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LINGULATUSIN, TWO EPIMERS OF AN UNUSUAL LINEAR DITERPENE FROM *ASTER LINGULATUS*

IN HONOUR OF PROFESSOR G. H. NEIL TOWERS 75TH BIRTHDAY

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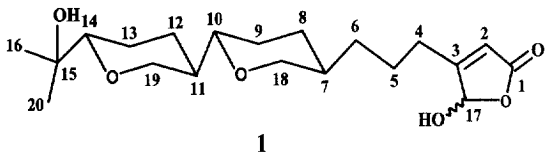
Abstract—A 1:1 mixture of two epimers of an unusual linear diterpene, lingulatusin, was isolated from the whole plant of *Aster lingulatus*. The structure was determined on the basis of IR, MS and extensive NMR spectral studies, and X-ray crystallographic data. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In previous papers we reported the isolation and structure elucidation of four cytotoxic triterpenoid saponins from the *n*-BuOH soluble fraction of a 70% ethanol extract of *Aster lingulatus* (Compositae) [1, 2]. In a continuing search for new bioactive natural substances in this plant, we undertook an examination of the EtOAc soluble fraction. Chromatography yielded the isolate **1** which was identified as a 1:1 mixture of two epimers of an unusual linear diterpene with a γ-hydroxy butenolide and two tetrahydropyran moieties. We herein describe the isolation and the structure elucidation of **1**, to which we have given the name lingulatusin, through extensive NMR spectroscopic techniques and X-ray crystallography data.



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RESULTS AND DISCUSSION

Lingulatusin (**1**) was obtained as colorless needles, mp 145–147°C, UV(MeOH) λ_{max} 207 nm (ε 11,270). The molecular formula, $C_{20}H_{32}O_6$, was derived from positive FAB MS ($[M+K]^+$ at m/z 407 and $[M+Na]^+$ at m/z 391), and ^1H and ^{13}C NMR data, and indicated five degrees of unsaturation. The IR absorptions at 3423 and 1745 cm^{-1} indicated the presence of alcohol and ester functions, and the DEPT and APT ^{13}C NMR spectra (Table 1) showed the signals for a total of twenty carbons, including two tertiary methyls, nine methylenes, six methines, and three quaternary carbons, suggesting a diterpenoid structure.

The ^{13}C resonances of two olefinic carbons at δ 172.2 (s, C-3) and 117.7 (d, C-2), a carbonyl at δ 174.0 (s, C-1) and a hemiacetal at δ 100.9 (d, C-17) led us to propose the presence of a hydroxy butenolide moiety (partial structure A) [3]. In the ^1H NMR spectrum of 1, two broad singlets at δ 5.88 and 5.99, assigned to H-2 and H-17, respectively, showed very small long-range coupling, and thus the butenolide moiety has a β-substitutent. This unit accounted for three of the five unsaturations and three oxygen atoms of the six oxygen atoms in the molecular formula. Therefore, **1** must contain two additional ring structures and three additional oxygen atoms.

The ^1H-^1H COSY, with aid of TOCSY, established the major proton system from H-4 to H-14. The isolated ^1H NMR signal (H-4) resonating at δ 2.38 was chosen as the starting point for the analysis of COSY and TOCSY spectra. The correlations between H-4 (δ 2.38) and H-5 (δ 1.61), H-5 and H-6 (δ 1.18), H-6 and H-7 (δ 1.51), H-7 and H-8 (δ 1.93 and 1.11), H-7 and H-18 (δ 2.97 and 3.88), H-8 and H-9 (δ 1.24 and 1.71),

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Table 1. NMR spectral data of lingulatusin (**1**) in CD_3OD

Position	δ_C	position	δ_H	HMBC
1	174.0 (s)	1		
2	117.7 (d)	2	5.88 (s)	C-1, C-4, C-17
3	172.2 (s)	3		
4	28.7 (t)	4	2.38 (m)	C-3, C-5
5	24.8 (t)	5	1.61 (m)	C-4, C-6
6	33.1 (t)	6	1.18 (m)	C-5, C-7
7	37.0 (d)	7	1.51 (m)	C-5, C-6, C-9, C-18
8	31.2 (t)	8α	1.93 (m)	C-10, C-18
		8β	1.11 (m)	C-10
9	30.1 (t)	9α	1.24 (m)	C-7, C-8, C-11
		9β	1.71 (m)	C-7, C-8
10	81.1 (d)	10	2.96 (m)	C-8, C-11, C-12, C-18, C-19
11	42.5 (d)	11	1.53 (m)	C-10
12	27.3 (t)	12α	1.83 (m)	C-13, C-14, C-19
		12β	1.23 (m)	C-10
13	26.4 (t)	13α	1.32 (m)	C-11, C-12, C-14, C-15
		13β	1.71 (m)	C-11, C-12
14	85.6 (d)	14	2.98 (dd, 11.2, 4.0)	C-12, C-15, C-16, 19, C-20
15	72.8 (s)	15		
16	25.6 (q)*	16	1.12 (s)*	C-14, C-15, C-20
17	100.9 (d)	17	5.99 (s)	C-1, C-2
18	74.4 (t)	18α	3.88 (dd, 11.2, 4.0)	C-7, C-8, C-10
		18β	2.97 (dd, 11.2, 11.2)	C-6, C-8, C-10
19	72.3 (t)	19α	4.20 (dd, 11.2, 3.9)	C-11, C-12, C-14
		19β	3.13 (dd, 11.2, 11.2)	C-10, C-11, C-12, C-14
20	25.7 (q)*	20	1.14 (s)*	C-14, C-15, C-16

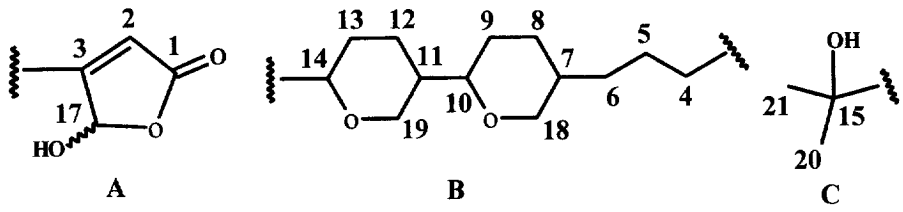
*Values may be interchanged in the same column. Coupling constants (parentheses) are in Hz.

H-9 and H-10 (δ 2.96), H-10 and H-11 (δ 1.53), H-11 and H-12 (δ 1.23 and 1.83), H-11 and H-19 (δ 3.13 and 4.20), H-12 and H-13 (δ 1.32 and 1.71), and H-13 and H-14 (δ 2.98) led to the assignment of the protons continuing from H-4 to H-14. The ^{13}C NMR data which were assigned by the HMQC experiment showed that C-10 (δ 81.1), C-14 (δ 85.6), C-18 (δ 74.4) and C-19 (δ 72.3) were attached to oxygen atoms. In the HMBC spectrum, H-14 (δ 2.98) was correlated with C-19 (δ 72.3), and H-19 (δ 4.20 and 3.13) was correlated with C-14 (δ 85.6). The above correlations suggested that C-14 and C-19 shared an oxygen atom to form a tetrahydropyran ring. Similarly, the long-range correlations between H-10 (δ 2.96) and C-18 (δ 74.4), and H-18 (δ 2.97 and 3.88) and C-10 (δ 81.1) indicated that C-10 and C-18 shared another oxygen atom to form a second tetrahydropyran ring. These data taken together are in accordance with the partial structure B.

Taking into account the molecular formula and the two partial structures A and B, two methyls and a hydroxy-bearing quaternary carbon (δ 72.6) remained to be assigned. The two methyls (Me-16 and Me-20), appearing as singlets (δ 1.12 and 1.14), were correlated with C-15, thus, establishing the partial structure C.

HMBC correlations were used to assemble the three partial structures. The correlation between C-15 (δ 72.8) and H-14 (δ 2.98) established the linkage of the partial structures B and C from C-14 to C-15, and the correlation between H-4 (δ 2.38) and C-3 (δ 172.2) established the linkage of the partial structures A and B from C-3 to C-4. These considerations yielded the gross structure of **1**, without stereochemical implications.

The relative stereochemistry of **1** was deduced using a combination of the ^1H-^1H coupling constants and NOE interactions from a NOESY experiment. The large couplings observed between H-14 and axial H-



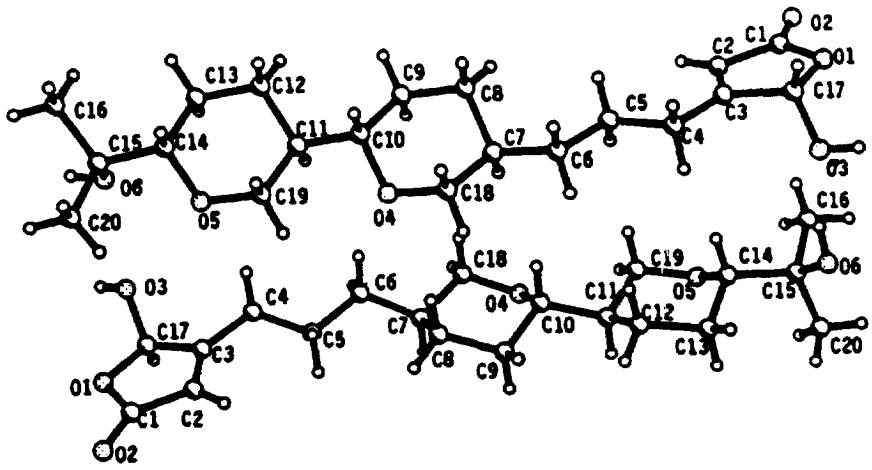


Fig. 1. ORTEP drawing of the crystal structure of lingulatusin (**1**).

13 ($J_{14,13ax}=11.2$ Hz), and H-11 and axial H-19 ($J_{11,19ax}=11.2$ Hz) required that H-14 and H-11 were located in *trans*-diaxial positions (β and α, respectively). It could be determined that the tetrahydropyran ring from C-14 to C-19 adopted a regular chair conformation with the aid of Dreiding stereo models. The observed NOEs from H-14 to H_{ax}-12, H-14 to H_{ax}-19, H_{ax}-12 to H-19, H-11 to H_{ax}-13, H-11 to equatorial H-19 favored the above deductions. Likewise, the assignment of a β-configuration for H-10 and an α-configuration for H-18 was based on the coupling constant ($J_{18ax,7}=11.2$ Hz) and observed NOEs between H-10 and H_{ax}-8, H-10 and H_{ax}-18, H-7 and H_{eq}-18. The tetrahydropyran ring from C-10 to C-18 was also present in a chair conformation.

The relative stereochemistry at C-17 could not be determined by NMR data. Thus, in order to firmly establish the structure and stereochemistry, **1** was subjected to single crystal crystallography.* The absolute configuration of **1** was finally established and lingulatusin (**1**) was found to be a C-17 epimeric (17α or 17β-hydroxy) mixture in the ratio 1:1. The isolate crystallized in a triclinic space group P1 with $z=2$ molecules in the unit cell. The statistic of $|E^*E-1|$ pointed clearly to a centrosymmetric space group, but all attempts to solve the structure in P-1 failed. A trial in the most common space group P1 revealed two separate epimeric molecules in the asymmetric unit, i.e. the unit cell was as shown in Fig. 1. The molecules differed only in the configuration of the hydroxy substituent at the C-17, one being up and the other down of the ring. This explained the unusual statistical value and the crystallization behavior. The refinement converged anisotropically with hydrogens indicated at $R=5.1\%$ for all 2850 measured data. It is noteworthy that the NMR data of **1** did not show two sets of signals for the two epimers. Hence, it was proposed that the conversion of two epimers in solution was very fast.

Lingulatusin represents a new class of linear diterpenoid. Even though several natural products bearing the γ-hydroxy butenolide moiety have been isolated from marine sources [4–12] and some of them have exhibited potent anti-inflammatory [11, 12] and antitumor activities [8], to our knowledge, no naturally occurring compound of this type has been reported from higher plants.

EXPERIMENTAL

General

Mps were determined on a Kofler apparatus and are uncorrected. IR spectrum was obtained on a Mattson CYGNUS 100 Fourier-transform infrared spectrometer. FABMS spectrum was recorded on a Finnigan MAT-90 instrument. ^{1}H-NMR and ^{13}C-NMR spectra were measured on a Nicolet NT-360 (360 MHz for ^{1}H, 90 MHz for ^{13}C) spectrometer in CD_3OD with TMS as int. standard. The two-dimensional NMR spectra (^{1}H-^{1}H COSY, HMQC, TOCSY and HMBC) were recorded on a General Electric GN Omega 500 MHz spectrometer operating at 500 MHz for ^{1}H NMR and at 125 MHz for ^{13}C NMR. ^{13}C-NMR multiplicity was determined using DEPT and APT experiments.

Plant material

The plant material of *Aster lingulatus* was collected in August 1992, from Li-Jiang County, Yunnan province, China. A voucher specimen was identified by Professor Z. W. Lu and is deposited in the Herbarium of Kunming Institute of Botany, Academia Sinica, Kunming, China.

*All X-ray structure calculations were performed with SHELXTL. The crystal data are deposited at the Cambridge Crystallographic Data centre.

Extraction and isolation

The dried whole plants of *A. lingulatus* (7 kg) were percolated five times with 70% EtOH (each 1 week) at room temperature. The combined extracts were evaporated to dryness *in vacuo* to afford a residue (1 kg) that was suspended in H_2O and then partitioned successively with petroleum ether, EtOAc, and H_2O-saturated *n*-BuOH to yield three corresponding fractions (145, 280, and 470 g, respectively). The EtOAc fraction (280 g) was subjected to column chromatography on silica gel eluting with chloroform–acetone (10:1–1:1) gradient. The chloroform–acetone (6:1) eluent (520 mg) was further separated by Sephadex LH-20 (MeOH) followed by crystallization from ethanol to afford **1** (235 mg, 0.0034%, dry wt.).

Lingulatusin (**1**)

Colorless needles; mp 145–147°C; UV (MeOH) λ_{max} 207 nm (ε 11270); $C_{20}H_{32}O_6$; positive-ion FABMS *m/z*: 407 $[M+K]^+$, 391 $[M+Na]^+$, 369 $[M+H]^+$, 351 $[M-H_2O+1]^+$, 309 $[M-C_3H_7O]^+$, and 225 $[M-C_8H_{15}O_2]^+$; IR (KBr) γ_{max} 3423 (OH), 1745 (COOR), 1640 and 1545 (C=C), 1000–1100 (C–O) cm^{-1}; ^1H and ^{13}C NMR: see Table 1.

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