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GERANYLATED FLAVONOIDS FROM *DORSTENIA POINSETTIFOLIA*

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Abstract—Two new geranylated flavonoids, poinsettifolins A and B, were isolated from the extracts of the herb *Dorstenia poinsettifolia*, and the structures were determined with NMR spectroscopy and mass spectrometry. In addition, the flavone 5,7,4-trihydroxy-8-prenylflavone (licoflavone C), the chalcones 4,2′,4′-trihydroxy-3′-prenylchalcone (isobavachalcone) and isobavachromene, the triterpene butyrospermol, and the carotenoid lutein were isolated. © 1998 Elsevier Science Ltd. All rights reserved

The leaves of *Dorstenia poinsettifolia* var. *angusta* Engl. (Moraceae), a scrambing herb indigenous to the humid forests of Cameroon [1], are used in folk medicine for the treatment of yaws and infected wounds [2]. No phytochemical study of this species has been reported, although flavonoids have been isolated from the related species *D. multiradiata* [M. Iwu, pers. comm.] while benzofurans have been reported from *D. barnimiana* [3]. In the course of our continuing search for new antiparasitic agents from Cameroonian medicinal plants [4], we have investigated the whole plant extracts of *D. poinsettifolia*. Five flavonoids, including two new geranylated compounds (**1** and **2**), a triterpene, and a carotenoid were isolated and characterised.

RESULTS AND DISCUSSION

The EIMS spectrum of poinsettifolin A (**1**) showed a molecular ion at *m/z* 504, while the ^{1}H NMR (data given in Table 1) and ^{13}C NMR (data given in Table 2) spectra indicated the presence of 28 non-exchangeable protons and 30 carbons. This is consistent with the elemental composition $C_{30}H_{32}O_7$, giving **1** an unsaturation index of 15. The ^{1}H NMR spectrum of **1** revealed the presence of a 3-methyl-2-butenyl substituent [δ 1.62 (3H, *s*), 1.75 (3H, *s*), 3.28 (2H, *s*, J = 7.2 Hz) and 5.18 (1H, *t*, J = 7.2 Hz)], a 4-methyl-3-pentenyl substituent [δ 1.51 (3H, *s*), 1.60 (3H, *s*), 1.62/1.76 (2H, *m*), 2.05 (2H, *m*) and 5.05 (1H, *t*, J = 7.2 Hz)], a methyl group [δ 1.38 (3H, *s*)], a *cis* double bond with protons resonating at δ 5.48 and 6.81 (1H each, *d*, J = 10.0 Hz), and a trisubstituted benzene [ring B, δ 6.90 (1H, *d*, J = 8.5 Hz), 7.59 (1H, *dd*, J = 8.5 and 2.1 Hz), and 7.72 (1H, *d*, J = 2.1 Hz)]. No protons are directly attached to rings A and C, and in order to satisfy the unsaturation index **1** must contain a fourth ring. The positioning of the substituents on the flavonol skeleton could be made following the correlations observed in the HMBC spectrum (summarised in Fig. 2). Especially important are the correlations between 1″-H and C-7, C-8 and C-9, and between 1‴-H and C-5, C-6 and C-7, which together with the correlations from 2″-H to C-8 and from 2‴- H to C-6 establish that ring A has three oxygen substituents. The chemical shifts of the carbons to which no long-range correlations could be observed in the NMR-experiment performed (C-3, C-4 and C-10) are in agreement with those reported previously for similar flavonols, e.g. petalostemumol G [5], confirming that **1** is a flavonol. The proposed structure was further supported by the correlations observed in a NOESY spectrum, for example the correlation between 2′-H and 1″-H.

The elemental composition of poinsettifolin B (**2**) is by EIMS and 1D NMR spectra suggested to be $C_{30}H_{34}O_5$. The presence of 10 C/C double bonds and one carbonyl group is indicated by the ^{13}C NMR data (see Table 2), showing that the structure of poinsettifolin B (**2**) contains 3 rings. The major difference

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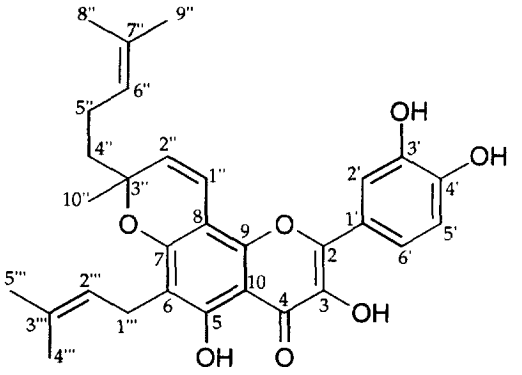

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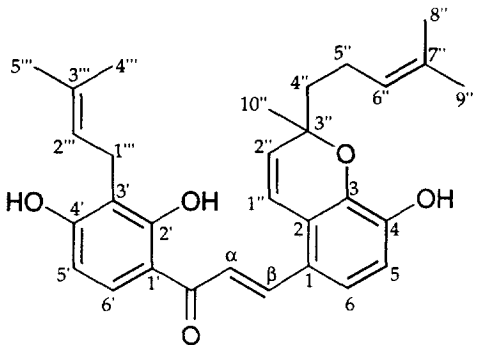

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Fig. 1.

Table 1. ^{1}H (500 MHz) NMR data (δ; multiplicity; J) for poinsettifolins A (**1**) and B (**2**) in $CDCl_3$: CD_3OD 9:1, with the $CHCl_3$ signal (7.26 ppm) as reference. The coupling constants J are given in Hz

H	1		2
2	—	β	7.99, *d*, 15.2
3	—	α	7.32, *d*, 15.2
5	—	6′	7.55, *d*, 9.0
6′	—	5′	6.31, *d*, 9.0
2′	7.72, *d*, 2.1		—
5′	6.90, *d*, 8.5	5	6.68, *d*, 8.5
6	6.58, *dd*, 2.1, 8.5	6	7.12, *d*, 8.5
1″	6.81, *d*, 10.0		6.74, *d*, 10.2
2″	5.48, *d*, 10.0		5.65, *d*, 10.2
4″	1.62/1.76, *m*		1.60/1.66, *dd*, 7, 11
5″	2.05, *m*		1.99, *m*
6″	5.03, *t*, 7.1		4.96, *t*, 7.2
8″	1.51, *s*		1.44, *s*
9″	1.60, *s*		1.53, *s*
10″	1.38, *s*		1.32, *s*
1‴	3.28, *d*, 7.2		3.26, *d*, 7.2
2‴	5.17, *t*, 7.2		5.14, *t*, 7.2
4‴	1.75, *s*		1.68, *s*
5‴	1.62, *s*		1.56, *s*

between the two poinsettifolins is that the latter contains a 1,2-*trans*-disubstituted double bond, and long-range ^{1}H-^{13}C correlations (summarised in Fig. 2) show that this is conjugated with a keto function and situated between two aromatic systems as in a chalcone derivative. As with poinsettifolin A (**1**), the presence of a 3-methyl-2-butenyl group, 4-methyl-3-pentenyl substituent, a methyl group, and a *cis* double bond is indicated by the ^{1}H NMR data (see Table 1), as well as two 1,2,3,4-tetrasubstituted benzene rings. As can be seen in Fig. 2, HMBC correlations show how the various parts fit together. The major MS fragments (at *m/z* 391 and 205) are explained by the loss of a 4-methyl-3-butenyl group (-C_6H_{11}, 83) and by the scission of the bond between the carbonyl group and C-α, and the latter fragment shows that the C-2′ and C-4′ oxygens are free hydroxyl groups.

The poinsettifolins A (**1**) and B (**2**) are new additions to a small group of cyclic C-geranylated flavonoids reported to date, mostly from species belonging to Moraceae. In addition to the two new compounds, the flavone 5,7,4-trihydroxy-8-prenylflavone (licoflavone C) [16], the chalcones 4,2′,4′-trihydroxy-3′-prenylchalcone (isobavachalcone) [7], and isobavachromene [8], the triterpene butyrospermol [9], and the carotenoid lutein [10] were isolated from the same extract, and identified by comparison of their spectroscopic and physical data with those reported in the literature.

EXPERIMENTAL

Plant material

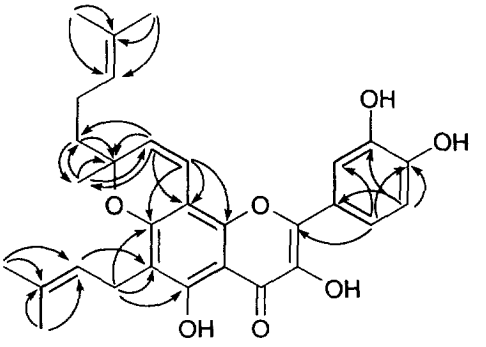
The whole plants of *Dorstenia poinsettifolia* var. *angusta* Engl., were collected in Kribi, Cameroon, in November 1995. A voucher specimen (PM No. 138) was authenticated by Mr Paul Mizili at the Cameroon National Herbarium, Yaoundé, Cameroon, where it is deposited.

Extraction and isolation

The powdered dried plant material (900 g) was extracted by percolation with a mixture of CH_2Cl_2–MeOH (1:1). The crude organic extract was partitioned between hexane and 80% MeOH, the MeOH–H_2O phase was diluted with water to 60% MeOH and extracted with CH_2Cl_2. The solvents were evaporated from the hexane and the CH_2Cl_2 extracts, to yield 14 g and 24 g, respectively. A TLC analysis showed that the two extracts were qualitatively very similar. They were thus combined, and a portion (30 g) was subjected to CC on silica gel (70–200 Mesh) eluted with mixture of hexane–Me_2CO (from 9:1, via 4:1, to

Table 2. ^{13}C (125 MHz) NMR data (δ, multiplicity) for poinsettifolins A (**1**) and B (**2**) in $CDCl_3$: CD_3OD 9 : 1, with the $CDCl_3$ signal (77.0 ppm) as reference

C	1		2
2	145.6, *s*	*β*	139.6, *d*
3	135.4, *s*	*α*	119.5, *d*
4	175.2, *s*	C=O	191.7, *s*
5	157.0, *s*	6′	128.8, *d*
6	111.5, *s*	5′	107.1, *d*
7	157.4, *s*	4′	162.0, *s*
8	100.6, *s*	3′	115.4, *s*
9	149.1, *s*	2′	163.6, *s*
10	103.1, *s*	1′	113.2, *s*
1′	123.0, *s*	1	122.6, *s*
2′	114.4, *d*	2	121.5, *s*
3′	144.3, *s*	3	139.8, *s*
4′	146.7, *s*	4	146.8, *s*
5′	115.1, *d*	5	115.3, *s*
6′	120.5, *d*	6	119.4, *d*
1″	115.5, *d*		118.7, *d*
2″	125.4, *d*		131.2, *d*
3″	80.4, *s*		78.3, *s*
4″	41.5, *t*		40.2, *t*
5″	22.6, *t*		22.5, *t*
6″	123.6, *d*		123.5, *d*
7″	131.8, *s*		131.7, *s*
8″	17.4, *q*		17.2, *q*
9″	25.5, *q*		25.3, *q*
10″	26.8, *q*		25.5, *q*
1‴	21.1, *t*		21.4, *t*
2‴	121.8, *d*		121.8, *d*
3‴	131.5, *s*		131.8, *s*
4‴	17.7, *q*		17.4, *q*
5‴	25.6, *q*		25.4, *q*



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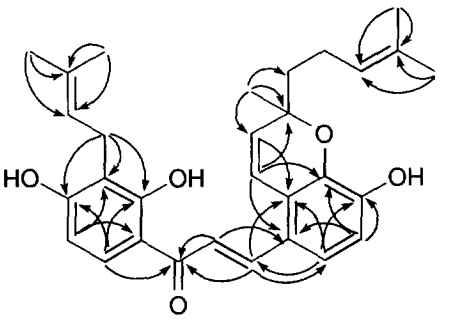

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Fig. 2. Pertinent HMBC correlations observed with poinsettifolin A (**1**) (top) and poinsettifolin B (**2**) (bottom).

3 : 2), and finally with pure Me_2CO. Four successive frs were obtained, I (7.0 g, hexane–Me_2CO 9 : 1), II (1.2 g, hexane–Me_2CO 4 : 1), III (1.9 g, hexane–Me_2CO 3 : 2) and IV (5.2 g, Me_2CO). Further purification of these frs was achieved by chromatography on Baeckström AB Separo columns with a continuous gradient of hexane–Me_2CO. Fraction I yielded the triterpene butyrospermol (259 mg) and a series of straight-chain aliphatic esters, while fraction II yielded poinsettifolins A (**1**) (85 mg) and B (**2**) (52 mg), isobavachalcone (208 mg) and the carotenoid lutein (18 mg). Frn III yielded licoflavone C (47 mg) and isobavachromene (38 mg), while frn IV yielded a mixture of inseparable products. For each of the compounds, an additional purification by CC on LH-20 gel eluted with hexane–CH_2Cl_2 1 : 1 was required to obtain analytically pure samples (this removed the last traces of chlorophyll). TLC experiments were performed on silica gel GF_{254} precoated plates, and detection was accomplished with an UV lamp at 254 nm or by spaying with 50% H_2SO_4 followed by heating to 120°.

Spectroscopy

^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) were recorded at room temp. with a Bruker ARX500 spectrometer with an inverse multinuclear 5 mm probehead equipped with a shielded gradient coil. The spectra were recorded in $CDCl_3$ or $CDCl_3$–CD_3OD 9 : 1, and the $CHCl_3/CDCl_3$ signals (7.26 and 77.0 ppm, respectively) were used as reference. The chemical shifts (δ) are given in ppm, and the coupling constants (J) in Hz. COSY, HMQC and HMBC experiments were recorded with gradient enhancements using sine shaped gradient pulses. For the 2D heteronuclear correlation spectroscopy the refocusing delays were optimised for $^1J_{CH} = 145$ Hz and $^nJ_{CH} = 10$ Hz. The raw data were transformed and the spectra were evaluated with the standard Bruker UXNMR software (ref. 941001). Mass spectra were recorded with a Jeol SX102 spectrometer.

Poinsettifolin A (**1**). Compound **1** was obtained as yellow crystals from hexane–EtOAc, mp 201–203°. $[\alpha]_D^{22}$ −7.2° ($CHCl_3$; c {0.53}). Analysis: found: C, 71.41; H, 6.39. $C_{30}H_{32}O_7$ requires: C, 71.40; H, 6.40%. UV λ_{max}^{MeOH} nm (log ε): 235 (4.16), 250 (4.19), 285 (4.10), 347 (3.88), 3.92 (3.84); +$AlCl_3$ 230 (5.00), 265 (4.97), 303 (4.92), 484 (4.93) and +$AlCl_3$+HCl:

232 (4.14), 253 (4.23), 295 (4.00), 383 (3.80), 457 (4.08). See Tables 1 and 2 for ^1H and ^{13}C NMR data. EIMS (probe) 70 eV, *m/z* (rel. int.): 504 $[M]^+$ (27), 489 (8), 449 (6), 435 (6), 421 (100), 391 (5), 365 (11), 215 (7), 189 (2), 183 (2), 137 (5), 69 (2), 41 (2).

Poinsettifolin B (**2**). Compound **2** was obtained as yellow crystals from hexane–EtOAc, mp 168–170°. $(\alpha)_D^{22}$ ($CHCl_3$; c {0.63}). Analysis: Found: C, 75.92; H, 7.22. $C_{30}H_{34}O_5$ requires: C, 75.91; H, 7.23%. UV λ_{max}^{MeOH} nm (log ε): 282 (3.93), 382 (4.35) and $+AlCl_3$: 272 (4.88), 440 (4.86). See Tables 1 and 2 for ^1H and ^{13}C NMR data. EIMS (probe) 70 eV, *m/z* (rel int.): 474 $[M]^-$ (34), 459 (4), 391 (100), 205 (41), 203 (9), 187 (9), 149 (40), 69 (5), 41 (4).

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