TABLE III

DATA ON 4-ALKYLCYCLOHEXANECARBOXYLIC ACIDS AND ETHYL ESTERS

Configuration	Alkyl	Compd	Mp or bp (mm), °C	$n^{25}D$	d^{25}_4	Formula	Calcd, % C	Calcd, % H	Found, % C	Found, % H
cis	Ethyl	Ester	227 (760)	1.4438	0.9358	$C_{11}H_{20}O_2$	71.69	10.94	71.83	10.84
trans	Ethyl	Ester	231 (760)	1.4413	0.9267		71.69	10.94	71.70	10.88
cis	Ethyl	Acid	127 (5)	1.4619		$C_9H_{16}O_2$	69.19	10.32	69.40	10.07
trans	Ethyl	Acid	49–49.8				69.19	10.32	68.90	10.18
cis	Isopropyl	Ester	243 (760)	1.4472	0.9345	$C_{12}H_{22}O_2$	72.68	11.18	72.56	11.43
trans	Isopropyl	Ester	245 (760)	1.4457	0.9263		72.68	11.18	72.62	11.08
cis	Isopropyl	Acid	41–42			$C_{10}H_{18}O_2$	70.55	10.66	70.53	10.69

Ethyl *trans*-4-Ethylcyclohexanecarboxylate.—A 2.8-g sample of the *trans* acid was esterified by dissolving in 25 ml of absolute ethanol containing 0.5 ml of concentrated sulfuric acid and heating the solution under reflux for 18 hr. The cooled solution was poured into water, and the product was extracted with ether. The ether extracts were washed with dilute sodium bicarbonate solution and water, and the solvent was evaporated. Distillation of the residue gave 2.44 g (74%) of the ester.

The corresponding series of 4-isopropylcyclohexanecarboxylic acids and esters were prepared similarly, and all the compounds were isomerically pure to the extent of at least 99%. The data are given in Table III.

The 4-methyl and 4-*t*-butyl compounds and the 4-*trans*-isopropylcyclohexanecarboxylic acid had properties in agreement with those reported in the literature.[16]

Equilibration Studies.—Temperatures were measured with calibrated thermometers or with a Leeds and Northrup potentiometer with an iron–constantan thermocouple, and are accurate to 1°. The absolute ethanol used in all equilibrations was used as obtained from the Commercial Solvents Corp.

For each temperature, equilibrium was attained starting from both the *cis* and *trans* esters. The time required to ensure that equilibrium was reached was determined by preliminary experiments. A typical run follows.

In 8 × 200 mm Pyrex combustion tubes were placed 0.25-ml samples of ester, one of which was 80% *cis*, the other 10% *cis*. To each tube was added 5.0 ml of alcohol containing 0.028 g of sodium. The tubes were flushed with nitrogen, sealed, and encased in metal cylinders, and were then placed in an electrically heated furnace thermostated at 366 ± 1°K. The samples were heated for 24 hr and were then quenched by removing the metal cylinders and plunging them into ice and water. The ester was isolated by extraction with 25 ml of ether. The ether was washed with ether-saturated sodium bicarbonate solution and dried over sodium sulfate. After removal of the ether by distillation through a 20-cm Vigreux column, the residue, a pale yellow liquid, was immediately analyzed at least three times by vapor phase chromatography on a Tide column. Analysis of *cis* and *trans* equilibration samples of a given compound were done consecutively.

The column used was a 7-mm Pyrex U, 190 cm in length, supported in a vertical heating jacket, and packed with 40–60 mesh Tide (Procter and Gamble). The column was operated at temperatures ranging from 170° for ethyl 4-*t*-butylcyclohexanecarboxylate to 110° for ethyl 4-methylcyclohexanecarboxylate.

The *cis* isomer was first eluted with each compound and had a retention time of 30–45 min. The isomers were all cleanly separated under the conditions described.

Equilibrium constants were calculated from the ratio of band areas which were calculated from peak-height, half-width measurements. The method of analysis was calibrated with samples of known composition, and it was found that the *trans* to *cis* ratio was consistently 0.97 of that observed. The observed values were correspondingly corrected.

(16) Subsequent to the completion of the present investigation, the 4-isopropylcyclohexanecarboxylic acids (having identical properties with those obtained herein) were reported by H. Van Bekkum, A. A. B. Kleis, D. Medema, P. E. Verkade, and B. M. Wepster, *Rec. Trav. Chim.*, **81**, 833 (1962).

The Preparation and Structure of Hetacillin

G. A. HARDCASTLE, JR., D. A. JOHNSON,[1] C. A. PANETTA,

Chemical Development Division, Bristol Laboratories, Division of Bristol-Myers Company, Syracuse 1, New York

A. I. SCOTT, AND S. A. SUTHERLAND

Chemistry Department University of British Columbia, Vancouver, British Columbia, Canada

Received September 27, 1965

Hetacillin (2), a derivative of penicillanic acid with a unique structure, was prepared by the reaction of 6-[D-(−)-α-aminophenylacetamido]penicillanic acid (1) with acetone. The structure of hetacillin methyl ester hydrobromide was determined unequivocally by X-ray diffraction analysis.

We wish to report on hetacillin (2), a unique derivative of 6-aminopenicillanic acid in which the 6-amino group is part of an imidazolidinone ring. This compound can be prepared by the reaction of acetone with 6-[D-(−)-α-aminophenylacetamido]penicillanic acid (1), the amphoteric and clinically important semisynthetic penicillin known generically as ampicillin.

Hetacillin, which is the generic name for 6-(2,2-dimethyl-5-oxo-4-phenyl-1-imidazolidinyl)penicillanic acid, can be conveniently prepared from the reaction mixture of 6-aminopenicillanic acid with D-(−)-α-aminophenylacetyl chloride hydrochloride in aqueous acetone and is best isolated by crystallization from an aqueous medium at pH 2–3. It exhibits properties expected for an amino acid, forming both a crystalline potassium and a crystalline hydrochloride salt. It is soluble in neutral or strongly acidic aqueous solutions and sparingly soluble at pH 2–3. Its isoelectric point of 2.5 is somewhat lower than that of ampicillin (4.7), indicating reduced basicity of the side chain. Its stability toward acid is superior to that of ampicillin. Thus, for dilute aqueous solutions at pH 1 and 25°, the half-life of hetacillin was found to be 27–42 hr *vs.* 10–11 hr for ampicillin as measured by bioassay.

(1) To whom correspondence should be addressed.

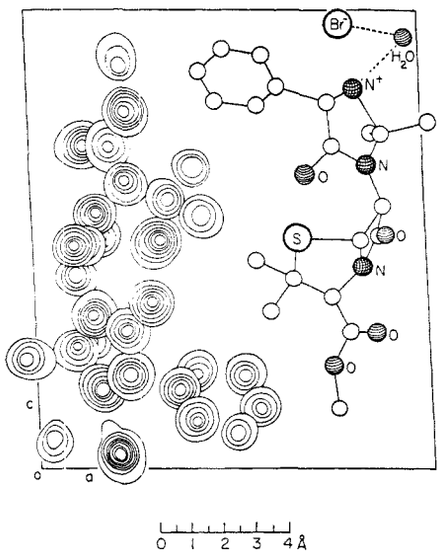


Figure 1.—Three-dimensional electron-density distribution shown by sections near atomic centers. Contours at intervals of 1 electron/A^3 except for S, 2e/A^3, and Br, 5e/A^3.

Of the three structures (**2**, **3**, **4**, Chart I) considered for hetacillin, the Schiff base **3** was rejected because hetacillin did not exhibit the expected lability to aqueous acid,[2] it failed to give a positive test for acetone with 2,4-dinitrophenylhydrazine reagent,[3] its infrared spectrum showed no amide II band,[4] and it failed to add hydrogen under conditions known to be effective for reducible penicillins.

The reduced basicity of hetacillin along with the absence of bands in its infrared spectrum typical of a monosubstituted amide indicated that both the amino and amide groups of ampicillin were involved in the condensation with acetone as illustrated by either the imidazolidinyl **2** or the oxazolidinylidene **4** structures. Davis and Levy[5] have described unsubstituted analogs in the sequence shown in Chart II.

CHART I

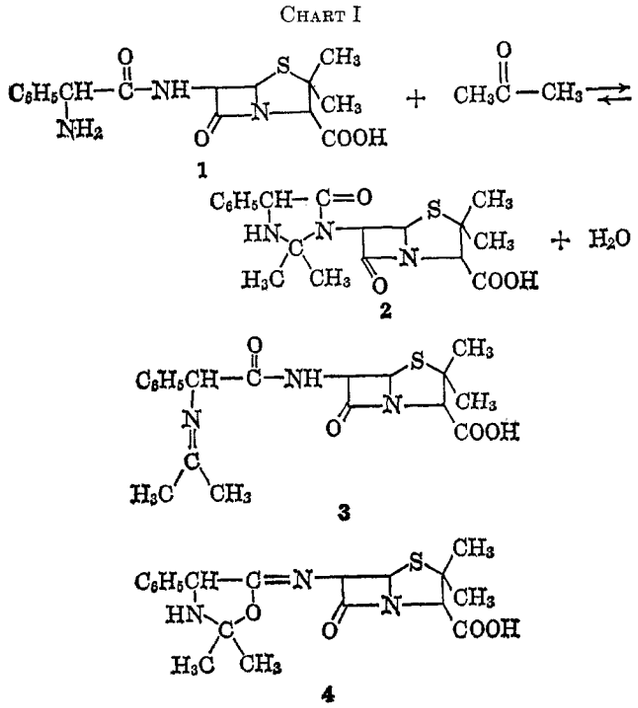
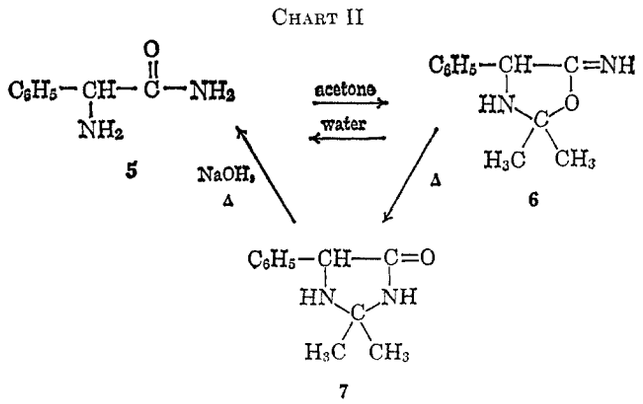


CHART II



α-Amino-α-phenylacetamide (**5**) reacted with acetone in basic solution to form 5-imino-2,2-dimethyl-4-phenyloxazolidine (**6**) which was easily hydrolyzed with cold water back to the amide and which gave an immediate precipitate with 2,4-dinitrophenylhydrazine reagent. On heating in dry pyridine, the oxazolidine **6** rearranged to 2,2-dimethyl-5-phenyl-4-imidazolidinone (**7**). The latter compound was hydrolyzed only on heating in alkali and gave no qualitative test for acetone. 4-Imidazolidinones are known to be quite stable in aqueous acid solution.[6] Thus, hetacillin is similar to the oxazolidine in its ease of formation, but more like the imidazolidinone in its conditions for hydrolysis. Attempts to prepare an isomerization product of hetacillin using conditions reported for preparing the imidazolidinone **7** were unsuccessful.

At this point it was decided that, since a choice between the oxazolidinylidene **4** and imidazolidinyl **2** structures based on the usual physical measurements, chemical behavior, and comparison with model compounds would be difficult and probably uncertain, X-ray diffraction analysis should be attempted. Some difficulty was encountered in incorporating a heavy atom into the hetacillin structure, but the hydrobromide salt of the methyl ester was finally obtained in nicely crystalline form and was used for the X-ray analysis. The results of this analysis showed clearly that the side-chain ring system and the thiazolidine-β-lactam are joined by a single bond without any intervening atoms as shown in Figure 1. Hetacillin is therefore assigned the imidazolidinylpenicillanic acid structure **2**.

Experimental Section[7]

D-(−)-α-Aminophenylacetyl Chloride Hydrochloride.—Hydrogen chloride was passed into a suspension of 100.0 g (0.66 mole) of D-(−)-α-aminophenylacetic acid in 2.0 l. of dry methylene chloride at 5–10° for 20 min. Phosphorus pentachloride, 200.0 g (0.96 mole), was added and the mixture was stirred at 0–10° for 2 hr. The acid chloride hydrochloride was collected by fil-

(2) T. W. J. Taylor and W. Baker, "Sidgwick's The Organic Chemistry of Nitrogen," Oxford University Press, London, 1937, p 65.

(3) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," 4th ed, John Wiley and Sons, Inc., New York, N. Y., 1956, pp 111, 112.

(4) (a) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," 2nd ed, John Wiley and Sons, Inc., New York, N. Y., 1958, pp 205, 217–220; (b) H. W. Thompson, R. R. Brattain, H. M. Randall, and R. S. Rasmussen, "The Chemistry of Penicillin," H. T. Clarke, J. R. Johnson, and R. Robinson, Ed., Princeton University Press, Princeton, N. J., 1949, p 388.

(5) A. C. Davis and A. L. Levy, *J. Chem. Soc.*, 3479 (1951).

(6) U. Zehavi and D. Ben-Ishai, *J. Org. Chem.*, **26**, 1097 (1961).

(7) All melting points are corrected. Microanalyses were by Mr. R. M. Downing, and the infrared and molecular weight measurements were by Mr. D. F. Whitehead.

tration, washed with 700 ml of dry methylene chloride, and dried. The yield was 124.0 g (91%).

Hetacillin (2). A. From D-(−)-α-Aminophenylacetyl Chloride Hydrochloride and 6-Aminopenicillanic Acid.—To a cold solution of 15.0 g (0.069 mole) of 6-aminopenicillanic acid in 200 ml of water at pH 7.0–7.5 was added 800 ml of cold acetone. While agitating the resulting solution vigorously at 0–10°, the pH was quickly adjusted to 2.5–3.0 with 6 *N* hydrochloric acid and the incremental addition of 18.2 g (0.088 mole) of D-(−)-α-aminophenylacetyl chloride hydrochloride was started immediately. The pH was maintained between 2.5 and 3.0 by the addition of 10% aqueous sodium hydroxide solution, and the temperature was held at 0–10°. Agitation was continued for 20 min after the addition with the pH and temperature at the same levels. The pH was then adjusted to 7.5 with triethylamine, and the solution was stored at 5–10° for 20 hr. It was extracted with 1 l. of methyl isobutyl ketone. The methyl isobutyl ketone solution was washed with 200 ml of cold water, and the aqueous solutions were combined and passed through a Dicalite precoated funnel. Methyl isobutyl ketone, 200 ml, was added to the aqueous solution and the pH was adjusted to 2.5–3.0 with 6 *N* hydrochloric acid. Rectangular plates separated, and the mixture was stirred in an ice bath for 3 hr. The crystals were collected by filtration, washed with cold water and methyl isobutyl ketone, and dried at 40°: 13.4 g (50%); mp 182.8–183.9° dec; γ_{max}^{KBr} 3240 (NH), 2920, 3060 (CH), 1795 (β-lactam carbonyl), 1725 (γ-lactam carbonyl), 1705 (sh, carboxyl carbonyl), 1405, and 1355 cm^{-1} (*gem*-dimethyls). This compound gave no precipitate when treated with acidic 2,4-dinitrophenylhydrazine reagent.[3] The isoelectric point, determined by electrophoresis, was 2.5; its specific rotation was $[\alpha]^{25}D$ +366° (*c* 1, pyridine).

Anal. Calcd for $C_{19}H_{23}N_3O_4S$: C, 58.60; H, 5.94; N, 10.79; S, 8.23; mol wt, 389.46. Found: C, 58.50; H, 6.02; N, 10.50; S, 8.05; mol wt (in pyridine), 389, 393

Hetacillin was insoluble in most organic solvents and water; it was soluble in very dilute aqueous sodium hydroxide solution (pH 7–8), N,N-dimethylformamide, dimethyl sulfoxide, and pyridine. It was also soluble in methanol but decomposed in this solvent.

Hetacillin can easily be distinguished from ampicillin by chromatography on silica gel coated plates (Camag D-5, 0.25 mm thick). Three microliters of a 1% solution in N,N-dimethylformamide was applied to the plate which was developed in 95% acetone–5% glacial acetic acid. Spraying with a 0.5% aqueous potassium permanganate solution immediately gave yellow zones on a purple background (approximate R_f values: hetacillin, 0.65; ampicillin, 0.15).

B. From Ampicillin.—To a slurry of 10 g (0.027 mole) of ampicillin trihydrate in 50 ml of acetone was added 7.0 ml of triethylamine. After stirring for 20 hr at 25°, the solution was clarified by filtration through a pad of filter aid and added slowly to 50 ml of water maintained at 0–10° and at pH 2.5–3.0 by simultaneous addition of dilute sulfuric acid. The slurry was stirred for 3–4 hr at pH 2.5–3.0 and then filtered to collect crystalline hetacillin which was washed with 20 ml of cold water and dried at 40–50°. The yield was 5.0 g (52%).

Hetacillin Potassium Salt.—Hetacillin, 100.0 g (0.257 mole), was dissolved in 1 l. of acetone and 40.0 ml of triethylamine, and the resulting solution was passed through a Dicalite precoated funnel. The filtrate was treated with 250 ml of a 30% solution of potassium 2-ethylhexanoate in acetone, and crystals soon separated. The mixture was stirred for 4 hr and then filtered, and the crystals were washed with 500 ml of dry acetone and dried at 40°: 95.0 g (86.5%); γ_{max}^{KBr} 1760 (β-lactam carbonyl), 1695 (γ-lactam carbonyl), 1620, 1610, and 1380 cm^{-1} (carboxylate group).

Anal. Calcd for $C_{19}H_{22}KN_3O_4S$: C, 53.40; H, 5.19; K, 9.15; N, 9.83. Found: C, 53.40; H, 5.20; K, 8.90; N, 9.60.

Hetacillin Hydrochloride.—Hetacillin, 20.0 g (0.051 mole), was dissolved in 400 ml of 1 *N* hydrochloric acid, and the resulting solution was stored at 5° for 16 hr. During this time large irregularly shaped crystals separated. They were collected by filtration, washed with acetone, and air dried: 12.7 g (58%); γ_{max}^{KBr} 3480, 3380 (NH), 2950 (CH), 2740, 2580, 2410 (NH_2^+), 1775 (β-lactam carbonyl), 1715 (γ-lactam carbonyl), 1700 (carboxyl carbonyl), and 1400 cm^{-1} (*gem*-dimethyls).

Anal. Calcd for $C_{19}H_{24}ClN_3O_4S$: C, 53.80; H, 5.70; Cl, 8.13; N, 9.89. Found: C, 53.80; H, 5.78; Cl, 8.25; N, 10.08.

Unchanged hetacillin was recovered from the aqueous filtrate when the pH was adjusted to 2.5.

Hetacillin Methyl Ester.—Diazomethane was prepared by the dropwise addition of 6.0 ml of 50% aqueous potassium hydroxide solution to a cold mixture of 3.1 g (0.03 mole) of N-nitroso-N-methylurea[8] and 60 ml of ether while the temperature was maintained at 5–10°. The resultant yellow ethereal solution was decanted into a suspension of 3.9 g (0.01 mole) of hetacillin, 160 ml of ether, and 40 ml of methanol (in later experiments, tetrahydrofuran replaced these solvents) while the temperature was held below 10°. The hetacillin completely dissolved and much gas was evolved. Addition was discontinued when the yellow color of diazomethane persisted. Most of this color was destroyed by addition of a few drops of glacial acetic acid. The solution was dried over anhydrous sodium sulfate and the filtrate was distilled under reduced pressure until the solvent was removed. The residue, a yellow glass, weighed 3.7 g. It was crystallized and then recrystallized several times from carbon tetrachloride and Skellysolve B (bp 60–70°) in order to obtain material suitable for analysis. The white crystals finally obtained melted at 101.5–102.0°: γ_{max}^{KBr} 3400 (NH), 3000 (CH), 1785 (β-lactam carbonyl), 1760 (ester carbonyl), 1710 (γ-lactam carbonyl), 1380 (*gem*-dimethyls), and 1215 cm^{-1} (ester C–O–C).

Anal. Calcd for $C_{20}H_{25}N_3O_4S$: C, 59.54; H, 6.25; N, 10.42; S, 7.92; mol wt, 403.4. Found: C, 59.00; H, 6.03; N, 10.47; S, 8.05; mol wt (in 95% ethanol), 402, 395.

The methyl ester was soluble in most organic solvents. Its approximate R_f value was 0.30 in benzene–ethyl acetate (20:80) system when 3.0 μl of a 1% solution in acetone was applied to a silica gel coated plate.

Hetacillin Methyl Ester Hydrobromide Monohydrate.—Hydrobromic acid (47%), 11.3 ml (0.095 mole of HBr) was added in one portion to a solution of 35.5 g (0.088 mole) of hetacillin methyl ester in 800 ml of methyl isobutyl ketone while the temperature was held at 0°. An oil separated which quickly crystallized. The mixture was stirred 15 min in an ice bath and filtered. The crystals were washed with methyl isobutyl ketone and ether and dried in a 42° vacuum oven: weight, 39.6 g (89.6%). It was homogeneous according to a thin layer chromatogram (same R_f value as that of the methyl ester free base).

Five grams of the above crystalline solid was dissolved in 72 ml of acetone and 8 ml of water and the resulting pale yellow solution was diluted with 120 ml of ether and cooled to −12°. After 1.5 hr, the crystals which separated were collected by filtration, washed with ether, and air dried: 1.40 g; γ_{max}^{KBr} 2700, 2500 (NH_2^+), 1780 (β-lactam carbonyl), 1755 (ester carbonyl), 1740 (γ-lactam carbonyl), 1590 (NH_2^+), 1385 (*gem*-dimethyls), and 1220 cm^{-1} (ester (C–O–C).

Anal. Calcd for $C_{20}H_{26}BrN_3O_4S \cdot H_2O$: C, 47.85; H, 5.63; Br, 15.91; N, 8.34; H_2O, 3.59. Found: C, 47.59; H, 5.70; Br, 15.78; N, 8.25; H_2O (by Karl Fischer), 3.4.

The methyl ester hydrobromide monohydrate was soluble in acetone or water, slightly soluble in methyl isobutyl ketone, and insoluble in ether or Skellysolve B (bp 60–70°).

X-Ray Crystal Structure Analysis.[9]—The crystal of hetacillin methyl ester hydrobromide monohydrate chosen for analysis was a clear, well-formed needle (0.1 × 0.3 × 1.5 mm). The unit cell is monoclinic with dimensions a = 12.37, b = 6.43, and c = 14.67 A, β = 92.6°. The space group is P_{2_1} with two molecules of $C_{20}H_{26}BrN_3O_4S \cdot H_2O$ in the cell. Three dimensional X-ray intensity data were recorded photographically with a Nonius equiinclination Weissenberg camera. The 2006 observed reflections were estimated visually.

The coordinates of the bromine atom were determined from a Patterson synthesis, and the false mirror plane was eliminated by the choice of coordinates for one sulfur atom from the first three-dimensional electron-density map. Coordinates of the other atoms were determined from further electron-density calculations. The molecule was found to have the structure outlined in Figure 1. This corresponds to structure **2** for hetacillin. A least-squares refinement of the coordinates with isotropic Debye temperature factors lowered the discrepancy to 14%. The calculations were performed on an IBM 7040 computer.[10]

A full account of the X-ray investigation will be published separately.

(8) F. Arndt, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p 461.

(9) Performed by A. I. Scott and S. A. Sutherland at the University of British Columbia.

(10) Programs were prepared by Dr. J. Trotter, University of British Columbia.