NEW ACRIDONE ALKALOIDS FROM <u>CITRUS YUKO</u>[1]

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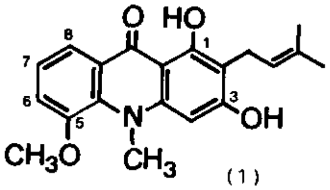
Abstract —— From the root barks of *Citrus yuko* (Rutaceae), two new acridone alkaloids, named yukomine and citracridone-III, were isolated and their structures were elucidated as 1 and 2 on the basis of spectroscopic data.

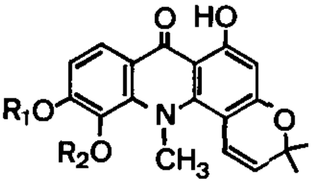
In the course of our studies on the constituents of *Citrus* plants, we have reported the isolation of many coumarins and acridone alkaloids.[2] In this paper, we wish to report the isolation and structure elucidation of two new acridone alkaloids, yukomine and citracridone-III, from the root barks of *Citrus yuko* Hort. ex. Tanaka.

Yukomine (1) was isolated as yellow needles, mp 214-217 °C and the molecular formula was determined as $C_{20}H_{21}NO_4$ by high resolution ms. The uv spectrum showed maximum absorptions at 233, 264, 285 and 335 nm, characteristic of the 9-acridone nucleus.[3] The ir bands at 3400 and 1630 cm^{-1} together with signals at δ 14.87 and 9.45 (each 1H) in the ^1H nmr spectrum indicated the presence of hydroxyl groups in 1 with at least one of them being hydrogen bonded. The ABX type signals at δ 7.97 (1H, dd, J= 7.81, 1.46 Hz), 7.38 (1H, dd, J= 7.81, 1.46 Hz), and 7.25 (1H, t, J= 7.81 Hz) were observed in the ^1H nmr spectrum, the lowest signal was assignable to H-8 which was effected by the deshielding of the 9-carbonyl moiety and, consequently, these three protons could be assigned to H-8, H-6, and H-7. A lone aromatic proton signal appeared at δ 6.49 as singlet which was assumed to be due to H-4 from its chemical shift.[4] The presence of prenyl group was inferred by the signals at δ 5.32 (1H, m), 3.38 (2H, d, J= 7.33 Hz), 1.80 (3H, s) and 1.65 (3H, d, J= 1.46 Hz) in the ^1H nmr spectrum together with the mass fragment ions at m/z 284 $[M-CH=C(Me)_2]^+$ and 270 $[M-CH_2-CH=C(Me)_2]^+$. The singlets at δ 4.01 and 3.93 in the ^1H nmr and the quartets at δ 57.06 and 41.97 in the ^{13}C nmr spectrum indicated the presence of each one O- and N-methyl groups. The locations of the O-methyl and prenyl groups were determined by nOe experiment. Irradiation of the signal at δ 4.01 afforded 13 % increments at the signal of δ 7.38, while irradiation of the signal at δ 3.93 showed 14% enhancements at the signal of δ 6.49. Thus O-methyl and prenyl groups were determined to locate at C-5 and C-2, respectively. From the above results, yukomine should be represented by the formula 1.

Citracridone-III (2) was isolated as yellow cubes, mp 135-140 °C, the molecular formula $C_{19}H_{17}O_5$ was established by high resolution ms. The uv spectrum showed the characteristic absorptions of 9-acridone alkaloids.[3] The ^1H nmr spectrum indicated the presence of hydrogen bonded hydroxyl group at δ 14.60, and three aromatic protons at δ 7.76, 6.99 (each 1H, d, J= 8.79 Hz) and δ 6.09 (1H, s). Bacause of characteristic low shift, the signal at δ 7.76 could be assigned to H-8, while the signal at δ 6.09 was assigned to H-2 from its chemical shift.[4] The remaining signals at δ 6.76, 5.68 (each 1H, d, J= 9.77 Hz), and 1.51 (6H, s) indicated the presence of 1,1-dimethylpyran ring. The signals at δ 3.85 (3H, s) in the ^1H nmr spectrum and at δ 48.87 (q) in the ^{13}C nmr spectrum showed the presence of an N-methyl group. From these results, this compound was assumed to be 1,3,5,6-tetraoxygenated acridone alkaloid. Further informations of structure were obtained by nOe differential spectrum. Irradiation of the N-methyl signal at δ 3.85 showed 4.5% increments on the signal at δ 6.76 (1H, d). Thus, the benzylic proton of 1,1-dimethylpyran ring was determined to locate at spacially near to N-methyl group. The comparisons of ^1H and ^{13}C nmr data of 2 with those of citracridone-I (3) and -II (4)[5] showed close resemblance to each others indicating the same substitution patterns of acridone skeleton. The relative locations of two phenolic hydroxyl groups and 1,1-dimethylpyran ring were confirmed by transformation of citracridone-III to citracridone-II (4). Treatment of citracridone-III with diazomethane as usual manner afforded O,O'-dimethyl compound which was identical with authentic citracridone-II (4). The above evidence established the structure 2 for citracridone-III.



(1)



(2) $R_1=R_2=H$
(3) $R_1=H$, $R_2=CH_3$
(4) $R_1=R_2=CH_3$

EXPERIMENTAL

Extraction and Separation : The root barks (3.2 kg) of Citrus yuko collected in Katsura, Tokushima, were refluxed with acetone (8 l)(50 h x 2), then with MeOH (8 l)(50 h x 2). The solvents were evaporated under reduced pressure and the residues were combined. The quarter of extracts (164.3 g) was fractionated by silica gel column chromatography using solvent systems of toluene, CH_2Cl_2, acetone and MeOH. The CH_2Cl_2 eluate was further submitted to ptlc (silica gel) using acetone : hexane (2:8) to afford yukomine (1) (25.7 mg), citracridone-III (2) (548.6 mg) and many known coumarins and acridone alkaloids.[6]

Yukomine (1)[7]: Yellow needles (from CH_2Cl_2), mp 214-217 °C; high resolution ms m/z 339.1476 (M^+, calcd for $C_{20}H_{21}NO_4$ 339.1471); ei-ms m/z 339 (M^+), 324, 309, 297, 296, 285, 284 (base peak), 271, 270, 256, 155; ir ($CHCl_3$) 3400, 1630, 1600, 1560 cm^{-1}; uv λ_{max} (log ε, EtOH) 233 (3.90), 264 (4.21), 276 (sh, 4.16), 285 (4.26), 335 (3.58) nm; ^1H nmr (acetone-d_6) δ 14.87 (1H, s), 9.45 (1H, br), 7.97 (1H, dd, J= 7.81, 1.46 Hz), 7.38 (1H, dd, J= 7.81, 1.46 Hz), 7.25 (1H, t, J= 7.81 Hz), 6.49 (1H, s), 5.32 (1H, m), 4.01, 3.93 (each 3H, s), 3.38 (2H, d, J= 7.33 Hz), 1.80 (3H, s), 1.65 (3H, d, J= 1.46 Hz); ^{13}C nmr (acetone-d_6) δ 181.17 (s), 163.33 (s), 162.88 (s), 150.72 (s), 146.81 (s), 136.07 (s), 131.30 (s), 124.42 (s), 123.82 (d), 122.74 (d), 118.70 (d), 117.07 (d), 109.05 (s), 105.67 (s), 91.90 (d), 57.06 (q), 41.97 (q), 25.88 (q), 21.98 (t), 17.92 (q).

Citracridone-III (2): Yellow cubes (from CH_2Cl_2), mp 135-140 °C; high resolution ms m/z 339.1111 (M^+, calcd for $C_{19}H_{17}NO_5$ 339.1107); ei-ms m/z 339 (M^+), 325, 324 (base peak), 322, 310, 309, 294, 255, 226; ir (Nujol) 3350, 1620, 1580 cm^{-1}; uv λ_{max} (log ε, EtOH) 273 (4.56), 295 (sh, 4.39), 320 (sh, 4.23), 350 (3.85), 386 (3.86) nm; ^1H nmr (acetone-d_6) δ 14.60 (1H, s), 8.50

(2H, br), 7.76 (1H, d, J= 8.79 Hz), 6.99 (1H, d, J= 8.79 Hz), 6.76 (1H, s, J= 9.77 Hz), 6.09 (1H, s), 5.68 (1H, d, J= 9.77 Hz), 3.85 (3H, s), 1.51 (6H, s); ^{13}C nmr (acetone-d_6) δ 182.54 (s), 165.67 (s), 161.77 (s), 151.64 (s), 148.98 (s), 139.04 (s), 135.62 (s), 124.85 (d), 124.81 (d), 121.80 (d), 118.60 (s), 112.91 (d), 107.16 (s), 103.28 (s), 98.13 (d), 77.28 (s), 48.87 (q), 27.32 (qx2).

O-Methylation of citracridone-III : Ethereal diazomethane solution was added to the methanolic solution (5 ml) of citracridone-III (14.1 mg) as usual manner. After standing overnight, solvent was distilled off and the residue was purified by ptlc (silica gel, iso-propyl ether) to afford the methyl ether (4) (13 mg). Yellow needles, mp 161-163 °C. ^{1}H nmr ($CDCl_3$) δ 14.35 (1H, s), 6.26 (1H, s), 3.74 , 3.90, 4.01 (each 3H, s), 7.01 (1H, d, J= 9 Hz), 8.13 (1H, d, J= 9 Hz), 6.62 (1H, d, J= 10 Hz), 5.58 (1H, d, J= 10 Hz), 1.51 (6H, s). The methyl ether was identified with citracridone-II (4) by comparison of ^{1}H nmr, ir spectra and tlc behavior.

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6 Isolation and characterization of known compounds from this plant will be reported elsewhere.

7 This alkaloid was also obtained from the roots of Citrus Wilsonii Tanaka. Unpublished our data.

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