Giffordene, 2Z, 4Z, 6E, 8Z-undecatetraene, is the odoriferous principle of the marine brown alga *Giffordia mitchellae*[1]

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Summary. Giffordene, 2Z, 4Z, 6E, 8Z-undecatetraene, and five stereoisomers are new odoriferous $C_{11}H_{16}$-hydrocarbons from the marine brown alga *Giffordia mitchellae*. Their isolation and identification is described.
Key words. Giffordene; 2,4,6,8-undecatetraenes; 1,3,5-undecatrienes; *Giffordia mitchellae;* chemotaxis; 1,7-sigmatropic hydrogen shift; biosynthesis of giffordene.

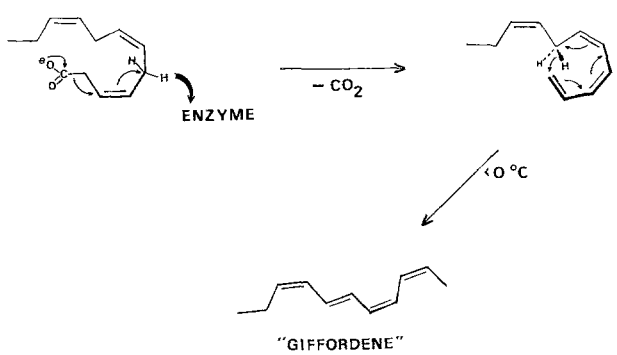
A number of marine brown algae produce odoriferous substances which – inter alia – contribute to the typical 'ocean smell'. Members of the brown algal genus *Dictyopteris* are particularly rich in odoriferous compounds[3–5]. Some of them are linear or alicyclic $C_{11}H_{14}$ to $C_{11}H_{18}$ hydrocarbons which serve as mating pheromones in the sexual cycles of various other brown algae[6,7].

An example of such an odoriferous compound with biological activity as a sexual pheromone is ectocarpene, (+)-(6S)-(1Z-butenyl)-2,5-cycloheptadiene, secreted by female gametes of the primitive brown alga *Ectocarpus siliculosus*[8]. *Giffordia mitchellae* (Harvey) Hamel is a closely related, but monoecious and anisogamous species. Laboratory cultures of gametophytes and sporophytes develop an aromatic odor, and large numbers of spermatozoids are attracted by female gametes (fig. 1). These observations suggested the search for a low-molecular volatile pheromone in female gametes of *G. mitchellae*.

Sporophyte and predominantly unisexual gametophyte cultures were selected from isolates collected in North Carolina (USA) in 1963[9], and grown in enriched natural seawater (Provasoli-ES[10]). Volatiles were collected from these cultures over a two week period by the 'closed-loop-stripping' technique[7,11,12]. Eluates from the carbon filters (30 µl CH_2Cl_2) were resolved by gas-chromatography into 9 distinct substances, amounting to about 10 µg per extract (fig. 2).

The major constituent and the closely eluting minor compounds exhibited virtually identical mass fragmentation patterns m/e = 148 (28% M^+), 133(9), 119(41), 105(32), 91(100), 79(32), 77(31), 65(17), 55(33), 41(68) similar to other $C_{11}H_{16}$-pheromones from marine brown algae[6]. Microhydrogenation with Pt/C indicated four double bonds in these compounds and yielded n-undecane (M^+ = 154) as the only reaction product. GC/FTIR-analysis proved the major constituent to be an unsaturated hydrocarbon with E- and Z-double bonds. Its UV-spectrum with maxima at 289, 302 and 317 nm, respectively indicated a conjugated tetraene system. However, Kovàts-indices[13] and the UV spectrum did not correspond to any of the known 1,3,5,7-undecatetraenes[14]. Instead, the more bathochromic absorption of the *Giffordia* hydrocarbon pointed to a 2,4,6,8-undecatetraene. We therefore selectively synthesized 10 of the 16 possible stereoisomeric 2,4,6,8-undecatetraenes[15]. Gas-chromatographic comparison of the natural compounds with the synthetic references on two columns of different polarity gave evidence that the main product of *G. mitchellae* is the 2Z,4Z,-6E,8Z-undecatetraene, for which we propose the trivial name 'giffordene'. The stereoisomers **5**, **7**, **8**, **9**, **13** and the trienes **1–3** are present but in traces (table and fig. 2).

Bioassays with male gametes of *G. mitchellae* and the synthetic 2,4,6,8-undecatetraenes or the original carbon eluates did not



Gaschromatographic identification of isomeric 2,4,6,8-undecatetraenes and 1,3,5-undecatrienes from *Giffordia mitchellae*

Compound[a] No.		Occurrence in *Giffordia*	Retention indices SE 30 110 °C	OV 17[b] 90 °C
1	1,3E,5Z	+	1164.2 ± 0.1 (1164.3 ± 0.1)	1224.2 ± 0.5 n.r.
2	1,3E,5E	+	1173.5 ± 0.1 (1173.6 ± 0.1)	1215.9 ± 0.3 (1215.5 ± 0.5)
3[c]	1,3Z,5Z	+	1217.9 ± 0.10 (1217.9 ± 0.10)	1275.8 ± 0.3 (1275.5 ± 0.1)
4	2E,4E,6E,8E	–	1270.2 ± 0.1 (1270.2 ± 0.1)	1347.3 ± 0.4
5	2E,4E,6E,8Z	+	1270.2 ± 0.1 (1270.2 ± 0.1)	1345.0 ± 0.2 (1345.3 ± 0.2)
6	2E,4Z,6E,8Z	+	1272.4 ± 0.1 (1272.4 ± 0.1)	1353.3 ± 0.3 n.r.
7	2E,4E,6Z,8Z	–	1274.7 ± 0.1	1351.4 ± 0.4
8	2E,4Z,6E,8E	+	1275.0 ± 0.1 (1275.1 ± 0.1)	1353.0 ± 0.2 (1352.6 ± 0.4)
9	2Z,4E,6E,8Z	+	1279.2 ± 0.1 (1279.2 ± 0.1)	1355.9 ± 01. (1355.8 ± 0.2)
10	2Z,4E,6E,8E	–	1282.8 ± 0.1	1358.4 ± 0.1
11	2Z,4Z,6E,8Z Giffordene	+ + +	1283.2 ± 0.1 (1283.2 ± 0.1)	1362.9 ± 0.2 (1362.7 ± 0.3)
12	2Z,4E,6Z,8Z	–	1284.6 ± 0.1	1363.0 ± 0.3
13	2Z,4Z,6E,8E	+	1285.1 ± 0.1 (1285.1 ± 0.1)	1362.9 ± 0.2 (1362.7 ± 0.3)

Kovàts-indices were determined at properly adjusted isothermal temperature levels indicated in the table. [a] Compounds are numbered according to their elution order on SE 30 (c.f. fig 2). [b] The separation on the more polar OV 17 column is incomplete for the isomeric 2,4,6,8-undecatetraenes. Poorly resolved compounds are indicated as n.r. = not resolved. [c] Kovàts-index corresponding to the rearranged 2Z,4Z,6E-isomer.

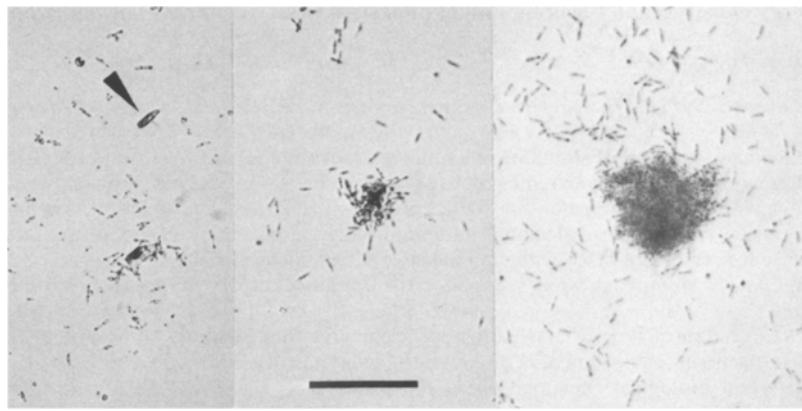


Figure 1. Sperm chemotaxis in *Giffordia mitchellae*. Motile female gamete (arrow, left) attracts increasing numbers of spermatozoids. After 4 min (center) and 8 min (right). Scale bar 100 µm.

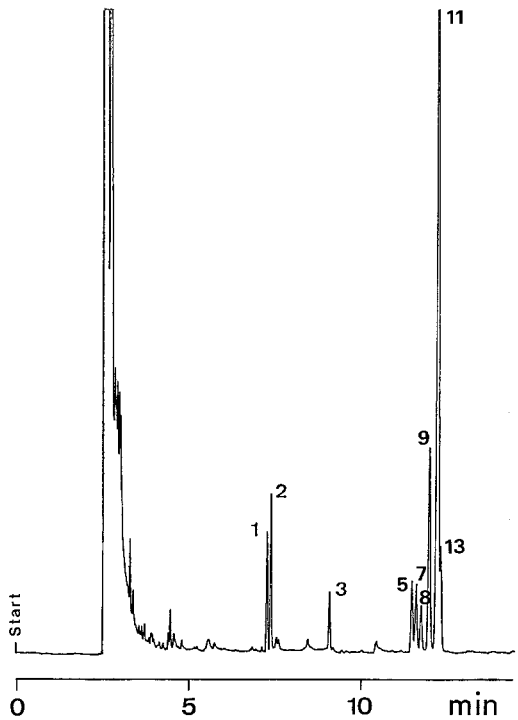


Figure 2. Gas-chromatographic separation of the *Giffordia* eluate. Compounds were separated on SE 30 (50 m × 0.31 mm) and are numbered according to their elution order (cf. table for identification). Conditions: 110 °C isotherm; H_2 = 0.6 bar (60 kPa); flame ionization detector (FID) 1 × 32, 220 °C; sample size 1 µl.

show chemotactic activity. Thus, the new C_{11} polyenes do not seem to act as sex attractants in *Giffordia*, and the structure of the extremely potent sperm attractant in *G. mitchellae* (fig. 1) is as yet unknown. In agreement sporophyte and gametophyte cultures with male or female gametes secrete the same bouquet of compounds without connection to reproductive events. 2,4,6,8-Undecatetraenes have to date not been isolated from marine brown algae or other sources, and the origin of the peculiar and very unfavorable 2Z,4Z,6E,8Z-geometry of giffordene is not immediately evident from current biogenetic concepts[16].

However, the same basic mechanism would apply for the biosynthesis of giffordene, if the newly formed double bond in position 3 of the enzymatically attacked precursor (3Z,6Z,9Z-dodecatrienoic acid) adopts Z-geometry as depicted in the scheme. The resulting 1,3Z,5Z,8Z-undecatetraene is thermally unstable (proven by independent synthesis[15], and easily, even below 0 °C, undergoes a thermally allowed antarafacial 1,7-sigmatropic hydrogen shift[18] to the 2Z,4Z,6E,8Z-isomer. This is additionally supported by the related, but thermally more stable 1,3Z,5Z-undecatriene[18] **3** (precursor: 3Z,6Z-dodecadienoic acid). This hydrocarbon elutes from the polar OV 17 column largely as the original, non-rearranged 1,3Z,5Z-isomer (90 °C isotherm; injection port: 150 °C) and confirms an 1,3Z,5Z,8Z-undecatetraene as a possible intermediate in the biosynthesis of giffordene.

The other isomers **5, 7, 8, 9** and **13** probably originate from giffordene by simple isomerization caused by the culture medium and illumination etc., since the synthetic reference also shows a comparable isomer distribution to that seen in *Giffordia* extracts after storage at r.t. and exposure to light. In contrast, adsorption to the charcoal filter during the 'stripping'-procedure (14 days!) almost completely retained the geometry of the adsorbed polyenes. This stabilization effect strongly recommends the versatile 'closed-loop-stripping' as a reliable and non-destructive extraction technique in natural product chemistry. The odor quality of giffordene may be characterized as 'sea algae' whereas the all-trans isomer **4** exhibits an almost oily, fatty linseed-like character. In general, the odor intensity of the 2,4,6,8-undecatetraenes is much lower than that of their corresponding 1,3,5,8-isomers.

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2 Author to whom correspondence should be addressed.
3 Pettus, J. A. Jr, and Moore, R. E., Chem. Commun. *1970*, 1093.
4 Kajiwara, T., Kodama, K., and Hatanaka, A., Bull. Jap. Soc. scient. Fish. *46* (1980) 771.
5 Moore, R. E., Accts chem. Res. *10* (1977) 40.
6 Jaenicke, L., and Boland, W., Angew. Chem. *94* (1982) 659; Angew. Chem. int. Ed. *21* (1982) 643.
7 Maier, I., and Müller, D. G., Biol. Bull. *170* (1986) 145.
8 Müller, D.G., Jaenicke, L., Donike, M., and Akintobi, T., Science *171* (1971) 815.
9 Müller, D. G., Naturwissenschaften *56* (1969) 220.
10 Starr, R. C., J. Phycol. *14*, Suppl. (1978) 47.
11 Grob, K., and Zürcher, F., J. Chromat. *117* (1976) 285.
12 Boland, W., Ney, P., Jaenicke, L., and Gassmann, G., in: Analysis of volatiles, p. 371. Ed. P. Schreier. Walter de Gruyter & Co., Berlin, New York 1984.
13 Kovàts, E., Helv. chim. Acta *41* (1958) 1915.
14 Näf, F., Decorzant, R., and Escher S.D., Tetrahedron Lett. *23* (1982) 5043.
15 Boland, W., Sieler, C., and Feigel, M., manuscript in preparation.
16 Müller, D. G., Z. PflPhysiol. *80* (1976) 120.
17 Boland, W., and Mertes, K., Eur. J. Biochem. *147* (1985) 83.
18 Näf, F., Decorzant, R., Thommen, W., Willhalm, B., and Ohloff, G., Helv. chim. Acta *58* (1975) 1016.

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