Formation of a Direct Mutagen, Diazo-*N*-nitrosoetilefrin, by Interaction of Etilefrin with Nitrite

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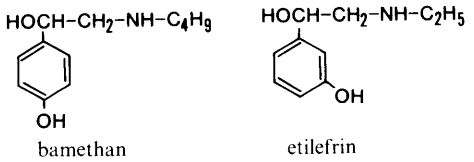
Reaction of an antihypotensive drug, etilefrin [α-{(ethylamino)methyl}-*m*-hydroxybenzyl alcohol], with nitrite under mildly acidic conditions produced *N*-nitrosoetilefrin [α-{(*N*-nitrosoethylamino)methyl}-*m*-hydroxybenzyl alcohol] (a mixture of *syn* and *anti* forms) (Iab) and diazo-*N*-nitrosoetilefrin [1-(4-diazo-3-oxo-1,5-cyclohexadienyl-2-(*N*-nitrosoethylamino)ethanol] (a mixture of *syn* and *anti* forms) (IIab). Treatment of etilefrin with an equivalent amount of nitrite at pH 3 and 37 °C for 4 h gave Iab (yield, 30%) and IIab (yield, 5%). Treatment of etilefrin with 4 eq of nitrite under the same conditions gave Iab (23%) and IIab (53%). Compounds Iab and IIab were each composed of two isomers due to the configuration of the *N*-nitroso group. While compound Iab was not mutagenic, compound IIab showed mutagenicity to *Salmonella typhimurium* TA98 and TA100 strains without metabolic activation. Specific mutagenic activity of IIab was 300 *his*$^+$ revertant colonies for both TA98 and TA100 strains with a dose of 0.1 μmol. Addition of a microsomal activation system little affected the activity. It is noteworthy that this orally administered drug can produce a direct-acting mutagen by reaction with nitrite, which is present in the digestive tract.

Keywords etilefrin; *N*-nitrosoetilefrin; diazo-*N*-nitrosoetilefrin; mutagenicity

The interaction of orally administered drugs with nitrite under mildly acidic conditions needs to be considered from a safety point of view,[1,2] because nitrite supplied by bacterial and salivary reduction of nitrate present in vegetables[3,4] can react with orally administered drugs to form mutagenic or carcinogenic compounds in the stomach. Several drugs have been shown to produce mutagenic compounds by interaction with nitrite.[5-10] Among them, bamethan [1-(4-hydroxyphenyl)-1-hydroxy-2-butylaminoethane] produced non-mutagenic *N*-nitrosobamethan and highly mutagenic 3-diazo-*N*-nitrosobamethan.[10] 3-Diazo-*N*-nitrosobamethan had potential tumor-initiating and -promoting activities in rat stomach mucosa.[11]

Bamethan bears a secondary amino group and a phenolic function in the molecule. *N*-Nitrosation occurs on the secondary amino group and diazotization occurs on the phenolic function.[10] Etilefrin [α-{(ethylamino)methyl}-*m*-hydroxybenzyl alcohol], which is used as an antihypotensive drug and may be administered orally over a long period, has a structure similar to that of bamethan (Fig. 1). Thus, we have investigated the interaction of etilefrin with nitrite under mildly acidic conditions, and we report here the formation of a direct-acting mutagenic diazoquinone.



bamethan etilefrin

Fig. 1. Structures of Bamethan and Etilefrin

Experimental

Materials Etilefrin hydrochloride used in the present experiment was the product of Tokyo Kasei Kogyo Co., Ltd., Tokyo. The preparation was used without further purification.

Analytical Methods Spectrophotometric Measurements: Absorption spectra were recorded with a Hitachi 557 dual-wavelength double-beam spectrophotometer. Infrared (IR) spectra of the samples dissolved in chloroform were measured with a JASCO A-302 infrared spectrophotometer. ^{1}H-Nuclear magnetic resonance (^{1}H-NMR) spectra of the samples dissolved in [$^2H_6$]dimethylsulfoxide were measured with a Bruker AM-400 NMR spectrometer. Mass spectra (MS) were measured with a Hitachi M-80 double focusing mass spectrometer by a chemical ionization (CI) technique using isobutane as the reagent gas.

Chromatographic Analysis: Thin-layer chromatography (TLC) was performed on Wakogel B5-F (Wako Pure Chemical Industries, Ltd., Osaka) with chloroform ethyl alcohol (9 : 1, v/v). Spots were visualized by irradiation with ultraviolet (UV) light at 254 nm or visible light. For column chromatography, 100-mesh silica-gel (Kanto Chemical Co., Inc., Tokyo) was used with chloroform ethyl alcohol (9 : 1, v/v) and ethyl acetate *n*-hexane (4 : 1, v/v). High-pressure liquid chromatography (HPLC) was carried out with a Shimadzu LC-6A liquid chromatograph equipped with a YMC A-303 ODS (particle size: 5 μm) column (4.6 mm i.d. × 25 cm: Yamamura Chemical Laboratories Ltd., Kyoto). Elution was carried out with methyl alcohol–0.1% acetic acid (1 : 4, v/v) at a flow rate of 0.8 ml/min. The peaks were detected at 260 nm by the use of a Shimadzu SPD-6A UV spectrophotometric detector.

Mutagenicity Test Mutagenicity was assayed by the preincubation method of Yahagi *et al.*[12] using *Salmonella typhimurium* TA98 and TA100 strains.[13] The microsomal S-9 system was prepared from liver microsomes of a rat that had been treated with polychlorinated biphenyl. Samples were dissolved in or diluted with 0.1 M phosphate buffer (pH 7.4) for the assay, 100 μl of the solution being used per plate. All the experiments were performed at least in duplicate. The numbers of *his*$^+$ revertants obtained with the positive controls dissolved in dimethylsulfoxide were 196/0.1 μg 4-nitroquinoline 1-oxide (4NQO) for TA98 without S-9 mix, 448/0.1 μg 3-amino-1,4-dimethyl-5*H*-pyrido[4,3-*b*]indole (Trp-P-1) for TA98 with S-9 mix, 586/0.1 μg 4NQO for TA100 without S-9 mix and 320/2.5 μg benzo[*a*]pyrene for TA100 with S-9 mix. Data are expressed after subtraction of spontaneously formed *his*$^+$ revertant colonies, the background numbers of *his*$^+$ revertants/plate being 18–21 for TA98 without S-9 mix, 16–22 for TA98 with S-9 mix, 73–85 for TA100 without S-9 mix and 80–92 for TA100 with S-9 mix.

Isolation and Identification of the Reaction Products of Etilefrin and Nitrite A mixture of 2.0 mmol of etilefrin and 8.0 mmol of sodium nitrite in 50 ml of water was adjusted to pH 3.0 with hydrochloric acid. After incubation at 37 °C for 4 h, the reaction was stopped by adding an equivalent amount of ammonium sulfamate to nitrite. The reaction mixture became orange. The reaction mixture was concentrated into about 1 ml, then ethyl alcohol (50 ml) was added and the whole was concentrated to about 0.5 ml. TLC analysis revealed two major yellow spots (*Rf* 0.26 and 0.31), a minor yellow spot (*Rf* 0.53) and two major UV-absorbing spots (*Rf* 0.57 and 0.60). In HPLC analysis, seven UV-absorbing peaks were detected at retention times of 6.8, 8.0, 9.4 (IIa), 12.2 (IIb), 40.2 (Ia), 48.0 (Ib) and 53.5 min. Among these peaks, four peaks corresponding to Ia, Ib, IIa and IIb were more prominent. When the major yellow spots in TLC were excised and analyzed by HPLC, two peaks appeared at retention times of 9.4 (IIa) and 12.2 (IIb) min. When the major UV-absorbing spots in TLC were analyzed by HPLC, two peaks appeared at retention times of 40.2 (Ia) and 48.0 (Ib) min. The concentrated reaction mixture was applied to a silica-gel column (2.5 i.d. × 20 cm). The column was eluted with chloroform–ethyl alcohol (9 : 1, v/v) and the fractions containing the major UV-absorbing product (fraction I), and the fractions containing the major yellow product (fraction II) were separately evaporated to dryness.

Fraction I was rechromatographed on a silica-gel column (1.5 i.d. × 22 cm) with ethyl acetate–*n*-hexane (4:1, v/v) and the fraction containing the product was evaporated to dryness, giving a colorless oil (90 mg). The product revealed two spots on a thin-layer chromatogram and two peaks on a high-pressure liquid chromatogram. ^{1}H-NMR ppm: 0.98 and 1.26 (total 3H, each t, CH_3 *syn* and *anti*), 3.49—3.75 and 4.01—4.18 (total 4H, m and m, CH_2–N–CH_2), 4.61 and 4.84 (total 1H, each m, CHOH *syn* and *anti*), 6.63—6.83 (3H, m, phenyl C^2H, C^4H and C^6H), 7.12 (1H, m, phenyl C^5H), 9.33 (br s, phenyl OH). UV λ_{max}^{MeOH} nm (ε): 224 (8890), 274 sh (2340). CI-MS *m/z* (relative intensity): M+H$^+$ 211 (95), M$^+$−17[·OH] 193 (100), and M$^+$−87 [·CH_2–N(NO)–C_2H_5] 123 (63). From the spectral data, the compound was determined to be a mixture of two isomers of *N*-nitrosoetilefrin [α-{(*N*-nitrosoethylamino)methyl}-*m*-hydroxybenzyl alcohol] (Ia and b).

Fraction II was rechromatographed on a silica-gel column (2.5 i.d. × 20 cm) with chloroform–ethyl alcohol (9:1, v/v) and the fraction containing the product was evaporated to dryness, giving an orange oil (120 mg). This oily product revealed two yellow spots on a thin-layer chromatogram, and two peaks on a high-pressure liquid chromatogram. ^{1}H-NMR ppm: 0.99 and 1.30 (total 3H, each t, CH_3 *syn* and *anti*), 3.41—3.79 and 4.12—4.21 (total 4H, m and m, CH_2–N–CH_2), 4.52 and 4.77 (total 1H, each m, CHOH *syn* and *anti*), 6.29 and 6.38 (total 1H, each d, J=9.0 Hz, phenyl C^6H *syn* and *anti*), 6.45 and 6.56 (total 1H, each s, phenyl C^2H *syn* and *anti*), 7.52 and 7.53 (total 1H, d, J=9.0 Hz, phenyl C^5H *syn* and *anti*). UV λ_{max}^{MeOH} nm (ε): 226 (20100), 272 sh (4300), 400 (4200). IR: 2100 (C≡N$_2$), 1620 (C=O) cm^{-1}. CI-MS *m/z*: M+H$^+$ 237. When 1 mg of the compound was treated with 10 mg of resorcinol in 5 ml of water at room temperature for 30 min, the color of the solution became red. From the spectral data and the chemical properties, the compound was determined to be a mixture of two isomers of diazo-*N*-nitrosoetilefrin [1-(4-diazo-3-oxo-1,5-cyclohexadienyl)-2-(*N*-nitrosoethylamino)ethanol] (IIa and b).

When a mixture of 0.3 mmol of Iab and 1.2 mmol of sodium nitrite in 10 ml of water was treated at pH 3.0 and 37 C for 4 h, Iab was converted to IIab as determined by HPLC.

Results

Etilefrin was treated with nitrite under the conditions recommended by the World Health Organization.[14] Etilefrin was allowed to react with 1 and 4 eq of nitrite at pH 3 and 37 C for 4 h and the mutagenicity of the reaction mixtures was tested in *S. typhimurium* TA98 and TA100 strains with and without metabolic activation. Dose-response curves derived from the assays are shown in Fig. 2. The reaction mixtures showed mutagenicity to both *S. typhimurium* TA98 and TA100 strains without S-9 mix, and the mutagenicity was slightly increased in the presence of S-9 mix. The mutagenicity of the reaction mixtures with 4 eq of nitrite was higher than that of the reaction mixtures with one equivalent of nitrite. The mutagenicity increased with increasing amount of etilefrin, up to 0.4 μmol of etilefrin. The maximal number of *his*$^+$ revertant colonies obtained with the reaction mixture with 4 eq of nitrite was about 460/0.4 μmol etilefrin in TA98 and TA100 strains with S-9 mix.

TLC of the reaction mixture of etilefrin with 4 eq of nitrite (Fig. 3) revealed two major yellow spots at *Rf* 0.26 and 0.31 and two major UV-absorbing spots at *Rf* 0.57 and 0.60. HPLC of the reaction mixture revealed four major UV-absorbing peaks at retention times of 9.4 (IIa), 12.2 (IIb), 40.2 (Ia) and 48.0 (Ib) besides three minor peaks (Fig. 4). The former two peaks were detected when the extract of the major yellow TLC spots was analyzed. The latter two peaks were detected when the extract of the major UV-

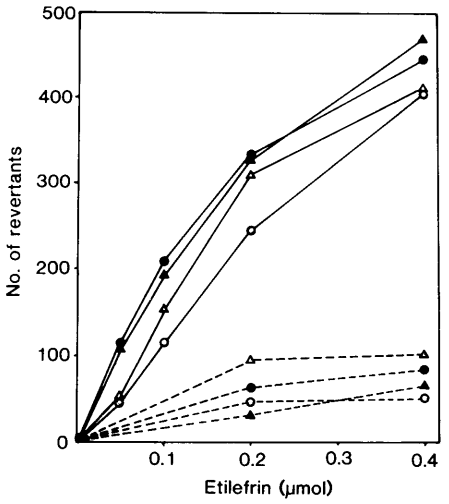


Fig. 2. Mutagenicity of the Reaction Mixtures of 2 mmol of Etilefrin with 2(------) or 8 (——) mmol of Sodium Nitrite in 50 ml of Water at pH 3 and 37 C for 4 h

The reaction was stopped by adding an equivalent amount of ammonium sulfamate to nitrite. For each reaction mixture, 10 ml was diluted to 100, 200 or 400 ml with 0.1 M phosphate buffer (pH 7.4) and the solution was assayed at 100 μl/plate. The mutagenicity was tested with *S. typhimurium* TA98 without S-9 mix (○), TA98 with S-9 mix (●), TA100 without S-9 mix (△) and TA100 with S-9 mix (▲). Each point represents the mean value of duplicate experiments. The number of *his*$^+$ revertants colonies/plate was plotted against the amount of etilefrin originally present in the sample used for the assay. Solutions of etilefrin (0–0.4 μmol) alone and nitrite (0—0.4 μmol) alone in the phosphate buffer showed no mutagenicity.

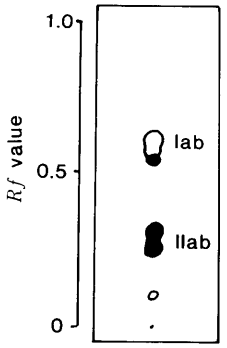


Fig. 3. TLC of the Reaction Mixture of 2 mmol of Etilefrin and 8 mmol of Sodium Nitrite in 50 ml of Water at pH 3 and 37 C for 4 h

The spots were detected under visible light (solid mark) and under irradiation with UV-light (open mark).

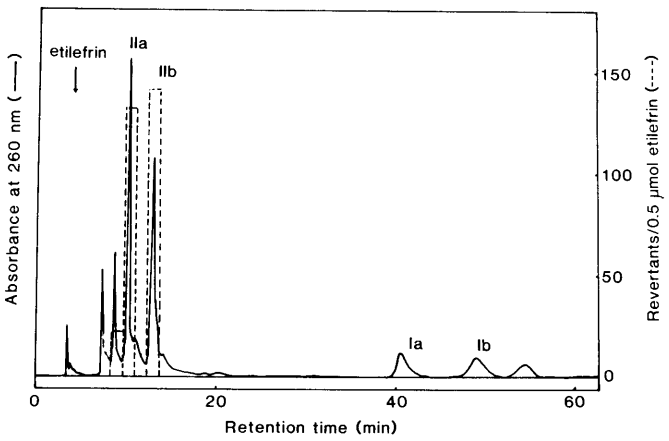


Fig. 4. HPLC of the Reaction Mixture of 2 mmol of Etilefrin and 8 mmol of Sodium Nitrite in 50 ml of Water at pH 3 and 37 C for 4 h

The reaction mixture (12.5 μl: corresponding to 0.5 μmol of etilefrin) was subjected to HPLC. The peaks were detected at 260 nm. For measurement of the mutagenicity, 1.6-ml HPLC fractions were evaporated to dryness, dissolved in 100 μl of 0.1 M phosphate buffer (pH 7.4) and tested against *S. typhimurium* TA98 without S-9 mix. The recovery of the mutagenic activity was 47%. This low recovery might be due to decomposition of the active fractions during the work-up.

absorbing TLC spots was analyzed. Attempts to isolate these four compounds failed, since each purified HPLC fraction containing Ia or Ib gave the same HPLC profile as that of the mixture of Ia and Ib, and each purified fraction containing IIa or IIb gave the same chromatographic profile as that of the mixture of IIa and IIb. Among the UV-absorbing peak fractions only the fractions containing IIa and IIb showed mutagenicity, and 47% of the mutagenicity loaded was recovered in these two peaks.

Compounds Iab (a mixture of Ia and Ib) and compounds IIab (a mixture of IIa and IIb) were obtained as a colorless oil and a yellow oil, respectively, by successive silica-gel column chromatographies. CI-MS of compound Iab revealed an ion peak at m/z 211 ($M+H^+$), indicating that molecular weight of Iab was 210 and that an NO function had been introduced into the secondary amino function. Compound Iab showed a typical ^{1}H-NMR spectrum of a mixture of two isomers of an *N*-nitroso derivative. It has been demonstrated that the *syn* α- and β-methylene protons to the nitroso oxygen in *N*-dialkylnitrosamines resonate at higher fields than the *anti* α- and β-methylene protons, respectively.[15] The *syn* and *anti* β-methyl proton signals are thus at 0.98 and 1.26 ppm, and the *syn* and *anti* hydroxymethylene proton signals at 4.61 and 4.84 ppm, respectively. The structure of Iab must be *N*-nitrosoetilefrin [α-{(*N*-nitrosoethylamino)methyl}-*m*-hydroxybenzyl alcohol] with two isomers, Ia and Ib.

When compound Iab was incubated with an excess of nitrite at pH 3, it was converted to IIab by interaction with nitrite. Compound IIab showed a molecular ion peak at 237 m/z ($M+H^+$) in CI-MS. The molecular weight of IIab was increased by 26 from that of Iab. The ^{1}H-NMR spectrum of IIab was different from that of Iab with respect to the signals only at the aromatic proton regions. In the *syn* form, *ortho*-coupled protons appeared at 6.29 and 7.52 ppm ($J=9.0$ Hz) and a non-coupled proton at 6.45 ppm. In the *anti* form, *ortho*-coupled protons appeared at 6.38 and 7.53 ppm ($J=9.0$ Hz) and a non-coupled proton at 6.56 ppm. The NMR spectra suggested that one of the aromatic protons of Iab was substituted. Compound IIab exhibited a characteristic absorption spectrum in methyl alcohol with maxima at 226, 272 (shoulder) and 400 nm, whereas Iab revealed absorption maxima at around 224 and 274 nm. The absorption maxima of IIab were similar to those of 3-diazo-*N*-nitrosobamethan.[10] The IR spectrum of IIab showed bands at 2100 and 1620 cm^{-1}, which are characteristic of a quinone diazo grouping.[16]

When IIab was treated with resorcinol in water, the color of the solution became red, indicating that azo coupling reaction due to the aromatic diazo grouping[17] had taken place. These spectral data and chemical properties of IIab established the structure of IIab to be 4-diazo-*N*-nitrosoetilefrin [1-(4-diazo-3-oxo-1,5-cyclohexadienyl)-2-(*N*-nitrosoethylamino)ethanol] with two isomers, IIa and IIb.

The time course of the formation of Iab and IIab in the reaction of etilefrin with 1 or 4 eq of nitrite was followed (Fig. 5). In the reaction with one equivalent of nitrite, Iab was predominant throughout the reaction period up to 4 h. After the reaction for 4 h, the yields of Iab and IIab were 30 and 5%, respectively. In the reaction with 4 eq of nitrite, the amount of Iab was the largest at 1 h and decreased thereafter. The amount of IIab progressively increased up to 4 h.

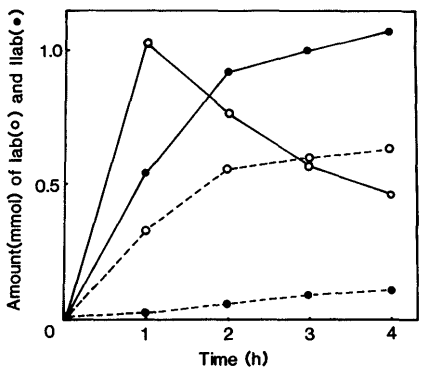


Fig. 5. Time Course of the Formation of Iab (○) and IIab (●) from a Mixture of 2 mmol of Etilefrin, and 2 (------) or 8 (——) mmol of Sodium Nitrite in 50 ml of Water at pH 3 and 37°C

The reaction was stopped by adding an equivalent amount of ammonium sulfamate to nitrite. The mixtures were analyzed by HPLC (Fig. 4) and the amounts of compounds Iab and IIab were determined by comparing the sum of the two peak heights due to Iab or IIab of the reaction mixtures with those of standard Iab or IIab. Calibration curves for standards Iab and IIab were linear.

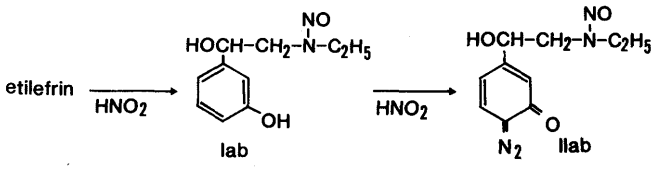


Fig. 6. Reaction of Etilefrin with Nitrite to Produce *N*-Nitrosoetilefrin (Iab) and Diazo-*N*-nitrosoetilefrin (IIab)

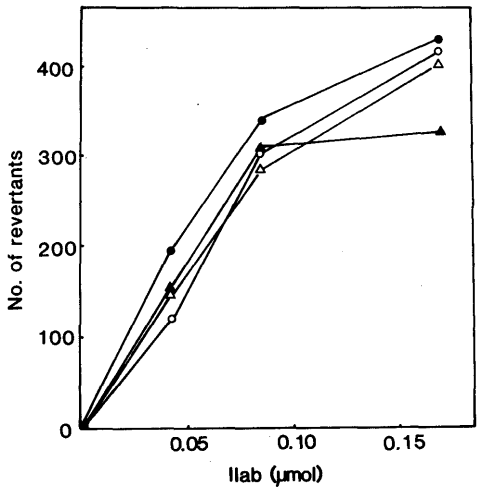


Fig. 7. Mutagenicity of Diazo-*N*-nitrosoetilefrin (IIab) Dissolved in 100 μl of 0.1 M Phosphate Buffer (pH 7.4), in *S. typhimurium* TA98 without S-9 Mix (○), TA98 with S-9 Mix (●), TA100 without S-9 Mix (△) and TA100 with S-9 Mix (▲)

Each point represents the mean value of duplicate experiments.

The decrease of Iab may be due to the reaction of Iab with an excess amount of nitrite to produce IIab. After the reaction for 4 h, the yields of Iab and IIab were 23 and 53%, respectively.

The reaction of etilefrin with nitrite in an acidic solution is shown in Fig. 6. Etilefrin was reacted with nitrite to form *N*-nitrosoetilefrin (Iab), which was in turn reacted with nitrite to produce diazo-*N*-nitrosoetilefrin (IIab).

The mutagenicity of diazo-*N*-nitrosoetilefrin (IIab) was tested in *S. typhimurium* TA98 and TA100 with and without metabolic activation (Fig. 7). The compound was

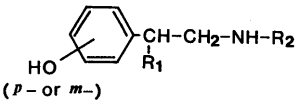
active in both strains without metabolic activation, indicating that it is a direct mutagen. The maximum number of *his*$^+$ revertant colonies with TA98 and TA100 strains was about 300 at a dose of 0.1 μmol. *N*-Nitrosoetilefrin (Iab) was similarly tested but no significant mutagenicity was detected.

Discussion

It has been suggested that certain orally administered drugs are derivatized into mutagenic and/or carcinogenic compounds by interaction with nitrite present in the digestive tract.[18] Pyrazolones,[5] pyridinol carbamate,[7] penicillin G[9] and bamethan[10] were converted into potent mutagens by reaction with nitrite under mildly acidic conditions. Etilefrin is a widely used antihypotensive drug and may be administered orally over a long period. Formation of mutagenic diazo-*N*-nitrosoetilefrin (IIab) may thus be important as a health risk. The potency of the mutagenicity of IIab was about 1/3 of that of the mutagenicity of the bamethan reaction product.

Previously we have demonstrated that *p*- and *o*-diazoquinones are produced in the reaction of phenol and nitrite under mildly acidic conditions.[19] *p*-Diazoquinone showed significant but low mutagenicity to *S. typhimurium* TA98 and TA100 strains without metabolic activation. In contrast, *o*-diazoquinone did not show any significant mutagenicity. Tyramine was converted into 3-diazotyramine[20] and bamethan into 3-diazo-*N*-nitrosobamethan,[10] and both these *ortho* diazoquinone compounds showed mutagenicity to *S. typhimurium* TA98 and TA100 strains without metabolic activation. It was found in the present experiments that etilefrin produced the mutagenic *ortho* diazoquinone IIab by reaction with nitrite. The compound showed similar mutagenicity to both TA98 and TA100 strains without metabolic activation. The direct mutagenic activity is presumably due to the *ortho* diazoquinone function in the molecule.

Tyramine, bamethan and etilefrin have a common partial structure, as shown in Fig. 8; a phenolic structure with an aminoethyl group at the *p*- or *m*-position. All three compounds have been shown to be diazotized by reaction with nitrite into *ortho* diazoquinone derivatives regardless of whether the amino group is nitrosated, and the diazoquinone derivatives with aminoethyl groups were highly mutagenic. There are several other drugs that have this common structure and are administered orally for long periods. For instance, octopamine, denopamine, norfenefrine and phenylephrine have the same common partial structure, and they are frequently used for treatment of cardiovascular diseases by oral administration for long periods. Phenolic drugs with aminoethyl groups, *i.e.*, isoxsuprine, nylidrin, labetalol and formoterol, are also given orally. These drugs with closely related structures might be derivatized into *ortho* diazoquinones with aminoethyl groups, which might act as potential mutagens. Our preliminary test on the reaction of isoxsuprine with nitrite showed that the reaction mixture was mutagenic, although the mutagenicity was lower than that of the reaction mixture of bamethan.[10] It is important to take account of possible formation of mutagenic diazoquinones with aminoethyl groups by reaction with nitrite in assessing the risk to human health from drugs.



octopamine: HO(*p*-), R_1=OH, R_2=H
denopamine: HO(*p*-), R_1=OH, R_2=CH_2CH_2-phenyl-$(OCH_3)_2$
norfenefrine: HO(*m*-), R_1=OH, R_2=H
phenylephrine: HO(*m*-), R_1=OH, R_2=CH_3

Fig. 8. Drugs with an Aminoethyl-Substituted Phenol Partial Structure

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References

1) W. Lijinsky, *Cancer Res.*, **34**, 255 (1974).
2) W. Lijinsky, M. D. Reuber and B.-N. Blackwell, *Science*, **209**, 817 (1980).
3) A. Ayanaba and M. Alexander, *Appl. Microbiol.*, **25**, 862 (1973).
4) H. Ishiwata, P. Boriboon, Y. Nakamura, M. Harada, A. Tanimura and M. Ishidate, *Shokuhin Eiseigaku Zasshi*, **16**, 19 (1975).
5) M. Arisawa, M. Fujiu, Y. Suhara and H. B. Maruyama, *Mutat. Res.*, **57**, 287 (1978).
6) G. Rao and G. Krishna, *J. Pharm. Sci.*, **64**, 1579 (1979).
7) H. Kanaya and Y. Takeda, *Cancer Lett.*, **18**, 143 (1983).
8) A. W. Andrews, W. Lijinsky and S. W. Snyder, *Mutat. Res.*, **135**, 105 (1984).
9) P. N. Gillatt, R. C. Palmer, P. L. R. Smith and C. L. Walters, *Food Chem. Toxicol.*, **23**, 849 (1985).
10) K. Kikugawa, T. Kato and Y. Takeda, *Mutat. Res.*, **177**, 35 (1987).
11) C. Furihata, A. Yamakoshi, T. Matsushima, T. Kato and K. Kikugawa, *Mutagenesis*, **3**, 299 (1988).
12) T. Yahagi, M. Nagao, Y. Seino, T. Matsushima, T. Sugimura and M. Okada, *Mutat. Res.*, **48**, 121 (1977).
13) B. N. Ames, J. McCann and E. Yamasaki, *Mutat. Res.*, **31**, 347 (1975).
14) World Health Organization, *Drug Information*, **2**, 4 (1978).
15) G. J. Kanabatsos and R. A. Taller, *J. Am. Chem. Soc.*, **86**, 4373 (1964).
16) R. J. W. L. Fèvre, J. B. Sousa and R. L. Werner, *J. Chem. Soc.*, **1954**, 4686.
17) A. R. Forrester and J. L. Wardell, "Rodd's Chemistry of Carbon Compounds," ed. by S. Coffey, Vol. III, Elsevier, Amsterdam, 1971, Part A, p. 363.
18) W. Lijinsky and S. S. Epstein, *Nature* (London), **225**, 21 (1970).
19) K. Kikugawa and T. Kato, *Food Chem. Toxicol.*, **26**, 209 (1988).
20) M. Ochiai, K. Wakabayashi, M. Nagao and T. Sugimura, *Gann*, **75**, 1 (1984).