190. *Lanosterol. Part V. Hydrogenation of the Inert Double Bond in Lanosterol Derivatives.*

By Charles Dorée, J. F. McGhie, and Frederick Kurzer.

The " inert " double bond of lanosterol, when situated between two carbonyl groupings, forming the chromophore —CO—C=C—CO—, can undergo reduction without difficulty. Diketodihydrolanosteryl acetate (Ruzicka *et al.*, *Helv. Chim. Acta*, 1944, **27**, 472) and diketolanostene (see experimental part), for example, have been converted smoothly by several methods into the fully saturated compounds *diketolanostanyl acetate* and *diketolanostane* respectively. The substances are of interest in being the first lanosterol derivatives recorded in which the " inert " double bond of the ring system has been hydrogenated. Whether this inert linkage migrates during the oxidation of dihydrolanosteryl acetate to the diketo-derivative, and takes up a position more favourable to hydrogenation cannot, at present, be decided.

Lanosterol, a tetracyclic alcohol of triterpenoid character, contains two double bonds, one of which is reactive and readily hydrogenated, while the other is chemically inert. Reduction of the active unsaturated centre, situated in the terminal *iso*propylidene grouping of the side chain, yields dihydrolanosterol, which still contains the inert double bond. Agnosterol, with an identical carbon skeleton, differs from lanosterol only in the possession of a third double bond, also of the inert type. Our previous attempts to reduce the inert unsaturated linkage directly have so far been unsuccessful. Catalytic hydrogenation of dihydrolanosteryl acetate, using Raney nickel under pressures up to 150 atmospheres at 200°, and the action of hydriodic acid under severe conditions, have not produced a saturated derivative. A study of certain oxidation products of dihydrolanosterol and dihydroagnosterol, in which the inert linkage is still present, has shown, however, that in these cases the unsaturated centre can readily be reduced, giving derivatives corresponding to the, so far, unknown saturated hydrocarbon lanostane.

Dihydrolanosteryl acetate, when oxidised with chromium trioxide in glacial acetic acid, yields monoketo-, diketo-, and triketo-dihydrolanosteryl acetate (Marker *et al.*, *J. Amer. Chem. Soc.*, 1937, **59**, 1368; Ruzicka *et al.*, *Helv. Chim. Acta*, 1944, **27**, 472). The diketo-acetate, $C_{32}H_{50}O_4$, forms the main product of the reaction and is also produced by the oxidation of dihydroagnosteryl acetate (Ruzicka *et al.*, *loc. cit.*; Dorée and McGhie, *Nature*, 1944, **153**, 148). Diketodihydrolanosteryl acetate forms deep-yellow plates, and gives an absorption curve in the ultra-violet with a well defined maximum at $\lambda = 275$ mμ (log $\varepsilon = 3{\cdot}94$), which suggests the presence of the chromophoric grouping —CO—C=C—CO—, the two carbonyl groups being formed by the oxidation of the methylene groups adjacent to the inert double bond. The displacement of the absorption maximum towards the longer wave-lengths of the spectrum in the case of monoketodihydrolanosteryl acetate ($\lambda_{max} = 255$ mμ) indicates, in accordance with Woodward's rule (*J. Amer. Chem. Soc.*, 1941, **63**, 1123), the location of the inert double bond between quaternary carbon atoms. The question whether the inert double bond is situated in the same position as in dihydrolanosteryl acetate, or has migrated to a new position during oxidation, cannot yet be decided.

In an attempt to reduce the ketonic groups of diketodihydrolanosterol and compare the reaction product with the original dihydrolanosterol, diketodihydrolanosteryl acetate was subjected to the Clemmensen reduction. The reaction, however, took an abnormal course, yielding saturated colourless *diketolanostanyl acetate*, $C_{32}H_{52}O_4$. This composition and the absence of the high-intensity maximum characteristic of the original chromophoric grouping suggested that hydrogenation of the ethenoid double bond of the chromophore system —CO—C=C—CO— had taken place, the " inert " unsaturated linkage being activated by the adjacent ketonic groups, so that reduction becomes possible. The abnormal course of the Clemmensen reduction was further apparent from the fact that diketodihydrolanosteryl acetate was reduced with equal facility by other reduction methods normally without effect on ketonic groupings. Thus, catalytic hydrogenation, using platinum oxide prepared according to Adams, and the action of zinc and acetic acid, gave high yields of diketolanostanyl acetate. Reduction with sodium and *iso*propyl alcohol gave, owing to simultaneous hydrolysis, diketolanostanol, $C_{30}H_{50}O_3$, the acetate of which was identical with the product obtained by the other methods. *Diketolanostanol* gives on oxidation the corresponding *diketolanostanone*. Owing to the absence of the chromophoric system, these saturated compounds are colourless and show no high-intensity absorption spectrum in the near-ultra-violet; diketolanostanyl acetate, for example,

produces only an absorption maximum, of very low intensity, at $\lambda = 298$ mμ. The keto-groups are unreactive and yield no dioxime or semicarbazone under the usual conditions.

Similar results were obtained with hydrocarbons of the lanosterol series. "α"-Lanostene ("dihydrolanostene"), $C_{30}H_{50}$, and agnostadiene ("γ"-lanostene), $C_{30}H_{48}$, corresponding to dihydrolanosterol and dihydroagnosterol, respectively, have been prepared by the Wolff–Kishner reduction of the ketone-semicarbazones (Ruzicka *et al.*, *loc. cit.*) and by direct reduction of the ketones (Dorée, McGhie, and Kurzer, *J.*, 1947, 1467). Chromic acid oxidation of lanostene, under controlled conditions, gave a diketone, *diketolanostene*. The yellow colour of this, and the occurrence of the absorption maximum at $\lambda = 275$ mμ, indicate the presence of the same chromophore system as in diketodihydrolanosteryl acetate. Chromic acid oxidation of agnostadiene ("γ"-lanostene) also gave diketolanostene, identical with the product from "α"-lanostene. The production of the same oxidation product from both "α"-lanostene and agnostadiene recalls the behaviour of dihydrolanosterol and dihydroagnosterol, both of which are oxidised to the same diketo-derivative, and suggests the same distribution of the chromophore in the respective oxidation products. This view receives further support from the observation that, as in diketodihydrolanosterol, the double bond of the chromophore was readily reduced by the methods described above, yielding the saturated colourless *diketolanostane*.

The ease with which the double bond contained in analogous chromophores can be reduced has been recorded in related compounds. Wieland *et al.* (*Annalen*, **1939**, **539**, 219, 242), for example, when oxidising the dimethylcarbinol of pyroquinovic acid, obtained a yellow diketone, $C_{30}H_{44}O_4$, showing maximum absorption at $\lambda = 270$ mμ, which was readily reduced with zinc and acetic acid to the saturated colourless compound $C_{30}H_{46}O_4$. Similarly, diketones with absorption maxima $\lambda = 273$—275 mμ, containing the chromophore —CO—C=C—CO, have also been obtained from "α" and "β"-elemolic acids by Ruzicka *et al.* (*Helv. Chim. Acta*, 1942, **25**, 1375, 1403): acetyldihydro-"α"-elemolic acid, for example, gave a yellow diketone, $C_{32}H_{48}O_6$ ($\lambda_{max} = 275$ mμ), which yielded colourless reduction products on catalytic hydrogenation.

The similarity between the behaviour of the oxidation products of lanosterol, quinovic acid, and the elemolic acids confirm the analogies already apparent between those compounds. Further reduction of diketolanostanol and diketolanostane should yield a series of saturated compounds corresponding to the unknown lanostane, and experiments in this direction are in progress.

Experimental.

Melting points are uncorrected. Optical rotations were determined in chloroform solution at 17°.

Diketodihydrolanosteryl Acetate.—This was prepared by Ruzicka's method (*Helv. Chim. Acta*, 1944, **27**, 472). Dihydrolanosteryl acetate (50 g.) dissolved in glacial acetic acid (1 l.). when oxidized with a solution of chromium trioxide (50 g.) in 90% acetic acid (250 ml.) for 3 hours, gave 60—70% yields of the diketo-compound, m. p. 157—158°; absorption maximum $\lambda = 275$ mμ ($\log \varepsilon = 4{\cdot}28$).

Diketolanostanyl Acetate.—(a) *By Clemmensen reduction.* A solution of diketodihydrolanosteryl acetate (5 g.; 1 mol.) in glacial acetic acid (200 ml.) was added to amalgamated zinc (40 g.; 60 atoms), and to the boiling mixture concentrated hydrochloric acid (40 ml.; 30 mols.) was added dropwise within 5 minutes. Vigorous reaction set in and the yellow liquid was decolourised within 10 minutes. Refluxing was continued for a total of 1 hour, and the colourless liquid poured off the zinc, which was extracted once again with boiling acetic acid. The combined acetic acid extracts were poured into water (600 ml.), and the crude product was isolated by extraction with ether, and purified by filtration of its light petroleum solution through a small column of alumina (Brockmann, 15 g.). After 2 crystallisations from chloroform–methyl alcohol *diketolanostanyl acetate*, m. p. 222—224°, was obtained in white lustrous plates. No high-intensity absorption in the near-ultra-violet region of the spectrum; low-intensity maximum at $\lambda = 298$ mμ ($E^{1\%}_{1\,cm.} = 2$) (Found: C, 76·82; H, 10·37. $C_{32}H_{52}O_4$ requires C, 76·80; H, 10·40%); $[\alpha]_D + 54{\cdot}6°$ (*c*, 0·535).

(b) *By reduction with zinc and acetic acid.* To a boiling solution of diketodihydrolanosteryl acetate (2 g.; 1 mol.) in glacial acetic acid (100 ml.), zinc dust (12 g.) was added in portions over a period of 10 minutes and refluxing continued for 1 hour. The colourless acetic acid extracts were poured into water and the product isolated and crystallised as described above. Diketolanostanyl acetate, m. p. 222—224° (1·2 g.), was obtained in colourless lustrous plates (Found: C, 76·70; H, 10·25%), and was further identified by hydrolysis to diketolanostanol, m. p. 183—184°, and oxidation to diketolanostanone, m. p. 165—167° (see below).

(c) *By catalytic hydrogenation.* To diketodihydrolanosteryl acetate (2 g.; 1 mol.) dissolved in glacial acetic acid (120 ml.), platinum oxide (0·5 g.), prepared according to Adams, was added, and the reaction mixture shaken at 70° in an atmosphere of hydrogen for 30 minutes. Hydrogen (80 ml.; 95%) was smoothly absorbed, and the initially deep yellow solution turned colourless within 15 minutes. After removal of the platinum, the product was isolated and gave lustrous crystals of diketolanostanyl acetate, m. p. 222—224° (1·8 g.), from which the alcohol, m. p. 183—184°, and the ketone, m. p. 164—167°, were prepared.

(d) *By reduction with sodium and* iso*propyl alcohol.* To a boiling solution of diketodihydrolanosteryl acetate (3 g.; 1 mol.) in anhydrous *iso*propyl alcohol (180 ml.), sodium (8 g.; 60 mols.) was added in portions over ½ hour, and the mixture refluxed for a total of 1½ hours. The deep green solution obtained

was evaporated to small bulk under reduced pressure, diluted with water, and neutralised with hydrochloric acid. The crude product separating was extracted with benzene, the combined extracts filtered through a small column of alumina (Brockmann, 6 g.), and the residue, obtained after removal of the solvent, repeatedly crystallised from chloroform–acetone. A small quantity (0·4 g.) of diketolanostanol (see below), m. p. 183—184°, was obtained in minute felted needles (Found : C, 78·70; H, 10·73. $C_{30}H_{50}O_3$ requires C, 78·60; 10·92%).

The combined mother liquors from the alcohol were evaporated under reduced pressure and the crude diketolanostanol acetylated by heating it for 1 hour to 100° with pyridine (20 ml.) and excess of acetic anhydride (12 ml.). Isolation of the product gave colourless plates of diketolanostanyl acetate, m. p. 222—224°. A small quantity of unchanged yellow diketodihydrolanosteryl acetate (m. p. 154—158°) separated from the mother liquors.

Diketolanostanol.—Diketolanostanyl acetate (2 g.) when boiled under reflux for 2 hours with a solution containing potassium hydroxide (8 g.) in ethanol (100 ml.) gave *diketolanostanol*, m. p. 183—184°, in white felted needles (Found : C, 78·42; H, 10·53%); $[\alpha]_D$ + 25·7°, 25·3° (*c*, 0·560, 0·380).

Diketolanostanone.—Diketolanostanol (2 g.; 1 mol.) was dissolved in a mixture of benzene (60 ml.) and glacial acetic acid (10 ml.). To the clear yellow solution, Kiliani's 10% chromic acid solution (20 ml.) was added dropwise, with efficient mechanical stirring during 10 minutes, the temperature of the mixture being maintained below 5° by external cooling. After vigorous shaking for another 15 minutes, the excess of oxidising agent was removed by means of sulphur dioxide. After separation of the benzene layer the aqueous phase was extracted thrice with light petroleum, and the combined extracts washed with sodium hydroxide solution and water, but no acidic oxidation products were removed. The dried light petroleum extracts were filtered through alumina, the solvent removed under reduced pressure, and the residue crystallised thrice from chloroform–methyl alcohol. *Diketolanostanone*, m. p. 165—167°, was obtained in lustrous colourless plates (Found : C, 78·73; H, 10·48. $C_{30}H_{48}O_3$ requires C, 78·95; H, 10·53%); $[\alpha]_D$ + 121·1 (*c*, 0·385).

Diketolanostene.—(a) *From "α"-lanostene ("dihydrolanostene").* A solution of lanostene (5 g.; 1 mol.) (prepared from dihydrolanosterol according to Ruzicka *et al.*, *Helv. Chim. Acta*, 1944, **27**, 472; and Dorée *et al.*, *loc. cit.*), in glacial acetic acid (100 ml.), was treated, at 85—90°, with excess of chromium trioxide (5 g.; 4 mols.) in 90% acetic acid (30 ml.), and maintained at 90° for 2½ hours. The excess of oxidising agent was removed with sulphur dioxide, the liquid poured into water (400 ml.), and the yellow product extracted with ether. The combined ethereal extracts were washed with sodium hydroxide solution and water, the solvent removed, and the oily residue, after filtration of its light petroleum solution through a column of alumina (Brockmann, 12 g.), repeatedly crystallised from methyl alcohol. *Diketolanostene*, m. p. 119—120°, was obtained in deep yellow lustrous plates (Found : C, 81·85; H, 10·39. $C_{30}H_{46}O_2$ requires C, 82·19; H, 10·50); ultra-violet absorption spectrum : maximum absorption λ_{max} = 273 mμ; ($E^{1\%}_{1\,cm.}$ = 410).

(b) *From agnostadiene ("γ"-lanostene).* Agnostadiene (3 g.; 1 mol.), prepared from dihydroagnosterol (see refs. above), was oxidised with chromium trioxide (3 g., 4 mols.) as before. Crystallisation from methyl alcohol gave diketolanostene, m. p. 119—120°, identical with the product obtained from "α"-lanostene, with which it gave no m. p. depression (Found : C, 81·92; H, 10·45%).

Diketolanostane.—A solution of diketolanostene (2 g.) in glacial acetic acid (80 ml.) was added to amalgamated zinc (15 g.), and to the boiling mixture concentrated hydrochloric acid (15 ml.) added dropwise. After refluxing for 1 hour the product was isolated as described under diketolanostanyl acetate, and gave colourless crystals of *diketolanostane*, m. p. 140—142°. Low-intensity absorption at λ = 298 ($E^{1\%}_{1\,cm.}$ = 3) (Found : C, 81·60; H, 10·72. $C_{30}H_{48}O_2$ requires C, 81·81; H, 10·91%); $[\alpha]_D$ + 104° (*c*, 0·573).

CHELSEA POLYTECHNIC, LONDON, S.W. 3. [*Received, July 1st*, 1947.]