143. Separation of Enantiomeric Algal Pheromones and Related Hydrocarbons by Gas-Liquid Chromatography on Modified Cyclodextrins as Chiral Stationary Phases. Biosynthetic Relevance of Racemic By-products

by **Wilhelm Boland***

Institut für Organische Chemie der Universität, Richard-Willstätter-Allee, D-7500 Karlsruhe

and **Wilfried A. König*** and **Ralph Krebber**

Institut für Organische Chemie der Universität, Martin-Luther-King-Platz 6, D-2000 Hamburg 13

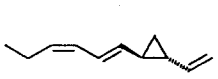
and **Dieter G. Müller**

Fakultät für Biologie der Universität, Postfach 5560, D-7750 Konstanz

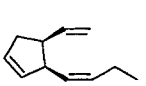
(8. VI. 89)

The separation of the enantiomers of the olefinic hydrocarbons multifidene **3** and aucantene **5** from the pheromone blend of the two seaweeds *Cutleria multifida* and *Chorda tomentosa* is achieved by gas chromatography on modified cyclodextrins as chiral stationary phases. The method is also applicable for the separation of enantiomeric alkyl-, alkenyl- or alkynyl-substituted cyclooctadienes and cyclopentenes. A plausible rational is presented for the biosynthesis of racemic by-products of algal pheromone blends.

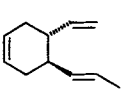
1. Introduction. – Capillary gas chromatography on chiral stationary phases is one of the most sensitive and reliable tools for the determination of absolute configurations and enantiomeric excesses (e.e.) of volatile natural products. This is particularly important, if ng to µg quantities have to be characterized as is often the case with biologically active compounds. Still lower concentrations (pg) of pheromones are released from individual female gametes of marine brown algae [1] [2]. Using efficient stripping procedures and mass cultures, the amount of available material can be raised to *ca.* 1–10 µg, but even this amount is by far too low for chiroptical measurements or degradation experiments. Also, transformation of algal pheromones with chiral reagents and subsequent chromatographic separation is impossible because of lacking functional groups (see **1**–**8**).



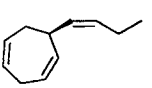
(−)-(1*R*,2*R*)-**1**



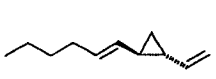
(+)-(3*S*,4*S*)-**3**



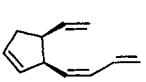
(+)-(4*R*,5*R*)-**5**



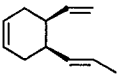
(+)-(6*S*)-**7**



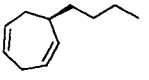
(+)-(1*R*,2*R*)-**2**



(+)-(3*R*,4*S*)-**4**



rac-**6**



(−)-(6*R*)-**8**

The first separation of enantiomeric algal pheromones was achieved with the UV-active hydrocarbons hormosirene **1** and viridiene **4** by HPLC using inclusion effects on cross-linked triacetyl cellulose [3] or cellulose tribenzoate coated on silica [4]. In both cases, the conjugated diene moiety of **1** and **4** allowed for a highly sensitive UV-detection of the compounds ($\varepsilon \approx 30000$) at the lower µg level. Since very recently, modified cyclodextrins turned out to be powerful chiral stationary phases for the separation of enantiomeric olefines by GLC [5] [6], this prompted us to extend this new separation technique to the field of algal pheromones, particularly to those which can not be monitored by UV. The separation of most of the biologically relevant compounds from the bouquets of the two phaeophytes *Cutleria multifida* and *Chorda tomentosa* and some analogues are reported.

2. Experimental. – Heptakis(3-*O*-methyl-2,6-di-*O*-pentyl)-β-cyclodextrin and heptakis(2,3,6-tri-*O*-pentyl)-β-cyclodextrin were prepared as described previously [7]. The preparation and coating of *Pyrex* glass capillaries was performed according to [8]. Separation conditions were as follows: column, 41 m × 0.25 mm i.d.; carrier gas H_2 at 1 bar. Elution of compounds was achieved at an isothermal level at 70° or under programmed conditions as indicated in *Fig. 2* (see below). Extracts from *Cutleria multifida* were obtained by the 'closed-loop-stripping' technique [2] from freshly harvested fertile male and female gametophytes from the harbour of Beaulieu (Cote d'Azur, France). Mass release of male or female gametes occurred during the 24 h stripping period. *Chorda tomentosa* was cultured as described [9] and extracted by the same technique after mass release of eggs.

3. Results. – *Fig. 1a* shows the successful separation of the two major hydrocarbons of the *Cutleria* extract, namely (±)-multifidene ((±)-**3**) and (±)-aucantene ((±)-**5**). Baseline separation of the enantiomers is achieved for both compounds and warrants an exact determination of the e.e. for each constituent. The elution order of the enantiomers follows from coinjection with synthetic (+)- or (−)-references [10] [11]. When the extract of female plants with freshly released gynogametes from *Cutleria multifida* is analyzed under identical conditions, (+)-(3*S*,4*S*)-multifidene ((+)-(3*S*,4*S*)-**3**) elutes as a single enantiomer (> 99% e.e., *Fig. 1b*).

Although the absolute configuration of multifidene **3** is already known from biological activity tests with pure samples of (+)-(3*S*,4*S*)- and (−)-(3*R*,4*R*)-**3** [10], this approach provides the first exact and reliable data for the e.e. of the secreted pheromone. Multifidene from extracts of fertile gametophytes of *Chorda tomentosa* with released eggs has the same absolute configuration [11] and is likewise optically pure (> 99% e.e.). This corresponds to previous findings with the more unsaturated cyclopentene pheromone viridiene (+)-(3*R*,4*S*)-**4** (> 99% e.e.) from gynogametes of *Syringoderma phinneyi* [3]. Aucantene **5**, the second prominent, but biologically inactive hydrocarbon which is emanated from male and female gametophytes of *Cutleria multifida* shows a slightly different picture. According to *Fig. 1b*, its absolute configuration is (+)-(4*R*,5*R*) and thereby confirms the earlier tentative assignment which was based on the optical rotation of a very small and impure sample only [11] [12]. In contrast to multifidene **3**, aucantene **5** is not released as an enantiomerically pure compound (e.e. 97.5 ± 0.05%). Furthermore, careful analysis of the by-products in the *Cutleria* bouquet reveals the presence of a second aucantene isomer **6** (2.7% with respect to **5**) which was identified as *cis*-4-[(1*E*)-prop-1-enyl]-5-vinylcyclohexene (**6**) [11]. Unlike the major *trans* isomer **5**, the new *cis*-aucantene **6** is completely racemic.

Other by-products, like *e.g.* hormosirene **1**, ectocarpene **7**, or dictyotene **8** can not be resolved by GLC as yet and have to await further development of appropriate chiral stationary phases.

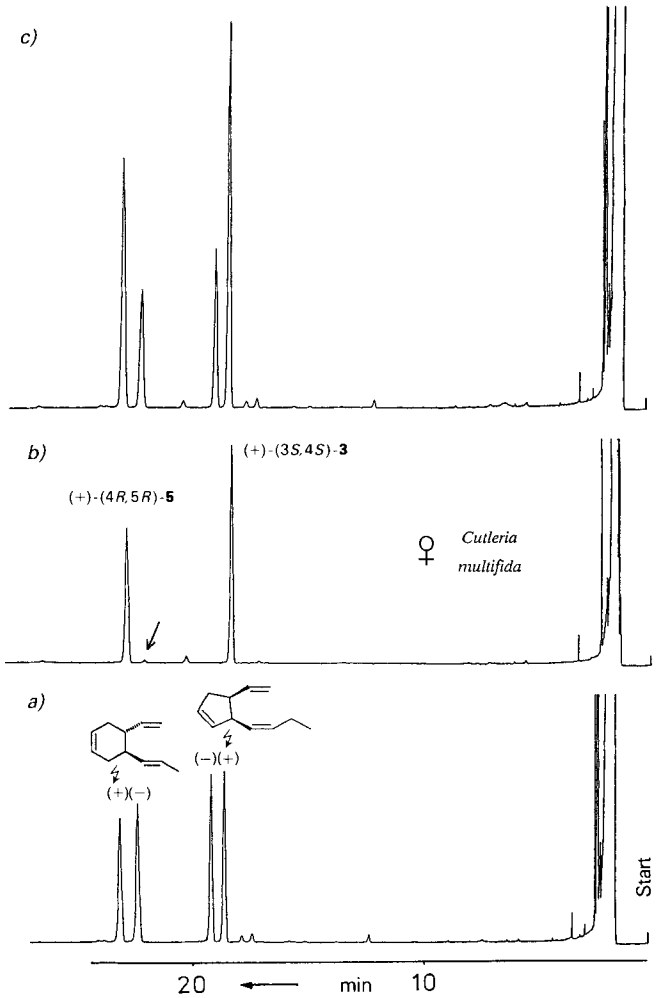


Fig. 1. a) *GLC separation of (±)-multifidene* ((±)-**3**) *and (±)-aucantene* ((±)-**5**) *on a* Pyrex *capillary column with heptakis(3-*O*-methyl-2,6-di-*O*-pentyl)-β-cyclodextrin.* 70°, 1 bar H_2; column, 41 m × 0.25 mm i.d. The elution order of the enantiomers was determined by coinjection with synthetic references. b) *GLC analysis of an extract from female gametophytes of* Cutleria multifida *with released gametes under identical conditions.* Arrow: (−)-(4*S*,5*S*)-aucantene ((−)-(4*S*,5*S*)-**5**). c) *Extract from* Cutleria multifida *after admixture of a small amount of racemic multifidene* (±)-**3** *and aucantene* (±)-**5** to establish the identity of the eluting compounds.

To explore the limits of the method, some pheromone analogues or trace constituents of pheromone bouquets of marine brown algae are also analyzed. The results are shown in *Fig. 2.* The separation of enantiomeric *cis*-disubstituted cyclopentenes turns out to be good to excellent throughout. Even if only a single double bond is left in the molecule, there is still a near baseline separation. Similar encouraging results are obtained for some enantiomeric alkynyl- or alkenylcyclooctadienes [13] as exemplified in *Fig. 2.* Thus, the new technique is a very promising tool to unravel absolute configurations and optical

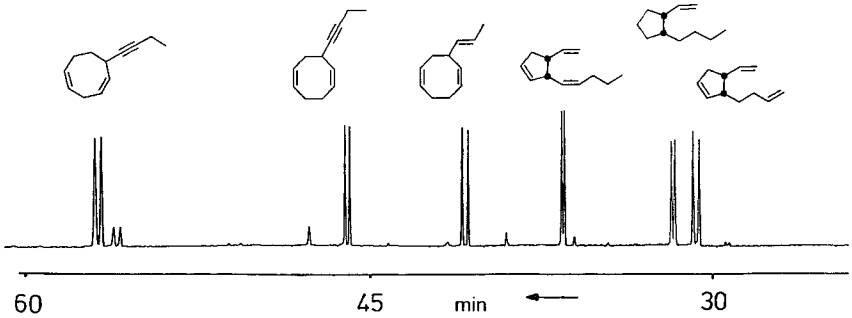
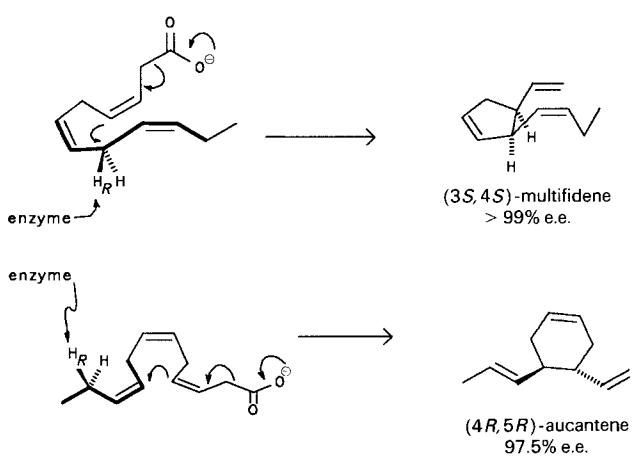


Fig. 2. *GLC separation of some enantiomeric pheromone analogues and trace constituents of algal pheromone bouquets on a* Pyrex *glass capillary column with heptakis(2,3,6-tri-O-pentyl)-β-cyclodextrin* (available from *Macherey & Nagel*, D–5160 Düren, FRG). Conditions: column, 41 m × 0.25 mm i.d.; H_2, 1 bar; elution programmed from 50 to 130° at 2°/min.

purities of algal pheromones and will be further applied to establish biosynthetic interrelations between the various classes of these bioactive compounds.

4. Biosynthetic Considerations. Significance of Racemic By-products. – Multifidene **3** and aucantene **5**, like all other alicylic $C_{11}H_{16}$ hydrocarbons, are metabolites of (3*Z*,6*Z*,9*Z*)-dodeca-3,6,9-trienoic acid, as has been shown in a model study with the flowering plant *Senecio isatideus* (Asteraceae) [14]. Accordingly, the pheromones are formed from this precursor by an enzymatic attack onto a H-atom at C(8) followed by cyclization of the reactive intermediate and subsequent fragmentation into an olefin and CO_2 as depicted in the *Scheme*. Taking into account the now known absolute configurations and optical purities of the secreted compounds together with the recently established enantiospecificity (*Re*-attack) of one of such enzyme(s) [15], a more detailed

Scheme. *Biosynthesis of (3S,4S)-Multifidene* ((+)-(3*S*,4*S*)-**3**) *and (4R,5R)-Aucantene* ((+)-(4*R*,5*R*)-**5**) *from (3Z,6Z,9Z)-Dodeca-3,6,9-trienoic Acid*[a])



[a]) The absolute configuration of both products is in agreement with abstraction of a *pro-R* H-atom from C(8) or C(11), respectively, of the precursor acid and corresponds to the stereochemical events observed with the flowering plant *Senecio isatideus* [15].

picture of the biosynthesis of these compounds can be drawn. In the case of multifidene **3**, removal of the *pro-R* H–C(8) from dodeca-3,6,9-trienoic acid in combination with 'backside' cyclization and fragmentation of the fatty acid readily accounts for the absolute configuration and high optical purity of the product.

The same mechanism, based on enzymatic removal of a *pro-R* H–C(11) from dodeca-3,6,9-trienoic acid and 'backside' cyclization with participation of the C(3)=C(4) bond yields (+)-(4*R*,5*R*)-aucantene ((+)-(4*R*,5*R*)-**5**) by analogy. The model also explains the presence of racemic by-products. If, *e.g.*, in the case of aucantene, the aliphatic terminus of the attacked fatty acid 'escapes' the control of the active center of the enzyme after removal of the *pro-R* H–C(11) while being still fixed to it with its carboxyl group, random cyclization to racemic *cis*- or *trans*-aucantenes can occur with approximately equal chance. Since the *racemic cis*-isomer **6** *(vide supra)* contributes with 2.7% to the total amount of aucantenes, the degree of racemization of *trans* aucantene should be roughly of the same order. This is indeed the case, since the e.e. of *trans*-aucantene **5** was found to be to 97.5%. Similar considerations should be valid for multifidene **3**, which can yield an additional *trans*-isomer by random cyclization. And, as a matter of fact, a *trans*-multifidene is found, but it contributes to not more than 0.17% to the total amount of cyclopentenes, which is below the threshold of the analytical method and is further consistent with the very high e.e. of the major isomer.

Random cyclization should give raise to additional isomers which are indeed present among the trace constituents of the *Cutleria* bouquet. Further work is necessary, to clarify this interesting aspect of fatty-acid cyclization in marine brown algae.

Financial support by the *Deutsche Forschungsgemeinschaft* and the *Fonds der Chemischen Industrie*, Frankfurt am Main, is gratefully acknowledged. We thank Mrs. *C. Schmidt* and Mrs. *B. Stache*, University of Konstanz, for the collection of plant material.

REFERENCES

[1] C. Schmid, D.G. Müller, *Biol. Chem. Hoppe-Seyler* **1988,** *369*, 647.
[2] a) W. Boland, *Biologie in unserer Zeit* **1987,** *17*, 176; b) I. Maier, D.G. Müller, *Biol. Bull.* **1986,** *170*, 145.
[3] W. Boland, L. Jaenicke, D.G. Müller, *Naturwissenschaften* **1985,** *72*, 147.
[4] W. Boland, U. Flegel, G. Jordt, D.G. Müller, *Naturwissenschaften* **1987,** *74*, 448.
[5] J. Ehlers, W.A. König, S. Lutz, G. Wenz, H. tom Dieck, *Angew. Chem. Int. Ed.* **1988,** *27*, 1556.
[6] W.A. König, S. Lutz, M. Hagen, R. Krebber, G. Wenz, K. Baldenius, J. Ehlers, H. tom Dieck, *HRC CC, J. High Resolut. Chromatogr. Chromatogr. Commun.* **1988,** *11*, 621.
[7] W.A. König, S. Lutz, G. Wenz, *Angew. Chem. Int. Ed.* **1988,** *27*, 979.
[8] W.A. König, S. Lutz, P. Mischnick-Lübbecke, B. Brassat, E. von der Bey, G. Wenz, *Starch* **1988,** *40*, 472.
[9] I. Maier, D.G. Müller, G. Gassmann, W. Boland, F.-J. Marner, L. Jaenicke, *Naturwissenschaften* **1984,** *71*, 48.
[10] W. Boland, K. Mertes, L. Jaenicke, D.G. Müller, E. Fölster, *Helv. Chim. Acta* **1983,** *66*, 1905.
[11] W. Boland, U. Niedermeyer, L. Jaenicke, H. Görisch, *Helv. Chim. Acta* **1985,** *68*, 2062.
[12] L. Jaenicke, D.G. Müller, R.E. Moore, *J. Am. Chem. Soc.* **1974,** *104*, 4659.
[13] N. Schroer, Dissertation, University of Cologne, 1989.
[14] W. Boland, K. Mertes, *Eur. J. Biochem.* **1985,** *147*, 83.
[15] C. Neumann, W. Boland, in preparation.