differences between the spectra of IIa and IIb are found here than between the analogous compounds tigogenin acetate and 3-desoxytigogenin.[23]

3-Desoxyhecolic Acid (IIIb) (13ξ-Hydroxy-12,13-seco-5α,-22a-spirostan-12-oic Acid).—Saponification of IIb gave 3-desoxyhecolic acid. The product melted at 178° on a preheated Kofler stage, solidified on further heating, and remelted at 238.0–241.4°, $[\alpha]^{25}D$ −63.8°.

Anal. Calcd. for $C_{27}H_{44}O_5$: C, 72.28; H, 9.89. Found: C, 71.84; H, 9.99.

Infrared spectrum ($CHCl_3$ solution) included: broad carboxyl–hydroxyl absorption in the 3000 cm.$^{-1}$ region, carboxyl–carbonyl band at 1707 cm.$^{-1}$, and F-ring bands at 868 (m), 901 (s), 923 (m), 984 (s) cm.$^{-1}$ with the usual intensity pattern for "iso" F-rings.

Conversion of Methyl 3α-Carbethoxyoxy-12-ketocholanate to Methyl 3α-Carbethoxyoxy-13ξ-hydroxy-12-carboxy-12,13-secocholanate 12,13-Lactone (X).—A sample of methyl 3α-carbethoxyoxy-12-ketocholanate, m.p. 155–159°, 5 g., was oxidized during a ten-day reaction period at 25° using as oxidant 60 ml. of perbenzoic acid in chloroform solution (50 mg./ml.) containing 2 ml. of 10% sulfuric acid in acetic acid. The product was obtained after the usual washing with water, dilute sodium hydroxide and water, and evaporation of solvent as a glassy residue which crystallized on rubbing with pentane. This procedure gave 5.04 g. of a crystalline crude product, m.p. 98–102°. A portion chromatographed on $SiO_2 \cdot xH_2O$ was eluted with chloroform to give the analytical sample, m.p. 105–106.5°, $[\alpha]^{25}D$ +2.95°. No other steroidal materials were formed in the reaction.

Anal. Calcd. for $C_{28}H_{44}O_7$: C, 68.26; H, 9.00. Found: C, 68.23; H, 8.94.

The lactonic product, without isolation of intermediates, was sequentially saponified, esterified with diazomethane, and reduced with $LiAlH_4$ to a polyhydroxy compound which on acetylation showed unacetylated hydroxyl infrared bands. The infrared spectra of the intermediate crudes were more difficult to interpret than the corresponding sapogenin analogs because of the overlapping of the absorption bands of the functional groups.

Acknowledgment.—The authors are indebted to Miss Harriet Cooper for technical assistance, to Miss Mary Anne Morris and Mr. C. S. Fenske for assistance with infrared curves, and to Dr. C. L. Ogg, Mrs. M. J. Bythrow and Mrs. R. B. Kelley for microanalyses. We greatly appreciate the kindness of Dr. R. Sifferd of the Bio Process Corp., Joliet, Ill., for a generous gift of bile acid samples.

PHILADELPHIA 18, PA.

(23) M. E. Wall, S. Serota and C. R. Eddy, paper presented at A.C.S. Meeting-in-Miniature, Philadelphia, Pa., January 29, 1953. Abstract of Papers p. 12.

[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Nitration of Unsaturated Steroids

BY CONSTANTINE E. ANAGNOSTOPOULOS AND LOUIS F. FIESER

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Cholesteryl acetate reacts with dinitrogen tetroxide in ether to give the 6β-nitro-5α-nitrate (V), convertible by the action of ammonia into 6-nitrocholesteryl acetate, prepared conveniently by nitration in ether. The 6-nitro acetates of epicholesterol and stigmasterol were obtained similarly; diosgenin suffered both nitration and cleavage of the side chain to the lactone. The acetates of Δ^{7}- and $\Delta^{9(11)}$-enes and of a $\Delta^{7,9(11)}$-diene gave products regarded as the 7,8-dinitro, 9-nitro-11-nitrate and Δ^{8}-7,11-dinitro derivatives, respectively. Nitroalkane, nitroölefin, and nitrate groups are distinguishable by characteristic infrared absorption bands.

In 1903, Windaus[1,2] and Mauthner and Suida[3] independently discovered that cholesteryl acetate and the free sterol on nitration afford 6-nitrocholesteryl acetate and the 6-nitro-3-nitrate, respectively, and that both substances on reduction with zinc and acetic acid give cholestane-3β-ol-6-one, as acetate or as free alcohol, in high yield. Δ^{4}-Cholestene, by the same transformations, affords cholestane-4-one[4]; Δ^{5}-cholestene[4] and Δ^{5}-stigmastene[5] (sitostene) have also been nitrated. High-yield procedures have been reported for nitration of cholesterol[6] in acetic acid according to Windaus and for nitration of cholesteryl acetate[7] with nitric acid–sodium nitrite according to Mauthner and Suida.

In a systematic investigation of the reaction of sinple olefins with oxides of nitrogen, Levy, Scaife and co-workers[8] found that difficulties encountered by earlier workers could be eliminated by use of pure dinitrogen tetroxide, that addition of this reagent to form *vic*-dinitroalkanes and nitro-nitrites or nitrates proceeds particularly well in ether or ester solvents, and that added oxygen prevents interference by dinitrogen trioxide (and probably aids in oxidation of nitro-nitrites to nitro-nitrates). In the present work, we investigated application to unsaturated steroids of one of the general procedures of the British investigators and also explored nitration with fuming nitric acid in ether solution. Sterol acetates proved to be better starting materials than the free alcohols, since introduction of a nitrate group can then be recognized from the infrared spectrum.

Treatment of cholesteryl acetate (I) in ether solution at 0° with fuming nitric acid afforded pure 6-nitrocholesteryl acetate (III) in 72% yield. When a 2:1 mixture of gaseous dinitrogen tetroxide and oxygen was passed into a chilled ethereal solution of cholesteryl acetate, a crystalline reaction product was obtained having the composition of a nitro-nitrate. The nitro group is located at C_6 by the observation that the substance was converted smoothly by reaction with ammonia in ether into 6-nitrocholesteryl acetate (III), and hence the nitrate group must be at position 5, as in formula V. It seems very likely that the two products of nitration, III and V, are formed in ionic reactions from

(1) A. Windaus, "Uber Cholesterin," Habilitationschrift, Freiburg i. B., 1903.

(2) A. Windaus, *Ber.*, **36**, 3752 (1903).

(3) J. Mauthner and W. Suida, *Monatsh.*, **24**, 648 (1903).

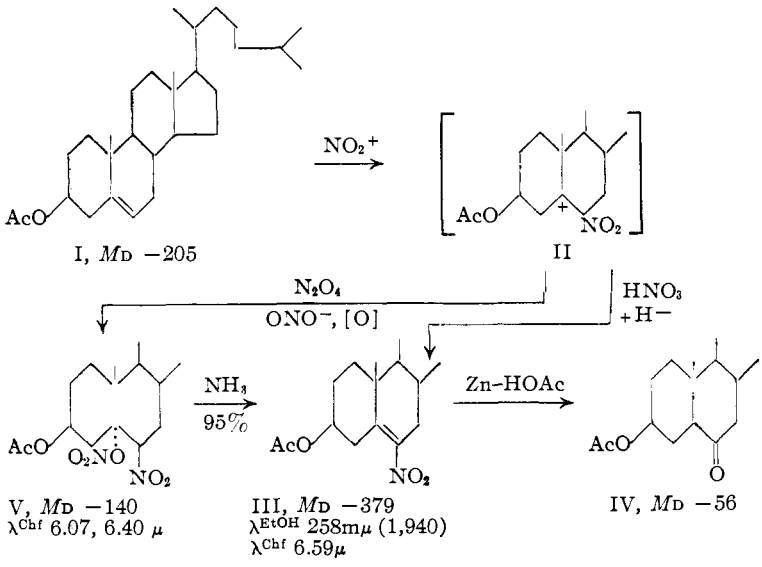
(4) A. Windaus, *Ber.*, **53**, 488 (1920).

(5) A. Windaus and C. Brunken, *Z. physiol. Chem.*, **140**, 52 (1924).

(6) I. M. Heilbron, H. Jackson, E. R. H. Jones and F. S. Spring, *J. Chem. Soc.*, 102 (1938).

(7) R. M. Dodson and B. Riegel, *J. Org. Chem.*, **13**, 424 (1948).

(8) N. Levy, C. W. Scaife and co-workers, *J. Chem. Soc.*, 1093, 1096, 1100 (1946); 52 (1948); 2678 (1949).



I, M_D −205

II

V, M_D −140
λ^{Chf} 6.07, 6.40 μ

III, M_D −379
λ^{EtOH} 258mμ (1,940)
λ^{Chf} 6.59μ

IV, M_D −56

the same intermediate carbonium ion II and hence that the nitro-nitrate has the 5α,6β-orientation characteristic of cholesterol dibromide.[9] This inference is supported by the fact that the molecular rotation of the nitro-nitrate (−140 Chf) is of the same sign and order of magnitude as that of cholestane-3β,5α,6β-triol triacetate (−192 Chf).[10] 6-Nitrocholesteryl acetate (III) is characterized by an ultraviolet absorption maximum of low intensity at 258 mμ; the infrared spectrum contains, in addition to the usual bands at 5.79 and 8.00 μ characteristic of the acetate function, a band at 6.59 μ attributable to the olefinic nitro group (Fig. 1). The nitro-nitrate V shows bands at 6.07 and 6.40 μ that appear to be attributable, respectively, to the nitrate (C_5) and nitroalkane (C_6) functions (Fig. 2).

Shoppee and Summers[11] have recently reported the preparation of cholestane-3α-ol-6-one from epicholesteryl acetate by nitration with concentrated nitric acid in the presence of potassium nitrate, reduction of the crude product, saponification and chromatography. We found that the reaction of epicholesteryl acetate with nitric acid in ether affords in low yield a crystalline product identified as the 6-nitroölefin by its absorption characteristics: λ^{EtOH} 257 μ (1,170), λ^{Chf} 6.59μ. An identical substance was obtained in satisfactory over-all yield through the 6-nitro-5-nitrate, prepared by reaction with dinitrogen tetroxide and identified by its characteristic absorption at 6.07 and 6.40 μ.

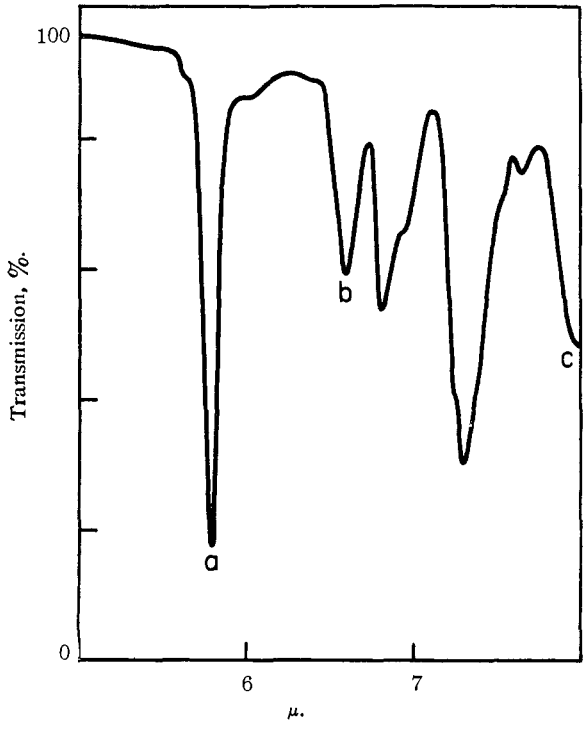


Fig. 1.—6-Nitrocholesteryl acetate: a, acetate carbonyl, 5.79 μ; b, nitroölefin, 6.59 μ; c, acetate, 8.0 μ.

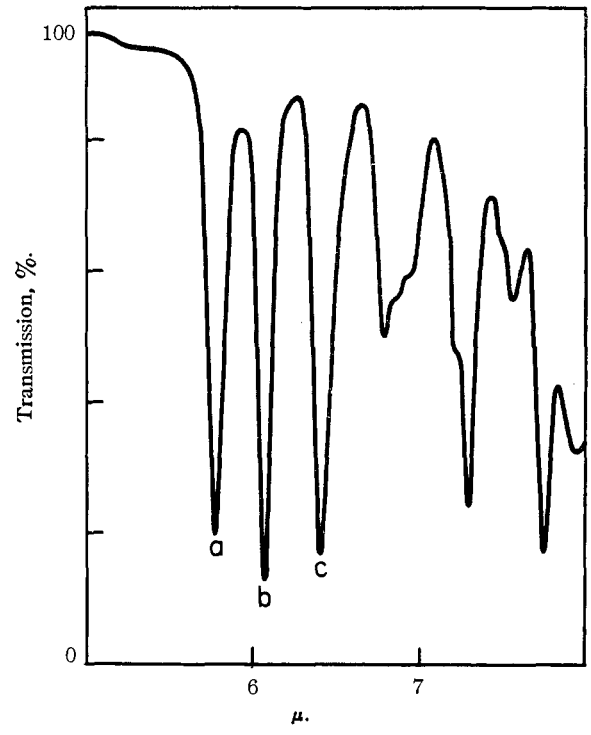
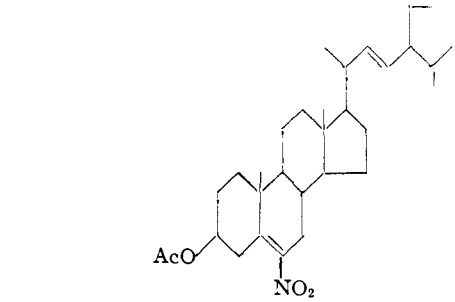


Fig. 2.—6β-Nitrocholestane-3β,5α-diol 3-acetate 5-nitrate; a, acetate carbonyl, 5.79 μ; b, nitrate, 6.07 μ; c, nitroalkane, 6.40 μ.

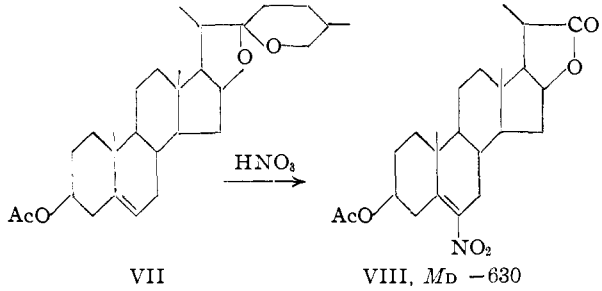
Nitric acid nitration of stigmasteryl acetate (M_D −253) in ether by the usual procedure was incomplete; the sole nitrogen-containing product, isolated by chromatography, was a mononitroolefin (M_D −490) showing the same ultraviolet and infrared absorption as 6-nitrocholesteryl acetate and reducible with zinc and acetic acid to a ketone (M_D −147). The molecular rotation differences between the starting material and its nitro deriva-

(9) D. H. R. Barton and E. Miller, THIS JOURNAL, **72**, 1066 (1950).
(10) Observation by E. J. Tarlton.
(11) C. W. Shoppee and G. H. R. Summers, *J. Chem. Soc.*, 1790 (1952).

tive (−137) and ketone (+106) are sufficiently close to those found in the cholesterol series (−174 and +149) to establish the structure as 6-nitrostigmasteryl acetate (VI). Furthermore, the substance adds bromine only slowly and the infrared spectrum shows a sharp band at 10.30μ, characteristic of the *trans*-22,23-double bond. The course of the nitration thus supplements other evidence[12] of the greater reactivity of the nuclear double bond.



VI, M_D −490



VII VIII, M_D −630

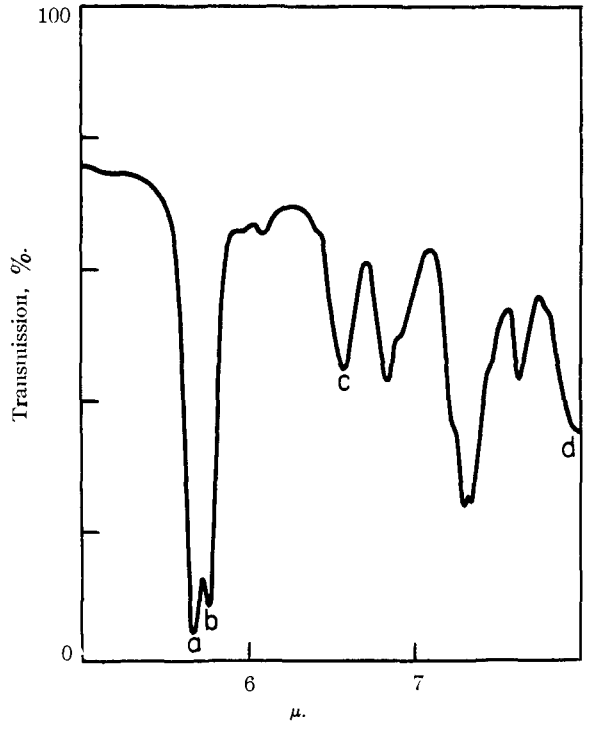
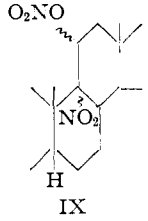


Fig. 3.—6-Nitro-3β,16β-dihydroxy-Δ^5-bisnorcholenic-22 → 16-lactone 3-acetate (VIII): a, lactone, 5.68 μ; b, acetate carbonyl, 5.79 μ; c, nitroölefin, 6.59 μ; d, acetate, 8.0 μ.

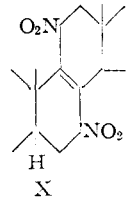
(12) E. Fernholz, *Ann.*, **507**, 128 (1933).

Treatment of diosgenin acetate (VII) with nitric acid in ether–chloroform gave a product of analysis and absorption characteristics indicative of both introduction of a 6-nitro group and degradation of the side chain to a lactone ring (VIII, see Fig. 3). Identical treatment of tigogenin acetate gave a product corresponding in constants to the known tigogenin lactone acetate, one of the products of chromic acid oxidation of the sapogenin acetate.[13] Reduction of the nitrolactone VIII gave a ketone of M_D −327; the M_D increment for this transformation of +303 corresponds to the increments of +323 and +343 found in the cholesterol and stigmasterol series between the nitroölefin and the ketone.

Nitric acid nitration of Δ^7-cholestenyl acetate gave a mixture from which only one product could be isolated. From the analysis and from the presence of a band at 6.45 μ in the infrared spectrum, this appears to be a dinitrocholestanyl acetate. Treatment of the substance with base gave an oily product that appeared to contain a nitroolefin, since on reduction it afforded a small amount of cholestane-3β-ol-7-one. Hence the product of nitration probably is a 7,8-dinitro derivative. Similar nitration of methyl 3α-acetoxy-$\Delta^{9(11)}$-cholenate gave only a small amount of nitrogen-containing, non-crystalline material that on reduction afforded the corresponding 11-ketocholanate. The reaction with dinitrogen tetroxide proceeded better and gave a crystalline product showing absorption characteristics of nitrate (6.08 μ) and nitro (6.42 μ) groups. Unlike the 6-nitro-5-nitrate V, the substance is stable to ammonia and hence is probably the 9-nitro-11-nitrate IX. Nitration of



IX



X

methyl 3α-acetoxy-$\Delta^{7,9(11)}$-choladienate gave, after removal of 25% of starting material and chromatography, a crystalline product that appears to be the Δ^8-7,11-dinitro derivative X. The substance is transparent to ultraviolet light and the infrared spectrum is that of a nitroalkane (λ^{Chf} 6.42 μ). Treatment with either ammonia in ether or alcoholic alkali failed to effect elimination of nitrous acid and hence the substance cannot be a 7,8- or 9,11-dinitro derivative and must be the product of 1,4-addition (X). Attempts to replace the nitro groups by oxygen functions were unsuccessful.

Experimental

6-Nitrocholesteryl Acetate (III).[1,3]—A solution of 2.5 g. of cholesteryl acetate in 35 cc. of absolute ether was stirred mechanically in a flask fitted with a condenser and dropping funnel and cooled by a salt–ice-bath, and 15 cc. of fuming nitric acid was added dropwise during 30 min. (reaction temperature, 5–10°). After stirring in the ice-bath for 1 hr. more the solution was transferred to a funnel and washed with three 50-cc. portions of 5% sodium hydroxide solution. Since complete removal of acid by further washing with base

(13) R. Tscheesche and A. Hagedorn, *Ber.*, **68**, 1412 (1935).

had been found to result in marked darkening of the solution and destruction of product, the solution was next washed with successive portions of saturated sodium chloride solution until the water layer was neutral to litmus. The ethereal solution was then dried and evaporated to dryness under reduced pressure; the semi-solid residue when triturated with methanol gave 2.2 g. (79%) of 6-nitrocholesteryl acetate, m.p. 96–101°. Crystallization from methanol (2.0 g., 72%, m.p. 101–102°) and recrystallization gave material of m.p. 103–104°, α_D −80° Chf, λ^{EtOH} 258 mμ (1,940), λ^{Chf} 5.79, 6.59, 8.00 μ.

Anal. Calcd. for $C_{29}H_{47}O_4N$ (473.67): C, 73.53; H, 10.00; N, 2.96. Found: C, 73.59; H, 9.96; N, 3.17.

Reduction of 1 g. of 6-nitrocholesteryl acetate (m.p. 101–102°) with zinc and acetic acid according to Dodson and Riegel[7] and crystallization from methanol gave 780 mg. (73%) of **cholestane-3β-ol-6-one acetate**, m.p. 127–129°. After two recrystallizations the substance melted at 129–130°, α_D −12.5° Chf, λ^{Chf} 5.79, 5.84, 7.96 μ (calcd.: C, 78.32; H, 10.88; found: C, 78,43; H, 11.04). Saponification and crystallization from methanol gave **cholestane-3β-ol-6-one**, m.p. 142–144°; after further purification by chromatography the substance formed fine needles from methanol, m.p. 150–151° (softened at 144°), α_D −3.0° Chf, λ^{Chf} 5.85 μ (calcd.: C, 80.54; H, 11.52; found: C, 80.39; H, 11.64). Dodson and Riegel[7] report m.p. 142–143°, α_D −5.1° Chf.

6β-Nitrocholestane-3β,5α-diol 3-Acetate 5-Nitrate (V).—The nitration was conducted in a cylindrical flat-bottomed tower cooled in salt–ice and having gas inlet and outlet tubes fused into the ground glass stopper. The inlet tube extended to the bottom of the reaction vessel and had a sintered glass spoon sealed at the end. Gaseous dinitrogen tetroxide, drawn from a tank of liquid tetroxide which was at room temperature, and commercial oxygen, dried by passage through calcium chloride, were passed through mineral oil bubblers for approximate estimation of the rate of flow, and then led through a T-tube to the common delivery tube. At the beginning of a run the reaction cylinder was charged with 2 g. of cholesteryl acetate and 50 cc. of absolute ether and dry oxygen was bubbled through the mixture to effect solution. Then the flow of dinitrogen tetroxide and of oxygen were adjusted to an estimated 2:1 ratio, and this mixture was passed through the chilled ethereal solution until absorption of tetroxide was complete (about 1 hr.). The end-point can be detected from the depth of color of the effluent gas, which was passed into a receiver kept at −70° to trap tetroxide and ether. The reaction mixture was now let stand in the ice-bath with only oxygen bubbling through it for an additional 2 hr. and then poured into a large beaker containing 100 cc. of 5% sodium hydroxide solution. The mixture was stirred until no more dark fumes filled the beaker, and then the ethereal solution was separated and shaken with two 75-cc. portions of the same alkali solution and next washed with sodium chloride solution until the washings reacted neutral to litmus. The dried ethereal solution on evaporation at reduced pressure left an oily residue that on crystallization from methanol gave 0.9 g. of crystals, m.p. 126–128°, and a second crop of 0.35 g., m.p. 123–125°; total yield 50%. Two further crystallizations from methanol–ether gave fine white needles, m.p. 132–133° dec., α_D −26° Chf, λ^{Chf} 5.79, 6.07, 6.40, 7.75, 7.98 μ.

Anal. Calcd. for $C_{29}H_{48}O_7N_2$ (536.69): C, 64.90; H, 9.02; N, 5.22. Found: C, 65.17; H, 9.19; N, 5.38.

The 6-nitro-5-nitrate was converted into **6-nitrocholesteryl acetate** by bubbling gaseous ammonia through a solution of 1 g. of material in 30 cc. of ether, cooled in a salt–ice-bath. After about 1 hr. the solution turned cloudy and then ammonium nitrate separated. This was removed by suction filtration (m.p. 167–170°) and the filtrate evaporated to dryness. Crystallization of the residual oil gave 700 mg. of prismatic needles of 6-nitrocholesteryl acetate, m.p. 101–102°, undepressed by admixture with an authentic sample. A second crop from the mother liquor raised the total yield to 830 mg. (95%). When the 6-nitro-5-nitrate was treated in ether with nitric acid at 0° for 24 hr. no reaction occurred and the starting material was recovered unchanged.

6β-Nitrocholestane-3α,5α-diol 3-Acetate 5-Nitrate.—A solution of 2 g. of epicholesteryl acetate in 50 cc. of ether was saturated with 2:1 dinitrogen tetroxide–oxygen and the mixture worked up as described above. Treatment of the semi-solid reaction product with methanol afforded 1.85 g. (74%) of crude material, m.p. 122–125°. Two recrystallizations from methanol–water raised the m.p. to 133.5–134.5°, α_D −29° Chf, λ^{Chf} 5.78, 6.08, 6.40, 7.75, 7.98 μ.

Anal. Calcd. for $C_{29}H_{48}O_7N_2$ (536.69): C, 64.90; H, 9.02. Found: C, 65.02; H, 9.15.

6-Nitroepicholesteryl Acetate.—On nitration of 4.5 g. of epicholesteryl acetate in ether by the procedure described for nitration of cholesteryl acetate and crystallization of the product from methanol–ether, 3 g. of starting material was recovered (m.p. 84–85°, identified by mixed m.p. determined). A brown oil recovered from the mother liquor on treatment with ammonia gas in a chilled ethereal solution gave a precipitate of ammonium nitrate (m.p. 167–170°), and evaporation of the filtered ethereal solution gave an oil that on crystallization from methanol afforded 0.8 g. of crystals of the 6-nitro-3-acetate, m.p. 95–98°. Three recrystallizations from methanol gave material of m.p. 109–110°, α_D −51° Chf, λ^{EtOH} 257 mμ (1,170), λ^{Chf} 5.79, 6.59, 8.00 μ.

Anal. Calcd. for $C_{29}H_{47}O_4N$ (473.67): C, 73.53; H, 10.00. Found: C, 73.46; H, 10.23.

6-Nitroepicholesteryl acetate was obtained more readily by passing ammonia gas into a chilled ethereal solution of 1.7 g. of 6β-nitrocholestane-3α,5α-diol 3-acetate 5-nitrate. Initial crystallization from methanol afforded 1.1 g. (80%) of crystals, m.p. 106–109°; after two recrystallizations, m.p. and mixed m.p. 109–110°.

Cholestane-3α-ol-6-one acetate[7] was obtained by adding 3.6 g. of zinc dust in portions over a period of 3 hr. to a refluxing solution of 900 mg. of 6-nitroepicholesteryl acetate (m.p. 106–109°) in 40 cc. of acetic acid and 5 cc. of water. The solution was cooled, filtered, diluted, and the product extracted with ether. A solution of the oily residue in methanol was treated with a few drops of hydrochloric acid and refluxed for 15 min., then diluted with a little water, and let stand, when 260 mg. of crystals separated; without the treatment with acid, no crystalline product could be secured, even on chromatography. Two crystallizations from methanol gave material m.p. 106–107°, α_D −6° Chf, λ^{Chf} 5.78, 5.84, 7.96 μ (Dodson and Riegel[7]: m.p. 107–108°, α_D −3.7° Chf).

Anal. Calcd. for $C_{29}H_{28}O_3$ (444.67): C, 78.32; H, 10.88. Found: C, 78.18; H, 10.89.

6-Nitrostigmasteryl Acetate (IV).—Crude stigmasteryl acetate (m.p. 134–139°) kindly supplied by Dr. Percy L. Julian of Glidden Co. was saponified in 1:1 ethanol–benzene and the free sterol refluxed with 30–35° petroleum ether for 30 min. and the undissolved portion collected and converted to the acetate, m.p. 140.5–142°. A stirred suspension of 5 g. of acetate in 100 cc. of ether was treated at 0° with 45 cc. of fuming nitric acid, added in 30 min. Further processing as described for the nitration of cholesteryl acetate afforded, after recrystallization from 95% ethanol, 2.8 g. of fine needles, m.p. 124–125°. This apparently homogeneous product proved to contain starting material and the 6-nitro derivative. On chromatography (acid-washed alumina) of 0.6 g. of the product, petroleum ether eluted a total of 200 mg. of stigmasteryl acetate (after crystallization, m.p. and mixed m.p. 140.5–142°). Petroleum ether–ether then eluted three fractions, m.p. 132–133°, that when combined and crystallized twice from ethanol gave 180 mg. of fine needles of the 6-nitro compound, m.p. 133–134°, α_D −98° Chf, λ^{EtOH} 258 mμ (1,930), λ^{Chf} 5.78, 6.59, 7.95 μ.

Anal. Calcd. for $C_{31}H_{49}O_4N$ (499.71): C, 74.51; H, 9.88. Found: C, 74.64; H, 10.03.

Δ^{22}-Stigmastene-3β-ol-6-one Acetate.—Reduction of 1 g. of the above 6-nitro compound with zinc and acetic acid and crystallization of the product twice from methanol–ether gave 0.78 g. of 6-ketone as fine needles, m.p. 145–146°, α_D −31.2° Chf, λ^{Chf} 5.79, 5.83, 7.98 μ; λ^{CS_2} 5.80, 5.83, 8.06, 10.30 μ.

Anal. Calcd. for $C_{31}H_{50}O_3$ (470.71): C, 79.10; H, 10.71. Found: C, 79.18; H, 10.87.

6-Nitro-3β,16β-dihydroxy-Δ^5-bisnorcholenic-22 → 16-lactone 3-Acetate (VIII).—A solution of 7.5 g. of diosgenin acetate (m.p. 191–192°, kindly supplied by Dr. F. Giral) in 50 cc. of chloroform was diluted with 150 cc. of ether and the solution was stirred in a salt–ice-bath during the dropwise addition in the course of 1 hr. of 100 cc. of fuming nitric acid. After an additional hour of stirring in the ice-bath,

the solution was washed with three 200-cc. portions of 5% potassium hydroxide and then with successive portions of sodium chloride solution until neutral, dried and evaporated. Addition of methanol to the residue gave a granular solid that on recrystallization from ethanol–acetone afforded 1.7 g. of nitrolactone, m.p. 250–251° dec., α_D −146° Chf, λ^{EtOH} 258 mμ (1,850), λ^{Chf} 5.68, 5.79, 6.59, 8.00 μ.

Anal. Calcd. for $C_{24}H_{33}O_6N$ (431.51): C, 66.80; H, 7.71; N, 3.25. Found: C, 66.69; H, 7.81; N, 3.78.

6-Keto-3β,16β-dihydroxybisnorallocholanic-22 → 16-lactone 3-Acetate.—Reduction of 700 mg. of the above nitrolactone with zinc and acetic acid was conducted as described previously and the washed and dried ethereal solution of the reaction product on concentration afforded in three crops a total of 460 mg. (61%) of long, fine needles, m.p. 239–242 dec. Two recrystallizations from methanol gave material of m.p. 242–243° dec., α_D −81° Chf, λ^{Chf} 5.67, 5.79, 5.82, 7.98 μ.

Anal. Calcd. for $C_{24}H_{34}O_5$ (402.51): C, 71.61; H, 8.51. Found: C, 71.43; H, 8.61.

3β,16β-Dihydroxybisnorallocholanic-22 → 16-lactone 3-Acetate.—A solution of 1.4 g. of tigogenin acetate (m.p. 193–195°, prepared by hydrogenation of diosgenin acetate in acetic acid over Adams catalyst) in 14 cc. of chloroform and 56 cc. of ether was treated at 0° with 25 cc. of fuming nitric acid, added during one-half hr. Further processing as in the above nitrations and crystallization of the product from 95% ethanol gave 0.5 g. of small white needles, m.p. 200–212°. On digestion of this material with ether and crystallization of the undissolved residue twice from methanol–water, colorless needles separated, m.p. 215–216°, α_D −50° Chf, λ^{Chf} 5.68, 5.79, 7.99 μ. Tschesche and Hagedorn[13] report for tigogenin lactone the constants m.p. 219°, α_D −49.5° Chf.

Nitration of Δ⁷-Cholestenyl Acetate.—Nitric acid nitration of 4 g. of acetate gave a brown oil that on chromatography afforded first a series of fractions eluted by 9:1 and 7:3 petroleum ether–benzene. The major component appeared to be a nitronitrate, but rechromatography and attempted crystallization yielded only a non-crystalline flaky solid, m.p. 75–77°, α_D −101° Chf, λ^{Chf} 5.79, 6.10, 6.34, 7.95 μ. Petroleum ether–benzene (1:3) and benzene eluted five fractions of solid that on crystallization from ethanol and a few drops of ether gave 250 mg. of crystals, m.p. 147–157°. Repeated crystallizations from ethanol gave plates of what appears to be **7,8-dinitrocholestanyl acetate**, m.p. 180–181°, α_D +114°, λ^{Chf} 5.79, 6.45, 7.95 μ.

Anal. Calcd. for $C_{29}H_{48}O_6N_2$ (520.69): C, 66.89; H, 9.29; N, 5.38. Found: C, 67.17; H, 9.10; N, 5.42.

A solution of 150 mg. of the dinitro-acetate in 5 cc. of 2% alcoholic potassium hydroxide was let stand overnight at 25°, carefully neutralized with dilute hydrochloric acid, diluted, and extracted with ether. The oily product, which could not be obtained crystalline, appeared from the constants: λ^{EtOH} 257 mμ, λ^{Chf} 6.60 μ, to contain a nitroölefin. It was reduced with zinc and acetic acid and the oily product chromatographed on 6 g. of alumina. Benzene–ether (4:1) eluted an oil that solidified on addition of methanol. Recrystallization from methanol–chloroform gave 18 mg. of plates of **cholestane-3β-ol-7-one**, m.p. 165–168°, α_D −34° Chf, λ^{Chf} 5.83 μ; no depression in mixed m.p.

Nitration of Methyl 3α-Acetoxy-Δ⁹⁽¹¹⁾cholenate.—Nitration in ether led to recovery of 65% of the starting material. Nitration of 600 mg. of ester with fuming nitric acid alone at 0° gave 440 mg. of a yellow powder that could not be crystallized or purified by chromatography. Reduction of this material with zinc and acetic acid, chromatography, and crystallization from methanol of a solid fraction eluted by 9:1 petroleum ether–ether gave 13 mg. of **methyl 3α-acetoxy-11-ketocholanate**, m.p. 130–132°, α_D +67° An, λ^{Chf} 5.80, 5.84, 7.97 μ, undepressed in m.p. on admixture with an authentic sample.

Nitration of 3 g. of the Δ⁹⁽¹¹⁾-cholenate with dinitrogen tetroxide in ether and chromatography of the reaction mixture afforded 480 mg. of unreacted starting material (3:7 benzene–ether) and, in four fractions eluted by 1:1 benzene–ether and after crystallization from ether, 760 mg. of material of the probable structure **methyl 3α,11-dihydroxy-9-nitrocholanate 3-acetate-11-nitrate**, m.p. 187–188°, recrystallized, m.p. 187.5–188.5°, α_D +66° Chf, λ^{Chl} 5.79, 6.08, 6.42, 7.80, 7.98 μ; no ultraviolet absorption.

Anal. Calcd. for $C_{27}H_{42}O_9N_2$ (538.62): C, 60.20; H, 7.87; N, 5.20. Found: C, 60.38; H, 8.09; N, 5.38.

A solution of 35 mg. of this substance in 10 cc. of ether was saturated with ammonia at 0°, let stand, concentrated to a small volume and cooled. The product that separated (28 mg.) melted at 186–188° and was identified as starting material by mixed m.p. determination.

Nitration of Methyl 3α-Acetoxy-Δ⁷,⁹⁽¹¹⁾-choladienate.—The reaction of 4 g. of this ester with fuming nitric acid in ether by the above procedure gave an ethereal solution of reaction mixture that when concentrated deposited 1 g. of starting material, m.p. 142–146°. The oily material from the mother liquor was chromatographed and the fractions crystallized from methanol. Early fractions of the melting range 132–154° on further crystallization afforded 25 mg. of an unidentified substance (yellowish needles), m.p. 150–151°, λ^{EtOH} 234 mμ, λ^{Chf} 5.78, 6.57, 7.96 μ. Then four fractions eluted by benzene and 99:1 benzene–ether gave, after two recrystallizations from aqueous methanol, 0.8 g. of colorless needles of a substance of the probable structure **methyl 3α-acetoxy-7,11-dinitro-Δ⁸-cholenate**, m.p. 161.5–163.5°, α_D −2° Chf, λ^{Chf} 5.78, 6.42, 7.96 μ; no ultraviolet absorption. Two later fractions gave 0.6 g. more product, m.p. 155–160°.

Anal. Calcd. for $C_{27}H_{40}O_8N_2$ (520.61): C, 62.29; H, 7.74. Found: C, 62.64, 62.54, H, 7.78, 7.89.

The dinitro compound failed to eliminate nitrous acid on treatment with ammonia in ether or with dilute alcoholic potassium hydroxide and it resisted attempted bromination with N-bromosuccinimide.

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