[Chem. Pharm. Bull. 13(9)1148~1150(1965)]

UDC 615.779.92-011

Semisynthetic Penicillins. I. α-Methylthio-2-chlorocinnamylpenicillin*1

Since the isolation of 6-aminopenicillanic acid by Batchelor and Doyle, *et al.*,[1] numerous penicillinase-resistant penicillins have been synthesized. One of these, 2,6-dimethoxyphenylpenicillin (methicillin),[2] has been proved to be useful in the treatment of benzylpenicillin-resistant Staphylococcal infections. 2-Biphenylylpenicillin (ancillin)[3] and (2-ethoxy-1-naphtyl)penicillin (nafcillin),[4] which have the related chemical structures to methicillin, have been reported to be effective against resistant Staphylococci. The isoxazolyl penicillins, such as 5-methyl-3-phenyl-4-isoxazolylpenicillin (oxacillin)[5] and 5-methyl-3-*o*-chlorophenyl-4-isoxazolylpenicillin (cloxacillin)[6] are also highly penicillinase-resistant and are now widely used clinically.[7]

This paper reports the preparation and properties of new penicillins, the *trans* and *cis**2 forms of (α-methylthio-2-chlorocinnamyl)penicillin.

(*trans*-α-Methylthio-2-chlorocinnamyl)penicillin (I) and (*cis*-α-methylthio-2-chlorocinnamyl)penicillin (II) were respectively prepared by condensing 6-aminopenicillanic acid (6APA) with the *trans*- and *cis*-α-methylthio-2-chlorocinnamoyl chlorides.

On treatment with thionyl chloride, *trans*-α-methylthio-2-chlorocinnamic acid[8a,b] (III) gave its chloride (IV), which was purified by vacuum distillation: b.p. 133°/2 mm. Hg. The structure of the compound (IV) was confirmed by converting to its acid amide (V), m.p. 112~113° (*Anal.* Calcd. for $C_{10}H_{10}ONSCl$: C, 52.74; H, 4.43; N, 6.15. Found: C, 52.86; H, 4.43; N, 6.05). The acid chloride (IV) was coupled with 6APA in aqueous acetone containing sodium bicarbonate. The penicillin was purified by extration in ether as its free acid and then back into water by adding the requisite quantity of aqueous sodium hydroxide. The lyophylization of the aqueous phase gave the sodium salt of (*trans*-α-methylthio-2-chlorocinnamyl)penicillin (I), which was sufficiently pure for preliminary biological and pharmacological tests. It showed $[\alpha]_D^{24}$ +167° (c=0.3, H_2O). (*Anal.* Calcd. for $C_{18}H_{18}O_4N_2S_2ClNa \cdot H_2O$: C, 46.30; H, 4.32; N, 6.00. Found: C, 46.05; H, 4.29; N, 5.55). N,N'-Dibenzylethylenediamine salt of I was obtained as needle crystals. m.p.130~150°. $[\alpha]_D^{23}$ +100° (c=0.4, acetone) (*Anal.* Calcd. for $C_{52}H_{58}O_8N_6S_4Cl_2 \cdot H_2O$: C, 56.15; H, 5.44; N, 7.56. Found: C, 56.06; H, 5.31; N, 7.15).

Treatment of *cis*-α-methylthio-2-chlorocinnamic acid (VI)[8b] with thionyl chloride afforded its chloride (VII), which was thermally unstable and converted to the *trans* form (IV) by the vacuum distillation at 100~150°. The undistilled chloride (VII) gave *cis* amide (VIII) by the usual procedure. m.p. 150~151°. (*Anal.* Found: C, 53.52; H, 4.43; N, 6.06).

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*1 Paper 3 in the series, Studies on Semisynthetic Penicillins. Paper 1 and 2: Agr. Biol. Chem., **29**, 728, 732 (1965).

*2 The prefixes *trans* and *cis* refer to relationship between the carboxylic acid function and β-phenyl group.

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7) M. Barber, P. M. Waterworth: British Med. J., 8, 344 (1964), described a comparative study of the antibacterial activity of five penicillinase-resistant penicillins.
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Preparation of (*cis*-α-methylthio-2-chlorocinnamyl)penicillin (Ⅱ) from the undistilled chloride (Ⅶ) and 6APA was carried out by the similar procedure as the *trans* isomer. The sodium salt of penicillin Ⅱ showed $[\alpha]_D^{21}$ +147° (c=0.4, H_2O) and N,N′-dibenzylethylenediamine salt of Ⅱ showed m.p. 120~130°. (*Anal.* Calcd. for $C_{52}H_{58}O_8N_6S_4Cl_2$: C, 57.08; H, 5.34; N, 7.68. Found: C, 57.32; H, 5.02; N, 7.41).

The rate of inactivation of penicillin I, Ⅱ, benzylpenicillin, (α-phenoxyethyl)penicillin and cloxacillin by *Bacillus cereus* penicillinase was determined. The penicillins and penicillinase were dissolved in 1/15*M* phosphate buffer at pH 7, kept at 37° and the remaining activity of samples was determined by the cylinder method (Table I). It was observed that the penicillin I and Ⅱ were considerably more resistant to *B. cereus* penicillinase than was benzylpenicillin. The penicillin Ⅱ appeared to be slightly more stable than the penicillin I.*3

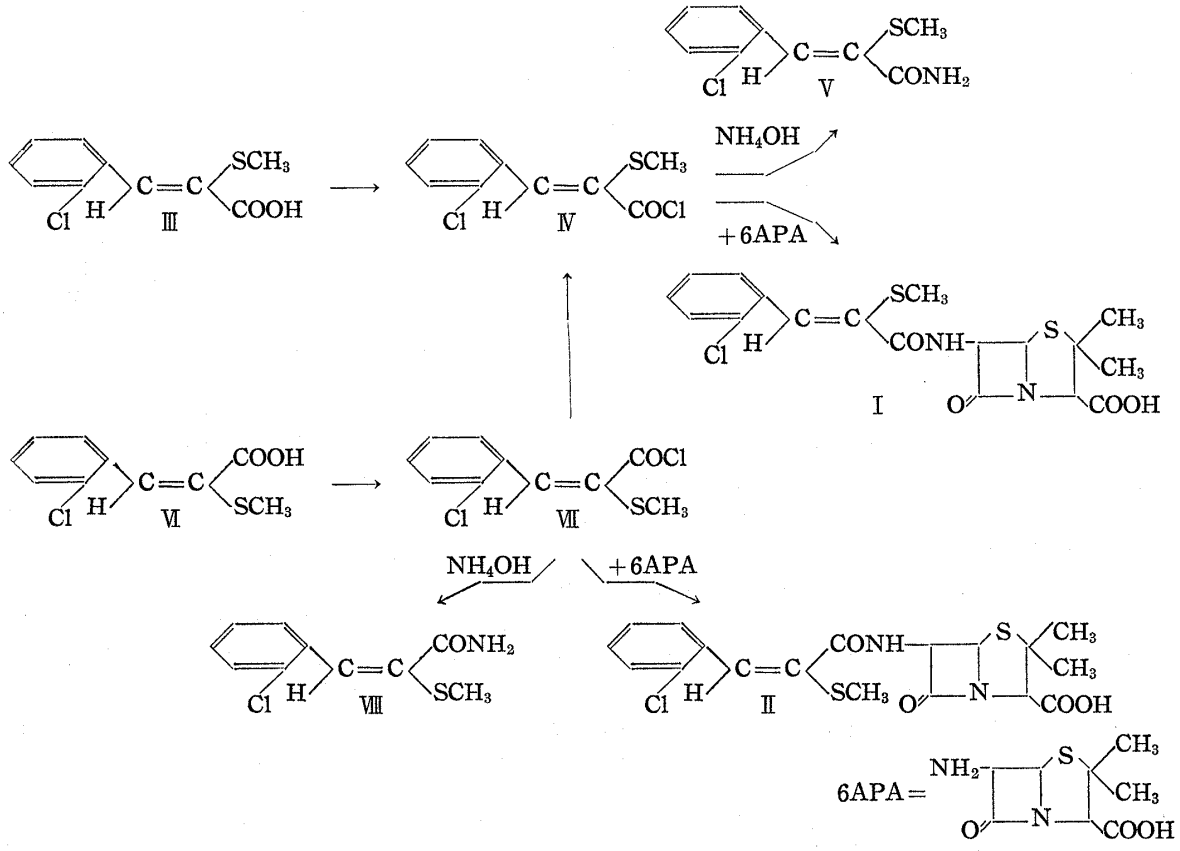

Table I. Stability of Penicillins to *B. cereus* Penicillinase

Time (min.)	Residual penicillins (%)				
	Penicillin I Na salt	Penicillin Ⅱ Na salt	Benzyl penicillin K salt	Phenoxy ethyl penicillin K salt	Cloxacillin Na salt
0	100	100	100	100	100
30	90	92	35	42	90
60	40	60	0	0	60
90	32	55	0	0	55

*3 Details of the preparation of many analogous α-alkylthio-cinnamylpenicillins and the relationship between the chemical structures and the stability towards penicillinase will be reported later.

Table II. Comparison of Minimum Inhibitory Concentrations of Penicillins

Organism	Minimum inhibitory concentration (μg./ml)			
	Penicillin I Na salt	Penicillin II Na salt	Benzylpenicillin K salt	Cloxacillin Na salt
Staphylococcus aureus 209 P	0.078	0.09	0.039	0.156
Staphylococcus aureus resistant[a]	0.625	3.12	12.5	0.312
Streptococcus haemolyticus Cook	0.004	0.005	0.004	0.078
Diplococcus pneumoniae Type 1	0.009	0.005	0.019	0.156
Escherichia coli Communis	>100	>100	25	>100

a) The laboratory-trained benzylpenicillin-resistant strain

Table III. Comparison of Minimum Inhibitory Concentrations of Penicillins using *Staph. aureus* Benzylpenicillin Resistant Strains of Clinical Origin

Staph. aureus Strain No.[a]	Minimum inhibitory concentration (μg./ml.)			
	Penicillin I Na salt	Benzyl penicillin K salt	Methicillin Na salt	Cloxacillin Na salt
S-1	1.56	625 (1000 u)	2.5	0.31
S-2	1.56	625	>100	10
S-3	1.56	625	2.5	0.31
S-4	1.56	625	2.5	0.31

a) The strains have been isolated from patients in Showa Medical College. The strain numbers are temporary ones.

The *in vitro* antimicrobial activity of sodium salt of penicillin I and II are presented in Table II. The minimum inhibitory concentrations were determined against the clinically isolated resistant staphylococcal strains (Table III). As shown in Table II and III, the penicillin I was effective *in vitro* against benzylpenicillin-sensitive microorganisms and also active against benzylpenicillin-resistant Staphylococci.

It was found that the penicillin I was more highly bound to serum proteins than methicillin and benzylpenicillin. However, the penicillin I was more acid-stable than methicillin and benzylpenicillin and was absorbed by the oral route.

Bacteriological and pharmacological investigation of this new penicillin I, which has been given the generic name "thiocillin," will be reported elsewhere.

The authors are very grateful to Dr. H. Ogawa, the chief director of this laboratory, for his kind guidance and encouragement throughout the work. The authors wish to express their thanks to Dr. B. Nomiya and Mr. K. Kagino of this laboratory and the members of Pharmaceutical Division of Kawasaki Factory of this Company for their microbiological works.

Research Laboratories of
Meiji Seika Kaisha Ltd.,
Morooka-cho, Kohoku-ku, Yokohama

Teiichiro Ito (伊藤定一郎)
Tadao Ishii (石井忠男)
Motohiro Nishio (西尾元宏)

Received June 16, 1965