A New Synthesis of Ampicillin and Related Investigations (1)

F. Kajfež, T. Kovač, M. Mihalić, B. Belin and V. Šunjić

Department of Biomedical and Biochemical Research, CRC, 33 048 S Giovanni al Natisone (UD) Italy, and Institute of Organic Chemistry and Biochemistry, University of Zagreb, Zagreb, Croatia, Yugoslavia

Received December 22, 1975

Quaternization of (S)-α-bromophenylacetic acid amide (**8**) with hexamethylentetramine (hexamine) preceded with *ca.* 80% inversion of configuration. Accordingly, starting from the trimethylsilylester of *N*-(S)-(α-bromo-α-phenylacetyl)-6-aminopenicillanic acid (**4**) quaternization with hexamine and subsequent hydrolysis afforded *N*-(R)-α-phenylglycyl-6-aminopenicillanic acid (**1**, ampicillin). Some other model reactions have been investigated.

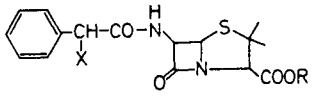
J. Heterocyclic Chem., 13, 561 (1976).

Introduction

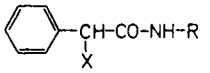
Recently, Kajfež and Blažević reported (2-3) a new method for cyclization of 2-(*N*-α-bromoacetyl)amino-5-substituted benzophenones into 1,4-benzodiazepin-2-ones using hexamine as a mild reagent for ammonolysis of a reactive halogen. The intermediate quaternary salt has been isolated in some instances along with some side products in the cyclization step (4). A similar sequence of reactions has been proposed to be useful in the synthesis of ampicillin (**1**), semisynthetic penicillin derived from 6-aminopenicillanic acid (6-APA) by acylation with (R)-α-phenylglycine. A number of procedures for preparation of **1** have already been described (5-8) and its benificial therapeutic (9,10) and pharmacodynamic properties (11,12) have repeatedly been established. The present paper relates to our investigations on the synthesis of ampicillin based on our earlier work (2-4,13) and on some new model reactions as well. At the same time the procedure which has been developed represents a new possibility for the preparation of amoxycillin (14); which pharmacological (15) and clinical (16) properties have recently been described.

Results and Discussion

The originally planned reaction sequence leading to ampicillin consisted of the preparation of *N*-α-bromophenylacetyl-6-APA (**2**) and its conversion into **1** *via* the quaternary hexaminium salt **3**. Three questions had to be answered before experimentation upon the chemically sensitive penicillinic derivative **2**, could be undertaken; a) the reaction rate, b) the stereochemical course of the quaternization with hexamine, and c) the conditions for selective hydrolytic cleavage of the hexaminium salt in the presence of the acid sensitive (17-19) β-lactam ring.



	X	R
1	(R)-NH_2	H
2	(S)-Br	H
3	(R)-$(CH_2)_6N_4^+Br^-$	H
4	(S)-Br	$Si(Me)_3$

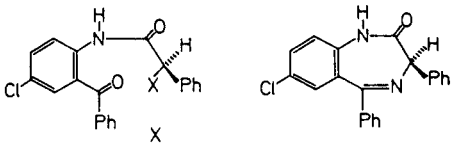


	X	R
5	Br	CH_3
6	$(CH_2)_6N_4^+Br^-$	CH_3
7	(R)-Br	(R)-$CH(CH_3)Ph$
8	(S)-Br	(R)-$CH(CH_3)Ph$
9	$(CH_2)_6N_4^+Br^-$	(R)-$CH(CH_3)Ph$
10	(S)-NH-Cbo	(R)-$CH(CH_3)Ph$
11	(R)-NH-Cbo	(R)-$CH(CH_3)Ph$
12	(R)-NH-Cbo	$N(C_6H_{11})\cdot CO\cdot NH(C_6H_{11})$
13	(S)-NH_2	(R)-$CH(CH_3)Ph$
14	(R)-NH_2	(R)-$CH(CH_3)Ph$
15	Br	(R)-$CH(CH_3)COOH$
16	Br	CH_2CH_2COOH

From some earlier papers, and own experience, we knew that reactive halides can be quaternized with hexamine under very mild conditions (20,21) which are compatible with the high sensitivity of the penicillanic nucleus to various nucleophiles.

The semi-quantitative reaction rate was determined by nmr techniques (13) for quaternization of **5** into **6**. It was found that the rate, as measured in deuteriochloroform, was dependent upon the concentration of hexamine, revealing its bimolecularity, the half-time at 35±0.1° being between *ca.* 40-60 minutes. No H/D exchange for the benzylic proton was observed on addition of methanol-d_4 to the nmr probe, eliminating this possibility of racemization.

To follow the stereochemical course of quaternization, and consequently to obtain the correct diastereomer of ampicillin, a simple and highly effective resolution of racemic α-bromophenylacetic acid was required. Earlier methods (22,23) made use of morphine as the resolving base, whereby racemic acid was used in a molar excess what allows isolation of (-)-enantiomer which crystallizes as the first salt. We found that equimolar amount of brucine in ethanol led to highly effective resolution, allowing easy separation of the second, (+)-enantiomer, as well.



	X
18	Br
19	NH-Cbo

20

The stereochemical course of quaternization was initially attempted on the model compound **18**. Its quaternization, hydrolysis and cyclization led to **20** which proved to be fully racemized. The racemic **20** was obtained from the chiral precursor **19** as well. This result was particularly surprising in view of the recently established (24) optical stability of a number of chiral 1,4-benzodiazepin-2-ones. Compound **20**, however, underwent fast H/D exchange in methanol-d_4 ($t_{1/2}$ about 8 minutes at 35±0.1°). This finding excluded compounds **18** and **20** as the models for investigation of the stereochemical course of ammonolysis of the α-bromophenacetyl moiety by hexamine.

The second way to establish the stereochemical course of quaternization was to prepare diastereomers **7** and **8** and to follow their conversion into diastereomers **13** and/or **14** *via* the quaternary hexaminium salts. Diastereomers **7** and **8** were conveniently prepared from racemic acid and subsequently separated by partial crystallization from diisopropyl ether. For determination of the unknown configuration at the second chiral center in the diastereomers **7** and **8**, (R)-(-)-α-bromopropionic acid (25,26) was converted into **7** (R,R-diastereomer). Stereochemically pure standards of diastereomers **13** and **14** have independently been prepared from *N*-carbobenzoxy-(S)- and (R)-phenylglycine *via* intermediates **10** and **11**.

Quaternization of compound **8**, and subsequent hydrolysis of **9** gave a mixture of **13** and **14** with $[\alpha]_D$ + 57.8°. This rotation value indicates a composition of about 80% of the R,R-diastereomer **14**, and of 20% of S,R-diastereomer **13**, thus about 60% of net inversion resulted in going from **8** to **13/14**. This stereochemical result can be a consequence of various factors, such as a high incidence of the bimolecular S_N2 mechanism, and/or stereoselective substitution from one diastereotopic face on the intermediate ion-pair in the rate-determining step (27,28) and/or partial inversion (29) in unimolecular reactions as well. Retention through two inversions, one of them being α-lactone formation, is a well known pathway for solvolysis of α-halocarboxylic acids and their chlorides (30,31). However, this possibility is excluded in case of the solvolytically stable amides **7** and **8**, however.

Repeating the same procedure on **2** prepared from the racemic α-bromoacid using an equimolar amount of hexamine, an unexpected separation of hexaminium hydrobromide occurred, giving up to 80% of the theoretical amount. Elimination of one mole of hydrobromic acid from **2** leading to some decomposition products had to be proposed (32).

Therefore, we turned back to protection of the carboxylic group in 6-APA using the procedure of Glombitze (33). The resulting ester was then acylated with (S)-(+)-α-bromophenylacetic acid and quaternized with hexamine. It was found that these reactions could most conveniently be performed without isolation of intermediates. Intermediate ester **4** was an amorphous powder which decomposed in significant amounts into **2** upon any attempt at crystallization from carefully dried solvents. The same applied to the quaternary salt, therefore optimal conditions for its hydrolysis were found by a trial and error method in a number of experiments, taking into account the known stability of the product **1** under acidic conditions (17,34). All attempts to replace hexamine with aqueous or ethanolic ammonia as a halogen displacing reagent led to severe decomposition of the β-lactam ring. The best procedure for purification of the crude ampicillin (**1**) was found to be ion-exchange chromatography on Amberlite XAD-2, recently applied for purification of some other semisynthetic penicillins (35,36), followed by crystallization from aqueous acetone. The total yield of pure **1**, which exhibits over 90% of antimicrobial activity in standard tests, varied vetween 15-20%. Although chemical and chiroptical properties of pure **1** corresponded to those described in the literature for the (R)-α-phenylblycyl derivative (37), the crude diastereomeric mixture contained some quantity of the other diastereomer. Since on recrystallization from aqueous acetone the diastereomeric composition could be

changed along with an increase in chemical purity, no precise information about the stereoselectivity of quaternization could be obtained in this particular case (38).

In conclusion, it can be stated from the studies on the model α-bromophenylacetylamides that α-phenylglycylamides were formed with inversion of configuration on ammonolysis with hexamine. This reaction offers a new approach to semisynthetic penicillins as herein described for the preparation of ampicilline **1**.

EXPERIMENTAL

Melting points were determined on a Kofler hot-stage apparatus. Ir spectra were recorded on a Perkin-Elmer M-257 spectrophotometer (potassium bromide pallets). Nmr spectra were measured on a Varian T-60 spectrometer with TMS as the internal reference. Tlc was performed on a silica gel HF_{254} (Fertigplatten Merck). For column chromatography silica gel Merck 0.05-0.2 mm was used, fractions being collected automatically by Ultra Rac-7000 fraction collector and tlc controlled for their content. Ion-exchange chromatography was performed on Amberlite XAD-2 (100-200 mesh) resin. Rotations were measured on a Perkin-Elmer M 141 apparatus. All organic solutions were dried over anhydrous sodium sulphate and evaporated *in vacuo* using a Rotavapor-R (Büchi) unless otherwise stated. The light petroleum used corresponded to the fraction with b.p. 40-60°.

Resolution of (±)-α-Bromophenylacetic Acid *via* the Salt with Brucine.

In 25 ml. of 99.8% ethanol brucine tetrahydrate (4.05 g., 8.7 mmoles, Aldrich) was dissolved with gentle heating. A clear solution was gradually cooled to 25 ± 1° and finely powdered (±)-α-bromophenylacetic acid (1.88 g., 8.8 mmoles) was added all at once. The resulting solution was cooled in ice for 24 hours, the crystals which separated were filtered with suction, and washed with ethanol (2 x 5 ml.). After drying *in vacuo* 2.64 g. (92%) of the first diastereomeric salt was obtained, m.p. 185-190°, $[\alpha]_{578}$ -19.5° (c 2.0 in methanol-water 1:1).

Anal. Calcd. for $C_{13}H_{33}BrN_2O_6$ (609.53): C, 61.09; H, 5.46; N, 4.59. Found: C, 60.96; H, 5.79; N, 4.78.

This salt (2.50 g.) was dissolved in ice-cooled 5% sulfuric acid (20 ml.), extracted with ether (3 x 30 ml.), and the extracts dried in the refrigerator. On evaporation these remained 0.95 g. of an oil which slowly crustallized on standing in 3 ml. of light petroleum. There was obtained 652 mg. of the (-)-enantiomer with $[\alpha]_{578}$ -136° [c 2.0 in benzene, 92.5% optical purity (41), 74% yield].

The ethanolic mother liquors after crystallization of the first diastereomeric salt were evaporated to dryness, and a residual amorphous powder was obtained and dried *in vacuo* yielding 2.86 g. of the second diastereomeric salt, m.p. 150-155°, $[\alpha]_{578}$ +14.2° (c 2.0 in methanol-water 1:1). On treatment with cold, diluted sulfuric acid, as described for the (-)-enantiomer, 0.35 g. of the (+)-enantiomer with $[\alpha]_{578}$ +111° was obtained. About 0.6 g. of oily (+)-enantiomer was obtained from mother liquors. Brucine was recovered in about 80% yield according to the procedure described (42).

N-(R)-α-Bromophenylacetyl-(R)-phenylethylamine (**7**).

To (R)-(+)-phenylethylamine (2.45 g., 20 mmoles, Fluka puriss), dissolved in methylene chloride (10 ml.), dicyclohexylcarbodiimide (DCC, 4.52 g., 22 mmoles) and (-)-α-bromophenylacetic acid (4.2 g., 19 mmoles), both dissolved in 20 ml. of methylenechloride, were added dropwise. After standing on ice overnight, dicyclohexylurea separated and was removed by suction filtration. The filtrate was evaporated and crude **7** recrystallized thrice from ether-light petroleum, m.p. 117-119°, $[\alpha]_{578}$ +2.78° (c 1.6 in chloroform).

Anal. Calcd. for $C_{16}H_{16}BrNO$ (318.22): C, 60.39; H, 5.07; N, 4.40. Found: C, 60.04; H, 4.86; N, 4.22.

N-(S)-α-Bromophenylacetyl-(R)-phenylethylamine (**8**).

(R)-(+)-Phenylethylamine (2.35 g., 19.5 mmoles) in 10 ml. of 15% sodium hydroxide was cooled in ice-bath, and under vigorous stirring, (±)-α-bromophenacylchloride (4.0 g., 17.5 mmoles) dissolved in ether (15 ml.) was added dropwise, during 0.5 hour. Stirring was prolonged at -5° to 0° for 2 hours, and then 3 hours at room temperature. Thereafter, 150 ml. of ice water was added and the resulting suspension extracted with ethyl acetate (3 x 70 ml.). It was dried, evaporated, and the residual oil crystallized from 20 ml. of diisopropyl ether. A mixture of **7** and **8** was obtained, 3.74 g. (69.5%) as pale-yellow crystals. The diastereomeric mixture was dissolved in 300 ml. of hot diisopropyl ether and allowed to stand on ice overnight. There was obtained 2.40 g. (70% of starting material) of crystals which consisted of a 7:3 mixture of **8/7**. It was observed by nmr on addition of 5% $Eu(fod)_3$, that the singlet for Ar-C*H*Br- was shifted to 6.5 ppm for **8** and to about 6.3 ppm for **7** allowing estimation of the **8/7** ratio. The mother liquors contained 840 mg. of a 6:4 mixture of **7/8**.

Two additional crystallizations of 2.40 g. of the 7:3 mixture of **8/7** gave 1.25 g. of **8** which contained less than 3% of **7**, as determined by nmr. This sample had m.p. 140-143°; nmr (deuteriochloroform): δ 1.51 ppm (d, J = 10.5 Hz, 3H), 5.08 (q, J = 10.5 Hz, 1H), 5.40 (s, 1H), 6.9-7.2 (broad s, N*H*), 7.3-7.5 (m, 10H); $[\alpha]_{578}$ +99.2°, $[\alpha]_{546}$ +119° (c 1.64 in chloroform).

Anal. Calcd. for $C_{16}H_{16}BrNO$ (318.22): C, 60.39; H, 5.07; N, 4.40. Found: C, 60.63; H, 5.29; N, 4.20.

Isolation of Compound **7**.

The mother liquors of the three first crystallizations of **8** afforded on evaporation 980 mg. of crude **7**. This sample was thrice recrystallized from ether-light petroleum yielding 280 mg. of **7** which was about 87% stereochemically pure. This was proved by nmr on addition of $Eu(fod)_3$, and by comparison of $[\alpha]_{578}$ -value to those obtained for **7** in the preceding experiment: m.p. 116-119°. The nmr spectrum could not be differentiated from that of **7**; $[\alpha]_{578}$ +2.44°, $[\alpha]_{546}$ +2.30° (c 1.34 in chloroform).

N-α-Hexaminiumphenylacetyl-(R)-phenylethylamine (**9**).

Hexamine (420 mg., 3.0 mmoles) was dissolved in chloroform (10 ml., dried over molecular sieve A4), and compound **8** (954 mg., 3.0 mmoles) was added with stirring. After 4 hours stirring at ambient temperature, the solvent was evaporated *in vacuo*, and the remained amorphous powder slurried in light petroleum and removed by suction filtration. On drying (at 0.02 mm Hg over phosphorus pentoxide) a quantitative yield of **9**, as a hygroscopic white powder, was obtained, m.p. 123-126° dec.; nmr (deuteriochloroform): δ 1.57 ppm (d, J = 7 Hz, 3H), 4.52 (s, 6H), 5.00 and 5.53 (dd, J = 12 Hz, 6H), 6.86 (s, 1H), 7.1-8.0 (m, 10H).

Anal. Calcd. for $C_{22}H_{28}BrN_2O$ (458.41): C, 57.53; H, 6.16; N, 15.28. Found: C, 57.94; H, 5.90; N, 15.06.

Hydrolysis of **9**.

Compound **9** (300 mg.) was dissolved in a 1:1 mixture of ethanol and 0.1*N* aqueous hydrochloric acid (20 ml.) and stirred for 8 hours at ambient temperature. Thereafter, the solution was

made neutral using 10% aqueous sodium acetate, and the ethanol was evaporated *in vacuo*. The residual slurry was extracted with ethyl acetate (3 x 10 ml.), the organic extracts were dried and evaporated. The residual oil was purified by passing through a silica gel column (10 g.) (chloroform-ether 1:1 as eluant). The unseparated diastereomeric mixture of **13**/**14** (oil) was dried (0.02 mm Hg/phosphorus pentoxide/overnight); $[\alpha]_D$ +57.8° (c 1.42 in chloroform).

(R)-α-*N'*-Carbobenzoxyaminophenylacetyl-(R)-phenylethylamine (**11**).

The solutions of *N*-Cbo-(R)-phenylglycine (2.85 g., 10.0 mmoles), and (R)-(+)-α-phenylethylamine (1.22 g., 10.0 mmoles), each in 10 ml. of methylene chloride, have been mixed with ice-cooling, and then a solution of DCC (2.26 g., 11.0 mmoles) in methylenechloride (10 ml.) was added immediately. It was stirred for 8 hours at room temperature, the urea which precipitated was filtered with suction, the filtrate evaporated *in vacuo*, and the residual oil placed on a silica-column (70 g.) and eluated with methylene chloride. Fractions 32-45 (10 ml. per fraction) contained 459 mg. of crude side product **12**, fractions 54-72 contained 2.45 g. of crude **11**, which on recrystallization from ethyl acetate melted at 180-182°; nmr (deuteriochloroform): δ 1.39 ppm (d, J = 6.8 Hz, 3H), 5.10 (q, J = 6.8 Hz, 1H), 5.33 (d, J = 7.2 Hz, 1H), *ca.*, 6.3 (two d, superimposed, N*H* each), 6.9-7.5 (m, 15H).

Anal. Calcd. for $C_{24}H_{24}N_2O_3$ (388.47): C, 74.20; H, 6.32; N, 7.21. Found: C, 74.32; H, 6.43; N, 7.30.

N-α-Carbobenzoxyaminophenylacetyl-*N,N'*-dicyclohexylurea (**12**).

Crude **12** (459 mg. in fractions 54-72) was recrystallized from methylene chloride-light petroleum affording the pure compound with m.p. 141-143°; $[\alpha]_{578}$ -87.5° (c 1.28 in chloroform); nmr (deuteriochloroform): δ 0.7-2.2 ppm (broad signal, 20H), 3.4-4.3 (broad m, 2H-each on the cyclohexyl carbon α-to the nitrogen atom), 5.10 (s, 2H), 5.63 (d, 1H), 5.93 (d, 1H), 6.9-7.2 (broad s, 1H), 7.3 (broad signal, 10H).

Anal. Calcd. for $C_{29}H_{37}N_3O_4$ (491.64): C, 70.85; H, 7.59; N, 8.54. Found: C, 70.69; H, 7.46; N, 8.72.

(R)-α-Aminophenylacetyl-(R)-phenylethylamine (**14**).

Compound **11** (730 mg.) was dissolved in 4.0 ml. of 48% hydrobromic acid in acetic acid, and stirred for 1 hour at room temperature. The solvent was evaporated *in vacuo*, the residual oil dissolved in ice-water (5 ml.), the *p*H adjusted to 7-7.5, and extracted with ethyl acetate (3 x 10 ml.). After drying and evaporation the oily residue was crystallized on ice from ether-light petroleum. Pure **14** melted at 90-91°, $[\alpha]_{578}$ +75.8° (c 2.0 in chloroform): nmr (deuteriochloroform): δ 1.42 ppm (d, J = 8 Hz, 3H), 2.2-2.4 (broad s, NH_2), 4.49 (s, 1H), 5.08 (q, J = 8 Hz, 1H), 7.21 (s, 5H), 7.28 (s, 5H).

Anal. Calcd. for $C_{16}H_{18}N_2O$ (254.33): C, 75.56; H, 7.13; N, 11.02. Found: C, 75.32; H, 7.41; N, 10.88.

The stereochemical purity of this compound was checked on successive addition of Eu(fod)$_3$ to an nmr probe in deuteriochloroform. No splitting of the singlet at 4.49 ppm (for Ar-C*H*NH_2-CO) was observed, which indicates that no epimerization occurred during the preparation of **14**.

(S)-α-*N'*-Carbobenzoxyaminophenylacetyl-(R)-phenylethylamine (**10**).

Starting from (S)-*N*-Cbo-phenylglycine, **10** was isolated as an intermediate in the preparation of **13** following the analogous procedure as described for **11**. On recrystallization from ethyl acetate, **10** melted at 170-173°; $[\alpha]_{578}$ +83° (c 1.40 in chloroform).

Anal. Calcd. for $C_{24}H_{24}N_2O_3$ (388.47): C, 74.20; H, 6.23; N, 7.21. Found: C, 74.05; H, 6.50; N, 7.29.

(S)-α-Aminophenylacetyl-(R)-phenylethylamine (**13**).

After purification by column chromatography the analytically pure sample of **13** was obtained on recrystallization from chloroform-light petroleum, m.p. 86-88°; $[\alpha]_{578}$ -14.0° (c 2.0 in chloroform).

Anal. Calcd. for $C_{16}H_{18}N_2O$ (254.33): C, 75.56; H, 7.13; N, 11.02. Found: C, 75.71; H, 7.04; N, 11.21.

N-α-Bromophenylacetyl-(R)-alanine (**15**).

(R)-Alanine (3.55 g., 40.0 mmoles) was dissolved in 25 ml. of 20% potassium hydroxide, the solution was cooled to -5°, and the racemic α-bromophenylacetic acid (8.2 g., 36.7 mmoles) dissolved in ether (30 ml.), was added dropwise over 0.5 hour. After additional stirring for 1 hour at room temperature, the organic layer was separated, the aqueous phase washed with ether (2 x 30 ml.), and then the *p*H adjusted to 2. Crude **15** separated as an oil, which was extracted with 3 x 25 ml. of chloroform. After drying and evaporation the oily residue was crystallized on addition of diisopropyl ether (10 ml.). It was filtered off, dried, redissolved in aqueous bicarbonate, and filtered with charcoal. The filtrate was acidified (*p*H 1), the pure **15** which separated was collected on a filter and dried; m.p. 132-135°; nmr (methanol-d_4): δ 1.44 ppm (d, J = 7.2 Hz, 3H), *ca.*, 4.5 (q, J = 7.2 Hz, 1H, overlapped with CD_3OH), 5.48 (s, 1H), 7.2-7.7 (m, 5H).

Anal. Calcd. for $C_{11}H_{12}BrNO_3$ (286.14): C, 46.18; H, 4.23; N, 4.90. Found: C, 45.92; H, 4.44; N, 4.75.

N-α-Bromophenylacetyl-β-alanine (**16**).

Compound **16** has been prepared as described for **15** starting from 7.1 g. (80 mmoles) of β-alanine and 16.4 g. (73.4 mmoles) of racemic α-bromophenylacetyl chloride. The crude product (4.9 g.) was purified by crystallization from acetone-light petroleum (1:1), m.p. 117-119°; nmr (acetone-d_6): δ 2.57 ppm (t, 2H), 3.53 (q, 2H on addition of deuterium oxide becomes a triplet), 5.70 (s, 1H), 7.3-7.4 (m, 7H- on addition of deuterium oxide, the intensity falls to 5H, *i.e.*, -N*H* and -O*H* disappeared).

Anal. Calcd. for $C_{11}H_{12}BrNO_3$ (286.14): C, 46.18; H, 4.23; N, 4.90. Found: C, 46.26; H, 4.49; N, 4.95.

2-Phenyl-1,4-oxazepine-3,7-dione (**17**).

Compound **16** (2.50 g., 8.75 mmoles) was dissolved in chloroform (100 ml.) and hexamine (1.22 g., 8.75 mmoles) was added. The resulting solution was stirred for 4 hours at ambient temperature, the hexaminium bromide which separated was filtered off, and the filtrate was washed with water (3 x 20 ml.). The organic layer was dried, evaporated and the residual oil crystallized from ether-chloroform. There was obtained 177 mg (10%) of pure **17**, m.p. 90-94°; ir: 1738 cm^{-1} (7-membered lactone), 1670 (7-membered lactam), 1540 (amide II band); nmr (deuteriochloroform): δ 2.62 ppm (t, 2H), 3.60 (q, 2H), 5.45 (s, 1H), 7.2-7.6 (m, 5H), 8.1 (s, 1H).

Anal. Calcd. for $C_{11}H_{11}NO_3$ (205.22): C, 64.38; H, 5.41; N, 6.83. Found: C, 64.12; H, 5.09; N, 6.61.

2-*N'*-Carbobenzoxy-(R)-phenylglycylamino-5-chlorobenzophenone (**19**).

This compound has been prepared from 2-amino-5-chlorobenzophenone (2.42 g., 10.4 mmoles) and *N*-Cbo-(R)-phenylglycine (2.83 g., 10.0 mmoles) using DCC (2.32 g., 11.0 mmoles) in methylene chloride as described for **11**. The crude product (3.55 g., 71.5%), was recrystallized from cyclohexane-chloroform (2:1) giving pure **19**, m.p. 157-159° (c 2.0, chloroform).

Anal. Calcd. for $C_{29}H_{23}ClN_2O_4$ (498.97): C, 69.81; H, 4.65; N, 5.61. Found: C, 69.80; H, 4.69; N, 5.82.

(R)-(-)-2-α-Bromophenylacetylamino-5-chlorobenzophenone (**18**).

2-Amino-5-chlorobenzophenone (2.3 g., 10.0 mmoles), and (R)-(-)-α-bromophenylacetic acid (2.2 g., 10.0 mmoles) were dissolved in methylene chloride (10 ml.) under cooling in an ice-bath. To this solution DCC (2.06 g., 10.0 mmoles), dissolved in 10 ml. of methylene chloride was added dropwise over 1 hour. After 3 hours, stirring at room temperature, the DC-urea was removed by suction filtration, and crude **18** was obtained on evaporation of the filtrate and crystallization from 20 ml. of ether (2.89 g., 67.5%, m.p. 128-131°). On recrystallization from ethyl acetate the pure compound melted at 129-131°; $[\alpha]_{578}$ -107.6° (c 2.0, chloroform); nmr (deuteriochloroform): δ 5.50 ppm (s, 1H), 7.2-7.9 (m, 12H), 8.60 (d, 1H for C(3)-H on substituted benzophenone), 11.0 (s, N*H*).

Anal. Calcd. for $C_{21}H_{15}BrClN$ (428.72): C, 58.83; H, 3.53; N, 3.27. Found: C, 59.08; H, 3.49; N, 3.04.

Attempted Preparations of Optically Active 3,5-Diphenyl-7-chloro-1,4-benzodiazepin-2-one (**20**).

a) Compound **18** (0.85 g., 2.0 mmoles) was dissolved in chloroform (20 ml.) and 1.0 g. of hexamine was added. The solution was stirred 4 hours at ambient temperature, then the solvent was evaporated *in vacuo*, and the residue dissolved in 10 ml. of ethanol and 10 ml. of aqueous 1*N* hydrochloric acid. The solution was stirred 8 hours, then neutralized and partially evaporated at ambient temperature. The aqueous phase was diluted with 50 ml. of water, extracted with chloroform (3 x 50 ml.) and the organic layers were dried and evaporated. The residual oil was taken up in 5 ml. of DMF and cyclization into **20** was followed by tlc. Pure product (0.28 g.) exhibited no rotation (c 2.2, in DMF), as measured at 578 and 546 nm.

b) Compound **19** (3.0 g.) was dissolved in 30 ml. of 48% hydrobromic acid in acetic acid and stirred for 0.5 hour at ambient temperature. Thereafter 3 x 50 ml. of light petroleum was added and each time separated by decantation, a crystalline slurry remaining. It was dissolved in 100 ml. of ice-cold water, the *p*H adjusted to 7.0-7.5 and extracted under cooling with ice, using ethyl acetate (3 x 100 ml.). After drying and evaporation the residue was dissolved in DMF (50 ml.) and cyclization into **20** was followed by tlc. After 48 hours at ambient temperature, DMF was evaporated *in vacuo* and crude **20** recrystallized from methanol, affording 1.7 g. with m.p. 270-272 (lit. 43) m.p. 269-270°; $[\alpha]_{578}$ 0 ± 0.1° (c 2.0 in DMF).

C(3) H/D exchange rate measurement on **20** was performed in methanol-d_4/DMF-d_7 (2:1) at 35 ± 0.1° whereby a signal at 5.03 ppm was integrated at 30 second intervals.

N-α-Bromophenylacetyl-6-aminopenicillanic Acid (**2**).

α-Bromophenylacetyl chloride (8.2 g., 36.7 mmoles), dissolved in ether (50 ml.), was added dropwise to the solution of 6-APA (7.58 g., 35.0 mmoles) in aqueous bicarbonate (10.1 g., 120 mmoles of sodium bicarbonate, and 80 ml. of water), cooled to -10°. After additional stirring at -10° for 1 hour, the ethereal layer was separated and discarded. The aqueous layer was washed with ether, made acidic (*p*H 2) with dilute hydrochloric acid, and then extracted with chloroform (3 x 30 ml.). This extract was dried 2 hours at ambient temperature, then filtered and left on ice for crystallization. Yellow crystals separated overnight, sometimes seeding was required. The product was removed by suction filtration and dried *in vacuo* to give 7.9 g. (54.5%) of pure **2**, m.p. 122-125°. On dilution of the mother liquors with light petroleum an additional 2-4 g. of **2** could be obtained, which is, according to the nmr spectrum, contaminated with some degradation products; nmr (pyridine-d_5): δ 1.66 ppm (s, 3H), 1.95 (s, 3H), 4.85 (s, 1H), 6.47 (s, 1H), 6.66 (s, 1H), 7.2-7.6 (m, 3H-*meta*-and-*para*- aromatic protons), 7.6-8.0 (m, 2H-*ortho*- protons); ir (potassium bromide): 1790, 1730, 1655, 1557, 1392, 1378, 1220, 1202, 755 and 690 cm^{-1}.

Anal. Calcd. for $C_{16}H_{13}BrN_2O_4S$ (413.30): C, 46.51; H, 4.15; N, 6.77. Found: C, 46.36; H, 4.37; N, 6.47.

(R)-α-Phenylglycyl-6-aminopenicillanic Acid (**1**).

6-APA (13.0 g.) was slurried in chloroform (110 ml., dried over molecular sieve A4), and hexamethyldisilazane (25 ml., 98% pure-Fluka) was added. Brief heating under reflux, and exclusion of moisture, led to dissolving of 6-APA in about 40 minutes. Chloroform was evaporated and the residual oil distilled at 90-92°/0.05 mm Hg (lit (27) b.p. 114°/0.03 mm), yield *ca.*, 12 g. Freshly distilled 6-APA-trimethylsilyl ester (3.9 g., 13.7 mmoles) was dissolved in methylene chloride (20 ml.), dicyclohexylcarbodiimide (DCC, 2.90 g., 14.1 mmoles) was added, and to the resulting solution (S)-(+)-bromophenylacetic acid (3.0 g., 13.9 mmoles) was added dropwise. The reaction was proceeded for 1 hour at ambient temperature and 3 hours in the refrigerator. On suction filtration of the urea which separated (2.45 g., 80%), hexamine (2.8 g., 20.0 mmoles) was added. The resulting solution was stirred for 4 hours at room temperature, and the solvent evaporated affording the crude quaternary hexaminium salt of **1**. This material exhibited microbiological activity according to standard test (31,37) of *ca.*, 30-35% inhibition. The crude mixture was dissolved in 40 ml. of 25% aqueous ethanol and the solution acidified to *p*H 1.5. After 4 hours stirring between 15-20° the reaction solution was neutralized with sodium bicarbonate to *p*H 6.0-6.5 and liophylized. The dry material exhibited biological activity and produced a spot on tlc (acetone-acetic acid 9:1 as eluent) corresponding to the standard of **1** (Rf 0.25). This product (2 g), dissolved in 120 ml. of water, was applied to an Amberlite XAD-2 column (60 x 2 cm). Elution with distilled water afforded pure **1** (0.69) in fractions 11-26 (20 ml. per fraction). This material was crystallized from acetone-water giving a sample with m.p. 200-202°, and with a microbiological activity of 90-95% inhibition.

REFERENCES AND NOTES

(1) Presented in part at the 5th International Congress of Heterocyclic Chemistry (Ljubljana, July 13-18, 1975), section Penicillins and Cephalosporins, Abstracts, p. 30.

(2) F. Kajfež and N. Blažević, *J. Heterocyclic Chem.*, 7, 1175 (1970).

(3) F. Kajfež and N. Blazevic, *ibid.*, 8, 845 (1971).

(4) N. Blažević, D. Kolbah, I. Crvelin, V. Sunjić and F. Kajfež, *ibid.*, 9, 531 (1972).

(5) G. V. Kaiser and S. Kukolja, in "Cephalosporines and Penicillins", E. H. Flynn, Ed., Academic Press, New York and London, 1972, pp. 74-131.

(6) British Patent 1,341,921; *Chem. Abstr.*, 80, 133,237f (1974).

(7) I. Isaka, T. Kashiwagi, N. Nakano, N. Kawahara, A. Koda, Y. Numesaki, S. Kawahara and M. Murakami, *Yakugaku Zasshi*, 92, 454 (1972).

(8) A. Koda, K. Takanabu, I. Isaki, T. Kashiwagi, K. Takahashi, S. Kawahara and M. Murakami, *ibid.*, 92, 459 (1972).

(9) G. T. Stewart, "The Penicillin Group of Drugs," Elsevier Publishing Co., Amsterdam-Longon-New York, 1965, pp. 46-64.

(10) D. A. Kulikova, T. P. Radkewich and E. A. Rudzith, *Anti-*

biotiki, 9, 835 (1973).

(11) G. T. Stewart and M. P. Harrison, *Br. J. Pharmacol.*, 17, 414 (1961).

(12) H. Knoth, B. Lauer and K. Fabricius, *Arzneim.-Forsch.*, 24, 951 (1974).

(13) V. Šunjić, F. Kajfež, M. Oklobdžija, D. Kolbah and M. Stromar, *Bull. Acad. Sci. Yugoslavia, Sect. A*, 18, 226 (1973).

(14) Swiss. Patent Application 6855/1974.

(15) E. A. P. Croydon and R. Sytherland in, "Advances in Antimicrobial and Antineoplastic Chemotherapy," M. Hejzlar, M. Semonsky and S. Mosak, Eds., Urban and Schwarzenberg, München-Berlin-Wien, 1972, Vol. I/2, p. 975.

(16) A. M. Geddes, J. D. Williams, J. Cosmidis, J. A. D. Goodall and J. Andrews, *ibid.*, p. 981.

(17) F. P. Doyle, J. H. C. Nayler, H. Smith and E. R. S. Tove, *Nature*, 191, 1092 (1961).

(18) J. P. Hou and J. W. Poole, *J. Pharm. Sci.*, 58, 447 (1969).

(19) T. Yamana, A. Tsuji and Y. Mizukami, *Chem. Pharm. Bull.*, 22, 1186 (1974).

(20) C. Mannich and F. L. Hahn, *Ber.*, 44, 1542 (1911).

(21) W. A. Jacobs and M. Heidelberger, *J. Am. Chem. Soc.*, 37, 459 and 465 (1915).

(22) A. McKinzie and N. Walker, *J. Chem. Soc.*, 107, 1685 (1915).

(23) A. M. Ward, *ibid.*, 1184 (1926).

(24) M. Stromar, V. Šunjić, T. Kovač, L. Klasinc and F. Kajfež, *Croat. Chem. Acta*, 46, 265 (1974).

(25) P. Brewster, E. D. Hughes, C. K. Ingold and P. A. D. Rao, *Nature*, 166, 178 (1950).

(26) W. Klyne and J. Buckingham, "Atlas of Stereochemistry," Chapman and Hall, London 1974, pp. 5 and 17.

(27) R. A. Sneen and J. W. Larsen, *J. Am. Chem. Soc.*, 88, 2593 (1966).

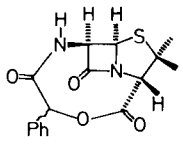
(28) R. A. Sneen, J. V. Carter and P. S. Kay, *ibid.*, 88, 2595 (1966).

(29) J. Steigman and L. P. Hammett, *ibid.*, 59, 2536 (1937).

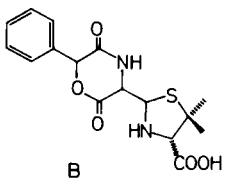
(30) E. S. Gould, "Mechanism and Structure in Organic Chemistry," H. Holt and Co., New York, N.J., 1959, p. 270.

(31) W. Reeve, R. J. Bianchi and J. R. McKee, *J. Org. Chem.*, 40, 339 (1975).

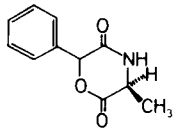
(32) *Note.* The structure of a product isolated from hydrogen bromide elimination was erronously reported (1) as the nine-membered lactone *A*. A reconsideration of all spectral data revealed the incorrectness of the proposed structure. Of other possible



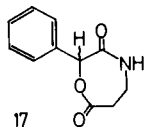
A



B



C



17

structures, that of *B* was found to best fit the spectral and elemental analytical data. Reinforcing this interpretation, cyclization of the model compound **15** did not give a trace of the corresponding oxazine-dione *C*, however, while the 7-membered homolog **17** could be obtained from **16** in only 10% yield. Therefore, we hesitate to offer any conclusion as to the structures of the hydrogen bromide elimination products of **2**. We are indebted to Drs. J. H. C. Nayler, Beecham Pharmaceuticals, Betchworth, Surrey, England, and N. G. Steinberg, Merck Sharp and Dohme Research Laboratories, Rahway, New Jersey, for their comments and suggestions regarding this point, expressed after our communication cited under reference 1.

(33) K. W. Glombitza, *Ann. Chem.*, 673, 166 (1974).

(34) T. Yamana, A. Tsuji and Y. Mizukami, *Chem. Pharm. Bull*, 22, 1186 (1974).

(35) S. Morimoto, H. Nomura, T. Ishiguro, T. Fugono and K. Maeda, *J. Med. Chem.*, 15, 1105 (1972).

(36) S. Morimoto, H. Nomura, T. Fugono, T. Axama, I. Minami, M. Hori and T. Masuda, *ibid.*, 15, 1108 (1972).

(37) E. Ivashkiv in, "Analytical Profiles of Drug Substances," K. Florey, Ed., Academic Press, New York-London, 1973, pp. 2-61.

(38) *Note.* After this work was finished we become aware of two French patents (39,40) discolsing the preparation of ampicillin and pivampicillin. The use of ammonia, hexamine *as a source of ammonia*, or hydroxylamine have been claimed for replacement of a halogen by an amino group, and a keto group by the ketoxime group, respectively. To the best of our knowledge, all reagents cited decompose the β-lactame ring under the reaction conditions claimed.

(39) French Patent 2,211,009; *Chem. Abstr.*, 82, 57682w (1975).

(40) French Patent 2,211,010; *Chem. Abstr.*, 82, 57677y (1975).

(41) A. McKenzie and R. Smith, *J. Chem. Soc.*, 125, 1588 (1924).

(42) A. I. Vogel, "Practical Organic Chemistry," 3rd ed., Longmans, London, 1957, p. 507.

(43) L. H. Sternbach, R. I. Fryer, W. Metlesics, E. Reeder, G. Sach and A. Stempel, *J. Org. Chem.*, 27, 3788 (1962).