Identification of the Oxidative Decomposition Products of the Boll Weevil Pheromone, Grandlure, and the Determination of the Fate of Grandlure in Soil and Water

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The oxidation products of grandlure were isolated by column and thin-layer chromatography. The compounds were identified by a combination of gas chromatography, mass spectrometry, ir, and proton magnetic resonance analysis. Two acids, two esters, and one aldehyde were identified as oxidation products of the grandlure aldehydes; the grandlure alcohols were stable. Soil and water persistence studies at 32 and 21°C showed that 98% of all components were lost from soil within 24 h at 32°C and that no grandlure remained in soil after 32 h at either 32 or 21°C. Losses of grandlure from water were less rapid than soil; all components were dissipated from water within hours at 32°C. The physical barrier grandlure formulations currently in use showed no tendencies toward excessive soil and water contamination even under extreme conditions.

Male boll weevils, *Anthonomus grandis* Boheman, emit a complex of volatile compounds that acts as a sex-aggregation pheromone (Keller et al., 1964; Cross and Mitchell, 1966; Cross and Hardee, 1968; Hardee et al., 1969). The isolation, identification, and synthesis of the boll weevil pheromone (Tumlinson et al., 1969, 1971) presented researchers with a potentially useful tool for managing natural populations of the insect. As a result, numerous field trapping experiments and laboratory evaluations have centered on the development of controlled-release formulations and studies of the biological activity of the pheromone complex (Hardee et al., 1972; Coppedge et al., 1973; Bull et al., 1973a,b). The resulting formulations of an effective and long-lasting mixture of pheromone and extenders led to revisions in trap design (Leggett and Cross, 1971; Mitchell and Hardee, 1974) and dispenser size, shape, and composition (McKibben et al., 1971; Bull et al., 1973a,b; Coppedge et al., 1973), but the emphasis was usually on the ensuing insect behavior, and little attention was paid to decomposition products or environmental contamination.

However, the use of the pheromone to monitor or control field populations of boll weevils involves the distribution of grandlure dispensers in trap crops or in traps placed in or around cotton fields. It was therefore important to determine any possible contamination by grandlure and to determine the longevity and fate of the compounds if they should reach soil and water. This determination was made possible by the development of a gas chromatographic technique developed by Bull et al. (1971).

EXPERIMENTAL SECTION

Chemicals. The four components of the pheromone (I, *cis*-2-isopropenyl-1-methylcyclobutaneethanol; II, (*Z*)-3,3-dimethyl-$\Delta^{1,\beta}$-cyclohexaneethanol; III, (*Z*)-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde; and IV, (*E*)-3,3-dimethyl-$\Delta^{1,\alpha}$-cyclohexaneacetaldehyde) were purchased from commercial sources. Compounds I and II were supplied and held as neat liquids; compounds III and IV were supplied in pentane solutions of various concentrations. All solvents used were redistilled and held in glass containers.

Thin-Layer Chromatography (TLC). Plates were prepared with 50-μ layers of silica gel G on 20 × 20 cm glass supports. Development systems for grandlure were pentane–diethyl ether (95:5, v/v) and pentane–diethyl ether (90:10, v/v). Visualization of separated components was achieved by spraying the developed plates with vanillin–sulfuric acid reagent (Stahl, 1965) and heating the plates in an oven at 110°C until spots appeared. Grandlure components appeared as either blue or blue-green spots, while the decomposition products were of various colors.

Column Chromatography. Columns of Florisil (100–200 mesh) were used for crude separations of grandlure components and oxidation products. Developing gradient solvent systems consisted of pentane with increasing concentrations of diethyl ether.

Instrumentation. *Gas Chromatograph.* An F&M 402 instrument equipped with a hydrogen flame ionization detector was used with a 1-mV strip chart recorder. The column was 185 cm × 4 mm i.d. glass packed with 10% QF-1 on 80–100 mesh Chromosorb W. The carrier gas was nitrogen at 100 ml/min. Column, injector, and detector temperatures were 110, 135, and 190°C, respectively.

Infrared Spectrophotometer, Beckman IR-18A. Samples were run in CCl_4.

Nuclear Magnetic Resonance Spectrophotometer (NMR), JEOL MH-100. Samples were run in $CDCl_3$.

Mass Spectrometer (MS). A Varian-Mat-CH-7 coupled with a Varian 2700 gas chromatograph and 620L Varian computer were used.

Water Studies. The longevity of grandlure in water was determined by placing 10 mg of the individual components in 250-ml beakers and adding 100 ml of distilled water. Groups of such beakers were held in constant temperature chambers at 32 and 21°C for 0, 4, 8, 12, 24, 32, and 48 h. At the end of each interval, three beakers were removed from each group and extracted three times with diethyl ether. The ether solutions were dried over sodium sulfate, concentrated on a rotary evaporator, dissolved in 1 ml of heptane, and analyzed for grandlure by GLC according to the procedure of Bull et al. (1971). An ether solution of grandlure was carried through the evaporation procedure as a control measure to monitor loss in manipulation. Quantitation of the control sample showed 5% loss of aldehydes from 10 mg and no measurable loss of alcohols.

Also, tests were designed to determine whether the formulation of grandlure currently in use can be washed from the filter dispensers. The grandlure mixture (I, 30%; II, 40%; III + IV, 30%) was injected into ordinary cigarette filters by using the carrier solution developed by Hardee

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et al. (1972) and modified by Bull et al. (1973b). Both bare filters and filters encased in cardboard physical barriers were used. The filter was placed in a glass column equipped with a stopcock to regulate flow. A 100-ml buret was positioned above the tube so that water from the buret dripped directly onto the impregnated filter at a rate of 100 ml in 30 min. The water solution was collected, extracted three times with diethyl ether, concentrated under vacuum, and analyzed by GLC.

Soil Studies. Soil (fine sandy loam) was weighed (100 g) into 250-ml beakers, and 20 ml of water was added to simulate moist field conditions. The individual grandlure components (10 mg of each) in 1 ml of pentane solution were applied evenly to the surface of the soil, and the beakers were held in constant temperature chambers at 32 or 21°C for 0, 4, 8, 12, 24, 32, and 48 h. Three replicates of each compound at each interval and temperature were analyzed. The amount of material remaining in the soil at the end of each interval was determined by extracting the soil samples three times with ether, concentrating the extract, and analyzing the residue by GLC.

The question of whether grandlure could escape from the filters into surrounding soil was investigated by imbedding physical barrier filters containing 10 mg of grandlure mixture in 100 g of moist soil (fine sandy loam) for 24 h at 21°C. Then the filters were removed and the soil was extracted three times with diethyl ether, and the extract was analyzed for grandlure components by GLC.

Also, since the physical barrier formulation is used in Leggett traps (Leggett and Cross, 1971) in the field, it is important to know whether volatilized grandlure contaminates the surrounding soil. Therefore, a Leggett trap containing two cardboard physical barrier filters impregnated with 10 mg of grandlure was placed in a container filled with soil and left in the field for 24 h. Three 100-g samples of the soil around the trap were collected and analyzed for grandlure by ether extraction and GLC.

RESULTS AND DISCUSSION

Compounds I and II. GLC and TLC on the two alcohols revealed no evidence of breakdown products when the compounds were held as solutions (pentane, heptane) or as neat liquids. Only one GLC peak and one TLC spot were observed for each of the compounds, which suggests purity. The conclusion is that both compounds are stable to oxidative and reductive degradation in normal use.

Compounds III and IV. A preliminary GLC analysis of the aldehydes that had been stored in pentane solution in a freezer revealed the presence of four major volatile impurities (Figure 1). A sample of the solution was applied to a Florisil column and subjected to a gradient elution with increasing concentrations of diethyl ether in pentane. Oxidation products A and B eluted together in the pentane–ether (95:5, v/v) fraction and the aldehydes III and IV eluted in the 90:10 and 80:20 fractions; the components C and D eluted with 100% diethyl ether. This behavior on the silica column indicated that in terms of polarity: A + B < III + IV < C + D.

When the purified aldehydes were held in pentane solution at concentrations above 20 mg/ml, the same impurities were formed over a period of 2 days, even when the solution was stored in the freezer. The decomposition did not occur to any measurable extent when the purified aldehydes were held in pentane solution at concentrations less than 10 mg/ml. This information suggests that the pheromone aldehydes should be stored in hydrocarbon solutions at concentrations less than 10 mg/ml until ready for use.

Identification of C and D. The shape of the GLC peaks and the behavior of oxidation products C and D on silica columns and TLC indicated that both contained a highly polar functional group. That both compounds could be extracted from pentane or ether solution with 10% aqueous sodium bicarbonate or 1 N aqueous KOH suggested the presence of a carboxylic acid group. A GLC–MS analysis indicated that the parent ion of both compounds was at *m/e* 168 (Figures 2 and 3). Other significant peaks were: *m/e* 153 (P − 15), loss of CH_3; 135 (P − 33), loss of CH_3 and H_2O; 123 (P − 45), loss of COOH, and base peaks at *m/e* 69 attributable to cleavage of the six-membered ring allylic to the double bond. These results suggested structures C and D.

The assignment of C as the *Z* acid and D as the *E* acid was based on the following observations. The retention time of C vs. D on the QF-1 GLC column parallels the assigned polarity of aldehydes III and IV. The more facile

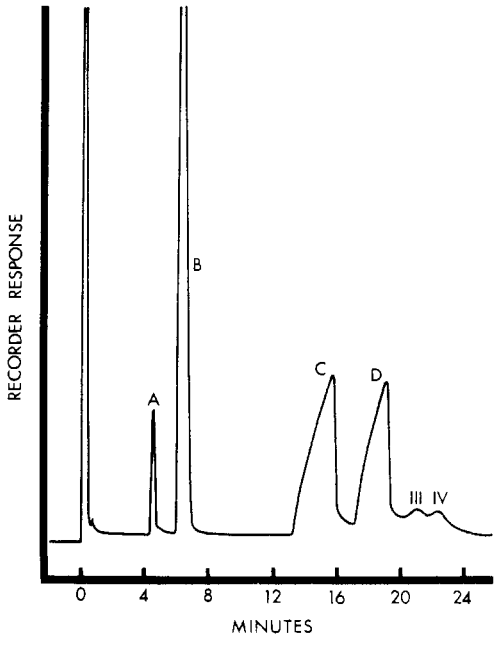


Figure 1. Gas chromatographic trace of boll weevil pheromone aldehyde oxidation products. A, B, C, and D represent oxidation products; III and IV are the original aldehydes. Column conditions are outlined in the text.

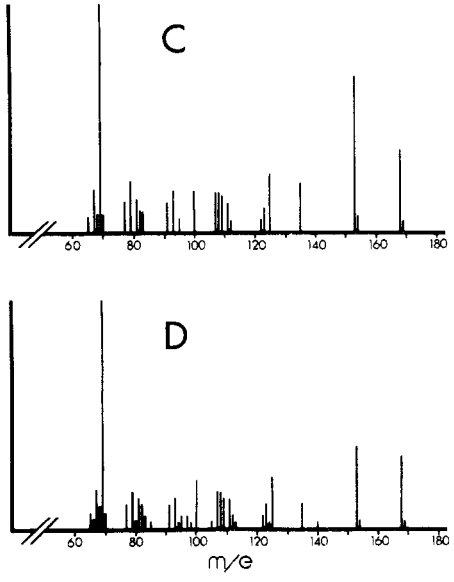


Figure 2. Mass spectrum of compounds C and D, oxidation products of the boll weevil pheromone aldehydes.

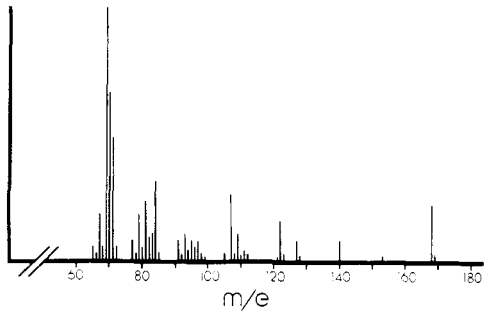


Figure 3. Mass spectrum of compound B, an oxidation product of the boll weevil pheromone aldehydes.

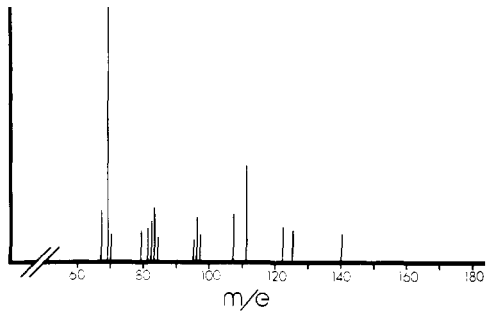
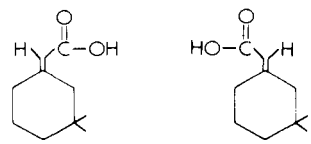


Figure 4. Mass spectrum of compound A, an oxidation product of the boll weevil pheromone aldehydes.



C D

loss of CH_3 (m/e 153) would occur in the Z acid as shown in Figure 2 for compound C; the same is true for loss of $CH_3 + H_2O$ (m/e 135).

Further support of these structures was obtained by treating the compounds with diazomethane and observing the m/e 182, the expected increase when a methyl ester is formed from a carboxylic acid. The fragmentation patterns were characteristic of methyl esters.

Identification of B. The GLC–MS analysis of product B showed the parent ion at m/e 168 (Figure 4) and peaks at m/e 153 (P – 15), loss of CH_3; 140 (P – 28), loss of CO; 122 (P – 46), loss of HCOOH; 107 (P – 61), loss of HCOOH + CH_3, and a base peak at m/e 69. B could not be extracted from pentane with aqueous sodium bicarbonate. The ir spectrum showed a strong absorption at 1730 cm^{-1}, indicative of a carbonyl group, and a strong band at 1150 cm^{-1}, characteristic of a C–O functional group. The ir data coupled with the fact that the compound can be hydrolyzed with KOH indicate the presence of an ester. Also, the hydroxamic acid test for esters gave a strong positive reaction. Results of the NMR analysis also lend support to the ester hypothesis. A doublet at 7 ppm represents the olefinic protons associated with the two unsaturated isomers of the compound. An unresolved doublet at 8.1 ppm is characteristic of the formate ester protons of the Z/E mixture. The identification of C and D as acids of the original aldehydes and the tentative identification of B as the isomers of the formate ester suggests that the original aldehydes were oxidized first to the respective intermediate peroxy acids (Backstrom, 1934), which in turn reacted (Baeyer-Villiger oxidation) with the remaining original aldehydes (Figure 5) to produce the formate esters (Baeyer and Villiger, 1900).

Identification of A. The GLC–MS analysis indicated

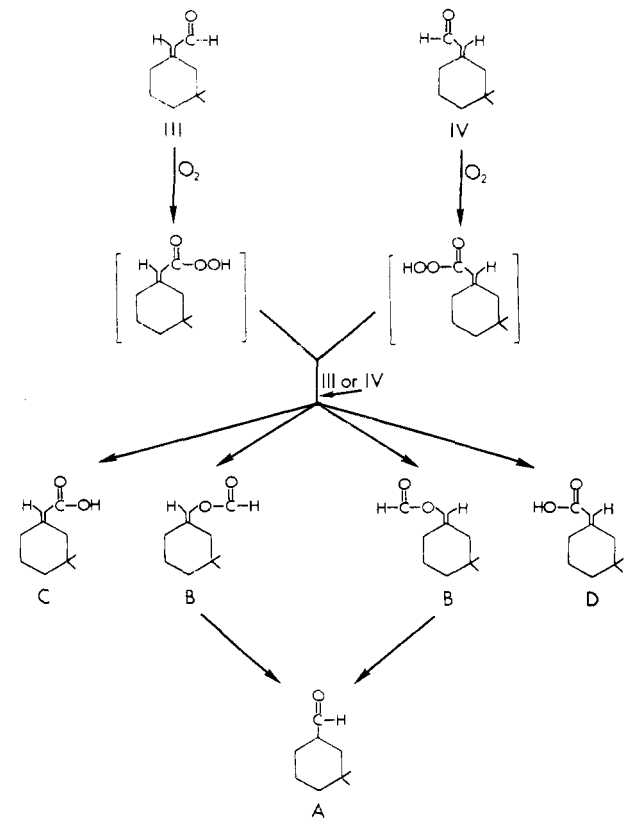
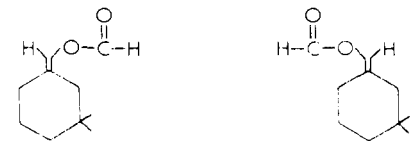


Figure 5. Oxidative degradation scheme of grandlure aldehydes.

Table I. Loss of Grandlure Components from Water at Two Constant Temperatures; Expressed as Percent Loss

Time, h	I		II		III + IV	
	21°C	32°C	21°C	32°C	21°C	32°C
0	0	0	0	0	0	0
4		19.2		32.0		56.1
8	30.8	40.0	26.7	48.0	59.3	74.0
12	38.7	59.5	41.3	74.0	79.8	91.1
24	67.2	90.9	55.1	95.4	90.7	100.0
32	81.6	95.3	74.5	100.0	96.4	
48	86.8	100.0	84.6		100.0	



B

product A had a m/e of 140 with peaks at m/e 125 (P – 15), loss of CH_3; 123 (P – 18), loss of H_2O, and a base peak at m/e 69. A strong carbonyl absorption at 1700–1720 cm^{-1} and the lack of absorption in the 3590–3650-cm^{-1} region of the ir suggested that the P – 18 fragment was the result of loss of H_2O from an aldehyde rather than from an alcohol; also, the NMR peak far downfield (9.3 ppm) is indicative of an aldehydic proton. The compound gave a positive reaction on TLC plates with 2,4-dinitrophenylhydrazine spray reagent. That A can be formed from B by treatment with KOH and the overwhelming spectral evidence in favor of an aldehyde functional group strongly indicate the following structure for A.

Saponification of the ester in B leads to the formation of the unstable vinyl alcohol (>CHOH), which immediately forms the aldehyde. The proposed overall oxidation scheme of the grandlure aldehydes is shown in Figure 5.

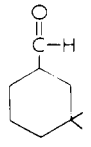
Water Studies. The results of studies of the fate of the

Table II. Percentage of Grandlure Components Washed from Dispensers with 100 ml of Water

Filter	I	II	III + IV
Bare	94.3	92.1	100.0
Physical barrier	8.7	8.4	12.0

Table III. Loss of Grandlure Components from Soil at Two Constant Temperatures; Expressed as Percent Loss

Time,	I		II		III + IV	
h	21°C	32°C	21°C	32°C	21°C	32°C
0	0	0	0	0	0	0
4	44.0	57.2	57.0	81.0	77.8	89.6
8	70.5	74.8	62.5	85.8	85.6	92.9
12	79.0	81.4	79.3	95.2	90.3	96.5
24	92.2	97.6	94.8	97.7	96.8	97.9
32	100.0	100.0	100.0	100.0	100.0	100.0



A

grandlure components in water are shown in Table I. At 32°C the aldehydes (III and IV) were lost within 24 h. No evidence of the oxidation of these compounds to products A, B, C, and D was observed probably because they were present in low concentration and were lost rapidly. Alcohol II was depleted in 32 h, and alcohol I disappeared in 48 h. At 21°C the order of volatilization was the same, though the rates were different. However, even at this lower temperature, the two aldehydes were no longer present after 48 h.

When the two types of grandlure dispensers (bare filters and cardboard physical barrier filters) were washed with dripping water, they showed a marked difference in loss of the compounds (Table II). The loss from the bare filters was nearly quantitative; the filters enclosed in cardboard cylinders lost only 8–12%. The advantage of the enclosed filters is plain; not only does the physical barrier prolong the effectiveness of the pheromone, but it also minimized leaching of the components into the soil.

Soil Studies. The grandlure components volatilized more rapidly from soil than from water (Table III). At 32°C almost 98% of all components were lost within 24 h, and no grandlure remained after 32 h at either 32 or 21°C. Apparently soil particles provide a relatively large surface area that spread the organic volatiles into thin films, and the absorptive effect of the soil is not great enough to overcome the dispersion of the molecules into the surrounding air. Although soil would seem to provide an excellent support for degradation of the compounds, no traces of the oxidation products were found. Perhaps the short duration of contact did not provide ample opportunity for reaction.

Neither grandlure nor the oxidative degradation products of grandlure were detected in the soil around the Leggett trap. The volatility of the compounds apparently forced them into the atmosphere rather than into the soil. The same was true of the soil containing the imbedded grandlure dispensers. A longer period of exposure could possibly show some contamination, and then the degradation compounds would volatilize.

In a subsequent preliminary study of the longevity of the oxidation products A, B, C, and D in soil, A and B (aldehyde and ester) completely volatilized after 24 h at 32°C. However, more than 95% of compounds C and D remained after 24 h.

CONCLUSIONS

The four-component grandlure system is a volatile complex that dissipates rapidly from soil and water at constant temperatures of 21 and 32°C. Thus, contamination of soil and water in the field by the grandlure in present formulations is highly unlikely. The components apparently can be washed from the bare filter dispensers but not from the cardboard physical barrier dispensers currently used in field experiments. Also, no evidence of the decomposition of alcohols I and II was detected and though the aldehydes undergo moderately fast oxidation in storage solutions, no products of decomposition were found in soil or water, probably because of their short residence in those media.

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LITERATURE CITED

Backstrom, H. L. J., *Z. Phys. Chem. Abt. B* **25**, 99 (1934).
Baeyer, A. V., Villiger, V., *Chem. Ber.* **33**, 1569 (1900).
Bull, D. L., Coppedge, J. R., Hardee, D. D., Rummel, D. R., McKibben, G. H., House, V. S., *Environ. Entomol.* **2**, 905 (1973a).
Bull, D. L., Coppedge, J. R., Ridgway, R. L., Hardee, D. D., Graves, T. M., *Environ. Entomol.* **2**, 829 (1973b).
Bull, D. L., Stokes, R. A., Hardee, D. D., Gueldner, R. C., *J. Agric. Food Chem.* **19**, 202 (1971).
Coppedge, J. R., Bull, D. L., House, V. S., Ridgway, R. L., Bottrell, D. G., Cowan, C. B., *Environ. Entomol.* **2**, 837 (1973).
Cross, W. H., Hardee, D. D., *USDA Coop. Econ. Insect Rep.* **18**, 430 (1968).
Cross, W. H., Mitchell, H. C., *J. Econ. Entomol.* **59**, 1503 (1966).
Hardee, D. D., Cross, W. H., Mitchell, E. B., *J. Econ. Entomol.* **62**, 165 (1969).
Hardee, D. D., McKibben, G. H., Gueldner, R. C., Mitchell, E. B., Tumlinson, J. H., Cross, W. H., *J. Econ. Entomol.* **65**, 97 (1972).
Keller, J. C., Mitchell, E. B., McKibben, G. H., Davich, T. B., *J. Econ. Entomol.* **57**, 609 (1964).
Leggett, J. E., Cross, W. H., *USDA Coop. Econ. Insect Rep.* **21**, 773 (1971).
McKibben, G. H., Hardee, D. D., Davich, T. B., Gueldner, R. C., Hedin, P. A., *J. Econ. Entomol.* **64**, 317 (1971).
Mitchell, E. B., Hardee, D. D., *J. Econ. Entomol.* **67**, 506 (1974).
Stahl, E., Ed., "Thin-Layer Chromatography", Academic Press, New York, N.Y., 1965.
Tumlinson, J. H., Gueldner, R. C., Hardee, D. D., Thompson, A. C., Hedin, P. A., Minyard, J. P., *Science* **166**, 1010 (1969).
Tumlinson, J. H., Gueldner, R. C., Hardee, D. D., Thompson, A. C., Hedin, P. A., Minyard, J. P., *J. Org. Chem.* **36**, 2616 (1971).

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