Synthesis and Biologic Activity of *p*-Hydroxylaminopropiophenone†

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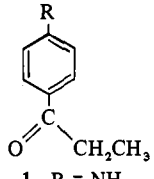
The radioprotective ability of *p*-aminopropiophenone (1) has been recognized since the experiments of Storer and Coon[1] in 1950. Since these investigators did not obtain radioprotective effects with $NaNO_2$ at dosage levels which produced the same or higher methemoglobin (metHb) levels, they concluded that metHb production *per se* was not the primary mechanism for the radioprotective effect of 1. Since it is well known that 1 produces metHb *in vivo* but not *in vitro*, it was of interest to determine whether the metHb-producing metabolite of 1 also exhibits radioprotective activity. Recently Graffe, *et al.*,[2] have shown that the high activity of 1 in producing metHb is due to the formation of *p*-hydroxylaminopropiophenone (2). It was of interest therefore to examine the radioprotective properties of this compound. The purpose of this communication, then, is to report a detailed method for the preparation of 2 and a study of some of its biological properties, especially its radioprotective ability.

Results and Discussion

Chemistry. Graffe, *et al.*,[2] prepared 2 by partially reducing *p*-nitropropiophenone (3), obtained through use of Sugimoto's method[3] which involved decomposition of the diazonium salt of 1 with $CuNO_2$ formed *in situ* by mixing $CuSO_4$, Na_2SO_4, and $NaNO_2$. This method of preparation of 3 was unsuccessful in our hands, but when the diazonium fluoroborate salt of 1 was isolated in relatively pure form the desired compound, 3, was obtained in good yield upon reaction of the fluoroborate with $NaNO_2$ and Cu.



1, R = NH_2
2, R = NHOH
3, R = NO_2

Biological Activity. Toxicity. Mice treated with either compound 1 or 2 showed obvious symptoms of oxygen starvation—fast breathing followed by gasping and convulsions—and exhibited the characteristic cyanosis of methemoglobinemia. The LD_{50} (ip, 10 days) for 2 is 134 ± 10 mg/kg; that of 1 is 277 ± 26 mg/kg. Since the compounds have molecular weights of 165 and 149, respectively, on a molar basis 2 is twice as toxic as is 1.

In 1970, Goldstein[4] reported that the LD_{50} of 1 in rats was not affected by a 2-hr exposure to 4 atm of abs O_2, but that Methylene Blue raised the LD_{50} significantly. In similar experiments, mice receiving an LD_{50} dose of 2 showed 100% survival when simultaneously injected with 30 mg/kg of Methylene Blue. Half of such mice died within a hyperbaric chamber while being exposed to 3 atm of abs O_2 for 2 hr after the treatment with 2; 90% died within 0.5 hr after removal from the chamber. These results indicate similarity in the actions of 1 and 2 despite their differences in toxicity.

Standard Assay. The Bratton-Marshall[5] assay method (diazotiazation and coupling with 1-naphthylethylenediamine to give a blue color) has been used routinely to assay for 1 in blood and urine. Although it was not expected that this method would work for a hydroxylamine, it was applied to 2 to determine whether this substance would interfere with the assay for 1. The results indicate either that 2 is 100-fold less sensitive to this method than is 1 or that our supply of 2 is contaminated by, at most, 1% of 1. No satisfactory analytical method for 2 was found.

In Vitro **metHb.** From the work of Graffe, *et al.*,[2] it was expected that 2 would produce a one-to-one conversion of Hb to metHb. Addition of various amounts of 2 in DMSO to lysed red blood cells gave an approximatley linear conversion of Hb to metHb, and maximal conversion was noted when 2 was present in an equimolar ratio with respect to iron (Figure 1). It is interesting, however, that blood drawn by cardiac puncture and not necessarily 100% oxygenated showed a lower maximal metHb response than similar blood exposed to 5 atm of abs O_2 for 2 hr prior to exposure to 2. The amine (1) produced no metHb *in vitro*.

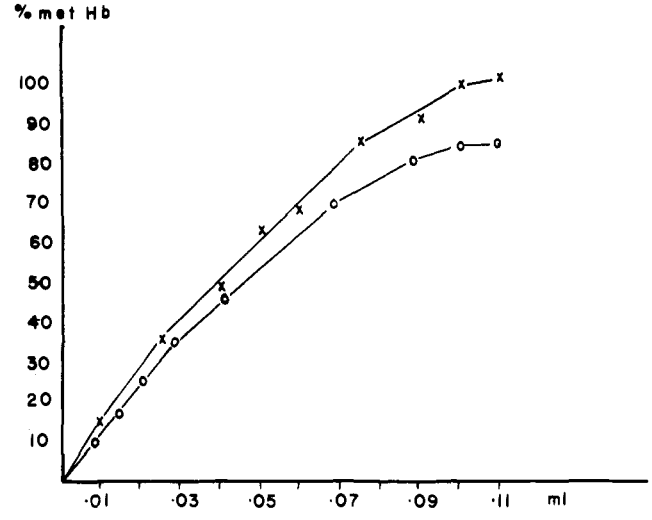


Figure 1. Milliliters of PHAPP (2) solution (calculated such that 0.1 ml of solution will convert 100% of the Hb in a 0.1-ml sample of blood of Hb titer of 15 g/100 ml to metHb) *vs.* % metHb observed; x = blood exposed to 5 atm of abs O_2 for 2 hr; o = blood freshly drawn by cardiac puncture.

In Vivo **metHb.** If 1 is first converted to a hydroxylamine to produce metHb, as suggested by Kiese,[6] then it would be expected that the peak effect of 1 would be somewhat delayed when compared to the peak effect of 2 and that a dose of the hydroxylamine (2) would give a somewhat stronger response than an equimolar dose of the amine (1). Our results indicate that this is indeed the case: the peak response of 2 occurs within the first 3 min, while that of 1 occurs at about 10–15 min (Figure 2). Dose-response curves, on the other hand, are very similar when the response is measured at the peak of action, although the hydroxylamine uniformly shows a slightly stronger response.

Radioprotection. Groups of mice were injected ip with various doses of 1 or 2 in propylene glycol, exposed to 750 R of whole-body irradiation, and observed for 30 days. No control mice survived, while significant numbers of mice treated with either drug did survive (Table I). The mean survival time for control mice is about 10 days while the

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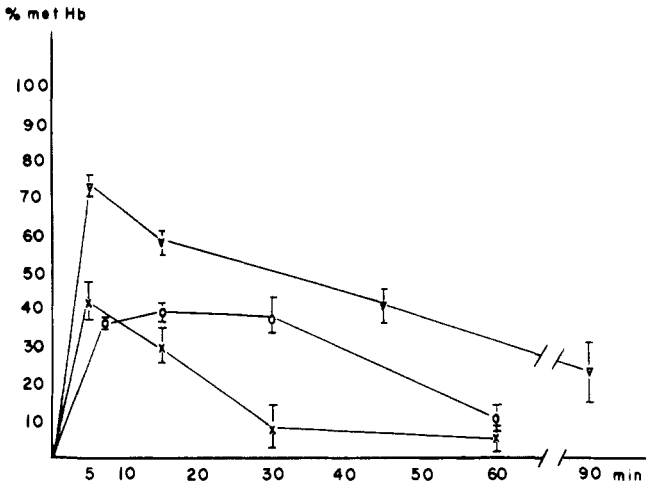

Figure 2. *In vivo* metHb time response for Sutter mice: x = PHAPP (**2**), mg/kg ip; o = PAPP (**1**), 20 mg/kg ip; ▽ = PHAPP (**2**), 40 mg/kg ip.

Table I. Radiation Survival (30 Day) after 750 R[a]

Compd[b] (dose, mg/kg)	Survivors/ total	% survival	Compd[b] (dose, mg/kg)	Survivors/ total	% survival
PG (control)	0/48	0	PAPP (**5**)	1/15	6
PHAPP (**5**)	2/15	13	PAPP (**15**)	3/15	20
PHAPP (**15**)	4/15	27	PAPP (30)	6/31	19
PHAPP (33)	10/31	32	PAPP (60)	12/30	40
PHAPP (**66**)	6/23	26	PAPP (90)	4/22	18

[a]Vehicle: propylene glycol (PG)–0.15 ml/30-g mouse. [b]PHAPP, *p*-hydroxylaminopropiophenone (**2**); PAPP, *p*-aminopropiophenone (**1**).

mean survival time for mice treated with either compound is about 15 days; a plot of daily survival (Figure 3) exemplifies the similarity of action of the two compounds.

Evidence presented in the literature[7,8] suggests that the radioprotective activity of *p*-aminopropiophenone (**1**) is not directly associated with its metHb-forming ability, thereby leading to the speculation that different compounds are the active agents for these two activities. Our data indicate that while **2** is the active metHb-forming agent of **1**, it does not seem to be significantly more effective than **1** as a radioprotective agent and therefore is probably not directly responsible for the radioprotection effect of **1**. The possibility exists, of course, that the amine (**1**) itself is radioprotective.

Experimental Section

Chemistry. Elemental analyses were performed by the Schwarzkopf Microanalytical Laboratory, Woodside, N. Y. Melting points were obtained on a Fisher-Johns melting point apparatus and are uncorrected. Ir spectra were recorded on a Perkin-Elmer Model 257 spectrophotometer. All reagents were of AR grade.

p-Hydroxylaminopropiophenone (**2**). Five g (33 mmoles) of **1** (Eastman) was stirred into a soln of 48% HBF_4 (20 ml; 110 mmoles) in 10 ml of H_2O in an ice-water bath. A soln of $NaNO_2$ (6 g; 90 mmoles) in 10 ml of H_2O was added to the fluoroborate suspension in portions over a 0.5-hr period with constant stirring. The yellow amorphous slurry changed rather sharply to a suspension of yellow needles (a small amount of the crystalline suspension was filtered, washed with Et_2O, and dried: mp 81–84° dec; ir consistent with the structure of the diazonium fluoroborate intermediate). The diazonium fluoroborate suspension was filtered, washed with Et_2O, and added in portions to a soln of $NaNO_2$ (60 g; 900 mmoles) and Cu powder (12 g; 190 mg-atoms) in 250 ml of H_2O at room temperature with vigorous stirring–a small amount of Et_2O was added to retard frothing. The soln was extracted with 60 ml of Et_2O which was evaporated to yield 4.5 g of crude *p*-nitropropiophenone (**3**). A sample of **3** was purified for analysis by elution from a silica gel column with benzene: mp 86–88°. *Anal.* ($C_9H_9NO_3$) C, H, N. Crude **3** was weighed and dissolved in 95% EtOH (60 ml/g). A 1 *M* excess of NH_4Cl and 10 mg/g of H_2O were added followed by a 10 *M* quantity of Zn dust, which was added in portions. The mixture was allowed to react at room temperature with vigorous stirring for 45 min. The soln was filtered and evaporated *in vacuo* to about 0.05 original volume, Et_2O was added, and the ethereal layer was separated and evaporated. Crude **2** was purified on a silica gel column (15 g of silica gel/g); the column was first eluted with EtOAc–$CHCl_3$ (1:9) until the initial dark band came off, then eluted with a 1:4 mixture of EtOAc and $CHCl_3$ which removed the hydroxylamine: mp 93–95° (lit.[2] 96–97°). *Anal.* ($C_9H_{11}NO_2$) C, H, N. Overall yield for both reactions was 25%. The ir of **2** was consistent with the presence of the hydroxylamine function; furthermore, reduction of **2** with Sn and HCl regenerated **1** as indicated by an ir spectrum identical with the starting material.

Biological Activity. Adult, male Sutter mice (pseudomonas free) were obtained weighing 16–18 g. After a 2-week isolation period, those mice between 24 and 30 g were selected (nearly 100%). The mice were housed 8/cage; Purina lab chow and water were supplied *ad libertum*.

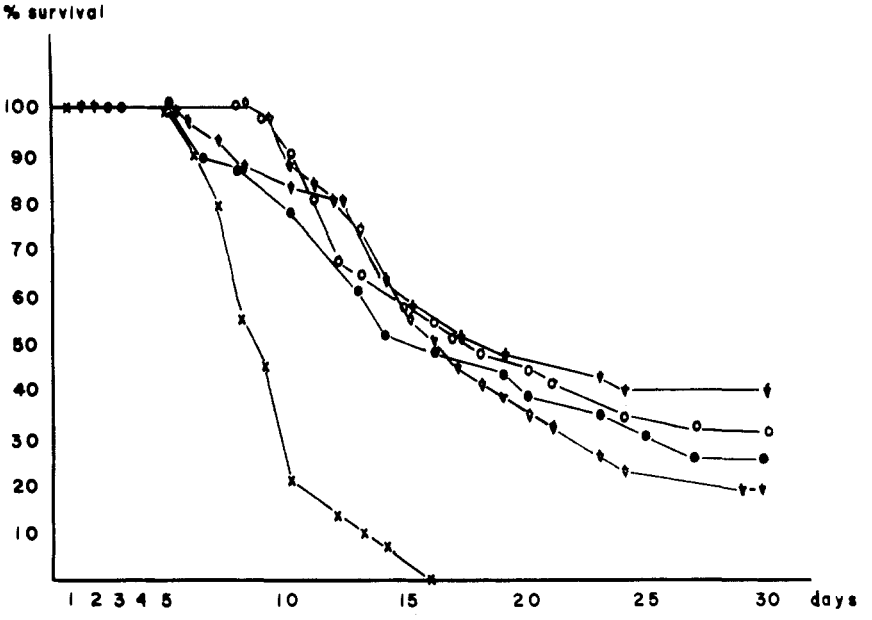

Figure 3. Radiation survival (daily) of Sutter mice after 750-R whole-body X irradiation: x = control (PG); ○ = 33 mg/kg of PHAPP (**2**); ● = 66 mg/kg of PHAPP (**2**); ▽ = 30 mg/kg of PAPP (**1**); ▼ = 60 mg/kg of PAPP (**1**).

Groups of 5 mice were injected ip with increasing doses of 1 and 2 in DMSO (0.1 ml/30-g mouse). After 10 days the LD_{50} was calculated using a maximum likelihood probit analysis method programmed for digital computation.

Groups of 16 mice were injected ip with increasing doses of 1 and 2 in propylene glycol (0.15 ml/30-g mouse). The mice were placed in individual, perforated, plastic centrifuge tubes, positioned on a rotating turntable, and (15 min after injection) exposed to 750 R of whole-body X irradiation (250 DVP, 15 mA, target-skin distance 50 cm, dose rate 45 R/min). Controls were exposed simutaneously with test doses of drugs. Mortality from day 3 to day 30 was tabulated. Propylene glycol was used as drug solvent for radiation studies instead of DMSO since the latter solvent has radioprotective properties of its own.

Relative metHb levels were determined with the Storer and Coon[1] modification of the method of Evelyn and Malloy[9] on a Spectronic 20 colorimeter.

Absolute Hb was determined on a Beckman DU using the Hycel reagent.

Animals were exposed to hyperbaric O_2 in a Bethelehem Corporation Hyperbaric Chamber Model 615 HP.

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Absolute Configuration of (+)- and (−)-*trans*-2-Phenylcyclopropylamine Hydrochloride†

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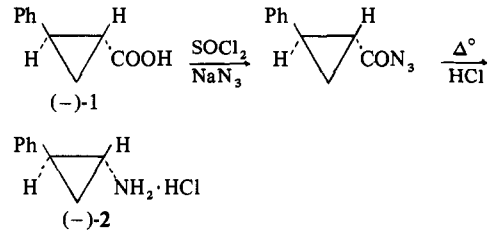
Tranylcypromine·HCl (**2**, *trans*-2-phenylcyclopropylamine·HCl) is a clinically useful agent in the treatment of mental depression and certain phobic-anxiety states. The therapeutic actions of this drug have been ascribed to its ability to inhibit monoamine oxidase. The inhibition of MAO by 2-phenylcyclopropylamine·HCl is characterized by a significant but low order of stereoselectivity with the trans isomer being three times more potent than the cis isomer and (+)-**2** being four times more potent than (−)-**2**.[1]

In view of the utility of the knowledge of absolute configuration in elucidating the nature of drug-biomolecule interactions,[2] we have undertaken the determination of absolute configuration of the tranylcypromine enantiomers.

(−)-Tranylcypromine·HCl was unequivocally shown to possess 1*R*:2*S* stereochemistry by virtue of its synthesis from (−)-1*R*:2*R*-2-phenylcyclopropanecarboxylic acid[3]



(**1**). The synthetic sequence employed is that of Burger and Yost.[4] It is of interest to note that the Curtius rearrangement of the acid azide to the isocyanate involves a transformation affecting the C-1 asymmetric center. However, the retention of the configuration of the migrating group in this rearrangement is well documented.[5]

Experimental Section

Melting points were taken on a Mel-Temp and are uncorrected. Optical rotations were obtained using a Perkin-Elmer 114 polarimeter and a 1-dm cell. Ir (Perkin-Elmer 257) and nmr (Jeolco C-60HL) for all compounds were as expected.

Resolution of *trans*-2-Phenylcyclopropanecarboxylic Acid. A literature procedure[6] for the resolution of racemic **1** involves the use of brucine as a resolving agent. Experiments in our laboratory indicated that (+)-dehydroabietylamine was a superior resolving agent with regard to obtaining (−)-**1** of high optical purity. The following procedure is illustrative of a typical resolution.

A warm soln of 8 g (0.049 mole) of (±)-**1** in 50 ml of MeOH was slowly added to 13.9 g (0.049 mole) of (+)-dehydroabietylamine in 40 ml of warm MeOH. The mixt was allowed to sit at room temp, and the crystals were filtered, mp 159–167°. After three recrystn from aqueous MeOH (10–15%), 5.8 g of (−)-dehydroabietylamine 2-phenylcyclopropanecarboxylate was obtd, mp 174–174.5°; $[\alpha]^{25}D$ −80.2° (*c* 1, MeOH). The free acid was liberated from the salt by treatment with a satd soln of Na_2CO_3, extn with Et_2O, acidification of the aqueous fraction with cold, concd HCl, and filtration of the ppt to yield 1.3 g of (−)-**1** after one recrystn from Me_2CO, mp 47–49°; $[\alpha]^{25}D$ −381.1° (*c* 1, $CHCl_3$) (lit.[6] mp 51–52°; $[\alpha]^{24}D$ −368° (*c* 0.931, $CHCl_3$)).

(−)-1*R*:2*S*-2-Phenylcyclopropylamine·HCl.[4] Thionyl chloride (5.96 g, 0.050 mole) was added dropwise to (−)-**1** (4 g, 0.025 mole) at 0°. The soln was stirred at room temp for 24 hr, and the excess $SOCl_2$ was evapd *in vacuo*. The residue was dissolved in 60 ml of dry Me_2CO, and 10 ml of an aqueous soln of NaN_3 (2.88 g, 0.044 mole) was added dropwise with stirring at 0°. The mixt was stirred for 30 min and extd with toluene. The toluene soln was dried (Na_2SO_4) and dropped into a flask heated on a steam bath. After N_2 evolution had ceased the toluene was evapd *in vacuo*. The residue (isocyanate) was dissolved in 35% HCl, refluxed for 13 hr, concd *in vacuo*, and basified with 50 ml of 8 *N* NaOH. The mixt was extd three times with Et_2O, the ethereal fraction dried (Na_2SO_4) and concd *in vacuo*, and the residue distilled to afford 2.4 g of the amine, bp 68–70° (0.9 mm), $[\alpha]^{25}D$ −115.8° (*c* 1.13, $CHCl_3$). Treatment of the amine with ethereal HCl provided (−)-**2**, mp 176–178°; $[\alpha]^{25}D$ −67.7° (*c* 0.882, H_2O) (lit.[1] mp 180–181°; $[\alpha]^{25}D$ −75.5° (*c* 1, H_2O)).

Results and Discussion

The synthesis of (−)-2-phenylcyclopropylamine·HCl from (1*R*:2*R*)-2-phenylcyclopropanecarboxylic acid, as described, unequivocally establishes the absolute configuration of the enantiomers of tranylcypromine·HCl. Hence the more active MAO-inhibitory enantiomer of this agent possesses the 1*S*:2*R* configuration.

It is of interest to note that the weak MAO inhibitor, amphetamine (**3a**), and the potent inhibitor, 1-phenyl-2-propylhydrazine (**3b**, pheniprazine), possess the basic β-phenethylamine moiety and a chiral center α to the amino group as found in tranylcypromine. Further, as in the case of tranylcypromine, studies have shown that the greater MAO-inhibitory activity of amphetamine[7] and phenipraz-

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