Notes

Experimentally Induced Phenylketonuria. 4. Potential Inhibitors of Phenylalanine Hydroxylase

Joseph I. DeGraw,* Vernon H. Brown, W. A. Skinner, Susan LeValley, and Chozo Mitoma

Departments of Pharmaceutical Chemistry and Biochemistry, Stanford Research Institute, Menlo Park, California 94025.
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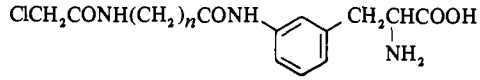
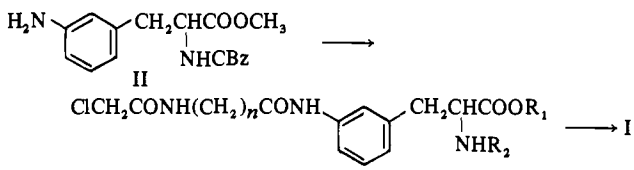
In earlier communications[1,2] we reported our investigations to find a potent inhibitor of phenylalanine hydroxylase for the purpose of creating a condition of phenylketonuria. As a continuation of this work we have continued to seek potent irreversible inhibitors of the enzyme. Previous results[2] showed that introduction of large groups in the 4 position of phenylalanine drastically lowered activity. 3-Chloroacetamidophenylalanine was also ineffective as an inhibitor. However, it can be hypothesized that an extended 3 side chain, containing an alkylating function, may achieve alkylation at a location distant from the active site of the enzyme.[3]

To test the above thesis we have synthesized some alkylating agents derived from *m*-aminophenylalanine. These compounds are represented by formula I and contain chloroacetamido groups that have been extended through amide linkage to the nuclear amino group.



Ia, $n = 1$
b, $n = 2$
c, $n = 3$

The synthesis of the compounds involved amide coupling, *via* the mixed anhydride method, of *N*-chloroacetylglycine, -β-alanine, and -4-aminobutyric acid with methyl α-*N*-carbobenzoxy-3-aminophenylalanate (II). The amido ester intermediates III were carefully hydrolyzed with 1 *N* KOH in MeOH to afford the carboxylic acids IV. Treatment with 30% HBr in HOAc readily cleaved the carbobenzoxy group to yield the amino acids I as the crystalline HBr salts.



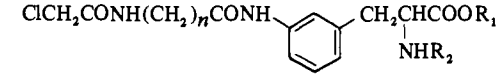
$n = 1, 2, 3$
III, $R_1 = CH_3$; $R_2 = CBz$
IV, $R_1 = H$; $R_2 = CBz$

The compounds Ia-c were evaluated for their inhibitory activity against phenylalanine hydroxylase by the technique previously described.[1] All three showed no inhibition at a ratio of substrate to inhibitor of 2:1, whereas 4-fluorophenylalanine gave 50% inhibition at a ratio of 10:1.

Experimental Section

Analyses are indicated by symbols of the elements and the results obtained for those elements were within ±0.4% of the theoretical values. Physical data are recorded in Table I.

Table I



Compd No.	*n*	R_1	R_2	Mp, °C	Formula	*Anal.*
Ia·HBr	1	H	H	134–137	$C_{13}H_{16}ClN_3O_4 \cdot HBr \cdot H_2O$	C, H, N
Ib·HBr	2	H	H	176–184	$C_{14}H_{18}ClN_3O_4 \cdot HBr \cdot 0.5H_2O$	C, H, N
Ic·HBr	3	H	H	195–199	$C_{15}H_{20}ClN_3O_4 \cdot HBr$	C, H, N
IIIa	1	CH_3	CBz	158–160.5	$C_{22}H_{24}ClN_3O_6$	C, H, N
IIIb	2	CH_3	CBz	138–140	$C_{23}H_{26}ClN_3O_6$	C, H, N
IIIc	3	CH_3	CBz	123–126	$C_{24}H_{28}ClN_3O_6$	C, H, N
IVa	1	H	CBz	173–175	$C_{21}H_{22}ClN_3O_6$	C, H, N
IVb	2	H	CBz	125–128	$C_{22}H_{24}ClN_3O_6$	C, H, N

***N*-Chloroacetyl Amino Acid Amides of Methyl *N*-α-Carbobenzoxy-3-aminophenylalanates (III).** *N*-Chloroacetylglycine was prepared by the method of Ronwin;[4] *N*-chloroacetyl-β-alanine and -4-aminobutyric acid by the method of Hanson and Smith.[5] Equimolar amts of *N*-chloroacetyl acid and Et_3N in THF were stirred 1 hr at room temp, cooled to 0°, and treated with 1 equiv of *i*-C_4H_9OCOCl. After 2 hr at 0°, 1 equiv of II[2] in THF was added dropwise and the mixt kept at ambient temp for 15 hr. The solvent was evapd *in vacuo* and the residue partitioned between EtOAc and H_2O. The EtOAc ext was washed (2 *N* HCl, then satd $NaHCO_3$), dried ($MgSO_4$), and evapd to leave the crude amido ester which was recrystd (EtOAc); yield 25–35%.

***N*-Chloroacetyl Amino Acid Amides of *N*-α-Carbobenzoxy-3-aminophenylalanine (IV).** Equimolar amounts of the ester III and 1 *N* KOH in MeOH were stirred at 25–30° for 6 hr and evapd *in vacuo*. The residue was partitioned (EtOAc–satd $NaHCO_3$) and the aqueous portion acidified (6 *N* HCl to pH 2) to ppt the crude CBz acid in 70–80% yield; compd IVa recrystd (EtOH); IVb (EtOAc); IVc was a gum.

***N*-Chloroacetyl Amino Acid Amides of 3-Aminophenylalanine (I).** A mixt of the CBz acid IV and 4 vol of 30% HBr in HOAc was stirred for 15 min. The solvent was removed *in vacuo* and the gummy HBr salt crystd (50–60% yield) when treated with 2-PrOH. Recrystn from EtOH afforded analytical samples.

References

(1) J. I. DeGraw, M. Cory, W. A. Skinner, M. C. Theisen, and C. Mitoma, *J. Med. Chem.*, **10**, 64 (1967).
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(4) E. Ronwin, *J. Org. Chem.*, **18**, 127 (1953).
(5) H. T. Hanson and E. L. Smith, *J. Biol. Chem.*, **175**, 842 (1948).

Steroids and Related Natural Products. 70. Conversion of Cardenolides to Isocardanolides†,[1]

F. Warren Villaescusa and George R. Pettit*

Department of Chemistry, Arizona State University, Tempe, Arizona 85281. *Received September 30, 1971*

Previously[2] we described methods for obtaining the γ-type isocardanolides. In order to further evaluate the biological effects of modifying the cardenolide lactone ring we have

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