Solubility, Stability, and Dissociation Constants of (2*RS*,4*R*)-2-Substituted Thiazolidine-4-carboxylic Acids in Aqueous Solutions

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(2*RS*)-2-Alkyl, 2,2-dialkyl, and 2-aryl derivatives of (4*R*)-thiazolidine-4-carboxylic acid were studied from the viewpoint of their solubility, stability, and dissociation constants in aqueous solutions using the potentiometric titration and HPLC method. Solubilities vary with the size of a molecule, position, and number of polar groups rather regularly. The stability decreases in the order: 2-carboxyl derivative, the acid (both stable), 2-alkyl, 2-aryl, and 2,2-dialkyl derivatives. It is likely that the more bulky and/or the electron-donating is a substituent, the deeper and faster is the decomposition. Their alkaline salts are more stable (intact up to 3 h). The dissociation constant of the imino nitrogen protolytic equilibrium is significantly influenced by the character of aryl substituents, while only a little by the alkyl ones.

In the paper [1] there is described the interaction of (4*R*)-thiazolidine-4-carboxylic acid (*I*) and its three (2*RS*)-2-alkyl derivatives with a series of the trivalent metal ions, only which gave the sufficiently reliable and reproducible results; those for *I* and divalent cations are published elsewhere (see relevant Refs. in [1]). The study of other compounds substituted with various aryl radicals due to their low (metal ion complexes) solubilities, and mainly because of their instability in aqueous solutions was practically impossible. Decomposition of thiazolidines as a main or subsidiary aim has been studied by several authors [2—8] at various conditions using several methods. *Reimschneider* and *Hoyer* [2] have studied a number of 2-alkyl-, 2-aryl-, and 2,2-dialkyl-substituted thiazolidines by the polarimetry in order to determine the hydrolytic equilibrium and rate constants (the latter only for 2,2-dialkyl derivatives) and some other dependences on pH (3 levels), two temperatures, and number of carbon atoms in the alkyl chains. Mainly their results helped us to plan our study, since a palette of 2-substituted thiazolidines were synthesized at our workplace primarily for the study of the new potential radio pharmaceuticals labelled with ^{99m}Tc [9, 10] and that described in [1], at which we frequently have encountered the behaviour that has called for its deeper investigation.

This paper reports on the determination of the solubility, stability, and dissociation constants for the imino nitrogen protolytic equilibrium in dependence on the nature of substituents attached to the C-2 atom in *I* by the potentiometry and HPLC. The aim was to confirm the known and add some new and useful information about behaviour of this class of heterocyclic compounds in aqueous solutions. The attractivity and biological effectivity of thiazolidines in biochemistry and pharmacology [3, 5, 11—15] is evident.

EXPERIMENTAL

The derivatives of *I* were synthesized by the method described in [1, 2], *I* was a commercial product (Avocado Research Chemicals, Ltd., U.S.A.). Their identity was, in majority, confirmed by comparison of the measured and known melting points. These and other characterizing data gained at our workplace have been [9] or are being published elsewhere [1, 10, 16] with the exception of 2-ethyl-2-methyl derivative *IV* (m.p. = 130—132 °C; Ref. [2] gives m.p. = 132—134 °C).

The procedure for the determination of solubilities was based on the preparation of saturated solutions at ambient temperature (θ = (21 ± 0.5) °C) by dissolving a thiazolidine derivative in 15 cm^3, 25 cm^3 or 250 cm^3 of redistilled water (assessed according to preliminary tests) until a sufficient amount of undissolved material was observed. After 10 min mixing the solid residue was filtered off with a sintered glass funnel (or centrifuged), and then the portions of the filtrate (supernatant) were titrated with the sodium hydroxide solution (c(NaOH) = 0.0848 mol dm^{-3}, containing 0.4 mass % of carbonates) immediately, and at se-

lected time intervals. All measurements were carried out with an automated assembly for the potentiometric titrations; $\theta = 25\,°C$, ionic strength $I = 0.1$ mol dm^{-3} (NaCl). The other details are described in [1].

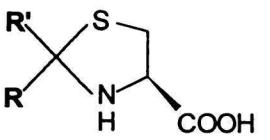
The solubilities were calculated from the dissolved mass amounts (mmol) found from the titrant consumption at the first (2-alkyl derivatives) or second inflection (end) points (2-aryl and 2,2-dialkyl ones) upon the titration curves, and the initial volumes of water. In some uncertain cases these were determined gravimetrically.

The stability was judged from the shifts of the first end points with aging of saturated stock solutions and from several HPLC experiments (at $c = 20$ μg cm^{-3}, *i.e.* $c < 0.1$ mmol dm^{-3}, $\theta = 25\,°C$, UV detection at $\lambda = 235$ nm). The HPLC equipment, other working conditions (mobile and stationary phase, columns, pressures), manipulation with the samples, and manner of evaluation was published in this journal [16].

The selected titration curves were treated by the PKAS program [17] to evaluate the dissociation constants. Reliability of the system and the program was firstly tested by the determination of the pK_a of cysteine and proline.

RESULTS AND DISCUSSION

The compounds studied have a general structure



where R = R′ = H (*I*), R = CH_3 and R′ is methyl and ethyl radical in *III* and *IV*, respectively, in all other cases R = H and R′ is alkyl or aryl substituent (Table 1).

Solubility

This was for each compound determined twice, averaged, and rounded to the two most significant digits (Table 1). In general, the solubilities quite regularly reflect the chemicophysical properties of thiazolidines studied (size of the whole molecule, type, number and displacement of all polar groups). Higher solubility of *III* and *IV* than *I* is probably connected with their extreme lability [8]. On the other hand, surprising is the low solubility of *XIII* and *XX*. Their 2-OH and mainly 4-COOH groups on the benzene ring should enhance the solubility when compared with that of *X*. The solubilities of these two acids and those of *XVII* and *XXI*, due to not sufficient evaluation from the titration data, were also determined gravimetrically. The results obtained by both methods agreed rather well (SD of titrimetry was < 0.04, the gravime-

Table 1. Solubility and Dissociation Constants of 2-Substituted Thiazolidine-4-carboxylic Acids

Compound	Substituent	M_r	Solubility	pK_a^{NH}
			mmol dm^{-3}	
I	H	133.17	220[a]	6.19(3)
II	Methyl	147.19	210[b]	6.17(8)
III	Dimethyl(gem)	161.22	(300)[d]	(5.86)
IV	Ethyl-methyl	175.25	(260)[d]	(5.73)
V	Propyl	175.25	85[b]*	6.12(6)
VI	Carboxyl	177.18	43[a]	5.86(8)
VII	Butyl	189.27	57[b]*	6.08(7)
VIII	Isobutyl	189.27	49[b]	6.10(2)
IX	Hexyl	217.33	2.8[b]	5.94(8)
X	Phenyl	209.27	4.5[c]	5.31(9)
XI	Thienyl	215.28	4.9[c]	4.91(0)
XII	Tolyl	223.30	1.8[c]	5.50(0)
XIII	2-Hydroxyphenyl	225.27	2.1[c,e]	5.67(2)
XIV	4-Hydroxyphenyl	225.27	7.0[c]	5.51(4)
XV	Styryl	235.30	3.7[b]	5.35(9)
XVI	4-Methoxyphenyl	239.29	0.4[c]	5.80(0)
XVII	2-Chlorophenyl	243.71	2.1[c,e]	4.95(5)
XVIII	4-Chlorophenyl	243.71	5.9[c]	5.24(4)
XIX	4-Dimethylaminophenyl	252.33	2.7[c]	5.83(3)
XX	4-Carboxyphenyl	253.27	0.6[c,e]	5.01(0)
XXI	3-Nitrophenyl	254.26	5.3[c,e]	4.70(0)
XXII	2-Hydroxy-3-methoxyphenyl	256.30	6.0[c]	5.39(1)
XXIII	5-Bromo-2-hydroxyphenyl	304.16	1.2[c]	5.53(7)
XXIV	1,4-Phenylenebis(thiazolidine-4-carboxylic acid)	340.41	1.8[c]	5.17(0)

a) Stable, *b*) partial decomposition, *c*) fast decomposition, *d*) very fast and extent decomposition, *intact for 2 h, *e*) results from gravimetric determination, ( ) uncertain values.

try with SD < 0.02 gave higher results from 7 to 11 %, Table 1). *II*, *III*, and *XV* at the preparation of their saturated solutions formed the turbid ones which resisted the filtering, so they were centrifuged (5 min at 4000 min^{-1}) in order to get a clear supernatant (its subsequent titrations gave the results only at SD ≅ 0.07).

An attempt to support (speed up) the process or a degree of saturation by ultrasound has failed. Raising the temperature increases the solubility, but remarkably affects the stability [2, 6]. To increase the stability and concentration too, it is advisable to prepare directly the solutions of monosodium (potassium) salts, unless they do not interfere with a planned type of study.

Stability

A base for the stability investigation was recording the shifts of end points belonging to the declared compounds with aging of corresponding saturated solutions. As the zero time the moment of removal of the undissolved residue was taken. The first end points were attained up to 12 min. The scan of entire titration curves lasted from 9 to 15 min. The fraction of an original (declared) compound was calculated as the ratio of the titrant consumption of the first and second end point (the latter represents the overall consumption for a compound and cysteine released). Due to the extreme low solubility of *XVI* and *XX* it was impossible to read out correctly the end point shifts and hence the evaluation of their decomposition.

The behaviour of thiazolidines in aqueous solutions within 24 h may be divided into the four groups: (A) stable, *i.e.* *VI* and *I*, (B) partial decomposition of 2-alkyl derivatives and *XV*, (C) deep decomposition of all 2-aryl and 2,2-dialkyl derivatives, and (D) a "partial renovation" of *XVII* and *XXI*. Thus the *VI* and *I* solutions exhibited no measurable changes during four weeks and two weeks, respectively. Decreased stability was observed for the 2-alkyl derivatives. Among them *II* seems to be the most unstable (Figs. 1 and 6), the same should be valid for the 2-ethyl derivative [2]. Derivatives *V* and *VII* did not exert measurable changes till the second hour after dissolution, but with further aging (up to 24 h) the shapes of their titration curves were changing similarly as plotted in Fig. 1 (*VIII* behaved as *II*). Entirely, all the 2-alkyl derivatives and *XV* after 6 h were partly hydrolyzed, their solutions contained about 90 % of starting substances (Fig. 6). This state remains seemingly constant for a few days. After longer time (up to 18 d) their titration curves have been gradually changing until a final equilibrium of deep decomposition was reached. The rate of the decomposition has been found to be independent of concentration for an arbitrary *I* derivative [2], our experiments have confirmed it.

2,2-Dialkylthiazolidines decomposed with the highest rates (Figs. 2 and 6). Although we did not calculate the corresponding rate constants as in [2], the course of their decomposition curves unambiguously confirmed it. The time necessary for deep destruction of *III* was several times shorter than that of *IV* (0.3 % was still found after 24 h). Initial pH of aqueous solutions of these compounds was 4.1—4.2. Based on the corresponding rate constants/min^{-1} of *III*

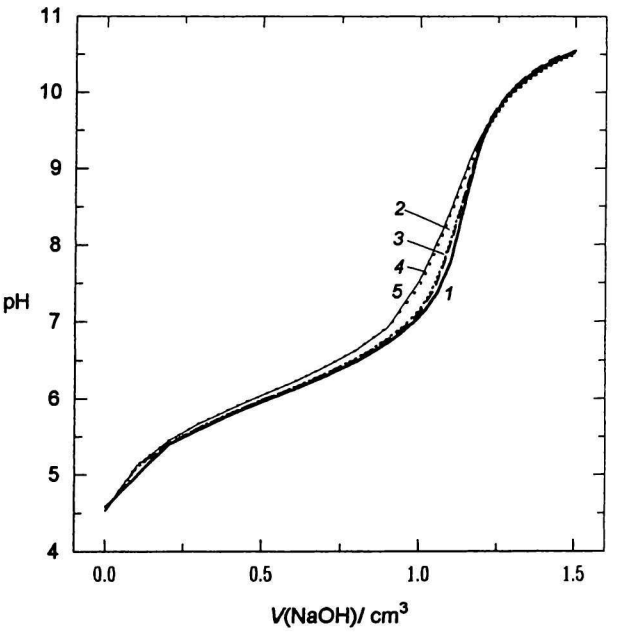


Fig. 1. Titration curves of *II* in dependence on time. *1.* 0.2 h, *2.* 0.5 h, *3.* 1.0 h, *4.* 3.0 h, *5.* 5.0 h, 8.0 h, and 24 h.

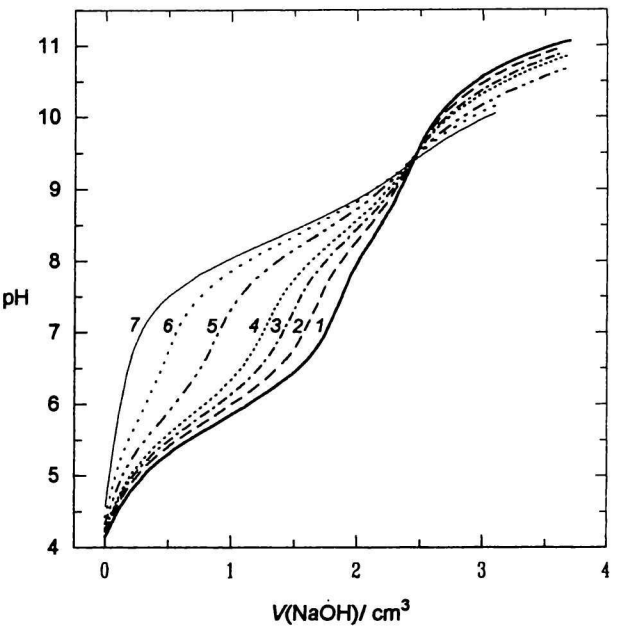


Fig. 2. Titration curves of *IV* in dependence on time. *1.* 0.2 h, *2.* 0.75 h, *3.* 1.25 h, *4.* 1.75 h, *5.* 3.0 h, *6.* 5.0 h, *7.* 24 h.

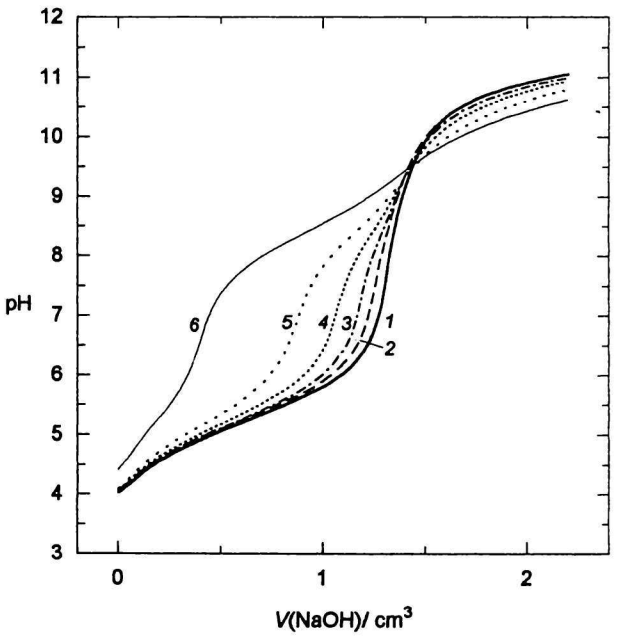


Fig. 3. Titration curves of *X* in dependence on time. *1.* 0.2 h, *2.* 0.75 h, *3.* 1.5 h, *4.* 3.0 h, *5.* 6.0 h, *6.* 24 h.

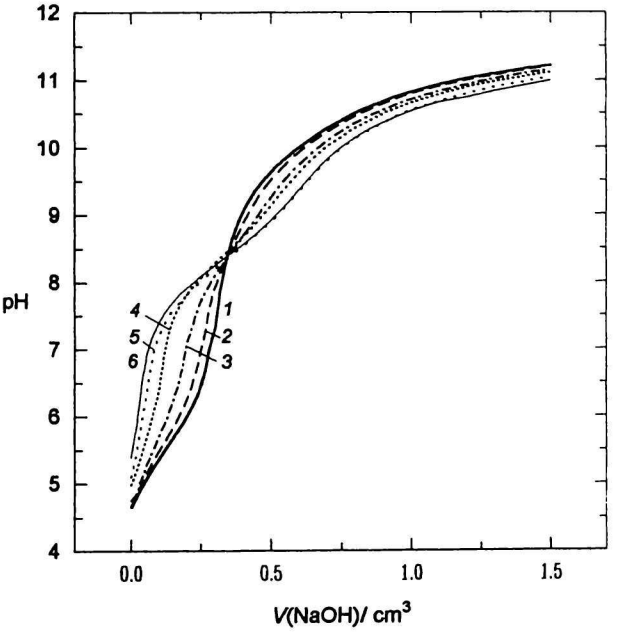


Fig. 4. Titration curves of *XIII* in dependence on time. *1.* 0.2 h, *2.* 0.75 h, *3.* 1.5 h, *4.* 3.0 h, *5.* 6.0 h, *6.* 24 h.

(0.0106), *IV* (0.0055), 2,2-diethyl derivative (0.0037), 2,2-pentamethylene one (0.0023) [2], and our observations, we have adopted an assumption that the greater is the (overall) bulkiness of the first fragment(s) of alkyl radical(s) directly bound to the C-2 atom, the sooner (more rapidly) is a thiazolidine derivative decomposed. Moreover, we suppose that the radical bulkiness contributes to the decomposition of 2-aryl-substituted thiazolidines in the same direction. A decrease of the initial concentration of *III* and *IV* from that of saturated solutions has significantly reduced the time of their deep hydrolysis; for all the other thiazolidines it was much less significant.

The decomposition curves of 2-aryl derivatives without an OH group, *i.e. X, XI, XII, XVIII, XIX,* and *XXIV* are illustrated in Fig. 7. (The set of titration curves of *X* is shown in Fig. 3.) The courses of the decomposition curves are rather similar with the exception of those of *XIX* and *XXIV*. The former had to be strongly decomposed already at the preparation of its saturated solution, the course of decay of the latter is probably somehow connected with mutual influencing of its two thiazolidine parts.

Derivatives *XIII* (its titration curves are in Fig. 4), *XIV, XXII,* and *XXIII* decompose visibly faster than the preceding compounds (Fig. 8). These derivatives possess the phenolic OH group, regarded as an electron-donating (activating) one. The 2-OH-substituted *XIII* and *XXII* decompose faster than *XIV* (4-OH) probably due to the steric effect (increased bulkiness). If an additional substituent was in an opposite position to 2-OH (Br), such a derivative (*XXIII*) decayed almost linearly; the degree of its

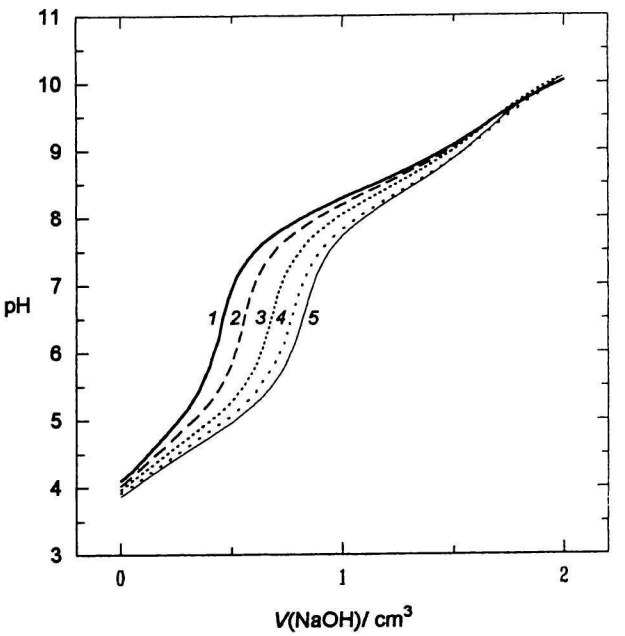


Fig. 5. Titration curves of *XVII* in dependence on time. *1.* 0.2 h, *2.* 0.75 h, *3.* 3.0 h, *4.* 6.0 h, *5.* 24 h.

hydrolysis was, however, high.

Unusual is the behaviour of *XVII* (its titration curves are in Fig. 5) and *XXI* (Fig. 7). Cl atom and especially NO_2 group are known to be as strongly electron-withdrawing (deactivating). But when the halogen (Cl) is in the 4-position, corresponding derivative *XVIII* hydrolyzed "normally" The maximum fraction of *XVII* and *XXI* persisted during the sec-

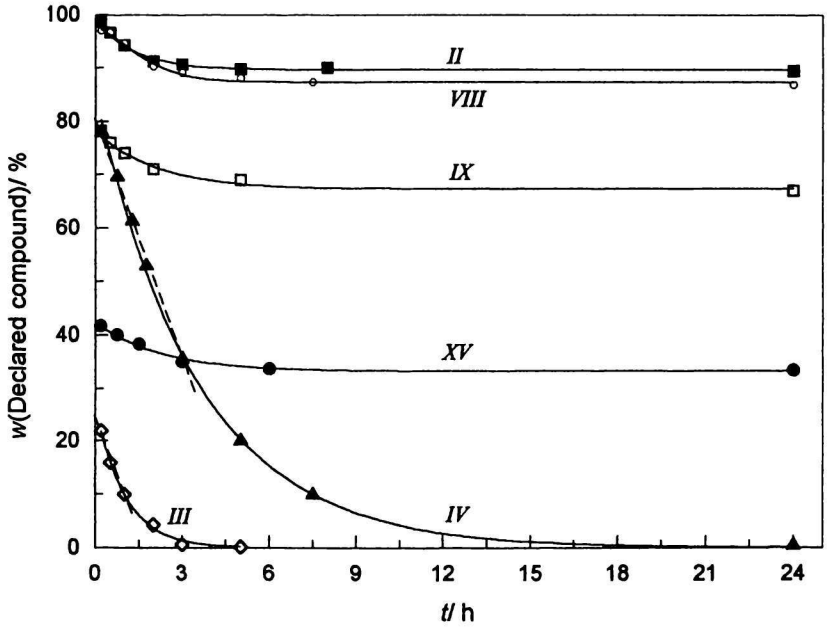


Fig. 6. Curves of the decomposition of 2-alkyl- (*II, VIII, IX*), 2,2-dialkyl- (*III, IV*), and 2-styryl-substituted (*XV*) thiazolidines.

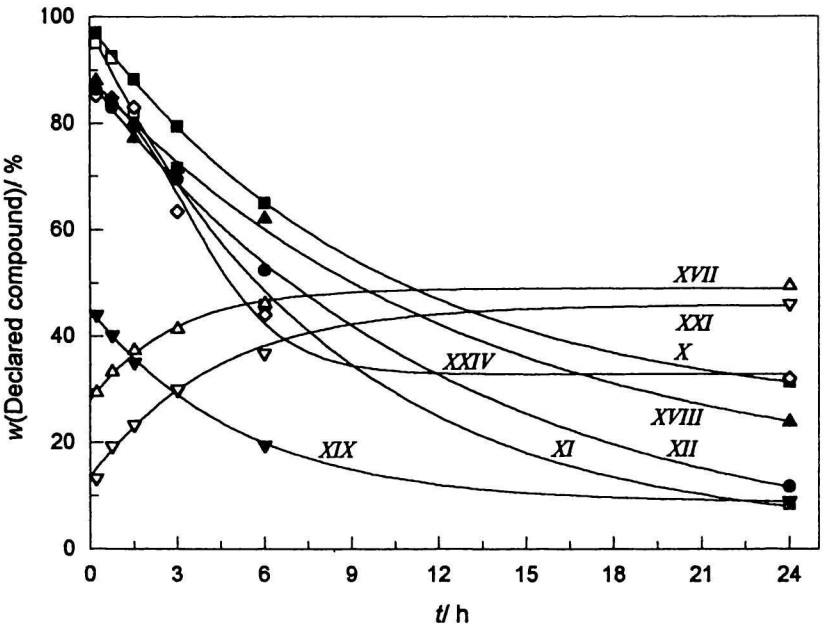


Fig. 7. Curves of the decomposition of 2-aryl-substituted thiazolidines without Ar—OH.

ond day, and then their relative content was decreasing again. Since the search for something analogical was unsuccessful, at this moment we are unable to discuss that behaviour. The decay profiles of *X*, *XIII*, *XIV*, *XVIII*, and *XXI* obtained from the titration (Figs. 7 and 8) and HPLC experiments (Fig. 9, the bottom set of curves) are mutually rather inconsistent. The biggest difference exerted *XXI*, for which the latter method indicated no "renovation", but a normal course of decomposition while all the "HPLC" curves began at approx. 100 %. The relative content of the *I* derivatives after 24 h has not fallen below 40 % (*XIV*), and the curve of *XXIII* was not linear, but sigmoidal one ending at 65 %. A common feature might be seen in that the major portion of thiazolidines had been decomposed in about 6 h. It is believed that most of the observed differences are due to the species studied. While the titrimetric results were directly the decrements in % of an original thiazolidine with time, the HPLC ones before recalculation were the increments of released aromatic aldehydes (determined by the cal-

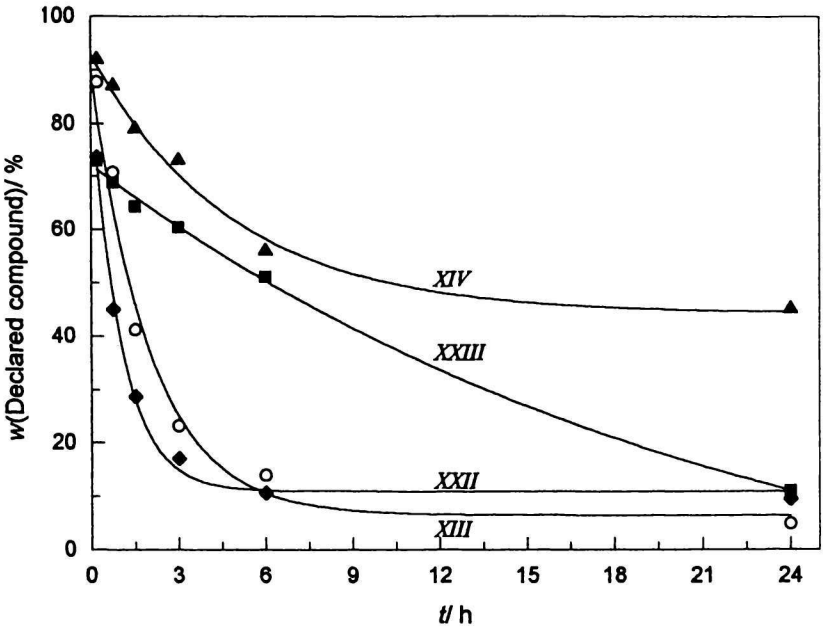


Fig. 8. Curves of the decomposition of 2-aryl-substituted thiazolidines with Ar—OH.

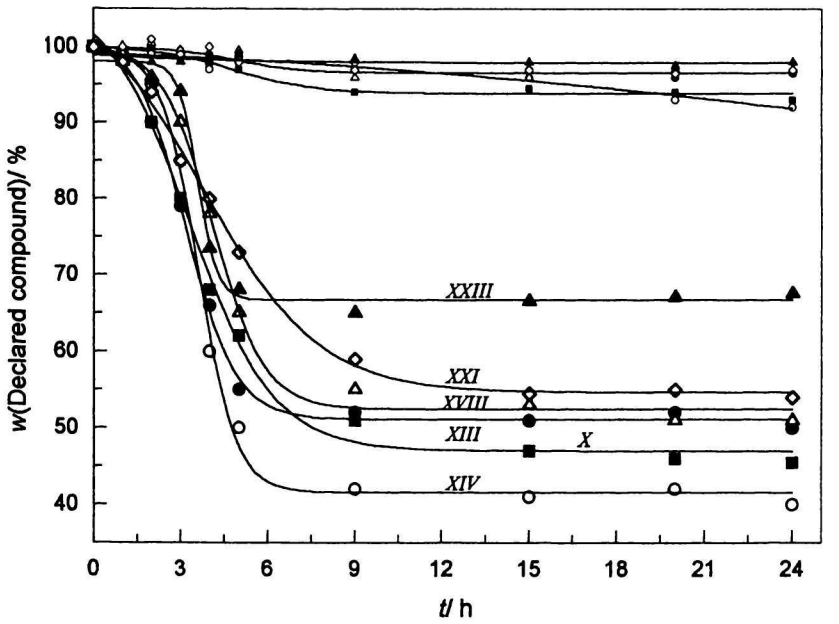


Fig. 9. Curves of the decomposition of selected 2-aryl-substituted thiazolidines obtained from the HPLC experiments.

ibration curve method). Subtraction of the fractions gained from the HPLC and those from titrimetry was always higher than zero, which confirms that the thiazolidine hydrolysis proceeds through certain intermediate products [3—5, 7, 20] undetectable by either method. Further it means that the rates of decomposition of intermediates into cysteine and a carbonyl compound are comparable with those of the starting substances.

The HPLC method (a typical set of recorded curves therefrom is shown in Fig. 10), however, enabled to find an important fact that the monosodium salts of given thiazolidines, existing in solution at pH of 7.4—7.8, are much more stable and remain intact for approx. 3 h (Fig. 9, the upper set of curves). It should be true for an arbitrary 2-aryl derivative; it was impossible to test 2-alkyl or 2,2-dialkyl ones by HPLC (unavailability of suitable type of detector). It is noteworthy to mention here that at this value of pH the 2,2-dialkylated thiazolidines exhibited the highest hydrolytic rates [2].

In general, the stabilities of free thiazolidines dis-

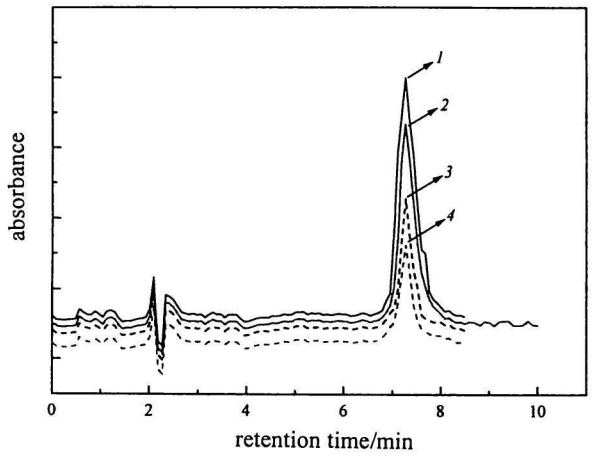


Fig. 10. The HPLC curves of the released 5-bromo-2-hydroxyphenylcarbaldehyde from *XXIII* in dependence on time. *1.* 24 h, *2.* 4 h, *3.* 2 h, *4.* 1 h.

solved in aqueous media are: *VI* > *I* > 2-alkyl derivatives ending with *II* > 2-aryl derivatives > 2,2-dialkyl ones. Taking into account the information from the literature it is apparent that the degree (thermodynamic equilibrium) and rate of the decomposition is closely related to the electronic properties, polarizing and steric effects (bulkiness) of the substituents. With respect to the highest stability of *VI* (direct bonding of the electron-attracting —COOH group) and *I* itself, it might be stated that the more bulky (mainly alkyl radicals) and/or more electron-donating is the substituent (aryl ones), the deeper and faster is the decomposition of a 2-substituted derivative of *I*. Electron-attracting substituents, as in the case of *VI*, should stabilize the corresponding 2-substituted thiazolidines in aqueous solutions.

Dissociation Constants

The dissociation constants were evaluated from the first titration curves by the PKAS program, frequently verified from those of the second batches of saturated solutions, and those obtained in suitable time intervals. The mean values were differing on the third decimal place (indicated by round brackets, Table 1, the last column), individual SD's were not greater than 0.03. Thiazolidines possess two pK_a values describing the protolytic equilibrium at the COOH (with its value around 2 [1]) and at —NH— group in the fundamental heterocycle. Compounds *VI*, *XIII*, *XX*, *XXII*, and *XXIII* have the third pK_a. The magnitude of the lowest (first) pK_a is steady [1]; this was not subjected to the examination here. The second pK_a depended more or less on the nature of the substituent (Table 1).

Starting with *VI*, one can see that the additional COOH group (pK_a = 2.31(7)) decreased the "sensible" dissociation constant only a little when compared with that of *I*. In the series of 2-alkyl derivatives this pK_a is slightly influenced in the same direction [1]. The remarkably lower values of 2,2-dialkyl-substituted thiazolidines than those of *I*, *V*, *VII*, *VIII*, and even *VI*, are rather surprising and difficult to explain.

All the pK_a values of 2-aryl-substituted thiazolidines are clearly under 6, the highest are 5.7 (*XIII*) and 5.8 (*XXII*); these derivatives bear a 2-OH group (with pK_a about 10, determined spectrophotometrically [18]; titrimetry gave unrelevant results). The lowest pK_a (4.7) was found for *XXI* with 3-nitrophenyl group. This reflects an electron-attracting effect of the aromatic substituents mediated through two σ-bonds, and further, that the more deactivating is the group (atom) attached to the phenyl substituent in 2, 3 or 4 position, the lower is the pertinent pK_a and *vice versa*. In case of *XV*, there occurs a visible transfer of the substituent effect of even more distant benzene ring (through four bonds including the second, conjugated double one); the corresponding pK_a is practically the same as that for *X*, but its decay profile is as those of 2-alkyl derivatives. The pK_a of *XXIV* is valid for both *I* parts, their two dissociable protons are titrated simultaneously.

The abnormally high pK_a values of *III* and *XIII* reported in [1] are indeed incorrect, because they were obtained for their solutions prepared much below the saturated state and titrated a few hours after dissolution (a requirement for *XIII* to be completely dissolved).

The second end point upon titration curves has occurred in all cases, mainly when the solutions have been aging (Figs. 3—5). The corresponding pK_a was initially always a little higher than that of expected cysteine (8.18 [19]), but after sufficiently long time (several or tens of hours) both became identical. The constant, while higher, should be ascribed to the protonation equilibrium of the primary amino nitrogen of a semimercaptal being an intermediate hydrolysis product of the thiazolidines as proposed in [3]. Since there have been suggested also further mechanisms of the thiazolidine hydrolysis involving other intermediate compounds [4, 5, 7, 20], it complicates the interpretation. The only common feature of all the published solvolyses is the initial and final step, *i.e.* the heterocycle ring opening by cleavage of the C-2—N bond and the presence of the released cysteine (and a carbonyl compound).

After judging all the obtained results and data available from literature it is evident that a study of thiazolidines in any type of interaction in aqueous media, which requires well defined chemical individuals, is practically restricted to *I*, its stable derivatives (substituted with an electron-attracting group), and to some lower 2-alkyl analogues. The disadvantage of their instability can be overcome by use of their (mono)alkaline salts.

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REFERENCES

1. Butvin, P., Světlík, J., and Taligová, D., *Chem. Papers 52*, 205 (1998).
2. Reimschneider, R. and Hoyer, G. A., *Z. Naturforsch. 18b*, 25 (1963); Reimschneider, R. and Hoyer, G. A., *Z. Naturforsch. 17b*, 765 (1962).
3. Wlodek, L. B. and Wróbel, M., *Neoplasma 43*, 259 (1996).
4. Fulop, F., Mattinen, J., and Pihlaja, K., *Tetrahedron 46*, 6545 (1990).
5. Nagasawa, H. T., Goon, D. J. W., Muldoon, W. P., and Zera, R. T., *J. Med. Chem. 27*, 591 (1984).
6. Luhowy, R. and Meneghini, F., *J. Am. Chem. Soc. 101*, 420 (1979).
7. Pesek, J. J. and Frost, J. H., *Tetrahedron 31*, 907 (1973).
8. Woodward, G. E. and Schroeder, E. F., *J. Am. Chem. Soc. 59*, 1690 (1937).
9. Havránek, E., Bumbálová, A., Beláková, M., Kettmann, V., and Světlík, J., *J. Radioanal. Nucl. Chem. 212*, 69 (1996).
10. Bumbálová, A., Havránek, E., Al-Ja'Afreh, J., Kettmann, V., Světlík, J., and Štroffeková, O., *Česká a slovenská farmacie*, in press.
11. Rathbun, W. B., Killen, C. E., Hollesachau, A. M., and Nagasawa, H. T., *Biochem. Pharmacol. 51*, 1111 (1996).
12. Johnson, D. J., Graham, D. G., Amarnath, V., Amarnath, K., and Walentine, W. H., *Chem. Res. Toxicol. 9*, 910 (1996).
13. Magni, A., Signorelli, G., and Bochciola, G., *Arzneim. Forsch. 44*, 1402 (1994).
14. Roberts, J. C., Nagashawa, H. T., Zera, R. T., Fricke, R. F., and Goon, D. J. W., *J. Med. Chem. 30*, 1891 (1987).
15. Oya, M., Baba, T., Kato, E., Kawashima, Y., and Watanabe, T., *Chem. Pharm. Bull. 30*, 440 (1984).
16. Al-Ja'Afreh, J., Hatrík, Š., and Havránek, E., *Chem. Papers 53*, 89 (1999).
17. Martell, A. E. and Motekaitis, R. J., *Determination and Use of Stability Constants*. Second Edition. VCH Publishers, Weinheim, 1992.
18. Capková, I. and Kopecká, B., unpublished results.
19. Martell, A. E. and Smith, R. M., *Critical Stability Constants* (Amino Acids). P. 47. Plenum Press, New York, 1974.
20. Refouvelet, B. and Robert, J. F., *J. Heterocycl. Chem. 31*, 77 (1994).