Δ^3-THIAZOLINES, Δ^4-THIAZOLINES AND THIAZOLES FROM PENEM ANTIBIOTICS

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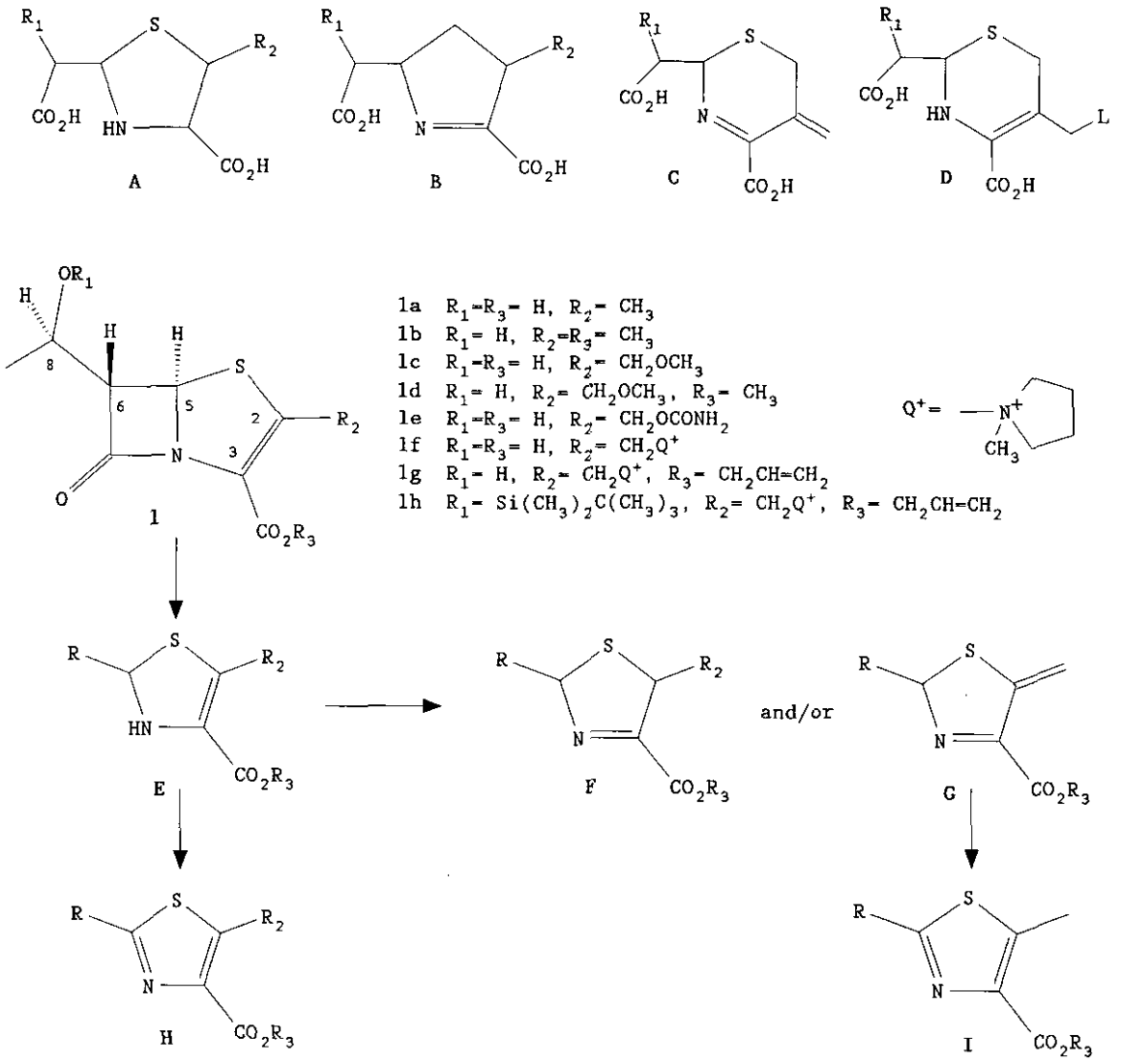
Abstract - Upon cleavage of the β-lactam ring, penems are converted to unstable Δ^4-thiazolines, which epimerize at the ex-C-5 position, lose a nucleofugal group at C-2′ (if originally present), tautomerize to Δ^3-thiazolines, or suffer hydrolytic decomposition. Other chemical events, observed on some substrates under determinate conditions, include protomeric and oxidative aromatization to thiazoles (followed by side-chain reactions, such as epimerization at C-6, decarboxylation, dehydration, retro-aldol condensation), and conjugate addition of external and internal thiols. Mention is made of the chemical, biochemical and pharmacological significance of these findings.

The products arising from cleavage of the azetidinone ring of bioactive β-lactam compounds are of interest in many ways. They interest the biochemist, since are likely to reproduce part of the structure of the acylenzyme intermediate responsible for inactivation of bacterial peptidoglycan transpeptidases,[1] β-lactamases,[2] or human leukocyte elastase.[3] They interest the pharmacologist, since most metabolic pathways of β-lactam drugs, including those potentially leading to adverse reactions, are triggered by β-lactam cleavage.[4] They obviously interest the chemist, who has the opportunity of studying and possibly exploiting[5] highly reactive species difficult to obtain otherwise. Among these products, heterocyclic structures (A~D) have been object of extensive investigation. Thiazolidines (A) are known since the early years of penicillin research[6] but essential questions on their structure and reactivity were debated until recently.[7] The enamine-imine tautomerization leading to pyrrolines (B) was associated with the β-lactamase inhibitory activity of olivanic acids.[2] The origin and fate of *exo*-methylenedihydrothiazines (C) has intrigued

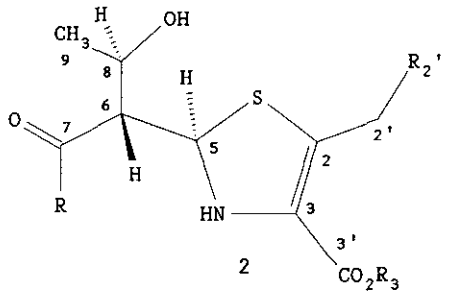
Scheme 1



medicinal chemists for years; the favourable properties of cephalosporins bearing good leaving groups at the C-3′ position were ascribed either to a mechanism of β-lactam cleavage concerted with expulsion of such groups,[8,9] or to the imine substructure[10] and Michael-acceptor ability[3,11] of the resulting species (C). We wish here to report on related species (unsaturated analogs of A, sulfur analogs of B, nor-analogs of C and D) observed after hydrolysis of penem antibiotics (1), i.e. Δ^4-thiazolines (E; compounds 2~4), Δ^3-thiazolines (F; 5,6), *exo*-methylene-Δ^3-thiazolines (G; 7,8), and the derived thiazoles (H,I; 9~15).

Δ^4-THIAZOLINES

β-Lactam cleavage of penems is expected to afford Δ^4-thiazolines (**2**) as the primary product. Surprisingly, these 'penemoic acids' (analogs of the penicilloic and cephalosporoic acids) have never been reported in 15 years of intensive research on penems. We identified these species in the alkaline hydrolysis of penems devoid of good leaving groups at C-2' (**1a~d**), and gained unequivocal evidence of their intermediacy in the hydrolysis of other penems (**1e~h**).



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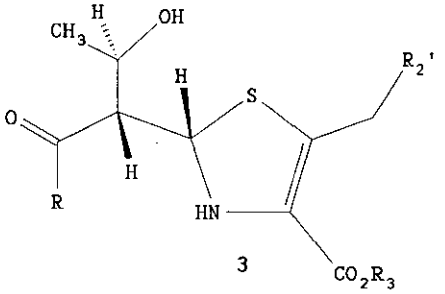
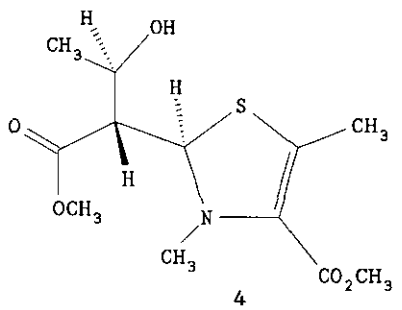


3

a R= OH, R_2'=R_3= H
b R= OH, R_2'= OCH_3, R_3= H
c R= OCH_3, R_2'= H, R_3= CH_3
d R= OCH_3, R_2'= OCH_3, R_3= CH_3



4

^{1}H Nmr spectroscopy was the essential tool to observe the Δ^4-thiazoline products. Monitoring the alkaline hydrolysis (1 mol equiv. of NaOD in D_2O) of the 2-methylpenem (**1a**) revealed the development of partially superimposing nmr signals attributable to two thiazoline isomers (**2a** and **3a**, ca. 4:1). The proportion of the two components did not change in the course of hydrolysis and after its completion, but degradation to a complex mixture of products occurred within 2 days. The CD spectrum of the mixture (positive Cotton effect for the main transition at 257 nm, negative for the minor one at 302 nm; Figure 1) was impressively close to that of the penem precursor (**1a**). Analogous correspondence of uv spectra (**1a**, λ_{max}= 262 and 302 nm; **2a+3a**, λ_{max}= 266 and 302 nm) indicates that the chromophore of penems is confined to the thiazoline ring. Preservation

of signs for both experimentally accessible Cotton effects proves that the major component (**2a**) retained the original configuration (*R*) at the relevant asymmetric center (C-2 or, according to the penem numbering used hereinafter for convenience, C-5).

Hydrolysis of the 2-alkoxymethylpenem (**1c**) under identical conditions also produced two Δ^4-thiazoline isomers (**2b** and **3b**, ca. 3:1), but these compounds were progressively converted into two Δ^3-thiazoline pairs (**5a**-I,II and **5a**-III,IV). Since **2b**,**3b** were never present alone in reaction mixtures and degraded when isolation was attempted, CD spectra could not be acquired. Deuterium incorporation did not occur in the formation of the Δ^4-thiazolines, suggesting retention of the (6*S*,8*R*) configuration and the occurrence of a non-protomeric epimerization at C-5 analogous to that reported for penicilloates.[7] The major isomer (**2b**) was characterized by a H-5, H-6 coupling constant similar to that of **2a** (see Experimental). Thence, **2b** was provisionally assigned the (5*R*,6*S*,8*R*) configuration, while **3b** was considered to be its (5*S*,6*S*,8*R*) diastereoisomer.

Attempts to isolate the Δ^4-thiazolines were frustrating. For example, **2b** and **3b** were destroyed in short times when the alkaline solution resulting from hydrolysis of **1c** was brought to pH 7.2 by addition of a MOPS buffer; by contrast, the Δ^3-thiazoline isomers (**5a**) survived, and were isolated in separate experiments (see below). On the reasonable assumption that ester derivatization of the thiazoline carboxyl would stabilize the N-C_5-S thioaminal moiety of the ring, the 4:1 solution of **2a**,**3a** was freeze-dried and treated with excess CH_3I in DMF. This procedure did not afford the expected dimethyl ester, but a sample of the stable *N*-methyl derivative (**4**) was isolated (10% yield) from a mixture of unstable products and degradates. The CD spectrum of **4** was quite similar (Figure 1) to that of the penem methyl ester (**1b**), proving that the former compound belongs to the (5*R*) series as well. Encouraged by this result, we examined the methanolysis of penem esters as an alternative way to obtain esters of the Δ^4-thiazoline products. β-Lactam cleavage of the 2-methylpenem methyl ester (**1b**) by a catalytic amount of sodium methoxide in methanol afforded two inseparable Δ^4-thiazoline esters (**2c** and **3c**, ca. 3:1). By addition of chloranil, this mixture was converted to a single thiazole product (**9a**). Since a single chiral center (C-5) is lost in the aromatization process, this result unequivocally proves that the original thiazolines were C-5 epimers, and confirms that they did not differ for the configuration at C-6 and C-8. Similarly, a mixture of thiazoline dimethyl esters (**2d**,**3d**; ca. 3:1) was obtained from methanolysis of the 2-methoxymethylpenem methyl ester (**1d**) in methanol. These products could not be isolated because of their rapid conversion (analogous to that observed on the corresponding acids **2b**,**3b**) to two pairs of Δ^3-thiazolines (**5b**) and several minor unidentified degradates.

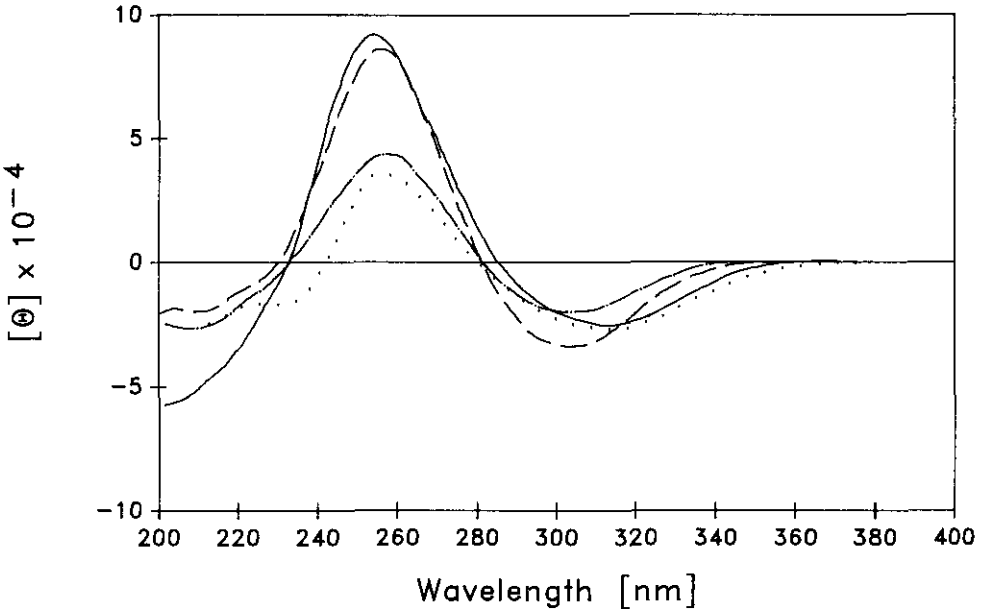


Figure 1 - Circular dichroism spectra of penems (sodium salt 1a in water, — — — ; ester 1b in CH_3CN, ————) and Δ^4-thiazolines (4:1 mixture of disodium salts 2a,3a in water, — · — · ; ester 4 in CH_3CN, ·····).

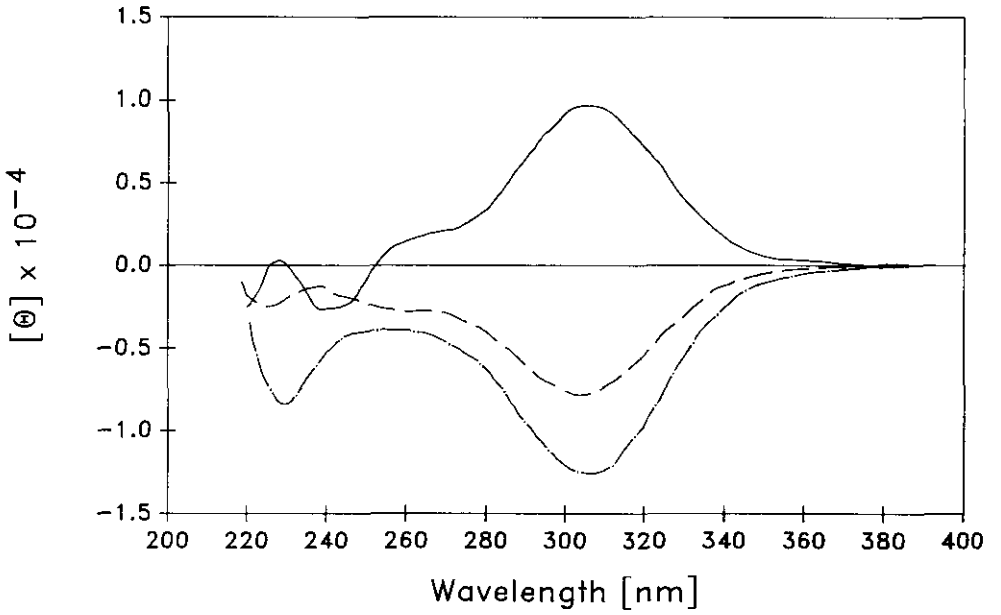


Figure 2 - Circular dichroism spectra of *exo*-methylene-Δ^3-thiazolines disodium salts (7a, — · — · ; 7b, — — — ; 8a, ————) in aqueous solution.

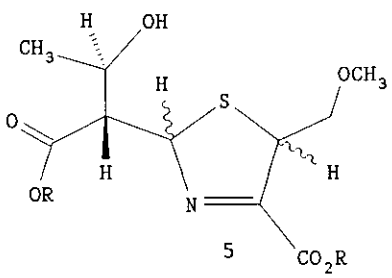
A different outcome was observed from penems possessing nucleofugal C-2' substituents. Monitoring (hplc, nmr) the hydrolysis of **1e** and **1f** did not reveal the hypothetical primary Δ^4-thiazoline products at any time. None the less, their involvement is betrayed by the formation of *exo*-methylene-Δ^3-thiazolines (**7a**,**8a**), which arise from expulsion of the leaving group *after* partial or complete epimerization at C-5 (see Discussion). A potential way to obtain back the elusive Δ^4-thiazolines from this type of compounds was probed without success but with interesting results. In the past, observation of cephalosporoic acids (species D) proved possible in a few selected cases by adding an excess of a Michael-donor HL to a solution of the *exo*-methylenedihydrothiazines (*C*).[11,12] We were unable to observe Δ^4-thiazolines by adding 2-mercaptoethanol, sodium mercaptoethanesulfonate or thiolacetic acid to solutions of the (5*R*) isomer (**7a**); none the less, the last reagent did induce formation of its (5*S*) epimer (**8a**). Apparent equilibrium (ca. 1.6:1 in favour of **8a**) was achieved within few hours under unoptimized conditions (1 mol equiv. of thiolacetic acid, DMSO); further exposure led to progressive degradation. Chloroacetic acid, a reagent with comparable pK_a but with poor nucleophilic ability, was unable to promote equilibration, and that supports the hypothesis that epimerization at C-5 is a non-protomeric process occurring at the level of a thermodynamically disfavoured Δ^4-thiazoline adduct (see Discussion). Addition of thiolacetic acid was experimented also to an aqueous solution of the (5*R*)-Δ^3-thiazoline amide (**7b**). Here again, epimerization was observed; the final mixture (20 hours) contained the (5*S*) amide (**8b**) as the major component (ca. 1.4:1), though this isomer was not formed at all in the direct ammonolysis of FCE 22101 (**1e**).

Δ^3-THIAZOLINES

Woodward reported the isolation of a Δ^3-thiazoline ester when prolonged (2 days) alkaline hydrolysis of the racemic 3-carboxypenem nucleus was followed by alkylation with 4-nitrobenzyl bromide.[13] Since the intermediate Δ^3-thiazoline sodium salt must have arisen by tautomerization of the initial Δ^4-thiazoline product under the reaction conditions, the fate of solutions of **2a**,**3a** and **2b**,**3b** left aside for several hours was monitored by nmr and hplc. As mentioned above, no definite result apart from degradation was obtained from **2a**,**3a**, but progressive depletion of **2b**,**3b** in favour of four diastereomeric Δ^3-thiazolines (**5a**) was observed, with full deuterium incorporation at C-2. These products came in two pairs (ca. 2.5:1 to each other and 1:1 within each pair: **5a-I**,**5a-II** major; **5a-III**,**5a-IV** minor); further, minor amounts ($\leq$ 10%) of the (5*S*) *exo*-methylene-Δ^3-thiazoline (**8a**) were detected. Repetition of the hydrolysis of **1c** with NaOH in H_2O on a preparative scale,

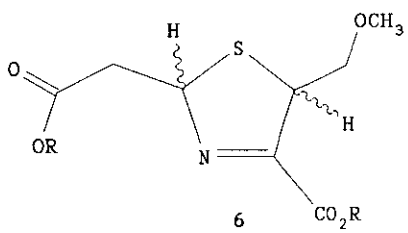
followed after 24 hours by reversed phase chromatography and freeze-drying, led to isolation of **5a** (mixture of isomers) in 84% yield, while **8a** was lost. After isolation, enriched fractions of the individual isomers did not equilibrate, nor deuterium exchange at any position (C-2 included) was observed, even under mild basic (pyridine) or acid (TFA) catalysis, where degradation eventually occurred.

The stereochemistry of the products (**5a**) was tentatively inferred as follows. First, the (6*S*,8*R*) configuration, the same as that of the original penem (**1c**) and of the intermediate Δ^4-thiazolines (**2b**,**3b**), is warranted by the reaction conditions and by the absence of deuterium incorporation at these positions. The configuration at C-5 was assigned by comparison with the *exo*-methylenethiazolines (**7a**,**8a**), whose stereochemistry had been ascertained after CD studies (see below). A distinguished feature of the nmr spectrum of the (5*S*) isomer (**8a**), shared by the (5*S*) penem metabolite reported by Suntory,[15] is the abnormally large C-5-H, C-6-H coupling constant, suggestive of a conformational preference promoted by intramolecular hydrogen bonding. Since the components of the major pair (**5a-I**, **5a-II**) were characterized by $J_{5,6}$ values of 9.7 and 10.0 Hz, respectively, while corresponding values for **5a-III**, **5a-IV** were 7.5 and 7.3 Hz, the former two were attributed the (5*S*) configuration. The stereochemistry at C-2 was provisionally assigned as shown in the figure on the basis of small NOE enhancements of H-5 observed upon irradiation of H-2 in **5a-II** but not in **5a-I**.



5

a R= H
b R= CH_3
c R= $CH_2C_6H_4NO_2$-*p*



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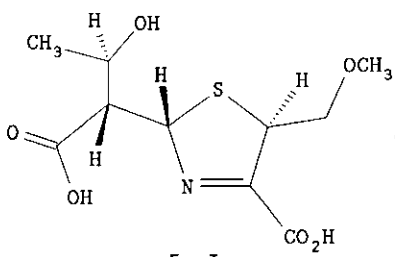
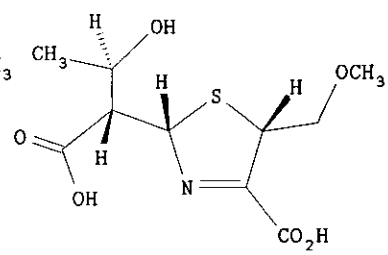
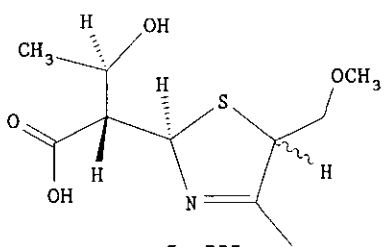


5a-I



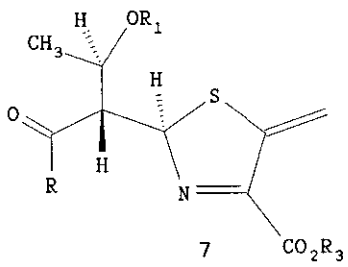
5a-II



5a-III
5a-IV

Esters of Δ^3-thiazolines were obtained either by alcoholysis of penem esters or by hydrolysis of penem acids followed by alkylation. As reported above, methanolysis of 1d produced minor amounts of four Δ^3-thiazoline dimethyl esters (5b), which progressively increased at the expense of the initially formed Δ^4-isomers (2d,3d). Similarly, alkylation with *p*-nitrobenzyl bromide in DMSO of 5a (as obtained from the alkaline hydrolysis of 1c, left for 24 hours under nitrogen and freeze-dried) produced the expected four di-*p*-nitrobenzyl esters (5c) in two distinct pairs (54% overall yield), accompanied by a minor amount (10%) of retro-aldol products (6c). A component of the major pair of 5c could be isolated and its structure was confirmed by ^{13}C nmr.

The remaining Δ^3-thiazolines identified in the present study were the *exo*-methylene compounds (7,8). In accord with our original rationale[16] presiding the design and evaluation of penems possessing good leaving groups at the C-2′ position, experimental observation of the alkaline hydrolysis of FCE 22101[17] (1e) and of the quaternary ammonium derivative (1f)[18] confirmed that release of such groups occurs upon β-lactam cleavage to produce a solution of a common *exo*-methylene-Δ^3-thiazoline. Beside the expected (5*R*) product (7a), however, a minor amount of its (5*S*) epimer (8a) was detected. When the hydrolysis was run with diluted ammonia or ammonium carbonate, the (5*R*) amide (7b) was the exclusive thiazoline product. Concentrated solutions of FCE 22101 under mild alkaline conditions also led to the formation of minor amounts of 7b; clearly, under such conditions the ammonia liberated from the expelled carbamic acid competes with hydroxide ions for β-lactam cleavage. Preliminary structural evidences have been anticipated[19] for 7a, which is also the major product of FCE 22101 degradation by renal dehydropeptidases (DHP) and the major urinary metabolite of FCE 22101 in humans.[20]



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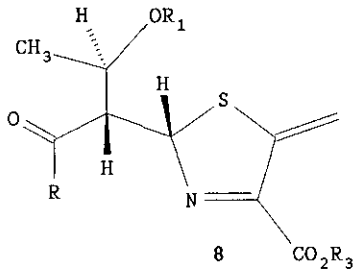


8

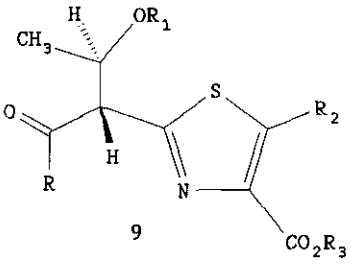
a R= OH, R_1=R_3= H
b R= NH_2, R_1=R_3= H
c R= OCH_3, R_1= H, R_3= CH_3
d R= OH, R_1= $Si(CH_3)_2C(CH_3)_3$, R_3= $CH_2CH{=}CH_2$
e R= OH, R_1= $Si(CH_3)_2C(CH_3)_3$, R_3= H

Separation of **7a** from **8a** could be achieved either by anion exchange chromatography (Sephadex DEAE-A25) or by reversed phase hplc (μBondapack C18); these techniques provided aqueous solutions of the two components as diammonium or disodium salts, free from organic contaminants but impure from external salts. Evaporation or freeze-drying led to extensive decomposition of the solutes, though **7a** proved stable once isolated in the solid form by precipitation with acetone. In spite of these difficulties, a method for the accurate quantification of **7a** in biological fluids could be set down.[21] The diastereomeric relationship of **7a** with **8a** was evident from the very beginning on the basis of the very close (^{1}H and ^{13}C nmr) or identical (ir, uv) spectral data. Further, partial conversion of **7a** into **8a** (accompanied by extensive degradation) was observed when a very concentrated solution of the former compound was let aside for several hours.[22] Examination of the CD spectra (Figure 2) showed a maximum negative Cotton effect centered at 307 nm for **7a** ($[\Theta]$= -12,600) and the opposite at 305 nm for **8a** ($[\Theta]$= +10,000). Like **7a**, the amide **7b** (304 nm, $[\Theta]$= -8,000) and all of the (5*R*) penems (e.g., **1a** and **1b**, Fig. 1) are characterized by negative Cotton effects for the absorption band at long wavelength.[23] Thus, **7a** and **7b** were attributed the (*R*) configuration at the C-5 position, and **8a** the opposite one (*S*).[24] Although the CD spectra gave no indication on the other two chiral centers, the stereochemistry at the carbon atoms of the thiazoline side-chain was considered to be the same as that of the penem precursor (6*S*,8*R*). In fact, the isolated products did not interconvert under the conditions of their generation and, when hydrolysis was run with NaOD in D_2O, deuterium incorporation was not observed.

The epimerization of the (5*R*) amide (**7b**) to its (5*S*) isomer (**8b**) in the presence of thiolacetic acid has been detailed in the previous section. The isolation of **7e** and the formation of *exo*-methylene-Δ^3-thiazoline esters (**7c,d**), (**8c,d**) is reported herebelow.

THIAZOLES

Thiazoles unsubstituted at C-2 (C-5 according to the penem numbering used in the present work) are well-known 'vertical splitting' decomposition products of penems, originating from a reverse [2+2] cycloaddition catalyzed by acids[25] or photolysis.[26] Thiazoles retaining the framework elements of the cleaved azetidinone ring, with one exception,[27] have never been reported. In the present study, a first set of these products was obtained from base-catalyzed aromatization of the *exo*-methylene-Δ^3-thiazolines (**7,8**) arising from hydrolysis of penems possessing nucleofugal groups at C-2'; this reaction was fast and almost quantitative upon esterification of the thiazoline carboxyl group.



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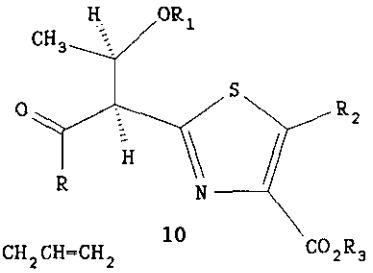
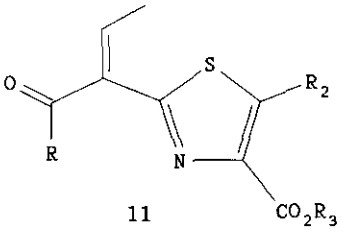


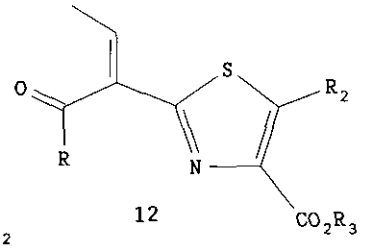
10

a R= OCH_3, R_1= H, R_2=R_3= CH_3
b R= OCH_3, R_1= H, R_2= CH_3, R_3= $CH_2CH{=}CH_2$
c R= OH, R_1= $Si(CH_3)_2C(CH_3)_3$, R_2= CH_3, R_3= $CH_2CH{=}CH_2$

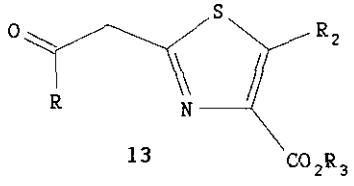


11

a R= OCH_3, R_2=R_3= CH_3
b R= OH, R_2= CH_3, R_3= $CH_2CH{=}CH_2$

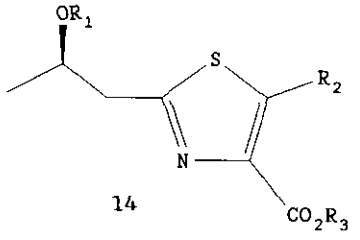


12



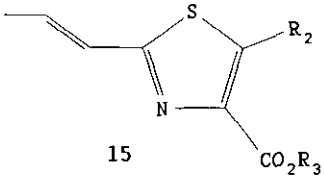
13

a R= OCH_3, R_2=R_3= CH_3
b R= OCH_3, R_2= CH_3, R_3= $CH_2CH{=}CH_2$



14

a R_1= $Si(CH_3)_2C(CH_3)_3$, R_2= CH_3, R_3= $CH_2CH{=}CH_2$



15

a R_2= CH_2OCH_3, R_3= CH_3

In a typical experiment, **7a** and **8a** were alkylated with excess CH_3I in DMSO to provide the unstable ester products (**7c**,**8c**). Exposure to triethylamine of a solution ($CHCl_3$) of the individual compounds afforded in both cases two epimeric thiazoles (**9a**,**10a**) and a product of further transformation (**13a**). The thiazoles were epimers at C-6, and since **9a** was the exclusive product of oxidative aromatization of the Δ^4-thiazolines (**2c**,**3c**) by chloranil, it has the same configuration (*S*) as that of the original penem. Epimerization at this

center, activated by the simultaneous presence of the neighbouring aromatic nucleus and carboxylic ester moiety, must have been promoted by triethylamine *after* the protomeric aromatization of 7c,8c to the common primary thiazole product (**9a**). Upon standing, the original mixture (**9a**,**10a**) was converted into a similar mixture of dehydration products (**11a**,**12a**, ca. 1:1), while the isolated (6*S*) epimer (**9a**) gave the two alkenes (**11a**,**12a**) in a 5:1 relative ratio. Dehydration of **9a** through an E2 mechanism is expected to afford the (*Z*) alkene stereospecifically. Thus, **11a** was assigned the (*Z*) configuration as shown.

Aromatization of transient *exo*-methylene-Δ^3-thiazolines to methylthiazoles was directly observed in hydrolysis or alcoholysis experiments, when the original penems were derivatized as esters. Thus, in the silica gel catalyzed methanolysis of **1g**[18] three thiazoles (**9b**,**10b**, inseparable 1:1 mixture, and **13b**) were identified. In another experiment, when β-lactam cleavage (equivalent amount of 1.0*N* NaOH in CH_3CN) was run on the silyl-protected derivative (**1h**), the primary Δ^3-thiazoline product (**7d**) underwent competitive ester hydrolysis and aromatization. The former reaction led to separation of the disodium salt (**7e**) as a filterable solid, the latter gave the primary thiazole (**9c**) and products of further transformation, i.e. loss of silanol (**11b**,**12b**, ca. 5:1) and decarboxylation (**14a**), which were characterized after silica silica gel chromatography of the mother liquors.

Formation of the 2-methylthiazole (**9a**) was also observed when hydrolysis of the 2-methylpenem (**1a**) was followed by methylation with excess CH_3I in DMSO. In this case, aromatization must have entailed an oxidative process. Consistently, an authentic sample of **9a** was prepared by treatment of the Δ^4-thiazoline methyl esters (**2c**,**3c**) with chloranil.

DISCUSSION

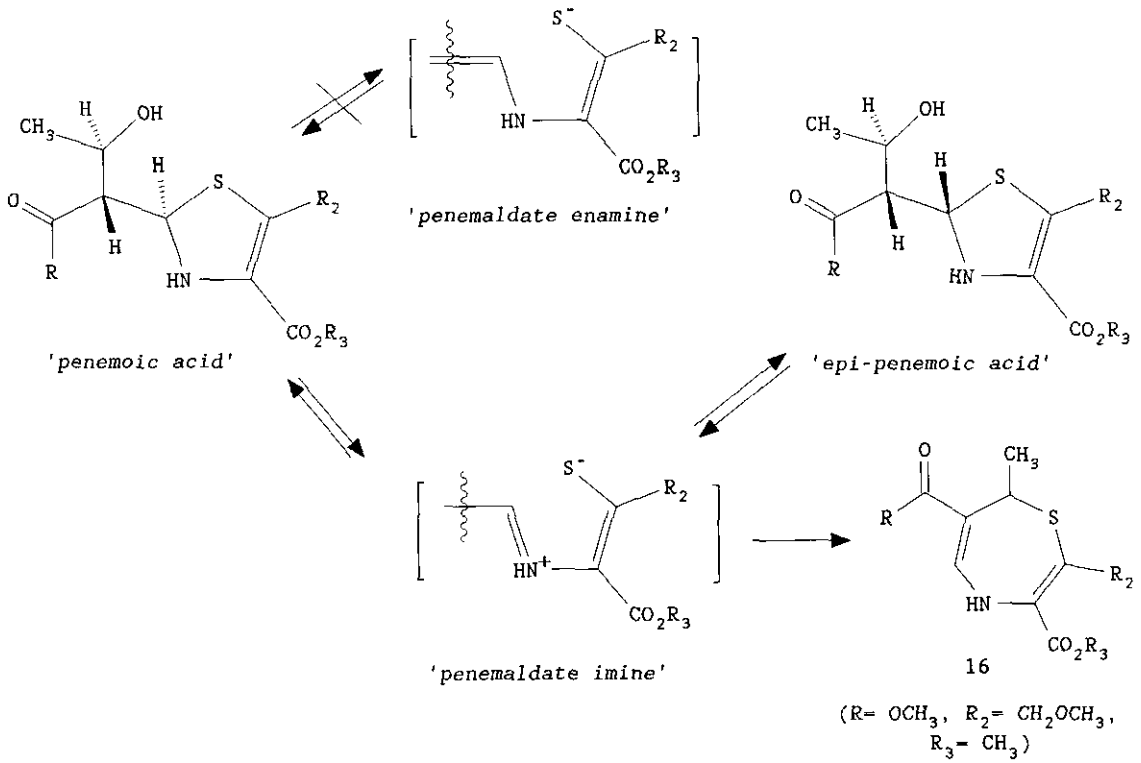
Recognized degradation pathways of β-lactam antibiotics consequent to hydrolytic cleavage are (*a*) epimerization at the C-5 position (penams),[7,28~30] (*b*) enamine → imine tautomerization (carbapenems),[2,13,31,32] and (*c*) expulsion of a leaving group attached to the allylic methylene (cephems).[8~11] All of them, sometimes in competition, have been observed in our study on penems. Thus, a first general comment is that penems are structural hybrids of penams, carbapenems and cephems both in concept[16,25] and in fact. In addition, the results acquired in the present study lend themselves to a more detailed discussion of the origin and fate of the observed Δ^4-thiazolines, Δ^3-thiazolines and thiazoles.

The Δ^4-thiazolines reported so far in the literature either bear at C-2 (thiazole numbering) a protomeric group (=NR, =O, =S, =CHR), or are substituted at nitrogen, or are stabilized by a fused aromatic ring (benzothiazolines).[33,34] Thus, the new Δ^4-thiazolines

(2,3) are the first representatives of the fundamental heterocyclic structure (E in Scheme 1). Compounds of this type are expected to be thermodynamically disfavoured over their Δ^3-tautomers, hydrolytically unstable, and easily susceptible to oxidation. Overall, the elusiveness of 2,3 is not surprising; as a matter of fact, the *N*-methyl derivative (4) was the only isolable Δ^4-thiazoline observed in the present study. The most interesting point about the Δ^4-thiazolines is the epimerization at C-2 (C-5 according to penem numbering). This is reminiscent of the analogous process which occurs on penicilloic acids, and poses questions about the reaction mechanism, rate and isomeric ratio at equilibrium.

Two different mechanisms have been proposed for the epimerization of penicilloic acids, i.e. β-elimination across either *C*-6,*C*-5 or *N*,*C*-5, with the intermediacy of 'penamaldate enamines' or 'penamaldate imines,' respectively.[29,35] These possibilities for the 'penemoic acids' (Δ^4-thiazolines) are depicted in Scheme 2. It has been proved that penicilloic acids epimerize through the latter mechanism[28] unless the carboxyl group is derivatized as an ester,[29] and the same unimolecular mechanism can be anticipated for the penemoic acids, which in addition lack the electron-withdrawing acylamino substituent at C-6.

Scheme 2



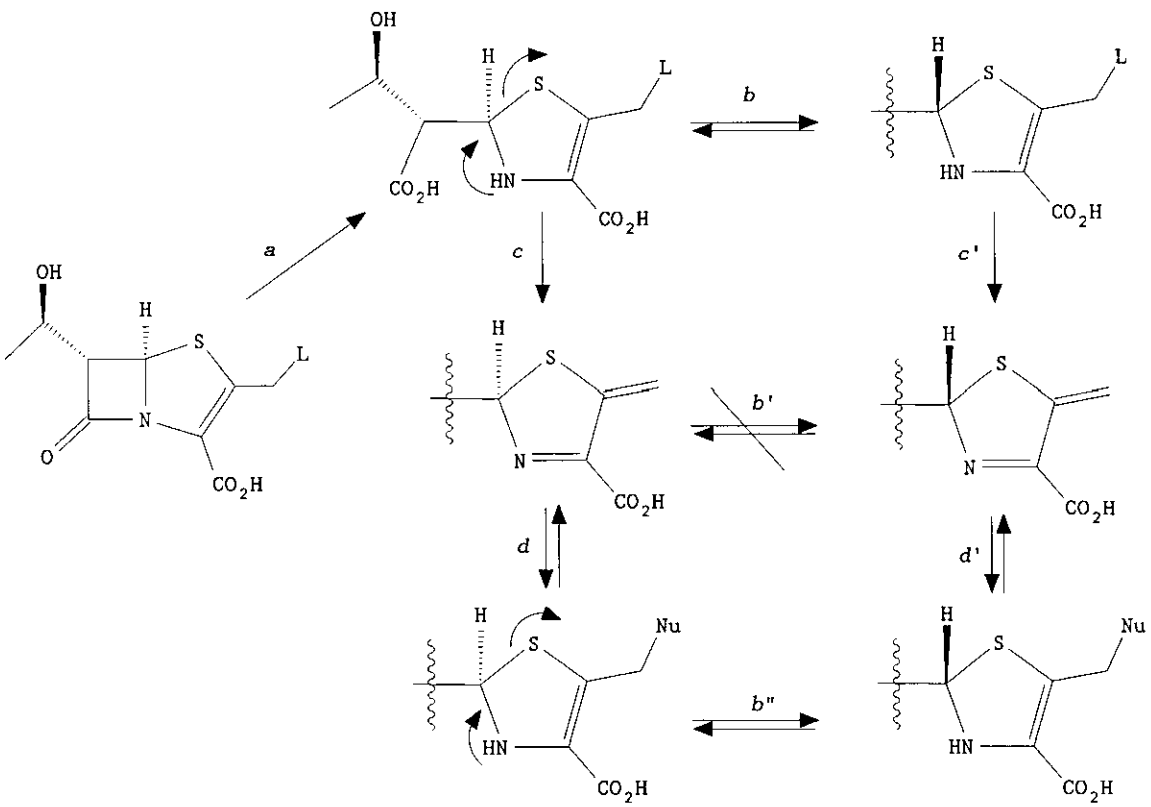
The observation that epimerization at C-5 is not accompanied by deuterium incorporation or epimerization at C-6 confirms this prediction. The thiol nucleophile characterizing the ring-opened species might be susceptible of trapping by external or internal electrophiles.[36] In this connection it is interesting to note that the 1,5-dihydro-1,4-thiazepine (**16**) was observed upon heating the mixture of 'penemoic esters' (**2d,3d**) in the nmr tube, and the same product was isolated by silica gel chromatography (27% yield) from the methanolysis of **1d** under refluxing conditions (MeOH - DMSO 2:1, 4 hours). The simplest interpretation of this finding is dehydration at C-6,C-8 and internal trapping of the thiol by the resulting acrylic moiety.

The epimerization rate of penicilloic acids was reported to be sensitive to minor modifications of the C-6 side-chain; it may take days (benzylpenicillin) or be immediate (carbenicillin).[30] It can be predicted that the conjugation existing in penemoic acids would stabilize the ring-opened species (an enethiolate), making epimerization much faster. Thiazolinones arising from anhydropenicillin,[37] which epimerize through a thiol-carboxylate, are observable only as the final equilibrium mixture of C-5 epimers; similarly the relative proportion of the Δ^4-thiazolines epimers (**2a~c** and **3a~c**) did not change in the course and after completion of the hydrolysis (or methanolysis) of their penem precursors. Thus, in contrast with penicilloates,[38] the kinetic products of penem hydrolysis appear to be also the preferred Δ^4-thiazoline isomers at equilibrium.

The Δ^3-thiazolines are a relatively recent discovery; previously, they were obtained as the condensation products of ketones or aldehydes with a mixture of sulfur and ammonia.[33] The new set of compounds of this type obtained in the course of our study adds new information. A first interesting aspect is their tautomerism with the Δ^4-isomers. It is known that secondary enamines are thermodynamically unstable, since the corresponding imines are usually the only detectable form in protic media.[39] Consequently, Δ^4-thiazolines (kinetic products of the β-lactam cleavage of penems) should be converted to Δ^3-thiazolines (thermodynamic products) under their formation conditions. On this basis, it was gratifying to follow by nmr and hplc the progressive depletion of Δ^4-thiazolines (**2b,3b** and **2d,3d**), with accumulation of the corresponding Δ^3-isomers (**5a** and **5b**). The tautomerization appeared to favour the (5*S*) epimers and to be irreversible, since the final products (**5**) did not interchange, nor deuterium exchange at C-2 (penem numbering) took place *after* their formation.[40] The apparent reluctance of the Δ^4-thiazolines derived from the 2-methylpenems (**2a,3a** and **2c,3c**) to undergo the same tautomerization may surprise, but has precedents in related structures.[41,42]

The formation of different relative amounts of C-5 epimers of the same *exo*-methylene-thiazoline (**7a**,**8a**) from different penems (**1c**,**1e**,**1f**) is one of the most intriguing aspects of penem hydrolysis. The relevant results can be summarized as follows. The penem with the most nucleofugal C-2′ substituent (**1f**) yielded the (5*R*) epimer (**7a**) almost exclusively; FCE 22101 (**1e**) gave **7a**,**8a** in a constant ratio[43] of about 7:1 both at pH 9-12 (alkaline hydrolysis) and at pH 7.1 under the catalysis of porcine dehydropeptidase (DHP, MOPS buffer),[19] but yielded **7a** exclusively with the oxoid β-lactamase SR 113;[44] finally from the penem with the least nucleofugal substituent (**1c**)[45] only the (5*S*) epimer (**8a**) was identified, apart from the major Δ^4- and Δ^3-thiazoline products (**2b**,**3b**,**5a**). The general mechanism of this process, anticipated in Scheme 1, is represented in more detail in the Scheme 3 below.

Scheme 3



The first step (*a*, β-lactam hydrolysis) is obviously irreversible. The transient primary product, the (5*R*) penemoic acid, equilibrates with the *epi*-penemoic acid (step *b*), but this process co-exists with elimination of HL from both (steps *c* and *c*′), which yields the

observed *exo*-methylene-Δ^3-thiazolines (**7a**,**8a**). The sp^2-hybridized nitrogen atom of these species is not expected to assist the unimolecular ring-opening (step *b'*) as the sp^3 nitrogen of the Δ^4-precursors does. Consistently with this prediction, dilute solutions of the separate *exo*-methylene-Δ^3-thiazolines (**7a**,**8a**) do not equilibrate in the whole pH interval investigated (2.5 - 12), and the same holds true for enriched fractions of the individual methoxymethyl-Δ^3-thiazolines (**5a**); i.e. step *b'* is not viable. Steps *c* and *c'*, at least for the investigated penems (**1e**,**1f**), also appeared to be irreversible; predictably, the carbamic acid freed from **1e** decomposes, while the *N*-methylpyrrolidine released from **1f** is not an effective Michael-donor. In fact, were steps *c*,*c'* reversible, **7a**,**8a** would be present as their thermodynamic equilibrium mixture. This event was accomplished by addition of thiolacetic acid (a good Michael-donor[46]) to the isolated (5*R*) epimer (**7a**), and is represented in the bottom part of the Scheme. The equilibration observed (reversible steps *d*,*b"*,*d'*; NuH= CH_3COSH) favoured the (5*S*) epimer (**8a**), in sharp contrast with the predominance of **7a** from hydrolysis of **1f** and FCE 22101. Similarly, the (5*S*) amide (**7b**) equilibrated to its epimer (**8b**), while the latter was not present at all in the direct ammonolysis of FCE 22101. At this point in discussion, it is tempting to postulate that with excellent leaving groups (*N*-methylpyrrolidine) step *c* is faster than the equilibration process *b*, and thence the 'natural' isomer (**7a**) is produced, while with leaving groups of intermediate ability (carbamate) the equilibrium *b* is frozen at midway, which results in a mixture of **7a** and **8a**. According to the alternate interpretation, the ratio of products (**7a**,**8a**) observed reflects differences in the rates of steps *c*, *c'*.[47] The contrasting results obtained when FCE 22101 was hydrolyzed by DHP and a β-lactamase can be matter of further speculation; obviously, more work is needed to clarify these points.

The third interesting topic of the chemistry of Δ^3-thiazolines concerns their stability and the nature of decomposition products. The dramatic influence of acidity can be appreciated by inspection of Figure 3, which reports the stability profile of **7a** in dilute solution (0.64 mM in 0.1*M* phosphate buffers) as a function of pH. Protonation of the nitrogen atom of Δ^3-thiazolines would be followed by hydrolysis of the thioaminal moiety, with release of ammonia, the latent aldehyde (a 'left-hand fragment' common to all of the penems bearing the hydroxyethyl side-chain), and a mercaptoketone component (the 'right-hand fragment'). Both fragments, in turn, are expected to be prone to further degradation; in particular, one might anticipate decarboxylation,[48] polymerization,[49] dehydration and retro-aldol fission on the former (an α-formyl-β-hydroxyacid), and self-condensation reactions[33] on the latter.

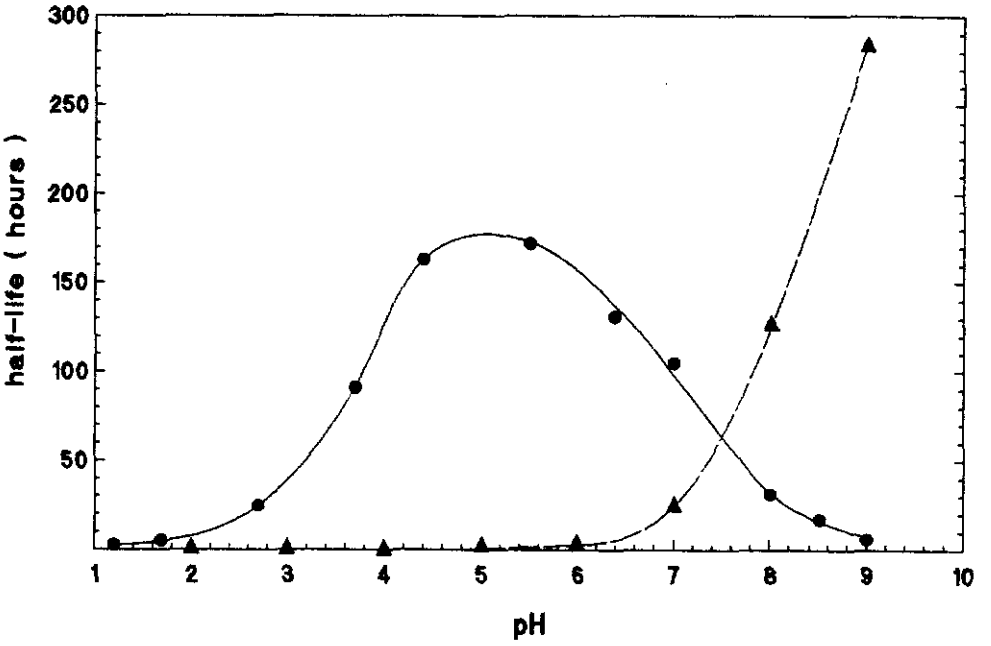
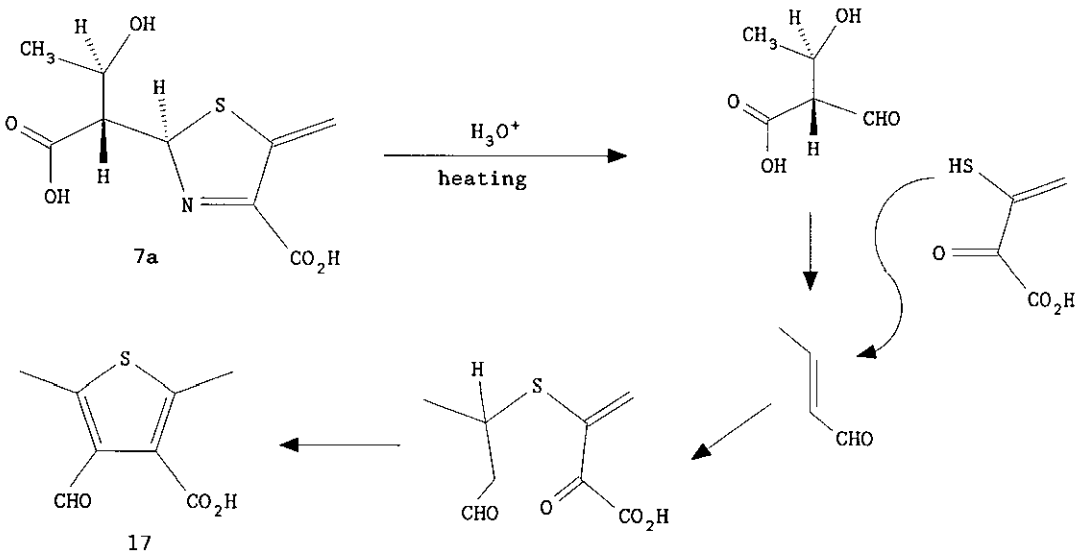


Figure 3 - Stability - pH profile (0.1*N* phosphate buffers, 37°C) of the *exo*-methylene-Δ^3-thiazoline (7a) (- - - -) and of its penem precursor FCE 22101 (————) in dilute solutions (0.64 mM).

Indeed, a plethora of products was apparent by hplc and nmr after mild acidic hydrolysis of **7a**, but a simpler result was obtained under forcing conditions. When acid hydrolysis (pH 4.0) was immediately followed by heating up to 90°C, 2,5-dimethyl-4-formyl-3-thiophenecarboxylic acid (17) was isolated as a stable final product (40%), and hydrogen sulfide, formaldehyde, acetaldehyde and crotonaldehyde were identified among the volatile by-products. The mechanism for the formation of 17 (Scheme 4) exposes the structure of the 'right-hand' fragment, even if its direct isolation has hitherto failed. As far as the 'left-hand' fragment is concerned, the reactions leading to crotonaldehyde (decarboxylation and dehydration) were clearly accelerated by the particular conditions of the experiment. Under milder condition (pH 5.0 - 6.0, 37°C) the amount of crotonaldehyde present at any time in the hydrolysis mixture (hplc) never exceeded 7% of that theoretically predictable from the amount of **7a** hydrolyzed; neither crotonaldehyde nor the thiophene (17) were ever identified in the hydrolysis of FCE 22101 at pH 7.4. A second factor affecting the stability of *exo*-methylenethiazolines was apparent. As mentioned before, evaporation or freeze-drying of solutions of **7a**,**8a** led almost invariably to total degradation of the solutes, in sharp contrast with their remarkable stability in dilute alkaline solutions. A similar sensitiveness to high concentration conditions and freeze-

drying[50] is proper of their homo-counterparts, the *exo*-methylenedihydrothiazines derived from cephalosporins, and of the conjugated thiazolinones derived from anhydropenicillin,[37] whereas decompositions following a second-order kinetics were not reported for their saturated analogs (the penicilloic acids). Thus, it seems likely that the *exo*-methylene-Δ^3-thiazolines act as Michael acceptors toward their own thiol degradates. It is interesting to recall that degradation of the sodium salt (**7a**) in concentrated solution was accompanied by its partial conversion to **8a**, in analogy with the cleaner result observed upon addition of thiolacetic acid, and to mention that its ester derivative (7c) gave a dimer (whose structure could not be ascertained because of further events) upon storage for one day in the refrigerator.

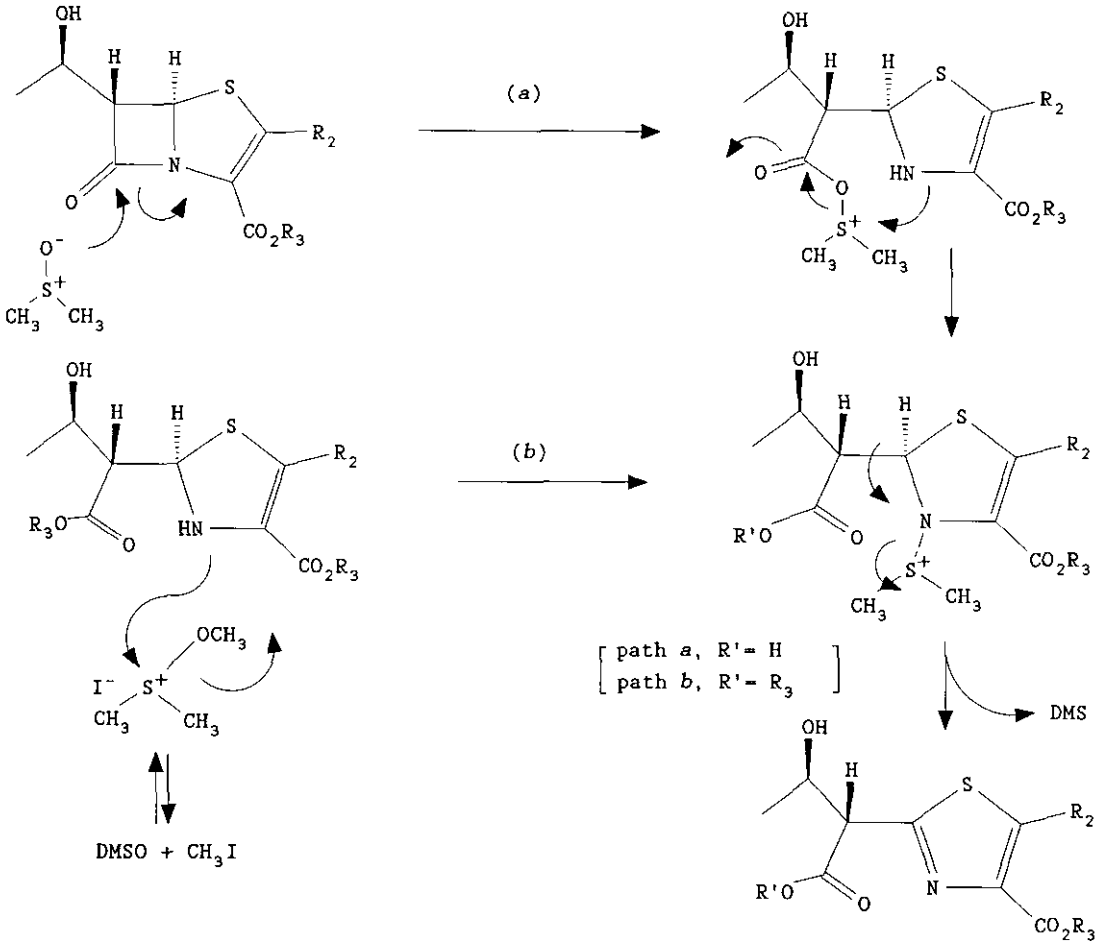
Scheme 4



Thiazoles were obtained in the present study either from protomeric aromatization of *exo*-methylene-Δ^3-thiazolines or from oxidative aromatization of Δ^4-thiazolines. The protomeric process (e.g., **7c,8c** → **9a,10a**), promoted by base catalysis and by derivatization of the thiazoline carboxylic function as an ester, does not require particular comment.[51] The oxidative aromatization of Δ^4-thiazolines promoted by chloranil (**2c,3c** → **9a**) has a close literature precedent for Δ^3-thiazolines.[52] A related aromatization (**2a,3a** → **9a**) was observed on Δ^4-thiazolines when their alkylation (CH_3I) was attempted in a DMSO solution. To probe the possibility that aromatization could have involved DMSO as an oxidant, direct

cleavage of the penem ester (**1d**) with neat DMSO was attempted.[53] The main component (27% yield) of the crude reaction mixture was identified as the propenylthiazole (**15a**), but the reaction occurred at a reasonable rate (few hours at 70°C) only in a few occasions difficult to reproduce, and appeared to be sensitive to the water content of DMSO.[54] The mechanisms proposed for the oxidative aromatizations involving DMSO are collectively presented in Scheme 5. A common Δ^4-thiazoline-*N*-sulfonium derivative would arise either from nucleophilic attack of the oxygen atom of DMSO at the β-lactam carbonyl followed by transfer of sulfur from oxygen to nitrogen (path *a*), or by direct electrophilic attack of the thiazoline nitrogen atom to the methoxysulfonium species ('activated DMSO')[55] reversibly generated from DMSO and methyl iodide (path *b*). In the former case, the β-lactam carbonyl is converted to a carboxyl (R'= H) and further events occur (decarboxylation and dehydration) finally leading to the observed product (**15a**).

Scheme 5



These and other 'side-chain reactions' complicate the identification of the thiazole end-products. Epimerization at C-6 (**9a,b** → **10a,b**) was observed on thiazole diesters under base catalysis. Retro-aldol fragmentation was observed on diesters of thiazoles (**9a,b** → **13a,b**) and Δ^3-thiazolines (**5c** → **6c**), and was promoted by silica gel chromatography. Decarboxylation is known to occur readily on the thiazoleacetic acids; it was observed on an isolated derivative (**9c** → **14a**) and, en route, in the cleavage of penem esters mediated by DMSO (**1d** → **15a**). Eliminations observed at C-6,C-8 included loss of silanol (**9c** → **11b,12b**) and dehydration (**9a,10a** → **11a,12a**).

The structure and reactivity of the primary products of β-lactam cleavage bear biochemical and pharmacological consequences. The identification of the (5*S*)-*exo*-methylene-Δ^3-thiazoline (**8a**) proves that β-lactam cleavage and expulsion of a leaving group at C-2′ are not concerted, so that the rate of the former reaction can depend on the C-2′ substituent for its inductive effect[16] *but not* for its nucleofugality. Thus, no advantage is expected from the presence of a leaving group at C-2′ as far as the ability of penems to acylate the serine hydroxyl of the bacterial enzymes is concerned. However, with enzymes of poor catalytic efficiency (as the known bacterial transpeptidases are),[5,6] expulsion of the leaving group might be fast enough to occur at the level of the acyl-enzyme intermediate. Then, the original acylenzyme incorporating the unstable Δ^4-thiazoline moiety would convert to one incorporating the *exo*-methylene-Δ^3-thiazoline, and that might result in a slower reactivation rate.

This mechanistic dichotomy between C-2′ substituted penems and other penems may also be relevant to their metabolism. Most penems are susceptible to renal degradation promoted by dehydropeptidases, and the currently available results[15,20] confirm the prediction that the primary metabolites are identical to those resulting from alkaline hydrolysis. One common product (**7a**, accompanied by minor amounts of the C-5 epimer **8a**) is produced from the penems substituted at C-2′ with a good leaving group, e.g. FCE 22101, whereas Δ^4-thiazolines differing for the original C-2 side-chain are generated from other penems. The present study indicates that the latter type of products are highly unstable at the physiological pH of blood and urine, while the former can survive for a few hours. Actually, **7a** is the major metabolite found in the urine of humans following administration of FCE 22101.[20] Preliminary toxicological results support the hypothesis that intact **7a** is a safe metabolite, but little is known on the tolerability of the plethora of products which can arise from degradation of the Δ^4-thiazolines originated from other penems.

EXPERIMENTAL

General. The ^{1}H- and ^{13}C-nmr spectra were taken at 400 and 100 MHz, respectively, on a Varian VXR-400S spectromer, or, as indicated, at 200 and 50 MHz on a Varian VXR-200 spectrometer, at 27°C unless otherwise stated. Internal references for reported chemical shift values were: $CHCl_3$, 7.25 δ; DMSO, 2.49 δ; CH_3OD, 3.31 δ; HDO (27°C), 4.81 δ; HDO (45°C), 4.62 δ. The ir spectra were obtained on a Perkin-Elmer 1420 spectrophotometer, and the uv spectra were recorded on a Farmitalia Carlo Erba Strumentazione UV Spectracomp 301 spectrophotometer. The FD mass spectra were recorded on a Varian Mat 311/A instrument equipped with a combined EI/FI/FD ion source using benzonitrile activated emitters. The CD spectra were acquired on a Jobin-Yvon Mark V dichrograph. Melting points are uncorrected. The hydrolysis, methanolysis and ammonolysis experiments were monitored by hplc and by ^{1}H-nmr spectroscopy in parallel experiments run with deuterated solvents in the nmr tube. A Kontron 400 hplc apparatus equipped with a Whatman Partisphere C18 column (5 μm, 4.7×110 mm) was used under the following conditions: mobile phase A, 0.05*M* KH_2PO_4 brought to pH 2.5 with conc. H_3PO_4; mobile phase B, CH_3CN/phase A 60:40; elution, gradient from 0% B to 100% B; flow rate, 1 ml/min; detection, uv at 220 and 300 nm. Separation of **7a** and **8a** on a semi-preparative scale was performed by a Hewlett-Packard HP 1090 M chromatographer equipped with a μBondapack C18 column (10 μm, 7.8×300 mm). Column flash chromatograpy was performed either under reversed phase conditions (Merck LiChroprep C18, elution with H_2O and H_2O/CH_3CN mixtures) or with a 230-400 mesh, 60Å silica gel stationary phase. The 'penem-numbering' (shown in formula **2**) was followed for the sake of simplicity in the attribution of the ^{1}H and ^{13}C nmr signals of thiazolines and thiazoles.

(2*S*,3*R*)-2-[(*R*)-2-(4-Carboxy-5-methyl-2,3-dihydrothiazolyl)]-3-hydroxybutanoic acid (2a): Aqueous solutions of **2a** disodium salt (4:1 admixture with **3a**) were obtained from hydrolysis of **1a** sodium salt with 0.1*N* NaOH (slight molar excess, 0°C). ^{1}H Nmr (400 MHz, D_2O) 1.18 (d, J= 6.4 Hz, CH_3-9), 2.17 (s, CH_3-2'), 2.68 (dd, J= 9.1, 9.9 Hz, H-6), 3.97 (dq, J= 6.4, 9.1 Hz, H-8), 4.96 (d, J= 9.9 Hz, H-5). Uv (H_2O; 4:1 admixture with **3a**), λ_{max} 266 (ε= 3,850) and 302 nm (ε= 4,900). CD (H_2O; 4:1 admixture with **3a**), [Θ]= +44,000 (257 nm) and -20,000 deg×cm^{2}×decimole^{-1} (302 nm).

(2*S*,3*R*)-2-[(*R*)-2-(4-Carboxy-5-methoxymethyl-2,3-dihydrothiazolyl)]-3-hydroxybutanoic acid (2b): Aqueous solutions of **2b** disodium salt (3:1 admixture with **3b**, contaminated by increasing amounts of **5a**) were obtained at the early stages of hydrolysis (0.1*N* NaOH slight molar excess, 0°C) of **1c** sodium salt. ^{1}H Nmr (400 MHz, D_2O) 1.21 (d, J= 6.0 Hz, CH_3-9), 2.74 (dd, J= 9.4, 9.7 Hz, H-6), 3.15 (s, CH_3O-2'), 3.99 (dq, J= 6.0, 9.4 Hz, H-8),

4.58 and 4.64 (each d, J= 12.9 Hz, CH_2-2'), 5.08 (d, J= 9.7 Hz, H-5).

Methyl (2*S*,3*R*)-2-[(*R*)-2-(4-methoxycarbonyl-5-methyl-2,3-dihydrothiazolyl)]-3-hydroxybutanoate (2c): To a solution of **1b** (51 mg, 0.21 mmol) in methanol (3 ml) a solution of sodium methoxide (1.7 mg, 0.03 mmol) in the same solvent (0.05 ml) was injected under stirring. An immediate reaction took place (hplc), leading to the almost quantitative formation of **2c** and **3c** (ca. 3:1). The products decomposed when isolation and silica gel chromatography was attempted. Compound **2c**: ^{1}H Nmr (400 MHz, CD_3OD; sample obtained by methanolysis in the nmr tube) 1.10 (d, J= 6.2 Hz, CH_3-9), 2.19 (s, CH_3-2'), 2.84 (dd, J= 8.2, 9.4 Hz, H-6), 3.71 (s, CH_3O-3'), 4.01 (dq, J= 6.2, 8.2 Hz, H-8), 5.19 (d, J= 9.4 Hz, H-5).

Methyl (2*S*,3*R*)-2-[(*R*)-2-(4-methoxycarbonyl-5-methoxymethyl-2,3-dihydrothiazolyl)]-3-hydroxybutanoate (2d): Identified in admixture with **3d** (ca. 3:1) in the early stages of methanolysis (catalytic amount of CH_3ONa in methanol) of **1d**; spontaneously converts to **5b**. ^{1}H Nmr (400 MHz, CD_3OD; sample obtained by methanolysis in the nmr tube) 1.12 (d, J= 6.2 Hz, CH_3-9), 2.88 (dd, J= 8.3, 9.0 Hz, H-6), 3.29 (s, CH_3O-2'), 3.73 (s, CH_3O-3'), 4.01 (dq, J= 6.2, 8.3 Hz, H-8), 4.49 (s, CH_2-2'), 5.25 (d, J= 9.0 Hz, H-5).

(2*S*,3*R*)-2-[(*S*)-2-(4-Carboxy-5-methyl-2,3-dihydrothiazolyl)]-3-hydroxybutanoic acid (3a): Aqueous solutions of **3a** disodium salt (1:4 admixture with **2a**) were obtained as indicated above. ^{1}H Nmr (400 MHz, D_2O) 1.20 (d, J= 6.4 Hz, CH_3-9), 2.14 (s, CH_3-2'), 2.75 (dd, J= 7.5, 7.5 Hz, H-6), 5.15 (d, J= 7.5 Hz, H-5); H-8 obscured.

(2*S*,3*R*)-2-[(*S*)-2-(4-Carboxy-5-methoxymethyl-2,3-dihydrothiazolyl)]-3-hydroxybutanoic acid (3b): Aqueous solutions of **3b** disodium salt (1:3 admixture with **2b**) were obtained as indicated above. ^{1}H Nmr (400 MHz, D_2O) 1.26 (d, J= 6.0 Hz, CH_3-9), 2.81 (dd, J= 8.2, 8.2 Hz, H-6), 3.15 (s, CH_3O-2'), 3.99 (dq, J= 6.0, 8.2 Hz, H-8), 4.3 - 4.5 (m, 2H, CH_2-2), 5.23 (d, J= 8.2 Hz, H-5).

Methyl (2*S*,3*R*)-2-[(*S*)-2-(4-methoxycarbonyl-5-methyl-2,3-dihydrothiazolyl)]-3-hydroxybutanoate (3c): Identified in admixture with **2c** (ca. 1:3), as indicated above. ^{1}H Nmr (400 MHz, CD_3OD; sample obtained by methanolysis in the nmr tube) 1.13 (d, J= 6.5 Hz, CH_3-9), 2.16 (s, CH_3-2'), 2.85 (dd, J= 7.3, 7.3 Hz, H-6), 3.70 (s, CH_3O-3'), 5.31 (d, J= 7.3 Hz, H-5); H-8 obscured.

Methyl (2*S*,3*R*)-2-[(*S*)-2-(4-methoxycarbonyl-5-methoxymethyl-2,3-dihydrothiazolyl)]-3-hydroxybutanoate (3d): Identified in admixture with **2d** (ca. 1:3), as indicated above; spontaneously converts to **5b**. ^{1}H Nmr (400 MHz, CD_3OD; sample obtained by methanolysis in the nmr tube) 1.14 (d, J= 6.2 Hz, CH_3-9), 2.84 (dd, J= 7.3, 7.3 Hz, H-6), 3.29 (s, CH_3O-2'), 3.72 (s, CH_3O-3'), 4.47 (s, CH_2-2'), 5.38 (d, J= 7.3 Hz, H-5); H-8 obscured.

Methyl (2*S*,3*R*)-2-[(*R*)-2-(3,5-dimethyl-4-methoxycarbonyl-2,3-dihydrothiazolyl)]-3-hydroxybutanoate (4): A sample of **1a** sodium salt (90 mg, 0.36 mmol) was dissolved in H_2O (5.8 ml) and treated dropwise at 0°C with 0.1*N* NaOH (3.2 ml, 0.32 mmol). After standing for 2 h, the solution was freeze-dried and the residue was suspended in DMF (6 ml). Methyl iodide (3 ml, 48 mmol) was added and stirring was continued for 2 h. The reaction mixture was partitioned between ethyl acetate and brine, the organic layer was washed twice with brine, dried over Na_2SO_4 and evaporated. Fractionation by flash chromatography over silica (ethyl acetate/hexane mixtures) gave the title compound (**4**; syrup, 10 mg; 10% yield), the methyl ester of the unreacted penem (**1b**, 9 mg), and a complex mixture of unidentified products (10 mg). ^{1}H Nmr (400 MHz, $CDCl_3$) 1.12 (d, J= 6.2 Hz, CH_3-9), 2.37 (s, CH_3-2'), 2.66 (s, CH_3-N), 2.80 (dd, J= 8.3, 10.8 Hz, H-6), 3.70 and 3.75 (each s, CH_3O-3' and CH_3O-7), 3.98 (dq, J= 6.2, 8.3 Hz, H-8), 4.79 (d, J= 10.8 Hz, H-5), 5.73 (br s, OH-8). ^{13}C Nmr (100 MHz, $CDCl_3$) 14.8 (C-2'), 21.0 (C-9), 40.8 ($\underline{C}H_3$-N), 51.8 and 51.9 ($O\underline{C}H_3$-3' and $O\underline{C}H_3$-7), 59.4 (C-6), 69.6 (C-8), 77.4 (C-5), 128.7 (C-3), 147.2 (C-2), 161.5 (C-3'), 171.5 (C-7). Uv (CH_3CN), λ_{max} 313 nm. CD (CH_3CN), [Θ]= +36,000 (255 nm) and -26,000 deg×cm^2×decimole^{-1} (314 nm). FD-ms (EHC= 17 mA), m/z 289 ($M^{+\cdot}$); EI-ms, m/z 289 (2%), 172 (100%).

(2*S*,3*R*)-2-[(2*S*,5*R*)-2-(4-Carboxy-5-methoxymethyl-2,5-dihydrothiazolyl)]-3-hydroxybutanoic acid (5a-I): A sample of **1c** sodium salt (175 mg, 0.62 mmol) was dissolved in H_2O (6.2 ml) and treated dropwise at 0°C under nitrogen with 0.1*N* NaOH (6.2 ml, 0.62 mmol). After stirring for 10 h at 0°C and for further 38 h at room temperature, the solution was concentrated in vacuo and fractionated by reversed phase chromatography. Two fractions were collected and freeze-dried separately. Fraction A (125 mg, 63%) contained almost exclusively **5a-I**,**5a-II** (ca. 1:1) as their disodium salts; fraction B (42 mg, 21%) consisted mainly of **5a-III**,**5a-IV** disodium salts (ca. 1.5:1). Isomer **5a-I**: ^{1}H Nmr (400 MHz, D_2O) 1.26 (d, J= 6.2 Hz, CH_3-9), 2.60 (dd, J= 7.9, 9.7 Hz, H-6), 3.39 (s, CH_3O-2'), 3.79 (m, 2H, CH_2-2'), 4.25 (m, 1H, H-8), 4.93 (m, 1H, H-2), 5.90 (dd, J= 4.9, 9.7 Hz, H-5). Ir (KBr; in admixture with **5a-II**), ν_{max} 3420, 1630, 1590, 1395 cm^{-1}. Uv (H_2O; in admixture with **5a-II**), 240 nm end absorption. FAB-ms (in admixture with **5a-II**), m/z 322 $[M+H]^+$, 344 $[M+Na]^+$.

(2*S*,3*R*)-2-[(2*S*,5*S*)-2-(4-Carboxy-5-methoxymethyl-2,5-dihydrothiazolyl)]-3-hydroxybutanoic acid (5a-II): Obtained (disodium salt) in admixture with **5a-I** as described above. ^{1}H Nmr (400 MHz, D_2O) 1.27 (d, J= 6.2 Hz, CH_3-9), 2.66 (dd, J= 7.3, 10.7 Hz, H-6), 3.41 (s, CH_3O-2'), 3.71 (dd, J= 6.4, 10.2 Hz, C$\underline{H}$(H)-2'), 3.83 (dd, J= 4.4, 10.2 Hz, CH($\underline{H}$)-2'), 4.25 (m, 1H, H-8), 4.87 (ddd, J= 2.3, 4.4, 6.4 Hz, H-2), 5.88 (dd, J= 2.3, 10.0 Hz, H-5).

(2*S*,3*R*)-2-[(2*R*,5*RS*)-2-(4-Carboxy-5-methoxymethyl-2,5-dihydrothiazolyl)]-3-hydroxybutanoic acids (5a-III and 5a-IV): Obtained as a 1.5:1 mixture of isomers as described for 5a-I. Major component, **5a-III** disodium salt: ^{1}H Nmr (400 MHz, D_2O) 1.25 (d, J= 6.2 Hz, CH_3-9), 2.66 (dd, J= 7.5, 9.1 Hz, H-6), 3.39 (s, CH_3O-2'), 3.67 (dd, J= 6.7, 10.3 Hz, CH(H)-2'), 3.85 (dd, J= 4.2, 10.3 Hz, CH(H)-2'), 4.16 (m, 1H, H-8), 4.90 (m, 1H, H-2), 5.86 (dd, J= 3.0, 7.5 Hz, H-5). Minor component, **5a-IV** disodium salt: ^{1}H Nmr (400 MHz, D_2O) 1.25 (d, J= 6.2 Hz, CH_3-9), 2.63 (dd, J= 7.3, 9.8 Hz, H-6), 3.37 (s, CH_3O-2'), 3.76 (m, 2H, CH_2-2'), 4.16 (m, 1H, H-8), 4.85 (m, 1H, H-2), 5.88 (dd, J= 5.3, 7.3 Hz, H-5).

4-Nitrobenzyl (2*S*,3*R*)-2-[(2*RS*,5*RS*)-2-(5-methoxymethyl-4-(4-nitrobenzyl)oxycarbonyl-2,5-dihydrothiazolyl)]-3-hydroxybutanoate (5c): A sample of **1c** (174 mg, 0.67 mmol) was dissolved in H_2O (6.7 ml) and treated dropwise at 0°C under nitrogen with 0.1*N* NaOH (13.4 ml, 1.34 mmol). After standing for 2 h, the solution was freeze-dried and the residue was suspended in DMF (15 ml). 4-Nitrobenzyl bromide (290 mg, 13.4 mmol) was added and stirring was continued for 3 h. The reaction mixture was diluted with ethyl acetate and washed in sequence with 5% aqueous $NaHCO_3$, water and brine. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography over silica (ethyl acetate/hexane mixtures) gave a first fraction consisting of the retro-aldol product (**6c**; syrup, 32 mg, 10% yield), and a second fraction containing the title compound (**5c**; syrup, 197 mg, 54% yield). A fraction enriched in isomer I (15 mg) was collected separately.

Isomer I: ^{1}H Nmr (400 MHz, $CDCl_3$) 1.18 (d, J= 6.2 Hz, CH_3-9), 3.01 (dd, J= 7.4, 10.2 Hz, H-6), 3.28 (s, CH_3O-2'), 3.45 (dd, J= 3.3, 10.0 Hz, C<u>H</u>(H)-2'), 3.76 (br s, OH-8), 3.87 (dd, J= 4.4, 10.0 Hz, CH(<u>H</u>)-2'), 4.31 (m, 1H, H-8), 4.82 (ddd, J= 2.0, 3.3, 4.4 Hz, H-2), 5.37 and 5.25 (each ABq, 4H, OCH_2-3' and OCH_2-7), 6.09 (dd, J= 2.0, 10.2 Hz, H-5), 7.52 (m, 4H, *meta*-NO_2Ar), 8.20 (m, 4H, *ortho*-NO_2Ar). ^{13}C Nmr (100 MHz, $CDCl_3$) 20.6 (C-9), 58.2 (C-2), 59.4 (O<u>C</u>H_3-2'), 63.5 (C-6), 65.3 and 66.3 (2×<u>C</u>H_2Ar), 70.0 (C-8), 73.6 (C-2'), 81.7 (C-5), 123.8 and 124.0 (4×*ortho*-NO_2Ar), 128.5 and 128.7 (4×*meta*-NO_2Ar), 141.7 and 142.5 (2×*para*-NO_2Ar), 148.0 and 148.1 (2×<u>C</u>-NO_2), 160.6 (C-3'), 163.7 (C-3), 170.4 (C-7). Ir ($CHCl_3$), ν_{max} 1735 cm^{-1}. Uv (CH_3CN), λ_{max} 266 nm (ε= 20,040). FD-ms, m/z 584 $[M+H]^+$.

Isomer II: ^{1}H Nmr (400 MHz, $CDCl_3$) 1.20 (d, J= 6.2 Hz, CH_3-9), 2.92 (dd, J= 7.3, 9.2 Hz, H-6), 3.28 (s, CH_3O-2'), 3.53 (dd, J= 3.8, 9.9 Hz, C<u>H</u>(H)-2'), 3.83 (dd, J= 4.6, 9.9 Hz, CH(<u>H</u>)-2'), 4.33 (m, 1H, H-8), 4.77 (m, 1H, H-2), 5.2 - 5.4 (m, 4H, OCH_2-3' and OCH_2-7), 6.11 (dd, J= 4.3, 9.2 Hz, H-5), 7.52 (m, 4H, *meta*-NO_2Ar), 8.20 (m, 4H, *ortho*-NO_2Ar).

Isomer III: ^{1}H Nmr (400 MHz, $CDCl_3$) 1.24 (d, J= 6.5 Hz, CH_3-9), 3.20 (dd, J= 6.2, 7.2 Hz, H-6), 3.26 (s, CH_3O-2'), 3.4 - 3.9 (m, 2H, CH_2-2'), 4.40 (m, 1H, H-8), 4.90 (m, 1H, H-2), 5.2 - 5.4 (m, 4H, OCH_2-3' and OCH_2-7), 6.06 (dd, J= 3.2, 6.2 Hz, H-5), 7.50 (m, 4H, *meta*-

NO_2Ar), 8.20 (m, 4H, *ortho*-NO_2Ar).

Isomer IV: ^1H Nmr (400 MHz, $CDCl_3$) 1.30 (d, J= 6.3 Hz, CH_3-9), 3.00 (dd, J= 7.3, 7.3 Hz, H-6), 3.27 (s, CH_3O-2'), 3.4 - 3.9 (m, 2H, CH_2-2'), 4.30 (m, 1H, H-8), 4.77 (m, 1H, H-2), 5.2 - 5.4 (m, 4H, OCH_2-3' and OCH_2-7), 6.12 (dd, J= 5.5, 7.3 Hz, H-5), 7.50 (m, 4H, *meta*-NO_2Ar), 8.20 (m, 4H, *ortho*-NO_2Ar).

4-Nitrobenzyl 2-[(2*RS*,5*RS*)-2-(5-methoxymethyl-4-(4-nitrobenzyl)oxycarbonyl-2,5-dihydrothiazolyl)]acetate (6c): Obtained as a 1:1 mixture of two diastereoisomers (A and B; enantiomeric mixture each), as described in the preparation of 5c (syrup, 10% isolated yield). ^1H Nmr (400 MHz, $CDCl_3$) 2.85 and 2.91 (each dd, J= 9.5, 16.6 and 8.6, 16.6 Hz, CH(H)-6 of isomers A and B), 3.26 and 3.28 (each s, CH_3O-2' of A and B), 3.30 (m, CH(H)-6 of A and B), 3.46 (dd, J= 3.2, 9.8 Hz, CH(H)-2' of A), 3.56 (dd, J= 4.2, 9.8 Hz, CH(H)-2' of B), 3.79 (dd, J= 4.7, 9.8 Hz, CH(H)-2' of B), 3.87 (dd, J= 4.1, 9.8 Hz, CH(H)-2' of A), 4.84 (m, H-2 of A), 4.86 (m, H-2 of B), 5.2-5.4 (m, OCH_2-3' and OCH_2-7), 6.09 (m, H-5 of A and B), 7.53 (m, 4H, *meta*-NO_2Ar), 8.20 (m, 4H, *ortho*-NO_2Ar). Ir ($CHCl_3$), ν_{max} 1735 cm^{-1}. Uv (CH_3CN), λ_{max} 265 nm; FAB-ms, m/z 504 $[M+H]^+$.

(2*S*,3*R*)-2-[(*R*)-2-(4-Carboxy-5-methylene-2,5-dihydrothiazolyl)]-3-hydroxybutanoic acid (7a) This compound was never isolated pure. A sample of the diammonium salt of 7a impure from external salt (73% titre by hplc) was obtained once as described in ref. 19. The following procedure was used to obtain solutions of disodium salts of the separate components (**7a**,**8a**). A solution of **1e** (44 mg, 0.15 mmol) in distilled water (100 ml) was treated with 0.1*N* NaOH (100 ml, 10 mmol). After 3 min enough Amberlite CG50 (acid form) was added to the solution as to lower the pH down to 8.5. After filtration and concentration in vacuo to a final volume of 10 ml, the solution was repeatedly injected at 150 µl portions, bracketed by two 50 µl layers of 1*M* H_3PO_4, into the hplc apparatus (see General). A linear gradient of pH 3.5 0.05*M* phosphate buffer/CH_3CN from 0% to 12% organic solvent in 7.5 min at a 4 ml/min flow allowed separate elution of **8a** first and **7a** second. The product-containg fractions were collected, cooled in an ice bath, and immediately neutralized with microliter additions of a saturated Na_2CO_3 solution (final pH 8.5). The entire procedure was made automatic through the instrument data station. The resulting solutions were concentrated in vacuo, cooled on ice, and filtered to remove precipitated salts. The amount of **7a** and **8a** was estimated from optical density at 300 nm, on the basis of ε= 4,200 $M^{-1}cm^{-1}$ for both compounds. Samples for nmr spectroscopy were prepared by solvent exchange with several cycles of dilution in D_2O followed by concentration in vacuo.

^1H Nmr (400 MHz, D_2O) 1.16 (d, J= 6.2 Hz, CH_3-9), 2.56 (dd, J= 7.5, 8.5 Hz, H-6), 4.01 (dq, J= 6.2, 8.5 Hz, H-8), 5.49 and 5.54 (each dd, J= 1.5, 1.5 Hz, CH_2-2'), 5.93 (ddd, J=

1.5, 1.5, 7.5 Hz, H-5). ^{13}C Nmr (100 MHz, D_2O) 22.3 (C-9), 64.5 (C-6), 69.1 (C-8), 82.6 (C-5), 108.4 (C-2'), 147.7 (C-2), 170.1 (C-3), 171.6 (C-3'), 179.7 (C-7). Ir (KBr; diammonium salt), ν_{max} 1620 cm^{-1} (br). Uv (H_2O), λ_{max} 300 nm (ε= 4,200). CD (H_2O), [Θ]= -12,600 (307 nm) deg×cm^2×decimole^{-1}.

(2*S*,3*R*)-2-[(*R*)-2-(4-Carboxy-5-methylene-2,5-dihydrothiazolyl)]-3-hydroxybutanamide (7b): A solution of **1e** free acid (600 mg; 2.1 mmol) in 3% ammonium hydroxyde (2 ml) was kept for 1 h at 20°C. After addition of a second portion of ammonium hydroxide (1 ml), the disappearance of **1e** was checked by hplc. The solution was evaporated in vacuo to dryness to afford the title product (ammonium salt) as a white powder (550 mg, 100%). ^{1}H Nmr (400 MHz, D_2O) 1.34 (d, J= 6.4 Hz, CH_3-9), 2.83 (dd, J= 8.2, 8.2 Hz, H-6), 4.24 (dq, J= 6.4, 8.2 Hz, H-8), 5.65 and 5.71 (each dd, J= 1.6, 1.6 Hz, CH_2-2'), 6.15 (ddd, J= 1.6, 1.6, 8.2 Hz, H-5). ^{13}C Nmr (100 MHz, D_2O) 20.3 (C-9), 60.2 (C-6), 67.3 (C-8), 80.3 (C-5), 108.2 (C-2'), 146.2 (C-2), 169.9 (C-7), 170.2 (C-3), 175.7 (C-3'). Ir (KBr; ammonium salt), ν_{max} 1670, 1610 cm^{-1}. Uv (H_2O), λ_{max} 300 nm (ε= 3,850). CD (H_2O), [Θ]= -8,000 (304 nm) deg×cm^2×decimole^{-1}. FAB-ms, m/z 262 [M ammonium salt + H]$^+$, 245 [M+H]$^+$.

Methyl (2*S*,3*R*)-2-[(*R*)-2-(4-methoxycarbonyl-5-methylene-2,5-dihydrothiazolyl)]-3-hydroxybutanoate (7c): A sample of **7a** diammonium salt (50 mg, titre 73%; 0.13 mmol) was dissolved in DMSO (2 ml) and treated with methyl iodide (0.5 ml, 8 mmol). After 15 min, ethyl acetate (30 ml) was added, the solution was washed twice with brine, dried for a short time over Na_2SO_4, concentrated to a small volume and poured onto a silica gel column. Elution with ethyl acetate/cyclohexane mixtures afforded the title product (syrup, 25 mg, ca. 70% yield), slightly impure from a dimer of unknown structure. The latter contaminant increased at the expense of **7c** upon storage but degraded when further fractionation by flash chromatography was attempted. ^{1}H Nmr (200 MHz, $CDCl_3$) 1.30 (d, J= 6.5 Hz, CH_3-9), 3.00 (dd, J= 6.5, 6.5 Hz, H-6), 3.71 (s, CH_3O-7), 3.91 (s, CH_3O-3'), 4.30 (dq, J= 6.5, 6.5 Hz, H-8), 5.60 and 5.96 (each dd, J= 1.8, 1.8 Hz, CH_2-2'), 6.26 (ddd, J= 1.8, 1.8, 6.5 Hz, H-5). Ir ($CHCl_3$), ν_{max} 1735, 1700 cm^{-1}. FD-ms, m/z 546, 273 [M]$^{+\cdot}$.

(2*S*,3*R*)-2-[(*R*)-2-(4-Carboxy-5-methylene-2,5-dihydrothiazolyl)]-3-(*tert*-butyldimethylsilyloxy)butanoic acid (7e): A solution of **1h** (1.3 g, 1.95 mmol) in acetonitrile (30 ml) was treated at 0°C dropwise with 1*N* NaOH (2 ml, 2 mmol). After stirring for 2 h, the separated solid was collected by filtration, triturated with acetone and dried to afford **7e** disodium salt as a tan powder (300 mg, 38%). Work-up of the mother liquors gave **9c**,**11b**,**12b**,**14a** (see below). ^{1}H Nmr (200 MHz, DMSO-d_6) 0.04 (s, 6H, $Si(CH_3)_2$), 0.86 (s, 9H, Si-*t*-C_4H_9), 1.12 (d, J= 6.0 Hz, CH_3-9), 2.24 (dd, J= 7.0, 8.9 Hz, H-6), 4.21 (dq, J= 6.0, 8.9 Hz, H-8), 5.29 and 5.93 (each s, CH_2-2'), 5.86 (d, J= 7.0 Hz, H-5). Ir (KBr), ν_{max} 1620 cm^{-1} (br).

(2*S*,3*R*)-2-[(*S*)-2-(4-Carboxy-5-methylene-2,5-dihydrothiazolyl)]-3-hydroxybutanoic acid (8a)

A) Solutions of this compound (disodium salt) in H_2O and D_2O were obtained as described in the preparation of **7a**. ^1H Nmr (400 MHz, D_2O, 45°C) 1.04 (d, J= 6.4 Hz, CH_3-9), 2.49 (dd, J= 7.0, 9.7 Hz, H-6), 4.01 (dq, J= 6.4, 7.0 Hz, H-8), 5.41 and 5.44 (m, 2H, CH_2-2'), 5.83 (d, J= 9.7 Hz, H-5). ^{13}C Nmr (100 MHz, D_2O) 20.7 (C-9), 65.0 (C-6), 69.6 (C-8), 83.4 (C-5), 109.4 (C-2'), 146.5 (C-2), 170.4 (C-3), 171.2 (C-3'), 179.2 (C-7). Uv (H_2O), λ_{max} 300 nm (ε= 4,200). CD (H_2O), [Θ]= +10,000 deg×cm^2×decimole^{-1} (305 nm).

B) A solution of **7a** diammonium salt (13 μmol) in DMSO-d_6 (2ml) was mixed with a solution of thiolacetic acid (14 μmol) in CD_3CN (0.1 ml). The **8a**:**7a** ratios were (hplc, ^1H nmr): time zero, 0; 2 min, 0.4; 15 min, 0.9; 1 hour, 1.3; 4 hours, 1.6.

(2*S*,3*R*)-2-[(*S*)-2-(4-Carboxy-5-methylene-2,5-dihydrothiazolyl)]-3-hydroxybutanamide (8b):

A solution of **7b** ammonium salt (5 mg, 19 μmol) in D_2O (2 ml) was mixed with a solution of thiolacetic acid (21 μmol) in CD_3CN (0.1 ml). The **8b**:**7b** ratios were (hplc, ^1H nmr): time zero, 0; 15 min, 0.2; 1 h, 0.9; 20 h, 1.4. Compound **8b**: ^1H Nmr (400 MHz, D_2O) 1.36 (d, J= 7.0 Hz, CH_3-9), 3.04 (dd, J= 7.9, 7.9 Hz, H-6), 4.31 (dq, J= 7.0, 7.9 Hz, H-8), 5.71 and 5.75 (each dd, J= 1.6, 1.6 Hz, CH_2-2'), 6.23 (ddd, J= 1.6, 1.6, 7.9 Hz, H-5).

Methyl (2*S*,3*R*)-2-[2-(4-methoxycarbonyl-5-methylthiazolyl)]-3-hydroxybutanoate (9a):

A) A solution of **2c**,**3c** (ca. 3:1; estimated 0.2 mmol) in methanol (3 ml) was obtained from methanolysis of **1b** (51 mg, 0.21 mmol) as described above. Chloranil (49 mg, 0.2 mmol) was added and the suspension was warmed up to 30°C. After 5 min the reaction mixture was concentrated to half volume and poured onto a short silica gel column. Fast elution with ethyl acetate/hexane mixtures (from 1:4 to 1:1) afforded a main fraction consisting of the title product (syrup, 25 mg; 45% yield). ^1H Nmr (400 MHz, $CDCl_3$) 1.17 (d, J= 6.4 Hz, CH_3-9), 2.74 (s, CH_3-2'), 3.13 (br s, OH-8), 3.73 (s, OCH_3-7), 3.91 (s, OCH_3-3'), 4.18 (d, J= 4.4 Hz, H-6), 4.51 (dq, J= 4.4, 6.4 Hz, H-8). Ir ($CHCl_3$), ν_{max} 1725 cm^{-1} (br). Uv (95% EtOH), λ_{max} 245 nm. FD-*ms*, *m/z* 273 $[M]^{+\cdot}$.

B) A solution of **2a**,**3a** (ca. 4:1; estimated 0.12 mmol) in water (5 ml) was obtained from hydrolysis of **1a** sodium salt (43 mg, 0.17 mmol) with 0.1*N* NaOH (2 ml, 0.2 mmol), as reported above. This solution was freeze-dried, the residue was suspended in DMSO (4 ml) and treated with methyl iodide (1.5 ml, 24 mmol). After 1 h the reaction mixture was partitioned between ethyl acetate and water. The organic phase was washed with water, dried over Na_2SO_4 and evaporated in vacuo. The residue was purified by silica gel chromatography as indicated above to afford a main fraction (syrup, 10 mg), consisting of an inseparable mixture of **9a** and an unidentified product, which degraded during the acquisition of a ^{13}C nmr spectrum.

C) A solution of **7c** (25 mg, 0.09 mmol) in dichloromethane (20 ml) was treated with triethylamine (0.4 ml, 2.8 mmol). After 2 h the reaction mixture was washed with 3% HCl in water, washed again with brine, dried over Na_2SO_4, concentrated in vacuo to a small volume (1 ml) and poured onto a silica gel column. Rapid elution with ethyl acetate/hexane mixtures (from 1:4 to 1:1) and evaporation of the main fraction afforded an inseparable mixture of **9a**,**10a** (ca. 1.2:1) as a syrup (20 mg, 80% yield), contaminated by a minor amount of **13a** (ca. 10%).

D) A solution of **8a** disodium salt (estimated 7 μmoles), obtained as described above, was concentrated to a small volume in vacuo, diluted with DMSO (1 ml), and treated with methyl iodide (0.5 ml, 8 mmol) to obtain a solution of the crude dimethyl ester (**8c**). After 0.5 h the reaction mixture was partitioned between ethyl acetate and brine, and triethylamine (0.1 ml, 0.7 mmol) was added to the dried (Na_2SO_4) organic layer. Work-up and chromatography as described above under heading C afforded a mixture of **9a**,**10a** (ca. 1.1:1 by nmr integration) as a syrup (1 mg, 52% yield).

Methyl (2*S*,3*R*)-2-[2-(4-allyloxycarbonyl-5-methylthiazolyl)]-3-hydroxybutanoate (9b):
A solution of **1g** trifluoromethanesulfonate salt (200 mg, 0.4 mmol) in 80% aqueous methanol (20 ml) was let aside for 1 day. The solvent was removed in vacuo and the residue was fractionated by silica gel chromatography (ethyl acetate/hexane mixtures) to obtain **13b** (first-eluting, 50 mg), and an inseparable mixture of **9b**,**10b** (1.2:1; syrup, 39 mg; 33% yield). Compound **9b**: ^{1}H Nmr (200 MHz, $CDCl_3$) 1.17 (d, J= 6.7 Hz, CH_3-9), 2.72 (s, CH_3-2'), 3.73 (s, OCH_3-7), 4.16 (d, J= 4.4 Hz, H-6), 4.57 (dq, J= 4.4, 6.7 Hz, H-8), 4.82 (d, J= 5.7 Hz, allyl $C\underline{H}_2$-CH=), 5.26 (d, J= 10.0 Hz, *cis*-CH=CH($\underline{H}$)), 5.37 (d, J= 17.2 Hz, *trans*-CH=C$\underline{H}$(H)), 5.99 (ddt, J= 10.0, 17.2, 5.7 Hz), CH_2-C$\underline{H}$=CH_2). Ir ($CHCl_3$; in admixture with **10b**), ν_{max} 1735 (sh), 1715 cm^{-1}. Uv (95% EtOH; in admixture with **10b**), λ_{max} 245 nm (ε= 7,950). FD-ms (in admixture with **10b**), m/z 299 $[M]^{+\cdot}$, 255.

(2*S*,3*R*)-2-[-2-(4-Allyloxycarbonyl-5-methylthiazolyl)]-3-(*tert*-butyldimethylsilyl)oxybutanoic acid (9c): This compound was identified among the products of alkaline hydrolysis of **1h**, as described above for the preparation of **7e**. ^{1}H Nmr analysis of the mother liquors of **7e** revealed the presence of **9c**, **11b**, **12b**, and **14a** in the relative ratio of 43:27:5:25. Work-up and chromatography allowed the separation of **14a**, while the other components could not be isolated pure. Compound **9c**: ^{1}H Nmr (200 MHz, $CDCl_3$) 0.04 (s, 6H, $Si(CH_3)_2$), 0.83 (s, 9H, Si-*t*-C_4H_9), 1.10 (d, J= 6.2 Hz, CH_3-9), 2.79 (s, CH_3-2'), 4.27 (d, J= 3.4 Hz, H-6), 4.77 (dq, J= 3.4, 6.2 Hz, H-8), 4.81 (d, J= 5.7 Hz, $C\underline{H}_2$CH=), 5.2-5.4 (m, CH=C$\underline{H}_2$), 5.97 (m, $CH_2C\underline{H}$=CH_2).

Methyl (2*R*,3*R*)-2-[2-(4-methoxycarbonyl-5-methylthiazolyl)]-3-hydroxybutanoate (10a): Isolated in admixture with **9a** in the reactions reported above under headings C and D. ^{1}H Nmr (400 MHz, $CDCl_3$) 1.22 (d, J= 6.3 Hz, CH_3-9), 2.75 (s, CH_3-2'), 3.17 (br s, OH-8), 3.75 (s, OCH_3-7), 3.91 (s, OCH_3-3'), 4.17 (d, J= 6.0 Hz, H-6), 4.33 (dq, J= 6.0, 6.3 Hz, H-8).

Methyl (2*R*,3*R*)-2-[2-(4-allyloxycarbonyl-5-methylthiazolyl)]-3-hydroxybutanoate (10b): Isolated in admixture with **9b** as reported above. ^{1}H Nmr (200 MHz, $CDCl_3$) 1.20 (d, J= 6.7 Hz, CH_3-9), 2.72 (s, CH_3-2'), 3.75 (s, OCH_3-7), 4.17 (d, J= 6.0 Hz, H-6), 4.37 (dq, J= 6.0, 6.7 Hz, H-8), 4.82 (d, J= 5.7 Hz, allyl C$\underline{H}_2$-CH=), 5.26 (d, J= 10.0 Hz, *cis*-CH=CH($\underline{H}$)), 5.37 (d, J= 17.2 Hz, *trans*-CH=C$\underline{H}$(H)), 5.99 (ddt, J= 10.0, 17.2, 5.7 Hz, CH_2-C$\underline{H}$=CH_2).

Methyl 2-[2-(4-methoxycarbonyl-5-methylthiazolyl)]-(*Z*)-2-butenoate (11a):

A) Compound **9a** (10 mg), obtained as reported above under heading B, was let aside for 1 day in chloroform solution. After flash-chromatography (ethyl acetate/hexane mixtures), the title compound, contaminated by a minor amount (ca. 1:5) of the inseparable (*E*) isomer (**12a**), was isolated as a syrup (7.5 mg, 80% yield). ^{1}H Nmr (200 MHz, $CDCl_3$) 2.15 (d, J= 7.5 Hz, CH_3-9), 2.79 (s, CH_3-2'), 3.80 (s, OCH_3-7), 3.92 (s, OCH_3-3'), 7.43 (q, J= 7.5 Hz, H-8). Ir ($CHCl_3$; in admixture with **12a**), ν_{max} 1715 cm^{-1} (br). FD-ms, m/z 255 $[M]^{+\cdot}$.

B) The mixture of **9a**,**10a** (ca. 1.2:1), obtained as reported above for the preparation of **9a**, heading C, was let aside for 1 day in $CDCl_3$ solution. ^{1}H Nmr analysis revealed the almost complete disappearance of **9a**,**10a** and the formation of **11a**,**12a** (ca. 1:1).

2-[2-(4-Allyloxycarbonyl-5-methylthiazolyl)]-(*Z*)-2-butenoic acid (11b): Identified among the products of alkaline hydrolysis of **1h**, as described above for the preparation of **9c**. ^{1}H Nmr (200 MHz, $CDCl_3$) 2.22 (d, J= 7.6 Hz, CH_3-9), 2.74 (s, CH_3-2'), 4.81 (d, J= 5.7 Hz, allyl C$\underline{H}_2$-CH=), 5.2-5.4 (m, CH=C$\underline{H}_2$), 5.97 (m, CH_2-C$\underline{H}$=CH_2), 7.73 (q, J= 7.6 Hz, H-8). Most distinguished signal of the minor (*E*) isomer (**12b**; ca. 1:5): 2.35 (d, J= 7.5 Hz, CH_3-9).

Methyl 2-[2-(4-methoxycarbonyl-5-methylthiazolyl)]-(*E*)-2-butenoate (12a): Obtained in the preparations of **11a** reported above, as an inseparable mixture with the latter (ca. 1:5, heading A; ca. 1:1, heading B). ^{1}H Nmr (200 MHz, $CDCl_3$) 2.20 (d, J= 7.5 Hz, CH_3-9), 2.74 (s, CH_3-2'), 3.89 (s, CH_3-7'), 3.92 (s, CH_3-3'), 7.54 (q, J= 7.5 Hz, H-8).

Methyl 2-[2-(4-methoxycarbonyl-5-methylthiazolyl)]ethanoate (13a): This compound was a minor side-product in the preparation of **9a**,**10a** (headings C and D). The amount of **13a** increased at their expense after silica gel chromatography. Conversion of **9a**,**10a** to **13a** was almost quantitative under slow flow elution conditions, in which case **13a** was isolated pure as a syrup. ^{1}H Nmr (400 MHz, $CDCl_3$) 2.76 (s, CH_3-2'), 3.72 (s, OCH_3-7), 3.92 (s, OCH_3-3'), 4.06 (s, CH_2-6). Ir ($CHCl_3$), ν_{max} 1735, 1715 cm^{-1}. Uv (95% EtOH), λ_{max} 245 nm. FD-ms, m/z 229 $[M]^{+\cdot}$.

Methyl 2-[2-(4-allyloxycarbonyl-5-methylthiazolyl)]ethanoate (13b): This compound was obtained as described above for the preparation of **9b** by methanolys of **1g** trifluoromethanesulfonate salt (200 mg, 0.4 mmol), followed by silica gel chromatography (ethyl acetate/hexane mixtures). Syrup (50 mg; 49% yield). ^{1}H Nmr (200 MHz, $CDCl_3$) 2.73 (s, CH_3-2'), 3.73 (s, OCH_3-7), 4.06 (s, CH_2-6), 4.82 (d, J= 5.8 Hz, allyl $C\underline{H}_2$-CH=), 5.24 (d, J= 10.0 Hz, *cis*-CH=CH(H)), 5.35 (d, J= 17.3 Hz, *trans*-CH=C$\underline{H}$(H)), 6.00 (ddt, J= 10.0, 17.3, 5.8 Hz, CH_2-C$\underline{H}$=CH_2). ^{13}C Nmr (50 MHz, $CDCl_3$) 13.2 (C-2'), 38.4 (C-6), 52.6 (C-7'), 65.8 ($\underline{C}H_2$-CH=), 119.0 (CH=$\underline{C}H_2$), 132.1 ($\underline{C}$H=CH_2); C-2, C-3, C-3', C-5: not seen. Ir ($CHCl_3$), ν_{max} 1720 cm^{-1} (br). Uv (95% EtOH), λ_{max} 245 nm (ε= 7,500). FD-ms, m/z 255 $[M]^{+\cdot}$.

Allyl 2-[(*R*)-2-(*tert*-butyldimethylsilyloxy)propyl]-5-methylthiazole-4-carboxylate (14a): This compound was detected in the alkaline hydrolysis mixture of **1h** trifluoromethanesulfonate salt, as described above for the preparation of **7e** and **9c**, and was isolated after silica gel chromatography (ethyl acetate/hexane mixtures) as a syrup in 10% yield. ^{1}H Nmr (200 MHz, $CDCl_3$) 0.04 (s, 6H, $Si(CH_3)_2$), 0.83 (s, 9H, Si-*t*-C_4H_9), 1.15 (d, J= 6.1 Hz, CH_3-9), 2.70 (s, CH_3-2'), 3.04 (m, CH_2-6), 4.23 (m, H-8), 4.81 (d, J= 5.7 Hz, $C\underline{H}_2$CH=), 5.2-5.4 (m, CH=C$\underline{H}_2$), 5.97 (m, $CH_2C\underline{H}$=CH_2). Ir ($CHCl_3$), ν_{max} 1715 cm^{-1}. Uv (95% EtOH), λ_{max} 243 nm. FD-ms, m/z 355 $[M]^{+\cdot}$, 299.

Methyl 5-methoxymethyl-2-[(*E*)-1-propenyl]thiazole-4-carboxylate (15a): A solution of **1d** (31 mg, 0.11 mmol) in DMSO (0.4 ml) was kept at 70°C for 6 h, after which period only traces of the starting material could be detected (hplc). The solution was diluted with distilled water and freeze-dried. Silica gel chromatography (ethyl acetate/hexane mixture, from 1:4 to 1:1) afforded the title product (7 mg, 27% yield) as a waxy solid. ^{1}H Nmr (400 MHz, $CDCl_3$) 1.90 (dd, J= 1.5, 6.5 Hz, CH_3-9), 3.48 (s, CH_3O-2'), 3.91 (s, OCH_3-3'), 4.96 (s, CH_2-2'), 6.52 (dq, J= 6.5, 15.8 Hz, H-8), 6.62 (dq, J= 1.5, 15.8 Hz, H-6). Ir ($CHCl_3$), ν_{max} 1720 cm^{-1}. Uv (CH_3CN), λ_{max} 220, 276 nm (ε= 4,400). FD-ms, m/z 227 $[M]^{+\cdot}$.

Methyl 2-methoxycarbonyl-6-methoxymethyl-7(*R,S*)-methyl-4,7-dihydro-1,4-thiazepine-3-carboxylate (16): A solution of **1d** (32 mg, 0.12 mmol) in CH_3OH/DMSO (2:1, 0.3 ml) was heated at 70°C for 8 h. The reaction mixture was diluted with ethyl acetate, washed with water and dried over Na_2SO_4. The solvent was removed in vacuo and the residue was fractionated by silica gel chromatography (ethyl acetate/hexane mixtures), obtaining the title compound (syrup, 9 mg; 27% yield) and the unreacted penem (9.5 mg; 30% recovery). ^{1}H Nmr (400 MHz, $CDCl_3$) 1.46 (d, J= 7.0 Hz, CH_3-7), 3.34 (s, $CH_2OC\underline{H}_3$), 3.69 and 3.88 (each s, CO_2CH_3), 4.22, 4.57 (ABq, J= 11.7 Hz, CH_2O), 4.50 (dq, J= 1.0, 7.0 Hz, H-7), 7.17 (br d, J= 8.2 Hz, NH), 7.38 (dd, J= 1.0, 8.2 Hz, H-5). Ir ($CHCl_3$), ν_{max} 1725 cm^{-1} (br). FD-ms, m/z 287 $[M]^{+\cdot}$.

2,5-Dimethyl-4-formylthiophene-3-carboxylic acid (17): A sample of **1e** free acid (600 mg, 2.1 mmol) in water (150 ml) was hydrolized by the dropwise addition of 0.1*N* NaOH (150 ml, 15 mmol). The resulting solution of **7a**,**8a** was treated with enough 1*M* H_3PO_4 to stabilize the pH at 4.0. After 30 min, the temperature was progressively raised to 90°C, and heating at this temperature was continued for 0.5 h. The solution was cooled and extracted twice with ethyl acetate. The extracts were dried over Na_2SO_4 and the solvent was removed in vacuo. Silica gel chromatography (CH_2Cl_2/CH_3OH 95:5 as eluants) afforded **17** (150 mg, 39%) as crystals, mp 138-140°C. ^{1}H Nmr (200 MHz, $CDCl_3$) 2.68 and 2.70 (each s, CH_3), 10.10 (s, CHO). ^{13}C Nmr (50 MHz, $CDCl_3$) 14.6 and 16.1 (2 × $\underline{C}H_3$), 134.2 (C-2 and C-5, superimposing), 149.5 and 152.5 (C-3 and C-4), 165.0 ($\underline{C}O_2H$), 188.2 ($\underline{C}HO$). Ir ($CHCl_3$), λ_{max} 1715, 1680, 1630 cm^{-1}. Uv (CH_3OH), λ_{max} 225, 265 nm (ε= 10,200). FD-ms, m/z 184 $[M]^{+\cdot}$.

ACKNOWLEDGEMENTS

We thank Piergiuseppe Orezzi for a generous gift of **7a** and helpful discussions, Giuseppe Cassinelli for his interest, and IMI (Istituto Mobiliare Italiano) for financial support.

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23. Although the precise nature of the long wavelength transition of the chromophores of penems and *exo*-methylene-Δ^3-thiazolines is not known, the geometrical position of the substituents at the relevant chiral center (C-5) is preserved with respect to possible symmetry elements in π-π* or n-π* transitions of these chromophores. We may thus speculate that the sign of the corresponding Cotton effect should be maintained for compounds with identical stereochemistry at C-5.

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Received, 3rd December, 1991