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A new triterpenoid from *Azadirachta indica*

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Abstract

A new triterpenoid, 1α,7α-diacetoxyapotirucall-14-ene-3α,21,22,24,25-pentaol (**1**), and the two known compounds odoratone (**2**) and 2β,3β,4β-trihydroxypregnan-16-one (**3**) were isolated from a methanolic extract of the seed kernels of *Azadirachta indica.* Their structures were elucidated on the basis of spectral methods.

Keywords: Azadirachta indica; Triterpenoid; Steroids

1. Introduction

Azadirachta indica A. Juss (syn. *Melia azadirachta* L.), known in the vernacular as Neem and Nimba, belongs to the Meliaceae family. It is widely distributed in Asia, Africa and other tropical parts of the world. Almost every part of the plant is used in the indigenous system of medicine for the treatment of a variety of human ailments, particularly against diseases of bacterial and fungal origin [1–3]. In view of the attributed therapeutic and pesticidal importance of this plant, comprehensive investigations on its different parts have been carried out by various groups, leading to the isolation and structure elucidation of more than 100 compounds [3–7]. In continuation of investigation on the constituents of *A. indica* [8], three

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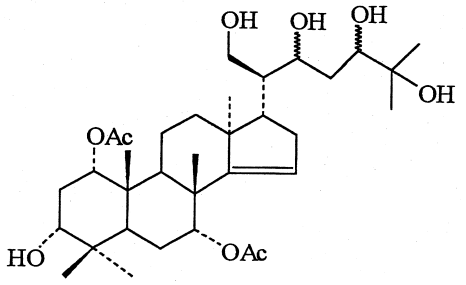
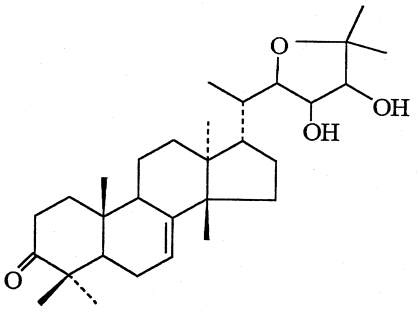
compounds, 1α,7α-diacetoxyapotirucall-14-ene-3α,21,22,24,25-pentaol (**1**), odoratone (**2**) [9], and 2β,3β,4β-trihydroxypregnan-16-one (**3**) [10] were isolated from a methanolic extract of the seed kernels. Compound **1** was new and compounds **2** and **3** were obtained primarly from this species.

2. Results and discussion

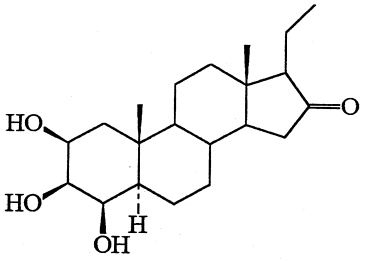
Compound **1** was obtained as white powder. The negative-ion HRFABMS *m/z* 607.3853 $[M-H]^-$, together with ^{13}C-NMR and DEPT spectral data, indicated the molecular formula as $C_{34}H_{56}O_9$. The ^{1}H- and ^{13}C-NMR spectra showed seven tertiary methyls, seven methylenes, one of which was oxygenated, nine methynes, five of which were oxygenated, one trisubstituted double bond and two acetates. The ^{13}C-NMR spectrum of **1** also showed four quaternary carbon signals and one hydroxytertiary carbon signal. These data suggested that **1** belonged to an apotirucallol (euphol) skeleton [11,12].



1



2



3

Three oxygenated methine protons, appearing in the ^{1}H-NMR spectrum at δ 4.63 (*t*, *J* = 2.8 Hz), 3.39 (*t*, *J* = 2.8 Hz) and 5.11 (*t*, *J* = 2.2 Hz), were placed at the C-1, C-3 and C-7 positions, respectively, based on the observations of cross peaks between C-1 and H-19, C-3 and H-28, H-29′ and C-7 to H-30 in the HMBC spectrum. Small coupling constants of the three protons suggested for all of them an α orientation. From the chemical shift values of H-1 (δ 4.63) and H-7 (δ 5.11), we could assume that C-1 and C-7 were acetylated. The assumption was supported by the HMBC spectrum, in which cross signals could be observed for δ_C 172.3 (*s*) to H-1 (δ_H 4.63) and δ_C 172.4 to H-7 (δ_H 5. 11). Furthermore, cross peaks between C-14 (δ_C 161.1) to H-18, and between C-15 (δ_C 120.5) to H-16 in HMBC spectrum indicated that the double bond was located between C-14 and C-15. These data suggested that **1** is a 1α,7α-diacetoxy-3α-hydroxyapotirucallol-14-ene with a 17-α side chain.

The structure of the side chain was determined by analysis of HMBC spectrum. Two methyl group protons H-26 [δ_H 1.27 (*s*)] and H-27 [δ_H 1.21 (*s*)], showed cross peaks to δ_C 74.7 (C-25), and this suggested that a hydroxyl is connected to C-25. The oxymethylene was attributed to C-21 also based on the analysis of HMBC spectrum. That the C-20 configuration belongs to the apotirucallane rather than the apoeuphane series is suggested by ROESY spectrum, which revealed a H-20α (C-20R). The other two hydroxy groups were placed at C-22 and C-24, respectively, on the basis of cross peaks between H-26 and H-27 to δ_C 78.6 (C-24), δ_H 4.07 (H-22) to δ_C 40.6 (C-20), and 38.0 (C-23), in the HMBC spectrum. So, the structure of **1** was determined as 1α,7α-diacetoxyapotirucall-14-ene-3α,21,22,24,25-pentaol.

The known compound odoratone (**2**) [9] was identified by detailed analysis of its previously unreported NMR spectra. 2β,3β,4β-Trihydroxypregnan-16-one (**3**) was identified by direct comparison with the published data [10].

3. Experimental

3.1. Plant material

Seeds of *A. indica* were collected in Mandalay, Myanmar in August 1994, where the plant is cultivated. The plant material was identified by Prof. Tianlu Ming, Kunming Institute of Botany, Academia Sinica, Kunming, Yunnan, PR China, where the specimen was deposited.

3.2. Extraction and isolation

The dehulled and air-dried Neem seed kernels (1.3 kg) were extracted with petrol at room temp., then with MeOH at room temp. The MeOH extract was evaporated in vacuo, the residue was suspended in water, and then extracted with petrol, EtOAc, and *n*-BuOH. The EtOAc and *n*-BuOH layers were concentrated in vacuo to give 32 and 45 g of residues, respectively. The EtOAc extract was repeatedly Si-gel CC to yield **2** (18 mg). The *n*-BuOH extract was fractionated on

D-101, eluting with aqueous MeOH. The fraction eluted with 70% MeOH was Si-gel CC eluting with $CHCl_3$–MeOH (4:1–2:1) to yield **1** (8 mg) and **3** (8 mg).

1α,7α-Diacetoxyapotirucall-14-ene-3α,21,22,24,25-pentaol (**1**). White powder (MeOH), mp 132–134°C; IR bands (KBr): 3434, 2956, 1727, 1439, 1379, 1253, 1052 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz): δ 4.63 (1H, *t*, *J* 3.1 Hz, H-1), 2.29, 1.90 (each 1H, *dd*, *J* 13.4, 3.1 Hz, H-2), 3.39 (1H, *t*, *J* 3.0 Hz, H-3), 2.28 (1H, *m*, H-5), 1.72, 1.88 (each 1H, *m*, H-6), 5.11 (1H, *t*, *J* 2.2 Hz, H-7), 2.59 (1H, *dd*, *J* 12.2, 3.1 Hz, H-9), 1.27, 1.50 (each 1H, *m*, H-11), 1.50, 1.60 (each 1H, *m*, H-12), 5.27 (1H, *t*, *J* 2.2 Hz, H-15), 2.06, 2.25 (each 1H, *m*, H-16), 1.35 (1H, *m*, H-17) 1.10 (3H, *s*, H-18), 0.97 (3H, *s*, H-19), 1.75 (1H, *m*, H-20), 3.74 (1H, *dd*, *J* 10.9, 2.9 Hz, H-21a) , 3.40 (1H, *dd*, *J* 11.0, 4.8 Hz, H-21b), 4.07 (1H, *m*, H-22), 2.55, 2.20 (each 1H, *m*, H-23), 3.15 (1H, *brs*, H-24), 1.27 (3H, *s*, H-26), 1.21 (3H, *s*, H-27), 0.90 (3H, *s*, H-28), 0.87 (3H, *s*, H-29), 1.15 (3H, *s*, H-30), 2.09, 1.98 (each 3H, C$\underline{H}_3$COO); ^{13}C-NMR (Table 1); EIMS (70 eV) *m/z*: 608 $[M]^+$ (1), 546 (2), 518 (3), 488 (5), 470 (10), 452 (5), 381 (10), 365 (6), 293 (12), 279 (7), 173 (13), 161 (15), 147 (21), 133 (23), 119 (30), 105 (35), 83 (47), 69 (64), 55 (100); HRFAB-MS *m/z*: 607.3853 $[M-H]^-$ (calculated for $C_{34}H_{55}O_9$, 607.3846).

Odoratone (**2**). Colorless needles (Me_2CO), mp 201–203°C; IR bands (KBr): 3490, 3370, 2910, 1720, 1680, 1450, 1380, 1360, 1230, 1120, 810 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 400 MHz): δ 5.27 (1H, *d*, *J* 3.0 Hz, H-7), 3.94 (1H, *dd*, *J* 6.3, 6.4 Hz, H-23), 3.79 (1H, *d*, *J* 6.4 Hz, H-22), 3.62 (1H, *d*, *J* 6.3 Hz, H-24), 2.72 (1H, *dt*, *J* 14.5, 5.5 Hz, H-17), 0.81 (3H, *d*, *J* 6.6 Hz, H-21), 0.79 (3H, *s*, Me), 0.97 (3H, *s*, Me), 1.01 (6H, *s*, Me), 1.08 (3H, *s*, Me), 1.19 (6H, *s*, Me); ^{13}C-NMR (see Table 1); EIMS (70

Table 1
^{13}C-NMR spectral data for compounds **1** and **2** (100 MHz)[a]

C	**1**	**2**	C	**1**	**2**
1	75.3 *d*	33.6 *t*	18	20.8 *q*	12.4 *q*
2	29.2 *t*	38.6 *t*	19	16.6 *q*	21.3 *q*
3	76.2 *d*	210.2 *s*	20	40.6 *d*	37.5 *d*
4	38.0 *s*	47.9 *s*	21	65.2 *t*	12.8 *q*
5	37.6 *d*	49.4 *d*	22	71.1 *d*	83.8 *d*
6	24.1 *t*	24.4 *t*	23	38.0 *t*	77.5 *d*
7	77.5 *d*	117.8 *d*	24	78.7 *d*	72.9 *d*
8	43.5 *s*	146.0 *s*	25	74.7 *s*	80.9 *s*
9	36.9 *d*	48.6 *d*	26	26.4 *q*	27.7 *q*
10	41.6 *s*	35.1 *s*	27	24.1 *q*	27.7 *q*
11	17.4 *t*	18.4 *t*	28	28.6 *q*	24.6 *q*
12	36.1 *t*	34.1 *t*	29	22.6 *q*	21.8 *q*
13	47.8 *s*	43.6 *s*	30	27.5 *q*	21.6 *q*
14	161.2 *s*	51.2 *s*	C$\underline{H}_3$COO	21.3 *q*	
15	120.5 *d*	34.6 *t*		21.6 *q*	
16	35.7 *d*	27.1 *t*	$CH_3\underline{C}OO$	172.3 *s*	
17	57.3 *d*	52.5 *d*		172.4 *s*	

[a]Compound **1** was measured in CD_3OD, compound **2** in $CDCl_3$; chemical shifts are in ppm, with TMS as internal standard.

eV) *m/z*: 472 $[M]^+$, 457 (55), 439 (45), 421 (11), 395 (5), 357 (13), 339 (26), 325 (25), 313 (15), 299 (42), 271 (19), 257 (13), 245 (18), 185 (16), 173 (22), 155 (55), 145 (30), 131 (60), 109 (54), 95 (73), 71 (100).

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