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BIOSYNTHESIS OF PHEROMONES IN FEMALE GAMETES OF MARINE BROWN ALGAE (PHAEOPHYCEAE).

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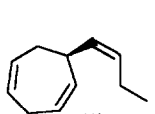
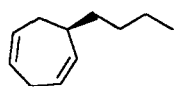
Abstract: Female gametes of the brown algae *Ectocarpus siliculosus* and *Sphacelaria rigidula* as well as thalli of *Giffordia mitchellae* metabolise externally added [^2H_n]icosanoic acids into the hydrocarbon pheromones ectocarpene (1), dictyotene (2) and finavarrene (5). The series of the $C_{11}H_{16}$ hydrocarbons originates from *all-cis*-5,8,11,-14,17-icosapentaenoic acid (7); the $C_{11}H_{18}$ compound dictyotene (2) is produced from *all-cis*-5,8,11,14-icosatetraenoic acid (8) (arachidonic acid). The key step in the biosynthesis of giffordene (6) is a thermally allowed [1,7]-hydrogen shift of an 1,3*Z*,5*Z*,8*Z*-undecatetraene (21) intermediate derived from 7.

The sexual reproduction of marine brown algae (Phaeophyceae) typically involves the fusion of a motile male and a sessile female sexual cell. This process is assisted by chemical signals which are released from the female to attract the conspecific males. In some cases the secreted compounds have a dual function: *i)* release of the male gametes from their gametangia (temporal synchronisation) and *ii)* attraction of the liberated males towards the calling female[1,2]. Most of these plant pheromones are simple acyclic or cyclic hydrocarbons with the molecular formulas $C_{11}H_{14}$, $C_{11}H_{14}O$, $C_{11}H_{16}$ and $C_{11}H_{18}$. For example, fertile female gametes of the cosmopolitan brown alga *Ectocarpus siliculosus* produce a bouquet of $C_{11}H_{16}$ compounds which consists of ectocarpene **(1)** (90.8%), dictyotene **(2)** (5.3%), multifidene **(3)** (1.7%), hormosirene **(4)** (0.7%) and finavarrene **(5)** (1.5%)[3] (cf. also Figure 1). Ectocarpene **(1)** and hormosirene **(4)** are the most attractive compounds showing threshold concentrations down to 0.1 → 1 nmol[4].



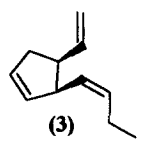
(1)

ectocarpene



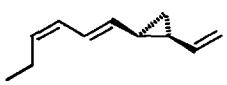
(2)

dictyotene



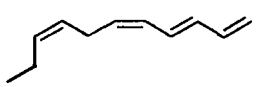
(3)

multifidene



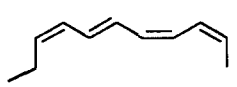
(4)

hormosirene



(5)

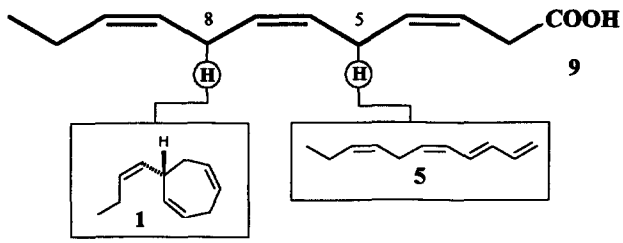
finavarrene



(6)

giffordene

The structures of **1** → **6** suggest a common biogenetic origin from fatty acids. For example the terminal (5*Z*,-8*Z*)-heptadienyl segment of finavarrene (**5**) matches the corresponding structural element of α-linolenic acid or icosa-5,8,11,14,17-pentaenoic acid (**7**) (Scheme 4). The (*Z*)-butenyl moiety of **1**, **3** and **4** fits with the same precursors, and the aliphatic terminus of the $C_{11}H_{18}$ hydrocarbon dictyotene (**2**) could originate from linoleic acid or arachidonic acid (**8**). However, irrespective of these very conspicuous relationships, previously the somewhat difficult culture conditions to obtain eggs or female gametes hampered biosynthetic studies with the reproductive cells of the seaweeds. Instead, the terrestrial plant *Senecio isatideus* (Asteraceae) which produces in its leaves large amounts of ectocarpene (**1**) accompanied by small amounts of dictyotene (**2**) and finavarrene (**5**)[5] served as the first model system for studying the biosynthesis of algal pheromones. Feeding studies with deuterium labelled precursors and freshly cut plantlets of *S. isatideus* confirmed, indeed, unsaturated fatty acids as the source for the biosynthesis of the C_{11} hydrocarbons. The immediate precursor for the two $C_{11}H_{16}$ hydrocarbons **1** and **5** is 3*Z*,6*Z*,9*Z*-dodeca-3,6,9-trienoic acid (**9**), and the $C_{11}H_{18}$ compound **2** originates from the corresponding 3*Z*,6*Z*-dodeca-3,6-dienoic acid[6].

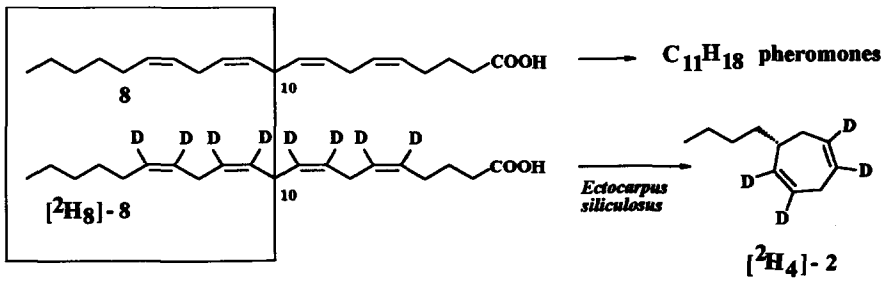


The unsaturated C_{12} fatty acids are derived from the corresponding C_{18} precursors by three ß-oxidation cycles[7]. Although the actual mechanism of the oxidative decarboxylation of **9** yielding the C_{11} hydrocarbons and a C_1 fragment is, as yet, not fully understood, it was demonstrated that linear hydrocarbons like finavarrene (**5**) are produced from **9** by loss of C(1) and one of the two enantiotopic hydrogen atoms from C(5). The biosynthesis of the cyclic hydrocarbon **1** is accomplished by decarboxylation and loss of the C(8)-H_R hydrogen atom[8]. A unified mechanism and a product genealogy in relation to the corresponding C_{12} precursor has been developed and published[9] (cf. Scheme 1). However, all attempts to reproduce these results with female gametes of brown algae failed. We now report that the biosynthesis of C_{11} hydrocarbons in marine brown algae does, indeed, not follow the same pathway as in the terrestrial plant *S. isatideus* but represents a novel, hitherto not known metabolism of icosanoids[10].

Feeding experiments with female gametes of *Ectocarpus siliculosus*.- Unlike higher plants and most other marine algae, the archetype brown algae are known for their rich content of icosanoids[11]. Recent analyses have shown that this is also true for the fatty acid contents of membranes of male- and female gametes of *E. siliculosus*. In particular, certain phospholipids of the plasma membrane of the female sexual cells contain up to 60% of the icosapentaenoic acid (**7**) and ca. 20% of arachidonic acid (**8**)[12]. Since the positions of double bonds within these fatty acids match the requirements as precursors for the C_{11} pheromones, [$^2H_8$]-arachi-

donic acid (**8**) was administered to a suspension of female gametes of *E. siliculosus*. Following 30 min of pre-incubation, the released volatiles were continuously trapped onto activated carbon by air circulation within a closed system (ca. 15 ml total volume; see experimental). Mass spectroscopic analysis of the collected volatiles revealed that [$^2H_8$]-arachidonic (**8**) acid is, indeed, metabolized and yields [$^2H_4$]-dictyotene (**2**). The efficiency of this transformation is unusually high, since the *de novo* synthesis of [$^2H_4$]-(**2**) exceeds the level of the natural trace constituent [^1H]-**2** by ca. 800% (calculated from the relative abundance of the molecular ions at 150 and 154 Da of the coeluting hydrocarbons). Due to the positions of the double bonds in the precursor acid and the preservation of only four deuterium atoms in the product, the aliphatic segment C(10) → C(20) of [$^2H_8$]-**8** must have been incorporated into [$^2H_4$]-**2** as outlined in Scheme 1.

Scheme 1

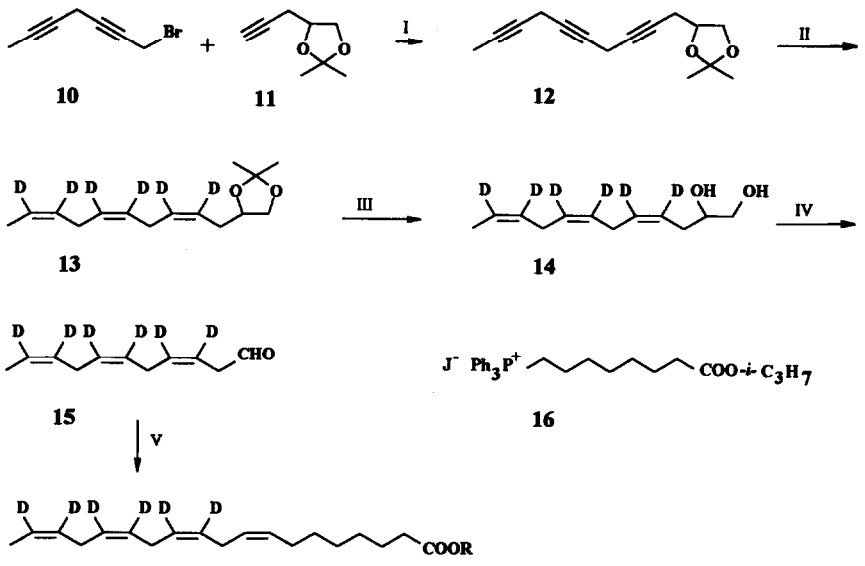


Accordingly, the more highly unsaturated $C_{11}H_{16}$ hydrocarbon ectocarpene (**1**) should arise from the aliphatic segment C(10) → C(20) of the higher unsaturated *5Z,8Z,11Z,14Z,17Z*-icosapenta-5,8,11,14,17-enoic acid (**7**). However, due to the very large amount of [^1H]-**1** within the blend of the signal compounds released from the female gametes of *E. siliculosus* (cf. Figure 1A), it is advisable to use a structurally modified [^2H]-precursor which avoids superposition of the usually very small amounts of the artificial metabolite(s) (0.1 → 0.5 μg) by the natural product(s). The required structural label is most conveniently achieved by modification of the aliphatic terminus of the natural substrate leading to *homo-* or *nor*-fatty acids possessing the same arrangement of double bonds. The strategy has been already successfully employed to unravel the biosynthesis of ectocarpene (**1**) in leaves of the higher plant *S. isatideus*[6] (*vide supra*). The shortened aliphatic terminus of the precursor results in the production of the labelled *nor*-ectocarpene (**19**) which is readily separated from **1** by gas chromatography. Moreover, in the case of the $C_{11}H_{16}$ hydrocarbons of the type **1**, **3** and **4** the deuterium label of a precursor like **18** is of particular advantage, since the metabolites exhibit a unique mass fragmentation pattern which allows for a positional analysis of the deuterium isotopes (cf. Scheme 3; mass spectra of $C_{11}H_{18}$ compounds are much less informative[6]). Since the C(5)=C(6) double bond of arachidonic acid [$^2H_8$]-**8** appears to be not essential for the conversion into dictyotene **2**, the tetraenoic C_{19} acid **18** was designed as a substitute for the natural substrate icosapentaenoic acid (**6**).

The synthesis of deuterium labelled **18** is briefly outlined in Scheme 2. The central intermediate, namely [$^2H_6$]-dodeca-3,6,8-trienal (**15**), is readily available *via* the acetylenic approach. Thus, the copper catalysed alkylation of the magnesium salt of **11**[13,14] with **10** provides the 1.3-dioxolane **12** in 62% yield. Introduc-

tion of deuterium is achieved with D_2 and *Lindlar's* catalyst (≥ 95% $^2H_6$; 68% yield). Removal of the protective group and cleavage of the resulting diol **14** with $NaIO_4$ under neutral conditions generates **15**. Subsequent *Wittig*-olefination of **15** with the ylid derived from the phosphonium salt **16**[15] (*t*-BuLi; THF, -78°) furnishes the ester **17**, and saponification with MeOH/NaOH yields the acid **18** in high configurational purity (> 95% according to GC; 70% yield).

Scheme 2



10 **11** **12** **13** **14** **15** **16**

17 R = -i-C_3H_7 I) EtMgBr, Cu(I). II) D_2, *Lindlar's cat.* III) H^+ IV) $NaIO_4$.

18 R = H V) 1. t-Buli, 2. MeOH / NaOH.

The volatiles produced during the feeding experiment with **18** and female gametes of *E. siliculosus* were collected, and the resulting extract was analysed by gas chromatography. Besides the natural C_{11} hydrocarbons **1**, **2**, **4** and **5** two additional compounds were identified by mass spectroscopy as the *nor*-ectocarpene (**19**) and the *nor*-finavarrene (**20**), respectively. The amount of the two labelled C_{10} metabolites **19** and **20** is about 10% in comparison to the natural C_{11} hydrocarbon **1** (cf. Figure 1A). The mass spectrum of **19** (Figure 1B) displays an intense molecular ion at m/z = 140 Da indicating, that all six deuterium atoms of the precursor **18** have been incorporated into the *nor*-ectocarpene (**19**). The fragment ions **(a)** (m/z = 68 Da), **(b)** (m/z = 72 Da) and **(d)** (m/z = 96 Da), which originate from **19** by defined pathways (cf. Scheme 3; Figure 1B) and without scrambling of the isotopes, are in agreement with the positioning of the deuterium atoms at the double bonds of the metabolite. **(a)** and **(b)** arise from all $C_{11}H_{16}$ compounds of the type **1** and **3** *via* a cyclopropyl intermediate like **4** followed by a [1,5]-hydrogen shift and ß-cleavage. Dependent on the isotopic substitution, either hydrogen- or deuterium atoms are transferred from the cyclopropane moiety towards fragment **(b)** and, hence, these fragments can be reliably used for positioning of the isotopes. Fragment **(d)** is the product of a 1,3-hydrogen shift within the C_4 side chain followed by ß-cleavage. The validity of this concept has been

proven with ca. 30 structural analogues of **1**, **3** and **4** which consistently decay into fragment ions corresponding to **(a)**, **(b)**, **(c)** and **(d)**[16]. Moreover, at low electron impact (17 eV) the fragmentation pathway outlined in Scheme 3 becomes the dominant reaction channel and demonstrates that the sequence of ring contraction, [1,5]- and [1,3]-hydrogen shifts in conjunction with ß-cleavage is energetically favoured.

Scheme 3

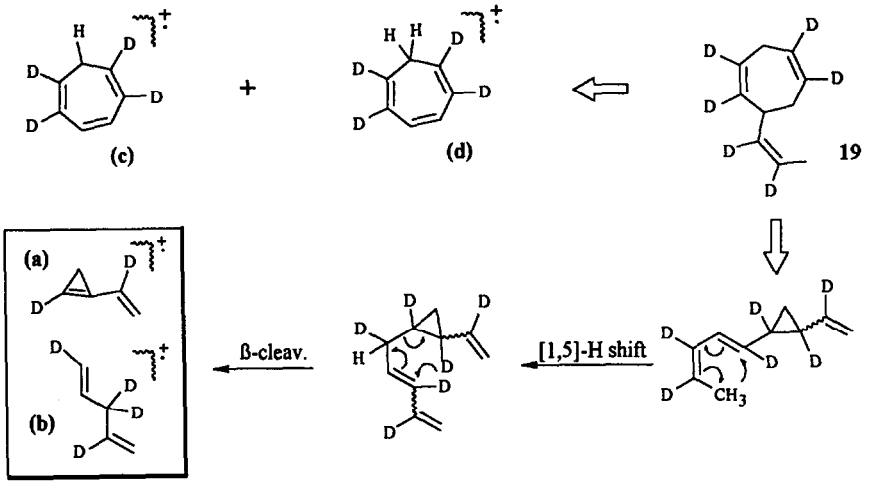


Figure 1

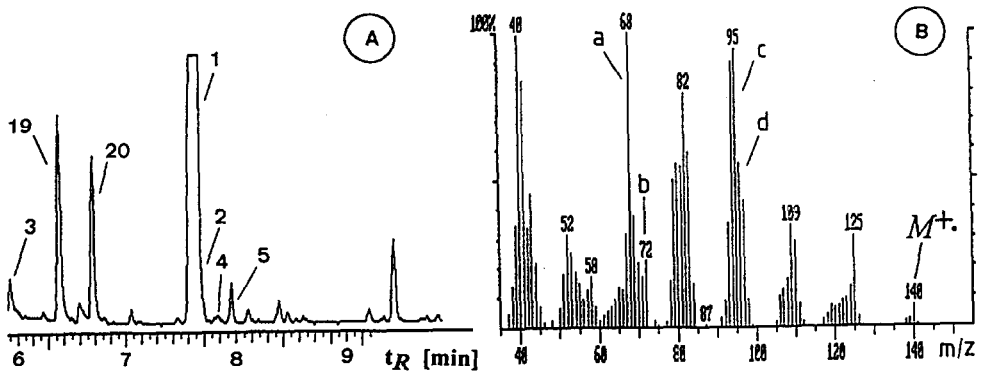
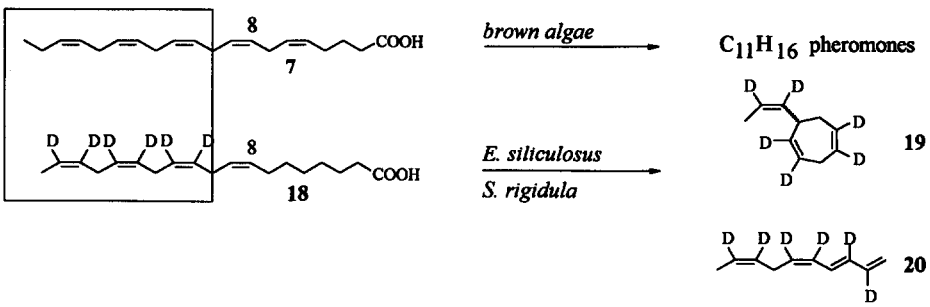


Figure 1. **(A)**: Section of the gas chromatogram of the volatiles obtained from female gametes of *E. siliculosus* after incubation with **18**. **1**, **2**, **3** and **5** are natural C_{11} compounds. *Nor*-ectocarpene **(19)** and *nor*-finavarrene **(20)** are labelled metabolites of **18**. A similar profile of products is observed after administration of **18** to female gametes of the marine brown alga *Sphacelaria rigidula*. GC conditions: *RSL 300* (30 m x 0.32 mm; *Alltech*, Munich (FRG)) under programmed conditions (40°C for 2 min, then at 10°C min^{-1} to 250 °C). Detection and identification of compounds: *Finnigan* ion trap *ITD 800* (transfer line: 270°, scan range: 35 - 250 Da/sec). **(B)**: Mass spectrum of the metabolite **19**. The fragments **(a)**, **(b)**, **(c)** and **(d)** are indicated and correspond to the fragmentation pathway outlined in Scheme 3.

In agreement with Scheme 3, the mass numbers of the fragment ions **(a)**, **(b)**, **(c)** and **(d)** of **19** are consistent with an incorporation of the C(10) → C(20) segment of **18** into the metabolite. Formally, this is achieved by bond cleavage between C(9) and C(10) of the acid **18** and ring closure between C(10) and C(16) of the precursor. The second metabolite **20** also displays a molecular ion at m/z = 140 Da corresponding to the molecular formula $C_{10}H_8{}^2H_6$. However, the spectrum is different from that of **19** and reflects the typical fragmentation pattern[6] of acyclic olefins like finavarrene (**2**). The reason for the over proportional production of the acyclic tetraene **20** (compare the ratio of natural **1/5**; Figure 1) is as yet unknown, but corresponds to previous findings with the terrestrial plant *S. isatideus* and deca-3,6-dienoic acid instead of the natural C_{12} acid **9**. As a matter of fact, in the higher plant the short chain C_{10} acid yields 3*E*,5*Z*-nona-1,3,5-triene as the only product; the expected 6-ethylcyclo-1,4-heptadiene was not found[6].

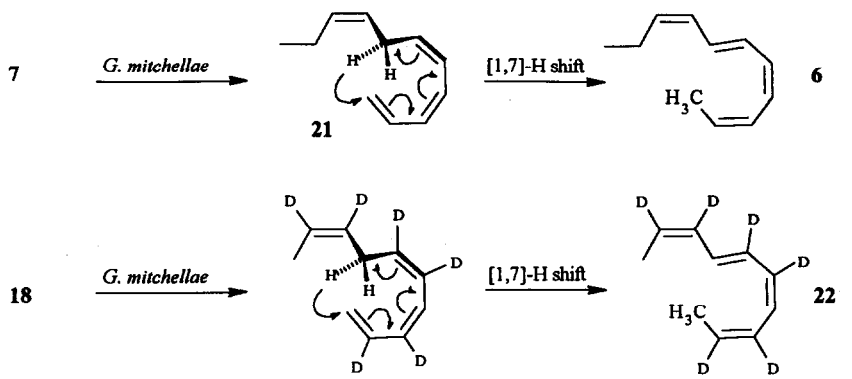
Feeding experiments with female gametes of *Sphacelaria rigidula*, another brown alga[17], and **18** resulted in the same pattern of labelled metabolites **19** and **20** and, hence, the enzymatic equipment of both plants appears to be closely related (cf. Figure 1A, Scheme 4).

Scheme 4



The metabolism of labelled **18** in fertile gametophytes of *Giffordia mitchellae*[18] is particularly interesting, since thalli of this alga release the uncommon C_{11} hydrocarbon 2*Z*,4*Z*,6*E*,8*Z*-undeca-2,4,6,8-tetraene (**6**) (= giffordene) as the major product[19].

Scheme 5

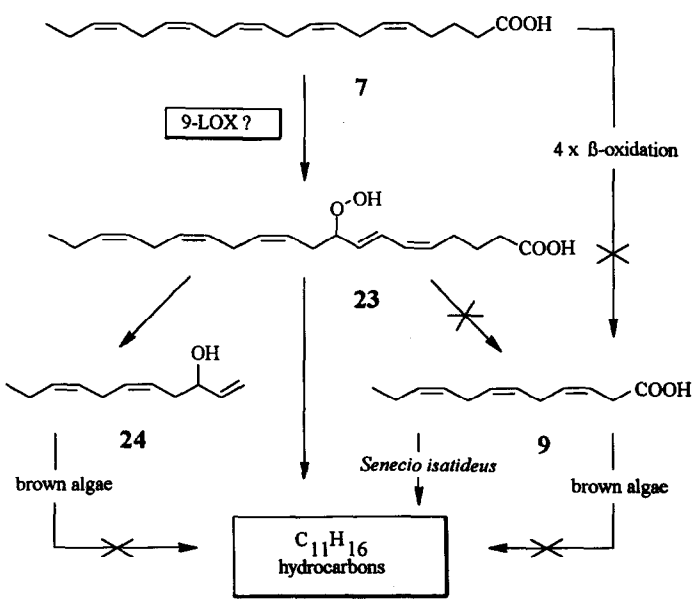


The unique configuration of giffordene (**6**) has been previously explained[19,20] as the result of a naturally oc-

curring, thermally allowed antarafacial [1,7]-hydrogen shift of the primarily formed 3*Z*,5*Z*,8*Z*-undeca-1,3,5,8-tetraene **(21)** (Scheme 5). This hypothesis is now strongly supported by our finding that the segment C(10) → (C19) of **18** is incorporated into the *nor*-giffordene **(22)** which has been identified by mass spectroscopic analysis. Following this concept, the biosyntheses of the postulated intermediate **21** and that of finavarrene **(5)** appear to be directly related. Both compounds demand for an enzymatic attack onto one of the two enantiotopic hydrogen atoms at C(13) of the precursor acid **7**, but different transition state structures result in (*E*)- or (*Z*)-configuration at the C(3)=C(4) double bond. While **5** is thermally stable, **21** rearranges spontaneously to yield giffordene **(6)** as a moderately stable hydrocarbon which subsequently isomerizes into a number of isomeric $C_{11}H_{16}$ compounds[18,19].

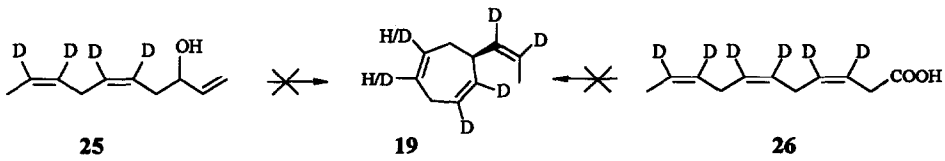
Biosynthetic considerations. In the terrestrial plant *Senecio isatideus* (Asteraceae) the C_{11} hydrocarbons are produced from unsaturated C_{12} fatty acids. This is different in the case of the marine brown algae. Incubation experiments with female gametes of *E. siliculosus* and labelled C_{12} or C_{11} precursors like **9** and **26** failed to give labelled C_{11} or C_{10} hydrocarbons, respectively. Hence, it follows that the unsaturated C_{20} acids are not degraded to C_{12} acids (by ß-oxidation; Scheme 6) prior to the transformation into the C_{11} hydrocarbons. In connection with the isolation of novel C_{11}hydrocarbons from the mediterranean brown alga *Cutleria multifida*, the undeca-1,5,8-trien-3-ol **(24)** has been discussed by *Jaenicke* and *Moore*[21] as a plausible link between unsaturated fatty acids and the C_{11} hydrocarbons. This assumption appears to be particularly reasonable since 1-alken-3-ols like **24** have been isolated from brown algae[22,23]. Their biosynthesis can be rationalized assuming a consecutive action of a 9-lipoxygenase onto **7** yielding **23** and a peroxide lyase which cleaves **23** into the C_{11} trienol **24** and a dicarbonyl fragment analogous to the release of oct-1-en-3-ol from linoleic acid in fungi[24]. Moreover, a "biomimetic" synthesis of the C_{11} hydrocarbon **4** using a phosphate ester[25] of **24** has been reported.

Scheme 6



However, incubation experiments with the *nor*-trienol [$^2H_4$]-**25** or the acid [$^2H_6$]-**26** and female gametes of *E. siliculosus* following the protocol used for the incorporation of the C_{20} acid **18** failed to give [$^2H_4$]- or [$^2H_6$]-**19** and thereby disprove the intermediacy of 1-alken-3-ols like **24** (Scheme 6 and Scheme 7).

Scheme 7



25 **19** **26**

Since the C_{20} precursors are also not degraded by ß-oxidation (Scheme 6), it is obvious that the biosynthesis of the C_{11} hydrocarbons (6*S*)-**1** and finavarrene **(5)** follows two different routes in the higher and the lower plants. We conclude, that **1** and probably all other C_{11} hydrocarbons are in brown algae produced from 9-OOH icosanoids like **23** by the action of a hitherto not characterised hydroperoxide lyase. This enzyme probably directly cleaves the reactive intermediate **23** into the olefinic C_{11} hydrocarbon and a C_9 dicarbonyl fragment. Following this concept, a single precursor may be forced by the individual enzyme(s) into various transition state structures, each of which may yield a certain C_{11} hydrocarbon characteristic for the active center of the involved enzyme. Some of the resulting hydrocarbons are thermally labile and suffer spontaneous sigmatropic and electrocylic reactions[9]. Besides the [1,7]-sigmatropic hydrogen shift in the biosynthesis of giffordene **(6)**, a [3,3]-sigmatropic rearrangement of an intermediary *cis*-disubstituted cyclopropane appears to be responsible for the production of ectocarpene **(1)**[8,26]. An electrocyclic ring closure of a linear 3*Z*,5*Z*,7*E*-nona-1,3,5,7-tetraene has been recently postulated for the generation of 7-methylcycloocta-1,3,5-triene and its valence tautomers within the hydrocarbon blend of mature gametophytes of *Cutleria multifida*[9]. The present work provides the first valuable experimental platform for the validation of all of the hitherto postulated transition state structures and rearrangement reactions. In particular, the modes of the activation and fragmentation of the C_{12} and C_{20} acids appear to be interesting problems which deserve further investigations.

Acknowledgements

Financial support by the *Deutsche Forschungsgemeinschaft*, Bonn, and the *Fonds der Chemischen Industrie*, Frankfurt, is gratefully acknowledged. We also thank the *BASF*, Ludwigshafen, and the *Bayer AG*, Leverkusen, for generous supply with chemicals and solvents..

Experimental:

General remarks. Reactions were performed under Ar. Solvents and reagents were purified and dried prior to use. Anh. $MgSO_4$ was used for drying. Boiling points are not corrected. The following spectroscopic and

analytical instruments were used: ^1H- and ^{13}C NMR: *Bruker Cryospec WM 250* and *Bruker WM 400*; $CDCl_3$, TMS as internal standard. IR: *Perkin-Elmer-882* IR spectrophotometer. MS: *Finnigan MAT 90* GLC/MS system and *Finnigan ITD 800* combined with a *Carlo-Erba* gas chromatograph, model *Vega*, equipped with a fused-silica capillary *SE 30*, (10m x 0.32 mm); carrier gas, He at 30cm/s; scan range: 35-249 Dalton/s. Analytical GLC: *Carlo-Erba* gas chromatograph, *HRGC 5300, Mega* series, equipped with fused silica capillaries, *SE 30* (10m x 0.32mm); H_2 at 30 cm/s as carrier. Isolation of volatiles: A miniaturised version of the instrumentation given in ref. 27 was used. Silica gel, *Si 60*, (0.040-0.063 mm, *E. Merck*, Darmstadt, FRG) was used for column chromatography. [$^2H_8$]-arachidonic acid was from *Campro scientific* (Emmerich, FRG).

(4*RS*)-4-(Deca-2,5,8-triyn-1-yl)-2,2-dimethyl-1,3-dioxolane (12)

A soln. of the Grignard-reagent prepared from **11** (20.78 g, 0.148 mol)[13,14] in 120 ml dry THF was treated at *rt* with 250 mg CuCN, and 1-bromo-2,5-heptadiyne **(10)** (25 g, 0.146 mol) was added slowly. The mixture was refluxed for 5 hours, cooled and hydrolysed by addition of H_2O (75 ml) and satd. aq. NH_4Cl (150 ml). Extractive workup and chromatography (SiO_2, Et_2O/pentane (2:3 v/v)) afforded **(12)** as a colourless viscous oil (20.8 g, 62%). ^1H-NMR (250 MHz, $CDCl_3$): δ 4.22 (quint, J= 6.4 Hz, 1H), 4.11 (dd, J= 5.4, 8.2 Hz, 1H), 3.77 (dd, J= 5.4, 8.2 Hz, 1H), 3.12 (m, 4H), 2.52 (ddt, J= 16.4, 5.4, 2.5 Hz, 1H,), 2.40 (ddt, J= 16.4, 5.4, 2.5 Hz, 1H), 1.80 (t, J= 2.5 Hz, 3H), 1.44 (s, 3H,), 1.38 (s, 3H); IR (neat): 2986, 2919, 1450, 1415, 1369, 1318, 1253, 1213, 1155, 1102, 1070, 887, 834 cm^{-1}; MS (%): 215 (M^+-CH_3, 40), 173 (4), 153 (22), 128 (7), 115 (5), 101 (100), 73 (12). HR-MS m/z calcd. for $C_{14}H_{15}O_2$ (M^+-CH_3): 215.1072, found 215.1023.

4-((4*RS*,2*Z*,5*Z*,8*Z*)-[4,5,7,8,10,11-$^2H_6$]-Deca-2,5,8-trien-1-yl)-2.2-dimethyl-1,3-dioxolane (13)

Triyne **12** (20.8 g, 0.09 mol) in dry THF/acetone (250 ml (4/1; v/v)) and *Lindlar's* catalyst (Fluka & Buchs, Switzerland, 2 g) were rapidly stirred under an atmosphere of $^2H_2$-gas until 3 equ. were consumed. Usual workup and chromatography (SiO_2, Et_2O/ pentane (1:4, v/v)) afforded **13** (14.85 g, 68 %). ^1H-NMR (250 MHz, $CDCl_3$): δ 4.14 (quint, J= 6.4 Hz, 1H), 4.03 (dd, J= 5.4, 8.2 Hz, 1H), 3.77 (dd, J= 5.4, 8.2 Hz, 1H), 2.82 (s, 2H), 2.80 (s, 2H), 2.46 (dd, J= 16.2, 8.2 Hz, 1H), 2.40 (dd, J= 16.2, 8.2 Hz, 1H), 1.64 (s, 3H), 1.43 (s, 3H), 1.38 (s, 3H); IR (neat): 2989, 2938, 2878, 2251, 1370, 1323, 1251, 1214, 1172, 1157, 1111, 1067, 846 cm^{-1}; MS (%): 242 (M^+, 0.2), 227 (5), 184 (2), 140 (2), 125 (2), 112 (3), 101 (100), 95 (4), 83 (10), 72 (8); HR-MS m/z calcd. for $C_{15}H_{18}O_2{}^2H_6$: 242.2152, found 242.2140.

(4*Z*,7*Z*,10*Z*)-[4,5,7,8,10,11-$^2H_6$]-Dodeca-4,7,10-trien-1,2-diol (14)

A soln. of **13** (14.85 g, 0.061 mol) in THF (65 ml) was treated with 0.15 N H_2SO_4 (105 ml), and the mixture was stirred over night. The heterogeneous mixture was neutralised by slow addition of solid $NaHCO_3$, and **14** was extracted with Et_2O (5 x 50 ml). Drying and evaporation of solvents yielded crude **14** (12.39 g, 100%) which was used in the next step without further purification. ^1H-NMR (250 MHz, $CDCl_3$): δ 3.74 (m, 1H), 3.66 (dd, J= 3.1, 11.2 Hz(1)), 3.47 (dd, J= 7.2, 11.1 Hz, C(1)), 2.83 (s, 2H, C(6)), 2.80 (s, 2H,

C(9)), 2.27 (t, J= 6.9 Hz, 2H, C(3)), 1.63 (s, 3H, C(12)); IR (neat): 3379, 2922, 2250, 1630, 1435, 1375, 1348, 1172, 1094, 1037, 934, 882, 858, 498 cm^{-1}.

(3*Z*,6*Z*,9*Z*)-[3,4,6,7,9,10-$^2H_6$]-Undeca-3,6,9-trienal (15)

A soln. of $NaIO_4$ (13.74 g, 0.064 mol) in water (100 ml) is slowly added (90 min) to a well stirred and chilled emulsion of **14** (12.39 g, 0.061 mol) in the same solvent (100 ml). Stirring is continued for 30 min, and the product is extracted with Et_2O. Removal of solvents and distillation yielded **15** as a pale yellow liquid (8.44g, 81%). B.p.: 46°C/3·10^{-2} torr; ^{1}H-NMR (250 MHz, $CDCl_3$): δ 9.67 (t, J= 1.2 Hz, 1H), 3.20 (s, 2H), 2.82 (s, 2H,), 2.80 (s, 2H), 1.62 (s, 3H); ^{13}C-NMR ($CDCl_3$): δ 198.7 (C(1)), 132.6 (t, J_{CD}= 23.8 Hz, C(3)), 128.49 (t, J_{CD}= 23.8 Hz, C(4), 124.3-129.5 (m, C(6)+(7)+(9)), 118.2 (t, J_{CD}= 23.8 Hz, C(10)), 42.3 (C(2)), 25.6 (C(5)), 25.01 (C(8)), 12.62 (C(11)); IR (neat): 2920, 2828, 2730, 2251, 1727, 1629, 1440, 1377, 1170 cm^{-1}; MS (%): 170 (M^+, 0.1), 155 (0.2), 125 (26), 110 (14), 98 (31), 85 (100), 70 (34). HR-MS *m/z* calcd. for $C_{11}H_{10}{}^2H_6O$: 170.1577, found 170.1569.

1-Methylethyl-(8Z,11Z,14Z,15Z)-[11,12,14,15,17,18-$^2H_6$]-nonadeca-8,11,14,17-tetraenoate (17)

Into a cold (0°C) suspension of the phosphonium iodide **16** [2] (5.15 g, 0.898 mol) in dry THF (50 ml) was injected with stirring 1.5*M* *t*-BuLi in pentane (5.99 ml, 8.98 mmol). Stirring was continued for 15 min, and the red soln. was cooled to - 78°C. Then, a soln. of **15** (1.39g, 8.2 mmol) in the same solvent (15 ml) was slowly added, and the mixture was allowed to come to rt after 30 min. Usual workup and chromatography (SiO_2 , Et_2O/pentane (98:2 v/v)) afforded **17** (0.935 g, 34 %). ^{1}H-NMR (250 MHz, $CDCl_3$): δ 5.35 (m, J = 10.96 Hz, 2H), 4.99 (sept., J= 6,3 Hz, 1H), 2.78 (s(b), 6H), 2.25 (t, 7.2 Hz, 2H), 2.04 (m, 2H), 1.55-1.63 (m, 5H), 1.28-1.32 (s(b), 6H), 1.22 (d, J= 6.3 Hz, 6H); IR (neat): 2981, 2934, 2858, 2251, 1732, 1466, 1373, 1252, 1173, 1143, 1109 cm^{-1}; MS (%): 338 (M^+, 31), 295 (26), 279 (23), 239 (13), 209 (13), 153 (11), 123 (20), 109 (35), 98 (79), 83 (100), 69 (52).HR-MS *m/z* calcd. for $C_{22}H_{30}O_2{}^2H_6$: 338.3091, found 338.3088.

(8*Z*,11*Z*,14*Z*,15*Z*)-[11,12,14,15,17,18-$^2H_6$]-Nonadeca-8,11,14,17-tetraenoic acid (18)

The ester **17** (705 mg, 2.09 mmol) and NaOH (600 mg, 15 mmol) in MeOH/ H_2O (50 ml, (9:1 v/v)) were refluxed for 2 h. After cooling to r.t., water (50 ml) was added and unpolar by-products were extracted with pentane (3 x 20 ml). Acidification (pH 3, 0.1N HCl) and extraction with Et_2O/pentane (1:9 v/v) gave pure **18** as a colourless oil (435 mg, 70%). ^{1}H-NMR (250 MHz, $CDCl_3$): δ 5.35 (m, 2H), 2.78 (s(b), 6H), 2.34 (t, 7.4 Hz, 2H), 2.04 (m, 2H), 1.56-1.68 (m, 5H), 1.25-1.34 (s(b), 6H); ^{13}C-NMR ($CDCl_3$): δ = 180.18 (C(1)), 130.10 (C(8)), 127.79 (C(9)), 128.12/127.88/127.51/127.27 (C(11)/(12)/(14)/(15)/(17)), 123.43 (C(18)), 34.03 (C(2)), 29,39/28.96/28.88 (C(4)/(5)/(6)), 27.14 (C(7)), 25.48/25.35/25.02 (C(10)/ (13)/(16)), 24.6 (C(3)), 12.63 (C(19)); IR (neat): 3013, 2932, 2858, 2251, 1709, 1413, 1287, 1220, 1171, 935 cm^{-1}. HR-MS *m/z* calcd. for $C_{19}H_{24}O_2{}^2H_6$: 296.2622, found 296.2606.

Administration of fatty acids to female gametes of marine brown algae.

Collection of volatile metabolites

Fatty acids were added as solns. in DMSO (0.2 mg in 2µL) to suspensions of approximately 10^8 gynogametes in 15 ml sea water[3] in a round bottomed flask attached to a trapping device consisting of a miniature circulation pump (Fa. *E. Fürgut*, D-W-7971 Aitrach, FRG) and a charcoal "filter" (1.5 mg; *CLSA*-Filter, CH-8405 Winterthur, Switzerland). Flask, pump and filter holder were joined together forming a closed system (air volume: ca 15 ml). After 30 min at 18°C, the air above the surface of the sea water was circulated for 24h, and during this time the produced volatiles were adsorbed to the charcoal trap[27]. Following desorbtion[28] from the carbon traps with CH_2Cl_2 (2 x 15 µl) the compounds were directly analysed by GC/MS.

Mass spectra of deuterium labelled metabolites

6-Butyl-[1,2,4,5-$^2H_4$]-cyclohepta-1,4-diene ([$^2H_4$]-(2)). *M/z* (%): 154 (M^+,9), 111 (13), 94 (70), 82 (100), 67 (40).

(3*E*,5*Z*,8*Z*)-[2,3,5,6,8,9-$^2H_6$]-Deca-1,3,5,8-triene (20): *M/z* (%): 140 (M^+, 46), 125 (16), 109 (39), 95 (67), 82 (100), 69 (36), 55 (17).

(2*Z*,4*Z*,6*E*,8*Z*)-[2,3,5,6,8,9-$^2H_6$]-Deca-2,4,6,8-triene (22): *M/z* (%): 140 (M+, 49), 125 (55), 109 (31), 95 (100), 82 (38), 80 (38), 68 (22), 53 (21), 41 (57), 40 (57).

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