be very similar to fibrinogen molecules in size and shape. But when the urea concentration is reduced to 2.35 *M*, two peaks appear in the sedimentation diagram, with constants of 9 and 25 *S*.

The presence of the fast peak, both in partly polymerized fibrinogen inhibited by glycol or urea, and in depolymerized fibrin in urea, is always accompanied by a high viscosity which depends markedly on the rate of shear. The reduced specific viscosity falls rapidly with dilution of the protein, however, approaching that characteristic of the original fibrinogen, indicating dissociation of long linear aggregates.

The behavior of the intermediate represented by the fast peak is thus the same whether it is formed from fibrinogen by the action of thrombin or from urea-depolymerized fibrin by decreasing the concentration of urea.[4]

We are much indebted to Professor J. W. Williams for use of the Svedberg oil turbine ultracentrifuge.

(4) In general agreement with the experience of Mihályi,[5] there was no evidence, under the conditions of our experiments, of the denaturation which is observed[5] at somewhat higher urea concentrations or temperatures (or lower *p*H). Fibrinogen in 2.35 *M* urea had the same intrinsic viscosity as in the absence of urea; and the solubility of fibrinogen was not impaired by contact for 18 hours with 3.5 *M* urea, at *p*H 7.5, room temperature, and subsequent removal of the urea by dialysis. These criteria are of course not applicable to fibrin, but the susceptibilities of fibrinogen and fibrin to denaturation should be similar. Also, the viscosity of a fibrin solution in 3.5 *M* urea at *p*H 6.3 showed no change with time for two days, indicating that no progressive changes were taking place.

(5) E. Mihályi, *Acta Chem. Scand.*, **4**, 317 (1950).

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RECEIVED FEBRUARY 12, 1951

DIETHYL CHLOROARSENITE AS A REAGENT FOR THE PREPARATION OF PEPTIDES

Sir:

In the course of an investigation in these Laboratories of methods of peptide synthesis, new reagents for forming the peptide linkage at either the amino or carboxylic function of an aminoacid or peptide chain have been found. The use of diethyl chlorophosphite has been reported recently.[1] Similarly, diethyl chloroarsenite[2] reacts readily with aminoacid esters and with N-substituted aminoacids to give highly reactive amides and anhydrides, respectively. The new reagent has advantages over the phosphite analog in being stable and readily prepared. Comparable yields are obtained with either reagent.

Both the amides, $(C_2H_5O)_2AsNHCH(R)COOR'$, and the anhydrides, $R'NHCH(R)COOAs(OC_2H_5)_2$, are non-distillable oils which are conveniently prepared and reacted without isolation. The reactions are accomplished in an inert solvent in the presence of an equivalent of triethylamine as the acid acceptor. After removal of the precipitated triethylamine hydrochloride, the solution of the intermediate diethylarsenite amide or anhydride is refluxed one hour with an equivalent of a second N-substituted aminoacid or aminoacid ester, respectively. The by-product, presumably diethyl arsenite in both cases, is precipitated quantitatively as arsenic trioxide by addition of water.

The N-substituted peptide ester prepared by either of these procedures is obtained crystalline by first extracting the reaction solution successively with dilute sodium bicarbonate and dilute hydrochloric acid and then concentrating in an air stream. One crystallization from ethanol–water or ethyl acetate–petroleum ether generally has given pure products.

Prepared by the intermediate amide method were carbobenzoxyglycine anilide[3] (79%), m.p. 144–145°; carbobenzoxyglycine morpholide[4] (70%), m.p. 144–145°; ethyl carbobenzoxyglycyl-DL-phenylalanate[5] (59%), m.p. 91–92°; ethyl phthalyl-DL-alanyl-DL-valinate[4] (71%), m.p. 121–123°; and ethyl carbobenzoxyglycyl-L-tyrosinate (74%), m.p. 125–126° (a mixed m.p. with an authentic sample[6] was not depressed).

Prepared by the anhydride method were carbobenzoxyglycine anilide[3] (63%), m.p. 146–147°; ethyl carbobenzoxyglycyl-DL-phenylalanate[5] (52%), m.p. 92–93°; ethyl carbobenzoxy-DL-alanyl-DL-phenylalanate[4] (60%), m.p. 104–106°; ethyl carbobenzoxy-L-leucyl-DL-phenylalanate[4] (74%), m.p. *ca.* 90° $[\alpha]^{24}D$ −9.2° (*c* = 5, 95% ethanol) and ethyl carbobenzoxyglycylglycyl-DL-phenylalanate monohydrate[4] (30%), m.p. 80–82° (from ethyl phenylalanate and the diethyl arsenite anhydride from carbobenzoxyglycylglycine).

(3) Wieland and Sehring, *Ann.*, **569**, 122 (1950).
(4) Carbon, hydrogen and nitrogen analyses were satisfactory.
(5) Neurath, *et al.*, *J. Biol. Chem.*, **170**, 222 (1947).
(6) Bergmann and Fruton, *ibid.*, **118**, 412 (1937).

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RECEIVED FEBRUARY 7, 1951

CRYSTALLINE XYLOBIOSE AND XYLOTRIOSE

Sir:

Charcoal chromatography of partially hydrolyzed xylan permits the separation and isolation of a considerable amount of crystalline xylobiose and xylotriose. This is the first isolation of crystalline di- and trisaccharides composed only of pentose sugar units.

In one instance a 2% solution of xylan in 42% hydrochloric acid was hydrolyzed at 0° until the reaction was 66% complete as indicated by reducing value and by optical rotation. The hydrolyzate was neutralized with sodium bicarbonate and chromatographically separated on charcoal columns following the method of Whistler and Durso.[1] After washing the column with water, xylobiose was removed with 5% ethanol. The sirupy concentrate from this extraction was dissolved in a small amount of warm water and hot methanol added. On cooling, crystallization occurred. The yield was 4.8% of the xylan used, m.p. 186–187°; $[\alpha]D^{25}$ −32.0→−25.5 (1 hour) (*c*, 1 in water).

(1) Anderson, Welcher and Young, THIS JOURNAL, **73**, 501 (1951).
(2) McKenzie and Wood, *J. Chem. Soc.*, **117**, 406 (1920).

(1) Roy L. Whistler and Donald F. Durso, THIS JOURNAL, **72**, 677 (1950).

Anal. Calcd. for $C_{10}H_{18}O_9$: C, 42.53; H, 6.42. Found: C, 42.5; H, 6.4.

Iodimetric titration gave the expected equivalent value for a disaccharide and on hydrolysis the final reducing value and specific rotation were those calculated for D-xylose. On acetylation of the disaccharide, there was produced the same crystalline hexaacetyl xylobiose as reported by Bachrach and Whistler.[2] Thus, the structure of the disaccharide is 4-(β-D-xylopyranosyl)-β-D-xylopyranose.

In another instance a 2% xylan solution in 42% hydrochloric acid solution was hydrolyzed to 50% of completion. It was neutralized and chromatographically separated as before. After washing the column with water and 5% ethanol, a trisaccharide fraction was removed with 9% ethanol. The concentrated sirup was dissolved in a small amount of warm water and hot absolute ethanol was added to produce a solution of 80–85% alcohol concentration. Upon cooling, crystallization occurred. The yield was 8.0% of the original xylan; m.p. 205–206°; $[\alpha]_D^{25}$ 46.96 (1.06% in water).

Anal. Calcd. for $C_{15}H_{26}O_{13}$: C, 43.48; H, 6.33. Found: C, 43.4; H, 6.4.

Iodimetric titration gave the expected value for a trisaccharide and on hydrolysis the reducing value and specific rotation were those calculated for D-xylose.

(2) J. Bachrach and R. L. Whistler, paper presented before the Division of Sugar Chemistry, 116th meeting of American Chemical Society, Atlantic City, 1949.

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RECEIVED FEBRUARY 3, 1951

ADRENAL CONVERSION OF C^{14} LABELED CHOLESTEROL AND ACETATE TO ADRENAL CORTICAL HORMONES[1]

Sir:

It previously has been demonstrated that beef adrenals, perfused with blood containing added ACTH, synthesize and release into the perfusion medium a mixture of corticosteroids, the principal components of which are 17-hydroxycorticosterone (I) and corticosterone (II).[2,3] We wish to report that when similar experiments are carried out in the presence of either C^{14} labeled acetate or cholesterol, the I and II isolated from adrenal perfusates are radioactive, and have approximately the same number of counts per mg. per min. (c.m.m.) as determined under identical conditions.

Groups of 5 glands were perfused in parallel from a manifold with 1 liter of homologous citrated blood containing 25 mg. of ACTH (Armour) for four hours, the perfusate being recycled through the glands. The corticosteroids were extracted from perfusates with activated carbon (Darco G-60),[4] and fractionated by paper partition chromatography. The compounds were characterized by the method of mixed chromatograms,[5] both of the free steroids and of their esters, and by measuring the chromogen produced by H_2SO_4.[6] All counts were made using a thin-window Geiger counter with 0.1–0.7 mg. samples (diluted with non-isotopic compound when necessary) plated as a thin film. Ten milligrams of carboxyl labeled sodium acetate having radioactivity of 5.8×10^6 c.m.m. was added to the medium at the initiation of the perfusion. I and II were isolated in **3.0** and **1.0** mg. amounts, and had activity of 319 and 305 c.m.m. respectively, in one experiment; in a second similar experiment, the c.m.m. for each were 219 and 208 and the total amounts isolated were **4.5** and **1.5** mg. for I and II, respectively. The counts were made with 0.09 to 0.13 mg. samples; with our technique the c.m.m. of I or II remains constant in the range 0.09 to 0.7 mg.

A similar perfusion of cholesterol labeled in position 3 with C^{14} prepared from radio-cholestenone[7] by Drs. Schwenk, Gut and Belisle[8] was conducted in which 90 mg. of radiocholesterol (300 c.m.m.) was used. I and II were isolated in **1.0** and **0.4** mg. amounts and had activities of 25 and 18 c.m.m., respectively.

The data of Table I indicate that the radioactivity is a property of the compounds isolated since (a) rechromatography on paper and (b) the preparation of two derivatives led to no significant alteration of the specific activity. It is recognized that the method of mixed chromatograms of the free compounds and their esters plus determination of the H_2SO_4 chromogen does not constitute a classical characterization of I and II. In our experience, however, no substance proved to be homogeneous by this method, has failed to meet classical criteria of purity and composition.

TABLE I

The specific activities of cortical hormones and their derivatives isolated from an adrenal perfusion experiment with $CH_3C^{14}OONa$.

		mg.	c.m.m.
1	Free 17-hydroxycorticosterone (I)[a]	0.125	319
2	I after rechromatography	.115	340
3	I acetate[b]	.130	362
4	I propionate[b]	.098	332
1	Free corticosterone (II)[a]	.130	305
2	II after rechromatography[a]	.090	326
3	II acetate[b]	.123	294
4	II propionate[b]	.110	342

[a] Isolated from paper following partition chromatography using the propylene glycol–toluene system. [b] Isolated from paper following partition chromatography using the formamide–benzene system. The samples were then rechromatographed on paper.

These data indicate that both acetate and cholesterol can be transformed by the isolated adrenal

(1) Aided by United States Public Health Service Grant GG-2742 and G. D. Searle and Company.

(2) Hechter, Zaffaroni, Jacobsen, Levy, Jeanloz, Schenker and Pincus, *Recent Progress in Hormone Research*, in press.

(3) Pincus, Hechter and Zaffaroni, *2nd Clin. ACTH Conf.*, The Blakiston Co., Philadelphia, Pa., 1951, in press.

(4) Hechter, Jacobsen, Jeanloz, Levy, Marshall, Pincus and Schenker, *Arch. Biochem.*, **25**, 477 (1950).

(5) Zaffaroni, Burton and Keutman, *Science*, **111**, 6 (1950).

(6) Zaffaroni, THIS JOURNAL, **72**, 3828 (1950).

(7) Turner, THIS JOURNAL, **69**, 726 (1947).

(8) Schwenk, Gut and Belisle, in press.