to be a general property of all steroid compounds having a β hydroxyl at C_3 and a double bond at C_5–C_6.

Recently we have succeeded in preparing the epimer of *i*-chloesterol, which we shall now designate as *epi-i*-cholesterol by the reduction of *i*-cholestane-6-one with lithium aluminum hydride. This compound melts at 80.5–81.5° and has a specific rotation, $[\alpha]^{24}D$ +80.9, *c*, 1.83 ($CHCl_3$) (*Anal.* Calcd. for $C_{27}H_{46}O$: C, 83.85; H, 11.92. Found: C, 84.08; H, 11.90). Our experimental results show that on *i*-cholesterol is formed in the reduction. Chromic acid oxidation of *epi-i*-cholesterol in the cold gives *i*-cholestanone (m. p. 96.5–97.5°), $[\alpha]^{22}D$ +44.9, *c*, 0.7 ($CHCl_3$), showing no depression in melting point when admixed with an authentic sample of *i*-cholestan-6-one.

The result of the acid rearrangement of *epi-i*-cholesterol supports the concept of a unimolecular mechanism as developed in this Laboratory and elsewhere.[4] The rearrangement in acetic acid solution in the presence of sulfuric acid followed by alkaline hydrolysis of the acetate and separation by digitonin yields cholesterol in 97% yield. No *epi*-cholesterol is produced.

Further investigation of the properties of this new and highly interesting compound is being continued and the results will be reported at a later date.

(4) Winstein and Adams, THIS JOURNAL, **70**, 838 (1948); Hafey, Halsey and Wallis, *Science*, **110**, 474 (1949); see also C. W. Shoppee, *J. Chem. Soc.*, **149**, 1147 (1946).

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BIOCYTIN, A NATURALLY-OCCURRING COMPLEX OF BIOTIN

Sir:

The biotin content of natural products has been studied by Wright and Skeggs[1] employing *Lactobacillus casei* and *Lactobacillus arabinosus* as assay organisms. In the case of some soluble natural products, especially those originating from the autolysis of actively metabolizing material such as, for example, yeast extract, the results with *Lactobacillus casei* were considerably higher than were those obtained with *Lactobacillus arabinosus*. After acid hydrolysis the results obtained with *Lactobacillus casei* were unchanged but the results obtained with *Lactobacillus arabinosus* then equalled those obtained with *Lactobacillus casei*. The existence of a naturally-occurring complex of biotin is thus indicated. The term biocytin (Gr. Κύτος, cell) has been used to designate this biologically active compound, which appears to contain biotin as a moiety.

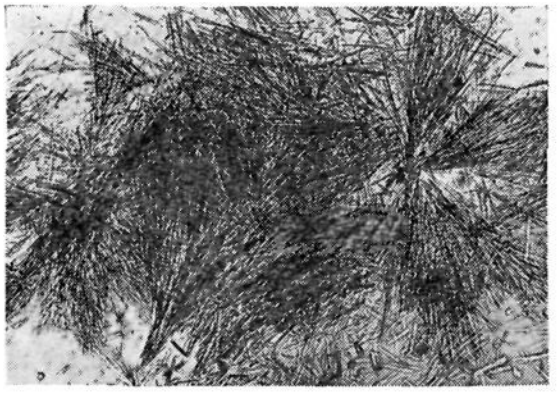

Fig. 1.—Crystalline biocytin.

Biocytin is available as a source of biotin to *Lactobacillus delbrückii* LD_5, *Lactobacillus acidophilus*, *Streptococcus fecalis R*, *Neurospora crassa*, and *Saccharomyces carlsbergensis* in addition to *Lactobacillus casei*. Biocytin is unavailable prior to hydrolysis to *Lactobacillus pentosus*, and *Leuconostoc mesenteroides P-60* as well as *Lactobacillus arabinosus*. Digestion with pepsin, trypsin, papain, takadiastase, mylase or polidase does not affect the availability of biocytin for *Lactobacillus arabinosus*. Biocytin is heat stable, avidin combinable, and readily dialyzable. When acid is employed as a means of hydrolyzing biocytin to biotin or its microbiological equivalent for *Lactobacillus arabinosus*, mineral acid of at least 3 *N* at 120° for one hour is essential for anything approaching quantitative hydrolysis.

A process for the purification of biocytin from yeast extract that depends largely on adsorption and elution techniques, solvent partition, and counter-current distribution has been developed. The extracts derived from over ten tons of yeast have been processed in this research on biocytin purification. We were successful in isolating a few milligrams of crystalline material. Difficulties were then encountered in obtaining more crystalline material, and when attempts were made to recrystallize the available material contaminating gummy impurities were hard to remove and resulted in low yields of recrystallized material. The substance itself does not crystallize readily. Now we have obtained 1.5 mg. of recrystallized material (Fig. 1) which melts at 230–240° (dec.). This material on hydrolysis yields 40 ± 4% of biotin by microbiological assay, in addition to exhibiting microbiological properties characteristic of biocytin. Countercurrent distributions of highly purified material show the presence of only one biologically active substance. Thus, the crystalline material appears to be biocytin. When a larger amount of crystalline material becomes available, attempts will be made to complete the chemical and biological characterization of the compound.

(1) Wright and Skeggs, *Proc. Soc. Expt. Biol. Med.*, **56**, 95 (1944).

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