INSECT SEX ATTRACTANTS

IV. THE DETERMINATION OF GYPLURE IN ITS MIXTURES BY ADSORPTION AND GAS CHROMATOGRAPHY*

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INTRODUCTION

Following the reported synthesis of gyplure [(+)-12-acetoxy-*cis*-9-octadecen-1-ol][2,3], a sex attractant for the male gypsy moth (*Porthetria dispar* (L.)), many samples of this material prepared commercially and in our laboratories were bioassayed under laboratory and field conditions. The results were inconsistent. Some gyplure samples, particularly those prepared commercially, demonstrated only weak attraction and others were completely devoid of activity. Previous findings[4] showed that gyplure could be rendered unattractive by admixture with at least 20% of its crude *trans* isomer; thus, it appeared likely that loss of activity was probably due to the presence of unattractive contaminants formed by failure to rigidly control production conditions. Before we could proceed with confidence, we needed a satisfactory method to determine the gyplure content of such mixtures and, if possible, to identify the major contaminants. These needs were met by the simple adsorption and gas-chromatographic methods described in this report.

MATERIALS**

Solvents

The isooctane and ethyl acetate used were obtained by distilling the technical-grade solvents.

Adsorbents and packings

Merck reagent-grade silicic acid was used for the adsorption chromatography. Commercial SE-30, Carbowax 20M, and Chromosorb W (acid-washed, 60–80 mesh) were used for gas chromatography.

Gyplure

Gyplure for this study was prepared in our laboratories according to the procedure previously reported,[3] except that the ricinoleyl alcohol (b.p. 145–8°/0.15 mm, n_D^{25} 1.4711) used as starting material was obtained by careful fractional distillation of a

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** The mention of trade names or products does not constitute endorsement by the Department of Agriculture over those not named.

commercial product (Archer-Daniels-Midland Adol 40) by use of a spinning-band column. Commercial batches of gyplure had been prepared by acetylating distilled Adol 40, selectively saponifying the undistilled diacetate, distilling the final product and decolorizing it by filtration through charcoal.

Standard reference substances

Ricinoleyl alcohol was obtained as described above. Oleyl and stearyl alcohols were commercial materials obtained from Archer-Daniels-Midland Co. and Eastman Kodak Co. (White Label, No. 4053, m.p. 56.5–8.0°), respectively. Oleyl acetate was prepared by acetylation of the commercial alcohol with acetyl chloride in dry benzene, and was not distilled. Pure 1,12-diacetoxy-*cis*-9-octadecene (b.p. 167–8°/0.1 mm, n_D^{26} 1.4515) was prepared in 90% yield.[3]

Gas-chromatographically pure gyplure was prepared by selectively saponifying the diacetate[3] and chromatographing the distilled product (b.p. 163–4°/0.1 mm, n_D^{26} 1.4595; 89% yield) on a column of silicic acid (see below); elution with 10% ethyl acetate in isooctane following elution with 5% ethyl acetate in isooctane gave a 76% yield of the pure product.

Analysis. Calculated for $C_{20}H_{38}O_3$: C, 73.56; H, 11.74. Found: C, 74.57; H, 11.97.

Gas-chromatographically pure 1-acetoxy-*cis*-9-octadecen-12-ol was prepared by the following procedure. A solution containing 10 g ricinoleyl alcohol (b.p. 145–8°/0.15 mm), 2 g glacial acetic acid, 0.3 ml sulfuric acid, and 100 ml dry benzene was refluxed for 2 h with a trap to continuously distill the water as it was formed. Ether was added to the cooled solution and the separated organic layer was washed successively with 5% hydrochloric acid, 5% potassium hydroxide, and water, then dried over sodium sulfate. Removal of the solvent left 10 g yellow oil, 1.132 g of which was chromatographed on a column of 80 g silicic acid. The column was eluted with 400 ml of isooctane containing 2% ethyl acetate and then with 400 ml of isooctane containing 5% ethyl acetate; removal of solvent from the latter eluate gave 0.661 g of the desired product (n_D^{25} 1.4607).

Analysis. Calculated for $C_{20}H_{38}O_3$: C, 73.56; H, 11.74. Found: C, 72.94; H, 11.23.

METHODS

Column chromatography

The column used for isolating gyplure from its mixtures was prepared by the following procedure. A slurry of 80 g silicic acid was poured into a glass tube (38 × 2.5 cm) fitted with a stopcock and glass wool plug at the lower end and a 24/40 female joint at the top. Air pressure of 3 p.s.i. was applied to the column to pack the adsorbent, which was then prewashed successively with 150 ml of 70% ethyl acetate in isooctane and 200 ml of isooctane.

Commercial gyplure (1 g) dissolved in a few milliliters of isooctane was placed on the column and washed in with a few portions of isooctane. The column was eluted successively with 400 ml of isooctane (fraction 1), 400 ml of 5% ethyl acetate in isooctane (fraction 2), 400 ml of 10% ethyl acetate in isooctane (fraction 3), and 400 ml of 25% ethyl acetate in isooctane (fraction 4). Eluates were collected in 100-ml portions. Table I gives the percentage content of each fraction found in four replicates of the same commercial sample; the average contents of these fractions were 8.6, 34.3, 30.4, and 21.2%, respectively.

TABLE I

PERCENTAGE OF FRACTIONS 1–4 OBTAINED BY COLUMN CHROMATOGRAPHY OF COMMERCIAL GYPLURE

Sample No.	*Fraction 1*	*Fraction 2*	*Fraction 3*	*Fraction 4*
1	9.1	32.8	31.2	20.4
2	6.8	31.5	32.7	22.0
3	8.4	33.5	29.8	21.7
4	10.0	39.5	28.0	20.5
		Average		
	8.6	34.3	30.4	21.2

Fraction 1 (8.6% of the original commercial sample) was shown, by gas chromatography described below, to contain oleyl acetate (peak 3) and two unidentified minor components (peaks 4 and 7), and was not investigated further.

Fraction 2 (34.3%) was shown by gas chromatography to contain oleyl alcohol (peak 1), stearyl alcohol (peak 2), 1-acetoxy-*cis*-9-octadecen-12-ol (peak 9), 1,12-diacetoxy-*cis*-9-octadecene (peak 10), and an unidentified component (peak 5). Fraction 2 dissolved in a few milliliters of 1% ethyl acetate in isooctane was placed on an 80-g silicic acid column (prepared and prewashed as above) and washed in with a few portions of the same solvent. The column was then eluted successively with 400 ml of this solvent (fraction 2A), 400 ml of 2% ethyl acetate in isooctane (fraction 2B, collected in sixteen 25-ml portions), 400 ml of 3% ethyl acetate in isooctane (fraction 2C), and 400 ml of 5% ethyl acetate in isooctane (fraction 2D, collected in 25-ml portions). Fraction 2A, comprising 8% of fraction 2, contained 21% oleyl acetate (peak 3) and 60% unidentified material (peak 7). Fraction 2B, comprising 56% of fraction 2, contained 16% oleyl alcohol (peak 1), 5% stearyl alcohol (peak 2), 10% unidentified material (peak 5), and 66% 1,12-diacetoxy-*cis*-9-octadecene (peak 10); its elution pattern is shown in Fig. 1. The eighth 25-ml portion of fraction 2B, after removal of solvent at 20 mm pressure, consisted of 95% pure 1,12-diacetoxy-*cis*-9-octadecene (peak 10).

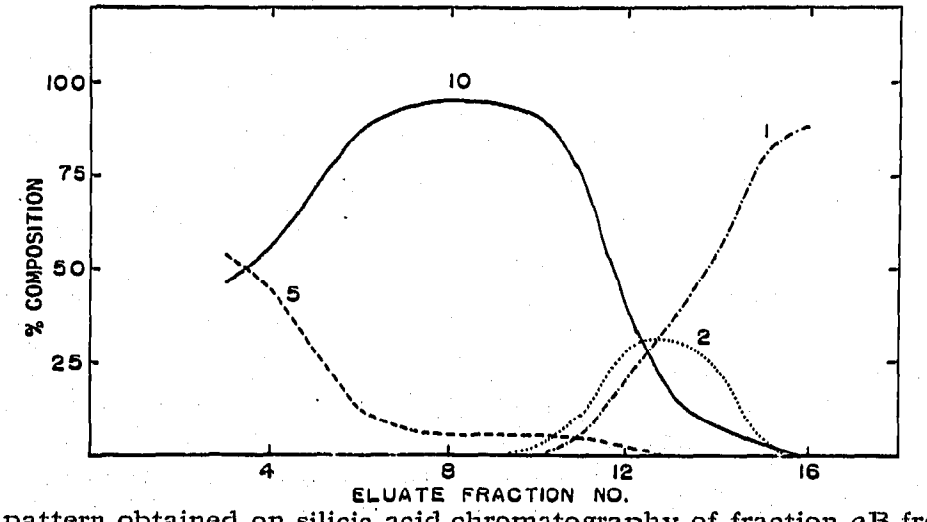

Fig. 1. Elution pattern obtained on silicic acid chromatography of fraction 2B from commercial gyplure. Numbers correspond with the peak numbers given for the compounds in Table II.

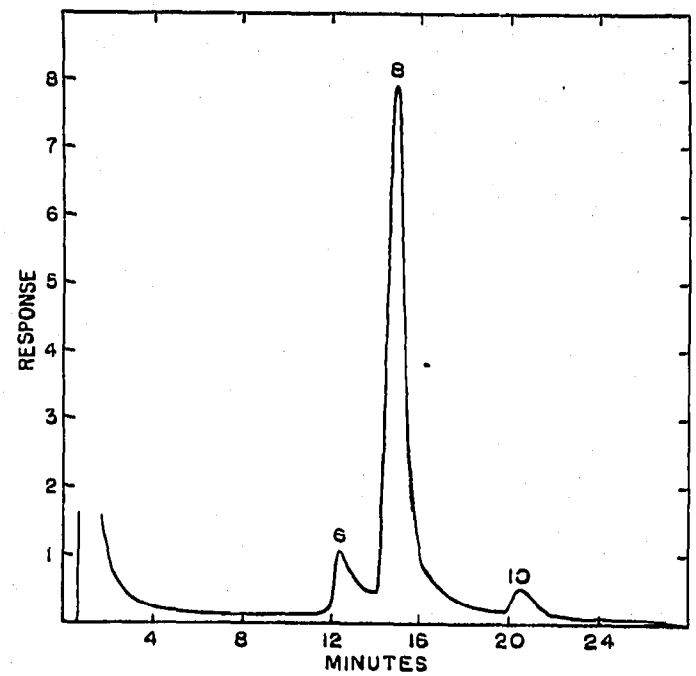

Fig. 2. Gas chromatogram of laboratory-synthesized gyplure. Sample size, 1 μl of a 3% solution in acetone; temperature, 200°; nitrogen flow rate, 22 ml/min; stationary phase, SE-30 on a support of acid-washed Chromosorb W (60–80 mesh), 5% by weight; stainless steel column 8ft. by 1/8 in. O.D. Peak numbers correspond with those given for the compounds in Table II.

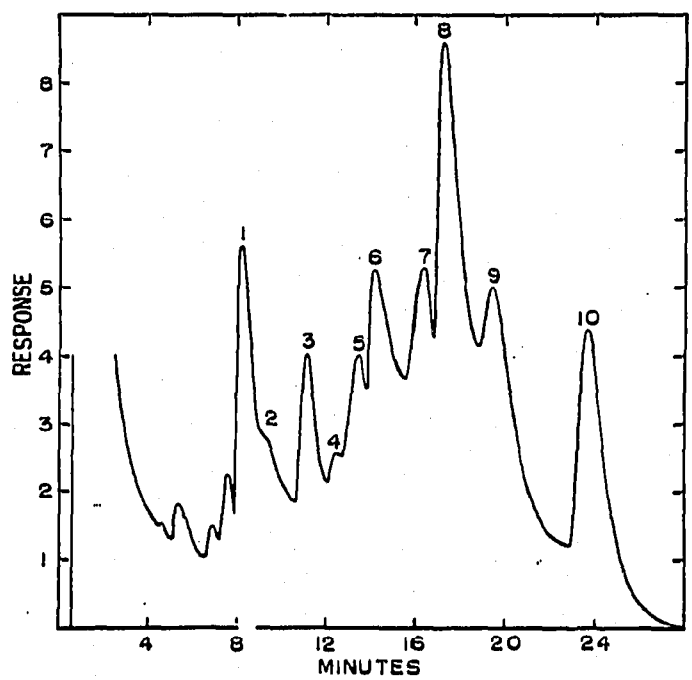

Fig. 3. Gas chromatogram of commercial gyplure. Conditions as given for Fig. 2, except that the nitrogen flow rate was 20 ml/min. Peak numbers correspond with those given for the compounds in Table II.

Analysis. Calculated for $C_{22}H_{40}O_4$: C, 71.69; H, 10.94. Found: C, 71.43; H, 10.71.

Fraction 2C, comprising 19% of fraction 2, contained 84% oleyl alcohol (peak 1). Fraction 2D, comprising 17% of fraction 2, consisted of pure 1-acetoxy-*cis*-9-octadecen-12-ol (peak 9) after removal of solvent.

Analysis. Calculated for $C_{20}H_{38}O_3$: C, 73.56; H, 11.74. Found: C, 74.13; H, 12.12.

Fraction 3 (30.4%) consisted of pure 12-acetoxy-*cis*-9-octadecen-1-ol (gyplure) (peak 8 by gas chromatography).

Analysis. Calculated for $C_{20}H_{38}O_3$: C, 73.56; H, 11.74. Found: C, 73.85; H, 11.90.

Fraction 4 (21.2%) consisted of pure ricinoleyl alcohol (peak 6 by gas chromatography).

Analysis. Calculated for $C_{18}H_{36}O_2$: C, 75.99; H, 12.76. Found: C, 75.72; H, 12.30.

Gas chromatography

All chromatograms were obtained with an F & M Model 1609 gas chromatograph equipped with a flame ionization detector, with nitrogen as the carrier gas. Columns used were 8-ft., 1/8-in. diameter stainless steel packed with 5% SE-30 on 60–80 mesh, acid-washed Chromosorb W, and 10-ft., 1/4-in. diameter aluminum packed with 5% Carbowax 20M on 60–80 mesh, acid-washed Chromosorb W.

Gyplure samples prepared in these laboratories by the procedure described above contained predominantly gyplure (peak 8) with small amounts of ricinoleyl alcohol (peak 6) and 1,12-diacetoxy-*cis*-9-octadecene (peak 10), as shown in Fig. 2. In contrast with this, commercial samples tested were found to contain varying amounts of the 10 substances obtained by column chromatography, as shown in Fig. 3. Retention times of these substances with their peak numbers and their relative retention ratios based on gyplure for both type columns are given in Table II.

TABLE II

RETENTION TIMES AND AVERAGE RELATIVE RETENTION RATIOS (GYPLURE = 1.00) OF SUBSTANCES OBTAINED BY GAS CHROMATOGRAPHY OF COMMERCIAL GYPLURE

Peak No.	*Compound*	*Retention times (min) on*		*Average relative retention ratios on*	
		SE-30	*Carbowax 20M*	*SE-30*	*Carbowax 20M*
1	Oleyl alcohol	7.4	10.7	0.49	0.31
2	Stearyl alcohol	7.9	9.8	0.54	0.28
3	Oleyl acetate	9.8	9.8	0.66	0.28
4	Unidentified	10.5	14.4	0.73	0.41
5	Unidentified	11.8	21.8	0.80	0.63
6	Ricinoleyl alcohol	12.3	41.4	0.83	1.19
7	Unidentified	14.0	18.0	0.96	0.52
8	12-Acetoxy-*cis*-9-octadecen-1-ol	14.7	34.8	1.00	1.00
9	1-Acetoxy-*cis*-9-octadecen-12-ol	16.7	36.0	1.13	1.03
10	1,12-Diacetoxy-*cis*-9-octadecene	20.2	30.0	1.37	0.86

Conditions: For SE-30, columns were 8 ft. stainless steel, 1/8 in. O.D., 5% on acid-washed Chromosorb W (60–80 mesh), N_2 flow rate 22 ml/min., temperature 200°. For Carbowax 20M, columns were 10 ft. stainless steel, 1/4 in. O.D., 5% on acid-washed Chromosorb W (60–80 mesh), N_2 flow rate 27 ml/min., temperature 210°.

DISCUSSION

The chromatographic methods described permit the ready determination of actual gyplure content in laboratory and commercial pilot-plant samples of gyplure, as well as the separation of the pure *cis* isomer from its contaminants. Samples prepared in the laboratory showed an approximate content of 80% or more *cis*-gyplure, contaminated with unchanged ricinoleyl alcohol and its diacetate. On the other hand, commercial samples contained approximately 30–35% *cis*-gyplure, the contaminants being stearyl, oleyl, and ricinoleyl alcohols, oleyl acetate, ricinoleyl alcohol diester, 1-acetoxy-*cis*-9-octadecen-12-ol, and three unidentified substances. It is apparent that, in order to obtain a satisfactory grade of gyplure, it is necessary to usé a pure grade of ricinoleyl alcohol and to distill the product obtained at each stage.

Although satisfactory resolution of the samples by gas chromatography is obtained with both types of column packing, SE-30 permits much shorter retention times than Carbowax 20M.

SUMMARY

Adsorption and gas-chromatographic methods are described for determining the content of *cis*-gyplure in samples of gyplure prepared in the laboratory and the pilot plant, as well as for separating the *cis* isomer from its contaminants.

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