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# TETRANORTRITERPENOIDS FROM *AZADIRACHTA INDICA*

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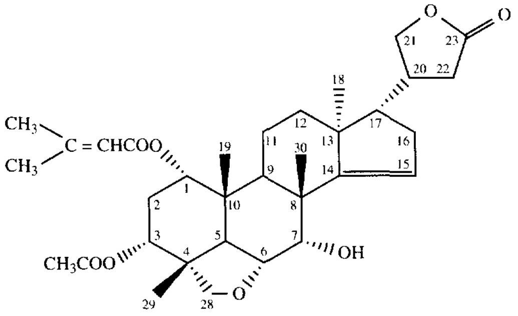
**Key Word Index**—*Azadirachta indica*; Meliaceae; neem tree; tetranortriterpenoid; azadirachtolide; deoxyazadirachtolide.

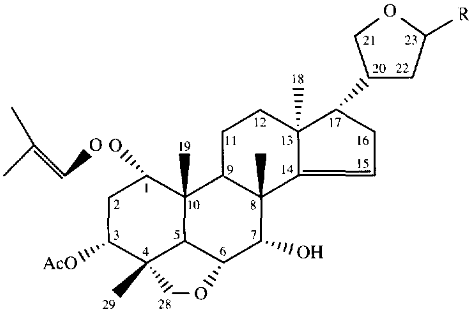
**Abstract**—*Azadirachta indica* afforded two novel tetranortriterpenoids by gravity column chromatography. Their structures were elucidated by extensive 1D and 2D NMR techniques. © 1997 Elsevier Science Ltd

## INTRODUCTION

*Azadirachta indica* is used as an anti-malarial, anti-inflammatory, anti-pyretic, antitumour and anthelmintic, a febrifuge, and an astringent. It is also used for the treatment of ulcers and skin diseases. The plant is known for its insecticidal and pesticidal properties [1–2]. Earlier studies reported on the isolation of tetranortriterpenoids and triterpenoids [3–24] from the plant which are of relevance to our present report. We now report two additional tetranortriterpenoids (**1**–**2**) from the same species.



**1**



**1** R = =O
**2** R = H

## RESULTS AND DISCUSSION

The $CHCl_3$ extract of the air-dried leaves of *A. indica* afforded compounds **1** and **2**. The structures of which were elucidated by NMR and FT-IR spectroscopy and mass spectrometry.

The mass spectrum of **1** contained a $[M]^+$ peak at $m/z$ 570 which corresponds to a molecular formula of $C_{33}H_{46}O_8$. The $^1H$ NMR spectrum showed resonances for a senecioyloxy substituent [$\delta$ 1.88 (3H, *d*, $J = 1.2$ Hz), $\delta$ 2.20 (3H, *d*, $J = 1.2$ Hz), $\delta$ 5.70 (1H, *s*, *br*)] [25], an acetate [$\delta$ 1.96 (3H, *s*)], four additional methyl singlets ($\delta$ 0.97, $\delta$ 1.0, $\delta$ 1.05, $\delta$ 1.19), two olefinic hydrogens [$\delta$ 5.50 (*dd*, $J = 1.74$, 3.0 Hz), 5.70 (*s*, *br*)], methylene hydrogens bonded to oxygenated carbons [$\delta$ 4.4 (1H, *t*, $J = 7.7$ Hz), $\delta$ 3.95 (1H, *t*, $J = 7.7$ Hz), $\delta$ 3.63 (1H, *d*, $J = 7.6$ Hz), $\delta$ 3.56 (1H, *d*, $J = 7.6$ Hz)], and methine hydrogens bonded to oxygenated carbons [$\delta$ 4.12 (1H, *dd*, $J = 3.3$, 12 Hz), $\delta$ 4.14 (1H, *s*, *br*), $\delta$ 4.70 (1H, *t*, $J = 3.0$ Hz), $\delta$ 4.90 (1H, *t*, $J = 3.0$ Hz)] (Table 1). The $^{13}C$ and DEPT NMR spectra gave the following functionalities: a carbonyl of a lactone at $\delta$ 176.0, a carbonyl of an acetate at $\delta$ 171.0, a carbonyl of a conjugated ester at $\delta$ 166.0, four oxygenated methine carbons at $\delta$ 74.0, 72.8, 71.8 and 71.2, two oxygenated methylene carbons at $\delta$ 77.9 and 72.4, two non-protonated olefins at $\delta$ 157.4 and 160.0, two

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Table 1. 300 MHz $^1$H NMR spectral data of compounds **1** and **2** in $CDCl_3$

| Proton | **1** (δ) | **2** (δ) |
|---|---|---|
| 1 | 4.70 (*t*, 3.0 Hz) | 4.70 (*t*, 3.0 Hz) |
| 2,2′ | 2.15 (*m*) | 2.15 (*m*) |
| 3 | 4.90 (*t*, 3.0 Hz) | 4.90 *t*, 3.0 Hz) |
| 5 | 2.65 (*m*) | 2.65 (*m*) |
| 6 | 4.12 (*dd*, 3.3, 12 Hz) | 4.11 (*dd*, 3.3, 12 Hz) |
| 7 | 4.14 (*s*, *br*) | 4.14 (*s*, *br*) |
| 9 | 1.65 (*m*) | 1.65 (*m*) |
| 11, 11′ | 1.38 (*m*), 1.55 (*m*) | 1.38 (*m*), 1.55 (*m*) |
| 12, 12′ | 1.60 (*m*), 2.55 (*m*) | 1.60 (*m*), 2.55 (*m*) |
| 15 | 5.50 (*dd*, 1.74, 3.0 Hz) | 5.50 (*dd*, 1.74, 3.0 Hz) |
| 16 | 2.25 (*m*), 2.20 (*m*) | 2.25 (*m*), 2.20 (*m*) |
| 17 | 1.80 (*m*) | 1.80 (*m*) |
| 18 | 1.00 (*s*) | 1.00 (*s*) |
| 19 | 0.97 (*s*) | 0.97 (*s*) |
| 20 | 2.70 (*m*) | 2.70 (*m*) |
| 21, 21′ | 3.95 (*t*, 7.7 Hz), 4.40 (*t*, 7.7 Hz) | 4.14 (*t*, 7.7 Hz), 3.78 (*t*, 7.7 Hz) |
| 22 | 2.25 (*m*), 2.52 (*m*) | 2.25 (*m*), 2.52 (*m*) |
| 23 | | 3.45 (*m*), 3.55 (*m*) |
| 28,28′ | 3.56 (*d*, 7.6 Hz), 3.63 (*d*, 7.6 Hz) | 3.56 (*d*, 7.6 Hz), 3.63 (*d*, 7.6 Hz) |
| 29 | 1.19 (*s*) | 1.19 (*s*) |
| 30 | 1.05 (*s*) | 1.05 (*s*) |
| OSen | 5.70 (*s*, *br*) | 5.70 (*s*, *br*) |
| | 1.88 (*s*, *br*) | 1.88 (*s*, *br*) |
| | 2.20 (*s*, *br*) | 2.20 (*s*, *br*) |
| OAc | 1.97 (*s*) | 1.96 (*s*) |

protonated olefins at δ 116.0 and 120.0, five methylene, four methine, four quaternary and seven methyl carbons (Table 2). The DEPT spectrum indicated 45 protons attached to carbons, while the FT-IR spectrum indicated a hydroxyl at 3439 cm$^{-1}$ to account for 46 hydrogens. From the molecular formula, the index of hydrogen deficiency is 11. With three carbonyls [1774, 1732 and 1724 cm$^{-1}$] and two double bonds in **1**, the rest of hydrogen deficiency is accounted for by six ring systems.

The COSY spectrum of **1** enabled quite extensive chains of coupling to be delineated. Thus, the proton at δ 4.70 (H-1) was coupled to the methylene hydrogens at δ 2.15 (H-2, H-2′), which were in turn coupled to the proton at δ 4.90 (H-3). The coupling chain was then blocked by non-proton-bearing carbons. Further short coupling chains linked the proton at δ 3.63 (H-28) to the hydrogen to δ 3.56 (H-28′) and the proton at δ 2.65 (H-5) to the hydrogen at δ 4.10 (H-6), which was further coupled to the proton at δ 4.14 (H-7). Further coupling chains linked the hydrogen at δ 1.55 (H-9) to the methylene protons at δ 1.38 (H-11) and 1.65 (H-11′), which were in turn coupled to the methylene protons at δ 2.55 (H-12) and 1.60 (H-12′). Another coupling chain linked the olefinic proton at δ 5.50 (H-15) to the methylene hydrogens at δ 2.25 (H-16) and 2.20 (H-16′), which were in turn coupled to the proton at δ 1.80 (H-17), which was further coupled to the hydrogen at δ 2.70 (H-20). The last hydrogen was coupled to the methylene protons at δ 2.52 (H-22) and 2.25 (H-22′) and the methylene protons at δ 4.40 (H-21) and 3.95 (H-21′).

The $^{13}$C and $^1$H NMR assignments for **1** (Tables 1 and 2) were verified by HMQC and connectivity was verified by an inverse long-range heteronuclear experiment HMBC optimized for $J = 8$ Hz (Table 3). The relative stereochemistry of **1** was determined by NOESY which indicated correlation through space of the $^1$H nuclei in the molecule (Table 4). The trivial name azadirachtolide is suggested for **1**.

The structure of **2** was determined by comparing the $^1$H and $^{13}$C NMR spectral data of **2** with those of **1** (Table 1). Compound **2** indicated similar resonances to those in **1**. The differences are the shielding of the methylene protons (H-21) attached at an oxygenated carbon at δ 3.90 and 4.40 in **1** to δ 3.75 and 4.15 in **2** and the appearance of another pair of hydrogens attached to an oxygenated carbon at δ 3.45 and 3.55 in **2**. The $^{13}$C NMR spectral data of **2** (Table 2) indicated similar resonances to those in **1**. The differences are the absence of the carbonyl carbon of the lactone at δ 175.0 and the oxygenated methylene carbon at δ 72.4 in **2**. However, two oxygenated methylene carbons appeared at δ 64.2 and 60.8 in **2**. This indicated that the carbonyl carbon of the lactone in **1** was converted to an oxygenated methylene carbon in **2** which results in the shielding of the methylene carbon at δ 72.4 in **1**. This change in structure also caused the shielding of the methine carbon in δ 58.1 in **1** to move to δ 54.4 in **2** and the deshielding of the methine carbon at δ

Table 2. 75 MHz $^{13}$C NMR data for compounds **1*** and **2** in $CDCl_3$

| C | **1** ($\delta$) | **2** ($\delta$) |
|---|---|---|
| 1 | 71.2 | 71.2 |
| 2 | 27.6 | 27.5 |
| 3 | 71.8 | 71.8 |
| 4 | 42.3 | 42.3 |
| 5 | 39.8 | 39.8 |
| 6 | 74.0 | 74.1 |
| 7 | 72.8 | 73.0 |
| 8 | 45.5 | 45.7 |
| 9 | 33.2 | 33.4 |
| 10 | 39.3 | 39.7 |
| 11 | 15.2 | 15.6 |
| 12 | 34.0 | 34.4 |
| 13 | 46.7 | 46.7 |
| 14 | 160.0 | 160.5 |
| 15 | 120.0 | 120.6 |
| 16 | 34.7 | 34.8 |
| 17 | 58.1 | 54.4 |
| 18 | 20.1 | 19.9 |
| 19 | 15.4 | 15.4 |
| 20 | 37.5 | 39.3 |
| 21 | 72.4 | 64.2 |
| 22 | 33.8 | 33.4 |
| 23 | 176.0 | 60.8 |
| 28 | 77.9 | 77.8 |
| 29 | 19.6 | 19.6 |
| 30 | 26.3 | 26.3 |
| OSen | 166.0 | 165.7 |
| | 116.0 | 116.0 |
| | 157.4 | 157.5 |
| | 27.4 | 27.5 |
| | 20.4 | 20.5 |
| OAc | 171.0 | 170.5 |
| | 21.0 | 21.0 |

* Assignments based on HMQC, HMBC and COSY spectroscopy.

Table 3. 300 MHz $^{13}$C-$^{1}$H long-range correlation data of compound **1** in $CDCl_3$

| C | Long-range het. corr. expt. |
|---|---|
| 1 | H2, H3, H19 |
| 2 | H1, H3 |
| 3 | H1, H2, H29 |
| 4 | H3, H5, H28, H29 |
| 5 | H19, H28, H29 |
| 6 | H5, H7, H19 |
| 7 | H6, H30 |
| 8 | H9, H15, H30 |
| 9 | H5, H7, H12, H19, H30 |
| 10 | H1, H19 |
| 11 | H9, H12 |
| 12 | H11, H18 |
| 13 | H15, H16, H18 |
| 14 | H18, H30 |
| 15 | H16 |
| 16 | H15, H17, H20 |
| 17 | H15, H16, H18, H21 |
| 18 | H12, H17 |
| 19 | H1, H5, H9 |
| 20 | H15, H17, H21, H22 |
| 21 | H17, H20, H22 |
| 22 | H20 |
| 23 | H21, H22 |
| 28 | H29 |
| 29 | H3, H5, H28 |
| 30 | H7, H9 |
| OSen | |
| –C= | $2CH_3$, CH= |
| –C=O | CH= |
| OAc | |
| –C=O | H3, $CH_3$ |

Table 4. NOESY correlation data of compound **1** in $CDCl_3$

| H | NOESY correlation |
|---|---|
| 3 | H1, H2, H29 |
| 6 | H7, H19, H29, H30 |
| 7 | H6, H15, H30 |
| 18 | H12′, H20, H21′ |
| 19 | H1, H2, H3, H6, H29 |
| 29 | H2, H3, H6, H19 |
| 30 | H7, H11, H29, H29 |

37.5 in **1** to move to $\delta$ 39.3 in **2**. The trivial name deoxyazadirachtolide is suggested for **2**.

## EXPERIMENTAL

*General.* IR; KBr pellets NMR; $CDCl_3$ sols on a 300 MHz NMR spectrometer. Silica gel 60 (70–230 mesh); CC plastic backed plates coated with silica gel $F_{254}$ TLC. Plates were visualized by spraying with vanillin–$H_2SO_4$ and warming.

*Plant material and extraction. Azadirachta indica* was collected at the Pampanga Agricultural College, Magalang, Pampanga in June. The air-dried leaves (200 g) were extracted with $CHCl_3$ (2 l) to afford a crude extract (10 g). The crude extract (10 g) was chromatographed in a column of silica gel (70–230 mesh) using $Me_2CO$ in $CHCl_3$ (10% increments) as eluent. The 20% $Me_2CO$ in $CHCl_3$ fr. afforded **1** [crystals, mp 269°, $\alpha_D = -48°$ ($CHCl_3$, $c = 4.25$), 16.5 mg] after rechromatography (twice) in 20% $Me_2CO$ in $CHCl_3$. The 30% $Me_2CO$ in $CHCl_3$ fr. afforded **2** (crystals, mp 118–120°, 10.5 mg) after rechromatography (twice) in 25% $Me_2CO$ in $CHCl_3$.

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