A Revised Structure for (−)-Dihydropertusaric Acid, a γ-Butyrolactone Acid from the Lichen *Punctelia microsticta*

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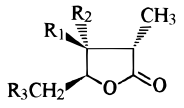
The γ-butyrolactone acid (**1**) and two known compounds, (−)-isomuronic acid and the tridepside gyrophoric acid, have been isolated from the lichen *Punctelia microsticta*. The structure and stereochemistry of compound **1** were determined on the basis of spectroscopic evidence and molecular modeling. Spectroscopic and physical data of **1** and (−)-dihydropertusaric acid, previously isolated from the lichen *Pertusaria albescens*, showed that both are the same compound, although for the latter the epimeric structure **2** has been proposed.

Naturally occurring chiral-substituted γ-butyrolactones represent about 10% of all known natural products, and many of these compounds are biologically significant.[1] Stereocontrolled syntheses of γ-butyrolactones and in particular of their 4-carboxy derivatives (paraconic acids) isolated from lichens have recently been described.[2−5] These syntheses have served to establish the absolute stereochemistry of these natural products.

In 1982, Krog[6] reported the presence of unknown medullary fatty acids in *Punctelia microsticta* (Muell. Arg.) Krog (Parmeliaceae). So far the structures of these compounds have not been elucidated. We report here on the isolation and structure elucidation of the two major compounds present in this lichenized fungus, namely, the γ-butyrolactone acid (**1**) and the known (−)-isomuronic acid,[7,8] as well as the tridepside gyrophoric acid.[8]



1 $R_1 = CO_2H$, $R_2 = H$, $R_3 = CH_3CO(CH_2)_{12}$
1a $R_1 = CO_2CH_3$, $R_2 = H$, $R_3 = CH_3CO(CH_2)_{12}$
2 $R_1 = H$, $R_2 = CO_2H$, $R_3 = CH_3CO(CH_2)_{12}$
3 $R_1 = CO_2H$, $R_2 = H$, $R_3 = C_{12}H_{25}$
4 $R_1 = H$, $R_2 = CO_2H$, $R_3 = C_{12}H_{25}$
5 $R_1 = CO_2H$, $R_2 = H$, $R_3 = CH_2CH_3$

The known compounds [(−)-isomuronic acid and gyrophoric acid] were identified by comparison of their ^1H, ^{13}C NMR, MS, and optical rotation data with published data.[7,8] Compound **1** was isolated as a white solid. The EIMS of **1** showed a molecular ion at *m*/*z* 368, while the negative-ion FABMS exhibited a $[M - H]^-$ ion at *m*/*z* 367, and the positive-ion FABMS showed the $[M + H]^+$ ion at *m*/*z* 369. These data are consistent with a molecular formula of $C_{21}H_{36}O_5$. The ^{13}C NMR spectrum of **1** showed 13 signals corresponding to 21 carbons, and DEPT measurements revealed the presence of two methyl groups, 13 methylenes, three methines (one bearing oxygen), and three oxygenated quaternary carbons at δ 173.6, 177.6, and 209.9, assigned to carboxylic acid, lactone, and ketone carbonyls, respectively. The ^1H NMR spectrum of **1** showed signals at δ 3.04 (1H, dq, J = 9.8, 7.2 Hz, H-3), 3.22 (1H, dd, J = 9.8, 8.3 Hz, H-4), and 4.64 (1H, ddd, J = 8.7, 8.3, 4.6 Hz, H-5), typical for a trisubstituted γ-butyrolactone acid.[8] The presence of signals at δ 2.11 (3H, s, H-22) and 2.39 (2H, t, J = 7.3 Hz, H-20) were indicative of a methyl ketone at the side chain. The methyl ester of **1** (**1a**) showed a molecular ion peak $[M]^+$ at *m*/*z* 382 in the EIMS, compatible with the molecular composition of $C_{22}H_{38}O_5$. The ^1H and ^{13}C NMR spectra of **1a** showed signals at δ 3.73 (3H, s, OCH_3) and 52.3 (OCH_3), characteristic of a methyl ester.

Examination of NMR, MS, and physical data for compound **1** and its methyl ester (**1a**) showed that they are very similar to the data of (−)-dihydropertusaric acid, isolated by Huneck et al.[9] from the lichen *Pertusaria albescens*, to which structure **2** has been ascribed. On the other hand, the values of the coupling constants of the methine ring protons (H-3, H-4, and H-5) were coincident with those reported for nephromopsinic acid (**3**), which differs from compound **1** in the side chain only.[3] Furthermore, on comparison of the coupling constant between methine ring protons H-4 and H-5 reported for (−)-dihydropertusaric acid[9] (J = 8 Hz) and for synthetic (−)-dihydroprotolichesterinic acid[2] (**4**) (J = 6 Hz), which has the same side chain as nephromopsinic acid (**3**), we decided to investigate the relative stereochemistry of the ring substituents in compound **1**.

The relative stereochemistry of **1** was assigned on the basis of the 2D NOESY spectrum, which exhibited the presence of NOEs, indicating that the Me-6, H-4, and H-5 are oriented on the same side of the molecule, while H-3 has the same orientation as the lipophilic side chain (Figure 1), suggesting that **1** is either the 3*S*,4*S*,5*S* isomer or the 3*R*,4*R*,5*R* isomer. Taking into account the presence of (−)-isomuronic acid in *P. microsticta*, we assume the same configuration at C-5 (5*S*) for compound **1** and propose the absolute configuration 3*S*,4*S*,5*S* shown in Figure 1.

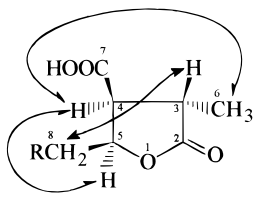


Figure 1. Key NOE interactions of compound **1**.

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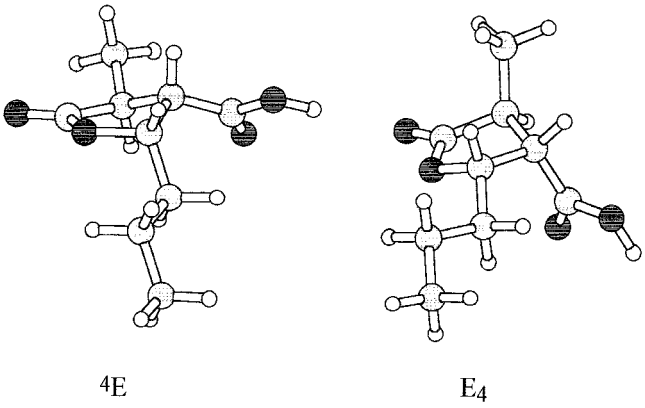

Figure 2. Minimum energy conformations (HF/6-31G level, in $CHCl_3$) of compound **5**.

Conformational analysis of the five-membered lactone ring in compound **1** was carried out by performing molecular mechanics and quantum mechanical calculations and by comparison with the experimentally obtained coupling constants. An analogue (**5**) of compound **1**, in which the 15-carbon side chain on C-5 has been replaced by an *n*-propyl group, was used for the sake of simplicity. The ab initio molecular orbital calculations at the HF/6-31G level, with inclusion of chloroform as a polarizable continuum,[10] yielded only two stable envelope conformers, which exhibit the flat region of the envelope around the lactone bond.[11] Although cyclopentane conformers interconvert easily by a pseudorotational pathway,[12] it is known that other substituted five-membered rings exhibit only a few conformational minima.[13−15]

With the energies and geometries, the Boltzmann-averaged coupling constants can be calculated. Although for five-membered rings, many combinations of conformers or even the presence of a single one could match experimental coupling constants,[16] accurate molecular modeling makes it possible to predict their conformational features. Figure 2 depicts a model of the most populated (^4E) and less populated (E_4) conformers of **5**, whose Boltzmann-averaged coupling constants are $J_{H3,H4} = 11.0$ Hz and $J_{H4,H5} = 8.3$ Hz (cf. with experimental values of 9.8 and 8.3 Hz, respectively). Those geometries agree with the NOE interactions observed and reinforce the suggestion that both the product reported in this paper and that from *P. albescens* correspond to the 3*S*,4*S*,5*S* configuration depicted in **1** and not to the 3*S*,4*R*,5*S* configuration indicated by structure **2**.[9]

Experimental Section

General Experimental Procedures. Melting points were determined on a Fisher−Johns apparatus and are uncorrected. ^1H and ^{13}C NMR spectra (chemical shifts in ppm) were recorded on Bruker ACE-200 and -500 instruments. EIMS were measured on a TRIO-2 VG mass spectrometer. FABMS were obtained on a VG-ZAB mass spectrometer. The optical rotations were determined on a Perkin-Elmer 343 polarimeter. The UV spectra were measured on a Hewlett-Packard 8451A diode array spectrophotometer. The CD spectrum was obtained on a JASCO J-20 spectropolarimeter. TLC was performed on precoated Si gel F_{254} plates.

Plant Material. Lichen samples of *P. microsticta* were collected on fruit trees at General Rodríguez, Buenos Aires Province, Argentina, in September 1997. The specimens were identified by one of us (M.T.A), and voucher specimens are housed at the Herbarium of the Department of Biological Sciences of the Faculty of Exact and Natural Sciences BAFC, under registration no. 39013.

Extraction and Isolation. Air-dried lichen thallus (19 g) was extracted with hexane in a Soxhlet extractor for 8 h and this solvent discarded. The residual lichen material was then re-extracted with Et_2O. The crude extract (0.3 g) was subjected to vacuum−dry column chromatography on Si gel C_{18} reversed-phase (35−75 μm) material using as eluents H_2O, H_2O−MeOH mixtures with increasing amounts of MeOH, and finally MeOH. Fractions of 100 mL were collected and analyzed by TLC (Si gel, toluene−HOAc, 17:3) and detected by spraying with 2% vanillin in H_2SO_4. Fractions containing aliphatic acids were purified by repeated Si gel H column chromatography (CH_2Cl_2−EtOAc−HOAc, 90:9:1) to afford the pure compounds **1** (56.4 mg), (−)-isomuronic acid (23.8 mg), and gyrophoric acid (7.0 mg).

(3*S*,4*S*,5*S*)-4-Carboxy-3-methyl-2-oxo-5-(14-oxopentadecyl)tetrahydrofuran (1): obtained as white crystals (MeOH); mp 107−108 °C; $[\alpha]^{25}_D$ −72.0° (*c* 1.45, MeOH); UV ($CHCl_3$) λ_{max} (log ϵ) 216 (2.40) nm; CD (EtOH) λ_{max} (nm) ($\Delta\epsilon$) 217 (+0.02); ^1H NMR ($CDCl_3$, 500.1 MHz) δ 1.23 (22H, s, H-9−19), 1.27 (3H, d, J = 6.6 Hz, Me-6), 1.55 (2H, br s, H-8), 2.11 (3H, s, Me-22), 2.39 (2H, t, J = 7.3 Hz, H-20), 3.04 (1H, dq, J = 9.8, 7.2 Hz, H-3), 3.22 (1H, dd, J = 9.8, 8.3 Hz, H-4), 4.64 (1H, ddd, J = 8.3, 4.6, 8.7 Hz, H-5); ^{13}C NMR ($CDCl_3$, 50.3 MHz) δ 209.9 (s, C-21), 177.6 (s, C-2), 173.6 (s, C-7), 77.5 (d, C-5), 51.7 (d, C-4), 43.9 (t, C-20), 36.6 (d, C-3), 31.1 (t, C-8), 29.5 (q, C-22), 29.1 (t, C-10-C-18), 25.6 (t, C-9), 23.8 (t, C-19), 14.5 (q, C-6); EIMS (70 eV) *m*/*z* 368 $[M]^+$ (2), 353 (0.5), 339 (1), 323 (1), 293 (3), 275 (3), 237 (2), 95 (15), 81 (17), 69 (29), 55 (47), 43 (100); *anal.* C 68.38%, H 9.77%, calcd for $C_{21}H_{36}O_5$, C 68.45%, H 9.85%.

Methylation of Compound 1. Compound **1** (4.5 mg) was treated with CH_2N_2 in Et_2O at 20 °C for 3 h. After this time the solvent was removed under N_2, affording 4.6 mg of the methyl ester **1a**: mp (MeOH) 60−61 °C; $[\alpha]^{25}_D$ −70.5° (*c* 0.95, $CHCl_3$); UV ($CHCl_3$) λ_{max} (log ϵ) 206 (2.12) nm; ^1H NMR ($CDCl_3$, 500.1 MHz) δ 1.23 (22H, s, H-9−19), 1.27 (3H, d, J = 6.6 Hz, Me-6), 1.55 (2H, br s, H-8), 2.10 (3H, s, Me-22), 2.38 (2H, t, J = 7.3 Hz, H-20), 3.04 (1H, dq, J = 9.8, 7.2 Hz, H-3), 3.12 (1H, dd, J = 9.8, 8.3 Hz, H-4), 3.73 (3H, s, OCH_3), 4.61 (1H, ddd, J = 8.3, 4.6, 8.7 Hz, H-5); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 209.9 (s, C-21), 177.5 (s, C-2), 170.1 (s, C-7), 77.5 (d, C-5), 51.8 (d, C-4), 52.3 (q, C-23), 43.8 (t, C-20), 36.4 (d, C-3), 31.2 (t, C-8), 29.8 (q, C-22), 29.6−29.2 (t, C-10-C-18), 25.6 (t, C-9), 23.9 (t, C-19), 14.4 (q, C-6); EIMS (70 eV) *m*/*z* 382 $[M]^+$ (2), 367 (0.4), 351 (2), 339 (1), 323 (3), 307 (2), 293 (3), 279 (3), 275 (7), 251 (3), 95 (11), 81 (15), 69 (38), 55 (38), 43 (100); *anal.* C 68.99%, H 9.92%, calcd for $C_{22}H_{38}O_5$, C 69.08%, H 10.01%.

Calculation Methods and Procedures. The calculations were carried out in either a Sun SparcStation 10 workstation, or a Pentium II-based PC computer. Ab initio calculations were performed with the Gaussian 98 program (Revision A.3, Gaussian Inc., Pittsburgh, PA) at the HF/6-31G level. The polarizable continuum solvent model of Barone et al.[11] was also applied, using $CHCl_3$. MM3(92) (QCPE, Indiana University, Bloomington)[17] molecular mechanics calculations were used to test the 10 possible envelope conformations of **5**. The two final conformers were used as startpoints. Coupling constants were calculated with the Karplus equation using the parametrization of Hassnoot et al.[18]

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Supporting Information Available: Calculated geometries and relative energies for the conformers of **5** by molecular mechanics (MM3) and ab initio procedures. This material is available free of charge via the Internet at http://pubs.acs.org.

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