

AN INVESTIGATION ABOUT THE FORMATION OF N-NITROSOMAPROTILINE UNDER  
PHYSIOLOGICAL CONDITIONS

FİZYOLOJİK KOŞULLARDA N-NİTROZOMAPROTİLİN OLUŞUMUNUN ARAŞTIRILMASI

BUKET ALPERTUNGA, NURAN MEŞECİ

Marmara University, Faculty of Pharmacy, Department of Pharmaceutical Toxicology, 81010 Haydarpaşa,  
Istanbul, Turkey

*In this study first N-nitroso derivative from maprotiline hydrochloride which is a therapeutic agent comprising secondary amine group in its structure, was synthesized and characterized by IR-, <sup>1</sup>H NMR spectroscopy and Mass spectrometry and secondly the formation of N-nitrosomaprotiline under simulated stomach conditions was examined and the amount of N-nitrosomaprotiline was determined colorimetrically. Under physiological conditions the yield of N-nitrosomaprotiline was found to be 0.35%. Ascorbic acid added to the reaction medium is able to inhibit the formation of NOM up to 80.6%.*

*Çalışmanın ilk aşamasında sekonder amin grubu içeren bir ilaç maddesi olan maprotilin hidroklorürün N-nitrozo türevinin sentezi yapıldı. N-nitrozomaprotilinin (NOM) yapısı, IR-, <sup>1</sup>H NMR spektroskopisi ve MS spektrometrisi ile aydınlatıldı. İkinci aşamada fizyolojik şartlarda NOM oluşumu incelenerek miktarı kolorimetrik bir yöntem ile tayin edildi. Fizyolojik koşullarda N-nitrozomaprotilin verimi %0.35 olarak bulundu. İn-kübasyon ortamına ilave edilen askorbik asidin NOM oluşumunu %80.6 ya varan bir oranda inhibe ettiği görüldü.*

**Keywords :** Drug-nitrite interaction; N-nitroso compounds; N-nitrosomaprotiline

**Anahtar kelimeler:** İlaç-nitrit etkileşimi; N-nitrozo bileşikleri; nitrozomaprotilin

### Introduction

It is now clearly established that N-nitroso compounds are carcinogenic in a variety of organs and many animal species (1,2). N-nitroso compounds have been shown to occur in food products, water, alcoholic beverages, industrial products, agricultural products, cosmetics, drugs, tobacco products, and human biological fluids (3,4). N-nitroso compounds are formed by a reaction between nitrite and secondary, tertiary amines or amides at acid pH. The human stomach provides an acidic milieu that favors the formation of nitrosamines endogenously. Drinking water and food are common sources for nitrite and nitrate.

Because of the presence of amine or amide groups many orally administered drugs were found to react with sodium nitrite in vitro under simulated human stomach conditions to yield N-nitroso compounds (5-10). Only some of N-nitroso compounds formed could be isolated as the pure compounds (11-16).

In this work we first synthesized and characterized the N-nitrosomaprotiline (NOM) from maprotiline hydrochloride which is comprising a secondary amino group and secondly we examined the formation of NOM under simulated stomach conditions.

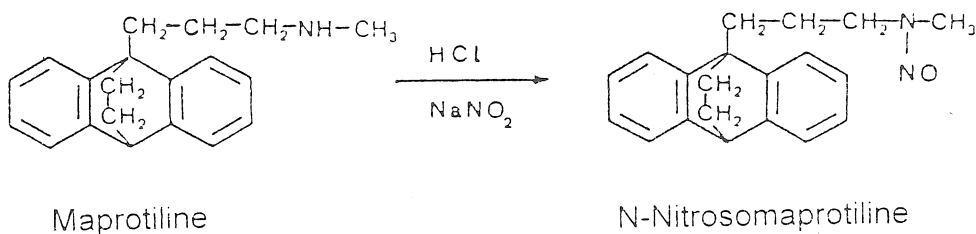
Maprotiline [3-(9,10-dihydro-9,10-ethanoanthracen-9-yl)-N-methylpropylamine]

hydrochloride is widely used for the treatment of major depression, often for long period and orally (17). Therefore it is interesting to evaluate the possibility that small amounts of N-nitrosomaprotiline might arise in the stomach of patients taking these drugs.

### Material and Methods

Melting point was determined on a Buchi 530 melting point apparatus. pH determinations were performed at a Phillips pH Meter pW 9421. IR spectrum was recorded on a Perkin Elmer 240 infrared spectrophotometer. <sup>1</sup>H NMR spectrum was determined by using a Bruker AC 200L (200 MHz). Mass spectrometry was performed on Hewlett Packard AEI MS-30 spectrometer. A Varian Series 634 double-beam spectrophotometer was used for spectrophotometric determinations. Pharmaceutical grade maprotiline hydrochloride was obtained from Ciba-Geigy, Istanbul. Other chemicals were purchased from Merck. All solvents were of analytical-reagent grade.

Synthesis of N-nitrosomaprotiline: A solution of maprotiline hydrochloride (1.0 mmol) in 20 ml of distilled water was slowly added to the stirred solution over a period of 15 min. The mixture was incubated for 2h at room temperature. At the end of the incubation, the mixture was extracted several times with dichloromethane. After the pooled organic phases were dried with anhydrous sodium sulfate, the solvent was evaporated. The extract was purified by preparative TLC on silikagel GF<sub>254/366</sub> using dichloromethane as solvent system, and crystallized from methanol-dichloromethane



Scheme. Nitrosation of maprotiline hydrochloride

(1:1).

N-nitrosomaprotiline (NOM) (Scheme 1.), yellow crystals 78% yield, m.p 119-120°C..

Rf of N-nitrosomaprotiline	: 0.76
Rf of free base	: 0.00

Its structure was confirmed by IR-, <sup>1</sup>H NMR spectroscopy and Mass spectrometry:

IR (KBr) :  $\bar{\nu}$  = 1440 cm<sup>-1</sup> (NO), <sup>1</sup>H NMR(CDCl<sub>3</sub>):  
 $\delta$  1.61, 1.81 (2 m, -CH<sub>2</sub>-CH<sub>2</sub>-,4H), 2.24 (m, >-CH<sub>2</sub>-  
 ,2H), 2.48 (m, >-CH<sub>2</sub>-CH<sub>2</sub>-,2H), 3.17 (s, -CH<sub>3</sub>,3H),  
 4.29 (t, anthracene C-10H, 1H), 4.44 (t, -NNOCH<sub>3</sub>,  
 3H), 7.20-7.38 (m, arom. H, 8H), MS (EI): m/z 306  
 (15.6%), 278 (46.3%), 248 (100%), 204 (42%), 191  
 (38.7%), 178 (17.2%), 165 (5.1%), 44 (34%).

Nitrosation of maprotiline hydrochloride under simulated stomach conditions: One-fifth of the usual maximum dose of maprotiline hydrochloride (10 mg) was dissolved in 100 ml of distilled water. 0.1 ml of freshly prepared 0.64 M aqueous sodium nitrite solution was added. Adjustments of different pH 2.0 to 5.0 were effected by the addition of NaOH and HCl. The mixture was incubated at 37°C for 1h. Incubations were conducted in dark, with shaking. At the end of the incubation the reaction was terminated by the addition of an excess of sulfamic acid in order to decompose the residual nitrite. The mixture was extracted twice with 20 ml of dichloromethane. After the combined organic phases were dried with anhydrous sodium sulfate, the solvent was evaporated. The amount of NOM was calculated from the regression equation of the calibration graph.

N-nitrosomaprotiline was determined colorimetrically according to the method of Eisenbrand and Preussmann (18). For this purpose, the residue was dissolved in 5 ml of dichloromethane. One ml of this solution was added to 2 ml of 0.24 M hydrogen bromide in glacial acetic acid. After waiting at room temperature for 15 min the mixture was made up to 5 ml with the color reagent. The color reagent is a mixture of equal volumes of sulfanilic acid [1%; in glacial acetic acid (30%)] and N-naphthyl-(1)-ethylenediamine dihydrochloride [0.1%; in glacial acetic acid (30%)] After waiting for 10 min, the absorbance of the solution was read at 520 nm against the blank. A calibration graph was prepared

using NOM between 0.5-5  $\mu\text{g ml}^{-1}$  (A=0.146C+0.012, r=0.9989).

To determine the effect of the nitrite concentration on the formation of NOM, the drug solution was adjusted to pH 3 and stirred at 37°C. 0.1 ml of aqueous sodium nitrite of varying concentrations was then added. After incubation for 1h the NOM amount was determined as described above.

To estimate the degree of inhibition of nitrosation by ascorbic acid maprotiline hydrochloride was nitrosated under simulated stomach conditions at pH 3 described above in the presences of 5-40 mg ascorbic acid.

## Results and Discussion

N-nitrosomaprotiline, m.p. 119-120°C, was synthesized in 78% yield by nitrosating maprotiline hydrochloride with a 10 fold molar excess of sodium nitrite for 2h at 37°C in the presence of hydrochloric acid. <sup>1</sup>H NMR spectrum and IR-spectrum of the NOM differ from those of maprotiline hydrochloride for the disappearance of the NH signal (19). The m/z peak in MS-spectrum confirmed the molecular weight of NOM.

The amount of NOM was determined according to the method of Eisenbrand and Preussmann (18). The method based on the cleavage of the N-nitroso group by means of hydrogen bromide in glacial acetic acid. The liberated NO<sup>+</sup> is captured by diazotation of sulfanilic acid and the resulting diazonium ion is coupled with N-(naphthyl-(1)-ethylene diamine.

The conditions such temperature, pH, reaction time and sodium nitrite concentration are closer to those occurring in the stomach. The volume of a full stomach is normally about 500 ml. We used 1/5 of this volume, 1/5 of the maximum daily dose of maprotiline

hydrochloride and 1/5 of the amount of the daily nitrite intake. The time of emptying the stomach depends on the nature of diet. Following the intake of a normal meal it's about 60 min. The optimum nitrosation of maprotiline hydrochloride occurs between pH 2,5 and pH 3.0 (Table 1). The pH optimum found for formation of NOM is in agreement with that for N-nitrosation of secondary amines, which has been described in detail by Mirvish (20). Maximum nitrosation was observed at pH 3.0 with a yield of 0.35% (33.1 µg). The formation of NOM was measured at pH 3.0 and 37°C in the presence of varying concentrations of sodium nitrite because of the variability of nitrite concentration occur in the normal fasting stomach and after consumption of meals containing nitrite. The yield of NOM increased as the concentration of sodium nitrite was raised (Table 2). Ascorbic acid is known to reduce the endogenous synthesis of N-nitroso compounds (21,22). In order to examine to what extent the nitrosation of maprotiline hydrochloride was inhibited, the experiments were carried out in the presence of ascorbic acid. As shown in Table 3, ascorbic acid inhibited the nitrosation up to 80.6%.

Table 1. Effect of pH on the formation of NOM (3.2x10<sup>-5</sup> mol maprotiline hydrochloride, 6.4x10<sup>-5</sup> mol sodium nitrite in 100 ml)

N-nitrosoprotiline			
pH	µg*	mol	Yield(%)**
2.0	21.2	6.9x10 <sup>-8</sup>	0.22
2.5	25.5	8.3x10 <sup>-8</sup>	0.26
3.0	33.1	1.1x10 <sup>-7</sup>	0.35
3.5	18.2	5.8x10 <sup>-8</sup>	0.19
4.0	16.7	5.4x10 <sup>-8</sup>	0.17
4.5	12.7	4.2x10 <sup>-8</sup>	0.13
5.0	nd	nd	nd

\* The given values are mean values of at least two experiments.

\*\* Yields are calculated on the basis of initial maprotiline hydrochloride concentration.

It is reasonable to assume that small amounts of NOM can be formed in the human stomach. But even if the levels of N-nitrosocompounds

are small, the daily intake of such drugs over a long period represents an increase in the carcinogenic risk. Further studies are needed to asses the potentially hazard of drug-nitrite interactions in humans, especially those on chronic medication where readily nitrosatable drugs and high dietary intake of nitrite may be involved. The readily nitrosatable drugs might be employed in formulations containing sufficient amounts of ascorbic acid, which is known to hinder intragastric formation of nitrosamine if the pharmacokinetic datas of drugs are suitable.

Table 2. Effect of sodium nitrite concentration on the formation of NOM (3.2x10<sup>-5</sup> mol maprotiline hydrochloride, 6.4x10<sup>-5</sup> mol sodium nitrite in 100 ml, pH 3.0)

NaNO <sub>2</sub> mol	N-nitrosoprotiline		
	µg	mol	Yield (%)
1x10 <sup>-3</sup>	287.5	0.9x10 <sup>-6</sup>	2.96
7.5x10 <sup>-4</sup>	166.5	0.5x10 <sup>-6</sup>	1.72
5x10 <sup>-4</sup>	72.2	2.3x10 <sup>-7</sup>	0.74
1x10 <sup>-4</sup>	48.4	1.6x10 <sup>-7</sup>	0.50
7.5x10 <sup>-5</sup>	36.5	1.2x10 <sup>-7</sup>	0.37
5x10 <sup>-5</sup>	18.5	6x10 <sup>-8</sup>	0.19
1x10 <sup>-5</sup>	10.2	3x10 <sup>-8</sup>	0.10

Table 3. The inhibition of the nitrosation of maprotiline hydrochloride by ascorbic acid (3.2x10<sup>-5</sup> mol maprotiline hydrochloride, 6.4x10<sup>-5</sup> mol sodium nitrite in 100 ml, pH 3.0)

Ascorbic acid (mg)	N-nitrosoprotiline µg	Inhibition (%)
-	33.1	-
5	23.4	29.1
10	17.7	46.3
20	12.5	62.0
30	6.8	79.5
40	6.4	80.6

## References

- Williams, G.M., Weisburger, J.H.: Chemical Carcinogenesis. In: Amdur, M.O, Doull, J., Klaassen, C.D., The Basic Science of Poisons, pp. 178-180, Pergamon Press, New York, 1991

2. Shank, R.C.: *Toxicol. Appl. Pharmacol.* 31, 361 (1975)
3. Tannenbaum, S.R.: *Lancet* 629 (1983)
4. Tricker, A.R., Kumar, R., Siddiqi, M., Khuroo, M.S., Preussmann, R.: *Carcinogenesis* 12, 1595(1981)
5. Ohshima, H., Bartsch, H.: *Cancer Res.* 41, 3658 (1981)
6. Brambilla, G., Martelli, A., Sottofattori, E.: *Carcinogenesis* 16, 1239 (1995)
7. Ziebarth, D., Schramm, T., Töppel, A.: *Arch. Geschwulstforsch.* 59, 257 (1989)
8. Ziebarth, D.: *Arch. Pharm.* 327, 381 (1994)
9. Eisenbrand, G., Schmähl, D.: Nitrosation of Drugs. In: Eisenbrand, G., Bozler, G., v.Nicolai, H., The Significance of N-Nitrosation of Drugs, pp. 291-304, Gustav Fischer Verlag, Stuttgart, New York (1990)
10. Ziebarth, D., Teichmann, B.: *IARC Scientific Publ.* 31, 231 (1980)
11. Robbiano, L., Martelli, A., Allavena, A., Mazzel, M., Gazzaniga, M.G., Braubilla, G.: *Cancer Res.* 51, 2273 (1991)
12. Chen, J., Raisfeld-Danse, I.A.: *J.Pharmacol. Exp. Ther.* 225, 702 (1983)
13. Kinawi, A., Onken, A., Rozyczka, B., König, § W.: *Arzneim.- Forsch./Drug Res.* 27, 363 (1977)
14. Kinawi, A., Schuster, T.: *Arzneim.- Forsch./Drug Res.* 28, 219 (1978)
15. Abou-Gharbia, M.A., Pylypiw, H., Harrington, G.W., Swern, D.: *J. Org. Chem.* 46, 2193 (1981)
16. Mazzei, M., Sottofattori, E., Balbi, A.: *Il Farmaco* 46, 1403 (1991)
17. Katzung, B.G.: *Basic and Clinical Pharmacology*, pp. 327-335, Appleton&Lange, Beirut, (1987)
18. Eisenbrand, G., Preussmann, R.: *Arzneim.-Forsch./Drug Res.* 20, 1513 (1970)
19. Mills, T., Roberson J.G.: *Instrumental Data for Drug Analysis*, Vol: 2, pp. 1038-1039, Elsevier, New York, Amsterdam, London, (1987)
20. Mirvish, S.S.: *Toxicol. Appl. Pharmacol.* 31, 325 (1975)
21. Ziebarth, D., Schramm, T.: *Arch. Geschwulstforsch.* 55, 81 (1985)
22. Tannenbaum, S.R., Mergens, W.: *Ann. N.Y. Acad. Sci.* 355, 267 (1980)

Accepted: 06.01.1997