Lindleyanin and Bergapten-8-yl Sulfate from *Pleurospermum lindleyanum*

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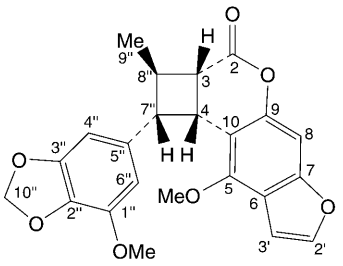
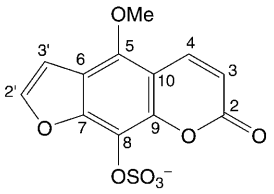
A novel cyclobutane-type lignan with a six-membered lactone ring, named lindleyanin (**1**), and a novel furocoumarinyl sulfate, named bergapten-8-yl sulfate (**2**), along with 7 known compounds were isolated from the whole plants of *Pleurospermum lindleyanum*. Their structures were established on the basis of spectral techniques and X-ray crystallographic analysis of **2**.

Introduction. – *Pleurospermum lindleyanum* (LIPSKY) B. FEDTSCH (Umbelliferae) is a Chinese folk medicine used for the treatment of hypertension, coronary heart disease, high-altitude sickness, and hepatitis in Xinjiang province of China [1]. Previously phytochemical studies on this plant reported the isolation of 11 compounds [1][2]. In the course of our phytochemical investigation on the whole plants, lindleyanin (**1**), a novel cyclobutane-type lignan with a six-membered lactone ring, and bergapten-8-yl sulfate (**2**), a novel furocoumarinyl sulfate, together with 7 known compounds, bergapten (=4-methoxy-7*H*-furo[3,2-*g*][1]benzopyran-7-one), isopimpinellin, isoimperatorin [1], lasidiol angelate [3][4], (*E*)-isoelemicin [5], isobutyl β-D-glucopyranoside [6], benzyl β-D-glucopyranoside [7], were isolated and identified. Herein, we describe the isolation and structural elucidation of the two new compounds **1** and **2**.[1])



1[1])



2[1])

[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*

Results and Discussion. – Lindleyanin (**1**) was obtained as optically inactive colorless gum. Its molecular formula $C_{23}H_{20}O_7$ was deduced from the quasimolecular-ion peak at m/z 431.1137 ($[M+Na]^+$) in the HR-ESI-MS. The IR absorption at 1751 cm^{-1} suggested the presence of a lactone group. The structure and relative configuration of **1**, *i.e.*, (3β,4β,7″β,8″α) were established by ^{1}H- and ^{13}C-NMR (*Table*) and 2D-NMR data. The relative configuration is in agreement with a biogenetic hypothesis that **1** is biosynthesized from bergapten and (*E*)-isomyristicin (=4-methoxy-6-[(1*E*)-prop-1-enyl]-1,3-benzodioxol) through a [2+2] cycloaddition reaction [9][10].

Table. *^{1}H- and ^{13}C-NMR Data of* **1** *and* **2**[a]); δ in ppm, J in Hz.

	1[1])		**2**[1])	
	δ(H)	δ(C)	δ(H)	δ(C)
C(2)	–	167.4 (*s*)	–	159.9 (*s*)
H–C(3)	3.17 (*dd*, *J*=8.7, 8.9)	39.7 (*d*)	6.31 (*d*, *J*=9.8)	112.4 (*d*)
H–C(4)	4.13 (*dd*, *J*=8.5, 8.7)	35.9 (*d*)	8.18 (*d*, *J*=9.8)	139.6 (*d*)
C(5)	–	151.0 (*s*)	–	145.1 (*s*)
C(6)	–	112.1 (*s*)	–	114.1 (*s*)
C(7)	–	156.2 (*s*)	–	151.4 (*s*)
H–C(8) or C(8)	6.94 (*d*, *J*=1.0)	93.4 (*d*)	–	121.1 (*s*)
C(9)	–	149.7 (*s*)	–	144.7 (*s*)
C(10)	–	102.9 (*s*)[b])	–	106.5 (*s*)
H–C(2′)	7.47 (*d*, *J*=2.3)	143.7 (*d*)	8.04 (*d*, *J*=2.3)	146.2 (*d*)
H–C(3′)	6.71 (*dd*, *J*=2.3, 1.0)	104.9 (*d*)	7.33 (*d*, *J*=2.3)	105.2 (*d*)
*Me*O–C(5)	3.42 (*s*)	57.9 (*q*)[c])	4.18 (*s*)	60.7 (*q*)
*Me*O–C(1″)	3.42 (*s*)	55.8 (*q*)[c])	–	–
C(1″)	–	142.8 (*s*)	–	–
C(2″)	–	133.9 (*s*)	–	–
C(3″)	–	148.0 (*s*)	–	–
H–C(4″)	6.15 (*d*, *J*=1.5)	102.9 (*d*)[b])	–	–
C(5″)	–	134.2 (*s*)	–	–
H–C(6″)	5.74 (*d*, *J*=1.5)	106.5 (*d*)	–	–
H–C(7″)	3.50 (*dd*, *J*=8.5, 10.2)	53.4 (*d*)	–	–
C(8″)	2.87 (*m*)	42.8 (*d*)	–	–
Me(9″)	1.27 (*d*, *J*=6.6)	19.5 (*q*)	–	–
CH_2(10″)	5.85, 5.88 (2*d*, *J*=1.4)	101.2 (*t*)	–	–

[a]) **1** in $CDCl_3$, at 500 (^{1}H) and 125 MHz (^{13}C); **2** in (D_6)DMSO, at 400 (^{1}H) and 100 MHz (^{13}C). [b]) Overlapping signals. [c]) Assignment may be interchanged.

The ^{13}C-NMR spectrum of **1** showed 23 C-signals (1 Me, 2 OMe, 1 OCH_2O, 4 CH (sp^3), 5 CH (sp^2), and 10 C (sp^2)). In the ^{1}H-NMR spectrum, signals for a 1,2,3,5-tetrasubstituted benzene ring (δ 6.15 and 5.74 (each *d*, *J*=1.5 Hz, 1 H)), a benzofuran moiety (δ 6.94, 6.71, and 7.47 (each 1 H; *J*(2′,3′)=2.3, *J*(3′,8)=1.0 Hz)) [8], an OCH_2O (δ 5.88 and 5.85 (each *d*, *J*=1.4 Hz, 1 H)), 2 MeO (δ 3.42 (*s*)), and 1 Me group (δ 1.27 (*d*, *J*=6.6 Hz)), as well as for four aliphatic protons (δ 4.13, 3.50, 3.17, and 2.87) were observed. The ^{1}H,^{1}H-COSY and HMQC data disclosed a methylcyclobutane unit (*Fig. 1*). The HMBC cross-peaks established the skeletal structure of **1** as shown in *Fig. 1* and the relationship of the proton spin system of the methylcyclobutane unit with the benzofuran moiety, the benzene ring, and the lactone group. Furthermore, the HMBC correlations confirmed the assignments of the OCH_2O and the 2 MeO groups. The relative configuration of **1** was elucidated by the NOESY correla-

tions. Me(9″)/H–C(3), H–C(3)/H–C(4), H–C(4)/H–C(7″), H–C(7″)/Me(9″), and H–C(8″)/H–C(4″) and H–C(6″)[1]), demonstrating the *cis*-orientation of the proton(s) at C(3), C(4) and C(7″) and of Me–C(8″).

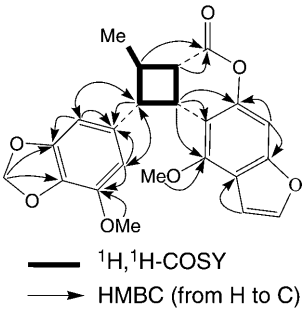


Fig. 1. *Significant ^{1}H,^{1}H COSY and HMBC correlations of* **1**

Compound **2** was obtained as yellowish prisms. Its IR bands at 835 and 1254 cm^{-1} indicated the presence of an S=O group [11][12]. The NMR data of **2** (*Table*) suggested that it possesses the structure of 8-hydroxy-5-methoxypsoralen (=9-hydroxy-4-methoxy-7*H*-furo[3,2-*g*][1]benzopyran-7-one) with OH–C(8) being sulfated which is, in accord with the quasi molecular ions at *m*/*z* 313 and 311 in the ESI-MS (positive- and negative-ion mode).

The ^{13}C-NMR spectrum of **2** displayed signals of 11 olefinic C-atoms (4 CH and 7 C) and one MeO group. In the ^{1}H-NMR spectrum, a MeO *s* (δ 4.18), and two diagnostic *AB* patterns (δ 8.04 and 7.33 (each *d*, *J* = 2.3 Hz, 1 H)); 8.18 and 6.31 (each *d*, *J* = 9.8 Hz, 1 H)) were observed, suggesting a 'linear' 5,8-dioxy-substituted furanocoumarin structure [13]. The ^{1}H- and ^{13}C-NMR of **2** were similar to those of 8-hydroxy-5-methoxypsoralen [14][15], with the exception of the downfield shift of the signal of C(5), C(7), and C(9) ($\Delta\delta$ = +4.0, +4.4, and +5.2, resp.) and the upfield shift of the signal of C(8) ($\Delta\delta$ = –4.3) in **2**.

To confirm the structure of **2**, we performed an X-ray diffraction analysis of **2** (*Fig. 2*)[2]). The results showed that 0.5 molecule of calcium cation hydrated with 2.5 molecule of H_2O are bonded to the sulfate group. In addition, 0.25 molecule of free MeOH and 0.5 molecule of Cl^- are present in the crystal. The structure of **2**, therefore, was defined to be hemicalcium bergapten-8-yl sulfate[1]).

Taking into account that in this case all processing conditions of the extraction and isolation were mild and no reagent containing Ca^{2+} or SO_4^{2-} was used, it is apparent that **2** should be natural products. To the best of our knowledge, **2** is the first naturally occurring coumarinyl sulfate.

Experimental Part

General. Column chromatography (CC): silica gel (200–300 or 400 mesh; *Qingdao Haiyang, Co.*, China). Optical rotation: *Perkin-Elmer-341* polarimeter. UV Spectra: λ_{max} in nm. IR Spectra: *Nicolet-Magna-750-FTIR* spectrometer, KBr pellets; in cm^{-1}. NMR Spectra: *Bruker-AV-500* (500 (^{1}H) or 125

[2]) CCDC 236307 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the *Cambridge Crystallographic Data Centre via* www.ccdc.cam.ac.uk/data_request/cif.

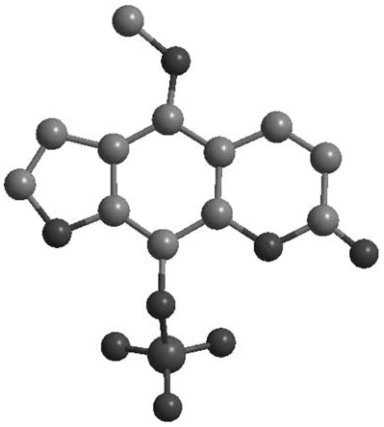


Fig. 2. *OPRTEP View of* **2**. Protons and the ion part are not shown.

MHz (^{13}C)) or *Bruker-DRX-400* (400 (^{1}H) or 100 MHz (^{13}C)) instruments; $CDCl_3$ or (D_6)DMSO solns.; δ in ppm rel. to $SiMe_4$, *J* in Hz. EI-MS, ESI-MS, and HR-ESI-MS: *MAT-95*, *LCQ-Deca*, and *Q-Tof-Ultima* mass spectrometer, resp.; in *m*/*z* (rel. int.).

Plant Material. The dried whole plants of *P. Lindleyanum* were collected in Tashikurgan, Xinjiang Province, China, in October 2000, and identified by Prof. *Guang-Xing Yang* of the Kashi Institute of Drug Research of Xinjiang. A voucher specimen (No. 0064) was deposited in our laboratory.

Extraction and Isolation. The dried and powdered whole plants of *P. Lindleyanum* (10 kg) were extracted with 95% EtOH (3×20 l) at r.t. The concentrated extract was suspended in H_2O (3 l) and partitioned successively with petroleum ether, $CHCl_3$, AcOEt, and BuOH (each 5×3 l). The $CHCl_3$-soluble fraction (77.4 g) was subjected to CC (SiO_2, 2.2 kg); petroleum ether/acetone 100:1, 50:1, 30:1, 20:1, 10:1, 5:1, 3:1, 2:1, 3:2, 1:1 gradient), eluting until the eluent became colorless: *Fr. 1–10*. *Fr. 4* (obtained with petroleum ether/acetone 20:1; 3.23 g) was further subjected to CC (SiO_2, petroleum ether/$CHCl_3$ 3:2): *Fr. 4.1–4.5*. After filtering the crude crystals ($CHCl_3$/Et_2O) from *Fr. 4.2*, the mother liquor (387 mg) was separated by CC (SiO_2, $CHCl_3$/AcOEt 10:1): *Fr. 4.2.1–4.2.4*. *Fr. 4.2.1* (45 mg) was further purified by CC (SiO_2, petroleum ether/AcOEt 3:1): **1** (6 mg). Bergapten (43 mg) and isopimpinellin (80 mg) were obtained from the crude crystal of *Fr. 4.2* after purification by CC (SiO_2, $CHCl_3$). *Fr. 3* yielded crude crystals that were recrystallized from petroleum ether/acetone 1:1: isoimperatorin (1.24 g). *Fr. 2* (1.1 g) was repeatedly subjected to CC (SiO_2, petroleum ether/AcOEt 30:1): lasidiol angelate (101 mg; $[\alpha]_D^{20} = -216$ ($c = 1.12$, $CHCl_3$)) and (*E*)-isoelemicin (48 mg).

The BuOH-soluble fraction (145 g) was subjected to CC (*D1400* macropore resin (∅ 10×65 cm; *Yangzhou Pharmaceutical Factory,* Yangzhou, China), H_2O, then 10, 20, 40, 70, and 95% (*v*/*v*) EtOH). The 10% EtOH fraction (6.66 g) was resubjected to CC (*Sephadex LH-20* (∅ 6×50 cm), H_2O, then 50 and 95% (*v*/*v*) EtOH): *Fr. A–F*. *Fr. E* (0.45 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 10:1, 6:1, 3:1, 3:2 gradient): *Fr. E_1–E_4*. *Fr. E_2* (62 mg) was subjected to CC (SiO_2, $CHCl_3$/MeOH 10:1): **2** (35 mg) as prisms. *Fr. C* (1.85 g) was subjected to CC (SiO_2, $CHCl_3$/MeOH 10:1, 6:1 and 3:1): *Fr. C_1–C_5*). *Fr. C_4* (676 mg) was subjected to CC (SiO_2, $CHCl_3$/acetone 2:1, 3:2, 1:1, 1:2, 0:1 gradient): *Fr. C_{4a}–C_{4d}*. *Fr. C_{4b}* (79 mg) was purified by two CC (*RP C-18* (35 g), H_2O/MeOH 5:1); then silica gel (30 g, AcOEt): isobutyl β-D-glucopyranoside (6 mg) and benzyl β-D-glucopyranoside (9 mg).

Lindleyanin (=rel-*(1*R,*2*R,*2a*S,*9b*R*)-1,2,2a,9b-Tetrahydro-9-methoxy-1-(7-methoxy-1,3-benzodioxol-5-yl)-2-methyl-3*H-*cyclobuta[*c*]*furo[3,2-*g*][1]benzopyran-3-one;* **1**): Colorless gum. $[\alpha]_D^{20} = \pm 0$ ($c = 0.065$, $CHCl_3$). UV ($CHCl_3$): 192, 207, 240. IR: 2924, 2850, 1751 (lactone C=O), 1599, 1510, 1470 (arom.), 1435, 1450, 1350, 1321, 1279, 1252, 1198, 1180, 1122, 1090, 1041 (ether), 995, 966, 922, 899, 831, 800, 766, 737, 721. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS (70eV): 408 (1, M^+), 216 (2), 192 (100), 119 (2), 91 (2). HR-ESI-MS: 431.1137 ($[M+Na]^+$, $C_{23}H_{20}O_7Na^+$; calc. 431.1107).

Hemicalcium Bergapten-8-yl Sulfate (=*4-Methoxy-9-(sulfooxy)-7*H-*furo[3,2-*g*][1]benzopyran-7-one Calcium Salt (2:1)*; **2**): Yellowish prisms from $CHCl_3$/MeOH 10:1. IR: 3450, 1705 (lactone C=O), 1605,

1483 (arom.), 1437, 1360, 1254 (S=O), 1169, 1047, 1007, 835 (S=O), 752, 669, 573. ^{1}H- and ^{13}C-NMR: *Table*. EI-MS (70eV): 232 (81), 217 (100), 203 (2), 189 (53), 175 (2), 161 (28), 143 (3), 133 (16), 116 (3), 105 (15), 95 (8), 77 (12), 51 (7). HR-ESI-MS: 334.9866 ($[C_{12}H_7O_5SO_3+H+Na]^+$, $C_{12}H_8NaO_8S^+$; calc. 334.9838).

X-Ray Crystal-Structure Analysis of **2**. $C_{12}H_7O_5\cdot SO_3^-\cdot 0.5[Ca(H_2O)_5]^{2+}\cdot Cl-\cdot 0.25$ MeOH, (M_r 402.06). Monoclinic $P2(1)/m$: $a=7.8980(17)$ Å, $b=25.854(6)$ Å, $c=8.7185(17)$ Å, $\beta=115.015(5)^\circ$, $V=1613.3(6)$ Å^3, $Z=4$; $D_{calc}=2.260$ Mg·m^{-3}; T 293(2) K, wavelength 0.71073 Å; absorption coefficient 0.453 mm^{-1}; $F(000)$ 980; crystal size $0.253\times0.147\times0.052$ mm; θ range for data collection: $1.58^\circ\leq\theta\leq$ to 28.31°; limiting indices: $-10\leq h\leq10$, $-30\leq k\leq33$, $-6\leq l\leq11$; reflections collected: 9938; unique reflections: 3827 (R(int)=0.1263); completeness to $\theta=28.31$: 93.3%; absorption correction: empirical; max. and min. transmission: 1.00000 and 0.87871; refinement method: full-matrix least-squares on F^2; data: 3827; restraints: 3; parameters: 249; goodness-of-fit on F^2: 0.879; final R indices ($I>2\sigma(I)$): $R_1=0.0785$, $wR_2=0.1592$; R indices (all data): $R_1=0.2034$, $wR_2=0.2030$; extinction coefficient: 0.0076 (16); largest diff. peak and hole: 0.788 and -0.329 e·Å^{-3}; audit creation method: SHELXL-97.

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