GAS–LIQUID CHROMATOGRAPHY OF DERIVATIVES OF NATURALLY-OCCURRING MIXTURES OF LONG-CHAIN POLYISOPRENOID ALCOHOLS*

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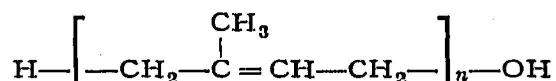
INTRODUCTION

There is present in nature a wide variety of polyisoprenoid alcohols (prenols) with the general structure:



These alcohols differ not only in the number of isoprenoid units present in the molecule, but also in the *cis-trans* nature of each of these units. Closely related compounds exist in which one or more of the isoprenoid units is saturated.

LINDGREN[1] has reported on the nature of the prenols present in the wood of *Betula verrucosa* (the silver birch). More recently[2] he has used gas–liquid chromatography to establish that the mixture isolated was of four alcohols containing six, seven, eight and nine isoprene units respectively, with the C_{35} and C_{40} compounds predominating. Using essentially the same experimental approach, the present authors have shown the presence of five polyisoprenoid alcohols containing nine, ten, eleven, twelve and thirteen isoprene units respectively in leaves of four higher plants. A mixture of alcohols isolated from *Saccharomyces cerevisiae* (baker's yeast) yielded three prenols containing fourteen, fifteen and sixteen isoprene units respectively.

EXPERIMENTAL

Chromatography

The acetates or hydrccarbons, dissolved in cyclohexane, were injected onto silanised Chromosorb W which had been coated with SE-30 (1 % w/w) and had been packed in a 4 ft. long silanised stainless-steel tube having an internal diameter of 0.125 in. A dual column F & M Model 810 gas chromatograph fitted with a flame ionization detector was used isothermally at a temperature of either 300 or 340°. The carrier gas was argon at a flow rate of 60 ml/min.

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Materials

A sample of the mixture of prenols from *Betula verrucosa* and referred to as betulaprenols, was provided by Dr. B. O. LINDGREN. The mixture of prenols from the leaves of *Aesculus hippocastanum* (the horse-chestnut) and referred to as castaprenols, was prepared by the authors[3]. The mixtures of prenols from the leaves of *Beta vulgaris* var. *crassa* (sugar beet) and of *Ficus elasticus* (rubber plant) were prepared in essentially the same manner in conjunction with Dr. J. F. PENNOCK. Mr. P. J. DUNPHY and Dr. J. F. PENNOCK provided the mixture of prenols derived from *Arum maculatum* (Lords and Ladies). The isolation of the prenols from *Saccharomyces cerevisiae* was carried out in conjunction with Drs. J. BURGOS and J. F. PENNOCK. Solanesol was provided by Hoffmann-La Roche.

A sample (10–20 μg) of solanesol or of the prenol mixtures appeared to chromatograph as a single spot on thin layers of silica gel G, using methanol in benzene (1:99, v/v) as developing solvent and staining the developed chromatogram with phosphomolybdic acid.

Acetylation and hydrogenation

Samples of solanesyl acetate and of the acetates of the prenol mixtures were prepared by mixing and leaving overnight, solutions of the alcohols (10–20 mg) in benzene (5 ml) with acetic anhydride (5 ml) and pyridine (3 drops). Ice cold 2 *N* HCl (10 ml) was then added and the acetates were extracted with ether.

Small portions of these acetates were hydrogenated at room temperature and pressure in a Towers microhydrogenation apparatus using platinum oxide as catalyst and cyclohexane as solvent. The resulting mixtures contained perhydroprenyl acetates and the corresponding saturated hydrocarbons resulting from elimination of the acetyl residue during hydrogenation. The hydrocarbons and acetates were separated by preparative thin-layer chromatography. Silica gel G was used as adsorbent and light petroleum (b.p. 40–60°) as developing solvent.

When samples of the perhydroprenyl acetates were required with minimal losses to hydrocarbon, it was found best to first hydrogenate the free prenols. The perhydroprenols were then acetylated.

Solanesyl trimethyl silyl ether

The trimethyl silyl ether of solanesol was prepared in essentially the same method as described by LUUKKAINEN *et al.*[4]. Solanesol (20 mg) was dissolved in tetrahydrofuran (2 ml) containing trimethyldichlorosilane (3 drops) as catalyst. Hexamethyl-disilazine (0.3 ml) was then added slowly, drop by drop, and the mixture allowed to stand at room temperature for 0.5 h. The precipitate produced was removed by centrifugation and the supernatant solution was taken to dryness. This material was triturated for 2 min with hexane and again centrifuged. The product in the supernatant solution was recovered by evaporating the solvent and it was dissolved in isooctane for gas chromatography.

Solanesyl trifluoroacetate

Solanesol was esterified with trifluoroacetic anhydride, using pyridine as catalyst, according to the method described by VANDENHEUVEL *et al.*[5]. The reaction was complete in only a few minutes at room temperature. Solvent (tetrahydrofuran) and excess reagent were removed by evaporation.

RESULTS AND DISCUSSION

Perhydroprenyl acetates

The chromatographic mobility of fully hydrogenated polyisoprenoid compounds is uncomplicated by problems arising from the *cis* or *trans* nature of the isoprene units. In this form one might expect the retention times of the series of perhydroisoprenologues to be related in the same way as are the retention times of a series of homologues. In fact LINDGREN[2] has produced good evidence that this does happen in the case of the perhydrohexa-, perhydrohepta-, perhydroocta- and perhydronona-prenyl acetates. Because the present authors required to investigate larger polyisoprenoid alcohols, they have run their gas chromatograph at higher temperatures than did LINDGREN. It was reassuring to find that the relationship between these perhydroprenyl acetates described by LINDGREN still held.

In Table I are recorded the retention times at 300° of the four components of the mixture of perhydrobetulaprenyl acetates. These retention times were reproducible over numerous runs on the same day but changed slightly from one day to the next. When a sample of perhydrosolanesyl acetate was chromatographed, either

TABLE I

GAS-CHROMATOGRAPHIC DATA FOR THE MIXTURE OF PERHYDROBETULAPRENYL ACETATES AND FOR PERHYDROSOLANESYL ACETATE AT 300°

Component	*Retention time (min)*	*Peak area (% of total)*
1	0.35	4.2
2	1.05	52.2
3	2.58	41.0
4	5.90	2.6
Perhydrosolanesyl acetate*	5.90	

* The perhydrobetulaprenyl acetates mixture was chromatographed both with and without added perhydrosolanesyl acetate.

with the mixture or separately but on the same day as the mixture, its retention time coincided exactly with that of the fourth peak in the chromatogram of the mixture (see Table I.) Fig. 1 shows that if the four components of the mixture are assumed to contain six, seven, eight and nine saturated isoprene residues respectively, then a plot of log retention time against the number of saturated isoprene residues gives four points, all of which fall on a straight line. This is precisely the situation described by LINDGREN[2] for lower temperatures. It is good evidence that the original mixture of prenols contained four compounds containing respectively six, seven, eight and nine isoprene residues. The C_{35} and C_{40} isoprenologues were the major components (see Table I). Mass spectrometry and nuclear magnetic resonance spectroscopy support this conclusion[2].

Most of the available evidence[3] indicated that "castaprenol" was a C_{60} prenol containing rather more *cis* than *trans* isoprene residues. However, mass spectrometry suggested that while the C_{60} prenol predominated, C_{55} and C_{65} prenols were also pre-

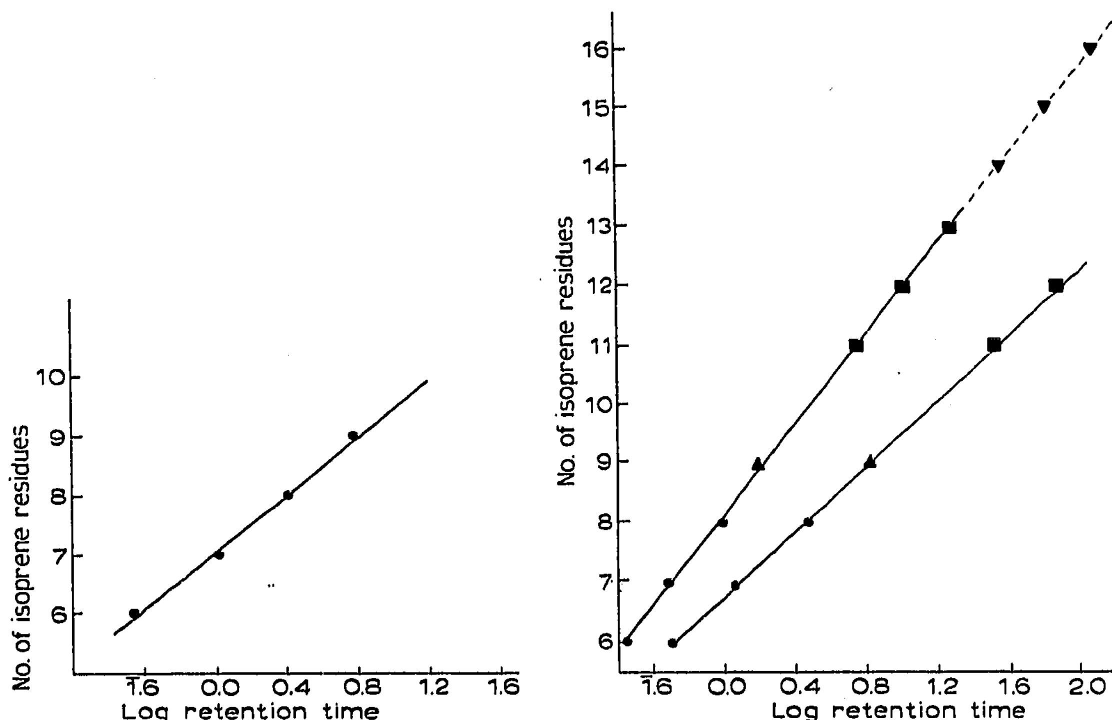


Fig. 1. Relationship between $\log_{10}$ retention time at 300° and the number of saturated isoprene residues per molecule of each component of the mixture of perhydrobetulaprenyl acetates.

Fig. 2. Relationship between $\log_{10}$ retention time at 300° (lower line) and 340° (upper line), and the number of saturated isoprene residues per molecule of each component of a mixture of perhydrobetulaprenyl acetates (—●—●—), perhydrosolanesyl acetate (—▲—), perhydrocastaprenyl acetates (—■—■—) and the perhydroprenyl acetates from *Saccharomyces cerevisiae* (--▼--▼--).

sent. As expected, the relative intensities of the three molecular ions varied with the temperature at which the mass spectrum was obtained and it was not possible to calculate the precise composition of the original mixture. Gas–liquid chromatography of the mixture of perhydrocastaprenyl acetates revealed three clear components, the middle peak of the three on the chromatogram being of largest area (see Table II). When a sample of the mixture was chromatographed together with samples of the mixture of perhydrobetulaprenyl acetates and of perhydrosolanesyl acetate (see Table II) it was clear that peak 1 corresponded in position to perhydroprenyl acetate made up of eleven isoprene residues, peak 2 to one made up of twelve isoprene residues and peak 3 to one of thirteen isoprene residues. This is made clearer in Fig. 2, in which a plot of log retention time against the number of saturated isoprene units gives points, all of which fall on a straight line at both 300 and 340°. As forecast by mass spectrometry, the major constituent of the mixture was the C_{60} isoprenologue. In some cases, when relatively large quantities of the mixture were applied to the column, a small peak corresponding to the C_{50} isoprenologue could be observed. This material accounted for less than 0.5 % of the mixture.

In Table III are collected together the chromatographic data for the perhydroacetates of the prenols derived from *Ficus elasticus*, *Beta vulgaris* and *Arum maculatum*. The retention times for the different components differed slightly from those obtained with the mixture of perhydrocastaprenyl acetates on different days.

TABLE II

GAS-CHROMATOGRAPHIC DATA FOR THE MIXTURES OF PERHYDROCASTAPRENYL ACETATES, PERHYDROBETULAPRENYL ACETATES AND PERHYDROSOLANESYL ACETATE CHROMATOGRAPHED TOGETHER AT 300 AND 340°

Component	*Retention time (min)*		*Peak area* (% of total)*
	at 300°	*at 340°*	
Betula-1**	0.48	0.29	
Betula-2**	1.28	0.47	
Betula-3**	2.86	0.82	
Perhydrosolanesyl acetate**	6.47	1.47	
Casta-1	32.2	5.48	16.2
Casta-2	69.0	10.0	81.7
Casta-3	— ***	18.2	2.1

* These figures refer to the mixture of perhydrocastaprenyl acetates chromatographed at 340°.
** The mixture of perhydrocastaprenyl acetates was chromatographed with and without adding the mixture of perhydrosolanesyl acetate and perhydrobetulaprenyl acetates. The peak for Betula-4 was hidden under that for perhydrosolanesyl acetate.
*** Insufficient sample was applied to the column for this peak to be observed. A larger sample was applied at 340°.

TABLE III

GAS-CHROMATOGRAPHIC DATA FOR THE PERHYDRO-ACETATES OF THE PRENOLS ISOLATED FROM THE LEAVES OF *Ficus elasticus* AND *Beta vulgaris* AND THE SPADICES OF *Arum maculatum* AND CHROMATOGRAPHED AT 300° AND 340°

Probable isoprenologue	*Retention time (min)*		*Peak area (% of total)**		
	at 300°	*at 340°*	*Ficus*	*Beta*	*Arum*
C_{45}	5.9– 6.0	1.40–1.62	2.1	1.3	—
C_{50}	13.6–14.6	2.63–2.82	7.4	3.3	11.5
C_{55}	29.2–31.0	5.29–5.56	61.2	51.5	36.5
C_{60}	61.8–63.1	9.7 –10.1	28.3	42.6	41.4
C_{65}	— **	17.8 –18.1	1.0	1.3	10.6

* These figures refer to those samples chromatographed at 340°.
** Insufficient sample was applied to the column for this peak to be observed. A larger sample was applied at 340°.

TABLE IV

GAS-CHROMATOGRAPHIC DATA FOR THE MIXTURE OF PERHYDROPRENYL ACETATES ISOLATED FROM *Saccharomyces cerevisiae* AND CHROMATOGRAPHED AT 340°

Component	*Retention time (min)*	*Peak area (% of total)*
1 (C_{70})	36.1	13.5
2 (C_{75})	64.8	73.4
3 (C_{80})	119.1	13.1

However, when a further sample of each was chromatographed with a sample of mixed perhydrobetulaprenyl acetates and perhydrosolanesyl acetate retention times were observed which when plotted as in Figs. 1 and 2 gave points all of which fell on a straight line. This was taken as good evidence that the components of the mixtures had chain lengths as indicated in Table III. No other accurate evidence concerning the size of these compounds is available yet but thin-layer and paper chromatographic data indicate that the figures in Table III for the major components of each mixture are probably correct.

The relevant data for the fully hydrogenated acetates of the prenols isolated from *Saccharomyces cerevisiae* are recorded in Table IV. It can be seen in Fig. 2 that the retention times of the three components are consistent with the mixture containing a C_{70}, a C_{75} and a C_{80} perhydroprenyl acetate. It is clear that the three points on Fig. 2 corresponding to these three components fall remarkably close to the line extrapolated from a plot of the results reported in Table II, bearing in mind that the samples were chromatographed on different days and that the peaks in the *Saccharomyces* chromatogram were rather broad. The major component of the mixture proved to be the C_{75} compound (see Table IV) and this was accompanied by much smaller quantities (about one eighth of the total in each case) of the C_{70} and C_{80} compounds. This conclusion is supported by mass spectrometry and it is also relevant that the mean equivalent weight of the original mixture of alcohols, as determined by the specific activity of the acetates formed from [1-^{14}C] acetic anhydride, corresponded to a C_{75} polyisoprenoid monohydric alcohol[6].

Saturated hydrocarbons

It was indicated in the Experimental section that hydrogenation of the prenyl acetates resulted in the formation of saturated hydrocarbons produced by the replacement of the acetyl residue with a hydrogen atom. In most cases a small sample of the hydrocarbon(s) was recovered by thin-layer chromatography and this was subjected to gas chromatography under the same conditions as were the perhydroprenyl acetates.

The retention times of the components of each sample of hydrocarbons are recorded in Table V. Fig. 3 illustrates that if one assumes that these hydrocarbons correspond in chain length to the prenyl acetates present in the mixture from which

TABLE V

RETENTION TIMES FOR SATURATED HYDROCARBONS DERIVED FROM PRENYL ACETATE MIXTURES AND CHROMATOGRAPHED AT 340°

Component	*Retention time (min) and source*			
	Solanesol	*Aesculus*	*Ficus*	*Beta*
1	0.78		0.75	0.74
2		1.42	1.42	1.45
3		2.80	2.81	2.82
4		5.42	5.33	5.41
5		[illegible]	—*	[illegible]

Insufficient sample was applied to the column for this peak to be observed.

they were derived, then a plot of the number of saturated isoprene residues against log retention time produces a good straight line. As expected the retention times of the hydrocarbons were considerably shorter than were those of the corresponding perhydro-acetates. The peaks on the hydrocarbon chromatograms were also sharper than those on chromatograms of the perhydroprenyl acetates when the recorder was operating under the same conditions.

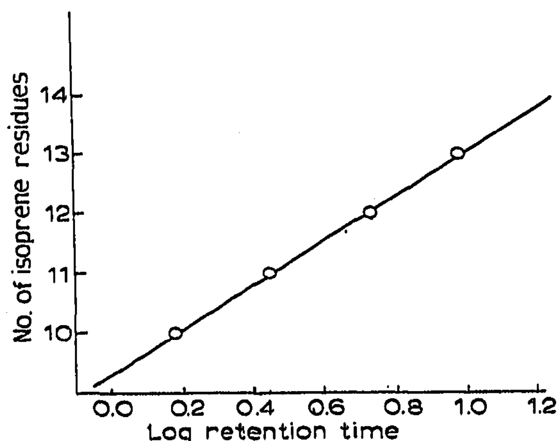
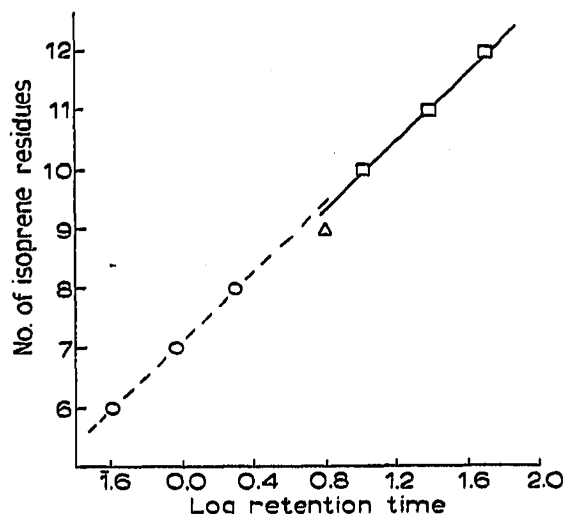


Fig. 3. Relationship between $\log_{10}$ retention time at 340° and the number of saturated isoprene residues per molecule of each component of the saturated hydrocarbons derived from the mixture of castaprenyl acetates.

The relative areas under each hydrocarbon peak of the different mixtures were essentially the same as for the corresponding perhydroprenyl acetates. The peak with a retention time of 1.42 min in the sample derived from *Aesculus hippocastanum* accounted for less than 0.5 % of the total mixture. Its presence suggests that the original mixture contained small quantities of a decaprenol. A hint of its presence was gained from some of the chromatograms of the castaprenyl acetates and of the perhydrocastaprenyl acetates.

Prenyl acetates

When the unsaturated prenyl acetates were chromatographed the same number of peaks was obtained as appeared in the chromatograms of the corresponding perhydroprenyl acetates and saturated hydrocarbons. It appeared that the order in which the unsaturated acetates left the column was the same as for the corresponding perhydro-acetates and saturated hydrocarbons. The relative areas under the respective peaks were essentially the same for the three types of compounds. The retention times for those mixtures containing a number of *cis*-isoprenoid residues were somewhat smaller than for the corresponding perhydroprenyl acetates but in the case of all-*trans*-solanesyl acetate the difference was less marked. The peaks were broadened when compared with those of the saturated hydrocarbons and the perhydroprenyl acetates and retention times varied a little from one chromatogram to the next. Nevertheless, this variation was relatively minor and it was quite obvious that marked *cis*:*trans* differences affected the retention times. It was no longer possible to fit all of the points to a straight line when the number of isoprene residues was plotted against log retention time. Retention times for a number of the mixtures are recorded



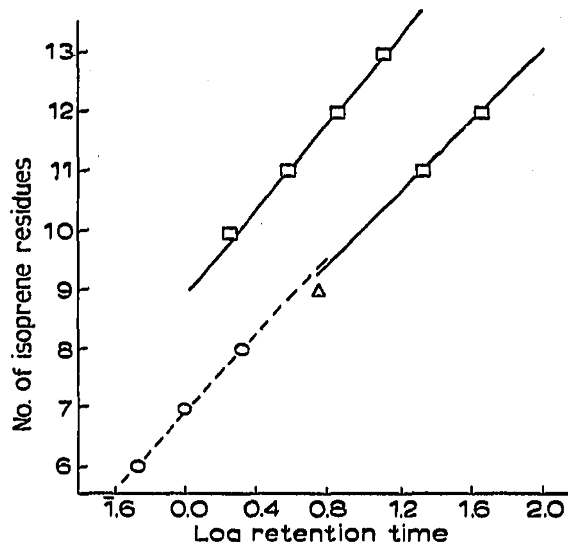


Fig. 4. Relationship between $\log_{10}$ retention time at 300° and the number of isoprene residues per molecule of each component of a mixture of betulaprenyl acetates (--⊙--⊙--), solanesyl acetate (△) and prenyl acetates from *Beta vulgaris* (—⊡—⊡—).

Fig. 5. Relationship between $\log_{10}$ retention time at 300° (lower lines) and 340° (upper line), and the number of isoprene residues per molecule of each component of a mixture of betulaprenyl acetates (--⊙--⊙--), solanesyl acetate (△) and castaprenyl acetates (—⊡—⊡—).

in Table VI. Log retention time against the number of isoprene residues for each component of the *Betula* plus *Beta* and *Betula* plus *Aesculus* plus solanesyl mixtures is plotted in Figs. 4 and 5, respectively.

Nuclear magnetic resonance studies indicate that in the betulaprenol mixture[1,2], in the castaprenol mixture[3] and in the mixture of prenols isolated from *Ficus elasticus* and *Beta vulgaris*[6] more than half of the isoprene residues are in the *cis* configuration. It is not known, however, if one component of the mixtures is predominantly *trans* and the others predominantly *cis* or if each component contains a small excess of *cis* over *trans* residues. Work is in fact progressing in this laboratory on the separation and

TABLE VI

RETENTION TIMES OF SOME PRENYL ACETATE MIXTURES AT 300 AND 340°

Number of isoprene residues	*Retention time (min) and source*					
	Solanesol (300°)	*Betula* (300°)	*Betula + Aesculus + Solanesol* (300°)	*Aesculus* (340°)	*Betula + Beta* (300°)	*Betula + Ficus* (300°)
6		0.47	0.57		0.41	—*
7		0.98–1.17	1.10		0.92	0.94
8		2.01–2.25	2.15		2.01	1.91
9	5.85–6.40	5.82–6.35	5.85		6.40	—*
10			—*	1.85	10.3	10.0
11			22.0	3.82	24.2	22.4
12			47.2	7.4	48.0	**
13			—*	13.1	—*	**

* Insufficient sample was applied to the column for this peak to be observed.
** Not recorded.

collection of the individual components of each mixture with a view to settling this point. Nevertheless, at this stage it is interesting to note that in Fig. 4 the points for the betulaprenyl acetates fall on a straight line as do the points for the prenyl acetates derived from *Beta vulgaris*, whereas the point for solanesyl acetate (all-*trans*-nonaprenyl acetate) is well clear of either line (see also Fig. 5). It is reasonable to argue that if one of the mixtures had contained a predominantly all-*trans* component then the point for this component also would not have fallen on the same straight line as that produced by the predominantly *cis* components. The evidence of Table VI and Figs. 4 and 5 would support the idea that the *cis*:*trans* ratio is not drastically different in each of the components of any one of the mixtures. It may also be that the prenols of *Aesculus hippocastanum* are similar, in this respect, to those of corresponding chain length derived from *Beta vulgaris* and *Ficus elasticus*.

It is important to the student of the biogenesis of these compounds to know, for instance, if the distribution of *cis* and *trans* residues in the C_{60} component of the *Beta vulgaris* mixture is precisely the same as in the ω-C_{50} portion of the C_{55} component and, further, if the distribution of *cis* and *trans* residues in the C_{55} component is precisely the same as in the ω-C_{55} portion of the C_{60} component. Such a situation would be a strong pointer to the shorter components being biogenetic precursors of the longer components (assuming *cis*–*trans* isomerases are not involved). Unfortunately, while the gas-chromatographic data do not rule this situation out, they cannot be quoted as evidence in favour of it. Although this technique has proved to be a powerful tool in studying long chain prenols, it cannot be used to supply useful information regarding relatively small differences in distribution of *cis* and *trans* isoprene residues.

Solanesyl trimethyl silyl ether and trifluoroacetate

It was found that the retention times (at 300°) of solanesyl trimethyl silyl ether and solanesyl trifluoroacetate were 8.9 min and 5.0 min compared with 5.9 min for solanesyl acetate. Since it appeared that the use of even the trifluoroacetates would offer no substantial advantage over the use of the acetates, no further work was carried out with these derivatives.

General

The foregoing results and discussion show that gas–liquid chromatography is a valuable tool not only for detecting the presence of small quantities of prenologues in natural mixtures of prenols, but also for forecasting the chain length of the components of these mixtures. In this way it has been possible to show that samples of prenol acetates that chromatographed on thin layers of silica gel as if one compound are in fact mixtures of prenol acetates of varying chain-length. The gas chromatograms can also be used to indicate the approximate percentage composition of the mixtures. The accurate composition of these mixture will be uncertain until the response of the flame ionisation detector to each component has been checked.

Previous reports have described the gas–liquid chromatography of alcohols varying in size from C_{16} (ref. 7) to C_{45} (ref. 2). It is perhaps remarkable that it has been found possible to extend the range to the acetate of C_{80} perhydroprenol. C_{100} and C_{110} prenols have also been isolated from natural sources[8,9]. It may be that, given a stationary phase of sufficient thermostability, it will be possible to chromatograph derivatives of these compounds.

SUMMARY

Gas–liquid chromatography has been used to confirm that the leaves of *Aesculus hippocastanum* contain prenols ranging from C_{45} to C_{65} with the C_{60} component predominating. Leaves of *Beta vulgaris* var. *crassa*, *Ficus elasticus* and *Arum maculatum* were shown to yield a mixture of prenols with the same range in chain length. The prenols from *Saccharomyces cerevisiae* were found to range from C_{70} to C_{80}. Retention times for prenol acetates, perhydroprenyl acetates and the derived saturated hydrocarbons are reported, together with the approximate quantitative composition of each mixture.

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