Table III. Antileukemic Activity (L1210 Leukemic Mice) of Ara-Cytidine Esters[a]

Compd	ara-C derivative	Median Survival (days)	%ILS[b]
	Control	9.0	
7b	2',3'-Distearate	8.5	0
7a	2',3'-Dipalmitate	10.5	17
7c	2',3'-Dibenzoate	8.5	0
6b	2'-Stearate	12.5	39
6c	2'-Benzoate	10.0	11
5c	3'-Benzoate	13.0	45
5b	3'-Stearate		292[c]
	5'-Benzoate		185[c]
	5'-Stearate		216[c]
	5'-Palmitate		>300[d]

[a] Single dose treatment (200 mg/kg) ip, 1 day after tumor inoculation. [b] % increase in life-span calculated from median survivals. [c] Data from other experiments (G. L. Neil, unpublished data and ref 9). [d] 4/8 mice treated were cured (45-day survivors) at 100 mg/kg (ref 9).

tecting groups, the 2'-*O*- or 3'-*O*-monoesters were crystd from various solvents as indicated in Table I. The nmr data for the 2'-*O*- and 3'-*O*-esters are compiled in Table II.

Biological Testing of 2'- and 3'-Esters. Methods used in the testing of these compounds for antileukemic activity and immunosuppressive activity were similar to those used for 5'-esters of ara-cytidine.[9] Test data for the leukemic mice are indicated in Table III. All compounds were administered intraperitoneally (ip) as suspensions in 0.2 ml of aqueous methylcellulose to female BDG mice, one day after ip inoculation with 1×10^5 L1210 cells/mouse. The dose employed was 200 mg/kg. Groups of 8 mice were used. Per cent increase in life-span (% ILS) was calculated from median survivals of treated and control (untreated) groups. Data for some of the 5'-esters are included for comparison. The immunosuppressive activities of all of the compounds prepared in this work were consistently lower than the activities of the 5'-*O*-esters. Consequently no detailed results are included.

References

(1) D. T. Gish, G. L. Neil, and W. J. Wechter, *J. Med. Chem.*, 14, 882 (1971) (paper 12).
(2) G. L. Neil, P. F. Wiley, R. C. Manak, and T. E. Moxley, *Cancer Res.*, 30, 1047 (1970).
(3) D. T. Gish, R. C. Kelly, G. W. Camiener, and W. J. Wechter, *J. Med. Chem.*, 14, 1159 (1971).
(4) G. D. Gray, M. M. Mickelson, and J. A. Crim, *Biochem. Pharmacol.*, 18, 2163 (1969).
(5) G. D. Gray and M. M. Mickelson, *Transplantation*, 9, 177 (1970).
(6) G. D. Gray and M. M. Mickelson, *Immunology*, 19, 419 (1970).
(7) L. N. Nikolenko, W. N. Nesawibatko, A. F. Usatiy, and N. M. Semjenowa, *Tetrahedron Lett.*, 5193 (1970).
(8) T. B. Windholz and D. B. R. Johnston, *ibid.*, 2555 (1967).
(9) G. D. Gray, F. R. Nichol, M. M. Mickelson, G. W. Camiener, D. T. Gish, R. C. Kelly, W. J. Wechter, T. E. Moxley, and G. L. Neil, *Biochem. Pharmacol.*, 21, 465 (1972).

Synthesis of Some Analogs of Angiotensin II as Specific Antagonists of the Parent Hormone†

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Syntheses of [8-cyclohexylalanine]-, [Val[8]]-, [Leu[8]]-, [Phe[4], Ala[8]]-, [Suc[1], Phe[4], Tyr[8]]-, and [Sar[1], Ile[8]]-angiotensin II was carried out by solid-phase technique to study factors responsible for agonistic or antagonistic properties in angiotensin II. While pressor response decreased, when position 8 (Phe) of angiotensin II was replaced with 3-amino-4-phenylbutyric acid (10%), DL-3-amino-2-benzylpropionic acid (1%), cyclohexylalanine (20%), valine (0.5%), or leucine (0.3%), antagonistic effect enhanced in the same order; analogs with valine and leucine were found to be very potent and specific antagonists of angiotensin II. The antagonistic properties of [Ile[8]]angiotensin II increased when aspartic acid in position 1 was substituted with sarcosine. However, antagonistic activity decreased when position 1 (Asp) in [Phe[4],Tyr[8]]angiotensin II was replaced with the succinic acid residue or when Tyr in position 4 of [Ala[8]]-angiotensin was replaced with Phe.

Structural modification of angiotensin II (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe) indicates that replacement of the aromatic residue (Phe) by an aliphatic group in position 8 invokes antagonistic activity.[2-7] To further evaluate the potential of this modification, phenylalanine in position 8 was replaced by: (a) cyclohexylalanine (V), to delineate the contribution of aromaticity or ring size for myotropic response; (b) valine and leucine, to investigate the nature of aliphatic side chain required for potent antagonism; (c) 3-amino-4-phenylbutyric acid (III) or DL-3-amino-2-benzylpropionic acid (VI), to study the importance of the position of the aromatic side chain in relation to the remainder of the peptide chain for myotropic response. Transposition of the amino acid residues of positions 4 (Tyr) and 8 (Phe) in angiotensin II also invoked antagonistic activity to the parent hormone;[8] [Phe[4], Ala[8]] angiotensin II was synthesized to investigate the contribution of position 4 in relation to position 8 for the antagonistic effect. [Sar[1], Ile[8]]-, and [Suc[1], Phe[4], Tyr[8]]angiotensin II were synthesized to possibly improve the antagonistic properties.

Synthesis and Purification of Analogs. The solid-phase procedure of peptide synthesis[9] suffers from several shortcomings; the most serious of these is the formation of failure sequences and truncated sequences.[10,11] These are caused by incomplete deblocking or incomplete coupling. Also, mechanical shaking may break polymer beads, thereby exposing dormant sites, where coupling could take place to give rise to shortened chains. To avoid these difficulties, the Boc group was deblocked with CF_3COOH in CH_2Cl_2,[12,13] each coupling step was carried out twice,[14,15] and the poly-

†This investigation was partially supported by Grant HL-6835 from the National Heart and Lung Institute and partially by General Research Support Grant FR-5674. Presented in part at the 162nd National Meeting of the American Chemical Society, Washington, D. C., Sept 12–17, 1971.[1] Abbreviations used are: L-cyclohexylalanine = Cha; 3-amino-4-phenylbutyric acid = Apb; 3-amino-2-benzyl propionic acid = Apib. (In earlier publications[2-7] this compound was referred to as 3-amino-3'-phenylisobutyric acid.) Unless stated otherwise, all amino acids are of the L variety.

mer was stirred by bubbling N_2 through the mixture.[16-18] In spite of these modifications, the initial product obtained still showed a number of side products. Purification of the desired peptides was carried out by chromatography on AG-1 (AcO^-) ion-exchange resin using buffers of varying pH, either by a stepwise or by a gradient procedure. If necessary, the compound was then further purified by partition chromatography on Sephadex G-25. The optical homogeneity, as determined by the procedure of Jorgensen, *et al.*,[19] indicated that the purified peptides had all L-amino acids. No attempt was made to isolate and study the structure of various side-products formed.

Biological Results.‡ The pressor activity was determined by pressor assay in vagotomized, ganglion-blocked rat.[20] Isolated rabbit aortic strips were used to study inhibition of myotropic activity, and inhibition of pressor activity was studied *in vivo* with anesthetized rats and cats. The results obtained indicate that [Val^8]- and [Leu^8]angiotensins II have 0.5 and 1.3% pressor activity of angiotensin II, respectively, and that both these analogs have a specific, competitive antagonistic effect against angiotensin II which is comparable to [Ile^8]angiotensin II[6] both *in vitro* and *in vivo* studies. The pressor response of [Sar^1, Ile^8]angiotensin II is approximately 1.0% of angiotensin II and preliminary studies suggest an increase in the antagonistic properties of this compound as compared to [Ile^8]angiotensin II.

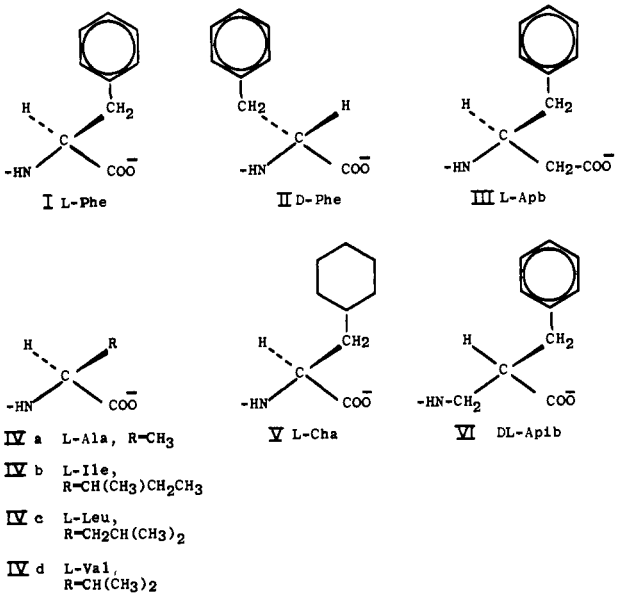
[Cha^8]angiotensin II gave agonistic activity which was equal to approx 20% of angiotensin II, both *in vitro* and *in vivo*. Continued administration, however, completely blocked the myotropic effect due to angiotensin II and [Cha^8]angiotensin II on rabbit aortic strips. In intact rats, [Cha^8]angiotensin II antagonized the pressor response to angiotensin II at a dose level of 0.9 ng/g body weight. Similar antagonistic activity was also observed in dogs and cats. This antagonistic activity may possibly be related to tachyphylaxis.

[Apb^8]angiotensin II showed approx 10% pressor activity of angiotensin II but no antagonistic activity, whereas [DL-Apib^8]angiotensin II had 1% pressor activity and it antagonized the myotropic response of angiotensin II on rabbit aortic strips at a dose level of approx 5 μg/ml. The duration of action of this analog was very short and the antagonistic effect was overcome in 5 min.

[Phe^4, Ala^8]angiotensin II showed 0.001% pressor activity of angiotensin II and negligible myotropic activity. When tested on rabbit aortic strips this compound blocked the myotropic response to angiotensin II at a dose level above 1 μg/ml. At a concentration of 10 μg/ml it decreased the myotropic effect of 16 ng/ml of angiotensin II by about 64% and at 20 μg/ml concentration, it completely blocked 7.6 ng/ml of angiotensin II. The antagonistic effect of this compound was readily reversed, and the responses returned to control level within 30 min.

When tested on rabbit aortic strips, [Suc^1, Phe^4, Tyr^8]-angiotensin II antagonized the myotropic effect of angiotensin II above a concentration of 10 μg/ml. It had 0.19% pressor response and negligible antagonistic effect in the *in vivo* pressor assays on rats.

Structure–Activity Relationships. The low pressor response obtained by the replacement of position 8 of angiotensin II with D-Phe^5 (II), L-Apb (III), or DL-Apib (VI) indicate that for a good pressor and myotropic response, the



amino acid residue in this position should have the L configuration, an aromatic ring in the side chain, and a free carboxyl group. Further, the length of the peptide backbone and the juxtaposition of the carboxyl group in relation to the aromatic side chain should be retained. On the other hand, for antagonistic properties to myotropic response, the aromatic ring in position 8 should be either shifted by 1 C atom (VI), dislocated through spatial orientation (II), or replaced with an H atom (IVa). Present information suggests that to produce potent antagonistic properties to both the myotropic and pressor responses of angiotensin II, the aromatic ring in position 8 should be replaced either with an alicyclic ring (V) or an aliphatic side chain branced at the β or γ position (IV). Since [Cha^8]-angiotensin II shows agonistic properties it appears that cyclohexyl ring may be able to partially mimic the aromatic ring of phenylalanine but once the receptor has been occupied, the peptide no longer acts as an agonist. The decrease in antagonistic properties of [Phe^4, Tyr^8]-angiotensin II when position 1 (Asp) in this peptide is replaced with succinic acid or when Tyr in position 4 of [Ala^8]angiotensin is replaced with Phe, indicates that the biological activity of the compounds, due to variation in position 8, is influenced by substituents in positions 1 and 4. It is of interest to note that the replacement of aspartic acid in position 1 with sarcosine in [Ile^8]angiotensin II appears to improve its antagonistic properties.

Experimental Section

Solvents used for ascending paper chromatography (pc) on Whatman No. 1 filter paper and tlc were: (a) *n*-BuOH–AcOH–H_2O (BAW) (4:1:5); (b) *n*-BuOH–AcOH–H_2O–Pyr (BAWP) (30:6:24:20); (c) *n*-BuOH–AcOEt–AcOH–H_2O (BEAW) (1:1:1:1); (d) *n*-BuOH–Pyr–H_2O (BPW) (65:35:65); (e) MeCOEt–Pyr–H_2O (MPW) (40:20:16). Ascending tlc was conducted on silica gel or cellulose supported on glass plates (Merck AG). Ionophoresis was carried out on filter paper strips on S & S 2043A filter paper strips in Beckman electrophoresis cell (Durrum type) Model R, series D at 400 V, using HCO_2H–AcOH buffer prepared by diluting 60 ml of HCO_2H and 240 ml of AcOH to 2 l. with distd H_2O (pH 1.9) and Beckman barbiturate buffer B-2 (pH 8.6). Glutamic acid was used as a reference compound and *E*(Glu) indicates the electrophoretic mobility relative to glutamic acid = 1.00. Detection of the compound on chromatograms was carried out with ninhydrin and/or with diazotized sulfanilic acid. The free peptides were hydrolyzed in 6 *N* HCl at 110° for 36 hr. Amino acid analyses were performed

‡Pressor activities were determined by Dr. S. Sen, and antagonistic activities by Dr. R. K. Türker, Cleveland Clinic, Research Division.

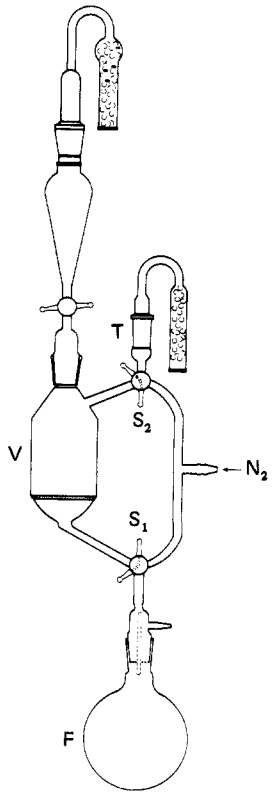


Figure 1. Apparatus for solid-phase peptide synthesis.

on Jeolco-5AH amino acid analyzer. Melting points were taken on a Leitz Wetzlar hot-stage apparatus and are uncorrected. Elemental analyses were performed by Micro-Tech Laboratories, Skokie, Ill., where analyses are indicated only by symbols of the elements or functions, analytical results obtained for those elements or functions were within ±0.4% of the theoretical values.

Manual apparatus for synthesis (Figure 1) consists of a cylindrical reaction vessel (V) (5 × 12 cm) fitted at the bottom with a coarse glass fritted disk of 5 cm diameter. The tube below the fritted coarse disk was connected to a 4-mm, 3-way stopcock S_1 with 1:5 Teflon plug (Corning Glass Works 7451), which could connect the reaction vessel either to N_2 supply or to a receiving flask F. On the top, the vessel was fitted with a dropping funnel (through (ξ 24/40 male and female joints) and the side arm of another 3-way stopcock S_2. For percolation, stopcock S_1 was connected to N_2 supply and stopcock S_2 was connected to outlet tube T. While for filtration stopcock $S_2$was connected to N_2 supply and stopcock S_1 was connected to the filter flask F. During cleavage of the peptide from the polymer, N_2 was replaced by HBr.

Procedure for solid-phase peptide synthesis consists of esterification with C-terminal amino acid[21] followed by coupling of each amino acid residue utilizing the cycle of reactions given below. Unless specified, all washings were carried out three times for 3 min. The esterified polymer was: (1) washed with glacial acetic acid; (2) washed with CH_2Cl_2; (3) the Boc group removed by treatment with 40% (v/v) CF_3CO_2H in CH_2Cl_2 for 30 min; a prewash with this reagent for 3 min was necessary to avoid diln of CF_3CO_2H by the previous CH_2Cl_2 wash; (4) washed with $CHCl_3$ (5 times for 2 min); (5) the free amino group liberated by treatment with 10% Et_3N in $CHCl_3$ for 7 min; (6) washed with $CHCl_3$; (7) washed with CH_2Cl_2; (8) Boc-amino acid (2-fold excess) in CH_2Cl_2 was added and mixed for 10 min. Boc-Arg (NO_2) and Boc-His(Bzl) were first dissolved in DMF, any insoluble material filtered, and the filtrate mixed with 0.33 vol of CH_2Cl_2; both these derivs were added in 3-fold excess. (9) DCI (2-fold excess) in CH_2Cl_2 was added and mixed for 2.5 hr, except in the case of Boc-Arg (NO_2) and Boc-His(Bzl) where 3-fold excess of DCI was used and the mixt mixed for 8 hr. The polymer was: (10) washed with DMF; (11) washed with a 1:1 mixt of DMF-CH_2Cl_2; (12) steps 8 and 9 were repeated using a 1:1 mixt of DMF-CH_2Cl_2 as the solvent;[14] (13) washed with 1:2 mixt of MeOH-$CHCl_3$ to remove dicyclohexyl urea;[22] (14) washed with DMF. Completeness of coupling at intermediate stages was checked by the color test[23] or by hydrolysis.[24] At the end of synthesis, the peptide was cleaved from the polymer and hydrogenated over Pd Black in the usual way.[21]

The crude products were purified on a column (5 × 100 cm) of AG-1 × 2 (200–400 mesh) in acetate form by eluting with 0.25 *M* pyridine–0.05 *M* NH_4OAc buffers of varying pH. The resin was conditioned by washing with a soln 1 *M* in both AcOH and AcONa, deionized H_2O, pyridine–ammonium acetate buffer (pH 7.5), and pyridine–ammonium acetate buffer (pH 8.0). Crude peptide (approx 500 mg) was dissolved in pyridine–ammonium acetate buffer, and pH of the soln adjusted to 8.4 with NH_4OH. This soln was filtered onto wet resin (30 ml), pH readjusted to 8.4 with NH_4OH, and the resin slurry applied to the column. The column was then eluted with pyridine–NH_4OAc buffers in the following order: pH 8.0 (500 ml); linear gradient[25,26] pH 8.0–6.0 (2 l.); pH 6.0 (1 l.). Alternatively, the column was eluted with buffers of pH 8, 7, 6, and 5.5 in a step-wise manner. Both these procedures gave comparable results. Fractions (10 ml) were collected at a flow rate of 80 ml/hr. The effluent was monitored at 280 mμ, and fractions containing the major component, as evidenced by uv absorbance and thin-layer chromatography, were pooled and lyophilized. In general, the peptides obtained were homogeneous but, if necessary, the compound was further purified by partition chromatography on Sephadex G-25 using BuOH–pyridine–H_2O (10:1:5, upper phase) as the solvent. [Apb[8]]-, and [Apib[8]]angiotensin were synthesized as reported earlier.[27]

L-Cyclohexylalanine HCl salt was prepd by a modified procedure of Wassar and Brauchli.[28] A soln of L-tyrosine (10 g) in 1.8 *M* HCl (200 ml) was hydrogenated over PtO_2 (5 g) at 50 psi for 48 hr. The ppt formed was dissolved by the addn of H_2O, the catalyst filtered, and the filtrate evapd *in vacuo*. Tlc (silica gel) in MeOH indicated one major spot (R_f 0.53) and a minor spot (R_f 0.45). The crude product was purified by passing through a column (5 × 25 cm) of silica gel ARCC-4 (100–200 mesh) using a mixt of 4:1 $CHCl_3$–MeOH as eluant to give 7.5 g of cyclohexylalanine HCl salt; ir and uv were in accordance with the desired product. The compd was homogeneous on tlc, and amino acid analysis gave only one peak with a retention time greater than phenylalanine.

Boc-L-cyclohexylalanine was prepd from L-cyclohexylalanine HCl salt (5 g, 24.1 mmoles) and Boc-N_3 (3.6 ml, 25.2 mmoles) in 50% dioxane by the addn of 4 *N* NaOH to maintain pH at 9.8: yield, 3 g; mp 45–50°; $[\alpha]^{23}D$ −13.25° (*c* 2, AcOH). *Anal.* ($C_{14}H_{25}NO_4 \cdot H_2O$) C, H, N.

[Cha[8]]angiotensin II had mp 220° dec; $[\alpha]^{23}D$ −37° (*c* 0.54, 1 *N* AcOH); pc R_f 0.40 (BAW), R_f 0.53 (BAWP); tlc (cellulose) R_f 0.76 (BEAW), R_f 0.71 (BAWP), R_f 0.25 (BPW); *E*(Glu) 1.18 (pH 1.9), *E*(Glu) 0.675 (pH 8.4). Amino acid ratios found were: Asp, 0.87; Arg, 1.02; Val, 1.00; Tyr, 0.86; Ile, 0.96; His, 0.99; Pro, 1.00; Cha, 0.98. *Anal.* ($C_{50}H_{77}N_{13}O_{12} \cdot CH_3CO_2H \cdot 2H_2O$) N. Loss at 100° was 4.12%, but it varied with each sample or with different drying conditions.

[Val[8]]angiotensin II had mp 247°dec; $[\alpha]^{27}D$ −70° (*c* 0.5, H_2O); pc R_f 0.35 (BAW), R_f 0.55 (BAWP); tlc (silica gel) R_f 0.13 (BAW), R_f 0.37 (BAWP), R_f 0.12 (BPW); *E*(Glu) 1.26 (pH 1.9). Amino acid ratios found were: Asp, 1.00; Arg, 0.97; Val, 2.00; Tyr, 0.95; Ile, 0.93; His, 0.99; Pro, 1.01. *Anal.* ($C_{46}H_{71}N_{13}O_{12} \cdot CH_3CO_2H \cdot H_2O$) C, H, N.

[Leu[8]]angiotensin II had mp 253° dec; $[\alpha]^{27}D$ −66° (*c* 0.5, 1 *N* AcOH); pc R_f 0.53 (BAW), R_f 0.57 (BAWP); tlc (silica gel) R_f 0.12 (BAW), R_f 0.39 (BAWP), R_f 0.25 (BPW); *E*(Glu) 1.13 (pH 1.9). Amino acid ratios found were: Asp, 0.92; Arg, 1.00; Val, 1.00; Tyr, 0.99; Ile, 0.95; His, 0.97; Pro, 0.93; Leu, 1.06. *Anal.* ($C_{47}H_{73}N_{13}O_{12} \cdot CH_3CO_2H \cdot H_2O$) C, H, N.

[Phe[4], Ala[8]]angiotensin II had mp 245–50° dec; $[\alpha]^{23}D$ −82° (*c* 0.5, 1 *N* AcOH); pc R_f 0.42 (BAW), R_f 0.53 (BAWP); tlc (cellulose) R_f 0.48 (BAW), R_f 0.8 (BEAW), R_f 0.58 (BAWP); tlc (silica gel), R_f 0.12 (BAW), R_f 0.35 (BAWP); *E*(Glu) 1.06 (pH 1.9), *E*(Glu) 0.65 (pH 8.6). Amino acid ratios found were: Asp, 1.00; Arg, 0.92; Val, 1.01; Phe, 0.99; Ile, 0.96; His, 0.92; Pro, 0.98; Ala, 1.00. *Anal.* ($C_{44}H_{67}N_{13}O_{11} \cdot H_2O \cdot CH_3CO_2H$) C, H, N.

[Suc[1], Phe[4], Tyr[8]]angiotensin II was synthesized as usual, except that succinic anhydride, in the presence of 1 equiv of NEt_3 was used to introduce the N-terminal succinyl residue. The peptide obtained did not give color test with ninhydrin, but gave positive tests with Pauly and Sakaguchi reagents: mp 214–218°; $[\alpha]^{23}D$ −90° (*c* 0.5, 1 *N* AcOH); pc R_f 0.80 (BAW), R_f (0.83) (BAWP); tlc (cellulose) R_f 0.91 (BEAW), R_f 0.86 (BAWP), R_f 0.61 (MPW); *E*(Glu) 0.935 (pH 1.9), *E*(Glu) 0.756 (pH 8.4). Amino acid ratios found were: Arg, 1.09; Val, 1.08; Phe, 1.10; Ile, 1.00; Pro, 1.12; His, 1.06; Tyr, 0.94. *Anal.* ($C_{50}H_{70}N_{12}O_{12} \cdot 2H_2O$) C, H, N.

[Sar[1], Ile[8]]angiotensin II had mp 215° dec; $[\alpha]^{27}D$ −36° (*c* 0.5,

DMF); pc R_f 0.54 (BAW), R_f 0.65 (BAWP); tlc (silica gel) R_f 0.13 (BAW), R_f 0.43 (BAWP), R_f 0.09 (BPW); E(Glu) 1.35 (pH 1.9). Amino acid ratios found were: Sar, 1.09; Arg, 0.954; Val, 1.00; Tyr, 0.91; Ile, 2.04; His, 0.956; Pro, 0.935. *Anal.* ($C_{46}H_{73}N_{13}O_{10}\cdot 2H_2O$) C, H, N.

References

(1) M. C. Khosla, R. R. Smeby, and F. M. Bumpus, Abstracts of the 162nd National Meeting of the American Chemical Society, Sept 1971, MEDI 64.
(2) W. K. Park, R. R. Smeby, and F. M. Bumpus, *Biochemistry*, **6**, 3458 (1967).
(3) P. A. Khairallah, A. Toth, and F. M. Bumpus, *J. Med. Chem.*, **13**, 181 (1970).
(4) R. K. Turker, M. Yamamoto, P. A. Khairallah, and F. M. Bumpus, *Eur. J. Pharmacol.*, **15**, 285 (1971).
(5) D. Gagnon, W. K. Park, and D. Regoli, *Brit. J. Pharmacol.*, **43**, 409 (1971).
(6) M. C. Khosla, S. Kumar, R. R. Smeby, and F. M. Bumpus, *J. Med. Chem.*, **15**, 627 (1972).
(7) M. Yamamoto, R. K. Turker, F. M. Bumpus, and P. A. Khairallah, *Eur. J. Pharmacol.*, in press.
(8) G. R. Marshall, W. Vine, and P. Needleman, *Proc. Nat. Acad. Sci. U. S.*, **67**, 1624 (1970).
(9) R. B. Merrifield, *J. Amer. Chem. Soc.*, **85**, 2149 (1963).
(10) E. Bayer, H. Eckstein, K. Hagele, W. A. Konig, W. Bruning, H. Hagenmaier, and W. Parr, *ibid.*, **92**, 1735 (1970).
(11) E. C. Jorgensen, S. R. Rapaka, and G. C. Windridge, *J. Med. Chem.*, **14**, 904 (1971).
(12) F. C. H. Chou, R. K. Chawla, R. F. Kibler, and R. Shapira, *J. Amer. Chem. Soc.*, **93**, 267 (1971).
(13) B. Gutte and R. B. Merrifield, *ibid.*, **91**, 501 (1969).
(14) F. C. Westall and A. B. Robinson, *J. Org. Chem.*, **35**, 2842 (1970).
(15) M. Manning, E. Coy, and W. H. Sawyer, *Biochemistry*, **9**, 3925 (1970).
(16) L. C. Dorman and L. D. Markley, *J. Med. Chem.*, **14**, 5 (1971).
(17) H. C. Beyerman, *Peptides: Chem. Biochem., Amer. Peptides Symp., 2nd*, **1971** (1971).
(18) J. D. Young, W. Voelter, M. Shimizu, C. Y. Leung, W. J. Peterson, and E. Benjamini, *ibid.*, **1971** (1971).
(19) E. C. Jorgensen, S. R. Rapaka, G. C. Windridge, and T. C. Lee, *J. Med. Chem.*, **14**, 899 (1971).
(20) P. T. Pickens, F. M. Bumpus, A. M. Lloyd, R. R. Smeby, and I. H. Page, *Circ. Res.*, **17**, 438 (1965).
(21) M. C. Khosla, R. R. Smeby, and F. M. Bumpus, *Biochemistry*, **6**, 754 (1957).
(22) G. Flouret, *J. Med. Chem.*, **13**, 843 (1970).
(23) E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. I. Cook, *Anal. Biochem.*, **34**, 595 (1970).
(24) J. Scotchler, R. Lozier, and A. B. Robinson, *J. Org. Chem.*, **35**, 3151 (1970).
(25) C. W. Parr, *Biochem. J.*, **56**, XXVII (1954).
(26) C. J. O. R. Morris and P. Morris, "Separation Methods in Biochemistry," Sir Isaac Pitman and Sons Ltd., London, 1964.
(27) N. C. Chaturvedi, W. K. Park, R. R. Smeby, and F. M. Bumpus, *J. Med. Chem.*, **13**, 177 (1970).
(28) E. Wasser and E. Brauchli, *Helv. Chim. Acta*, **7**, 740 (1924).

Sulfur Analogs of Dopamine and Norepinephrine. Inhibition of Catechol-*O*-methyltransferase

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3-Mercaptotyrosine, 3-mercaptotyramine, *N*-acetyl-3-mercaptotyramine, 2-mercapto-5-hydroxyphenethylamine, and 2-mercapto-5-hydroxyphenethanolamine have been synthesized from phenolic precursors by reaction with thiocyanogen chloride followed either by direct reduction to the desired mercapto derivative with hydrosulfide ion or mercaptoethanol, or by acid hydrolysis of the cyclization product, a 2-imino-1,3-benzoxathiole, to a 2-oxo-1,3-benzoxathiole, which is further hydrolyzed or hydrazinolyzed to the desired *o*-mercaptophenol, a relatively stable compound. 3-Mercaptotyramine, the mercapto analog of dopamine, is not a substrate for catechol-*O*-methyltransferase, but irreversibly inhibits the enzyme, presumably by formation of a disulfide bridge to a reactive mercapto group in the active site. Studies with 3-mercaptotyramine in the anesthetized dog indicate only minimal effects on blood pressure and cardiac contractility, and little if any effect on renal blood flow. This mercapto analog of dopamine appears to be a very weak, indirectly acting, sympathomimetic amine without significant agonist or antagonist activity for norepinephrine or dopamine receptors.

Catecholamines, such as epinephrine, norepinephrine, and dopamine, are important neurohormones whose structure-activity relationships have been extensively studied (for pertinent references see ref 1). Both alterations in the substitution on the terminal nitrogen, and, to a lesser extent, the effects of substituents on the aromatic ring have been investigated.[1] Thus, one of the phenolic groups may be replaced by a methanesulfonamide[2] or a hydroxymethyl group[3] with significant retention of biological activity. Another logical chemical alteration of the catechol moiety would be the replacement of either or both of the phenolic groups by a mercapto group. Such compounds might prove to be site-directed sulfhydryl reagents. Replacement of hydroxyl groups by mercapto groups in biologically active compounds has been reported for the estrogen, hexestrol, the thiophenol isostere of which was synthesized and found to be biologically inactive.[4] A mercapto analog of norepinephrine in which the β-hydroxy group was replaced by a mercapto group was only $^1/_{240}$ as active a pressor agent as norepinephrine.[5]

This report describes the synthesis of analogs of L-dopa, dopamine, and norepinephrine in which the *m*-hydroxy group of the catechol moiety has been replaced by a mercapto group. The effects of the dopamine analog on catechol-*O*-methyltransferase (COMT) and on cardiovascular responses in the anesthetized dog have been investigated.

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