with values of 91 and 9%, respectively, obtained for the "chair" and "boat" conformations of cyclohexane-1,4-dione by LeFèvre and LeFèvre (see above) in the same solvent. The relatively smaller contribution of the boat structure of cyclohexane-1,4-dione may be attributed, at least in part, to dipole repulsions between the two carbonyl groups, which in this case are proximate in space. On the basis of the above arguments any appreciable concentration of the high energy "2-boat" structure V ($\sigma = 148°$, $\mu = 1.70$ *D*) would appear to be excluded.

Similar consideration of the conformations of androstane-3,17-dione indicates that the ring A "boat" structure (III) in this case ($\varphi = 105°$) should possess a dipole moment of 3.58 *D*. The presence of 10–15% of this form will hence be much less effective in weighting the average dipole moment than is the corresponding "boat" structure IV of etiocholane-3,17-dione. This conclusion is in accord with the experimental findings.

Acknowledgment.—The authors are grateful to Prof. Robert H. Cole for assistance and advice. H. R. N. is indebted to the Corporation of Brown University for a Faculty Summer Research Grant which made possible a portion of this study.

PROVIDENCE, R. I.
HOUSTON, TEXAS

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Permanganate Oxidation of Ergosterol

BY MARY FIESER, ADOLFO QUILICO,[1] ALEX NICKON,[2] WILLIAM E. ROSEN, E. JAMES TARLTON AND LOUIS F. FIESER

RECEIVED APRIL 18, 1953

A product reported by Reindel in 1928 to be formed in high yield by oxidation of ergosterol in methylcyclohexane with aqueous permanganate has been separated into an O_3- and an O_5-component. The O_3-product has been identified as $\Delta^{7,14,22}$-ergostatriene-3β,5α,6α-triol (II); it forms a diacetate, a maleic acid adduct, and on reduction it affords $\Delta^{8(14)}$-ergostene-3β,5α,6α-triol (III), identical with the product of hydrogenation of $\Delta^{7,22}$-ergostadiene-3β,5α,6α-triol (VII). Further evidence that the triol VII has a 6α-oriented hydroxyl group was obtained by comparison of the triol with the 6β-epimer VI, resulting with VII on reduction of the 6-ketone V: epimer VII forms an acetonide, whereas VI does not. The O_5-oxidation product has not yet been identified.

When Reindel initiated a program of independent research at Munich, Windaus turned over to him the problem of investigating the then little studied ergosterol.[3] Shortly afterwards, the sterol rose to a position of key interest with the discovery by Windaus, Hess and Pohl at Göttingen[4] and by Rosenheim and Webster in England[5] that on irradiation it acquires high antirachitic potency, but Windaus only authorized work at Göttingen on the structure of ergosterol at the urgent insistence of members of his vitamin D research group.[6] In Reindel's third paper on ergosterol,[7] he reported experiments conducted with A. Frölich that resulted in isolation in good yield of a crystalline, neutral product of oxidation. The nature of the product was not established, but the results were communicated with the idea that "they might later be of use in elucidation of the structure of ergosterol." This hope was not realized, and a quarter of a century has gone by with no further reference in the literature to the oxidation product.

We were intrigued with the novel method of oxidation used and the high yield of crystalline product. Reindel and Frölich[7] added aqueous permanganate solution to a hot solution of ergosterol in methylcyclohexane, shook the mixture for 12 hours, and processed the resulting three-phase system by a briefly described procedure that is not clear to us. They reported isolation of a small amount of an unidentified lower fatty acid derived from fission of the side-chain double bond and of a crystalline neutral product for which they suggested the formulas $C_{26}H_{40-42}O_4$, on the basis of the formula $C_{27}H_{42}O$, at the time attributed to ergosterol (now $C_{28}H_{44}O$); the analytical data also fit the formulas $C_{28}H_{46-48}O_4$. Several ester derivatives were prepared and analyzed and hydrogenation was studied, but some of the results appeared anomalous and no definite conclusions were reached.

After considerable experimentation (A.Q.), a reproducible procedure was worked out for oxidation of ergosterol in 4-g. lots in hot methylcyclohexane with a hot aqueous solution of three oxygen equivalents of permanganate (Reindel and Frölich used six equivalents); when less oxidizing agent was used, starting material was found in the reaction mixture. The precipitated manganese dioxide was reduced with sulfur dioxide, the methylcyclohexane was eliminated by steam distillation and evaporation, and the reaction product was obtained in a crystalline filterable form by digestion with methanol. Under the best conditions found, crystalline, ergosterol-free product corresponding approximately to that of Reindel and Frölich was regularly obtained in 80% yield by weight. This material, however, was found to be unhomogeneous, and by fractional crystallization it was separated into a main O_3-component and a secondary O_5-component, of composition and properties indicated in Table I. The analyses and optical rotations indicate that the oxidation affords a mixture of about

(1) On leave of absence from the Politecnico di Milano.
(2) National Institutes of Health Predoctoral Fellow, 1950–1952.
(3) F. Reindel, E. Walter and H. Rauch, *Ann.*, **452**, 34 (1927), footnote 1. See also F. Reindel and E. Walter, *ibid.*, **460**, 212 (1928).
(4) A. Windaus and A. F. Hess, *Nachr. Ges. Wiss., Göttingen*, 175 (1926); R. Pohl, *ibid.*, 185 (1926).
(5) O. Rosenheim and T. A. Webster, *Biochem. J.*, **21**, 389 (1927).
(6) Incident related by Dr. Werner Bergmann.
(7) F. Reindel, *Ann.*, **466**, 131 (1928).

7 parts of the dextrorotatory substance $C_{28}H_{44}O_3$ and 3 parts of the levorotatory product $C_{28}H_{46}O_5$.

TABLE I

PRODUCTS OF PERMANGANATE OXIDATION OF ERGOSTEROL

	Hydroxy compound			Acetyl derivative		
	Composition	M.p., °C.	α_D Chf	M.p., °C.	α_D Chf
Reindel-Frölich product	$C_{28}H_{44-46}O_4$	199–201	−24.4	148–150	−5.5
Main component	$C_{28}H_{44}O_3$	223–224	+24.9	183–184	+34
Secondary component	$C_{28}H_{46}O_5$	177–179	−50.0	167–168	−17

That two of the three oxygen atoms of the main oxidation product are present as secondary alcoholic groups was established by formation of a diacetate, from which the original material was regenerated by either alkaline hydrolysis or cleavage with lithium aluminum hydride. Reindel and Frölich had prepared a di-*p*-nitrobenzoate that on saponification yielded a substance different from their starting material and regarded by them as an anhydro derivative; actually their derivative corresponds in melting point and analysis to the di-*p*-nitrobenzoate prepared from the pure O_3-product, and hence separation from the contaminating derivative of the O_5-product evidently had been effected in the course of the purification. We also found that purification of the O_3-component is best accomplished through a derivative, such as the diacetate. Reindel and Frölich also isolated a product of hydrogenation that they thought was an anhydro derivative but this also corresponds to the product of hydrogenation of the pure O_3-component.

Reindel and Frölich observed that the total oxidation product is inert to carbonyl reagents, and this is true also of both pure components. The third oxygen function of the O_3-product was identified as a tertiary or hindered hydroxyl group from the infrared spectrum of the diacetate. The free triol shows strong absorption in the ultraviolet characteristic of a diene system, with a maximum at 245 mμ, E 12,000–13,500.

The oxidation product is thus an ergostatrienetriol, and evidence to be presented indicates the specific structure and configuration shown in formula II. The 3,6-diacetate, on prolonged contact with platinum catalyst and hydrogen, absorbed only two moles of gas and gave a product corresponding in constants to a diacetate obtained by Windaus and Lüttringhaus[8] by hydrogenation of the diacetate of a substance known at the time as as ergostadienetriol-II, which on present evidence can be assigned the formula VII. Direct comparison of samples prepared from II and from VII established their identity. Heilbron, Morrison and Simpson[9] showed that the ergostenetriol diacetate of Windaus and Lüttringhaus can be transformed by the action of hydrogen chloride into a substance identified as the Δ^{14}-isomer because it is hydrogenable to ergostane-3β,5α,6α-triol 3,6-diacetate (VIII), and these transformations were confirmed in the present work. The British investigators assumed that the product of hydrogenation of VII is the $\Delta^{8(14)}$-isomer III, but a double bond initially at the 7,8-position could also migrate to the 14,15-position under the influence of hydrogen chloride. Indeed Bladen, *et al.*,[10] report an instance where the usual isomerization of a Δ^7- to a $\Delta^{8(14)}$-stenol is retarded when the 5α-hydrogen atom is replaced by a 5α-hydroxyl group. However, evidence that the hydrogenation product actually is the $\Delta^{8(14)}$-isomer III is afforded by the observation of Achtermann[11] that the diacetate can be distilled in high vacuum without change, whereas on similar treatment the diacetate of the allylic ergostadienetriol-II (VII) loses water and acetic acid and affords dehydroergosteryl acetate.

Conversion of the O_3-product to $\Delta^{8(14)}$-ergostenetriol does not reveal the location of the original diene system, since the same substance could arise, by 1,2- or 1,4-addition with or without bond migration, from any of the possible systems: D-type $\Delta^{7,9(11)}$-, B_1-type ($\Delta^{8,14}$), or B_3-type ($\Delta^{7,14}$). In absorption characteristics, the substance conforms most nearly to the B_3-type, although the extinction coefficient is somewhat higher than that of simple B_3-dienes. Isolation of a maleic anhydride adduct affords convincing evidence in support of the B_3-formulation II, since the reaction is specific to homoannular and B_3-type dienes and since the wave length of ultraviolet absorption (245 mμ) clearly shows that the diene is not homoannular. A supporting observation is that the triol diacetate is stable to hydrogen chloride at 0°, conditions under which D-, B_1- and B_2-dienes are isomerized to B_3-dienes.[12] The dextrorotation may appear anomalous, since B_3-dienes usually are strongly levorotatory, but the presence in II of a new center of asymmetry at C_6 adjacent to one of the diene systems vitiates comparison; possibly the hydroxyl group at C_6 also affects the extinction coefficient.

The O_3-product of oxidation clearly corresponds in the orientation of the three hydroxyl groups to ergostadienetriol-II, and the only point of uncertainty in respect to this compound is the configuration at C_6. Triol-II was first prepared by Windaus and Lüttringhaus[8] by the action of perbenzoic acid on ergosterol, followed by saponification of the resulting triol 6-monobenzoate. Peroxide hydroxylation of monoenes normally affords *trans*-glycols,[13] and the product of hydroxylation of cholesterol is the 3β,5α,6β-triol. However, the same triol-II results from ergosterol by oxidation with lead tetraacetate and hydrolysis,[14] or by oxidation with osmium tetroxide.[15] The strong indication that the product of osmium tetroxide hydroxylation is a *cis*-5,6-glycol was substantiated by the observation of Criegee, Marchand and Wannowius[15] that triol-II reacts with osmium tetroxide to form a cyclic ester. The evidence falls short of conclusive proof only in that no comparison was made of the behavior of the C_6-epimer of triol-II. Thus certain *trans*-gly-

(8) A. Windaus and A. Lüttringhaus, *Ann.*, **481**, 119 (1930).

(9) I. M. Heilbron, A. L. Morrison and J. C. E. Simpson, *J. Chem. Soc.*, 302 (1933).

(10) P. Bladen, D. B. Clayton, G. W. Greenhalgh, H. B. Henbest, E. R. H. Jones, B. J. Lovell, G. Silverstone, G. W. Wood and G. F. Woods, *ibid.*, 4883 (1952).

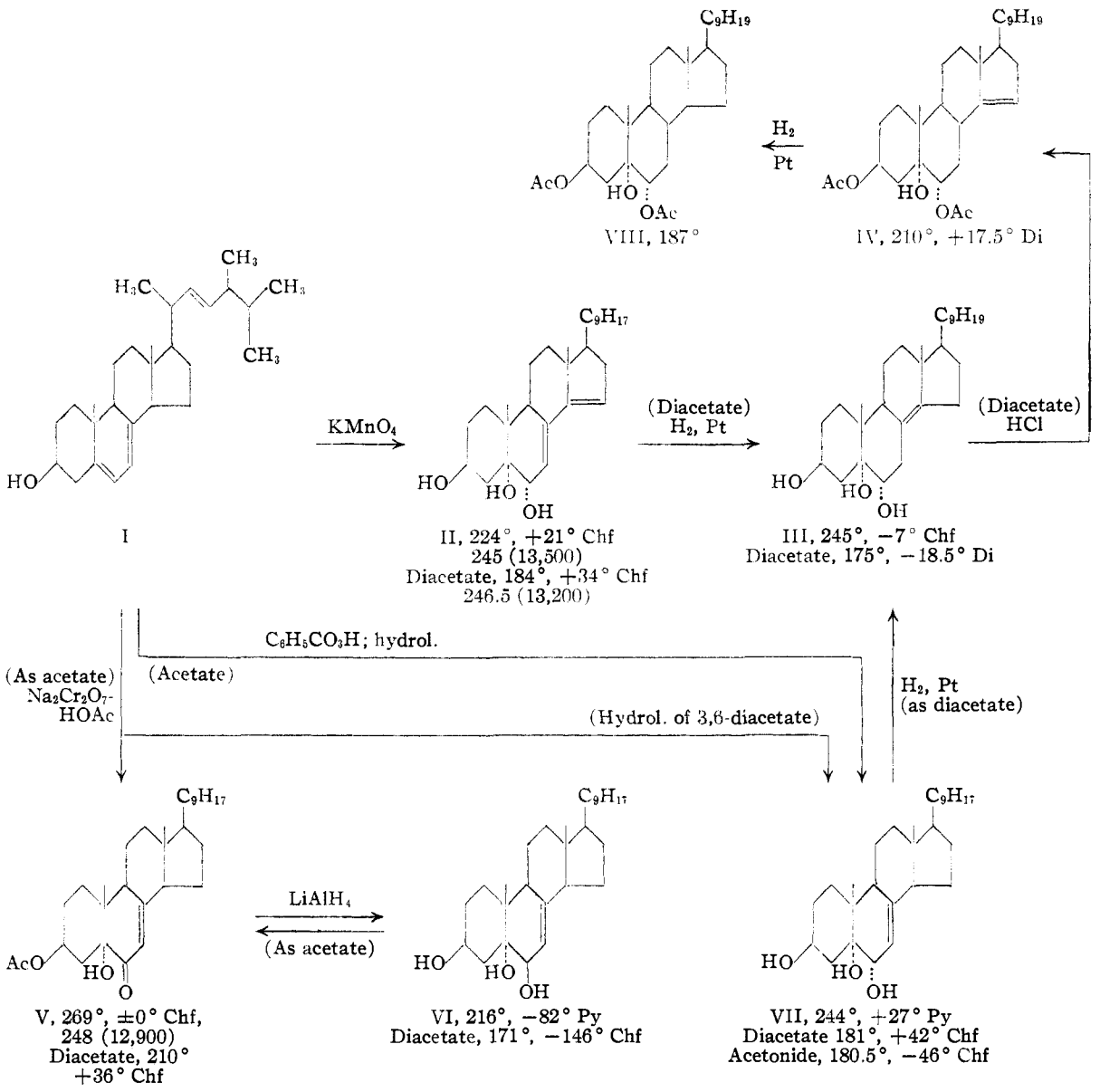
(11) Th. Achtermann, *Z. physiol. Chem.*, **217**, 281 (1933).

(12) D. H. R. Barton and T. Brunn, *J. Chem. Soc.*, 2728 (1951).

(13) D. Swern, *Chem. Revs.*, **45**, 1 (1949).

(14) A. Windaus and U. Riemann, *Z. physiol. Chem.*, **274**, 206 (1942).

(15) R. Criegee, B. Marchand and H. Wannowius, *Ann.*, **550**, 114 (1942).



cols have been observed to form acetonides, if less readily than the *cis*-epimers.[16] Independently of Alt and Barton,[17] we found that the C_6-epimer of ergostadienetriol-II can be obtained by lithium aluminum hydride reduction of $\Delta^{7,22}$-ergostadiene-3β,-5α-diol-6-one 3-acetate[18] (V). The structure of the product (VI) was established by dichromate oxidation to $\Delta^{7,22}$-ergostadiene-3,6-dione.[18] The two C_6-epimeric triols were then heated gently in acetone containing a trace of hydrochloric acid. Since the long-known triol-II formed an acetonide while the epimer was recovered unchanged, the former substance is definitely the 3β,5α,6α-triol VII and the epimer is VI. In less highly substituted compounds a 5α- but not a 5β-hydroxyl group can be acylated[19] by the action of acetyl chloride in dimethylaniline. The test was negative in the case of the O_3-triol 3,6-diacetate, but the failure to react may well be due to hindrance by the adjacent *cis* acetoxyl group at C_6.

That oxidation of ergosterol with neutral permanganate results in part in *cis* hydroxylation of the 5,6-double bond corresponds to the observation of Windaus[20] that oxidation of cholesterol with alkaline permanganate in a benzene–water system affords as the sole neutral product (4–5% yield) cholestane-3β,5α,6α-triol. Oxidation of cholesterol with permanganate in acetic acid leads to cholestane-3,6-dione-5α-ol[21] and cholesterol α,β-oxide.[22] In analogy with recent inferences regarding the oxidation of Δ^7-stenols with selenium dioxide, chromic acid and perbenzoic acid,[23] it seems likely that the B_3-diene system of the O_3-product results from allylic hydroxylation. Burawoy[18] demon-

(16) W. R. Christianson, C. J. Gogler and C. B. Purnes, *Can. J. Research*, **29**, 911 (1951).

(17) G. W. Alt and D. H. R. Barton, *Chemistry and Industry*, **45**, 1103 (1952).

(18) A. Burawoy, *J. Chem. Soc.*, 409 (1937).

(19) Pl. A. Plattner, Th. Petrzilka and W. Lang, *Helv. Chim. Acta*, **27**, 513 (1944).

(20) A. Windaus, *Ber.*, **40**, 257 (1907).

(21) R. Marker and E. Rohrmann, THIS JOURNAL, **62**, 516 (1940).

(22) M. Ehrenstein and M. T. Decker, *J. Org. Chem.*, **5**, 544 (1940).

(23) L. F. Fieser and G. Ourisson, THIS JOURNAL, in press.

strated that ergosteryl acetate on oxidation with chromic acid affords $\Delta^{7,22}$-ergostadiene-3β,5α-diol-6-one 3-acetate (V). We repeated the oxidation but employed sodium dichromate in benzene–acetic acid and isolated, in addition to V, a substance identified as $\Delta^{7,22}$-ergostadiene-3β,5α,6α-triol 3,6-diacetate (VII-diacetate). Possibly the latter product arises by acetolysis of an intermediate oxide,[22,24] in analogy to the formation of the 6-benzoate of the triol VII on perbenzoic acid oxidation of ergosterol.

The structure of the O_5-oxidation product is under investigation. The empirical formula suggests that the substance is formed from the O_3-triol by addition of two hydroxyl groups to the diene system. The O_5-product is transparent to ultraviolet light, shows no infrared carbonyl absorption, and forms an anhydro derivative diacetate.

Experimental

Permanganate Oxidation of Ergosterol (A.Q.).—Four grams of ergosterol was dissolved in 100 cc. of methylcyclohexane at the boiling point, and 75–80 cc. of 4% aqueous potassium permanganate, preheated to 90–100°, was added at once to the hot solution of the sterol. The flask was stoppered and shaken vigorously, with frequent interruption for removal of the stopper for release of pressure. The formation of manganese dioxide was soon evident, and the initially thick mass soon changed to a thin suspension of brown powder suspended between the aqueous and hydrocarbon phases. The permanganate was all consumed in 8–10 min., and at this stage the reaction mixture was combined with those of two other similarly conducted oxidations. Sulfur dioxide was bubbled through the mixture to reduce the manganese dioxide, and the resulting suspension of white solid was transferred to a separatory funnel and shaken gently with successive portions of water to extract inorganic salts; vigorous shaking is to be avoided since emulsions result. The milky organic layer consisting in a suspension of the sparingly soluble oxidation products was then steam-distilled for separation and recovery of solvent and to eliminate volatile fragments derived from the side chain. The distillation was stopped when foaming became excessive, and the mixture was then transferred to a large porcelain dish and evaporated on the steam-bath to a thick gelatinous curd. On addition of 250 cc. of methanol and digestion at the boiling point for a few minutes, the gelatinous product was converted (without appreciably going into solution) into crystalline, readily filterable material. After cooling, this was collected and washed with methanol; yield of light tan material, 10–12 g. (m.p. indefinite).

$\Delta^{7,14,22}$-Ergostatriene-3β,5α,6α-triol, II (A.Q.).—This triol was obtained crystalline only as a hemihydrate; attempted crystallizations from anhydrous solvents were unsuccessful. Thus 10 g. of the crude oxidation product on crystallization from benzene saturated with water gave 5.2 g. of yellow-tinged material that on one recrystallization from moist methanol gave shiny plates of the triol, m.p. 212–214°. Three further crystallizations from methanol raised the melting point to the constant value 223–224° (the mother liquors were saved and processed for recovery of the O_5-product as described below); α_D +24.9° Chf (*c* 1.47), +27.5° Di (*c* 1.49); λ^{EtOH} 245 mμ (6,800), λ^{Chf} 2.82–2.92 μ (minute peak at 6.21 μ).

Anal. Calcd. for $C_{28}H_{44}O_3 \cdot {}^1/_2H_2O$ (437.64): C, 76.87; H, 10.36; H_2O, 2.05. Found: C, 76.74; H, 10.53; H_2O, 2.13.

The low value of the extinction coefficient suggests that this material still contained a trace of the O_5-product. The triol dissolves in concentrated sulfuric acid with a red-brown coloration.

The **diacetate of II** was prepared by letting a solution of 2.4 g. of the triol in 18 cc. of pyridine and 12 cc. of acetic anhydride stand overnight at room temperature and adding 200 cc. of water. The product initially separated in a pasty form but after several hours became brittle and crystalline and could be collected by filtration (yield 2.6 g.). One crystallization from 95% ethanol afforded large prisms, m.p. 180–181°; repeated recrystallization gave material of constant m.p. 183–184°, α_D +34.0° Chf (*c* 1.55), +35.3° Di (*c* 1.42), λ^{Chf} 246–247 mμ (13,200), λ^{Ether} 244 mμ (13,400), λ^{Chf} 2.8–2.9, 5.83, 8.03 μ.

Anal. Calcd. for $C_{32}H_{48}O_5$ (510.70): C, 74.96; H, 9.44. Found: C, 74.76; H, 9.59.

The pure diacetate can be obtained more readily by acetylation of the crude oxidation product and crystallization from 95% ethanol; II-diacetate is very sparingly soluble in cold ethanol and crystallizes nearly completely, whereas acetylated companion substances remain in solution. Thus three crystallizations afforded 3.5 g. of pure II-diacetate, m.p. 183°, from 6 g. of crude oxidation product.

Treatment of the diacetate (200 mg.) in chloroform solution at 0° with hydrogen chloride for 10 min. (E.J.T.), a procedure shown[12] to effect isomerization of ergosteryl acetate to the B_3-acetate, resulted in some changes in optical constants but did not afford an evidently different substance. The solution, which had turned deep purple, was neutralized with ice-cold bicarbonate solution and the product was extracted with ether and crystallized from methanol. The orange-tinged product (120 mg.) melted at 184–185° (no depression on admixture with II-diacetate), α_D +49° Chf, λ^{EtOH} 244 mμ (16,400).

Saponification of II-diacetate (2.5 g.) by refluxing it for 1 hr. with 10% methanolic potassium hydroxide and acidification with dilute hydrochloric acid gave a precipitate (2.2 g.) that after three crystallizations from moist methanol afforded pure $\Delta^{7,14,22}$-ergostatriene-3β,5α,6α-triol (II), as shiny colorless leaflets, m.p. 222–224°, α_D +20.7° Chf, +24.6° Di, λ^{EtOH} 245 mμ (13,500).

Anal. Calcd. for $C_{28}H_{44}O_3 \cdot {}^1/_2H_2O$ (437.64): C, 76.87; H, 10.36. Found: C, 76.85; H, 10.30.

Cleavage of the diacetate with lithium aluminum hydride gave less pure triol in about 50% yield: m.p. 231–232°, α_D +29.5° Di, λ^{EtOH} 245 mμ (7,300). The mother liquors afforded a levorotatory by-product.

Isolation of the O_5-Oxidation Product (A.Q.).—Four grams of the crude product from the oxidation of ergosterol was dissolved in 700 cc. of boiling anhydrous benzene and the clear solution let stand overnight. Beautiful thin needles separated and on recrystallization from methanol afforded 0.8 g. of nearly pure O_5-product, m.p. 175–179° dec. The benzene mother liquor can be processed as described above for recovery of the O_3-product. Further purification of the secondary product was effected by crystallization from methanol, benzene and finally methanol–benzene, from which the substance separated as a mat of thin, white needles, m.p. 177–179° dec., α_D −50.0° Chf (*c* 1.25), −45.5° Di (*c* 1.43), λ^{Chf} 2.95, 6.22 (minute) μ.

Anal. Calcd. for $C_{28}H_{46}O_5$ (462.65): C, 72.69; H, 10.02. Found: C, 73.03; H, 10.50.

The sample showed low intensity absorption at 245 mμ (*E* 740), and hence was not completely pure.

A substance that appears to be an **anhydro derivative diacetate** was obtained from the methanol mother liquors remaining from crystallization of the O_3-oxidation product II. The residue was acetylated with pyridine–acetic anhydride at room temperature and the acetate mixture crystallized from 95% ethanol. The sparingly soluble II-diacetate separated and was collected. On spontaneous evaporation of the filtrate the anhydro diacetate separated as snow-white needles slightly contaminated with oily material. Four recrystallizations from 80% ethanol afforded material that appeared to be homogeneous: m.p. 167–168°, α_D −10.2° Chf (*c* 1.57), −17.4° Di (*c* 1.58); λ^{Chf} 2.82, 5.81, 8.0 μ; no absorption between 220 and 330 mμ.

Anal. Calcd. for $C_{32}H_{48}O_6 \cdot {}^1/_2H_2O$ (537.71): C, 71.47; H, 9.19; sapon. equiv., 269. Found: C, 71.25, 71.58; H, 9.62, 9.17; sapon. equiv., 237.

Maleic Anhydride Adduct of $\Delta^{7,14,22}$-Ergostatriene-3β,5α,-6α-triol (E.J.T.).—The procedure was that of Barton and Bruun.[12] A solution of 200 mg. of II-diacetate and 80 mg. of freshly distilled maleic anhydride in dry benzene was refluxed for 90 min., cooled, and diluted with petroleum ether, when 60 mg. of adduct separated. Crystallization from acetone–ligroin gave silky needles, m.p. 280–281° dec., α_D −30.3° Chf, λ^{Chf} 2.9, 5.46, 5.67, 5.81, 8.0 μ.

Anal. Calcd. for $C_{36}H_{50}O_8$ (610.76): C, 70.79; H, 8.25. Found: C, 70.91; H, 8.28.

(24) L. F. Fieser, *ibid.*, in press.

$\Delta^{7,14,22}$-Ergostatriene-3β,5α,6α-triol Di-*p*-nitrobenzoate (E.J.T.).—A mixture of 150 mg. of II, 300 mg. of *p*-nitrobenzoyl chloride and 3 cc. of pyridine was heated on the steam-bath for 3 hr., cooled, diluted with water and extracted with ether. The solution, washed free of pyridine and acid and dried, gave on evaporation an oily product that was purified by chromatography. Fractions eluted by 1:1 petroleum ether–benzene and by benzene were combined and on crystallization from chloroform–ethanol afforded di-*p*-nitrobenzoate, m.p. 193–194°, α_D +98° Chf. Reindel[7] obtained material of the same composition, m.p. 197°.

Anal. Calcd. for $C_{42}H_{50}O_8N_2$ (726.84): C, 69.40; H, 6.93; N, 3.86. Found: C, 69.67; H, 7.15; N, 3.78.

$\Delta^{8(14)}$-Ergostene-3β,5α,6α-triol 3,6-Diacetate; III-Diacetate (A.N.).—A solution of 200 mg. of $\Delta^{7,14,22}$-ergostatriene-3β,5α,6α-triol (II) diacetate in 15 cc. of acetic acid was added to a suspension prepared by reduction of 7.5 mg. of platinum oxide in 15 cc. of acetic acid, and hydrogenation was conducted at room temperature at a slight positive pressure. Two moles of hydrogen was consumed in 30–45 min., and no further reaction was observed on continuing the shaking for 3 hr. The filtered solution was evaporated to dryness in vacuum; crystallization of the residue from methanol gave 156 mg. (78%) of tiny prisms, m.p. 172–173.5°. On further crystallization the substance was obtained as long, thin needles, m.p. 174.5–177.5°, α_D −18.5° Di (*c* 1.54), λ^{Chf} 2.8–2.9, 5.82, 7.99, 12.0 (weak) μ; unsaturated to tetranitromethane.

Anal. Calcd. for $C_{32}H_{52}O_5$ (516.74): C, 74.37; H, 10.14. Found: C, 74.37; H, 10.31.

The substance did not depress the m.p. of a sample of III (m.p. 173.5–174.5°, α_D −14° Di) prepared according to Windaus and Lüttringhaus[8] (172–173°, α_D −9.8° Chf), and the infrared spectra were identical.

$\Delta^{8(14)}$-Ergostene-3β,5α,6α-triol, III (A.N.).—Hydrogenation of free triol II under the same conditions gave, in 78% yield, a product that crystallized from aqueous ethanol in glistening plates, m.p. 244–245°, α_D −7° Chf (*c* 1.07).

Anal. Calcd. for $C_{28}H_{48}O_3 \cdot {}^1/_2H_2O$ (441.67): C, 76.14; H, 11.18. Found: C, 75.93; H, 11.10.

The triol is too sparingly soluble in chloroform to permit test for unsaturation with tetranitromethane in the usual way. Acetylation gave material identical with the 3,6-diacetate described above. A comparison sample of the triol was prepared by hydrogenation of $\Delta^{7,22}$-ergostadiene-3β,5α,6α-triol with platinum oxide in acetic acid according to Windaus and Lüttringhaus,[8] who report that the substance crystallizes as the hemihydrate, m.p. 234°, α_D −4.6° Py. Our sample melted at 232–235° and a mixture with the above triol showed no depression in m.p.

Δ^{14}-Ergostene-3β,5α,6α-triol 3,6-Diacetate, IV (A.N).—Treatment of $\Delta^{8(14)}$-ergostene-3β,5α,6α-triol 3,6-diacetate with hydrogen chloride by the low-temperature procedure of Barton and Brooks[25] afforded in 70% yield, a substance corresponding to that of Heilbron, Morrison and Simpson[9] (m.p. 209–210°) of the following constants: m.p. 207–210°, α_D +17.5° Di (*c* 1.24), λ^{Chf} 2.83, 2.95, 5.80, 7.99, 11.90 (weak) μ; unsaturated to tetranitromethane. Hydrogenation afforded **ergostane-3β,5α,6α triol 3,6-diacetate (VIII)**, which on crystallization from ethanol–water melted at 184–187° (reported[9] 191°).

Dichromate Oxidation of Ergosteryl Acetate (W.E.R.).—A solution of 9 g. of sodium dichromate dihydrate in 20 cc. of acetic acid was added at room temperature to a solution of 30 g. of ergosteryl acetate (m.p. 174.5–175.5°, α_D −90° Chf) in 200 cc. of benzene diluted with 200 cc. of acetic acid. After 24 hr. the mixture was diluted with water, the benzene removed by steam distillation, and the precipitated solid was collected, washed with water and with bicarbonate solution, dried in vacuum, powdered, and dried further; yield 22 g. Ten grams of this material was chromatographed on 300 g. of acid-washed alumina with the following results. Benzene eluted 2.2 g. of **ergosteryl acetate**, which on crystallization from dioxane–methanol formed white plates, m.p. and mixed m.p. 174–175°. Benzene–ether (9:1) eluted a total of 2.0 g. of material that on three crystallizations from methanol afforded pure **$\Delta^{7,22}$-ergostadiene-3β,5α,6α-triol 3,6-diacetate** (VII-diacetate) as white cubes, m.p. 180.5–181°, α_D +42° Chf (*c* 1.39), no ultraviolet absorption; no depression in m.p. on admixture with an authentic sample.

Anal. Calcd. for $C_{32}H_{50}O_5$ (514.72): C, 74.67; H, 9.79. Found: C, 74.70; H, 10.05.

Saponification, effected by heating the diacetate with Claisen alkali for 10 min., gave a solid (m.p. 241–242°) that crystallized from ethyl acetate in needles, m.p. 244°, α_D +27° Py (*c* 2.23); selenium dioxide test (25°) negative. The constants correspond to those reported[8,11] for **$\Delta^{7,22}$-ergostadiene-3β,5α,6α-triol (VII)** (m.p. 239–242°, α_D 26–29° Py; diacetate m.p. 182°, α_D +41° Chf).

Anal. Calcd. for $C_{28}H_{46}O_3$ (430.65): C, 78.09; H, 10.77. Found: C, 78.26; H, 10.80.

Benzene–ether (1:1) then eluted 2.8 g. of **$\Delta^{7,22}$-ergostadiene-3β,5α-diol-6-one 3-acetate (V)**, which after two crystallizations from ethyl acetate (Norit) was obtained as thick white plates, m.p. 267.5–269°, α_D ±0° Chf (*c* 0.59), 3.2° Di (*c* 0.94), λ^{EtOH} 248 mμ (12,900); Burawoy[18]: m.p. 264°, α_D −5° Chf, λ^{EtOH} 251.5 mμ (13,570).

Anal. Calcd. for $C_{30}H_{46}O_4$ (470.67): C, 76.55; H, 9.85. Found: C, 76.72; H, 9.93.

Further elution with chloroform yielded 0.7 g. of a yellow semisolid that on repeated crystallization from acetone gave a small amount of colorless microcrystals, m.p. 221–223°, α_D +11° Chf, λ^{EtOH} 245–250 mμ (3,000); found: C, 75.38; H, 10.28. Elution with methanol gave 1.5 g. of a brown oil that afforded a few mg. of needles, m.p. 125–126.5°, found: C, 70.88; H, 9.83.

$\Delta^{7,22}$-Ergostadiene-3β,5α-diol-6-one 3,5-Diacetate (W.E.R.).—This derivative was obtained in low yield by reaction of the 3-monoacetate either with ketene in chloroform containing a trace of sulfuric acid or with acetic anhydride-boron fluoride etherate. Crystallized from either acetone or methanol, it formed colorless needles, m.p. 208–210°, α_D +36° Chf (*c* 2.47).

Anal. Calcd. for $C_{32}H_{48}O_5$ (512.70): C, 74.96; H, 9.44. Found: C, 74.81; H, 9.40.

$\Delta^{7,22}$-Ergostadiene-3β,5α,6α-triol 5,6-Acetonide (W.E.R.).—A solution of 108 mg. of the triol in 17 cc. of hot acetone was treated with two drops of 36% hydrochloric acid, boiled for two minutes, and let stand overnight. The solution was then neutralized with solid sodium carbonate and the solvent evaporated in a stream of air. The solid residue was digested with benzene and the suspension poured onto a column of alumina. Elution with benzene and benzene–ether removed 60 mg. of acetonide, which crystallized from chloroform–methanol as microcrystals, m.p. 179–180.5°, α_D −46° Chf (*c* 1.09).

Anal. Calcd. for $C_{31}H_{50}O_3$ (470.71): C, 79.10; H, 10.71. Found: C, 78.79; H, 10.72.

$\Delta^{7,22}$-Ergostadiene-3β,5α,6β-triol 3,6-Diacetate, VI-Diacetate (W.E.R.).—$\Delta^{7,22}$-Ergostadiene-3β,5α-diol-6-one 3-acetate, V (1.5 g.), was added to a suspension of 2.5 g. of lithium aluminum hydride in 125 cc. of redistilled tetrahydrofuran and the mixture was refluxed for 4 hr., cooled, decomposed with the required amount of aqueous sodium sulfate. The product was extracted from the granular lithium aluminate by several extractions with dioxane–ether and the dried solution was evaporated to a volume of 80 cc., treated with 80 cc. of acetic anhydride and 15 cc. of pyridine, heated on the steam-bath for one-half hour, and let stand overnight. Addition of water precipitated 1.3 g. of solid, m.p. 158–162°. Two crystallizations from methanol (Norit) gave colorless needles, m.p. 170–171°, α_D −146° Chf (*c* 1.15), −150° Py (*c* 1.15); constants reported[17]: m.p. 168–170°, α_D −149° Chf.

Anal. Calcd. for $C_{32}H_{50}O_5$ (514.72): C, 74.67; H, 9.79. Found: C, 74.77; H, 9.90.

When the crude reduction product was processed as such rather than after acetylation, repeated crystallization gave a small amount of material, m.p. 244°, that appeared to be the 3β,5α,6α-triol, the minor product of reduction. The major product is thus best isolated as the diacetate.

$\Delta^{7,22}$-Ergostadiene-3β,5α,6β-triol, VI (W.E.R.).—The above diacetate was heated with methanol–Claisen alkali for 45 min. and the solution cooled and diluted with water. The solid precipitate crystallized from ethyl acetate in small white needles, m.p. 214–216°, α_D −82° Py (*c* 0.94); reported[17] 253–256°, α_D −84° Py.

Anal. Calcd. for $C_{28}H_{46}O_3$ (430.65): C, 78.09; H, 10.77. Found: C, 78.37; H, 10.53.

Under the conditions employed for conversion of the 6α-

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

epimer into the acetonide, the 3β,5α,6β-triol was recovered unchanged. Oxidation of the 3β,5α,6β-triol with sodium dichromate in acetic acid gave, after chromatography and in 27% yield, $\Delta^{7,22}$-ergostadiene-3,6-dione-5α-ol,[9] identical with a sample prepared by oxidation of the 3β,5α,6α-triol; m.p. 254–255° dec., α_D +46° Chf (*c* 0.5), λ^{EtOH} 249 mμ (11,750).

Anal. Calcd. for $C_{28}H_{42}O_3$ (426.62): C, 78.82; H, 9.92. Found: C, 78.65; H, 10.24.

Perbenzoic Acid Oxidation of Ergosteryl Acetate (W.E.R.).—An ice-cold chloroform solution of 7.2 g. of perbenzoic acid (1 equiv.) was added during 45 min. to an ice-cold solution of 22 g. of ergosteryl acetate in 500 cc. of chloroform. The solution was let stand at 0–5° for 20 hr. and then washed with aqueous carbonate solution, dried and evaporated below 60° in vacuum to a light yellow solid. The relative intensity of ultraviolet absorption at 231 mμ (benzoate) and at 324 mμ ($\Delta^{5,7,9(11)}$-triene) indicated the presence of about 2 parts of $\Delta^{7,22}$-ergostadiene-3β,5α,6α-triol 3-acetate 6-benzoate to 1 part of dehydroergosteryl acetate, and these two products were separated by chromatography. Dehydroergosteryl acetate (less soluble in acetone–methanol), was identified by analysis, comparison with an authentic sample, and its physical constants: m.p. 144.5–145.5°, α_D +184° Chf (*c* 2.02), λ^{EtOH} 311, 324, 340 mμ (9,600; 10,800; 6,670). $\Delta^{7,22}$-Ergostadiene-3β,5α,-6α-triol 3-acetate 6-benzoate, m.p. 190–191° from acetone–methanol (lit.[8] 186–187°), on hydrolysis with Claisen alkali gave the triol, which crystallized from ethyl acetate as white, rectangular prisms, m.p. 243.5–244.5°, α_D +30° Py (*c* 0.97); lit.[8] m.p. 241–242°, α_D +29° Py. The 3,6-diacetate melted at 180–181°, α_D +42° Chf (*c* 1.23), +10° Py (*c* 1.34); lit.[11] m.p. 181–182°, α_D +41° Chf.

CAMBRIDGE, MASSACHUSETTS

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, MONTANA STATE UNIVERSITY]

Synthetic Estrogens. II.[1] Derivatives of 2-Phenylphenanthrene and 1-Ethyl-2-benzylnaphthalene

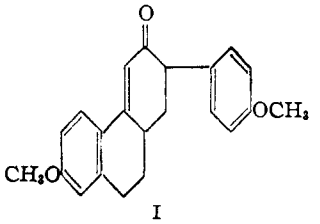
BY RICHARD E. JUDAY

RECEIVED MARCH 9, 1953

Derivatives of 2-phenylphenanthrene have been made using the method of DuFeu, McQuillan and Robinson starting with 6-methoxy-α-tetralone and anisylacetone. The derivatives of 1-ethyl-2-benzylnaphthalene were prepared by alkylating 6-methoxy-α-tetralone followed by reaction with ethylmagnesium bromide. Three of the four compounds tested showed activity at the 1 mg. level.

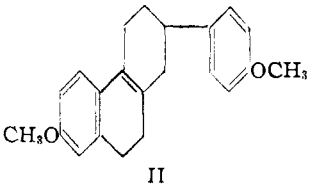
In connection with work on derivatives of 1-phenylphenanthrene,[1] it was desired to compare their biological activity with corresponding 2-substituted isomers and with compounds in which ring C had been opened.

The reaction between 6-methoxy-α-tetralone and 1-dimethylamino-2-(*p*-methoxyphenyl)-3-butanone methiodide using the method of DuFeu, McQuillan and Robinson[2] was not successful. The modification of Shunk and Wilds[3] using the 2-hydroxymethylene derivative of 6-methoxy-α-tetralone was successful, giving a yield of 46% of product, m.p. 187–188.5°. This compound had an ultraviolet absorption spectrum closely resembling that of the 1-substituted isomer[1] with a maximum at 330 mμ, indicating that it was the expected 2-(*p*-methoxyphenyl)-3-keto-7-methoxy-1,2,3,9,10,-10a-hexahydrophenanthrene (I). Reduction of I using the modification of the Wolff–Kishner tech-

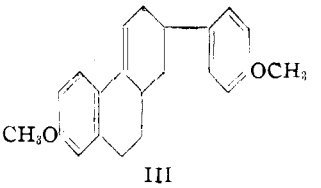


I

nique previously outlined[1] produced a mixture, which was separated with some difficulty by fractional crystallization and chromatographing on alumina, into II, m.p. 148–151°, and III, m.p. 121–123.5°. Consideration of the ultraviolet absorption



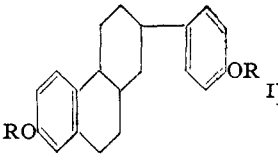
II



III

spectra indicated that II, with a maximum at 272.5 mμ has the double bond in the endo position, while in III, with a maximum at 264.5 mμ, the double bond is located in the exo position.[1,4]

Catalytic hydrogenation of a mixture of II and III produced a single isomer of IV, m.p. 91.5–93°, in good yield. Cleavage of the methoxyl groups of IV with hydrobromic acid produced V, m.p. 194–196°.



IV, R = CH_3
V, R = H

Since only the one isomer was obtained from the reduction of II and III, the most likely B:C configuration of IV is *cis*, because reduction of the endo double bond should give rise to that configuration exclusively. If the results found by Linstead and

(1) R. E. Juday, Paper I, THIS JOURNAL, **75**, 3008 (1953).

(2) E. C. DuFeu, F. J. McQuillan and R. Robinson, *J. Chem. Soc.*, 53 (1937).

(3) C. H. Shunk and A. L. Wilds, THIS JOURNAL, **71**, 3946 (1949).

(4) J. Heer and K. Miescher, *Helv. Chim. Acta*, **31**, 219 (1948).