

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

SIEGFRIED HUNECK, JOSEPH D. CONNOLLY,* DAVID S. RYCROFT* and AKIHIKO MATSUO†

Institute of Plant Biochemistry, Research Centre for Molecular Biology and Medicine, GDR Academy of Sciences, DDR-401
Halle/Saale, Weinberg, German Democratic Republic; * Department of Chemistry, University of Glasgow, Glasgow G12 8QQ,
U.K.; † Department of Chemistry, Faculty of Science, Hiroshima University, Hiroshima 730, Japan

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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

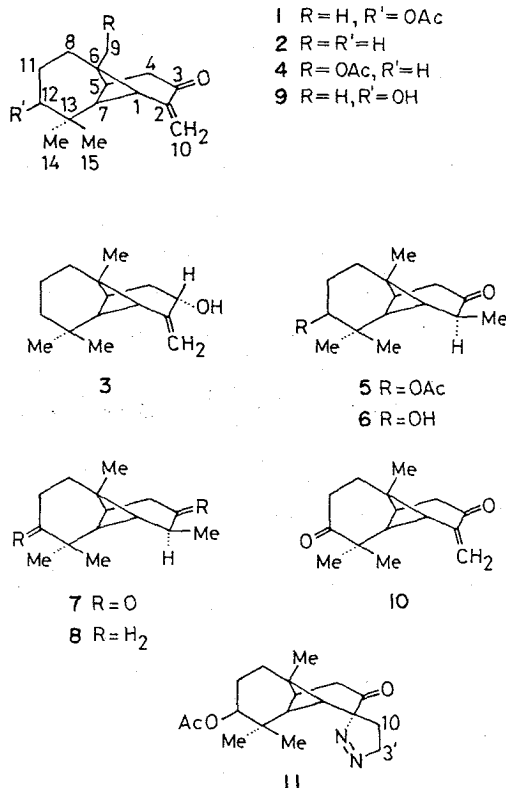
Extraction of the air-dried and ground liverwort with Et₂O and subsequent chromatography of the green oily extract gave, besides oily fractions, (–)-ent-12β-acetoxylongipin-2(10)-en-3-one (**1**) in cubes, mp 95–96°, $[\alpha]_D^{24} - 17.1^\circ$. The MS of **1** has an M⁺ peak at *m/z* 276, corresponding to the formula C₁₇H₂₄O₃, and an intensive peak at *m/z* 234 [M – CH₂=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=CH₂—CO—) and 1735 cm^{–1} (–OAc). The ¹H NMR spectrum (360 MHz, CDCl₃, 25°, TMS as internal standard assuming CHCl₃ 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, H₂-8, 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 ¹³C 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. ¹³C 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 |
| CH ₃ —CO— | 21.3 | | 21.2 |
| CH ₃ —CO— | 170.6 | | 170.5 |
| 3' | | | 78.9 |



the liverwort *Mannia fragrans* (Balbis) Frye et Clark [2]. On treatment of 7 with CD₃OD in the presence of CD₃ONa five atoms of deuterium are incorporated; this is further evidence for the 12-position of the acetoxy 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 CH₂N₂ 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 Et₂O for 3 days. The Et₂O was removed *in vacuo* and the resulting dark green oil chromatographed on Si gel (with 5% H₂O) using a *n*-hexane–Et₂O gradient to yield six fractions. The first five fractions gave oils, the analysis of which is under way. Fraction 6, eluted with *n*-hexane–Et₂O (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°, [α]_D²⁴ – 17.1° (CHCl₃, *c* 4.90) and *R_f* 0.37 (*n*-hexane–Et₂O–HCO₂H (20:12:3), detection with AcOH + SO₃HCl at 150°: brown-violet spot). C₁₇H₂₄O₃ (276). MS *m/z* (rel. int.): 276.1726 ([M]⁺, calc. 276.1725) (52), 261 [M – Me]⁺ (6), 258 (8), 248 (6), 234 [M – CH₂=C=O]⁺ (80), 219 (40), 216

[M – AcOH]⁺ (77), 206 [M – CH₂=C=O – CO]⁺ (27), 201 [M – CH₂=C=O – H₂O – 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 *v*_{max}^{CCl}, 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): [θ]₁₉₇ – 2690, [θ]₂₀₂ – 4040, [θ]₂₂₁ 0, [θ]₂₄₀ – 2500, [θ]₂₇₅ 0, [θ]₂₉₉ + 120, [θ]₃₁₀ + 270, [θ]₃₂₂ + 480, [θ]₃₃₅ + 700, [θ]_{349.5} + 700, [θ]_{365.5} + 430, [θ]₃₈₀ + 130, [θ]₃₉₅ 0; ORD (MeOH): [α]₂₇₀ 0°, [α]₃₀₈ – 180°, [α]₃₄₈ 0°, [α]₃₆₈ + 30°, [α]₃₉₀ 0°, [α]₄₄₀ – 20°.

(–)-Ent-12β-acetoxylongipin-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 [α]_D²⁴ – 33.8° (CHCl₃, *c* 2.18). C₁₇H₂₆O₃ (278). MS *m/z* (rel. int.): 278 [M]⁺ (22), 236 [M – CH₂=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 *v*_{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): [α]₂₂₂ 0°, [α]₂₃₂ – 65°, [α]₂₄₄ 0°, [α]₂₇₂ + 132°, [α]₂₉₀ 0°, [α]₃₁₆ – 260°, [α]₃₅₄ – 100°, [α]₄₀₀ – 60°.

(–)-Ent-12β-hydroxylongipin-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–H₂O gave 6 as needles, mp 92–94° and [α]_D²⁴ – 66.5° (CHCl₃, *c* 1.66). C₁₅H₂₄O₂ (236). MS *m/z* (rel. int.): 236 [M]⁺ (78), 221 [M – Me]⁺ (32), 218 [M – H₂O]⁺ (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 *v*_{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-longipinane-3,12-dione (7). 6 (0.15 g) in Me₂CO (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 Et₂O. The Et₂O was evapd and the residue recrystallized from MeOH–H₂O to give 7 as rhombic plates, mp 55–56° and [α]_D²⁴ – 8.8° (CHCl₃, *c* 0.84). C₁₅H₂₂O₂ (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 *v*_{max}^{KBr}, cm^{–1}: 840, 908, 938, 1028, 1084, 1106, 1122, 1152, 1196, 1224, 1320, 1364, 1408, 1426, 1460, 1698, 3000; ORD (MeOH): [α]₂₃₀ – 80°, [α]₂₄₈ – 56°, [α]₂₈₀ – 60°, [α]₂₉₆ 0°, [α]₃₁₀ + 29°, [α]₃₂₀ + 27°, [α]₃₈₄ 0°, [α]₄₃₀ – 1°.

(–)-Ent-longipinane (8). A soln of 7 (90 mg) in diethylene-glycol (15 ml) was heated with N₂H₄ (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 N₂H₄ and H₂O were removed at 230° and this temp. was maintained for 3 hr. After cooling to room temp. and dilution with H₂O the soln was extracted with Et₂O 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 [α]_D²⁴ – 13.6° (CHCl₃, *c* 1.66). C₁₅H₂₆ (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 *v*_{max}^{film}, cm^{–1}: 860, 890, 980, 1064, 1140, 1160, 1188, 1264, 1360, 1370, 1390, 1460, 2970.

2,4,4,11,11-Pentadeuterio-ent-longipinane-3,13-dione. 7 (3 mg) was heated with a soln of Na (50 mg) in CD₃OD (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-12 β -hydroxy-longipin-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-Et₂O (1:1) (100 ml) yielded **9** which was recrystallized from *n*-hexane to afford prisms, mp 91–92° and $[\alpha]_D^{24} - 50.4^\circ$ (CHCl₃, *c* 1.425), $C_{15}H_{22}O_2$ (234). MS m/z (rel. int.): 234 [M]⁺ (62), 219 [M - Me]⁺ (72), 216 [M - H₂O]⁺ (65), 201 [M - Me - H₂O]⁺ (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 CH₂Cl₂ (4 ml) was added a soln of CrO₃ (0.15 g) in CH₂Cl₂ (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-Et₂O-HCO₂H (90:75:18). Treatment with I₂ vapour revealed two bands. The upper one yielded, after extraction with Et₂O 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-12 β -acetoxy-3-oxolongipinan-2(R),5'-spiropyrzolin-(1') (11). **1** (0.2 g) was treated with an excess of CH₂N₂ in Et₂O for 12 hr. Recrystallization from Et₂O-*n*-hexane gave **11** as needles, mp 133° (dec.) and $[\alpha]_D^{24} + 231.3^\circ$ (CHCl₃, *c* 1.29). $C_{18}H_{26}N_2O_3$ (318). MS m/z (rel. int.): 319 [M + H]⁺ (19), 290 [M - N₂]⁺ (60), 275 [M - N₂ - Me]⁺ (31), 248 [M - N₂ + CH=C=O]⁺ (73), 247 (64), 230 [M - N₂ - 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): $[\alpha]_{250} - 2600^\circ$, $[\alpha]_{260} - 3000^\circ$, $[\alpha]_{282} - 2500^\circ$, $[\alpha]_{322} - 7600^\circ$, $[\alpha]_{330} 0^\circ$, $[\alpha]_{344} + 7200^\circ$, $[\alpha]_{450} + 600^\circ$.

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