SOME APPROACHES TO THE SYNTHESIS OF SULBACTAM

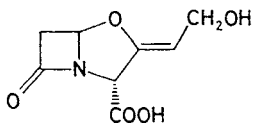
Suren HUSINEC*[a], Deana DRAGOVIĆ[a,b], Zdenka STOKIĆ[c] and Josipa KOJIĆ[c]

[a] *Institute of Chemistry, Faculty of Science, University of Belgrade, Studentski trg 16, P.O. Box 550, YU-11001, Belgrade, Yugoslavia*
[b] *Institute of Chemistry Technology and Metallurgy, Faculty of Science, University of Belgrade, Studentski trg 16, P.O. Box 550, YU-11001, Belgrade, Yugoslavia and*
[c] *Research Department, Galenika Pharmaceutical Co., 29 Novembra 111, 11000 Belgrade, Yugoslavia*

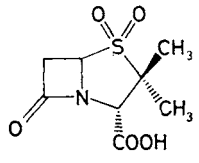
Received July 8, 1988
Accepted September 28, 1988

A study on the synthesis of penicillanic acid 1,1-dioxide, Sulbactam, was carried out. Two reliable convergent synthetic routes to this β-lactamase inhibitor starting from penicillin G potassium salt and 6-aminopenicillanic acid have been developed.

Since the discovery that the spectrum of activity of enzyme sensitive penicillins and cephalosporins can be extended by the use of β-lactamase inhibitors[1,2] there has been a considerable body of work directed towards the development of new compounds to serve this function. The first breakthrough in this field was made in 1976 with the discovery of clavulanic acid (*I*), a metabolite of *Streptomyces clavuligerus*[3]. Subsequently English and coworkers[4] demonstrated that penicillanic acid 1,1-dioxide (*II*, Sulbactam), a synthetic molecule, also showed useful β-lactamase inhibitory properties.



I



II

We became interested in developing a practical synthesis of Sulbactam which would permit multigram quantities to become available for pharmacological evaluation. To the present time two approaches, both starting from 6-aminopenicillanic acid, have found application. Deamination of 6-aminopenicillanic acid by diazotiza-

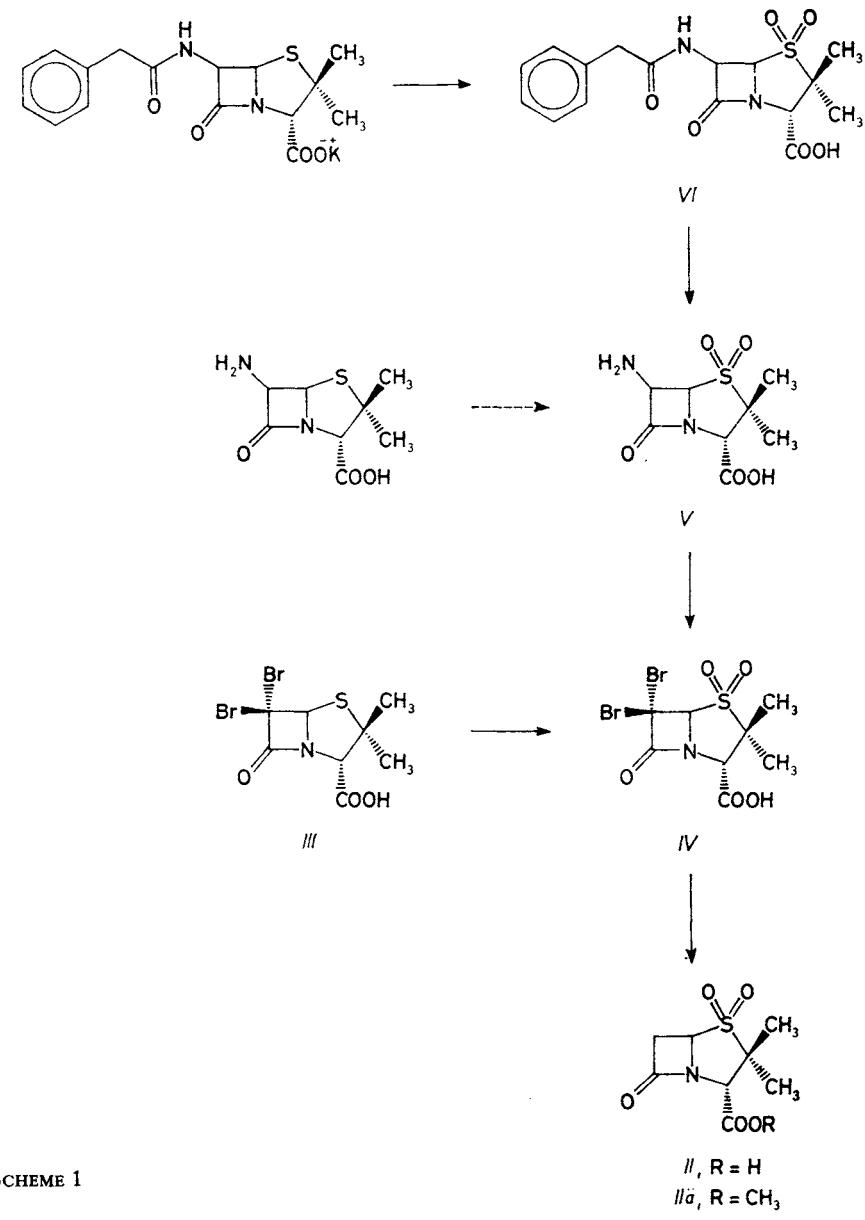
tion/bromination was first reported by Clayton[5] but both yield and purity of the 6,6-dibromopenicillanic acid (*III*) were unsatisfactory. Subsequently Volkmann and coworkers[6] introduced a two phase system, dichloromethane/water using a large excess of bromine and reported that 80% yields of 6,6-dibromopenicillanic acid were obtained. Oxidation of the 6,6-dibromopenicillanic acid to the sulfone *IV* was accomplished by aqueous potassium permanganate and catalytic reduction of *IV*, over 5% palladium on carbon at 0·2–0·5 MPa gave an overall reported yield of Sulbactam *II* of 54–65%.

Kapur and Fasel[7] have reported a procedure based on diazotization/bromination of 6-aminopenicillanic acid 1,1-dioxide (*V*). Attempts to carry out diazotization of (*V*) under the conditions described by Clayton[5] or Volkmann[6] resulted in impractically low yields and a new experimental procedure based on diazotization/bromination in the presence of methanol was reported to give high yields of *IV* which was reduced to *II* using magnesium under acidic conditions in high overall yields.

In our hands all of the existing literature procedures were plagued with the problem of extremely variable yields and we have directed our attention specifically to developing reproducible reactions. In the first series of experiments, (Scheme 1), the potassium salt of penicillin G was oxidized with potassium permanganate at pH 7 to give the corresponding sulfone *VI* in 85% yield. Deacylation of *VI* was performed using the iminoester method, at low temperature with the protection of the carboxyl group using dimethyl dichlorsilane in the presence of N,N-dimethyl aniline. The yield of *V* was 61%.

Diazotization/bromination of *V* was carried out in acidic medium with sodium nitrite and bromine and brown colored dibromo sulfone *IV* was isolated in 69% yield. The spectral characteristic and microanalytical data confirmed the structure and the mass spectrum confirmed the presence of two bromine atoms in molecule. This observation is particularly interesting as it is known that mass spectrometry is not the method of choice for the analysis of β-lactam antibiotics[9]. By using the electron impact technique (70 eV) the molecular ions characteristic of the dibromo-β-lactam (*IV*) with M^+, $M^+ + 2$, $M^+ + 4$ appeared in a ratio of 1 : 2 : 1. Reduction of *IV* was effected using zinc powder at pH 3·4–4·0 in acetonitrile and Sulbactam *II* was obtained in 60% yield. Alternatively the reduction step can be carried out at subambient temperature using magnesium powder as described in patent literature[8], but this does not significantly improve the yield. The overall yield of this sequence, starting from the potassium salt of penicillin G, was 21%.

Several steps on this sequence may prove problematic on a large scale, in particular the deacylation step. It should be carried out at low temperature, −40 to −50°C, and traces of N,N-dimethyl aniline that is used in the reaction are detected in the precipitated 6-aminopenicillanic acid 1,1-dioxide. There is no simple technique to purify the product. We have developed an alternative route.



SCHEME 1

Attempts to oxidize 6-aminopenicillanic acid directly with potassium permanganate only resulted in a very low yields of the sulfone *V*. (Scheme 1, dotted line). Alternatively, diazotization/bromination of 6-aminopenicillanic acid was carried out under the same experimental conditions as the diazotization/bromination of *V*,

and 6,6-dibromopenicillanic acid (*III*) was obtained in 70% yield. Oxidation of *III* with potassium permanganate and treatment of the crude reaction product with sodium bisulphite followed by extraction into ethyl acetate resulted in the formation of the brown product identified as *IV*. This product was rather impure and proved troublesome to handle. However, by simple expedient of changing the solvent a remarkable change occurred. When ethyl acetate was substituted with dichloromethane (in which *IV* is insoluble) and sodium bisulfite added, a colorless crystalline solid was precipitated and on filtration this product was identified as the dibromo sulfone *IV* and was isolated in an analytically pure state.

Reduction of *IV* to Sulbactam (*II*) was carried out as described above, and using this procedure the overall yield from 6-aminopenicillanic acid was 33%.

EXPERIMENTAL

Melting points were determined on a Boetius apparatus and are uncorrected. Infrared spectra were recorded by using Perkin–Elmer model 457 grafting spectrophotometer. Proton NMR spectra (80 MHz) were obtained with a Varian FT-80A, with tetramethylsilane as internal standard. Mass spectra were taken with Finnigan model 8230 (70 eV) mass spectrometer. TLC was carried out using precoated silica plates Kieselgel 60 F_{254}, Merck.

Sulbactam (*II*) was identified on the basis of its spectral and microanalytical data, by comparison with an authentic sample (Gist Brocades) and by conversion to its methyl ester and its sodium salt.

Benzylpenicillin 1,1-Dioxide (*VI*)

Benzylpenicillin potassium salt (5 g, 0·0134 mol) was dissolved in water (70 ml) and the pH adjusted to 7·1 with 2% NaOH. The reaction flask was cooled to 5°C. A premixed solution containing potassium permanganate (2·9 g, 0·0183 mol), 85% H_3PO_4 (0·7 ml) and water (55 ml) was added dropwise and the temperature maintained at 0—10°C and the pH maintained at 7·2 with 2% NaOH. After the addition was complete the reaction mixture was stirred for 1 h at a temperature 0—10°C. The reaction mixture was filtered through Celite. Solution of 0·5M sodium bisulphite was added to the filtrate until the disappearance of purple permanganate color. The pH was adjusted to 2·0—2·5 with H_3PO_4 (8%) and ethyl acetate (25 ml) was added. The organic layer was separated and the aqueous phase extracted with ethyl acetate (2 × 25 ml). Combined ethyl acetate layers were dried (Na_2SO_4), filtered and the solvent evaporated. The yield of the title compound was 4·28 g (85%). M.p. 124—126°C (ref.[10], m.p. 128°C). IR spectrum (KBr): ν_{max} 1 815, 1 320, 1 125 cm^{-1}. ^1H NMR spectrum ($(CD_3)_2SO$): 1·25 s, 3 H (CH_3), 1·40 s, 3 H (CH_3); 3·55 s, 2 H (CH_2); 4·30 s, 1 H (H-3); 5·25 d, 1 H (H-5); 5·75 q, 1 H (H-6); 7·20 s, 5 H (C_6H_5); 8·55 d, 1 H (NH). For $C_{16}H_{18}N_2O_6S$ (366·4) calculated: 52·45% C, 4·92% H, 7·65% N, 8·74% S; found: 52·62% C, 5·18% H, 7·80% N, 8·65% S.

6-Aminopenicillanic Acid 1,1-Dioxide (*V*)

Benzylpenicillin 1,1-dioxide *IV* (3·9 g, 0·0107 mol) was suspended in dichloromethane (33 ml), and N,N-dimethylaniline (4·8 ml) was added at once. Dichlorodimethylsilane (1·19 ml, 0·097 mol) was added dropwise. The reaction mixture was stirred at room temperature for 45 min then it was cooled to —50°C. Phosphorus pentachloride (2·4 g, 0·0113 mol) was added in one portion

and the reaction mixture was stirred for 2 h at −35°C. The reaction mixture was cooled to −50°C, isobutyl alcohol (16·7 ml) was added and stirred for 1 h at −35°C. The reaction mixture was poured into cold water (20 ml), the pH adjusted to 3·3 with ammonium hydroxide (8%), and stirred for 30 min. The precipitate was filtered and washed with cold acetone–water mixture (1 : 1). The yield of the title compound was 1·6 g (60·6%). M.p. 150°C (ref.[11], m.p. 154–155·5°C). IR spectrum (KBr) ν_{max} 3 600, 3 440, 1 800, 1 320, 1 125 cm^{-1}. ^1H NMR spectrum ($CDCl_3$): 1·30 s, 3 H (CH_3); 1·40 s, 3 H (CH_3); 4·25 s, 1 H, (H-3); 4·70 d, 1 H (H-6); 5·10 d, 1 H (H-5).

6,6-Dibromopenicillanic Acid 1,1-Dioxide (*IV*) from *V*

Bromine (1·54 ml, 0·03 mol), followed by 1M-H_2SO_4 (16 ml) was added to dichloromethane (20 ml) while the temperature was maintained below 5°C and $NaNO_2$ (1·375 g, 0·02 mol) was added. Penicillanic acid 1,1-dioxide *V* (2·5 g, 0·01 mol) was added in small portions during 30 min and the temperature was maintained below 5°C. The reaction mixture was stirred for an additional 30 min, and 1M sodium bisulphite (16·5 ml) was added dropwise over 20 min, while the temperature was maintained at 5–15°C. Insoluble material was filtered and layers were separated, the aqueous phase extracted with dichloromethane (2 × 20 ml). Combined dichloromethane layers were washed with brine, dried (Na_2SO_4), filtered and the solvent evaporated. Insoluble material and the compound isolated from the dichloromethane layer were identical and the yield of the title compound was 5·5 g (69%). M.p. 207°C (ref.[10], m.p. 201°C). IR spectrum (KBr) ν_{max} 1 810, 1 740, 1 340, 1 125 cm^{-1}. ^1H NMR spectrum (CD_3CN): 1·15 s, 3 H (CH_3), 1·30 s, 3 H (CH_3); 4·60 s, 1 H (H-3); 5·35 s, 1 H (H-5); 8·25 broad s, 1 H (O-H). Mass spectrum M^+ 390, $M^+ + 2$ 392, $M^+ + 4$ 394. For $C_8H_9Br_2NO_5S$ (391·0) calculated: 24·55% C, 2·30% H, 3·58% N, 8·18% S; found: 24·72% C, 2·53% H, 3·70% N, 8·18% S.

Penicillanic Acid 1,1-Dioxide (*II*)

6,6-Dibromopenicillanic acid 1,1-dioxide *IV* (2·5 g, 0·0064 mol) was suspended in a cooled (ice bath) mixture of acetonitrile (20 ml) and water (30 ml). The pH was adjusted to 5·2 with 4M-NaOH, and activated zinc powder was added in small portions with vigorous stirring, while the temperature was maintained at 0–10°C. Upon the completion of the addition of zinc, the pH was adjusted to 3·5–4 with 4M-HCl. After 30 min the reaction was completed, the excess of zinc was filtered, washed with little cold water and extracted with dichloromethane (2 × 20 ml). Combined organic layers were dried (Na_2SO_4), filtered and the solvent evaporated at temperature below 30°C. The yield of the title compound was 0·9 g (60·4%). M.p. 170°C (ref.[6], m.p. 170°C). IR spectrum (KBr) ν_{max} 3 500, 1 770, 1 755, 1 740, 1 320, 1 120 cm^{-1}. ^1H NMR spectrum (CD_3CN): 1·40 s, 3 H (CH_3), 1·50 s, 3 H (CH_3); 3·35 d, 1 H (H-6); 3·45 d, 1 H (H-6), 4·35 s, 1 H (H-3); 4·75 q, 1 H (H-5); 7·25 broad s, 1 H (O-H). For $C_8H_{11}NO_5S$ (233·2) calculated: 41·20% C, 4·72% H, 6·00% N, 13·73% S; found: 41·38% C, 4·92% H, 6·16% N, 13·42% S.

Penicillanic Acid 1,1-Dioxide Methyl Ester (*IIa*)

The methyl ester was obtained in 41% yield treating the dichloromethane layer from the reduction step with an 0·02M etheral solution of diazomethane. On evaporation of the solvent a crystalline compound was isolated. M.p. 117°C (ref.[12], m.p. 118–120°C). IR spectrum (KBr) ν_{max} 1 785, 1 730, 1 320, 1 120 cm^{-1}. ^1H NMR spectrum (CD_3CN): 1·70 s, 3 H (CH_3); 1·85 s, 3 H (CH_3), 3·70 dd, 2 H (H-6); 4·10 s, 3 H (CH_3-O); 4·70 s, 1 H (H-3); 5·10 q, 1 H (H-5). For $C_9H_{13}NO_5S$ (247·3) calculated: 43·72% C, 5·26% H, 5·67% N, 12·96% S; found 43·50% C, 5·36% H, 5·61% N, 13·17% S.

6,6-Dibromopenicillanic Acid (*III*)

Bromine (99·5 g, 0·62 mol), 1·25M-H_2SO_4 (167 ml) and sodium nitrite (28·8 g, 0·42 mol) were added to a cooled (5°C) dichloromethane (400 ml). 6-Aminopenicillanic acid (45 g, 0·21 mol) was added over a period of 30 min while the temperature was maintained at 4—10°C. Upon the addition of 6-aminopenicillanic acid the reaction mixture was stirred for an additional 30 min. Sodium bisulfite (1M) (340 ml) was added dropwise over 30 min at temperature 4—10°C. The organic layer was separated and the aqueous phase extracted with dichloromethane (2 × 150 ml). Combined dichloromethane layers were dried (Na_2SO_4), filtered and the solvent evaporated. The yield of the title compound was 53 g (70·6%). M.p. 123°C. IR spectrum (KBr) ν_{max} 3 280, 1 750, 1 330, 1 170 cm^{-1}. ^1H NMR spectrum (CD_3CN): 1·37 s, 3 H (CH_3), 1·42 s, 3 H (CH_3), 4·45 s, 1 H (H-3); 5·65 s, 1 H (H-5), 8·75 broad s, 1 H (O-H). For $C_8H_9Br_2NO_3S$ (359·1) calculated: 26·74% C, 2·51% H, 3·90% N, 8·91% S; found: 26·57% C, 2·78% H, 3·93% N, 8·28% S.

6,6-Dibromopenicillanic Acid 1,1-Dioxide (*IV*) From *III*

6,6-Dibromopenicillanic acid (*III*) (20 g, 0·056 mol) was dissolved in dichloromethane (300 ml) and water (300 ml) was added. 3M-NaOH was added dropwise until the pH stabilized at 7·0. Layers were separated, and the organic layer was extracted with water (2 × 100 ml). Combined aqueous layers were cooled to —5°C and a premixed solution containing potassium permanganate (59·25 g), H_3PO_4 (18 ml) and water (600 ml) was added until the purple color of permanganate was persistent (approximately 200 ml). The reaction mixture was filtered, dichloromethane (300 ml) was added and the pH was adjusted to 1·2 with 6M-HCl. Solution of 1M sodium bisulphite (410 ml) was added and white crystals precipitated. The solid was filtered. The yield of the title compound was 14·7 g (67%). Spectral characteristics were identical with the sample obtained previously.

6,6-Dibromopenicillanic acid 1,1-dioxide (*IV*), can be directly obtained from 6-aminopenicillanic acid without isolating the 6,6-dibromopenicillanic acid (*III*), in 55% yield.

We are grateful to Belgrade's Research Council for the financial assistance and for a studentship (to D. D.), to Gist Brocades for a sample of Sulbactam and to the Instrumental Organic Department of the Faculty of Science for recording spectra.

REFERENCES

1. Behrens O. K., Garrison L.: Arch. Biochem. Biophys. *27*, 94 (1950).
2. Housewright R. D., Henry R. J.: J. Bacteriol. *53*, 241 (1947).
3. Howarth T. T., Brown A. G., King T. J.: J. Chem. Soc., Chem. Commun. *1976*, 266.
4. English A. R., Retsma J. A., Girard A. E., Lynch J. E., Barth W. E.: Antimicrob. Agents Chemother. *14*, 414 (1978).
5. Clayton J. P.: J. Chem. Soc. *1969*, 2123.
6. Volkmann R. A., Carroll R. D., Drolet R. B., Eliott M. L., Moore B. S.: J. Org. Chem. *47*, 3344 (1982).
7. Kapur J. C., Fasel H. P.: Tetrahedron Lett., *1985*, 3875.
8. U.S. 4,554,104 (Appl. 659,088); Chem. Abstr. *103*, 123262 q (1985).
9. Muller M. D., Seibl J., Simon W.: Anal. Chim. Acta., *100*, 263 (1987).
10. Gudall E., Morch P., Tybring L.: Tetrahedron Lett. *1962*, 381.
11. U.S. 4,234,579 (Appl. 17,700); Chem. Abstr. *90*, 121589 r, (1979).
12. Micetic R. G., Maiti S. N., Tanaka M., Yamazaki T., Ogawa K.: J. Org. Chem. *51*, 858 (1986).