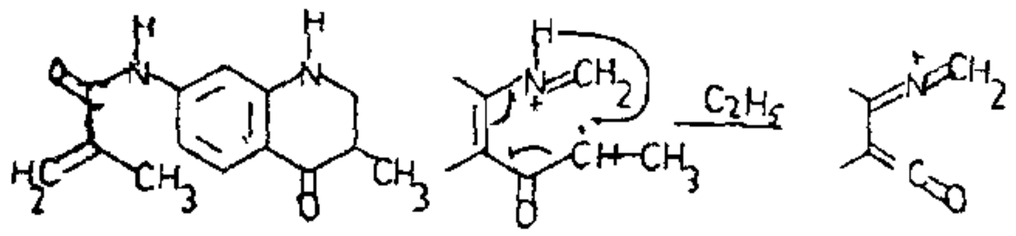
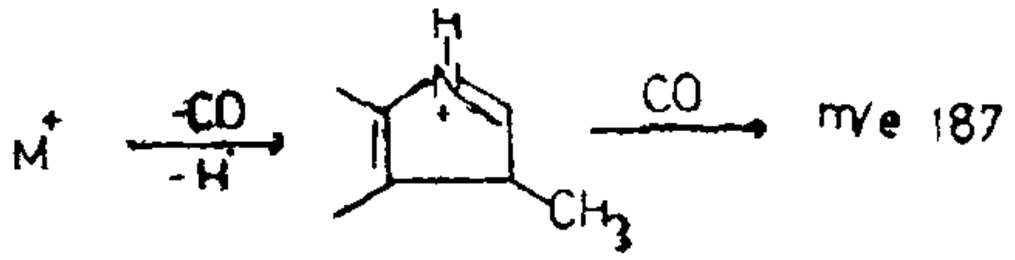
MASS SPECTRAL FRAGMENTATION PATTERN OF SOME COMPOUNDS OBTAINED BY REACTION OF PHENYLENEDIAMINES WITH ACRYLIC ACIDS

IN a previous communication[1] we have reported the formation of some new nitrogen heterocycles obtained by the reaction of phenylenediamines with acrylic and methylacrylic acids in the presence of PPA. The structures of these compounds were assigned on the basis of their spectral analytical evidence. Their mass spectra apart from giving the molecular weights present some interesting fragmentation patterns which are discussed here.

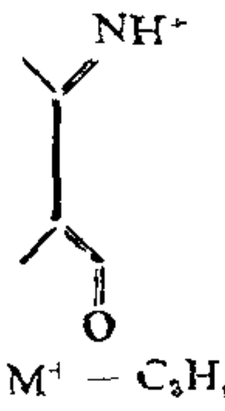
The mass spectrum of I, (M 244) shows a peak at m/e 229 ($M-CH_3$) and at m/e 201 due to loss of carbon monoxide. Further, I undergbes α cleavage as shown followed by loss of C_2H_5 to give a fragment corresponding to m/e 215 which loses CO to give the peak at m/e 187.



Alternatively, the fragmentation could occur as follows.



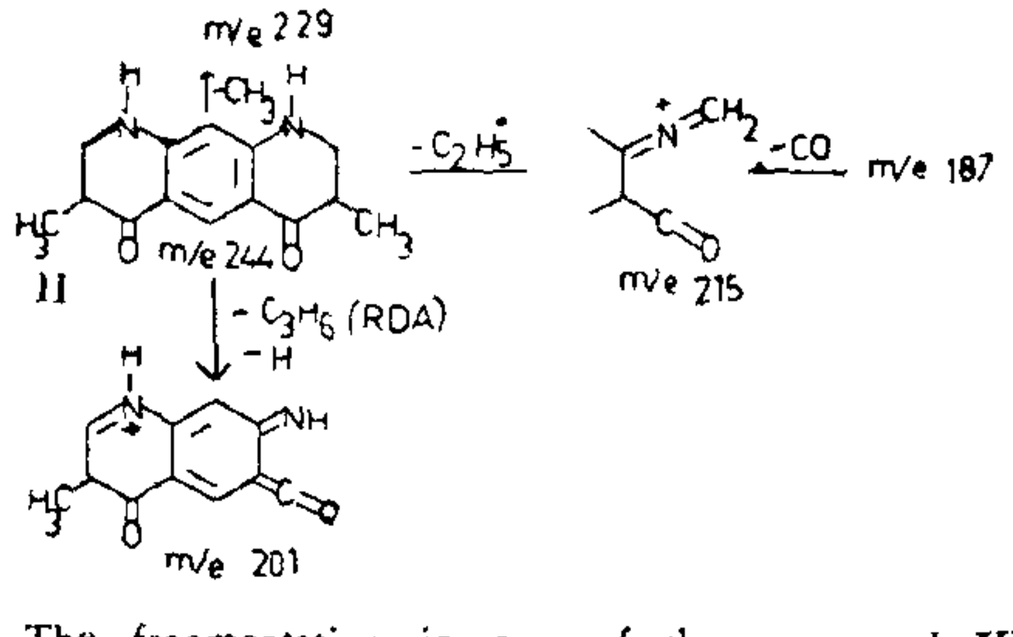
A retro Diels Alder reaction possibly occurs giving NH^+ which by loss of H° accounts for the peak at



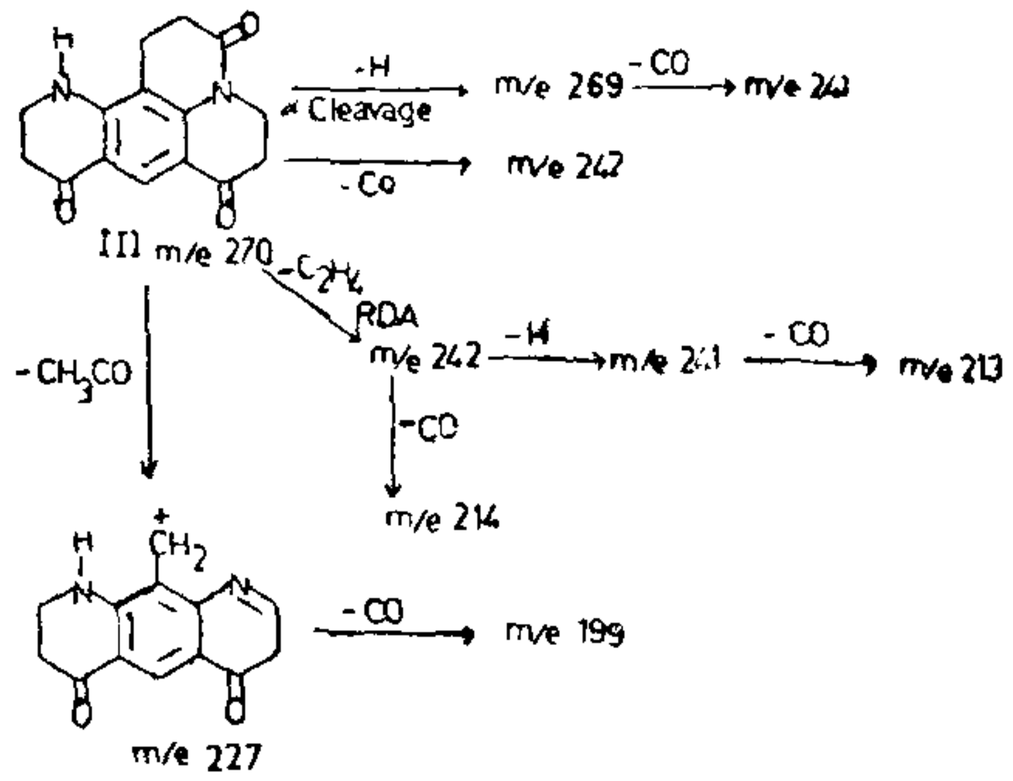
$M^+ - C_3H_5$

m/e 201 which on loss of CO gives the peak at m/e 172 and loss of $CH_2^\bullet$ gives m/e 187. The peak at m/e 69 corresponds to the fragment $CH_3-\underset{\underset{CH_2}{\|}}{C}-CO$ which eliminates CO to give the C_3H_5 peak (m/e 41).

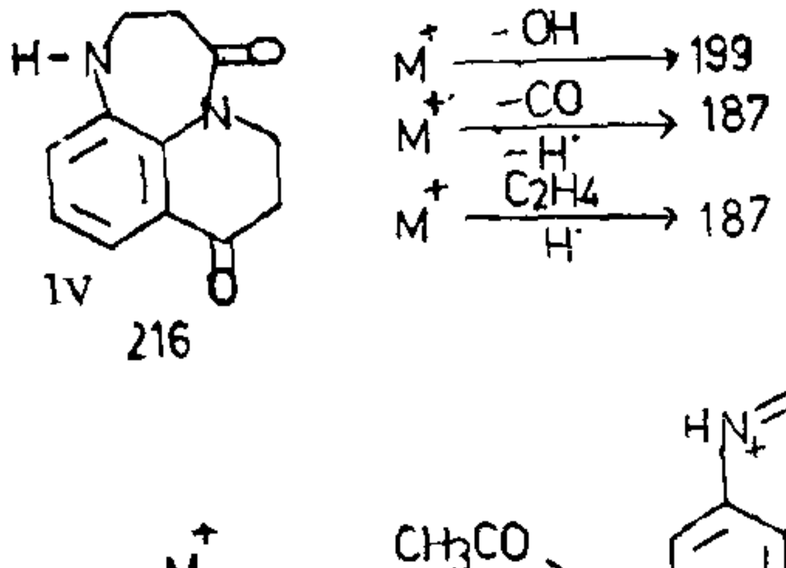
The appearance of the various peaks in the mass spectrum of II could be rationalised by the following fragmentation pattern,

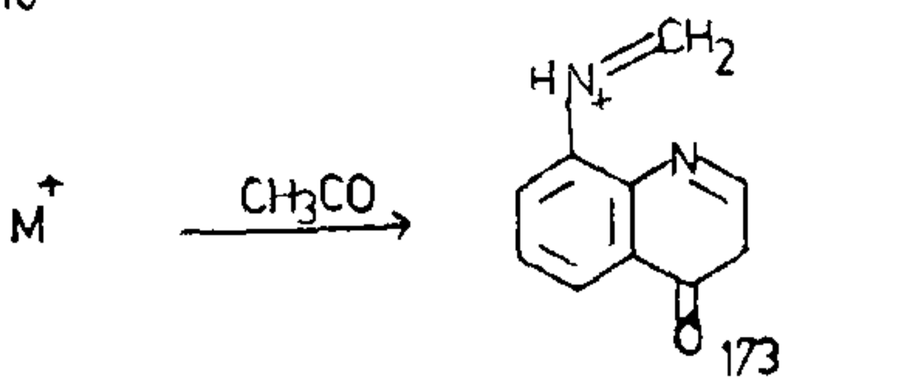


The fragmentation in case of the compound III could be explained as indicated below :



In the case of the compound IV the following fragmentation scheme could be envisaged.





Thanks are due to Professor H. Budzikiewicz, W. Germany, for the mass spectra.

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Bombay 400 032,
January 20, 1978.

1. Merchant, J. R. and Chothia, D. S., *Indian J. Chem.*, 1975, **13**, 814.

INDIRECT QUANTITATIVE COMPLEMENT CONSUMPTION ASSAY

Introduction

ONE of the major advances in the area of complement fixation has been the development of improved complement fixation procedures for the detection and estimation of antigen and antibody. The main stream of contribution has been based on 50% hemolytic unit of complement ($C'H_{50}$), which has been defined as the reciprocal of the volume of antiserum which fixes 50 out of 100 $C'H_{50}$ employed in the optimally reactive dilution of antigen[1]. However, the unit thus defined has relevance with reference to the test system used and has not been conducive to be considered on absolute terms. In addition, one or both of the antigen or antiserum are often anticomplementary and give rise to non-specific complement fixation. The above definition does not clearly exclude these events of complement consumption. In our work, we have established a test system which gives rise to quantitative complement fixation analysis and this is conducive to a definition of complement fixation unit, which, though not absolute, is qcantitative. In wost immunological systems, the antigen is often anticomplementary; the antibody is sometimes anticomplementary and at times both antigen and the antibody have anticomplementary activity. In such cases, the use of our system makes feasible quantitation of complement consumption due to antigen alone, due to antibody and due to antigen plus antibody and thereby we can determine the specific consumption due to antigen antibody interaction.

Materials and Method

Diluent : Isotonic Veronal buffer with 0·0005 M $MgCl_2$, 0·00015 M $Cacl_2$ and 0·1% Bovine Serum Albumin.

Complement (C) : Lyophilized guinea pig serum, obtained from Cappel Laboratories. The lyophilized complement was reconstituted to ihe original volume using the diluent purchased from Cappel Laboratories. Aliquot volumes were stored at –20° C.

Hemolytic antibody (*A*) : Rabbit antisheep erythrodyte serum was purchased from Colorado Serum Company Laboratories.

Sheep Erythrocytes (*E*) : Sheep Red Blood Cells (SRBC) preserved in Alsevers solution was obtained from Colorado Serum Company Laboratories.

Well washed SRBC are suspended 2% in diluent. Hemolysin is diluted 1 : 32 to 1 : 8192 by serial dilution. To each tube is added an equal amount of Sheep Red Blood Cells to obtain a 1% suspension. After incubation for 30 minutes at room temperature, one agglution unit of sheep cell hemolysin titration is determined (Table I).

Quantitative complement fixation analyses were performed as follows. Four sets of serial dilution of complement were incubated overnight at 4° C: one set with known amount of antigen in all the tubes: the second set with appropriate dilutions of antibody; the third set with both antigen and antibody in the same dilutions as used in the first two sets; and the fourth set with diluent as control. The next day, to all the tubes were dispensed a fixed amount of sensitized (SSRBC) to one agglutinating unit and reaction mixtures were incubated for 30 minutes at 37° C. At

TABLE I

Determination of units of agglutination of 1% SRBC titrated against anti-SREC

	Dilution of anti-SRBC						
	1 : 32	1 : 64	1 : 228	1 : 256	1 : 512	1 : 1024	1 : 2048
SRBC + anti-SRBC	+	+	+	+	⊕	–	–
Units of Agglutination	16	8	4	2	1	½	¼

⊕ indicated 1 agglutinating unit,