nancial support for this study from the National Institute of General Medical Sciences (GM 20022).

References and Notes

(1) K. Asghar and L. J. Roth, *J. Pharmacol. Exp. Ther.*, **176**, 83 (1971).
(2) O. Wassermann, *Naunyn-Schmiedebergs Arch. Pharmacol.*, **270**, 419 (1971).
(3) H. Shindo, I. Takahashi, and E. Nakajima, *Chem. Pharm. Bull.*, **19**, 1876 (1971).
(4) Other compounds which bind to cartilage and, under certain conditions, can be used to visualize cartilage have been described: P. Torok, K. O. Raker, and E. Habermann, *Arzneim.-Forsch.*, **22**, 2110 (1972); A. M. Love and T. H. Vickers, *Stain Technol.*, **47**, 7 (1972).
(5) S. C. J. Olivier, *Recl. Trav. Chim. Pays-Bas*, **42**, 516 (1923).
(6) J. Hebky and M. Karasek, *Collect. Czech. Chem. Commun.*, **29**, 3108 (1964).
(7) P. Ruggli, B. B. Bussemaker, W. Muller, and A. Staub, *Helv. Chim. Acta*, **18**, 1388 (1935).
(8) H. Suzuki, K. Nakamura, and R. Goto, *Bull. Chem. Soc. Jpn.*, **39**, 128 (1966).
(9) G. D. Olsen, E. M. Chan, and W. K. Riker, *J. Pharmacol. Exp. Ther.*, **195**, 242 (1975).
(10) S. Kosay, W. K. Riker, and S. Guerrero, *J. Pharmacol. Exp. Ther.*, **180**, 255 (1972).
(11) V. M. Solev'ev and A. P. Skoldinov, *Zh. Obshch. Khim.*, **33**, 1821 (1963).

Synthesis and Antihypertensive Activity of Some Imidazoindole Derivatives

P. K. Adhikary,* S. K. Das,

Department of Biochemistry and Nutrition, Meharry Medical College, Nashville, Tennessee 37208

and B. A. Hess, Jr.

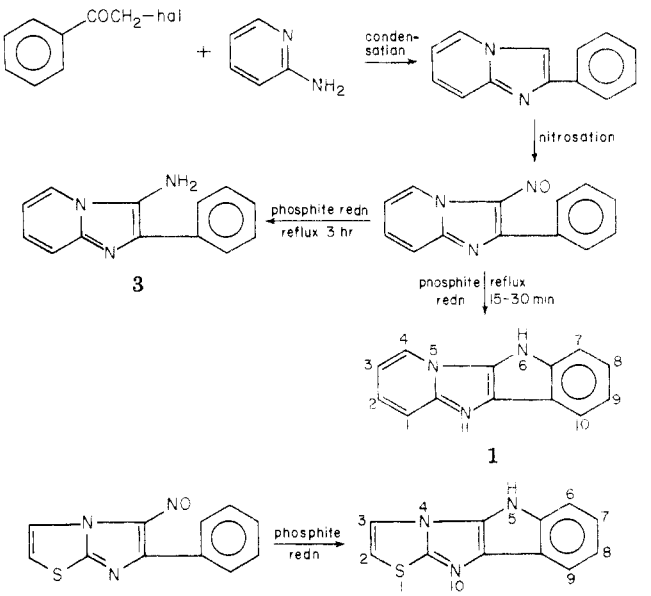
Department of Chemistry, Vanderbilt University, Nashville, Tennessee 37235. *Received December 12, 1975*

The synthesis of pyridino[1,2-*a*]imidazo[5,4-*b*]indole (**1**) and thiazolo[3,2-*a*]imidazo[5,4-*b*]indole (**2**) has been achieved by phosphite reduction of 3-nitroso-6-phenylimidazo[1,2-*a*]pyridine and 5-nitroso-6-phenylimidazo[2,1-*b*]thiazole. Compound **1** has shown strong antihypertensive activity in spontaneously hypertensive rats while compound **2** showed similar bioactivity both in spontaneously hypertensive rats and in normotensive dogs. A tricyclic amino derivative, 3-amino-2-phenylimidazo[1,2-*a*]pyridine, which has structural resemblance to compound **1**, showed no hypotensive activity.

Some known antihypertensive agents like hydralazine, catapres, and the amidines[1-3] have the –N=CNH– group common to their structures which we believe is the basis for the bioactivity of these compounds. We wanted to know if other compounds, in which –N= of the above-mentioned bioactive group was replaced by a bridgehead nitrogen, would show some kind of bioactivity. We were also interested to ascertain the role of higher cyclic order in the bioactivity of such compounds. The objective of the following research was to synthesize some tetracyclic imidazoindole derivatives and to compare the bioactivity of these tetracyclic compounds with a tricyclic compound of similar structure.

Chemistry. The reduction of a nitroso compound by triethyl phosphite, as described by Cadogan,[4] was adopted by us for the reduction of 3-nitroso-2-phenylimidazo-[1,2-*a*]pyridine and 5-nitroso-6-phenylimidazo[2,1-*b*]-thiazole. The nitroso intermediates of pyridine and thiazole derivatives were synthesized by condensation of ω-bromoacetophenone respectively with 2-aminopyridine and with 2-aminothiazole as described by Almirante et al.[5,6] and then nitrosation of the resulting bases with sodium nitrite and acetic acid as described by LaRocca et al.[7] In the case of the pyridine derivative, the phosphite reduction of the nitroso intermediate to the tetracyclic indole derivative, pyridino[1,2-*a*]imidazo[5,4-*b*]indole (**1**), was complete with 15–30 min of refuxing. Further heating yielded gradual decomposition of compound **1** and simultaneous formation of the tricyclic amine, 3-amino-2-phenylimidazo[1,2-*a*]pyridine (**3**), which was the single product obtained after 3 h of refluxing. It could not be ascertained at this stage whether **3** was formed due to the cleavage of the indolic bond of **1** or by the triethyl phosphite reduction of the nitroso intermediate. Compound **3** is the zinc–acetic acid reduction product of 3-nitroso-2-phenylimidazo[1,2-*a*]pyridine[5] and its formation by phosphite reduction is rather unexpected. (The hypothesis that the phosphite reduction occurs via formation of a nitrene intermediate does not favor such a reduction product. We are in the process of conducting additional experiments to resolve this problem.) The phosphite reduction of the nitroso intermediate of thiazole yielded the expected tetracyclic indole derivative, thiazolo[3,2-*a*]imidazo[5,4-*b*]indole (**2**), and no further change of product or yield occurred by prolonging the reaction period (Scheme I).

Scheme I. Synthesis of Indole Derivatives



The assumed structures of **1** and **2** are consistent with their elemental analysis and ir spectra shown in the Experimental Section. In addition, the compounds show

Table I. Hypotensive Effects of the Synthesized Compounds

Test compd	Test animals	Dosage and route of admin	Initial blood pressure in mmHg	Max redn of blood pressure in mmHg	Time required for max redn
1	SHR	20 mg/kg sc	190 ± 3.5[a]	40 ± 3.0[c]	3 h
1	SHR	30 mg/kg sc	185 ± 5.0[a]	32 ± 3.5	3 h
2	SHR	50 mg/kg sc	190 ± 2.5[a]	30 ± 2.0	2 h
2	SHR	100 mg/kg sc	165 ± 3.0[a]	50 ± 2.5	2 h
2	Dog	10 mg/kg iv	150 ± 5.0[b]	50 ± 3.0	15-30 min
3	SHR	50 mg/kg sc	170 ± 2.5[a]		
3	SHR	100 mg/kg sc	175 ± 5.0[a]	Inactive[d]	Time of observation, 3 h
3	Dog	10 mg/kg iv	155 ± 7.5[b]		
	SHR	Solvent, sc	170 ± 3.5[a]	Inactive[d]	Time of observation, 3 h
	Dog	Solvent, iv	155 ± 5.0[b]		

[a] Mean systolic blood pressure ± SD of three animals. [b] Mean arterial blood pressure ± SD of three animals. [c] Mean reduction of blood pressure ± SD of three animals. [d] Indicates variation of blood pressure by less than ±10 mmHg. SHR = spontaneously hypertensive rats.

proper molecular ion distribution in their mass spectra and their structures were further confirmed by a detailed examination of their proton magnetic resonance spectra. While both contain an indole ring, 1 contains a pyridine ring and 2 a thiazole ring. In agreement with this, both their spectra contain two areas of absorption which are essentially identical in appearance, presumably arising from the indole ring; 1 has multiplets at δ 8.24 and 7.54 in a ratio of two to three and 2 at δ 8.22 and 7.52 in the same ratio. They correspond to the four aromatic protons of the indole ring and the NH. The remaining absorptions can be assigned to the pyridine ring of 1 and the thiazole ring of 2. Complete assignments could not be made for the pyridine ring protons in 1 from the normal spectrum due to overlapping of the absorption of two protons. A doublet of doublets was observed at δ 8.60 (1 H, H-4, J = 6, 2 Hz), a triplet of doublets at δ 7.82 (1 H, H-2, J = 8, 2 Hz), and a complex multiplet centered at δ 7.26 (2 H, H-3 and H-1). However, use of the NMR shift reagent Euroshift F (Pierce) separated the complex multiplet at δ 7.26 into a doublet of doublets (1 H, H-1, J = 8, 2 Hz) and a multiplet (1 H, H-3, J = 8, 6, 2 Hz). The assigned coupling constants are in good agreement for those reported for 1-azaindolizine.[8] Finally the two protons of the thiazole ring in 2 appear as a typical AB quartet at δ 7.88 (1 H, H-3, J = 3.5 Hz) and 7.50 (1 H, H-2, J = 3.5 Hz).

Compound 3 was identified as 3-amino-2-phenylimidazo[1,2-*a*]pyridine by elemental analysis, ir, and mixture melting point with a fresh sample of 3-amino-2-phenylimidazo[1,2-*a*]pyridine synthesized by zinc–acetic acid reduction of 3-nitroso-2-phenylimidazo[1,2-*a*]-pyridine.[5]

Biological Activity. The imidazoindole derivatives of pyridine and thiazole together with the tricyclic amine (compound 3) were tested for antihypertensive activity in our laboratories at Meharry Medical College and by McNeil Laboratories Inc., Fort Washington, Pa. Antihypertensive activity was evaluated in unanesthetized spontaneously hypertensive rats (SHR, Taconic Farms, Inc., Germantown, N.Y. 12526) and in anesthetized normotensive dogs. Rats with closely similar systolic blood pressure were divided into groups of three each and the systolic blood pressure of each rat was determined by taking the average of three consecutive blood pressure readings (tail cuff). Initial blood pressure of each rat was determined on three separate days prior to dosing and the average was noted as the mean initial blood pressure. The test compounds were dissolved in a minimum volume of 50% alcohol and known concentrations were administered subcutaneously twice at 7-day intervals. The control group was administered with a equal volume of the solvent (50% alcohol). Systolic blood pressure of the rats was checked hourly after dosing and the reduction of blood pressure as well as the time elapsed was noted for each rat. Mean reduction of blood pressure was determined by taking the average of two separate days' reduction for each rat. The time elapsed for the maximum depression of blood pressure after dosing was noted as the time required for maximum reduction.

Normotensive dogs, anesthetized with pentabarbitol, were used in other experiments. The dogs were divided in groups of three and the mean arterial blood pressure was determined from six arterial blood pressure readings over a period of 3 weeks prior to dosing. The test compounds were dissolved in 50% alcohol and a known concentration was administered intravenously. The control group was administered with a equal volume of the solvent in the same manner. The blood pressures of the animals were checked continuously after dosing and the experiments were repeated with the same animals after 3 days. The mean reduction of the blood pressure was determined by taking the average of two separate days' maximum reduction of the arterial blood pressure after dosing. The time elapsed for the maximum depression of the blood pressure was noted as the time required for maximum reduction of blood pressure.

Compounds 1 and 2 were found to reduce the systolic blood pressure of spontaneously hypertensive rats by approximately 40 mmHg. For 1 maximum hypotensive effect was achieved at a dose level of 20 mg/kg of body weight and for 2 it was achieved at a dose level of 100 mg/kg of body weight. Compound 2 reduced the arterial blood pressure of normotensive dogs by 50 mmHg at a dose level of 10 mg/kg of body weight. Compound 3 did not show any hypotensive activity in test animals (Table I).

The site and mode of action of the bioactive compounds 1 and 2 are being evaluated at this time and the findings of that study will be reported in a later communication.

Structure–Activity Relationship. The compound 1,2,3,5-tetrahydroimidazo[2,1-*b*]quinazoline and some of its derivatives with three-ring systems have structural similarity to our compounds with four-ring systems and have been reported by Loev et al.[1] and Jen et al.[2,3] as effective antihypertensive agents in animals. The same groups of investigators have also found that a structurally similar bicyclic compound, trihydropyrimidinoimidazoline, has no antihypertensive activity. Our tetracyclic compounds 1 and 2 are expected to have planar structures due to their fused aromatic ring systems. The bioactivity shown by our compounds conforms with the finds of Jen et al.[2,3] that a higher cyclic order and linear configuration are required for such bioactivity. The lack of bioactivity shown by the tricyclic compound 3 further corroborates the above theory.

A similar structural analogy may be drawn between the standard hypotensive drugs hydralazine and catapres and the synthesized compounds 1 and 2. Both hydralazine and catapres have the common group -N=CNH- as against >NCNH- in compounds 1 and 2. Apparently the substitution -N= by a bridgehead nitrogen in our compounds did not retard or alter the bioactivity.

Experimental Section

All melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. NMR spectra were taken in $CDCl_3$ on a JEOL MH-100 spectrometer using Me_4Si as internal standard. Mass spectra were recorded on LKB Model 9000. Ir spectra were obtained in KBr on a Beckman IR-12.

Synthesis of the Nitroso Intermediates. General Method. 3-Nitroso-2-phenylimidazo[1,2-*a*]pyridine and 5-nitroso-6-phenylimidazo[2,1-*b*]thiazole were obtained by a combination of methods described by Almirante et al.[5,6] and LaRocca et al.[7] ω-Bromoacetophenone was condensed respectively with 2-aminopyridine and 2-aminothiazole. The condensation products were suspended in 10% NaOH solution, extracted with $CHCl_3$, and evaporated to dryness to obtain the respective free bases. These free bases were finally dissolved in acetic acid and nitrosated with sodium nitrite solution. 3-Nitroso-2-phenylimidazo[1,2-*a*]pyridine after one recrystallization from ethanol had mp 165–167 °C and 5-nitroso-6-phenylimidazo[2,1-*b*]thiazole after one recrystallization from acetone had mp 175–177 °C.

Pyridino[1,2-*a*]imidazo[5,4-*b*]indole (1). A mixture of 9.0 g of analytically pure 3-nitroso-2-phenylimidazo[1,2-*a*]pyridine (0.04 mol) and 10 ml of 97% triethyl phosphite (0.05 mol) in 50 ml of anhydrous toluene was refluxed for 15–30 min with stirring and under a constant flow of dry nitrogen gas. The temperature of the oil bath was kept between 110 and 120 °C. After cooling, the solvent and excess triethyl phosphite were removed by vacuum distillation at 0.2 Torr. The temperature of the oil bath was kept under 120 °C also during the distillation. The residue which was a thick oily liquid was kept overnight at 0 °C during which time it solidified. The solid was washed on a glass filter with cold CCl_4. The residue was taken in a small quantity of $CHCl_3$ and was eluted over a column of activated alumina (80–325 mesh) presoaked with CCl_4. $CHCl_3$ was used as eluent and the first colored zone was collected and evaporated to dryness. The residue after one recrystallization from 2-propanol had mp 78–80 °C; crude yield ~4 g; ir 3410, 3080, and 2580 cm^{-1}. Anal. ($C_{13}H_9N_3$) C, H, N.

Thiazolo[3,2-*a*]imidazo[5,4-*b*]indole (2). About 8.5 g of analytically pure 5-nitroso-6-phenylimidazo[2,1-*b*]thiazole (0.04 mol) was taken with 10 ml of triethyl phosphite and 50 ml of toluene and was refluxed for 3 h with stirring and under constant flow of nitrogen. The rest of the procedure was the same as above. The product after one recrystallization from 2-propanol had mp 102–104 °C; crude yield ~3 g; ir 3120, 3080, and 1560 cm^{-1}. Anal. ($C_{11}H_7N_3S$) C, H, N, S.

3-Amino-2-phenylimidazo[1,2-*a*]pyridine (3). The procedure was the same as for compound 1 except that the reflux was continued for 3 h. The product had mp 212–214 °C after recrystallization from ethanol; crude yield ~3 g; ir 3340, 3080, and 1560 cm^{-1}; mmp (with a synthesized sample of 3-amino-2-phenylimidazo[1,2-*a*]pyridine) 212–214 °C. Anal. ($C_{13}H_{11}N_3$) C, H, N.

Acknowledgment. The authors wish to thank Dr. L. E. Burgess and Dr. M. T. Scott of Meharry Medical College, Nashville, Tenn., for their assistance in measuring blood pressure of rats. We appreciate the help rendered by McNeil Laboratories Inc., Fort Washington, Pa., by performing the hemodynamic studies and providing us with the data. This research was partly supported by Grant No. DE03191 from the National Institute of Health, Bethesda, Md.

References and Notes

(1) B. Loev, P. E. Bender, H. Bowman, A. Helt, R. McLean, and T. Jen, *J. Med. Chem.*, **15**, 727 (1972).
(2) T. Jen, B. Dienel, H. Bowman, J. Petta, A. Helt, and B. Loev, *J. Med. Chem.*, **15**, 727 (1972).
(3) T. Jen, P. Bender, H. Van Hoeven, B. Dienel, and B. Loev, *J. Med. Chem.*, **16**, 407 (1973).
(4) J. I. G. Cadogan, *Synthesis*, **1**, 11 (1969).
(5) L. Almirante, L. Polo, A. Mugnaini, E. Provinciali, P. Rugarli, A. Biancotti, A. Gamba, and W. Murmann, *J. Med. Chem.*, **8**, 305 (1965).
(6) L. Almirante, L. Polo, A. Mugnaini, E. Provinciali, P. Rugarli, A. Gamba, A. Olivi, and W. Murmann, *J. Med. Chem.*, **9**, 29 (1966).
(7) J. P. LaRocca, C. A. Gibson, and B. B. Thompson, *J. Pharm. Sci.*, **60**, 74 (1971).
(8) P. J. Black, M. L. Heffernan, L. M. Jackman, Q. N. Porter, and G. R. Underwood, *Aust. J. Chem.*, **17**, 1128 (1964).

Communications to the Editor

A Circular Dichroism Study of the Interaction of Sodium and Potassium Ions with Methionine-Enkephalin[1]

Sir:

Several laboratories have shown that a group of peptides found in brain tissue[2-4] and the pituitary gland[5,6] possesses binding characteristics and biological activity similar to that of morphine.[2-12] One of these substances is the pentapeptide, Tyr-Gly-Gly-Phe-Met, known as methionine-enkephalin (Met-enkephalin).[12] As the stereospecific binding of Met-enkephalin to rat-brain homogenate is known to be reduced in the presence of Na^+,[6,9] we have investigated the possibility that the reduced affinity might in part be related to a sodium-induced conformational change of the ligand due to complexation. In this communication we report on the interaction of Na^+ and K^+ with Met-enkephalin using circular dichroism (CD) as a means of detecting conformational changes.

The use of CD to assess oligopeptide conformations in solution is based mainly on empirical comparisons of standard curves obtained from the model peptide, poly-L-lysine.[13] We have extended the use of this model using an indirect approach[14] based on the assumption that the aromatic side chain and carbonyl contributions to the CD spectrum of Met-enkephalin are additive[15] and that aromatic contributions are relatively constant[16] in different conformational states of the peptide chain. Under such conditions conformational changes of the peptide chain can be detected by difference curves.[17]

The CD spectrum of Met-enkephalin in water is characterized by a group of ^1L_b aromatic transitions of weak positive ellipticities from 290 to 254 nm (Figure 1). An inflection at 225 nm and a peak at 218 nm (Figure 2) fall in the diagnostic area of the tyrosine and phenylalanine residues.[18] An additional band is detected at 212 nm and has been tentatively assigned to the terminal carboxylate on the basis of its position and the increasing ellipticity