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A Racemic Diterpene from the Marine Bryozoan *Flustra foliacea*, Natural Product or Artefact?

Pia Bachmann Holst,[a] Uffe Anthoni,[a] Carsten Christophersen,*[,a] Per Halfdan Nielsen[a] and Klaus Bock[b]

[a]Marine Chemistry Section, The H.C. Ørsted Institute, University of Copenhagen, Universitetsparken 5, DK-2100 Copenhagen, Denmark and [b]Carlsberg Laboratory, Department of Chemistry, Gamle Carlsberg Vej 10, DK-2500 Valby, Denmark

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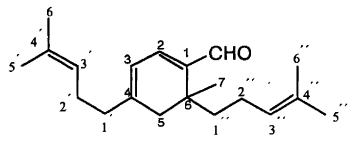
A monocyclic diterpene aldehyde, 4,6-bis(4-methylpent-3-en-1-yl)-6-methylcyclohexa-1,3-diene-1-carbaldehyde (**1**), was isolated from the marine bryozoan *Flustra foliacea* (L.) by gas phase extraction. The structure was established from extended spectroscopic studies allowing a complete assignment of ^{1}H and ^{13}C NMR spectra. Three isomeric trace diterpenes were detected in the extract. Since **1** was racemic it might arise from condensation of citral occurring during work-up of the extract. In support of this hypothesis, **1** was formed as the main product on base-catalyzed dimerisation of citral *in vitro* and the three isomeric compounds were formed as by-products or arose from decomposition of **1**.

The presence of terpenoids has so far been established in four species of marine bryozoans. The reported compounds include four monoterpenes in *Flustra foliacea* (L.),[1] five di- and two tri-terpenes in *Bugula turrita*,[2] and five sterols from *Myriopora truncata*.[3] More than one hundred compounds, mainly terpenoids, are present in *Conopeum seuratum*,[4] and of these 14 monoterpenes, six diterpenes and one triterpene have been identified. The terpenes may not be true bryozoan metabolites, but could originate (directly or by transformation) from the diet of these filter-feeding organisms[4] or could be synthesized by associated microorganisms.[5] We have reinvestigated the volatile terpene fraction from *F. foliacea*. We report the isolation and structure elucidation of **1**, the characterization of three diterpenoids isomeric with **1** and the characterization of 6-methyl-5-hepten-2-one. All these compounds are also known from base-catalyzed condensation of citral.



An ether extract of *F. foliacea* was obtained using a Likens and Nickerson apparatus.[6] This extraction method is a continuous steam distillation in a closed system at ambient pressure, and with concurrent continuous extraction of the distillate with ether. The concentrated ether extract was subjected to column chromatography. This led to isolation of a diterpene, assigned the structure of 4,6-bis(4-methylpent-3-en-1-yl)-6-methylcyclohexa-1,3-diene-1-carbaldehyde (**1**), based on spectroscopic studies. The same product was isolated by room temperature extraction of initially frozen bryozoan followed by chromatographic separation. The molecular formula was inferred from HREIMS (found m/z 286.2294, calc. for $C_{20}H_{30}O$ 286.2296). A ^{13}C NMR signal at 193.2 ppm characteristic of an aldehyde carbon together with signals for further eight sp^2 hybridized carbon atoms indicate a monocyclic diterpene aldehyde. The UV spectrum of **1** suggested the aldehyde group to be doubly conjugated in agreement with the proton chemical shifts of CHO, H-2 and H-3. Owing to coinciding chemical shifts a COSY experiment served only to confirm the sequence H-2–H-3, reveal the geminal couplings for the H-5 methylene protons, and connect H-1'' with H-2''. A COSYLR experiment identified the structural unit 3–4–5–1' (correlation H-3/H-5 and H-3/H-1'), and both terminal isoprene groups (correlation H-3''/H-5'', H-3''/H-2'', H-3''/H-6'', and H-3'/H-2', H-3'/H-5', H-3'/H-6', respectively). These results were confirmed and the total skeletal sequence CHO–1–2–3–4(–1'–2'–3')–5–6(–7)–1''–2''3'' established in an INADEQUATE experiment. COLOC results gave the final sequence CHO–1–6 defining unambiguously the structure of **1**.

* To whom correspondence should be addressed.

Although chiral, **1** was devoid of optical activity as determined by optical rotation and circular dichroism measurements. To prove the presence of both enantiomers in the product the ^{1}H NMR behavior with addition of the optically active chemical shift reagent Eu(fod)$_3$ was studied. The results proved beyond doubt the presence of diastereomeric complexes thus confirming the racemic nature of the product. In addition three isomeric diterpenes and 6-methyl-5-hepten-2-one was detected in the extract by GC–MS and ^{1}H NMR analyses.

In different collections the previously reported monoterpenes,[1] (*Z*)- and (*E*)-citral, geraniol and citronellol were always present while nerol was sometimes absent. In some collections citronellol was the major monoterpene in contrast with geraniol in others. A few terpenes were tentatively identified by GC–MS and comparison with library MS data: 3-methyl-2-(3-methyl-2-butenyl)furan (rosefuran), 3-(4-methyl-3-pentenyl)furan and 3,7-dimethyl-6-octenal (citronellal). Some of the terpenes isolated from *F. foliacea* might originate from an associated green alga, *Epicladia flustra*.[7]

The terpene **1** has been available since 1898 as a synthetic product formed by condensation of citral.[8] Later **1** was obtained from condensation of citral with potassium hydroxide in toluene in 60% yield.[9] The latter compound had ^{1}H and ^{13}C NMR data almost identical with those of our sample. Taneja *et al.*[10] using sodium hydride in ether reported an almost quantitative yield in the same reaction of two compounds one of which (5% of the mixture) was believed to have structure **1**. The latter compound was not isolated in a pure state. In a later paper[11] the same authors isolated **1** as a by product in the reaction between citral, methyl acrylate and sodium hydride. However, the ^{1}H NMR data differ considerably from those of **1**. To clarify the course of this reaction we reproduced the condensation of citral with sodium hydride in dry ether and obtained **1** as the main product confirming the earlier data and structure.[9] Examination of the crude product revealed the presence of three diterpenes isomeric with **1** and 6-methyl-5-hepten-2-one, all identical with the compounds detected in the bryozoan extract.

In conclusion, therefore, we cannot unambigously decide whether **1** is an artefact formed from citral during collection, freezing, storage and extraction of the bryozoan or one of the rare examples of a racemic natural product. Although several examples of more or less racemic metabolites[14–14] are known the absence of optical activity in a chiral natural product is usually a strong indication of artefact formation.

Experimental

GC–MS results were recorded at 70 eV, 250 mA, 1s scan^{-1} at 250°C on a VG 7070F instrument and GC traces at 248°C on a Hewlett Packard 5890A instrument, equipped with a flame ionisation detector. Both GC–MS and GC were programmed from 35°C to 60°C at 30°C min^{-1} and from 30°C to 300°C (5 min) at 15°C min^{-1} using a cross-linked 5% phenyl methyl silicone column (25 m × 0.2 mm i.d.) and helium as the carrier gas. Mass spectra originate from a JEOL JMS-HX/HX110A tandem mass spectrometer. High resolution data were obtained by peak matching. ^{1}H and ^{13}C NMR spectra were recorded on a Varian 400 FT-NMR spectrometer at 400 MHz and 100.6 MHz for ^{1}H and ^{13}C, respectively, HETCOR, COSY, COSYLR, NOEDIFF, COLOC, DEPT and INADEQUATE experiments were recorded on a Bruker 600 MHz instrument. All NMR spectra were recorded in $CDCl_3$ and chemical shifts are reported downfield from internal TMS. UV spectra were recorded on a Hewlett Packard 8452A diode array spectrophotometer. TLC was performed on silica gel 60 F_{254} Merck plates using petroleum ether–EtOAc (99.5:0.5) as the eluent.

Biological material. The bryozoan was collected in the North Sea near Harboøre Tange on the Danish west coast in April 1993 and kept frozen until use.

Extraction and isolation. Frozen *F. foliacea* (wet wt. ca. 6 kg) was subjected in portions (10 × 600 g) to gas phase extraction with diethyl ether (10 × 15 ml) for 4 h using a Likens and Nickerson apparatus modified as described previously.[6] The combined extracts (150 ml) were dried ($MgSO_4$) and concentrated with nitrogen to give an oil (ca. 1 g). This was separated into seven fractions by column chromatography (silica gel, Lobar Merck, size C; petroleum ether–EtOAc 99.5:0.5). Column chromatography of the first fraction (silica gel, Lobar Merck, size B; petroleum ether–EtOAc 99.5:0.5) yielded 149.2 mg (0.0025% of wet wt.) pure **1** as a yellow oil in addition to three isomers and 6-methyl-5-hepten-2-one. The same result was obtained by room temperature EtOAc extraction of frozen material.

4,6-Bis(4-methylpent-3-en-1-yl)-6-methylcyclohexa-1,3-diene-1-carbaldehyde (**1**). MS *m/z* (% rel. int.): 286 (6, M^+), 271 (2, $M^+ - CH_3$), 257 (1, $M^+ - CHO$), 243 (2, $M^+ - C_3H_7$), 217 (4, $M^+ - C_5H_9$), 203 (100, $M^+ - C_6H_{11}$), 134 (20), 119 (14), 105 (68), 91 (23), 83 (17), 69 (50), 55 (29). For ^{1}H and ^{13}C NMR data see Table 1. UV λ_{max}/nm (EtOH) (log ε) = 318 (6.3), 262 (5.9). Optical rotation, $[\alpha]_D^{20}$ 0° and CD, Δε = 0 between 1190 and 500 nm, $c = 0.055$ g l^{-1}. Several ^{1}H NMR spectra were recorded of a $CDCl_3$ solution of **1** with Eu(fod)$_3$ added in cumulative amounts. The series of spectra revealed a doubling of several signals, e.g., the aldehydic proton resonance, owing to the formation of two diastereomeric complexes.

Condensation of citral. A suspension of oil-free sodium hydride (700 mg) in dry diethyl ether (100 ml) was stirred at room temperature while citral [(Z)- + (E)-3,7-dimethyl-2,6-octadienal, Fluka, 5.6 ml] was added. The mixture was boiled under reflux for 0.5 h and stirring was

Table 1. ^{1}H and ^{13}C NMR data of **1**.

Position	^{13}C [a]	INADEQUATE [b]	COLOC [b]	^{1}H [c]	COSY [b]	COSYLR [b]	NOE [b]
CHO	193.15 d	141.38	9.43	9.43 (1 h, s)			6.68
1	141.38 s	193.15, 145.75	9.43				
2	145.75 d	141.38, 117.88	9.43, 5.94	6.68 (1 H, d)[d]	5.94		9.43, 5.43
3	117.88 d	145.75, 150.70	5.94	5.94 (1 H, d)[d]	6.68	2.37, 2.03, 2.20	6.68, 2.20
4	150.70 s	117.88, 37.67, 41.85					
5a	41.85 t	150.70, 36.15		2.37 (1 H, d)[e]		5.94, 2.03	2.03, 5.94
5b				2.03 (1 H, d)[e]		5.94, 2.37	2.37, 1.20
6	36.15 s	41.85, 38.28, 25.13	9.43				
7	25.13 q	36.15		1.20 (3 H, s)			2.03, 1.90/ 1.89,[g] 1.39
1′	37.67 t	150.70, 25.54		2.20 (2 H, s)		5.94	5.94
2′	25.54 t	37.67, 123.18		2.20 (2 H, s)		5.10	1.63
3′	123.18 d	25.54		5.10 (1 H, t)[f]		2.20, 1.69, 1.63	1.69, 2.20
4′	132.22 s						
5′	25.54 q			1.69 (3 H, s)		5.10	5.10
6′	17.65 q			1.63 (3 H, s)		5.10	2.20
1″a	38.28 t	36.15, 25.54		1.90 (1 H, m)	1.39, 1.84, 1.89		
1″b				1.39 (1 H, m)	1.90, 1.84, 1.89		1.90, 1.84
2″a	25.54 t	38.28, 124.70		1.89 (1 H, m)	1.39, 1.90, 1.84	5.07	
2″b				1.84 (1 H, m)	1.39, 1.90, 1.89	5.07	2.37
3″	124.70 d	25.54		5.07 (1 H, t)[f]		1.65, 1.84, 1.89, 1.56	1.65
4″	131.02 s						
5″	25.54 q			1.65 (3 H, s)		5.07	5.07
6″	17.47 q			1.56 (3 H, s)		5.07	

[a] Measured at 100.6 MHz in $CDCl_3$. s, d, t or q was determined by a DEPT experiment and refers to singlet, doublet, triplet and quartet, respectively. [b] Connectivity. [c] Measured at 400.0 MHz in $CDCl_3$. [d] J=5.5 Hz. [e] J=17.5 Hz. [f] J=6.8 Hz. [g] Either 1″a or 2″a.

continued for a further 2 h at room temperature. A 10% solution of NH_4Cl (2 ml) was added. The ether phase was washed to neutrality with water, dried ($MgSO_4$) and evaporated *in vacuo* yielding 4.01 g residue. Investigations of this residue revealed the presence of the same four isomers as reported above and 6-methyl-5-hepten-2-one (GC–MS, ^{1}H NMR) as detected in the extract of the bryozoan. A sample (500 mg) was purified by preparative column chromatography (LiChroprep Si 60 40–63 mm, EtOAc–heptane 9:1) yielding pure (GC) **1** (160 mg, 80%).

Isomeric diterpenes. Three diterpenes were characterized from the bryozoan extract and the synthetic product. The diterpenes from the natural source and from the synthetic preparation were identical. Since the compounds were present in only small amounts they were not purified to homogeneity. They all contain the conjugated double bond system and the aldehyde group. Chemical shifts and coupling constants for these structural elements are given below. The following data was obtained from GC–MS and ^{1}H NMR spectroscopy on partly purified mixtures:

Diterpene. MS [*m/z* (% rel. int.)] 286 (5, M^+), 203 (100, $M^+ - C_6H_{11}$), 134 (25), 119 (29), 105 (79), 91 (36), 69 (97), 55 (32). ^{1}H NMR: δ 9.41 (1 H, s), 6.67 (1 H, d, J = 5.7 Hz), 5.95 (1 H, d, J = 5.7 Hz).

Mixture of two diterpenes. From the NMR integration the ratio between the two components was determined to be 60:40. One component, MS [*m/z* (% rel. int.)] 286 (18, M^+), 203 (8), 175 (12), 159 (13), 147 (19), 135 (23), 119 (26), 105 (35), 91 (42), 83 (70), 69 (100), 55 (79) the other component 286 (4, M^+), 217 (4, $M^+ - C_5H_9$), 203 (52, $M^+ - C_6H_{11}$), 189 (5), 175 (8), 161 (8), 145 (15), 133 (18), 119 (19), 105 (68), 91 (37), 83 (29), 69 (100), 55 (51). ^{1}H NMR: δ one compound 9.48 (1 H, s), 7.22 (1 H, d, J = 11.7 Hz), 6.17 (1 H, d, J = 11.7 Hz), the other compound 9.42 (1 H, s), 7.07 (1 H, d, J = 11.5 Hz), 6.32 (1 H, d, J = 11.5 Hz).

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