Certain Quinolyl and Acridyl Derivatives of β-Alanine

N-(7-Chloro-4-quinolyl)-β-alanine Monohydrate.—A mixture of 10 g. (0.05 mole) of 4,7-dichloroquinoline (m.p. 84.5–85.5°), 8.9 g. (0.1 mole) of β-alanine (m.p. 198–200°) and 40 g. of phenol was heated on a steam-bath for 12 hours. The phenol was removed from the reaction mixture by steam distillation and the clear aqueous residue decanted from a small amount of an oil. The aqueous residue yielded a precipitate on standing overnight at room temperature. The white crystals were collected, m.p. 248–250°; yield 9.0 g. (72%) of crude product. Recrystallization from 1500 ml. of 95% ethanol gave 7.7 g. of the monohydrate; m.p. 250.5°.

Anal. Calcd. for $C_{12}H_{11}ClN_2O_2 \cdot H_2O$: C, 53.63; H, 4.88; N, 10.43; neut. equiv., 269. Found: C, 53.48; H, 4.89; N, 10.34; neut. equiv., 270.

Drying over phosphorus pentoxide *in vacuo* for five hours at 80° failed to give anhydrous product.

N-(2-Methoxy-6-chloro-9-acridyl)-β-alanine Monohydrate.—Similarly 8.3 g. (0.03 mole) of 2-methoxy-6,9-dichloroacridine (m.p. 163–164°) and 2.9 g. (0.033 mole) of β-alanine gave yellow crystals, m.p. 216°; yield 7.1 g. (68%). Recrystallization failed to raise the melting point.

Anal. Calcd. for $C_{17}H_{15}ClN_2O_3 \cdot H_2O$: C, 58.54; H, 4.91; N, 8.03. Found: C, 58.31; H, 5.19; N, 7.96.

DEPARTMENT OF CHEMISTRY WILBERT J. HUMPHLETT
DUKE UNIVERSITY CHARLES R. HAUSER
DURHAM, NORTH CAROLINA

RECEIVED FEBRUARY 24, 1951

COMMUNICATIONS TO THE EDITOR

PURIFICATION OF CORTICOTROPIN WITH OXYCELLULOSE[1]

Sir:

Previous observations[2] showing that a ten-fold concentration of corticotropin could be achieved by adsorption of the crude glacial acetic acid extract of pig anterior pituitary powder on a fifty-fold weight of powdered cellulose from 0.1 *N* acetic acid and elution therefrom by 0.1 *N* hydrochloric acid suggested that the cellulose acted as a cation-exchange medium by virtue of its constituent carboxyl groups. Experiments with oxidized cellulose[3] showing it to possess a much larger capacity for the active fraction supported this view; it proved to be more selective for the active component in the extracts and its use provided a simple method for the preparation of highly active material in virtually quantitative yield.

Experiments such as the first three shown in Table I led to the use of nearly optimal conditions in experiment 4 when the quantity of oxycellulose was 8% of the weight of crude extract.

In experiment 4, the 4 g. of oxycellulose (10.4% COOH) was washed just before use with water, 1 *N* hydrochloric acid, water, and 0.1 *N* acetic acid. Fifty grams of corticotropin powder[4] was dissolved in 0.1 *N* acetic acid, filtered, and diluted to 2 liters with 0.1 *N* acetic acid. The washed oxycellulose was added and the mixture was stirred at room temperature for 24 hours. The oxycellulose was collected on a 30 mm. diameter sintered glass filter and washed with 0.1 *N* acetic acid until the effluent was negative to the biuret reagent. After a final washing with water the funnel was stoppered and the oxycellulose was stirred in 20 cc. of 0.1 *N* hydrochloric acid. After standing for an hour the hydrochloric acid was allowed to drip through by gravity and the evenly settled oxycellulose was washed with 0.1 *N* hydrochloric acid until the effluent contained a negligible amount of material (as measured by the biuret reaction or optical density at 275 mμ.). The first 68 cc. contained 987 mg., the second 68 cc., 34 mg., and the third 60 cc., a negligible quantity. The 1021 mg., containing all but 10% of the activity, was approximately 40 times as active as the starting material (80 times as active as preparation La-1-A). This potency was confirmed by the finding that doses of 0.1 to 0.5 mg. thrice daily were fully effective in the treatment of patients suffering from rheumatic and allergic diseases.

Thus, crude corticotropin of potency twice La-1-A prepared by the glacial acetic acid method[2] was readily purified some forty-fold in a single step by a simple efficient procedure. The technique should be adaptable to cruder extracts and to extracts of the less potent but more abundant pituitary glands

TABLE I

ADSORPTION OF CORTICOTROPIN IN 0.1 *N* ACETIC ACID ON OXYCELLULOSE AND ELUTION BY 0.1 *N* HYDROCHLORIC ACID

Exp.	Crude corticotropin, g.	Oxycellulose,[a] g.	Ad-sorbed,[b] %	Unad-sorbed, %	Est. potency,[c] Mg.	Activ. recov., %
Crude corticotropin					2	100
1[d]	10	40	7.4		20	74
				94	0.1	4.5
2	25	1st 5	3.3		40	66
		2nd 95	8.2		2	8.2
				89	0.1	4.5
3	25	1st 1	1.2		100	60
		2nd 4	2.2		40	44
				96	0.04	2
4	50	4	2.04		80	80
				98	0.2	10

[a] Weight before washing. [b] Quantity eluted by 0.1 *N* HCl. [c] One unit, as here defined, represents the activity of 0.5 mg. of crude corticotropin or 1 mg. of preparation La-1-A when tested by the method of M. Sayers, G. Sayers, and L. A. Woodbury, *Endocrinology*, **42**, 379 (1948). [d] 1% solution and stirred for only one-half hour; all other solutions 2.5% and stirred for 24 hours.

(1) Supported in part by grants from the National Institutes of Health, U. S. Public Health Service, and from the American Cyanamid Company.

(2) R. W. Payne, M. S. Raben and E. B. Astwood, *J. Biol. Chem.*, **187**, 719 (1950).

(3) Kindly supplied by the Tennessee Eastman Corporation, Kingsport, Tennessee; E. C. Yackel and W. O. Kenyon, THIS JOURNAL, **64**, 121 (1942).

(4) The extract used in these experiments was generously supplied by Dr. David Klein of the Wilson Laboratories, Chicago, Illinois.