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Short communication

Indications of the chemical structure of oxygen-containing constituents of volatile oils by capillary gas chromatography using one or two modified β- and α-cyclodextrin phases

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Abstract

Three modified β-cyclodextrin (CD) and two α-CD phases were used at 125 and 150°C to gas chromatograph 22 diverse oxygen-containing substances from volatile oils. Percentage changes with temperature in retention times relative to *n*-undecane were determined. Solutes showing significant decreases include acyclic geraniol and citronellol, plus three cyclic alcohols. A smaller decrease than the average 90%, or even an increase in relative retention on some phases was shown by three non-alcohol bicyclics and menthone, all saturated molecules. At 125°C, results are compared for pairs of β-CDs, the dipentyl modification proving very useful. Percentage increases in relative retentions observed against hydroxypropyl-dimethyl- or trimethyl-CDs could indicate solutes to be an alcohol (bicyclic, monocyclic or acyclic), another type of bicyclic, an aromatic or a carbonyl compound. The two methods offer good structure indications for unknown solutes using either temperature changes on one CD phase, or on two phases at one temperature.

Keywords: Oils; Stationary phases, GC; Cyclodextrins

1. Introduction

The author is in the course of a series of studies to see if retention behaviour on modified cyclodextrin (CD) stationary phases can indicate the chemical nature of diverse unknown solutes, such as those found in volatile oils, by gas chromatography. Chromatography is now a classical separation and identification technique (by R_F values and modified retention times), but it can also provide biomedical information (by R_M values [1,2]), and physical parameters such as the hydrophile–lipophile balance of surfactants by inverse gas chromatography [3]. The present work attempts to fill the "chemical gap" in scientific information available from chromatographic processes, an interest of this author since 1970 [4]. We first used three columns packed with conventional phases over the temperature range 160–220°C. Relative retention times with respect to linalol were used, and although for most solutes the values decreased with increase in temperature, some showed rises in relative retention on polar phases, viz., cineole, camphor and isomenthone. Some others gave "constant" values, including estragole, pulegone and iso-

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borneol, on polyethylene glycol 20M. Most alcohols exhibited the highest relative retentions on this phase of the three tested.

The use of capillaries at lower temperatures [5] confirmed the increases in camphor and isomenthone relative retentions with temperature increase, but not the other changes. With commercial availability of modified CDs in capillaries, a few solutes were examined previously over the temperature range 110–140°C [6]. Estragole again gave a "constant" result, whereas the relative retention of geraniol decreased considerably to about 80% of its initial value. Reviewing subsequent studies on different α-CDs [7] reveals that acyclic citronellol also showed this large decrease, whilst two bicyclics gave distinctive increases in relative retention to 112% or more of the original value. There is thus evidence for indication of the structure of some solutes by following changes in their relative retention time on a single α-CD phase. This topic was further investigated in this work, including studies with β-CDs. Only oxygenated substances were studied in this work since hydrocarbon constituents of volatile oils have extremely short retentions at 150°C. This study did not include γ-CDs, which were found to be unsatisfactory at 150°C. They give very short retention times, and consequently imprecise relative times. They do not handle some bicyclic solutes well, yielding broad peaks. In fact, few of the CD phases perform very reliably at 150°C. However, patterns of change emerged in relation to results at 150°C compared with those obtained at 25°C lower for each of two α- and three β-CDs. Results from the latter three phases were also compared with each other, in pairs, at 125°C.

Modified CD phases have received extensive gas chromatographic use for chiral resolutions since 1990 [8,9], although enantiomeric separations are not readily obtained with any given phase and racemic solute. We have studied pairs of these phases as potential indicators of structure. Using undecane as a standard, the response of the 2,6-dipentyl(per α-glucose)-β-CD was compared with that of its α-CD analogue [10], with that of the stable ester 3-propionyldipentyl-γ-CD [11] at 125°C, and with that of 2-hydroxypropyl-3,6-dimethyl-α-CD. Also compared was the last phase with its β-CD analogue [11]. Thus, a review of pairing the stable β-CD modifications had not been made, and this is done here, with some fresh work and use of previous data. The new studies involved the supposedly fully trimethyl-β-CD, which is also commercially available.

Undecane should be a better standard than linalol for relative retention times as it should show low CD affinity and is representative of the *n*-alkanes used in retention index determinations. Some results are already available using undecane at 125°C. With dipentyl α-CD, the relative retention changes are found to be similar whether using undecane or linalol as standard. Relative retention changes were seen to be indicative of retention index changes for another chirally selective phase, Chirasil-Val [12].

2. Experimental

2.1. Apparatus

A Hewlett-Packard Model 5790A gas chromatograph was used, fitted with a capillary control unit and a splitter injection port. This latter, and the flame ionization detector, were maintained at 215°C. Helium was used as the mobile phase at a flow-rate of about 0.9 ml min^{-1} and as the make-up gas to the detector. Oven temperatures of 125 and 150°C were used.

A Cyclodex-B (CDX-B) capillary was purchased from J & W Scientific (Folsom, CA, USA) and 10 m of the initial 30 m × 0.25 mm I.D. capillary were used; the film thickness of the heptakistrimethyl-β-CD was given as 0.25 μm.

Results obtained previously at 125°C [10,11] and used here were acquired with Chiraldex capillaries. B-PH is heptakis-2-hydroxypropyl-3,6-dimethyl-β-CD and was kindly donated by ASTEC (Whippany, NJ, USA). The others were purchased. B-DA is heptakis-2,6-dipentyl-β-CD and A-PH and A-DA are the hexakis-α-CD analogues. The capillary dimensions were 10 m × 0.25 mm I.D. with a film thickness given as 0.125 μm ± 10%. All capillaries were heated and

cooled at less than 10°C min^{-1} to avoid damaging the phases.

2.2. Methods

Solutes found in volatile oils from various commercial sources were used, injecting trace residues from an "emptied" microsyringe. Hold-up times, obtained by extrapolating to methane the retention times for *n*-heptane and *n*-hexane on semi-logarithmic graph paper, were deducted from the observed retention times. Relative retention times with respect to *n*-undecane were used, mostly being very reproducible.

3. Results and discussion

New relative retention times of 22 oxygen-containing solutes on five modified CD phases are given in Table 1 for Cyclodex-B and for three Chiraldex phases at 150°C. Earlier results obtained at 125°C are also given, with some new values. Inspection suggests that on α-CDs, borneol and cuminal elute more rapidly but citral and estragole relatively more slowly, and therefore alter their positions in the sequence.

Fig. 1 plots some of the data from Table 1, and illustrates the "cross-over" effect with linalol (L) on four phases—it emerges ahead of some other solutes which have slightly shorter retention at

Table 1
Relative retention times (*n*-undecane = 1.00) on various α- and β-CD phases at 125 and 150°C

Solute	Cyclodex-B[a]		Chir.B-DA[a] [11]		Chir.B-PH[a] [11]		Chir.A-PH[a] [11]		Chir.A-DA[a] [10]	
	125°C	150°C	125°C	150°C	125°C	150°C	125°C	150°C	125°C	150°C
Cineole	1.06	1.12	1.43	1.39	1.80	1.73	0.99	1.12	0.76[b]	0.88[b]
Fenchone	1.96	2.00	2.05[b]	2.06	3.14	3.05	1.87	2.03	1.16	1.29
Linalol	2.08	1.91	2.32[b]	2.06	4.09	3.55	3.31	2.95	1.57	1.56
Thujone[c]	2.28	2.26	2.81	2.55	3.59	3.42	2.22	2.23	1.24	1.36
Citronellal	2.45	2.40	2.26	2.15	3.42	3.15	3.01	2.73	1.81	1.79
Menthone[c]	2.75	2.73	2.80	2.72	3.92	3.92	3.16	3.10	1.73	1.85
Estragole	3.24	3.07	2.95[b]	2.76	5.53	5.40	5.76	5.15	3.10[b]	3.00[b]
Camphor	3.39	3.11	4.1[b]	3.80	6.98	6.08	3.24	3.35	1.86	2.04
Pulegone	4.62	4.23	3.83	3.56	6.63	6.35	6.11	5.50	2.77	2.84
4-Terpineol	3.57	3.24	5.26	4.80	7.36	6.54	5.10	4.50	2.85	2.88
Menthol	4.10	3.46	5.92	5.13	9.22	7.60	6.20	5.5	3.73	3.33
Piperitone	5.69	5.10	4.96	4.44	8.77	7.93	7.60	6.8	3.70	3.59
Citral[c]	6.00	5.10	5.27	4.57	9.24	8.08	8.50	7.20	5.93	5.02
Carvone	5.36	4.84	5.30	4.85	9.47	8.65	7.32	6.5	3.54	3.54
α-Terpineol	4.45	3.75	6.96	5.80	10.28	8.66	7.32	6.2	3.47	3.19
Citronellol	4.97	4.08	6.40	4.94	11.65	9.25	10.16	7.75	5.11	4.27
Anethole	5.65	4.97	4.9	4.26	10.09	8.85	11.16	9.3	5.97[b]	5.31[b]
Cuminal	5.79	5.17	5.9	5.37	11.98	10.8	9.54	8.4	5.05[b]	4.62[b]
Safrole	5.85	5.25	5.9[b]	5.16	13.0	11.55	12.36	10.4	6.00[b]	5.45[b]
Geraniol	5.91	4.80	6.60	5.30	15.3	11.75	13.25	9.9	6.36	5.04
Borneol	5.0	4.10	8.36	6.87	16.1	12.4	6.95	6.12	3.84	3.57
Cinnamal	8.87	7.70	6.85[b]	6.10	24.4	19.7	21.5	17.3	10.87[b]	9.62[b]

Usual adjusted retention times for undecane obtained with the above capillaries were 0.58, 0.28; 0.44, 0.26; 0.23, 0.12; 0.48, 0.20; and 0.66, 0.32 min.

[a] The suffix A or B to the Chiraldex or other phase indicates α- or β-CD, respectively. DA and PH modifications are given in Section 2.1.

[b] New value, not in Ref. [10] or [11].

[c] Main peak from impure solute.

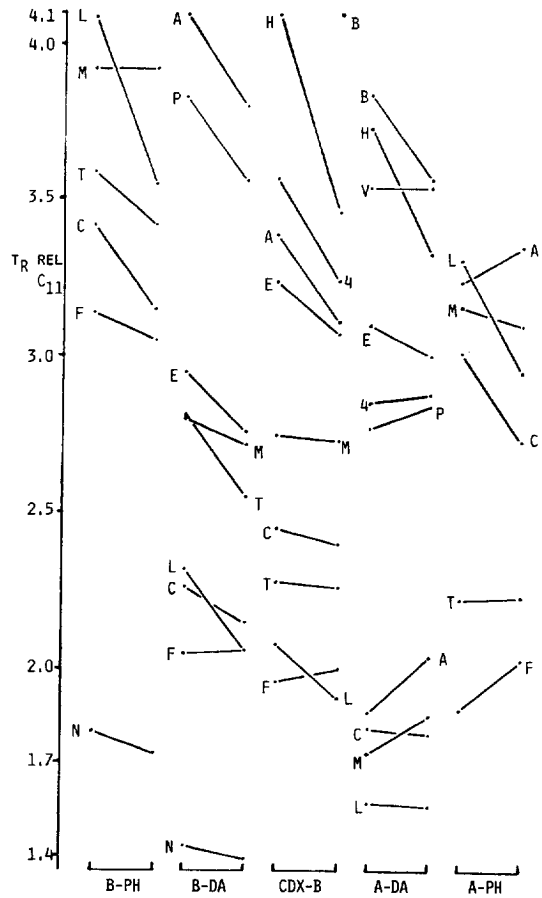

Fig. 1. Connected plots of relative retention times (*n*-undecane = 1.00) for some solutes in Table 1 at two temperatures on five modified CD capillaries. Points to the left of connected lines were obtained at 125°C and to the right at 150°C. Capillary stationary phases: CDX-B = Cyclodex-B (methyl); others are Chiraldexes: A- = $-\alpha$-CD; B- = β-CD; -DA = dipentyl; -PH = hydroxypropyl (dimethyl). Solutes: A = camphor; B = borneol; C = citronellal; E = estragole; F = fenchone; H = menthol; L = linalol; M = menthone; N = cineole; P = pulegone; T = thujone; V = carvone; 4 = 4-terpineol.

125°C, as the temperature increases. Hence it shows a greater loss of affinity than the other solutes for these CDs. This also occurs with geraniol, another acyclic substance (not shown). Some solutes clearly do not show the typical decrease in relative retention, but remain roughly constant or increase in value as the temperature increases (thujone, camphor and fenchone; T, A and F, respectively, in Fig. 1).

Chiraldex B-PH comes nearest to the typical solute elution sequence for the β-CDs used in Table 1, apart from a strong preference for linalol, which emerges after thujone, and also after menthone, at 125°C (see Fig. 1). B-DA exhibits a relatively strong retention for the terpineols; 4-terpineol emerges after piperitone and anethole on this phase, whilst α-terpineol lags behind the acyclics citronellol and geraniol and after the aromatics anethole and safrole (and cinnamal at 125°C). Cyclodex-B shows affinity for carbonyl solutes, e.g., piperitone and citral (the latter is the penultimate eluate at 125°C), and lower retentions of alcohols, e.g., borneol and geraniol (the latter is only 15th in elution sequence at 150°C, instead of almost last).

Comparing the three β-CDs on the left in Table 1, Chiraldex B-PH always exhibits the largest relative retention values, particularly with aromatics. Cyclodex-B shows smallest values of the three for about half of the solutes studied, whilst B-DA is lowest for aromatics (except for cuminal with its branched side-chain), for carbonyl acyclics and for monocyclics (pulegone and piperitone). Menthone and carvone give nearly the same relative retention times on B-DA as on Cyclodex-B. These are two keto(mono)cyclic solutes. Differences between α-CDs have been discussed previously [7]. Values on the right of Table 1 confirm that fenchone and camphor showed a distinct increase in relative retention times with increase in temperature (F and A in Fig. 1). This can also be seen for menthone (M) and some other solutes on the Chiraldex A-DA stationary phase.

In Table 2 the relative retention times are listed as percentages of initial values on changing from 125 to 150°C, so that 100% indicates no change. This response ±3% is seen for one third of the solutes on Chiraldex A-DA. Apart from this phase, from the almost perfect average solute piperitone, a typical response is a decrease to 89–90% of the relative retention time at 125°C. A few solutes on some phases, particularly A-DA, show an increase in value.

Mean values for the 22 solutes were deter-

Table 2
Changes in relative retention times (*n*-undecane = 1.00) as percentages at 150°C of values at 125°C on some modified CD phases

Solute	Chemistry[a]	A-DA	A-PH	B-DA	B-PH	Cyclodex-B	Mean
Cineole	sBet	*116*	*113*	*97*	*96*	*105*	*105*
Fenchone	sBco	*111*	*109*	*100*	*97*	*102*	*104*
Menthone[b]	sMco	*107*	98 ↓	*97*	*100*	*99*	*100*
Thujone[b]	sBco	*110*	*100*	91	*95*	*99*	*99*
Camphor	sBco	*110*	*103*	93	87	92	*97*
Citronellal	1Nco	99	91	95	92	*98* ↑	95
Pulegone	1Mco	103	90	93	*96* ↑	92	95
Estragole	1Aet	97	89	94	*98* ↑	95	95
4-Terpineol	1Mol	101	88	91	89	91	92
Carvone	2Mco	100	89	91	91	90	92
Linalol	2Nol	99	89	89	87	92	91
Piperitone	1Mco	97	89	89	90	90	91
Cuminal	sAco	91	88	91	90	89	90
Safrole	1Aet	91	84	87	89	90	88
Anethole	1Aet	92	83	87	85	88	87
α-Terpineol	1Mol	92	85	*83* ↓	84	*84*	*86*
Citral[b]	2Nco	*85* ↓	85	87	87	*85*	*86*
Menthol	sMol	89	89	87	*82*	*84*	*86*
Cinnamal	1Aco	88	*80*	89	*81*	87 ↑	*85*
Borneol	sBol	93	88 ↑	*82*	*77*	*82*	*84*
Citronellol	1Nol	*84*	*76*	*77*	*79*	*82*	*80*
Geraniol	2Nol	*79*	*75*	*80*	*77*	*81*	*78*
Mean		97	90	90	89	91	

100 means no change in value. Results in italics represent greatest decreases at the foot of the table and least decreases or increases at the top of the table (regions separated by stepped lines). Arrows show solutes "out" of region.

[a] A = aromatic; B = bicyclic; M = monocyclic; N = acyclic; co = carbonyl; et = ether; ol = alcohol; s = saturated; 1 = monoene; 2 = diene. Monoterpenoids if not aromatics.

[b] Main peak of impure solute used.

mined, then using plus or minus one standard deviation as a guide, results for each phase were grouped into three categories of "most decrease" (three to six solutes) or "least decrease or increase", leaving 11–15 solutes "in between". Solutes that show "most" and "least" decreases are placed at the bottom and top, respectively, of Table 2, which has solutes in reducing order of mean change. The greatest decrease on all five phases is shown by two flexible-molecular acyclic alcohols with terminal oxygen functions, each with three results less than 80%. Three cyclic alcohols near the bottom of Table 2, including menthol, give similar large decreases in relative times on two or three phases, as also do citral and cinnamal.

Ten solutes fall "in between", with mean decreases of 87–95%, including four aromatics, which show this response on at least four phases. They are associated with a mixed collection of monocyclic and acyclic terpenoids, all unsaturated. The "least decrease or increase" in relative retention time with increase in temperature is shown on at least four phases by three saturated bicyclics and menthone, including three carbonyl solutes. Menthone thus behaves differently from menthol, but in a similar way to the monoene acyclics citronellal and citronellol. For these four compounds, the carbonyl/alcohol feature dominates their response, rather than the rest of the solute molecule. The reverse is true for the diene acyclics, citral and geraniol, which behave similarly as if the identical non-oxygen part of their molecules is critical. Acyclic compounds with a terminal alcohol or aldehyde function used here seem to respond to the phase

being a cyclodextrin rather than its various modifications, as each of the four give a set of similar (but different) relative retention times on all five phases. The results for citral, for example, all fall within a narrow 85–87% decrease, with those for geraniol being 75–81% (Table 2). In contrast, the CD modification is important to the five bicyclics; with camphor the changes range widely over 87–110%, cineole 96–116% and borneol 77–93%. These are for solutes with three different oxygen functions.

Comparing the new CD results with the conventional phase work from 1970 [4], an increase in relative retention time with temperature is seen again for cineole on three CDs and for camphor and menthone on α-CDs. Roughly constant values of 103–92% are also found again on four CDs for estragole and pulegone. In contrast, borneol shows strong decreases on four CDs. If molecular shape is involved in this effect, CD phases should detect it more readily than conventional stationary phases.

Having previously compared pairs of a selected set of modified α-, β- and γ-CDs [11] at 125°C, the data here permitted a similar comparison of pairs of three β-CDs for structure–retention characteristics. This is presented in Table 3 as a percentage increase/decrease in order of reverse elution sequence from Cyclodex-B, and includes values from some hydrocarbon, fast-emerging solutes which had previously been studied on two β-CDs [11]. Here, zero indicates no difference in relative retention time for a solute between a pair of phases, whilst 100% shows that one phase yields double the value of the other phase.

The strong alcohol preference expected of Chiraldex B-PH is shown against Cyclodex-B values. A 125% or greater increase in relative retention times indicates an alcohol (or the aromatic cinnamal), although linalol and 4-terpineol do not increase this much. Oxygen-containing aromatics show a 70% increase or more along with some other types of solutes. Smaller increases of less than 42% indicate citronellal or a hydrocarbon, apart from two bicyclics. Such relationships are to be expected from the highest polarity cyclodextrin phase (B-PH) used here [13,14].

An alcohol-favouring response is also seen for Chiraldex B-DA (a phase with hydroxy groups remaining) values against those of Cyclodex-B, in Table 3 (B-DA/CDX-B). This combination only detects cyclic alcohols, which give increases more than 40% in relative retention times, with a bicyclic alcohol being as high as 67%. Flexible acyclic alcohol molecules are much less favoured by B-DA, giving only 9–29% increases, as do cyclic hydrocarbons. Although the Chiraldex B-DA molecules favour cyclic alcohols, they do not respond to aromatics, as do those of B-PH. The majority of aromatic solutes here favoured Cyclodex-B, by showing negative increases with respect to B-DA, like some carbonyl-containing substances, which tend to exhibit little change between the two phases.

Comparing Chiraldex B-PH results against B-DA also gives the expected alcohol response (over 80% increase), but only for the bicyclic and acyclic alcohols. This combination indicates that the concealed B-DA hydroxy group has most affinity for monocyclic alcohols, whilst the more exposed hydroxy group of B-PH favours the other types of alcohols. B-PH again shows its affinity for oxygen-containing aromatics, with some increases being greater than those for the favoured alcohols. The B-DA relative affinity for terpene hydrocabons is shown by the results with the lowest percentage increases in values, which are all under 30%, along with low-polarity ether cineole. B-DA has the lowest polarity of the three β-CDs [13,14], so this response is to be expected.

From Fig. 2, the increase of over 80% relative retention time using Chiraldex B-PH vs. B-DA could be due to a bicyclic alcohol (detected by a >60% increase with B-DA/CDX-B), an acyclic alcohol (only an 8–40% increase with B-DA/CDX-B) or an oxygen-containing aromatic (less than 8% increase or even a decrease with B-DA/CDX-B). A lower B-PH vs. B-DA increase of 30–80% could be due to a carbonyl-containing terpenoid (bicyclic if showing an 8–40% increase with B-DA/CDX-B, and probably mono- or

Table 3
Comparisons between relative retention times (*n*-undecane = 1.00) on pairs of modified β-CD phases at 125°C

Solute	% increase B-PH/CDX-B			% increase B-DA/CDX-B			% increase B-PH/B-DA[a]		
	<42	42–124	>124	<8	8–40	>40	<30	30–80	>80
Cinnamal			*175*	−22					251
Citral[b]		54		−12				75	
Geraniol			159		12				132
Safrole		122		−13					155
Cuminal		107		2					103
Piperitone		54		−13				77	
Anethole		79		−13					106
Carvone		77		−1				79	
Borneol			222			67			93
Citronellol			134		29				82
Pulegone		43		−17				73	
α-Terpineol			131			56		48	
Menthol			125			44		56	
4-Terpineol		*106*				47		40	
Camphor		106			18			74	
Estragole		71		−7					84
Methone[b]		43		2				40	
Citronellal	*40*			−8				51	
Thujone[b]		57			18			33	
Linalol		*97*			9				81
Fenchone		60		*3*				55	
γ-Terpinene[c]	30				11		17		
Cineole		70			35		*26*		
p-Cymene[c]	31			−1				*33*	
Limonene[c]	32				21		9		
α-Terpinene[c]	30			*5*			24		
3-Carene[c]	40				29		9		
Camphene[c]		*72*			34			28	
Myrcene[b,c]	14			−11				28	
α-Pinene[c]		*45*			20		21		

Percentage increases are given, in groups, so that zero means no change. Values in italic are not in their typical group. Solute sequence is in decreasing order of relative retention on Cyclodex-B (CDX-B).

[a] Chiraldex B-DA is β-dipentyl and B-PH is β-hydroxypropyl (dimethyl).

[b] Main peak of impure solute used.

[c] Using newly obtained relative retention times on Cyclodex-B of 1.07 for γ-terpinene, 0.96 for *p*-cymene, 0.91 for limonene, 0.84 for α-terpinene, 0.79 for 3-carene, 0.67 for camphene, 0.65 for myrcene and 0.55 for α-pinene.

acyclic if less than this), or to a monocyclic alcohol (B-DA/CDX-B increase greater than 40%). An increase of less than 30% with B-PH vs. B-DA indicates cineole or a monoterpene hydrocarbon, which is cyclic if giving an 8–40% increase with B-DA/CDX-B.

Previously [11], the identifications resulting from a procedure similar to that in Fig. 2 stressed the overall molecular features of the possible solutes—type of cyclic or aromatic structure. This new work stresses the functional groups of the possible solutes—alcohol, carbonyl, hydrocarbon. Chiraldex B-DA is the key phase, used twice in the present and previous studies. Originally it was used with Chiraldex G-PN and A-PH [11]. Here, with B-PH and Cyclodex-B, it leaves less ambiguity, with only the carbonyl-containing mono- and acyclics being unresolved, whereas

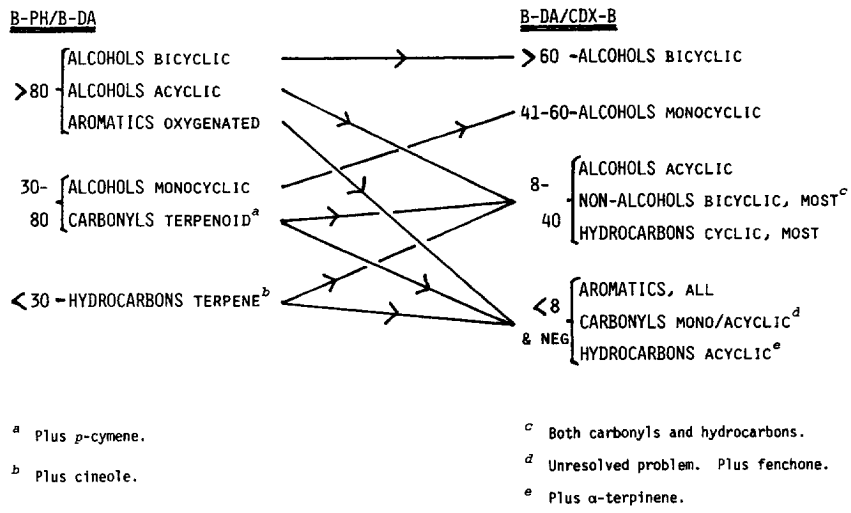

Fig. 2. Solute groupings found for various percentage increases in relative retention times (n-undecane = 1.00) on changing from Cyclodex-B (CDX-B) to other modified β-CD phases (B-PH or B-DA). These can aid in the identification of unknown peaks from volatile oils which yield ambiguous data by other means , e.g., GC–MS.

previously other acyclics were also included in this grouping.

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