272. *The Application of the Method of Molecular Rotation Differences to Steroids. Part VI.* neo*Sterol.*

By D. H. R. BARTON and J. D. COX.

Experimental evidence is given to prove that *neo*sterol is a mixture of ergosterol and α-dihydroergosterol.

*neo*STEROL was first isolated from yeast fat by Wieland and Asano (*Annalen*, 1929, **473**, 300), who characterised it by the formation of an acetate and a benzoate. Somewhat later, Callow (*Biochem. J.*, 1931, **25**, 87) suggested that the sterol was simply a mixed crystal of ergosterol and α-dihydroergosterol. More recently, Wieland, Rath, and Hesse (*Annalen*, 1941, **548**, 34) have reported on a further examination of *neo*sterol and have again asserted its homogeneity. In Parts I and II of this series (*J.*, 1945, 813; 1946, 512) the evidence of the German authors was critically examined and shown to be incompatible with their views as to the constitution of this sterol. It is not necessary to repeat the discussion in detail here, but it may be said that all published physical evidence is in apparent agreement with Callow's original suggestion. Accordingly, we have prepared a mixture of 75·5% of ergosterol and 24·5% of α-dihydroergosterol corresponding, from the calculated optical rotation, with *neo*sterol. The properties of this mixture and its derivatives have been examined in detail as indicated below.

Table I compares m. p.s and optical rotations of *neo*sterol and its derivatives, as reported by Wieland and his collaborators, with those observed for this "synthetic neosterol". In Table II

TABLE I.

	*neo*Sterol.			" Synthetic *neo*sterol."		
	M. p.	$[\alpha]_D$ in $CHCl_3$.	Mixed m. p.*	M.p.	$[\alpha]_D$ in $CHCl_3$.	Mixed m. p.*
Sterol	164—165° †	−105° †	161—162° †	164—165° §	−107° (*c*, 1·67) §	159—161° §
	164—165 ‡	−104 ‡	—			
Acetate	173—174 †	—	—	173—175	− 73 (*c*, 1·28)	173—175
	173—174 ‡	− 67 ‡	—			
Benzoate	173—175 †	− 51 †	171—173 †	173—175	− 52 (*c*, 1·43)	168—172
	171—173 ‡	− 42 ‡				

* With corresponding ergosterol derivatives.

† Wieland and Asano, *loc. cit.*

‡ Wieland, Rath, and Hesse, *loc. cit.*

§ After conversion into the benzoate and then back into the alcohol (see text). The original mixture had m. p. 164—165°, $[\alpha]_D$ − 105°, as made up from the components.

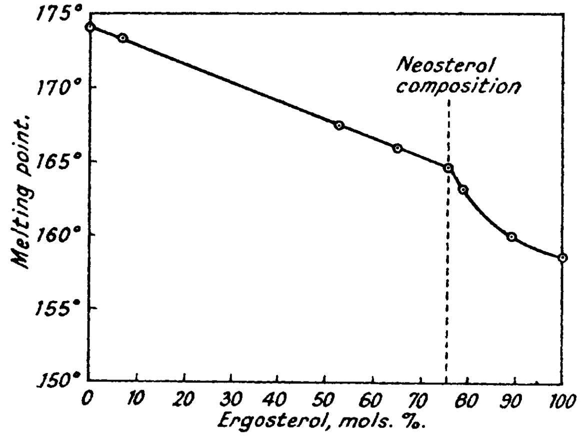
TABLE II.

Substance.	$\lambda_{max.}$, mμ.			$\epsilon_{max.}$.		
	1.	2.	3.	1.	2.	3.
Ergosterol (this paper) * ...	271	282	292	11,900	12,500	5,950
Ergosterol †......................	271	283	293	12,600	12,600	6,500
*neo*Sterol †	271	282	294	11,400	11,400	6,900
" Synthetic *neo*sterol " * ...	271	281	291	9,500	10,100	5,000

* In alcohol. † Solvent unspecified; data from Wieland and Gough, *Annalen*, 1930, **482**, 36.

a similar comparison is made for light-absorption data. There is such good agreement between the figures for *neo*sterol and " synthetic neosterol " as to leave no doubt as to the correctness of the view that we have advocated.

Wieland, Rath, and Hesse (*loc. cit.*) rejected the hypothesis that *neo*sterol was a mixture because they claimed to be able to fractionate mixtures of ergosteryl benzoate and α-dihydroergosteryl benzoate on crystallisation; *neo*steryl benzoate was said to be unchanged by such treatment. On the other hand, we have prepared " synthetic *neo*sterol ", converted it into the benzoate, recrystallised this, and then hydrolysed it back again to the alcohol without any significant change in properties (see Table I).



The accompanying diagram shows a plot of m. p. against the molar composition of various mixtures of ergosterol and α-dihydroergosterol. It is of interest that there is a slight but distinct inflection at the composition corresponding to " synthetic *neo*sterol " (compare, however, Lettré, *Annalen*, 1932, **495**, 41).

Experimental.—The constants for the ergosterol and α-dihydroergosterol used in this work have already been reported in Parts IV and V (this vol., pp. 783, 1354). Optical rotations were taken in chloroform solution for the Na_D line and at room temperature (see Experimental, Part IV). The experimental conditions for acetylation, benzoylation, and alkaline hydrolysis were also as in the experimental section, Part IV.

Melting point–composition diagram. Weighed amounts of ergosterol and α-dihydroergosterol were dissolved in a few drops of hot dioxan and precipitated by adding water. The m. p.s recorded in the diagram are the mid-points of the 12° melting-point ranges observed.

We thank Dr. E. A. Braude for the absorption spectra. This work was carried out during the tenure of an I.C.I. Fellowship by one of us (D. H. R. B.).

IMPERIAL COLLEGE OF SCIENCE AND TECHNOLOGY, S.W.7. [*Received, October* 21*st*, 1947.]