*Anal.* Calcd. for $C_{11}H_{21}NO_4$: N, 6.06. Found: (kjeldahl) N, 6.08.

This diester hydrolyzes in water in two successive steps to the monoester and to N-methyldiethanolamine, respectively, $k_1 = 0.0079$ min.$^{-1}$ and $k_2 = 0.0037$ min.$^{-1}$ at *p*H 7.4, 37°.

DEPARTMENT OF PHYSIOLOGICAL CHEMISTRY
JOHNS HOPKINS SCHOOL OF MEDICINE BARNETT COHEN
BALTIMORE 5, MD. ERVIN R. VAN ARTSDALEN

RECEIVED SEPTEMBER 21, 1951

### S-(2-Methyl-1,4-naphthoquinonyl-3)-β-mercaptopropionic Acid

To a solution of 35.2 g. (0.2 mole) of 2-methyl-1,4-naphthoquinone in 800 ml. of 95% ethanol was added 21 g. (0.2 mole) of β-mercaptopropionic acid. The mixture was allowed to stand at 20° for two days at which time all of the starting material went into solution. The red brown solid remaining after removal of the solvent *in vacuo* was dissolved in 500 ml. of hot 50% alcohol. On cooling for one week an orange precipitate formed which was collected, washed with cold ether, and recrystallized from benzene to give 10 g. of bright orange needles, m.p. 161° (cor.).

The filtrate was concentrated, diluted with ether, and extracted with 10% sodium carbonate until color was no longer extracted. The alkaline extracts were extracted with 100 ml. of ether, which removed 2 g. of the starting quinone. The cooled alkaline solution was neutralized with 20% acetic acid and extracted with ether until the extract was colorless (*ca.* 500 ml.). The dark residue from this extract solidified on standing, and was dissolved in hot benzene, treated with charcoal, and twice crystallized to yield 7 g., m.p. 161° (cor.). A mixed melting point with the needles from the original reaction mixture showed no depression. The total yield of S-(2-methyl-1,4-naphthoquinonyl-3)-β-mercaptopropionic acid was 17 g. (30%). *Anal.* Calcd. for $C_{14}H_{12}O_4S$; C, 60.85; H, 4.37. Found: C, 61.23; H, 4.38. The compound is soluble in chloroform and ether but less soluble in ethanol (5 g./liter in 95% ethanol), ligroin and benzene and insoluble in water. It has no odor when pure.

DEPARTMENT OF PHARMACOLOGY
STATE UNIVERSITY OF IOWA
IOWA CITY, IOWA CALVIN HANNA

RECEIVED DECEMBER 26, 1951

### Di-(diazoacetyl) and 3,5-Dinitro-ω-diazoacetophenone

**Di-(diazoacetyl).**—A solution of 3 g. of oxalyl chloride in 30 ml. of absolute ether was added dropwise with ice-cooling and stirring to a solution of diazomethane (from 21.5 g. of N-nitrosomethylurea) in 250 ml. of absolute ether. After the vigorous reaction had subsided the ether was removed *in vacuo*. A red, highly lachrymatory oil with suspended yellow crystals remained. After filtration and washing with a little ether the crystals were crystallized from benzene; yield 1.2 g. (37%), m.p. 122–123° (dec.).

*Anal.* Calcd. for $C_4H_2N_4O_2$: C, 34.8; H, 1.5; N, 40.6. Found: C, 35.1; H, 1.8; N, 40.7.

The substance is soluble in methanol and ethanol, insoluble in water.

The nature of the remaining red oil is being investigated.

**3,5-Dinitro-ω-diazoacetophenone.**—A solution of 5 g. of 3,5-dinitrobenzoyl chloride in 140 ml. absolute ether was added slowly with stirring and cooling to a solution of diazomethane (from 10 g. N-nitrosomethylurea) in 100 ml. of absolute ether. After three hours standing at room temp. the reaction mixture was cooled to −10°. The yellowish crystals obtained were recrystallized from methanol; yield 2.0 g. (39%), m.p. 106° (dec.).

*Anal.* Calcd. for $C_8H_4N_4O_5$: C, 40.7; H, 1.7. Found: C, 41.2; H, 1.7.

DEPARTMENT OF ORGANIC CHEMISTRY
THE HEBREW UNIVERSITY MAX FRANKEL
JERUSALEM, ISRAEL M. HARNIK

RECEIVED NOVEMBER 8, 1951

# COMMUNICATIONS TO THE EDITOR

## PITUITARY HORMONES. III.[1] THE ISOLATION OF CORTICOTROPIN-B

*Sir:*

Fractionation of pepsin digests of corticotropin with oxycellulose and by countercurrent distribution has yielded a product which is approximately 300 times as active as Armour Standard La-1-A ACTH. This material, which has the highest adrenocorticotropic activity yet reported, behaves as a pure substance. It is designated corticotropin-B, since its properties are different from those of corticotropin.

Swine pituitary gland extracts of activities of about 2 to 5 u./mg.[2] were purified with oxycellulose (10% carboxyl)[3] to give corticotropin fractions active at approximately 60 to 100 u./mg. These were then digested with pepsin (3.7 mg./g.) at *p*H 2.5 for twenty-four hours at 37°. Material insoluble in 5% trichloroacetic acid solution was discarded, and after removal of excess trichloroacetic acid by ether extraction, corticotropin-B concentrates were isolated by lyophilization.

When corticotropin-B concentrates of potencies about 100 u./mg. or higher were subjected to countercurrent distributions of 200 transfers, using the system *s*-butyl alcohol/0.5% aqueous trichloroacetic acid, corticotropin-B was obtained as a major component and reproducibly characterized by a distribution coefficient of about 0.6. The acetate salt of corticotropin-B was isolated from this fraction as an amorphous white solid by the use of Amberlite IRA-400 on the acetate cycle and lyophilization. A sample was redistributed through twenty transfers in the same solvent system and the fractions analyzed by their ultraviolet absorption

(1) The first two papers of this series are, I, N. G. Brink, M. A. P. Meisinger and K. Folkers, THIS JOURNAL, **72**, 1040 (1950); and II, N. G. Brink, F. A. Kuehl, Jr., M. A. P. Meisinger, M. N. Bishop and K. Folkers, *ibid.*, **74**, 480 (1952).

(2) Preparations were assayed by a modification of the adrenal ascorbic acid depletion method of M. A. Sayers, G. Sayers and L. A. Woodbury, *Endocrinol.*, **42**, 379 (1948). Results are expressed in U. S. P. units per milligram. Because of the variations in the assay, all values must be regarded as approximate. However, key samples of highest potency were assayed repeatedly at different dose levels to eliminate large errors in the estimation of activity.

(3) E. B. Astwood, M. S. Raben, R. W. Payne and A. B. Grady, THIS JOURNAL, **73**, 2969 (1951).

at 2775Å. Comparison of the observed and calculated curves[4] showed an approximate purity of 95%. As a further evidence of purity, distribution coefficients calculated on the basis of the contents of adjacent pairs of tubes[4] were: $K_{4,5} = 0.53$; $K_{5,6} = 0.55$; $K_{6,7} = 0.53$; $K_{7,8} = 0.52$; and $K_{8,9} = 0.53$. More recently, a countercurrent distribution of a highly purified corticotropin-B concentrate carried through 450 transfers in the *s*-butyl alcohol/trichloroacetic acid system revealed no indication of inhomogeneity in the corticotropin-B component.

It was early noted that the frequently observed loss of activity which has been most marked in highly purified preparations can be minimized by the presence of antioxidant during processing. Thus, corticotropin-B concentrates of 100 u./mg. activity were purified on oxycellulose columns to give material of the order of 200 to 300 u./mg. activity, provided that all solutions contained hydrogen sulfide. In its absence, the products had activities no greater than about 200 u./mg. Activities of *ca.* 300 u./mg. were observed for samples of corticotropin-B purified by distribution, when they were exposed to aqueous hydrogen sulfide solution prior to assay. Without this step, the material showed assay values in the range of 200–250 u./mg. In no case were antioxidants used in making up assay solutions of the solid products reported here. Others have recently reported[5,6] the use of reducing agents to prevent inactivation prior to assay.

Corticotropin at an activity level of about 100 u./mg. was clearly differentiated from corticotropin-B by its entirely different solubility behavior in the countercurrent distribution system, the non-digested material being excessively soluble in the organic phase.

The corticotropin-B described here has shown no evidence of inhomogeneity by the criteria of countercurrent distribution behavior which were applied. Further examination of the absolute purity of corticotropin-B is in progress, together with studies on its chemical, physical and biological properties, to be reported later.

(4) B. Williamson and L. C. Craig, *J. Biol. Chem.*, **168**, 687 (1947).
(5) W. F. White, W. L. Fierce and J. B. Lesh, *Proc. Soc. Exptl. Biol. Med.*, **78**, 616 (1951).
(6) H. B. F. Dixon, S. Moore, M. P. Stack-Dunne and F. G. Young, *Nature*, **168**, 1044 (1951).

NORMAN G. BRINK
FREDERICK A. KUEHL, JR.
JOHN W. RICHTER
ALVA W. BAZEMORE
MELVIN A. P. MEISINGER
DONALD E. AYER
KARL FOLKERS

RESEARCH LABORATORIES
MERCK & CO., INC.
RAHWAY, NEW JERSEY

RECEIVED MARCH 24, 1952

## PURIFICATION OF ADRENOCORTICOTROPIC HORMONE BY CELLULOSE COLUMN CHROMATOGRAPHY[1]

*Sir:*

Since the introduction, by Astwood, *et al.*,[2] of oxycellulose for the batchwise purification of adrenocorticotropic hormone (ACTH), attempts to utilize this adsorbent in a column procedure have been unsuccessful,[3] probably because of the slow rate of attainment of equilibrium in such a system. Nevertheless, it is possible to employ such columns to obtain a reproducible purification by working under *non-equilibrium* conditions, where it is required that the amount of material placed on the column, the quantity of adsorbent, the column length and the rate of flow be carefully controlled.

In a typical experiment 10 mg. of purified sheep ACTH Preparation E[4] was pressed into a column (7 × 115 mm.) containing a mixture of 200 mg. oxycellulose[5] and 600 mg. cellulose powder,[6] washed according to the procedure of Astwood, *et al.*[2] Development was accomplished by means of a discontinuous *p*H gradient with 40 ml. 0.3 *N* $ClCH_2COOH$, followed by 40 ml. 0.7 *N* $ClCH_2COOH$, and finally by 20 ml. 0.1 *N* HCl. The flow rate was maintained at 4 ± 1 ml. per hour. The distribution of emerging material was obtained by the method of Lowry, *et al.*[7] Three peaks are obtained from such an analysis (Fig. 1). The average distribution of material in these three peaks, as

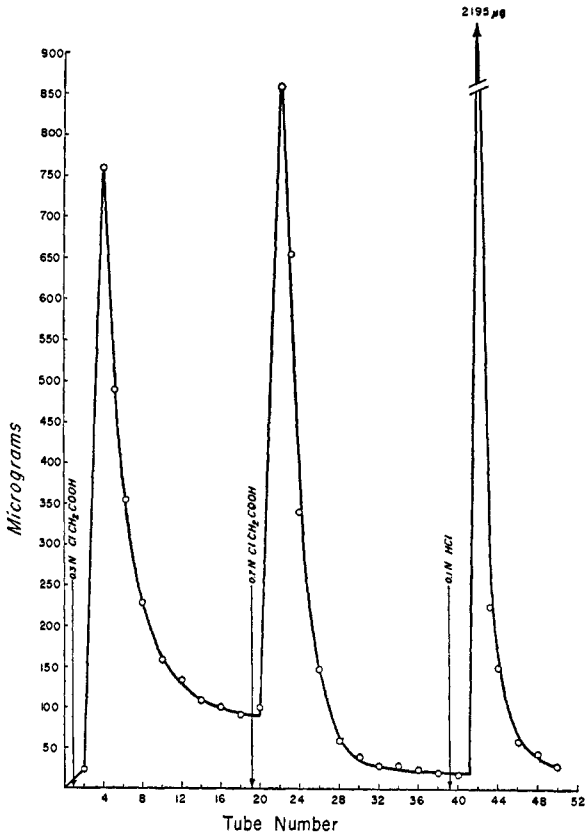


Fig. 1.—Chromatography of 10 mg. of an ACTH Preparation E (L2287E). The flow rate was 4 ml. per hour and the effluent was collected in 2 ml. fractions.

(1) This work is supported in part by grants from the U. S. Public Health Service, the Eli Lilly Laboratories, Merck and Company, Inc., the Armour Laboratories, and the Rockefeller Foundation.
(2) E. B. Astwood, M. S. Raben, R. W. Payne and A. B. Grady, THIS JOURNAL, **73**, 2969 (1951).
(3) E. B. Astwood, M. S. Raben and R. W. Payne, *Recent Prog. Hormone Res.*, **7**, in press (1952).
(4) C. H. Li, THIS JOURNAL, **74**, 2124 (1952).
(5) 11.0% carboxyl, obtained from the Tennessee Eastman Corp., Kingsport, Tennessee.
(6) Solka-Floc, obtained from the Brown Co., Berlin, New Hampshire.
(7) O. H. Lowry, N. J. Rosebrough, L. A. Farr and R. J. Randall, *J. Biol. Chem.*, **193**, 265 (1951).