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In Vitro Microsomal Metabolism of N,N-Dibenzylmethylamine, N,N-Dibenzyl-4-Nitro and N,N-Dibenzyl-2,4-Dichlorobenzylamine

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In Vitro Microsomal Metabolism of N,N-Dibenzylmethylamine, N,N-Dibenzyl-4-Nitro and N,N-Dibenzyl-2,4-Dichlorobenzylamine

Summary : *In the present study, model compounds, ie. N,N-dibenzylmethylamine (T1), N,N-dibenzyl-4-nitro (T2) and N,N-dibenzyl-2,4-dichlorobenzylamine (T3), were chosen as substrates and their in vitro hepatic microsomal metabolic studies were carried out. The aim was to establish whether N-oxidation and N-dealkylation reactions occurred following the metabolism of these benzylic tertiary amines. The proposed substrates and their potential metabolites, ie. the corresponding N-oxides and secondary amines were synthesized. The structures of these compounds were confirmed by UV, IR, ^{1}H-NMR and MASS spectroscopic techniques. The separation of substrates from potential metabolites was achieved by thin layer chromatography (TLC) and high pressure liquid chromatography (HPLC). Substrates were incubated with the microsomes isolated from rat liver in the presence of co-factors including NADPH. The unchanged substrates and metabolites were then extracted into dichloromethane. Metabolites occurred following metabolic reactions were compared with their authentic standards. The results indicated that only T1 formed an N-oxide metabolite and all the substrates produced N-dealkylated metabolites.*

Key words: *Benzylic tertiary amines, in vitro metabolism, microsomes.*

N,N-Dibenzilmetilamin, N,N-Dibenzil-4-Nitro ve N,N-Dibenzil-2,4-Diklorobenzilamin'in İn Vitro Mikrozomal Metabolizması

Özet : *Bu çalışmada substrat olarak N,N-dibenzilmetilamin (T1), N,N-dibenzil-4-nitrobenzilamin (T2) ve N,N-dibenzil-2,4-diklorobenzilamin (T3) model bileşikleri seçilerek in vitro karaciğer mikrozomal metabolizma çalışmaları yapıldı. Bu çalışmanın amacı, bu tip benzilik tersiyer aminlerde metabolik N-oksidasyon ve N-dealkilasyon reaksiyonlarının gerçekleşip gerçekleşemeyeceğini açığa çıkartmaktı. Substrat ve olası metabolitleri uygun metodlarla sentez edildi ; yapıları UV, IR, ^{1}H-NMR ve kütle spektroskopisi ile aydınlatıldı. Substrat ve olası metabolitleri daha sonra ince tabaka kromatografisi (İTK) ve yüksek basınçlı sıvı kromatografisi (HPLC) kullanarak birbirlerinden ayrıldı. Substratlar, NADPH ve diğer ko-faktörlerle birlikte sıçan karaciğerinden izole edilen mikrozomal preparatlarla inkübasyona tabi tutuldu. Substrat ve oluşan metabolitler diklorometana çekildi. Oluşan metabolitler standartlarla karşılaştırılarak açığa çıkartıldı. Sonuçlar, sadece T1 bileşiğinin N-oksit metaboliti oluşturduğunu gösterdi. Tüm substratlar N-dealkilasyon metabolitlerini verdi.*

Anahtar kelimeler: *Benzilik tersiyer aminler, metabolizma, mikrozomlar*

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INTRODUCTION

Tertiary amines are present in the majority of drugs, a number of toxicants and a multiplicity of enviromental and industrial chemicals[1]. Therefore, both *in vivo* and *in vitro* metabolism studies on their nitrogen functions are of great importance. It is well known that N-dealkylation is the most frequently occurring metabolic reaction of secondary and tertiary amines. McMahon, who studied the relationship between lipid solubility and the rate of demethylation, showed that there was a direct correlation between

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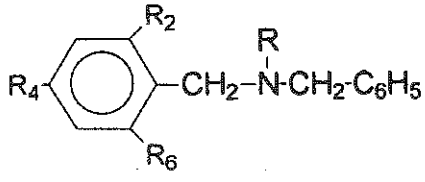
lipid solubility and substrate activity[2]. In the metabolism of tertiary amines, two mechanisms have been suggested for N-dealkylation. The first proposal is that the initial a-carbon oxidation directly forms the carbinolamine which rearranges to from the carbonyl compound and the dealkylated amine[3,4]. Alternatively, tertiary amine N-oxide formed as an intermediate metabolite can undergo iron catalyzed rearrangements to yield dealkylated amines[5]. N-oxide formation was first demonstrated *in vivo* in dogs and rabbits after trimethylamine administration[6]. A major metabolite of N-dimethylamphentamine in humans is the corresponding N-oxide[6]. Several antihistaminic drugs form N-oxides[7]. Methylephedrine has been shown to be metabolized to methylephedrine-N-oxide and ephedrine by rat liver microsomes. The formation of ephedrine, however, could not be detected by incubation of methylephedrine-N-oxide with rat liver. Ephedrine was formed directly by initial C-oxidation[8]. The local anaesthetic Lignocaine possesses a tertiary amine group and it was shown to produce an N-oxide metabolite *in vitro*[9]. QSAR study on species differences in microsomal N-oxygenation of N,N-dimethylalkylamines showed that the biological N-oxygenation of these amines is controlled by lipophilicity, stereochemistry, electron effects and species differences[10].

Although a number of metabolic studies using tertiary amines are available, as indicated above, there is no report on the *in vitro* microsomal metabolism of tertiary benzylic amines in the literature. In our study, *in vitro* hepatic microsomal metabolism of benzylic tertiary amines (T1, T2 and T3) was carried out (Figure 1). The aim was to establish whether N-oxidation and N-dealkylation reactions occurred following the metabolism of these benzylic tertiary amines (Figure 2)



Compound	R	R2	R4	R6
T_1	CH_3-	H-	H-	H-
T_2	C_6H_5-CH_2-	H-	NO_2-	H-
T_3	C_6H_5-CH_2-	Cl-	Cl-	H-

Figure 1. Structures of substrates used in this study.

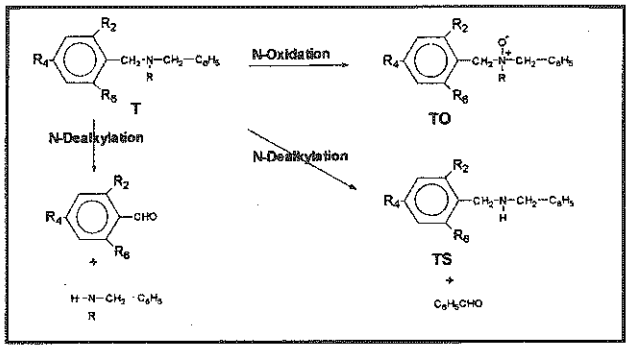


Figure 2. Proposed metabolic pathways for N,N-dibenzyl substituted tertiary amines used as substrates in this study (T= tertiary amine, TO= N-oxide, TS= Secondary amine)

MATERIALS and METHODS

Chemicals: Dibenzylamine, benzylchloride, N-benzylmethylamine, α,2,4-trichlorotoluene, and 2,4-dichlorobenzaldehyde were purchased from Aldrich Chemical Company, UK. p-Nitrobenzylchloride, p-nitrobenzoylchloride, p-nitrobenzaldehyde; and calcium, sodium and potassium chlorides were purchased from British Drug Houses (B.D.H.), Poole Dorset UK. Benzaldehyde, glacial acetic acid, hydrogen peroxyde (30 %) and sodium hydroxide were obtained from E. Merck (Darmstadt, Germany). m-Chloroperoxybenzoic acid (m-CPBA) was purchased from Sigma. Acetonitrile (HPLC grade) was obtained from Merck. All other solvents were purchased from Lab-Scan. Plastic backed TLC plates precoated with silica-gel $60F_{254}$ were obtained from E. Merck (Darmstadt, Germany). Glucose-6-phosphate dehydrogenase was purchased from the Boehringer Mannheim Corporation (London) Ltd. Nicotinamide adenine dinucleotide phosphate mono sodium salt (NADP) and glucose-6-phosphate disodium salt were obtained from Sigma Ltd. Potassium dihydrogenorthophosphate and disodium hydrogen phosphate hydrate were both purchased from B.D.H. $MgCl_2$. $6H_2O$ was obtained from FSA Laboratory, UK.

Animals : Albino Wistar rats (200-250 g) were used in this study. The animals were deprived of food overnight prior to sacrifice, but were allowed water *ad libitum*. They were previously fed on a balanced diet. Hepatic washed microsomes were prepared using calcium chloride precipitation method as described by Schenkman and Cinti[11].

Instrumentation: Melting points were determined with a Buchi (B-530) apparatus and were uncorrected. Spectroscopic data were recorded with the following instruments: UV spectra was recorded on a Shimadzu-260 spectrophotometer, IR with a Perkin Elmer Model 240 spectrophotometer, MS : Mass spectrometer with an ionisation potential of 70eV. C,H,N analyses were carried out on a model 240XY Control and 1106 Carlo Erba Equipments, TUBİTAK Instrumental Analysis Lab, Gebze Turkey.

High Performance Liquid Chromatography (HPLC): HPLC column (Spherisorb C185 µm (25cm length x 4.6mm i.d.) was purchased from Phase Separations Limited, Deeside, UK. The guard column packing material (Whatman Pellicular ODS) was purchased from Whatman International Ltd., Maidstone, Kent, UK. The HPLC chromatograph consisted of an isocratic system comprising one LCD analytical constaMetric 3200 solvent delivery system, a Rheodyne syringe loading sample injector valve (model 7125) fitted with a 20µL sample loop, a Milton ROY spectroMonitor-3100 Variable wavelength UV detector, and a Milton ROY integrator. The mobile-phase compositions were as follows:

A : acetonitrile : 20 mM phosphate buffer (50:50,v/v pH:4) Flow rate : 2 ml/min

B : acetonitrile : 20 mM phosphate buffer (60:40,v/v pH:6) Flow rate : 2 ml/min

C : acetonitrile : 20 mM phosphate buffer (50:50,v/v pH:7) Flow rate :1 ml/min

HPLC retention times of the substrates and their potential metabolites are shown in Table 1.

Thin Layer Chromatographic Analysis : TLC was carried out using plastic-backed TLC plates precoated with silicagel $60F_{254}$ with the following solvent systems: ie. 1. chloroform : methanol (95:5,v/v) ; 2. chloroform : methanol (80:20,v/v) 3. petroleum ether (b.p. 40-60°C) : acetone (50:50,v/v), 4. petroleum ether (b.p. 40-60°C) : acetone (70:30,v/v). The plates, after development, were examined under UV light (254 nm) and then sprayed with detection reagents, ie Ehrlichs reagent (for primary and secondary amines) and Dragendorff reagent (for N-oxides) (Table 1).

Table 1. Chromatographic properties of substrates (T1, T2 and T3) and their potential metabolites used in this study

Compound (Abbreviation)	Rt (min) (HPLC Solvent System)	Rfx100 (TLC Solvent) System)
Dibenzylamine (DBA)	3.17 (A), 4.87 (B), 8.24 (C)	60 (1), 62 (2), 66 (3), 34 (4)
N,N-dibenzyl-4-nitrobenzylamine (T2)	27.95 (A)	75 (1), 80 (3)
N-(4-nitrobenzyl)-N-benzylamine (T2S)	4.01 (A)	38 (1), 42 (3)
N,N-dibenzyl-4-nitrobenzylamine-N-oxide (T2O)	7.36 (A)	43 (1), 3 (3)
4-nitrobenzaldehyde (4NB)	2.54 (A)	65 (1), 72 (3)
N,N-dibenzyl-2,4-chloro dibenzylamine (T3)	27.13 (B)	76 (1), 62 (4)
N-(2,4-dichlorobenzyl)-N-benzylamine (T3S)	8.20 (B)	70 (1), 49 (4)
N,N-dibenzyl-2,4-dichloro benzylamine-N-oxide (T3O)	16.72 (B)	52 (1), 3 (4)
2,4-dichlorobenzaldehyde (24DCB)	3.19 (B)	72 (1), 56 (4)
N,N-dibenzylmethylamine (T1)	11.33 (C)	71 (1), 74 (2)
N-methylbenzylamine (T1S)	5.13 (C)	8 (1), 13 (2)
N,N-dibenzylmethylamine-N-oxide (T1O)	12.73 (C)	20 (1), 25 (2)
Benzaldehyde (B)	2.50 (A), 2.11 (B), 5.07 (C)	–

Incubation and Extraction Procedures : Incubations were carried out in a shaking water-bath at 37°C using a standard co-factor solution consisting of NADP (2µmol), G-6-P (10 µmol), G-6-P dehydrogenase suspension (1 unit) and aqueous $MgCl_2$ (50 %,w/w) (20 µmol) in phosphate buffer (0.2M, pH:7.4, 2 ml) at pH 7.4. Co-factors were pre-incubated for 5 min to generate NADPH, before the addition of microsomes (1 ml equivalent to 0.5 g original liver) and substrate (5 µmol) in methanol (5 µl). The incubation was continued for 30 min, terminated and extracted with dichloromethane (2x5 ml). The organic extracts were evaporated. The residues were reconstituted in 200 µl of methanol for HPLC and 50 µl of methanol for TLC (Figure 3).

Synthesis of substrates ie. N,N-dibenzylmethylamine (T1), N,N-dibenzyl-4-nitrobenzylamine (T2) and N,N-Dibenzyl-2,4-dichlorobenzylamine (T3) : The method

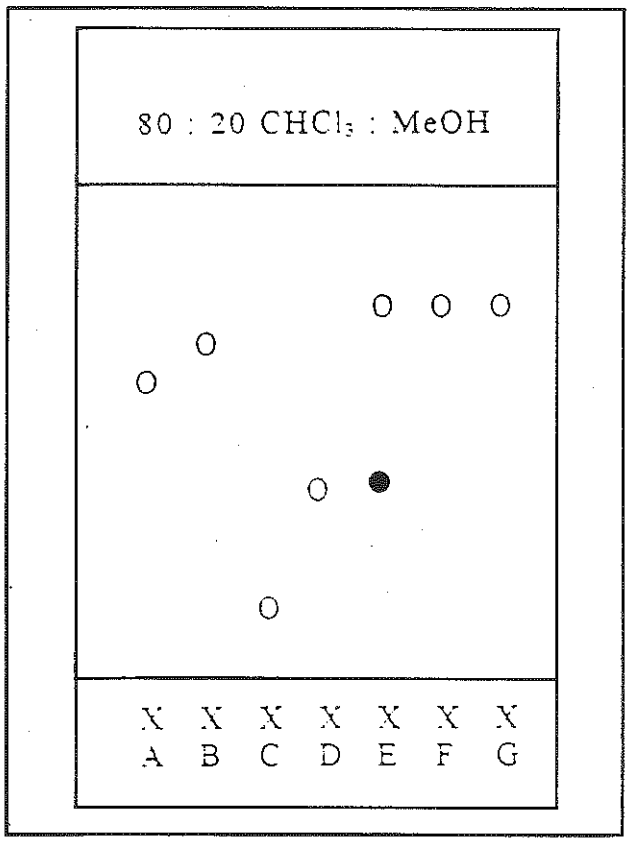


Figure 3. TLC chromatogram of standard T1, its potential metabolites and T1 metabolic extract
A=DBA, B=MDBA, C=MBA, D=T3O, E=Test, F=Control (without co-factors), G=Control (Denaturated microsomes) (see text for abbreviations and TLC conditions)

employed was benzylation of aliphatic secondary amines[12]. Dibenzylamine (0.01 mol) for T2 and T3 or N-methylbenzylamine (0.01 mol) for T1, NaOH solution (10%) and potassium iodide were refluxed in a small amount of acetone while stirring vigorously. When the temperature rose to 90°C, substituted benzylchlorides (in equimolar amounts) were added to the mixture dropwise over an hour. The reaction mixture was refluxed for 2 days.

The resulting precipitates for T2 and T3 were filtered off and, washed with water. T2 was recrystallized from petroleum ether (b.p. 40-60°C) and its hydrochloride salt was prepared. T3 was recrystallized from ethanol. Since T1 had some impurities, it was purified by the use of preparative TLC using petroleum ether (b.p. 40-60°C) : dichloromethane (50:50,v/v) as a solvent system. Yield and description of products are given in Table 2.

Synthesis of N-(4-nitrobenzyl)benzylamine (T2S) and N-(2,4-dichlorobenzyl)benzylamine (T3S) :

T2S: The reduction of the corresponding amide, N-(4-nitrobenzoyl)benzylamine by $NaBH_4$ was performed as described by Vogel (1989)[13]. For this reaction, the amide (0.0015 mol, 0.384 g), which was prepared by Schotten-Baumann method[14], and $NaBH_4$ (0.0075 mol, 0.283 g) were refluxed in glacial acetic acid for two hours. The resulting precipitate was extracted with $CHCl_3$. Evaporation of the organic phase gave pure T2S as a brown liquid.

T3S: This was prepared by the $NaBH_4$ reduction of corresponding imine, N-(2,4-dichlorobenzylidene) benzylamine as described by Vogel (1980)[15]. To prepare the imine, 2,4-dichlorobenzaldehyde and benzylamine (in equimolar amounts) were heated at 90°C with constant stirring for 6h[16]. Then, the imine (0.0015 mol, 0.386 g) and an equimolar amount of $NaBH_4$ (in methanol) were refluxed for 4h during constant stirring. T3S was obtained as a yellow liquid following the preparative TLC using petroleum ether (b.p. 40-60°C) : acetone (80:20,v/v) as a solvent system (Table 2).

Synthesis of N-oxides ie. N,N-dibenzylmethylamine-N-oxide (T1O), N,N-dibenzyl-4-nitrobenzylamine-N-oxide (T2O) and N,N-Dibenzyl-2,4-dichlorobenzylamine-N-oxide (T3O) :

To synthesize T2O and T3O, the corresponding benzylic tertiary amines, T2 or T3 were reacted with m-CPBA (in dichloromethane) at room temparature for 2h with stirring[17,18]. The mixture was cooled and washed with Na_2SO_3 (10%), Na_2CO_3 (10%) and distilled water respectively to remove m-chlorobenzoic acid, unreacted amine and excess of m-CPBA. Following the extraction and evaporation of dichloromethane phase, the precipitate formed was washed with petroleum ether. Finally, T2O and T3O were obtained pure as yellow and white powders respectively (Table 2). T1O was prepared by oxidation with H2O2 (30%) solution in glacial acetic acid as described by Davis and Hetzer[19]. The desired product was extracted with dichloromethane and the organic phase was evaporated. The crude T1O was washed with petroleum ether and obtained as brown crystals (Table 2).

Table 2 - Some physico-chemical and spectral characteristics of substrates and potential metabolites used in this study (see text for abbreviations)

COMPOUND	MOLECULAR FORMULA (M.W.)	m.p. (°C) and Physical appearance	YIELD %	ELEMENTAL ANALYSIS Calc./Found C	H	N	UV (MeOH) λmax (nm)	Mass spectral fragmentations m/z (% relative abundance)
T3	$C_{21}H_{19}Cl_2N$ (356.29)	77-78 White crystals	42	70.79 (70.54)	5.37 (5.29)	3.93 (3.89)	205	355(17.46) , 278(15.88), 196(13.60), 91(100), 65 (14.96)
T3S	$C_{14}H_{13}Cl_2N$ (266.16)	- Yellow liquid	28	63.18 (62.67)	4.92 (4.73)	5.26 (5.00)	204	266(85), 188(25), 176(76), 161(100), 106(35), 91(51), 77(23), 65(41)
T3O	$C_{21}H_{19}Cl_2NO.H_2O$ (390.307)	86-89 White powder	43	64.62 (64.54)	5.42 (5.22)	3.59 (3..52)	204	371(9), 355(10), 280(18), 212(100), 264(10), 159(67), 91(63), 77(20)
T2	$C_{21}H_{20}N_2O_2.HCl$ (368.86)	182-185 (HCl) Yellowish solid	29	75.88 (75.48)	6.06 (5.97)	8.43 (8.50) (Base)	205, 270	332(53.61), 255(33.95), 241(37.75), 196(34.5), 136(61), 106(68.73), 91(100), 65(25.6)
T2S	$C_{14}H_{14}N_2O_2$ (242.27)	- Brown liquid	33	69.41 (69.36)	5.82 (6.17)	11.56 (10.36)	206,250	241.2(76), 195.2(16), 165(37), 151(86), 106(62), 91(100), 65(32.5), 78(31)
T2O	$C_{21}H_{20}N_2O_3.\ 1/2H_2O$ (357.41)	114 Yellow powder	41	70.57 (70.16)	5.92 (5.52)	7.84 (7.88)	204, 263	348(10), 332(18), 212(100), 136(7), 106(32)
T1	$C_{15}H_{17}N$ (211.32)	- Light brown liquid	40	85.26 (85.21)	8.11 (8.13)	6.63 (6.84)	206	211(82), 196(9), 183(42.5), 165(25), 120(43), 91(100), 77(10), 65(33)
T1O	$C_{15}H_{17}NO.\ 3/2H_2O$	80-85 Brown crystals	26	70.83 (70.11)	7.92 (7.79)	5.50 (5.47)	207	227(26), 211(21), 196(5.5), 136(50), 120(29), 106(27), 92(82), 65(100)

RESULTS AND DISCUSSION

The methods employed for the synthesis of substrates and potential metabolites yielded the desired substrates and potential metabolites. Mass spectral analysis of T2, T2S and T2O showed the correct fragmentation patterns and the molecular ion peaks ie. m/z 332, 242 and 348 respectively. (Table 2). Elemental analysis was consistent with the required structures (Table 2).

Following the metabolism studies using rat microsomal preparations fortified with NADPH, no metabolite of T2 and T3 detected by TLC. However, following the metabolism of T1, a metabolite was observed by TLC which had the same Rfx100 value as that of standard N-oxide using TLC system 2 (see text) (Figure 3). Unfortunately, a number of different HPLC mobile phase combinations were tried but they did not contribute to a complete separation of T1O from T1. This was the reason for not detecting T1O as a metabolite of T1 by HPLC. It was also observed by HPLC that T1, T2 and T3 were only converted to N-dealkylation products. Figures 4a, 5a and 6a show HPLC separation of T1, T2, T3 and their potential metabolites while figures 4b, 5b and 6b represent HPLC chromatograms from in vitro hepatic microsomal metabolism of T1, T2 and T3 respectively.

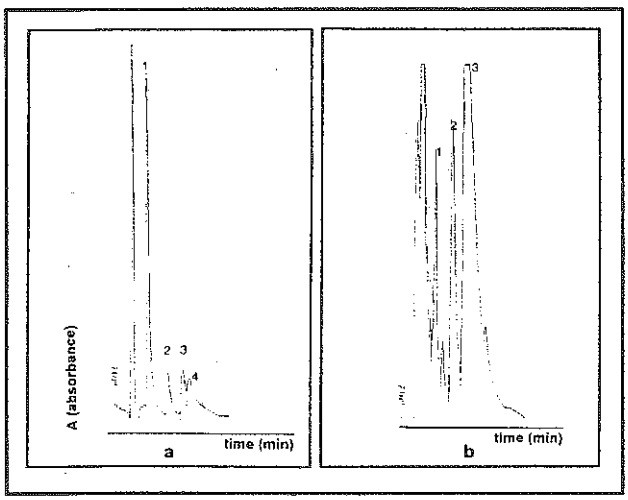


Figure 4. HPLC chromatogram of
(a) Standard T1 and its potential metabolites
(b) T1 metabolic extract
1=B, T1S; 2=DBA; 3=T1 (Substrate); 4=T1O
(see text for abbrevations and HPLC conditions)

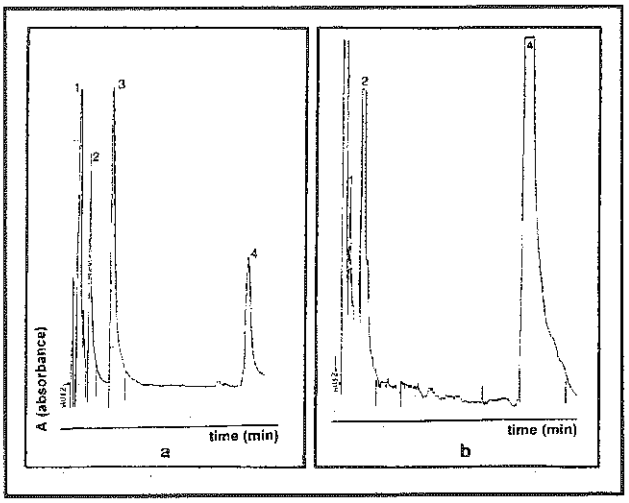


Figure 5. HPLC chromatogram of
(a) Standard T2 and its potential metabolites
(b) T2 metabolic extract
1=B, 4NB; 2=T2S, DBA; 3 = T2O; 4=T2 (Substrate)
(see text for abbreviations and HPLC conditions)

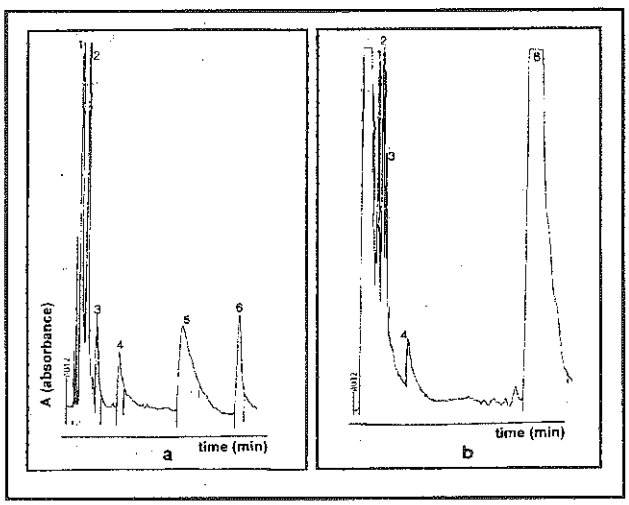


Figure 6. HPLC chromatogram of
(a) Standard T3 and its potential metabolites
(b) T3 metabolic extract
1=B, 2=24DCB, 3=DBA, 4=T3S, 5=T3O, 6=T3 (Substrate)
(see text for abbreviations and HPLC conditions)

In conclusion, the benzyl groups seem to prevent N-oxidation of T2 and T3 because of the electronic and/or steric influence which decreases the pKa of the constituent nitrogen. However, in the case of T1, the methyl group increases the basicity of nitrogen and hence the pKa[2]. This may allow the formation of the corresponding N-oxide, T1O, as a metabolite of

this substrate. As Meishenheimer rearrangement of the benzylic N-oxides yielding alkoxyamines is a well known reaction[20], this reaction may also account for the failure to detect T2O and T3O, the corresponding N-oxides of T2 and T3 in this study. In order to fully understand which mechanism proposed above is operative, the metabolic experiments on all these N-oxides are now under investigation in our laboratory.

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REFERENCES

1. Gorrod, JW, Chemical Determinants of the Enzymology of Organic Nitrogen Oxidation, *Drug Met. and Disp.*, 13, 283-286, 1985.
2. McMahon, RE, Microsomal Dealkylation of Drugs Substrate Specificity and Mechanism, *J.Pharm.Sci.*, 55, 457-465,1966.
3. Lindeke, B, Cho, AK, N-Dealkylation and Deamination, in W.B.Jakoby, J.R. Bend, J. Caldwell (eds), *Metabolic Basis of Detoxication*, New York, Academic Press, pp.105-125, 1982
4. Testa, B, Jenner, P, Phase I reactions. Oxidative N-Dealkylation, in B.Testa, P. Jenner (eds), *Chemical Aspects of Drug Metabolism*, New York, Basel, M.Dekker, pp. 82-97, 1976.
5. Ferris, JP, Gerwe, RD, Gapski, GR, Detoxication Mechanims III. Scope and Mechanism of the iron catalyzed dealkylation of tertiary amine oxides, *J.Org.Chem.*, 33, 3493-3498, 1968.
6. Damani, LA, Oxidation at Nitrogen Centers, in W.B. Jakoby, J.R. Bend, J. Caldwell (eds), *Metabolic Basis of Detoxication*, New York, Academic Press, pp. 127-149, 1982.
7. Testa, B, Jenner, P, Phase I Reactions. Oxidation of Nitrogen-Containing Functional Groups, in B.Testa, P. Jenner (eds), *Chemical Aspects of Drug Metabolism.* New York , Basel, M. Dekker, pp.61-73, 1976.
8. Inoue, T, Tanaka, K, N-oxidation and N-demethylation of methylephedrine by rat liver microsomes, *Xenobiotica*, 20 , 265-271, 1990.
9. Patterson, LH, Hall, G, Nijjar, BS, Khatra, PK, Covan, DA, In-vitro metabolism of lignocaine to its N-oxide, *J. Pharm. Pharmacol.* 38, 326, 1986.
10. Devinsky, F, Gorrod, JW, QSAR study on species differences in microsomal N-oxygenation of N,N-dimethylalkylamines, *J. Pharm. Pharmacol.*, 41, 57-59, 1988.
11. Schenkman, JB, Cinti, DL, Preparation of microsomes with calcium, *Methods in Enzymol.*, 52, 83-89, 1978.
12. Ulgen, M, Metabolic and Chemical Determinants of Amide Formation from Benzylic Amines, Ph.D. Thesis, University of London (1992).
13. Furniss, BS, Hannaford, AJ, Smith, PWG, Tatchell, AR, *Vogel's Textbook of Practical Organic Chemistry*, Fifth Edition, New York, John Wiley & Sons, p. 774, 1989.
14. Ergenç, N, Ateş, Ö, Gürsoy, A, *Eczacılar için Organik Kimya*, İstanbul Üniversitesi Yayınları No: 3596, Eczacılık Fak. No: 57, pp. 359-380, 1990.
15. Smith, BV, Waldron, NM, *Vogel's Elementary Practical Organic Chemistry I Preparations*, Third Edition, New York, John Wiley & Sons, p. 547, 1980.
16. Low, CM, Ulgen, M, Gorrod, JW, The recognotion of a diarylimine as a metabonate produced during incubation of N-benzyl-4-chloroaniline with hepatic microsomal preparations, *J. Pharm. Pharmacol.*, 46, 585-590, 1994.
17. Qchiai, E, *Preparation of Aromatic Amine Oxides, in Aromatic Amine Oxides,* Amsterdam, Elseiver Publishing Company, pp. 19-24, 1967.
18. Culvenor, CCJ, Amine Oxides, *Rev. Pure Appl. Chemi*, 3, 83-114, 1953.
19. Davis, MM, Hetzer, HB, Relative basicities of tribenzylamine and tribenzylamine-N-oxides in benzene and water, *J. Am. Chem. Soc.*, 76, 4247-4260, 1954.
20. Brauman, JI, Sanderson, WA, Rearrangement of A Neopentylamine Oxide, *Tetrahedron*, 23, 37-44,1967.