[CONTRIBUTION FROM DIAMOND ORDNANCE FUZE LABORATORIES]

# PREPARATION OF LANOSTEROL FROM BROMOLANOSTEROL

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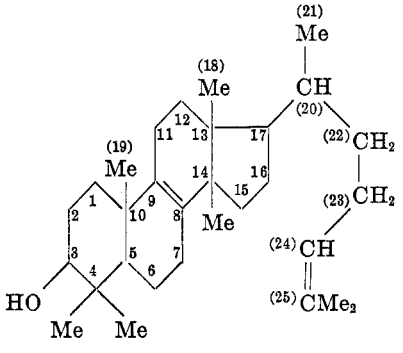
Lanosterol is a triterpenoid with the empirical formula $C_{30}H_{50}O$. It is a secondary alcohol with two double bonds. The double bond between the 8 and 9 positions is inert.

The starting material in preparing this triterpenoid is lanolin which is a wool fat. Lanolin contains a non-saponifiable fraction called "iso-cholesterol", or sometimes incorrectly "lanosterol." The use of the name "lanosterol" will be restricted in this paper to $\Delta^{8,\ 24}$-lanostadienol. The name "iso-cholesterol" as used herein refers only to the wool fat fractions. For other uses see Elsevier's Encyclopedia of Organic Chemistry (1).

The lanostone group of triterpenoids is closely related to the sterols. This can be seen by comparing their structure with that of cholesterol. The numbering system used in this paper is the same as that adopted by Elsevier. It is based on the numbering system for the sterols which is illustrated by the following structural formula for lanosterol:




$\Delta^{8,24}$-lanostadien-3-ol or lanosterol (a triterpenoid)

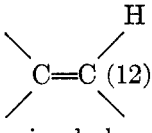
Prior to 1945 iso-cholesterol was known only to be a mixture of sterols and sterol-like materials. In 1945 Ruzicka, *et al.* (2) found that iso-cholesterol is mainly a mixture of approximately 25 percent lanosterol (lanostadienol), 25 percent dihydrolanosterol (lanostenol), 20 per cent dihydroagnosterol, and 3–10 percent cholesterol. Prior to 1945 the reactions described for lanosterol were carried out with iso-cholesterol.

Attempts to separate iso-cholesterol into its component parts by means of chromatography were made unsuccessfully by Ruzicka (3). He also found that fractional crystallization of the mixture of dihydrolanosterol and lanosterol acetates yielded mixed crystals of the two in equimolar proportions. Separation of dihydrolanosterol and lanosterol is further complicated by their nearly iden-

tical melting points, 139–141° and 138–140°, respectively. While this mixture is easily hydrogenated to pure dihydrolanosterol acetate, it does not readily lend itself to the preparation of pure lanosterol. For example, after hydrolysis and oxidation of the mixture of acetates, Ruzicka separated lanostadien-3-one by fractional crystallization and then reduced it to lanosterol (2).

In seeking a method for isolating lanosterol from iso-cholesterol without recourse to tedious fractional crystallizations and chromatographic separations, bromolanosterol (24-bromo-8,24-lanostadien-3-ol) which had first been prepared by Curtis and Silberman (4) was investigated in this laboratory. This derivative was reported easily obtainable in high purity directly from the wool-wax triterpenes; Curtis and Silberman reduced it catalytically to dihydrolanosterol. If lanosterol is to be prepared from bromolanosterol, the problem remains to reduce the bromine from its relatively inert vinyl position without saturating the C-24 double bond. Existing reports of the reactivity of vinyl halides suggested the use of metallic reducing agents. For example, although Gilman (5) was unable to obtain vinyllithium by exchange of vinyl bromide with butyllithium, Braude and his co-workers (6–8) have obtained the lithium alkenyls in satisfactory yields from lithium metal in ether. Results with the magnesium derivatives are conflicting; Krestinsky (9) reported formation of the Grignard reagents from vinyl halides and magnesium, but Braude was not able to obtain satisfactory reactions of this kind with magnesium. Little information is available on the use of lithium aluminum hydride for reduction of vinyl halides except for the reduction of ω-bromostyrene in low yield (10).

In this laboratory it was found that of the various reagents applied to the reduction of bromolanosterol, lithium in tetrahydrofuran converts the halide entirely to the lithium derivative at room temperature. Hydrolysis of the latter compound gave lanosterol in 83 per cent yield from the halide. Lithium metal in ether was much less effective and lithium aluminum hydride or butyllithium in ether were completely inactive.

The infrared spectrum was taken on a disk of the lanosterol pelleted with KBr. Absorption maxima occurred at wave lengths of 6.124, 9.69$_0$, and 12.222 μ. These maxima were interpreted as due to the double-bond stretching (11), the hydroxyl group (11) at position 3, and the group  (12), respectively.

Since the acetate of bromolanosterol had not previously been reported, it was prepared, recrystallized from alcohol and ethyl acetate, and found to have a melting point of 181–182°.

## EXPERIMENTAL

*Materials.* A sample of commercial quality iso-cholesterol was obtained from the Sprague Electric Company; it had been made by Botany Mills. A 50-g. sample of iso-cholesterol produced 16 g. of 24-bromolanosterol, using the method of Curtis and Silberman (4). The

product melted at 196–198° (reported 196–198°);

$[\alpha]_D^{20}$ = 46.4 (*c*, 2.89 in $CHCl_3$),

reported $[\alpha]_D^{20}$ = 51.4 (*c*, 3.0 in $CHCl_3$) (4).

*Anal.* Calc'd for $C_{30}H_{49}BrO$: C, 71.3; H, 9.8; Br, 15.8.

Found: C, 71.2; H, 9.7; Br, 15.7.

The tetrahydrofuran was dried and distilled over calcium hydride. Water-pumped argon was passed through a drying tube before being run into the reaction flask.

*Preparation of lanosterol from bromolanosterol.* The reaction was carried out in a 1000-ml., three-necked, round-bottomed flask fitted with a sealed, mechanically-driven stirrer. The flask was flushed with dry argon. Into a solution containing 13.6 g. (0.028 mole) of 24-bromo-$\Delta^{8,24}$-lanostadien-3-ol in 500 ml. of dry tetrahydrofuran, there was introduced 2.1 g. (0.3 gram-atom) of lithium ribbon which had been cut into small strips. After the solution had been stirred for two hours at room temperature it became cloudy, the lithium gradually acquiring a grey coating. At the end of 24 hours 1.5 g. (0.15 gram-atom) of fresh lithium was added and stirring was continued for a total of 72 hours. Pieces of unreacted lithium were picked out of the flask and 500 ml. of water was added with stirring. Most of the tetrahydrofuran was removed by distillation and the precipitated product was collected, washed with water, and dried overnight in a vacuum desiccator. The white product weighed 12 g. and melted at 135–139°. Recrystallization from a mixture of 75 ml. of benzene and 225 ml. of methyl alcohol yielded 9.5 g. of lanosterol (82.5% yield) melting at 138–140°, $[\alpha]_D^{20}$ = 59.5 (*c*, 8.2, in $CHCl_3$)

*Anal.* Calc'd for $C_{30}H_{50}O$: C, 84.4; H, 11.8.

Found: C, 84.2; H, 11.8.

*Preparation of the acetate of bromolanosterol.* A solution of 2 g. of bromolanosterol and 3 g. of anhydrous sodium acetate in 75 ml. of acetic anhydride was refluxed for 5 hours. The solution then was poured into water and the excess acetic anhydride was hydrolyzed. The crystalline acetate was collected, washed with water, and recrystallized from alcohol and ethyl acetate. It melted at 181–182°.

*Anal.* Calc'd for $C_{32}H_{51}BrO_2$: C, 70.2; H, 9.4; Br, 14.6.

Found: C, 69.8; H, 9.2; Br, 14.6.

## SUMMARY

Synthesis of lanosterol was accomplished by reacting bromolanosterol with lithium in tetrahydrofuran, and hydrolyzing the lithium derivative with water. The synthetic material was analyzed and found to conform to the chemical formula of lanosterol, $C_{30}H_{50}O$; it melted at 138–140°. The yield was 82.2 percent of theoretical. The infrared spectra was taken on a disk of the lanosterol pelleted with KBr. Absorption maxima occurred at wave lengths of 6.124, 9.69$_0$, and 12.22 $\mu$.

Acetylation of bromolanosterol yielded the acetate which melted at 181–182°.

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