b.p. 70–72° at 1 mm., $n^{20}D$ 1.4349, m.p. of *p*-toluidide 79°)[2]; and (6) methyl tridecanoate (6.69 g., b.p. 90–95° at 1 mm., $n^{20}D$ 1.4405, m.p. of *p*-toluidide 87°, m.p. of mixture with authentic sample 87°).

Anal. Calcd. for $C_{20}H_{33}ON$: C, 79.15; H, 10.96; N, 4.62. Found: C, 78.85; H, 11.02; N, 4.73.

Tridecanoic acid for comparison was prepared by the reaction of carbon dioxide with *n*-dodecylmagnesium bromide. After the distillation of the above products, a residue (33.84 g., average mol. wt. 397, 12 ethylene : 1 methyl formate) remained.

A reaction mixture containing methyl formate (540 g., 9.0 moles), 1-hexene (25.2 g., 0.30 mole) and *t*-butyl peroxide (4.4 g., 0.031 mole) was held at 130–135° in the above apparatus for 24 hours. After the removal by distillation of unreacted methyl formate, 1-hexene and *t*-butyl alcohol, the following fractions were obtained: (1) methyl enanthate (4.71 g., b.p. 112–115° at 100 mm., $n^{22}D$ 1.4100, m.p. of *p*-toluidide 79.5–80°, m.p. of mixture with authentic sample 79.5–80°); (2) a mixture of 1:1 and 2:1 products (1.02 g., b.p. 120–130° at 20 mm., $n^{22}D$ 1.4303, average mol. wt. 188); (3) mixture as in 2 (1.23 g., b.p. 130–149° at 20 mm., $n^{22}D$ 1.4323); and (4) 2:1 product, presumably methyl β-*n*-butylpelargonate (2.34 g., b.p. 135–140° at 12 mm., $n^{22}D$ 1.4372, mol. wt. 236, calcd. mol. wt. 228). A residue (10.3 g., average mol. wt. 385) remained.

Further work to determine the scope of this reaction and other inducing agents for it is continuing.

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RECEIVED JULY 27, 1953

STUDIES ON PITUITARY ADRENOCORTICOTROPIN. VII. A C-TERMINAL SEQUENCE OF CORTICOTROPIN-A

Sir:

Corticotropin-A[1] has been treated with carboxypeptidase and the release of amino acids has been followed by paper chromatography. In a preliminary experiment, a sample of Corticotropin was incubated at 37° with 1% by weight of carboxypeptidase[2] for 24 hours in *p*H 7.5 ammonium acetate buffer. Acetic acid was added to stop the reaction and the buffer was removed by lyophilization. The residue was divided into two parts and chromatographed in the two solvent systems which we employ to resolve the principal amino acids.[3] Only phenylalanine, glutamic acid and leucine were detected. In order to determine the sequence of these amino acids, another sample of Corticotropin-A was incubated with carboxypeptidase under the same conditions and aliquots were removed at suitable intervals. These aliquots were chromatographed in one of the solvent systems (*s*-butyl alcohol/3% ammonia) in comparison with standard quantities of the three amino acids. By photometric measurement of the ninhydrin colors,[4] quantitative values were obtained. The results are shown in Fig. 1. It is apparent that the order of release is: phenylalanine, glutamic acid, leucine. Thus, in the nomenclature of Sanger,[5] the C-terminal sequence appears to be: . . Leu.Glu.Phe.

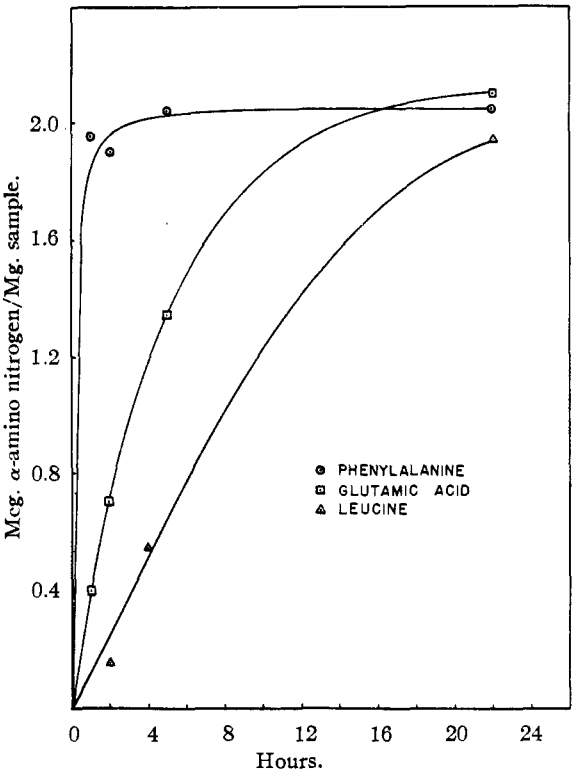

Fig. 1.—Rate of liberation of amino acids from corticotropin-A by treatment with carboxypeptidase.

Additional evidence for this sequence has been obtained from a study of three of the peptide fragments resulting from peptic[6] hydrolysis of Corticotropin-A. Table I shows the data which have been obtained on these fragments. In the first two columns are given the rates of movement in the two solvent systems used to separate the fragments.

TABLE I

THREE FRAGMENTS ISOLATED FROM PEPTIC DIGESTS OF CORTICOTROPIN-A

Rates of movement			
Partridge system[a]	*s*-Butyl alcohol ammonia	Isatin test	Amino acid composition (complete acid hydrolysis)
0.81	Tyr	+	Pro, Leu, Glu, Phe
.73	Thr	+	Pro, Leu, Glu
.68	Phe	−	Phe

[a] *n*-Butyl alcohol–acetic acid–water (80:20:100).

(1) W. F. White, THIS JOURNAL, **75**, 503 (1953).

(2) A six-times recrystallized material prepared by Miss E. Dickinson of The Armour Laboratories. Although free from chymotryptic contamination, the enzyme was incubated with di-isopropyl fluorophosphate before use.

(3) Phenol (72%)/water and *s*-butyl alcohol/3% ammonia, the latter used in an extended run with an absorbent pad at the bottom.

(4) Measurements were made with a photoelectric transmission densitometer (Densichron, W. M. Welch Mfg. Co., Chicago, Illinois). Amino acid standards were run at levels of 0.1, 0.25 and 0.4 μg. α-amino nitrogen.

(5) "Advances in Protein Chemistry," Vol. VII, Academic Press, Inc., New York, N. Y., p. 5.

(6) The pepsin treatment was done at 37° with 1% enzyme (pepsin crystallized Armour) in 0.1 *N* formic acid (*p*H 2.2–2.3). The fragments described appear in both 6-hour and 24-hour digests in addition to other fragments not mentioned in this paper.

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.

Acknowledgment.—The author wishes to acknowledge the technical assistance of Mr. A. M. Gross.

(7) R. Acher, J. Chauvet and P. Fromageot, *Biochem. Biophys. Acta*, **9**, 471 (1952).

(8) E. L. Smith, "The Enzymes," Vol. I, Part 2, Academic Press, Inc., New York, N. Y., p. 802.

(9) J. S. Fruton and M. Bergmann, *J. Biol. Chem.*, **127**, 627 (1939).

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TOTAL SYNTHESIS OF RACEMIC METHYL 3-KETOETIOCHOLANATE

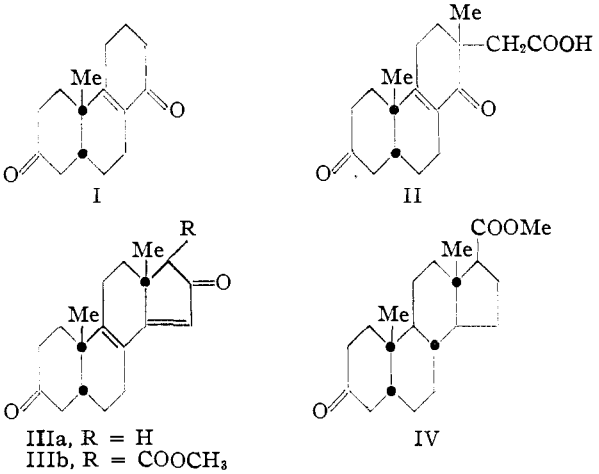
Sir:

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



(1) M. Steiger and T. Reichstein, *Helv. Chim. Acta*, **20**, 1040 (1937); T. Reichstein and H. G. Fuchs, *ibid.*, **23**, 658 (1940).

(2) A. L. Wilds, J. W. Ralls, W. C. Wildman and K. E. McCaleb, THIS JOURNAL, **72**, 5794 (1950).

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