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NEW ACRIDONE ALKALOID AND COUMARIN FROM CITRUS PLANTS[1]

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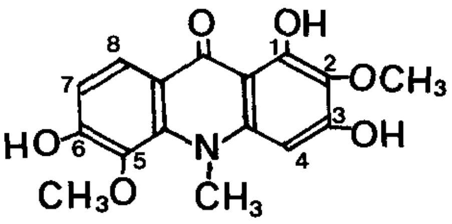
Abstract ——— A new acridone alkaloid citramine (1) and a new coumarin osthenon (2) were isolated from the root of the hybrid seedlings of Ogonkan x Hyuganatsu and their structures were elucidated on the basis of spectroscopic data.

Our extensive investigations of the constituents of the genus Citrus have resulted in the isolation of many kind of acridone alkaloids and coumarins.[1,2] We now wish to report the isolation and structure elucidation of a new acridone alkaloid, citramine (1) and a new coumarin, osthenon (2) from the roots of several hybrid seedlings[3] resulting from a cross of Ogonkan[4] (Citrus spp.) x Hyuganatsu (C. tamurana Tan.) and also from other Citrus plants.

Citramine (1), light yellow prisms, mp 277-279°C, had the molecular formula $C_{16}H_{15}NO_6$. The uv [λ_{max}^{EtOH} nm (log ε): 222 (4.25), 274 (4.76), 335 (4.12), 384 (3.84)] and ir spectra (3400, 1620, 1605, 1580 cm^{-1}) showed the characteristic absorptions of the 1-hydroxy-9-acridone system.[5] The existence of an N-methyl and two methoxy groups was clear from the ^{13}C-nmr signals (δ 39.51, 59.80, 60.68) and ^{1}H-nmr signals (δ 4.04, 3.87, 3.78). The presence of three phenolic hydroxy groups was apparent from the ir (3400 cm^{-1}) and ^{1}H-nmr spectra (δ 15.03, 9.04, 8.91). In the aromatic proton region of the ^{1}H-nmr spectrum, *ortho*-coupled doublets at δ 8.05 and 6.95 (each 1H, J= 8.79 Hz) and one proton singlet at δ 6.49 were observed. The lower field signal at δ 8.05 was characteristic of H-8 and higher field signal at δ 6.49 was assignable to H-4 in the 9-acridone skeleton,[6,7] thus suggesting the location of these three aromatic protons at C_4, C_7, and C_8. In NOE experiments, irradiation at the frequency corresponding to the N-methyl proton at δ 4.04 gave enhancements of 19.5 % of the signal at δ 6.49 (H-4). On the other hand, irradiation of the methoxy proton signals at δ 3.87 and 3.78 showed no enhancements at any aromatic proton signals. The above results indicated the location of the two methoxy groups at C_2 and C_5, respectively. Consequently, the structure of citramine was established as formula 1 and this alkaloid was also isolated from *C. natsudaidai* Hayata.[8] Many types of acridone alkaloids have been known so far,[9] but the 1,2,3,5,6-pentaoxygenated type compound is rare and citramine is the fourth example from natural sources.[10-12]

Osthenon (2) was obtained as slightly yellow prisms, mp 141-142°C. The molecular formula $C_{14}H_{12}O_4$ was determined by mass spectrometry and elemental analysis. The uv spectrum (λ_{max}^{EtOH} 216 and 306 nm) showed the characteristic absorption of 7-oxygenated coumarins.[13] The ^{1}H-nmr spectrum showed the presence of one methoxy (δ 4.00, 3H, s) and one acetyl (δ 2.43, 3H, s) groups. In the aromatic proton region, two pairs of signals at δ 7.65, 6.30 (each 1H, d, J= 9.52 Hz) and 7.46, 6.91 (each 1H, d, J= 8.79 Hz) were observed and these signals were diagnostic to C_4-H, C_3-H, C_5-H and C_6-H of the coumarin nucleus.[14] Two olefinic protons at δ 7.98 and 7.34 (each 1H, d, J= 16.6 Hz) were assumed to *trans*-oriented α,β-unsaturated carbonyl moiety. These spectral data suggested the presence of *trans*-but-3-on-1-enyl side chain at C_8 and this speculation was confirmed by ^{13}C-nmr spectrum [δ 27.68 (q), 131.22 (d), 132.56



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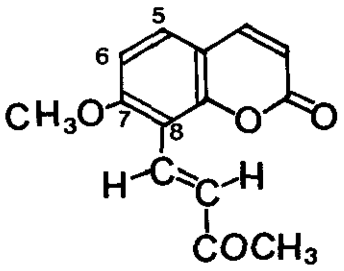

(2)

(d) and 199.40 (s)]. From the above spectral data, the structure of osthenon was confirmed as formula 2. Osthenon was also isolated from *C. natsudaidai*, *C. tachibana*, *C. rugulapsa*, *C. tamurana* x *C. kinokuni*, and *C. hassaku* x *C. grandis*.[1]

EXPERIMENTAL

Extraction and Isolation : The dried roots (800 g) of hybrid seedlings resulting from a cross of Ogonkan x Hyuganatsu were extracted with acetone. Evaporation of the solvent yielded 120.8g of residue. The residue was chromatographed over silica gel and eluted with hexane, benzene, 50% benzene-AcOEt, AcOEt, CH_2Cl_2, acetone and MeOH. Each eluate was further separated with repeated PTLC using the solvent systems of acetone-$CHCl_3$ (1:19), iso-propyl ether and acetone-hexane (1:1) to afford citramine (1) (6 mg) and osthenon (2) (86.1 mg) as well as known compounds. Detailed procedure of separation and structures of known compounds will be reported elsewhere.

Citramine (1): light yellow prisms, mp 277-279°C, high ms: m/z 317.0895 (M^+, found), 317.0899 (calcd. for $C_{16}H_{15}NO_6$); ir ν_{max}^{KBr}: 3400, 1620, 1605, 1580 cm^{-1}; uv λ_{max}^{EtOH} nm (log ε): 222 (4.25), 274 (4.76), 335 (4.12), 384 (3.84); ^{1}H-nmr (acetone-d_6, δ): 15.03 (1H, s), 9.04 (1H, s), 8.91 (1H, s), 8.05 (1H, d, J= 8.79 Hz), 6.95 (1H, d, J= 8.79 Hz), 6.49 (1H, s), 4.04, 3.87, 3.78 (each 3H, s); ^{13}C-nmr (DMSO-d_6, δ): 39.51 (q), 59.80 (q), 60.68 (q), 91.86 (d), 103.69 (s), 112.68 (d), 115.28 (s), 121.91 (d), 129.03 (s), 135.31 (s), 138.58 (s), 142.55 (s), 155.28 (s), 156.10 (s), 157.44 (s), 179.37 (s); ei-ms m/z: 317 (M^+), 302 (base peak), 287, 274, 272, 263, 205, 189.

Osthenon (2): slightly yellow prisms, mp 141-142°C, high ms: m/z 244.0763 (M^+, found), 244.0753 (calcd. for $C_{14}H_{12}O_4$); ir $\nu_{max}^{CHCl_3}$: 1730, 1595 cm^{-1}; uv λ_{max}^{EtOH} nm (log ε): 216 (sh, 4.35), 306 (4.46); ^{1}H-nmr ($CDCl_3$, δ): 7.98 (1H, d, J= 16.6 Hz), 7.65 (1H, d, J= 9.52 Hz), 7.46 (1H, d, J= 8.79 Hz), 7.34 (1H, d, J= 16.6 Hz), 6.91 (1H, d, J= 8.79 Hz), 6.30 (1H, d, J= 9.52 Hz), 4.00, 2.43 (each 3H, s); ^{13}C-nmr ($CDCl_3$, δ): 27.68 (q), 56.35 (q), 107.72 (d), 111.77 (s), 112.97 (s), 113.56 (d), 130.02 (d), 131.22 (d), 132.56 (d), 143.46 (d), 153.88 (s), 159.92 (s), 161.71 (s), 199.40 (s); ei-ms m/z: 244 (M^+), 229, 213 (base peak), 201, 186, 173, 158.

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