concentration of the filtrate to 100 ml, gave 16.9 g (67%) of colorless product, mp 199–200°.

Acknowledgments.—We are indebted to Dr. J. Bernstein for his interest and encouragement during this investigation, to Dr. J. Burke and his associates for the summary of pharmacological data, to Dr. A. Cohen for interpretation of the nmr spectra, to Miss B. Keeler for the infrared data, and to Mr. J. Alicino and his associates for the analyses reported herein.

Aziridine Derivatives as Potential Monoamine Oxidase Inhibitors[1]

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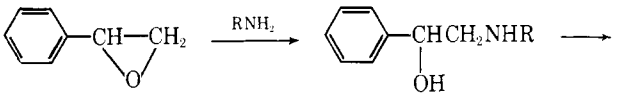
A series of 1-substituted phenylaziridines has been prepared and tested for *in vitro* MAO-inhibition activity. The most active compounds were studied to assure that a hydrolysis product of the aziridine ring was not responsible for MAO inhibition.

Zeller, *et al.*,[2] postulated that the most suitable inhibitors of monoamine oxidase (MAO) contained a two-carbon chain between an aromatic ring and an amino group. Work by Paget and Davis indicated that the high π-electron density, presumably present in heterocyclic systems analogous to cyclopropane, may give rise to MAO inhibition.[3] The latter work also showed that, in the diaziridine series, a free amino group was not necessary for MAO inhibition.

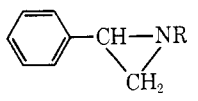
In this paper the preparation and *in vitro* MAO-inhibition activity of 1-substituted derivatives of 2-phenylaziridine are reported. These derivatives are essentially phenethylamine structures with the amine incorporated in a three-membered ring. Such a configuration offers a unique advantage of increasing the π-electron density between the two carbon atoms of the alkyl chain. The MAO activity of 2-phenylaziridine has been demonstrated.[4] Our interest was to determine the effect of increased electron density in the aziridine ring and also the effect of bulk in the 1-position on MAO-inhibition activity.

Treatment of styrene oxide with alkyl or aralkyl primary amines gave the desired 2-amino-1-phenylethanol derivatives (I).[5] Compound IIa was prepared by the method of Brois[6] using 70% sulfuric acid to prepare the O-sulfate ester. Compounds IIb, c, and d were prepared according to the method of Taguchi and Kojimi.[7]



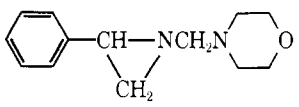
I

a, R = CH_3 c, R = $CH(CH_3)_2$
b, R = C_2H_5 d, R = $CH_2CH_2C_6H_5$



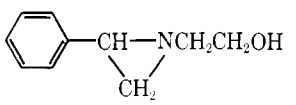
II

The hydrogen on the aziridine nitrogen was reactive enough to undergo aminomethylation.[8] Reaction between 2-phenylaziridine, formaldehyde, and morpholine gave 1-morpholinomethyl-2-phenylaziridine (III).

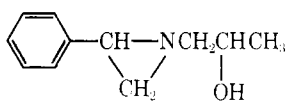


III

The method used for the preparation of 1-(2-hydroxylalkyl)-2-phenylaziridines (IV and V) was a slight modification of that reported by Funke and Benoit[9] in which 2 equiv of the appropriate epoxide was treated with 1 equiv of 2-phenylaziridine.



IV



V

A convenient preparation of 2-[(2-phenethyl)amino]-ethanol consisted of refluxing 2-phenyl-1-bromoethane with an excess of 2-aminoethanol. 2-[(2-Phenethyl)-amino]ethanol was converted to 1-(2-phenethyl)-aziridine (VI).[7] Compound VII, 2-[(2-phenethyl)-amino]-2-phenylethanol, was prepared by refluxing 2-phenyl-1-bromoethane with a large excess of 2-amino-2-phenylethanol.

In Vitro **Studies.**—Inhibition of monoamine oxidase *in vitro* was assessed by the method of Wurtman and Axelrod[10] which consists of incubating the potential MAO inhibitor and the substrate, 2-C^{14}-tryptamine, with rat liver homogenate. After a suitable length of time the deaminated product (C^{14}-indoleacetic acid) was extracted and counted for its radioactive content. The compounds here were screened at 5×10^{-4} *M* concentration as the free base, which was dissolved in phosphate buffer (pH 7.4) containing not more than 4% *p*-dioxane. The degree of inhibition was compared to a 5×10^{-4} *M* iproniazid standard. The results are given in Table I.

(1) From the Ph.D. Thesis of Ajit V. Shirodkar, Purdue University.
(2) E. A. Zeller, S. Sarkar, R. Banerjee, and M. S. Ise, *Helv. Chim. Acta*, **43**, 439 (1960).
(3) C. J. Paget and C. S. Davis, *J. Med. Chem.*, **7**, 626 (1964).
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(6) S. J. Brois, *J. Org. Chem.*, **27**, 3532 (1962).
(7) T. Taguchi and M. Kojimi, *Chem. Pharm. Bull.* (Tokyo), **7**, 103 (1959).
(8) F. F. Blicke, *Org. Reactions*, **1**, 303 (1942).
(9) A. Funke and G. Benoit, *Bull. Soc. Chim. France*, 1021 (1953).
(10) R. J. Wurtman and J. Axelrod, *Biochem. Pharmacol.*, **12**, 1439 (1963).

TABLE I

RESULTS OF *in Vitro* SCREENING FOR MAO INHIBITION USING RAT LIVER HOMOGENATES

Compd (5×10^{-4} *M*)	% inhib
Iproniazid	100
IIa	22[a]
IIb	28[a]
IIc	62 ± 6[b]
Ic	36[a]
IId	64 ± 4[b]
Id	>100[b,c]
III	35[a]
IV	11[a]
V	Inactive
VI	20[a]
VII	Inactive

[a] Single determination. [b] Duplicate determinations reported as the mean and average deviation of an individual result from the mean value. [c] $ID_{50} = 9.25 \times 10^{-5}$ *M*.

Experimental Section[11]

1-Methyl-2-phenylaziridine (IIa).—Styrene oxide (60.0 g, 0.50 mole) and 40% aqueous methylamine (2.0 moles) were heated in a steel bomb at 95–100° for 5.5 hr with constant stirring. The resulting mixture was cooled to room temperature and excess methylamine and water were removed *in vacuo*. The residue was distilled to yield Ia, bp 105–107° (0.2 mm); yield from *n*-hexane 47.5 g (70.5%), mp 71–73° (lit.[12] 75–76°). This Ia (10.0 g, 0.07 mole) was neutralized to methyl red end point with 70% aqueous H_2SO_4, followed by the addition of an equal volume of the H_2SO_4 solution. Water was removed on a steam bath at 15–20 mm. The sulfate ester, a semisolid, was dried in a vacuum oven and dissolved in 2 *N* NaOH (310 ml) at 0°. The temperature was slowly increased until the product appeared as an upper layer at 90–95°. The cooled mixture was extracted with three 100-ml portions of ether. The combined ether extract was dried (Na_2SO_4) and evaporated to leave a yellow residue which was distilled. The fraction boiling at 35–37° (0.3 mm) weighed 2.8 g (31.8% yield), $n^{20}D$ 1.5321.

Anal. Calcd for $C_9H_{11}N$: C, 81.16; H, 8.32. Found: C, 81.00; H, 8.35.

1-Ethyl-2-phenylaziridine (IIb).—The amino alcohol Ib (11.0 g, 0.07 mole)[5] was ice-cooled and treated (mechanical stirring) dropwise with chlorosulfonic acid (4.4 ml, 0.07 mole) during 45 min. After the addition, the solid mass was broken up and heated in an oil bath at slightly less than 150° for 1.5 hr at reduced pressure (15–20 mm). To the ester dissolved in water (35 ml) was added a solution of KOH (20.0 g in 20 ml of water). The mixture was heated on a steam bath for 30 min, cooled to room temperature, and extracted with three 100-ml portions of ether. The combined ether extract was dried (Na_2SO_4), and the ether was distilled *in vacuo*. The residue gave 3.15 g (32.2%) of IIb, bp 68–70° (2.5 mm), $n^{20}D$ 1.5220.

Anal. Calcd for $C_{10}H_{13}N$: C, 81.58; H, 8.90. Found: C, 81.57; H, 9.03.

1-Isopropyl-2-phenylaziridine (IIc).—The method used for the preparation of Ia was employed in the preparation of Ic, using styrene oxide (47.5 g, 0.44 mole) and isopropylamine (51.4 g, 0.87 mole). Ic was recrystallized from *n*-hexane–benzene; yield 55.0 g (73.8%), mp 88–89°. The above amino alcohol (40.0 g, 0.22 mole) was esterified with chlorosulfonic acid (14.7 ml, 0.22 mole) as in the preparation of IIb. Ring closure of the inner salt, 2-isopropylamino-1-phenethyl sulfate, was accomplished by dissolving in KOH (44.0 g in 44 ml of water). The basic product was distilled to give 18.5 g (51.5%) of colorless liquid, bp 70° (2.4 mm), $n^{20}D$ 1.5090, ν_{max} 1381 and 1369 cm^{-1} [$C(CH_3)_2$].

Anal. Calcd for $C_{11}H_{15}N$: C, 81.94; H, 9.38. Found: C, 82.17; H, 9.46.

(11) Melting points were determined on a Büchi apparatus with open capillary tubes and are uncorrected. The infrared absorption spectra (neat or as a KBr pellet) were determined with a Perkin-Elmer Model 21 infrared spectrophotometer. Compounds IIa–d, III, IV, V, and VI showed strong bands at 1204–1213 and at 1080–1087 (aziridine deformation).

(12) J. F. Hyde, E. Browning, and R. Adams, *J. Am. Chem. Soc.*, **50**, 2287 (1928).

1-(2-Phenethyl)-2-phenylaziridine (IId).—Styrene oxide (22.8 ml, 0.20 mole) was added to refluxing 1-amino-2-phenylethane (50.4 ml, 0.40 mole) during 1 hr. The mixture was refluxed for an additional 2.5 hr. The solid which precipitated upon cooling was collected and recrystallized from *n*-hexane–benzene (4:1) to give 35 g (72.5%) of Id, mp 90°, lit.[13] 89.5–90°. The amino alcohol (10.0 g, 0.04 mole) was treated with chlorosulfonic acid (4.62 g, 0.04 mole) and KOH (9.0 g in 9 ml of water) as described in the preparation of IIb; yield of IId 3.1 g (33.5%), bp 110° (0.17 mm), $n^{20}D$ 1.5670.

Anal. Calcd for $C_{16}H_{17}N$: C, 86.05; H, 7.67. Found: C, 86.13; H, 7.95.

1-Morpholinomethyl-2-phenylaziridine (III).—A mixture of 2-phenylaziridine (6.7 g, 0.06 mole), morpholine (7.3 g, 0.09 mole), and water (80 ml) was cooled to −5°. Aqueous (37%) formaldehyde (6.9 ml, 0.09 mole) was added dropwise to the mixture during 30 min with constant stirring. The solution was gradually brought to room temperature, at which time the mixture turned white. It was refluxed for 30 min and saturated with Na_2SO_4. The cooled mixture was extracted with three 100-ml portions of ether. The combined ether extract was dried (Na_2SO_4) and evaporated *in vacuo*. The residue was distilled through a 10-in. Vigreux column to give 7.1 g (58%) of III bp 113–116° (0.55 mm), ν_{max} 1115 cm^{-1} (COC).

Anal. Calcd for $C_{13}H_{18}N_2O$: C, 71.53; H, 8.31. Found: C, 71.54; H, 8.66.

1-(2-Hydroxyethyl)-2-phenylaziridine (IV).—To a cold solution of 2-phenylaziridine (5.0 g, 0.35 mole) and water (2 ml) was added cold ethylene oxide (3.08 g, 0.70 mole) in one portion. Using a Dry Ice–acetone condenser, the mixture was refluxed for 4 hr. On distilling, the fraction boiling at 97–100° (0.65 mm) was collected and redistilled to give 3.0 g (43%) of IV, bp 87–88° (0.19 mm), $n^{20}D$ 1.5516, ν_{max} 1063 cm^{-1}.

Anal. Calcd for $C_{10}H_{13}NO$: C, 73.59; H, 8.03. Found: C, 73.55; H, 8.05.

1-(2-Hydroxypropyl)-2-phenylaziridine (V).—To a cold solution of 2-phenylaziridine (6.75 g, 0.06 mole) in water (2.0 ml) was added propylene oxide (5.4 g, 0.09 mole), and the mixture was refluxed for 4 hr. The resulting thick mixture was distilled to give 2.75 g of liquid, bp 104° (1.7 mm). This was again distilled through a 10-in. Vigreux column to give 2.25 g of V (22.5%), bp 83–84° (0.2 mm), $n^{20}D$ 1.5357. The infrared spectrum displayed strong bands at 3420 and 1136 cm^{-1} (OH).

Anal. Calcd for $C_{11}H_{15}NO$: C, 74.54; H, 8.53. Found: C, 74.87; H, 8.60.

1-(2-Phenethyl)aziridine (VI).—Chlorosulfonic acid (3.9 ml, 0.05 mole) was added over a period of 20 min, with constant stirring, to a solution of 2-(2-phenethyl)aminoethanol[14] (8.75 g, 0.05 mole) in 20 ml of water. As described in the preparation of IIb, 2.1 g (28%) of VI, bp 45° (0.4 mm), $n^{20}D$ 1.5190, was obtained.

Anal. Calcd for $C_{10}H_{13}N$: C, 81.58; H, 8.90. Found: C, 81.46; H, 8.73.

2-(2-Phenethyl)amino-2-phenylethanol (VII).—A mixture of 2-amino-2-phenylethanol (10.0 g, 0.07 mole) and 1-bromo-2-phenylethane (6.65 g, 0.04 mole) was heated under reflux for 6 hr. The mixture was then heated with three 100-ml portions of ether to extract the unchanged starting materials. The ether was decanted to leave a semisolid which was recrystallized from boiling benzene. The recrystallized solid was dissolved in water, and the solution was saturated with NaCl. The aqueous solution was made alkaline with NH_4OH and extracted with three 100-ml portions of ether. The combined ether extract was dried (Na_2SO_4) then concentrated *in vacuo* to give a solid residue which was recrystallized from benzene to give 6.0 g (63.5%) of white solid, mp 122–125°.

Anal. Calcd for $C_{16}H_{19}NO$: C, 79.63; H, 7.94. Found: C, 79.69; H, 8.19.

Discussion

1-Alkylaziridines appear to show a gradual increase in MAO-inhibition activity with increase in alkyl chain length. Activity increased considerably with isopropyl and phenethyl derivatives. Since the in-

(13) A. L. Allewelt and A. R. Day, *J. Org. Chem.*, **6**, 384 (1941).

(14) J. Barbiere, *Bull. Soc. Chim. France*, **11**, 470 (1944).

stability of the aziridine ring is well documented,[15] there was some doubt as to whether the relatively high activity observed with compounds IIc and IId was due to the intact molecule or to one of the hydrolysis products. A study of the stability of these two compounds under *in vitro* testing conditions in phosphate buffer (pH 7.4), containing not more than 4% *p*-dioxane, indicated that the isopropyl derivative was stable whereas the phenethyl derivative underwent slight hydrolysis as detected by thin layer chromatography using ethyl acetate–benzene (1:3) as solvent.

Experiments were designed to determine whether the MAO-inhibitory action of 1-(2-phenethyl)-2-phenylaziridine was due to the intact molecule or a hydrolysis product. Both 2-(2-phenethyl)amino-1-phenylethanol and 2-(phenethyl)amino-2-phenylethanol were tested along with 1-(2-phenethyl)-2-phenylaziridine for *in vitro* MAO inhibition. Although the results indicated that 2-(2-phenethyl)amino-1-phenylethanol has considerable activity at 5×10^{-4} *M* concentration, its ID_{50} was found to be 9.25×10^{-5} *M*. This indicated that the above amino alcohol, in the amounts formed during *in vitro* testing could not be solely responsible for the MAO inhibition observed with compound IId. Thin layer chromatograms with a solution of Id (9.25×10^{-5} *M*) and of IId which gave approximately corresponding *in vitro* activity showed that, whereas the amino alcohol was easily detectable on the plates, the parent aziridine showed only a very faint spot of hydrolysis product after subjecting the two to the *in vitro* testing conditions.

It is possible that the inductive effect of an alkyl substituent would serve to increase the availability of the unshared pair of electrons on the aziridine nitrogen, making it favored as a center of high nucleophilic reactivity.[16] This does not, however, explain why compound IId is considerably more active than compound IIb. The inductive effect should be about the same from both substituents, which leaves a bulk effect as a possible explanation. The compounds which are most active (IIc and IId) have in common at the 1-position, nonpolar, hydrocarbon moieties which have considerable bulk.

Compound III which contains a polar group also shows MAO-inhibition activity. However, the observation that IV and V showed very little activity as compared to III leads to the conclusion that even in the series of polar substituents in the 1-position, greatest activity is shown by the compounds that have a bulky substituent.

Although 2-amino-1-phenylethanol is a good substrate of MAO,[17] no phenethylamine derivatives containing groups larger than methyl or dimethyl on the nitrogen have been found to be good substrates of MAO. One may speculate that due to the presence of the phenethyl unit, compounds Ic and Id are able to establish their presence near the active site of MAO and thus may partially prevent the enzymatic oxidation of the substrate. Here again it would seem that the large hydrocarbon substituent of the 1-position is responsible for inhibitory activity.

(15) J. E. Early, C. E. O'Rourke, L. B. Clapp, J. O. Edwards, and B. C. Lawes, *J. Am. Chem. Soc.*, **80**, 3458 (1958).

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

(17) N. Weiner, *Arch. Biochem.*, **91**, 182 (1960).

Synthesis of Chelating Compounds to Be Used as Potential Bone Seekers[1,2]

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A number of new derivatives of iminodiacetic acid, of the general formula $RN^+H(CH_2COOH)CH_2COO^-$ have been prepared for evaluation as potential bone seekers. Radical R stands for a chain containing, *inter alia*, an oxygen atom belonging to a hydroxy, carbonyl, amide, carboxyl, or ether function. In this way, tridentate or quadridentate chelating agents are formed. These compounds were prepared mainly by introducing the iminodiacetic acid group *in toto* into different molecules by the action of various alkyl halides on iminodiacetic acid dimethyl ester in nonpolar solvents, followed by saponification and precipitation of the imino acids at their isoelectric points. In order to study their biological behavior, the imino acids were labeled with one of the radiohalogens F^{18}, Br^{82}, or I^{131}. The labeling methods were based on exchange reactions, direct halogenation, or recoil labeling. Acid dissociation constants of the imino acids and the chelate stability constants of the corresponding anions with divalent metal ions were determined potentiometrically. With calcium ions, the major metallic constituent of bone mineral, each of the amino acids forms a single stable 1:1 metal chelate, its stability ranging from $\log K_{S_1} = 3$ to $\log K_{S_1} = 5$ ($t = 30°$, $\mu = 0.100$). Biological studies have proved that the affinity of the different synthetic imino acids for bone can be correlated with their chelating ability with calcium ions.

Bone seekers as a group comprise a heterogeneous list which by broad definition includes any substance localizing in the skeleton.[4] Most of the research work on bone seekers has been concerned with radioactive elements, because of the radiation hazard arising from the deposition of such elements in the skeleton, and because they can be used to study the physiological activities of bone, qualitatively and quantitatively. Bone-seeking radioelements have also found extensive use in the study of both localized lesions in bone (*e.g.*, fractures and primary or metastatic neoplasms) and metabolic disorders of the skeleton (*e.g.*, Paget's

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(2) Part of a thesis submitted by G. Shtacher to the Senate of the Hebrew University, Jerusalem, 1965, in partial fulfillment of the requirements for the Ph.D. degree.

(3) The Hospital for Special Surgery, New York, N. Y.

(4) P. S. Chen, Jr., A. R. Terepka, and H. C. Hodge, *Ann. Rev. Pharmacol.*, **1**, 369 (1961).