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IDENTIFICATION OF ORGANIC COMPOUNDS BY CHROMATOGRAPHY AFTER DECOMPOSITION AND DEGRADATION

INTRODUCTION

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SUMMARY

Identification procedures based on degradation of the original molecule under investigation and chromatographic analysis of the degradation products are reviewed and discussed. The examples given for illustration are divided into three groups.

(a) Identification of functional groups based on splitting off the functional group in the form of a very simple chemical compound which is then analyzed by a suitable chromatographic technique.

(b) Degradation of complex molecules or macromolecules to basic units of which the original molecules have been built up and chromatographic identification of these degradation products.

(c) Total degradation achieved mostly by pyrolysis yielding chromatographic patterns characteristic for a given compound (fingerprint).

INTRODUCTION

Each chromatographic technique has its specific requirements as to the properties of the compounds to be chromatographed and the differences in their structure essential to obtain the desired separation and identification. It is usually the too high volatility of compounds of lower molecular weight and the instability, low solubility, or even insolubility, or low separation effect of larger molecules or macromolecular compounds that make them unsuitable for paper or thin-layer chromatography. Whenever this is the case, the compounds must be converted into substances suitable for the chromatographic techniques. Organic compounds can be chromatographed in the following three ways: directly, after derivatization, and after degradation. The latter will be discussed in this contribution.

The identification of organic compounds by chromatography of their chemical, enzymatic or any other degradation products represents a common procedure, many examples of which could be found in the literature.

These examples can be easily divided into three groups, *viz.*: identification of functional groups; degradation to simple basic units; and total degradation (pyrolysis).

IDENTIFICATION OF FUNCTIONAL GROUPS

Identification of functional groups can be achieved by splitting off the functional group from the original molecule in the form of a very simple chemical compound which is then analyzed by means of a suitable chromatographic technique. GC is the proper technique in the case of gaseous fission products. PC or TLC can be used with success in the case of non-volatile fission products or of such volatile products that can be easily converted into non-volatile derivatives.

The following identification procedures can be used for illustration.

O- and N-alkyl groups can be split off in the form of the corresponding alkyl iodides by the action of hydroiodic acid. The resulting alkyl iodides can be identified by GC[1-5] or after conversion into the corresponding alkyl 3,5-dinitrobenzoates by PC or TLC[6].

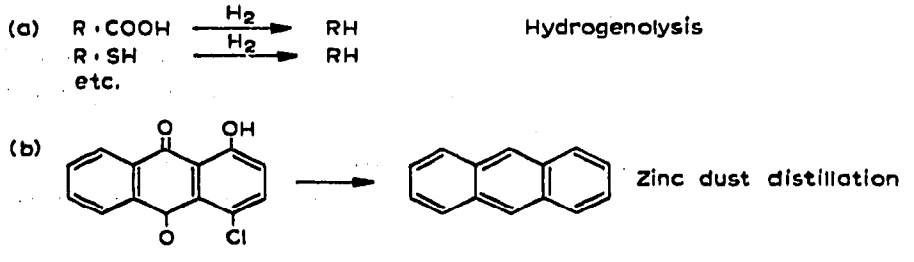
Secondly, identification of different oxyethylene or oxypropylene adducts of alkylphenols can be achieved by TLC using silica gel[7] or by PC using paper impregnated with silicic acid[8]. Under these conditions the compounds are separated according to the number of oxyalkylene groups (n) irrespective of the alkyl group (R), *i.e.*, compounds with the same n but different R have the same R_F values. Complete identification can be achieved by fission with hydroiodic acid, followed by PC of the resulting alkylphenol[9]. Furthermore, differentiation of the oxyalkylene groups is carried out by pyrolysis with $KHSO_4$, followed by identification of the acetaldehyde or propionaldehyde formed[10]. Quantitative analysis of the oxyalkylene groups is also possible[11]. After treatment with hydrobromic acid the cleavage products are determined by GC.

Alkyl groups bound to sulfur or silicone can be split off in a similar manner by reaction with Raney nickel in butanol[12] or sulfuric acid in the presence of a vanadium catalyst[13]. In both cases hydrocarbons result, which can be readily identified by GC. Alkyl groups bound to carbon in an aromatic or cycloaliphatic ring can be split off by oxidation with chromic acid and the resulting aliphatic acids can be identified by PC[14]. Another procedure makes use of catalytic reductive cleavage and identification of the resulting hydrocarbons by GC[15].

Lastly, O-acyl groups react with hydroxylamine. The identification of the corresponding hydroxamic acids can be achieved by PC or TLC[16].

In all these cases the functional groups were split off and identified. It is, however, possible to split off all functional groups present in the molecule that contain oxygen, nitrogen, sulfur or halogens and identify the parent compound or the basic skeleton[17-20] (*e.g.*, hydrogenolysis and GC or zinc dust distillation and PC):

It must be emphasized here that many modern instrumental methods are available at present to solve the above mentioned problems. NMR spectroscopy, *e.g.*,



represents a very efficient method for the identification of alkyl groups, provided the compounds under investigation are soluble in solvents suitable for this technique. When this instrument is not available or the investigated compounds are not soluble enough, the mentioned reactions combined with chromatographic procedures can be successful.

DEGRADATION TO SIMPLE BASIC UNITS

Identification procedures based on degradation to basic units and identification of these by chromatographic methods are very often used in the case of complex molecules or macromolecules. Such substances are usually slightly soluble or even quite insoluble in water or in organic solvents involved in the separation processes in PC or TLC. Poor separation efficiency can often be observed even if the compounds can be chromatographed. It is caused by small or insufficient structural differences.

It is therefore necessary to degrade the compounds to the basic simple molecules out of which the complex molecule has been built up. Thus, the complex molecule is characterized by its degradation products and identification is achieved by identification or comparison of these products. This procedure is also used in the case of elucidation of unknown structures.

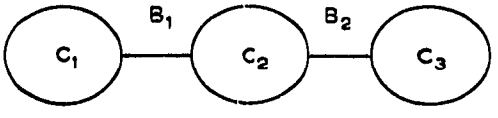
A complex organic molecule can be represented schematically and in a very simple way as is shown in the next scheme. Two, three or even a large number of the simple molecules C_1, C_2, C_3, etc., can be linked to chains by bonds B_1, B_2, etc., to form macromolecules.

Two groups of complex molecules can be observed, *viz.*:

(1) $B_1 = B_2$ etc., *i.e.* all the units of the macromolecule are of the same type;

(2) $B_1 \neq B_2$ etc., *i.e.* the complex molecule is built up of compounds of different chemical classes.

This differentiation is very important for the degradation procedures to be considered. The bonds B_1, B_2 etc. represent such centers in the macromolecule that can be attacked by a chemical or any other agent which must not attack other bonds in the molecule. If, by a suitable chemical or enzymatic reaction, the bonds between the individual linked-up units are cleaved, a mixture of basic units is formed. The individual compounds in the mixture can then be identified by PC or TLC and from the



(1) $B_1 = B_2$

Peptides	(amino acids)	$-CO \cdot NH-$
Polysaccharides	(sugars)	$-O-$
Polyazo dyes	(amines)	$-N = N-$
	etc.	

(2) $B_1 \neq B_2$

Azo dyes: $B_1 = -N = N-$, $B_2 = -CO \cdot NH-$

Fats: $B_1 = -CO \cdot OR$, $B_2 = -CH = CH-$

etc.

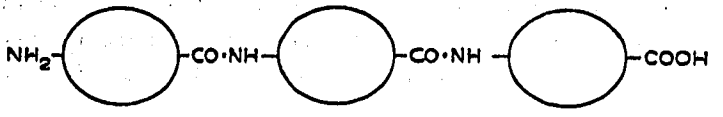
information thus obtained, the composition of the original molecule can be inferred.

The reactions commonly used to obtain bond cleavage are:

—CO·NH—	hydrolysis
glycosidic —O—	hydrolysis
—N=N—	reduction
—CO·O·R	hydrolysis
—CH=CH—	oxidation

Let us now direct our attention to such complex molecules that are built up of compounds of the same chemical group, *e.g.* peptides, polysaccharides, polyazo dyes. There are some problems which are common to all these groups.

In the case of peptides[21] (the next scheme represents a tripeptide built up of glycine and alanine), total hydrolysis results in the cleavage of all carboxamide groups and a mixture of amino acids is obtained. The identification of these basic units is the first step which is to be carried out. But further information is necessary. Since one amino acid can be present several times in one peptide molecule, it is important to carry out a quantitative analysis to get information about the ratio of the individual amino acids in the hydrolysate. Furthermore it is necessary to determine which amino acid is N- and which is C-terminal, *i.e.* which has a free amino or



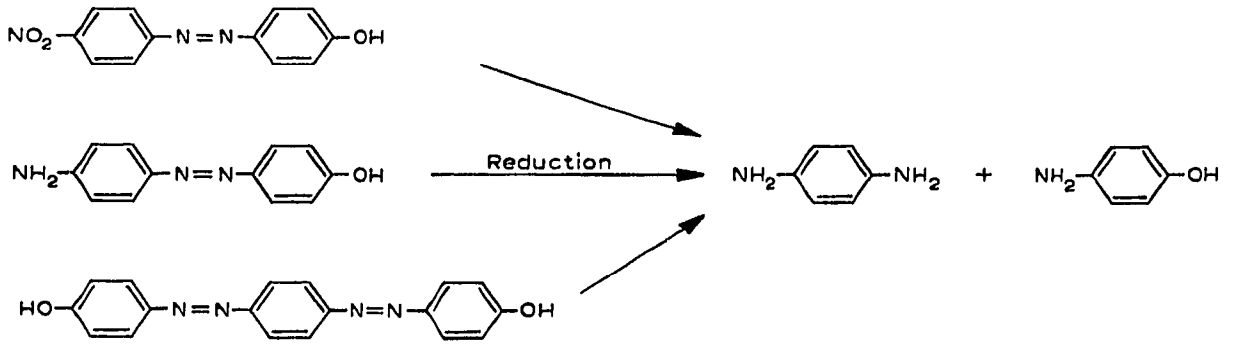
Tripeptide	*Components*	*Ratio*	*N-terminal*	*C-terminal*
Gly Gly Ala	Gly + Ala	2:1	Gly	Ala
Gly Ala Gly	Gly + Ala	2:1	Gly	Gly
Ala Gly Gly	Gly + Ala	2:1	Ala	Gly
Ala Ala Gly	Gly + Ala	1:2	Ala	Gly
etc.				

a carboxyl group, respectively. This problem is solved usually by derivatization of the group under consideration and the derivatized amino acid is found in the hydrolysate provided the new bond has not been destroyed during hydrolysis. The problem is of course more complicated and its solution includes partial hydrolysis, consecutive degradation, etc.

The identification of polysaccharides[22] is achieved in a similar manner. The following analytical steps are involved: (1) identification of monosaccharides after total hydrolysis; (2) determination of the proportion of the individual monosaccharides after total hydrolysis; (3) determination of the sequence of the glucose units after partial hydrolysis; (4) determination of the nature of branching; and (5) determination of the position of the glycosidic linkage (methylation and hydrolysis).

A similar example is represented by polyazo dyes though the problem is slightly different. Azo dyes are formed by diazotation of the active coupling component, a primary aromatic amine, and the diazo compound formed is coupled with the so-called passive coupling component, usually a phenol or an aromatic amine. Thus, diazotized aniline coupled with phenol yields *p*-hydroxyazobenzene. The reduction cleavage of the azo bond results in the formation of aniline and *p*-aminophenol. This simple

example is characteristic for the reductive cleavage of azo dyes: the passive component contains a new amino group which has not been present before. Azo dyes very often contain nitro groups and these are also reduced to amino groups. Thus we can see from the above scheme that all the three azo compounds yield identical products.



This is also the case with peptides. The number of amino acids and simple sugars of which the naturally occurring peptides or polysaccharides are built up, resp., is usually limited, but the number of amines that can be used for the preparation of azo dyes exceeds several hundreds. This may cause some difficulties in the identification of the products of reductive cleavage. Another complication is caused by the fact that some aromatic amines obtained by reductive cleavage contain sulfo groups. This must be taken into consideration since different solvent systems will have to be chosen for the identification of the water-soluble and the water-insoluble amines by PC.

The problems involved in the identification of complex molecules built up of units of different chemical classes will be demonstrated in the case of fats and azo dyes.

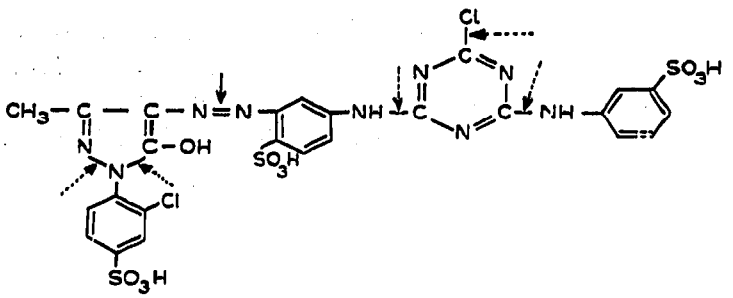
The main components of naturally occurring fats are triglycerides, the esters of a variety of fatty acids and glycerol. Since glycerol is a trihydric alcohol, triglycerides can be simple or mixed, *i.e.* they may contain one, two or three different fatty acid species. Two primary and one secondary hydroxy group are present in the glycerol molecule, giving rise to positional isomers of the same fatty acid composition and enantiomorphic forms, respectively. The natural fats always represent complex mixtures of triglycerides. Let us therefore simplify the problem and consider only one chemically pure compound:

$$\begin{array}{l} CH_2 \cdot O \cdot COC_{17}H_{35} \\ | \\ CH \cdot O \cdot COC_{17}H_{35} \\ | \\ CH_2 \cdot O \cdot CO(CH_2)_7CH{=}CH(CH_2)_7CH_3 \end{array}$$

(Distearoolein)

The following analytical steps involving chromatographic methods must be carried out[23,24]: (1) identification of the individual fatty acids after total hydrolysis; (2) differentiation of fatty acids bound to the primary and secondary hydroxyl groups (by partial hydrolysis); and (3) location of the double bond (by oxidation).

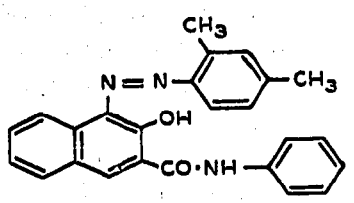
When the identity of the following dye is to be checked the following reactions must be carried out: (1) reduction by $SnCl_2$ under mild conditions (azo bond cleavage);



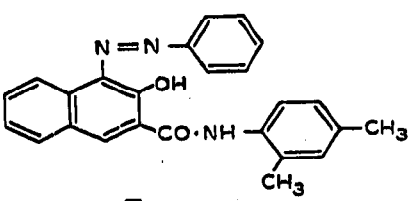
(2) reduction by $SnCl_2$ at 180° (azo bond and –NH– bond cleavage); (3) mild hydrolysis (cyanuric acid formation); and (4) reaction with zinc and ammonia (pyrazolone ring cleavage).

Let us now compare the following two dyes, I and II:

Three reactions can be carried out to achieve cleavage of different bonds, *viz.*: (1) reduction with zinc in acetic acid (azo bond cleavage); (2) reduction with $SnCl_2$ at 180° (azo and amidic bonds cleavage); and (3) alkaline hydrolysis (amidic bond cleavage).



I



II

Reaction	*Products*	
	I	*II*
Reduction with Zn + AcOH	2,4-Xylidine	Aniline
Reduction by $SnCl_2$ at 180°	2,4-Xylidine + aniline	2,4-Xylidine + aniline
Alkaline hydrolysis	Aniline	2,4-Xylidine

These three examples should show that the presence of different types of bonds in a molecule which can be attacked and cleaved enables us to find a system of reactions by which the complex molecules can be subsequently degraded and the basic units in the molecule determined.

TOTAL DEGRADATION (PYROLYSIS)

Total degradation is achieved mostly by pyrolysis, yielding products characteristic for each compound. The pyrolysis products are usually volatile compounds and therefore the combination of pyrolytic breakdown and chromatography is mainly the domain of GC.

Patterns characteristic for each compound (fingerprints) are obtained from the recorder provided the pyrolysis has been carried out under carefully controlled conditions. Identification is achieved by comparison of these patterns with the patterns

of known standard compounds obtained under the same experimental conditions. Several degradation procedures have been described in the literature and several instruments consisting of a pyrolyzer coupled with a gas chromatograph are commercially available[25–27].

We can summarize that the combination of degradation procedures and chromatographic methods is a very efficient tool for identification of organic compounds. The degradation procedures are usually very simple and can be carried out using very simple equipment, *e.g.* glass ampoules or even sealed capillaries, and sometimes they can even be carried out on the start of the chromatogram.

The problems presented here for illustration have been simplified so as to give information only on essential principles of this type of work and to encourage other authors to take advantage of it in their field of interest.

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