

SYSTEMATIC ANALYSIS OF NITROGEN COMPOUNDS USING CLEAVAGE REACTIONS AND GAS CHROMATOGRAPHY

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INTRODUCTION

The application of gas chromatography combined with cleavage reactions has proved advantageous for elucidating the structure of organic compounds.

In the last two years, several papers¹⁻⁷ have appeared, which describe the detection and quantitative determination of various functional groups by means of a gas chromatographic investigation of the various gaseous products from a reaction performed in a suitably modified microreactor which is placed immediately ahead of the column.

One important and extensive group of organic substances comprises the compounds containing nitrogen, which is bound in the molecule in various ways. In many cases it is possible to determine how this nitrogen is bound by classical methods; however, these are sometimes tedious, often nonspecific, and mostly require an appreciable amount of sample. Alternatively, various modern methods such as nuclear magnetic resonance, and in some cases, infrared spectroscopy can also be used; these methods, however, need relatively expensive apparatus.

The present work describes the systematic analysis of the nitrogen bonds in organic molecules by using different combinations of cleavage reactions; in most cases it is nitrogen or nitrogen oxide that is determined in the gaseous mixture formed. The reagents for the reactions were chosen in such a way that, as far as possible, the various types of nitrogen bond could be differentiated. This was not successful, however, when the types of bonds were very similar. The method described is mainly advantageous in cases where several nitrogen atoms bonded in different ways are present in the same molecule. Hence this method is a useful supplement to the present classical methods.

EXPERIMENTAL

The cleavage reaction

Oxidation with chromic-sulphuric acid. The oxidation is carried out in a microreactor with 5 N chromic-sulphuric acid in three stages.

(1) 1 ml of 5 N H_2CrO_4 is added to 20-30 mg of the compound under investigation.

(2) If a reaction does not occur after 1-2 min (*i.e.* no gas formation occurs), 1 ml of concentrated sulphuric acid is added. The sulphuric acid is added with a syringe.

(3) If a reaction still does not occur after a further 1–2 min, the whole mixture is heated to 160° for about 5 min.

Especially N₂, as well as H₂, CH₄, CO and CO₂ are the by-products looked for in the gases evolved during the reaction.

Oxidation with bromide–bromate mixtures. 20–30 mg of solid KBr + KBrO₃ mixture is added to the sample (20–30 mg) in the microreactor, and 1.5 ml of glacial acetic acid is added. The reactor is closed with a stopper, such as in used for penicillin bottles, and flushed with hydrogen carrier gas. The suspension is dissolved by heating mildly, and the reaction is started by further heating up to boiling. Any nitrogen evolved is determined.

Van Slyke reaction (modified by Kainz). About 1 ml of 40% sodium acetate is placed in the reactor, and about 0.5 ml of 10% potassium iodide and 0.5 ml of a saturated solution of sodium nitrite is added, the microreactor is flushed with carrier gas (H₂). Then 20–30 mg of substance is dissolved in glacial acetic acid and placed in the reactor by means of a syringe. After the reaction has proceeded at 40–50° the nitrogen formed is identified.

The Kainz method. The sample (20–50 mg) is dissolved in 0.5 ml of glacial acetic acid in the reactor and is covered with a layer of a solution of nitrosyl bromide, which can be prepared from 15 ml of acetic acid, 3 ml of bromide and 2 g of sodium nitrite. The reactor is closed, flushed with carrier gas (H₂), and 0.1 ml of formic acid–sodium formate mixture (1:1) is added by means of the syringe. Nitrogen formation can be expected over a period of 20–30 min; care must be taken that the temperature does not exceed 30°.

Reaction with phenylhydrazine. 20–30 mg of substance is mixed with about the same amount of phenylhydrazine hydrochloride, and dissolved in acetic acid. The mixture is placed in the reactor and after closing the reactor it is flushed with the carrier gas (H₂). The mixture is heated to its boiling point for about 2 min. Formation of nitrogen is checked.

Reaction with copper(II) sulphate. 20–30 mg of substance is mixed in the reactor with the same amount of copper(II) sulphate, dissolved in glacial acetic acid, and brought to boiling. The process is then the same as in the preceding case.

Reaction with α -nitroso- β -naphthol. (1) 20–30 mg of substance is mixed in the reactor with the same amount of α -nitroso- β -naphthol, and dissolved in acetic acid. After closing the reactor the mixture is heated to boiling. Nitrogen is looked for. (2) If the reaction is positive, 0.2–0.5 ml of nitrosyl bromide solution (see above) is added (the temperature must be kept below 30°). Nitrogen may be evolved.

Reaction with ferrous sulphate. About 20–30 mg of ferrous sulphate and several drops of concentrated hydrochloric acid are added to 20–30 mg of sample in the microreactor which is then closed, flushed with carrier gas and heated to about 60–80°. The gaseous products formed are examined for nitrogen oxide.

Reaction with platinum black. 1–2 ml of concentrated sulphuric acid is added to 20–30 mg of sample, and a platinum mesh with a deposit of platinum black is inserted in the mixture. The reactor is heated to 300° for about 10 min. The nitrogen formed is observed.

The platinum black is prepared in the following manner: a 2% solution of platinum tetrachloride, with a small addition of lead(II) acetate is placed in a beaker. The minus pole of a 6 V accumulator is connected to the platinum mesh on to which

the platinum black is deposited. The second electrode is also of platinum. The platinum black separates out within a few minutes.

Thermal decomposition of oximes. Paraffin oil and about 5 mg of CuO are added to 20–30 mg of sample and heated to 180°. Nitrogen formation is checked.

Reaction with sulphur. 20–30 mg of substance is mixed with sulphur, and the suspension put on a platinum mesh which is placed in the closed reactor. The mesh is heated by an electric current for 1–2 sec to a temperature of about 500°. The gases evolved are led into a Fe³⁺ solution.

The microreactor

The microreactor is essentially an improved version of the type used previously². It is substantially made of glass and has a volume of 3 ml (see Fig. 1). The reactor is closed with a rubber stopper, which is perforated by a syringe (10 ml). This syringe is used for washing (with carrier gas) and it also serves as reservoir for the gases formed by the reaction, which press out the piston by their own pressure. The lower part of the reactor is mounted in an electrically heated brass block, the temperature

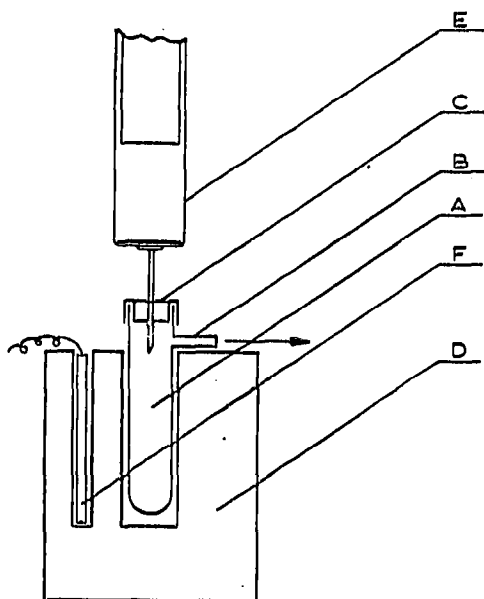


Fig. 1. A = Glass microreactor; B = outlet of reaction products to the gas chromatograph; C = rubber closure of reactor; D = heating block of reactor; E = syringe, reservoir for reaction products; F = heating element.

of which is measured with a thermometer. The set up is connected through a stopcock to the gas chromatograph by means of a capillary tube and an injection needle which perforates the column closure. The sample (*e.g.* in solution) and the reaction medium can be measured into the closed reactor and rinsed with carrier gas by means of a second syringe. Before proceeding with the reaction it is necessary to verify that the space of the reactor does not contain nitrogen from the air (blank assay of the chromatograph).

Gas chromatography conditions

The microreactor is connected to a gas chromatography column with the di-

mensions 300×0.4 cm; the packing is molecular sieve 5 A. The working temperature is 25° ; hydrogen is used as carrier gas (flow rate 3 l/h). Detection is carried out by a thermal conductivity detector, a 1 mV recorder being used. Nitrogen or nitrogen oxide are detected by their elution times. In cases where H_2 , CH_4 , CO and CO_2 are studied the column is 150 cm long, filled with active charcoal; nitrogen is the carrier gas (flow rate 3 l/h). The other conditions are identical.

Selection of compounds for study

The compounds that have been studied for their cleavage reactions were selected in such a way that, apart from the basic structure, as wide a range of additional substituents as possible was included. Nine triazoles, one tetrazole, twelve amines, four amides, five oximes, eleven nitro-compounds, seven nitroso-compounds, nine hydrazo-compounds, seventeen azo-compounds, ten nitramines, fourteen nitriles, eleven compounds with heterocyclic nitrogen, three ammonium salts of organic acids and one urethane were cleaved. Most of the compounds had various functional groups. Altogether 114 compounds were studied.

DISCUSSION AND RESULTS

Various reactions mentioned in the literature, which yield gaseous products, have been used in our experiments. One of the main reactions, which seemed to be very advantageous, was the oxidation in chromic-sulphuric acid medium. However it is known⁸⁻¹¹ that substances containing nitrogen cleave in chromic-sulphuric acid in different ways, and this fact had been employed by JUREČEK and his collaborators for the quantitative determination of various nitrogenous functional groups in the presence of each other. For example, all substances having -N-N- groups give nitrogen, while the nitro-group is converted to nitric acid, and primary or secondary amino-groups yield ammonia. We employed this reaction to determine the various nitrogen bonds in a quantitative manner, namely by combining the reaction with gas chromatography.

However, it is evident from Table I that the whole range of nitrogenous compounds studied will yield nitrogen as a result of this oxidation process, though evolution of nitrogen was not always found to be uniformly easy. Therefore three different working conditions were chosen, which would serve to facilitate further differentiation. The cleavage was first effected in the cold, with the addition of 5 N chromic acid, whereby nitrogen was released from azo- and azoxy-compounds, and from oximes. After addition of conc. sulphuric acid nitrogen is formed from hydrazines and hydrazones, tetrazoles and some azo-compounds, which have not already been cleaved in the cold. If the reaction is also negative in the second case, the mixture is heated to 160° , and the nitrogen formation is watched once more. Under these conditions the majority of triazoles, and some further azo-compounds are cleaved. It was found that the unequal speed of nitrogen formation in the case of the azo-compounds was caused by steric hindrances. For example, compounds with -OH or -NH₂ groups in the *ortho*-position to the azo-group are split much more slowly owing to hydrogen bridges. Nitrogen formation can at times be inhibited in the cold by the low solubility of some azo-compounds in chromic-sulphuric acid. In Table I the other groups shown do not produce elementary nitrogen on cleavage.

TABLE I

REVIEW OF THE REACTIONS OF VARIOUS FUNCTIONAL GROUPS CONTAINING NITROGEN: FISSION PRODUCTS

| Functional group | N_2 formation | | | | | Phenylhydrazide | |
|---|------------------|-------------------------------------|-------------------|----------------------------|----------------------------------|-----------------|------|
| | 5 N chromic acid | | | KBr + KBrO ₃ | Van Slyke (HNO ₂) | | NOBr |
| | In the cold | + H ₂ SO ₄ | heated to 160° | | | | |
| Amines primary | — | — | — | — | + | + | |
| other | — | — | — | — | | (sec. NO) | |
| Nitroso- —C—NO | — | — | — | — | | + | |
| Nitro- —NO ₂ | — | — | — | — | | — | |
| aromatic | | | | | | | |
| Nitramines —N—NO ₂ cyclic | — | — | — | — | | — | |
| linear | — | — | — | — | | — | |
| Nitriles —CN | — | — | — | — | | — | |
| Heterocyclic nitrogen | — | — | — | — | | — | |
| Azo- —N=N— | + | + | + | + | | | |
| aromatic | | | | | | | |
| Hydrazo- —NH—NH— | — | + | — | + | | | |
| aromatic | | | | | | | |
| Hydrazines —NH·NH ₂ | — | + | — | + | | | |
| Hydrazones =N—NH ₂ | (+) | + | — | + | | | |
| Azoxy- $\begin{array}{c} \text{—N—N—} \\ \diagdown \quad / \\ \text{O} \end{array}$ | + | — | — | + | | | |
| Oximes —C=N—OH | + | — | — | + | | | |
| Triazoles $\begin{array}{c} \text{—C—N—} \\ \parallel \quad \parallel \\ \text{—C—N—N—} \\ \\ \text{H} \end{array}$ | — | (+) | + | — | | | |
| Tetrazoles $\begin{array}{c} \text{N=N—} \\ \quad \diagdown \\ \text{N—C—N—} \\ \\ \text{H} \end{array}$ | — | + | — | + | | | |
| Amides and thioamides $\begin{array}{c} \text{—C—NH}_2 \\ \parallel \\ \text{O(S)} \end{array}$ | — | — | — | — | + | — | |
| Ammonium carboxylates and sulphonates —COONH ₄ —SO ₃ NH ₄ | — | — | — | — | | | |

+ = present; — = absent.

A range of gaseous products, besides nitrogen, was also formed during the oxidative cleavage with chromic-sulphuric acid. These were detected by gas chromatography and include H₂, CH₄, CO and CO₂. At first we thought that the molecular structure could be deduced from them. It was found, however, that during the cleavage the formation of this mixture is also influenced very substantially by other groups, so that only limited conclusions could be drawn (e.g., amides and oximes are cleaved with carbon monoxide formation only).

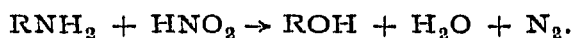
A bromide-bromate mixture¹² also oxidizes some nitrogenous compounds with nitrogen formation. There is a positive reaction with azo-compounds, hydrazines, hydrazones, and oximes. On the whole the results of the cleavage are similar to those

BY GAS CHROMATOGRAPHY

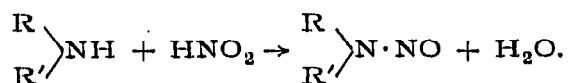
| Reaction | | | HSCN | H ₂ , CH ₄ , CO, CO ₂ formation | | | | NO formation | | |
|----------|-------------------|------------------|----------|--|--|-----------------|----|-----------------|-------------------|---|
| sol | CuSO ₄ | Paraffin at 190° | Pt-black | S | 5 N chromic-sulphuric acid + H ₂ SO ₄ ; 160° | | | | FeSO ₄ | 5 N chromic-sulphuric acid + H ₂ SO ₄ |
| | | | | | H ₂ | CH ₄ | CO | CO ₂ | | |
| | | | | | + | — | + | (+ +) | | |
| | | | | | (—) | — | + | (—) | | |
| | | | | | ± | — | + | + | | |
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with chromic-sulphuric acid, except with the triazoles where nitrogen is not formed.

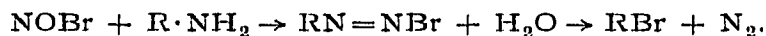
To find primary amino-groups, the VAN SLYKE method¹³ modified by KAINZ¹⁴, was used. The reaction is as follows:



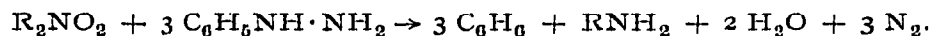
Secondary and tertiary amines yield nitrosamines:



However it is indicated in KAINZ's paper that the reaction is also positive in some cases when the compound has no primary groups; for example some isonitroso-compounds, compounds with an active methyl group, monohalogenated acids, phenols, nitrosophenols, sulphur compounds that are wholly oxidizable with nitric acid, indoles, and oxindole compounds, five-membered heterocycles with urea groups, hydrolytically fissible compounds, and lactams that form unstable nitroso-compounds, can react. On the other hand, the reaction can be negative if the molecule contains primary amino groups that are not easily diazotized, or if relatively stable diazo-salts or poorly soluble compounds¹⁵⁻¹⁷ are formed. In spite of these numerous anomalies we have used this method because the reaction proceeds very quickly. Further corroboration of the presence of primary amino groups, in the case of a positive reaction, could be obtained by the KAINZ method with nitrosyl bromide, which removes the anomalies of the VAN SLYKE method. The KAINZ method was not used alone because it requires a relatively long reaction time, namely about 30 min. The reaction scheme with nitrosyl bromide is as follows:



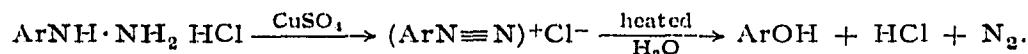
To resolve the nitro- and nitroso-compounds, and nitramines the reaction with phenylhydrazine was used:



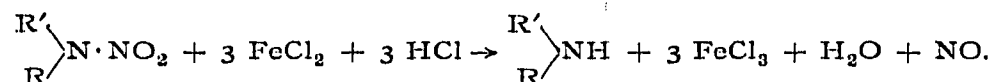
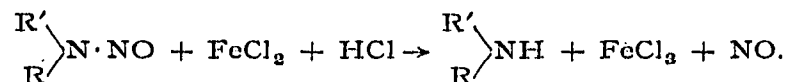
This reaction does not proceed with nitroso-compounds and N-nitrosamines. Aromatic nitro-compounds react only after a longer time and under pressure at 200° (WALTER's method¹⁸).

The reaction of phenylhydrazines with α -nitroso- β -naphthol is reciprocal.

Aromatic hydrazines also react with cupric sulphate with nitrogen formation¹⁹:



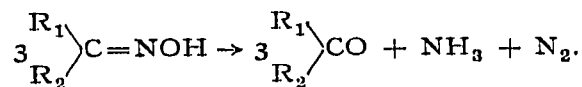
The reactions presented up to now were so chosen that elementary nitrogen is formed. Another possibility is to observe the formation of nitrogen oxide. The reaction of nitrosamines and nitroamines with ferrous chloride or ferrous sulphate²⁰ is an example of this:



However, it was shown that the linear nitramines also yield nitrogen oxide with chromic-sulphuric acid mixture, and it is necessary to keep this in mind.

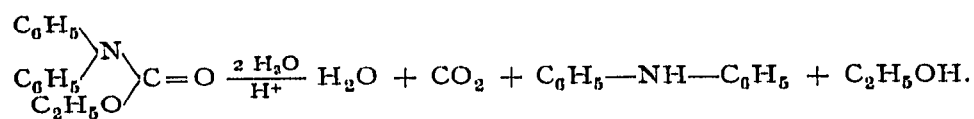
Oximes will submit to thermal decomposition²¹, namely at a temperature of

about 190°, with nitrogen formation. This reaction is not given by azo-, hydrazo- and azoxy-compounds.



As a supplement to the reactions observed by gas chromatography, pyrolytic cleavage with sulphur²² was used as a proof of nitriles, and the HSCN formed was confirmed by the reaction with Fe³⁺.

In Table I we do not show the hydrolytic decomposition of urethanes yielding CO₂, because only one compound (diphenylurethane) has been tested. The cleavage proceeds as follows:



In conclusion it is necessary to mention that the aim of this work was to present a series of reactions that can be used in conjunction with gas chromatography for the systematic analysis of nitrogenous compounds. For this reason, the results for all the compounds enumerated in the experimental section and used to compile the table, are not presented individually as it would make the whole work too voluminous.

The fact that the formation of fissile products from compounds with many substituents is of an additive character must be borne in mind when assessing results as these are not always explicit.

However, this work may be considered as an acceptable supplement to other methods of identification.

SUMMARY

For the systematic analysis of organic compounds containing nitrogen cleavage reactions with chromic-sulphuric acid, bromide-bromate, nitrous acid, nitrosyl bromide, phenylhydrazine, α -nitroso- β -naphthol, copper(II) sulphate, iron(II) sulphate and platinum black were used, and the reaction products were identified by gas chromatography. The behaviour of 14 various nitrogenous functional groups is summarized.

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