SYNTHESIS OF 6-AMINOPENICILLANIC ACID DERIVATIVES. III[1]

6-(3-SUBSTITUTED-SYDNONE-4-CARBOXAMIDO)-PENICILLANATES

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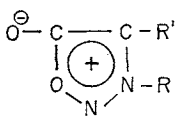
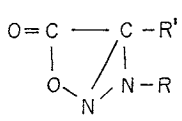
The preparation and properties of a new series of penicillins, 6-(3-substituted sydnone-4-carboxamido)penicillanates, are described. Those of the new penicillins having an aryl group at 3-position of sydnone ring showed activity against a penicillinase-producing organism. These penicillins are, however, not very resistant to penicillinase when compared with oxacillin.

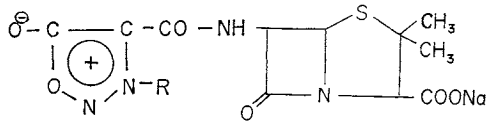
The first mesoionic compounds known as sydnones (I) were synthesized in 1935 by EARL and MACKENEY[2] at the University of Sydney, who originally postulated the incorrect bicyclic structure (II) for their products. Since then, studies on the synthesis and structure of sydnones have been reported by many workers[3,4]. However, the pharmacological aspects of such compounds have been investigated only recently[5~8]. This paper reports the preparation and antimicrobial activity of penicillins (III) derived from 3-substituted sydnone-4-carboxylic acids.



(I)



(II)



(III)

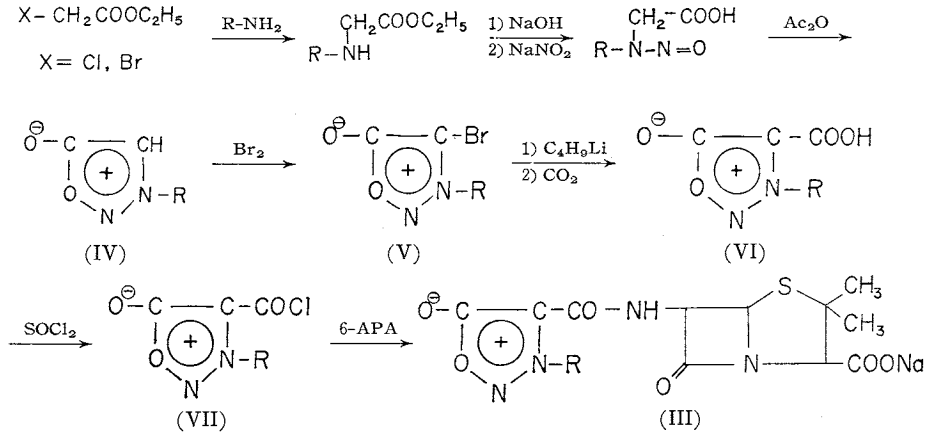
Synthesis

3-Substituted sydnones (IV) have been prepared by the procedure illustrated in Fig. 1, which is the original method of EARL and MACKNEY[2] and is still the only general route to sydnones.

Introduction of a carboxyl group to the 4-position of 3-phenylsydnone was carried out successfully by KATO and OHTA[9] by bromination with bromine in acetic acid and lithiation of the resulting 4-bromo derivative with *n*-butyl lithium followed by treatment with carbon dioxide. Subsequently, KIER *et al.*[7c] applied this procedure to 3-isopropyl- and 3-*sec*-butylsydnones. All of the 3-substituted sydnones prepared in our laboratory afforded the corresponding 4-carboxylic acids (VI) by this procedure with the exception of 3-*tert*-butylsydnone which reacted readily with bromine to give the 4-bromosydnone but did not give the desired acid.

The 3-substituted sydnone-4-carboxylic acids (VI) were converted into the corresponding acid chlorides (VII) with thionyl chloride and condensed with 6-amino-

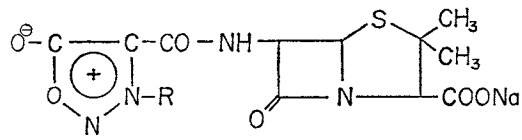
Fig. 1. Preparation of 6-(3-substituted sydnone-4-carboxamido)penicillanates



penicillanic acid (6-APA) triethylammonium salt in dry methylene chloride (Method A) or with 6-APA sodium salt in acetone-water (Method B) to give the 6-(3-substituted sydnone-4-carboxamido)penicillanates (III) listed in Table 1.

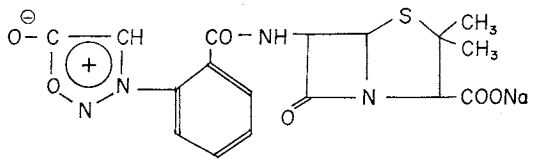
Two related compounds were also prepared. 2-(3-Sydnone)benzoic acid[10] was converted into the corresponding acid chloride with phosphorus pentachloride and condensed with 6-APA to give sodium 6-[2-(3-sydnone)benzamido]penicillanate (VIII), which is a heterocyclic analog of biphenylylpenicillin[11]. 3-Phenylsydnone-4-carbonyl chloride was reacted with 7-aminocephalosporanic acid (7-ACA) by a similar procedure

Table 1. 6-(3-Substituted sydnone-4-carboxamido)penicillanates

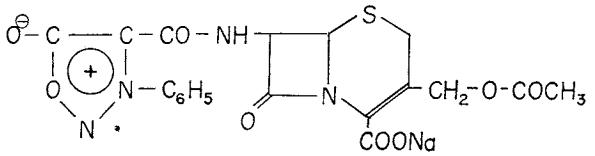


R	Melting point ℃ (dec.)	Yield (%)	Method	$\lambda_{max}^{H_2O}$ (mμ)	ε	Formula	C (%)		H (%)	
							Calcd.	Found	Calcd.	Found
C_6H_5	180~190	41	A	228 311	10,800 11,700	$C_{17}H_{15}N_4O_6SNa\cdot\frac{1}{2}H_2O$	46.89	46.82	3.70	3.76
o-Cl-C_6H_4	180~195	65	B	313	10,000	$C_{17}H_{14}ClN_4O_6SNa\cdot H_2O$	42.63	42.99	3.37	3.59
p-Cl-C_6H_4	195~210	23	A	219 312	17,500 11,400	$C_{17}H_{14}ClN_4O_6SNa\cdot H_2O$	42.64	42.74	3.37	3.99
o-OCH_3-C_6H_4	175~185	94	B	310	10,300	$C_{18}H_{17}N_4O_7SNa$	47.36	47.17	3.75	4.60
p-OCH_3-C_6H_4	210~220	97	B	226 312	16,000 12,300	$C_{18}H_{17}N_4O_7SNa\cdot\frac{1}{2}H_2O$	46.45	46.62	3.90	4.04
o-CH_3-C_6H_4	170~177	11	A	225 308	10,000 10,500	$C_{18}H_{17}N_4O_6SNa\cdot\frac{1}{2}H_2O$	48.10	47.98	4.04	5.00
p-CH_3-C_6H_4	170~180	92	B	310	11,000	$C_{18}H_{17}N_4O_6SNa\cdot\frac{1}{2}H_2O$	48.10	48.19	4.04	4.48
$(CH_3)_2CH-$	203~206	85	B	226 300	9,000 12,300	$C_{14}H_{17}N_4O_6SNa\cdot\frac{1}{2}H_2O$	41.89	42.31	4.52	5.02
$(C_2H_5)(CH_3)CH-$	209~211	6.6	A	211.5 300	10,000 10,500	$C_{15}H_{19}N_4O_6SNa\cdot H_2O$	42.45	42.37	4.99	5.26

to afford sodium 7-(3-phenylsydnone-4-carboxamido)cephanosporanate (IX) which is the 7-ACA derivative corresponding to sodium 6-(3-phenylsydnone-4-carboxamido)-penicillanate (X).



(VIII)



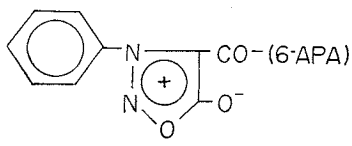
(IX)

The ultraviolet spectra of the penicillins prepared here show an absorption maxima in the same range as those of the corresponding sydnone carboxylic acids——at about 310 mμ for penicillins bearing an aryl substituent and at about 300 mμ for those bearing an alkyl substituent. In the infrared spectra they have strong absorption bands at 1,760~1,770 cm^{-1} (β-lactam and sydnone C-O^-), 1,670~1,690 cm^{-1} (amide carbonyl), 1,600~1,620 cm^{-1} and 1,400~1,410 cm^{-1} (carboxylate). As the carbonyl stretching vibration of the β-lactam in the penicillin nucleus overlaps with that of the sydnone ring, it is difficult to estimate the purity of the penicillins by the intensity of the β-lactam carbonyl band in dimethylsulfoxide solution[12].

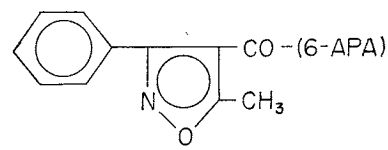
Antimicrobial Activity

The minimum inhibitory concentrations (MIC) and the median curative doses (CD_{50}) of the penicillins were determined with *Staphylococcus aureus* SMITH (benzylpenicillin-sensitive) and *Staphylococcus aureus* BX-1633-2 (benzylpenicillin-resistant) by the procedures described in the previous paper[1]. The *in vitro* and *in vivo* antibacterial data obtained with the new series of penicillins are shown in Table 2. The compounds are grouped according to the structure of the substituents on the sydnone ring. As can be seen in the table, the series of 3-arylsydnone derivatives (Group 1) showed activities against both benzylpenicillin-sensitive and resistant strains. Substituents on the phenyl group at the 3-position of the sydnone did not improve the biological activity. The 3-alkylsydnone derivatives (Group 2) were virtually inactive against the resistant strain.

The inhibitory activity of the 3-arylsydnone penicillins against resistant staphylococcus seems to correlate with the fact that the sydnone ring is planar and has aromatic character, and accordingly, the aryl group at the 3-position of the sydnone ring might exert a steric effect on reactions involving the β-lactam carbonyl of the penicillin nucleus. Thus, 6-(3-phenylsydnone-4-carboxamido)penicillanate (X) is considered to have a certain sterical similarity to oxacillin (XI), a typical penicillinase-resistant penicillin.

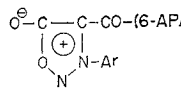
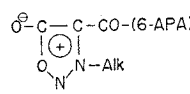
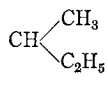
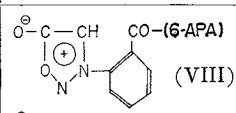
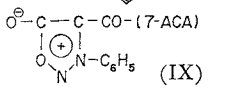


(X)

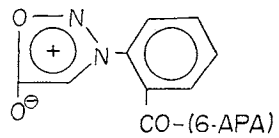


(XI)

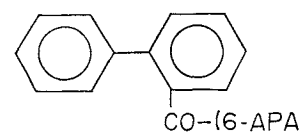
Table 2. Antimicrobial activities of 6-(3-substituted sydnone-4-carboxamido)-penicillanates and the related compounds

Group	Compounds	Code No.	Minimum inhibitory concentrations (mcg/ml)				CD_{50} (im, mg/kg)	
			S. aureus Smith		*S. aureus* BX-1633-2		*S. aureus* Smith	*S. aureus* BX-1633-2
			no serum	with 50% serum	no serum	with 50% serum		
(1) 3-Aryl derivatives 	Ar=C_6H_5(X)	BBP-359	0.4	1.6	0.8	3.1	9.6	150
	o-Cl-C_6H_4	BBP-380	0.8	6.3	1.6	12.5	50	330
	p-Cl-C_6H_4	BBP-373	0.8	6.3	1.6	12.5	3.1	160
	o-OCH_3-C_6H_4	BBP-375	1.6	6.3	3.1	12.5	5.4	200
	p-OCH_3-C_6H_4	BBP-376	0.8	6.3	3.1	12.5	20.5	230
	o-CH_3-C_6H_4	BBP-372	0.8	3.1	3.1	12.5	30	260
	p-CH_3-C_6H_4	BBP-377	0.8	6.3	1.6	12.5	11.2	250
(2) 3-Alkyl derivatives 	Alk.=$CH(CH_3)_2$	BBP-430	3.1	12.5	100	>100	16	>800
		BBP-426	1.6	6.3	50	>100	—	—
(3) Related compounds	 (VIII)	BBP-362	0.8	1.6	12.5	25	0.7	600
	 (IX)	BBP-433	6.3	12.5	25	50	100	>800
Control	Benzylpenicillin		0.05	0.1	>100	>100	0.3	—
	Oxacillin		0.1	0.8	0.4	1.6	4.0	32
	Cephalothin		0.2	0.4	0.4	0.8	1.3	100

Similarly, 2-sydnonephenylpenicillin (VIII) of Group 3 is related structurally to biphenylylpenicillin (XII). The new penicillin showed, however, only a modest activity against the penicillinase-producing organism, suggesting that a sydnone ring is not an efficient substitute for the distal phenyl group[13] in biphenylylpenicillin.



(VIII)



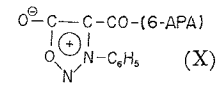
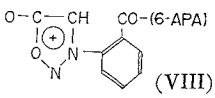
(XII)

The degree of resistance to the action of staphylococcal penicillinase was studied with two of the new penicillins, VIII and X. A simple way obtaining this information is to determinet he MIC in presence of increasing inoculum size of the penicillinase-producing organism, *S. aureus* BX-1633-2. As shown in Table 3, the MIC values of the new penicillins increased markedly at higher inoculum levels, indicating that these penicillins are not very resistant to penicillinase.

The acid stabilities of these new penicllins were determined by allowing the penicillins to stand in 0.05 M pH 2.0 citrate buffer at 37°C. In order to calculate the

half life of a penicillin under the defined conditions, aliquots of the test solution were taken at 0.5, 1, 2, 3 and 5 hours, and assayed for the remaining activity. The half lives of benzylpenicillin and oxacillin under these conditions were shorter than 10 minutes and about 2 hours, respectively, whereas all the penicillins listed in Table 2 showed half lives of longer than 5 hours, indicating high resistance to acid.

Table 3. Effect of inoculum size of resistant organism

Penicillins	Inoculum size (dilutions) and MIC (mcg/ml) of *S. aureus* BX-1633-2	
	10^4	10^2
Oxacillin (control)	0.4	0.8
 (X)	0.8	>100
 (VIII)	12.5	>100

Experimental

3-Substituted sydnones (IV)

3-Phenyl-[2], 3-(*p*-chlorophenyl)-[14], 3-(*o*-methoxyphenyl)-[15], 3-(*p*-methoxyphenyl)-[15], 3-(*o*-tolyl)-[4], 3-(*p*-tolyl)-[14], 3-isopropyl-[7a], 3-*sec*-butyl-[7a] and 3-*tert*-butyl-[7a] sydnones were prepared by the general method of EARL and MACKENEY[2]. The preparation of 3-(*o*-chlorophenyl)-sydnone, which has not been hitherto described, is given below as a representative example.

A mixture of 127.5 g (1.0 mole) of *o*-chloroaniline, 122.5 g (1.0 mole) of ethyl chloroacetate and 136 g (1.0 mole) of sodium acetate trihydrate was refluxed for 18 hours. The reaction mixture was poured into 1 L of water. The organic layer was separated, dried with anhydrous sodium sulfate and distilled under reduced pressure to give 77 g (36 %) of ethyl N-(*o*-chlorophenyl)glycinate boiling at 160~167°C/3 mm.

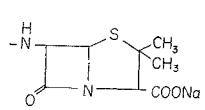
A suspension of 77 g (0.36 mole) of ethyl N-(*o*-chlorophenyl)glycinate in 200 ml of 10 % aqueous sodium hydroxide was mixed with 20 ml of ethanol to obtain a solution. The solution was refluxed for 2 hours. The ethanol was evaporated under reduced pressure and the residual solution was adjusted to pH 2.0 with dil. hydrochloric acid to afford 53 g (80 %) of N-(*o*-chlorophenyl)glycine melting at 161~163°C.

A solution of 20 g (0.3 mole) of sodium nitrite in 50 ml of water was added dropwise at 0~5°C to a stirred solution of 53 g (0.28 mole) of N-(*o*-chlorophenyl)glycine in 1.3 L of 25 % hydrochloric acid. The precipitate which separated was collected by filtration, washed with water and dried *in vacuo* to give N-nitroso-N-(*o*-chlorophenyl)glycine melting at 78 ~79°C in a yield of 49 g (80 %).

A mixture of 49 g (0.23 mole) of N-nitroso-N-(*o*-chlorophenyl)glycine and 210 ml of acetic anhydride was stirred for 4 days at room temperature. The reaction mixture was poured into 500 ml of water. After the acetic anhydride was completely decomposed, the solution was made slightly alkaline with 300 ml of 28 % ammonium hydroxide. The 3-(*o*-chlorophenyl)sydnone which separated was collected by filtration, washed with water and dried *in vacuo*. The analytical sample was recrystallized from hot water. Yield 14.3 g (32 %). M. p. 87~88°C. ν_{max}^{KBr} 3,180 (ring C-H), 1,785 (C-O$^{\ominus}$) cm^{-1}. λ_{max}^{EtOH} 303 mμ (ε 6,200).

Anal. Calcd. for $C_8H_5ClN_2O_2$: C 48.87, H 2.56, N 14.25.
Found : C 49.00, 48.80; H 2.57, 2.84; N 14.17, 14.15.

In this article, 6-APA and 7-ACA represent the following structures :

-6-(APA) = 

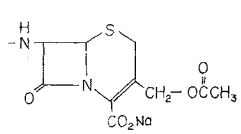
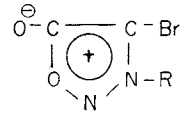
-(7-ACA) = 

Table 4. 4-Bromo-3-substituted sydnones



R	Melting point (°C)	Yield (%)	λ_{max}^{EtOH} (mμ)	ε	$\nu_{C=O}$ (cm^{-1})	C (%)		H (%)		N (%)	
						Calc.	Found	Calc.	Found	Calc.	Found
C_6H_5	140 (141)[a]	80	241.5 321	5,430 7,140	1790						
o-Cl-C_6H_4	109~110	84	318	7,600	1780	34.87	35.02	1.46	1.08	10.16	10.17
p-Cl-C_6H_4	115~118	84	247.5 322	7,260 6.250	1770[c] 1740	34.87	35.20	1.46	1.80	10.16	10.37
o-OCH_3-C_6H_4	121~123	82	315	7,900	1770[c] 1745	39.87	40.36	2.60	2.92	10.34	10.10
p-OCH_3-C_6H_4	102~104	94	223 318	9,800 8,100	1790	39.87	39.83	2.60	2.13		
o-CH_3-C_6H_4	109~110	92	231 316	2,870 7,760	1775	42.38	42.17	2.77	2.79	10.98	11.05
p-CH_3-C_6H_4	124~127	96	247 322	6,150 7,500	1780	42.38	42.88	2.77	2.81	10.98	10.97
$(CH_3)_2CH$-	78~79.5 (80~81.5)[b]	50	310	8,370	1750						
C_2H_5>CH_3>CH-	59~60.5 (61~62.5)[b]	48			1750						
$(CH_3)_3C$-	145~147	86	310	7,550	1750	32.60	32.86	4.10	4.05	12.67	12.47

a : see, ref. 9). b : see, ref. 7c). c : split into two bands

4-Bromo-3-substituted sydnones (V)

To a stirred mixture of 0.1 mole of the appropriate 3-substituted sydnone (IV) and 0.2 mole of anhydrous sodium acetate in about 100 ml of acetic acid was added dropwise 0.1 mole of bromine at 0~10°C. The reaction mixture was stirred for an hour at about 10°C and poured into a large quantity of water. The resulting precipitate was collected by filtration, washed with water and recrystallized from ethanol. Table 4 shows the yield and physical properties of the 4-bromo-3-substituted sydnones.

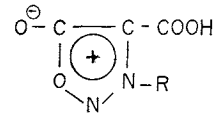
3-Substituted sydnone-4-carboxylic acids (VI)

A solution of *n*-butyl lithium prepared from 0.15 atom of lithium metal and 0.087 mole of butyl bromide in dry ether was added dropwise at −50°C under nitrogen atmosphere to a stirred suspension of 0.05 mole of 4-bromo-3-substituted sydnone (V) in dry ether. The reaction mixture was stirred for 1~2 hours, the temperature being allowed to rise to −10°C. The mixture was poured onto about 60 g of powdered Dry Ice. After the excess Dry Ice had evaporated, the mixture was decomposed with water. The aqueous layer was washed with benzene and acidified with 10 % hydrochloric acid. The resulting precipitate was collected by filtration, washed with water and recrystallized with ethanol or aqueous ethanol. Table 5 shows the yield and physical properties of 3-substituted sydnone-4-carboxylic acids.

3-Substituted sydnone-4-carbonyl chlorides (VII)

A mixture of 0.018 mole of 3-substituted sydnone-4-carboxylic acid (VI) and 5 ml of thionyl chloride was refluxed for one hour. The excess thionyl chloride was distilled off under reduced pressure to give the solid product, which was recrystallized from benzene-petroleum ether. Table 6 shows the yield and physical properties of the acid chlorides. As the 3-*p*-methoxyphenyl and 3-*sec*-butyl derivatives were not obtained as solids, their structures were confirmed by infrared spectra and they were used for the next reactions without further purification.

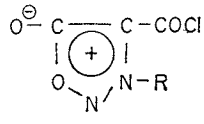
Table 5. 3-Substituted sydnone-4-carboxylic acids



R	Melting point (°C)	Yield (%)	λ_{max}^{EtOH} (mμ)	ε	$\nu_{C-O^{\ominus}}$ (cm^{-1})	ν_{COOH} (cm^{-1})	C (%)		H (%)		N (%)	
							Calc.	Found	Calc.	Found	Calc.	Found
C_6H_5	189~192 (193)[a]	61	322 313	12,400 7,750	1,810	1,680	52.43	52.90	2.93	3.03	13.59	13.66
o-Cl-C_6H_4	180~181	65	314	7,200	1,830	1,680	44.92	45.33	2.09	1.99	11.63	11.49
p-Cl-C_6H_4	184~185	50	219 314	19,000 7,950	1,810	1,680	44.92	44.61	2.09	2.19	11.63	11.78
o-OCH_3-C_6H_4	185~186	39	311	8,700	1,770	1,740	50.85	51.44	3.41	3.69	11.86	11.37
p-OCH_3-C_6H_4	176~177	47	223.5 312	19,900 10,400	1,810	1,690	50.85	51.15	3.41	3.49	11.86	11.53
o-CH_3-C_6H_4	178~179	78	310.5	8,400	1,810	1,675	54.55	54.52	3.66	4.34	12.72	12.70
p-CH_3-C_6H_4	196~197	40	312	8,300	1,810	1,680	54.55	54.98	3.66	3.70	12.72	12.48
$(CH_3)_2CH$	139~141 (143~144)[b]	60	222 304	11,700 9,500	1,760	1,680	39.78[c]	39.54	5.01[c]	5.48	15.47[c]	15.25
C_2H_5 CH_3>CH	88~89.5 (88~89)[b]	75	211.5 304	9,600 8,100	1,755	1,675	43.07[d]	43.02	5.68[d]	6.48	14.35[d]	14.47

a : see, ref. 9). b : see, ref. 7c). c : Calc. for $C_6H_8N_2O_4 \cdot \frac{1}{2}H_2O$ d : Calc. for $C_7H_{10}N_2O_4 \cdot \frac{1}{2}H_2O$

Table 6. 3-Substituted sydnone-4-carbonyl chlorides



R	Melting point (°C)	Yield (%)	$\nu_{C-O^{\ominus}}$[b] (cm^{-1})	$\nu_{C=O}$[b] (cm^{-1})	C (%)		H (%)		N (%)	
					Calc.	Found	Calc.	Found	Calc.	Found
C_6H_5	135 (133~134)[a]	80	1,820 1,800	1,750 1,720	48.12	48.65	2.24	8.28	12.47	12.46
o-Cl-C_6H_4	118~120	92	1,820 1,785(Sh)	1,745(Sh) 1,710	41.73	42.09	1.56	1.81	10.81	10.90
p-Cl-C_6H_4	96~97	95	1,815 1,790(Sh)	1,745 1,715	41.73	42.12	1.56	1.87	10.81	11.07
o-OCH_3-C_6H_4	80	89	1,800 1,785	1,750 1,720						
p-OCH_3-C_6H_4	oil	94	1,810 1,785(Sh)	1,750 1,730						
o-CH_3-C_6H_4	123~124	94	1,810 1,785(Sh)	1,745(Sh) 1,720	50.33	49.68	2.96	2.62	11.74	11.49
p-CH_3-C_6H_4	89~90	97	1,820 1,790(Sh)	1,745(Sh) 1,710						
$(CH_3)_2CH$	60.5~61.5	74	1,820 1,790	1,755 1,710						
C_2H_5 CH_3>CH	oil	83	1,820 1,800	1,760 1,720						

a : see, ref. 16). b : split into two bands.

2-(3-Sydnone)-benzoyl chloride

A mixture of 4.0 g (0.0195 mole) of 2-(3-sydnone)-benzoic acid[10] and 7 g (0.033 mole) of phosphorus pentachloride in 40 ml of dry methylene chloride was refluxed for an hour to give a clear solution, to which 200 ml of petroleum ether was added. Scratching induced crystallization of the chloride and the mixture was stored for 2 hours in a refrigerator. The crystals which separated were collected by filtration, washed with methylene chloride-petroleum ether (1 : 5) and dried *in vacuo*. Yield 2.4 g (55 %). M. p. 79~80°C. ν_{max}^{Nujor} 3,180, 1,785, 1,765, 1,740, 885 cm^{-1}.

Anal. Calcd. for $C_8H_5ClN_2O_3$: C 48.12, H 2.24, N 12.47.
Found : C 47.66, 48.06, H 2.28, 2.42, N 12.87, 12.95, 12.92.

Sodium 6-(3-substituted sydnone-4-carboxamido)penicillanates (III)

The sodium 6-(3-substituted sydnone-4-carboxamido)penicillanates described in Table 1 were prepared by two general methods. Method A was carrid out by treating the 3-substituted sydnone-4-carbonyl chloride (VII) with 6-APA in dry methylene chloride containing excess triethylamine. Method B consisted of neutralizing 6-APA with aqueous sodium hydroxide and treating it in aqueous acetone containing sodium bicarbonate with the sydnone acid chloride (VII). The following are representative example of each of the two method.

Method A: Sodium 6-(3-phenylsydnone-4-carboxamido) penicillanate (X)——A mixture of 3.55 g (0.0158 mole) of 3-phenylsydnone-4-cabonyl chloride in 5 ml dry methylene chloride was added dropwise to a stirred solution of 3.42 g (0.0158 mole) of 6-APA and 3.2 g (0.032 mole) of triethylamine in 50 ml of dry methylene chloride at 0~5°C. The reaction mixture was stirred for one hour at 15°C, then extracted with one 50-ml and two 20-ml portions of water. The combined aqueous solutions were washed with 50 ml of ether, layered with 50 ml of ethyl acetate and, with stirring, adjusted to pH 2 with 10 % hydrochloric acid. The ethyl acetate layer was separated and the aqueous layer extracted with two 50-ml portions of ethyl acetate. The combined ethyl acetate solutions were washed with two 30-ml portions of cold water, dried with anhydrous sodium sulfate and filtered. The filtrate was treated with 3 ml of a 39 % sodium 2-ethylhexanote (SEH) solution in methyl isobutyl ketone (MIBK) and concentrated to about 30 ml under reduced pressure. The concentrate was poured into 300 ml of dry ether to give a precipitate which was collected by filtration and dried *in vacuo*.

Method B: Sodium 6-[3-(*o*-chlorophenyl)sydnone-4-carboxamido]penicillanate—— To a stirred solution of 4.5 g (0.021 mole) of 6-APA, 0.8 g (0.02 mole) of sodium hydroxide, 1.74 g (0.021 mole) of sodium bicarbonate in 60 ml of water and 60 ml of acetone was added dropwise a solution of 4.9 g (0.019 mole) of 3-(*o*-chlorophenyl)sydnone-4-carbonyl chloride in 10 ml of dry acetone at 0~5°C. The reaction mixture was stirred for one hour at 10°C, then washed with 100 ml of ether. The water layer was covered with 100 ml of ethyl acetate and adjusted to pH 2.0 with dilute hydrochloric acid with stirring. The ethyl acetate layer was separated and the aqueous layer was extracted twice with 100-ml portions of ethyl acetate. The combined ethyl acetate solutions were washed with 50 ml of cold water and dried with anhydrous sulfate. The filtrate was treated with 7 ml of 39 % SEH solution in MIBK and concentrated to about 50 ml under reduced pressure. The concentrate was poured into 100 ml of petroleum ether to afford a precipitate, which was filtered, dried *in vacuo* and recrystallized from methanol-ether.

Sodium 6-[2-(3-sydnone)benzamido]penicillanate (VIII)

A solution of 3.0 g (0.0133 mole) of 2-(3-sydnone)benzoyl chloride in 5 ml of dry methylene chloride was added dropwise to a stirred solution of 3.0 g (0.0138 mole) of 6-APA and 3.0 g (0.03 mole) of triethylamine in 50 ml of dry methylene chloride at 0~5°C. The reaction mixture was stirred at 10~15°C for an hour and then extracted with three 50-ml portions of water. The combined water solutions were washed with 50 ml of ether, layered with 100 ml of ethyl acetate and adjusted to pH 2.0 with 10 % hydrochloric acid

at 5°C. After separation of the phases the aqueous phase was extracted with 100-ml and 50-ml portions of ethyl acetate. The combined ethyl acetate extracts were washed with 50 ml of cold water, dried with anhydrous sodium sulfate, filtered and treated with 4 ml of 39 % SEH solution in MIBK to give an oily precipitate, which was triturated with dry acetone and dry ether to afford an amorphous powder. Yield 2.4 g (42 %). M. p. 210~215°C (dec.). $\lambda_{max}^{H_2O}$ 301 mμ (ε 6,300). ν_{max}^{KBr} 1,760, 1,670, 1,610, 1,400 cm^{-1}.

Anal. Calcd. for $C_{17}H_{15}N_4O_6SNa \cdot \frac{1}{2}H_2O$: C 46.89, H 3.60.
Found : C 47.08, 47.25, H 4.08, 4.36.

Sodium 7-(3-phenylsydnone-4-carboxamido)cephalosporanate (IX)

To a stirred solution of 1.7 g (0.0062 mole) of 7-ACA in a mixture of 50 ml of water and 25 ml of acetone containing 1.6 g (0.019 mole) of sodium bicarbonate was added dropwise at about 10°C a solution of 1.4 g (0.0062 mole) of 3-phenylsydnone-4-carbonyl chloride in 25 ml of anhydrous acetone. After the addition was completed, the mixture was stirred for 0.5 hour at room temperature, then washed with two 30-ml portions of ether. The aqueous solution was covered with 50 ml of ethyl acetate, stirred vigorously in the cold and acidified with dilute hydrochloric acid. The aqueous layer was separated and extracted with two 30-ml of ethyl acetate. The combined ethyl acetate extracts were washed twice with cold water and dried with anhydrous sodium sulfate and calcium chloride. The solution was concentrated under reduced pressure to about 50 ml, and 39 % SEH solution (2.3 ml) was added to afford a precipitate, which was collected by filtration, washed with ether and dried *in vacuo*. Yield 2.3 g (77 %). The product could be recrystallized from methanol-ether. On heating it colored slowly to brown at above 160°C, but did not melt up to 300°C. $\lambda_{max}^{H_2O}$ 229 mμ (ε 13,300), 263 mμ (ε 10,300), 310 mμ (ε 9,900). ν_{max}^{KBr} 1,760, 1,680, 1,610, 1,410 cm^{-1}.

Anal. Calcd. for $C_{19}H_{15}N_4O_8SNa$: C 47.30, H 3.18.
Found : C 47.45, H 3.48.

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