

Summary

1. Pure β -ergosterol (m. p. 141° , $[\alpha]_D +21.2^\circ$) and β -ergosterol acetate (m. p. 114° , $[\alpha]_D +10.0^\circ$) have been isolated and their properties described.

2. Both chloroacetyl chloride and chloroacetic anhydride reacting on β -ergosterol give a chloroacetyl derivative consisting of a mixture of the α - and β -forms. Approximately this same mixture of α - and β -ergosterol chloroacetates is obtained by the reaction of both chloroacetyl chloride and chloroacetic anhydride on α -ergosterol.

3. α -Ergosterol acetate isomerized by the method of Mauthner consists of a mixture of α - and β -ergosterol acetate with the acetate of at least a third isomer having a higher specific rotation than either α - or β -ergosterol.

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[CONTRIBUTION FROM THE CHEMICAL RESEARCH LABORATORY, THE UPJOHN COMPANY]

THE STEROLS OF ERGOT. III. THE OCCURRENCE OF AN ISOMER OF ALPHA-DIHYDROERGOSTEROL

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Recently¹ we described the isolation of α -dihydroergosterol from the ether-petroleum ether filtrate from crude ergosterol. Not many fractions below the head fraction pure α -dihydroergosterol separated in the process of fractionation with ethyl acetate.

The subsequent fractions show a gradual lowering of the levorotation and from this series there can be repeatedly obtained magnificent plates which greatly resemble the description given for "fungisterol." We are, in fact, convinced that the "fungisterol" of Tanret ($[\alpha]_D 15.9^\circ$) represents one of these fractions.

From any of these fractions showing $[\alpha]_D -6.0^\circ$ or greater, persistent fractionation, especially of the acetate from a mixture of ether and alcohol, will yield α -dihydroergosterol.

Evidence indicates that the third sterol is optically inactive and that it is practically impossible to separate it in quantity by fractional crystallization of the sterols or of the acetates.

Our purest fraction of the sterol melted at $133-134^\circ$. The corresponding acetate, $C_{27}H_{43}OCOCH_3$, melted at $143-145^\circ$ and had $[\alpha]_D -0.5^\circ$.

It is unsaturated, giving the Liebermann-Burchard test and quantitatively absorbs two atoms of hydrogen. To color tests it responds exactly as α -dihydroergosterol, with which in fact it appears to be isomeric.

Incidentally the presence of a hydrocarbon, hentriacontane, and of an alcohol, very probably myricyl alcohol, has been observed in the unsaponifiable material from ergot fat.

¹ Heyl and Swoap, THIS JOURNAL, 52, 3688 (1930).

Neither α -dihydroergosterol nor the new sterol gives an insoluble dibromide, but bromination of the very soluble acetates yielded a minute amount of an insoluble dibromide, m. p. 173–174°. This is evidence of the presence of a fourth sterol. Slight yields of dextrorotatory fractions also indicate the existence of very small quantities of a fourth sterol. We cannot state whether or not the dextrorotatory sterol is the same as the one yielding the insoluble dibromide.

Experimental

A quantity of crystalline material (386 g.) separating from the petroleum ether filtrates from the preparation of "ergosterol" from ergot was fractionally crystallized from ethyl acetate and separated into thirty-three fractions. Fractions 6, 7 and 8 yielded pure α -dihydroergosterol; and the next seven fractions also yielded the same material but less readily. Fractions 15–22 showed great tendency to separate Tanret's fungisterol.²

CRYSTALLIZATIONS FROM FRACTION 23

Fraction	Weight, g.	[α] _D	Recrystallized	
			M. p., °C.	[α] _D
V ₆	0.6	-3.9	137-141	-2.8
V ₇	.8	-2.3	134-136	-0.7
V ₈	.6	-4.7	133-136	-1.6
V ₉	.8	-2.2	133-134	-0.0
V ₁₀	.2 (discarded)	...	124-132	

The fractionation resulted in a sharp lowering of the specific rotation between V₅ and V₆ and this is characteristically obtained in similar fractions. This was accompanied here by the separation of sharply defined sterol crystals having a homogeneous appearance. Upon recrystallization of each of these fractions separately from ether the levorotation is not increased and this is taken as proof of the absence of an excessive quantity of α -dihydroergosterol. If we accept [α]_D -20° for α -dihydroergosterol and assume that the third sterol has no optical activity, then these recrystallized fractions have in no case more than 14% of the predominant sterol. The rotations of the recrystallized fractions are given in the fifth column of the table.

Fractions V₇ and V₉ recrystallized from ether were selected as the purest material and acetylated. This acetate melted at 141–142°.

Another less pure acetate was prepared as follows. After recrystallizing V₇ from ether and obtaining the above described material ([α]_D -0.7°), a second crop was obtained showing ([α]_D -1.1°). In the same way a crop was removed from the ethereal filtrate from V₉ recrystallized. It showed ([α]_D -1.5°). These were joined to V₆ and V₈ recrystallized from ether.

Two fractions of the purest acetate (m. p. 143–145°) upon recrystallization gave [α]_D -0.5° and the subsequent crops from the less pure acetate gave in order -0.6, -1.5, -2.3, -3.3, -4.0, -4.0 and -5.0°.

Since there is no evidence of any dextrorotatory material in this fraction and since the specific rotation of the top fraction continuously approaches zero, we conclude that we have here an almost pure specimen of the third sterol. The acetate was analyzed.

Anal. Calcd. for C₂₇H₄₈OCOCH₃: C, 81.6; H, 10.87. Found: C, 82.1; H, 10.54.

² Tanret gives [α]_D -15.9° in chloroform, *Compt. rend.*, **147**, 75 (1908).

Color Tests on the New Sterol.—These resembled those previously given for α -dihydroergosterol. With the Rosenheim trichloroacetic acid reagent, a completely negative test resulted. With the Salkowski test, the chloroform layer was colorless and the sulfuric acid orange-yellow. With the Liebermann-Burchard test the color transitions were as follows: slight pink, transitory lavender, deep blue, slowly changing to green. The Heilbron and Spring modification of the Tortelli-Jaffe reaction was positive.

Reduction of the New Sterol.—Quantitative catalytic reductions by the method of Hyde and Scherp³ showed conclusively the presence of one reducible double bond when using palladium black.⁴

Anal. 19.8 mg. of acetate absorbed 0.1166 mg. of H_2 . Found: 1.2 double bond. 16.83 mg. of acetate absorbed 0.0681 mg. of H_2 . Found: 0.9 double bond.

0.12 g. of the acetate reduced at room temperature as previously described⁵ using, however, palladium black, gave a top fraction (0.07 g.) melting at 103–104°. Upon recrystallization it melted at 110° and a mixed melting point with α -ergosterol acetate was not depressed. Upon saponification with 3% methyl alcoholic potash, α -ergosterol melting at 129–131° was obtained. A mixed melting point with α -ergosterol showed no depression.

Fraction 24 failed to yield the third sterol of the purity arrived at above due to the appearance in this fraction of a fourth sterol with dextrorotatory properties. A specific rotation of +0.9° was observed in the more soluble part of the series.

Fraction 25, obtained by precipitating the ethyl acetate mother liquors with ethyl alcohol, upon repeated crystallization from large volumes of ethyl alcohol, gave a characteristic top fraction melting at 66–75° which was a mixture of myricyl alcohol (m. p. 84–88°) and hentriacontane (m. p. 65–66°).

Anal. Calcd. for $C_{31}H_{64}O$: C, 82.2; H, 14.25. Found: C, 82.1; H, 14.0.

Anal. Calcd. for $C_{31}H_{64}$: C, 85.3; H, 14.7. Found: C, 84.5; H, 14.6.

The material in the filtrate was acetylated, fractionated and treated with bromine in the usual manner, when a slight yield of a dibromide, crystallizing from alcohol in plates, was obtained (m. p. 173–174°).

Anal. Calcd. for $C_{27}H_{48}OCOCH_3Br_2$: Br, 27.25. Found: Br, 25.3.

Summary

1. The filtrates from α -dihydroergosterol yielded a third ergot sterol $C_{27}H_{48}OH$, melting at 133–134°. This sterol forms an acetate melting at 143–145° and $[\alpha]_D -0.5^\circ$.

2. The new sterol absorbs two atoms of hydrogen and corresponds to color reactions in the same manner as α -dihydroergosterol.

3. The so-called fungisterol of Tanret is a mixture of α -dihydroergosterol and this new sterol.

4. Myricyl alcohol, $C_{31}H_{64}O$, and hentriacontane, $C_{31}H_{64}$, are shown to be present in ergot fat.

5. The presence of a fourth sterol is definitely indicated by the fact that dextrorotatory fractions were separated from extremely soluble parts of the fractionation. From these an insoluble sterol dibromide was separated.

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³ Hyde and Scherp, *THIS JOURNAL*, **52**, 3359 (1930).

⁴ Willstätter and Waldschmidt-Leitz, *Ber.*, **54**, 123, 137 (1921).

⁵ Hart, Speer and Heyl, *ibid.*, **52**, 2017 (1930).