A GERMACROLIDE FROM *GOCHNATIA HYPOLEUCA* AND ITS TRANSFORMATION INTO A MELAMPOLIDE*

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Abstract—Chemical analysis of *Gochnatia hypoleuca* subsp. *obtusata* afforded, in addition to several known compounds, the new sesquiterpene lactone, gochnatolide. Hydrolysis and subsequent allylic oxidation of gochnatolide gave a germacrolide which was easily isomerized into a melampolide under acid treatment. Thermal isomerization of gochnatolide was also carried out.

INTRODUCTION

During our chemical research on *Gochnatia* genus (Compositae, Mutisieae, Gochnatinae) [1], we have studied *Gochnatia hypoleuca* (DC) A. Gray subsp. *obtusata* (Blake) Cabrera. In previous works on these species, the subspecies were not specified [2, 3]. Several triterpenes were isolated from the roots [2] while guaianolides, germacranolides and triterpenes were extracted from aerial parts [3]. Now we have isolated from *G. hypoleuca* subsp. *obtusata*, a new germacr-8,12-olide, which we have named gochnatolide (1). A derivative of this compound was chemically and thermally transformed into a melampolide. So far only photochemical [4] and chemical methods [5, 6] to induce this transformation had been described.

RESULTS AND DISCUSSION

Column chromatography of the extracts from aerial parts of *G. hypoleuca* afforded the known triterpenes erythrodiol, β-amyrin and oleanolic acid, as well as the flavone luteolin. They were characterized by comparison of their physical constants with those described in the literature and also by formation of some derivatives (see Experimental).

Gochnatolide (1), $C_{19}H_{24}O_6$ (MS), mp 107–109°, was an α,β-unsaturated-γ-lactone (IR bands at 1758 and 1655 cm^{-1}) possessing two hydroxyl groups (IR 3611 cm^{-1}) as was demonstrated by formation of the diacetyl derivative 2. The ^{1}H NMR spectrum of 1 (CDCl$_3$, room temp.) showed unresolved signals indicative of a *trans*-fused germacr-8,12-olide or a *cis,cis*-germacranolide, both of which can exist as a mixture of conformational isomers in solution [7]. The similarity of the ^{1}H NMR spectrum mentioned before with that of the 14-acetoxydesacetyl laurenobiolide 4 [8], which was also isolated for us from a *Perymenium* species [9] constituted
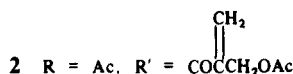
an indication that the new lactone differed from 4 only in the nature of the ester group. The ^{1}H NMR spectrum of 1 determined in DMSO-d_6 solution at 140° (Table 1) was clear and also was quite similar to that of 4 determined in
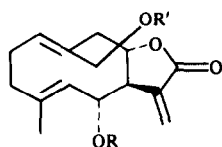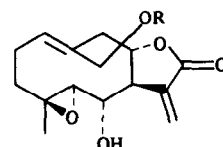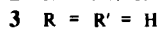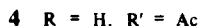

1   R = H, R' = COCCH₂OH

2   R = Ac, R' = COCCH₂OAc
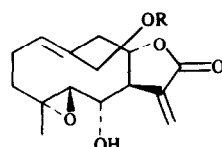3   R = R' = H
4   R = H, R' = Ac

5   R = COCCH₂OH
6   R = Ac

7   R = COCCH₂OH

8

9
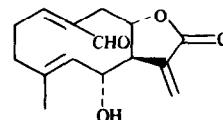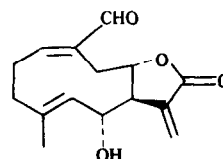
the same conditions [9]. This, together with the above mentioned data allowed us to propose the structure of gochnatolide as is depicted in 1.

Compound 1 upon oxidation with m-chloroperbenzoic acid gave a mixture of the epoxides 5 and 7 which arise as a consequence of its conformational flexibility. Compound 5 was an ester analogous to 14-acetoxy-4β,5α-epoxydesacetyl laurenobiolide (6) [8] as was demonstrated by comparison of their respective ¹H NMR spectra. Although other authors stated that 6 [8] and its metacryloyl, tigloyl and isobutyroyl analogues [3] were conformationally flexible at room temperature, we found that 5 gave a clear ¹H NMR spectrum (in CDCl₃) at this temperature thus indicating the presence of a single conformer. The same is true for 6 [9]. Furthermore, the ¹H NMR spectrum of 5 was determined in CDCl₃ at 57° in order to compare it with that of 6 reported under these conditions [3]. In our case no changes were observed confirming the rigidity of the molecule. In these spectra the $J_{5,6} = 4$ Hz and the $J_{6,7} = 12$ Hz indicated an equatorial–axial and axial–axial relationship between H-5, H-6 and H-6, H-7, respectively. On the other hand, a trans-diaxial disposition of H-5, H-6 and H-6, H-7 was deduced from the $J_{5,6} = 9$ Hz and $J_{6,7} = 10$ Hz values exhibited by compound 7 in its ¹H NMR spectrum. It also demonstrated a single conformer at room temperature. From these results it is clear that the presence of a 4,5-epoxy group restrains the conformational mobility in compounds 5–7.

Alkaline hydrolysis of 1 afforded the diol 3, which was oxidized with manganese dioxide to give the hydroxy aldehyde 8. Its ¹H NMR spectrum (CDCl₃, room temp.) showed three aldehydic proton signals whose chemical shifts (δ9.99, 9.94 and 9.89) indicated the presence of three conformations for a molecule with an aldehydic function conjugated with a trans-double bond, as depicted in 8. An additional singlet signal at δ9.46 was observed in the same spectrum and this revealed an incipient isomerization of the germacrolide 8 into the melampolide 9, probably due to the acidity of the CDCl₃.

The ¹H NMR spectrum of 8 in DMSO-d₆ at room temp. confirmed the existence of three conformations for this compound (three aldehydic proton signals at δ 10.01, 9.93 and 9.88 and three vinylic methyl signals at 1.65, 1.54 and 1.35). The above data revealed that the chemical shift of the aldehydic protons was not substantially affected with the solvent change. Thus, the spectrum determined at 140°, which showed a single set of signals, confirmed the proposed structure 8. The E-geometry of the 1(10)-double bond was inferred from the chemical shifts of H-1 (δ6.58, t, $J = 9$ Hz) and H-14 (9.93,s).

When the temperature of the probe reached 160°, a new aldehydic proton signal appeared indicating a partial isomerization of the 1(10)-double bond. After 20 min the transformation of the germacrolide 8 into the melampolide 9 was completed. In the ¹H NMR spectrum of the melampolide 9 (DMSO-d₆, 160°) the signal for H-1 was triplet at δ6.68 ($J = 8$ Hz) while H-14 appeared at 9.47 as a singlet. The recovered sample of the previous experiment gave a well defined ¹H NMR spectrum in CDCl₃ solution at room temperature. In this spectrum the signals for H-1 and H-14 showed the chemical shifts characteristic for an aldehyde conjugate with a cis-double bond (Table 1). The isomerization also occurred when a CHCl₃ solution of 8 was left to stand for an hour and it was accelerated upon heating. Finally, chemical transform-

ation of 8 into 9 was achieved upon addition of hydrochloric acid to an acetone solution of 8.

EXPERIMENTAL

Gochnatia hypoleuca (DC) A. Gray subsp. *obtusata* (Blake) Cabrera, was collected in Oaxaca, México (specimen deposited at the Herbarium of the Instituto de Biología, U.N.A.M., MEXU 215836). The ground dried aerial parts (1 kg) were extracted with hexane (2 l.), CHCl₃–Me₂CO 4:1 (3 l.) and Me₂CO (2 l.) to give 32.6, 34.5 and 29 g of extracts, respectively. The hexane residue was adsorbed on celite and fractioned by CC (silica gel, Merck G, 8 cm diameter × 15 cm height, operated with vacuum) using as eluent hexane-EtOAc gradient solvent system. The first fractions eluted with hexane-EtOAc (9:1) yielded after crystallization 1.249 g of β-amyrin, mp 191–193° [lit.[10] mp 197°], which was further characterized as the acetyl derivative, mp 235–236° [lit. [10] mp 236°]. The subsequent fractions eluted with hexane-EtOAc (9:1) afforded, after crystallization, 0.204 g of erythrodiol, mp 222–224° [lit. [10] mp 232°]. This compound was converted into its diacetate, mp 183–185° [lit. [10] mp 187°] and correlated with oleanolic acid through the formation of 3-oxo-oleanolic acid. The CHCl₃–Me₂CO extract was adsorbed on celite and fractioned as above. Fractions eluted with hexane-EtOAc (17:3) afforded 0.21 g of erythrodiol. Fractions eluted with hexane-EtOAc (4:1) gave 0.353 g of oleanolic acid, mp 299–301° [lit. [10] mp 306–308°]. The Me ester of this acid was obtained and compared with an authentic sample. Fractions eluted with hexane-EtOAc (11:9) yielded, after crystallization from Me₂CO-hexane, 5.48 g of gochnatolide (1), mp 107–109°, $[\alpha]_D^{25}$ +8.89° (c, 0.169, CHCl₃); UV (EtOH) 211 nm (ε17300); IR $\nu_{max}^{CHCl_3}$ cm⁻¹: 3611, 1758, 1711, 1655, 1633, 1273, 1143, 1025, 962. CIMS (CH₄) m/z (rel. int.): 349 [M + 1]⁺ (C₁₉H₂₄O₆, 1), 331 (56), 247 (30), 229 (100), 201 (20), 183 (17), 151 (26), 133 (74), 121 (13).

The Me₂CO extract was fractioned in the same way as above. Fractions eluted with hexane-EtOAc (3:2) gave 78 mg of 1. From fractions eluted with hexane-EtOAc (11:9), 72 mg of luteolin (mp 330°, lit. [11] mp 331°) were isolated.

Diacetyl gochnatolide (2). To a soln of 100 mg of 1 in 1 ml of pyridine were added 2 ml of Ac₂O. The reaction mixture was kept for 12 hr at room temp. After the usual work up 81.6 mg of 2 were obtained as a yellow oil. IR $\nu_{max}^{CHCl_3}$ cm⁻¹: 1760, 1737, 1657, 1644, 1442, 1371, 1272, 1148, 1020, 960. MS m/z (rel.int.): (432 [M]⁺, C₂₃H₂₈O₈, unobserved), 390 (0.1), 373 (2), 331 (0.4), 289 (6), 228 (16), 213 (7), 200 (6), 127 (28), 43 (100).

Hydrolysis of gochnatolide 1. A mixture of 100 mg of 1 and 180 mg NaOH in 10 ml H₂O was stirred by 1 hr at room temp. neutralized with 10% H₂SO₄ and extracted with EtOAc. The organic layer was washed with H₂O, dried and concd in vacuo to yield 63 mg of 3 as a yellow gum which could not be induced to crystallize. UV (MeOH) 204 nm (ε = 12700), $[\alpha]_D^{25}$ +29.8° (c, 0.179, EtOH), IR $\nu_{max}^{CHCl_3}$ cm⁻¹: 3600, 1756, 1653, 1600, 1270, 1140, 1070, 958. MS m/z (rel. int.): 264 [M]⁺ (C₁₅H₂₀O₄, 1), 246 (5), 228 (7), 213 (7), 200 (7), 188 (12), 133 (14), 117 (37), 105 (46), 91 (58), 84 (100), 83 (87), 67 (47), 55 (67).

Epoxidation of 1. To a soln of 110 mg of 1 in 10 ml CHCl₃ were added 75 mg m-CPBA. After 5 min the solvent was evapd and the residual gum (two spots on TLC) was chromatographed over a silica gel column using as eluent CHCl₃-Me₂CO (9:1). The less polar product 5 was obtained as a colourless oil (70 mg). IR $\nu_{max}^{CHCl_3}$ cm⁻¹ 3572, 1762, 1712, 1656, 1628, 1358, 1275, 1141, 955 and 987; MS m/z (rel. int.): 364 [M]⁺ (C₁₉H₂₄O₇, 0.5), 280 (4), 262 (2), 215 (5), 177 (12), 109 (22), 105 (16), 91 (21), 85 (100), 84 (39), 69 (37), 67 (30), 55 (24), 43 (41). The more polar compound 7

Table 1. ¹H NMR spectral data of compounds 1-3,5,7-9 (80 MHz, DMSO-d_6, 140° TMS as internal standard)

H	1	2	3	5*	7*	8	9	9*
1	5.35 t br (8)†	5.37 t br (7.5)	5.06 t br (7.5)	5.57 t br (8)	5.68 t br (8)	6.58 t (9)	6.68 t (8)	6.58 t br (8)
5	4.97 d br (8)	4.96 d br (8)	4.94 d br‡ (8)	2.76 d (4)	2.67 d (9)	4.75 m‡	5.09 d br (10)	4.98 d br (10)
6	4.06 dd (9.5,8)	4.19 dd (9.5,8)	4.14 dd‡ (10,8)	4.10 dd (11,4)	3.44 dd (10,9)	4.00 m‡	4.17 t (10)	4.25 t (10)
7	2.89 m	3.17 m	2.92 m	3.05 m	3.08 m			2.51 m‡
8	4.15‡	4.32 dt (11,4)		4.58 ddd (12,5,3.5)	4.12 ddd (11.5,3)	4.75 m‡	3.81 dd (12,6)	3.87 dd (13,7)
9	2.76 dd (14,4)		2.73 dd‡ (13,3.5)		2.93 d br (14)			
13	6.10‡	6.13 dd (3,1)	6.14 dd‡ (3,1.8)	6.39 dd (3,1.5)	6.44 dd (3,1.5)	6.14 dd‡ (3,1.5)		6.31 dd (3.5,1)
							6.08 d(2H) (3)	
13	6.10‡	5.80 dd (2.4,1)	6.06 dd‡ (2.5,1.8)	6.08 dd (2.5,1.5)	6.25 s	6.06 dd‡ (2.5,1.5)		6.10 dd (3,1)
14	4.63 s	4.68 s	3.93 s	4.71 s	4.75 s	9.93 s	9.47 s	9.46 s
15	1.62 d (1.5)	1.65 d (1.5)	1.59 d (1.5)	1.46 s	1.29 s	1.57 d (1.5)	1.70 d (1.5)	1.74 d (1.5)
OCOR	4.15 t br (1.5)	4.72 dd (1.5,1)		4.29 s	4.33 s			
	5.22 dt (2,1.5)	5.85 dt (1.5,1)		5.87 dt (1.5, 1)	5.88 dt (1.5, 1)			
	6.10‡	6.21 dt (1,1)		6.19 d br (1)	6.27 d br‡ (1)			
OAc		2.23 s(6H)						

*Run in CDCl₃ at room temp.
†J values (Hz) are given in parentheses.
‡Superimposed signal.

was also obtained as a colourless oil (41 mg). IR $\nu_{max}^{CHCl_3}$ cm⁻¹: 3592, 1762, 1712, 1655, 1635, 1272, 1142, 1037, 964 and 894. CIMS (CH₄) m/z (rel. int.): 365 [M + 1]⁻ C₁₉H₂₄O₇, 23), 321 (8), 281 (48), 263 (59), 245 (100), 227 (37), 217 (34), 179 (24), 167 (26), 149 (69), 121 (41).

Allylic oxidation of 3. To a soln of 90 mg of 3 in 15 ml of CHCl₃ were added 900 mg of MnO₂. After 10 min the reaction mixture was filtered off and the solvent was eliminated under vacuum. From the residue compound 8 was crystallized. Mp 163–165° (Me₂CO-hexane); IR ν_{max}^{KBr} cm⁻¹: 3503, 1739, 1656, 1454, 1276, 1153, 1033, 972, 874; $\nu_{max}^{CHCl_3}$ cm⁻¹: 3600, 1758, 1708, 1668, 1270, 1135, 1025, 955, 878 cm⁻¹. MS m/z (rel. int.): 262 [M]⁺ (C₁₅H₁₈O₄, 1), 244 (6), 234 (10), 215 (19), 179 (17), 133 (22), 105 (36), 91 (28), 84 (100), 83 (63), 67 (23), 55 (28).

Acid isomerization of 8. Traces of conc HCl were added to a soln of 8 (11.4 mg) in Me₂CO (3 ml). After 5 min the solvent was eliminated and 11 mg of 9 were obtained as a yellow oil. IR $\nu_{max}^{CHCl_3}$ cm⁻¹: 3610, 1760, 1711, 1687, 1639, 1449, 1364, 1271, 1238, 1144, 1023, 961, 876. MS m/z (rel. int.): 262 [M]⁺ (C₁₅H₁₈O₄, 1) 244 (1), 233 (5), 215 (3), 179 (13), 161 (27), 133 (17), 105 (36), 91 (33), 84 (100), 83 (54), 55 (61), 39 (94).

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