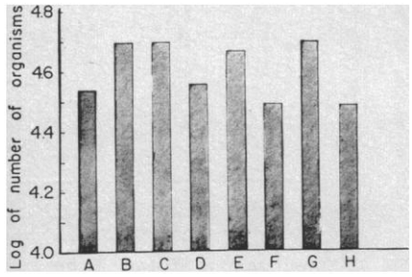

Fig. 2. Effects of different kinds of treatment on the probiotic factor produced by *Colpidium campylum*. Maximum growth of *Tetrahymena* after 6 days. *A*, Control (no factor added); *B*, conditioned medium autoclaved 20 minutes; *C*, nonfilterable factor (with membrane filter of 0.1-μ porosity); *D*, filtrate (with membrane filter of 0.1-μ porosity); *E*, factor hydrolyzed with 1*N* HCl at 100°C for 60 minutes; *F*, factor autoclaved at 20 lbs (9 kg) for 2 hours; *G*, factor separated by means of Sephadex G-25; *H*, factor hydrolyzed with 1*N* NaOH at 100°C for 30 minutes.

to prolonged autoclaving (1 hour or more) or to Seitz filtration. The active material was retained by membrane filters (Millipore and Gelman types) of fine grades with porosities of 0.1 μ, but not by filters of average porosity. Preliminary tests indicated that the separated materials contained considerable protein. Ninhydrin tests were positive after acid hydrolysis of the residue (obtained by filtration) in 1*N* HCl for 1 hour at 100°C, but this treatment did not destroy the probiotic effect. On the other hand, alkaline hydrolysis with 1*N* NaOH for 30 minutes at 100°C resulted in complete destruction of the factor. By the use of a Sephadex column (G-25) it was possible to obtain active fractions after elution with 3 to 6 ml of phosphate buffer (Fig. 2).

We could neither increase the probiotic effect more than 50 percent by concentrating the active material, nor could we dilute the original concentration of the material by more than a factor of 50. When probiotic materials produced by different species of ciliates were used together, no additive effect could be observed. Typical proteins, such as ovalbumin and casein, did not increase growth to any appreciable extent.

At present, the chief significance of probiotics is their possible mode of action in growth regulation. Since the logarithmic phase of growth in microorganisms has been significantly prolonged by the action of these products of ciliates, perhaps similar but more potent growth regulators will be found with even more striking effects. The fact that no potentiation or synergistic action of probiotics of different origin has yet been demonstrated may simply mean that these have a common mode of action. The somewhat analogous relation between the protozoan probiotic effects and the response of target cells to hormones in higher animals suggests another biological area of interest. The failure to increase the probiotic effect to the dramatic order of magnitude seen in the action of antibiotics might be explained by low responsiveness, in comparison with the better understood control mechanisms in inhibition, of the control mechanism that accelerates growth, or extends the period of growth, of microorganisms. Perhaps the current interest among bacteriologists in peptides with strepogenin activity will result in more information about stimulatory mechanisms in microorganisms generally. At least the protozoan probiotics offer an approach to the problem of growth regulation from the positive or accelerative aspect, rather than the more usual inhibitory or decelerative aspect.

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12. The numerous counts necessary for this investigation were facilitated by the use of a Coulter counter, Model A. The samples for counting were diluted 1 : 10 in a counting fluid containing 0.2 percent sodium chloride and 0.1 percent formalin.
13. We thank G. W. Kidder for the original cultures of ciliates used in this work. Supported by NSF grant GB-1121.

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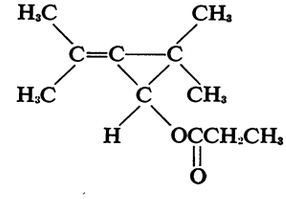
American Cockroach Sex Attractant

Abstract. *The structure (2,2-dimethyl-3-isopropylidenecyclopropyl propionate) previously assigned to the sex attractant of the American cockroach has now been shown by additional physical and chemical data and biological inactivity of the synthetic preparation to be incorrect. The structure of this attractant remains to be determined.*

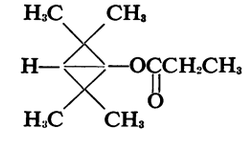
Widespread interest has been exhibited in the natural sex attractant of the American cockroach, *Periplaneta americana* (L.), whose structure we reported earlier (*1*) to be (I).



(I)

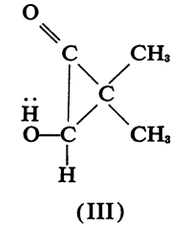
A compound having this structure has now been synthesized by Day and Whiting (*2*) and, recently, also in our laboratory by another procedure (*3*); it does not elicit a sexual response in *P. americana* males and is therefore not the sex attractant.

Day and Whiting suggest structure (II) as the correct one.



(II)

II has the same formula ($C_{11}H_{18}O_2$) as I but differs from it in the position of one of the bonds with the corresponding transfer of a hydrogen atom. We have now obtained additional evidence that reflects on the structure of the sex attractant and that of one of its oxidative degradation products, whose melting point is 55°C, for which we had suggested structure (III) (*1*).

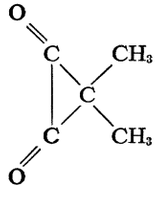


(III)

Mass spectra (*4*), previously not available to us, have confirmed the

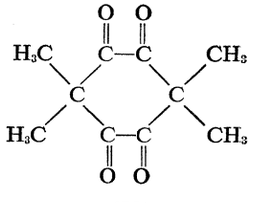
molecular weight of the roach attractant as 182, and peak matching on a double-focusing high resolution instrument has confirmed the elemental formula as $C_{11}H_{18}O_2$. A prominent peak, corresponding with C_3H_5O by peak matching (mass/electron charge, $m/e = 57$), is consistent with a propionate. However, the nuclear magnetic resonance (NMR) spectrum at 100 mcy/sec (*5*) (Fig. 1) contradicts this assignment even though propionic acid was obtained as one of the degradation products in both the saponification and oxidation experiments. The $-CH_2-$ group of a propionate should appear as a quadruplet equivalent to two protons at about 7.7 τ (τ being 10 minus the parts per million); instead we found at 7.68 τ a multiplet equivalent to only one proton. Decoupling attempts indicated that the triplet at 9.14 τ was coupled with the 8.75 τ peak or the 8.39 τ multiplet. Irradiating at 7.68 τ did not collapse the 9.14 τ triplet to a singlet.

We were also able to obtain a mass spectrum of the oxidation product, m.p. 55°C. An intense peak appearing at m/e 98 gave a molecular formula of $C_5H_6O_2$ (Beynon's tables, *6*) and suggests 1,1-dimethylcyclopropanedione (IV) (*7*) as a possibility.



(IV)

Other peaks of the mass spectrum were also consistent with structure IV (*8*). Considering that dimethylmalonic acid was obtained by periodic acid oxidation of the degradation product, that the infrared spectrum shows two carbonyl bands (1715 and 1680 cm^{-1}), and that a red color test for *o*-diketones (*9*) is positive, structure IV is possible for the degradation product; but the dimer whose structure is shown (V) is not ruled out if the 98 peak is not the parent one.



(V)

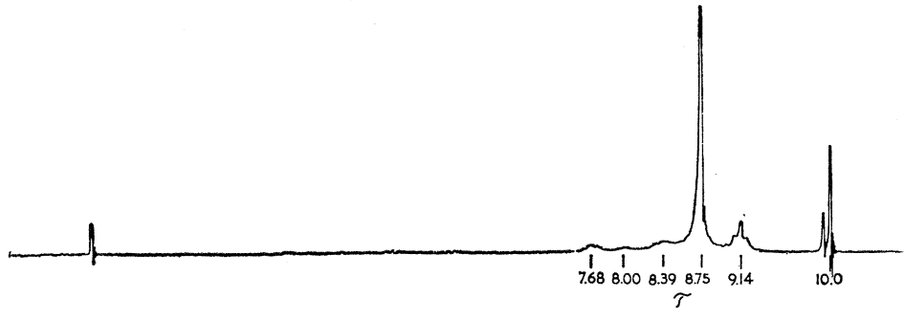


Fig. 1. Nuclear magnetic resonance spectrum of naturally occurring cockroach sex lure in $CDCl_3$.

Structure I had been based partially on the identity of infrared spectra of hydrogenated I (one mole hydrogen uptake) and a synthetic saturated preparation, and on their gas chromatographic analysis by the method of Wharton *et al.* (*10*). Both preparations were subsequently found to be mixtures. Gas chromatography (*11*) of each mixture gave five peaks in nearly identical proportion with corresponding retention times. Infrared spectra of the synthetic mixture run before and after chromatography were identical, and this indicates that decomposition had not occurred on the column. Two of the constituents of the mixture obtained by synthesis were identified as diethyl maleate and diethyl fumarate. Saponification of the hydrogenated attractant mixture and of the synthetic saturated product gave propionic acid in both cases. These results indicated that the sex attractant readily undergoes a complex rearrangement whose nature and mechanism we do not yet comprehend.

The roach attractant is exceptionally unstable and will decompose in a few days if kept undiluted, even in the cold. If kept under nitrogen and well diluted in cold hydrocarbon solution, the compound is stable for at least 6 months. During the isolation the active fraction was always stored in this manner. Just before any of the spectra were run or oxidations carried out, the compound was chromatographed on silicic acid and steam-distilled (*1*) to remove any material that might have polymerized or that might have been otherwise degraded. This procedure, which presumably prevents intermolecular reactions, is useful for sensitive compounds.

Although we had considered II as a possible structure for the natural attractant, because (i) the strong peak at 8.75 τ is roughly equivalent to 12 protons, (ii) the compound lacked optical activity, (iii) the strong C–H stretching vibrations in the infrared spectrum (2960 and 2873 cm^{-1}), and (iv) the unusual instability of this highly strained structure made this choice seem logical, the nuclear magnetic resonance spectrum and decoupling experiments do not support this possibility. However, we have been attempting to synthesize a compound having structure II. The structure of the sex attractant remains to be determined.

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4. Kindly run by Dr. A. K. Bose, Stevens Institute of Technology, Hoboken, New Jersey, and by Dr. H. Fales, National Institutes of Health, Bethesda, Maryland.
5. Kindly run by D. Hollis, Varian Associates, at Pittsburgh, Pennsylvania. The original spectrum (*1*) was obtained with a solution which was too dilute to be useful for learning much beyond the fact that no protons were present on a double-bond carbon. The peak at 140.5 cy/sec, absent on the rerun at 100 mcy/sec, was due to a trace of acetone inadvertently introduced by the NMR operator. A repeat of the oxidation confirmed the validity of the acetone obtained by oxidation as reported in (*1*).
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7. The broad hydroxyl band previously reported in the infrared spectrum (*1*) may have been due to water of crystallization [K. Nakanishi, *Infrared Absorption Spectroscopy* (Holden-Day, San Francisco, 1962), p. 30], which would be lost in the high vacuum of the mass spectrometer.
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