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GAS CHROMATOGRAPHY AND THE STRUCTURAL ELUCIDATION OF LEPIDOPTERAN PHEROMONES*

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SUMMARY

Retention data are presented for standards which can be used in the structural elucidation of lepidopteran sex attractants. The use of gas chromatography in the analysis of the geometrical isomers of Δ^{11}-tetradecenal, the sex pheromones of the spruce budworm, *Choristoneura fumiferana*, is discussed.

INTRODUCTION

The majority of lepidopteran sex pheromones which have been isolated and fully characterized are either monounsaturated alcohols or their acetates, containing twelve, fourteen or sixteen carbon atoms in the chain[1]. Such compounds have been shown to function as sex attractants of species belonging to several families, including Pyralidae, Tortricidae, Olethreutidae, and Noctuidae. Diversity of pheromone structure is a factor in maintaining species integrity. The variation in pheromone structure may be realized through differences in functional group, positional isomerism, geometrical isomerism, and by the formation of "blends" of two or more compounds. The blends may consist of two compounds differing in function group, as in *Argyrotaenia citrana*[2], or of two compounds which are positional isomers, as in *Adoxophyes orana*[3,4]; or, as exemplified by the pheromone of *Choristoneura fumiferana*, the blend is composed of geometrical isomers[5]. A fourth possible variation is utilized by *Heliothis virescens*, the blend being composed of two aldehydes which differ in chain length and double bond position[6]. The proposed use of sex pheromones in insect control has contributed to much interest in this area of insect chemistry. This paper presents retention data for standards which may be used in the structural elucidation of lepidopteran sex attractants, and discusses the use of gas chromatography (GC) in the analysis of the geometrical isomers of Δ^{11}-tetradecenal, the sex pheromone of the spruce budworm, *C. fumiferana*.

* Contribution No. 303.

TABLE I

RETENTION DATA FOR VARIOUS STANDARD C_{12}, C_{14} AND C_{16} ALCOHOLS USED IN LEPIDOPTERAN PHEROMONE STUDIES

t_r = Retention time; r_{ol} = retention relative to tetradecan-1-ol; r_{ac} = retention relative to tetradecan-1-ol acetate.

No.	*Compound*	*DEGS*			*OV-25*			*OV-1*		
		t_r (sec)	r_{ol}	r_{ac}	t_r (sec)	r_{ol}	r_{ac}	t_r (sec)	r_{ol}	r_{ac}
1	Dodecan-1-ol	218	0.50	0.64	143	0.34	0.19	177	0.34	0.18
2	Tetradecan-1-ol	436	1.00	1.28	415	1.00	0.54	515	1.00	0.52
3	Hexadecan-1-ol	863	1.98	2.54	1130	2.72	1.48	930	1.81	0.94
4	(*Z*)-4-Dodecen-1-ol	271	0.62	0.80	151	0.36	0.20	155	0.30	0.16
5	(*E*)-4-Dodecen-1-ol	271	0.62	0.80	148	0.36	0.19	153	0.30	0.15
6	(*E*)-5-Dodecen-1-ol	273	0.63	0.80	155	0.37	0.20	158	0.31	0.16
7	(*Z*)-6-Dodecen-1-ol	288	0.66	0.85	155	0.37	0.20	155	0.30	0.16
8	(*Z*)-7-Dodecen-1-ol	290	0.67	0.85	163	0.39	0.21	162	0.31	0.16
9	(*E*)-7-Dodecen-1-ol	275	0.63	0.81	173	0.42	0.23	165	0.32	0.17
10	(*Z*)-8-Dodecen-1-ol	285	0.65	0.84	163	0.39	0.21	156	0.30	0.16
11	(*Z*)-9-Dodecen-1-ol	308	0.71	0.91	172	0.41	0.22	164	0.32	0.17
12	(*Z*)-7-Tetradecen-1-ol	544	1.25	1.60	423	1.02	0.55	477	0.93	0.48
13	(*Z*)-9-Tetradecen-1-ol	571	1.31	1.68	579	1.40	0.76	475	0.92	0.48
14	(*E*)-9-Tetradecen-1-ol	522	1.20	1.53	430	1.04	0.56	522	1.01	0.53
15	(*Z*)-10-Tetradecen-1-ol	579	1.33	1.70	575	1.39	0.75	489	0.95	0.49
16	(*Z*)-11-Tetradecen-1-ol	597	1.37	1.75	472	1.14	0.62	534	1.04	0.54
17	(*E*)-11-Tetradecen-1-ol	548	1.26	1.61	463	1.11	0.60	519	1.01	0.52
18	(*Z,E*)-9,12-Tetradecadien-1-ol	809	1.86	2.38	565	1.36	0.74	510	0.99	0.51
19	(*Z*)-7-Hexadecen-1-ol	1013	2.32	2.98	845	2.04	1.10	1322	2.57	1.33
20	(*Z*)-9-Hexadecen-1-ol	1026	2.36	3.02	1182	2.85	1.54	1338	2.60	1.35
21	(*E,Z*)-10,12-Hexadecadien-1-ol	2081	4.78	6.12	1935	4.66	2.53	1750	3.40	1.76

MATERIALS AND METHODS

The compounds used as standards in this study were obtained as follows: Nos. 1, 2, 3, and 20 from Applied Sciences Labs. (College Park, Pa., U.S.A.); Nos. 4, 5, 6, 7, 10, 11, 13, 14, and 15 were prepared by Dr. J. A. Findlay (University of New Brunswick, Fredericton, Canada). Nos. 8, 9, 12, 18, 19, 25, 26, 27, 28, 29, 30, 36, and 37 were purchased from Farchan Division, Story Chemical Corp. (Willoughby, Ohio, U.S.A.). Compounds 22, 23, and 24 were prepared by the acetylation of Nos. 1, 2 and 3[7]. The remaining compounds were gifts from the following sources: No. 21, from Dr. K. Eiter (Bayer, Leverkusen, G.F.R.), Nos. 31, 32, 33, and 34 from Dr. W. L. Roelofs (New York State Agricultural Experiment Station, Geneva, N.Y., U.S.A.), and No. 35 from Dr. Y. Tamaki (National Institute of Agricultural Science, Tokyo, Japan). Compounds 16 and 17 were both the gift of Ayerst Research Labs. (Montreal, Canada), who also donated samples of (*E*)- and (*Z*)-11-tetradecenal.

The spruce budworm wash used for the stereoisomer analysis was obtained as previously described by Weatherston *et al.*[8].

GC was performed on a Perkin-Elmer Model 990 gas chromatograph fitted with flame ionization detectors. The following columns were used: (a) a 6 ft. × 0.125 in. I.D. stainless-steel column filled with 15% DEGS* on Chromosorb W HP (80–100 mesh), temperature 160°, helium flow-rate 40 ml/min; (b) a 6 ft. × 0.125 in. I.D. stainless-steel column filled with 3% OV-25 on Chromosorb W HP (80–100 mesh) temperature 130°, helium flow-rate 40 ml/min; (c) a 6 ft. × 0.125 in. I.D. stainless-steel column filled with 5% OV-1 on Chromosorb W HP (80–100 mesh) temperature 140°, helium flow-rate 40 ml/min; (d) a 15.75 ft. × 0.125 in. I.D. stainless-steel column filled with 2.5% PDEAS* on Chromosorb W AW DMCS (100–120 mesh), temperature 140°, helium flow-rate 20 ml/min; (e) a 50 ft. × 0.02 in. I.D. stainless-steel support-coated open-tubular (SCOT) column, liquid phase DEGS, temperature 155°, helium flow-rate 3.3 ml/min; (f) a 50 ft. × 0.02 in. I.D. stainless-steel SCOT column, liquid phase PDEAS, temperature 145°, helium flow-rate 3.3 ml/min.

RESULTS AND DISCUSSION

Tables I and II give retention data for thirty-one unsaturated and six saturated compounds that can serve as synthetic standards in the elucidation of lepidopteran pheromones. The relative retention values (*r*) have proven useful during identification of areas of activity in insect extracts. Split effluent fractions are collected from both a polar and a non-polar column and the active fraction(s) are determined by bioassay. The *r* values are then used to predict (a) the length of the carbon chain, (b) the number of double bonds, and (c) whether the activity was caused by an alcohol or an acetate.

The geometrical isomer composition of both natural and synthetic sex pheromones has received much attention since it has been shown that the isomer ratio may have profound effects on the attractancy of the pheromones[9]. Various techniques may be used to effect such analyses, *e.g.*, IR spectrometry, GC, low-pressure liquid chromatography, thin-layer chromatography, and spinning band distillation[10]. In our investigations of the *Z*:*E* ratio of the 11-tetradecenals in the pheromone blend of the

* Abbreviations: DEGS = diethylene glycol succinate; PDEAS = phenyl diethanolamine succinate.

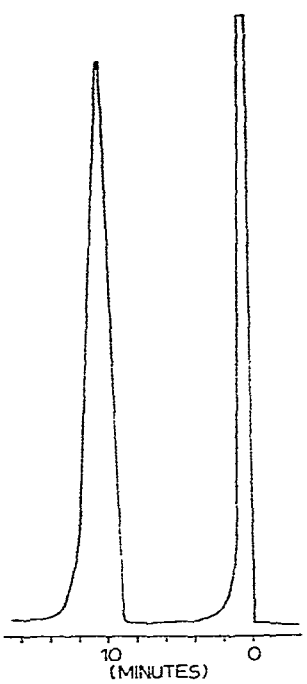
TABLE II

RETENTION DATA FOR VARIOUS STANDARD C_{12}, C_{14} AND C_{16} ALCOHOL ACETATES USED IN LEPIDOPTERAN PHEROMONE STUDIES

No.	*Acetate of*	*DEGS*			*OV-25*			*OV-1*		
		t_r *(sec)*	r_{ol}	r_{ac}	t_r *(sec)*	r_{ol}	r_{ac}	t_r *(sec)*	r_{ol}	r_{ac}
22	Dodecan-1-ol	173	0.40	0.51	276	0.67	0.36	366	0.71	0.37
23	Tetradecan-1-ol	340	0.78	1.00	766	1.85	1.00	992	1.93	1.00
24	Hexadecan-1-ol	680	1.56	2.00	2150	5.18	2.81	2770	5.38	2.79
25	(*Z*)-7-Dodecen-1-ol	224	0.51	0.66	307	0.74	0.41	358	0.70	0.36
26	(*E*)-7-Dodecen-1-ol	214	0.49	0.63	303	0.73	0.40	349	0.68	0.35
27	(*Z*)-8-Dodecen-1-ol	225	0.52	0.66	314	0.76	0.41	356	0.69	0.36
28	(*Z*)-9-Dodecen-1-ol	232	0.53	0.68	319	0.77	0.42	367	0.71	0.37
29	(*Z*)-7-Tetradecen-1-ol	410	0.94	1.21	815	1.96	1.06	910	1.77	0.92
30	(*Z*)-9-Tetradecen-1-ol	427	0.98	1.26	821	1.98	1.07	900	1.75	0.91
31	(*Z*)-11-Tetradecen-1-ol	460	1.06	1.35	875	2.11	1.14	1027	1.99	1.04
32	(*E*)-11-Tetradecen-1-ol	429	0.99	1.26	880	2.12	1.15	1015	1.97	1.02
33	(*Z*)-12-Tetradecen-1-ol	513	1.18	1.51	993	2.39	1.30	1049	2.04	1.06
34	(*E*)-12-Tetradecen-1-ol	466	1.07	1.37	817	1.97	1.07	986	1.92	0.99
35	(*Z*,*E*)-9,11-Tetradecadien-1-ol	819	1.88	2.41	1344	3.24	1.75	1131	2.20	1.14
36	(*Z*,*E*)-9,12-Tetradecadien-1-ol	625	1.43	1.83	1126	2.71	1.46	970	1.88	0.97
37	(*Z*)-7-Hexadecen-1-ol	774	1.78	2.28	2182	5.26	2.85	2266	4.40	2.28

spruce budworm several GC conditions were tried before satisfactory separation was achieved.

Weatherston and MacLean[11] were able to distinguish between (*Z*)-11-tetradecen-1-ol and the (*E*) isomer using a 6 ft. × 0.125 in. stainless-steel column filled with 15% PDEAS on Chromosorb W HP (80–100 mesh). Analyses of (*E*)-11-tetradecenal and the (*Z*) isomer gave retention times of 9.8 and 10.5 min; however, a 1:1 mixture of these compounds was not resolved (Fig. 1). Recently Persoons *et al.*[12] have reported the use of a 4.8-m × 4-mm column filled with 2.1% PDEAS on Varaport 30 to separate various tetradecen-1-ol acetate isomers. Using an almost identical column (column d) it was found that mixtures of (*E*)- and (*Z*)-11-tetradecenal were separable, though only partially, as long as the (*Z*) content exceeded 15% (Figs. 2a and b).



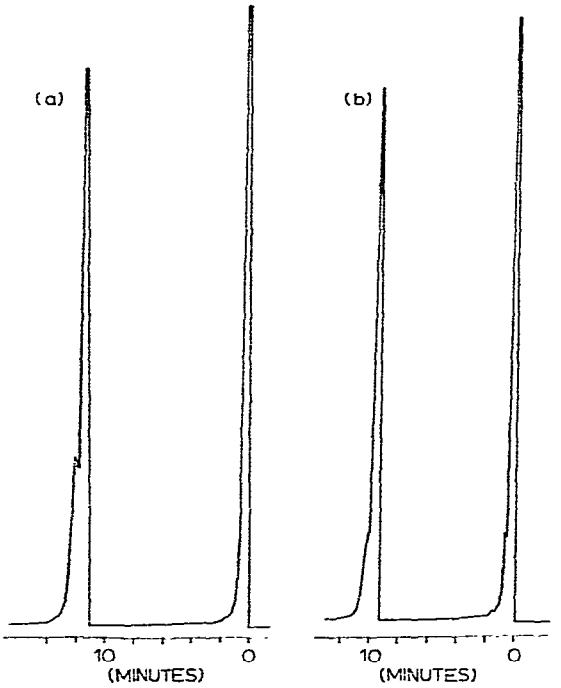


Fig. 1. Chromatogram of a 1:1 mixture of (*Z*)- and (*E*)-11-tetradecenal. Column, 6 ft. × 0.125 in. stainless steel, filled with 15% PDEAS on Chromosorb W HP (80–100 mesh).

Fig. 2 Separation of Δ^{11}-tetradecenal isomers. Column, 15.75 ft. × 0.125 in. stainless steel, filled with 2.5% PDEAS on Chromosorb W AW DMCS (100–120 mesh). (a) *E*:*Z* = 75:25; (b) *E*:*Z* = 85:15.

The method adopted for the routine analysis of isomeric mixtures of Δ^{11}-tetradecenal used in field work was capillary chromatography. Using column e isomers are separable in all proportions, Figs. 3a and b show the separation of 70:30 and 98:2 mixtures of (*E*)- and (*Z*)-11-tetradecenal, respectively. The use of column f extended the reproducibility of the analyses of mixtures containing as little as 0.5% of one of the isomers. Utilizing this latter SCOT column, the natural sex pheromone

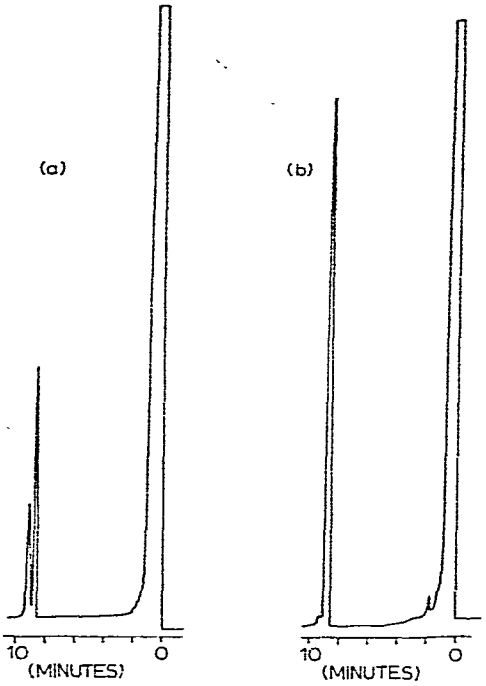


Fig. 3. Separation of Δ^{11}-tetradecenal isomers on a 50-ft. × 0.02-in. SCOT column employing DEGS as the liquid phase. (a) $E{:}Z = 70{:}30$; (b) $E{:}Z = 98{:}2$.

secreted by virgin female spruce budworm was determined to contain 3.9% (*Z*)-11-tetradecenal and 96.1% of the (*E*) isomer. Reproducible assays were determined on twenty FN aliquots of budworm wash.

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