Norlignans from *Sequoia sempervirens*

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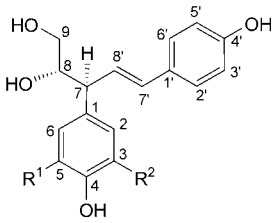
Six new norlignans, named sequosempervirins B–G (**1**–**6**), together with three known norlignans, agatharesinol (**7**), agatharesinol acetonide (**8**), and sugiresinol (**9**), were isolated from the branches and leaves of *Sequoia sempervirens*. Their structures were determined mainly by high-resolution mass spectroscopy (HR-MS), and various 1D- and 2D-NMR methods, as well as, in the case of **1**, by means of X-ray diffraction. Compound **8** showed anticancer activity towards the A549 non-small-cell lung-cancer cell line (IC_{50} = 27.1 μM). The acetone extract of *S. sempervirens* was found to be antifungal towards *Candida glabrata* (IC_{50} = 15.98 μg/ml), and both the acetone and MeOH extracts inhibited the proteolytic activity of cathepsin B (IC_{50} = 4.58 and 5.49 μg/ml, resp.).

Introduction. – According to the literature, many types of compounds have been isolated from Taxodiaceae plants, including terpenoids [1], lignans [2][3], and flavonones [4], some of which show antifungal [5], antibacterial [6], and antitumor [7][8] activities. However, chemical constituents of *Sequoia sempervirens* (LAMB.) ENDL. have, so far, rarely been reported. As part of our investigations on bioactive compounds from Taxodiaceae, we have carried out extensive chemical and biological studies on *S. sempervirens*. In a previous communication [9], we published the structure of sequosempervirin A, the first naturally occurring norlignan with a spirocyclic $C_6-C_2-C_3-C_6$ skeleton. Here, we report the structure elucidation of six novel norlignans (**1**–**6**), isolated together with three known compounds (**7**–**9**), as well as selected biological activities.

Results and Discussion. – *Isolation and Structure Elucidation*. – Compounds **1**–**4**, **7**, and **8** were isolated from the acetone extract of *S. sempervirens*; and compound **9** was obtained from the corresponding MeOH extract. Interestingly, compounds **3** and **4** were found by TLC to be converted to **5** and **6**, respectively, upon standing in MeOH solution (see below).

Compound **1** had the molecular formula $C_{18}H_{20}O_5$, as derived by HR-TOF-MS ($[M-1]^+$ at m/z 315.1220; calc. 315.1232) and confirmed by ^{13}C-NMR (DEPT) spectroscopy. The IR spectrum of **1** showed absorption bands for OH (3441 cm^{-1}) and C=C groups (1609 cm^{-1}). Its UV spectrum revealed the presence of aromatic (Ph) groups (λ_{max} 204, 265 nm). The ^{1}H- and ^{13}C-NMR spectra (*Table 1*) showed the presence of one Me, one CH_2 and eleven CH groups, as well as five quaternary C-atoms



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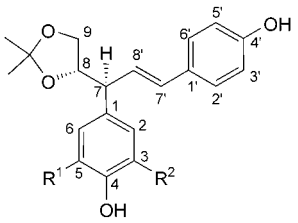

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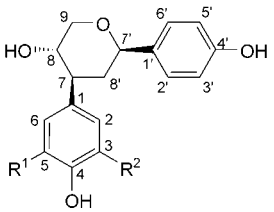
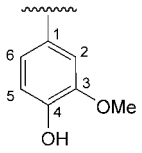


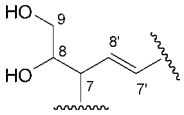
5 R^1 = H, R^2 = MeO
6 R^1 = R^2 = MeO
9 R^1 = R^2 = H

Arbitrary atom numbering

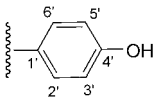
(C_q). On the basis of HMQC, ^{1}H,^{1}H-COSY, and HMBC spectra, fragments **A** – **C** were identified.



A



B



C

In the HMBC spectrum of **1**, the resonance at δ(C) 134.6 (*s*, C(1))[1]) showed cross-peaks with the signals at δ(H) 3.46 (*m*, H−C(7)), 3.94 (*m*, H−C(8)), and 6.25 (*dd*, J = 6.8, 12.7 Hz, H−C(8′)), which indicated a link between fragments **A** (at C(1)) and **B** (at C(7)). The signal at δ(C) 130.6 (*s*, C(1′)) showed a cross-peak with that at δ(H) 6.25 (*dd*, J = 6.8, 12.7 Hz, H−C(8′)), indicating that fragments **C** and **B** were linked at C(1′) and C(7′), respectively. Finally, the solid-state structure of compound **1** (*Fig. 1*), which was recrystallized from MeOH as colorless, plate-like crystals, unequivocally settled the absolute configuration of the compound as (7*S*) and (8*S*)[1]). From all these data, the structure of **1** was, thus, determined as (2*S*,3*S*,4*E*)-3-(4-hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)pent-4-ene-1,2-diol, named *sequosempervirin B*.

Compound **2** had the molecular formula $C_{19}H_{22}O_6$, as deduced from HR-FAB-MS ($[M-1]^-$ at *m*/*z* 345.1335, calc. 345.1338) and confirmed by ^{13}C-NMR (DEPT). Optical rotation, IR and UV data indicated that both **1** and **2** were structurally very similar. Relative to **1**, the ^{1}H- and ^{13}C-NMR spectra of **2** (*Tables 1* and *2*, resp.) showed only differences in the resonances of one of the Ph rings (C(1)−C(6)), as well as an additional MeO group. From the above data, combined with HMQC, ^{1}H,^{1}H-COSY and HMBC spectra, compound **2** was, thus, identified as (2*S*,3*S*,4*E*)-3-(4-hydroxy-3,5-dimethoxyphenyl)-5-(4-hydroxyphenyl)pent-4-ene-1,2-diol, and named *sequosempervirin C*.

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[1]) Arbitrary atom numbering. For systematic compound names, see text and *Exper. Part*.

Table 1. *^{13}C- and ^{1}H-NMR Data of Sequosempervirin B* (**1**). At 100/400 MHz, resp., in CD_3OD; chemical shifts δ in ppm, coupling constants J in Hz.

Position[1])	δ(C)	δ(H)	^{1}H,^{1}H-COSY	HMBC
1	134.6 (*s*)			H-2,5,7,8,8′
2	122.1 (*d*)	6.76 (*s*)		H-5,7
3	148.7 (*s*)			H-5,6, MeO
4	145.9 (*s*)			H-2,5
5	113.5 (*d*)	6.91 (*s*)		H-7
6	116.1 (*d*)	6.76 (*s*)		H-5
7	53.6 (*d*)	3.46 (*m*)	H-8,8′	H-2,5,7′,8′,9
8	76.2 (*d*)	3.94 (*m*)	H-7, 9	H-8′,9
9	65.7 (*t*)	3.46 (*m*), 3.64 (*dd*, J = 2.9, 9.1)	H-8	H-7,8
1′	130.6 (*s*)			H-3′,5′,7,8′
2′	128.4 (*d*)	7.19 (*d*, J = 6.8)	H-3′	H-7′
3′	116.2 (*d*)	6.70 (*d*, J = 6.8)	H-2′	H-2′
4′	157.8 (*s*)			H-2′,3′,5′,6′
5′	116.2 (*d*)	6.70 (*d*, J = 6.8)	H-6′	H-6′
6′	128.4 (*d*)	7.19 (*d*, J = 6.8)	H-5′	H-7′
7′	131.6 (*d*)	6.34 (*d*, J = 12.6)	H-8′	H-2′,6′
8′	129.1 (*d*)	6.25 (*dd*, J = 6.8, 12.7)	H-7′	
MeO	56.4 (*q*)	3.83 (*s*)		

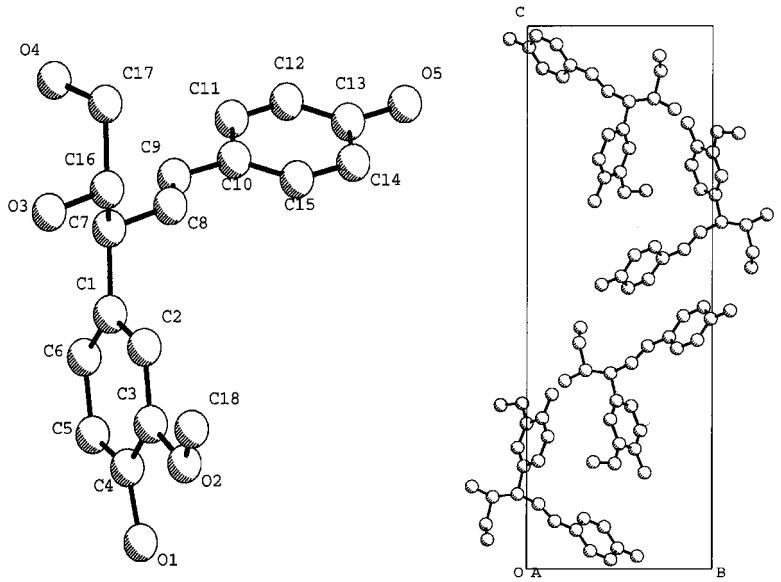


Fig. 1. *X-Ray single-crystal structure of compound* **1**

Compound **3** had the molecular formula $C_{21}H_{24}O_5$ ($[M-1]^-$ at m/z 355.1550, calc. 355.1545), as confirmed by ^{13}C-NMR (DEPT), and structurally also resembled **1** and **2**. Compared with **1**, the ^{1}H- and ^{13}C-NMR spectra of **3** showed differences at C(8) and C(9) due to an acetonide. The structure of **3** was identified as 4-[(1*S*,2*E*)-1-[(4*S*)-

Table 2. *^{13}C-NMR Data of Compounds* **2**–**6**. In $CDCl_3$ (**2**–**4**) or CD_3OD (**5**, **6**); chemical shifts δ in ppm.

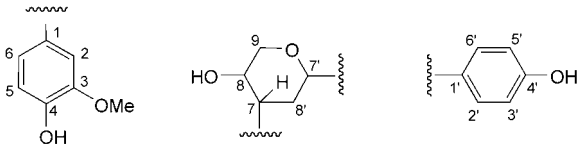
Position	**2**[a])	**3**[b])	**4**[a])	**5**[b])	**6**[a])
1	133.4	132.8	131.9	135.6	135.6
2	104.9	120.7	104.9	121.3	106.6
3	147.3	146.5	146.8	149.0	149.4
4	131.9	144.2	133.3	146.2	134.9
5	147.3	111.1	146.8	112.9	149.4
6	104.9	114.5	104.9	116.3	106.6
7	52.9	52.3	52.7	51.3	51.8
8	74.4	78.5	78.4	71.0	70.9
9	64.4	67.6	67.6	73.9	73.9
1′	128.7	129.1	128.9	134.2	134.3
2′	127.3	127.4	127.4	128.5	128.5
3′	115.1	115.3	115.3	116.0	116.1
4′	156.2	155.8	155.8	158.0	158.0
5′	115.1	115.3	115.3	116.0	116.1
6″	127.3	127.4	127.4	128.5	128.5
7″	131.0	131.2	131.2	81.2	81.2
8′	126.4	126.3	126.0	42.6	42.5
3-MeO	56.0	55.7	56.0	56.5	57.0
5-MeO	56.0		56.0		57.0
Me_2C		109.5	109.4		
Me_2C		26.6, 25.3	26.6, 25.3		

[a]) Recorded at 100 MHz. [b]) Recorded at 125 MHz.

2,2-dimethyl-1,3-dioxolan-4-yl]-3-(4-hydroxyphenyl)prop-2-en-1-yl]-2-methoxyphenol, named *sequosempervirin D*.

According to HR-TOF-MS ($[M + Na]^+$ at *m/z* 409.1630; calc. 409.1627) and ^{13}C-NNR (DEPT; *Table 2*), compound **4** had the molecular formula $C_{22}H_{26}O_6$. Spectral analysis as above corroborated that **4** was an analogue of **3** with an additional MeO group. Thus, **4** was identified as 4-[(1*S*,2*E*)-1-[(4*S*)-2,2-dimethyl-1,3-dioxolan-4-yl]-3-(4-hydroxyphenyl)prop-2-en-1-yl]-2,6-dimethoxyphenol, named *sequosempervirin E*.

Compound **5** had the molecular formula $C_{18}H_{20}O_5$, as deduced by HR-TOF-MS ($[M-1]^-$ at *m/z* 315.1227; calc. 315.1232) and ^{13}C-NMR (DEPT; *Table 2*). Its IR spectrum indicated absorption bands for OH (3441 cm^{-1}) and C=C (1630 cm^{-1}) groups. Its UV spectrum revealed the presence of phenyl groups (λ_{max} 205, 226, 278 nm). The ^{1}H- and ^{13}C-NMR spectra showed the presence of one Me, two CH_2, and ten CH groups, together with five C_q-atoms. On the basis of HMQC, ^{1}H,^{1}H-COSY, and HMBC spectra, fragments **D**–**F** were identified.



D **E** **F**

In the HMBC spectrum of **5**, the resonance at δ(C) 135.6 (*s*, C(1))[1]) showed a cross-peak with the signal at δ(H) 2.72 (*m*, H–C(7)), which indicated that fragments **D** and **E** were linked at C(1)–C(7) (see *Fig. 2,a*). The signal at δ(C) 134.2 (*s*, C(1′)) showed a cross-peak with that at δ(H) 4.42 (*dd*, $J = 1.3$, 10.8 Hz, H–C(7′)), suggesting a C(1′)–C(7′) linkage between fragments **E** and **F**. By means of a ROESY experiment (*Fig. 2,b*), H–C(7′), H_a–C(8′), H_b–C(8′), H–C(7), H–C(8), H_a–C(9), and H_b–C(9) were assigned axial, equatorial, axial, axial, axial, axial, and equatorial positions, respectively. Therefore, the structure of **5** was determined as (3*S*,4*S*,6*R*)-3,4,5,6-tetrahydro-4-(4-hydroxy-3-methoxyphenyl)-6-(4-hydroxyphenyl)-2*H*-pyran-3-ol, named *sequosempervirin F.*

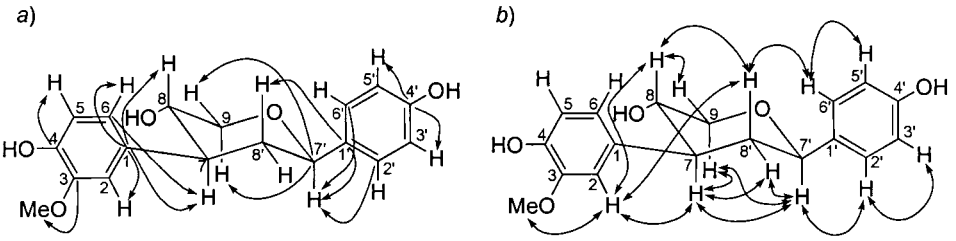


Fig. 2. a) *Observed key HMBC and* b) *key ROESY correlations* for compound **5**

By similar analysis, compound **6** was found to be the 3,5-dimethoxy variant of **5**, and was named *sequosempervirin G.*

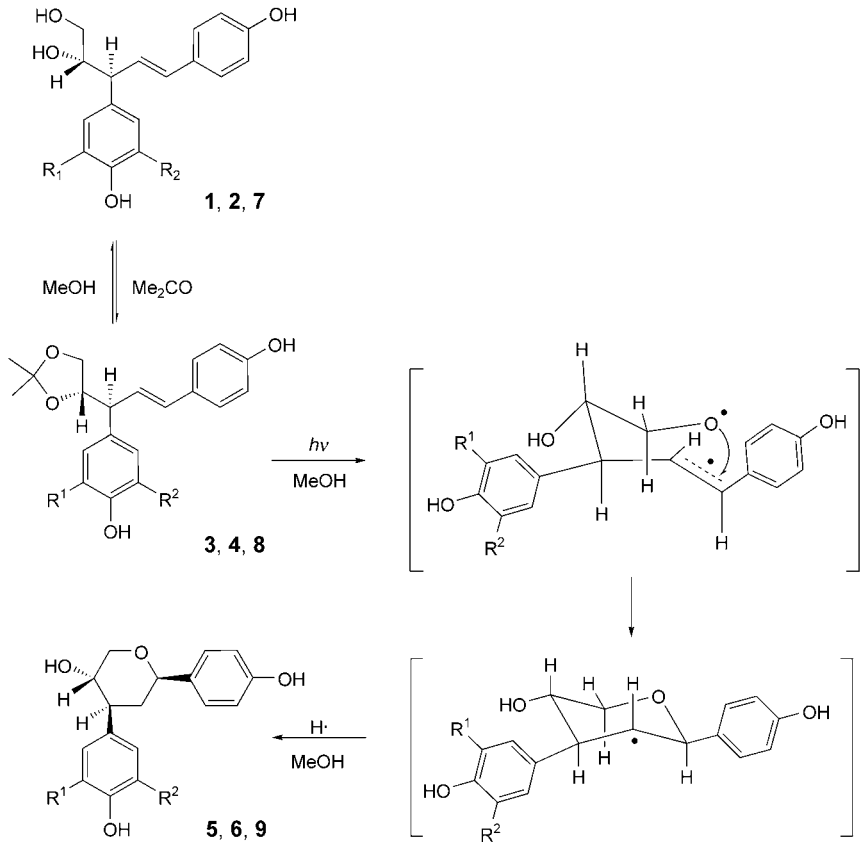
The three known compounds were determined to correspond to agatharesinol (**7**), agatharesinol acetonide (**8**), and sugiresinol (**9**), as inferred from melting point, optical rotation, IR, UV, MS, and 1D- and 2D-NMR data [3][10][11]. Since agatharesinol acetonide (**8**) is known to be formed from agatharesinol (**7**) during acetone extraction [3], it seems to be likely that sequosempervirins D (**3**) and E (**4**) are analogous artifacts of **1** and **2**, respectively (acetone extraction!).

Interestingly, when CD_3OD solutions of compounds **3** or **4** were left standing for 20 d, three TLC spots were detected, corresponding to mixtures of **1**/**3**/**5** and **2**/**4**/**6**, respectively. Analogously, sugiresinol (**9**) was detected by TLC in CD_3OD solution of agatharesinol acetonide (**8**) after 20 d. Apparently, these compounds, thus, are interconvertible in solution. A possible mechanism for this process is proposed in the *Scheme* below.

Compounds **1**, **2**, and **7** readily form the corresponding acetonides in the presence of acetone, giving rise to compounds **3**, **4**, and **8**, respectively, which are cleaved back to the starting materials in the presence of MeOH or a similar protic solvent. However, **3**, **4**, and **8** in MeOH may also react under the influence of light *via* free-radical addition of the 9-O-atom to the C(7′)=C(8′) bond, thus forming the tetrahydropyran rings of **5**, **6**, and **9**, respectively. In summary, compounds **3**–**6**, **8**, and **9** might be artificial products. The assumption of *Enzell et al.* [10] that **9** is a true natural product might, thus, be revised.

Biological Properties. – Compounds **1**, **2**, **5**, **8**, and **9** were randomly tested for *in vitro* activity in five bioassays, including CCLT (anticancer), CDC25 (anticancer), CAT-B (anti-osteoporosis), CA-II (anti-osteoporosis), and PP1 (metabolism) assays. The

Scheme. *Possible Mechanism for the Solvent-Dependent Interconversion of Structurally Related Compounds*



concentrations of the compounds were 10, 25, 5, 10, and 61 μg/ml, respectively. The original petroleum ether (PE), acetone, and MeOH extracts of *S. sempervirens* were also randomly tested for *in vitro* activity in six bioassays, including CDC25, YNG (antifungus), CAT-B, CA-II, TS (metabolism), and PP1, at concentrations of 100, 16, 10, 12.5, 10, and 244 μg/ml, respectively.

The positive results of these investigations are summarized in *Table 3*. Compound **8** showed anticancer activity on the A549 non-small-cell lung-cancer cell line, with an IC_{50} value of 27.1 μM (taxol as positive control). The acetone extract showed antifungal activity on *Candida glabrata*, with an IC_{50} value of 15.98 μg/ml (fluconazole as positive control). The acetone and MeOH extracts were active towards cathepsin B, with IC_{50} values of 4.58 and 5.49 μg/ml, respectively (leupeptin as positive control).

Some norlignans with the same skeleton as compounds **1**–**9** have been isolated from conifer plants, where they readily occur [10][12][13]. These structural analogues have been reported to show antifungal activities, inhibitory effects on cyclic AMP phosphodiesterase and against *Cortinellus shiitake* hyphae growth and fruiting body formation, as well as vinyl polymerization inhibitory activities [11]. However,

Table 3. *Biological Activities* (in terms of IC_{50}) *of Compound* **8**, *and of the Acetone and MeOH Extracts of* Sequoia sempervirens. The concentrations required for 50% inhibition (IC_{50}) were calculated from dose-response curves by recording changes in optical and emissive densities at four different concentrations each.

Substance	CCLT-A549[a])	YNG[b])	CAT-B[c])
8	27.10 μM	–	–
Taxol[d])	33.72 nM	–	–
Acetone extract	–	15.98 μg/ml	4.58 μg/ml
Methanol extract	–	–	5.49 μg/ml
Leupeptin[d])	–	–	47.21 nM

[a]) Anticancer assay with the A549 non-small-cell lung-cancer cell line. [b]) Antifungal assay with *Candida glabrata*; positive control: fluconazole (data not shown). [c]) Assay for the determination of inhibition of cathepsin B. [d]) Positive control.

compound **8** was the first compound of this type to show anticancer activity (A549 non-small-cell lung cancer). Our studies, in turn, show for the first time that the acetone and MeOH extracts of *S. sempervirens* display inhibitory activity on cathepsin B.

The authors are grateful to members of the analytical group in the State Key Laboratory of Phytochemistry and Plant Resources in West China (SKL-PPRWC), Kunming Institute of Botany (KIB), Chinese Academy of Sciences (CAS), for spectral measurements. We also thank Mrs. *Rui-Rui Jia*, Mrs. *Guang-Zhi Zeng*, Mr. *Xiang Fu*, Mr. *Chang-Jiu Ji*, and Mr. *Huo-Qiang Huang* from the Screening Laboratory of SKL-PPRWC (KIB, CAS) for bioactivity tests. This work was supported by grants from the *Natural Science Fund of Yunnan Province* (2001B0055M), SKL-PPRWC (KIB, CAS), the agreement between KIB (CAS) and *Bayer AG* (Germany) (BK-9501), and CAS (KSCX1-09-03-1, KSCX1-SW-11).

Experimental Part

General. Silica gel (200–300 mesh) for column chromatography (CC) and TLC silica-gel plates were from *Qindao Marine Chemical Factory* (Qindao, People's Republic of China); *Sephadex LH-20* gel was from *Pharmacia* (Uppsala, Sweden). As TLC eluants, $CHCl_3$/MeOH or petroleum ether (PE)/AcOEt mixtures were used; visualization with 5% H_2SO_4 in EtOH. Melting points (m.p.) were measured on a *SEISAKUSHO-1240* micro-melting-point apparatus; uncorrected. Optical rotations were measured on a *Horiba SEAP-300* polarimeter. IR Spectra (KBr): *Bio-Rad FTS-135* spectrophotometer; in cm^{-1}. UV Spectra: *2401-PC* spectrophotometer, λ_{max} [nm] ($\log \varepsilon$); in MeOH. ^{1}H-, ^{13}C-, and 2D-NMR Spectra: *Bruker AM-400* or a *DRX-500* spectrometers; δ in ppm rel. to Me_4Si (= 0 ppm) as internal standard, *J* in Hz. MS: *VG Autospec-3000* mass spectrometer; in *m*/*z* (rel. %).

Plant Material. The branches and leaves of *S. sempervirens* (Lamb.) Endl. were collected in Kunming Botany Garden, Yunnan Province, People's Republic of China, in August 2002, and identified by Prof. *Zhong-Shu Yue* (Kunming Botany Garden). A voucher specimen (No. 0040453) was deposited at the herbarium of the Kunming Institute of Botany.

Extraction and Isolation. The dried and powdered branches and leaves (11.9 kg) of *S. sempervirens* were extracted with petroleum ether (PE), acetone, and MeOH under reflux, step by step. The extracts were concentrated *in vacuo*, affording 156 g of PE extract, 214 g of acetone extract, and 524 g of MeOH extract. The acetone extract was purified by CC (2.2 kg SiO_2; $CHCl_3$/Me_2CO mixtures of increasing polarity), giving fractions *Fr. 1 – Fr. 23*. *Fr. 18* was eluted with $CHCl_3$/MeOH 9:1 to afford **1** (65 mg). *Fr. 19* was subjected to repeated CC (SiO_2; 1. $CHCl_3$/MeOH 6:1; 2. PE/AcOEt 1:3) to yield **2** (52 mg), **3** (84 mg), **4** (98 mg), **7** (28 mg), and **8** (24 mg). By TLC detection, **3** and **4** were separately converted to three spots in CD_3OD within 20 d after their NMR measurements. These mixtures were subjected to CC (SiO_2; $CHCl_3$/MeOH 6:1) to afford **5** (19 mg) from **3**, and **6** (11 mg) from **4**, respectively. The dry MeOH extract was dissolved in anh. EtOH and re-concentrated *in vacuo* to obtain 250 g of residue, which was subjected to CC (2 kg SiO_2; $CHCl_3$/MeOH mixtures of increasing polarity) to give three major fractions. *Fr.2* was subjected to repeated CC (SiO_2; 1. $CHCl_3$/MeOH 6:1; 2. *Sephadex LH-20*; MeOH/H_2O 1:1) to yield compound **9** (36 mg).

Sequosempervirin B (=*(2S,3S,4E)-3-(4-Hydroxy-3-methoxyphenyl)-5-(4-hydroxyphenyl)pent-4-ene-1,2-diol*; **1**). Colorless crystals. M.p. 150–154° (MeOH). $[\alpha]_D^{25} = -25.3$ ($c = 1.75$, MeOH). UV (MeOH): 203.8 (4.45), 264.8 (4.20). IR (KBr): 3441, 1609, 1513, 1448, 1373, 1249, 1122, 1029, 969, 851, 826, 806. ^{1}H- and ^{13}C-NMR: see *Table 1*. FAB-MS (neg.): 315 (100, $[M-1]^-$), 281 (21), 258 (8), 160 (4), 80 (9). HR-TOF-MS (neg.; $C_{18}H_{20}O_5$): 315.1220 ($[M-1]^-$, $C_{18}H_{19}O_5^-$; calc. 315.1232). X-Ray crystal structure: see *Fig. 1* and X-ray section (below).

Sequosempervirin C (=*(2S,3S,4E)-3-(4-Hydroxy-3,5-dimethoxyphenyl)-5-(4-hydroxyphenyl)pent-4-ene-1,2-diol*; **2**). Colorless crystals. M.p. 145–147° (MeOH). $[\alpha]_D^{26} = -63.3$ ($c = 0.10$, MeOH). UV (MeOH): 205.0 (4.81), 265.2 (4.46). IR (KBr): 3443, 1612, 1515, 1460, 1326, 1239, 1120, 1017, 965, 826, 805. ^{1}H-NMR ($CDCl_3$, 400 MHz)[1]): 3.35 (*t*, $J = 8.7$, H–C(7)); 3.48 (*dd*, $J = 7.0$, 11.5, H_a–C(9)); 3.70 (*dd*, $J = 3.0$, 11.5, H_b–C(9)); 3.82 (2 MeO); 3.89 (*m*, H–C(8)); 6.07(*dd*, $J = 8.9$, 15.7, H–C(8′)); 6.32 (*d*, $J = 15.7$, H–C(7′)); 6.48 (*s*, H–C(2,6)); 6.68 (*d*, $J = 8.6$, H–C(3′,5′)); 7.13 (*d*, $J = 8.5$, H–C(2′,6′)). ^{13}C-NMR: see *Table 2*. EI-MS ($C_{19}H_{22}O_6$): 346 (6, M^+), 285 (100), 253 (27), 225 (9), 210 (7), 181 (8), 131 (24), 111 (17), 107 (9). HR-FAB-MS (neg.): 345.1335 ($[M-1]^-$, $C_{19}H_{21}O_6^-$; calc. 345.1338).

Sequosempervirin D (=*4-[(1S,2E)-1-[(4S)-2,2-Dimethyl-1,3-dioxolan-4-yl]-3-(4-hydroxyphenyl)prop-2-en-1-yl]-2-methoxyphenol*; **3**). Colorless oil. $[\alpha]_D^{26} = -19.1$ ($c = 0.90$, MeOH). UV (MeOH): 204.0 (4.70), 265.0 (4.42). IR (KBr): 3406, 2985, 1610, 1514, 1451, 1434, 1372, 1270, 1216, 1171, 1154, 1127, 1060, 1033, 968, 853, 824. ^{1}H-NMR ($CDCl_3$, 500 MHz)[1]): 1.35, 1.41 (2*s*, Me_2C); 3.49 (*m*, H–C(7)); 3.77 (*m*, H_a–C(9)); 3.82 (*s*, MeO); 4.06 (*m*, H_b–C(9)); 4.44 (*m*, H–C(8)); 6.10 (*dd*, $J = 8.5$, 15.8, H–C(8′)); 6.37 (*d*, $J = 15.7$, H–C(7′)); 6.72 (*d*, $J = 8.6$, H–C(3′,5′)); 6.78 (*s*, H–C(2,6)); 6.84 (*s*, H–C(5)); 7.17 (*d*, $J = 8.6$, H–C(2′,6′)). ^{13}C-NMR: see *Table 2*. FAB-MS (neg.; $C_{21}H_{24}O_5$): 355 (100, $[M-1]^-$), 341 (6), 253 (8), 239 (14), 119 (9). HR-TOF-MS (neg.): 355.1550 ($[M-1]^-$, $C_{21}H_{23}O_5^-$; calc. 355.1545).

Sequosempervirin E (=*4-[(1S,2E)-1-[(4S)-2,2-Dimethyl-1,3-dioxolan-4-yl]-3-(4-hydroxyphenyl)prop-2-en-1-yl]-2,6-dimethoxyphenol*; **4**). Colorless oil. $[\alpha]_D^{24} = -19.1$ ($c = 1.45$, MeOH). UV (MeOH): 206.0 (4.69), 265.4 (4.33). IR (KBr): 3417, 2934, 1611, 1515, 1460, 1371, 1326, 1216, 1153, 1115, 1061, 968, 916, 853, 825, 806. ^{1}H-NMR ($CDCl_3$, 400 MHz): 1.34, 1.41 (2*s*, Me_2C); 3.46 (*t*, $J = 8.1$, H–C(7)); 3.80 (*m*, H_a–C(9)); 3.82 (*s*, 2 MeO); 4.05 (*dd*, $J = 6.1$, 8.2, H_b–C(9)); 4.44 (*dd*, $J = 6.8$, 13.7, H–C(8)); 6.09 (*dd*, $J = 8.5$, 15.8, H–C(8′)); 6.37 (*d*, $J = 15.8$, H–C(7′)); 6.51 (*s*, H–C(2,6)); 6.72 (*d*, $J = 8.5$, H–C(3′,5′)); 7.16 (*d*, $J = 8.4$, H–C(2′,6′)). ^{13}C-NMR: see *Table 2*. FAB-MS (pos.; $C_{22}H_{26}O_6$): 386 (16, M^+), 285 (100), 101 (47). HR-TOF-MS (pos.): 409.1630 ($[M+Na]^+$, $C_{22}H_{26}NaO_6$; calc. 409.1627).

Sequosempervirin F (=*(3S,4S,6R)-3,4,5,6-Tetrahydro-4-(4-hydroxy-3-methoxyphenyl)-6-(4-hydroxyphenyl)-2H-pyran-3-ol*; **5**). Colorless crystals. M.p. 93–95° (MeOH). $[\alpha]_D^{29} = +2.2$ ($c = 0.15$, MeOH). UV (MeOH): 205.2 (4.77), 225.6 (4.53), 278.2 (3.98). IR (KBr): 3441, 1630, 1518, 1243, 1072, 1033. ^{1}H-NMR (CD_3OD, 500 MHz): 1.84 (*q*, $J = 12.4$, H_a–C(8′)); 1.94 (*m*, H_b–C(8′)); 2.72 (*m*, H–C(7)); 3.40 (*t*, $J = 10.5$, H_a–C(9)); 3.81 (*m*, H–C(8)); 3.85 (*s*, MeO); 4.12 (*dd*, $J = 4.9$, 10.8, H_b–C(9)); 4.42 (*dd*, $J = 1.3$, 10.8, H–C(7′)); 6.73 (*d*, $J = 8.7$, H–C(3′,5′)); 6.75 (*s*, H–C(2,6)); 6.88 (*s*, H–C(5)); 7.20 (*d*, $J = 8.5$, H–C(2′,6′)). ^{13}C-NMR: see *Table 2*. FAB-MS (neg.; $C_{18}H_{20}O_5$): 315 (100, $[M-1]^-$), 292 (9), 267 (11), 235 (8), 173 (9), 115 (12), 92 (8). HR-TOF-MS (neg.): 315.1227 ($[M-1]^-$, $C_{18}H_{19}O_5^-$; calc. 315.1232).

Sequosempervirin G (=*(3S,4S,6R)-3,4,5,6-Tetrahydro-4-(4-hydroxy-3,5-dimethoxyphenyl)-6-(4-hydroxyphenyl)-2H-pyran-3-ol*; **6**). Colorless oil. $[\alpha]_D^{20} = 0.0$ ($c = 0.23$, MeOH). UV (MeOH): 207.6 (4.63), 275.6 (3.58), 359.4 (2.48). IR (KBr): 3431, 1616, 1519, 1460, 1217, 1114, 1077, 833. ^{1}H-NMR (CD_3OD, 400 MHz): 1.85 (*m*, H_a–C(8′)), 1.95 (*m*, H_b–C(8′)); 2.71 (*m*, H–C(7)); 3.39 (*t*, $J = 10.5$, H_a–C(9)); 3.79 (*m*, H–C(8)); 3.84 (*s*, 2 MeO); 4.13 (*dd*, $J = 5.0$, 10.8, H_b–C(9)); 4.41 (*dd*, $J = 1.8$, 11.0, H–C(7′)); 6.59 (*s*, H–C(2,6)); 6.74 (*d*, $J = 8.6$, H–C(3′,5′)); 7.20 (*d*, $J = 8.5$, H–C(2′,6′)). ^{13}C-NMR: see *Table 2*. FAB-MS (neg.; $C_{19}H_{22}O_6$): 345 (100, $[M-1]^-$), 311 (13), 293 (16), 238 (25), 219 (43), 203 (19), 177 (17), 127 (12), 90 (19). HR-TOF-MS: 345.1328 ($[M-1]^-$, $C_{19}H_{21}O_6^-$; calc. 345.1338).

Agatharesinol (**7**). $[\alpha]_D^{24} = -26.7$ ($c = 0.45$, MeOH).

Agatharesinol Acetonide (**8**). $[\alpha]_D^{25} = -13.3$ ($c = 1.20$, MeOH).

Sugiresinol (**9**). $[\alpha]_D^{20} = -18.7$ ($c = 0.25$, MeOH).

X-Ray Crystallography. A colorless plate-like crystal of **1** ($0.15 \times 0.30 \times 1.00$ mm) was subjected to X-ray diffraction on a *MAC DIP-2030K* diffractometer, with MoK_α radiation and graphite monochromator at a maximum 2θ value of 50.0°. The total number of independent reflections was 1826, of which 1810 were observed ($I \geq 2\sigma(I)$). Crystal data: molecular formula, $C_{18}H_{20}O_5$ (M_r 316.35); orthorhombic system, space group $P2_12_12_1$, $a = 5.628(1)$, $b = 9.776(1)$, $c = 29.199(4)$ Å; $V = 1606.5(3)$ Å^3, $Z = 4$, $D_c = 1.308$ g/cm^3. The structure was solved by the direct methods (SHELXS-97) [14], and expanded with difference *Fourier* techniques, refined by the full-matrix least-squares method (SHELXL-97) [15]. H-Atoms were fixed at calculated positions. The final indices were $R_f = 0.065$, $R_w = 0.066$ ($w = 1/[\sigma^2(F_0^2) + (0.2000\,P)^2 + 0.0000\,P]$, where $P = (F_0^2 + 2F_c^2)/3$). Crys-

tallographic data (excluding structure factors) for the structure reported in this paper have been deposited with the *Cambridge Crystallographic Data Centre* (*CCDC*) as publication number CCDC-233462. These data can be obtained, free of charge, via http://www.ccdc.cam.ac.uk/products/csd/request or from the *CCDC*, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 1223 336033; e-mail: data_request@ccdc.cam.ac.uk).

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