Since the *s*-butyl alcohol/3% ammonia system is used in an extended run with an absorbent pad at the bottom, the rate is referred to an amino acid mixture run in parallel as reference. The third column shows the reaction to isatin. The formation of a blue spot is considered positive indication of N-terminal proline.[7] The last column shows the amino acid composition as determined by complete acid hydrolysis (6 *N* hydrochloric acid for 16 hours at 105° in a sealed tube). In order to confirm the sequence of the amino acids in the first two fragments in the table, they were treated with carboxypeptidase for 24 hours and the products were chromatographed in the *s*-butyl alcohol/3% ammonia system. In the first case only phenylalanine and glutamic acid were detected with ninhydrin, although isatin gave a blue spot, presumably due to Pro.Leu. In the second case only glutamic acid was detected with ninhydrin, although isatin again gave the same blue spot.

Thus it appears that Corticotropin-A has the C-terminal sequence: . . Pro.Leu.Glu.Phe. On prolonged treatment, carboxypeptidase splits off the last three amino acids quantitatively, stopping at proline. This result is consistent with classical concepts of carboxypeptidase action.[8] Pepsin splits off the entire tetrapeptide and then apparently makes a secondary split between glutamic acid and phenylalanine. A secondary split such as this is consistent with the work of Fruton and Bergmann,[9] who found that carbobenzoxyglutamylphenylalanine was hydrolyzed slowly by pepsin at the *p*H used in our experiment, although the reaction went more rapidly at higher *p*H's.

Due to the specific requirements of carboxypeptidase, it is not possible to conclude from this work that Corticotropin-A is made up of a single, unbranched chain. Further work with the more generally applicable chemical techniques is in progress.

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RECEIVED JUNE 22, 1953

TOTAL SYNTHESIS OF RACEMIC METHYL 3-KETOETIOCHOLANATE

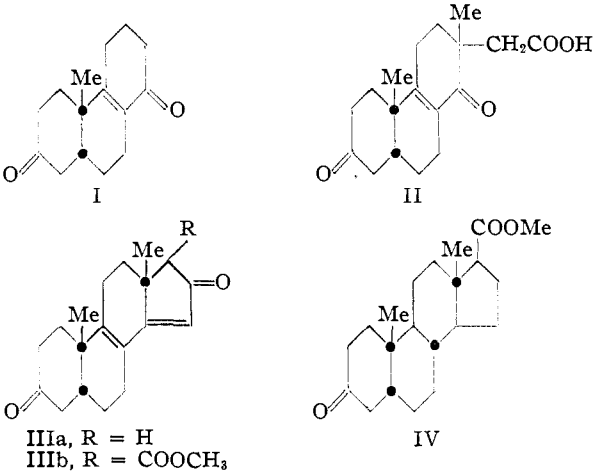
Sir:

We wish to report completion of the total synthesis of methyl 3-ketoetiocholanate (IV), a steroid first prepared by Reichstein and co-workers[1] from stigmasterol in connection with the partial synthesis of desoxycorticosterone.

cis - 4b - Methyl - Δ^{10a-4a} - dodecahydrophenanthrene-1,7-dione (I)[2] was methylated as the 2-hydroxymethylene-7-ethylene ketal derivative and cleaved to the 2-methyl-7-ethylene ketal, which on treatment with triphenylmethylsodium and methyl bromoacetate followed by hydrolysis gave the epimeric diketo acids II, isomer A, m.p. 117–118° (Found: C, 71.0; H, 7.82) and isomer B, m.p. 149.5–151.5° (Found: C, 71.1; H, 7.95). Reaction of each as the acid chloride with the sodium derivative of *t*-butyl malonate, acid hydrolysis to the methyl ketone and sodium methoxide cyclization gave the two tetracyclic diketones IIIa epimeric at C-13, isomer A, m.p. 176–177°, λ_{max}^{EtOH} 289 mμ (ϵ = 25,400) (Found: C, 80.2; H, 8.62), isomer B, m.p. 138.5–139.5°, λ_{max}^{EtOH} 289 mμ (25,100) (Found: C, 80.2; H, 8.71).[3]

The same isomers of the tetracyclic ketone IIIa were also obtained from a similar sequence using the derivative of I having an additional 8-8a double bond, the latter being reduced by alkaline palladium hydrogenation at the stage of the crystalline doubly unsaturated methyl triketones.

Selective ketal formation at C-3, reaction with methyl carbonate and sodium hydride, and selective cleavage with dilute methanolic acid gave the crystalline 17-carbomethoxy derivative IIIb in each series. Vigorous hydrogenation with platinum oxide in acetic acid containing hydrochloric acid gave hydrogenolysis of the 16-oxygen function as well as hydrogenation to the fully saturated derivative (accompanied by some of the 3-hydroxy ester retaining a double bond). After purification as the hydroxy acid, chromic acid oxidation of the methyl ester gave a mixture from which the saturated 3-keto-17-carbomethoxy derivative IV could be isolated. The crystalline *dl*-keto ester obtained from isomer A differed in infrared spectrum from both the natural derivative and its 14β,17α-isomer. The infrared spectrum of the ketoester IV prepared from isomer B, m.p. 122.5–125.5°, determined both on the Baird double beam and Perkin–Elmer single beam double pass instruments, showed it to be the *dl*-methyl ester corresponding in configuration to natural 3-ketoetiocholanic acid. Since this acid has been converted into desoxycorticosterone, progesterone, and into intermediates which have been interrelated with most of the other steroids, this



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(3) For this general method see A. L. Wilds and T. L. Johnson, *ibid.*, **70**, 1166 (1948).

completes another route for the total synthesis of these natural products.

Further details will be reported in future papers for these transformations.

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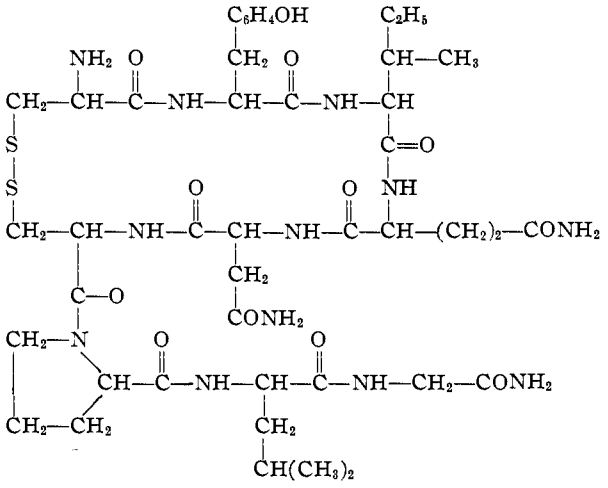
THE SYNTHESIS OF AN OCTAPEPTIDE AMIDE WITH THE HORMONAL ACTIVITY OF OXYTOCIN

Sir:

Highly purified preparations of oxytocin, the principal uterine-contracting and milk-ejecting hormone of the posterior pituitary, have been obtained in this laboratory, which upon hydrolysis gave 1 equivalent each of leucine, isoleucine, tyrosine, proline, glutamic acid, aspartic acid, glycine and cystine, and 3 equivalents of ammonia.[1,2,3,4] The active principle appeared to be a polypeptide of molecular weight approximately 1000.[3,5] Degradative studies indicated some type of cyclic disulfide.[6,7] On the basis of further degradative studies[5,8,9,10] along with the assumption that glutamine and asparagine residues were present rather than their isomers, the following structure was postulated[10] for oxytocin, the amino acids having the L configuration.



It was known from the work of Sealock and du Vigneaud[11] that oxytocin could be reduced and reoxidized without appreciable inactivation and that treatment of the reduced material with benzyl chloride resulted in loss of activity. If oxytocin could be regenerated from benzylated oxytocin and if the proposed structure be correct, a total synthesis of the hormone should follow from the preparation of the nonapeptide derivative, N-carbobenzoxy - S - benzyl - L - cysteinyl - L - tyrosyl - L-isoleucyl - L - glutaminyl - L - asparaginyl - S - benzyl-L-cysteinyl-L-prolyl-L-leucylglycine amide (I).

The preparation of S,S′-dibenzyloxytocin from the natural hormone and its possible reconversion to oxytocin were therefore explored. Oxytocin, with sodium in liquid ammonia, followed by benzyl chloride, has given the desired benzyl derivative from which the hormone can be regenerated by debenzylation by the sodium–liquid ammonia method[12] followed by oxidation with air.

Synthesis of I was accomplished by coupling N-carbobenzoxy-S-benzyl-L-cysteinyl-L-tyrosine (II) with the heptapeptide amide L-isoleucyl-L-glutaminyl - L - asparaginyl - S - benzyl - L - cysteinyl-L-prolyl-L-leucylglycine amide (V), prepared in turn from tosyl-L-isoleucyl-L-glutaminyl-L-asparagine (IV), and the tetrapeptide amide, S-benzyl-L-cysteinyl-L-propyl-L-leucylglycine amide (III).

Ethyl L-leucylglycinate was condensed with carbobenzoxy-L-proline by the isovaleryl mixed anhydride procedure[13] to give ethyl carbobenzoxy-L-prolyl-L-leucylglycinate, m.p. 148–149°, $[\alpha]^{22.5}D$ −79.8° (*c* 2.5, ethanol) (calcd. for $C_{23}H_{33}O_6N_3$: C, 61.7; H, 7.43; N, 9.39. Found: C, 61.8; H, 7.65; N, 9.24). The latter was reduced catalytically and then coupled with biscarbobenzoxy-L-cystinyl bischloride. The saponified product was reduced and benzylated in liquid ammonia to give S-benzyl-L-cysteinyl-L-prolyl-L-leucylglycine, which was esterified to the corresponding benzyl ester hydrochloride, m.p. 193–194° dec. (calcd. for $C_{30}H_{41}O_5N_4SCl$: N, 9.26; S, 5.30. Found: N, 9.17; S, 5.36). Treatment of the benzyl ester with methanolic ammonia gave tetrapeptide amide III.

1-Tosylpyrrolid-5-one-2-carboxyl chloride, from tosyl-L-glutamic acid and phosphorus pentachloride, was coupled with L-asparagine and the resulting N-(1′-tosylpyrrolid-5′-one-2′-carbonyl)-L-asparagine, m.p. 150–151° (calcd. for $C_{16}H_{19}O_7N_3S$: C, 48.4; H, 4.82; N, 10.6. Found: C, 47.9; H, 5.04; N, 10.4), was treated with aqueous ammonia. After removal of the tosyl group from the tosyl-L-glutaminyl-L-asparagine, m.p. 197–198° (calcd. for $C_{16}H_{22}O_7N_4S$: C, 46.4; H, 5.35; N, 13.5; amide N, 6.7. Found: C, 46.3; H, 5.55; N, 13.2; amide N, 6.6), by sodium in liquid ammonia,[14] the dipeptide, m.p. 210–211° dec., $[\alpha]^{21}D$ +17.1° (*c* 1.5, water) (calcd. for $C_9H_{16}O_5N_4$: C, 41.5; H, 6.20; N, 21.5. Found: C, 41.2; H, 6.60; N, 21.3) was coupled with tosyl-L-isoleucyl chloride to give tosyl-L-isoleucyl-L-glutaminyl-L-asparagine (IV), m.p. 225–226°, $[\alpha]^{22}D$ −28.9° (*c* 1.76, 0.5 *N* $KHCO_3$) (calcd. for $C_{22}H_{33}$-

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