

279. *Lanosterol. Part IV. Hydrocarbons formed by the Action of Dehydrating Agents.*

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Phosphorus oxychloride and pentachloride have proved effective dehydrating agents in the lanosterol group. Thus, dihydrolanosterol, according to the reagent and conditions employed, yields two isomeric hydrocarbons of the formula $C_{30}H_{60}$, *α -lanostadiene*, and *isolanostadiene*, respectively. Dihydroagnosterol (*γ -lanosterol*) and other members of this series are dehydrated with equal facility to the corresponding hydrocarbons. A new alternative method for the preparation of *α -lanostene* (Ruzicka *et al.*, *Helv. Chim. Acta*, 1944, **27**, 479) is also described, and the relationship between these hydrocarbons is discussed in connection with the position of the unsaturated centres in the molecule.

THE experimental facts established in previous researches in regard to lanosterol are the formula, $C_{30}H_{50}O$, and the presence of one secondary hydroxyl group and two double bonds—one reactive and one inert—in a tetracyclic, triterpenoid nucleus, with a side-chain of at least four carbon atoms terminating in an *isopropylidene* group, in which the reactive double bond is incorporated. It was known that the lanosterol fraction obtained from wool-grease contained approximately 10% of the analogous triply unsaturated alcohol agnosterol, $C_{30}H_{48}O$. More recently, Ruzicka *et al.* (*Helv. Chim. Acta*, 1944, **27**, 479; *ibid.*, 1945, **28**, 759) have recognized the presence of both dihydrolanosterol and dihydroagnosterol, and lanosterol has been shown to be identical with the cryptosterol of yeast. The agnosterol compounds can be removed by exhaustive crystallization, leaving a non-separable mixture of lanosterol and its dihydro-derivative, which hitherto has been described as lanosterol. Lanosterol (cryptosterol) has been obtained from this by oxidation, followed by separation and reduction of the ketones. The non-separable mixture, on hydrogenation, yields pure dihydrolanosterol which is suitable for experimental work.

An examination of the hydrocarbons derived from lanosterol and its analogues was undertaken to obtain evidence of the relationship between lanosterol and other tetracyclic triterpenes, and to make available new compounds for structural investigation. The fully saturated parent hydrocarbon lanostane has not yet been obtained. Lanostene, corresponding to lanosterol, and hydrocarbons derived from dihydrolanosterol, dihydroagnosterol (γ -lanosterol), and isodihydrolanosterol have been prepared by Ruzicka *et al.* (*loc. cit.*). The procedure employed in each case was to prepare the ketone, the semicarbazone of which was reduced, according to the Wolff-Kishner method, with sodium and ethanol under pressure. A simple and convenient modification has now been found in the direct reduction of ketone to hydrocarbon, using a modified form of the Clemmensen reaction with amalgamated zinc and hydrochloric acid. Under the conditions of experiment the hydrochloric acid does not cause bond-movement, and good yields of the desired hydrocarbons are readily obtained.

A systematic examination of the action of dehydrating agents on dihydrolanosterol has resulted in a new approach to hydrocarbons containing an additional double bond. In the dehydrations studied, the unsaturated linkage would be introduced by the removal of the hydroxyl group and the hydrogen of an adjacent carbon atom of the ring system. Provided that bond-movement does not take place, therefore, the location of the newly formed double bond is limited with great probability to a single position in the ring containing the hydroxyl group.

Most of the dehydrating agents that have been successfully employed in the case of the sterols and triterpenes were found unsuitable for dehydration in the lanosterol series. Anhydrous copper sulphate in xylene, activated alumina at temperatures up to 250°, *p*-toluenesulphonyl chloride in boiling pyridine, and acetic anhydride-sulphuric acid mixtures failed to yield products of dehydration. On the other hand, the halides and oxyhalides of phosphorus, particularly the oxychloride and pentachloride, proved highly satisfactory, giving hydrocarbons smoothly and in excellent yield.

Phosphorus oxychloride, applied by Heilbron (*J.*, 1935, 1223) in the conversion of lumisterol into lumistatetraene, when acting on dihydrolanosterol under analogous conditions gave a non-homogeneous reaction product from which α -lanostadiene was isolated. γ -Lanosterol (dihydroagnosterol) similarly gave a trebly unsaturated hydrocarbon, γ -lanostatatriene. α -Lanostadiene showed no characteristic absorption in the ultra-violet, but γ -lanostatatriene gave absorption maxima almost identical with those of γ -lanosteryl acetate (Bellamy and Dorée, *J.*, 1941, 178), indicating that the system of conjugated double bonds is still present. The newly formed unsaturated linkage is remote from this system, as the characteristic absorption curve is not altered, though the intensities are higher in the case of the hydrocarbon, as shown by the following measurements:

	$\lambda_{\max.}$	$E_{1\text{ cm.}}^{1\%}$	$\lambda_{\max.}$	$E_{1\text{ cm.}}^{1\%}$	$\lambda_{\max.}$	$E_{1\text{ cm.}}^{1\%}$
γ -Lanosteryl acetate	238	300	245	380	250	250
γ -Lanostatatriene	237	400	243	440	254	320

Lanostadiene has an increased reactivity due to the additional double bond which, unlike the inert double bond in dihydrolanosterol, shows a reactivity approaching that of an ethylenic linkage. Its probable location in the terminal ring of the triterpene molecule, already suggested by its mode of formation, is supported by the properties of the hydrocarbon. Thus α -lanostadiene readily adds two atoms of hydrogen in the presence of contact catalysts forming α -lanostene, which is identical with Ruzicka's "dihydrolanostene", obtained by Wolff-Kishner reduction of dihydrolanostenone semicarbazone. Similarly, α -lanostene rapidly absorbs two atoms of bromine: the addition product is highly labile, however, and decomposes at once forming a halogen-free compound, provisionally designated isomer X. Lanostadiene is readily isomerized by hydrogen chloride yielding β -lanostadiene, and it can be dehydrogenated to yield products of still higher unsaturation. The action of either selenium dioxide in anhydrous ethanol or *N*-bromosuccinimide afford excellent yields of α -lanostatatriene. This triene shows no absorption in the ultra-violet, indicating the absence of a system of conjugated double bonds, and its non-identity with γ -lanostatatriene.

Opening of the terminal ring of α -lanostadiene by controlled oxidative fission under various conditions has been attempted, but no crystallisable substances have so far been isolated from the large yields of acidic reaction products obtained.

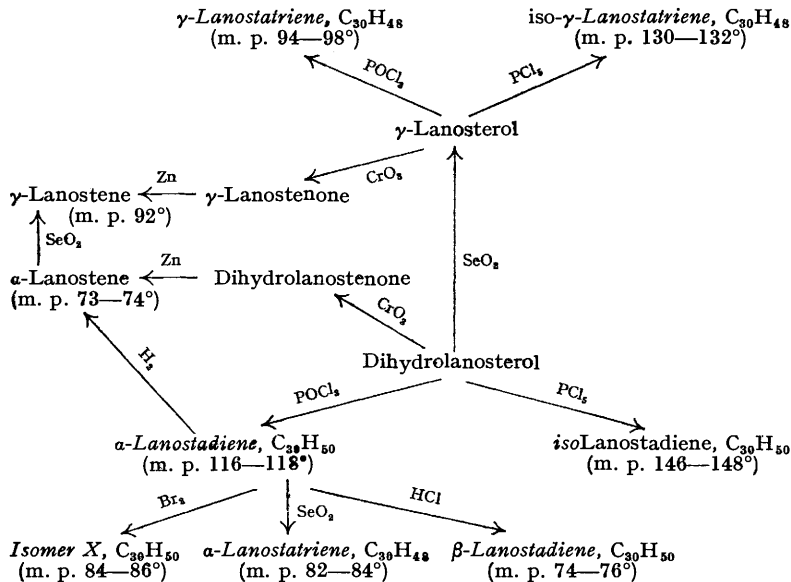
Phosphorus pentachloride has also proved a useful dehydrating reagent. α -Amyranonol, for example, in light petroleum solution is converted into α -amyradienone (Spring and Vickerstaff, *J.*, 1937, 251), while 3(β)-acetoxycholestan-7-" β "-ol can be similarly dehydrated using the reagent in the presence of calcium carbonate in anhydrous media (Wintersteiner and

Moore, *J. Amer. Chem. Soc.*, 1943, **65**, 1507). Phosphorus pentachloride, acting on dihydrolanosterol in boiling light petroleum solution, gave excellent yields of a doubly unsaturated hydrocarbon, *isolanostadiene*. Wieland, when dehydrating dihydrocryptosterol by high vacuum distillation with boron trioxide at 350° (Wieland and Joost, *Annalen*, 1941, **546**, 119) obtained a cryptostadiene, $C_{30}H_{48}$. Since the identity of lanosterol and cryptosterol has been established by Ruzicka *et al.* (*Helv. Chim. Acta*, 1945, **28**, 759) and by ourselves, there seems little doubt that *isolanostadiene* is identical with Wieland's cryptostadiene.

*iso*Lanostadiene and α -lanostadiene, prepared from dihydrolanosterol by the action of phosphorus pentachloride and oxychloride respectively, are isomeric. Unlike α -lanostadiene, *isolanostadiene* does not undergo hydrogenation, does not readily absorb bromine, and is considerably more resistant to chromic acid oxidation. The double bond in *isolanostadiene* is therefore far less reactive than that in α -lanostadiene, and its absence from the six-membered terminal ring (presumably owing to bond movement) can be assumed. This view is supported by spectrometric evidence. While α -lanostadiene is transparent over the spectral range in the ultra-violet, *isolanostadiene* shows a distinct absorption maximum at 235 $m\mu$, suggesting the presence of a system of conjugated double bonds and consequently the close proximity of the centres of unsaturation. If the isomerism of the two products, however, was entirely due to the migration of the reactive double bond of α -lanostadiene under the influence of hydrochloric acid, α -lanostadiene should be readily convertible into the *iso*-compound by treatment with anhydrous hydrogen chloride. As already mentioned, however, α -lanostadiene when treated with either hydrogen chloride or phosphorus pentachloride does not yield *isolanostadiene*, but an entirely new isomer, β -lanostadiene. The observed facts can therefore not be accounted for by a consideration of geometrical isomerism alone. A retropinacoline rearrangement of the type recently described by Ruzicka *et al.* (*Helv. Chim. Acta*, 1945, **28**, 767, 942, 1628; 1946, **29**, 210), occurring when α -amyranonol, lupeanol, and oleanolic acid lactone are treated with phosphorus pentachloride, may possibly take place in the analogous formation of *isolanostadiene*. Experiments to obtain further information on the nature of these isomers are in progress.

Phosphorus pentachloride has proved equally satisfactory in the dehydration of related compounds. When applied to γ -lanosterol (dihydroagnosterol), for example, the procedure furnishes good yields of *iso*- γ -lanostatriene.

The relationships of these hydrocarbons are shown in the scheme below :



EXPERIMENTAL.

Melting points are uncorrected. The rotation measurements, for which the authors are indebted to Mr. H. Heath, were made in chloroform solution at 15°.

α -Lanostene ("Dihydrolanostenone").—A hot solution of dihydrolanostenone (5 g.; 1 mol.) in glacial acetic acid (150 ml.) was added to amalgamated zinc filings (40 g.; 50 mols.) and the mixture boiled. On

addition of concentrated hydrochloric acid (40 ml.; 30 mols.) vigorous reaction took place, and refluxing was continued for 1½ hours. The clear acetic acid solution was poured into water (600 ml.) and the crude dihydrolanostene, separating as a white flocculent precipitate, isolated by extraction with ether. The extracts were washed with sodium hydroxide solution and with water until neutral, and the colourless oily residue obtained on removal of the solvent further purified by filtration of its petroleum solution through alumina. After 3 recrystallisations from chloroform-methyl alcohol (1:5), *α*-lanostene (2–2.5 g.), m. p. 73–74°, $[\alpha]_D + 66.2^\circ$ (*c*, 0.90), was obtained in lustrous plates (Found: C, 87.58; H, 12.40. Calc. for $C_{30}H_{52}$: C, 87.38; H, 12.62%).

γ-Lanostene.—(a) *γ*-Lanostenone, when treated with amalgamated zinc as described above, gave 40–50% yields of *γ*-lanostene, forming shining plates, m. p. 89–91°, $[\alpha]_D + 77^\circ$ (*c*, 1.04) (Found: C, 87.99; H, 12.05. Calc. for $C_{30}H_{50}$: C, 87.81; H, 12.19%).

(b) A solution of dihydrolanostene (3 g.; 1 mol.) was refluxed with excess of selenium dioxide (3 g.; 4 mols.) in glacial acetic acid (50 ml.) for 2 hours. The separated selenium was removed, and the filtrate deposited crystalline *γ*-lanostene on cooling and standing. After two recrystallisations from chloroform-ethyl acetate-methyl alcohol (1:1:4), pure *γ*-lanostene, m. p. 90–92°, was obtained.

α-Lanostadiene.—To a solution of dihydrolanosterol (10 g.; 1 mol.) in pyridine (100 ml.) excess of phosphorus oxychloride (15 ml.; 7 mols.) was added slowly with shaking. The temperature of the mixture rose sharply to 80°. After being heated on the steam-bath for 1½ hours, the whole was boiled gently for 3 minutes and allowed to cool to room temperature. The dark brown liquid was then slowly treated with water under cooling; the crude reaction product then appeared in suspension. After repeated extraction with ether, the combined extracts were shaken with concentrated hydrochloric acid to remove pyridine, followed by repeated washings with distilled water until nearly neutral. The yellow mobile oil obtained, on removal of the solvent, was taken up in light petroleum (500 ml.) and filtered through a 25 g. alumina column. A clear colourless eluate was obtained. The yellow oil remaining after distillation of the solvent was crystallised from chloroform-acetone-methyl alcohol (30, 30, and 100 ml. respectively) to give good yields (*ca.* 7 g.) of a white microcrystalline mass. The material melted between 95° and 112° (signs of sintering at 85°). After repeated crystallisation from chloroform-methyl alcohol, *α*-lanostadiene, m. p. 114–117°, was obtained in minute white needles (2.5–3 g.). From the mother liquors there resulted a further crop (3–4 g.) of inferior material. The final fractions separated as an uncrystallisable oil (1–2 g.).

α-Lanostadiene forms microcrystalline white needles, m. p. 116–118°, $[\alpha]_D + 113^\circ$ (*c*, 1.040), slightly Beilstein-positive, giving a brown colour with tetranitromethane. It is soluble in chloroform, ethyl acetate, and acetone, sparingly soluble in methyl alcohol and hot glacial acetic acid (Found: C, 87.73; H, 12.26. $C_{30}H_{50}$ requires C, 87.81; H, 12.19%). Lanostadiene is optically transparent in the ultra-violet. It yields no addition product in the Diels-Alder reaction, and the absence of a system of conjugated double bonds may be inferred. It is readily reduced to *α*-lanostene.

A solution of *α*-lanostadiene (2.5 g., 1 mol.) in glacial acetic acid (150 ml.) was treated with hydrogen at 100° at ordinary pressure in the presence of finely divided platinum. Absorption of hydrogen took place rapidly, a total of 115 ml. being taken up within 10 minutes (theory, 130 ml.). Shaking was continued for another 30 minutes. After removal of the platinum the filtrate was distilled in a vacuum to small bulk (20 ml.) and the crude reaction product isolated from the residue, previously diluted with water (100 ml.), by extraction with ether. The ethereal extracts were washed with sodium hydroxide solution and with water and the solvent was removed. The residual oil, after filtration of its light petroleum solution through alumina, was crystallised thrice from chloroform-ethyl acetate-methyl alcohol, when *α*-lanostene was obtained in minute white plates, m. p. 70–74°, $[\alpha]_D + 65.7^\circ$ (*c*, 0.556), showing no depression of the melting point in admixture with *α*-lanostene obtained by the reduction of dihydrolanostenone (Found: C, 87.62; H, 12.40%).

Isomer X.—*α*-Lanostadiene (3 g.; 1 mol.), dissolved in anhydrous ether (60 ml.) was treated at 0°, within 5 minutes, with the theoretical amount of bromine dissolved in anhydrous ether (3.7 ml. of 10% v/v solution; 2 g.-atoms). The bromine solution was decolourised instantaneously; after 10 minutes' standing at 0° the ether was removed under reduced pressure, the slightly fuming yellow oil taken up in light petroleum (*b. p.* 60–80°; 200 ml.) and purified chromatographically. After three crystallisations from ethyl acetate-acetone-methyl alcohol, a halogen-free product, *isomer X*, m. p. 84–86°, $[\alpha]_D + 124^\circ$ (*c*, 0.372), was obtained in colourless lustrous elongated plates; ultra-violet absorption spectrum showed no absorption between 230 and 500 $m\mu$ (Found: C, 87.78; H, 12.21. $C_{30}H_{50}$ requires C, 87.81; H, 12.19%).

α-Lanostatriene.—(a) *α*-Lanostadiene (4 g.; 1 mol.) dissolved in ethanol (350 ml.) was treated with excess of selenium dioxide (3 g.; 3 mols.) and refluxed gently for 8 hours. The solvent was removed in a vacuum, the dark brown residue taken up in ether, and freed from traces of selenium by extraction with sodium cyanide solution. The crude reaction product in light petroleum solution was filtered through a comparatively large alumina column (30 g.). The intense yellow colour of the eluate, however, was retained after two such purifications. On crystallisation of the residue from chloroform-acetone-methyl alcohol, *α*-lanostatriene, m. p. 82–84°, $[\alpha]_D + 138^\circ$ (*c*, 0.796), was obtained in pale yellow needles; no characteristic absorption between 230 and 500 $m\mu$ (Found: C, 88.12; H, 11.83. $C_{30}H_{48}$ requires C, 88.23; H, 11.76%).

(b) *α*-Lanostadiene (2.5 g.; 1 mol.), dissolved in anhydrous carbon tetrachloride (60 ml.), was refluxed with excess of *N*-bromosuccinimide (1.5 g.; 1.6 mols.) for 1½ hours. Hydrogen bromide was given off throughout the reaction. After cooling, the succinimide was removed, the filtrate washed until neutral and concentrated. The oily residue was purified through an alumina column, and, after three recrystallisations from chloroform-acetone-methyl alcohol, gave *α*-lanostatriene in lustrous needles, m. p. 82–84°, $[\alpha]_D + 136^\circ$ (*c*, 0.362), identical with the product obtained by selenium dioxide dehydrogenation.

β-Lanostadiene.—*α*-Lanostadiene (2.5 g.), dissolved in anhydrous chloroform (50 ml.), was boiled under reflux, and hydrogen chloride passed through the solution for 3 hours. The clear green liquid obtained was washed until neutral and concentrated under reduced pressure. The residual oil, after

purification through alumina of its light petroleum solution, was crystallised thrice from chloroform-acetone-methyl alcohol, when β -lanostadiene was obtained in white needles, m. p. 74–76°, $[\alpha]_D + 128^\circ$ (c , 0.806); no characteristic absorption between 230 and 500 $m\mu$ (Found: C, 87.75; H, 12.28. $C_{30}H_{50}$ requires C, 87.81; H, 12.19%).

γ -Lanostatriene.— γ -Lanosterol (3 g.; 1 mol.), dissolved in pyridine (30 ml.) was treated with excess of phosphorus oxychloride (6 ml.; 10 mols.) at 100° for 1½ hours. On isolation of the product as described under α -lanostadiene, colourless lustrous plates of γ -lanostatriene were obtained in 60% yield, m. p. 94–98°, $[\alpha]_D + 133^\circ$ (c , 0.568) (Found: C, 88.06; H, 11.93. $C_{30}H_{48}$ requires C, 88.23; H, 11.76%). Ultra-violet absorption spectrum: Maxima at 237, 243, 254 $m\mu$; ($E_{1\text{cm.}}^{1\%} = 400, 440, \text{ and } 320$ respectively), giving an absorption curve practically identical with that of γ -lanosteryl acetate (*J.*, 1941, 178).

isoLanostadiene.—Dihydranosterol (10 g.; 1 mol.) dissolved in light petroleum (150 ml., b. p. 60–80°), was treated with excess of phosphorus pentachloride (10 g.; 2 mols.). Reaction took place with warming, and, after 2 hours at room temperature, dehydration was completed by refluxing for 1 hour on the steam-bath. The phosphorus pentachloride dissolved rapidly, hydrogen chloride being evolved throughout the reaction. The clear liquid was allowed to cool, diluted with an equal volume of ether (200 ml.), and freed from excess of acid by several washings with water. After drying, the solution of the crude reaction product in light petroleum was filtered through alumina and the residue obtained after evaporation of the solvent crystallised three times from chloroform-methyl alcohol. Yield, 75–85%.

isoLanostadiene forms lustrous needles, m. p. 146–148°, $[\alpha]_D + 66.4^\circ$ (c , 0.950), slightly Beilstein-positive, and giving a brown colouration with tetranitromethane (Wieland and Joost, *loc. cit.*, give m. p. 141–142° for cryptostadiene). It is soluble in chloroform, acetone, and ethyl acetate, sparingly soluble in methyl alcohol; like α -lanostadiene, and in contrast to the other members of the lanosterol group, it is sparingly soluble in hot glacial acetic acid (Found: C, 87.80; H, 12.18. Calc. for $C_{30}H_{50}$: C, 87.81; H, 12.19%). Ultra-violet absorption spectrum: maximum at 235 $m\mu$.

iso- γ -Lanostatriene.— γ -Lanosterol (6 g.; 1 mol.), dissolved in light petroleum (120 ml.; b. p. 60–80°) and treated with excess of phosphorus pentachloride (6 g.; 2 mols.) as described for isolanostadiene, gave good yields (60%) of iso- γ -lanostatriene, m. p. 130–132°, $[\alpha]_D + 11^\circ$ (c , 1.100), in colourless lustrous plates (Found: C, 88.14; H, 12.01. $C_{30}H_{48}$ requires C, 88.23; H, 11.76). Ultra-violet absorption spectrum: maxima at 235, 242, and 251 $m\mu$ ($E_{1\text{cm.}}^{1\%} = 420, 450, \text{ and } 340$ respectively). Apart from higher intensities, the absorption curve was identical with that of γ -lanosteryl acetate.

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[Received, January 14th, 1947.]