

Lamoxirene. Structural Proof of the Spermatozoid Releasing and Attracting Pheromone of Laminariales

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Mature female gametes of several Laminariales excrete a substance which acts interspecifically to release and to attract spermatozooids from ripe antherids. This pheromone was identified by chemical degradation and by synthesis of the natural compound as 1-(1',2'-*cis*-epoxy-but-3'-enyl)-cyclohepta-2,5-diene (**3**). An oxirane is a novel feature among the mating substances of thallophytes. It is therefore suggested to name the active hormone "Lamoxirene".

Sexual reproduction in brown algae and its mediation by messenger substances have been the subject of intensive studies during the last decade [1]. The pheromones identified so far have been nonfunctionalized highly unsaturated C₈- or C₁₁-hydrocarbons [2].

A chemical interaction was also observed in the sexual reproduction of several Laminariales [3]. The pheromone is secreted by the female gametes. Upon contact with ripe antheridia it causes explosive discharge of spermatozooids and after that acts as attractant to direct the male gamete towards the egg [4, 5]. It proved identical in six different Laminariales and to cross-react.

Ectocarpene (**2**) (Fig. 1) and *n*-pentadecane always accompany the *Laminaria*-pheromone in various amounts [4]. Due to the small quantity (at the ng-level) available of this mixture, it was impossible to purify enough material to apply NMR-spectroscopy. The structural formula was established by high-resolution GC/MS as C₁₁H₁₄O, showing *m/e* 162.1059 (M⁺, C₁₁H₁₄O requires 162.1040) [6, 7].

From the relatively high polarity of the signal compound [4] and the presence of ectocarpene (**2**) in the algal product-mixture, we first assumed a carbonyl-substituted cycloheptadiene-skeleton to be present in the pheromone. Synthesis of all possible

C₁₁H₁₄O-ketones containing the 2,5-cycloheptadiene-moiety and the carbonyl-group in a C₄ side-chain (unpublished) disproved this assumption: the ketones were even more polar than the pheromone. Also the completely different fragmentation pattern in low-resolution MS (Finnigan MAT 4510 GC/MS) ruled out these structures.

On hydrogenation with Pt- or Pd-catalysts, two products showing molecular ions at *m/e* 168 and one at *m/e* 170 were obtained. Thus, of the five unsaturation sites implied by the sum-formula, at least one had to belong to a ring-system that could not be cleaved by hydrogenation. – The reasons mentioned above favoured the presence of a cycloheptadiene-ring which – considering the fragmentation behaviour – apparently did not contain the oxygen-function. Its nature became clearer by the compound's failing to react with NaBH₄/alumina [8] and with silylating agents. This proved the absence of a carbonyl- as well as a hydroxy-group. – Acid treatment destroyed the compound, indicating by this that the oxygen had to be part of an acid-labile cyclic ether such as a vinyl-ether or an epoxide.

An epoxide was more probable since reduction of the natural compound with LiAlH₄ lead to the

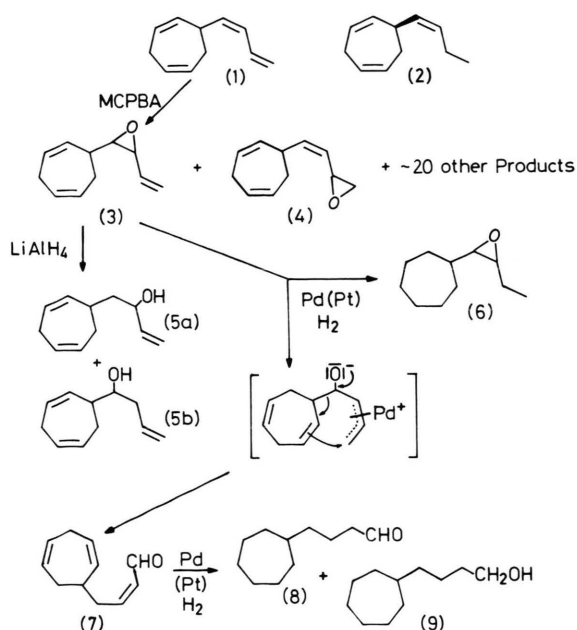


Fig. 1. Structure, synthesis, and reactions of lamoxirene (**3**), the sex-attractant of Laminariales, and of related compounds (MCPBA = *m*-chloroperbenzoid acid).

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Table I. Physical properties of the natural and the synthetic pheromone.

	Mass-spectrum 70 eV <i>m/e</i> [%]	Kováts-Indices		IR-spectrum CCl ₄ -soltn. [cm ⁻¹]	¹ H-NMR-spectrum (CCl ₄) [ppm] (δ)
		50 m OV1 [130 °C]	40 m Ucon 75 H 90000 [150 °C]		
Natural product	39 (35), 41 (17), 51 (10), 52 (5), 53 (7), 65 (15), 66 (7), 67 (8), 69 (5), 77 (37), 78 (55), 79 (30), 91 (100), 92 (20), 93 (26), 105 (19), 117 (1), 118 (1), 129 (3), 143 (0.5), 147 (0.5), 161 (M ⁺ -1, 0.1)	1284.5 $\pm$ 0.3	1662.4 $\pm$ 0.5		
Synthetic Epoxide 3	39 (45), 41 (25), 51 (14), 52 (7), 53 (10), 65 (21), 66 (11), 67 (13), 69 (9), 77 (47), 78 (71), 79 (39), 91 (100), 92 (26), 93 (34), 105 (23), 117 (2), 118 (2), 129 (4), 143 (0.5), 147 (0.5), 161 (0.1), 162 (M ⁺ , 0.05)	1284.7 $\pm$ 0.2	1662.1 $\pm$ 0.3	3095, 3020, 2965, 2930, 2860, 1635, 1445, 1405, 1265, 1100, 1020, 985, 925	2.1–2.6 (m, 3 H), 2.7–3.0 (m, 3 H), 3.3 (dd, 1 H), 5.2–6.0 (m, 7H)

formation of two isomeric alcohols with a molecular ion of *m/e* 164. They reacted with common silylating agents to form the corresponding trimethyl-silyl-ethers showing *m/e* 236 for M⁺.

From these details and from the structure of sex-attractants found in related brown algae such as ectocarpene (**2**) and desmarestene (**1**) [9] we proposed either one of the compounds (**3**) or (**4**) to be the pheromone of the Laminariales, assuming *cis*-geometry for the side-chain. For confirmation the isomeric epoxides were prepared following the route outlined in Figure 1.

Desmarestene (**1**), synthesized as described previously [10], was treated with *m*-chloroperbenzoic acid in methylene chloride at 0 °C to yield, besides about 20 minor products, the four possible mono-epoxides. From this mixture, 1-(1',2'-*cis*-epoxi-but-3'-enyl)-cyclohepta-2,5-diene (**3**) was isolated by column chromatography on silicagel and on 5% AgNO₃-impregnated silica using a pentane/ether gradient as the eluant. — Epoxide (**3**) has properties identical to the natural compound (Table I), thus proving it to be the *Laminaria* release and luring pheromone which we will name henceforth "lamoxirene", referring to source, structure, and action.

The chemical reactions of the natural compound leading to the products found during elucidation of the structure are outlined in Figure 1.

Reduction of the epoxide with LiAlH₄ yields the two isomeric alcohols (**5a**) and (**5b**); catalytic hydrogenation with Pt or Pd, on the one side leads to the corresponding saturated epoxide (**6**) which was verified by comparison with synthetic (**6**); on the other side, a rearrangement, presumably by means of the complexing Pd or Pt(0)-metal [11], takes place forming the α,β -unsaturated aldehyde (**7**) which then is reduced to the saturated aldehyde (**8**) or alcohol (**9**).

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