

### 160. Studies in the Sterol Group. Part XLVI. The Isolation of a New Form of $\Delta^4$ -Cholestenone.

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Oxidation of cholesterol by Oppenauer's method and careful chromatographic analysis of the product gives  $\Delta^4$ -cholestenone in two forms, m. p.'s  $88^\circ$  and  $82^\circ$ . They are interconvertible and give ketonic derivatives with identical m. p.'s. The optical rotations and light-absorption intensities of both forms are slightly higher than those previously recorded.

CHOLESTEROL has been oxidised to  $\Delta^4$ -cholestenone by Oppenauer's method (*Rec. Trav. chim.*, 1937, **56**, 137) by a number of workers and in each case the m. p. recorded has been in the range  $80$ — $82^\circ$ . Recently one of us (Jones, Wilkinson, and Kerlogue, J., 1942, 391) prepared the ketone in this manner and reported m. p.  $81$ — $82^\circ$ . The experience gained in chromatographic analysis (see previous paper) induced us to repeat this work, with the result that on careful chromatography of the crude  $\Delta^4$ -cholestenone, two different forms, m. p.'s  $88^\circ$  and  $82^\circ$ , were isolated. Their optical rotations and light-absorption intensities (see table) are identical, and they give the same semicarbazone and 2 : 4-dinitrophenylhydrazone.

The haphazard manner in which fractions of different m. p.'s were isolated from the chromatogram (see Experimental) suggested that they might be interconvertible, and this was readily established. A mixture of the two forms has m. p.  $88^\circ$ , and samples which melt at  $82^\circ$  when cooled and reheated often have m. p.  $88^\circ$ . In general, both forms can be recrystallised from alcohol without undergoing conversion, but occasionally the form of m. p.  $82^\circ$  gave the higher-melting form on crystallisation. It is probably purely fortuitous that the converse change has never been noted, for if a saturated alcoholic solution of either of the two forms is seeded with the other form, then in every case the form with m. p. corresponding to the seeding crystals is deposited. Thorough drying in high vacuum failed to alter the constants of either form, and this and the facts recorded above preclude the possibility that the phenomena observed can be explained by hydrate or alcoholate formation.

#### Constants of $\Delta^4$ -cholestenone.

Reference.	M. p.	$[\alpha]_D^{20}$ in $\text{CHCl}_3$ .	$\epsilon_{\text{max.}}$ in EtOH.
Diels and Abderhalden, <i>Ber.</i> , 1904, <b>37</b> , 3092 .....	$78^\circ$	—	—
Windaus, <i>Ber.</i> , 1906, <b>39</b> , 518 .....	$81$ — $82$	—	—
Heilbron and Sexton, J., 1928, 347, 2825 .....	80	—	—
Menschick, Page, and Bossert, <i>Annalen</i> , 1932, <b>495</b> , 227 .....	—	—	16,200
Grasshof, <i>Z. physiol. Chem.</i> , 1934, <b>223</b> , 249 .....	—	+83.5°	—
Butenandt and Wolff, <i>Ber.</i> , 1935, <b>68</b> , 2091 .....	$79$ — $80$	+88.6	—
Mohler, <i>Helv. Chim. Acta</i> , 1937, <b>20</b> , 289 .....	—	—	15,000
Galinovsky, <i>Ber.</i> , 1941, <b>74</b> , 1048 .....	$80$ — $81$	+88.7	—
Jones, Wilkinson, and Kerlogue, <i>loc. cit.</i> .....	$81$ — $82$	—	18,000
This paper .....	$82$ and $88$	+92.2	19,500

The discovery of this new form of  $\Delta^4$ -cholestenone may be due to the high state of purity in which the ketone was obtained by the chromatographic technique. This is borne out by the somewhat higher rotations and extinction coefficients of the two forms as compared with those previously reported (see table).

Closely related to the phenomena now described is the behaviour of progesterone, the two forms of which were originally believed to be different substances (Slotta, Ruschig, and Blanke, *Ber.*, 1934, 67, 1947; Butenandt and Schmidt, *ibid.*, p. 2088).

## EXPERIMENTAL.

Chromatogram fractions were crystallised from alcohol before the m. p. was determined. Other conditions were as noted under "Experimental" in Part XLV (preceding paper).

*Chromatographic Analysis of  $\Delta^4$ -Cholestenone.*—Cholesterol (5 g.) was oxidised as described by Jones, Wilkinson, and Kerlogue (*loc. cit.*); the crude  $\Delta^4$ -cholestenone (4.3 g.) after one crystallisation had m. p. 78°. Its solution in light petroleum was adsorbed on a 2.9 × 45 cm. column of "Birlec" alumina, and the chromatogram developed with benzene. The following is a typical chromatogram (50 c.c. fractions). (Fractions 2 and 3 were contaminated with resinous substances.)

Fraction number.	M. p.	M. p. after recrystn. from EtOH.	Fraction number.	M. p.	M. p. after recrystn. from EtOH.
2	86.5—87.5°	—	8	87.5°	88°
3	87	—	9	82	82
4	87.5	88°	10	88	88
5	87.5	88	11	82	82
6	82	82	12	87.5	88
7	88	88	13	88	88

The various fractions with m. p. 88° were combined and recrystallised without any change in m. p. being observed. The new form separated from alcohol in needles (Found: C, 84.6; H, 11.8.  $C_{27}H_{44}O$  requires C, 84.3; H, 11.6%);  $[\alpha]_D^{20} +92.2^\circ$  ( $c = 2.560$ ),  $+91.9^\circ$  ( $c = 1.350$ ). The form with m. p. 82° separated from alcohol in needles,  $[\alpha]_D^{20} +92.2^\circ$  ( $c = 4.510$ ),  $+93.6^\circ$  ( $c = 3.095$ ). The semicarbazone, prepared from either form, had m. p. 237° (decomp.) (lit., m. p. 237°, decomp.), and the 2:4-dinitrophenylhydrazone m. p. 238° (decomp.) (lit., m. p. 238°, decomp.).

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