HORRIDINE, A NEW ISOEUONYMINOL SKELETON ALKALOID

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Abstract - The structure of a new alkaloid, horridine, isolated from the ethanol extract of the root bark of Maytenus horrida Reiss (Celastraceae) was established from spectral and degradation data.

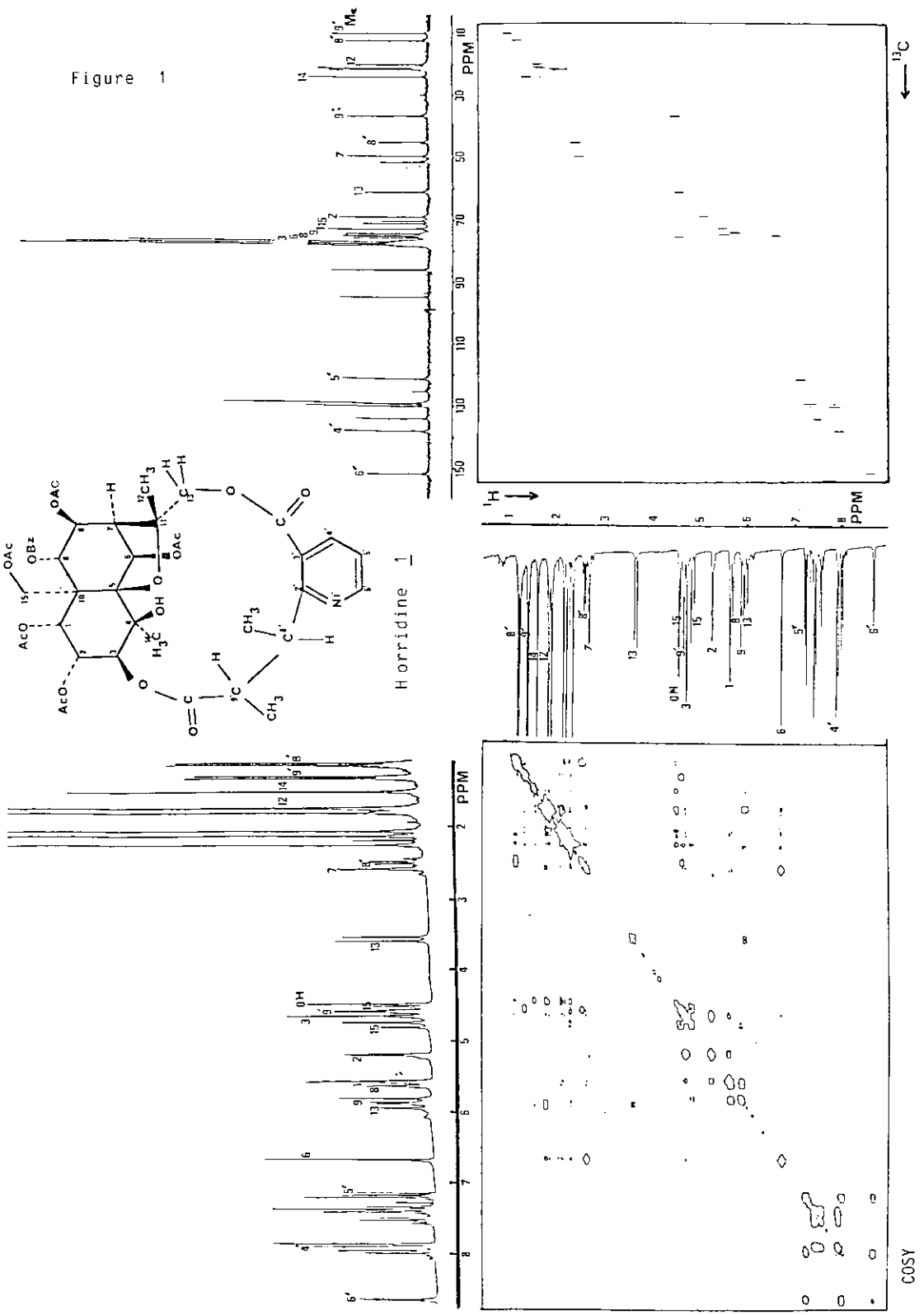
As part of our work on Celastraceae species[2,3] we analysed the more polar fraction of the ethanol extract of the root bark of Maytenus horrida Reiss[4] (habitat El Chaco, Paraguay), isolating triterpenes which are being studied and an alkaloid (1), 40mg, extremely difficult to purify, to which we gave the name horridine[5] and the structure shown, based on the following data: MS, molecular ion at m/z 867 which, together with the elemental analysis[6], suggested the formula $C_{43}O_{18}H_{49}N$, with fragments indicating a benzoate at m/z 105, an acetate at m/z 43 and evoninate residues[7,8,9] at m/z 206, 178, 160 and 107; IR shows a hydroxyl group at 3675 cm^{-1} and an ester function and aromatic grouping were discerned at 1745, 1719, 1598, 1581, 1562, 1450, 1430 cm^{-1}; and at 202, 229, 265 and 280 nm in UV.

^{1}H and ^{13}C NMR spectra, shown in Figure 1, are complex and a long-range coupling COSY and a 2-D ^{13}C-^{1}H were taken. In conjunction with the data given above, these spectra confirmed the presence of a benzoate, evoninate and five acetates for which a provisional partial assignment was made of four acetates at C-1, C-6, C-8 and C-15, the evoninate at C-3 and C-13, with C'-10 close to C-3 due to biogenetic and spectroscopic considerations[10,11,12]. One acetate and a benzoate remained to be assigned to C-2 and C-9, most probably on a sesquiterpene skeleton of isoeuonyminol. When (1) was reduced with $LiAlH_4$, chromatography of the acetylated gross reaction product[13] separated isoeuonyminol octa-acetate[14] and also an evoninic acid reduction product. Isoeuonyminol octa-acetate proved identical with a sample provided for us by Professor Yamada and the skeleton was thus established.

The positioning of the benzoate at C-9 and the remaining acetate at C-2 was made by comparison of the ^{1}H NMR data with those given for: isoeuonyminol octa-acetate[15], isoeuonyminol hepta-acetate methyl ester[15], euonyminol octa-acetate[15], wilfordine[13], neo-alatamine[16], alatamine[15] and mono-benzoates at C-1, C-2 and C-15 derived from evonine[13], ensuring unambiguous assignments.

^{13}C NMR provided significant information[17,18,19,20,21] which, together with the data obtained

Figure 1



from the 2-D spectrum, is set out in the Table.

EXPERIMENTAL

Plant Collection The plant was gathered at El Chaco, Paraguay and identified by Professors Pavetti and Zaldivar of the Botany Department, Faculty of Chemistry, Universidad Nacional de Asunción, Paraguay where a voucher specimen, No. 1232, is lodged.

Extraction and Isolation 2 kg of root bark of M. horrida yielded 45g of extract after boiling with ethanol. The soluble part of this extract in $CHCl_3$ was repeatedly chromatographed on silica gel, and 40mg of horridine (1) was obtained as a white, amorphous powder which would not crystallize. ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 3675, 2970, 3018, 2965, 1745, 1719, 1598, 1581, 1562, 1450, 1430, 1366, 1314, 1274, 1215, 1176, 1166, 1117, 1105, 1090, 1062. ^{1}H nmr (200MHz , $CDCl_3$) δ: 1.21 (3H, d, J=7.1Hz), 1.40 (3H, d, J=6.9Hz), 1.60 (3H, bs, J=1.0Hz), 1.83 (6H, s), 1.90 (3H, s), 2.16 (3H, s), 2.22 (3H, s), 2.35 (3H, s), 2.60 (1H, q, J=7.1Hz), 2.69 (1H, d, J=3.0Hz), 3.66, 6.00 (2H, d_{AB}, J=11.6Hz), 4.57 (1H, bs, J=1.0Hz), 4.63 (1H, q, J=6.9Hz), 4.64, 4.87 (2H, d_{AB}, J=13.2Hz), 4.74 (1H, d, J=3.0 Hz), 5.27 (1H, t, J=3.0Hz), 5.65 (1H, d, J=3.0Hz), 5.69 (1H, dd, J=3.0, J=9.7Hz), 5.92 (1H, d, J=9.7Hz), 6.75 (1H, bs), 7.25 (1H, dd, J=7.8, J=4.8Hz), 7.44 (2H, q, J=7.0Hz), 7.57 (1H, q, J=7.0Hz), 7.94 (2H, d, J=7.0Hz), 8.03 (1H, dd, J=7.8, J=1.8Hz), 8.69 (1H, dd, J=4.8, J=11.8Hz). ^{13}C nmr (50Hz , $CDCl_3$): see Table. uv λ_{max}^{EtOH} nm: 202, 229, 265, 280. ms m/z: 867 (M^+), 852, 839, 824, 808, 794, 781, 764, 748, 736, 635, 634, 436, 305, 288, 280, 279, 262, 253, 247, 245, 241, 238, 236, 233, 231, 220, 218, 213, 206, 204, 192, 178, 161, 160, 150, 134, 132, 107, 105. Calc: $C_{43}O_{18}H_{49}N$, C 59.50%, N 1.61%, H 5.70%; found: C 58.76%, N 1.59%, H 5.90%.

Total Reduction of Horridine 23.6mg (0.027 mmol) of (1) dissolved in dry THF (3ml) was added to a suspension of $LiAlH_4$ (40.8mg, 0.75 mmol) in dry THF (8ml) and Et_2O (3ml), chilled to 0°C, while stirred under argon atmosphere. The reaction continued at this temperature for 30 min and at rt. for 30 min, then at rt.for 7h more with no change. 150mg (2.78 mmol) of $LiAlH_4$ were added and the reaction at rt.was prolonged until the original substance had disappeared according to TLC (24h). EtOAc was added carefully in an ice bath to eliminate excess hydride and after evaporation at reduced pressure the substance was acetylated with Ac_2O in Py at rt.for 24h and at 70°C for 48h longer. The reagents were removed by repeated evaporation under reduced pressure with THF-CH_3OH-C_6H_6 and the gross acetylation product was repeatedly chromatographed on si gel, yielding two major products.

Evoninic Acid Reduction Product 8.6mg of this reduction product was obtained. It retained slight impurities. ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 3625, 2996, 2950, 2920, 2863, 1723, 1687, 1594, 1456, 1428, 1359, 1309, 1228, 1154, 1116. ^{1}H nmr (200MHz , $CDCl_3$) δ: 1.17 (3H, d, J=4.5Hz), 1.36 (3H, d, J=2.8Hz),

1.63 (2H, m, W½=5Hz), 1.98, 2.20 (3H each, s), 4.06 (4H, m, W½=5.2Hz), 6.90 (3H, s). MS m/z: 220 (M^+-59), 205, 189, 177, 165, 161, 149, 145, 115, 105, 81.

Isoeuonyminol octa-acetate This substance was isolated from the more polar fractions of the total reduction product after acetylation. 4mg were obtained (0.0057 mmol, R=21.1%). Under TLC it proved identical to an authentic sample of isoeuonyminol.ir $\nu_{max}^{CHCl_3}$ cm^{-1}: 3440, 3008, 2947, 2918, 1740, 1420, 1364, 1230, 1148, 1089, 1065, 1038. ^1H nmr(200MHz , $CDCl_3$) δ: 1.49 (3H, d, J=1.2Hz), 1.61 (3H, s), 1.89, 1.98, 2.01, 2.13, 2.14, 2.17, 2.29 (3H each, s), 2.12 (6H, s), 2.45 (1H, d, J=2.6Hz), 3.94, 4.90 (2H, d_{AB}, J=11.8Hz), 4.23 (1H, d, J=1.2Hz), 4.66 (2H, dd, J=13.2Hz), 4.80 (1H, d, J=2.6Hz), 5.27 (1H, t, J=2.6Hz), 5.60 (3H, m,W½=12Hz), 6.50 (1H, s). ms m/z: 685 (M^+-17), 672, 629, 569, 509, 467, 425, 407, 365, 347, 323, 305, 287, 275, 263, 245, 233, 217, 215, 203, 191, 175, 173, 163, 161, 153, 149, 137.

T A B L E: ^{13}C AND ^1H NMR SPECTRA OF HORRIDINE

^{13}C (50MHz) $CDCl_3$ (ppm)			
1	72.53	$\underline{CH_3}$-OCO-	21.63
2	68.74	$\underline{CH_3}$-OCO-	21.10
3	75.39		
4	70.78		
5	94.65		
6	74.85	$\underline{CH_3}$-OCO-	20.66
7	49.53		
8	74.66	$\underline{CH_3}$-OCO-	20.85
9	73.98		
10	51.52		
11	85.81		
12	19.74		
13	60.92		
14	23.96		
15	70.03	$\underline{CH_3}$-OCO-	21.34
2'	425.19		
3'	129.14		
4'	137.82		
5'	121.18		
6'	151.65		

^{13}C (50MHz) $CDCl_3$ <continued>			
8'	45.00	$\underline{CH_3}$	12.17
9'	36.62	$\underline{CH_3}$	9.94
Benzoate	133.84, 129.86(2), 128.89(2)		
Ester Carbonyls	165.58, 168.53, 168.69,169.80, 169.92, 170.13, 174.11		

^1H (200MHz) $CDCl_3$ TMS (ppm)	
$H_{4'}$	8.04, 8.01, dd, $J_{4',5'}$=7.8Hz, $J_{4',6'}$=1.8Hz
$H_{5'}$	7.22, 7.26, dd, $J_{5',4'}$=7.8Hz, $J_{5',6'}$=4.8Hz
$H_{6'}$	8.68, 8.70, dd, $J_{6',5'}$=4.8Hz, $J_{6',4'}$=1.8Hz
H_1	5.65, d, $J_{1,2}$=3.0Hz
H_2	5.26, 5.28, dd, $J_{2,1}$=3.0Hz, $J_{2,3}$=3.0Hz
H_3	4.74, d, $J_{3,2}$=3.0Hz
H_6	6.75, bs, $J_{6,7}$=could not be measured
H_7	2.69, d, $J_{7,8}$=3.0Hz, $J_{7,6}$=could not be measured
H_8	5.66, 5.71, dd, $J_{8,7}$=3.0Hz, $J_{8,9}$=9.7Hz
H_9	5.92, d, $J_{9,8}$=9.7Hz
H_{13}	3.66, 6.00, dd, AB, J=11.6Hz
H_{15}	4.64, 4.87, dd, AB, J=13.2Hz
Me-C_8'	2.59, 2.63, q, J=7.2Hz
Me-C_9'	4.59, 4.67, q, J=6.9Hz

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