CI-867, A NEW BROAD-SPECTRUM SEMISYNTHETIC PENICILLIN

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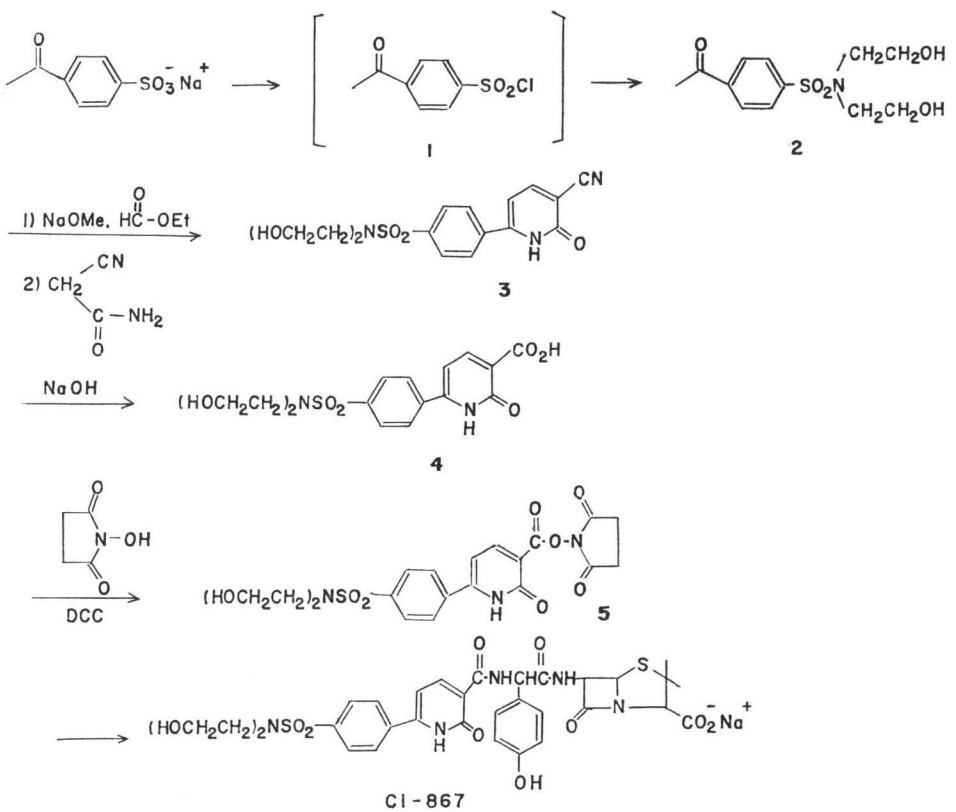
The synthesis and antimicrobial activity of a new semisynthetic penicillin are described. Both *in vitro* and *in vivo*, the compound shows promising antibacterial activity when compared with piperacillin and ticarcillin. High activity is shown against *Pseudomonas* and other Gram-negative bacteria.

Among the more troublesome bacteria involved in hospital infections are those caused by species of *Pseudomonas, Enterobacter*, and *Klebsiella*. A recent report[1] from these laboratories described a series of semisynthetic penicillins containing the sulfonamidophenyl-substituted pyridone

Chart 1.



moiety. This series showed a broad spectrum of antibacterial activity, including good activity against the above microorganisms. Because of its high activity and low level of toxicity, a member of this series, CI-867 has been selected for further development, and we report here its synthesis and preliminary microbiological evaluation.

Synthesis

The synthesis of CI-867 was carried out as outlined in Chart 1 and is described in detail in the Experimental Section. Conversion of 4-acetyl-N,N-bis(2-hydroxyethyl)benzenesulfonamide (**2**) to 4-(5-cyano-1,6-dihydro-6-oxo-2-pyridinyl)-N,N-bis(2-hydroxyethyl)benzenesulfonamide (**3**) was an adaptation of a method described by MARIELLA[2]. Hydrolysis of **3** to the corresponding acid **4** with 5% NaOH solution proceeded without significant hydrolysis of the sulfonamide grouping. Use of the N-hydroxysuccinimide ester[3] as an activator for the carboxyl group avoided any interference by the primary hydroxyl group present in the side chain.

Antimicrobial Activity

The *in vitro* antibacterial activities were determined by microtitration dilution[4] and were done in Trypticase soy broth with an inoculum of 10^3CFU (colony forming units). The *Staphylococcus* and *Streptococcus* tests were run using an inoculum of 10^5CFU. The minimum inhibitory concentrations (MIC) were determined after 16~18-hour incubation at 37°C. Values were determined on the basis of complete inhibition of visible growth. The results of these tests are listed in Table 1.

Table 1. *In vitro* antibacterial activity of CI-867, piperacillin, and ticarcillin.

Organism	MIC, mcg/ml		
	CI-867	Piperacillin	Ticarcillin
Staphylococcus aureus UC-76	0.8	0.4	0.4
Streptococcus faecalis MGH-2	0.2	1.6	25
Klebsiella pneumoniae MGH-2	6.3	1.6	>50
Serratia marcescens IMM-16	6.3	0.8	3.1
Enterobacter cloacae IMM-11	3.1	0.8	3.1
Pseudomonas aeruginosa #28	1.6	3.1	12.5
Pseudomonas aeruginosa BRK-12-4-4	3.1	3.1	25
Pseudomonas aeruginosa UI-18	1.6	1.6	12.5
Escherichia coli Vogel	0.4	0.8	1.6
Escherichia coli Brig	0.8	0.8	3.1
Proteus vulgaris #1810	3.1	0.2	0.8

Table 2. *In vivo* activity of CI-867, piperacillin, and ticarcillin.

Organism	Route	PD_{50} (mg/kg, mice)		Total of 2 doses
		CI-867	Piperacillin	Ticarcillin
Pseudomonas aeruginosa UI-18	s.c.	70	150	240
Pseudomonas aeruginosa BRK-12-4-4	s.c.	62	100	186
Enterobacter cloacae IMM-11	s.c.	42	20	12
Klebsiella pneumoniae MGH-2	s.c.	90	38	3,200

The median protective dose (PD_{50}) is the cumulative result of at least two tests each employing 8 mice per dose level. These tests were performed in groups of Charles River female mice weighing 18~22 g. The intraperitoneal challenge consisted of approximately 100 median lethal doses (LD_{50} of the bacterium in question) and therapy was by a single dose at the time of challenge and a second dose 2 hours post challenge. The results of these tests are listed in Table 2.

From Table 1 it can be seen that *in vitro* CI-867 has an activity profile similar to piperacillin[5,6] and is distinctly more active than ticarcillin. The *in vivo* results versus *Pseudomonas* sp. (Table 2) show the advantage of CI-867 compared to both piperacillin and ticarcillin.

Single intravenous doses as high as 4,000 mg/kg showed no toxic manifestations in mice.

Fig. 1. NMR Spectrum of CI-867.

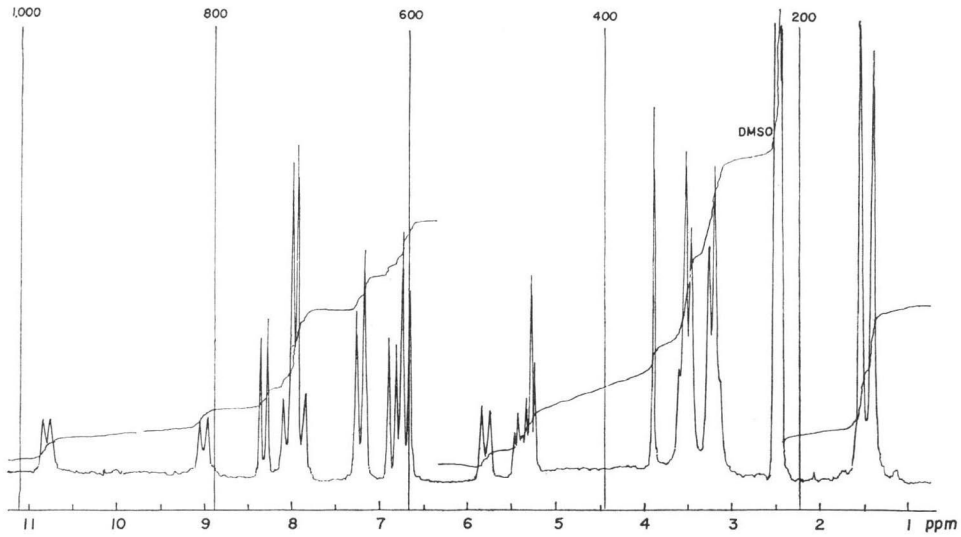


Fig. 1 shows the NMR spectrum of CI-867 as determined with a Bruker B-23367, 90 Hz instrument with DMSO-d_6 as solvent. The peaks and assignments are as follows:

Peak(s)	Assignment
1.4, 1.52 ppm (singlets)	*gem*-dimethyl
2.45 ppm (broad singlet)	DMSO solvent
3.2, 3.5 ppm (multiplet)	CH_2-CH_2-OH (all 3 proton types)
3.89 ppm (singlet)	C_3 hydrogen
5.3 ppm (multiplet centered here)	C_5-C_6 hydrogen
5.75 ppm (doublet)	CH of hydroxyphenylglycine
6.65, 7.18 ppm (pair of doublets, AB pattern)	hydroxyphenyl group
6.82, 8.25 ppm (pair of doublets, AB pattern)	pyridone CH protons
7.92 ppm (center of quartet, AB pattern)	phenyl carrying the SO_2 group
8.95 ppm (doublet)	N-H
10.74 ppm (doublet)	N-H

Experimental Section

4-Acetylbenzenesulfonyl Chloride (**1**)

A suspension of 100 g (0.45 mole, a 5% excess) of sodium 4-acetylbenzenesulfonate in 225 ml DMF was treated all at once with 31 ml (0.428 mole) of $SOCl_2$ and the mixture stirred at room temperature for 5 minutes, then poured into ice-water. The solid was collected and washed with cold water. The wet product was used directly in the next reaction.

4-Acetyl-N,N-bis(2-hydroxyethyl)benzenesulfonamide (**2**)

The wet sulfonyl chloride (from 300 g sodium salt, 1.35 mole) was added in portions to a cooled solution of 500 ml (5.24 mole) of diethanolamine in 750 ml H_2O, and then left stirring at room temperature overnight. The mixture was cooled and the product collected. Recrystallization from 1 liter H_2O gave 190.3 g of a tan solid, mp 87.5~89.5°C.

Anal. Calcd. for $C_{12}H_{17}NO_5S$: C, 50.16; H, 5.97; N, 4.87
Found: C, 50.35; H, 6.13; N, 4.98

4-(5-Cyano-1,6-dihydro-6-oxo-2-pyridinyl)-N,N-bis(2-hydroxyethyl)benzenesulfonamide (**3**)

A suspension of 117.7 g (2.18 mole; 3 equiv plus 10% excess) of $NaOCH_3$ in 1 liter THF and under nitrogen was cooled in ice and treated all at once with 178 ml (2.18 mole, 3 equiv plus 10% excess) of ethyl formate. The mixture was then treated dropwise with a solution of 190.1 g (0.662 mole) of 4-acetyl-N,N-bis(2-hydroxyethyl)benzenesulfonamide in 1.75 liters THF. When the addition was complete, the cooling was removed and the mixture allowed to stir at room temperature overnight. The suspension was diluted with 500 ml Et_2O and the brown solid collected and allowed to air dry.

A solution of this salt in 2.5 liters H_2O was adjusted to pH 9 with HOAc and 84 g (1.0 mole) of cyanoacetamide added. The solution was heated at 90°C for 6 hours, then allowed to stand at room temperature overnight. The solution was brought to pH 6 with HOAc, cooled and 171 g of a brown solid collected. Recrystallization from DMF - MeOH - H_2O gave 138.2 g of product, m.p. 226~228°C.

Anal. Calcd. for $C_{16}H_{17}N_3O_5S$: C, 52.89; H, 4.72; N, 11.56
Found: C, 52.76; H, 4.85; N, 11.43

1,2-Dihydro-6-[4-[[bis(2-hydroxyethyl)amino]sulfonyl]phenyl]-2-oxo-3-pyridinecarboxylic acid (**4**)

A solution of 86.9 g (0.239 mole) of 4-(5-cyano-1,6-dihydro-6-oxo-2-pyridinyl)-N,N-bis(2-hydroxyethyl)benzenesulfonamide in 1.5 liters of a 5% NaOH solution was heated at 105°C for 42 hours in a stainless steel autoclave, then poured onto ice and HCl. The pH was adjusted to give a color change to Congo Red, cooled and the solid collected and dried. Recrystallization from DMF - MeOH - H_2O gave 60 g of a yellow solid, m.p. 252~254°C.

Anal. Calcd for $C_{16}H_{18}N_2O_7S$: C, 50.26; H, 4.74; N, 7.32
Found: C, 50.15; H, 4.90; N, 7.26

4-[5-[[(2,5-Dioxo-1-pyrrolidinyl)oxy]carbonyl]-1,6-dihydro-6-oxo-2-pyridinyl]-N,N-bis(2-hydroxyethyl)-benzenesulfonamide (**5**)

A solution of 45.0 g (0.118 mole) of 1,2-dihydro-6-[4-[[bis(2-hydroxyethyl)amino]sulfonyl]phenyl]-2-oxo-3-pyridinecarboxylic acid and 14.0 g (0.118 mole) of N-hydroxysuccinimide in 450 ml DMF was cooled in ice and treated dropwise with a solution of 26.8 g (0.13 mole) of dicyclohexylcarbodiimide in 30 ml DMF over a 15-minute period. The ice bath was allowed to melt, and the mixture stirred at room temperature overnight. The mixture was recooled and the dicyclohexylurea removed by filtration. Addition of 1.5 liters 2-propanol to the filtrate precipitated the product. There was obtained 40.8 g of the activated ester, m.p. 213~215.5°C.

Anal. Calcd. for $C_{20}H_{21}N_3O_9S$: C, 50.10; H, 4.42; N, 8.76
Found: C, 50.11; H, 4.54; N, 9.00

CI-867: [2S-[2α,5α,6β(S*)]]-6-[[[[[6-[4-[[Bis(2-hydroxyethyl)amino]sulfonyl]-phenyl]-1,2-dihydro-2-oxo-3-pyridinyl]carbonyl]amino](4-hydroxyphenyl)acetyl]amino]-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid, monosodium salt

A solution of 8.0 g (0.01668 mole) of the activated ester in 80 ml N,N-dimethylacetamide was cooled in ice and treated with 7.7 g (0.0183 mole) of amoxicillin·$3H_2O$. Solution occurred in 5 minutes. The solution was kept at 0°C for 3 hours, then allowed to warm to room temperature over 1 hour. The solution was poured into 800 ml of ice water, and the pH brought to 2.0 with dil.HCl. The solid was collected and washed with H_2O. The solid was resuspended twice in cold water and collected. The solid was then suspended in cold water and the pH brought to 6.2 with 1 N NaOH.

A few particles were filtered off and the filtrate freeze-dried to give 9.8 g of the sodium salt. $[\alpha]_D^{23}$ +140° (1.02% in MeOH). Iodometric Assay/Blank: 93.4%/3.8%.

Anal. Calcd. for $C_{32}H_{34}N_5O_{11}S_2Na \cdot 1.5\ H_2O$: C, 49.35; H, 4.79; N, 8.99; S, 8.23
Found: C, 48.82; H, 4.51; N, 8.92; S, 8.02

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