the effect of test compounds on traction (Tr), dish (D), nicotine-induced tonic extensor convulsions (TE) and death (D), thiosemicarbazide- and strychnine-induced lethality, and electroshock convulsions have been described previously.[21] The antagonism of pentylenetetrazole-induced clonic convulsions was also described previously.[1a] ED_{50} values were calculated by the method of Spearman and Karber.[22]

Acknowledgment. The authors are indebted to Mr. A. Koning and Mr. W. Friis for laboratory assistance, to Dr. L. Baczynskyj, Ms. B. Zimmer, Mr. Paul Meulman, and Mr. R. C. Anderson for physical and analytical data, and to Mrs. Helen Branch for preparing this manuscript.

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Conversion of *N*-Alkylaminobenzophenones to Benzodiazepines in Vivo

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The results of this study suggest that 5-chloro-2-[3-methyl-5-(dimethylamino)methyltriazol-4-yl]benzophenone can undergo N-dealkylation and ring closure in vivo to form the corresponding benzodiazepine. The in vivo conversion was found to occur in mice, rats, and monkeys. A variety of substituted aminobenzophenone compounds were also able to undergo these conversions. The conversions to benzodiazepines were confirmed by a comparison of retention times on a gas chromatograph as well as through the use of a GC–mass spectrometer. The results obtained did not prove that the *N*-alkylaminobenzophenones were devoid of activity, but they do suggest that their observed pharmacological activity may be due to the formation of the corresponding benzodiazepines.

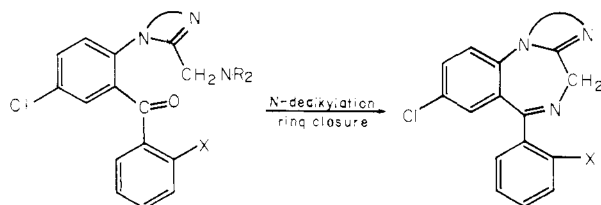
A new series of benzodiazepines, the triazolobenzodiazepines,[1–3] has been prepared which has many interesting pharmacological properties.[3] During the course of preparing this new series of compounds, a number of aminobenzophenone analogues were prepared which may be considered open ring analogues of these benzodiazepines[4] and were found to possess antimetrazole activity (Table I), a possible indicator of anxiolytic activity.[3] These "open-ring benzodiazepines" were so designated because the imine bond was absent and the molecule contained a carbonyl group and an alkyl-substituted nitrogen (see the last four structures in Table I).

The biological activity of these *N*-alkylaminobenzophenones was of great interest since the possibilities existed that they may be active in their own right, or they may be active by being converted to benzodiazepines of known

Table I. Antimetrazole Activity of Various Open- and Closed-Ring Benzodiazepines

No.	Compd	Structure	Antimetrazole ED_{50}
1	8-Chloro-1-methyl-6-phenyl-4*H*-*s*-triazolo[4,3-*a*][1,4]-benzodiazepine (alprazolam)		0.20
2	8-Chloro-6-(*o*-chlorophenyl)-1-methyl-4*H*-*s*-triazolo[4,3-*a*]-[1,4]benzodiazepin-1-one		2.8
3	8-Chloro-6-(*o*-chlorophenyl)-1-methyl-4*H*-*s*-triazolo[4,3-*a*]-[1,4]benzodiazepine (triazolam)		0.03
4	5-Chloro-2-[3-methyl-5-(morpholinomethyl)-4*H*-1,2,4-triazol-4-yl]benzophenone		1.8
5	5-Chloro-2-[3-[(dimethylamino)-methyl]-5-methyl-4*H*-1,2,4-triazol-4-yl]benzophenone		1.1
6	5-Chloro-2-[1-methyl-3-[(methylamino)methyl]-5-oxo-Δ^2-1,2,4-triazolin-4-yl]benzophenone hydrochloride		5.6
7	2′,5-Dichloro-2-[3-[(dimethylamino)-methyl]-5-methyl-4*H*-1,2,4-triazol-4-yl]benzophenone		0.28

activity. The latter assumption seemed reasonable since N-dealkylation followed by ring closure could occur in vivo to form the benzodiazepines, as shown below.



N-alkylaminobenzophenone benzodiazepine

The experiments described in this report were carried out to determine whether or not the *N*-alkylaminobenzophenones undergo N-dealkylation and ring closure to form benzodiazepines in vivo. Such data could be used to evaluate the assumption that this metabolic conversion could account for their biological activity.

Table II. Compound 1 and 3 Levels in Mouse Brain Following Administration of 1, 3, and Their Precursor *N*-Alkylaminobenzophenones 5 and 4, and 7, Respectively[a]

Drug administered	Sacrifice time, h	Drug determined	Amt found, ng/g ± SE, in brain
1	0.5	1	1060 ± 88
1	1.0	1	625 ± 37
1	2.0	1	245 ± 55
5	0.5	1	224 ± 35
5	1.0	1	135 ± 14
5	2.0	1	120 ± 45
4	0.5	1	97 ± 15
4	1.0	1	83 ± 6
4	2.0	1	82 ± 5
3	0.5	3	106 ± 29
3	1.0	3	
3	2.0	3	27 ± 10
7	0.5	3	193 ± 40
7	1.0	3	73 ± 22
7	2.0	3	49 ± 10

[a] One mouse brain per determination and three determinations per time period. Doses were 20 mg/kg ip.

Results and Discussion

The amount of compounds **1** and **3** in mouse brains was determined at various time intervals following the administration of their precursor *N*-alkylaminobenzophenones, **4** and **5**, and **7**, respectively. Compound **1** was found to be formed from its *N*-dimethylamino- (**5**) and morpholino- (**4**) triazolylbenzophenones. The *N*-dimethyl derivative **5** was found to provide higher levels of **1** in the brain than did the morpholino derivative **4**. For comparative purposes, the amount of compound **1** in the brain at various times following its administration was determined. It was found that compound **5** gave brain levels 21% of those found when **1** was administered in the same dose (20 mg/kg), whereas the morpholino compound **4** gave brain levels 9% of those found with **1** (Table II). The differences in brain levels of **1** found in the above three cases relate very well to the antimetrazole data reported in Table I.

In the dichlorotriazolobenzodiazepine series (**3**), the *N*-dimethylaminobenzophenone **7** generated higher mouse brain levels of **3** than did the same dose of **3**. In this series of compounds, the antimetrazole data did not reflect the brain drug levels; no sound explanation is readily available.

The 0.5-h brain levels, presented in Table II, indicate that the *o*-chloro analogue **7** was N-dealkylated and closed almost as efficiently as was the non-*o*-chloro derivative **5**. The possibility that an oxotriazolinylbenzophenone **6** could also undergo N-dealkylation and ring closure to the benzodiazepine **2** was next evaluated. From the data presented in Table III, one can conclude that in the oxotriazolo series N-dealkylation and ring closue occur to a significant degree, as judged by a comparison of the brain drug levels shown in Tables II and III.

The ability of a species other than mice to carry out the metabolic conversions was next evaluated. Studies in the rhesus monkey where blood–drug levels were determined provided evidence that the N-dealkylation ring closure process is not restricted to the mouse, as shown in Table IV where administered compound **5** was converted to **1**. The measured blood levels of **1** in the monkey corre-

Table III. Levels of Compound 2 in Mouse Brain Following Administration of Compound 6[a]

Sacrifice time, h	Amt of 2, ng/g ± SE, in brain
0.5	131 ± 4
1.0	69 ± 1
2.0	86 ± 7

[a] One mouse brain per determination and three determinations per time period. Dose was 20 mg/kg ip.

Table IV. Blood Levels of Compound 1 in Rhesus Monkeys Following Administration of Compound 5[a]

Amt of drug received, po	Blood sampling time, h	Amt of 1, ng/ml, in blood
3.0 mg/kg	1.0	14.1 ± 3.0
	4.0	7.2 ± 4.5

[a] Dose of compound 5 was 3.0 mg/kg. Single determinations were made per blood sample.

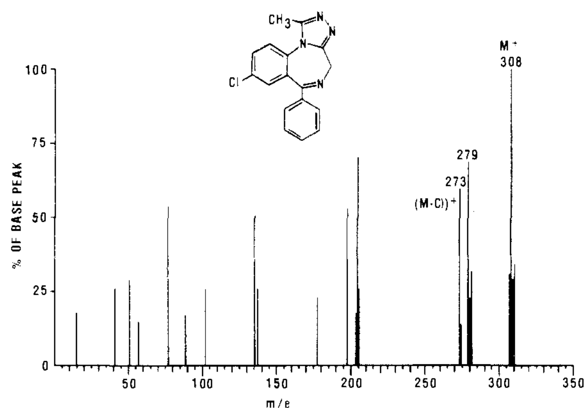


Figure 1. Mass spectrum of authentic compound 1.

sponded to the pharmacological profile in that the greatest overt effects were observed at 1 h and minimal effects were seen at 4 h.

Although the evidence is fairly strong that the described chemical conversions are taking place since similar results were obtained for three different structural types (a monochlorotriazolyl-, dichlorotriazolyl-, and a monochloroxotriazolylbenzophenone), the possibility still remains that the results may be due to some other metabolite with a similar retention time. Therefore, these results were confirmed using the LKB gas chromatograph–mass spectrometer (GC–MS).

Adult male rats were injected with 30 mg/kg of the appropriate drug and blood samples were taken after 30 min. Extraction, isolation, and GC–MS methods were carried out as described in the Experimenal Section, B. The mass spectrographic data obtained for compound **1** (Figure 1) and for the material found in blood of rats treated with **4** (Figure 2) and **5** (Figure 3) appear to be identical. The "extra" ions in Figures 2 and 3 probably represent background from previous compounds entering the spectrometer. The parent ion m/e 308 was the most abundant peak for this compound, followed by an M − 29 peak at m/e 279. The peak at m/e 273 represents M − 35 or loss of chlorine which is evident since the m/e 275 peak due to the iostopic abundance of chlorine is missing.

Further identification of the materials found in blood was obtained by using thin-layer chromatographic methods. Using silica gel G plates with acetone as the solvent, compound **1** had an R_f of 0.17 and **5** had an R_f of 0.10 as did **4**. The material obained from blood of treated rats had R_f values identical with those of authentic materials. When

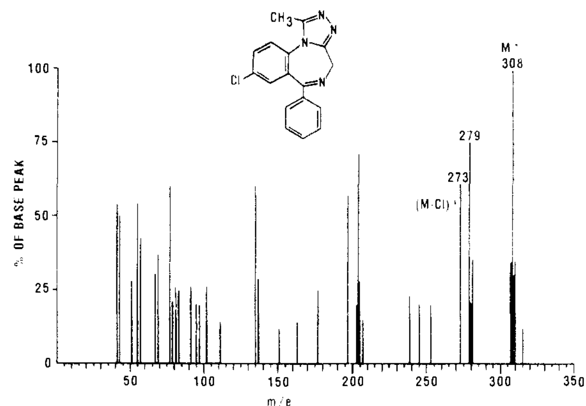


Figure 2. Mass spectrum of "apparent" compound 1 obtained from rats treated with compound 4.

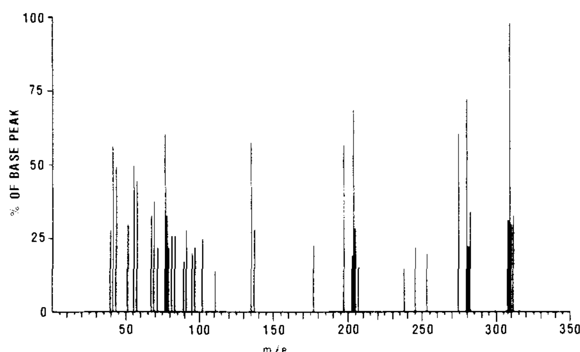


Figure 3. Mass spectrum of "apparent" compound 1 obtained from rats treated with compound 5.

the material was eluted from the TLC plate with acetonitrile and checked on the gas chromatograph using electron-capture techniques, the retention times were identical with that of the authentic material.

The results obtained in the GC–MS studies adequately demonstrate that the material determined in the gas chromatographic studies was indeed compound **1**. The net result of the studies does not prove that the *N*-alkylaminobenzophenones are devoid of activity in their own right. The brain level data do indicate that much of their observed pharmacological activity is due to the formed benzodiazepine since, in several cases, brain–drug levels correlate well with the pharmacological activity.

With regard to the mechanism involved in these transformations, it would be rational to assume that the N-dealkylation step is an enzymatic process[5] since these N-substituted derivatives apparently are stable compounds. The ring-closure step is probably nonenzymatic since intramolecular condensation reactions between amines and carbonyl compounds readily take place.

Experimental Section

A. Gas Chromatographic Studies. Each mouse brain was homogenized in 3 ml of water; 3 ml of 1 M K_3PO_4, pH 6.0, was then added to the homogenate and mixed. Hexane (10 ml) was added to the homogenate and the mixture was shaken mechanically for 10 min and then centrifuged and a 7.5-ml aliquot of hexane was removed. Extraction with hexane was repeated once more and the two hexane extracts were combined and reduced to dryness with nitrogen. Samples were taken up in a small volume of hexane containing the internal standard and transferred to a 1-dram vial containing 0.3 ml of 1 M K_3PO_4, pH 6.0. Samples were shaken and centrifuged and an aliquot of the hexane layer was used for analysis.

The blood samples were extracted by adding 5 ml of 1 M K_3PO_4 at pH 6.0 to the 10-ml blood sample, then adding 20 ml of hexane,

and shaking mechanically for 10 min. A 15-ml aliquot of hexane was removed, the hexane extraction was repeated twice more, and the hexane fractions were combined and reduced to dryness with nitrogen. Samples were taken up in 0.3 ml of hexane containing compound **3** as an internal standard, and the samples were used as described above.

A Microtek gas chromatograph equipped with a ^{63}Ni electron-capture detector was used for analysis. A 3-ft glass column packed with 3% OV-225 on 100–120 mesh Gas Chrom Q was used. Nitrogen was used as carrier gas at 60 cc/min with 8 cc/min purge. Column temperature was 260° for compounds **1** and **3** and a temperature of 230 °C was used for compound **2** determinations. Inlet temperature was 295 °C and detector was 310 °C. The area under the peaks was calculated by xeroxing the chromatograms, cutting them out, and weighing them.

Retention times were **1** = 7 min; **3** = 9.5 min; and **2** = 7.25 min. Compound **3** was used as an internal standard for **1** and **1** was used for **3**. 9-Chloro-1-methyl-7-phenylpyrimido[1,2-*a*][1,4]-benzodiazepin-3(5*H*)-one was the internal standard for compound **2** determinations.

One unit of compound **3** gave a response equivalent to 1.81 units of compound **1**, and one unit of 9-chloro-1-methyl-7-phenylpyrimido[1,2-*a*][1,4]benzodiazepin-3(5*H*)-one gave a response equivalent to 1.92 units of **2**. Results were not corrected for percent recovery.

B. Gas Chromatograph–Mass Spectrograph Studies. Essentially the same procedure was used for GC–MS studies of both compounds **5** and **4**. Twenty-four male Sprague–Dawley rats, 200–225 g, were used in each study. Rats were injected with 30 mg/kg of the appropriate drug via the ip route as a suspension in 0.5 ml of sterile vehicle no. 122. After the drug was administered (30 min), the rats were anesthetized with halothane and 4–5 ml of blood was removed via heart puncture. The blood from two similarly treated rats was placed in a 100-ml glass-stoppered flask containing 50 ml of benzene and 4 ml of 0.1 M Tris buffer at pH 8.0. Flasks were mechanically shaken for 10 min and centrifuged, and the benzene layer was removed and combined with other samples. The benzene extraction was repeated a second time and combined with the first extraction. The total benzene extracts, approximately 900 ml from the 12 samples, were reduced to approximately 100 ml using a Rotovapor. The benzene solution was transferred to a separatory funnel and washed with 25 ml of 0.1 M Tris buffer, pH 8.0. The benzene layer was then reduced to dryness; the residue was dissolved in 50 ml of acetone, transferred to a clean flask, and reduced to dryness. This residue was dissolved in 50 ml of acetonitrile, transferred to a clean flask, and reduced to dryness. The final residue was taken up in 25 ml of benzene and placed on a 30 × 1 cm glass column containing silica gel (70–325 mesh) to a height of 4.5 cm. The effluent was collected and the column was washed with 5 ml of benzene. The materials were then eluted from the column with acetone and 5-ml fractions were collected and saved. The entire procedure was monitored for compounds **1**, **5**, or **4** by using gas chromatographic methods described earlier. Compound **1** was found in acetone fractions 4–7, **5** in fractions 7–10, and **4** in fractions 7–10. The respective fractions were combined and reduced to dryness with a stream of N_2. In the compound **5** study, the final sample was dissolved in 0.15 ml of acetonitrile, and in the compound **4** study, 0.1 ml of acetone was used as the final solvent.

Analysis on the LKB was carried out using a 4-ft glass column packed with 3% OV-17 on Gas Chrom Q, with carrier gas flow rate at approximately 20 ml/min. Temperatures were: column, 255 °C in the compound **5** study and 245 °C in the **4** study; flask heater, 290 °C; ion source, 250 °C; separator, 280 °C; and electron energy of 70 eV. In the compound **5** study, the retention time of **1** at 255 °C was 20.6 min and for **5** it was 11 min. In the compound **4** study, the retention time of **1** at 245 °C was 33 min and for **4** it was 62.4 min. Mass spectra were obtained for authentic samples of **1**, **5**, and **4** and for the materials extracted from rat blood which had retention times comparable to those of the authentic compounds.

C. Pharmacology Methods. Antimetrazole activity was determined as previously described.[3]

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Notes

Synthesis of 3-Iodo-L-thyronine and Its Iodinated Derivatives

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3-Iodo-L-thyronine has been synthesized from iodo-L-tyrosine by coupling with an anisyliodonium salt. Further iodination yields the 3,3′-diiodo and the 3,3′,5′-triiodo derivatives. Chromatographic systems for separating these compounds and establishing their purity are described.

Interest in 3-iodo-L-thyronine (**1**) and its 3′-iodo (**2**) and 3,5′-diiodo (**3**) derivatives has recently been stimulated by the finding that appreciable amounts of these metabolites of the thyroid hormones may be formed in the developing fetus. Fetal metabolism of thyroxine in the human, implied from analysis of amniotic fluid, differs from that during extrauterine life in the predominance of inner ring deiodination to produce high levels of **3**.[1] Within a few hours of birth, serum levels of 3,5,3′-triiodothyronine rise rapidly from very low levels to those found in adults, accompanied by a more gradual fall in levels of **3**.[2] The early appearance of **3** in the readily accessible amniotic fluid provides the first means for early detection and potential treatment of congenital or drug-induced hypo-