New Coumarins from *Pilocarpus goudotianus*

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Received: May 12, 1989

Abstract

The leaves of *Pilocarpus goudotianus* afforded in addition to eight known coumarins three new ones, donatin, alloxanthoxyletol, and (+)-elisin. The structures were elucidated on the basis of spectroscopic evidence and chemical derivatives.

Key words

Pilocarpus guodotianus, Rutaceae, coumarins, donatin, alloxanthoxyletol, (+)-elisin.

Introduction

The genus *Pilocarpus* (Rutaceae) comprises some twenty species widely spread over tropical and subtropical America (1); they are commonly known as "jaborandi" and their preparations were used in the treatment of many diseases (2–4). In this paper we report the isolation of eleven coumarins from the extract of the leaves of *Pilocarpus goudotianus*, a shrub growing in Colombia and Venezuela. This is the first report on this type of compound in a *Pilocarpus* species.

Materials and Methods

Plant material

Pilocarpus goudotianus Tulasne (leaves) was collected in Peninsula de Paraguaná, Edo. Falcón, Venezuela, in October 1984. A voucher specimen is deposited in the Herbarium MERF, López Figueiras et al. N° 31080, Universidad de los Andes, Mérida, Venezuela.

Instruments

^{1}H-NMR and ^{13}C-NMR: in $CDCl_3$ except where noted, Varian XL-200 or Bruker AM-300, chemical shifts on the δ (ppm) scale, coupling constants (*J*) in Hz, TMS as in internal standard; IR: in film, Perkin Elmer 881; UV: in MeOH, Shimadzu MPS 2000; optical rotation: in MeOH, Perkin Elmer 214; MS: 70 eV, VG 12250; m.p.: uncorrected.

Isolation of products

Dried leaves (7.2 kg) were extracted in a Soxhlet with EtOH. 140 g of extract were chromatographed on a silica gel column (Kieselgel 60, 0.2–0.063 mm, Merck) eluted with petroleum ether (5 l), $CHCl_3$ (27 l), AcOEt (8 l), and acetone (10 l). 50 (1 l) fractions were collected. These were analysed by TLC and joined into the groups (1–15), (16–25), (26–32), (33–40), and (41–50).

The fraction (16–25) was rechromatographed on a silica gel column (Kieselgel 60, < 0.063 mm, Merck) under N_2 overpressure, eluted with petroleum ether/AcOEt mixtures. 195 fractions were collected, and after TLC analysis, combined into five groups: E-1 to E-5. Repeated CC under N_2 overpressure interchanging petroleum ether/AcOEt and CH_2Cl_2/MeOH mixtures as eluents, yielded:

- From E-3: **1** (2.06 g), **2m** (24 mg), **3** (104 mg), and **4** (100 mg).
- From E-4: **6** (255 mg), **7** (346 mg), **8** (10 mg), and **9** (30 mg).

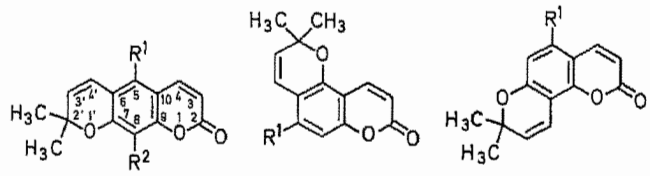
The fraction (26–32) was rechromatographed on a silica gel column under N_2 overpressure, using petroleum ether/AcOEt mixtures as eluent. 134 fractions were collected, analysed by TLC, and joined into six groups: F-1 to F-6. Further purification of F-2, F-4, and F-6 by preparative TLC yielded **5** (7 mg), **11** (15 mg), and **10** (4 mg), respectively.

Characterization of new products

Donatin (**4**): UV λ_{max} nm (lg ε): 347 (3.98), 268 (4.38), 227 (4.33), 205 (4.25). IR ν_{max} cm^{-1}: 2970, 2950, 1709, 1602, 1550. ^{13}C-NMR: 160.59 (C-2), 149.65 (C-7), 148.70 (C-9), 143.51 (C-4), 139.11 (C-4″), 134.05 (C-8), 130.92 (C-3′), 120.97 (C-5), 119.98 (C-3″), 119.02 (C-4′)*, 118.73 (C-6)*, 112.94 (C-10)#, 112.90 (C-3)#, 76.91 (C-2′), 69.69 (C-2″), 28.19 (C-2′(Me)$_2$), 25.71, 17.91 (C-5″, C-6″). (*, #, these signals may be interchangeable). MS *m/z* (% rel. int.): 313 $[M+1]^+$ (1), 312 $[M]^+$ (0.1), 244 (71), 229 (100), 201 (10), 172 (3), 144 (3), 115 (13), 69 (15).

Alloxanthoxyletol (**5**): UV λ_{max} nm (lg ε): 330 (3.99), 282 (4.14), 225 (4.11), 203 (3.98). IR ν_{max} cm^{-1}: 3300, 2925, 2856, 1700, 1610, 1593. ^{1}H-NMR (acetone-d_6): 7.93 (d, J = 9.6, H-4), 6.61 (d, J = 10.0, H-4′), 6.29 (s, H-8), 6.03 (d, J = 9.6, H-3), 5.63 (d, J = 10.0, H-3′), 1.42 (6H, s, C-2′(Me)$_2$). MS *m/z* (% rel. int.): 244 $[M]^+$ (15), 229 (100), 201 (15), 173 (1), 145 (1), 115 (4).

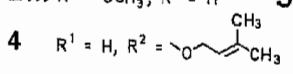
Alloxanthoxyletin (**5m**): A mixture of **5** (4 mg), K_2CO_3 (8 mg), iodomethane (0.02 ml), and acetone (3 ml) was refluxed for 15 min. Usual work up and purification by preparative TLC gave **5m** (3 mg); m.p. 106–108 °C (petroleum ether/AcOEt). IR ν_{max} cm^{-1}: 2927, 1724, 1591, 1430. ^{1}H-NMR: 7.89 (d, J = 9.5, H-4),



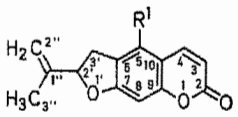
2 R^1 = OH, R^2 = H
2a R^1 = OAc, R^2 = H
2m R^1 = OCH_3, R^2 = H

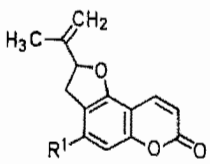
5 R^1 = OH
5a R^1 = OAc
5m R^1 = OCH_3

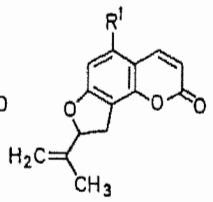
12 R^1 = OH
12a R^1 = OAc
12m R^1 = OCH_3



4 R^1 = H, R^2 =







11 R^1 = OH
11a R^1 = OAc

13

14

6.54 (d, J = 9.9, H-4'), 6.28 (s, H-8), 6.09 (d, J = 9.5, H-3), 5.48 (d, J = 9.9, H-3'), 3.80 (3H, s, OMe), 1.40 (6H, s, C-2'$(Me)_2$). MS *m/z* (% rel. int.): 258 $[M]^{+\cdot}$ (14), 243 (100), 228 (12), 200 (11).

Alloxanthoxyletol acetate (**5a**): A solution of **5** (3 mg), Ac_2O (0.1 ml), and pyridine (0.2 ml) was kept at room temperature for 5 min. Evaporation under reduced pressure gave **5a** (3 mg): m.p. 160–162 °C (petroleum ether/AcOEt). IR ν_{max} cm^{-1}: 2982, 2931, 1733, 1616, 1589, 1461, 1429. ^{1}H-NMR: 7.92 (d, J = 9.7, H-4), 6.56 (s, H-8), 6.25 (d, J = 10.1, H-4'), 6.22 (d, J = 9.7, H-3), 5.58 (d, J = 10.1, H-3'), 2.29 (3H, s, $OCOCH_3$), 1.51 (6H, s, C-2'$(Me)_2$). MS *m/z* (% rel. int.): 286 $[M]^{+\cdot}$ (15), 271 (14), 244 (5), 229 (100), 215 (1), 201 (6).

(+)-*Elisin* (**11**): $[\alpha]_D^{25}$: +12.8° (MeOH, *c* 0.1). UV λ_{max} nm (lg ε): 333 (4.09), 259 (3.51), 250 (3.56), 209 (4.40). IR ν_{max} cm^{-1}: 3340, 1700, 1620, 1605. ^{13}C-NMR (acetone-d_6): 165.19 (C-7), 161.33 (C-2), 157.86 (C-9), 151.17 (C-5), 144.73 (C-1''), 139.79 (C-4), 112.53 (C-2''), 110.16 (C-3), 108.95 (C-6), 104.35 (C-10), 90.60 (C-8), 88.08 (C-2'), 32.00 (C-3'), 17.06 (C-3''). MS *m/z* (% rel. int.): 244 $[M]^{+\cdot}$ (23), 229 (100), 201 (17).

(+)-*Elisin acetate* (**11a**): A solution of **11** (3 mg), Ac_2O (0.1 ml), and pyridine (0.2 ml) was kept at room temperature for 5 min. Evaporation under reduced pressure gave **11a**: IR ν_{max} cm^{-1}: 2932, 1760, 1726, 1625, 1563, 1446. ^{1}H-NMR: 7.57 (d, J = 9.7, H-4), 6.62 (s, H-8), 6.15 (d, J = 9.7, H-3), 5.25 (br. t, J = 8.5, H-2'), 5.02 (br. s, H-2''a), 4.88 (br. s, H-2''b), 3.19 (dd, J = 15.8, 9.4, H-3'a), 2.86 (dd, J = 15.8, 7.8, H-3'b), 2.33 (3H, s, $OCOCH_3$), 1.69 (3H, s, 3''-Me). MS *m/z* (% rel. int.): 286 $[M]^{+\cdot}$ (32), 244 (31), 229 (100), 215 (2), 201 (9).

Results and Discussion

The isolated coumarins have been identified by their physical, spectroscopic, and chemical properties as the pyranocoumarins xanthyletin (**1**) (5), xanthoxyletin (**2m**) (6), luvangetin (**3**) (7), donatin (**4**), and alloxanthoxyletol (**5**), the furanocoumarins bergapten (**6**) (5, 6, 8), xanthotoxin (**7**) (8), isopimpinellin (**8**) (5), imperatorin (**9**) (9), and trichoclin (**10**) (10), and the dihydrofuranocoumarin (+)-elisin (**11**). Trichoclin (**10**) has only been reported once from *Trichocline incana* (10). Compounds **4**, **5**, and **11** were not found in the literature.

Donatin (**4**), $C_{19}H_{20}O_4$ ($M^{+\cdot}$: 312), was obtained as a colourless oil. Its UV and IR spectra, and the presence in its ^{1}H-NMR spectrum of two doublets, 1H each, at δ = 7.54 and 6.20 ppm with J = 9.5 Hz (H-4 and H-3) established the presence of a coumarin nucleus without an oxygenated function on C-5 (11). A singlet, 6H, at δ = 1.49 ppm and two doublets, 1H each, at δ = 6.30 and 5.67 ppm (J = 9.9 Hz) are characteristic of two geminal methyls on C-2', and H-4' and H-3' protons, respectively. A singlet, 1H, at δ = 6.80 ppm is assigned to the benzene proton H-5.

All these data are close to those of luvangetin (**3**) (7), but the ^{1}H-NMR spectrum of donatin (**4**) shows, instead of the methoxyl singlet, two singlets, 3H each, at δ = 1.69 and 1.73 ppm, a 2H doublet at δ = 4.63 ppm (J = 7.3 Hz), and a broad triplet, 1H, at δ = 5.58 ppm. These signals and the mass spectral fragment at m/z = 244 ($M^{+\cdot}$ – 68), reveal the presence of a 3,3-dimethylallyloxy moiety in the molecule. The above data, and the ^{13}C-NMR spectrum, are in agreement with structure **4** {10-[(3-methyl-2-butenyl)-oxy]-8,8-dimethyl-2*H*,8*H*-benzol[1,2,-*b*:5,4-*b*']dipyran-2-one} for donatin.

Alloxanthoxyletol (**5**); $C_{14}H_{12}O_4$ ($M^{+\cdot}$: 244), was obtained as yellowish prisms (m.p. 217–219 °C, dec. from AcOEt). Its spectroscopic data (UV, IR ^{1}H-NMR) were consistent with a pyranocoumarin hydroxylated on the benzene ring, possessing a 5,7-dioxygenated pattern (11); hence structures **5**, **2** (m.p. > 200 °C, dec., from AcOEt) (12), and **12** (m.p. > 210 °C, dec. from AcOEt) (12), had to be considered.

Methylation of **5** yielded **5m**. Its m.p. was closely similar to that reported for alloxanthoxyletin (**5m**), a natural product isolated from *Zanthoxylum americanum* (13), and quite different from the m.p.s of xanthoxyletin (**2m**) (11) and 5-methoxyseselin (**12m**) (14). Nevertheless, as spectroscopic data for alloxanthoxyletin (**5m**) were not available, further confirmation was necessary.

From the ^{1}H-NMR data, the most significant feature is a deshielding effect for H-8 (Δ δ = –0.3) when **2** and **2m** are compared, whilst δ (H-6) for **12** (12) and **12m** (14), as well as for **5** and **5m** are closely similar. Consequently, structure **2** could be excluded.

Acetylation of **5** yielded **5a**. It is worth noting that the acetyl derivatives **2a**, **5a**, and **12a** are easily distinguishable by ^{1}H-NMR. The linear isomer **2** shows upfield shift of the H-4 and H-4' signals (Δ δ = +0.5) on acetylation to give **2a** (12). Acetylation of **12**, to obtain **12a**, causes upfield shift of the H-4 signal (Δ δ = +0.4) and downfield shift of the H-4' signal (Δ δ = –0.2) (12).

The ^{1}H-NMR spectrum of alloxanthoxyletol acetate (**5a**), showed no variation of the position of the H-4 signal in relation to the spectrum of **5**, thus precluding the hydroxyl group at C-5, and an upfield shift of the H-4' signal

($\Delta\ \delta = +0.36$), indicating an *ortho* relationship between the hydroxyl group and the pyranic ring. Hence, structure **5** (5-hydroxy-2,2-dimethyl-2*H*,8*H*-benzo[1,2-*b*-3,4-*b'*]-dipyran-8-one) is assigned to alloxanthoxyletol. An NOE experiment with the methyl ether **5m** furnished a further argument for structure **5**.

(+)-Elisin (**11**), $C_{14}H_{12}O_4$ ($M^{+\cdot}$ 244), was obtained as colourless needles (m.p. 238–240 °C, from AcOEt). The presence of a hydroxyl group is evidenced by an IR band at 3340 cm^{-1}. Its ^{1}H-NMR spectrum shows two doublets, 1H each, at $\delta = 8.07$ and 6.07 ppm ($J = 9.6$ Hz) corresponding to the H-4 and H-3 protons of a coumarin with an oxygenated function on C-5 (11). A 1H singlet at $\delta = 6.31$ ppm is assigned to a benzene proton (H-8). Two double doublets, 1H each, at $\delta = 3.46$ (H-3'a, $J = 15.6$, 9.6 Hz) and $\delta = 3.03$ ppm (H-3'b, $J = 15.6$, 7.5 Hz) together with a broad triplet, 1H, at $\delta = 5.38$ ppm (H-2', $J = 8.5$ Hz), are attributable to an ABX system of a dihydrofuranocoumarin substituted at C-2' (15, 16). On irradiation at the frequency corresponding to the H-2' proton, an NOE effect of the signals of H-3'a and H-3'b was observed, in agreement with a preferential conformation in which the H-2' displays an equidistant position from both H-3' protons.

The presence of two broad singlets, 1H each, at $\delta = 5.10$ (H-2''a) and 4.94 ppm (H-2''b), a 3H singlet at $\delta = 1.76$ ppm, and the MS fragment at $m/z = 201$ ($M^{+\cdot} - 43$) are characteristic of an isoprenyl group (15, 16). These data are in agreement with structures **11**, **13**, and **14**.

Acetylation of **11** yielded **11a** and causes upfield shift of the H-4 doublet ($\Delta\ \delta = +0.5$), indicating that the hydroxyl group is at C-5. Hence, structure **13** can be excluded. The signals corresponding to the protons H-3'a and H-3'b experience upfield shifts ($\Delta\ \delta = +0.27$ and $+0.17$, respectively) closely similar to those observed on acetylation of the phenolic hydroxyl of 5-hydroxymarmesin (17). These data are consistent with the linear isomer **11**.

The ^{13}C-NMR spectrum (assignments elucidated by ^{1}H-^{13}C hetcor spectrum and DEPT) confirmed this structure on the basis of γ_{syn} and δ effects on δ (C-3') and δ (C-8), respectively (18). Hence, structure **11** (8,9-dihydro-8-(1-methylethenyl)-5-hydroxy-2*H*-furo[2,3-*h*]-1-benzopyran-2-one) is assigned to (+)-elisin.

Acknowledgements

This research was supported in part by the Comision Asesora de Investigación Cientifica y Técnica (CAICYT n° PR84-0247). The authors are grateful to the Dirección General de Investigación Cientifica y Técnica (DGICYT) (proyect n° PB87-0965) for support of this research and to Prof. Dr. A. García Granados, Dpto. Química Orgánica, Universidad de Granada (Spain) for NOE experiments and ^{13}C-NMR spectrum of (+)-elisin.

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