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THREE NEW SIMPLE INDOLE ALKALOIDS FROM *LIMONIA ACIDISSIMA*

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Limonia acidissima L. (Rutaceae) is a small tree commonly known as the elephant-apple or wood-apple. Alternate botanical names for it are *Limonia crenulata* (1) and *Hesperthrusa crenulata* (1). It has an interesting use as a cosmetic by the women of Burma and immediately adjoining countries. To this day, Burmese women paint their faces yellow with the powder derived from the stems, commonly known in that country under the name of "tanaka."

Several chemical studies have been reported on *L. acidissima*. The species is particularly rich in coumarins, including umbelliferone (2), geranyl umbelliferone (2), marmesin (3), xanthotoxine (2), luvangetin (2,4), suberosin (3), epoxysuberosin (3), suberenol (3), dihydrosuberenol (1), crenulin (2), and crenulatin (5,6). The ubiquitous sterol β-sitosterol is also present (3,7), as well as the triterpene lupeol (7) and the limonoid limonin (2). The only alkaloid found was 4-methoxy-1-methyl-2-quinolone (3,8).

Our *L. acidissima* sample consisted of approximately 400 g of the dry woody, stems purchased in the main market in the town of Pagan in north central Burma. In our hands, this material was powdered and extracted with cold EtOH. The extracts were partitioned between H_2O and petroleum ether, and then between H_2O and $CHCl_3$. The petroleum ether and $CHCl_3$ extracts were combined, since tlc indicated they were nearly identical in composition. Additionally, in the present investigation, only the relatively polar compounds were investigated. Final separation of the compounds relied upon silica gel column and thin layer chromatography;acid treatment was not used at any stage during the separation-purification process. A wide range of natural products was thus obtained including the known quinolones 4-methoxy-2-quinolone (9), edulitine (≡ 4,8-dimethoxy-2-quinolone) (10), as well as 4-methoxy-1-methyl-2-quinolone (3,8) which had previously been reported to be present in the plant (3). Present also were the yellow anthraquinone physcion (11), the limonoids limonin (2,12) and methyl deacetylnominilate (13), the amides tembamide (14) and *N*-(*p*-hydroxy-β-phenethyl)-*p*-hydroxycinnamide (15), and the lignan (±)-syringaresinol (16). The remaining known compounds consisted of indole derivatives, namely *N,N*-dimethyltryptamine, 3-formylindole (17), and 2-methyltetrahydro-β-carboline (18).

Our first new natural product was the alkaloid $N_{(b)}$-acetyl-$N_{(b)}$-methyltryptamine (**1**), $C_{13}H_{16}N_2O$, ν max ($CHCl_3$) 1630 and 3485 cm^{-1}. It is rather surprising that this simple compound has not previously been reported as a natural product. The main values of its ^{1}H-nmr spectrum (200 MHz, $CDCl_3$) are given around expression **1**. Final proof of structure was forthcoming from acetylation of $N_{(b)}$-methyltryptamine, which furnished material identical with the natural product.

Our second and third new compounds were the terpenoid indoles (+)-tanakine (**2**), $C_{13}H_{17}NO_2$, and (+)-tanakamine (**4**) of the same molecular composition.

The uv spectrum of tanakine (**2**) (Experimental) is typical of simple indole derivatives and showed no bathochromic shift upon addition of base. The mass spectrum includes a molecular ion peak at *m/z* 219 (20%) and a base peak at *m/z* 130 due to benzylic cleavage with

loss of part of the side chain. Another prominent fragment is *m/z* 160 (21%) formed by loss of $(CH_3)_2COH$ from the molecular ion.

The ^{1}H-nmr spectrum of **2** shows two three-proton singlets at δ 1.33 and 1.36 for the two C-methyl groups. The protons at C-α and C-β appear as three doublets of doublets centered at δ 2.76, 3.08, and 3.75. H-2 is represented by a broad singlet at δ 7.12.

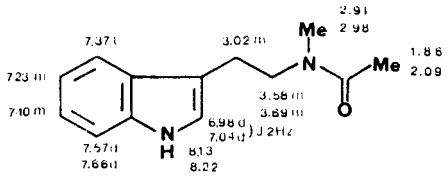
Acetylation of **2** afforded monoacetyl derivative **3**, $C_{15}H_{19}NO_3$, whose mass spectrum shows a molecular ion at *m/z* 261 (2%). The base peak is still *m/z* 130. Other prominent peaks are *m/z* 201 $(M-HOAc)^+$ (79%) and *m/z* 186 $(M-HOAc-CH_3)^+$ (86%). The ^{1}H-nmr spectrum of acetate **3** incorporates an acetoxyl singlet absorption at δ 1.86. The C-α protons appear as a multiplet at δ 3.09, while H-β is in evidence as a quartet centered further downfield at δ 5.14.

Tanakamine (**4**) exhibits a uv spectrum similar to that of its structural isomer **2** (Experimental). The mass spectrum again shows a molecular ion at *m/z* 219 (8%). However, the base peak is now *m/z* 143 due to loss of $(CH_3)_2COH$ and OH from the molecular ion.

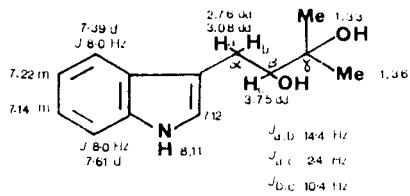
The ^{1}H-nmr spectrum of **4** includes two C-methyl singlets at δ 1.28 and 1.33. H-β appears as a triplet at δ 3.30, and the two C-α protons are observed as a doublet at δ 4.12. The H-2 absorption is buried under the signals due to H-5 and H-6.

The ^{13}C-nmr spectrum of **4** displayed thirteen peaks which were duly assigned as indicated in expression **4a**. A telling feature of this spectrum is the two resonances at δ 113.9 and 122.3 representing C-3 and C-2, respectively. These values are comparable with those reported for the corresponding carbons in 3-methylindole (19) but differ markedly from those for 2-methylindole (19), thus confirming the position of the side chain in species **4**.

Acetylation of **4** supplied monoacetyl derivative **5**, $C_{15}H_{19}NO_3$, with mass spectral molecular ion *m/z* 261. The base peak is *m/z* 143, as with tanakamine itself. The nmr spectrum showed an acetyl



1



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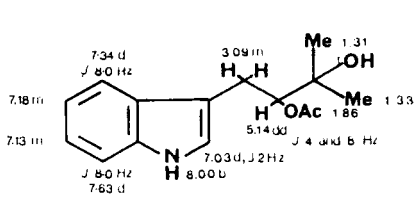

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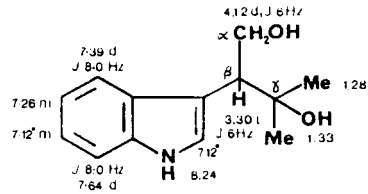

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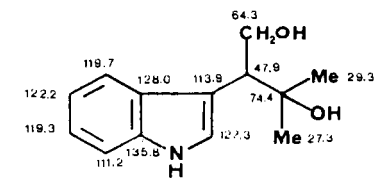
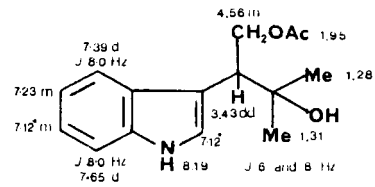


4a



5

singlet at δ 1.95. Significantly, the C-α protons now appeared downfield as a multiplet at δ 4.56.

It is worth noting, in conclusion, the wide variety of nitrogenous products present in *L. acidissima*, including specifically phenethylamine, quinoline, and indole derivatives.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—^{1}H-nmr spectra were obtained at 200 MHz in $CDCl_3$ solution. Column chromatography was on silica gel 60 (70-230 mesh). Final purification of the compounds was by tlc using silica gel 60 F-254 glass plates.

PLANT MATERIAL.—*L. acidissima* was purchased in the market of Pagan, Burma, and identified by Dr. Mong Mong Gale, Director, Pharmaceutical Research Department, Central Research Organization, Rangoon, Burma.

EXTRACTION AND CHROMATOGRAPHY.—The milled, dry stems (400 g) were extracted with cold EtOH. The solvent was evaporated, and the residue (24 g) was suspended in H_2O and extracted first with petroleum ether and then with $CHCl_3$. The residues from both extracts were combined, following evaporation of the solvents. The combined residue (5.4 g) was chromatographed on a column (500 g) packed in CH_2Cl_2. Elution was with CH_2Cl_2 and then with CH_2Cl_2/MeOH mixtures of increasing polarity. Elution was terminated when a 50:50 mixture of CH_2Cl_2-MeOH had been passed through the column. Some 86 fractions (250 ml) were collected, which were consolidated into 17 fractions on the basis of tlc patterns.

Fractions 1-8, which contained the relatively non-polar components, were not investigated. The remaining distribution was as follows: Fraction 9, physcion (1.3 mg); fraction 10, 4-methoxy-1-methyl-2-quinolone (16 mg), limonin (68 mg), 3-formylindole (1.7 mg); fraction 11, 4-methoxy-1-methyl-2-quinolone (4.1 mg), tembamide (1 mg); fraction 12, (±)-syringaresinol (7 mg); fraction 13, (±)-syringaresinol (8 mg), edulitine (10 mg), methyl deacetylnomilinate (7 mg), $N_{(b)}$-acetyl-$N_{(b)}$- methyltryptamine (**1**) (11 mg); (+)-tanakine (**2**) (11 mg), (+) tanakamine (**4**) (12 mg); fraction 14, 4-methoxy-2-quinolone (4 mg); fraction 15, *N*-(*p*-hydroxy-β-phenethyl)-*p*-hydroxycinnamide (2 mg); fraction 16, 2-methyltetrahydro-β-carboline (14 mg); fraction 17, *N,N*-dimethyltryptamine (18 mg). All known compounds were identified through their nmr, mass, ir, and uv spectra.

$N_{(b)}$-ACETYL-$N_{(b)}$-METHYLTRYPTAMINE (**1**).—Amorphous; cims *m/z* 216 (M^+, 9), 143 (100), 130 (97), 115 (10), 103 (15), 77 (21), 69 (10), 57 (18), 44 (93); uv λ max (MeOH) 221, 273 sh, 281, 290 nm (log ε 4.41, 3.61, 3.64, 3.59); ir ν max ($CHCl_3$) 1630, 3485 cm^{-1}.

(+)-TANAKINE (**2**).—Amorphous; $[\alpha]^{25}D$ +38° (*c* 0.51, MeOH); cims *m/z* 219 (M^+, 20), 201 (2), 160 (21), 143 (12), 130 (100), 117 (23), 97 (19), 83 (22), 69 (33), 57 (48); hrms calcd. for $C_{13}H_{17}NO_2$ 219.1260, found 219.1263; uv λ max (MeOH) 222, 273 sh, 281, 290 nm (log ε 4.31, 3.70, 3.71, 3.66).

(+)-TANAKAMINE (**4**).—Amorphous; $[\alpha]^{25}D$ +7.6° (*c* 0.69, MeOH); cims *m/z* 219 (M^+, 8), 201 (3), 186 (10), 173 (21), 160 (42), 143 (100), 130 (89), 118 (77), 97 (20), 83 (26), 77 (22), 69 (32), 57 (45), 43 (72); hrms calcd. for $C_{13}H_{17}NO_2$ 219.1260, found 219.1251; uv λ max (MeOH) 221, 277 sh, 281, 290 nm (log ε 4.31, 3.37, 3.56, 3.49).

SYNTHESIS OF **1**.—Tryptamine HCl (2 g) was basified with NH_4OH and extracted into $CHCl_3$. The residue after evaporation of the organic solvent was treated with ethyl formate (3 ml) and K_2CO_3 (0.2 g). The mixture was refluxed for 10 h under N_2. Work-up furnished an oil which was reduced with $LiAlH_4$ (0.5 g) in THF. The *N*-methyltryptamine obtained was acetylated with Ac_2O (2 ml) in pyridine (5 ml). Work-up afforded an amorphous material (1 g) identical with **1**.

ACETYLATION OF **2** AND **4**.—The two compounds were acetylated at room temperature using Ac_2O in pyridine.

ACETATE **3**.—Amorphous; cims *m/z* 261 (M^+, 37), 201 (78), 186 (86), 160 (21), 143 (17), 130 (100), 117 (40), 103 (26), 77 (30), 59 (28), 43 (83).

ACETATE **5**.—Amorphous, cims *m/z* 261 (M^+, 1), 201 (6), 186 (8), 160 (38), 143 (100), 130 (47), 115 (21), 59 (18), 43 (59).

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