[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Cholesterol and Companions. IV. Oxidation of Δ^7-Stenols with Selenium Dioxide

BY LOUIS F. FIESER AND GUY OURISSON[1]

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Oxidation of Δ^7-cholestenyl acetate with selenium dioxide in acetic acid–benzene at 0° affords $\Delta^{8(14)}$-cholestene-3β,7α-diol diacetate (II). The corresponding diol (III), obtained by deacetylation with lithium aluminum hydride, is reconvertible into II by acetylation and also gives a dibenzoate. The structure was indicated by conversion to derivatives of $\Delta^{7,14}$-cholestadiene-3β-ol and the corresponding 3-ketone and was fully established by oxidation of III with perbenzoic acid to the known 8α,14α-oxidocholestane-3β,7α-diol (XVIIa). The product obtained by Callow and Rosenheim by oxidation of 5-dihydroergosterol with selenium dioxide in ethanol has been characterized by Dr. E. F. Schoenewaldt of Merck and Co., Inc. as an ethoxy compound and not an oxide as originally formulated, and the present evidence shows it to be 3β-hydroxy-7α-ethoxy-$\Delta^{8(14),22}$-ergostadiene (XIa). Its 3-acetate is readily converted into ergosterol-B_3 acetate (XV) both by phosphorus oxychloride–pyridine and by acetic acid at 25°, and on chromic acid oxidation it yields the known 8α,14α-oxido-Δ^{22}-ergostene-3β-ol-7-one acetate (XIV). $\Delta^{8(14)}$-Cholestene-3β,7α-diol diacetate (II) was similarly oxidized to 8α,14α-oxidocholestane-3β-ol-7-one acetate. Selenium dioxide oxidation of 5-dihydroergosteryl acetate in acetic acid gives $\Delta^{8(14),22}$-ergostadiene-3β,7α-diol diacetate, which corresponds in M_D to the analogous product in the cholesterol series (II). The configurations of all of the new compounds and of the 8,14- and 8,9-oxido-7-ketones and 7-alcohols of Stavely and Bollenback and of Wintersteiner and Moore, as well as of a cholestane-3,7,8-triol described by the latter authors, are deduced from M_D relationships. All known facts regarding the oxidation of Δ^7-stenyl derivatives with selenium dioxide, peracids and chromic acid seem interpretable on the postulate of initial allylic hydroxylation at C_{14} and C_9. According to conditions, this may be the end result, or it may be followed by allylic rearrangement and acetylation or alkylation of a resulting hydroxyl group at C_7, by dehydration to D- and B_3-type dienes, or by oxidation to ketoxides. Inferences regarding the configurations and mode of formation of products of oxidation of $\Delta^{7,9(11)}$-dienes reported by Heusser, *et al.*, are presented in a concluding note.

The observation[2] that Δ^7-cholestenol (lathosterol) is extraordinarily sensitive to attack by selenium dioxide in acetic acid solution and that ready reaction with the reagent at 0–25° is specific to allo- or Δ^5-steroids having a double bond or diene system adjacent to a 14α-hydrogen atom, prompted the present investigation of the course of the oxidation of Δ^7-stenols. In the only previous study, Callow and Rosenheim[3] oxidized the substance now known to be a Δ^7-stenol, 5-dihydroergosterol, and isolated a reaction product that they regarded as an oxide. Results of a reinvestigation of this product are presented below.

Of practical significance is the discovery of a simple and effective expedient for removal of otherwise persistent and troublesome colloidal or bound selenium with use of precipitated silver. A solution of the crude reaction mixture in alcohol, benzene or ether is refluxed or stirred with the metal, or a ground mixture of alumina and precipitated silver is used as a bottom layer of a chromatogram column.

Structure of Products.—We studied first the oxidation of Δ^7-cholestenyl acetate in acetic acid-benzene at 0–5° and isolated in 55% yield a diacetate, m.p. 139°, that is unsaturated to tetranitromethane. Hydrolysis with alkali gave a rather poor product, but deacetylation to the pure diol was effected quantitatively by reaction with lithium aluminum hydride. The infrared spectrum of the diacetate showed a small band at 12.0 μ, which is regarded as characteristic of a tertiary–secondary double bond[4] and which thus suggested that the 7,8-double bond is still intact and hence that the substance is the product of allylic acetoxylation at C_{14}. The result of Oppenauer oxidation of the diol might seem to point in the same direction, since the product was not a diketone, which might be expected to arise from a disecondary diol such as III, but a monoketone with a second double bond derived from elimination of the hydroxyl group introduced in the oxidation. However, the diol on acetylation in pyridine yielded the original diacetate, and on benzoylation it gave a dibenzoate. This evidence that the diol is disecondary seems to us to outweigh the above suggestions to the contrary.

Actually the elimination of water from the secondary allylic alcohol grouping in the Oppenauer oxidation of III is analogous to the elimination of secondary hydroxyl groups at C_{16} and C_{20} in the Oppenauer oxidation of allopregnane-3,16,20-triol to the Δ^{16}-ene-3,20-dione and of pregnanetriol to the Δ^{17}-ene-3,16-dione.[5] Our oxidation product (V) has a diene system not conjugated with the 3-keto group. The position of the ultraviolet absorption maximum (242 mμ), the low extinction coefficient (9,700), and the strong levorotation (M_D −563 Chf) all point to the presence of a $\Delta^{7,14}$, or B_3-type, diene system, as in formula V. Derivatives of $\Delta^{7,14}$-cholestadienol (VIIIa, b) were then obtained in the following two ways. Cathylation of the diol III, as in other instances,[6] proved more selective than acetylation or benzoylation and afforded a 3-monocathylate, which was not isolated in pure form because of partial dehydration, but which was converted by reaction with phosphorus oxychloride in pyridine into $\Delta^{7,14}$-cholestadienyl cathylate, VIIIa. Saponification and benzoylation afforded the corresponding benzoate, VIIIb, which was also obtained by refluxing the diol dibenzoate in dimethylaniline. Schenck, Buchholz and Wiese[7] report for the benzoate of "dehydrocholesterol-B_3," prepared by acid isomerization of 7-dehydro-

(1) On leave of absence from the École Normale Supérieure (Paris) on a scholarship from the Direction des Relations Culturelles and as Attaché de Recherches of the Centre National de la Recherche Scientifique (France).

(2) L. F. Fieser, THIS JOURNAL, **75**, 4395 (1953).

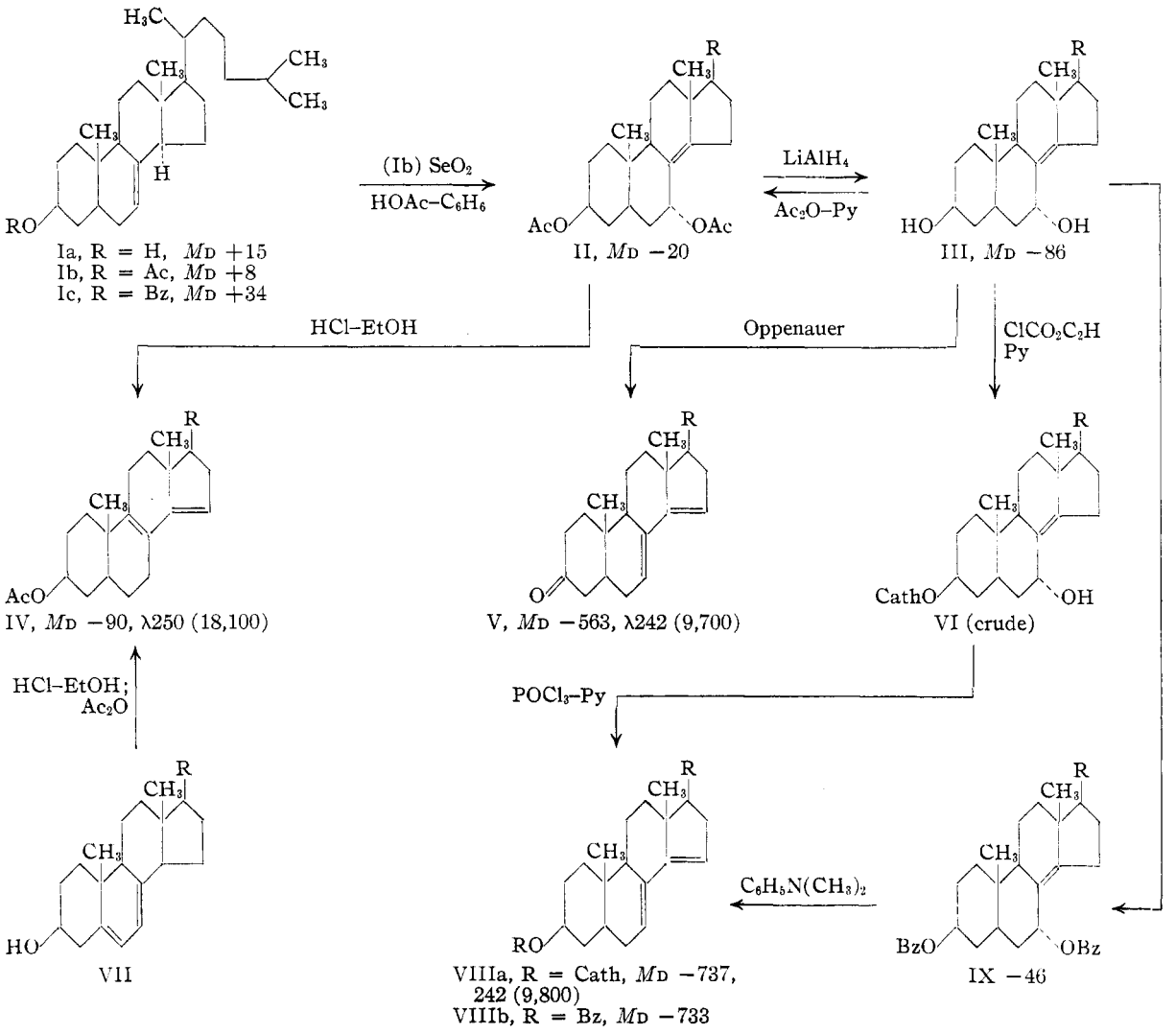
(3) R. K. Callow and O. Rosenheim, *J. Chem. Soc.*, 387 (1933); see also R. K. Callow, *ibid.*, 462 (1936).

(4) P. Bladon, J. M. Fabian, H. B. Henbest, H. P. Koch and G. W. Wood, *ibid.*, 2402 (1951).

(5) R. E. Marker and D. L. Turner, THIS JOURNAL, **62**, 2540 (1940).

(6) L. F. Fieser, J. E. Herz, M. W. Klohs, M. A. Romero and T. Utne, *ibid.*, **74**, 3309 (1952).

(7) Fr. Schenck, K. Buchholz and O. Wiese, *Ber.*, **69**, 2696 (1936).



(Rotations in chloroform; absorption maxima, mμ, in ethanol.)

cholesterol, a rotation (α_D −115° Chf) much lower than we find (−150° Chf). We believe our material to be purer than theirs because the molecular rotation difference for introduction into Δ^7-cholestenyl benzoate (Ic) of the 14,15-double bond calculated from our data, namely, −733 − 34 = −767, corresponds much more closely to the M_D difference of −885 for ergosterol-B_3 benzoate.[8] Furthermore, the rotations found for the cathylate VIIIa and the 3-ketone V are consistent with that found for the benzoate (see chart). The formation of the $\Delta^{7,14}$-diene system under non-acidic conditions, coupled with the evidence that the allylic alcoholic function introduced is secondary and therefore at C_7 rather than at C_{14}, affords substantial evidence for formulation of the product of selenium dioxide oxidation as $\Delta^{8(14)}$-cholestene-3β,7-diol diacetate (II). Conclusive evidence will be cited later in a discussion of the configuration at C_7.

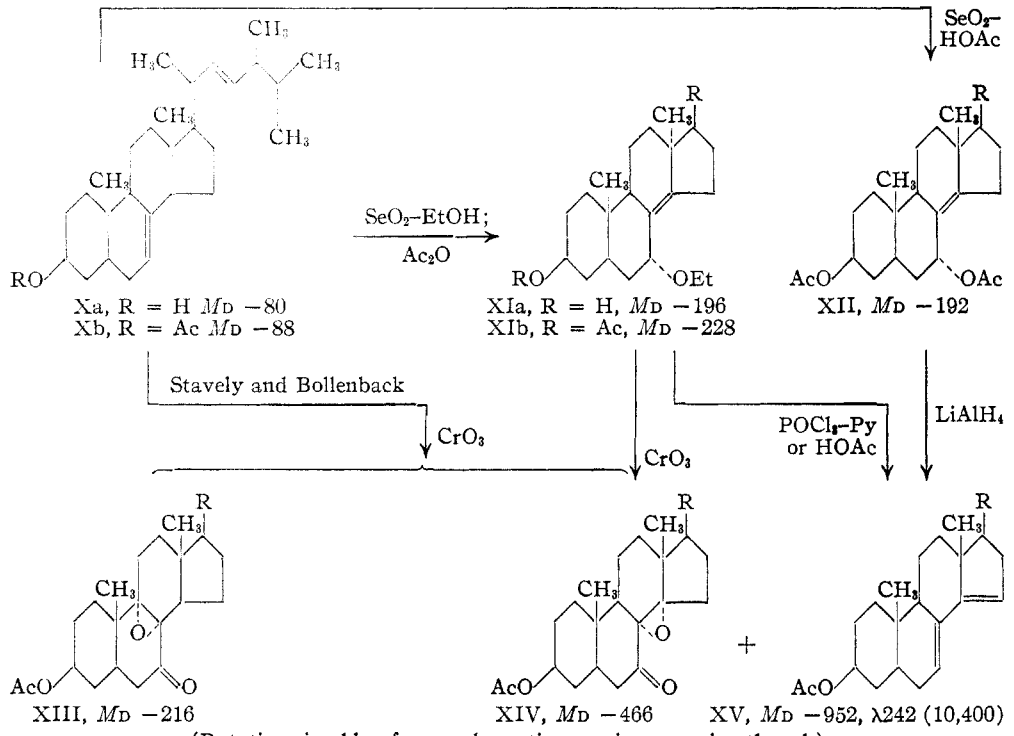
The $\Delta^{7,14}$-diene system is labile to acid, and when $\Delta^{8(14)}$-cholestene-3,7-diol diacetate (II) was refluxed with ethanol and hydrochloric acid, followed by acetylation, it was converted smoothly into a cholestadienyl acetate isomeric with B_3 but corresponding closely in the constants of the acetate and free alcohol with those reported by Adams, Petrow and Royer[9] for the acetate of the $\Delta^{8,14}$-isomer, cholestadienol-B_1 (IV). Equally pure B_1-acetate was obtained readily from 7-dehydrocholesterol (VII) by the same method or with hydrogen chloride in acetic anhydride in yields up to 80%. Since application of the same method to ergosterol[10] gave material rich in ergosterol-B_1 but not fully pure, it appears that in an acidic medium at about 80° the $\Delta^{8,14}$-system is favored more in the cholestadienol series than in the ergosterol series.

In the earlier experiments on the oxidation of Δ^7-cholestenyl acetate the reagent was made by dissolving commercial selenous acid in a volume of water such that the final solution in benzene–acetic acid contained 0.6% of water. Oxidations with this reagent at 0–5° consistently gave a reaction product that crystallized readily from methanol to give II in 50–55% yield. Oxidation

(8) D. H. R. Barton and C. J. W. Brooks, *J. Chem. Soc.*, 257 (1951).

(9) W. J. Adams, V. Petrow and R. Royer, *ibid.*, 678 (1951).

(10) M. Fieser, W. E. Rosen and L. F. Fieser, THIS JOURNAL, **74**, 6321 (1952).



(Rotations in chloroform; absorption maxima, mμ, in ethanol.)

in the same way but at 25° afforded no II but a mixture found to consist of the acetates of $\Delta^{7,9(11)}$-cholestadienol (D) and $\Delta^{7,14}$-cholestadienol (B_8). The benzoate likewise yielded a diene mixture at 25°. Callow and Rosenheim[3] in their study of the oxidation of 5-dihydroergosterol isolated, beside the supposed oxide, a product identified as ergosterol-D. In later oxidations at 0° with reagent made from selenium dioxide instead of selenous acid the product consistently failed to crystallize until it had been acetylated. Addition of acetic anhydride speeded up the reaction and gave crystalline diacetate directly but in poor yield. Addition of water to a content of 6% in the reaction mixture retarded oxidation to such a marked extent that only a trace of selenium was deposited in 15 hr. at 0°; the reaction proceeded satisfactorily at 25° and afforded II directly in 50–55% yield. The observations emphasize the sensitivity of the reaction.

On repeating the oxidation[3] of 5-dihydroergosterol (Xa) in ethanol–benzene at 35° for 20 hr. we obtained the "oxide" and found the properties of this substance and its 3-acetate and 2,4-dinitrobenzoate to be as recorded by Callow and Rosenheim. The formulation of the substance as an oxide seemed to us questionable in view of Wintersteiner and Moore's[11] observation that even perbenzoic acid in chloroform solution reacts with Δ^7-cholestenol to give an oxido alcohol and not the simple oxide.[12] An indication of the presence of a nuclear double bond is that the substance gives a strong test for unsaturation with tetranitromethane comparable to the starting material (Xa); in comparison, the ketoxide XIV, which has only the double bond in the side chain, gives a very feeble test. The free hydroxy compound was recovered unchanged after being refluxed with lithium aluminum hydride in ether, but the result does not exclude the 7,8-oxide formulation, since the 9α,11α-oxido group is stable to this reagent.[13] Callow and Rosenheim treated the "oxide" 3-acetate with mineral acid and identified the product as ergosterol-B_8 acetate (XV). For better differentiation, we treated the 3-acetate with phosphorus oxychloride in pyridine at 0–25° and worked up the mixture under strictly non-acidic conditions; the product, isolated in 80% yield, corresponded closely in melting point, rotation, position and intensity of ultraviolet absorption, with pure ergosterol B_8-acetate as described by Barton and Brooks.[8] This observation seemed to us to suggest that the substance is an allylic alcohol, with the hydroxyl group at C_{14} and the double bond at the original 7,8-position. However, infrared spectra of the 3-acetate taken in solution and in mulls on Baird and on Perkin–Elmer instruments showed no evidence of the presence of a hydroxyl group.

The apparent paradox was resolved by observations made at the Merck laboratories by Dr. Erwin F. Schoenewaldt, who very kindly has let us report the results. A thoroughly dried sample of the "oxide" 3-acetate was found by Zerewitinoff and deuterium exchange analyses to contain only 0.3 and 0.05 active hydrogen, respectively. Zeisel determination, on the other hand, revealed the presence of one alkoxyl group. Henbest and Jones[14] have shown that 7-hydroxy derivatives of cholesteryl esters undergo alkylation very readily in alcoholic solution in the presence of acids, even acetic acid, and an analogous reaction evidently has taken place in the case at hand. The samples

(11) O. Wintersteiner and M. Moore, THIS JOURNAL, **65**, 1507 (1943).

(12) Formation of the 7,8-oxide has been observed by Dr. E. F. Schoenewaldt with use of perbenzoic acid in benzene and by Dr. D. H. R. Barton with use of perphthalic acid.

(13) L. F. Fieser and S. Rajagopalan, THIS JOURNAL, **73**, 118 (1951).

(14) H. B. Henbest and E. R. H. Jones, *J. Chem. Soc.*, 1798 (1948).

prepared by Callow and Rosenheim, Schoenewaldt and us were all crystallized from methanol (and agreed in m.p.), but since the oxidation itself involved prolonged contact with ethanol in the presence of selenous acid there is little doubt that the substance is the ethyl rather than the methyl ether; the specific formulation XI is suggested on the basis of further evidence to be cited. Callow and Rosenheim's analyses of the alcohol and its acetate and benzoate agree as well with the theory for $C_{30}H_{50}O_2$ as with that for $C_{28}H_{46}O_2$; two of three reported carbon values for the dinitrobenzoate (69.2, 69.6, 69.5) agree better with the theory for an ethoxy derivative (69.78) than with that for an oxide or hydroxy compound (69.05).

The experiments thus far cited refer to oxidation of Δ^7-cholestenyl acetate in acetic acid–benzene and of 5-dihydroergosterol in ethanol. Oxidations of Δ^7-cholestenol in ethanol according to Callow and Rosenheim and of 5-dihydroergosteryl acetate in acetic acid–benzene–0.6% water (0°) afforded no crystalline products, but correlation between the two series was finally effected by oxidation of 5-dihydroergosteryl acetate in acetic acid–benzene–6% water at 25°, which gave about 40% of crystalline material consisting in a readily separable mixture of ergosterol-D acetate and a diacetate (XII) analogous to that in the cholestenol series (II). Deacetylation with lithium aluminum hydride was attempted under conditions that proved too drastic, for the product was ergosterol-B_3, identified as the acetate XV. The dehydration is analogous to that observed in the cholesterol series on Oppenauer oxidation of III. The two series can also be correlated by molecular rotations. The effect of introduction of the acetoxyl group into $\Delta^{8(14)}$-cholestenyl acetate (M_D +41 Chf) is $\Delta^{7\text{-OAc}} = -61$. Laubach and Brunings[15] report the M_D value for $\Delta^{8(14),22}$-ergostadiene-3β-ol acetate of −117 Chf (the value calculated from the data of Barton[16] is −98); hence in this series $\Delta^{7\text{-OAc}} = -75$, a value sufficiently close to −61 to warrant assignment of the structure XII to the ergosterol derivative.

The molecular rotations of the ethoxy and acetoxy derivatives of 5-dihydroergosterol acetate, −228 and −192, appear consistent with the postulate that the substances are similarly constituted, but no basis is available for more than a qualitative evaluation of the data. The case of 7α-methoxycholesteryl acetate[14] (M_D −569) compared to 7α-acetoxycholesteryl acetate (M_D −846) is not strictly analogous, since here the substituted asymmetric center is adjacent to a second center of asymmetry (C_8). The best evidence that the ethoxy and acetoxy compounds have the analogous structures XIb and XII is that they are produced in similar oxidations, behave in analogous fashion in the eliminations to B_3-diene systems already cited, and respond in the same way to a surprising oxidation reaction encountered first in the ergosterol series. At the time we were attempting to differentiate between the 7,8-oxide and Δ^7-ene-14-ol formulations for the Callow and Rosenheim substance, we investigated oxidation of the 3-acetate with chromic acid and isolated a product identical with the 7-keto-8,14-oxide (XIV), which Stavely and Bollenback[17] isolated, along with the 7-keto-8,9-oxide XIII, as products of chromic acid oxidation of 5-dihydroergosteryl acetate (Xb). We later oxidized $\Delta^{8(14)}$-cholestene-3,7-diol diacetate (II) in the same way (chromic acid in acetic acid at 25°) and isolated 8,14-oxidocholestane-3β-ol-7-one 3-acetate.[2]

Configurations.—Wintersteiner and Moore[11] found that Δ^7-cholestenyl acetate reacts with two moles of perbenzoic acid to give a substance that they proved to be a 7-hydroxy-8,14-oxide (XVIIb) and, in smaller amount, an isomer recently shown in our laboratory[18] to be the 7-hydroxy-8,9-oxide (XVIa). From the reaction of perbenzoic acid with our cholestenediol formulated as III we obtained in high yield a product identified by direct comparison as diol and as diacetate as identical with Wintersteiner and Moore's 8,14-oxidocholestane-3β,7-diol (XVII). This result fully substantiates the structure III deduced on other grounds.

A reasonable inference regarding the configurations of the Wintersteiner and Moore oxidocholestanediols and of our cholestenediol can be made from consideration of molecular rotation relationships. The molecular rotation increment for 3,7-diacetylation of the 8,14-oxidodiol XVIIa is −94, a value close to that (−117) for diacetylation of cholestane-3β,7α-diol[19] (XIXa), but quite different from that (+53) for diacetylation of cholestane-3β,7β-diol[19] (XXa); hence the hydroxyl group at C_7 is α-oriented in XVIIa and also in our enediol III. The same conclusion regarding III is reached, independently of the correlation with the oxidodiol, by the following less secure but evidently valid evidence: the M_D increment for 7-acetoxylation of $\Delta^{8(14)}$-cholestenyl acetate (M_D +41 Chf, $\Delta = -61$) is of the same sign as that for 7α-acetoxylation of cholestanyl acetate ($\Delta = -144$) and of sign opposite that for 7β-acetoxylation ($\Delta = +207$). The 7α-configuration of III means that the 7-hydroxyl group is polar, and hence so oriented as to be somewhat resistant to acylation but susceptible to elimination, as noted, respectively, in the selective 3-cathylation of the diol and in the various conversions to B_3-derivatives.

The 7-hydroxyl group of the 3-acetoxy-8,9-oxido alcohol XVIa must also be α-oriented, since 7-acetylation is attended with a strong levorotatory shift (−147), corresponding to that for 7-acetylation of 7α-hydroxycholestanyl acetate (−84) and not for acetylation of the β-epimer (+109). On hydroxylation of Δ^7-cholestenyl acetate with osmium tetroxide and hydrolysis at C_3, Wintersteiner and Moore[11] obtained a product which must be the 3β,7α,8α-triol XVIIIa since the M_D increment for formation of the 3,7-diacetate is strongly negative (−147), as in the comparison diol XIXa.

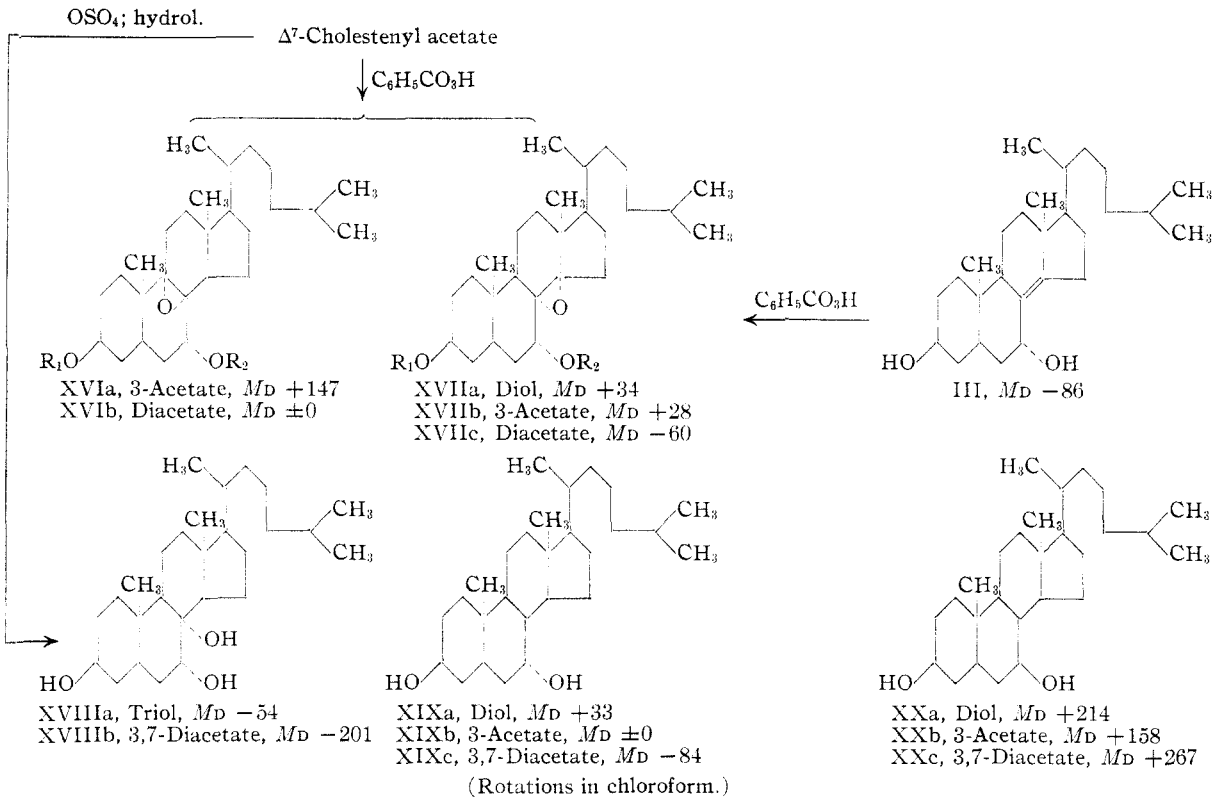
(15) G. D. Laubach and K. J. Brunings, THIS JOURNAL, **74**, 705 (1952).

(16) D. H. R. Barton, *J. Chem. Soc.*, 813 (1945); D. H. R. Barton, J. D. Cox and N. J. Holness, *ibid.*, 1771 (1949).

(17) H. E. Stavely and G. N. Bollenback, THIS JOURNAL, **65**, 1290, (1943).

(18) L. F. Fieser, K. Nakanishi and W.-Y. Huang, *ibid.*, in press.

(19) O. Wintersteiner and M. Moore, *ibid.*, **65**, 1503 (1943); for configuration, see H. Heymann and L. F. Fieser, *Helv. Chim. Acta*, **35**, 63 (1952).



In this instance, oxidative attack at C_8 has occurred exclusively from the rear, in accordance with the general rule[20]; hence it is justifiable to assume α-attack at C_8 in the stereospecific oxidation with perbenzoic acid leading to the 8,9- and 8,14-oxido alcohols, which are both α-oriented at C_7 and whose formation involves two points of attack in common. Thus the oxygen functions introduced in the oxidations under discussion at positions 7, 8, 9 and 14 are all α-oriented.

The 7-keto oxides,[2,11] corresponding to the 3-acetoxyoxidocholestane-7α-ols XVIa and XVIIb differ in M_D by an increment (−172) sufficiently close to that for the ketoxides XIII and XIV of the dihydroergosterol series (−250) for assignment of the α-configuration to the oxido groups in the latter series. Stavely and Bollenback's ketoxides of the "α"-spinasterol series[21] are correlated with XIII and XIV by M_D relationships.

In the 5-dihydroergosterol series the diacetate (XII) formed on selenium dioxide oxidation in acetic acid is identified as 7α-oriented by the M_D correlation with the corresponding cholestenediol derivative II, and the 7-ethoxyl group of the product of oxidation in ethanol (XIa) is identified as being also α-oriented by the M_D relationship and by oxidation of the 3-acetate to the ketoxide XIV, shown above to be the 8α,14α-oxide.

Eck and Hollingsworth[22] isolated a product of oxidation of Δ^7-cholestene[23] that they regarded as a diketone but that may well be a ketoxide. In the case of Δ^7-cholestenyl acetate, oxidation to the 8α,9α-oxido-7-ketone is attended with an M_D increment of −156, and the increment for oxidation to the 8α,14α-oxido-7-ketone is −328. The product of Eck and Hollingsworth differs from their starting material by the M_D increment −256 (CCl_4), which suggests that the material is a mixture of about equal amounts of the two ketoxides. Berner, Lardon and Reichstein[25] isolated from the reaction of perbenzoic acid on methyl 3α-acetoxy-12α-hydroxy-Δ^7-cholenate (M_D +451 Chf) a product of M_D +356 Chf tentatively formulated as the 7-hydroxy-8,14-oxide; in this case comparison with the M_D relationships in the cholesterol series is vitiated by the many differences and by the presence in one series of a center of asymmetry at C_{12} close to the oxide function.

Mechanism.—The mechanism that we favor for the observed selenium dioxide oxidations of the Δ^7-stenols (A) is allylic hydroxylation (B), allylic rearrangement, and acetylation or alkylation (D); if ethylation occurs in the one case under very feeble acid catalysis, it is reasonable to postulate acetylation by acetic acid in the other. The rearrangement of B into C is in accord with the known tendency of the 7,8-double bond to migrate to the 8,14-position under hydrogenating conditions. The hydroxyl group in the intermediate B is formulated as α-oriented because the rearrangement to C proceeds in a single steric sense and hence probably by a concerted mechanism, as in the case of Δ^5-cholestene-3β,4β-diol → Δ^4-cholestene-3β,6β-

(20) L. F. Fieser, *Experientia*, **6**, 312 (1950); T. F. Gallagher and T. H. Kritchevsky, THIS JOURNAL, **72**, 882 (1950).

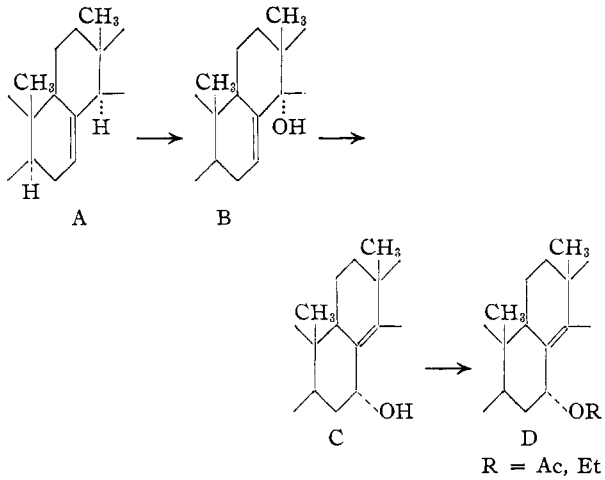
(21) H. E. Stavely and G. N. Bollenback, *ibid.*, **65**, 1600 (1943).

(22) J. C. Eck and E. W. Hollingsworth, *ibid.*, **63**, 2986 (1941).

(23) Originally described as "Δ^8-cholestene," for assignment of the Δ^7-structure, see Phenanthrene book,[24] p. 248.

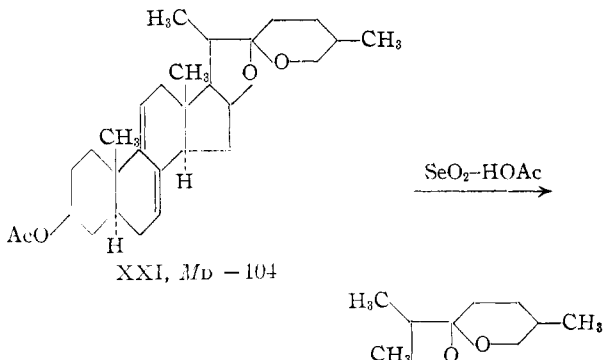
(24) L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," 3rd Edition, Reinhold Publ. Corp., New York, 1949.

(25) E. Berner, A. Lardon and T. Reichstein, *Helv. Chim. Acta*, **30**, 1542 (1947).

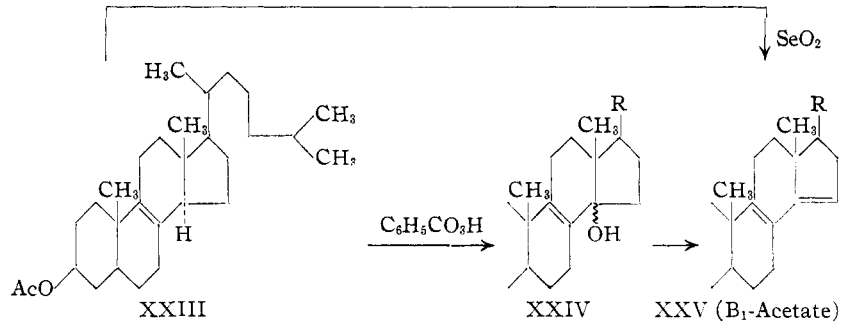


diol. The alternate mechanism of an initial attack of A at C_7 with direct formation of C (or D) seems less plausible, since it would entail expulsion of an α-hydrogen atom at C_{14} on entrance of a hydroxyl group at C_7 on the same (α) side of the molecule. On the A → C postulate a 14β-hydrogen atom would seem more favorable for elimination than a 14α-atom, but it has been found that in the highly specific selenium dioxide test[2] compounds of the allo series having an 8β-hydrogen atom activated by a double bond at either the 9,11- or 14,15-position give a negative response. The observation that oxidation of dehydroepiandrosterone acetate dibromide with chromic anhydride under anhydrous conditions affords a 14-hydroxy derivative[26] shows that the 14α-hydrogen atom is vulnerable to oxidative attack even in the absence of an activating double bond.

A substance of the type B was produced in a similarly conducted reaction of the $\Delta^{7,9(11)}$-diene of the diosgenin series, XXI.[27] Oxidation of XXI with selenium dioxide in acetic acid–benzene at 0° gave a product having the composition of a monohydroxy derivative or oxide. Since the substance retains the original ultraviolet absorption characteristics of the D-diene system it is evidently not an oxide, and the infrared spectrum shows a small but definite hydroxyl band. Since the substance is not acylable, it very probably is the 14-hydroxy derivative XXII. The reaction with selenium dioxide is unique, since a variety of other oxidizing agents all attack the $\Delta^{7,9(11)}$-diene system with obliteration of the characteristic spectrum. The diene system in the reaction product is so stable that the substance is not altered by hot acetic acid.



The conversion of Δ^7-cholestenyl acetate by oxidation first with selenium dioxide and then with peracid to an 8,14-oxido-7-ol identical with that resulting from the action of peracid alone on the stenyl indicates that the peracid oxidation proceeds by initial allylic hydroxylation at C_{14} (B) followed by allylic rearrangement (C) and oxide formation, as postulated by Fieser and Fieser.[28] That perbenzoic acid can effect hydroxylation of olefins of specific structural types is established by Windaus, Linsert and Eckhardt's[29] oxidation of a preparation rich in Δ^8-cholestenyl acetate (XXIII) to a tertiary alcohol characterized by dehydration with acetic anhydride to a product designated "cholestadienol-D acetate" but actually corresponding closely in constants to the B_1-isomer XXV. These facts have been interpreted as indicating that the product is the 14-hydroxy derivative XXIV.[30] The observation of Adams, Petrow and Royer[9] that the Δ^8-stenyl acetate XXIII affords B_1-acetate (XXV) on reaction with selenium dioxide in hot ethanol establishes a further correlation between the action of the two oxidizing agents.



Fieser and Fieser[31] formulated the oxidation of Δ^7-stenols with chromic acid as involving initial formation of a 7,8-oxide,[32] oxidation to an 8-hydroxy-7-ketone, dehydration to the Δ^8- and $\Delta^{8(14)}$-7-ketones, and oxide formation. Barton[33] postu-

(26) A. F. St. André, H. B. MacPhillamy, J. A. Nelson, A. C. Shabica and C. R. Scholz, THIS JOURNAL, **74**, 5506 (1952).

(27) The initial experiments in the diosgenin series were conducted by one of us (L.F.F.) at the laboratory of Syntex S.A., Mexico City, through the courtesy of Drs. George Rosenkranz and Carl Djerassi and with the assistance of Rosa Yashin.

(28) Reference 24, pp. 245–246.

(29) A. Windaus, O. Linsert and H. J. Eckhardt, *Ann.*, **534**, 22 (1938).

(30) Reference 24, p. 226; the name under formula XVIII of the book should be Cholestadienol-B_1 acetate.

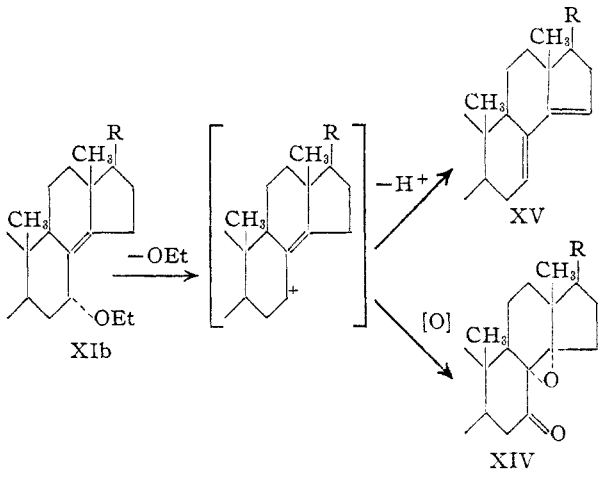
(31) Reference 24, pp. 227–233, 283–285.

(32) Stavely and Bollenback[17] has postulated initial oxide formation.

(33) D. H. R. Barton, *J. Chem. Soc.*, 813 (1945); 512 (1946).

lated the same intermediate 8-hydroxy-7-ketone but suggested its formation *via* an initial hydroxylation of the 7,8-double bond. These views do not now appear tenable. α-Hydroxy ketones of the type postulated, for example cholestane-3β,5α-diol-6-one,[34] are very resistant to acid dehydration and are stable to chromic acid; Δ^4-cholestene-3,6-dione, in which the double bond is reactive enough to be reducible with zinc and acetic acid, is not convertible to a ketoxide by chromic acid. The α,β-unsaturated ketonic group of cholestenone is not altered in the course of oxidative fission of the side chain to give progesterone and androstenedione.[35]

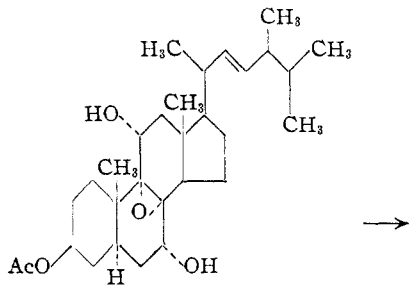
It was reported above that both the 7α-ethoxy-$\Delta^{8(14)}$-stenol acetate XIb and the 7α-acetoxy-$\Delta^{8(14)}$-stenol acetate II are convertible by chromic acid oxidation into the corresponding 8α,14α-oxido-7-ketones. A clue to the mechanism of the oxidation is provided by the observation that the 7α-ethoxy derivative XIb is converted by acetic acid at room temperature into the B_3-type diene (XV). Such a diene can hardly be an intermediate to the 8α,14α-



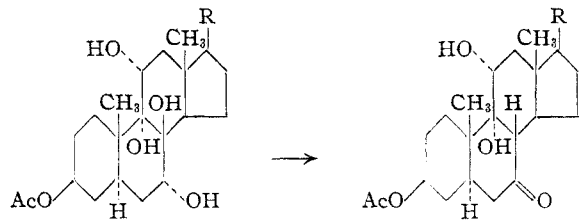
oxido-7-ketone (XIV), and the 7α-acetoxy-$\Delta^{8(14)}$-stenol acetate resulting from oxidation in acetic acid–benzene is obviously less susceptible to B_3-elimination under mild acid catalysis. However, the ready conversion of the ethoxy compound XIb to a diene (XV) reveals a lability of this type of system that perhaps is expressed in a tendency toward expulsion of an ethoxide or acetate anion with formation of a carbonium ion with the charge at C_7. This ion perhaps can either expel a proton to give the B_3-diene (XV) or, under oxidizing conditions, give rise to the 8α,14α-oxido-7-ketone XIV. The chromic acid oxidation of a Δ^7-stenol to the oxidoketone XIV may involve merely allylic hydroxylation at C_{14} and direct oxidation of the Δ^7-ene-14α-ol to the 8α,14α-oxido-7-ketone. The concurrent formation of the 8α,9α-oxido-7-ketone can be interpreted as involving a similar process starting with allylic hydroxylation at C_9; abundant evidence of oxidative attack at this position is provided by the dehydrogenation of Δ^7-stenols to $\Delta^{7,9(11)}$-dienes by mercuric acetate,[36] perbenzoic acid,[37] bromine,[38,39] N-bromosuccinimide[40] and selenium dioxide.[3] Selenium dioxide is the only reagent other than chromic acid demonstrated to attack Δ^7-stenols at both positions 9 and 14, as evidenced by formation of $\Delta^{7,9(11)}$- and $\Delta^{7,14}$-dienes at 25°, cited above.

Acknowledgments.—We are greatly indebted to the du Pont Company for supplies of 7-dehydrocholesterol, to Drs. Max Tishler and John Chemerda of Merck and Drs. George Rosenkranz and Carl Djerassi of Syntex for supplies and general coöperation, to Dr. Erwin F. Schoenewaldt of Merck and Co., Inc., for his important contribution, and to Dr. Derek H. R. Barton for a stimulating discussion of certain aspects of the problem.

Note.—The present observations are of interest in relation to the question of the configurations of a series of products of oxidation of 3β-acetoxy-$\Delta^{7,9(11),22}$-ergostatriene described by Heusser, *et al.*[41] One of the key substances was characterized as an 8,9-oxido-7,11α-diol of probable α-orientation of the oxide linkage, as in XXVI. The Swiss investigators draw the further reasonable inference that in a tetrol derivative resulting from hydrolytic fission of the oxide ring the newly formed hydroxyl groups



XXVIa, 3-Acetate, M_D +78
XXVIb, 3,7,11-Triacetate, M_D +34



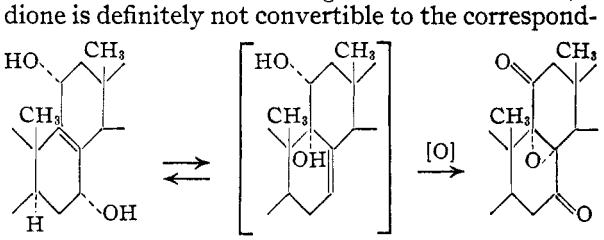
XXVIIIa, 3-Acetate, M_D −288
XXVIIIb, 3,11-Diacetate, M_D −239

have the orientations 8β,9α, as in XXVII. From the observation that acidic dehydration of the 7,8-glycol grouping of this substance affords the 7-keto grouping of XXVIII, they tentatively conclude that the 7-hydroxyl group is *cis* to the hydroxyl at C_8, and hence β, on the postulate that the dehydration must involve *trans*-elimination of the 8β-OH with a 7α-H. The M_D increment for 7-acetylation of a derivative of XXVI can be estimated as follows. In the case of the 7-ketone XXVIII, conversion of the 3-acetate to the 3,11-

(34) Attempted dehydration of this substance with phosphorus oxychloride in pyridine resulted only in recovery of starting material.
(35) W. Dirscherl and F. Hanusch, *Z. physiol. Chem.*, **252**, 49 (1938).
(36) A. Windaus and E. Auhagen, *Ann.*, **472**, 185 (1929).
(37) A. Windaus and A. Lüttringhaus, *ibid.*, **481**, 119 (1930).
(38) J. C. Eck and E. W. Hollingsworth, THIS JOURNAL, **64**, 140 (1942).
(39) R. C. Anderson, R. Budziarek, G. T. Newbold, R. Stevenson and F. S. Spring, *Chemistry and Industry*, 1035 (1951).
(40) L. F. Fieser, THIS JOURNAL, **73**, 5007 (1951).
(41) H. Heusser, R. Anliker, K. Eichenberger and O. Jeger, *Helv. Chim. Acta*, **35**, 936,(1952).

diacetate is attended with a shift in M_D of +49; hence the M_D predicted for the unknown 3,11-diacetate of XXVI is +78 + 49 = +127. The effect of acetylation at C_7 is then given by the difference in M_D of the triacetate XXVIb (+34) and the hypothetical 3,11-diacetate, +34 − 127 = −93. The figure agrees in sign and magnitude with that (−84) for 7-acetylation of cholestane-3β,7α-diol 3-acetate (+109 for the 7β-OH), and hence the compounds are most likely 7α-hydroxy derivatives, as formulated in XXVI and XXVII. Conversion to the 7-ketone XXVIII is then a *cis*-dehydration of a 7,8-*trans*-glycol grouping, exactly analogous to the *cis*-dehydration of a 9,11-*trans*-glycol grouping observed in this Laboratory[42] and interpreted as involving neighboring group participation of the tertiary hydroxyl function.

Heusser, Jeger and co-workers[43] report the chromic acid oxidation of $\Delta^{8,22}$-ergostadiene-3β,7α,11α-triol 3-acetate (XXIX) to a mixture of the expected Δ^8-ene-7,11-dione and the 8,9-oxido-7,11-dione XXXI. The analogous Δ^4-cholestene-3,6-dione is definitely not convertible to the correspond-



XXIX XXX XXXI

ing oxide by oxidation with hexavalent chromium[44] and, in reply to our inquiry on the point, Drs. Heusser and Jeger state: "we presume that the enedione and its ketoxide (XXXI) are produced by different mechanisms from the same starting material." In analogy with the above postulated oxidation of a Δ^8-stenol through the Δ^7-ene-14α-ol to an 8α,14α-oxido-7-one (XIV), we suggest that the Δ^8-ene-7,11-diol XXIX undergoes allylic rearrangement to XXX, or to the $\Delta^{9(11)}$-ene-7,8-diol, either or both of which is then oxidized to the 8,9-oxido-7,11-dione XXXI.

Experimental[45]

Δ^7-Cholestenol.—The procedure[46] of purifying methanol-moist 7-dehydrocholesterol prior to hydrogenation was advantageously modified by dissolving 50 g. of material in 100 cc. of chloroform, filtering and adding methanol to the point of saturation at the boiling point. The first crystallizate was washed with methanol and the second crop obtained on concentration of the mother liquor; yield 40 g. (80%), m.p. 151–153° vac.

Oxidation of Δ^7-Cholestenyl Acetate at 0–5°: $\Delta^{8(14)}$-Cholestene-3β,7α-diol Diacetate (II, L.F.F.,G.O.).—A solution of 5 g. of acetate in benzene (50 cc.)–acetic acid (100 cc.) was treated at 0–5° with 60 cc. of 0.1 *M* selenous acid[47] in acetic acid and after 16 hr. ether and water were added and the benzene–ether layer washed free of acid with water and then with bicarbonate solution. The dried solution was evaporated and a solution of the residue in ether was filtered to remove coagulated selenium and refluxed with precipitated silver for about 1 hr. The filtered solution (yellow) was diluted with a little methanol until crystals started to separate, and evaporated in a stream of air with addition of more methanol. The crystals had a yellow tinge, removed by a second treatment with silver. Recrystallization from ether–methanol then gave 3.1 g. (55%) of colorless, transparent prisms, m.p. 134–136°. This yield was duplicated in several runs and the result was the same when the product was isolated by chromatography (eluted by 4:6 petroleum ether–benzene); in this case removal of selenium was effected by filling a bottom section of the column with a ground 1:1 mixture of alumina and precipitated silver. Repeated recrystallization of the diacetate from ether–methanol gave material of m.p. 138.8–139.2°, α_D −4.4° Chf, λ^{CS_2} 5.77, 8.02, 12.0 μ.

Anal. Calcd. for $C_{31}H_{50}O_4$ (486.71): C, 76.50; H, 10.36. Found: C, 76.38, 76.21, 76.59; H, 10.40, 10.35, 10.11.

The first experiments (L.F.F.) were conducted as described above at 0° but without use of silver (introduced by G.O.), and the washed and dried ether–benzene extract was evaporated to dryness; the residual, selenium-containing glass when triturated with methanol promptly afforded solid diacetate, m.p. in the range 132–136°, in 45–55% yield. In later experiments (L.F.F.), in which the 0.1 *M* selenous acid solution was made by dissolving 1.1 g. of selenium dioxide in 2 cc. of water and diluting to 100 cc. with acetic acid, the extracted reaction product failed to crystallize from methanol, even after treatment with silver, until it had been reacetylated (pyridine–acetic anhydride, one-half hour on the steam-bath). Since commercial samples of selenous acid such as that initially employed usually smell of nitric acid, the difference may have been due to the presence of this acid in the first experiments. Attempts to promote and to prevent acetylation of a possible alcoholic intermediate were not decisive but led to a procedure by which crystalline cholestenediol diacetate could be isolated reproducibly without reacetylation, and which also proved applicable in the 5-dihydroergosterol series, where the other procedure failed. Addition of excess acetic anhydride speeded up the oxidation but gave an inferior product; addition of water slowed down the reaction so much that only a trace of selenium separated in 15 hr. at 0°.

The new procedure was as follows. A solution of 2 g. of Δ^7-cholestenyl acetate in 50 cc. of absolute ether and 40 cc. of acetic acid was treated at 25° with a mixture of 40 cc. of 0.1 *M* selenous acid in acetic acid (made from selenous acid) and 8 cc. of water. The solution (25°) turned yellow in a minute or two and when left overnight had deposited a large amount of red selenium. The solution was filtered, diluted with water, extracted with ether, and the extract washed with soda solution, dried, and stirred for 2 hr. at 25° with precipitated silver. The filtered solution was light yellow but completely free from selenium. Evaporation gave a yellow glass that gave a solid at once when rubbed with methanol. The material was brought into solution and let crystallize; there resulted 1.01 g. of slightly yellow diacetate, m.p. 134–137°. Short treatment with Norit in ether removed the color, and crystallization from methanol gave 0.70 g., m.p. 138.5–139.5°, α_D −2.1° Chf. (Found: C, 76.19; H, 10.33).

$\Delta^{8(14)}$-Cholestene-3β,7α-diol (III).—A solution of 0.95 g. of the above diacetate in absolute ether (50 cc.) was treated with 20 cc. of 0.5 *N* lithium aluminum hydride in ether, refluxed for 1 hr. and left overnight at 25°. Addition of a little ethyl acetate, then methanol and water, gave a solid product (0.8 g., m.p. 149–155°). The diol crystallized from methanol in long needles, m.p. 157–158°, α_D −21° Chf, λ^{Nujol} 2.9–3.1, 12.0 μ.

Anal. Calcd. for $C_{27}H_{46}O_2$ (402.64): C, 80.54; H, 11.52. Found: C, 80.25; H, 11.49.

For reacetylation, the diol was let stand in pyridine–acetic anhydride at 25° overnight. Addition of water caused separation of crystals, m.p. 134–136°, and on recrystallization from ether the substance formed heavy needles, m.p. 141–141.5°, α_D ±0° Chf. The substance did not depress the m.p. of the starting 3β,7α-diacetate and had an identical infrared spectrum.

Anal. Calcd. for $C_{31}H_{50}O_4$ (486.71): C, 76.50; H, 10.36. Found: C, 76.37; H, 10.26.

Saponification also afforded the diol, but in less pure form.

(42) H. Heymann and L. F. Fieser, THIS JOURNAL, **73**, 5252 (1951).

(43) H. Heusser, K. Eichenberger, P. Kurath, H. R. Dällenbach and O. Jeger, *Helv. Chim. Acta*, **34**, 2106 (1951).

(44) L. F. Fieser, Paper II, THIS JOURNAL, **75**, 4386 (1953).

(45) All melting points are corrected. Rotations were determined at 25° in 1–2% solutions in chloroform.

(46) L. F. Fieser and J. E. Herz, THIS JOURNAL, **75**, 121 (1953).

(47) Selenous acid (1.29) g. is dissolved in 2 cc. of hot water and the solution diluted to 100 cc. with acetic acid.

Thus a solution of 305 mg. of diacetate, m.p. 131–133°, in 20 cc. of methanol was treated at 25° with a mixture of 1 cc. each of methanol and 50% potassium hydroxide, let stand for 1 hr., filtered from a little fluff, and diluted. A white solid separated, m.p. 143–146° (276 mg.); found: C, 80.64; H, 11.55. Acetylation in pyridine (1/2 hr. on the steam-bath) and crystallization from methanol gave the original diacetate, m.p. 135–137°, α_D −2.1° Chf (identified by mixed m.p. and infrared).

The diol dibenzoate was prepared with pyridine and benzoyl chloride (overnight at 25°) and crystallized from ethyl acetate, chloroform–methanol, and chloroform–acetone: long needles, m.p. 152.5–153.5°, α_D −7.5° Chf, λ^{CS_2} 5.80, 6.20, 7.85 (benzoate); 12.0 μ.

Anal. Calcd. for $C_{41}H_{54}O_4$ (610.84); C, 80.61; H, 8.91. Found: C, 80.81; H, 8.88. (A monobenzoate, $C_{34}H_{50}O_3$, would require C, 80.58; H, 9.95.)

$\Delta^{7(14)}$-Cholestadienyl Cathylate (VIIIa)—A solution of 0.42 g. of $\Delta^{8(14)}$-cholestene-3β,7α-diol in benzene (5 cc.)–pyridine (5 cc.) was treated at 0° with a cooled solution of 2 cc. of ethyl chlorocarbonate in 5 cc. of benzene. After 1/2 hr. at 0° and 2 hr. at 25°, the pink solution was slowly warmed to 60° until evolution of CO_2 ceased. The solution was then treated at 0° with a solution of 0.2 cc. of phosphorous oxychloride in 5 cc. of benzene and left at 25° about 1 hr. The mixture containing precipitated pyridinium chloride was poured onto ice and potassium bicarbonate solution and the product was collected by ether extraction and chromatographed on 15 g. of alumina. Petroleum ether–benzene (8:2) eluted about 0.2 g. of a colorless oil that solidified slowly in the cold room. Four crystallizations from ether–methanol gave long needles of solvated product, m.p. 81–82°, α_D −156° Chf, λ^{EtOH} 242 mμ (9,800), λ^{Chf} 5.76, 7.9 (cathylate); 6.07 μ (double bond).

Anal. Calcd. for $C_{30}H_{48}O_3 \cdot 1/2CH_3OH$ (472.70): C, 77.54; H, 10.66. Found: C, 77.70, 77.79; H, 10.50, 10.41.

Attempts to isolate the intermediate Δ^7-cholestene-3β,14α-diol 3-cathylate before treatment with phosphorous oxychloride led only to low melting material that could not be crystallized satisfactorily from the usual solvents. Some absorption at 242 mμ indicated that partial dehydration had occurred during cathylation.

$\Delta^{7,14}$-Cholestadienyl Benzoate (VIIIb). (a) From the Cathylate.—A solution of 20 mg. of the cathylate in 5 cc. of ethanol was treated with one drop of 25% sodium hydroxide and a trace of phenolphthalein. The pink color did not fade when the solution was heated on the steam-bath, and dilution with water precipitated a highly solvated crystallizate. This was combined with a little oily product obtained by ether extraction, dried by distillation with benzene, and treated in benzene (2 cc.)–pyridine (10 drops) with benzoyl chloride (5 drops). The solution was warmed on the steam-bath and then treated with potassium bicarbonate solution and extracted with ether. The washed and dried extract on evaporation left an oil; this was taken up in petroleum ether–benzene (8:2) and the solution filtered through a short column of alumina. Evaporation gave a colorless oil that crystallized from methanol in small, off-white needles (15 mg.), m.p. 142–143°, α_D −159° Chf.

(b) From the Diol Dibenzoate.—A solution of 150 mg. of dibenzoate, m.p. 152–153.5°, in 1 cc. of dimethylaniline was heated overnight in a bath of boiling dimethylaniline and cooled. By addition of ether, water and acetic acid in suitable relative proportions, it was possible to produce a two-phase system, the aqueous layer of which was sufficiently acidic so that four such washings removed all the dimethylaniline. The ethereal layer was then washed with bicarbonate, dried and evaporated with addition of methanol. The initially dark brown crystallizate when clarified with charcoal and recrystallized from chloroform–methanol gave 56 mg. (47%) of small colorless platelets, m.p. 151–152° (strongly depressed by starting material), α_D −150° Chf, λ^{EtOH} 232 mμ (18,400; benzoate absorption), λ^{Chf} 5.82, 6.20, 7.82 (benzoate); 6.07 (double bond). A mixture of this $\Delta^{7,14}$-cholestadienyl benzoate with the less pure sample (a) melted at 145–150°.

$\Delta^{7,14}$-Cholestadiene-3-one (V).—A solution of 2 g. of $\Delta^{8(14)}$-cholestene-3β,7α-diol (m.p. 152–156°) in freshly distilled cyclohexanone (10 cc.) and toluene (150 cc.) was dried by distillation of 80 cc. of liquid, and then 2.5 g. of aluminum isopropoxide in 50 cc. of toluene was added during 35 min. while slow distillation was maintained. After 35 min. more the mixture was steam distilled, first at atmospheric pressure and then at reduced pressure. The residual yellow oil was extracted with ether and the product chromatographed on 100 g. of alumina. Petroleum ether–benzene (1:1) eluted the cholestadienone, which crystallized from methanol–acetone in short needles (about 0.1 g.), m.p. 129–129.4°, α_D −147° Chf., λ^{EtOH} 242 mμ (9,700), λ^{Chf} 5.85 μ.

Anal. Calcd. for $C_{27}H_{42}O$ (382.61): C, 84.79; H, 10.94. Found: C, 84.75; H, 11.07.

Later oily fractions showed only weak or no absorption at 242 mμ and could not be crystallized. The infrared and ultraviolet spectra of the total reaction product, and of the different fractions, showed the absence of α,β-unsaturated ketone.

$\Delta^{8,14}$-Cholestadienol and Acetate (IV). (a) From $\Delta^{8(14)}$-Cholestene-3β,7α-diol Diacetate.—A solution of 0.35 g. of the diacetate in 15 cc. of 95% ethanol containing 1 cc. of 36% hydrochloric acid was refluxed for 2 hr. and the solvent was then evaporated at 80°, first in a current of air and then in vacuum. The residue was taken up in 5 cc. each of benzene, pyridine and acetic anhydride and the solution let stand for 2 hr. at 25°. The benzene was then evaporated in a current of air, and enough water was added to decompose the excess anhydride and give a slightly cloudy solution. Thin platelets separated on cooling, and two recrystallizations from ether–methanol yielded 167 mg. (55%) of pure **$\Delta^{8,14}$-cholestadienyl acetate (cholestadienyl-B_1 acetate)**, m.p. 99–99.4°, α_D −21.6° Chf, λ^{EtOH} 246, 249.5 mμ (18,050; 18,200).

Anal. Calcd. for $C_{29}H_{46}O_2$ (426.66): C, 81.63; H, 10.87. Found: C, 81.86; H, 11.06.

Adams, Petrow and Royer[9] report: m.p. 101–102°, α_D −22.9° Chf, λ^{EtOH} 250 mμ (log E 4.3).

(b) From 7-Dehydrocholesterol.—A solution of 8 g. of crude methanol-moistened 7-dehydrocholesterol (du Pont) in 95% ethanol (150 cc.)–benzene (20 cc.) was treated with 36% hydrochloric acid (20 cc.) and refluxed for 1.5 hr. Evaporation of the deep green liquid at 80°, eventually in vacuum, gave a dry residue that absorbed strongly at 250–251 mμ, with no absorption near 280 mμ due to starting diene. One part, washed with potassium bicarbonate, was purified as free **$\Delta^{8,14}$-cholestadienol;** it crystallized from methanol in needles, m.p. 116–117°, α_D −12° Chf, λ^{EtOH} 250 mμ (18,100). Adams, Petrow and Royer[9] report: m.p. 119–120°, α_D −13°, λ^{EtOH} 250 (log E 4.3). The **acetate** formed large elongated plates (up to 3 cm. long) from methanol, m.p. 99–100°, α_D −21° Chf, λ^{EtOH} 250 mμ (18,100).

$\Delta^{8,14}$-Cholestadienyl acetate of exactly the same constants was also obtained as follows. A solution of crude 7-dehydrocholesterol (10 g.) in acetic acid (170 cc.)–benzene (50 cc.) containing 36% hydrochloric acid (3 cc.) was heated and the benzene allowed to evaporate. A mixture prepared by cautiously adding 2 cc. of 36% hydrochloric acid to 30 cc. of acetic anhydride was then added slowly. The solution was refluxed for 1 hr. and then sodium acetate (3 g.) was added to neutralize the mineral acid (a sample of distillate gave a negative test for chloride ion). The mixture was then cooled and diluted with water. The product, extracted with ether as a yellow oil, crystallized from methanol in long plates and was obtained pure after a second crystallization from ether–methanol; yield 8 g. (80%).

8α,14α-Oxidocholestane-3β,7α-diol[11] (XVIIa) from $\Delta^{8(14)}$-Cholestene-3β,7α-diol (III).[48]—The total diol obtained from 0.8 g. of diacetate, m.p. 137–139°, with lithium aluminum hydride (the ultraviolet spectrum indicated the presence of about 3% of the $\Delta^{8,14}$-diene) was treated with 2.2 equivalents of perbenzoic acid in chloroform and let stand at 3° for 8 days. The solution was diluted with chloroform, washed with carbonate solution, water and brine, and the product recovered and crystallized from methanol. A first crop of 237 mg. had m.p. 186–187°, α_D +7.0 Chf (Wintersteiner and Moore,[11] m.p. 186–187°, α_D +8.1 Chf), not depressed in m.p. on mixing with an authentic sample prepared by K. Nakanishi,[18] second crop, 137 mg., m.p. 180–182°. Acetylation of 157 mg. of pure material in pyridine–acetic anhydride (48 hr. at 25°) gave 160 mg. of crude **diacetate,** m.p. 160–162°, and one crystallization from methanol gave pure material, m.p. 162–163°, α_D −13.4° Chf. (W. and M.,

(48) Experiment by Dr. Wei-Yuan Huang.

m.p. 162–163°, α_D −11.9 Chf), also undepressed in mixed m.p. with authentic diacetate.

Crude $\Delta^{8(14)}$-Cholestene-3β,7α,β-diol.—A solution of 290 mg. of $\Delta^{8(14)}$-cholestene-3β-ol-7-one acetate (prepared by Dr. W.-Y. Huang) in 20 cc. of ether was refluxed with 150 mg. of lithium aluminum hydride. Processing as described for the preparation of $\Delta^{8(14)}$-cholestene-3β,7α-diol gave 250 mg. of white solid melting indefinitely between 160 and 170°. The material gave gels on attempted crystallization and afforded a low-melting acetate mixture. The diol mixture showed λ^{Nujol} 3.0 μ, the acetate mixture λ^{CS_2} 5.77–5.80, 8.0 μ; in neither case was there any absorption in the region 11.7–12.4μ.

Oxidation of Δ^7-Cholestenyl Acetate at 25°.—A solution of Δ^7-cholestenyl acetate (5 g., 11.7 millimoles) in benzene (75 cc.)–acetic acid (100 cc.) was treated at room temperature with 60 cc. of 0.1 *M* selenous acid in acetic acid (6 millimoles). After 15 hr. the solution was filtered through cotton to remove the bulk of the precipitated selenium, diluted with ether and washed three times with water. The benzene–ether layer, still containing a little acetic acid, was shaken with brine, filtered through anhydrous sodium sulfate, and refluxed with precipitated silver for a few hours until pale yellow. Evaporation of the filtered solution left an oily residue that afforded 1.5 g. of crystals from acetone–methanol. Crystallization from methanol gave needles, m.p. 85–88°, α_D −45° Chf, consisting of a mixture of the **acetates of $\Delta^{7,9(11)}$-cholestadienol (D) and of $\Delta^{7,14}$-cholestadienol (B_3).** A sample crystallized further from ether–methanol melted at 102–103.5°, λ^{EtOH} 242 mμ (11,400), shoulders at 236 and 250 mμ.

Anal. Calcd. for $C_{29}H_{46}O_2$ (426.66): C, 81.63; H, 10.87. Found: C, 81.68; H, 10.75.

Another portion of crude mixture, m.p. 85–88°, was chromatographed on alumina, and separated into four fractions, all eluted by petroleum ether–benzene (9:1) and all having an absorption band at 242 mμ: (1) m.p. 101–103°, α_D +2°, *E* 14,000; (2) m.p. 96–97°, α_D −23°, *E* 13,000; (3) m.p. 95–96°; (4) m.p. 91–93°, α_D −105°, *E* 11,000. Analyses of the first two fractions corresponded to the formula $C_{29}H_{46}O_2$ (found: C, 81.52, 81.62; H, 11.05, 10.94). Mixtures of the fractions showed no depression in m.p. The constants of fraction 4 indicate that it is very rich in $\Delta^{7,14}$-cholestadienyl acetate (B_3).

The B_3-component was removed from another portion of the 85–88° mixture by refluxing 0.5 g. with 0.2 g. of maleic anhydride in 10 cc. of benzene for 4 hr. The solvent was evaporated completely and the residue dissolved in hot acetic acid. On cooling, long thin needles crystallized, m.p. 106–107°, α_D +28° Chf, λ^{EtOH} 242 mμ (10,000). The rotation corresponds to a content of about 80% $\Delta^{7,9(11)}$-cholestadienyl acetate (D).

Oxidation of Δ^7-Cholestenyl Benzoate at 25°.—A solution of 1 g. of benzoate in benzene (15 cc.)–acetic acid (5 cc.) was treated with selenium dioxide (0.6 g.) in acetic acid (20 cc.). After 19 hr. at 25° the reaction mixture was worked up as above. The initial crystallizate (about 0.1 g.) on recrystallization from ethyl acetate–methanol and from chloroform–methanol formed heavy needles, m.p. 145–147°, α_D −74° Chf; the substance showed benzoate absorption at 232 mμ but no shoulder at 250 mμ. The material appears to be a mixture of cholestadienyl benzoates.

Anal. Calcd. for $C_{34}H_{48}O_2$ (488.72): C, 83.55; H, 9.90. Found: C, 83.43; H, 9.93.

Chromic Acid Oxidation of $\Delta^{8(14)}$-Cholestene-3β,7α-diol Diacetate (L.F.F.).—A solution prepared by dissolving 100 mg. of chromic anhydride in a few drops of water and diluting with 20 cc. of acetic acid was poured on 200 mg. of the diacetate II. The solid dissolved on brief shaking and after standing overnight the solution was diluted with water and the product extracted with ether and dissolved in a little methanol. After standing for a few hr. at 5° the solution deposited a few round tufts of small needles of 8α,14α-oxidocholestane-3β-ol-7-one acetate, m.p. 139–140°, yield in the first crop, 43 mg. On recrystallization from methanol–water the substance formed large, thin plates, m.p. and mixed m.p. 142–142.5°.

Anal. Calcd. for $C_{29}H_{46}O_4$ (458.66): C, 75.94; H, 10.11. Found: C, 75.92; H, 10.01.

Chromatography of the mother liquor gave more of the same material, m.p. and mixed m.p. 140.5–141.5°, as the only solid product encountered.

3β-Acetoxy-7α-ethoxy-$\Delta^{8(14),22}$-ergostadiene (XIb).—A sample of 5-dihydroergostenyl acetate kindly supplied by Merck and Co., Inc., had the constants: m.p. 183–185°, α_D −20° Chf; saponification and crystallization from chloroform–methanol gave 5-dihydroergosterol, m.p. 177–177.5°, α_D −20° Chf.

A solution of 1 g. of 5-dihydroergosterol in 22 cc. of benzene was added to a solution of 1 g. of selenium dioxide in 62 cc. of 95% ethanol and the mixture was heated at 35° for 20 hr. The solution was diluted with water and the benzene layer washed with potassium bicarbonate solution, water, and saturated brine and evaporated to dryness. Treatment with 3,5-dinitrobenzoyl chloride (1 g.) in benzene (50 cc.)–pyridine (10 cc.) overnight, followed by decomposition with water, extraction with ether, and crystallization from ethyl acetate yielded 1.2 g. (77%) of crude 3β-hydroxy-7α-ethoxyergosteryl 3,5-dinitrobenzoate, m.p. 195–198°. The alcohol obtained on saponification, $\Delta^{8(14),22}$-ergostadienediol (XIa), separated from methanol as highly solvated needles, which when dried overnight at 80° over phosphorus pentoxide melted at 110–111°. Acetylation gave the **3-acetate**, which formed solvated needles from methanol melting, after thorough drying, at 108–109°, as reported by Callow and Rosenheim[3] for the supposed 7,8-oxide. Our sample gave a strong positive test for unsaturation with tetranitromethane, comparable to that given in a parallel test by 5-dihydroergosteryl acetate. Infrared spectra of the substance in chloroform solution or a Nujol mull on Baird and Perkin–Elmer instruments revealed no hydroxyl band.

Stability of the oxygen function to lithium aluminum hydride was tested as follows. A solution of 0.2 g. of the alcohol XIa in 50 cc. of ether was refluxed for 3 hr. with 0.2 g. of lithium aluminum hydride. The excess reagent was decomposed with ethyl acetate, then methanol and a little water. The solution was decanted, the residue washed several times with acetone, and the solution and washings on evaporation gave 0.18 g. of crystalline product. Recrystallized from acetone–methanol, the substance formed needles, m.p. 110–112° (after thorough drying) and gave no depression with the starting alcohol. Acetylation in pyridine–acetic anhydride gave the monoacetate as needles, m.p. 105–106°; this showed no depression in m.p. on admixture with 3β-acetoxy-7α-ethoxy-$\Delta^{8(14),22}$-ergostadiene, 107–108°, and the infrared spectra in carbon bisulfide were identical. The alcohol, its acetate and its dinitrobenzoate all had in common: λ^{CS_2} 10.24 μ (CH=CH), 12.05 μ.

3β-Acetoxy-7α-methoxy-$\Delta^{8(14),22}$-ergostadiene (Experiment by E. F. Schoenewaldt).—Substitution of methanol for ethanol in the reaction mixture resulted in a 76% yield of product, m.p. 114–115.5° (from methanol), $\alpha^{24}D$ −35.7° Chf (*c* 0.76).

Anal. Calcd. for $C_{31}H_{50}O_3$ (470.71): C, 79.10; H, 10.71. Found: C, 79.34; H, 10.91.

This substance depressed the m.p. of the 7α-ethoxy derivative. The infrared spectra of the two compounds were very similar in the region 2–8 μ but significantly different in the fingerprint region.

Ergosterol-B_3 Acetate (XV).—A solution of 50 mg. of 3β-acetoxy-7α-ethoxy-$\Delta^{8(14),22}$-ergostadiene in benzene (3 cc.)–pyridine (2 cc.) was treated at 0° with 0.5 cc. of phosphorus oxychloride in 2 cc. of benzene and let stand overnight at 25°. The liquid, containing crystals of pyridinium chloride, was poured onto a mixture of excess potassium bicarbonate solution and chopped ice and the benzene layer was separated and concentrated in a current of air at reduced pressure. The product separated as platelets, which were collected, washed thoroughly with water, then with a little methanol, and dried. The yield of crude product, m.p. 138–139.5°, α_D −208° Chf, λ^{EtOH} 242 mμ (10,400), was 30 mg. (80%). Recrystallized from chloroform–methanol, the acetate melted at 138–140°, α_D −217° Chf, λ^{Chf} 5.78, 7.95 (acetate), 6.08 (double bond), 10.25 μ (*trans* CH=CH).

A sample of pure 3β-acetoxy-7α-ethoxy-$\Delta^{8(14),22}$-ergostadiene was dissolved in acetic acid in the cold and the solution was let stand overnight at 25° and diluted to the point of saturation at the boiling point. Crude ergosterol-B_3 acetate separated in thin plates, m.p. 138–140°, α_D −194° Chf (*c* 1.56), λ^{Chf} 5.75, 6.08 μ, λ^{EtOH} 243 mμ (9,250).

8α,14α-Oxido-Δ^{22}-ergostene-3β-ol-7-one Acetate (XIV).—A solution of 170 mg. of 3β-acetoxy-7α-ethoxy-$\Delta^{8(14),22}$-ergostadiene in 10 cc. of acetic acid was treated at 25° with a

solution of 100 mg. of chromic anhydride in a few drops of water, diluted with 10 cc. of acetic acid. Oxidation proceeded rapidly; after standing overnight, the solution was diluted with water and extracted with ether. The total, oily reaction product showed weak ultraviolet absorption, λ^{EtOH} 259.5 mμ (590), suggesting the presence of 5–6% of $\Delta^{8(14)}$-cholestene-3β-ol-7-one acetate. On chromatography, a main fraction of 100 mg. was eluted by 1:1 petroleum ether–benzene. Crystallization from petroleum ether, attended with considerable loss, afforded long, soft needles, m.p. 150–151°, α_D −90°, λ^{Chf} 5.80, 8.0, 5.85 μ. In the standard test with tetranitromethane, the substance gave only a very feeble yellow color.

A comparison sample was prepared (L.F.F.) by oxidation of 2.5 g. of 5-dihydroergosteryl acetate with chromic acid according to Stavely and Bollenback.[17] Addition of a limited amount of water to the resulting solution gave a product that when collected, washed with methanol and dried, proved to be nearly pure **8α,9α-oxido-Δ^{22}-ergostene-3β-ol-7-one acetate** (380 mg., m.p. 209–220°); recrystallized from 95% ethanol, in which it is sparingly soluble, the substance formed plates, m.p. 220–221°, α_D −43.7° Chf (*c* 2.54); S. and B.[17]: m.p. 223–225°, α_D −16° Chf. On chromatography of the mother liquor material, 4:1 and 1:1 petroleum ether–benzene eluted, in fractions 4–16, successive crops of solid product that were combined and crystallized from 95% ethanol (very soluble, slow crystallization from concentrated solution) and then from methanol to give 76 mg. of **8α,14α-oxido-Δ^{22}-ergostene-3β-ol-7-one acetate**, m.p. 152–155°, α_D −98° Chf (*c* 1.47); S. and B.[17]: m.p. 155°, α_D −99° Chf. A mixture of this substance with the above product of oxidation of the Δ^7-ene-14α-ol melted at 152–155°.

$\Delta^{8(14),22}$-Ergostadiene-3β,7α-diol Diacetate (XII, L.F.F.).—A solution of 1 g. of 5-dihydroergosteryl acetate in 25 cc. of benzene and 20 cc. of acetic acid was treated at 25° with a mixture of 4 cc. of water and 20 cc. of the 0.1 *M* solution prepared from selenium dioxide as described above. The solution turned yellow in a few minutes and was let stand overnight and filtered from red selenium. The mixture was extracted with ether and the solution was washed neutral, dried, and let stand over powdered silver for 2 hr. (selenium was not later found in the products). The ether-benzene solution was evaporated, boiled down with petroleum ether a few times to remove the benzene, and the residual oil taken up in a little methanol. The solution slowly set to a stiff paste of crystals; 380 mg., m.p. 112–115°. On recrystallization from methanol a small part of the material was of different appearance than the rest, and the latter could be brought back in solution and separated sharply from the sparingly soluble product. This was recrystallized and identified by its constants as **ergosterol-D acetate**. It formed short needles, m.p. 168–170°, λ^{EtOH} 236, 242, 251 mμ (13,800, 14,600, 10,700). (A sample available from a previous research had turned yellow and the m.p. had dropped about 15°).

The more soluble fraction slowly separated from a small volume of methanol in large, soft needles, 121–122°, and a recrystallized sample of the dienediol diacetate melted at 123.5–124.5°, α_D −38.4 Chf.

Anal. Calcd. for $C_{32}H_{50}O_4$ (498.72): C, 77.06; H, 10.11. Found: C, 76.95, 77.24; H, 10.09, 10.36.

Another oxidation of 3 g. of acetate was let proceed at 9° for 15 hr., when a fair crop of selenium had separated, and then the solution was filtered and left at 25° and occasionally filtered from further crops of selenium that separated in the course of about 8 hr. The yield of ergosterol-D acetate, m.p. 162–163° and of recrystallized XII, m.p. 122–123°, 678 mg. (20%).

An attempt to deacetylate this material with lithium aluminum hydride as done (by G.O.) in the cholesterol series afforded ergosterol-B_3, m.p. 135–136°, α_D −204 Chf; acetate, m.p. and mixed m.p. 138–139°. The only difference was that in this experiment suspended reagent was present throughout whereas in the other one a solution was employed.

Oxidation of γ-Diosgenin Acetate.—A solution of 1.2 g. of γ-diosgenin acetate in 20 cc. each of benzene and acetic acid was treated at 0° with 13 cc. of 0.1 *N* selenous acid in acetic acid and left 15 hr. at 0–5°. Processing as in the Δ^7-cholestenyl acetate series gave a yellow oil that crystallized slowly when triturated with cold methanol. The crude product (1 g., m.p. 155–160°) was recrystallized repeatedly from ether–methanol and then acetone, but did not afford material of constant m.p. The final sample formed small needles that appear to consist of a mixture of the $\Delta^{7,9(11)}$- and $\Delta^{7,14}$-dienes: m.p. 206–208°, α_D −52° Chf, λ^{EtOH} 242 mμ (14,700), with shoulders at 236 and 250 mμ.

Anal. Calcd. for $C_{29}H_{42}O_4$ (454.62): C, 76.61; H, 9.31. Found: C, 76.59; H, 9.56.

The constants reported for the $\Delta^{7,9(11)}$-diene by Rosenkranz, *et al.*,[49] are: m.p. 200–203°, α_D −23° Chf, λ^{EtOH} 236; 242 mμ (13,500; 14,800).

In another experiment chromatography of the crude product afforded one series of fractions eluted by 9:1 petroleum ether–benzene and another series (more abundant) eluted by an 8:2 mixture. Samples prepared by several recrystallizations of the early and late fractions, however, appeared to be essentially the same: m.p. 210–211°, α_D −36.5° Chf (found: C, 76.53; H, 9.37); and m.p. 210–211°, α_D −34° Chf (found: C, 76.77; H, 9.39. Mixtures with the $\Delta^{7,9(11)}$-diene (below) showed no depression in m.p.

Oxidation of $\Delta^{7,9(11)}$-22-Isospirostadiene-3β-ol Acetate (L.F.F.; G.O.).—A solution of 1.2 g. of the diene acetate in benzene (25 cc.)–acetic acid (50 cc.) was treated at 0–5° with 39 cc. of 0.1 *N* selenous acid in acetic acid for 17 hr. Processing as usual gave a pale yellow oil that was chromatographed on 40 g. of alumina. Petroleum ether eluted a little starting material and then 8:2 petroleum ether–benzene eluted 0.32 g. (26%) of crystals, m.p. 179–186°. Recrystallization from methanol gave constant melting material of the probable structure **$\Delta^{7,9(11)}$-22-isospirostadiene-3β,14-diol 3-acetate (XXII)**, m.p. 195–197°, α_D −11° Chf, λ^{EtOH} 242 mμ (14,800); λ^{Chf} 2.9 μ (weak).

Anal. Calcd. for $C_{29}H_{42}O_5$ (470.62): C, 74.01; H, 9.00. Found: C, 74.50, 73.99, 73.65; H, 9.17, 9.13, 9.14.

The substance was recovered unchanged after being heated for 1 hr. in acetic acid on the steam-bath; it also was recovered unchanged after attempted acetylation.

$\Delta^{7,9(11)}$-22-Isoallaspirostadiene-3β,14-diol.—Saponification of 300 mg. of the above compound gave 290 mg. of crude product m.p. 170–175°, which was recrystallized from ethyl acetate. The analytical sample had the constants m.p. 195–198°, α_D −13°, λ^{EtOH} 242 mμ (17,000).

Anal. Calcd. for $C_{27}H_{40}O_4$ (428.59): C, 75.66; H, 9.41. Found: C, 75.98; H, 9.77.

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