NEW ACRIDONE ALKALOIDS FROM *CITRUS* PLANT[1]

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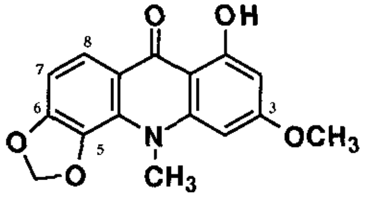
Abstracts Two new acridone alkaloids, named marshdine (1) and marshmine (2), were isolated from the root of Marsh grapefruit (*Citrus paradisi* Macf.) and their structures were elucidated by spectroscopic method.

On continuing our phytochemical studies on the constituents of *Citrus* plants,[2] we studied the roots of Marsh grapefruit (*Citrus paradisi* Macf.) and isolated two new acridone alkaloids named marshdine (1) and marshmine (2). In this paper we wish to report the structure elucidation of these alkaloids. The acetone extract of the roots was subjected to column, centrifugal and preparative thin layer chromatographies using silica gel.

Marshdine (1) was isolated as yellow cubes. The molecular formula $C_{16}H_{13}NO_5$ was established by HR ms [m/z 299.0797]. The uv [224 (sh), 265, 297, 335, 390 nm] and ir (1635, 1590 cm^{-1}) spectra showed characteristic absorptions of 1-hydroxy-9-acridone moiety.[3] The ^{1}H nmr spectrum indicated the presence of hydrogen-bonded hydroxyl group (δ 14.75), *ortho*-coupled protons [δ 8.12, 6.88 (each 1H, d, J= 8.5 Hz)], *meta*-coupled protons [δ 6.30, 6.24 (each 1H, d, J= 2.4 Hz)],



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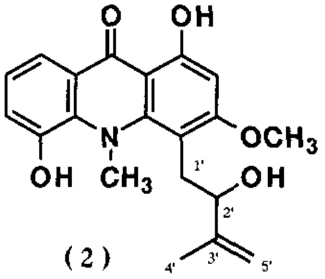

(2)

and methylene protons [δ 6.11 (2H, s)]. The lowest signal at δ 8.12 is deshielded by a carbonyl function and could be assigned to H-8. Therefore, *ortho*-coupled protons were concluded to be located at C-7 and C-8. Two singlets at δ 3.98 and 3.90 in ^{1}H nmr and at δ 37.03 and 55.60 in ^{13}C nmr indicated the presence of *N*-methyl and *O*-methyl groups. The location of methoxy group was determined by nOe experiments. Irradiation of the signal at δ 3.90 showed 10% and 5% increments of the signals at δ 6.30 (H-2) and 6.24 (H-4), respectively. When the signal at δ 3.98 was irradiated, 14% increments were observed on the signal at δ 6.24. Thus, the methoxy group was determined to locate at C-3. The two proton singlet at δ 6.11 in the ^{1}H nmr and triplet signal at δ 101.20 in the ^{13}C nmr spectra indicated the presence of methylenedioxy group and the location of this group was concluded to C-5 and C-6. From the above results, the structure of marshdine was assigned as 1. Although acridone alkaloids having methylenedioxy group have been known,[4] the location was only C-ring and marshdine is the first example containing this moiety on the A-ring.

Marshmine (2) was isolated as a yellow oil, the molecular formula $C_{20}H_{21}NO_5$ was obtained by HR ms [m/z 355.1420]. The ir (3550, 1625, 1590, 1570 cm^{-1}) and uv (225, 269, 336 nm) showed characteristic absorptions of 1-hydroxy-9-acridone. The ^{1}H nmr spectrum showed the presence of characteristic hydrogen-bonded hydroxyl group (δ 13.95), ABC type [δ 7.83 (1H, d, J= 7.3 Hz), 7.15 (1H, d, J= 7.3 Hz), 7.11 (1H, t, J= 7.3 Hz)] and a lone (δ 6.39) aromatic protons. These signal patterns suggested that C-2 (C-4), C-6, C-7 and C-8 were unsubstituted. The presence of 2-hydroxy-3-methylbut-3-enyl group was assumed by the ^{1}H nmr signals at δ 4.83, 4.73 (each 1H, s), 3.21 (1H, dd, J= 15.3, 4.9 Hz), 3.15 (1H, dd, J= 15.3, 8.6 Hz), 4.40 (1H, dd, J= 4.9, 8.6 Hz), 2.50 (1H, br s), 1.77 (3H, s) and the ^{13}C nmr signals at δ 17.72 (q), 32.38 (t), 77.63 (d), 110.70 (t), 158.88 (s) together with the fragment ion at m/z 284 $[M-C_4H_7O]^+$. In the nOe experiments, on irradiation of the signal at δ 3.91 (3H, s) induced 14% increments of the signal at δ 6.39. On the other hand, irradiation of the signal at δ 3.52 (3H, s) showed 4% increments of one of the methylene signal at δ 3.21. These results indicated the location of methoxy group was at C-3 and the alkyl group was located at C-4. From the above results, the structure of marshmine was assigned as 2 except for the stereochemistry of hydroxyl group.

EXPERIMENTAL

Extraction and Isolation : The dried roots (1.2 kg) of Marsh grapefruit cultivated and collected at the Fruit Tree Research Station was extracted with acetone for 40 h under reflux (3 x 2 l).The acetone extract (138 g) was chromatographed over silica gel with successive elution with hexane, benzene, CH_2Cl_2, acetone-CH_2Cl_2, acetone and MeOH. The benzene and CH_2Cl_2 eluates were subjected to repeated preparative thin layer chromatography [solvent: isopropyl ether, acetone:hexane (1:1), $CHCl_3$:MeOH (9:1), benzene:MeOH (9:1), benzene:AcOEt (7:3), hexane:acetone (7:3)] to give 1 (1.9 mg) and 2 (3.1 mg), respectively along with many kind of known coumarins and acridone alkaloids.[5]

Marshdine (1): Yellow cubes, mp 210-213°C; high ms m/z: 299.0797 (M^+, found), 299.0794 (calcd for $C_{16}H_{13}NO_5$); eims m/z: 299 (M^+, base peak), 298, 271, 270, 269, 256, 226, 136; ir ν_{max} *($CHCl_3$) cm^{-1}: 1635, 1590; uv λ_{max} (EtOH) nm: 224 (sh), 265, 297, 335, 390;* ^{1}H nmr ($CDCl_3$, δ): 14.75 (1H, s), 8.12 (1H, d, J= 8.5 Hz), 6.88 (1H, d, J= 8.5 Hz), 6.30 (1H, d, J= 2.4 Hz), 6.24 (1H, d, J= 2.4 Hz), 6.11 (2H, s), 3.98, 3.90 (each 3H, s),. ^{13}C nmr ($CDCl_3$, δ): 180.11 (C-9), 166.17 (C-3), 166.06 (C-1), 152.08 (C-6), 145.53 (C-4a), 133.38 (C-5), 130.00 (C-10a), 122.81 (C-8), 117.98 (C-8a), 104.96 (C-7), 104.47 (C-9a), 101.20 (-CH_2-), 94.25 (C-4), 89.84 (C-2), 55.60 (OCH_3), 37.03 (NCH_3).

Marshmine (2): Yellow oil, $[\alpha]_D$ -28° (c= 0.15, $CHCl_3$), high ms: m/z 355.1420 (M^+, found), 355.1419 (calcd for $C_{20}H_{21}NO_5$); ei ms m/z: 355 (M^+), 285, 284 (base peak), 269, 268, 252, 240; ir ν_{max} ($CHCl_3$, cm^{-1}): 3550, 1625, 1590, 1570; uv λ_{max} (EtOH, nm): 225, 269, 336; ^{1}H nmr ($CDCl_3$, δ): 13.95 (1H, s), 7.83 (1H, d, J= 7.3 Hz), 7.15 (1H, d, J= 7.3 Hz), 7.11 (1H, t, J= 7.3 Hz), 6.39 (1H, s), 4.83, 4.73 (each 1H, s), 4.40 (1H, dd, J= 4.9, 8.6 Hz), 3.91, 3.52 (each 3H, s), 3.21 (1H, dd, J= 15.3, 4.9 Hz), 3.15 (1H, dd, J= 15.3, 8.6 Hz), 2.50 (1H, br s, OH), 1.77 (3H, s); ^{13}C nmr ($CDCl_3$, δ): 186.17 (C-9), 164.97 (C-3), 163.64 (C-1), 158.88 (C-3'), 151.15 (C-4a), 147.48 (C-

5), 133.23 (C-10a), 126.07 (C-8a), 123.66 (C-7), 120.57 (C-6), 118.22 (C-9), 110.70 (C-5'), 108.13 (C-4), 106.75 (C-9a), 94.64 (C-2), 77.63 (C-2'), 56.03 (OCH_3), 48.06 (NCH_3), 32.38 (C-1'), 17.72 (C-4').

ACKNOWLEDGEMENT

The authors express their deep gratitude to Misses K. Suwa and S. Horiyama, Mukogawa Women's University, for measurements of ms and nmr spectra.

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

Received, 27th January, 1994