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(−)-*ENT*-12β-ACETOXYLONGIPIN-2(10)-EN-3-ONE, AN *ENT*-LONGIPINANE DERIVATIVE FROM THE LIVERWORT *MARSUPELLA AQUATICA*

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Key Word Index—*Marsupella aquatica*; Hepaticae; sesquiterpenoid; (−)-*ent*-12β-acetoxylongipin-2(10)-en-3-one.

Abstract—The structure of a new *ent*-longipinane type of sesquiterpenoid, (−)-*ent*-12β-acetoxylongipin-2(10)-en-3-one from the liverwort *Marsupella aquatica*, has been elucidated by spectroscopic and chemical methods.

INTRODUCTION

In the course of our investigations on the chemistry of liverworts we have analysed *Marsupella aquatica* (Lindenb.) Schiffn. (family Marsupellaceae) and isolated a new *ent*-longipinane derivative, (−)-*ent*-12β-acetoxylongipin-2(10)-en-3-one (**1**), the structural elucidation of which is described in this paper. Recently, Matsuo *et al.* [1] found, in the closely related species *Marsupella emarginata* (Ehrh.) Dum. ssp. *tubulosa* (Steph.) N. Kitag., three *ent*-longipinane sesquiterpenoids, (−)-marsupellone (**2**), (+)-marsupellol (**3**) and an isomer of **1**, (+)-9-acetoxymarsupellone (**4**).

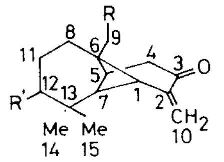
RESULTS AND DISCUSSION

Extraction of the air-dried and ground liverwort with Et_2O and subsequent chromatography of the green oily extract gave, besides oily fractions, (−)-*ent*-12β-acetoxylongipin-2(10)-en-3-one (**1**) in cubes, mp 95–96°, $[\alpha]_D^{24}$ − 17.1°. The MS of **1** has an M^+ peak at *m*/*z* 276, corresponding to the formula $C_{17}H_{24}O_3$, and an intensive peak at *m*/*z* 234 $[M - CH_2{=}C{=}O]^+$ formed by loss of an acetoxyl group. The UV spectrum has a maximum at 243 nm (log ε 3.72), typical for an α-methylene ketone. The presence of both these functional groups is confirmed by bands in the IR spectrum at 945, 1628, 1710 ($C{=}CH_2$—CO—) and 1735 cm^{-1} (−OAc). The ^1H NMR spectrum (360 MHz, $CDCl_3$, 25°, TMS as internal standard assuming $CHCl_3$ 7.250, digital resolution 0.244 Hz/data point) of **1** also indicates the presence of these functional groups and reveals that the OAc group is secondary: 0.783 (3H, *s*, Me-9), 0.928 and 0.978 (each 3H, *s*, Me-14 and Me-15), 1.441 (1H, *s*, H-7), 1.75 (3H, *m*, H_2-8, H-11), 1.96 (1H, *m*, includes *J* = 10.4 Hz, H-11), 2.071 (3H, *s*, OAc), 2.306 (1H, *ddd*, *J* = 2.7, 3.2 and 7.0 Hz, H-5), 2.487 (1H, *dd*, *J* = 3.2 and 19.0 Hz, H-4), 2.720 (1H, *ddd*, *J* = 0.5, 2.7 and 19.0 Hz, H-4), 2.985 (1H, *d*. *J* = 7.0 Hz, H-1), 4.781 (1H, *dd*, *J* = 1.3 and 10.4 Hz, H-12), 4.986 (1H, *d*, *J* = 1.6, H-10), 5.923 (1H, *d*, *J* = 1.6, H-10). The ^{13}C NMR data of **1** and **2** are in excellent agreement (Table 1) and support the attachment of the acetate group to C-12. Thus C-11 and C-13 show characteristic downfield shifts while C-8, C-15 and, to a much smaller extent, C-14 and C-7 move upfield in **1** with respect to **2** on the introduction of the acetate function. The β configuration of the acetate follows from the large (7.5 ppm) shift of C-15. The CD spectra of **1** and **2** are very similar and suggest that both sesquiterpenoids have the same absolute configuration as shown in structures **1** and **2**.

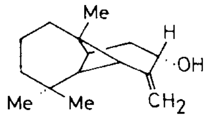
Hydrogenation of **1** with Pd–C gives (−)-*ent*-12β-acetoxylongipinan-3-one (**5**). On steric grounds, addition of hydrogen should occur from the α-face of the molecule resulting in the formation of a β-10-Me group. Saponification of **5** followed by oxidation of the resulting alcohol **6** yields the diketone **7** which gives the hydrocarbon (−)-*ent*-longipinane (**8**) on Wolff–Kishner reduction. It is interesting to note that the diketone **7** has a very pleasant odour resembling that of grimaldone from

Table 1. ^{13}C NMR data of compounds **1**, **2** and **11**

Carbon	**1**	**2**	**11**
1	46.0	47.8	46.1
2	150.0	150.5	106.6
3	200.3	200.6	208.2
4	44.7	44.7	46.1
5	37.8	37.3	37.4
6	42.5	42.5	41.0
7	58.7	59.3	56.1
8	36.4	39.4	36.7
9	22.8	23.1	23.4
10	116.6	115.4	29.5
11	26.6	21.4	26.7
12	78.8	41.4	78.6
13	36.4	33.1	36.1
14	26.6	27.5	26.6
15	20.3	27.8	19.7
$\underline{C}H_3$—CO—	21.3		21.2
CH_3—$\underline{C}$O—	170.6		170.5
3′			78.9



1 R = H, R' = OAc
2 R = R' = H
4 R = OAc, R' = H
9 R = H, R' = OH



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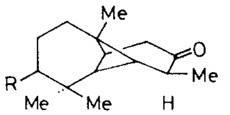
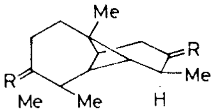
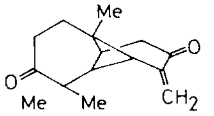


5 R = OAc
6 R = OH



7 R = O
8 R = H_2



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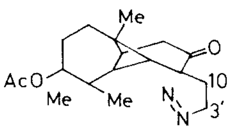

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the liverwort *Mannia fragrans* (Balbis) Frye et Clark [2]. On treatment of **7** with CD_3OD in the presence of CD_3ONa five atoms of deuterium are incorporated; this is further evidence for the 12-position of the acetoxyl group in **1**. Hydrolysis of **1** gives (−)-*ent*-12β-hydroxylongipin-2(10)-en-3-one (**9**) which is oxidized to (−)-*ent*-longipin-2(10)-3,12-dione (**10**) with Jones' reagent. Addition of CH_2N_2 to **1** gives the spiro-pyrazoline **11** whose ORD curve supports the configuration shown [3].

The occurrence of (−)-*ent*-12β-acetoxylongipin-2(10)-en-3-one in *M. aquatica* confirms the close relationship of this species and *M. emarginata*. Furthermore, it seems that *ent*-longipinanes of the marsupellone type are chemical markers of the family *Marsupellaceae*.

EXPERIMENTAL

Extraction of M. aquatica. Air-dried and ground material (319 g), collected and identified by PD Dr. R. Mues in the Dombach near Zysch, Hunsrück Mountains, Federal Republic of Germany, 11.10.1979 (voucher specimen in the herbarium of S.H.), was extracted with Et_2O for 3 days. The Et_2O was removed *in vacuo* and the resulting dark green oil chromatographed on Si gel (with 5% H_2O) using a *n*-hexane–Et_2O gradient to yield six fractions. The first five fractions gave oils, the analysis of which is under way. Fraction 6, eluted with *n*-hexane–Et_2O (4:1) (500 ml) solidified after some hours and was twice recrystallized from *n*-hexane to yield (−)-ent-12β-*acetoxylongipin*-2(10)-*en*-3-*one* (**1**) (1.44 g) in cubes, mp 95–96°, $[\alpha]_D^{24}$ − 17.1° ($CHCl_3$, *c* 4.90) and R_f 0.37 (*n*-hexane–Et_2O–HCO_2H (20:12:3), detection with AcOH + SO_3HCl at 150°: brown-violet spot). $C_{17}H_{24}O_3$ (276). MS *m/z* (rel. int.): 276.1726 ($[M]^+$, calc. 276.1725) (52), 261 $[M - Me]^+$ (6), 258 (8), 248 (6), 234 $[M - CH_2{=}C{=}O]^+$ (80), 219 (40), 216 $[M - AcOH]^+$ (77), 206 $[M - CH_2{=}C{=}O - CO]^+$ (27), 201 $[M - CH_2{=}C{=}O - H_2O - Me]^+$ (63), 190 (88), 173 (69), 165 (53), 160 (56), 148 (100), 134 (62), 127 (73), 123 (77), 109 (60), 108 (64), 107 (69), 95 (61); IR $\nu_{max}^{CCl_4}$, cm^{-1}: 910, 920, 945, 970, 990, 1005, 1020, 1040, 1106, 1142, 1164, 1230, 1280, 1322, 1370, 1378, 1408, 1432, 1452, 1465, 1628, 1710, 1735, 2950. CD (*n*-heptane): $[\theta]_{197}$ − 2690, $[\theta]_{202}$ − 4040, $[\theta]_{221}$ 0, $[\theta]_{240}$ − 2500, $[\theta]_{275}$ 0; $[\theta]_{299}$ + 120, $[\theta]_{310}$ + 270, $[\theta]_{322}$ + 480, $[\theta]_{335}$ + 700, $[\theta]_{349.5}$ + 700, $[\theta]_{365.5}$ + 430, $[\theta]_{380}$ + 130, $[\theta]_{395}$ 0; ORD (MeOH): $[\alpha]_{270}$ 0°, $[\alpha]_{308}$ − 180°, $[\alpha]_{348}$ 0°, $[\alpha]_{368}$ + 30°, $[\alpha]_{390}$ 0°, $[\alpha]_{440}$ − 20°.

(−)-Ent-12β-*acetoxylongipinan*-3-*one* (**5**). **1** (0.3 g) was hydrogenated with 5% Pd–C (60 mg) in EtOH (15 ml) under normal conditions for 2 hr. The usual work-up and crystallization from MeOH afforded **5** as plates, mp 82–83° and $[\alpha]_D^{24}$ − 33.8° ($CHCl_3$, *c* 2.18). $C_{17}H_{26}O_3$ (278). MS *m/z* (rel. int.): 278 $[M]^+$ (22), 236 $[M - CH_2{=}C{=}O]^+$ (24), 218 $[M - AcOH]^+$ (86), 203 (24), 190 (48), 175 (51), 167 (59), 161 (43), 153 (74), 149 (96), 147 (65), 135 (68), 125 (57), 122 (87), 109 (84), 108 (72), 107 (81), 95 (100), 93 (83); IR ν_{max}^{KBr}, cm^{-1}: 840, 872, 906, 910, 960, 980, 1016, 1030, 1092, 1006, 1124, 1138, 1166, 1242, 1314, 1368, 1380, 1418, 1440, 1460, 1700, 1714, 2990, 3500; ORD (MeOH): $[\alpha]_{222}$ 0°, $[\alpha]_{232}$ − 65°, $[\alpha]_{244}$ 0°, $[\alpha]_{272}$ + 132°, $[\alpha]_{290}$ 0°, $[\alpha]_{316}$ − 260°, $[\alpha]_{354}$ − 100°, $[\alpha]_{400}$ − 60°.

(−)-Ent-12β-*hydroxylongipinan*-3-*one* (**6**). A soln of **5** (0.18 g) in MeOH (5 ml) was refluxed with KOH (0.5 g) for 1 hr. The usual work-up yielded an oil which crystallized after addition of a few drops of *n*-pentane. Recrystallization from MeOH–H_2O gave **6** as needles, mp 92–94° and $[\alpha]_D^{24}$ − 66.5° ($CHCl_3$, *c* 1.66). $C_{15}H_{24}O_2$ (236). MS *m/z* (rel. int.): 236 $[M]^+$ (78), 221 $[M - Me]^+$ (32), 218 $[M - H_2O]^+$ (26), 208 (30), 193 (24), 175 (24), 165 (24), 154 (24), 149 (34), 138 (64), 125 (94), 111 (96), 109 (94), 108 (98), 107 (70), 97 (100), 96 (94), 95 (83); IR ν_{max}^{KBr}, cm^{-1}: 842, 904, 930, 962, 990, 1010, 1020, 1034, 1054, 1074, 1100, 1122, 1136, 1200, 1220, 1230, 1244, 1300, 1318, 1378, 1402, 1416, 1434, 1454, 1686, 2960, 3520.

(−)-Ent-*longipinan*-3,12-*dione* (**7**). **6** (0.15 g) in Me_2CO (6 ml) was oxidized with Jones' reagent for 5 min. Excess oxidant was destroyed with MeOH, the solvent removed *in vacuo* and the residue extracted with Et_2O. The Et_2O was evapd and the residue recrystallized from MeOH–H_2O to give **7** as rhombic plates, mp 55–56° and $[\alpha]_D^{24}$ − 8.8° ($CHCl_3$, *c* 0.84). $C_{15}H_{22}O_2$ (234). MS *m/z* (rel. int.): 234 $[M]^+$ (97), 219 $[M - Me]^+$ (64), 206 (28), 201 (14), 191 (42), 178 (58), 166 (85), 149 (38), 138 (55), 125 (96), 124 (88), 123 (73), 111 (80), 110 (77), 109 (73), 97 (85), 96 (100); IR ν_{max}^{KBr}, cm^{-1}: 840, 908, 938, 1028, 1084, 1106, 1122, 1152, 1196, 1224, 1320, 1364, 1408, 1426, 1460, 1698, 3000; ORD (MeOH): $[\alpha]_{230}$ − 80°, $[\alpha]_{248}$ − 56°, $[\alpha]_{280}$ − 60°, $[\alpha]_{296}$ 0°, $[\alpha]_{310}$ + 29°, $[\alpha]_{320}$ + 27°, $[\alpha]_{384}$ 0°, $[\alpha]_{430}$ − 1°.

(−)-Ent-*longipinane* (**8**). A soln of **7** (90 mg) in diethyleneglycol (15 ml) was heated with N_2H_4 (2 ml) at 170–180° for 5 hr. KOH (1 g) was added and the mixture heated at 180° for 1 hr. and at 220° for another hour. Excess N_2H_4 and H_2O were removed at 230° and this temp. was maintained for 3 hr. After cooling to room temp. and dilution with H_2O the soln was extracted with Et_2O and the oily residue, after evaporation of the solvent, chromatographed on Si gel (5 g). *n*-Hexane (100 ml) eluted **8** as oil (30 mg) of $[\alpha]_D^{24}$ − 13.6° ($CHCl_3$, *c* 1.66). $C_{15}H_{26}$ (206). MS *m/z* (rel. int.): 206 $[M]^+$ (75), 191 $[M - Me]^+$ (56), 177 (44), 163 (67), 150 (53), 149 (54), 138 (57), 137 (57), 136 (64), 135 (73), 125 (63), 124 (96), 123 (69), 122 (70), 121 (80), 110 (77), 109 (100), 108 (63), 107 (81), 96 (82), 95 (90), 94 (62), 93 (77); IR ν_{max}^{film}, cm^{-1}: 860, 890, 980, 1064, 1140, 1160, 1188, 1264, 1360, 1370, 1390, 1460, 2970.

2,4,4,11,11-*Pentadeuterio*-ent-*longipinan*-3,13-*dione*. **7** (3 mg) was heated with a soln of Na (50 mg) in CD_3OD (1 ml) under reflux for 3 hr. After usual work-up the resulting crystals showed in the MS a molecular ion peak at *m/z* 239 corresponding to the formula $C_{15}H_{17}D_5O_2$.

(−)-Ent-*12β-hydroxylongipin*-2(10)-*en*-3-*one* (**9**). **1** (100 mg) in MeOH (5 ml) was heated with KOH (0.5 g) under reflux for 1 hr. The resin obtained after the usual work-up was chromatographed on Si gel (5g). Elution with *n*-hexane–Et_2O (1:1) (100 ml) yielded **9** which was recrystallized from *n*-hexane to afford prisms, mp 91–92° and $[\alpha]_D^{24}$ − 50.4° ($CHCl_3$, *c* 1.425)). $C_{15}H_{22}O_2$ (234). MS *m/z* (rel. int.): 234 $[M]^+$ (62), 219 $[M - Me]^+$ (72), 216 $[M - H_2O]^+$ (65), 201 $[M - Me - H_2O]^+$ (61), 191 (65), 187 (44), 173 (80), 168 (38), 160 (52), 150 (76), 149 (87), 148 (98), 135 (81), 127 (98), 126 (76), 123 (94), 121 (83), 109 (83), 108 (92), 107 (100), 95 (97), 93 (79); IR ν_{max}^{KBr}, cm^{-1}: 842, 890, 910, 948, 970, 990, 1002, 1010, 1050, 1070, 1096, 1130, 1166, 1268, 1310, 1380, 1400, 1436, 1452, 1616, 1688, 2980, 3600.

(−)-Ent-*longipin*-2(10)-*en*-3,12-*dione* (**10**). To a soln of **9** (59 mg) in CH_2Cl_2 (4 ml) was added a soln of CrO_3 (0.15 g) in CH_2Cl_2 (4 ml) and pyridine (0.5 ml) and the mixture shaken at room temp. for 15 min. The resulting resin was separated by prep. TLC on Si gel PF (5 × 20 × 20 × 0.1 cm) with *n*-hexane–Et_2O–HCO_2H (90:75:18). Treatment with I_2 vapour revealed two bands. The upper one yielded, after extraction with Et_2O and recrystallization from *n*-pentane, the dione **10** (13.5 mg) as needles, mp 43–45°. $C_{15}H_{20}O_2$ (232). MS *m/z* (rel. int.): 232 $[M]^+$ (55), 217 $[M - Me]^+$ (86), 214 (18), 204 (7), 199 (16), 189 (46), 176 (31), 175 (29), 164 (28), 161 (40), 149 (49), 147 (59), 137 (69), 125 (94), 123 (100), 109 (59), 107 (58), 105 (55), 95 (91), 94 (75), 93 (65); IR ν_{max}^{KBr}, cm^{-1}: 746, 920, 950, 998, 1014, 1030, 1074, 1100, 1124, 1140, 1170, 1228, 1280, 1322, 1364, 1390, 1410, 1470, 1620, 1696, 2980; CD (*n*-heptane): $[\theta]_{203}$ − 2600, $[\theta]_{217}$ 0, $[\theta]_{238}$ + 1670, $[\theta]_{261}$ + 680, $[\theta]_{290}$ + 2140, $[\theta]_{296}$ + 2320, $[\theta]_{305}$ + 1960, $[\theta]_{317}$ + 1060, $[\theta]_{336}$ + 710, $[\theta]_{350.5}$ + 710, $[\theta]_{367}$ + 430, $[\theta]_{382}$ + 120, $[\theta]_{395}$ 0.

(+)-Ent-*12β-acetoxy*-3-*oxolongipinan*-2(R),5′-*spiropyrazolin*-(1′) (**11**). **1** (0.2 g) was treated with an excess of CH_2N_2 in Et_2O for 12 hr. Recrystallization from Et_2O–*n*-hexane gave **11** as needles, mp 133° (dec.) and $[\alpha]_D^{24}$ + 231.3° ($CHCl_3$, *c* 1.29). $C_{18}H_{26}N_2O_3$ (318). MS *m/z* (rel. int.): 319 $[M + H]^+$ (19), 290 $[M - N_2]^+$ (60), 275 $[M - N_2 - Me]^+$ (31), 248 $[M - N_2 - CH{=}C{=}O]^+$ (73), 247 (64), 230 $[M - N_2 - AcOH]^+$ (96), 215 (85), 204 (56), 201 (62), 187 (95), 179 (69), 174 (73), 173 (74), 161 (95), 147 (95), 137 (95), 121 (100), 107 (95), 95 (94), 93 (96); IR ν_{max}^{KBr} cm^{-1}: 800, 818, 850, 892, 908, 950, 970, 990, 1020, 1036, 1060, 1094, 1140, 1244, 1318, 1370, 1382, 1410, 1430, 1460, 1550, 1700, 1718, 2960; ORD (MeOH): $[\alpha]_{250}$ − 2600°, $[\alpha]_{260}$ − 3000°, $[\alpha]_{282}$ − 2500°, $[\alpha]_{322}$ − 7600°, $[\alpha]_{330}$ 0°, $[\alpha]_{344}$ + 7200°, $[\alpha]_{450}$ + 600°.

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