Scopolamine was much more potent; a good effect has been observed even at 0.2 mg/kg. This superiority of scopolamine, taken together with the demonstrated (relative) inactivity of the quaternary compounds, is compelling evidence that anticholinergic drugs inhibit drinking by a central effect.

Table 1 also shows that all compounds strongly depressed food intake. In the feeding experiments, however, the quaternary compounds were slightly more potent than their tertiary analogs. In addition, the potencies of atropine and scopolamine tended to converge; note that atropine at 1 mg/kg had about the same effect as scopolamine at 0.4 mg/kg. These observations indicate that anticholinergic drugs inhibit food intake by a peripheral effect. Possibly feeding is inhibited because it is aversive to chew dry food when salivation is blocked.

The results on water intake complement recent findings of Grossman (*6*) and Stein and Seifter (*7*). Grossman induced drinking in water-satiated rats by applying minute quantities of cholinergic substances directly into the hypothalamus. Stein and Seifter confirmed and extended this work by showing that the effect was muscarinic rather than nicotinic (*8*). These studies suggest strongly that, at least in the rat, muscarinic synapses in the hypothalamus are part of a brain system that regulates water intake. Such hypothalamic synapses, and perhaps other muscarinic synapses at different levels, provide likely sites of action for the drug effects reported here (*9*).

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24 October 1962

Isolation and Identification of the Sex Attractant of the American Cockroach

Abstract. *The highly potent sex attractant of the female American cockroach,* Periplaneta americana *(L.), has been isolated in pure form and identified as 2,2-dimethyl-3-isopropylidenecyclopropyl propionate. The hydrogenated form of the attractant has been synthesized.*

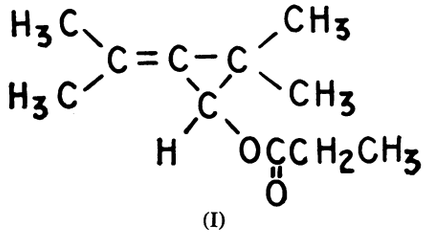
The virgin female American cockroach, *Periplaneta americana* (L.), emits a powerful attractant which elicits intense excitement and characteristic wing-raising in the males of this species (*1*). Wharton *et al.* (*2*) showed that filter paper over which these females had crawled was highly attractive to males, and these investigators have very recently reported that they obtained from such paper 28 μg of a pure attractant which they were not able to identify (*3*). As the result of an independent investigation, we report the successful isolation and identification of the natural sex attractant.

Extraction of filter papers with which virgin female roaches had been in contact gave extremely small amounts of an attractive mixture from which the abundant contaminants were very difficult to separate. Much larger amounts of fairly pure attractant were obtained by passing air continuously over many virgin females in metal containers, condensing the vapors in a dry ice cooled flask containing a little 0.1-percent hydrochloric acid (*4*), and extracting the condensate with distilled hexane (*5*). The hexane solution was washed with water, dried over sodium sulfate, and freed of solvent at 20 mm-Hg pressure (bath below 40°C). The residual yellow semisolid was chromatographed on a column of silicic acid [Bio Rad Laboratories, Richmond, Calif., specially treated to conform with that described by Hirsch and Ahrens (*6*)] by successive elution with spectral-grade hexane and 3 percent ether in hexane; elution with 10 percent ether in hexane then removed a highly active yellow liquid from which the pure attractant was obtained by steam distillation. In this way there was obtained, from the equivalent of approximately 10,000 females "milked" continuously over a 9-month period, 12.2 mg of the pure attractant as a yellow liquid with a characteristic odor; it elicits a response from males at levels below 10^{-14} μg.

Gas chromatography of the attractant on a 4-foot Chromosorb W column (80 to 100 mesh) coated with 5-percent Apiezon M, under conditions identical with those reported by Wharton *et al.* (*3*), gave a single peak with an elution time of 6 minutes as contrasted with 105 or 145 minutes reported by them. In a single determination it analyzed for $C_{11}H_{18}O_2$, showed no optical rotation at a concentration of 1.8 percent ($CHCl_3$), and was free of absorption in the ultraviolet range. Its infrared spectrum showed it to be an ester, which was supported by its inactivation through refluxing with dilute alkali or concentrated hydrochloric acid; the presence of an isopropylidene group at 12.5 μ was also indicated (*7*). Catalytic hydrogenation of 2.2 mg of the attractant with platinum oxide catalyst resulted in an uptake of hydrogen sufficient for 1.1 double bonds, giving a colorless, inactive oil whose infrared spectrum lacked isopropylidene absorption and showed splitting at 7.25 μ characteristic of an isopropyl group. Hydrogenolytic chromatography of the attractant by the method of Beroza (*8*) gave ethane and 2,2,4-trimethylpentane. A nuclear magnetic resonance spectrum at 60 mcy/sec on the limited amount of attractant in deuterochloroform showed no hydrogen attached to a double bond and disclosed, among other features, two sharp prominent peaks: one at 75.5 cy/sec from internal tetramethylsilane was roughly equivalent to six hydrogen atoms (two methyl groups) and one at 140.5 cy/sec was equivalent to one hydrogen atom. Alkaline saponification of 2.2 mg of the saturated compound showed a saponification equivalent of 182, yielding 1.5 mg of a liquid alcohol, whose infrared spectrum showed a secondary hydroxyl group, and 0.85 mg of an acid identified by paper chromatography and its infrared spectrum as propionic acid. Oxidation of 4 mg of the attractant with periodate-permanganate reagent (*9*) gave propionic acid (identified by infrared spectrum and paper chromatography), acetone [2,4-dinitrophenylhydrazone, mp 127°C, undepressed by an authentic sample, λ_{max} 349 mμ (EtOH)], and 2.2 mg of a neutral substance that formed colorless crystals, mp 55°C (from ethyl acetate), λ_{max} 277 mμ (EtOH), whose 2,4-dinitrophenylhydrazone melted at 232°C, λ_{max} 355, 358 mμ ($CHCl_3$). The infrared spectrum of this substance indicated the presence of a chelated hydroxyl and showed two carbonyl bands. Further

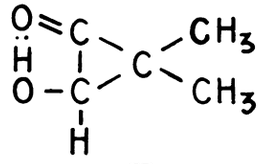
oxidation with periodic acid converted the neutral substance to a crystalline acid, mp 197° to 198°C, identified as dimethylmalonic acid by paper chromatography, infrared spectrum, and mixed melting point with a pure synthetic sample.

The only structure for the attractant consistent with the foregoing data is 2,2-dimethyl-3-isopropylidenecyclopropyl propionate (I).



(I)

The neutral substance formed on oxidation probably possesses the structure shown in (II).



(II)

That the attractant does indeed possess structure I was proved by synthesizing its hydrogenation product according to the following procedure. The reaction of 2,4-dimethyl-2-pentene with diazoacetic ester in the presence of copper powder (*10*) gave ethyl 2,2-dimethyl-3-isopropylcyclopropanecarboxylate (54 percent; bp 95° to 100°C at 20 mm-Hg; n_D^{25} 1.4315), which was saponified with ethanolic potassium hydroxide to the corresponding acid (66 percent), a colorless, viscous oil. The undistilled acid was decarboxylated with lead tetraacetate and iodine (*11*) to give 2,2-dimethyl-1-iodo-3-isopropylcyclopropane (66 percent; bp 52°C at 20 mm-Hg). Reaction of the iodide with silver propionate in dry benzene gave 2,2-dimethyl-3-isopropylcyclopropyl propionate (35 percent; bp 112°C at 20 mm-Hg; n_D^{25} 1.4352; analyzed exactly for $C_{11}H_{20}O_2$), whose infrared spectrum and elution time (5.9 minutes) by the previously cited gas chromatographic procedure were identical with those of the hydrogenated attractant.

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14 December 1962

Giant Muscle Fibers in a Barnacle, Balanus nubilus Darwin

Abstract. *Cross-striated muscle fibers of very large size have been found in the scutal-tergal adductor and depressor muscles of the large barnacle* B. nubilus. *Adductor muscle fibers are up to 2 mm thick. They are innervated by separate nerves, each supplying one end, but not the central region, with terminals; each fiber receives two or three excitor axons. Depressor muscle fibers are up to 1.4 mm thick and receive multiterminal innervation along their entire length; they are innervated by two excitor axons. Postsynaptic potentials are of small or large size and lead to small or large twitches; they do not show facilitation. The muscle fibers shorten to as little as one-sixth resting length.*

The study of the physiology of specialized cells has often been greatly aided by the use of examples of unusually large size. Giant muscle fibers might be expected to be of considerable value in muscle physiology. Fibers up to 600 μ in diameter, present in crab muscle, enabled Caldwell to study intracellular *p*H changes (*1*) but these fibers cannot be readily isolated for study and have not been widely used.

We have found fibers of at least twice this thickness in the scutal-tergal adductor and depressor muscles of the large barnacle *Balanus nubilus* Darwin. They are almost completely free from connective tissue and can very readily be isolated in good condition, with resting potentials from 78 to 86 mv. They can be partially isolated with the nerve supply intact.

The adductor contains about 25 giant fibers having maximum thickness ranging from 500 to 2000 μ. These are innervated by two separate nerves entering close to the two margins where the fibers are attached to the scutes. Each fiber receives several terminals from each nerve, and these are concentrated at the ends. In no case does the nerve cross the midline. Four axons in each nerve were stained clearly by methylene blue.

Intracellular recordings were made during graded electrical stimulation applied to the nerves. These gave similar results on each side. Most of the muscle fibers were doubly innervated, although some were triply innervated. The fibers were all excitatory. No evidence for inhibitory fibers has been found.

The smallest responses were postsynaptic potentials of 10 to 15 mv when recorded 3 mm from the attachment (peak zone); these were not present in all the muscle fibers. A second kind of postsynaptic potential of about 25 mv was observed in many muscle fibers. All gave a giant postsynaptic potential, 40 to 50 mv in magnitude, following maximal stim-

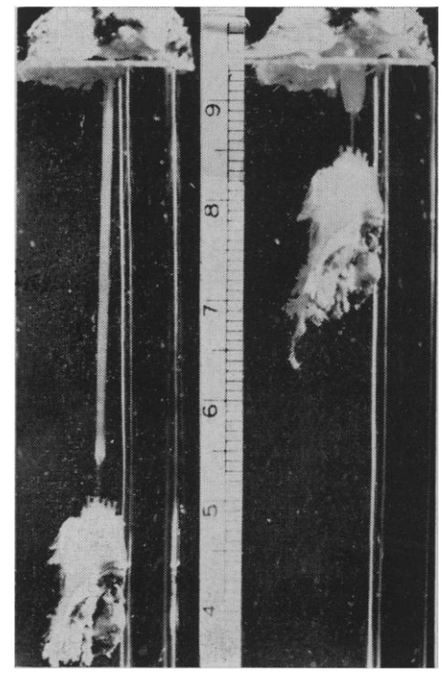

Fig. 1. Isolated single giant muscle fiber of m. depressor scutorum rostralis of *Balanus nubilus*. A portion of the shell is seen at the upper end, and a scute at the lower. The cut tendons of other giant fibers can be seen. The fiber was immersed first in barnacle Ringer solution (left) and then placed in barnacle Ringer (right) containing 200 mmole of potassium in place of sodium. Scale in centimeters.