one step from the sulphonyl chloride to the final product instead of the several steps required by the old Edinger's method.

Best results were afforded when a 20% excess of lithium aluminum hydride was used. More or less hydride gave lower yields. The decomposition of the reduction complex and the excess lithium aluminum hydride with a calculated amount of water and 20% sodium hydroxide (2) (or 15% sodium hydroxide (5)) is not advised in the synthesis of 8-mercaptoquinoline, because it gives poor yields due to the formation of the corresponding sodium salt of the product. It is worth noting that no 8-mercaptoquinoline could be isolated by following the suggestion (1) to employ a 0.5 : 1 mole ratio for hydride to sulphonyl chloride.

EXPERIMENTAL

Quinoline-8-sulphonyl Chloride

A mixture of quinoline-8-sulphonic acid (20 g) and phosphorus pentachloride (25 g) was ground together for 5 minutes, and heated under reflux for 3 hours in an oil bath at 130° C. At the end of this period the contents of the flask became brown and almost all liquefied. After cooling, the crude product was poured onto crushed ice (180 g), neutralized with sodium bicarbonate, and extracted with ether. The ether extract was decolorized with charcoal (2 g) and filtered. After evaporation of the ether, the colorless crystalline sulphonyl chloride (21.1 g, 97%) was dried *in vacuo*. It melted at 118–122° (lit. 122° (4), 128.5–129° (3)).

8-Mercaptoquinoline

A solution of 0.55 g of lithium aluminum hydride in 250 ml of anhydrous ether was added, over a period of 1½ hours, to a solution of 1.83 g of quinoline-8-sulphonyl chloride and 600 ml of anhydrous ether in a 1-liter three-necked round-bottomed flask, fitted with a motor-driven stirrer and a condenser. Ice water was circulated through the condenser. The yellow intermediate was decomposed by the addition of 3 ml of water. Inorganic material was removed by filtration from the yellow ether solution. After the removal, by evaporation, of the solvent, some bluish red residue appeared. 8-Mercaptoquinoline dihydrate, shiny red needles (m.p. 58–59°; lit. 58–59° (4), 58.5° (6)), was obtained by dissolving the residue in dilute sodium hydroxide solution, and then neutralizing the mixture by adding dry ice. Anhydrous 8-mercaptoquinoline, a non-viscous blue liquid, was obtained by drying the red product in a nitrogen atmosphere over sodium hydroxide pellets. The yield was 0.78 g (61%).

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STUDIES ON PIGMENTS OF PENICILLIUM FUNICULOSUM
I. PRODUCTION OF CHOLESTEROL[1]

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During investigations on pigments from an aberrant strain (PRL 1724) of *Penicillium funiculosum* Thom, the authors found that petroleum ether extracts of culture material

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showed strong sterol color reactions. The material responsible for these reactions was isolated by column chromatography and was found to be a white crystalline substance (white plates from acetone), m.p. 148–149° C. This melting point was not depressed by admixture with cholesterol. The substance sublimed and was readily soluble in ether, acetone, chloroform, benzene, ethyl acetate, petroleum ether, and hot alcohol, and was slightly soluble in water and cold alcohol. It was optically active, $[\alpha]_D^{20}$ +39.8° (*c*, 2.123 in chloroform). Its color reactions in a series of seven tests were the same as those obtained with authentic cholesterol.

From the results of elementary analysis and of the osmometric method for molecular weight determination, it was confirmed that the molecular formula corresponded to $C_{27}H_{46}O$.

The substance gave a positive precipitation reaction with digitonin (8). The infrared spectrum in KBr and the ultraviolet spectrum (9) in ethanol were identical with those of cholesterol.

The substance formed the following derivatives: a monomethyl ether, $C_{28}H_{48}O$, m.p. 84° C, $[\alpha]_D^{20}$ −46.6° (*c*, 1.356 in chloroform); a monoacetate, $C_{29}H_{48}O_2$, m.p. 115° C, $[\alpha]_D^{20}$ −47.3° (*c*, 1.985 in chloroform); a benzoate, $C_{34}H_{50}O_2$, m.p. 146° and 180° C, $[\alpha]_D^{20}$ −13.9° (*c*, 1.562 in chloroform); a dibromide (8, 10, 11), $C_{27}H_{46}OBr_2$, m.p. 113° C, $[\alpha]_D^{20}$ −44.6° (*c*, 1.568 in chloroform).

All of the above properties of the substance and its derivatives are the same as those of cholesterol and its derivatives; therefore there seems to be no doubt that the substance is cholesterol.

There have been reports on the isolation of fungal sterols (12). The most abundant in microorganisms is ergosterol, with yields depending both on the species and on the conditions of cultivation (13). Among species of *Penicillium* (14) only traces of ergosterol could be found in mycelium grown in submerged culture, while the felts from surface cultures contained over 1% of the steroid. However, there has been no report on the isolation of cholesterol from fungal metabolic products. Indeed, among plants, only certain of the marine Rhodophyceae have been reported to produce this substance (15). It is very interesting, then, to find that *P. funiculosum* (PRL 1724) is capable of producing cholesterol.

EXPERIMENTAL

Organisms

Penicillium funiculosum Thom (PRL 1724) was used throughout this work. Its identity was confirmed by Miss Dorothy Fennel, who considered it to be an aberrant strain of *P. funiculosum*. Its culture and characteristics are being described elsewhere.

Cultural Methods for Sterol Production

The liquid medium used for shake-flask culture contained glucose 30 g, Difco Bacto-peptone 5 g, distilled water 1000 ml.

The medium was dispensed into 250-ml Erlenmeyer flasks in the amount of 25 ml per flask. The flasks containing the medium were plugged with cotton and autoclaved at 15 lb pressure for 15 minutes. Liquid cultures were grown for approximately 7 days at 24° C on a rotary shaker with a 1/2-in. radius of motion, operating at about 240 r.p.m.

Inoculum

Inoculum for shake-flask cultures was usually prepared from 7-day shake-flask cultures which had been originally inoculated with a suspension of a potato dextrose agar culture blended in distilled water.

Recovery of Sterol from Mycelium

When the wine-red pigmentation of the cultures visually appeared to have reached a maximum, the cultures were bulked and freeze-dried. The resulting solids were extracted with petroleum ether (b.p. 35–60° C) for 48 hours in Soxhlet apparatus. A pale yellow "oil" was recovered from the extract by removal of the petroleum ether by evaporation.

Column Chromatography

A slurry of aluminum oxide (30 g Brockman Grade 1) in benzene was used to prepare a column 15 cm long and 2.2 cm in diameter. The sample (1 g) of pale yellow "oil" was dissolved in a minimum amount of benzene and added to the column. The column was eluted, first with benzene and then with benzene – ethyl acetate (4:1). A white crystalline substance (24 mg) was left after evaporation of the solvent from the benzene – ethyl acetate eluates. This substance was recrystallized repeatedly from acetone to give white plates, m.p. 149° C, $[\alpha]_D^{20}$ +39.8 (*c*, 2.123 in chloroform). Found: C, 83.96; H, 12.01; OMe, 0. Calculated for $C_{27}H_{46}O$: C, 83.87; H, 11.99.

Characteristics of Crystalline Material

Solubility.—The substance sublimed and was readily soluble in ether, acetone, chloroform, benzene, ethyl acetate, petroleum ether, and hot alcohol and was slightly soluble in water and cold alcohol.

Molecular weight determination by osmometric method.—Found: 391, 385. Calculated for $C_{27}H_{46}O$: 386.44.

Precipitation reactions with digitonin (8).—One hundred milligrams of digitonin was dissolved in 10 ml of 90% alcohol. A small amount of the crystalline metabolic product was dissolved in alcohol and when added to digitonin alcoholic solution immediately gave a perceptible precipitate.

Color reactions.—The color reactions of the substance obtained from *P. funiculosum* and of authentic cholesterol were positive for the Liebermann (1), Liebermann–Burchard (2), Salkowski (3), Lifschütz (4), and Tshugajeff (5) reactions but negative for the Rosenheim (6) and Tortelli–Jaffe (7) reactions.

Derivatives

Monomethyl ether.—The substance (100 mg) was refluxed with dimethyl sulphate (2 ml) and 5% sodium hydroxide solution (15 ml) for 3 hours. The solution was cooled, neutralized, and extracted with ether. Purification by recrystallization from acetone gave white crystals (66 mg), m.p. 84° C, $[\alpha]_D^{20}$ −46.6° (*c*, 1.356 in chloroform). Found: C, 83.95; H, 12.1. Calculated for $C_{28}H_{48}O$: C, 83.93; H, 12.08.

Monoacetate.—The substance (200 mg) taken in freshly distilled acetic anhydride (5 ml) containing anhydrous sodium acetate (500 mg) was refluxed for 4 hours. The contents were poured onto broken ice, and white crystals separated. Purification by recrystallization from acetone gave white needles (142 mg), m.p. 115° C, $[\alpha]_D^{20}$ −47.3° (*c*, 1.985 in chloroform). Found: C, 81.28; H, 11.30. Calculated for $C_{29}H_{48}O_2$: C, 81.25; H, 11.29.

Benzoate.—The substance (100 mg) was dissolved in anhydrous pyridine (3 ml), and benzoyl chloride (500 mg) was added. The mixture was refluxed for 1 hour and poured onto broken ice. The precipitate was extracted with ether, washed with 5% sodium carbonate solution, and purified by recrystallization from 95% ethanol to give white prisms (52.5 mg), m.p. 146° C (the melting becomes clear at about 180° C), $[\alpha]_D^{20}$ −13.9° (*c*, 1.562 in chloroform). Found: C, 83.18; H, 10.3. Calculated for $C_{34}H_{50}O_2$: C, 83.21; H, 10.27.

Dibromide (8, 10, 11).—The substance (200 mg) in 5 ml of ether was gently warmed and 4 ml of a solution of bromine and sodium acetate in acetic acid added. The dibromide began to crystallize out in a few minutes. The crystalline paste was cooled in an ice bath and stirred with a rod for about 10 minutes to ensure complete crystallization. The crystals were collected on a small suction funnel and washed with an ice-cold ether – acetic acid (3:7) solution to remove the yellow mother liquor. The crystals were finally washed with a little methanol, and dried (111 mg), m.p. 113° C, $[\alpha]_D^{20}$ −44.6° (*c*, 1.568 in chloroform). Found: C, 59.42; H, 8.3; Br. 29.8. Calculated for $C_{27}H_{46}O_2Br_2$: C, 59.36; H, 8.40; Br, 29.28.

Each of these derivatives was prepared from authentic cholesterol with similar results.

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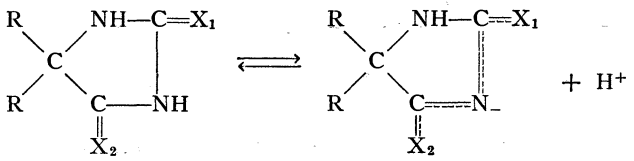
THIOHYDANTOINS
V. IONIZATION OF 2,4-DITHIOHYDANTOINS AS ACIDS

J. T. EDWARD AND O. J. CHIN

Thioamides are more acidic than amides (1) because the thiocarbonyl group is more polarized than the carbonyl group (2). Similarly, 2-thiohydantoin (I; R = H, X_1 = S, X_2 = O) (pK_a ~ 8.5 (3)) is more acidic than hydantoin (I; R = H, X_1 = X_2 = O) (pK_a 9.12 (4)), and so 2,4-dithiohydantoin (I; R = H, X_1 = X_2 = S) would be expected to be more acidic yet, with a pK_a of about 8. We have confirmed this expectation by determining the dissociation constant of the readily available 5,5-dimethyl-2,4-dithiohydantoin (I; R = Me, X_1 = X_2 = S); this should be only very slightly weaker as an acid than 2,4-dithiohydantoin (3).



The absorption spectrum of 5,5-dimethyl-2,4-dithiohydantoin in weakly acidic aqueous solution shows a strong peak at 296 mμ (ϵ 27,600). A peak in this region has been found characteristic of 2,4-dithiohydantoins generally (5). In solutions more alkaline than pH 10 the peak has shifted to 318 mμ (Fig. 1A). In buffer solutions of pH between 5 and 10

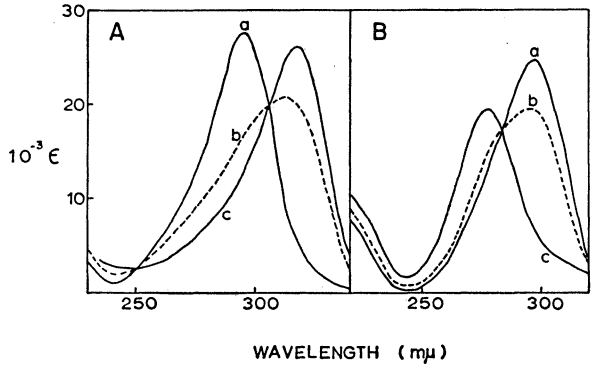


FIG. 1. Ultraviolet absorption of (A) 5,5-dimethyl-2,4-dithiohydantoin at pH (*a*) 2.05, (*b*) 8.00, (*c*) 10.00. (B) 3-methyl-5,5-pentamethylene-2,4-dithiohydantoin at pH (*a*) 6.5, (*b*) 9.71, and in (*c*) 0.1 *N* sodium hydroxide.