Stimulation and inhibition. The rate of decarboxylation of fluoro-Dopa was increased by PLP (Table II). It was decreased by α-methyl-Dopa (Table III).

Discussion. Our observations show that fluoro-Dopa is decarboxylated to fluoro-dopamine by dopa decarboxylase, an enzyme known to have a broad specificity[4]. As might be expected the rate of this reaction was increased by the addition of PLP, the coenzyme for dopa decarboxylase[5] and inhibited by α-methyl-Dopa, a known competitive inhibitor[6].

The K_m value for fluoro-Dopa was slightly lower than, but of the same order as that obtained with Dopa. Our estimate of the K_m for the latter compound was similar to that reported by HARTMAN et al.[7] and YUWILER et al.[8]. It is concluded that [^{18}F] fluoro-Dopa is an analogue of Dopa which could be used to investigate the intracerebral metabolism of Dopa and dopamine. Such investigations could make use of peripheral decarboxylase inhibitors to enhance the penetration of the gamma-emitting analogue into the brain.

Table II. Stimulation of fluoro-Dopa decarboxylation by pyridoxal-5′-phosphate (PLP)

PLP (μmol)	Fluoro-dopamine formed (nmol)
0	6.06
10	25.9
30	24.4

Incubation conditions: 0.1 ml hss of hog kidney cortex (equivalent to 2.69 mg protein); iproniazid phosphate, 10^{-5} mol/l; fluoro-Dopa (as the L-isomer), 1.28×10^{-4} mol/l; in a volume of 1.0 ml/10 min at 37°.

Table III. Inhibition of decarboxylation by α-methyl-Dopa

Enzyme preparation from rabbit brain	α-Me-Dopa	Fluorodopamine (μmol) formed (g wet wt. of brain)$^{-1}$ h^{-1}
crude	—	3.72
crude	+	0.12
hss	—	2.30
hss	+	0.32

Incubation conditions: Enzyme preparation (corresponding to 0.5 g of brain tissue, wet weight), 2.0 ml; PLP, 5×10^{-5} mol/l; nialamide, 2.14×10^{-5} mol/l; fluoro-Dopa (as L-isomer), 1.41×10^{-4} mol/l; L-α-methyl-Dopa, 2.38×10^{-4} mol/l (final concentrations), in a volume of 3.12 ml.; 1 h at 37 °C. The amounts of fluorodopamine tabulated have been corrected for non-enzymic decarboxylation.

Summary. [^{18}F]-5-fluoro-Dopa is a substrate for dopa decarboxylase of kidney and brain. Its potential use in brain studies is proposed.

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[4] W. LOVENBERG, H. WEISSBACH and S. UDENFRIEND, J. biol. Chem. *237*, 89 (1962).
[5] D. E. GREEN, L. F. LELOIR and V. NOCITO, J. biol. Chem. *161*, 559 (1945).
[6] T. L. SOURKES, Arch. Biochem. Biophys. *51*, 444 (1954).
[7] W. J. HARTMAN, R. I. AKAWIE and W. C. CLARK, J. biol. Chem. *216*, 507 (1955).
[8] A. YUWILER, E. GELLER and S. EIDUSON, Arch. Biochem. Biophys. *89*, 143 (1960).
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Synthesis of Histidine^2-Angiotensin II Analogues[1]

Histidine^2-angiotensin II, an analogue of Ile^5-angiotensin II, was found essentially inactive as a pressor agent, but it displayed minor oxytocic activity[2]. For the purpose of comparison, it seemed worthwhile to determine the effects of a protected imidazole nucleus and the β-aspartyl bond of the aforementioned analogue, since Asp^1-β-Val^5-angiotensin II shows an increased and prolonged activity[3]. Towards this end, synthetic steps were conducted in part by stepwise addition via the carbodiimide method or by fragment condensation via the azide route in order to avoid the danger of racemization[4]. Thus, Z-Val-Tyr-Ile-$NHNH_2$ (I), prepared from the corresponding methyl ester[5], was coupled via the azide method with H-(im-Bzl)His-Pro-Phe-OCH_3 dihydrobromide[6], desalted, prior its use, by column chromatography on DOWEX 2-X8 using MeOH as an eluent. The resulting hexapeptide, Z-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OCH_3 (II) gave comparable or higher values than those reported[7]. Compound II was subjected to selective catalytic hydrogenation[8] over palladium black for 1 h affording H-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OCH_3 (III), which was then condensed with Z-(im-Bzl)His-OH via the carbodiimide method to give Z-(im-Bzl)His-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OCH_3 (IV). Saponification of IV afforded the corresponding acid, Z-(im-Bzl)His-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OH (V), which by selective catalytic hydrogenation, as described before, produced the substituted heptapeptide, H-(im-Bzl)His-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OH (VI). The latter was ester-

[1] The amino acids are of L-configuration.
[2] A. METALLIDIS and D. THEODOROPOULOS, Chim. Chronika, New series *1*, 154 (1972).
[3] B. RINIKER and R. SCHWYZER, Helv. chim. Acta *47*, 2357 (1964).
[4] The physical data of intermediate peptides and final products are listed in the Table.
[5] H. SCHWARZ and F. M. BUMPUS, J. Am. chem. Soc. *81*, 890 (1959).
[6] D. THEODOROPOULOS and J. GAZOPOULOS, J. chem. Soc. *1960*, 3861.
[7] R. H. MAZUR, Can. J. Chem. *40*, 1098 (1962).
[8] D. THEODOROPOULOS, J. org. Chem. *21*, 1550 (1956). – D. THEODOROPOULOS and G. FÖLSCH, Acta chem. scand. *12*, 1955 (1958).

Synthetic histidine²-angiotensin II analogues and related intermediate peptide fragments

Compound	Yield (%)	Mp. (°C)	$[\alpha]_D^{25}$ values[a]
I[b]	83	245–247	−14.1° (c 2.0, DMF)
II[c,d]	55	213–214	−67.5° (c 1.0, MeOH)
III[e]	91	208–210	−51.0° (c 1.0, MeOH)
IV[e]	65	168–170	−29.4° (c 1.0, AcOH)
V[f]	85	150–151	−48.5° (c 1.0, MeOH)
VI	90	151–154	−44.1° (c 1.0, MeOH)
VII	85	140–145	−31.7° (c 0.5, MeOH)
VIII	86	135–140	
IX	90	156–160	−48.3° (c 0.5, MeOH); −29.7° (c 0.25, DMF)
X	97	152–157	−47.1° (c 0.5, MeOH); −31.1° (c 0.25, DMF)
XI	87	157–159	−49.1° (c 0.5, MeOH); −33.2° (c 0.25, DMF)
XII	95	159–162	−42.3° (c 0.5, MeOH); −28.8° (c 0.25, DMF)

[a] Unless otherwise stated; [b] $[\alpha]_D^{23}$; [c] $[\alpha]_D^{22}$; [d] reported[7] mp 192–194; $[\alpha]_D^{23}$ −66.0° (c 1.0, MeOH); [e] $[\alpha]_D^{24}$; [f] $[\alpha]_D^{20}$.

ified with benzyl alcohol/*p*-toluenesulfonate/benzene to give H(im-Bzl)His-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OBzl as the tri-*p*-toluenesulfonate (VII). The product VII was desalted on a DOWEX 2-X8 column and the resulting heptapeptide ester, H-(im-Bzl)His-Val-Tyr-Ile-(im-Bzl) His-Pro-Phe-OBzl (VIII) was coupled with Z-(β-benzyl)-L-aspartate[9] via the carbodiimide method in DMF solution. The obtained octapeptide derivative, Z-(β-OBzl)-Asp-(im-Bzl) His-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OBzl (IX) afforded, by selective catalytic hydrogenation, the desired product, H-Asp-α-(im-Bzl)His-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OH (X), which was isolated in pure form by gel filtration on Sephadex LH-20, using MeOH as the eluent.

Finally, the substituted Asp¹-β-His²-Ile⁵-angiotensin II analogue was synthesized by condensation of the heptapeptide benzyl ester VIII with Z-(α-benzyl)-L-aspartate via the carbodiimide method. The resulting octapeptide derivative, Z-(α-OBzl)-Asp-(im-Bzl)His-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OBzl (XI), after removal of the N-carbobenzoxy and *O*-benzyl groups by selective catalytic hydrogenation, gave the analogue H-Asp-β-(im-Bzl)His-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OH (XII).

In the course of this work, α-benzyl-L-aspartate was synthesized by a new route, using N-trityl-L-aspartate di-benzyl ester (XIII), [mp 103–104°; $[\alpha]_D^{25}$ + 12.8° (c 2,0, CH_2Cl_2)] as the starting material. Due to the steric hindrance of the trityl group[10], the alkaline hydrolysis of the ester XIII proceeds with removal of the β-benzyl group selectively. Thus, the obtained crude product of N-trityl-α-benzyl-L-aspartate (86% yield) was further detritylated with acetic acid[10] to give α-benzyl-L-aspartate[9] in 85% yield. [mp 173–174°; $[\alpha]_D^{25}$ −15.7° (c 5.0, HCl)]. The latter upon carbobenzoxylation gave Z-α-benzyl-L-aspartate[9] [mp 84–85°; $[\alpha]_D^{25}$ −15.1° (c 5.0, acetone)] in 72% yield.

The biological activities of the new synthetic analogues X and XII will be reported in a forth-coming communication. All new compounds, reported here, gave satisfactory elemental analysis.

Summary. The synthesis of histidine²-angiotensin II analogues, namely H-Asp-α-(im-Bzl)His-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OH and H-Asp-β-(im-Bzl)His-Val-Tyr-Ile-(im-Bzl)His-Pro-Phe-OH, are described. Also a new route leading to the synthesis of α-benzyl-L-aspartate, using N-trityl-L-aspartate di-benzyl ester as the starting material, is reported.

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Laboratory of Organic Chemistry, University of Patras (Greece), 23 June 1975.

[9] P. H. BRYANT, R. H. MOORE, P. J. PIMLOTT and G. T. YOUNG, J. chem. Soc. *1959*, 3868.

[10] L. ZERVAS and D. THEODOROPOULOS, J. Am. chem. Soc. *78*, 1359 (1956).

Distribution of Glycerophosphorylcholine Diesterase in Rat Brain

The enzyme glycerophosphorylcholine diesterase (L-3-glycerylphosphorylcholine glycerophosphohydrolase EC 3.1.4.2., GPC diesterase) has been shown to occur in liver[1], kidney[2] and brain[3] of mammals. Its action releases choline from glycerophosphorylcholine, which in turn is released by the breakdown of phosphatidylcholine. Its action in the brain could make a substantial contribution to the pool of free choline which has been thought to be the major source of choline for ACh synthesis. Potentially, therefore, GPC diesterase could influence the synthesis of ACh, but if that were the case it would be expected that its regional distribution in the brain would be closely associated with that of ACh and choline acetyltransferase (EC 2.3.1.6). Both regional and subcellular distributions of the enzyme and their relation to choline acetyltransferase are examined in the present paper.

Materials and methods. L-3-glycerophosphorylcholine (1-^{2}^{14}C-choline, GPC), as the cadmium chloride complex was obtained from ICN radiochemicals and was diluted to a suitable specific activity (approx. 400 dpm/nmol)

[1] R. M. C. DAWSON, Biochem. J. *62*, 689 (1956).

[2] J. J. BALDWIN and W. E. CORNATZER, Biochim. biophys. Acta *164*, 195 (1962).

[3] G. R. WEBSTER, E. A. MARPLES and R. H. S. THOMPSON, Biochem. J. *65*, 374 (1957).