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Short Communication

Use of esterified and unesterified dipentylated γ-, β- and α-cyclodextrins as gas chromatographic stationary phases to indicate the structure of monoterpenoid constituents of volatile oils

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Abstract

"Chiraldex-G-DA", unesterified dipentylated γ-cyclodextrin, yields a twenty-nine solute sequence distinctively different to other cyclodextrin modifications and to conventional stationary phases, particularly retaining bicyclic molecules. Percentage increases in relative retention times to *n*-undecane from the α-cyclodextrin corresponding phase are very indicative of solute structure: acyclics less than 60%, bicyclics over 150%, with monocyclics between. Percentage increases are also discussed from α- to γ-ester dipentylated cyclodextrins; from unesterified to esterified "G-PN" γ-phases; and between the two β-phases. With the lattermost pair, carbonyl solutes show increased values on the β-ester "B-TA" if not bicyclic, whilst alcohols increase on the unesterified phase except for some acyclics. Plots of values for some solutes on the three phases (both the esterified and unesterified) suggest that only sometimes may inclusion complexing be involved with the cyclodextrin rings —usually the largest. A solute is likely to have a different pattern on the esterified phases.

1. Introduction

Some modified cyclodextrins were introduced for the gas chromatography of enantiomers by Armstrong and Jin [1] in 1990. They used dipentylated cyclodextrins initially ("DA" meaning dialkyl), and then esterified the third glucose hydroxyl group to prepare dipentylated cyclodextrins with a 3-O-trifluoroacetyl ("TA") ester on each α-glucose unit in the rings [2]. The present author has used some of these phases since 1991 for non-enantiomeric studies of the constituents of volatile oils; on the dipentylated-hydroxy smallest ring α-cyclodextrin "Chiraldex-A-DA" [3–5], the similar intermediate-sized ring β-cyclodextrin ("B-DA") [4] and the esterified derivative "A-TA" [5].

Having noted some structurally informative graphic response patterns for terpenoids by comparing results for the dipentylated α- and β-cyclodextrins [4], it was of interest to try the largest eight-glucose (γ-) cyclodextrins for these solutes. The biggest ring phases are available as dipentylated Chiraldex-G-DA and -G-PN, where instead of the one free hydroxyl per α-glucose unit of DA, the PN phase is the 3-O-propionate. "This phase was designed to extend the scope of the (temperature-limited) Chiraldex-G-TA" phase, with some "enhanced selectivity" and "increased sensitivity" [6]. The β-cyclodextrin-

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TA phase was also studied, so that results could be compared on both sets of three dipentylated cyclodextrins, either with the remaining glucose hydroxyl present, or with this esterified.

2. Experimental

2.1. Materials

A Hewlett-Packard 5790A gas chromatograph was used, fitted with a capillary control unit, and a splitter injection port and flame ionisation detector, both set at 235°C. A Hewlett-Packard 3380A recorder/integrator was attached.

"Chiraldex" capillaries were obtained from Advanced Separation Technologies (ASTEC, Whippany, NJ, USA). A-TA and G-PN were purchased and G-DA and B-TA were kindly donated. They were all 10 m × 0.25 mm I.D. with film thickness given as 0.125 mm ± 10%. They were heated and cooled at 10°C min^{-1} to preserve the phases. Helium was the mobile phase, used at flow-rates of 0.55–0.95 ml min^{-1}, and also as the "makeup" gas to the detector.

Monoterpenoid solutes used were from various commercial sources.

2.2. Methods

Monoterpenoid solutes were injected as trace residues from a microsyringe which had been filled and "emptied". A few aromatic constituents found in some volatile oils were used for comparison. Main peaks were used if solutes were found to be impure. Holdup times were deducted, obtained by extrapolating to methane the retention times for *n*-heptane and *n*-hexane plotted on semi-logarithmic graph paper.

3. Results and discussion

Average results are presented in Table 1 and in Figs. 1 and 2, involving comparison with previous reports [4,7,8]. Ranges of values are quoted on Chiraldex-G-DA, being dose-dependent, in column 3 of Table 1. Berthod *et al.* [9] noted a group of solutes which "should not tolerate overloading . . . (as these) compounds or one of their enantiomers may be forming a dominant enantioselective inclusion complex. The large entropy decrease of the group . . . may be due to the loss of degrees of freedom for the compound included in the (cyclodextrin) cavity". Camphor gives unsharp peaks below 170°C; and the similarly bicyclic borneols emerge even more slowly, without giving good peaks, even at 190°C. The bicyclic sesquiterpene hydrocarbon caryophyllene also gives broad peaks. Relative retention times are used, having previously [5] proved more valuable than retention indices. Six solutes, citral to cuminal in the table, emerge in close sequence, and show ranges of values which overlap. Noteworthy features, which are special for Chiraldex-G-DA, are that (i) cineole is retained more strongly than linalol and citronellal, (ii) fenchone is retained after estragole and menthone, as well as the previous two solutes, (iii) menthol emerges after citral and pulegone and (iv) camphor is more strongly retained than all the other solutes (except borneols) in the table —yet on conventional phases, it would be no later than sixth in sequence after linalol (see column 16). The whole solute sequence on Chiraldex-G-DA is distinctive, compared to the other phases in Table 1, in showing relatively less retention of acyclic aldehydes citral and citronellal, and of aromatics estragole and *p*-cymene. Stronger retention is apparent for bicyclics, and monocyclic monoene ketones piperitone and pulegone.

The percentage increase in relative retention times on changing to the γ (G) from the α-dipentylated (A-DA) *hydroxy*cyclodextrin (Table 1, column 4) at 150°C seems spectacularly indicative of terpenoid structure. Acyclics (N) examined all show less than 60% increase with citral being as low as 13%. The monocyclics (M) at 150°C have increases of 65–91%; but the bicyclics (B) show much greater values, 157% and more. At 125°C bicyclic cineole also has a much higher relative retention value on the G-phase than limonene or other hydrocarbons, including γ-terpinene (see Table 1, column 6). Also the other rigid bicyclics, the pinenes and

camphene, emerge after γ-terpinene —they come first from conventional phases (see column 16 in Table 1). The bicyclic 3-carene, which is not rigid as it has a three-carbon side chain that can rotate, still shows a three figure percentage increase in relative retention times going to the G phase from A-DA (column 8). This is less than, but like other bicyclics (B) at 125°C (column 7). The two oxygenated aromatics show increases of only 20% or less at 150°C (cuminal and estragole, in column 4, Table 1).

In Fig. 1, a number of solutes show a virtual linear increase in relative retention time on changing from α- to β- to γ-*hydroxy*dipentylated cyclodextrins. Thus so for thujone, linalol, citronellal, menthone, menthol, carvone and citronellol (and the borneols?) on the unesterified phases (T, L, A, K, –, V, O, B, respectively in Fig. 1). In contrast, the upward angled plots of fenchone, pulegone, citral, geraniol, camphor (and piperitone?) reveal evidence of special interaction with the γ-phase, as their slope increases sharply from β-cyclodextrin values (F, P, C, G, H –, respectively). The manufacturers of these phases say that "inclusion complexing . . . is the basic driving mechanism" [6] for the dipentylhydroxycyclodextrins —not, it would appear, for monoterpenoids by the α-rings except possibly for citral and geraniol which are flexible acyclics and have higher values on this phase than on the larger B-DA rings. Fenchone, pulegone and camphor have rigid molecules, which could facilitate interaction with the γ-rings whereas thujone, menthone and menthol possess a rotatable three-carbon side chain (like piperitone!) and do not. The pattern in Fig. 1 for acylic citral (and geraniol?) suggests that the β-phase cavities may reject it, unlike citronellol (C, G, O, respectively); whilst this intermediate size ring phase could accept α-terpineol (and 4-terpineol?) equally as readily as the larger γ-ring phase (α, 4 in Fig. 1). Although monocyclic, α-terpineol is distinctive in having its oxygen "concealed" within the rotatable side chain. α-Terpineol and menthol enantiomers were separated by Kobor and Schomburg [10] on both the α- and β-rings (but not the γ-) of a different unesterified cyclodextrin (dimethyl, tertiary-butyldimethylsilyl) derivative. Their β modification gave highest α value separation for α-terpineols and thus agrees with the observation here, except that my unesterified γ-cyclodextrin is just as useful; but not the α-ring. My results for menthol indicate no favoured ring-size (see Fig. 2, M) and Kobor and Schomburg agreed by finding the same α-values on both the α- and β-cyclodextrins. They only obtained enantiomeric separation of linalols using their β-phase.

The γ-cyclodextrin with an *esterified* hydroxyl along with the dipentyl substitution (G-PN) shows some of the features seen above, but not all in Table 1, column 9. The dose-dependence is not a factor as it is for G-DA. Considering the solute elution sequence, citral and citronellal are relatively more strongly retained (acyclic aldehydes); but camphor, menthol, fenchone, thujone, cineole (mostly bicyclics) and some hydrocarbons less so than on G-DA. The change from the α-(A-TA) to γ-(G-PN) esterified cyclodextrins at 125°C (column 10) sees a three-figure percentage increase for oxygenated bicyclics other than cineole. This, and some hydrocarbon bicyclics still increase by 83% or more; but so do monocyclics piperitone and pulegone. Increases of less than 40% are typical of terminally oxygenated acyclics (*i.e.* not acyclics linalol and myrcene) particularly citral, which is thus distinctive on both esterified and non-esterified γ-cyclodextrin modifications. Fast-emerging solutes examined at 125°C have lower relative retention times on the esterified γ-cyclodextrin (PN) than on the corresponding hydroxyl DA phase (columns 6 and 9). The two α-cyclodextrin phases show closely similar values (columns 8 and 11).

Fig. 2, for α-, β- and γ-*esterified* dipentylated cyclodextrins shows similarities and differences to Fig. 1. As before, a number of solutes show a virtual linear increase in relative retention times. Menthol and citronellol again do this (but not the other solutes mentioned before) and also fenchone, α-terpineol and some more (M, O, F, α, respectively). There are upwardly angled plots, as before, now for camphor and the borneols (but not the others previously listed) and for some faster emerging bicyclics like

Table 1
Relative retention times (*n*-undecane = 1.00) on modified dipentyl cyclodextrin capillaries at two temperatures

Solute	Type[a]	Column No. in this table					
		3: Chiraldex-G-DA at 150°C	4: % increase ← α- to γ-CD	5: Chiraldex-A-DA at 150°C [4]	6: Chiraldex-G-DA at 125°C	7: % increase ← α- to γ-CD	8: Chiraldex-A-DA at 125°C [4]
Borneol	B	>12 at 170°C		*3.57*[b]			*3.84*
Isoborneol	B			*3.33*			*3.45*
Camphor	B	9.00 (±0.07)	341	*2.04*			*1.86*
Geraniol	N	7.15 (±0.18)	42	5.04			6.36
Piperitone	M	6.85 (±0.18)	91	*3.59*			*3.70*
Cuminal	A	6.00 (±0.11)	20	5.01[c]			5.09[c]
Citronellol	N	5.90 (±0.27)	38	4.27			5.11
Carvone	M	5.85 (±0.22)	65	3.54			3.54
Menthol	M	5.80 (±0.23)	74	3.33			↑ *3.73*
α-Terpineol	M	5.75 (±0.32)	80	3.19			3.47
Citral	N	5.70 (±0.20)	13	↑ *5.02*			↑ *5.93*
Pulegone	M	5.35 (±0.12)	88	*2.84*			2.77
4-Terpineol	M	4.95 (±0.25)	72	2.88			2.85
Fenchone	B	4.00 (±0.13)	210	*1.29*			*1.16*
Estragole	A	3.55 (±0.09)	12	↑ *3.18*[c]			↑ *3.19*[c]
Thujone	B	3.50 (±0.17)	157	*1.36*	3.76	203	*1.24*
Menthone	M	3.35 (±0.10)	81	1.85			1.73
Cineole	B	2.80 (±0.10)			2.95 (±0.16)	288	*0.76*
Linalol	N	2.40	54	1.56	2.72	73	1.57
Citronellal	N	2.40	34	↑ *1.79*	2.60 (±0.09)	44	↑ *1.81*
β-Pinene	B				1.84	268	*0.50*
Camphene	B				1.75	349	*0.39*
γ-Terpinene	M				1.27	69	0.75
3-Carene	B				1.27	115	*0.59*
Limonene	M				1.22	85	0.66
α-Pinene	B				1.12	203	*0.37*
p-Cymene	A				1.10	67	0.66
α-Terpinene	M				0.97	73	0.56
Myrcene	N				0.74	76	0.42

Using γ-(G), β-(B) or α-(A) cyclodextrins (CDs) with either one remaining hydroxyl group per α-glucose unit (-DA) or else esterified with trifluoracetic (-TA) or propionic (-PN) acid. Average values, with range for G-DA phase.
[a] A = Aromatic; B = bicyclic; M = monocyclic; N = acyclic.
[b] Values in italics are not in the sequence for Chiraldex-G-DA (lower, unless with an upward pointing arrow).
[c] Estimated from ref. 5.
[d] Direction of increase shown by arrow.

cineole, camphene and β-pinene which may be due to inclusion complexing with the γ-rings (H, B, N, E, –, respectively, in Fig. 2). A new feature is the large number of solutes on the β-esterified phase (B-TA) showing values almost as large as on the corresponding γ-cyclodextrin; hydrocarbons like α-terpinene and myrcene; linalol, menthone, pulegone and piperitone (R, Y, L, K, P, –, respectively, in Fig. 2). Several solutes are distinctive in exhibiting highest relative retention values preferentially for the β-esterified cyclodextrin (see Table 1, columns 9,

Column No. in this table							
9: Chiraldex-G-PN at 125°C	10: % increase ← α- to γ-CD	11: Chiraldex-A-TA at 125°C	12: Chiraldex-B-TA at 125°C	13: % increase[d] β-TA/DA-CD	14: Chiraldex-B-DA at 125°C [4]	15: % increase γ-PN to DA	16: Elution sequence [7,8] on methyl-polysiloxane
10.46	189	*3.62*	*4.68*	79→	8.36		18
9.78	187	*3.41*	*4.22*	84→	7.78		17
6.04	208	*1.96*	*3.19*	26→	*4.01*		14
7.69	36	5.66	7.13	← 8	6.60		28
7.17	99	*3.60*	*6.54*	←32	*4.96*		27
6.55	34	4.88	7.13				24
6.54	38	4.74	*5.81*	10→	6.40		23
5.98	74	3.43	6.34	←20	5.30		25
5.54	54	*3.59*	*4.45*	33→	↑ *5.92*		19
5.75	75	3.29	4.87	43→	↑ *6.96*		22
↑ *6.33*	5	↑ *6.03*	↑ *8.25*	←56	5.27		29
5.04	85	*2.73*	↑ *4.90*	←28	*3.83*		26
4.39	58	2.77	3.80	38→	5.26		20
2.59	123	*1.16*	*1.91*	6→	*2.02*		11
3.93	39	↑ *2.82*	3.78				21
2.85	126	*1.26*	*2.54*	6→	*2.69*	32	13
3.12	81	1.72	2.90	← 4	2.80		16
1.39	83	*0.76*	*0.98*			112	8
2.42	68	1.44	2.30	← 2	2.26	12	12
↑ *2.46*	39	↑ *1.77*	↑ *2.74*	←21	2.26	6	15
0.95	83	*0.52*	*0.69*			94	3
0.82	95	*0.42*	*0.56*			113	2
1.14	54	0.74	1.09			11	10
0.91	54	*0.59*	*0.82*			40	5
0.99	48	0.67	0.94			23	9
0.64	73	*0.37*	*0.48*			75	1
↑ *1.04*	53	↑ *0.68*	↑ *1.03*			6	7
0.87	55	0.56	0.86			11	6
0.67	56	0.43	0.63			10	4

11, 12) —citronellal, carvone, cuminal and citral (A, V, –, C, respectively) which have the common feature of a carbonyl-group that may facilitate inclusion in these medium-sized rings. Some other ketones exhibit a tendency towards this, but not all (not fenchone, nor camphor, which may have this effect counteracted by their bicyclic structures promoting enhanced γ-ring retention). The previously observed superiority of their β-cyclodextrin modification by Kobor and Schomburg [10] is seen here on the esterified phase with carbonyl-terpenoids; but not with alcoholic terpenoids, as they found. Their explanation for hydrocarbons was that their α-cyclodextrin "is too small to be entered by the pinene (and camphene) molecules whereas the cavity of (their γ-phase) is too big". No such preference was detected here for camphene (E) or other hydrocarbons (R, Y in Fig. 2). However, interactions with solutes must be influenced by the chemical modifications made to each set of three cyclodextrin phases. Andrews *et al.* [11] considered "with the larger γ cavity (of the trifluoroacetyl ester) all isomers (of naphthalene)

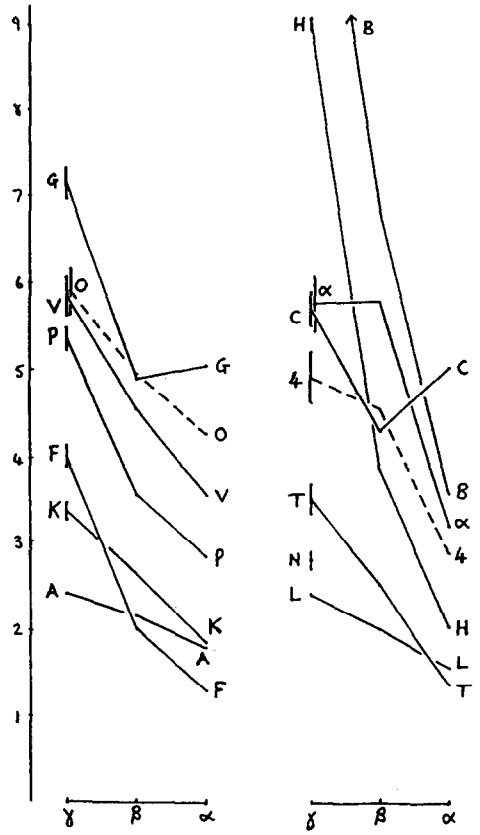

Fig. 1. Linked plots of average relative retention times (*n*-undecane = 1.00) (*y* axis) of some solutes on γ-, β- and α-hydroxy dipentylated cyclodextrins (Chiraldex DA phases) at 150°C. Ranges of results on the γ-phase shown by vertical lines. A = Citronellal; B = borneol; C = citral; F = fenchone; G = geraniol; H = camphor; K = menthone; L = linalol; N = cineole; O = citronellol; P = pulegone; T = thujone; V = carvone; α = α-terpineol; 4 = 4-terpineol. β-phase values come from ref. 4 and are not in Table 1.

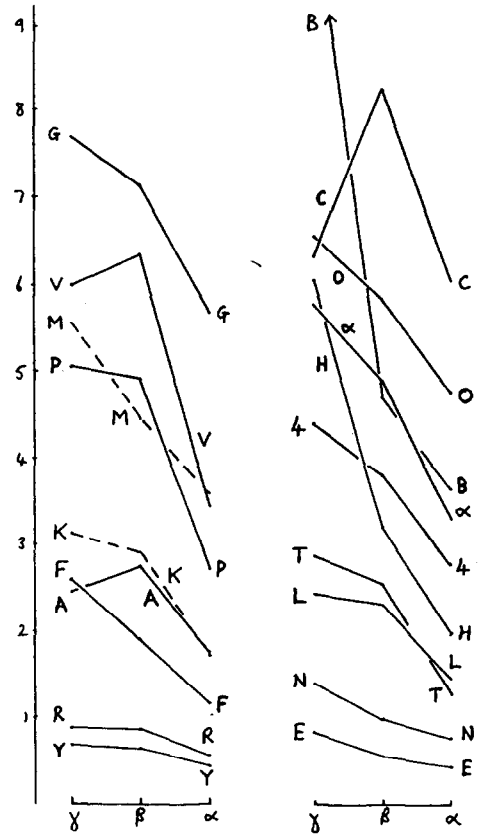

Fig. 2. Linked plots of average relative retention times (*n*-undecane = 1.00) (*y* axis) of some solutes on γ-(G-PN), β- and α-(TA) esterified dipentylated cyclodextrins (Chiraldex phases) at 125°C. Solute abbreviations as for Fig. 1 plus E = camphene; M = menthol; R = α-terpinene; Y = myrcene.

are equally able to fit into the cavity . . . However with the smaller β cavity (of permethylated, unesterified cyclodextrin!) some isomers are sterically hindered from fitting".

Bicyclic structure can also be detected by considering the percentage increase in relative retention time on changing γ-cyclodextrin modifications from Chiraldex-G-PN (column 9) to G-DA (column 6) in table 1, see column 15. At 125°C, considering the faster-emerging solutes, rigid-molecule bicyclics (four) show 75% or more increase (camphene and cineole being over 110%), greater than 3-carene which has the rotable side-chain. This solute still shows 40% increase, unlike the acyclic and monocyclics, which range from just 6 to 23%. By my *c* ratio method [12] the G-PN phase rates as more polar than G-DA (0.40 at 125°C against 0.26 at 170°C, respectively —"low" polarity values).

Considering the changes between the two β-cyclodextrin phases (Table 1, column 13) it is apparent that the increases are not all in one direction as previously found. Percentage increases from the ester (B-TA) to the alcohol (B-DA) phase of 84–10% are shown by alcohol solutes which should obviously have affinity for DA (apart from two acyclic alcohols); and by bicyclic ketones which increase by 26–6%. The

reverse increases, DA to TA, of 56–4% are given by non-bicyclic carbonyl-containing solutes. The *c* ratios of these intermediate size cyclodextrins confirm the ester phase as the more polar (0.61 for B-TA *versus* 0.28 for B-DA at 125°C. These "low" ratings are supported by considering the elution sequence of three of McReynolds' probe solutes, which is butanol, pyridine, then octyne [13].

In summary, for dipentylated cylcodextrin phases:

(i) The γ-phases can indicate molecular shape. They show high retention of many bicyclic monoterpenoids. There may also be delay-causing interaction with the acyclic dienes geraniol and citral. The esterified phase (G-PN) gives sharper, more reliable peaks, which are not dose-dependent as are those from the alcohol phase (G-DA).

Compared to the corresponding α-phase, the hydroxycyclodextrin (DA) gives over 100% increase in relative retention times to undecane for bicyclics, with some increases over 340%. In contrast, acyclics increase less than 55%, with citral only by 13%. Monocyclics fall in between, less than 100% increase.

(ii) The β-phases can indicate functional groups. The hydroxy phase (B-DA) shows equal relative affinity for α-terpineol to the corresponding γ-phase, and equally for geraniol to the α-phase —probably alcoholic responses. Citral is not favoured. The ester phase (B-TA) shows relatively higher affinity than the corresponding larger or smaller cyclodextrins for citral, carvone, citronellal (and cuminal) —probably carbonyl responses.

Comparing relative retention times on the ester with the alcoholic phase, carbonyl compounds show an increase unless bicyclic. Alcohols give a decrease unless possibly acyclic.

Acknowledgement

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References

[1] D.W. Armstrong and H.L. Jin, *J. Chromatogr.*, 502 (1990) 154.
[2] W-Y. Li, H.L. Jin and D.W. Armstrong, *J. Chromatogr.*, 509 (1990) 303.
[3] T.J. Betts, *J. Chromatogr.*, 606 (1992) 281.
[4] T.J. Betts, *J. Chromatogr.*, 639 (1993) 366.
[5] T.J. Betts, *J. Chromatogr. A*, 653 (1993) 167.
[6] *Chiraldex Capillary GC Columns*, ASTEC, Whippany, NJ, 1993.
[7] N.W. Davies, *J. Chromatogr.*, 503 (1990) 1.
[8] T. Shibamoto, in P. Sandra and C. Bicchi (Editors), *Capillary Gas Chromatography in Essential Oil Analysis*, Hüthig, New York, 1987, p. 270.
[9] A. Berthod, W. Li and D.W. Armstrong, *Anal. Chem.*, 64 (1992) 873.
[10] F. Kobor and G. Schomburg, *J. High Resolut. Chromatogr.*, 16 (1993) 693.
[11] A.R.J. Andrews, Z. Wu and A. Zlatkis, *Chromatographia*, 34 (1992) 163.
[12] T.J. Betts, *J. Chromatogr.*, 628 (1993) 138.
[13] T.J. Betts, *J. Chromatogr.*, 354 (1986) 1.