A Method for Trapping Disparlure from Air and Its Determination by Electron-Capture Gas Chromatography

Joseph H. Caro,* Barbara A. Bierl, Horatio P. Freeman, and Philip E. Sonnet[1]

An efficient and sensitive method has been developed for determination of airborne concentrations of disparlure (*cis*-7,8-epoxy-2-methyloctadecane), the gypsy moth [*Lymantria dispar* (L.)] sex attractant. Disparlure is collected by drawing a measured volume of air through a bed of type 4A molecular sieves. For quantitative analysis, the sieves are extracted with 1:1 hexane–ether, and the disparlure in the extract is derivatized with triphenylphosphine dibromide. After cleanup with activated Florisil, the brominated product is determined by electron-capture gas chromatography. The method yields recoveries of 80% or more over a wide range of concentrations. It has been successfully applied in the field to disparlure air concentrations as low as 0.2 ng/m^3.

Disparlure (*cis*-7,8-epoxy-2-methyloctadecane) was identified in 1970 as the chemical emitted by the female gypsy moth to attract the male for mating purposes (Bierl et al., 1970). A method for its synthesis was developed (Bierl et al., 1972), and the synthetic material was subsequently subjected to extensive field testing in experimental programs for monitoring of gypsy moth infestations or for reducing the population of the insect by air permeation or mass trapping. In the air permeation technique, an area of low-level infestation is aerially treated with the synthetic pheromone so that the pheromone emitted by the females is masked; the males have difficulty finding the scent of the females in an artificially created lure atmosphere and mating is thereby reduced. Effectiveness of this approach in field research conducted to date has only been evaluated by comparing extent of mating of tethered females in treated and untreated areas or by corresponding eggmass counts. Actual air concentrations of disparlure have not been measured, principally because a suitable analytical procedure is lacking. Such quantitative information is needed to optimize disparlure management in the field and to aid in the process of its registration for both air permeation and trapping applications.

Bierl and DeVilbiss (1975) reported a method for quantifying disparlure in air by trapping it in hexane, then determining the disparlure content of the hexane by flame ionization gas chromatography. The method was suitable for the intended purpose: laboratory measurement of emission rates from controlled-release formulations. However, it was not sufficiently sensitive for general use in field studies, because of both the limited air flow rates achievable with the liquid trap and the limited sensitivity of the flame ionization detector. Solid adsorbents, generally beds of porous polymers such as the Tenaxes or Chromosorbs, trap most pesticides efficiently while permitting relatively high flow rates through the adsorbent and are consequently now being widely used in air studies in lieu of liquid traps (Seiber and Woodrow, 1976). We report here an electron-capture gas chromatography method having high sensitivity and which we have already successfully used in field experiments (Caro et al., 1977). We used a bed of molecular sieves as the solid adsorbent in preference to a bed of porous polymer because of (1) lower cost of the material, (2) ease of desorption of disparlure from the sieves, and (3) availability of a pelletized form of the adsorbent that allowed high air flow rates while minimizing pressure drop through the system.

Agricultural Environmental Quality Institute, Beltsville Agricultural Research Center, U.S. Department of Agriculture, Beltsville, Maryland 20705.

[1]Present address: Honey Bee Pesticides/Diseases Laboratory, U.S. Department of Agriculture, Laramie, Wyo. 82071.

EXPERIMENTAL SECTION

Derivatization Reagent. The analytical method depends on the conversion of disparlure to a derivative giving a strong electron-capture response. The reagent triphenylphosphine dibromide was selected to effect the conversion because its reaction with 1,2-disubstituted epoxides had earlier been found to proceed virtually quantitatively to produce *vic*-dibromides (Sonnet and Oliver, 1976). It is readily prepared from triphenylphosphine and bromine (Wiley et al., 1964), and, in dichloromethane solvent, it reacts with disparlure to produce a mixture of the diastereomeric 7,8-dibromo-2-methyloctadecanes, which are analyzed together. Batches of 0.25 M triphenylphosphine dibromide solution were prepared by stirring 2.56 g of triphenylphosphine with 0.5 mL of bromine in 39 mL of dichloromethane in an ice bath. The reagent was stored in the dark and was freshly prepared each week because it slowly decomposed on standing.

Trapping System. In the field, disparlure was collected from the air by drawing a measured volume of air by vacuum pump through a 1.5-cm (about 8 g) bed of 14 to 30 mesh type 4A molecular sieves (Fisher M-516). The assembly is shown in Figure 1. In use, the tube is oriented horizontally and connected by Tygon tubing to the vacuum pump. Air flow rates ranged from 2 to 3 m^3/h, as measured by a rotameter connected momentarily at the inlet of the tube. We used duplicate sets of tubes at each sampling point to permit rapid exchange of the entire tube after each sampling period.

Analytical Procedure. The molecular sieves were transferred from the sampling tube into a 20-mL glass scintillation vial having a foil-lined cap and was hand shaken vigorously with 10.0 mL of 1:1 *n*-hexane–ethyl ether for 1 min. The mixture was allowed to stand at least 1 h, and a measured aliquot of the liquid (generally 5.5 to 6.5 mL) was removed with a pipet. The aliquot was then evaporated to 1 mL in a graduated 10-mL Kuderna-Danish receiver, using a micro Snyder column on a steam bath. After removal from the steam bath, a gentle stream of high-purity gas was used for final evaporation to 0.2 mL. Next, 0.5 mL of the 0.25 M triphenylphosphine dibromide reagent was added to the concentrated extract, and the mixture was placed in a water bath at 37 to 40 °C for 1 h. Excess reagent generally precipitated on addition because of its insolubility in hexane, but this did not

Table I. Recovery of Disparlure from Doped Air

Disparlure on watch glass source, ng	Approximate disparlure volatilized, ng	% disparlure recovered in replicate no.					Av recov, %
		1	2	3	4	5	
100	80	91.6	100.1	86.4	100.9	90.9	94.0
500	450	75.1	86.6	77.9			79.9
2500	2300	82.9	81.6	89.3			84.6

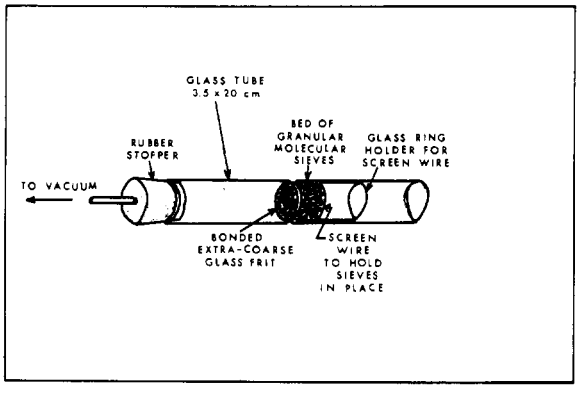

Figure 1. Field air sampler.

interfere with the completion of the reaction.

The reacted mixture was removed from the water bath, brought to 0.5 mL volume with dichloromethane, and allowed to stand 1 h longer at room temperature. It was then cleaned up by column chromatography: 10 g of Florisil PR activated at 130 °C was placed into a 22 mm × 330 mm chromatographic column and topped with a 1-cm layer of anhydrous Na_2SO_4. To minimize possible interferences, the column was washed with dichloromethane and hexane (30 mL of each) before the reaction mixture was added. Transfer of the latter to the column was completed with one or two 100-μL rinses of dichloromethane. The column was eluted with 50 mL of hexane and the eluate, containing the dibromo derivative of disparlure, was concentrated to proper volume for injection of an aliquot into the gas chromatograph.

Gas–Liquid Chromatography. Analyses were conducted on a Tracor 222 gas chromatograph fitted with a 180 cm × 2 mm i.d. glass column and a ^{63}Ni electron-capture detector. Column packing was 3% DC-200 silicone on 100–120 mesh Gas-Chrom Q. Oven temperature and carrier gas (95:5 argon–methane) flow rate were adjusted to give optimum resolution of the dibromo derivative peak (see discussion of Figure 3, below), and the electrometer attenuation was set so that 0.4 ng of the derivative gave 40 to 50% full-scale deflection.

Recovery Tests. A laboratory airflow assembly was designed to measure recoveries of disparlure from air with the described method (Figure 2). The air sampling tube used in the field experiments (Figure 1) was positioned vertically, an 8-g bed of type 4A molecular sieves was poured onto the glass frit, and a short length of glass sleeve slightly smaller in diameter than the tube was set onto the sieves. To provide a source of disparlure, a 2.5-cm watch glass, containing a surface film of disparlure deposited from a hexane solution of known concentration, was suspended vertically on the glass sleeve, as shown in Figure 2. The watch glass was held in place by opposing glass nipples fused onto the upper edge of the sleeve.

After this assembly was completed, a second air-sampling tube was placed above and abutting the first tube. A 10-g bed of molecular sieves was dropped into this tube to serve as a prescrubber for removal of contaminants from incoming laboratory air. The two tubes were sealed together with Teflon tape, and the bottom of the lower tube

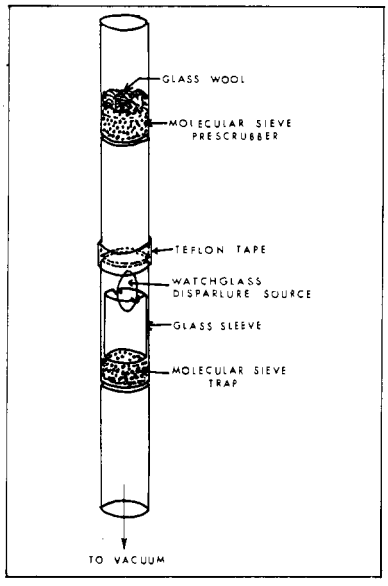

Figure 2. Assembly for laboratory recovery tests.

was connected to a source of vacuum.

In use, air was drawn through the assembly for a given period of time at a rate measured by momentarily attaching a rotameter to the upper tube. Disparlure volatilized from the watch glass as air passed over the surface and was trapped in the underlying bed of molecular sieves. After at least 3 m^3 of air had passed through the system, air flow was stopped, and the molecular sieves were removed for analysis. To strike a material balance, the watch glass, sleeve, and lower tube were rinsed well in 1:1 *n*-hexane–ethyl ether and the rinsings were combined and analyzed.

RESULTS AND DISCUSSION

When we used this method to determine disparlure concentrations in air samples, field air produced much cleaner chromatograms than did laboratory air, even when samples were taken under forest canopies. Laboratory air was so contaminated that disparlure recoveries could not be quantitated unless we inserted the prescrubber in the apparatus, as shown in Figure 2. Although field air was cleaner, chromatograms of samples taken before application of the chemical often exhibited a peak with a retention time very near that of the dibromo derivative. This peak was large enough to introduce a substantial error at low disparlure concentrations. However, the interfering peak could be separated from the derivative peak by adjustment of oven temperature and carrier gas flow rate in the gas chromatograph, as shown in Figure 3. The interference was completely masked under conditions of high temperature and low flow rate (Figure 3A), producing an erroneous single peak, but was resolved by lowering the temperature and simultaneously increasing the flow rate (Figure 3B). We used the latter set of conditions for routine analyses.

We conducted some 50 individual experiments in the laboratory to develop the details of the analytical method. Disparlure recoveries obtained with use of the resulting method in replicated laboratory tests over a wide range

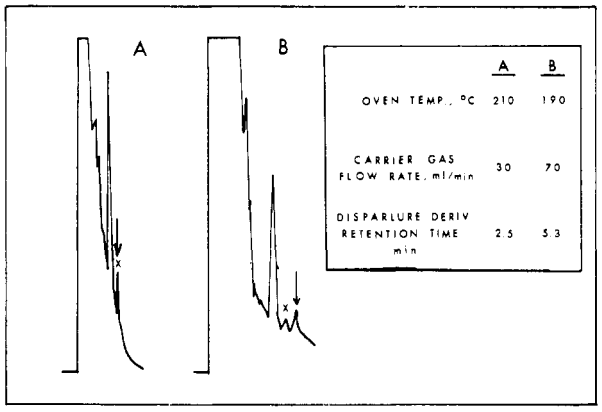

Figure 3. Separation of a background peak from the disparlure derivative peak in chromatograms of an air sample taken from a disparlure-treated Maryland field. The arrows indicate the derivative peak and the crosses indicate the background peak.

of concentrations in air are shown in Table I. Air flow through the system in individual tests ranged from 3.6 to 6.5 m^3, corresponding to disparlure concentrations in the fortified air ranging from 2.7 to 506 ng/m^3. Recoveries among replicates varied considerably, but part of the variability was undoubtedly caused by the experimental technique, which involved a material balance for the entire system. Results for field samples would therefore be expected to be less variable, with slightly higher recoveries than those for laboratory samples.

With both field air and prescrubbed laboratory air, as much as 30 m^3 of air were drawn through the system without appreciable effect on the cleanliness of the chromatograms or on disparlure recovery. This shows not only that the molecular sieves strongly sorb the chemical, but also that field air does not contain components that in themselves, or possibly in reaction with triphenylphosphine dibromide, are sensitive to electron-capture detection. In one set of analyses of forest air that contained very low levels of disparlure, we were able to quantitate only 6 ng of the chemical in 30 m^3 of air, so that the limit of quantitation of the method is about 0.2 ng of disparlure/m^3 of air.

LITERATURE CITED

Bierl, B. A., Beroza, M., Collier, C. W., *Science* **170**, 87 (1970).
Bierl, B. A., Beroza, M., Collier, C. W., *J. Econ. Entomol.* **65**, 659 (1972).
Bierl, B. A., DeVilbiss, D., Proceedings of the International Controlled Release Pesticide Symposium, Dayton, Ohio, 1975 p 230.
Caro, J. H., Bierl, B. A., Freeman, H. P., Glotfelty, D. E., Turner, B. C., *Environ. Entomol.* **6**, 877 (1977).
Seiber, J. N., Woodrow, J. E., Proceedings of the International Conference on Environmental Sensing and Assessment, Institute of Electrical and Electronics Engineers, New York, 7–2, 1 (1976).
Sonnet, P. E., Oliver, J. E., *J. Org. Chem.* **41**, 3279 (1976).
Wiley, G. A., Hershkowitz, R. L., Rein, B. M., Chung, B. C., *J. Am. Chem. Soc.* **86**, 964 (1964).

Received for review August 1, 1977. Accepted November 21, 1977. Presented at the 11th Middle Atlantic Regional Meeting, American Chemical Society, Newark, Delaware, April 20, 1977. Reference to a company or product does not imply approval or recommendation of the product by the U.S. Department of Agriculture to the exclusion of others that may be suitable.

Determination of Antibiotic Residues of Plauracin in Swine Tissues

Hassan G. Fouda* and Martin J. Lynch

A bioautographic method is described for the quantitative determination of the antibiotic complex plauracin at levels down to 20 ppb in edible tissues of swine. The method involves extraction, partial purification, concentration, and application to silica gel plates followed by incubation with *Sarcina lutea* seeded agar. Measuring areas of inhibition zones by the method of Xerox copying provided a basis for quantitation. A group of 16 pigs were maintained on plauracin in feed at 110 ppm for 29 days. Following withdrawal of the medication, groups of three pigs were slaughtered at timed intervals, and edible tissues of each were analyzed for plauracin residues by the method described. With no withdrawal of the antibiotic, fat contained 25 ppb plauracin, and was the only tissue showing measurable (>20 ppb) activity. One day after withdrawal of the medication, measurable residues were not present in fat or any of the major edible tissues.

Plauracin is a novel antibiotic complex produced by a new strain of *Actinoplanes* (*Amorphosphorangium*) *auranticolor* (Celmer et al., 1975). It belongs to the same family as PA-114, virginiamycin, mikamycin, and others. Each of these is a combination of several macrocyclic lactone and depsipeptide components. Separately, each component exhibits a bacteriostatic effect, but in combination they are synergistic and bactericidal. Preliminary indications are that plauracin possesses good growth promoting and antidysentery activities combined with a low toxicity profile. It is presently under evaluation as a feed additive for growth promotion of swine and for the prophylaxis and treatment of swine dysentery.

A tissue residue method for the analysis of the antibiotic virginiamycin in swine tissue has previously been described (DiCuollo et al., 1973). In our hand, efforts to extend the limit of detection of this method to levels below 0.1 ppm were not successful. We report here the development of a sensitive microbiological assay for plauracin antibiotic residues in swine tissues. Application of the method to

Central Research, Pfizer Inc., Groton, Connecticut 06340.

0021-8561/78/1426-0463$01.00/0