Sex Pheromones: (E,E)-8,10-Dodecadien-1-ol in the Codling Moth

Abstract. *Although* (E,E)*-8,10-Dodecadien-1-ol was reported to be a sex pheromone of the codling moth* [Laspeyresia pomonella *(L.)*], *its presence in the moth was questioned, mainly because it has not been isolated. A computerized search of data from gas chromatography–mass spectrometry of a partially purified extract equivalent to 45 abdominal tips of female moths produced a mass spectrum that matched that of the authentic compound. Other data also confirmed the presence of the compound.*

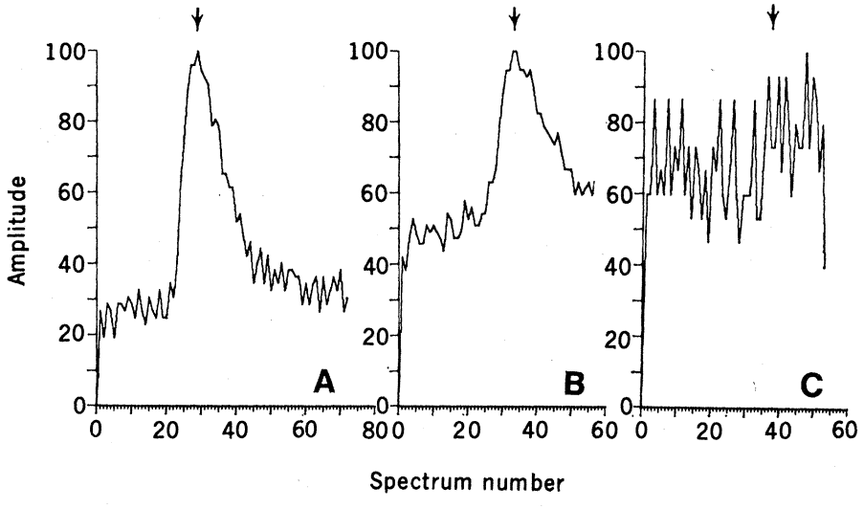
Roelofs *et al.* (*1*) proposed (*E,E*)-8-10-dodecadien-1-ol as the sex pheromone of the codling moth, primarily on the basis of electroantennograms of model compounds and of the pheromonal extract. They found that the retention times of the synthetic chemical (hereafter referred to as codlelure) on polar and nonpolar gas chromatographic columns coincided with those of the active material in a pheromonal extract (monitored by electroantennograms at 0.5-minute intervals), and that codlelure was a potent attractant of the male codling moth in the field. Because chemical characterization of the natural attractant was meager, the presence of the compound in the moth was still open to question. This doubt was reinforced when McDonough and co-workers (*2*) were not able to isolate the compound from codling moth extracts.

By a computerized search of mass spectra obtained from the gas chromatographic effluent of a codling moth extract subjected to minimum cleanup, we obtained at the proper retention time a mass spectrum that closely duplicated that of codlelure. This result, plus supplementary data, provides confirmatory evidence for the presence of codlelure in the moth.

We partially purified an extract of female codling moth tips by silica gel chromatography (*3*) and noted that synthetic codlelure, when chromatographed identically, eluted in the first and second 30 percent ether-in-hexane fractions. After concentrating with a stream of nitrogen one half of each of these two fractions of the extract and the succeeding one to a small volume (about 20 μl), we injected amounts equivalent to 45 insect tips into the gas chromatograph–mass spectrometer (GC-MS) (*4*) and recorded mass spectra every 2 seconds from about 1 minute before to 1 minute after the retention time of codlelure. The computer plots of the *m/e*–182 amplitude (molecular ion of codlelure) as a function of spectrum number from the three fractions showed a peak at the retention time of codlelure in the plots from the first two fractions eluting with 30 percent ether in hexane (Fig. 1, A and B); as would be expected from the chromatographic behavior of codlelure, no peak appeared in the plot from the third fraction (Fig. 1C). A differential mass spectrum was then prepared by subtracting a mass spectrum taken at the side of the peak from one at the peak apex (*5*). The very good agreement of this differential spectrum with the mass spectrum of an authentic sample of codlelure (*6*) is apparent in Fig. 2.

Gas chromatography of the second fraction eluting with 30 percent ether in hexane on a column of 5 percent Carbowax 20M (*7*) produced a peak at the retention time of codlelure. From the increase in peak height after the addition of 20 ng of codlelure, we estimated that the fraction contained about 3 ng of codlelure per abdominal tip. With apparently somewhat less codlelure in the first fraction, the original extract was estimated to contain roughly 5 ng of codlelure per abdominal tip.

The remaining halves of the two fractions eluting with 30 percent ether in hexane mentioned above were combined and chromatographed on a silica gel–silver nitrate column (Adsorbosil CABN), which separates compounds on the basis of the number of double bonds and their configuration. The fraction having the same elution volume as



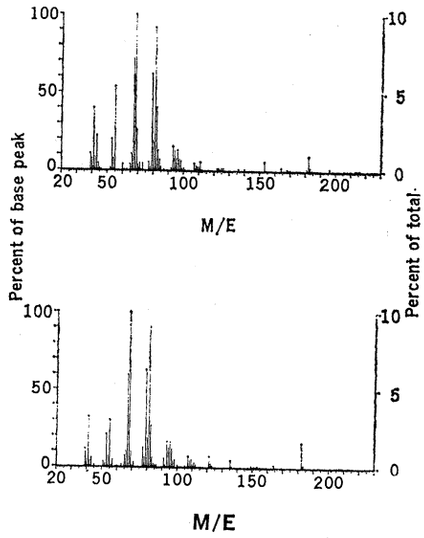


Fig. 1 (left). Computerized plots derived from gas chromatographic–mass spectral data on a partially purified extract of female codling moth tips. Spectra were taken every 2 seconds. Curves show *m/e*–182 amplitude as a function of spectrum number from the following fractions (30 percent ether in hexane) from silica gel chromatography: (A) first fraction, (B) second fraction, and (C) third fraction. Arrow shows retention time of codlelure in each spectrum. Fig. 2 (right). (Top) Differential mass spectrum obtained from partially purified extract (from Fig. 1B, spectrum No. 33–spectrum No. 26); (bottom) mass spectrum of synthetic codlelure.

codlelure showed a peak in the GC-MS plot of m/e–182 amplitude as a function of spectrum number at the same retention time as authentic codlelure.

Although mass spectral data do not establish positions of double bonds, the agreement between the retention times of codlelure and the natural pheromone on the Carbowax column (*7*) as well as the agreement in elution volumes on the silica gel–silver nitrate column provide strong confirmatory evidence for the assigned positions of the double bonds and the configurations, which were fixed by the data of Roelofs *et al.* (*1*).

The presence of codlelure in the moth extract raises the question of why it has not been isolated from the moth. We ascribe this difficulty to the exceptional instability of the pure compound when exposed to air and light during isolation. For example, virtually no codlelure was detectable as such when we allowed a thin film of it in a test tube to stand for a few days at room temperature exposed to air and light.

Sex attractants have been found by empirical screening of chemicals (*8*) and by the antennogram technique of Roelofs *et al.* The technique of computerized searching of GC-MS data can supplement these procedures by verifying or rejecting the presence of specific chemicals in the extract of the natural pheromone. The procedures are compatible because they can be used with minute amounts of chemical, rigorous purification of insect extracts is not necessary, and the number of insects needed is few compared with the number needed to elucidate chemical structure by conventional methods of isolation and identification.

The codling moth is a major and worldwide pest of apples. With the presence of the compound in the insect established, we now have a firm basis for proceeding with the use of codlelure in control efforts against this highly injurious insect.

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References and Notes

1. W. L. Roelofs, A. Comeau, A. Hill, G. Milicevic, *Science* **174**, 297 (1971).
2. L. M. McDonough, D. A. George, B. A. Butt, J. M. Ruth, K. R. Hill, *ibid.* **177**, 177 (1972).
3. A total of 500 abdominal tips from 3-day-old female codling moths was ground in a mortar with acetone and then hexane, and the slurry was filtered. The filtrate was extracted with water (to remove the acetone) and with saturated aqueous NaCl, and then dried (over Na_2SO_4). The concentrated extract was then chromatographed on a 25-g column of silica gel (J. T. Baker No. 3404) used as received and eluted successively with 100-ml portions of 0, 2, and 5 percent ether in hexane, two 100-ml portions of 15 percent ether in hexane, one 100-ml and six 50-ml portions of 30 percent ether in hexane, and 100 ml of ether.
4. We used a Finnigan Corp. (Palo Alto, Calif.) 1015 quadrupole mass spectrometer interfaced with a glass Gohlke separator to a gas chromatograph equipped with a glass column 1.5 m by 2 mm (inside diameter) containing 3 percent OV-1 on Varaport at 135°C and operated with a helium flow rate of 15 ml/min. With these conditions, the retention time of codlelure was 9.8 minutes. A Systems Industries (Palo Alto, Calif.) 150 computer system was used to control the scan of the mass spectrometer, record the mass spectra on magnetic tape, and retrieve the data.
5. Background interference is removed by this procedure.
6. Supplied by T. P. McGovern.
7. The column was 3.6 m by 6 mm (outside diameter); the temperature was 165°C, and the helium flow rate was 70 ml/min. The retention time of codlelure was 11.4 minutes.
8. W. L. Roelofs and A. Comeau, *J. Econ. Entomol.* **63**, 969 (1970).

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Creutzfeldt-Jakob Disease: Focus among Libyan Jews in Israel

Abstract. *A countrywide search for Creutzfeldt-Jakob disease in Israel disclosed 29 cases with onset between 1963 and 1972. Incidence in various ethnic groups varied in the narrow range of 0.4 to 1.9 per million population except among Jewish immigrants from Libya, among whom the incidence was 31.3 per million. An extraordinary excess of Creutzfeldt-Jakob disease exists in this ethnic group.*

Creutzfeldt-Jakob disease (CJD) is a rapidly progressive, fatal "degenerative" disease of the central nervous system which is now classified, along with kuru, among the unconventional slow virus diseases of man (*1*). Passage of the disease from affected individuals to certain primates has been accomplished (*2*), but no conventional viral agent has been isolated. A genetic cause or at least a familial predisposition has been recognized in some instances (*3*), but even familial cases are transmissible (*4*). With both an unconventional virus and a genetic (familial) predisposition in mind as possible etiologic factors, we report a focus of CJD among Libyan Jewish immigrants to Israel.

Israel has a heterogeneous population composed of immigrants from many different countries, and analysis of the ethnic distribution of different diseases can easily be performed. All ethnic groups have equal access to Israel's excellent medical facilities. Behar *et al.* (*5*) reported a series of six cases of CJD in Israel, two of whom were Libyan immigrants. Goldhammer *et al.* (*6*) reported their experience with CJD in Israel, and five of their twelve cases were Jewish immigrants from Libya. Cases of CJD have been reported in widely scattered regions of the world (*4*), but no ethnic focus has previously been recognized.

We thought that the observations by Braham's group (*6*) and by Behar *et al.* (*5*) might indicate an unusual predilection to CJD among Libyans in

Table 1. Creutzfeldt-Jakob disease among ethnic groups in Israel. The numbers in parentheses exclude possible CJD cases.

Country of birth	Patients (No.)	Population at risk	Average annual incidence per million population (1963 to 1972), age-adjusted to population of	
			Israel, 1968	U.S., 1970*
Libya	13 (13)	30,792	31.3 (31.3)	33.0
Iraq	3 (2)	117,587	1.9 (1.3)	2.5
West and Central Europe	4 (3)	199,300	1.0 (0.6)	1.1
Israel	2 (1)	1,020,411	1.0 (0.5)	1.0
Morocco, Algeria, Tunisia	2 (1)	275,432	0.8 (0.3)	0.9
East Europe	5 (3)	509,317	0.4 (0.2)	0.5
Total	29 (23)			

* Based on total CJD cases.