Insect Sex Attractants: XI. Analysis of *Cis* and *Trans* Isomers of Fatty Alcohol Acetates by Gas Chromatography

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Abstract

A gas chromatographic procedure was devised for the analysis of geometric isomers of dodecenyl acetates, tridecadienyl acetates, tetradecenyl acetates and hexadecenyl acetates. Use of diethylene glycol succinate in a 300 ft capillary column permitted the separation of nine pairs of *cis-trans* isomers and four geometric isomers of 5,9-tridecadienyl acetate. Data are presented on the isomeric composition of alkenyl acetates prepared by three different stereospecific reactions and by elaidinization.

Introduction

During the past several years, many acetates of unsaturated fatty alcohols have been prepared in our laboratory for evaluation as insect sex attractants (1,2). Among these are the sex attractant for the cabbage looper, *Trichoplusia ni* (Hübner) (3); the pheromone for the fall armyworm, *Spodoptera frugiperda* (J. E. Smith) (4); *cis*-7-hexadecen-1-ol acetate (5), the attractant of the pink bollworm, *Pectinophora gossypiella* (Saunders); and numerous isomers and homologs. Since attractancy of insect attractants is dependent upon structure (6), it was essential that these candidate attractants be of known isomeric composition and purity.

In the synthesis of these esters, the *cis* double bond was introduced by hydrogenation of a triple bond by using a catalyst of poisoned Pd-$CaCO_3$, a reduction reported to yield the *cis* configuration almost exclusively (7). The *trans* double bond was generated by the following three reactions: reduction of a triple bond by metallic sodium in liquid ammonia, reported to yield only *trans* double bonds (8,9); ring scission of 2-alkyl-3-chlorotetrahydropyrans, reported to yield only *trans*-4-alken-1-ols (10); and elaidinization with nitrous acid of a *cis* double bond, reported to produce 75–80% *trans* double bond (11).

In our search for a good analytical method, we looked at known procedures for analyzing mixtures of geometric isomers. Infrared analysis for *trans* content is a standard method (12) routinely used by many laboratories. However, the unavailability of correct standards and the inability to detect isomeric impurity in a *cis* isomer precluded the use of the method. Thin-layer chromatography using an Eastman chromatogram pretreated with silver nitrate has been employed to separate methyl oleate from methyl elaidate but the inability to detect isomeric impurity precluded the use of this method also. Since none of these methods were convenient or accurate enough for our purpose, we decided to investigate the possibilities of gas liquid chromatography (GLC).

A preliminary test was made with the *cis* and *trans* isomers of 7-dodecen-1-ol acetate by using a 5 ft × 1/8 in. column packed with 20% diethylene glycol succinate (DEGS) on 60–80 mesh Chromosorb W. Although the separation achieved was not satisfactory, it was sufficiently promising to warrant further study of a GLC method.

After the introduction of capillary columns in 1958, many workers reported the use of these columns in high resolution gas chromatography to distinguish

TABLE I
Analysis of C-12 and C-13 Acetates by GLC at a Column Temperature of 160 C

Acetate	Retention time, min	Δ Rt	% Impurity	Double bond formation, Ref.
trans-5-Dodecen-1-ol	27.24		No trace	8,9
		0.44		
cis-5-Dodecen-1-ol	27.68		4.4 t	7
trans-6-Dodecen-1-ol	27.12		16.5 c	11
		0.56		
cis-6-Dodecen-1-ol	27.68		1.8 t	7
trans-7-Dodecen-1-ol	27.52		1.3 c	10
		0.80		
cis-7-Dodecen-1-ol	28.32		9.5 t	7
trans-5,*trans*-9-Tridecadien-1-ol	37.60		No trace	8,9
		1.28		
cis-5,*trans*-9-Tridecadien-1-ol	38.88		2.52 < 3.2 c,c 2.7 t,t	7,8,9
trans-5-,*cis*-9-Tridecadien-1-ol	40.12		1.96 < 1.3 c,c 4.5 t,t	7,8,9
		0.72		
cis-5-,*cis*-9-Tridecadien-1-ol	40.84		< 7.3 t,c 6.1 c,t	7

TABLE II
Analysis of C-14 and C-16 Acetates by GLC at a Column Temperature of 185 C

Acetate	Retention time, min	Δ Rt	% Impurity	Double bond formation, Ref.
trans-5-Tetradecen-1-ol	22.36		.....	11
		0.16		
cis-5-Tetradecen-1-ol	22.52		.....	7
trans-7-Tetradecen-1-ol	22.28		21 c	11
		0.44		
cis-7-Tetradecen-1-ol	22.72		7.2 t	7
trans-9-Tetradecen-1-ol	22.36		17 c	11
		0.52		
cis-9-Tetradecen-1-ol	22.88		9.3 t	7
trans-5-Hexadecen-1-ol	29.44		.....	11
		0.24		
cis-5-Hexadecen-1-ol	29.68		.....	7
trans-6-Hexadecen-1-ol	29.44		.....	11
		0.24		
cis-6-Hexadecen-1-ol	29.68		.....	7
trans-7-Hexadecen-1-ol	29.52		.....	11
		0.48		
cis-7-Hexadecen-1-ol	30.00			7

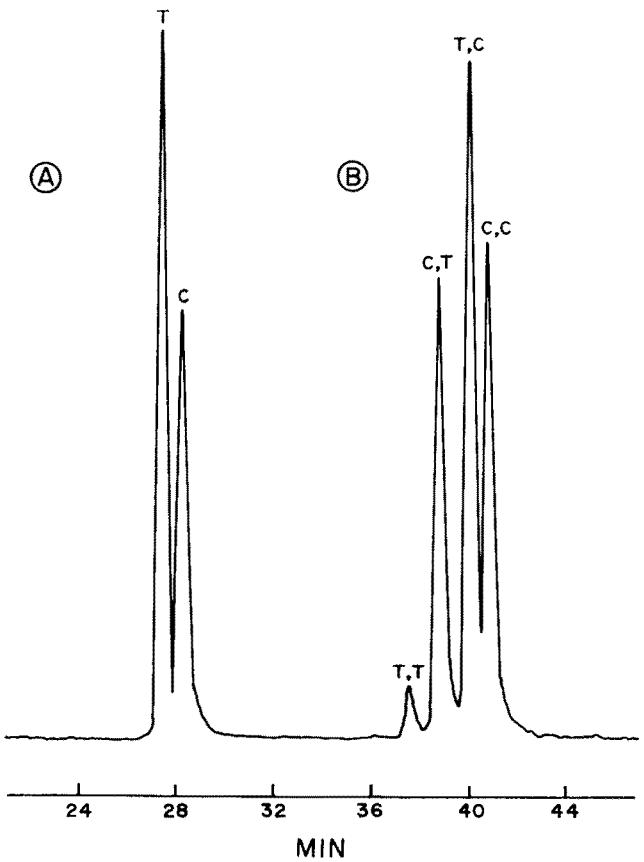

FIG. 1. Separation of the geometric isomers of (A) 7-dodecen-1-ol acetate and (B) 5,9-tridecadien-1-ol acetate on 300 ft of capillary column coated with DEGS polyester, at 160 C.

between *cis* and *trans* double bonds. Apiezon, polyester, or nitrile silicone liquid phases were employed by Litchfield (13,14), Kaufman (15), and Lipsky (16,17) to make these separations. We find a capillary column provides satisfactory separations of our *cis* and *trans* isomers while the packed column did not.

In this paper, we discuss the isomeric analyses of the four geometric isomers of the 5,9-tridecadien-1-ol acetate and the acetates of *cis-* and *trans-* 5-, 6- and 7-dodecen-1-ol; 5-, 7- and 9-tetradecen-1-ol; and 5-, 6- and 7-hexadecen-1-ol. The relative geometric purity of synthetic isomers prepared by four different methods is also compared. However, the separation of positional isomers is outside the scope of this paper and will not be covered.

Experimental Procedures

Materials

The preparation of acetates of unsaturated fatty alcohol isomers used in this study has been previously described (1–5).

Gas Chromatography. An Aerograph Gas Chromatograph Model 204-1B equipped with a capillary splitter, needle valves, make-up gas adapter and a flame ionization detector was used for all analyses. A 300 ft (91.5 m) stainless steel capillary column with an internal diameter of 0.01 in. (0.245 mm) was coated and conditioned as described by Litchfield (14). The coating was effected by passing 10 ml of a 10% solution of DEGS in chloroform through the column under 15 to 20 lb nitrogen/in^2.

Temperatures employed were: injection port 225 C, detector oven 225 C, column 160 C for C-12 and C-13

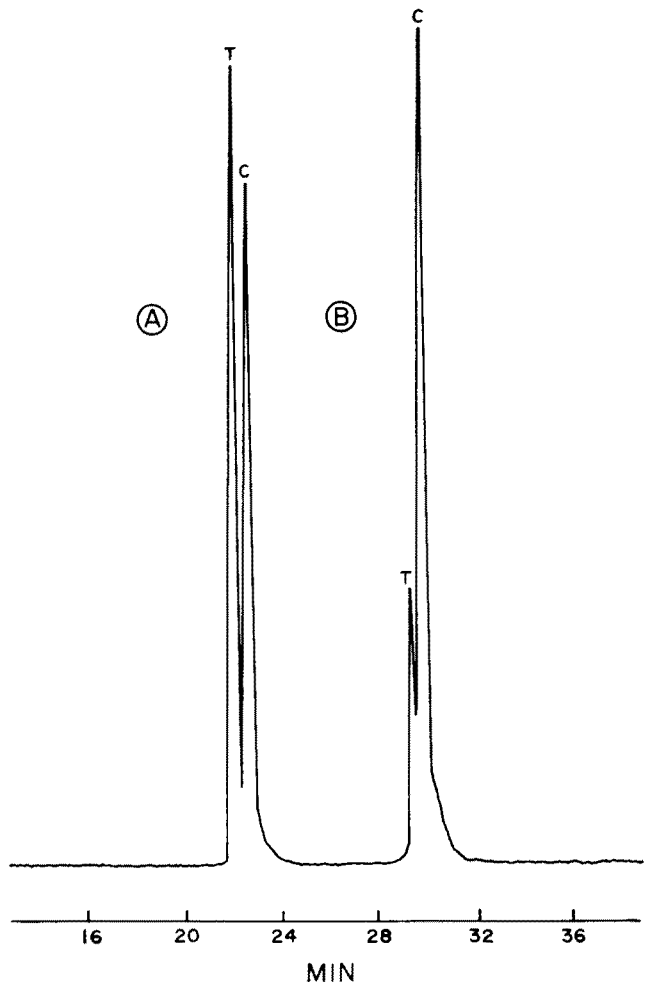

FIG. 2. Separation of the geometric isomers of (A) 9-tetradecen-1-ol acetate and (B) 7-hexadecen-1-ol acetate on 300 ft of capillary column coated with DEGS polyester, at 185 C.

acetates and 185 C for C-14 and C-16 acetates. Helium flow through the column was 0.8 ml/min for both column temperatures. At a column temperature of 160 C and a capillary split ratio of 89:1, 14.5 ml H_2/min and 26.3 ml He/min make-up gas passed directly through the detector. At a column temperature of 185 C and a capillary split ratio of 91:1, 15 ml H_2/min and 28.2 ml He/min make-up gas passed directly through the detector. Samples were dissolved in an equal volume of acetone and 0.1 μliter of this solution was injected by using a 1.0 μliter/syringe. With the split ratio used, approximately 0.001 μliter of solution, roughly 0.5 μg of isomer, was placed on the column; this amount was found to give maximum resolution of *cis* and *trans* isomers.

The retention times were measured from the point of injection. The percentage impurity of the isomers was determined by computation of the areas under the GLC curves by the triangulation method.

Results and Discussion

It has been reported that the separation of geometric isomers on a DEGS capillary column appears to be only a matter of efficiency rather than any olefin π complex interaction (14). In each instance the *trans* isomers of the monounsaturated esters eluted before the *cis* isomers (see Tables I and II and Fig. 1 and 2). This order of elution parallels that reported

on a nitrile silicone capillary column (13), which possibly produces a π complex interaction with the double bond.

The difference in retention time of the *cis* and *trans* monounsaturated isomers was greater with shorter alcohol chains and with larger distances between the double bond and acetate moiety. These retention time differences are evident in Tables I and II. In the 5,9-diunsaturated C-13 acetates, the double bond further from the acetate has a greater influence on elution time than the double bond closer to the acetate. This is shown in Table I by the elution of *cis*-5, *trans*-9-tridecadien-1-ol acetate before *trans*-5,*cis*-9-tridecadien-1-ol acetate. A change in the configuration at the 9 position caused a much greater difference in retention times (2.52 and 1.96 min) than a change in the 5 position (1.28 and 0.72 min) when the other bond configuration remained constant.

The *cis-trans* C-12 to C-14 alkenol acetates were well resolved by the capillary column. However, the isomeric hexadecenyl acetates were not separated well enough to permit an estimate of isomeric impurity by the triangulation method.

The purity of monounsaturated isomers varied according to the method by which the double bond was introduced. The purest isomer was *trans*-5-dodecen-1-ol acetate whose double bond was generated by sodium-liquid ammonia reduction (8,9) of a triple bond; no perceptible amount of *cis* impurity was found in this acetate. *Trans*-7-dodecen-1-ol acetate containing a double bond resulting from ring scission of 2-butyl-3-chlorotetrahydropyran (10) possessed 1.3% *cis* impurity. *Cis* isomers, containing double bonds produced by reduction of a triple bond over poisoned Pd-$CaCO_3$ (7), possessed from 1.8–9.5% *trans* impurity. The least pure isomers were those containing *trans* double bonds generated by elaidinization (11) with nitrous acid; these isomers contained from 16.5–21% *cis* impurity.

Of the four geometric isomers of 5,9-tridecadien-1-ol acetate (1), the *trans,trans* isomer was the purest. Double bonds of this isomer resulted from sodium-liquid ammonia reduction. The least pure isomer was the *cis,cis* which had 13.4% impurity consisting of *trans,cis* and *cis,trans* isomers, but no perceptible amount of *trans,trans* isomer was present. The *cis, trans* and *trans,cis* isomers were of equal purity, containing 5.9% and 5.8% impurity respectively, consisting of *cis,cis* and *trans,trans* isomers. No perceptible amount of *trans,cis* impurity appeared in the *cis,trans* isomer nor did any *cis,trans* impurity appear in the *trans,cis* isomer.

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