10-*epi*-Olguine from *Rabdosia ternifolia*

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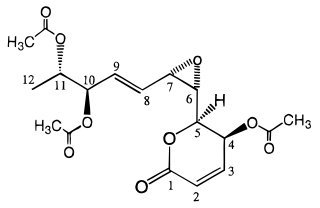
An unsaturated lactone, 10-*epi*-olguine (**1**), has been isolated from *Rabdosia ternifolia* (D. Don) Hara. The structure was established by spectroscopic and X-ray crystallographic analyses. The compound displayed modest cytotoxicity in several human cancer cell lines.

The genus *Rabdosia* (Labiatae) has been known to contain diterpene compounds, many of which possess cytotoxic, antitumor and other biological activities.[1] One of the species, *R. ternifolia* (D. Don) Hara, a shrub growing in the southwestern region of China and Indochina, is used in folkloric medicine as an antibacterial and antiinflammatory remedy to treat infectious diseases such as enteritis, laryngopharyngitis, hepatitis, nephritis, and common cold.[2] The leaf part of *R. ternifolia* has been reported to contain diterpene compounds,[3−8] including the antitumor constituent oridonin.[9−13] Considering the contribution of this plant species to local folk medicine, we have reinvestigated the leaf part for new chemical constituents. The present report describes the isolation and structural elucidation of an unsaturated lactone (**1**).



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Compound **1** was isolated from an acetone extract of the dried leaves as colorless needles, mp 147−148 °C; $[\alpha]^{24}_D$ +149° (*c* 0.03, $CHCl_3$). A molecular formula of $C_{18}H_{22}O_9$ was deduced for **1** by HRCIMS and corroborated by ^{13}C NMR data. The IR spectrum of **1** exhibited absorptions at 1735−1742 (C=O) cm^{-1}, and the UV spectrum was transparent beyond 216 nm. The ^1H NMR spectrum of **1** (Table 1) displayed three acetyl methyl singlets at δ 1.95, 2.00, and 2.06, which could be confirmed by the corresponding carbons at δ 20.81, 21.16, and 21.36. The connectivity of the remaining protons was established by the coupling information obtained from the ^1H,^1H-COSY, and COLOC spectra.

Table 1. ^1H and ^{13}C NMR Data for **1**

position	δ_C[a]	δ_H[a] (multiplicity, *J* in Hz)
1	161.5	
2	125.1	6.15 (d, 9.6)
3	140.7	7.01 (d, 5.7, 9.6)
4	62.8	5.21 (dd, 3.0, 5.5)
5	75.0	4.14 (dd, 3.0, 8.4)
6	54.7	3.41 (dd, 4.2, 8.4)
7	55.8	3.61 (dd, 4.2, 4.5)
8	127.2	5.71 (dd, 4.5, 15.6)
9	130.8	5.80 (dd, 5.7, 15.6)
10	74.4	5.26 (dd, 3.9, 5.7)
11	70.4	4.98 (dq, 6.6, 4.0)
12	15.5	1.04 (d, 6.6)
$OCOCH_3$	170.0	
	170.2	
	170.5	
$OCOCH_3$	20.8	1.93 (s)
	21.2	2.01 (s)
	21.3	2.04 (s)

[a] Assignments were aided by ^1H,^1H-COSY, ^1H,^{13}C-COSY, and COLOC results.

On the basis of the ^1H,^{13}C-COSY data (Table 1), the C-8 and C-9 could be assigned to a *trans*-oriented olefinic group (J = 15.6 Hz), whereas C-2 and C-3 constituted another olefinic group in *cis*-orientation (J = 9.6 Hz). In addition, H-6 and H-7 showed a vicinal coupling of 4.2 Hz, consistent with a *cis*-epoxy functional group. The remaining part of the molecule was therefore a lactone involving the carbonyl at C-1 (δ 161.50). The analysis led to two plausible structures for **1**, i.e., a five-membered lactone between C-1 and C-4 and a six-membered lactone between C-1 and C-5. Inspection of the EI and FAB mass spectra of the compound revealed base peaks at m/z 227 and 154, respectively. The former fragment corresponded to a loss of C-1 through C-5 from **1**, whereas the latter fragment peak represented the six-membered lactone part.

A search in the literature revealed a compound called olguine, previously isolated from a *Hyptis* species, possessing the same planar structure **1**.[14] In order to unambiguously establish the identity of the isolated compound, a suitable single crystal was chosen for X-ray crystallographic analysis.

The crystal structure (Figure 1) confirmed the *cis*-stereochemistry about the oxirane ring (C-6 and 7) as

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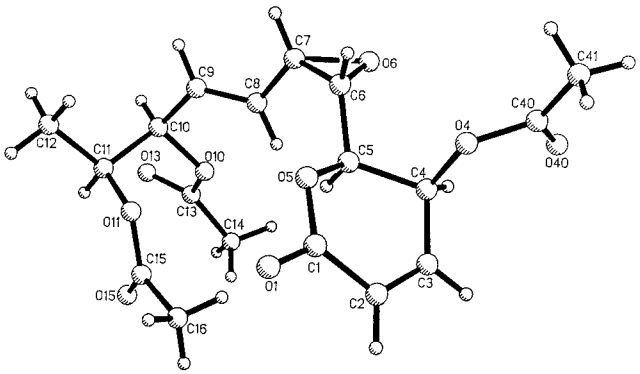

Figure 1. Crystal structure of **1**.

Table 2. Cytotoxic Activity of **1**

cell line[a]	ED_{50}, μg/mL
BC-1	1.8
Lu-1	4.8
Col-2	2.1
KB	1.8
KB-V (-VLB)	2.1
LNCaP	1.2
KB-V (+VLB)	2.2

[a] See ref 15 for full details of cell lines.

well as the *trans* vinyl group (C-8 and 9). In the solid state the H-7 and H-8 protons located in between the epoxide−vinyl linkage are essentially coplanar and *trans*. The proton H-10 is also nearly coplanar with H-9, albeit in a *syn*-relationship, since the C-10 substituents are present in an eclipsed manner to the vinyl carbon C-9. Closer inspection showed that compound **1** was isomeric with olguine[14] and possessed the *R*-configuration at C-10 compared with the *S*-configuration for olguine itself. The assumption was made that all other chiral centers conform to the stereochemistry of olguine, since an absolute configuration determination was unsuccessful. Compound **1** was thus determined to be 10-*epi*-olguine.

A 2D-NOESY experiment indicated that, in $CDCl_3$ solution, the bond between C-5 and C-6 rotates freely to allow the observation of NOE between H-5/H-6 and H-5/H-8. Full ^1H and ^{13}C NMR assignments (Table 1) were made on the basis of the ^1H,^1H-COSY, ^1H,^{13}C-COSY, and COLOC results.

Compound **1** was evaluated for cytotoxic activity in a panel of cultured human cancer cells using established protocols.[15] As shown in Table 2, 10-*epi*-olguine exhibited modest cytotoxicity (ED_{50} < 4 mg/mL) in a number of cell lines.

Experimental Section

General Experimental Procedures. The melting point was determined on an Electrothermal digital melting point apparatus and was uncorrected. Optical rotation was measured using a Perkin-Elmer 241 polarimeter. IR spectra were obtained as a KBr pellet on a Perkin-Elmer 16 PC FTIR spectrometer and UV spectra on a Milton Roy Spectronic 3000 array spectrophotometer. ^1H and ^{13}C NMR spectra were recorded on a Bruker ARX-300 NMR spectrometer. All NMR experiments were run in $CDCl_3$ at room temperature with TMS as internal standard. EIMS and FABMS were recorded on a Finnigan TSQ-7000 triple quadrupole mass spectrometer, whereas HRCIMS was measured on a Kratos MS80RFAQ instrument. The X-ray structure was obtained using a Siemens P4/RA diffractometer.

Plant Material. *R. ternifolia* was collected by Wenwu Li of the Chengdu Institute of Biology from the Yunnan Province in November 1994. The plant was authenticated by Prof. Xiwen Li of the Kunming Institute of Botany. A voucher specimen has been deposited in the herbarium of the School of Pharmacy, West China University of Medical Sciences.

Extraction and Isolation. Air-dried, powdered leaves (1.2 kg) were extracted with petroleum ether (boiling range 35−60 °C) at 45 °C for 10 h, followed by extraction with acetone for 12 h at the same temperature. After evaporation of acetone under reduced pressure, the residue (125 g) was dissolved in MeOH (2 L) and filtered. The filtrate was decolorized over active charcoal and concentrated under reduced pressure to 500 mL. A yellowish precipitate (28 g) was obtained overnight. The crude isolate was purified by chromatography over Si gel (type H, 160−200 mesh, Qingdao Marine Chemical Factory), eluted with increasing polarity of cyclohexane−acetone mixtures. An analytically pure product of **1** (360 mg) was obtained following recrystallization from MeOH. The compound revealed an R_f value of 0.49 on TLC in a solvent system containing 10% acetone in $CHCl_3$.

10-*epi*-Olguine (1): obtained as colorless crystals; mp 147−148 °C; UV (95% EtOH) λ_{max} 216 nm; IR (KBr) ν_{max} 1735−1742, 1256 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz), see Table 1; ^{13}C NMR ($CDCl_3$, 75 MHz), see Table 1; HRCIMS *m/z* 383.1327 (calcd for $C_{18}H_{23}O_9$ 383.1342); EIMS (70 eV) *m/z* $[M]^+$ 382 (5), 323 (8), 280 (4), 227 (100); FABMS *m/z* 383 $[M + H]^+$, 154.

Crystal data: $C_{18}H_{22}O_9$, $M = 382.4$, colorless needle, 0.35 × 0.1 × 0.1 mm, monoclinic, space group $P2_1$, $T = 298$ K, $a = 8.518(2)$ Å, $b = 9.074(2)$ Å, $c = 13.187(2)$ Å, $\beta = 103.64(2)°$, $V = 995.7(4)$ Å, $D = 1.275$ Mg/m^3, $Z = 2$, $F(000) = 404$, μ (Cu Kα) = 0.878 mm^{-1}, $\lambda = 1.541\,78$ Å.

Data Collection and Structure Refinement. Intensities of 1592 reflections within $3 \le 2\theta \le 108°$ were collected on a Siemens P4-RA diffractometer using Cu Kα radiation. Of these, 1186 were unique and observed $F \ge 4\sigma(F)$. Structure solution was by direct methods and refinement by full-matrix least-squares methods using the SHELXTL-PLUS suite of X-ray programs.[16] All non-hydrogen atoms were refined anisotropically, and hydrogen atoms were found by difference Fourier method and then placed in geometrically idealized positions ($\delta_{C-H} = 0.96$ Å). Final discrepancy indices were $R = 0.054$, wR = 0.051, and the GOOF (goodness of fit) = 2.17. An empirical extinction parameter was successfully refined. Residual electron-density was ±0.25 e^- $Å^{-3}$.

Cytotoxicity Assay. Compound **1** was evaluated for cytotoxic activity using previously described protocols.[15]

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