Lignan Oligosaccharide Esters from *Eritrichium rupestre*

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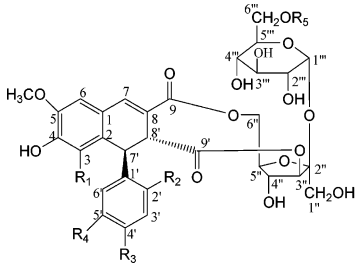
Three new lignan oligosaccharide esters, rupestrin A (**1**), rupestrin B (**2**), and rupestrin C (**3**), were isolated from the ethanol extract of *Eritrichium rupestre*. Their structures were elucidated on the basis of spectroscopic and chemical evidence.

Eritrichium rupestre (Pall.) Bunge is widely distributed in the northwest and northeast of China. As a Chinese folk medicine, it is extensively used to treat headache, angeitis, and anthrax. Especially, it is the preferred medicine prescribed for flu cure in Inner Mongolia of China.[1] An EtOH extract of the aerial part of *E. rupestre* was concentrated and partitioned with solvents further into petroleum ether-, $CHCl_3$-, EtOAc-, *n*-BuOH-, and H_2O-soluble fractions. The EtOAc fraction was subjected to column chromatography with silica gel and finally purified by Sephadex LH-20 to yield compounds **1** (20 mg), **2** (14 mg), and **3** (9 mg).



1 R_1=OCH_3 R_3=OH R_2=R_4=H R_5=$COCH_3$

2 R_1=OCH_3 R_3=OH R_2=R_4=R_5=H

3 R_1= OH R_3= H R_2=R_4= OCH_3 R_5=$COCH_3$

Compound **1** was obtained as a colorless powder, and its molecular formula was deduced as $C_{34}H_{38}O_{18}$ from HRFABMS (*m*/*z* 757.1956 $[M + Na]^+$). The molecular formula indicated 16 degrees of unsaturation. Its UV spectrum exhibited absorption bands at 202, 225, 254, and 355 nm. The IR spectrum showed absorptions of hydroxyls (3440, 3280 cm^{-1}), carbonyl groups (1740, 1720 cm^{-1}), and phenyl rings (1636, 1517 cm^{-1}).

The ^1H NMR spectrum (Table 1) of **1** exhibited two aromatic protons at δ 8.15 (1H, s) and 6.89 ppm (1H, s) and a set of AA′BB′ resonance protons at δ 7.37 (2H, d, J = 8.0 Hz) and 7.19 (2H, d, J = 8.0 Hz), the latter suggesting the presence of a 1,4-disubstituted aromatic ring. The signals at δ 3.74, 3.65, and 1.72 showed the presence of three tertiary methyl protons. The ^{13}C NMR spectrum (Table 1) revealed 34 carbons, and the signals at δ 174.6, 170.9, and 167.4 represented three carbonyl groups. The analysis of ^{13}C NMR, DEPT, and HMQC spectra revealed three methyls, three methylenes, 16 methines, and 12 quaternary carbons. Hydrolysis of **1** with 0.2% NaOH indicated the presence of sucrose under detection by TLC,[2] which was further supported by the HMBC, $^1H-^1H$ COSY, and TOCSY data. Furthermore, the cross-peaks between the carbonyl carbon at δ 170.9 and the methyl protons at δ 1.72 and between the carbonyl carbon at δ 170.9 and the proton at δ 4.88 (H-6‴) were observed in the HMBC spectrum (Figure 1), indicating an acetyl group at C-6‴ of sucrose.

Table 1. ^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) Data of **1**−**3** (in pyridine-d_5)[a]

	1		**2**		**3**	
position	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C	δ_H (J in Hz)	δ_C
1		122.1		122.1		121.8
2		124.8		124.8		125.3
3		147.0		147.0		147.2
4		144.9		144.7		144.9
5		148.6		148.5		148.6
6	6.89 s	109.4	6.86 s	109.4	6.89 s	109.4
7	8.15 s	141.5	8.03 s	141.3	8.13 s	141.7
8		122.5		122.6		122.6
9		167.4		167.4		167.4
1′		136.1		136.2		136.7
2′	7.37 d (8)	129.4	7.45 d (8.5)	129.5		146.9
3′	7.19 d (8)	116.5	7.22 d (8.5)	116.5	7.23 d (8.0)	116.8
4′		157.9		157.6	7.06 d (8.5)	121.0
5′	7.19 dd (8)	116.5	7.22 dd (8.5)	116.5		148.7
6′	7.37 dd (8)	129.4	7.45 dd (8.5)	129.5	7.11 s	112.4
7′	5.22 br s	38.8	5.29 br s	38.7	5.19 br s	39.3
8′	4.78 br s	50.4	4.73 br s	50.7	4.76 br s	50.1
9′		174.6		174.4		174.5
1″	4.37 br d (9.5)	63.2	4.44 br d (12)	63.2	4.37 br s	63.3
	4.73 br d (7)		4.70 br s		4.71 m	
2″		109.9		109.9		109.8
3″	5.66 br s	80.5	5.65 br s	80.5	5.67 br s	80.3
4″	4.63 br s	87.7	4.62 br s	87.6	4.62 br s	87.5
5″	4.68 br s	74.7	4.64 br s	74.7	4.65 br s	74.7
6″	4.40 br d (12.5)	65.8	4.27 br d (12)	65.9	4.40 br s	65.9
	5.27 dd (12.5, 2.5)		5.23 br dd (12.5, 2.5)		5.29 dd (12, 2)	
1‴	6.13 br d (4)	94.5	6.17 br d (3.5)	94.7	6.13 d (3.5)	94.5
2‴	4.27 br d (9.5)	73.6	5.05 br d (9.5)	74.8	4.26 dd (8.5, 3)	73.6
3‴	4.81 t (9)	75.5	4.27 t (9)	75.8	4.75 m	75.4
4‴	5.23 t (9)	72.2	4.46 t (9)	71.4	5.13 t (8)	72.2
5‴	4.02 br t (9)	72.1	4.29 m	73.8	4.00 t (9)	72.0
6‴	4.76 d (7)	65.8	4.38 dd (12, 2.5)	62.2	4.68 m	62.2
	4.88 br d (11)		4.50 dd (12, 4)		4.83 br d (11)	
OCH_3-3	3.65 s	60.0	3.69 s	60.0		
OCH_3-5	3.74 s	56.2	3.74 s	56.1	3.72 s	56.1
OCH_3-2′					3.66 s	59.9
OCH_3-5′					3.86 s	56.2
CH_3CO		170.9				170.8
CH_3	1.72 s	20.4			1.77 s	20.4

[a] All assignments based on HMQC, HMBC, DEPT, $^1H-^1H$-COSY, and TOCSY experiments.

In addition to a sucrose and an acetyl group of **1**, the molecular formula of the remaining moiety was $C_{20}H_{18}O_6$, including two carbonyls and two methoxyl groups. Additionally, interpretation of the $^1H-^1H$ COSY spectrum led to assignment of two vicinal methine protons [δ 5.22 (H-7′) and 4.78 ppm (H-8′)]. Thus, **1** possesses a 2,7′-cycloligna-7-ene skeleton. The signals at δ 174.6 and 167.4 of the ^{13}C NMR were deduced as carbonyl carbons at C-9′ and C-9, respectively.[3] One methoxyl group was linked to C-3 and the other methoxyl group was linked to C-5, which was supported by the correlation of 3-OCH_3 (δ 3.65) and C-3 (δ 147.0)

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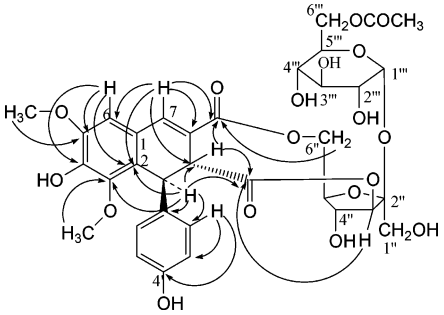

Figure 1. Principal HMBC correlations of **1**.

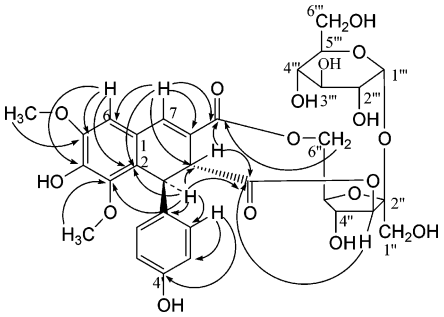

Figure 2. Principal HMBC correlations of **2**.

and the correlation of 5-OCH_3 (δ 3.74) and C-5 (δ 148.6) in the HMBC spectrum. One hydroxyl group was assigned to C-4 (δ 144.9). The other hydroxyl group was linked to C-4′ (δ 157.9), which was in accordance with four phenyl protons of the AA′BB′ system correlated with C-4′ (δ 157.9) in HMBC spectrum.

The above results suggested that **1** possesses a 4,4′-dihydroxy-3,5-dimethoxy-2,7′-cycloligna-7-ene-9,9′-dicarbonyl moiety and 6‴-acetyl sucrose unit. Besides these, the cross-peaks between C-9′ (δ 174.6) and H-3″ (δ 5.66) and between C-9 (δ 167.4) and H-6″ (δ 5.27) in the HMBC spectrum indicated that the sucrose moiety was connected at C-9′ and C-9 by ester bonds with the 3″- and 6″-hydroxyl groups, respectively. All proton and carbon signals were assigned by HMQC, DEPT, HMBC, ^{1}H−^{1}H COSY, and TOCSY data.

In the NOESY spectrum of **1**, the cross-peaks between H-2′ and H-7′ and between H-7′ and H-8′ were observed. The CD spectrum of **1**, which was similar to (1*S*,2*R*)-1,2-dihydro-6,7-dihydroxy-1-(3′,4′-dihydroxyphenyl)naphthalene-2,3-dicarboxylic acid dimethyl ester, [4,5] showed a positive Cotton effect at 357 nm and a negative Cotton effect at 256 nm. Thus, the structure of compound **1** was established as cyclic 3″→9′:6″→9-[(7′*S*,8′*R*)-4,4′-dihydroxy-3,5-dimethoxy-2,7′-cycloligna-7-ene-9,9′-dicarbonyl]-6‴-acetylsucroside and given the trivial name rupestrin A.

Compound **2** was obtained as a yellow powder. The molecular formula was determined as $C_{32}H_{36}O_{17}$ by HRFABMS (*m*/*z* 715.2131 $[M + Na]^+$). The UV spectrum showed strong absorptions at 202, 224, 253, and 353 nm, similar to compound **1**. The IR spectrum showed absorption bands for hydroxyls (3381 cm^{-1}), carbonyls (1736, 1720 cm^{-1}), and aromatic rings (1630, 1510 cm^{-1}). Hydrolysis of **2** with 0.2% NaOH also indicated the presence of sucrose via detection by TLC.

The characteristics of **2** in the ^{1}H NMR and ^{13}C NMR (Table 1) spectra were very similar to those of **1**. The ^{13}C NMR spectrum of **2** showed 32 carbons. Comparing the ^{13}C NMR spectrum with **1**, the signals for one methyl (δ 20.4) and the carbonyl (δ 170.9) carbon disappeared. Correspondingly, the methyl proton at δ 1.72 in **1** disappeared in the ^{1}H NMR spectrum of **2**. This suggests that **2** does not possess the acetyl group at C-6‴ as in **1**. HMQC and HMBC spectra (Figure 2) permitted the complete assignment of the proton and carbon signals of **2**. The CD spectrum of **2** was similar to that of **1**, showing a positive Cotton effect at 357 nm

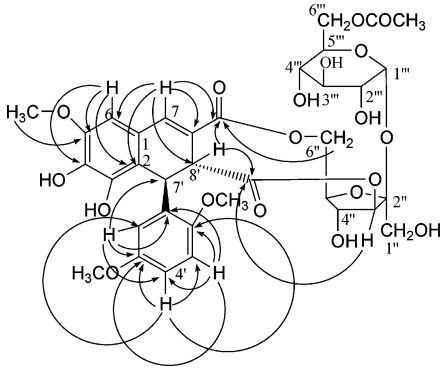

Figure 3. Principal HMBC correlations of **3**.

and a negative Cotton effect at 229 nm. Thus, the structure of compound **2**, rupestrin B, was elucidated as cyclic 3″→9′:6″→9-[(7′*S*,8′*R*)-4,4′-dihydroxy-3,5-dimethoxy-2,7′-cycloligna-7-ene-9,9′-dicarbonyl]sucroside.

Compound **3** was obtained as a light green powder. The molecular formula was deduced as $C_{35}H_{40}O_{19}$ from HRFABMS (*m*/*z* 787.2104 $[M + Na]^+$). Its UV data showed strong absorptions at 203, 255, 276, and 352 nm. The IR spectrum showed the presence of hydroxyls (3406 cm^{-1}), carbonyls (1730, 1715 cm^{-1}), and aromatic groups (1630, 1514 cm^{-1}). Hydrolysis of **3** with 0.2% NaOH indicated the presence of sucrose via detection by TLC.

The ^{1}H NMR spectrum of **3** showed five aromatic protons and three methoxyl protons. Correspondingly, the ^{13}C NMR of **3** also exhibited three methoxyl carbons. The ^{1}H NMR and ^{13}C NMR data (Table 1) were similar to those of **1**. Compared with **1**, compound **3** lacked an aromatic proton instead of a methoxyl group, which was consistent with its molecular formula. Three aromatic protons at δ 7.06 (d, J = 8.5 Hz), 7.23 (d, J = 8.0 Hz), and 7.11 (br, s) in the ^{1}H NMR spectrum, together with the correlation of H-6′ (δ 7.11) and C-1′ (δ 136.7) and of H-3′ (δ 7.23) and C-1′ (δ 136.7) in the HMBC (Figure 3), suggested the presence of a 1,2,5-trisubstituted phenyl ring. One methoxyl (δ 59.9) was linked to C-2′ and the other methoxyl (δ 56.2) to C-5′, which was supported by the cross-peaks between 2′-OCH_3 (δ 3.66) and C-2′ (δ 146.9) and between 5′-OCH_3 (δ 3.86) and C-5′ (δ 148.7).

On the basis of the molecular formula and NMR spectrum, **3** had two hydroxyls and one methoxyl group linked to the other phenyl ring. Additionally, the cross-peaks between H-6 (δ 6.89) and C-5 (δ 148.6); H-6 (δ 6.89) and C-4 (δ 144.9); and 5-OCH_3 (δ 3.72) and C-5 (δ 148.6) were observed in the HMBC, which suggested that the methoxyl at δ 56.1 was linked to C-5, and two hydroxyls were linked to C-3 and C-4. All signals were assigned on the basis of HMQC and HMBC spectra and comparison with **1**. The CD spectrum of **3** was similar to **1**, displaying a positive Cotton effect at 362 nm and a negative Cotton effect at 256 nm. Compound **3** was established as cyclic 3″→9′:6″→9-[(7′*S*,8′*R*)-3,4-dihydroxy-2′,5,5′-trimethoxy-2,7′-cycloligna-7-ene-9,9′-dicarbonyl]-6‴-acetylsucroside and named rupestrin C.

Experimental Section

General Experimental Procedures. Melting points were determined on an X4 micro melting point apparatus and are uncorrected. Optical rotations were measured on a Perkin-Elmer 341 digital polarimeter (Perkin-Elmer, Norwalk, CT) at 589 nm. UV spectra were recorded on a Hitachi UV-2201 spectrophotometer (Shimadzu, Kyoto, Japan) and CD data on a JASCO J-810 spectropolarimeter. IR spectra were recorded in KBr disks on an Impact 410 FTIR spectrophotometer. NMR data were acquired on INOVA-500 and -125 MHz spectrometers (Varian, San Francisco, CA) in pyridine-d_5 using tetramethylsilane (TMS) as an internal standard. FABMS were obtained on a VG-Autospec-3000 spectrometer (Thermo Electron, Manchester, UK). Silica gel (100−200, 300−400 mesh) and silica gel GF_{254} sheets (both from

QingDao Haiyang Chemical Group Co., Qingdao, Shandong Province, China) were used for column chromatography and TLC, respectively.

Plant Material. *E. rupestre* was collectted in June 2002 from DaQingShan of Inner Mongolia of China and identified by Dr. XiuSheng Pang (Pharmacy Department, Inner Mongolia Medical College). A voucher specimen (No. YA-02-0726) was deposited in the Herbarium of the Institute of Medicinal Plant Development, Chinese Academy of Medical Sciences and Peking Union Medical College.

Extraction and Isolation. Air-dried *E. rupestre* (5.0 kg) was extracted twice with 95% EtOH under reflux conditions, each for 2 h. Removal of the solvent in vacuo gave 110 g of dark syrup, which was suspended in H_2O and successively extracted with petroleum ether, $CHCl_3$, EtOAc, and *n*-BuOH. The EtOAc solubles (23 g) were subjected to silica gel (100−200 mesh) column chromatography, eluting with a $CHCl_3$−MeOH gradient to afford 45 fractions. Fractions 10 and 11 were combined and separated by chromatography ($CHCl_3$−MeOH = 15:1) on silica gel (300−400 mesh) to afford 30 subfractions. Subfraction 5 was repeatedly purified using silica gel (300−400 mesh) ($CHCl_3$−MeOH = 15:1) to give **1** (20 mg). Subfraction 8 was repeatedly fractionated on silica gel (300−400 mesh) ($CHCl_3$−MeOH = 10:1) and purified by Sephadex LH-20 column chromatography (MeOH) to give **3** (9 mg). Fraction 17 was repeatedly separated using silica gel (300−400 mesh) ($CHCl_3$−MeOH = 4:1) and purified by Sephadex LH-20 column chromatography (MeOH) to yield **2** (14 mg).

Rupestrin A (1): colorless powder (MeOH); $[\alpha]^{20}_D$ +79.4 (*c* 0.61, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.39), 225 (4.24), 254 (4.29), and 355 (4.10); IR ν^{KBr}_{max} (cm^{-1}) 3440, 3280, 1740, 1720, 1636, 1517, 1444, 1256, 1218, 1190, 1103, 1020; ^1H and ^{13}C NMR data, see Table 1; FABMS (positive) *m*/*z* 735 $[M + H]^+$; HRFABMS (positive) *m*/*z* 757.1956 $[M + Na]^+$ (calcd for $C_{34}H_{38}O_{18}Na$, 757.1955).

Rupestrin B (2): yellow powder (MeOH); $[\alpha]^{20}_D$ +101.7 (*c* 0.60, MeOH); UV (MeOH) λ_{max} (log ϵ) 202 (4.40), 224 (4.24), 253 (4.28), and 353 (4.08); IR ν^{KBr}_{max} (cm^{-1}) 3381, 1736, 1720, 1630, 1510, 1458, 1352, 1259, 1205, 1105, 1020, and 847; ^1H and ^{13}C NMR data, see Table 1; FABMS (positive) *m*/*z* 715.1 $[M + Na]^+$; HRFABMS (positive) *m*/*z* 715.2131 $[M + Na]^+$ (calcd for $C_{32}H_{36}O_{17}Na$, 715.2122).

Rupestrin C (3): light green powder (MeOH); $[\alpha]^{20}_D$ +78.5 (*c* 0.39, MeOH); UV (MeOH) λ_{max} (log ϵ) 203 (4.62), 255 (4.34), 276 (4.00), and 352 (3.99); IR ν^{KBr}_{max} (cm^{-1}) 3406, 1730, 1715, 1630, 1514, 1458, 1269, 1201, 1107, 1026, and 970; ^1H and ^{13}C NMR data, see Table 1; FABMS (positive) *m*/*z* 787 $[M + Na]^+$; HRFABMS (positive) *m*/*z* 787.2104 $[M + Na]^+$ (calcd for $C_{35}H_{40}O_{19}Na$, 787.2061).

Alkaline Hydrolysis of 1−3. To a MeOH (1 mL) solution of each compound (1 mg) was added 0.2% NaOH (0.2 mL), and the mixture was stirred for 20 h at room temperature. The reaction mixture was acidified by 1 M HCl solution and extracted with EtOAc. From the H_2O layer, sucrose was detected by TLC ($CHCl_3$−MeOH−H_2O, 3:3:0.2) in accordance with the standard sample.

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References and Notes

(1) *Chinese Medical Encyclopedia Meng Medical Branch*; Science Technology Press: Shanghai, 1990; p 212.
(2) Fukuyama, Y.; Sato, T.; Miura, I. *Phytochemistry* **1983**, *22*, 549−552.
(3) Sakakibara, I.; Ikeya, Y.; Hayashi, K. *Phytochemistry* **1992**, *9*, 3219−3223.
(4) Kobayashi, W.; Miyase, T.; Suzuki, S. *J. Nat. Prod.* **2000**, *63*, 1066−1069.
(5) Nishizawa, M.; Tsuda, M.; Hayashi, K. *Phytochemistry* **1990**, *29*, 2645−2649.

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