In Vitro Inhibition Studies with Homogeneous Monoamine Oxidases

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The inhibition of the deamination of five substrates by highly purified beef liver mitochondrial monoamine oxidase was measured using several known MAO inhibitors. Inhibition values for the deamination of benzylamine by pure beef plasma MAO were also determined. The relative potencies of the inhibitors varied with the substrates used and did not always follow literature data obtained with less homogeneous MAO preparations. They did not run parallel for the two enzymes used. Harmine and harmaline were much less potent as inhibitors of the purified enzymes than observed previously with tissue homogenates.

The often conflicting mechanistic and quantitative literature data on inhibitors of monoamine oxidase (MAO) have been reviewed ably and critically by Zirkle and Kaiser and by Biel, *et al.*, in a recent monograph.[2] The difficulties of obtaining reasonably matching inhibition values encountered in various studies are illustrated for harmine, tranylcypromine (I), and N-benzyl-N-methyl-N-propargylamine (III) in Table I. The nature of the inhibition, the need for preincubation with the inhibitor, the presence of oxygen during preincubation, and especially the source of the enzyme, its state of purity, and the substrate to be deaminated all determine the relative potency of the inhibitor. In turn, the nature of the inhibition (competitive *vs.* noncompetitive, reversible *vs.* nonreversible) depends on the substrate,[3] on a combination of this factor with source and purity of the enzyme,[4-6] and on experimental conditions.[7]

As a result of diverse procedures, potency values varying from each other by factors as high as 1400 have been reported.[2a] The enzyme preparations used in all but one[5] of these studies were crude tissue homogenates, or, at best, solubilized mitochondria.[7] This, and species specificity, may have accounted for much of the observed discrepancies. These differences should be minimized by use of homogeneous enzymes from the same species. The purification of mitochondrial MAO, the enzyme which is responsible for the deamination of important natural neuromodulatory substrates, has been attempted several times.[8] A 20-fold concentration of mitochondrial MAO from beef liver has been described,[5] but this preparation was insoluble and could be sedimented easily. Indeed, the pH activity curves of this preparation alone and of the enzyme plus iproniazid were parallel, but with another inhibitor (I) a second maximum appeared at pH 7.0, suggesting to Barbato and Abood the presence of two monoamine oxidases.[5] A recent short announcement[8] of MAO prepared by sonication of rat liver mitochondria and by chromatography to an over-all 350-fold purification has not been followed up further.

We had available MAO from beef liver mitochondria which had been purified highly by a new fractionation procedure by Nara and Yasunobu.[9] The crude enzyme preparation was extracted with Triton X-100 and fractionated twice with ammonium sulfate. This was followed by absorption on alumina C_γ and then by DEAE-cellulose column chromatography to an approximately over-all 50-fold concentration. The resulting enzyme had a specific activity of more than 3000.

It appeared interesting to use this enzyme in a study of the inhibitory effect of several 2-phenylcyclopropylamines[10] and some other structurally different MAO inhibitors on the deamination of several natural substrates: dopamine, norepinephrine, tyramine, tryptamine, 5-hydroxytryptamine (5-HT), as well as benzylamine. It was hoped that this study would complement previous measurements with less pure catalysts and with a narrower spectrum of substrates. In addition, significant differences in inhibition values may indicate different mechanisms by which the inhibitors block the deamination of various substrates in their natural surroundings.

A relative lack of stereospecificity of inhibitors of MAO has been observed by several investigators. We had at hand the (±)-, (+)-, and (−)-*trans* and (±)-*cis* isomers of 2-phenylcyclopropylamine and felt that a further examination of the inhibitory potencies of these stereoisomers,[11] using homogeneous enzymes and the substrates listed above, would illustrate more clearly the unspecificity of these isomers at the *same* active enzyme site.

Beef plasma MAO has been obtained pure.[12] Since this enzyme does not dehydrogenate amines of the phenethylamine and indolylethylamine type, we tested the effect of the same series of inhibitors on the deamination of benzylamine by this enzyme. Interest in this enzyme was augmented by the observation of Yamada,

(1) (a) On leave of absence from the University of Virginia, Charlottesville, Va., where inquiries should be sent. (b) Supported by a Special Fellowship from the National Institute of General Medical Sciences, 1-F3-GM-24,405-01, National Institutes of Health, U. S. Public Health Service. (c) On leave of absence from Hokkaido Gakugei University, Hakodate, Japan. (d) Supported by a grant from the National Institute of General Medical Sciences, GM-06733-05, National Institutes of Health, U. S. Public Health Service.

(2) (a) C. L. Zirkle and C. Kaiser in "Psychopharmacological Agents," Vol. I, M. Gordon, Ed., Academic Press Inc., New York, N. Y., 1964, pp. 445–554; (b) J. H. Biel, A. Horita, and A. E. Drucker, *ibid.*, pp. 359–443.

(3) R. F. Long, *Biochem. J.*, **82**, 3P (1962).

(4) A. R. Maass and M. J. Nimmo, *Nature*, **184**, 547 (1959).

(5) L. M. Barbato and L. G. Abood, *Biochim. Biophys. Acta*, **67**, 531 (1963).

(6) B. Belleau and J. Moran, *J. Med. Pharm. Chem.*, **5**, 215 (1962).

(7) E. A. Zeller and S. Sarkar, *J. Biol. Chem.*, **237**, 2333 (1962).

(8) S. R. Guha and C. R. Krishna Murti, *Biochem. Biophys. Res. Commun.*, **18**, 350 (1965), and references contained therein.

(9) S. Nara and K. T. Yasunobu, *Biochem. Biophys. Res. Commun.*, to be published

(10) C. Kaiser, B. M. Lester, C. L. Zirkle, A. Burger, C. S. Davis, T. J. Delia, and L. Zirngibl, *J. Med. Pharm. Chem.*, **5**, 1243 (1962).

(11) For a summary, see ref. 2a, pp. 523–524.

(12) H. Yamada and K. T. Yasunobu, *J. Biol. Chem.*, **237**, 1511 (1962).

TABLE I

In Vitro INHIBITORY ACTIVITY OF FOUR STRUCTURALLY DIVERGENT COMPOUNDS ON THE DEAMINATION OF TYRAMINE AND 5-HYDROXYTRYPTAMINE BY CRUDE MAO PREPARATIONS

Inhibitor	Nature of inhib.	$[I]_{50}$, M	Substrate	Relative potency (iproniazid = 1)	Source of MAO	Ref.
Harmine	Competitive, reversible	1.2×10^{-7}	5-HT	7250	Rat brain	*a*
		Ca. 10^{-6}	5-HT	300	Rat liver	*b*
		10^{-6}	5-HT	7	Rat liver	*c*
		Ca. 2×10^{-6}	5-HT	500	Rat liver	*d*
(±)-*trans* I	Competitive, ? reversible	9.7×10^{-7}	5-HT	900	Rat brain	*e*
		6×10^{-7}	5-HT	67	Mouse brain	*f*
		3.6×10^{-7}	Tyramine	44	Rat brain	*g*
		10^{-7}	Tyramine	100	Beef liver	*h*
		3×10^{-6}	Tyramine	5	Mouse liver	*h*
		3×10^{-6}	Tyramine	5	Rabbit liver	*h*
		2.8×10^{-6}	5-HT	250	Rat brain	*i*
		10^{-5}–10^{-6}	5-HT	100–1000	Rat liver	*d*
		10^{-5}–10^{-6}	5-HT	100	Guinea pig liver	*j*
III	Competitive	9×10^{-7}	5-HT	8	Rat liver	*c*
		7.3×10^{-6}	5-HT	120	Rat brain	*k*
		2.3×10^{-6}	5-HT	17	Mouse brain	*f*
IV		2×10^{-6}	5-HT	20 (reserpine reversal in mice *in vivo*)	Guinea pig liver	*l*

[a] Ref. 19. [b] S. Udenfriend, B. Witkop, B. G. Redfield, and H. Weissbach, *Biochem. Pharmacol.*, **1**, 160 (1958). [c] J. D. Taylor, A. A. Wykes, Y. C. Gladish, and W. B. Martin, *Nature*, **187**, 941 (1960). [d] M. Ozaki, H. Weissbach, A. Ozaki, B. Witkop, and S. Udenfriend, *J. Med. Pharm. Chem.*, **2**, 591 (1960). [e] H. Green and R. W. Erickson, *J. Pharmacol. Exptl. Therap.*, **129**, 237 (1960). [f] K. Stock and E. Westermann, *Arch. Exptl. Pathol. Pharmakol.*, **243**, 44 (1962). [g] D. R. Maxwell, W. R. Gray, and E. M. Taylor, *Brit. J. Pharmacol.*, **17**, 310 (1961). [h] Ref. 7. [i] Ref. 4. [j] P. A. Shore and V. H. Cohn, Jr., *Biochem. Pharmacol.*, **5**, 91 (1960). [k] H. Green, unpublished results. [l] W. Schuler and E. Wyss, *Arch. intern. pharmacodyn.*, **128**, 431, 439 (1960).

et al.,[13] that the crystalline MAO from *Aspergillus niger* does not only oxidize the typical aliphatic amines affected by serum MAO, but also tyramine, tryptamine, 5-HT, mescaline, phenethylamine, and norepinephrine. The fungal enzyme, moreover, contains Cu^{2+} and resembles beef plasma MAO in other respects.

Experimental Section

Materials.—Substrates, listed at final concentrations used in the experiments, included tyramine hydrochloride, 5×10^{-3} M; dopamine hydrochloride, 10^{-2} M; tryptamine hydrochloride, 2×10^{-3} M; 5-hydroxytryptamine hydrogen oxalate,[14] 2×10^{-3} M; (±)-norepinephrine hydrochloride, 10^{-3} M; and benzylamine sulfate, 1.6×10^{-3} M. The inhibitors studied were (±)-*trans*-, (+)-*trans*-, (−)-*trans*-, and (±)-*cis*-2-phenylcyclopropylamine (as hydrochlorides) (2-phenylcyclopropylamine = I), (±)-*trans*-N,N-dimethyl-N-(2-phenylcyclopropyl)amine (II) hydrochloride, N-benzyl-N-methyl-N-(2-propynyl)amine (III) hydrochloride, α-methylphenethylhydrazine (IV) hydrochloride, 7-methoxy-1-methyl-9H-pyrido[3,4-*b*]indole (harmine) hydrochloride, 4,9-dihydro-7-methoxy-1-methyl-3H-pyrido[3,4-*b*]indole (harmaline), and 4,9-dihydro-7-hydroxy-1-methyl-3H-pyrido-[3,4-*b*]indole (harmalol).[15]

Substrate Specificity.—The relative substrate specificity of highly purified beef liver mitochondrial MAO was determined by the method of Conway.[16] All substrates used were 5×10^{-3} M. The results are shown in Table II.

TABLE II

SUBSTRATE SPECIFICITY OF BEEF LIVER MITOCHONDRIAL MAO

Substrate	Relative rate of deamination
Tyramine	100
Benzylamine	73
Dopamine	60
Tryptamine	53
5-Hydroxytryptamine	46
Norepinephrine	17

Methods.—Enzyme activity was measured by the method of Tabor, *et al.*,[17] using benzylamine as the substrate. Protein concentration was determined colorimetrically by the biuret reaction measuring absorbancy at 540 mμ. The potencies of the inhibitors were assayed spectroscopically[17] on the deamination of benzylamine, but for all other substrates a simplified *in vitro* volumetric method was evolved.

Two-chamber porcelain Conway dishes were charged with 1.00 ml. of 2% boric acid, to which 1 drop of brom cresol green indicator was added. In the outer chamber was placed an amount of 0.1 M potassium phosphate buffer (pH 7.4), chosen to make a total of 1.00 ml. after addition of enzyme, inhibitor, and substrate. Enzyme (0.05–0.3 ml., depending on its activity and on the substrate) and inhibitor (0.1 ml.) were added, and the mixture was allowed to stand under cover for 15 min. Then 0.1 ml. of substrate solution was admixed, and the dish was covered with a polished glass plate fastened with High Vacuum Silicon vacuum grease. After 30 min. at 20–24°, a saturated K_2CO_3 solution (1 ml.) was added rapidly, and the tightly covered dish was placed in an oven at 33° for 1 hr. The borate solution was titrated with 7.15×10^{-3} N H_2SO_4. Each determination was run in duplicate. A blank of enzyme and buffer alone, and a control of enzyme, buffer, and substrate were run similarly each day. Inhibitor concentrations were chosen to bracket 50% inhibition values, and $[I]_{50}$ was extrapolated from these points. None of the inhibitors evolved NH_3 or amines under the experimental conditions. Of the substrates used, only norepinephrine and benzylamine gave appreciable amounts of NH_3 which were deducted from the reaction product values.

(13) H. Yamada, O. Adachi, H. Kumagai, and K. Ogata, *Mem. Res. Inst. Food Sci., Kyoto Univ.*, **No. 26**, 21 (1965); H. Yamada, O. Adachi, and K. Ogata, *Agr. Biol. Chem.* (Tokyo), **29**, 117 (1965). After conclusion of our work we learned of the studies of C. M. McEwen, Jr., *J. Biol. Chem.*, **240**, 2003, 2011 (1965), on MAO from human plasma. This enzyme is probably also a copper protein and deaminates primary amines, among them, benzylamine, tryptamine, tyramine, dopamine, and kynuramine, but not histamine and 5-HT.

(14) Because the experiments, except those with benzylamine, depended of the titration of NH_3 liberated with K_2CO_3 from the enzymatically deaminated substrates, a common complex of 5-HT with creatine sulfate could not be used.

(15) We are grateful to Dr. Bernard Witkop of the National Institutes of Health for samples of harmaline and harmalol.

(16) E. J. Conway, "Microdiffusion Analysis and Volumetric Error," 4th Ed., Crosby, Lockwood and Son, Ltd., London, 1957.

(17) C. W. Tabor, H. Tabor, and S. M. Rosenthal, *J. Biol. Chem.*, **208**, 645 (1954).

TABLE III

In Vitro INHIBITION OF HIGHLY PURIFIED BEEF LIVER MITOCHONDRIAL MAO

Inhibitor	$[I]_{50}$, M				
	Benzylamine[a]	Tyramine[b]	Dopamine[c]	Tryptamine[d]	5-Hydroxytryptamine[e]
(±)-*trans* I	1.4×10^{-7}	5.6×10^{-7}	3.9×10^{-6}	6.3×10^{-7}	1.6×10^{-6}
(+)-*trans* I	7.1×10^{-8}	3.2×10^{-7}	1.8×10^{-6}	2.5×10^{-7}	7.1×10^{-7}
(−)-*trans* I	4.4×10^{-6}	2.5×10^{-6}	1.8×10^{-5}	5.6×10^{-6}	4.5×10^{-6}
(±)-*cis* I	8.9×10^{-8}	5.6×10^{-7}	8×10^{-7}	3.5×10^{-7}	8.9×10^{-7}
II	7.1×10^{-8}	5.6×10^{-7}	6.3×10^{-7}	3.2×10^{-7}	7.1×10^{-7}
III	7.1×10^{-8}	2.5×10^{-6}	4×10^{-7}	2.2×10^{-7}	7.1×10^{-7}
IV	5.0×10^{-7}	3.2×10^{-5}	2.2×10^{-5}	1.8×10^{-6}	4.5×10^{-6}

[a] Benzylamine sulfate, 1.6×10^{-3} M; determined spectrophotometrically at 250 mμ by the method of Tabor, *et al.*[17] [b] Tyramine·HCl, 5×10^{-3} M. [c] Dopamine·HCl, 1×10^{-2} M. [d] Tryptamine·HCl, 2×10^{-3} M. [e] 5-Hydroxytryptamine hydrogen oxalate, 2×10^{-3} M.

Results

The *in vitro* inhibitory concentrations, $[I]_{50}$, of seven inhibitors of beef liver mitochondrial MAO are listed in Table III. They emphasize the significance of the substrate in such inhibition experiments. For example, (±)-*cis* I has been reported[18] to be as potent as the (±)-*trans* isomer for the inhibition of the deamination of tyramine by MAO in a beef liver mitochondrial preparation; under other conditions (rat brain homogenate, 5-HT) the *cis* isomer was about 6 times more potent than (±)-*trans* I.[19] Comparative values in Table III range from equipotency of the two isomers for the deamination of tyramine, to approximately the double potency for the *cis* isomer with other substrates. In accord with the literature,[19,20] (+)-*trans* I was 10–20 times more potent than the (−) isomer. These data confirm the relative nonspecificity of purified mitochondrial MAO toward geometrical, but not toward optical isomers.

The tertiary amine, (±)-*trans* II, is a powerful inhibitor of the mitochondrial enzyme, ranging in potency with (±)-*cis* I.

N-Benzyl-N-methyl-N-(2-propynyl)amine (III) was found to be one of the most potent inhibitors of our mitochondrial MAO for all substrates used, while α-methylphenethylhydrazine (IV) was from 7–55 times less active than III. This relation was reversed with pure beef plasma MAO (*vide infra*).

In view of the low rate of deamination of norepinephrine (Table II), only one inhibitor was tested with this substrate; (±)-*trans* I inhibited the reaction of norepinephrine 60% at a concentration of 10^{-5} M.

The most unexpected finding was the significant lack of potency of harmine and related alkaloids in our experiments. Harmine and harmaline have been reported as being among the most potent *in vitro* inhibitors of MAO in rat brain and rat liver homogenates, using 5-HT as a substrate,[21] or guinea pig liver homogenate using tyramine.[22] As an inhibitor of the deamination of four substrates by highly purified beef liver mitochondrial MAO (Table IV), harmine was from 10^{-3} to 10^{-4} times as potent as in the tests with the above tissue homogenates from other species. The chance that harmine may have been metabolized to a more potent inhibitor by some factor in the tissue homogenates, by reduction or O-demethylation, was discounted when harmaline and harmalol were found to exhibit equally low activity under our *in vitro* conditions. The fact that these condensed-ring indole alkaloids show only even low-grade activity (in the 10^{-3} M region) in inhibiting the deamination of 5-HT by the beef liver enzyme points to a possible difference in mechanism[23] between the action of these alkaloids and of those inhibitors which do not contain an indole system.

TABLE IV

In Vitro INHIBITION OF HIGHLY PURIFIED BEEF LIVER MITOCHONDRIAL MAO BY HARMALA ALKALOIDS

Inhibitor	Concn., M	Substrate	% inhib.
Harmine	1.6×10^{-4}	Benzylamine	0
	1×10^{-3}	Dopamine	11
	1×10^{-3}	Tryptamine	17
	1×10^{-3}	5-HT	46
Harmaline	1.6×10^{-5}	Benzylamine	0
	1×10^{-3}	5-HT	69
Harmalol	1.6×10^{-5}	Benzylamine	0

The effect of some of our inhibitors on pure beef plasma MAO was measured spectroscopically[17] using benzylamine as a substrate. None of the other substrates used with the mitochondrial liver enzyme could be tested because they are not deaminated by plasma MAO.[12] A comparison of the inhibition values in Table V with those in the benzylamine column in Table III reveals the uniformly higher inhibitor concentrations needed to affect the reactions of the plasma enzyme. A notable exception was the hydrazine IV which was the most potent inhibitor in our series for plasma MAO, while it did not occupy such a position with the mitochondrial enzyme. Plasma MAO has been shown to be inhibited by carbonyl reagents of the hydrazine class, apparently by reaction with the pyridoxal cofactor of the enzyme.[24] It is possible that IV inhibits the enzyme in this fashion.

TABLE V

In Vitro INHIBITION OF PURE BEEF PLASMA MAO

Inhibitor	$[I]_{50}$, M[a]
(±)-*trans* I	6.3×10^{-6}
(+)-*trans* I	3.2×10^{-6}
(−)-*trans* I	1.4×10^{-4}
(±)-*cis* I	2.8×10^{-4}
IV	1.6×10^{-7}

[a] Benzylamine as the substrate.

(18) S. Sarkar, Dissertation, Northwestern University, Evanston, Ill., 1961.

(19) C. L. Zirkle, C. Kaiser, D. H. Tedeschi, R. E. Tedeschi, and A. Burger, *J. Med. Pharm. Chem.*, **5**, 1265 (1962).

(20) J. F. Moran, Dissertation, University of Ottawa, Ottawa, Canada, 1962.

(21) See Table I, footnotes *a–d*.

(22) A. Pletscher, H. Besendorf, H. P. Bächtold, and K. F. Gey, *Helv. Physiol. Pharmacol. Acta*, **17**, 202 (1959).

(23) *Cf.* ref. 19; for a review of this situation, see ref. 2a, p. 531.

(24) H. Yamada and K. T. Yasunobu, *J. Biol. Chem.*, **238**, 2669 (1963).

(±)-*cis*-2-Phenylcyclopropylamine was less active in inhibiting the plasma enzyme than the (±)-*trans* isomer. Harmine again made a poor showing. It inhibited the deamination of benzylamine by beef plasma MAO to only 10% at 3.2×10^{-5} *M*. Higher concentrations could not be measured spectrophotometrically[17] because of the interference of optical absorption by the alkaloid.

The tertiary 2-phenylcyclopropylamine derivative II and the propargylamine III, which inhibit the mitochondrial enzyme at very low concentrations (Table III), did not affect the deamination of benzylamine by beef plasma MAO below 8 and 4×10^{-4} *M*, respectively. At higher concentrations of these two compounds, an apparent reversal of inhibition values was observed repeatedly in our test system.[17]

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Notes

Potential Antiradiation Agents.[1a] Preparation and Polymerization of Monomeric Thiazolidines[1b,c]

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Because the major shortcoming of present radioprotective agents is their relatively short-lived protection, we have undertaken to prepare compounds that may act as sources of molecular entities of proven protective capacity; that is, compounds so designed that they would slowly release moieties such as 2-mercaptoethylamine and cysteine under physiological conditions, and in so doing provide a long-lasting source of radioprotective agent in nontoxic amounts.

Since the thiazolidine heterocycle is readily cleaved to α-amino-β-thiols under mild hydrolytic conditions,[2] we herein report the preparation of polymeric thiazolidines of 2-mercaptoethylamine and cysteine. The synthetic method employed is outlined in Scheme I.

2,2-Dimethylthiazolidine (I) was prepared by treating a solution of ethyleneimine in acetone with gaseous hydrogen sulfide according to the method of Bestian.[3] 4-Carbomethoxy-2,2-dimethylthiazolidine (VII) was prepared by the more conventional method of condensing cysteine methyl ester hydrochloride with acetone, followed by liberation of the free amine with aqueous sodium carbonate.[4]

N-Acrylyl-2,2-dimethylthiazolidine (II) and N-acrylyl-4-carbomethoxy-2,2-dimethylthiazolidine (VIII) were prepared by acylation of I and VII, respectively, with acrylyl chloride in the presence of trimethylamine as the acid acceptor. The yields of the acrylamide were 61.5 and 60%, respectively. Treatment of I with S-β-chloroethyl chlorothiolformate[6] afforded a 71.5% yield of IV which readily underwent dehydrochlorination with 1 molecular equiv. of potassium *t*-butoxide in *t*-butyl alcohol to afford the S-vinylmonothiolcarbamate V.

The monomeric thiazolidines (II, V, and VIII) thus prepared were homopolymerized to high conversion using α,α′-azobisisobutyronitrile as initiator. While the polymeric acrylamides III and IX may conceivably act as a source of 2-mercaptoethylamine and cysteine, respectively, the polymeric monothiolcarbamate VI may be expected to undergo metabolic hydrolysis with the formation of polyvinylmercaptan (itself a radioprotective agent[7,8]) as well as resulting in the liberation of 2-mercaptoethylamine.

It has been determined that polyvinylpyrrolidone is capable of complexing toxic radiation products and hastening excretion in the urine.[9] The monomeric thiazolidines were therefore copolymerized with N-vinylpyrrolidone in the hope that the copolymers might combine effects with the absorptive ability exhibited by polyvinylpyrrolidone. A further desirable feature of the copolymers is their water solubility.

It is worthy of note that after this research was begun a report was published[10] relating to the fact that some thiazolidines were as effective in protecting against ionizing radiation as is 2-mercaptoethylamine. Indeed, it has been found[11] that a copolymer consisting of 18 mole % V and 82 mole % N-vinylpyrrolidone was effective in protecting experimental rats at a dosage of 150 mg./kg. of body weight.

(1) (a) Supported by Contract No. DA-49-193-MD-2032 from the United States Army Medical Research and Development Command, Office of the Surgeon General. (b) Presented at the 141st National Meeting of the American Chemical Society, Washington, D. C., March 1962. (c) This is 28th in a series of papers concerned with the preparation and properties of new monomers and polymers; for the previous paper in this series, see C. G. Overberger, H. Ringsdorf, and B. Aychen, *J. Org. Chem.*, **30**, 232 (1965). (d) This article is taken from the dissertation of B. Aychen submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy (Chemistry).

(2) E. D. Bergmann, *Chem. Rev.*, **53**, 309 (1953).

(3) H. Bestian, *Ann.*, **566**, 210 (1950).

(4) Acetone was chosen as the condensing agent in each case because of the reported[5] greater ease of fission of 2,2-dimethylthiazolidines (as opposed to 2-phenylthiazolidines, for example).

(5) "The Chemistry of Penicillin," H. T. Clarke, J. R. Johnson, and R. Robinson, Ed., Princeton University Press, Princeton, N. J., 1949, Chapter 25.

(6) H. Ringsdorf and C. G. Overberger, *Makromol. Chem.*, **44**, 418 (1961).

(7) C. G. Overberger and A. Lebovits, *J. Am. Chem. Soc.*, **77**, 3675 (1955).

(8) C. G. Overberger, H. Biletch, and R. G. Nickerson, *J. Polymer Sci.*, **27**, 381 (1958).

(9) L. Sukyasyan, *Probl. Gematol. i. Perelic. Krovi*, **4**, 48 (1959).

(10) A. Kaliszyner, P. Czerniak, and E. D. Bergmann, *Radiation Res.*, **14**, 23 (1961).

(11) Private communication to the authors from Dr. P. Coad, Walter Reed Army Institute of Research, Department of Medicinal Chemistry.