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Anticonvulsant and Monoamine Oxidase Inhibitory Activities of some Triazene N^1-Oxides

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Abstract: Thirty-three N^3-2-, -3- or -4-substituted aryl-N^1-(alkyl/aryl/substituted aryl)-triazene N^1-oxides were synthesized and evaluated for their anticonvulsant and monoamine oxidase (MAO) inhibitory activities. Most of the compounds exhibited MAO inhibitory activity *in vitro*, and kinetic studies conducted with N^3-4-chlorophenyl-N^1-methyltriazene N^1-oxide, the most potent inhibitor, showed that the inhibition is non-competitive in nature. The MAO inhibiting activity of the compounds correlated well with their anticonvulsant effect against maximal electroshock-induced seizures in rats. Acute toxicity studies indicate that the compounds have a wide margin of safety.

Monoamine oxidase inhibitors are known to exhibit significant anticonvulsant activity against experimentally induced seizures, and a significant correlation, between these two activities has been established (1–4), although reports to the contrary also exist (5, 6). Triazene N^1-oxides have been recently reported to exhibit significant anticonvulsant activity against maximal electroshock-induced seizures in rats (7). In this communication we report the *in vitro* MAO inhibitor activity of a series of triazene N^1-oxides, synthesized in this laboratory, together with their anticonvulsant effects, as a function of their chemical structures.

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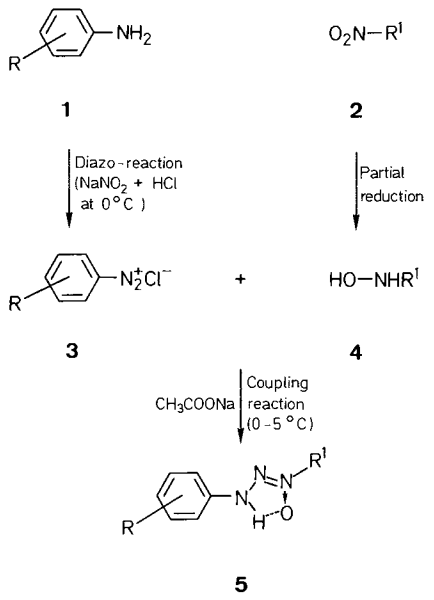
The compounds were synthesized as per the route shown in Fig. 1. The diazonium salt (**3**), prepared from the corresponding primary aryl amine (**1**), when coupled with hydroxylamine (**4**), prepared from the corresponding nitro-compound (**2**), in acetate buffered solution, yielded the corresponding triazene N^1-oxide (**5**). The structures of the compounds were established by elemental analysis, IR and NMR spectral data. The analytical results for elements were within ± 0.4 % of the theoretical values. The prepared compounds are listed in Table I, along with their physical constants.



Materials and Methods

Synthesis of N^3-phenyl-N^1-methyl-triazene N^1-oxide:

Freshly distilled aniline (0.06 mole) was diazotized, the reaction taking approximately 30 min for completion, and nitromethane (0.15 mole) was reduced with zinc dust (22.7 g) and ammonium chloride (9.1 g) to methylhydroxylamine (0.10 mole), in an ice bath with constant stirring. The diazotized product was slowly added to this latter solution, with occasional addition of a saturated solution of sodium acetate (40 g), to maintain the pH at 4.5 to 5.5, and crushed ice to keep the temperature between 0 to 5° C. A creamy yellow mass separated, which was filtered under suction, washed with ice cold water and crystallized from aqueous alcohol. It was finally decolorized with charcoal to yield silky white crystals. M.p.: 71° C; yield: 4 g (44 %); IR (KBr cm^{-1}) : 3160 (–NH), 1520 (N→0), 1440 (–N=N–), 1320 (–N=N–NH–); NMR ($CDCl_3$ δ scale): 3.9 (s, 3H, N–CH_3), 6.8 to 7.4 (m, 5H, Ar-H), 10.1 (bs, 1H, –NH).

The other substituted triazene N^1-oxides were prepared similarly, using appropriate molar quantities of the corresponding amino and nitro compounds. Benzocaine derivatives were unstable at pH 4.5 to 5.5 and were prepared at a lower pH (1.0 to 2.5).

Enzymatic and Pharmacological Assays

MAO inhibitory activity was determined by a radiometric enzyme assay technique (8). The enzyme source was rat liver homogenate and the substrate used was ^{14}C-tyramine. The test drugs were dissolved in propylene glycol, and the MAO inhibition afforded by graded concentrations of the compounds was determined with the respect to controls, where only equivalent amount of propylene glycol was added to the reaction mixture. The I_{50} values of the test compounds were calcualted as the concentration required to induce 50 % inhibition of the enzyme activity. In a separate study, the nature of enzyme inhibition,

Table I. Physical constants, MAO-inhibiting and anticonvulsant properties of N^3 -2-, -3- or -4-substituted aryl -N^1-(alkyl/aryl/substituted aryl)-triazene N^1-oxides.

Compound	R	R^1	M.p.* (°C)	Yield (%)	Elemental analysis** (%)	C	H	N	MAO-inhibiting activity*** ($I_{50}\times10^{-4}$ M)	Anticonvulsant activity (ED_{50}, mg/kg, i.p.)
1	H	$-CH_3$	71 (Lit.[12] 72–73)	44	Found	65.51	5.82	27.94	1.60	65
					$C_7H_8N_3O$ Calcd.	65.63	5.96	27.80		
2	H	n-C_3H_7	61 (Lit. [13] 61)	40	Found	59.90	7.62	23.54	1.76	70
					$C_9H_{13}N_3O$ Calcd.	60.33	7.26	23.46		
3	H	$-C_6H_5$	119 (Lit. [14] 119.5)	86	Found	67.20	4.92	19.91	2.60	170
					$C_{12}H_{11}N_3O$ Calcd.	67.72	5.16	19.71		
4	4-OC_2H_5	$-CH_3$	137	41.6	Found	55.49	6.51	21.77	1.25	33
					$C_9H_{13}N_3O_2$ Calcd.	55.38	6.66	21.53		
5	4-OC_2H_5	$-C_2H_5$	111	45	Found	57.52	7.15	20.31	1.90	70
					$C_{10}H_{15}N_3O_2$ Calcd.	57.41	7.17	20.09		
6	4-OC_2H_5	n-C_3H_7	85	55	Found	59.31	7.66	18.91	2.90	170
					$C_{11}H_{17}N_3O_2$ Calcd.	59.19	7.62	18.83		
7	4-OC_2H_5	$-C_6H_5$	112 (Lit. [15] 112–113)	54	Found	65.45	5.39	16.46	5.00	> 200
					$C_{14}H_{14}N_3O_2$ Calcd.	65.62	5.46	16.40		
8	4-NO_2	$-CH_3$	231 (Lit.[16] 231)	47	Found	42.61	394	28.62	3.64	115
					$C_7H_8N_4O_3$ Calcd.	42.85	4.08	28.57		
9	4-NO_2	$-C_2H_5$	169	58	Found	45.61	4.72	26.79	4.40	> 200
					$C_8H_{10}N_4O_3$ Calcd.	45.71	4.76	26.67		
10	4-NO_2	n-C_3H_7	124	46	Found	48.04	5.28	25.21	4.70	> 200
					$C_9H_{12}N_4O_3$ Calcd.	48.21	5.35	25.00		
11	4-NO_2	$-C_6H_5$	156 (Lit. [14] 157)	45	Found	55.64	3.81	22.08	> 5.00	–
					$C_{12}H_{10}N_4O_3$ Calcd.	55.81	3.87	21.70		
12	4-Cl	$-CH_3$	142 (Lit.[13] 141.5)	46	Found	45.15	4.38	22.73	1.21	27
					$C_7H_8N_3OCl$ Calcd.	45.29	4.28	22.64		
13	4-Cl	$-C_2H_5$	102 (Lit. [13] 102)	38	Found	48.25	4.83	20.87	1.40	38
					$C_8H_{10}N_3OCl$ Calcd.	48.12	5.01	21.05		
14	4-Cl	n-C_3H_7	83 (Lit.[13] 81.5)	30	Found	50.38	5.49	19.78	2.54	76
					$C_9H_{12}N_3OCl$ Calcd.	50.58	5.62	19.67		
15	4-Cl	$-C_6H_5$	140 (Lit.[14] 140)	71	Found	58.02	3.97	17.12	3.50	150
					$C_{12}H_{10}N_3OCl$ Calcd.	58.18	4.04	16.96		
16	4–Cl	4-Cl-C_6H_4	139	76	Found	50.92	3.12	14.97	4.78	> 200
					$C_{12}H_9N_3OCl_2$ Calcd.	51.06	3.19	14.80		
17	4–Cl	4-CH_3-C_6H_4	135	52	Found	59.52	4.51	16.23	5.00	> 200
					$C_{13}H_{12}N_3OCl$ Calcd.	59.65	4.58	16.06		

Table I. (Continued)

Compound	R	R^1	M.p.* (°C)	Yield (%)	Elemental analysis** (%)	C	H	N	MAO-inhibiting activity*** ($I_{50}\times10^{-4}$ M)	Anticonvulsant activity (ED_{50}, mg/kg, i.p.)
18	4-SO_2NH_2	-CH_3	204	65	Found	38.74	4.56	19.56	2.70	76
					$C_7H_{10}N_4O_3S$					
					Calcd.	38.88	4.63	19.44		
19	4-SO_2NH_2	-C_2H_5	198	71	Found	39.47	4.10	23.90	2.80	115
					$C_8H_{12}N_4O_3S$					
					Calcd.	39.34	4.91	22.95		
20	4-SO_2NH_2	*n*-C_3H_7	175	43	Found	41.84	5.34	21.64	3.40	140
					$C_9H_{14}N_4O_3S$					
					Calcd.	41.86	5.42	21.70		
21	4-SO_2NH_2	-C_6H_5	180	40	Found	49.19	4.02	19.31	> 5.00	> 200
					$C_{12}H_{12}N_4O_3S$					
					Calcd.	49.31	4.11	19.17		
22	4-SO_2NH_2	4-COOH-C_6H_4	165	48	Found	46.26	3.44	16.82	3.02	123
					$C_{13}H_{12}N_4O_5S$					
					Calcd.	46.42	3.57	16.66		
23	4-SO_2NH_2	4-CH_3-C_6H_4	172	53	Found	50.84	4.50	18.46	> 5.00	–
					$C_{13}H_{14}N_4O_3S$					
					Calcd.	50.98	4.57	18.30		
24	4-$COOC_2H_5$	-CH_3	144	52	Found	53.66	5.79	18.89	3.70	200
					$C_{10}H_{13}N_3O_3$					
					Calcd.	53.81	5.83	18.83		
25	4-$COOC_2H_5$	-C_2H_5	98	45	Found	55.43	6.28	17.94	2.43	140
					$C_{11}H_{15}N_3O_3$					
					Calcd.	55.96	6.33	17.72		
26	4-$COOC_2H_5$	*n*-C_3H_7	124	92	Found	57.13	6.84	16.82	1.60	112
					$C_{12}H_{17}N_3O_3$					
					Calcd.	57.37	6.77	16.73		
27	4-$COOC_2H_5$	4-CH_3-C_6H_4	90	52	Found	64.04	5.57	14.26	> 5.00	–
					$C_{16}H_{17}N_3O_3$					
					Calcd.	64.21	5.68	14.04		
28	4-COOH	-CH_3	199	85	Found	49.11	4.58	21.66	> 5.00	–
					$C_8H_9N_3O_3$					
					Calcd.	49.23	4.61	21.53		
29	4-COOH	-C_2H_5	200	79	Found	51.61	5.10	20.21	4.40	> 200
					$C_9H_{11}N_3O_3$					
					Calcd.	51.67	5.26	20.09		
30	3-CH_3	-C_6H_5	117	36	Found	68.58	5.64	18.75	> 5.00	–
					$C_{13}H_{13}N_3O$					
					Calcd.	68.72	5.72	18.50		
31	3-CH_3	4-Cl–C_6H_4	145	46	Found	59.71	4.54	16.23	> 5.00	xp> –
					$C_{13}H_{12}N_3OCl$					
					Calcd.	59.65	4.58	16.06		
32	2-CH_3	4-Cl-C_6H_4	151	53	Found	59.75	4.51	16.26	> 5.00	–
					$C_{13}H_{12}H_3OCl$					
					Calcd.	59.65	4.58	16.06		
33	4-OCH_3	-C_6H_5	116 (Lit.[17] 116–117)	60	Found	64.02	5.23	17.42	> 5.00	–
					$C_{13}H_{13}N_3O_2$					
					Calcd.	64.19	5.35	17.28		

induced by the most potent compound (compound 12) was determined (9). The MAO inhibitory activity was determined against two concentrations of the substrate, ^{14}C-tyramine (150 and 300 μM). The inhibitor concentrations were plotted against the reciprocal of the enzyme activity (Fig. 2).

The general behavior profile of the test compounds (200 mg/kg, ip) was observed in albino rats (100 to 150 g) of either sex (10).

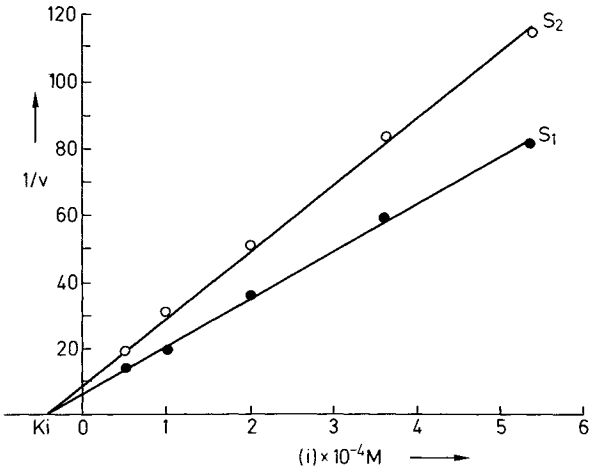


Fig 2. MAO-inhibition studies with compound 12 in the presence of 150 μm (S_2) and 300 μm (S) ^{3}H-tyramine. The mean of three experiments performed in duplicate are plotted.

The anticonvulsant activity of the compounds was assessed in albino rats (100 to 150 g) of either sex against maximal electroshock-induced seizures (11). The test compounds, suspended in 2% Tween-80, were administered ip in graded doses to groups of 10 rats each. The anticonvulsant activity was determined 1 h later and the ED_{50} dose was calculated.

Acute toxicity studies were conducted in albino mice (20 to 30 g), of either sex. Graded doses (200, 500 and 1000 mg/kg, ip) of the test compounds, suspended in 2% Tween-80, were administered to groups of 10 mice for each dose. Mortality and the presence of any overt toxic sign were determined over a 24 h period.

Results and Discussion

The results are summarized in Table I. All the test compounds exhibited concentration-dependent MAO inhibitory activity, and their I_{50} values were recorded. N^3-4-Chlorophenyl-N^1-methyltriazene N^1-oxide (compound 12) produced maximal inhibition, and the enzyme inhibition was found to be non-competitive in nature. The Ki (inhibitor constante) value, obtained graphically, was found to be 0.4×10^{-4} M (Fig. 2).

All the compounds, except 27, 29, 30 and 33, produced signs of sedation in rats. The animals showed reduction in spontaneous motor activity and ptosis. Grooming, irritability and startle response was markedly inhibited in the treated rats. Compounds 8 and 13 produced motor paralysis, with loss of righting reflex. There was no discernible effects on respiration, urination or defecation.

The anticonvulsant ED_{50} values of the test compounds were well correlated with their respective MAO inhibitory I_{50} concentrations. The data indicate that the presence of alkyl groups made the compounds more effective as anticonvulsants and MAO inhibitors, in comparison to aryl or substituted aryl groups at the N^1 end of the triazene N^1-oxide moiety. Chloro- or ethoxy-groups at position 4 of the N^3-phenyl nucleus resulted in augmentation of both biological activities. On the contrary, nitro-, sulphonamido-, carbethoxy-, carboxy-, methoxy- or methyl-substitutions at this position, or methyl-substitution at position 3 of this nucleus, resulted in decrease of both anticonvulsant and MAO inhibitory activities. Some triazene N^1-oxides, like N^3-2 or 3-tolyl-N^1-chlorophenyltriazene N^1-oxide, had weak MAO inhibitory activity and were devoid of anticonvulsant action. It is evident that structural modifications at both ends of the triazene-N^1-oxide moiety affect either biological activity equally. This again indicates that the anticonvulsant action of the title compounds is dependent on their MAO inhibitory activity.

Apart from compounds 8 and 13, which induced motor paralysis, the other compounds did not induce any overt toxicity or death up to a dose of 1000 mg/kg ip, over a 24 h observation period.

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