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THOMANDERSINE AND ISOTHOMANDERSINE, 2-INDOLINONE ALKALOIDS FROM *THOMANDERSIA LAURIFOLIA*

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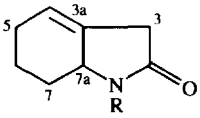
Key Word Index—*Thomandersia laurifolia*; Acanthaceae; leaves; 2-indolinone alkaloids; thomandersine and isothomandersine.

Abstract—In addition to the usual phytosterol mixture, taraxeryl palmitate and maslinic acid, two new 2-indolinone alkaloids, thomandersine and isothomandersine, have been isolated from a methanol extract of the leaves of *Thomandersia laurifolia*. Their structures were elucidated by spectroscopic methods and chemical correlations.

INTRODUCTION

Thomandersia laurifolia, a small tree of the rainforest, is a folkloric medicinal plant. A decoction of the leaves is used in Cameroon and Zaire as a remedy for diarrhoea and colitis [1]. Previous work on the twigs of *T. laurifolia* [2] reported four known pentacyclic triterpenoids, which included taraxeryl palmitate, 2α,3α-dihydroxyurs-12-en-28-oic acid, maslinic and epimaslinic acids, as well as the new one, thomandertriol. The known carotenoid, zeaxanthin, was also isolated. On the basis of taxonomic and pharmacological considerations, it became of interest to search for further constituents of this species. We report herein the isolation and structural elucidation of two new 2-indolinone alkaloids, thomandersine (**1**) and isothomandersine (**2**), isolated as the corresponding acetates, from the extracts of the leaves of *T. laurifolia*.



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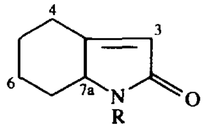

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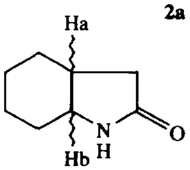
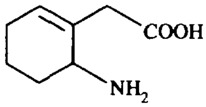


3 α-Ha, α-Hb
4 α-Ha, β-Hb



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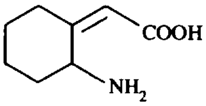

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RESULTS AND DISCUSSION

A methanol extract of the finely powdered leaves of *T. laurifolia* upon column chromatography and recrystallization afforded the two pentacyclic triterpenoids, taraxeryl palmitate [3] and maslinic acid [4] and a typical sterol mixture composed of β-sitosterol, campesterol, stigmastanol and stigmasterol. A mixture of two alkaloids was also isolated. Attempts to separate this mixture were unsuccessful. However, the mixture was finally resolved after acetylation followed by column and preparative TLC chromatography into two acetates (**1a**) and (**2a**).

Compound **1a**, obtained as a brown oil, was provisionally named thomandersine acetate. Its molecular composition was found to be $C_{10}H_{13}NO_2$ from NMR and confirmed by high resolution mass spectroscopy. The IR spectrum of **1a** showed strong vibrations at ν_{max} 1705 and 1740 cm^{-1} indicative of the presence of a γ-lactam and acetate carbonyl groups, respectively. Its ^{1}H NMR was well resolved and typical of that of a *cis* (**3**)- and *trans* (**4**)-lactam. The spectrum contained, amongst other signals, those for four methylene groups, three occurring as multiplets at δ 1.70–2.40 and the fourth as a sharp singlet at δ3.0. Also clearly discernible was one deshielded methine and one acetate group resonating at δ4.95 (*m*) and δ2.03 (*s*) respectively. The presence of the acetate group was confirmed by the appearance of a singlet in the ^{13}C NMR at δ170.9. Furthermore, the NMR spectrum showed one trisubstituted double bond {δ_C 130.4 (*s*), 122.9 (*d*) and δ_H 5.50 (*m*)}. The above data are best accommodated by structure **1a** for thomandersine acetate, a new compound. This structure was further confirmed by

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Table 1. ^{13}C NMR data for thomandersine acetate (**1a**) and isothomandersine acetate (**2a**) recorded at 50 MHz in $CDCl_3$

C	**1a**	**2a**
2	177.3 (*s*)	171.7 (*s*)
3	42.5 (*t*)	113.7 (*d*)
3a	130.4 (*s*)	163.2 (*s*)
4	122.9 (*d*)	33.9 (*t*)
5	27.2^a (*t*)	32.1^a (*t*)
6	26.1 (*t*)	25.7 (*t*)
7	30.8^a (*t*)	31.4^a (*t*)
7a	69.1 (*d*)	70.3 (*d*)
Ac	170.9 (*s*)	170.6 (*s*)
	21.4 (*q*)	21.3 (*q*)

Assignments based on chemical shift rules and multiplicities obtained from DEPT spectra.

^{a}Values interchangeable in the same column.

^{13}C NMR data (Table 1) which was fully assigned using chemical shift rules and DEPT spectra.

The second new alkaloid (**2a**) has the same molecular formula $C_{10}H_{13}NO_2$ as **1a** and recrystallized as needles from hexane–ethyl acetate, mp 92°. It exhibited in its IR spectrum, absorptions for an α,β unsaturated γ-lactam and one acetate carbonyl group at ν_{max} 1710 and 1750 cm^{-1}, respectively. Its ^{1}H NMR spectrum (see Experimental) showed one vinyl proton and one acetate group at δ5.68 and 2.05, respectively. One methine and four methylene protons were also observed in this spectrum at δ5.00 and at δ1.70–2.30, respectively. The ^{13}C NMR (Table 1) displayed four methylenes at δ33.9, 32.1, 31.4 and 25.6, two carbonyls at δ171.7 and 170.6, one methine at δ70.3 and one trisubstituted double bond [δ163.2 (*s*) and 113.7 (*d*)]. On the basis of the above spectral data, structure **2a** was assigned to isothomandersine acetate. The proposed structure was confirmed by ^{13}C NMR (Table 1), the signals of which were fully assigned.

Base hydrolysis of lactam acetates **1a** and **2a** afforded the amino acids, 2-aminocyclohexenyl acetic (**5**) and its isomer (**6**), respectively. The relative stereochemistry of the two new alkaloids remains to be determined.

EXPERIMENTAL

General. Mps: uncorr. UV were obtained in MeOH. IR were recorded in $CHCl_3$ soln or as KBr discs. NMR were run in $CDCl_3$ soln, unless otherwise mentioned, at 25°, 200 MHz for ^{1}H (shifts relative to $CDCl_3$ at δ_H 7.25) and 50 MHz for ^{13}C (shifts relative to $CDCl_3$ at δ_C77.0). DEPT experiments were carried out with $\theta = 45°$, 90° and 135°. Quaternary carbons were determined by substraction of these spectra from the broad-band ^{13}C NMR. EIMS: 70 eV, direct insert. Silica gel 60 (70–230 mesh Merck) was used for CC.

Plant material. Leaves of *T. laurifolia* (T. Anders ex. Benth.) Bail were collected at Edea Littoral Province in Cameroon in April 1986. Identification was carried out at the National Herbarium in Yaounde, where voucher specimen (No 9120) has been deposited.

Extraction and isolation. Powdered sun-dried leaves (1 kg) were extracted with MeOH in a Soxhlet. Removal of solvent gave a dark green residue (35 g) which was chromatographed over silica gel (360 g). Elution started with petrol (bp 60°) and continued stepwise through petrol–EtOAc and EtOAc–MeOH mixts. Frs (250 ml) were combined on the basis of TLC composition using appropriate solvent systems from the chromatographic sepn described above. After recrystallization, sterol (100 mg) and two pentacyclic triterpenoids, taraxeryl palmitate (40 mg) and maslinic acid (25 mg) were isolated, together with a mixt. of 2 alkaloids (800 mg). Known compounds were identified by ^{1}H and ^{13}C NMR and comparison with authentic samples. GC of the sterols identified a mixt. of stigmasterol, stigmastanol, campesterol and β-sitosterol. Attempts to separate the mixt. of the 2 alkaloids (**1** and **2**) were unsuccessful and it was finally acetylated with cold Ac_2O–pyridine (1:1).

2-Indolinone acetates. The alkaloid mixt. (400 mg) was dissolved in pyridine (20 ml) and Ac_2O (25 ml) added. After 24 hr at room temp., the reaction mixt. was diluted (H_2O) and extracted with $CHCl_3$. The acetylated residue upon repeated CC and prep. TLC yielded 2 acetates (**1a**, 20 mg) and (**2a**, 250 mg).

Thomandersine acetate (**1a**). Brown oil. IR ν_{max} $CHCl_3$ cm^{-1}: 1740, 1705, 1670, 1440, 1400, 1380, 1240, 1150, 1050. UV λ_{max} MeOH nm: 237. ^{1}H NMR (200 MHz, $CDCl_3$): δ5.50 (1H, *m*, H-4), 4.95 (1H, *m*, CHN), 3.00 (2H, *s*, H-3), 2.03 (3H, *s*, Me), 1.70–2.40 (6H, *m*, 2H-5, 2H-6 and 2H-7). ^{13}C NMR data see Table 1. EIMS *m/z* (rel. int.) 179 $[M]^+$ (1), 156 (12), 153 (10), 140 (11), 139 (68), 138 $[M - Ac + 2H]^+$ (72), 122 (12), 121 (62), 120 (70), 97 (24), 96 (14), 95 (13), 94 (13), 93 (58), 92 (13), 91 (27), 81 (13), 79 (32), 77 (16), 69 (15), 60 (31), 43 (100). Found $[M]^+$ *m/z* 179.0985, $C_{10}H_{13}NO_2$ requires 179.0946.

2-Aminocyclohexenyl acetic acid (**5**). A mixt. of thomandersine acetate (**1a**, 15 mg) EtOH (3 ml) and 1M NaOH (10 ml) was heated at reflux for 24 hr. The mixt. was dissolved in H_2O (10 ml) and the resulting soln deposited on an ion-exchange column (15 g). Elution with 1M NH_4OH and evapn of appropriate frs gave the 2-aminocyclohexenyl acetic acid (**5**, 8 mg) as an oil. ^{1}H NMR (200 MHz, D_2O): δ5.70 (1H, *m*, vinyl proton), 3.80 (1H, *m*, CH NH_2) 1.5–3.0 (8H, *m*).

Isothomandersine acetate (**2a**). Fine brown needles from hexane–EtOAc, mp 92°, IR ν_{max} KBr cm^{-1}: 1750, 1710, 1660, 1450, 1400, 1340, 1300, 1270, 1240, 1190, 1060. UV λ_{max} MeOH nm: 249 (ε 8600) and 289sh (ε 7500). ^{1}H NMR (200 MHz, $CDCl_3$): δ5.68 (1H, *br s*, H-3), 5.00 (1H, *m*, CHN), 3.10 (1H, *m*), 2.75 (1H, *m*), 2.45 (1H, *m*), 2.05 (3H, *s*, MeCO), 1.70–2.30 (5H, *m*). ^{13}C NMR data see Table 1. EIMS *m/z* (rel. int.) 179 $[M]^+$ (1) 138 $[M - Me\ CO + H]^+$ (81), 120 $[138 - CO]^+$ (12), 110 (18), 99 (10), 97 (12), 93 (65), 92 (12), 91 (22), 85 (27), 83 (11), 79 (19), 77 (14), 71 (37), 70 (11), 69 (15), 60 (17), 57 (48), 55 (20), 43 (100).

Found $[M]^+$ m/z 179.0990, $C_{10}H_{13}NO_2$ requires 179.0946.

Amino acid **6**. Isothomandersine acetate (**2a**, 20 mg) was hydrolysed in a similar manner to **1a** using 1M NaOH (15 ml) and EtOH (4 ml). Similar work-up and sepn, yielded **6** (10 mg) as an oil. ^{1}H NMR (200 MHz, D_2O): δ6.5 (1H, $=C\underline{H}COOH$), 3.95 ($C\underline{H}NH_2$) and 1.80–3.10 (8H, *m*).

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