

A New Diepoxy-*ent*-kauranoid, Rugosinin, from *Isodon rugosus*

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Abstract

A new diterpenoid, rugosinin (**1**), isolated from *Isodon rugosus*, with absolute configuration was proved by single-crystal X-ray diffraction analysis, to be the member of a rare class of C-20/C-7 and C-20/C-14 diepoxy-*ent*-kauranoids. Effusanin A (**2**), effusanin B (**3**), effusanin E (**4**), lasiokaurin (**5**) and oridonin (**6**) were found as known constituents of the genus *Isodon* with C-20/C-7 epoxy function. These compounds have exhibited DNA-damaging activity in assay which employed DNA-repair deficient (RAD52Y) and repair proficient (RAD⁺) yeast strains.

Many *ent*-kaurane diterpenoids have been isolated from plants belonging to the genus *Isodon* (syn. *Rabdosia*), most of which have significant biological activities [1], [2], [3], [4]. The present phytochemical investigation on the methanolic extracts of *Isodon rugosus* of Pakistani origin has led to isolation of six *ent*-kauranoids (**1–6**). Compound **1** was obtained from the aerial part of the plant along with known epoxy-*ent*-kauranoids, effusanin A (**2**) [5], effusanin B (**3**) [5], effusanin E (**4**) [5], lasiokaurin (**5**) [6] and oridonin (**6**) [7]. The new compound, rugosinin (**1**) had a unique structure in which two epoxy units are present as ether bridges between C-20/C-7 and C-20/C-14. The structure was confirmed by X-ray crystallographic analysis.

Rugosinin (**1**) is a sixth example of diepoxy containing *ent*-kauranoid. Among five of known congeners of this rare class [8], [9], [10], only the structure of xerophyllusin C, has been determined by the X-ray diffraction analysis [10]. The new compound, rugosinin (Fig. 1) was found to be a 6-*O*-acetyl derivative of xerophyllusin A [10]. The present work, therefore, provides further confirmation of the diepoxy-containing structures in this class of compounds.

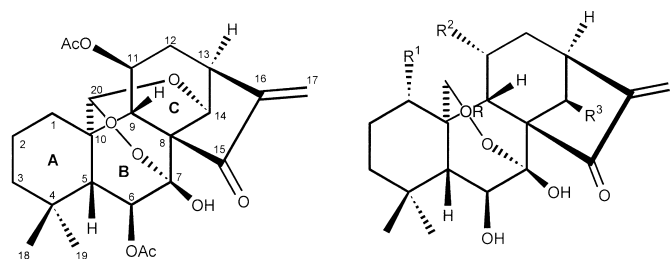
The structure **1** established by X-ray diffraction analysis clearly explains the NMR spectral pattern. The diepoxy linkages present between C-7/C-20 and C-14/C-20 forces the molecule to acquire

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Rugosinin (1)

Effusanin A (2)	R ¹ =OH, R ² =H, R ³ =H
Effusanin B (3)	R ¹ =OAc, R ² =H, R ³ =H
Effusanin E (4)	R ¹ =OH, R ² =OH, R ³ =H
Lasiokaurin (5)	R ¹ =OAc, R ² =H, R ³ =OH
Oridonin (6)	R ¹ =OH, R ² =H, R ³ =OH

a rigid shape with a chair conformation for ring A, boat for ring B, chair for ring C (C8-9-11-12-13-14) and boat for the seven-membered ring (C8-9-11-12-13-15-16). Apparently there is a flexibility only in ring A. Thus, the dihedral angles between H-5 β and H-6 α , H-9 β and H-11 α , and H-11 α and H-12 β become almost 90°, exhibiting very small *J* values (0–2 Hz).

Compounds **1–5** exhibited significant cytotoxic activity in the bioassay employing DNA repair-deficient (RAD 52Y, mutant) yeast strains [11]. This may be due to the presence of conjugated exomethylene system in an *ent*-kaurane skeleton [1]. Interestingly, these compounds were invariably less toxic to the repair-proficient (RAD⁺, wild type) yeast strains. Compound **6** was not tested due to insufficient quantity.

Material and Methods

Melting points were determined on a Yanaco apparatus. UV spectra were measured on a Shimadzu UV 240 spectrophotometer. IR spectra were recorded on a Jasco A-302 spectrophotometer. ¹H- and ¹³C-NMR spectra were recorded on a Bruker AM-400 spectrometer with tetramethylsilane (TMS) as an external standard. 2D NMR spectra were recorded on a Bruker AMX 500 NMR spectrometer. Optical rotations were measured on Jasco DIP-360 digital polarimeter by using 10 cm cell tube. Mass spec-

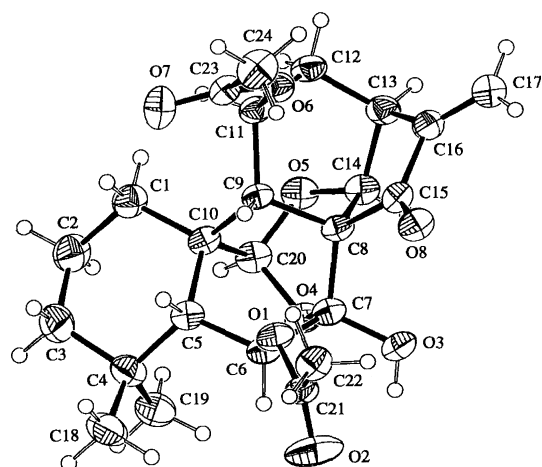


Fig. 1 The computer generated ORTEP drawing of rugosinin (1) (showing absolute stereochemistry).

tra (EI and HREIMS) were measured in an electron impact mode on Finnigan MAT 311 or MAT 312 spectrometers and ions are given in *m/z* (%). Fast atom bombardments (FAB) MS were measured on Jeol HX110 mass spectrometer. TLC was performed on a pre-coated silica gel card (E. Merck). For column chromatography, silica gel (E. Merck, 230–400 mesh) was used.

The aerial parts of *I. rugosus* Wall. were collected from Shahrig, Balochistan, Pakistan, in December 1998 by two of us (R. B. T. and M. A. Z.). A voucher specimen (No. 1422) was deposited in the Herbarium of the University of Balochistan.

Air-dried and ground aerial parts of *I. rugosus* (7.5 kg) were extracted three times (each one week) with MeOH (20 L) at room temperature. The resulting MeOH extract was concentrated under vacuum. The dried extract (ca. 500 g) was adsorbed on silica gel (ca. 1.5 kg) and eluted with hexane, hexane-CHCl₃ (1 : 1, 2 × 500 mL), CHCl₃ (100%, 2 × 750 mL), CHCl₃-MeOH (1 : 1, 2 × 750 mL) and MeOH (100%, 2 × 500 mL). The CHCl₃ (300 g) fraction was chromatographed over 900 g of silica gel and eluted with mixtures of CHCl₃ in hexane (50% to 100%, ca. 2 × 750 mL each of the five fractions) and then with acetone (2 × 1L). The CHCl₃ (100%) eluant (1.5 g) afforded compounds **1** (ca. 50 mg, 7.1 × 10⁻⁴% yield) and **3** (ca. 40 mg, 5.7 × 10⁻⁴%). The acetone eluant was concentrated and the residue (163 g) was extracted successively with hexane, ether, benzene, EtOAc, and CHCl₃-MeOH (ca. 2 × 500 mL each eluant). The ether extract (39 g) was chromatographed on silica gel (90 g) with CHCl₃-MeOH (5% to 10%, 2 × 250 mL) to obtain nine fractions. The fractions 6–8 (750 mg) were combined and chromatographed over 2.5 g silica gel using EtOAc-hexane-MeOH (30 : 65 : 5), stepwise elutions (12 × 50 ml) with increasing only EtOAc from 30–50% to obtain compounds **2**, **4**, **5**, and **6** in ca. 20 mg (2.8 × 10⁻⁴% yield), 45 mg (6.4 × 10⁻⁴% yield), 35 mg (5 × 10⁻⁴% yield) and 15 mg (2.1 × 10⁻⁴% yield), respectively. Compounds **2–6** were identified by comparison of their physical and spectral data with that reported in the literature.

Rugosinin (1): Colorless prisms (EtOAc-hexane); m.p. 156–158 °C; [α]_D²⁵: –127° (MeOH, *c* 0.4); UV (MeOH): λ_{\max} log (ϵ) = 232 nm (4.10); IR (CHCl₃): ν_{\max} = 3350, 1722, 1640 cm⁻¹; EIMS: *m/z* = 386 [(M - AcOH)⁺, 23], 326 [(M-2×AcOH)⁺ 16], 298 (53), 280 (100); FABMS (-ve): *m/z* = 537 [M+glycerol-1]⁻; HREIMS *m/z* = 386.1752, calcd: for (C₂₄H₃₀O₈ - AcOH) 386.1729. ¹H-NMR (400 MHz, CDCl₃): δ = 6.04, 5.39 (each 1H, s, CH₂ = C <), 5.12 (1H, d, *J*_{11 α 12 α} = 5.6 Hz, H-11 α), 5.11 (1H, s, H-20), 4.86 (1H, d, *J*_{6 α 5 β} = 1.5 Hz, H-6 α), 4.59 (1H, br d, *J*_{14 β 13 α} = 6.1 Hz, H-14 β), 3.19 (1H, ddd, *J*_{13 α 14 β} = 6.1 Hz, *J*_{13 α 12 β} = 3.1 Hz, *J*_{13 α 12 α} = 3.1, H-13 α), 2.48 (1H, ddd, *J*_{12 α 12 β} = 14.6 Hz, *J*_{12 α 11 α} = 5.6 Hz, *J*_{12 α 13 β} = 3.1 Hz, H-12 α), 2.17 (1H, br s, H-9 β), 2.23, 1.90 (each 3H, s, OAc), 0.89 (6H, s, 2 × Me). ¹³C-NMR (100 MHz, CDCl₃) δ 27.1 (C-1), 18.4 (C-2), 40.0 (C-3), 34.5 (C-4), 60.0 (C-5), 74.0 (C-6), 101.0 (C-7), 55.0 (C-8), 53.0 (C-9), 44.0 (C-10), 67.0 (C-11), 31.7 (C-12), 38.5 (C-13), 68.5 (C-14), 199.0 (C-15), 150.0 (C-16), 117.5 (C-17), 30.9 (C-18), 23.1 (C-19), 97.1 (C-20), 171.6, 171.9 (2 × OCOCH₃), 21.4, 21.2 (2 × OCOCH₃).

Effusanin A (2): Colorless needles (EtOH); m.p. 252–256 °C; [α]_D²⁵: –53.8° (EtOH, *c* 0.4) {lit. [6] 262–265 °C; [α]_D: –76.0° (EtOH, *c* 0.05)}.

Effusanin B (3): Colorless prisms (acetone-hexane); m.p. 254–256 °C; $[\alpha]_D^{23}$: –60° (MeOH, c 0.1) {lit. [6] 258–260 °C; $[\alpha]_D$: –66.7° (EtOH, c 0.027)}.

Effusanin E (4): Colorless prisms (EtOH); m.p. 231–235 °C (lit. [5] 240–242 °C); $[\alpha]_D^{23}$: –28.2° (EtOH, c 0.06) {lit. [5] $[\alpha]_D$: –81.3° (C₅H₅N, c 0.28)}.

Lasiokaurin (5): Colorless needles (MeOH); m.p. 237–241 °C; $[\alpha]_D^{25}$: –57° (EtOH, c 0.2) {lit. [6] 223–226 °C; $[\alpha]_D$: –68° (EtOH, c 0.05)}.

Oridonin (6): Colorless prisms (EtOH); m.p. 254–260 °C; $[\alpha]_D^{25}$: –51° (EtOH, c 0.1) {lit. [6] 250–252 °C; $[\alpha]_D$: –54.6° (EtOH, c 0.097)}.

X-Ray crystallography of rugosinin (1)

X-Ray diffraction data of compound **1** was collected on a Enraf-Nonius CAD-4 diffractometer with graphite monochromated Cu-K α radiations at a temperature of 20 \pm 1° C using the ω -2 θ scan at variable speeds 5° < 2 θ < 136°. A total of 4338 reflections were collected, 4011 were found unique (R_{int} = 0.33). Freidal pairs of reflections were also measured and both enantiomers were identified by statistical methods. The structure **1** was solved by direct methods (SIR92) [13] and expanded using the Fourier technique. The non-hydrogen atoms were refined anisotropically. Hydrogen atoms were included but not refined. The final cycle of full-matrix least-squares refinement using F² with the aid of SHELXL97 [14] was based on 3614 observed reflections above I > 2 σ (I) level and 295 variable parameters. The final unweighted and weighed agreement factors (largest parameter shifts was 0.001 times its ESD) were R = 0.059 and wR = 0.169. All calculations for data reduction were performed by using teXsan [15] crystallographic software package. The ORTEP drawing of the molecule with absolute configuration is given in Fig. 1. Crystal Data: Approximate crystal size, 0.42 \times 0.37 \times 0.18 mm³. C₂₄H₃₀O₈, M_r = 446.48, orthorhombic, a = 10.7404(16), b = 19.191(3), c = 10.7464(10) Å, V = 2215.0(5) Å³, D_c = 1.339 g/cm³, Z = 4. Space group, P2₁2₁2₁ (No. 19). R = 0.059, wR = 0.169. The X-ray data are deposited in Cambridge Crystallographic Data Center (12 Union Road, Cambridge, CB2 1EZ, UK).

Assay for growth inhibition of *Saccharomyces cerevisiae*

Assay was performed according to the method reported by Gunatilaka et al. [12]. Strains of genetically engineered yeast (*Saccharomyces cerevisiae*) were provided by Mr. Leo Faucette of SmithKline Beecham Pharmaceuticals. The strains RS322YK (RAD 52) were plated on YPD agar (7 mm layer) in 170 \times 170 mm plate. Wells (6 mm diameter) were dug in the plates (9 wells in 170 \times 170 mm plate) with a sterile cork borer. Test compounds were dissolved in DMSO-MeOH (1 : 1) and 100 μ L samples were placed in each well. Streptonigrin was used as a positive control for RS322YK yeast strain. Plates were read after incubation for 48 h at 30 °C. IC₁₂ value were determined as the dose that gives an inhibition zone of 12 mm using a 6 mm diameter. The data on RAD 52Y (mutant strain) and RAD* (wild type) are given in Table 1.

Copies of the original spectra of all the compounds and positional parameters (X-ray diffraction data) for **1** can be obtained from the corresponding author on request.

Table 1 Bioactivity of compounds 1–5

Compound	Yeast Strain	
	RAD 52 Y ^a	RAD ^a
Rugosinin (1)	25	45
Effusanin A (2)	20	50
Effusanin B (3)	12	35
Effusanin E (4)	95	> 100
Lasiokaurin (5)	90	> 100
Streptonigrin ^b	0.4	1.0

^a Results are expressed as IC₁₂ values in μ g/mL.

^b Standard reference compound.

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