# NEW SYNTHESIS OF OXIME-TYPE BETA-LACTAM ANTIOBIOTICS

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Reaction of anhydrous acids *II* with phosphorus pentachloride afforded hydrochlorides of chlorides *III* which were used in acylations of N,O-bis(trimethylsilyl) derivatives of 6-aminopenicillanic and 7-aminodeacetoxycephalosporanic acid. Change of the (*Z*)-configuration of the alkoxyimino group during the synthesis was observed only in the methoxyimino series. The prepared penicillins *IV* are effective against gram-positive as well as gram-negative bacteria.

Investigation of β-lactam antibiotics has led to the synthesis of a series of oxime-type cephalosporins containing a (Z)-2-alkoxyimino-2-(2-amino-4-thiazolyl)acetamido group in position 7 of the cephem nucleus, e.g. cefotaxime[1] *Ia*, ceftazidime[2] *Ib* (carboxyl as anion), ceftizoxime[3] *Ic* and other. These antibiotics were prepared predominantly by acylation of the amino group in 7-aminodeacetoxycephalosporanic or 7-aminocephalosporanic acid or their derivatives with reactive derivatives of (Z)-2-alkoxyimino-2-(2-amino-4-thiazolyl)acetic acids (*II*) in which the amino group was protected e.g. with a triphenylmethyl[4] or chloroacetyl[5] group. The antibacterial activity of the oxime-type antibiotics is conditioned by (Z)-configuration of the alkoxyimino group[6]; the antibacterial effects of the corresponding (*E*)-isomers are sometimes several orders of magnitude lower. In this communication we report the new method of preparing some β-lactam antibiotics which does not require protection of the thiazole amino group.

The acids *II* were prepared as described in the literature[4,7] and were obtained anhydrous by boiling in ethanol or by removal of water by azeotropic distillation from their suspension in benzene. Reaction with phosphorus pentachloride in anhydrous acetonitrile (in some cases catalyzed with small amount of dimethylformamide) or in anhydrous dichloromethane (catalyzed with small amount of conc. hydrochloric acid[8]) afforded hydrochlorides of chlorides *III*. We have found that the configuration of hydrochloride of *IIIa* (*E* or *Z*, or a mixture of both) depends on the reaction conditions used for its preparation (Table I). The configurational assignment to the alkoxyimino group in compounds *IIa* and *IIIa* was based on the observation that the thiazolyl H-5 proton signal in (Z)-isomers usually appears 0·3 – 0·6 ppm upfield.

The relative population of the isomers, given in Table I, was determined by integration of the signals.

As shown in Table I, higher temperature, greater excess of phosphorus pentachloride and the presence of dimethylformamide (when Vilsmeier–Haack reagent was used as the chlorination reagent) or concentrated hydrochloric acid led preferentially to products with (*E*)-configuration of the methoxyimino group. On the other hand, the (*Z*)-isomer of hydrochloride of *IIIa* was obtained at lower temperatures, in the absence of catalyst and with 1·2 equivalent of phosphorus pentachloride. Hydrochlorides of *III* are moisture-sensitive and very sparingly soluble substances which decompose on heating in dimethyl sulfoxide; therefore the data in Table I are only approximate.

Conversion of the (*Z*)-configuration of the methoxyimino group to the thermodynamically more stable (*E*)-configuration has been described for the preparation of antibiotics of the type *I* as well as their intermediates. Thus, e.g. ethyl ester of acid *IIa* was obtained by reaction of thiourea with ethyl (*Z*)-2-methoxyimino-3-oxobutanoate[1,9]. The (*Z*)-isomer was obtained in aqueous medium only, in the presence of only one equivalent of thiourea and at not very high temperature. Also configurational change of the methoxyimino group in the preparation of amine-protected chlorides *IIIa* has been reported[1,9–12]. The isomerization in the last step of preparation of some oxime-type antibiotics during acylation with reactive derivatives of acid *IIa* is mentioned e.g. by Ochiai and collaborators[12]. These authors also state[11]

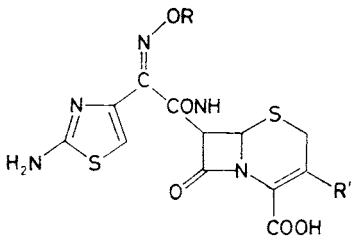
TABLE I

Composition of reaction product in the reaction of acid *IIa* with phosphorus pentachloride, as determined by $^1$H NMR spectra

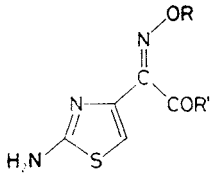
| Reaction conditions | | | | | Product composition, % | | | |
|---|---|---|---|---|---|---|---|---|
| method | equiv. of $PCl_5$ | medium | catalyst | temperature range, °C | *IIa*.HCl | | *IIIa*.HCl | |
| | | | | | *Z*[a] | *E*[b] | *Z*[c] | *E*[d] |
| *A* | 1·66 | $CH_2Cl_2$ | HCl, $H_2O$ | −10 to 0 | — | — | 2 | 98 |
| *B* | 2·1 | $CH_3CN$ | DMF | −20 to 20 | — | 6 | — | 94 |
| *B* | 1·2 | $CH_3CN$ | — | −5 | 65 | — | 35 | — |
| *B* | 1·2 | $CH_2Cl_2$ | DMF | −20 | — | 33 | — | 77 |
| *B* | 1·2 | $CH_2Cl_2$ | DMF | −12 | 55 | 13 | 28 | 4 |

[a] 7·05 ppm (hydrochloride of (*Z*)-isomer of *IIa*); [b] 7·60 ppm (hydrochloride of (*E*)-isomer of *IIa*); [c] 7·20 ppm (hydrochloride of (*Z*)-isomer of chloride *IIIa*); [d] 7·50 ppm (hydrochloride of (*E*)-isomer of chloride *IIIa*).

that in acylations the presence of base is a necessary condition for preservation of the (*Z*)-configuration of the alkoxyimino group. On the other hand, Takaya and coworkers[7] described acylations that proceed without isomerization even in the absence of any base. We did not confirm the assumption[13] that configuration of the methoxyimino group is changed in an acidic medium (no configurational change was observed on boiling the acid *IIa* in ethanolic hydrogen chloride or in conc. hydrochloric acid).



*Ia*, R = $CH_3$; R' = $CH_2OCOCH_3$
*Ib*, R = $C(CH_3)_2COOH$; R' = $CH_2$–N$^{(+)}$(pyridinium)
*Ic*, R = $CH_3$; R' = H



*IIi*, R' = OH
*III*, R' = Cl

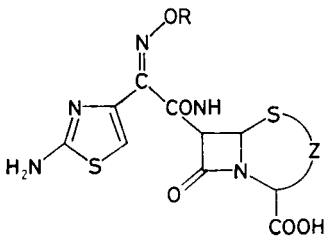
In formulae *II*–*V*: *a*, R = $CH_3$ *b*, R = $CH_2CH_3$ *c*, R = $CH(CH_3)_2$
*d*, R = cyclo-$C_5H_9$ *e*, R = cyclo-$C_6H_{11}$

Interesting in this respect is the observation of Vtorov[14] that (*Z*)-isomer of hydrochloride of chloride *IIIa* is methanolyzed to give the ester *IIa* with (*E*)-configuration of the methoxyimino group whereas in ethanolysis the (*Z*)-configuration is preserved.

Surprisingly enough, the $^1$H NMR spectra of the synthesized oxime-type antibiotics (*IVb*–*IVe* and *Vb*–*Vc*) have shown that their preparation (including the preparation of hydrochlorides of chlorides *IIIb*–*IIIe*) is not accompanied by any discernible configurational change at the alkoxyimino group.

The mentioned cases of configurational change at the methoxyimino group in various stages of the oxime-type β-lactam antibiotics (most of the effective com-

pounds of this type are methoxyimino derivatives) are somewhat contradictory, particularly as concerns conditions under which the isomerization was observed[1,7,9–13]. Therefore, also the reason why in the synthesis via hydrochlorides of chlorides *III* only the methoxyimino derivatives are isomerized is not quite apparent.




*IV*, Z = $\begin{matrix} CH_3 \\ CH_3 \end{matrix}$ >C<

*V*, Z = $\begin{matrix} \\ CH_3 \end{matrix}$ >C=

6-Aminopenicillanic acid or 7-aminodeacetoxycephalosporanic acid were acylated in the form of their N,O-bis(trimethylsilyl) derivatives obtained by heating a suspension of the corresponding acid in a mixture of dichloromethane with hexamethyldisilazane under catalysis with small amount of concentrated sulfuric acid. This solution was treated with dimethylaniline and the corresponding hydrochloride of chloride *III* under cooling. With some derivatives, at room temperature, after several hour's stirring the reaction mixture deposited the silylated intermediate. In two cases (*IVa* and *Va*) this intermediate was isolated and converted into the sodium salt by precipitation with sodium capronate solution. Other antibiotics (*IVb–IVe* and

TABLE II

Minimum inhibitory concentrations (MIC) of penicillins *IV*, determined by dilution micromethod on plates (Dynatech)

| Microorganism (State collection of strains, Prague) | MIC, mg $l^{-1}$ | | | | |
|---|---|---|---|---|---|
| | *IVa*[a] | *IVb* | *IVc* | *IVd* | *IVe* |
| *Streptococcus pyogenes* 4/49 | 100 | 50 | 50 | 12·5 | 50 |
| *Streptococcus faecalis*, sk. D 16/66 | >100 | 25 | 50 | 12·5 | 50 |
| *Staphylococcus aureus* 1/45 | 12·5 | 25 | 1·25 | 1·5 | 25 |
| *Pseudomonas aeruginosa* 26/56 | >100 | >100 | >100 | >100 | >100 |
| *Escherichia coli* 326/71 | >100 | 3·1 | 6·2 | 12·5 | 50 |
| *Proteus vulgaris* 2/35 | 25 | 50 | 12·5 | 3·1 | 12·5 |

[a] (*E*)-izomer.

*Vb*–*Ve*) were isolated by decomposition of the reaction mixture with sodium hydrogen carbonate solution, separation of the organic phase and acidification of the aqueous solution of the antibiotics to pH 2–3·5 with dilute (1 : 1) hydrochloric acid. Under these conditions, the antibiotics separated as solids. The infrared and $^1$H NMR spectra of cephalosporins *Vb*–*Ve* and penicillin *IVa* corresponded to the published data[1,11,15,16].

For the antibacterial activity tests we prepared sodium salts (*IVa*–*IVd* and *Va*–*Ve*) and potassium salt (*IVe*) of the synthesized antibiotics by precipitation with sodium capronate in butanol and potassium acetate, in ethanol, respectively. The purity of these salts (HPLC) was 71–98% for compounds *IV* and 95–99% for compounds *V*.

The antibacterial activity of the prepared antibiotics was tested by the dilution micromethod on plates using a Dynatech instrument. Salts of the evaluated antibiotics were dissolved in dimethyl sulfoxide or a phosphate buffer. The synthesized cephalosporins *V* were less active than described in the literature[4,17], the new penicillins *IVb*–*IVe* exhibited a good antibacterial activity against both gram-negative and gram-positive bacteria (Table II). Penicillins *IVc* and *IVd* showed an excellent activity against clinically significant strains of *Staphylococcus aureus*; such activity was so far observed only for oxacillin and its derivatives.

## EXPERIMENTAL

The melting points were determined on a Kofler block and are uncorrected. IR spectra were recorded on a Perkin–Elmer 557 spectrometer in KBr pellets. $^1$H NMR spectra were taken on a BS-487 C (80 MHz) and a BS-567 A (100 MHz) instrument (Tesla), using tetramethylsilane or trimethylsilyl pentadeuteriopropionic acid; the NMR data are given in the $\delta$-scale.

The content of the prepared hydrochlorides of chlorides *III* was estimated by argentometric determination of chlorine; no melting point determination was possible since these compounds decomposed slowly in the interval 230–260°C without melting. The content of the synthesized antibiotics *IV* and *V* was determined by high performance liquid chromatography, using a Waters chromatograph equipped with a Waters 440M detector; column Micro Bondapac C 18, mobile phase acetonitrile–water–acetic acid (40 : 60 : 1). The analyses were evaluated by the internal normalization method on a Waters data model using an Apple 2+ computer.

### 2-(2-Amino-4-thiazolyl)-(*Z*)-2-methoxyiminoacetic Acid (*IIa*)

A suspension of hydrated form[7] of acid *IIa* (20 g) in absolute ethanol (100 ml) was refluxed for 30 min. After standing at room temperature for 2 h, the solid was filtered and washed with acetone and hexane to give 17 g (97%) of *IIa*, m.p. 202–204°C (decomp.). Its $^1$H NMR spectrum was identical with that of the hydrated form[4,7], m.p. 140–150°C.

### 2-(2-Amino-4-thiazolyl)-(*Z*)-2-ethoxyiminoacetic Acid (*IIb*)

Hydrated form[4] of acid *IIb* (28 g) was suspended in benzene (100 ml) and a part (40 ml) of the benzene was distilled off. Filtration and drying afforded 27 g (98%) of *IIb*, m.p.154–157°C. $^1$H NMR spectrum corresponded to that of the hydrated form[4], m.p. 151°C.

2-(2-Amino-4-thiazolyl)-(*Z*)-2-isopropoxyiminoacetic Acid (*IIc*)

Hydrated form[4] (75 g) of acid *IIc* was converted into anhydrous *IIc* (73 g; 99%), m.p. 194–195°C (decomp.), as described for the preparation of *IIb*. The $^1$H NMR spectrum was identical with that of the hydrated form[4], m.p. 161°C (decomp.).

2-(2-Amino-4-thiazolyl)-(*Z*)-2-cyclopentyloxyiminoacetic Acid (*IId*)

Hydrated form[17] of the acid *IId* (110 g) was converted into *IId* (108 g; 99%), m.p. 193–195°C, as described for *IIa*. The $^1$H NMR spectrum of the product was identical with that of the hydrated form[17], m.p. 186°C.

2-(2-Amino-4-thiazolyl)-(*Z*)-2-cyclohexyloxyiminoacetic Acid (*IIe*)

Hydrated form[7] of acid *IIe* (48 g) was converted into anhydrous *IIe* (46·7 g; 99%), m.p. 154 to 157°C, as described for the preparation of *IIb*. The $^1$H NMR spectrum was identical with that of the hydrated form[4], m.p. 148°C.

2-(2-Amino-4-thiazolyl)-2-methoxyiminoacetyl Chloride Hydrochloride (*IIIa*)

Method *A*). Phosphorus pentachloride (19·5 g; 0·094 mol) was added at −10°C to a stirred mixture of anhydrous dichloromethane (90 ml) and conc. hydrochloric acid (0·48 ml). After 10 min, compound *IIa* (12 g; 0·06 mol) was added at −10°C. The mixture was stirred at 0°C for 1·5 h, the solid was filtered, washed with anhydrous benzene and hexane and dried under diminished pressure to give 12 g (79%) of *IIIa*. For $C_6H_7Cl_2N_3O_2S$ (256·1) calculated: 27·68% Cl; found: 27·26% Cl, i.e. 99% of the calculated amount.

Method *B*). Phosphorus pentachloride (5 g; 0·025 mol) was added at −20°C to a stirred suspension of *IIa* (4 g; 0·02 mol) in anhydrous acetonitrile (20 ml) containing a drop of dimethylformamide. The reaction mixture was stirred without cooling for 0·5 h, the separated product was collected and washed with anhydrous benzene and hexane. Drying under diminished pressure afforded 3·3 g (65%) of the product, containing ($^1$H NMR spectrum) hydrochlorides of (*E*)-*IIIa* and (*E*)-*IIa* in the ratio 94 : 6. (*E*)-*IIIa*. HCl ($(CD_3)_2SO$): 7·55 s, 1 H (thiazole H); 4·12 s, 3 H ($OCH_3$). (*E*)-*IIa*. HCl ($(CD_3)_2SO$): 7·64 s, 1 H (thiazole H); 4·02 s, 3 H ($OCH_3$). For $C_6H_7Cl_2N_3O_2S$ (256·1) calculated: 27·68% Cl; found: 27·21% Cl, i.e. 98·5% of calculated amount.

Hydrochlorides of 2-(2-Amino-4-thiazolyl)-(*Z*)-2-alkoxyiminoacetyl Chlorides *IIIb*–*IIIe*

The title hydrochlorides of *IIIb*–*IIIe* were obtained as described for the preparation of hydrochloride of *IIIa* (Method *B*); see Table III.

6-[2-(2-Amino-4-thiazolyl)-(*E*)-2-methoxyiminoacetamido]penicillanic Acid (*IVa*)

Hexamethyldisilazane (10·4 g; 0·065 mol) was added to a suspension of 6-aminopenicillanic acid (12·6 g; 0·059 mol) in dichloromethane (115 ml) containing one drop of conc. sulfuric acid. The reaction mixture was refluxed for 8 h, filtered and cooled to room temperature. After addition of dimethylaniline (7·4 g; 0·06 mol), the mixture was cooled to −30°C and hydrochloride of *IIIa* (15 g; prepared according to method *A*) was added under stirring. The mixture was stirred without cooling for 2 h, mixed with anhydrous benzene (50 ml), set aside at 0°C for 2 h and the leaflets of the silylated intermediate were filtered. Drying at 50°C under diminished pressure afforded 14·2 g of colourless material. Most of it (12 g) was dissolved in water (200 ml) and the

solution was acidified with dilute hydrochloric acid (1 : 1) to pH 3. The separated product was collected on filter, washed with water and dried at room temperature to give 12 g of acid *IVa*, m.p. 193–200°C (decomp.). IR spectrum, $cm^{-1}$: 1 030, 1 630–1 660, 1 770 (β-lactam), 3 300. Owing to insolubility of *IVa*, its $^1H$ NMR spectrum could not be measured.

*Sodium salt of* IVa. A solution of sodium capronate (3·14 g of 41·4% solution in butanol, diluted with 20 ml of 2-propanol) was added to a vigorously stirred solution of the acid *IVa* (3·0 g; 0·0075 mol) in 2-propanol (15 ml). The separated sodium salt of *IVa* was filtered, washed with 2-propanol and diethyl ether and dried at room temperature; yield 2·7 g (85%) of crystalline product, decomposing above 260°C; content 72·9%. IR spectrum, $cm^{-1}$: 1 030, 1 630, 1 770 (β-lactam), 3 300. $^1H$ NMR spectrum ($(CD_3)_2SO$): 9·20 bd, 1 H (CONH, $J = 8·0$ Hz); 7·48 s, 1 H (thiazole H); 7·20 bs, 2 H ($NH_2$); 5·68 dd, 1 H (H-6, $J = 8·0$; 4·0 Hz); 5·52 d, 1 H (H-5, $J = 4·0$ Hz); 4·30 s, 1 H (H-2); 4·00 s, 3 H ($OCH_3$); 1·64 s and 1·51 s, 6 H (2 $CH_3$).

In an alternative procedure, the sodium salt of *IVa* was prepared by dissolving the silylated intermediate (8 g) in 2-propanol (45 ml) and adding 3·5 g of 41·4% solution of sodium capronate in butanol. Filtration, washing with 2-propanol and diethyl ether and drying at room temperature afforded 7·0 g of the same product.

General Procedure for Preparation of Penicillins *IVb–IVe*

6-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-ethoxyiminoacetamido]penicillanic Acid (*IVb*)

Hexamethyldisilazane (2·0 g; 0·012 mol) was added to a suspension of 6-aminopenicillanic acid (2·4 g; 0·011 mol) in dichloromethane (20 ml) containing a drop of conc. sulfuric acid. The reaction mixture was refluxed for 8 h, filtered and cooled to −30°C. Dimethylaniline (1·35 g; 0·011 mol) and hydrochloride of *IIIb* (2·8 g) were added at −30°C under stirring. The stirring was continued for 2 h at room temperature and then a solution of sodium hydrogen carbonate (3·0 g) in water (80 ml) was added under vigorous stirring. The aqueous layer was extracted with dichloromethane (50 ml), filtered with charcoal and acidified to pH 2·7 with dilute (1 : 1) hydrochloric acid. The separated colourless precipitate was collected on filter, washed with water to neutrality and then with diethyl ether. Drying at 35°C afforded 3·2 g (87%) of the acid *IVb*, decomposition above 190°C. IR spectrum, $cm^{-1}$: 1 210–1 300 (COOH), 1 390 (thiazole), 1 580 (>C=NO–), 1 656 (CONH), 1 780 (β-lactam), 3 200, 3 320 ($NH_3^+ \cdot COO^-$). $^1H$ NMR spectrum ($(CD_3)_2SO$): 8·48 bd, 1 H (CONH, $J = 8·0$ Hz); 6·75 s, (thiyzole H); 5·60 m, 2 H (H-6, H-5);

TABLE III

Hydrochlorides of 2-(2-amino-4-thiazolyl)-(*Z*)-alkoxyiminoacetyl chlorides

| Compound | Formula | % Cl | | Purity[a] % | Yield % |
|---|---|---|---|---|---|
| | | Calculated | found | | |
| *IIIb*.HCl | $C_7H_8Cl_2N_3O_2S$ | 26·35 | 26·32 | 99·9 | 53 |
| *IIIc*.HCl | $C_8H_{11}Cl_2N_3O_2S$ | 24·94 | 24·80 | 99·4 | 52 |
| *IIId*.HCl | $C_{10}H_{13}Cl_2N_3O_2S$ | 22·86 | 22·33 | 97·7 | 85 |
| *IIIe*.HCl | $C_{11}H_{15}Cl_2N_3O_2S$ | 21·87 | 21·17 | 96·8 | 66 |

[a] Calculated from the analysis for Cl.

4·24 s, 1 H (H-2); 4·10 q, 2 H ($CH_2$, $J$ = 7·0 Hz); 1·63 s and 1·51 s, 6 H (2 $CH_3$); 1·25 t, 3 H ($CH_3$ in ethyl, $J$ = 7·0 Hz).

General Procedure for Preparing Salts of *IV*

*Sodium salt of* IVb. A solution of sodium capronate (1·3 g, as 3·1 g of 41·4% solution in butanol, diluted with 20 ml of acetone) was added to a vigorously stirred solution of *IVb* (3 g; 0·0074 mol) in acetone. Filtration, washing with acetone and drying at 35°C afforded 2·8 g (89%) of the amorphous salt decomposing above 215°C. Content 98·2%. IR spectrum, $cm^{-1}$: 1 388 (thiazole), 1 534 (thiazole + >C=NO—), 1 605 (COONa), 1 656 (CONH), 1 767 (β-lactam), 3 300 ($NH_2$, NH). $^1$H NMR spectrum ($(CD_3)_2SO$): 9·30 bd, 1 H (CONH, $J$ = 8·0 Hz); 7·90 bs and 7·25 bs, ($NH_2$ + H$a$O); 6·75 s, 1 H (thiazole H), 5·48 bm, 2 H (H-6 + H-5), 4·10 q, 2 H ($CH_2$, $J$ = = 7·0 Hz), 4·0 s, 1 H (H-2), 1·58 s and 1·50 s, 6 H (2 $CH_3$); 1·21 t, 3 H ($CH_3$ in ethyl, $J$ = 7·0 Hz).

6-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-isopropoxyiminoacetamido]penicillanic Acid (*IVc*)

The title acid was obtained in 91% yield, m.p. 227—235°C (decomp.). IR spectrum, $cm^{-1}$: 1 380 (thiazole), 1 520, 1 660 (CONH), 1 770 (β-lactam), 2 920—2 980 (isopropyl), 3 320 ($NH_2$, NH). $^1$H NMR spectrum ($(CD_3)_2SO$): 9·41 d, 1 H (CONH, $J$ = 6·5 Hz); 6·78 s, 1 H (thiazole H); 5·60 dd, 1 H (H-6, $J$ = 6·5, 4·0 Hz); 5·52 d, 1 H (H-5, $J$ = 4·0 Hz); 4·38 m, 1 H (CH); 4·25 s, 1 H (H-2); 1·60 s and 1·48 s, 6 H (2 CH2); 1·21 d 6 H (2 $CH_3$); 1 21 d 6 H (2 $CH_3$ in isopropyl, $J$ = 6·0 Hz).

*Sodium salt of* IVc. Obtained in 63% yield, decomposition above 230°C, content 88%. IR spectrum, $cm^{-1}$: 1 385—1 330 (thiazole), 1 570 (>C=NO—), 1 600 (COONa), 1 660 (CONH), 1 770 (β-lactam), 2 980—2 930 (isopropyl), 3 460—3 280 ($NH_2$, NH). $^1$H NMR spectrum ($(CD_3)_2SO$): 6·75 s, 1 H (thiazole H), 5·50 bm, 2 H (H-6 + H-5), 4·33 m, 1 H (CH), 4·05 s, 1H (H-2); 1·60 s and 1·55 s, 6 H (2 $CH_3$); 1·25 d, 6 H (2 $CH_3$ in isopropyl, $J$ = 6·0 Hz).

6-[-2-(2-Amino-4-thiazolyl)-(*Z*)-2-cyclopentyloxyiminoacetamido]penicillanic Acid (*IVd*)

Obtained in 76% yield, decomposition above 205°C. IR spectrum, $cm^{-1}$: 1 320, 1 390 (thiazole), 1 590 (thiazole), 1 660 (CONH + COOH), 1 772 (β-lactam), 2 870 and 2 965 (isopropyl), 3 360 ($NH_2$, NH). $^1$H NMR spectrum ($(CD_3)_2SO$): 9·40 bd, 1 H (CONH, $J$ = 8·0 Hz); 7·20 bs, ($NH_2$); 6·70 s, 1 H (thiazole H); 5·52 m, 2 H (H-6 + H-5); 4·65 bm, 1 H (CH in cyclopentyl), 4·22 s, 1 H (H-2); 1·30—2·00 m, (cyclopentyl), 1·60 s and 1·50 s, 6 H (2 $CH_3$).

*Sodium salt of* IVd. Obtained in 70% yield, decomposition above 235°C, content 92·7%. IR spectrum, $cm^{-1}$: 1 320, 1 390 (thiazole), 1 530 (>C=NO—), 1 660 (CONH), 1 770 (β-lactam), 2 960, 2 870 (cyclopentyl), 3 280—3 420 ($NH_2$, NH). $^1$H NMR spectrum ($(CD_3)_2SO$): 9·60 bd, 1 H (CONH, $J$ = 8·0 Hz); 7·25 bs, 2 H ($NH_2$); 6·75 s, 1 H (thiazole H); 5·50 m, 2 H (H-6 + H-5); 4·70 bm 1 H (CH in cyclopentyl); 4·05 s, 1 H (H-2); 1·70 bs, 1·60 s and 1·50 s, 11 H (cyclopentyl + 2 $CH_3$).

6-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-cyclohexyloxyiminoacetamido]penicillanic Acid (*IVe*)

Obtained in 97% yield as colourless product decomposing above 190°C. IR spectrum, $cm^{-1}$: 1 320, 1 380 (thiazole), 1 530 (>C=NO—), 1 630—1 660 (NHCO), 1 778 (β-lactam), 2 880, 2 950 (cyclohexyl), 3 480—3 380 ($NH_2$, NH). Because of insolubility, no $^1$H NMR spectrum could be taken.

*Potassium salt of* IVe. A solution of potassium acetate (0·66 g; 0·0067 mol) in ethanol (3 ml) was added to a solution of *IVe* (3·3 g; 0·0067 mol) in methanol (50 ml). After evaporation to half of the original volume and addition of diethyl ether (10 ml), the separated salt of *IVe* was filtered, washed and dried; yield 2·6 g (75% of amorphous solid, decomposing above 210°C; content 76·4%. IR spectrum, $cm^{-1}$: 1 300–1 400 (thiazole), 1 620 (COOK), 1 770 (β-lactam), 2 860, 2 940 (cyclohexyl), 3 320, 3 430 ($NH_2$, NH). $^1H$ NMR spectrum ($(CD_3)_2SO$): 9·25 bd, 1 H (CONH); 6·70 s, 1 H (thiazole H); 5·50 m, 2 H (H-6 + H-5); 4·08 s and 4·10 bs, 2 H (H-2 + CH in cyclohexyl); 1·60 s and 1·51 s, (2 $CH_3$ + unseparated m of cyclohexyl).

7-[2-(2-Amino-4-thiazolyl)-(*E*)-2-methoxyiminoacetamido]-deacetoxycephalosporanic Acid (*Va*)

Hexamethyldisilazane (8·5 g; 0·05 mol) and one drop of conc. sulfuric acid were added to a suspension of 7-aminodeacetoxycephalosporanic acid (10 g; 0·047 mol) in dichloromethane (80 ml). After reflux for 8 h and filtration, dimethylaniline (5·9 g; 0·049 mol) was added. The mixture was cooled to −30°C and a solution of hydrochloride of *IIIa* (12·5 g; 0·049 mol) in anhydrous dichloromethane (100 ml) was added under stirring. After stirring at room temperature for 1 h, the separated solid was filtered, washed with dichloromethane and dried in vacuo at 40°C to give 18·5 g of colourless silylated intermediate. A suspension of this intermediate (6·0 g) in water (100 ml) was adjusted to pH 3·5 with dilute (1 : 1) aqueous ammonia and the colourless acid *Va* was filtered, washed with water and dried; yield 3·1 g (75%), decomposition above 190°C. IR spectrum, $cm^{-1}$: 1 660 (COOH), 1 760 (β-lactam), 3 300 ($NH_2$, NH). $^1H$ NMR spectrum ($(CD_3)_2SO$): 7·48 s, 1 H (thiazole H); 5·70 dd, 1 H (H-7, $J$ = 8·0, 4·0 Hz), 5·10 d, 1 H (H-6, $J$ = 4·0 Hz); 4·00 s, 3 H ($CH_3$); 3·65 and 3·28 ABq, 2 H ($CH_2$ in dihydrothiazine, $J$ = 13·0 Hz); 2·02 s, 3 H ($CH_3$ on dihydrothiazine ring).

*Sodium salt of* Va. Acetone (30 ml) and sodium capronate (1·3 g, 0·0078 mol, as 41·4% solution in butanol) were added to a solution of *Va* (3·1 g; 0·0076 mol) in dimethylformamide (5 ml). Sodium salt of *Va* was obtained in 92% yield; decomposition above 210°C; content 99·0%. IR spectrum, $cm^{-1}$: 1 600 (COONa), 1 760 (β-lactam), 3 300 ($NH_2$, NH). $^1H$ NMR spectrum ($(CD_3)_2SO$): 7·46 s, 1 H (thiazole H); 5·65 bdd, 1 H (H-7); 5·00 d. 1 H (H-6, $J$ = 4·0 Hz); 4·00 s, 3 H ($OCH_3$); 3·50 and 3·10 (ABq, 2 H ($CH_2$ in dihydrothiazine, $J$ = 13·0 Hz); 1·95 s, 3 H ($CH_3$ on dihydrothiazine ring).

7-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-ethoxyiminoacetamido]-deacetoxycephalosporanic Acid (*Vb*)

Hexamethyldisilazane (3·4 g; 0·021 mol) was added to a suspension of 7-aminodeacetoxycephalosporanic acid (4·0 g; 0·019 mol) in anhydrous dichloromethane (40 ml). The mixture was refluxed for 8 h, filtered and dimethylaniline (2·4 g; 0·019 mol) was added to the filtrate. After cooling to −30°C, hydrochloride of *IIb* (4·6 g; 0·017 mol) was added under stirring. The reaction mixture was stirred at room temperature for 2 h and then added to a stirred solution of sodium hydrogen carbonate (6 g) in water (100 ml). The separated aqueous layer was washed with dichloromethane, mixed with charcoal, filtered and adjusted to pH 2·7 with dilute (1 : 1) hydrochloric acid. The separated material was collected on filter, washed with water and dried at 40°C to give 5·1 g (66%) of crystalline acid *Vb*, m.p. 217–219°C (decomp.). IR spectrum, $cm^{-1}$: 540, 1 380, 1 540 (thiazole), 1 630 (>C=NO–), 1 665 (CONH), 1 770 (β-lactam), 3 280 ($NH_2$, NH). $^1H$ NMR spectrum ($(CD_3)_2SO$): 11·50 broad signal (COOH); 9·55 bd, 1 H (CONH); 7·60 (broad signal, $NH_2$); *g* 75 s, 1 H (thiazole H); 5·70 dd, 1 H (H-7, $J$ = 8·0, 4·0H z); 5·10 d, 1 H (H-6, $J$ = 4·0 Hz);

m·12 q, 2 H ($CH_2$ in ethyl, $J$ = 7·0 Hz); 3·63 and 3·28 ABq, 2 H ($CH_2$ in dihydrothiazine, $J$ = = 13·0 Hz); 2·05 s, 3 H ($CH_3$ on dihydrothiazine ring); 1·25 t, 3 H ($CH_3$ in ethyl, $J$ = 7·0 Hz).

*Sodium salt of* Vb. Acetone (25 ml) and sodium capronate (1·4 g; 0·008 mol, as 41·4% solution in butanol) were added to a stirred solution of *Vb* (3·0 g; 0·0078 mol) in dimethylformamide (5 ml). After standing for one hour, the product was filtered, washed with acetone and dried at 40°C. Yield 3·0 g (95%) of colourless salt of *Vb*, decomposing above 225°C; content 95·2%. IR spectrum, $cm^{-1}$: 1 384 (thiazole), 1 530, 1 602 (COONa), 1 660 (CONH), 1 752 (β-lactam), 3 320 (NH*a*, NH). $^1H$ NMR spectrum ($(CD_3)_2SO$): 9·50 bd, 1 H (CONH, $J$ = 8·0 Hz); 7·65 bs and 7·20 bs ($NH_2 + H_2O$); 6·70 s, 1 H (thiazole H); 4·97 d, 1 H (H-6, $J$ = 4·0 Hz); 4·12 q, 2 H ($CH_2$ in ethyl, $J$ = 7·0 Hz); 3·50 and 3·10 ($CH_2$ in dihydrothiazine, $J$ = 13·0 Hz); 1·98 s, 3 H ($CH_3$ on dihydrothiazine ring); 1·21 t, 3 H ($CH_3$ in ethyl, $J$ = 7·0 Hz).

7-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-isopropoxyiminoacetamido]-deacetoxycephalosporanic Acid (*Vc*)

Hexamethyldisilazane (1·7 g; 0·011 mol) and one drop of conc. sulfuric acid were added to a suspension of 7-aminodeacetoxycephalosporanic acid (2·0 g; 0·0095 mol) in anhydrous dichloromethane (18 ml). After refluxing for 8 h and filtration, hydrochloride of *IIIc* (2·7 g; 0·0095 mol) was added at −30°C under stirring. The mixture was then stirred at room temperature for 1 h, the separated intermediate was filtered, washed with dichloromethane and dried at 40°C. The intermediate (2·8 g) was dissolved in a solution of sodium hydrogen carbonate (2 g) in water (100 ml), the impurities were extracted with dichloromethane and the mixture was adjusted to pH 2·0 with dilute (1 : 1) hydrochloric acid. On standing for 1 h, the obtained colourless solution deposited the acid *Vc* which was collected on filter, washed with water and dried at 40°C; yield 1·5 g (40%), m.p. 203—204°C (decomp.). IR spectrum, $cm^{-1}$: 1 200, 1 280, 1 556 (thiazole), 1 660 (CONH), 1 772 (β-lactam), 3 270 (NH), 3 540 ($NH_2$). $^1H$ NMR spectrum ($(CD_3)_2SO$): 9·50 d, 1 H (CONH, $J$ = 8·0 Hz); 7·25 bs, 2 H ($NH_2$); 6·70 s, 1 H (thiazole H); 5·72 dd, 1 H (H-7, $J$ = 8·0, 5·0 Hz); 5·12 d, 1 H (H-6, $J$ = 5·0 Hz); 4·31 m, 1 H (CH in isopropyl); 3·62 and 3·28 ABq, ($CH_2$ in dihydrothiazine, $J$ = 18·0 Hz); 2·03 s, 3 H ($CH_3$ on dihydrothiazine ring); 1·25 d, 6 H (2 $CH_3$, $J$ = 6·0 Hz).

*Sodium salt of* Vc. Sodium capronate (0·55 g; 0·0015 mol, as 41·4% solution in butanol) was added to a solution of *Vc* (0·5 g; 0·0013 mol) in ethanol (10 ml). After standing for 1 h at room temperature, the crystalline product was filtered and washed with ethanol. Drying at 40°C afforded 0·5 g of sodium salt of *Vc*, decomposing above 230°C; content 99·1%. IR spectrum, $cm^{-1}$: 1 360 (thiazole), 1 590 (COONa), 1 660 (CONH), 1 760 (β-lactam), 3 300—3 420 ($NH_2$, NH). $^1H$ NMR spectrum was not measured because of insolubility of the salt.

7-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-cyclopentyloxyiminoacetamido]-deacetoxycephalosporanic Acid (*Vd*)

The title acid was prepared in 63% yield as described for the preparation of *Vc*; decomposition above 205°C. IR spectrum, $cm^{-1}$: 1 320, 1 380 (thiazole), 1 530, 1 660 (CONH), 1 770 (β-lactam), 2 870 (cyclopentyl), 3 260—3 420 ($NH_2$, NH). $^1H$ NMR spectrum ($(CD_3)_2SO$): 9·50 d, 1 H (CONH, $J$ = 8·0 Hz); 7·35 broad signal ($NH_2$); 6·70 s, 1 H (thiazole H); 5·70 dd, 1 H (H-7, $J$ = 8·0, 5·0 Hz); 5·10 d, 1 H (H-6, $J$ = 5·0 Hz); 4·10 bm, 1 H (CH in cyclopentyl); 3·62 and 3·28 ABq, ($CH_2$ in dihydrothiazine, $J$ = 13·0 Hz); 2·05 s, 3 H ($CH_3$ on dihydrothiazine ring); 1·00—2·00 unseparated m (cyclopentyl).

*Sodium sat of* Vd. The salt was obtained in 81% yield as described for the preparation of *Vc*; decomposition above 225°C, content 98%. IR spectrum, $cm^{-1}$: 1 370 (thiazole), 1 530 (>C=NO—), 1 600 (COONa), 1 660 (CONH), 1 760 (β-lactam), 2 960—2 880, 3 300—3 460 ($NH_2$, NH). $^1H$ NMR spectrum ($(CD_3)_2SO$): 9·45 bs, 1 H (CONH, $J$ = 8·0 Hz); 7·30 bs, 2 H ($NH_2$); 6·70 s, 1 H (thiazole H); 5·59 dd, 1 H (H-7, $J$ = 8·0, 5·0 Hz); 5·00 d, 1 H (H-6, $J$ = 5·0 Hz); 4·70 bm, 1 H (CH in cyclopentyl); 3·50 and 3·10 ABq, 2 H ($CH_2$ in dihydrothiazine, $J$ = 13·0 Hz); 1·99 s and 1·70 bs, 11 H ($CH_3$ on dihydrothiazine ring + cyclopentyl).

7-[2-(2-Amino-4-thiazolyl)-(*Z*)-2-cyclohexyloxyiminoacetamido]-deacetoxycephalosporanic Acid (*Ve*)

The acid *Ve*, m.p. 188—196°C (decomp.) was prepared in 73% yield as described for the preparation of acid *Vc*. IR spectrum, $cm^{-1}$: 1 370, 1 330 (thiazole, 1 530 (>C=NO—), 1 630, 1 660 (NHCO), 1 770 (β-lactam), 2 860, 2 940 (cyclohexyl), 3 295—3 430 ($NH_2$, NH). $^1H$ NMR spectrum: ($(CD_3)_2SO$): 9·48 bd, 1 H (CONH, $J$ = 8·0 Hz); 7·20 bs, 2 H ($NH_2$); 6·70 s, 1 H (thiazole H); 5·70 dd, 1 H (H-7, $J$ = 8·0, 5·0 Hz); 5·10 d, 1 H (H-6, $J$ = 5·0 Hz); 4·68 bm, 1 H (CH in cyclohexyl); 3·63 and 3·88 ABq, 2 H ($CH_2$ in dihydrothiazine, $J$ = 13·0 Hz); 2·06 s, 3 H ($CH_3$ on dihydrothiazine ring); 1·20—2·00 unseparated m.

*Sodium salt of* Ve. Prepared in 86% yield as described for the preparation of *Vc*; decomposition above 200°C, content 97·7%. IR spectrum, $cm^{-1}$: 1 370 (thiazole), 1 530 (>C=NO—), 1 630 to 1 660 (CONH), 1 768 (β-lactam), 1 860, 1 940 (cyclohexyl). $^1H$ NMR spectrum ($(CD_3)_2SO$): 9·50 bd, 1 H (CONH, $J$ = 8·0 Hz); 7·20 bs, 2 H ($NH_2$); 6·69 s, 1 H (thiazole H); 5·56 dd, 1 H (H-7, $J$ = 8·0, 4·0 Hz); 4·98 s, 1 H (H-6, $J$ = 4·0 Hz); 4·05 bm, 1 H (CH in cyclopentyl); 3·50 and 3·10 ABq, 2 H ($CH_2$ in dihydrothiazine, $J$ = 13·0 Hz); 1·86 s, 3 H ($CH_3$); 1·10—2·00 unseparated m (cyclohexyl).

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