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ENT-TRACHYLOBANE DITERPENOIDS FROM THE LIVERWORT *MASTIGOPHORA DICLADOS*

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Key Word Index—*Mastigophora diclados*; Hepaticae; diterpenoid; trachylobane; *ent*-trachyloban-18-oic acid; *ent*-trachyloban-19-oic acid; 18-hydroxytrachyloban-19-oic acid.

Abstract—The Malaysian liverwort *Mastigophora diclados* afforded *ent*-trachyloban-18-oic acid, *ent*-trachyloban-19-oic acid and the novel *ent*-18-hydroxytrachyloban-19-oic acid. The compounds were identified using ^{1}H and ^{13}C NMR spectroscopy. © 1997 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Mastigophora diclados (Brid.) Nees is a primitive liverwort which is found in tropical Asiatic areas. It is common in the mountains of Malaysia and a previous study of material collected in East Malaysia yielded herbertane sesquiterpenoids such as α-herbertenol (**1**) and mastigophorene A (**2**) [1, 2]. We have now analysed the constituents of a collection of *M. diclados* made in West Malaysia and have isolated three trachylobane diterpenoids.

RESULTS AND DISCUSSION

Separation of the chloroform extract by CC and PTLC afforded none of the herbertane sesquiterpenoids which characterised *M. diclados* collected in East Malaysia [1, 2]. Instead, small amounts of three diterpenoids were isolated. From their chromatographic behaviour, all were acidic in nature. The two least polar compounds were isomeric (each $C_{20}H_{30}O_2$) and showed only a resonance due to a carboxyl group in the sp^2 region of their ^{13}C NMR spectrum. They were therefore pentacarbocyclic compounds. In addition, the appearance of signals due to three tertiary methyls and a tetrasubstituted cyclopropane ring in their ^{1}H and ^{13}C NMR spectra suggested that the compounds were trachylobanoic acids. Comparison of their physical data and those of their methyl esters with literature values identified the compounds as *ent*-trachyloban-18-oic acid (**3**) [3] and its C-4 epimer *ent*-trachyloban-19-oic acid (**4**) [4, 5].

The third compound, *ent*-18-hydroxytrachyloban-19-oic acid (**5**) $C_{20}H_{30}O_3$ (*m/z* 318.2187) also possessed a carboxylic acid group [ν_{max} 3500–2500 and 1698 cm^{-1}, δ_C 181.3 (*s*, COOH)] and two protons attached to a cylopropane ring [δ_H 0.57 (1H, *br d*, J = 7.7 Hz, H-12) and 0.81 (1H, *dd*, J = 3.1 and 7.7 Hz, H-13)] which were consistent with a trachylobanoic acid structure. ^{1}H and ^{13}C NMR resonances typical of the C-10 and C-16 methyl groups of a trachylobane [δ_H 1.12 (3H, *s*, H_3-17) and 0.91 (3H, *s*, H_3-20); δ_C 20.5 (*q*, C-17) and 12.7 (*q*, C-20)] were observed whilst the two other methyls had been oxidised to an acid (see above) and a hydroxymethyl group [δ_H 4.02 and 3.40 (each 1H, *d*, J = 10.5 Hz, H_2-18); δ_C 71.4 (*t*, C-18)]. Comparison of the ^{13}C NMR shifts for **5** with those of trachyloban-19-oic acid (**4**) revealed no major differences except for the resonances due to C-3, C-4, C-5, C-18 and C-19. This established both that the compound was a trachylobane and that it was C-18 and C-19 which were oxidised. C-4 and C-18 were both deshielded by the additional hydroxyl group whereas the carbons γ to the hydroxyl group (C-3, C-5 and C-19) showed the expected shielding. The C-4 stereochemistry was assigned initially by considering the chemical shift of C-20. In trachyloban-19-oic acid (**4**), C-20 resonates at δ_C 12.5 whereas in trachyloban-19-ol (**6**) C-20 is more deshielded and appears at δ_C 15.1 [5]. In this case, the chemical shift of C-20 (δ_C 12.7) suggested that C-19 was the carboxylic acid group whilst C-18 was the hydroxymethyl. In addition, methylation of **5** caused the expected shielding of H_3-20 ($\Delta\delta$ 0.11) in the methyl ester (**7**) [4]. Confirmation of the proposed structure and stereochemistry came from NOE difference spectroscopy. When the more deshielded methyl (H_3-17) was saturated, enhancements were observed for both of the cyclopropane protons whereas saturation of the other methyl group (H_3-20) showed a strong enhancement of H-14*S*.

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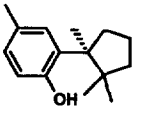

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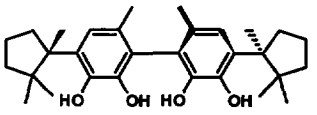

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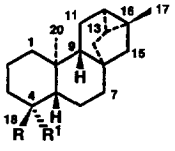
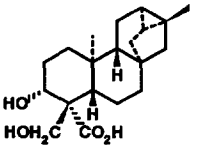


(3) R = CO_2H, R^1 = CH_3
(4) R = CH_3, R^1 = CO_2H
(5) R = CH_2OH, R^1 = CO_2H
(6) R = CH_3, R^1 = CH_2OH
(7) R = CH_2OH, R^1 = CO_2CH_3



(8)

Irradiation of the more shielded hydroxymethyl methylene proton (H-18*R*) enhanced the resonance assignable to H-5 [δ_H 1.14 (1H, *br d*, J = 10.4 Hz, H-5)] whilst an enhancement of H-6eq was observed upon irradiation of H-18*S*. The absolute configuration of **5** has not been determined but the compound is assumed to belong to the *enantio*-series of absolute configuration in common with the co-metabolites and other naturally-occurring trachylobanes [6, 7]. Trachylobanes are rarely found in Nature and only one example, *ent*-3β,18-dihydroxytrachyloban-19-oic acid (**8**), has been reported from a liverwort [8].

EXPERIMENTAL

General. Mps uncorr. Unless stated otherwise, the following conditions were used for spectroscopic and chromatographic analyses. CC: silica gel (40 μm particle size). Isocratic HPLC was carried out with RI detection. UV: EtOH. IR: CCl_4, EIMS: 70 eV. NMR: In $CDCl_3$ at 500 MHz (^{1}H) or 125 MHz (^{13}C) relative to TMS at δ = 0.00. ^{13}C multiplicities were determined using the DEPT pulse sequence and difference NOE experiments were carried out using the NOEMULT pulse program. Proton detected HMQC experiments were optimized for $^1J_{CH}$ = 140 Hz. The relaxation delay was 2.5 sec. In t_1, 512 increments were used with zero-filling to 1K before 2D Fourier transformation. In t_2, 2K points were used with no zero-filling. Gaussian multiplication was used in both dimensions to improve the signal to noise ratio and to suppress truncation errors.

Plant material. Mastigophora diclados was collected at an altitude of 1500 m on Gunung Reskit, Pahang, West Malaysia in 1993 and was identified by Prof. R. Grolle, University of Jena, Germany. A herbarium sample (MD1) is retained in the Chemistry Department, National University of Singapore.

Extraction and isolation. CC (silica, MeOH–$CHCl_3$ step gradient) of the $CHCl_3$ extract (2.4 g) from the air-dried, ground material (92 g) gave two diterpenoid-containing frs. The first fr. was passed through Sephadex LH-20 (MeOH–$CHCl_2$, 1:1) to give two crude compounds. The less polar compound was purified using PTLC (silica gel, EtOAc–hexane, 5:95) to give (**3**) (9.4 mg) whilst identical treatment of the more polar compound gave (**4**) (9.2 mg). The second column fr. yielded (**5**) (9.6 mg) after CC on Sephadex LH-20 (MeOH–$CHCl_3$, 1:1) and final HPLC purification (C-18, 70% Me_2CO–H_2O).

ent-*Trachyloban*-18-*oic acid* (**3**). Mp 132–134°, $[\alpha]_D$ −36° (*c* 0.9 in $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3200–2500 (COOH), 2910, 2845, 1697 (C=O), 1450, 1381, 1278 (C—O). HRMS: $[M]^+$ *m/z* 302.2231 ($C_{20}H_{30}O_2$ requires *m/z* 302.2246). EIMS *m/z* (rel. int.): 302 $[M]^+$ (7), 246 (13), 231 (20), 187 (16), 131 (24), 105 (38), 91 (100). ^{1}H NMR: δ 2.04 (1H, *br d*, J = 11.8 Hz, H-14), 1.88 (1H, *m*, H-11), 1.73 (1H, *dt*, J = 4.2 and 13.8 Hz, H-3ax), 1.66 (1H, *ddd*, J = 2.3, 7.2 and 12.8 Hz, H-11), 1.61 (1H, *m*, H-9), 1.58 (1H, *m*, H-3eq), 1.57 (1H, *m*, H-2), 1.45 (1H, *m*, H-6), 1.45 (1H, *m*, H-2), 1.45 (1H, *m*, H-1), 1.38 (1H, J = 11.3 Hz, H-15), 1.35 (1H, *m*, H-1), 1.26 (1H, *d*, J = 11.3 Hz, H-15), 1.21 (1H, *m*, H-5), 1.14 (1H, *m*, H-14), 1.14 (3H, *s*, H_3-19), 1.12 (3H, *s*, H_3-17), 0.97 (3H, *s*, H_3-20), 0.83 (1H, *m*, H-7), 0.81 (1H, *dd*, J = 3.1 and 7.7 Hz, H-13), 0.57 (1H, *br d*, J = 7.7 Hz, H-12). ^{13}C NMR: δ 185.0 (*s*, C-18), 53.2 (*d*, C-5), 50.3 (*t*, C-15), 50.2 (*d*, C-9), 47.2 (*s*, C-4), 40.9 (*s*, C-8), 38.4 (*t*, C-1), 38.3 (*t*, C-7), 37.6 (*s*, C-10), 37.0 (*t*, C-3), 33.5 (*t*, C-14), 24.2 (*d*, C-13), 23.0 (*t*, C-6), 22.5 (*s*, C-16), 20.5 (*d*, C-12), 20.5 (*q*, C-17), 19.6 (*t*, C-11), 17.2 (*t*, C-1), 16.2 (*q*, C-19), 14.9 (*q*, C-20).

Methyl ent-*trachyloban*-18-*oate*. Treatment of an ethereal soln of the corresponding acid with CH_2N_2 gave the methyl ester, mp 107–109° (MeOH) (lit. 110–112° [9]), $[\alpha]_D$ −39° (*c* 0.3 in $CHCl_3$) (lit. −41 [9]). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2924, 2862, 1726, 1244. HRMS: $[M]^+$ *m/z* 316.2414 ($C_{21}H_{32}O_2$ requires *m/z* 316.2402). EIMS : (rel. int.): 316 $[M]^+$ (62), 301 (44), 260 (100), 241 (56), 201 (33), 159 (39), 121 (66), 105 (93), 91 (66). ^{1}H NMR: 3.63 (3H, *s*, COOMe), 2.03 (1H, *d*, J = 11.7 Hz, H-14), 1.88 (1H, *ddd*, J = 3.1, 11.3 and 14.5 Hz, H-11), 1.13 and 1.12 (each 3H, *s*, H_3-17 and H_3-19), 0.96 (3H, *s*, H_3-20), 0.81 (1H, *dd*, 4.4 and 7.7 Hz, H-13), 0.57 (1H, *d*, 7.7 Hz, H-12).

ent-*Trachyloban*-19-*oic acid* (**4**). Mp 125–127° (lit. 126–129° [10]). $[\alpha]_D$ −52° (*c* 0.8 in $CHCl_3$), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3200–2500 (COOH), 2910, 2845, 1697 (C=O), 1450, 1381, 1278 (C—O). HRMS: $[M]^+$ *m/z* 302.2236 ($C_{20}H_{30}O_2$ requires *m/z* 302.2246). EIMS *m/z* (rel. int.): 302 $[M]^+$ (7), 246 (13), 231 (20), 187 (16), 131 (24), 105 (38), 91 (100). ^{1}H NMR: comparable with lit. values [4]. ^{13}C NMR: see Table 1.

Methyl ent-*trachyloban*-19-*oate*. Methylation of *ent*-trachyloban-19-oic acid with ethereal CH_2N_2 gave the methyl ester, mp 95–96° (lit. 98–100° [10]), $[\alpha]_D$ −61° (*c* 0.9 in $CHCl_3$) (lit. −70.5 [11]). IR, MS, ^{1}H and ^{13}C NMR comparable with lit. values [4, 5].

Table 1. NMR shifts (^{1}H: 500 MHz; ^{13}C: 125 MHz; $CDCl_3$ for *ent*-trachyloban-19-oic acid (**4**) and *ent*-18-hydroxy-trachyloban-19-oic acid (**5**) (*J* in parentheses)

Position	δ_H (**5**)	δ_C (**5**)	δ_C (**4**)
1	1.31 *m*	38.8	39.5
	1.41 *m*		
2	1.44 *m*	18.0	18.7
	1.47 *m*		
3ax	1.05 *dt* (4.4, 13.3)	32.1	37.8
3eq	2.32 *br d* (13.3)		
4		49.6	43.7
5	1.14 *br d* (10.4)	51.6	57.0
6	1.63 *m*	21.6	21.8
	1.66 *m*		
7ax	0.77 *dt* (4.0, 13.2)	38.9	39.2
7eq	1.58 *br d* (13.2)		
8		40.4	40.8
9	1.09 *m*	52.7	52.2
10		38.6	38.9
11	1.64 *m*	19.8	19.7
	1.89 *m*		
12	0.58 *br d* (7.5)	20.5	20.6
13	0.82 *dd* (3.1, 7.5)	24.2	24.3
14*R*	1.18 *br d* (11.0)	33.1	33.1
14*S*	2.03 *br d* (11.0)		
15	1.22 *d* (11.3)	50.3	50.4
	1.38 *d* (11.3)		
16		22.4	22.4
17	1.12 *s*	20.5	20.6
18*R*	3.40 *d* (10.5)	71.4	28.9
18*S*	4.02 *d* (10.5)		
19		181.3	184.7
20	0.91 *s*	12.7	12.5

^{1}H NMR assignments were made with the assistance of HMQC spectroscopy.

ent-18-*Hydroxytrachyloban*-19-*oic acid* (**5**). Mp 180–181.5°, $[\alpha]_D$ −57° (*c* 0.9 in $CHCl_3$). IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3580, 3500–2500, 2905, 2845, 1698, 1440, 1259, 1050. HRMS: $[M]^+$ *m/z* 318.2187 ($C_{20}H_{30}O_3$ requires *m/z* 318.2194); EIMS *m/z* (rel. int.): 318 $[M]^+$ (7), 285, (13), 270 (21), 244 (18), 232 (12), 133 (54), 119 (59), 105 (87), 91 (100). ^{1}H and ^{13}C NMR: see Table 1.

Methyl ent-18-*hydroxytrachyloban*-19-*oate* (**7**). Methylation of the acid (**5**) with ethereal CH_2N_2 gave the corresponding methyl ester (**7**) as a gum which could not be induced to crystallise, $[\alpha]_D$ −44° (*c* 0.2 in $CHCl_3$), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 2927, 2856, 1728, 1460, 1373, 1163, 1052. HRMS: $[M]^+$ *m/z* 332.2370 ($C_{21}H_{32}O_3$ requires *m/z* 332.2351). EIMS *m/z* (rel. int.): 332 $[M]^+$ (32), 314 (27), 302 (28), 276 (46), 255 (30), 241 (25), 187 (30), 147 (52), 91 (100). ^{1}H NMR: 3.95 (1H, *d*, *J* = 9.9 Hz), 3.68 (3H, *s*, OCH_3), 3.41 (1H, *d*, *J* = 9.9 Hz), 2.30 (1H, *br d*, *J* = 13.3 Hz), 2.01 (1H, *d*, *J* = 11.7 Hz), 1.12 (3H, *s*, H_3-17), 0.82 (1H, *dd*, *J* = 3.2 and 7.5 Hz, H-13), 0.80 (3H, *s*, H_3-20), 0.57 (1H, *br d*, *J* = 7.5 Hz, H-12). ^{13}C NMR: 176.1 (*s*, C-19), 71.3 (*t*, C-18), 52.7 (*d*, C-9), 51.6 (*d*, C-5), 51.4 (*q*, OCH_3), 50.3 (*t*, C-15), 49.8 (*s*, C-4), 40.5 (*s*, C-8), 39.0 (*t*, C-1), 38.9 (*t*, C-7), 38.4 (*s*, C-10), 33.1 (*t*, C-14), 32.2 (*t*, C-3), 24.2 (*d*, C-13), 22.4 (*s*, C-16), 21.7 (*t*, C-6), 20.5 (*d*, C-12), 20.5 (*q*, C-17), 19.8 (*t*, C-11), 18.2 (*t*, C-2), 12.6 (*q*, C-20).

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