80, H, 9 71, $C_{31}H_{50}O_6$ requires C,-71.78, H, 9.73).

Bellenic acid methyl ester tetraacetate (**4**) Bellenic acid methyl ester (**3**, 80 mg) was dissolved in pyridine (1 ml) and treated with Ac_2O (1 ml) at water bath temp for 3 hr After work-up as usual, the product was purified by chromatography Fractions having the same R_f values with solvent system (C) were mixed together and crystallized from MeOH to furnish needles of **4**, mp 224–225°, $[\alpha]_D$ +37° (*c* 0.24; $CHCl_3$), IR ν_{max}^{KBr} cm^{-1}. 1735, 1730, 1240, 1160 and 1040, MS *m/z* (rel int): 626 $[M-HOAc]^+$ (42), 594 $[M-HOAc-MeOH]^+$ (3), 567 $[M-HOAc-COOMe]^+$ (7), 566 $[M-2\,HOAc]^+$ (5), 534 $[M-2\,HOAc-MeOH]^+$ (12), 507 $[M-2\,HOAc-COOMe]^+$ (10), 423 [**a**, 5], 363 [**a** – HOAc, 4], 303 (**a** – 2 HOAc, 6], 262 [**b**, 78], 203 [**b** – COOMe, 100] and 202 (**b** – COOMe – H, 92); ^{1}H NMR (300 MHz). δ0.72 (*s*, 3H), 0 90 (*s*, 3H), 0 93 (*s*, 3H), 1 09 (*s*, 3H), 1 1 (*s*, 6H) (together 6 × Me), 1 98 (3H, *s*, –OAc), 2 02 (3H, *s*, –OAc), 2 04 (3H, *s*, –OAc), 2 07 (3H, *s*, –OAc), 3.62 (3H, *s*, –COOMe), 3.91 (*d*, J = 12 Hz, H-23), 4.16 (*d*, J = 12 Hz, H-23), 4.26 (*d*, J = 12 Hz, H-24), 4.32 (*d*, J = 12 Hz, H-24), 5.14 (*d*, J = 10 Hz, H-3), 5 18 (*m*, H-2) and 5 28 (*t*-like, H-12) (Found: C, 68 15; H, 8.44, $C_{39}H_{58}O_{10}$ requires C, 68 19, H, 8 51)

Isopropylidene derivative (**8**). Bellenic acid methyl ester (**3**, 100 mg) was dissolved in pyridine (4 ml) and to this soln was added freshly crystallized dry trityl chloride (400 mg) The reaction mixture was kept at water bath temp for 20 hr After cooling, pyridine (2 ml) and Ac_2O (3 ml) were added and the mixt. kept at 100° for 2 hr After work-up as usual, the product obtained was hydrolysed with 80% HOAc (10 ml) for 3 hr. The residue obtained was dried *in vacuo* and treated for 24 hr at room temp. with 10 ml of reagent (50 ml Me_2CO, 2 ml Et_2O and 0.1 ml conc H_2SO_4). Work-up in the usual way gave the acetonide **8**, amorphous powder, MS *m/z* 642 $[M]^+$ (Found C, 70 80; H, 9 12; $C_{38}H_{58}O_8$ requires: C, 70.99, H, 9 09)

Isolation of arjunglycoside I (**9**) *and bellericoside* (**10**). The *n*-BuOH fraction on removal of the solvent under red. pres gave a viscous dark brown mass (25 g). This extract was chromatographed on silica gel (400 g) with petrol, petrol–$CHCl_3$ (1·1), $CHCl_3$, $CHCl_3$–MeOH (19:1, 9 1, 22 3 and 17·3) as eluents The early fractions eluted with $CHCl_3$–MeOH (22 3) furnished crude arjunglucoside I, which on further purification by re-chromatography and crystallization from MeOH yielded pure arjunglucoside I (250 mg), mp 235–236°, $[\alpha]_D$ +17° (pyridine; *c* 0.3), [lit [4] mp 231°, $[\alpha]_D$ + 11° (EtOH)] (Found C, 64 72, H, 8.80, Calc for $C_{36}H_{58}O_{11}$ C, 64 83, H, 8.76)

The later fractions of the $CHCl_3$–MeOH (22 3) eluent were found to be a mixture of arjunglucoside I (**9**) and bellericoside (**10**) A part (350 mg) was subjected to prep. TLC on silica gel G using solvent system (A). The bellericoside thus separated was crystallized from MeOH as fine needles (150 mg), mp 238° (dec.), $[\alpha]_D$ +45° (pyridine, *c* 0 26) (Found C, 64 81, H, 8.72, $C_{36}H_{58}O_{11}$ requires: C, 64 83; H, 8.76).

Alkaline hydrolysis of bellericoside (**10**). Bellericoside (**10**, 120 mg) was hydrolysed with 5% methanolic KOH (aq) under reflux for 2 hr and worked-up in the usual way The residue, on

chromatographic purification followed by crystallization from MeOH, yielded belleric acid (**10**, 60 mg) The aq layer was passed through a column of Dowex 1-X2 (OH^-) and 50W-X8 (H^+) respectively and concd under red pres The residue obtained was tested for sugar by PC, D-glucose was identified by comparison with an authentic sample The presence of D-glucose was also confirmed by GC (column 1) after preparation of the alditol acetate

Permethylation of **10** *and hydrolysis* Bellericoside (**10**, 50 mg) in hexamethyl–phosphoramide (5 ml) was treated with NaH (200 mg) and MeI (5 ml), at room temp for 3 hr The reaction mixture was extracted with Et_2O, evapd to dryness and purified on silica gel eluting with petrol–EtOAc (2 1) to give the permethylate (**11**) as a white amorphous powder (no OH absorption in the IR) ^{1}H NMR δ0 72 (s, 3H), 0 92 (s, 3H), 0 94 (s, 3H), 1 02 (s, 3H), 1 12 (s, 3H), 1 26 (s, 3H) (together 6 × Me), 3 2, 3 36, 3 42, 3 52, 3 62 (all s, together 8 × –OMe) and 5 3 (*t-like*, *H*-12)

The permethylate (**11**) was hydrolysed by refluxing with 5% MeOH–HCl (aq) for 3 hr The reaction mixture was diluted with water and extracted with $CHCl_3$ Evapn of the solvent gave crude **12** which was purified by chromatography to yield tetra-*O*-methyl belleric acid (**12**) as a colourless powder, mp 235–237° (dec), $[\alpha]_D$ + 59° ($CHCl_3$, *c* 0 35) ^{1}H NMR δ0 72 (*s*, 3H), 0 91 (s, 3H), 0 93 (s, 3H), 1 00 (s, 3H), 1 08 (*s*, 3H), 1 24 (*s*, 3H) (together 6 × Me), 3 22 (3H, *s*,–OMe), 3 40 (3H, s,-OMe), 3 42 (6H, s, 2 × –OMe) and 5 28 (*t*-like, H-12)

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