NEW ACRIDONE ALKALOIDS FROM CITRUS PLANTS[1]

Yuko Takemura, Hiromi Uchida, Motoharu Ju-ichi,*,[a]
Mitsuo Omura,[b] Chihiro Ito, Kiyomi Nakagawa,
Tomohisa Ono, and Hiroshi Furukawa[c]

Faculty of Pharmaceutical Sciences, Mukogawa Women's University,[a] Nishinomiya, Hyogo 663, Japan, Fruit Tree Research Station,[b] Ministry of Agriculture, Forestry and Fisheries, Okitsu, Shimizu, Shizuoka 424-02, Japan and Faculty of Pharmacy, Meijo University,[c] Tempaku, Nagoya 468, Japan

Abstracts ——The spectroscopic structure elucidations of five new acridone alkaloids named yukocitrine (1), yukodine (3), yukodinine (4), grandisine-III (6) and pummeline (7) from some Citrus plants were described.

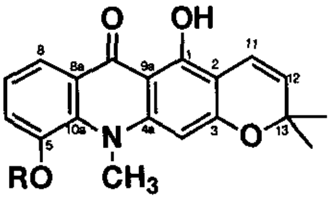
Our studies of the constituents of several species of genus Citrus plants (Rutaceae) have resulted in the isolation of many kinds of acridone alkaloids and coumarins.[2] We wish to describe the isolation and structure elucidation of five new acridone alkaloids, yukocitrine (1), yukodine (3), yukodinine (4), grandisine-III (6) and pummeline (7) from the roots of some Citrus plants.

Yukocitrine (1) was obtained as a yellow oil. The molecular formula $C_{19}H_{17}NO_4$ was determined by high resolution ms. The uv absorptions [λ_{max} 225(sh), 266(sh), 295, 306, 340(sh)] and ir bands (3300, 1630, 1595, 1560 cm^{-1}) suggested the 1-hydroxy-9-acridone skeleton.[3] The ^{1}H nmr spectrum showed four aromatic protons at δ 8.01 (1H, dd, J=1.84, 7.95 Hz), 7.08 (1H, t, J=7.95 Hz), 7.05 (1H, dd, J=1.84, 7.95 Hz) and 6.29 (1H, s). The lowest signal at δ 8.01 was assignable to H-8 deshielded by C-9 carbonyl group and, consequently, ABX type signals could be assigned as H-8, H-7 and H-6. The olefinic proton signals at δ 6.79 and 5.58 (each 1H, d, J=9.78 Hz) and the methyl signal at δ 1.49 (6H, s) represented the presence of 1,1-dimethylpyran ring. The signal at δ 4.00 in the ^{1}H nmr and δ 41.00 in the ^{13}C nmr spectra indicated the presence of an N-methyl group. In nOe experiment, irradiation of N-methyl signal at δ 4.00 showed 6% increments on the signal at δ 6.29. The result suggested the location of this proton at C-4, and this alkaloid revealed to have linear type structure. From the above results, the remaining hydroxyl group was considered to locate at C-5. Further confirmation of the structure was performed by treatment of 1 with diazomethane as usual manner to give junosidine (2)[4] and which was identified with authentic samples. On the basis of these results, the structure of yukocitrine was determined as 1.

Yukodine (3) was obtained as yellow needles, mp 246-250°C. The molecular formula $C_{15}H_{13}NO_4$ was confirmed by high resolution ms. The uv and ir spectra (see Experimental) indicated the characteristic absorptions of 1-hydroxy-9-acridone skeleton.[3] The ^{1}H nmr spectrum showed the presence of ABX type [δ 7.96, 7.40 (each 1H, dd, J=1.22, 7.93 Hz) and 7.26 (1H, t, J=7.93 Hz)] and <u>meta</u> coupled [δ 6.42 and 6.16 (each 1H, d, J=2.44 Hz)] aromatic proton signals. The signals at δ 4.02 and 3.96 in ^{1}H nmr and at δ 57.09 and 42.28 in ^{13}C nmr spectra indicated the presence of each one O-methyl and N-methyl group. The location of O-methyl group was determined by nOe experiments. Irradiation of the N-methyl signal (δ 3.96) showed 8.8% increments on the signal at δ 6.42 (H-4), meanwhile irradiation of the O-methyl signal (δ 4.02) afforded 9.4% increments on the signal at δ 7.40 (H-6). From the above data, the structure of yukodine was established as 3.

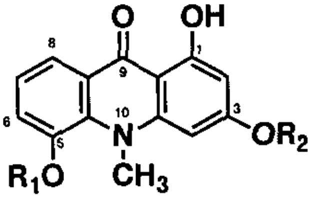
Yukodinine (4) was isolated as a pale yellow oil. The molecular formula $C_{16}H_{15}NO_4$ was established by high resolution ms. The presence of 1-hydroxy-9-acridone skeleton was demonstrated by the typical uv and ir spectra[3] (see Experimental). The ^{1}H nmr spectrum of 4 showed ABX type [δ 7.96 (1H, dd, J=8.07, 1.35 Hz), 7.43 (1H, dd, J=8.07, 1.35 Hz), 7.29 (1H, t, J=8.07 Hz)] and *meta* coupled [δ 6.50 (1H, d, J=2.01 Hz), 6.23 (1H, d, J=2.01 Hz)] aromatic protons, one N-methyl (δ 3.95) and two O-methyl (δ 4.03 and 4.01) signals similar to that of yukodine (3), except for the presence of an additional O-methyl signal and the absence of one hydroxyl signal. Treatment of citrusamine (5)[5] isolated from *Citrus* plant with diazomethane gave a O-methyl ether and it was identified with yukodinine (4). Thus the structure of yukodinine was deduced to 4.

Grandisine-III (6) was obtained as a yellow amorphous powder. The molecular formula $C_{15}H_{13}NO_5$ was determined by high resolution ms. The uv and ir spectra (see Experimental) showed characteristic absorptions of



1. R=H

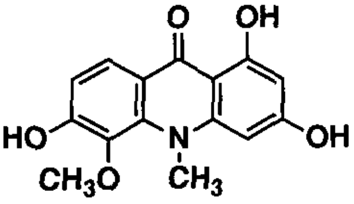
2. R=CH3



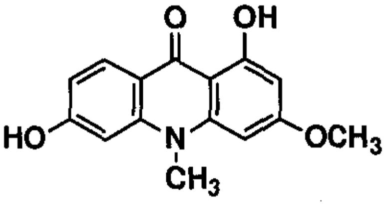
3. $R_1=CH_3$, $R_2=H$

4. $R_1=R_2=CH_3$

5. $R_1=H$, $R_2=CH_3$



6



7

1-hydroxy-9-acridone skeleton.[3] The ^1H nmr spectrum showed the presence of _ortho_ coupled [δ 8.04 (1H, d, J=8.79 Hz) and 6.95 (1H, d, J=8.79 Hz)] and _meta_ coupled [δ 6.41 (1H, d, J=1.95 Hz) and 6.14 (1H, d, J=1.95 Hz)] aromatic protons. The lowest signal at δ 8.04 was typical signal of H-8, so remaining signals were assigned to H-7, H-4 and H-2, respectively. The signals at δ 4.02 and 3.79 in the ^1H nmr spectrum and the signals at δ 40.63 and 62.16 in the ^{13}C nmr spectrum showed the presence of each one N-methyl and O-methyl groups. The location of O-methyl group was determined by nOe experiment. Irradiation of the N-methyl signal (δ 4.02) showed the 4.1% increments on the signal at δ 6.41 (H-4), on the other hands, irradiation of the O-methyl signal (δ 3.79) showed no increments on any aromatic proton signals. The results indicated the location of O-methyl group at C-5 and the structure of grandisine-III was assigned as **6**.

Pummeline (**7**) was obtained as yellow cubes, mp 235-240°C. The molecular formula $C_{15}H_{13}NO_4$ was established by high resolution ms. The uv and ir spectra (see Experimental) indicated the presence of 1-hydroxy-9-acridone skeleton.[3] The ^1H nmr spectrum showed ABX type signals at δ 8.23 (1H, d, J=8.55 Hz), 7.03 (1H, d, J=1.84 Hz) and 6.87 (1H, dd, J=8.55, 1.84 Hz). The lowest signal at δ 8.23 could be assigned to H-8, so remaining signals were deduced to H-5 and H-7. Two more aromatic proton signals observed at δ 6.50 (1H, d, J=2.44 Hz) and 6.21 (1H, d, J=2.44 Hz) were assignable to H-4 and H-2. The ^1H nmr signals at δ 3.94 and 3.82 and the ^{13}C nmr signals at δ 55.56 and 34.11 indicated the presence of each one O-methyl and N-methyl groups respectively. In the nOe experiments, irradiation of the signal at δ 3.94 showed 7.2% and 4.2% increments on the signals at δ 6.50 and 6.21. On the other hand, when the signal at δ 3.82 was irradiated, each 6.9% increments were observed on the signal at δ 7.03 and

6.50. These results indicated the location of O-methyl group at C-3. From the above mentioned results, the structure of pummeline was determined as 7. Many kinds of acridone alkaloids have been isolated so far, oxygen substituents on the A-ring are usually situated at C-5 or C-5 and C-6.[6] Pummeline is the first example which have oxygenated substituent only at C-6 and lacks at C-5 on the A ring.

EXPERIMENTAL

Isolation of Acridone Alkaloids The dried roots of each *Citrus* plants were extracted with acetone (1~2 l x 2) under reflux for 8 h. The acetone extract was evaporated under reduced pressure to give oily residue, which was subjected to silica gel column chromatography and eluted with benzene, CH_2Cl_2, acetone and MeOH, successively. Each fraction was subjected repeatedly to preparative tlc developed with appropriate combinations of acetone, $CHCl_3$, benzene, hexane, isopropyl ether, and MeOH to yield new acridone alkaloids as well as known components.[7] Yukocitrine (1)(5.0 mg) and yukodine (3)(9.9 mg) were obtained from the CH_2Cl_2 eluate of the extract of the roots (3.2 Kg) of *Citrus yuko* Hort. ex. Tanaka collected at Katsura, Tokushima. Yukodinine (4)(2.0 mg) was obtained from the benzene eluate of the extract of the roots of "Swingle citrumelo" (*Poncirus trifoliata* x *C. paradisi*)[8] (250 g) collected at Fruit Tree Research Station, Okitsu, Shizuoka. Grandisine-III (5)(3.3 mg) and pummeline (6)(31.9 mg) were isolated from the CH_2Cl_2 eluate of the extracts of the roots (500 g) of several hybrid seedlings resulting from a cross of Pummelo (*C. grandis* (L.) Osbeck) cv. May Pummelo x Marsh grapefruit (*C. paradisi* Macf.) cultivated at Fruit Tree Research Station, Okitsu, Shizuoka.

Yukocitrine (1) Yellow oil, hrms m/z 323.1156 (M^+, calcd for $C_{19}H_{17}NO_4$

323.1158); eims m/z 323 (M^+), 308 (base peak), 293, 264, 203, 154; ir ($CHCl_3$) 3300, 1630, 1595, 1560 cm^{-1}; uv λ_{max} (EtOH) 225 (sh), 266 (sh), 295, 306, 340 (sh) nm; ^1H nmr ($CDCl_3$) δ 12.09 (1H, s), 8.01 (1H, dd, J= 1.84, 7.95 Hz), 7.08 (1H, t, J= 7.95 Hz), 7.05 (1H, dd, J= 1.84, 7.95 Hz), 6.79 (1H, d, J= 9.78 Hz), 6.29 (1H, s), 5.69 (1H, s, OH), 5.58 (1H, d, J= 9.78 Hz), 4.00 (3H, s), 1.49 (6H, s); ^{13}C nmr ($CDCl_3$)[9] δ 180.61 (C-9), 133.60 (C-10a), 126.81 (C-12), 124.35 (C-8a), 122.00 (C-7), 120.18 (C-6), 118.94 (C-8), 115.97 (C-11), 105.38 (C-9a), 102.61 (C-2), 92.06 (C-4), 77.95 (C-13), 41.00 (NMe), 28.47 (13-Me x2).

O-Methylation of Yukocitrine (1) Yukocitrine (1)(2.5 mg) was dissolved in MeOH (5ml) and treated with excess ethereal diazomethane solution. After as usual work up, the product was purified with ptlc to give 1.0 mg of junosidine (2). The identity with authentic sample was confirmed by comparisons of ^1H nmr spectrum and co-tlc behaviors.

Yukodine (3) Yellow needles, mp 246-250°C, hrms m/z 271.0845 (M^+, calcd for $C_{15}H_{13}NO_4$ 271.0845); eims m/z 271 (base peak), 257, 246, 242, 241, 228, 214, 135, 121; ir ($CHCl_3$) 3580, 1630 cm^{-1}; uv λ_{max} (EtOH) 229 (sh), 260, 274 (sh), 281, 301, 330 (sh) nm; ^1H nmr (acetone-d_6) δ 14.56 (1H, s), 9.41 (1H, br s), 7.96 (1H, dd, J= 1.22, 7.93 Hz), 7.40 (1H, dd, J= 1.22, 7.93 Hz), 7.26 (1H, t, J= 7.93 Hz), 6.42 (1H, d, J= 2.44 Hz), 6.16 (1H, d, J= 2.44 Hz), 4.02, 3.96 (each 3H, s); ^{13}C nmr (acetone-d_6) δ 181.26 (C-9), 166.20 (C-3), 165.64 (C-1), 150.79 (C-5), 149.12 (C-4a), 136.23 (C-10a), 124.52 (C-8a), 123.03 (C-7), 118.61 (C-6), 117.31 (C-8), 105.68 (C-9a), 96.84 (C-2), 92.47 (C-4), 57.09 (OMe), 42.28 (NMe).

Yukodinine (4) Pale yellow oil, hrms m/z 285.1006 (M^+, calcd for $C_{16}H_{15}NO_4$ 285.1000); eims m/z 285 (M^+, base peak), 270, 256, 227, 199, 167, 149, 137; ir ($CHCl_3$) 3400 (br), 1640, 1600, 1560 cm^{-1}; uv λ_{max} (MeOH) 214 (sh.), 261, 272 (sh), 281, 302, 326 (sh), 399 nm;

^{1}H-nmr (acetone-d_6) δ 14.56 (1H, s, OH), 7.96 (1H, dd, J= 8.07, 1.35 Hz), 7.43 (1H, dd, J= 8.07, 1.35 Hz), 7.29 (1H, t, J= 8.07 Hz), 6.50 (1H, d, J= 2.01 Hz), 6.23 (1H, d, J= 2.01 Hz), 4.03, 4.01, 3.95 (each 3H, s.).

O-Methylation of Citrusamine Citrusamine (5)(2.0 mg)[5] was dissolved in MeOH (5 ml) and treated with excess ethereal diazomethane solution. After as usual work up, the product was purified with ptlc to give 1.5 mg of yukodinine (4). The identity with authentic sample was performed by comparisons of ^{1}H nmr, uv, mass spectra and co-tlc.

Grandisine-III (6) Yellow amorphous powder, hrms m/z 287.0785 (M^+, calcd for $C_{15}H_{13}NO_5$ 287.0794); eims m/z 287 (M^+, base peak), 273, 272, 244, 230, 121; ir (KBr) 3400, 1640, 1600 cm^{-1}; uv λ_{max} (MeOH) 210, 220 (sh), 268, 295 (sh), 329, 380 (sh) nm; ^{1}H nmr (acetone-d_6) δ 14.72 (1H, s), 9.2 (2H, br), 8.04 (1H, d, J= 8.79 Hz), 6.95 (1H, d, J= 8.79 Hz), 6.41 (1H, d, J= 1.96 Hz), 6.14 (1H, d, J= 1.96 Hz), 4.02, 3.79 (each 3H, s). ^{13}C nmr (acetone-d_6) δ 183.39 (C-9), 167.32 (C-3), 165.64 (C-1), 157.37 (C-6), 142.00 (C-4a), 135.82 (C-5), 131.06 (C-10a), 124.12 (C-8), 117.00 (C-8a), 113.41 (C-7), 103.31(C-9a), 97.18 (C-2), 93.05 (C-4), 62.16 (OMe), 40.63 (NMe).

Pummeline (7) Yellow cubes, mp 235-240°C, hrms m/z 271.0846 (M^+, calcd for $C_{15}H_{13}NO_4$ 271.0845); eims m/z 271 (M^+, base peak), 270, 243, 242, 241, 228, 213, 198, 185, 121; ir ($CHCl_3$) 3300, 1720, 1630, 1600 cm^{-1}; uv λ_{max}(EtOH) 225 (sh), 256, 270, 326 nm; ^{1}H nmr (acetone-d_6) δ 15.09 (1H, s), 10.00 (1H, br s), 8.23 (1H, d, J= 8.55 Hz), 7.03 (1H, d, J= 1.84 Hz), 6.87 (1H, dd, J= 8.55, 1.84 Hz), 6.50 (1H, d, J= 2.44 Hz), 6.21 (1H, d, J= 2.44 Hz), 3.94, 3.82 (each 3H, s); ^{13}C nmr (acetone-d_6) δ 178.89 (C-9), 165.24 (C-3), 164.66 (C-1), 163.38 (C-6), 144.53 (C-4a), 144.43 (C-10a), 127.91 (C-8), 113.15 (C-8a), 112.03 (C-7), 103.67 (C-9a), 99.75 (C-2), 94.11 (C-4), 89.90 (C-5), 55.56 (OMe), 34.11 (NMe).

ACKNOWLEDGEMENT

The authors thanks Misses K. Suwa and S. Horiyama (Mukogawa Women's University) for measurements ^{1}H, ^{13}C nmr and mass spectra.

REFERENCES AND NOTES

1 Part XIII of "Studies on the Constituents of Domestic Citrus Plants"; Part XII: M. Ju-ichi, Y. Takemura, C. Ito, and H. Furukawa, Heterocycles, 1991, **32**, 1781.

2 C. Ito, K. Fujiwara, M. Kajita, M. Ju-ichi, Y. Takemura, Y. Suzuki, K. Tanaka, M. Omura, and H. Furukawa, Chem. Pharm. Bull., 1991, **39**, 2509.

3 J. Reisch, K. Szendri, E. Minker, and I. Novak, Pharmazie, 1972, **27**, 208; R.D. Brown and F.N. Lahey, Aust.J.Sci.Res., 1950, **A3**, 593.

4 M. Ju-ichi, M. Inoue, K. Sakiyama, M. Yoneda, and H. Furukawa, Heterocycles, 1987, **26**, 2077.

5 M. Ju-ichi, M. Inoue, C. Ito, M. Matsuoka, H. Furukawa, and I. Kajiura, Heterocycles, 1987, **26**, 1873.

6 J. P. Michael, Nat. Prod. Rep., 1992, **9**, 30 and references cited therein.

7 Isolation and characterization of known compounds will be reported elsewhere.

8 "Swingle citrumelo" is a name of one of Citrus plants collected in Taiwan, and cultivated at Fruit Tree Station. The scientific name has not been identified.

9 Because of low solubility, other quaternary carbon signals could not be detected.

Received, 29th June, 1992