a thick sirup. The sirup was redissolved in 100 ml. of alcohol-free chloroform and shaken, first, with an ice-cold half-saturated aqueous sodium bicarbonate solution (50 ml. × 2) and then with ice-water (50 ml. × 2) and dried over anhydrous sodium sulfate. Concentration of the chloroform solution at 30–40° under reduced pressure yielded a solid colorless glassy substance $[\alpha]^{21}D$ +11.3° (alcohol-free $CHCl_3$, *c* 6).

Anal. Calcd. for $C_{15}H_{21}O_{10}Br$: C, 40.84; H, 4.80; Br, 18.12. Found: C, 40.90; H, 4.75; Br, 18.1.

Preparation of *aldehydo*-D-Arabinose Hexa-*O*-acetate.—The sirup (1 g.) obtained by the reaction of the acid chloride described above was dissolved in 10 ml. of toluene and added to a suspension of silver acetate (1 g.) in boiling toluene.[12] The mixture was immediately allowed to cool to room temperature and after standing at room temperature overnight filtered under suction through a fritted glass filter. Petroleum ether (b.p. 30–60°) was added to the filtrate and the gummy precipitate which resulted removed by decantation. After washing with ice-cold half-saturated aqueous sodium bicarbonate solution, the gum was crystallized from ethanol. Constants found were: m.p. 89–90°, $[\alpha]^{21}D$ +30° ($CHCl_3$, *c* 2) in accord with the values reported for *aldehydo*-D-arabinose hexa-*O*-acetate.[13,14] The compound showed no depression in melting point when mixed with an authentic sample of *aldehydo*-D-arabinose hexa-*O*-acetate prepared from D-arabinose after the manner of Wolfrom.[14]

(12) R. S. Tipson, *J. Biol. Chem.*, **130**, 55 (1939).

(13) E. M. Montgomery, R. M. Hann and C. S. Hudson, THIS JOURNAL, **59**, 1124 (1937).

(14) M. L. Wolfrom, *ibid.*, **57**, 2498 (1935).

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Preparation of N-Substituted 1-Amino-1-deoxy-D-arabino-hexuloses[1] of Arginine, Histidine and Lysine.[2] II

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The preparation of N-substituted 1-amino-1-deoxy-D-arabino-hexuloses of L-arginine, L-histidine and L-lysine is described. The parent sugar was D-glucose. Like the derivatives of the neutral and acidic amino acids, they reduce ferricyanide in 0.1 *N* alkali rapidly at room temperature, give low values for sugar with the anthrone reagent, nearly equivalent values for sugar in boiling alkaline solution with ferricyanide and stimulate incorporation *in vitro* of labeled amino acids into the proteins of rabbit reticulocytes.

We have reported previously the isolation from hog liver of compounds of the N-substituted 1-amino-1-deoxy-2-ketohexose type, their ability to stimulate incorporation *in vitro* of amino acids into the proteins of rabbit reticulocytes[3] and the synthesis of such compounds by condensation of hexoses with L-alanine, L-aspartic acid, L-glutamic acid, glycine, L-leucine, L-serine, L-threonine and L-valine.[4]

These compounds may be viewed as the Amadori rearrangement products of the N-glycosylamino acids. The amino acid-deoxy-fructoses of the neutral and acidic amino acids had been separated from the parent sugar and amino acid by adsorption on Dowex-50 (H^+ form) resin, removal of the sugar by elution with water and of the amino acid-deoxy-fructose with trichloroacetic acid whose concentration was varied from 0.05 to 0.50 molar depending on the constituent amino acid.[4] This chromatographic procedure was unsatisfactory for isolation of the amino acid-deoxy-fructoses of arginine, histidine and lysine from the respective reaction mixtures. These compounds are adsorbed so strongly on Dowex-50 (H^+ form) resin that, to elute them, 4 *N* hydrochloric acid was necessary. This also eluted the parent amino acid without sufficient separation from the amino acid-deoxy-fructose. The following procedure was successful. The reaction mixture was passed through a column of Amberlite IRC-50 (H^+ form); the sugar passed through and was completely removed with water, the amino acid-deoxy-fructose and the free basic amino acid were retained on the column from which both together were eluted with acetic acid, 1 *N* for the arginine and lysine, 0.1 *N* for the histidine preparations. The separation of the amino acid-deoxy-fructose from the corresponding free amino acid was achieved by adsorption on a cellulose column and elution with pyridine–water (3:1).

After drying by lyophilization, the amino acid-deoxy-fructoses were dissolved in absolute methanol from which they were precipitated by dry ether. All three compounds were thus obtained as white powders. L-Arginino-deoxy-fructose and L-histidino-deoxy-fructose can be dried to constant weight at 80° *in vacuo*. L-Lysino-deoxy-fructose begins to brown after 45 minutes at 80° *in vacuo*. We have not determined which nitrogen atom of the dibasic amino acids is linked to the carbohydrate; furthermore, the anomeric configuration and the ring structure of the sugar moiety have not been ascertained.

Most of the amino acid-deoxy-fructoses begin to brown on the melting stage at about 120° when they give off a pleasant and characteristic odor, which is different from caramel and reminiscent of freshly baked cereal foods. On further heating all the compounds darken and decompose.

(1) These compounds have been called N-substituted 1-amino-1-deoxy-2-ketohexoses and for convenience fructose amino acids, *e.g.*, fructose alanine. To comply with the Rules of Carbohydrate Nomenclature [*Chem. Eng. News*, **31**, 1776 (1953)], we now use the generic name N-substituted 1-amino-1-deoxy-D-arabino-hexulose. We designate the compound with a specific amino acid, *e.g.*, 1-(L-alanino)-1-deoxy-D-fructose. As a shorter generic name we use the term amino acid-deoxy-fructose and as a short specific name, *e.g.*, alanino-deoxy-fructose.

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(3) H. Borsook, A. Abrams and P. H. Lowy, *J. Biol. Chem.*, **215**, 111 (1955).

(4) A. Abrams, P. H. Lowy and H. Borsook, THIS JOURNAL, **77**, 4794 (1955).

Table I is a summary of some characteristic properties of amino acid-deoxy-fructoses compared with those of fructose and glucose. The methods used were as previously described.[3,4] It is seen that the arginino-, histidino- and lysino-deoxy-fructoses do not differ significantly in comparable properties from other members of this class of compounds, of which alanino-deoxy-fructose is cited as a typical example.

TABLE I

COMPARISON OF SOME CHARACTERISTIC PROPERTIES OF AMINO ACID-DEOXYFRUCTOSE COMPOUNDS WITH D-FRUCTOSE AND D-GLUCOSE

The following sizes of aliquots were used: hot reduction of ferricyanide, 1.0 ml. of 4×10^{-4} *M*; anthrone, 1.0 ml. of 4×10^{-3} *M*, except lysino-deoxy-fructose of which 1.0 ml. of 4×10^{-2} *M* was used; room temperature reduction of ferricyanide, 0.1 ml. of 4×10^{-4} *M*. The tests on the incorporation of leucine were carried out at 38° for 4 hr.

Compound	Total sugar: Hot reduction of ferricyanide, % of glucose	Total sugar: Anthrone % of glucose	Reduction of ferricyanide at room temperature, % of alanino-deoxy-fructose	Stimulation of incorporation *in vitro* of leucine into reticulocyte proteins, % of blank; concn., 5×10^{-4} *M*
D-Fructose	90	95	3	0
D-Glucose	100	100	0	0
Alanino-deoxy-fructose	99	20	100	134
Arginino-deoxy-fructose	97	20	95	149
Histidino-deoxy-fructose	116	50	102	144
Lysino-deoxy-fructose	134	4	111	141

Experimental

1-(L-Arginino)-1-deoxy-D-fructose.—L-Arginine hydrochloride, 2.8 g., and 20 g. of D-glucose in 560 ml. of dry methanol were refluxed for 2.25 hr. The clear, slightly brown solution was dried *in vacuo*. The residue was dissolved in 100 ml. of water. Chloride was removed by the addition of Dowex-1 (OH^- form) until the *p*H of the supernatant solution was 10; the resin was filtered off and washed with 80 ml. of water. The combined filtrate and washings were then passed slowly through an Amberlite IRC-50 (H^+ form) column (43 × 410 mm., hold-up volume 150 ml.). Water, 2.5 liters, was then passed through the column to remove uncombined glucose and compounds other than the free and combined arginine. The latter were then eluted with 1 *N* acetic acid; they were detected in the eluate by making filter paper chromatograms of aliquots of eluate fractions and spraying with the following reagents: ninhydrin in pyridine; alkaline nitroprusside ferricyanide (guanidino reagent)[5] and alkaline ferricyanide.[3] Arginine reacts positively only with the first two of these reagents; arginino-deoxy-fructose reacts with all three.

(5) H. K. Berry, H. E. Sutton, L. Cain and J. S. Berry, Univ. Texas Publication (Austin), No. 5109, p. 22 (1951).

Those fractions of acetic acid eluate which were found to contain the arginino-deoxy-fructose were dried at room temperature under diminished pressure, the residue dissolved in 20 ml. of water and enough Whatman cellulose powder was added to soak up all the liquid. The mixture was dried under reduced pressure and then placed on top of a column, 43 × 440 mm. of dry-packed Whatman cellulose powder and eluted with pyridine–water (3:1). Fractions of 40 ml. each were collected. Arginino-deoxy-fructose emerged in fractions 5 to 7 and was free of arginine. The latter began to emerge at fraction 8. The combined fractions 5 to 7 were dried under reduced pressure, the glassy residue was dissolved in 20 ml. of dry methanol from which it was precipitated by the addition of 40 ml. of dry ether. The precipitate was washed on a filter with ether. The yield was 400 mg. A sample was prepared for analysis by reprecipitation from methanol with ether and drying to constant weight at 80°; R_f in pyridine water, 65:35, ascending: arginino-deoxy-fructose 0.47; arginine 0.12.

Anal. Calcd. for $C_{12}H_{24}N_4O_7$ (336.34): C, 42.8; H, 7.2; N, 16.6. Found: C, 42.5; H, 7.1; N, 16.0.

1-(L-Histidino)-1-deoxy-D-fructose.—L-Histidine (free base), 1.6 g., and 20 g. of D-glucose were refluxed in 560 ml. of dry methanol for 3 hr. The reaction mixture was then distilled to dryness under reduced pressure. The residue was dissolved in 150 ml. of water and then passed through a column (43 × 300 mm.) of IRC-50 (H^+ form). Two liters of water were passed through. The original eluate and the water washings were discarded. Histidine and histidino-deoxy-fructose were eluted with 0.1 *N* acetic acid. The fractions containing the histidino-deoxy-fructose, located by the reduction of ferricyanide,[3] were pooled and evaporated to dryness under reduced pressure. The residue was dissolved in 15 ml. of pyridine–water (65:35) and chromatographed on a column (43 × 410 mm.) of cellulose powder. The column was prepared from a suspension of Whatman cellulose powder in acetone and was washed with 1 liter of acetone, followed by 700 ml. of pyridine–water (65:35). The histidino-deoxy-fructose was eluted with the above pyridine–water mixture. After 480 ml. of eluate had been collected, material emerged in the next 50 ml. which reduced ferricyanide at room temperature and was positive to the ninhydrin reagent. This fraction after drying *in vacuo* weighed 820 mg. It was dissolved in absolute methanol from which it was precipitated by ether. The product thus obtained, when dried, weighed 440 mg.; it decomposed at 120–130°. When dried to constant weight at 80° *in vacuo* (4 hr.), the weight loss was 4.4%; R_f in pyridine water 65:35, ascending: histidino-deoxy-fructose 0.74; histidine 0.46.

Anal. Calcd. for $C_{12}H_{19}N_3O_7$ (317.29): C, 45.6; H, 6.04; N, 13.26. Found: C, 45.4; H, 6.28; N, 13.50.

1-(L-Lysino)-1-deoxy-D-fructose.—L-lysine·HCl (1.6 g.) and 20 g. of D-glucose were refluxed in 560 ml. of dry methanol for 1.5 hr. The reaction mixture was then submitted to the identical procedure described above for arginino-deoxy-fructose. The product after precipitation from methanol by ether weighed 450 mg. When first prepared the product was white; after storage for a month *in vacuo* at room temperature it had become orange-yellow. The sample prepared for analysis by drying *in vacuo* at 80° for 45 minutes became slightly brown; R_f in pyridine water 3:1, ascending: lysino-deoxy-fructose 0.52; lysine 0.13.

Anal. Calcd. for $C_{12}H_{24}N_2O_7$ (308.33): C, 46.7; H, 7.8; N, 9.1. Found: C, 46.3; H, 8.0; N, 8.6.

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