15-Acetoxypinguisone and a Cyclocuparanol from the Liverwort *Cryptothallus mirabilis* Malmb.†‡

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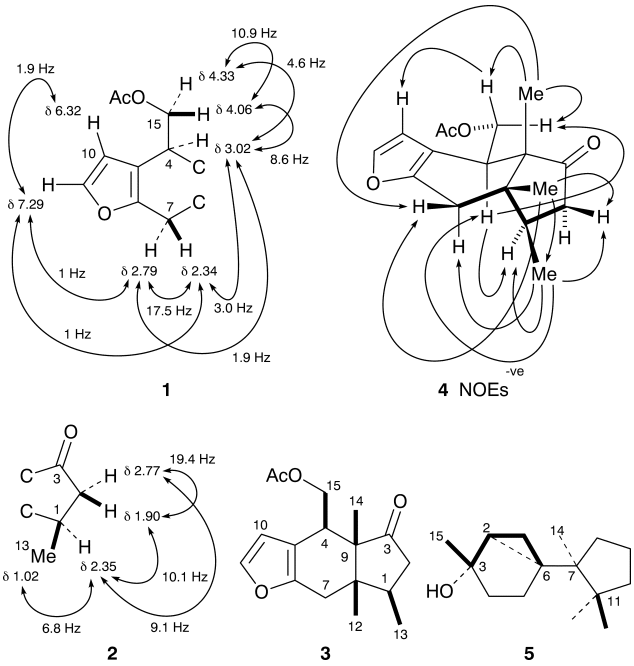
The new sesquiterpenoid 15-acetoxypinguisone has been found as the dominant constituent in the $CDCl_3$ extract of a specimen of the liverwort *Cryptothallus mirabilis* and the structure elucidated using ^1H and ^{13}C NMR spectroscopy together with GC–MS and HRMS; a very minor constituent was assigned as (2*S*,6*S*)-cyclo-(7*S*)-cuparan-(3*S*)-ol, but the major/minor roles were reversed in a second specimen.

A characteristic difference of liverworts (Hepaticae) from mosses (Musci) is their ability to form intracellular oil bodies containing a wide variety of terpenoid and aromatic compounds, some of which have unusual carbon skeletons that are unique to liverworts. *Cryptothallus mirabilis* Malmb. (Aneuraceae) is a rare but under-recorded thalloid liverwort of oceanic north-western Europe that remarkably grows below the surface layer of vegetation, often beneath other bryophytes (especially *Sphagnum*) and near birch trees. It lacks chlorophyll and is parasitic on an endophytic fungus. A second, closely related member of the genus, *Cryptothallus hirsutus*, was described recently from Costa Rica.[1] The plants grow hidden from view and are not readily available in other than small quantities. A study of the lipophilic secondary metabolites (the oil body compounds) is possible however using the method we have been developing for studying small samples of liverworts. This involves the concerted application of the complementary techniques of NMR fingerprinting and GC–MS.[2,3] Here, we report the characterization and structural elucidation of a new pinguisane sesquiterpenoid from a sample of *C. mirabilis* collected near Glasgow. Pinguisanes are unique to liverworts and the non-isoprenoid carbon skeleton poses an interesting biosynthetic problem that has been investigated recently using ^{13}C-labelling.[4] The NMR fingerprint of a second sample of the liverwort was very different and the major lipophilic constituent has been characterized as a 2,6-cyclocuparan-3-ol.

The ^1H NMR spectrum[5] of the $CDCl_3$ extract of the first specimen of *C. mirabilis* (no. 96097) suggested the presence of one dominant compound (concentration 7 mM by comparison with the residual $CHCl_3$ signal[3]) in addition to small amounts of compounds containing long, partially unsaturated hydrocarbon chains. Analysis of the ^1H data, assisted by the results of homodecoupling experiments, suggested the presence of fragments **1** and **2** together with two tertiary methyl groups. These could be assembled readily into the pinguisane structure **3**. The ^{13}C NMR spectrum showed signals for sixteen of the carbons of **3** (the ketonic carbonyl signal was lost in the noise) and the chemical shifts agreed closely with the data reported[4] for pinguisone except as expected, for C-15, C-4 and C-5. Observation of the fragment $(M^+ - CH_3CO_2H)$ at *m/z* 230.1307 by HREIMS confirmed that the molecular formula is $C_{17}H_{22}O_4$. The NOEs shown in **4** were observed in NOE difference experiments and demonstrate that C-12, -13, -14 and -15 are all on the β-face of the molecule (the absolute configuration is assumed to be the same as that of pinguisone).



The $CDCl_3$ extract of the second specimen of *C. mirabilis* examined (no. 96271) gave a very different ^1H NMR spectrum, although **3** was still present to the extent of 2% according to GC–MS TIC integration. The major component (3 mM by NMR, 88% by GC–MS, M^+ 222) showed four tertiary methyl groups (one geminal to oxygen; none attached to sp^2 carbons), and a highly shielded proton (dd, *J* 5.4, 3.6) consistent with the *endo* methylene proton of the trisubstituted cyclopropane moiety of a bicyclo[*n*.1.0]alkane, but no olefinic protons. This compound could not be acetylated using acetic anhydride and pyridine. However GC/GC–MS investigation of the product of treatment with thionyl chloride and pyridine showed that dehydration had occurred. The major components in the GC–MS TIC trace of the dehydration product were two sesquiterpenes with M^+ 204 as well as a third sesquiterpene, M^+ 202, identified as cuparene by comparison with the published retention index (allowing for column differences) and MS fragmentation pattern.[6] Cuparene was not detectable initially (although the two sesquiterpenes with M^+ 204 were) and the conclusion is that it resulted from oxidation and aromatization of an initial, intermediate dehydration product. These observations together with the molecular formula $C_{15}H_{26}O$ show that the parent compound is a tricarbocyclic sesquiterpenoid tertiary alcohol. Cyclocuparanol **5** is the only tricyclic structure (apart from stereochemistry) consistent with the evidence that, following dehydration, could result in the formation of cuparene without rearrangements requiring

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the shifting of carbon atoms having to be invoked. An isomer of **5**, (2*R*,6*R*)-cyclo-(7*R*)-cuparan-(3*S*)-ol, has been assigned[7] as the structure of microbiotol from *Microbiota decussata*. The NMR data reported for a $CDCl_3$ solution of microbiotol are distinctly different from those of the *C. mirabilis* cyclocuparanol: whereas in microbiotol the most deshielded methyl signal is a doublet (*J* 0.9) arising from an antiperiplanar relationship with 4-*pro*-S-H, that in the *C. mirabilis* cyclocuparanol is sharp (even sharper than the other three methyl singlets) implying a difference in the configuration of C-3 relative to that of C-2. The *C. mirabilis* cyclocuparanol has therefore been assigned structure **5**, (2*S*,6*S*)-cyclo-(7*S*)-cuparan-(3*S*)-ol, the absolute configurations of positions 2, 6 and 7 being assumed by analogy with those of another cyclocuparane derivative, grimaldone[8] from the Marchantialean liverwort *Mannia fragrans*. GC–MS showed 2% of **5** in the TIC trace of extract 96097.

Comparison of characteristic signals in the ^1H NMR spectrum of the *C. mirabilis* cyclocuparanol **5** with data[9] for a compound reported,[10,11] with the name (−)-cyclopropanecuparenol, from *Marchantia polymorpha* demonstrated clearly that the two compounds are the same. Although the stereochemistry has been shown previously[11] as (7*S*,6*R*,2*R*,3*R*), the only direct evidence of relative configuration comes from NOE experiments[9] showing that 3-Me is on the same face of the molecule as 1-H-*endo* and is equally consistent with structure **5**.

GC–MS and retention index data[6] suggested the presence of *β*-acoradiene (9% in extract 96271 and detectable in the extract of a sample of no. 96097 that had been dried before extraction). Furan signals indicative of a second pinguisane derivative (8% of **3**) were present in the ^1H NMR spectrum of extract 96097; GC–MS showed this component had M^+ 232 (suggestive of pinguisone or an isomer) and was also detectable in extract 96271.

The frequent production of spores by the dioecious *C. mirabilis* demonstrates that exchange of genetic material can occur readily (unlike the situation in liverwort species that are apparently completely sterile) and the dramatic difference in the pattern of secondary metabolites between the two samples is not as surprising as it might seem at first sight. The discovery of a pinguisone derivative in *C. mirabilis* is of chemosystematic interest because pinguisone itself was first isolated[12] from *Aneura pinguis*, another member of the family Aneuraceae. Cyclocuparanes are rare and this is the first report of a cyclocuparane derivative from the order Metzgeriales.

Experimental

GC, GC–MS, HREIMS, ^1H and ^{13}C NMR (360 and 90 MHz respectively, $CDCl_3$; *J* values are given in Hz) were performed as described previously.[13] ^{13}C multiplicities were obtained from DEPT experiments.

Plant material.—*Cryptothallus mirabilis* was collected by D.S.R. and kept refrigerated till used. The liverwort was extracted by maceration with sufficient $CDCl_3$ to produce *ca.* 0.7 ml of a filtered solution. (i) Ref. no. Rycroft 96097, collected 8th August 1996 at Dougalston, Milngavie, near Glasgow; 688 mg (not dried) extracted 30th August 1996 (a sample that had been dried soon after collection and then extracted on 14th March 1997 gave similar results). (ii) Ref. no. Rycroft 96271, collected 28th September 1996 in Glen Creran, Argyll; 139 mg (not dried) extracted 6th October 1996.

Extracts 96097 *and* 96271.—GC–MS: R_i (% in 96097, % in 96271 for components ⩾1% in at least one of the extracts): 1443 (0, 9.2) *m/z* 204 (M^+, 20%), 119 (100); **5**, *1541* (1.5, 88.2) *m/z* 222 (M^+, 1%), 111 (100); *1576* (5.1, 0) *m/z* 220 (M^+, 2%), 150 (100); *1605* (1.3, trace) *m/z* 222 (M^+, 83%), 152 (100); *1672* (2.3, trace) *m/z* 232 (M^+, 14%), 108 (100); *1932* (2.2, 0) *m/z* 244 (M^+, 2%), 174 (100); **3**, *1998* (77.0, 1.8) *m/z* 230 ($M^+ - 60$, 100%); 2222 (6.6, trace) *m/z* 262 (M^+, 10%), 43 (100); *2279* (4.0, trace) *m/z* 304 (M^+, 1%), 174 (100).

15-*Acetoxypinguisone* **3**.—Compound **3** was present (77% by GC–MS) in the gum obtained by evaporation of the $CDCl_3$ extract 96097; R_i (GC) 1998; δ_H 0.85 (3 H, s, 12-H_3), 0.86 (3 H, s, 14-H_3), 1.02 (3 H, d, *J* 6.8, 13-H_3), 1.90 (1 H, dd, *J* 19.4, 10.1, 2*β*-H), 2.08 (3 H, s, OAc), 2.34 (1 H, ddd, *J* 17.5, 3.0, 1.0, 7*β*-H), 2.35 (1 H, obsc ddq, *J* 10.1, 9.1, 6.8, 1-H), 2.77 (1 H, dd, *J* 19.4, 9.1, 2*α*-H), 2.79 (1 H, br dd, *J* 17.5, 1.9, 7*α*-H), 3.02 (1 H, dddd, *J* 8.6, 4.6, 3.0, 1.9, 4-H), 4.06 (1 H, dd, *J* 10.9, 8.6, 15-*pro-S*-H), 4.33 (1 H, dd, *J* 10.9, 4.6, 15-*pro-R*-H), 6.32 (1 H, d, *J* 1.9, 10-H), 7.29 (1 H, dt, *J* 1.9, 1.0, 11-H); δ_C 9.2 (C-14), 13.7 (C-13), 18.4 (C-12), 21.0 (CH_3CO), 28.2 (C-7), 31.9 (C-1), 34.2 (C-3), 41.4 45.8 (C-8), 55.4 (C-9), 64.6 (C-15), 109.4 (C-10), 113.9 (C-5), 141.3 (C-11), 148.6 (C-6), 170.9 (CH_3CO); *m/z* (intensities from GC–EIMS but high resolution data measured using a direct probe) 230.1307 (100%) ($M^+ - CH_3CO_2H$. $C_{15}H_{18}O_2$ requires 230.1307), 215.1066 (4) ($M^+ - CH_3CO_2H - CH_3$. $C_{14}H_{15}O_2$ requires 215.1072), 187 (9), 173.0981 (9) ($M^+ - CH_3CO_2H - CH_3 - CH_2CO$. $C_{12}H_{13}O$ requires 173.0966), 160.0889 (56) ($M^+ - CH_3CO_2H - CH_3CHCH_2CO$. $C_{11}H_{12}O$ requires 160.0888), 159 (38), 145.0675 (30) ($M^+ - CH_3CO_2H - CH_3 - CH_3CHCH_2CO$, $C_{10}H_9O$ requires 145.0653), 124.0885 (27) [$M^+ - CH_3CO_2H - (CH_2C_4H_2OC{=}CH_2)$. $C_8H_{12}O$ requires 124.0888], 119 (14), 106 (9), 95 (9), 91 (10), 77 (10), 69 (6), 43 (41).

(2*S*,6*S*)-*Cyclo*-(7*S*)-*cuparan*-(3*S*)-*ol* **5**.—Compound **5** was present (88% by GC–MS) in the gum obtained by evaporation of the $CDCl_3$ extract 96271; R_i (GC) 1541; δ_H 0.202 (1 H, dd, *J* 5.4, 3.6, 1*β*-H), 0.913 (1 H, ddd, *J* 8.7, 3.6, 1.4, 2-H), 0.959 (3 H, s, Me), 0.973 (1 H, ddd, *J* 8.7, 5.3, 1.7, 1*α*-H), 1.002 (3 H, s, Me), 1.057 (3 H, s, Me), 1.302 (3 H, s, 15-Me), 2.116 (ddd sd, *J* 12.6, 11.1, 8.6, 1.7, 5*α*-H); *m/z* (GC–EI) 222 (1%), 207 (11), 204 (6), 189 (12), 161 (12), 151 (23), 137 (23), 121 (36), 119 (36), 111 (100), 94 (91), 79 (35), 69 (79), 55 (66), 43 (98), 41 (65).

Dehydration of **5**.—Thionyl chloride (30 *μ*l) was added to a solution of **5** in pyridine (50 *μ*l) at −20 °C. The reaction mixture was brought to room temperature, quenched with ice-cold saturated aqueous $NaHCO_3$ (1 ml) and extracted with CH_2Cl_2 (2 × 0.5 ml). The extract was dried (Na_2SO_4), concentrated and analysed using GC/GC–MS: R_i 1474, *m/z* 204 (35%), 119 (100); cuparene, R_i 1475, *m/z* 202 (28%), 132 (100); R_i 1506, *m/z* 204 (22%), 119 (100).

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