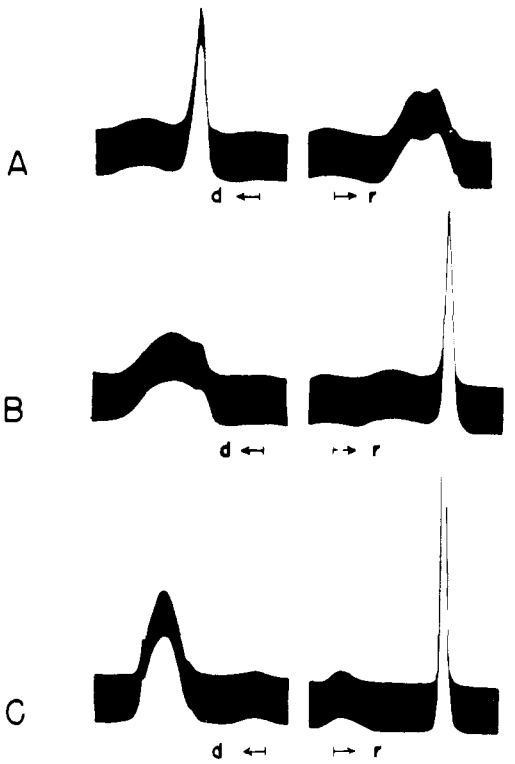

Fig. 2.—Electrophoretic patterns of Armour bovine serum albumin in various buffer solvents at *p*H 4.0: A, 0.002 ionic strength NaAc–HAc + 0.198 ionic strength NaCl; B, 0.005 NaAc–HAc + 0.195 NaCl; C, 0.20 NaAc–HAc. Protein migrated toward the cathode. The product of field strength and time is approximately the same in all these experiments.

aries, *i.e.*, those corresponding to more positively charged components, at the expense of slower moving ones. That this represents an effect of increasing Ac^- and/or HAc rather than decreasing Cl^- is indicated by the observation that the patterns obtained in 0.01 ionic strength NaAc–HAc, *p*H 4.7, are essentially the same as those obtained in 0.01 NaAc–HAc + 0.015 NaCl (the chief difference being that the more rapidly moving boundary in the rising pattern was not resolved into two peaks at the lower ionic strength).[2]

While binding of acetate has been either demonstrated or postulated in a number of protein systems, this is the first demonstration that such binding has a major effect on protein structure. (Such binding does not, however, change the sedimentation constant.) Recognition of this effect increases our understanding of the structure of BSA. The non-enantiographic nature of the electrophoretic patterns of this protein has been interpreted by Longsworth and Jacobsen[3] to indicate continual readjustment of an equilibrium across the moving boundaries. But other workers assume that BSA is a simple mixture of proteins.[4] A solution of BSA may well contain molecules which differ chemically.[5] However, we conclude from our experiments that BSA is indeed an equilibrium mixture of electrophoretic components; that any chemically different molecules participate in this equilibrium; and that the equilibrium composition depends upon the composition of the supporting medium, particularly the acetate concentration.[6] Two independent observations support these conclusions, first, if at the end of the usual moving-boundary electrophoresis experiment of duration *t*, the polarity of the electrodes is reversed and the experiment continued for another length of time *t*, then the final boundaries which have now returned to their initial positions are not single symmetrical ones as expected of a simple mixture of proteins but are complex showing as many as three distinct, although poorly resolved peaks. This suggests that an equilibrium was being continuously readjusted as the boundaries returned to their initial positions. Experiments of this type were carried out at several ionic strengths and concentrations of NaAc-HAc. The second piece of evidence is afforded by small differences in the ultraviolet absorption spectra of BSA in 0.1 NaAc–HAc and in 0.002 NaAc–HAc + 0.098 NaCl. These differences are confirmed by a differential spectrum which shows two main bands at 2350–2400 and 2900Å., and indicate that binding of Ac^- and/or HAc by BSA produces structural changes in the protein, perhaps due to making or breaking of hydrogen bonds.

The effect described above has also been observed with ovalbumin and γ-globulin and accounts for the complex electrophoretic patterns observed[7] for these proteins in certain buffers. Experiments are in progress to elucidate the nature of the effect of various buffer constituents (such as homologous series of aliphatic acids) on protein structure.

(2) The possibilities that the complex patterns arise from electrical anomalies or as a result of poor buffering action, have also been eliminated.

(3) L. G. Longsworth and C. F. Jacobsen, *J. Phys. Colloid Chem.*, **53**, 126 (1949).

(4) R. A. Alberty, *ibid.*, **53**, 114 (1949).

(5) H. A. Sober, F. J. Gutter, M. M. Wyckoff and E. A. Peterson, THIS JOURNAL, **78**, 756 (1956).

(6) The electrophoretic patterns of BSA are also dependent on whether the buffer cation is Na^+ or K^+, *p*H, and to some extent ionic strength.

(7) A. Saifer and H. Corey, *J. Biol. Chem.*, **217**, 23 (1955); Abstracts of Papers, 128th Meeting American Chemical Society, Minneapolis, Minn., Sept. 11–16, 1955, p. 40-C.

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SYNTHESIS AND ANABOLIC ACTIVITY OF 4-SUBSTITUTED TESTOSTERONE ANALOGS

Sir:

We wish to make a preliminary announcement of the synthesis and anabolic activity of 4-substituted testosterone analogs.

Treatment of testosterone with alkaline hydrogen peroxide gave a mixture of the β- and α-epoxides, which could be separated by crystallization: 4β,5-oxidoetiocholane-17β-ol-3-one, m.p. 156–157°, $(\alpha)_D$ +145° and 4α,5-oxidoandrostane-17β-ol-3-one, m.p. 147–148°, $(\alpha)_D$ −33° [Calcd. for $C_{19}H_{28}O_3$: C, 74.96; H, 9.27. Found: C, 74.86; H, 9.35 and C, 75.16; H, 9.28]; acetates m.p. 155–157°, $(\alpha)_D$ +130° ($CHCl_3$) and m.p. 172–173°, $(\alpha)_D$

$-62°$ ($CHCl_3$) [Calcd. for $C_{21}H_{30}O_4$ C, 72.80; H, 8.73. Found C, 72.74; H, 8.88 and C, 72.86; H, 8.74]. The assignment of configuration was based on rotatory power, taking as reference 4β,5-oxido coprostane-3-one, whose structure has been demonstrated.[1]

Rearrangement of the β- or α-epoxide with boron trifluoride in benzene yielded Δ^4-androstene-4,17β-diol-3-one, m.p. 222–223°, strong ferric chloride test, while acetic acid containing 2% of H_2SO_4 gave Δ^4-androstene-4,17-β-diol-3-one 17-acetate (4-hydroxytestosterone 17-acetate) m.p. 194–196°., $(\alpha)_D$ +83°, λ_{max} 277 mμ, ϵ = 12,100 [Calcd. for $C_{21}H_{30}O_4$: C, 72.80; H, 8.73; Found: C, 72.40; H, 8.80].

The latter compound formed with O-phenylenediamine a quinoxaline derivative and with acetic anhydride in pyridine a 4,17-diacetate, m.p. 170–172°, $(\alpha)_D$ +105°, λ_{max} 246 mμ, ϵ = 15,500 [Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.03; H, 8.25].

The 4β,5-oxidoetiocholane-17β-ol-3-one in acetone containing dil. H_2SO_4 rearranged to 2α-hydroxytestosterone, m.p. 160–162°, $(\alpha)_D$ +166° (reported[2]: m.p. 161–162°, $(\alpha)_D$ +120°), while the 4α,5-oxidoandrostane-17β-ol-3-one acetate gave the normal product of epoxide fission androstane-4β,5α,17β-triol-3-one 17-acetate, m.p. 213–215° [Calcd. for $C_{21}H_{32}O_5$: C, 69.20; H, 8.85; Found: C, 69.07; H, 9.18].

Treatment of the 4β,5-oxidoetiocholane-17β-ol-3-one acetate with the requisite hydrogen halide in acetic acid formed the 4-bromotestosterone acetate, m.p. 196–197°, λ_{max} 261 mμ, ϵ = 11,600 [Calcd. for $C_{21}H_{29}O_3Br$: C, 61.61; H, 7.14. Found: C, 61.66; H, 7.18], the 4-chlorotestosterone acetate (3), m.p. 228–230°, $(\alpha)_D$ +118°, λ_{max} 255 mμ, ϵ = 13,300 [Calcd. for $C_{21}H_{29}O_3Cl$: C, 69.11; H, 8.01; Found: C, 69.37; H, 8.22] and the 4-fluorotestosterone acetate, m.p. 178–180°, λ_{max} 241 mμ, ϵ = 11,670 [Calcd. for $C_{21}H_{29}O_3F$; C, 72.38; H, 8.38. Found: C, 72.50; H, 8.61].

The reaction of the 4α,5-oxidoandrostane-17β-ol-3-one acetate with hydrogen chloride in acetic acid produced the 4β-chloroandrostane-5α,17β-diol-3-one-17-acetate, m.p. 202–204° [Calcd. for $C_{21}H_{31}O_4Cl$: C, 65.86; H, 8.15. Found: C, 65.68; H, 8.32] with on prolonged heating eliminated water to give 4-chlorotestosterone acetate.[3]

In the same way the α-epoxide acetate on reaction with hydrogen bromide in acetic acid yielded directly 4-bromotestosterone acetate.

The β- and α- epoxides reverted to testosterone on treatment with aqueous hydrogen iodide in chloroform or by heating with potassium iodide in acetic acid. The corresponding derivatives were obtained also from 11β-hydroxytestosterone, 17α-methyltestosterone, 19-nortestosterone, progesterone, 11β-hydroxyprogesterone, desoxycorticosterone, cortisone and other steroids and will be described in forthcoming papers.

(1) Pl. A. Plattner, H. Heusser and A. B. Kulkarni, *Helv. Chim. Acta*, **31**, 1822 (1948).

(2) F. Sondheimer, St. Kaufmann, J. Romo, H. Martinez and G. Rosenkranz, THIS JOURNAL, **75**, 4712 (1953).

(3) 4-Chlorotestosterone acetate was prepared also by direct chlorination of testosterone acetate.

Most of the described compounds showed remarkable anabolic activity and low androgenic effect. The following anabolic androgenic ratios, determined according to Hershberger, Shipley and Meyer,[4] at 500 γ daily doses, were obtained:

Testosterone propionate	0.28
4-Chlorotestosterone acetate	.88
4-Hydroxytestosterone acetate	.61
4-Fluorotestosterone acetate	.35
19-Nortestosterone cyclopentylpropionate	.72
4-Chloro-19-nortestosterone cyclopentylpropionate	1.82
4-Chloro-19-nortestosterone acetate	1.60

We are indebted to Dr. Sala and G. Baldratti of our Department of Pharmacology for the data on anabolic and androgenic potency and to Dr. F. Canal for the microanalyses.

(4) L. G. Hershberger, E. G. Shipley and R. K. Meyer, *Proc. Soc. Exp. Biol. Med.*, **83**, 175 (1953).

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A NEW PANCREATIC CARBOXYPEPTIDASE

Sir:

A hitherto unreported carboxypeptidase has been found in the euglobulin fraction obtained from bovine pancreas after autolysis. It manifests a rapid activity in releasing lysine and arginine from the carboxyl terminal position of the synthetic substrates, α-N-benzoylglycyl-L-lysine (BGL) and benzoylglycyl-L-arginine (BGA), which is not ascribable to the known carboxypeptidase (CP). The new enzyme is tentatively designated basic carboxypeptidase (BCP) because of its apparent requirement for a basic carboxyl terminal amino acid. Like CP it exists in fresh frozen pancreas as a proenzyme which is liberated in active form upon treatment of pancreas extract with trypsin.

In Table I are shown the proteolytic coefficients of some of the fractions obtained during a typical

TABLE I

PROTEOLYTIC COEFFICIENTS[a] OF CARBOXYPEPTIDASE PREPARATIVE FRACTIONS WITH DIFFERENT SUBSTRATES

	Substrate[b] CGP[c]	BGL[d]	BGA[e]
Crude pancreas extract	1.12	0.85	0.64
Crude euglobulin precipitate	3.26	2.55	1.90
Barium hydroxide extract	3.62	3.00	2.85
Once crystallized carboxypeptidase	18.00	2.85	2.10
Three times crystallized carboxypeptidase	19.40	[f]	[f]
Six times crystallized carboxypeptidase	19.10	[g]	[g]

[a] First order proteolytic coefficient defined as the first order velocity constant, $Kt = \log (a/a - x)$, per mg. protein N/ml. The extent of hydrolysis was measured on 0.2-ml. samples by the alcoholic KOH titration procedure of Grassman and Heyde, *Z. physiol. Chem.*, **183**, 32 (1929). [b] Substrates employed at 0.025 *M* concentration with 0.025 *M* "tris" buffer *p*H 7.65 at 25°. [c] Hofmann and Bergmann, *J. Biol. Chem.*, **134**, 225 (1940). [d] Hofmann and Bergmann, *ibid.*, **134**, 225 (1940); Folk, *Arch. Biochem. Biophys.*, in press. [e] Folk, unpublished data. [f] Five to 10% hydrolysis in 24 hours at a substrate–enzyme molar ratio of 300 to 1. [g] Very little hydrolysis, only detectable by paper chromatography after 24 hours at a substrate–enzyme molar ratio of 30 to 1.