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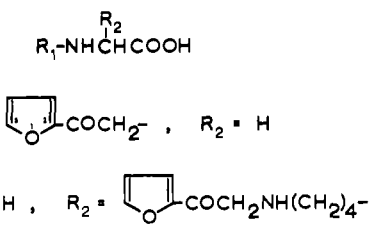
Further Chemical Studies of *N*-(2-Furacyl)glycine

Samuel H. Lipton* and C. E. Bodwell

In chemical studies related to the Maillard reaction, a complex mixture of new *N*-hydroxyalkylglycine derivatives was obtained by the hydrogenation of *N*-(2-furacyl)glycine·HCl, a furan amino acid obtained by the acid-catalyzed dehydration of fructose–glycine. These stable reduction products were detectable with ninhydrin and were resolved in an amino acid analyzer. By oxidation with periodic acid, the reduction products were deduced to include *N*-mono-, di-, and trihydroxyhexylglycines. Their formation is discussed, as well as their relation to products of the glucose–glycine browning reaction.

A furan amino acid, *N*-(2-furacyl)glycine (I), derived from fructose–glycine by an acid-catalyzed dehydration, was recently identified and synthesized (Lipton *et al.*, 1971). I is structurally related to N^{ϵ}-furoylmethyl-L-lysine (II) (called furosine) which had been previously identified (Finot *et al.*, 1968; Heyns *et al.*, 1968) as the post-arginine amino acid first observed in acid-hydrolyzed dried skim milk (Erbersdobler and Zucker, 1966; Brueggemann and Erbersdobler, 1968). These *N*-furacyl amino acids appear to be



Maillard reaction intermediates which, like the fructose–amino acids from which they are derived, precede an eventual destruction of the amino acids themselves. The *N*-furacyl amino acids may have eluded earlier recognition due to their extreme lability, except under conditions of high acidity. This report describes a complex mixture of stable amino acids produced by the hydrogenation of I and a study of the oxidation of these reduction products by periodic acid. Their formation as well as their relation to glucose–glycine browning products is also discussed.

EXPERIMENTAL

Materials and Methods. Fructose–glycine and *N*-(2-furacyl)glycine·HCl (I·HCl) were prepared as previously described (Lipton *et al.*, 1971). Platinum oxide (Adam's oxide), periodic acid (H_5IO_6), and iminodiacetic acid were commercial products. Quantitative amino acid analyses were obtained on a Phoenix Biolyzer, Model 3000. The various N-substituted glycine derivatives obtained by the reduction of I were detectable and resolved in the amino acid analyzer, with no modification of the buffers and procedure previously used (Lipton *et al.*, 1971).

Hydrogenation Studies. A Parr hydrogenation apparatus was used for hydrogenation of I at room temperature. Initially the reduction was undertaken to convert I into an alcohol in order to chemically confirm the ketone function of I (efforts to obtain semicarbazone and 2,4-dinitrophenylhydrazone derivatives of I had not been successful). I·HCl was reduced (1 mg/ml of solution) in either 95% ethanol or in aqueous solution, to which was added in either case 0.01 volumes of 1 *N* HCl. The weight of platinum oxide used in the reduction was equal to that of the I·HCl. Time of hydrogenation varied from 2 min to 18 hr; pressure was either 25 or 50 lb per in.^{2} (psi). Reduction was evidenced by pressure drop and an observed decrease in absorbance at 280 nm (final value after complete reduction was approximately 0.1 that of the initial value). The catalyst was removed by centrifuging prior to evaluating the reduction products in the amino acid analyzer.

Periodate Oxidation Studies. Oxidations were carried out by the addition of an excess of solid periodic acid (25 mg/ml) to the sample either in aqueous acid or in bicarbonate-neutralized solution at either 0 or 24°C. The concentration of the sample was about 1 mg per ml. For time studies, oxidation was stopped by the addition of glycerol to consume the excess periodate. The composition of oxidized samples was determined on the amino acid analyzer. Experiments on the oxidation of hydrogenated I, I, and of fructose–glycine were carried out.

RESULTS AND DISCUSSION

Hydrogenation Products. As seen in Figure 1, a complex mixture of amino acids, which were resolved in the amino acid analyzer, was obtained upon hydrogenation of I. After 20 min at 50 psi (Figure 1A), the hydrogenation was essentially

Protein Nutrition Laboratory, Human Nutrition Research Division, Agricultural Research Service, United States Department of Agriculture, Beltsville, Maryland 20705.

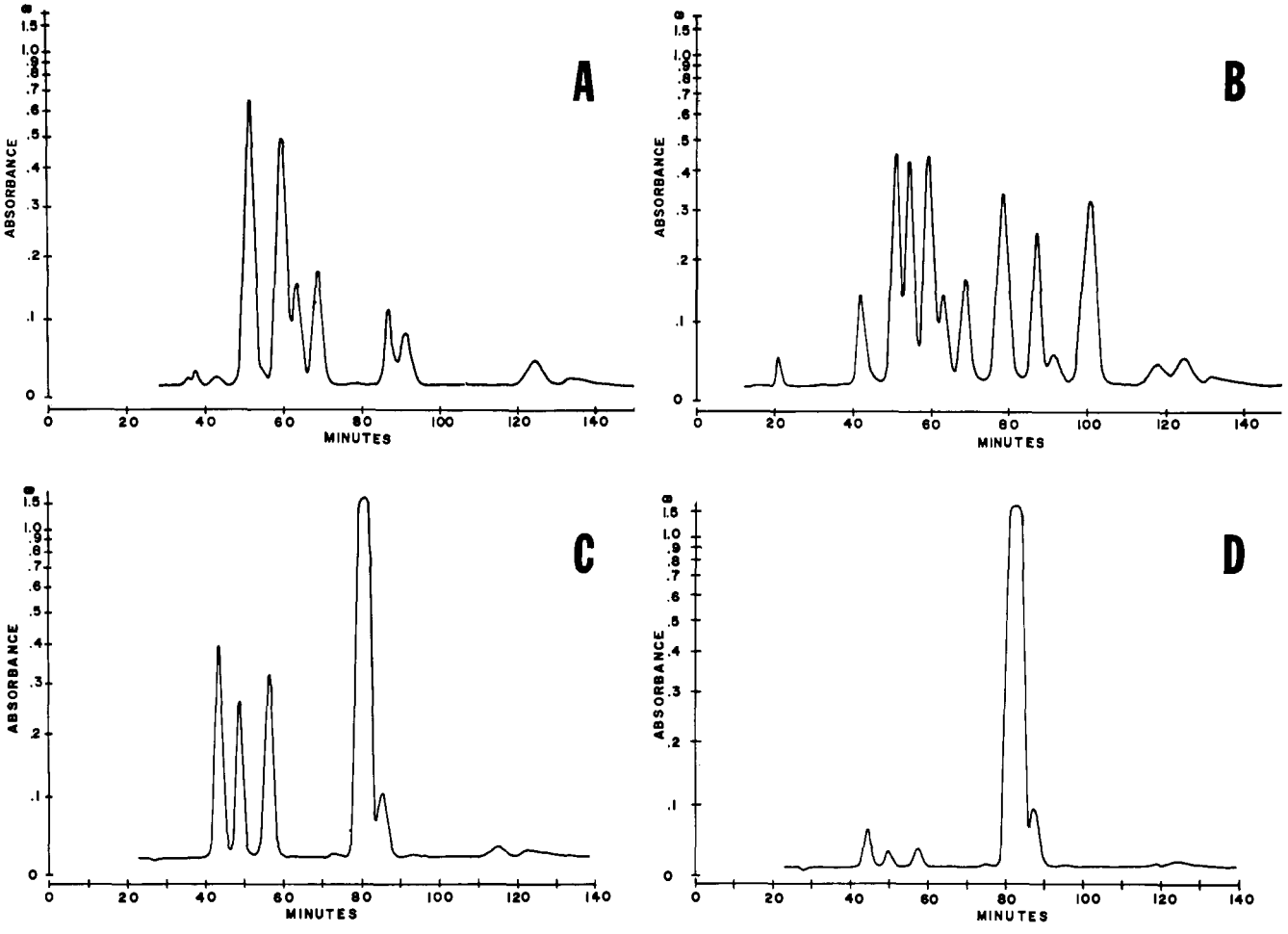
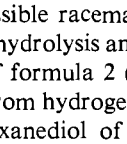


Figure 1. Chromatograms from amino acid analyzer of hydrogenation mixture from *N*-(2-furacyl)glycine (I). Curve A, 18 hr reduction of I at 50 psi; Curve B, 2 min reduction of I at 25 psi; Curve C, hydrogenated (I) mixture, following oxidation with periodate for 0.5 hr; Curve D, same as Curve C, but after 3 hr periodate oxidation

complete, since the composition was unchanged when the sample was reduced 18 hr at 50 psi. However, an incomplete hydrogenation of I, for 2 min at 25 psi (Figure 1B), afforded an even more complex mixture. In addition to unreduced I (100 min peak) other additional major peaks were observed at 42, 55, and 79 min, as well as several minor peaks. In Table I probable structures are shown of the fully hydrogenated products, which would be expected on the basis of earlier work on the hydrogenation of furans (Dunlop and Peters, 1953; Rylander, 1967). Compounds designated by formulas numbers 1–4 would result from hydrogenation accompanied by either ring hydrolysis or hydrogenolysis. The substituted hexanetriols (two possible racemates) of formula 1 would be produced by ring hydrolysis and hydrogenation. The substituted hexanediols of formula 2 (again two racemates are possible) would result from hydrogenolysis of the 1,5 bond of the furan ring. The hexanediol of formula 3 (only one racemate is possible) would result from hydrogenolysis of the 1,2 bond of the furan ring. Hydrogenolysis of both the 1,2 and 1,5 bonds would yield the substituted hexanol, designated by formula 4. Hydrogenation of I without ring cleavage would produce the compound represented by formula 5 (the peak at 117 min, last to emerge and the least abundant, is probably this product, since ring compounds tend to emerge from a polystyrene ion-exchange column later than open chain compounds). Thus, a total of seven different amino acids are described by the formulas of Table I.

Table I. Structural Formulas of Some Possible Hydrogenation Products of *N*-Furacylglycine (I)

R–CHOHCH$_2$NHCH$_2$COOH

Formula[a] no.	R group	Name
1	HOCH$_2$CH$_2$CH$_2$CHOH–	*N*-(2,3,6-trihydroxyhexyl)-glycine
2	CH$_3$CH$_2$CH$_2$CHOH–	*N*-(2,3-dihydroxyhexyl)-glycine
3	HOCH$_2$CH$_2$CH$_2$CH$_2$–	*N*-(2,6-dihydroxyhexyl)-glycine
4	CH$_3$CH$_2$CH$_2$CH$_2$–	*N*-(2-hydroxyhexyl)glycine
5		*N*-(2-hydroxy-2-tetrahydrofurylethyl)-glycine

[a] Formulas shown are of fully reduced products (hydrogen uptake either 3 or 4 mol). The additional peaks in Figure 1B represent partially hydrogenated components where either the ring or the side chain was not fully reduced. Five additional products would be obtained by merely considering those compounds in which the keto group of the side chain remained unreduced.

Periodate Oxidation Studies. This work was initiated for the purpose of obtaining structural evidence, prior to our recognition of the structure of I (Lipton *et al.*, 1971). We first observed that, upon periodate oxidation in bicarbonate solution at room temperature, both I itself and all the components of reduced I were quickly (within 1.5 min) converted into free glycine. An immediate powerful butyraldehyde

Table II. Amino Acid Composition of Hydrogenated *N*-Furacylglycine (I) Following Periodate Oxidation

Amino acid peak time, min	Molar composition at various oxidation[a] times, min			
	0, %[b]	1.5, %	30, %	180, %
44	0	27	9.5	1.0
50	31	21	5.9	0.3
58	45	32	8.7	0.4
61	5.2	0	0	0
66	8.5	0	0	0
84	6.5	14	75	96
89	4.6	3.5	2.7	2.3

[a] The cleavage was carried out at 24°C by addition of solid periodic acid (25 mg per ml) to an aqueous solution of hydrogenated I·HCl (12.5 mg per ml). At the desired time, glycerol was added (0.1 ml per ml of oxidation solution) to consume the excess periodate. The sample was then placed immediately on the amino acid analyzer. [b] The mole percentage distribution values were calculated from individual runs on the amino acid analyzer of appropriately oxidized samples, assuming all components had identical peak areas equal on a molar basis to that of glycine. The trace components early in the chromatogram and those following the 89 min peak were not used in these calculations.

odor, observed upon the addition of periodate to reduced I, but not upon oxidation of I itself, was evidence of structures 1 and 2 of Table I. Upon cleavage through oxidation of the vicinal hydroxyl groups, 4-hydroxybutyraldehyde and butyraldehyde, respectively, would be produced from structures 1 and 2.

By oxidation with periodic acid in acid solution, a slower change in the amino acid composition of reduced I was easily observable. As shown in Table II, the glycine (84 min peak) increased gradually to a near maximum value during 3 hr. An interesting feature of this study was the appearance of a transient (44 min peak) intermediate. Although totally absent from the original reduction mixture of I, this intermediate accounted for 27% of the total amino acid, after periodic acid oxidation for 1.5 min, and it subsequently gradually disappeared (see Figures 1C and 1D). The intermediate was interpreted to be the semialdehyde of iminodiacetic acid, $CHOCH_2NHCH_2COOH$, since it was converted to iminodiacetic acid (22 min peak) by moist silver oxide. It would arise from structures 1 and 2 simultaneously with the formation of the butyraldehydes. A short-time oxidation of fructose–glycine by periodate directly yielded iminodiacetic acid, observed as an identical 22 min peak. A further oxidation of iminodiacetic acid by periodate to yield glycine and formaldehyde has been reported (Anet, 1959).

The decline in the peaks at 50, 58, 61, and 66 min with increased time of oxidation (Figures 1A, 1C, and 1D) is consistent with structures 1 and 2 of Table I. As pointed out above, each of these formulas may represent two amino acid peaks. For each structure, the racemate with cis vicinal hydroxyl groups would be expected to undergo a more rapid oxidation than that with the trans configuration. The abundant peaks at 50 and 58 min (Table II), which account for 75% of the amino acid products, represent components which probably have vicinal hydroxyl groups. In order to distinguish between these structures, it would be necessary to obtain each component in pure form. However, it is likely that the trihydroxy compound (formula 1) would precede the dihydroxy compound (formula 2) in the elution sequence. As seen in Table I and Figure 1D, the most resistant component to periodate oxidation was the 89 min peak. This peak and the 117 min peak may correspond to formulas 4 and 5, respectively. Regarding products produced by hydrogenolysis (formulas 2, 3, and 4) under acidic conditions with platinum catalysts, these would be expected to predominate among reduction products of furans (Rylander, 1967). Our study of the reduction of I thus offered a unique opportunity to assess the extent of the various ring cleavages which occur during the reduction of furans, since the products were resolvable in the amino acid analyzer.

At least 24 different products have been observed chromatographically in a glucose–glycine reaction mixture (Hodge, 1953). The structure of most of these is not known. The various hydroxyhexyl glycine derivatives produced in the hydrogenation of I and similar derivatives of other amino acids may be among the browning reaction products of foods. These may arise by an intermolecular oxidation–reduction of the *N*-furacyl–amino acids. The very strong reducing properties of I which we have observed may be explained on the basis of the diendiol structure obtained upon a hydrolytic opening of the furan ring (Hodge, 1953). Milder conditions than those described in this paper were effective for the reduction of I (after only 30 sec at 10 psi of hydrogen in the presence of PtO_2, the mixture of reduction products was already evident). Although we have not specifically identified these hexyl glycine derivatives in browned foods, our study may contribute to an understanding of the complexity of ninhydrin-reactive products which are known to be produced.

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