isomer. Attention is directed to an apparent lack of correlation between geometrical isomers of the furan series and their sweet taste.

AMES, IOWA

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THE STEROLS OF ERGOT

BY MERRILL C. HART AND FREDERICK W. HEYL

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In recent years considerable literature has developed about the chemistry of the ergosterol of yeast and its accompanying sterols, though but one paper[1] on the chemistry of the ergosterol of ergot since the original work by Tanret[2] has come to our attention. In this paper Rosenheim and Webster report on the irradiation of a "fungisterol" which, however, differs widely from that described by Tanret, and at the same time differs from any of the sterols accompanying the ergosterol of yeast as described by Wieland and Asano.[3]

Having at hand a considerable quantity of the ergosterol from ergot, it seemed desirable to us (1) to repeat and confirm if possible Tanret's isolation of fungisterol, and (2) to prepare a number of derivatives of our ergosterol, some previously described and some new, with a view to establishing more rigorously, or to disestablish, the complete chemical identity of the sterol from the two sources. That differences might be expected is indicated by the experience of Heilbron, Sexton and Spring,[4] who found two different specimens of ergosterol from yeast which behaved quite differently upon hydrogenation. In this paper we present our work on the fractionation of the ergot sterols; we hope in subsequent papers to report on a number of derivatives of the ergosterol of ergot.

Experimental

Preparation of Ergosterol.—Spanish ergot was exhaustively extracted with benzene and, after recovery of the solvent, the residue boiled with an excess of 20% alcoholic potassium hydroxide. The unsaponifiable fraction was removed in the usual way, and crude ergosterol precipitated by the addition of petroleum ether (b. p. 60–80°) to the concentrated ether extract. The yield of this crude averaged 0.13% of the ergot used. When twice crystallized from ethyl alcohol, plates melting at 152–157° were obtained. Filtration of a chloroform solution of this material removed a small amount of amorphous substance and subsequent crystallization of the soluble portion raised the melting point to 160–162°. Acetylation, crystallization of the acetate from acetic acid, ethyl and methyl alcohols, and subsequent hydrolysis, failed to raise this. $[\alpha]_D$ varied from -112.2 to $-100°$ in various runs, with the yield averaging about 60% of the crude.

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[1] Rosenheim and Webster, *Biochem. J.*, **22,** 1426 (1928).

[2] Tanret, *Compt. rend.*, **108,** 98 (1889); *ibid.*, **147,** 75 (1908).

[3] Wieland and Asano, *Ann.*, **473,** 300 (1929).

[4] Heilbron, Sexton and Spring, *J. Chem. Soc.*, 926 (1929).

Crystallization from the alcohol–benzene mixture of Bills and Honeywell[5] gave in poor yield a product melting at 163–164.5° and having $[\alpha]_D$ of −123°, which is the closest we have been able to approach Tanret's values (m. p. 165°, $[\alpha]_D$ −132°) by these methods. Acetylation and hydrolysis of this preparation only served to lower the melting point to 160–162° and $[\alpha]_D$ to −112°.

Separation of Fungisterol.—The combined filtrates from the purification of about 250 g. of crude ergosterol were concentrated and fractionated from ethyl acetate. A large top fraction with $[\alpha]_D$ of −64° was discarded, and the rest refractionated twice with the results shown in Table I.

TABLE I

SEPARATION OF THE FUNGISTEROL FRACTION

Fraction no.	Weight, g.	$[\alpha]_D$	M. p., °C.
1	0.3	−20.8	130–140
2	.2	−19.1	130–135
3	.2	−20.8	125–130
4	.2	−20.8	130–132
5	.2	−2.6	116–120
6	..	....	Sirup

Each of the first four fractions when recrystallized from ethyl alcohol gave a small amount of fungisterol melting at 144–146°, which yielded an acetate melting at 156–157°. Tanret gives 144° for the sterol and 158.5° for the acetate. There was not enough material to establish the elementary analysis, but it probably contains at least two more hydrogen atoms than ergosterol, since it no longer gives the Rosenheim[6] color test with trichloro-acetic acid, which is regarded as specific for the $\Delta^{1,2}$ (or $\Delta^{1,13}$) linkage in sterols. The Liebermann–Burchard test was positive, showing that fungisterol is not completely saturated. The Salkowski reaction gave in the acid layer a yellow color which reddened with extreme slowness.

Isolation of a Third Sterol.—It was evident from Table I that there was also present a third compound of even lower rotatory power than fungisterol. Fraction 5 was acetylated, yielding an acetate melting at 121–124°. Several recrystallizations from acetic anhydride and from ethyl alcohol raised this to 122–125°.

Anal. Calcd. for $C_{27}H_{45}OCOCH_3$: CH_3CO, 10.0. Found: CH_3CO, 10.0.

The sterol recovered from this hydrolysis crystallized from alcohol in plates melting at 120–125°.

Anal. Calcd. for $C_{27}H_{46}O$: C, 83.85; H, 12.01. Found: C, 83.46; H, 11.55.

The Rosenheim color test was negative; the Liebermann–Burchard test positive; the Salkowski test gave a yellow color in the acid layer, which slowly changed to orange.

The petroleum ether filtrates from the separation of the crude ergosterol deposited on long standing considerable further crystalline material which is a mixture of the three ergot sterols. It is being worked up at present to prepare larger quantities of fungisterol and the third sterol.

Summary

1. The association of fungisterol (m. p. 144–146°, $[\alpha]_D$ −20°, acetate, m. p. 156–157°) with the ergosterol of ergot, as described by Tanret, is confirmed.

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[5] Bills and Honeywell, *J. Biol. Chem.*, **80**, 15 (1928).

[6] Rosenheim, *Biochem. J.*, **23**, 47 (1929).

2. The presence of a third sterol (m. p. 120–125°, $[\alpha]_D$ −2°, acetate, m. p. 121–124°) is established.

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SOME NEW ESTERS OF ERGOSTEROL

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In continuation of our study of the sterols of ergot, we have prepared several new esters of ergosterol with acid derivatives which have frequently been used to characterize alcohols, namely, phthalic anhydride, phenyl isocyanate, and chloro-acetyl *o*- and *p*-nitrobenzoyl chlorides. Since it was found that the action of the acid chlorides in the absence of an agent to absorb the hydrogen chloride evolved yielded derivatives of isoergosterol, it was necessary to work in pyridine solution.

Experimental

Ergosteryl Acid Phthalate.—Ergosterol (2 g.) was refluxed with phthalic anhydride (3 g.) in 5 cc. of pyridine. The resulting product appeared in the form of prisms melting at 169° after several recrystallizations from alcohol; $[\alpha]_D$ was −51°.

Anal. Calcd. for $C_{35}H_{46}O_4$: C, 79.2; H, 8.7; mol. wt., 530. Found: C, 79.1; H, 8.9; mol. wt., 532 (by titration).

Upon hydrolysis with alcoholic potassium hydroxide, this gave ergosterol identical with the starting material.

The silver salt was made by adding a slight excess of an alcoholic solution of silver nitrate to the portion neutralized in titrating for the molecular weight. It separated as white crystals melting with decomposition at 170–180°, and blackening upon standing.

Anal. Calcd. for $C_{35}H_{45}O_4Ag$: Ag, 16.9. Found: Ag, 17.1.

The copper salt was prepared from cupric acetate in an analogous manner and separated as a green amorphous precipitate.

Anal. Calcd. for $(C_{35}H_{45}O_4)_2Cu$: Cu, 7.06. Found: Cu, 7.17.

Ergosteryl Phenylurethan.—Ergosterol (1.6 g.) was refluxed with 5 cc. of phenylisocyanate and 25 cc. of benzene for three hours. The solvent and excess reagent were removed by distillation at 15 mm. and the residue was recrystallized by dissolving in hot benzene and adding alcohol to incipient precipitation. It crystallizes in short hard needles melting at 236.5–238° (corr.).

Ergosteryl *m*-Nitrobenzoate was prepared by refluxing 1 g. of ergosterol with 0.6 g. of *m*-nitrobenzoyl chloride in 5 cc. of pyridine for one hour. The reaction mixture was poured into hot alcohol and allowed to crystallize. It was purified by recrystallization from alcohol, forming tiny plates melting at 151°; $[\alpha]_D$ was −71°.

Ergosteryl *p*-Nitrobenzoate was made in a similar manner. It formed clusters of plates melting at 182°; $[\alpha]_D$ was −49.5°. Both this and the meta compound yielded ergosterol upon hydrolysis.

The *m*- and *p*-nitrobenzoates of isoergosterol were formed by melting together ergosterol and the corresponding acid chloride either in toluene or without a solvent. Upon crystallization from alcohol they melted at 172° (*m*-) and 189° (*p*-), respectively, and were both optically inactive.