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Studies on Peroxidized Lipids. I. Interaction of Malondialdehyde with Secondary Amines and Its Relevance to Nitrosamine Formation

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Malondialdehyde (MDA) reacted with secondary amines (dimethylamine, diethylamine, piperidine, pyrrolidine and morpholine) at 37° under mild acidic or neutral conditions to yield β-dialkylaminoacroleins (**1**—**5**) of *trans*, *s-trans* conformation. The optimal pH of the reaction was 3—5, and the yields were 15—55% in a 6 hr incubation. The acroleins (**1**—**5**) were unstable under acidic and alkaline conditions, and produced a pink color on reaction with 2-thiobarbituric acid. β-Dimethylaminoacrolein (**1**) could be readily nitrosated in the acidic pH range to produce N-nitrosodimethylamine, and the rate of nitrosamine formation from **1** at pH 5.0 was much higher than that from dimethylamine; this is consistent with earlier observations of the stimulating effect of MDA on nitrosamine formation.

Keywords——malondialdehyde; β-dimethylaminoacrolein; β-diethylaminoacrolein; β-piperidinoacrolein; β-pyrrolidinoacrolein; β-morpholinoacrolein; β-dialkylaminoacrolein formation; N-nitrosodimethylamine formation; 2-thiobarbituric acid test

It has been demonstrated that lipids readily undergo peroxidation by oxygen to be transformed into hydroperoxides, which in turn degrade into a mixture of secondary products, such as aldehydes, epoxides, ketones and other products. Malondialdehyde (MDA) has been recognized as a secondary product of peroxidation of polyunsaturated fatty acids.[2] Early investigations showed that MDA reacts with proteins,[3–5] and the reaction was interpreted as involving the primary free amino groups to form an enamine linkage, N-substituted-3-aminopropenals[6] and fluorescent N,N′-disubstituted-1-amino-3-iminopropenes.[7,8] The fluorescence of lipofuscin pigments accumulated in aging organisms as a result of *in vivo* lipid peroxidation is considered to be due to this type of MDA-Schiff base.[9]

In the previous report,[10] we showed that MDA exerted stimulatory effects on the formation of carcinogenic nitrosamines from the corresponding secondary amines and nitrite under mild acidic conditions. The reasons for this effect of MDA were not elucidated at that time. This study was undertaken to characterize the reactions of MDA with several secondary amines under mild conditions and to determine their relevance to the formation of nitrosamines by the reaction of secondary amines with nitrite.

Experimental

Materials——Dimethylamine hydrochloride, pyrrolidine and morpholine were obtained from Kanto Chemical Company, Inc. Diethylamine hydrochloride was obtained from Wako Pure Chemical Industries,

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2) W.H. Gardner, *J. Agric. Food Chem.*, **27**, 220 (1979).
3) T.W. Kwon and W.D. Brown, *Fed. Proc.*, **24**, 592 (1965).
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7) E. Sawicki, T.W. Stanley, and H. Johnson, *Anal. Chem.*, **35**, 199 (1963).
8) K.S. Chio and A.L. Tappel, *Biochemistry*, **8**, 2821 (1969).
9) A.L. Tappel, *Fed. Proc.*, **32**, 1870 (1973).
10) T. Kurechi, K. Kikugawa, and M. Ozawa, *Fd Cosmet. Toxicol.*, **18**, 119 (1980).

Ltd. Piperidine hydrochloride and 2-thiobarbituric acid were products of Tokyo Kasei Kogyo Company, Ltd.

Malondialdehyde (MDA) was prepared according to the method described by Chio and Tappel,[8] and the solution was preincubated for complete hydrolysis of the acetal.[10] Thus, 1.64 g (10 mmol) of malonaldehyde bis(dimethylacetal) (Tokyo Kasei Kogyo Company, Ltd.) was mixed with 0.90 ml of 1.0 N HCl and the heterogeneous mixture was shaken at 40° until it became homogeneous. The solution was made up to 10 ml with water and the acidic solution was incubated at 37° for 1 hr for use (1 M MDA solution).

Methods——Absorption spectra were measured with a Shimadzu UV-200S double beam spectrometer. Mass spectra were obtained with a Hitachi RMU-7L double focusing mass spectrometer. Nuclear magnetic resonance spectra were taken in d_6-dimethylsulfoxide or deuterochloroform with a JEOL SP-100 machine with tetramethylsilane as an internal standard.

Thin-layer chromatography was performed on Wakogel B-5F (Wako Pure Chemical Industries, Ltd.) with a solvent system of $CHCl_3$–MeOH (9: 1). Paper chromatography was done on Toyo Roshi No. 51 paper with *n*-BuOH–H_2O (84: 16). Spots were located by irradiation with an ultraviolet (UV) lamp at 254 nm (Superlight, Nikko Sekiei Works). Silica gel column chromatography was performed on silica gel for chromatography (above 100 mesh, Kanto Chemical Company, Inc.).

A Yanaco G80 gas chromatograph, equipped with a hydrogen flame ionization detector and a glass column (3 mm ID × 2 m) packed with polyethylene glycol 6000 (25%) on 80—100 mesh Chromosorb W AW, was used to determine N-nitrosodimethylamine. The chromatograph was operated isothermally at 120° (column temperature) and 140° (injection temperature) with a nitrogen carrier-gas flow of 25 ml/min. Under these conditions, retention times of 2.7 min for the internal standard (ethyl caproate), and 4.3 min for N-nitrosodimethylamine were obtained. The amount of the nitrosamine was determined by comparing the peak area of the sample (5 μl) with that of authentic standard solution in $CHCl_3$ (5 μl of 0.5 mg/ml). Recovery of the nitrosamine in the control was 93%.

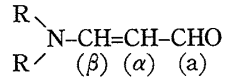
β-Dialkylaminoacroleins——A solution (9.0 ml) of 1 M MDA (9.0 mmol) and a solution (18.0 ml) of 1 M secondary amine (free base or hydrochloride form) (18 mmol) were added to 180 ml of 0.05 M sodium citrate–HCl buffer (pH 5.0), and the mixture was incubated at 37° for 24 hr (43 mM MDA + 87 mM secondary amine). The mixture was extracted with 100 ml of $CHCl_3$ four times after basification by addition of 40 ml of 5 N NaOH. The combined extracts were evaporated to dryness and the residue was made up to 50 ml with ethanol. Each reaction produced one major product with an absorption maximum at around 290 nm (pH 7.0), when the extract was checked by thin-layer chromatography and UV-absorption measurement. The extracts were chromatographed on a column of silica gel (20 g) by a stepwise elution with mixed solvents composed of $CHCl_3$ and MeOH (39: 1, 19: 1 and 9: 1). The fractions containing the UV-absorbing substance were rechromatographed in the same way. The products derived from dimethylamine (**1**), diethylamine (**2**), piperidine (**3**) and pyrrolidine (**4**) were obtained as yellow oils; **1** and **2** were further purified by distillation *in vacuo*. The product derived from morpholine (**5**) crystallized after complete removal of the solvent. The yields and the physicochemical properties of the β-dialkylaminoacroleins (**1**—**5**) are listed in Table I.

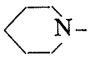
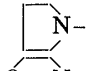
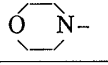
pH Dependence of the Formation of β-Dialkylaminoacroleins——A mixture of 43 mM MDA and 87 mM secondary amine in 0.1 N HCl (pH 1.0), 0.2 M sodium citrate–HCl (pH 3.0), 0.1 M sodium acetate (pH 5.0), 0.1 M sodium phosphate (pH 7.0) or 0.05 M sodium borate (pH 9.5) was incubated at 37° for 45 hr. Aliquots of 10 ml were withdrawn and mixed with 5.0 ml of 1 N NaOH and 2.0 g of NaCl, and the mixtures were extracted with 20 ml of $CHCl_3$. The extracts were evaporated to dryness and made up to 10 ml with EtOH. The ethanolic solutions were subjected to thin-layer chromatography and UV-absorption determination after 1: 500 dilution with 0.1 M phosphate buffer (pH 7.0). Thin-layer chromatography of each reaction solution revealed one major UV-absorbing spot. Absorbances at the absorption maximum of each β-dialkylaminoacrolein (Table I) were measured, and the yields of the acroleins were calculated (Figure 3 and Table II).

Thiobarbituric Acid Test of β-Dialkylaminoacroleins——A mixture of 0.30 ml of 20 mM of one of **1**—**5** or malonaldehyde bis(dimethylacetal), 1.0 ml of 20 mM 2-thiobarbituric acid (TBA) and 1.0 ml of glacial acetic acid was heated on a water bath for 20 min. After cooling it was made up to 500 ml with water, and the spectra between 400—600 nm were recorded. The compounds (**1**, **4** and **5**) and malonaldehyde bis-(dimethylacetal) were treated with TBA at various ratios (total concentration, 10 mM) in glacial acetic acid. Thus, a mixture of x ml of 20 mM each compound or malonaldehyde bis(dimethylacetal), $1-x$ ml of 20 mM TBA, and 1.0 ml of glacial acetic acid, was heated on a water bath. The spectra were recorded in the same way (Figure 4).

Formation of N-Nitrosodimethylamine from β-Dimethylaminoacrolein (1)——A mixture of 1.0 ml of 0.50 M dimethylamine hydrochloride, 50 mg of β-dimethylaminoacrolein (**1**), and 1.0 ml of 2.0 M sodium nitrite was made up to 10.0 ml with 0.1 M sodium citrate-HCl buffer (pH 3.0 and pH 5.0). The mixture was incubated in a stoppered tube at 37° for 3.5 hr. It was then mixed with 5.0 ml of 5 N NaOH and 2.0 g of NaCl to be extracted with 40 ml of $CHCl_3$. The extracts were analyzed by gas chromatography. Gas chromatography of the control mixture, which contained dimethylamine or **1** alone, revealed no peaks near the peak corresponding to the nitrosamine (Table V).

TABLE I. β-Dialkylaminoacroleins obtained by Reaction between Secondary Amines and Malondialdehyde at 37° and pH 5.0 for 24 Hours



No.	Compound[a] $\begin{matrix}R\\R\end{matrix}$>N–	Starting amine	% Yield (isolation)	Appearance	Formula	Analysis (%) Calcd (found) C	H	N	Mass spectrum m/e M+	M+-1	M+-17	M+-29
1	$\begin{matrix}CH_3\\CH_3\end{matrix}$>N–	Dimethylamine	19	Yellow oil (bp_{15}, 150°)	C_5H_9ON	60.58 (60.42	9.15 9.33	14.13 14.32)	99	98	82	70
2	$\begin{matrix}C_2H_5\\C_2H_5\end{matrix}$>N–	Diethylamine	7	Yellow oil (bp_{15}, 155°)	$C_7H_{13}ON\cdot 0.2H_2O$	65.30 (65.18	10.36 9.96	10.65 10.49)	127	126	110	98
3		Piperidine	25	Yellow oil	$C_8H_{13}ON\cdot 0.5H_2O$	64.83 (64.87	9.52 9.85	9.45 8.89)	139	138	122	110
4		Pyrrolidine	21	Yellow oil	$C_7H_{11}ON\cdot 0.5H_2O$	62.66 (62.60	9.01 8.57	10.44 10.46)	125	124	108	96
5		Morpholine	19	Yellow crystals (mp 69—70°)	$C_7H_{11}O_2N\cdot 0.25H_2O$	57.71 (57.65	7.96 7.79	9.62 9.23)	141	140	124	112

No.	UV: nm (ε ×10⁻³) $\lambda_{max}^{pH\ 7}$	$\lambda_{max}^{pH\ 1}$	$\lambda_{max}^{pH\ 12}$	NMR[b] ppm H_a (1H, d)	H_β (1H, d)	H_α (1H, dd)	Others	Hz $J_{a\alpha}$	$J_{\alpha\beta}$	Rf value TLC[c]	PPC[d]	pKa value
1	288(41)	272(29)	288(41)	8.87	7.32	4.95	{3.07 (3H, s, CH_3) 2.80 (3H, s, CH_3)}	8	13	0.32	0.52	2.4
				9.05*	7.05*	5.12*	{3.10 (3H, s, CH_3)* 2.85 (3H, s, CH_3)*}	8*	13*			
2	289(41)	273(29)	289(41)	8.90	7.28	5.00	{3.3 (4H, bm, CH_2) 1.1 (6H, bm, CH_3)}	8	13	0.21	0.67	2.4
3	290(40)	273(29)	290(40)	8.90	7.13	5.07	{3.4 (bs) 1.6 (bs)}	8	13	0.32	0.64	2.4
4	292(41)	275(30)	292(41)	8.90	7.50	4.90	{3.5 (bm) 3.13 (2H, t) 1.9 (5H, bm)}	8	13	0.21	0.59	2.4
5	290(45)	280(30)	289(41)	8.95	7.28	5.15	{3.6 (bm) 3.3 (bm)}	8	13	0.32	0.45	2.1

a) Compounds were prepared by reaction between propargyl aldehyde and the corresponding secondary amines: **1**, bp_{10}, 143—144°; **2**, bp_{15} 150—153°; **3**, $bp_{0.1}$, 124°; **5**, mp, 69—71° (references 11 and 12).

b) NMR spectra were taken in d_6-dimethylsulfoxide or in $CDCl_3$ (*) with an internal standard (tetramethylsilane).

c) Thin-layer chromatography on Silica gel with $CHCl_3$–MeOH (9: 1).

d) Paper chromatography with *n*-BuOH–H_2O (84: 16).

Results

Formation of β-Dialkylaminoacroleins from Malondialdehyde and Secondary Amines

Dialkylamines such as dimethylamine, diethylamine, piperidine, pyrrolidine and morpholine were treated with malondialdehyde (MDA) at pH 5.0 and at 37° for 24 hours. The reaction products were extracted with chloroform after basification of the reaction mixture, and purified by passage through a silica gel column. They were isolated as non-fluorescent yellow oils or crystals. The yields and the physicochemical properties of **1**—**5** are summarized in Table I, and all the products were shown to be β-dialkylaminoacroleins.

Several studies on the preparation of β-aminoacrolein derivatives have been done in the industrial and pharmaceutical fields. These compounds were principally prepared by the reaction of amines with propargyl aldehyde,[11,12] and by several slight modifications of this approach.[13–15] The compounds (**1**, **2**, **3** and **5**) obtained here by reaction of MDA with secondary amines were identical with those prepared earlier[11,12] with respect to boiling point or melting point.

The mass spectrum of β-dimethylaminoacrolein (**1**) (Figure 1) revealed a molecular ion peak M^+ (99 *m/e*) and fragment ion peaks of M^+-1 (98 *m/e*), M^+-17 (82 *m/e*), M^+-29 (70 *m/e*) and 55 *m/e*; all these peaks appeared in the spectra of **2**—**5** (Table I). In the spectrum of **1** an intense molecular ion (M^+) and M^+-1 ion were observed together with an intense ion at *m/e* 82 [$(CH_3)_2N^+$=CH–C≡CH], and less intense ions at *m/e* 70 [$(CH_3)_2N^+$–CH=CH] and *m/e* 55 [CH_2=CH–C≡O^+]. The appearance of an intense molecular ion may be due to the mesoionic resonance form of the structure **1** and the consequent stability of its molecular ion.

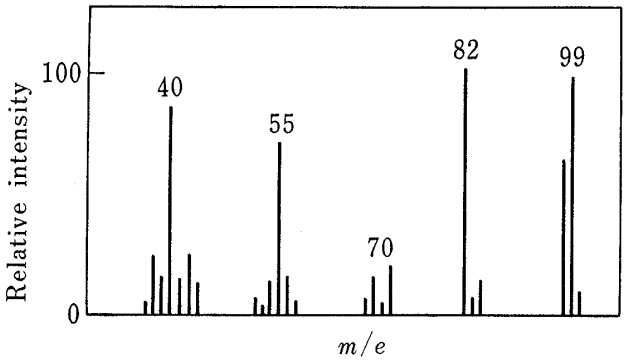


Fig. 1. Mass Spectrum of β-Dimethylaminoacrolein (**1**)

Ultraviolet (UV) absorption spectra of β-dimethylaminoacrolein (**1**) in aqueous solution were compared with those of MDA (Figure 2). MDA showed absorption maxima at 243 (pH 1) and 267 (pH 7) nm (Figure 2A), as has been reported by Mashio and Kimura,[16] who indicated that deprotonation occurred at the O-position of an enolate form of MDA with a pK_a value of 4.6. The compound (**1**) showed absorption maxima at 272 (pH 1) and 288 (pH 4.5—12) nm (Figure 2B), and a hypo- and hypsochromic shift of the maximum with decreasing pH was observed. The spectrum of the neutral form of **1**, which coincided with that already described,[17] showed a change with a pK_a value of 2.4 into the O-protonated iminium form, whose structure was suggested by the nuclear magnetic resonance (NMR) spectrum of the protonated form of **1**.[18] The spectra of **2**—**5** were similar to those of **1**, and their pK_a values

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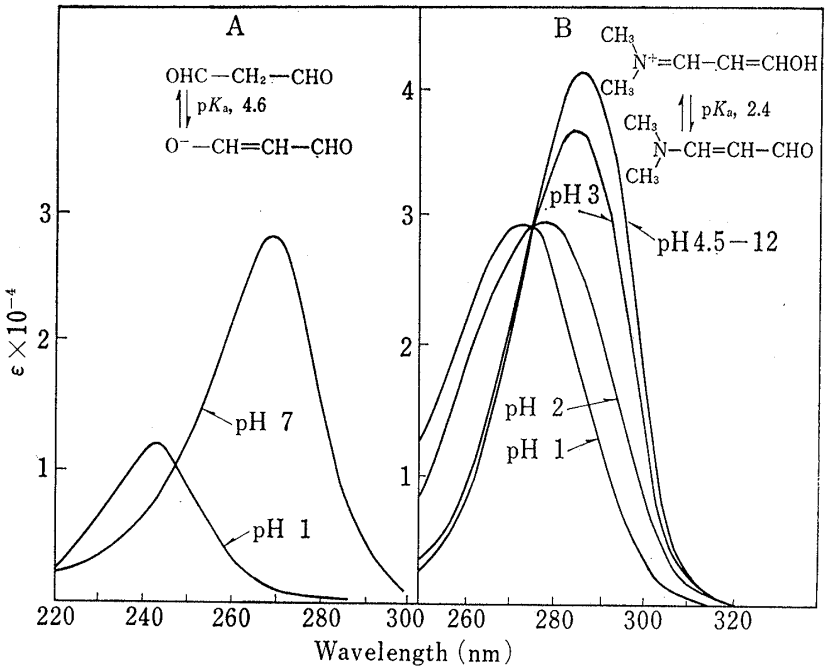

Fig. 2. Ultraviolet Absorption Spectra

A: Malondialdehyde; and B: β-Dimethylaminoacrolin (**1**).

were identical with that of **1**, except for **5**, which showed a rather lower pK_a value of 2.1 (Table I).[19]

NMR spectra of **1**—**5**, shown in Table I, taken in d_6-dimethylsulfoxide or deuterochloroform, revealed identical AMX system signals with an aldehyde proton (H_a) and two olefinic protons (H_α and H_β), and were similar to the spectrum of **1** taken in carbon tetrachloride.[20] The coupling constants, $J_{\alpha\beta}$=13 Hz and $J_{a\alpha}$=8 Hz, indicated that the conformation of **1**—**5**

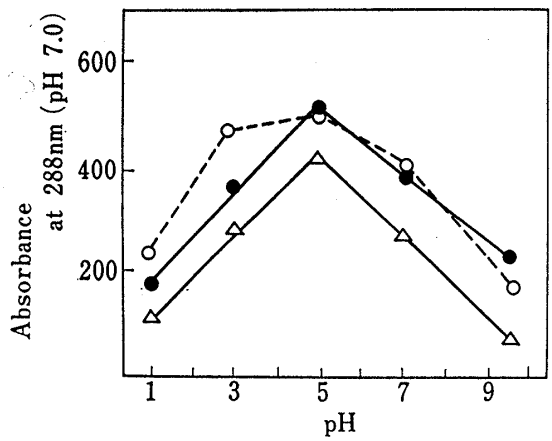

Fig. 3. pH Dependence of the Formation of β-Dimethylaminoacrolein (**1**)

A mixture of 43 mM MDA and 87 mM dimethylamine was treated at 37° for 8 hours (△), 20 hours (○) and 45 hours (●). The absorbance at 288 nm (pH 7.0) of the chloroform extract is plotted.

Table II. Yields of β- Dialkylaminoacroleins in the Reaction of 43 mM MDA and 87 mM Secondary Amines at 37° for 6 Hours

Compound	pH	β-Dialkylaminoacrolein formed in the reaction mixture	
		mM	Yield (%)
1	5[a]	10	23.3
	7	7	16.3
2	3[a]	6.3	14.7
	7	3.3	7.7
3	5[a]	15.8	36.8
	7	8.5	19.8
4	3[a]	23.8	55.3
	7	7.3	17.0
5	3[a]	23.8	55.3
	7	9.0	20.9

a) pH of maximum formation.

19) The pK_a values of the amines are 10.08 (dimethylamine), 11.10 (diethylamine), 11.20 (piperidine), 11.11 (pyrrolidine) and 8.40 (morpholine).

20) H.E.A. Kramer and R. Gompper, *Z. Physik. Chem.* (Frankfurt/M), **43**, 292 (1964).

was the same *trans*, *s-trans* form[21,22] regardless of the solvent used.

None of these products (**1**—**5**) reduced Fuchsin–bisulfite or Fehling's solution, which indicates that the terminal aldehyde group in the compounds was not active.

pH Dependence of the Formation of β-Dialkylaminoacroleins

A reaction mixture containing 43 mM MDA and 87 mM secondary amine was incubated in the pH range of 1.0 to 9.5. Thin-layer chromatography of the chloroform extract from every reaction mixture showed no strongly UV-absorbing sponts other than β-dialkylaminoacroleins (**1**—**5**), indicating that disubstituted compounds of MDA were not produced under these conditions. The profiles of the pH dependence of the formation of **1**, measured spectrophotometrically, are shown in Figure 3, which indicates that **1** was formed over the whole pH range and that the rate of formation was greatest at pH 5.0. The formation of **1** at pH 5.0 increased with time and reached a maximum after 24 hours, but at lower pH values such as 1.0 and 3.0, the amount of **1** in the reaction mixture decreased after 48 hours, indicating lability of **1** in these more strongly acidic media. The yields of **1**—**5** at the optimal pH's and at pH 7.0 on incubation for 6 hour at 37° are summarized in Table II. The compounds were produced in yields of 8—21% at pH 7.0 and in yields of 15—55% under the optimal conditions at pH 3.0 or 5.0. The rates of formation depended on the nature of the amine, and decreased in the order, morpholine>pyrrolidine>piperidine>dimethylamine>diethylamine. The differences in the rate of formation and the optimal pH may be due to the pK_a values of the starting amines,[19] and also to the relative stabilities of the products.

Stability and 2-Thiobarbituric Acid Test of β-Dialkylaminoacroleins

The compounds (**1**—**5**) at low concentrations were treated at pH 7.0, in 0.1 N HCl and in 0.1 N NaOH at 37° for 3 hours. Decreases in absorbance at the absorption maximum of every compound are shown in Table III, which demonstrates that all the compounds were stable at pH 7.0 and labile in both the acidic and alkaline media. The lability decreased in the order **5**, **1**, **3**, **2** and **4** in the acidic medium and in the order of **5**, **3**, **1**, **4** and **2** in the alkaline medium. When β-dimethylaminoacrolein (**1**) was treated in water, concentrated ammonia, 1 N NaOH, 1 N HCOOH and 1 N HCl at 37° overnight, the mixtures in 1 N NaOH, 1 N HCOOH and 1 N HCl turned brown and viscous, and those in concentrated ammonia, 1 N HCOOH and 1 N HCl became slightly fluorescent. Paper chromatography of the mixtures (Table IV) revealed many UV-absorbing spots. The fluorescent spot (X) with the absorption maximum at 308 nm (neutral, H^+, OH^-) appeared on the chromatograms after **1** had been treated with concentrated ammonia, 1 N HCOOH and 1 N HCl. The spot (Y) with absorption maxima at 388 (neutral, H^+) and 346 (OH^-)nm appeared on the chromatogram after **1** had been treated in 1 N HCOOH. The UV-absorption spectra of some other spots were similar to those of **1** or MDA.

Like MDA,[23,24] the compounds (**1**—**5**) were found to be positive in the 2-thiobarbituric acid (TBA) test. The spectra between 400—600 nm of the reaction mixture of each compound with TBA were identical with those of the mixture of MDA and TBA; all exhibited a maximum at 532 nm, a shoulder at around 502 nm and a minimum at 420 nm. The compounds (**1**, **4** and **5**) and MDA were treated with TBA in various ratios in aqueous acetic acid (Figure 4) in order to compare the maximum ratios of coloration. In all cases the absorbances at 532 nm were the highest at the same ratio of TBA: compound (**1**, **4** and **5**) and MDA. This result

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Table III. Stability of 10^{-5} M β-Dialkylaminoacroleins (**1**—**5**) at 37° for 3 Hours

Compound	% Decrease in absorbance at the maximum		
	0.05 M phosphate buffer (pH 7.0)	0.1 N HCl	0.1 N NaOH
1	2	46	32
2	1	17	9
3	4	33	37
4	2	7	21
5	3	100	92

Table IV. Analysis by Paper Chromatography of the Degradation Products of β-Dimethylaminoacrolein (**1**)

Reaction medium[a]	*Rf* value[b]	UV: λmax, nm[c]			Identity
		H_2O	H^+	OH^-	
H_2O	0.54	288	277	—	**1**
Concentrated NH_4OH	0.54	288	276	—	**1**
	0.49 (weak)	—	—	—	
	0.24	308	308	308	X
1 N NaOH	0.54	288	274	—	**1**
	0.31 (weak)	—	—	—	
	0.09	267	245	267	
1 N HCOOH	0.71	250	250	—	
	0.45	388	388	346	Y
	0.36	250	250	—	
	0.24	308	308	308	X
1 N HCl	0.66	250	250	—	
	0.49	288	286	284	
	0.43	250	250	—	
	0.24	308	308	308	X
	0.0 (weak)	—	—	—	

a) Compound (**1**) was treated at 37° overnight in the medium described.
b) Spots were detected by UV irradiation (254 nm).
c) UV spectra of the water extract of the spot.

Table V. Formation of N-Nitrosodimethylamine from Dimethylamine and β-Dimethylaminoacrolein (**1**) by Incubation with Nitrite at 37° for 3.5 Hours

Reaction mixture				Formation of N-nitrosodimethylamine	
pH	Dimethylamine mM	**1** mM	Sodium nitrite mM	mg/ml	mM
3	50	—	200	2.16	29
3	—	50	200	2.16	29
3	50	50	200	4.32	58
5	50	—	200	0.24	3.2
5	—	50	200	1.20	16
5	50	50	200	2.08	28

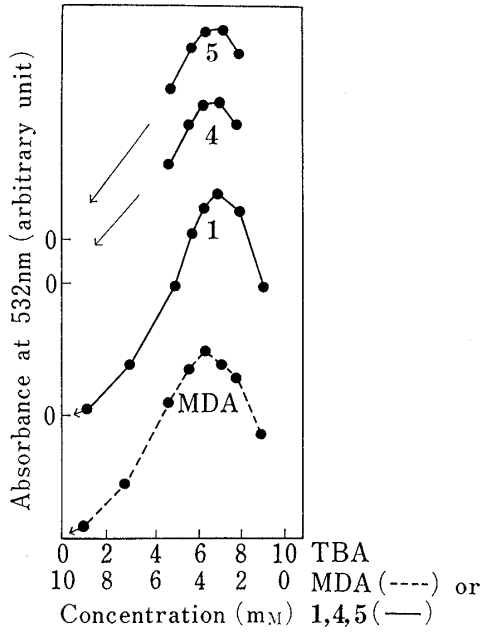

Fig. 4. Coloration of MDA and β-Dialkylaminoacroleins with 2-Thiobarbituric Acid (TBA)

Various concentrations (0—10 mM) of TBA, and MDA or β-dialkylaminocroleins (**1**, **4** or **5**) in 50% glacial acetic acid was treated at 100° for 20 min. Color intensity at 532 nm was estimated as described in "Experimental."

may indicate that the coloration of the compounds by TBA was due to the MDA liberated from the compounds.

N-Nitrosodimethylamine Formation from β-Dimethylaminoacrolein (1) by Reaction with Nitrite

Dimethylamine and β-dimethylaminoacrolein (**1**) were treated with sodium nitrite at pH 3.0 and 5.0 at 37° for 3.5 hours. Formation of N-nitrosodimethylamine, as determined by gas chromatography, is summarized in Table V, which indicates that N-nitrosodimethylamine was readily produced from **1**. The rate of formation of the nitrosamine from **1** at pH 3.0 was comparable to that from dimethylamine, while the rate from **1** at pH 5.0 was 5 times as high as that from dimethylamine. It is apparent that the formation of N-nitrosodimethylamine from **1** was greater than that from dimethylamine at pH 5.0. When a mixture of **1** and dimethylamine was treated with nitrite at pH 3.0, the rate of the nitrosamine formation was as expected. However, when the mixture was treated at pH 5.0, an unexpectedly high rate of formation of the nitrosamine was observed.

Discussion

It was found that MDA, which can be formed *via* peroxidation of polyunsaturated fatty acids,[2] readily underwent reaction with secondary amines under mild conditions close to physiological conditions. The major reaction products were non-fluorescent β-dialkylaminoacroleins (**1**—**5**) of *trans*, *s-trans* conformation. Zinnitz *et al.*[12,25] demonstrated that β-aminoacroleins synthesized from propargyl aldehyde and amines have pharmacological activity, such as central stimulating action. Since β-dialkylaminoacroleins such as **1**—**5** were readily formed under mild conditions, it is conceivable that such kinds of acroleins of biologically important secondary amines might be formed from MDA under physiological conditions and serve as pharmacologically active agents.

While β-dialkylaminoacroleins were stable under neutral conditions, they were readily hydrolyzed into several UV-absorbing substances with loss of the absorbance under acidic and alkaline conditions.

Kohn and Leversedge[26] described the formation of a characteristic pink color by the reaction of TBA with an unknown substance formed during the aerobic incubation of tissue homogenates. Since the principal TBA reaction was recognized to be a 2:1 reaction between TBA and MDA,[24] the reaction with TBA has been widely adopted as a sensitive assay method for MDA in peroxidized lipid. β-Dialkylaminoacroleins obtained in the present study were reactive with TBA and gave the same pink color with the same reaction ratio as MDA. The results were similar to those of Buttkus and Bose,[27] who reported that adducts such as N-substituted-3-aminopropenals[6] and N,N′-disubstituted-1-amino-3-iminopropenes[7,8] produced

25) F. Zinnitz and K. Heuwing, *Arch. Exptl. Pathol. Pharmakol.*, **213**, 30 (1951).
26) H.I. Kohn and M. Liversedge, *J. Pharmacol. Exp. Ther.*, **82**, 292 (1942).
27) H. Buttkus and R.J. Bose, *J. Am. Oil Chemist's Soc.*, **49**, 440 (1972).

a pink color with TBA. It should be emphasized that β-dialkylaminoacroleins as well as other adducts of MDA gave positive TBA coloration, and thus the color formation from tissue homogenates and biomaterials with TBA is due not only to MDA but also to its reaction products with several kinds of amines.

In the previous paper,[10] it was reported that MDA promoted the reaction of dimethyl (and diethyl)amine with nitrite to produce the corresponding nitrosamines at pH 4.0 and 5.0, while the agent inhibited the reaction at pH 3.0. In the present study, it was shown that β-dimethylaminoacrolein (**1**) was readily nitrosated to yield N-nitrosodimethylamine. Production of the nitrosamine from **1** at pH 3.0 was comparable to that from dimethylamine, whereas its production from **1** at pH 5.0 was much higher than that from dimethylamine. The mixture of dimethylamine and **1** produced a much greater amount of the nitrosamine at pH 5.0 than expected. The promotion of the formation of the nitrosamine by MDA at pH 5.0[10] could be explained by ready formation of **1** from MDA and dimethylamine under the conditions used, and by the greater rate of production of the nitrosamine from **1**, or a mixture of **1** and dimethylamine, than from dimethylamine alone. The reason for the inhibitory effects of MDA on the nitrosamine formation at pH 3.0[10] remains unclear.

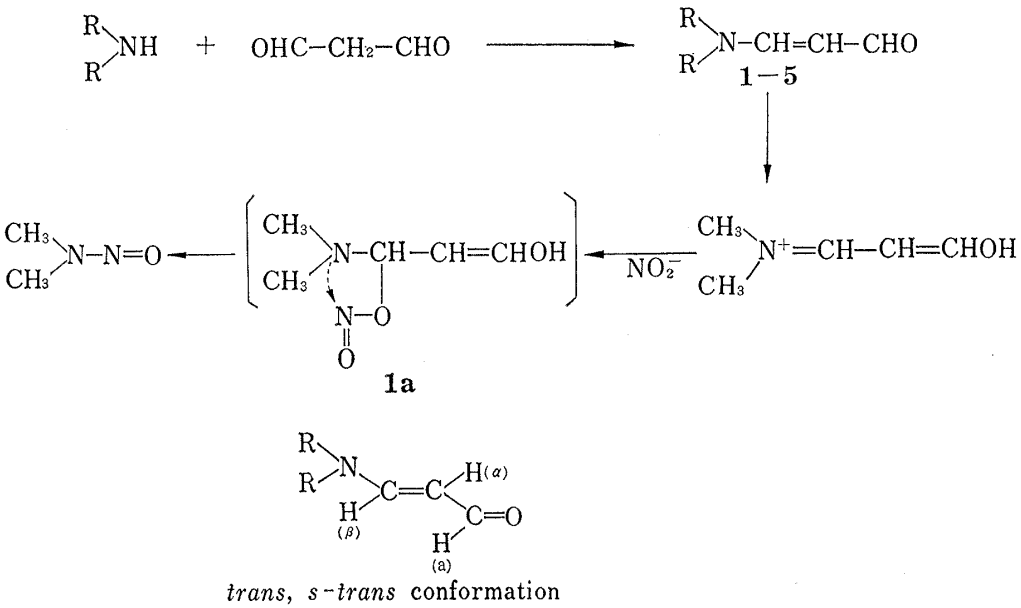

Chart 1

The formation of the nitrosamine from **1** by reaction with sodium nitrite might be explained as follows. The compound (**1**) was considered to be converted into iminium form under acidic conditions. Since iminium ions such as dimethylformamidinium ions are thought to be highly reactive with nitrite,[28] nucleophilic addition of nitrite to the iminium ion may yield the adduct (**1a**), in which the nitroso group migrates to produce N-nitrosodimethylamine as a result of an intramolecular attack of the nitrogen atom. When dimethylamine is present together with this adduct (**1a**), intermolecular attack of the nitrogen atom of dimethylamine may occur to form the nitrosamine.

In conclusion, MDA readily produced β-dialkylaminoacroleins by reaction with secondary alkylamines under mild acidic or neutral conditions; these compounds were relatively unstable, produced a pink color in the TBA test and were readily converted into the nitrosamine by reaction with nitrite. Studies on the formation of β-dialkylaminoacroleins *in vivo* and their physiological significance are clearly required.

28) L.K. Keefer and P.P. Roller, *Science*, **181**, 1245 (1973).