

## CEFAZOLIN, A NEW SEMISYNTHETIC CEPHALOSPORIN ANTIBIOTIC. I

### SYNTHESIS AND CHEMICAL PROPERTIES OF CEFAZOLIN\*

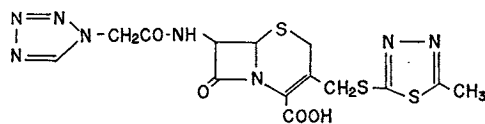
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Cefazolin, 7-[1-(1 H)-tetrazolylacetamido]-3-[2-(5-methyl-1, 3, 4-thiadiazolyl-thiomethyl)]- $\Delta^8$ -cephem-4-carboxylic acid, is a new semisynthetic antibiotic having a broad spectrum of antibacterial activity. A synthesis of cefazolin and its chemical properties are described.

Cefazolin, 7-[1-(1 H)-tetrazolylacetamido]-3-[2-(5-methyl-1, 3, 4-thiadiazolyl-thio-methyl)]- $\Delta^8$ -cephem-4-carboxylic acid (I), is a new semisynthetic antibiotic having a broad spectrum of antibacterial activity. Against Gram-positive bacteria the activity of cefazolin is nearly equal to the activities of cephalothin (CET) and cephaloridine (CER), and against Gram-negative bacteria cefazolin is more active than CET and CER<sup>2)</sup>.



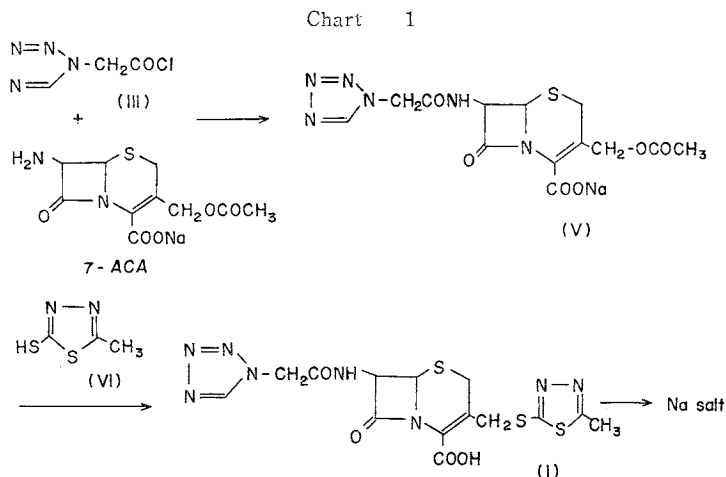
Cefazolin (I)

Cefazolin is characterized by superior activity *in vitro* to clinically isolated *Escherichia coli* and *Klebsiella pneumoniae*, which are strongly resistant to CET and CER<sup>1)</sup>.

### Synthesis

1-(1 H)-Tetrazolylacetic acid (II) was prepared by the hydrolysis of its ethyl ester<sup>2)</sup>. Treatment of tetrazolylacetic acid with phosphorus pentachloride in benzene solution afforded the acid chloride (III). The acid chloride could not be distilled without decomposition. Acylation of 7-aminocephalosporanic acid (7-ACA) with the above acid chloride (III) was carried out by modified SCHOTTEN-BAUMMAN's method with sodium 7-ACA in aqueous acetone, when 7-[1-(1 H)-tetrazolylacetamido]-cephalosporanic acid (V) was produced. Cefazolin was obtained from V by displacement of the acetoxy group by 2-mercapto-5-methyl-1, 3, 4-thiadiazole<sup>3)</sup> (VI) in buffer solution.

\* Studies on cephalosporin. III.



### Properties of Cefazolin (CEZ) and its Sodium Salt (Sodium CEZ)

CEZ crystallizes in colorless needles (m. p. 198~200°C, dec.,  $\lambda_{\text{max}}^{\text{buffer pH } 6.4}$  272 m $\mu$ ,  $\epsilon$  13,150).

The sodium salt of CEZ crystallizes in several forms according to the conditions used. The  $\alpha$ -form containing five moles of crystalline water is obtained by crystallization from aqueous ethanol as colorless fine needles (m. p. 185~186°C, dec.,  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  272 m $\mu$ ,  $\epsilon$  13,250). Treatment of the  $\alpha$ -form with 99% methanol gives the  $\beta$ -form which contains  $3/2$  mole of crystallization water (m. p. 188~189°C, dec.,  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  272 m $\mu$ ,  $\epsilon$  13,200). The  $\beta$ -form can be reconverted to the original  $\alpha$ -form by recrystallization as described

Fig. 1. NMR spectrum of sodium CEZ.

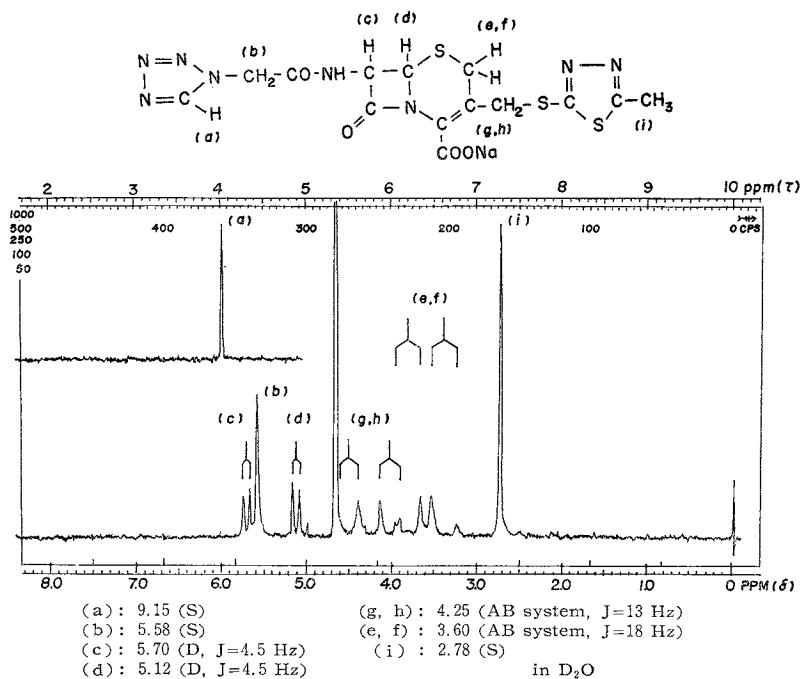


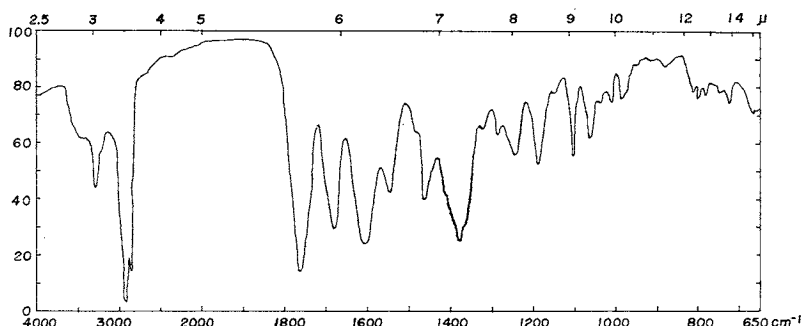
Fig. 2. IR spectrum of sodium CEZ ( $\alpha$ -form)

Fig. 3. IR spectrum of CEZ

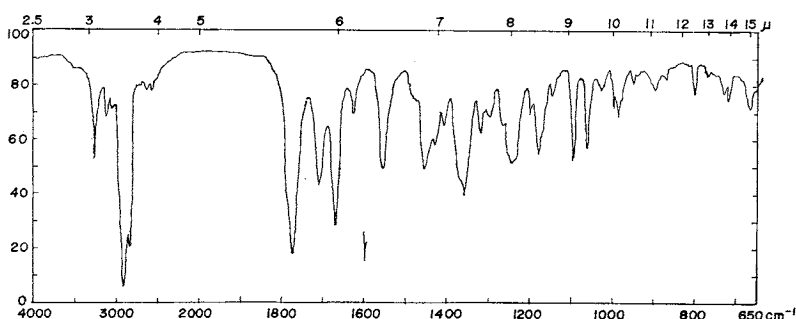


Table 1. Solubility of CEZ and sodium CEZ

	CEZ	Sodium CEZ
easily soluble	DMF, pyridine	H <sub>2</sub> O
soluble	aqueous acetone, aqueous dioxane, aqueous EtOH	DMF, pyridine
hardly soluble	MeOH, EtOH, dioxane, acetone	MeOH, EtOH
insoluble	CHCl <sub>3</sub> , benzene, ether	CHCl <sub>3</sub> , acetone

above or exposure to 100% humidity. The  $\gamma$ -form contains one mole of ethyleneglycol and can be obtained by recrystallization from ethyleneglycol as colorless needles (m. p. 172~174°C, dec.,  $\lambda_{\max}^{\text{H}_2\text{O}}$  272  $\mu$ ,  $\epsilon$  13,200).

The  $\alpha$ - and  $\beta$ -forms were characterized by IR spectra: the  $\alpha$ -form showed a broad absorption band due to water at 3350~3250  $\text{cm}^{-1}$  and an amide carbonyl band at 1665  $\text{cm}^{-1}$ , the  $\beta$ -form showed simple bands due to water at 3430 and 3560  $\text{cm}^{-1}$  and an amide carbonyl band at 1685  $\text{cm}^{-1}$ .

Anhydrous sodium CEZ ( $\lambda_{\max}^{\text{H}_2\text{O}}$  272  $\mu$ ,  $\epsilon$  13,200) was obtained as a hygroscopic colorless amorphous powder by drying *in vacuo* or lyophilizing from either the  $\alpha$ - or

Table 2. Thin-layer chromatography (TLC) and paper chromatography (PPL) of sodium CEZ

		Rf	Solvent	Indicator
TLC	Silica-gel	0.53 ~0.60	<i>n</i> -BuOH 6 HOAc 3 H <sub>2</sub> O 2	KMnO <sub>4</sub>
PPC	Toyo filter paper No. 51A	0.69 ~0.71	<i>n</i> -BuOH 5 HOAc 1 H <sub>2</sub> O 4 (upper layer)	KMnO <sub>4</sub>

Table 3. Stability of sodium CEZ after storing 1 year

	Residual activity (%)		
	0°C	15°C	25°C
$\alpha$ -Form (H <sub>2</sub> O 15.9%)	97	99	99
Amorphous (H <sub>2</sub> O 6.2%)	95	93	92
$\beta$ -Form (H <sub>2</sub> O 5.4%)	97	98	93

Residual activity was determined by agar dilution method using *B. subtilis* ATCC 6633.

Table 4. Stability of CEZ and sodium CEZ at 80°C

	Residual activity (%)								
	Initial	1 hr.	5 hrs.	10 hrs.	20 hrs.	40 hrs.	50 hrs.	100 hrs.	150 hrs.
CEZ	100						99	103	95
Sodium CEZ									
$\alpha$ -Form (H <sub>2</sub> O 15.9%)	100	94	54	39	13	0.5	0	0	0
Amorphous (H <sub>2</sub> O 13.0%)	100						0.8	0	0
Amorphous (H <sub>2</sub> O 8.0%)	100						38	12	2
$\beta$ -Form (H <sub>2</sub> O 5.4%)	100	96	100	93	89	81	78	67	55
Amorphous (H <sub>2</sub> O 2.0%)	100						95	89	84

Residual activity was determined by agar dilution method using *B. subtilis* ATCC 6633.

$\beta$ -forms. Sodium CEZ is easily soluble in water and up to 50% solution can be obtained at 20°C. The solubilities of CEZ and sodium CEZ are shown in Table 1.

The NMR and IR spectra of sodium CEZ showed the corresponding signals and absorption bands as shown in Figs. 1 and 2, and the IR spectrum of CEZ in Fig. 3. CEZ and sodium CEZ were identified by TLC and PPC (Table 2).

The stabilities of CEZ and sodium CEZ in solid forms were examined in a sealed vial under various conditions, their activities being determined by bioassay using an agar dilution method. The results are shown in Tables 3 and 4.

These observations showed that the  $\alpha$ - and  $\beta$ -forms as well as the lyophilized powder were very stable at 25° or 15°C after storing for one year. At 80°C, CEZ was more stable than its sodium salt. The stability of sodium CEZ increases as the water content is decreased: *i.e.*, anhydrous powder >  $\beta$ -form (5.4% of water) >  $\alpha$ -form (15.9%).

The aqueous solution of sodium CEZ was much less stable than the solid form as shown in Table 5. The aqueous solution could be stored without decomposition for two or three days in a cold dark place.

### Experimental\*

#### 1-(1H)-Tetrazolylacetyl chloride (III)

To a suspension of 1-(1H)-tetrazolylacetic acid (II) (7.7 g) in 60 ml of absolute benzene, fine powdered phosphorus pentachloride (25.0 g) was added in one portion with stirring at room temperature. The reaction mixture first became clear for a short while, then it became cloudy with the separation of the acid chloride. After the reaction mixture had been stirred for 2 hours, the precipitated acid chloride was collected, washed with benzene, and dried *in vacuo*. It was employed in the next step without further purification.

IR: acid chloride carbonyl band 1800 cm<sup>-1</sup>

#### 7-[1-(1H)-Tetrazolylacetamido]-cephalosporanic acid (V)

To a solution of 7-ACA (2.7 g) and NaHCO<sub>3</sub> (2.7 g) in water (50 ml)-acetone (35 ml),

\* All melting points are uncorrected. The infrared spectra were recorded on Hitachi EPI S<sub>2</sub>. The nuclear magnetic resonance spectra were measured with a Varian A-60 spectrometer using tetramethylsilane as an internal standard.

Table 5. Stability of sodium CEZ aqueous solution (25%)

	Residual activity (%)				
	Initial	1 day	2 days	3 days	5 days
at 5°C	100	98.9	97.7	96.6	91.6
at 25°C	100	93.4	90.7	88.1	84.2

Residual activity was determined by pulp disk method using *B. subtilis* ATCC 6633.

an acetone solution of the above chloride (2.2 g in 18 ml) was added dropwise with stirring at  $-3\sim 0^{\circ}\text{C}$ . The reaction mixture, which was kept at pH 7.0~8.0 by saturated  $\text{NaHCO}_3$ , was stirred for 1 hour at  $0\sim 5^{\circ}\text{C}$  and for an additional 2 hours at room temperature. It was washed with ether to remove the acetone. The aqueous layer was acidified to pH 1.0 with 5% HCl and was extracted with EtOAc. The organic layer was evaporated *in vacuo*. The residue (10.8 g) and  $\text{NaHCO}_3$  (2.3 g) were dissolved in 20 ml of water, filtered, and 80 ml of 99% EtOH was added to the filtrate. Sodium 7-[1-(1 H)-tetrazolylacetamido]-cephalosporanate was crystallized (m. p.  $176\sim 179^{\circ}\text{C}$ , dec.,  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  262  $\text{m}\mu$ ,  $\epsilon$  8,350).

*Anal.* Calcd. for  $\text{C}_{13}\text{H}_{13}\text{O}_6\text{N}_6\text{SNa}\cdot\frac{1}{2}\text{H}_2\text{O}$ : C 40.83, H 3.69, N 21.98, S 8.39.

Found: C 40.63, H 3.56, N 22.18, S 8.34.

7-[1-(1 H)-Tetrazolylacetamido]-3-[2-(5-methyl-1, 3, 4-thiadiazolyl)-thiomethyl]-2<sup>8</sup>-cephem-4-carboxylic acid (I, Cefazolin CEZ)

A solution of V (10 g),  $\text{NaHCO}_3$  (2.1 g) and 2-mercapto-1,3,4-thiadiazole (VI, 3.8 g) in phosphate buffer (pH 6.4, 200 ml) was stirred for 5.5 hours at  $60^{\circ}\text{C}$ . The reaction mixture was acidified to pH 2.0 with 5% HCl and was extracted with EtOAc. The organic layer was washed with aq. NaCl solution, dried over  $\text{MgSO}_4$ , then evaporated *in vacuo* at below  $40^{\circ}\text{C}$  to give crude cefazolin (9.2 g). The crude cefazolin was recrystallized from aqueous acetone to produce colorless fine needles (m. p.  $198\sim 200^{\circ}\text{C}$ , dec.,  $\lambda_{\text{max}}^{\text{buffer pH } 6.4}$  272  $\text{m}\mu$ ,  $\epsilon$  13,150).

*Anal.* Calcd. for  $\text{C}_{14}\text{H}_{14}\text{O}_4\text{N}_8\text{S}_3$ : C 36.99, H 3.10, N 24.66, S 21.17.

Found: C 36.55, H 3.04, N 24.37, S 21.43.

Sodium cefazolin ( $\alpha$ -form)

To a solution of CEZ (7.35 g) and  $\text{NaHCO}_3$  (1.3 g) in 15 ml of water, 70 ml of 99% EtOH were added. The solution was allowed to stand in a cold dark place. Sodium CEZ was collected by filtration, washed with 20 ml of 95% EtOH, dried over silica-gel under reduced pressure for one day, and then exposed to the atmosphere for one day to give the  $\alpha$ -form (m. p.  $185\sim 186^{\circ}\text{C}$ , dec.,  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  272  $\text{m}\mu$ ,  $\epsilon$  13,250).

*Anal.* Calcd. for  $\text{C}_{14}\text{H}_{13}\text{O}_4\text{N}_8\text{S}_3\text{Na}\cdot 5\text{H}_2\text{O}$ :

C 29.68, H 4.09, N 19.78, S 16.98, Na 4.06,  $\text{H}_2\text{O}$  15.90.

Found: C 29.78, H 4.12, N 19.66, S 16.84, Na 4.03,  $\text{H}_2\text{O}$  15.75.

Sodium cefazolin ( $\beta$ -form)

Sodium CEZ (2.0 g,  $\alpha$ -form) was recrystallized from 10 ml of MeOH. The resulting crystalline product was filtered, dried over silica-gel under reduced pressure for one day and exposed to the atmosphere to give  $\beta$ -form (1.3 g, m. p.  $188\sim 189^{\circ}\text{C}$ , dec.,  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  272  $\text{m}\mu$ ,  $\epsilon$  13,200).

*Anal.* Calcd. for  $\text{C}_{14}\text{H}_{13}\text{O}_4\text{N}_8\text{S}_3\text{Na}\cdot\frac{3}{2}\text{H}_2\text{O}$ : C 33.39, H 3.20, N 22.26, S 19.10,  $\text{H}_2\text{O}$  5.40.

Found: C 33.65, H 3.40, N 22.29, S 19.26,  $\text{H}_2\text{O}$  5.27.

Sodium cefazolin ( $\gamma$ -form)

Pure CEZ (0.5 g) and NaOAc (91 mg) were dissolved in 4 ml of ethyleneglycol. To this solution, acetone (100 ml) was added. The resulting crystalline product was collected by filtration (0.3 g, m. p.  $172\sim 174^{\circ}\text{C}$ , dec.,  $\lambda_{\text{max}}^{\text{H}_2\text{O}}$  272  $\text{m}\mu$ ,  $\epsilon$  13,200).

*Anal.* Calcd. for  $\text{C}_{14}\text{H}_{13}\text{O}_4\text{N}_8\text{S}_3\text{Na}\cdot\text{HOCH}_2\text{-CH}_2\text{OH}$ : C 35.68, H 3.56, N 20.81,  $\text{H}_2\text{O}$  0.00.

Found: C 35.60, H 3.70, N 20.63,  $\text{H}_2\text{O}$  0.00.

Transformation of  $\beta$ -form to  $\alpha$ -form

The  $\beta$ -form was converted to the  $\alpha$ -form by keeping it in an atmosphere of 100% humidity for 3 days or by recrystallization from aqueous alcohol as described above.

**References**

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