

STUDY OF *SALVIA PALAEOFOLIA*: ABSOLUTE CONFIGURATION OF GLECHOMAFURAN

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ABSTRACT.—The aerial part of *Salvia palaeifolia* yielded squalene, lupeol, taraxerol, taraxerone, β -amyrin, olean-12-en-3-one, oleanonic acid, oleanolic acid, and ursolic acid, together with 5-hydroxy-7,6,8,4'-tetramethoxyflavone, caryophyllene oxide, and an enantiomer of glechomafuran. The compounds were identified by spectroscopy, X-ray analysis, and, when applicable, comparison with authentic samples.

As part of an intensive study of the medicinal flora of South America, *Salvia palaeifolia* H.B.K. (1), an Andean species collected in Colombia, where it is extensively used as an antihypertensive agent (2), was analyzed.

An extract of the aerial part of the plant yielded the known triterpenes squalene, lupeol, taraxerol, taraxerone, β -amyrin, olean-12-en-3-one, oleanonic acid, oleanolic acid, and ursolic acid, identified by spectroscopic study and comparison with authentic samples. 5-Hydroxy-7,6,8,4'-tetramethoxyflavone, previously obtained from other Labiatae (3,4), was isolated as a major product, as were two sesquiterpenes. One was characterized as caryophyllene oxide (5), and the other, a solid crystalline product, mp 110°, $[\alpha]^{20}_D - 137^\circ$ ($c = 0.8$, CHCl_3), $\text{C}_{15}\text{H}_{20}\text{O}_3$, coincided in all respects except optical activity with glechomafuran isolated (6) from another plant of the Labiatae, *Glechoma hederacea* L. Glechomafuran has long been known; its structure (7) and relative configuration (8,9) have all been published.

A detailed spectroscopic study revealed that this compound is structurally identical to glechomafuran, but its optical activity indicates that it is enantiomeric to the previously described compound (6). Its absolute configuration was established by X-ray single-crystal diffraction analysis as (1*R*,4*R*,5*R*,10*R*)-1(10),4(5)diepoxy-7,8-furangermacrane. Glechomafuran crystallized in the monoclinic space group $\text{P}2_1$. The asymmetric unit consists of two independent molecules. The molecules do not show large variations in their conformation as shown by selected torsion angles (Table 1). The epoxides are trans-attached, and the methyl groups on C-10 and C-4 are β -positioned syn to each other. The β -methylfuran ring fused to the ten-membered main ring is essentially flat, as the torsion angles show (Table 1).

Figure 1 shows a PLUTO-generated view of the correct enantiomer for one of the two independent molecules, with the β face upwards.

According to our records this is the first time that a sesquiterpene with this type of skeleton, most commonly found in the Umbelliferae of the genus *Smyrnia* (11,12) and Labiatae of the genus *Glechoma*, has been isolated from the Salviaeae (10); its occurrence in *S. palaeifolia* may thus be significant from a chemotaxonomical point of view.

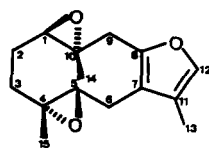


TABLE 1. Atomic Coordinates of the Two Independent Molecules of Glechomafuran [1].

Atom		x	y	z
O-1	A*	0.52777(61)	1.09008(157)	0.78678(21)
O-2	A	-0.12424(65)	1.17310(160)	0.51524(26)
O-3	A	0.01414(67)	0.83367(160)	0.72505(28)
C-1	A	-0.07897(77)	0.91524(174)	0.66936(35)
C-2	A	-0.17661(91)	0.88246(171)	0.58533(41)
C-3	A	-0.24004(85)	0.98544(178)	0.53596(42)
C-4	A	-0.06807(87)	1.05901(174)	0.52362(34)
C-5	A	-0.00253(80)	1.13772(170)	0.58993(32)
C-6	A	0.19887(102)	1.19331(174)	0.60334(43)
C-7	A	0.34776(79)	1.15796(170)	0.67548(30)
C-8	A	0.35065(80)	1.08438(165)	0.73486(30)
C-9	A	0.21817(104)	0.99654(168)	0.76161(32)
C-10	A	0.13861(80)	0.91909(177)	0.69579(31)
C-11	A	0.53494(79)	1.21792(167)	0.69119(31)
C-12	A	0.63334(93)	1.17436(178)	0.75878(37)
C-13	A	0.59530(88)	1.31009(175)	0.64146(42)
C-14	A	0.28270(86)	0.87456(178)	0.64245(40)
C-15	A	0.06082(113)	1.02065(182)	0.46289(35)
O-1	B	0.43275(58)	0.27147(154)	0.11402(21)
O-2	B	0.98738(58)	-0.07963(151)	0.23783(21)
O-3	B	0.88028(56)	0.12671(160)	-0.05547(21)
C-1	B	0.96560(84)	0.06982(169)	0.01756(32)
C-2	B	1.02622(105)	-0.04793(175)	0.01183(37)
C-3	B	1.08881(83)	-0.09183(167)	0.09844(35)
C-4	B	0.92109(80)	-0.09176(168)	0.15013(32)
C-5	B	0.88330(81)	0.00900(172)	0.19438(32)
C-6	B	0.69168(102)	0.03814(172)	0.22574(39)
C-7	B	0.57357(75)	0.13397(167)	0.18782(29)
C-8	B	0.58519(78)	0.19525(167)	0.12207(29)
C-9	B	0.71758(83)	0.21105(173)	0.05534(35)
C-10	B	0.75800(72)	0.10859(167)	0.01084(28)
C-11	B	0.40359(77)	0.17149(163)	0.22464(31)
C-12	B	0.32453(86)	0.25143(170)	0.17828(34)
C-13	B	0.33505(86)	0.12959(164)	0.30181(31)
C-14	B	0.58136(78)	0.03452(169)	-0.01464(30)
C-15	B	0.76729(117)	-0.17947(176)	0.12896(41)

*molecule designation.

EXPERIMENTAL

The plant material was collected in Cundinamarca (Bogotá plain), Colombia in December 1987. A voucher specimen (No. 293387) is stored in the Herbario Nacional Colombiano and was identified by Dr. Clara Inés Orozco of the Instituto de Ciencias Naturales of the Universidad Nacional de Colombia. Another voucher specimen has been lodged in the Department of Botany of the Universidad de La Laguna where its identification was duly confirmed.

GENERAL TECHNIQUES APPLIED.—Mp's were determined on a Kofler-type block and are uncorrected. Uv spectra were obtained on a Perkin-Elmer 550 SE. Ir spectra were recorded on a Perkin-Elmer 681 spectrometer. High and low

resolution ms were recorded on a VG-Micromass ZAB-2F spectrometer at 15 or 70 eV. ¹H-nmr spectra were obtained on a Bruker AC 80 WP-200 SY spectrometer and ¹³C-nmr spectra on a Bruker WP-200 SY. Chemical shifts are given in δ. Merck (0.2–0.5 mm) and type G Si gel were used for wet cc and the same Si gel (0.2–0.063 mm) for dry cc. Tlc was carried out on type G Si gel plates. A Harrison Research CHROMATOTRON apparatus, model 7924T, was used for Chromatotron chromatography and a P-E 900 with flame ionization detector for gc.

PLANT EXTRACTION.—The leaves of *S. palaeifolia* were shredded and extracted with cold Me₂CO giving 105 g of extract which was dry-column chromatographed with *n*-hexane as eluent followed by differing mixtures of *n*-

TABLE 2. Selected Torsion Angles in the Two Independent Molecules (estimated standard deviations in parentheses).

	Molecule A	Molecule B
C-4-O-2-C-5-C-6	118.7 (1.3)	118.9 (1.3)
C-5-O-2-C-4-C-3	108.6 (1.3)	109.6 (1.2)
C-5-O-2-C-4-C-15	-116.0 (1.3)	-115.8 (1.3)
C-1-O-3-C-10-C-14	-114.2 (1.3)	-114.8 (1.2)
C-1-O-3-C-10-C-9	110.7 (1.3)	109.9 (1.2)
C-10-O-3-C-1-C-2	116.0 (1.4)	115.5 (1.4)
C-2-C-1-C-10-O-3	-107.1 (1.5)	-107.7 (1.4)
O-3-C-1-C-10-C-14	98.3 (1.4)	98.9 (1.3)
O-3-C-1-C-10-C-9	-100.8 (1.3)	-100.9 (1.3)
O-3-C-1-C-2-C-3	-173.2 (1.2)	-173.5 (1.1)
C-2-C-1-C-10-C-14	-8.7 (2.1)	-8.8 (2.0)
C-2-C-1-C-10-C-9	152.1 (1.3)	151.3 (1.3)
C-10-C-1-C-2-C-3	-100.5 (1.5)	-100.4 (1.5)
C-1-C-2-C-3-C-4	61.3 (1.5)	63.1 (1.4)
C-2-C-3-C-4-O-2	-151.5 (1.2)	-150.9 (1.1)
C-2-C-3-C-4-C-5	-85.7 (1.5)	-85.2 (1.5)
C-2-C-3-C-4-C-15	73.7 (1.5)	74.8 (1.5)
C-3-C-4-C-5-O-2	-102.1 (1.3)	-101.9 (1.3)
C-3-C-4-C-5-C-6	160.2 (1.3)	159.3 (1.2)
O-2-C-4-C-5-C-6	-97.7 (1.4)	-98.8 (1.4)
C-15-C-4-C-5-O-2	100.3 (1.4)	100.0 (1.4)
C-15-C-4-C-5-C-6	2.6 (2.1)	1.1 (2.1)
C-4-C-5-C-6-C-7	-107.7 (1.5)	-114.2 (1.5)
O-2-C-5-C-6-C-7	-175.3 (1.1)	176.9 (1.1)
C-5-C-6-C-7-C-8	0.9 (2.3)	10.7 (2.3)
C-6-C-7-C-8-C-9	2.4 (2.9)	5.4 (3.0)
C-7-C-8-C-9-C-10	50.7 (2.3)	52.4 (2.3)
C-8-C-9-C-10-C-1	-115.7 (1.4)	-116.3 (1.3)
C-8-C-9-C-10-O-3	178.7 (1.1)	177.9 (1.1)
C-8-C-9-C-10-C-14	46.0 (1.7)	44.3 (1.6)

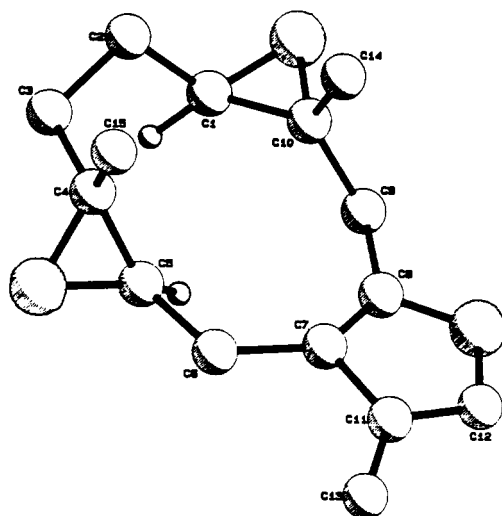


FIGURE 1. PLUTO-generated view of (-)-glechomafuran [1].

hexane/EtOAc, yielding, in order of increasing polarity: squalene (2.8 g), taraxerone (1.5 g), olean-12-en-3-one (1 g), β -amyryn (0.5 g), caryophyllene oxide (2.3 g), lupeol (1 g), taraxerol (0.8 g), glechomafuran (2.5 g), ursolic acid (4 g), oleanonic acid (1 g), oleanolic acid (0.5 g), and 5-hydroxy-7,6,8,4'-tetramethoxyflavone (2 g).

GLECHOMAFURAN.—Glechomafuran [1] was crystallized with MeOH/Et₂O: mp 110°; $[\alpha]_D -137^\circ$ ($c=0.8$, CHCl₃); ir (CHCl₃), 3090, 1610, 1550, 1450, 1250, 980, 930, 830, 750, 730 cm⁻¹; uv λ max (EtOH) 220 nm; ¹H nmr (200 MHz, CDCl₃) δ 1.16 (1H, s), 1.89 (3H, br s), 2.32 (1H, d, $J=16$ Hz), 2.53 (2H, d, $J=17$, 5 Hz), 2.80 (1H, d, $J=1$ Hz), 3.00 (1H, dd, $J=2, 17, 5$ Hz), 3.17 (1H, dd, $J=2.5, 9.7$ Hz), 3.38 (1H, d, $J=16$ Hz), 7.06 (1H, br s); ¹³C nmr (CDCl₃) δ 8.6, 16.4, 17.4, 23.9, 24.7, 36.6, 38.2, 59.8, 61.2, 61.6, 68.2, 116.2, 122.0, 136.7, 146.4; ms m/z (%) [M]⁺ 248 (18), 233 (2), 205 (3), 191 (4), 161 (10), 177 (2), 147 (10), 91 (40), 79 (50), 77 (40), 69 (24), 68 (4). Hrms calcd for C₁₅H₂₀O₃, 248.1432; found 248.1422.

X-RAY DATA. ¹-C₁₅H₂₀O₃, (MW = 248.3), monoclinic, space group P2₁, $a=6.787(1)$, $b=12.312(1)$, $c=16.390(1)$ Å, $\beta=97.87(13)^\circ$, $V=1353$ Å³, $z=4$, $D_c=1.21$ g·cm⁻³, $F(000)=536$. Crystal dimension $0.3 \times 0.4 \times 0.6$ mm. Data were collected with a computer-controlled four-circle Siemens AED diffractometer using graphite monochromated CuK α radiation ($\lambda=1.5418$ Å) and $\omega\theta$ scan mode. The intensities of two standard reflections measured every h showed no significant intensity decay with time. The cell parameters were established by least squares adjustment of 24 reflections in the range $20^\circ < 2\theta < 40^\circ$.

Of 1245 unique measured reflections, 1235 with $I > 2\sigma(I)$ were considered as observed and, after corrections for Lorentz and polarization factors, used for structure analysis. No absorption correction ($\mu=6.35$ cm⁻¹) was made. The structure was solved by direct methods (13) and successive Fourier syntheses. Some of the hydrogen atoms were found on a difference synthesis map, and the remainder were placed in calculated positions (C-H 1.0 Å). Refinement by least-squares analysis using anisotropic thermal parameters for nonhydrogen atoms and fixed isotropic contribution for hydrogen atoms with a weighting scheme chosen to obtain flat dependence of

$\langle \Delta^2 F \rangle$ vs. $\langle F_0 \rangle$ and $\langle \sin \theta / \lambda \rangle$ converged to final discrepancy indices of $R=0.048$ and $R_w=0.057$.

At this point the *hkl* part of the spectrum was included in the analysis and the structure refined again as above. The absolute configuration was established by using 53 Bijvoet pairs with $F_o \geq 10\sigma(F_o)$, $\Delta F_c > 0.06$, in the ranges $5 \leq F_o \leq 50$ and $0.1 < \sin \theta / \lambda < 0.45$. The averaged Bijvoet differences are 0.386 for the correct enantiomer vs. 0.405 for the wrong one (14).

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LITERATURE CITED

1. V. Vareschi, "Flora de los Páramos de Venezuela," Universidad de Los Andes/Ediciones del Rectorado, Mérida, 1970, p. 360.
2. H. García Barriga, "Flora Medicinal de Colombia," Imprenta Nacional, Bogotá, 1975, Vol. III, p. 33.
3. G.H. Stout and V.F. Stout, *Tetrahedron*, 296 (1961).
4. B.P. Chaliha, G.P. Sastry, and P.R. Rao, *Tetrahedron*, 1441, (1965).
5. E. Cabrera, A. García Granados, and M.A. Quecuya, *Phytochemistry*, 27, 183 (1988).
6. E. Stahl and S.N. Datta, *Liebigs Ann. Chem.*, 757, 23 (1972).
7. H. Budzikiewicz, C. Djerassi, and D.H. Williams, "Structural Elucidation of Natural Products by Mass Spectroscopy," Holden-Day, San Francisco, 1961, pp. 151-154.
8. A. Ulubelen, S. Oksüz, J.D. Korps, I. Bernal, D.A. Gage, J. Gershenzon, and T.J. Mabry, *J. Nat. Prod.*, 46, 490 (1983).
9. J.D. Korps and I. Bernal, *J. Crystallogr. Spectrosc. Res.*, 15, 191 (1985).
10. R. Wunderlich, *Oesterr. Bot. Z.*, 114(4/5), 383 (1967).
11. A. Ulubelen, S. Oksüz, and N. Tanker, *Phytochemistry*, 23, 1793 (1984).
12. A. Ulubelen, N. Goren, F. Bohlmann, J. Jakupovic, M. Grenz, and N. Tanker, *Phytochemistry*, 24, 1305 (1985).
13. P. Main, S.J. Fisque, S.E. Hull, L. Lessniger, G. Germain, J.P. Declercq, and M.M. Woolfson, "MULTAN 80: A System of Computer Programs for the Automatic Solution of Crystal Structures from X-Ray Diffraction Data," Universities of York, England and Louvain, Belgium, 1980.
14. M. Martínez-Ripoll and J. Fayos, *Z. Kristallogr.*, 152, 189 (1980).

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¹Atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre and can be obtained from Dr. Olga Kennard, University Chemical Laboratory, Lensfield Road, Cambridge CB2 1EW, UK.