with 250 ml. of ether for 18 hr. On cooling the ether solution deposited an additional 330 mg. of D-galactose diethyl mercaptal (m.p. 140–142°) bringing the total yield of this material to 2.61 g.

The resulting ether solution was concentrated and deposited crystalline material on cooling; yield 520 mg., m.p. 110–112°. This material was recrystallized from ethyl acetate containing a little petroleum ether; m.p. 112–113°, undepressed on admixture with an authentic specimen of 3,6-anhydro-D-galactose diethyl mercaptal, synthesized as described below, $[\alpha]^{25}D$ −10° (*c* 1.0, water), +26.8° (*c* 1.0, pyridine); final yield 1.48 g.

Anal. Calcd. for $C_{10}H_{20}O_4S_2$: C, 44.75; H, 7.51; S, 23.9. Found: C, 44.93; H, 7.36; S, 24.1.

3,6-Anhydro-*aldehydo*-D-galactose.—One hundred mg. of 3,6-anhydro-D-galactose diethyl mercaptal, dissolved in 5 ml. of water, was treated with 175 mg. of mercuric chloride and 300 mg. of cadmium carbonate for 6 hr. at 50°. The resulting mixture was filtered and the excess mercuric chloride was removed by extraction with ether. Concentration of the solution by distillation under reduced pressure yielded a sirup which reduced Fehling solution at 25°, gave the Seliwanoff reaction and restored the color to Schiff solution. On treatment in aqueous solution with phenylhydrazine hydrochloride and sodium acetate for 2 hr. at 80° it yielded a phenylosazone, which on recrystallization from methyl alcohol had m.p. 216–217°, $[\alpha]^{24}D$ + 71° (*c* 0.3, methyl alcohol). The melting point was undepressed on admixture with authentic 3,6-anhydro-D-galactose phenylosazone prepared from methyl 3,6-anhydro-α-D-galactopyranoside. Percival[26] reports m.p. 215° and $[\alpha]^{16}D$ +71° for this compound.

Anal. Calcd. for $C_{18}H_{20}O_3N_4$: C, 63.5; H, 5.9; N, 16.5. Found: C, 63.35; H, 5.66; N, 16.41.

2,4,5-Tri-*O*-*p*-nitrobenzoyl-3,6-anhydro-D-galactose Dimethyl Acetal.—3,6-Anhydro-*aldehydo*-D-galactose was converted through the sirupy dimethyl acetal into the crystalline 2,4,5-tri-*O*-*p*-nitrobenzoyl derivative according to the method of Haworth.[23] The compound melted at 111–112° in agreement with the value previously reported, and was undepressed on admixture with authentic material prepared in the same manner from methyl 3,6-anhydro-α-D-galactopyranoside.

Methyl 6-*O*-*p*-Tolylsulfonyl-α-D-galactopyranoside.—By the method described in reference 23, anhydrous methyl α-D-galactopyranoside (20 g.) was treated with 22 g. (1.1 moles) of *p*-toluenesulfonyl chloride. The yield of desired product was 5.6 g., m.p. 172–174° dec., $[\alpha]^{27}D$ +106° (*c* 1.2, pyridine).

Anal. Calcd. for $C_{14}H_{20}O_8S$: C, 48.3; H, 5.8; S, 9.2. Found: C, 48.48; H, 5.68; S, 8.9.

Methyl 3,6-Anhydro-α-D-galactopyranoside.—The above 6-tosyl derivative (3.0 g.) was converted into this compound by treatment in ethanol solution with sodium hydroxide,[23] to give 1.34 g. of crystalline methyl 3,6-anhydro-α-D-galactopyranoside, m.p. 139–140°, $[\alpha]^{26}D$ +80° (*c* 1.0, water).

Anal. Calcd. for $C_7H_{12}O_5$: C, 47.7; H, 6.8. Found: C, 47.93; H, 6.80.

3,6-Anhydro-D-galactose Diethyl Mercaptal.—Methyl 3,6-anhydro-α-D-galactopyranoside (500 mg.) was dissolved in 0.75 ml. of concentrated hydrochloric acid precooled to 0°. Ethyl mercaptan (0.5 ml.) was added and the mixture shaken at 0° for 90 min. The solution was diluted with ice and water and the crystalline 3,6-anhydro-D-galactose diethyl mercaptal filtered and washed with cold water. It was dried *in vacuo* over potassium hydroxide and recrystallized from ethyl acetate–petroleum ether; yield 485 mg., m.p. 112–113°, $[\alpha]^{26}D$ −9.1° (*c* 1.15, water), +27.0° (*c* 1.1, pyridine).

Anal. Calcd. for $C_{10}H_{20}O_4S_2$: C, 44.75; H, 7.51; S, 23.9. Found: C, 44.87; H, 7.55; S, 23.8.

Quantitative Determination of 3,6-Anhydro-D-galactose.—Ten 25-mg. samples of the polysaccharide were dissolved in 2 ml. of 0.15 *N* hydrochloric acid and hydrolyzed in sealed tubes at 100°. Tubes were removed at intervals during a 24-hr. period, the contents neutralized with barium carbonate and filtered quantitatively into 10-ml. volumetric flasks. The solutions were made up to volume and diluted 1 → 50. Measurements of optical density were made on these dilutions at 2850 Å. in a Beckman model DU spectrophotometer. Maximum production of H.M.F. was found at 8 hr. To account for the first-order decomposition[29] of H.M.F. to formic and levulinic acids during the hydrolysis, the log of the optical density was plotted against time and the straight line portion of the curve concerned with this decomposition was extrapolated to zero time. From the value of the intercept (0.09—corresponding to an optical density of 1.23) and the molar extinction coefficient (16,500)[30] it was calculated that the H.M.F. formed represented 18.7% of the κ-carrageenin which corresponds to 24% of 3,6-anhydro-D-galactose.

(29) H. P. Teunissen, *Rec. trav. chim.*, **49**, 784 (1930); **50**, 1 (1930).

(30) M. L. Wolfrom, R. D. Schultz and L. F. Cavalieri, THIS JOURNAL, **70**, 514 (1948).

HALIFAX, N. S., CANADA

[CONTRIBUTION FROM THE RESEARCH LABORATORIES, GENERAL MILLS, INC.]

Reactions of Long Chain Amines. V. Reactions with Sugars[1,2]

BY JOHN G. ERICKSON

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Aldohexoses react with primary long chain alkylamines at room temperature, giving N-alkylglycosylamines in good yields. Observations are offered regarding the stability and ease of hydrolysis of these compounds. Ketohexoses react with primary long chain amines at room temperature to form the corresponding N-alkylketosylamines, as well as substantial amounts of products formed from one mole of sugar and two moles of amine. At higher temperatures several moles of amine may react with one mole of either aldohexose or ketohexose. Reducing disaccharides may also react with more than one mole of primary amine. Possible structures for these products are discussed.

Hodge's[3] comprehensive survey of the browning reaction discusses many types of products formed by reactions of amines with sugars. The present paper deals with the reactions between primary long chain aliphatic amines and various kinds of sugars. Some of the products are of types not generally heretofore recognized.

Sorokin[4] prepared N-arylglycosylamines by heating amines and sugars in ethanol. When Mitts and Hixon[5] used this method to prepare glucosyl derivatives of primary long chain amines yields fell noticeably short of theoretical and there was much decomposition, as shown by the very dark color developed. Pigman, Cleveland, Couch and Cleveland[6] modified the procedure by using hot methanol

(1) Paper No. 173, Journal Series, General Mills, Inc., Research Dept.

(2) A preliminary announcement of some of these findings has been made: J. G. Erickson, THIS JOURNAL, **75**, 2784 (1953).

(3) J. E. Hodge, *J. Agr. Food Chem.*, **1**, 928 (1953).

(4) W. Sorokin, *Ber.*, **20**, 783R (1887).

(5) E. Mitts and R. M. Hixon, THIS JOURNAL, **66**, 483 (1944).

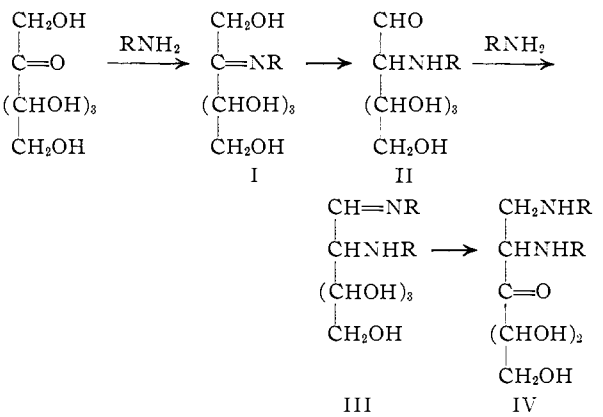
(6) W. Pigman, E. A. Cleveland, D. H. Couch and J. H. Cleveland, *ibid.*, **73**, 1976 (1951).

in place of ethanol and in this way obtained N-alkylglycosylamines in higher yields and with less discoloration. We have found that very good yields of white, or very nearly white, N-alkylglycosylamines may be obtained by reaction at room temperature. Equimolar amounts of long chain amine and aldose sugar are dissolved in the minimum amount of aqueous alcohol. After a day or two, formation of the product is nearly complete and, because of the moderate conditions employed, little or no decomposition and darkening occur. N-Alkylglycosylamines are known[5-7] to be easily hydrolyzed but this factor can be eliminated by removal of the product from the arena of reaction. If the proper ratio of alcohol to water is chosen, the yield is virtually quantitative, for the product precipitates as it is formed.

It has been reported[6] that N-dodecylglucosylamine is completely hydrolyzed by 0.5 *N* hydrochloric acid at 30°. We have confirmed this and have also found that the compound may be recovered quantitatively after several days in 0.4 *N* alcoholic-aqueous hydrochloric acid at 25°. Such stability in acid, surprising though it is, is consistent with findings in the earlier paper.[6] The long chain N-alkylglucosylamines are unchanged after two years storage at 0°. Some preparations decomposed in a few months at 25°, becoming dark and tarry, with a caramel-like odor. Others were little affected by standing for many months at 25°.

At room temperature glucose reacts with amines to form only N-alkylglucosylamines, even when a 100% excess of amine is used. A different picture is found with fructose. It has been known for many years that fructose is less reactive than glucose toward aromatic amines. Indeed, Barry and Honeyman[8] found it necessary to catalyze reactions of fructose with several aromatic amines by the use of ammonium chloride or amine hydrochloride. These workers have summarized the experiences of earlier studies in this direction. Not until recently has anyone studied the corresponding reactions with aliphatic amines. As reported in our earlier communication,[2] we found that fructose reacts readily with primary long chain amines. One of the products in each case is either an N-alkylfructosylamine or, since it reduces methylene blue readily in alkaline methanol solution, perhaps the Amadori rearrangement product of the fructosylamine.[9] Most of the product, however, is formed by a reaction of two moles of amine with one of ketose, wherein two moles of water are eliminated. This is true even when equimolar amounts of fructose and amine are used. Thus, octadecylamine and fructose give a white solid product, m.p. 107.5–109°, analyzing closely for $C_{42}H_{86}N_2O_4$. This material, "fructose-bisoctadecylamine," is noticeably more stable than N-octadecylglucosylamine. Upon microhydrogenation in butanol with palladium on charcoal at room temperature, one mole of fructose-bisoctadecylamine (calculated on the basis of the formula $C_{42}H_{86}N_2O_4$) absorbs 2.4 atoms of hydrogen. This fact would seem to exclude the formula $C_{21}H_{43}NO_2$, corresponding to products of chain scission, for which twice as much hydrogen absorption would be expected. Scission seems excluded also by the fact that our product has a low but definite optical activity.

The most plausible way to explain the formation of such compounds is to assume that the initial reaction product, the ketosylamine (I) undergoes an Amadori rearrangement. The new carbonyl group so formed (II) then reacts with another mole of amine, giving a compound III similar to an osazone. Since fructose-bisoctadecylamine reduces methylene blue in alkaline methanol readily, a second rearrangement may have created a new carbonyl group (IV). These structures are written in the open-chain form for simplicity but, of course, the corresponding cyclic forms may be the ones actually involved.



Carson[10] has very recently reported that fructose reacts with isopropylamine and cyclohexylamine at 25° or lower temperatures. Only one mole of isopropylamine reacts, giving the rearranged aldose derivative II. One or two moles of cyclohexylamine may react, forming the aldose derivative II and another product, assigned the cyclic form of structure III. The products from cyclohexylamine were reported to be interconvertible, the monoamino compound reacting with cyclohexylamine to form the diamino compound; the latter material was hydrolyzed by acid back to the monoamino compound. It is of interest in this connection that Mitts and Hixon[5] found that glucose reacts with two molecules of cyclohexylamine at 75°, one molecule of water being lost. The product so formed apparently had both amino groups attached to the number 1 carbon. Such behavior has apparently not been observed with any other primary amine. Attempts to prepare the condensation product of one molecule of glucose and one molecule of cyclohexylamine were not successful.

The reaction of a ketose with the second mole of amine is apparently faster than the reaction with the first mole. Further, fructose undergoes such reactions much more readily than glucose. These facts underscore the similarity of this reaction to

(7) J. C. Irvine, R. F. Thomson and C. S. Garrett, *J. Chem. Soc.*, **103**, 238 (1913).

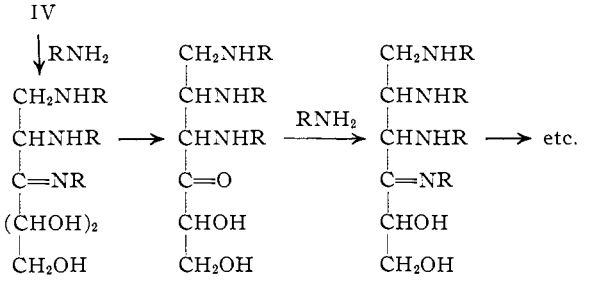
(8) C. P. Barry and J. Honeyman, *ibid.*, 4147 (1952).

(9) The original Amadori rearrangement involved the conversion of N-arylaldosylamines to 1-arylamino-1-desoxy-2-ketoses in the presence of acids. The term has recently been used in a more general sense to cover similar rearrangements of products from aliphatic amines.[3] The use of acid is not necessarily implied in the newer meaning.

(10) J. F. Carson, Abstracts of Papers, 126th Natl. Meeting, Amer. Chem. Soc., p. 17-D; THIS JOURNAL, **77**, 1881 (1954).

osazone formation, where fructose is known to be more reactive than glucose.[11,12] In fact, the formation of ketose-bisalkylamines, osazone formation, the Amadori rearrangement, and the Lobry de Bruyn–Alberda van Ekenstein reaction are quite similar in a formal sense, despite the different reaction conditions used. Each of these reactions can be considered as depending on isomerizations of hydroxy-carbonyl compounds or of their nitrogen analogs. Subsequent reactions, such as oxidation in osazone formation or loss of water in certain anomalous Amadori rearrangements,[13] may occur. Weygand and Reckhaus[14] have used the Amadori rearrangement in explaining osazone formation.

In our earlier communication,[2] we have shown that sugars apparently react with more than two moles of primary long chain amine when these reagents are warmed together in alcohol. Thus, sorbose and such aldohexoses as glucose and galactose react with octadecylamine, giving products which contain from three to five $C_{18}H_{37}N$ groupings. Analytical values are consistent with the picture of a series of Amadori rearrangements, each followed by reaction of the carbonyl group with amine. To illustrate this, we may continue the series of reactions shown above. A similar series could be written for amine–aldose reactions, of course. It has not been possible to confirm this mechanism by molecular weight determinations because the molecular weights are very high and the products are both somewhat unstable and insufficiently soluble in suitable solvents. Nevertheless, scission of the sugar chain appears to be excluded by the fact that picrates of these products analyze correctly for monopicrates. Picrates of products derived by degradation of the sugar chain would have carbon and hydrogen values quite different from those observed.



Werntz[15] has described the preparation of glycosylamines from maltose and long chain amines. Pigman, Cleveland, Couch and Cleveland[6] have also prepared these compounds as well as N-dodecyllactosylamine. We have found that such reducing disaccharides as lactose and maltose can react further when warmed with long chain amines. At least two molecules of amine will react and probably still more. These reactions have not been studied very extensively but presumably the same mode of reaction prevails here as with the monosaccharides. As would be expected, sucrose does not react with amines.

(11) E. Fischer, *Ber.*, **17**, 579 (1884).
(12) J. Ashmore and A. E. Renold, THIS JOURNAL, **76**, 6189 (1954).
(13) R. H. Anderson, *J. Org. Chem.*, **19**, 1238 (1954).
(14) R. Weygand and M. Reckhaus, *Ber.*, **82**, 438 (1949).
(15) J. H. Werntz, U. S. Patent 2,181,929 (Dec. 5, 1939).

The products described in this paper are readily isolated and purified because of the long alkyl groups which give the products desirable solubility properties. Similar products from most primary aliphatic amines of shorter chain length should also be formed but may crystallize with more difficulty. Perhaps it is for this reason that some of these reactions have not been observed before. That such reactions do occur, for instance, in biological systems seems quite possible. They may take place with amino acids or with free amino groups in proteins or polypeptides. It is possible that they are involved in the browning or Maillard reaction. We have pointed out that fructose is more reactive than glucose toward amines. It is of interest to find that fructose may, in biological systems, play roles that are apparently closed to glucose. One example of this is the discovery by Baker, Chaikoff and Schusdek[16] that the synthesis of fatty acids from two-carbon intermediates like lactic acid or acetic acid fails in the liver of glucose-fed alloxan-diabetic rats but is restored by the feeding of fructose to these animals. Another example is the recent report by Fochem[17] that fructose is effective in treating radiation sickness. The cause of the so-called X-ray illness lies in a protein poisoning brought about by products of a continuing breakdown of the body cells. The removal of these toxic materials from the tissues and blood is accomplished by the liver which may suffer damage in the process. Fochem found that fructose is very effective in preventing these toxins from injuring the liver. He points out that other people have found fructose to be a valuable means of protecting the liver in various liver illnesses. There are, no doubt, several explanations for such observations. However, it is at least possible that such effects may be based on the greater ability of fructose to react with more than one amino group, thereby changing enzyme or hormone action or otherwise modifying the nature of proteins. We do not have the time or facilities to explore such points and do not contemplate any further work on these reactions.

Acknowledgments.—The author wishes to thank Dr. Ray H. Anderson and Prof. Fred Smith for helpful discussions. James Kerns performed the microanalyses and did the microhydrogenation of fructose-bisoctadecylamine.

Experimental

Reagents.—The preparation of the amines has already been described.[18]

N-Dodecyl-D-glucosylamine.—A homogeneous mixture of dodecylamine (18.5 g., 0.10 mole), D-glucose (9.0 g., 0.05 mole), ethanol (50 ml.) and water (35 ml.) was allowed to stand at room temperature for 15 days, then filtered. Weight of dry white crude product was 16.0 g. (93%), m.p. 88–100°. After two recrystallizations from ethanol, m.p. was 107.5–109°; reported[6] m.p. 105.5°.

Anal. Calcd. for $C_{18}H_{37}NO_5$: C, 62.22; H, 10.73; N, 4.03. Found: C, 62.45; H, 10.45; N, 4.32.

After two years at 25°, a sample had developed a slight tinge of brown color. Another sample was unchanged after two years at 0°.

It was found that N-dodecyl-D-glucosylamine is completely

(16) N. Baker, I. L. Chaikoff and A. Schusdek, *J. Biol. Chem.*, **194**, 435 (1952).
(17) K. Fochem, *Strahlentherapie*, **93**, 466 (1954).
(18) J. G. Erickson and J. S. Keps, THIS JOURNAL, **77**, 485 (1955).

hydrolyzed by 0.5 *N* HCl at 25° in 70 hours or less. However, the compound withstood hydrolysis under the following conditions. Three grams was dissolved in a mixture of ethanol (100 ml.), water (50 ml.) and concentrated hydrochloric acid (5 ml.). This solution, which was *ca.* 0.4 *N* HCl, stood for two days at 25°, was neutralized with excess dilute potassium hydroxide solution, diluted with water, and filtered. This gave 3.0 g. of white dry product, which melted at 106.5–108° after recrystallization from ethanol.

The infrared absorption spectrum and analysis showed it to be unchanged starting material.

Anal. Calcd. for $C_{18}H_{37}NO_5$: C, 62.22; H, 10.73; N, 4.03. Found: C, 62.37; H, 10.98; N, 4.52.

Two grams of N-dodecyl-D-glucosylamine was mixed with tributylamine (6 ml.) and absolute ethanol (50 ml.). After five days at 25°, the mixture was heated at 60° for three hours, chilled and filtered. Analysis and infrared absorption spectrum of the recrystallized product showed it to be unchanged starting material.

N-Octadecyl-D-glucosylamine.—A mixture of octadecylamine (26.9 g., 0.10 mole), D-glucose (18.0 g., 0.10 mole), isopropyl alcohol (200 ml.) and water (100 ml.) stood for two days at 25°. It was then filtered and the light yellow precipitate was washed with 2:1 (by volume) isopropyl alcohol–water, giving 34.9 g. (100%) of dry product. Two recrystallizations from absolute ethanol gave a white solid, m.p. 104.3–105° dec.; reported[5] m.p. is 106–107°.

Anal. Calcd. for $C_{24}H_{49}NO_5$: C, 66.79; H, 11.44; N, 3.25. Found: C, 66.80; H, 11.14; N, 3.25.

In the course of a few months, this compound turned to a dark tar, with an odor like that of caramel.

N-Dodecyl-D-fructosylamine (or an Amadori Rearrangement Product) and D-Fructose-bisdodecylamine.—A solution of dodecylamine (11.1 g., 0.06 mole) and D-fructose (5.4 g., 0.03 mole) in 40 ml. of 1:1 (by volume) water–isopropyl alcohol was allowed to stand at 25° for eight days. It was then chilled at 0° for two days and filtered, yielding 2.9 g. of a gummy semisolid (A). Acetonitrile was added to the filtrate until an oil came out of solution. The upper layer was decanted, more acetonitrile was added to the oily lower layer and the upper layer was again decanted. The lower layer was mixed with benzene (50 ml.), chilled and filtered to yield 1.5 g. of almost white solid (B), not at all g mmy or waxy.

Fraction A was recrystallized from Skellysolve B, giving 1.0 g. of white powder, m.p. 101.5–104.5°. It was recrystallized again from Skellysolve B–ethyl acetate, m.p. 103.5–104.5°. Analysis showed it was formed from one mole of sugar and two moles of amine by loss of two moles of water.

Anal. Calcd. for $C_{30}H_{62}N_2O_4$: C, 69.98; H, 12.14; N, 5.44. Calcd. for $C_{30}H_{64}N_2O_5$: C, 67.64; H, 12.11; N, 5.26. Found: C, 70.35; H, 11.99; N, 5.54.

Fraction B was recrystallized from Skellysolve B, then from benzene–ethyl acetate, giving a white product, m.p. 103–104° dec. A mixed melting point with purified fraction A was depressed. Analysis showed that B was N-dodecyl-D-fructosylamine or an isomer of this compound.

Anal. Calcd. for $C_{18}H_{37}NO_5$: C, 62.22; H, 10.73; N, 4.03. Found: C, 62.01; H, 10.68; N, 4.09.

N-Octadecyl-D-fructosylamine (or an Amadori Rearrangement Product) and D-Fructose-bisoctadecylamine.—A homogeneous mixture of D-fructose (9.0 g., 0.05 mole), octadecylamine (26.9 g., 0.10 mole), water (100 ml.) and isopropyl alcohol (200 ml.), after standing four days at 25°, had deposited much light yellow solid. It was filtered, giving 18.8 g. of crude fructose-bisoctadecylamine. Three recrystallizations from benzene gave a white solid, m.p. 107.5–109°. It reduced methylene blue readily in alkaline methanol solution. Analysis showed clearly that this compound is formed by reaction of one mole of fructose with two moles of amine, with elimination of two moles of water.

Anal. Calcd. for $C_{42}H_{86}N_2O_4$: C, 73.81; H, 12.69; N, 4.10. Calcd. for $C_{42}H_{88}N_2O_5$: C, 71.92; H, 12.65; N, 4.00. Found: C, 73.70; H, 12.65; N, 4.25.

Determination of the optical activity was somewhat difficult because of the insolubility of the material in most solvents at convenient temperatures. It was finally done in a 1:1 mixture by weight of acetic acid and benzene. The optical activity appeared to be zero or very small at first but increased after a few minutes to a readable value, presumably because of mutarotation. At the same time the solution was darkening and this made observation increasingly difficult. The value we present is in no sense an equilibrium value but it does seem to establish the presence of optical activity in the compound, $[\alpha]^{30}D$ $-6 \pm 2°$ (*c* 6.2).

The filtrate from the isolation of D-fructose-bisoctadecylamine, upon standing, deposited 5.0 g. of crude N-octadecyl-D-fructosylamine (or isomer). Several recrystallizations from benzene gave a white solid, m.p. 101–103°. It reduced methylene blue readily in alkaline methanol solution.

Anal. Calcd. for $C_{24}H_{49}NO_5$: C, 66.79; H, 11.45; N, 3.25. Found: C, 67.32; H, 11.80; N, 4.06.

In other experiments, wherein equimolar amounts of octadecylamine and fructose were employed, we found that the product is largely fructose-bisoctadecylamine.

Reaction of D-Galactose with Three Moles of Octadecylamine.—A mixture of D-galactose (3.6 g., 0.02 mole), octadecylamine (32.3 g., 0.12 mole), isopropyl alcohol (150 ml.) and water (35 ml.) was prepared. It was warmed to 60°, then allowed to cool to 25° once for each of seventeen working days during the next four weeks. Filtration gave 22.9 g. of almost white product. After two recrystallizations from absolute ethanol, m.p. was 64.5–67°.

Anal. Calcd. for $C_{60}H_{123}N_3O_3$: C, 77.10; H, 13.27; N, 4.50. Found: C, 77.12; H, 12.62; N, 4.72.

Reaction of D-Glucose with Four Moles of Octadecylamine.—A mixture of D-glucose (3.6 g., 0.02 mole), octadecylamine (32.3 g., 0.12 mole), isopropyl alcohol (150 ml.) and water (35 ml.) was warmed to 60–70°, then allowed to cool to 25°, once on each of ten working days during two weeks. Filtration gave 23.3 g. of brownish solid; m.p., after two recrystallizations from absolute ethanol, 66.5–68°.

Anal. Calcd. for $C_{78}H_{160}N_4O_2$: C, 77.96; H, 13.60; N, 4.72. Found: C, 78.66; H, 13.37; N, 4.41.

This product reduced methylene blue in alkaline methanol. A monopicrate was prepared by heating 0.10 g. of the product with picric acid (0.15 g.) in absolute ethanol (8 ml.). Cooling and filtration gave a small amount of brown solid, m.p. 50–95° dec.

Anal. Calcd. for $C_{84}H_{163}N_7O_9$: C, 71.29; H, 11.61; N, 6.93. Found: C, 71.83; H, 11.77; N, 6.70.

Reaction of L-Sorbose with Two to Three Moles of Octadecylamine.—A mixture of octadecylamine (26.9 g., 0.10 mole), L-sorbose (9.0 g., 0.05 mole), isopropyl alcohol (200 ml.) and water (100 ml.) stood at 25° for four days. Filtration gave 16.4 g. of light-colored product. Recrystallization from ethanol and ethyl acetate gave a product with m.p. 69–71° dec.

Anal. Calcd. for $C_{42}H_{86}N_2O_4$: C, 73.81; H, 12.69; N, 4.10. Calcd. for $C_{60}H_{123}N_3O_3$: C, 77.10; H, 13.27; N, 4.50. Found: C, 75.33; H, 12.54; N, 4.67.

Reaction of L-Sorbose with Five Moles of Octadecylamine.—A mixture of octadecylamine (26.9 g., 0.10 mole), L-sorbose (2.7 g., 0.015 mole), isopropyl alcohol (200 ml.) and water (50 ml.) was heated to 60–70°, then allowed to cool, once on each of the twenty working days during four weeks. It was then filtered and washed, giving 12.7 g. of very light tan product. A portion was recrystallized from absolute ethanol, m.p. 63.5–65°.

Anal. Calcd. for $C_{96}H_{197}N_5O$: C, 80.19; H, 13.81; N, 4.87. Found: C, 79.73; H, 12.91; N, 5.03.

A mixture of 3.0 g. of the crude product above and 3.0 g. of octadecylamine in 50 ml. of benzene was heated at 80° for 8 hours, cooled to 25° and filtered. The filtrate was chilled to 5° and filtered, giving 2.0 g. of tan solid. Recrystallization from absolute ethanol gave a product with m.p. 67–69.5°. It rapidly decolorized methylene blue in alkaline ethanolic solution.

Anal. Calcd. for $C_{96}H_{197}N_5O$: C, 80.19; H, 13.81; N, 4.87. Found: C, 80.24; H, 13.25; N, 4.66.

A picrate of the material melting at 67–69.5° was prepared. After recrystallization from ethanol, the m.p. was 45–47°. Analysis showed it to be a monopicrate.

Anal. Calcd. for $C_{102}H_{200}N_8O_8$: C, 73.50; H, 12.10; N, 6.72. Found: C, 72.95; H, 11.43; N, 6.73.

Reaction of Maltose with Two Moles of Dodecylamine.—A mixture of dodecylamine (18.5 g., 0.10 mole), maltose (7.2 g., 0.02 mole) and absolute ethanol (50 ml.) was heated

at 75–80° for six hours. It became dark and the sugar dissolved after three hours. The mixture was evaporated at 25° and atmospheric pressure yielding 24.5 g. of dark brown, partially crystalline paste. Recrystallization from Skellysolve B gave 0.25 g. of faintly tan solid, m.p. 210–220° dec., insoluble in water. Analysis indicated it was a mixture of N-dodecylmaltosylamine (or an isomer) with a larger amount of the reaction product derived from two moles of amine.

Anal. Calcd. for $C_{24}H_{47}NO_{10}$: C, 56.58; H, 9.30; N, 2.75. Calcd. for $C_{36}H_{73}N_2O_9$: C, 63.77; H, 10.85; N, 4.13. Found: C, 61.33; H, 10.19; N, 4.42.

N-Octadecyllactosylamine.—Lactose monohydrate (19.8 g., 0.055 mole) and octadecylamine (26.9 g., 0.10 mole) were dissolved in a mixture of isopropyl alcohol (200 ml.) and water (120 ml.). After a day at 25°, it had largely solidified. It was warmed to 60°, becoming a clear solution, allowed to cool and crystallize, and filtered, giving 36.9 g. of white solid, m.p. 106.5–108.5°. It was recrystallized twice from absolute ethanol, m.p. 119–121.5° dec.

Anal. Calcd. for $C_{30}H_{59}NO_{10}$: C, 60.67; H, 10.02; N, 2.36. Found: C, 59.60; H, 10.46; N, 2.67.

Reaction of Lactose with Two Moles of Octadecylamine.—A mixture of octadecylamine (26.9 g., 0.10 mole), lactose (7.2 g., 0.02 mole), water (25 ml.) and isopropyl alcohol (75 ml.) was heated at 70° for one hour. The sugar soon dissolved and the mixture became colored. After cooling, it could not be filtered so the entire mass was air-dried, 31.9 g., m.p. 70–85°. Three recrystallizations from alcohol gave a yellow solid, m.p. 116–117°. Analysis indicated it was a mixture of N-octadecyllactosylamine (or isomer) and a product derived from two moles of amine.

Anal. Calcd. for $C_{30}H_{59}NO_{10}$: C, 60.67; H, 10.02; N, 2.36. Calcd. for $C_{48}H_{96}N_2O_9$: C, 68.19; H, 11.44; N, 3.31. Found: C, 63.80; H, 10.34; N, 3.07.

MINNEAPOLIS 13, MINNESOTA

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

Isolation, Structure and Synthesis of a Lathyrus Factor from *L. Odoratus*[1,2]

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The crystalline substance $C_8H_{13}O_3N_3$, isolated from *Lathyrus odoratus* seeds, which produces in rats the skeletal deformities characteristic of lathyrism, has been shown by degradation and synthesis to be β-(N-γ-L-glutamyl)-aminopropionitrile.

The isolation from *Lathyrus odoratus* seeds of a crystalline substance capable of producing skeletal abnormalities characteristic of lathyrism in rats has recently been accomplished.[3–6] The substance I, obtained in this Laboratory, fine white needles, m.p. 193–194° dec., gave analytical values agreeing with the formula $C_8H_{13}O_3N_3$.[4]

The compound gave one well-defined ninhydrin-positive spot, with no evidence of admixture with any ninhydrin-positive impurity, when subjected to paper chromatography in three different solvent systems according to published procedures. After strong acid hydrolysis this spot disappeared and was replaced by two others. This behavior plus the amphoteric and strongly polar character of the substance suggested that I could well be a dipeptide. Concentration of the hydrolysis mixture yielded a crystalline degradation product which was identified as L-glutamic acid hydrochloride. When the filtrate was made alkaline, the odor of a volatile base became evident. The volatile material was aerated into dilute hydrochloric acid and the resulting hydrochloride found to be free of carbon. The volatile base, therefore, was ammonia. Since the non-volatile portion of the hydrolysate still showed two ninhydrin spots in paper chromatograms, at least one other nitrogenous degradation product remained to be identified.

Calculations based on the $C_8H_{13}O_3N_3$ formula revealed unsaturation in I equivalent to four double bonds. Since the glutamic acid accounted for two of these, it was obvious that some other structure, probably in the unidentified three carbon portion of I, contained the remaining unsaturation. Furthermore, it appeared that in the original molecule this three-carbon portion must have contained either one or two nitrogen atoms.

Consideration was therefore directed to type structures for the remaining fragment which would contain three carbon atoms, one or two nitrogen atoms, two double bonds or the equivalent, and a grouping which would yield ammonia on hydrolysis. In this connection it was observed that the infrared spectrum of I showed a sharp band at 4.45 μ characteristic of a triple bond. This infrared band, the production of ammonia on hydrolysis, and the expected degree of unsaturation were all compatible with the presence of a nitrile function in I. On the assumption that a nitrile group was in fact present, the unidentified hydrolysis product would have had to be either alanine, β-alanine or sarcosine. Comparison with known samples on paper chromatograms clearly pointed to β-alanine as the actual degradation product, and its presence in the hydrolysate of I was then verified by direct isolation of a crystalline derivative.

In the light of the above results it seemed probable that I was a peptide-like combination of either glutamic acid plus β-aminopropionitrile or β-alanine plus a glutamonitrile. Of the six possible structures of this type, that of β-(N-γ-L-glutamyl)-aminopropionitrile (II) was selected as the first to be tested by synthesis, because a good synthetic method was available,[7] because the pK values of I (2.2 and 9.14) resembled those of glutamic acid, and because II contained the labile γ-glutamyl

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(2) Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part by grants from the Division of Research Grants and Fellowships of the National Institutes of Health, United States Public Health Service.

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