Spontaneous formation of diastereoisomeric 2-methylthiazolidine-2,4-dicarboxylates from cystine esters and related compounds

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Dialkyl esters of cystine and lanthionine undergo conversion to *cis*- and *trans*-2-methylthiazolidine-2,4-dicarboxylates at 25–80 °C in protic solvents.

The thiazolidine ring system is an important and widespread feature among natural products (*e.g.* the penicillins).[1] In particular, the amino acid, 2-methylthiazolidine-2,4-dicarboxylic acid **1** is a ubiquitous metabolite,[2] a product observed in the alkaline degradation of cysteine and cystine[3,4] and, recently, the subject of considerable interest as an immunomodulator[5] and hepatoprotective antioxidant.[6] In studies on the comparative thermolability of protein amino acid sidechains,[7] we have observed the extremely facile transformation of simple esters of cystine **2a–c**, lanthionine **3a–b**, and cysteine **4a–b**, into esters of **1**,‡ whose stereochemistry is now clarified also for the first time.§

Amino acid esters were prepared from commercially available precursors using standard procedures. Thermolyses were effected with 0.1 mol dm^{-3} substrate solutions in anhydrous solvents under an argon atmosphere as outlined in Table 1. Following dilution in aqueous phosphate buffer solution,¶ thermolysate samples were analysed by reverse phase HPLC (typically: SP, Jones Apex II ODS 5 μ, 250 × 4.6 mm; MP, 2–40% MeCN in 0.05 mol dm^{-3} aq. KH_2PO_4, pH 2.6; FR, 1.0 cm^3 min^{-1}; λ, 210 nm) directly or, in the case of toluene solutions, after gentle solvent evaporation and reconstitution in buffer solution. Thermolysis products of **3b** were isolated by silica column chromatography using a petroleum ether (bp 40–60 °C)–chloroform–methanol eluent system.

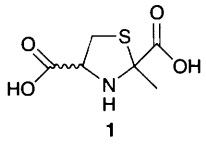

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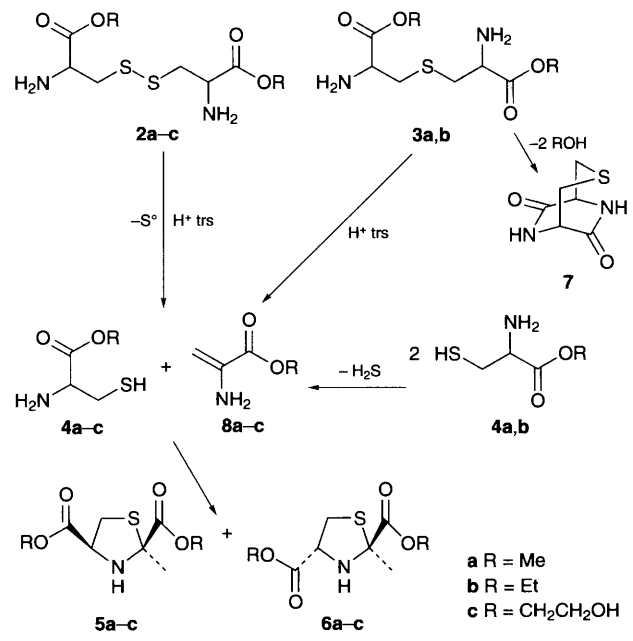
Table 1 Conditions and outcomes of thermolyses

Substrate	Solvent	*T*/°C	*t*/h	Conversion (%)	Observed products (HPLC)[a]
2a[b]	Toluene	110	15	10[c]	**5a** 0.09, **6a** 0.09
2a[d]	Toluene	110	15	10[c]	**5a** 0.18, **6a** 0.28
2b[d]	Ethanol	79	6	>99	**5b** 0.43, **6b** 0.53
2c[b]	Ethane-1,2-diol	25	24	>99	**5c** + **6c** 0.49
3a[e]	Toluene	110	15	10[c]	**4a** 0.77,[c] **5a** <0.001, **7** 0.16
3b[e,f]	Ethanol	79	6	75	**5b** 0.65, **6b** 0.45, **7** 0.06
4a	Toluene	110	15	10[c]	**5a** 0.05, **6a** 0.09
4b	Ethanol	79	6	20	**5b** 0.45, **6b** 0.25, complex mixture

[a] Molar ratio to substrate consumed (theoretical maximum for **4a–b** = 0.5). [b] (*R,R*)-Diastereoisomer. [c] Maximum value. [d] Meso-diastereoisomer. [e] Mixture of (*R,R*)-, (*S,S*)- and meso-diastereoisomers. [f] 0.04 mol dm^{-3} solution.

The thermolysis of **3b** in dry ethanol generates a strong ammoniacal odour and, after 17 h at 79 °C, affords **5b** and **6b** (81% combined yield), plus the more direct condensation product, and new compound **7** (10%). Stereochemical assignment was achieved using DIFNOE spectroscopy in deuteriochloroform solution, where the *cis* diastereoisomer **5b** shows a clear NOE from the 4-H atom to the 2-methyl group. Both compounds were confirmed by independent synthesis of *cis*- and *trans*-2-methylthiazolidine-2,4-dicarboxylic acid from cysteine and pyruvic acid,‖ followed by esterification with ethanolic hydrogen chloride. While **4b** was not detected during the course of this thermolysis, a minor component was observed in HPLC analysis whose UV–VIS spectrum (obtained by diode-array detection) was distinctive in possessing a λ_{max} at 220 nm, consistent with an α,β-unsaturated carbonyl group. Moreover, the relative yields of this compound fall with increasing conversion of the substrate, implying that it is a primary product of the reaction, possibly **8b**. Its apparent lability precluded further characterisation, however. Thermolysis was extended to a range of related substrates in ethanol, ethane-1,2-diol and toluene, and the outcomes are summarised in Table 1. Ready formation of 2-methylthiazolidine-2,4-dicarboxylates is observed under mild conditions in protic solvents such as ethanol and ethane-1,2-diol, but their yield in toluene is poor. Despite being a well-documented thermolysis product of cystine and cysteine, no lanthionine derivatives were detected in the thermolysates of **2a–c** or **4a–b**. However, the presence of elemental sulfur (TLC) and **4a** in the thermolysates of **2a** and **3a** respectively support the reaction sequence summarised in Scheme 1.



Scheme 1

Thus, we suggest the two-stage process begins in all solvents with a β-elimination producing **4a–c** and the dehydroalanine derivative **8a–c**. This is fully consistent with the known chemistry of cystine and related amino acids.[3,4,9] The next stage, however, is strongly influenced by solvent. The cysteinyl thiol group can add to the imine tautomer of **8** to give a transient thioaminal, but the subsequent displacement of ammonia by unshared electrons on the adjacent sulfur atom needs the participation of a protic solvent. Cyclisation is then completed by intramolecular attack of the remaining amino group on the residual electrophilic carbon atom.

The ease of 2-methylthiazolidine-2,4-dicarboxylate formation from cystine derivatives and related compounds invites consideration of the implications wherever these amino acids are subjected to thermal stress. In particular, this chemistry may reduce the availability of cysteine, and provide a potentially significant source of thiazolidine derivatives, in both cooked food and, under milder conditions, in living systems.

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Footnotes

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‡ All new compounds were fully characterised spectroscopically and by microanalysis or HRMS.

§ Two distinct forms of diethyl 2-methylthiazolidine-2,4-dicarboxylate hydrochloride have been isolated,[4] but their stereochemical significance has not been recognised.

¶ Aqueous potassium dihydrogen orthophosphate (0.05 mol dm^{-3}) adjusted to pH 2.6 with concentrated phosphoric acid.

‖ Modification of the original procedure[8] (concentration of the ethanolic mother liquors) allows isolation of each diastereoisomer by fractional crystallisation. Details will be given in the full account of this study.

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