was dried over sodium hydroxide and distilled under reduced pressure.

All of the fluoroisoquinolines and 1-hydroxyisoquinoline were prepared in this Laboratory[7] and were redistilled until the absorption spectra of successive distillates were identical.

Acknowledgment.—This work is part of a study of the preparation and properties of heterocyclic fluorine compounds being carried out in this laboratory, and was supported in part by the Office of Naval Research, Contract No. N8onr-69900.

(7) A. Roe and C. E. Teague, THIS JOURNAL, **73**, 687 (1951).

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Sterols of Algae. III.[1] The Occurrence of Ergosterol in *Chlorella pyranoidosa*[2]

BY MARYLIN KLOSTY AND WERNER BERGMANN

It was reported in the first communication of this series,[3] that chondrillasterol is the principal sterol of the green alga, *Scenedesmus obliquus*. This sterol is of special interest because it is a $\Delta^{7,22}$-diene-3-ol and therefore potentially suitable for conversion into cortisone by methods now being investigated in several laboratories. The amount of chondrillasterol obtainable from *Scenedesmus*, however, appears too small to be of practical significance at this time. The studies on algae sterols have now been extended to other primitive algae of the class of *Chlorophyceae*, and in particular to those, whose commercial cultivation is contemplated by several organizations.

Through the courtesy of the Lederle Laboratories Division[4] the authors obtained several pounds of freeze-dried cells of a pure culture of the green alga, *Chlorella pyranoidosa*. Upon acetone extraction, the algae yielded 12.5% of lipoid material, of which 10% was unsaponifiable. The sterol content of the unsaponifiable fraction was approximately 20%, corresponding to 0.15–0.2% of the dry alga. A more efficient extraction was achieved when the cells were first triturated with warm glacial acetic acid and then exhaustively extracted with acetone. Cells so treated yielded a lipoid and sterol fraction corresponding, respectively, to 20 and 0.4%. The sterols were isolated from the unsaponifiable fraction either by precipitation with digitonine, by direct crystallization or better by way of their benzoates. The high negative rotation and the ultraviolet absorption spectra of the crude fractions indicated the presence of $\Delta^{5,7}$-sterols in excess of 75% of the mixture. Repeated recrystallizations of the benzoates eventually afforded ergosteryl benzoate, m.p. 169°; $[\alpha]^{25}D$ −72°. It was converted to ergosterol, m. p. 164°; $[\alpha]^{25}D$ −128°, and ergosteryl acetate, m.p. 176°; $[\alpha]^{25}D$ −88°.

Chlorella pyranoidosa appears to be the first organism other than fungi and lichens in which ergosterol has been shown to be the principal sterol.[5] As a minor component, 0.1–5%, ergosterol has been found in the sterol mixtures from cocksfoot,[6] cottonseed oil,[7] scopolia root oil[7] and wheat germ oil.[8] More substantial amounts of ergosterol have been found in the sterol mixtures from certain animals,[9] in particular invertebrates.[10]

Experimental

The following extraction procedure was found to be the most efficient. Fifty grams of freeze-dried cells of *Chlorella pyranoidosa*, which contained about 10% of moisture, was heated for one hour at 70° with 100 ml. of glacial acetic acid. The acid was then removed by freeze-drying, and the residue ground, and extracted in a Soxhlet apparatus with acetone for 24 hours. The extract was filtered to remove some amorphous, gray solid (1.5 g.), and the solvent was removed first by distillation and finally by freeze-drying. The extract thus obtained, 10 g., was saponified under nitrogen with 45 g. of a 20% solution of potassium hydroxide in 80% ethanol. After 24 hours 150 ml. of water was added, and the solution extracted seven times with 100-ml. portions of peroxide-free ether. The combined ether layers were washed with water and concentrated under nitrogen. After freeze-drying, the residue weighed 1.5 g. The sterol content of the residue, as determined by the digitonide method, was 13.4%, corresponding to 0.4% of the algae.

The unsaponifiable fraction obtained from several hundred grams of algae was dissolved in a minimum amount of boiling methanol. Upon cooling a waxy, crystalline material was obtained in a yield of 23%. It was dissolved in anhydrous pyridine and treated with an excess of benzoyl chloride for 24 hours at room temperature. The mixture was then poured into methanol, and the precipitate, m.p. 144–156°, recrystallized from ether–methanol; yield 11% of unsaponifiable fraction; m.p. 156–162°; $[\alpha]^{25}D$ −58. Several recrystallizations from dioxane–methanol and ethyl acetate afforded ergosteryl benzoate, m.p. 169°; $[\alpha]^{25}D$ −72° in chloroform. The ultraviolet absorption spectra indicated a purity in excess of 95%.

Anal. Calcd. for $C_{35}H_{44}O_2$: C, 84.00; H, 9.60. Found: C, 83.75; H, 9.93.

The benzoate was refluxed for one hour with a 3% solution of potassium hydroxide in ethanol in an atmosphere of nitrogen. The solution was then diluted with water, and the precipitated ergosterol was recrystallized several times from acetone and ethyl acetate, m.p. 162°; $[\alpha]^{25}D$ −128° (chloroform). Acetylation by reflux with acetic anhydride afforded ergosteryl acetate, m.p. 176°; $[\alpha]^{25}D$ −92° (chloroform). None of the products gave depressions of melting points when mixed with authentic material.

(1) Paper II, THIS JOURNAL, **73**, 2395 (1951).

(2) This investigation was supported by a research grant from the National Institute of Health, Public Health Service.

(3) W. Bergmann and R. J. Feeney, *J. Org. Chem.*, **15**, 812 (1950).

(4) American Cyanamid Co., Pearl River, New York.

(5) The statement made in Elsevier's "Encyclopaedia of Organic Chemistry," Vol. **14**, 69 (1940), that ergosterol is present in the brown alga, *Fucus crispus*, is in error. It is based on a brief note by Gérard (*Compt. rend.*, **126**, 909 (1898)) which states that the sterol of *Fucus* gives color reactions reminiscent of those shown by sterols from cryptogams. Since then it has been shown that fucosterol is the principal sterol of this alga.

(6) A. Pollard, *Biochem. J.*, **30**, 382 (1936).

(7) A. Windaus and F. Bock, *Z. physiol. Chem.*, **250**, 258 (1937).

(8) A. Windaus and F. Bock, *ibid.*, **256**, 47 (1938).

(9) A. Windaus and O. Stange, *ibid.*, **244**, 218 (1936)

(10) F. Bock and F. Wetter, *ibid.*, **256**, 33 (1938).

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[FROM THE CHEMICAL LABORATORY OF THE ACADEMY OF COMMERCE IN VIENNA]

Bromination of Resorcinol Monomethyl Ether and Debromination of Tribromoresorcinol Monomethyl Ether

BY MORITZ KOHN

The bromination of resorcinol monomethyl ether with two molecules of bromine yields a crys-