Notes

Experimentally Induced Phenylketonuria. 4. Potential Inhibitors of Phenylalanine Hydroxylase

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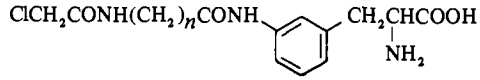
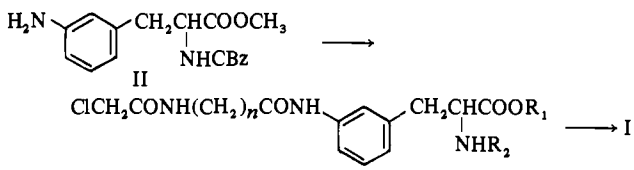
In earlier communications[1,2] we reported our investigations to find a potent inhibitor of phenylalanine hydroxylase for the purpose of creating a condition of phenylketonuria. As a continuation of this work we have continued to seek potent irreversible inhibitors of the enzyme. Previous results[2] 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.



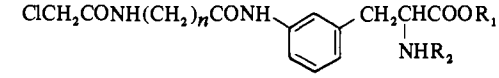
$n = 1, 2, 3$
III, $R_1 = CH_3$; R_2 = CBz
IV, $R_1 = H$; R_2 = CBz

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.

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

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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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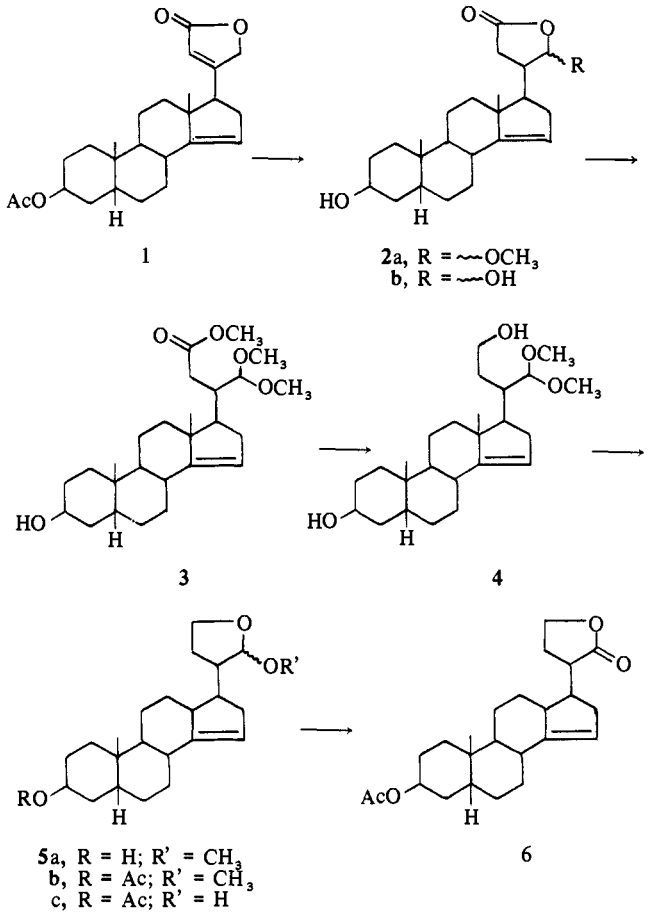

developed a route to isocardanolides in which the steroid D ring is joined to the butenolide α position. The new synthesis was also devised to complete a method for conversion of cardenolides to isocardanolides. Transformation of digitoxigenin to 3β-acetoxy-23-hydroxy-24-nor-5β-chol-14-en-21-oic acid γ-lactone (**6**) was chosen for study. The cardenolide was acetylated and dehydrated to yield 3β-acetoxy-5β-carda-14,20(22)-dienolide (**1**).[3] Treatment of lactone **1** with NaOMe in refluxing MeOH followed by acidification afforded acetal **2a**. The corresponding hemiacetal **2b** can also be obtained using similar reaction conditions.[3] The mixture of C-21 isomers (**2a**) exhibited two methoxy group singlets in the pmr spectrum at δ 3.45 and 3.50 integrating for a total of 3 protons. After separation by chromatography, the individual isomers gave signals at δ 3.45 for the less polar and δ 3.50 for the more strongly adsorbed acetal. No attempt was made to stereochemically define the acetals at position C-20.

Treatment of acetal **2a** with MeOH containing *p*-TSA yielded methyl ester **3** which was converted to alcohol **4** by reduction with $LiAlH_4$. Cyclization of the γ-hydroxy-acetal **4** to acetal **5a** occurred readily in $CHCl_3$ containing a trace of *p*-TSA. Acetylation of **5a** gave acetate **5b** which was converted to cyclic hemiacetal **5c** by heating in acetic acid solution. Oxidation of hemiacetal **5c** with Jones' reagent gave the required isocardanolide (**6**). Acetal **5a** and lactone **6** were studied[‡,4] in respect to their effect on the Na^+- and K^+-activated, Mg^{2+}-dependent ATPase of cardiac muscle membrane which has been shown to be the molecular point of attack of cardioactive steroids. Concentrations as high as 100 μ*M* inhibited the enzyme (guinea pig) to 45% and 10%, respectively, with **5a** and **6**. The Mg^{2+}-dependent ATPase, also present in the membrane, was inhibited to almost the same extent. Thus, the isocardanolides exhibit a rather low and nonspecific effect like hormone-type steroids.

‡These preliminary biological results were kindly provided by Professor K. R. H. Repke, Deutsche Akademie Der Wissenschaften Zu Berlin, Forschungszentrum fur Molekularbiologie und Medizin, Berlin.

Experimental Section[§]

3β-Hydroxy-21ξ-methoxy-5β,20ξ-card-14-enolide (2a). To a stirred suspension of 3β-acetoxy-14-dehydrodigitoxigenin[3,5] (**1**, 4.0 g) in MeOH (270 ml) was added a soln of NaOMe (4.0 g of Na in 130 ml of MeOH) in a slow stream (under nitrogen). The mixt was stirred 7 hr and chilled (ice bath), and cold 3 *N* HCl (146 ml) was added in a slow stream. The reaction mixt was extd with $CHCl_3$ (400, 200, and 100 ml). The combined ext was dried and the $CHCl_3$ removed *in vacuo* to yield crude methoxy lactone **2a** (3.9 g) as a pale yellow solid giving two very close spots on tlc (2:1 ligroin–EtOAc mobile phase). A 0.70-g portion of **2a** was chromatographed (powder loading technique) on silica gel (21 g) and eluted with ligroin–EtOAc (2:1) to yield 150 mg of less polar isomer, 160 mg of more polar isomer, and 239 mg of combined isomers. Each of the individual isomer fractions was further purified by plc (1:1 ligroin–EtOAc mobile phase).

The more mobile isomer was recrystd twice from Me_2CO–hexane to yield one of the C-21 isomers of **2a**, mp 230–233°. *Anal.* ($C_{24}H_{36}O_4$ C, H.

The less mobile isomer recrystd from EtOAc–hexane gave mp 170–182°. *Anal.* ($C_{24}H_{36}O_4$) C, H.

Methyl 3β-Hydroxy-21-dimethoxy-5β,20ξ-norchol-14-enic Acid (3). A soln of lactone **2a** (1.0 g) in MeOH (50 ml) containing *p*-TSA (100 mg) was heated (reflux) for 3 hr, cooled to room temp, poured into H_2O (50 ml) containing $NaHCO_3$ (100 mg), and extd with $CHCl_3$ (3 × 25 ml). The combined extract was washed with H_2O (50 ml) and dried, and solvent removed *in vacuo* to yield 1.08 g of an orange solid. The crude product **3** was combined with a previously prepd 2.2-g quantity and was chromatographed (powder loading technique) on silica gel (170 g). Elution with ligroin-EtOAc (3:1) yielded ester **3** (3.9 g) as a tacky white solid which could not be recrystd. The structure was supported by ir, nmr, and mass spectra.

The same reaction was conducted with both the C-21 isomers of acetal **2a** (300 mg of each) using MeOH (30 ml) and *p*-TSA (30 mg). The yield from each isomer after purification by plc (1:1 ligroin–EtOAc mobile phase) was 0.22 g of **3**, mutually identical[#] with the product described above from the isomeric mixt **2a**.

3β,23-Dihydroxy-21-dimethoxy-5β,20ξ-norchol-14-ene (4). To a stirred, ice-cold suspension of $LiAlH_4$ (1.50 g) in dry Et_2O (200 ml) was added dropwise (under nitrogen) a soln of methyl ester **3** (3.2 g) in dry Et_2O (100 ml). After stirring the reaction mixture 1 hr, H_2O (100 ml) was added (cautiously at first), the phases sepd, and the aqueous part extd with Et_2O (100 ml). The combined Et_2O phase was washed with H_2O (2 × 100 ml) and dried, and solvent removed *in vacuo* to yield crude alcohol **4** (2.9 g). The product (**4**) was combined with 0.2 g prepd earlier and chromatographed (powder loading technique) on silica gel (100 g). Elution with ligroin–EtOAc (1:1) gave 2.88 g of alcohol **4** which could not be recrystd without cyclization occurring to produce very small amounts of acetal **5a**. Spectral properties viewed immediately after chromatography, when the product was the purest (one spot on tlc), supported the assigned structure.

3β-Hydroxy-21ξ-methoxy-23-deoxy-5β,20ξ-card-14-enolide (5a). A soln of alcohol **4** (2.3 g) in $CHCl_3$ (50 ml) containing *p*-TSA (20 mg) was allowed to stand at room temp for 15 min. The reaction mixt was then washed with dilute $NaHCO_3$ soln (30 ml) and dried, and solvent removed *in vacuo* to provide acetal **5a** (1.95 g, 95%). Three recrystallizations from hexane afforded an analytical sample as needles, mp 157–173°. *Anal.* ($C_{24}H_{38}O_3$) C, H.

§ Melting points were determined on a Kofler melting point apparatus and are uncorrected. Elemental microanalyses were performed by the laboratory of Dr. A. Bernhardt, Mikroanalytisches Laboratorium, 5251 Elbach Uber Engelskirchen, West Germany, and were within 0.5% of theoretical values. The structures of all compounds were supported by ir, nmr, and mass spectra determined on Beckman IR-12, Varian A-60, and Atlas CH-4B spectrometers, respectively. Other general experimental techniques, reagents and chromatographic methods have been summarized by Villaescusa and Pettit.[1]

#The samples gave identical ir, nmr, and mass spectra and exhibited identical R_f values on tlc.

3β-Acetoxy-23-hydroxy-24-nor-5β-chol-14-en-21-oic Acid γ-Lactone (6). After 22 hr at room temp, a mixt of alcohol **5a** (1.9 g, 5.1 mmoles), pyridine (9 ml), and Ac_2O (4.5 ml) was poured into crushed ice. The ppt was collected and dried (vacuum) to yield acetate **5b** (1.96 g). The crude acetate **5b** was heated (steam bath, 2 hr) with AcOH–H_2O (2:1), poured into H_2O (250 ml), and extd with $CHCl_3$ (3 × 100 ml). The combined ext was washed with H_2O (100 ml), satd $NaHCO_3$ soln (100 ml), H_2O (100 ml), and dried and solvent removed *in vacuo* to yield 1.7 g of a brown solid. Chromatography in ligroin–EtOAc (17:3) on silica gel (50 g) and elution with the same solvent gave purified hemiacetal **5c** (0.84 g, 46%). To a soln of the hemiacetal (**5c**, 0.165 g) in Me_2CO (15 ml) was added dropwise with stirring 8 *N* Jones' reagent[6] until an orange color persisted. After 5 min, a few drops of *i*-PrOH was added and the ppt collected and washed with Me_2CO. The combined Me_2CO soln was poured into H_2O (20 ml) and the Me_2CO removed *in vacuo*. The resultant solid and aqueous soln was extd with $CHCl_3$ (2 × 10 ml) and the combined ext dried. Removal of solvent *in vacuo* yielded isocardanolide **6** (0.16 g, 96% from **5c**). Three recrystn from Me_2CO–hexane afforded large needles, mp 203–206°. *Anal.* ($C_{25}H_{36}O_4$) C, H.

References

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New Compounds

Synthesis and Antiinflammatory Activity of Betamethasone 17-Benzoate

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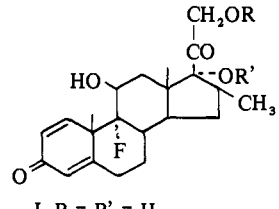
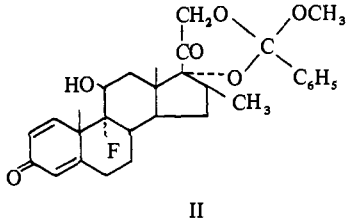
Corticosteroid 17-esters were first prepared some years ago in our laboratory by acid hydrolysis of the corresponding cyclic 17,21-alkyl ortho esters.[1,2]

Many 17-alkanoates of various corticosteroids have been found to display enhanced antiinflammatory activity after local application.[3] Here, we wish to report the synthesis and some biological properties of betamethasone 17-benzoate (III).†

The compound was prepared from betamethasone (I) through epimeric cyclic 17,21-methyl orthobenzoates II (for the stereoisomery of corticosteroid 17,21-alkyl ortho esters see ref 4) and subsequent hydrolysis of the latter in buffered medium.[5,6] Base-catalyzed rearrangement[2] of III gave betamethasone 21-benzoate (IV), identical with the product obtained by conventional benzoylation of I. A





II

I, R = R' = H
III, R = H; R' = C_6H_5CO
IV, R = C_6H_5CO; R' = H
V, R = H; R' = *n*-C_4H_9CO

benzoyl group cannot be introduced at 17α-O by direct acid-catalyzed acylation.[7]

Betamethasone 17-benzoate (III) was compared with I and betamethasone 17-valerate (V)[8,‡] in five assays for the antiinflammatory, thymolytic, and vasoconstrictive activity after different administration routes.§ The relative potencies are given in Table I. Compound III displayed the highest ratio between topical and systemic activities.

Table I. Topical (T) and Systemic (S) Activities of III and V

Assay	Route	Activity	Potency[a] III	Potency[a] V
1[b]	Oral	Antigranulomatous (S)	<1	<1
2[c]	Oral	Antiexudative (S)	<1	<1
3[c]	Intracavitary	Antiexudative (T)	100	1
	Intracavitary	Thymolytic (S)	3	1
4[d]	Percutaneous	Antiedematous (T)	1	<1
	Percutaneous	Thymolytic (S)	1	1
5[e]	Percutaneous	Vasoconstrictive (T)	500	450[f]

[a] Betamethasone (I) = 1. [b] On rat.[10] [c] On rat.[11] [d] On rat.[12] [e] On man.[13] [f] Data from ref 8.

Experimental Section#

Betamethasone 17-Benzoate (III). To a boiling solution of I (10 g) in dioxane (400 ml) and C_6H_6 (1,000 ml) under anhyd conditions, trimethyl orthobenzoate (10 ml) was added, followed by Py·TsOH (1 g). Heating was pursued for 1 hr, about two-thirds of the solvent being removed by distn. After addn of a few drops of Py and complete removal of the solvent under reduced pressure, the residue was triturated with petr ether to give II, cryst isomeric mixt (12 g). Recrystallization from CH_2Cl_2–Et_2O gave the analytical sample; mp 169–172°; tlc, R_f 0.56; $[\alpha]D$ +91°. *Anal.* ($C_{30}H_{35}O_6F$) H, C.

To a solution of crude II in MeOH (2,000 ml), sodium acetate

†Beben.

‡The compound prepared in our laboratory showed mp 195–198°; $[\alpha]$ +77°. *Anal.* ($C_{27}H_{37}O_6F$) C, H.

§For other biological assays on compound III see ref 9.

#Melting points were taken in a capillary apparatus and are uncorrected. Optical rotations were detd in dioxane at 24°, $c \sim 1$. Uv were detd in 95% EtOH and ir in Nujol mull. Absorption bands of these spectra were as expected. Tlc were done using 250-μ thick layers (Fluorosil G) and 8:2 C_6H_6–Me_2CO. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values.