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CONSTITUENTS OF THE SEED PODS OF *EPERUA PURPUREA*

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ABSTRACT.—From the seed-pods of *Eperua purpurea* (Leguminosae), several diterpenoids were isolated and their structures determined on the basis of spectroscopic evidence. A new clerodane-type diterpene whose structure we assign as (–)-cleroda-7,13*E*-dien-15-oic acid [2], a known clerodane (–)-2-oxo-10*α*-*cis*-cleroda-3,13*E*-dien-15-oic acid [3], and two known labdane compounds, (–)-7-oxolabda-8,13*E*-dien-15-oic acid [4] and copalic acid [5], were obtained.

Continuing our interest in the structures of secondary metabolites present in *Eperua* species that could aid in the establishment of phylogenetic and chemotaxonomic relations with the genus, and the Leguminosae in general, we report in this paper the chemical study of the seed pods of *Eperua purpurea* Benth. (Leguminosae).

## RESULTS AND DISCUSSION

From the petroleum ether extract of the seed pods, the acidic fraction was separated as a complex mixture. Attempts to isolate the main constituent through successive recrystallizations of its cyclohexylamine salt were unsuccessful. Therefore, the mixture was methylated and the methyl ester derivative purified by preparative tlc. The ir spectrum of the resulting colorless oil showed a low frequency band due to carbonyl absorption, suggesting an  $\alpha,\beta$ -unsaturated methyl ester ( $\nu_{C=O}$  1721  $\text{cm}^{-1}$ ,  $\nu_{C=C}$  1644  $\text{cm}^{-1}$ ).

The  $^1\text{H}$ -nmr spectrum exhibited the resonance of one MeO group at  $\delta$  3.66 which confirmed the presence of a single carbomethoxy moiety. It also showed a broad singlet at  $\delta$  5.68 and a multiplet at  $\delta$  5.32, which combined with  $^{13}\text{C}$ -nmr evidence ( $\delta$  161.2, 141.2, 122.1, and 114.3) indicated the presence of two double bonds. Furthermore, there were signals for two tertiary methyl groups ( $\delta$

1.15 and 1.03), a doublet for a secondary Me group ( $\delta$  0.86,  $J=7$  Hz), and signals corresponding to two Me groups attached to double bonds ( $\delta$  1.63 and 2.18).

Our experimental findings and information from the literature (1–3) about compounds from some *Eperua* species led us to consider a labdane-diterpenic-bicyclic structure with a side chain containing an  $\alpha,\beta$ -unsaturated carboxylic acid. In decoupling experiments the signal for an Me group on a double bond at  $\delta$  2.18 showed coupling ( $J=1.2$  Hz) with that at  $\delta$  5.68 for an olefinic proton. According to the literature (4) this is typical of bicyclic diterpenoid acids with an *E* configuration on the side chain, while the *Z* isomers show the signal for the methyl groups at higher fields.

Furthermore, the signal at  $\delta$  5.32 for the other olefinic proton was coupled ( $J=1.2$  Hz) to a broad singlet at  $\delta$  1.63, reminiscent of the signals for H-7 and Me-17 respectively, in cativic acid [1] previously obtained from *Eperua leucantha* (3). The  $^{13}\text{C}$ -nmr spectrum exhibited signals for five quaternary carbons (two of them olefinic and one belonging to the carbonyl group), four methine carbons (two of them olefinic), five Me carbons, an MeO, and six methylene carbons, consistent with the molecular ion at  $m/z$  318 in the mass spectrum and with a molecular formula  $\text{C}_{21}\text{H}_{34}\text{O}_2$ .

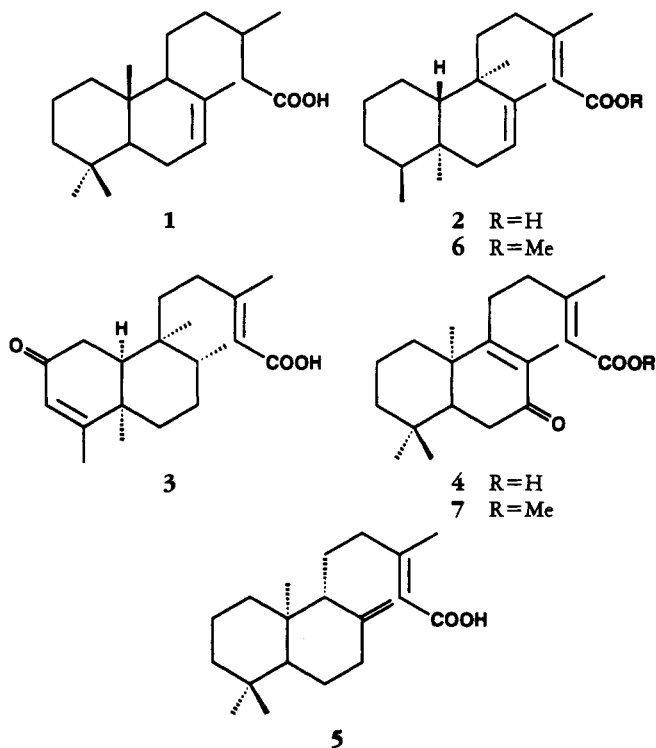
Assuming the existence of a double bond between C-7 and C-8 and an Me group on C-8, it is not possible to accommodate a secondary and two tertiary Me groups in a labdane-type skeleton. Thus, we proposed a rearranged, clerodane-type skeleton. This assumption was confirmed by the mass spectrum where the base peak appeared at  $m/z$  95, resulting from a retro-Diels-Alder type fragmentation in ring B, directed by the C-7-C-8 unsaturation. If it were a labdane skeleton, such a fragmentation in ring B would give rise to a base peak at  $m/z$  109.

The stereochemistry at C-4 and the A/B fusion are inferred from the  $^{13}\text{C}$ -nmr data. The observed resonance for C-18 at  $\delta$  14.9 indicates that the Me group should be  $\beta$ -oriented such as is asserted in the literature (5) for some norlabdanes. On the other hand, the resonance for C-19, at  $\delta$  20.8, is indicative of an A/B-trans junction. According to previous reports (6) about A/B junctions in steroids, the chemical shift for the signal corresponding to an Me group on C-5 in A/B-cis

fusions is deshielded by some 12 ppm, resulting in resonance around  $\delta$  32.0.

The negative optical rotation ( $[\alpha]^{25}\text{D}$   $-40^\circ$ ) typical of the normal clerodane series (7,8), in addition to all the data given above, allows us to propose the structure of (-)-cleroda-7,13E-dien-15-oic acid [**2**] for the original compound, which represents a new natural product.

From the  $\text{C}_6\text{H}_6$  extract, other diterpenic acids were identified; namely, a *cis*-clerodane, whose structure we previously assigned (9) as (-)-2-oxo-10 $\alpha$ -*cis*-cleroda-3,13E-dien-15-oic acid [**3**], and (-)-7-oxolabda-8,13E-dien-15-oic acid [**4**], identified by partial synthesis and comparison of its spectroscopic data with those of the natural compound obtained previously from *Cistus libanotis* (10). In addition to these compounds, a colorless oil, which exhibited spectral data identical to those of the racemic product isolated from the oleoresin of this plant (11), ( $\pm$ )-8(17),13E-labdadien-15-oic acid, was obtained. The optical rotation of this oil,  $[\alpha]^{25}\text{D}$   $-34.3^\circ$ , indicated that it is the



levorotatory isomer, copalic acid [**5**] [lit. (12)  $[\alpha]^{25}_D -45^\circ$ ].

## EXPERIMENTAL

**PLANT MATERIAL.**—Original sample identified by R. Liesner, 1979, (Missouri Botanical Garden). Present sample identified by E. Medina (Centro de Ecología, IVIC). The seed pods of *E. purpurea* were collected in March 1987 from San Carlos de Río Negro (Venezuelan Amazonas). A voucher specimen is deposited under N° M-243 at the Botanical Garden, Caracas, Venezuela.

**GENERAL PROCEDURES.**—Mp's are uncorrected. Optical rotations were measured in a Carl-Zeiss polarimeter in  $\text{CHCl}_3$ ; elemental analysis was performed in a Leco CHN-800 analyzer. The ir spectra were recorded in a Nicolet 5DX FTIR spectrophotometer;  $^1\text{H}$ - and  $^{13}\text{C}$ -nmr spectra were recorded in a Bruker AM300 instrument operating at 300 and 75.5 MHz, respectively. In all cases TMS was used as internal standard. Ms were obtained from a Kratos MS25RFA spectrometer operating at 70 eV.

**EXTRACTION AND FRACTIONATION.**—The dried and ground seed pods (4.0 kg) were extracted in a Soxhlet using petroleum ether (40–60°) and  $\text{C}_6\text{H}_6$  as solvents. The petroleum ether extract (120 g) was partitioned with 10% aqueous KOH; the organic layer was dried over  $\text{MgSO}_4$ , and evaporation of solvent left the neutral fraction (9.6 g). The aqueous phase was adjusted to pH 3 with HCl and extracted (3 $\times$ ) with  $\text{Et}_2\text{O}$ . Evaporation of the solvent yielded the acidic fraction (96 g). A portion (12 g) of this fraction was chromatographed on Si gel (Merck®, 70–230 mesh, 500 g) using  $\text{Et}_2\text{O}$ /petroleum ether mixtures of increasing polarity as eluent. The  $\text{C}_6\text{H}_6$  extract (40 g), by similar treatment, afforded neutral and acidic fractions (6.4 and 32.0 g, respectively). A portion (6.5 g) of the acidic fraction was also chromatographed on Si gel (240 g), using  $\text{C}_6\text{H}_6$  as the eluent, and the polarity was increased by addition of  $\text{Et}_2\text{O}$ . In both cases fractions of 200 ml were collected.

(-)-*Cleroda-7,13E-dien-15-oic methyl ester* [**6**].—From fractions 11–32 (10%  $\text{Et}_2\text{O}$ /petroleum ether) eluted by chromatography of the acidic fraction from the petroleum ether extract, a yellowish oil was separated. Treatment with  $\text{CH}_2\text{N}_2$  and further purification by preparative tlc afforded a colorless oil (**6** (80.0 mg):  $[\alpha]^{25}_D -18.4^\circ$  ( $\text{CHCl}_3$ ,  $c=1.0$ ); found: C 79.1, H 10.9% ( $\text{C}_{21}\text{H}_{34}\text{O}_2$  requires C 79.0, H 10.8%); uv  $\lambda$  max (EtOH) nm ( $\epsilon$ ) 230 (2000); ir  $\nu$  max  $\text{cm}^{-1}$  2947, 2882, 1721, 1648, 1448, 1359;  $^1\text{H}$  nmr  $\delta$  0.86 (d,  $J=7$  Hz, 3H H-18), 1.03, 1.14 (2s, 3H each, H-19, H-20), 1.63 (d,  $J=1.2$  Hz, 3H, H-17), 2.18 (d,  $J=1.2$  Hz, 3H, H-16), 5.32 (m, 1H, H-7), 5.68 (br s, 1H, H-14);  $^{13}\text{C}$  nmr  $\delta$  166.6 (C-15), 161.2 (C-13), 141.2

(C-8), 122.1 (C-7), 114.3 (C-14), 50.1 (OMe), 44.2 (C-10), 38.2 (C-9, C-5), 36.9 (C-4), 35.5, 34.7 (C-6, C-12), 31.8 (C-11), 26.8, 25.3, 19.5 (C-1, C-2, C-3), 20.8 (C-19), 18.9 (C-16, C-17), 18.6 (C-20), 14.9 (C-18); eims  $m/z$  (%)  $[\text{M}]^+$  318 (7), 303 (11), 287 (2), 275 (3), 271 (3), 205 (16), 191 (35), 189 (46), 135 (39), 109 (82), 95 (100).

(-)-*Oxo-10 $\alpha$ -cis-cleroda-3,13E-dien-15-oic acid* [**3**].—Fractions 45–55 from chromatography of the  $\text{C}_6\text{H}_6$  extract (10%  $\text{Et}_2\text{O}/\text{C}_6\text{H}_6$ ) yielded **3** (250 mg), needles: mp 144–147° (from  $\text{Et}_2\text{O}$ );  $[\alpha]^{25}_D -62^\circ$  ( $\text{CHCl}_3$ ,  $c=0.5$ ); spectroscopic data see Avila and Medina (9).

(-)-*7-Oxolabda-8,13E-dien-15-oic acid* [**4**].—Fractions 27–34 (10%  $\text{Et}_2\text{O}/\text{C}_6\text{H}_6$ ) eluted from the chromatography above, yielded a solid on evaporation of the solvent. Recrystallization from  $\text{Et}_2\text{O}$  afforded colorless needles of **4** (75 mg): mp 184–186°;  $[\alpha]^{25}_D -43^\circ$  ( $c=1.2$ ;  $\text{CHCl}_3$ ). By treatment with  $\text{CH}_2\text{N}_2$ , the methylester **7** was obtained. Both compounds showed spectral features identical to those of the natural compounds (10).

*Synthetic (-)-methyl-7-oxolabda-8,13E-dien-15-oate.*—Copalic acid [**5**] (600 mg) was dissolved in MeOH (40 ml) containing 10% v/v of concentrated  $\text{H}_2\text{SO}_4$  and refluxed 4 h. The reaction mixture was diluted with  $\text{H}_2\text{O}$  (160 ml) and extracted with petroleum ether (3 $\times$ ). The crude product was filtered through Si gel. The methyl ester obtained was dissolved in HOAc (10 ml) with  $\text{K}_2\text{Cr}_2\text{O}_7$  (600 mg) and refluxed 6 h. After most of the HOAc was removed under reduced pressure, the mixture was diluted with  $\text{NaHCO}_3$  solution and extracted (3 $\times$ ) with  $\text{Et}_2\text{O}$ . After purification by chromatography, the synthetic product showed spectral features identical with those of the methyl ester of the natural compound (10).

*Copalic acid* [**5**].—Fractions 3–9 from chromatography of the  $\text{C}_6\text{H}_6$  extract (10%  $\text{Et}_2\text{O}/\text{C}_6\text{H}_6$ ) yielded a colorless oil (800 mg),  $[\alpha]^{25}_D -34.3^\circ$ . All spectral evidence was identical with that reported for **5** (12).

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