[CONTRIBUTION FROM THE FRICK CHEMICAL LABORATORY, PRINCETON UNIVERSITY]

MOLECULAR REARRANGEMENTS IN THE STEROLS. IX. THE STRUCTURE DETERMINATION OF 3,5-CYCLO-$\Delta^{6,8(14),22}$-ERGOSTATRIENE

JOSEPH J. CAHILL JR.,[1a, b] NIKOLAUS E. WOLFF, AND EVERETT S. WALLIS

Received December 23, 1952

In an attempt to prepare *i*-ergosterol by the rearrangement of ergosteryl *p*-toluenesulfonate, m.p. 115–116°, $[\alpha]_D^{25}$ $-58°$, a mixture of isomeric hydrocarbons of the formula $C_{28}H_{42}$ was obtained. This mixture of hydrocarbons, on treatment with acetic anhydride, maleic anhydride, or activated alumina yields a single hydrocarbon (I), m.p. 102–103°, $[\alpha]_D^{25}$ $+93°$. This substance is identical with hydrocarbon "B" prepared by Stoll (1) by refluxing ergosterol with *p*-toluenesulfonyl chloride in pyridine. Hydrocarbon "B" was first isolated by Rygh (2) by reacting ergosterol with phosphorus oxychloride in pyridine and subsequent treatment of the products with acetic anhydride. It has also been reported as a by-product in the acetylation of ergosterol (3). None of these investigators have proposed a structure for hydrocarbon "B". However, it was believed to contain four double bonds. The fact that hydrocarbon I was isolated under conditions favorable for *i*-steroid rearrangement (4) indicated the possibility of a 3,5-cyclo-bond in the molecule.

To substantiate this we have reinvestigated the original work on hydrocarbon I and confirmed the following facts: perbenzoic acid titration of I results in the absorption of three equivalents of oxygen corresponding to three double bonds. Treatment of I with dry hydrochloric acid in chloroform yields a mixture of ergosteryl chlorides (5), whereas a few drops of sulfuric acid in glacial acetic acid rearrange hydrocarbon "B" to a mixture of ergosteryl acetates of uncertain structure. All these reactions are analogous to the rearrangement of 3,5-cyclosteroids.[2]

Hydrogenation of hydrocarbon I in glacial acetic acid at 70° and 150 atm., using a platinum oxide catalyst, yields ergostane (II) (2). We have now succeeded in selectively reducing I with Raney nickel in dioxane solution to 3,5-cyclo-$\Delta^{8(14),22}$-ergostadiene (III), m.p. 69.5–70.5°, $[\alpha]_D^{25}$ $+43°$. The removal of the 6,7-double bond in this reduction is in good agreement with the work of Laubach (6) who selectively reduced $\Delta^{6,8(14),22}$-ergostatrien-3β-ol acetate to $\Delta^{8(14),22}$-ergostadien-3β-ol acetate under the same conditions. Compound III gives a positive *Tortelli-Jaffé* test, indicative of the 8,14-double bond.

Perbenzoic acid titration of III shows that two double bonds are present in the molecule. In a separate experiment we have treated hydrocarbon III with an excess of perbenzoic acid in benzene solution at 10° for 20 hours. The resulting

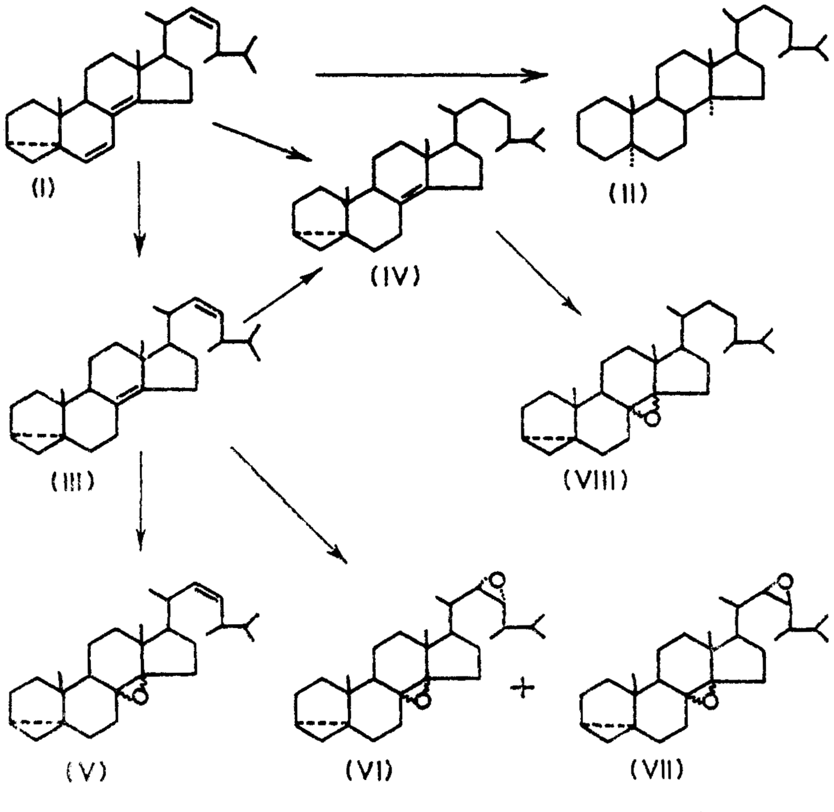
---

[1a] Standard Brands Fellow in Organic Chemistry, 1950–1952.

[1b] This paper is based in part upon a thesis submitted by Mr. Joseph J. Cahill Jr. to the Faculty of the Graduate School of Princeton University in partial fulfillment of the requirements for the degree of Doctor of Philosophy.

[2] For complete references see: Wolff and Wallis, *J. Org. Chem.*, **17**, 1361 (1952).

crystalline epoxide was found to be 3,5-cyclo-Δ^{22}-ergosten-8(14)-oxide (V), m.p. 91–92°, $[\alpha]_D^{20}$ +50°, on the basis of its analysis, absorption spectra, and a negative *Tortelli-Jaffé* test. Construction of molecular models shows that the C-9 hydrogen must have the α-configuration in 3,5-cyclo-$\Delta^{6,8(14),22}$-ergostatriene (I). Assuming that the configuration of the C-9 hydrogen is not affected during the reduction to compound III (or IV), we can then have the following epoxides: an α-oxide with rings A/B *trans*, B/C *cis*, and C/D *trans*; or a β-oxide with rings A/B *trans*, B/C *trans*, and C/D *cis*. Only one mono-epoxide from III (or IV) was isolated. It is not possible at the moment to assign a configuration to the 8,14-epoxides.



When compound III is allowed to react with perbenzoic acid for three days, two isomeric dioxides are produced, m.p. 107.5–108.5°, $[\alpha]_D^{20}$ +53°, and m.p. 136–137°, $[\alpha]_D^{20}$ +26°. Of the four possible structures for these epoxides we have obtained 3,5-cycloergostan-8(14), 22α-dioxide (VI), and3,5-cycloergostan-8(14), 22β-dioxide (VII). Again it is not possible to decide which of the physical constants have to be assigned to the respective structures.

When hydrocarbon I is hydrogenated in ethyl acetate solution with platinum oxide as catalyst, two moles of hydrogen are absorbed at which point the reaction stops. A clear oily product has been isolated which forms crystals with difficulty. The pure, chromatographed sample melts at 84.5–85.5°, $[\alpha]_D^{20}$ +75°. A positive

Tortelli-Jaffé test, analysis, and absorption spectra, as well as molecular rotation calculations indicate that this hydrocarbon is 3,5-cyclo-$\Delta^{8(14)}$-ergostene (IV). The same hydrocarbon is obtained from III on hydrogenation with platinum oxide in ethyl acetate solution. Under those conditions it is not possible to reduce an 8,14-double bond (7).

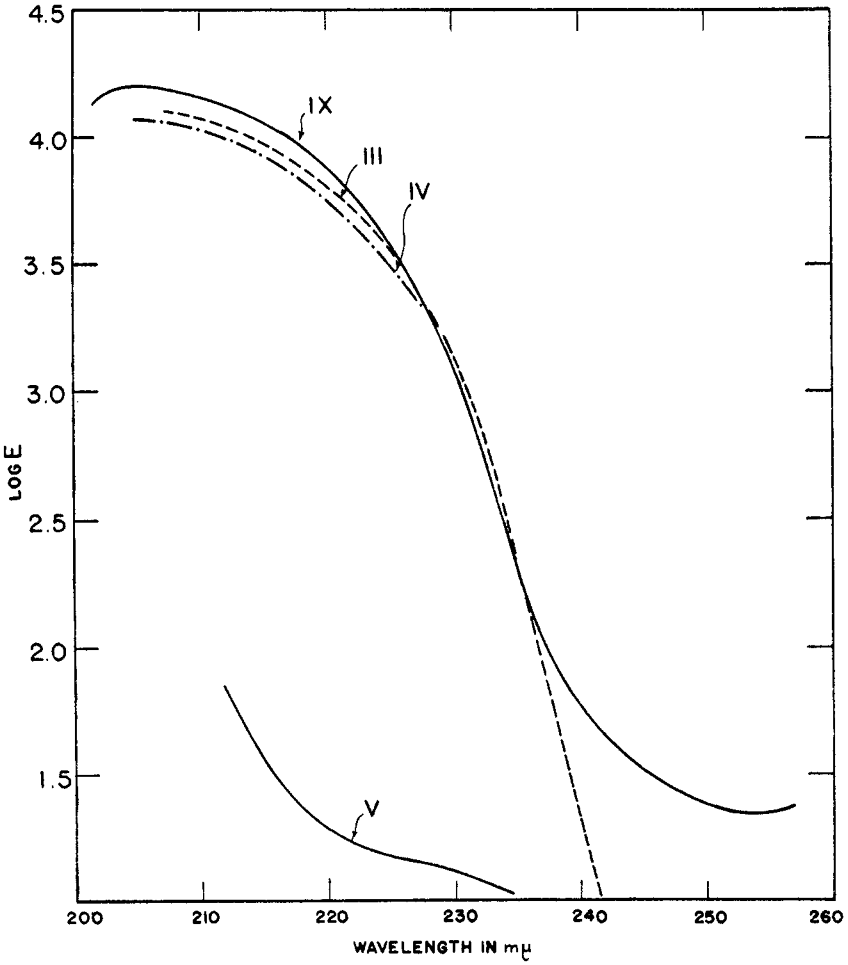


FIG. 1 ULTRAVIOLET ABSORPTION SPECTRA OF COMPOUNDS III, IV, V, AND IX

Hydrocarbon IV on perbenzoic acid titration takes up one equivalent of oxygen. The resulting epoxide (VIII), 3,5-cycloergostan-8(14)-oxide, m.p. 72–73°, $[\alpha]_D^{20}$ +67° was characterized by analysis. The *Tortelli-Jaffé* test is negative. The formation of the partially hydrogenated hydrocarbons III and IV, the conversion of III into IV, as well as the resulting epoxides V, VI, VII, and VIII clearly indicate that hydrocarbon I has the assigned structure.

In a recent publication by Fieser (8) the same structure was proposed for

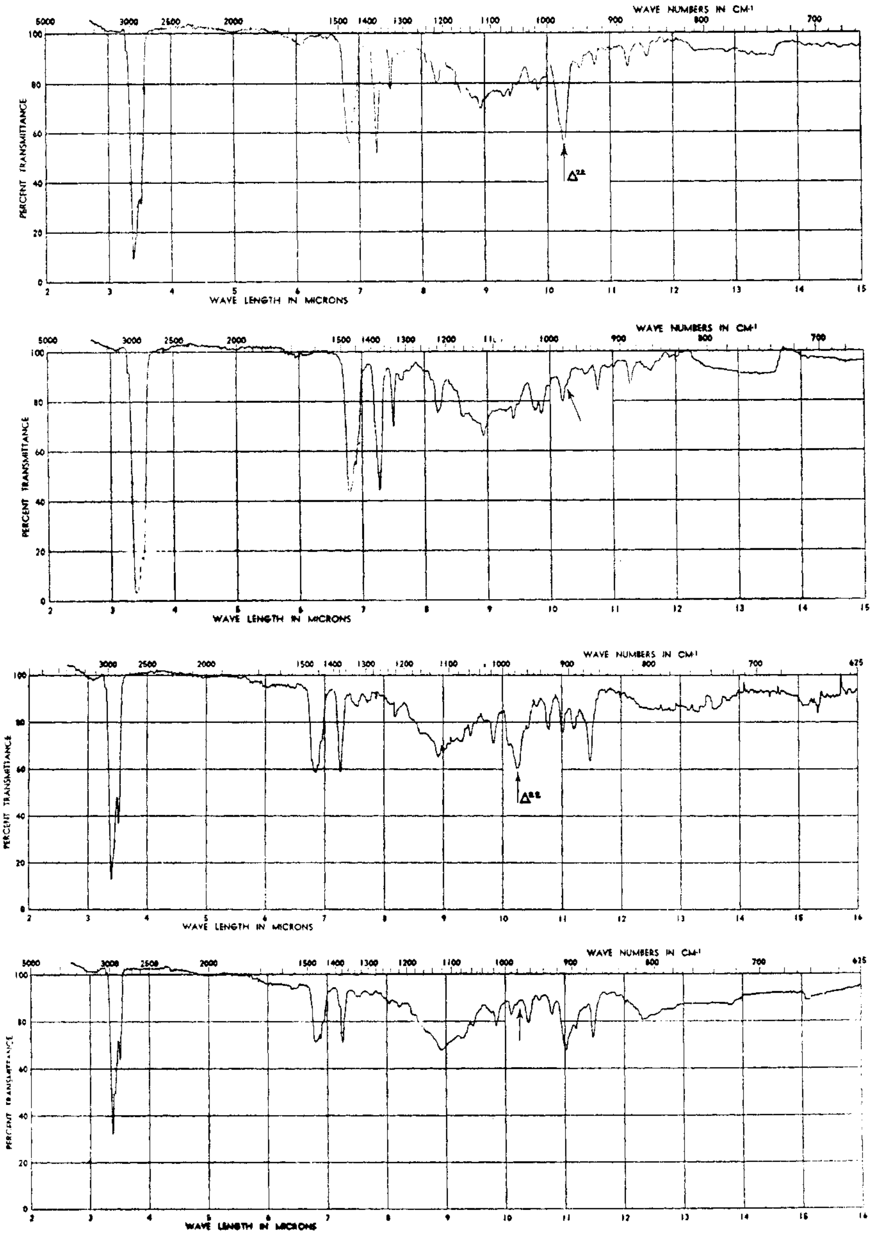


Fig. 2. Infrared Spectra. Top to bottom: 3,5-cyclo-$\Delta^{8(14),\,22}$-ergostadiene(III); 3,5-cyclo-$\Delta^{8(14)}$-ergostene (IV); 3,5-cyclo-Δ^{22}-ergosten-8(14)-oxide (V); 3,5-cycloergostan-8(14),22-dioxide VI (or VII)

hydrocarbon "B" partly on the basis of its ultraviolet absorption maximum at 261 mμ. His reasoning was based on the absorption of 3,5-cyclo-Δ^6-cholestene (IX), reported in an earlier paper by Klotz (9) who pointed out that there is a strong indication of an absorption maximum of hydrocarbon IX[3] at about 210 mμ. Fieser stated that an additional double bond at the 8,14-position would shift the absorption maximum by 50 mμ toward longer wave lengths, and hence arrived at a value of 260 mμ for hydrocarbon I. In order to determine the true absorption maximum of 3,5-cyclo-Δ^6-cholestene (IX), we have prepared this hydrocarbon, m.p. 72.5–73°, $[\alpha]_D^{20}$ −56°, (*cf.* 9) from 3,5-cyclocholestan-6β-ol (10) by a process analogous to that described by Riegel (11) who prepared hydrocarbon IX from *i*-cholesteryl methyl ether. The spectrum has been taken in especially purified cyclohexane (12) between 202 mμ, the lower limit of the instrument, and 300 mμ (Figure 1). An absorption maximum is found at 204 mμ. On the same figure we have plotted the UV absorption spectra of compound III and IV. Both curves are identical and correspond to that of $\Delta^{8(14)}$-ergostene (13). The UV spectra of the 8,14-epoxides, however, do not show the characteristics of their parent hydrocarbons.

TABLE I

MOLECULAR ROTATION CALCULATIONS FOR THE HYDROGENATION PRODUCTS

COMPOUND	CONTRIBUTING SYSTEM				M_D		$[\alpha]_D$	
	Ergostane	$\Delta^{8(14)}$-[a]	Δ^{22}-[b]	3,5-cyclo-[c]	Calc'd	Found	Calc'd	Found
3,5-Cyclo-$\Delta^{8(14),22}$-ergostadiene (III)	+66	−16	−94	+207	+163	+166	+42°	+43°
3,5-Cyclo-$\Delta^{8(14)}$-ergostene (IV)	+66	−16	—	+207	+257	+286	+67°	+75°

[a] Barton and Klyne, *J. Soc. Chem. Ind.* (*London*), 755 (1948); [b] Barton and Brooks, *J. Am. Chem. Soc.*, **72**, 1633 (1950); [c] 3,5-cyclocholestane minus cholestane.

The infrared spectra of hydrocarbon III and IV, the epoxides V, and VI (or VII) are given in Figure 2. All compounds show absorption peaks in the 860, 890, and 1020 cm^{-1} region, indicative of the 3,5-cyclo- structure (14). A strong peak at 978 cm^{-1} (arrow) in the spectrum of 3,5-cyclo-$\Delta^{8(14),22}$-ergostadiene (III) can be regarded as additional proof that the 22,23-double bond has not been reduced (15). The presence of the same double bond in the epoxide V can be seen on comparing its infrared spectrum with that of the di-epoxide VI (or VII).

In Table I we have calculated the rotations for hydrocarbon III and IV which are in good agreement with the observed values.

EXPERIMENTAL[4]

Ergosteryl p-toluenesulfonate. To a solution of 11 g. of ergosterol in 100 ml. of anhydrous pyridine was added 11 g. of pure *p*-toluenesulfonyl chloride in small amounts over a period of half an hour. The mixture was protected from moisture and the reaction temperature was kept between 0° and 20°. The solution was allowed to stand at room temperature

---

[3] Also known as "Riegel's hydrocarbon" or *i*-cholestadiene.

[4] All melting points are uncorrected and were determined on a Kofler Block; all rotations were taken in chloroform in a 1-dm. tube unless otherwise noted. Analyses reported in this paper were performed by Mr. J. F. Alicino, Metuchen, New Jersey.

overnight. It was poured into a mixture of ice and water with stirring and the resulting precipitate was filtered and washed with water. The material was thoroughly dried *in vacuo* and crystallized from anhydrous acetone. Yield: 10 g. of material, m.p. 106–107° (dec.), $[\alpha]^{25}_{D}$ −44° (*c*, 2.00). Recrystallization from anhydrous ether gave stout prisms, m.p. 115–116° (dec.), $[\alpha]^{25}_{D}$ −58° (*c*, 0.48, ether).

Anal. Calc'd for $C_{35}H_{50}O_3S$ (550.81): C, 76.31; H, 9.15; S, 5.92.

Found: C, 76.49; H, 9.12; S, 6.07.

Our constants are in good agreement with those recently reported by Karrer and Asmis (16): m.p. 104–105°, $[\alpha]^{20}_{D}$ −45.6°.

3,5-Cyclo-$\Delta^{6,8(14),22}$-ergostatriene (I). This hydrocarbon was obtained as a rearrangement product from ergosteryl *p*-toluenesulfonate.[5] It was also prepared by the method of Stoll (1) with the following modifications: ergosterol (32 g.) was dried *in vacuo* and dissolved in 400 ml. of anhydrous pyridine. Pure *p*-toluenesulfonyl chloride (50 g.) was added to the solution and the mixture was refluxed for six hours. The solution was allowed to stand overnight in the cold. The crystalline material formed was filtered and washed with cold methanol. It was taken up in ether, washed with 5% sodium bicarbonate solution and water, and the ether layer separated and dried over sodium sulfate. The filtered ether solution was concentrated to a small volume and hot ethanol was added. On cooling 13.5 g. of long needles were obtained, m.p. 100–101°, $[\alpha]^{25}_{D}$ +100°. This material was purified by shaking it with activated alumina in benzene solution for two hours; the pure sample melted at 102–103°, $[\alpha]^{25}_{D}$ +93° (*c*, 1.00). (λ^{alc}_{max} 261 mμ, log E, 4.4). The yield varied from 45 to 60%.

Anal. Calc'd for $C_{28}H_{42}$ (378.62): C, 88.81; H, 11.18.

Found: C, 89.03; H, 11.17.

Reaction of HCl-gas on I. 3,5-Cyclo-$\Delta^{6,8(14),22}$-ergostatriene (0.5 g.) was dissolved in 50 ml. of chloroform. Dry hydrogen chloride gas was passed through the solution for one hour. The reaction mixture was washed with bicarbonate solution, water, and the chloroform layer separated. Upon concentrating to dryness and recrystallizing the residue several times from ethanol, 40 mg. of material, m.p. 107–108°, $[\alpha]^{25}_{D}$ −158°, was obtained. (λ^{alc}_{max} 248 mμ, log E 4.07). The compound gave a positive *Beilstein* test. (Mixture of ergosteryl chlorides.)

Rearrangement of I *in acid solution.* The same hydrocarbon I (0.2 g.) was dissolved in 12 ml. of glacial acetic acid. Two drops of concentrated sulfuric acid were added (positive *Liebermann* test) and the mixture was heated for 30 min. on the steam-bath. Concentration of the solution *in vacuo* and crystallization of the residue from methanol-acetone gave a small amount of material, m.p. 133.5–135.5°, $[\alpha]^{25}_{D}$ −90°. (Mixture of ergosteryl B-acetates.)

3,5-Cyclo-$\Delta^{8(14),22}$-ergostadiene (III). A solution of 6 g. of hydrocarbon I in 50 ml. of dioxane was hydrogenated over one gram of Raney nickel at an initial pressure of 3 atm. at room temperature. After shaking for six hours the theoretical amount of hydrogen for one double bond was absorbed at which point the reaction stopped. The catalyst was removed, the solution was concentrated to dryness *in vacuo*, and the residual oil was crystallized from acetone. It formed needles (3.5 g.), m.p. 67–68°. Recrystallization from acetone-ether raised the melting point to 69.5–70.5°, $[\alpha]^{20}_{D}$ +43° (*c*, 1.04). Yield: 57%.

Anal. Calc'd for $C_{28}H_{44}$ (380.64): C, 88.35; H, 11.65.

Found: C, 88.13; H, 11.77.

3,5-Cyclo-$\Delta^{8(14)}$-ergostene (IV). (*A*). A solution of 13 g. of 3,5-cyclo-$\Delta^{6,8(14),22}$-ergostatriene (I) in 180 ml. of ethyl acetate was hydrogenated over one gram of platinum oxide at an initial pressure of 3 atm. at room temperature. After shaking for two hours the theoretical amount of hydrogen for two double bonds was absorbed at which point the reaction stopped. The reaction mixture was worked up as described above and the residual

---

[5] Details of the rearrangement of ergosteryl *p*-toluenesulfonate will be published shortly.

oil was taken up in petroleum ether (30–60°). Chromatography on acid-washed, activated (300°, 16 hrs.) alumina gave the following fractions (50 ml. each, petroleum ether):

FRACTION	AMOUNT, G.	FORM
1	6.4	Oil
2	2.8	Oil
3	0.3	Oil
4	0.03	Crystalline
5	Nil	—

Fraction 4 on recrystallization from acetone yielded a small amount of material, m.p. 80–83°. Fraction 1 was rechromatographed on alumina and produced a colorless oil which finally crystallized from acetone on cooling in a Dry Ice-acetone mixture. Yield: 4 g. of fine needles, m.p. 80–81°, $[\alpha]_D^{20}$ +67°. Recrystallization from ether-methanol raised the melting point to 84.5–85.5°, $[\alpha]_D^{20}$ +75°.

Anal. Calc'd for $C_{28}H_{46}$ (382.65): C, 87.88; H, 12.12.

Found: C, 87.64; H, 12.09.

(*B*). A solution of 0.48 g. of 3,5-cyclo-$\Delta^{8(14),22}$-ergostadiene (III) in 30 ml. of ethyl acetate was hydrogenated under the same conditions as described under (*A*). The reaction stopped after one mole of hydrogen had been absorbed and the product was isolated in the usual manner. Repeated crystallization from acetone-ether gave a product, m.p. 84–85° which was identical in all respects with hydrocarbon IV. Yield: 42%.

3,5-Cyclo-Δ^{22}-ergosten-8(14)-oxide (V). To a solution of 0.2 g. of hydrocarbon III in 15 ml. of benzene was added 10 ml. of a 0.5 *N* benzene solution of perbenzoic acid. This mixture was allowed to stand for 20 hours at 10°, and washed successively with 2% sodium hydroxide solution and water. The separated benzene layer was dried over sodium carbonate and concentrated *in vacuo*. Recrystallization of the residue gave needles, m.p. 91–92°, $[\alpha]_D^{20}$ +50° (*c*, 1.00). Yield: 89%.

Anal. Calc'd for $C_{28}H_{44}O$ (396.64): C, 84.78; H, 11.18.

Found: C, 84.95; H, 11.05.

3,5-Cycloergostan-8(14),22-dioxide (VI) *and* (VII). To a solution of 0.4 g. of hydrocarbon III in 25 ml. of benzene was added 25 ml. of a 0.5 *N* benzene solution of perbenzoic acid. The mixture was allowed to stand for three days at 10° and then was worked up as described for V. Careful fractionation of the residue from ethanol afforded 50 mg. of stout prisms, m.p. 136–137°, $[\alpha]_D^{20}$ +26° (*c*, 0.74). The mother liquors on recrystallization afforded 200 mg. of fine needles, m.p. 107.5–108.5°, $[\alpha]_D^{20}$ +53° (*c*, 1.00); (isomeric oxides VI and VII).

Anal. Calc'd for $C_{28}H_{44}O_2$ (412.64): C, 81.49; H, 10.75.

Found: C, 81.44; H, 10.82. (m.p. 136°).

C, 81.55; H, 10.82. (m.p. 107°).

3,5-Cyclo-ergostan-8(14)-oxide (VIII). To a solution of 0.2 g. of hydrocarbon IV in 10 ml. of benzene was added 10 ml. of a 0.5 *N* benzene solution of perbenzoic acid. After standing for 20 hours at 10° the reaction mixture was worked up as described for V. Recrystallization from ethanol afforded short needles, m.p. 72–73°, $[\alpha]_D^{20}$ +67° (*c*, 1.00). Yield: 85%.

Anal. Calc'd for $C_{28}H_{46}O$ (398.65): C, 84.35; H, 11.63.

Found: C, 84.21; H, 11.75.

Perbenzoic acid titrations. The perbenzoic acid was prepared by the method of Braun (17) with modifications by Kolthoff (18). The initial normality of the perbenzoic acid was 0.488 *N* and this did not change appreciably. The titer was checked prior to each titration. Solutions of about 100 mg. of hydrocarbon in 10 ml. of benzene were treated with 5 ml. of perbenzoic acid solution. Immediately before titration 10 ml. of glacial acetic acid and an excess of potassium iodide were added. The liberated iodine was titrated with 0.1 *N* thiosulfate solution.

COMPOUND	NO. OF DOUBLE BONDS	FOUND
I	3	2.83
III	2	1.90
IV	1	1.14

Ultraviolet absorption spectra. All spectra were taken on a Beckman spectrophotometer, Model DU. Spectra below 214 mμ were taken in cyclohexane (12) in a 0.05-cm. cell.

Infrared absorption spectra. All spectra were taken on a Baird Double Beam spectrophotometer in carbon tetrachloride.

Acknowledgment. We wish to thank Merck and Company, Inc., Rahway, New Jersey, for recording the infrared spectra and for carrying out the catalytic hydrogenations reported in this paper. We also wish to thank Standard Brands Inc., for a generous supply of ergosterol.

SUMMARY

Hydrocarbon "B" which can be obtained as a rearrangement product from ergosteryl *p*-toluenesulfonate is shown to be 3,5-cyclo-$\Delta^{6,8(14),22}$-ergostatriene (I). Its selective hydrogenation affords two new hydrocarbons, 3,5-cyclo-$\Delta^{8(14),22}$-ergostadiene (III) and 3,5-cyclo-$\Delta^{8(14)}$-ergostene (IV). The structure of these hydrocarbons was elucidated by conversion of III into a mono-epoxide V and two isomeric di-epoxides VI and VII. Hydrocarbon IV affords a single epoxide VIII. Perbenzoic acid titrations confirm the number of double bonds in the respective hydrocarbons. The presence of the 3,5-cyclo-bond in the compounds described is demonstrated by their infrared spectra as well as their behavior towards perbenzoic acid. Calculated molecular rotation data are in good agreement with the observed values. A reinvestigation of the ultraviolet absorption spectrum of 3,5-cyclo-Δ^6-cholestene shows that there is an absorption maximum at 204 mμ.

PRINCETON, NEW JERSEY

REFERENCES

(1) STOLL, *Z. physiol. Chem.*, **202**, 232 (1931).
(2) RYGH, *Z. physiol. Chem.*, **185**, 99 (1929).
(3) STANSBURY, *J. Am. Chem. Soc.*, **65**, 1243 (1943).
(4) BEYNON, HEILBRON, AND SPRING, *J. Chem. Soc.*, 1459 (1937).
(5) GUITERAS, *Z. physiol. Chem.*, **215**, 196 (1933).
(6) LAUBACH AND BRUNINGS, *J. Am. Chem. Soc.*, **74**, 705 (1952).
(7) SCHENCK, BUCHHOLZ, AND WEISE, *Ber.*, **69**, 2696 (1936).
(8) FIESER, ROSEN, AND FIESER, *J. Am. Chem. Soc.*, **74**, 5397 (1952).
(9) KLOTZ, *J. Am. Chem. Soc.*, **66**, 88 (1944).
(10) WAGNER, WOLFF, AND WALLIS, *J. Org. Chem.*, **17**, 529 (1952).
(11) RIEGEL, HAGER, AND ZENITZ, *J. Am. Chem. Soc.*, **68**, 2562 (1946).
(12) PESTEMER, *Angew. Chem.*, **63**, 118 (1951).
(13) BLADON, HENBEST, AND WOOD, *J. Chem. Soc.*, 2737 (1952).
(14) JOSIEN, FUSON, AND CAREY, *J. Am. Chem. Soc.*, **73**, 4445 (1951); DERFER, PICKETT, AND BOORD, *J. Am. Chem. Soc.*, **71**, 2482 (1949).
(15) GÜNTHARD AND RUZICKA, *Helv. Chim. Acta*, **31**, 642 (1948); THOMPSON AND WHIFFEN, *J. Chem. Soc.*, 1412 (1948).
(16) KARRER AND ASMIS, *Helv. Chim. Acta*, **35**, 1926 (1952).
(17) BRAUN, in *Org. Syntheses*, Coll. Vol. I, 2nd Ed., 431 (1941).
(18) KOLTHOFF, LEE, AND MAIRS, *J. Polymer Sci.*, **2**, 199 (1947).