SYNTHESES AND ANTIBACTERIAL ACTIVITIES OF PENICILLINS FROM (+)- AND (−)-α-AMINO-4-ISOTHIAZOLYLACETIC ACIDS

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α-Amino-4-isothiazolylacetic acid has been prepared by reaction of α-bromo-4-isothiazalylacetic acid with ammonium hydroxide and by catalytic hydrogenation of α-azido-4-isothiazolylacetic acid. The α-azido acid has been resolved into its two optical isomers from which the optically active amino acids were prepared. The absolute configurations of these amino acids are tentatively assigned. The dextrorotatory isomer can also be prepared directly by resolution of the racemic amino acid with *d*-camphor-10-sulfonic acid. From the optically active amino acids the penicillins were synthesized by the activated ester method using the *p*-nitrocarbobenzoxy protecting group. Some MIC and CD_{50} values against Gram-positive and Gram-negative bacteria are given. The introduction of an α-amino group into 4-isothiazolylmethylpenicillin produces only a minimal effect in the Gram-negative activity.

In a previous publication[1] we have reported on the syntheses of isothiazolylacetic acids and their penicillin derivatives. The introduction of an α-amino group in arylmethylpenicillins has produced penicillins such as α-aminobenzylpenicillin (ampicillin)[2] and α-amino-3-thienylpenicillin[3] which not only show a substantial improvement in the activity against Gram-negative bacteria but which are also highly effective when taken orally. As the Gram-negative activities of 4- and 5-isothiazolylmethylpenicillins are similar to those of ampicillin, which is higher than any other penicillin with a simple arylacetic acid side-chain, it was of definite interest to synthesize the α-aminoisothiazolylmethylpenicillins. In the following we report our work on the two epimers of α-amino-4-isothiazolylmethylpenicillin, including the synthesis of the optically active α-amino-4-isothiazolylacetic acids.

α-Bromo-4-isothiazolylacetic acid (**2**) was obtained in good yield by bromination of methyl 4-isothiazolylacetate** with N-bromosuccinimide, followed by acid hydrolysis of the bromo ester (Scheme 1). The amino acid **3** was prepared, in 60 % yield, by treatment of the bromo acid with concentrated ammonium hydroxide. α-Azido-4-isothiazolylacetic acid (**4**) was obtained quantitatively from **2** by reaction with sodium

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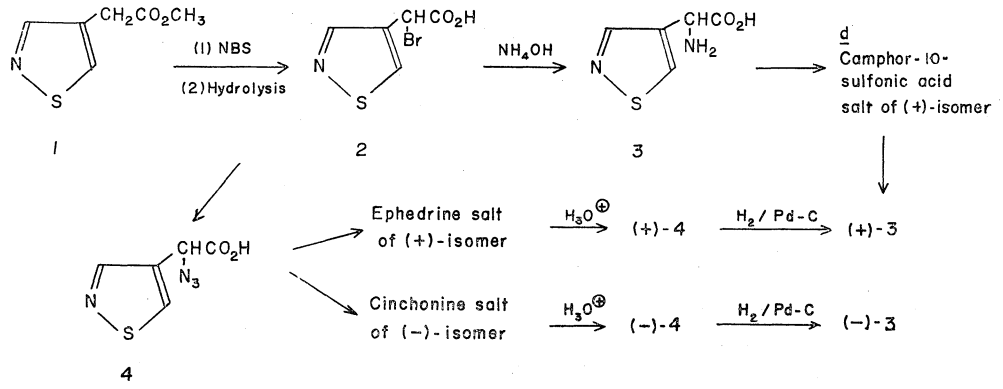
** Previously[1] we described the preparation of methyl 4-isothiazolylacetate from isothiazole-4-carboxylic acid by the ARNDT-EISTERT synthesis. This ester can more conveniently be prepared from 4-methylisothiazole by bromination with N-bromosuccinimide followed by cyanation and methanolysis.

Scheme 1



azide in aqueous acetone. A good resolution procedure for this acid was found and consisted of adding an aqueous solution cinchonine hydrochloride to an aqueous solution of sodium α-azido-4-isothiazolylacetate. The precipitated cinchonine salt was optically pure after one or two recrystallizations from methanol. The levorotatory azido acid was liberated from this salt by acidification. The partially resolved dextrorotatory acid isolated from the aqueous solution was obtained optically pure after two recrystallizations of its ephedrine salt from ethyl acetate. The α-azido-4-isothiazolacetic acids could be hydrogenated in good yields to the optically active α-amino-4-isothiazolylacetic acids. The direction of rotation was maintained in these reductions. The dextrorotatory amino acid was also obtained directly from racemic **3** by fractional recrystallization of the *d*-camphor-10-sulfonic acid salt from isobutyl alcohol.

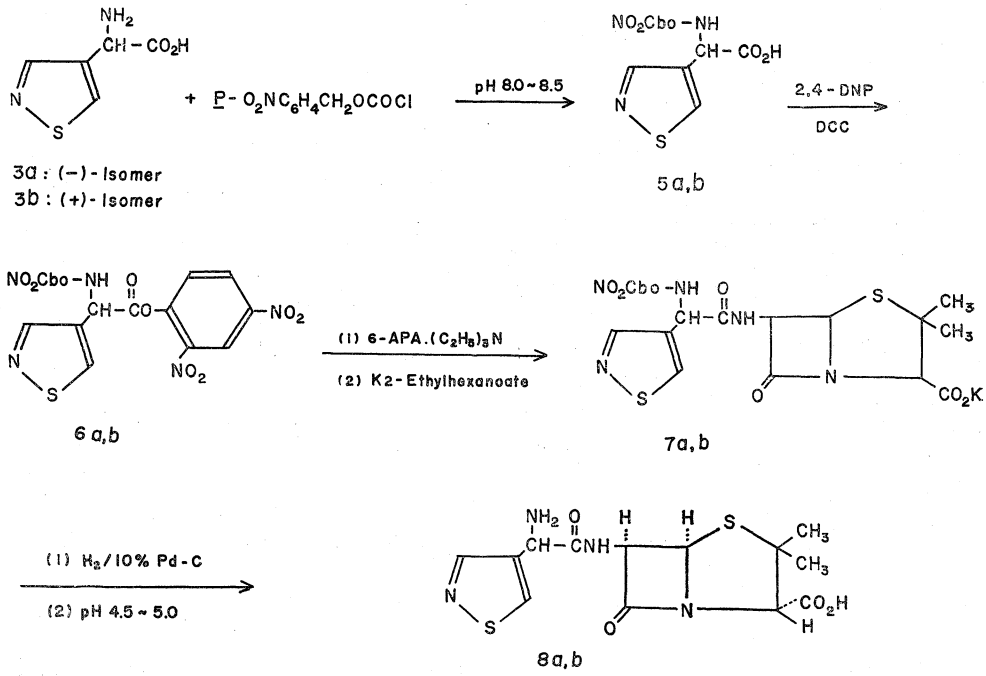
The absolute configurations of the optically active forms of **3** were not established. It is however believed that the levorotatory isomer, with a specific rotation $[\alpha]_D$ in water of −76°, has the D-configuration since the D-configurations of the structurally closely related α-aminophenylacetic acid and α-amino-3-thienylacetic acid are both also levorotatory with specific rotations $[\alpha]_D$ in water of −114° and −100° respectively[4,5]. The activities of the penicillins provide additional support for this assignment.

Optically active **4** racemizes very rapidly in dilute sodium hydroxide. In 0.12 N aqueous sodium hydroxide at 25°C the first order rate constant was found to be 0.0146 min^{-1} (half-life time: 32 minutes). This is considerably faster than the rate of racemization of the structurally related α-azido-3-thienylacetic acid and α-azidophenylacetic acid, for which first order rate constants of 0.0088 and 0.0038 min^{-1} respectively in 1.0 N aqueous sodium hydroxide have been reported[5]. An interesting reaction was discovered when **4** was dissolved in 3 N aqueous sodium hydroxide. A rapid gas evolution took place and sodium 4-isothiazolylglyoxylate precipitated. 4-Isothiazolylglyoxylic acid could thus readily be prepared from the azido acid in 77 % yield*.

Scheme 2 outlines the procedure by which the two epimers of 6-(α-amino-4-isothiazolylacetamido)penicillanic acid (**8**) were synthesized from the optically active isomers of α-amino-4-isothiazolylacetic acid. The application of the *p*-nitrocarbobenzoxy protecting group in peptide syntheses has first been reported by CARPENTER

* The preparation of arylglyoxylic acids by this procedure has been reported separately[6].

Scheme 2



and GISH[7] and the method has since been used in the synthesis of cephaloglycin[8]. It proved to be a very useful protecting group in the present work. The use to the carbobenzoxy protecting group was less satisfactory as its removal was difficult, giving rise also to decomposition of the penicillin. The 2, 4-dinitrophenyl esters were used as the activating esters instead of the *p*-nitrophenyl esters as the latter ones reacted rather slowly with triethylammonium 6-aminopenicillanate. In the reaction between triethylammonium 6-aminopenicillanate and a 2, 4-dinitrophenyl ester generally an equivalent amount of triethylamine is added to neutralize the dinitrophenol. However in the preparation of the protected penicillins **7a** and **7b** the dinitrophenol had to be neutralized after the reaction was finished because the addition of even a small amount of free triethylamine to the optically active esters **6a** and **6b** resulted in very rapid racemization.

Epimers **8a** and **8b** can readily be distinguished by nuclear magnetic resonance (n. m. r.) spectroscopy as is shown in Table 1. The β-lactam hydrogens appear to be slightly more shielded in **8a**. A more characteristic difference is displayed by the *geminal* dimethyl group which appears as two singlets in **8a** and as one sharp singlet in **8b**.

Table 1. N. m. r. chemical shifts (τ) of 6-[α-amino-4-isothiazolylacetamido]penicillanic acids in D_2O (25°C)

Epimer	C^3H	$C^2(CH_3)_2$	β-Lactam H's*	Isothiazole H's	α-H
8a	5.82	8.55, 8.60	4.46, 4.58	0.82, 1.32	4.43
8b	5.78	8.50	4.37, 4.50	0.83, 1.33	4.43

* The β-lactam protons appeared in both epimers as two doublets of an AB quartet, with spacings of 3.8 and 4.5 cps for **8a** and **8b** respectively.

Antibacterial Activity

The compounds were

Table 2. MIC and CD_{50} values of 6-(α-amino-4-isothiazolylacetamido)penicillanic acids

Microorganism		MIC μg/ml				CD_{50} mg/kg		
		8a	8b	Ampicillin	4-Isothiazolylmethylpenicillin	8a	8b	Ampicillin
Gram-positive	*D. pneumoniae*	0.031	0.125	0.016	0.016			
	S. pyogenes	0.016	0.031	0.008	0.008			
	S. aureus (Smith) −serum	0.125	0.25	0.062	0.062	0.8	>4.0	0.5
	〃 +serum	0.125	0.4	0.062	0.062			
Gram-negative	*E. coli*	6.2	12.5	6.2	12.5	60	>200	62
	S. enteritidis	0.4	1.6	0.125	0.125			
	S. typhosa	0.8	3.1	0.8	0.8			
	K. pneumoniae	0.8	3.1	0.8	0.8	80	—	52

tested for antibacterial activity by the Microbiology Department of Bristol Laboratories, Syracuse, N. Y., using published techniques[9]. Minimum inhibitory concentrations (MIC) were determined using a twofold serial dilution technique in heart infusion broth in the absence of serum and in the presence of 50 % pooled human serum.

In Table 2 the MIC values of **8a** and **8b** against certain microorganisms are compared with the values for ampicillin and 4-isothiazolylmethylpenicillin[1]. Also included in Table 2 are some CD_{50} values for **8a**, **8b** and ampicillin, when administered orally to mice infected with *Staphylococcus aureus* (Smith), *Escherichia coli* or *Klebsiella pneumoniae*. The data presented in Table 2 permit the following conclusions: (1) The antibacterial activity of **8a** and ampicillin is very similar both *in vitro* and *in vivo*. (2) The stereochemistry of the side chain acid has a definite effect on the *in vitro* and *in vivo* activity; *in vitro* **8a** is approximately four times more active than **8b** against Gram-negative microorganisms and approximately twice as active against Gram-positive organisms. (3) In contrast to the case of benzylpenicillin and 3-thienylpenicillin, the introduction of an α-amino group into 4-isothiazolylmethylpenicillin produces only a minimal effect in the Gram-negative activity; it is possible that the effect of the amino group is offset by the increase in polarity of the molecule. (4) As in the reported instances[2,3] the penicillin derived from a D-amino acid shows superior activity than the epimer derived from the L-amino acid, the difference in activity between **8a** and **8b** further supports the contention that **3a** and **3b** have the D and L configuration respectively.

Experimental

All temperatures are uncorrected. The infrared spectra were obtained on a Perkin-Elmer "Infracord" spectrophotometer model 137. Nuclear magnetic resonance (n. m. r.) spectra were measured with a Varian Associates model A-60 spectrometer, using tetramethylsilane as a reference. Optical rotations were measured in 1-dm tubes using a Perkin-Elmer 141 automatic polarimeter. Microanalyses were performed by Dr. F. B. STRAUSS, Microanalytical Laboratory, Oxford, England.

Methyl 4-Isothiazolylacetate (**1**)

To a stirred and refluxing solution of 4-methylisothiazole (200 g, 2.02 mole) and benzoyl peroxide (1.0 g) in 3 liters of CCl_4 was added a mixture of NBS (420 g, 2.36 mole) and benzoyl peroxide (2.0 g) in portions over a 1-hour period, while the reaction flask was

exposed to a 750 watt light source. The reaction mixture was heated under reflux for an additional 1 hour whereafter it was cooled and filtered. The filtrate was concentrated to a volume of approximately 700 ml and added over a 45-minute period to a refluxing mixture of NaCN (300 g, 6.1 mole), water (500 ml) and methanol (3 liters). The mixture was heated under reflux for an additional 1 hour and concentrated to a volume of 1 liter. The solution, after saturation with NaCl, was extracted with six 1-liter portions of ethyl acetate. The combined extracts were dried and the solvent removed. The crude 4-cyanomethylisothiazole was dissolved in 2 liters of methanol and heated under reflux while a rapid stream of HCl was passed through the solution for 1.5 hour. Next, 400 ml of concentrated hydrochloric acid was added and the heating under reflux continued for 1 hour. The mixture was concentrated to a volume of 1.5 liter followed by the addition of 2 liters of water, saturation with NaCl and extraction with seven 1-liter portions of CH_2Cl_2. The residue obtained after drying and removal of the CH_2Cl_2 was fractionally distilled to give 104 g (33 %) of **1**, b.p. 75~76°C (0.1 mm), identical with the material prepared from isothiazole-4-carboxylic acid by the ARNDT-EISTERT synthesis[1].

Methyl α-Bromo-4-isothiazolylacetate

A mixture of **1** (102.0 g, 0.65 mole), NBS (116.0 g, 0.65 mole), benzoyl peroxide (1.0 g) and CCl_4 (2 liters) was heated under reflux for 1.5 hour, while exposed to a 750 watt light source. The succinimide was filtered off and the solvent removed from the filtrate, leaving 136.0 g of slightly colored liquid residue which was used directly. In a small-scale experiment the residue was distilled (with some decomposition) to give the bromo ester as a pale yellow liquid, b.p. 104~106°C (0.5 mm), in 77 % yield. The n.m.r. spectrum (in CCl_4) contained singlets at τ 1.20, 1.47, 4.52 and 6.21 with an integrated area ratio of 1 : 1 : 1 : 3 respectively.

α-Bromo-4-isothiazolylacetic Acid (**2**)

A mixture of methyl α-bromo-4-isothiazolylacetate (136.0 g, 0.576 mole), acetic acid (275 ml) and 6 N hydrochloric acid (550 ml) was kept at 25°C for 16 hours. The solvent was removed and ether (400 ml) and water (100 ml) were added to the residue. The ether solution was extracted with 360 ml of cold 3 N aqueous sodium carbonate. The carbonate extract was cooled, acidified with 120 ml of concentrated hydrochloric acid and **2** extracted with ether. After drying and removal of the ether there remained a pale brown oily residue which slowly solidified. The crude product, m. p. 71~90°C, amounted to 90.9 g (70 %) and was used directly.

α-Amino-4-isothiazolylacetic Acid (**3**)

α-Bromo-4-isothiazolylacetic acid (90.9 g, 0.41 mole) was added to 1,300 ml of concentrated NH_4OH. The solution was kept at 25°C for 2 days. The mixture was concentrated to dryness and methanol (250 ml) was added to the residue. The amino acid was filtered off and recrystallized from water (250 ml) to give 39.0 g (60 %) of white crystalline material, m.p. 199~200°C (decomp.), n.m.r. spectrum (CF_3CO_2H): 1-proton singlets at τ 0.60, 0.96 and 4.13. The elemental analyses were performed on the N-acetyl derivative, m.p. 177~180°C.

Anal. Calcd. for $C_7H_8N_2O_3S$ (mol. wt., 200): C 41.99, H 4.03, N 14.00, S 16.01.
Found: C 41.83, H 4.22, N 13.80, S 16.02, neutralization equivalent, 196.

α-Azido-4-isothiazolylacetic Acid (**4**)

A mixture of **2** (56.0 g, 0.25 mole), NaN_3 (16.3 g, 0.25 mole), Na_2CO_3 (14.7 g, 0.14 mole) and 300 ml of 90 % aqueous acetone was stirred at 25°C for 15 hours. The solvent was removed and the residue taken up in 100 ml of water. The aqueous solution was washed with ether, acidified and extracted with ether. The ether extract was dried and concentrated to give 45.5 g (99 %) of **4** as a yellow solid, m.p. 68~74°C; neutralization equivalent, 180 (calcd., 184). This material was used directly for optical resolution.

Optical Resolution of α-Azido-4-isothiazolylacetic Acid

A solution of cinchonine (50.0 g, 0.17 mole) in 340 ml of warm 0.5 N hydrochloric acid was carefully added, with scratching, to a solution of **4** (46.0 g, 0.25 mole) in 500 ml of 0.5 N aqueous NaOH. The mixture was left at 25°C for 30 minutes, then the salt was filtered off and washed with 300 ml of water. The azido acid was liberated from the salt and again precipitated from an aqueous solution by the addition of an equivalent amount of cinchonine hydrochloride solution in the same manner. This process was repeated until the optical activity of the acid remained constant, with the following results:

Crystallization	1	2	3	4
g of **4** dissolved	46.0	20.4	14.8	0.46
g of **4** recovered	20.4	15.9	12.5	0.19
$[\alpha]_D^{25}$ of recovered **4**	−44.9°	−54.5°	−56.3°	−55.9°
m. p. (°C) of **4**	oil	56~58	67~71	67~70

In another experiment, using the same quantities as above, the cinchonine salt that precipitated in the first crystallization was air-dried and recrystallized from methanol. For optical rotation measurements, the acid was liberated from 0.5 g of the salt. The results of this resolution were as follows:

Crystallization	1	2	3
g of salt dissolved	—	52.6	36.8
g of salt recovered	53.2	37.3	29.2
ml of methanol	—	700	500
$[\alpha]_D^{25}$ of recovered **4**	−45.9°	−54.2°	−55.7°
m. p. (°C) of **4**	60~75	67~72	66~69

To obtain the dextrorotatory acid, the combined filtrate and washing of the first crystallization were acidified and continuously extracted with ether. The azido acid (21.7 g, 0.118 mole), $[\alpha]_D^{25}$ +42.7°, was dissolved in 100 ml of ethyl acetate and treated with a solution of L(−)-ephedrine (19.4 g, 0.118 mole) in 100 ml of ethyl acetate. After cooling, the ephedrine salt was collected and recrystallized from ethyl acetate. After each recrystallization the azido acid was liberated from 0.4 g of the salt. The progress of this resolution was as follows:

Crystallization	1	2	3
g of salt dissolved	—	29.6	1.0
g of salt recovered	30.0	24.2	0.8
ml of ethyl acetate	200	300	25
$[\alpha]_D^{25}$ of recovered **4**	+50.9°	+52.1°	+52.2°
Decomp. point (°C) of salt	117~118	122~123	121~122

(−)-α-Azido-4-isothiazolylacetic Acid

The cinchonine salt of (−)-**4** (28.7 g, 0.060 mole), m.p. 176~177°C (decomp.), was suspended in 125 ml of water and treated with 25 ml of 6 N hydrochloric acid. The mixture was shaken until all the solid had dissolved, followed by extraction with four 150 ml portions of ether. The combined ether extracts were dried and the solvent removed giving 10.5 g (95 %) of white solid, m.p. 66~69°C; ν_{max}^{Nujol} 2120 and 1705 cm^{-1}. The n.m.r. spectrum (in $CDCl_3$) consisted of four singlets with the same intensity at τ −1.86 ($CO_2\underline{H}$), 1.28 and 1.42 (isothiazole protons) and 4.74 (α-hydrogen). An analytical sample was obtained by recrystallization from an ethyl acetate-*n*-hexane (1 : 3) mixture: m.p. 68~71°C; $[\alpha]_D^{25}$ −56.2° (*c* 2.5, abs.EtOH).

Anal. Calcd. for $C_5H_4N_4O_2S$: C 32.60, H 2.19, N 30.42.
Found: C 32.70, H 1.85, N 30.10.

(+)-α-Azido-4-isothiazolylacetic Acid

The (+)-azido acid was liberated in 95 % yield from its L(−)-ephedrine salt in the same manner as the (−)-isomer; m.p. 68~71°C; $[\alpha]_D^{25}$ +56.2° (*c* 2.3, abs.EtOH).

Anal. Calcd. for $C_5H_4N_4O_2S$: C 32.60, H 2.19, N 30.42.
Found: C 32.75, H 1.99, N 30.21.

(−)-α-Amino-4-isothiazolylacetic Acid (**3a**)

A mixture of (−)-**4** (9.2 g, 0.050 mole), 10 % Pd on charcoal (3.5 g), methanol (150 ml) and 0.5 N hydrochloric acid (100 ml) was hydrogenated at 50 p. s. i. for 12 hours, followed by filtration. The combined filtrates of two batches were concentrated to a volume of approximately 50 ml and extracted with ether. From the ether extract 2.4 g of starting material was recovered. The aqueous solution was cooled in ice and brought to pH 5.0 with concentrated NH_4OH. The white solid precipitate was dried *in vacuo* over P_2O_5; yield: 11.5 g (84 %, based on unrecovered starting material), m. p. 180~181°C (decomp.); $[\alpha]_D^{25}$ −76.3° (*c* 1.5, water). The elemental analyses were performed on the N-*p*-nitrocarbobenzoxy derivative.

(+)-α-Amino-4-isothiazolylacetic Acid (**3b**)

(1) By reduction of (+)-α-azido-4-isothiazolylacetic acid:

(+)-Azido-4-isothiazolylacetic acid was hydrogenated in the same manner as the (−)-isomer to give **3b** in 70 % yield: m.p. 181~182°C (decomp.), $[\alpha]_D^{25}$ +76.4° (*c* 1.3, water). The elemental analyses were performed on the N-*p*-nitrocarbobenzoxy derivative.

(2) By resolution of D, L-α-amino-4-isothiazolylacetic acid:

A mixture of **3** (20.0 g, 0.126 mole) and *d*-camphor-10-sulfonic acid (29.4 g, 0.126 mole) was dissolved in the minimum amount of boiling isobutyl alcohol. The solution was cooled for 3 days at 0°C. The fluffy precipitate was filtered off, washed with cold isobutyl alcohol and recrystallized from isobutyl alcohol until no further increase in optical rotation was observed, with the following results:

Crystallization	1	2	3	4
g of salt dissolved	—	21.6	9.6	5.3
g of salt recovered	21.6	9.6	5.3	3.6
ml of isobutyl alcohol	175	265	160	90
$[\alpha]_D^{25}$ of salt in water	+34.8°	+45.4°	+47.0°	+46.0°

A solution of optically pure *d*-camphor-10-sulfonic acid salt (4.9 g, 0.00129 mole, m.p. 166~167°C (decomp.)) in 15 ml of water was brought to pH 5.0 with 3 N aqueous NaOH and cooled. The white crystals (1.45 g, 73 %) were dried *in vacuo* over P_2O_5: m.p. 181~182°C (decomp.); $[\alpha]_D^{25}$ +76.3° (*c* 1.2, water). The infrared spectrum was identical with that of the material obtained by reduction of the (+)-azido acid.

(−)- and (+)-α-(N-*p*-Nitrocarbobenzoxyamino)-4-isothiazolylacetic Acid (**5**)

To a cooled suspension of **3a** (21.0 g, 0.133 mole) in 300 ml of water was added 133 ml of 1 N aqueous NaOH, followed by 300 ml of THF. The mixture was cooled in ice and a solution of *p*-nitrocarbobenzoxy chloride (35.7 g, 0.166 mole) in 300 ml of THF was added dropwise with stirring in 30 minutes. The pH of the reaction mixture was maintained at 8.0~8.5 by the simultaneous addition of 1 N aqueous NaOH. After washing with two 150 ml portions of ether, the aqueous solution was layered with 300 ml of ethyl acetate and acidified with 3 N hydrochloric acid. The ethyl acetate layer was dried and the solvent removed to give a quantitative amount (46.0 g) of **5a**, m. p. 155~158°C, which could be recrystallized from ethyl acetate - *n*-hexane (1 : 1): m.p. 159~160°C, $[\alpha]_D^{25}$ −58.5° (*c* 2.8, abs.EtOH).

Anal. Calcd. for $C_{13}H_{11}N_3O_6S$ (mol. wt., 337): C 46.29, H 3.29, N 12.46.
Found: C 46.47, H 3.50, N 12.19,
neutralization equivalent, 321.

Compound **5b** was prepared in the same way as the (−)-isomer: m.p. 158~159°C; $[\alpha]_D^{25}$ +58.0° (*c* 2.7, abs.EtOH).

Anal. Calcd. for $C_{13}H_{11}N_3O_6S$ (mol. wt., 337): C 46.29, H 3.29, N 12.46.
Found: C 46.20, H 3.40, N 12.51,
neutralization equivalent, 331.

Potassium 6-[(−)- and (+)-α-(N-*p*-Nitrocarbobenzoxyamino)-4-isothiazolylacetamido]-penicillanate (**7**)

N,N'-Dicyclohexylcarbodiimide (13.2 g, 0.064 mole) was added under stirring to a solution of **5a** (21.6 g, 0.064 mole) and 2,4-dinitrophenol (11.8 g, 0.064 mole) in 200 ml of THF, cooled in ice. After 1 hour at 0°C, the N,N'-dicyclohexylurea was filtered off and the solvent was removed from the filtrate to give the crude activated ester **6a**. A sample was recrystallized from methylene chloride: m. p. 92~93°C (decomp.); $[\alpha]_D^{25}$ −18.5° (*c* 3.3, dioxane). Triethylammonium 6-aminopenicillanate was prepared as a white solid by concentrating a solution of triethylamine (12.4 g, 0.123 mole) and 6-APA (13.3 g, 0.0615 mole) in 125 ml of CH_2Cl_2 to dryness. The crude activated ester was dissolved in 150 ml of CH_2Cl_2 and the mixture was cooled in ice (most of the ester crystallized). A solution of the triethylammonium 6-aminopenicillanate in 125 ml of CH_2Cl_2 was added dropwise in 20 minutes to the stirred suspension. The reaction mixture was left at 25°C for 2 hours, after which an additional amount of triethylamine (6.4 g, 0.064 mole) was added with stirring and cooling in ice. The triethylammonium salt of the penicillin was precipitated as an oil by the addition of ether. It was twice redissolved in CH_2Cl_2 (80 ml) and reprecipitated with ether (400 ml). The material, which crystallized in the final precipitation, was dissolved in 70 ml of methanol and this solution was treated with 26 ml of a 2.4 M solution of potassium 2-ethylhexanoate in *n*-butyl alcohol. The potassium penicillanate **7a** was precipitated by the addition of ether (500 ml) to give 30.0 g (85 %) of yellow solid of 90 % purity (as estimated from the t. l. c. and the n. m. r. spectrum); ν_{max}^{Nujol} 3300, 1770, 1720, 1680, 1600 and 1520 cm^{-1}.

The protected penicillin **7b** was prepared in the same manner (69 % yield) *via* the intermediate ester **6b**, m.p. 91~92°C, $[\alpha]_D^{25}$ +18.4° (*c* 3.9, dioxane).

6-[(−)- and (+)-α-Amino-4-isothiazolylacetamido]penicillanic Acid (8)

A mixture of **7a** (15.0 g, 0.0262 mole), 10 % Pd on charcoal (2.5 g) and water (150 ml) was hydrogenated at 20 p.s.i. for one hour. The mixture was filtered through Celite and, under ice cooling, the filtrate brought to pH 2.5 with 3 N hydrochloric acid. The precipitate was removed by another filtration through Celite and the pale yellow clear filtrate was adjusted to pH 4.5 with 3 N NaOH. The solvent was removed under reduced pressure at approximately 25°C, giving 9.5 g of solid residue. The combined material of four batches (37.3 g) was extracted with three 200 ml portions of methanol (5.0 g of insoluble material) and the combined methanol extracts were treated with 1 of ether. The precipitated solid (21.7 g) was washed with one 40 ml portion and two 15 ml portions of cold methanol. The product (15.5 g) was dissolved in 100 ml of water and this solution was freeze-dried to give 14.6 g (42 %) of pale yellow penicillin **8a**; $[\alpha]_D^{25}$ +215° (*c* 1.4, water); ash value <2 %. The i. r. spectrum contained bands at 1770 and 1690 cm^{-1} ascribed to the β-lactam and amide carbonyl respectively. The n.m.r. spectrum is reported in Table 1. The purity of the material was estimated (by n.m.r. and t.l.c.) at 85 %.

Epimer **8b** was prepared in a similar way. Hydrogenation of **7b** (12.3 g) yielded 5.7 g of crude penicillin which had a better solubility in methanol than **8a**. It was purified by dissolving it in 75 ml of methanol (0.7 g of inorganic salt was filtered off), followed by treatment with ether. The precipitate was filtered off and dissolved in 40 ml of water. Freeze-drying of the aqueous solution yielded 3.0 g (40 %) of pale yellow penicillin with a purity estimated at 85~90 %; $[\alpha]_D^{25}$ +184° (*c* 1.0, water). The n.m.r. spectrum is reported in Table 1.

Acknowledgements

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