in the above procedure. The glycol amounted to 9.64 g. (67%); b.p. 120–121° (10 mm.), $n^{25}D$ 1.4843, d^{25}_4 1.0422.

Anal. Calcd. for $C_8H_{16}O_2$: C, 66.62; H, 11.18; M_D, 40.00. Found: C, 66.63; H, 11.09; M_D, 39.61.

A **mono-3,5-dinitrobenzoate** was prepared in a manner similar to that of I, above. This substance was recrystallized from 95% ethanol to give fine, white needles, m.p. 122.5–123.5°.

Anal. Calcd. for $C_{13}H_{18}N_2O_7$: C, 53.25; H, 5.36; N, 8.28. Found: C, 53.46; H, 5.33; N, 8.35.

Oxidation of Glycols with Lead Tetraacetate.—A sample of 0.52 g. (0.004 mole) of III dissolved in 20 ml. of glacial acetic acid was dropped upon dry lead tetraacetate in an apparatus so arranged that a slow stream of dry air could be passed over the mixture to sweep out acetaldehyde[19] (anticipated as one of the products) into a receiver containing 300 ml. of 2 *N* hydrochloric acid saturated with 2,4-dinitrophenylhydrazine. The removal of acetaldehyde was completed by heating the mixture at 60° for one-half hour, while the flow of dry air was continued through the apparatus.

The crude acetaldehyde 2,4-dinitrophenylhydrazone was filtered off and air-dried; 0.53 g. (68%), m.p. 146–149°.[20] When admixed with an authentic sample of acetaldehyde 2,4-dinitrophenylhydrazone, the melting point showed no depression, m.p. 146–150°. After one recrystallization, from dilute ethanol, the product amounted to 0.45 g. and melted at 153.5–154°.

The mixture left in the reaction flask, after the oxidation had been completed, was distilled to dryness under reduced pressure. The distillate was added to 200 ml. of 2 *N* hydrochloric acid solution saturated with 2,4-dinitrophenylhydrazine. The crude cyclopentanone 2,4-dinitrophenylhydrazone which formed was removed by filtration and air-dried and amounted to 0.76 g. (72%), m.p. 142.5–145°.[17] When admixed with the 2,4-dinitrophenylhydrazone prepared from an authentic sample of cyclopentanone, the melting point showed no depression, m.p. 143–146°. After one recrystallization from dilute ethanol the product amounted to 0.59 g., m.p. 144.5–145.5°.

The glycol of the cyclohexane series (VII) was treated with lead tetraacetate by identically the same procedure. Crude acetaldehyde 2,4-dinitrophenylhydrazone was obtained in a 70% yield and cyclohexanone 2,4-dinitrophenylhydrazone in a 74% yield.

Reaction of 1-Acetylcyclopentanol (II) with Sodium Hypobromite.—To a solution of 8.4 g. of sodium hydroxide in 70 ml. of water at 0° was added 12.0 g. of bromine. To this solution at 0° was slowly added 2.62 g. (0.020 mole) of II. The mixture was stirred in an ice-bath for 30 minutes and then at room temperature for three hours. The bromoform was separated by means of a separatory funnel and the aqueous layer carefully acidified with 10 ml. of concentrated sulfuric acid. The solution was extracted with several portions of ether; the combined extracts were then washed with several portions of saturated sodium bisulfide solution and dried over anhydrous magnesium sulfate. The solvent was removed and the residue recrystallized from toluene–petroleum ether (b.p. 35–50°) to give 1.15 g. (43%) of 1-hydroxycyclopentanecarboxylic acid (V), m.p. 102–103.5°.[21] On admixture with an authentic specimen prepared from the cyanohydrin of cyclopentanone, no depression was observed, m.p. 104–105°.

Acknowledgment.—This investigation was supported in part by a research grant from the National Institute of Arthritis and Metabolic Diseases, of the National Institutes of Health, Public Health Service, and in part by the State College of Washington Research Fund.

We are indebted to Dr. Edward L. Wagner and Mr. David E. Little (deceased) for determination of the infrared adsorption spectra.

(19) The portion of the procedure dealing with the determination of acetaldehyde is adapted from that of R. C. Hockett, *et al.*, THIS JOURNAL, **68**, 922 (1946).

(20) One of the melting points reported for acetaldehyde 2,4-dinitrophenylhydrazone is 148°; another form is reported to melt at 157°—W. M. D. Bryant, *ibid.*, **60**, 2814 (1938).

(21) O. Wallach, *Ann.*, **414**, 296 (311) (1918), reported m.p. 103–104°.

PULLMAN, WASHINGTON

[CONTRIBUTION FROM THE EASTERN REGIONAL RESEARCH LABORATORY[1]]

Steroidal Sapogenins. XI.[2] Steroidal C-Ring Lactones

BY EDWARD S. ROTHMAN, MONROE E. WALL AND C. ROLAND EDDY

RECEIVED SEPTEMBER 19, 1953

Contrary to reports by other workers we have found that acid-catalyzed perbenzoic acid and peracetic acid oxidation of steroidal C_{12}-ketosapogenins results in oxidative attack on the C-ring with ε-lactone formation. The introduction of oxygen led to only one of the two possible isomeric lactones, *viz.*, the lactone of the 12,13-secospirostane series. The sapogenin side chain was unaffected by the reagent. The bile acid, methyl 3α-carbethoxyoxy-12-ketocholanate, gave similar results, but C_{11}-ketosteroids resisted attack.

Oxidation of steroid carbonyl groups at C_3, C_7, C_{17} and C_{20} by peracids has been reported by various workers.[3–7] On the other hand, the less reactive carbonyl groups at C_{11} and C_{12} are described as inert[8] to this type of reagent. We have found conditions for reaction not only of the C_{12} keto group of methyl 3α-carbethoxyoxy-12-ketocholanate, but also for reaction of the more hindered C_{12} keto group of the sapogenin, hecogenin acetate (5α,22α-spirostan-3β-ol-12-one 3-acetate). Under non-anhydrous reaction conditions using perbenzoic acid or peracetic acid in the presence of a catalytic amount of sulfuric acid and a reaction period of several days, seven-membered-C-ring lactones were obtained in high yield. In this manner the oxidation of hecogenin acetate led to the formation of a single lactonic product

(1) One of the laboratories of the Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, United States Department of Agriculture. This work was done as part of a coöperative arrangement between the Bureau of Plant Industry, Soils, and Agricultural Engineering and the Bureau of Agricultural and Industrial Chemistry (United States Department of Agriculture), and the National Institutes of Health (Department of Health, Education and Welfare). Article not copyrighted.

(2) Paper X, E. S. Rothman, M. E. Wall and C. R. Eddy, THIS JOURNAL, **75**, 6325 (1953).

(3) L. Ruzicka, V. Prelog, *et al.*, *Helv. Chim. Acta*, **28**, 618, 1651 (1945).

(4) H. Heymann and L. F. Fieser, *ibid.*, **35**, 631 (1952).

(5) R. P. Jacobsen, *et al.*, *J. Biol. Chem.*, **171**, 61, 71, 81 (1947).

(6) H. Heusser, A. Segre and P. A. Plattner, *Helv. Chim. Acta*, **31**, 1183 (1948).

(7) T. F. Gallagher and T. H. Kritchevsky, THIS JOURNAL, **72**, 882 (1950); R. B. Turner, *ibid.*, **72**, 878 (1950).

(8) V. Burckhardt and T. Reichstein, *Helv. Chim. Acta*, **25**, 821, 1434 (1942); *cf.* L. F. Fieser and M. Fieser, "Natural Products Related to Phenanthrene," Reinhold Publ. Corp., New York, N. Y., 1949, pp. 237, 402.

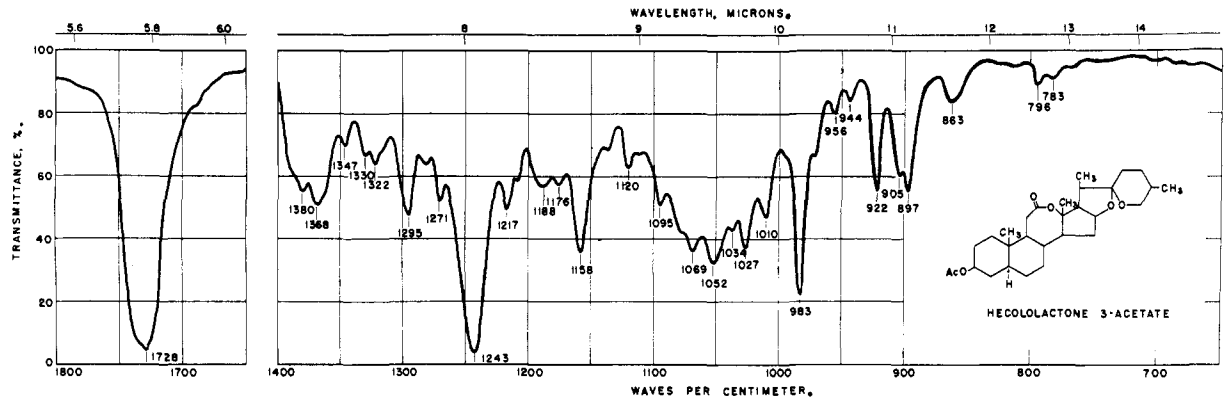
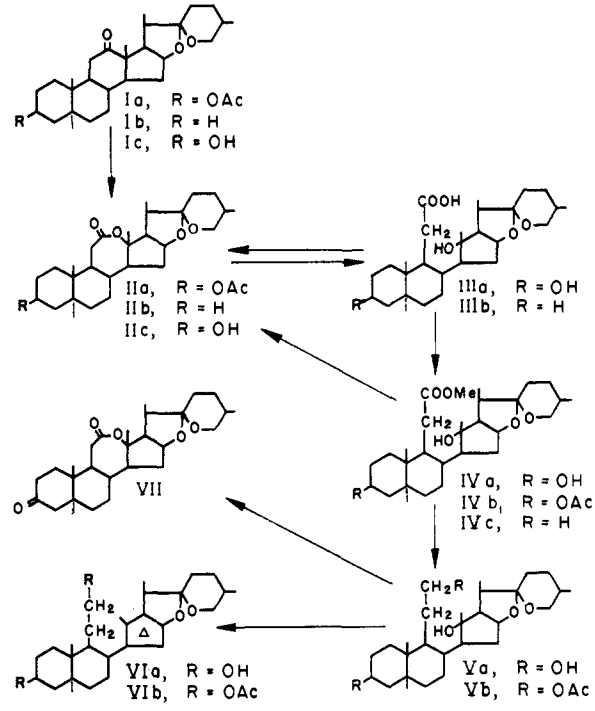


Fig. 1.—Infrared absorption spectrum of hecololactone 3-acetate (IIa) (CS_2 solution, 6.7 g./l.; 1.0 mm. cell).

unaltered in the spiroketal side chain. We formulate this product as IIa and assign it the trivial name of hecololactone 3-acetate. The systematic name[9] is 3β,13ξ-dihydroxy-12,13-seco-5α,22a-spirostan-12-oic-acid 12,13-lactone 3-acetate. Under the same conditions of reaction C_{11}-ketosteroids failed to react in 30 days.

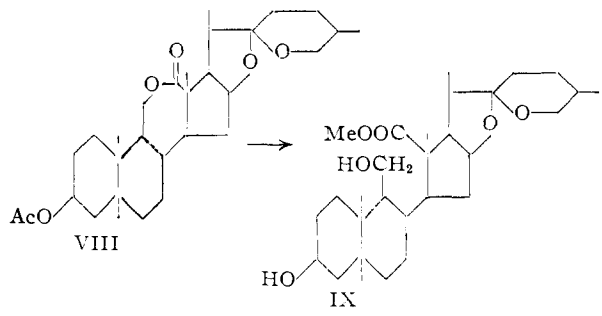


Hecololactone 3-acetate was best prepared by reaction of hecogenin acetate, Ia, with perbenzoic acid in chloroform solution containing 0.3 volume percentage of sulfuric acid. The reaction proceeded smoothly at room temperature giving 80% yields in 60 hours and 98% yields in 12 days. Hecololactone 3-acetate also could be prepared in lower yields using a mixture of 30% aqueous commercial hydrogen peroxide in acetic acid medium. Use of pre-formed commercial peracetic acid in acetic acid gave poor yields. Jacobsen[5] reported similar difficulties with this reagent in oxidizing C_{17}-ketones.

(9) Nomenclature recommended by International Union of Pure and Applied Chemistry, Division of Chemistry and Chemical Technology, National Research Council.

Proof of Structure of Hecololactone 3-Acetate

The sharp melting points, failure to obtain two sets of derivatives, and behavior on chromatography indicate that the oxidation product IIa is a single compound rather than a mixture. Saponification of IIa gave hecolic acid, IIIa (3β,13ξ-dihydroxy-12,13-seco-5α,22a-spirostan-12-oic acid). All attempts to acetylate hecolic acid resulted in ring closure and formation of hecololactone 3-acetate. When hecolic acid was heated *in vacuo* hecololactone, IIc, (3β,13ξ-dihydroxy-12,13 seco-5α,22a-spirostan-12-oic acid 12,13-lactone) was formed. Esterification of hecolic acid with diazomethane gave methyl hecolate (IVa) (methyl 3β,-13ξ-dihydroxy-12,13-seco-5α,22α-spirostan-12-carboxylate). Acetylation by the analytical method of Ogg, Porter and Willits[10] showed that only one of the two hydroxyl groups present was acetylatable. The product, methyl hecolate 3-acetate (IVb) (methyl 3β,13ξ-dihydroxy-12,13-seco-5α,22a-spirostan-12-carboxylate 3-acetate) showed infrared absorption bands in the 3500 cm.$^{-1}$ region characteristic of hydroxyl groups and a band at 1245 cm.$^{-1}$ of strength corresponding to only one acetate group per molecule. An alternative formulation of hecololactone 3-acetate as VIII must be rejected since saponification and treatment with diazomethane to form IX leaves no explanation for (1) failure to acetylate completely, (2) ease of dehydration (see below).



Lithium aluminum hydride reduced methyl hecolate in high yield to triol Va, hecolyl alcohol,

(10) C. L. Ogg, W. L. Porter and C. O. Willits, *Anal. Ed., Ind. and Eng. Chem.*, **17**, 394 (1945).

(12,13-seco-5α-22a-spirostan-3β,12,13ξ-triol) which had one non-acetylatable hydroxyl group. This compound formed, on acetylation the hydroxy diacetate Vb (12,13-seco-5α,22a-spirostan-3β,12,13ξ-triol 3,12-diacetate). Final proof of the tertiary nature of the C-13 hydroxyl group of hecolyl alcohol was its facile dehydration by traces of mineral acid or by heat to a dihydroxy olefin, VIa, anhydrohecolyl alcohol (12,13-seco-5α-22a-spirost-ξ-ene-3β,12-diol).

Figure 2 shows a portion of the infrared spectra of Va (dotted line) with VIa (solid line). In the conversion of Va to VIa there is a marked loss of hydrogen-bonded hydroxyl groups (absorbing near 3400 cm.$^{-1}$), with only a small change in non-bonded hydroxyl groups (absorbing near 3600 cm.$^{-1}$). This suggests that the C_{13}-hydroxyl of VIa is hindered from intermolecular hydrogen bonding and that in Va, this hydroxyl forms primarily intramolecular hydrogen bonds with the C_{12}-hydroxyl. The diacetate VIb was free of hydroxyl groups.

The double bond in VI has not been definitely located, but the following evidence indicates its presence in the D-ring. The olefin gave a negative Tortelli–Jaffe test and a yellow color with tetranitromethane. The olefinic bond resisted catalytic hydrogenation (Adams catalyst in acetic acid, atmospheric pressure) and resisted oxidation by potassium permanganate both in pyridine and in aqueous potassium hydroxide–dioxane media. Addition of one mole of bromine occurred, however, under non-substitution conditions,[11] and about one atom of oxygen was added by epoxidation with perbenzoic acid to form a product giving no color reaction with tetranitromethane. Unless inversion or racemization at C_{13} occurred in the oxidation step, the rule of *trans* (ionic) elimination, if valid in this system, would locate the bond between C_{13} and C_{18}; however, the lack of reactivity of the double bond is behavior typical of hindered ethylenic linkages.[12] This fact and the lack of terminal methylene vibrations in the infrared spectrum require the placing of the double bond at C_{13}–C_{14} or C_{13}–C_{17} in the D-ring. The authors in reference 7 have shown that perbenzoic acid oxidation of the 1-acetyl-2-methylcyclohexanes and 20-ketosteroids proceeds without configurational change.

The ease of lactonization of hecolic acid involving the internal esterification of the carboxyl group with a tertiary non-acetylatable hydroxyl group may appear unusual; however, Jacobsen[5] and Westerfeld[13] report such behavior for the methyl ester of estrolic acid wherein a C_{13}-tertiary hydroxy-C_{17} carbomethoxy compound lactonized in several attempts to remove the tertiary hydroxyl group by dehydration. We obtained lactone formation on attempts to acetylate both hecolic acid and its methyl ester. Oxidation of hecolyl alcohol under

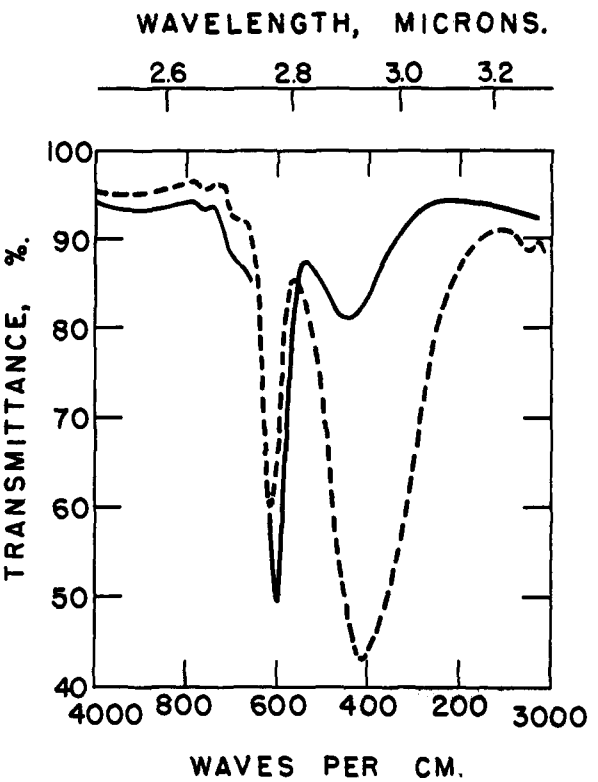


Fig. 2.—Infrared absorption spectra of: (1) anhydrohecolyl alcohol (VIa), (solid line) chloroform solution, 19.2 g./kg. (*ca.* 28 g./l.), 0.5 mm. cell; (2) hecolyl alcohol (dotted line) chloroform solution, 25 g./l., 0.5 mm. cell.

mild conditions using chromium trioxide in pyridine[14,15] also led to lactone formation.[16]

Evidence for unchanged E and F rings in hecololactone and its derivatives is found in the infrared spectra. Open C-ring derivatives showed the usual spiroketal bands[17,18] with the 900 cm.$^{-1}$ band much stronger than the 920 cm.$^{-1}$ band, indicating an "iso" configuration. Lactonized C-ring derivatives showed these two bands nearly equal in strength, with the 900 band split into two components. See Fig. 1 as an example. This is not due to partial isomerization, since opening of the lactone ring by saponification gave a product with the usual "iso" type spectral bands, while the bands of the re-lactonized product were identical with those of the original lactone.

Rough estimates of the areas of these bands showed that, although the areas of the 900 and 920 bands of IIa were less and greater, respectively, than the corresponding bands of hecogenin acetate,[18] the sum of the areas of the two bands was roughly the same for both compounds. This suggests that the altered 900 and 920 bands of the

(11) The brominated product contained no bromine in the side chain since it showed the usual spiroketal infrared absorption bands.[17-18] 23-Bromohecogenin and 23,23-dibromosarsasapogenin showed marked perturbation of these bands (unpublished observations, this Laboratory.)

(12) See *e.g.*, R. B. Turner in "Natural Products Related to Phenanthrene," L. F. Fieser and M. Fieser, Reinhold Publ. Corp., New York, N. Y., 1949, p. 624.

(13) W. W. Westerfeld, *J. Biol. Chem.*, **143**, 177 (1942).

(14) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, THIS JOURNAL, **75**, 422 (1953); H. H. Sisler, J. D. Bush and O. E. Accountius, *ibid.*, **70**, 3827 (1948).

(15) Under these conditions we found that the 11α-hydroxy group of 11α-hydroxyprogesterone was nearly quantitatively converted to 11-keto-progesterone.

(16) The reaction possibly proceeded *via* the intermediate steps of aldehyde and cyclic hemiacetal formation.

(17) M. E. Wall, C. R. Eddy, M. L. McClennan and M. A. Klumpp, *Anal. Chem.*, **24**, 1337 (1952).

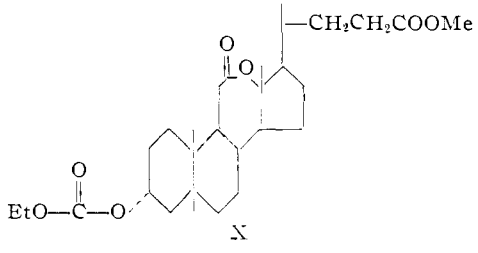
(18) C. R. Eddy, M. E. Wall and M. K. Scott, *ibid.*, **25**, 266 (1953).

C-lactones are due to the same intrinsic vibration as in ordinary sapogenins, rather than due to superposition of new bands from other centers in the molecule. Solvent environment also affects the relative intensities of these bands, non-polar solvents giving curves similar to Fig. 1 whereas polar solvents give curves midway between Fig. 1 and those of ordinary "iso" sapogenins.

From these facts, we conclude that the sapogenin lactone derivatives contain the same E and F ring structure as the natural "iso" sapogenins and that the alteration of the 900 and 920 bands is due to internal and external perturbation. Possibly the seven-membered ring distorts the spiroketal skeleton enough to add some new constraints to the vibrational modes of the spiroketal system. A similar splitting of the 900 band into two components is also observed with pennogenin 3-acetate,[19,20] in which the EF ring vibrations are perturbed by a hydroxyl at C_{17}.

Characterization of the Perbenzoic Acid Oxidation Product of Methyl 3α-Carbethoxyoxy-12-ketocholanate

We have found that the 12-keto group of a typical bile acid is also oxidizable to a lactone. Although we have not fully characterized derivatives of the lactone obtained from methyl 3α-carbethoxyoxy-12-ketocholanate we have evidence that the lactone has the same 12,13-secosteroid structure described above for the hecogenin derivatives. Thus saponification to an acid, esterification with diazomethane, and lithium aluminum hydride reduction of the resulting ester gave a polyhydroxy product which upon acetylation gave a compound whose infrared spectrum showed the presence of one unacetylated, *i.e.*, probably tertiary, hydroxyl group. On this basis the structure X, methyl 3-α-carbethoxyoxy-13ξ-hydroxy-12-carboxy-12,13-secocholanate 12,13-lactone, is tentatively assigned to the oxidation product of the bile acid.



X

We plan further experiments involving the degradation of the sapogenin C-ring lactones to analogs of progesterone and cortisone. Such C-ring oxygenated products might show interesting physiological activity.

Experimental

Hecololactone 3-Acetate, IIa (3β,13ξ-Dihydroxy-12,13-seco-5α,22a-spirostan-12-oic-acid 12,13-Lactone 3-Acetate).—Hecogenin acetate (Ia), $[\alpha]^{25}D$ −0.75,[21] m.p. 236–240°, 5 g. was placed in a 125-ml. erlenmeyer flask surrounded with a melting-ice bath, and was covered with 58 ml. of a chloroform solution of perbenzoic acid (52 mg./ml.) prepared as described in reference 22 and often turbid with emulsified water. Two ml. of 10% sulfuric acid in acetic acid was added, the temperature allowed to rise to 25°, and the flask kept in the dark for the desired period. The progress of the oxidation was followed by removal of aliquots, which were washed with aq. sodium hydroxide and water, evaporated to dryness, and examined in the infrared for disappearance of the ketonic band at 1712 cm.$^{-1}$ and increase in the absorption near 1730 cm.$^{-1}$. The exact position of the latter band shifts with relative amounts of 3-acetate and lactone. The main reaction mixture was washed with water and dilute sodium hydroxide containing a little methanol to break emulsions. Evaporation gave a white crystalline residue of low solubility in methanol so that the product could be obtained in high purity from a single crystallization from that solvent. Purification of large scale preparations was effected by solution in hot chloroform, dilution with methanol and distillation of the chloroform. Alternatively purification could be effected by saponification to the water-soluble potassium salt of hecolic acid, *vide infra*, washing the aqueous alkaline solution with benzene to free of unreacted hecogenin, precipitation of hecolic acid with hydrochloric acid and acetylation to the lactone 3-acetate. The yield was 80% when the time allotted for the oxidation was 60 hours, and was 98% in 12 days. The product, rhombic tablets from methanol or acetone, melted at 292.0 −292.5° after transition to regular hexagonal plates, $[\alpha]^{25}D$ −65.1°.

Anal. Calcd. for $C_{29}H_{44}O_6$: C, 71.21; H, 9.21. Found: C, 71.28; H, 9.08.

Its infrared spectrum is shown in Fig. 1. The ratio of the area of the split 900 cm.$^{-1}$ band to that of the 920 cm.$^{-1}$ band is roughly 1.3 (on a scale of absorptivity *vs.* wave number), whereas the corresponding ratio for the spectrum of hecogenin acetate[21] is roughly 2.5. The sum of the areas of the 900 and 920 cm.$^{-1}$ bands is roughly the same in the two compounds. When IIa was dissolved in carbon disulfide, carbon tetrachloride or benzene, the appearance of these two bands was similar to Fig. 1. When chloroform, bromoform or pyridine were used as solvents, or when the solid sample was mulled in mineral oil, the 900 cm.$^{-1}$ band was a little stronger than the 920 cm.$^{-1}$ band, and the second component of the 900 cm.$^{-1}$ band was not as well resolved. The effect of solvent environment on other lactones of this series was similar. The ester band at 1728 cm.$^{-1}$ in Fig. 1 includes both lactone and 3-acetate carbonyls. On the other hand the band at 1243 cm.$^{-1}$ has only an intensity comparable to that of the 3-acetate group of simple sapogenin monoacetates.[21]

Hecololactone 3-Acetate by Hydrogen Peroxide–Acetic Acid Oxidation.—Five grams of hecogenin acetate in 75 ml. of alcohol-free chloroform was dissolved in 30 ml. of glacial acetic acid, 3 ml. of 30% hydrogen peroxide (aqueous) and 2 ml. of 10% sulfuric acid in acetic acid (chilling was not necessary). After 12 days the product was largely diluted with water, was boiled to expel chloroform, and the aqueous layer was decanted from the resulting crystalline crust. The crust was triturated with methanol to give 4.5 g. of hecololactone 3-acetate, m.p. 287–289°, m.p. on recrystallization from acetone 292°. Use of preformed commercial peracetic acid gave a low yield of crude product.

Hecolic Acid (IIIa) (3-β,13ξ-Dihydroxy-12,13-seco-5α,-22a-spirostan-12-oic Acid).—Saponification of hecololactone 3-acetate by refluxing in aqueous methanolic potassium hydroxide for 1.5 hours gave the potassium salt of hecolic acid. The solution was diluted with water, washed with benzene and acidified to precipitate hecolic acid (IIIa) which was crystallized from chloroform to give large needles with a double m.p. 187.5, 253–255°, $[\alpha]^{23.5}D$ −66.3° (dioxane).

Anal. Calcd. for $C_{27}H_{44}O_6$: C, 69.79; H, 9.55. Found: C, 69.76; H, 9.63.

Attempts to acetylate this product gave ring reclosure to hecololactone 3-acetate. The infrared spectrum of the acid

(19) Unpublished observations.

(20) "Collected Infrared Absorption Spectra of Steroid Sapogenins," by R. N. Jones, E. Katzenellenbogen and K. Dobriner, Division of Information Services, National Research Council, Ottawa, Canada, and Sloan-Kettering Institute for Cancer Research, New York, N. Y.

(21) All rotations were determined in a 1-dm. tube in chloroform solution 20 mg./2 ml. unless otherwise noted. All melting points were determined on a Kofler block and are therefore corrected. Infrared spectra were obtained with a Perkin-Elmer model 21 spectrophotometer, using a sodium chloride prism.

(22) G. Braun in "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1946, p. 431.

(solid film on NaCl plate) included strong broad carboxyl band, 2300–3700 cm.$^{-1}$, with peak at 3400 cm.$^{-1}$; strong, carboxyl carbonyl band at 1700 cm.$^{-1}$ with shoulder at 1720 cm.$^{-1}$; F-ring bands at 866 (m), 901 (s), 922 (m), 983 (s) cm.$^{-1}$, with the usual intensities corresponding to the "iso" configuration.

Hecololactone (IIc) (3β,13ξ-Dihydroxy-12,13-seco-5α,-22a-spirostan-12-oic Acid 12,13-Lactone).—A sample of hecolic acid was heated for 2 hours at 200° (0.15 mm.), to give a neutral product, m.p. 256–258°, $[\alpha]^{25}D$ −52.0°.

Anal. Calcd. for $C_{27}H_{42}O_5$: C, 72.61; H, 9.48. Found: C, 72.49; H, 9.79.

Infrared spectum ($CHCl_3$ solution) included: hydroxyl bands at 3650 cm.$^{-1}$ (unbonded) and 3450 cm.$^{-1}$ (broad, hydrogen bonded) with the 3650 band much stronger than the 3450 band; lactone carbonyl band at 1705 cm.$^{-1}$; F-ring bands at 868 (m), 898 (m), 906 (m), 923 (m), 984 (s) cm.$^{-1}$. The 898–906 cm.$^{-1}$ band combination is a little stronger than the 923 cm.$^{-1}$ band, but the relative intensities are much closer to those of Fig. 1 than to those usually found with "iso" F-ring bands.

Methyl Hecolate (IVa).—Hecolic acid (IIIa), 6.8 g., was dissolved in 75 ml. of tetrahydrofuran (freshly filtered through activated aluminum to remove peroxides and water). The solution was cooled to 0°, a 1.5 *M* excess of diazomethane in 150 ml. of ether was added and allowed to react for 3 hours during which time the temperature was allowed to rise slowly. A little dilute acetic acid was added to decompose the excess reagent, the solution was diluted with water, extracted with ether, washed with water and the solvents were evaporated on the steam-bath. The glassy residue was crystallized by solution in a minimum of methanol, dilution with cyclohexane and removal of the methanol by distillation of the azeotrope. The product, 6.4 g., melted at 164–165°. Occasionally a metastable form melted about 79°, resolidified on further heating, and remelted at 164–165°, $[\alpha]^{23.5}D$ −63.6°.

Anal. Calcd. for $C_{28}H_{46}O_6$: C, 70.26; H, 9.68. Found: C, 70.26; H, 10.14.

Infrared spectrum ($CHCl_3$ solution) included: hydroxyl bands at 3620 cm.$^{-1}$ (unbonded) and 3460 cm.$^{-1}$ (broad, hydrogen bonded) of strength corresponding approximately to two hydroxyls per molecule; ester band at 1725 cm.$^{-1}$; F-ring bands at 866 (m), 900 (s), 922 (m), 982 (s) cm.$^{-1}$ corresponding to the usual "iso" F-ring intensities.

Only one of the two hydroxyl groups was acetylatable. The monohydroxy monoacetate, IVb, was obtained as long silky needles, m.p. 99–101°, from pentane, only after slow crystallization, $[\alpha]^{25}D$ −62.7°.

Anal. Calcd. for $C_{30}H_{48}O_7$: C, 69.20; H, 9.29. Found: C, 69.02; H, 9.33.

Infrared spectrum (CS_2 solution) included: hydroxyl bands at 3600 cm.$^{-1}$ (unbonded) and 3510 cm.$^{-1}$ (broad, with shoulder at 3450 cm.$^{-1}$, hydrogen bonded) of strength corresponding approximately to one hydroxyl per molecule; F-ring bands at 864 (m), 901 (s), 922 (m), 983 (s) cm.$^{-1}$ corresponding to the usual "iso" F-ring intensities; ester band at 1731 cm.$^{-1}$ of strength corresponding to two ester groups per molecule; acetate band at 1245 cm.$^{-1}$ of strength corresponding to one acetate group per molecule. No band of comparable strength occurs in the 1250 cm.$^{-1}$ region of the spectra of IIb, IIc, IIIa, IIIb, IVa, Va, VIa or VII showing that this band must be due to the acetate group. On the other hand, sapogenins known to have two acetate groups have much stronger bands in this region.[21]

Reduction of Methyl Hecolate to Hecolyl Alcohol (Va) (12,13-Seco-5α-22a-spirostan-3β,12,13ξ-triol).—Methyl hecolate, 2.49 g., in 50 ml. of tetrahydrofuran was slowly added to a slurry of 1 g. of $LiAlH_4$ in 50 ml. of tetrahydrofuran, and the stirred mixture was refluxed for an additional two hours. The excess reagent was decomposed with water and dissolved in dilute hydrochloric acid, and the product was extracted with ether. The ether layer was washed with water, with 3% sodium hydroxide, and with water. If the sodium hydroxide wash was omitted, traces of hydrochloric acid caused olefin formation during the evaporation of the ether–tetrahydrofuran mixture. The solvents were evaporated to give a colorless glass which formed 2.4 g. of a white solid foam on drying at 80° *in vacuo*. The foam was dissolved in chloroform, and crystalline rhombs precipitated at once. The analytical sample melted at 141° with effervescence, $[\alpha]^{26}D$ −71.4°.

Anal. Calcd. for $C_{27}H_{46}O_5$: C, 71.96; H, 10.29. Found: C, 72.35; H, 10.09.

Infrared spectrum ($CHCl_3$ solution) included: hydroxyl bands at 3610 and 3410 cm.$^{-1}$ as shown in Fig. 2, dotted curve (see text for discussion); no bands in the 1700 cm.$^{-1}$ region. F-ring bands at 868 (m), 902 (s), 923 (m), 983 (s) cm.$^{-1}$ corresponding to the usual "iso" F-ring intensities. Dehydration did not occur when the triol was acetylated since hydroxyl bands were observed in the spectrum of the diacetate Vb at 3600 cm.$^{-1}$ (unbonded) and 3510 cm.$^{-1}$ (bonded). It is interesting to note that the unbonded band of Vb was unusually weak, showing that hydrogen bonding to the acetoxyl group is favored in this molecule. The spectrum showed acetate bands at 1720 and 1258 cm.$^{-1}$ and F-ring bands at 865 (m), 899 (s), 919 (m), and 981 (s) cm.$^{-1}$.

3-Dehydrohecololactone (VII) (13ξ-Hydroxy-12,13-seco-5α,22a-spirostan-3-one-12-oic Acid 12,13-Lactone).—The triol Va, 2.5 g., was dissolved in 25 ml. of pyridine and was treated with 2.5 g. of CrO_3 dissolved in 40 ml. of cold pyridine and let stand overnight at room temperature. The dark solution was diluted with water and ether, was filtered free of reddish solids, was separated and the ether layer washed well with water, dilute hydrochloric acid, dilute sodium hydroxide and with water. Evaporation of the ether left a colorless, semi-crystalline residue which on trituration with hexane gave 2.0 g. (86% yield) of crystalline product. The analytical sample, hexagonal scales, was crystallized from acetone and from ether, m.p. 250–252°, $[\alpha]^{26}D$ −51.6°.

Anal. Calcd. for $C_{27}H_{40}O_5$: C, 72.94; H, 9.07. Found: C, 73.13; H, 9.19.

The infrared spectrum (CS_2 solution) included: a single carbonyl band at 1720 cm.$^{-1}$ of strength corresponding to two carbonyl groups per molecule (lactone and ketone); F-ring bands at 865 (m), 896 (m), 905 (m), 921 (m), 982 (s) cm.$^{-1}$ with intensity pattern resembling that of Fig. 1.

Dehydration of Hecolyl Alcohol (Va) to Anhydrohecolyl Alcohol (VIa) (12,13-Seco-5α,22a-spirost-ξ-ene-3β,12-diol.—The triol Va was heated a few degrees above its melting point under a pressure of about 20 mm. and was held at that temperature until effervescence had subsided. The product, a hard colorless glass, was crystallized from acetone to form blades, m.p. 174–176°, $[\alpha]^{25}D$ −46.1°.

Anal. Calcd. for $C_{27}H_{44}O_4$: C, 74.95; H, 10.25. Found: C, 74.63; H, 10.33.

Infrared spectrum ($CHCl_3$ solution) included: hydroxyl bands at 3600 cm.$^{-1}$ and 3450 cm.$^{-1}$ as shown in Fig. 2, solid line (see text for discussion). The strength of the bonded hydroxyl band is roughly the same as we have observed in natural sapogenins containing only one hydroxyl group at C3. F-ring bands occurred at 867 (m), 903 (s), 921 (m), 983 (s) cm.$^{-1}$. No new strong bands were found in the 900 cm.$^{-1}$ region where $R_1R_2C{=}CH_2$ should absorb strongly. This suggests that the elimination of the elements of water did not occur between C_{13} and C_{18}, but that the double bond lies in the D-ring.

Acetylation gave a diacetate, VIb, not crystallized, $[\alpha]^{24}D$ −35.9° showing no hydroxyl bands in the infrared spectrum and reconvertible on saponification to crystalline VIa. VIb had acetate bands at 1732 and 1243 cm.$^{-1}$ of strength corresponding to two acetate groups per molecule, and F-ring bands at 863 (w), 901 (s), 922 (m), 982 (s) cm.$^{-1}$.

Anal. Calcd. for $C_{31}H_{48}O_5$: C, 72.06; H, 9.36. Found: C, 71.84; H, 9.44.

3-Desoxyhecololactone (IIb) (13ξ-Hydroxy-12,13-seco-5α,22a-spirostan-12-oic Acid 12,13-Lactone).—3-Desoxyhecogenin (Ib) (5α,22a-spirostan-12-one), 3.8 g., m.p. 190–195°, in 70 ml. of chloroform, was oxidized at room temperature with 70 ml. of a solution of perbenzoic acid in chloroform (50 mg./ml.) in the presence of 4 ml. of 10% sulfuric acid in acetic acid. After 64 hours the chloroform solution was washed with water, dilute sodium hydroxide, and water, and was evaporated on the steam-bath. Crystallization from acetone gave 3 g. of the pure compound, m.p. 240–241°, $[\alpha]^{25.5}D$ −5.85°.

Anal. Calcd. for $C_{27}H_{42}O_4$: C, 75.31; H, 9.83. Found: C, 75.14; H, 9.51.

Infrared spectrum (CS_2 solution) included: lactone band at 1727 cm.$^{-1}$ and F-ring bands at 864 (m), 898 (m), 906 (m), 923 (m) and 983 (s) cm.$^{-1}$ with intensity pattern resembling that of Fig. 1. Assignment of a band to the lactone function in the 1200–1300 cm.$^{-1}$ region is difficult since greater

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.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF HARVARD UNIVERSITY]

Nitration of Unsaturated Steroids

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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

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