Sulfemodin 8-*O*-*β*-D-Glucoside, a New Sulfated Anthraquinone Glycoside, and Antioxidant Phenolic Compounds from *Rheum emodi*

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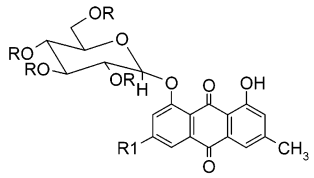
Received March 28, 2003

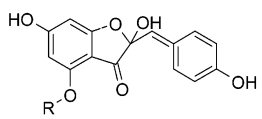
A sulfated emodin glucoside, emodin 8-*O*-*β*-D-glucopyranosyl-6-*O*-sulfate (**1**), was isolated from the roots of *Rheum emodi* in an investigation of the active constituents of this Nepalese medicinal plant, and its structure was determined by spectroscopic and chemical methods. Additionally, two rare auronols, carpusin (**2**) and maesopsin (**3**), besides other anthraquinones and phenolics, were isolated and identified. Compounds **2** and **3** showed significant antioxidant activity in the DPPH assay, while chrysophanol, physcion, and emodin and their 8-*O*-glucosides were found to be inactive.

Rheum emodi Wall. (syn. *Rheum australe* D. Don, Polygonaceae) is a stout herb, distributed in the alpine and sub-alpine zones of the Himalayas. The roots of this species are used widely in Ayurvedic medicine and in Asian folk medicine as a stomachic, purgative, astringent, and tonic, as well as in certain skin diseases. The drug is also administered in biliousness, lumbago, piles, chronic bronchitis, and asthma.[1−3] Only limited information is available concerning the chemical composition of this rhubarb species, with common anthraquinones such as emodin, chrysophanol, physcion, and rhein and the flavonoid rutin having been identified.[4,5] More recently, rheinal, rhein-11-*O*-*β*-D-glucoside, revandchinone 3, and the nonpolar oxanthrones revandchinones 1, 2, and 4 have been reported.[6,7] We now present the results of a detailed study of the polar compounds of *R. emodi* roots.

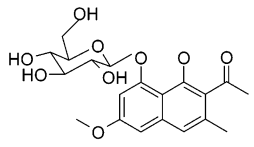
For the present investigation, the plant material was pre-extracted with petroleum ether to remove nonpolar substances. The resulting petroleum ether extract contained large amounts of emodin, chrysophanol, and physcion. The pre-extracted drug was then refluxed with methanol and purified by chromatography to yield the 8-*O*-glucopyranosides of emodin, chrysophanol, and physcion and the 8-*O*-gentiobiosides of chrysophanol, and physcion as the main anthraquinones besides some minor anthraquinone and anthrone glycosides.[8,9] In addition, an unknown, very polar anthracene derivative (**1**) and two rare auronols, carpusin (=marsupsin, **2**) and maesopsin (**3**), as well as torachrysone 8-*O*-*β*-D-glucoside (**4**) and epicatechin (**5**), were isolated. The known anthraquinones, their glycosides, and **5** were identified by co-chromatography with authentic substances, while for the structure elucidation of **1** and the identification of **2**−**4**, spectroscopic methods (^{1}H, ^{13}C NMR, COSY, HSQC, HMBC, HSQC-TOCSY, and selective NOE) and electrospray ionization mass spectrometry in the negative mode were applied.



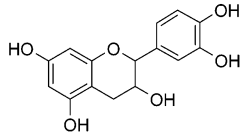
1 R = H, R1 = OSO_3H
6 R = OAc, R1 = OH



2 R = CH_3
3 R = H



4



5

The negative-ion ESIMS of **1** showed as base peak the pseudomolecular ion $[M^- - H]^-$ at *m*/*z* 511 and three fragment ions at *m*/*z* 431 ($[M^- - H^- - 80$ u$]^-$; 12%), *m*/*z* 349 ($[M^- - H^- - 162$ u$]^-$; 1%), and *m*/*z* 269 ($[M^- - H^- - 80$ u$^- - 162$ u$]^-$; 2%); the collisionally activated dissociation (CAD) MS of *m*/*z* 511 gave ions at *m*/*z* 431 (100%), *m*/*z* 349 (2%), and *m*/*z* 269 (12%). The molecular formula of the $(\mathbf{1}^- - H)^-$ ion was determined by negative-ion HRFABMS to be $C_{21}H_{19}SO_{13}$ (calcd 511.0546, found 511.0542).

By sequential ^{1}H and ^{13}C NMR as well as 2D NMR studies, including COSY, HSQC, HMBC, HSQC-TOCSY, and selective NOE experiments, the structure of **1** was shown to be a derivative of emodin glucoside. The anomeric proton at *δ* 5.10 exhibited a long-range correlation with the carbon atom at *δ* 161.8 (C-8) in an HMBC experiment, thus proving the linkage of the sugar moiety at C-8 of the aglycon. In addition, a NOE was observed between this anomeric proton and H-7. Compared with the resonances of authentic emodin 8-*O*-glucoside,[8] the signals of H-5 and H-7 were affected by marked substituent chemical shifts

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10.1021/np0301442 CCC: $25.00
Published on Web 07/19/2003

Table 1. ^{1}H and ^{13}C NMR Data of **1** and **6** (CD_3OD, δ, J in Hz)

	1		**6**	
position	δ_C	δ_H	δ_C	δ_H
1	164.1		163.9	
2	125.7	7.10 s	125.5	7.06 s
3	149.5		148.3	
4	121.4	7.56 s	120.7	7.50 s
5	115.0	7.87 d (2.2)	112.5	7.32 d (2.5)
6	160.4		170.0	
7	116.4	7.79 d (2.2)	111.8	6.90 d (2.5)
8	161.8		162.3	
9	189.4		188.1	
10	183.3		184.5	
1a	116.4		116.4	
4a	134.1		134.3	
5a	138.1		138.6	
8a	119.4		114.1	
11	22.3	2.44 s	22.2	2.41 s
1′	104.1	5.11 d (7.6)	100.8	5.38−5.42 o
2′	75.2	3.68−3.72 m	72.8	5.35−5.40 m
3′	77.9	3.54−3.61 m	74.6	5.37−5.43 m
4′	71.2	3.51−3.58 m	70.0	5.17 t (9.5)
5′	78.9	3.54−3.61 m	73.6	4.14−4.18 m
6′	62.5	3.81 dd (12.1, 3.7)	63.4	4.22 dd (12.3, 2.1)
		3.98 br d (12.1)		4.35 dd (12.3, 5.5)
Ac/Me			3 × 20.9,	2.01 s, 2.03 s,
			1 × 21.2	2.04 s, 2.09 s
Ac/CO			2 × 171.6,	
			1 × 171.9,	
			1 × 172.7	

of +0.83 and +1.08, respectively. The other signals in the ^{1}H NMR spectrum were in good agreement with those of emodin 8-*O*-glucoside. The assignment of the ^{13}C NMR resonances (Table 1) matched those of emodin 8-*O*-glucoside with the exception of C-5 to C-10 and C-8a. These findings suggested the presence of a strongly electronegative OH-6 substituent. Its inorganic nature was deduced from the lack of further carbon resonances. Thus, in accordance with the HRFABMS data and the polarity of the compound, the C-6-OSO_3H substitution was confirmed.

Upon acetylation[10] of **1**, the attachment of four acetate moieties to the sugar hydroxy groups and hydrolysis of the sulfate were established. The phenolic OH remained unacetylated, and emodin 8-*O*-(2′,3′,4′,6′-tetraacetyl)glucoside (**6**) was afforded. The ^{1}H and ^{13}C NMR shifts of the anthraquinone moiety of **6** showed good correlation with those of emodin 8-*O*-glucoside. Consequently, the structure of **1** was proven to be emodin 8-*O*-*β*-D-glucosyl-6-*O*-sulfate, and the substance was named sulfemodin-8-*O*-*β*-D-glucoside. To our knowledge, this is the first characterization of a sulfated anthraquinone glycoside. Until now, only two sulfated anthraquinone derivatives have been detected in *Rumex pulcher*, with a suggested sulfate substitution in the sugar moiety.[11]

Auronols are rarely occurring natural compounds of the flavonoid family.[12] In this study two such substances, **2** and **3**, were isolated. Compound **2** was the less polar substance. From the FABMS the molecular weight of 302 and the molecular formula of $C_{16}H_{14}O_6$ were deduced. The ^{1}H and ^{13}C NMR data were in excellent accordance with those reported for carpusin.[13] This substance, the synonym of which is marsupsin, was previously isolated only from *Pterocarpus marsupium*,[14] *Berchemia racemosa*,[13] and *Glycyrrhiza uralensis*.[15] It possesses antihyperglycemic activity.[16]

By NMR spectroscopic studies and FABMS the structure of **3** was elucidated as maesopsin.[17] This substance is restricted to very few species exclusively of the family Rhamnaceae.[17−21] Accordingly, this is the first report of auronols from the Polygonaceae.

Table 2. Free-Radical-Scavenging Activity of Compounds from *R. emodi*[a,b]

compound	IC_{50} (μg/mL)
carpusin (**2**)	4.7
maesopsin (**3**)	5.3
torachrysone 8-*O*-glucoside (**4**)	18.5
epi catechin (**5**)	1.7
ascorbic acid[a]	3.9

[a] Ascorbic acid was used as the positive control substance. [b] Chrysophanol, physcion, emodin, chrysophanol 8-*O*-glucoside, physcion 8-*O*-glucoside, and emodin 8-*O*-glucoside were inactive (IC_{50} >100 μg/mL).

Recently the radical-scavenging activity of rhubarb was shown not to be caused by anthraquinones, but by other phenolics, mainly stilbenes.[22] The scavenging effects of **2** and **3** on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical have never been investigated before. To determine a possible contribution of these compounds to the effects of *R. emodi* roots, their activity in the DPPH assay was studied. Both substances as well as **5** showed excellent radical-scavenging activity (Table 2), comparable to that of ascorbic acid. Torachrysone 8-*O*-*β*-D-glucoside (**4**) was about an order of magnitude less effective. In contrast, the anthraquinones tested were inactive in this assay, in a manner consistent with published data.[22] On the basis of the radical-scavenging activity of **2** and **3**, it can be inferred that auronols contribute to the antiinflammatory effect of *R. emodi*.

Experimental Section

General Experimental Procedures. UV spectra were recorded on a Beckman DU 640 spectrophotometer. IR spectra were determined as film spectra on silica disks on a Perkin-Elmer Spectrum 2000 FT-IR spectrometer. NMR spectra were recorded on a Varian Unity Inova 400 MHz NMR spectrometer in 5 mm sample tubes with TMS as internal standard. COSY, HSQC, HMBC, HSQC-TOCSY, and selective NOE experiments were obtained using conventional pulse sequences. FABMS were recorded on a Finnigan MAT 95 A mass spectrometer in the negative- and positive-ion modes, with an acceleration voltage of 5.0 kV in a glycerol matrix. ESIMS were recorded on a PE Sciex API 150 EX single quadrupole instrument configurated for negative ionization, the orifice plate voltage set at −20 and −80 V. Full-scan spectra were acquired over the range m/z 200−700. Scan time: 2 s. Semipreparative HPLC was carried out on two ISCO 2350 HPLC pumps with a linear UVIS-205 absorbance detector on a Nucleosil 100−7 C_{18}, 20 × 250 mm column. Silica DCC for vacuum-liquid chromatography (VLC) and Sephadex-LH-20 and silica gel 60 for column chromatography were obtained from ICN Pharmaceuticals (Eschwege, Germany), Pharmacia Biotech (Uppsala, Sweden), and Merck (Darmstadt, Germany), respectively. TLC was performed on precoated plates (silica gel 60 F_{254}, RP-2 F_{254}, RP-18 F_{254}, Merck, Germany) with the following systems. A: toluene−ethyl formate−formic acid (75:24:1). B: $CHCl_3$−MeOH−H_2O (90:5:5). C: $CHCl_3$−MeOH−H_2O (75:20:2). D: $CHCl_3$−MeOH−H_2O (65:30:6). E: MeOH−H_2O (1:1). F: MeOH. After drying, the plates were examined under UV_{366} light. 1,1-Diphenyl-2-picrylhydrazyl radical was obtained from Sigma-Aldrich (Vienna, Austria) and epicatechin (**5**) from Carl Roth (Karlsruhe, Germany). Chrysophanol, physcion, emodin, chrysophanol 8-*O*-glucoside, physcion 8-*O*-glucoside, emodin 8-*O*-glucoside, chrysophanol 8-*O*-gentiobioside, and physcion 8-*O*-gentiobioside were isolated from a commercial *Rheum* extract earlier.[23]

Plant Material. Roots of *Rheum emodi* were collected from Gorkha district, Nepal, in October 1996 and authentified by

Senior Botanist T. M. Shresta, Department of Natural Resources, Ministry of Forest and Soil Conservation, His Majesty's Government, Kathmandu, Nepal. A specimen (No. Rhemsa 6/96) is deposited in the Institute of Pharmacognosy, University of Vienna.

Extraction and Isolation. The air-dried, powdered roots (600 g, 3.6% total anthraquinone content, determined according to the monograph "Rhubarb" in the European Pharmacopoeia) were pre-extracted three times in portions of 150 g with petroleum ether (1.5 L) under reflux. The remaining drug was dried and then extracted four times with methanol (1.5 L) under the same conditions. The extraction yielded 8 g of petroleum ether extract (40% total anthraquinone content) and 200 g of methanol extract (9.1% total anthraquinone content). The latter was fractionated by VLC on silica DCC 60 (54 × 12.5 cm) using $CHCl_3$−MeOH mixtures of increasing polarity to yield fractions VM 1−VM 12. From the three most nonpolar fractions (7 g) and the petroleum ether extract the genins chrysophanol (1.5 g), physcion (1.4 g), and emodin (3.2 g) were obtained by crystallization. Five fractions of medium polarity (34.5 g) were separated by column chromatography on silica 60. For the separation of the four most polar fractions (134 g) VLC on silica DCC 60 with ethyl acetate−MeOH−H_2O mixtures of increasing polarity was applied. From different fractions emodin 8-*O*-glucoside (6.1 g), chrysophanol 8-*O*-glucoside (3.1 g), physcion 8-*O*-glucoside (3.2 g), chrysophanol 8-*O*-gentiobioside (0.4 g), physcion 8-*O*-gentiobioside (0.5 g), **4** (0.4 g), and **5** (0.2 g) were isolated.

Sulfemodin 8-*O*-*β*-D-Glucoside (1). From the VLC fractionation (100 × 8.2 cm) of fraction VM 10 (58.4 g) on silica DCC 60A with ethyl acetate−MeOH−H_2O mixtures as mobile phase, seven subfractions were obtained. Subfraction 5 (16.3 g) was further separated by VLC (60 × 4.6 cm) eluting the same stationary phase with $CHCl_3$−MeOH−H_2O mixtures to yield 16 fractions. Fraction 13 (4.6 g) was purified by column chromatography (100 × 4 cm) on Sephadex LH 20 with methanol−water mixtures, and the resulting subfraction 7 (185 mg) was separated by semipreparative HPLC. This purification yielded 6.3 mg of **1**: dark orange amorphous substance; UV (MeOH) λ_{max} 326, 410 nm; IR (silica disk) ν_{max} 3434, 2919, 1632, 1593, 1367, 1301, 1263, 1069 cm^{-1}; ^1H NMR and ^{13}C NMR data, see Table 1; ESIMS (negative ion) *m*/*z* 511 $[M - H]^-$ (100), 431 (12), 349 (1), 269 (2); CADMS *m*/*z* 431 (100), 349 (2), 269 (12); HRFABMS (negative ion) *m*/*z* 511.0542 (calcd for $C_{21}H_{19}SO_{13}$, 511.0546).

Carpusin (=marsupsin, 2). Column chromatography (100 × 3 cm) of fraction VM 4 (2.3 g) on silica 60 with ethyl acetate−MeOH−H_2O mixtures as mobile phase yielded eight subfractions. From subfraction 2 (648 mg) 169 mg of **2** was crystallized: whitish crystals, freely soluble in methanol.

Maesopsin (3). Column chromatography (80 × 5 cm) of fraction VM 8 (8.5 g) on silica 60 with ethyl acetate−MeOH−H_2O mixtures as mobile phase yielded nine subfractions. Subfraction 6 (172 mg) was further separated by column chromatography (100 × 1.5 cm) on silica, using $CHCl_3$−MeOH−H_2O mixtures as mobile phase, resulting in seven fractions. Fraction 5 (65 mg) after another purification step (column chromatography 65 × 1 cm) with the same stationary and mobile phase yielded 40 mg of **3**: amorphous, whitish powder, soluble in methanol.

Emodin 8-*O*-(2′,3′,4′,6′-Tetraacetyl)glucoside (6). An aliquot (4 mg) of **1** was acetylated with 0.4 mL of acetic anhydride and 4-(dimethylamino)pyridine (6 mg) in 0.2 mL of pyridine for 4 h at room temperature. After a cleanup by preparative TLC on silica using ethyl acetate−methanol−water (100:16.5:13.5) as mobile phase 2.1 mg of **6** were isolated. ESIMS (negative ion) *m*/*z* 599 $[M - H]^-$ (100), 557 (12), 539 (8), 395 (18), 377 (2), 252 (26).

DPPH Assay. The free-radical-scavenging capacity of test compounds was determined with DPPH. A 100 μM solution of DPPH was mixed with different concentrations of the compounds tested and the absorbance read at 515 nm.[24]

Acknowledgment. The authors are grateful to Mag. E. Pemp and Dr. I. Werner for technical assistance.

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NP0301442