SYNTHESIS OF 6-AMINOPENICILLANIC ACID DERIVATIVES. IV

6-(SYDNONE-3-ACETAMIDO)PENICILLANATES AND 7-(SYDNONE-3-ACETAMIDO)CEPHALOSPORANATES

TAKAYUKI NAITO, SUSUMU NAKAGAWA, KIYOSHI TAKAHASHI, KEI-ICHI FUJISAWA and HIROSHI KAWAGUCHI

Bristol-Banyu Research Institute, Ltd., Meguro, Tokyo, Japan

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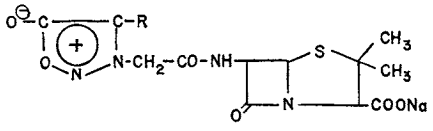
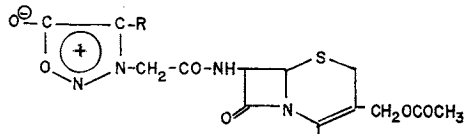
6-(Sydnone-3-acetamido)penicillanates and 7-(sydnone-3-acetamido)cephalosporanates were prepared by N-acylation of 6-APA or 7-ACA with sydnone-3-acetyl chloride. The new series of semi-synthetic antibiotics were compared with ampicillin and cephalothin. Sydonylmethylpenicillin (BBP-425) and sydnonylmethylcephalosporin (BBP-428) were active against gram-positive and gram-negative microbes both *in vitro* and *in vivo*.

In the preceding paper[1] we described the preparation and properties of 6-(3-substituted sydnone-4-carboxamido)penicillanates. The aromaticity of the sydnone ring has prompted us to prepare heterocyclic analogs in which the aromatic ring of benzylpenicillin and cephalothin has been replaced by this ring system.

It was interesting to find that a series of penicillins of this type showed inhibitory activity against gram-negative bacteria as well as gram-positive. The paper describes the preparation and antimicrobial activity of the compounds represented by the following general formulas, (I) and (II).



I



II

Synthesis

Sydnone-3-acetic acid[2] and 4-phenylsydnone-3-acetic acid[3] were prepared by the procedure reported in the literature. Sydnone-3-acetic acid was readily brominated with bromine in water to give 4-bromosydnone-3-acetic acid. The position of bromine atom was confirmed by the nuclear magnetic resonance spectrum (60 Mc; in D_2O). Sydnone-3-acetic acid shows a single-proton singlet at δ 7.11 for the ring proton and a two-proton singlet at δ 5.49 for the α-methylene group. In the brominated product, the former singlet completely disappears and the latter remains with a slight shift to δ 5.53 (the chemical shifts are reported in ppm from the methyl protons of sodium 2,2-dimethyl-2-silapentane-5-sulfonate[4] as an internal reference).

4-Phenylsydnone-3-acetic acid was converted into the corresponding acid chloride with phosphorus pentachloride and condensed with 6-aminopenicillanic acid (6-APA)

in dry methylene chloride with triethylamine to give a penicillin (I, R=C_6H_5). Both sydnone-3-acetic acid and 4-bromosydnone-3-acetic acid reacted readily with phosphorus pentachloride to give the acid chlorides which, however, decomposed during distillation. Therefore, the acid chlorides were used without purification for the N-acylation of 6-APA and 7-aminocephalosporanic acid (7-ACA) to give the corresponding penicillins (I, R=H, Br) and cephalosporins (II, R=H, Br), respectively.

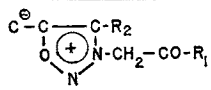
Biological Properties

In vitro Antibacterial Spectrum

The minimum inhibitory concentrations (MIC) of the antibiotics which were prepared in the present study were determined by a two-fold serial dilution method using heart infusion broth. The test organisms employed were two staphylococcal strains (benzylpenicillin-sensitive and resistant) in both the presence and absence of pooled human serum, and five gram-negative bacteria including three strains of *Escherichia coli* and one each of *Klebsiella pneumoniae* and *Salmonella typhosa*. The results are shown in Table 1.

The penicillins (I) of this series are highly active against *Staphylococcus aureus* Smith, a benzylpenicillin-sensitive strain, with a low order of serum binding. The penicillinase-producing organism is not sensitive to these penicillins. One of the notable characteristics of this series of penicillins, as examplified by sydnonylmethylpenicillin (I, R=H, BBP-425), is the considerable activity against gram-negative bacteria. Two strains of *E. coli* (ATCC-8739 and BX-1373) exhibit the same order of

Table 1. Antibacterial spectra and acid stability of 6-(sydnone-3-acetamido)-penicillanates and 7-(sydnone-3-acetamido)cephalosporanates



		R_1=	6-APA			7-ACA		Controls		
		R_2=	H	Br	C_6H_5	H	Br	Benzyl-Penicillin	Ampicillin	Cephalothin
		Code No.	BBP-425	BBP-431	BBP-368	BBP-428	BBP-434			
			mcg/ml							
Test organism	*S. aureus* Smith		0.19	0.19	0.19	0.39	0.39	0.025	0.05	0.19
	S. aureus Smith +50 % serum		0.39	0.39	0.39	0.78	1.56	0.05	0.1	0.39
	S. aureus BX-1633-2		100	100	100	0.78	1.56	100	100	0.39
	S. aureus BX-1633-2 +50 % serum		>100	>100	>100	1.56	3.13	>100	>100	0.78
	E. coli NIHJ		6.25	6.25	6.25	3.13	12.5	31.3	0.78	3.13
	E. coli ATCC 8739		6.25	25	100	3.13	25	50	6.25	12.5
	E. coli BX-1373		6.25	50	>100	3.13	25	25	6.25	12.5
	Kl. pneumoniae Julianelle A		6.25	12.5	100	3.13	25	12.5	1.56	3.13
	Sal. typhosa NIHJ B-26		3.13	6.25	50	6.25	25	3.13	0.78	3.13
Acid stability	Half life at pH 2.0, 37°C		>5 hr.	>5 hr.	>5 hr.	>5hr.	>5hr.	<0.25 hr.	>5 hr.	>5 hr.

susceptibility to BBP-425 as to ampicillin, a typical penicillin having gram-negative activity. Another strain of *E. coli* (NIHJ) and the other two gram-negative bacteria are less sensitive to BBP-425 than to ampicillin. Sydnone ring substitution at the 4-position did not produce any effect on the activity of the penicillin against staphylococci but had an adverse effect on its activity against gram-negative organisms. This structure-activity relationship also held true with the cephalosporins in the present study.

The cephalosporins (II) of the sydnonylmethyl series were compared with cephalothin, a commercial semi-synthetic cephalosporin. The antibacterial spectrum of sydnonylmethylcephalosporin (II, R=H, BBP-428) is essentially the same as that of cephalothin, and they are active against both types of staphylococci and gram-negative bacteria. In terms of MIC values, BBP-428 was slightly less active than cephalothin against staphylococci and slightly more active against *E. coli*.

In vivo Activity

The median curative doses (CD_{50}) of sydnonylmethylpenicillin (BBP-425) and sydnonylmethylcephalosporin (BBP-428) were determined with three experimental mice infections of *S. aureus* Smith, *E. coli* BX-1373 and *K. pneumoniae* by the procedure described in the previous report[5]. The antibiotics were given subcutaneously or orally just before the bacterial challenge, and ampicillin and cephalothin were used as controls. The results are summarized in Table 2.

Table 2. *In vivo* activity of sydnonylmethylpenicillin (BBP-425) and sydnonylmethylcephalosporin (BBP-428)

Challenge organism	Route	Mediam curative does (CD_{50}) in mg/kg			
		BBP-425	BBP-428	Ampicillin	Cephalothin
S. aureus Smith	i. m.	0.63	6.2	0.35	3.8
	p. o.	2.5	20	2.9	17
E. coli BX-1373	i. m.	24	5.6	23	11
	p. o.	80	>300	75	120
K. pneumoniae	i. m.	35	26	13	35
	p. o.	160	>300	40	280

Sydnonylmethylpenicillin showed almost the same degree of *in vivo* activity as ampicillin against *S. aureus* and *E. coli* by both routes of administration and somewhat less acivity against *K. pneumoniae*. As expected from the *in vitro* activity, sydnonylmethylcephalosporin was more potent than cephalothin against gram-negative infections by parenteral administration, but judging from the oral CD_{50} values obtained, the new cephalosporin was less absorbed orally than cephalothin.

Acid Stability

The acid stabilities of the new series of semi-synthetic antibiotics were determined by the procedure reported previously[1]. As can be seen in the bottom row of Table 1, all compounds prepared in the present study were fairly stable in acidic medium. It is interesting to note that sydnonylmethylpenicillin (BBP-425) which is structurally

related to benzylpenicillin shows a half life of longer than 5 hours under the condition that quickly destroy the latter.

Experimental

4-Bromosydnone-3-acetic acid

To a stirred solution of 4.2 g (0.034 mole) of sydnone-3-acetic acid[2] and 2.9 g (0.034 mole) of sodium bicarbonate in 20 ml of water was added dropwise 5.5 g (0.034 mole) of bromine at room temperature. The reaction mixture was stirred for 10 minutes, then evaporated to one-fifth volume under reduced pressure. The concentrate was extracted with three 20-ml portions of ethyl acetate. The combined ethyl acetate solutions were dried with anhydrous sodium sulfate and the solvent was evaporated to give the crude acid which was recrystallized from ethyl acetate and benzene. Yield 5.7 g (75 %). M. p. 110~111°C (dec.). $\nu_{C=O}^{KBr}$ 1765~1735 cm^{-1} (broad). λ_{max}^{EtOH} 310 mμ (ε 8,400).

Anal. Calcd. for $C_4H_3BrN_2O_4$: C 21.54, H 1.74, N 12.56.
Found : C 22.17, 22.28, H 1.50, 1.45, N 12.44.

4-Phenylsydnone-3-acetyl chloride

A mixture of 3.9 g (0.0185 mole) of 4-phenylsydnone-3-acetic acid[3] and 6.3 g (0.03 mole) of phosphorus pentachloride in 10 ml of dry methylene chloride was refluxed for 2 hours on a water bath. The reaction mixture was filtered and 10 ml of petroleum ether was added to the filtrate to afford 2.8 g (66 %) of 4-phenylsydnone-3-acetyl chloride. M. p. 94~96°C. $\nu_{C=O}$ 1800 cm^{-1} (sydnone ring C-O$^-$), 1720 cm^{-1}, (COCl).

Anal. Calcd. for $C_{10}H_7ClN_2O_3$: C 50.53, H 2.96, N 11.74.
Found : C 49.74, 49.58, H 3.30, 3.44, N 11.51, 11.45.

Sodium 6-(4-phenylsydnone-3-acetamido)penicillanate (I, R=C_6H_5)

To a stirred solution of 2.5 g (0.0115 mole) of 6-APA and 4 ml of triethylamine in 50 ml of dry methylene chloride was added dropwise a solution of 2.8 g (0.0117 mole) of 4-phenylsydnone-3-acetyl chloride in 5 ml of dry methylene chloride at 5°C. The reaction mixture was stirred for one hour at 10~15°C, then extracted with 100-ml and 50-ml portions of water. The combined aqueous extracts were washed with two 200-ml portions of ether, covered with 100 ml of ethyl acetate and adjusted to pH 2.0 with 10 % hydrochloric acid with vigorous stirring in the cold. The ethyl acetate layer was separated and the aqueous layer was extracted with another 100 ml of ethyl acetate. The combined ethyl acetate solutions were washed with two 50-ml portions of water, dried over anhydrous sodium sulfate and evaporated to *ca.* 50 ml under reduced pressure below 30°C. To the concentrate was added 5 ml of 39 % sodium 2-ethylhexanoate (SEH) solution in methyl *iso*butyl ketone (MIBK) to give a white precipitate, which was collected by filtration with suction and dried over phosphorus pentoxide *in vacuo*. Yield 1.73 g (32 %). M. p. 198~205°C (dec.). ν_{max}^{KBr} 1750, 1690, 1610, 1410 cm^{-1}. $\lambda_{max}^{H_2O}$ 240 mμ (ε 5,700), 312 mμ (ε 7,400).

Anal. Calcd. for $C_{18}H_{17}N_4O_6SNa \cdot 1\frac{1}{2}H_2O$: C 46.26, H 4.31.
Found : C 46.02, 45.62, H 4.06, 4.11.

Sodium 6-(sydnone-3-acetamido)penicillanate (I, R=H)

A suspension of 1.4 g (0.01 mole) of sydnone-3-acetic acid in 25 ml of dry methylene chloride was treated with 2.5 g (0.012 mole) of phosphorus pentachloride and allowed to react at room temperature for half an hour with occasional shaking. The isolation of sydnone-3-acetyl chloride was unsuccessful, therefore the resulting acid chloride solution was used directly for the acylation of 6-APA.

A solution of 2.2 g (0.01 mole) of 6-APA and 4.2 g (0.05 mole) of sodium bicarbonate in 40 ml of water was chilled and covered with 50 ml of ethyl acetate and the acid chloride solution was added slowly with vigorous stirring at 0°C. The reaction mixture was stirred for 10 minutes at 10°C, the organic layer was separated and the aqueous layer was extracted with 20 ml of ethyl acetate. The combined extracts were washed

with water, dried with anhydrous sodium sulfate, filtered and treated with 4 ml of 35 % SEH solution in MIBK to give a precipitate, which was collected by filtration, washed with ethyl acetate and dried *in vacuo* over phosphorus pentoxide. Yield 1.0 g (28 %). M. p. 180~200°C (dec.). ν_{max}^{KBr} 1780~1740 (broad), 1680, 1600, 1400 cm^{-1}. $\lambda_{max}^{H_2O}$ 293 mμ (ε 4,400).

Anal. Calcd. for $C_{12}H_{18}N_4O_6SNa$: C 39.56, H 3.60.
Found : C 39.51, 39.01, H 4.37, 4.51.

Sodium 6-(4-bromosydnone-3-acetamido)penicillanate (I, R=Br)

This compound was prepared by the procedure as that of sodium 6-(sydnone-3-acetamido)penicillanate (I, R=H). The 4-bromosydnone-3-acetyl chloride solution was prepared from 2 g (0.009 mole) of 4-bromosydnone-3-acetic acid and 2.25 g (0.010 mole) of phosphorus pentachloride in 60 ml of dry methylene chloride. The resulting solution was reacted with a stirred mixture of 1.95 g (0.009 mole) of 6-APA and 3.8 g (0.0045 mole) of sodium bicarbonate in 30 ml of water and 50 ml of ethyl acetate to give 2.8 g (70 %) of sodium 6-(4-bromosydnone-3-acetamido)penicillanate. M. p. 180~190°C (dec). ν_{max}^{KBr} 1780~1740 (broad), 1690, 1605, 1380 cm^{-1}. $\lambda_{max}^{H_2O}$ 309 mμ (ε 7,400).

Anal. Calcd. for $C_{12}H_{12}BrN_4O_6SNa \cdot H_2O$: C 31.25, H 3.06, N 12.15.
Found : C 31.63, H 3.02, N 11.87.

Sodium 7-(sydnone-3-acetamido)cephalosporanate (II, R=H)

A mixture of 1 g (0.007 mole) of sydnone-3-acetic acid and 2 g (0.0095 mole) of phosphorus pentachloride in 50 ml of dry methylene chloride was occasionally shaken for half an hour at room temperature. The resulting sydnone-3-acetyl chloride solution was added slowly at 0°C to a stirred mixture of 1.9 g (0.007 mole) of 7-ACA and 6 g (0.07 mole) of sodium bicarbonate in 20 ml of water and 30 ml of ethyl acetate. The reaction mixture was stirred at 10°C for 20 minutes. The organic layer was discarded. The aqueous layer was covered with 50 ml of ethyl acetate and adjusted to pH 2.0 with 20 % hydrochloric acid with stirring at 5°C. The ethyl acetate layer was separated and the aqueous layer was extracted twice with 20-ml portions of ethyl acetate. The combined ethyl acetate solutions were washed with water, dried with anhydrous sodium sulfate, filtered and treated with 3.3 ml of 35 % SEH solution in MIBK. The precipitated product was collected by filtration, washed with 5 ml of ethyl acetate and dried *in vacuo* over phosphorus pentoxide. Yield 0.6 g (20 %). M. p. 180~200°C (dec.). ν_{max}^{KBr} 1780~1740 (broad), 1640, 1610, 1405 cm^{-1}. $\lambda_{max}^{H_2O}$ 269 mμ (ε 10,000).

Anal. Calcd. for $C_{14}H_{13}N_4O_8SNa \cdot H_2O$: C 38.36, H 3.45.
Found : C 38.65, 39.12, H 4.31, 4.30.

Sodium 7-(4-bromosydnone-3-acetamido)cephalosporanate (II, R=Br)

This compound was prepared by the same procedure as that of sodium 7-(sydnone-3-acetamido)cephalosporanate (II, R=H). The acid chloride solution prepared from 1.5 g (0.0067 mole) of 4-bromosydnone-3-acetic acid and 1.7 g (0.008 mole) of phosphorus pentachloride in 50 ml of dry methylene chloride was allowed to react with a stirred mixture of 1.85 g (0.0067 mole) of 7-ACA and 6 g (0.07 mole) of sodium bicarbonate in 50 ml of water and 50 ml of ethyl acetate to yield 1.4 g (42 %) of sodium 7-(4-bromosydnone-3-acetamido)cephalosporanate. M. p. 155~175°C (dec.). ν_{max}^{KBr} 1770~1730 (broad), 1630~1600 (broad), 1380 cm^{-1}. $\lambda_{max}^{H_2O}$ 270 mμ (ε 7,200), 307 mμ (ε 9,450).

Anal. Calcd. for $C_{14}H_{12}BrN_4O_8SNa \cdot 3H_2O$: C 30.39, H 3.28, N 10.13.
Found : C 30.28, H 2.54, N 10.14, 10.18.

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