Iso-dehydroergosterol as A Component of Iso-neoergosterol, A Molecular Compound Obtained from the Pyrolysis of Ergopinacone.

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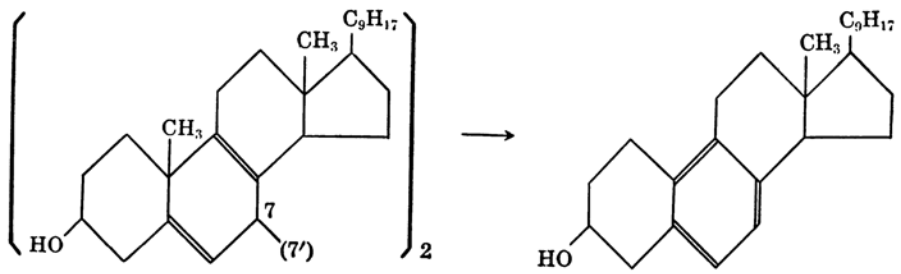
Ergosterol, $C_{28}H_{44}O$, as found by Windaus and Borgeaud,[1] undergoes dehydrogenation when its alcoholic solution containing eosin is exposed to light in the absence of oxygen. The product, $C_{56}H_{86}O_2$, has been unappropriately called ergopinacone. Ergopinacone suffers a characteristic decomposition on submitting it to distillation in vacuum or on heating it with acetic anhydride.[2] In this decomposition methane is eliminated, and neoergosterol, $C_{27}H_{40}O$, has been the only other fragment to be characterized, although Inhoffen[3] suggested that ergopinacone and its decomposition might be formulated as follows:



(I) Ergopinacone, $C_{56}H_{86}O_2$. (II) Neoergosterol, $C_{27}H_{40}O$.

$+ CH_4 + (C_{28}H_{42}O ?)$.

Some time ago Urushibara and the present author[4] obtained a new compound besides neoergosterol from the pyrolysis followed by distillation in vacuum of ergopinacone, and named it iso-neoergosterol, since it then appeared to be an isomeride of neoergosterol. Subsequently,[5] on acylation with 3,5-dinitrobenzoyl chloride and pyridine it gave equal quantities of two different dinitrobenzoates, one being neoergosteryl 3,5-dinitrobenzoate melting at 227–228° (corr.) with decomposition and the other a 3,5-dinitrobenzoate with the presumable composition $C_{35}H_{44}O_6N_2$ melting at 187.5°–189.5° (corr.) with decomposition. The latter corresponded in composition to the dinitrobenzoate of a compound with the formula $C_{28}H_{42}O$. However, the dinitrobenzoate was not identical with dehydroergosteryl dinitrobenzoate with the same composition and the same melting point. Thus the author was led to presume that iso-neoergosterol was a

(1) A. Windaus and P. Borgeaud, *Ann.*, **460** (1928), 235.
(2) H. H. Inhoffen, *Ann.*, **497** (1932), 130.
(3) H. H. Inhoffen, *Naturwissenschaften*, **25** (1937), 125.
(4) Y. Urushibara and T. Ando, this Bulletin, **11** (1936), 757.
(5) T. Ando, *ibid.*, **13** (1938), 371.

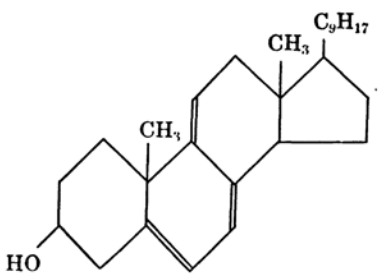
molecular compound of neoergosterol and an isomeride of dehydroergosterol. The latter component has now been isolated in free state. The author proposes the name "iso-dehydroergosterol" for this new sterol derivative, because it is most probably an isomeride of dehydroergosterol.

Careful hydrolysis of the 3,5-dinitrobenzoate melting at 187.5–189.5° with 1% alcoholic potash and recrystallization of the product from methanol gave free iso-dehydroergosterol in colourless aggregated needles with a melting point 128–129.5° (corr.) and a specific rotation $[\alpha]_D^{10°} = -102°$ in chloroform. The substance is easily soluble in organic solvents, and gives instantly an insoluble addition compound with digitonin. It is thus shown that iso-neoergosterol owes its pronounced precipitability with digitonin to this component.

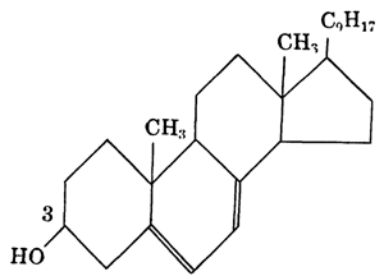
On account of great difficulty in drying completely the substance due to unstability to heat and of the hygroscopic nature of the dried substance, analyses gave no satisfactory results. However, it was possible to decide between two alternative formulas, $C_{28}H_{44}O$ and $C_{28}H_{42}O$: By taking advantage of the fact that the hydrogen content is hardly affected by the addition of water (H_2O requires H = 11.19%) to the ergosterol formula ($C_{28}H_{44}O$ requires H = 11.19%) and of a molecule or a fraction of water to the dehydroergosterol formula ($C_{28}H_{42}O$ requires H = 10.74 and $C_{28}H_{42}O+H_2O$ H = 10.75%), it could be shown that the dehydroergosterol formula is preferable to the ergosterol formula (Found: H = 10.96, 10.85%). The analyses of the dinitrobenzoate also corresponded to the dehydroergosterol formula more satisfactorily than to the ergosterol formula.[5] Lack of the material made it unable to determine the number of double bonds by any other method.

Iso-dehydroergosterol is not stable in air nor to heat, gives distinctly the colour reactions of Liebermann-Burchard and of Rosenheim, and shows sharp absorption maxima at 280, 270 mμ, and indistinct one at 252 mμ.[6]

Comparison of the free alcohols also indicates clearly that iso-dehydroergosterol is different from dehydroergosterol (III). The latter melts at 146° and gives $[\alpha]_D^{18°} = +149°$ and an absorption maximum at 320 mμ corresponding to the three conjugate double bonds.[7]



(III) Dehydroergosterol.



(IV) Ergosterol.

(6) The author thanks Dr. K. Yamasaki for taking absorption spectra.
(7) A. Windaus and O. Linsert, *Ann.*, **465** (1928), 148.

As for the constitution of iso-dehydroergosterol, its properties as described above indicates that a hydroxyl group is situated at carbon atom 3 with the same configuration as in ergosterol (IV), and further may suggest that, of the three dobule bonds in the ring system, two are conjugated probably in one ring and another is isolated in one of the other rings. However, it is hardly possible to infer the positions or the distribution of these three double bonds from the data of absorption spectra, rotatory power, and colour reactions, because there are no sufficient materials to formulate any rules for compounds with three double bonds in the ring system. Even if Inhoffen's formula (I) for ergopinacone is assumed, it would give no reference for knowing the positions of the double bonds in the product from such a destructive change as pyrolysis.

Iso-neoergosterol can be synthesized from components: When neoergosterol and iso-dehydroergosterol in equal quantities were dissolved in and crystallized from acetone, the molecular compound separated out. Recrystallization gave colourless needles melting at 138–139° (corr.) alone and in admixture with the specimen obtained from the pyrolysis of ergopinacone. The synthetic compound showed a specific rotation $[\alpha]_D^{11°} = -66.1°$ while the other $[\alpha]_D^{29°} = -59.1°$.[5] The synthetic iso-neoergosterol, as a molecular compound, showed a depression in the melting point when mixed with a small amount of either component.

The isolation of iso-dehydroergosterol, $C_{28}H_{42}O$, from the pyrolysis of ergopinacone substantiates Inhoffen's hypothetical equation for this reaction. On recrystallizing the crude product from this reaction neoergosterol crystallizes out first, and then iso-neoergosterol follows. Iso-neoergosterol is the molecular compound of neoergosterol and iso-dehydroergosterol, but cannot be separated into components by mere recrystallization. Hence, it is clear that in this reaction more neoergosterol is formed than iso-dehydroergosterol, while Inhoffen's equation requires equimolecular amounts of neoergosterol and a compound or a group of compounds with the formula $C_{28}H_{42}O$. It seems, therefore, that iso-dehydroergosterol either is formed by a secondary change from the direct product of the pyrolysis or suffers partly a transformation during distillation. Or otherwise, a number of compounds with the formula $C_{28}H_{42}O$ may be formed side by side. In any case, at least one more compound with the formula $C_{28}H_{42}O$ may be expected, unless the primary product suffers such a change as to alter its composition.

Experimental.

The Isolation of Iso-dehydroergosterol. Iso-dehydroergosteryl 3,5-dinitrobenzoate was obtained from iso-neoergosterol in the same way as described in the previous paper.[5] For the experiments recorded in this paper 87 mg. of a specimen of the dinitrobenzoate melting at 187–189.5° (corr.) and 91 mg. of another melting at 184.5–187° (corr.) were available. The dinitrobenzoate was carefully saponified as follows:

The dinitrobenzoate (87 mg.) of the melting point 187–189.5° was dissolved in benzene (14 c.c.), and the solution was added drop by drop to boiling 1% alcoholic potash (0.06 g. potassium hydroxide in 6 g. alcohol), while the benzene was instantly distilled off. The resulting brown solution was concentrated under a slightly diminished pressure and water was added to the concentrate, when a dark brown viscous matter separated. It was extracted with ether, and the ethereal solution was washed with dilute acetic acid and with water, dried with anhydrous sodium

sulphate, and evaporated. The remaining orange-coloured mass, after dried over caustic potash in vacuum, was crystallized from hot methanol in almost colourless needles (46 mg.) melting at 130–131.5° (corr.). Repeated recrystallization from methanol containing a small amount of water gave pure iso-dehydroergosterol in colourless aggregated small needles (21 mg.). It melts at 126.5–128° (uncorr.) or 128–129.5° (corr.) to a colourless liquid, and shows $[\alpha]_D^{10°} = -102°$ (7.35 mg. in 1 c.c. chloroform solution, $l = 1$ dm., $\alpha_D^{10°} = -0.75°$) and ultraviolet absorption maxima in 0.036% hexane solution at 280, 270 (both sharp), and 252 mμ (indistinct). An additional yield (9 mg.) was obtained from the mother liquors of recrystallization.

In the same way the other specimen (91 mg.) of the dinitrobenzoate melting at 184.5–187° gave another crop of iso-dehydroergosterol (35 mg.) in colourless aggregated small needles showing melting point 125.5–128° (uncorr.) or 127–129.5° (corr.), and ultraviolet absorption maxima in 0.035% hexane solution at 280, 270 (both sharp), and 254 mμ (indistinct), an additional yield (11 mg.) being obtained from the mother liquors.

The substance is unstable in air and must be kept in vacuum or in the atmosphere of carbon dioxide. It rapidly became yellow when it was dried at 100° in vacuum over phosphorus pentoxide. For analysis the substance was dried in vacuum at room temperature over calcium chloride.

Found:		(1)	C, 82.69; H, 10.96
		(2)	C, 81.49; H, 10.85
Calculated for	$C_{28}H_{42}O$:		C, 85.21; H, 10.74
	$C_{28}H_{42}O+\frac{1}{2}H_2O$:		C, 83.30; H, 10.75
	$C_{28}H_{42}O+H_2O$:		C, 81.47; H, 10.75
	$C_{28}H_{44}O$:		C, 84.76; H, 11.19
	$C_{28}H_{44}O+\frac{1}{2}H_2O$:		C, 82.87; H, 11.19
	$C_{28}H_{44}O+H_2O$:		C, 81.08; H, 11.19%

Reactions of Iso-dehydroergosterol. In the Liebermann-Burchard test iso-dehydroergosterol gives red, violet, and deep blue colours, while in the Rosenheim test instantly pink, light violet after five minutes, then blue, and finally dark violet colours.

When iso-dehydroergosterol (3 mg.) dissolved in a few drops of 95% alcohol was mixed with a 1% alcoholic solution of digitonin (10 mg.), precipitation of the digitonide took place instantly and was completed in a few minutes.

The Synthetic Preparation of Iso-neoergosterol from Neoergosterol and Iso-dehydroergosterol. When equal quantities (12 mg. each) of neoergosterol (m.p. 153.5–154.5°, corr.) and iso-dehydroergosterol (m.p. 127–129.5°, corr.) were dissolved together in a small amount of acetone with warming and the solution was cooled, there occurred separation of fine colourless needles (19 mg.) melting at 138.5–140° (corr.). Recrystallization gave iso-neoergosterol in colourless fine needles melting at 138–139° (corr.) alone and in admixture with the specimen obtained from the pyrolysis of ergopinacone. The synthetic product showed a specific rotation $[\alpha]_D^{11°} = -66.1°$ (6.8 mg. in 1 c.c. chloroform solution, $l = 1$ dm., $\alpha_D^{11°} = -0.45°$).

A depression of the melting point was observed when the synthetic iso-neoergosterol was melted with a very small quantity of either neoergosterol (mixed melting point 135–138°, corr.) or iso-dehydroergosterol (mixed melting point 128.5–134.5°, corr.).

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