New Coumarins from *Pterocaulon polystachyum*

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Abstract: **Aerial parts of *Pterocaulon polystachyum* from Chaco province, Argentina, afforded the known coumarins ayapin, isoscopoletin, prenyletin, prenyletin methyl ether, virgatenol, obtusinin, 5-methoxy-6,7-methylenedioxycoumarin, 5-(3,3-dimethylallyloxy)-6,7-methylenedioxycoumarin, 5-(2′,3′-dihydroxy-3-methylbutanoxy)-6,7-methylenedioxycoumarin, haplopinol methyl ether, 6-(1,1-dimethyl-2-propenyl)-7-hydroxycoumarin and demethylnieshoutin; the last two are new as natural products while the five new coumarins from the collection were isovirgatenol, 3′-deoxyobtusinin, 6-methoxy-7-(2′-hydroxyethoxy)-coumarin, 5-(2′-hydroxyethoxy)-6,7-methylenedioxycoumarin and a substance tentatively identified as 5-hydroxy-6,7-methylenedioxy-8-(3,3-dimethylallyl)-coumarin.**

The genus *Pterocaulon* (Asteraceae, tribe Plucheae) comprises 18 species distributed over North and South America, Australia and adjacent areas (1). Reports exist on the chemistry of nine species. Characteristic constituents of the aerial parts are oxygenated coumarins containing relatively simple isoprenoid chains on the aromatic ring.

Pterocaulon polystachyum DC of southern Brazil, Paraguay, Uruguay and northeastern Argentina (2) is used in Argentine traditional medicine (3). In a recent note Palacios et al. (4) reported isolation from an Entre Rios province collection of eight known coumarins aesculetin, scopoletin, ayapin (**2a**), **2b**, **2c**, prenyletin (**1a**), prenyletin methyl ether (**1b**) and virgatenol (**1d**) (5); in the table listing the constituents on p. 295 of ref. 4 the substituents R^2 and R^3 of prenyletin, prenyletin methyl ether and virgatenol were unfortunately interchanged.

Our own work has dealt with a collection of *P. polystachyum* from Chaco province, Argentina. Extraction of the flowers and leaves and extensive fractionation of the extract by our usual procedure (column chromatography followed by HPLC of the various fractions) yielded as the main constituents the coumarins prenyletin (**1a**), prenyletin methyl ether (**1b**), obtusinin (**1f**) (6), (7), ayapin (**2a**) and 5-methoxy-6,7-methylenedioxycoumarin (**2b**), the latter previously reported from *Quisivisianthe papinue* (Meliaceae) (8) and *Pterocaulon virgatum*. (9). Prenyletin methyl ether was distinguished from its isomer 6-(3,3-dimethylallyloxy)-7-methoxycoumarin by the method of Cardona et al. (10). Known coumarins isolated in smaller

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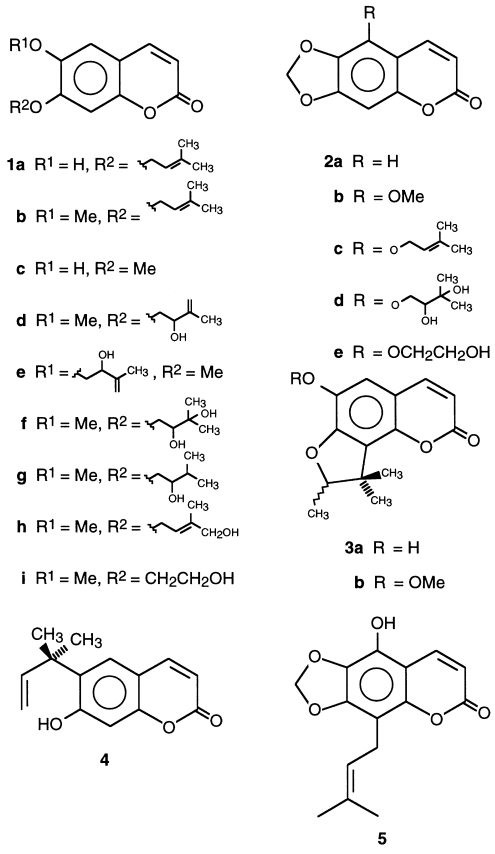

amounts were isoscopoletin (**1c**), virgatenol (**1d**) (5), haplopinol methyl ether (**1h**), (7), (11), **2c** and **2d**, both previously reported from *P. virgatum* (4), (12) demethylnieshoutin (**3a**), new as a natural product but previously obtained by treatment of nieshoutin (**3b**) with acetic acid, (13), and 6-(1,1-dimethyl-2-propenyl)-7-hydroxycoumarin (**4**) also previously known only as a product of synthesis (14), (15).

Five new coumarins found as very minor constituents of our *P. polystachyum* extract were isovirgatenol (**1e**), 3′-deoxyobtusinin (**1g**), 6-methoxy-7-(2′-hydroxyethoxy)-coumarin (**1i**), 5-(2′-hydroxyethoxy)-6,7-methylenedioxycoumarin (**2e**), and a substance tentatively identified as 5-hydroxy-6,7-methylenedioxy-8-(3,3-dimethylallyl)-coumarin (**5**). The extract also contained the degraded terpenoids loliolide, 7-epiloliolide, vomifoliol and vomifolione.

Structure assignment of **1e** of which only 1 mg was isolated was based on the ^{1}H NMR spectrum (see Materials and Methods section) which showed that C-5 and C-8 were unsubstituted and that the substance contained the same substituents on C-6 and C-7 as virgatenol (**1d**) although it was clearly isomeric with it. Irradiation at the frequency of the OMe signal enhanced the frequency of H-8 which was long-range coupled to H-4, thus placing the OMe group on C-7 and the five-carbon side chain on C-6. In the ^{1}H NMR spectrum of **1 g**, $C_{15}H_{18}O_5$, the frequencies of H-3, H-4, H-5 and H-8 coincided with those of its congener obtusinin (**1f**) also present in the extract while in the five carbon side chain the 3′-hydroxy of **1f** was replaced by hydrogen.

In the ^{1}H-NMR spectrum of **1i**, only 1 mg of which was isolated, the H-5 and H-8 signals were very close, at δ 6.848 and δ 6.852 respectively, but could be differentiated because of long-range coupling between H-4 at δ 7.59 and H-8. Irradiation at the frequency of the OMe signal at δ 3.89 enhanced the signal of H-5 as well as the two proton signals of H-1′a,b at δ 4.02, thus showing that the two carbon ether side chain was on C-7.

β-Hydroxyethoxy ether **2e** was unsubstituted on C-8 as shown by long range coupling between H-4 at δ 7.97, whose chemical shift indicated presence of an oxygen atom on C-5, and H-8 at d 6.55. In fact the frequencies of H-3, H-4 and H-8 corresponded to those of H-3, H-4 and H-8 in **2b** – **2d** and irradiation at the frequency of H-4 caused enhancement of the two proton multiplet at δ 3.95 (H-1′a,b) thus confirming structure **2e**.

The remaining new coumarin containing a phenolic hydroxy, a methylenedioxy and a prenyl group on the aromatic ring was formulated as **5** because of the pronounced similarity of its ^{1}H-NMR spectrum to that of 5,7-dihydroxy-6-methoxy-8-prenylcoumarin (13), the chemical shift of H-4 at δ 7.96 indicating presence of an oxygen function on C-5, and because of the absence of an NOE between H-4 and the protons of the methylenedioxy group. The observed pronounced benzene-induced chemical shift of the -OCH_2O- frequency (δ 6.02 in $CDCl_3$ vs. 5.06 in C_6D_6) which earlier might have suggested location of the methylenedioxy function at C-5, C-6 has more recently been shown to be misleading (12), (16), (17). The ^{13}C-NMR spectrum was too weak to permit verification of the proposed allocation of the oxygen containing functional groups by the usual techniques.

Materials and Methods

General: ^{1}H-NMR spectra were run in $CDCl_3$ on a Bruker AC 300 or Varian Inova 500 MHz NMR spectrometer. ^{13}C-NMR spectra were run on an IBM/Bruker WP270SY NMR spectrometer at 67.5 MHz. Mass spectra were run on a Finnigan MAT 90 instrument. For separation of mixtures HPLC equipment with a differential refractometer was used. Columns were A, a Beckman ultrasphere C 18 (5 μm, 10 mm i.d. × 250 mm), B, a Beckman ultrasphere C8 (5 μm, 10 mm i.d. × 250 mm) and C, a Merck Lobar Lichoprep Si 60 (40 – 635 μm, 10 mm i.d. × 240 mm). Retention times were measured from the solvent peak. The Merck silicia gel used for chromatography was 70 – 230 mesh, particle size 0.063 – 0.200 mm. Known compounds were identified by mass and ^{1}H-NMR spectrometry.

Plant material: Aerial parts of *Pterocaulon polystachyum* DC were collected at the flowering stage in March 1996 in Chaco province, Argentina. A voucher speciment (LIL 603 564) is on deposit at the herbarium of the Fundación Miguel Lillo, Tucumán.

Extraction and isolation: Flowers and leaves (337 g) were extracted at ambient temperature successively with $CHCl_3$ (2 × 3 l) to give 22 g (yield 6.5 %) and with MeOH to give 14 g (yield 4.1 %) of crude extracts. The $CHCl_3$ extract was suspended in EtOH (200 ml) at 55 °C, diluted with H_2O (150 ml) and extracted successively with hexane (3 × 150 ml) and $CHCl_3$ (3 × 150 ml). The second $CHCl_3$ extract on evaporation at reduced pressure furnished a residue (10 g) which was subjected to CC (silicia gel) using $CHCl_3$ containing increasing amounts of EtOAc (0 – 100 %) and finally MeOH to give 10 frs (Frs I-X). The

residue from the methanolic extract was suspended in 210 ml of a 1 : 1 mixture of *n*-butanol and water and the butanol extract was then evaporated at reduced pressure. CC of the residue over silicia gel using $CHCl_3$ with increasing amounts of MeOH gave four fractions A-D.

Fr. II (2.06 g) was chromatographed over silicia gel using hexane with increasing amounts of EtOAc (0 – 100%), four frs being collected. The second fraction, eluted with 150 ml of 4 : 1 hexane-EtOAc, gave on evaporation 11.8 mg of residue which on HPLC (column A, MeOH-H_2O 4 : 1, 1.5 ml min^{-1}) afforded 0.5 mg of a mixture of **1b** and **3a** and 0.8 mg of **2c**. The third fraction, a combination of frs eluted with 3 : 1 hexane-EtOAc (350 ml) and 2 : 1 hexane-EtOAc (480 ml), on evaporation gave 1.27 g of residue which on HPLC (column B, MeOH-H_2O 2 : 1, 1.5 ml min^{-1}) yielded 61.9 mg of **2a**, 162.5 mg of a mixture of **2b** and **1a**, and 393 mg of **1b**. Fr. III (348.2 mg) on HPLC (column B, MeOH-H_2O 2 : 1, 1.5 ml min^{-1}) gave 25.3 mg of **1a**, 3.1 mg of a fraction whose ^{1}H-NMR spectrum exhibited signals suggestive of 3,4-dihydroxy-6-methoxy-7-(3,3-dimethylallyloxy)-coumarin at δ 6.55 (s, H-8), 6.17 (s, H-5), 5.39 (t sept, J = 7, 1 Hz, H-2), 4.22 and 4.12 (each brt, J = 8.5 Hz and mutually coupled), 3.81 (s, 3p, OMe), 1.73 and 1.67 (each brs and 3p, H-4′, H-5′) but whose MS was unsatisfactory, and three further fractions which on separate rechromatography by HPLC (column A, MeOH-H_2O 2 : 1, 1.5 ml min^{-1}) gave from the first fraction 1.7 mg of **3a**, calcd. for $C_{14}H_{14}O_4$: 246; found: EIMS 246 (100), 231 (57), 203 (33), 190 (33), 178 (24), 147 (42), 84 (54); ^{1}H-NMR (500 MHz, $CDCl_3$) δ = 7.53 (d, 9.5 Hz, H-4), 6.80 (s, H-5), 6.20 (d, 9.5 Hz, H-3), 4.56 (9, 6.5 Hz, H-2′), 1.57 (s, 3p, H-3′), 1.41 (d, J = 6.5 Hz, H-5′), 1.29 (s, 3p, H-4′), in agreement with the literature (13). The second fraction consisted of 4 mg of **1a** and a mixture further purified by TLC ($CHCl_3$-MeOH, 12 : 1) to give 7.2 mg of **5**. The third fraction was a mixture containing mainly **1b**.

Fr. IV (73 mg) on HPLC (column B, MeOH-H_2O 2 : 1, 2 ml min^{-1}) gave 4.2 mg of **1c**, 1 mg of **1e** and mixtures of coumarins not resolved by further attempts at HPLC. Fr. V (45 mg) on HPLC (column B, MeOH-H_2O 3 : 2, 1.5 ml min^{-1}) gave mixtures of coumarins some of which contained **1d** followed by 4.9 mg of pure **1d**. Fr. VI (24 mg) on HPLC (column B, MeOH-H_2O 4 : 3, 1.5 ml min^{-1}) gave 1.4 mg of vomifolione, 1.8 mg of a coumarin which decomposed, 3.2 mg of **1d** and 0.7 mg of **1 g**. Fr. VII (64 mg) on HPLC (column A, MeOH-H_2O 4 : 3, 1.5 ml min^{-1}) gave 5.8 mg of loliolide, 5.2 mg of **1d**, 3.4 mg of **1 g** and a fraction which on resubmission to HPLC (column B, MeOH-H_2O 1 : 1, 1.5 ml min^{-1}) furnished 1.8 mg of vomifolione.

Fr. VIII (225 mg) on HPLC (column A, MeOH-H_2O 4 : 3, 1.5 ml min^{-1}) gave 1.1 mg of material exhibiting ^{1}H NMR signals at δ = 6.41 s, 3.86 (OMe), 3.67 (t, J = 6.3 Hz) coupled to 1.88 (m) which was in turn coupled to 2.63 (t, J = 7.9 Hz) and mixtures; one broad peak, that however, on resubmission to HPLC (column A, MeOH-H_2O 1 : 1, 1.5 ml min^{-1}) gave 2.3 mg of epiloliolide, 1.7 mg of loliolide, 4.5 mg of **2e** and 1.7 mg of **1b**. HPLC of fr. IX (74 mg) afforded only 0.5 mg of **1b**, 2.3 mg of **4** and unidentified material while fr. X (280 mg) on HPLC (column A, MeOH-H_2O 1 : 1, 1.5 ml min^{-1}) yielded 1 mg of **1i**, 0.5 mg of vomifoliol, 35.6 mg of **1f**, 7.8 mg of **2d** and 20.5 mg of a noncrystalline substance of unknown structure which decomposed on keeping. The polar fractions A-D yielded no identifiable substances.

6-(2-Hydroxy-3-methyl-3-butenyloxy)-7-methoxycoumarin (isovirgatenol) (**1e**): Gum which decomposed after removal of solvent before the mass spectrum could be run; ^{1}H-NMR (500 MHz) δ = 7.59 (brd, J = 9 Hz, H-4), 6.86 (s, H-5), 6.84 (brs long range coupled to H-4, H-8), 6.28 (d, J = 9.5 Hz, H-3), 5.18 (brs, H-4′a), 5.07 (t, J = 1.5 Hz, H-4′b), 4.76 (t, J = 7 Hz, H-2′), 4.27 (2H, m, H-1′ab), 3.87 (s, 3H, OMe), 2.34 (broad, 1H, OH), 1.88 (s, 3H, vinyl Me).

6-Methoxy-7-(2-hydroxy-3-methylbutyloxy)-coumarin (3′-deoxyobtusinin). (**1g**): Gum. Analyzed for $C_{15}H_{18}O_5$: 278 (M). Found : EI-MS (*m/z*, %): 278 (M^+, 100), 220 (51), 192 (93), 177(67); ^{1}H-NMR (300 MHz) δ = 7.60 (brd, J = 9.5 Hz, H-4 slightly coupled to H-8), 6.84 (brs, 2H, H-5, H-8), 6.28 (d, J = 9.5 Hz, H-3), 4.12 (dd, J = 9.5, 2.7 Hz, H-1′a), 3.95 (dd, J = 9.5, 8 Hz, H-1′b), 3.87 (s, 3H, OMe), 3.81 (m, 2H, H-2′a,b), 1.87 (sept, J = 6.8 Hz, H-3′), 1.03 and 0.99 (each d and 3H, J = 6.8 Hz, H-4′, H-5′).

6-Methoxy-7-(2-hydroxyethoxy)-coumarin (**1i**): Gum. Calcd. for $C_{12}H_{12}O_5$: 236 (M). Found EI-MS (*m/z*, %): 236 (M^+, 68), 192 (100), 177 (63), 164 (43), 149 (35); ^{1}H-NMR (500 MHz): δ = 7.59 (d, J = 9.5 Hz, H-4 long range coupled to H-8), 6.852 (2, H-8), 6.848 (s, H-5), 6.28 (d, J = 9.5 Hz, H-3) 4.16 (2H, t, J = 4.5 Hz, H-2′a,b), 4.02 (2H, t, J = 4.5 Hz, H-1′a,b), 3.89 (s, 3H, OMe). Irradiation of the H-1′ signal enhanced the signal of H-8; irradiation of the -OMe signal enhanced the signal of H-5. The partial ^{13}C-NMR spectrum ($CDCl_3$, 67.5 MHz) exhibited significant signals at δ = 161.2 (C-2), 149.9 (C-7 or C-8a), 143.1 (C-4), 113.9 (C-3), 111.9 (C-4a), 108.4 (C-5), 101.7 (C-8), 70.9 (C-1′) and 61.0 (C-2′).

5-(2-Hydroxyethoxy)-6,7-methylenedioxycoumarin (**2e**): Gum. Analyzed for $C_{12}H_{10}O_6$: 250 (M). Found : EI-MS (*m/z*, %): 250 (M^+, 100), 206 (77), 192 (64, 178 (85), 164 (19); ^{1}H-NMR (500 MHz) δ = 7.94 (brd, J = 9.5 Hz, H-4, slightly coupled to H-8), 6.55 brs (H-8), 6.22 (d, J = 9.5 Hz, H-3), 6.00 (s, 2H, -OCH_2O-), 4.55 (2H, t, J ~5 Hz, H-2′a,b), 3.95 (2H, t, J ~5 Hz, H-1′a,b).

5-Hydroxy-6,7-methylenedioxy-8-(3,3-dimethylallyl)-coumarin (**5**). Gum. Analyzed for $C_{15}H_{14}O_5$: 274 (M). Found: EI-MS (*m/z*, %): 274 (M^+, 100), 259 (60), 231 (39), 219 (22), 69 (21); ^{1}H NMR (300 MHz): δ = 7.96 (d, J = 9.5 Hz, H-4), 6.20 (d, J = 9.5 Hz, H-3), 6.02 (s, 2p, -OCH_2O-), 5.45 (broad, -OH), 5.25 (tquint, J = 7, 1.5 Hz, H-2′), 3.39 (2H, brd, J = 7.5 Hz, H-1′a,b), 1.76 and 1.66 (both brs and 3H, H-4′, H-5′);^{1}H-NMR (C_6D_6): δ = 7.33 (d, J = 9.5 Hz, H-4), 5.85 (d, J = 9.5 Hz, H-3), 5.06 (s, 2H, -OCH_2O), 5.48 (t quint, J = 7, 1.5 Hz, H-2′), 3.47 (2H, brd, J = 7.5 Hz H-1′a,b), 1.83 and 1.61 (both brs and 3H, H-4′, H-5′). The partial ^{13}C-NMR (spectrum, $CDCl_3$, 67.5 MHz) exhibited significant signals at δ = 161.6 (C-2), 138.4 (C-4), 133.2 (C-3′), 120.5 (C-2′), 111.4 (C-3), 102.2 (-OCH_2O-), 25.7 (C-4′), 22.0 (C-1′), 17.8 (C-5′), but signals of the aromatic ring were too weak to be identified satisfactorily.

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