Notes

Isocentratherin

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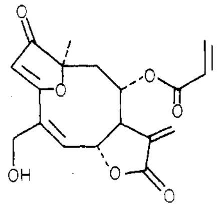
The discovery that certain germacranolide sesquiterpenes possessing the α-methylene-γ-butyrolactone moiety show potent cytotoxic and antitumor effects, and their frequent use as chemotaxonomic markers, mainly of the Compositae, has led to the isolation of a plethora of these compounds from plant sources.[1] However, in a number of cases the structure determination has been equivocal, particularly with respect to the orientation of the lactone group. Our own work on the neurolenins,[2] for instance, has led to stereostructural reassignments of some related compounds.[3] More recently, Herz[4] has shown that goyazenzolide should be **1**, rather than **2**, and has conjectured that the cytotoxic germacranolide isocentratherin[5] should be **3**, rather than **5**. In this paper we confirm Herz's suggestion that isocentratherin should be **3**, in which the lactone group is oriented toward C-6, rather than toward C-8.



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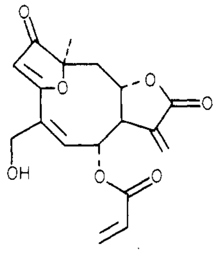

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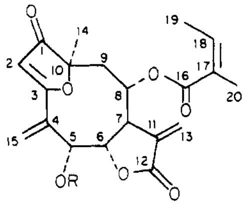
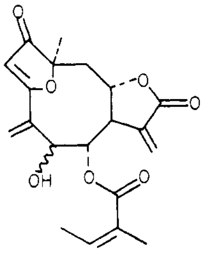


3, R = H
4, R = CH_3CO



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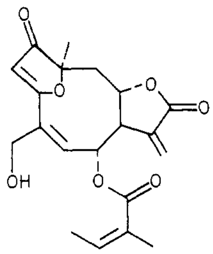
During a reinvestigation of a plant sample of *Centratherum punctatum* Cass. ssp. *punctatum* (Compositae) from Trinidad,[6] we encountered a sesquiterpene, $C_{20}H_{22}O_7$, mp 225–227 °C, $[\alpha]_D{}^{25}$ −48.5° ($CHCl_3$), whose structure and stereochemistry were unambiguously established by spectral and X-ray crystallographic methods as **3**, i.e., the structure proposed by Herz[4] for isocentratherin. A stereoscopic view of **3** (relative stereochemistry) as determined from the X-ray crystallographic analysis is shown in Figure 1. Listings of final atomic parameters, final anisotropic thermal parameters, bond lengths, bond angles, and torsion angles are given in Tables I–V as supplementary material.

Although the spectral properties of our compound were virtually identical with those reported[5] for isocentratherin, the physical constants for the two compounds and their acetates were completely different. Thus, the melting points for this compound and its acetate were 225–227 and 253–254 °C, respectively, while those reported for isocentratherin and its acetate were 187–188 and 149 °C, respectively.[7] Similarly, the optical rotation for our material was $[\alpha]_D{}^{25}$ −48.5° ($CHCl_3$, *c* 1.00), whereas that reported for isocentratherin was $[\alpha]_D{}^{25}$ −28.5° ($CHCl_3$, *c* 0.5). Herz[4] has emphasized that melting points in this series of compounds are quite unreliable as a criterion of identity.

Despite the differences in physical constants, a direct comparison (UV, IR, NMR, MS, TLC, cell constants) of our compound with isocentratherin isolated from Brazilian *C. punctatum*[5a] leads us to conclude that the two compounds are identical and that **3** is indeed the correct structure for isocentratherin. Again, in agreement with Herz,[4] we suspect that the structure of centratherin[8] should be revised from **6** to **7**, i.e., it should be identical with lychnophorolide A whose structure has been established by X-ray crystallography.[9]



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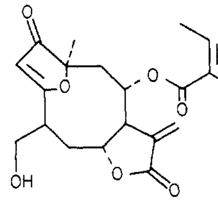

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Experimental Section

General Procedure. Melting points were determined in capillaries on a Thomas-Hoover melting point apparatus and are uncorrected. Unless otherwise indicated, infrared (IR) and nuclear magnetic resonance spectra (NMR) were determined in $CHCl_3$ and $CDCl_3$, respectively, ^1H and ^{13}C NMR spectra were recorded

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[1] (a) "Terpenoids and Steroids"; Specialist Periodical Reports, Royal Society of Chemistry: London, 1982; Vol. 11 and preceding volumes. (b) Fischer, N. H.; Oliver, E. J.; Fischer, H. D. *Fortschr. Chem. Org. Naturst.* **1979**, *38*, 47.

[2] Manchand, P. S.; Blount, J. F. *J. Org. Chem.* **1978**, *43*, 435.

[3] Herz, W.; Kumar, N. *Phytochemistry* **1980**, *19*, 593 and references cited therein.

[4] Herz, W.; Goedken, V. L. *J. Org. Chem.* **1982**, *47*, 2798.

[5] (a) Bevelle, C. A.; Handy, G. A.; Segal, R. A.; Cordell, G. A.; Farnsworth, N. R. *Phytochemistry* **1981**, *20*, 1605. (b) Bohlmann, F.; Zdero, C.; Robinson, H.; King, R. M. *Ibid.* **1982**, *21*, 1087.

[6] Kirkman, L. K. *Rhodora* **1981**, *83* (833), 1.

[7] The melting point reported[5a] previously was obtained on material crystallizing from $CHCl_3$–3% MeOH. Reisolation from Brazilian *C. punctatum* and crystallization from ethyl acetate afforded **3**, mp 225–228 °C (uncorrected), $[\alpha]_D{}^{25}$ −33° ($CHCl_3$). Bohlmann et al. report[5b] mp 172 °C, $[\alpha]_{24}{}^D$ −43° ($CHCl_3$, *c* 1) for **3** isolated from *Lychnophora sellowii*, but a comparison of this sample with ours was not carried out.

[8] Ohno, N.; McCormick, S.; Mabry, T. J. *Phytochemistry* **1979**, *18*, 681.

[9] LeQuesne, P. W.; Menachery, M. D.; Pastore, M. P.; Kelley, C. T.; Brennan, T. F.; Onan, K. D.; Raffauf, R. F. *J. Org. Chem.* **1982**, *47*, 1519.

0022-3263/83/1948-4388$01.50/0

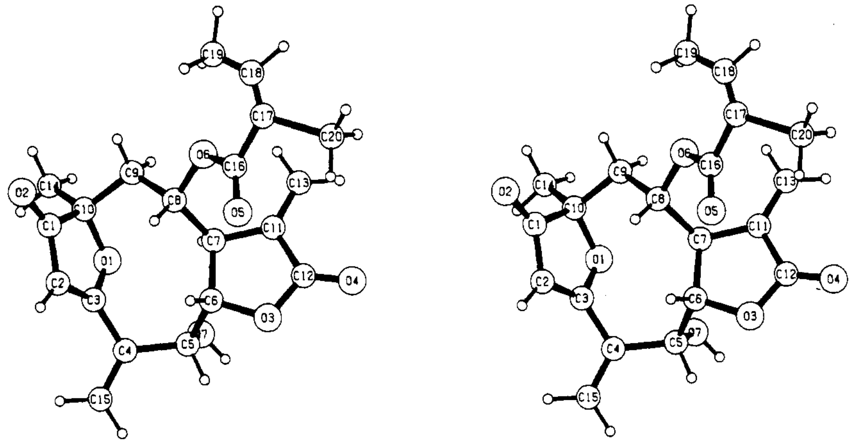

Figure 1. An ORTEP stereoscopic drawing of isocentratherin (**3**).

at 200 and 50.4 MHz, respectively. Chemical shifts are expressed in parts per million (ppm) relative to tetramethylsilane, and coupling constants (*J*) are expressed in hertz (s = singlet, d = doublet, t = triplet, m = multiplet). Mass spectra (MS) were determined with a direct inlet system with ionization energy of 70 eV; *m/z* values are given with relative intensities (%) in parentheses. Thin-layer chromatograms (TLC) were prepared from Merck (Darmstadt) silica gel G, and spots were made visible by spraying with 10% phosphomolybdic acid in ethanol and heating the plates to 110 °C.

Isolation of 3. One kilogram of air-dried, finely ground leaves of *Centratherum punctatum* Cass. ssp. *punctatum* (Compositae),[6] collected in Trinidad (July 1981), were steeped in 4 L of methylene chloride for 4 days. The mixture was filtered and the filtrate was evaporated to give 19.2 g of a green gum, which was dissolved in 500 mL of methanol and stirred with 2 g of neutral charcoal for 4 h. The mixture was filtered and the filtrate evaporated to afford 10.8 g of a light brown gum. A 5-g portion of this material was dissolved in 10 mL of hexane–ethyl acetate (3:1), and the solution was left at 0 °C overnight. The solid was collected by filtration and was washed with cold (0 °C) hexane–ethyl acetate (3:1) to yield 735 mg of crude **3**, mp 173–176 °C; crystallization from hot ethyl acetate gave 410 mg of **3**: mp 225–227 °C; $[\alpha]_D^{25}$ −48.5° ($CHCl_3$, *c* 1.00); CD (CH_3OH) $[\theta]_{304}$ +1390, $[\theta]_{281}$ −630, $[\theta]_{250}$ +26700; UV (EtOH) 211 nm (ε 27900), 282 (ε 10000); IR ($CHCl_3$) 3600, 3400, 1760, 1700, 1640, 1582, 945 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.55 (3 H, s, H-14), 1.78 (3 H, br s, H-20), 1.88 (3 H, dd, *J* = 6, 1, H-19), 2.35 (1 H, d, *J* = 12, H_α-9), 2.54 (1 H, dd, *J* = 12, H_β-9), 4.25 (1 H, m, H-7), 4.33 (1 H, br d, *J* = 12, H-8), 4.83 (1 H, d, *J* = 5, H-6), 4.92 (1 H, s, H-5), 5.47 (1 H, d, *J* = 3, H_A-13), 5.85 (1 H, s, H-2), 5.91 (1 H, s, H_A-15), 5.93 (1 H, s, H_B-15), 6.07 (1 H, q, *J* = 5, H-18), 6.19 (1 H, d, *J* = 3, H_B-13); ^{13}C NMR ($CDCl_3$) δ 15.67 (q, C-19), 20.07 (q, C-20), 21.25 (q, C-14), 44.45 (t, C-9), 44.50 (d, C-7), 71.64 (d, C-8), 75.10 (d, C-5), 86.15 (d, C-6), 90.47 (s, C-10), 106.66 (d, C-2), 124.08 (t, C-15), 126.47 (s, C-17), 126.76 (t, C-13), 134.22 (s, C-11), 137.92 (s, C-4), 140.56 (d, C-18), 167.25 (s, C-12), 169.63 (s, C-16), 186.06 (s, C-3), 204.53 (s, C-1); MS, *m/z* (relative intensity) 374 (M^+, 3), 356 (7), 330 (14), 312 (3), 291 (2), 274 (4), 246 (5), 152 (22), 83 (100).

In a mixed TLC (silica gel, 70% ethyl acetate in hexane), **3** was inseparable from isocentraterin isolated from *Centratherum punctatum* obtained from Brazil.[5a] Furthermore, the two substances had identical cell constants and virtually indistinguishable IR, NMR, and MS parameters.

Acetylation of 3. A solution of 37.4 mg (0.1 mmol) of **3** in 0.5 mL of pyridine was treated with 0.1 mL of acetic anhydride and the mixture was left at room temperature overnight. It was diluted with 20 mL of ice-cold water and extracted with ethyl acetate (2 × 25 mL). The extract was washed with 25 mL of ice-cold 0.1 N HCl followed by saturated brine, dried ($MgSO_4$), and evaporated to give 33 mg of a gum. Crystallization from ethyl acetate–hexane gave 32 mg of **4** as colorless prisms: mp 253–254 °C; $[\alpha]_D^{25}$ −57.8° ($CHCl_3$, *c* 0.86); UV (EtOH) 212 nm (ε 22255), 281 (ε 8735); IR ($CHCl_3$) 1763, 1748, 1707, 1646, 1590 cm^{-1}; Raman (neat) 1700 (conjugated ester C═O), 1646 (double bond), 1584 (dienone double bond) cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.56 (3 H, s, H-14), 1.79 (3 H, br s, H-20), 1.88 (3 H, dd, *J* = 6, 1, H-19), 2.10 (3 H, s, $OCOCH_3$), 2.36 (1 H, d, *J* = 12, H_α-9), 2.56 (1 H, dd, *J* = 12, H_β-9), 4.18 (1 H, m, H-7), 4.33 (1 H, d, *J* = 12, H-8), 4.90 (1 H, d, *J* = 5, H-6), 5.49 (1 H, d, *J* = 3, H-13), 5.86 (1 H, s, H-2), 5.92 (1 H, s, H-5), 6.07 (1 H, s, H_A-15), 6.08 (1 H, q, *J* = 5, H-18), 6.17 (1 H, s, H_B-15), 6.25 (1 H, d, *J* = 3, H-13); ^{13}C NMR ($CDCl_3$) δ 15.7 (q, C-19), 20.0 (q, C-20), 21.1 (q, $OCOCH_3$), 21.3 (q, C-14), 44.6 (t, C-9), 46.0 (d, C-7), 71.7 (d, C-8), 74.9 (d, C-5), 83.8 (d, C-8), 90.4 (s, C-10), 107.0 (d, C-2), 124.0 (t, C-13), 126.3 (s, C-17), 130.7 (t, C-15), 133.6 (s, C-4), 133.8 (s, C-11), 140.9 (d, C-18), 167.3 (s, C-12), 168.5 (s, $OCOCH_3$), 169.6 (s, C-16), 185.3 (s, C-3), 204.2 (s, C-1); MS, *m/z* (relative intensity): 416 (M^+, 2). Anal. Calcd for $C_{22}H_{24}O_8$: C, 63.45; H, 5.81. Found: C, 63.60; H, 5.76.

X-ray Crystallographic Analysis of 3. **3** belongs to space group $P2_12_12_1$, with *a* = 10.239 (3) A, *b* = 11.728 (2) A, *c* = 15.558 (4) A, *Z* = 4, d_{calcd} = 1.331 g cm^{-3}, μ(Cu K_α = 8.5 cm^{-1}. The intensity data, uncorrected for absorption, were measured on a Hilger–Watts diffractometer (Ni-filtered CuK_α radiation, θ–2θ scans, pulse height discrimination) using a crystal of approximately 0.12 × 0.25 × 0.30 mm that was grown from ethyl acetate. Of 1461 independent reflections for $\theta < 57°$, 1223 were considered to be observed [$I > 2.5\sigma(I)$]. The structure and relative stereochemistry of **3** were solved by a multiple-solution procedure and was refined by full-matrix least squares.[10] In the final refinement, anisotropic thermal parameters and isotropic temperature factores were used for non-hydrogen and hydrogen atoms, respectively. The hydrogens were included in the structure factor calculations, but their parameters were not refined. The final discrepancy indices were *R* = 0.046 and R_w = 0.052 for the 1223 observed reflections. The final difference map had no peaks greater than ±0.3 e A^{-3}. Listings of final atomic parameters, final anisotropic thermal parameters, bond lengths, bond angles, and torsion angles are given in Tables I–V as supplementary material.

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Registry No. **3**, 80377-52-2; **4**, 87391-92-2.

Supplementary Material Available: Listings of final atomic parameters, final anisotropic thermal parameters, bond lengths, bond angles, and torsion angles for **3** are given in Tables I–V (5 pages). Ordering information is given on any current masthead page.

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(10) Germain, G.; Main, P.; Woolfson, M. M. *Acta Crystallogr., Sect. A* 1971, *27*, 368.