Absolute Stereochemistry of Puupehenone and Related Metabolites

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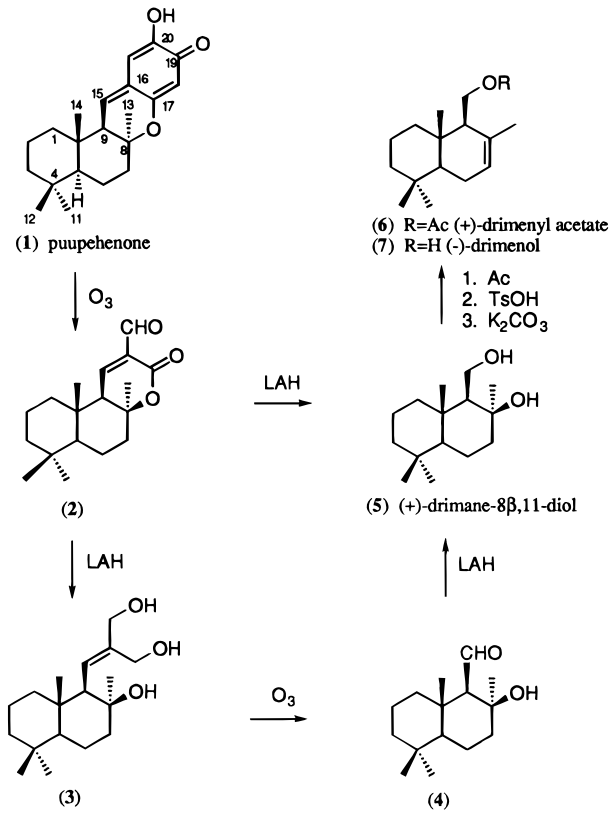
Degradative studies have permitted an absolute stereochemistry to be assigned to the marine natural product puupehenone (**1**). On biosynthetic grounds 10 related co-metabolites were assigned to the same antipodal series.

Puupehenone (**1**) was first reported in 1979 from a Hawaiian sponge tentatively identified as *Chondrosia chucalla*[1] and the structure proof supported by ozonolytic degradation to the crystalline derivative **2**, which was subjected to X-ray analysis. At that time neither spectroscopic analysis nor degradative studies permitted assignment of an absolute stereochemistry to puupehenone (**1**). Although exhibiting a range of interesting biological properties[1,2] and the subject of a total synthesis,[3] the absolute stereochemistry of puupehenone remained unresolved. In more recent years 10 related metabolites have been reported to co-occur with puupehenone (**1**),[4−7] all of which are chiral and none of which have been assigned an absolute stereochemistry. Our prior experience in exploring both structural and stereochemical aspects of marine natural products derived from mixed sesquiterpene and quinone/quinol biosynthesis,[8] together with a fortuitous re-isolation of puupehenone (**1**) from a southern Australian marine sponge, *Dysidea* sp., prompted an attempt at solving the absolute stereochemistry of puupehenone (**1**).

Ozonolysis of puupehenone (**1**) yielded a single isolable product identical in all respects to the previously described aldehyde **2**.[1] It was envisaged that reduction of **2** with lithium aluminium hydride (LAH) would yield **3**, which on repeating the ozonolysis and reduction sequence would proceed via the aldehyde **4** to drimane-8β,11-diol (**5**) (see Scheme 1). In the event, anomalous LAH reduction of **2** proceeded directly to (+)-drimane-8β,11-diol (**5**) spectroscopically identical with data published for synthetically derived (±) **5**.[9] This anomalous reduction was presumably initiated by the doubly activated nature of the double bond in **2**. Protection of the hydroxy group in **5** as the acetate followed by dehydration of the tertiary hydroxy group with *p*-TsOH yielded the known metabolite (+)-drimenyl acetate (**6**) ([α]$_D$ +6°, lit,[10] [α]$_D$ +9.7°). Hydrolysis of the acetate functionality in **6** yielded the known terrrestrial natural product (−)-drimenol (**7**) ([α]$_D$ −14°, lit.[9,11] [α]$_D$ −20°). Consequently, the absolute stereochemistry of puupehenone (**1**) can be assigned as shown, while on biosynthetic grounds the absolute stereochemistry of the co-metabolites chloropuupehenone,[1,4] bromopuupehenone,[1] puupehedione,[4] 21-chloropuupehenol,[5] dipuupehetriol,[4] 15-cyanopuupehenol,[6] 15-cyanopuupehenone,[4] 15-oxopuupehenol,[5] bispuupehenone,[7] and molokinenone[5] could also be assigned to the same antipodal series.

Scheme 1



Experimental Section

General Experimental Procedures. For general experimental procedures, see Butler and Capon.[12]

Collection, Extraction, and Isolation. A specimen of *Dysidea* sp. (type specimen registry number F77032, lodged with the Museum of Victoria) was collected during scientific trawling operations on the RV *Franklin* in 1994, at a depth of approximately 150 m, at a location 50 km south of Ceduna in the Great Australian Bight, along the edge of the continental shelf. The crude EtOH extract of this sponge displayed growth inhibitory properties against *Staphylococcus aureus* and *Micrococcus lutea* in a standard antibiotic disk assay, as well as antifungal activity against *Candida albicans*. The crude EtOH extract was partitioned into CH_2Cl_2-soluble and CH_2Cl_2-insoluble fractions. The former fraction was subjected to rapid silica filtration (10% stepwise elution from petroleum spirits (40−60 °C) to CH_2Cl_2 to EtOAc), followed by normal-phase HPLC [2.0 mL/min, either 20% or 30% EtOAc/petroleum spirits (60−80 °C) on a Phenomenex Spherex 5 μ 10 × 250-mm column] to yield puupehenone (**1**) (630 mg, 1.22%), chloropuupehenone

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(16 mg, 0.03%), bromopuupehenone (isolated as a mixture with chloropuupehenone) (19 mg, 0.04%), puupehedione (8 mg, 0.02%), 21-chloropuupehenol (50 mg, 0.09%), and dipuupehetriol (7 mg, 0.01%). All compounds were identified by comparison of their spectroscopic data with literature data.[1,4,5]

Puupehenone (1): a stable yellow oil with spectroscopic data (^{1}H NMR, IR, UV) identical to that recorded in the literature[1] [literature ^{13}C NMR ($CDCl_3$) assignments 39.8 (s) and 33.2 (t) should be revised to 39.8 (t) and 33.2 (s), respectively]; $[\alpha]_D$ +189° (*c* 1.08, CCl_4), lit.[4] $[\alpha]_D$ +297° (*c* 0.44, CCl_4);[4] $[\alpha]_D$ +113° (*c* 1.79, $CHCl_3$), authentic[1] $[\alpha]_D$ +117° (*c* 0.42, $CHCl_3$).

Ozonolysis of Puupehenone (1). A dry-ice/Me_2CO-cooled solution of puupehenone (**1**) (41 mg) in CH_2Cl_2 (25 mL) was treated with a 60% stream of O_3/O_2 for 0.5 h. The resulting ozonide was quenched with Jones' reagent (2 drops), and the stirred solution was allowed to warm to room temperature. After dilution with H_2O (40 mL), the reaction mixture was extracted with CH_2Cl_2 (3 × 50 mL) and the combined organic layers dried with anhydrous $MgSO_4$ and concentrated under reduced pressure. Purification of the crude reaction mixture was achieved by normal-phase HPLC [Phenomenex Spherex 5 μ 250 × 10-mm column, 20% EtOAc/petroleum spirits (60−80 °C) at 2 mL/min], to yield aldehyde **2** as a stable colorless oil (12 mg, 33%) for which the spectroscopic data (^{1}H NMR and MS) were identical to those reported;[1] $[\alpha]_D$ +109° (*c* 0.35, $CHCl_3$).

(+)-Drimane-8β,11-diol (5). A crude sample of aldehyde **2** was subjected to a LAH reduction using the following procedure: LAH (4 g), dry Et_2O (20 mL), and a solution of **2** (420 mg) in dry Et_2O (20 mL) were refluxed under dry N_2 for 1 h, after which time the reaction was quenched (100 mL, 15% H_2SO_4/H_2O), diluted with H_2O (50 mL), and allowed to stand overnight. The reaction product was extracted with Et_2O (3 × 50 mL) and the combined organic layers dried with anhydrous $MgSO_4$ and concentrated under reduced pressure to yield a crude mixture containing **5** (57 mg, 18%). Purification of the crude product was achieved by normal phase HPLC [Phenomenex Spherex 5 μ 250 × 10-mm column, 50% EtOAc/petroleum spirits (60−80 °C) at 2 mL/min] to yield (+)-drimane-8β,11-diol (**5**) as a stable colorless oil (27 mg, 8%): $[\alpha]_D$ +12° (*c* 1.05, $CHCl_3$); IR (film) 3357, 1459, 1364, 1023 cm^{-1}; UV (EtOH) 202 (ϵ 4000), 232 sh (ϵ 1400), 323 (ϵ 1000) nm; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.85, 0.87, and 1.23 (3s, Me-11, Me-12, and Me-14), 1.34 (s, Me-13), 4.03−4.15 (m, H_2 15), 4.31 (br s, OH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 74.1 (s, C-8), 60.0 (t, C-15), 58.3 (d, C-9), 55.8 (d, C-5), 42.8 (t, C-7), 41.9 (t, C-3), 40.0 (t, C-1), 38.3 (q, C-13), 33.6 (s, C-10), 33.3 (s, C-4), 30.9 (q, C-11), 21.6 (q, C-12), 18.4 (2t, C-2 and C-6), 16.9 (q, C-14); EIMS (70 eV) *m*/*z* 222 ($M^+ - H_2O$, 19), 207 ($C_{14}H_{23}O^+$, 15), 189 ($C_{14}H_{21}^+$, 14), 177 ($C_{13}H_{21}^+$, 13), 164 (30), 149 (19), 109 (91), 95 (89), 81 (74), 69 (100); HREIMS *m*/*z* 222.1986; calcd for $C_{15}H_{26}O$, 222.1983.

(+)-Drimenyl Acetate (6). A sample of drimane-8β,11-diol (**5**) (12 mg) was treated with anhydrous pyridine and Ac_2O (1:1) (4 mL) at room temperature overnight, after which the reaction mixture was evaporated to dryness under reduced pressure to give a quantitative yield of the corresponding monoacetate. The monoacetate (12 mg, 0.05 mmol), together with anhydrous *p*-TsOH (45 mg, 0.24 mmol) in dry C_6H_6 (3 mL) was refluxed for 0.5 h, after which the reaction mixture was concentrated under reduced pressure and the product subjected to reversed-phase HPLC (Phenomenex Spherex 5 μ C_{18} 250 × 10-mm column, 100% MeOH at 2 mL/min) to yield (+)-drimenyl acetate (**6**) as a stable colorless oil (2 mg, 15%): $[\alpha]_D$ +6° (*c* 0.05, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.81, 0.86, and 0.88 (3s, Me-11, Me-12, and Me-14), 1.66 (s, Me-13), 2.03 (s, OAc), 4.08 (dd, J = 6, 12 Hz, H-15$_a$), 4.25 (dd, J = 3, 12 Hz, H-15$_b$), 5.50 (br s, H-7); EIMS (70 eV) *m*/*z* 264 (M^+, 3), 221 (M − Ac, 3), 205 (M − OAc, 4), 191 (M − CH_2OAc, 9), 155 (38), 109 (26), 56 (100).

(−)-Drimenol (7). Drimenyl acetate (**6**) (2 mg), together with K_2CO_3 (26 mg) and MeOH (2 mL), was stirred at room temperature under N_2 for 3 h, after which time the contents were evaporated under reduced pressure. Extraction with petroleum spirits (60−80 °C) resulted in a quantitative yield of (−)-drimenol (**7**) as a stable colorless oil: $[\alpha]_D$ −14° (*c* 0.28, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.85, 0.86, and 0.88, (3s, Me-11, Me-12, and Me-14), 1.78 (s, Me-13), 3.85 (dd, J = 5, 11 Hz, H-15$_a$), 3.74 (dd, J = 3, 11 Hz, H-15$_b$), 5.54 (br s, H-7); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 132.8 (s, C-8), 124.2 (d, C-7), 60.9 (t, C-15), 57.3 (d, C-5), 49.9 (d, C-9), 42.1 (t, C-3), 39.9 (t, C-1), 33.4 (2s, C-4 and C-10), 32.9 (q, C-11), 23.6 (q, C-13), 22.1 (t, C-6), 21.9 (q, C-12), 18.8 (t, C-2), 14.9 (q, C-14); EIMS (70 eV) *m*/*z* 222 (M^+, 31), 207 ($M^+ - CH_3$, 17), 205 ($M^+ - OH$, 9), 192 ($M^+ - 2 \times CH_3$, 10), 176 ($C_{13}H_{20}^+$, 6), 147 ($C_{11}H_{15}^+$, 16), 132 ($C_{10}H_{12}^+$, 26), 51 (100); HREIMS *m*/*z* 222.1977; calcd for $C_{15}H_{26}O$, 222.1983.

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