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Piscidenone and Piscidinol G, Two New Protolimonoids from *Walsura piscidia*

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Abstract – Two new protolimonoids, piscidenone (**1**) and piscidinol G (**2**) were isolated from the leaves of the plant *Walsura piscidia* together with piscidinol A and C. The structurers were assigned based on spectral evidences (IR, ^{1}HNMR, ^{13}CNMR and MS).
Keywords – *Walsura piscidia*; Meliaceae; protolimonoids; piscidenone, piscidinol G.

Introduction

Walsura piscidia Roxb. (Fam: Meliaceae) (syn: *Heynea trifoliata* A. Juss., *Trichilia coriacea* Wall. and *T. trifoliata* Wall.) is used in the traditional medical practices of India. The bark of the plant is reported to possess stimulant, expectorant, emmenagogue and emetic properties. The fruit pulp is used as fish poison (Anonymous, 1976). Earlier workers have reported the isolation of tirucallane and apotirucallane compounds, picidinol A-F from the leaves (Purushothaman *et al.*, 1985; Govindachari *et al.*, 1995), piscidofuran from the fruits (Govindachari *et al.*, 1995), ß-sitosterol, lup-20 (29)-ene-3 ß, 30-diol and 5-hydroxy-7, 3', 4', 5'-tetramethoxy flavone from the aerial parts (Balakrishna *et al.*, 1995) of this plant. In this communication, the isolation of two new protolimonoids, piscidenone and piscidinol G in addition to piscidinol A and C from the leaves of the plant is reported.

Experimental

M.p.s are uncorrected. IR spectrum was recorded on Perkin-Elmer instrument in KBr disc. ^{1}H NMR (300 MHz) and ^{13}C NMR (75 MHz) were obtained on Bruker while the mass spectrum was recorded on Shimadzu GC-MS instrument operating at 70 eV.

Plant materials – The leaves of *W. piscidia* were collected from Kodikkarai forests near Vedaranyam (Nagapattinam district, Tamil Nadu, India) and authenticated by Dr. S. Usman Ali, Dept. of Pharmacognosy, Central Research Institute for Siddha, Chennai, India where voucher specimen has been deposited.

Extraction and isolation – Shade dried and coarsely powdered plant material (*ca* 2.5 kg) was extracted with $CHCl_3$ in cold (72 h). The solvent was distilled off *in vacuo* and the extract (*ca* 30g) was column chromatographed over silica gel (100-200 mesh). The column was eluted with *n*-hexane, benzene and ethyl acetate in varying proportions. Elution of the column with *n*-hexane gave a waxy mass and with benzene yielded β-sitosterol (m.p. 136°).

Isolation of pisicidenone (1). Elution of the column with benzene ethyl acetate (9:1) gave compound **1** (102 mg) crystallized from methanol-ether, m.p: 270°; $(\alpha)_D$-80° ($CHCl_3$); Found: C, 72.41; H, 8.27% $C_{35}H_{48}O_7$ requires C, 72.42; H 8.26%; IR ν_{max} (cm^{-1}): 2950, 2850, 1730, 1700, 1660, 1620, 1450, 1380, 1270, 1240, 1110, 1080, 1030, 860, 820.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.10, 1.22, 1.25, (3H, each, s, 3xC-Me), 1.05, 1.40 (6H each, s, 4x C-Me), 0.90 (3H, t, J=7 Hz, 4'-Me) 1.07 (3H, d, J=7 Hz, 5'-Me), 3.92 (1H,s, H-24), 5.52 (1H, bs, $W_{1/2}$=3 Hz, H-21), 5.65 (1H, m, $W_{1/2}$= 20 MHz, H-11), 5.98(1H, bt, H-15), 7.15 (1H, d,J=10Hz, H-1), 5.77(1H, d, J=10 Hz, H-2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 156.8(C-1), 126.7(C-2), 207.7(C-3), 40.2(C-4), 42.0(C-5), 38.3(C-6), 206.1(C-7), 44.8(C-8), 49.8(C-9), 46.6(C-10), 69.5(C-11), 35.8(C-12), 51.8(C-13), 151.4(C-14), 124.2(C-15), 34.8(C-16), 55.1(C-17), 51.7(C-20), 102.7(C-21), 44.0 (C-22), 202.6(C-23), 85.9(C-24), 80.1(C-25), 175.7(C-1'), 41.7(C-2'), 26.3(C-3'), 11.3(C-4'), 16.1(C-5'), 30.3, 27.4, 25.9, 21.6, 20.9, 21.1, 19.6 (7x C-Me). EIMS *m/z*: 580 (M^+), 534, 522, 510, 495[M^+-CH_3-CH_2-$CH(CH_3)$-CO], 480, 478(M^+-102), 462, 452, 446, 433, 425(M^+-side chain), 407, 392, 390, 378, 363, 350, 335, 322, 157, 145, 135, 105, 101 [CH_3-CH_2-$CH(CH_3)$-COO^+], 93, 85[CH_3-CH_2-$CH(CH_3)$-

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CO^+], 83, 72, 57[CH_3-CH_2-CH^+ (CH_3)].

Isolation of piscidinol A. Elution of the column with benzene-ethyl acetate (4:1) afforded piscidinol A (60 mg), crystallized from methanol, mp 195°.

Isolation of piscidinol C. Elution of the column with benzene-ethyl acetate (1:1) yielded piscidinol C (70 mg), crystallized from methanol, mp 215°.

Isolation of piscidinol G (2). Later part of the elution with benzene ethyl acetate (1:1) gave **2**, purified by rechromatography over silica gel and crystallized from methanol (yield: 80 mg), mp 220; Found: C, 71.70; H, 19.15, $C_{30}H_{46}O_6$ requires C, 71.71; H, 19.13%; IR ν_{max} (cm^{-1}): 3450, 2900, 2850, 1660, 1650, 1450, 1380, 1240, 1145, 1080, 1040, 980, 820. ^{1}HNMR (300 MHz, $CDCl_3$); δ 1.00, 1.07, 1.10, 1.26, 1.28(3H each, s, 5x Me), 1.14(6H,s, 2x CHMe), 3.17(1H, bs, H-24), 3.97(1H,t, J=3.0Hz, H-7), 4.57(1H, M, H-23), 5.30(1H, d, J=3.0 Hz, H-21), 5.50(1H, bt, H-15), 7.15(1H, d, J=10.0 Hz, H-1), 5.80(1H, d, J=10.0 Hz, H-2). ^{13}C NMR(75 MHz, $CDCl_3$): δ 158.3(C-1), 125.5(C-2), 205.2 (C-3), 40.2(C-4), 44.6(C-5), 24.2(C-6), 71.6(C-7), 44.2(C-8), 44.6(C-9), 44.8(C-10), 16.3(C-11), 32.9(C-12), 46.6(C-13), 161.4(C-14), 120.1(C-15), 35.0(C-16), 52.1(C-17), 44.2 (C-20), 96.7(C-21), 30.0(C-22), 78.6(C-23), 74.8(C-24), 73.7 (C-25), 27.6, 27.1, 26.8, 26.7, 21.5, 20.1, 18.9(7x C-Me). EIMS *m/z*: 502(M^+), 487 (M^+-CH_3), 469(M^+-CH_3-H_2O), 429 (M^+-CH_3-acetone), 397[M^+-CH_3-CHOH-C(CH_3)(CH_3)-OH], 326(M^+-side chain-H), 308(M^+-side chain-H-H_2O).

Acetylation of piscidinol G. Compound **2** (100 mg) acetylated with acetic anhydride (2 ml) in pyridine (1 ml) at room temp. (16 h). After usual work up, the diacetate **3** was obtained as a gum. IR: ν_{max} (cm^{-1}): 3500, 3010, 2920, 2840, 1730, 1660, 1620, 1470, 1380, 1100, 940, 820; ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.00-1.30 (7x C-Me), 3.9 (1H, t, J=3.0 Hz, H-7), 4.5 (1H,,m, H-23), 4.85 (1H,d, J=20.0, Hz, H-24), 5.50 (1H, bt, H-15), 6.17(1H, d, J=3.0 Hz, H-24), 5.82, 7.20(1H, d, J=10.0Hz, H-2 and H-1), 2.05,2.10 (2xOAc).

Results and Discussion

Piscidenone G (**1**), $C_{35}H_{48}O_7$, (M^+ 580) showed IR absorptions at 1730 and 1700cm^{-1} corresponding to an ester and cyclohexanone moieties respectively. The absorption at 1660 cm^{-1} corresponded to α, β-unsaturated cyclohexenone while that at 1620 and 820 cm^{-1} indicated the pressure of trisubstituted double bond. There was no absorption in the hydroxyl region.

The ^{1}H and ^{13}C NMR spectra also confirmed the presence at the above groups. The ^{1}H NMR showed the presence of 7-tertiary methyls in the region δ 1.05-2.40. The AB pattern at δ 7.15 and 5.77 (J=10 Hz) has been assigned to Δ^{1-3} ketone (Chan *et al.*, 1967). The one-proton triplet at δ 5.98 has been assigned to the olefinic proton at H-15 in an apotirucallane system with the ketone group at C-7 (Ayafar *et al.*, 1981). Out of the three keto groups, one was placed at C-3 while the second at C-7 as stated earlier. The third keto group must be in the side-chain.

The ester present was identified as 2'-methylbutyrate on the basis of ^{13}C NMR signals (Pettit *et al.*, 1978). The presence of a strong peak in the mass spectrum at M^+-102 due to McLafferty rearrangement also suggested the presence of this ester moiety. Peaks at *m/z* 85 and 57 are due to α-cleavage on either side of the ester carbonyl function. The ester function was placed at C-11. H-11 appeared at δ 5.65 as a broad multiplet ($W_{1/2}$=20 Hz) showing that two diaxial interactions are involved. Hence H-11 must be axial.

In limonoid chemistry, the methyl shifts are more dependable in structure elucidation and the use of Karplus equation may lead to wrong conclusions (Taylor, 1984). Thus, in 11 α and 11 β-acetoxy azadirones, the prediction on the configuration at C-11 was not possible since both gave broad multiplets of similar bandwidth. The α-configuration of the ester function at C-11 was justified by comparing the methyl shifts with those of 11 α and 11β-acetoxy azadirones. Examination of models have shown that 11α-OAc will affect the C-10 methyl moderately while 11β-OAc will affect C-8 and C-10 methyls strongly (Halsall and Troke, 1975). The tertiary methyl signals of compound **1** were comparable with those of 11 α-acetoxy azadirone.

The side chain was found to be a seven-membered cyclic hemiacetal as in piscidinol C. But in **1**, C-21 is further linked to C-24 by an ether linkage. This is evident by comparison of the NMR data of **1** with piscidinol C. Peak at *m/z* 425 correspond to the loss of side chain. Thus **1** is the first protolimonoid to be reported with this type of side-chain. Isoturraeanthin, which was obtained by treating turraeanthin with H_2SO_4 in ether, has a similar side-chain but with hydroxyl group at C-23 instead of the keto group (Beavan *et al.*,1967) The ^{1}H NMR values of the side chain protons of **1** are comparable with those of isoturraeanthin.

Piscidinol G (**2**), $C_{30}H_{46}O_6$, (M^+502) showed IR bands at 1660 cm^{-1} corresponding to α, β-unsaturated cyclohexenone and trisubstituted double bond at 1650 and 820 cm^{-1}. The ^{1}H and ^{13}C NMR spectra indicated seven tertiary methyl groups, one trisubstituted double bond, one tertiary and three secondary hydroxyl groups as well as, α, β-unsaturated cyclohexenone moiety. Placement of the above functional groups in the apotirucallane skeleton suggested structure **2** for piscidinol G. The structure was also confirmed by comparison of the spectral data with those of melianodiol

(Merrien and Polonsky, 1971) and compound C isolated from *Chisocheton paniculatus* of Meliaceae (Connolly *et al.*, 1979).

Acetylation of **2** gave diacetate **3**, in which signals for H-21 and H-24 shifted to δ 6.17 (d, J=3.0 Hz) and 4.85 (d, J=2.70 Hz) while the signals for H-7 and H-15 did not change showing that the hindered 7α-hydroxyl was not acetylated. The configuration at C-21 gives the angle between the two hydrogens H-20 α and H-21 β of about 110° [13]. This accounted for a small coupling constant of H-21. The configuration at C-23 has been fixed as *R* similar to melianodiol based on biogenetic considerations. However, the configuration at C-24 has not been fixed.

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