A NEW SEMISYNTHETIC 7α-METHOXYCEPHALOSPORIN, CS-1170: 7β-[[(CYANOMETHYL)THIO]ACETAMIDO]-7α-METHOXY-3-[[(1-METHYL-1H-TETRAZOL-5-YL)THIO]METHYL]-3-CEPHEM-4-CARBOXYLIC ACID

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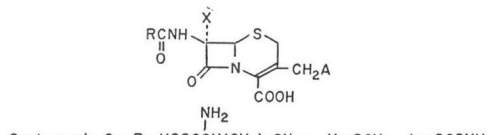
(Received for publication January 9, 1976)

The synthesis and antimicrobial activity of a new semisynthetic 7α-methoxycephalosporin, 7β-[[(cyanomethyl)thio]acetamido]-7α-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]-methyl]-3-cephem-4-carboxylic acid (CS-1170), are described. This compound shows interesting antibacterial activity when compared to cefoxitin and cephalothin.

The recent discovery of a new family of antibiotics, the cephamycins[1~3] and the synthesis of biologically active cefoxitin,[4~7] a semisynthetic analogue of cephamycin C with marked resistance to the action of β-lactamases from gram-positive and gram-negative organisms, stimulated efforts to synthesize various 7α-methoxycephalosporins.

Previously we reported a novel method[8] for synthesizing 7β-amino-7α-methoxycephalosporanate which is an important key intermediate for preparing various 7β-acylamino-7α-methoxycephalosporins. Applying this method in a search for more active agents, we have synthesized a number of 7β-substituted-thioacetamido-7α-methoxycephalosporins. Among them, the title compound (CS-1170) was the most active derivative with more activity than cefoxitin. This paper describes the preparation and antimicrobial activity of this compound (CS-1170).



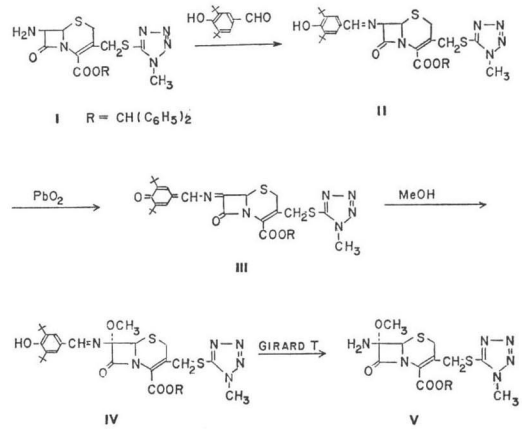
Cephamycin C : R = HOOCCH(CH2)2CH2-, X = OCH3, A = OCONH2

Cefoxitin : R =  , X = OCH3, A = OCONH2

Cephalothin : R =  , X = H, A = OCOCH3

CS-1170 : R = NCCH2SCH2-, X = OCH3, A = 

Chart 1



Synthesis

The title compound was synthesized by acylation of diphenylmethyl 7β-amino-7α-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylate (**V**), which was prepared from the corresponding 7α-hydrogen compound (**I**) by

a previously reported method, with (cyanomethylthio)acetyl chloride (**VIII**). Subsequently the diphenylmethyl protecting group was removed with trifluoroacetic acid as shown in Chart 1.

(Cyanomethylthio)acetic acid was prepared by coupling chloroacetonitrile with ethyl mercaptoacetate in the presence of sodium ethoxide in ethanol followed by hydrolysis of the ester (**VI**). Treatment of the acid (**VII**) with phosphorus pentachloride in ether afforded the acid chloride (**VIII**).

Chart 2

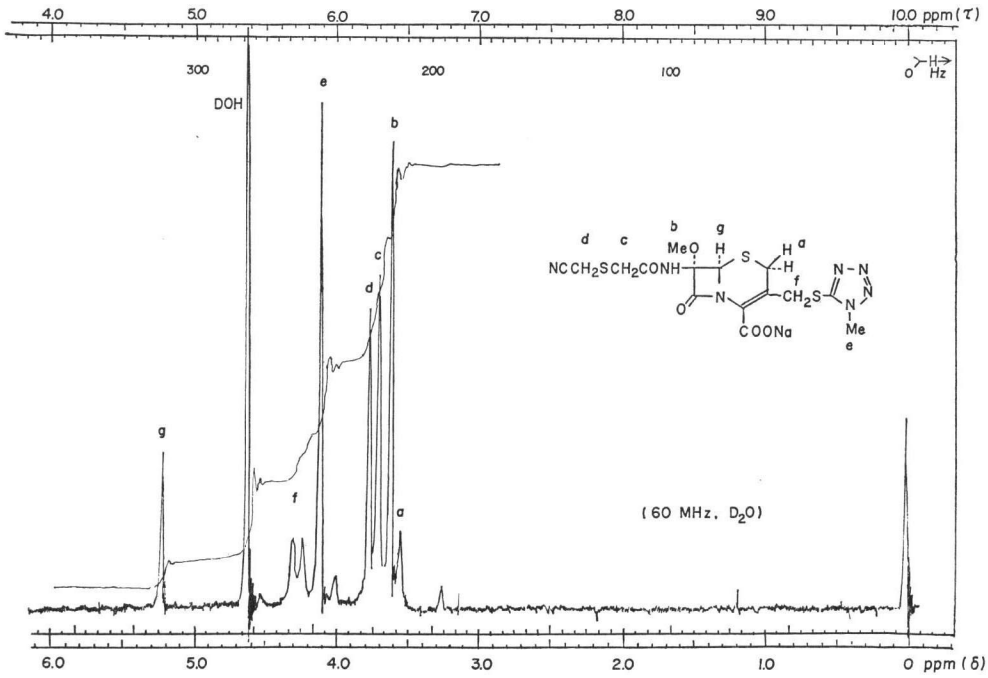
$$NCCH_2Cl + HSCH_2COOC_2H_5 \longrightarrow \underset{\textbf{VI}}{NCCH_2SCH_2COOC_2H_5}$$

$$\xrightarrow{KOH} \underset{\textbf{VII}}{NCCH_2SCH_2COOH} \xrightarrow{PCl_5} \underset{\textbf{VIII}}{NCCH_2SCH_2COCl}$$

The final 7-methoxycephalosporin (CS-1170) was non-crystalline but the dicyclohexylamine salt was crystallized from ethanol as colorless needles. The NMR spectrum of the sodium salt of CS-1170 is shown in Fig. 1.

Fig. 1. NMR spectrum of sodium CS-1170

a: 3.46 (1H, d, J=18 Hz), 3.84 (1H, d, J=18 Hz) b: 3.63 (3H, s)
c: 3.71 (2H, s) d: 3.76 (2H, s) e: 4.12 (3H, s)
f: 4.17 (1H, d, J=13 Hz), 4.38 (1H, d, J=13 Hz) g: 5.22 (1H, s)



Antimicrobial Activity

The *in vitro* antibacterial activity of CS-1170 was tested by the serial agar dilution method. The minimal inhibitory concentrations (MIC) against a variety of gram-positive and gram-negative bacteria are compared with those of cefoxitin and cephalothin in Table 1. From the data it can be seen that CS-1170 has good antimicrobial activity, is 2~4 times more active than cefoxitin, and is

far more active than cephalothin against the β-lactamase-producing *Escherichia coli*. Further details on the biological properties will be reported in near future.

Table 1. Antibacterial activity of CS-1170

Organism	(MIC, mcg/ml)		
	CS-1170	Cefoxitin	Cephalothin
Bacillus subtilis PCI 219	0.2	0.2	0.05
Staphylococcus aureus FDA 209P	0.2	0.4	0.05
Staphylococcus aureus (R)*	0.8	1.5	0.2
Escherichia coli NIHJ	0.8	3.1	6.2
Escherichia coli 609 (R)**	0.8	3.1	50
Klebsiella 806	0.8	3.1	3.1
Proteus vulgaris	1.5	3.1	6.2
Salmonella enteritidis	0.4	1.5	3.1
Shigella flexneri 2a	0.8	3.1	12.5
Pseudomonas aeruginosa	>200	>200	>200

* Penicillinase producer.
** Cephalosporinase producer.
Nutrient agar: Inocula were diluted 100-fold after overnight culture.

Experimental Section

Ethyl (cyanomethylthio)acetate (**VI**)

To a solution of 4.6 g of sodium in 100 ml of EtOH was added dropwise 24 g of ethyl mercaptoacetate followed by 16 g of chloroacetonitrile with stirring at 10~20°C and the mixture was stirred for 3 hours at room temperature. After removal of EtOH *in vacuo*, 100 ml of water was added and the mixture was extracted with ether. The extract was dried ($MgSO_4$) and concentrated; the residue was distilled to give 24 g of colorless liquid, bp 105°C (3 mm).

Anal. Calcd. for $C_6H_9O_2NS$: C, 45.28; H, 5.70, N, 8.80.
Found: C, 45.44; H, 5.86; N, 8.57.

(Cyanomethylthio)acetic Acid (**VII**)

A mixture of 20 g of **VI** and 35 ml of 25% aqueous KOH was stirred at 5~10°C for 1.5 hours. The resulting clear solution was washed with ether, then adjusted to pH 1.5~2 with concentrated HCl at 5~15°C, saturated with NaCl and extracted with ether. The extract was dried ($MgSO_4$) and evaporated to give a crude product (12 g) of **VII** as a viscous liquid, which was employed in the next step without further purification.

(Cyanomethylthio)acetyl Chloride (**VIII**)

To a cold solution of 2.4 g of **VII** in 15 ml of anhydrous ether was added 3.8 g of phosphorus pentachloride and the mixture was stirred for 2 hours at 5~15°C. The reaction mixture was concentrated at room temperature and diluted with 10 ml of CCl_4 then concentrated at 35~40°C. This procedure was repeated once more to remove phosphorus oxychloride. The resulting residue was distilled to give 2 g of **VIII** as an almost colorless liquid, bp 110°C (4 mm).

Anal. Calcd. for C_4H_4ONSCl: C, 32.11; H, 2.69; N, 9.36.
Found: C, 31.82; H, 2.41; N, 9.10.

This chloride is unstable, so should be used immediately or stored at very low temperature.

Diphenylmethyl 7-(3,5-di-*tert*-butyl-4-hydroxybenzylideneamino)-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylate (**II**)

A mixture of 10 g of diphenylmethyl 7-amino-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-3-cephem-

4-carboxylate (**I**) and 5 g of 3,5-di-tert-butyl-4-hydroxybenzaldehyde in 200 ml of benzene was heated under reflux for 40 minutes using a water separator. The reaction mixture was concentrated to about 25 ml *in vacuo* and the residue was dissolved in 150 ml of MeOH and allowed to stand overnight in a refrigerator. The separated crystals were collected, washed with MeOH and dried to give 12 g of colorless crystals, mp 175°C.

Anal. Calcd. for $C_{38}H_{42}O_4N_6S_2$: C, 64.21; H, 5.96; N, 11.83.
Found: C, 64.21; H, 5.97; N, 11.79.

Conveniently the above reaction mixture in benzene is employed in the next step without separation of **II**.

Diphenylmethyl 7-[(3,4-di-*tert*-butyl-4-oxo-2,5-cyclohexadien-1-ylen)methylimino-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylate (**III**)

To a solution of **II** in benzene prepared from 10 g of **I** by the above-described procedure was added 50 ml of 1,2-dichloroethane. After cooling with ice-water, 20 g of freshly prepared PbO_2 was added and the mixture was stirred for 1 hour and then filtered by suction. The red-brown filtrate was concentrated to about half volume *in vacuo* and addition of 300 ml of *n*-hexane yielded a precipitation, which was collected and dried *in vacuo* to give 13 g of **III** as a brown powder. NMR ($CDCl_3$) δ 1.33 (18H, s, *tert*-butyl), 3.82 (2H, br s, C-2), 3.84 (3H, s, N–CH_3), 4.22, 4.53 (2H, d-d, J=14 Hz, CH_2S–), 5.42 (1H, s, C-6), 7.05 (2H, d, J=3 Hz, cyclohexadienyl), 7.07 (1H, s, diphenylmethyl), 7.41 (10H, br s, diphenyl), 7.94 (1H, d, J=3 Hz, =CH–N=).

Diphenylmethyl 7β-(3,5-di-*tert*-butyl-4-hydroxybenzylideneamino)-7α-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylate (**IV**)

To a solution of 13 g of **III** in 100 ml of benzene or the above-described red-brown filtrate was added 400 ml of MeOH. The mixture was allowed to stand overnight at room temperature, then concentrated *in vacuo*. The resulting residue was recrystallized from MeOH to give 7 g of pale yellow crystals. An analytical sample melted at 170°C.

Anal. Calcd. for $C_{39}H_{44}O_5N_6S_2$: C, 63.22; H, 5.99; N, 11.34; S, 8.66.
Found: C, 63.04; H, 6.02; N, 11.08; S, 8.93.

NMR ($CDCl_3$) δ 1.42 (18H, s, *tert*-butyl), 3.59 (3H, s, OCH_3), 3.60 (2H, ABq, J=18 Hz, C-2), 3.80 (3H, s, N–CH_3), 4.20, 4.40 (2H, d-d, J=14 Hz, CH_2S–), 5.03 (1H, s, C-6), 5.60 (1H, s, OH), 6.96 (1H, s, diphenylmethyl), 7.3 (10H, m, diphenyl), 7.65 (2H, s, aromatic), 8.55 (1H, s, –CH=N–).

Diphenylmethyl 7β-amino-7α-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylate (**V**)

To a solution of 5 g of **IV** in 50 ml of EtOAc was added a solution of 2 g of GIRARD T reagent in 40 ml of MeOH. The mixture was stirred for 2.5 hours at room temperature, then concentrated *in vacuo*. To the residue was added 50 ml of EtOAc and 30 ml of water and the separated aqueous layer was extracted with EtOAc. The combined organic layers were washed two times with water, dried ($MgSO_4$) and evaporated *in vacuo*. To the residue was added ether to precipitate a yellow powder, which was collected and dried. Yield, 3 g, mp 95~120°C (dec.).

Anal. Calcd. for $C_{24}H_{24}O_4N_6S_2$: C, 54.96; H, 4.61; N, 16.03.
Found: C, 54.53; H, 4.46; N, 15.45.

7β-[[(Cyanomethyl)thio]acetamido]-7α-methoxy-3-[[(1-methyl-1H-tetrazol-5-yl)thio]methyl]-3-cephem-4-carboxylic Acid (CS-1170)

(a) Acylation of **V**: To a cold solution of 1.9 g of **V** in 30 ml of 1,2-dichloroethane was added dropwise 670 mg of N,N-diethylaniline followed by a solution of 670 mg of cyanomethylthioacetyl chloride (**VIII**) in 2 ml of 1,2-dichloroethane. The mixture was stirred for 30 minutes under cooling, then washed with water, 3% aqueous $KHSO_4$ solution and water successively. The dried ($MgSO_4$) organic layer was concentrated to dryness *in vacuo* to give 2.1 g of the diphenylmethyl ester of CS-1170 as a yellowish amorphous powder, which was employed in the next step without purification.

(b) Hydrolysis of ester: To a chilled solution of the above ester (2.1 g) in 12 ml of 1,2-dichloroethane and 2.1 ml of anisole was added dropwise 3 ml of trifluoroacetic acid with stirring.

After 30 minutes of stirring, the mixture was evaporated *in vacuo* below 35°C to remove excess trifluoroacetic acid. The resulting residue was dissolved in 30 ml of EtOAc, washed with water and then extracted with 10% aqueous K_2HPO_4 solution. The extract was washed two times with EtOAc, then covered with 50 ml of EtOAc and adjusted to pH 2.0 with 10% HCl with stirring. After separation of the organic layer, the aqueous layer was extracted two more times with EtOAc. The combined organic layers were washed with water, dried ($MgSO_4$) and evaporated *in vacuo* to give 1 g of crude acid as a yellowish powder.

(c) Dicyclohexylamine and sodium salts: The above crude acid (1 g) was dissolved in 5 ml of acetone, a solution of 450 mg of dicyclohexylamine in 10 ml of EtOH was added under cooling and the mixture was evaporated *in vacuo*. To the residue was added a mixture of ether and EtOH. The resulting crystals were collected and recrystallized from EtOH to give 0.7 g of dicyclohexylamine salt of CS-1170 as colorless needles, mp 158°C (dec).

Anal. Calcd. for $C_{27}H_{40}O_5N_8S_3$: C, 49.67; H, 6,18; N, 17.16.
Found: C, 49.45; H, 6.40; N, 16.99.

To a solution of 540 mg of the above dicyclohexylamine salt in 15 ml of dichloromethane was added a solution of 0.55 ml of 2 M sodium 2-ethylhexanoate in *n*-butanol. Dilution with 22 ml of cyclohexane yielded a precipitate, which was collected and washed with cyclohexane - dichloromethane (1: 1) mixture to give 350 mg of sodium salt of CS-1170 as colorless powders. UV $\lambda_{max}^{H_2O}$ 272 nm, ε 10,900. IR (Nujol) cm^{-1}: 3200~3500 (NH, H_2O), 2240 (CN), 1755 (β-lactam C=O), 1685 (CONH), 1600 (COO^-). The NMR spectrum is shown in Fig. 1.

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