17-Aminoacylamido-5-androsten-3β-ols

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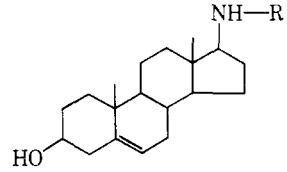
A series of 17-aminoacylamido-5-androsten-3β-ols was made *via* active ester condensations. The aminoacyl groups used were L-leucyl, L-tyrosyl, L-seryl, D-seryl, glycyl, and glycylglycyl. In the case of the L-seryl group, both 17α and 17β isomers are described. The aminoamides show antidepressant activity in mice, but neither the blocked intermediates nor the related 17β-glycolamido-5-androsten-3β-ol are active.

Various amides derived from steroid amines and amino acids have been made and tested for possible endocrine and pharmacological activity. The discovery of interesting neuropharmacological activity in some of these prompts us to describe their preparation and properties.

Blocked intermediates were made by coupling of the steroid amine with an active ester of the suitably blocked amino acid. The active ester method[1] was selected as the most convenient after several trials in which carbonyldiimidazole[2] and dicyclohexylcarbodiimide[3] coupling methods were employed. We found the active ester method to give good yields with a variety of amino acids.

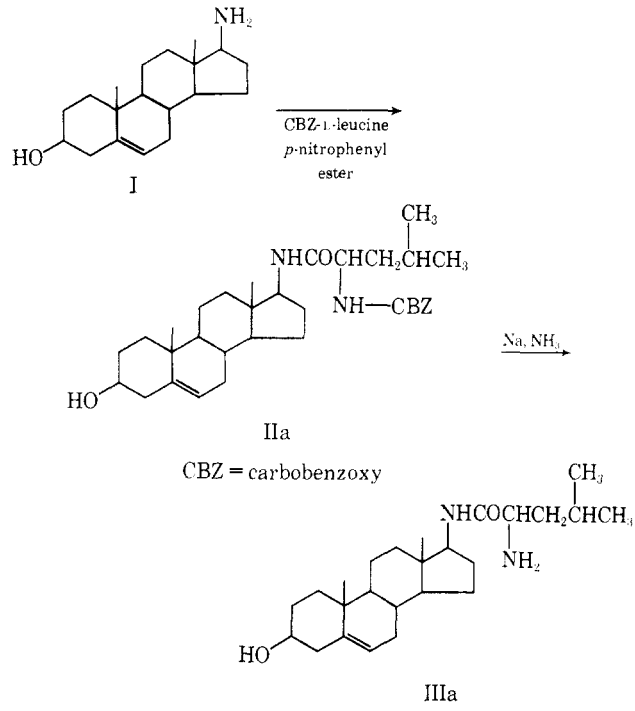
For example, 17β-L-leucinamido-5-androsten-3β-ol (IIIa) was obtained by condensation of carbobenzoxy-L-leucine *p*-nitrophenyl ester with the steroid amine I to obtain the intermediate IIa in 88% yield, followed by deblocking with sodium and liquid ammonia[4] in 58% yield (Scheme I). Similar couplings led to analogous intermediates containing L-tyrosine (IIb), L-serine (IIc), glycine (IId), glycylglycine (IIe), and D-serine (IIf). Reductions of these with sodium and liquid ammonia yielded the amino amides IIIb, IIIc, IIId, and IIIf as well as the peptide amide IIIe.



IIa, R = CBZ-L-leu	IIIa, R = L-leu
IIb, R = N-CBZ-O-Bzl-L-tyr	IIIb, R = L-tyr
IIc, R = N-CBZ-L-ser	IIIc, R = L-ser
IId, R = CBZ-gly	IIId, R = gly
IIe, R = CBZ-glygly	IIIe, R = glygly
IIf, R = N-CBZ-D-ser	IIIf, R = D-ser

In the case of L-tyrosine the *p*-nitrophenyl ester of N-carbobenzoxy-O-benzyl-L-tyrosine[5] was used in the preparation of IIb, and the blocking groups were removed simultaneously during the sodium and ammonia treatment. The *p*-nitrophenyl ester was used as the active ester in all except the serine cases, where the 2,4-dinitrophenyl esters of N-carbobenzoxy-L-serine[6] and N-carbobenzoxy-D-serine were more readily prepared.

SCHEME I



With the discovery of interesting biological activity in these products the question of stereospecificity was studied. At first this involved the preparation of the D-serine derivative IIIf, which is an epimer of IIIc. Subsequently we chose to make also another epimer, 17α-L-serinamido-5-androsten-3β-ol (IV). Coupling of the 2,4-dinitrophenyl ester of N-carbobenzoxy-L-serine with 17α-amino-5-androsten-3β-ol and deblocking as described above proceded in good yield to give IV.

For biological comparison the neutral 17β-glycolamido-5-androsten-3β-ol was made by coupling glycolic acid N-hydroxysuccinimide ester with the steroid amine I.

Related steroidal peptides have been described by Pettit and coworkers.[7] These authors made the L-proline derivative of amine I using Woodward's reagent K for the coupling. Also they have described a number of blocked amides and peptides involving glycine, L-tryptophan, and L-arginine. We began our work unaware of these studies by Pettit's group and did not try the same methods. The only overlap of Pettit's work and ours appears to be in the preparation (by different methods) of 17β-carbobenzoxyglycinamido-5-androsten-3β-ol (IId).

(1) M. Bodanszky, *Nature*, **175**, 685 (1955).
(2) G. W. Anderson and R. Paul, *J. Am. Chem. Soc.*, **80**, 4423 (1958).
(3) J. C. Sheehan and G. P. Hess, *ibid.*, **77**, 1067 (1955).
(4) R. H. Sifferd and V. du Vigneaud, *J. Biol. Chem.*, **108**, 753 (1935).
(5) M. Bodanszky and V. du Vigneaud, *J. Am. Chem. Soc.*, **81**, 5688 (1959).
(6) F. Marchiori, R. Rocchi, and E. Scoffone, *Gazz. Chim. Ital.*, **93**, 834 (1963).
(7) (a) G. R. Pettit, R. L. Smith, A. K. DasGupta, and J. L. Occolowitz, *Can. J. Chem.*, **45**, 501 (1967); (b) G. R. Pettit, R. L. Smith, and H. Klinger, *J. Med. Chem.*, **10**, 145 (1967), and references cited therein.

Table I
Antidepressant Activity in Mice

Compd	Dose, mg/kg	Act. rating
IIIa	20 *po*	+1
	50 *po*	+2
	20 ip	+2
IIIb	50 *po*	+1.5
IIIc	5 *po*	+1
	10 *po*	+2
	20 *po*	+2
IIId	50 *po*	+2
	10 ip	±
	30 ip	+2
IIIe	50 *po*	+3
IIIf	20 *po*	+1
	50 *po*	+2
IV	20 *po*	+1
	50 *po*	+1
Positive control	25 *po*	+2

The new amino amides were tested for antidepressant activity in mice, using a procedure described by Everett.[8] The compounds were given to groups of four mice, and the effects were recorded at the 4-hr period. Amitriptyline hydrochloride was used (at 25 mg/kg *po*) as a positive reference. The activities are recorded in Table I.

Variation of the amino acid component changes the intensity of action but not the type of response. In the serine cases the epimers IIIf and IV were less active than IIIc, leaving the 17β-L-serinamido derivative as one of the more orally active compounds. The glycylglycinamido derivative (IIIe) also showed good activity. The related amide, 17β-glycolamido-5-androsten-3β-ol, showed no activity in the above test. Likewise the blocked intermediate IIc gave a barely detectable response.

Endocrine testing with two of the compounds (IIIb and IIIe) did not show significant activity as measured on the male sex accessory glands of the rat after 12-day treatment at subcutaneous doses up to 1.5 mg/rat/day.[9]

Experimental Section

Tlc was used to determine purity of intermediates and products. Silica gel G plates were used and were developed with combinations of MeOH and $CHCl_3$. A Thomas–Hoover apparatus was used for melting point determinations in capillary tubes. All compounds gave ir spectra consistent with the structures. All compounds gave elementary analyses for C, H, and N within 0.4% of the theoretical values.

17β-(N-Carbobenzoxy-L-leucinamido)-5-androsten-3β-ol (IIa).—A solution of 17β-amino-5-androsten-3β-ol (435 mg, 0.0015 mole) in 6 ml of $CHCl_3$ was treated with *p*-nitrophenyl carbobenzoxy-L-leucinate (639 mg, 0.00165 mole) for 16 hr and then evaporated to dryness *in vacuo*. Crystallization from EtOH–hexane yielded 0.9 g of crude product. Recrystallization (Me_2CO–heptane) afforded white rosettes, 710 mg (88%), mp 165–167°, $[\alpha]^{26}D$ −77.6° (*c* 1.0, $CHCl_3$). *Anal.* ($C_{33}H_{48}N_2O_4$).

17β-(N-Carbobenzoxy-O-benzyl-L-tyrosinamido)-5-androsten-3β-ol (IIb).—To a solution of the amine I (435 mg, 0.0015 mole) in 8 ml of $CHCl_3$ was added *p*-nitrophenyl N-carbobenzoxy-O-benzyl-L-tyrosinate (800 mg, 0.00153 mole). After a few minutes a clear solution resulted, which became milky after 15 min. After 16 hr the mixture was evaporated to dryness *in vacuo*, the residue was washed with ether, and the resulting white solid (1.02 g) was crystallized from $CHCl_3$–hexane, affording white rosettes (0.91 g), mp 202–204°, $[\alpha]^{26}D$ −26.7° (*c* 1.0, $CHCl_3$). *Anal.* ($C_{43}H_{52}N_2O_5$).

17β-(N-Carbobenzoxy-L-serinamido)-5-androsten-3β-ol (IIc).—To a solution of the amine I (867 mg, 0.003 mole) in 13 ml of THF was added 2,4-dinitrophenyl N-carbobenzoxy-L-serinate (1.239 g, 0.00302 mole) in 5 ml of THF. After a few minutes crystallization of the product ensued. After 6 hr ether was added to increase crystallization, and 1 hr later the product was separated; yield 1.35 g. After recrystallization from dioxane–hexane the yield was 1.25 g, mp 133–136° (polymorphic forms, mp 125–127° and mp 110–113°, were also observed), $[\alpha]^{24}D$ −59.6° (*c* 1.0, DMF). *Anal.* ($C_{30}H_{42}N_2O_5$).

17β-Carbobenzoxyglycinamido-5-androsten-3β-ol (IId).—A solution of the amine I (145 mg, 0.0005 mole) in 2 ml of hot $CHCl_3$ was cooled to room temperature and treated with *p*-nitrophenyl carbobenzoxyglycinate (176 mg, 0.00055 mole) for 16 hr. Dilution with hexane caused crystallization; yield 222 mg (92%) of white needles, mp 197–199°. Recrystallization (EtOH–$CHCl_3$) gave mp 200–201°, $[\alpha]^{25}D$ −65° (*c* 1.0, $CHCl_3$); lit.[7b] mp 201–202°, $[\alpha]^{20}D$ −66.7° (*c* 0.87, $CHCl_3$). *Anal.* ($C_{29}H_{40}N_2O_4$).

17β-Carbobenzoxyglycylglycinamido-5-androsten-3β-ol (IIe).—A solution of *p*-nitrophenyl carbobenzoxyglycylglycinate (0.852 g, 0.0022 mole) in 5 ml of dioxane was added to a solution of the amine I (0.578 g, 0.002 mole) in 4 ml of dioxane. The mixture became cloudy and crystallization began after 2 min. At 2 hr the product was separated, washed with $CHCl_3$ and Et_2O; yield 965 mg of white needles, mp 223–225°. Recrystallization of 1.442 g of material of this quality from 40 ml of dioxane gave 1.343 g, white needles, mp 228–229°, $[\alpha]^{26}D$ −55.4° (*c* 1.0, DMF). *Anal.* ($C_{31}H_{43}N_3O_5$).

17β-(N-Carbobenzoxy-D-serinamido)-5-androsten-3β-ol (IIf).—The intermediate 2,4-dinitrophenyl N-carbobenzoxy-D-serinate was first prepared as follows. 2,4-Dinitrophenol (3.5 g, 0.019 mole) was added to a solution of N-CBZ-D-serine (4.1 g, 0.017 mole) in 30 ml of THF. The mixture was cooled to 0°, dicyclohexylcarbodiimide (3.56 g, 0.017 mole) was added, and the mixture was kept cold 16 hr. The solution was clarified by filtration and then concentrated to an oily residue, which was crystallized from EtOH–hexane; yield 3.6 g of white fluffy needles, mp 115–116°, $[\alpha]^{25}D$ +32.5° (*c* 1, DMF); lit.[6] L isomer, mp 116–117°, $[\alpha]^{20}D$ −32.7° (*c* 2, DMF).

The above 2,4-dinitrophenyl ester (1.239 g, 0.00303 mole) was added to a solution of the amine I (0.867 g, 0.003 mole) in 9 ml of dioxane. After 16 hr the solution was concentrated to a solid, which was suspended in ether and collected on a filter. The product (1.48 g) was recrystallized from MeOH–$CHCl_3$–hexane to give 1.2 g, mp 168–170°, $[\alpha]^{25}D$ −49° (*c* 1.0, EtOH). *Anal.* ($C_{30}H_{42}N_2O_5$).

17α-(N-Carbobenzoxy-L-serinamido)-5-androsten-3β-ol.—A solution of 2,4-dinitrophenyl N-carbobenzoxy-L-serinate (826 mg, 0.00202 mole) in 3 ml of dioxane was added to a solution of 17α-amino-5-androsten-3β-ol[10] (578 mg, 0.002 mole) in 6 ml of dioxane. After 8 hr, Et_2O–hexane was added to induce crystallization. The product was washed with Et_2O; yield 672 mg, mp 174–176°. Recrystallization ($CHCl_3$–MeOH–hexane) gave 792 mg, mp 178–180°, $[\alpha]^{24}D$ −11° (*c* 1.0, EtOH), $[\alpha]^{26}D$ −36° (*c* 1.0, DMF). *Anal.* ($C_{30}H_{42}N_2O_5$).

Reduction of the Intermediates. General Procedure.—A solution of the carbobenzoxy intermediate in dioxane (20–50 ml) was added to 350 ml of liquid NH_3 (freshly distilled from Na). The stirred reaction mixture, at the NH_3 boiling point, was touched with a glass tube containing an exposed sodium tip until a pale blue color persisted for about 30 sec. The solvent was evaporated, and the residue was dissolved in $CHCl_3$, MeOH, and water. The $CHCl_3$ extract was washed with saturated NaCl, dried (Na_2SO_4), and evaporated. The solid residue was then

(8) G. M. Everett, Proceedings of the First International Symposium on Antidepressant Drugs, Milan, 1966; *Excerpta Med. Intern. Congr. Ser.*, **No. 122**, 164 (1967). See also G. M. Everett, F. Will, and A. Evans, *Fed. Proc.*, **23**, 198 (1964). In rating the test compounds, +1 denotes increased activity, +2 denotes markedly increased activity plus irritability, and +3 is maximal response including aggressive fighting.

(9) Endocrine testing was done by Dr. R. Oslapas and associates. The test used was similar to the intact male rat assay described by others; see R. A. Edgren, *Acta Endocrinol., Suppl.* **87**, 11 (1963).

(10) The 17α-amino-5-androsten-3β-ol (mp 195°) was made from NH_3 and 17β-*p*-toluenesulfonoxy-5-androsten-3β-ol at 125° under pressure; J. W. Cole, French Patent 1,365,225 (1964); *Chem. Abstr.*, **62**, 5319 (1965); see also C. H. Robinson, C. Ermann, and D. P. Hollis, *Steroids*, **6**, 509 (1965).

TABLE II
AMINOAMIDE DERIVATIVES OF 5-ANDROSTEN-3β-OL

Compd	Formula	Yield, %	Mp, °C	$[\alpha]^{25}D$, deg	Recrystn solvents
IIIa	$C_{25}H_{42}N_2O_2$	57	194–196	−39 (*c* 1, EtOH)	EtOH–hex
IIIb	$C_{28}H_{40}N_2O_3$	63	258–260	−37 (*c* 0.95, EtOH)	EtOH–$CHCl_3$–hex
IIIc	$C_{22}H_{36}N_2O_3$	50	204–206	−117 (*c* 1, DMF)	MeOAc
IIId	$C_{21}H_{34}N_2O_2$	56	255–259	−107.5 (*c* 0.92, EtOH)	EtOH–hex
IIIe	$C_{28}H_{37}N_3O_3$	57	289–291	−78.6 (*c* 1, DMF)	EtOH–hex
IIIf	$C_{22}H_{36}N_2O_3$	30	257–260	−120 (*c* 1, AcOH)	MeOH–THF
IV	$C_{22}H_{36}N_2O_3$	49	199–202	−17.6 (*c* 1, EtOH)	THF–EtOH–Et_2O–hex

recrystallized to obtain the product as indicated in Table II.

N-Hydroxysuccinimidyl Glycolate.—Dicyclohexylcarbodiimide (1.237 g, 0.006 mole) was added to a cold (0°) solution of N-hydroxysuccinimide (0.691 g, 0.006 mole) and glycolic acid (0.456 g, 0.006 mole) in 12 ml of dioxane. After stirring 2 hr at 0° and 20 hr at 25°, the solution was filtered to remove dicyclohexylurea. The combined filtrate and washings (dioxane) were evaporated *in vacuo;* the residue was recrystallized (EtOH–hexane) to yield 0.85 g, mp 135–136°. *Anal.* ($C_6H_7NO_5$).

17β-Glycolamido-5-androsten-3β-ol.—The hydroxysuccinimidyl glycolate (173 mg, 0.001 mole) was added to a solution of the amine I (289 mg, 0.001 mole) in 3 ml of dioxane. A precipitate which formed almost immediately was redissolved by warming. After 16 hr the crystalline product was separated (yield 317 mg, mp 266–270°) and recrystallized (EtOH–$CHCl_3$–Et_2O) to give 258 mg, mp 270–273°, $[\alpha]^{26}D$ −109° (*c* 1.0, AcOH). *Anal.* ($C_{21}H_{33}NO_4$).

Acknowledgment. The authors are indebted to Dr. E. B. Goodsell for the pharmacological testing and the evaluation of antidepressant activity, to Dr. Ray Oslapas and Mr. T. Kallal for endocrine assays, to Mr. W. Washburn and associates for ir spectra, and to Mr. V. Rauschel and associates for elemental analyses.

Notes

The Mitomycin Antibiotics. Synthetic Studies. XX.[1] N-Substituted Carbamoyl and Acyl Esters of a Related 3-Hydroxymethyl-4,7-indoloquinone

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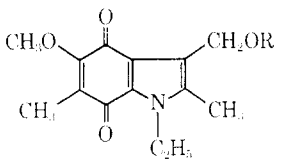
The finding that the mitomycin-related indoloquinone **1** possessed interesting *in vitro* and *in vivo* antibacterial activity[2] prompted the preparation of a variety of congeners. In the present paper we report the synthesis and antibacterial properties of compounds wherein the carbamoyl moiety present in **1** is variously

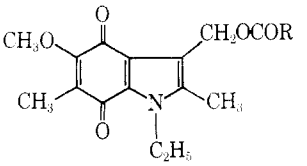


1, R = $CONH_2$
2, R = H
3, R = $COOC_6H_5$

substituted on the nitrogen atom or replaced by other acyl groups. Variations of the group attached to the 3-methylene carbon are of special interest in the delineation of the structure–activity relationships, inasmuch as this carbon is one of the sites for biological alkylation, which may be implicated in the mechanisms by which the mitomycins exert their effect.[3]

Acylation of the indoloquinone-3-carbinol **2**[2] with certain acid anhydrides or acyl halides in pyridine gave the ester derivatives described in Table I. For the

TABLE I
CARBOXYLIC ESTERS OF 1-ETHYL-3-HYDROXYMETHYL-5-METHOXY-2,6-DIMETHYL-4,7-INDOLOQUINONE[a]



Compd	R	Yield, %[b]	Mp, °C[c]	Formula
5	CH_3	91	144–145[e]	$C_{16}H_{19}NO_5$
6	C_2H_5	85	127–128	$C_{17}H_{21}NO_5$
7	*i*-C_3H_7	82	113–114[f]	$C_{18}H_{23}NO_5$
8	C_6H_{11}	70	127–128	$C_{21}H_{27}NO_5$
9	C_6H_5	71	139–141	$C_{21}H_{21}NO_5$[g]
10	2-C_4H_4O	91	156–157	$C_{19}H_{19}NO_6$
11	$C_6H_5CH{=}CH$	66	123–125	$C_{23}H_{23}NO_5$
12	C_6H_5CHOAc	20	100–101	$C_{24}H_{25}NO_7$
13	CH_3O	80	137–138	$C_{16}H_{19}NO_6$
14	C_2H_5O	80	112–113	$C_{17}H_{21}NO_6$

[a] Prepared by method A. [b] Material of analytical purity. [c] Unless noted otherwise, all compounds were recrystallized from CH_2Cl_2–petroleum ether (bp 30–60°). [d] Analyses for C, H, and N were within ±0.4% of the theoretical values except where noted. [e] Recrystallized from Me_2CO–hexane. [f] Recrystallized from EtOAc–hexane. [g] N: calcd, 3.81; found, 4.32.

(1) Paper XIX: J. F. Poletto, G. R. Allen, Jr., and M. J. Weiss, *J. Med. Chem.*, **10**, 95 (1967).

(2) G. R. Allen, Jr., and M. J. Weiss, *ibid.*, **10**, 1 (1967).

(3) (a) W. Szybalski and V. N. Iyer, *Fed. Proc.*, **23**, 946 (1964); (b) A. Weissbach and A. Lisio, *Biochemistry*, **4**, 196 (1965).