Isomeric Triterpenoids from *Peritassa campestris*

Luciano M. Lião[a,*], Paulo C. Vieira[b], Edson Rodrigues-Filho[b], João B. Fernandes[b] and Maria F. G. F. Silva[b]

[a] Instituto de Química, Universidade Federal de Goiás, C.P. 131, 74001-970, Goiânia, Goiás, Brazil. Fax: +55 62 521 11 67. E-mail: luciano@quimica.ufg.br

[b] Departamento de Química, Universidade Federal de São Carlos, C.P. 676, 13565-905, São Carlos, São Paulo, Brazil

* Author for correspondence and reprint requests

Z. Naturforsch. **57 c,** 403–406 (2002); received October 31, 2001

Peritassa campestris, Hippocrateaceae, Campestrine, *Seco*-Quinonemethide Triterpenoids

An investigation of the MeOH extract from the roots of *Peritassa campestris* (Hippocrateaceae) afforded two isomeric *seco*-A-ring quinonemethide triterpenoids, campestrine-I (**1**) and -II (**2**). This appears to be the first report of a C_{26}-type triterpene carbon skeleton from the Celastraceae or Hippocrateaceae families. The structures were elucidated on the basis of spectral data, particularly HMQC, HMBC and NOE experiments.

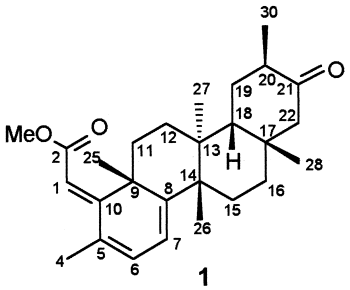
Introduction

In the course of our continuing search for bioactive compounds from plants, following their toxicity to brine shrimp larvae (Alkofahi *et al.*, 1989), several bioactive quinonemethide triterpenoids were isolated previously (Lião, 1997). These compounds, known as celastroloids, constitute a relatively small group of natural products encountered exclusively in plants of the families Celastraceae and Hippocrateaceae. Celastroloids have been of interest due to their antitumour, antileukemic, antimicrobial (Brüning and Wagner, 1978; Gunatilaka, 1996), antimalarial (Pavanand *et al.*, 1989), and trypanocidal activities (Goijman *et al.*, 1985). In this paper, we describe the isolation and identification of two new isomeric triterpenes, campestrine-I (**1**) and -II (**2**), which are *seco*-A-ring quinonemethide triterpenoid derivatives, as well as six celastroloids, tingenone, 22-hydroxytingenone, 20-hydroxy-20-*epi*-tigenone, celastrol, pristimerin, and netzahualcoyene (Gunatilaka, 1996). These compounds were isolated from the roots of *Peritassa campestris* Cambess. (Hippocrateaceae), a plant that occurs in the Brazilian Savannah, popularly known as "bacupari do campo", and used in folk medicine for wound healing in the form of a decoction (Septímio, 1994).

Results and Discussion

The MeOH extract from the roots of *P. campestris* afforded, through droplet countercurrent chromatography (DCCC) and repeated chromatographic separation, the isomeric triterpenoids **1** and **2** in low yield, besides the known quinonemethide triterpenoids.

Campestrine-I (**1**) showed a molecular ion peak at *m/z* 410 $[M]^+$ in the EI-mass spectrum. The UV spectrum of **1** displayed absorption bands at 243 and 287 nm, and the IR spectrum showed a ketone band at 1708 cm^{-1} and an ester band at 1735 cm^{-1}, indicating that the ester carbonyl group in C-2 was distorted, avoiding the steric crowding with C-11, in contrast with the spectral data for **2** (*vide infra*). The ^1H NMR spectrum displayed signals for six methyl groups, one methoxy, three olefinic protons, and one methine. These signals were very similar to those described for tingenone (Gunatilaka, 1989), particularly the signals for the E-ring, indicating a ketone group at C-21. The main differ-



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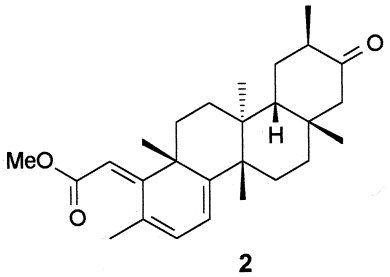

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ences observed were in the protons of the A- and B-rings, wherein H-1, H-6 and H-7 were shielded in comparison with those in tingenone. The multiplicities were identical, however. In quinonemethide triterpenoids like tingenone, typical signals are the singlets at δ 7.00 (1H, s) and 2.23 (3H, s), characteristic of the hydroxy and 23-Me groups, attached to C-3 and C-4, respectively. These signals were not observed in **1**, but a singlet signal was observed at δ 1.76, reinforcing the differences observed in the protons of the A-ring. The long-range coupling observed between H-1 and H-6 (J = 2.0 Hz) indicated the coplanarity of the bonds involved. The ^{13}C NMR spectrum of **1** revealed a carbonyl signal at δ 213.7, a carbomethoxy at δ 163.7, carbon signals associated with three double bonds at δ 103.7 (*s*), 110.7 (*d*), 115.9 (*d*), 126.4 (*d*), 161.1 (*s*) and 165.2 (*s*), one methoxyl, six methyl, six methylene, two methine, and four quaternary carbon signals. The absence of the methyl group attached to C-4 in quinonemethide triterpenolids, observed near δ 10.3 (23-Me), along with the presence of only 8 sp^2 carbons, corroborated the information that the differences between these compounds were in the A- and B-rings. The ^{13}C NMR, HMQC and HMBC spectra supported the six-membered B-ring and a *seco*-A-ring, by the cross-peaks of the proton signal at δ 5.86 (1H, *d, J* = 2.0 Hz, H-1) with the carbon resonances at δ 110.7 (C-1) and 126.4 (C-6); the proton signal at δ 6.38 (1H, *dd, J* = 6.8 and 2.0 Hz, H-6) with the carbon resonances at δ 103.7 (C-5), 126.4 (C-6), 161.1 (C-8) and 165.2 (C-10); the proton signal at δ 6.06 (1H, *d, J* = 6.8 Hz, H-7) with the carbon resonances at δ 126.4 (C-6), 39.6 (C-9) and 44.1 (C-14); the proton signal of the methyl at δ 1.53 (25-Me) with the cabon resonances at δ 161.1 (C-8) and 165.2 (C-10); the proton signal of the methyl at δ 1.30 (26-Me) with the carbon resonance at δ 161.1 (C-8); and proton signal of the methyl at δ 1.76 (4-Me) with the carbon resonances at δ 103.7 (C-5) and 126.4 (C-6).

Campestrine-II (**2**) showed a ketone band at 1706 cm^{-1} and a conjugated ester band at 1685 cm^{-1} in its IR spectrum. The UV spectrum contained absorption maxima at 244 and 306 nm, also suggesting a conjugated chromophore, in contrast with **1**. This triterpenoid showed NMR spectra very similar to those of **1**. The ^{1}H NMR spectrum indicated significant differences only in the hydrogen signals for the A- and B-rings. In compound **2**, the signals for H-1 and H-6 were more deshielded than in **1**, and H-7 was more shielded. The tertiary methyl group attached to C-4, observed at δ 1.65 is more shielded (Δδ 0.11) than in **1**. This information, and the differences observed for the carbon shifts of A- and B-rings in the ^{13}C NMR spectrum, suggested that **1** and **2** are stereoisomers, and the difference in their structure is only in the stereochemistry of the $\Delta^{1,10}$ double bond. This isomeric relationship was also suggested by the EI-mass spectrum of the mixture which showed the same fragmentation pattern.

The *cis* or *trans* stereochemistry for the $\Delta^{1,10}$ double bond in **1** and **2**, was determined by NOE experiments, which showed correlations in **1** of the proton signal at δ 5.86 (H-1) with the proton signals at δ 1.76 (H-4) and 3.32 (OMe), indicating the *trans* configuration for this compound. Correlations of the proton signal at δ 5.84 (H-1) with the proton signals at δ 1.50 (H-25) and δ 3.33 (OMe) indicated the *cis* configuration for **2**.

This appears to be the first report of a C_{26}-type carbon skeleton from the Celastraceae or Hippocrateaceae families (Brüning and Wagner, 1978; Gunatilaka, 1996). Regelone, isolated from *Tripterygium wilfordii* var. *Regelii* (Takaishi *et al.*, 1997) and celastranhydride, obtained from *Kokoona zeylanica* (Gamlath *et al.*, 1990), which are also *seco*-A-ring quinonemethide triterpenoid derivatives, are C_{27}-type examples. Campestrine-I (**1**) and -II (**2**), which lack the typical quinonemethide chromophore, probably are formed by oxidative cleavage of A-ring, resulting in the two isolated stereoisomers.

Experimental

General experimental procedures

NMR: on a Bruker DRX 400, in $CDCl_3$ and TMS as int. standard; GC-MS: low resolution on a HP-2576 instrument; EIMS: low resolution on a VG Platform II (Fisons) instrument; HRMS were obtained on a Fisons VG Autospec; $[\alpha]_D$: Perkin Elmer 241 instrument; DCCC: Tokyo Rikakikai Co. with 300 columns (40 cm x 2 mm i.d.).

Plant material

Roots of *P. campestris* were collected in a Savannah reserve at Universidade Federal de São

Carlos, in October, 1992 and identified by Dr. Maria Helena de O. Antunes, Departamento de Botânica, Universidade Federal de São Carlos, São Paulo, Brazil, where a voucher specimen (no. 2845) is deposited.

Extraction and isolation

The roots were dried, powdered (2.8 kg) and successively extracted with hexane, CH_2Cl_2, and MeOH. The combined extracts were submitted to liquid-liquid partition, resulting in hexane, MeOH, EtOAc, 1-butanol and aqueous extracts. The MeOH extract (3.5 g) was submitted to droplet countercurrent chromatography (DCCC) using the aqueous layer of hexane: MeOH:H_2O:EtOAc (5:4:1:2 v/v/v/v) as the stationary phase and the organic layer as the mobile phase, in a flow rate of 0.6 ml/min. The collected fractions were monitored by TLC and grouped in 22 fractions. Fractions 13 and 14 were flash chromatographed on silica gel, eluting with hexane: EtOAc (8:2 v/v), to afford tingenone and a mixture of triterpenoids. The latter was then purified by prep. TLC (silica gel; hexane: CH_2Cl_2:MeOH, 8:2:0.5 v/v/v) yielding campestrine-I (**1**, 2.1 mg) and a mixture containing **1** and **2**. This mixture was rechromatographed on recycling HPLC using a Shim-pack column (silica 5 μm; 2.0×25 cm; hexane: CH_2Cl_2:MeOH-8:2:0.5 v/v/v; flow rate: 3 ml/min; detection; UV 254 nm), affording campestrine-II (**2**, 1.0 mg).

Spectroscopic data

Campestrine-I (**1**) was obtained as pale amorphous yellow powder, $[\alpha]_D^{20}$ +20.6° (*c* 0.002, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε) 243 (3.11), 287 (2.71), 363 (2.38) nm; IR (KBr) ν_{max} 2928, 2853, 1735, 1708, 1421, 891 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.00 (3H, *s*, CH_3-27), 1.00 (3H, *d, J* = 6.4 Hz, CH_3-30), 1.04 (3H, *s*, CH_3-28), 1.30 (3H, *s*, CH_3-26), 1.53 (3H, *s*, CH_3-25), 1.76 (3H, *s*, CH_3-4), 1.85 (1H, *d, J* = 14.4 Hz, H-22β), 2.19 (1H, *dd, J* = 15.2 and 6.8 Hz, H-19α), 2.51 (1H, *ddq, J* = 13.5, 7.0 and 6.5 Hz, H-20), 2.91 (1H, *d, J* = 14.4 Hz, H-22α), 3.32 (3H, *s*, O–CH_3), 5.86 (1H, *d, J* = 2.0 Hz, H-1), 6.06 (1H, *d, J* = 6.8 Hz, H-7), 6.38 (1H, *dd, J* = 6.8 and 2.0 Hz, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.1 (C-30), 19.7 (C-27), 22.5 (C-26), 24.7 (C-4), 28.4 (C-15), 29.7 (C-12), 32.0 (C-19), 32.5 (C-28), 33.4 (C-11), 35.5 (C-16), 36.8 (C-25), 38.2 (C-17), 39.6 (C-9), 40.5 (C-13), 41.9 (C-20), 43.4 (C-18), 44.1 (C-14), 50.5 (O–CH_3), 52.5 (C-22), 103.7 (C-5), 110.7 (C-1), 115.9 (C-7), 126.4 (C-6), 161.1 (C-8), 163.7 (C-2), 165.2 (C-10), 213.8 (C-21); EIMS *m/z* 410 $[M]^+$ (1), 395 (12), 379 (7), 220 (61), 189 (61), 188 (78), 160 (100), 109 (68), 107 (58), 105 (61); HRMS *m/z* 410.2842 (calcd. for $C_{27}H_{38}O_3$, 410.2821).

Campestrine-II (**2**) was obtained as pale amorphous yellow powder, $[\alpha]_D^{20}$ +39.2° (*c* 0.0021, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ε): 244 (3.36), 306 (3.08), 363 (2.76) nm; IR (KBr) ν_{max} 2926, 2851, 1707, 1685, 1459, 896 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 1.00 (3H, *s*, CH_3-27), 1.00 (3H, *d, J* = 6.4 Hz, CH_3-30), 1.04 (3H, *s*, CH_3-28), 1.30 (3H, *s*, CH_3-26), 1.50 (3H, *s*, CH_3-25), 1.65 (3H, *s*, CH_3-4), 1.85 (1H, *d, J* = 14.4 Hz, H-22β), 2.19 (1H, *dd, J* = 15.2 and 6.8 Hz, H-19α), 2.51 (1H, *ddq, J* = 13.5, 7.0 and 6.5 Hz, H-20), 2.91 (1H, *d, J* = 14.4 Hz, H-22α), 3.33 (3H, *s*, O–CH_3), 5.84 (1H, *d, J* = 2.0 Hz, H-1), 6.08 (1H, *d, J* = 6.8 Hz, H-7), 6.34 (1H, *dd, J* = 6.8 and 2.0 Hz, H-6); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 15.1 (C-30), 19.8 (C-27), 22.5 (C-26), 28.2 (C-4), 28.4 (C-15), 29.7 (C-12), 32.1 (C-19), 32.6 (C-28), 33.3 (C-11), 35.5 (C-16), 36.5 (C-25), 38.2 (C-17), 39.5 (C-9), 40.3 (C-13), 41.9 (C-20), 43.4 (C-18), 44.1 (C-14), 51.0 (O–CH_3), 52.5 (C-22), 105.6 (C-5), 110.6 (C-1), 115.9 (C-7), 125.8 (C-6), 160.4 (C-8), 163.7 (C-2), 166.1 (C-10), 213.7 (C-21); EI-MS *m/z* 410 $[M]^+$ (1), 395 (6), 379 (3), 220 (38), 189 (38), 188 (66), 160 (100), 109 (43), 107 (39), 105 (33); HRMS *m/z* 410.2835 (calcd. for $C_{27}H_{38}O_3$, 410.2821).

Acknowledgement

The authors thank Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), Fundação de Apoio à Pesquisa da UFG (FUNAPE) for the financial support, Michael Murgu for EIMS, and Marcos Eberlin, IQM-UNICAMP for HRMS.

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