also of the two well known lipotropic factors, choline and methionine, on the activity of the enzyme at a pH of 8·2 in the presence of Ca^{++} ions was tested. The results, presented in Fig. 1, show clearly that both cystine and cyanide increase the lipolytic activity of the enzyme under the conditions specified. This effect of cystine may be related to its known influence on the accumulation of fat in the liver of rats fed on a diet low in lipotropic factors (Beeston & Channon, 1936; Tucker & Eckstein, 1937; Mulford & Griffith, 1942). The significance of results obtained when zinc and cyanide were added is not at present clear and merits further investigation.

SUMMARY

1. The changes in the metabolism of nitrogen, phosphorus and sulphur and in urinary excretion of uric acid and total creatinine (creatine and creatinine) in rats fed on a high-fat diet supplemented with zinc have been shown to be similar to those observed for a stock diet. It has also been shown that the decreased assimilation of phosphate from the intestines is not due to its being precipitated as insoluble zinc phosphate.

2. Zinc supplements at the levels investigated affected phosphatase activity of the tissues. While the activity of intestinal alkaline phosphatase was lowered, there was an increase in activity in the liver and kidneys in the supplemented groups.

3. Though zinc supplements lowered the fat content of the liver and the assimilation of phosphate from the intestines, there appeared to be no change in the excretion of fat in the faeces.

4. The influence of zinc and other substances such as choline, methionine, L-cystine and cyanide on the lipolytic activity of pancreatic lipase of the rat was also investigated.

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Some Biochemically Relevant Properties of *N*-Substituted Fructosamines Derived from Amino-Acids and *N*-Arylglucosylamines

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N-Substituted glucosamines (*N*-acetyl-D-glucosamine, *N*-formyl-D-glucosamine, *N*-methyl-L-glucosamine), derived from 2-amino-2-deoxyglucose, are well known as components of polysaccharides and mucoproteins produced by animal cells, fungi and bacteria. Fructosamine (1-amino-1-deoxyfructose) (also called *iso*glucosamine) and *N*-substituted fructosamines have not been found among the hydrolytic products of polysaccharides and mucoproteins. It was, however, recently shown (Gottschalk, 1951) that an enzyme present at the surface of the influenza virus splits off from a homogeneous mucoprotein, isolated from human urine (Pye, to be published), an *N*-substituted fructosamine characterized as a condensation product of a ketose and an amino-acid.

N-Substituted fructosamines (III, p. 459) are prepared chemically from the so-called *N*-glucosides of primary aromatic amines (*N*-arylglucosylamines, I, p. 459) through the Amadori rearrangement. Thus *N*-arylglucosylamines from *p*-toluidine, *p*-phenetidine and 3:4-dimethylaniline are transformed to the corresponding fructosamines when heated for a few hours in ethanolic solution with a weak acid as catalyst (Kuhn & Weygand, 1937; Weygand, 1939, 1940). The isomerization of glucosylamines to fructosamines, i.e. the rearrangement of a D-glucose to a D-fructose derivative, has been observed with *N*-arylglucosylamines only. So far attempts to induce the Amadori rearrangement in *N*-alkylglucosylamines have failed (Mitts & Hixon, 1944). While continuing our investigations into the inter-

action between simple sugars and amino-acids (Gottschalk & Partridge, 1950), with the aim of preparing in quantity *N*-glucosylamino-acids (so-called amino-acid *N*-glucosides) it became evident that by boiling for several hours anhydrous D-glucose and DL-phenylalanine in thoroughly dried methanol the *N*-substituted fructosamine, fructose-phenylalanine was formed. (III, $R=CH(CO_2H).CH_2.C_6H_5$; the systematic name, '*N*-(1'-carboxy-2'-phenylethyl)amino-1-deoxyfructose' is so clumsy that the trivial name 'fructose-phenylalanine' is used in this paper.) The compound was practically free from contamination by *N*-glucosyl-phenylalanine which must be assumed to be the first product of reaction, its carboxyl group providing the acidic catalyst required for the Amadori rearrangement; it is known that glucosylamino-acids are stronger acids than the respective free amino-acids (Micheel & Klemer, 1951). Earlier preparations of *N*-glucosylamino-acids obtained by a similar technique probably also consisted mainly of the isomeric fructosamine without it having been recognized. Fructose-phenylalanine has not been crystallized and it was not possible to free it completely from impurities. The chromatographic and analytical results, however, suggest that the amount of contaminants is small. Moreover, *N*-*p*-tolyl-D-fructosamine, a crystalline *N*-arylfructosamine, was found to have in common with the phenylalanine analogue certain very characteristic reactions. On examination of their behaviour towards mineral and organic acids the *N*-substituted fructosamines tested revealed properties differentiating them clearly from *N*-substituted glucosamines and from the parent D-fructose thus providing a tool for the detection of such structures in natural products.

EXPERIMENTAL

Chromatography

General. Paper partition chromatography was carried out in the usual way with Whatman no. 1 paper. The chromatograms were developed for 16 hr. with the upper phase of a mixture of *n*-butanol, acetic acid and water in the ratio 40:10:50 (by vol.), the lower phase serving as stationary phase (Partridge, 1948). The assays were applied to the paper with a calibrated capillary pipette (0·005 ml.). After development, the paper strips or sheets were freed of the solvent by exposing them to a strong draught in the fume chamber for several hours at room temperature followed by 10 min. heating at 95° in the drying chamber. Owing to variations in the room temperature and to an uncontrolled degree of esterification of the solvent, the R_F values quoted were not constant and refer only to a given set of conditions.

Colour reactions used on paper. Phenylalanine was located with ninhydrin, glucose with aniline hydrogen phthalate (Partridge, 1949), 5-(hydroxymethyl)-2-furaldehyde with a solution consisting of 10 ml. 1% (w/v) resorcinol in ethanol and 90 ml. 2N-HCl. To locate *p*-toluidine use was made of the observation that *p*-dimethylaminobenzaldehyde (*p*-DAB) in the presence of HCl gives an intense yellow colour with *p*-toluidine. For use on paper a solution was prepared containing 80 mg. *p*-DAB (recrystallized) in a mixture of 3·0 ml. conc. HCl and 3·0 ml. ethanol; for spraying 5·0 ml. solution were mixed with 15·0 ml. purified *n*-butanol. The *N*-substituted fructosamines reacted on paper with both aniline hydrogen phthalate and the appropriate reagent for the amine. The Schiff's bases of 5-hydroxymethylfurfuraldehyde were traced by their colour reactions with resorcinol and with the reagent for the amine.

Semiquantitative analysis. For the semiquantitative determination by chromatography of glucose, phenylalanine, *p*-toluidine, 5-hydroxymethylfurfuraldehyde, *N*-*p*-tolyl-D-fructosamine and fructose-phenylalanine the assays were run and compared with serial dilutions of the appropriate reference substance. These dilutions were adjusted in such a way that the concentration of the critical standard and that of the assay did not differ by more than 10% as judged by the area of the spot and by the colour intensity. When the colour given by the assay was intense, a dilution was made so as to bring the colour intensity into a range where a 10% difference could be appreciated. All chromatograms for quantitative evaluation were run at least twice on successive days. For this procedure an accuracy greater than $\pm 10\%$ is not claimed. Because of the volatility of *p*-toluidine at higher temperatures this compound both as standard and in the assay was run on paper as *p*-toluidine hydrochloride.

Other methods used. Nitrogen determinations were made by the micro-Kjeldahl method using the apparatus of Parnas & Wagner; the period of combustion was 8 hr. after clearing; the catalyst mixture consisted of K_2SO_4, $CuSO_4.5H_2O$ and sodium selenate as described by Chibnall, Rees & Williams (1943). The reducing power was determined by the Hagedorn-Jensen micromethod.

Purification of n-*butanol.* The commercial product was refluxed for 24 hr. over NaOH, then distilled and the distillate after drying over anhydrous Na_2SO_4 fractionated in a column; the fraction boiling at 117–118° was collected.

Drying of methanol. Methanol (200 ml.) was slowly added to a mixture of 10 g. powdered Mg and 0·5 g. I_2; when the reaction ceased, further 800 ml. methanol were added, the mixture refluxed for 6 hr. and the dry methanol separated by distillation.

N-p-*Tolyl*-D-*glucosylamine.* This was prepared according to Weygand (1939); *N*-*p*-tolyl-D-fructosamine according to Kuhn & Dansi (1936) and 5-hydroxymethylfurfuraldehyde according to Haworth & Jones (1944).

Fructose-phenylalanine

Preparation. Dried methanol (140 ml.) was added to 700 mg. DL-phenylalanine and boiled under reflux for 1 hr., when only part of the amino-acid was dissolved. Anhydrous D-glucose (5 g.) was then added and the mixture refluxed for 4–5 hr.; 1 hr. after the addition of glucose nearly all phenylalanine was dissolved indicating the progress of the condensation reaction. After a while the methanolic solution turned yellow and later on light brown; at this stage heating was interrupted. After cooling, the solvent was removed *in vacuo* at 35°. The brown residue was dissolved in 75 ml. water, 5 g. thoroughly washed and freshly compressed baker's yeast added and the suspension stirred mechanically at 26° for 7 hr., when the excess glucose was completely fermented as

ascertained by chromatography. The yeast was centrifuged off, the supernatant filtered and the filtrate concentrated under reduced pressure and finally dried in a desiccator over P_2O_5. The resulting brittle material which could easily be powdered with a glass rod was dissolved in 10·0 ml. methanol, a white precipitate (phenylalanine) removed, the solvent evaporated by a fan at 20° and the residue dried again in the desiccator. This procedure was repeated once more. Yield 1·34 g. Attempts to crystallize the substance failed.

Chemical analysis. A stock solution was prepared by dissolving 1·3 g. of dried material in water to a final volume of 10 ml.; pH was 4·0. After removal of some insoluble dark matter this solution was analysed qualitatively and semi-quantitatively by chromatography and quantitatively for N and reducing power. Chromatography revealed (1) a spot with R_F 0·43 reacting with both ninhydrin and aniline hydrogen phthalate, the ninhydrin spot becoming more marked when the paper was dried at 95° instead of at room temperature, (2) a ninhydrin-reacting spot with R_F 0·59 coinciding with the phenylalanine standard and equivalent to a total content of 33 mg. phenylalanine in the original preparation and (3) a faint spot reacting with aniline hydrogen phthalate (brown colour) and trailing from R_F 0·31 to R_F 0·41. No glucose spot was detectable though even 5 mg. (in total) would have been traced. N content: 45·0 mg. Reducing power (as glucose): 532 mg. (both for total original solution). N and reducing power were determined in an appropriate dilution of the stock solution. The stock solution strongly reduced Benedict's reagent on heating and gave a positive Seliwanow test; in alkaline medium *o*-dinitrobenzene and methylene blue were reduced in the cold.

Since the chromatograms indicated the presence only of two ninhydrin-reacting spots and as ninhydrin is an effective reagent not only for amino-acids but also for primary aliphatic amines and even for some secondary aliphatic amines (Bremner & Kenten, 1951), the total amount of fructose-phenylalanine can be computed from the chromatographic and analytical data. Since 33 mg. free phenylalanine containing 2·8 mg. N were recovered, there are present in total original solution $42·2 \times 327/14 = 986$ mg. fructose-phenylalanine, i.e. 71% of theory. 986 mg. compound contain the equivalent of 543 mg. glucose; the experimental figure of 532 mg. glucose compares well with that to be expected from the N analysis. It may be mentioned that in another preparation free of the trailing spot described under (3)—but containing more free phenylalanine—the reducing power of the compound was 92% referred to its glucose equivalent, whereas in the above preparation it is 98%; apparently the substance responsible for the trailing spot contributes about 5% to the total reducing power. The reducing power of the glucose residue in *N*-acetyl-D-glucosamine was found to be 89% (Hagedorn-Jensen; glucose = 100).

Colour reaction with p-*DAB.* When 1·0 ml. of a solution containing 5 mg. compound was heated with acetylacetone in alkaline medium followed by the addition of *p*-DAB (Elson & Morgan, 1933), a purple colour was produced identical in shade with that given by a glucosamine standard but of only about 10% its intensity, if equimolar amounts are compared. Spectroscopic examination (Hartridge spectrometer) disclosed that the positions of the absorption bands produced with D-glucosamine and with the *N*-substituted fructosamine were practically identical: they were 537 and 539 mμ. respectively.

N-p-*Tolylfructosamine.* A 2% (w/v) solution of *p*-tolylfructosamine (in dilute acetic acid) was submitted to chromatography: a spot with R_F 0·74 reacting with both aniline hydrogen phthalate and *p*-DAB was obtained.

The substance strongly reduced Benedict's reagent in the hot and *o*-dinitrobenzene and methylene blue (in alkali) in the cold, as described (Kuhn & Weygand, 1937; Kuhn & Birkofer, 1938). The Seliwanow test, if carried out as is usually done in 12% final concentration of HCl was negative; when performed in ethanol saturated with HCl gas in the cold, the test was positive. In the Elson-Morgan test the compound gave a red colour immediately on adding *p*-DAB; however, on shaking, the yellow colour formed by the reagent with *p*-toluidine prevailed resulting finally in a brownish colour.

N-p-*Tolyl*-D-*glucosylamine.* A 2% (w/v) solution of the glucoside (in water) was submitted to chromatography; there were observed a spot with R_F 0·18 trailing to R_F 0·27 reacting with aniline hydrogen phthalate (glucose standard R_F 0·18) and a spot with R_F 0·85 reacting with *p*-DAB and coinciding with the spot given by *p*-toluidine. Obviously the *N*-glucoside is completely hydrolysed under these conditions.

N-*p*-Tolyl-D-glucosylamine (0·3% w/v in water) containing glucose and *p*-toluidine residues corresponding to 0·201% glucose and 0·119% *p*-toluidine were found to have a reducing power corresponding to 0·239% of glucose; since 0·2% (w/v) *p*-toluidine (in water) has a reducing power corresponding to 0·072% of glucose, the corrected figure for the glucose present in the glucoside is $0·239 - 0·043 = 0·196$%, i.e. 97·5% of the glucose equivalent of *N*-*p*-tolyl-D-glucosylamine.

The *N*-glucosylamine derivative reduced Benedict's reagent in the hot, but not *o*-dinitrobenzene in the cold; it gave a positive Seliwanow test in the modification mentioned above. In the Elson-Morgan test the compound showed a behaviour similar to that of *N*-*p*-tolylfructosamine, though the intensity of the colour formed was less.

Treatment of N-*substituted fructosamines and of* N-p-*tolylglucosylamine with acid*

Experiment 1. To 3·0 ml. of a solution containing approx. 100 mg. substance were added 0·33 ml. conc. HCl and 0·40 ml. glacial acetic acid, the tubes were immediately sealed and submerged for 2 hr., in a boiling water bath. The solutions, after cooling, were neutralized with a measured quantity of 10 N-NaOH, filtered and submitted to semi-quantitative analysis by chromatography. The results are shown in Table 1 and Fig. 1.

As indicated in Fig. 1 the acetic acid-treated sample of *N*-*p*-tolylfructosamine contained a small amount of the Schiff's base of 5-hydroxymethylfurfuraldehyde. In the case of fructose-phenylalanine the corresponding Schiff's base (R_F 0·62) was found occasionally as impurity in the preparation of the original compound but not after acid treatment, probably due to hydrolysis of the Schiff's base under these conditions.

A better separation of 5-hydroxymethylfurfuraldehyde and *p*-toluidine than shown in Fig. 1, *D*5 and *D*6, was obtained when the assay was subjected to electrophoresis on paper using an apparatus similar to that described by Cremer & Tiselius (1950). The analysis was carried out in 0·1 N-acetic acid at 20° applying a potential of 180 V. for 4 hr.

Table 1. *Action of* HCl *and acetic acid on* N-*substituted fructosamines and an isomeric* N-*glucosylamine derivative*

Substrate and treatment	Substrate initial (mg.)	Substrate left at end of experiment	D-Glucose liberated (mg.)	5-Hydroxymethylfurfural formed (mg.)	Amine released (mg.)	Insoluble humin formed
Fructose-phenylalanine:						
N-HCl, 100°, 2 hr.	98·6	42·9	0	6·8	25·9	+
2N-Acetic acid, 100°, 2 hr.	98·6	9·9	2·5	20·4	25·9	Trace
N-p-Tolylfructosamine:						
N-HCl, 100°, 2 hr.	100·0	50·0	0	6·6	20·0	+
2N-Acetic acid, 100°, 2 hr.	100·0	0	0	17·0	20·4	+ + +
N-p-Tolylglucosylamine:						
N-HCl, 100°, 2 hr.	100·0	0	59·9	<1·5	40·2	Trace
2N-Acetic acid, 100°, 2 hr.	100·0	0	16·7	13·3	25·4	+ + +

Experiment 2. 70·0 mg. D-Fructose, dissolved in 3·40 ml. 2N-acetic acid, were heated in a sealed tube for 2 hr. at 100°. This treatment did not result in the formation of enough 5-hydroxymethylfurfuraldehyde to be detectable by chromatographic analysis.

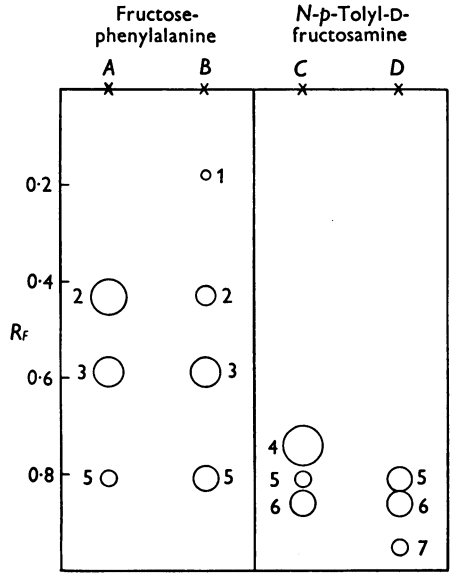


Fig. 1. Chromatograms of acid-treated *N*-substituted fructosamines. *A* and *C*: compounds treatedwith N-HCl. *B* and *D*: compounds treated with 2N-acetic acid. (1) glucose; (2) fructose-phenylalanine; (3) phenylalanine; (4) *N-p*-tolylfructosamine; (5) 5-hydroxymethylfurfuraldehyde; (6) *p*-toluidine; (7) Schiff's base of (5) with (6).

Experiment 3. In two experiments were added to 3·0 ml. 0·6% (w/v) solution of 5-hydroxymethylfurfuraldehyde 0·33 ml. conc. HCl and 0·40 ml. glacial acetic acid respectively and the mixtures heated in sealed tubes for 2 hr. at 100°. Recoveries of the compound by chromatography were 25 and 100% respectively.

Alkaline treatment of N-*substituted fructosamines*

Experiment 4. A solution containing approx. 300 mg./100 ml. (w/v) fructose-phenylalanine (in water) was tested for its reducing power before and after treatment with 0·1N-Na_2CO_3 (final concn.) at 100° for 20 min.; the same was done with a solution containing 140 mg./100 ml. (w/v) D-glucose (in water). With the former compound the reducing power decreased by 72·9%, with glucose by 77·5%.

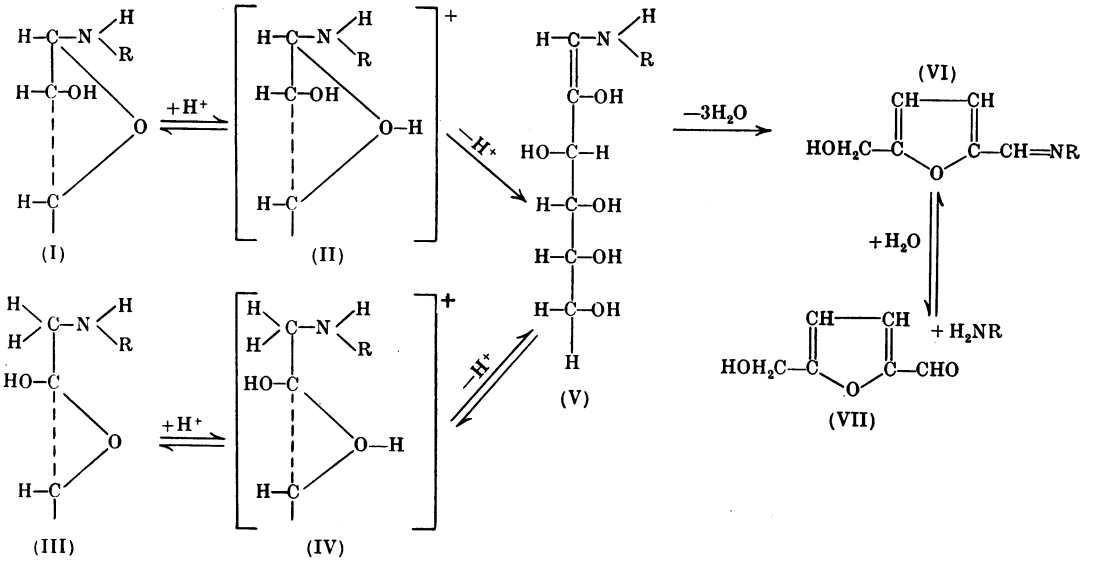
Experiment 5. Solutions containing approx. 5% (w/v) fructose-phenylalanine and 1% (w/v) D-glucose respectively in water and adjusted to pH 11·0 with alkali were heated at 100° for 10 min. and after neutralization run on paper. Only in the glucose experiment was glucose detectable; the other solution contained free phenylalanine and some residual fructosamine.

Experiment 6. A 0·1N-Na_2CO_3 solution containing 0·36% (w/v) *N-p*-tolylfructosamine was heated for 20 min. at 100° and after neutralization tested chromatographically; there was no residual fructosamine and a practically complete recovery of the liberated base.

DISCUSSION

It would appear that the compound formed in hot methanol from D-glucose and DL-phenylalanine can be identified as fructose-phenylalanine (*N*-substituted fructosamine) by its strong reducing power (*o*-dinitrobenzene in the cold), the fact that it cannot be hydrolysed by acid or alkali, by its colour reaction with *p*-DAB after pretreatment with acetylacetone and by its conversion to 5-hydroxymethylfurfuraldehyde and phenylalanine; no other structure would fit these properties. The inability of the compound to be hydrolysed by acid contrasts with the ready hydrolysis by weak acids of the *N*-glucosylamino-acids prepared by Micheel & Klemer (1951) and by Pigman, Cleveland, Couch & Cleveland (1951). The reduction in the cold of *o*-dinitrobenzene was found by Kuhn & Weygand (1937) to be a characteristic property of fructosamines distinguishing them from the isomeric *N*-substituted glucosylamines. The colour reaction

with *p*-DAB is known to be given only by sugars with an amino group attached to $C_{(1)}$ or $C_{(2)}$ (Elson & Morgan, 1933). The ready transformation to 5-hydroxymethylfurfuraldehyde of the compound by acid is incompatible with the displacement by an NHR group of the hydroxyl group at $C_{(2)}$ since this hydroxyl group is essential for the conversion of a hexose to 5-hydroxymethylfurfuraldehyde; thus D-glucosamine cannot be converted to the furfuraldehyde derivative even by strong mineral acid. In our compound, therefore, the amino group must be linked to $C_{(1)}$ and there must be a free reducing group at $C_{(2)}$ because the compound, though not hydrolysable, is strongly reducing.



Fructose-phenylalanine shows no tendency to crystallize, a property which it shares with the *N*-glucosylamino-acids and with a number of *N*-arylfructosamines which were found previously to be uncrystallizable (Micheel & Klemer, 1951; Weygand, 1940).

The outstanding feature of the *N*-substituted fructosamines (III) investigated, the crystalline *N*-*p*-tolyl-D-fructosamine and the amorphous phenylalanine analogue, is the ease with which in the presence of weak acid they rearrange and give off water to form the Schiff's base (VI) of 5-hydroxymethylfurfuraldehyde (VII) which in turn is hydrolysed to VII and the free amine. The degradation of (III) to (VII) has a pH optimum; it proceeds more quickly with 2N-acetic acid than with N-hydrochloric acid. Presumably the reaction involves a preliminary acid-base equilibrium yielding the conjugate acid (IV) followed by transformation of (IV) into an activated complex (of unknown structure) which by loss of a proton changes reversibly to the enol form (V). It is of interest that *N*-*p*-tolylglucosylamine (I), when quickly dissolved (otherwise hydrolysis prevails) and heated in 2N-acetic acid, is also largely degraded to (VII), the concomitant hydrolysis to glucose and base playing a minor role. As indicated in the diagram the conversion of the secondary *N*-glucosylamine (I) into the enol (V) must be assumed to be irreversible; whereas *N*-arylglucosylamines are convertible to the corresponding fructosamines, the reverse reaction has not been observed; the same seems to hold for the *N*-glucosylamino-acids.

The facility with which compounds of the general structure (I) and (III) produce (VII) on mild acid treatment is conditioned (*a*) by the basicity of the group NHR thus favouring the formation of the respective conjugate acids (II) and (IV) and in turn of the enol form (V), and (*b*) by the presence in the enol form of the tautomeric mobile vinylamine structure. D-Fructose, lacking the basic N atom at $C_{(1)}$, did not yield (VII) in appreciable amounts under conditions where fructose-phenylalanine formed (VII) up to 63% of theory; yet of the hexoses D-fructose is the sugar most sensitive to acid probably owing to the presence in its solution of considerable quantities of the furanose form (Gottschalk, 1943). Compound (VII) is the main product of the degradation of fructose-phenylalanine when treated with 2N-acetic acid. Its ready formation under such mild conditions reflects the marked effect of the replacement of the primary hydroxyl group at $C_{(1)}$ of a ketohexose by the basic NHR group on the reactivity of the sugar molecule. A useful tool is thus provided for the detection of the

fructosamine structure in natural products like mucoproteins and mucopolysaccharides.

The observation by Akabori (1933) that amino-acids when heated with furfuraldehyde or with (VII) undergo oxidative deamination and decarboxylation may account for the incomplete recovery of the amino-acid in an experiment in which fructose-phenylalanine was treated with acetic acid. With *p*-toluidine as the amine the loss in (VII) and in *p*-toluidine upon acetic acid treatment of the parent compounds (I) and (III) is explained by the presence among the reaction products of the Schiff's base (VI) and of considerable amounts of insoluble humin (Table 1, Fig. 1). When treated with N-HCl the *N*-substituted fructosamines yield only small quantities of (VII) due to secondary reactions of (VII) induced by the mineral acid (Exp. 3).

N-Substituted fructosamines just like glucosamine and glucosylamine form a deep colour with *p*-DAB only after pretreatment with acetylacetone; the identity of the absorption bands of the coloured compounds from glucosamine and the *N*-alkylfructosamine suggests closely related chromophoric structures. Most probably, as proposed by Elson & Morgan (1933), these chromophores are pyrrol derivatives.

SUMMARY

1. The *N*-substituted fructosamine (*iso*glucosamine) fructose-phenylalanine (*N*-(1′-carboxy-2′-phenylethyl)amino-1-deoxyfructose) has been prepared. The product, which showed no tendency to crystallize, was not completely free of impurities; the contaminants, however, were present in small amounts only.

2. The compound's characteristic reactions were also given by *N*-*p*-tolyl-D-fructosamine, a crystalline *N*-arylfructosamine. The outstanding property of *N*-substituted fructosamines tested is the ease with which upon mild acid treatment (2N-acetic acid) they form 5-hydroxymethylfurfuraldehyde, in the case of the phenylalanine compound up to 63% of the theory.

3. Since under the same conditions even the acid-labile D-fructose does not yield appreciable amounts of 5-hydroxymethylfurfuraldehyde, its production by dilute acetic acid may be used as a tracer for *N*-substituted fructosamines in natural products.

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Studies of Sebum

3. METHODS FOR THE COLLECTION AND ESTIMATION OF SMALL AMOUNTS OF SEBUM

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In the course of investigations into the nature and functions of sebum it was necessary to measure the 'sebum levels' (i.e. the amount of sebum which covers 1 sq.cm. of skin) and rates of secretion of different areas of the human body. This necessitated the collection of samples of sebum from an accurately measured area of skin and the estimation of the quantity of sebum thus obtained. The methods that have been used for the collection of sebum have been reviewed elsewhere (Wheatley, 1952); of these only one can be used for the quantitative collection of sebum from a measured area of skin. This method entails the extraction with a fat solvent of an area of skin demarcated by means of a cup or cylinder held against the skin by manual pressure, or by suction applied to an outer chamber, and has