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TRANSFORMATION OF 3-THIAZOLIOMETHYLCEPHALOSPORIN INTO 3-SPIROCEPHALOSPORIN BY INTRAMOLECULAR MICHAEL ADDITION [1]

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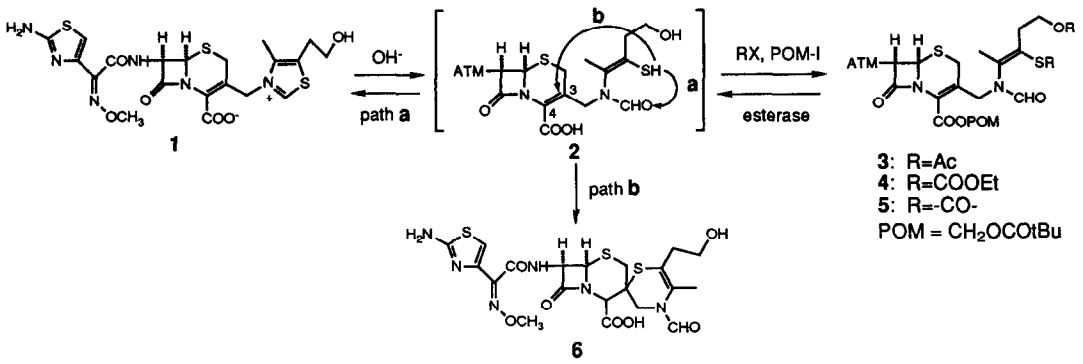
Abstract. 3-Thiazoliomethylcephalosporin **1** was transformed under alkaline conditions into 3-spirocephalosporin **6**. The structures of three isolated stereoisomers were elucidated by NMR experiments.

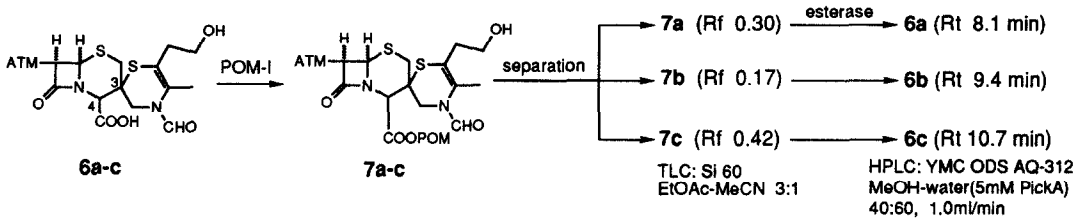
Introduction

Quaternary ammonium-type cephalosporin **1**, 7β-[2-(2-aminothiazol-4-yl)-(z)-2-methoxyiminoacetamido]-3-[4-methyl-5-(2-hydroxyethyl)thiazoliomethyl]-3-cephem-4-carboxylate, is a new parenteral cephalosporin antibiotic.[2] It shows a potent and broad antimicrobial activity against both Gram-positive and Gram-negative bacteria. In our study of an orally active prodrug of cephalosporin **1**, the thiazoliomethyl moiety at the C-3 position was converted to an open-ring thiolate **2** under alkaline conditions, and the resulting thiol group was protected by biologically labile functions to obtain three derivatives **3**, **4** and **5**.[3] This approach had succeeded in some orally active prodrugs of thiamine.[4] In the case of our derivatives **3-5**, however, practical enhancement of oral bioavailability could not be achieved in mice. Analysis of metabolites in urine and feces revealed that the orally administered derivatives **3-5** were mainly transformed into 3-spirocephalosporin **6** (path **b**), and conversion to the original 3-thiazoliomethylcephalosporin **1** was slight (path **a**). This paper describes the chemical transformation of cephalosporin **1** into 3-spirocephalosporin **6** and the structure elucidation of **6**.



Results and Discussion

Preparation of 3-spirocepham 3-Thiazoliomethylcephalosporin **1** was treated under alkaline conditions (pH 12-13) to generate an open-ring thiolate **2**, and was kept under ice-cooling for 2 hours. The thiolate **2** was almost quantitatively transformed into a more lipophilic product which was a mixture of three compounds **6a-c** (**6a**:**6b**:**6c** = 32:30:38). These compounds were separated as pivaloyloxymethyl esters **7a-c** by silica-gel chromatography. Treatment of these esters **7a-c** with esterase successfully produced the corresponding acids **6a-c**, respectively as single compounds.



Isolation procedure of 3-spirocephams **6a-c** and **7a-c**

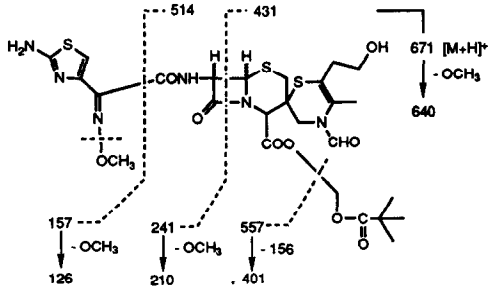
Table 1. ^{1}H- and ^{13}C-NMR chemical shifts of 3-spirocephams **6** and **7**.

Proton	6			7			Carbon	7		
	a	b	c	a	b	c		a	b	c
2a	2.88	2.65	2.64	3.07	2.70	2.70	2	36.41	30.33	31.13
2b	2.95	3.70	3.86	3.01	3.78	4.00	3	49.50	41.11	45.85
4	3.89	4.05	4.16	4.15	4.48	4.58	4	60.28	54.88	54.84
6	5.02	5.28	5.28	5.13	5.29	5.33	6	57.20	55.22	55.47
7	5.36	5.40	5.39	5.76	5.68	5.78	7	59.24	59.08	58.87
5'a	4.08	3.65	3.31	4.09	3.67	3.09	8	165.37	164.12	166.34
5'b	3.83	4.09	4.30	3.97	4.52	4.80	9	163.84	165.48	165.51
6'a	2.30	2.30	2.17	2.30	2.26	2.14	2'	116.48	109.40	109.85
6'b	2.30	2.30	2.40	2.55	2.54	2.70	3'	127.15	126.43	125.22
7'a	3.51	3.50	3.50	3.68	3.66	3.64	5'	44.89	43.42	45.92
7'b	3.51	3.50	3.50	3.72	3.66	3.70	6'	35.76	35.89	35.99
8'	1.95	1.96	1.98	2.18	2.18	2.17	7'	60.94	61.00	60.91
9'	8.40	8.42	8.42	8.54	8.64	8.65	8'	17.47	16.82	16.08
							9'	160.81	160.67	160.09

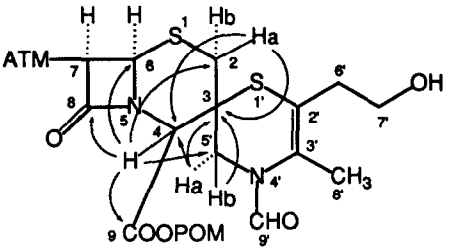
*Spectra of **6** and **7** were measured in D_2O and $CDCl_3$, respectively.

Structure determination The structures of these compounds **6** and **7** were determined using IR, MS, and NMR technique. First, we determined the structure of ester **7c**. The IR spectrum shows absorption at 1780 cm^{-1} due to the β-lactam carbonyl function. On FAB-MS(*m/e* 671)/MS spectrum fragment peaks at *m/e* 241 and 431 were observed, which are characteristic for the β-lactam cleavage. These results show that the ester **7c** contains a β-lactam structure after treatment under alkaline condition. ^{1}H-NMR spectrum of ester **7c** shows a new singlet at 4.58 ppm assigned to H-4. The ^{13}C-NMR spectrum lacks olefinic carbons at the C-3 and C-4 positions of original cephalosporin **1**, but shows a quaternary carbon at 45.85 ppm and a tertiary carbon at 54.84 ppm assigned

to C-3 and C-4, respectively. ^{13}C-^{1}H COSY spectrum detects the connection between H-4 and C-4. HMBC spectrum[5] reveals long-range couplings between the new quaternary carbon C-3 and the protons of H-2 and H-5'. Similar spectra were observed in **7a-b** and **6a-c**. These experimental results support the 3-spirocepham structure of compounds **6** and **7**. 3-Spirocepham **6** would be formed via intramolecular Michael attack of the thiol function to the C3=C4-C9=O moiety in the intermediate **2**. The compounds **6a**, **6b**, and **6c** must be diastereoisomers at C-3 and C-4 chiral centers formed in this reaction.

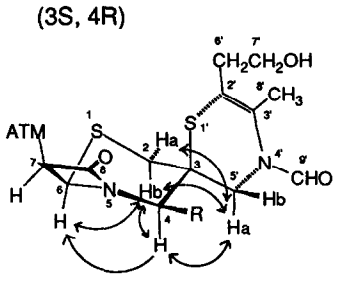


MS fragmentation of 3-spirocepham **7a-c**

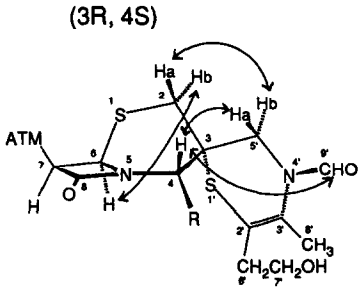


HMBC around C-3 and C-4 of 3-spirocepham **7a-c**
The arrow points from ^{1}H to ^{13}C.

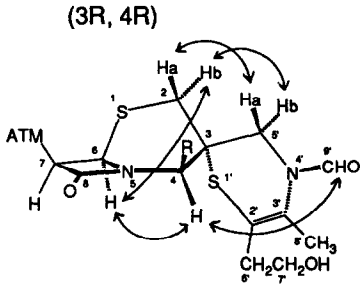
Stereochemical analysis In order to get stereochemical information around the C-3 and C-4 positions, NOE experiments were applied to 3-spirocephams **6a-c** and **7a-c**. NOEs observed in these 3-spirocephams are summarized as shown below. These NOEs suggest that **6a** has (3S, 4R)-configuration, **6b** has (3R, 4S), and **6c** has (3R, 4R). The 1,5-thiazine ring in the isomer **6a** has a chair conformation, because NOEs are observed among H-2b, H-4 and H-6 which occupy axial positions. In the isomer **6b** and **6c**, on the other hand, the 1,5-thiazine ring has a skew-boat conformation which is supported by the NOE between H-4 and H-9' and by the W-type long-range coupling between H-2b and H-4 observed in HOHAHA spectra[6] of **7c**.



6a and **7a**



6b and **7b**



6c and **7c**

Structure of 3-spirocephams **6a-c** and **7a-c**
The double-headed arrows indicate the NOEs essential for defining the ring conformations. **6a-c**: R=COOH, **7a-c**: R=COOPOM.

Although there should theoretically be four stereoisomers from a couple of new chiral centers, only three isomers **6a**, **6b** and **6c** were actually detected and isolated. The remaining (3S, 4S)-isomer could not be detected. When these isomers were treated under alkaline conditions, each isomer was transferred into a mixture of **6a-c**. This shows that the retro-Michael reaction and subsequent recyclization can occur under alkaline conditions, and epimerization at the C-4 position is possible in the (3R)-configuration and not in the (3S)-configuration. These results are explained by conformational analysis as follows. Isomers **6b** and **6c** of the (3R)-configuration have an energetic disadvantage in the chair conformation, where a large 5'-CH_2 group occupies the axial space. The skew-boat conformation can overcome this disadvantage by placing the 5'-CH_2 group in an equatorial space. In addition, the skew-boat conformation of **6b** can release steric energy due to the axial 4-COOH group in the chair conformation. Therefore, both isomers **6b** and **6c** can exist in energetically stable conformations. In the (3S)-configuration, on the other hand, the chair conformation of (3S, 4R)-isomer **6a** can exist stably because it has no factor of lability. The (3S, 4S)-isomer which was not detected, however, cannot release structural energy arising from the axial 4-COOH group in the chair conformation, because the possible skew-boat conformation has also a high energy level due to the steric interaction between the 5'-CH_2 group and the H-6 hydrogen atom. Therefore, the (3S, 4S)-isomer is energetically unstable compared to the (3S, 4R)-isomer **6a**. The (3S, 4S)-isomer seems to be eliminated by thermodynamic control in the 3-spirocepham-producing reaction.

In order to obtain a theoretical interpretation of these conformations of stereoisomers and the stereochemical control of the 3-spirocepham-producing reaction, molecular mechanics and molecular orbital calculations are now being investigated.

Acknowledgment

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