and normal bone marrow (NBM), (ii) normal thymus (NT) and tolerant bone marrow (TBM), and (iii) normal thymus (NT) and normal bone marrow (NBM). The size of each group at individual points varied from four to eight mice. The percentage of suppression in thymus cells or bone marrow cells from tolerogen treated donors was calculated at each point of the curve in the following manner:

$$\% \text{ suppression} = 100 - \left[\frac{\frac{\Sigma \text{ PFC in TT, NBM (or NT, TBM)}}{\text{No. mice treated}}}{\frac{\Sigma \text{ PFC in NT, NBM}}{\text{No. mice treated}}} \times 100 \right]$$

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Sex Attractant in a Brown Alga: Chemical Structure

Abstract. *The male-attracting substance produced by the female gametes of the marine brown alga* Ectocarpus siliculosus *was identified as allo-*cis*-1-(cyclohepta-dien-2′,5′-yl)-butene-1.*

Cellular chemotaxis is widely distributed in lower plants. Many cases of interaction on the gamete level are known, but so far there is only one system, the active compound of which is known; this compound is sirenine, which is produced by the female gametes of the water mold *Allomyces* (*1*). We shall report on the identification of the substance which is secreted by the female gametes of the marine brown alga *Ectocarpus siliculosus* (Dillw.) Lyngb. as an attractant for the male gametes. The sexual reaction in this species has been observed by many workers. Motile gametes are isomorphic. Female gametes, when settled on a surface, attract numerous male gametes which become attached to the female cell with the tip of their front flagellum. After one male gamete has fused with the female, the zygote loses its attraction for the other male gametes and they disperse. At the time that the attractant was first detected and isolated (*2*) only microgram quantities could be isolated by gas chromatography and assayed for its biological activity. By increasing the capacity of the cultivation apparatus, we have now obtained sufficient quantities of the gamone to identify its chemical structure.

For the production of the gamone, the female gametophytic clone D-A2 was used and grown in large quantities, essentially by the procedure described in (*2*). Between June 1968 and August 1970, 14,900 culture dishes were inoculated and harvested. The total harvest during this time was 1041 g of fresh gametophyte material, which corresponds to 154 g dry weight. Because of its high volatility, the gamone could be removed from the cultures by means of a stream of purified air, and condensed in a stainless steel trap at −80°C. This condensate was then flushed with a stream of N_2 through a drying tube containing $CaCl_2$ and into a glass trap at −78°C; the dry condensate was then dissolved in CCl_4. This procedure yielded a starting material of already good purity. The few contaminant compounds were then removed by preparative gas chromatography (Fig. 1A). The resulting pure gamone fraction was used for the analytical procedures. Quantitative assay of the gamone was made by gas chromatographic comparison with *n*-nonanal (*2*). During the 2 years of mass production, 92 mg of gamone were obtained. Our results indicate that the female gamone of *Ectocarpus siliculosus* is allo-*cis*-1-cycloheptadien-2′,5′-yl)-butene-1. This conclusion is based on the following evidence.

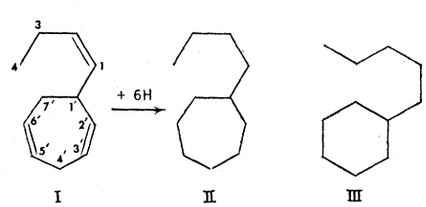
Table 1. Kovats indices of hexahydrogamone and authentic hydrocarbons for comparison. Columns used were: (A) 3.8 percent UCW 98 (methyl vinyl silicone gum); (B) 10 percent OV 225 (cyano silicone gum); (C) 2.5 percent Apiezon L; and (D) Apiezon L plus 2 percent Igepal.

Column	Temperature (°C)	Kovats indices		
		Hexahydrogamone	Amylcyclohexane	Butylcycloheptane
A	100	1165.14	1136.20	1165.01
B	60	1210.98	1166.88	1210.76
C	100	1191.24	1167.80	1191.15
D	172	1216.83	1176.43	1216.72

Mass spectrometry (Fig. 1B) shows that the mass of M^+ is 148. Comparison of the intensities of the masses 148 and 149 gives values between 10.2 and 11.6 for the number of carbon atoms per molecule. This means that the empiric formula is $C_{11}H_{16}$. The peaks at mass-to-charge ratios (m/e) of 133, 119, 105, and 91 indicate the successive loss of C_1 fragments. Peak 91 possibly represents the tropylium ion. The peak at m/e 146 arises from dehydrogenation in the inlet system. It is absent if the sample is introduced directly into the ion source. Elementary analysis suggests a hydrocarbon of the composition $C_{11}H_{16}$, and thus confirms the mass spectrometric data.

Proton magnetic resonance spectroscopy (Fig. 1C) confirms the presence of 16 protons per molecule. The following structural details are evident: one methyl triplet with chemical shift (τ) equal to 9.0 ppm, one tertiary proton (τ =6.6 ppm), six olefinic protons (τ = 4.4 to 4.8 ppm), two methylene protons between two double bonds (τ = 7.2 ppm, **VI**), and four methylene protons (τ = 7.9 to 8.0 ppm, **VII**). These data imply that the four unsaturation equivalents corresponding to the empiric formula $C_{11}H_{16}$ are represented by three double bonds and one ring system. The tertiary proton occupies the branched carbon atom holding the side chain.

The infrared spectrum (ultramicrocell, 50 μm path length, microilluminator) confirms the absence of functional groups. There is no band around 970 cm^{-1}, which means that there is no *trans* configuration at any of the double bonds. Two bands of medium intensity at 730 and 690 cm^{-1} indicate



I II III

$CH_3-C{=}C-$ **IV**

$CH_3-C-C{=}C-$ **V**

$=C-CH_2-C=$ **VI**

$-CH_2-C{=}C-$ **VII**

the presence of *cis* configuration of the unsaturated side chain. In the ultraviolet spectrum of the gamone in *n*-hexane (0.3 percent, weight per volume; path length, 1 mm) end absorption begins below 220 nm. There is no absorption above this wavelength. This means that the double bonds are nonconjugated. Identical results are obtained with the gamone before and after gas chromatography, thus indicating that no ring isomerization has taken place during purification.

When the gamone was hydrogenated in the presence of platinum in methylacetate, the resulting product, assayed by mass spectrometry, showed M^+ to be 154. This increase of six mass units as a result of hydrogenation confirms the existence of three double bonds per molecule. Moreover, upon saturation of the double bonds the characteristic odor of the genuine gamone is lost. Measurements of carbon isotope peaks gave values ranging from 10.5 to 11.5 for the number of carbon atoms per molecule.

The spectroscopic methods described so far could not give information on the size of the ring. Therefore, monocyclic C_{11} hydrocarbons with an unbranched side chain were synthesized and compared by gas chromatography with the hydrogenated gamone (*3*). Amyl-cyclohexane (**III**) is different from the hydrogenated gamone, whereas butyl-cycloheptane (**II**) proved to be identical with the hexahydrogamone, as indicated by the Kovats indices (Table 1). Furthermore, mass spectrograms of the hydrogenated gamone and butyl-cycloheptane are identical. Our results clearly show that the gamone is a threefold unsaturated hydrocarbon with a seven-membered carbon ring and an unbranched side chain. According to the data obtained by nuclear magnetic resonance (NMR) spectroscopy and ultraviolet spectroscopy two double bonds are located in the ring; the third one is located in the side chain. The location of the double bond in the side chain can be derived from the position of the methyl signal in the NMR spectrum; a double bond between carbon atoms 2 and 3 ($\Delta 2$) as indicated in (**IV**) would correspond to τ of 8.4 $\pm$ 0.2 ppm. The observed value of τ equal to 9.0 ppm matches most closely the theoretical value of τ equal to 8.9 $\pm$ 0.2 ppm for $\Delta 1$ (**V**). The location is confirmed by the fact that the methyl signal is a triplet. The NMR signal at τ equal to 7.2 ppm, which is attributed to two protons of the divinylmethane system, localizes the double bonds within the ring at positions 2′ and 5′. Thus, the compound has the structure of (**I**).

This is the first case in which the chemical structure of an algal gamone has been determined. Because it acts upon male gametes and attracts them to the female cells, the compound may be named *Ectocarpus* sirenine (*4*).

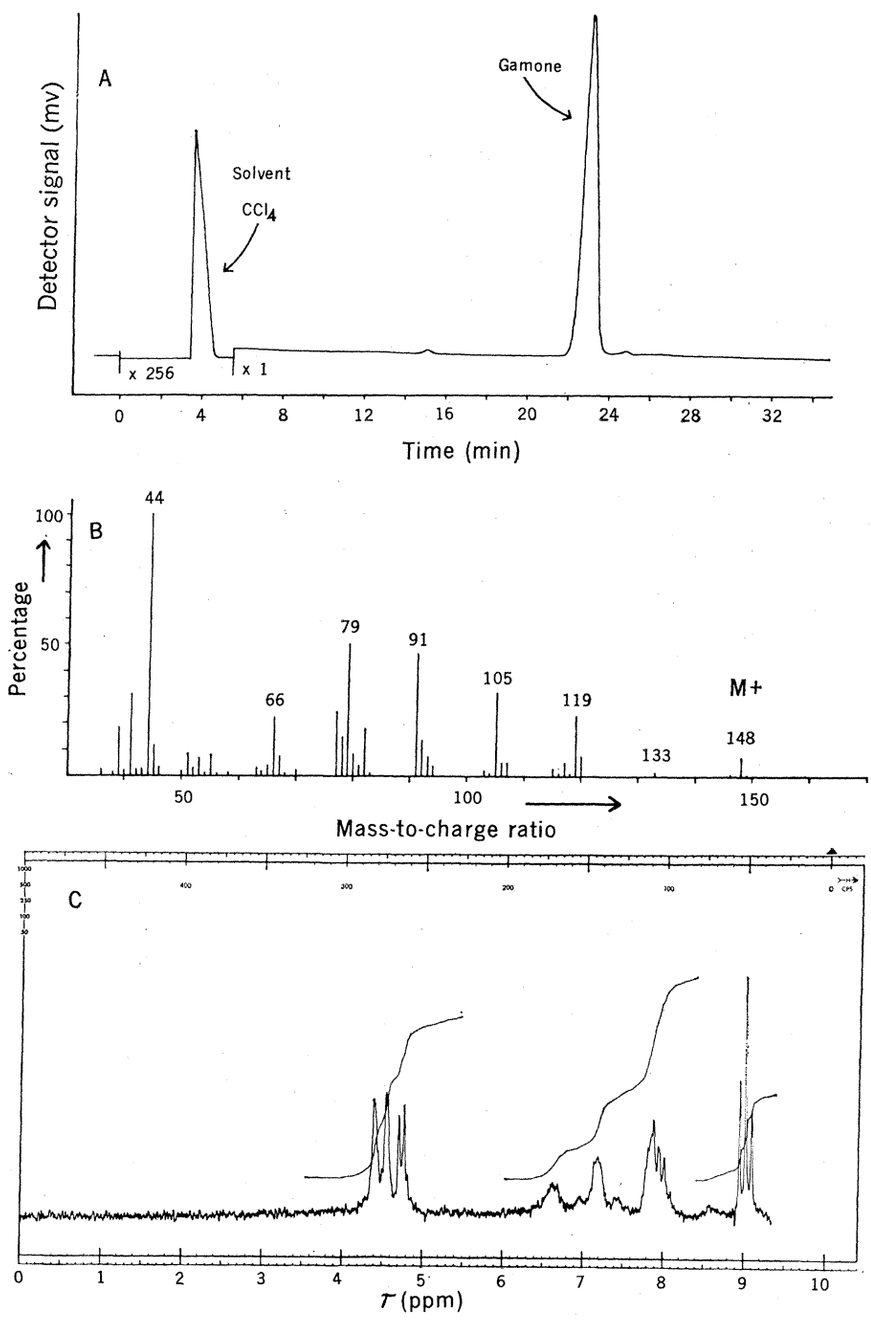


Fig. 1. (A) Preparative gas chromatography. Injection of 0.6 mg of crude gamone dissolved in 50 μl of CCl_4. The column (0.63 cm by 6.08 m) contained 5 percent SE 30 (silicone gum rubber) at 150°C. The temperature of the injection block was 170°C; that of the thermal conductivity detector was 175°C. Carrier gas was H_2 at 60 ml/minute. (B) Mass spectrum of gamone at 70 ev. The sample was injected as vapor at a temperature of 90° to 100°C. (C) Proton nuclear magnetic resonance spectrum of 6.2 mg of gamone in CCl_4 at 100 Mhz.

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Visual Patterned Reflex Present during Hypothalamically Elicited Attack

Abstract. *A cat from which attack is elicited by electrical stimulation of the hypothalamus lunges more frequently toward a mouse presented to the eye contralateral to the stimulated site than it does to a mouse presented to the ipsilateral eye. This differential effect does not appear to be attributable to a temporary or permanent defect in the ipsilateral eye.*

It has been reported that the attack behavior of a cat elicited by electrical stimulation of the brain is mediated, at least in part, by mechanisms that are like reflexes (*1*). However, unlike such a reflex as pupillary constriction to light, which is regularly present, these patterned reflexes become operative when the cat is electrically stimulated at sites in the brain from which attack is elicited, provided that an adequate stimulus is present in the environment. We report here the discovery of another such patterned reflex (that is, a reflex present during stimulation) which consists of a cat's lunge toward a mouse visually presented during electrical stimulation of the brain at hypothalamic "attack" sites (*2*).

Observations were made on five cats. Each cat was fitted under aseptic conditions with several electrode guides mounted stereotaxically on the skull over the hypothalamus. After each cat recovered from surgery (5 to 7 days), it was placed in a large test cage with a deeply anesthetized rat. None of the cats spontaneously attacked the rats. Sterile calibrated monopolar electrodes were then advanced in small steps through the guides into the brain tissue of each animal. Stimulation was carried out at each step, and the animal's behavior was noted. The stimulation consisted of 1-msec biphasic pulses repeated at a frequency of 62.5 per second whose intensity ranged from 0.2 to 0.9 ma. When a biting attack upon the rat occurred, the exploring electrodes were cemented into place.

The experiment was conducted with the cat restrained in a loose-fitting canvas sack from which its head protruded. The cat in its sack was further enclosed in a plastic box that left its head but not its body free to move. No signs of discomfort were observed. The cats purred, ate, and even slept while being restrained.

For each experimental trial a hypothalamic attack site was stimulated for 30 seconds. During this time a deeply anesthetized mouse attached to one end of a tongue depressor was repeatedly moved toward the cat's head and directed toward the mouth. The experimenter varied the angle of presentation, exploring the cat's visual field. Movements of the cat's head introduced additional variations in the angles of presentation. The number of presentations within a 30-second period varied from five to ten, depending on the cat's success in reaching the mouse. First one eye was covered and then the other, and the occurrence of lunging by the cat toward the mouse was noted. A blinder along the midline further restricted the visual fields. At least 5 minutes intervened between each pair of 30-second trials. No more than ten trials were carried out on the same day at any one attack site.

In the unstimulated cat the visual presentation of the mouse produced no observable activity beyond a tendency in some cats to withdraw the head from the approaching stimulus. During hypothalamic stimulation, however, the cat lunged (that is, rapidly moved its head forward) and opened its mouth at the sight of the mouse. The maximum distance at which the mouse elicited the lunge and jaw-opening was 2 to 2.5 inches (5.1 to 6.4 cm) from the cat's mouth. The lunge and jaw-opening were always followed by vicious biting of the mouse if the probe was not quickly withdrawn.

There was a higher likelihood of a lunge occurring when the mouse was presented to the eye contralateral to the site stimulated than when the mouse was presented to the ipsilateral eye. This main result for each of the five cats is shown in Fig. 1. For one cat (cat 5), however, if one site (site b) was stimulated, no difference between the two sides was found, although from this same animal the general result was obtained if a second site (site a) was stimulated (*3*).

The finding that the contralateral eye is more effective in mediating attack (that is, lunge and jaw-opening) is not attributable to a consistent defect in the ipsilateral eye of the cats since in

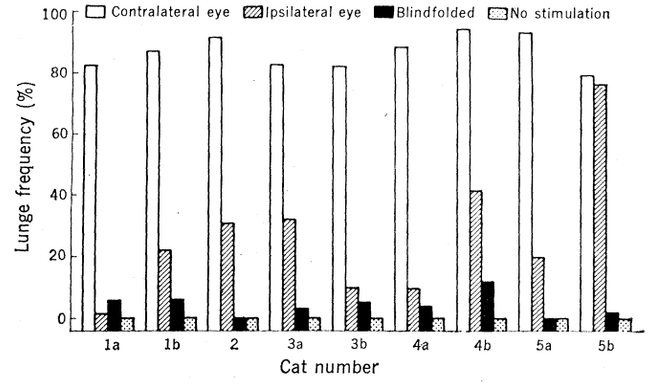

Fig. 1. The frequency of a cat's lunge toward a mouse during electrical stimulation of hypothalamic attack sites was greater when the mouse was presented to the contralateral eye than when the mouse was presented to the ipsilateral eye. The cat is indicated by a number, and the stimulated site by a letter. Sites a and b for cats 1, 4, and 5 were on opposite sides of the brain. The lunge frequency percentages for the contralateral and ipsilateral eyes were based on at least 200 presentations of the mouse to each eye, with the exception of cat 4, for which the frequencies were based on 110 presentations to each eye. All lunge frequency percentages for the unstimulated and blindfolded conditions were based on at least 50 presentations of the mouse. The differences between the contralateral and ipsilateral eye (except for cat 5, site b) were significant at $P < .001$ on the basis of a χ^2 test.