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A triterpenoid saponin possessing antileishmanial activity from the leaves of *Careya arborea*

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Abstract

Bioguided-fractionation of the methanol extract of the leaves of *Careya arborea* led to isolation of a triterpenoid saponin, designated arborenin, and characterized as 3-*O*-β-D-glucopyranosyl(1→2)-β-D-glucopyranosyl-2α,3β-dihydroxy-taraxast-20-en-28-oic acid (**1**), together with desacylescin III (**2**). The structures were determined on the basis of extensive 2D NMR spectroscopic analysis. The saponin showed in vitro antileishmanial activity against *Leishmania donovani* (strain AG 83).

Keywords: *Careya arborea*; Triterpenoid saponin; Arborenin; Desacylescin III barringtogenol C; Barringtogenol D; 2D NMR

1. Introduction

Careya arborea is a handsome deciduous tree, widely distributed throughout the greater part of India. The different parts of the plant enjoy considerable reputation in Indian medicine as astringent and tonic, and are used as antipyretic and antipruritic in eruptive fevers (Phondke, 2000). Previous phytochemical investigations on the leaves and seeds of the plant were limited to the isolation of few sterols and rearranged triterpenes (Row and Sastry, 1964; Das and Mahato, 1982). In continuation of our work on chemical studies on naturally occurring bioactive saponins (Sahu et al., 2002) we report herein the isolation and characterization of a novel triterpenoid saponin, designated as arborenin, with antileishmanial activity, from the methanolic extract of the leaves of this medicinal plant, along with a known triterpenoid glycoside, desacylescin III.

2. Results and discussion

The *n*-BuOH soluble fraction obtained from defatted MeOH extract of *C. arborea* leaves showed moderate in vitro antileishmanial activity against *Leishmania donovani* (strain AG 83). Bioguided fractionation of the active fractions eluted with MeOH–H_2O mixture from the Diaion HP-20 chromatography led to isolation of two triterpenoid saponins.

Arborenin (**1**) has the molecular formula $C_{42}H_{68}O_{14}$ as determined from its high resolution positive ion FABMS (*m*/*z* 819.4457 $[M + Na]^+$). Of the 42 carbon signals displayed in the ^{13}C NMR spectrum, 12 could be assigned to the carbohydrate moiety (10 methine and 2 methylene signals), whereas the aglycone accounted for seven singlets, eight doublets, eight triplets and seven quartets. The ^1H NMR spectrum displayed seven methyl signals, of which six were singlets (δ 0.87, 1.03, 1.04, 1.18, 1.31, 1.73) and one doublet (δ 1.11, $J = 6.6$ Hz). Additional signals observed include those ascribed to an olefinic proton (δ 5.48, *t*, $J = 7.8$ Hz), two oxy methine protons (δ 3.24, *d*, $J = 9.4$ Hz; 4.04, *m*) and two anomeric protons (δ 4.93, *d*, $J = 7.6$ Hz; 5.49, *d*, $J = 7.6$ Hz). These data suggested that

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the aglycone part of **1** was likely to be a pentacyclic triterpene with two hydroxyl groups and a trisubstituted double bond. Comparison of ^{13}C NMR data (Table 1) with those of known triterpenes (Dai et al., 2001; Reynolds et al., 1986) suggested that **1** belongs to the taraxastane series. The ^{1}H-^{1}H COSY spectrum of **1** showed a coupling interaction between the signals of δ 3.24 and δ 4.04, which were assigned to two hydroxyl groups at C-3 and C-2 positions. The HETCOR spectrum showed that the corresponding carbon signals were at δ 95.8 and 66.9, respectively. The assignment of hydroxyl groups to C-2 and C-3 was further confirmed from the HMBC correlations (Fig. 1) between the signal for C-2 and those of H-1, H-3 and H-23; similarly, the signal for C-3 showed correlation with those for H-1, H-2, H-23 and H-24. Both the hydroxyl groups at C-2 and C-3 must be equatorial as evident from the large coupling constant ($J = 9.4$ Hz) for H-3. Furthermore, the H-2 signal showed cross peaks in the NOESY spectrum with H-24 (δ 1.18), H-25 (δ 0.87), and H-1β (δ 2.37) signals, while that of H-3 was correlated with signals for H-1α (δ 1.12) and H-5 (δ 0.82). The ^{13}C signal at δ 178.2, assigned to a carboxyl group, showed HMBC cross peaks with H-18, H-16 and H-22 signals, justifying its assignment to C-28. The HMBC correlations of the signals for C-20 with H-19, H-22, H-29 and H-30 signals, and C-21 with H-19, H-22 and H-30 signals suggested the position of the double bond in the triterpene core (Fig. 1). The relative orientations of the remaining protons of **1** were established from coupling patterns and cross peaks generated from the phase sensitive NOESY spectrum. For example, the NOESY connectivities for H-5α with H-9 and H-1α, and for H-27 with H-18 and H-15α showed the spatial proximity between these protons. Using COSY, HOHAHA, HMBC and NOESY information, assignments for α and β protons of other carbons were also made. From the foregoing evidences it was concluded that the triterpene core of **1** was 2α,3β-dihydroxy-taraxast-20-en-28-oic acid.

Table 1
^{13}C NMR chemical shifts[a] of **1**, **3** and **5** in pyridine-d_5

Carbon no.	**1**	**3**	**5**		**1**
1	47.8 *t*	48.2 *t*	39.1 *t*	Glucose-1	
2	66.9 *d*	68.9 *d*	28.1 *t*	1′	104.6 *d*
3	95.8 *d*	83.8 *d*	78.1 *d*	2′	82.5 *d*
4	41.2 *s*	39.9 *s*	39.4 *s*	3′	78.6 *d*
5	55.8 *d*	56.1 *d*	55.7 *d*	4′	71.3 *d*
6	18.5 *t*	18.7 *t*	18.8 *t*	5′	78.4 *d*
7	34.6 *t*	34.3 *t*	32.5 *t*	6′	62.4 *t*
8	41.0 *s*	40.9 *s*	40.3 *s*	Glucose-2	
9	51.0 *d*	50.9 *d*	47.3 *d*	1″	105.7 *d*
10	38.0 *s*	38.7 *s*	37.3 *s*	2″	76.7 *d*
11	22.1 *t*	22.3 *t*	23.9 *t*	3″	78.2 *d*
12	29.6 *t*	25.4 *t*	122.6 *d*	4″	72.0 *d*
13	39.4 *d*	43.2 *d*	144.4 *s*	5″	78.3 *d*
14	42.3 *s*	41.4 *s*	41.1 *s*	6″	63.0 *t*
15	27.9 *t*	27.7 *t*	30.8 *t*		
16	33.7 *t*	28.1 *t*	76.6 *d*		
17	49.1 *s*	42.2 *s*	49.7 *s*		
18	49.4 *d*	48.4 *d*	41.1 *d*		
19	37.9 *d*	42.4 *d*	41.0 *t*		
20	143.1 *s*	83.9 *s*	38.1 *s*		
21	117.9 *d*	27.3 *t*	90.7 *d*		
22	38.5 *t*	32.3 *t*	76.4 *d*		
23	28.3 *q*	29.2 *q*	28.8 *q*		
24	17.7 *q*	17.5 *q*	16.6 *q*		
25	17.6 *q*	17.8 *q*	15.5 *q*		
26	16.4 *q*	15.9 *q*	17.2 *q*		
27	15.0 *q*	14.4 *q*	29.0 *q*		
28	178.2 *s*	176.7 *s*	61.0 *t*		
29	23.7 *q*	18.6 *q*	29.8 *q*		
30	22.2 *q*	24.1 *q*	28.8 *q*		

[a] Assignments based on ^{13}C, DEPT, HSQC, HMBC experiments and multiplicities as determined by DEPT.

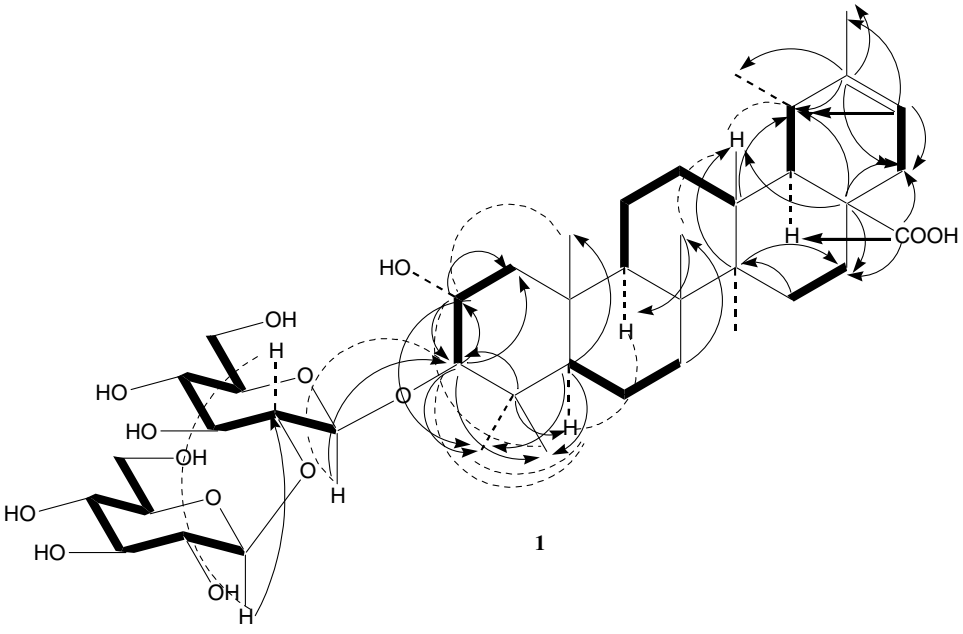

Fig. 1. COSY (▬) HMBC (→) NOESY (-----) correlations of **1**.