Three Novel Terpenoids from *Schisandra pubescens* var. *pubinervis*

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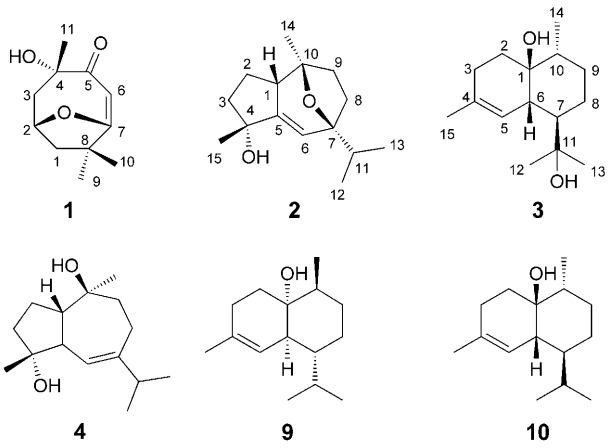
Three new terpenoids, pubinernoids A–C (**1**–**3**), together with six known sesquiterpenoids, were isolated from the leaves and stems of *Schisandra pubescens* var. *pubinervis*. The structures of the new compounds were established on the basis of extensive spectroscopic analyses, including application of 2D-NMR spectroscopic techniques. A plausible formation of the sesquiterpenoid **2** is proposed (*Scheme 2*), starting from guaianediol (**4**) as the biogenetic precursor, which was also present in the extract.

1. Introduction. – Plants of the genus *Schisandra* (Schisandraceae) have attracted much attention owing to a variety of medicinal properties and biological activities such as antihepatitis, antitumor, anti-HIV properties, as well as inhibition of cholesterol biosynthesis [1–5]. One of the most-distinguishing features of *Schisandra* species studied recently is the discovery of rearranged triterpenoid derivatives endowed with a different carbon skeleton, most of which can be related biogenetically to cycloartane precursors [6–10]. As part of our continuing investigations of triterpenoid constituents from *Schisandra* species, we phytochemically investigated the leaves and stems of *Schisandra pubescens* var. *pubinervis*. Interestingly, we did not obtain any triterpenoid isolated previously from other species of this genus, but isolated a novel compound, pubinernoid A (**1**) and two new sesquiterpenoids, pubinernoids B (**2**) and C (**3**), together with five known compounds: guaianediol (**4**) [11], 10-*O*-methylalismoxide [12], eudesm-4(15)-ene-1,6-diol [13], voleneol [14], and clovane-2,9-diol [15]. This paper deals with the isolation and extensive structure elucidation of the new compounds. Additionally, a biosynthetic pathway of **2** from **4** is hypothesized to further corroborate the configuration of the epoxide bridge in the molecule.

2. Results and Discussion. – Pubinernoid A (**1**), obtained as an amorphous powder, showed a quasi-molecular ion peak at *m*/*z* 219 in its positive ESI mass spectrum. The molecular formula of **1** was revealed as $C_{11}H_{16}O_3$ by HR-ESI-MS (*m*/*z* 219.0992 ($[M+Na]^+$; calc. 219.0997)). The ^{1}H-NMR spectrum showed three *singlet*s at δ(H) 1.28 (3 H), 1.47 (3 H), and 1.78 (3 H), which indicated the presence of three Me groups. The ^{13}C-NMR and DEPT spectra displayed eleven C-atom resonances, including a keto



group (δ(C) 183.2 (*s*)), one trisubstituted C=C bond (δ(C) 171.6 (*s*) and 113.0 (*d*)), one oxygenated quaternary C-atom (δ(C) 86.6), one oxygenated CH (δ(C) 66.8), one regular quaternary C-atom (δ(C) 35.9), two CH_2 (δ(C) 45.7, 47.4), and three Me groups (δ(C) 27.0, 26.5, 30.6). These observations, in combination with the molecular formula, indicated one OH group and two rings.

Extensive 2D-NMR (^{1}H,^{1}H-COSY, HMQC, and HMBC) experiments allowed us to define the molecular connectivity. As shown by bold lines in *Fig. 1*, the partial structure **1a** was deduced by the ^{1}H,^{1}H-COSY correlations of CH_2(1) with H–C(2), and of H–C(2) with CH_2(3). The ^{1}H,^{13}C long-range couplings (^2J and ^3J) in the HMBC spectrum gave the following results: the cross-peaks from Me(9) and Me(10) to C(1) and C(8), and from H–C(6) to C(4), C(5), C(7), and C(8), respectively, suggested the presence of partial structure **1b** (*Fig. 1*). Furthermore, HMBC correlations of CH_2(3) with both C(4) and C(5), of H–C(2) with both C(4) and C(8), and of CH_2(1) with both C(8) and Me(9) required direct connections of C(1) to C(8), and of C(3) to C(4), respectively, so that **1a** and **1b** could be joined to the planar structure of **1**.

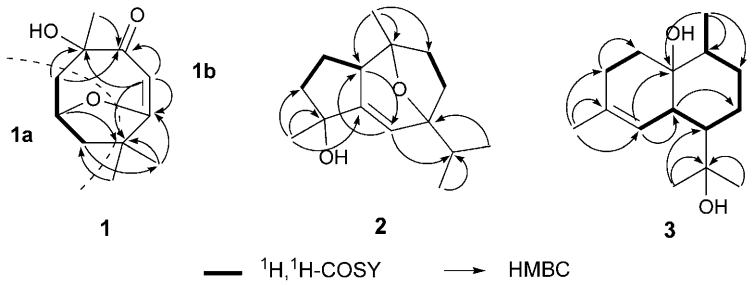


Fig. 1. *^{1}H,^{1}H-COSY and HMBC Correlations for* **1**–**3**

The relative configuration of **1** was deduced from a ROESY experiment, and the computer-generated three-dimensional (3D) representation (energy-minimized with

the PCFF force field) is shown in *Fig. 2*. ROEs were observed from H–C(2) to Me(9), H_α–C(1), and H_α–C(3), and from H–C(6) to Me(9), as further supported by the calculated interatomic distances of <3 Å (*Fig. 2*), suggesting that H–C(2), H_α–C(1), H_α–C(3), H–C(6), and Me(9) were all on the same side of the eight-membered carbon ring. Furthermore, the ROESY correlation of H_β–C(3) with Me(11) indicated β-orientation of Me(11). Thus, the relative configuration of **1** was determined as shown in *Fig. 2*, and the compound was identified as (4*S**,6*R**)-4-hydroxy-4,8,8-trimethyl-9-oxabicyclo[4.2.1]non-1-en-3-one.

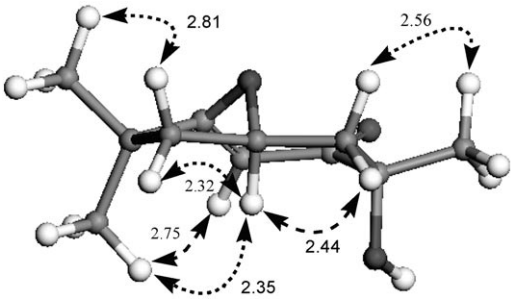
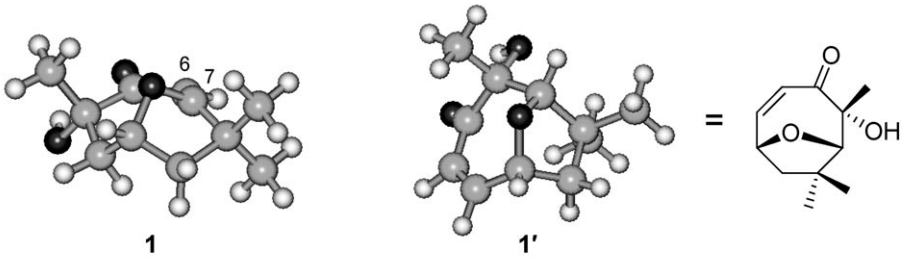


Fig. 2. *Key ROESY Correlations for* **1**. Interatomic distances in Å.

Bredt's rule is a consequence of the fact that a double bond at a bridgehead is geometrically equivalent to an (*E*)-configured double bond in a ring, which is not possible for small rings (less than eight atoms) due to ring strain, especially angle strain. Pubinernoid A (**1**) is a borderline case, (*E*)-C=C bond in an eight-membered ring). Thus, a comparison of the calculated energies between **1** and the hypothetical constitutional isomer **1′** (*Fig. 3*) with an essentially unstrained C=C bond, would be of interest to rationalize the stability of **1**. The computed energies, at different levels of theory, showed that strained **1** is a little higher in energy than the isomer **1′** (*Fig. 3*) [16], but more stable than expected.

Pubinernoid B (**2**) was obtained as a colorless solid. The molecular formula of **2** was established as $C_{15}H_{24}O_2$ by HR-ESI-MS (*m*/*z* 259.1674 ([*M* + Na]$^+$; calc. 259.1673)) and



1 **1′**

PM3	–13.5	0
HF	–10.4	0
DFT	– 7.8	0

Fig. 3. *Energy of* **1** *relative to its isomer* **1′** (=0 kcal/mol) *at different levels of theory*

Table. *^{1}H- and ^{13}C-NMR Data of* **2** *and* **3**. At 400/100 MHz, resp., in $CDCl_3$; δ in ppm, J in Hz. Arbitrary atom numbering. Assignments were confirmed by ^{1}H,^{1}H-COSY, HMQC, and HMBC experiments.

Position	**2**		**3**	
	^{1}H	^{13}C	^{1}H	^{13}C
1	2.03 (*dd*, *J* = 12.1, 6.6)	51.6 (*d*)	–	72.7 (*s*)
2	1.60–1.67 (*m*), 1.38–1.48 (*m*)	24.7 (*t*)	1.70–1.78 (*m*)[a]), 1.57–1.66 (*m*)[b]),	21.9 (*t*)
3	1.69–1.80 (*m*)[a]), 1.76–1.83 (*m*)[b])	40.0 (*t*)	2.07–2.21 (*m*, 2 H)	26.6 (*t*)
4	–	76.3 (*s*)	–	136.3 (*s*)
5	–	151.5 (*s*)	5.65 (*d*, *J* = 5.4)	122.5 (*d*)
6	6.07 (br. *s*)	122.5 (*d*)	1.87 (*dd*, *J* = 9.0, 5.4)	48.0 (*d*)
7	–	84.4 (*s*)	1.33–1.40 (*m*)	54.9 (*d*)
8	1.69–1.80 (*m*)[a]), 1.76–1.83 (*m*)[b])	36.6 (*t*)	1.72–1.80 (*m*)[a]), 1.02–1.11 (*m*)	28.9 (*t*)
9	1.67–1.72 (*m*), 1.69–1.80 (*m*)[a])	39.9 (*t*)	1.58–1.65 (*m*)[b]), 1.14–1.22 (*m*)	31.1 (*t*)
10	–	78.9 (*s*)	1.60–1.65 (*m*)[b])	41.5 (*d*)
11	1.88–1.95 (*m*)	34.5 (*d*)	–	74.0 (*s*)
12	0.97 (*d*, *J* = 6.8)	17.1 (*q*)	1.20 (*s*)	24.8 (*q*)
13	0.94 (*d*, *J* = 6.8)	17.9 (*q*)	1.16 (*s*)	30.2 (*q*)
14	1.32 (*s*)	23.3 (*q*)	0.97 (*d*, *J* = 6.8)	15.2 (*q*)
15	1.34 (*s*)	29.1 (*q*)	1.73 (*s*)	23.4 (*q*)

[a]) [b]) Signals were overlapped.

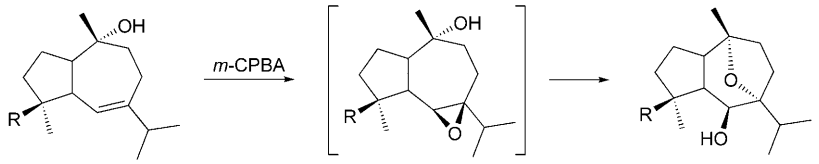
NMR spectroscopy (*Table*). Thus, compound **2** possesses four degrees of unsaturation. The presence of an OH group was revealed by the IR absorption band at 3441 cm^{-1}. The ^{13}C-NMR spectrum showed signals of 15 C-atoms, including those of four Me groups and three quaternary sp^3 C-atoms (δ(C) 76.3, 78.9, 84.4), suggesting an oxygenated sesquiterpenoid. Furthermore, the signals at δ(C) 122.5 (*d*) and 151.5 (*s*) indicated the presence of one trisubstituted C=C bond. Thus, the tricyclic nature of **2** was revealed.

From the ^{1}H,^{1}H-COSY and HMBC correlations, a guaiane-type skeleton of **2** was established (*Fig. 1*), with the OH group being located at a Me-bearing C-atom, C(4), based on the HMBC correlations from Me(15) (δ(H) 1.34 (*s*)) to the quaternary C-atoms at δ(C) 76.3 (C(4)) and 151.5 (C(5)). Therefore, the additional O-atom had to form an oxy bridge. The ^{1}H,^{13}C long-range correlations found from Me(14) (δ(H) 1.32 (*s*)) to C(1) (δ(C) 51.6), C(10) (78.9), and C(9) (39.9), and from both Me(12) and Me(13) (δ(H) 0.97, 0.94 (2*d*)) to C(7) (δ(C) 84.4) and C(11) (34.5), respectively, led to the establishment of a 7,10-epoxide bridge.

The relative configuration of **2** was established by a ROESY experiment. The α-orientation of the 4-OH group was in agreement with the ROE between H–C(1) and Me(15). The ROESY correlation observed between H_α–C(3) and Me(14), but not between H–C(1) and Me(14), reflected the β-orientation of the epoxide bridge between C(7) and C(10).

Since the 7,10-epoxy guaiane sesquiterpenoids **5** and **6** can be synthesized through epoxidation of **7** and **8**, respectively (*Scheme 1*) [17], it is reasonable to assume that the ether bond in **2** could be built up by epoxidation of compound **4**, followed by intramolecular S_N2 reaction and dehydration, as proposed in *Scheme 2*. This biosynthetic assumption, and the co-occurrence of compounds **2** and **4** in the same plant, further supported the β-orientation of the ether bond in **2**. Therefore, the structure of pubinernoid B (**2**) was elucidated as (1*R**,3a*S**,4*S**,7*S**)-4,7-epoxy-1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethyl)azulen-1-ol.

Scheme 1

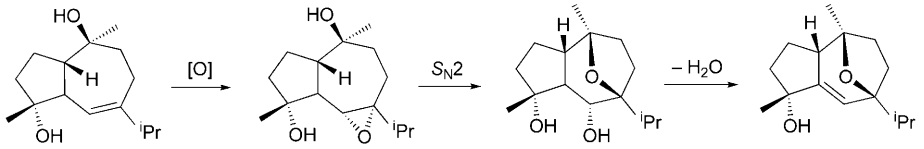


7 R = OH
8 R = SO_3H

m-CPBA = Metachloroperbenzoic acid

5 R = OH
6 R = SO_3H

Scheme 2



4 **2**

Pubinernoid C (**3**) was assigned the molecular formula $C_{15}H_{26}O_2$, as deduced from the positive HR-ESI-MS (*m*/*z* 261.1938 ($[M+Na]^+$)). Careful analysis of 1D- and 2D-NMR data showed that **3** was a cadinene sesquiterpenoid. Comparison of the spectroscopic data of **3** with those of two known compounds (+)-*ent*-epicubenol (**9**) and (−)-epicubenol (**10**) revealed that they were quite similar, except for the moiety at C(11) [18][19]. Observation of the presence of an oxygenated quaternary C-atom (δ(C) 74.0) and the absence of a CH in the ^{13}C-NMR spectrum of **3** indicated an 11-OH group for **3** instead of a nonoxygenated methine at this position in **9** or **10**. This deduction was corroborated by the HMBC correlations of both Me(12) and Me(13) with C(11) (*Fig. 1*). The same sign of optical rotation of **3** ($[\alpha]_D = -60.7$ (MeOH) and **10** ($[\alpha]_D = -95.7$ ($CHCl_3$)) suggested that **3** should have the same absolute configuration as **10** [18]. Thus, the structure of **3** was determined as (1*R**,4*R**,4a*S**,8a*S**)-1,3,4,5,6,8a-hexahydro-1-(1-hydroxy-1-methylethyl)-4,7-dimethylnaphthalen-4a(2*H*)-ol.

Experimental Part

General. Petroleum ether (PE) for chromatography had a b.p. range of 60–90°. Column chromatography (CC) was performed on silica gel (100–200 mesh; *Qingdao Marine Chemical, Inc*., China) and

silica gel *H* (10–40 μm; *Qingdao*). Fractions were monitored by TLC, and spots were visualized by spraying with 10% H_2SO_4 in EtOH, followed by heating. UV Spectra: *Shimadzu 210A* double-beam spectrophotometer; λ_{max} log (ε) in nm. Optical rotations: *Horiba SEPA-300* spectropolarimeter. IR Spectra: *Bio-Rad FTS-135* spectrophotometer, KBr discs; in cm^{-1}. 1D- and 2D-NMR Spectra: *Bruker AM-400* and *DRX-500* instruments; chemical shifts δ in ppm rel. to residual solvent signals, *J* in Hz. ESI-MS and HR-ESI-MS: *VG AutoSpec-3000* spectrometers; in *m*/*z*.

Plant Material. Plants of *S. pubescens* var. *pubinervis* were collected in Erlang Mountain, Sichuan Province, China, in July 2004, and were identified by Prof. *Xi-Wen Li*, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen was deposited at the State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, P. R. China.

Extraction and Isolation. The air-dried leaves and stems of *S. pubescens* var. *pubinervis* (6.4 kg) were extracted with 70% aq. acetone (3×40 l, 2 d, each) at r.t. After evaporating the solvents *in vacuo* at 45°, a residue (320 g) was obtained, which was dissolved in H_2O, and then extracted successively with PE and AcOEt. The AcOEt extract (140 g) was chromatographed on *MCI* gel *CHP 20P* (MeOH/H_2O 9:1, then MeOH). The 90% MeOH fraction (125 g) was subjected to CC (SiO_2, 200–300 mesh, 1 kg) eluting with $CHCl_3$/Me_2CO 1:0 → 0:1 to afford fractions (*Fr.*) A–E. *Fr. C* (24.5 g) was subjected to CC (SiO_2; $CHCl_3$/Me_2CO 30:1) to afford **2** (8 mg), 10-*O*-methylalismoxide (8 mg) [12] and eudesm-4(15)-ene-1, 6-diol (75 mg) [13]. *Fr. D* (15.6 g) was subjected to CC (*RP-18*; MeOH/H_2O 30:70 → 100:0) to afford *Fr. D1–D4*. *Fr. D1* (0.4 g) was subjected to CC (*Sephadex LH-20*; then SiO_2, PE/Me_2CO 10:1) to give **1** (4 mg) and voleneol (12 mg) [14]. *Fr. D3* (3.5 g) was subjected to CC (*RP-18*, *Sephadex LH-20*, SiO_2) to give a residue (223 mg), which was purified by dry-CC (silica gel *H*; PE/i-PrOH 15:1) to afford **3** (12 mg), **4** (65 mg), and clovane-2,9-diol (13 mg) [15].

Pubinernoid A (=*(4S*,6R*)-4-Hydroxy-4,8,8-trimethyl-9-oxabicyclo[4.2.1]-non-1-en-3-one*; **1**). Amorphous powder. UV (MeOH): 260 (3.21). $[\alpha]_D^{19} = +57.8$ ($c = 0.12$, MeOH). ^{1}H-NMR ($CDCl_3$, 400 MHz): 5.69 (*s*, H–C(6)); 4.27–4.38 (*m*, H–C(2)); 2.45 (*dt*, *J* = 13.8, 2.6, H_β–C(3)); 1.94–2.02 (*m*, H_β–C(1)); 1.75–1.81 (overlapped, H_α–C(3)); 1.54 (*dd*, *J* = 14.5, 3.9, H_α–C(1)); 1.78 (*s*, Me(11)); 1.47 (*s*, Me(9)); 1.28 (*s*, Me(10)). ^{13}C-NMR ($CDCl_3$, 100 MHz): 183.2 (*s*, C(5)); 171.6 (*s*, C(7)); 113.0 (*d*, C(6)); 86.6 (*s*, C(4)); 66.8 (*d*, C(2)); 47.4 (*t*, C(1)); 45.7 (*t*, C(3)); 35.9 (*s*, C(8)); 30.6 (*q*, C(10)); 27.0 (*q*, C(11)); 26.5 (*q*, C(9)). HR-ESI-MS (pos.): 219.0992 ($[M+Na]^+$, $C_{11}H_{16}NaO_3^+$; calc. 219.0997).

Pubinernoid B (=*(1R*,3aS*,4S*,7S*)-4,7-Epoxy-1,2,3,3a,4,5,6,7-octahydro-1,4-dimethyl-7-(1-methylethyl)azulen-1-ol*; **2**). Colorless solid. UV (MeOH): end absorption. $[\alpha]_D^{19} = +15.1$ ($c = 0.10$, MeOH). IR (KBr): 3441, 2959, 2927, 1631, 1462, 1376, 1174. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS (pos.): 259.1674 ($[M+Na]^+$, $C_{15}H_{24}NaO_2^+$; calc. 259.1673).

Pubinernoid C (=*(1R*,4R*,4aS*,8aS*)-1,3,4,5,6,8a-Hexahydro-1-(1-hydroxy-1-methylethyl)-4,7-dimethylnaphthalen-4a(2H)-ol*; **3**). Colorless solid. UV (MeOH): end absorption. $[\alpha]_D^{19} = -60.7$ ($c = 0.15$, MeOH). IR (KBr): 3431, 2957, 2924, 1630, 1449, 1375, 1152, 1024. ^{1}H- and ^{13}C-NMR: see the *Table*. HR-ESI-MS (pos.): 261.1938 ($[M+Na]^+$, $C_{15}H_{26}NaO_2^+$; calc. 261.1933).

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