A New Phenanthrene Glycoside and Other Constituents from *Dioscorea opposita*

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Phytochemical investigation of the rhizome of *Dioscorea opposita* has led to the isolation of a new phenanthrene glycoside, 3,4,6-trihydroxyphenanthrene-3-*O*-β-D-glucopyranoside (1), and five known compounds, soyacerebroside I (2), adenosine (3), β-sitosterol (4), palmitic acid (5) and palmitoyloleoylphosphatidylcholine (6). Their structures were determined by spectroscopic methods, including 1D- and 2D-NMR. Compounds 1—6 exhibited no antifungal activity against the human pathogenic yeasts *Candida albicans*, *C. glabrata* and *C. tropicalis*.

Key words   *Dioscorea opposita*; Dioscoreaceae; phenanthrene glycoside; cerebroside, 2D-NMR

The *Dioscorea* genus includes an important group of tropical food yams, the edible portion of which is the tuber, or occasionally the bulbil. This is the case of the Chinese yam *D. opposita* Thunb (=*D. batatas* Decne) (Dioscoreaceae), which is widely cultivated in Asia as food.[1,2] This plant is also included in the Pharmacopoeia of the People's Republic of China and is widely used in traditional medicine for the treatment of anorexia, chronic diarrhoea, diabetes, seminal emission and excessive leukorrhea.[3] Previous phytochemical investigations carried out on *D. opposita* have resulted in the obtention of various constituents such as diosgenin,[4] gibberellins,[5] allantoin,[6] mucilages,[2] phytoalexin,[7] dioscorin,[8] phenanthrene and bibenzyl derivatives (batatasins).[9] As a part of our ongoing studies of the plants from the Dioscoreaceae family,[10] a new phenanthrene glycoside, 3,4,6-trihydroxyphenanthrene-3-*O*-β-D-glucopyranoside (1), was isolated together with five known compounds (2—6) from the rhizome of *D. opposita*. This paper describes the isolation and characterization by 2D-NMR of these compounds.

Six compounds were isolated from the Hexane and MeOH extracts of the rhizome of *D. opposita*, as described in Experimental. Compound 1, an amorphous solid, showed UV absorptions maxima at 222, 239 and 341 nm. The IR spectrum exhibited absorptions at 3330 (OH), 2850 (OMe), 1737 and 1435 cm^{-1} (aromatic rings). The high-resolution ESI mass spectrometry (HR-ESI-MS) (positive-ion mode) of 1 exhibited a pseudomolecular ion peak at *m/z* 411.1075 [M+Na]$^+$ (Calcd 411.1056), consistent with a molecular formula of $C_{20}H_{20}O_8Na$. The ^{1}H-NMR spectrum of 1 (Table 1) showed the presence of seven aromatic protons at δ 7.04, 7.05, 7.06, 7.25 (d, *J*=8.6 Hz), 7.42 (d, *J*=7.6 Hz), 7.59 (s) and 7.94 (d, *J*=7.4 Hz), one anomeric proton at δ 5.29 (d, *J*=7.9 Hz), and six protons at δ in the 3.93—4.37 ppm range due to the sugar residue. The ^{13}C-NMR spectrum combined with the DEPT experiment of 1, showed the existence of 20 carbons, including one methylene carbon at δ 61.7, four methine carbons at δ 70.7, 74.3, 77.5 and 77.8 and one anomeric carbon at δ 102.3 from the sugar moiety, seven aromatic methine carbons at δ in the 111.8—124.8 ppm range, and seven quaternary aromatic carbons at δ 109.5, 118.8, 127.8, 137.0, 144.0,

151.1 and 153.3, the last three being oxygenated. The presence of a sugar residue was determined to be a β-glucopyranosyl moiety by comparison of the ^{13}C-NMR chemical shifts with literature values,[10,11] and its absolute configuration was determined to be D by GC analysis, as described in Experimental. The analysis of COSY, TOCSY, NOESY, HMQC and HMBC spectra was used to elucidate the structure and establish the ^{1}H- and ^{13}C-NMR assignments of 1. Three pairs of *ortho*-coupled aromatic protons were observed through their ^{1}H–^{1}H COSY cross peaks between δ 7.05 (H-1) and δ 7.25 (H-2), between δ 7.04 (H-7) and δ 7.94 (H-8) and between δ 7.06 (H-9) and δ 7.42 (H-10). In the HMBC spectrum (Fig.1), H-1 (δ 7.05) showed correlations with two aromatic carbons, C-3 (δ 151.1) and C-10 (δ 111.8), and H-2 (δ 7.25)

Table 1. ^{1}H- and ^{13}C-NMR Data of 1 (in Pyridine-d_5),[a,b] δ in ppm, *J* in Hz

No.	δ_C	δ_H	HMBC correlations
1	116.2	7.05	
2	118.5	7.25 d (*J*=8.6)	
3	151.1		H-1, H-2, H-1′
4	153.3		H-2
4a	109.5		H-5
4b	137.0		H-5, H-8, H-9
5	124.8	7.59 s	
6	144.0		H-7
7	119.0	7.04	
8	118.7	7.94 d (*J*=7.4)	
8a	127.8		H-5, H-7, H-10
9	121.5	7.06	H-8
10	111.8	7.42 d (*J*=7.6)	H-1
10a	118.8		H-9, H-10
1′	102.3	5.29 d (*J*=7.9)	
2′	74.3	4.14	
3′	77.5	4.26	
4′	70.7	4.14	
5′	77.8	3.93	
6′	61.7	4.20, 4.37	

a) The assignments were based on DEPT, HMQC, and HMBC experiments (150 MHz for ^{13}C-NMR, 600 MHz for ^{1}H-NMR). *b*) Overlapping ^{1}H-NMR signals are reported without designated multiplicities.
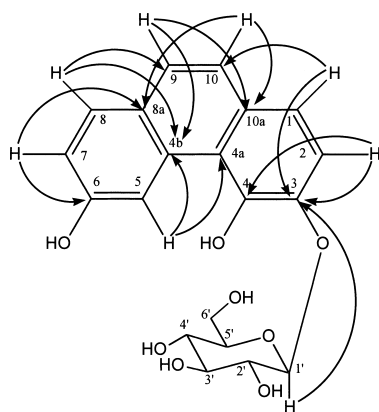

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Fig. 1

correlated with C-3 (δ 151.1) and C-4 (δ 153.3), respectively. H-5, an isolated proton (δ 7.59, s), showed HMBC correlations with three quaternary carbons C-4a (δ 109.5), C-4b (δ 137.0) and C-8a (δ 127.8). Cross peaks were equally observed between H-7 (δ 7.04) and C-6 (δ 144.0) and C-8a (δ 127.8). Additional HMBC correlations were observed between H-8 (δ 7.94) and C-4b (δ 137.0) and C-9 (δ 121.5), between H-9 (δ 7.06) and C-4b (δ 137.0) and C-10a (δ 118.8), and between H-10 (δ 7.42) and C-8a (δ 127.8) and C-10a (δ 118.8). All these data suggested the presence of a 3,4,6-trisubstitued phenanthrene.[12] The HMBC correlation between H-1′ (δ 5.29, d, $J=7.9$ Hz) of the glucopyranosyl residue and C-3 (δ 151.1), and the NOESY correlation between H-1′ (δ 5.29) and H-2 (δ 7.25), indicated the attachment of a glucopyranosyl moiety at C-3 of the phenanthrene residue. Thus, the structure of **1** was established to be 3,4,6-trihydroxyphenanthrene-3-*O*-β-D-glucopyranoside (Fig. 1), a new natural compound.[13]

The only reports of the isolation of phenanthrene glycosides concerned plants from the Orchidaceae family[12,14]; it is, to the best of our knowledge, the first report of the isolation of such secondary metabolites from the Dioscoreaceae family.

Compounds **2**—**6** were identified by interpretation of their spectral data, mainly FAB-MS and 2D-NMR (COSY, NOESY, HMBC, HMQC), as well by comparison with literature data as soyacerebroside I (**2**),[15] adenosine (**3**),[16] β-sitosterol (**4**),[17] palmitic acid (**5**),[16] and 1-*O*-palmitoyl-2-*O*-oleoyl-glycero-3-phosphocholine or 1-*O*-oleoyl-2-*O*-palmitoyl-glycero-3-phosphocholine (**6**),[13] respectively.

Soyacerebroside I (**2**) has been previously isolated from *Glycine max* (Leguminoseae),[18] *Prunus jamasakura* (Rosaceae),[19] and *Dimocarpus fumatus* and *Longan arillus* (Sapindaceae).[15,20] Plant cerebrosides are of interest because they have been shown to be cytotoxic,[21] anti-ulcerogenic,[22] and hepatoprotective.[23] To our knowledge, the isolation of **2** from *Dioscorea opposita* is the first account of the isolation of a cerebroside from the Dioscoreaceae family.[13]

Previous phytochemical investigations carried out on *Dioscorea opposita* have resulted in the obtention of antifungal compounds such as phytoalexins.[7] Nevertheless, compounds **1**—**6** were devoid of antifungal activity against *Candida albicans*, *C. glabrata* and *C. tropicalis* with MICs >200 μg/ml.

Experimental

General IR spectra were recorded on a Perkin-Elmer 281 spectrophotometer. UV spectra were performed using a Uvikon 931 spectrophotometer. GC analysis was carried out on a Termoquest gas chromatograph using a DB-1701 capillary column (30 m×0.25 mm, i.d.) (J & W Scientific), detection by FID, with the initial temperature maintained at 80 °C for 5 min and then raised to 270 °C at the rate of 15 °C/min, carrier gas: He. Optical rotations were taken with a Perkin-Elmer 241 polarimeter. The HR-ESI-MS was measured in positive ion mode on a Q-TOF 1-micromass spectrometer. 1D- and 2D-NMR spectra (^{1}H–^{1}H COSY, TOCSY, NOESY, HMQC and HMBC) were measured on a UNITY-600 spectrometer at the operating frequency of 600 MHz on a Varian INOVA 600 instrument equipped with a SUN 4 L-X computer system (600 MHz, for ^{1}H and 150 MHz for ^{13}C-NMR spectra). All chemical shifts (δ) are given in ppm, and the compounds were solubilized in pyridine-d_5. Column Chromatography (CC) was carried out on a Sephadex LH-20 (Pharmacia), silica gel column (63—200 μm). Vacuum Liquid Chromatography (VLC) was performed on C$_{18}$ reversed-phase (12×3 cm). Medium-Pressure Liquid Chromatography (MPLC) was carried out using a silica gel 60 (Merck, 15—40 μm) with a Gilson pump M 305, and eluted with CHCl$_3$–MeOH–H$_2$O (13 : 7 : 2, lower phase). TLC was performed on silica gel 60 F$_{254}$ (Merck), detected by spraying with Komarowsky reagent, a (5 : 1) solution of 2% 4-hydroxybenzaldehyde in MeOH and 50% H$_2$SO$_4$.

Plant Materials The rhizomes of *D. opposita*, harvested in April 1998 in Hexan province (China), were identified by Pr. Dr. H. Wagner. A voucher specimen was deposited under the number 3533 in the herbarium of the Laboratory of Pharmacognosy, University of Dijon, France.

Extraction and Separation Dried powdered rhizomes (400 g) were submitted to successive extractions in a Soxhlet by hexane (3 l, 24 h), CH$_2$Cl$_2$ (3 l, 24 h) and MeOH (4 l, 48 h). After evaporation of the solvent, the corresponding hexane (1.08 g), CH$_2$Cl$_2$ (1.31 g) and MeOH (24.2 g) extracts were obtained. The MeOH extract was dissolved in 400 ml H$_2$O and partitioned with *n*-BuOH (3×300 ml). One part of the *n*-BuOH extract (2 g) was subjected to VLC, then eluted with H$_2$O containing increasing amounts of MeOH. The fraction eluted with MeOH–H$_2$O (1 : 4) was finally submitted to MPLC to yield **1** (6 mg). Another part of the *n*-BuOH extract (4 g) was submitted to CC (Sephadex LH-20, MeOH) to give 7 fractions. Fraction 2 (1 g) was submitted to MPLC, as previously described, yielding **2** (12 mg). Fraction 6 (82 mg) was treated in the same way to give **3** (10 mg). The hexane extract was chromatographed on a silica gel column (63—200 μm), and eluted with CHCl$_3$ containing increasing amounts of MeOH, to give 7 fractions. The fraction CHCl$_3$–MeOH (98 : 2) (125 mg) was rechromatographed in the same way to yield compounds **4** (15 mg) and **5** (18 mg). The fraction CHCl$_3$–MeOH (92 : 8) (160 mg) was treated in the same way to yield **6** (13 mg).

3,4,6-Trihydroxyphenanthrene-3-*O*-β-D-glucopyranoside (**1**): Amorphous solid, HR-ESI-MS (positive ion-mode) *m/z*: 411.1075 [M+Na]$^+$, (Calcd for C$_{20}$H$_{20}$O$_8$Na: 411.1056). [α]$_D^{20}$ $-16°$ (*c*=0.25, MeOH). IR ν_{max} (CHCl$_3$) cm^{-1}: 3330, 2850, 1737 and 1435. UV λ_{max} (MeOH) nm: 222, 239, 341. ^{1}H- and ^{13}C-NMR: see Table 1.

Acid Hydrolysis A solution of compound **1** (3 mg) in 2 N aqueous CF$_3$COOH (5 ml) was refluxed on a water bath for 3 h. After extraction with CH$_2$Cl$_2$ (3×5 ml), the aqueous layer was repeatedly evaporated to dryness with MeOH until neutral, then glucose was identified by TLC with a standard using CHCl$_3$–MeOH–H$_2$O (8 : 5 : 1). Furthermore, a silylated derivate of the sugar was prepared according to the procedure previously described.[11] L-Cysteine methyl ester hydrochloride (0.06 mol/l) and HMDS-TMCS (hexamethyldisilazane–trimethylchlorosilane, 3 : 1) were added to the aqueous residue. After centrifugation of the precipitate, the supernatant was concentrated and partitioned between *n*-hexane and H$_2$O, and the hexane layer was analyzed by GC. D-Glucose was detected.

Antifungal Bioassay Minimum inhibitory concentrations (MICs) were performed using the broth dilution test.[24] For these bioassays, three human pathogenic yeasts were used: *Candida albicans* (IP 1180-79), *C. glabrata* and *C. tropicalis* (clinical isolates).

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