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Short Communication

Potential of three different α-cyclodextrin modifications for the gas chromatographic evaluation of constituents of volatile oils

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ABSTRACT

Twenty-two typical constituents of volatile oils were studied by gas chromatography at 110 and 140°C using capillaries of three different α-cyclodextrin modifications. The monotrifluoracetyl, dipentyl form gave the highest relative retention times against linalol for monoterpene carbonyls and hydrocarbons. It was used for the analysis of dill oil, containing such constituents, which is illustrated, with methofuran as a specific feature. The dipentyl (monohydroxy) derivative previously studied gave highest relative retention times for monoterpenols and ethers (including aromatics). The permethylated hydroxypropyl α-cyclodextrin tested as the most polar phase gave lowest values of the three modifications for most solutes except four alcohols and two others. It was otherwise non-discriminatory over structure, but gave best resolution of certain solute pairs, which emerge close together from some conventional phases.

INTRODUCTION

"Chiraldex" modified cyclodextrins in capillaries have previously been used as stationary phases for the gas chromatography of volatile oil constituents [1,2] and some oils [3]. 2,6-O-Dipentyl monohydroxy (DA) cyclodextrins gave some structurally informative response patterns for monoterpenoids such as bicyclic or acyclic molecules, when relative retention times to *n*-undecane were compared on different sized (α- and β-) modified cyclodextrins [2]. Temperature change on the α-modification also gave results suggesting the position of polar groups in the monoterpenoids. It was now of interest to compare their behaviour and that of aromatics on two other different α-cyclodextrins to see if more information could be obtained. These are available from the same commercial source in the 3-O-monotrifluoroacetyl, dipentyl (TA) and the permethylated-hydroxypropyl (PH) modifications. These cyclodextrin derivatives were originally devised to resolve enantiomeric pairs, but may offer advantages for the gas chromatographic separation of the mixtures which form some volatile oils, even though only one enantiomer usually occurs naturally. They were introduced in 1990 by Armstrong and co-workers, DA first [4], then TA which resolved carvones and various other substances [5], and PH which handled the limonene enantiomers as well [6].

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EXPERIMENTAL

Apparatus

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.

The "Chiraldex" capillaries were purchased from Advanced Separation Technologies (Whippany, NJ, USA) and were 10 m × 0.25 mm I.D. with film thickness given as 0.125 μm ± 10%. They were heated and cooled at less than 10°C min^{-1} to preserve the phases. Helium was the mobile phase, used at 0.9–2.1 ml min^{-1}, and as "makeup" gas to the detector.

The GC–MS apparatus used as an adjunct has been recorded before [3]. The capillary used with this was from J & W Scientific (Folsom, CA, USA) and was 30 m × 0.25 mm I.D. with a film thickness of 0.15 μm DB-23 (cyanopropylmethylpolysiloxane).

Materials and methods

Solutes used were from various commercial sources including Aldrich, BDH, Dragoco (Holzminden, Germany), Eastman, Fritzsche-D.O., Koch-Light, Sigma and T.C.I. (Tokyo, Japan). The dill oil was of unspecified geographic origin, from Faulding (Perth, Australia). Injections of solutes were made with the trace residue in a microsyringe which had been filled, then "emptied"; apart from dill oil, where 0.2 μl was injected. Holdup times were deducted, obtained by extrapolating to methane the retention times for *n*-heptane and *n*-hexane plotted on semilogarithmic graph paper.

RESULTS AND DISCUSSION

Results are presented in Table I, arranged in descending sequence, so far as possible. Fig. 1, where the A-PH phase is not shown, indicates some changes in relative retention times to linalol, from A-TA to A-DA. (The A prefix indicates α-cyclodextrins, modified chemically as described in the Introduction.) Only citronellol, estragole, 4-terpineol, citronellal and fenchone values fit perfectly at both the temperatures used into this arbitrary arrangement. Four solutes do not "fit" into the sequence at the same position for both temperatures, even after ignoring one result in a set of three. Only α-terpineol and citronellol have two sets of relative retentions to linalol at 110 and 140°C which are each quite similar on all three phases, with low standard deviations.

Inspection of Table I reveals that relative retention values on Chiraldex-A-PH are usually the lowest of the three phases. The exceptions to this are the solutes safrole, pulegone and the alcohols geraniol, citronellol and α-terpineol, together with borneol at 140°C. Alcohols are polar and this phase responds as the most polar of the three modified α-cyclodextrins, even though it is only of intermediate polarity, similar to OV-225. The table shows that PH favours the retention at 110°C of polar α-terpineol (in relation to the arranged sequence of solutes) but does not show affinity for non-polar caryophyllene, as is to be expected. More than half the seventeen solutes showed an unusual response of an increase in relative retention times on going to the higher temperature —not only the quicker emerging substances, but also caryophyllene, piperitone and carvone. The PH phase gave the best resolution here of the diverse solute pairs safrole/anethole, piperitone/carvone, α-terpineol/menthol and *p*-cymene/cineole, which are aromatics and terpenoids. Lemberkovics [7] records that this second terpenone pair is not resolved on polyethylene glycol (PEG) 20M, nor the lattermost pair on OV-17.

Chiraldex-A-TA gave highest values in the sets of three phase results in Table I for carbonyl-containing solutes (ketones and aldehydes) and hydrocarbons. This is displayed in Fig. 1 (in relation to the A-DA phase) where these solutes are marked "CO" and "H", respectively. A-TA particularly favours the retention of cuminal (aromatic aldehyde), caryophyllene and *p*-cymene. It is of intermediate to low polarity, apparently decreasing with temperature increase (as does A-PH). Here only the bicyclic ketones fenchone and camphor showed a distinct increase in relative retention times with temperature, but they gave this on all three Chiraldexes.

TABLE I

RELATIVE RETENTION TIMES (LINALOL = 1.0) ON THREE MODIFIED α-CYCLODEXTRIN "CHIRALDEX" CAPILLARIES "DA", "PH" AND "TA" AT TWO TEMPERATURES (°C)

Average results in best possible decreasing order. Suffix arrows indicate values out of sequence and the direction where they occur. Four solutes, named at the sides, do not fit a common sequence for both temperatures.

Solute	Chiraldex phases at 110°C			Solute	Chiraldex phases at 140°C			Solute
	PH	TA	DA		PH	TA	DA	
Geraniol	4.44	4.12	4.60[a]					
	3.84	3.68↓	4.17[a]	Safrole	3.70	3.32	3.82[a]	
	3.49	3.81↓	4.03[a]	Anethole	3.31↓	3.33	3.72[a]	
					3.62	3.16	3.64[a]	Geraniol
	2.19↓	4.03	3.77[a]	Caryophyllene	2.41↓	3.80↑	3.61[a]	
					2.95	3.72↑	3.22[a]	Cuminal
	3.42	3.31	3.66	Citronellol	2.87	2.72	3.00[b]	
Cuminal	3.00	4.46↑	3.25[a]					
	2.30	2.90	2.34↓	Piperitone	2.40	2.82↑	2.31[b]	
	2.14	2.82	2.23[b]↓	Carvone	2.25	2.69	2.26[b]	
Borneol	1.96	2.00↓	2.49					
	1.98↑	2.20	2.44	Menthol	1.90↓	2.04	2.26[a]	
	2.21↑	2.20	2.29[a]	α-Terpineol	2.14	2.04	2.18[a]	
					2.05	1.99	2.38[b]↑	Borneol
	1.81	2.18	1.74[b]↓	Pulegone	1.89	2.20↑	1.79[b]↓	
	1.71	1.89	2.03[a]	Estragole	1.77	1.84	2.04[a]	
	1.50	1.77	1.81[b]	4-Terpineol	1.58	1.78	1.83[b]	
					1.08	1.35↓	1.26[b]	Camphor
	0.89	1.43	1.16[b]	Citronellal	0.94	1.39	1.14[b]	
Camphor	0.87	1.21	1.12[b]					
	0.51	0.73	0.67[b]	Fenchone	0.64	0.89	0.79[b]	
γ-Terpinene	0.36	0.45	0.44[a]					
p-Cymene	0.34	0.42	0.38[a]					
Limonene	0.28	0.39	0.39[a]					
Cineole (1,8)	0.25	0.39	0.44[a]↑					
α-Terpinene	0.24	0.34	0.32[a]					
Polarity[c]	1.03	0.83	0.65		0.92	0.73	0.67	

[a] Literature value [1].

[b] Graphic interpolation from ref. 3.

[c] Expressed by *c* ratio corrected retention times 3(cuminal)/4(caryophyllene) [16]. Values below 0.8 indicate low polarity phases; those above this to 1.2 are of intermediate polarity. Thus the TA phase changes from minimal intermediate to the upper region of low polarity on heating from 110 to 140°C.

TA gave best resolution of the terpenoid solute pair piperitone/α-terpineol, which emerge close together from 20M [7].

Chiraldex-A-DA has shown highest values in the sets of three results for ethers ("E" in Fig. 1) and also for alcohols ("OH"), despite rating as the lowest polarity Chiraldex. A-DA is particularly unfavourable for the retention of the monocyclic ketones carvone, piperitone and pulegone —the lattermost is distinctive in giving the lowest relative retention times in both sets of three on this phase, as does piperitone at 140°C. It gave best resolution of the solute pairs borneol/α-terpineol, menthol/pulegone, γ-terpinene/*p*-cymene and cineole/limonene. Lemberkovics [7] records these four pairs being unresolved by PEG 20M.

The value of using relative retention times

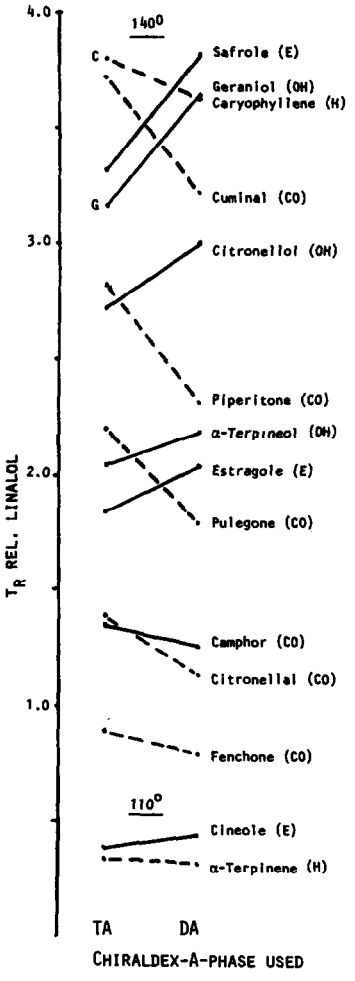

Fig. 1. Relative retention time (T_R REL.) (linalol = 1.00) changes on Chiraldex-A phases going from TA to DA at 140°C, except for plots below 0.5 which are at 110°C. CO = Carbonyl group (terpenoid ketones and aldehydes including an aromatic); E = ether (aromatic or terpenoid); H = hydrocarbon (mono- or sesquiterpene); OH = terpene alcohol.

against the polar solute linalol for this work was confirmed by determining some retention indices by graphical means. Indices for the phases Chiraldex-A-TA and -DA were similar, and only values for the PH phase were distinctly bigger, confirming that it is of higher polarity than the other two (see the bottom line of Table I). Thus no useful structural indications emerged from a consideration of retention indices, which are based on non-polar *n*-alkanes. Illustrative indices for citronellol on the phases Chiraldex-A-PH, -TA and -DA respectively are at 110°C 1500, 1360 and 1357, and at 140°C 1505, 1354 and 1360.

Chiraldexes-A-DA and -TA provide most discrimination in relation to solute structure, and so are displayed in Fig. 1. The implications are that DA should be chosen for ether-containing oils (*e.g.* anise, cajuput, eucalyptus, fennel, mace, nutmeg and sassafras) or those with alcohols (*e.g.* coriander, geranium, lavender, peppermint, rose, rosemary and teatree); whilst TA should be best for aldehyde- or ketone-containing oils (*e.g.* caraway, dill, lemongrass and spearmint) or those with hydrocarbons (*e.g.* ginger, juniper, lemon). The higher affinities of these phases for the types of solutes indicated should ensure the best results. The manufacturers of the Chiraldex capillaries record the resolution of *dextro-* and *laevo*-isomers of carvone on A-TA phase, and of 4-terpineols on A-DA, which agree with the above selectivity conclusions. "Unique selectivity has been noted on the TA series for carbonyl-containing molecules" [8]. I have reported the analysis of sweet fennel and mace oils on Chiraldex-A-DA [1], which was thus appropriately selected. Fig. 2 shows the temperature-programmed analysis of an Indian dill oil using Chiraldex-A-TA, although good isothermal results can be obtained in five minutes at 150°C. The main values obtained correspond well with average results from conventional and liquid crystal capillaries —43.6% impure "limonene", 3.1% anethofuran and 48.9% carvone. A polyethylene glycol 20M or a DB23 capillary was needed to resolve the 41.2% limonene from only 2.1% *p*-cymene, and to separate 0.4% dillapiole from other late peaks. This lattermost was not seen on methyl-polysiloxane, which also failed to resolve a trace of dihydrocarvone from anethofuran —Chiraldex-A-TA achieved both these resolutions (Fig. 2).

Anethofuran was named in 1979 by Goeckeritz *et al.* [9], and is an interesting constituent of dill fruit oil, which serves to distinguish it from caraway oil. This "tetrahydrocoumaran" derivative was detected in dill oils by two independent groups in 1977 [10,11], and found to form about 3% of American source oils a year later, where it was also referred to as a dimethyl-

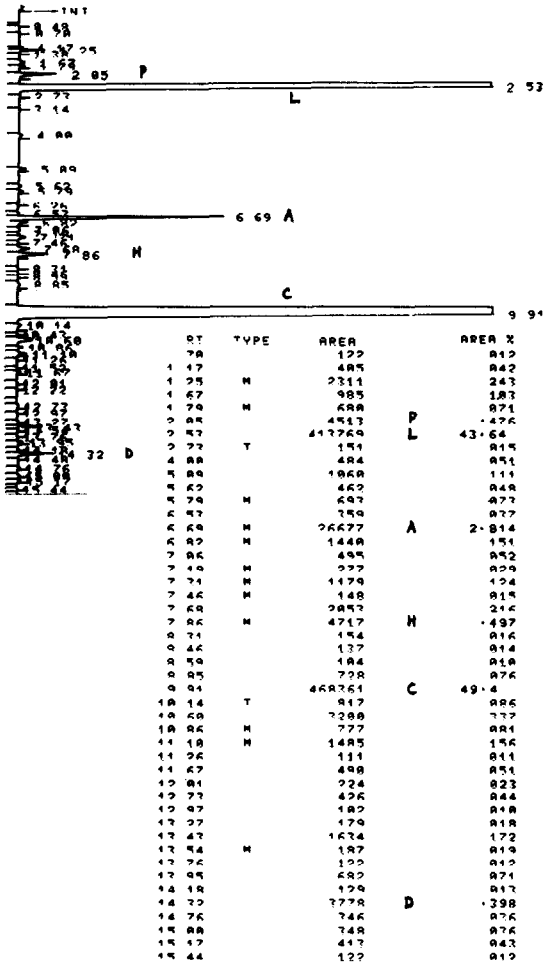

Fig. 2. Chromatogram of 0.2 μl dill oil on Chiraldex-A-TA capillary with 1.6 ml min^{-1} helium flow. Conditions 75°C initially for 1 min followed by programming at 4°C min^{-1} to 110°C (at 9.75 min), then at 10°C min^{-1} to 150°C (at 13.75 min) with concluding isothermal period. RT = retention time in min; peak TYPE = good unless merged (M) with previous one or tangent (T) skimmed baseline. Peaks: A = anethofuran; C = carvone; D = dillapiole; H = *E*-dihydrocarvone; L = limonene (including about 2% unresolved *p*-cymene); P = α-phellandrene. These named constituents form over 97% of the dill oil.

hexahydrobenzofuran [12]. This confusion was compounded in 1981 when it was recorded in dill herb as an "epoxy" (incorrect!)-menthene [13]. Although the benzofuran derivation is the preferred chemical name, anethofuran is clearly a monoterpenoid, best considered as 3-9-(furan)-oxy-*p*-menth-1-ene. It has been found to be a major component of the oil from the flowering umbels, declining as the fruits develop [14], forming 0.4–11.9% of Tasmanian dill oils [15]. I observed an anethofuran peak in each of several other samples of dill oil. Its identity was confirmed by GC–MS [principal ions *m/z* 137, 69, 109 and (trace) 152] from a DB23 capillary —a procedure also used to check other major and minor peaks. The anethofuran peak gave approximately 40% increase in relative retention times to undecane at 150°C on Chiraldex-B-DA compared to A-DA, indicating its cyclic structure [2]. On A-TA it gave almost exactly the same relative retention to linalol as on A-DA, a response not seen here for any other type of solute, and indicating a different structure to those examined in Table I, where there are no furans. The peak identified as dihydrocarvone by GC–MS gave an increase in relative retention times to linalol from 1.53 on A-DA to 1.87 on A-TA at 110°C, very appropriate for a carbonyl compound.

Two of the three α-cyclodextrin modifications used here thus appear to be of value for the analysis of selected volatile oils, and together can suggest the chemical nature of an unknown peak. Under the operating conditions used, there was no possibility of any chiral separations. Chiraldex-A-TA was best able to achieve this for carvones or 4-terpineols when programmed up to about 100°C.

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