SEPARATION OF A SERIES OF POSITIONAL AND GEOMETRICAL ISOMERS OF OLEFINIC ALIPHATIC PRIMARY ALCOHOLS AND ACETATES BY CAPILLARY GAS CHROMATOGRAPHY

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SUMMARY

A general procedure has been developed for the separation of the isomers of many of the olefinic aliphatic insect pheromones using glass capillary gas–liquid chromatographic columns. Four stationary phases containing varying amounts of cyano-propyl and two polyoxyethylene phases were investigated for the separation of positional and geometrical isomers of a series of mono-unsaturated primary acetates and alcohols. Based on the data obtained a procedure has been developed which permits the prediction of elution order and resolution for many di-unsaturated pheromones.

INTRODUCTION

Olefinic aliphatic primary alcohols and their corresponding acetates are important in the chemistry of insect pheromones. Analytical methods capable of resolving the geometrical and positional isomers of these compounds are crucial to the successful isolation, identification and subsequent stereoselective synthesis of unsaturated pheromones[1,2]. The separation of these isomers varies in difficulty depending on the position and the configuration of the olefinic bond and may become complex when di-unsaturated compounds are involved.

Numerous methods have been published for separating geometrical isomers of the biologically important mono- and diolefinic fatty acid methyl esters[3–5]. Efforts to extend these methods so that structurally similar pheromone isomers can be separated by capillary gas chromatography have been limited. A few known pheromones were separated with one particular column, but no data on column performance were reported[6,7]. Information on the separation of specific compounds is useful but may not be applicable to the separation of new pheromones.

We now report an investigation of the separation of positional isomers and geometrical isomers of pheromones (PIP and GIP) and pheromone-like structures by glass capillary gas chromatography. An analogous series of mono-unsaturated alcohols and acetates were used to evaluate several stationary phases used for the

separation of olefinic isomers. Based on this data simple calculations enabled the prediction of the elution order and resolution of the isomers of several di-unsaturated compounds. All but one of the liquid phases tested are commonly used, and all were coated onto the columns by the dynamic method.

EXPERIMENTAL

Column preparation

Glass capillary columns, approximately 35 m long ,were drawn from soft (soda-lime) glass with a Shimadzu®-type GDM-1 glass-drawing machine (Shimadzu, Tokyo, Japan). The final I.D. of the columns were 0.25–0.30 mm, and the O.D. 0.55–0.60 mm.

The glass columns were etched according to the method of Alexander and Rutten[8] with dry hydrogen chloride gas sealed in the capillary for 24 h at 350°.

All columns were coated by the dynamic method described by Dijkstra and DeGoey[9]. The capillary was attached to a nitrogen-pressurized reservoir containing ca. 0.8 ml of the dissolved stationary phase (see Reagents) and a small amount of mercury. A buffer capillary was attached to the exit end to minimize the pressure change which occured when the coating plug exited the column. The pressure was adjusted so that the velocity of the coating plug was 2 cm/sec. After the stationary phase was in the column, ca. 1 coil of the column was filled with mercury. Nitrogen was allowed to flow 10 h after the coating plug had exited the column.

Reagents*

The stationary phases SP-2300, SP-2310, SP-2330 and SP-2340 (having cyanopropyl contents of 35, 55, 68 and 75%, respectively) and SP-100 were commercial products from Supelco (Belefonte, Pa, U.S.A.). Each phase was used as a 20% (w/v) solution in acetone and coated as described. By the method of Blomberg[10], Carbowax 20M (Supelco) was coated as a 5% (w/v) solution in dichloromethane onto a previously deposited thin layer of non-extractable Carbowax 20M. A nitrile silicone polymer made by General Electric® (GE 238-149-99) and described by Litchfield et al.[11] was used as a reference phase. It is an experimental polymer with a limited operating temperature and lacks long term stability. The coating solution consisted of 20% (w/v) of the GE polymer and 5% (w/v) of Igepal CO-880 (Supelco) in equal volumes of acetone and ethyl acetate.

The saturated acetates were obtained from Chemical Samples (Columbus, Ohio, U.S.A.). The (Z)-7-tetradecen-1-ol acetate, (Z)- and (E)-9-tetradecen-1-ol acetates and (Z)- and (E)-11-tetradecen-1-ol acetates were obtained from the Farchan Division, Storey Chemical, Willoughby, Ohio, U.S.A.). The remaining samples were synthesized in our laboratory. The alcohols were obtained by saponification of the acetates. When required, the acetate geometrical isomers were purified by AgNO₃ liquid chromatography[12].

Chromatographic procedure

The instrument was a Varian 1400 chromatograph equipped with a flame-ionization detector (make-up gas flow-rate = 30 ml/min) and an injector splitter. The splitter, which was constructed in this laboratory, was similar to those sold commercially and had a split ratio of *ca.* 50:1. The linear flow velocity of the helium carrier gas was 18 cm/sec for all analyses. The gas chromatograph was interfaced to a Nicolet® 1180 computer (Nicolet Science, Madison, Wisc., U.S.A.) and all retention times from the various columns were verified on this system. All data reductions and plots were done on this computer with author written programs.

The mono-unsaturated isomers contained two internal standards which were saturated and one and four carbons shorter in length. Use of the standards not only facilitated the determination of system errors but also provided a reference for phase interaction. The column temperature was adjusted to give a k' (partition ratio)* of *ca.* 1.6 for the saturated acetate or alcohol of the corresponding unsaturated acetate or alcohol being investigated.

RESULTS AND DISCUSSION

Seven stationary phases were evaluated as to their usefulness in separating geometrical and positional isomers of acetates and alcohols. The ability of these phases to separate the compounds available for this study could be defined through the use of well-known expressions[13], based on column efficiency and resolution. We used compounds of 14-carbon chain length in this study because of their availability from previous investigations. When mono-unsaturated acetates, aldehydes and alcohols having olefinic bonds in various positions and chain lengths of 12, 16 or 18 carbons only marginal differences in the chromatographic properties were found when compared to the 14-carbon analogous series used in this study. Each phase was first evaluated for its ability to separate geometrical isomers of the analogous series of the 14-carbon acetates. Table I lists the k' values and separation factors ($\alpha = k'$ of second eluted isomer/k' of the first eluted isomer) for isomeric pairs of 14-carbon acetates differing in position of double bond. The liquid phases were the SP-2300 series and the GE material. Table II lists the values obtained for the 14-carbon acetates on the Carbowax 20M and SP-1000 columns; they were identical and therefore are listed only once.

Generally, the separation factor for a given pair of E and Z isomers increased with increasing cyanopropyl content of the liquid phase (Table I). However, even the SP-2340 phase only marginally separated the isomeric pair with unsaturation at carbon 4 (Fig. 1).

The SP-1000 and Carbowax 20M columns separated the E and Z isomers with C-4 unsaturation more effectively than any other isomeric pair in the analogous series of acetates (Table II). Moreover, the elution order of the E and Z isomers with unsaturation at carbon numbers 7 and lower was different from that of the isomers with unsaturation or carbon numbers greater than 7.

Investigation of the tetradecen-1-ol alcohols was somewhat limited because of

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* k' = elution time of sample (T_s) minus the elution time of an unretained solute (T_0) divided by the elution time of an unretained solute, $k' = (T_s - T_0)/T_0$.

TABLE I

PARTITION RATIO (k') AND SEPARATION VALUES (α) FOR THE (E)- AND (Z)-TETRADECEN-1-OL ACETATES ON THE CYANO-SILICONE PHASES

Phase	Bond position	k'		α
		E isomer	Z isomer	
SP-2300 (36%)	Saturated	1.63	1.63	
	3	1.69	1.72	1.01
	4	1.74	1.74	1.00
	5	1.80	1.80	1.00
	6	1.81	1.82	1.01
	7	1.81	1.87	1.03
	9	1.87	1.94	1.04
	11	1.92	2.00	1.04
SP-2310 (55%)	Saturated	1.61	1.61	
	3	1.66	1.69	1.02
	4	1.69	1.69	1.00
	5	1.79	1.79	1.00
	6	1.78	1.81	1.02
	7	1.78	1.81	1.02
	9	1.84	1.93	1.05
	11	1.90	2.03	1.07
SP-2330 (68%)	Saturated	1.62	1.62	
	3	1.68	1.74	1.03
	4	1.72	1.76	1.02
	5	1.79	1.85	1.03
	6	1.81	1.86	1.03
	7	1.81	1.90	1.05
	9	1.87	1.99	1.06
	11	1.92	2.08	1.08
SP-2340 (75%)	Saturated	1.60	1.60	
	3	1.63	1.69	1.03
	4	1.69	1.72	1.02
	5	1.74	1.80	1.03
	6	1.75	1.81	1.03
	7	1.78	1.87	1.05
	9	1.85	1.96	1.06
	11	1.90	2.06	1.08
GE	Saturated	1.60	1.60	
	3	1.62	1.69	1.04
	4	1.68	1.73	1.03
	5, 6	1.70	1.77	1.04
	7	1.72	1.81	1.05
	9	1.76	1.89	1.06
	11	1.81	1.97	1.09

our inability to reduce tailing on the SP phases having low cyano content. Table III lists the compiled data obtained with the SP-2310, SP-2340 and SP-1000 columns. Again, the values for the Carbowax and SP-1000 columns were the same. Analysis of this table indicates that with exception of the separation factors for the alcohols with C-3 unsaturation, the factors for the unsaturated alcohols and unsaturated

TABLE II

PARTITION RATIO (k') AND SEPARATION VALUES (α) FOR THE Z AND E ISOMERS OF TETRADECEN-1-OL ACETATES ON THE POLYOXYETHYLENE PHASES

Phase: Carbowax 20M and SP-1000.

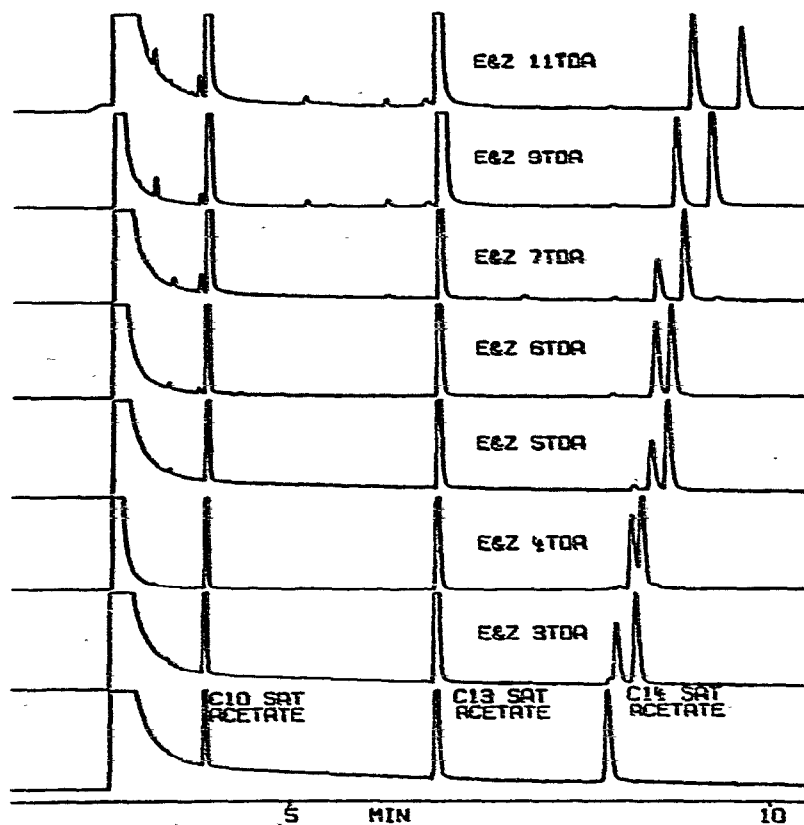| Bond position	k'		α
	First eluted isomer	Second eluted isomer	
Saturated	1.63	1.63	1.00
3	1.75 (Z)	1.75 (E)	1.00
4	1.71 (Z)	1.79 (E)	1.05
5	1.77 (Z)	1.81 (E)	1.02
6	1.76 (Z)	1.79 (E)	1.02
7	1.83 (Z)	1.83 (E)	1.00
9	1.88 (E)	1.91 (Z)	1.02
11	1.93 (E)	2.00 (Z)	1.04

Fig. 1. Chromatograms of a series of positional and geometrical isomers of tetradecen-1-ol acetate on SP-2340 column; length = 35 m; column temperature = 135°; split ratio = 50:1; helium carrier gas flow-rate = 18 cm/sec.

TABLE III

PARTITION RATIO (k') AND SEPARATION VALUES (a) FOR THE (Z)- AND (E)-TETRADECEN-1-ALCOHOLS ON THE CYANO-SILICONE AND POLYOXYETHYLENE PHASES

Phase	Bond position	k'		a
		E isomer	Z isomer	
SP-2310	Saturated	1.81	1.81	1.00
	3	1.81	2.00	1.11
	4	2.02	2.07	1.02
	5	2.20	2.28	1.04
	9	2.21	2.33	1.05
	11	2.32	2.49	1.07
SP-2340	Saturated	1.66	1.66	1.00
	3	1.67	1.90	1.14
	4	1.89	1.96	1.04
	5	1.97	2.09	1.06
	9	2.04	2.19	1.06
	11	2.04	2.24	1.10
SP-1000	Saturated	1.57	1.57	1.00
	3	1.55	1.71	1.10
	4	1.73	1.37	1.00
	5	1.79	1.79	1.00
	9	1.79	1.82	1.02
	11	1.86	1.94	1.05

acetates were generally similar. The elution order of the isomeric unsaturated alcohols from the SP-1000 column did not exhibit a crossover as noted with the unsaturated

Using the data in Tables I, II and III, we developed a method for predicting the k' values of the four positional and geometric isomers of many diolefinic primary alcohols and their acetates. The method is based on the concept that the k' of an unsaturated isomer equals the k' of corresponding saturated compound plus additivity constants ($\Delta k'$ values) that are specific for the double bonds in the isomer with respect to position and geometry. (Details on the use of the method are provided in examples below.) The sequence of elution could be predicted from the calculated k' values, and the separation factor for any two sequentially eluted isomers could then be predicted. Also, by use of appropriate k' and a values, the number of theoretical plates required (n req.) to separate the four isomers could be predicted. In the examples that follow, calculations for estimating n req. are based on the k' for the isomer eluted third from the column, the minimum predicted a value, and a resolution (R_s) of 1.2. Use of the last two parameters in the calculation assures 99% separation of the isomers[14]. To test the method we developed we used two dienes that are pheromone acetates and one diene that is a pheromone alcohol. These three compounds were used because all four isomers of each compound were available.

Example A shows the calculations used to predict the elution characteristics of the four isomers of 3,13-octadecadien-1-ol acetate when chromatographed on the SP-2340 column. The E,Z and Z,Z have been reported as pheromones[1]. These acetates are four carbons longer than the mono-unsaturated acetates. Therefore, in

EXAMPLE A

CALCULATION OF PREDICTED ELUTION ORDER (k') AND SEPARATION (α) OF THE
GEOMETRICAL ISOMERS OF 3,13-OCTADECADIEN-1-OL ACETATE ON SP-2340

Values for additivity constant ($\Delta k'$) due to double bonds, as calculated from experimental k' values
for 14-carbon acetates (Table I).

Bond geometry	Bond position	$k' - k'_{sat} = \Delta k'$
E	3	$1.63 - 1.60 = 0.03$
E	9	$1.85 - 1.60 = 0.25$
Z	3	$1.69 - 1.60 = 0.09$
Z	9	$1.96 - 1.60 = 0.36$

Sum of $\Delta k'$ ($\Delta k''$) to be added to 1.81, the k' of saturated 18-carbon acetate.

GIP	PIP	$\Delta k' + \Delta k' = \Delta k''$
E,E	3-9	$0.03 + 0.25 = 0.28$
E,Z	3-9	$0.03 + 0.36 = 0.39$
Z,E	3-9	$0.09 + 0.25 = 0.34$
Z,Z	3-9	$0.09 + 0.36 = 0.45$

k' predicted	α predicted	k' experimental	α experimental
E,E = 2.09	1.029	2.09	1.029
Z,E = 2.15	1.023	2.15	1.028
E,Z = 2.20	1.027	2.21	1.032
Z,Z = 2.26		2.28	

Number of theoretical plates (N) required ($N_{req.}$) to achieve resolution (R_s) of 1.2 for 3,13-octade-
cadien-1-ol acetate isomers on SP-2340 capillary column.

$k' = 2.2, \alpha = 1.023$.

$$N_{req.} = 16R_s^2 \left(\frac{\alpha}{\alpha - 1} \right)^2 \left(\frac{k' + 1}{k'} \right)^2 = 96,500 \text{ (ref. 13)}$$
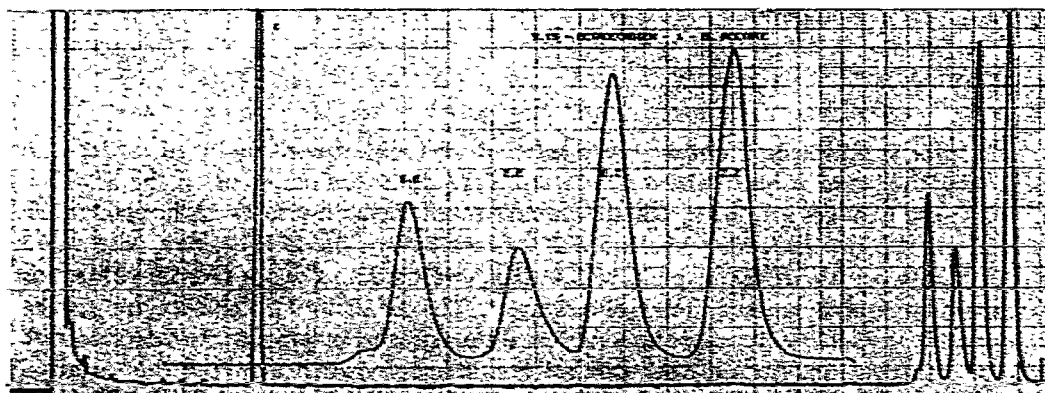

Fig. 2. Chromatogram of the four isomers of 3,13-octadecadien-1-ol acetate on SP-2340 column;
length = 35 m; column temperature = 185°; split ratio = 50:1; Helium carrier gas flow-rate = 18
cm/sec.

determining $\Delta k'$ values due to the C-13 double bond in the diunsaturate, we used data for the mono-unsaturate with double bond at C-9, because both these double bonds are five carbons from the terminal carbon. The predicted elution or (k' predicted) allowed us to predict the separation factors (α predicted) for the four isomers. We next determined that the number of theoretical plates (n) needed to separate the two isomers having a minimum predicted separation factor of 1.023 with a resolution of 1.2 is *ca.* 96,500. Fig. 2 is the chromatogram of the 4 isomers obtained with the SP-2340 column. The k' and α values for the four isomers are close to the predicted values.

EXAMPLE B

CALCULATION OF PREDICTED ELUTION ORDER (k') AND SEPARATION (α) OF THE GEOMETRICAL ISOMERS OF 3,13-OCTADECADIEN-1-OL ON SP-1000

Values for additivity constant ($\Delta k'$) due to double bonds, as calculated from experimental k' value for 14-carbon alcohols (Table III).

Bond geometry	Bond position	$\Delta k'$
E	3	−0.02
E	9	0.22
Z	3	0.14
Z	9	0.25

Sum of $\Delta k'$ ($\Delta k'$) to be added to 1.44, the k' of saturated 18-carbon alcohol.

GIP	PIP	$\Delta k'$ to be added
E,E	3-9	0.20
E,Z	3-9	0.23
Z,E	3-9	0.36
Z,Z	3-9	0.39

k' predicted	α predicted	k' experimental	α experimental
E,E = 1.64	1.018	1.64	1.024
E,Z = 1.67	1.078	1.68	1.065
Z,E = 1.80	1.017	1.79	1.011
Z,Z = 1.83		1.81	

$N_{req.}$ for $R_s = 1.2$, $k' = 1.80$, $\alpha = 1.017$
$N_{req.} \approx 199,545$

Similarly, example B shows the predicted and experimental elution order and α values for the separation of the 4 isomers of 3,13-octadecadien-1-ol on the SP-1000 column. These isomers are the synthetic precursors of the isomeric 18-carbon acetates. As predicted the elution order was the E,Z isomer before the Z,E isomer, with good separation between them. Example C shows the predicted and experimental values for the isomers of 7,11-hexadecadien-1-ol acetate[15]. The additivity constants due to the double bond at C-11 were calculated from data for the mono-unsaturated acetate with C-9 unsaturation. Again, both double bonds are five carbons from the terminal carbon. The predicted number of theoretical plates required to separate the Z,E and the E,Z is quite high and suggests that for a resolution of 1.2, the column must be highly efficient (3000 plates/m) and of considerable length ($\approx$90 m).

EXAMPLE C

CALCULATION OF PREDICTED ELUTION ORDER (k') AND SEPARATION (α) OF THE GEOMETRICAL ISOMERS OF 7,11-HEXADECADIEN-1-OL ACETATE ON SP-2340

Values for additivity constants ($\Delta k'$) due to double bonds, as calculated from experimental k' value for 14-carbon acetates (Table I).

Bond geometry	Bond position	$\Delta k'$
E	7	0.18
E	9	0.25
Z	7	0.27
Z	9	0.36

Sum of Δk ($\Delta k'$) additivity constant to be added to 2.07, the k' of saturated 16-carbon acetate.

GIP	PIP	$\Delta k'$ to be added
E,E	7-9	0.43
E,Z	7-9	0.54
Z,E	7-9	0.52
Z,Z	7-9	0.63

k' predicted	α predicted	k' experimental	α experimental
E,E $= 2.50$	1.037	2.5	1.040
Z,E $= 2.59$	1.008	2.60	1.004
E,Z $= 2.61$	1.035	2.61	1.041
Z,Z $= 2.70$		2.72	

$$N_{req.} \text{ for } R_s = 1.2, \; k' = 2.6, \; \alpha = 1.008$$
$$N_{req.} = 247,000$$

The good agreement between the predicted and experimental chromatographic parameters point out the importance of double-bond position and geometry in the chromatographic characteristics of isomeric unsaturated compounds. For expediency the columns we used were of minimal length and numbers of theoretical plates, and were operated for fast elution, rather than efficiency. Thus, even better results should be obtained if column performance is optimized. Also, increases in the availability of, and data on, homologous mono-unsaturated compounds should increase the accuracy of the predictions; and testing the accuracy of predictions would be facilitated by increased availability of homologous di-unsaturated compounds. The most important aspect of the procedure we developed is that it allows selection of the optimal column and conditions to separate a set of isomers of a given unsaturated aliphatic alcohol or acetate with minimum experimentation.

REFERENCES

1 J. H. Tumlinson, C. E. Yonce, R. E. Doolittle, R. R. Heath, G. R. Gentry and E. R. Mitchell, *Science*, 185 (1974) 614.
2 M. W. Barry, P. G. Nielsen, F. F. Purrington and J. H. Tumlinson, *Environ. Entomol.*, 7 (1978) 1.
3 E. S. Van Vleet and J. G. Quinn, *J. Chromatogr.*, 151 (1978) 396.
4 H. Heckers, F. W. Melcher and A. Schloeder, *J. Chromatogr.*, 136 (1977) 311.
5 H. Jaeger, H. U. Klör, G. Blos and H. Ditchuneit, *Chromatographia*, 8 (1975) 507.
6 W. Schäfer, *J. High Resolut. Chromatogr. Chromatogr. Commun.*, 1 (1978) 71.
7 J. D. Warthen, R. M. Waters and D. J. Voaden, *Chromatographia*, 10 (1977) 720.
8 G. Alexander and G. A. F. Rutten, *J. Chromatogr.*, 99 (1974) 81.

9 G. Dijkstra and J. DeGoey, in D. H. Desty (Editor), *Gas Chromatography*, Butterworth, London, 1958, pp. 56–58.
10 L. Blomberg, *J. Chromatogr.*, 115 (1975) 365.
11 C. Litchfield, R. Reiser, C. A. F. Isbell and G. L. Feldman, *J. Amer. Oil Chem. Soc.*, 41 (1964) 52.
12 R. R. Heath, J. H. Tumlinson, R. E. Doolittle and J. H. Duncan, *J. Chromatogr. Sci.*, 15 (1977) 10.
13 L. S. Ettre, *J. Gas Chromatogr.*, 36 (1963) 36.
14 L. R. Snyder, *J. Gas Chromatogr.*, 10 (1972) 200.
15 H. E. Hummel, L. K. Gaston, H. H. Shorey, R. S. Kaae, K. J. Byrne and K. M. Silverstein, *Science*, 181 (1973) 873.