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The Neutral Constituents of the Cactus *Lophocereus schottii*. The Structure of Lophenol—4α-Methyl-Δ⁷-cholesten-3β-ol—A Link in Sterol Biogenesis[1-3]

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The neutral fraction of the cactus *Lophocereus schottii* was found to contain *n*-octyl alcohol, lupeol, an alcohol tentatively identified as Δ⁷-stigmastenol and a new sterol, named lophenol. Degradation experiments are reported which demonstrate that lophenol is 4α-methyl-Δ⁷-cholesten-3β-ol and the biogenetic significance of the occurrence in a plant of a 4-monomethyl sterol is emphasized.

Our interest in the constituents of giant cacti was stimulated originally by the report[4] that the cactus[5] *Lophocereus schottii* (Engelmann) Britton and Rose, was rich in alkaloids and subsequent work in our laboratory led to the isolation[6] and structure elucidation[7] of several new alkaloids. As a result, an extensive investigation[8] of over fifty species of giant cacti was undertaken and this indicated that triterpenoid glycosides—which were found in many of these cacti[8]—did not occur together with alkaloids. In agreement with this postulate was the observation that *Lophocereus schottii* was essentially devoid of glycosides, but while processing rather large amounts of this cactus for our alkaloid studies,[7] it was observed that it contained an appreciable neutral fraction. The present paper is concerned with a description of the separation and identification of the constituents of this mixture.

Initial experiments on the chromatographic fractionation of the neutral fraction were complicated by the presence of large amounts of oily material until it was realized that this was due to the presence of esters. Consequently, the neutral cactus extract was first subjected to saponification followed by steam distillation which furnished nearly 1% (based on dry cactus) of *n*-octyl alcohol. Chromatography of the resulting, semi-solid residue gave a small amount of lupeol (I) and 0.36% (based on dry cactus) of a sterol-like fraction, which was separated into two alcohols called "lophenol" and "schottenol."

The Structure of Lophenol.—The analytical data for lophenol, m.p. 149–151°, $[\alpha]D$ +5°, were consistent with formulations ranging from $C_{27}H_{46}O$ (Calcd.: C, 83.87; H, 11.99) to $C_{30}H_{52}O$ (Calcd.: C, 84.04; H, 12.23) and the correct molecular weight and hence empirical formulas were established only at an advanced stage of our degradation work. The presence of one double bond (*vide infra*) indicated that the substance was tetracyclic and therefore most likely a sterol or triterpene. The alcoholic function was characterized by formation of an acetate (m.p. 119–121°, $[\alpha]D$ +28°) and a benzoate (m.p. 161–163°, $[\alpha]D$ +43°) and by oxidation to a ketone (m.p. 122–124°, $[\alpha]D$ +12°) named lophenone. The infrared spectrum of the ketone was typical of a cyclohexanone derivative and since it was unaffected by base, the double bond could not be located in an isomerizable β,γ-position. Reduction of lophenone with either lithium aluminum hydride or with sodium and alcohol regenerated lophenol. It follows, therefore, that the hydroxyl group of lophenol is secondary, equatorially oriented and attached to a six-membered ring.

Lophenol gave a yellow color with tetranitromethane and its absorption in the far ultraviolet[9] was consistent with the presence of a trisubstituted double bond.[10] Several lophenol samples exhibited also low ultraviolet absorption (log ε 2.6) near 246 mμ, typical of a heteroannular diene impurity and this in turn suggested that lophenol might be triterpenoid,[11] since many naturally occurring tetracyclic triterpenes are found admixed with the corresponding $\Delta^{7,9(11)}$-diene. With one exception,[12] all tetracyclic triterpenes are characterized by a 3-hydroxy-4,4-dimethyl grouping (II) (or the derived ketone) and the equatorially oriented 3β-hydroxy triterpenes undergo a characteristic *retro*-pinacolinic change (III) upon treatment with phosphorus pentachloride.[11] When lophenol was subjected to these conditions, a chloride—subsequently shown to be XI—was isolated, which implied that the grouping II could not be present in lophenol.

More precise information about the nature of the double bond could be adduced by hydrogenation experiments. Lophenol acetate was not reduced by platinum oxide in glacial acetic acid solution but rather was transformed into an isomer, m.p. 79–81°, which in turn could be isomerized with hydrogen chloride in chloroform to an acetate, m.p. 133–136°. The latter substance could also be obtained in one step by exposing lophenol acetate to hydrogen chloride—chloroform and the

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(2) The contents of this paper were first presented in a lecture at the University of Osaka, Osaka, Japan, on March 11, 1958.

(3) For preliminary communication on the structure of lophenol see C. Djerassi, J. S. Mills and R. Villotti, THIS JOURNAL, **80**, 1005 (1958).

(4) G. Heyl, *Arch. Pharm.*, **239**, 451 (1901).

(5) At that time the cactus was referred to as *Pilocereus sargentianus* Orcutt.

(6) C. Djerassi, N. Frick and L. E. Geller, THIS JOURNAL, **75**, 3632 (1953).

(7) C. Djerassi, S. K. Figdor, J. M. Bobbitt and F. X. Markley, *ibid.*, **78**, 3861 (1956); **79**, 2203 (1957); C. Djerassi, T. Nakano and J. M. Bobbitt, *Tetrahedron*, **2**, 58 (1958).

(8) For review see C. Djerassi in "Festschrift Arthur Stoll," Birkhäuser, Basel, 1957, pp. 330–352.

(9) We are indebted to Dr. G. D. Meakins (University of Manchester) and Prof. T. Reichstein (University of Basel) for measurements in the 195–220 mμ region which were conducted with a Unicam SP 500 spectrophotometer.

(10) P. Bladon, H. B. Henbest and G. H. Wood, *J. Chem. Soc.*, 2737 (1952); T. G. Halsall, *Chemistry & Industry*, 867 (1951).

(11) For leading references see E. R. H. Jones and T. G. Halsall in L. Zechmeister's "Progress in the Chemistry of Organic Natural Products," Springer, Vienna, 1955, Vol. XII, pp. 44–130.

(12) J. S. G. Cox, F. E. King and T. J. King, *Proc. Chem. Soc.*, 290 (1957).

double bond was now reducible with platinum oxide catalyst. Saturation of the double bond of lophenol could be accomplished in one step by conducting the hydrogenation in the presence of hydrochloric or perchloric acid. This behavior is typical of steroidal 5α-Δ^7- or Δ^8-alcohols,[13] the reaction proceeding *via* the non-reducible $\Delta^{8(14)}$-isomer to the Δ^{14}-stenol, whose double bond can now be hydrogenated. The possibility that lophenol may have a double bond in position 8–9 rather than 7–8 was already rendered unlikely by the above-mentioned ultraviolet spectral data[9,10] and could be eliminated completely by oxidation evidence mentioned below. Furthermore, lophenol acetate was found to react with mercuric acetate[14,15] in the manner characteristic[16] of Δ^7-stenols to yield the corresponding $\Delta^{7,9(11)}$-diene (*e.g.*, XII) with the typical triple ultraviolet absorption maxima at 234, 242 and 250 mμ. As was to be expected, lophenol gave a positive Tortelli–Jaffé[17] and selenium dioxide[18] test.

By anticipating the evidence presented below for structure IV for lophenol and its functional derivatives, the platinum oxide–acetic acid rearrangement product can be assigned structure VI, the hydrogen chloride–chloroform promoted isomer is represented by VII and the saturated alcohol, lophanol, by VIII.

While the above-mentioned reactions were all in agreement with a Δ^7-sten-3β-ol formulation for lophenol, molecular rotation calculations (Table I) demonstrated a striking difference. Assuming an intact sterol skeleton, the stereochemistry of the A/B ring juncture could not be implicated since the optical rotatory dispersion curves[19] of lophenone (V) and the saturated ketone lophanone (IX) are characterized by a single, positive Cotton effect[20] and hence bear the same relationship to each other as do Δ^7-unsaturated[21] and saturated[22] 3-keto steroids of the cholestane series.

At this point of the investigation, there appeared a paper by de Mayo and Reed[23] in which was reported a method for the mass spectrographic determination of molecular weights of hydrocarbons of the steroid and triterpene series. Consequently, the saturated alcohol lophanol (VIIIa) was oxidized to the ketone lophanone (IX) and the latter converted by Huang-Minlon's modification[24] of the Wolff–Kishner reduction to the parent hydrocarbon, lophane (X). The molecular weight of lophane (X), obtained[25] by the mass spectrographic procedure,[23] corresponded exactly to $C_{28}H_{50}$ (calcd. 386.7, found[25] 386.3 ± 0.6), identical with that of ergostane. The physical constants of lophane (X) and ergostane[26] were quite similar, but identity was already precluded from the great rotation differences of the precursors. Furthermore, the observation was made[25] that at low electron potential, lophane (in contrast to ergostane) yielded a mass corresponding to 372 and the loss of methyl was further corroborated by detecting its mass.

Since the grossly abnormal molecular rotation calculations (Table I) of members of the lophenol series could not possibly have been due to differences in the side chain (as compared to the analogous ergostane derivatives), a number of oxidation experiments were conducted with lophenol acetate (IVb) and its isomers VI and VII. Such studies were expected to shed more light upon the immediate environment of the unsaturated system and at the same time provide additional ketones suitable for rotatory dispersion analysis[19] and determination of the stereochemistry of centers in close proximity to such newly formed carbonyl groups.

Oxidation at 60° of lophenol acetate (IVb) in acetic acid solution with chromium trioxide furnished in poor yield two isomeric acetoxy keto-epoxides, m.p. 190–192°, $[\alpha]_D$ ±0°, and m.p. 175–176°, $[\alpha]_D$ −45°. By analogy to similar oxidation products in the $\Delta^{7,22}$-ergostadien-3β-ol[27] and Δ^7-cholesten-3β-ol[28] series, the more dextrorotatory isomer is assigned the 7-keto-8,9-oxido structure XIII, while the more levorotatory product is represented by the 7-keto-8,14-oxide XIV. The two keto epoxides XIII and XIV were obtained in improved yield by conducting the oxidation of lophenol acetate (IVb) stepwise[28] with perbenzoic acid followed by chromium trioxide; treatment with zinc[27] furnished the Δ^8-7-ketone (XVa) and $\Delta^{8(14)}$-7-ketone (XVI), respectively. The characteristic ultraviolet absorption spectra of these two unsaturated ketones served to confirm the structures assigned to the precursor keto epoxides XIII and XIV. Either one of these keto epoxides was transformed by means of hydrochloric acid to the identical $\Delta^{8(9),14}$-dien-7-one XVII with the characteristic[28] ultraviolet absorption maxima at 223.5 and 299 mμ.

Since the analogous transformation products of Δ^7-ergosten-3β-ol acetate (XXIb) were unknown, these were prepared by the procedures already employed earlier[27,28] with the corresponding $\Delta^{7,22}$-dienes and led to the two keto epoxides and the $\Delta^{8,(9),14}$-dien-7-one corresponding to XIII, XIV and XVII. The ultraviolet and infrared carbonyl

(13) For a complete survey of the literature see H. P. Sigg and T. Reichstein, *Helv. Chim. Acta*, **39**, 1507 (1956).

(14) Using the conditions of G. Saucy, P. Geistlich, R. Helbling and H. Heusser, *ibid.*, **37**, 250 (1954).

(15) The bromination sequence of R. C. Anderson, R. Stevenson and F. S. Spring, *J. Chem. Soc.*, 2901 (1952), also was examined, but the resulting diene was found to be contaminated with some starting material.

(16) A. Windaus and E. Auhagen, *Ann.*, **472**, 185 (1929).

(17) See R. Tschesche and G. Snatzke, *Ber.*, **88**, 511 (1955).

(18) L. F. Fieser, THIS JOURNAL, **75**, 4395 (1953), noted that a positive reaction with selenium dioxide is given by Δ^7- as well as by Δ^8-stenols with the 5α- but not 5β-orientation, thus affording some evidence for the stereochemistry of lophenol at that center.

(19) For leading references and introduction to use of rotatory dispersion data see C. Djerassi, *Bull. soc. chim. France*, 741 (1957).

(20) For nomenclature see C. Djerassi and W. Klyne, *Proc. Chem. Soc.*, 55 (1957).

(21) C. Djerassi, O. Halpern, V. Halpern and B. Riniker, THIS JOURNAL, **80**, 4001 (1958).

(22) C. Djerassi, W. Closson and A. E. Lippman, *ibid.*, **78**, 3163 (1956).

(23) P. de Mayo and R. I. Reed, *Chemistry & Industry*, 1481 (1956).

(24) Huang-Minlon, THIS JOURNAL, **71**, 3301 (1949).

(25) We are greatly indebted to Drs. de Mayo and Reed (University of Glasgow) for the mass spectrographic results.

(26) E. Fernholz, *Ber.*, **69**, 1792 (1936).

(27) (a) H. E. Stavely and G. N. Bollenback, THIS JOURNAL, **65**, 1290 (1943); (b) H. Heusser, G. Saucy, R. Anliker and O. Jeger, *Helv. Chim. Acta*, **35**, 2090 (1952); (c) L. F. Fieser and G. Ourisson, THIS JOURNAL, **75**, 4404 (1953).

(28) L. F. Fieser, K. Nakanishi and W. Y. Huang, *ibid.*, **75**, 4719 (1953); see also O. Wintersteiner and M. Moore, *ibid.*, **65**, 1507 (1943).

bands of these transformation products of the two series—lophenol and Δ^7-ergosten-3β-ol (XXI)—were identical but the actual compounds differed. It follows that lophenol and Δ^7-ergosten-3β-ol (XXIa), though isomeric, could not just differ at C-9 and/or C-14 since these centers were destroyed in the diene XVII.

Additional oxidations were performed on $\Delta^{8(14)}$-(VIb) and Δ^{14}-lophenol acetate (VIIb) except that *t*-butyl chromate[27b,29] was selected as the oxidizing agent. The principal identified oxidation products of $\Delta^{8(14)}$-lophenol acetate (VIb) were the 7-keto-8,14-epoxide XIV and the 7,15-diketo-8,14-diol XVIII. These two ketones also were produced in the *t*-butyl chromate oxidation of Δ^{14}-lophenol acetate (VIIb) together with the desired 16-keto-Δ^{14}-lophenol acetate (XIX).

The above oxidation products together with lophenone (V) offered a sufficient number of diversely substituted ketones so that an assignment of configuration could be made at positions 5, 9, 10, 13, 14 and 17 by comparisons of optical rotatory dispersion curves. As indicated before, the positive Cotton effect curves (see Experimental) of lophenone (V) and lophanone (IX) imply a 5α,10β-orientation. Comparison of the optical rotatory dispersion curve (see Experimental) of 16-keto-Δ^{14}-lophenol acetate (XIX) with that[30] of 16-keto-Δ^{14}-cholesten-3β-ol benzoate (XX)[31,32] showed such striking similarities that the orientation of C-13 and C-17 had to be identical[19] in both compounds. There remained only the determination of the orientation of C-9 and C-14. Since the optical rotatory dispersion curves of the two keto epoxides XIII and XIV closely resembled (see Experimental and Fig. 1) those of the corresponding keto epoxides in the Δ^{22}-ergosten-3β-ol acetate series,[27b] a similar resemblance also applying to the dispersion curves (Fig. 1) of 7-keto-Δ^8-lophenol acetate (XVa) and 7-keto-Δ^8-cholesten-3β-ol acetate (XVb), it seems safe to assign the 9α,14α-orientation to lophenol.

The above chemical and rotatory dispersion results can be summarized by stating that lophenol must be related, structurally and stereochemically, to a typical Δ^7-3β-ol of the 5α-steroid series. There remained only the question of the abnormal rotation behavior (Table I) of lophenol and its esters. A decisive clue was provided by the elegant side chain determination procedure of Jones and collaborators[33] which demonstrated clearly that lophenol could only possess a C_8-side chain of the cholestane type, since no evidence for the presence of a C_9-fragment (as found in C_{28} steroids of the ergostane series) could be detected. The coincidence of the various rotatory dispersion curves with those of standard steroid ketones virtually established the presence of an unrearranged steroid skeleton (requiring 19 carbon atoms) and since the mass spectrographic examination[28,25] had proved a molecular weight based on C_{28}, an extra methyl group had to be present in the ring. Positions 9 and 14 were excluded as possible sites because of the formation of a 7-keto-8,14-diene (XVII) and this also applied to C-5 because of the regeneration of lophenol (IVa) upon reduction of lophenone (V) with both lithium aluminum hydride and sodium–ethanol.[34]

TABLE I

MOLECULAR ROTATION DIFFERENCES[a]

3β-OH-5α	Δ_1 ROAc–ROH	Δ_2 ROBz–ROH	Δ_3 R=O–ROH
-ane	− 29	+ 5	+73
7-ene	− 10	+ 18	+96
8(14)-ene	− 40	− 44	+75
14-ene	− 30	+ 28	+71
Lophane	+ 78	...	− 9
—7-ene	+104	+192	+28
—8(14)-ene	+106	+126	..
—14-ene	+ 75	...	..

[a] The standard steroidal values are taken from W. Klyne in E. A. Braude and F. C. Nachod "Determination of Organic Structures by Physical Methods," Academic Press, Inc., New York, N.Y., 1955, p. 112.

If an extra methyl substituent were indeed responsible for the abnormal rotation values (Table I), then it clearly had to be in close vicinity to the hydroxyl group. Early attempts to establish this point by determining the number of hydrogen atoms adjacent to the carbonyl group of lophanone (IX) by exhaustive bromination[35] failed, apparently due to spontaneous dehydrobromination and consequent excessive bromine up-take.[36] The course of the monobromination of lophanone is recorded in the Experimental section.

Concurrent work in this Laboratory[37] on the detection and stereochemical implications of hemiketal formation by rotatory dispersion means[38] has demonstrated that hemiketal formation (with methanol) is largely inhibited when (a) a new 1,3-diaxial interaction is set up in the hemiketal or (b) when the ketone is flanked by even one methyl group. Thus while a 3-keto-5α-steroid such as

(29) R. V. Oppenauer and H. Oberrauch, *Anal. Asoc. Quim. Argent.*, **37**, 246 (1949); see also K. Heusler and A. Wettstein, *Helv. Chim. Acta*, **35**, 284 (1952).

(30) C. Djerassi, J. Osiecki and W. Herz, *J. Org. Chem.*, **22**, 1361 (1957), Fig. 3.

(31) K. Tsuda and R. Hayatsu, THIS JOURNAL, **78**, 4107 (1956).

(32) A change in the nature of the ester function (acetate in XIX *vs.* benzoate in XX) has no particular effect upon the rotatory dispersion curve (see C. Djerassi and W. Closson, *ibid.*, **78**, 3761 (1956)).

(33) J. S. G. Cox, L. B. High and E. R. H. Jones, *Proc. Chem. Soc.*, 234 (1958). This pyrolytic vapor phase chromatographic procedure permits the determination of the size of the side chain in steroids and terpenoids. We are very grateful to Prof. Jones for acquainting us with this method prior to publication and to Dr. L. B. High of Oxford University for the actual determinations.

(34) If a methyl group had been located at C-5, then lithium aluminum hydride would have produced a different (axial) alcohol as has already been observed in the friedelin series (see G. Brownlie, F. S. Spring, R. Stevenson and W. S. Strachan, *J. Chem. Soc.*, 2419 (1956)).

(35) C. S. Barnes, D. H. R. Barton, A. R. H. Cole, J. S. Fawcett and B. R. Thomas, *ibid.*, 571 (1953), employed this technique with certain steroid and triterpenoid ketones which, however, did not possess an alkyl group at an adjacent replaceable center.

(36) Recently (C. Djerassi and S. Burstein, THIS JOURNAL, **80**, 2593 (1958)) it was observed in the steroid and sesquiterpene series that bromination of a ketone such as 4α-methyldihydrotestosterone proceeds by bromination, spontaneous dehydrobromination and rebromination in the allylic position.

(37) C. Djerassi, L. A. Mitscher and B. J. Mitscher, *ibid.*, **81**, in press (1959).

(38) This is done simply by measuring the rotatory dispersion of a ketone in methanol solution, adding a drop of hydrochloric acid and repeating the dispersion measurement. Since the hemiketal would be expected to give a plain dispersion curve, similar to that of the corresponding alcohol, the extent to which the amplitude of the anomalous dispersion curve of the ketone is reduced is a semi-quantitative indication of the extent of hemiketal production.

cholestan-3-one suffers a reduction in rotatory dispersion amplitude by about 65%, lophanone (IX) was only affected to the extent of about 17%.

The position of the carbonyl group of lophenone (and lophanone), and hence of the hydroxyl group of lophenol (IVa), at C-3 was already indicated by the rotatory dispersion curves. Consequently, the additional methyl group had to be located at C-2 or C-4 and direct comparison of lophanone (IX) with synthetic[39] 4α-methylcholestanone by means of mixture melting point determination, infrared comparison and coincidence of optical rotatory dispersion curves established the C-4 location. Lophenone (V) proved to be stable to treatment with either base or acid, thus indicating the equatorial character of the C-4 methyl group. However, since there exists some indication[39b] that axial 4β-methyl groups are not readily inverted, further confirmation was provided by converting synthetic[39] 4α-methylcholestan-3-one by means of lithium aluminum hydride into 4α-methylcholestan-3β-ol and thence by acetylation to the corresponding acetate. These two specimens proved to be identical with lophanol (VIIIa) and its acetate (VIIIb) and since the formation of these two substances from lophenol did not involve conditions which could have caused inversion of the original methyl group of lophenol (IVa), this alcohol can now be given the systematic name 4α-methyl-Δ^7-cholesten-3β-ol. Quite recently,[40] this alcohol has been synthesized from cholesterol and comparison of the natural and synthetic specimens demonstrated their identity.

The Structure of "Schottenol."—As indicated in the introduction to this paper, lophenol was always accompanied by a companion of similar polarity from which it could be separated only with difficulty. This companion alcohol, which was named "schottenol," was slightly more polar and even after rechromatography and eighteen recrystallizations, still exhibited a 10° melting point range. Finally, it was noted fortuitously that selective precipitation with hexane from benzene solution effected purification and material which had been so treated could then be recrystallized to a constant and sharp melting point. The alcohol was characterized further as the acetate, benzoate and ketone and the relevant constants are summarized in Table II together with those of the literature values for Δ^7-ergosten-3β-ol (XXI) and values found in our laboratory for a synthetic specimen of XXI and its derivatives. The analytical results were compatible with a $C_{28}H_{50}O$ formulation and mixture melting point determinations and infrared comparisons suggested that schottenol was indeed Δ^7-ergosten-3β-ol (XXI).

However, when a sample of the derived ketone, "schottenone" was transformed by Wolff–Kishner reduction into the unsaturated hydrocarbon "schottene" and the latter subjected to mass spectrographic analysis,[23,25] a molecular weight of 398 was obtained in four runs. This result definitely excluded Δ^7-ergosten-3β-ol (XXI) from further consideration (Δ^7 ergostene = 384.6) and strongly pointed toward stigmastene (XXIV) ($C_{29}H_{50}$ = 398.7) or an isomer thereof. The presence of an "extra" methyl group at C-4 in schottenone (XXIII) was excluded by the observation[38] that the amplitude of its rotatory dispersion curve in methanol solution was reduced by *ca.* 65% upon addition of hydrochloric acid, a behavior typical[37] of ring A unsubstituted 3-keto-5α-steroids. Furthermore, vapor phase chromatographic determination[33] of the size of the side chain indicated a C_{10}-fragment, as was also observed in a parallel run with stigmasterol.

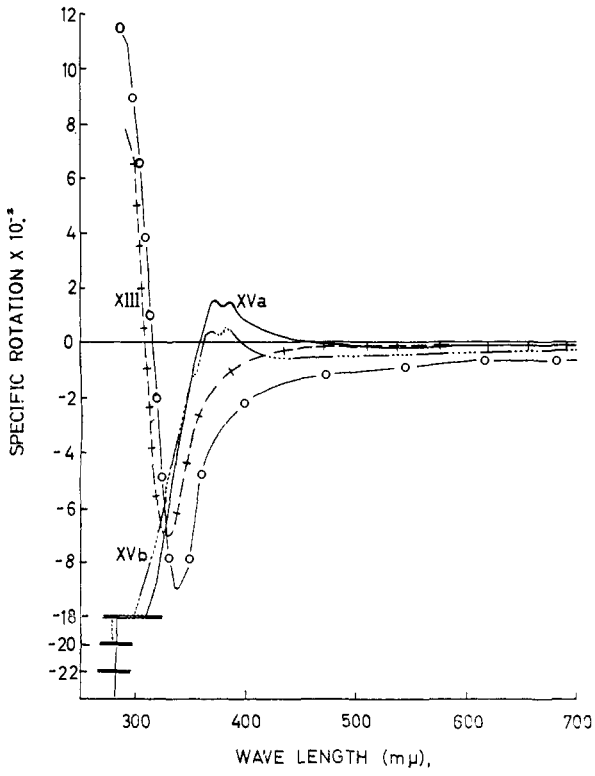


Fig. 1.—Optical rotatory dispersion curves of 8α,9α-oxidolophan-3β-ol-7-one acetate (XIII) (methanol), 8α,9α-oxido-Δ^{22}-ergosten-3β-ol-7-one acetate (—O—O—O—), (dioxane), Δ^8-lophen-3β-ol-7-one acetate (XVa) (dioxane) and Δ^8-cholesten-3α-ol-7-one acetate (XVb) (dioxane).

The mass spectrographic[25] and vapor phase chromatography[33] results require that "schottenol" be Δ^7-stigmasten-3β-ol (XXIIa) or an isomer thereof. In Table II are included the reported constants for Δ^7-stigmasten-3β-ol ("γ-spinastenol") and its derivatives. The melting points of "schottenol" and Δ^7-stigmasten-3β-ol and their respective benzoates are in good agreement and a mixture melting point determination of "schottenol benzoate" with an authentic sample of Δ^7-stigmasten-3β-ol benzoate (XXIIc)[41] was undepressed. Nevertheless, there exists a consistent difference in the specific rotations of the various pairs (see Table II) which points toward the presence of an

(39) (a) G. D. Meakins and O. R. Rodig, *J. Chem. Soc.*, 4679 (1956), (b) J. L. Beton, T. G. Halsall, E. R. H. Jones and P. C. Phillips, *ibid.*, 753 (1957).

(40) Y. Mazur and F. Sondheimer, THIS JOURNAL, **80**, 6293 (1958). We are indebted to Dr. Sondheimer (Weizmann Institute of Science) for informing us of his unpublished results and for carrying out the comparison in his laboratory.

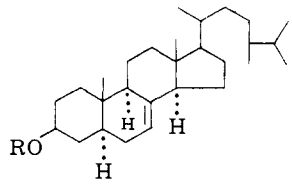
(41) D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 1354 (1948); we are indebted to Prof. Barton (Imperial College of Science and Technology) for this sample.

TABLE II

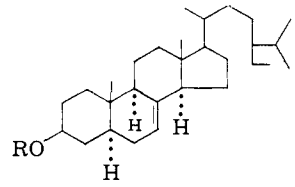
Derivative	"Schottenol" M.p., °C.	"Schottenol" $[\alpha]_D^a$	Δ^7-Stigmasten-3β-ol (XXII) Lit. values[41,b] M.p., °C.	Δ^7-Stigmasten-3β-ol (XXII) Lit. values[41,b] $[\alpha]_D^a$	Δ^7-Ergosten-3β-ol (XXI) Lit. values[65] M.p., °C.	Δ^7-Ergosten-3β-ol (XXI) Lit. values[65] $[\alpha]_D^a$	Δ^7-Ergosten-3β-ol (XXI) Exptl. this paper M.p., °C.	Δ^7-Ergosten-3β-ol (XXI) Exptl. this paper $[\alpha]_D^a$
Alcohol	148–150	+ 4°	144–145	+11°	148	− 4°	149–151	± 0°
Acetate	168–170	− 3.6	156–157	+ 8	157–159	− 2	166–168	+ 4.3
Benzoate	183–185	+ 3.5	180.5	+13	180.5	+ 2	180–182	− 1.9
Ketone	152–154	+30	.....	...	159	+22	159–161	+20

[a] All rotations in chloroform solution. [b] D. R. Idler, A. A. Kandutsch and C. A. Baumann, THIS JOURNAL, **75**, 4325 (1953).

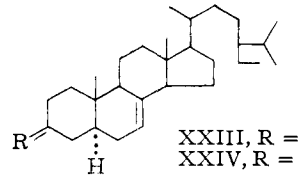
impurity or a slight stereochemical change. The 3β-orientation of the alcohol follows from the molecular rotations and the ready precipitability of "schottenol" with digitonin. The location of the double bond at 7–8 was not established with the same degree of certainty as was done with lophenol, but a micro-experiment with mercuric acetate led to a $\Delta^{7,9(11)}$-diene, recognized by the triple ultraviolet absorption maxima at 235, 242.5 and 251 mμ. We conclude tentatively, therefore, that "schottenol" is probably identical with Δ^7-stigmasten-3β-ol ("γ-spinastenol") and that the divergence in the rotation values (Table II) can be ascribed to an impurity.



XXIa, R = H
b, R = CH_3CO
c, R = C_6H_5CO



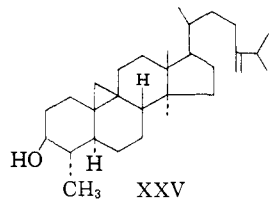
XXIIa, R = H
b, R = CH_3CO
c, R = C_6H_5CO



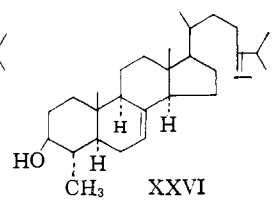
XXIII, R = O
XXIV, R = H_2

Biogenetic Implications.—Until recently,[42] cholesterol has not been encountered in the plant kingdom[43] and the isolation from a cactus of lophenol—a C_{28}-member of the cholestane group—is, therefore, unusual. More important, however, is the occurrence of a 4-monomethyl sterol in nature. Prior to our structure elucidation[3] of lophenol, the only related authentic example was cycloeucalenol (XXV),[12] a 4-monomethyl triterpenoid. Concurrently with our preliminary communication,[3] there appeared one by Mazur, Weizmann and Sondheimer[44a] in which it was shown that citrostadienol, isolated[44b] from orange and grapefruit peel-oil, possessed structure XXVI. It is noteworthy that just as in the presently studied cactus *Lophocereus schottii*, which contains a triterpene (lupeol (I)), a sterol (schottenol (XXIIa)) and a 4-monomethyl sterol (lophenol (IVa)), citrostadienol (XXVI)[44a] is accompanied[44b] in orange peel-oil by the triterpene friedelin and the sterol β-sitosterol.

At present, these two observations represent the most direct evidence for the suggestion[45] that squalene or a squalenoid precursor is also concerned with sterol biogenesis in plants. In contrast to the impressive experimental evidence[45] for such a biogenetic course in animals, which is based largely on the incorporation of radioactive labeled squalene and other precursors into cholesterol and thence the other animal steroids, such evidence is not yet available in plants. Indeed, the likeliness of squalenoid precursors operating in the biogenesis of plant triterpenoids, which has led to the present highly unified picture[46]—consistent with current stereochemical and mechanistic views—was largely the result of isolating and establishing the structures of "missing links." These "missing links" appear to be products of incomplete or alternate cyclization and/or methyl migration schemes, which can all be derived from squalene.[47] As pointed out by Robinson in another connection,[48] biogenetic speculation "must be judged at this stage by the degree of coincidence which called for it." This "degree of coincidence" seems quite adequate with the large body of diverse triterpenoid types[12,46,47] but still very deficient with respect to plant sterols. To that extent, the isolation and structure elucidation of lophenol (IV)[3] and citrostadienol (XXVI)[44a] and their co-occurrence with sterols and triterpenes is important, since in the absence of direct biochemical experimentation, this represents the kind of circumstantial evidence which contributes greatly to the "degree of coincidence" and permits the inclusion of plant sterols in this unifying picture[45–47] by assuming demethylation of squalenoid cyclization products as already demonstrated[45] in animal tissues.



XXV



XXVI

While additional "missing links" undoubtedly will

(42) K. Tsuda, S. Akagi and Y. Kishida, *Science*, **126**, 927 (1957), have reported the isolation of cholesterol from several algae.

(43) A detailed survey of sterols encountered in plants is given by A. Stoll and E. Jucker in K. Paech and M. V. Tracey "Moderne Methoden der Pflanzenanalyse," Springer, Berlin, 1955, Vol. III, pp. 142–148.

(44) (a) Y. Mazur, A. Weizmann and F. Sondheimer, THIS JOURNAL, **80**, 1007 (1958); (b) A. Weizmann and Y. Mazur, *J. Org. Chem.*, **23**, 832 (1958).

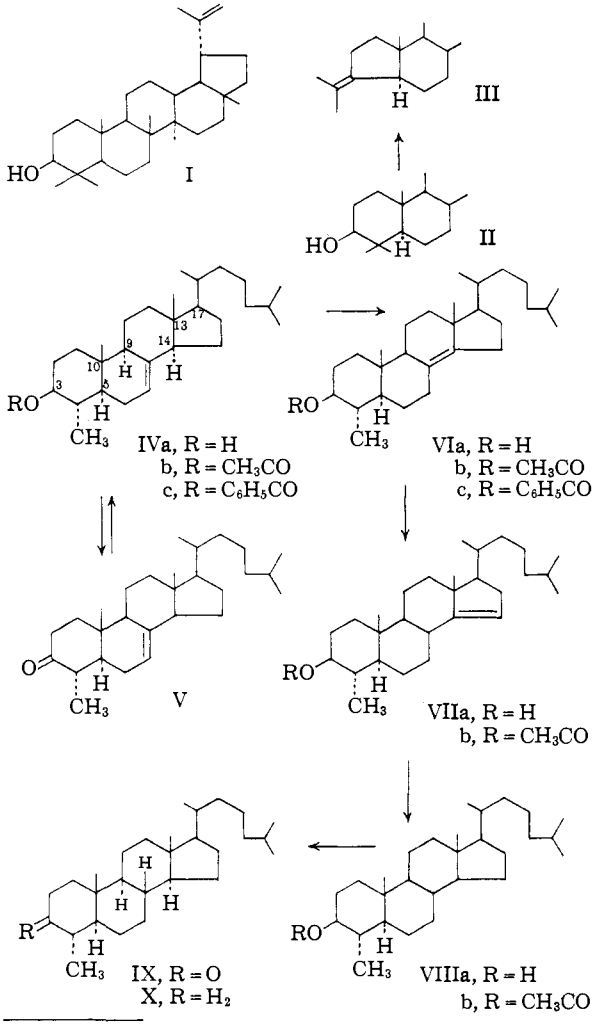
(45) For a recent review see K. Bloch, "Vitamins and Hormones," Academic Press, Inc., New York, N. Y., 1957, Vol. XV, pp. 119–150.

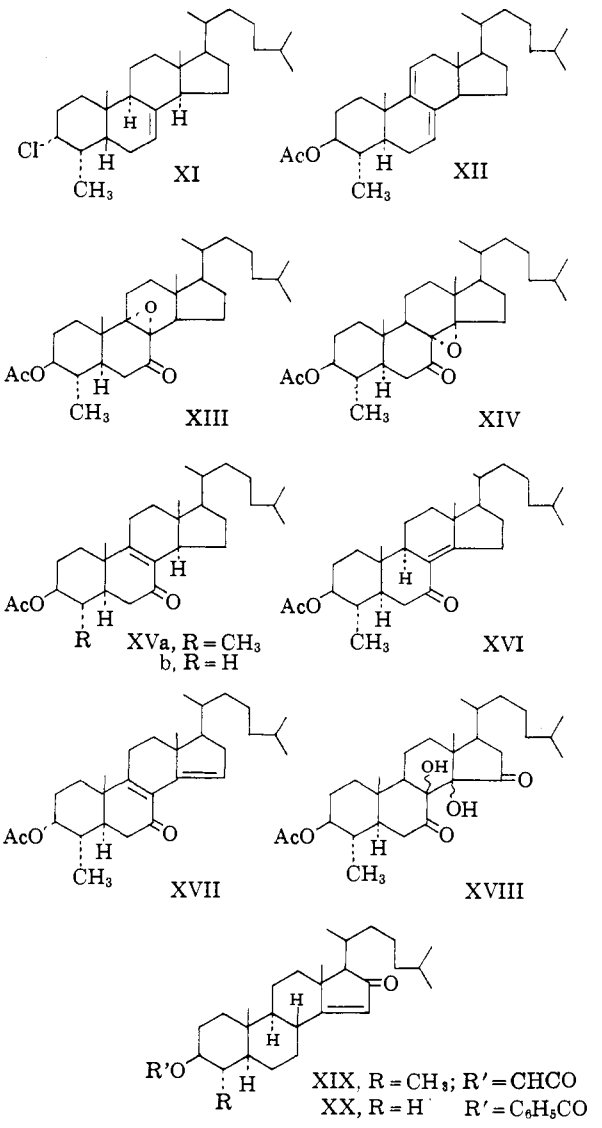
(46) L. Ruzicka, *Experientia*, **9**, 357 (1953); A. Eschenmoser, L. Ruzicka, O. Jeger and D. Arigoni, *Helv. Chim. Acta*, **38**, 1890 (1955).

(47) For a recent review see P. Crabbé and G. Ourisson, *Ind. chim. Belg.*, **22**, 1309 (1957).

(48) R. Robinson, *Nature*, **162**, 155 (1948).

be found in nature,[49] direct biochemical verification for the role of squalenoid precursors in the biogenesis of plant sterols seems essential.[50] Once the technical aspects of such biochemical experiments are solved, the cactus *Lophocereus schottii* might represent an excellent substrate. Not only does it offer the possibility of studying the biosynthesis of a triterpene and a sterol at the same time, but it might also be interesting to examine more closely the origin of the isopentyl fragment in the alkaloids[7] pilocereine and piloceredine by using suitable precursors of the mevalonate type.





Experimental[51]

Isolation of Neutral Components of *Lophocereus schottii* (Engelmann) Britton and Rose.—Extraction[52] of the dried and powdered cactus (36 kg.) with ethanol yielded 5.5 kg. of extract which was partitioned between ether and 10% hydrochloric acid. Any insoluble material was discarded while the examination of the alkaloidal fraction, soluble in 10% hydrochloric acid, already has been reported.[6,7] The dark olive-green ether soluble material (1250 g.) was saponified by heating under reflux for 4 hr. with 5% methanolic potassium hydroxide solution (10 l.) to yield 600 g. of an orange colored, semi-solid neutral fraction. A portion (200 g.) was subjected to steam distillation for 10 hr. whereupon 110 g. (0.9%) of ***n*-octyl alcohol** (b.p. 190–194°) was isolated. A sample was redistilled, b.p. 195°, and submitted for analysis.

Anal. Calcd. for $C_8H_{18}O$: C, 73.78; H, 13.92; O, 12.29. Found: C, 73.60; H, 13.63; O, 13.01.

(49) After submission but prior to the publication of the original communications on lophenol (ref. 3) and citrostadienol (ref. 44a), there appeared a communication by W. W. Wells and D. H. Neiderhiser (THIS JOURNAL, **79**, 6569 (1957)) in which it was suggested that a sterol from rat feces was 4α-methyl-Δ⁷-cholesten-3β-ol. While several of the constants were in good agreement with those found by us for lophenol, there were several discrepancies, the most serious being in the respective acetates. Mixture melting point determinations of lophenol and lophenol acetate with the corresponding substances kindly supplied by Dr. Wells exhibited a depression and there were slight differences in the infrared spectra. Nevertheless it appears that the sterol from rat feces is probably impure lophenol and more detailed comparisons of the sterol with lophenol and synthetic specimens will be reported by Mazur and Sondheimer (ref. 40). The rat sterol has been named methostenol (*Fed. Proc.*, **17**, 333 (1958).

(50) During the typing of this manuscript there appeared an important paper by D. Arigoni (*Experientia*, **14**, 153 (1958)) in which was presented direct experimental evidence for the incorporation of labeled mevalonate into a plant triterpene and sterol.

(51) Melting points were determined on the Kofler block. Unless noted otherwise, rotations were measured in chloroform solution. We are indebted to Mrs. R. Riniker, Mrs. V. Halpern and Mrs. L. Crabbé for the rotatory dispersion measurements and to Mrs. Dolores Phillips for the infrared and ultraviolet spectral determinations. The microanalyses were performed by Dr. A. Bernhardt, Mülheim, Germany.

(52) We are indebted to Chas. Pfizer and Co., Brooklyn, N. Y., and to Parke, Davis and Co., Detroit, Mich., for putting facilities of their pilot plants at our disposal.

The 3,5-dinitrobenzoate exhibited m.p. 59–60°, undepressed upon admixture with an authentic specimen of *n*-octyl alcohol 3,5-dinitrobenzoate.

Anal. Calcd. for $C_{15}H_{20}N_2O_6$: C, 55.55; H, 6.22; N, 8.64; O, 29.60. Found: C, 55.62; H, 6.25; N, 8.95; O, 29.35.

The semi-solid residue from the steam distillation, isolated with ether, weighed 80 g. and was chromatographed on 2.0 kg. of unwashed Alcoa alumina, collecting a total of twenty-two 2-l. eluates. Fractions 3–9 (hexane–benzene, 4:1) yielded a semi-solid orange wax which after acetylation and several wasteful recrystallizations from ethanol provided 2.4 g. (0.02% based on dry cactus) of **lupeol acetate**, m.p. 213–215°, $[\alpha]_D$ +41°; saponification of a sample afforded lupeol (I), m.p. 211–213°, $[\alpha]_D$ +24°. Identity was established in each instance by mixture melting point determination and infrared spectral comparison.

Fractions 10–16 (benzene–hexane, 1:1 and 3:1) after crystallization from methanol yielded 27 g. (0.23% based on dry cactus) of **lophenol (IVa)**, m.p. 140–144°.

Crystallization of fractions 17–22 (benzene–hexane, 3:1; benzene; and benzene–ether, 3:1) led to 15 g. (0.13%) of impure **schottenol**, m.p. 115–130°. Attempts to purify this material by careful chromatography or by chromatography of the derived acetate or benzoate were unsuccessful. Repeated recrystallization also failed, 18 crystallizations from methanol giving material of m.p. 135–145°. Purification was finally effected as follows:

The crude schottenol (10 g.) was dissolved in the minimum quantity of benzene (*ca.* 20 cc.) and hexane (100 cc.) was added until precipitation occurred. The precipitate was collected and 110 cc. of hexane was added to the filtrate which then was filtered through Celite. This operation was performed four times until no further precipitation occurred. Evaporation of the final filtrate and crystal'ization from acetone and from methanol–ethyl acetate yielded 7.5 g. of long prisms of schottenol, m.p. 146–148°.

Lophenol (IVa) and Derivatives.—Several recrystallizations of the above-described lophenol fraction from methanol afforded the analytical sample of IVa, m.p. 149–151°, $[\alpha]_D$ +5°; apparent $\lambda_{max}^{cyclohexane}$ 204 mμ, log ε 3.87[9]; log ε at 210, 215, 220, 225 and 230 mμ: 3.80, 3.65, 3.24, 2.81 and 2.55; λ_{max} 246 mμ, log ε 2.66.

Anal. Calcd. for $C_{28}H_{46}O$: C, 83.93; H, 12.08; O, 3.99. Found: C, 83.82; H, 11.89; O, 3.90.

Acetylation with acetic anhydride and pyridine at room temperature and then chromatography and recrystallization from ethanol led to **lophenol acetate (IVb)**, m.p. 119–121°, $[\alpha]_D$ +28°, yellow color with tetranitromethane. Saponification with 5% ethanolic potassium hydroxide regenerated lophenol.

Anal. Calcd. for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38; O, 7.23. Found: C, 81.49; H, 11.10; O, 7.52.

Lophenol benzoate (IVc) (benzoyl chloride–pyridine, 25°, 20 hr.) was obtained in microcrystalline form after chromatography and recrystallization from ethanol–ethyl acetate, but sublimation at 155° and 0.005 mm. produced colorless needles, m.p. 161–163°, $[\alpha]_D$ +43°.

Anal. Calcd. for $C_{35}H_{52}O_2$: C, 83.28; H, 10.38. Found: C, 83.78; H, 10.22.

Lophenone (V).—To a solution of 700 mg. of lophenol (IVa) in 35 cc. of purified acetone (distilled over potassium permanganate) and 2 cc. of glacial acetic acid was added at room temperature dropwise over a period of 10 min. 0.4 cc. of 8 *N* chromium trioxide–sulfuric acid solution.[53] After stirring for 40 min., water was added and the product was isolated with ether. Two recrystallizations of the crude product from methanol gave 600 mg. of lophenone (V), m.p. 120–122°, while further purification was effected by chromatography on 25 g. of alumina, elution with hexane–benzene (8:2) and recrystallization from methanol; yield 530 mg., m.p. 122–124°, $[\alpha]_D$ +12°, $\lambda_{max}^{CHCl_3}$ 5.88 μ; R.D. (*c* 0.115 in methanol): $[\alpha]_{700}$ +18°, $[\alpha]_{589}$ +22°, $[\alpha]_{305}$ +695°, $[\alpha]_{265}$ − 980°, $[\alpha]_{260}$ −925°.

Anal. Calcd. for $C_{28}H_{46}O$: C, 84.35; H, 11.63. Found: C, 84.80; H, 11.31.

(53) See K. Bowden, I. M. Heilbron, E. R. H. Jones and B. C. L. Weedon, *J. Chem. Soc.*, 39 (1946), as well as references cited by C. Djerassi, R. R. Engle and A. Bowers, *J. Org. Chem.*, **21**, 1547 (1956).

Lophenone (V) was recovered unchanged after being heated under reflux for 1 hr. with either 10% ethanolic sodium hydroxide or with 15 cc. of ethanol containing 0.2 cc. of 20% sulfuric acid.

Reduction of 135 mg. of lophenone (V) with lithium aluminum hydride in boiling ether led to 115 mg. of lophenol (IVa), m.p. 148–150°, $[\alpha]_D$ +5.8°; acetate IVb, m.p. 118–120°, $[\alpha]_D$ +26°. When the reduction of 90 mg. of lophenone (V) was performed with 1 g. of sodium in 20 cc. of absolute ethanol (2.5 hr., steam-bath), there was isolated 75 mg. of lophenol (IVa), m.p. 148–150°.

3α-Chlorolophene (XI).[54]—Phosphorus pentachloride (650 mg.) was added to a solution of 500 mg. of lophenol (IVa) in 150 cc. of hexane and nitrogen gas was bubbled through the solution while stirring at room temperature for 6 hr. After diluting with aqueous sodium chloride solution, the mixture was let stand overnight, the hexane solution was separated, washed with bicarbonate and water, dried and evaporated. Chromatography of the residue on 15 g. of neutral alumina, elution with hexane and recrystallization from methanol provided 495 mg. of the chloro derivative XI, m.p. 86–88°,[55] $[\alpha]_D$ −3°.

Anal. Calcd. for $C_{28}H_{45}Cl$: C, 80.47; H, 11.23; Cl, 8.49. Found: C, 80.80; H, 11.38; Cl, 7.95.

$\Delta^{8(14)}$-Lophenol (VIa).—A solution of 500 mg. of lophenol acetate (IVb) in 40 cc. of acetic acid was shaken in an atmosphere of hydrogen with 100 mg. of pre-reduced platinum oxide catalyst. No hydrogen was consumed in one hr., whereupon the catalyst was filtered, the filtrate evaporated to dryness *in vacuo* and the residue recrystallized from methanol to give 395 mg. of the **acetate VIb**, m.p. 79–81°, $[\alpha]_D$ +41°, apparent $\lambda_{max}^{cyclohexane}$ 207 mμ, log ε 3.93[9]; log ε at 210, 215, 220, 225 and 230 mμ: 3.90, 3.82, 3.68, 3.50 and 3.18.

Anal. Calcd. for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.44; H, 11.69.

Saponification of the acetate VIb with 5% methanolic potassium hydroxide (2.5 hr., steam-bath) and recrystallization from methanol yielded **$\Delta^{8(14)}$-lophenol (VIa)** as colorless needles, m.p. 160–163°, $[\alpha]_D$ +19°.

Anal. Calcd. for $C_{28}H_{46}O$: C, 83.93; H, 12.08. Found: C, 84.04; H, 12.06.

The benzoate VIc (benzoyl chloride–pyridine, 20°, 22 hr.) was purified by chromatography and recrystallization from ethanol; colorless plates, m.p. 140–142°, $[\alpha]_D$ +40°.

Anal. Calcd. for $C_{35}H_{52}O_2$: C, 83.28; H, 10.38; O, 6.34. Found: C, 82.60; H, 10.16; O, 5.98.

Δ^{14}-Lophenol (VIIa).—Lophenol acetate (IVb) (300 mg.) in 30 cc. of chloroform was treated with dry hydrogen chloride gas at 0° for a period of 2 hr. Removal of the solvent and several recrystallizations from methanol led to 114 mg. of the **acetate VIIb**, m.p. 133–136°, $[\alpha]_D$ +45°; log ε[9] at 205, 210, 215, 220, 225 and 230 mμ (cyclohexane solution): 3.90, 3.73, 3.47, 3.21, 2.95 and 2.73.

Alternatively, 150 mg. of $\Delta^{8(14)}$-lophenol acetate (VIb) was treated with hydrogen chloride in chloroform in an identical manner to produce 65 mg. of the acetate VIIb, m.p. and mixture m.p. 132–135°, $[\alpha]_D$ +45°. Identity also was confirmed by coincidence of the respective X-ray diffraction patterns.[56]

Anal. Calcd. for $C_{30}H_{50}O_2$: C, 81.39; H, 11.38. Found: C, 81.65; H, 11.48.

The free alcohol VIIa was obtained by saponification with 5% methanolic potassium hydroxide and recrystallization from methanol containing a few drops of water, m.p. 156–158°, $[\alpha]_D$ +31°.

Anal. Calcd. for $C_{28}H_{46}O$: C, 83.93; H, 12.08. Found: C, 84.08; H, 12.04.

Lophanol (VIIIa).—Lophenol acetate (IVb) (2.5 g.) was hydrogenated with 1.0 g. of platinum oxide catalyst in 100 cc. of acetic acid and 20 cc. of ethyl acetate containing 3

(54) The α-orientation is assumed by analogy to the reaction of cholestan-3β-ol with phosphorus pentachloride (for leading references see C. W. Shoppee, *J. Chem. Soc.*, 1138 (1946)).

(55) After drying for analysis in high vacuum, the m.p. was 87.5–91°.

(56) We are grateful to Dr. Reuben Jones (Eli Lilly and Co., Indianapolis, Ind.) for arranging for the X-ray measurements.

drops of perchloric acid.[57] After shaking for 48 hr. at room temperature and atmosphere pressure, 1 g. of platinum oxide was added and hydrogenation was continued for 72 hr. The catalyst was filtered, the solvent was removed *in vacuo* and since the residue still gave a positive Liebermann-Burchard reaction, it was dissolved in 200 cc. of carbon tetrachloride and shaken for 15 min. with 50 cc. of acetic anhydride and 5 cc. of concd. sulfuric acid. To the dark colored solution was added 100 cc. of water, the aqueous phase was discarded and this procedure was repeated until practically no more color was produced. The solid residue, obtained on evaporation of the carbon tetrachloride, was saponified directly by heating under reflux for 3 hr. with 300 cc. of 5% methanolic potassium hydroxide solution. Recrystallization from methanol yielded 1.85 g. of colorless **lophanol (VIIIa)**,[58] m.p. 166–168°, $[\alpha]_D$ +27°, which gave no more color with tetranitromethane.

Anal. Calcd. for $C_{28}H_{50}O$: C, 83.51; H, 12.52. Found: C, 83.49; H, 12.72.

Lophanol acetate (VIIIb),[58] m.p. 131–133° (recrystallized from methanol), $[\alpha]_D$ +42°, was prepared by conventional acetylation (acetic anhydride–pyridine, 20°, 24 hr.) of lophanol (VIIIa), by hydrogenation of lophenol acetate (IVb)[57] or by similar hydrogenation of Δ^{14}-lophenol acetate (VIIb).

Anal. Calcd. for $C_{30}H_{52}O_2$: C, 81.02; H, 11.79. Found: C, 81.28; H, 12.23.

Lophanone (IX).—The oxidation of 1.37 g. of lophanol (VIIIa) was conducted exactly as described above for lophenol (IVa) and yielded, after recrystallization from methanol, 1.30 g. of the ketone, m.p. 121–123°, $[\alpha]_D$ +25°, $\lambda_{max}^{CHCl_3}$ 5.87 μ. The rotatory dispersion curve (*c* 0.0886 in methanol): $[\alpha]_{700}$ +17°, $[\alpha]_{583}$ +27°, $[\alpha]_{305}$ +707°, $[\alpha]_{265}$ −605°, $[\alpha]_{250}$ −538° was virtually indistinguishable from that[21] of synthetic 4α-methylcholestan-3-one; furthermore, the respective infrared spectra were completely superimposable and no depression of a mixture melting point was encountered.

Anal. Calcd. for $C_{28}H_{48}O$: C, 83.93; H, 12.08. Found: C, 84.29; H, 12.14.

Polybromination experiments[35,36] with lophanone (IX) led to oily products, but monobromination in glacial acetic acid produced **2α-bromolophan-3-one**, which was purified by rapid chromatography on silica gel (elution with hexane–benzene 1:1) and recrystallization from ether–methanol, whereupon it exhibited m.p. 90–92°, $\lambda_{max}^{CS_2}$ 5.75 μ,[59] $[\alpha]_D$ +28°. In accordance with the assignment[60] of an equatorial orientation to the bromine atom, its rotatory dispersion peak ($[\alpha]_{315}$ +570° in dioxane) was not displaced[61] with respect to that of the bromine-free ketone IX nor was the amplitude increased.

Anal. Calcd. for $C_{28}H_{47}BrO$: C, 70.10; H, 9.88. Found: C, 70.70; H, 9.91.

The location of the bromine atom at C-2 was established by dehydrobrominating 18 mg. of the bromo ketone with 8 mg. of 2,4-dinitrophenylhydrazine by the acetic acid technique[62] and recrystallizing the red 2,4-dinitrophenylhydrazone from chloroform–methanol, m.p. 246–247°; $\lambda_{max}^{CHCl_3}$ 384 mμ, log ϵ 4.47[63] as compared to 367 mμ for lophanone (IX) 2,4-dinitrophenylhydrazone (not analyzed).

Anal. Calcd. for $C_{34}H_{50}N_4O_4$: C, 70.55; H, 8.71. Found: C, 69.96; H, 8.45.

Lophane (X).—Lophanone (IX) (295 mg.) was heated under reflux for 1 hr. with 12 cc. of diethylene glycol and 1 cc. of 85% hydrazine hydrate. A solution of 0.6 g. of sodium in 12 cc. of diethylene glycol was added, the water was boiled off by heating the mixture until the temperature of the vapor rose to 195° and heating under reflux then was continued for 5 hr. The product was isolated with ether in the conventional manner and filtered in hexane solution through a short column of alumina. Trituration with ethanol afforded crystals, m.p. 70–75°, raised upon further recrystallization from ethanol–ethyl acetate to 75–77.5°, $[\alpha]_D$ +20°. The analytical sample was sublimed at 135° in a high vacuum.

Anal. Calcd. for $C_{28}H_{50}$: C, 86.93; H, 13.13; mol. wt., 386.7. Found: C, 86.97; H, 13.03; mol. wt., 386.3 ± 0.6.[25]

9(11)-Dehydrolophenol Acetate (XII).—A solution of 7.0 g. of mercuric acetate in hot, glacial acetic acid was added over a period of 12 min. in a current of nitrogen to a boiling solution of 3.56 g. of lophenol acetate (IVb) in 55 cc. of dioxane. Precipitation of mercurous acetate commenced within 5 min. and at the end of the addition, the mixture was diluted with 50 cc. of boiling water and kept at 0° overnight. The precipitate was filtered, washed well with water and a small quantity of cold methanol and the solid triturated thoroughly with ether. The ether solution was washed with 5% sulfuric acid and water, dried, evaporated and the oily residue was passed in benzene solution through 30 g. of neutral alumina. Elution with the same solvent and recrystallization from methanol afforded 1.32 g. of colorless solid, m.p. 88–91°, $[\alpha]_D$ +84°; λ_{max}^{EtOH} 234, 242 and 250 mμ, log ϵ 4.16, 4.17 and 4.01.

Anal. Calcd. for $C_{30}H_{48}O_2$: C, 81.76; H, 10.98. Found: C, 82.49; H, 11.02.

8α,9α-Oxido- (XIII) and 8α,14α-Oxido-lophan-3β-ol-7-one Acetate (XIV). (a) **With Chromium Trioxide.**—Lophenol acetate (IVb) (1.6 g.) in 75 cc. of acetic acid was maintained at 60° and a solution of 1.2 g. of chromium trioxide in 5 cc. of water and 10 cc. of acetic acid was added dropwise with stirring over a period of 20 min. After stirring for an additional 10 min., the mixture was poured into dilute sodium bisulfite solution, the product isolated with ether and divided into acidic (165 mg.) and neutral (1.45 g.) fractions. The latter was chromatographed on 50 g. of Merck neutral alumina, hexane–benzene (3:1) removing 350 mg. of unreacted starting material. Elution with benzene gave **8α,9α-oxidolophan-3β-ol-7-one acetate (XIII)** which crystallized from methanol as plates (108 mg.) and after recrystallization had m.p. 190–192°, $[\alpha]_D$ ±0°; its rotatory dispersion curve together with that of 8α,9α-oxido-Δ^{22}-ergosten-3β-ol-7-one acetate[27b] is reproduced in Fig. 1.

Anal. Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24. Found: C, 75.96; H, 10.35.

Further elution with benzene-ether (9:1) and recrystallization from methanol afforded 85 mg. of colorless needles of **8α,14α-oxidolophan-3β-ol-7-one acetate (XIV)**, m.p. 175–176°, $[\alpha]_D$ −45°; R.D. in methanol (*c* 0.13): $[\alpha]_{700}$ −8°, $[\alpha]_{589}$ −35°, $[\alpha]_{327.5}$ −930°, $[\alpha]_{310}$ −527°. For comparison, the rotatory dispersion (*c* 0.107 in dioxane) of 8α,14α-oxido-Δ^{22}-ergosten-3β-ol-7-one acetate[27b] also was measured: $[\alpha]_{700}$ − 66°, $[\alpha]_{589}$ −91°, $[\alpha]_{335}$ −907°, $[\alpha]_{295}$ +349°.

Anal. Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24; O, 13.54. Found: C, 76.19; H, 10.37; O, 13.54.

(b) **With Perbenzoic Acid.**—Better yields were realized when 2.0 g. of lophenol acetate (IVb) was dissolved in 25 cc. of chloroform containing 1.35 g. (2.25 equivalents) of perbenzoic acid and leaving the solution in the refrigerator for one week. Washing with dilute sodium bicarbonate solution and water, drying and evaporation gave a pale yellow oil which was dissolved in 40 cc. of purified acetone and oxidized with 8 *N* chromium trioxide solution.[53] Isolation with ether gave an oil which was chromatographed on 80 g.

(57) In a small scale experiment, the hydrogenation was conducted with 0.2 g. of IVb, 20 cc. of acetic acid, 1.2 cc. of concd. hydrochloric acid and 0.1 g. of platinum oxide catalyst and lophanol acetate (VIIIb) was isolated directly.

(58) Lithium aluminum hydride reduction of 50 mg. of a synthetic sample (ref. 39) of 4α-methylcholestan-3-one (kindly supplied by Prof. E. R. H. Jones and Dr. T. G. Halsall of Oxford University) furnished 44 mg. of 4α-methylcholestan-3β-ol, m.p. 164–166°, and acetylation of a portion of it gave 4α-methylcholestan-3β-ol acetate, m.p. 131–133°. Identity of these synthetic specimens with lophanol (VIIIa) and lophanol acetate (VIIIb) was established by mixture melting point determination and infrared spectral comparison.

(59) Thus exhibiting the expected shift (lophanone has $\lambda_{max}^{CS_2}$ 5.83 μ) for an equatorial α-bromo ketone (R. N. Jones, D. A. Ramsay, F. Herling and K. Dobriner, THIS JOURNAL, **74**, 2828 (1952)).

(60) C. Djerassi, J. Osiecki, R. Riniker and B. Riniker, *ibid.*, **80**, 1216 (1958).

(61) Taking into consideration the usual correction factor of 8 mμ in going from methanol to dioxane (C. Djerassi and W. Closson, *ibid.*, **78**, 3761 (1956); C. Djerassi, R. Riniker and B. Riniker, *ibid.*, **78**, 6377 (1956)).

(62) C. Djerassi, *ibid.*, **71**, 1003 (1949).

(63) C. Djerassi and E. Ryan, *ibid.*, **71**, 1000 (1949), have shown that 2,4-dinitrophenylhydrazones of Δ^1-3-keto steroids exhibit a maximum at 382–384 mμ as compared to 367–369 mμ for a saturated ketone and 390 mμ for a Δ^4-3-ketone.

of Merck neutral alumina. Elution with benzene led to 230 mg. of the 8α,9α-oxide XIII, m.p. 190–192°, while further development of the column with benzene and with benzene–ether (9:1) gave 305 mg. of the 8α,14α-epoxide, m.p. 174–175°.

Δ⁸-Lophen-3β-ol-7-one Acetate (XVa).—The above 8,9-oxide XIII (100 mg.) was heated for 30 min. under reflux with 15 cc. of acetic acid and 100 mg. of zinc dust, five further 50-mg. portions of zinc dust then being added in 10-min. intervals. The solution was filtered, the product collected and crystallized from methanol to give 65 mg. of plates, m.p. 162–169°. Further recrystallization yielded the analytical sample, m.p. 168–171°; $\lambda_{max}^{CHCl_3}$ 5.74, 6.00 and 6.15 μ; λ_{max}^{EtOH} 254 mμ, log ε 3.98; $[\alpha]_D$ −17° (dioxane), rotatory dispersion curve reproduced in Fig. 1.

Anal. Calcd. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.59; O, 10.51. Found: C, 78.38; H, 10.39; O, 10.05.

Δ⁸⁽¹⁴⁾-Lophen-3β-ol-7-one Acetate (XVI).—Zinc dust reduction of the 8,14-oxide XIV was carried out exactly as described above for the 8,9-oxide and provided, after recrystallization from aqueous methanol, the Δ⁸⁽¹⁴⁾-7-ketone XVI, m.p. 168–170° (strongly depressed upon admixture with XVa), λ_{max}^{EtOH} 263 mμ, log ε 3.90; R.D. in dioxane (*c* 0.085): $[\alpha]_{700}$ −12°, $[\alpha]_{589}$ −19°, $[\alpha]_{375}$ − 532°, $[\alpha]_{312.5}$ +635°, $[\alpha]_{307.5}$ + 259°.

Anal. Calcd. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.59; O, 10.51. Found: C, 78.59; H, 10.52; O, 10.23.

Δ⁸,¹⁴-Lophadien-3β-ol-7-one Acetate (XVII).—A solution of 130 mg. of 8α,9α-oxidolophan-3β-ol-7-one acetate (XIII) was heated under reflux for 2 hr. with 13 cc. of 95% ethanol containing 5% of hydrochloric acid. The product was extracted with ether, re-acetylated with acetic anhydride–pyridine and crystallized from methanol to give 50 mg. of colorless needles, m.p. 188–190°. An additional 25 mg. was isolated by chromatography of the mother liquors and the melting point remained unchanged after two recrystallizations, $[\alpha]_D$ +3°; $\lambda_{max}^{CHCl_3}$ 5.75, 5.96 and 7.95 μ; λ_{max}^{EtOH} 223.5 and 299 mμ,[64] log ε 4.14 and 3.65. The identical product was isolated when starting with the 8,14-oxide XIV.

Anal. Calcd. for $C_{30}H_{46}O_2$: C, 79.24; H, 10.20. Found: C, 79.23; H, 10.34.

By an analogous series of reactions, 2.0 g. of Δ⁷-ergosten-3β-ol acetate was treated with perbenzoic acid and then chromium trioxide to provide after chromatography and recrystallization from methanol 320 mg. of **8α,9α-oxidoergostan-3β-ol-7-one acetate**, m.p. 216–217°, $[\alpha]_D$ −41°, and 370 mg. of **8α,14α-oxidoergostan-3β-ol-7-one acetate**, m.p. 133–134°, $[\alpha]_D$ − 83°.

Anal. Calcd. for $C_{30}H_{48}O_4$: C, 76.22; H, 10.24; O, 13.54. Found (8,9-oxide): C, 76.02; H, 9.94; O, 14.06; (8,14-oxide): C, 75.76; H, 10.10; O, 13.59.

Treatment of the 8,9-oxide with ethanolic hydrochloric acid and then reacetylation gave **Δ⁸,¹⁴-ergostadien-3β-ol-7-one acetate**, m.p. 178–179°, $[\alpha]_D$ −22°; $\lambda_{max}^{CHCl_3}$ 5.75, 5.96 and 7.95 μ, but differing significantly in the 10–12 μ region from the corresponding lophadienolone derivative XVII, λ_{max}^{EtOH} 223 and 299 mμ, log ε 4.22 and 3.64.

Anal. Calcd. for $C_{30}H_{46}O_3$: C, 79.24; H, 10.20. Found: C, 79.01; H, 10.14.

***t*-Butyl Chromate Oxidation of Δ⁸⁽¹⁴⁾-Lophenol Acetate (VIb).**—Δ⁸⁽¹⁴⁾-Lophenol acetate (1.0 g.) in 15 cc. of benzene and 8 cc. of acetic acid was treated for 3 days at room temperature with *t*-butyl chromate (prepared[29] from 3.7 g. of chromium trioxide and 10 cc. of *t*-butyl alcohol) in carbon tetrachloride. Chromatography on 60 g. of Merck neutral alumina yielded the following products in order of elution: a substance (50 mg.), m.p. 120–132°, whose m.p. could not be improved and whose analysis indicated that it was a mixture of a $C_{30}H_{50}O_2$ and a $C_{30}H_{50}O_3$ compound; 71 mg. of **8α,14α-oxidolophan-3β-ol-7-one acetate (XIV)**, m.p. 171–175°; and finally 40 mg. of a substance, m.p. 215–219° (from methanol), $[\alpha]_D$ +77°, which on the basis of its formation from the Δ¹⁴-isomer VIIb, its analytical composition and its infrared spectrum ($\lambda_{max}^{CHCl_3}$ 2.92, 5.68–5.72 (broad) and 5.80 μ) is assumed to be **lophane-7,15-dione-3β,8,14-triol 3-acetate (XVIII)**.

Anal. Calcd. for $C_{30}H_{48}O_6$: C, 71.39; H, 9.59; O, 19.02. Found: C, 71.37; H, 9.44; O, 19.05.

***t*-Butyl Chromate Oxidation of Δ¹⁴-Lophenol Acetate (VIIb).**—The oxidation of 1.0 g. of Δ¹⁴-lophenol acetate (VIIb) was conducted in exactly the same fashion as described above for the Δ⁸⁽¹⁴⁾-isomer VIb and yielded the following substances:

Δ¹⁴-Lophen-3β-ol-15-one acetate (XX) (52 mg.), m.p. 120–123°, λ_{max}^{EtOH} 235 mμ, log ε 4.22; R.D. in dioxane (*c* 0.15): $[\alpha]_{700}$ +71°, $[\alpha]_{589}$ +111°, $[\alpha]_{360}$ +1446°, $[\alpha]_{307.5}$ − 1250°, $[\alpha]_{290}$ −840°.

Anal. Calcd. for $C_{30}H_{48}O_3$: C, 78.89; H, 10.59; O, 10.51. Found: C, 78.38; H, 10.36; O, 10.87.

Immediately thereafter was eluted 20 mg. of the oxido ketone XIV and finally 10 mg. of the diketone XVIII.

"Schottenol" and Derivatives.—Schottenol, m.p. 146–148° as obtained in the isolation scheme described above, was purified further by conversion to the acetate, recrystallization, saponification and three additional recrystallizations from methanol–ethyl acetate, whereupon it exhibited m.p. 148–150°, $[\alpha]_D$ +4°. The substance readily gave a precipitate with an alcoholic solution of digitonin and when treated with mercuric acetate on a microscale as reported above for XII yielded a solid with ultraviolet absorption maxima at 235, 242.5 and 251 mμ.

Anal. Calcd. for $C_{29}H_{50}O$: C, 83.99; H, 12.15; O, 3.86. Found: C, 83.76; H, 12.10; O, 4.39.

The acetate was prepared with acetic anhydride–pyridine and recrystallized from chloroform–methanol; large plates, m.p. 168–170°, $[\alpha]_D$ −3.6°.

Anal. Calcd. for $C_{31}H_{52}O_2$: C, 81.52; H, 11.48; O, 7.01. Found: C, 81.54; H, 11.21; O, 7.22.

The benzoate crystallized as plates from chloroform–methanol, m.p. 183–185°, undepressed upon admixture with a sample of Δ⁷-stigmasten-3β-ol benzoate[41] (XXIIc), $[\alpha]_D$ +3.5°.

Anal. Calcd. for $C_{36}H_{54}O_2$: C, 83.34; H, 10.49; O, 6.17. Found: C, 83.53; H, 10.06; O, 6.33.

Oxidation of 330 mg. of "schottenol" with chromium trioxide in acetone solution[58] and recrystallization from acetone–methanol yielded 310 mg. of colorless crystals of **schottenone (XXIII)**, m.p. 152-154°, $\lambda_{max}^{CHCl_3}$ 5.88 μ, $[\alpha]_D$ +30°; R.D. (*c* 0.0665 in methanol): peak at $[\alpha]_{310}$ +565°, reduced[37,38] to 197° after 35 min. upon addition of one drop of concd. hydrochloric acid.

Anal. Calcd. for $C_{29}H_{48}O$: C, 84.40; H, 11.72; O, 3.88. Found: C, 83.92; H, 11.26; O, 4.27.

Wolff-Kishner reduction[24] of 170 mg. of schottenone (XXIII) according to the procedure given above for lophane (X) afforded 125 mg. of colorless plates (from methanol–ethyl acetate) of **schottene (XXIV)**, m.p. 100–102°, $[\alpha]_D$ +11°.

Anal. Calcd. for $C_{29}H_{50}$: C, 87.36; H, 12.64; mol. wt., 398.7. Found: C, 87.57; H, 12.55; mol. wt., 398.0.[25]

Δ⁷-Ergosten-3β-ol (XXIa) and Derivatives.—Prior to the mass spectrographic molecular weight determination[25] and the side chain determination,[33] it was assumed that schottenol was in fact Δ⁷-ergosten-3β-ol (XXIa) because of the fairly close coincidence of its physical constants and those of its derivatives with the literature values[65] for the corresponding derivatives of Δ⁷-ergosten-3β-ol (see Table II). The only serious discrepancy was noted in the melting points of the respective acetates and, consequently, a sample was prepared by hydrogenating 3.0 g. of Δ⁷,²²-ergostadien-3β-ol acetate (m.p. 183–185°[66]) in ethyl acetate solution with 3 g. of W-2 Raney nickel catalyst at room temperature and atmospheric pressure, the theoretical amount of hydrogen being consumed in 2.5 hr. Recrystallization from chloroform–methanol and from ethyl acetate–methanol afforded 2.3 g. of large plates of the **acetate XXIb**, m.p. 166–168°, $[\alpha]_D$ +4.3°. Δ⁷-Ergosten-3β-ol (XXa) and the corresponding benzoate XXc and ketone were prepared from this sample of acetate by standard methods and the pertinent constants are listed in Table II. Mixture melting point comparison between "schottenol" and Δ⁷-ergosten-3β-ol (XXa) and their respective derivatives showed no depression except for the acetates where a 2° depression was noted.

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(64) Due to a typographical mistake, this 299 mμ maximum was reported as 229 mμ in our preliminary communication (ref. 3).

(65) D. H. R. Barton and J. D. Cox, *J. Chem. Soc.*, 783 (1948).

(66) Kindly supplied by Merck and Co., Rahway, N. J.