STRUCTURAL ANALYSIS OF ORGANIC SUBSTANCES BY MEANS OF HYDROGENATION COMBINED WITH GAS CHROMATOGRAPHY

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In recent years gas chromatography has been employed advantageously to the solution of problems involved in structural analysis of organic compounds. This development has been used as an adjunct to methods which in various ways modify the substance under test prior to its chromatographic treatment, so that from its behaviour before and after modification conclusions can be drawn on the arrangement of at least a part of the organic compound.

One such method is hydrogenation, which is usually carried out on a mixture of *e.g.* saturated and unsaturated compounds. The presence of unsaturated bonds is indicated by the disappearance of some chromatographic peaks[1–3]. In later work, hydrogenation of pure substances has been employed directly for identification purposes if they contain oxygen, nitrogen, or halogen[4–7], or for direct determination of the respective carbon skeleton[8–10]. In none of these procedures, however, has any attempt been made so far to compare the elution times of the hydrogenated and the non-hydrogenated product in such a way that from their relationship conclusions on the structural arrangement of the molecule of the substance under investigation may be drawn.

In the present work we have attempted to ascertain whether there was any relationship between the elution time and the number of double bonds in the molecule. In the chromatography of sterols, CLAYTON[11] has already found that a double bond causes a constant difference in the relative elution time. ACKMAN[12], who studied the relation between structure and elution volume of unsaturated fatty acids, found that the elution time changes depending on the position of the double bond; the larger the number of double bonds, the greater is this change.

It is generally known that saturated compounds exhibit shorter elution times than non-saturated compounds on polar stationary phases. This fact is frequently utilized, *e.g.*, in the separation of aromatic and aliphatic hydrocarbons, unsaturated and saturated acids, etc. If such separations are carried out and the relative elution time is calculated by dividing the elution time of the saturated substance by that of the unsaturated one, it was found that the relative elution time is proportional to the number of double bonds in the molecule.

For convenient and, at the same time, exact comparison of the elution times of these two types of substances in a single experiment, we have designed a simple hydrogenation apparatus which permits a simultaneous chromatographic record of both components.

EXPERIMENTAL

The principal part of the hydrogenation equipment is shown in Fig. 1 and consists of a layer of platinum catalyst C contained in tube A, which is heated in block B by means of the bodies I and J. The top end is fitted with a rubber closure E, through which the substance to be tested is fed by an injection syringe. Under this closure there is the hydrogen inlet CH. The hydrogen simultaneously serves as carrier gas. At the bottom end of the tube a needle, F, from an injection syringe is cemented so that it pierces into closure G of the chromatographic column H.

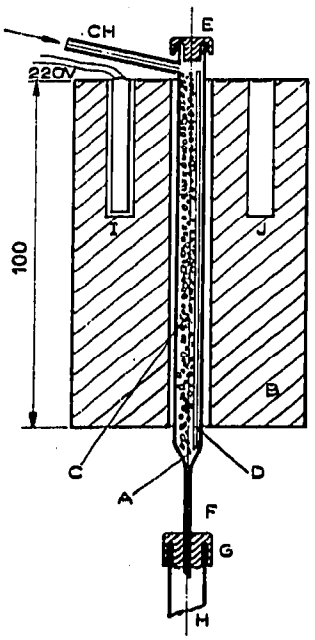

Fig. 1. Schematic drawing of the equipment for hydrogenation preceding chromatography, permitting simultaneous determination of the introduced non-hydrogenated and hydrogenated substances.

In order to obtain a simultaneous chromatograph of both the hydrogenated and the non-hydrogenated substance under the same conditions, one (or if required more) glass capillary of about 1 mm in diameter, extending from the top to the bottom end, is inserted into the catalyst layer. This arrangement permits about one half of the sample to pass through the catalyst and be hydrogenated, while the other half passes through the glass capillary without being affected.

Preparation of the catalyst

1 g of platinum is dissolved in aqua regia, evaporated to dryness, then HCl is added to remove HNO_3 and finally the evaporation to dryness is repeated. The residue is dissolved in about 15 ml of water and 10 g of pumice are poured into the solution contained in a porcelain dish. The mixture is dried to a brown coloration with constant agitation. The dried pumice is placed into a quarz tube, sealed with asbestos and reduced with hydrogen at a temperature of about 400°. The tube is emptied when cold.

Working conditions

It was found that hydrogenation proceeds best at a temperature of about 180°, and all the experiments described were performed at this temperature.

Gas chromatography was carried out in a CHROM I apparatus on a column of 85 cm in length, filled with Rysorb carrier with a stationary phase of 25 % of 3,5-dinitrobenzoyl ester of the butyl ether of triethylene glycol. Depending on the requirements, a column temperature of 68° and/or 150° was selected.

The flow rate of the carrier gas H_2 was 3.0 l/h. The relative elution time was calculated from the ratio of both elution times:

$$r_H = \frac{V_1 \text{ of non-hydrogenated substance}}{V_2 \text{ of hydrogenated substance}}.$$

DISCUSSION

From Tables I–VI it is obvious that the relationship between the number of –C=C– double bonds in the molecule and the logarithm of the relative elution time of the hydrogenated and non-hydrogenated substance is approximately linear

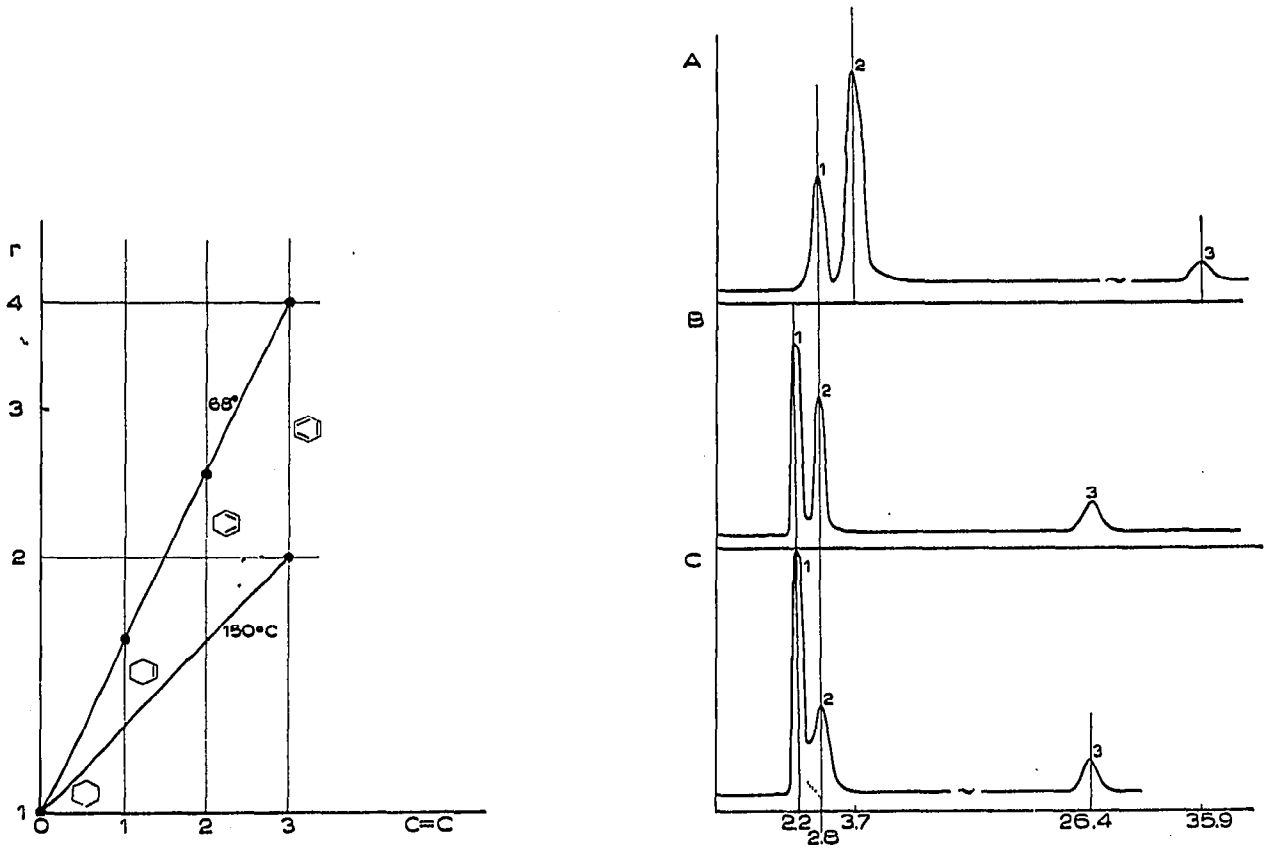

Fig. 2. Representation of the relationship between log r_H and the number of double bonds of benzene and its hydrogenation product at the various temperatures of the chromatographic column. Column filling as given in Table I.

Fig. 3. Hydrogenation of xylenes. A = *o*-xylene; B = *p*-xylene; C = *m*-xylene; (1) = *trans*-dimethylcyclohexane; (2) = *cis*-dimethylcyclohexane; (3) = xylene. Temperature of chromatographic column 68°. Filling 3,5-dinitrobenzoyl ester of butyl ether of triethyleneglycol.

TABLE I

RELATIVE ELUTION TIMES (r_H) OF MISCELLANEOUS HYDROCARBONS AND ESTERS AT VARIOUS TEMPERATURES ON A COLUMN WITH 3,5-DINITROBENZOYL ESTER OF BUTYL ETHER OF TRIETHYLENEGLYCOL

	$t = 68°$ r_H	$t = 150°$ r_H	*Number of double bonds reduced*
Cyclohexane	1.0	1.0	0
Cyclohexene	1.6	1.25	1
Methylcyclohexene	1.55		1
Dodecene		1.2	1
Cyclohexadiene	2.5		2
Thiophene	2.2		2
Benzene	4.0	2.0	3
Toluene	6.6	2.3	3
Ethylbenzene	4.8	2.3	3
o-Xylene	9.7	3.7	3
	12.4		
p-Xylene	9.4	3.6	3
	12.2		
m-Xylene	9.4	3.6	3
	12.2		
Cumene	3.4	2.4	3
p-Cymene	4.6	2.3	3
	5.2		
1,2,4-Trimethylbenzene	12.2		3
	[illegible].0		
Butylbenzene	4.3	2.2	3
1,3,5-Trimethylbenzene		3.8	3
Methyl benzoate		2.6	3
Methyl phenylacetate		2.4	3
Styrene	12.0	4.1	4
α-Methylstyrene	8.8	4.3	4
p-Ethylvinylbenzene		3.8	4
Naphthalene		8.0	5
		10.5	
Methylnaphthalene		13.8	5
1,6-Dimethylnaphthalene		14.3	5
p-Divinylbenzene		6.3	5
1,6-Dimethyltetralin		2.9	3
		3.6	
Tetralin		3.1	3
		4.1	
Methyltetralin		2.9	3
		2.9	

(Fig. 2); however, the slope of this linear relationship depends on the temperature of the chromatographic column and the type of stationary phase. For this reason, two temperatures, 68° and 150°, were selected. The relatively greater scattering of the values obtained is usually due, on the other hand, to the occurrence of various isomers, whose elution time is influenced by the magnitude of the permanent dipole moment[13], and, on the other hand, to the formation of various isomers of the hydrogenated product (*cis*, *trans*). This circumstance, however, can be utilized for a more exact identification of the substance under test. For instance, *m*-xylene and *p*-xylene yield the respective *cis*- and *trans*-dimethylcyclohexanes, which, although they are

derived from the two different isomers, exhibit the same elution times. The ratio between the *cis* and the *trans* isomers, however, varies considerably (as is evident from Fig. 3), but is constant under the same working conditions, thus permitting the semi-quantitative estimation of the two components in the case of mixtures.

Despite all the effects mentioned, the variations in the relative elution times (r_H) are so small that the number of double bonds in various hydrocarbons can be reliably determined in the majority of cases (Fig. 4).

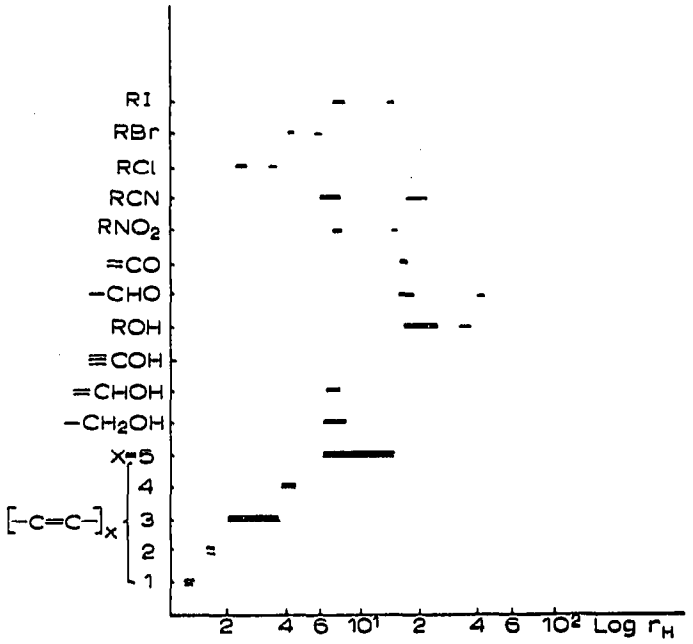

Fig. 4. Graphical representation of the magnitude of the relative elution time (r_H) for different numbers of double bonds and different functional groups that can be hydrogenated. Thin line: complete hydrogenation. Bold line: intermediate hydrogenation product corresponding to hydrogenation of the functional group.

The situation is sometimes complicated when the molecule contains functional groups, as well as double bonds, which can be hydrogenated, or groups which are split off under given conditions. For this reason, the analytical method described was applied to a series of compounds containing the groups $-CH_2OH$, $-OH$, $-CHO$, $=CO$, $-NO_2$, $-CN$, $-Cl$, $-I$ and $-Br$. The contribution of these groups to the relative elution time can also be investigated quantitatively since in the majority of cases it was possible to ascertain the intermediate products of the hydrogenation. The elution times are markedly increased by the presence of the above-mentioned functional groups. Tables II to IV present values showing how hydrogenation of the group or its splitting off influences the r_H. The diagram (Fig. 4) summarizes these results in such a way that according to the value of r_H conclusions can be drawn as to the number of double bonds as well as to the types of individual functional groups; thus, the substance under test can be identified in more detail. It is evident from the diagram that in certain cases the r_H's are equal for two different compounds. Further information can be obtained from the intermediate hydrogenation products present, or from the absolute elution time of the non-hydrogenated substance, which is usually longer for compounds with a larger molecule.

TABLE II

RELATIVE ELUTION TIMES (r_H) OF ALCOHOLS AND PHENOLS

	$t = 68°$ r_H	$t = 150°$ r_H	*Number of double bonds reduced*	*Group subjected to hydrogenation*	*Ratio corresponding to double bonds*
Methanol	15.2		0	$-CH_2OH$	
Ethanol	22		0		
Propanol	39		0		
n-Butanol	37		0		
Amyl alcohol	67		0		
Iso-amyl alcohol	38		0		
Hexyl alcohol	55	6.0	0		
Octyl alcohol	74	8.0	0		
3-Methylcyclohexanol		7.3 9.2	0	$=CHOH$	
4-Methylcyclohexanol		7.5 9.0	0		
Cyclohexanol	48	7.0	0		
tert.-Butanol	12.0	1.0	0	$\equiv COH$	
sec.-Butanol		6.1	0	$=CHOH$	
Phenol		16.6*	0	$-OH$	
o-Cresol		16.8*	0		
m-Cresol		22.1*	0		
p-Cresol		20.0*	0		
Phenol		33	3	$-OH$	2.0
o-Cresol		32	3		1.9
m-Cresol		35	3		1.6
p-Cresol		35	3		1.7
Furyl alcohol	7.4		2		
Allyl alcohol		1.1	1		
Furfural	2.2		2		

* Related to the elution time of benzene.

It must be pointed out, however, that hydrogenation of pyridine and its derivatives could not be achieved at the usual working temperature. The required effect was attained only after increasing the temperature up to 225°.

The presence of sulfur and halogens leads to a poisoning of the catalyst, usually requiring renewal of the reactor filling after a completed hydrogenation. Halogens are split off in this hydrogenation.

The hydrogenation of alcohols, which can be very easily differentiated, is also of interest. While primary and secondary alcohols have a comparatively high relative elution time, this time is essentially lower for tertiary alcohols. The comparatively great differences in the case of lower alcohols are caused by the fact that their hydrogenation results in highly volatile, paraffinic hydrocarbons, which exhibit short elution times, and the negligible differences in their elution times lead to considerable errors in their determination.

Ester groups are not hydrogenated and thus esters behave like hydrocarbons; however, they differ from hydrocarbons with the same number of double bonds by their essentially longer elution time. In the case of halogenated hydrocarbons, the

TABLE III

RELATIVE ELUTION TIMES (r_H) OF ALDEHYDES AND KETONES

	t = 150° r_H	*Number of double bonds reduced*	*Group subjected to hydrogenation*	*Ratio corresponding to double bonds*
Propionaldehyde	18.4	0	–CHO	
Octylaldehyde	18.0	0		
Nonylaldehyde	18.6	0		
Decylaldehyde	26.4	0		
Benzaldehyde	16.1*	0	–CHO	
Cinnamaldehyde	16.0*	0		
o-Hydroxybenzaldehyde	16.6*	0		
p-Tolylaldehyde	17.0*	0		
Benzaldehyde	36.7	3	–CHO	2.3
Cinnamaldehyde	51.0	4		3.2
o-Hydroxybenzaldehyde	41.3	3		2.5
p-Tolylaldehyde	40.3	3		2.4
Cyclohexanone	17.0	0	=CO	
3-Methylcyclohexanone	16.3	0		
4-Methylcyclohexanone	17.5	0		
Methyl phenyl ketone	18.0	3	=CO	
Methyl phenyl ketone	8.0	0		
Cyclohexanone	2.2	0	=CO → –CHOH	
4-Methylcyclohexanone	2.0	0		
	2.4			
3-Methylcyclohexanone	1.9	0		
	2.4			

* Related to the respective aromatic hydrocarbon.

TABLE IV

RELATIVE ELUTION TIMES (r_H) OF NITRO COMPOUNDS AND NITRILES

	t = 150° r_H	*Number of double bonds reduced*	*Group subjected to hydrogenation*	*Ratio corresponding to double bonds*
Nitrobenzene	7.5*	0	$-NO_2$	
Nitrotoluene	7.7*	0		
m-Tolunitrile	8.5*	0	–CN	
o-Tolunitrile	6.2*	0		
m-Tolunitrile	22.5	3	–CN	2.6
o-Tolunitrile	17.5	3		2.8

* Related to the elution time of aniline.

TABLE V

RELATIVE ELUTION TIME (r_H) OF HALOGENO-DERIVATIVES

	$t = 150°$ r_H	*Number of double bonds reduced*	*Group subjected to cleavage*
Chlorobenzene	3.4	3	–Cl
Chlorobenzene	2.3*	0	–Cl
Dichlorobenzene	6.5	3	2 –Cl
Bromobenzene	5.7	3	–Br
Bromobenzene	4.2*	0	–Br
Iodobenzene	13.2	3	–I
Iodobenzene	7.6*	0	–I
1,3,5-Trichlorobenzene	25.0	3	3 –Cl
1,2,4-Trichlorobenzene	25.0	3	3 –Cl

* Related to the elution time of benzene.

halogen present can be easily determined from the substantial differences in the relative elution times.

As already pointed out previously, the graph in Fig. 4 applies only to one temperature and to a certain stationary phase. If the experiments were carried out in parallel on different stationary phases, a further differentiation could be achieved, as is obvious from Table VI, which gives relative elution times on various stationary phases.

Another advantage of the method is the easy identification of intermediate hydrogenation products and the determination of the substance in question from the ratios of the elution times, and hence the mechanism of the reaction may be ascertained both qualitatively and quantitatively. This is clearly illustrated by Fig. 5.

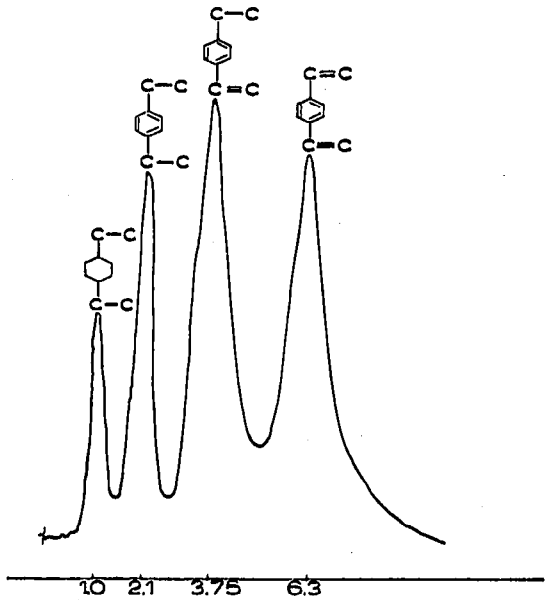

Fig. 5. Example of hydrogenation with identification of all intermediate products of hydrogenation of divinyl benzene.

TABLE VI

RELATIVE ELUTION TIME ON VARIOUS STATIONARY PHASES
t = 150°; Rysorb BLK.

	3,5-Dinitrobenzoyl ester 20% r_H	*Slovamin 7* 10% r_H	*Polywax 12.000* 20% r_H	*Asfalt EXP* 20% r_H
Benzene	2.0	2.2	1.9	0.0
Toluene	2.3	2.3	2.2	0.0
p-Xylene	3.6	2.6	2.7	0.0
Ethylbenzene	3.5	2.2	2.2	0.0
p-Cymene	2.3	2.3	2.3	0.0
Methyl benzoate	2.6	2.6	2.6	1.6
o-Tolunitrile	6.2	4.0	6.3	2.8
α-Methylstyrene	4.3	—	3.3	—
Cyclohexene	—	—	1.2	—
Cyclohexanol	7.0	7.8	13.0	7.2
p-Cresol	33.0	13.1	8.4	13.0
Phenol	33.0	14.6	10.2	19.1
3-Methylcyclohexanone	16.3	4.6	14.9	4.8
Mesitylene	3.8	2.6	—	—

SUMMARY

A method involving hydrogenation of a substance prior to chromatography has been developed and permits conclusions to be drawn on the presence of a certain number of double bonds, or certain functional groups simply from the ratio of the elution times of the hydrogenated and the non-hydrogenated substance. The elution times of both these substances are ascertained in a single experiment by means of a simple apparatus which is described in the paper.

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