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3α,18-DIHYDROXYTRACHYLOBAN-19-OIC ACID FROM THE LIVERWORT *JUNGERMANNIA EXSERTIFOLIA* SUBSP. *CORDIFOLIA*

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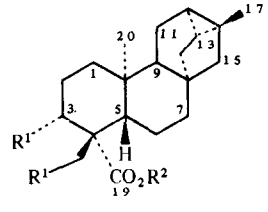
Key Word Index—*Jungermannia exsertifolia* subsp. *cordifolia*; Hepaticae; diterpenoids; 3α,18-dihydroxytrachyloban-19-oic acid.

Abstract—Extraction of the liverwort *Jungermannia exsertifolia* subsp. *cordifolia* afforded 3α,18-dihydroxytrachyloban-19-oic acid, isolated as its diacetate methyl ester.

INTRODUCTION

Although a number of *Jungermannia* species within the Hepaticae have been shown to contain diterpenoids, little work has been done on *Jungermannia exsertifolia* Steph. subsp. *cordifolia* (Dum.) Váňa apart from studies of the mono- and sesquiterpene hydrocarbons which are present [1–3]. We have now investigated the polar constituents of this species and have isolated a novel trachylobane diterpenoid, 3α,18-dihydroxytrachyloban-19-oic acid (**1**), as its diacetate methyl ester.

The liverwort *Jungermannia exsertifolia* Steph. subsp. *cordifolia*, collected in Wales, U.K., was extracted with diethyl ether. Column chromatography of the crude extract, followed by methylation and acetylation of a polar fraction which was further purified by preparative thin layer chromatography, yielded a novel diterpenoid, methyl 3α,18-diacetoxytrachyloban-19-oate (**2**), $C_{25}H_{36}O_6$ (*m/z* 432.2518), $[\alpha]_D$ −37.1°, $\nu_{max}^{CCl_4}$ cm^{-1}: 1755, 1740 and 1725. ^{1}H and ^{13}C NMR spectroscopy revealed the presence of a carbomethoxy group [δ_H 3.69 (3H, *s*, OMe); δ_C 51.40 (*q*, OMe) and 171.55 (*s*, C-19)], two acetates [δ_H 2.02 and 2.05 (both 3H, *s*); δ_C 20.83 (*q*), 21.25 (*q*), 170.52 (*s*), and 170.66 (*s*)], one of which is secondary [δ_H 4.81 (*dd*, *J* = 4.5 and 11.9 Hz, H-3)] and the other primary [δ_H 4.24 (*s*, 2H-18)], a cyclopropane ring [δ_H 0.82 (*dd*, *J* = 2.9 and 7.8 Hz, H-13) and 0.59 (*br d*, *J* = 7.8 Hz, H-12); δ_C 20.37 (*d*, C-12), 22.45 (*s*, C-16), and 24.09 (*d*, C-13)], and two tertiary methyl groups [δ_H 1.13 (*s*, 3H-17) and 0.86 (*s*, 3H-20); δ_C 12.64 (*q*, C-20) and 20.45 (*q*, C-17)] which, together with seven methylenes, two methines, and three fully-substituted carbon atoms, constitute a pentacarbocyclic system. These data, in particular the presence of a tetrasubstituted cyclopropane, suggest that the compound is a diterpenoid of the trachylobane class. Comparison of the ^{13}C shifts with published values [4] for methyl trachyloban-19-oate (**3**) (see Table 1) indicates that the oxidzed methyl groups are C-18 and C-19 and that the secondary acetate is at C-3. The values of the couplings of H-3 indicate that the secondary acetate group is equatorial. The relative stereochemistry at C-4 was determined by the use of NOE difference spectroscopy. Saturation of the oxygenated methylene resonance (2H-18) enhanced the signals due to H-3 (4.3%) and H-6eq (9.7%). Models indicate that the acetoxymethyl group must be equatorial to allow these effects. Thus, compound **2** is methyl 3α,18-diacetoxytrachyloban-19-oate and the original natural product must be 3α,18-dihydroxytrachyloban-19-oic acid (**1**). Treatment of **2** with potassium carbonate–methanol afforded the corresponding diol (**4**), the spectroscopic values of which are in accord with the proposed structure. Thus the ^{1}H NMR resonances of H-3 and 2H-18 have moved upfield [4.81→3.49 and 4.24→4.17 and 3.63 (ABq), respectively]. The seemingly anomalous deshielding of C-3 and C-18 which occurs upon deacetylation of **2** (Table 1) is an effect of hydrogen bonding and has previously been observed in the case of triterpenoids which possess analogous diol groupings [5]. The diol was esterified with *p*-bromobenzoyl chloride to give the bis-bromobenzoate (**5**). However this could not be induced to crystallize in a form suitable for X-ray crystallographic analysis. Although the chirality of dibenzoates can be determined by the exciton



1 R^1 = OH, R^2 = H
2 R^1 = OAc, R^2 = Me
3 R^1 = H, R^2 = Me
4 R^1 = OH, R^2 = Me
5 R^1 = $O_2CC_6H_4Br$, R^2 = Me

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Table 1. ^{13}C Chemical shifts of trachylobanes

C	2	3*	4
1	37.11	39.5	37.86
2	23.25	18.8	27.84
3	73.16	38.1	79.38
4	51.84	43.7	53.28
5	49.18	57.0	50.61
6	21.33	21.8	21.62
7	38.48	39.3	38.59
8	40.35	40.8	40.36
9	52.75	52.7	52.47
10	38.04	38.6	38.00
11	19.85	19.7	19.83
12	20.37	20.5	20.38
13	24.09	24.2	24.08
14	33.05	33.1	32.97
15	50.00	50.4	50.00
16	22.45	22.4	22.41
17	20.45	20.5	20.41
18	62.46	28.7	71.32
19	171.55	177.8	176.09
20	12.64	12.3	12.58
OMe	51.40	51.0	51.55
OAc	170.66		
	170.52		
	20.83		
	21.25		

Measured at 100.5 MHz in $CDCl_3$ solution. Chemical shifts in ppm relative to TMS at δ_c 0.00. Multiplicities were determined using the INEPT pulse sequence.

*Taken from ref. [4].

chirality method, in the case of **5** the conformation cannot be accurately predicted and this method is not applicable [6]. Thus, the absolute configuration of **1** remains undetermined.

This is the first example of a trachylobane from the Hepaticae. Studies of other *Jungermannia* species [7] have led to the isolation of diterpenoids belonging to the labdane, pimarane and kaurane classes which are biogenetically related to the trachylobanes. However, not enough *Jungermannia* species have been studied for a clear picture of the characteristic metabolites of this large genus to have emerged.

EXPERIMENTAL

IR spectra were determined for CCl_4 solns. Optical rotations were measured in $CHCl_3$. NMR spectra were recorded for $CDCl_3$ solns (^1H, 400 MHz; ^{13}C, 100.5 MHz). Chemical shifts were measured relative to TMS at δ0.00. CC was performed using Kieselgel 60 (70–230 mesh, Merck) and prep TLC was carried out on precoated Kieselgel 60 GF_{254} plates (Merck) of 0.5 mm thickness. Organic solutions were dried over $MgSO_4$.

Extraction and isolation. *J. exsertifolia* ssp. *cordifolia* (427 g) was collected in the Snowdonia National Park, Wales in August 1978. Extraction with Et_2O afforded a crude extract (11.2 g) which was chromatographed over a column of silica gel using an EtOAc–hexane gradient. The fraction which was eluted by EtOAc was acetylated and methylated using Ac_2O–pyridine and ethereal CH_2N_2, respectively. Prep. TLC (5% EtOAc–C_6H_6) then afforded methyl 3α,18-diacetoxytrachyloban-19-oate (**2**) (41 mg) as an oil, MS: *m/z* 432.2518 ($C_{25}H_{36}O_6$ requires *m/z* 432.2512), $[\alpha]_D$ −37.1° (*c* 0.34), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 1755, 1740 and 1701; ^1H NMR: δ 4.78 (*dd*, *J* = 4.8 and 12.1 Hz, H-3), 4.24 (*s*, 2H-18), 3.69 (*s*, OMe), 2.05 and 2.02 (each *s*, AcO), 1.13 (*s*, 3H-17), 0.86 (*s*, 3H-20), 0.82 (*dd*, *J* = 2.9 and 7.8 Hz, H-13), and 0.59 (*br d*, *J* = 7.8 Hz, H-12); ^{13}C NMR: see Table 1.

Methanolysis of the diacetate. To a stirred soln of **2** (35 mg) in dry MeOH (2 ml) was added dry K_2CO_3 (50 mg). Stirring was continued overnight, after which the solvent was removed. The residue was dissolved in EtOAc, washed with H_2O, dried and evapd to afford the crude product. This was purified by prep TLC (15% EtOAc–C_6H_6) to give the diol (**4**), (22 mg), mp 134–135° (from hexane), MS: *m/z* 348.2332 ($C_{21}H_{32}O_4$ requires *m/z* 348.2301), $[\alpha]_D$ −60.8° (*c* 0.75), IR $\nu_{max}^{CCl_4}$ cm^{-1}: 3540 and 1701; ^1H NMR: δ 4.17 and 3.63 (AB*q*, J_{AB} = 10.7 Hz, 2H-18), 3.72 (*s*, OMe), 3.49 (*dd*, *J* = 4.7 and 12.1 Hz, H-3), 2.05 (*dq*, *J* = 3.9 and 13.7 Hz, H-2*ax*), 1.10 (*s*, 3H-17), 0.80 (3H-20), and 0.56 (*br d*, *J* = 11.7 Hz, H-12); ^{13}C NMR: see Table 1.

Bromobenzoylation of the diol. The diol **4** (15 mg) and freshly recrystallized *p*-bromobenzoyl chloride (50 mg) were dissolved in dry pyridine (0.5 ml). After 24 hr, the mixture was dissolved in Et_2O, washed with aq. $CuSO_4$ and brine, and dried. The residue was purified by prep TLC (5% EtOAc–C_6H_6) to give the bis-bromobenzoate **5** (22 mg), mp 125–127°, ^1H NMR: δ 8.50 and 7.95 (4H, A_2B_2 system, J_{AB} = 8.8 Hz), 8.45 and 7.80 (4H, A_2B_2 system, J_{AB} = 9.1 Hz), 4.97 (*dd*, *J* = 4.6 and 13.5 Hz, H-3), 4.32 (*s*, 2H-18), 3.70 (*s*, OMe), 1.15 (*s*, 3H-17) and 0.85 (*s*, 3H-20).

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