situation has also been proposed by Glickson, *et al.* (ref 17, p 563), for Ang in aqueous solution. As yet there is no evidence to indicate that the histidine side chain plays a role in determining the conformation of Ang under physiological conditions or at the receptor. For these reasons we have attempted to interpret our results with the 6-position analogs in terms of receptor interaction rather than conformation. Combined with other studies of pH effects on smooth muscle response to Ang (ref 17, p 521) and of alkylating derivatives of Ang,[18] this interpretation has led to a better understanding of hormone-receptor interaction and a new interpretation of tachyphylaxis.[19]

In summary, these results indicate that a structure resembling the imidazole side chain of His^6 is required for good biological activity of Ang. Both aromatic and nucleophilic character are useful properties of residues at this position. In contrast, substitution of strongly basic or acidic residues results in essentially total inactivation of the peptide.

Experimental Section

All *tert*-butyloxycarbonylamino acids were purchased from Schwarz BioResearch except Boc-Pro, Boc-Orn(Z), and Boc-Glu(Bzl) which were purchased from Fox Chemical Co. *tert*-Butyloxycarbonylthienylalanine was a gift of Dr. Floyd W. Dunn. Other special amino acid derivatives used were Boc-Tyr(Bzl), Boc-Arg(NO_2), Boc-His(Tos), and Boc-Asp(Bzl). Reagent grade $CHCl_3$ was used as obtained.

Solid-Phase Synthesis of Peptides. The essentials of the procedures used have been published previously.[12] Boc-Phe was esterified to 1% crosslinked chloromethyl resin (Bio-Rad Laboratories, 1.7 mequiv of Cl/g) by the standard procedure. Hydrolysis and amino acid analysis of an aliquot of the resin showed the degree of substitution to be 0.52 mmol/g. All syntheses were carried out on an improved model of the automatic instrument previously described.[20] For each synthesis an amount of Boc-Phe resin (0.77 g) was used which contained a total of 0.4 mmol of Phe. The Boc-protecting group was removed by a 30-min treatment with 25% CF_3CO_2H in $CHCl_3$ (v:v) (also containing 1 mg/ml of 2-mercaptoethanol or indole) following a prewash with the same reagent. After six $CHCl_3$ washes the trifluoroacetate form of the α-NH_2 group was neutralized with 10% Et_3N in $CHCl_3$ (v:v) for 10 min following a prewash with the same reagent. The resin was again washed six times with $CHCl_3$ and the coupling of the next *tert*-butyloxycarbonylamino acid was carried out with DCI in $CHCl_3$. The DCI and *tert*-butyloxycarbonylamino acids were present in 2.5-fold excess (1.0 mmol total) and the coupling time was 2 hr. Because of its limited solubility in $CHCl_3$, Boc-Arg(NO_2) was coupled in 1:1 DMF–$CHCl_3$. After the last residue was coupled, the peptide-resin was subjected to a final deprotection step. The resin was then washed with EtOH and CH_2Cl_2 and dried *in vacuo*. Cleavage of the remaining blocking groups and removal of the peptide from the resin were accomplished in anhydrous HF (10 ml per g of resin) in the presence of 50 equiv of anisole for 30 min at 0°. After evaporation of the HF, the resin and cleaved peptide were dried *in vacuo* and then washed with EtOAc to extract the remaining anisole and its reaction products. The peptides were extracted from the resin with glacial AcOH and dried by lyophilization.

The crude peptides were purified by countercurrent distribution in *n*-BuOH–AcOH–H_2O (4:1:5). Paper electrophoresis was carried out at either pH 5 (pyridine–AcOH; 0.1 *M* in pyridine) or pH 2.8 (1 *M* AcOH). Tlc was done on commercially prepared (Brinkmann or Eastman) cellulose plates developed in solvents F and I of ref 12, p 59. Electropherograms and tlc plates were developed with ninhydrin, Sakaguchi, and Pauly reagents.[12] Quantitative amino acid analyses were carried out on samples hydrolyzed in sealed N_2-flushed tubes for 72 hr at 110° in 6 *N* HCl containing 1 mg/ml each of PhOH and 2-mercaptoethanol. Partial destruction of thienylalanine occurs even in the presence of these protective reagents. The analyses were carried out on a Beckman 120C amino acid analyzer.

Bioassays. Rat uterus assays were carried out in modified de Jalon's solution containing 20 mg/l. of $CaCl_2$. Guinea pig ileum assays were carried out in Tyrode's solution containing 0.1 μg/ml of atropine sulfate. The details of the procedure were as described earlier[21] except that both solutions were bubbled with 98% O_2–2% CO_2 to give a final pH of 7.4. All assays were done at a bath temperature of 28°.

Rat blood pressure assays were carried out as previously described.[14] The peptides were administered by intrajugular injection. In those experiments where ganglion blockade was used, pentolinium tartrate was administered at a dose of 5 mg/kg ip.

Acknowledgments. The authors thank Misses Martha Knight, Alice Gordon, and Katherine Hill and Mrs. Edith McConnell for their expert technical assistance and Mrs. Roberta Tudor for the amino acid analyses. The research was aided by NIH Grant HL-12325 and the Population Council.

References

(1) *J. Biol. Chem.*, 241, 2491 (1966).
(2) L. T. Skeggs, K. E. Lentz, N. P. Shumway, and K. R. Woods, *J. Exp. Med.*, 104, 193 (1956).
(3) E. Schröder and K. Lübke, "The Peptides," Vol. 2, Academic Press, New York, N. Y., p 4.
(4) F. M. Bumpus, R. R. Smeby, and P. A. Khairallah in "Peptides: Chemistry and Biochemistry," B. Weinstein and S. Lande, Ed., Marcel Dekker, New York, N. Y., p 127.
(5) E. C. Jorgenson, G. C. Windridge, W. Paton, and T. C. Lee, ref 4, p 113.
(6) F. M. Bumpus in "Structure-Activity Relationships of Protein and Polypeptide Hormones," M. Margoulies and F. C. Greenwood, Ed., Excerpta Medica, Amsterdam, 1971, p 18.
(7) A. C. M. Paiva and T. B. Paiva. *Biochim. Biophys. Acta*, 48, 412 (1961).
(8) E. Schröder, *Justus Liebigs Ann. Chem.*, 680, 142 (1964).
(9) E. Schröder and R. Hempel, *ibid.*, 684, 243 (1965).
(10) M. C. Khosla, S. Kumar, R. R. Smeby, and F. M. Bumpus, *J. Med. Chem.*, 15, 627 (1972).
(11) R. Andreatta and K. Hofmann, *J. Amer. Chem. Soc.*, 90, 7334 (1968).
(12) J. M. Stewart and J. D. Young, "Solid-Phase Peptide Synthesis," W. H. Freeman, San Francisco, Calif., 1969.
(13) V. B. Schatz in "Medicinal Chemistry," A. Burger, Ed., Interscience, New York, N. Y., p 79.
(14) F. W. Dunn and J. M. Stewart, *J. Med. Chem.*, 14, 779 (1971).
(15) R. J. Weinkam and E. C. Jorgensen, *J. Amer. Chem. Soc.*, 93, 7038 (1971).
(16) S. Fermandjian, J.-L. Morgat, and P. Fromageot, *Eur. J. Biochem.*, 24, 252 (1971).
(17) J. Meienhofer, Ed., "Chemistry and Biology of Peptides," Ann Arbor Science Publishers, Ann Arbor, Mich., 1972, pp 545–581.
(18) T. B. Paiva, A. C. M. Paiva, R. J. Freer, and J. M. Stewart, *J. Med. Chem.*, 15, 6 (1972).
(19) J. M. Stewart and R. J. Freer, *Intra-Sci. Chem. Rep.*, in press.
(20) R. B. Merrifield, J. M. Stewart, and N. Jernberg, *Anal. Chem.*, 38, 1905 (1966).
(21) R. J. Freer and J. M. Stewart, *J. Med. Chem.*, 15, 1 (1972).

L-5-Oxo-2-pyrrolidinecarboxamides from Phenethylamines

Lewis A. Walter,* Robert J. Gyurik, Wei Chang,

Medicinal Chemistry Research Department

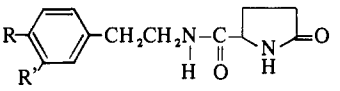
and Allen Barnett

Department of Pharmacology, Schering Corporation, Bloomfield, New Jersey 07003. *Received October 10, 1972*

The L-pyroglutamide of *d-trans*-2-phenylcyclopropylamine recently was found inactive as a monoamine oxidase (MAO) inhibitor *in vitro*, though readily cleaved by a rat brain enzyme to the free amine.[1] The latter is a clinically useful antidepressant acting *via* MAO inhibition.

It thus seemed possible that the L-pyroglutamide of dopamine and/or similar compounds might cross the blood-brain barrier more readily than its usual amino acid precursor and that subsequent enzymatic cleavage of the amide might give

Table I. L-5-Oxo-2-pyrrolidinecarboxamides



No.	R	R'	Mp, °C[a]	Crystn solvent[b]	Yield, %	$[\alpha]^{26}D$	1% in solvent	Formula[c]
1	H	OH	133-134	AE	60	−16.7	E	$C_{13}H_{16}N_2O_3$
2	OH	OH	182.5-184.5[d]	E	77	−4.2	D	$C_{13}H_{16}N_2O_4$
3	MeO	OH	151-151.5	A	74	−1.3	D	$C_{14}H_{18}N_2O_4$
4	OH	MeO	150-151	A	65	−5.3	D	$C_{14}H_{18}N_2O_4$
5	MeO	MeO	131-133	A	36	−39.7	C	$C_{15}H_{20}N_2O_4$
6	H	$PhCH_2O$	138-140	A	73	−29.6	C	$C_{20}H_{22}N_2O_3$
7	$PhCH_2O$	MeO	95-97	A	63	−28.8	C	$C_{21}H_{24}N_2O_4$
8	MeO	$PhCH_2O$	144.5-145.5	EA	42	−28.7	C	$C_{21}H_{24}N_2O_4$
9	$PhCH_2O$	$PhCH_2O$	104-106	A	89	−24.7	C	$C_{27}H_{28}N_2O_4$

[a] Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. [b] A, MeCN; EA, EtOAc; E, EtOH; C, $CHCl_3$; D, DMF. [c] Analyses for C, H, and N were within ±0.4% of the theoretical values. [d] Subsequent to this work, this compound has been described in German Patent 2,153,825 issued to Hoffmann-La Roche (May 1972).

increased brain levels of the amine. Such an increase with dopamine purportedly is helpful in the treatment of Parkinson's disease.[2] Accordingly, the compounds described in Table I were prepared and tested as follows.

Pharmacology. The compounds were screened in groups of three male CF No. 1 mice by the method of Irwin[3] using apomorphine, a dopamine-like compound that passes the blood-brain barrier as a standard.

Four types of stereotyped behavior were seen after apomorphine: continual sniffing, head-searching, rearing, or chewing movements. Because these movements were occurring simultaneously in various combinations, it was not possible to score the intensity or duration of each of the movements. Therefore, a quantal measure had to be used. A maximum combination of three of these movements was found after high doses of apomorphine (*e.g.*, sniffing, head-searching, and rearing) and this was used as the criterion for significant stereotypy. Any mouse that showed a combination of any three of the four movements (continual sniffing, rearing, head-searching, or chewing) was considered to have exhibited significant stereotypy. For apomorphine all three mice in each group receiving 30, 100, or 300 mg/kg po exhibited significant stereotypy whereas apomorphine was inactive at 10 mg/kg po in the three mice tested at this dose. The ED_{50} for apomorphine was estimated graphically as 54.0 mg/kg. No confidence limits could be calculated because of the steepness of the slope of the dose-response curve.

Groups of three mice received 100 mg/kg po of each test compound and none of the compounds reported met the defined criterion for significant stereotypy in any of the mice tested. The enzymatic hydrolysis of these compounds was not studied and whether or not they cross the blood-brain barrier is unknown but their lack of activity relative to apomorphine discouraged further work.

Experimental Section

The compounds in Table I were prepared by heating a 5% excess of the appropriate amine and methyl L-pyroglutamate at 60–100° until an ir spectrum of the mixture indicated the reaction was essentially complete. The product was recrystallized directly or, if necessary, was first purified by washing a $CHCl_3$ solution with dilute HCl, $NaHCO_3$, and H_2O. The benzyl groups were hydrogenolyzed with Pd/C in alcohol in the usual manner.

References

(1) J. J. McMonigle and A. Horita, *Arch. Int. Pharmacodyn. Ther.*, 178, 53 (1969).
(2) G. C. Cotzias, P. S. Papavasiliou, J. Ginos, A. J. Steck, and S. E. Duby, *Annu. Rev. Med.*, **22**, 305 (1971).
(3) S. Irwin, "Clinical Pharmacological Techniques," J. H. Nodine and P. S. Siegler, Ed., Yearbook Medical Publishers, Chicago, Ill., 1964, Chapter 4.

Antifertility Activity of Some β-Amino Alcohols[1]

Duane F. Morrow,* Porter C. Johnson, Houshang Torabi, David Williams, Donald L. Wedding, Joe W. Craig, Robert F. Majewski,

Department of Chemical Research

John P. Braselton, and Duane G. Gallo

Department of Pharmacology, Mead Johnson Research Center, Evansville, Indiana 47721. *Received July 24, 1972*

The addition of a basic ether side chain to a variety of both steroidal[2] and nonsteroidal estrogens[3] confers a significant degree of antifertility activity to these compounds. It has been shown that this effect is caused primarily by an antiestrogenic activity produced by the addition of the basic side chain.[3,4] However, no study of the antifertility effect of the amino alcohols themselves corresponding to these side chains had yet been reported and a study of the activity of these compounds was undertaken.

Some simple amino alcohols were prepared, and one of them, 2-(isopropylamino)ethanol (**2**), exhibited an unexpectedly high degree of antifertility activity. On this basis, the series was extended to include a variety of amino alcohols and related compounds in order to determine the structural features necessary for activity in this type of compound. Those compounds showing significant antifertility activity are listed in Table I, and those with weak activity as well as the inactive compounds which were previously unreported on the literature are tabulated in Table II.†

The structure of **2** can be considered as being comprised of four distinct "parts," each of which was varied in turn, keeping the other three constant in order to determine the relative importance of each to the antifertility activity.

Hydroxyl Group. The hydroxyl group was replaced by

†A complete listing of all compounds tested in this series will appear following these pages in the microfilm edition of this volume of the journal. Single copies may be obtained from the Business Operations Office, Books and Journals Division, American Chemical Society, 1155 Sixteenth St., N.W., Washington, D. C. 20036. Remit check or money order for $3.00 for photocopy or $2.00 for microfiche, referring to code number JMED-73-736.