

ASTROTRICHILIN, A LIMONOID FROM *ASTROTRICHILIA ASTEROTRICHIA*

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**Abstract**—The novel limonoid, astrotrichilin, has been isolated from the Madagascan Meliaceae plant *Astrotrichilia asterotricha*.

## INTRODUCTION

*Astrotrichilia asterotricha* (Harms) J. F. Leroy, is a tree belonging to the Meliaceae, growing on the island of Madagascar. Plant material was collected in Madagascar with the cooperation of Professor Raven of the Missouri Botanical Gardens, which holds voucher specimens. *Astrotrichilia* is a small genus of trees endemic to Madagascar which is thought to be closely related to *Owenia*, and less closely to *Ekebergia* and *Lepidotrichilia* [1]. *Owenia* has been found to contain simple limonoids of the havanensin type [2]. *Ekebergia* is distinguished by the occurrence of highly oxidized methyl angolensate derivatives [3] such as ekebergin (1), while *Lepidotrichilia* has not yet been examined chemically. The isolation from *A. asterotricha* of the novel compound astrotrichilin, which is related to ekebergin, suggests a relationship between *Astrotrichilia* and *Ekebergia*. The structure of astrotrichilin was determined using two-dimensional NMR and mass spectroscopic techniques and by comparison with spectra of related compounds from *Ekebergia capensis*.

## RESULTS AND DISCUSSION

Extraction of the bark of *A. asterotricha* with refluxing hexane, followed by column chromatography, yielded two dammaranes [4] and a complex mixture of limonoids. The limonoid mixture was very difficult to separate and required repeated column chromatography, which eventually yielded astrotrichilin as the major component.

The molar mass of astrotrichilin ( $855.3064 \text{ g mol}^{-1}$ ) indicated a molecular formula of  $\text{C}_{46}\text{H}_{49}\text{O}_{15}\text{N}$ . The  $^{13}\text{C}$  NMR and mass spectra indicated that the compound was pure. However, the  $^1\text{H}$  NMR spectrum was not well resolved, suggesting restricted rotation, in the

manner of priedurianin [5]. Resonances ascribable to the limonoid furan-ring protons were present at  $\delta$  7.60 (H-21), 7.53 (H-23) and 6.55 (H-22) in the  $^1\text{H}$  NMR spectrum. The corresponding  $^{13}\text{C}$  NMR resonances occurred at  $\delta$  140.0d, 108.5d and 143.9d. The C-20 singlet occurred at  $\delta$  121.4. The  $^{13}\text{C}$  NMR spectrum indicated the presence of a keto group ( $\delta$  207.8) and six ester or lactone carbonyl groups. These included a ring D lactone carbonyl group (confirmed by a sharp singlet at  $\delta$  6.68 ascribable to H-17), a  $12\alpha$ -acetate group indicated by the characteristic upfield position of the acetate methyl proton resonance at  $\delta$  1.58s [6], a second upfield acetate methyl group proton resonance at  $\delta$  1.50s, a carbomethoxyl group at C-7, (confirmed by the methoxyl group three-proton singlet at  $\delta$  3.66), a nicotinate ester and a cinnamate ester. Using HETCOR and COSY spectra, all the carbon and hydrogen resonances of the nicotinate and cinnamate esters could be assigned. Further evidence for the presence of these esters came from the mass spectrum. Fragment ions  $[\text{M}-60-131]^+$  and  $[\text{M}-106]^+$  corresponded to the loss of  $[\text{CH}_3\text{COOH}]$  and  $[\text{Ph}-\text{CH}=\text{CH}-\text{CO}]$ , and  $[\text{C}_5\text{H}_4\text{N}-\text{CO}]$ , respectively, and peaks were detected at  $m/z$  131 and 106.

The double-bond region of the  $^{13}\text{C}$  NMR spectrum contained 19 resonances, of which furan ring and nicotinate and cinnamate esters accounted for 17. The remaining unassigned singlet ( $\delta$  141.7) and triplet ( $\delta$  116.7) were ascribed to C-8 and C-30, respectively, of a ring B opened limonoid. The ring B opened nature of the compound was confirmed by singlets ascribable to the two vinyl H-30 protons at  $\delta$  5.38 and  $\delta$  5.06 in the  $^1\text{H}$  NMR spectrum and the presence of the carbomethoxyl group at C-7. The COSY spectrum indicated long-range coupling between one of the H-30 protons and H-9 ( $\delta$  3.63s) and coupling between H-5 ( $\delta$  3.10,  $J = 3.8 \text{ Hz}$ ) and the C-6 methylene protons. ( $\text{H}_{6\text{A}}$ , dd,  $\delta$  2.30,  $J = 3.8 \text{ Hz}$ , 18.5 Hz;  $\text{H}_{6\text{B}}$ , d,  $\delta$  2.87,  $J = 18.5 \text{ Hz}$ ). The COSY spectrum indicated that ring A was  $1\alpha$ ,  $2\alpha$ ,  $3\alpha$ -tri-substituted as in ekebergin. Reso-

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nances at  $\delta$  5.33, 5.57 and 4.54 were ascribed to H-1 $\beta$ , H-2 $\beta$  and H-3 $\beta$ , respectively. The assignment of the respective H-1 and H-3 resonances will be discussed later. The nicotinate and cinnamate esters were placed at C-1 and C-2 on the basis of the H-1 $\beta$  and H-2 $\beta$  chemical shifts. The sharp singlet at  $\delta$  5.88 was assigned to H-12 $\beta$ . As H-9 occurred as a singlet, the keto group was placed at C-11. A trijugin-type five-membered ring C was discounted because H-12 $\beta$  occurs at  $\delta$  5.58 in these compounds [7] and no coupling was apparent between H-9 and H-12 $\beta$ , as is usual in trijugin compounds. A triplet at  $\delta$  36.1, a singlet at  $\delta$  88.9, and a second upfield acetate group proton resonance remained to be assigned. The only place possible for the triplet was at C-15. The two H-15 protons occurred as a pair of doublets at  $\delta$  2.87 and  $\delta$  2.85 ( $J$  = 18.5 Hz). The chemical shift of H-3 ( $\delta$  4.54) ruled out the possibility of placing an acetate group here and indicated the presence of a hydroxyl group at this position. To confirm that the hydroxyl group was present at C-3 $\alpha$  and the ester at C-1 $\alpha$ , a NOESY spectrum was run. A positive nOe was observed between one of the H-30 protons and the proton resonating at  $\delta$  5.33. A molecular model showed that a positive nOe could be expected between H-30 and H-1 $\beta$ , but not between H-30 and H-3 $\beta$ . This confirmed the placement of the ester at C-1 $\beta$  and the hydroxyl group at C-3 $\beta$ . The singlet at  $\delta$  88.9 was assigned to C-14 and the second acetate group was placed here. A molecular model indicated that an acetate group at C-14 $\alpha$  was in the same position, on the  $\gamma$ -carbon atom, relative to the furan ring as the acetate group at C-12 $\alpha$ . This could account for the upfield chemical shift of this acetate group proton resonance. Acetylation of the diacetate yielded a tri-acetate, but the proton NMR spectrum was too weak for H-3 $\beta$  to be detected.

The 1, 14-oxide bridge in ekebergin-type compounds prevents rotation about the C-9/C-10 bond resulting in well-resolved  $^1\text{H}$  NMR spectra. However, in astrotrichilin, as in prieurianin, rotation is possible about this bond, but is restricted by the large substituents, resulting in the presence of isomers equilibrating on the NMR time scale and hence the poorly resolved  $^1\text{H}$  NMR spectrum. We were unable to ascertain which of the nicotinate and cinnamate esters were present at C-1 and C-2, respectively. However, astrotrichilin 2, iso-

lated in small amounts, only differed from astrotrichilin 1 in slightly different positions for H-2 and H-3 in the  $^1\text{H}$  NMR spectrum and had the same molar mass. Thus, in astrotrichilin 2, the cinnamate and nicotinate esters are interchanged.

## EXPERIMENTAL

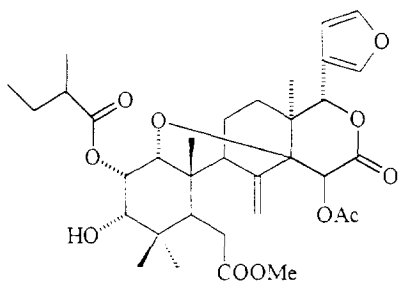
**General.** Ground bark (119 g) of *A. asterotricha* was extracted in a soxhlet apparatus with refluxing hexane yielding a gum (6.5 g). Repeated CC over silica gel (Merck 9385) yielded two dammaranes [5] and astrotrichilin.

NMR spectra were recorded on a Varian 300 MHz spectrometer in  $\text{CDCl}_3$  with TMS as an int. standard. IR spectra were recorded on a Mattison FT-IR. HR-MS and EI-MS were recorded at Cape Technikon on a Finnigan 1020 GC-MS Spectrophotometer.

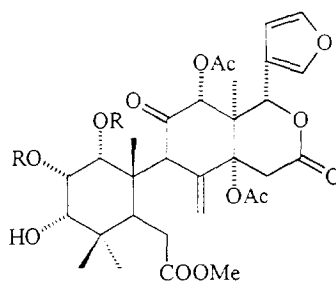
**Astrotrichilin 1 (2)** (11 mg), amorphous gum. HR-MS:  $\text{M}^+$  at  $m/z$  855.3064 ( $\text{C}_{47}\text{H}_{49}\text{NO}_{15}$  requires 855.3100). EI-MS: 855 [ $\text{M}$ ] $^+$ , 797 [ $\text{M} - \text{CH}_2\text{COO}$ ] $^+$ , 795 [ $\text{M} - \text{CH}_3\text{COOH}$ ] $^+$ , 664 [ $\text{M} - \text{CH}_3\text{COOH} - \text{PhCH} = \text{CHCO}$ ] $^+$ , 749 [ $\text{M} - \text{C}_5\text{H}_4\text{N} - \text{CO}$ ] $^+$ .

$^1\text{H}$  NMR:  $\delta$  9.23 (1H, s), 8.75 (1H, d,  $J$  = 7.6 Hz), 8.24 (1H, d,  $J$  = 7.6 Hz), 7.72 (1H, d,  $J$  = 7.6 Hz), (H-2, H-6, H-4, H-5, respectively, of nicotinate ester), 8.05 (2H, d,  $J$  = 6.8 Hz), 7.62 (1H, d,  $J$  = 15.2 Hz), 7.44 (2H, m), 7.30 (1H, m), 6.54 (1H, d,  $J$  = 15.2 Hz), (H-5/H-9, H-3, H-6/8, H-7, H-2 of cinnamate ester), 7.29 (1H, s, H-21), 7.16 (1H, d, H-23), 6.68 (1H, s, H-17), 6.22 (1H, d, H-22), 5.88 (1H, s, H-12 $\beta$ ), 5.75 (1H, m,  $W_{1/2}$  = 6.5 Hz, H-2 $\beta$ ), 5.38 (1H, s, H-30a), 5.33 (1H, bs, H-3 $\beta$ ), 5.06 (1H, s, H-30b), 4.54 (1H, bs, H-1 $\beta$ ), 3.66 (3H, s, -OCH<sub>3</sub>), 3.63 (1H, s, H-9), 3.10 (1H, d,  $J$  = 3.8 Hz, H-5), 2.87 (1H, d,  $J$  = 18.5 Hz, H-15<sub>A</sub>), 2.87 (1H, d,  $J$  = 18.4 Hz, H-6<sub>B</sub>), 2.85 (1H, d,  $J$  = 18.5 Hz, H-15<sub>B</sub>), 2.30 (1H, dd,  $J$  = 3.8, 18.4 Hz, H-6<sub>A</sub>), 1.58, 1.50 (ea 3H, s, -OCOCH<sub>3</sub>), 1.15, 0.97, 0.97, 0.92 (ea 3H, s, H-19, 28, 29, 18).

$^{13}\text{C}$  NMR:  $\delta$  207.8s (C-11), 174.2s (C-7), 169.6s, 169.6s ( $2 \times \text{OCOCH}_3$ ), 166.6s (C-16), 167.0s, 153.8d, 151.5d, 138.4d, 125.3s, 123.5d (nicotinate ester), 165.2s, 146.7d, 134.2s, 130.2d, 128.5d, 128.3 d, 116.6d (cinnamate ester), 143.9d (C-23), 141.7s (C-8), 140.0d (C-21), 121.4s (C-20), 116.7t (C-30), 108.5d (C-22), 88.9s (C-14), 79.8d (C-17), 76.1d (C-3), 75.4d



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2 R = cinnamate/nicotinate ester

(C-1), 69.8d (C-12), 67.2d (C-2), 58.9d (C-9), 56.2s (C-4), 52.0q (OCH<sub>3</sub>), 49.7s (C-13), 39.6s (C-10), 37.8d (C-5), 36.1t (C-15), 29.7t (C-6), 27.5q, 22.9q, 22.9q (C-19, 28, 29), 19.8 2q (OCOCH<sub>3</sub>), 18.5q (C-18).

IR:  $\nu_{\text{max}}^{\text{KBr}}$  cm<sup>-1</sup>: 3500, 3000, 1735, 1695, 1640, 1600, 1450, 1380, 1080.

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