[CONTRIBUTION FROM THE SCHOOL OF CHEMISTRY, GEORGIA INSTITUTE OF TECHNOLOGY]

Some Compounds Derived from Lanosterol

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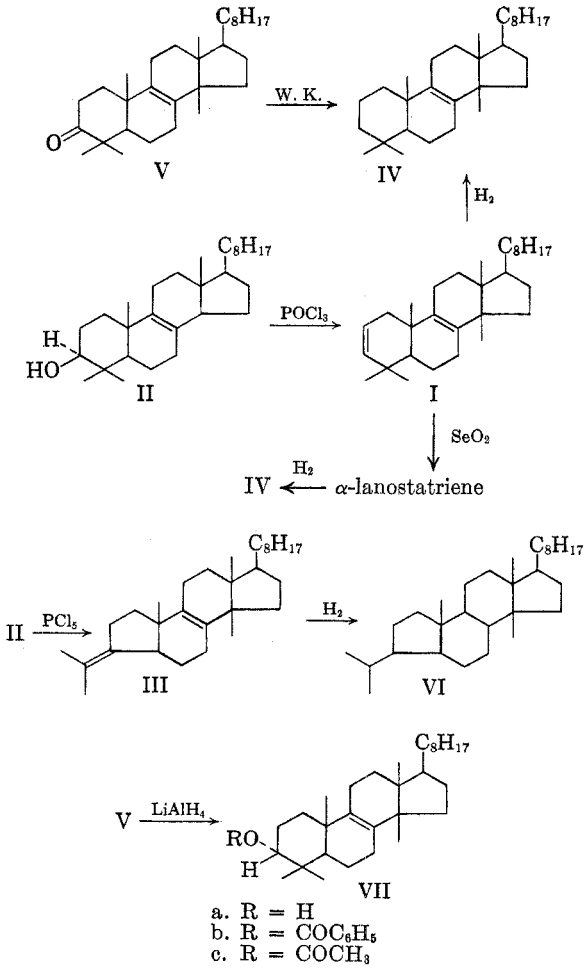
Received September 8, 1958

The selenium dioxide oxidation of $\Delta^{2,8}$-lanostadiene to α-lanostatriene has been reinvestigated, and α-lanostatriene has been shown to be an impure sample of $\Delta^{2,8}$-lanostadiene. Δ^{8}-Lanosten-3α-ol has been prepared and exists with the A ring in the boat form. This observation has been rationalized by conformational arguments.

During the course of the degradation of the tetracyclic triterpene lanosterol, it was reported that the hydrocarbon I, obtained by the dehydration of dihydrolanosterol, Δ^{8}-lanosten-3β-ol, (II) with phosphorus oxychloride in pyridine gave upon oxidation with alcoholic selenium dioxide a nonconjugated triene (α-lanostratriene) $C_{30}H_{48}$.[1]

In the time which has passed since the elucidation of the structure of the lanostane group of triterpenes,[2] and the confirmation of this degradative work by total synthesis,[3] the structure of α-lanostatriene has not been investigated.



In our hands, dehydration of dihydrolanosterol with phosphorus oxychloride in pyridine proceeded in 30% yield to give a hydrocarbon, $C_{30}H_{50}$, which after chromatography on alumina, and several recrystallizations, had m.p. 79–81°. The earlier workers reported that this material melted at 118°. It is known[4] that dehydration of lanostan-3β-ol with phosphorus oxychloride in pyridine affords Δ^{2}-lanostene which is contaminated with several per cent of the isomeric isolanostene. It is possible that our $\Delta^{2,8}$-lanostadiene is similarly contaminated with a small amount of isolanostadiene (III), accounting for our low melting point. Attempts to achieve a separation or purification of this substance, either by chromatography, crystallization, or by partial bromination[4] failed to give higher melting material. In order to confirm that our hydrocarbon was actually $\Delta^{2,8}$-lanostadiene, a portion of the lanostadiene was catalytically hydrogenated using platinum in acetic acid to Δ^{8}-lanostene, (IV) identical to an authentic sample prepared by the Wolff-Kishner reduction of Δ^{8}-lanosten-3-one(V).[5]

The hydrogenation of the diene proceeded smoothly with one mole of hydrogen being taken up. The product was somewhat difficult to crystallize and purify, lending more weight to the argument that our lanostadiene was not completely pure. Since the principal impurity in our diene was in all probability isolanostadiene[4] (III), a sample of isolanostadiene was hydrogenated under the same conditions as were used for $\Delta^{2,8}$-lanostadiene. Much to our surprise 1.8 moles of hydrogen were absorbed readily giving a saturated hydrocarbon, or more probably, a mixture of hydrocarbons, $C_{30}H_{54}$, m.p. 56–59°, VI. This is extremely unusual in view of the normal inertness to hydrogenation of the double bond in the 8:9 position in the lanostane ring system.[2]

This unusual reactivity can be explained as follows.

(1) C. Doree, J. F. McGhie, and F. Kurzer, *J. Chem. Soc.*, 1467 (1947).

(2) J. Simonsen and W. C. J. Ross, *The Terpenes*, Cambridge (1957), Vol. IV, pp. 39–116, give a complete account of the degradation of these compounds.

(3) D. H. R. Barton, D. A. J. Ives, R. B. Kelly, R. B. Woodward, and A. A. Patchett, *J. Am. Chem. Soc.*, **76**, 2852 (1954); *J. Chem. Soc.*, 1131 (1957).

(4) D. H. R. Barton, D. A. Lewis, and J. F. McGhie, *J. Chem. Soc.*, 2907 (1957).

(5) J. F. McGhie, M. K. Pradhan, and J. F. Cavalla, *J. Chem. Soc.*, 3176 (1952).

The steric strain introduced into the B ring of the lanostane skeleton, by the *trans* fusion of the five-membered A ring to it, could permit the relatively facile hydrogenation of the 8:9 double bond, which is twisted somewhat from its normal bond angles. This same strain could also permit the migration of the double bond to some other position in the molecule, (*e.g.* the Δ^6 olefin) by the action of the platinum catalyst.[6]

Hydrogenation of the isopropylidene double bond could then proceed normally, either by direct hydrogenation, or by migration of the isopropylidene double bond into the five-membered ring. Both of these phenomena have been observed in the hydrogenation of β-amyrilene.[7-9] A combination of these possible alternatives indicates that there are sixteen possible stereoisomers for the hydrocarbon VI. For this reason we do not imply any stereochemical relationships in the hydrocarbon VI.

A possible alternative to the explanation that the strained 8:9 double bond migrates during hydrogenation, is that the isolanostadiene does not have the structure assigned to it by earlier workers, but is in fact a double-bond isomer. There is some precedent for the migration of the 8:9 double bond under acid conditions,[1,2] and the preparation of the diene is carried out in acid medium. The position of the isopropylidene double bond has been established adequately by chemical means.[2]

The infrared spectrum of isolanostadiene (CS_2) shows a medium intensity band at 12.33μ which could be due either to the C–H out-of-plane deformation of a trisubstituted olefin, or a hydrogen in an isopropyl group (C-24).[10] However, the proton magnetic resonance spectrum of this compound lacks the peak at 60 to 80c ycles per second above chloroform which is usually present in compounds containing vinyl hydrogens.[11] Inasmuch as the existence of any but tetrasubstituted double bonds in isolanostadiene is excluded, the structure of this compound is as originally formulated, and the possibility of bond migration during dehydration must be ruled out.

Oxidation of I with selenium dioxide in alcohol gave a white compound, m.p. 79–81°, compared with the reported m.p. 82–84° for α-lanostatriene.[1] This material did not depress the melting point of the starting material, and had an identical infrared spectrum. By increasing the reaction time the amount of solid material obtained could be reduced to virtually none, and only yellow oils could be obtained. The analytical figures reported by the English workers for α-lanostatriene, while fitting the formula $C_{30}H_{48}$ better than $C_{30}H_{50}$ (a lanostadiene), are still within the acceptable range for a compound $C_{30}H_{50}$.[12]

Catalytic hydrogenation of our "α-lanostatriene" afforded the same Δ^8-lanostene (IV) obtained by hydrogenation of $\Delta^{2,8}$-lanostadiene or reduction of Δ^8-lanosten-3-one. It would seem very unusual for selenium dioxide, which reacts smoothly with Δ^8-lanostene to yield the conjugated $\Delta^{7,9}$-lanostadiene,[1] and with Δ^8-lanosten-3-yl acetate to yield $\Delta^{7,9}$-lanosten-3-yl acetate,[1,2] to give a nonconjugated triene on reaction with $\Delta^{2,8}$-lanostadiene. The oxidation to a nonconjugated triene would require oxidation at an unactivated carbon atom, *under conditions milder than those used for the oxidations which were cited above.* Doree[1] reported that oxidation of lanostadiene with *N*-bromosuccinimide gave the same "triene," however, in our hands this reaction gave only intractable oils. It is now apparent that "α-lanostatriene" probably does not exist as such but is unoxidized $\Delta^{2,8}$-lanostadiene, recovered from the reaction with selenium dioxide.

In an effort to prepare a sample of $\Delta^{2,8}$-lanostadiene, uncontaminated with any isolanostadiene, a suitable preparation for the C-3 epimer of dihydrolanosterol was sought. It is well known that triterpenoid alcohols, bearing an equatorial hydroxyl group at C-3, dehydrate readily to give a Ring A contracted product,[13] (II→III) while those compounds bearing axial hydroxyl group in this position react with phosphorus pentachloride to give a normal dehydration product.[13,14] It was felt that the best hope for obtaining a reasonable amount of *epi*-dihydrolanosterol, Δ^8-lanosten-3α-ol, was by the lithium aluminum hydride reduction of Δ^8-lanosten-3-one(V). Barton[15] has stated that reduction of sterically hindered ketones with metal hydrides affords axially oriented hydroxyl groups. However, there appear to be relatively few examples in the literature of the reduction of moderately hindered alcohols with metal hydrides. Fieser[16] has reduced 3β-acetoxycholestan-7-one to a mixture of the 3β, 7α, and 3β, 7β diols, while Corey[17] has

(6) The direct hydrogenation would lead to a *cis* B—C ring fusion, while the bond migration would be expected to lead to the more stable *trans* ring fusion. It is difficult however to make similar generalizations regarding the stereochemistry about the isopropyl group, or the A—B ring fusion.

(7) L. Ruzicka, H. Silbermann, and M. Furter, *Helv. Chim. Acta*, **15**, 482 (1932).

(8) L. Ruzicka, H. Silbermann, and P. Pieth, *Helv. Chim. Acta*, **15**, 1285 (1932).

(9) F. S. Spring, *J. Chem. Soc.*, 1345 (1933).

(10) L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, Methuen, London, 1954, pp. 24, 44.

(11) We would like to thank Prof. J. Goldstein and Dr. H. L. Clever of the Department of Chemistry, Emory University, for their kindness in carrying out and interpreting the proton magnetic resonance spectrum of isolanostadiene.

(12) Calcd. for $C_{30}H_{48}$: C, 88.23; H, 11.76. Calcd. for $C_{30}H_{50}$: C, 87.81; H, 12.19. Found: C, 88.12; H, 11.83.

(13) W. Klyne, *Progress in Stereochemistry*, Butterworth's, London, 1954, Vol. I, p. 70.

(14) R. Novak, O. Jeger, and L. Ruzicka, *Helv. Chim. Acta*, **32**, 323 (1949).

(15) D. H. R. Barton, *J. Chem. Soc.*, 1027 (1953).

(16) L. F. Fieser, M. Fieser, and R. N. Chakarvarti, *J. Am. Chem. Soc.*, **71**, 2226 (1949).

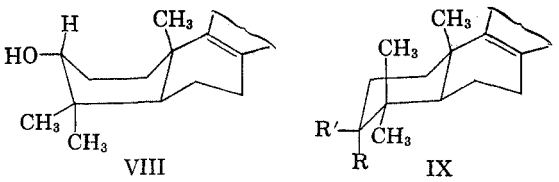
(17) E. J. Corey and J. J. Ursprung, *J. Am. Chem. Soc.*, **78**, 5041 (1956).

found that friedelin affords only the axial alcohol, *epi*-friedelanol, on reduction with lithium aluminum hydride. On the other hand, both β-amyranone[18] and 4,4-dimethylcholestan-3-one[3] afford the equatorial isomers on reduction with metal hydrides.

Reduction of Δ^8-lanosten-3-one with lithium aluminum hydride in ether gave 69% of an alcohol, (VIIa); m.p. 142–143. The melting point was not depressed on mixing with a sample of dihydrolanosterol, however the infrared spectrum (CS_2) showed a medium intensity peak at 12.28μ which was absent from the spectrum of dihydrolanosterol and there were also various other small differences in the spectra of these two materials. The benzoate of the alcohol (VIIb) was prepared and found to have m.p. and mixed m.p. of 193–194°. The acetate of the lithium aluminum hydride reduction product however has m.p. 133–135°, while several workers[2] have found dihydrolanosteryl acetate to melt at 121°. Marker[19] has reported that reduction of lanostenone by the Meerwein-Pondorff-Verley method yield a small amount of an *epi*-dihydrolanosterol, m.p. 139°, acetate m.p. 167.5°. Ruzicka's group,[20] however, found that the same conditions afforded only dihydrolanosterol. This observation coupled with the discrepancies in physical constants between our material and that of Marker make it appear rather doubtful that Marker actually obtained *epi*-dihydrolanosterol.

An interesting consequence of the great similarity of the infrared spectra of our *epi*-dihydrolanosterol and its acetate to that of dihydrolanosterol is the presence of the C—O stretching band at 9.76μ, in both compounds. Jones[21] has found that equatorial, steroidal hydroxyl groups have a C—O stretching band in the vicinity of 9.70μ, while axial substituents show absorption at 9.90μ. The acetates of the epimeric alcohols both have a single strong band at 8.06μ, which has been shown to be characteristic of equatorial acetoxyl groups.[21] Although these empirical rules have been set down for steroidal alcohols and their acetates, they have been applied in the decalin series,[22,23] and are certainly applicable to lanosterol derivatives.

The only explanation, compatible with this spectral evidence is that Δ^8-lanosten-3α-ol exists in the boat form VIII rather than the more conventional chair form IXa, as is also the case in some 2β-bromolanosterol derivatives.[4]



a. R = OH, R′ = H
b. R = H, R′ = OH

Additional evidence for this assignment of conformation can be found in the difference in the reaction of dihydrolanosterol and its epimer with phosphorus oxychloride in pyridine and with phosphorus pentachloride. Reaction of *epi*-dihydrolanosterol with phosphorus pentachloride under precisely the same conditions as used for the preparation of isolanostadiene (III) gave only a low yield of noncrystalline material, as contrasted to the dehydration of the axially substituted *epi*-lupanol to Δ^2-lupene.

The dehydration of the equatorially substituted Δ^8-lanosten-3β-ol with phosphorus oxychloride in pyridine on the steam bath gave a dark brown reaction mixture, from which could be easily isolated $\Delta^{2,8}$-lanostadiene. The same conditions applied to *epi*-dihydrolanosterol gave a colorless reaction mixture from which no organic material could be obtained in the usual manner. This is probably due to the formation of phosphate esters, a phenomenon recently observed in the attempted dehydration of some sterically hindered steroidal alcohols.[24]

If this reaction is carried out at the boiling point of pyridine, a low yield of solid material is obtained, which, while it is undoubtedly hydrocarbon in nature, (eluted readily from an alumina column by hexane) is certainly inhomogeneous, for even after chromatography and several recrystallizations it melts over a 17° range.

Since *epi*-lupanol can be inferred to exist in the normal chair conformation by virtue of its dehydration reactions, and the only significant difference in *epi*-lupanol and *epi*-dihydrolanosterol in the A and B rings is the presence of the 8:9 double bond, it is undoubtedly the presence of this double bond which forces *epi*-lanosterol into the boat form. It is well known that the presence of a 1:2 double bond in a *trans* fused decalin system is thermodynamically unstable.[25,26] The cause of this instability is the abnormal puckering of the saturated carbocyclic ring fused *trans* to the ring containing the double bond.[27]

In dihydrolanosterol (IXb) this puckering has the effect of increasing the distance between the axial

(18) T. R. Ames, T. G. Halsall, and E. R. H. Jones, *J. Chem. Soc.*, 450 (1951).
(19) A. F. Marker and E. L. Wittle, *J. Am. Chem. Soc.*, **59**, 2289 (1937).
(20) L. Ruzicka, R. Denss, and O. Jeger, *Helv. Chim. Acta*, **28**, 759 (1945).
(21) R. N. Jones, P. Humphries, and K. Dobriner, *J. Am. Chem. Soc.*, **73**, 3215 (1951).
(22) W. G. Dauben and E. Hoerger, *J. Am. Chem. Soc.*, **73**, 1504 (1951).
(23) W. G. Dauben, E. Hoerger, and N. K. Freeman, *J. Am. Chem. Soc.*, **74**, 5206 (1952).
(24) E. R. H. Jones, G. D. Meakins, and J. S. Stephenson, *J. Chem. Soc.*, 2158 (1958).
(25) R. B. Turner, W. R. Meador, and R. C. Winkler, *J. Am. Chem. Soc.*, **79**, 4122 (1957).
(26) E. J. Corey and R. A. Sneen, *J. Am. Chem. Soc.*, **77**, 2505 (1955).
(27) J. W. Huffman, Ph.D. thesis, Harvard University, 1957.

methyl groups at C-4 and C-10, and decreasing the distance between the axial hydrogens at C-1, C-3, and C-5. In the chair conformation of *epi*-dihydrolanosterol, the axial hydrogen at C-3 is replaced by the somewhat bulkier hydroxyl group, and in order to decrease the interaction between this hydroxyl group and the axial hydrogens, the A ring assumes the boat form. In the case of *epi*-lupanol, the A ring is not puckered by a double bond in the B ring and the axial-axial interactions are considerably less.

We have made several other attempts to prepare pure $\Delta^{2,8}$-lanostadiene both by pyrolysis of dihydrolanosteryl benzoate, and dehydration with alumina in xylene,[28] however both these methods failed to afford any crystalline material.

EXPERIMENTAL[29]

$\Delta^{2,8}$-Lanostadiene. To a slurry of 4.0 g. of Δ^{8}-lanosten-3-ol[20] in 75 ml. of dry pyridine was added slowly 6.0 ml. of phosphorus oxychloride. The resulting mixture was warmed on the steam bath for 1 hr., during which time the solution became homogeneous, and turned a deep brown color. The reaction mixture was cooled, poured into water, and extracted twice with ether. The ethereal extracts were combined, washed several times with water, and finally with 10% hydrochloric acid. The ether solution was dried, and the solvent removed at reduced pressure, affording a pale yellow oil which partially crystallized on standing. The impure hydrocarbon was dissolved in hexane and filtered through a column of Merck alumina. Removal of the solvent afforded a colorless oil which slowly crystallized. Two recrystallizations from chloroform-methanol gave 1.01 g. (29%) of fluffy white needles, m.p. 72–74°.

A solution of 0.222 g. of this material in hexane was chromatographed on 8.0 g. of neutral alumina, Brockmann activity I. The bulk of the material (0.214 g.) could be accounted for in the first fraction eluted with hexane. This fraction crystallized readily on removal of the solvent, and after recrystallization from chloroform-methanol had m.p. 79–81°. Repeated recrystallization from the same solvent pair did not alter the melting point. Doree and co-workers[1] report a melting point of 116–118° for this compound.

Anal. Calcd. for $C_{30}H_{50}$: C, 87.73; H, 12.27. Found: C, 87.98; H, 12.35.

"α-Lanostatriene." To a solution of 0.50 g. of $\Delta^{2,8}$-lanostadiene in 45 ml. of 95% ethanol was added 0.40 g. of selenium dioxide. The mixture was heated under reflux for 8 hr., cooled, and filtered through Filter-Cel. The resulting clear yellow solution was evaporated to dryness at reduced pressure, and the residual yellow oil taken up in hexane and chromatographed on Merck alumina. Elution with hexane afforded 0.069 g. of white solid, which on recrystallization from chloroform-methanol-acetone gave white crystals, m.p. 81–82°. Doree[1] reported that α-lanostatriene had m.p. 82–84°. Our material had an infrared spectrum (chloroform) identical to that of $\Delta^{2,8}$-lanostadiene, and the two materials on mixing showed no depression in melting point.

Elution of the column with benzene-pentane mixtures afforded only yellow oils from which no solid could be obtained.

Δ^{8}-Lanostene. (a) To a slurry of 0.01 g. of prehydrogenated platinum oxide in 10 ml. of glacial acetic acid was added a solution of 0.085 g. of $\Delta^{2,8}$-lanostadiene (m.p. 72–74°). The reaction mixture was hydrogenated at room temperature and atmospheric pressure, until the uptake of hydrogen ceased; 4.2 ml.[30] (87% for one mole) of hydrogen had been absorbed. The reaction mixture was filtered, water added, and the turbid mixture extracted twice with ether. The ethereal extracts were washed repeatedly with water, and finally with 10% sodium bicarbonate. On removal of the solvent at the water pump, a colorless oil was obtained which partially crystallized on standing. The semisolid was taken up in hexane and chromatographed on activity I alumina. Elution with hexane afforded 0.077 g. (85%) of colorless oil which slowly crystallized. Several recrystallizations from chloroform-methanol afforded white crystals, m.p. 67–69°, identical with an authentic sample of Δ^{8}-lanostene prepared by the Wolff-Kishner reduction of Δ^{8}-lanosten-3-one.[5]

(b) A 0.056 g. sample of α-lanostatriene was hydrogenated by the same method and found to absorb 3.7 ml. of hydrogen (115% for one mole).[30] The product was worked up as in part (a) and 0.034 g. of white crystals, m.p. 69–71° were obtained. This material was also identical to a sample of Δ^{8}-lanostene prepared from Δ^{8}-lanosten-3-one.

Hydrogenation of isolanostadiene. A 0.115 g. sample of isolanostadiene[4] in 10 ml. of glacial acetic acid was hydrogenated in the same manner as $\Delta^{2,8}$-lanostadiene. A total of 11.7 ml. (1.8 moles) of hydrogen was absorbed. On working up the reaction mixture a colorless oil was obtained, which was dissolved in hexane and chromatographed on activity I alumina. Elution with hexane gave 0.054 g. of colorless oil which partially crystallized on standing. Recrystallization from chloroform-methanol gave white crystals m.p. 51–55°. Several additional recrystallizations gave material m.p. 56–59°.

Anal. Calcd. for $C_{30}H_{54}$: C, 86.88; H, 13.12. Found: C, 86.98; H, 12.83.

Δ^{8}-Lanosten-3α-ol. To a stirred suspension of 0.20 g. of lithium aluminum hydride in 15 ml. of dry ether was added 0.35 g. of Δ^{8}-lanosten-3-one.[2] The reaction mixture was stirred at room temperature for 1 hr. The excess hydride was decomposed with a solution of ethyl acetate in dry ether. Water and 10% hydrochloric acid were added, and the aqueous layer drawn off. The ethereal solution was washed with successive portions of water, 5% sodium bicarbonate, and again with water, dried, and the solvent removed at reduced pressure, leaving a waxy solid. Recrystallization from ethyl acetate-methanol gave 0.24 g. (69%) of crystals, m.p. 141–143°. A mixed melting point with Δ^{8}-lanosten-3β-ol (dihydrolanosterol) gave no depression, although their infrared spectra differed. Additional recrystallizations from ethyl acetate-methanol gave material m.p. 142–143°.

Anal. Calcd. for $C_{30}H_{52}O$: C, 84.04; H, 12.23. Found: C, 84.05; H, 12.39.

The benzoate was prepared with benzoyl chloride in pyridine by the method of Wieland.[31] It formed silky needles from chloroform-ethyl acetate-methanol, m.p. 193–194°, undepressed on mixing with a sample of Δ^{8}-lanosten-3β-yl-benzoate.

Anal. Calcd. for $C_{37}H_{56}O_2$: C, 80.97; H, 10.28. Found: C, 81.08; H, 10.50.

The acetate was prepared by the method used for the preparation of Δ^{8}-lanosten-3β-yl acetate,[31] and formed small needles, m.p. 133–135°; from ethyl acetate-methanol, Δ^{8}-

(28) B. Riegel, G. P. Hager, and B. L. Zenitz, *J. Am. Chem. Soc.*, **68**, 2562 (1946).

(29) All melting points were determined on a Kofler hot stage and are uncorrected. Infrared spectra were recorded on a Perkin-Elmer model 137 spectrophotometer, using chloroform or carbon disulfide as a solvent for the sample. Analyses were carried out by Galbraith Analytical Laboratories, Knoxville, Tenn.

(30) The apparatus used was accurate to ±0.5 ml., precluding very precise measurements on a semimicro scale.

(31) H. Wieland, H. Pasedach, and A. Ballauf, *Ann.*, **529**, 68 (1937).

lanosten-3β-yl acetate has been reported to melt at 120–121°.[2]

Anal. Calcd. for $C_{32}H_{54}O_2$: C, 81.63; H, 11.56. Found: C, 82.12; H, 11.76.

Reaction of Δ^8-lanosten-3α-ol with phosphorus oxychloride. (a) To a solution of 0.10 g. of *epi*-dihydrolanosterol in 5.0 ml. of dry pyridine was added 0.20 ml. of phosphorus oxychloride. The homogeneous, colorless reaction mixture was warmed on the steam bath for 2 hr. After about 1 hr. the solution turned milky and deposited a colorless oil. (Compare dihydrolanosterol.) The reaction mixture was poured into water, and extracted twice with ether. The ethereal extracts were washed well with water, and finally 10% hydrochloric acid, and the solvent removed at the water pump. By this procedure approximately 1 mg. of organic material could be recovered. Additional extractions of the aqueous phases with chloroform afforded no organic material.

(b) To a solution of 0.05 g. of *epi*-dihydrolanosterol in 3 ml. of dry pyridine was added 0.10 ml. of phosphorus oxychloride and the mixture heated under reflux for 2 hr. The reaction was worked up as in (a) and a yellow oil obtained which was dissolved in hexane and filtered through an alumina column. On removal of the solvent, a colorless glass was obtained which was crystallized from chloroform methanol to afford 0.010 g. of semicrystalline solid, m.p. 87–94° with previous sintering. Several recrystallizations from the same solvents gave a minute amount of material, m.p. 95–112°.

Reaction of Δ^8-lanosten-3α-ol with phosphorus pentachloride. To a suspension of 0.05 g. of *epi*-dihydrolanosterol in 5.0 ml. of hexane was added 0.05 g. of phosphorus pentachloride. The reaction was stirred 2 hr. at room temperature, and then heated under reflux for an additional hour. The reaction mixture was diluted with ether, washed with successive portions of water, 5% sodium bicarbonate, and again with water, dried, and the solvent removed at reduced pressure. The resulting yellow oil was taken up in hexane and filtered through an alumina column. Removal of the hexane afforded a small amount of colorless oil, which could not be induced to crystallize. Under identical conditions dihydrolanosterol in our hands yields isolanostadiene.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, FORDHAM UNIVERSITY]

Studies on Some Oxidation and Reduction Products of Thiamine. II.[1] Thiamine Disulfide-Thioglycolic Acid Reaction.[2–4]

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Received October 13, 1958

Thioglycolic acid in aqueous solution at *p*H 5 reduces thiamine disulfide (I) to thiamine (IIa). When the reaction conditions are more vigorous, thioglycolic acid displaces the thiazole moiety of thiamine and of oxythiamine (IIb) to give (4-amino-2-methyl-5-pyrimidinylmethylthio)acetic acid (IIIa) and (4-hydroxy-2-methyl-5-pyrimidinylmethylthio)acetic acid (IIIb) respectively and 5-(β-hydroxyethyl)-4-methylthiazole (IV). The structures of IIIa and IIIb were established by Raney-nickel desulfurization to give 4-amino-2,5-dimethylpyrimidine (Va) and 2,5-dimethyl-4-hydroxypyrimidine (Vb) respectively and acetic acid. IIIa was converted to IIIb and Va was converted to Vb by 6*N*-hydrochloric acid at reflux temperature. IIIa was synthesized from 4-amino-5-bromomethyl-2-methylpyrimidine hydrobromide (VI) and thioglycolic acid.

The possibility of vitamin B_1 activity in natural products being due, at least in part, to the biologically active oxidation product thiamine disulfide[6,7] and other reversibly oxidized forms of thiamine[8] led us to modify the thiochrome assay[9] by including a reduction step in the procedure. The reduction of thiamine disulfide to thiamine is necessary because thiamine disulfide is not oxidized to thiochrome by alkaline ferricyanide. We used thioglycolic acid for the reduction of thiamine disulfide[10] in the thiochrome procedure. While investigating this reduction, it was observed that the recovery of thiamine disulfide as thiamine decreased when the thioglycolic acid concentration was too high.[10] This low recovery was thought to be caused by a further reaction between thiamine and thioglycolic acid following the reduction. To test this hypothesis, an aqueous solution of thiamine and three molar equivalents of thioglycolic acid was adjusted to *p*H 5, and refluxed for one hour. The crystalline product, which separated in 70–75% yield on cooling the reaction mixture, analyzed for a compound of empirical formula $C_8H_{11}N_3O_2S$ (IIIa or IIIc). The ether extract of the basified aqueous filtrate yielded 5-(β-hydroxyethyl)-4-methylthiazole (IV) in 65–70% yield, identified as the picrate and picrolonate salts.

(1) Paper I: G. E. Bonvicino and D. J. Hennessy, *J. Am. Chem. Soc.*, **79**, 6325 (1957).

(2) This work was aided by a grant from the Williams-Waterman Fund.

(3) From the dissertation submitted by G. E. Bonvicino in partial fulfillment of the requirements for the degree of Doctor of Philosophy, the Graduate School, Fordham University, 1952.

(4) Presented before the Division of Biological Chemistry, American Chemical Society, (a) 116th Meeting, Atlantic City, N. J., September, 1949; see Abstracts, p. 63C, (b) 117th Meeting, Philadelphia, Pa., April, 1950; see Abstracts, p. 49C.

(5) Present address: Organic Chemical Research Section, Research Division, American Cyanamid Co., Lederle Laboratories, Pearl River, N. Y.

(6) O. Zima and R. R. Williams, *Ber.*, **73**, 941 (1940).

(7) O. Zima, K. Ritsert, and T. Moll, *Z. physiol. Chem.*, **267**, 210 (1941).

(8) M. Fujiwana, H. Watanabe, and K. Matsui, *J. Biochem. (Japan)*, **41**, 29 (1954).

(9) D. J. Hennessy, *Biol. Symposia*, **12**, 111 (1947).

(10) G. E. Bonvicino and D. J. Hennessy, in preparation.