

MOLECULAR STRUCTURE OF IRAZUNOLIDE, A NEW EUDESMANOLIDE FROM *HIERACIUM IRAZUENSIS*

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ABSTRACT.—The isolation and structure determination of a new eudesmanolide, irazunolide (1), from *Hieracium irazuensis* is reported. The structure was inferred from nmr and mass spectral data, and the molecular structure of irazunolide was determined by single crystal X-ray diffraction.

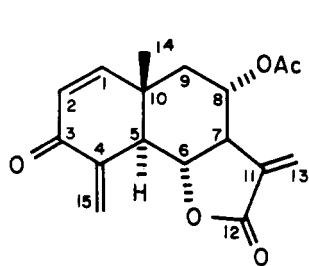
Because of the limited data on the chemistry of the tribe Lactuceae (Cichorieae) of the family Compositae (1), we wished to learn about the chemical constituents of members of the large genus *Hieracium*, which includes about 1000 species (2). Selected taxa of the genus *Hieracium* have previously been investigated for their content of secondary metabolites, but to our best knowledge no reports on the presence of sesquiterpene lactones in this genus have appeared.

In our biochemical systematic study combined with a search for biologically active natural products within the family Compositae, we wished to learn about the constituents of *Hieracium irazuensis* L., a species endemic to Costa Rica. This paper describes the isolation and structure determination of the first new eudesmanolide-type sesquiterpene lactone from *Hieracium*.

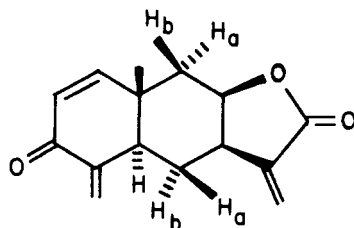
RESULTS AND DISCUSSION

Spectroscopic Characterization

Standard column chromatography procedures applied to extracts of dried aerial parts of *H. irazuensis* provided a colorless, crystalline compound which we named irazunolide (1), $C_{17}H_{18}O_5$, mp 202–204°. The uv (λ_{max} 239 nm) and ir (1670 and 1620 cm^{-1}) absorptions were characteristic of the cross-conjugated dienone chromophore present also in encelin (2) (3). 1H nmr signals at 6.74 (H-1), 6.02 (H-2), 5.72 (H-15a) and 6.28 (H-15b) (see table 1) also supported the presence of a cross-conjugated dienone moiety in irazunolide. The presence of an α -methylene lactone was indicated by an ir band at 1770 cm^{-1} . Three diagnostic one-proton signals, a multiplet at 2.90 (H-7) and doublets at 5.56 (H-13a) and 6.17 (H-13b), corroborated the ir assignment. A three-proton singlet at 2.17 together with a strong ms peak at m/z 242 (100%, M- CH_3COOH) supported the presence of an acetate moiety which must be attached to C-8 on the basis of chemical shift arguments. Further assignments of the basic skeleton of irazunolide were deduced from extensive double resonance experiments in $CDCl_3$, the results being summarized in table 1. Assuming that H-7 is α -oriented, as in all sesquiterpene lactones from higher plants (4), the configurations at C-5, C-6 and C-8 could be derived from 1H nmr couplings which were correlated with the dihedral angles obtained from stereomodel considerations. Large couplings ($J_{5,6} = J_{6,7} = J_{7,8} = 11.0$ Hz) indicated antiperiplanar arrangements of the protons at C-5 to C-8 suggesting H-5 α , H-6 β , H-7 α and H-8 β .

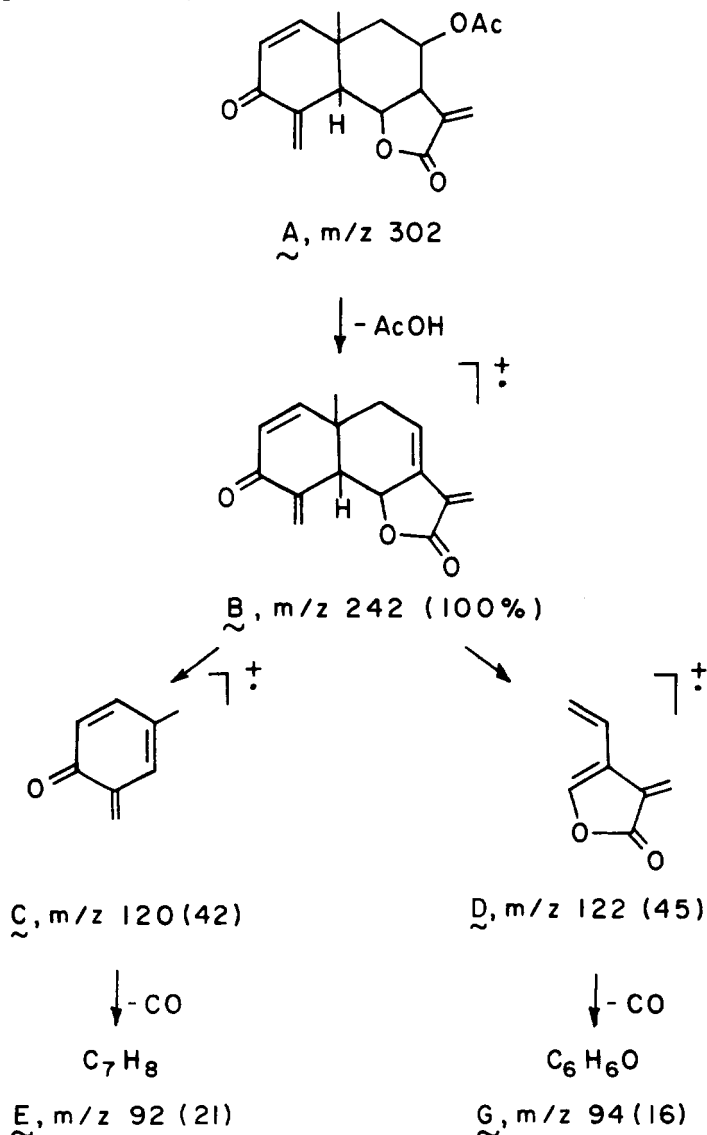


irazunolide (1)



encelin (2)

The high resolution mass spectrum of irazunolide exhibited a pattern which is in accord with fragmentations outlined in scheme 1. The base peak at m/z 242 must be due to ion **B** derived from irazunolide by the loss of acetic acid. From **B** the strong radical ions at m/z 120 (**C**) and 122 (**D**) would be formed by a retro-Diels-Alder process which by loss of CO give rise to ions **E** and **G**, respectively.



Scheme 1. Major mass spectral fragmentations of irazunolide (1).

A strong positive cd band at 245 nm was contrary to Geissman's lactone rule (5). However, the presence of a second chromophore, the cross-conjugated dienone, appears to be the main contributor to the positive band at 246 nm. This strong positive Cotton effect could be due to the chiral environment of the dienone moiety or, more likely, due to the inherent chirality of the chromophore.

Crystal structure analysis¹

A fragment was cleaved from a large, well-formed, clear crystal of irazunolide. Lattice constants were determined by least squares analysis of the positions of 25 random reflections. Diffraction symmetry and systematic absences identified the crystal system and space group as orthorhombic, $P2_12_12_1$. Periodic remeasurement of standard reflections revealed no machine instabilities or crystal decay. The structure was solved by use of the direct methods program MULTAN (6) and refined by use of full matrix, weighted least squares techniques. Due to the limited data, only exocyclic non-hydrogen atoms were allowed to exhibit anisotropic thermal motion in the refinement model. Hydrogen atoms were placed in calculated positions. Crystal data are listed in table 3, atomic positions are listed in table 4, and figure 1 shows the molecular structure. Structure factors, anisotropic thermal parameters, and calculated molecular dimensions are available from one of the authors (S.F.W.).

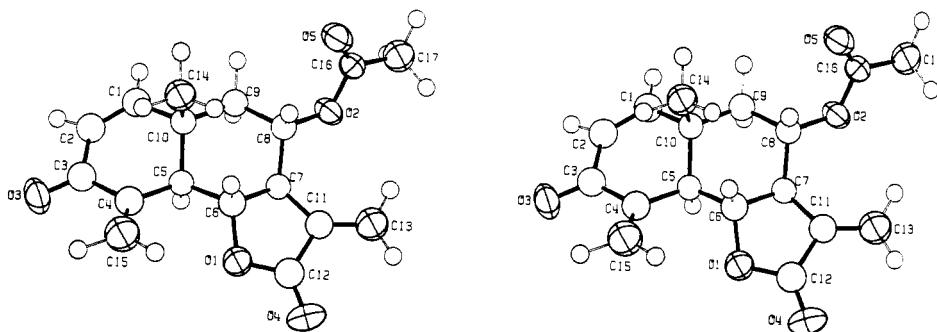


FIGURE 1. Stereoscopic diagram of the β -face of irazunolide.

TABLE 1. ¹H Nmr parameters^a of irazunolide (1) and encelin (2).

	1	2
H-1.....	6.74 d (10.0)	6.82 d (10.0)
H-2.....	6.02 } (5.92) ^b brd (10.0)	6.02 d (10.0)
H-5.....	3.03 (2.22) ddd (11.0;2.5;~2.5)	2.61 dddd (2.5;2.5;3.0;12.0)
H-6a.....	4.17 (3.27) dd (11.0;11.0)	1.56 ddd (12.0;14.0;14.0)
H-6b.....	—	2.04 ddd (3.0;7.5;14.0)
H-7.....	2.90 (2.13) dddd (11.0;3.0;3.2)	3.07 m ^c (5.0)
H-8.....	5.52 (4.81) ddd (11.0; 4.5)	4.60 ddd (5.0;5.0;2.0)
H-9a.....	1.62 (0.80) brdd (12.5;11.0)	1.79 dd (15.5;5.0)
H-9b.....	2.29 (1.68) dd (12.5;4.5)	2.41 dd (15.5;2.0)
H-13a.....	5.56 (5.28) d (3.0)	5.7 d (1)
H-13b.....	6.17 (6.14) d (3.2)	6.22 d (1.2)
H-15a.....	5.72 (5.55) brd (2.5)	5.23 brd (2.5)
H-15b.....	6.28 (6.46) dd (2.5;1.0)	6.14 dd (2.5; 1.0)
C-10-Me.....	1.14 (0.44) s	1.05 s
Ac.....	2.17 (1.64) s	—

^aSpectra were obtained at 200 MHz in $CDCl_3$ (C_6D_6). TMS was used as internal standard and values are recorded in ppm relative to TMS. Multiplets are given by the usual symbols: d=doublet; s=singlet; br=broadened.

^bData obtained in C_6D_6 .

^cSignal appeared as a broadened pentet.

¹X-ray diffraction data were collected on Enraf-Nonius CAD-4 diffractometer at ca. 21° by graphite monochromated $MoK\alpha$ radiation.

TABLE 2. ^{13}C Nmr spectral data^a for irazunolide (1) and encelin (2).

	1	2
C-1.....	156.8 d	159.3 d
C-2.....	127.7 d	126.6 d
C-3.....	187.2 s	188.5 s
C-4.....	139.7 s ^e	144.2 s ^e
C-5.....	52.1 d ^b	44.6 d ^b
C-6.....	76.6 d ^e	26.7 tr ^e
C-7.....	51.7 d ^b	39.5 d ^b
C-8.....	68.4 d ^e	76.0 d
C-9.....	42.2 tr	37.4 tr ^e
C-10.....	39.1 s	36.0 s
C-11.....	135.6 s ^e	141.1 s ^e
C-12.....	170.0 s ^d	169.8 s
C-13.....	120.1 tr ^e	121.1 tr ^d
C-14.....	20.9 q ^f	19.3 q
C-15.....	122.7 tr ^e	119.0 tr ^d
CH ₃ -CO-	20.7 q ^f	—
CH ₃ -CO-	169.1 s ^d	—

^aSpectra were obtained in CDCl_3 at ambient temperature at 50.32 MHz. Chemical shifts (δ) are in ppm relative to TMSi as internal standard as determined by proton noise decoupling. Peak multiplicity was obtained by off-resonance decoupling (3.5 ppm above TMSi). Multiplicities are designated by the following symbols: s = singlet, d = doublet, tr = triplet, q = quartet.

^{b-g}Assignments inter changeable.

TABLE 3. Crystal data and data collection parameters of irazunolide.

Molecular formula.....	$\text{C}_{17}\text{H}_{18}\text{O}_5$
Molecular weight.....	302.4
Crystal size.....	ca. 0.4 x 0.4 x 0.2 mm ³
Crystal System.....	Orthorhombic
Space group.....	$\text{P}2_12_12_1$
Lattice constants.....	$a = 7.793(1)\text{Å}$ $b = 9.394(1)\text{Å}$ $c = 21.439(3)\text{Å}$ $V = 1569.5(3)\text{Å}^3$
Molecules per cell (Z).....	4
Calculated density.....	1.28 g cm ⁻³
Wavelength (MoK α).....	0.71069 Å
Linear absorption coefficient.....	0.57 cm ⁻¹
Diffraction limits	
2θ	0 to 36°
$\sin\theta/\lambda$	0 to 0.43481
Data collection	
Unique reflections measured....	1004
Refinement data set ($F > 2\sigma(F)$)..	712
Reciprocal weights (w^{-1}).....	$\sigma^2(F) + 0.008 F^2$
Final residuals	
R.....	0.0451
R _w	0.0577
Residual density.....	< 0.3 e ⁻ /Å ³

EXPERIMENTAL²

²Melting points were performed in capillaries on a Thomas-Hoover melting point apparatus and are uncorrected. Infrared spectra were taken on a Perkin-Elmer model 621 spectrophotometer, and ultraviolet spectra were obtained on a Cary model 14 spectrophotometer. The cd spectra were determined on a Durrum-Jasco J-20 spectrometer. Low-resolution mass spectra were obtained on Hewlett-Packard GC MS-DS 5985, and the high-resolution mass spectra were run on a Varian MAT 711 instrument (70 eV ionizing voltage). The samples were introduced via the direct inlet tube. Nmr spectra were taken on a Bruker WH 200.

Table 4. Coordinates for non-hydrogen atoms* in irazuolide.

ATOM	X	Y	Z
C (1).....	2399 (12)	-1785 (8)	-3219 (4)
C (2).....	2371 (12)	-1789 (10)	-2598 (3)
C (3).....	782 (11)	-1743 (9)	-2237 (4)
C (4).....	-825 (12)	-1455 (8)	-2603 (3)
C (5).....	-533 (10)	-792 (7)	-3237 (3)
C (6).....	-2089 (9)	-591 (8)	-3653 (3)
C (7).....	-1611 (9)	234 (8)	-4229 (3)
C (8).....	-478 (10)	-720 (8)	-4621 (3)
C (9).....	1160 (10)	-1008 (8)	-4249 (3)
C (10).....	776 (9)	-1700 (7)	-3604 (3)
C (11).....	-3311 (10)	761 (8)	-4439 (4)
C (12).....	-4350 (12)	901 (9)	-3848 (4)
C (13).....	-3906 (14)	1173 (9)	-4988 (4)
C (14).....	183 (11)	-3248 (7)	-3711 (4)
C (15).....	-2321 (13)	-1876 (11)	-2366 (4)
C (16).....	320 (11)	-795 (10)	-5688 (4)
C (17).....	625 (14)	69 (11)	-6262 (4)
O (1).....	-3493 (7)	249 (5)	-3380 (2)
O (2).....	-44 (7)	24 (5)	-5195 (2)
O (3).....	760 (9)	-1933 (6)	-1669 (2)
O (4).....	-5707 (7)	1466 (6)	-3779 (3)
O (5).....	360 (9)	-2056 (6)	-5669 (2)

*Atomic positional parameters x 10⁴.

PLANT MATERIAL.—A bulk collection of *Hieracium irazuensis* was made in Costa Rica along the Interamerican Highway "Cerro de la Muerte" at km 64 on June 9, 1980. The voucher is deposited at the National Herbarium of Costa Rica under number 79325. Air dried plant material (845 g) was extracted with methylene chloride at ambient temperature providing 4 g of crude extract. Early fractions of a chromatography run over 125 g silica-gel, with petroleum ether-acetone (4:1) as eluent, provided a gum which solidified upon trituration with chloroform/cyclohexane (9:1). Recrystallization from ethyl acetate gave 180 mg of pure crystalline irazuolide (1), mp 202–204°; ir: 1770 (γ -lactone), 1735 (ester) 1670 and 1620 cm⁻¹ (double bonds); uv, λ_{\max} (MeOH): 239 (ϵ , 9,000), 205 nm (ϵ , 10,000); cd (c, 6.6 x 10⁻³, MeOH) $[\theta]_{215} = 1.1 \times 10^5$, $[\theta]_{245} = 2.0 \times 10^5$; ms, 70 eV, m/z (rel. int.): 302 (—, M⁺) 260.1035 (4.1, M-C₂H₂O), 242.0948 (100, M-CH₃COOH), 227.0707 (26, M-CH₃COOH-CH₃), 214.0859 (20, M-CH₃COOH-CO), 199.0751 (36, M-CH₃COOH-CH₃-CO), 185.0905 (28, C₁₂H₁₂O), 173.0595 (16, C₁₁H₉O₂), 135.0777 (63, C₉H₁₁O), 122.0720 (45, C₈H₁₀O), 120.0581 (42, C₈H₈O), 95.0511 (22, C₆H₇O), 91.0515 (58, C₇H₇); CI, isobutane, m/z 303 (M+1; C₁₇H₁₈O₅).

ACKNOWLEDGMENT

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