Dimeric Coumarin and Phenylpropanoids from *Clausena lenis*

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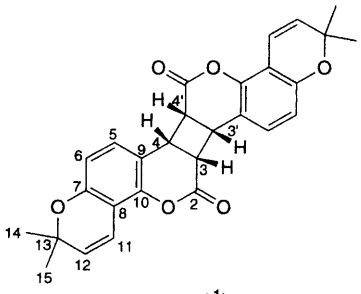
A new dimeric coumarin, diseselin B (**1**), and three new phenylpropanoids, lenisin A–C (**2**–**4**), together with eight known *O*-terpenoidal coumarins, were isolated from the aerial part of *Clausena lenis*. Lenisin A (**2**) was also isolated from the aerial part of *C. excavata*. The structures were elucidated on the basis of 1D- and 2D-NMR experiments (HMQC, HMBC, and ^{1}H,^{1}H-COSY).

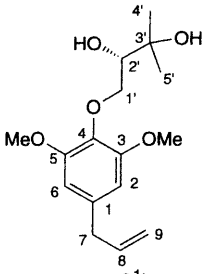
Introduction. – In our continuing chemical investigation of the genus *Clausena* [1–7], we previously reported a dimeric coumarin, diseselin A [5], from *Clausena lenis* DRAKE (Rutaceae). Leaves and barks of this plant have been used for the treatment of dysentery and arthritis. This paper describes the new dimeric coumarin **1**, named diseselin B, and three new phenylpropanoids, lenisin A-C (**2**–**4**), which were isolated from the aerial part of *C. lenis* collected in Xishuangbanna, Yunnan Province, together with eight known *O*-diterpenoidal coumarins, excavacoumarin A [1], C [2], F [2], and G [2], and excavatin D, E, K, and G [8]. Lenisin A (**2**) was also isolated from the aerial part of *C. excavata*. The structures of the new compounds were elucidated on the basis of 1D- and 2D-NMR experiments (HMQC, HMBC, and ^{1}H,^{1}H-COSY).

Results and Discussion. – The molecular formula of diseselin B (**1**) was determined to be $C_{28}H_{24}O_6$ by high-resolution mass spectrometry (M^+ at m/z 456.1517). In the ^{1}H- and ^{13}C-NMR spectra (*Table 1*) of **1**, the number of H- and C-atoms observed was only half of that corresponding to the molecular formula, suggesting that compound **1** was a completely symmetrical structure. The ^{1}H- and ^{13}C-NMR spectra of **1** were very similar to those of seselin (=8,8-dimethyl-2*H*,8*H*-benzo[1,2-*b*:3,4-*b*′]dipyran-2-one) [9–12], except for the signals of H–C(3), H–C(4), C(3), and C(4)[1]). The EI-MS of **1** exhibited an M^+ peak at m/z 456 and another peak at m/z 228 which corresponded to $C_{14}H_{12}O_3$. Analyzing the ^{13}C-NMR and EI-MS data suggested that **1** was a dimer of seselin [9–12]. The ^{1}H- and ^{13}C-NMR spectra suggested that **1** was a dimer composed of two molecules of seselin linked between rings A through C–C bonds to form a cyclobutane-type structure. Several forms of such a cyclobutane-type structure exist. By comparing the coupling constants of such cyclobutane-type structures [13–15], that

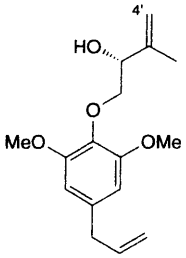
[1]) Arbitrary atom numbering; for systematic names, see *Exper. Part.*



1[1])



2[1])



3[1])

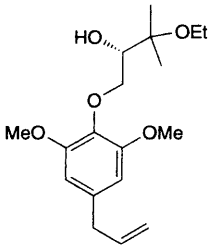


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Table 1. *^{1}H- and ^{13}C-NMR Data of* **1**[1]) *in $CDCl_3$*[a]). δ in ppm, J in Hz.

	δ(H)[b])	δ(C)	HMBC[c])
C(2), C(2′)	–	164.0	–
CH(3), CH(3′)	4.09 (*t*, *J*=8.2)	40.3	37.3 (*d*), 109.0 (*s*), 164.0 (*s*)
CH(4), CH(4′)	4.18 (*t*, *J*=8.2)	37.4	37.4 (*d*), 40.3 (*d*), 109.0 (*s*), 127.7 (*d*, w), 145.8 (*s*), 164.0 (*s*, w)
CH(5), CH(5′)	6.84 (*d*, *J*=8.4)	127.7	37.4 (*d*), 109.0 (*s*, w), 145.8 (*s*, w), 153.7 (*s*)
CH(6), CH(6′)	6.52 (*d*, *J*=8.4)	113.2	109.0 (*s*), 110.1 (*s*), 145.8 (*s*, w), 153.7 (*s*)
C(7), C(7′)	–	153.7	–
C(8), C(8′)	–	110.1	–
C(9), C(9′)	–	109.0	–
C(10), C(10′)	–	145.8	–
CH(11), CH(11′)	6.36 (*d*, *J*=10.0)	115.4	27.6 (*q*, w), 76.2 (*s*), 110.1 (*s*), 113.2 (*d*), 145.8 (*s*, w), 153.7 (*s*)
CH(12), CH(12′)	5.50 (*d*, *J*=10.0)	130.7	27.6 (*q*), 76.2 (*s*), 110.1 (*s*)
C(13), C(13′)	–	76.2	–
Me(14), Me(14′)	1.32 (*s*, 3H)	27.6	27.6 (*q*), 76.2 (*s*), 130.7 (*d*)
Me(15), Me(15′)	1.27 (s, 3H)	27.6	27.6 (*q*), 76.2 (*s*), 130.7 (*d*)

[a]) ^{1}H- and ^{13}C-NMR spectra were obtained at 400 and 100 MHz, resp. [b]) Coupling constants in Hz, unless otherwise indicated, all δ(H) integrate to 1 H. [c]) w=weak.

Table 2. *^{1}H-NMR Data of* **2**–**4**[*l*]*) in C_5D_5N at 400 MHz*. δ in ppm, *J* in Hz.

	2	**3**	**4**
CH(2)	6.59 (*s*)	6.40 (*s*)	6.39 (*s*)
CH(6)	6.59 (*s*)	6.40 (*s*)	6.39 (*s*)
CH_2(7)	3.37 (*d*, *J* = 6.7)	3.33 (*d*, *J* = 6.7)	3.32 (*d*, *J* = 6.7)
CH(8)	6.07 (*m*)	5.96 (*m*)	5.96 (*m*)
CH_2(9)	5.18 (*m*)	5.11 (*m*)	5.11 (*m*)
CH_2(1′)	4.86 (*dd*, *J* = 10.0, 3.2), 4.43 (*dd*, *J* = 10.0, 8.2)	4.29 (*t*, *J* = 10.0)	4.46 (*dd*, *J* = 10.5, 2.4), 3.69 (*dd*, *J* = 10.5, 7.0)
CH(2′)	4.28 (*dd*, *J* = 8.2, 3.2)	3.67 (*t*, *J* = 10.0)	3.80 (*dd*, *J* = 7.0, 2.4)
Me(4′) or CH_2 (4′)	1.52 (*s*)	4.90, 5.07 (2*s*, each 1 H)	1.15 (*s*)
Me(5′)	1.54 (*s*)	1.72 (*s*)	1.20 (*s*)
2 MeO	3.72 (*s*)	3.84 (*s*)	3.83 (*s*)
EtO	–	–	3.46 (*q*, *J* = 6.9, 2 H), 1.12 (*t*, *J* = 6.9, 3 H)

Table 3. *^{13}C-NMR Data of* **2**–**4**[*l*]*) in C_5D_5N at 100 MHz*. δ in ppm.

	2	**3**	**4**
C(1)	136.5 (*s*)	136.2 (*s*)	136.0 (*s*)
C(2)	106.5 (*d*)	105.3 (*d*)	105.3 (*d*)
C(3)	153.8 (*s*)	152.9 (*s*)	152.9 (*s*)
C(4)	136.3 (*s*)	135.0 (*s*)	136.0 (*s*)
C(5)	153.8 (*s*)	152.9 (*s*)	152.9 (*s*)
C(6)	106.5 (*d*)	105.3 (*d*)	105.3 (*d*)
C(7)	40.7 (*t*)	40.5 (*t*)	40.5 (*t*)
C(8)	136.5 (*d*)	137.0 (*d*)	137.1 (*d*)
C(9)	115.9 (*t*)	116.1 (*t*)	116.1 (*t*)
C(1′)	76.5 (*t*)	77.9 (*t*)	75.8 (*t*)
C(2′)	77.4 (*d*)	73.5 (*d*)	75.0 (*d*)
C(3′)	71.5 (*s*)	143.0 (*s*)	75.6 (*s*)
C(4′)	25.8 (*q*)	112.0 (*t*)	21.2 (*q*)
C(5′)	27.4 (*q*)	19.0 (*q*)	22.9 (*q*)
MeO	56.2 (*q*)	56.0 (*q*)	56.0 (*q*)
EtO	–	–	56.8 (*t*) 16.1 (*q*)

of compound **1** was established to be a head-to-tail form, with the four protons at C(3), C(4), C(3′), and C(4′) on the same side of the cyclobutane-ring plane.

The HR-EI-MS (*m*/*z* 296.1619 (M^+)) of **2** was consistent with the molecular formula $C_{16}H_{24}O_5$. In the ^{13}C-NMR, only 13 C-atoms appeared, which suggested that **2** had a symmetrical moiety. Comparison of its spectral data (*Tables 2* and *3*) with literature data established the structure **2** which was named lenisin A.

The NMR signals of **2** at δ(H) 6.59 (*s*, 2 H) and δ(C) 106.5 (*d*), 136.3 (*s*), 136.5 (*s*), and 153.8 (*s*) were typical of a symmetrical tetrasubstituted benzene moiety [16–18]. In the HMBC plot, the ^{1}H,^{13}C long-range correlations between two equivalent MeO groups at δ(H) 3.72 (*s*, 6 H) and the C-atoms at δ(C)

153.8 (*s*), revealed that the two MeO were at C(3) and C(5)[1]). In the ^{1}H,^{1}H-COSY plot of **2**, the correlations between δ(H) 5.18 and 6.07, and between δ(H) 6.07 and 3.37 (*d*, J = 6.7, 2 H) showed the presence of an allyl group. The HMBC correlations between the protons at δ(H) 3.37 (CH_2(7)) and C(1), C(2), and C(6), indicated the allyl group was linked at C(1). The ^{1}H,^{13}C long-range correlations between δ(H) 1.52 (*s*, Me(4′)) and δ(C) 27.4 (*q*, C(5′)), 71.5 (*s*, C(3′)), and 77.4 (*d*, C(2′)), between δ(H) 1.54 (*s*, Me(5′)) and δ(C) 25.8 (*q*, C(4′)), 71.5 (*s*, C(3′)), and 77.4 (*d*, C(2′)), and between δ(H) 4.28 (*dd*, J = 8.2, 3.2, CH(2′)) and δ(C) 25.8 (C(4′)), 27.4 (C(5′)), 71.5 (C(3′)), and 76.5 (*t*, C(1′)), showed the presence of an $OCH_2CH(OH)C(OH)Me_2$ moiety. This moiety was determined to be linked to C(4) *via* an O-atom by means of the HMBC correlations between δ(H) 4.86 (*dd*, J = 10.0, 3.2, 1 H) and δ(C) 136.3 (*s*, C(4)), and between δ(H) 4.43 (*dd*, J = 10.0, 8.2, 1 H) and δ(C) 136.3. The configuration at C(2′) was determined to be (*S*) by comparison of the $[\alpha]_D$ value of **2** with that of a furoquinoline alkaloid, (2*S*)-1-[(6,7-dimethoxyfuro[2,3-*b*]quinolin-4-yl)oxy]-3-methylbutane-2,3-diol [6], which has the same 2,3-dihydroxy-3-methylbutane-2,3-diol moiety and stereogenic C-atom as **2**.

By means of the HR-EI-MS (M^+ at *m*/*z* 278.1516), the molecular formula of **3** was determined to be $C_{16}H_{22}O_4$. Further spectral data (*Tables 2* and *3*) were consistent with the proposed structure **3** which was named lenisin B.

The NMR signals of **3** at δ(H) 6.40 (*s*, 2 H)) and δ(C) 105.3 (*d*), 135.0 (*s*), 136.2 (*s*), and 152.9 (*s*) indicated that **3** had a symmetrical tetrasubstituted benzene moiety like **2**. The ^{1}H- and ^{13}C-NMR data of **3** showed the presence of a same phenylpropanoid moiety as in **2**, which was also supported by the HMBC correlations of **3**. The ^{1}H,^{13}C long-range correlations between δ(H) 4.90 and 5.07 (2*s*, CH_2(4′)), and δ(C) 19.0 (*q*, C(5′)), 73.5 (*d*, C(2′)), and 143.0 (*s*, C(3′)), between δ(H) 1.72 (*s*, Me(5′)) and δ(C) 73.5 (*s*, C(2′)), 112.0 (*t*, C(4′)), and 143.0 (*s*, C(3′)), and between δ(H) 3.67 (*t*, J = 10.0, CH(2′)) and δ(C) 19.0 (C(5′)), 77.9 (*t*, C(1′)), 112.0 (C(4′)), and 143.0 (C(3′)) showed the presence of an $OCH_2CH(OH)C(Me)=CH_2$ moiety. This moiety was linked to C(4) *via* an O-atom as shown by the HMBC correlations between δ(H) 4.29 (*t*, J = 10.0, CH_2(1′)) and δ(C) 135.0 (*s*, C(4)).

The molecular formula of **4** was determined to be $C_{18}H_{28}O_5$ based on the HR-EI-MS (M^+ at *m*/*z* 324.1928). The ^{1}H- and ^{13}C-NMR of **4** (*Tables 2* and *3*) were very similar to those of **2**, except for the presence of an additional EtO group in **4** (δ(H) 3.46 (*q*, J = 6.9, 2 H) and 1.12 (*t*, J = 6.9, 3 H); δ(C) 56.8(*t*) and 16.1(*q*). This EtO group was linked to C(3′) as suggested by the downfield shift of the C(3′) NMR signal. Thus, the structure **4** was established which was named lenisin C.

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Experimental Part

General. CC = Column chromatography. UV Spectra: *UV-210A* spectrophotometer; λ_{max} in nm. NMR Spectra: *Bruker AM-400* (for 1D) and *Bruker DRX-500* spectrometer (for 2D). MS: *Autospec-3000* spectrometer, 70 eV; in *m*/*z* (rel. %).

Plant Material. The aerial part of *Clausena lenis* was collected in Yunnan, China. A voucher specimen (No. H98041802-2) of this plant was deposited in Kunming Institute of Botany, Kunming, China.

Extraction and Isolation. The powdered aerial part of *C. lenis* (500 g) was extracted with 90% EtOH (4 × 2 l) under reflux, each for 5 h. The extract (60 g) was separated into five fractions by CC (porous resin *D101*, EtOH gradient 20% → 100%). *Fr. 4* (3.3 g) was subjected to CC (silica gel, $CHCl_3$/Me_2CO 10 : 0.5, 9 : 1, 8 : 2, 7 : 3, 6 : 4): *Fr. 4A–4F*). *Fr. 4B* was purified by repeated CC: **1** (7 mg), **3** (8.2 mg), and **4** (6.4 mg). *Fr. 4* was subjected to CC (silica gel, *Sephadex LH-20*, resp.): **2** (10.1 mg). Compound **2** (95 mg) also was isolated from the aerial part of *C. excavata.*

Diseselin B (= *(6aR,6bS,14aR,14bS)-6a,6b,14a,14b-Tetrahydro-2,2,10,10-tetramethyl-pyrano[2,3-h]2H,6H,10H,14H-pyrano[2″,3″:7′,8′][1]benzopyrano[3′,4′:3,4]cyclobuta[1,2-c][1]benzopyran-6,14-dione*; **1**)[2]). $[\alpha]_D^{19} = -3.33$ ($c = 0.30$, $CHCl_3$). UV: 228.6, 241.6, 316.6. ^{1}H- and ^{13}C-NMR: *Table 1*. EI-MS: 456 (2, M^+), 228 (12), 213 (100), 199(2), 185 (16), 128 (9), 115 (4), 55 (8). HR-EI-MS: 456.1517 (M^+, $C_{28}H_{24}O_6^+$; calc. 456.1573).

(2S)-1-[2,6-Di(methoxy-4-(prop-2-enyl)phenoxy]-3-methylbutane-2,3-diol (**2**)[2]). $[\alpha]_D^{30.3} = -17.09$ ($c = 1.18$, MeOH). ^{1}H- and ^{13}C-NMR: *Tables 2* and *3*. EI-MS: 296 (63), 281 (4), 237 (7), 194 (100), 179 (23), 163 (17), 147 (14), 133 (11), 119 (12), 91 (16), 59 (38); HR-EI-MS: 296.1619 (M^+, $C_{16}H_{24}O_5^+$; calc. 296.1624).

(2R)-1-[2,6-Dimethoxy-4-(prop-2-enyl)phenoxy]-3-methylbut-3-en-2-ol (**3**)[2]). $[\alpha]_D^{28.9} = -12.67$ ($c = 0.90$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Tables 2* and *3*. EI-MS: 278, 194, 163, 147, 119, 91. HR-EI-MS: 278.1516 (M^+, $C_{16}H_{22}O_4^+$; calc. 278.1518).

(2S)-1-[2,6-Dimethoxy-4-(prop-2-enyl)phenoxy]-3-ethoxy-3-methylbutan-2-ol (**4**)[2]). $[\alpha]_D^{29.9} = -27.41$ ($c = 1.07$, $CHCl_3$). ^{1}H- and ^{13}C-NMR: *Tables 2* and *3*. EI-MS: 324 (51), 309 (3), 291 (3), 278 (9), 252 (4), 238 (9), 222 (27), 207 (16), 194 (91), 179 (38), 163 (35), 147 (34), 131 (36), 119 (42), 87 (88), 59 (100). HR-EI-MS: 324.1928 (M^+, $C_{18}H_{28}O_5^+$; calc. 324.1937).

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[2]) The indicated absolute configurations were not established.