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Structure Elucidation (Part A)

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## FOREWORD

The tremendous advances made during the last two decades in spectroscopic techniques have accelerated the pace of research in the field of isolation and structure elucidation of complex natural products. These advances have been particularly spectacular in the fields of NMR and mass spectroscopy, which have considerably extended the capabilities of these techniques. For instance, the advent of pulse NMR techniques and mini-computers led to the development of two-dimensional NMR spectroscopy, which has now come to be routinely employed in laboratories around the world for unravelling complex structural problems. Less dramatic, though no less important, have been the advances in the field of mass spectroscopy, where the development of new ionization techniques such as negative and positive fast atom bombardment, field desorption and chemical ionization, etc., have followed the acquisition of mass spectra of non-volatile compounds which do not respond well to the standard electron impact ionization method.

These developments have provided a fresh impetus in the area of natural product chemistry, which is reflected by the rapidly growing number of new natural products being discovered from terrestrial and marine plant and animal kingdoms. In view of the growing importance of natural products to man, it was felt that there was a strong need for a series of volumes which would highlight the latest developments in natural product chemistry, with particular reference to new techniques being developed for isolation and structure elucidation and their applications to the solution of structural problems, and which would focus attention on other related areas such as biosynthesis, cell structure, biotechnology, etc. The field of stereoselective synthesis of natural products is deliberately avoided in this volume as a separate sub-series on this topic is being published under my editorship by Elsevier as a part of the same series.

The present volume covers developments on a broad front of natural product chemistry. The first several chapters are concerned with developments in mass spectroscopy, NMR spectroscopy and circular dichroism, highlighting their applications to structure elucidation of natural products. The next few chapters present work carried out on the isolation and structure elucidation of new natural products from medicinal plants and liverworts. The last three chapters are devoted to polysaccharides from protozoa, biotechnology, and

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biosynthetic studies in plant tissue culture. All the authors are well known for their prolific and important contributions in this field, and the articles should prove to be of interest to a large number of organic chemists, phytochemists, medicinal chemists and biochemists.

I wish to express my gratitude to Miss Khurshid Zaman for her assistance in preparing the index of the manuscript and Mr Mahmood Alam for secretarial assistance.

August 1988

Atta-ur-Rahman, Editor

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## Structure Elucidation





## CHEMICAL IONIZATION MASS SPECTROMETRY WITH NITRIC OXIDE (NO) AS REAGENT GAS\*

H. BUDZIKIEWICZ

## 1. INTRODUCTION

"Preliminary results indicate that it may be possible to identify the functional group present in many organic compounds from chemical ionization mass spectra recorded with nitric oxide as the reagent gas". This statement was made in 1972 by D.F. Hunt (ref. 2), one of the pioneers of the practical application of Chemical Ionization (CI) mass spectrometry. Now, 15 years later one can say that the importance of NO as a reagent gas does not lie in the identification of functional groups but rather in the location of double and triple bonds in aliphatic compounds. In addition, NO demonstrates perhaps more than any other gas used in CI the potential strengths and weaknesses of this technique.

## 2. PROPERTIES OF THE REAGENT GAS

NO produces the plasma ions  $\text{NO}^+$  and  $(\text{NO})_2^+$ . It has an ionization energy (IE) of 9.25 eV, the recombination energy of  $\text{NO}^+$  has, however, been estimated as 8.3 eV or possibly about 0.5 eV higher (ref. 3). Therefore, ionization by charge exchange (CE) is to be expected for compounds with an IE of about 9 eV or below (ref. 3). CE can be fostered by mixing NO with  $\text{N}_2$  (or another inert gas) (refs. 4-6) or by using  $\text{N}_2\text{O}$  (refs. 7,8) (which also produces  $\text{NO}^+$  but has a rather high IE of 12.9 eV). In this way  $\text{M}^+$  and characteristic EI-type fragment ions can be obtained. Examples have been reported from the steroid and alkaloid field.  $\text{NO}^+$  reacts as an electrophile; thus  $[\text{M} + \text{NO}]^+$  will usually be observed with compounds possessing either a  $\pi$ -system (refs. 2,7,9,12) or a nonbonding electron pair (refs. 2,9,17,18) (olefins, alkynes, alcohols, ethers, esters). The  $[\text{M} + \text{NO}]^+$  ions may, however, be rather unstable and, therefore, of low intensity or even missing in the mass spectrum. In addition, having a hydride affinity of 1028 kJ/mole (ref. 7) NO can abstract  $\text{H}^-$  (resulting in  $[\text{M} - \text{H}]^+$ ) even from alkanes.

NO has oxidizing properties, thus species arising from the loss of  $\text{H}_2$  have been reported frequently (e.g.,  $[\text{M} - 3\text{H}]^+$ , i.e.  $\text{C}_n\text{H}_{2n+2} \rightarrow \text{C}_n\text{H}_{2n} \rightarrow \text{C}_n\text{H}_{2n-1}^+$  from

\* Part XV of the series "Studies in Chemical Ionization Mass Spectrometry." For part XIV see (ref. 1).

alkanes) (ref. 10). It has been suggested that primary ( $-\text{CH}_2\text{OH} \rightarrow -\text{CHO} \rightarrow -\text{C}\equiv\text{O}^+$ , i.e.  $[\text{M} - 3\text{H}]^+$ ), secondary ( $>\text{CHOH} \rightarrow \text{C}=\text{O} \rightarrow [\text{M} - 2\text{H} + \text{NO}]^+$ ) and tertiary alcohols ( $\text{C}(\text{OH})_3 \rightarrow \text{C}^+$ , i.e.  $[\text{M} - \text{OH}]^+$ ) can be distinguished from each other in this way (refs. 2,17). These dehydrogenation reactions, however, depend strongly on experimental parameters (NO pressure, temperature, catalytic effects of metal surfaces), and with modern instruments frequently they are not observed at all.

### 3. REACTIONS OF $\text{NO}^+$ WITH FUNCTIONAL GROUPS

After a promising start (ref. 2) the reactions of the various functional groups have not been investigated systematically. From the limited literature data available the following conclusions can be drawn (the formation of  $\text{M}^+$  by charge exchange and ions formed by the fragmentation of  $\text{M}^+$  as well as oxidation reactions of the unionized compounds will not be mentioned):

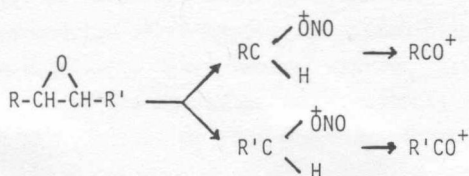
*Carboxylic acids* and their methyl esters (refs. 11-13) give  $[\text{M} + \text{NO}]^+$ ,  $[\text{M} + \text{NO} - \text{OH}(\text{OR})]^+$  and  $[\text{M} - \text{H}]^+$ , esters with higher alcohols also  $[\text{M} + \text{NO} - \text{ROH}]^+$ ,  $[\text{M} + \text{NO} - (\text{R} - \text{H})]^+$  and, interestingly,  $[\text{M} + \text{NO} - \text{H}_2\text{O}]^+$  (cf. Fig. 4).

*Aldehydes* (refs. 2,9,14) give  $[\text{M} + \text{NO}]^+$  and  $[\text{M} - \text{H}]^+$  (in most cases the H from the CHO-group is lost exclusively), while *ketones* (refs. 2,9) give  $[\text{M} + \text{NO}]^+$ .

*Alcohols* give  $[\text{M} - \text{H}]^+$  (primary and secondary) (refs. 9,11) and/or  $[\text{M} - \text{OH}]^+$  (secondary and tertiary) (refs. 2,9,17). A distinction by the dehydrogenation reactions mentioned above is not reliable (ref. 11).

*Dialkyl ethers* (ref. 9) give  $[\text{M} - \text{H}]^+$ , the only alkyl aryl ether mentioned in the literature, viz. anisole (ref. 9)  $[\text{M} + \text{NO}]^+$ .

Long chain aliphatic *epoxides* (refs. 15,16,18) besides the quasi molecular ions  $[\text{M} + \text{NO}]^+$  and  $[\text{M} - \text{H}]^+$  give two abundant fragments of the structure  $\text{R}-\text{C}\equiv\text{O}^+$ , the precursors of which are short-lived  $[\text{R}-\text{CH}=\text{ONO}]^+$  ions (which are structurally identical with the adducts of NO to aldehydes) as could be shown by constant neutral loss and collision activation studies. The presence of OH,  $\text{OCOCH}_3$  or CHO groups in the molecule does not interfere. CI (NO) may thus be used for the location of an epoxide ring in an aliphatic compound.



### 4. REACTIONS OF $\text{NO}^+$ WITH HYDROCARBONS

#### 4.1 General Remarks

Aromatic hydrocarbons (refs. 3,9,19) give  $[\text{M} + \text{NO}]^+$  and/or (by charge exchange)  $\text{M}^+$ , alkanes (ref. 10)  $[\text{M} - \text{H}]^+$  (only highly branched ones in addition

to  $M^+$  abundant fragment ions by cleavage of the most highly strained bond). From olefinic and acetylenic compounds, however, very characteristic fragments can be obtained and those compounds have, therefore, been investigated in considerable detail.

The reactions of  $NO^+$  which may be used for the location of double or triple bonds in aliphatic compounds typically consist in an electrophilic addition to the  $\pi$ -system with subsequent hydrogen migrations frequently accompanied by more than one bond formation and bond cleavage step which may even involve the intervention of a functional group present in the molecule (in such cases the distance between the latter and the site of unsaturation plays an important role). These complex sequences of reactions involving cyclic transition states are of necessity rather slow processes. There is tentative evidence (ref. 20) that the residence time in the ion source influences the relative abundance of such ions. A major problem is a competing fragment formation starting from  $M^+$  formed by CE. The recombination energy of NO (probably between 8.3 and 9.0 eV, *v. supra*) keeps CE and thus the formation of unspecific hydrocarbon ions at a relatively low level provided the source temperature is kept low. As can be seen from Fig. 1 by going from 60°C to 180°C the abundance of the N-containing ions formed from octadecene-5 drops almost to zero while that of the hydrocarbon ions goes up. Other examples will be mentioned below. The effect of the NO pressure is less pronounced; only at low values the rate of interaction of  $NO^+$  with the substrate decreases drastically. Collision activation seems to be a promising technique to suppress the undesirable hydrocarbon ions (ref. 23), but little has been done in this respect so far.

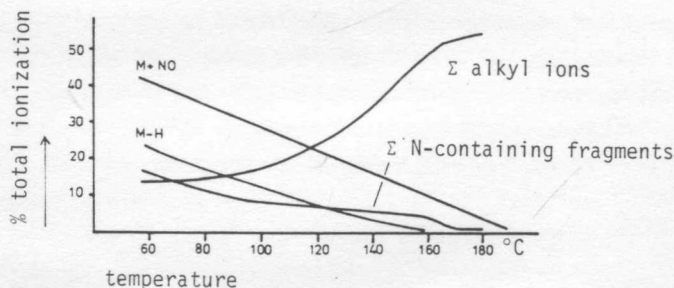


Fig. 1 Temperature dependance of the various types of ions in the CI(NO) spectrum of *n*-octadecene-5.

## 4.2 Compounds with double bonds

### 4.2.1 Alkenes with one double bond

For 1-alkenes a series of ions of the composition  $(\text{CH}_2)_n\text{NO}$  of high abundance can be observed the intensity maximum of which shifts from  $n = 4$  ( $m/z$  86) to  $n = 6$  ( $m/z$  114) with increasing chain length (refs. 7,21) (see Fig. 2). For the genesis of these ions Markovnikov-oriented addition of  $\text{NO}^+$  to the olefin followed by a series of hydride shifts, cyclisation and olefin elimination by a McLafferty rearrangement has been suggested (ref. 21).

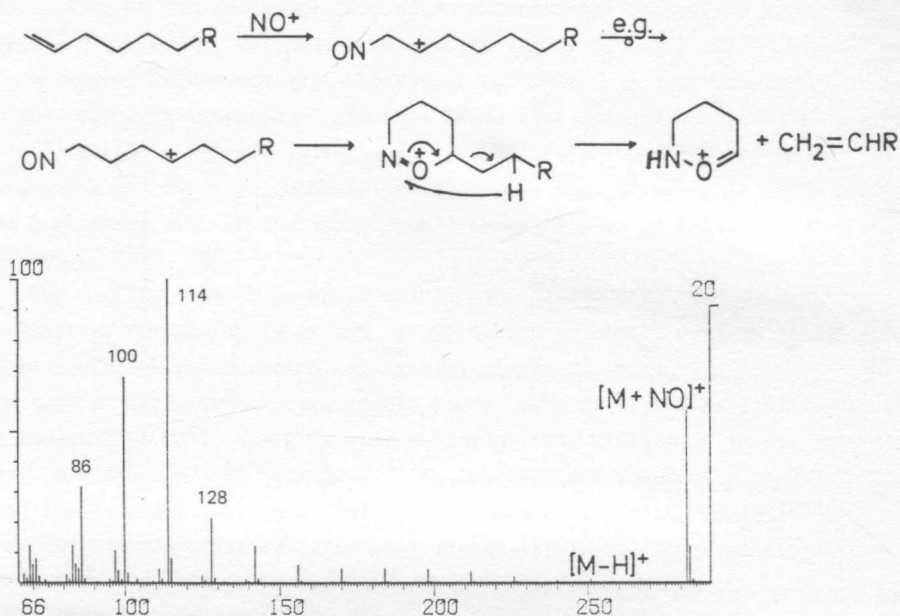


Fig. 2 CI(NO) spectrum of octadecene-1

This mechanism has not been substantiated by D-labelling (cf., however, below), but it is in agreement with the observation (ref. 21) that branching of the hydrocarbon chain results in a stabilization of the carbenium ion at the branching site which makes further hydride shifts less favourable. Thus, for 2-methyl alkenes the characteristic fragments are of low abundance (O-attack at C-2 in the cyclization step would result in a four-membered ring), for 3-methyl alkenes formation of a five-membered ring is enhanced, etc.

For alkenes with in-chain double bonds (ref. 7) (Fig. 3) by the same mechanism two series of  $(\text{CH}_2)_n\text{NO}$  ions are obtained by addition of  $\text{NO}^+$  to either end of the double bond. Here the high specificity of the H transfer during the olefin elimination has been demonstrated by labelling studies. Two further ions



also belong formally to the  $(\text{CH}_2)_n\text{NO}^+$  series; they are formed by highly specific McLafferty rearrangement reactions resulting in the cleavage of the vinylic bonds (a,  $m/z$  128 and 212 in Fig. 3).

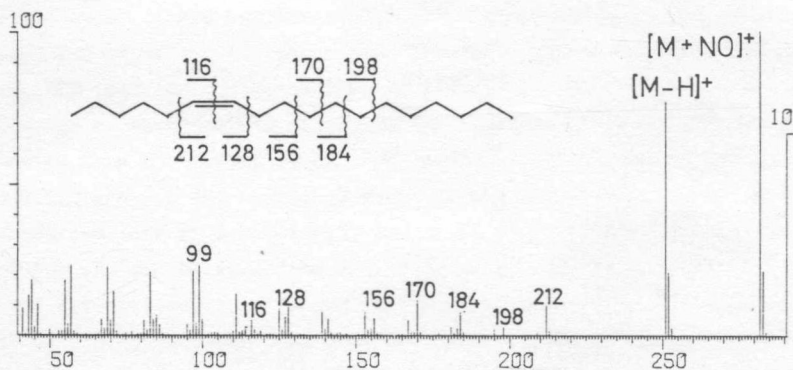
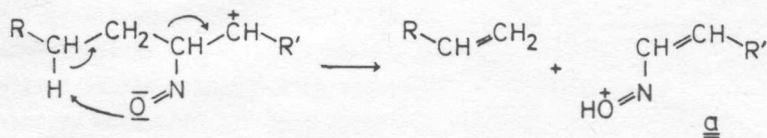
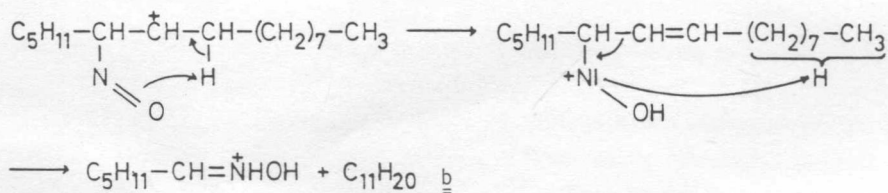


Fig. 3 CI(NO) spectrum of Z-octadecene-6

In addition, cleavage of the double bond by a rearrangement process with one specific and one unspecific H-transfer leads to a fragment of the composition  $\text{C}_n\text{H}_{2n+2}\text{NO}^+$  the bigger part of the molecule being lost preferentially (refs. 7,24) (b,  $m/z$  116 in Fig. 3).



The intensity of the fragments mentioned before is relatively low and, therefore, they are easily obscured by hydrocarbon ions at higher source temperatures.

One further ion characteristic for the position of the double bond has to be discussed here. It is formed by cleavage of the double bond and has the