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Atropurpuran, a novel diterpene with an unprecedented pentacyclic cage skeleton, from *Aconitum hemsleyanum* var. *atropurpureum*

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ABSTRACT

A novel diterpene with an unprecedented pentacyclic skeleton, designated as atropurpuran (**1**), featuring a unique motif to fuse two bicyclo[2.2.2]octane units, was isolated from the roots of *Aconitum hemsleyanum* var. *atropurpureum*. Its structure and relative configuration were established by NMR techniques and single-crystal X-ray diffraction. The biosynthetic origin of **1** was proposed to be Hetidane-type diterpenoid.

The genus *Aconitum,* comprising *ca.* 400 species, is widely distributed throughout the northern hemisphere region, such as Asia, Europe, and North America. Of the *ca.* 400 species, more than 200 are endemic to China.[1] Since antiquity, preparations of various species of *Aconitum* plants have been widely used by various civilizations as sources of both traditional medicines and arrow poisons.[2,3] There are around 76 species of *Aconitum* plants clinically used in China. For example, the traditional Chinese medicine 'Cao-Wu', the tubers of *Aconitum* species, has been extensively employed for the clinical treatment of pain, rheumatic, and neurological disorders.[4–6] Plants of *Aconitum* genus are most widely known for their production of diverse diterpenoid alkaloids, structurally classified as C_{18}, C_{19}, and C_{20}-diterpenoid alkaloids, with a wide range of fascinating bioactivities.[2,7] In contrast, diterpene is of rare occurrence in *Aconitum* genus.

Aconitum hemsleyanum var. *atropurpureum* (Hand.-Mazz.) W.T. Wang, growing in the mountain with an elevation between 2100 and 3100 m, taxonomically falls into the Ser. Volubilia Steinb. of Subgen. *Aconitum*.[8] Our previous investigation on the roots of *A. hemsleyanum* var. *atropurpureum* resulted in the isolation of two new C_{19}-diterpenoid alkaloids.[9] Gratifyingly, further chemical investigation on this plant, collected from Emei mountain of China, led to the discovery of a structurally unique diterpene, atropurpuran (**1**), with an unprecedented skeleton, which is the characteristic of a cage framework consisting of five six-membered rings (rings A, B, C, D, and E). Most interestingly, the moiety of rings B, C, D, and E is a unique fusion of two bicyclo[2.2.2]octane units. We presented herein the isolation and structure elucidation of the novel diterpene, atropurpuran (**1**), as well as its proposed biosynthetic pathway.

Air-dried and powdered roots of *A. hemsleyanum* var. *atropurpureum* (2.4 kg) were percolated with 0.05 mol/L hydrochloric acid (25 L). Wet resin (dry weight 25 kg) was added to the percolates, followed by rinsing repeatedly on a suction filter with deionized water. The air-dried resin was then alkalized with 10% aqueous ammonium hydroxide and was continuously eluted with 95% EtOH until no alkaloid could be detected. Removal of the organic solvents in vacuo afforded a black crude material, which was partitioned between Et_2O and H_2O. The ether extract was concentrated to yield a crude alkaloid (23.6 g), which was chromatographed over a silica gel (400 g) column eluting with a gradient petroleum ether-acetone (4:1→1:1) to furnish six fractions (A–F). Repeated column chromatography of Fraction F over silica gel employing cyclohexane–acetone–diethylamine (100:5:1) as eluent, followed by crystallization from methanol, afforded atropurpuran (**1**, 12 mg, Fig. 1).

Atropurpuran (**1**)[10] was isolated as colorless needles. Its IR spectrum suggested the presence of hydroxyl (3743 cm^{-1}) and carbonyl (1723, 1695 cm^{-1}) groups. Its positive-ion HRESIMS showed a quasimolecular ion peak at *m/z* 335.1625 $[M+Na]^+$, corresponding

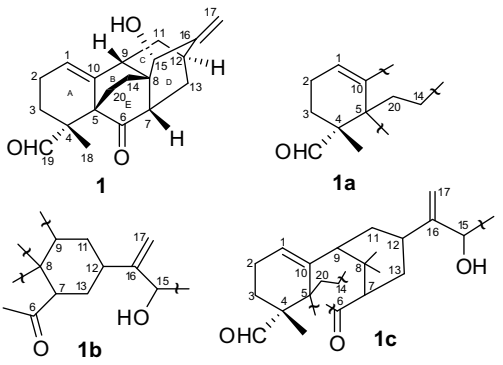

Figure 1. Structure and structural fragments of atropurpuran.

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Table 1
^{1}H and ^{13}C NMR data, and 2D NMR correlations of **1** (400 MHz for ^{1}H, 100 Mz for ^{13}C)

No.	δ_H Mult (J = Hz)	δ_C	^{1}H–^{1}H COSY	HMBC (H→C)
1	5.61 t (4.0)	122.2 d	2α, 2β, 3α, 9	3, 5, 9
2	1.95 m (α)	21.8 t	1	10
	2.08 dd (6.4, 2.8) (β)		1	10
3	1.57 m (α)	30.0 t	1, 2β, 3β	1, 5, 18, 19
	1.69 m (β)		3α	1, 5, 18, 19
4	—	45.1 s	—	—
5	—	54.9 s	—	—
6	—	215.6 s	—	—
7	2.22 dt (11.6, 2.4)	45.9 d	9, 13α, 13β	6, 9, 12, 14
8	—	39.1 s	—	—
9	2.73 m	34.0 d	1, 7, 11α, 11β	1, 5, 7, 12
10	—	142.3 s	—	—
11	1.21 td (7.2, 2.0) (α)	35.9 t	9, 11β, 12	8, 10, 13, 16
	2.11 m (β)		9, 11α, 12	8, 10, 13
12	2.37 m	36.1 d	11α,11β,13α, 13β	7, 9, 15, 17
13	1.98 m (α)	30.1 t	7, 12, 13β	6, 8, 11, 16
	2.04 m (β)		7, 12, 13α	6, 8, 13, 16
14	1.61 m (a)	25.2 t	20a, 20b	5, 9, 15
	1.65 m (b)		20a, 20b	5, 7, 9, 15
15	4.15 br s	72.7 d	17a, 17b	7, 9, 12, 14, 17
16	—	154.2 s	—	—
17	5.05 d (2.4) (a)	108.7 t	15	12, 15
	5.16 dd (2.4, 1.2) (b)		15	12, 15
18	1.04 s	17.3 q	19	3, 5, 19
19	9.67 s	205.3 d	18	5, 18
20	1.87 m (a)	26.1 t	14a, 14b	4, 6, 8, 10
	1.90 m (b)		14a, 14b	4, 6, 8, 10

to the molecular formula $C_{20}H_{24}O_3$ which required nine degrees of unsaturation. The ^{13}C NMR and DEPT NMR spectra of **1** (Table 1) showed 20 carbon resonances due to a methyl carbon, seven methylene carbons, six methine carbons, and six quaternary carbons. ^{1}H NMR, ^{13}C NMR, and HMQC spectra of **1** afforded evidence of an oxymethine (δ_C 72.7; δ_H 4.15 br s, CH-15), a ketone carbonyl (δ_C 215.6, C-6), an aldehyde carbonyl (δ_C 205.3; δ_H 9.67, s, CH-19), a disubstituted double bond [δ_C 154.2 (C-16), δ_C 108.7; δ_H 5.16 dd, J = 2.4, 1.2 Hz; 5.05, d, J = 2.4 Hz, (CH_2-17)], and a trisubstituted double bond [δ_C 142.3, C-10; δ_C 122.2; δ_H 5.61, t, J = 4.0 Hz, CH-1]. Since all these functional groups accounted for four degrees of unsaturation, the remaining five degrees of unsaturation suggested to be contributed by a pentacyclic skeleton.

The combination of ^{1}H–^{1}H COSY and HMBC spectra was critical in both assembling the fragments **1a** and **1b** (see Fig. 1), and linking two fragments together. Fragment **1a** was assembled via (i) COSY correlations between H-1/H_2-2/H_2-3 and H_2-14/H_2-20, and (ii) subsequent HMBC correlations from H_3-18 (δ_H 1.04 s) to C-3, C-5, and C-19, from H-19 (δ_H 9.67 s) to C-5 and C-18, from H-1 (δ_H 5.61 t) to C-3 and C-5, from H_2-2 (δ_H 1.95 m, 2.08 dd) to C-10, from H_2-3 (δ_H 1.57 m, 1.69 m) to C-1, C-5, C-18 and C-19, from H_2-14 (δ_H 1.61 m, 1.65 m) to C-5, and from H_2-20 (δ_H 1.87 m, 1.90 m) to C-4. Similarly, fragment **1b** was evidenced by (i) COSY correlations between H-9/H_2-11/H-12/H_2-13/H-7, and H-15/H-17, and (ii) HMBC correlations from H-9 (δ_H 2.73 m) to C-7 and C-12, from H_2-11 (δ_H 1.21 td, 2.11 m) to C-8, C-13 and C-16, from H-12 (δ_H 2.37 m) to C-7, C-9, C-15, and C-17, from H_2-13 (δ_H 1.98 m, 2.04 m) to C-6, C-8, C-11 and C-16, from H-7 (δ_H 2.22 dt) to C-6, C-9, and C-12, from H-15 (δ_H 4.15 br s) to C-12 and C-17, and from H_2-17 (δ_H 5.05 d, 5.16 dd) to C-12 and C-15. The linkage of fragment **1a** to fragment **1b** via C-9 and C-10 connection to form fragment **1c** (see Fig. 1) was determined on the basis of a HMBC correlation from H-1 to C-9, in addition to correlations from H-9 to C-1 and C-5, and from H_2-11 to C-10. Also, CH_2-14 was found to be connected to C-8 on the basis of HMBC correlations from H_2-14 to C-7 and C-9. In addition, the H-15 exhibited long-range correlations with C-7, C-9, and C-14 in its HMBC spectrum, indicating that CH-15 was also linked with C-8. At this point, C-5 can only be connected to C-6, which was confirmed by the correlations from H_2-20 to C-6 in its HMBC spectrum.

The relative configuration of atropurpuran was primarily established by the NOE correlations summarized in a three-dimensional diagram[11] as shown in Figure 2. In the NOEds experiment, correlations between H_3-18 and H_2-20, H_2-20 and H-7, H-7 and H-15, H-15 and H_2-14 (Fig. 2) indicated the β-orientation of CH_3-18, CH_2-20, H-7, H-15, and CH_2-14, respectively. Despite repeated efforts the relative positions of the remaining C-9 and C-12 could not be assigned by NMR techniques due to lack of NOESY correlations between the protons of these positions. It was highly desirable to obtain a single crystal for X-ray crystallography, which could unambiguously confirm the flat structure and relative configuration. Luckily, compound **1** was successfully recrystallized from methanol and was subjected to X-ray crystallography. The X-ray diffraction pattern (Fig. 3) placed the H-9 on the β-orientation, and the H-12 on the α-orientation, in addition to confirm the above-mentioned flat structure and relative stereochemistry of **1**. The structure of **1** was, therefore, characterized as shown in Figure 1, to which we gave a trivial name atropurpuran according to its originality. This new type of pentacyclic diterpene was designated as atropurpuran-type diterpene.

To the best of our knowledge, atropurpuran (**1**)[12] represents the first example of a novel 6/6/6/6/6-membered ring carbon framework, featuring a bis-bicyclo[2.2.2]octane system. A plausible biogenetic pathway from a hetidane skeleton to atropurpuran was proposed (Scheme 1). Recently, we reported the first hetidane-type diterpene from the whole herbs of *Delphinium campylocentrum*.[13] Diterpene is also of rare occurrence in *Delphinium* genus, an alternate major source of diterpenoid alkaloids. Atropurpuran (**1**) might

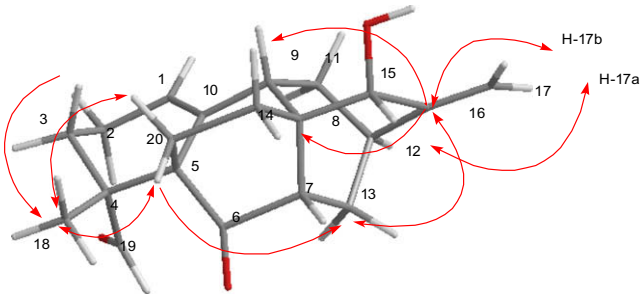


Figure 2. NOE correlations (red arrow) of atropurpuran (**1**).

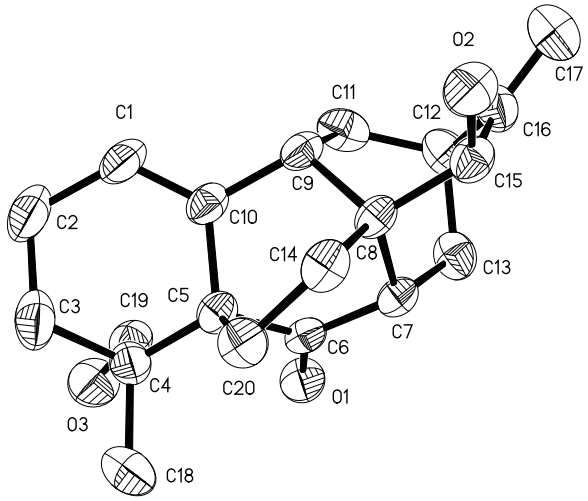
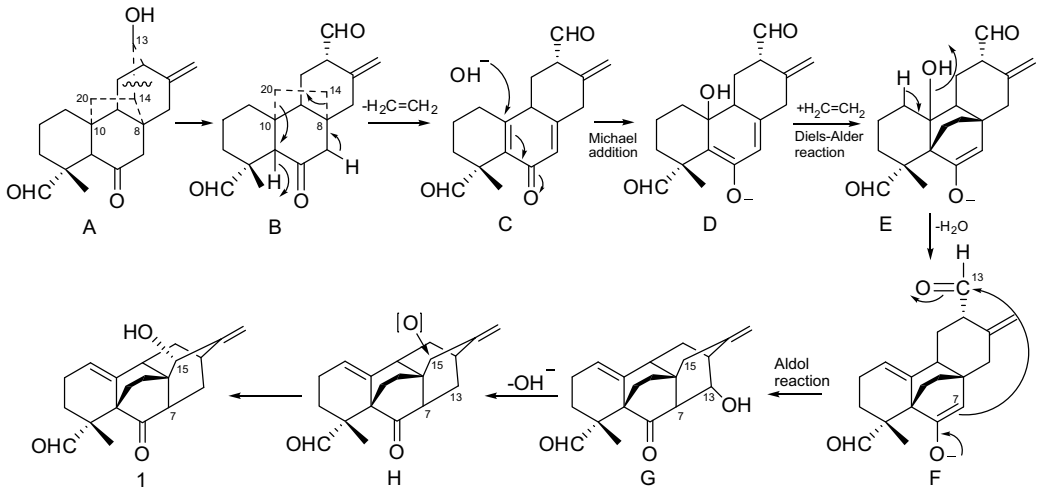


Figure 3. ORTEP drawing of atropurpuran (**1**).



Scheme 1. The plausible biogenetic pathway of atropurpuran (**1**).

be synthesized from a hetidane-type precursor **A**. Fragmentation of C(13)–C(14) of **A** yields a dialdehyde **B**, which can be converted to a hemi-quinone **C**, releasing a molecule of ethylene simultaneously, through fractures of C(14)–C(8) and C(20)–C(10). Michael addition of hemi-quinone **C** produces **D**, followed by a Diels–Alder cycloaddition of **D** with ethylene to yield **E**. Intermediate **G** already possessing the atropurpuran framework can be obtained via dehydration of **E** followed by intramolecular Aldol condensation of the corresponding product **F**. Finally, removal of hydroxyl group at C-13 followed by introduction of a new hydroxyl group at C-15 leads to the construction of atropurpuran (**1**). This compound is of great 1interest for its biogenetic pathway.

Acknowledgments

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Supplementary data

Experimental procedures ^{1}H, ^{13}C, DEPT, HMQC, HMBC, ^{1}H–^{1}H COSY, NOEds, HR-ESI Mass, and IR spectra of atropurpuran (**1**). X-ray crystallographic structure of **1**. CCDC reference number: CCDC 707737. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.11.028.

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10. Atropurpuran (**1**): colorless needle, mp 134-135 °C; $[\alpha]_D^{20}$ −4.8 (*c* 0.6, $CHCl_3$); IR(KBr) ν_{max} 3473, 1723, 1695, 1402, 1111, 1071 cm^{-1}; ^{1}H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 335.1625 ($[M+Na]^+$, calcd for $C_{20}H_{24}O_3Na$, 335.1623).
11. The 3D presentation of 1 was generated by the CambridgesoftTM Chem3D Ultra 2006. In such processing, MM2 energy minimization had been applied.
12. *Crystallographic data for* **1**: $C_{20}H_{24}O_3$, orthorhombic, space group $P2_12_12_1$, (a) 7.458 (2) Å, (b) 11.194 (2) Å, (c) 19.995 (4) Å, *V*) 1669.3(6) $Å^3$, *Z*) 4, (d) 1.243 g/cm3, crystal dimensions 0.01_0.15_0.60 mm was used for measurements on a MAC DIP-2030K diffractometer with a graphite monochromator (ω scans, 2θ max) 50.0°), Mo Kα radiation. The total number of independent reflections measured was 1690, of which 1382 were observed ($|F|^2 \geqslant 2\sigma|F|^2$). Final indices: *R*1, 0.0502; *wR*2, 0.1236; *S*, 1.066; (δ/σ)max, 0.038; $(\delta\rho)$min, −0.163 e/$Å^3$; $(\delta\rho)$max, 0.194 e/$Å^3$. The crystal structures were solved by direct methods using SHELXS-97 (Sheldrich, G. M. Univerisy of Gottingen: Gottingen, Germany, 1997) and were expanded using difference Fourier techniques, refined by SHELXL-97 (Sheldrich, G. M. University of Gottingen: Gottingen, Germany, 1997) and full-matrix least-squares calculations.
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