

## TERPENOIDS

## CXVI. EVALUATION OF POLYESTERS AS STATIONARY PHASES IN GAS-LIQUID CHROMATOGRAPHY OF TERPENOIDS\*

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In our previous communications<sup>1-3</sup> we have described the use of various polyesters as stationary phases in gas-liquid chromatography of terpenoids. Initially, polyesters from  $1,\omega$ -dicarboxylic acids with diethylene glycol were studied as GLC substrates<sup>1</sup>. It was found that the number of methylene groups in the acid fragment have a profound influence, giving an arithmetical relationship in the case of retention times of hydrocarbons. When the series of polyesters from various  $1,\omega$ -glycols and some fixed acids like adipic acid were used as substrates<sup>3</sup>, a similar effect due to the spacing of the methylene groups in the glycolic fragment was also observed. The effects of molecular weights and temperature gradient have also been reported<sup>2</sup>.

From these observations it was felt that polyesters having properties of both series may find wide applications. Since the long chain acids or glycols are not always easily accessible, it was thought that some of the hydroxy acids, commercially available from indigenous sources, might be useful for the synthesis of polyesters *via* self-condensation.

Previous workers<sup>4</sup> observed that esterified oleic acid, possibly because of its unsaturation, is useful in the separation of monoterpenic hydrocarbons and oxygenated compounds. This suggested that introduction of an "unsaturated centre" in a polyester may be advantageous in the separation of various terpenoids.

The present communication deals with some of the results obtained on polyester substrates prepared by self-polymerisation of hydroxy acids, and from saturated and unsaturated glycols with saturated dicarboxylic acids.

## EXPERIMENTAL

Two varieties of polyesters have been prepared and their solvent capability on GLC columns, have been studied.

*Polyesters from self-condensation of hydroxy acids*

Polyesters crosslinked with diglycerol or pentaerythritol are fairly stable substrates and are known to give good separations<sup>5</sup>. With a view to preparing a polyester from a hydroxy acid permitting self-crosslinking, we selected aleuritic acid  $\text{HOCH}_2\text{-(CH}_2\text{)}_5\text{-CHOH-CHOH-(CH}_2\text{)}_7\text{-COOH}$ . It is available in abundance in India from an indigenous raw material shellac. It possesses interesting structural features. Besides

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the terminal primary hydroxyl group, it contains two vicinal secondary hydroxyl groups at C-9 and C-10. Because of the presence of the vicinal hydroxyl groups in the central part of the molecule, it is capable of giving a cross-linked polymer.

Along with aleuritic acid, another straight chain monohydroxy acid,  $\omega$ -hydroxystearic acid,  $\text{HOCH}_2\text{-(CH}_2\text{)}_{15}\text{-CH}_2\text{-COOH}$  has also been used for self-polymerisation. Unlike aleuritic acid, it is only capable of forming straight-chain polymers on self-condensation.

#### *Preparation of polymers*

*Aleuritic acid.* This acid when polymerised according to our previous procedure<sup>1</sup>, gave a high-melting, rubber-like solid mass, which is almost insoluble in chloroform or ethyl acetate at their boiling points and nearly so in  $\beta$ -pinene at  $100^\circ$ .

Due to the insolubility of the polymer in most of the common solvents, it was not found practicable to use it to impregnate a solid support. Hence, a novel method of preparation of the polymer on the solid support was employed.

A known weight of aleuritic acid as such, was used to impregnate a fire-brick support by using ethyl alcohol as a solvent. The solvent was evaporated off on a water bath and the whole mass dried in an oven at  $80^\circ$  for 2 h. It was then placed in a 100 ml flask and heated at about  $140\text{--}145^\circ$  for 2 h, and then under vacuum (2 mm) for a further 2 h. This material was then used in the column.

A few initial runs on this column revealed some strange retention time data. When retention times of monoterpenic hydrocarbons were being recorded, it was found that, as time elapsed, the retention times of hydrocarbons went on increasing and at the same time, the peaks of the alcoholic and ketonic compounds showed considerable tailing. The base line stability was also poor. These observations indicated that the polymerisation of aleuritic acid was incomplete under the experimental conditions described above.

A number of experiments were therefore designed to trace this phenomenon. Each time a known weight of aleuritic acid was impregnated on the fire-brick support, dried and used.

Initially the column was stabilised at  $100^\circ$  or  $120^\circ$  and the retention time data for monoterpenic hydrocarbons and a few medium boiling nonterpenic compounds were recorded. The same column was then stabilised at  $163\text{--}164^\circ$  and the retention time data were recorded for monoterpenic oxygenated compounds and sesquiterpenic hydrocarbons. After this, the same column was conditioned at  $200^\circ$  for nearly 3 h and again the retention time data were recorded at  $100^\circ$  and  $120^\circ$  as before.

Based on the observations made during the last experiments described above, a further batch of polymer was prepared as follows: 2.425 g of aleuritic acid (on the whole column filling) were heated at  $200^\circ$  for 6 h with constant stirring; loss during heating was 0.381 g; the retention time data were then recorded.

*Polymerisation of  $\omega$ -hydroxystearic acid.* 2.435 g of the acid (on the whole column filling) were heated at  $200^\circ$  for 6 h with constant stirring; loss during heating was 0.219 g.

#### RESULTS AND DISCUSSION

In the case of earlier polyesters, derived from  $1,\omega$ -dicarboxylic acids and diols,

the polyesters are essentially linear with only a little cross-linking. But in the case of aleuritic acid polymer, it could be expected that because of the presence of two vicinal —OH groups in the central part of the molecule, the resulting polymer would possess cross-linking, representing a reticular structure. This explains the very high melting point of the final polymer. Obviously, the solubilities of hydrocarbons or comparatively non-polar compounds are expected to be high and the results tabulated in Table I are in agreement with it.

TABLE I

RETENTION TIME (IN MINUTES) ON ALEURITIC ACID POLYMER

<i>Temperature 120°</i>				<i>Temperature 163–164°</i>		
<i>Compound</i>	<i>Set No. I</i>	<i>Set No. III</i>	<i>Set No. IV</i>	<i>Compound</i>	<i>Set No. II</i>	<i>Set No. V</i>
$\alpha$ -Pinene	5.91	8.83	9.18	$\alpha$ -Terpineol	5.25	8.00
$\beta$ -Pinene	9.95	13.96	14.21	Camphor	11.67	17.1
<i>p</i> -Cymene	16.75	25.73	26.83	Borneol	19.0	30.8
1,8-Cineole	17.5	25.46	25.83	Longifolene	21.75	35.0
Cyclohexanone	14.00	18.25	17.00	Humulene	30.75	Broad peak

The retention time data were recorded as follows:

Set No. I — Monoterpenic hydrocarbons and other low boiling compounds at 120°.

Set No. II — Sesquiterpenic hydrocarbons and monoterpenic oxygenated compounds at 163–165°.

Set No. III — Again for monoterpenic hydrocarbons and other low boiling compounds at 120°.

Then the column was conditioned at 200° for several hours and further readings taken.

Set No. IV — Monoterpenic hydrocarbons at 120°.

Set No. V — Sesquiterpenic hydrocarbons and monoterpenic oxygenated compounds at 163–165°.

Comparison of the retention times of Sets No. III and IV, and II and V, shows that there is not much difference in the retention times of the former set, but a large difference is observed in the latter set. This can be attributed to the fact that the polymeric mass is becoming soft at the higher temperature (163–165°), thus allowing the sample vapours to dissolve better.

In the case of  $\omega$ -hydroxy-stearic acid polymer the same steps were followed for the determination of retention time data.

In the case of both the columns, mentioned above, some decomposition of tertiary alcohols was observed. In order to retard this acid-catalysed decomposition the following procedure was adopted.

#### *Aleuritic acid polymer column filling*

About 3 g of KOH were dissolved in 300 ml of alcohol and this solution was added to the above mentioned filling. The mixture was stirred from time to time and finally allowed to stand for 15 min. The supernatant liquid was decanted off. The

remaining column filling was again washed with aqueous alcohol till the pH of the washings was nearly 8. The mass was dried in an oven and again used as column filling to record the retention time data. It was noted that the retention times were very low when compared to the previous values. This indicated that the alcoholic alkali must have leached out the stationary phase; however, at the same time the decomposition of monoterpenic oxygenated compounds had nearly vanished.

#### *$\omega$ -Hydroxy-stearic acid polymer*

This column filling was suspended in 100 ml of alcohol, and with constant stirring, titrated with 0.4 *N* aqueous KOH solution till it was slightly alkaline. The whole mass was slowly heated on a water bath to dryness and the resulting column filling heated in an air oven for several h. This column filling was again placed in the column and the retention time data were recorded. At this stage chromatograms of sesquiterpenic hydrocarbons as well as monoterpenic oxygenated compounds did not show any tailing or decomposition (Figs. 1a and b), but the retention time values were less by nearly 20%. This may be due to the fact that some of the stationary phase

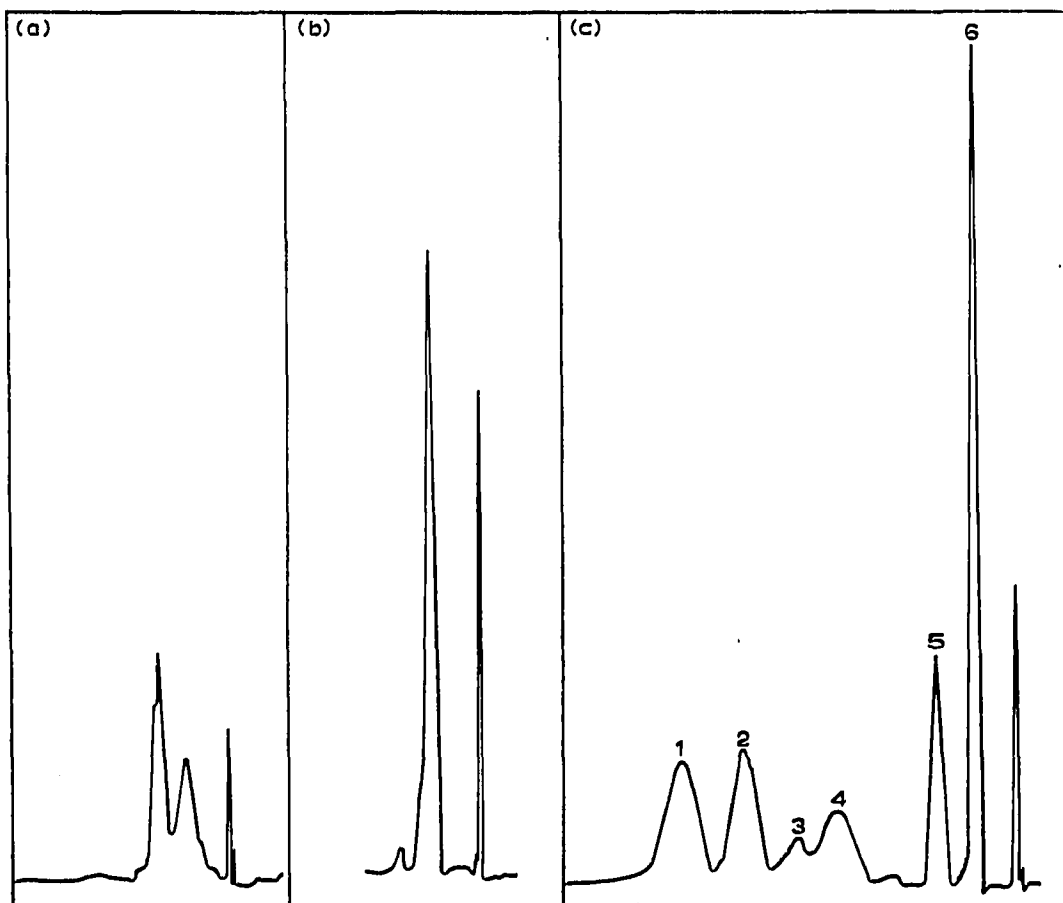


Fig. 1. Chromatogram of (a) and (b) linalool, and (c) a mixture of (1) humulene, (2) longifolene, (3) longicyclene, (4) citral, (5) linalool, and (6) methyl heptenone. Conditions: (a) temperature: 163–164°; stationary phase:  $\omega$ -hydroxy-stearic acid polymer; sample: linalool; flow rate: 4 l/h  $H_2$ ; (b) temperature: 163–164°; stationary phase:  $\omega$ -hydroxy-stearic acid polymer (neutralized); sample: linalool; flow rate: 4 l/h  $H_2$ ; (c) temperature: 180°; stationary phase:  $\omega$ -hydroxy-stearic acid polymer (neutralized); flow rate: 13.5 sec/10 ml  $H_2$ .

might have been lost during the above mentioned process; or neutralisation of the free carboxylic groups might have changed the general nature of the stationary phase to some extent. Analogous observations have been recorded by previous workers<sup>4,6</sup>.

The decomposition of monoterpenic oxygenated compounds seems to be retarded, in the present case at least, by neutralisation of the column<sup>7</sup> filling. It should be pointed out at this stage that some of the previous workers have incorporated 1% sodium bicarbonate<sup>7</sup> in the column fillings and have also used potassium or lithium salts of fatty acids as part of the stationary phase<sup>8</sup>. It is interesting to note that some of the popular support materials have been found to be slightly alkaline in nature<sup>9</sup>.

The present  $\omega$ -hydroxy-stearic acid polymer showed very high solubilities for C<sub>15</sub> hydrocarbons. A mixture of methyl heptenone, linalool, citral, longifolene, longicyclene and humulene was easily resolved as shown in Fig. 1, chromatogram (c). However, the geometrical isomers of citral are not resolved, though citral can easily be resolved into two geometrical isomers by using succinic acid-diethylene glycol polymer.

#### *Effect of unsaturation*

In order to study the effect of unsaturation a polyester was prepared from 1,4-butanediol (HOCH<sub>2</sub>-CH=CH-CH<sub>2</sub>OH) and adipic acid. For comparative evaluation, another polyester from 1,4-butanediol and adipic acid, was also synthesised. Both the columns were run under identical conditions. Relative retention time data recorded on these two columns are presented in Table II at 120° and Table III at 163°. Retention time data obtained on the two types of hydroxy acid polymers are also given. For comparison, retention time data compiled from our previous series of polyesters are also given in the same table for immediate reference.

Examination of the relative retention time data reveals some striking differences in the case of the stationary phases discussed above. Aromatic compounds have

TABLE II

RELATIVE RETENTION TIMES (WITH RESPECT TO LIMONENE) AT 120°

Compound	1,4-Butane- diol-adipic acid polyester	1,4-Butene- diol-adipic acid polyester	$\omega$ -Hydroxy- stearic acid polymer	Aleuritic acid polymer	Pentadecane- 1,15-dicarbo- xylic acid- diethylene glycol polyester	Apiezon
$\alpha$ -Pinene	0.4009	0.4140	0.4574	0.4355	0.4184	0.5052
$\beta$ -Pinene	0.6790	0.6719	0.6915	0.6755	0.640	0.5825
$\Delta^3$ -Carene	0.8091	0.7812	0.7939	0.8654	0.7703	0.8404
Ocimene	1.161	1.219	—	—	—	—
<i>p</i> -Cymene	1.416	1.50	1.151	1.196	1.144	0.9330
Limonene	9.22 $\equiv$ 1.0	6.4 $\equiv$ 1.0	9.4 $\equiv$ 1.0	12.6 $\equiv$ 1.0	12.5 $\equiv$ 1.0	19.4 $\equiv$ 1.0
Tricyclene	0.4122	0.4031	0.4468	0.3761	0.3960	0.4890
Camphene	0.5424	0.5671	0.5958	0.5859	0.5208	0.6081
1,8-Cineole	1.237	1.479	1.191	1.1630	1.1280	1.021
Cyclohexanone	1.728	2.369	0.9318	0.9506	0.8508	0.3299
Benzene	0.2332	0.2547	0.1329	0.1109	0.1312	0.0773
Toluene	0.4122	0.3751	0.2666	—	—	—
Methyl alcohol	0.1356	0.1719	0.046	—	—	—
Ethyl alcohol	0.1660	0.2266	0.065	—	—	—

TABLE III  
RELATIVE RETENTION TIMES (WITH RESPECT TO CAMPHOR) AT 163-164°

Compound	Succinic acid- diethylene glycol polyester	Azelaic acid- diethylene glycol polyester	Brassylic acid- diethylene glycol polyester	Adipic acid- 1,4-butanediol polyester	Adipic acid- 1,4-butanediol polyester	w-Hydroxy- stearic acid polymer*	Aleuritic acid polymer*
$\alpha$ -Terpineol	1.435	1.644	1.608	1.577	1.729	1.547	1.696
Linalool	0.6296	0.7568	0.7476	0.6893	0.7762	0.6373	0.7365
Borneol	—	—	—	1.496	1.646	1.418	1.798
Isoborneol	—	—	—	1.234	1.399	1.2019	1.560
Menthol	—	—	—	1.190	1.454	1.235	—
Camphor	5.40 = 1.0	9.25 = 1.0	10.3 = 1.0	11.1 = 1.0	—	5.75 = 1.0	3.95 = 1.0
Carvone	—	—	—	2.072	2.173	—	—
Dihydrocarvone	—	—	—	1.365	1.473	—	—
Longifolene	0.6685	1.329	1.971	1.0	1.338	2.28	1.63
Caryophyllene	—	—	—	—	1.427	2.423	—
Humulene	1.019	1.909	2.617	1.432	1.904	2.869	2.15
Longicyclene	—	—	—	0.7523	1.050	1.826	—
Cyclopentadecane	—	—	—	1.712	2.301	5.217	—

\* Relative retention time data were recorded on neutralised column filling.

higher relative retention values on unsaturated polyester, compared to those obtained on saturated polyester (*p*-cymene). Similarly, oxygenated compounds also show higher relative retention values on unsaturated polyester. The relative retention time of cyclohexanone is 2.37 on the unsaturated polyester, while on the saturated polyester, its relative retention value is only 1.73. In the case of the remaining terpenic hydrocarbons, there is no significant change in the relative retention values. 1,8-Cineole presents an interesting example. It has an oxygen atom in the form of "ether linkage". Its relative retention time (limonene = 1) on saturated polyester happens to be only 1.24, but rises to 1.48, when recorded on unsaturated polyester.

When we take into consideration the relative retention time data recorded at 163–165°, we find the same differences in the two cases. For example a mixture of camphor and longifolene can be easily resolved on the corresponding saturated polyester. But the same pair cannot be separated easily on the unsaturated polyester. The  $\omega$ -hydroxy-stearic acid polymer column seems to be the best for the separation of camphor and longifolene. Here the relative retention value for this pair happens to be 2.28. At the same time it is interesting to note the relative retention time value for this pair on succinic acid–diethylene glycol polymer. This value happens to be 0.669, which indicates that the order of emergence of these two compounds is reversed due to the change in the nature of the polyester. It must be noted that, on the succinic acid–diethylene glycol column, camphor and humulene would overlap. But this pair can be easily separated on  $\omega$ -hydroxy-stearic acid polymer. Therefore it seems that for overall separation of monoterpenic oxygenated compounds and C<sub>15</sub> hydrocarbons, the  $\omega$ -hydroxy-stearic acid polymer column would give a better performance.

It is interesting to observe the separation of the individual components of the monoterpenic oxygenated compounds. Separation of borneol and camphor may serve as a typical example. The relative retention time of borneol with respect to camphor, on aleuritic acid polymer, happens to be 1.80. This is the maximum value amongst all those recorded in Table III. This in turn may indicate that in the case of aleuritic acid polymer some of the –OH groups remain "free". These free groups in turn may have some strong interactions with alcohols.

Further studies are contemplated.

#### SUMMARY

Polyesters from  $\omega$ -hydroxy acids have been prepared by self polymerisation and evaluated as substrates for gas–liquid chromatography. Two model compounds, (1)  $\omega$ -hydroxy-stearic acid and (2) aleuritic acid, which are easily available from indigenous sources, were employed for polyesterification. In the case of aleuritic acid, where cross-linking is freely possible, the usual process of ester formation was not found useful. It gave a rubber-like mass, insoluble in common organic solvents. Therefore a novel method of polyester formation on the supporting material *in situ* is described for both the hydroxy acids. Such polymers, when applied to the analyses of the monoterpenic oxygenated compounds, showed acid catalysed isomerisation of the sample vapours. A successful attempt has been made to retard this process of isomerisation. These polyesters showed very high solubilities for the nonpolar sesquiterpenic hydrocarbons and can be used for the separation of monoterpenic oxygenated compounds and sesquiterpenic hydrocarbons.

Simultaneously, the effect of "unsaturation in the polyester" has also been studied by synthesising two polyesters, one from adipic acid and 1,4-butanediol and the other from adipic acid and 1,4-butenediol. The effect of "unsaturation" is found to be opposite to that which is observed in the case of long chain dicarboxylic acid diethylene glycol polyesters. The unsaturated polyester shows better solubilising powers for oxygenated compounds than for hydrocarbons.

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