and the first 15 ml of distillate was collected and extracted with ether, the solvent was removed from the ether extract under reduced (150 mm) pressure, and the residue was distilled twice (Kugelrohr), yield 1.15 g, $[\alpha]^{26}D$ −73.4° (*c* 2.7, acetonitrile). This material was in all respects (except rotation) identical with that obtained above. The ORD was measured on the material with $[\alpha]D$ −63.6°, *i.e.*, of optical purity 87–95% (as determined from the rotation of the starting material and of 1 purified by means of the complex).

A-Nor-2-thiacholestane (2).—By duplicating literature procedures,[19] cholesterol was converted into 2,3-secocholestane-2,3-dioic acid (mp 194–196°, lit.[19b] mp 196–197°) *via* cholestan-3β-ol. A modified Hunsdiecker reaction[20] was used to prepare **1,4-seco-1,4-dibromocholestane** from the acid. To 4.34 g (10 mmoles) of the dioic acid, dissolved in 200 ml of carbon tetrachloride at reflux temperature, was added red mercuric oxide (3.24 g, 15 mmoles) while stirring vigorously. The reaction mixture was shielded from light by wrapping the system in aluminum foil, and bromine (3.2 g, 20 mmoles) was added dropwise. After 1 hr, the reaction flask was allowed to cool and the dark mixture was filtered; the filtrate was concentrated under reduced pressure; and the petroleum ether (bp 30–60°) soluble portion of the concentrate was filtered through a short column of silica gel. The crude, oily product (1.7 g, 33% yield), homogeneous to tlc, could be crystallized from methanol, mp 91–92°, $[\alpha]^{27}D$ −1.5 ± 0.3° (*c* 0.65, chloroform). The material exhibited end absorption only in the ultraviolet spectrum: $\epsilon_{200}^{\text{isooctane}}$ 650; $\epsilon_{190}^{\text{acetonitrile}}$ 1650.

Anal. Calcd for $C_{25}H_{44}Br_2$: Br, 31.68; mol wt, 502, 504, 506. Found: Br, 31.14; mol wt (mass spectrum),[21] 502, 504, 506.

The dibromide was dissolved in 100 ml of refluxing ethanol. To this solution was added a tenfold excess of sodium sulfide nonahydrate dissolved in a minimal amount of water. Heating was continued for 24 hr; after this time, tlc (ligroin as developer) indicated complete conversion of the dibromide to product. The solvent was removed by distillation, and the residue was diluted with ether and water. In the usual work-up, a 70% yield of **A-nor-2-thiacholestane** (2) was obtained, mp 101–102°, $[\alpha]^{23}D$ +61° (*c* 1.04, chloroform), after recrystallization from methanol or acetonitrile.

Anal. Calcd for $C_{25}H_{44}S$: C, 79.71; H, 11.77; S, 8.51; mol wt, 376, 378. Found: C, 80.11; H, 11.75; S, 8.22; mol wt (mass spectrum),[21] 376, 378.

(19) (a) E. B. Hershberg, E. Oliveto, M. Rubin, H. Staeudle, and L. Kuhlen, *J. Am. Chem. Soc.*, **73**, 1144 (1951); (b) C. W. Shoppee and J. C. P. Sly, *J. Chem. Soc.*, 345 (1959).

(20) S. J. Cristol and W. C. Firth, Jr., *J. Org. Chem.*, **26**, 280 (1961).

(21) We thank Dr. D. A. Lightner of the University of California at Los Angeles for this measurement made with an AEI MS-9 mass spectrometer. The spectrum was determined with the direct inlet system and 70-ev ionizing energy.

Chemistry of Cephalosporin Antibiotics. IX. Synthesis of Cephaloridine[1]

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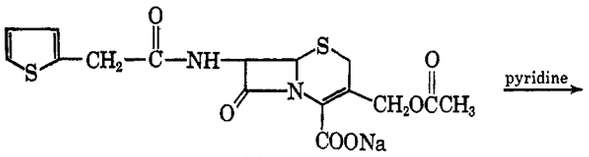
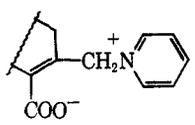
Displacement of the acetoxy group in cephalothin[2] by pyridine results in the formation of cephaloridine. This is analogous to the conversion of cephalosporin C to cephalosporin C_A (pyridine) as reported by Hale, Newton, and Abraham.[3] Initial laboratory evaluations[4,5] indicated this product to be a potentially useful, broad-spectrum antibiotic. These findings have been substantiated by further laboratory and clinical investigations.[6]



cephalothin



cephaloridine

The kinetics of this reaction have been studied by Taylor,[7] and on the basis of these data he proposed a mechanism involving a carbonium ion. We wish to report that addition of certain salts to the reaction solution enhanced the conversion of cephalothin to cephaloridine. In the absence of added inorganic salts, this conversion was only 20–25% because of primary decomposition of cephalothin and secondary reactions of the product, but was increased to 75–80% by addition of potassium thiocyanate or potassium iodide. The yield enhancement was directly proportional to the amount of salt added. In addition to increasing the yield of cephaloridine, these same salts were found to increase the solubility of cephaloridine in water. This made the isolation of cephaloridine more difficult and the removal of these salts mandatory. Exchange of the thiocyanate or iodide for another anion such as acetate which has less effect on the solubility of cephaloridine was accomplished by an anion-exchange resin. This had the added advantage of removing unreacted cephalothin and many of the decomposition products. Direct crystallization of cephaloridine was effected by chilling the solution following ion exchange. This procedure was limited to reaction mixtures containing relatively small amounts of conversion-promoting salts since relatively large amounts (greater than 5 moles/mole of cephalothin) of these adjuvants required impractically large quantities of resin. In addition, the solutions became saturated with inorganic acetates which, in turn, crystallized with cephaloridine.

We found that cephaloridine formed salts with hydrothiocyanic acid and with hydriodic acid which are sparingly soluble in water. This discovery permitted maximum utilization of the beneficial effect of added thiocyanate or iodide. Acidification of such reaction mixtures gave a facile separation of cephaloridine conjugate acids which were optimally converted to the betaine using an ion-exchange resin. Over-all yields of cephaloridine by this method were twice those previously attained.

The complete role of the added ions in this reaction is not yet clear. We have found that salts such as potassium thiocyanate stabilize cephaloridine under

(1) Cephaloridine is the generic name given to 7-[α-(2-thiophene)acetamido]-3-(1-pyridylmethyl)-3-cephem-4-carboxylic acid betaine. The trade name of cephaloridine is KEFLORDIN (cephaloridine, Lilly).

(2) R. R. Chauvette, E. H. Flynn, B. G. Jackson, E. R. Lavagnino, R. B. Morin, R. A. Mueller, R. P. Pioch, R. W. Roeske, C. W. Ryan, J. L. Spencer, and E. Van Heyningen, *J. Am. Chem. Soc.*, **84**, 3401 (1962); cephalothin is the generic name for the sodium salt of 7-[α-(2-thiophene)acetamido]-cephalosporanic acid. The trade name of cephalothin is KEFLIN (sodium cephalothin, Lilly).

(3) C. W. Hale, G. G. F. Newton, and E. P. Abraham, *Biochem. J.*, **79**, 403 (1961).

(4) P. W. Muggleton, C. H. O'Callaghan, and W. K. Stevens, *Brit. Med. J.*, **2**, 1234 (1964).

(5) W. E. Wick and W. S. Boniece, *Appl. Microbiol.*, **13**, 248 (1965).

(6) G. L. Hobby, Ed., "Antimicrobial Agents and Chemotherapy—1965," American Society for Microbiology, Ann Arbor, Mich., 1965, pp 879–937.

(7) A. B. Taylor, *J. Chem. Soc.*, 7020 (1965).

the reaction conditions but have little effect on the stability of cephalothin. Other factors such as stabilization of the intermediate carbonium ion[7] or change in the polar character of the solvent may be relevant. The proposed mechanism[7] precludes displacement of the acetoxy group by the added ion and subsequent displacement by pyridine.

We have established the generality of the findings described in this paper by applying them to the synthesis of substituted pyridinium derivatives of cephalothin as well as to several other 7-acylaminocephalosporanic acids. The products of this study and their antibacterial activity will be reported.[8]

Experimental Section

Cephaloridine (without Isolation of Salt).—A solution of 200 g (0.46 mole) of cephalothin,[2] 100 g (1.04 moles) of potassium thiocyanate, and 100 ml (1.25 moles) of pyridine in 500 ml of water was adjusted to pH 6.5 with 85% phosphoric acid and was heated with stirring at 60° for 6 hr. After cooling to room temperature, the solution was extracted with 25% Amberlite LA-1 (acetate form)[9] in methyl isobutyl ketone (MIBK) (six 1-l. portions) and washed with MIBK (500 ml). The aqueous solution was allowed to stand overnight in the cold (5°). The product which separated weighed 41 g (20%); $\lambda_{max}^{H_2O}$ 238 mμ (ε 15,200) and 251 mμ (ε 13,950). A sample was block dried at 100° for analysis.

Anal. Calcd for $C_{19}H_{17}N_3O_4S_2$: C, 54.92; H, 4.12; N, 10.11; S, 15.43. Found: C, 54.65; H, 4.36; N, 10.06; S, 14.70.

Cephaloridine Hydrothiocyanate.—A solution of 5.0 g (0.012 mole) of cephaloridine and 2.5 g (0.026 mole) of potassium thiocyanate in 100 ml of water was adjusted to pH 2.0 with 10% hydrochloric acid. The resulting salt weighed 5.2 g (91%); $\lambda_{max}^{H_2O}$ 236 mμ (ε 15,900) and 255 mμ (ε 14,300).

Anal. Calcd for $C_{19}H_{17}N_3O_4S_2 \cdot HSCN$: C, 50.61; H, 3.82; N, 11.81; S, 20.27; SCN^-, 12.3. Found: C, 50.87; H, 4.10; N, 11.42; S, 19.82; SCN^-, 12.9.

This same salt was obtained directly from cephalothin by the following procedure. A solution of 200 g (0.46 mole) of cephalothin, 908 g (9.5 moles) of potassium thiocyanate, 50 ml (0.75 mole) of pyridine, and 10 ml of 85% phosphoric acid in 200 ml of water (pH 6.5) was heated at 60° for 5 hr with stirring.

The reaction mixture was cooled to room temperature and diluted to 4 l. with water. It was extracted with chloroform (five 200-ml portions) and dissolved chloroform was removed under reduced pressure. After cooling to 0°, the aqueous layer was acidified to pH 2.0 by dropwise addition of 6 *N* hydrochloric acid with stirring. Maintaining the mixture at 0° for 3 hr with stirring afforded 163 g (75%) of product.

A portion of this salt from the reaction mixture was converted to cephaloridine in the following manner. A 25-g sample was slurried with 50 ml of water and 150 ml of 25% Amberlite LA-1 (basic form) in MIBK for 30 min. The resulting aqueous solution was extracted with 25% Amberlite LA-1 (acetate form) in MIBK (three 50-ml portions) and with MIBK (50 ml). After stirring at 5° for 1 hr, the betaine, 11.5 g (52%), was collected. This material was identical with that obtained without isolation of the salt.

Cephaloridine Hydriodide.—A solution of 10.0 g (0.024 mole) of cephaloridine and 5.0 g (0.03 mole) of potassium iodide in 200 ml of water was cooled to 5° and 10% hydrochloric acid was added dropwise until no further precipitate formed. The yield of product was 10.0 g (76%); $\lambda_{max}^{H_2O}$ 237 mμ (ε 24,350) and 255 mμ (ε 13,850).

Anal. Calcd for $C_{19}H_{17}N_3O_4S_2 \cdot HI$: C, 41.99; H, 3.34; I, 23.36; N, 7.73; S, 11.80. Found: C, 41.87; H, 3.98; I, 23.63; N, 7.46; S, 11.62.

This hydriodide salt (168 g, 65%) was obtained from 200 g (0.46 mole) of cephalothin, 300 g (1.80 moles) of potassium iodide, 50 ml (0.75 mole) of pyridine, 5 ml of 85% phosphoric acid, and 200 ml of water by reaction and work-up analogous to that of the hydrothiocyanate salt. A sample was converted to cephaloridine in 45% yield *via* the same method used on the hydrothiocyanate salt.

(8) J. L. Spencer, F. Y. Siu, E. H. Flynn, B. G. Jackson, M. V. Sigal, H. M. Higgins, R. R. Chauvette, S. L. Andrews, and D. E. Block, *Antimicrobial Agents Chemotherapy*, in press.

(9) Amberlite LA-1 is a high molecular weight, water-insoluble, liquid secondary amine, commercially available from Rohm and Haas Co. The acetate form used in this investigation was prepared as follows. To 1.0 l. of Amberlite LA-1 and 3.0 l. of methyl isobutyl ketone was added 120 ml of glacial acetic acid and the solution was stirred for 5 min. After stirring with 800 ml of water for 25 min, the organic layer was separated for use.

The Use of Benzene in Separating Aromatic Methoxyl Bands in Nuclear Magnetic Resonance Spectroscopy

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The effect of benzene in causing upfield shifts of various peaks in nmr spectra is well known[1] and has been explained as being due to its disk shape and the diamagnetism of regions above and below the plane of the ring,[2] but comparatively little use appears to have been made of the marked variation in diamagnetic shift experienced by methoxyl groups attached to aromatic rings. The technique appears to be particularly valuable in disentangling magnetically nonequivalent methoxyl peaks from one another. Thus, 1,2,3-trimethoxybenzene (1, Figure 1) is a singlet in deuteriochloroform but in benzene (1B, Figure 1), the 1- and 3-methoxyl groups are shifted upfield much more markedly than the 2-methoxyl,[3] presumably owing to steric inhibition of solvation of the latter.

The same effect is seen in 1-bromo-2,3,4-trimethoxybenzene (2 and 2B, Figure 1)[4] where only the 4-methoxyl is not seriously hindered; therefore, this might be assigned to the band at δ 2.70 (ν 60 Mc). However, this assignment must be treated with caution since the absolute magnitude of the shift is nearly twice that of the former case.

Some form of steric hindrance associated with one of the methoxyl groups is necessary in order for the effect to be seen. For example, the methoxyl peaks of 2,3-dimethoxybenzaldehyde (3 and 3B, Figure 1) separate considerably on solvent change while the unhindered methoxyl groups of the isomeric 3,4-dimethoxybenzaldehyde (4 and 4B, Figure 1) do not.

Examples 5–9 illustrate the same effect. Methyl reserpate in deuteriochloroform (8, Figure 1) shows a coincidence of three methoxyl groups at δ 3.92, two methoxyl groups at δ 3.82, and one methoxyl (presumably the aliphatic methoxyl) at δ 3.50. As expected from 1,2,3-trimethoxybenzene, the central methoxyl of the trimethylgallic acid ring is exposed at δ 3.83 in benzene while the two flanking methoxyl groups add to the A-ring methoxyl, presumably at δ 3.71. The ester and aliphatic methoxyl groups are now exposed at higher fields. Irrespective of the assignments, the

(1) For leading references, see K. Mislow, M. A. W. Glass, H. B. Hopps, E. Simon, and G. H. Wahl, Jr., *J. Am. Chem. Soc.*, **86**, 1718 (1964), footnote 38. See also D. H. Williams and N. S. Bhacca, *Tetrahedron*, **21**, 2021 (1965), regarding benzene shifts in the steroid series.

(2) J. R. Zimmerman and M. R. Foster, *J. Phys. Chem.*, **61**, 282 (1957).

(3) These assignments are made on the basis of symmetry.

(4) Traces of impurities are now visible at δ 2.88 and 3.25.