8-Phenylpropylguanine (III) was prepared in the same manner as IV; yield 54% of product that moved as a single spot on tlc in chloroform–ethanol (5:3). For analysis a sample was dissolved in 1.5 *N* NH_4OH and reprecipitated with glacial acetic acid: mp >250°; λ_{max} (pH 1) 253, 283 mμ; (pH 13) 278 mμ.

Anal. Calcd for $C_{14}H_{15}N_5O$: C, 62.4; H, 5.61; N, 26.0. Found: C, 62.2; H, 5.75; N, 26.0.

8-Phenylguanine (II) was prepared by the literature method[13] by treatment of 5-benzamido-2,6-diamino-4-pyrimidinol with $POCl_3$. The compound has λ_{max} (pH 2) 238, 268, 305 mμ; (pH 12) 238, 312 mμ, in agreement with those reported.[13] Ring closure with polyphosphoric acid at 150°[14] for 1.5 hr was a more consistent method than $POCl_3$ and gave a 60% yield of pure material. The polyphosphoric acid was not successful for preparation of III and IV.

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Experimentally Induced Phenylketonuria. 1. Inhibitors of Phenylalanine Hydroxylase

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The action on phenylalanine hydroxylase of a series of *o*-dihydroxy aromatic compounds and some substituted phenylalanine derivatives was studied. Chemical syntheses are reported for 3,4-difluoro-, 3-chloro-4-fluoro-, 3-bromo-4-fluoro-, 4-fluoro-α-methyl-, and 4-methyl-α-methylphenylalanine. The results of inhibition studies of phenylalanine hydroxylase confirm that 4-fluorophenylalanine is a good inhibitor. Introduction of a group larger than fluorine at the 4 position reduces the inhibition. Substitution about the amino acid moiety seems to be either without significant effect or detrimental to activity. Several *o*-dihydroxy compounds were inhibitors, but esculetin (6,7-dihydroxycoumarin) was the strongest inhibitor found.

Phenylketonuria (PKU) is a heritable metabolic disorder characterized by high plasma phenylalanine (PA), urinary excretion of phenyl ketones, and mental deficiency. The basic metabolic aberration lies in the inability of the diseased individual to oxidize PA to tyrosine. PA hydroxylase is known to be inhibited by a high concentration of PA, its own substrate,[1] thus the diseased state may be induced by a PA-enriched diet. A compound that can act as a specific inhibitor of PA hydroxylase would be desirable in studying experimental PKU. The disease state produced by such a compound not only would resemble PKU in its etiology, but would also be free of complications arising from the presently employed high PA diets.

Two structurally unrelated compounds, 4-fluorophenylalanine[2] and esculetin (6,7-dihydroxycoumarin)[3] are known to be reversible inhibitors of PA hydroxylase. The latter, by our observations, was about 20 times as potent as the former in this regard and was chosen as a starting point for our work. The 6- and 7-methoxy derivatives and dihydroesculetin were examined. In addition some other *o*-dihydroxy aromatic compounds were evaluated. A second broad class of potential inhibitors was comprised of substituted PA compounds. Various substituents were placed on the aromatic ring and some compounds were prepared with alterations about the amino acid portion of the PA molecule.

Enzyme Inhibition Studies.—Phenylalanine hydroxylase was prepared from rat liver by the method of Kaufman.[4] Purification was carried out up to step 2 of this method. The incubation mixture consisted of 100 μmoles of sodium phosphate buffer, pH 7.4, 20 μmoles of reduced nicotinamide–adenine dinucleotide, 10 μmoles of nicotinamide, approximately 10 mg of enzyme protein, 1.0 μmole of phenylalanine, and appropriate amounts of the test compound in a final volume of 2 ml. Incubation was carried out for 20 min at 37° in air. Tyrosine was assayed by the method of Udenfriend and Cooper.[5]

Biological Results and Discussion

The compounds tested as inhibitors of PA hydroxylase are listed in a decreasing order of potency in Table I. In general, *o*-dihydroxy-type compounds were potent inhibitors in agreement with the findings of Burkard, *et al.*,[6] and Ross and Haljasmaa.[3] As was pointed out by Fuller[7] in his inhibition studies on tryptophan hydroxylase, which may be identical with PA hydroxylase,[8] the inhibitory property of esculetin depended on the *o*-dihydroxy structure. It was inferred[7] that metal chelation by the *o*-dihydroxy moiety was responsible for the inhibition. In our studies 6-methyl-, 6-glucosyl-, and 7-methylesculetin showed considerably weaker inhibitory activity than esculetin. Also, the 3,4 double bond of the coumarin nucleus of esculetin is apparently required as evidenced by the greatly reduced potency of 3,4-dihydroesculetin as an inhibitor.

Udenfriend, *et al.*,[9] showed that 3,4-dihydroxyphenyl-α-propylacetamide inhibited tyrosine hydroxylase by competing with the cofactor, tetrahydropteridines. Since PA hydroxylase activity also is dependent on reduced pteridines,[10] the mechanism by which esculetin inhibits PA hydroxylase may be similar.

(1) S. Udenfriend and J. R. Cooper, *J. Biol. Chem.*, **194**, 503 (1952).
(2) H. H. Watt and J. P. Vandervoorde, *Federation Proc.*, **23**, 146 (1964).
(3) S. B. Ross and O. Haljasmaa, *Life Sci.*, **3**, 579 (1964).
(4) S. Kaufman, *Methods Enzymol.*, **5**, 809 (1962).
(5) S. Udenfriend and J. R. Cooper, *J. Biol. Chem.*, **196**, 227 (1952).
(6) W. P. Burkard, K. F. Gey, and A. Pletscher, *Life Sci.*, **3**, 27 (1964).
(7) R. W. Fuller, *ibid.*, **4**, 1 (1965).
(8) J. Renson, R. Weissbach, and S. Udenfriend, *Biochem. Biophys. Res. Commun.*, **6**, 20 (1962).
(9) S. Udenfriend, P. Zaltzman-Nirenberg, and T. Nagatsu, *Biochem. Pharmacol.*, **14**, 837 (1965).
(10) S. Kaufman, "Oxygenases," O. Hayaishi, Ed., Academic Press Inc., New York, N. Y., 1962, p 129.

TABLE I
INHIBITION OF RAT LIVER PHENYLALANINE HYDROXYLASE

Compd[a]	Ratio of substrate:inhibitor to give *ca.* 50% inhib
Esculetin	200:1
L-DOPA	15:1
4-Fluoro-PA	10:1
2,3-Dihydroxynaphthalene	10:1
1,2-Dihydroxyanthraquinone	10:1
Nordihydroguaiaretic acid	10:1
Esculetin 7-methyl ether	7:1
Esculin	3:1
Esculetin 6-methyl ether	2:1
3,4-Difluoro-PA	2:1
4-Fluoro-α-methyl-PA	2:1
3-Chloro-4-fluoro-PA	1.5:1
3,4-Dihydroesculetin	1:1
DL-α-Methyl-DOPA	1:1
3,4-Dihydroxycinnamic acid	1:1
3-Bromo-4-fluoro-PA	1:1
4-Chloro-PA	1:1

[a] The following compounds showed less than 50% inhibition at a ratio of 1:1: 2-bromo-, 2-fluoro-, 3-fluoro-, 3-chloro-, 3-bromo-, α-methyl-, 4-methyl-α-methyl-, and 4-nitrophenylalanine, α-propylphenylacetamide, 4-fluorophenylacetic acid, 4-fluorophenylpropionic acid, 4-methyl-, 4-fluoro-, and 4-hydroxyqinnamic acid, β-thienylalanine, mimosine, and 2,3-dihydroxyquinoxaline.

Among the non-*o*-dihydroxy compounds tested, 4-fluorophenylalanine was the most potent inhibitor. 4-Fluorophenylalanine was reported to act as a substrate for PA hydroxylase[10] and to inhibit the hydroxylation of phenylalanine competitively.[2] Our studies showed that α-methyl derivatives of DOPA and 4-fluorophenylalanine were considerably less active as inhibitors than their respective parent compounds. Furthermore, 4-fluorocinnamic, -phenylacetic, and -phenylpropionic acids showed essentially no inhibitory activity against PA hydroxylase. Indications are that disturbance of the amino acid side chain reduces the inhibition. Investigation of 3-substituted phenylalanine derivatives in the hope of obtaining an inhibitor based on hindrance of the 4 position were not fruitful. Substitution of fluorine, chlorine, or bromine in the 3 position of 4-fluorophenylalanine lowered the inhibitory activity progressively in the order listed. Phenylalanine derivatives without substituents in the 4 position were poor inhibitors as were derivatives substituted in the 4 position with groups other than fluorine, *e.g.*, chloro, bromo, nitro, or methyl.

These findings on the properties of PA hydroxylase inhibitors are in sharp contrast to those of tyrosine hydroxylase inhibitors.[9,11] Among the latter, the dihalogenated tyrosines were more potent inhibitors than monohalogenated tyrosines and α-methyl-halogenated tyrosines were more potent than the nonmethyl analogs. Finally, the relative activities of the 3-substituted α-methyltyrosine analogs were I > Br > Cl > H > F.

In attempting to design a potential *in vivo* inhibitor of an enzyme such as in the present study, one factor which must be reckoned with is the possibility that a candidate compound may be relatively inactive in an *in vitro* assay system but may be transformed into a potent inhibitor in an intact animal. The reverse could also happen. For example, 4-chlorophenylalanine was found to be a poor inhibitor of phenylalanine hydroxylase *in vitro*. However, in confirmation of the findings of Koe and Weissman,[12] the hydroxylase activity of a rat was found to be extensively inhibited 3 days after a single injection of the compound. The mechanism for this phenomenon is not understood. Esculin (6-glucosylesculetin), although considerably less potent than esculetin in an *in vitro* system, was found to be as active an inhibitor as esculetin when injected into rats.[13] The findings on intact animal studies will be a subject of another communication.

(11) M. Goldstein and Z. Weiss, *Life Sci.*, **4**, 261 (1965).

Experimental Section

The physical properties of the compounds are listed in Table II.

Substituted 5-Benzyl-5-methylhydantoins.—*p*-Tolylacetone, bp 80–81.5° (2.7 mm), lit.[14] bp 92–94° (3 mm), and *p*-fluorophenylacetone, bp 54–57° (0.5 mm), lit.[15] bp 120–130° (30 mm), were prepared according to the procedure of Cason and Prout.[16] The appropriate ketone was allowed to react with $(NH_4)_2CO_3$ and NaCN in 60% ethanol at 60° according to the procedure of Goodson, *et al.*,[17] to afford the hydantoin.

α-Methylphenylalanines.—A solution of 0.01 mole of the hydantoin and 0.10 mole of $Ba(OH)_2 \cdot 8H_2O$ in 75 ml of water was stirred at reflux for 72 hr, chilled to 0°, and treated with CO_2. The $BaCO_3$ was filtered and the aqueous solution was evaporated *in vacuo* to near dryness. The solution was acidified to pH 1 with concentrated HCl and chilled. The resulting products were collected and recrystallized.

3-Chloro-4-fluorotoluene.—To a hot solution of 10.6 g (43.4 mmoles) of $CuSO_4 \cdot 5H_2O$ in 35 ml of hot water was added 3.5 g (60.3 mmoles) of NaCl and 3.1 g (24.8 mmoles) of Na_2SO_3 in 25 ml of warm water. After chilling, the supernatant was decanted and the white solid was washed with 25 ml of cold water and dissolved in 13.1 ml of concentrated HCl. To this was added an ice-cold solution of the diazonium salt from 4.9 g (39.1 mmoles) of 2-fluoro-5-methylaniline in 75 ml of 3 *N* HCl and 2.8 g (41.3 mmoles) of $NaNO_2$ in 25 ml of water. The mixture was steam distilled and the distillate was extracted with three 50-ml portions of ether. The combined ether extracts were washed with three 30-ml portions of 5% NaOH, three 30-ml portions of water, dried over $MgSO_4$, and evaporated *in vacuo*. Distillation of the residue yielded 2.39 g (43%) of a colorless liquid, bp 85° (54 mm).

Anal. Calcd for C_7H_6ClF: C, 58.2; H, 4.18; Cl, 24.6. Found: C, 58.3; H, 4.36; Cl, 24.7.

Substituted Benzyl Bromides.—A mixture of 0.01 mole of the toluene, 0.01 mole of N-bromosuccinimide, 0.001 mole of benzoyl peroxide, and 30 ml of CCl_4 was stirred under reflux for 16 hr in the presence of a flood lamp. Succinimide was removed by filtration, and the solvent was evaporated *in vacuo*. The residual oils were extracted into petroleum ether (bp 60–120°) and the petroleum ether was evaporated *in vacuo*. The crude oil was distilled *in vacuo*.

Substituted Benzylacetamidomalonates.—Following the general procedure of Burckhalter and Stephens[18] 0.01 mole of the benzyl bromide in 10 ml of ethanol was added to a solution of 0.01 g-atom of sodium and 0.01 mole of diethyl acetamidomalonate in 60 ml of ethanol. The resulting solution was heated at reflux for 3 hr. The hot mixture was filtered and chilled to afford the product which was collected and recrystallized.

Phenylalanines.—One gram of the acetamidomalonate was boiled with 15 ml of concentrated HCl and 5 ml of HOAc for 16 hr. The hot solution was filtered, adjusted to pH 6, and chilled. The resulting product was collected and recrystallized. Com-

(12) B. K. Koe and A. Weissman, *Federation Proc.*, **25**, 452 (1966).

(13) M. C. Theisen and C. Mitoma, unpublished observation.

(14) T. I. Temnikova and V. I. Veksler, *Zh. Obshch. Khim.*, **19**, 1318 (1949).

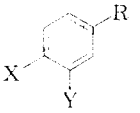
(15) T. Ando, *Yuki Gosei Kagaku Kyokai Shi*, 777 (1959); *Chem. Abstr.*, **54**, 4992*b* (1960).

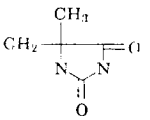
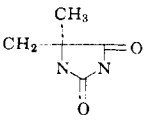
(16) J. Cason and F. S. Prout, "Organic Syntheses," Coll. Vol. III, John Wiley and Sons, Inc., New York, N. Y., 1955, p 601.

(17) L. H. Goodson, I. L. Honegberg, J. J. Lehman, and W. H. Burton, *J. Org. Chem.*, **25**, 1920 (1960).

(18) J. H. Burckhalter and V. C. Stephens, *J. Am. Chem. Soc.*, **73**, 56 (1951).

TABLE II



No.	X	Y	R	Yield, %	Mp or bp (mm), °C	Crystn solvent	Formula	Calcd, % C	Calcd, % H	Calcd, % N	Found, % C	Found, % H	Found, % N
1	CH_3	H		20	222–223	60% EtOH	$C_7H_8N_4O_2$	55.3	6.47	12.8	65.8	6.18	12.7
2	F	H		12	212–213.5	60% EtOH	$C_6H_5FN_4O_2$	59.5	1.99	12.5	59.3	4.90	12.5
3	F[a]	F	CH_2Br	57	59–61 (3.5)		[b]						
4	F	Cl	CH_2Br	73	63–65 (0.55)		[b]						
5	F	Br	CH_2Br	50	130 (0.5)		$C_7H_5BrFNO_2$	36.0	2.15	34.2[c]	35.8	2.30	34.3[c]
6	H	Br	$CH_2C(NHAc)(COOEt)_2$	66	97–99	EtOH	$C_{16}H_{20}BrNO_5$	49.8	5.22	3.63	49.9	5.31	3.56
7	F	F	$CH_2C(NHAc)(COOEt)_2$	41	145–146	EtOH	$C_{16}H_{19}F_2NO_5$	56.0	5.58	4.08	55.9	5.67	4.33
8	F	Cl	$CH_2C(NHAc)(COOEt)_2$	38	128	EtOH	$C_{16}H_{19}ClFNO_5$	53.4	5.32	3.90	53.6	5.34	4.01
9	F	Br	$CH_2C(NHAc)(COOEt)_2$	30	118–119.5	EtOH	$C_{16}H_{19}BrFNO_5$	47.5	4.74	3.47	47.4	4.47	3.51
10	CH_3	H	$CH_2C(CH_3)(NH_3^+Cl^-)COOH$	41	252–254	50% EtOH	$C_5H_9NO_2 \cdot HCl$	37.5	7.02	6.10	37.4	7.16	6.22
11	F	H	$CH_2C(CH_3)(NH_2)COOH$	43	265–268	Water	$C_9H_{12}FNO_2$	60.9	6.14	7.10	61.1	6.26	7.20
12	H	Br	$CH_2CH(NH_2)COOH$	61	226–228	50% EtOH	$C_9H_{10}BrNO_2$	44.1	4.13	5.74	44.1	4.19	5.69
13	F	F	$CH_2CH(NH_3^+Cl^-)COOH$	79	211–212		$C_9H_9F_2NO_2 \cdot HCl$	45.5	4.24	5.89	45.3	4.30	5.85
14	F	Cl	$CH_2CH(NH_3^+Cl^-)COOH$	85	202–204.5		$C_9H_9ClFNO_2 \cdot HCl$	42.5	3.97	5.51	42.8	4.12	5.30
15	F	Br	$CH_2CH(NH_2)COOH$	42	164.5–165.5	Water	$C_9H_9BrFNO_2$	41.2	3.46	5.35	41.4	3.47	5.29

[a] Prepared from 3,4-difluorotoluene: D. Robertson, *J. Org. Chem.*, **24**, 2051 (1959). [b] Compounds were found to be unstable and were used immediately in the next reaction. [c] Bromine analysis.

pounds **13** and **14** were obtained as the hydrochloride directly from the chilled acid solutions and did not require recrystallization.

3,4-Dihydro-6,7-dihydroxycoumarin (3,4-Dihydroesculetin).—To a suspension of 100 mg of PtO_2 in 10 ml of absolute ethanol was added 691 mg (3.98 mmoles) of esculetin. The mixture was hydrogenated at room temperature (22°) and atmospheric pressure, taking up 1 equiv of hydrogen in 4 hr. The catalyst was filtered off and the filtrate was evaporated *in vacuo* to dryness. Recrystallization of the residue from ethanol yielded 353 mg (60%) of white crystals, mp 204–206°. A second crop of 140 mg (20%) was obtained from the mother liquors: mp 200–203°; λ_{max}^{EtOH} 228 mμ (ε 12,000), 257 (5300), 300 (5300), 350 (9700).

Anal. Calcd for $C_9H_8O_4$: C, 60.0; H, 4.48. Found: C, 59.8; H, 4.49.

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Pyrazine Diuretics. II. N-Amidino-3-amino-5-substituted 6-Halopyrazinecarboxamides

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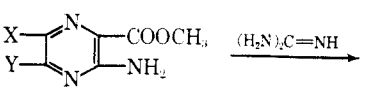
The synthesis of a series of N-amidino-3-amino-5-substituted 6-halopyrazinecarboxamides is described. In rats and dogs, these compounds cause diuresis and saluresis while potassium excretion is unaffected or repressed. Compounds with a variety of 5 substituents including hydroxy, alkoxy, mercapto, alkylmercapto, amino, and substituted amino were prepared. The latter two types embrace compounds with the highest activity. Several routes for the synthesis of methyl 3-amino-5,6-dichloropyrazinoate, a key intermediate, are presented.

The unique effect of the N-amidino-3-amino-6-halopyrazinecarboxamides[1] on renal electrolyte excretion prompted a thorough structure–activity study of this series and its congeners. It is the purpose of this paper to report the investigation of N-amidino-3-amino-6-halopyrazinecarboxamides (II) bearing various substituents at the 5 position and on one nitrogen of the amidino group.

Chemistry.—In general, the target compounds (II) were prepared by the interaction of the appropriate



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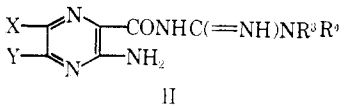

II

ester (I) with a guanidine. The reaction was usually carried out by heating the ester with a methanolic solution of the guanidine. Satisfactory results were achieved with guanidine itself and a variety of esters in-

(1) Paper I in this series: J. B. Bicking, J. W. Maspo, O. W. Woltersdorf, Jr., J. H. Jones, S. F. Kwong, C. M. Robb, and E. J. Cragoe, Jr., *J. Med. Chem.*, **8**, 638 (1965).