Table IV. New Aminotetralins Prepared for This Study

No.	Formula[a]	$n^{25}D$	Bp, °C (mm)	% yield
13	$C_{13}H_{19}NO_2$[b]	1.5593	183 (1.5)	23
15	$C_{14}H_{21}NO_2$[c]	1.5320	160–161 (1.5)	26
10	$C_{14}H_{21}NO$	1.5301	124 (0.75)	43
19	$C_{14}H_{18}N_2O$	1.547	168–170 (0.4)	27
11	$C_{17}H_{27}NO$	1.5203	161 (1.3)	48
20	$C_{18}H_{29}NO$	1.5182	171–174 (1.2–1.4)	48
4	$C_{13}H_{19}NO$	1.5375	124 (1.2)	64

[a]Analysis for C, H, and N within ±0.4% except where noted. [b]H, N; C: calcd, 70.55; found, 69.99. [c]H; N: calcd, 13.6; found, 14.05.

and 10 except that the reaction was accomplished in an autoclave (to prevent loss of low boiling amines) at 100° for 24 hr. The above Schiff bases were isolated by removal of the solvent and reduced with a 10–20% ratio of Raney nickel to compound in 100–200 ml of ethanol at 3 atm of H_2 until 1 equiv was absorbed.

Compound **19** was prepared from 6-methoxy-1-aminotetralin[1] and acrylonitrile. A 1:2 mixture of the compounds was refluxed neat with Triton B as a catalyst.

In all cases the solvent was removed by evaporation and the products were purified by distillation.

The analytical data for these compounds are summarized in Table IV.

MAO Inhibition *in Vitro.* Inhibition of mouse brain MAO was measured after preincubation of the enzyme with 10^{-4} *M* inhibitor for 10 min. Tryptamine (2×10^{-5} *M*) was used as substrate.[9]

Lack of Oxidation by MAO. The ability of compounds **2**, **3**, and **7** to serve as substrates for MAO was studied by monitoring the production of the ketone which would result from the oxidative deamination. A 1:10 homogenate of mouse liver in cold 0.15 *M* Na phosphate buffer, pH 7.4, was contrifuged at 0°, 2000g, for 10 min. The supernatant suspension (0.1 ml) was added to 2.9 ml of a 1×10^{-4} *M* solution of the amine equilibrated at 38°. The absorbance of the resulting suspension was followed in a Gilford multiple sample absorbance changer at 38° for 10 min. The wavelength was that of the potential ketone product. To assure activity in the MAO preparation the oxidation of benzylamine was assayed at 250 nm. All determinations were in triplicate.

MAO Inhibition *in Vivo.* The potentiation of the response to Dopa was used as an *in vivo* measure of the inhibition of MAO.[10] The drug was administered po as a suspension in carboxymethylcellulose to a group of four mice at a dose of 100 mg/kg. Four hr later 200 mg/kg of *dl*-Dopa was injected ip. The mice were observed for 1 hr; the severity of symptoms was scored 0 to 3+ according to the established criteria.[10]

Physical Properties. For hydrophobic properties the Π value (change in octanol-water partition coefficient) was calculated as the sum of Π for the substituent on the nitrogen plus Π for the nonaromatic carbon atoms of the ring. The Π values were taken from the compilation of Leo, *et al.*[11] For steric properties the Taft E_s values[12] corrected for hyperconjugation[13] were used.

Acknowledgment. The *in vitro* studies of monoamine oxidase inhibition were performed by Dr. Elizabeth B. Chappell.

References

(1) Y. C. Martin, C. H. Jarboe, R. A. Krause, K. R. Lynn, D. Dunnigan, and J. B. Holland, *J. Med. Chem.*, **16**, 147 (1973).
(2) (a) D. Morrison, "Multivariate Statistical Methods," McGraw-Hill, New York, N. Y., 1967, pp 130–133; (b) D. Morrison, *J. Marketing Res.*, **6**, 156 (1969).
(3) J. Service, "A User's Guide to the Statistical Analysis System (SAS)," Student Supply Stores, North Carolina State University, Raleigh, N. C., 1972, pp 190–195.
(4) R. E. Frank, W. F. Massy, and D. G. Morrison, *J. Marketing Res.*, **2**, 250 (1965).
(5) C. M. McEwen, Jr., G. Sasaki, and D. C. Jones, *Biochemistry*, **8**, 3952 (1969).
(6) E. A. Zeller, *Ann. N. Y. Acad. Sci.*, **107**, 811 (1963).
(7) C. F. Huebner, E. M. Donoghue, A. J. Plummer, and P. A. Furness, *J. Med. Chem.*, **9**, 830 (1966).
(8) W. J. Dixon, Ed., "BMD Biomedical Computer Programs," University of California Press, Berkeley, Calif., 1971, p 214a.
(9) R. J. Wurtman and J. Axelrod, *Biochem. Pharmacol.*, **12**, 1439 (1963).
(10) G. M. Everett and R. G. Wiegand, *Proc. Int. Pharmacol. Meet., 1st*, **8**, 85 (1962).
(11) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).
(12) R. W. Taft, Jr., in "Steric Effects in Organic Chemistry," M. S. Newman, Ed., Wiley, New York, N. Y., 1956, p 598.
(13) C. K. Hancock, E. A. Meyers, and B. J. Yager, *J. Amer. Chem. Soc.*, **83**, 4211 (1961).

Mouse Liver Glyoxalase I Inhibition by S-Substituted Glutathiones†

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A series of *S*-(ω-phthalimidoalkyl)glutathiones and of *S*-(ω-aminoalkyl)glutathiones has been prepared. Both of the series of compounds exhibit inhibitory activity toward the glyoxalase I of mouse liver. In general, inhibition increases with increasing length of the alkyl chain separating the phthalimido group or the amino group from the glutathione residue. The effect of chain length is quite pronounced with the aminoalkylglutathione series; an approximate 40-fold increase in inhibition activity results as the alkyl chain is lengthened from four to ten carbon atoms.

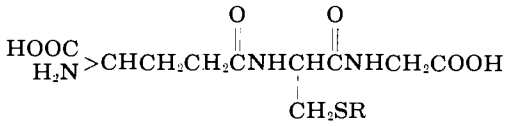
Previous studies have shown that *S*-alkyl- and *S*-arylglutathiones competitively inhibit glyoxalase I of the glyoxalase system of yeast[1,2] and that they are cytotoxic to mammalian cells in cell culture.[3] In the latter case cytotoxicity is substantially decreased by preincubating noninhibitory concentrations of methylglyoxal (pyruvaldehyde) with the cell cultures. Methylglyoxal has been shown to be carcinostatic as well as generally cytotoxic.[4,5] A consequence of the later observation has been the proposal that the widely distributed glyoxalase system is involved in cell growth regulation by controlling cellular levels of methylglyoxal.[1] Glutathione (GSH), implicated in the cell division process,[6] is a required cofactor for the enzyme system. The two-enzyme glyoxalase system catalyzes the following reaction.

$$CH_3COCHO + GSH \xrightarrow{\text{glyoxalase I}} CH_3CHOHCO{-}SG \xrightarrow{\text{glyoxalase II}} CH_3CHOHCOOH + GSH$$

Inhibitors of glyoxalase I activity have been proposed to elicit an endogenous buildup of methylglyoxal and thus give rise to a cytotoxic condition.[1]

†This investigation was supported by The Robert A. Welch Foundation of Texas, Grant No. B-133, and by Faculty Research of North Texas State University, Grant No. 35233.

Table I. Inhibition of Mouse Liver Glyoxalase I by S-Phthalimidoalkyl- and S-Aminoalkylglutathiones[a]



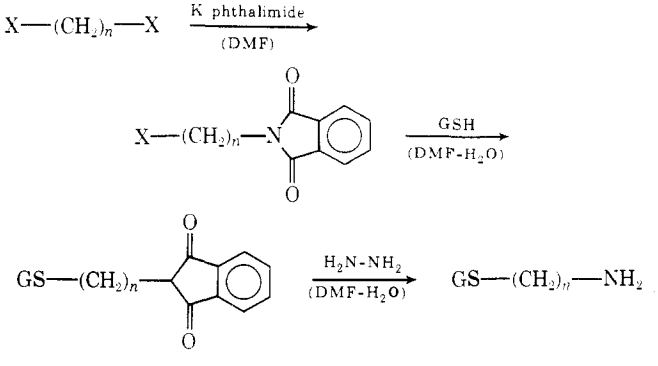
Compd no.	R	Concn giving 50% inhibn, m*M*
1	Phth-N-$(CH_2)_4$	0.13
2	Phth-N-$(CH_2)_6$	0.13
3	Phth-N-$(CH_2)_8$	0.06
4	Phth-N-$(CH_2)_{10}$	0.07
5	H_2N-$(CH_2)_4$	21
6	H_2N-$(CH_2)_6$	4.8
7	H_2N-$(CH_2)_8$	1.2
8	H_2N-$(CH_2)_{10}$	0.55

[a]Experimental details are given under routine enzyme assays in the Experimental Section.

Recent studies in this laboratory have been concerned with the glyoxalase system of enzymes from mouse and rat livers. The studies have included investigations of the effects of animal age, of tumors, and of the age of the tumors on the activities of this enzyme system.[7-9] The glyoxalase I activities from the livers of normal mice and from mice bearing a lymphosarcoma have been partially purified in these studies, and apparent differences were noted in the enzymes isolated from the two sources.[8] We have determined to purify this enzyme further from normal liver and from "abnormal" liver sources to ascertain the nature and hopefully the etiology of the apparent differences.

The purposes of this investigation were twofold. First, new and possibly cancerostatic inhibitors of glyoxalase I were to be prepared and studied *in vitro*. Second, the new inhibitors were to have use as ligands for the affinity chromatography purification of glyoxalase I from mammalian liver.[10] Thus, the inhibitors of choice were a series of *S*-(ω-aminoalkyl)glutathiones—compounds which, if having inhibitory properties, could readily be coupled to appropriate supports for affinity purification studies. Reported herein are the synthesis and preliminary enzymatic study of four *S*-(ω-phthalimidoalkyl)glutathiones and four *S*-(ω-aminoalkyl)glutathiones.

Chemistry. The preparation of the *S*-(ω-aminoalkyl)-glutathiones made use of the Gabriel synthesis and was accomplished according to the following scheme.



Although the synthesis of the *N*-(ω-haloalkyl)phthalimides prepared in this study has been described elsewhere,[11-14] the experimental methods vary greatly and conflicting physical data are sometimes presented. For these reasons the general method employed for preparing these compounds in the present investigation has been included. Both the dibromo- and the diiodoalkanes were used in the preparations (see the legend of Table II).

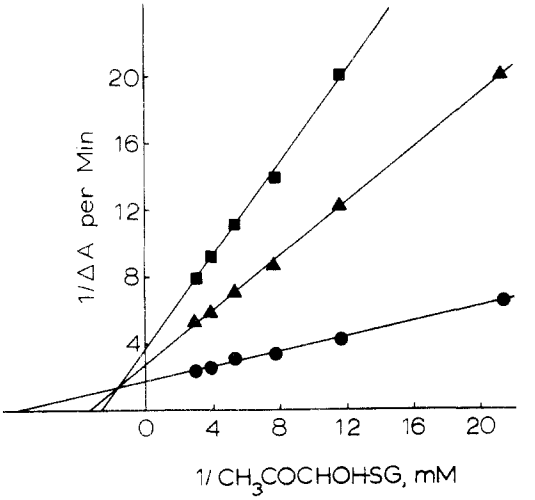


Figure 1. Double reciprocal plot showing inhibition by *S*-aminodecylglutathione of partially purified mouse liver glyoxalase I activity (300× purification).[8] Substrate concentrations, [CH_3COCHOH-SG], were calculated from the dissociation expression, K = [CH_3COCHO][GSH]/[CH_3COCHOH-SG], assuming K = 3.1.[2] The ratio, [CH_3COCHO]/[GSH], was maintained at 5.75 for all calculated substrate concentrations. *S*-Lactoylglutathione production was measured spectrophotometrically at 240 n*M* as described under routine enzyme assays in the Experimental Section: ● - - - ●, no aminodecylglutathione; ▲ - - - ▲, 0.5 m*M* aminodecylglutathione; ■ - - - - ■, 1.0 m*M* aminodecylglutathione.

For the synthesis of the *S*-(ω-phthalimidoalkyl)glutathiones, the methods described by Vince, *et al.*,[2] and by Kermack and Matheson[15] (in which the solvent systems were aqueous ethanol and liquid ammonia, respectively) were attempted; however, the reactant solubilities were poor and the yields were quite low with both methods. The dimethylformamide (DMF)-H_2O solvent ultimately employed proved much superior and no major solubility difficulties with the reactants were encountered. Considerable difficulty was initially encountered in obtaining clean hydrazinolyses of the phthalimidoalkylglutathione intermediates by following published procedures.[16,17] The reaction products were invariably contaminated with starting material and/or other undesired products as evidenced by paper chromatography. Successful removal of the phthalic acid group as the phthalhydrazide was finally achieved by the simple expedient of using DMF-H_2O as solvent under the conditions described in the Experimental Section. All four of the aminoalkylglutathiones prepared readily take up water, and only minimal amounts of water should be used in the recrystallization solvent if pure crystalline products are to be obtained.

Biochemistry. It has been reported previously[2] that glyoxalase I exhibits an apparent hydrophobic area in the region of its active center which may be occupied by alkyl and aryl groups substituted by thioether linkage to GSH. Further, it has been shown that there is a general correlation between the extent of glyoxalase I inhibition and the length of the substituent alkyl group and/or the nonpolar character of the S-substituted groups. Specifically, the longer the *S*-alkyl chain or the more nonpolar the *S*-aralkyl substituent, the greater is the probability of increased inhibition—presumably through tighter binding of the inhibitor with the active region of the enzyme. Similar correlations of inhibitory activity of the compounds prepared in this investigation have been found. For example, as shown in Table I, there is a significant increase in inhibi-

Table II. S-Substituted Glutathiones, GSR

No.	R	Mp, °C	Yield, %	Formula
1	Phthalimidobutyl[a]	213 dec	77	$C_{22}H_{28}N_4O_8S$
2	Phthalimidohexyl[a]	205.5–207 dec	68	$C_{24}H_{32}N_4O_8S$
3	Phthalimidooctyl[b]	190–192 dec	83	$C_{26}H_{36}N_4O_8S$
4	Phthalimidodecyl[a]	199–199.5 dec	83	$C_{28}H_{40}N_4O_8S$
5	Aminobutyl	200 dec	55	$C_{14}H_{26}N_4O_6S$
6	Aminohexyl	187.5–189 dec	49	$C_{16}H_{30}N_4O_6S \cdot 0.5H_2O$
7	Aminooctyl	203–204.5 dec	40	$C_{18}H_{34}N_4O_6S \cdot 0.5H_2O$[c]
8	Aminodecyl	197–198 dec	75	$C_{20}H_{38}N_4O_6S \cdot 0.5H_2O$[d]

[a]The *N*-(ω-iodoalkyl)phthalimides were employed in the synthesis of the corresponding *S*-(ω-phthalimidoalkyl)glutathiones. The syntheses of these iodo compounds are given a general description in the Experimental Section. [b]*N*-(8-Bromooctyl)phthalimide was synthesized from 1,8-dibromooctane (see the Experimental Section) and was employed in the synthesis of *S*-(8-phthalimidooctyl)glutathione. [c]H: calcd, 7.95; found, 7.52. [d]H: calcd, 8.34; found, 7.91.

tion of glyoxalase I as the length of the alkyl chain increases. Further, the relatively nonpolar phthalimido groups at the ends of the alkyl chains (compounds 1–4) impart significantly greater inhibitory activities than are given by the polar amino groups at the chain termini (compounds 5–8). It is also obvious that the further the polar amino groups are removed from the GSH residue, the less is their negative influence on inhibitory quality. Thus, the concentration of compound 8 (which has the *S*-aminodecyl chain) necessary for 50% inhibition of glyoxalase I is almost 40-fold lower than the concentration necessary for like inhibition by compound 5 (which has the *S*-aminobutyl chain). On the other hand, the length of the alkyl chain separating the glutathione residue from the bulky, relatively nonpolar phthalimido group has little influence on inhibitory quality (compounds 1–4).

The nature of the glyoxalase I inhibition by *S*-aminodecylglutathione (compound 8) was studied in some detail using partially purified mouse liver glyoxalase I (300× purification).[8] A double reciprocal plot of the kinetic data (Figure 1) indicates mixed type inhibition[18] (the extrapolated lines intersect at a point to the left of the ordinate and above the abscissa). There are many possible interpretations of the inhibitory processes whereby both K_m and V are altered by the inhibitor; however, until more information is available concerning the kinetic mechanism of uninhibited glyoxalase I, the analysis of the behavior of glyoxalase I inhibitors will remain complex.[18,19] The graphical determination of K_i by the method of Dixon[20] gives a value of 0.17 m*M* for *S*-aminodecylglutathione.

It is apparent that compound 7 or compound 8 should serve most effectively as an immobilized ligand for affinity chromatography applications.[21] Indeed, in preliminary studies employing compounds 5–8 immobilized on cyanogen bromide-activated Sepharose, both compounds 7 and 8 have proved highly effective as affinity chromatography ligands for the purification of glyoxalase I from mouse liver. Details of the purification of this enzyme by affinity chromatography will be presented elsewhere. Cytotoxicity studies of mammalian cells in cell culture are in progress with compounds 1–8.

Experimental Section

General. A Thomas-Hoover capillary melting point apparatus was employed for all melting point determinations, and the melting points reported are uncorrected. The enzymatic analyses were conducted on a Beckman DBG recording spectrophotometer. The dihaloalkanes were purchased from Aldrich Chemical Co. Analytical data for C, H, and N were obtained for all compounds listed in Table II and were within ±0.4% of the calculated values, except where indicated.

Routine Enzyme Assays. DBA/1J mice, 6–10 weeks old, were used as a source of glyoxalase I in this investigation. The mouse livers were routinely homogenized at 0° for 30 sec with a Virtis homogenizer in 2 vol of 0.01 *M* potassium phosphate, pH 7.5. The supernatant fraction obtained from a 100,000g centrifugation of the homogenate was used in the enzyme assays.

Glyoxalase I activity was monitored by a variation of the procedure of Racker.[22] The reaction mixture contained the following components: reduced glutathione, 1.07 m*M*; methyl glyoxal, 1.07 m*M*; imidazole–HCl buffer, pH 6.8, 100 m*M*; and $MgSO_4$, 16 m*M*. The reaction mixture was allowed to stand 10 min at room temperature to ensure equilibration. The hemimercaptal concentration ($CH_3COCHOH$-SG) at equilibrium was calculated to be 0.23 m*M*, which is equivalent to the K_m value for glyoxalase I from mouse liver.[8] The addition of the enzyme preparation to the quartz cell, giving a final volume of 3.0 ml, initiated the enzymatic reaction. A reference cell contained all reaction mixture components with the exception of the enzyme preparation. The enzymatic production of *S*-lactoylglutathione was followed at 240 nm for 2 min at 25° on a double beam recording spectrophotometer. The initial rate of the reaction was determined by the slope of the linear portion of the plot. When studying the inhibitor effects, varying amounts of the inhibitors were added to the equilibrated reaction mixture prior to the addition of the enzyme preparation. The concentration of the inhibitor required for 50% inhibition was determined from plots of velocity *vs.* inhibitor concentration.

Organic Syntheses. The methods of preparation of the compounds listed in Table II are given by the general procedures below. The haloalkylphthalimides have been prepared by a variety of procedures;[11-14] the general method employed in this study is also given.

***N*-(ω-Haloalkyl)phthalimides.** To approximately 80 ml of DMF was added 0.07 mol of the α,ω-dihaloalkane. Solid potassium phthalimide (0.035 mol) was then added, and the suspension was stirred at room temperature overnight. The reaction mixture was then concentrated to low volume *in vacuo*. Water was then added, and the resulting solution was extracted twice with ethyl acetate. The ethyl acetate extract was dried over sodium sulfate and concentrated to low volume *in vacuo*. Hexane was then added to the cloud point, and the solution was swirled in a Dry Ice–acetone bath. The white solid material which crystallized was filtered and dried. In most cases this material was suitable for use in further synthetic work;‡ sometimes, however, it was contaminated with the dicondensation product. This contaminant could be removed by briefly swirling the crystals in Et_2O and removing the less soluble contaminant by filtration.

***S*-(ω-Phthalimidoalkyl)glutathiones.** Glutathione (0.01–0.02 mol was employed in these reactions) was dissolved in a volume of 1.0 *N* NaOH to give 2 equiv of OH^- for each mole of glutathione. To the resulting solution was then added 2 vol of DMF. DMF solution (1 vol) of the appropriate haloalkylphthalimide (an amount equal to the number of moles of glutathione) was added dropwise to the stirring solution of sodium glutathionate. If the addition process was sufficiently slow, little precipitation occurred. The solution was then allowed to stir overnight. In some instances the solid reaction product precipitated from solution during this time period. The pH of the solution or slurry was then adjusted to 3.0–3.5 with concentrated HI, and the crystalline solid was collected by filtration, washed with cold water, and dried in a heated vacuum desiccator. The preparation was usually sufficiently pure for use in the next synthetic step. Only one ninhydrin-positive spot was observed for each compound after paper chromatography in 1-butanol–formic acid–water (100:30:25). Ana-

‡The melting points of the *N*-(ω-haloalkyl)phthalimides agreed closely with those reported in ref 11–14.

lytical samples of the phthalimidobutyl- and the phthalimidohexylglutathiones were prepared by recrystallization from ethanol–water. The phthalimidooctyl- and phthalimidodecylglutathiones were recrystallized from DMF–water.

S-(ω-Aminoalkyl)glutathiones. Hydrazinolysis of the phthalimido group was effected by the addition of 0.02 mol of 95% hydrazine to 0.01 mol of the phthalimido-substituted compound dissolved in 50 ml of DMF–water (50:50). The resulting solution was allowed to stir for 2 days at room temperature, by which time the solution had become quite turbid. After neutralization with concentrated HI the suspension was chilled, and the phthalhydrazide was removed by high-speed centrifugation. The supernatant portion was concentrated *in vacuo* to a low volume, and an additional volume of water was added. After chilling, more phthalhydrazide precipitated and was removed by filtration. The filtrate was then concentrated to very low volume, and the final product was crystallized from solution by the addition of ethanol. The hygroscopic solid was removed by filtration, washed with ethanol, and dried in a heated vacuum desiccator in the presence of phosphorus pentoxide. The final products gave a single ninhydrin spot in the solvent system above. Analytical samples were prepared by first dissolving the products in a minimum volume of hot water and then effecting crystallization by the addition of ethanol.

References

(1) R. Vince and W. B. Wadd, *Biochem. Biophys. Res. Commun.*, **35**, 593 (1969).
(2) R. Vince, S. Daluge, and W. B. Wadd, *J. Med. Chem.*, **14**, 402 (1971).
(3) R. Vince and S. Daluge, *J. Med. Chem.*, **14**, 35 (1971).
(4) F. E. Knock, *Lancet*, **1**, 824 (1966).
(5) M. A. Apple and D. M. Greenberg, *Cancer Chemother. Rep.*, **51**, 455 (1967).
(6) H. Stern, *Science*, **124**, 1292 (1956).
(7) R. A. Strzinek, G. R. Vela, V. E. Scholes, and S. J. Norton, *Cancer Res.*, **30**, 334 (1970).
(8) R. A. Strzinek, V. E. Scholes, and S. J. Norton, *Cancer Res.*, **32**, 2359 (1972).
(9) R. A. Strzinek and S. J. Norton, *Tex. J. Sci.*, **24**, 3 (1972).
(10) P. Cuatrecasas, *Annu. Rev. Biochem.*, **40**, 259 (1971).
(11) H. R. V. Arnstein, G. D. Hunter, H. M. Muir, and A. Neuberger, *J. Chem. Soc.*, 1329 (1952).
(12) H. B. Donahoe, R. J. Seiwald, Sr., M. M. Neumann, and D. E. Heitmeier, *J. Med. Chem.*, **12**, 906 (1969).
(13) W. Dirsherl and F. W. Weingarten, *Justus Liebigs Ann. Chem.*, **574**, 131 (1951).
(14) W. C. Austin, M. D. Potter, and E. P. Taylor, *J. Chem. Soc.*, 1489 (1958).
(15) W. O. Kermack and N. A. Matheson, *Biochem. J.*, **65**, 48 (1957).
(16) J. C. Sheehan and V. S. Frank, *J. Amer. Chem. Soc.*, **71**, 1856 (1949).
(17) F. E. King and D. A. A. Kidd, *J. Chem. Soc.*, 3315 (1949).
(18) M. Dixon and E. C. Webb, "*Enzymes*," 2nd ed, Academic Press, New York, N. Y., 1964, p 324.
(19) T. Bartfai, K. Ekwall, and B. Mannervik, *Biochemistry*, **12**, 387 (1973).
(20) M. Dixon, *Biochem. J.*, **55**, 170 (1953).
(21) P. Cuatrecasas and C. B. Anfinsen, *Methods Enzymol.*, **22**, 345 (1971).
(22) E. Racker, *J. Biol. Chem.*, **190**, 685 (1951).

Action of (*S*)- and (*R*)-Para-Substituted Amphetamine Hydrochlorides and (α*S*)- and (α*R*)-*p*-Chlorononephedrine and (α*S*)- and (α*R*)-*p*-Chlorononorpseudoephedrine Hydrochlorides on the Level of 5-Hydroxytryptamine and the Activity of Tryptophan Hydroxylase in Rat Brain†,‡

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Received August 15, 1973

Both (*S*)-(+)- and (*R*)-(−)-*p*-chloroamphetamine hydrochloride cause a reduction in the level of 5-hydroxytryptamine (serotonin, 5-HT) and the activity of tryptophan hydroxylase in rat brain. The *R* isomer induces a more rapid decline in 5-HT which is of shorter duration. The effect of this isomer on tryptophan hydroxylase activity disappeared 2 weeks after injection, but the effect of the *S* isomer persisted. These results indicate that different mechanisms are involved in the short- and long-term effects. (*S*)-(+)- and (*R*)-(−)-*p*-nitroamphetamine hydrochloride both reduce 5-HT levels and tryptophan hydroxylase activity 4 hr after injection, with some recovery after 2 weeks. Since (α*S*)-(−)- and (α*R*)-(+)-*p*-chlorononephedrine hydrochloride and (α*S*)-(+)- and (α*R*)-(−)-*p*-chlorononorpseudoephedrine hydrochloride do not have a significant effect on the level of 5-HT or the activity of tryptophan hydroxylase, β-hydroxylation is not an important factor in either the short- or long-term effects of *p*-chloroamphetamine hydrochloride on serotonergic neurons.

The administration of racemic *p*-chloroamphetamine hydrochloride [(±)-**1a**] to rats leads to a marked and long-lasting reduction of the levels of 5-hydroxytryptamine (serotonin, 5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) and the activity of tryptophan hydroxylase in the brain.[2] Although some recovery is observed, all three components are still significantly reduced 4 months after a single injection of (±)-**1a**. Tryptophan hydroxylase activity *in vitro*, however, was unaffected by addition of the racemic drug.[3]

Since it is known that enantiomers of a drug can produce different pharmacological effects,[4] we have now compared the effect of (*S*)-(+)- and (*R*)-(−)-*p*-chloroamphetamine hydrochloride§[6] [(*S*)-**1a** and (*R*)-**1a**] on the

†This is paper XV in the Department of Chemistry, Vanderbilt University series, Optically Active Amines. For paper XIV see ref 1.

‡Taken in part from the M.S. Thesis of C. D. M., Tennessee State University, May 1973.