Notes

Anthraquinone Derivatives from *Heterophyllaea pustulata*

Susana C. Núñez Montoya, Alicia M. Agnese, and José L. Cabrera*

Farmacognosia, Departamento de Farmacia, Facultad de Ciencias Químicas, Universidad Nacional de Córdoba, IMBIV-CONICET (Instituto Multidisciplinario de Biología Vegetal−Consejo Nacional de Investigaciones Científicas y Técnicas), Ciudad Universitaria, 5000 Córdoba, Argentina

Received May 26, 2005

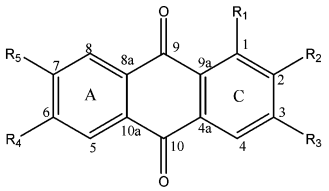
From the leaves of *Heterophyllaea pustulata* two new monomeric anthraquinones, heterophylline (1,6-dihydroxy-7-methoxy-2-methylanthraquinone, **1**) and pustuline (2-hydroxy-3-methoxy-7-methylanthraquinone, **2**), and one new bianthraquinone, (*S*)-5,5′-bisoranjidiol [(*S*)-5,5′-bis(1,6-dihydroxy-2-methylanthraquinone), **3**], were isolated. Furthermore, the iridoid glycoside asperuloside and three known flavonoids, quercetin, isoquercitrin, and quercetin-3-*O*-*β*-D-glucosyl-6″-acetate, were obtained. The structures were determined by analysis of their spectroscopic data and chemical evidence.

Heterophyllaea pustulata Hook. f. (Rubiaceae) is a shrub of 2 to 3 m high, popularly known as "cegadera". It grows in the Andean mountain range of the Northwest of Argentina and Bolivia between 2500 and 3000 m of altitude, where it is well known for its toxicity.[1] The animals that ingest this plant suffer from dermatitis and blindness (kerato-conjunctivitis), which are due to a typical photosensitization reaction, clinically presented without jaundice.[2]

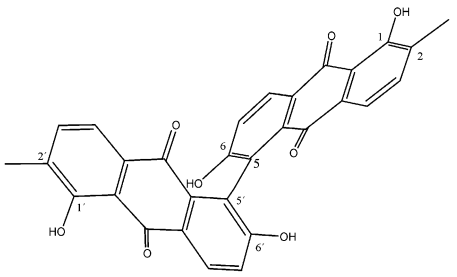
We have previously demonstrated the antibacterial and antifungal activity in vitro of different extracts of *H. pustulata* as well as their low acute toxicity in vivo.[3] The first chemical investigation of these bioactive extracts showed that the majority of the metabolites were 9,10-anthraquinone aglycones (AQs), among which soranjidiol, soranjidiol 1-methyl ether, rubiadin, rubiadin 1-methyl ether, 2-hydroxy-3-methylanthraquinone, damnacanthal, and damnacanthol were isolated and identified.[3] Herein, we report the isolation and identification of three new anthraquinone derivatives, 1,6-dihydroxy-7-methoxy-2-methylanthraquinone (heterophylline, **1**), 2-hydroxy-3-methoxy-7-methylanthraquinone (pustuline, **2**), and 5,5′-bis(1,6-dihydroxy-2-methylanthraquinone) (5,5′-bisoranjidiol, **3**), from the benzene leaves extract. In addition, the EtOAc extract afforded three known flavonoids and an iridoid glycoside. They were identified by comparison with literature data as quercetin,[4−6] isoquercitrin,[7,8] quercetin-3-*O*-*β*-D-glucosyl-6″-acetate,[5] and asperuloside.[9−11] The latter compound was previously reported from other genera of Rubiaceae.[12] The structures of the new AQ derivatives, related to the known soranjidiol, were elucidated on the basis of analysis of their spectroscopic/spectrometric properties.

Isolation and purification of the compounds from the leaves of *H. pustulata* were done by repeated combination of several chromatographic techniques (Sephadex column, preparative PC, and preparative TLC). Compounds **1**−**3**, which gave a positive Bortraëger test for anthraquinones, were obtained from the benzene extract. By contrast, the known flavonoids and the iridoid were isolated from the EtOAc extract.

The HREI mass spectrum of **1** gave a molecular ion at *m*/*z* 284.0675 (100%) that suggested the molecular formula $C_{16}H_{12}O_5$ (calcd 284.0685). The ^{1}H NMR spectrum contained signals attributable to four aromatic protons, a pair of doublets ($J = 7.7$ Hz) due to two *ortho*-positioned aromatic protons (H-3 and H-4), and a pair of singlets due to two *para*-positioned aromatic protons (H-5 and H-8). This spectrum also showed signals for three substituents, which were assignable to a methyl, an *O*-methyl group, and a phenolic hydroxyl *peri* to the carbonyl group.[13] The presence of this OH in the 1-position was confirmed by an intense IR absorption band at 1632 cm^{-1} together with a ^{13}C NMR signal at δ 188.1, which indicated the presence of a hydrogen-bonded carbonyl group (C-9).[13,14] Regarding the biosynthetic pathway leading to the anthraquinones in the Rubiaceae,[13] it was expected that the methyl group should be in the 2-position. NOE interactions between the methyl protons (δ 2.30) and OH-1/H-3 together with COLOC correlations of the methyl signal at δ 15.7 with H-3/H-4 confirmed the methyl group at C-2. The placement of the OCH_3 at C-7 was assigned by COLOC correlation of the OCH_3 to C-7, which showed a two-bond correlation with H-8, and this proton was also correlated with the hydrogen-bonded carbonyl group (C-9 at δ 188.1). In addition, the OCH_3 shows NOE correlation with H-8 (δ 7.56). Bearing in mind the molecular weight of this compound together with the fact that hydroxyl groups in 2-, 3-, 6-, or 7-positions (free



	R_1	R_2	R_3	R_4	R_5
Heterophylline (1)	OH	Me	H	OH	OMe
Pustuline (2)	H	OH	OMe	H	Me



(*S*)-5,5′-Bisoranjidiol (3)

* To whom correspondence should be addressed. Tel: +54-351-4334163. Fax: +54-351-4334127. E-mail: jcabrera@dqo.fcq.unc.edu.ar.

10.1021/np050181o CCC: $33.50 © 2006 American Chemical Society and American Society of Pharmacognosy
Published on Web 04/04/2006

Table 1. NMR Data (δ in ppm) for 5,5′-Bisoranjidiol (**3**) and Soranjidiol (**4**) in DMSO-d_6

3			**4**				
position	$^{13}C^a$	$^1H^b$	position	$^{13}C^a$	$^1H^b$	NOESY[c]	COLOC (C)[d]
1, 1′	159.6		1	159.9			
2, 2′	133.4		2	134.2			
3, 3′	136.9	7.34 (d, 7.6)	3	136.8	7.62 (d, 7.7)	4	1, 2, 4, 4a, 10, 9a, 2-Me
4, 4′	118.4	7.57 (d, 7.6)	4	118.6	7.68 (d, 7.7)	3	1, 2, 4a, 10
4a, 4a′	131.6		4a	131.1			
5, 5′	127.1		5	112.5	7.49 (d, 2.6)	7	5, 7, 8a, 10
6, 6′	161.5		6	163.8			
7, 7′	125.2	7.36 (d, 8.5)	7	124.5	7.27 (dd, 2.6, 8.5)	5, 8	7
8, 8′	128.6	8.27 (d, 8.5)	8	129.8	8.14 (d, 8.5)	7	6, 8a
8a, 8a′	120.1		8a	121.4			
9, 9′	187.8		9	187.6			
9a, 9a′	114.4		9a	114.7			
10, 10′	182.3		10	181.8			
10a, 10a′	132.5		10a	135.6			
1, 1′-OH		13.18 (s)	1-OH		13.13 (s)		
2, 2′-Me	15.5	2.29 (s)	2-Me	15.6	2.32 (s)	3	1, 2, 3, 2-Me

[a] At 50 MHz, referenced to DMSO-d_6 at 39.5. [b] At 200.13 MHz, referenced to DMSO-d_6 at 2.54. [c] NOESY correlations from H to H. [d] COLOC correlations from H to C.

OH) are not readily observable in ^{1}H NMR spectra,[13] it was possible to infer that the fourth substituent might be an aromatic hydroxyl. This was verified by the IR (3344 cm^{-1}, free OH) and EIMS ([M − OH]$^+$) spectra.[14,15] From the above data, compound **1** was identified as 1,6-dihydroxy-7-methoxy-2-methylanthraquinone (heterophylline).

Compound **2** showed a molecular ion [M]$^+$ at *m*/*z* 268.0734 (100%) in the HREIMS, consistent with the molecular formula $C_{16}H_{12}O_4$ (calcd 268.0736). Its ^{1}H NMR spectrum exhibited signals due to five aromatic protons, two one-proton singlets (H-1 and H-4), and an ABX pattern for three aromatic protons. This suggested that the C-ring was substituted in the 2- and 3-positions, whereas the A-ring had only one substituent, where either the 6- or 7-position is possible. In addition, signals for three substituents were observed, a methyl group, an *O*-methyl group, and a hydroxyl group.[13] Only one medium IR absorption band at 1670 cm^{-1} indicated that both carbonyl groups were non-hydrogen-bonded and together with the band at 3323 cm^{-1} corroborated a free hydroxyl group.[14] The location of this OH at C-2, adjacent to the *O*-methyl at C-3, was established by NOE correlations between OH-2 and H-1/OCH_3-3 and between OCH_3-3 and H-4/OH-2. The proximity of CH_3-7 to OH-2 was established by the following COLOC interactions: methyl protons (δ 2.50) with C-7 (δ 144.6), C-7 with H-8 (δ 7.94, d, J = 1.4), H-8 with C-9 (δ 181.9), and C-9 with H-1 (δ 7.53, s), together with a NOE correlation between H-1 and OH-2. On the other hand, C-10 correlated with H-6 (δ 7.69, dd, J = 1.4 and 7.9) and H-4 (δ 7.61, s). Thus, CH_3 was unequivocally located at C-7 from 2D NMR experiments. Combinations of these data defined compound **2** as 2-hydroxy-3-methoxy-7-methylanthraquinone (pustuline).

The positive-ion FAB-mass spectrum of **3** showed a pseudomolecular ion [M + H]$^+$ peak at *m*/*z* 507, which suggested a bianthraquinone derivative coincident with the molecular formula $C_{30}H_{18}O_8$ (calcd 506). The number of signals present in the ^{13}C NMR spectrum of **3** accounted for only half (15 peaks) of the carbon atoms of the molecule (Table 1). The ^{1}H NMR spectrum suggested a tetrasubstituted anthraquinone (Table 1) with the presence of an OH group in the *peri* position and a methyl group in the 2-position.[13] The principal peaks in the IR spectrum indicated a free OH group as the third substituent and confirmed the existence of a *peri*-positioned OH group by the carbonyl group absorptions.[14] On the basis of these data and the high molecular weight, it was suspected that **3** must be a symmetrical bianthraquinone.[16] Reductive cleavage of **3** with alkaline sodium dithionite[17] gave only one product, which was identified as soranjidiol (**4**) by UV−vis, IR, ^{1}H NMR, MS,[13] and co-chromatography with an authentic sample. The 2D NMR experiments (NOESY and COLOC) of **4** are recorded here for the first time (Table 1). Comparison of the NMR data of **3** and **4** proves that **3** is a symmetrical dehydro dimer of **4**. In the ^{1}H NMR spectrum of **3**, a *meta*-coupled doublet at δ 7.49 (assigned to H-5 in **4**) is absent, showing a C-5, C-5′ anthraquinone linkage. From the data obtained, it was concluded that compound **3** is 5,5′-bis-(1,6-dihydroxy-2-methylanthraquinone) (5,5′-bisoranjidiol). Compound **3** possesses axial chirality due to the C5−C5′ biphenyl bond. The CD spectrum revealed a positive Cotton effect centered at 243 nm, which correlated with an intense $\pi-\pi^*$ UV absorption. This indicates (*P*)-axial chirality, and hence an *S*-configuration of the biphenyl bond in **3**.[18] Figure 1 shows the 3D structure of **3** with minimized conformational energy, which was generated by AM1 calculation using MOPAC.

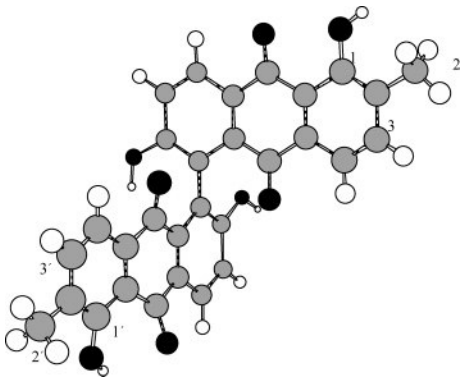


Figure 1. Molecular drawing of **3**.

Experimental Section

General Experimental Procedures. Melting points were determined on an Electrothermal 9100 melting point apparatus. The CD spectrum was recorded on a JASCO Model J-810 spectropolarimeter, which was calibrated with 10-camphorsulfonic acid. UV spectra were recorded on a Spectronic Genesis 5 UV−vis spectrophotometer. IR spectra were obtained with a Nicolet 5-sxc-FTIR infrared spectrophotometer. NMR spectra were measured on a Bruker AC 200 spectrometer. Chemical shifts (δ) are reported in ppm relative to TMS as internal standard and coupling constants (*J* values) in Hz. EIMS were obtained on a Variant Mat CH-7A at 70 eV. HRMS were recorded on a VG ZAB2SE (1996) spectrometer. FABMS were performed on a JEOL JMS-SX/SX102 spectrometer at 6 keV. Column chromatography (CC) was performed on Sephadex LH-20 (Pharmacia). TLC was performed on precoated silica gel 60 plates (Merck), and anthraquinones were revealed under UV light with NH_4OH vapors and by spraying the plates with a 10% KOH solution in EtOH. Paper chromatography (PC) was used to separate iridoids and flavonoids. These were visualized by using UV light and NH_4OH fumes.

Plant Material. Leaves of *H. pustulata* were collected in "La Almona", Jujuy Province, Argentina, in April 1996. Plant material was identified by Prof. Dr. Gloria Barboza (Instituto Multidisciplinario de Biología Vegetal, CONICET−Universidad Nacional de Córdoba). A voucher specimen has been deposited at Museo Botánico de Córdoba (U.N.C.) as M.E. Lázzaro s/n, CORD 305.

Extraction and Isolation. Dried and fragmented leaves (387.0 g) were extracted in a Soxhlet apparatus using solvents of different polarities in the following order: petrol, benzene, and EtOAc. The benzene extract (5.04 g) was dissolved in 10% aqueous $NaHCO_3$ and extracted with $CHCl_3$. The organic extract obtained was evaporated to dryness, and the residue was dissolved in 1 M NaOH and extracted with Et_2O. The aqueous alkaline extract was acidified with HCl and extracted with Et_2O. Thus, a final ether extract (I) rich in anthraquinones and without chlorophylls was obtained.[19] This extract (I) was submitted to CC eluted with $CHCl_3$, followed by a gradient of $CHCl_3$−EtOAc (3:7−7:3) and finally an acetone−EtOAc (3:7−7:3) gradient. The eluents were monitored by TLC with benzene−EtOAc (8:2) as mobile phase. Five major fractions were obtained (A−E). From fraction A, compounds **1** (20.1 mg) and **2** (6.4 mg) were separated by preparative TLC, developed first with $CHCl_3$ and then with benzene−EtOAc (1:1). Compound **3** (10.9 mg) was purified from fraction D by preparative TLC with benzene−EtOAc (1:1) as mobile phase.

The EtOAc extract (15.18 g) was dissolved in H_2O and partitioned with benzene, Et_2O (II), and EtOAc (III). The two last extracts (II and III) were separately subjected to CC eluted with benzene−EtOH (7:3) and increasing proportions of EtOH up to a 3:7 ratio. From II quercetin (13.0 mg) was obtained, which was purified by preparative PC using 40% HOAc as mobile phase. III provided isoquercitrin (16.1 mg) and quercetin-3-*O*-β-D-glucosyl-6″-acetate (28.7 mg); the former was purified by preparative PC with 15% HOAc and the latter by CC using the identical mobile phase. From the remaining aqueous extract, asperuloside (6.2 mg) was isolated by CC with EtOH as mobile phase. It was purified by preparative PC by using 15% HOAc as mobile phase.

Heterophylline (1): yellow crystals (acetone); mp 268−271 °C; UV (EtOH) λ_{max} (log ϵ) 287 (1.08), 398 (sh) (0.19), 410 (0.20), 431 (sh) (0.16) nm; (EtOH/MeONa) λ_{max} (log ϵ) 302 (0.97), 473 (0.39) nm; ($CHCl_3$) λ_{max} (log ϵ) 239 (0.34), 281 (1.17), 302 (sh) (0.35), 348 (sh) (0.06), 398 (sh) (0.16), 413 (0.17), 431 (sh) (0.13) nm; IR (KBr) ν_{max} 3344 (OH free), 2920, 2849, 1698 (C=O free), 1632 (C=O hydrogen-bonded), 1562, 1438, 1372 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 12.92 (1H, s, OH-1), 7.65 (1H, d, J = 7.7, H-4), 7.59 (1H, s, H-5), 7.59 (1H, d, J = 7.7, H-3), 7.56 (1H, s, H-8), 3.99 (3H, s, OMe-7), 2.30 (3H, s, Me-2); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 188.1 (C-9), 180.8 (C-10), 159.8 (C-1), 153.4 (C-7), 152.6 (C-6), 137.0 (C-3), 133.6 (C-2), 131.3 (C-4a), 126.8 (C-8a), 127.5 (C-10a), 118.5 (C-5), 114.9 (C-4), 112.2 (C-9a), 109.0 (C-8), 56.1 (OMe-7), 15.7 (Me-2); NOE correlations (H/H) OH-1/Me-2, H-3; H-3/OH-1, Me-2; OMe-7/H-8; Me-2/OH-1, H-3; COLOC correlations (H/C) H-3/C-1, C-2, C-4, C-4a, Me-2; H-4/C-2, C-4, C-4a; H-5/C-5, C-10; H-8/C-5a, C-6, C-7, C-8, C-8a, C-9, C-10a; Me-2/Me-2; OMe-7/C-7, OMe-7; EIMS m/z 284 $[M]^+$ (100), 269 $[M - CH_3]^+$ (12), 267 $[M - OH]^+$ (5), 255 $[M - COH]^+$ (11), 241 $[M - COCH_3]^+$ (19), 213 $[M - COCH_3 - CO]^+$ (11), 185 $[M - COCH_3 - 2CO]^+$ (6), 157 $[M - COCH_3 - 3CO]^+$ (2), 128 $[M - COCH_3 - 3CO - COH]^+$ (9), 115 (4); HREIMS m/z 284.0675 $[M]^+$ (calcd for $C_{16}H_{12}O_5$, 284.0685).

Pustuline (2): yellow-orange needles (acetone); mp 263−266 °C; UV (EtOH) λ_{max} (log ϵ) 248 (0.32), 287 (1.02), 338 (sh) (0.09), 386 (sh) (0.04) nm; (EtOH/MeONa) λ_{max} (log ϵ) 215 (0.47), 251 (0.54), 314 (0.76), 509 (0.08) nm; ($CHCl_3$) λ_{max} (log ϵ) 245 (sh) (0.29), 281 (1.28), 338 (0.11), 371 (sh) (0.06) nm; IR (KBr) $\nu_{max.}$ 3323 (OH free), 2922, 2851, 1670 (C=O free), 1592, 1517, 1462, 1381 cm^{-1}; ^1H NMR (DMSO-d_6, 200 MHz) δ 10.71 (1H, s, OH-2), 8.06 (1H, d, J = 7.9, H-5), 7.94 (1H, d, J = 1.4, H-8), 7.69 (1H, dd, J = 1.4 and 7.9, H-6), 7.61 (1H, s, H-4), 7.53 (1H, s, H-1), 3.99 (3H, s, OMe-3), 2.50 (3H, s, Me-7); ^{13}C NMR (DMSO-d_6, 50 MHz) δ 181.9 (C-9), 181.2 (C-10), 152.8 (C-2), 152.5 (C-3), 144.6 (C-7), 134.6 (C-6), 132.9 (C-8a), 130.9 (C-10a), 127.9 (C-4a), 126.6 (C-5), 126.6 (C-9a), 126.4 (C-8), 112.3 (C-1), 108.7 (C-4), 55.9 (OMe-3), 21.2 (Me-7); NOE correlations (H/H) OH-2/H-1, OMe-3, H-4; H-5/H-6, H-7, Me-7; H-8/H-5, H-6, Me-7; H-6/H-5, H-8, Me-7; H-4/OMe-3; H-1/OH-2; OMe-3/OH-2, H-4; Me-7/H-5, H-6, H-8; COLOC correlations (H/C) OH-2/C-1, C-3; H-5/C-6; H-8/C-6, C-7, C-9, C-10a; H-6/C-5, C-10, C-10a; H-4/C-1, C-3, C-4a, C-5a, C-9a, C-10; H-1/C-1, C-2, C-3, C-4a, C-9, C-9a; OMe-3/C-2, OMe-3; Me-7/C-6, C-7, Me-7; EIMS m/z 268 $[M]^+$ (100), 253 $[M - CH_3]^+$ (8), 239 $[M - COH]^+$ (20), 225 $[M - COCH_3]^+$ (29), 211 $[M - COH - CO]^+$ (5), 197 $[M - COCH_3 - CO]^+$ (17), 169 $[M - COCH_3 - 2CO]^+$ (9), 152 $[M - COCH_3 - 2CO - OH]^+$ (5), 139 (12), 115 (17); HREIMS m/z 268.0734 (calcd for $C_{16}H_{12}O_4$, 268.0736).

(*S*)-5,5′-Bisoranjidiol (3): orange amorphous powder (acetone); CD (c 0.15 mM, MeOH) $[\theta]_{243}$ + 2558; UV (EtOH) λ_{max} (log ϵ) 251 (sh) (0.08), 275 (0.07), 287 (sh) (0.07), 416 (sh) (0.04) nm; (EtOH/MeONa) λ_{max} (log ϵ) 239 (sh) (0.11), 317 (0.06), 431 (sh) (0.02), 521 (0.04) nm; ($CHCl_3$) λ_{max} (log ϵ) 236 (1.36), 272 (1.21), 287 (sh) (0.86), 409 (sh) (0.29), 419 (0.34), 437 (0.32) nm; IR (KBr) ν_{max} 3511 (OH free), 2921, 2847, 1672 (C=O free), 1623 (C=O hydrogen-bonded), 1572, 1455, 1430, 1363 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; NOE correlations (H/H) Me-2/H-4; H-3/OH-1, H-4; H-7/H-8; positive FABMS m/z 507 $[M + H]^+$ (calcd for $C_{30}H_{18}O_8$, 506.1).

Acknowledgment. The authors are thankful to S. Gohil (Chemistry Institute, Uppsala Faculty, Sweden) for measurements of FAB mass spectra, and to Prof. Dr. G. Barboza (Museo Botánico de Córdoba, Fac. Cs. Exactas y Naturales, Universidad Nacional de Córdoba, Argentina) for the identification of the species. This study was supported by Agencia Córdoba Ciencia, SeCyT (UNC), and FONCYT.

References and Notes

(1) Bacigalupo, N. M. In *Colección Científica INTA*; Cabrera, A. L., Ed.; INTA: Buenos Aires, 1993; pp 375−380.
(2) Hansen, E. W.; Martiarena, C. A. *Rev. Inv. Agropecuarias (INTA)* **1967**, serie 4: Patología Animal, *4*, 81−113.
(3) Núñez Montoya, S. C.; Agnese, A. M.; Pérez, C.; Tiraboschi, I. N.; Cabrera, J. L. *Phytomedicine* **2003**, *10*, 569−574.
(4) Harborne, J. B. *The Flavonoids−Advances in Research since 1986*; Chapman and Hall: London, 1994; pp 452−454, 487.
(5) Agrawal, P. K. *Carbon-13 NMR of Flavonoids*; Elsevier: New York, 1989; pp 154, 336−340.
(6) Mabry, T. J.; Markham, K. H.; Thomas, M. B. *The Systematic Identification of Flavonoids*; Springer-Verlag: New York, 1970.
(7) Bennini, B.; Chulia, A. J.; Kaouadji, M.; Thomasson, F. *Phytochemistry* **1992**, *31*, 2483−2486.
(8) Harborne, J. B. *Phytochemical Methods−A Guide to Modern Techniques of Plant Analysis*; Chapman and Hall: London, 1984; pp 69−76.
(9) Böjthe-Horváth, K.; Hetényi, F.; Kocsis, Á.; Szabó, L.; Varga-Balázs, M.; Máthé, I. Jr.; Tétényi, P. *Phytochemistry* **1982**, *21*, 2917−2919.
(10) Bailleul, F.; Delaveau, P.; Rabaron, A.; Plat, M.; Koch, M. *Phytochemistry* **1977**, *16*, 723−726.
(11) Briggs, L. H.; Cain, B. F.; Le Quesne, P. W.; Shoolery, J. N. *J. Chem. Soc.* **1965**, 2595−2601.
(12) Inouye, H.; Takeda, Y.; Nishimura, H.; Kanomi, A.; Okuda, T.; Puff, C. *Phytochemistry* **1988**, *27*, 2591−2598.
(13) Wijnsma, R.; Verpoorte, R. In *Progress in the Chemistry of Organic Natural Products*; Herz, W., Grisebach, H., Kirby, G. W., Tamm, Ch., Eds.; Springer-Verlag: New York, 1986; Vol. 49, pp 79−149.
(14) Bloom, H.; Briggs, L. H.; Cleverley, B. *J. Chem. Soc*. **1959**, 178−185.
(15) Beynon, J. H.; Williams, A. E. *Appl. Spectrosc*. **1960**, *14* (6), 156−160.
(16) Singh, J.; Singh, J. *Phytochemistry* **1986**, *25*, 1985−1987.
(17) Dagne, E.; Steglich, W. *Phytochemistry* **1984**, *23*, 1729−1731.
(18) Eliel, E. L.; Wilen, S. H. *Stereochemistry of Organic Compounds*; John Wiley & Sons: New York, 1994.
(19) Wijnsma, R.; Go, J. T. K. A.; Harkes, P. A. A.; Verpoorte, R.; Baerheim Svendsen, A. *Pytochemistry* **1986**, *25*, 1123−1126.

NP050181O