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TRITERPENOIDS FROM *WALSURA PISCIDIA*

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Key Word Index—*Walsura piscidia*; Meliaceae; triterpenoids; piscidinol F.

Abstract—Piscidinol F, a new apotirucallane, has been isolated from the leaves of *Walsura piscidia* in addition to the other piscidinols. The side chain configuration of piscidinol B has been established from ^{1}H NMR data.

INTRODUCTION

In the course of screening plants belonging to the family Meliaceae for insect control activity, it was observed that extracts from the leaves of *Walsura piscidia* Roxb. displayed significant antifeedant activity against some important insect pests. This plant has been investigated in some detail and shown to contain tirucallane and apotirucallane triterpenoids [1], but none of these compounds has been tested for biological activity. It was considered worthwhile to reinvestigate this plant and isolate the triterpenoidal compounds for testing as antifeedants.

A number of compounds were obtained, piscidinols, B–E, but piscidinol A was not present in our extract. A new compound, piscidinol F, was isolated and its structure elucidated on the basis of spectral data. The configuration of the side chain of piscidinol B was established, as this was not done earlier.

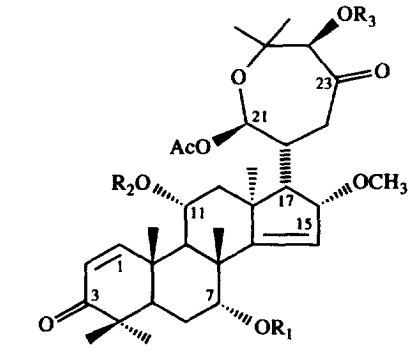
RESULTS AND DISCUSSION

Piscidinol F (**1**), $C_{33}H_{48}O_9$, contains seven tertiary methyls (δ_H 1.09, 1.11, 1.17, 1.22, 1.25, 1.28 and 1.44), one acetate (δ_H 2.17 *s*, 3H) and one methoxy (δ_H 3.23 *s*, 3H) group. Its spectroscopic properties indicate a close relationship to piscidinol D [1]. A ring-A enone system is shown by the presence of two well-separated doublets at δ8.05 (*d*, J = 10.2 Hz) and 5.80 (*d*, J = 10.2 Hz). The signals at δ6.37 (*d*, J = 2 Hz) and 3.88 (*s*, 1H) are assigned to H-21 and H-24, respectively, as in other piscidinols. The signals at δ4.04 (*t*, J = 3 Hz) and 4.39 (*m*, 1H) are assigned to H-7 and H-11, respectively. The coupling constant value of 3 Hz for H-7 shows the β, equatorial orientation of H-7 and thus the α, axial orientation of the OH-7 group. Irradiation of the H-11 signal at δ4.39 collapsed the H-9 doublet at δ2.38 (J = 8.8 Hz) into a singlet. The coupling value of 8.8 Hz shows the axial orientation of H-11 and thus the OH-11 group is α, equatorial. The signal at δ3.92 is assigned to the proton on the carbon bearing the methoxy group.

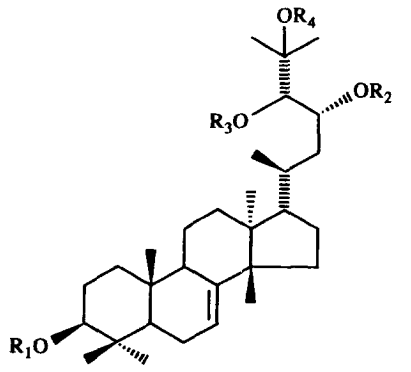
The double bond proton at δ5.93 which appears as a doublet (J = 2.9 Hz) is assigned to H-15 showing that C-16 is substituted. Confirmation of the siting of the OMe at C-16 is obtained from the ^{13}C NMR spectrum. The C-16 signal is shifted downfield ($\Delta\delta$8.2) and the C-17 signal shifted upfield ($\Delta\delta$2.1) [2], compared to piscidinol D which has a hydroxyl group at this position [1]. The signal for C-15 remains relatively unchanged.

On acetylation, piscidinol F formed a triacetate (**1a**). In the triacetate the H-7 and H-11 signals are shifted to δ5.35 and 5.4 but there is no change in the positions of H-24 (δ3.88) and H-16 (δ3.92), hence there is no free hydroxyl at C-16 and the hydroxyl at C-24 is not acetylated. All these assignments were confirmed by irradiation experiments. The configuration at C-21 and C-24 is assigned from NOE studies. Irradiation of H-21 at δ6.37 gave enhancements (NOE) of H-20 and H-22α (6%) and of H-24 (2%). Since the normal tirucallane configuration is H-20α, H-21 and H-24 can also be assigned α-configurations, OAc-21 group and the OH-24 group are β. Thus piscidinol F is assigned the structure **1**. That piscidinol F is not an artefact produced under chromatographic conditions was proved by demonstration of its presence in extracts obtained from leaves of *W. piscidia* with cold ethyl acetate and cold ethanol (TLC).

Confirmatory evidence for the side chain configuration of piscidinol B comes from the ^{1}H NMR spectral data. In piscidinol B there is no coupling between H-23 and H-24 but in triacetate (**2a**) a coupling of 1.5 Hz is observed as in other related tirucallanes, i.e. bourjotinolone [3], sapelin F and its tetraacetate [4, 5]. These assignments are confirmed by ^{1}H–^{1}H COSY and double irradiation experiments. Irradiation of the H-24 signal (at δ4.88 *d*, 1.5 Hz) in the acetate spectrum results in the removal of the 1.5 Hz coupling from the H-23 signal. Similarly, irradiation of the H-23 signal at δ5.43 simplified the H-24 signal to a singlet. These data indicate the similar configurations for the vicinal diol as in bourjotinolone and sapelin F. In the original paper [1], the configuration of the side chain in piscidinol B was not assigned, but from



1 $R_1 = R_2 = R_3 = H$
1a $R_1 = R_2 = Ac$; $R_3 = H$



2; $R_1 = R_2 = R_3 = R_4 = H$
2a; $R_1 = R_2 = R_3 = Ac$; $R_4 = H$

Table 1. ^{1}H NMR (400 MHz) data for piscidinols and their derivatives (δ in ppm, TMS as int. standard, J in Hz)

H	Piscidinol F (1) $CDCl_3$	Piscidinol B (2) (pyridine-d_5)	Piscidinol B acetate (2a) $CDCl_3$
1	8.05 (*d* (10.2)	—	—
2	5.80 *d* (10.2)	—	—
3	—	3.45 *m*	4.51 *dd* (4.4, 11.7)
5	2.50 *dd* (4.39, 11.71)	—	—
6	1.90 *m*	1.95 *m*	—
7	4.04 *t* (3)	5.29 *m*	5.22 *m*
9	2.38 *d* (8.8)	—	—
11	4.39 *m*	—	—
12α	1.75 *d* (13.3)	—	—
12β	1.66 *dd* (5.5, 13.3)	—	—
15	5.93 *d* (2.9)	—	—
16	3.92 *dd* (3.4, 5.8)	—	—
17	1.55 *dd* (5.8, 11.23)	—	—
18-Me	1.17 *s*	0.79 *s*	0.80 *s*
19-Me	1.22 *s*	0.87 *s*	0.76 *s*
20	2.81 *m*	—	—
21	6.37 *d* (2.0)	1.62 *d* (8.3)	0.93 *d* (5.9)
22α	2.74 *dd* (5.8, 16.1)	2.35 *m*	—
22β	2.45 *dd* (3.9, 16.1)	1.6 *m*	—
23	—	4.59 *m*	5.43 *ddd* (1.5, 5.0, 8.4)
24	3.88 *s*	3.67 *s*	4.88 *d* (1.5)
26-Me	1.25 *s*	1.61 *s*	1.19 *s*
27-Me	1.28 *s*	1.63 *s*	1.24 *s*
28-Me	1.09 *s*	1.15 *s*	0.97 *s*
29-Me	1.11 *s*	1.10 *s*	0.85 *s*
30-Me	1.44 *s*	0.99 *s*	0.94 *s*
OAc	2.17 *s*	—	2.05 *s*, 2.07 *s*, 2.19 *s*
OMe	3.23 *s*	—	—

Table 2. ^{13}C NMR (100 MHz) data for compounds **1** and **2**

C	Piscidinol F (1) ($CDCl_3$)	Piscidinol B (2) (pyridine-d_5)
1	161.1 *d*	36.7 *t*
2	123.6 *d*	27.7 *t*
3	204.7 *s*	77.4 *d*
4	40.7 *s*	38.6 *s*
5	44.1 *d*	50.2 *d*
6	24.3 *t*	23.5 *t*
7	71.6 *d*	117.5 *d*
8	44.2 *s*	145.1 *s*
9	45.2 *d*	48.4 *d*
10	44.4 *s*	34.3 *s*
11	66.3 *d*	17.5 *t*
12	45.6 *t*	33.5 *t*
13	46.5 *s*	42.9 *s*
14	168.8 *s*	50.5 *s*
15	119.6 *d*	33.3 *t*
16	82.0 *d*	27.9 *t*
17	56.7 *d*	53.4 *d*
18	24.6 *q*	12.5 *q*
19	20.0 *q*	21.2 *q*
20	36.0 *d*	33.5 *d*
21	91.0 *d*	18.6 *q*
22	38.6 *t*	41.4 *t*
23	208.6 *s*	68.5 *d*
24	80.7 *d*	75.8 *d*
25	72.2 *d*	72.9 *s*
26	24.3 *q*	26.3 *q*
27	26.2 *q*	26.9 *q*
28	21.5 *q*	27.4 *q*
29	26.8 *q*	14.6 *q*
30	29.7 *q*	26.5 *q*
COMe	169.8 *s*	—
COMe	20.9 *q*	—
OMe	56.2 *q*	—

Multiplicities determined by means of off-resonance decoupled spectra.

the above data it appears that piscidinol B is identical with hispidol B isolated from *Trichilia hispida* [6].

EXPERIMENTAL

Leaves of *Walsura piscidia* were collected from Guindy National Park. Their identity was confirmed by Dr Narasimhan, Dept of Botany, Madras Christian College and a voucher specimen is deposited in the Herbarium of Madras Christian College. The leaves were dried, powdered (1.325 kg) and extracted exhaustively with *n*-hexane and cold EtOH. The EtOH extract, following removal of the solvent, was suspended in H_2O and extracted with EtOAc. The EtOAc layer was concd and chromatographed on a column of silica gel using hexane, $CHCl_3$ and $CHCl_3$–MeOH mixtures as eluents. All the piscidinols were obtained in the $CHCl_3$–MeOH (19:1) fraction. This fr. was again chromatographed using $CHCl_3$–MeOH (49:1).

Isolation of piscidinol B (**1**). Frs 1–8 ($CHCl_3$–MeOH, 19:1) on further chromatography yielded piscidinol B (60 mg), which was recrystallized from MeOH. Mp 248–250°; $[\alpha]_D$ − 59.6 (MeOH; *c* 0.5); IR ν_{max} cm^{-1}: 3362, 2951, 1457, 1364, 1160, 1033, 798 ^1H NMR and ^{13}C NMR: Tables 1 and 2; EIMS *m/z* (rel. int.): 476 $[M]^+$ (6), 461 (2), 443 (11), 425 (18), 371 (50), 353 (34), 327 (15), 309 (11), 257 (7).

Acetylation of piscidinol B. A sample (15 mg) of **1** was treated with Ac_2O (1 ml) and pyridine (1 ml) and kept at room temp. for 24 hr. Usual work-up gave 12 mg of acetate (ex. MeOH). Mp 143°, IR ν_{max} cm^{-1}: 3600, 1728, 1380, 1229, 908; ^1H NMR: Table 1.

Isolation of piscidinol C. Frs. 9–13, on further chromatography, gave piscidinol C (350 mg), which was recrystallized from EtOAc–hexane (2:3), mp 230°.

Isolation of piscidinol F. The mother liquors, following removal of piscidinol C, were subjected to prep. HPLC (Shimadzu ODS column 20 mm × 25 cm, 220 nm, 10 ml min^{-1}) yielding piscidinol F (R_t 33.4 min) (200 mg) which was recrystallized from EtOAc–hexane. Mp 220°; IR ν_{max} cm^{-1}: 3468, 1749, 1715, 1655, 1558, 1457, 1386, 1223, 1083 1016, 951, ; $[\alpha]_D$ − 62.8 ($CHCl_3$; *c* 1); UV λ_{max} (EtOH) nm: 234 (ε 109.65), 213 (ε 134.75); Found: C, 66.89%: H, 8.04. $C_{33}H_{48}O_9$ requires C. 67.3, and H, 8.16%. MS (FAB) *m/z*: 589 $[M + H]^+$ (100%).

Acetylation of piscidinol F. Under standard conditions piscidinol F (50 mg sample, Ac_2O–pyridine room temp. for 16 hr), gave on usual work-up of the reaction mixture a product which on CC gave two compounds; acetate 1 (17 mg) and acetate 2 (6 mg), which was not characterized further. Acetate 1 had mp 182–184° (ex. MeOH); IR ν_{max} cm^{-1} 3618, 1730, 1668, 1379, 1223, 1084; ^1H NMR of acetate 1 (400 MHz, $CDCl_3$); δ1.06, 1.1, 1.21 (6H), 1.25, 1.26 1.4 (7-Me), 2.15, 2.13, 1.98 (3-OAc) 3.15 (*s*, 3H, OMe), 5.35 (*t*, H-7), 5.4 (*m*, H-11), 5.78 (*d*, *J* = 3 Hz), 5.82 (*d*, *J* = 11 Hz), 7.15 (*d*, *J* = 11 Hz), 3.88 (*s*, H-24)), 3.90 (*m*, H-16), 6.25 (*d*, *J* = 2 Hz).

Isolation of piscidinol E. Frs 14–16 on prep. TLC ($CHCl_3$–MeOH, 19:1) gave piscidinol E (200 mg) as a powder, mp 175–178°.

Isolation of piscidinol D. Frs. 17–20, on centrifugal chromatography (chromatotron) using $CHCl_3$–MeOH (97:3), gave piscidinol D (185 mg), mp 192°.

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