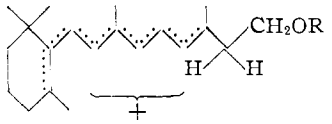
zation of IV to V proceeds through the conjugate acid Va. This intermediate seems reasonable in



Va

view of the well-defined proton acceptor tendencies of polyenes.[6]

Experimental

retro-Vitamin A Acetate (V, R = OAc).—Crystalline *trans*-vitamin A acetate (5.00 g.), m.p. 55–58°, λ_{max} 325 mμ, ϵ_{max} 51,000, was dissolved in 50 ml. of methylene chloride. The solution was mixed with 5 ml. of ice-cold concentrated hydrobromic acid. The mixture was shaken in a separatory funnel at 0° for 30 seconds and the layers permitted to separate. The organic layer was washed with 5% aqueous bicarbonate and water. The solvent was removed *in vacuo* at 20°. The resulting red-yellow oil, 4.85 g., had the typical absorption spectrum of *trans-retro*-vitamin A acetate,[2] $\lambda\lambda_{max}$ 333, **348**, 367 mμ, ϵ_{max} 56,800.

A petroleum ether solution of this oil (1.00 g. in 15 ml.) was chromatographed on a 2 × 25 cm. column of carefully neutralized alumina (60–200 mesh). On elution with ether–petroleum ether (1:9) a single band was obtained. A small red band remained essentially stationary at the top of the column. Evaporation of the solvent afforded 0.92 g. of a yellow oil which had the same spectrum as above.

Anal. Calcd. for $C_{22}H_{32}O_2$: C, 80.43; H, 9.82. Found: C, 80.57; H, 9.74; sapn. equiv., 320, 318.

(6) A. Wassermann, *J. Chem. Soc.*, 4329 (1954).

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Purification and Amino Acid Composition of Melanophore-expanding Hormone from Hog Pituitary Gland[1]

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Raben, *et al.*,[2] in 1952, reported that the melanophore hormone, after purification by adsorption on oxycellulose, can be separated from ACTH by partitition between certain organic acids and butanol. However, a detailed description of the procedure does not appear to have been published. Other workers,[3,4] more recently, have prepared highly potent ACTH preparations which retained a high degree of melanophore-expanding activity. The presence of the latter property in the purified product led these workers to conclude that the melanophore activity is a property of ACTH. The preparation from hog pituitary of a highly purified, if not pure, melanophore-expanding hormone, free from ACTH, recently has been reported by Lerner and Lee.[5]

The present authors with the aid of countercurrent distribution between 0.5 trichloroacetic acid and secondary butanol, have obtained a highly purified melanophore preparation free from ACTH activity. We have isolated also, from the same starting material in the same countercurrent run, an ACTH preparation which behaves like the unhydrolyzed corticotropin of Kuehl, *et al.*,[6] toward two solvent systems, namely, 0.5% trichloroacetic acid–secondary butyl alcohol and 0.1% trichloroacetic acid–secondary butyl alcohol. Our melanophore preparation behaves like the corticotropin-B (pepsin-hydrolyzed corticotropin-A) of Kuehl, *et al.*,[6] toward the 0.5% trichloroacetic acid–secondary butyl alcohol system. It would appear that, following the hydrolytic treatment used by Kuehl, *et al.*,[6] the partition ratios of our hog melanophore hormone and Kuehl's corticotropin-B are nearly the same for this solvent system, thus possibly accounting for the failure of these workers to separate the two materials by countercurrent distribution between 0.5% trichloroacetic acid and secondary butyl alcohol. The biological potencies of our preparations are given in Table I.

TABLE I

Partition ratio (K)	Melanophore activity (I.U./mg.)*	ACTH activity[7] (I.U./mg.)
0.64	3000	Less than 0.1
7.0	20	14–15

* This is 1500 times as potent as the International Standard Posterior Pituitary Powder (beef), as assayed in the intact frog. The potency of the purified product was between 400–500 times that of the initial hog posterior pituitary powder.

The extraction and purification of melanophore hormone from acetone-dried posterior pituitary powder (hog)[8] was achieved by extraction with acetic acid and adsorption on oxycellulose according to the method of Payne, *et al.*[9] Countercurrent distribution between 0.5% trichloroacetic acid and secondary butyl alcohol was carried out in an all-glass Craig apparatus,[10] using 100 ml. of each phase per tube with 180 transfers. The contents of the tubes, numbers 69 to 71, containing highest melanophore potency (K = 0.64) were concentrated *in vacuo* under nitrogen to a volume of about one ml. The concentrate was transferred to a 100-ml. cylinder with the aid of 4 ml. of methanol and the solute was precipitated by the addition of 85 ml. of ethyl acetate. A portion of the precipitate (14.6 mg.) was put through a 24-transfer countercurrent distribution in a small Craig machine using 0.5% trichloroacetic acid and secondary butyl alcohol as the solvent system. Countercurrent distribution was the only criterion of purity applied. The results of the spectrophotometric analysis are represented in Fig. 1.

(1) This work was supported in part by grants from the Hutchison Fund of McGill University and from Canada Packers Ltd., of Toronto (J. L. P.).

(2) M. S. Raben, I. N. Rosenberg and E. B. Astwood, *Federation Proc.*, **11**, 126 (1952).

(3) N. G. Brink, G. E. Boxer, V. C. Jelinek, F. A. Kuehl, Jr., J. W. Richter and K. Folkers, THIS JOURNAL, **75**, 1960 (1953).

(4) P. H. Bell, *ibid.*, **76**, 5565 (1954).

(5) A. B. Lerner and T. H. Lee, *ibid.*, **77**, 1066 (1955)

(6) F. A. Kuehl, Jr., M. A. P. Meisinger, N. G. Brink and K. Folkers, *ibid.*, **75**, 1955 (1953).

(7) The authors are indebted to M. Saffran and A. V. Schally for carrying out the ACTH assays according to their *in vitro* method (*Endocrinology* in press, 1955). Before assay the preparations were treated with hydrogen sulfide as a precaution against possible loss of activity.[6]

(8) The authors acknowledge the generous gift from Nordic Biochemicals Ltd., Montreal, of acetone-dried hog posterior pituitary powder.

(9) R. W. Payne, M. S. Raben and E. B. Astwood, *J. Biol. Chem.*, **187**, 719 (1950). E. B. Astwood, M. S. Raben, R. W. Payne and A. B. Grady, THIS JOURNAL, **73**, 2969 (1951).

(10) The authors are grateful to Ayerst, McKenna and Harrison, Ltd., Montreal, for making available their countercurrent machines.

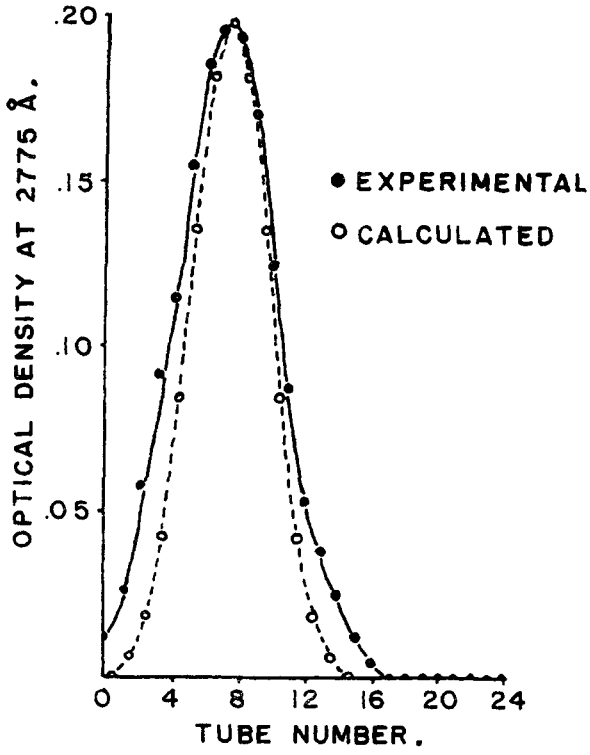

Fig. 1.—Analytical redistribution of hog melanophore hormone.

The contents of tubes numbers 166 to 168 in the larger countercurrent apparatus were found to have the highest ACTH potency ($K = 7.0$). The mixed contents of these tubes, after being concentrated and treated as previously described, were subjected to a 24-tube distribution between 0.1% trichloroacetic acid and secondary butyl alcohol. The ACTH preparation thus obtained was not pure and gave an approximate partition ratio of 1.0.

TABLE II

AMINO ACID COMPOSITION OF MELANOPHORE-EXPANDING HORMONE FROM HOG PITUITARY GLAND

The amino acids and amide ammonia account for 95.2% (uncorrected for moisture and ash) of the total nitrogen of the sample.

	Per cent.	Molar ratio	Minimum residues per molecule
Alanine	0.2	0.2	..
Arginine	4.2	1.9	2
Aspartic acid	5.8	3.5	4
Cystine	0.3	0.1	..
Glutamic acid	7.3	4.0	4
Glycine	2.2	2.3	2
Histidine	2.9	1.5	2
Isoleucine	..	..	..
Leucine	0.2	0.2	..
Lysine	6.7	3.7	4
Methionine	1.6	0.9	1
Phenylalanine	3.7	1.8	2
Proline	4.9	3.4	3
Serine	2.2	1.7	2
Threonine	..	..	..
Tryptophan	5.3	2.1	2
Tyrosine	3.8	1.7	2
Valine	0.5	0.3	..
Amide ammonia	0.5	4.4	4

The amino acid composition was determined according to the method of Wellington.[11] The acid hydrolysate of the melanophore material from the "peak" tubes of the 24-tube transfer was chromatographed on paper and the amino acids were stained with ninhydrin. The optical density of the respective eluates was read in a Beckman spectophotometer. Tryptophan was determined according to the method of Spies and Chambers.[12] Amide ammonia was determined by the method of Conway.[13] The results of the analyses are given in Table II.

The above amino acid analyses differ considerably from those reported by Lerner and Lee[5] for their purified hog melanophore preparation. The present authors find only trace amounts, or complete absence, of the following amino acids reported present by Lerner and Lee: alanine, cystine, leucine, threonine and valine. Furthermore, histidine and methionine are definitely present in our material whereas Lerner and Lee found the former to be absent from their preparation and did not analyze for the latter amino acid.

(11) E. F. Wellington, *Can. J. Chem.*, **30**, 581 (1952).
(12) J. R. Spies and D. C. Chambers, *Anal. Chem.*, **20**, 30 (1948).
(13) E. J. Conway, "Micro-Diffusion Analysis and Volumetric Error," Crosby Lockwood and Sons, Ltd., Great Britain, 1939.

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The Reaction of the Chloromagnesium Derivative of Chloromagnesium Phenylacetate with Basic Ketones

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It was found that the Mannich base, 4-diethylamino-2-butanone, reacted with the chloromagnesium derivative of chloromagnesium phenylacetate (an Ivanov reagent) to yield α-phenyl-β-methyl-β-hydroxy-δ-diethylaminovaleric acid.

When the Ivanov reagent was allowed to react with 1-methyl- and with 1-ethyl-4-piperidone, the products were α-phenyl-α-(1-alkyl-4-hydroxy-4-piperidyl)-acetic acids. The methyl esters of these acids were prepared.

Experimental

α-Phenyl-β-methyl-β-hydroxy-δ-diethylaminovaleric Acid.—Isopropylmagnesium chloride was prepared from 14.6 g. of magnesium, 70 cc. of isopropyl chloride and 100 cc. of ether. After the addition of 150 cc. of ether and 40.8 g. of phenylacetic acid, dissolved in 300 cc. of benzene, the mixture was refluxed for 18 hours. A solution of 28.6 g. of 4-diethylamino-2-butanone[1] in 100 cc. of benzene was added and the material was refluxed for 4 hours. The mixture was poured into an ice-cold solution of 60 cc. of concentrated hydrochloric acid in 300 cc. of water. The layers were separated and the organic layer was extracted with 10% hydrochloric acid. The combined aqueous layers were washed with ether and the solvent was removed under reduced pressure. The residue was extracted with chloroform at room temperature. After removal of the solvent under reduced pressure, the gummy residue was dissolved in 200 cc. of water and the solution was stirred with freshly prepared silver oxide which had been obtained from 51 g. of

(1) E. C. du Feu, F. J. McQuillin and R. Robinson, *J. Chem. Soc.*, 53 (1937).