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.

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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.

of other animals. Oxycellulose may prove useful as a fractionation medium for other purposes, where, for reasons of molecular structure or size, compounds are not amenable to separation with ion-exchange resins.

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RECEIVED MAY 7, 1951

SEDOHEPTULOSE IN PHOTOSYNTHESIS BY PLANTS[1]

Sir:

Although its function has not been ascertained, the general occurrence of sedoheptulose,[2] D-altroheptulose, in the succulent plants is well established. This sugar has not been identified in the majority of the members of the plant kingdom, but it now appears possible that its phosphate esters may perform a vital function during photosynthesis.

We have isolated labeled sedoheptulose monophosphate in $C^{14}O_2$ photosynthesis products of all the plants thus far studied in this laboratory (*Chlorella*, *Scenedesmus*, *Rhodospirillum rubrum*, and the leaves of barley seedlings, soybean, alfalfa, sugar beet, spinach and geranium). It is invariably found as monophosphate esters. At least two such esters have been observed in radiograms of C^{14}-labeled *Scenedesmus*. The major one is associated with fructose monophosphate while the minor one is inseparable, as yet, from glucose monophosphate. Sedoheptulose may be liberated enzymatically from its phosphates during the killing of the plant, but it has not been observed to accumulate in amounts exceeding the steady state concentrations of these phosphates. This suggests its participation only as a phosphate in most plants. These sedoheptulose phosphates are formed prior to hexose phosphates in the cases examined kinetically in this laboratory. In a typical experiment, one-second photosynthesis in $C^{14}O_2$ by barley seedling leaves, the distribution of radioactivity among the neutral compounds obtained upon phosphatase hydrolysis of the mixed phosphates was as follows: 43% in fructose, 47% sedoheptulose and 7% in glucose.

Sedoheptulose, isolated chromatographically[3] from phosphatase ("Polidase") hydrolysates of similarly separated phosphate esters,[3] was identified by the following tests. (1) Two-dimensional paper co-chromatography with authentic sedoheptulose[4] showed identical positions of the sugar and the radioactivity. The position of the authentic specimens was determined by resorcinol spray test. (2) The radioactive sugar in tracer concentrations is readily converted to sedoheptulosan by five-minute heating in 1 *N* hydrochloric acid. It was identified by co-chromatography with sedoheptulosan prepared similarly from an authentic specimen. (3) The equilibrium constant of the dehydration of the radioactive compound was found to be 4.0 as reported by LaForge and Hudson[2] for sedoheptulose. (4) Catalytic hydrogenation gave D-β-mannoheptitol, which was identified by co-chromatography with an authentic specimen prepared from sedoheptulose. (5) Periodate oxidation of both the hexose and the heptitol gave the expected amounts of products. The sedoheptulose obtained from five minutes $C^{14}O_2$ photosynthesis by soy bean leaves gave 14.4% of formaldehyde activity, 28% glycolic acid activity and 55% of formate activity. The heptitol obtained from this compound had a formate/formaldehyde ratio of 3.1 compared to an expected 2.5 for uniform labeling.

The examination of the kinetics of formation of the phosphate esters involved in $C^{14}O_2$ fixation[5] and a detailed description of the identification will be published.

This early synthesis of sedoheptulose in CO_2 fixation and its stereochemical deviation from that of glucose strongly suggests its participation in a C_2 regenerative system for the primary CO_2-acceptor rather than as a hexose precursor. The predominant role of malic acid in "succulent metabolism" may well be related to the accumulation of sedoheptulose in these plants.

(1) This work was sponsored by the United States Atomic Energy Commission.

(2) F. B. LaForge and C. S. Hudson, *J. Biol. Chem.*, 30, 61 (1917).

(3) A. A. Benson, J. A. Bassham, M. Calvin, T. Goodale, V. Haas and W. Stepka, THIS JOURNAL, 72, 1710 (1950).

(4) A sample was kindly supplied by Mr. F. W. Putman of the Division of Plant Nutrition of this University.

(5) A. A. Benson, S. Kawaguchi and M. Calvin, to be published.

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THE STRUCTURE OF JERVINE. II.[1] DEGRADATION TO PERHYDROBENZFLUORENE DERIVATIVES

Sir:

In a recent publication,[1] in which it was shown that the double bond conjugated with the inert keto group in jervine cannot occupy the 8,9-position as postulated by Jacobs and Sato,[2] we implied that this alkaloid does not have a normal steroid nucleus. We now present some of the evidence on which this assertion is based.

Jervine on treatment with acetic anhydride and zinc chloride at 140° yielded a dienone $C_{28}H_{30}O_3$, I (m.p. 186–188°,[3] $[\alpha]^{25}D$ −101°,[3] $\lambda_{max.}^{alc.}$ 300 mμ (4.4)[3]; calcd.: C, 78.02; H, 8.48; acetyl, 12.2; found: C, 78.14; H, 8.58; acetyl, 11.5), while isojervine[1] was merely acetylated under these conditions. I was cleaved by chromic acid into acetaldehyde and the yellow 1,4-diketone $C_{21}H_{26}O_4$, II (m.p. 181–183°; $[\alpha]^{22}D$ −234°; $\lambda_{max.}^{alc.}$ 267 mμ (4.16); 415 mμ (1.77); calcd.: C, 73.66; H, 7.62; acetyl, 12.6; found: C, 73.53; H, 7.87; acetyl, 13.5). Monoxime: (m.p. 243–245°). Alkali readily converted II into the phenol III (diacetate, m.p. 207–209°; $[\alpha]^{24}D$ −139°; calcd. for $C_{28}H_{26}O_5$: C, 72.23; H, 6.85; acetyl, 22.5; found: C, 72.58; H, 6.94; acetyl, 20.6), the ultraviolet charac-

(1) Paper I of this series: O. Wintersteiner, M. Moore, J. Fried and B. M. Iselin, *Proc. Nat. Acad. Science*, in press.

(2) W. A. Jacobs and Y. Sato (a) *J. Biol. Chem.*, **175**, 57 (1948); (b) **181**, 55 (1949).

(3) All melting points corrected; all rotations in chloroform; ultraviolet data; figures in parentheses denote log ϵ.