

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

QUANTITATIVE DETERMINATION OF ALPHA-DIHYDROERGOSTEROL IN ERGOSTEROL FROM ERGOT

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Callow¹ has recently showed that ergosterol from yeast contains α -dihydroergosterol. We previously showed this for ergosterol from ergot.² In the preceding paper we have found this substance to be the preponderant sterol in the filtrate from ergosterol preparations.

Previously it had been noted in this Laboratory that fine samples of α -ergosterol were regularly prepared from very impure samples of ergosterol. In fact there is no necessity for elaborate purification of ergosterol in proceeding with the preparation of α -ergosterol because, as we know, the impurity α -dihydroergosterol reduces to the same end-product.

In view of the incidental evidence which indicated that the sterols from ergot contained large proportions of α -dihydroergosterol, we have now carefully analyzed a typical ergosterol preparation and find that it contains as much as 30% of α -dihydroergosterol. A third sterol is perhaps present in minute quantity, judging by the slight tendency to impurity of the α -ergosterol prepared from it.

Experimental

A quantity of "ergosterol" from ergot was prepared in the usual manner³ and after recrystallization several times from alcohol, melted at 155–157°. The analysis of this material was carried out by first separating 100 g. fractionally into four crystalline crops and a final mother liquor.

We then determined the specific rotation of each fraction. From this value, and the assumption that we are dealing with a mixture of ergosterol for which $[\alpha] = -132^\circ$ and α -dihydroergosterol for which $[\alpha] = -20.4^\circ$, we can calculate the percentage composition.

In order to rule out as far as possible the presence of any appreciable quantity of a third sterol which would render the above calculation somewhat inexact, we reduced two samples of each fraction, in 3-g. quantities, in acetic acid with 0.3 g. of Adams' platinum oxide catalyst in the presence of 2 atmospheres of hydrogen. If the material is simply a mixture of the two sterols as assumed, we would expect a quantitative production of α -ergosterol. The quantitative procedure consisted in concentrating the filtrate from the catalyst to a small volume, saponifying with alcoholic potassium hydroxide, crystallizing from methyl alcohol and quantitatively collecting three successive crops of α -ergosterol. The third crop was separated from small volume. The purity of the α -ergosterol was proved by the melting points and the specific rotation.

Further quantitative recrystallizations of the third fractions enable us to report that the total yield of α -ergosterol in these four fractions is not less than 82, 89, 83 and 85%, respectively.

¹ Callow, *Biochem. J.*, **25**, 87 (1931).

² Heyl and Swoap, *THIS JOURNAL*, **52**, 3688 (1930).

³ Hart and Heyl, *ibid.*, **52**, 2013 (1930).

TABLE I
RESULTS OF REDUCTION OF ERGOSTEROL FRACTIONS

Ergosterol fractions			
Fraction	M. p., g.	Weight, °C.	Rotation, [α] _D
1	32	161	-123
2	30	159	-110
3	20	146-148	-96.5
4	4 ^a	123-127	-68

Ergostenol fractions									
Frac.	Ergostenol Frac. I			Ergostenol Frac. II			Ergostenol Frac. III		
	Wt., g.	M. p., °C.	Rot., [α] _D	Wt., g.	M. p., °C.	Rot., [α] _D	Wt., g.	M. p., °C.	Rot., [α] _D
1	3.75	133	+11.1	1.2	130	+ 9.7	0.45	110-120	+4.6
2	3.72	133	+10.4	0.8	130	+10.4	1.4	120-125	+9.2
3	4.12	133	+10.4	0.9	129	+ 9.02	0.55	120-125	+8.1
4	1.58	133	+10.4	1.82	132	+ 8.33	0.41	120-125	+6.9

^a 4-g. sample used for reduction.

We therefore conclude that from 100 g. of "ergosterol" from ergot, four fractions representing 86% of the original mixture are essentially mixtures of ergosterol and α -dihydroergosterol. We calculate that the top fraction is a mixture of ergosterol 92, α -dihydroergosterol 8%; the second fraction is 80 and 20%; the third is 68 and 32% and the fourth only 42% ergosterol. The total ergosterol from 86 g. is therefore 68.72 g. or about 70% of the total. By difference the α -dihydroergosterol amounts to 30% of the material.

Summary

"Ergosterol" from ergot consists of a mixture of ergosterol and α -dihydroergosterol, the latter amounting to not less than 30% of the material.

It is shown that the quantitative estimation of this mixture may be carried out polariscopically.

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