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Novel Anxiolytic Agents Derived from α-Amino-α-phenyl-*o*-tolyl-4*H*-triazoles and -imidazoles[1]

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Several new α-amino-α-phenyl-*o*-tolyltriazoles and -imidazoles have been prepared in one step by means of a novel reductive rearrangement of the corresponding benzodiazepines with hydrazine hydrate. These new triazoles were found to have moderate sedative and muscle relaxing activity in mice (i.e., these compounds depressed the traction and dish reflexes at higher doses than did diazepam) but were very potent antagonists of the clonic convulsions induced in mice by the administration of pentylenetetrazole. Furthermore, they antagonized the lethality induced by thiosemicarbazide. While these new compounds were very active in mice, most were inactive in rats. These results are discussed with reference to the metabolism of compound 13.

A recent report attributed potent muscle relaxant and anticonvulsant activity to a series of new triazoles, 1a, which may be considered to be ring-opened analogues of the corresponding triazolobenzodiazepines, **2**, wherein the

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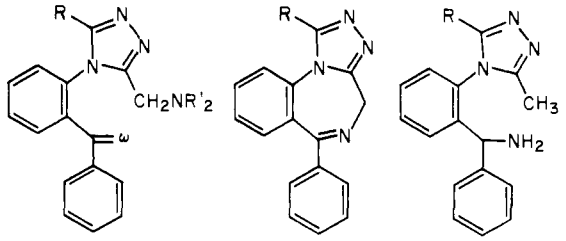


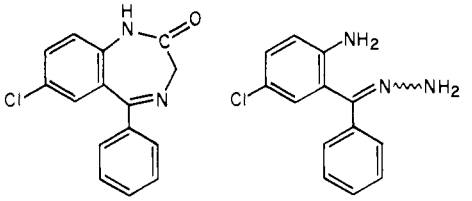
1a, ω = O
b, ω = H, H

2

3

C_6=N_5 imine bond is hydrolyzed.[1a] GLC and GLC–mass spectral data provided strong evidence that in mice, rats, and monkeys these triazolylbenzophenones, 1a, were metabolized to, and thereby functioned as prodrugs of, the corresponding benzodiazepines.[1b] Herein, we report the novel preparation and pharmacology of a second new class of triazoles, 3, which are ring opened analogues of triazolobenzodiazepines wherein the bond between C_4 and N_5 has been cleaved and the imine double bond reduced. In addition, we present GLC–mass spectral data which conclusively demonstrate that these new triazoles, 3, are also converted to benzodiazepines.

Chemistry. Derieg[2] and co-workers reported that demethyldiazepam, 4, was cleaved to the hydrazone, 5, on



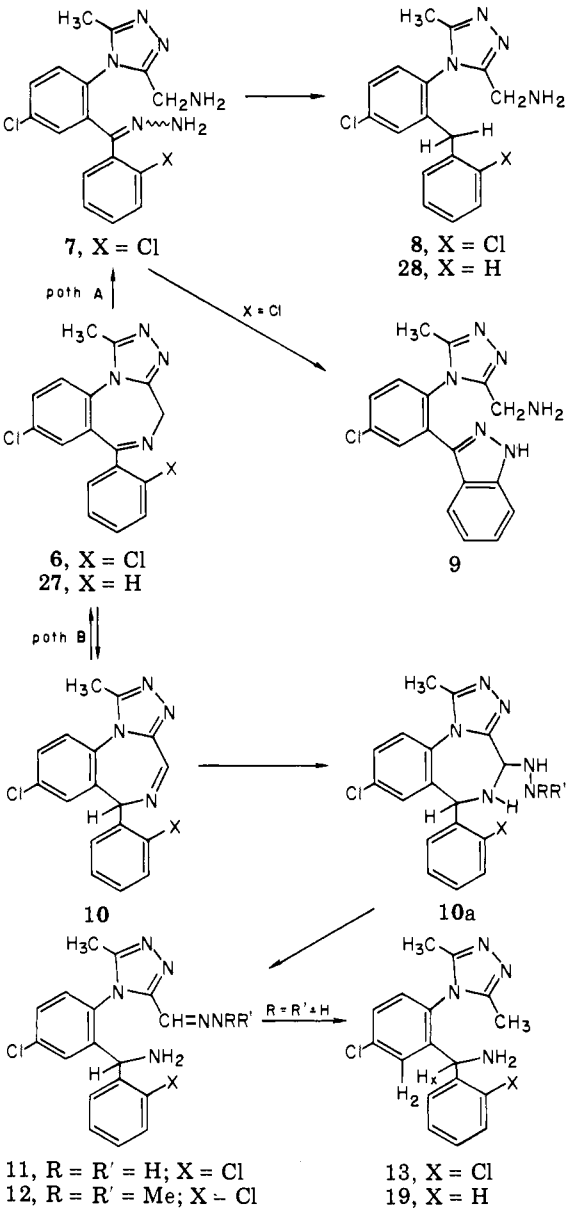
4

5

heating with a large excess of hydrazine in ethanol. We expected the reaction of triazolobenzodiazepine, 6 (triazolam), to follow an analogous course leading to amine 8 and indazole 9 via the benzophenone hydrazone 7 (path A in Scheme I). The formation of indazoles via the reaction of hydrazines with o-haloacetophenones and benzophenones is well documented,[3] and we have, in fact, found similar results with substituted triazolylbenzophenones related to 7.[3c] Our original objective had been to prepare and test indazole 9 in the expectation that it would be an analogue of triazolylbenzophenones of structure 1 which could not close to a benzodiazepine. This objective was temporarily set aside when it was discovered that 6 did not react at 80 °C with an excess of hydrazine hydrate in diethylene glycol but at 140 °C unexpectedly afforded the α-amino-α-phenyl-o-tolyltriazole, 13, as a single product isolated in 83% yield.[4] Assignment of the structure of the product as 13, rather than 8, was readily made by NMR spectroscopy which revealed the presence of *two sharp three-proton singlets* attributable to triazole methyl signals (vide infra).

When dimethylhydrazine was substituted for hydrazine in this reaction, *two* hydrazone products of general structure 12 were isolated. The two products were readily separable [R_f values were 0.54 and 0.61 by eluting the sample with methanol–chloroform (10:90%) mixtures on silica gel] and distinguishable (see Table I). X-ray analysis of racemate 12a (and 12c, see the Experimental Section) confirmed the gross structure as 12 and not 10a and also suggested an explanation for the observed large chemical shift differences between the triazole methyl NMR signals of 12a and 12b[5] (see Figure 1 and Table I). In the crystal the dihedral angle between the triazole ring and the trisubstituted benzene ring was about 82°, while the dihedral angle between the o-chlorophenyl ring and the trisubstituted benzene ring was 85° (and about 20° with the triazole ring). Assuming that the conformation of the molecule in solution approximated that of the crystal (vide infra) and the energy barrier to rotation of the triazole ring was quite high (i.e., $\Delta E > 20$ kcal/mol), it was clear that the chlorophenyl ring strongly shielded the triazole methyl signal of 12a (δ 1.46). [Analogously, the dimethylhydrazone NMR singlet of 12a appeared 0.21 ppm *downfield* from that of 12b (δ 2.75 vs. 2.54, respectively).] Compound 13, a single racemate, displayed the two types of triazole methyl signals within a single molecule (i.e., δ 1.48 and 2.25).

Scheme I



7, X = Cl

8, X = Cl
28, X = H

path A

X = Cl

6, X = Cl
27, X = H

9

path B

10

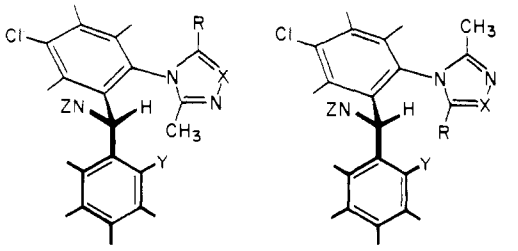
10a

11, R = R' = H; X = Cl
12, R = R' = Me; X = Cl

13, X = Cl
19, X = H

As might be anticipated from the above discussion, when a triazolobenzodiazepine bearing a group other than methyl (or hydrogen) was used as a substrate for the reaction with hydrazine, two *diastereomeric* α-amino-α-phenyl-o-tolyl-4*H*-triazoles were obtained which were readily distinguished by their NMR spectral properties (see Table I). Under the reaction conditions the two isomers were interconvertible. Thus when a pure sample of amino alcohol 23a was resubjected to the original reaction conditions and heated for 16.5 h at 145 °C, the NMR spectrum indicated that 23a and 23b were present in a

Table I. Physical Data for α-Amino-α-phenyl-o-tolyltriazoles and -imidazoles



type I type II

No.	Type	X	Y	Z	R[a]	δ, ppm, for triazole CH_3	% yield[b]	Mp, °C[c]	Formula	Analyses[d]
19	I or II	N	H	H, H	CH_3	2.28, 1.33	14	187-189	$C_{17}H_{17}ClN_4$	C, H, N, Cl
13	I or II	N	Cl	H, H	CH_3	2.25, 1.48	83	173-175[e]	$C_{17}H_{16}Cl_2N_4$	C, H, N, Cl
20	I or II	N	Cl	$=C(CH_3)_2$	CH_3			200-202[f]	$C_{20}H_{20}Cl_2N_4$	C, H, N, Cl
21a	I	N	Cl	H, H	c-C_3H_5[g]	1.42	7	144-147	$C_{19}H_{18}Cl_2N_4$	C, H, N, Cl
21b	II	N	Cl	H, H	c-C_3H_5	2.22	48	170-172[f]	$C_{19}H_{18}Cl_2N_4$	C, H, N, Cl
22a	II	N	Cl	H, H	CH_2NMe_2	2.21	51	142-144	$C_{19}H_{21}Cl_2N_5$	C, H, N, Cl
23a	I	N	Cl	H, H	CH_2OH	1.36	7	218-223[i]	$C_{17}H_{16}Cl_2N_4O$	C, H, N, Cl
23b	II	N	Cl	H, H	CH_2OH[g]	2.13	*h*	225-229[i]	$C_{17}H_{16}Cl_2N_4O$	C, H, N, Cl
12a	I	N	Cl	H, H	$CH^t{=}NNMe_2$[g]	1.46	46	217-219	$C_{19}H_{20}Cl_2N_6$	C, H, N, Cl
12b	II	N	Cl	H, H	$CH^t{=}NNMe_2$	2.22	22	197.5-198.5[i]	$C_{19}H_{20}Cl_2N_6$	C, H, N, Cl
12c	I	N	Cl	Me, Me	$CH^t{=}NNMe_2$	1.15		240-244	$C_{21}H_{22}Cl_2N_6$	C, H, N
24	I and II	N	Cl	H, H	$CH{=}NNHMe_2$	2.20, 1.50		173-184[f]	$C_{18}H_{18}Cl_2N_6$	C, H, Cl
25	I and II	CCH_3	Cl	H, H	H		81	159-161	$C_{18}H_{17}Cl_2N_3$	C, H, N, Cl
26	I and II	CH	H	H, H	H		13[j]	298-302	$C_{17}H_{16}ClN_3 \cdot HBr$	C, H, N

[a] For R = CH_3 and X = N, type I and type II are equivalent. [b] All values are of isolated crystallized yields. No effort was made to maximize these yields. [c] Unless otherwise noted, compounds were crystallized from ethyl acetate-hexane mixtures. [d] Satisfactory analyses have been obtained for the elements indicated. [e] Further recrystallization raised the melting point to 192-193 °C. This higher melting crystal form was used for the lanthanide shift studies (see text). [f] Crystallized from ethyl acetate. [g] This isomer eluted first on silica gel with a methanol-chloroform (3:97%) mixture. [h] A 46% yield of crystalline material was obtained containing an approximately 50/50 mixture of 23a and 23b. A small amount of 23b was obtained pure following high-pressure liquid chromatography. [i] Crystallized from methanol-ethyl acetate mixtures. [j] Following chromatography of the crude reaction mixture, 6.3 g of crude 26 was converted to a hydrobromide salt and crystallized from methanol-ethyl acetate mixtures to afford 2.29 g (13%) of solid 26. The second product of the reaction was 28a which resulted from partial reaction analogous to path A in Scheme I.

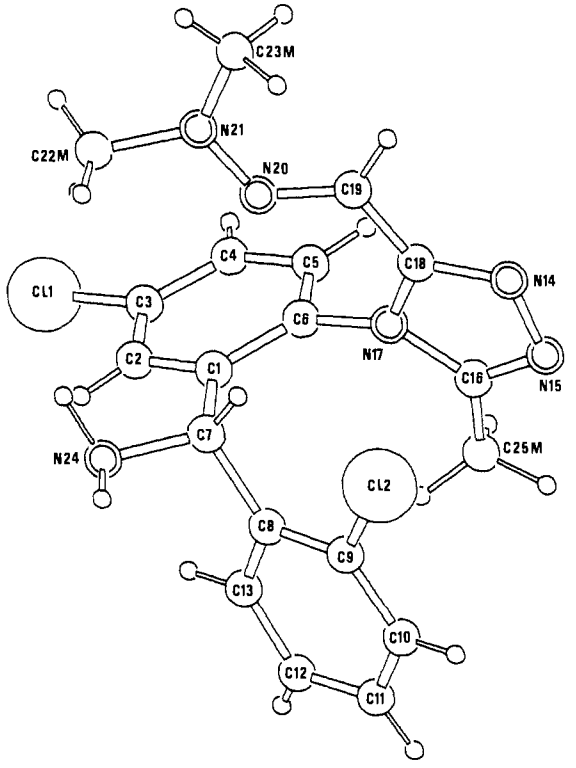


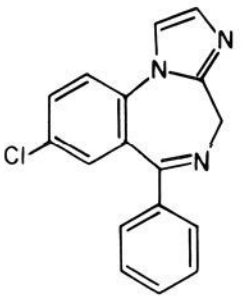
Figure 1. Computer drawing of **12a** obtained from x-ray data.

ratio of 2/1. (The NMR spectrum of the crude reaction mixture from the preparation of **23a** and **23b** indicated they had been formed in a ratio of about 1:1.)

We found that imidazobenzodiazepines underwent an analogous reaction with hydrazine. However, **25**, lacking a substituent larger than H at C_5 of the imidazole ring, was isolated as a *single*, rapidly equilibrating racemate. All signals in the NMR spectrum were broadened at room temperature because of the relatively low-energy barrier to imidazole ring rotation through the plane of the attached trisubstituted benzene ring.

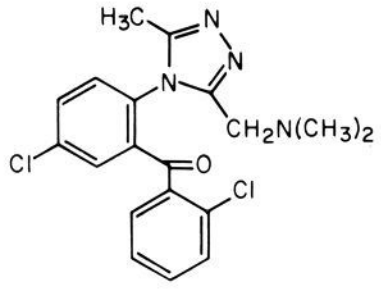
Every triazolo- or imidazobenzodiazepine bearing a 6-*o*-chlorophenyl ring which was subjected to reaction with hydrazine afforded products according to path B in Scheme I. Therefore, the discovery that **27**, lacking an *o*-chlorine in the 5-phenyl ring, underwent reaction by both paths leading to the formation of **19** and **28** in 14 and 21% crystallized yields, respectively, was not expected. Apparently the presence of the *o*-chlorine substituent significantly hindered the nucleophilic attack of hydrazine at the C_6 imine bond of **6**, thereby favoring attack at the C_4 imine bond of tautomer **10**. (The analogous imidazole products **26** and **28a** were formed from the corresponding imidazobenzodiazepine, **27a**.) It is difficult to avoid speculating that the *o*-chlorine also protects the imine bond of benzodiazepines from hydrolysis and subsequent metabolic deamination, thereby enhancing their biological potency relative to their non-ortho-halogenated congeners.

Lanthanide Shift Studies. In order to explain the large chemical shift differences observed for the methyl signals of **12a** and **12b** and for the two methyl signals of **13**, it was assumed that the conformation of these molecules in solution approximated that of **12a** in the crys-



27a

28a



29

talline state. However, CPK models indicated that **13** could exist in two rather similar conformations, **13a** and **13b**, shown in Figure 2. It is clear from the figure that the *o*-chlorophenyl ring can shield one methyl of the triazole ring in **13a** and shield the opposite triazole methyl in **13b**. To determine which conformation was correct in solution an $Eu(dpm)_3$ study was carried out on **13**. Despite the fact that four heteroatoms were present, straight-line plots of $\Delta\delta$ vs. (Eu)/(**13**) were obtained for five readily identifiable proton signals (see Figure 3). The shift data obtained on four protons of **13** were used as input to a computer program (LISC) for correlating molecular structure with the experimental lanthanide-induced shift.[6,7] The calculated shifts for **13a** and **13b** are provided in Table II and are compared with the actual experimental values obtained in the presence of 1 mole equiv of europium shift reagent. It is clear from the agreement factors that **13a** is the preferred conformation in solution as well as in the solid state.

Pharmacology. The data presented in Table III are the ED_{50} values obtained for the new compounds in several pharmacological tests which characterize sedative–hypnotic drugs. Several of the α-amino-α-phenyl-*o*-tolyltriazoles (e.g., **12**, **19**, **20**, **22**, **23a,b**, and **24**) displayed marked potency as anticonvulsants, with **22** being the most active antagonist of nicotine, thiosemicarbazide, and pentylenetetrazole. Interestingly, the data in the table reveal that triazolyldiphenylmethylamine **19** was about as active as the corresponding isomeric aminomethyltriazole **28** in antagonizing thiosemicarbazide-induced convulsions. Both were less potent than **13** which had activity comparable to that of the oxidized congeners **23a** and **23b**. The isomers of each diastereomeric pair seemed to be biologically equipotent (cf. **21a** and **21b** or **23a** or **23b**). Finally, the triazoles were more potent than the corresponding imidazoles.

Metabolism and Discussion. Table III shows comparative data of **13** in mice and rats. The results indicate a clear difference in activity of **13** in these two rodent species. Other test results have shown that **13** was inactive in the rat corticosteroid assay and in the Geller behavioral test.[8] In an attempt to discover whether the pharmacological activity of **13** was related to a difference in metabolism in rats vs. mice, brain extracts were analyzed (GLC, electron capture detector) for the presence of **13** (retention time 9 min) and **6** (triazolam, retention time 15 min). The results are shown in Table IV. It is clear that mice are capable of rapidly transforming **13** into a metabolite with the same GLC retention time as **6**. (An unidentified metabolite was also obtained.) No detectable metabolism of **13** to "**6**" was observed in the rat. These results appeared to support the supposition that the biological activity observed on administration of **13** to mice was the result of the conversion of **13** to a biologically active metabolite, viz., triazolam. Further evidence in support of this supposition was clearly desirable. However,

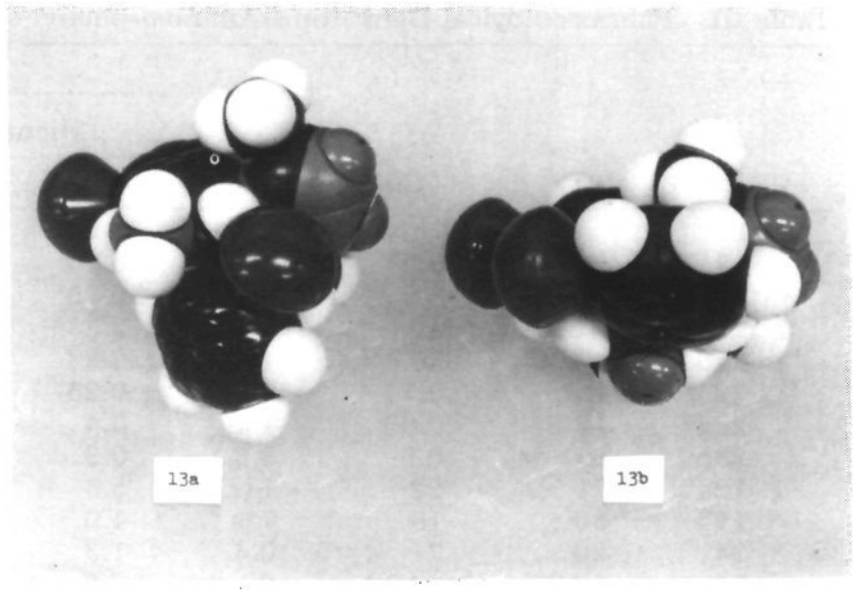


Figure 2. Conformation **13a** and **13b**.

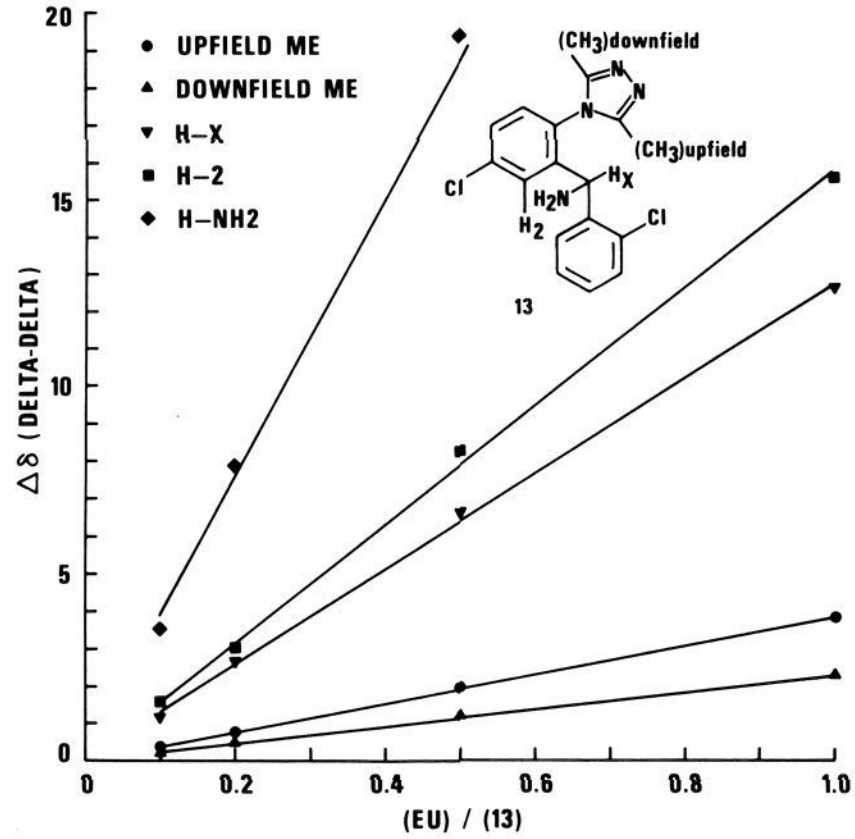


Figure 3. Plot of $\Delta\delta$ vs. (Eu)/(**13**).

Table II. Observed and Calculated Chemical Shifts of **13** in the Presence of $Eu(dpm)_3$

Proton	Obsd shift (Δδ, ppm)	Calcd shift (Δδ, ppm) for **13a**[a,c]	Calcd shift (Δδ, ppm) for **13b**[b,c]
Upfield CH_3	3.84	3.88	1.15
Downfield CH_3	2.28	2.25	1.27
H_2	15.60	15.58	13.97
H_x	12.60	12.60	15.00

[a] The coordinates for the atoms of conformation **13a** which were used to calculate the change in chemical shift (Δδ) caused by the lanthanide reagent were obtained by simulating the coordinates found for **12a** from the x-ray data. [b] The coordinates for the atoms of conformation **13b** which were used to calculate the change in chemical shift (Δδ) caused by the lanthanide reagent were obtained from the values of **13a** after a rotation of 133° in the benzyl-C_6-phenyl bond (equivalent to the C_1–C_7 bond in Figure 1). [c] The agreement factor (*R* factor) between the calculated and observed Δδ was 0.004 for **13a** and 0.268 for **13b**.

Table III. Pharmacological Data[a] for α-Amino-α-phenyl-o-tolyltriazoles and -imidazoles

				Antagonism					
				Nicotine		Thiosemi-	Electric	Pentylene-	Pentylene- tetrazole,
No.	Tr	Ch	D	TE	D	carbazide	shock	tetrazole	rat[c]
19								16	
13	>100	6.3	3.2	0.50	0.50	0.40	>100	1.2	>50
20	>30	6.0	6.6	0.75	0.85	0.34		1.2	
21a	>50	40	4.5	2.3	2.3			40	
21b	>100	56	40	2.8	2.0			>50	
22	1.4	0.71	0.56	0.25	0.18	0.09	40	0.2-1.9 (0.6)[b]	4.4
23a	16.0	0.63	1.1	0.4	0.50			1.2-2.2 (6.2)[b]	>50
23b	8.0	0.5	3.5	0.3	0.3			1.0-5.3	>50
12a	71	22	11.2	5.6	8.0		>100	8.9	
12b	50	16	7.1	4.0	4.0		>100	5.6	
24	20	7	0.4	1.3	1.4		>100	1.2	
25	>100	>100	20	3.6	3.6	1.8	>100		
26	32	18	16	9	9				
28[d]	25	18	14	7	6.2			12.5-22	
29	3.2		0.25	0.14	0.14	0.02	50	0.28	
30[e]	5.0		0.31	0.11	0.11	0.20	20	0.80	4.8
6	0.7	0.056	0.1	0.04	0.04	0.028	23	0.04	0.18

[a] See the Experimental Section for an explanation of the symbols and test procedures. Values in the table are ED_{50} values expressed in mg/kg. Except where indicated, all drugs were administered ip to mice. [b] The values in parentheses were determined after oral administration in mice. [c] The values were determined after oral administration in rats. [d] See Scheme I. [e] Compound 30 is diazepam.

Table IV. Presence of 13 and Metabolites in Extracts from Brains of Mice and Rats Administered 13[a]

Animal[b]	13,[c] ng/brain ± SEM	6,[c] ng/brain ± SEM
Mouse	65 ± 39	190 ± 36
Rat	272 ± 43	4 ± 4

[a] The animals received 20 mg/kg of 13 20 min prior to sacrifice. [b] Five animals were used per determination. [c] Assignments were made on the basis of the comparison of retention time of the products with authentic samples.

despite repeated attempts to characterize the material of retention time 15 min, we were not able to do so in a convincing manner by combination GC–mass spectrometric techniques. Our difficulties derived from the very small quantity of material available, even after pooling the extracts from 50 mice. In previous studies with aminobenzophenones, rats and monkeys provided the samples ultimately used to carry out the GC–MS studies. Because of the greater amount of sample isolated from these large animals, the background noise level posed no significant problem. In the present work the lack of biological activity of **13** in these larger animals precluded their use. Despite these difficulties, we have been able to obtain conclusive evidence that triazolobenzodiazepines are present in mice following the administration of **13**. Thus, by silylating the pooled blood extracts of mice and analyzing them on a new column with a flame detector (see the Experimental Section), we were able to detect and characterize by mass spectrometry the 4-OH metabolite of triazolam, i.e., **31a**. The mass spectral fragmentation data for authentic **31b** and "apparent **31b**" are presented in Figure 4. Except for extraneous ions at m/e 209, 253, 343, and 346, the two spectra are virtually indistinguishable.

While **31b** has been shown to be a metabolite of triazolam in man, dogs, and rodents,[13] it has a very low order of CNS sedative and anticonvulsant activity.[14] Unfortunately, our experiments do not permit us to conclude that **31a** was formed in mice from **13** only via triazolam **6**. Other metabolic routes could be envisioned for transforming **13** to **31a**. Clearly more detailed studies will be needed to answer this point. Finally, we note the fact that **22** was active in the rat. Does the rat convert **22** to **29**, which is known[1b] to be metabolized to **6**?

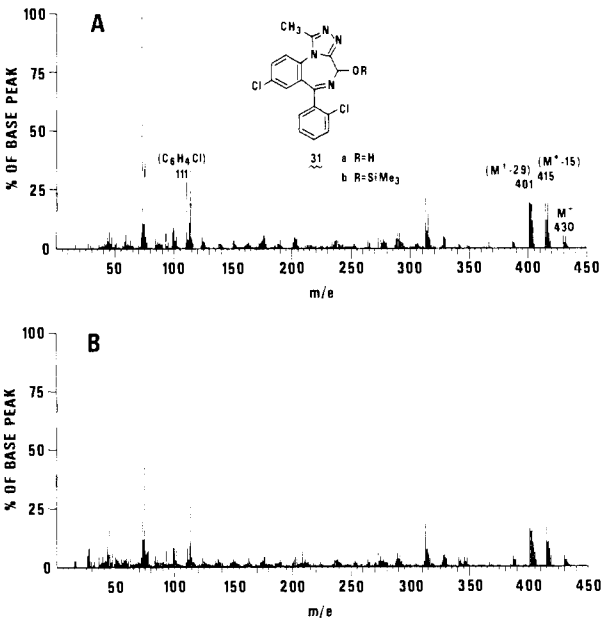


Figure 4. (A) Mass spectrum of authentic **31b**. (B) Mass spectrum of "apparent" **31b** obtained from mouse blood.

Conclusion

The new α-amino-α-phenyl-o-tolyl-4*H*-triazoles and -imidazoles reported herein are active sedative and anticonvulsant agents in mice but, with the exception of **22**, are inactive in the rat. The metabolic data conclusively demonstrate that, in mice, compound **13** is partly transformed into triazolobenzodiazepine **31a**, itself a known metabolite of triazolam **6**. Moreover, the data presented suggest that **6** is also present in the brain extracts of mice administered **13** but is absent from the extracts of rats administered the same compound. These results further suggest that **13** may serve as a prodrug of triazolobenzodiazepines in mice but not in rats.

Experimental Section

Chemistry. Melting points, taken with a Thomas-Hoover capillary melting point apparatus, are uncorrected. The structures of all compounds were supported by IR, UV, NMR, and mass spectral data. IR spectra were determined in Nujol with a

Perkin-Elmer Model 421 recording spectrophotometer. UV spectra were determined in 95% EtOH with a Cary Model 14 spectrophotometer. NMR spectra were recorded on a Varian Model A-60A; chemical shifts were recorded in δ (parts per million) downfield from tetramethylsilane. Mass spectra were obtained with a Varian MAT CH 7 or LKB. Starting materials for the reactions of hydrazine with benzodiazepines and benzophenones were described earlier.[1a]

General Procedure for the Reaction of Triazolo- or Imidazobenzodiazepines with Hydrazine Hydrate. A solution of triazolo- or imidazobenzodiazepine starting material (40.0 mmol) in 7.5 mL of diethylene glycol was treated with 100 mL of hydrazine hydrate and heated at 140–155 °C under nitrogen for from 6 to 22 h. After quenching the reaction mixture in a 5% aqueous sodium hydroxide solution, the products were extracted with chloroform, dried over magnesium or sodium sulfate, and concentrated in vacuo to an oil. If two diastereomeric products were obtained, the oil was chromatographed over silica gel by eluting with methanol–chloroform (3:97%) mixtures. The products were collected and crystallized in the yields reported in Table I. Pertinent physical data are also provided.

Preparation of 12c. A sample of 0.403 g (1.0 mmol) of **12a** in 1.4 g of 88% formic acid (15.0 mmol) was treated with 0.675 mL of a 37% aqueous formaldehyde solution (9.0 mmol of CH_2O) and heated to 110–120 °C for 1 h. The reaction mixture was quenched in a cold 5% aqueous NaOH solution, extracted with chloroform, dried, and concentrated in vacuo to an oil. The oil was chromatographed to afford, after crystallization from ethyl acetate–hexane mixtures, 40 mg of **12c**. A small amount of a second component had NMR spectral data identical with that obtained for authentic **29**.

X-ray Studies. Crystal data for racemic **12a** ($C_{19}H_{20}N_6Cl_2$) were as follows: monoclinic; space group $P2_1/c$; $Z = 4$; $a = 11.289$ (1) Å; $b = 12.687$ (1) Å; $c = 16.561$ (1) Å; $\beta = 124.08$ (1)°; $d_{calcd} = 1.36$ g/cm^3; $d_{measd} = 1.33$ g/cm^3; $\mu = 29.7$ cm^{-1}; 3946 reflections, of which 2742 were greater than one standard deviation. For racemic **12c** ($C_{21}H_{24}N_6Cl_2$), the corresponding data were as follows: triclinic; space group $P\bar{1}$; $Z = 2$; $a = 7.604$ (1) Å; $b = 10.704$ (1) Å; $c = 14.251$ (1) Å; $\alpha = 102.15$ (1)°; $\beta = 96.75$ (1)°; $\gamma = 91.46$ (1)°; $d_{calcd} = 1.27$ g/cm^3; $d_{measd} = 1.31$ g/cm^3; $\mu = 26.3$ cm^{-1}; 4465 reflections of which 3757 were greater than one standard deviation.

Data were automatically collected, to a maximum 2θ of 145°, on a Syntex $P\bar{1}$ diffractometer controlled by an IBM 1800 computer using graphite monochromatized Cu Kα radiation (λ = 1.5418°). The data were corrected for systematic errors, including absorption.[9] No crystal deterioration was observed. Trial structures were obtained easily by direct methods. Coordinates, including hydrogen coordinates, anisotropic thermal parameters for heavier atoms, isotropic temperature factors for hydrogens, and a scale factor were refined by multiple matrix least squares. The amine hydrogens in **12a** were disordered and were not included in the calculations. The function minimized was $\Sigma\omega(F_o^2 - F_c^2)^2$, where $\omega = 1/\sigma(F_o^2)$. The variance σ^2 was assigned at data reduction as $\sigma^2(F_o^2) = \sigma^2$ counting statistics + $(dI)^2$ where d was calculated from an analysis of variation among ten monitored reflections to be 0.012 for **12a** and 0.008 for **12c**. Final agreement indices ($R = \Sigma||F_o| - |F_c||/\Sigma|F_o|$) were 0.100 for **12a** and 0.127 for **12c**. The standard deviation of fit was 1.60 for **12a** and 1.73 for **12c**. Final coordinates and standard deviations for heavier atoms are listed in Tables Va and Vb.[10] Figure 1 is a computer drawing of **12a** showing the numbering scheme, the conformation, and the relative configuration. The N_{24} amine hydrogens were disordered; the figure shows hydrogens at the highest occupancy locations. Because C_7 is an asymmetric carbon and also rotation about the C_2–N_{17} bond is restricted, **12a** is one of two possible racemic mixtures. **12c** is not shown because it is identical with **12a** in relative configuration and almost identical in conformation. Numbering is the same except for the additional methyl substituents at N_{24}, $C_{26}M$, and $C_{27}M$. All calculations were done on an IBM 370 computer using the CRYM system of crystallographic programs written by one of the authors (D. J. Duchamp).

NMR Lanthanide Shift Study. Keeping the concentration of **13** constant (0.1 M), solutions were prepared containing 10, 20, 50, and 100 mol % $Eu(dpm)_3$. These solutions were heated and permitted to stand for 3 days. For the case of the high concentration of $Eu(dpm)_3$, it was difficult to maintain the solution homogeneous. The NMR spectra were recorded on a Varian HA-100, with frequency lock on $CDCl_3$. Chemical shifts were recorded (Table II) and used to obtain $\Delta\delta$ values for the five readily identifiable protons. The results are plotted in Figure 3. A regression analysis was made to find the slope of the best straight line through the points as shown in Figure 3. The value of $\delta(NH_2)$ at 100 mol % $Eu(dpm)_3$ (not shown on the graph) was 36 ppm. Calculated shifts for **13a** and **13b** were determined with a modified[7b] LISC program.[7a] The coordinates of **13a** were simulated on the basis of the x-ray data obtained for **12a**. The coordinates for **13b** were obtained from **13a**.

Pharmacology. Methods. Carsworth Farms male, albino mice (CF-1) weighing 18–22 g were used for the studies described in Table III. Unless otherwise indicated, the test compounds were dissolved or suspended in 0.25% aqueous methylcellulose solution and administered ip to groups of six mice per dose, at multiple dose levels distributed at 0.3 log intervals. Procedures for measuring the effect of test compounds on traction (Tr), chimney (Ch), dish (D), nicotine-induced tonic extensor convulsions (TE), death (d), and thiosemicarbazide-induced lethality end points have been described previously.[11] The antagonism of pentylenetetrazole-induced convulsions was also described.[12] The procedure for antagonizing pentylenetetrazole-induced convulsions in male Upjohn Sprague–Dawley rats weighing 130–150 g was carried out identically with that described for the studies in mice.

Metabolism Study. Male Upjohn mice and male Upjohn Sprague–Dawley rats (130–150 g) were injected ip with 20 mg/kg of **13** and sacrificed 0.5 h later. After homogenizing the mouse brain in 3 mL of cold water, 3 mL of 1 M potassium phosphate buffer (pH 6.0) was added. After the addition of 10 mL of benzene, the samples were shaken mechanically for 10 min and centrifuged. An 8-mL aliquot of the benzene layer was removed and the extraction was repeated. The benzene extracts were combined and dried under a stream of N_2 in a warm sand bath. The procedure was similar for rat brain using double quantities throughout. The dried mouse and rat samples were each taken up in 0.5 mL of benzene containing 100 ng of alprazolam as internal standard and transferred to 1-dram vials containing 0.3 mL of 1 M potassium phosphate buffer, pH 6.0. After mixing and centrifuging, an aliquot of the benzene layer was used for analysis.

A Microtek gas chromatograph equipped with a ^{63}Ni electron-capture detector and a 127-cm glass column packed with 3% OV-225 on 100–120 mesh Gas Chrom Q was used to analyze the extracts. The carrier gas was nitrogen at a flow rate of 60 cm^3/min with a methane–argon (10:90%) purge at 10 cm^3/min. The column temperature was 273 °C, inlet temperature 284 °C, and detector 310 °C. Integrator printout values were used to calculate results. Retention times: **13**, 9 min; **6**, 15 min; alprazolam, 12 min.

Supportive GC–MS studies were carried out using 50 male Carsworth Farms mice weighing 18–22 g. Mice were injected ip with 30 mg/kg of **13**. Thirty minutes later the mice were anesthetized with Metafane and 0.5 mL of blood was withdrawn from the surgically exposed heart. The blood from five mice was transferred to a tube containing 5 mL of 1 M NaOAc, pH 6.0, and 50 mL of benzene. The tubes were shaken and then centrifuged to separate the layers. Benzene was transferred to a round-bottom flask and the aqueous layer reextracted with 50 mL of benzene. The benzene extracts were combined and reduced to dryness in vacuo.

The dried samples were taken up in methylene dichloride and spotted on a silica gel GF TLC plate. The chromatogram was developed in a chloroform–methanol–95% ethanol–trimethylamine (121:2:7.4:1) solvent system. Samples were visualized using a UV lamp. R_f values for the standards were 0.34 for triazolam, 0.29 for **13**, and 0.20 for 4-hydroxytriazolam. Spots corresponding to triazolam **6** and certain hydroxylated metabolites of **6** were scraped from the plates and the samples eluted with several washings with methylene dichloride. The combined methylene chloride extracts were reduced to dryness with a stream of nitrogen. The residue was dissolved in 0.1 mL of chloroform and derivatized with Regisil RC-2 by heating on a sand–steam bath for 30 min.

The silylated samples were analyzed on an LKB Model 9000 GC–MS with a 3-ft 3% OV-17 on a Gas Chrom Q column under

the following conditions: flask heater, 290 °C; oven, 260 °C; separator, 285 °C; source, 290 °C; ionizing voltage, 70 eV; trap current, 60 μA; accelerating voltage, 3 keV; gas flow, approximately 20 cm^3/min of helium. The retention time for triazolam and its 4-hydroxy metabolite was 10.5 and 8.0 min, respectively. In most cases a background spectrograph was taken shortly after the desired peak passed, and this was subtracted (using computerized techniques) from the mass spectrograph data taken of the desired peaks. A representative spectrum of the material of retention time, 8 min, is shown in Figure 4 with a sample of authentic 4-hydroxytriazolam.

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Supplementary Material Available: Tables V–XV (12 pages). Ordering information is given on any current masthead page.

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Oxidative and Cardiovascular Studies on Natural and Synthetic Catecholamines

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The cyclic voltammometric behavior of epinephrine, norepinephrine, dopamine, epinine, α-methyldopamine, β-methyldopamine, β-methylepinine, and β-methoxyepinine has been examined in order to evaluate substituent effects on cyclization rates of the electrochemically generated quinones. We observed that α and β substituents caused a modest enhancement of cyclization rates while an *N*-methyl group dramatically increased cyclization rates. No correlation was observed between calculated amine pK_a values, suggesting that differences in cyclization rates between the primary and secondary amine series were due to inherent nucleophilicity, a measure of which would be gas-phase proton affinities. The acute pressor effects of the newly synthesized catecholamines were compared with the native amines.

The neurotransmitter catecholamines, dopamine (**1a**), norepinephrine (**1b**), and epinephrine (**1c**), undergo the series of oxidation and cyclization reactions summarized in Scheme I.[1] Following the facile two-electron oxidation of I to the *o*-quinones **2**, cyclization via intermediates **3** to the indolines **4** occurs. The indolines are further oxidized to iminoquinones **5** which rearrange via **6** to the dihydroxyindoles **7**. The 3-hydroxyindolines **4b** and **4c** may dehydrate to the corresponding indoles. Finally, the dihydroxyindoles **7** may undergo a third two-electron oxidation to the highly unsaturated species **8**. In an analogous fashion, 6-hydroxydopamine (**9**) is readily oxidized to the *p*-quinone **10** which, following cyclization to **5a**, shares the same fate of dopamine (**1a**).

The neurodestructive[2] and enzyme-inhibiting properties[3] of 6-hydroxydopamine (**9**) appear to be dependent upon its conversion to one or more of the reactive electrophiles, **5a**, **8a**, and **10**, which are capable of alkylating nucleophilic functionalities present on macromolecules.[4] Since the aberrant in vivo formation of these reactive electrophiles from the native catecholamines could result in biochemical lesions and neurological disorders, it is important to understand how structural parameters influence the susceptibility of the parent amines to autooxidation, as well as the subsequent fate of the resulting quinones. The elegant electrochemical[5] and structural analogue studies[6] of Adams and co-workers have contributed significantly to the current state of knowledge in this area. An interesting feature observed by Adams et al.[5] is the dramatic difference in cyclization rates of the quinones **2a** vs. **2c** derived from dopamine (**1a**) and epinephrine (**1c**), respectively. In order to evaluate the influence of N, α, and β substituents on the cyclization rates of **2**, we have compared the cyclic voltammometric (CV) characteristics of a selected series of catecholamines. If the rate-determining step in the conversion of **2** to **4** is cyclization to **3**, the cyclization rate constants can be determined by measuring the appropriate peak potential currents at different scan rates (see below). We have determined these rate constants and evaluated them in terms of steric factors

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