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Radioprotective Activity of p-Aminopropiophenone. A Structure-Activity Investigation†

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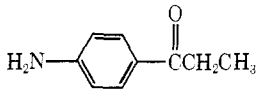
p-Aminopropiophenone (**1**) is well known for its radioprotective action which has generally been attributed to the anoxia associated with high levels of methemoglobin (metHb) following administration of **1** to animals.[1] However, certain lines of evidence suggest[2,3] that the radioprotection of **1** may not be related to metHb levels. Lack of any systematic structure-activity survey of the radioprotective effect of **1** prompted the present investigation of this relatively simple molecule. To this end a number of compounds related to **1** have been examined in an effort to sort out those features associated with radioprotection. The derivatives chosen for evaluation represent alterations in the amino function, variations in the acyl chain length, and a variety of para-substituted anilines. Within this group of substances, the only consistent structural feature required for radioprotection was the presence of a free amine or hydroxylamine group. While no evidence was obtained to support a general mechanism for the radioprotective action of **1**, it was found that in certain compounds (**12**, **13**, and **16**) metHb-forming ability was not necessary for radioprotective action.



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Results and Discussion

Biological. The structural relationships between **1** and the various derivatives investigated in this work may, for convenience, be divided into three groups: (**1**) changes in the nitrogen function (Table I); (2) changes in the alkyl chain length of the ketone function (Table II); (3) changes in the substituent para to the amino group (Tables III and IV). In these experiments usually more than one dose was tried, but only the dose giving maximum protection is shown in the tables.

Generally, alteration of the amino group led to less radioprotective compounds although appreciable toxicity characterized the nitroso (**3**) and *N,N*-dimethyl (**7**) derivatives (Table I). In view of the fact that free radicals have been implicated in radiation toxicity,[1] it was anticipated that the nitroso derivative **3** might show good radioprotection since the nitroso function is known to be a good radical scavenger.[4] However, this did not prove to be the case.

Several years ago *p*-aminoacetophenone (**8**) was tested to a limited extent and found to exhibit some radioprotection although less than *p*-aminopropiophenone.[5] Furthermore, it has been reported that *p*-aminobutyrophenone and *p*-aminovalerophenone (**9**) also showed radioprotection.[6,7] It was, therefore, of interest to examine the effect on radioprotective activity of extending the alkyl chain of the ketone moiety. Thus, compounds having an alkyl chain of 4, 5, and 6 carbons (as compared to 2 carbons in **1**) were prepared and evaluated. The results are shown in Table II. While all three compounds (**9**, **10**, and **11**) showed increased toxicity, only the *p*-pentanoyl- (**9**) and *p*-hexanoyl- (**10**) anilines exhibited radioprotective activity and, in fact, were more radioprotective than **1**.

Replacement of the ketone function by alkyl chains of different lengths gave a different pattern of radioprotective activity (Table III). In contrast to the ketones, the *p*-alkylanilines with shorter chains showed greater activity than the longer chain analogs. More interesting perhaps is the observation that *p*-methyl- and *p*-ethylaniline (**12** and **13**) and *o*-propylaniline (**16**) exhibited good radioprotection but produced essentially no metHb.‡ On the other hand, *p*-*n*-butylaniline (**15**), which produced high levels of metHb, was a poor radioprotective agent. These compounds, then, represent the first derivatives of **1** which effectively separate radioprotection and metHb-forming ability. Surprisingly, *p*-methoxyaniline (**20**), which is isosteric with *p*-ethylaniline (**13**), showed poor radioprotection as did *p*-ethoxyaniline (**21**). The *p*-trifluoromethyl- (**17**) and *p*-cyano- (**18**) anilines provided moderate protection against radiation. It is obvious, then, that the carbonyl function of **1** is not necessary for the molecule to be radioprotective. However, it is not possible at this time to be certain that **1** and the *p*-alkylanilines share a common mechanism for radioprotection.

Finally, it was of interest to compare the radioprotective activity of a series of *p*-aminobenzoic acid esters, which are isosteric with the *p*-aminophenones. The results are summarized in Table IV. The parent acid **22** gave poor protection, whereas the methyl and ethyl esters **23** and **24** were good radioprotectors. Activity dropped off with the propyl ester **25**, and the butyl and isobutyl esters **26** and **27** were poor protectors. These results appear to approximate more closely the trend seen with the *p*-alkylanilines (Table III) and are in contrast to the pattern observed with the ketones (Table II) in which radioprotection is associated with the corresponding longer chain lengths.

During the course of this work, it came to our attention that tetrahydrofolic acid (THFA) had been shown by three different groups of workers[8-10] to be an effective radioprotector even when administered to the animals after irradiation. The title compound (**1**), structurally similar to the *p*-aminobenzoyl moiety of THFA, is known to be effective only if given before radiation treatment. It seemed reasonable, then, to suspect that the radioprotective mechanism of **1** might be associated in some way with THFA metabolism. However, if THFA and the radioprotection of **1** were related, it would be reasonable to expect a mono-N-substituted derivative of **1**, being structurally closer to THFA, would retain good radioprotective action; in fact, the *N*-methyl derivative of **1** (**6**) was a poor protector. Furthermore, *p*-aminobenzoyl-L-glutamic acid (**28**), which even more closely resembles THFA, showed no radioprotective action in our experiments. It seems unlikely, then, that there is any obvious relationship between the radioprotective effect of **1** and THFA metabolism.

These results show no consistent structural requirements for good radioprotective activity of aniline derivatives save for a free amine or hydroxylamine function. Compared to **1**, the radioprotective activity of these compounds may be increased or decreased depending on the ortho or para substitutent, but with no apparent common

†A preliminary account of this work was presented at the 21st Annual Meeting of the Radiation Research Society in St. Louis, Mo., on May 1, 1973.

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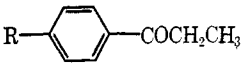
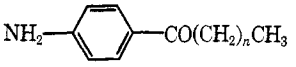
‡A complete investigation of the metHb-producing ability and toxicity of these compounds is in progress and results will be published later.

Table I. Radioprotective Activities of *p*-Aminopropiophenone Derivatives with an Altered Nitrogen Function



Compd	R	LD_{50} ± S.E., mg/kg	Dose, mg/kg	Survivors/total	% surviving
Control	No drug			33/277	11.9
1	NH_2	86 ± 3	30	25/59	42
2	NHOH	134 ± 10	33	10/31	30
3	NO	17 ± 3	4	3/16	20
4	NO_2	>600	360	1/16	7
5	$N{=}NO\text{-}C_6H_4\text{-}COCH_2CH_3$	>1000	100	0/16	0
6	$NHCH_3$	140–145	10	3/14	20
7	$N(CH_3)_2$	78–80	15	5/29	17

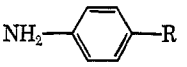
Table II. Radioprotective Activities of *p*-Acylanilines with Acyl Chains of Different Length



Compd	*n*	Total C's in acyl chain	LD_{50} ± S.E., mg/kg	Dose, mg/kg	Survivors/total	% surviving
1	1	3	86 ± 3	30	25/59	42
8[a]	0	2	420 ± 13	200	3/10	30
9	3	5	52 ± 8	15	26/46	56
10	4	6	35 ± 4	15	25/44	57
11[b]	5	7	35 ± 3	30	7/32	22

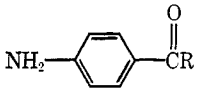
[a]The LD_{50} of **8** was determined in this work. The radioprotective data were taken from ref 10. [b]This compound was administered as the HCl salt. Other compounds in this table were given as the free base.

Table III. Radioprotective Activities of Various Para-Substituted Aniline Derivatives



Compd	R	LD_{50} ± S.E., mg/kg	Dose, mg/kg	Survivors/total	% surviving
1	$O{=}CCH_2CH_3$	86 ± 3	30	25/59	42
12	CH_3	326 ± 16	60	8/29	28
13	CH_2CH_3	133 ± 8	30	17/39	44
14	$CH_2CH_2CH_3$	201 ± 8	30	7/24	29
15	$CH_2CH_2CH_2CH_3$	81 ± 5	10	0/16	0
16	*o*-$CH_2CH_2CH_3$	~250	90	15/23	65
17	CF_3	101 ± 5	60	22/47	47
18	CN	155 ± 9	75	21/46	46
19	H	572 ± 10	300	6/30	20
20	OCH_3	806 ± 50	300	6/47	13
21	OCH_2CH_3	692 ± 34	300	4/31	13

Table IV. Radioprotective Activities of *p*-Aminobenzoic Acid Derivatives



Compd	R	LD_{50} ± S.E., mg/kg	Dose, mg/kg	Survivors/total	% surviving
1	CH_2CH_3	86 ± 3	30	25/59	42
22	OH		1000	5/31	16
23	OCH_3	237 ± 22	150	33/64	52
24	OCH_2CH_3	216 ± 8	150	22/46	48
25	$OCH_2CH_2CH_3$	126 ± 9	50	7/29	24
26	$OCH_2CH_2CH_2CH_3$	67 ± 7	20	0/16	0
27	$OCH_2CH(CH_3)_2$	48 ± 8	10	3/15	20
28	$NHCH(COOH)CH_2CH_2COOH$		750	1/14	7

factor. It is clear, however, that radioprotection is separable from metHb formation, at least in the *p*-alkylaniline series, although this may mean that the *p*-alkylanilines act through a mechanism different from that of the *p*-aminophenones (Table II). Any hypothesis of a common mechanism must explain the reversal of the effect on increasing the chain length on the radioprotective activity of the *p*-alkylanilines *vs.* the *p*-aminophenones.

Chemistry. Many of the compounds tested were purchased from commercial sources or have been described in the literature and were prepared by standard procedures or minor modifications thereof. For instance, a cleaner separation of *p*- and *o*-propylnitrobenzenes was achieved by column chromatography than by distillation.[11] The nitroso derivative **3** was prepared using the Ag_2CO_3–Celite oxidation described by Massen and DeBoer.[12] The azoxy derivative **1** (**5**) formed easily in DMSO solutions of the hydroxylamine **2** or the nitroso compound **3** but could be prepared in better yield by alkali treatment of **2**. The monomethyl derivative **1** (**6**) was synthesized by a modifica-

tion of the method of Johnstone, *et al.*[13] Friedel–Crafts acylation using *N*-methylaniline and propionyl chloride was unsuccessful in obtaining significant amounts of **6**.

Experimental Section

Elemental analyses were performed by Schwarzkopf Microanalytical Laboratories, Woodside, N. Y. Where analyses are indicated only by symbols of the elements, they are within ±0.4% of the theoretical values. Ir spectra in KBr were obtained with a Perkin-Elmer 257 spectrophotometer. Nmr spectra in $CDCl_3$ (Me_4Si) were obtained with a Varian T-60 instrument at ambient temperature in approximately 10% solutions. All spectra were consistent with the proposed structures. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. Tlc was carried out using Brinkmann silica gel precoated plastic sheets.

Unless otherwise noted, compounds were purchased from Aldrich Chemical Co. or K & K Laboratories, Inc. *p*-Aminopropiophenone (**1**) and methyl *p*-aminobenzoate (**24**) were purchased from Eastman Kodak Co. 4-Trifluoromethylaniline (**17**) was purchased from Research Organic/Inorganic Chemical Corp. Butyl and isobutyl *p*-aminobenzoates (**26** and **27**) were obtained from Matheson Scientific.

Compounds **9**, **10**, and **11** were prepared from aniline and the corresponding acyl chloride according to Clifford, *et al.*[14] Preparative methods for the hydroxylamine **2** and the nitro derivative **4** have been described.[15]

4-Nitrosopropiophenone (3). The hydroxylamine **2** (330 mg, 2 mmol) was dissolved in CH_2Cl_2 (20 ml) and treated with Ag_2CO_3–Celite reagent (1.7 g, 30 mmol of Ag_2CO_3). The mixture turned black at once, was stirred for 2 min, and filtered. Evaporation of the green filtrate gave a yellow, crystalline residue (301 mg, 92%) which was recrystallized from MeOH: mp 94–95°. *Anal.* ($C_9H_9NO_2$) C, H, N.

4,4'-Dipropionylazoxybenzene (5). A mixture of the hydroxylamine **2** (330 mg, 2 mmol), 0.2 ml of MeOH, and 10 ml of 0.4 *N* NaOH solution was stirred at room temperature for 16 hr at which time it was extracted twice with 20-ml portions of $CHCl_3$. The extracts were washed with 5 ml of H_2O, dried (Na_2SO_4), and evaporated to dryness to give 288 mg (93%) of **5** as an orange solid which was recrystallized from MeOH: mp 135–136°. *Anal.* ($C_{18}H_{18}N_2O_3$) C, H, N.

4-(*N*-Methylamino)propiophenone (6). A mixture of *p*-aminopropiophenone (6 g, 40 mmol), 40 ml of isopropyl alcohol, trifluoroacetic acid (4.56 g, 40 mmol), and 60 ml of CH_2Cl_2 was treated with a solution of dicyclohexylcarbodiimide (9.08 g, 44 mmol) in 20 ml of CH_2Cl_2 and stirred for 1 hr. Dicyclohexylurea was removed by filtration and the filtrate evaporated to give the crude trifluoroacetanilide which was used without further purification. The trifluoroacetanilide was dissolved in 200 ml of acetone to which was added CH_3I (13.64 g, 96 mmol). The mixture was heated almost to reflux for 10 min, evaporated to dryness, treated with 200 ml of H_2O, and refluxed for 15 min. The precipitate was filtered and the filtrate extracted with three 50-ml portions of $CHCl_3$. The precipitate was washed with 20 ml of warm $CHCl_3$ and filtered. This filtrate was combined with $CHCl_3$ extracts, dried (Na_2SO_4), and concentrated. The residue was dissolved in 20 ml of $CHCl_3$ and applied to a column (29 mm diameter) of silica gel (100 g) in $CHCl_3$. The column was eluted with $CHCl_3$ (fractions 1–9), 1% MeOH in $CHCl_3$ (10–12), 2% MeOH in $CHCl_3$ (13–15), and 5% MeOH in $CHCl_3$ (16–18). The first fraction was 200 ml, thereafter 50-ml fractions were taken. Fractions 4–12 were combined, on the basis of tlc, to yield 2.14 g (33%) 4-(*N*-methylamino)propiophenone (**6**). Recrystallization from EtOH–H_2O gave mp 129–131°. *Anal.* ($C_{10}H_{13}NO$) C, H, N. Fractions 14–18 gave 442 mg of **1**.

4-(*N*,*N*-Dimethylamino)propiophenone (7). A mixture of *p*-aminopropiophenone (750 mg, 5 mmol), Na_2CO_3 (1.6 g, 15.1 mmol), CH_3I (5.88 g, 41.4 mmol), and 15 ml of acetone was allowed to stir at room temperature for 24 hr. The solvent was evaporated, H_2O (35 ml) was added, and the mixture was extracted with three 50-ml portions of $CHCl_3$ which were dried (Na_2CO_3) and concentrated to give 770 mg (87%) yellow crystals: mp 90–92°. *Anal.* ($C_{11}H_{15}NO$) C, H, N.

4-Propylaniline (14) and 2-Propylaniline (16). Propylbenzene was nitrated according to Hurd and Jenkins.[11] The ortho and para isomers were separated on a silica gel column packed in hexane and eluted with 5% $CHCl_3$ in hexane. That the ortho isomer was eluted before the para isomer was shown by oxidation of a sample of the slower eluting material to *p*-nitrobenzoic acid. Both isomers were reduced in EtOH with 10% Pd/C and treated with HCl to give the salts.

Pharmacology. Adult male Sutter mice were used in all experiments. Radiation studies were carried out as before[15] except that a dose of 600 R was used. MetHb levels were obtained as previously described, as were LD_{50} values.[15]

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Synthesis of Seleno-Toluidine Blue

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Toluidine blue has been shown to have high specificity for the parathyroid, pancreas, and heart and has been useful in identifying parathyroid tissue at operation.[1] ^{35}S-Toluidine blue shows a similar distributional pattern[2] but since ^{35}S is an α-emitting isotope this compound is not efficient for scintiscanning. We report here an efficient synthesis of seleno-toluidine blue (**1**) by a procedure suitable for the incorporation of ^{75}Se, a γ-emitting isotope with a convenient half-life (121 days). This compound has physical and biological properties similar to those of toluidine blue and is thus an attractive candidate for diagnostic scintiscanning techniques.[3]

In an initial approach, *N*,*N*-dimethyl-*p*-nitrosoaniline was condensed with *o*-toluidine to give an unstable adduct