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STRUCTURAL ELUCIDATION OF 13-ACETOXYLICHESTERINIC AND 13-ACETOXYPROTOLICHESTERINIC ACIDS, TWO ALIPHATIC LICHEN METABOLITES FROM *NEUROPOGON TRACHYCARPUS*

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Key Word Index—*Neuropogon trachycarpus*; lichen; aliphatic acids.

Abstract—From the lichen *Neuropogon trachycarpus*, six aliphatic acids related to lichesterinic acid have been isolated: neuropogolic, murolic, isomuronic and muronic acids, as well as two new compounds, 13-acetoxylichesterinic [2-(13-acetoxytridecyl)-4-methyl-5-oxo-2,5-dihydro-furan-3-carboxylic acid] and 13-acetoxyprotolichesterinic acid [2-(13-acetoxytridecyl)-4-methylen-5-oxo-tetrahydro-furan-3-carboxylic acid]. The structures of these two acids were established by spectroscopic and chemical evidence.

INTRODUCTION

We earlier described the structural determination of isomuronic and neuropogolic acids from the lichen *Neuropogon trachycarpus* Stirt. where they occurred with the known usnic and norstitic acids [1]. Further studies on this same lichen enabled us to identify four other aliphatic acids, two of which are new: 13-acetoxylichesterinic acid **1** and 13-acetoxyprotolichesterinic acid **2**. The remaining two are murolic acid **6** isolated earlier by Huneck *et al.* [2] from *Lecanora muralis* and muronic acid **4** obtained by the same authors from the Jones oxidation of **6**. All these six long-chain acids are structurally related either to protolichesterinic acid **8** or to lichesterinic acid **7** which differ from each other in that former has an exocyclic double bond which in the latter is endocyclic.

RESULTS AND DISCUSSION

From the cyclohexane extract of *Neuropogon trachycarpus*, six compounds A–F crystallized, leaving a filtrate which contained usnic acid. These compounds were separated by TLC and examination of their spectroscopic parameters and some chemical reactions enabled us to determine their structures (see Table 1). Table 2 shows their physical properties.

Compounds B and E have been previously isolated [1] and assigned structures **3** and **5** respectively. Neuropogolic acid **5** has a planar structure identical to that of isomurolic acid which Huneck obtained [2] by isomerization of murolic acid, but also to that of constipatic acid isolated by Elix [3] from *Parmelia constipata* even though the latter has an inverse optical rotation $[\alpha]-24°$. Elix also isolated dehydroconstipatic acid from a *Parmelia* species and assigned it the same structure as that for isomuronic acid **3**, but with a negative optical activity and *S* configuration. Isomuronic acid obtained from *Neuropogon*, as well as that produced by isomerization of muronic acid has a positive optical rotation and thus has the *R* configuration on the only asymmetric centre.

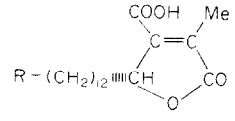
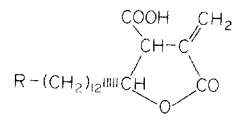
The products D and F were identified as muronic acid **4** and murolic acid **6**, respectively, in agreement with spectral data (IR, ^{13}C and ^{1}H NMR, mass spectroscopy) and by co-chromatography with authentic samples kindly provided by Huneck. Muronic acid (compound D) is easily isomerized to isomuronic acid by heating in Ac_2O under reflux. Compounds D and F are slowly transformed into **3** and **5** respectively when they are kept in solution for a long time.

Structure **1** is proposed for compound A on spectral and chemical evidence. HPMS showed the molecular ion at 382.235 corresponding to the formula $C_{21}H_{34}O_6$ which implies five unsaturated sites. Its IR spectrum displayed two intense carbonyl absorptions: the stronger at 1740 cm^{-1} (lactone and ester) and the other at 1705 cm^{-1}. An absorption at 1663 cm^{-1} was attributed to an olefinic group. The remaining unsaturation was assigned to the lactone ring.

The ^{1}H NMR spectrum of A was very similar to that of isomuronic acid **3**, with the same signals, but some chemical shift differences were observed. A sharp singlet (3H) at 2.08 ppm as well as a triplet (2H, $J = 6$ Hz) at 4.10 ppm in the spectrum of **1** replaced the signals at 2.17 (*s*, 3H) and 2.45 ppm (2H, *t*, $J = 7$ Hz) which corresponded to the acetonyl group in isomuronic acid.

Since the molecular formula of **1** has one oxygen more than that of isomuronic acid, the differences between the two metabolites could be due to a modification of the terminal side chain: the acetyl group in **3** must be replaced by an acetoxyl group in **1** for which the name 13-acetoxylichesterinic acid is proposed. This point was further corroborated by the mass spectrum of **1** which showed an abundant ion at

Table 1. Structures of aliphatic acids from *N. trachycarpus*

R		
Me	**7**	**8**
Me CHOH CH_2	**5**	**6**
Ac CH_2	**3**	**4**
Ac O CH_2	**1**	**2**

Table 2. Physical constants of the aliphatic acids

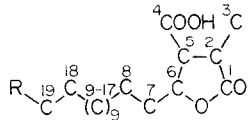
R_f		Compound	Structure	$[\alpha]_{589}^{D}(T)$	Concn ($CHCl_3$)	Mp (°)	(Solvent)
0.46	A	13-Acetoxylichesterinic acid	**1**	+18 (20°)	8.4×10^{-3}	98–99	(hexane)
0.43	B	Isomuronic acid	**3**	+24.7 (24°)	6×10^{-3}	108–109	(H_2O–Me_2CO)
0.40	C	13-Acetoxyprotolichesterinic acid	**2**			94–96	(H_2O–Me_2CO)
0.36	D	Muronic acid	**4**	+8.7 (20°)	8.5×10^{-3}	93–94	(H_2O–Me_2CO)
0.32	E	Neuropogolic acid	**5**	+22.4 (23°)	13×10^{-3}	111–112	(H_2O–Me_2CO)
0.30	F	Murolic acid	**6**			104–106	(H_2O–Me_2CO)

R_f are given for chromatography on Si plate (Merck 60F 254) in toluene–HOAc (17:3).

m/z 322 ($C_{19}H_{30}O_4$) arising from the terminal elimination of acetic acid from the molecular ion. Despite this structural difference, ion fragments concerning the remaining part of the molecule were similar to those observed in **3**.

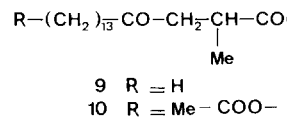
Further evidence in support of the proposed structure of 13-acetoxylichesterinic acid was obtained by comparing its ^{13}C NMR spectrum with that of isomuronic acid (Table 3). Slight modifications of the chemical shifts of the carbons of the common struc-

Table 3. ^{13}C NMR chemical shifts for compounds related to either lichesterinic or protolichesterinic acid



Carbon	**1**	**3**	**5**	**7**	**4**	**6**	**8**
1	173.0	172.3	172.5	172.8	173.5	173.3	174.4
2	139.2	139.0	138.5	140.3	133.1	133.3	132.6
3	10.4	10.5	10.5	11.0	125.6	125.5	125.9
4	165.3	165.1	164.5	167.0	168.5	168.7	168.2
5	147.4	146.6	146.9	146.9	50.0	49.7	49.6
6	81.5	81.0	81.0	81.5	79.2	79.3	78.9
7	32.3	32.2	32.1	32.8	35.8	35.7	35.8
8	24.6	24.2	24.0	24.8	24.8	24.7	24.8
9–17	29.4 29.1	29.1 28.8	29.4 28.9	29.4 31.9	29.2 29.4	28.1 29.4	29.2 29.4
18	24.6	23.4	25.1	22.7	24.0	25.6	22.7
19	60.6	43.4	38.6	14.1	44.0	39.0	14.0
20	174.7	210.1	68.4	—	210.3	68.7	—
21	30.9	29.1	22.8	—	29.1	23.3	—
R				H			H

The spectra were recorded in $CDCl_3$ at 20 MHz, chemical shifts (δ) from TMS.



9 R = H
10 R = Me–COO–

tural skeleton were within expected limits. The terminal methyl group of acetoxylichesterinic acid appeared at δ 30.9, while the $-CH_2-$ substituted by the acetoxy fragment was at δ 60.6 (instead of δ 29.1 and 43.4 respectively as is the case of isomuronic acid). A chemical shift value of δ 174.7 was also in good conformity with that of an ester carbonyl (C-20).

If the proposed structure of 13-acetoxylichesterinic acid is correct, alkaline hydrolysis will transform the acetoxy group into a hydroxyl function. However, it [4] has earlier been shown that under the same conditions, the lactone ring in lichesterinic acid is opened, followed by double bond migration giving an unstable β-ketonic acid intermediate which decarboxylates to give lichesterylic acid **9**. Under these conditions, the hydrolysis of 13-acetoxylichesterinic acid should give hydroxylichesterylic acid **10**. Effectively, the product obtained showed no optical activity and the IR spectrum showed intense bands at 3280 (ν_{OH}); 1710 and 1700 (ν_{CO}, ketone and acid); 1250 cm^{-1} (ν_{C-O}), which identified it as **10**. Its mass spectrum showed the molecular ion at *m/z* 314 and an intense M-18 peak due to the elimination of H_2O from the ionized molecule, a fact long established for long-chain alcohols [5]. The ketone carbonyl led to ions resulting from characteristic carbonyl fragmentation pathways analogous to that observed for lichesterylic acid. In the ^1H NMR spectrum, the methylene protons bearing the OH group appeared at δ 3.64 while the methine and the methylene protons α and β respectively to the COOH gave rise to a complex ABC system at 80 MHz which has been analysed at 250 MHz (cf. Experimental).

The compound corresponding to spot C has been identified as 13-acetoxyprotolichesterinic acid **2**. Its mass spectrum was identical to that of **1**, but its ^1H NMR spectrum though similar showed the absence of the vinyl methyl (CH_3–C=C–) doublet at δ 2.25 which was replaced by two ethylenic proton doublets (–C=CH_2–) at 6.05 (J = 2.7 Hz) and at 6.50 ppm (J = 3.2 Hz). Signals were also found at 3.65 (1H *dt*) and at 4.80 ppm (1H *dt*) which were present neither in 13-acetoxylichesterinic acid nor in lichesterinic acid, but in protolichesterinic acid. These values are characteristic of the methylene lactone group as earlier mentioned. Heating a solution of **2** in acetic anhydride, under reflux, led to isomerization of the carbon–carbon double bond from the *exo*-methylene position into the lactone ring thus yielding 13-acetoxylichesterinic acid **1**.

It is remarkable that these series of compounds related either to lichesterinic acid or to its isomer, protolichesterinic acid, have a variable oxidation state for the second last carbon of the alkyl chain. Isomuronic acid can be considered as being obtained from neuropogolic acid by oxidation of its secondary alcohol function into a ketone group, while a Bayer–Villiger oxidation of the former yields 13-acetoxylichesterinic acid. The same reasoning can be applied to the isomeric series.

EXPERIMENTAL

Well-crushed lichen (185 g) was continuously extracted with cyclohexane (2000 cm^3) under reflux in a Soxhlet for 6 hr. After cooling, this cyclohexane extract deposited microcrystals (2.5 g) which were filtered off. This crude material was chromatographed on TLC plastic plates (Kieselgel 60 F 254) in toluene–HOAc (17:3) leading to the isolation of **1–6**.

Neuropogolic acid (**5**). IR ν_{max}^{KBr} cm^{-1}: 3420, 3080, 2920, 2850, 1740, 1705, 1665, 1220, 720. ^1H NMR (80 MHz, $CDCl_3$): δ 1.20 (3H, *d*, J = 6 Hz, CH_3CH–), 1.29 [22H, *m*, $(-CH_2-)_{11}$], 1.52 (2H, *m*, CH_2CH_2CHO), 2.03 (2H, *m*, $-CHOHCH_2CH_2-$), 2.20 (3H, *d*, J = 1.7 Hz, $CH_3-C=$), 3.31 (2H, *s* large, OH), 3.81 [1H, *m*, $CH_3-CH(OH)-CH_2-$], 5.10 (1H, *m*, $-CH-O$). MS 70 eV, 200°, *m/z* (rel. int.): 368 (2), 367 (4), 366 (8), 353 (32), 350 (29), 332 (19), 324 (90), 307 (26), 279 (41), 261 (23), 168 (29), 155 (58), 142 (42), 139 (22), 123 (23), 111 (19), 109 (18), 98 (39), 83 (45), 57 (58), 55 (100), 45 (81), 43 (58), 41 (65).

Murolic acid (**6**). IR ν_{max}^{KBr} cm^{-1}: 3430, 3080, 2928, 2856, 1745, 1723, 1660, 1480, 1250, 720. ^1H NMR ($CDCl_3$): δ 1.18 (3H, *d*, J = 6 Hz, CH_3-CH-), 1.30 [22H, *m*, $-(CH_2)_{11}-$], 1.51 (2H, *m*, $-CH_2-CH_2-CHO-$), 3.23 (1H, *s* large, OH), 3.70 (1H, *dt*, J_d = 6 Hz, J_t = 3 Hz, $-HC-CO_2H$), 3.90 [1H, *m*, $CH_3-CH(OH)-CH_2-$], 4.88 (1H, *dt*, $J_d = J_t$ = 6 Hz, CH_2-CH-O), 6.01 (1H, *d*, J = 2.7 Hz), 6.47 (1H, *d*, J = 3.2 Hz, $C=CH_2$), 8.40 (1H, *s* large, COOH). MS 70 eV, 200°, *m/z* (rel. int.): 368 (3), 367 (4), 366 (9), 353 (38), 350 (29), 332 (16), 324 (82), 307 (17), 279 (43), 261 (17), 168 (24), 155 (72), 142 (41), 139 (19), 123 (21), 111 (21), 109 (21), 98 (40), 83 (55), 57 (55), 55 (100), 45 (97), 43 (67), 41 (72).

Isomuronic acid (**3**). IR ν_{max}^{KBr} cm^{-1}: 3080, 2920, 2850, 1740, 1715, 1705, 1665, 1475, 1430, 1215, 720. ^1H NMR ($CDCl_3$): δ 1.26 [24H, *m*, $(-CH_2-)_{12}$], 2.17 (3H, *s*, CH_3CO), 2.25 (3H, *d*, J = 2 Hz, $CH_3-C=$), 2.45 (2H, *t*, J = 7 Hz, $-COCH_2-CH_2-$), 5.13 (1H, *m*, CH_2-CH-O), 7.95 (1H, *s* large, COOH). MS 70 eV, 220°, *m/z* (rel. int.): 367 (7), 366 (28), 349 (6), 348 (25), 308 (25), 307 (15), 291 (6), 290 (11), 253 (18), 251 (11), 239 (18), 225 (24), 211 (18), 193 (6), 155 (57), 142 (12), 141 (11), 123 (14), 111 (14), 109 (20), 97 (31), 95 (35), 89 (17), 87 (29), 85 (31), 71 (49), 69 (62), 58 (61), 55 (71), 43 (100), 41 (31).

Muronic acid (**4**). $[\alpha]_D^{20°}$ + 8.7 (c 8.5 × 10^{-3}). IR ν_{max}^{KBr} cm^{-1}: 3080, 2928, 2856, 1745, 1723, 1660, 1460, 1250, 1220, 1165, 960, 815, 720. ^1H NMR ($CHCl_3$): δ 1.29 [24H, *m*, $-(CH_2)_{12}-$], 2.14 (3H, *s*, CH_3CO), 2.44 (2H, *t*, J = 6.5 Hz, $-CO-CH_2CH_2-$), 3.62 (1H, *dt*, J_d = 6 Hz, J_t = 3 Hz, $-HC-CO_2H$), 4.81 (1H, *dt*, $J_d = J_t$ = 6 Hz, $-CH_2-CH-O$), 6.01 (1H, *d*, J = 2.7 Hz), 6.46 (1H, *d*, J = 3.2 Hz, $C=CH_2$), 7.48 (1H, *s* large, COOH). MS 70 eV, 220°, *m/z* (rel. int.): 367 (5), 366 (23), 349 (8), 348 (28), 308 (21), 306 (10), 291 (5), 290 (10), 253 (15), 251 (8), 239 (15), 225 (18), 211 (13), 193 (10), 155 (35), 142 (8), 141 (8), 123 (8), 111 (8), 109 (10), 97 (18), 95 (20), 89 (10), 87 (23), 85 (18), 71 (25), 69 (35), 58 (38), 55 (50), 43 (100), 41 (40).

13-Acetoxylichesterinic acid (**1**). $[\alpha]_D^{20°}$ + 18° [c 8.4 × 10^{-3} ($CHCl_3$)], mp 98–99°, IR ν_{max}^{KBr} cm^{-1}: 3080, 2920, 2860, 1740, 1705, 1663, 1480, 1475, 1430, 1375, 1345, 1330, 1250, 1215, 1155, 1135, 1040, 1009, 980, 945, 880, 765, 760, 715, 705, 625. ^1H NMR ($CDCl_3$): δ 1.25 [24H, *m*, $-(CH_2)_{12}-$], 2.08 (3H, *s*, CH_3COO), 2.25 (3H, *d*, J = 2 Hz, $CH_3-C=$), 4.10 (2H, *t*, J =

6 Hz, $-O-CH_2-CH_2-$), 5.15 (1H, *tq*, $J_t = 6$ Hz, $J_q = 2$ Hz, $-CH-O-$), 8.38 (1H, *s* large, COOH). MS 70 eV, 180°, *m/z* (rel. int.): 383 (5), 382 (20)M^+, 364 (6), 340 (5), 339 (3), 322 (17), 309 (8), 304 (11), 277 (15), 239 (13), 225 (22), 211 (13), 209 (6), 207 (6), 206 (8), 193 (17), 192 (9), 181 (6), 179 (9), 178 (8), 168 (16), 155 (31), 142 (18), 125 (13), 123 (12), 111 (26), 109 (16), 97 (50), 95 (26), 83 (61), 81 (28), 69 (68), 67 (30), 57 (47), 55 (90), 43 (100), 41 (72).

13-*Acetoxyprotolichesterinic acid* (**2**). IR ν_{max}^{KBr} cm^{-1}: 3080, 2920, 2860, 1740, 1718, 1660, 1250. ^1H NMR ($CDCl_3$): δ 1.25 [24H, *m*, $(CH_2)_{12}$], 2.05 (3H, *s*, CH_3COO), 3.65 (1H, *dt*, $J_d = 6$ Hz, $J_t = 3$ Hz, $>CH-COOH$), 4.10 (2H, *t*, $J = 6$ Hz, $O-CH_2-CH_2$), 4.80 (1H, *dt*, $J_d = J_t = 6$ Hz, CH_2-CH-O), 6.05 (1H, *d*, $J = 2.7$ Hz) and 6.50 (1H, *d*, $J = 3.2$ Hz) ($>C=CH_2$), 8.40 (1H, *s* large, COOH). MS 70 eV, 220°, *m/z* (rel. int.): 383 (1.4), 382 (7) M^+, 364 (3), 340 (3), 339 (7), 322 (14) (M^+-CH_3COOH), 309 (7), 304 (12), 277 (17), 239 (10), 225 (9), 211 (17), 209 (10), 207 (7), 206 (10), 193 (17), 192 (10), 181 (14), 179 (10), 178 (21), 168 (14), 165 (24), 155 (34), 142 (12), 125 (14), 123 (14), 111 (17), 109 (17), 97 (34), 95 (28), 83 (45), 81 (31), 69 (69), 67 (58), 57 (55), 55 (83), 43 (100), 41 (52).

13-*Hydroxylichesterylic acid* (**10**). 20 mg of 13-acetoxylichesterinic acid in 10 ml EtOH and 20 ml 5% NaOH were added dropwise with stirring for 10 min. The reaction medium was heated under reflux for 4 hr, then cooled, acidified with dil. HCl and extracted with Et_2O (5 × 10 ml). The combined ethereal extracts were concentrated and chromatographed on Merck 0.2 mm Kieselgel 60 F 254 plastic TLC sheets using toluene–AcOH (17:3). The product (R_f: 0.38) was 13-hydroxylichesterylic acid. Mp 89–91°, Me_2CO. IR ν_{max}^{KBr} cm^{-1}: 3280, 2920, 2850, 1710, 1700, 1475, 1410, 1380, 1250, 1195, 1100, 1055, 1025, 934, 720. ^1H NMR ($CDCl_3$): δ 1.21 (3H, *d*, $J = 7$ Hz, $CH_3-CH<$), 1.26 [22H, *m*, $(-CH_2-)_{11}$], 2.41 (2H, *t*, $J = 7$ Hz, $-CH_2-CO-$), 2.40–3.00 [3H, *m*, $-CH_2-CH<$ (syst ABC)], 3.64 (2H, *t*, $J = 6.3$ Hz, $-CH_2OH$. MS 70 eV, 230°, *m/z* (rel. int.): 315 (1), 314 (4) M^+, 312 (1), 311 (3), 297 (13), 296 (36), 284 (12), 279 (7), 278 (10), 266 (7), 242 (13), 241 (54), 233 (12), 227 (23), 223 (15), 209 (48), 197 (15), 191 (50), 167 (34), 165 (46), 153 (52), 151 (27), 149 (42), 143 (83), 139 (77), 137 (75), 131 (94), 130 (98), 125 (99), 123 (63), 121 (79), 115 (96), 113 (94), 112 (100), 111 (96), 109 (85), 107 (65), 99 (83), 98 (83), 97 (96), 96 (79), 95 (90), 93 (69), 87 (96), 86 (85), 85 (85), 84 (92), 83 (83), 82 (85).

Lichesterylic acid (**9**) $C_{18}H_{34}O_3$. Lichesterinic acid (9 mg) subjected to the same conditions of saponification gave lichesterylic acid (6 mg). Mp 83°, $CHCl_3$ (lit. [6] 84°). IR ν_{max}^{KBr} cm^{-1}: 2960, 2920, 2850, 1710, 1700, 1475, 1410, 1260, 1250, 1205, 1090, 1050, 1005, 940, 930, 820, 720. ^1H NMR ($CDCl_3$, 250 MHz): δ 0.88 (3H, *t*, $J = 6$ Hz, CH_3-CH_2), 1.22 (3H, *d*, $J = 6.8$ Hz, CH_3-CH), 1.26 [20H, *m*, $(-CH_2-)_{10}$], 1.57 (2H, *m*, $-CH_2-CH_2-CO$), 2.42 (2H, *t*, $J = 6.2$ Hz, $-CH_2-CH_2-CO$), 2.49 (1H, *dd*, $J_{H_XH_A} = 5.0$ Hz, $J_{H_XH_B} = 17.5$ Hz, H_X), 2.88 (1H, *dd*, $J_{H_BH_X} = 17.5$ Hz, $J_{H_BH_A} = 7.6$ Hz, H_B), 2.99 (1H, *ddq*, $J_{H_AH_X} = 5.0$ Hz, $J_{H_AH_B} = 7.6$ Hz, $J_{H_AH_{Me}} = 6.8$ Hz, $-C(H_X)H_B-C(Me)H_A-$). MS 70 eV, 200°, *m/z* (rel. int.): 299 (7), 298 (35) M^+, 281 (11), 280 (13), 253 (9), 229 (13), 225 (6), 212 (17), 211 (100), 207 (12), 181 (8), 167 (9), 166 (9), 154 (11), 143 (17), 139 (14), 137 (12), 126 (15), 125 (17), 123 (16), 116 (14), 115 (17), 114 (12), 113 (18).

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