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Summary

NMR spectra of phenazine derivatives were completely analyzed by the theoretical treatment of observed signals. These assignments have shown the proton signals of phenazine as A_2B_2 type.

Various J values have been testified by the examination of the spectra of substituted derivatives. These analyses are expected to be effective tools for structural elucidation of synthetic unknown substituted phenazine derivatives arising from the ambiguity of their synthetic routes.

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59. Yutaka Morita : Studies on Phenazines. XXIX.*1 Mass Spectrometric and Chromatographic Studies.*2

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Recently, mass spectrometric studies on variety of compounds, such as petroleum components, alkaloids, terpenes, steroids and synthetic compounds, have been reported[1] and the accumulated generalization of their fragmentation pathways has been shown to be quite useful tool for clarifying the unknown substances further obtained. Moreover, the structural problems of natural products have been effectively studied by usage of mass spectrometry especially on the minor components of alkaloids, terpenoids and steroids with relation to their analogues.

While on polycyclic aromatic hydrocarbons, relatively little work has been done except principal source of information by the API-catalog.[2] Unsubstituted aromatic hydrocarbons such as naphthalene, chrysene, and pyrene have been dealt in the catalog indicating only very little fragmentations. The reason of which is understood by the stable aromatic cations.

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*1 This is one of the series of Studies on Phenazines (I. Yosioka). Part XXIII, preceding pages.

*2 This item was presented at the 85th Annual Meeting of the Pharmaceutical Society of Japan (Tokushima, October, 1965).

*3 Toneyama, Toyonaka, Osaka-Fu (森田 豊).

1) *a*) J. H. Beynon : "Mass Spectrometry and its Application to Organic Chemistry," (1960). Elsevier, Amsterdam. *b*) K. Biemann : "Mass Spectrometry, Organic Chemical Applications," (1962). MacGraw-Hill, New York. *c*) H. Budzikiewicz, C. Djerassi, D. H. Williams : "Interpretation of Mass Spectra of Organic Compounds," (1964). Holden-Day, San Francisco. "Structure Elucidation of Natural Products by Mass Spectrometry," (1964). Vol. I, Alkaloid; Vol. II, Steroid, Terpenoides, Sugars, and Miscellaneous Classes. Holden-Day, San Francisco.

2) American Petroleum Institute Research Project 44 : "Catalog of Mass Spectral Data" Carnegie Institute of Technology, Pittsburgh.

On heteroaromatic compounds including nitrogen atom, they are reported by Beynon,[3] Biemann[4] and others,[5] but there are upon the whole insufficient data available to be generalized on tricyclic N-containing aromatic compounds such as carbazols, acridines, and phenazines.

In this paper, the mass spectra of several phenazine derivatives are dealt with and their fragmentation paths are estimated showing the stability of phenazinium cation and simple fragmentation patterns which are quite similar to the other aromatic compounds such as anthraquinone[6] and other heteroaromatic tricyclic compounds.[7]

The following phenazine derivatives were analyzed; phenazine, phenazine-mono-N-oxide, phenazine-di-N-oxide, 1-methyl-, 1-methoxy-, and 2-methoxy-phenazines.

Table I. Methods of Chromatographies

a) Thin-layer chromatography (TLC)
Thickness of layer : 250 μ
Adsorbents : Silicic acid (Kiesel-gel nach Stahl, Merck)
Alumina (Aluminum-oxyd nach Stahl, Merck)
b) Gas liquid chromatography (GLC)
Instrument : YANAGIMOTO GCG-3DH
Stationary phase : SE-30 (1% on Chromosorb-W)
0.5×200 cm³ column
Detector : Super High Sensitive FID

Table II. Normalization of Figures

TLC : Rp; Rf-value relative to the Rf-value of phenazine
GLC : RTp; Retention time relative to the retention time of phenazine
All normalizations were carried out with many different conditions.

Then the chromatographic behaviors (thin-layer and gas liquid chromatographies) of various phenazines listed in Table III were examined. Since in the case of phenazine syntheses, adsorption chromatographic technique is almost inevitable for their purification and sometimes separation from their substituent isomers possibly arising from the synthetic ambiguity, the systematic information on their chromatographic movements must be quite useful.

Recently, many reports on gas chromatographic studies on aromatic hydrocarbons including heteroaromatic compounds have been reported,[8] however, no work on phenazine derivatives has been seen. Therefore, the present paper describes interrelation of various chromatographic data of phenazines aiming the clarification of relation between polarity of compounds and their chromatographic behaviors.

3) J. H. Beynon : page 397～403 of lit. 1 *a*).
4) K. Biemann : in F. W. MacLafferty ed. "Mass Spectrometry of Organic Ions," 534 (1963). Academic Press, New York.
5) J. Kontecky : Collection Czech. Chem. Commun., **24**, 1609 (1959); V. Hams, V. Cermack : *Ibid.*, **24**, 1602 (1959).
6) J. H. Beynon : page 272 of lit. 1 *a*).
7) API-catalog No. 633.
8) *a*) J. Hoigne, H. Widmer, T. Cäumann : J. Chromatog., **11**, 459 (1963). *b*) D. Schopov, N. Kotsev, K. Georgieva : Acta Chim. Acad. Sci. Hung., **37**, (2) 137 (1963). *c*) J. Januk, M. Hrivnac : Collection Czech. Chem. Commun., **25**, 1557 (1960). *d*) A. W. Decora, G. U. Dineen : Anal. Chem., **32**, 164 (1960).

Results and Discussion

A) Mass Spectrometric Studies

i) Phenazine and Its N-Oxides

In Fig. 1, the mass spectra of title compounds are drawn.

There is no marked difference among the spectra of these compounds. In the spectra of mono-N-oxide and di-N-oxide, deoxygenations take place in stepwise mode degradation to phenazinium cation, m/e 180 (base peak). These features disclose the stability of phenazinium cation as expected. The spectra of N-oxides are completely identical except their molecular peaks and present the same mass number peaks as seen in the spectrum of phenazine itself. Hence, the main path of fragmentation of these N-oxides seems to start from m/e 180 of phenazinium cation.

The first step for fragmentation of phenazine is the removal of nitrogen resulting M-28 ion (m/e 152). This fragmentation is certified by the existence of metastable peak at about m/e 128 presenting unordinary signal feature broadened in its foot of signal.

A peak at m/e 152 appears to be a signal of *o*-biphenylene which is known in the spectra of anthraquinone and fluorenone.[6] Towards smaller mass numbers, the same

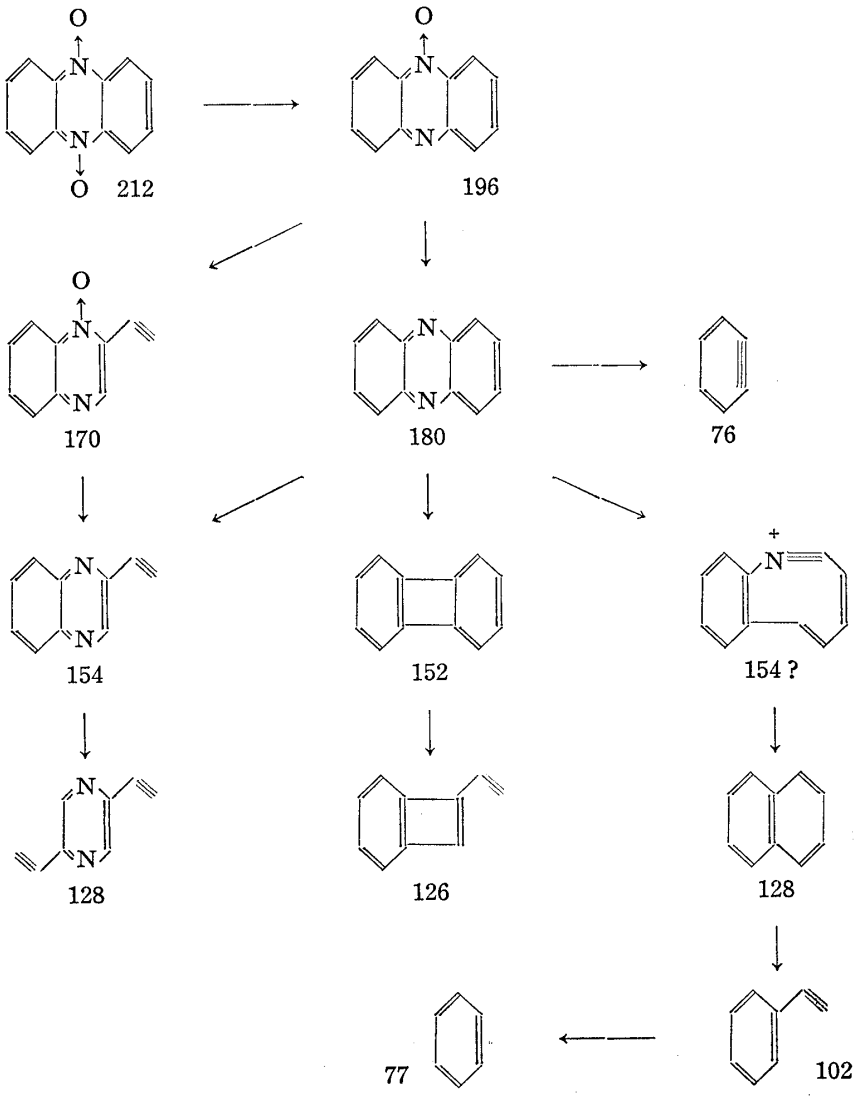

Chart 1. Fragmentations of Phenazine and Its Oxides

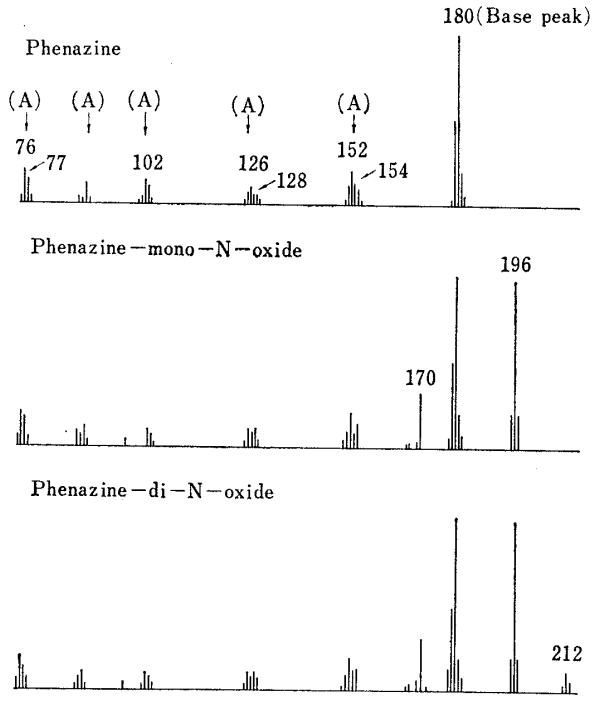

Fig. 1. Mass Spectra of Phenazine and Its Oxides

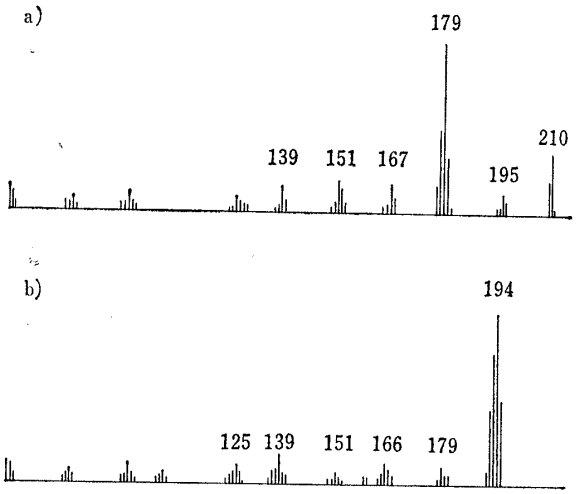

Fig. 2. Mass Spectra of Substituted Phenazines
a) 1-, and 2-methoxyphenazine
b) 1-methylphenazine

mass number peaks are found in Fig. 1 as for anthraquinone's, which are designated by arrows (A).

The only different peak in N-oxides comparing to phenazine is found at m/e 170 and considered to be caused by a loss of acetylene from mono-N-oxide ion. The existence of this fragmentation mode is justified by the metastable peak at m/e 147.

The tentative structural elucidation of the above mentioned fragmentations is afforded in Chart 1 with their mass numbers.

As a whole, it is pertinent to say that the base peak is phenazinium cation itself in any case of unsubstituted phenazines and then the loss of nitrogen molecule occurs at earlier stage suggested by the weak peaks of N-containing fragments.

ii) 1-Methyl-, 1-Methoxy- and 2-Methoxy-phenazines

In Fig. 2 mass spectra of these three compounds are presented. Comparing to the spectra of the above described unsubstituted phenazines, the following peaks are newly observed, m/e 195, 167, and 139 for 1-methoxy- and 2-methoxy-phenazines and m/e 193, 167, 166 and 139 for 1-methylphenazine in addition to their molecular ion. And as a whole, peaks of one mass unit smaller comparing to phenazine's are found in further fragmented peaks of these compounds suggesting fragmentations started with early loss of substituents.

The most probable explanations of those fragmentations are given in Chart 2 for methoxyphenazines and in Chart 3 for 1-methylphenazine respectively.

Unexpectedly, 1-methoxyphenazine and 2-methoxyphenazine exhibited entirely same fragmentation pattern, which could be rationalized by the rapid loss of substituent and the stability of m/e 179. On methylphenazine, ordinary ring enlargement and ring contraction are found as in alkylated benzene.[9]

9) J. H. Beynon : page 340 of lit. 1*a*).

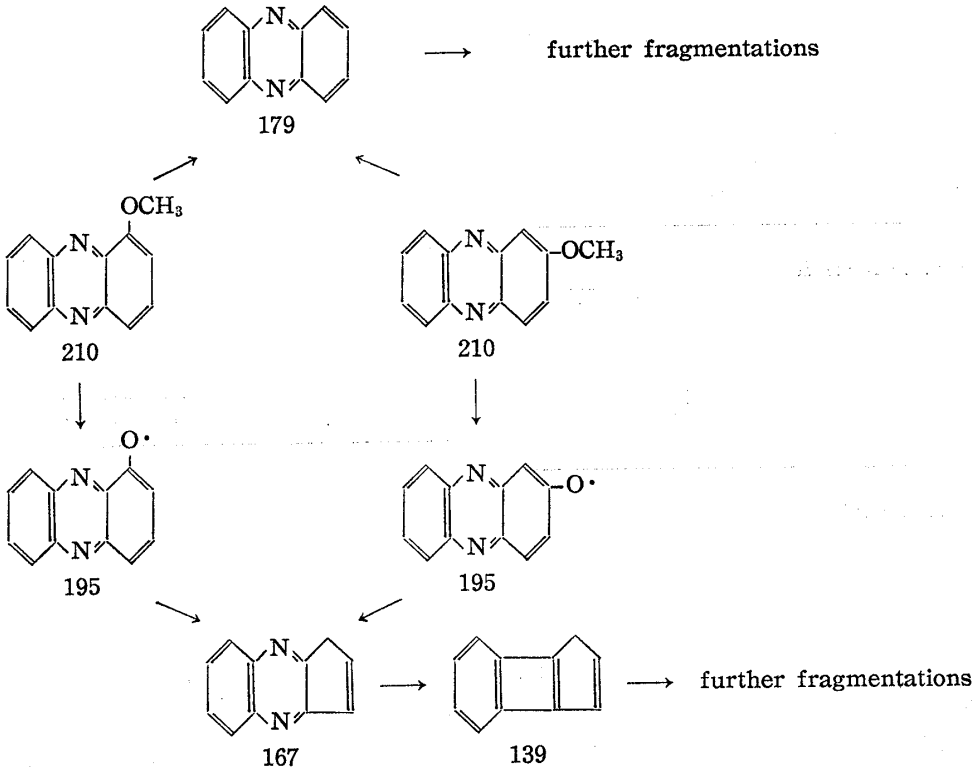

Chart 2. Fragmentations of Methoxyphenazines

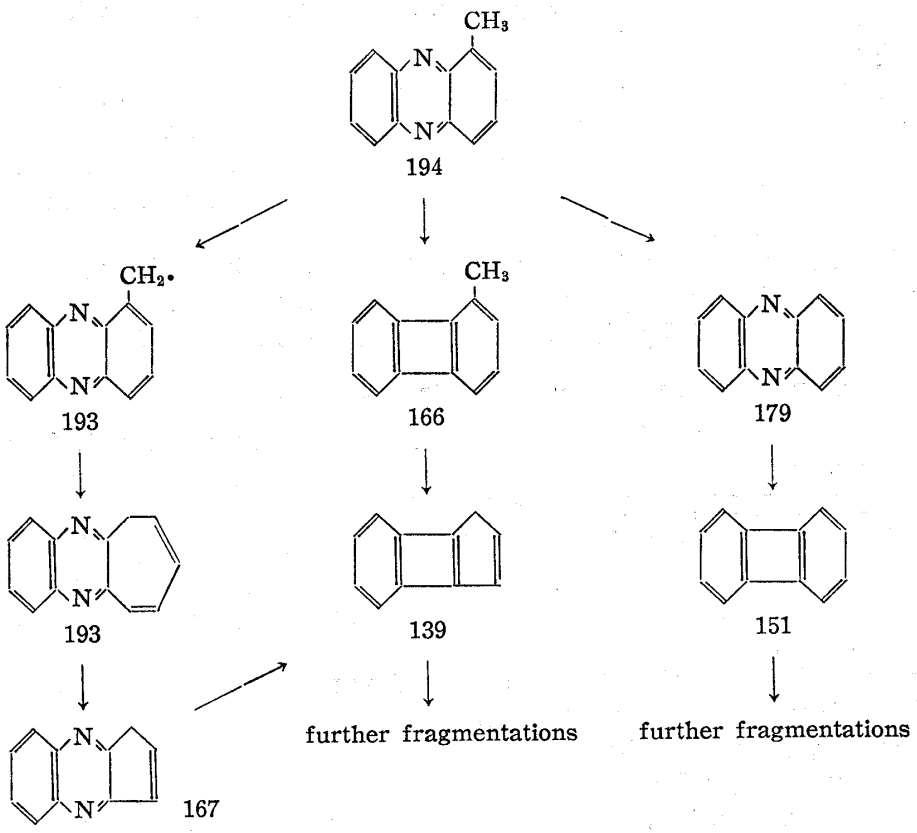

Chart 3. Fragmentations of 1-Methylphenazine

B) Chromatographic Studies

As is generally understood, the chromatographic technique for purification of synthesized phenazines is mainly based on their polarity arising from the kinds and location differences of substituents. By the methods shown in Table I the various chromatographies were performed. The data obtained are normalized as shown in Table II. Table III presents those data in the order of structural similarity. For comparison, there are demonstrated the dipole moments of substituted naphthalenes in Table IV.[10]

Table III. R_P and RT_P Values of Phenazines

Compound	R_P		RT_P
	Alumina	Silicic acid	SE-30
Phenazine	1	1	1
Mono-N-oxide	0.845	0.636	3.66
Di-N-oxide	0.213	0.109	1
1-CH_3O	0.635	0.305	3.72
2-CH_3O	0.911	0.622	3.03
1-CH_3	3.00	1.32	1.41
2-CH_3	2.50	0.903	1.62
1-Cl	1.45	1.65	2.42
1-Cl-5-oxide	1.24	1.12	2.47
2-Cl	1.39	1.45	1.80*
2-Cl-5-oxide	1.12	0.955	1.77
2-Cl-10-oxide	0.902	0.833	1.77
1,2-Di-Cl	1.61	2.26	3.80
1,3-Di-Cl	1.73	2.82	3.16
1,4-Di-Cl	1.82	3.36	4.18*
2,3-Di-Cl	1.65	2.30	3.88
1,9-Di-Cl	1.75	2.25	5.00
1,6-Di-Cl	2.02	3.10	4.99*
2,8-Di-Cl	1.89	2.69	3.25

Table IV. Dipole Moments of Halogeno-compounds

Compound	Dipolemoment (D)	Compound	Dipolemoment (D)
1,2-Dichlorobenzene	2.53	1,6-Dichloronaphthalene	1.44
1,3-Dichlorobenzene	1.67	1,7-Dichloronaphthalene	2.55
1,4-Dichlorobenzene	0	1,8-Dichloronaphthalene	2.82
1,2-Dichloronaphthalene	2.47	2,3-Dichloronaphthalene	2.55
1,3-Dichloronaphthalene	1.78	2,6-Dichloronaphthalene	0
1,4-Dichloronaphthalene	0.50	2,7-Dichloronaphthalene	1.53
1,5-Dichloronaphthalene	0		

The contribution of dipole moment of the compound has been believed to be a main factor for determining retention time in gas liquid chromatography (GLC).

In phenazine derivatives, relative retention times of N-oxides showed good agreement in this line, however, some of the presumed dipole moments (cf. Table IV) of chlorophenazine derivatives (asterisked) were shown inconsistent with the expected retention times. These facts seem to largely depend on the polarity of substituted

10) M. Kotake ed. : "Constants of Organic Compounds," 508 (1963). Asakura, Tokyo.

phenazine nuclei and adsorbing ability of those compounds. Further investigations on the subject is needed to clarify the abnormality of phenazine ring system, but these results obtained here are believed to be sufficient to predict the synthesized unknown phenazine's structure.

Experimental

Samples——All samples were synthesized by the known methods previously reported in the series of Studies on Phenazines (I. Yosioka, *et al.*) and in other's.[11]

Measurement of Mass Spectra——All mass spectra were measured at Hitachi Ltd., using a mass spectrometer HITACHI RMU-5B.

Measurement of Gas Liquid Chromatography——All GLC analyses were carried out by YANAGIMOTO GCG 3DH with Super High Sensitive FID.

The author is very indebted to Prof. I. Yosioka, Osaka University, for his kind encouragement throughout this work.

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Summary

Mass spectra of phenazine, phenazine mono-N-oxide, phenazine di-N-oxide, 1-methyl-, 1-methoxy-, 2-methoxy phenazines were analyzed. The results disclosed that their fragmentation pathways were comparable with the aromatic tricyclic compounds.

And chromatographic behaviors of phenazine derivatives were examined using mainly 20 kinds of chlorophenazines considering the polarisabilities of phenazine nuclei.

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11) G. A. Swan, D. G. I. Felton : "Phenazines," **69**, 622 (1957). Interscience, New York.