RATIBINOLIDE, A NEW SESQUITERPENE LACTONE FROM RATIBIDA LATIPALEARIS[1,2]

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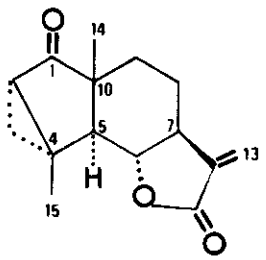
Abstract- Ratibinolide 1, a new sesquiterpene lactone has been isolated, using brine shrimp lethality for activity guided fractionation from Ratibida latipalearis. Its structure was established through spectral and X ray crystallographic analyses.

Continuing our search for novel bioactive compounds from Mexican medicinal plants we have investigated Ratibida latipalearis Richards (Asteraceae) [Tarahumara name: Chi'punuwa].

R. latipalearis is a yellow-flowered perennial herb restricted to the pine-oak forest of the Sierra Madre Occidental of western Chihuahua, México. The roots and leaves are heated and applied topically on skin wounds and inflamations. An infusion of the roots is drunk to alleviate headaches.

The methanol-chloroform extract of the whole plant of this species exhibited moderate activity in the brine shrimp lethality test[3] (BS LC_{50} = 366.61 µg/ml). Succesive column chromatography on silica gel, monitoring the fractions with thin layer chromatography and brine shrimp lethality led to the isolation of ratibinolide 1 (0.0014% yield) [BS LC_{50} = 62.75 µg/ml], a novel sesquiterpene lactone. Ratibinolide had the composition $C_{15}H_{18}O_3$. Its spectral properties were consistent with those of a geigerane type sesquiterpene[4] containing a carbonyl group and a C-6/C-7 trans fused γ-lactone. The stereochemistry at C-6/C-7 was clearly deduced from the coupling pattern observed for the signal at δ 4.20 (dd, $J_{6,7} = J_{5-6}$ = 11.5 Hz, H-6) which correlated in the 2D nmr spectra with the resonance at δ 1.72 (H-5) and δ 2.35 (H-7). The proton connectivities

between H-7 - H-9 and H-2 - H-3 were also established by the COSY experiment. The downfield shift observed for the methylene protons of the cyclopropane moiety [δ 1.08 ($H\text{-}3_A$) and 1.27 ($H\text{-}3_B$)] could be attributed to the anisotropic effect exerted by the carbonyl group at C-1. The ^{13}C nmr spectral data supported the proposed structure.



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The structure and relative stereochemistry of 1 were established unequivocally by X-ray analysis of a crystal. Figure 1 is a perspective drawing of the structure. The high level of activity of 1 in the brine shrimp bioassay clearly warrants further exploration for more complex biological activity.[3]

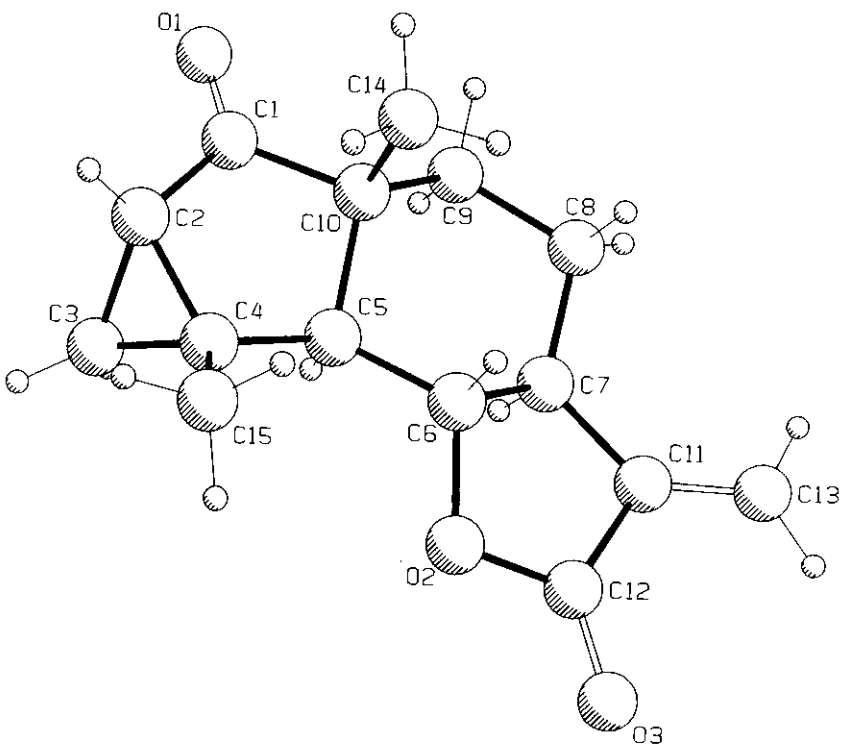

Figure 1. Stereoscopic view of ratibinolide.

EXPERIMENTAL

Ir spectrum was recorded on Perkin-Elmer 599B spectrophotometer. Ms was registered on Hitachi-Perkin-Elmer RMU-GD mass spectrometer. Nmr spectra were taken on a Varian VXR-300S spectrometer with TMS as internal reference. Silica gel 60 (70-230 mesh) Merck was used for column chromatography; tlc was done on silica gel 60 GF 254 plates (Merck). Two dimensional COSY-45° experiment was acquired at 300 MHz with sweep width of 1991 Hz. X ray data were collected on a Nicolet R 3m diffractometer with graphite monocromated Mo Kα radiation (λ = 0.7107 Å).

Isolation of Ratibinolide (1). The plant material (1447 g) was collected in Municipio Guachochic, Cusarase, Chihuahua, México (12 Nov. 1988). The voucher specimen (Bye and Ramamoorthy 16656) is deposited in the National Herbarium of México (MEXU), Instituto de Biología, Universidad Nacional Autónoma de México. The whole plant was air dried and extracted three times for 3 days periods with methanol:chloroform 1:1 (45 l) at room temperature. The extract was evaporated under reduced pressure and the resulting residue (125 g), was chromatographed over silica gel (1.25 Kg) and successively eluted with increasing polarities of mixture of hexane, chloroform, acetone and methanol. Active fractions 93-124 [BS LC_{50} = 65.9814], eluted with hexane:chloroform (1:1), afforded ratibinolide (1, 20 mg), mp 142-144°C.

Ir (KBr) cm^{-1}: 3040, 2925, 2850, 1770, 1730, 990. ^1H Nmr (300 MHz, $CDCl_3$): δ6.16 (d, $J_{7-13'}$=3 Hz, H-13'), 5.41 (d, J_{7-13}=3 Hz, H-13), 4.20 (dd, $J_{5-6}=J_{6-7}$= 11.5 Hz, H-6), 2.35 (m, J_{6-7}=11.5 Hz, $J_{7-8\beta}$=10 Hz, H-7), 2.06 (br d, $J_{8\alpha-8\beta}$= 14 Hz, H-8α), 1.81 (m, H-2), 1.72 (d, J_{5-6}=11.5 Hz, H-5), 1.67 (br d, $J_{9\alpha-9\beta}$= 14 Hz, H-9β), 1.60 (dddd, $J_{8\alpha-8\beta}$=14 Hz, $J_{7-8\beta}=J_{8\beta-9\alpha}$=11.5 Hz, $J_{8\beta-9\beta}$=4 Hz, H-8β), 1.46 (s, H-14), 1.42 (m, H-9α), 1.27 (s, H-15), 1.22-1.32 (m, H-3β), 1.08 (dd, $J_{3\alpha-3\beta}=J_{2-3\alpha}$= 4 Hz, H-3α). ^{13}C Nmr (75 MHz, $CDCl_3$): δ 212.22 (s, C-1), 170.42 (s, C-12), 138.07 (s, C-11), 111.22 (t, C-13), 79.85 (d, C-6), 59.26 (s, C-10), 55.07 (d, C-5), 52.04 (d, C-7), 32.96 (d, C-2), 29.68* (t, C-3), 29.92* (t, C-9), 25.90 (s, C-4), 20.54 (t, C-8), 19.32 (q, C-14), 16.81 (q, C-15, EIMS (70 eV, rel intensity): 246 (M^+, 10). $[\alpha]_D^{25}$ ($CHCl_3$)=+94°.

Single Crystal X-ray Analysis of Ratibinolide (1). The crystal is orthorhombic, space group $P2_12_12_1$ with a=6.779(4), b=11.052(6), c=17.760(9)Å. V=1331(2)A^3, Z=4, D_{calc}=1.23 g/cm^{-3}, F(000)=528, μ=0.79 cm^{-1}, and T=293 K. The crystal had dimensions 0.40 x 0.72 x 0.70 mm, and was mounted on a glass fiber. All

reflections in the hkl octant according to $3 < 2\theta < 50°$ with index range h 0/7, k 0/12 and ℓ 0/18 were collected. The total number of data collected was 1405, of which 1073 reflections has $I > 3.0\,\sigma(I)$ and these formed the basis of the structural solution and refinement. The crystal structure was solved by direct methods using the TEXSAN[5] structure analysis package and refined by full-matrix least-squares techniques with anisotropic temperature factor for non-hydrogen atoms and with fixed isotropic temperature factor, $U=0.06\text{Å}^2$, for the hydrogen atoms bonded to carbon atoms. The final R value is 0.038 (Rw=0.054). Atomic scattering factors International Tables for X-ray Crystallography.[6]

Bioassay. The brine shrimp bioassay for larvicidal activity was performed as previously described.[3] The LC_{50} was determined using Finney's probit analysis method with an extract having an LC_{50} of < 1000 µg/ml being considered active.[3]

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