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Short communication

Use of a trio of modified cyclodextrin gas chromatographic phases to provide structural information on some constituents of volatile oils

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Abstract

A trio of modified cyclodextrin (CD) gas chromatographic phases has been selected to provide structural information on some of the diverse constituents of volatile oils. Trifluoroacetyl-CDs were found to be unstable phases, which probably hydrolyse during use. A stable ester phase chosen is the propionyl-dipentyl-CD, used with an unesterified dipentyl-CD and a hydroxypropyl-dimethyl-CD, picking examples from the three CD ring sizes. They are used in three comparative pairs, when percentage increases in retention times relative to *n*-undecane clearly indicate solute structure such as bicyclic monoterpene, oxygenated aromatic, or non-carbonyl monocyclic. An acyclic monoterpene may not be distinguishable from certain carbonyl monocyclics.

1. Introduction

Various modified cyclodextrin (CD) gas-chromatographic phases have previously been used to study how comparative results might indicate the chemical structure of some solutes found in volatile oils [1–4] and hence be of use for unknowns. These may be cyclic or acyclic terpenes, or aromatics. In continuing this work with some hydroxypropyl-(dimethyl)-CDs, when new results were compared with those previously obtained with some esterified-(dipentyl)-CDs, anomalies became apparent. These are reported below. In attempting to select an optimum trio of modified CD phases to obtain structural information, a stable ester-CD is required, along with two other different modifications using the three CD-ring sizes (α , β and γ , with six, seven and eight cyclic α -glucose units, respectively).

The value of such a trio is reported here. The introduction of such CDs as chirally selective phases has been described previously [2], although no work of this type was involved here.

2. Experimental

2.1. Apparatus

A Hewlett-Packard 5790A gas chromatograph was used, fitted with a capillary control unit and a splitter injection port. The latter, and the flame ionisation detector, were both operated at 215°C.

The various “Chiraldex” modified CD capillaries were obtained from Advanced Separation Technologies (Whippany, NJ, USA) and were all 10 m \times 0.25 mm I.D. with film thickness of

0.125 $\mu\text{m} \pm 10\%$. Prefixes A, B and G refer to α , β and γ -CD, respectively. Suffix PH refers to 2-(*S*)-hydroxypropyl-dimethyl-CDs, DA refers to dipentyl-(3-hydroxyl remaining)-CDs, TA refers to dipentyl-3-trifluoroacetyl-CDs and PN to dipentyl-3-propionyl-CD, which is only available as G-PN. B-PH and B-TA capillaries used here were kindly donated. The others were purchased. All were used at 125°C.

Helium was used as the mobile phase at a flow-rate of ca. 0.9 ml min⁻¹, except for B-DA where a faster rate of ca. 1.5 ml min⁻¹ was used to produce narrower peaks from some solutes.

2.2. Methods

The 31 solutes studied are given in Table 1. They represent some of the various constituents found in natural volatile oils. Trace residues from an “emptied” syringe were injected. For the larger ring size CDs, unlike conventional phases, excessive amounts normally cause a typical reduction in retention time. Hold-up times, obtained by extrapolating to the retention times of *n*-heptane and *n*-hexane that of methane on semi-logarithmic graph paper, were deducted from observed retention times. Retention times relative to *n*-undecane were used, and percentage increases in these values on changing CD-phases.

3. Results and discussion

New observations (averages of two or more) at 125°C are reported in Table 1 from Chiraldexes B-PH, A-PH and, in part, from B-DA (columns 3, 5 and 8, respectively). They are compared in various pairs, and with G-PN values [3], in columns 4, 6, 7 and 9. Results are usually lowest on B-DA (of the three Chiraldex phases for which relative retention times are given in Table 1) and always highest on B-PH. Only one-third of the 31 solutes in Table 1 remain in the same descending order of values listed for B-PH, these being particularly non-alcoholic monocyclic monoterpenes. This implies a phase sensitivity to the various other molecular types of solutes.

Comparison of the relative retention times for Chiraldex B-PH vs. A-PH (column 4, Table 1) should indicate the influence of increasing the ring size of a hydroxypropyl-dimethyl-CD by one glucose unit. Most bicyclic solutes (not 3-carene) show a large increase of 62% or more. In contrast, four of six aromatic solutes (with low polarity) show less than 6% increase, with some having a small decrease – however, this group is “contaminated” by myrcene. Interestingly, all three monocyclic terpenols appear to fall in a narrow group with increases in relative retention times of 40–49%. Previously the B-DA and A-DA phases were compared [1]. Again, the highest increases (74–125%) were found for five bicyclic solutes, with two monocyclic terpenols also falling in this range.

When the results for B-PH were compared with a second set of observations on B-TA, it became apparent that the latter esterified phase had decomposed over a nine-month period. Values of the solute relative retention times had altered to 66–179% of those originally observed [3], with none remaining very close to its initial value. When these repeat results for B-TA were compared with values for B-DA (column 8, Table 1) they were found to be very similar (–4 to +12%), suggesting that the trifluoroacetyl (dipentyl) TA phase had hydrolysed to the hydroxy (dipentyl) phase, equivalent to B-DA. Thus values given previously in Ref. [3] only apply to a relatively new B-TA column. On checking the *c* ratio of $3(t_{\text{R}} \text{ cuminal})/4(t_{\text{R}} \text{ caryophyllene})$ [5] for B-TA, it was found to have decreased from the original 0.61 down to 0.32, which is practically identical to the *c* ratio of B-DA, itself [3]. The solute overloading responses of B-DA and (hydrolysed) B-TA were also the same—a reduced retention time for most solutes, except for some aromatics—which further confirmed the hydrolysis of B-TA. On B-DA, all aromatic solutes gave varying relative retention times which can only be expressed to one decimal, although their peaks are sharp. This phase gave repeat values deviating no more than +4% from those previously found [3], and was obviously stable.

The instability of B-TA provoked re-examina-

Table 1
Relative retention times (*n*-undecane = 1.00) on various modified cyclodextrin phases at 125°C, and percentage increases on changing from one to another. Values in italics are out of descending sequence

Solute	Type ^a	Column No.													
		3	4	5	6	7	8	9	8	9					
		Chiralindex B-PH	Increase B-PH/A-PH (%)	Chiralindex A-PH	Increase A-PH/ PN ^b (%)	Increase A-PH/G- PN ^b (%)	Increase A-PH/B-DA (%)	Chiralindex B-DA ^c [3]	Increase G-PN ^b / B-DA (%)						
		<6	6–38	39–55	>55	<0	0–35	>35	< -6	-6–10	11–61	>61	<0	0–23	>23
Cinnamal	Acco	24.4	13		21.5			100 ^b			209	6.95 ^c			55 ^b
Borneol	Bol	16.1		132	6.95	-34			-17			8.36			25
Geraniol	Nol	15.3	15		13.25			72			101	6.60		17	
Isoborneol	Bol	15.0		150	5.99	-39			-23			7.78			26
Safrrole	Act	13.0	5		12.36			61 ^b			142	5.1 ^c			51 ^b
Cuminal	Acco	11.98	26		9.54			46			62	5.9 ^c		11	
Citronellol	Nol	11.65	15		10.16			55		59		6.40		2	
α-Terpineol	Mol	10.28		40	7.32	27			5			6.96		-17	
Anethole	Act	10.09	-10		11.16			62 ^b			128	4.9 ^c			40 ^b
Carvone	Mco	9.47	29		7.32	22				38		5.30		13	
Citral ^d	Nco	9.24	9		8.50	34				61		5.27		20	
Menthol	Mol	9.22	49		6.20	12			5			5.92		-6	

Piperitone	Mco	8.77	15	44	7.60	6	53	4.96	45
4-Terpineol	Mol	7.36			5.10	16	-3	5.26	-17
Camphor	Bco	6.98	9		3.24	-46	-19	4.01	51
Pulegone	Mco	6.63			6.11	21		3.83	32
Estragole	Aet	5.53	-4		5.76		47	3.0 ^c	31
Linalol	Nol	4.09	24		3.31		37	2.26	7
Menthone	Mco	3.92	24		3.16	1		2.80	11
Thujone ^d	Bco	3.59			2.22	-22	-17	2.69	6
Citronellal	Nco	3.42	14		3.01	22		2.26	9
Fenchone	Bco	3.14			1.87	-28	-7	2.02	28
Cincole	Bct	1.80			0.99	29	-31	1.43 ^c	-3
γ -Terpinene	Mhc	1.39	8		1.29	13	8	1.19 ^c	-4
<i>p</i> -Cymene	Ahc	1.26	3		1.22	17		0.95 ^c	9
Limonene	Mhc	1.20	16		1.03	4	-6	1.10 ^c	10
Camphene	Bhc	1.15			0.54	-34	-40	0.90 ^c	-9
3-Carene ^d	Bhc	1.11	37		0.81	-11	-21	1.02 ^c	-11
α -Terpinene	Mhc	1.09	17		0.93	7	6	0.88 ^c	-1
α -Pinene	Bhc	0.80			0.44	31	-33	0.66 ^c	-3
Myrcene ^d	Nhc	0.74	4		0.71	6		0.58 ^c	16

^a Chemical type of solute. A = aromatic; B = bicyclic monoterpene; M = monocyclic; N = acyclic; co = carbonyl; et = ether; hc = hydrocarbon; ol = alcohol.

^b Previously unpublished relative retention times on ChiralDEX G+PN. Other values used are from Ref. [2]. Cinnamal 10.75; safrone 7.68; anethole 6.88.

^c Newly determined values, not in Ref. [3]. Most aromatics give variable results, and are only averaged to one decimal.

^d Impure solutes. Main peak used.

tion of the other two trifluoroacetyl (dipentyl) cyclodextrins, A-TA and G-TA. The c ratio of A-TA had also fallen, from an originally calculated 0.78 down to 0.59, a value below that of A-DA [2]. Reconsideration of earlier results revealed that 23 of 29 solutes had yielded relative retention times on A-DA which were within $\pm 5\%$ of those found on (already hydrolysed?) A-TA. The greatest difference was only $+13\%$, so that these phases were behaving very similarly and previous A-TA results should be deleted [2–4]. The c ratio of G-TA had also fallen, from an initial 0.53 down to about 0.2, below that of G-DA. On G-TA, after six months, eight of 21 solutes showed large changes in relative retention times, including increases by alcohols. This strongly suggested that the G-TA phase had also hydrolysed back to the alcohol, and confirmed that all three trifluoroacetyl-CD phases are inconsistent in behaviour and unreliable. In contrast, the propionyl ester phase G-PN had a constant c ratio of 0.40, and a dozen solutes gave values remaining at 98–104% of those determined nine months previously [4]—this ester had not hydrolysed. It was thus selected as one of a set of three modified CD phases which could provide structural information about solutes, being only available in the largest γ -CD form. To obtain maximum potential from such work, a diversity of CD-derivatives with the three ring-sizes is desirable, and B-DA and A-PH were chosen to give three pairings of optimum value.

An informative pairing of relative retention times is A-PH vs. G-PN in column 6 of Table 1. All 9 bicyclic monoterpenes, with or without oxygen, showed decreases from -11 to -46% , an exclusive set of values. Another valuable pairing is A-PH vs. B-DA (column 7), where six non-carbonyl monocyclics show very little change, and form an exclusive group of -6 to $+8\%$ increase. Bicyclics are identified again, exclusively, by considerable decreases (-40 to -7%); and oxygenated aromatics by big increases (209 – 62%), the only exception being geraniol. The final pairing possible of the three selected modified CDs is G-PN vs. B-DA, which gives no specific groupings in column 9. They all include more than one type of solute. The six

different acyclic monoterpenes are now all grouped together with 2–20% increase in relative time; however, this range also includes some other solute types.

In summary if G-PN vs. B-DA is used first to characterise an unknown solute, an increase greater than 23% could be due to an oxygenated aromatic or a polar bicyclic terpenoid, and such compounds are well distinguished by the other two CD-phase combinations. If not either of these, then the unknown should be a carbonyl-monocyclic monoterpene (See Fig. 1). If there is a negative increase (decrease) using G-PN vs. B-DA, the unknown could be a low-polarity bicyclic or non-carbonyl monocyclic monoterpene. (Monocyclic alcohols could be identified using B-PH vs. A-PH). These substances might also be differentiated by both other phase combinations although they are in adjacent groups. Finally, if the unknown gives a low increase in relative retention time—up to only 23%—it could be an acyclic substance, or possibly an aromatic or carbonyl-cyclic monoterpene. The A-PH vs. B-DA phase combination should then distinguish the aromatics (plus geraniol, unfortunately) and carbonyl bicyclics leaving the acyclics confused only by carbonyl monocyclics and *p*-cymene. An acyclic alcohol can then be confirmed using A-PH vs. G-PN. This procedure is outlined in Fig. 1, and provides good identification of aromatic solutes, and for bicyclic and non-carbonyl monocyclic monoterpenes. The method may also distinguish acyclics from carbonyl-monocyclics, although the latter were previously found to present a problem.

Examples of the use of this method are obtained by re-examining some volatile oils previously studied. Sweet fennel oil has a minor constituent just ahead of the main *trans*-anethole peak [6]. The G-PN vs. B-DA increase for this component is 35%, so that it might be an oxygenated aromatic. The A-PH vs. B-DA increase is 87%, so it clearly belongs to this chemical solute group and it was identified by a mass spectrometry (MS) as *cis*-anethole. The 39% A-PH vs. G-PN increase further complies with an aromatic structure. Dill oil has a minor constituent ahead of the main carvone peak [2].

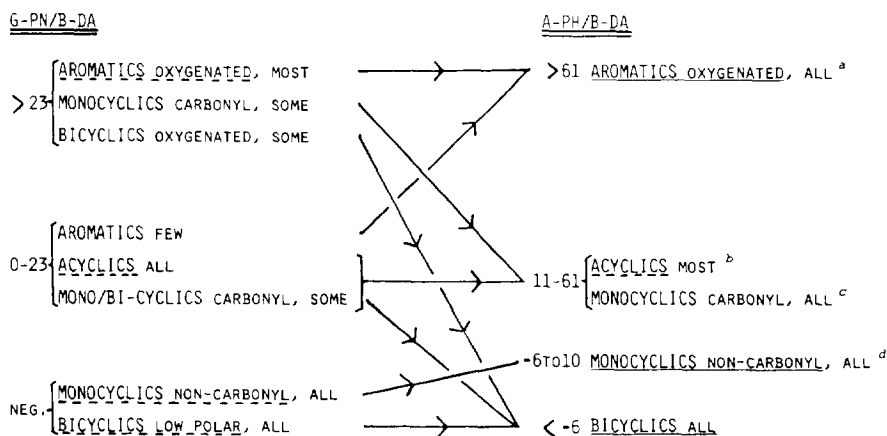


Fig. 1. Solute groupings found for various relative retention time (*n*-undecane = 1.00) percentage increases on changing from Chiraldex B-DA to the other phase indicated (G-PN or A-PH). ^aTogether with acyclic geraniol. ^bTogether with aromatic hydrocarbon *p*-cymene. Acyclic alcohols give over 35% increase A-PH/G-PN. ^cSome carbonyl monocyclics have been in a different group before. ^dMonocyclic alcohols can be detected using Chiraldex B-PH/A-PH, where they give 40–50% increase.

The G-PN vs. B-DA increase for this is 46%, so it could also be an oxygenated aromatic and cannot be acyclic. However, the A-PH vs B-DA increase is only 51% which shows that it cannot be aromatic, and must be a carbonyl monocyclic. The only 4% A-PH vs. G-PN increase agrees with this and MS identified it as dihydrocarvone. Note that it cannot be a carveol with these values. Another slightly bigger peak constitutes ca. 3% of the dill oil. G-PN vs. B-DA gave –5%, as did A-PH vs. B-DA. This indicates (just) a non-carbonyl monocyclic or possibly a low-polarity bicyclic. MS identified it as anethofuran, a bicyclic, non-carbonyl ether terpenoid. Interestingly, this substance eluted after dihydrocarvone from B-DA, unlike the results found for the other two phases. B-DA yields broader peaks for carbonyl compounds than the other phases, suggesting a special interaction.

Some other aromatic volatile oil constituents like eugenol and thymol (phenols), have excessively long retention times at 125°C. They can be studied at 150°C, and still fall in the A-PH vs. B-DA group of greater than 61% increase.

These two compounds give values of over 190% and 165%, respectively, whereas, in contrast, the bicyclic hydrocarbon sesquiterpene caryophyllene gives a decrease of more than –40%.

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