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TWO NEW ACRIDONE ALKALOIDS FROM GENUS CITRUS PLANTS[1]

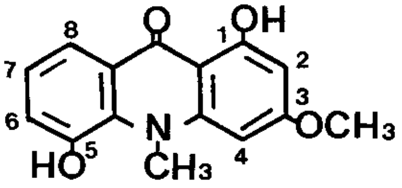
Motoharu Ju-ichi,[*,a] Mami Inoue,[a] Chihiro Ito,[b]
Miyuki Matsuoka,[b] Hiroshi Furukawa,[b] and Ichiro Kajiura[c]
Faculty of Pharmaceutical Sciences, Mukogawa Women's University,[a]
Nishinomiya, Hyogo 663, Japan; Faculty of Pharmacy, Meijo University,[b]
Tempaku, Nagoya 468, Japan; Okitsu Branch Fruit Tree Research
Station,[c] Minist. Agric. Forest. & Fisheries, Okitsu, Shizuoka
424-02, Japan

Abstract ——— Two new acridone alkaloids named citrusamine (1) and 1,3-dihydroxy-10-methylacridone (2) were obtained from the root of the hybrid seedlings of Okitsu No.17 x Hyuganatsu and also from Kawano-Natsudaidai.

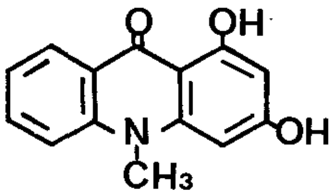
Extensive studies in our laboratories of the constituents of the genus Citrus have resulted in the isolation of a number of acridone alkaloids and coumarins.[1,2-8] In this paper we wish to report the isolation and structure elucidation of two new acridone alkaloids, citrusamine (1) and 1,3-dihydroxy-10-methylacridone (2), from the roots of several hybrid seedlings[9] resulting from a cross of Okitsu No.17[10] x Hyuganatsu (*Citrus tamurana* Tan.) and also from Kawano-Natsudaidai[9] (*C. natsudaidai* Hayata).

Citrusamine (1), yellow prisms, mp 243-246°C, $C_{15}H_{13}NO_4$. The uv [λ_{max}^{EtOH} nm (log ε) 264 (4.71), 285 (sh 4.35), 310 (4.16), 406 (3.87)] and ir spectra (3580, 1625, 1595 cm^{-1}) showed the characteristic absorptions of the 1-hydroxy-9-acridone system.[11] The presence of an N-methyl and a methoxy group was clear from the ^{13}C-nmr signals (δ 40.70 and 55.42) and ^{1}H-nmr signals (δ 4.11 and 3.95). The existence of two phenolic hydroxy groups was apparent from the ir (3580 cm^{-1}) and ^{1}H-nmr spectra (δ 14.67 and 9.34). In the aromatic proton region of the ^{1}H-nmr spectrum, *meta*-coupled two-proton signals at δ 6.22 and 6.49 (each 1H, d, J= 2.44 Hz) and ABC type three-proton signals at δ 7.91 (1H, dd, J= 7.81, 1.46 Hz), 7.31 (1H, dd, J= 7.81, 1.46 Hz) and 7.16 (1H, t, J= 7.81 Hz) were observed. The lower field signal at δ 7.91 was characteristic of H-8 in the 9-acridone skeleton, thus suggesting the location of these five aromatic protons at C_2, C_4, C_6, C_7 and C_8. In NOE experiments, irradiation at the frequency corresponding to the methoxy proton at δ 3.95 gave enhancements of 15.8% and 8.9% of the signals at δ 6.22 (H-2) and 6.49 (H-4), respectively. On the other hand, irradiation of the N-methyl proton signal at δ 4.11 showed 11.3% enhancement merely on the signal at δ 6.49 (H-4). The above results indicated the location of the methoxy and the hydroxy groups at C-3 and C-5, respectively. Consequently, the structure of citrusamine was established as formula 1.

1,3-Dihydroxy-10-methylacridone (2), mp $>$ 295°C, $C_{14}H_{11}NO_3$ was obtained as light yellow cubes. The presence of 1-hydroxy-9-acridone nucleus was suggested by the characteristic uv spectrum[11] [λ_{max}^{EtOH} nm (log ε) 249 (4.44), 262 (4.54), 271 (4.59), 328 (3.84), 394 (3.75)]. The existence of one N-methyl group was apparent from the ^{13}C-nmr signal at δ 32.59 and ^1H-nmr signal at δ 3.88. The ^1H-nmr showed signals *at δ 14.89 and 9.5 (each 1H, s) indicating* the presence of two hydroxy groups and in the aromatic proton region, two meta-coupled proton signals at δ 6.49 and 6.19 (each 1H, d, J= 1.95 Hz) and four proton signals at δ 8.38 (1H, dd, J= 8.79, 0.98 Hz), $\sim$7.8 (2H, m) and 7.33 (1H, ddd, J= 8.3, 1.95 Hz) were observed. These signal patterns suggested that C_5, C_6, C_7 and C_8 were unsubstituted. On the basis of these spectral results, the structure of this alkaloid could be proposed for 2 which had been known as a synthetic compound. To confirm this speculation, authentic 1,3-dihydroxy-10-methylacridone (2) was prepared by the procedure of Hlubucek et al.[12] and was identified with the natural compound by comparisons of nmr, ir spectra and TLC comparisons. This is the first isolation of this compound from natural sources.



(1)



(2)

EXPERIMENTAL

Extraction and Isolation : The dried root of plant material (hybrid seedlings resulting from a cross of Okitsu No.17 x Hyuganatsu, 500 g)[13] was extracted with acetone. Evaporation of the solvent yielded 54.4g of residue. The residue was chromatographed over silica gel and eluted successively with hexane, benzene, 50% CH_2Cl_2-benzene, CH_2Cl_2, AcOEt, acetone and MeOH. The acetone eluate was further separated with repeated PTLC using the solvent systems of iso-propyl ether, acetone-$CHCl_3$ (1:19) and acetone-hexane (1:1) to afford citrusamine (1) (19.8 mg) and 1,3-dihydroxy-10-methylacridone (2) (23.3 mg).

Citrusamine (1): yellow prisms, mp 243-246°C (from acetone); ms m/z: 271 (M^+, base peak), 256, 242, 228, 213; uv λ_{max}^{EtOH} nm (log ε): 264 (4.71), 285 (sh, 4.35), 310 (4.16), 406 (3.87); ir (cm^{-1}, $CHCl_3$): 3580, 1625, 1595; ^{1}H-nmr (δ, acetone-d_6): 14.67 (1H, s), 9.34 (1H, s), 7.91 (1H, dd, J= 7.81, 1.46 Hz), 7.31 (1H, dd, J= 7.81, 1.46 Hz), 7.16 (1H, t, J= 7.81 Hz), 6.49 (1H, d, J= 2.44 Hz), 6.22 (1H, d, J= 2.44 Hz), 4.11, 3.95 (each 3H, s); ^{13}C-nmr (δ, $CDCl_3$+ DMSO-d_6): 180.48 (s), 165.88 (s), 164.83 (s), 147.34 (s), 147.28 (s), 133.85 (s), 123.92 (s), 123.60 (s), 120.13 (d), 116.36 (d), 105.03 (s), 94.23 (d), 89.79 (d), 55.42 (q), 40.70 (q).

1,3-Dihydroxy-10-methylacridone (2): light yellow cubes, mp>295°C (from CH_2Cl_2). ms m/z: 241 (M^+, base peak), 226, 213, 212, 198, 184; uv λ_{max}^{EtOH} nm (log ε): 249 (4.44), 262 (4.54), 271 (4.59), 328 (3.84), 394 (3.75); ir (cm^{-1}, KBr): 3400, 1630, 1600; ^{1}H-nmr (δ, acetone-d_6): 14.89 (1H, s), 9.5 (1H, s), 8.38 (1H, dd, J= 8.79, 0.98 Hz), ~7.8 (2H, m), 7.33 (1H, ddd, J= 8.3, 1.95 Hz), 6.49 (1H, d, J= 1.95 Hz), 6.19 (1H, d, J= 1.95 Hz), 3.88 (3H, s); ^{13}C-nmr (δ, acetone-d_6)[14]: 165.71 (s), 134.90 (d), 126.99 (d), 122.05 (d), 116.21 (d), 96.82 (d), 91.77 (d), 32.59 (q).

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9) The plant was cultivated and collected in Fruit Tree Research Station, Okitsu Branch, Shizuoka.

10) Okitsu No.17 is a hybrid unnamed selection resulting from a cross of Hyuganatsu (C. tamurana Tan.) x Sweet orange (C. sinensis L. Osbeck cv. Fukuhara).

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13) Isolation procedures from the root of Kawano-Natsudaidai (C. natsudaidai Hayata) will be reported elsewhere.

14) Because of its low solubility in acetone, other quaternary carbon signals could not be observed.

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