dihydroisoquinoline in 40 ml. of dry cymene was added 4.0 g. of 5% palladium on deaerated charcoal.[24] The reaction mixture was then refluxed vigorously for 4 hr., filtered while hot, and the residue was washed thoroughly with chloroform. After combining the filtrates, 300 ml. of ether was added and the solution was extracted twice with 50-ml. portions of 20% hydrochloric acid.[25] The chilled acidic solution was neutralized cautiously with 40% sodium hydroxide solution and the product was extracted with two 100-ml. portions of ether. The ethereal extract was dried over potassium hydroxide and evaporated to yield the substituted isoquinoline. This material was then recrystallized or converted to a salt.

B.—A mixture of 0.5 g. of the crude substituted 3,4-dihydroisoquinoline and 0.5 g. of 5% palladium on charcoal[24] was placed under vacuum (0.1–0.3 mm.) and heated slowly to 150°. A temperature of 150–210° was maintained for 3 hr. The reaction mixture was cooled to 25° and extracted with ethanol. Concentration of the ethanolic solution yielded the crude substituted isoquinoline.

(24) R. Mozingo in "Organic Syntheses," Coll. Vol. III, E. C. Horning, Ed., John Wiley and Sons, Inc., New York, N. Y., 1955, p. 685.

(25) The substituted isoquinoline hydrochlorides were found to be very soluble in benzene-type solvents and it was necessary to dilute with ether before effective extraction with hydrochloric acid could be obtained.

Isolation of Cephalosporin C

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We wish to report a convenient procedure for the isolation of cephalosporin C,[1] which in turn may serve as a source of 7-aminocephalosporanic acid for the exploration of potentially useful semisynthetic cephalosporins.

Experimental

Cephalosporin C was measured by a cup agar diffusion assay *vs. Salmonella gallinarum* grown on pH 6 nutrient agar[2] medium containing penicillinase[3] (1250 μ/ml.) to destroy penicillin N.

A selected strain of *Cephalosporium acremonium* CMI 49,137 was grown at 28° in a 300-l. stainless steel fermentor with agitation and aeration on a medium composed of corn starch, 3%; soybean meal, 1%; calcium carbonate, 0.75%; DL-methionine, 0.1%; potassium chloride, 0.05%; and magnesium sulfate, 0.025%.

Following 3 days of fermentation, the culture mash was filtered. Activated carbon (3%) was added to the filtrate (275 l.) which was brought to pH 2.5 with hydrochloric acid. After stirring for 30 min., the carbon was filtered with the aid of diatomaceous earth and washed with water. The filter cake was stirred for 30 min. in 100 l. of acetone–water (3:2) maintained at pH 4.5 with ammonium hydroxide. The solids were removed by filtration and the eluate was concentrated to 60 l. under reduced pressure. The concentrate was adjusted to pH 2.5 with Dowex-50 (H^+) and stored at 4° overnight to destroy penicillin N, coproduced in the fermentation. After raising the pH to 4.5 with ammonium hydroxide, the solution was percolated through 6 kg. of moist IRA-401 ($HCOO^-$) (20 to 50 mesh) in a 15.3-cm. (i.d.) column (flow rate 20 l./hr.). The resin column was rinsed with water and developed with 30 l. of 0.2 *M* ammonium formate (flow rate 8 l./hr.). The eluate fractions containing cephalosporin C were combined and diluted with 1.5 vol. of acetone.

Aluminum oxide (2 kg., chromatographic grade, Merck) was equilibrated with aqueous formic acid pH 6, washed with acetone, and air-dried. This was packed in a 7-cm. (i.d.) column. The aqueous acetone solution was applied to the column (flow rate 12 l./hr.) followed by an acetone–water (3:2) wash. The column was developed with 0.05 *M* ammonium formate (flow rate 7 l./hr.) and the portion of the eluate containing cephalosporin C was lyophilized. The residue in water (250 mg./ml.) was chilled and 2 vol. of ethanol was slowly added. Cephalosporin C ammonium salt crystallized at 4° overnight and was recystallized from water–ethanol; $[\alpha]^{25}D$ +104° (*c* 2.2, water); λ_{max} 260 mμ (ϵ_{max} 9300, water).

Anal. Calcd. for $C_{16}H_{24}N_4O_8S \cdot H_2O$: C, 42.66; H, 5.77; N, 12.45; S, 7.11. Found: C, 42.82; H, 5.96; N, 12.11; S, 7.01.

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(1) (a) G. G. F. Newton and E. P. Abraham, *Biochem. J.*, **62**, 651 (1956); (b) E. P. Abraham and G. G. F. Newton, *ibid.*, **79**, 377 (1961).

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(3) Baltimore Biological Laboratory, Baltimore, Md.

Book Reviews

Vitamins and Coenzymes. By ARTHUR F. WAGNER and KARL FOLKERS, Merck Sharp and Dohme Research Laboratories. Interscience Publishers (John Wiley and Sons, Inc.), New York, N. Y. 1964. xvi + 532 pp. 23.5 × 16 cm. $17.50.

Although several annual progress-series are available in the field of vitamins and similar biocatalysts, few books have attempted to cover all of the vitamins and their activated forms in a compact volume. The last of these books (by Rosenberger) had been written over 10 years ago, and some of the more complex observations in this field have been made since then. Another large monograph has dealt with the nutritional aspects of the vitamins only. In 1960, Folkers and Wagner contributed a massive chapter on vitamins to "Medicinal Chemistry" (A. Burger, Ed., Interscience Publishers), and this chapter has now been expanded to the present volume.

The authors are organic chemists with a deep understanding of biochemistry, and this background colors their presentation. Methods of isolation, structural proof, and synthesis are reviewed broadly, leading from historical introductions up to the latest chemical data. However, biochemical theories and relations to coenzymes form a solid background to the chemical work, and nutritional and clinical applications are summarized clearly, concisely, and in depth. The complicated stereochemistry of some of the vitamins is presented masterfully as may be expected from an author such as Karl Folkers who has been a leading figure in this field for decades.

This book promises to become the "bible" on vitamins and their biochemical derivatives, and to remain so for years to come. Its publication at this moment is particularly timely because many—though by no means all—major problems of vitamin chemistry have been worked over extensively, and there seems to be a temporary hiatus in the dynamic progress of this field. By summarizing critically what has been done, and pointing out what needs to be done, the authors may well catalyze a revival of intensive activity in the remaining structural and biochemical problems of the vitamin field and in the largely untouched area of selective vitamin antagonists.

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