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Isopurpurasol, a coumarin from *Pterocaulon virgatum*

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Abstract

A new 5,6,7-trioxygenated coumarin, named isopurpurasol, was isolated from the chloroform extracts of the aerial parts of *Pterocaulon virgatum*. The structural elucidation of this regioisomer of purpurasol was performed by detailed spectroscopic analysis. © 1999 Elsevier Science Ltd. All rights reserved.

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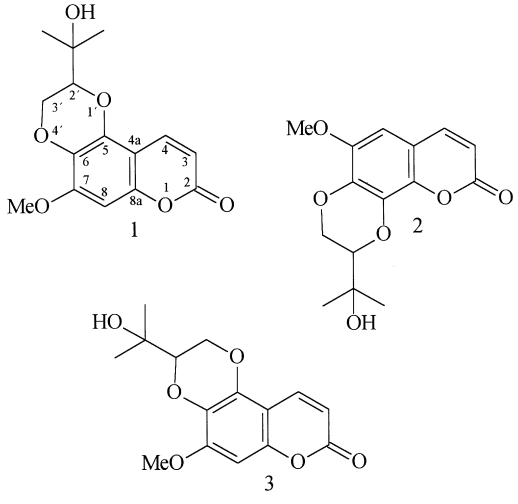
1. Introduction

Pterocaulon virgatum (L.) DC. is widely distributed in north eastern Argentina, southern Brazil and Paraguay. The aerial parts of this plant are used in Argentine traditional medicine as digestive, emenagogue, insecticide and as agent against snake bites (Schultez, 1976; Soraru & Bandoni, 1978; Arenas, 1981; Boelke, 1981).

In previous papers we have reported the isolation of four 5,6,7-trioxygenated coumarins (Debenedetti et al., 1994; Debenedetti, Boeykens, Coussio, & De Kimpe, 1997), five flavonoids (Debenedetti, Ferraro, & Coussio, 1983), caffeic acid, chlorogenic acid, isochlorogenic acid and 3,4-dicaffeoylquinic acid (Martino, Debenedetti, & Coussio, 1979; Debenedetti, Palacios, Wilson, & Coussio, 1993) from the aerial parts of *P. virgatum*. In a continuing investigation we have isolated and identified a new 5,6,7-trioxygenated coumarin, isopurpurasol (**1**) from the chloroform extract of the same source. The structure of the new compound was elucidated on the basis of spectroscopic studies. This is a new isomer of purpurasol (**2**), isolated from another *Pterocaulon* species, *Pterocaulon purpurascens* Malme (Debenedetti et al., 1992).



2. Results and discussion

The aqueous methanol extract of the aerial parts of *Pterocaulon virgatum* (L.) DC was subjected to succes-

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sive extraction with petrol, chloroform, diethyl ether and ethyl acetate.

The concentrated chloroform extract was submitted to CPC separation, yielding yellow needles of compound **1** (isopurpurasol). The UV spectrum of compound **1**, with absorption maxima at 230 and 320 nm, exhibited an extremely close resemblance with that of 5,6,7-trioxygenated coumarins, and further testified its oxygenation pattern (Murray, Mendez, & Brown, 1982). The IR spectra showed the presence of a lactone carbonyl, typical of coumarins, at 1710 cm^{-1} together with the bands of an aromatic ring at 1620 and 1575 cm^{-1}. Its ^1H NMR spectrum showed a typical pair of doublets at δ 6.22 and 7.89 (each 1H, d, $J=9.6$ Hz) for H-3 and H-4, respectively. The relative low field position of H-4 in coumarin **1** suggested already the presence of an oxygen substituent at C-5 (Steck & Mazurek, 1972). Additionally, the ^1H NMR spectrum indicated the presence of a methoxy group at δ 3.90 (3H, s) and three deshielded nonaromatic protons at 4.58 (1 H, dd, $J=1.5$, 11.0 Hz, O–C*H*(H)–CH–O), 4.11 (1H, dd, $J=9.2$, 11.0 Hz, O–CH(*H*)–CH–O) and 3.94 (1H, dd, $J=1.5$, 9.2 Hz, O–CH_2–C*H*–O) together with the appearance of a gem dimethyl group at δ 1.35 and 1.43. A $[M]^+$ at m/z 292 ($C_{15}H_{16}O_6$) in the mass spectrum, leads to the conclusion that coumarin **1** is an isomeric structure of purpurasol **2**. Compound **1** was also analyzed by LC-HRMS. The molecular ion of the electrospray mass spectrum revealed a protonated molecular ion of 293.10251, consistent with $C_{15}H_{17}O_6$ (calculated mass MH^+ 293.10252). NOE-difference spectra confirmed the positioning of the OMe group at C-7 of the coumarin nucleus. Thus, irradiation of the H-8 at δ 6.47 causes an enhancement of the methoxy signal at δ 3.90 with the same correlation being recorded in the reverse experiment. Irradiation at H-4 does not cause any enhancement of the methoxy signal. The methoxy group is hence situated at C-7. Therefore, the O–CH_2–CH–O moiety is attached to the coumarin unit at C-5 and C-6.

Two-dimensional heteronuclear shift correlation ($^1J_{CH}$-COSY, HETCOR) clearly established direct H–C connectivities and, together with the nondecoupled ^{13}C-spectrum, enabled the unequivocal assignment of the AMX-spin system at the 1,4-dioxa heterocyclic moiety. The methine proton H-2′ at δ 3.94 (1H, dd, $J_{AM}=1.5$, $J_{MX}=9.2$ Hz,) is correlated to C-2′ (δ 78.6), whereas the methylene protons H-3′a (1H, dd, $J_{AX}=11.0$, $J_{AM}=9.2$ Hz, δ 4.11) and H-3′b (1H, dd, $J_{AX}=11.0$, $J_{AM}=1.5$ Hz, δ 4.58) show cross-peaks with C-3′(δ 65.5). These findings are in agreement with the assignments made earlier and the commonly accepted consideration that a coupling constant of 9.2 Hz (the large coupling constant of H-2′) is insufficient to represent a geminal coupling of methylene protons, bearing part of an oxygen containing six-membered ring (Pistelli, Bertoli, Bilia & Morelli, 1996). By NOE difference experiments it was proved that H-2′ and H-3′a are positioned in a *cis* relationship on the 1,4-dioxa moiety. Similar to purpurasol, the methylene group unexpectedly resonated downfield from the methine proton. Long-range correlation $^3J_{CH}$-COSY, COLOC, optimized for 2 and 8 Hz, made it possible to differenciate between structure **1** or **3**. A three-bond correlation between H-2′ at δ 3.94 and C-5 (δ 139.1) of the AMX system was visible. From these experiments, it can be concluded unequivocally that isopurpurasol can be represented by structure **1**.

3. Experimental

3.1. General

Mps uncorr. UV spectra were recorded in MeOH. IR: KBr pellet. EIMS spectra were obtained by direct probe insertion at 70 eV. ^1H NMR and ^{13}C NMR were recorded at 270 and 67.8 MHz, respectively, in $CDCl_3$ and C_6D_6 using TMS as internal standard.

3.2. Plant material

Aerial parts of *P. virgatum* were collected from the Experimental Station INTA, Concepción del Uruguay, Entre Ríos, Argentina, during November 1989 and identified by Dr. B. Sorarú, 'Juan A. Dominguez' Museum, Faculty of Pharmacy and Biochemistry, University of Buenos Aires. A herbarium specimen No. SD-041 is being preserved in the Department of Pharmacology of the same institution.

3.3. Extraction and isolation

The air-dried and powdered aerial parts of *P. virgatum* (950 g) were extracted at room temperature with 25% aq. MeOH. The MeOH was removed under reduced pressure and the aqueous layer was partitioned with petrol, $CHCl_3$, Et_2O and EtOAc. The concentrated chloroform extract (1 g) was subjected to CC on silica gel and eluted successively with a petrol:EtOAc solvent mixture of increasing polarity. Fractions were collected (15 ml) and monitored by silica gel TLC. Fractions 25–27 (140 mg) were further chromatographied by CPC model CCC-1000, Pharma-Tech Research Corp., using hexane:EtOAc:MeOH:H_2O (1:1:1:1) reversed phase; the upper phase was used as mobile phase. Flow rate, 1 ml/min, pressure, 105 psi, rotation speed, 1050 rpm, run time, 270 min and fraction volume, 5 ml.

A total of 85 fractions was obtained. The fractions were joined, followed by TLC using silica gel, and the same mobile phase was used in the CPC. Fractions

26–30 were evaporated in vacuo and recrystallized from MeOH, yielding 14 mg of coumarin **1**.

3.4. Isopurpurasol (*1*)

Yellow needles (MeOH), mp 160–162°C. UV λ_{max} MeOH nm: 230, 320. IR ν_{max} cm^{-1}: 3400, 3080, 2975, 2910, 2850, 1710, 1620, 1575, 1505, 1470, 1380, 1370, 1330, 1310, 1260, 1200, 1140, 1080, 1020, 990, 960, 930, 890, 830, 750. ^{1}H NMR (270 MHz, $CDCl_3$): δ 7.89 (1H, d, $J=9.6$ Hz, H-4), 6.47 (1H, s, H-8), 6.22 (1H, d, $J=9.6$ Hz, H-3), 4.58 (1 H, dd, $J=1.5$, 11.0 Hz, O–C*H*(H)–CH–O), 4.11 (1H, dd, $J=9.2$, 11.0 Hz, O–CH(*H*)–CH–O), 3.94 (1H, dd, $J=1.5$, 9.2 Hz, O–CH_2–C*H*–O), 3.90 (3H, s, OMe), 1.43 and 1.35 (each 3H, each s, Me_2), OH was invisible. ^{13}C NMR (67.8 MHz, $CDCl_3$): δ 25.2 (q, Me), 26.2 (q, Me), 56.3 (q, OMe), 65.5 (t, OCH_2), 70.7 (s, Me_2C–OH), 78.6 (d, CH–O), 92.8 (s, C-8), 103.3 (s, C-4a), 112.0 (d, CH-3), 129.7 (s, C-6), 137.9 (d, CH-4), 139.1 (s, C-5), 149.6 (s, C-8a), 152.5 (s, C-7) and 161.5 (s, C=O). ^{1}H NMR (270 MHz, C_6D_6): δ 7.50 (1H, d, $J=9.6$ Hz, H-4), 6.14 (1H, s, H-8), 5.99 (1H, d, $J=9.6$ Hz, H-3), 4.07 (1H, dd, $J=2.1$, 11.2 Hz, O–C*H*(H)–CH–O), 3.64 (1H, dd, $J=9.2$, 11.2 Hz, O–CH(*H*)–CH–O), 3.38 (1H, dd, $J=2.1$, 9.2 Hz, O–CH_2–C*H*–O), 3.16 (3H, s, OMe), 0.96 and 1.07 (each 3H, each s, Me_2), OH was invisible. ^{13}C NMR (67.8 MHz, C_6D_6): δ 25.6 (q, Me_2), 55.6 (q, OMe), 65.4 (t, OCH_2), 70.1 (s, Me_2C–OH), 78.6 (d, CH–O), 93.0 (s, C-8), 103.4 (s, C-4a), 112.6 (d, CH-3), 129.8 (s, C-6), 137.0 (d, CH-4), 139.3 (s, C-5), 150.2 (s, C-8a), 152.6 (s, C-7), 160.6 (s, C=O). EIMS (probe) 70 eV, m/z (rel. int.): 292 [M^+] (76), 234 (100), 219 (18), 208 (13), 206 (16), 205 (58), 191 (12), 177 (13), 176 (16), 160 (10), 149 (13), 84 (17), 69 (34), 59 (72), 43 (73). $[\alpha]_{589}=+26$ ($CHCl_3$; $c=0.43$), $[\alpha]_{546}=+44$ ($CHCl_3$; $c=0.43$).

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