EXPERIMENTAL

Alkyl p-aminobenzoates—To a suspension of *p*-aminobenzoic acid (Eastman, White label) (6.9 g., 0.05 mole) in the appropriate alcohol (reagent grade) (40 ml., ~ 0.75 mole) was added boron trifluoride-ethyl ether (Eastman, White label) (12.6 ml., 0.1 mole) and the reaction mixture refluxed for 48 hr. The esters were precipitated by pouring the cooled, filtered solutions into an ice cold solution of sodium carbonate (5%, 400 ml.); these were sufficiently pure for most purposes and recrystallization increased the melting points only slightly (Table I). The compounds exhibited IR absorption frequencies characteristic of the C=O and NH_2 groups.

The methyl, *n*-propyl, and *n*-butyl esters were prepared using acid (Eastman, Practical grade) (0.05 mole), alcohol (0.5 mole), and boron trifluoride-ethyl ether (0.075 mole).

Under either experimental condition, when the reaction time was reduced to 24 hr. the yield was also reduced.

An attempt to prepare the methyl ester using diazomethane, yielded products with wide melting point ranges. Apparently, the diazomethane effects some *N*-methylation, giving rise to a mixture of products.

N-(2,4-Dichlorobenzylidene)-p-aminobenzoates—The Schiff base derivatives were prepared (1) by heating equimolar quantities of the *p*-aminobenzoic ester and the aldehyde in an ethanol solution (Table I).

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Inhibitors of Monoamine Oxidase V: Effect of Substitution on the Transport of Tetrahydro-β-Carboline Analogs to Mouse Brain

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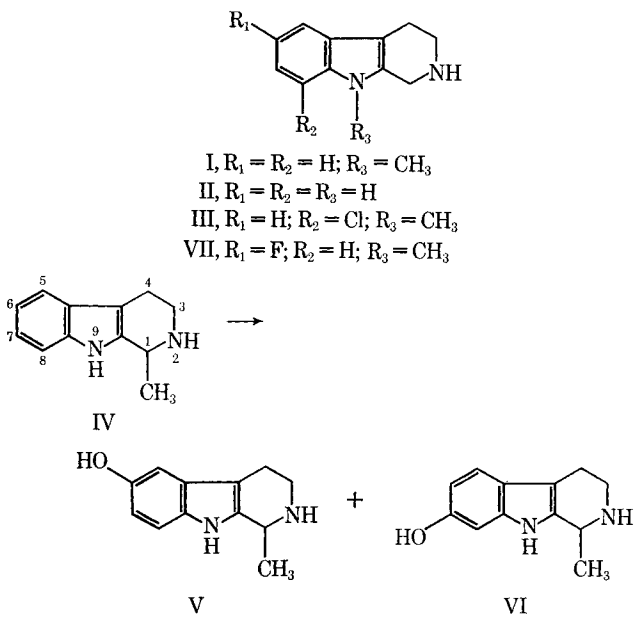
Abstract □ Methyl substitution on the indolic nitrogen and halogen substitution on the indole nucleus of tetrahydro-β-carbolines were found to facilitate the transport of compounds into the brain. A correlation between the brain accumulation, the pKa, and the partition coefficient has been made. The 8-chloro-9-methyl-1,2,3,4-tetrahydro-β-carboline was assayed with mitochondrial monoamine oxidase from mouse brain and liver. Its inhibitory activity was two-fold greater than that reported using bovine liver enzyme.

Keyphrases □ Monoamine oxidase inhibitors—tetrahydro-β-carboline analogs, ^{14}C-labeled □ Tetrahydro-β-carboline analogs—substitution effect □ Transport, mouse brain—tetrahydro-β-carboline substitution effect

A number of substituted tetrahydro-β-carbolines have been found to be potent inhibitors of monoamine oxidase (MAO) *in vitro* (1–3). Methyl substitution on the position N-9, the indolic nitrogen, in most cases, enhanced the inhibitory activity *in vitro*. For instance, 9-methyltetrahydro-β-carboline (I) is a 34-fold better inhibitor than tetrahydro-β-carboline (II) (1), and 8-chloro-9-methyltetrahydro-β-carboline (III) is nearly 100-fold better than II (3). It was of interest to determine whether the methyl group could increase the lipid solubility of the tetrahydro-β-carboline and thus facilitate its entry into the brain. Furthermore, from previous metabolic studies, 1-methyltetrahydro-β-carboline (IV) was found to have undergone hydroxylation forming 6-hydroxy-1-methyltetrahydro-β-carboline (V) and 7-hydroxy-1-methyltetrahydro-β-carboline (VI) (4), see Scheme I. Halogen substitution on the C-6 (see VII) or C-8 (see III) position might retard 6- or 7-hydroxylation of the tetrahydro-β-carboline, and possibly prolong the biological action.

The object of this study was to demonstrate the enhancement of brain absorption of tetrahydro-β-carbolines by the introduction of a methyl group to the indolic nitrogen and the additional effect when halogen substitution was placed on the indole ring. A correlation of brain accumulation of the four analogs of tetrahydro-β-carboline (I, II, III, and VII) with the partition coefficient and the pKa was also made. This finding would



Scheme I

establish a basis for further studies of the metabolism of these compounds and their inhibitory effect on MAO *in vivo* which will be published elsewhere (5).

EXPERIMENTAL

Chemicals—Compounds I–III and VII were synthesized as previously described (1, 3) except that ^{14}C-methyl iodide was used in the "methylation" step. They were recrystallized to a constant melting point and constant radioactivity. The specific activities of Compounds I, II, and VII ranged from 0.31–0.38 μc./mg., the specific activity of Compound III was 0.12 μc./mg.

Enzyme Assay—Mitochondrial monoamine oxidase from tissues was isolated and purified according to the previously reported method for bovine liver (1). For the assay, nonlabeled compounds were used, and the procedure was the same as that previously described (1).

Determination of pKa—A sample of 0.01 mole of each compound was dissolved in 25 ml. of 50% ethanol and, with constant stirring, this solution was titrated with 0.0227 *N* NaOH. The pKa value was calculated according to the method applied by Albert and Serjeant (6).

Partition Coefficient—An accurately weighed 4.0-mg. sample of each ^{14}C compound was partitioned between 4 ml. of *n*-heptane and 4 ml. doubly distilled water. Each tube was shaken for 1 hr. at room temperature (about 24°), then allowed to stand until there was a clear separation of two phases (about 90 min.). A 0.1-ml. aliquot of each phase was pipeted and assayed for ^{14}C in a liquid scintillation spectrometer. Partition coefficients were calculated as the ratio of radioactivity expressed as disintegration per minute (DPM) in the organic phase to that in the aqueous phase.

Brain Accumulation—Male BDF_1 mice weighing 20 g. were injected (i.p.) with 0.2 ml. of a 2.2×10^{-2} *M* aqueous solution. The mice were sacrificed by stunning and decapitation at varied time intervals of 15, 30, 60, and 120 min. Two mice were pooled for each time interval. The brains were removed and a 20% aqueous homogenate was prepared. A 0.1-ml. aliquot of this homogenate was added to 3 ml. of methanol and allowed to stand for 20 min., then assayed for ^{14}C in a liquid scintillation spectrometer.

Chromatographic studies showed no sign of demethylation of the compounds and no metabolite in the brain (5).

Plasma Level—Another group of mice was injected as previously described. Approximately 0.5 ml. of blood was obtained by heart puncture from each animal and placed in a heparinized tube. After separation from the blood by low speed centrifugation, plasma was assayed for ^{14}C.

RESULTS AND DISCUSSION

The results on the studies of pKa, partition coefficient, and brain accumulation of the four MAO inhibitors (I, II, III, and VII) are shown in Table I.

Among these four, the 8-chloro-9-methyl compound (III) was found to have the fastest and highest accumulation in brain. Methylation of indolic nitrogen facilitates the transport of I, III, and VII into the brain. Partition coefficients of compounds in organic solvent, such as heptane, are generally used to indicate the lipid solubility of the uncharged (nonprotonated) molecules. Compound II has a very low heptane solubility. With a pKa of 8.3 only 9% of the uncharged form is available at pH 7.3, and this may explain its having the slowest rate of entry (peak time 120 min.) into the brain and lowest accumulation in the brain (2.7%). In heptane, I is 1.5 times more soluble than VII; however, at pH 7.3 since its pKa is higher than VII, it resulted in a slightly lower accumulation in the brain. It would thus seem that solubility is not a decisive factor. The highest accumulation and fastest rate of entry of III is not entirely dependent on the availability of its uncharged molecules. With a pKa only slightly lower than I and VII, 19% of III exists as uncharged form, compared to 17 and 14% for VII and I, respectively. In this instance, the higher and faster level attained in plasma could account for the higher and faster accumulation of III in brain. The possible involvement of mechanisms other than simple diffusion for the transport of these tetrahydro-β-carbolines to the brain is also likely.

The above studies thus far indicate that the best MAO inhibitor, III, was able to penetrate into the brain faster and to a greater degree than Compounds I, II, and VII. It was noteworthy that III was also the best inhibitor of mitochondrial MAO from mouse brain, since in this study mice were used and the previous data on MAO inhibition were obtained using bovine liver enzyme (1–3). The results in Table I show that the order of inhibitory activity for

Table I—Accumulation of Tetrahydro-β-carboline Analogs in Mouse Brain and Their Inhibitory Activities on Monoamine Oxidase from Different Tissues

Compound	R_1	R_2	R_3	pKa	Partition Coefficient Heptane/H_2O	Brain Accumulation Peak Time, min.	Brain Accumulation DPM,[b] %	Plasma Level Time, min.	Plasma Level DPM,[b] %	I_{50}[a], mM Bovine Liver	I_{50}[a], mM Mouse Liver	I_{50}[a], mM Mouse Brain
II[c]	H	H	H	8.31 ± 0.02	0.00003	30	2.7	15	0.66	0.34[d]	0.13	0.032
						120	2.8	30	0.73			
I[c]	H	H	CH_3	8.10 ± 0.01	0.01030	30	3.4	15	1.09	0.01[d]	0.05	0.015
								30	1.21			
VII[c]	F	H	CH_3	8.01 ± 0.01	0.00740	30	3.8	15	0.80	0.12[e]	0.05	0.019
								30	0.73			
III[f]	H	Cl	CH_3	7.95 ± 0.02	0.00500	15	4.5	15	1.56	0.0055[e]	0.015	0.0028
								30	1.20			

[a] Concentration of the inhibitor giving 50% inhibition of the enzyme. [b] Disintegration per minute (See *Experimental*). [c] Hydrochloride salt. [d] Data from *Reference* 1. [e] Data from *Reference* 3. [f] Sulfate salt.

the four compounds when tested on bovine liver was also true for mouse brain and liver. Compound III, the most active of the four, demonstrated twice the activity in mouse brain as in bovine liver.

Substitution of a methyl group on the indolic nitrogen of II did not give a 34-fold increase in the inhibition of MAO from either mouse brain or mouse liver as it did with MAO from bovine liver. When the 9-H compound (II) and the 9-methyl compound (I) are compared in the two latter cases, I is only 2- and 2.6-fold more active than II, respectively. An additional example indicating species variation of inhibitory activity can be seen in VII. Fluorine substitution on the 6-position of I does not seem to affect the binding of VII on either mouse liver or brain MAO, while with bovine MAO this substituent causes a twelvefold decrease in inhibitory activity.

CONCLUSION

The methyl group on the indolic nitrogen (N-9) was found to facilitate the penetration of tetrahydro-β-carbolines into the brain. Judging from the low solubility of II in heptane, this group would seem to increase the solubility of this compound in lipids. Halogen substitution increased the amount of compound in the brain by lowering the pKa, thus providing more uncharged compound for passage into the brain. Compound III, substituted by the 8-chlorine atom and the 9-methyl group, was shown to be the best inhibitor of mouse brain MAO; its activity was even twofold greater than that previously reported using bovine liver as the enzyme source (3). This compound was also demonstrated to enter the brain not only to a greater extent, but also at a faster rate.

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Interaction of Sodium Erythrosin and Polyvinylpyrrolidone

JON C. ANDERSON and GERARD M. BOYCE

Abstract □ Sodium erythrosin and polyvinylpyrrolidone (PVP) will form a complex in aqueous solution. The effects of pH and PVP concentration have been investigated and a spectrophotometric method developed for the quantitation of the dye in oxymix, a PVP-containing pharmaceutical system. It has also been determined that PVP improves the color stability of an erythrosin solution over a broad pH range.

Keyphrases □ Polyvinylpyrrolidone, interaction—sodium erythrosin □ Spectrophotometry—quantitation of dye □ Stability—erythrosin solutions □ pH effect—erythrosin and erythrosin-PVP solutions

Numerous investigations have been conducted on the binding of polyvinylpyrrolidone (PVP) with various pharmaceutical agents and azo dyes in aqueous solution (1–8). A recent publication described the complex formation between sodium fluorescein, a phthalein dye, and this polymer (9).

In these laboratories, while investigating the chemistry of the excipients in oxymix,[1] Tarlin (10) observed that an association occurred between PVP and the 2,4,-

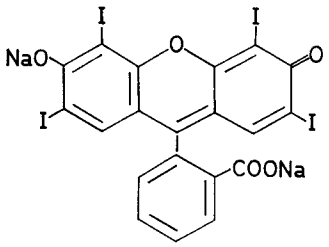

Figure 1—*Sodium erythrosin.*

5,7-tetraiodo derivative of sodium fluorescein, sodium erythrosin (Fig. 1). The complexation was indicated by a bathochromic shift of the $\lambda_{max.}$ of erythrosin.

In this communication, the effects of pH and PVP concentration on the complex are reported. In addition, a spectrophotometric method for the quantitation of erythrosin in oxymix is described.

EXPERIMENTAL

Materials—Polyvinylpyrrolidone,[2] average molecular weight 25,000; sodium erythrosin,[3] pharmaceutical grade; oxymix (gran-

[1] Ascoxal (Gum-ox, Ascumist), marketed for oral hygiene by Astra Läkemedel, Södertälje, Sweden.

[2] Kollidon, Badische Anilin und Soda Fabrik AG, Ludwigshafen am Rhein, Germany.

[3] Saturnus, Malmö, Sweden.