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165. Michiya Kimura and Toshihiro Nishina : Fundamental Studies on Clinical Chemistry. XIV.[*1] On Some Aspects in the Colorimetries of Steroidal Compounds using *p*-Nitrophenylhydrazine. (3). Light Absorption of *p*-Nitrophenylhydrazone in Alkaline Dimethylformamide.

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Bathochromic and hyperchromic absorption of *p*-nitrophenylhydrazones in alkaline dimethylformamide (DMF), that was shown in the colorimetric procedures for some steroids, was studied using several model compounds in various solvents. The color was ascribed mainly to the formation of quinoidal anion (I). The solvent effects of DMF as a dipolar aprotic solvent was discussed from the point of view of the cationic solvation and the increase in activities of hydroxide anion added as well as carbanion (I) formed was regarded as responsible for the hyperchromic absorption. The bathochromic effect of DMF was explained to be shown as a probable result of shielding the anion (I) from the cationic field by the solute-solvent interaction.

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In the previous papers[1~5] of this series, the present authors reported the novel methods for the colorimetry of some steroidal compounds, basing on the principle[6] that *p*-nitrophenylhydrazones in alkaline dimethylformamide (DMF) can afford the intensive colors having maximum absorptions in the region between 500 and 600 mμ. Another advantage of these methods is the favorable indifference of the hydrazine reagent employed, which in an alkaline DMF solution undergoes the base-catalyzed and oxidative decomposition to nitrobenzene and molecular nitrogen[7] so that the reagent blank becomes almost negligible. The reagent, *p*-nitrophenylhydrazine, gave *p*-nitrophenol when the solvent system was changed to methanol from DMF.[7] Although Isherwood, *et al.*[8] presented the possible structure of cyclohexadienide complex (II), the quinoidal form (I) would more reasonably be accepted as responsible for the bathochromic color of 2,4-dinitrophenylhydrazone in an alkaline solution.[9] Some of *p*-nitrophenylhydrazones have been reported as the pH indicator for strongly alkaline regions.[10,11] The present paper deals with bathochromic absorption of *p*-nitrophenylhydrazone caused by alkali and the role of DMF playing on the above-mentioned colorimetric procedures.

Structure of Anion Responsible for Bathochromic Absorption

The conversion of *p*-nitroaniline to *p*-nitrophenol has well been known to proceed quite easily in the presence of alkali, contrary to the *m*-isomer.[12] When the methanolic

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[*1] Part XIII : This Bulletin, **15**, 1242 (1967).

[*2] Nishi-6-chome, Kita-12-jo, Sapporo (木村道也, 仁科甫啓).

1) T. Nishina, Y. Sakai, M. Kimura : Steroids, **4**, 255 (1964).

2) T. Nishina, M. Kimura : This Bulletin, **12**, 521 (1964).

3) T. Nishina, I. Hariya, M. Kimura : *Ibid.*, **13**, 414 (1965).

4) *Idem* : Bunseki Kagaku (Japan Analyst), **14**, 125 (1965).

5) M. Kimura, T. Nishina : This Bulletin, **15**, 454 (1967).

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8) F.A. Isherwood, R.L. Jones : Nature, **175**, 419 (1955).

9) C.J. Timmons : J. Chem. Soc., **1957**, 2613.

10) R. O'connor, W. Rosenbrook, Jr., G. Anderson : Anal. Chem., **33**, 1283 (1961).

11) M. Kambe, Y. Hasegawa, E. Shindo : Bunseki Kagaku (Japan Analyst), **12**, 63 (1963); **13**, 1218 (1964).

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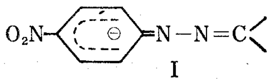
solution of *p*-nitrophenylhydrazine was made alkaline at room temperature, *p*-nitrophenol was obtained in the yield of about twenty per cent.[7] Parker[13] and other group[14] have recently studied on the behaviors of anions in the dipolar aprotic solvents such as DMF under the consideration of interactions between them. The rates of the nucleophilic reactions are generally higher in these solvents than in the protic ones.[13] The cyclohexadienide complex anions (II, III, IV and V) have been proposed[8,15~17] and some salts of such anions, for instance (VI, VII, and VIII), have been isolated when the nitrobenzene derivatives were treated with alkali.[18~20] The quinoidal structure (I), however, has generally been considered as responsible for the bathochromic color of *p*-nitrophenylhydrazones in an alkaline solution.[9] Thus, it should not be of little worth to examine whether the possible contribution of the structure (II) might be acceptable or not.

When different amounts of alkali were added to the DMF solution of cholest-4-en-3-one *p*-nitrophenylhydrazone, an isosbestic point was observed on the absorption curves similarly to the case of benzophenone as shown in Fig. 1 and 2; these color reactions thus seemed to be in a category of acid-base reaction. In a previous study,[5] more bathochromic shifts were observed on the *p*-nitrophenylhydrazones of α,β-unsaturated ketosteroids than those of the corresponding saturated ones; that increased further with increase of conjugation in steroids as have also been observed on some 2,4-dinitrophenylhydrazones.[9,21,22] The quinoidal structure (I) formed by the elimination of imino-hydrogen has ever been postulated[10,23] from the fact that no N-methylated nitrophenylhydrazone gave color with alkali. The model reactions were, therefore, reinvestigated on several hydrazones and nitroanilines, as well as their behaviors in DMF as a dipolar aprotic solvent and ethanol as a protic one. As summarized in Table I, none of these N-methyl derivatives was capable of giving such bathochromic absorption and the presence of intact imino-hydrogen was thus evidently indicated to be essential. The same table indicates that *p*-nitrophenol showed red-shift only under the presence of alkali in ethanol, in contrast to that in DMF which can act as a base. On the other hand, *p*-nitroanisol lacking in the dissociable proton was incapable of giving color



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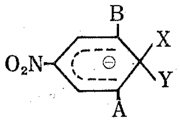
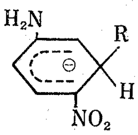


II[8] : A=H, $B=NO_2$, X=NH-N=C<, Y=OH
III[15] : A, $B=NO_2$, $X=N\langle^{CH_3}_{NO_2}$, Y=OH
V[17] : A, $B=NO_2$, X=H, Y=OH
VI[18] : A=H, $B=NO_2$, X=H, $Y=CH_2\cdot CO\cdot CH_3$
VII[19] : A, $B=NO_2$, X=H, $Y=CH_2\cdot CO\cdot CH_3$
VIII[20] : $A=SO_3H$, or COOH, $B=NO_2$, X=H, $Y=CH_2\cdot CO\cdot CH_3$



IV[16]

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[13] A. J. Parker : Quart. Rev., **16**, 163 (1962); Acta Chem. Scand., **16**, 855 (1962).

[14] N. Kornblum, R. Seltzen, P. Haberfield : J. Am. Chem. Soc., **85**, 1148 (1963).

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[16] C. C. Porter : Anal. Chem., **27**, 805 (1955).

[17] V. Gold, C. H. Rochester : J. Chem. Soc., **1964**, 1711.

[18] M. Kimura, M. Kawata, M. Nakadate : Chem. & Ind., **1965**, 2065.

[19] M. Kimura : Yakugaku Zasshi, **73**, 1219 (1953).

[20] M. Akatsuka : *Ibid.*, **80**, 378, 389 (1960).

[21] L. N. Ferguson, J. K. Robinson : J. Am. Chem. Soc., **71**, 742 (1949)

[22] H. H. Harry, H. J. Planisek : *Ibid.*, **72**, 4042 (1950).

[23] F. Bohlman : Chem. Ber., **84**, 490 (1951).

even in alkaline DMF, with the same result also on acetone *m*-nitrophenylhydrazone to form the quinoidal structure. It might thus be concluded that the dissociation of this hydrogen from *p*-nitrophenylhydrazone is mainly responsible for the bathochromic color caused by alkali forming the quinoidal structure (I) and that the nitrobenzene derivatives which are insufficiently activated with hydroxide ion are, in contrast to some polynitro compounds such as 1,3,5-trinitrobenzene and methyl picrate,[17] incapable of forming the cyclohexadienide complex (II). Recently, Crampton, *et al.*[24] reported also the elimination of imino-hydrogen by alkali such as sodium hydroxide and methoxide in dimethyl sulfoxide (DMSO), from the basis of nuclear magnetic resonance (NMR) studies on polynitro aniline derivatives.

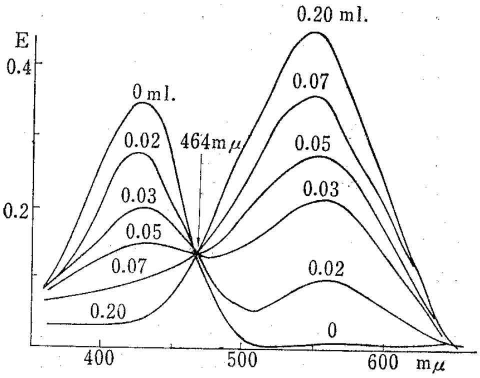

Fig. 1. Effect of Alkali Concentration (1)

Cholest-4-en-3-one *p*-nitrophenylhydrazone in DMF

The respective volume of 10% tetraethyl ammoniumhydroxide solution was added to each 5 ml. of DMF solution containing 20 μg. of cholest-4-en-3-one *p*-nitrophenylhydrazone.

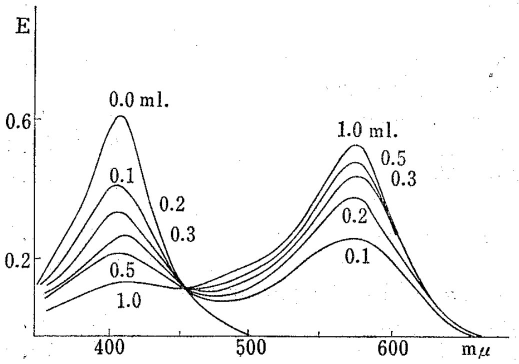

Fig. 2. Effect of Alkali Concentration (2)

Benzophenone *p*-nitrophenylhydrazone in methylcellosolve

The respective volume of 2.5*N* NaOH was added to each 5.0 ml. of methylcellosolve solution containing 29.3 μg. of benzophenone *p*-nitrophenylhydrazone.

It was of interest that contrary to the situation in alkaline alcoholic solution *o*-nitro derivatives of phenylhydrazones and aniline in DMF showed light absorption at longer wave lengths than those of the corresponding *p*-isomers (Table I); that is likely to be due to the ease of hydrogen bonding between *o*-nitro and amino groups.

Solvent Effects of DMF

The searches for the solvents having bathochromic and/or hyperchromic effects have long been carried out in the studies on colorimetry and the effects of dipolar aprotic solvents on the light absorptions of hydrazones as well as nitrobenzene derivatives have been observed.[16,25] Dipolar aprotic solvents such as DMF, DMSO, and dimethylacetamide clearly showed larger bathochromic and hyperchromic effects on cholest-4-en-3-one *p*-nitrophenylhydrazone than the ordinary solvents, as summarized in Table II.

1. Activity of Anion related to Hyperchromic Effect :

In the presence of alkali, the spectrum of benzophenone *p*-nitrophenylhydrazone varied with different amounts of alkali in the same solvent keeping the initial maximum, and with difference in the solvents employed, as are shown in Fig. 2 and 3, respectively. It might thus be considered in the case of some solvents that there still exists non-ionized hydrazone under the presence of highly excess alkali so that the additional peaks due to the contribution of the anion (I) formed are superimposed to give the resultant absorption curves. In other words, sufficient activities of alkali which was even of hundreds times in molar concentration than that of the hydrazone, might not

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24) M.R. Crampton, V. Gold : Chem. Commun., **12**, 256 (1965).
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Table I. Maximum Absorptions of Nitroaniline Derivatives and Nitrophenylhydrazones in Ethanol and Dimethylformamide

	EtOH					DMF				
	λ_{max} (mμ)	$\varepsilon\times10^{-3}$	λ_{max}^{NaOH} (mμ)	$\varepsilon\times10^{-3}$	$\Delta\lambda$ (mμ)	λ_{max} (mμ)	$\varepsilon\times10^{-3}$	λ_{max}^{NaOH} (mμ)	$\varepsilon\times10^{-3}$	$\Delta\lambda$ (mμ)
p-Nitroaniline	374	17.1	378	17.1	4	384	19.7	467	18.7	83
N-Methyl-*p*-nitroaniline	385	21.4	390	20.3	5	395	21.8	485	25.5	90
N-Dimethyl-*p*-nitroaniline	389	20.4	395	20.7	6	402	22.1	405	23.2	3
o-Nitroaniline	406	5.7	408	6.1	2	413	6.1	505	6.9	92
N-Dimethyl *o*-nitroaniline	418	2.6	423	3.1	5	428	3.1	432	3.1	5
p-Nitrophenol	320	11.4	403	23.8	83	433 316	22.4	427	31.2	−6
p-Nitroanisole	306	8.7	308	8.7	2	313		316		3
p-Nitrophenylhydrazone										
Cyclohexanone	395	24.7	488	26.4	83	407	24.5	520	36.4	113
Acetone	392	22.1	488	22.9	86	403	23.4	520	34.8	117
Benzaldehyde	403	35.8	535 392	38.0 24.3	132	414	34.7	557	55.8	133
Benzophenone	402	32.6	540 408	18.1 20.0	138	415	35.5	570	49.2	155
α-Methyl-α-*p*-nitrophenylhydrazone										
Benzaldehyde	400	31.7	403	34.9	3	414	31.6	415	31.4	1
Benzophenone	420	25.5	423	26.8	3	430	25.6	430	27.6	0
α-Methyl-α-2,4-dinitrophenylhydrazone										
Cyclohexanone	380	17.5	382	20.8	2	395	17.2	420	15.0a)	25
Acetone	380	17.6	382	28.0	2	394	17.1	393	11.2a)	1
o-Nitrophenylhydrazone										
Cyclohexanone	443	7.1	420	8.1	−23	452	7.1	565	7.7a)	113
Acetone	440	6.8	420	5.6	−20	450	5.7	560	6.9a)	110
m-Nitrophenylhydrazone										
Acetone	—	—	—	—	—	—	—	—	—	—

a) unstable
Alkaline solutions were made by adding 0.2 ml. of 2.5*N* NaOH.

Table II. Solvent Effect of Cholest-4-en-3-one *p*-Nitrophenylhydrazone

	Neutral		Alkalineb)	
	λ_{max} (mμ)	Indexa)	λ_{max} (mμ)	Indexa)
Carbontetrachloride	387	76		
Benzene	397	70		
Chlorobenzene	403	68		
Chloroform	405	69		
Methylcellosolve	415	79	530	62
Ethanol	418	76	510	73
Acetone	410	79	535	92
Pyridine	424	70		
Dimethylformamide	425	75	540	100
Dimethylacetamide	425	76	540	100
Dimethylsulfoxide	430	76	540	101

a) The relative extinction value at maximum was represented as DMF-Alkaline index, when the observed value with an equimolar amount of cholest-4-en-3-one *p*-nitrophenylhydrazone in alkaline DMF was taken as 100.
b) Alkaline solutions were made by adding 0.2 ml. of 2.5*N* NaOH.

be available in these solvents to release the conjugate base (I) entirely. Table III summarizes the differences within each pair of wave lengths at the maximum absorptions in neutral as well as alkaline solutions, measured on several *p*-nitrophenylhydrazones and other related compounds using different kinds of solvent. The rates of formation in these solvents containing a definite amount of alkali were calculated on the conjugate bases released from these nitro compounds, as summarized in Table IV. As has been reported by Murto,[26] it may, therefore, be concluded from these results that the activity of alkali added was fairly higher in the dipolar aprotic solvents, particularly in DMF, contrary to the protic solvents such as methanol.

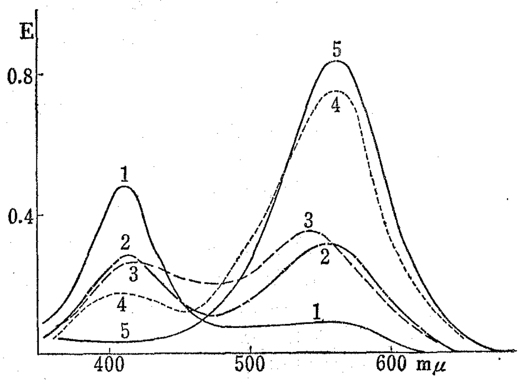

Fig. 3. Effects of Different Solvents

Benzophenone *p*-nitrophenylhydrazone (29.3 μg./5 ml.) with Alkali (1*N* NaOH, 0.5 ml.)
1. MeOH 2. Methylcellosolve 3. EtOH 4. Me_2CO 5. DMF

Kornblum, *et al.*[14] found that the O-alkylation proceeded to a larger extent than that at carbon atom when the reaction of β-naphtholate with alkyl chloride was carried out in the dipolar aprotic solvents such as DMF and DMSO, and this was ascribed to the larger solvation to the cation by them as the cationic solvent, the extent of which was dependent on the species of this ion. As is shown in Fig. 4 and as might be expected, the absorption curves in DMF were varied with the difference in cations, that were measured with different alkalis on N-methyl-*p*-nitroaniline as a suitable model

Table III. Maximum Absorptions of Some *p*-Nitrophenylhydrazones and Related Compounds in Different Solvents

		DMF		Acetone		MC[a)]		Ethanol		Methanol		Water	
		λmax (mμ)	Δλ	λmax (mμ)	Δλ	λmax (mμ)	Δλ	λmax (mμ)	Δλ	λmax (mμ)	Δλ	λmax (mμ)	Δλ
p-Nitrophenylhydrazone													
Acetone	N	403	117	390	120	396	107	393	97	392	—	405	38
	B	520		510		503		490		—		443	
Cyclohexanone	N	408	113	395	120	398	112	395	95	395	—	402	28
	B	520		515		510		490		—		430	
Benzaldehyde	N	414	147	400	150	408	135	402	133	402	—	—	—
	B	560		550		543		535		—			
Benzophenone	N	415	155	404	159	405	155	402	138	402	—	—	—
	B	570		563		560		540		—			
Cholestan-3-one	N	407	113	396	119	400	104	396	102	396	—	—	—
	B	520		516		504		498		—			
Cholest-4-en-3-one	N	425	120	414	126	420	115	414	110	413	—	—	—
	B	545		540		535		524		—			
p-Nitrophenol	N	316	111	415	—	316	87	314	87	314	80	320	82
	B	427		—		403		401		394		402	
p-Nitroaniline	N	384	83	368	—	386	—	374	—	374	—	—	—
	B	469		—		—		—		—			
N-Methyl *p*-nitroaniline	N	395	90	395	—	390	—	385	—	388	—	—	—
	B	485		—		—		—		—			

a) Methylcellosolve
N: Neutral B: Basic solution containing 0.2 ml. of 2.5*N* NaOH.

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[26] J. Murto: Acta Chem. Scand., **18**, 1043 (1964).

TABLE IV. Per Cent Ionization of Some *p*-Nitrophenylhydrazones and Related Compounds in Alkaline Solvents

	DMF	Acetone	MC[a]	Ethanol	Methanol
p-Nitrophenylhydrazone					
Benzaldehyde	100	90	79	81	16
Cyclohexanone	100	82	60	69	23
Acetone	100	79	50	68	10
Benzophenone	100	70	45	45	4.4
Cholestan-3-one	100	81	61	67	19
Cholest-4-en-3-one	100	83	64	70	12
p-Nitrophenol	100	—	100	100	100
p-Nitroaniline	80	0	0	0	0
N-Methyl-*p*-nitroaniline	89	0	0	0	0

a) Methylcellosolve
All solutions were made alkaline by adding 0.2 ml. of 2.5*N* NaOH.
Per cent was calculated as follows:

$$\% = \frac{\varepsilon_{\text{neutral}} - \varepsilon_{\text{alkaline}}}{\varepsilon_{\text{neutral}}} \times 100$$

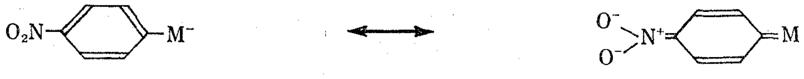
$\varepsilon_{\text{alkaline}}$: Molar extinction coefficient in alkaline solution observed at $\lambda_{\text{max}}^{\text{neutral}}$.

compound instead of too much chromogenic *p*-nitrophenylhydrazone. Thus the activity of hydroxyl anion in DMF may reasonably be considered to decrease in the following order of decreasing cationic solvation: $(Et)_4N^+ > K^+ > Na^+ > Li^+$, that agreed closely with the results already reported.[13,14,27] In the ethanolic solution of benzophenone *p*-nitrophenylhydrazone, on the contrary, nothing was observed on such differences, probably indicating the far less cationic solvation and consequently the far smaller activity in hydroxide anion.

Most anions are much more weakly solvated in aprotic dipolar solvent; the reverse is true of polarizable, charged transition state.[28] The activity of the carbanion is increased by coordination of the solvent with the cation.[29] In addition to the activity of hydroxide anion described above, it may, therefore, be reasonable to consider that *p*-nitrophenylhydrazone would become more acidic in dipolar aprotic solvents than in protic ones, as has been discussed on the ionization in various solvents.[25,30,31] The activity of the ambifunctional anion[32] of *p*-nitrophenylhydrazone (I) might thus be increased in DMF also giving the resultant hyperchromic absorption.

2. Bathochromic Effect:

A pronounced red shift (100 mμ) of the absorption maxima for the charge-transfer-transition (IX) occurred in the change from a protic to a dipolar aprotic solvent.[13] The marked blue shift of aryl oxide ion absorption in going from aprotic to hydroxylic solvents[25] was ascribed by Parker and Brody[33] to a general hydrogen bonded stabili-



IX M: Group VI atom

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[27] E. Tommila, M. L. Murto: Acta Chem. Scand., **17**, 1947 (1963).
[28] J. Miller, A. J. Parker: J. Am. Chem. Soc., **83**, 117 (1961).
[29] H. D. Zook, T. J. Russo: *Ibid.*, **82**, 1258 (1960).
[30] A. J. Parker, B. W. Clare, D. Cook, E. C. F. Ko, Y. C. Mac: *Ibid.*, **88**, 1911 (1966).
[31] V. I. Slovetskii, A. I. Ivanov, S. A. Shevelev, A. A. Fainsilberg, S. S. Novikov: Tetrahedron Lett., **1966**, 1745.
[32] R. Gompper: Angew. Chem. internatl. Edit., **3**, 560 (1964).
[33] A. J. Parker, D. Brody: J. Chem. Soc., **1963**, 4061.

zation[34,35] of the ground state by the protic solvent. It was reported that the cation interacts more strongly with the ground than with the excited state.[36,37] The spectral shift was considered to be produced primarily by the perturbation of the molecular energy levels of the anion due to the cationic field which is reduced in the magnitude by the solvation of cation with polar aprotic solvents; the direct anion solvation[38] was thus regarded as secondary.[35] Gompper[32] reported, on the contrary, that the aniondipole interaction due to mutual polarization leads to a shielding of the atoms with the highest electron density against attack by cations and the reactivity of the whole resonance system of the shielded anions in dipolar aprotic solvents thus approaches to that of the free compound. The solvent-separated carbanion pair[39] was also proposed as one of the possible state.

Cholest-4-en-3-one *p*-nitrophenylhydrazone showed bathochromic absorption in polar aprotic solvents as summarized in Table II. As is shown in Fig. 4 and was found similarly on the various *p*-nitrophenolates[37], the wave-length of maximum absorption did not alter with the difference of cations in DMF. The bathochromic effect of DMF might thus be due highly to the solvation of the cation and/or quinoidal anion (I) so that the shielding of the latter ion from the cationic field might subsequently be occurred. More detailed experiments should be required for further discussion.

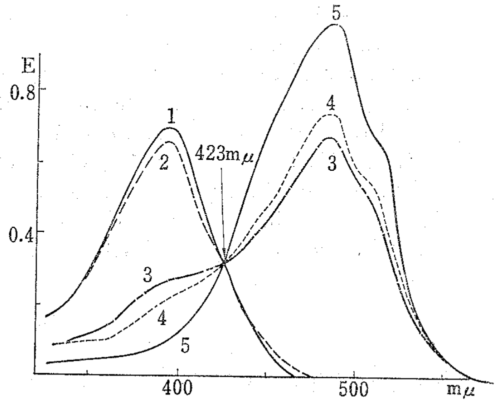

Fig. 4. Effects of Different Alkalis

N-methyl-*p*-nitroanilne (48.7 μg./10 ml. DMF) with alkali (1*N* 0.5 ml.)

1. None 2. LiOH (f=1.14)
3. NaOH (f=1.06) 4. KOH (f=1.17)
5. TEAH (f=0.64)

Experimental

Materials and Apparatus——All samples of hydrazones and aniline derivatives were prepared by the authors following the known methods or commercially available. Solvents were used with further purification.

Absorption spectra were measured using a Hitachi Model EPS 2U recording spectrophotometer.

Measurement Procedures in Tables I∼IV——An aliquot of 0.5 ml. containing a known quantity between 20∼30 μg. of sample in EtOH were pipetted into a glass-stopperd 15 i. d. × 200 mm. tubes and evaporated under reduced pressure. For measuring absorption spectra, the dried residues were dissolved in 10 ml. of various solvents. Spectra of alkaline solutions which were prepared by adding 0.2 ml. of 2.5*N* NaOH to the above solutions were also taken. Molecular extinction and per cent of ionization were calculated as an average of several determinations that showed generally good agreement with each other at the wave-length of maximum absorption for each sample.

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35) N. Kornblum, P. J. Berrigan, W. J. de Noble : J. Am. Chem. Soc., **85**, 1141 (1963).
36) J. F. Garst, D. Walmsley, C. Hewitt, W. R. Richards, E. R. Zabolotry : *Ibid.*, **86**, 412 (1964).
37) H. E. Zaugg, A. D. Schaefer : *Ibid.*, **87**, 1857 (1965).
38) J. Miller : *Ibid.*, **85**, 1628 (1963).
39) T. E. Hogen-Esch, J. Smid : *Ibid.*, **87**, 669 (1965).