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TWO NEW DITERPENE-BASED ALKALOIDS FROM *ICACINA GUESFELDTII*

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Key Word Index—*Icacina guesfeldtii*; Icacinaceae; leaf; root; lactonic alkaloids; pimarane skeleton.

Abstract—From *Icacina guesfeldtii* (leaves and roots), two new diterpene-based alkaloids have been isolated and identified as icaceine (**2**) and De-*N*-methylicaceine (**3**). Icacine (**1**) occurred both in the leaves and roots. Structure determination was performed by spectroscopic and chemical methods. As icacine (**1**), these two bases are the first alkaloids with a pimarane skeleton isolated from plants.

INTRODUCTION

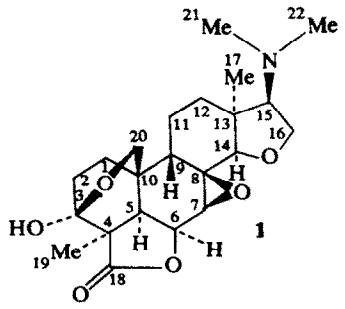
Icacina guesfeldtii Ascher is a shrub endemic to different regions of tropical Africa. Around Lodja (Zaire), the root decoction is used in popular medicine as an anticonvulsant. From a search for the active principle(s), alkaloids were detected in the tuberous roots and in the leaves. A previous study has revealed the presence of icacine (**1**) in the root; the structure of this new diterpenic alkaloid was established by X-ray analysis [1]. Further investigations, herein reported, led to the isolation and the identification of two additional alkaloids related to the same pimarane skeleton. These compounds, icaceine (**2**) and De-*N*-methylicaceine (**3**) occur almost exclusively in the leaves. Icacine was detected in larger quantities in the leaves than in the roots.

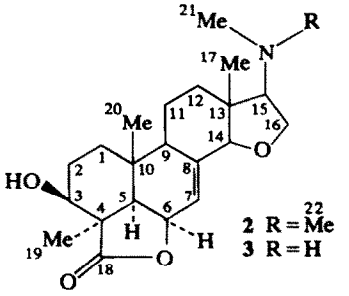
RESULTS AND DISCUSSION

A chloroform extract of the leaves obtained by a standard extraction procedure afforded a mixture of alkaloids; they were further purified by PLC on alumina to yield three bases.

1 was identical to icacine previously isolated from the root and identified by X-ray analysis [1]. Direct comparison with an authentic sample was carried out by GLC, TLC and by IR, MS and ^{1}H NMR spectroscopy.

2, icaceine, which afforded by Se dehydrogenation a mixture from which 1,7-dimethylphenanthrene (pimanthrene) was isolated and identified by UV spectroscopy [2], GLC, HPTLC and MS. As **1** and **2** afforded the same dehydrogenation product, a pimarane skeleton was assigned to **2**. High resolution MS established the formula as $C_{22}H_{33}N_1O_4$; the base peak (*m/e* 87, C_4H_9NO) was identical to that of icacine (**1**) and thus related to the fragmentation of an identical ring D. As in icacine (**1**), absorption at 1735 cm^{-1} suggested the presence of a γ-lactone. Unlike **1**, however, the formation of a monoacetate of **2** upon acetylation at room temperature indicated the presence of one secondary alcohol; furthermore, the IR spectrum of **2** showed absorption at 1655 cm^{-1} which suggested the presence of a double bond. The absorption at 3020 cm^{-1} observed in **1** (epoxide) did not appear in the IR of **2**. The ^{1}H NMR spectrum of **2** confirmed these assignments: it exhibited one olefinic proton at δ 5.89 (*dd*), one proton (−OH) at 3.64 and one proton on a carbon bearing a hydroxyl at 2.05





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(*m*), which moved downfield to 4.95 as a *dd* upon acetylation. The C-20 methylene protons shown by **1** (δ 4.76 and 3.56) were not present, but **2** exhibited a singlet at δ 1.01 (3H) which can be related to an additional angular methyl. Other signals from ring D were identical to those of icacine. The chemical shifts and couplings of H-14, H-7, H-6 and H-5 were in agreement with the structure proposed for **2**; they were similar to those observed for annonalide [**3**] and nomilactone [4] and further checked by double resonance experiments. Epoxidation of **2** or deoxidation of **1** to correlate both structures were unsuccessful.

However, the stereochemistry of (**2**) was not completely elucidated, but it was assumed that ring A presented a chair configuration and that the 3-OH was in the β-position: hydrogen bonding between the 3-OH and 18C=O was observed for **2** but not for **1**; molecular rotation differencies Δ(OAc) − Δ(OH) value (−19°) [5], as well as the comparison of the chemical shifts of H-19 exhibited by **1** (δ 1.38), icacine acetate (δ 1.42), icaceine (**2**) (δ 1.54) and icaceine acetate (δ 1.38) suggested a 3β-OH, 5α-H configuration. Furthermore, values of $J_{5,6}$ and $J_{6,7}$ identical to earlier published data [3, 4, 6] also supported the proposed stereochemistry of rings A and B.

3, De-*N*-methylicaceine, on methylation with MeI afforded **2** (MS, ^{1}H NMR, IR and TLC comparison). On treatment with CD_3I, **3** afforded a trideuterio derivative whose MS displayed a base peak at *m/e* 90: this result unequivocally confirmed the origin of the base peak *m/e* 87 shown by **1** and **2**, which is derived from the ring D fragmentation.

The largest accumulation of the alkaloids was found in the leaves, as shown in Table 1; it is reasonable to assume that they were synthetized in the aerial parts of the plant. The occurrence of annonalide in *Annona coriacea* [3] and momilactone in *Oryza sativa* [4] indicates that diterpenoids could be precursors of these lactonic alkaloids: further studies on their biogenesis and the isolation of diterpenoid precursors are in progress.

Table 1. Determination of alkaloids in *Icacina guesfeldtii* (as % dry out)

	Root	Leaf
Icacine (GLC)	0.070	0.180
Icaceine (GLC)	0.002	0.014
De-*N*-methylicaceine (GLC)	0.003	0.037
Total (titrimetry)	0.091	0.218

EXPERIMENTAL

Mps are uncorr. IR spectra were measured in KBr discs. NMR spectra were recorded at 270 MHz in $CDCl_3$, using TMS as internal reference; chemical shift values are reported in δ (ppm) units. MS were obtained by direct inlet, 70 eV.

Plant material. Roots and leaves of *I. guesfeldtii* were collected around Lodja (Zaire) in September 1977. Plants were identified by Dr. C. Evrard (Université Catholique de Louvain, Belgium). A voucher specimen has been deposited in the Botanical Laboratory of the National University of Zaire (Kinshasa).

Extraction and separation. The air-dried ground leaves or roots were extracted with EtOH. The EtOH-soluble residue was taken up by $CHCl_3$ and extracted with 2 N HCl. The combined aq. solns were basified by addition of NH_4OH and the liberated bases extracted with $CHCl_3$. The crude chloroform residue was stirred twice with Me_2CO at 4° and the yellow solvent discarded after decantation of the solid. Further purification was obtained by PLC on neutral Al_2O_3 (toluene–MeCO–EtOH–NH_4OH, 40:40:8:3; Dragendorff reagent was used for detection of spots. Three alkaloids were recovered from the leaves (1 kg): R_f 0.60 icacine (**1**) (1.5 g), R_f 0.79 icaceine (**2**) (95 mg) and R_f 0.66 De-*N*-methylicaceine (**3**) (50 mg).

Alkaloid assays were performed on the crude $CHCl_3$ extract by a standard volumetric method and GLC. GLC analysis was performed on 3% SE-30 on Chromosorb W (1 m × 2 mm column), using programmed temp. from 215 to 265° and a flow rate of 60 ml N_2/min; n-tetracosane was used as int. standard.

Icacine (**1**). The alkaloid crystallized from MeOH–$CHCl_3$ (4:1) as white needles, mp (decomp.) 250–280°, IR ν_{max}^{KBr} cm^{-1}: 3520, 3020 and 1758, $[\alpha]_D^{20}$ −51.3° (*c* 0.305, $CHCl_3$) ^{1}H NMR ($CDCl_3$): δ 1.16 (3H, *s*, H-17), 1.38 (3H, *s*, H-19), 2.11 (1H, *dd*, J_1 = 7.5, J_2 = 2.2 Hz, H-5), 2.21 (6H, *s*, H-21/22), 2.42 (1H, *t*, J = 8.6 Hz, H-15), 3.01 (1H, *s*, H-14), 3.34 (1H, *d*, J = 4.8 Hz, H-7), 3.56 (1H, *dd*, J_1 = 9.8, J_2 = 2.0 Hz, H-20), 3.86 (1H, *dd*, J_1 = 8.6, J_2 = 8.1 Hz, H-16), 3.88 (1H, *s*, −OH), 4.04 (IH, *dd*, J_1 = 8.1, J_2 = 8.1 Hz, H-16'), 4.76 (1H, *dd*, J_1 = 9.9, J_2 = 2 Hz, H-20'), 4.96 (1H, *dd*, J_1 = 7.5, J_2 = 4.5 Hz, H-6), MS: *m/e* 405.2143 (M^+ $C_{22}H_{31}NO_6$ requires 405.2150), 308.1854 ($C_{17}H_{26}NO_4$ requires 308.1861) and 87.0676 (base peak, C_4H_9NO requires 87.0684).

Icaceine (**2**). The alkaloid crystallized from MeOH–$CHCl_3$ (4:1) as white needles, mp (decomp. sublimation) 220–250°. IR ν_{max}^{KBr} cm^{-1}: 3490, 1785, 1737 and 1655 $[\alpha]_D^{20}$ −196.68° (*c* 0.995, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 1.01 (3H, *s*, H-17), 1.06 (3H, *s*, H-20), 1.54 (3H, *s*, H-19), 1.85 (1H, *d*, J = 4.4 Hz, H-5), 2.05 (1H, *m*, H-3), 2.22 (6H, *s*, H-21/22), 2.42 (1H, *t*, J = 8.4 Hz, H-15), 3.64 (1H, *s*, OH), 3.84 (1H, *s*, H-14), 3.89 (1H, *dd*, J_1 = 8.8, J_2 = 8.1 Hz, H-16), 4.07 (1H, *dd*, J_1 = 8.1, J_2 = 8.1 Hz, H-16'), 4.9 (1H, *dd*, J_1 = 5.1, J_2 = 4.4 Hz, H-6), 5.9 (1H, *dd*, J_1 = 5.1, J_2 = 1.5 Hz, H-7), MS: *m/e* 375.2404 (M^+ $C_{22}H_{33}N_1O_4$ requires 375.2409), 346.2371 ($C_{21}H_{32}N_1O_3$ requires 346.2381), 316.2271 ($C_{20}H_{30}N_1O_2$ requires 316.2276), 289.1806 ($C_{18}H_{25}O_3$ requires 289.1803), 271.1693 ($C_{18}H_{23}O_2$ requires 271.1697), 87 (base peak).

De-N-methylicaceine (**3**). The alkaloid crystallized from MeOH–$CHCl_3$ (4:1) as white needles, mp (decomp., sublimation 210–235°. IR ν_{max}^{KBr} cm^{-1}: 3220, 1770, 1737, 1655. $[\alpha]_D^{20}$ −165.79° (*c* 0.535, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 0.96 (3H, *s*, H-17), 1.06 (3H, *s*, H-20), 1.53 (3H, *s*, H-19), 1.85 (1H, *d*, J = 4.4 Hz, H-5), 2.06 (1H, *m*, H-3), 2.46 (3H, *s*, H-21), 3.04 (1H, *t*, J = 8.4 Hz, H-15), 3.66 (1H, *s*, −OH), 3.65 (1H, *dd*, J_1 = 8.8, J_2 = 8.1 Hz, H-16), 3.84 (1H, *s*, H-14), 4.21 (1H, *dd*, J_1 = 8.4, J_2 = 8.1 Hz, H-16'), 4.89 (1H, *dd*, J_1 = 5.5, J_2 = 4.4 Hz, H-6), 5.9 (1H, *dd*, J_1 = 5.1, J_2 = 1.4 Hz, H-7); MS: *m/e* 361.2251 (M^+ $C_{21}H_{31}N_1O_4$ requires 361.2252), 331.2141 ($C_{20}H_{29}N_1O_3$ requires 331.2146), 316.1906 ($C_{19}H_{26}N_1O_3$ requires 316.1911), 288.1716 ($C_{18}H_{24}O_3$ requires 288.1724), 260.1763 ($C_{17}H_{24}O_2$ requires 260.1775), 74 (base peak).

3-Acetylicaceine. A soln of **2** (20 mg) in 0.5 ml Py and 0.5 ml Ac_2O was allowed to stand overnight at room temp. After purification on neutral Al_2O_3 (toluene–Me_2CO–EtOH—NH_4OH, 40:40:8:3, R_f 0.92), the derivative crystallized from MeOH–$CHCl_3$ (4:1) as white needles, mp

(decomp., sublimation) 190–215°, IR ν_{max}^{KBr} cm^{-1}: 1770, 1735, 1660, $[\alpha]_D^{20}$ −180.34° (*c* 0.295, $CHCl_3$). ^1H NMR ($CDCl_3$): δ 1.01 (3H, *s*, H-17), 1.11 (3H, *s*, H-20), 1.38 (3H, *s*, H-19), 1.9 (1H, *d*, *J* = 4 Hz, H-5), 2.19 (3H, *s*, CH_3COO—), 2.22 (6H, *s*, H-21/22), 2.42 (1H, *t*, *J* = 8.4 Hz, H-15), 3.83 (1H, *s*, H-14), 3.88 (1H, *dd*, J_1 = 8.5, J_2 = 8.1 Hz, H-16), 4.07 (1H, *dd*, J_1 = 8.1, J_2 = 8.1 Hz, H-16'), 4.84 (1H, *dd*, J_1 = 4.8, J_2 = 4.4 Hz, H-6), 4.95 (1H, *dd*, J_1 = 11.0, J_2 = 7.0 Hz, H-3), 5.89 (1H, *dd*, J_1 = 5.1, J_2 = 1.5 Hz, H-7), MS: *m/e* 417 (M^+), 331, 271, 87 (base peak).

Methylation of De-N-methylicaceine (**3**). A soln of **3** (20 mg) in MeOH (1.1 ml) was treated at room temp. by 180 μl (30 μl every 30 min) of MeI or CD_3I and then left for 12 h at room temp. Unreacted **3** was removed by chromatography on neutral Al_2O_3 (toluene–Me_2CO–EtOH–NH_4OH, 40:40:8:3 (yield: 10 mg).

Se *dehydrogenation*. **1**, **2**, or **3** (5 mg) mixed with Se (25 mg) was heated at 300° for 7 hr in a capillary tube. The residue was taken up in *n*-hexane and purified by TLC on Si gel. Further comparison with 1,7-dimethylphenanthrene was achieved by UV spectroscopy, TLC on Si gel GF_{254} (HPTLC plates for the nano-TLC Merck, *n*-hexane, R_f 0.2), by GLC on a SE-52 column (50 m × 0.5 mm, carrier gas 6 ml He/min, oven temp. 220°, R_t phenanthrene 5.3 min, R_t 1,7-dimethylphenanthrene 10 min) and by MS. Authentic 1,7-dimethylphenanthrene was obtained by Se dehydrogenation of rimuene [7].

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