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Methylthiolated Cephem Derivatives*

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Methylthiolation reactions of 7-benzamido-3-methyl-3-cephem-4-carboxylic ester (3) *via* its carbanion gave 4-substituted derivatives, whereas its oxide (7) gave 2-substituted derivatives exclusively. In the presence of excess base, 7-substituted compounds were also obtained. In addition, some alkylation reactions and zinc reductions of 2-methylthio compounds thereby obtained were investigated.

Since observations of the marked stability of cephalosporin compounds towards base at low temperatures, much interest has been recently focused on substitution reactions *via* carbanions derived from cephalosporin compounds. For instance, benzaldehyde Schiff bases of 7-aminocephem derivatives (**1**) yield anions at C-7 with bases and further react with various electrophiles to afford 7α-substituted cephems,[2-4] wherein the 7-methylthio derivatives (**2**) provide important intermediates for the synthesis of certain naturally occurring 7α-methoxycephalosporins.[4] In addition, it was also reported that alkylation reactions *via* carbanions in case of 7β-acylamino-3-cephem-1-oxides proceed at the dihydrothiazine rings with a concomitant double bond shift to afford 4-alkyl-2-cephem-1-oxides.[5] This paper deals with analogous substitution reactions of 7-acylamino-cephem derivatives using methylthiolating agents wherein substitution occurs not only at C-4 but also at C-2 and/or C-7.

Methyl 7β-benzamido-3-methyl-3-cephem-4-carboxylate (**3**) and its 1β-oxide were utilized as the starting materials in this study. These cephem derivatives were treated with lithium diisopropylamide in tetrahydrofuran (THF) or THF–hexamethylphosphoramide (HMPA) or treated with sodium hydride in N,N-dimethylformamide (DMF) at low temperatures and subsequently the resulting anions reacted with methyl methanethiosulfonate[6] ($MsSCH_3$). The results of such methylthiolations are summarized in Table I.

Methylthiolation of the Sulfide 3

Similar to the preceding examples,[5] methylthiolation of the sulfide (**3**) occurred predominantly at C-4, giving just one of the two possible isomers at C-4, namely 4β-methylthio-2-cephem (**4**) (Run 1). When methylsulfenyl chloride[6] was used in place of $MsSCH_3$, the reaction did not proceed stereospecifically and formation of the other 4α-methylthio isomer (**5**)

* Dedicated to the memory of Prof. Eiji Ochiai.

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2) E.H.W. Böhme, H.E. Applegate, B. Toeplitz, J.E. Dolfini, and J.Z. Gougoutas, *J. Am. Chem. Soc.*, **93**, 4324 (1971).

3) R.A. Firestone, N. Schelechow, D.B.R. Johnston, and B.G. Christensen, *Tetrahedron Letters*, **1972**, 375; W.A. Spitzer, T. Goodson, R.J. Smithey, and I.G. Wright, *Chem. Commun.*, **1972**, 1138; E.H.W. Böhme, H.E. Applegate, J.B. Ewing, P.T. Funke, M.S. Puar, and J.E. Dolfini, *J. Org. Chem.*, **38**, 230 (1973).

4) W.A. Slusarchyk, H.E. Applegate, P.T. Funke, W. Koster, M.S. Puar, M. Young, and J.E. Dolfini, *J. Org. Chem.*, **38**, 943 (1973); W.A. Spitzer and T. Goodson, *Tetrahedron Letters*, **1973**, 273; T. Jen, J. Frazee and J.R.E. Hoover, *J. Org. Chem.*, **38**, 2857 (1973); H.E. Applegate, J.E. Dolfini, M.S. Puar, W.A. Slusarchyk, B. Toeplitz, and J.Z. Gougoutas, *ibid.*, **39**, 2794 (1974).

5) J.E. Dolfini, Ger. Offen., 2221361 (1972) [*C.A.* **78**, 43492*q* (1973)]. Also see D.O. Spry, *Tetrahedron* Letters, **1973**, 165; K. Kühlein and H. Jensen, *Ann. Chem.*, **1974**, 369.

6) I.B. Douglass, *J. Org. Chem.*, **24**. 2004 (1959).

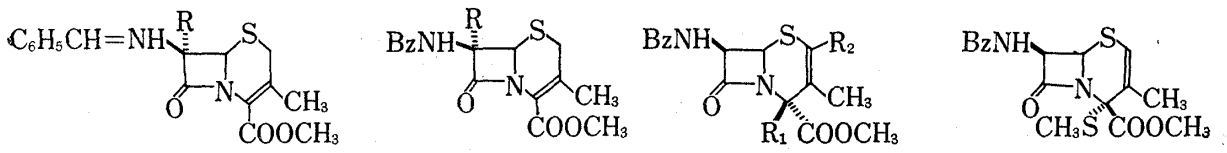
Table I. Substitution Reactions of Cephem Compounds

Run	Starting	Bases (moles; conditions[a])	Solvent	Substitution reagent (moles; conditions[b]-time)	Products[c]	Yields (%)
1	**3**	$LiN(iPr)_2$ (2.3; A)	THF	$MsSCH_3$ (3.2; A-10 min)	**4**[4β-SCH_3-Δ^2]	53
2	**3**	$LiN(iPr)_2$ (2.3; A)	THF	$ClSCH_3$ (2.4; A-10 min)	**4**[4β-SCH_3-Δ^2] **5**[4α-SCH_3-Δ^2]	30 13
3	**7**	$LiN(iPr)_2$ (2.1; B)	THF–HMPA	$MsSCH_3$ (1.6; B-10 min)	**8**[2α-SCH_3]	76
4	**7**	$LiN(iPr)_2$ (2.1; B)	THF–HMPA	$MsSCH_3$ (2.4; C-30 min)	**9**[2,2-$(SCH_3)_2$]	74
5	**7**	$LiN(iPr)_2$ (4.0; B)	THF–HMPA	$MsSCH_3$ (1.1; B-10 min)	**10**[7α-SCH_3] **11**[2α,7α-$(SCH_3)_2$]	22 40
6	**7**	$LiN(iPr)_2$ (4.0; B)	THF–HMPA	$MsSCH_3$ (2.5; B-10 min)	**11**[2α,7α-$(SCH_3)_2$]	71
7	**10**	$LiN(iPr)_2$ (2.1; B)	THF–HMPA	$MsSCH_3$ (2.5; B-10 min)	**11**[2α,7α-$(SCH_3)_2$]	72
8	**11**	NaH (1.2; C)	HMPA	$MsSCH_3$ (excess; D-3 hr)	**13**[2,2,7α-$(SCH_3)_3$]	90
9	**7**	$LiN(iPr)_2$ (2.1; B)	THF–HMPA	$C_6H_5SO_2SC_6H_5$ (2.0; B-10 min)	**17**[2α-SC_6H_5]	69
10	**7**	NaH (2.0; C)	DMF	$TsSCH_2CH_2CH_2STs$ (1.1; E-15 min)	**18**[2,2 ]	59
					19[bis-cephem]	32
11	**8**	NaH (1.1; C)	HMPA	CH_3I (excess; D-15 hr)	**20**[2-SCH_3-4β-CH_3-Δ^2] **21**[2α-CH_3-2β-SCH_3] **22**[2α-SCH_3-2β-CH_3]	24 33 23
12	**8**	NaH (1.1; C)	HMPA	$C_6H_5CH_2Br$ (excess; D-3 days)	**24**[2α-$CH_2C_6H_5$-2β-SCH_3]	75

a) Base solutions prepared: A at −78° allowed to warm to −10° and maintained for 10 min; B at −78° warmed to −23° and maintained for 5 min; C at 0° and maintained for 5 min.
b) Reagents added: A at −78° and maintained for 30 min then warmed to −10° and maintained during the time mentioned; B, at −78° and maintained; C, at −78° allowed to warm to −23° and maintained; D, at 0° and maintained; E, at −40° warmed to −10° and maintained.
c) Structures specially mentioned in parenthesis.

accompanied the formation of **4** (Run 2). Configurations of the methylthio groups in **4** and **5** were suggested on the basis of nuclear magnetic resonance (NMR) studies using the europium-shift reagent as follows. On addition of $Eu(fod)_3$[7] as a shift reagent, signals due to methyl protons of the C-4 ester groups in **4** and **5** exhibited different patterns according to the concentration of the reagent. As illustrated in Figures 1 and 2 and Table II, a high field shift of the ester methyl protons was observed in **4**, whereas a low field shift was prominant in **5**. Accordingly, these phenomena were compared with that of methyl 7β-benzamido-3-methyl-2-cephem-4α[8]-carboxylate (**6**) which was prepared from the sulfide **3** by treatment with bases according to Van Heyningen, *et al.*[9] As a result, the presence of the shift reagent caused the signal of ester methyl protons in **6** to shift to a higher field (Figure 3) just as in the case of the 4-methylthio compound (**4**), indicating that configurations of the ester groups in **4** and **6** are the same; and, subsequently, 4β- and 4α-configurations of the methylthio groups were assigned to **4** and to **5** respectively as shown below.[10]

7) Tris-(1,1,1,2,2,3,3-heptafluoro-7,7-dimethyl-4,6-octanedione)europium (III).
8) The 4α-ester configuration of **6** is plausible on the basis of the preceding structural determination. See R.M. Sweet and L.F. Dahl, *J. Am. Chem. Soc.*, **92**, 5489 (1970).
9) E.M. Van Heyningen and L.K. Ahern, *J. Med. Chem.*, **11**, 933 (1968).
10) As illustrated in Figures I and II, the proton at C-7 (H-7) exhibited the largest shift, indicating the europium complex coordinates almost exclusively with the nitrogen atom at C-7. This contrasts with the case of **5**, wherein a larger shift of methyl protons in the 4-methylthio group of **4** also suggests a β-orientation of the methylthio group adjacent to the 7β-amino group.



1: R=H
2: R=SCH$_3$

3: R=H
14: R=SCH$_3$

4: R$_1$=SCH$_3$, R$_2$=H
6: R$_1$=R$_2$=H
23: R$_1$=CH$_3$, R$_2$=SCH$_3$
25: R$_1$=H, R$_2$=SCH$_3$
26: R$_1$=H, R$_2$=CH$_3$

5

Chart 1

Predominat formation of the 4β-methylthio compound (**4**) indicates that an attack of the electrophile occurred from the β-side of the molecule at C-4, although an examination of the cephem model suggests an attack from the β-side is rather sterically unfavorable. One plausible reason for this phenomenon would be to assume that there is some electronic repulsion between the N-5 lone pair oriented towards the α-side and the forming C-4 carbanion electron pair. As a consequence, the latter rather assumes a β-orientation which affords the 4β-substituted compound (**4**) when undergoing electrophilic attack at C-4.

Methylthiolation of the Sulfoxide 7

Quite different from the case of the sulfide **3**, analogous methylthiolation of the 1β-oxide (**7**) proceeded without a shift of the double bond, giving a 2-substituted 3-cephem (**8**) (Run 3). The 3-cephem structure was shown by retention of ultraviolet absorption (270 nm, ε 8000) due to the $\alpha\beta$-unsaturated ester group. In this case, methylthiolation resulted in the formation of only one of the two possible isomers at C-2 wherein the α-configuration was assignaed on the basis of NMR studies as will be discussed later. Notwithstanding the presence of excess $MsSCH_3$, methylthiolation of **7** at a low temperature (−78°) gave only the 2-methylthio compound (**8**); but, when this reaction was effected at a higher temperature (−23°), a 2,2-dimethylthio compound[11] (**9**) was obtained in good yield (Run 4).

Thus, methylthiolation of the sulfoxide **7** *via* carbanion occurred principally at C-2. Meanwhile, in the presence of excess base (4 equivalents), treatment of **7** with 1.1 equivalent of $MsSCH_3$ resulted in a formation of a 7α-methylthio compound (**10**, 22%) and a 2α,7α-dimethylthio compound (**11**, 40%) (Run 5); and, a further excess of $MsSCH_3$ effected the exclusive formation of the latter compound (**11**) (Run 6).

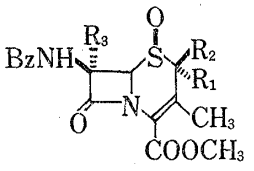
Supposedly, formation of the 7α-methylthio compound (**10**) in case of the presence of excess base suggests that there exists a possible intermediate, a trianion (**12**), involving a vicinal dianion system[12] with the most reactive center at C-7 and the attack of electrophiles occurs from the unhindered α-side. It is noteworthy that this is the first example of a substitution reaction at C-7 *via* a carbanion in case of 7-acylamino cephems. Moreover, methylthiolation of the 7α-methylthio compound (**10**) gave the 2α,7α-dimethylthio compound (**11**) (Run 7) and the latter was further methylthiolated to yield a 2,2,7α-trisubstituted compound[11] (**13**) (Run 8).

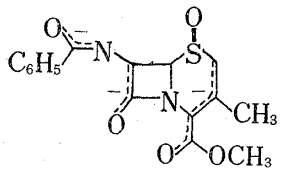
Herein, we would like to mention the configurations of the C-2 and C-7 methylthio groups in these products. The α-configuration of the 7-methylthio group in the compound **10** was synthetically verified as follows. Reduction of **10** with acetyl chloride and potassium iodide in DMF gave a sulfide (**14**) (See Chart 1). Compound **14** proved identical with the sample

11) These 2,2-dimethylthio derivatives (**9** and **13**) are not stable toward base, partially re-forming the corresponding 2α-monomethylthio compound (**8** and **11**) on prolonged standing with lithium diisopropylamide.
12) Koppel suggested the presence of this unusual dianion system in the epimerization of penicillins at C-6 with bases. See G.A. Koppel, *Tetrahedron Letters*, **1973**, 4233.

which was synthesized by removal of the benzylidene group from the known N-benzylidene-7β-amino-7α-methylthio cephem[4] (2) and successive benzoylation.

As for the α-configuration of the 2-methylthio group in **8**, analogous deoxygenation of compound **8** with acetyl chloride and potassium iodide was carried out, giving a mixture of the two isomeric sulfides (**15** and **16**) at C-2 in a ratio of 11 : 1. Application of the NMR analysis, including nuclear Overhauser effect (NOE) studies, suggested the configuration of the methylthio groups in these sulfides in the following way. The major sulfide (**15**) exhibited a marked five bonded coupling[13], J=1.0 Hz, between H-2 and H-7α as shown in Chart 2 and no coupling





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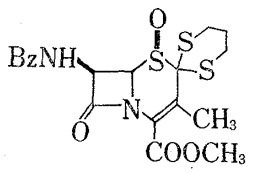

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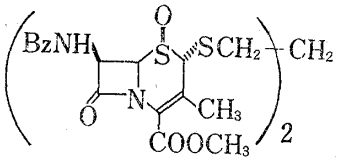
7: $R_1=R_2=R_3=H$
8: $R_1=SCH_3$, $R_2=R_3=H$
9: $R_1=R_2=SCH_3$, $R_3=H$
10: $R_1=R_2=H$, $R_3=SCH_3$
11: $R_1=R_3=SCH_3$, $R_2=H$
13: $R_1=R_2=R_3=SCH_3$
17: $R_1=SC_6H_5$, $R_2=R_3=H$
21: $R_1=CH_3$, $R_2=SCH_3$, $R_3=H$
22: $R_1=SCH_3$, $R_2=CH_3$, $R_3=H$
24: $R_1=CH_2C_6H_5$, $R_2=SCH_3$, $R_3=H$
27: $R_1=CH_3$, $R_2=R_3=H$
28: $R_1=R_3=H$, $R_2=CH_3$



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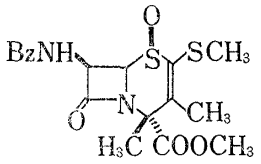

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Chart 2

between H-2 and H-6α, suggesting a β-configuration of the proton at C-2. On the other hand, the minor sulfide (**16**) did not show long range coupling between H-2 and H-7α and exhibited coupling between H-2 and H-6α, J=0.3—0.4 Hz. Additionally, a significant NOE between H-2 and H-6α in the latter sulfide (**16**) was observed in contrast to no NOE in case of **15**. These facts suggest that configuration of the proton at C-2 is α in **16** and β in **15**; subsequently, the structure of the major sulfide (**15**) was assignable as the 2α-methylthio compound and the minor one (**16**) as the 2β-isomer.

Oxidation of the 2α-methylthio sulfide (**15**) with *m*-chloroperbenzoic acid regenerated the sulfoxide (**8**) in good yield.[14] The NMR spectrum of **8** also shows a similar five bonded coupling between H-2 and H-7α, J=1.1 Hz, indicating that configuration of the methylthio group is α. In addition, this is supported by a marked NOE between H-2 and 3-methyl protons in **8** which is in accordance with the preceding observations.[13,15]

Summarizing these results, it would be worthy to note that, in contrast to the case of the sulfide (**3**), electrophilic attack on the dihydrothiazine ring in the sulfoxide (**7**) preferentially

13) D.O. Spry, *Tetrahedron Letters*, **1972**, 3717.
14) Oxidation of the isomeric sulfide (**16**) gave a complex product, indicating that oxidation is not limited at the sulfur atom at the 1-position in this case.
15) Reliable data of the corresponding NOE was not obtained in case of the 2α-methylthio sulfide (**15**) because of overlapping of signals due to C-3 methyl protons with those due to methyl protons of the C-2 methylthio group.

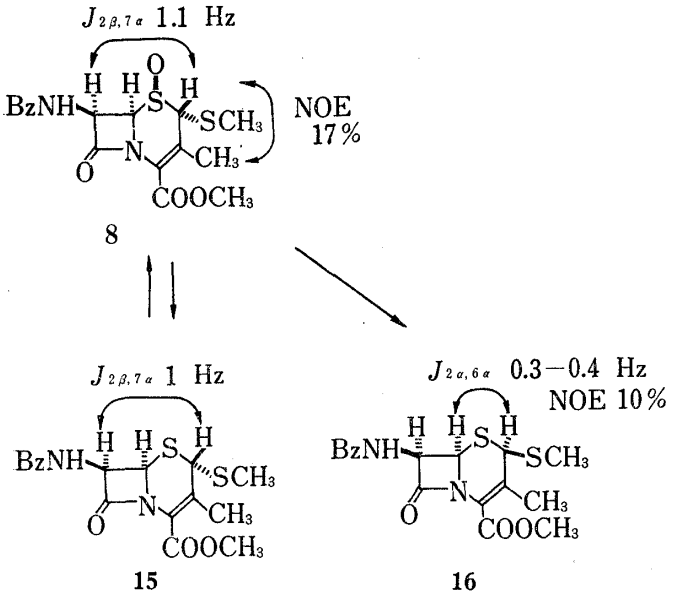

Chart 3

proceeds at C-2 possibly due to a centering of the electron density at C-2 effected by the vicinal S–O function, giving these 2-methylthio compounds. Prominant formation of 2α-isomers is presumably due to their base-stability.

Some extension of this methylthiolation reaction was analogously carried out; the base-treated sulfoxide (**7**) was converted into a 2α-phenylthio compound (**17**) ($J_{2\beta,7\alpha}$=1.0 Hz) with phenyl thiobenzenesulfonate[16] (Run 9), into a 2-dithiolated compound (**18**) and a biscephem compound (**19**) with trimethylene dithiotosylate[17] (Run 10).

Alkylation of the 2-Methylthio Sulfoxide 8

Methylation of **8** was effected with sodium hydride and methyl iodide in THF–HMPA, giving a mixture of 4β-methyl-2-methylthio-2-cephem (**20**, 24%), 2α-methyl-2β-methylthio-3-cephem (**21**, 33%), and 2β-methyl-2α-methylthio-3-cephem (**22**, 22%) (Run 11) which were separated by preparative thin-layer chromatography. The β-configuration of the methyl group in the 4-methyl-2-cephem (**20**) would be assignable on the basis of NMR study as follows. Treatment of **20** with acetyl chloride and potassium iodide afforded a sulfide (**23**) (See Chart 1), whose NMR spectrum, in the presence of Eu(fod)$_3$, exhibits an upfield shift of the ester methyl protons and a downfield shift of the 4-methyl protons as shown in Table II. This fact suggests that the methyl group is oriented β near the 7β-acylamino group and preferential methylation from the β-side occurred parallel to the case of the methylthiolation of the sulfide (**3**) as discussed earlier.

Configurations of the 2-methyl group in **21** and **22** were determined in the following way. In the NMR spectrum of the major 2-methylated compound (**21**), a significant upfield shift (δ 1.44 ppm) of the 2-methyl protons absorption was observed in contrast to that of its isomer (**22**, δ 1.85 ppm). This fact suggests that the methyl proton absorption in **21** is affected by a S–O bond anisotropy[18] and therefore **21** has a 2α-methyl group. In addition, this is also supported by an observation of a marked NOE (13%) between H-6 and the 2-methyl protons in the NMR spectrum of **21**. Prominant formation of the 2α-methylated cephem (**21**) would be ascribed to attack of the reagent from the unhindered α-side at C-2.

Analogous benzylation of the 2-methylthio compound (**8**) proceeded with high regio- and stereo-specificity, giving only a 2α-benzylated compound (**24**) (Run 12). The 2α-configuration of the benzyl group in **24** was assigned by noting an abnormal upfield shift of its H-6 absorption (δ 3.16 ppm) in the spectrum of **24** which could be explained by the shielding effect of the introduced benzyl group on the α-side of the molecule.

Zinc Reduction of the 2-Methylthio Sulfoxides

During this study, it was found that these 2-methylthiolated sulfoxides are labile to zinc. Treatment of the 2α-methylthio sulfoxide (**8**) with zinc in acetic acid regenerated the original

16) E. Müller, Houben-Weyl, "Die Methoden der Organischen Chemie," 4 Aufl. Bd. IX, 1955, p. 687.
17) R.B. Woodward, I.J. Pachter, and M.L. Scheinbaum, *J. Org. Chem.*, **36**, 1137 (1971).
18) P.V. Demarco and R. Nagarajan, "Cephalosporins and penicillins, Chemistry and Biology," ed. by E.H. Flynn, Academic Press, N.Y., 1972, p. 349.

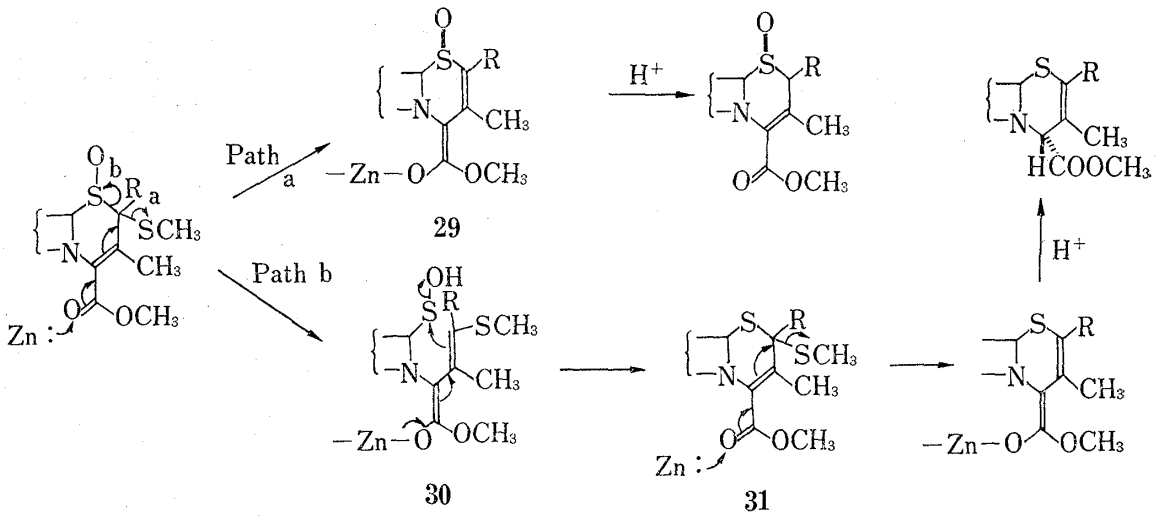

Chart 4

TABLE II. $Eu(fod)_3$ Induced Shift NMR Data[a] in ppm (60 MHz)

Compd.	3-CH_3	4-$COOCH_3$	4-(Substituent)	2-(Substituent)	H-6	H-7
4	0.61	−0.54	1.83(β-SCH_3)	0.94(H)	2.29	10.55
5	0.78	1.65	−0.12(α-SCH_3)	1.19(H)	2.39	11.20
6	0.82	−0.38	2.09(β-H)	1.21(H)	2.05	10.92
23	0.58	−0.59	1.75(β-CH_3)	0.32(SCH_3)	2.20	10.08

a) obtained by extrapolation of plots of the NMR shift *vs.* the concentration of $Eu(fod)_3$ to 1 molar equivalent of $Eu(fod)_3$. Concentration of compounds was about 0.3 moles in $CDCl_3$.

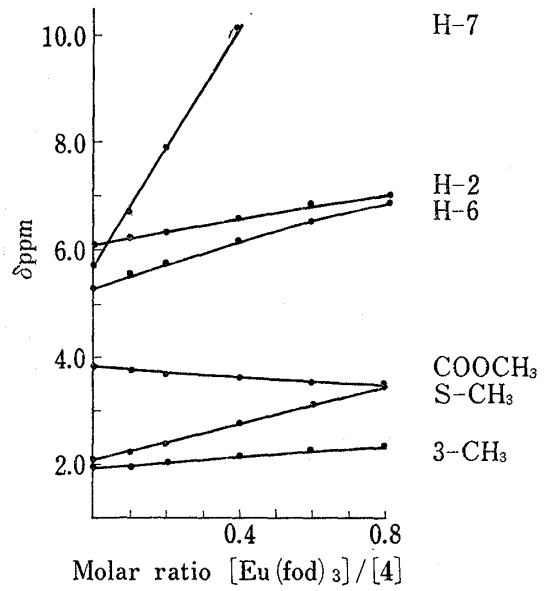

Fig. 1. Chemical Shifts of Methyl 7β-Benzamido-3-methyl-4β-methylthio-2-cephem-4α-carboxylate (**4**) in the Presence of $Eu(fod)_3$

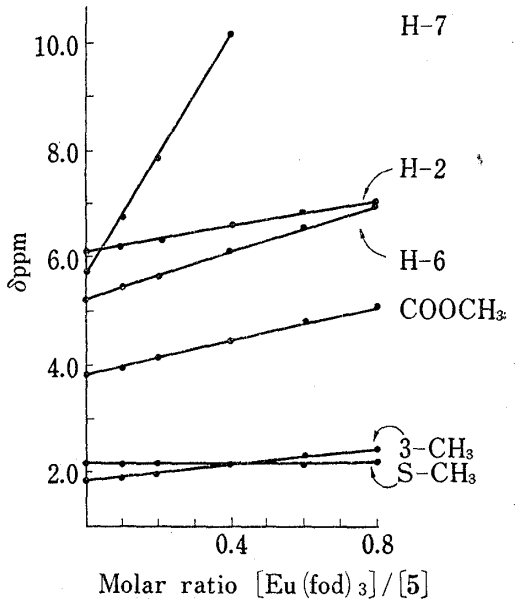

Fig. 2. Chemical Shifts of Methyl 7β-Benzamido-3-methyl-4α-methylthio-2-cephem-4β-carboxylate (**5**) in the Presence of $Eu(fod)_3$

sulfoxide (**7**). In this reaction, the 2-cephem (**6**) was formed in 14% yield under removal of the methylthio group accompanied by reduction of 1-oxide. The 2,2-dimethylthio sulfoxide (**9**) was also treated with zinc for a short time, affording the 2-methylthio-1-oxide (**8**) in 50% yield and a 2-methylthio-2-cephem[19] (**25**) in 23% yield. Either of the 2α-methyl-2β-methylthio-1-oxide (**21**) or its 2β-methyl-2α-methylthio isomer[20] (**22**) was analogously reduced with zinc and furnished a 2-methyl-2-cephem (**26**) in 54% yield along with a mixture of a 2α-methyl-1-oxide (**27**) and its 2β-methyl isomer (**28**) in 30% yield. The relative ratio of the latter isomers was determined as 2:5 by means of NMR analysis. The 2β-methyl-1-oxide (**28**) thus predominantly obtained was found to be a rather unstable compound; and the mixture of **27** and **28** thereby obtained were converted into an equilibrium mixture in a ratio of 5:1 with the 2α-methyl isomer (**27**) predominating on treatment with 1,4-diazabicyclo[2.2.2]octane (DABCO).[21] Moreover, peracid oxidation of the 2-methyl-2-cephem (**26**) gave a mixture of **27** and **28** whose ratio was 3:1.

Configurations of the 2-methyl group in **27** and **28** were determined as follows. In the NMR spectrum of the major product (**28**) obtained by reduction of **21** or **22**, a long range coupling (J=1.5 Hz) between H-2 and H-6 was observed and this is consistent with the foregoing data,[22] confirming a 2β-methyl structure in **28**; subsequently, the more stable isomer (**27**) is a 2α-methyl-1-oxide.

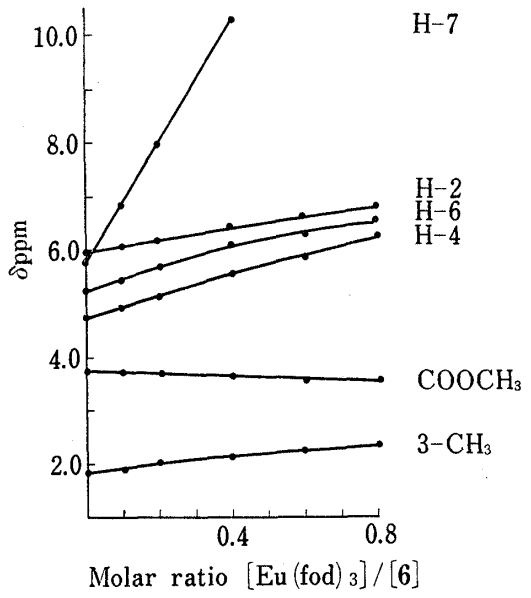

Fig. 3. Chemical Shifts of Methyl 7β-Benzamido-3-methyl-2-cephem-4α-carboxylate (**6**) in the Presence of Eu(fod)$_3$

Removal of the 2-methylthio groups in these zinc reductions would be explained by Path a as shown below and predominant formation of the 2β-methyl-1-oxide (**28**) in case of reduction of **21** or **22** might be assumed by a preferential protonization from the less hindered α-side of an intermediate[23] (**29**, R=CH_3). One of the plausible mechanisms for the formation of 2-cephems (**6**, **25**, and **26**) with deoxygenation at S-1 is shown by Path b (R=H, CH_3, and CH_3S) involving initial reductive cleavage of the bond between S-1 and C-2 and successive recyclization of the resultant sulfenic acid intermediate (**30**) into a cephem (**31**) which is further subject to reduction and gives a 2-cephem compound *via* a final protonization at C-4.

Thus, it might be mentioned that methylthiolation and successive alkylation of cephem-1-oxides at C-2 followed by zinc reduction provides an alternate method of synthesizing 2-alkylcephalosporins.[22]

19) The 2-cephem (**25**) was obtained on treatment of either of the 2α- and 2β-methylthio-3-cephems (**15** and **16**) with DABCO as an equilibrium mixture containing **15** and **25** in a ratio of 1:1.
20) A slightly slower reduction was observed in case of the 2α-methyl-2β-methylthio isomer (**21**).
21) Repeated chromatography over silica gel also caused an isomerization of **28** to **27** which made the separation of these two isomers difficult.
22) I.G. Wright, C.W. Achbrook, T. Goodson, G.V. Kaiser, and E.M. Van Heyningen, *J. Med. Chem.*, **14**, 420 (1971).
23) Exclusive formation of the 2α-methylthio compound (**8**) in reduction of the 2,2-dimethylthio-1-oxide (**11**) would be explained by assuming that a possible 2β-methylthio compound initially was also formed *via* path a (R=SCH_3) and its instability in acetic acid affects an easy isomerization into the stable 2α-methylthio-1-oxide (**8**).

Experimental

Melting points are not corrected. Infrared spectra (IR) were recorded on a JASCO A-2 or Perkin-Elmer Model 225 spectrometer, NMR spectra on a Hitachi Perkin-Elmer R-24, 60 MHz, or a Varian HA-100 spectrometer, 100 MHz, and mass spectra (MS) on a JEOL JMS-01SG mass spectrometer. Thin-layer chromatography (TLC) was performed on TLC-plates, Silica gel F_{254} precoated, layer thickness 0.25 mm (E. Merck) and spots were visualized by UV-irradiation or by spraying with vanadic acid–sulfuric acid followed by heating or with iodine. Columns for ordinary chromatography were prepared with Wakogel C-200 (Wako Pure Chemical Industries, Ltd.) and plates for preparative TLC with Silica gel $60F_{254}$ (E. Merck). Solvents were removed by a rotary flash evaporator at diminished pressure and usually at 15—35°. Physical constants and NMR data of cephem compounds are given in Table III and IV. The abbreviations used are as follows: s, singlet; d, doublet; q, quartet; m, multiplet; br., broad.

Methyl 7β-Benzamido-3-methyl-3-cephem-4-carboxylate (3)——To an ice-cold and stirred suspension of 8.56 g of 7-aminodeacetoxycephalosporanic acid in 200 ml of 50% aqueous THF was added dropwise 6.04 g of triethylamine and, then, 5.6 g of benzoyl chloride. After triethylamine was further added until the solid was dissolved, the mixture was stirred for further 2 hr at room temperature. The organic solvent was mostly evaporated *in vacuo* and the resulting aqueous solution was adjusted to pH 7.0 with dil. $NaHCO_3$. After washing with AcOEt, the mixture was acidified to pH 2.0 with dil. HCl and extracted twice with AcOEt. The combined extracts were washed with aq. NaCl, dried and concentrated to a half volume. Esterification in the usual manner with etheral diazomethane followed by dilution with *n*-hexane and gradual evaporation of the solvent resulted in a precipitation of 10.1 g (71%) of crystals which were recrystallized from MeOH. A second crop (0.4 g) was obtained from concentration of the mother liquor.

Methyl 7β-Benzamido-3-methyl-3-cephem-4-carboxylate-1β-oxide (7)——To an ice-salt cooled and well stirred solution of 6.65 g of 3 in 40 ml of $CHCl_3$ was added a solution of 3.74 g of *m*-chloroperbenzoic acid (85% purity, Aztec Chemicals) in 65 ml of $CHCl_3$. The resulting precipitates were collected and washed with AcOEt, giving 2.4 g of 7. Concentration of the remaining filtrate and washing the resulting crystals with AcOEt afforded 3.89 g of a second crop. The product was pure enough to use in the next reaction.

Methylthiolation of Methyl 7β-Benzamido-3-methyl-3-cephem-4-carboxylate (3) **and Its 1β-Oxide** (7) **through Carbanion and Related Substitution Reaction**——All reactions were carried out with vigorous stirring under an atomosphere of argon. THF was distilled over $LiAlH_4$ and HMPA and diisopropylamine were distilled over CaH_2 immediately before use. DMF was also distilled and stored over molecular sieves. Lithium diisopropylamide solution was prepared by adding a 20% butyllithium hexane solution (E. Merck) in a 0.1—0.2M solution of diisopropylamine in THF at 0° and successively by stirring for 5 min. After cooling to −78° witha Dry-Ice bath, a cephem compound was added in one portion. The mixture was diluted with 1/20 volume of HMPA in some cases and was allowed to warm to −10——23° to make the anion formation complete. Then, after cooling to −78°, $MsSCH_3$ or other reagent was added and the reaction was conducted as illustrated in Table I. Progress of reaction was monitored by periodic removal of aliquots and analysis on TLC. Then, after cooling quenching of the reaction was effected by adding a corresponding amount of AcOH at −78° and the mixture was poured onto ice-water and extracted with AcOEt. The extract was washed with H_2O, successively with dil. $NaHCO_3$ and with H_2O again, dried over Na_2SO_4 and evaporated *in vacuo*. The residue was separated and purified by preparative TLC or column chromatography using benzene–AcOEt or $CHCl_3$–AcOEt as eluent.

On the other hand, a NaH suspension was prepared by washing twice a 50% NaH-suspension in mineral oil with hexane and covering with DMF or HMPA at 0°. After adding a cephem compound, the mixture was maintained for 5 min at 0°, then the reagent was added under the condition described in Table I. Work-up described as above was carried out and products were isolated.

Methyl 7β-Benzamido-3-methyl-2-cephem-4α-carboxylate (6)——According to Van Heyningen, *et al.*,[9] 60 ml of 0.1N NaOH was added to an ice-cold solution of 1.99 g of 3 in a mixture of 100 ml of pyridine and 150 ml of H_2O with stirring. Then, the mixture was stirred for 3.5 hr at 0° and overnight at room temperature. After evaporation of organic solvent *in vacuo*, the mixture was diluted with H_2O, washed with AcOEt and, after acidification to pH 2 with dil. HCl, extracted with AcOEt. The extract was washed with aq. NaCl and evaporated *in vacuo*. The residue was crystallized from EtOH–hexane, giving 880 mg of a 2-cephem-4-carboxylic acid which was converted into a methyl ester (6) in the usual manner with etheral diazomethane. Recrystallization of the product from MeOH gave 747 mg of **6**.

Methyl 7β-Benzamido-3-methyl-7α-methylthio-3-cephem-4-carboxylate (14)——To an ice-cold and well stirred solution of 61 mg of **10** in 2 ml of DMF was added 103 mg of KI and then dropwise a solution of 25 mg of acetyl chloride in 0.5 ml of DMF. The mixture was stirred for 2.5 hr with cooling and poured onto an ice-cold saturated aq. $K_2S_2O_5$ and extracted with AcOEt. The extract was washed with dil. $NaHCO_3$ and with H_2O and dried. After evaporation of the solvent, the residue was chromatographed over 3 g of silica gel by using benzene–AcOEt (3: 1, v/v) as eluent, yielding 58 mg of **14**. The compound **14** was identical in IR and NMR spectra with the sample prepared by benzoylation of methyl 7β-amino-3-methyl-7α-methylthio-3-cephem-4-carboxylate[4] with 4 moles of benzoyl chloride and 4 moles of diethylaniline in $CHCl_3$.

Table III. Physical Data of Cephem Compounds

Compd.	mp[a] (°C)	IR[b] Nujol, cm^{-1}	Formula	Analysis % Calcd. (Found) C	H	N	S	Cl	MS
3	188—189 (MeOH)	1780, 1724, 1644	$C_{16}H_{16}O_4N_2S$	57.81 (57.53)	4.85 (4.79)	8.43 (8.19)	9.64 (9.76)		
4	foam	1770, 1738, 1657	$C_{17}H_{18}O_4N_2S_2$						378(M^+)
5	foam	1760, 1736, 1656	$C_{17}H_{18}O_4N_2S_2$						378(M^+)
6	182.5—183.5 (MeOH)	1780, 1738, 1642	$C_{16}H_{16}O_4N_2S$	57.81 (57.80)	4.85 (4.91)	8.43 (8.48)	9.64 (9.85)		332(M^+)
7	221—222 ($CHCl_3$–AcOEt)	1779, 1723, 1635	$C_{16}H_{16}O_5N_2S$	55.17 (54.94)	4.63 (4.53)	8.04 (8.23)	9.19 (9.49)		
8	160.5—162 (AcOEt)	1788, 1729, 1655[c]	$C_{17}H_{18}O_5N_2S_2$	51.78 (51.57)	4.61 (4.53)	7.10 (7.08)	16.23 (16.59)		
9	foam	1792, 1730, 1671[c]	$C_{18}H_{20}O_5N_2S_3$						423 (M^+-17)
10	192—198 (benzene)	1790, 1718, 1652	$C_{17}H_{18}O_5N_2S_2 \cdot 4/9C_6H_6$[d]	55.29 (55.36)	4.87 (4.85)	6.56 (6.31)	14.55 (14.56)		394(M^+)
11	169—170 d (MeOH)	1781, 1772, 1731, 1659[c]	$C_{18}H_{20}O_5N_2S_3$	49.07 (48.88)	4.58 (4.55)	6.36 (6.01)	21.83 (21.95)		
13	foam	1792, 1734, 1659[c]	$C_{19}H_{22}O_5N_2S_4 \cdot 0.59\ CCl_4$[e]	40.75 (41.01)	3.84 (3.85)	4.85 (4.99)	22.21 (22.55)	14.49 (14.76)	
14	foam	1775, 1726, 1668	$C_{17}H_{18}O_4N_2S_2$						378(M^+)
15	169.5—172 (MeOH)	1794, 1733, 1657	$C_{17}H_{18}O_4N_2S_2$	53.97 (54.08)	4.80 (4.70)	7.41 (7.48)	16.91 (16.86)		378(M^+)
16	161.5—164.5 (acetone–hexane)	1780, 1728, 1649	$C_{17}H_{18}O_4N_2S_2$	53.97 (54.36)	4.80 (5.01)	7.41 (7.05)	16.91 (17.29)		378(M^+)
17	179—180 d ($CHCl_3$–EtOH)	1783, 1736, 1682	$C_{22}H_{20}O_5N_2S_2$	57.88 (57.64)	4.42 (4.27)	6.13 (5.70)	14.05 (13.68)		456(M^+)
18	foam	1795, 1733, 1672	$C_{19}H_{20}O_5N_2S_3$						435 (M^+-17)
19	182—184 d ($CHCl_3$–acetone)	1786, 1729, 1665	$C_{35}H_{36}O_{10}N_4S_4 \cdot 1/4CHCl_3$	50.96 (50.81)	4.40 (4.28)	6.74 (6.76)	15.44 (15.65)		
20	foam	1778, 1743, 1671[c]	$C_{18}H_{20}O_5N_2S_2 \cdot 0.26\ CCl_4$[e]	48.90 (49.35)	4.49 (4.55)	6.25 (6.18)	14.30 (13.87)	8.22 (8.38)	
21	foam	1790, 1730, 1671[c]	$C_{18}H_{20}O_5N_2S_2 \cdot 0.42\ CCl_4$	46.76 (47.03)	4.26 (4.29)	5.92 (5.82)	13.55 (13.17)	12.59 (12.94)	
22	foam	1790, 1730, 1671[c]	$C_{18}H_{20}O_5N_2S_2 \cdot 0.3\ CCl_4$	48.35 (48.29)	4.43 (4.74)	6.16 (6.12)	14.11 (14.19)	9.36 (9.50)	
23	170—171 (benzene–hexane)	1775, 1743, 1643	$C_{18}H_{20}O_4N_2S_2$	55.10 (55.05)	5.14 (5.13)	7.14 (7.09)	16.31 (16.46)		392(M^+)
24	foam	1790, 1733, 1678	$C_{24}H_{24}O_5N_2S_2 \cdot 0.48\ CCl_4$	52.65 (52.77)	4.33 (4.20)	5.02 (4.98)	11.49 (11.41)	12.19 (12.10)	
25	196—197.5 (benzene–AcOEt)	1770, 1733, 1639	$C_{17}H_{18}O_4N_2S_2$	53.97 (53.70)	4.80 (4.80)	7.41 (7.44)	16.91 (16.82)		
26	215—219 (AcOEt)	1760, 1733, 1640	$C_{17}H_{18}O_4N_2S$	58.94 (58.65)	5.24 (5.19)	8.09 (7.91)	9.26 (9.55)		
27	210—211 d ($CHCl_3$–AcOEt)	1778, 1727, 1640	$C_{17}H_{18}O_5N_2S$	56.34 (56.25)	5.01 (4.98)	7.73 (7.45)	8.85 (8.96)		362(M^+)

a) recrystallization solvents in parenthesis; d, decomposition
b) run on a JASCO A-2
c) run on a Perkin-Elmer Model 225
d) contaminated with recrystallization solvents
e) contaminated with extraction solvent

Table IV. NMR Data of Cephem Compounds[a)]

Compd.	H-2	H-6	H-7	3-CH_3	$-SCH_3$	$-COOCH_3$	Others
3	3.18, 3.39 ABq(19)	4.95 d (5)	5.83dd (5, 7)	2.07 s		3.70 s	
4	6.27br. s	5.30 d (4)	5.57dd (4, 8)	1.92br. s	2.02 s	3.81 s	
5	6.10br. s	5.23 d (4)	5.72dd (4, 7)	1.81 d-like	2.18 s	3.79 s	
6	5.91m	5.29 d (4)	5.77dd (4, 9)	1.86br. s		3.75 s	4.72(1H, br. s, H-4)
7[b)]	3.66 s	4.91 d (5)	5.91dd (5, 9)	1.94 s		3.70 s	
8[c)]	4.25 d (1)	5.01 d (5)	6.33ddd (1, 5, 9.5)	2.31 s	2.37 s	3.89 s	
9		5.18 d (4.5)	6.23dd (4.5, 10.5)	2.21 s,	2.27 s[d)]	3.80 s	
10	3.39br. s	4.75 s		2.04 s	2.28 s	3.84 s	
11	4.11 s	4.96 s		2.19 s, 2.32 s	2.26 s,	3.87 s	
13		5.22 s		2.21—2.24, 2.30 s		3.81 s	
14	3.21br. s	4.96 s		2.08 s	2.30 s	3.69 s	
15[c)]	4.38 d (1)	5.39 d (5)	6.06ddd (1, 5, 8)	2.27 s	2.27 s	3.81 s	
16[c)]	4.18 d (0.3—0.4)	5.34dd (0.3—0.4, 4)	5.75dd (4, 9)	2.12 s	2.39 s	3.82 s	
17[c)]	4.49 d (1)	5.09 d (5)	6.38ddd (1, 5, 10)	2.44 s		3.92 s	
18		5.34 d (4.5)	6.31dd (4.5, 9.5)	2.23 s		3.84 s	1.9—2.3(2H, m, $-SCH_2\underline{CH_2}-CH_2S-$); 2.4—3.5(4H, m, $-S\underline{CH_2}CH_2\underline{CH_2}S-$)
19[b)]	4.98 s	4.98 d (5)	6.00dd (5, 8)	2.06 s		3.72 s	1.8—2.1(2H, m, $-SCH_2\underline{CH_2}-CH_2S-$); 2.6—3.0(4H, m, $-S\underline{CH_2}CH_2\underline{CH_2}S-$)
20		4.86 d (4.5)	6.13dd (4.5, 10)	2.00 s, 2.44 s[e)]	2.02 s,	3.76 s	
21[c)]		4.76 d (5)	6.28dd (5, 10)	2.28 s	2.25 s	3.90 s	1.44(3H,s,2-CH_3)
22		5.23 d (5)	6.31dd (5, 10)	2.09 s	2.09 s	3.84 s	1.85(3H,s,2-CH_3)
23		5.28 d (4)	5.64dd (4, 8)	1.89 s	2.32 s	3.77 s	1.98(3H, s, 4-CH_3)
24		3.16 d (5)	5.90dd (5, 11)	2.19 s,	2.31 s	3.82 s	2.89, 3.36 ABq(15)
25		5.39 d (4.5)	5.79dd (4.5, 9)	2.07 s,	2.27 s	3.77 s	4.84(1H, br. s, H-4)
26		5.36 d (4.5)	5.80dd (4.5, 9	1.83 s		3.76 s	1.83(3H, s, 2-CH_3) 4.75(1H, br. s, H-4)
27[b)]	3.7m	5.02 d (4.5)	6.05dd (4.5, 9)	2.02 s		3.77 s	1.23[3H, d(7.5), 2-CH_3]
28[b)]	3.7m	5.15dd (1.5, 4.5)	6.02dd (4.5, 8.5)	1.89 s		3.77 s	1.49[3H, d(7.5), 2-CH_3]

a) run on a Hitachi-Perkin-Elmer R-24 spectrometer 60 MHz in $CDCl_3$; coupling constants in parenthesis
b) run in d_6-DMSO
c) run on a Varian HA-100 spectrometer 100 MHz in $CDCl_3$.
d) Signals in a ratio of 2:1.
e) A singlet due to 4-CH_3 included.

Methyl 7β-Benzamido-3-methyl-2α- and 2β-Methylthio-3-cephem-4-carboxylates (15 and 16)——To an ice-cold and stirred solution of 1.182 g of **8** and 1.99 g of KI in 6 ml of DMF was added dropwise 942 mg of acetyl chloride. The mixture was stirred for 3 hr with cooling and worked-up as described above. The product was crystallized from MeOH to give 538 mg of the 2α-isomer (**15**). Preparative TLC of the mother liquor (benzene–AcOEt, 8: 1, v/v) afforded 361 mg of **15** and 90 mg of the β-isomer (**16**).

Peracid Oxidation of Methyl 7β-Benzamido-3-methyl-2α- and 2β-Methylthio-3-cephem-4-carboxylates (15 and 16)——To an ice-cold solution of 340 mg of **15** in 6 ml of $CHCl_3$ was added in portions 183 mg of *m*-chloroperbenzoic acid (85% purity) with stirring and the mixture was stirred for 1.5 hr with cooling. After dilution with AcOEt, the mixture was washed with dil. $NaHCO_3$ and with H_2O, dried and evaporated *in vacuo*. The residue was purified by preparative TLC (benzene–AcOEt, 3: 1, v/v), giving 213 mg of **8**.

Analogous oxidation of the 2β-isomer (**16**) afforded a complex product whose TLC revealed many spots.

Methyl 7β-Benzamido-3,4β-dimethyl-2-methylthio-2-cephem-4α-carboxylate (23)——To an ice-cold solution of 90 mg of **20** and 144 mg of KI in 1 ml of DMF was added 69 mg of acetyl chloride with stirring. The mixture was stirred with cooling for 2 hr and worked-up as above, giving 56 mg of **23** after purification by preparative TLC (benzene–AcOEt, 6: 1, v/v).

Zinc Reduction of Methyl 7β-Benzamido-3-methyl-2α-methylthio-3-cephem-4-carboxylate-1-oxide (8)——A mixture of 240 mg of **8**, 750 mg of zinc powder, and 3 ml of AcOH was stirred for 9 hr at room temperature. The solid was filtered and washed with $CHCl_3$. The combined filtrate and washing were evaporated *in vacuo*. The residue was dissolved in $CHCl_3$ and washed successively with H_2O, dil. $NaHCO_3$, and H_2O. After drying, the solvent was evaporated and the crystalline residue was recrystallized from $CHCl_3$–AcOEt to give 99 mg of **7**. Separation of the mother liquor by preparative TLC gave 23 mg of additional **7** (total yield, 57%) and 29 mg (14%) of **6**.

Zinc Reduction of Methyl 7β-Benzamido-3-methyl-2,2-dimethylthio-3-cephem-4-carboxylate-1-oxide (9)——A mixture of 150 mg of **9**, 0.45 g of zinc powder, and 1.5 ml of AcOH was stirred for 1 hr at room temperature. Work-up as above and purification by preparative TLC (AcOEt–benzene, 1: 3, v/v) gave 30 mg (23%) of the 2-methylthio-2-cephem (**25**), 67 mg (50%) of **8**, and a trace amount of **7**.

DABCO Treatment of Methyl 7β-Benzamido-3-methyl-2α(or 2β)-methylthio-3-cephem- or -2-Methylthio-2-cephem-4-carboxylate (15, 16, or 25)——A mixture of 144 mg of **15**, 10 mg of DABCO, and 7 ml of THF was stirred overnight at room temperature. The mixture was diluted with AcOEt and washed successively with dil. HCl and H_2O. After drying, the solvent was evaporated to give a product whose NMR spectrum showed the presence of **15** and **25** in a ratio of 1: 1.

Analogous treatment of **16** or **25** gave the same equilibrium mixture containing **15** and **25**.

Zinc Reduction of Methyl 7β-Benzamido-2β,3-dimethyl-2α-methylthio-3-cephem-4-carboxylate-1-oxide (22)—A mixture of 145 mg of **22**, 0.6 g of zinc powder, and 3 ml of AcOH was stirred for 15 hr at room temperature. The mixture was treated as described above and the product was separated by preparative TLC (AcOEt–benzene, 1: 3, v/v) to give 15 mg (10%) of recovered **22**, 60 mg (54% based on unrecovered **22**) of **26** and 34 mg (30% based on unrecovered **22**) of a mixture of **27** and **28**. The relative ratio of the last mixture of **27** and **28** was determined as 2: 5 by NMR analysis. An attempt to isolate pure **28** by repeated preparative TLC on silica gel resulted in its conversion to **27**. Moreover, treatment of the mixture of **27** and **28** with DABCO also gave a mixture rich in **27**.

A mixture of 24 mg of **26** thereby obtained, 16 mg of *m*-chloroperbenzoic acid (85% purity) and 1 ml of CH_2Cl_2 was stirred at −10° for 1 hr. The mixture was diluted with $CHCl_3$, washed with dil. $NaHCO_3$ and dried. Evaporation of the solvent and successive preparative TLC of the residue on silica gel with AcOEt–$CHCl_3$ (1: 3, v/v) gave 20 mg of a mixture of **27** and **28** in a ratio of 3: 1.

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