drug cations. The decreasing order of the Tp is O-benzoylthiamine > thiamine > N-methylephedrine > ephedrine > piperidine > N,N-dimethylaniline > N-methylaniline ≒ aniline > pyridoxine ≒ pyridine.

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UDC 547.864.03 : 543.422.25

58. Yutaka Morita : Studies on Phenazines. XXVII.*1 Nuclear Magnetic Resonance Studies. I.*2

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Recently, the dilution method and the double resonance technique have been applied to determine the chemical shift and coupling constant of ring protons in polycyclic aromatic compounds, such as triphenylene, chrysene, and pyrene.[1] The multiplicity of spin-spin coupling in these cases made unfavorable to analyse those data by using the ordinary theoretical treatment.

In some aromatic compounds, however, successful results have been obtained; naphthalene, lutidine, and alkylated benzenes.[2,3]

Among them, the NMR (nuclear magnetic resonance) spectrum of naphthalene has been studied by Pople, *et al.*[3] clarifying the existence of A_2B_2 system. In naphthalene nucleus, though eight protons look actually A_4B_4 type, they can approximately be dealt as a superposition of two sets of A_2B_2 type. In fact, the theoretical treatment of naphthalene protons assigned as A_2B_2 system has been shown in good agreement with the obtained data.

In the case of phenazine, which has also two pairs of equivalent protons in its molecule, the interrelation of protons appears quite similar to naphthalene. The present and following papers are dealing with the NMR analyses of phenazine and its derivatives with the complete assignment of their ring protons, thus expecting the possible elucidation of the chemical structures of phenazine derivatives, especially to determine the positions of ring substituents. First of all, the validity of A_2B_2 system in phenazine nucleus and the evaluation of their coupling constants (J) were examined using phenazine (I), phenazine mono-N-oxide (II), phenazine di-N-oxide (III). Secondly, by the inspections of the spectra of methoxy derivatives, the calculated various J values were demonstrated their correctness. These data are applicable to the analyses of numerous phenazine derivatives obtained by syntheses and from natural source. For these purposes, following methoxyphenazines were dealt with; *i.e.*, 1,3-dimethoxy- (V), 2,3-dimethoxy- (VI), 1,4-dimethoxy- (VII), 1,6-dimethoxy- (VIII), 2,8-dimethoxy- (IX), 1,9-dimethoxy- (X), and 1,6-dimethyl-4,9-dimethoxy-phenazine (IV). The latter compound

*1 This is one of the series of Studies on Phenazines (I. Yosioka). Part XXVI. Y. Kidani, K. Ukai: This Bulletin, **14**, 293 (1966).

*2 A part of this 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) R.H. Martin, N. Defay, F. Geerts-Evrard, S. Delavarenne : Tetrahedron, **20**, 1073 (1964).
2) H.J. Bernstein, J.A. Pople, W.G. Schneider : Can. J. Chem., **35**, 65 (1957).
3) J.A. Pople, W.G. Schneider, H.J. Bernstein : *Ibid.*, **35**, 1060 (1957).

of m.p. 250° was obtained by refluxing a benzene solution of 4-methoxy-2-nitrotoluene and 4-methoxy-2-aminotoluene under the existence of powdered potassium hydroxide and sodium amide.

Results and Discussion

A) Phenazine (I) and Its N-Oxides (II, III)

In Fig. 1, the NMR spectra of phenazine (I) and its N-oxides (II, III) are presented. As expected above, phenazine (I) gives a symmetrical pattern of characteristic A_2B_2 signals consisted of six significant peaks. In Fig. 1a, at the bottom of base line are given the energy values (c/s) corresponding to each transition shown in the parentheses.

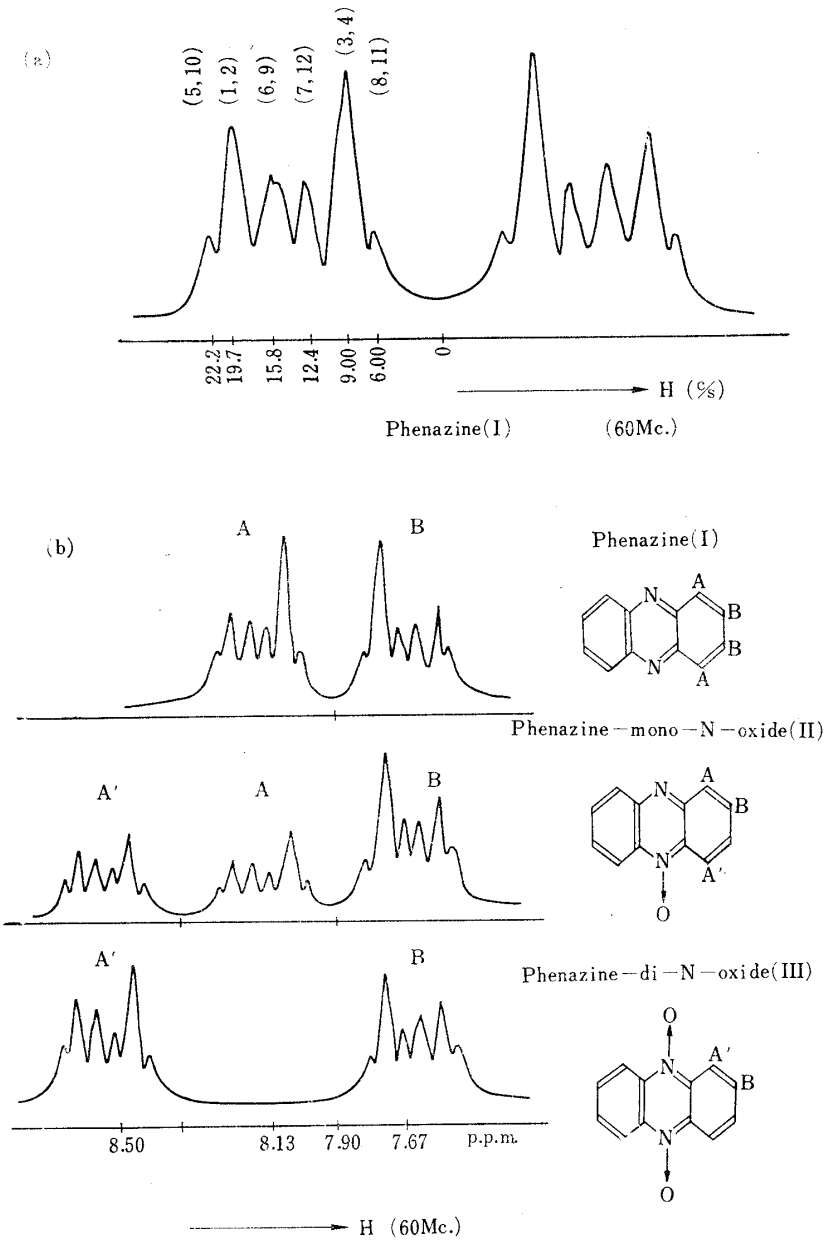


Fig. 1. Nuclear Magnetic Resonance Spectra of Phenazine and Its N-Oxides

These energy values were obtained by measuring the deviation of each signal from center of pattern.

Namely, the signals at 6.00 and 19.7 c/s are ascribed to the pairs of transition (3,4) and (1,2) respectively. There should be four separated signals between these two pairs from the theoretical point of view, but only two lines were observed. The two lines appearing at 12.4 and 15.8 c/s, however, look somewhat coupled respectively. Therefore, the assignment illustrated in Fig. 1a could be reasonable. On the basis of this assignment, it has become possible to calculate the values for the following parameters.

$$K = J_A + J_B, \qquad L = J - J'$$

$$M = J_A - J_B, \qquad N = J + J' \text{ (Fig. 2)}$$

Table I. Energy of A Transition for A_2B_2

Transition	Energy relative to $1/2\,(\nu_A+\nu_B)$	Transition	Energy relative to $1/2\,(\nu_A+\nu_B)$
1	$1/2\,N+1/2\,[(\nu_0\delta)^2+N^2]^{1/2}$	10	$1/2\,[(\nu_0\delta-M)^2+L^2]^{1/2}+1/2\,(M^2+L^2)^{1/2}$
3	$-1/2\,N+1/2\,[(\nu_0\delta)^2+N^2]^{1/2}$	11	$1/2\,[(\nu_0\delta+M)^2+L^2]^{1/2}-1/2\,(M^2+L^2)^{1/2}$
9	$1/2\,[(\nu_0\delta+M)^2+L^2]^{1/2}+1/2\,(M^2+L^2)^{1/2}$	12	$1/2\,[(\nu_0\delta-M)^2+L^2]^{1/2}-1/2\,(M^2+L^2)^{1/2}$

Conferring to Table I, the following values can be obtained.

$$N = E_1 - E_3 = 10.7 \text{ c/s}$$

$$(M^2+L^2)^{1/2} = E_9 - E_{11} = 9.80 \text{ c/s}$$

$$[(\nu_0\delta)^2+N^2]^{1/2} = E_1 + E_3 = 28.7 \text{ c/s}$$

$$[(\nu_0\delta)^2+M)^2+L^2]^{1/2} = E_9 + E_{11} = 21.8 \text{ c/s}$$

To get a complete set of the constants, a figure for K is needed. However, it seems impossible to obtain it in a direct manner by solving equations, In the case of naphthalene, Pople, *et al.*[3] have found J_A value to be zero, which manifested the negligible coupling between two protons existing in para-position.

In the present case, it could be reasonably assumed that $K=-M$, *i.e.*, $J_A=O$.

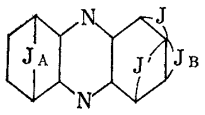
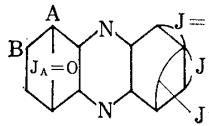


Fig. 2. Definement of Constants



$J=9.01$ %
$J_B=6.55$ %
$J'=1.67$%
$\nu_0\delta=27.1$ %
$\delta_A=8.13\pm0.02$ p.p.m.
$\delta_B=7.67\pm0.02$ p.p.m.

Fig. 3. Constants for Phenazine (I) (60 Mc.)

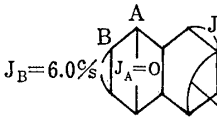


Fig. 4. Constants for Naphthalene (40 Mc.)

The complete set of constants can now be shown in Fig. 3. For comparison, the values for naphthalene presented by Pople, *et al.*[3] are given in Fig. 4.

Then the NMR spectra of phenazine mono-N-oxide (II) and phenazine di-N-oxide (III) were examined. These compound would show strong anisotropy caused by newly induced N-O linkage, which could shift the chemical shift of proton A to downfield as would be obtained in the case of aromatic nitro-compounds.[4]

The spectrum of mono-N-oxide exhibits two miniaturized sets (each corresponding to 2H) of six peaks in the area of A protons and entirely same pattern of B protons in phenazine itself (Fig. 1b). This indicates the chemical shift of two of A protons and four B protons are appeared at the original region. In the case of di-N-oxide (III), the spectrum obtained is quite similar shape to I except the both signal patterns of A and

4) P.L. Corio, B.P. Dailey: J. Am. Chem. Soc., 78, 3043 (1956).

B arisen apart with larger chemical shift difference (Fig. 1b). As is expected, J values of III are shown to be absolutely same as I. At the bottom of the base line, the calculated chemical shifts as for phenazine described above are presented.

Consequently, it has now become apparent that an introduction of N-O linkage on a phenazine nucleus causes downward shift of 0.37 p.p.m. to A proton (located in peri-position).

The confirmation of calculated J_B value (6.5 c/s in Fig. 3) was achieved by analysing the ring protons part in the spectrum of 1,6-dimethyl-4,9-dimethoxyphenazine (IV)

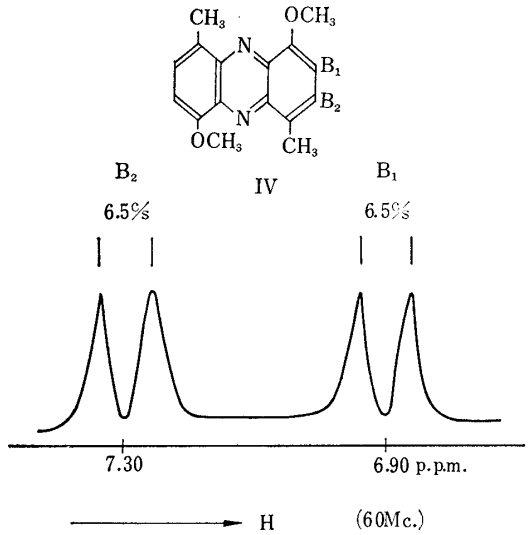


Fig. 5. Ring Proton Signals of 1,6-Dimethyl-4,9-dimethoxyphenazine (IV)

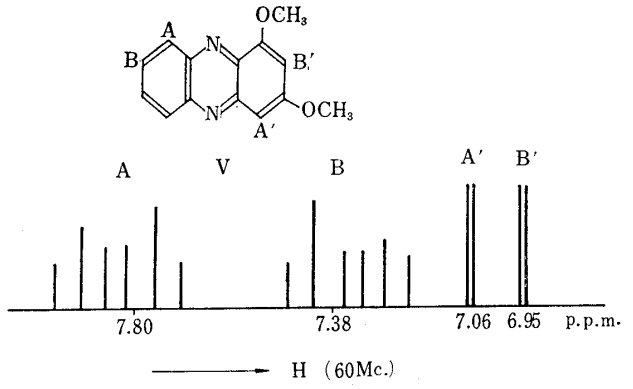


Fig. 6. Spectrum of 1,3-Dimethoxyphenazine (V)

(Fig. 5). Two separate doublets are found at 6.90 and 7.30 p.p.m. with J value of 6.5 c/s, which coincides nicely with the calculated value (J_B=6.55 c/s).

B) 1,3-Dimethoxyphenazine (V)

In Fig. 6, a schematic spectrum of the title compound is demonstrated. Considering its chemical structure, the signals due to ring protons of the left benzene moiety (designated as A, B) would appear as A_2B_2 type signals as in I and the signals ascribed to protons A′ and B′ may be observed at the separate higher field.

The spectrum of V exhibits one pair of characteristic A_2B_2 type pattern as was shown in I at slightly higher chemical shifts 7.80 and 7.38 p.p.m. for A and B protons, while there were obtained one pair of signals at 7.06 and 6.95 p.p.m., which looked to be doublet respectively with small J-value. The above described data (J′=1.67 c/s for phenazine (I)) would corroborate this findings.

Meta located methoxyl groups were found to afford marked influence on the both ring protons. In addition to the shielded signals of A′ and B′ protons, A, B protons are shown to be affected significantly appearing at higher field with approximate value 0.30 p.p.m. Furthermore, the noticeable feature of the signals ascribed to A,B protons is that $\nu_0\delta$ value and their pattern are fully preserved comparing to I.

C) 2,3-Dimethoxyphenazine (VI)

As is shown in Fig. 7, the compound shows one pair of six peaks (corresponding to 4H, A_2B_2 system) and a singlet (2H). The chemical shifts, δ_A and δ_B, can be obtained 7.92 and 7.62 p.p.m. The sharp peak at 7.21 p.p.m. also demonstrates somewhat influenced by the anisotropy of neighboring methoxyl function.

D) 1,4-Dimethoxyphenazine (VII)

In comparison with VI, VII contains in the substituted ring two equivalent B protons, which gave a single peak at 6.96 p.p.m. (Fig. 8).

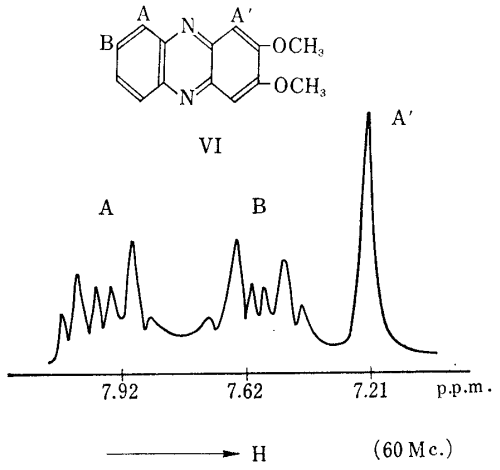


Fig. 7. Spectrum of 2,3-Dimethoxyphenazine (VI)

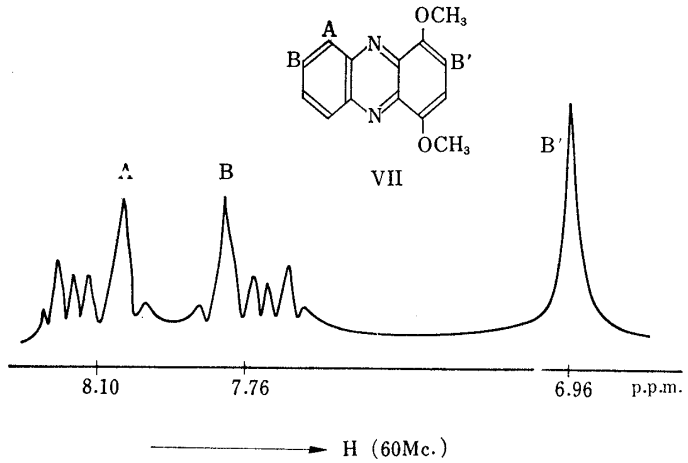
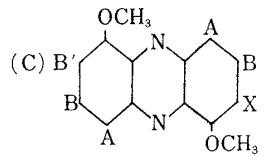


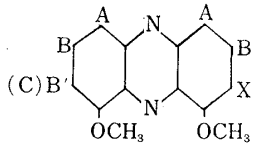
Fig. 8. Spectrum of 1,4-Dimethoxyphenazine (VII)

The unsubstituted ring protons, on the other hand, are found to be in A_2B_2 type, having δ_A and δ_B value at 8.10 and 7.76 p.p.m.

Three compounds (V, VI and VII) mentioned above contained their substituents only on one side of two benzene moiety in phenazine. Introduction of a methoxyl group on benzene ring causes generally some shielding effect as much as 0.23 p.p.m. in cyclohexane solution.[4] With infinite dilution in carbon tetrachloride, the value has been known to increase to 0.40 p.p.m.[5]



1,6—Dimethoxyphenazine
(VIII)



1,9—Dimethoxyphenazine
(X)

Fig. 9. Proton Type of VIII and X

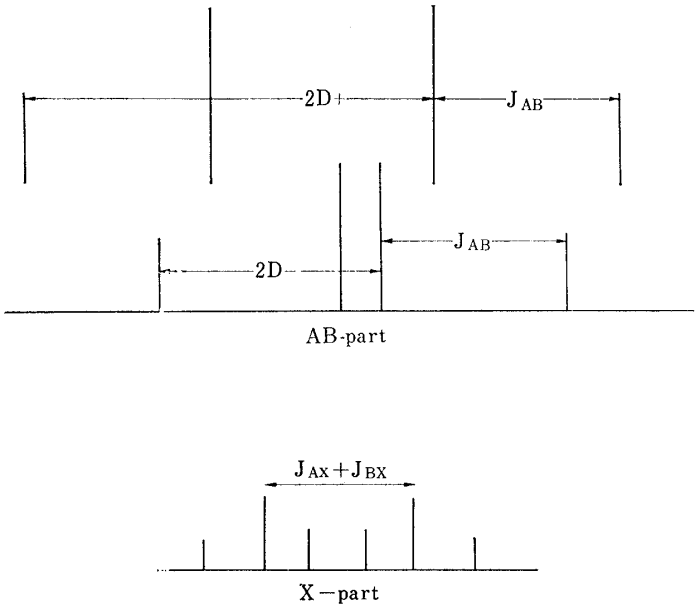


Fig. 10. Schematic Illustration of ABX Spectrum

5) A. A. Bothner-By, R. E. Glick : J. Chem. Phys., **26**, 651 (1957).

To obtain more distinct value of shielding effect of a methoxyl function, some phenazines with both benzene rings substituted with each one methoxyl group were thought to be appropriate examples.

E) 1,6-Dimethoxyphenazine (VIII) and 1,9-Dimethoxyphenazine (X)

These two derivatives (Ⅷ and X) have two pairs of ABC or ABX type ring protons as in Fig. 9. In Fig. 10, a schematic pattern of ABX type spectrum are illustrated and its transition energy can be given as shown in Table Ⅱ. The NMR spectra of Ⅷ

Table Ⅱ. Transition Energy for Three Nuclei ABX

Transition	Origin	Energy
1	B	$\nu_{AB}+1/4(-2J_{AB}-J_{AX}-J_{BX})-D_-$
2	〃	$\nu_{AB}+1/4(-2J_{AB}+J_{AX}+J_{BX})-D_+$
3	〃	$\nu_{AB}+1/4(2J_{AB}-J_{AX}-J_{BX})-D_-$
4	〃	$\nu_{AB}+1/4(2J_{AB}+J_{AX}+J_{BX})-D_+$
5	A	$\nu_{AB}+1/4(-2J_{AB}-J_{AX}-J_{BX})+D_-$
6	〃	$\nu_{AB}+1/4(-2J_{AB}+J_{AX}+J_{BX})+D_+$
7	〃	$\nu_{AB}+1/4(2J_{AB}-J_{AX}-J_{BX})+D_-$
8	〃	$\nu_{AB}+1/4(2J_{AB}+J_{AX}+J_{BX})+D_+$
9	X	$\nu_X-1/2(J_{AX}+J_{BX})$
10	〃	$\nu_X+D_+-D_-$
11	〃	$\nu_X-D_++D_-$
12	〃	$\nu_X+1/2(J_{AX}+J_{BX})$
13	Comb.	$2\nu_{AB}-\nu_X$
14	〃	$\nu_X-D_+-D_-$
15	〃	$\nu_X+D_++D_-$

$D_\pm=1/2\{[\nu_A-\nu_B\pm1/2(J_{AX}-J_{BX})]^2+J_{AB}^2\}^{1/2}$

and X were found to be entirely identical and exhibited the characteristic ABX features. In Fig. 11, the ring protons pattern of Ⅷ is reproduced.

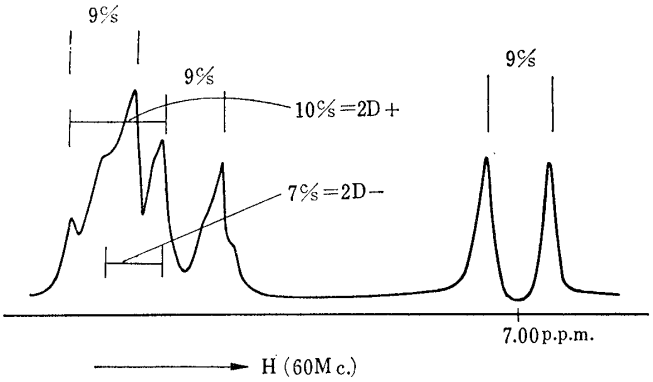


Fig. 11. Spectrum of 1,6-Dimethoxyphenazine (Ⅷ)

To secure the various constants as for phenazine derivatives mentioned above, the assignment of Fig. 11 was accomplished in the following manner. From the AB part of the spectrum, J_{AB} which corresponds to J value in I could be approved to be 9.00 c/s. The value coincides nicely with J=9.01 c/s of I as previously obtained. Distance between one pair of X part signals would give $|J_{AX}+J_{BX}|$ as 9.0 c/s, which fits approximately to J_B+J' (8.22 c/s) of I.

Besides these evidences, $\nu_0\delta$ values (difference between δ_A and δ_B) obtained by two kinds of calculations afforded a good agreement in their values as follows:

$\nu_0\delta$ calculated from $D_+=13.7$ c/s

$\nu_0\delta$ calculated from $D_-=13.6$ c/s

These data could support the correctness of previous calculations of various J values in I. The chemical shift at $\delta=7.00$ p.p.m. (center of X part quartet) indicates shielded character with about 0.67 p.p.m. based on the shift of B proton in I (7.67 p.p.m.). This would also reasonably be explicable by the anisotropic property of neighboring methoxyl function.

F) 2,8-Dimethoxyphenazine (IX)

The NMR spectrum of Ⅸ is reproduced in Fig. 12. On the basis of its chemical structure, signals at 7.95 p.p.m. (2H) with coupling constant 9 c/s can be assigned to

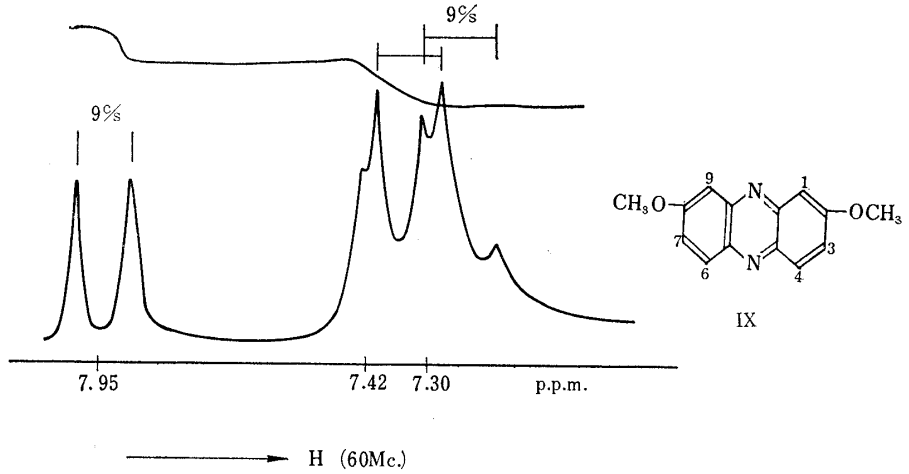


Fig. 12. Spectrum of 2,8-Dimethoxyphenazine (Ⅸ)

protons 4 and 6, and the upfield fairly complicated signals could be ascribed to protons 3 and 1 (also 7 and 9). These interpretations are supported by the integration curves cited therein. The detailed inspection of upfield signals has shown the following results:

a) The peak at lowest part looks somewhat coupled.

b) Deviation between the highest peak and the third peak can be read 9.0 c/s, which value is also recognized between the second and the fourth as are indicated in Fig. 12. These findings suggest to estimate that the upfield signals are combined pattern of AB type. Furthermore, the above described reason would lead to consider that the higher field quartet at 7.30 p.p.m. could be attributed to proton 3 (and 7) and a doublet at 7.42 p.p.m. to proton 1 (and 9).

Comparing with the usual shielding effect in phenazine derivatives caused by adjacent methoxyl group (cf. 0.67 p.p.m. in Ⅷ), the case of Ⅸ (B protons) was disclosed to be considerably less shielded, the reason for which has not been rationalized at the present moment.

Experimental

Samples——All samples used in this experiment were synthesized by known methods previously reported by Yosioka, *et al.* in the parts of this series[6] and other,[7] except newly synthesized compound shown below.

Measurement of Nuclear Magnetic Resonance Spectra——Each sample was measured with HITACHI H-60 in CCl_4 solution of about $M/200$ concentration at 60 Mc. Chemical shifts were obtained by measuring the deviations from the signal of tetramethylsilane in p.p.m. value.

Synthesis of 1,6-Dimethyl-4,9-dimethoxyphenazine (IV)——4-Methoxy-2-nitrotoluene (10 g.) and 4-methoxy-2-aminotoluene (10 g.) were condensed in benzene with existence of powdered KOH (10 g.) and $NaNH_2$ (10 g.) by refluxing 4 hr. After cooling, the filtrate of benzene solution was concentrated and chromatographed on alumina column, using benzene as eluant. The yellow needles were obtained by crystallizing from benzene, m.p. 250°. *Anal.* Calcd. for $C_{16}H_{16}O_2N_2$: C, 71.62; H, 6.01; N, 10.40. Found : C, 71.85; H, 6.06; N, 10.41.

6) I. Yosioka : This Bulletin, **2**, 25 (1954); I. Yosioka, H. Otomasu : *Ibid.*, **2**, 53 (1954); I. Yosioka : Yakugaku Zasshi, **72**, 1128 (1952).
7) H. McIlwain : J. Chem. Soc., **1943**, 322.

The author's thanks are due to Prof. I. Yosioka, Osaka University, for his generous encouragement and kind leading. And also his thanks are expressed to Miss M. Suzuki, Osaka University, for taking NMR spectra.

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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UDC 547.864.03 : 543.51

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

(*Faculty of Pharmaceutical Sciences, Osaka University**3)

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.