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α-(15-HYDROXYHEXADECYL)ITACONIC ACID FROM *USNEA ALIPHATICA*

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Key Word Index—*Usnea aliphatica*; Lichen; α-(15-hydroxyhexadecyl)itaconic acid; structural determination.

Abstract—The structure of a new aliphatic acid from *Usnea aliphatica* was established by spectroscopic and chemical methods as α-(15-hydroxyhexadecyl)itaconic acid.

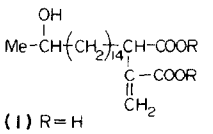
INTRODUCTION

The genus *Usnea*, comprising over 500 species and many intermediate forms, is well represented in the Venezuelan Andes above 1600 m. The role of chemistry in both the species-level and higher-level systematics of the lichens has already been discussed in detail [1–2]. As part of a chemotaxonomic study of the genus *Usnea* we have undertaken a phytochemical investigation of the Venezuelan species. A preliminary screening (TLC) showed the presence of a number of unidentified compounds and in this paper we report the structural elucidation of α (15-hydroxyhexadecyl)itaconic acid, a new aliphatic acid isolated from *Usnea aliphatica* Hale. The only other natural product of related structure is itaconic acid isolated from the submerged aerobic fermentation of carbohydrates by *Aspergillus terreus* [3].

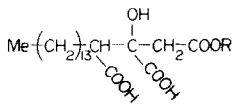
RESULTS AND DISCUSSION

TLC analysis of a fragment of the lichen thallus showed the presence of (**1**), usnic, norstictic, and salazinic acids. Extraction of the lichen with C_6H_6 afforded the aliphatic acid as optically active colourless crystals having a molecular formula of $C_{21}H_{38}O_5$ as determined by elemental analysis and MS. The solubility of the compound in $NaHCO_3$ and the formation of a Me ester on treatment with CH_2N_2 or $MeOH/H^+$ showed the compound to be a dicarboxylic acid. The presence of an OH group and a conjugated double bond was indicated by IR absorptions at 3480 and 1625 cm^{-1} respectively. Other absorptions at 2910, 2860, 1470 and 720 cm^{-1} were typical of an aliphatic chain. The PMR spectrum of (**1**) in Me_2CO-d_6 showed a doublet at δ 1.11 (J = 6 Hz) and a multiplet *ca* δ 3.75 (shifted to δ 4.92 in the acetate) characteristic of the 1-hydroxy-1-methylethyl group. Intense peaks at *m/e* 45 and 87 in the MS of the acid and its acetate, respectively, confirmed the presence of this function in the molecule. Two fine doublets at δ 5.82 and δ 6.35, (J = 1 Hz), which disappeared on reduction of (**1**) were assigned to a terminal —CH_2— group. The position of these signals and the formation of a pyrazoline on reaction of the compound with excess CH_2N_2 indicated that the double bond was conjugated with one of the carboxyl groups. The following reactions served to confirm the existence of the itaconic function in the molecule.

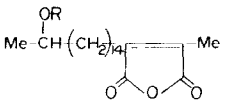
Treatment of (**1**) with Ac_2O/Py yielded the anhydride (**3**) as a colourless oil. The PMR spectrum showed a



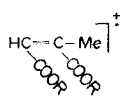
(**1**) R = H
(**2**) R = Me



(**5**) R = H



(**3**) R = Ac
(**4**) R = H



R = H *m/e* = 130
R = Me *m/e* = 158

multiplet at δ 4.92 and a singlet (3H) at δ 2.03 indicating that acetylation of the OH group had occurred. The absence of olefinic protons in the spectrum and the presence of IR absorptions (1850, 1820 and 1765 cm^{-1}), typical of an unsaturated cyclic anhydride [4], indicated that anhydride formation was accompanied by migration of the double bond. Other signals at δ 2.48 (t, 2H, J = 7 Hz) and δ 2.10 (s, 3H) were assigned to the vinylic CH_2 and Me groups, respectively. Pyrolysis of (**1**) gave the maleic anhydride derivative (**4**) whose PMR spectrum was similar to that of (**3**) showing signals for the 1-hydroxy-1-methylethyl function in place of those of the corresponding acetate. The spectrum of 2-methyl-3-tetradecyl maleic anhydride, prepared by pyrolysis of norcaperatic acid (**5**), showed the vinylic Me and CH_2 groups at δ 2.09 and δ 2.48, respectively. A comparison of these values and the IR spectrum (carbonyl region) with those of (**3**) and (**4**) confirmed the presence of the Me alkyl maleic anhydride group in the compounds, and thus confirmed the position of the two carboxyl groups in (**1**). The proton under the carboxyl of (1) appeared as a triplet at δ 3.53 (J = 7 Hz) partly overlapping with the multiplet at δ 3.75. The linear nature of the chain was indicated by the absence of a second Me in the PMR.

The high-resolution MS of (1) is consistent with the proposed structure and shows peaks which arise from the citraconic isomer as well as from (1) itself. A similar situation has been reported [5] for the MS of itaconic acid which showed fragments characteristic of mesaconic and citraconic acids. The highest ion recorded in the MS of (1) was M^+-H_2O–Me. The fragment at *m/e* 130 was produced by a McLafferty rearrangement of (1) and this in turn formed the ion at *m/e* 112 by loss of H_2O. An intense peak at *m/e* 280 probably arose from (1) by elimination of the OH group as H_2O followed by loss of a $C_3H_4O_2$ fragment. A similar fragmentation scheme has been proposed [5] to account for the ion at *m/e* 58 in the MS of itaconic acid. The base peak at *m/e* 126 was formed from the citraconic isomer by elimination of $2H_2O$ followed by a McLafferty rearrangment of the resulting *m/e* 334 ion. Loss of CO from the base ion gave *m/e* 98. The MS of the Me ester (2) showed an M^+ at *m/e* 398 and a base peak at *m/e* 157 which was formed by simple cleavage of the allylic C—C bond. The fragment at *m/e* 171 was formed in a similar manner from the citraconic isomer. A McLafferty rearrangement of (2) produced the fragment at *m/e* 158.

EXPERIMENTAL

Extraction and isolation. A preliminary analysis (Si gel HF_{254}; C_6H_6–dioxane–HOAc; 90:25:4) of a fragment of the thallus of *Usnea aliphatica* (MFK No 217 on deposit in the herbarium of the Facultad de Farmacia of this university and collected in San Eusebio, State of Merida) showed the presence of usnic, salazinic and norstictic acids as well as the aliphatic compound (1). Ground air-dried lichen (38 g) was succesively extracted with C_6H_6, Et_2O, and Me_2CO. On standing 18 hr, the C_6H_6 extract deposited a white solid which was recrystallised from HOAc–H_2O to afford (1) as colourless crystals, mp 77–79°. Found C, 68.25; H, 10.41%. Calc. for $C_{21}H_{38}O_5$ C, 68.10; H, 10.28%. γ_{max}^{KBr} (cm^{-1}): 3480, 3200–3040, 2910, 2860, 1680–1710, 1625, 1470, 1420, 1300, 1270, 1210, 1145, 1130, 1110, 1060, 1025, 1000, 970, 920, 850, 810, 755, 720, 675, 620. PMR 60 MHz (Me_2CO-d_6, δ): 1.11 (*d*, J = 6 Hz, 3H), 1.32 (CH_2XI4), 3.6 (*m*, 1H), 3.53 (*t*, 1H, J = 7 Hz), 5.82 (*d*, 1H, J = 1 Hz); 6.35 (*d*, 1H, J = 1H). MS, *m/e* (rel. int.): 337 (16), 334 (9), 308 (30), 290 (17), 289 (14), 280 (36), 263 (26), 182 (18), 168 (10), 154 (8), 151 (20), 150 (16), 140 (7), 139 (10), 130 (9), 126 ($C_6H_6O_3$, 100), 123 (16), 109 (35), 107 (14), 95 (73). Concn of the C_6H_6 extract afforded usnic acid identical (PMR, mmp) with an authentic sample. Extraction with Et_2O gave norstictic acid while extraction with Me_2CO yielded salazinic acid, both compounds being identified by comparison with authentic samples.

Pyrolysis of (1). The acid (25 mg) was maintained at 140° for 1 hr in a sublimation apparatus and then distilled onto a cold finger under red.press.to give (4) as a white solid, mp 40–42°. γ_{max}^{film} (cm^{-1}): 3460, 1855, 1820, 1765, 1670, 920, 730. PMR ($CDCl_3$, δ): 1.13 (half of doublet), 1.29 (CH_2XI3), 2.07 (s, 3H), 2.46 (*t*, 2H, J = 7 Hz), 3.78 (*m*, 1H). Found C, 71.42; H, 10.12%. Calc. for $C_{21}H_{36}O_4$ C, 71.59; H, 10.23%.

Dimethyl ester of (1). A soln of the acid (20 mg) in MeOH (2 ml) was refluxed in the presence of one drop of conc. H_2SO_4 for 6 hr. The reaction mixture was diluted with 5% $NaHCO_3$, extracted with Et_2O and the solvent evaporated to give the Me ester (2) as a colourless oil. γ_{max}^{film} (cm^{-1}): 3425, 1720, 1628, 1260, 1200, 1140, 950, 820, 720. PMR ($CDCl_3$, δ): 1.12 (half of doublet), 1.30 (CH_2XI4), 3.68 (*s*, 3H), 3.79 (*s*, 3H), 5.78 (*s*, H), 6.38 (*s*, 1H), 3.80 (*m*, 1H), 3.53 (*t*, 1H, J = Hz). MS (*m/e*): 398 (M^+), 383, 366, 351, 339, 322, 290, 263, 171, 158, 157 (100%), 149, 139, 126, 45.

Pyrolysis of norcaperatic acid. 60 mg compound prepared by hydrolysis of caperatic acid with 5% KOH, was maintained at 180° in a sublimation apparatus for 4 hr. The reaction mixture distilled at 150°/0.05 mm and the colourless liquid which condensed on the cold-finger dissolved in Et_2O. $NaHCO_3$ extraction of the soln followed by acidification gave a semi-solid which was resublimed to afford 2-methyl-3-tetradecyl-maleic anhydride, mp 27° (lit. [6] 27°). PMR ($CDCl_3$, δ): 0.88 (*t*, 3H), 1.29 (CH_2XI2), 2.09 (*s*, 3H), 2.48 (*t*, 2H, J = 7 Hz).

Reduction of (1). A soln of the acid (50 mg) in HOAc (3 ml) containing Pd/C (5%; 10 mg) was hydrogenated in a Parr apparatus at room temp and 50 kg for 4 hr to give the succinic acid derivative as a white solid, mp 66–68°, γ_{max}^{KBr} (cm^{-1}): 3480, 1690, 1470, 1420, 1190, 940, 720. PMR (Me_2CO-d_6, δ): 1.13 (*d*, J = 6Hz, 3H), 1.33 (CH_2XI4), 1.22 (*d*, J = 6 Hz, 3H), *ca* 2.67 (*m*, 2H), *ca* 3.65 (*m*, 1H).

Acetylation of (1). The acid (60 mg) was dissolved in a mixture of Py and Ac_2O (1:1, 4 ml) and maintained at room temp for 18 hr. Evaporation of the solvent afforded (3) as a colourless oil. γ_{max}^{film} (cm^{-1}): 2920, 2845, 1850, 1820, 1765, 1730, 1670, 1245, 1115, 1020, 920, 730. PMR ($CDCl_3$, δ): 1.16 (half of doublet), 1.30 (CH_2XI3), 2.03 (*s*, 3H), 2.1 (*s*, 3H), 2.48 (*t*, J = 7 Hz, 2H), 2.92 (*m*, 1H), MS (*m/e*): 394 (M^+), 379, 352, 351, 350, 289, 247, 233, 219, 205, 191, 126, 87, 61, 54.

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INHALTSSTOFFE SÜDAMERIKANISCHER *SENECIO*-ARTEN*

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