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RELATIONSHIPS BETWEEN STRUCTURE AND CHROMATOGRAPHIC RETENTION FOR *n*-ALKADIENES

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SUMMARY

The chromatographic behavior of *n*-alkadienes was studied by high-performance liquid chromatography on a silver nitrate-coated silica column and by gas chromatography on a non-polar capillary column. The retentions of 89 synthetic *Z,Z* and *E,Z* diene standards, of 18–38 carbons, were determined. Multiple regression models for retention were developed for both columns, based on double-bond configurations, sizes of the terminal alkyl groups, and the numbers of carbons between double bonds. The use of these chromatographic columns in the analysis of complex mixtures of dienes from natural sources is discussed.

INTRODUCTION

n-Alkadienes (throughout the article, simply "dienes") have been detected in the cuticular hydrocarbons of numerous arthropod species. It has been of interest to determine diene structures because these compounds have pheromonal activity in some species[1,2] and because they may provide useful chemotaxonomic characters[3]. Yet the structural analysis of dienes can be difficult because complicated mixtures of isomers are possible within any length of carbon chain. The intractability of the dienes of honey bees, *Apis mellifera*, has probably been due to such mixtures[4]. A similar situation existed with *Sitophilus* weevils[5]. Whenever more than one isomer occurred for a carbon chain length, unequivocal structure determination was very difficult.

High-performance liquid chromatography (HPLC) on a silver nitrate-coated silica column ($AgNO_3$ column) is a powerful tool for resolving diene mixtures. Heath and co-workers[6,7] and Heath and Sonnet[8] described the preparation and properties of these columns. Although originally intended for the separation of geometrical isomers, $AgNO_3$ columns are also capable of separating positional isomers, as demonstrated by Heath and Sonnet[8] for alkenes. We noted empirically that an $AgNO_3$ column would resolve the diene mixture from the fruit fly, *Drosophila virilis*, and through the use of this column, over 100 dienes, including 20 different pentatriacontadienes, were eventually identified[9]. In the present study, the separation characteristics of an $AgNO_3$ column were investigated more systematically, using synthetic diene standards.

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Capillary gas chromatography (GC) is also a useful method in the analysis of dienes, both for confirmation of purity and for structural information. As with alkenes[10,11], the GC retentions of dienes depend on the positions and configurations of the double bonds. Soják *et al.*[12] investigated the retentions of dienes with 8–10 carbons. Our study considers longer dienes, more typical of those found in arthropod cuticles.

For both the capillary GC and $AgNO_3$ HPLC columns, empirical multiple regression models were developed which related retention to diene structure. Predictive models had been presented previously for alkenes (*e.g.*, Papazova and Dimov[13]) and for alkanes (*e.g.*, Lombosi *et al.*[14]).

MATERIALS

Synthetic dienes

An abbreviated notation for diene names is used here; thus (*Z,Z*)-8,18-heptacosadiene is written as *Z,Z*-8,18-27. Chromatographic properties are discussed below in terms of the "middle" and "terminal" sections of the dienes. For example, *Z,Z*-8,18-27, with the structure, $C_8 \overset{Z}{=} C_{10} \overset{Z}{=} C_9$, has a middle section of ten carbons, and terminal sections of eight and nine carbons.

Seventy-two dienes with 18–38 carbons were synthesized for this study, and 17 additional dienes were available from other projects (Table I). Middle section sizes were 3, 4, 6, 8, 10 and 12 carbons. Most of the standards were symmetrical, or nearly symmetrical, with respect to double bond position (the two terminal sections having similar numbers of carbons), but some strongly asymmetrical dienes were also prepared, primarily with middle sections of 6 and 12 carbons. The *Z,Z* dienes were of greatest interest because the reported, naturally occurring compounds have had this configuration, but small amounts of *E,Z* isomers were unavoidably produced during synthesis, and the chromatographic properties of these were studied also.

The dienes were prepared by a Wittig synthesis, generally following Sonnet's[15] method for alkenes. Differences from his procedure were that the alkyltriphenylphosphonium salts were prepared from α,ω-dibromoalkanes —to allow for double-bond formation on both ends— and that the equivalents of other reactants (butyllithium and aldehyde) were doubled correspondingly. For symmetrical dienes, only one aldehyde was added to the reaction mixture. For asymmetrical dienes, equimolar amounts of the two appropriate aldehydes were added simultaneously. In these cases, two symmetrical dienes were also formed, one shorter and one longer than the desired asymmetrical diene. The molar ratios of products were *ca.* 2:1:1 in favor of the asymmetrical diene. Dimethylsulfoxide was used as a cosolvent to enhance the formation of *Z* double bonds. After workup[15], the dienes were passed through a column of silicic acid with hexane to remove polar by-products. Analysis by GC showed that the expected carbon chain lengths were always produced. These dienes, containing the *Z,Z*, *E,Z*, and traces of *E,E* isomers (as well as several chain lengths, in the cases of asymmetrical dienes), were used without further purification for HPLC analysis.

High-performance liquid chromatography

HPLC was conducted using a Waters Assoc. M6000 pump and 401 differential

TABLE I

SYNTHETIC DIENES AND THEIR CHROMATOGRAPHIC RETENTIONS ON THE $AgNO_3$ HPLC COLUMN AND THE CAPILLARY GC COLUMN

Diene	*Relative retention on $AgNO_3$ HPLC column**		*ECL on capillary GC column*	
	Z,Z diene	*E,Z diene*	*Z,Z diene*	*E,Z diene*
7,11-18	2.24	1.23	17.62	17.64
4,16-20	1.17	0.49	19.86	19.82
2,14-20	1.33	0.53	20.00	19.83, 20.00**
5,15-20	1.42	0.55	19.72	19.73
6,14-20	1.71	0.67	19.61	19.66
7,13-20	1.83	0.67	19.55	19.62
4,10-20	1.87	0.70	19.66	19.64, 19.74
8,12-20	1.84	1.05	19.54	19.58
9,12-21	2.82	0.43	20.54	20.68
4,14-23	1.32	0.51	22.64	22.60
6,18-24	0.91	0.39	23.65	23.66
9,12-24	2.40	0.38	23.49	23.65, 23.65
6,16-25	1.15	–	24.53	–
7,19-26	0.82	0.36	25.57	25.61
4,16-26	0.85	0.37	25.65	25.58, 25.72
8,18-26	1.05	0.44	25.49	25.54
7,17-26	1.06	0.42	25.50	25.54
10,16-26	1.40	0.53	25.39	25.50
7,13-26	1.47	0.55	25.47	25.52, 25.58
4,10-26	1.72	0.61	25.68	25.62, 25.76
11,15-26	1.38	0.79	25.43	25.48
8,18-27	1.00	0.40	26.47	26.53
7,11-27	1.58	0.93	26.55	26.55, 26.58
12,15-27	2.19	0.34	26.43	26.61
9,19-28	0.96	–	27.44	–
9,19-29	0.92	–	28.43	–
9,19-30	0.89	–	29.40	–
9,19-31	0.86	–	30.40	–
10,22-32	0.65	0.27	31.45	31.50
7,19-32	0.67	0.29	31.48	31.48, 31.56
4,16-32	0.75	0.32	31.62	31.54, 31.73
11,21-32	0.80	0.35	31.37	31.46
9,19-32	0.83	–	31.41	–
12,20-32	1.16	0.46	31.34	31.43
13,19-32	1.22	0.45	31.37	31.47
10,16-32	1.25	0.46	31.38	31.50, 31.50
14,18-32	1.17	0.70	31.41	31.43
9,19-33	0.81	–	32.40	–
9,19-34	0.79	–	33.40	–
9,19-35	0.77	–	34.41	–
9,19-36	0.76	–	35.42	–
16,20-36	1.14	0.71	35.40	35.43
9,19-37	0.75	–	36.42	–
13,25-38	0.56	0.25	37.38	37.45
16,22-38	1.10	0.40	37.35	37.48

* Relative retention, A, is the ratio of the capacity factor for the diene to that of the internal standard, *Z,Z*-8,18-27.

** When two ECLs are listed, the second refers to the *Z,E* isomer. On the $AgNO_3$ column, the *E,Z* and *Z,E* isomers did not separate.

refractometer detector and a Valco injector. The Adsorbosphere® silica column (25 cm × 4.6 mm I.D., with 5 μm particle size, Applied Science, Deerfield, IL, U.S.A.) was coated *in situ* with silver nitrate as described by Heath and Sonnet[8]. The solvent was 25% (v/v) toluene in hexane and was delivered at 1 ml/min. The solvents were HPLC grade. The column had been used *ca.* 50 h before the present study.

One practical difficulty was detector insensitivity. Although 10-mg diene samples were always detected by the refractometer, this amount overloaded the column, causing broad peaks which tailed severely. With samples of 200 μg or less, recorder peaks were symmetrical (efficiency was 2000–4000 theoretical plates at a capacity factor, k', of 2.5), but peaks were seen only for the larger dienes (over 30 carbons). In this study the sample sizes were kept small (< 200 μg/diene), and the effluent was collected in up to eighty 0.4-ml fractions, to be quantitated later by GC.

Quantitation of dienes in the HPLC fractions was done on a non-polar, packed GC column (3% Dexsil 300 on 100/120 Chromosorb WHP, 2 m × 2 mm I.D. glass column). The gas chromatograph was a Varian 3700, equipped with flame ionization detector and interfaced to a Hewlett Packard 3380A integrator. The carrier gas was helium. Quantitation was always relative to an internal GC standard, either *Z*-11-22 or *Z*-11-24, chosen so as not to interfere with the dienes in the samples. Although the HPLC fractions were collected every 0.4 ml, it was desirable to estimate the retention volume at least to the nearest 0.1 ml. It was possible to do this since a diene usually occurred in three or more consecutive HPLC fractions. The amounts in these fractions determined a Gaussian curve, from which the retention volume at the peak apex was obtained.

Each diene (Table I) was analyzed at least twice by HPLC. An internal standard, usually *Z,Z*-8,18-27, was included in each run so that relative retentions could be calculated. When a 27-carbon diene was being analyzed, the standard was *Z,Z*-7,17-26. The *E,Z* isomer of every diene eluted before the *Z,Z* isomer. The *Z,Z* dienes were recognized by their relatively greater amounts (being produced preferentially in the synthesis) and by spot checks of infrared spectra (*Z,Z*-dienes do not produce a peak at 970 cm^{-1}).

Capacity factors, k', were calculated for the dienes [$k' = (V_R - V_m)/V_m$, where V_R is the retention volume of the sample and V_m is the retention of an unretained solute, here 3.2 ml]. For *Z,Z*-8,18-27, k' had a mean of 2.6, with a standard deviation of 10%. The reasons for fluctuations in k' were unknown, but column temperature was not controlled, (the ambient laboratory temperature varied between 25 and 30°C), and slight variability among batches of solvent was possible. Nevertheless, by expressing the retentions of all dienes relative to the internal standard, *Z,Z*-8,18-27, the variability was reduced considerably. In this study, the relative retention, A, was the ratio of the capacity factor of the diene of interest to that of the internal standard. For dienes retained longer than *Z,Z*-8,18-27, A was equal to α, the separation factor; for those retained less than *Z,Z*-8,18-27, $A = 1/\alpha$, since by definition, α is never less than unity. The standard deviation of the relative retentions, A, was estimated from replicated analyses to be 4.4% [120 df (= degrees of freedom)]. A portion of this variability was undoubtedly due to the difficulty of determining the exact HPLC retentions of dienes.

Capillary gas chromatography

The HPLC fractions containing the *Z,Z* or *E,Z* dienes were further analyzed on a Durabond® DB-1 capillary column (30 m × 0.25 mm I.D., 0.25 μm film thickness). The column was installed in a Varian 3700 gas chromatograph, equipped with a Varian universal capillary injector and flame ionization detector. The helium pressure was 1.4 kg/cm² at the injector. The temperature program was 150–310°C at 5°C/min. The amount of each diene injected per run was 50 ng or less. *n*-Alkane standards (16–38 carbons) were always coinjected with the diene samples, and equivalent chain lengths (ECLs) were calculated by linear interpolation (ECL = Kováts Index/100). The ECL of each diene was determined at least twice.

For most asymmetrical dienes (those for which the two terminal sections had different numbers of carbons), the *E,Z* and *Z,E* isomers were separable by GC. The assignments as to which peak represented which isomer were based upon results for the symmetrical dienes (*E,Z* and *Z,Z*). For each pair of *E,Z* and *Z,E* isomers, the structure with the *E* double bond nearer the middle of the chain was assigned to the later GC peak. The structure–retention relationships derived from regression analysis supported these assignments completely.

Regression analysis

Multiple regression analysis was used to develop empirical relationships between chromatographic retention and structural features of *Z,Z* and *E,Z* dienes. For both the HPLC and GC columns, the retention model took the same general form. For a *Z,Z* diene, with the structural representation, $C_i^Z = C_j^Z = C_k$, the regression model was $Y = T_{Z,i} + M_{ZZ,j} + T_{Z,k}$. (In both the structure and the model, *j*, *i*, and *k* represent the numbers of carbons in the middle and two terminal sections of the diene.) Similarly, for an *E,Z* diene, $C_i^E = C_j^Z = C_k$, the model was $Y = T_{E,i} + M_{EZ,j} + T_{Z,k}$. In the models, *Y* represents chromatographic retention, and *T* and *M* are parameters estimated by regression techniques. For either type of column, the value of *M* depended only on the number of carbon atoms, *j*, in the middle section of the diene and on the configurations of the adjacent double bonds (hence, the subscripts in the models). Likewise, each *T* parameter depended only on the number of carbons in the terminal section (*i* or *k*) and on the configuration of the associated double bond.

The dependent variable, *Y*, took different forms for HPLC and GC. For HPLC, $Y = \log A$, where the relative retention, *A*, was the ratio of the capacity factor for the diene to that for the internal standard. The logarithmic form of the ratio was more amenable to linear modeling. For GC, $Y = \Delta ECL$, where $\Delta ECL = ECL_{diene} - ECL_{n\text{-alkane}}$, the diene and *n*-alkane having the same number of carbons. Since the ECL of an *n*-alkane is, by definition, equal to the number of carbons it possesses, $\Delta ECL = ECL_{diene} -$ (number of carbons in diene). This measure of GC retention was chosen because the decimal portion of the ECL contains the key information about double bond location.

The terminal-section parameters, *T*, and the middle-section parameters, *M*, were estimated by computer, using a multiple regression program. The data matrix included "dummy" variables[16], which were used to code for the presence (1) or absence (0) of the possible section sizes, adjacent to *Z* or *E* double bonds, in the

diene structures. For symmetrical *Z,Z* dienes, the code for the (identical) terminal sections was 2.

The additional constraints needed to fully determine the parameters, T and M, were: (1) $T_{Z,k}$ took the same values for *Z,Z* dienes and *E,Z* dienes and (2) $M_{ZZ,12} = M_{EZ,12} = 0$. In effect, the latter constraints specified how the "intercept" term generated by regression calculations was distributed among the T and M parameters. Any other comparable constraint would have led to identical fitted values for retentions, but we chose to interpret M as an "interaction" between double bonds, which was (arbitrarily) set at zero when the double bonds were separated as far as possible ($j = 12$).

RESULTS

Experimental data

The HPLC and GC retention data for the standard dienes are given in Table I. The relative retentions on the $AgNO_3$ column ranged from 0.25 × to 2.82 × the retention of the internal standard. That the *E,Z* dienes always eluted before the corresponding *Z,Z* dienes is obvious in Table I, but considerable differences in retention also occurred due to double bond location. No separation was observed between the *E,Z* and *Z,E* isomers of asymmetrical dienes. The individual HPLC fractions containing these had nearly equal amounts of *E,Z* and *Z,E* isomers, by capillary GC. Thus in Table I, the retentions for asymmetrical *E,Z* isomers apply to the *Z,E* isomers as well.

On the capillary GC column, the ECLs of the dienes ranged from 0.66 to 0.00 less than the corresponding *n*-alkanes. This variability indicated a large amount of structural information in the retentions, but the contributions of double-bond configuration and location to retention were not obvious from Table I. These contributions were evaluated by multiple regression analysis.

Regression analysis for $AgNO_3$ HPLC

The variability in retention on the $AgNO_3$ column was "explained" very well by the multiple regression model. R^2 for the *Z,Z* dienes was 0.987 and for the *E,Z* dienes, 0.980. Residual analysis indicated the variance was fairly homogenous throughout the data set, and no dienes were outliers from the model. The mean residual variability from the regression model amounted to 4.8% for any relative retention, A. The variability due to the chromatographic technique, obtained from replicated runs, was 4.4%. Thus the terminal- and middle-section terms accounted for virtually all of the variability in the data beyond that due to technique. Implicit in the model is the absence of interactions among the three diene sections; each exerts its effect independently. Furthermore, the section effects do not depend on overall chain length.

Since logarithms of relative retentions are not intuitively meaningful, the model was converted to its multiplicative form for presentation: $A = t_{Z,i} \cdot m_{ZZ,j} \cdot t_{Z,k}$ or $A = t_{E,i} \cdot m_{EZ,j} \cdot t_{Z,k}$, where $T = \log t$ and $M = \log m$. The factors for predicting relative retentions of dienes are listed in Table II. The relative retention, A, of a diene is estimated by multiplying the middle-section factor and the two terminal-section factors corresponding to the configuration and double bond locations of the diene.

TABLE II

PREDICTION MODELS FOR CHROMATOGRAPHIC RETENTION OF DIENES

Form of prediction models

Diene type	*$AgNO_3$ HPLC*	*Capillary GC*
$C_i^Z{=}C_j^Z{=}C_k$	$A = t_{Z,i} \cdot m_{ZZ,j} \cdot t_{Z,k}$	ECL = $T_{Z,i} + M_{ZZ,j} + T_{Z,k}$ + (number of carbons)
$C_i^E{=}C_j^Z{=}C_k$	$A = t_{E,i} \cdot m_{EZ,j} \cdot t_{Z,k}$	ECL = $T_{E,i} + M_{EZ,j} + T_{Z,k}$ + (number of carbons)

Middle-section parameters

Middle-section size (carbons)	*$AgNO_3$ factors*		*GC terms*	
	$m_{ZZ,j}$	$m_{EZ,j}$	$M_{ZZ,j}$	$M_{EZ,j}$
j = 3	3.86	1.35	0.065	0.165
4	2.46	3.37	0.030	0.015
6	2.24	1.90	−0.010	0.035
8	1.95	1.80	−0.030	−0.005
10	1.39	1.33	−0.020	−0.005
12	1.00	1.00	0.000	0.000

Terminal-section parameters

Terminal-section size (carbons)	*$AgNO_3$ factors*		*GC terms*	
	$t_{Z,i}$, $t_{Z,k}$*	$t_{E,i}$	$T_{Z,i}$, $T_{Z,k}$*	$T_{E,i}$
i,k = 2	1.39	0.56	0.180	0.010
4	1.09	0.46	−0.065	−0.115
5	1.01	0.41	−0.130	−0.135
6	0.95	0.39	−0.180	−0.170
7	0.92	0.39	−0.215	−0.195
8	0.87	0.37	−0.245	−0.200
9	0.84	0.35	−0.265	−0.205
10	0.80	0.34	−0.290	−0.230
11	0.76	0.32	−0.305	−0.230
12	0.76	0.33	−0.315	−0.240
13	0.73	0.32	−0.310	−0.245
14	0.69	0.30	−0.315	−0.250
15	0.68	–	−0.315	–
16	0.69	0.30	−0.305	−0.220
17	0.65	–	−0.305	–
18	0.65	–	−0.290	–

* For symmetrical *Z,Z* dienes, $i = k$, and the same terminal-section parameter is used twice.

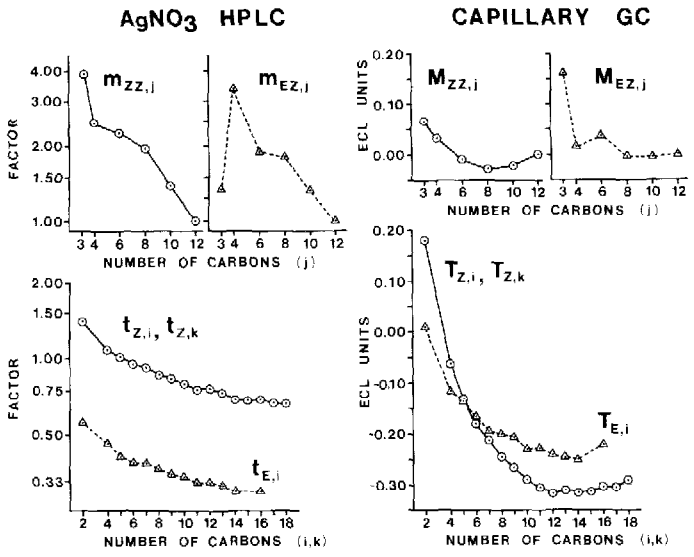

Fig. 1. Dependence of parameters on the numbers of carbons in the diene sections. In the retention model for $AgNO_3$ HPLC, the middle- and terminal-section factors were m and t, respectively, the subscripts denoting double-bond configuration and number of carbons. The axes for the factors are logarithmic; thus the relationships among these parameters appear as in the original linear model. In the model for capillary GC, middle- and terminal-section terms were M and T, respectively.

Thus for *E,Z*-5,15-20, the terminal-section factor for the *E* end (5 carbons) is 0.41, the middle-section factor (10 carbons) is 1.33, and the terminal-section factor for the *Z* end (5 carbons) is 1.01, making the prediction 0.55. The observed retention was 0.55 (Table I). The maximum standard error of prediction within the data set was 7%; thus few predictions in error by more than 14% would be expected.

Obviously, our internal standard (*Z,Z*-8,18-27), to which all the relative retentions were calculated, is of no general interest, but the ratio of any two calculated relative retentions gives the predicted separation factor, α, for the two chosen dienes. The table provides a way of predicting the separation factor for any pair of dienes within the scope of the study.

The influence of structural features on retention is summarized in Fig. 1. For *Z,Z* dienes, retention increased dramatically as the middle section decreased in size. The same trend was noted for *E,Z* dienes except that for a middle section with 3 carbons, the effect was far below the trend set by the other middle-section sizes, for unknown reasons.

The sizes of the terminal sections also affected retention. For both the *E,Z* and *Z,Z* dienes, retention decreased as the sizes of the terminal sections increased (Fig. 1). In the figure, the overall separation between *Z,Z* and *E,Z* dienes was reflected primarily in the difference between $t_{Z,i}$ and $t_{E,i}$, since the same $t_{Z,k}$ were used in both models and middle-section factors, $m_{ZZ,j}$ and $m_{EZ,j}$, were constrained to be equal for $j = 12$ carbons. The curves for t_Z and t_E were essentially parallel (Fig. 1), which must be true if the *E,Z* and *Z,E* isomers of asymmetrical dienes do not separate. However, the curvature in these plots did indicate a tendency for asymmetrical dienes to be retained longer than symmetrical ones, for a given chain length, middle-section size,

and double bond configuration. For example, *Z,Z*-4,10-26 was retained longer than *Z,Z*-10,16-26 (relative retentions were 1.72 and 1.40, respectively, Table I). The *E,Z* isomers showed the same trend, with retentions of 0.61 and 0.53, respectively.

Regression analysis for capillary GC

The GC retentions (ECLs) were also strongly related to structural features through the regression model. The R^2 values were 0.992 and 0.966 for the *Z,Z* and *E,Z* dienes, respectively. The root-mean-square errors from the regressions were 0.013 and 0.015 ECL units, respectively, which were only slightly larger than the "technique" error of 0.010 ECL units, calculated from replicated runs on the samples. The estimated values for the parameters are listed in Table II, and these may be used to predict the ECLs of dienes. The standard error of prediction was no larger than 0.016 ECL units, within the data set; thus deviations of more than 0.03 ECL units between prediction and experiment were "unusual".

The regression residuals for asymmetrical dienes with both double bonds close to one end of the molecule tended to be larger than for the symmetrical dienes. In fact, *Z,Z*-4,10-26 was an "outlier" from the regression model, with a predicted ECL of 25.62, but a measured ECL of 25.68. Our standards did not include many highly asymmetrical dienes, and more complicated models may be needed when both double bonds are near one end. One other diene, *E,Z*-16,20-36, was an outlier from the regression model. Its fitted ECL was 35.49, but the experimental value was 35.43. This error may be due in part to the estimate of $t_{E,16}$ being inaccurate, because it depended heavily on the highly asymmetrical dienes, *Z,E*-7,11-27 and *Z,E*-4,10-26.

The effects on ECL of various terminal-section sizes are shown in Fig. 1. For *Z,Z* dienes, the terminal-section parameters decreased rapidly as the carbon number increased from 2 to 10, but then changed relatively little with further increases in carbon number. The parameters for terminal sections adjacent to *E* double bonds showed a similar trend with change in carbon number, but the overall range was smaller. The *Z* and *E* curves crossed when the terminal section contained $i = 5$ carbons. When terminal sections contained fewer than 5 carbons, the *Z,Z* isomers tended to be retained longer than the *E,Z* isomers, but when the terminal sections had more than 5 carbons, the *E,Z* isomers tended to be retained longer, whenever the middle-section terms, $M_{ZZ,j}$ and $M_{EZ,j}$, were similar. Thus *Z,Z*-4,16-20 was retained longer than *E,Z*-4,16-20, but *Z,Z*-10,22-32 eluted more quickly than *E,Z*-10,22-32 (Table I). Heath *et al.*[11] observed an analogous reversal in the elution order of *E* and *Z* alkenes at the 5 double-bond position on a GC column of similar polarity (SP-2100).

As with the $AgNO_3$ parameters, the curvature in the plots of terminal-section parameters (Fig. 1) reflected the tendency for an asymmetrical diene to be retained longer than the symmetrical counterpart, given a constant chain length, middle-section size, and configuration of double bonds. This trend is seen in Table I. Since the plots for the *Z* and *E* end lengths are not parallel, the *E,Z* and *Z,E* isomers of the asymmetrical dienes would be expected to have different ECLs, and these isomers were usually separable on the DB-1 column. This situation contrasted with the $AgNO_3$ column, on which the *E,Z* and *Z,E* isomers eluted together.

Compared to the terminal-section parameters, middle-section parameters had relatively little impact on predicted ECLs except for *E,Z* dienes with a spacing of

$j = 3$ carbons (Fig. 1). As on the $AgNO_3$ column, these dienes departed markedly from the trend established by the other middle-section sizes.

DISCUSSION

The silver nitrate-coated HPLC column is a powerful tool for the analysis of diene mixtures from biological sources. For any carbon chain length within the range of this study, the $AgNO_3$ column separates dienes primarily on the size of the middle section and on double bond configuration. Separation by overall carbon chain length is easily achieved by another tool, preparative GC. By applying both methods consecutively, a complex diene mixture can be resolved into fractions, each of which will be fairly homogeneous with respect to double bond configuration, distance between double bonds, and carbon chain length. Ozonolysis[17] of these samples will lead to unambiguous determination of double bond location, even when a mixture of terminal-section sizes is present in the sample. If the carbon chain length and middle-section size are fixed, then the end pieces have to correspond in pairs to specific structures.

Although the $AgNO_3$ HPLC columns have not been commercially available, they can be prepared from a readily obtained silica column by using the *in situ* coating procedure described by Heath and Sonnet[8]. We have found such a column to give efficient and predictable separations of dienes. We have also noted, however, that the retentions of particular standards have gradually decreased over the life of the column. For that reason we have expressed our results as relative retentions. We do not know whether the relationships among the dienes would be the same on $AgNO_3$ columns prepared by a different method or even on the same column over time. We strongly recommend the use of standards to determine the properties of individual columns at the time of the diene analysis. Although the standards cannot generally be purchased, they are easily prepared as described above, and can be purified readily by preparative GC and $AgNO_3$ HPLC.

The capillary GC column is useful to check the purity and homogeneity of the collected fractions and gives information about the different "terminal-section" isomers in each HPLC fraction. Short terminal sections cause dramatic shifts in GC retention, so compounds with these are readily detected. This fact complements the ozonolysis procedure, because the smaller aldehydes are sometimes lost in handling or become obscured by the GC solvent front.

The dienes isolated from natural sources can not be expected to have structures included in the standards in this study, but the prediction model for ECL developed from our standards should work reasonably well for any dienes of *ca.* 18–38 carbons for which the asymmetry of double bond location is not too great. Additional research will be needed to assess the effects of terminal double bonds, conjugated double bonds, and highly asymmetrical systems, which were beyond the scope of the present study.

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