STABILITY OF THE PRODUCTS OF THE CONDENSATION OF L-CYSTEINE WITH CARBOHYDRATES

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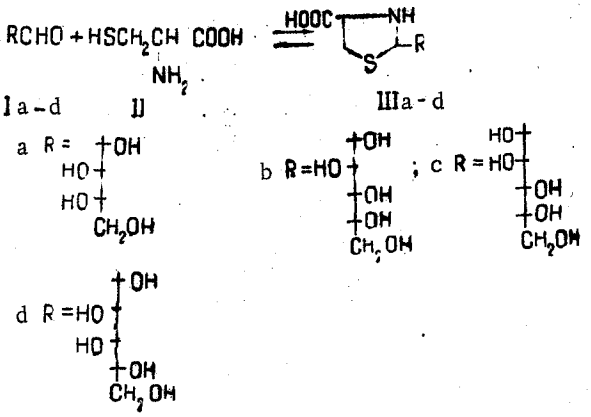
In a study of model reactions responsible for the origin of the "meaty" odors of food products [1], we have made a detailed investigation of the possibility of producing these odors by the interaction of carbohydrates with L-cysteine.

On the basis of published information [1–7] we consider that the products of the condensation of hydrocarbons (I) with L-cysteine (II) are 2-(polyhydroxyalkyl)thiazolidine-4-carboxylic acids (III). This name will be used in the present paper.



Compounds (IIIa–d) were obtained, using published methods [2, 5], by the reaction of L-cysteine (II) with the carbohydrates L-arabinose (Ia), D-glucose (Ib), D-mannose (Ic), and D-galactose (Id). These compounds are readily soluble in water and their aqueous solutions possess an acid reaction. They are dibasic acids whose ionization constants have been measured [5]. The results of the measurements that we have carried out for compound (IIIa) have confirmed these figures.

Solutions of compounds (IIIa–d) in distilled water with a concentration of 1% have pH ≈ 3–4. When solutions of this concentration, some of which are stable at ≈ 20° C or above, are rapidly heated they decompose with the liberation of the initial monosaccharide. Oxidation of the L-cysteine formed to L-cystine and its slight decomposition take place simultaneously. This decomposition leads to the formation of volatile substances taking part in the composition of the final imitator. It was interesting to ascertain whether the thiazolidinecarboxylic acids (IIIa–d) decompose readily, since, in our opinion, ease of the decomposition depends on the nature and intensity of the odor formed.

The decomposition of the thiazolidines takes place at a rate convenient for measurement at 50° C; at 70° C this rate is already difficult to measure. The carbohydrates (Ia–d) isolated as a result of the decomposition can be determined quantitatively. In this way it is possible to follow the course of the process. During the reaction, in addition to the liberation of carbohydrates, the concentration of hydrogen ions in the solution increases.

In measuring the kinetics of the liberation of the monose, we studied the comparative decomposition of compounds (IIIa–d) under deliberately nonstandard conditions, attempting only to approximate to the actual conditions for the preparation of the imitators.

Table 1 gives the melting points of compounds (IIIa–d), their R_f values in system 1 (see Experimental), the initial pH values of their 1% solutions, and also the half-period of the reactions ($\tau_{1/2}$) for the four thiazolidines investigated. To evaluate the influence of the carbohydrate residue on the ease of the reactions were determined at constant pH values in buffer solutions: acetate (pH 3.16) and phosphate (pH 4.99) (see Table 1). In an alkaline buffer solution (pH 9.85) the decomposition takes place extremely slowly and cannot be measured even for the extremely labile compound (IIIb) when it is heated at 50° for 8 hr 25 min.

As can be seen from Table 1, there is a marked difference in the stability of the derivatives of the hexoses (IIIb–d) and that of L-arabinose (IIIa). Moreover, in nonbuffered solutions the thiazolidines (IIIa–d) can be arranged in the following sequence of rates of decomposition: IIIa ≪ IIId < IIIc < IIIb. This sequence undergoes marked levelling out in buffer solutions, but the difference in the stability of the derivatives of the hexoses and of L-arabinose remains more clearly expressed.

Thus, a polyhydroxyalkyl side chain in position 2 of thiazolidine-4-carboxylic acids markedly increases their stability in an alkaline medium, since 2-alkyl- and 2,2-dialkylthiazolidine-4-carboxylic acids readily decompose under similar conditions [10]. The reason for the difference in the action of the substituents at C_2 on the stability of compounds (III) must evidently be sought in the +I effect of the alkyl groups and the −I effect of the polyhydroxyalkyl chain [11]. The −I effect is confirmed by the fall in the pK_2 value of compounds (III) to 5.2–5.7 as compared with the unchanged thiazolidine-4-carboxylic acid (pK_2 6.30) [5].

Table 1

Compound	mp, °C (decomp.)	R_f in system 1*	$\tau_{1/2}$ of the decomposition of (III), hr (pH)			Content of aldehyde form (Ia–d) in the aqueous solution [12], %
(IIIa)	151–153	0.72	19±3.0 (3.99)	28.9 (3.16)	29.1 (4.99)	0.400
(IIIb)	154–155	0.70	2.5±0.7 (3.13)	9.2 (3.16)	6.0 (4.99)	0.024
(IIIc)	150–151	0.72	5.1±0.5 (3.37)	9.9 (3.16)	6.0 (4.99)	0.068
(IIId)	135–136	0.71	7.9±0.9 (3.17)	11.5 (3.16)	6.6 (4.99)	0.082

* R_f of D-glucose 0.80.

A comparison of literature data [5, 7, 12, 13] and our results on the condensation of the carbohydrates (I) with L-cysteine (II) has enabled us to conclude that in acidic aqueous solutions there is an equilibrium I + II ⇄ III which determines the concentration of the aldehyde-form of the monose in the solution. This equilibrium is disturbed by the decomposition of the sulfur-containing compounds with the formation of odorous volatile substances, including hydrogen sulfide. With a rise in the temperature of the solution decomposition is markedly accelerated, in consequence of which even in boiling aqueous solution no appreciable amounts of compounds (III) can be detected. The experimental data do not enable us to determine unambiguously whether, in these circumstances, the thiazolidinecarboxylic acids (IIIa–d) or the L-cysteine (II) decompose by the Maillard reaction; the simultaneous decomposition of substances (II) and (III) is not excluded, either. An important result of this decomposition is the gradual increase of the constant of carbohydrates (I) in the solutions and the corresponding decrease in the concentration of compounds (IIIa–d). Consequently, by determining the concentration of monose we can find the half-period of the decomposition of compounds (IIIa–d).

In addition to establishing the half-period of the decomposition of compounds (IIIa–d) by the method mentioned, we have tested the possibility of using the polarographic method for this purpose. German workers have used polarography to determine the rate of the direct reaction I + II → III from the disappearance of the anode wave of cysteine ($E_{1/2}$ = = 0.45 V) [5]. Measurements of the stability of the thiazolidine-4-carboxylic acid (IIIa) that we have carried out on a precision polarograph have shown that rapid decomposition of the substances takes place not only in the ammonia buffer solution (pH 9.3) recommended by Weitzel et al. [5] but also in a strongly alkaline medium (pH 13–14). In the latter case, the complete decomposition of (IIIa) takes less than 30 min, as can be judged from the appearance of the anode wave of cysteine. It remains completely unclear how the authors mentioned were able to detect the "disappearance" of this wave. In order to find whether the anode wave of cysteine did not coincide with the wave of the thiazolidine-4-carboxylic acids themselves, we polarographed specially synthesized model compounds: unsubstituted thiazolidine-4-carboxylic acid (IV), the hydrochloride of its methyl ester (V), the N-acetyl derivative of IV (VI), and 2,2-dimethylthiazolidine-4-carboxylic acid (VII) [6, 10].

Compound (VII) proved to be extremely labile: Over the whole range of pH values (from 1 to 13), the anode wave of cysteine appeared instantaneously in the polarograms of its solutions, but this was not the case for the polarograms of solutions of compounds (IV) and (V) at pH 1. It appeared after about 30 min at pH 13–14. Finally, solutions of the N-acetyl derivative (VI) did not give this wave at any pH, which agrees well with the chemical stability of this substance (VI).

Thus, the polarographic method is very convenient for the rapid evaluation of the stability of various thiazolidine-4-carboxylic acids obtained by the condensation of L-cysteine with carbonyl compounds.

Experimental

All the thiazolidine-4-carboxylic acids and their derivatives (IIIa–d), (IV)–(VII) were obtained by published methods [2, 5, 6, 10] and were recrystallized to chromatographic purity. Chromatography was carried out with type M paper of the Leningrad No. 2 Mill in the solvent systems: 1) acetone–water (4:1), 2) butan-1-ol–acetone–water (2:7:1). All the chromatograms were made by the descending method.

The elemental analysis of substance (IIIa) and the value of its pK_1 and pK_2 determined by potentiometric titration agreed with literature data [5].

In each experiment, a solution of 0.1 g of one of compounds (IIIa–d) in 10 ml of water or a buffer was kept at 50 ± 0.5° C. Samples of the solutions under investigation were taken after different intervals of time for the different

Table 2

Relative Content of D-Glucose (C/C_∞, $C_\infty = 1$) in the Reaction Mixture

Time, min	Experiment No.							
	1	2	3	4	5	6	7	8
5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
35	0.09	0.11	0.14	0.15	0.0	0.0	0.0	0.0
75	0.20	0.30	0.32	0.34	0.15	0.0	0.0	0.13
95	0.27	0.38	0.41	0.43	0.22	0.0	0.08	0.19
125	0.38	0.52	0.55	0.57	0.33	0.10	0.20	0.28
185	0.73	0.77	0.83	0.85	0.56	0.31	0.43	0.47

thiazolidinecarboxylic acids: 0.1 ml each for solutions of (IIIa), and 0.05 ml each for solutions of (IIIb–d). The samples of the solutions were deposited on paper for chromatography, dried, and chromatographed in system 2. A marked separation of the initial compound (R_f 0) and the monose ($R_f \approx 0.2$) took place. Then the hexoses (Ib–d) were shown up by acid aniline phthalate [phenylammonium phthalate] and were determined colorimetrically by Baar's method [8] on a FEK-N-57 instrument with a No. 3 filter. L-Arabinose (Ia) was shown up by a mixture of 3,5-dinitrosalicylic acid and phenol [9]; the spots were cut out and eluted with 3 ml of water (≈20° C, 24 hr) and determined colorimetrically on the same instrument with a No. 4 filter.

All the measurements were carried out by similar methods. As an example, we give Table 2 containing kinetic data characterizing the decomposition of compound (IIIb) in aqueous solution at an initial pH of 3.13.

In each experiment the time was determined during which 50% of glucose (expressed as a fraction of its theoretically calculated amount) appeared in the solution. This time was also the half-period of the decomposition of compound (IIIb) (for further details, see above). Then from all the figures obtained we found the arithmetic mean half-period of the decomposition ($\tau_{1/2}$) (see Table 1).

The polarograms were taken on a LP-60 automatic electronic polarograph (Czech SSR). The cathode was a dropping mercury electrode with forced detachment of the drop and the anode was a calomel electrode. The measurements were carried out at 25° C in an atmosphere of nitrogen.

Summary

1. 2-(Polyhydroxyalkyl)thiazolidine-4-carboxylic acids (IIIa–d) decompose in aqueous solutions, their stability decreasing in the sequence IIIa ≫ IIId > IIIc > IIIb.

2. An equilibrium between the forward and back reactions evidently exists in aqueous solutions, being determined by the concentration of the aldehyde-form of the monose in the solution.

3. The stability of thiazolidine-4-carboxylic acids can easily be determined by the polarographic method.

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