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

Steroidal Sapogenins. LIII. Permanganate-Periodate Oxidation of Pseudosapogenins[2]

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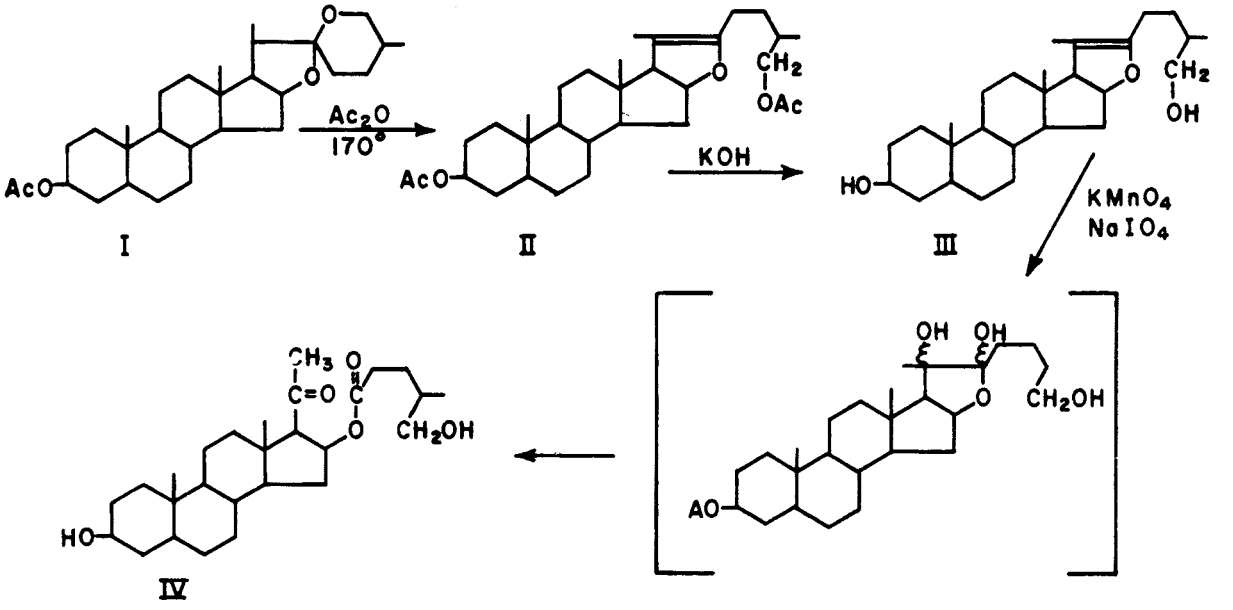
Pseudosapogenins and certain other steroid olefins with especially reactive double bonds can be smoothly oxidized with a permanganate-periodate reagent.

Modern steroid hormone technology is based in large part on 16-dehydro-20-oxo-pregnenes obtained by oxidative degradation of the steroidal sapogenin side chain. The classical three-step process was first developed by R. E. Marker and his associates[3] consisting of (1) conversion of sapogenins to pseudosapogenins, (2) oxidation of pseudosapogenins to give 16β-acyl esters of 20-oxo-pregnanes, and (3) alkaline cleavage of the 16β-ester followed by dehydration to give 16-dehydro-20-oxo-pregnanes. The over-all process has been studied in a number of laboratories[4a-h] leading to a degree of improvement in the classical procedure. However, in all of the cited references the oxidation agent has been chromic acid in acetic acid. As we have reported previously,[4c] the 16β-esters formed during oxidation of pseudosapogenins invariably undergo partial hydrolysis under the acidic reaction conditions giving 16-dehydro-20-oxo pregnenes which may then further react with excess oxidant. In order to avoid this undesirable hydrolysis we have studied the oxidation of pseudosapogenins under neutral or slightly alkaline conditions. A procedure developed by Lemieux and von Rudloff[5] involving permanganate-periodate oxidation of olefins soluble in slightly alkaline aqueous media seemed to offer promising possibilities. With some slight modifications the procedure was readily adapted to the oxidation of water insoluble pseudosapogenins by the route shown in Chart I.

The details of the procedure were developed



with speudosarsasapogenin, readily obtainable in pure, crystalline condition.[6] The conversion of

(1) Eastern Utilization Research and Development Division, Agricultural Research Service, U. S. Department of Agriculture.

(2) Previous paper in this series, "Steroidal Sapogenins. LII. Structure and Properties of the Acetyl Hypobromite Adduct from a Δ^{16}-Pregnen-20-one," S. G. Levine and M. E. Wall, *J. Am. Chem. Soc.*, in press.

(3) R. E. Marker *et al.*, *J. Am. Chem. Soc.*, **62**, 3350 (1940); *J. Am. Chem. Soc.*, **64**, 468 (1942); *J. Am. Chem. Soc.*, **69**, 2167 (1947).

(4) (a) D. H. Gould, H. Staeudle, and E. B. Hershberg, *J. Am. Chem. Soc.*, **74**, 3685 (1952); (b) W. G. Dauben and G. J. Fonken, *J. Am. Chem. Soc.*, **76**, 4618 (1954); (c) M. E. Wall, H. E. Kenney, and E. S. Rothman, *J. Am. Chem. Soc.*, **77**, 5665 (1955); (d) M. E. Wall and S. Serota, *J. Am. Chem. Soc.*, **79**, 6481 (1957); (e) A. F. B. Cameron, R. M. Evans, J. C. Hamlet, J. S. Hunt, P. G. Jones, and A. G. Long, *J. Chem. Soc.*, 2807 (1955).

(5) R. U. Lemieux and E. von Rudloff, *Can. J. Chem.*, **33**, 1710 (1955).

(6) M. E. Wall, S. Serota, and C. R. Eddy, *J. Am. Chem. Soc.*, **77**, 1230 (1955).

pseudosapogenins III to the corresponding 16β-ester, IV, was conveniently followed by noting the decrease or disappearance of the ultraviolet absorption band at 215 mμ found in pseudosapogenins,[3e] and by observing the concomitant appearance of strong infrared absorption bands at 1725 cm.$^{-1}$ and 1707 cm.$^{-1}$ due to 16β-ester and 20-ketone groups, respectively, found in IV. After some experimentation the correct conditions for conducting the oxidation of the water-insoluble pseudosapogenins were developed. It was found that the steroid, in the free hydroxyl form, must be dissolved in a water-miscible, inert solvent such as dioxane or tertiary butylalcohol. The steroid solution was then added to the aqueous solution of inorganic reagents and the mixture strongly agitated or shaken. The general reaction conditions of Lemieux and von Rudloff,[5] which utilize for 1 Mm. of olefin an oxidizing mixture of 3 Mm. of potassium carbonate, 8 Mm. of sodium metaperiodate, and 0.34 Mm. potassium permanganate at room temperature, were satisfactory under our experimental conditions and gave rapid oxidation of the C_{20},C_{22} double bond. Due possibly to their lower solubility in the aqueous dioxane or tertiary butyl alcohol solutions, pseudosapogenin diacetates were poorly oxidized under similar conditions. The yield was not improved by adding benzene as a co-solvent to the aqueous organic solutions. As shown in the experimental section the procedure was applicable to a variety of pseudosapogenins including those with unsaturation at C_5 or with a 12-ketone group.

Because of the noncrystalline nature of the pseudosapogenin oxidation products of structure IV it was difficult to ascertain yields. All oxidation products were checked by infrared spectroscopy and then subjected to alkaline hydrolysis[4c] to the corresponding 16-dehydro-20-keto-pregnenes, all of which were known crystalline compounds. We have not made a thorough comparison of yields of 16-dehydro-20-ketopregnenes by the chromium trioxide[4c] and the present procedure. However, with simple cases, i.e. no Δ^5-unsaturation or absence of 12-keto groups, higher yields are obtained by the present method. Thus from pseudosmilagenin we obtained a 40% yield of 3β-acetoxy-16-pregnen-20-one and 70% by the permanganateperiodate procedure, using potassium hydroxide in *t*-butyl alcohol[4c] in both cases.

Because of the mild, smooth oxidation action of the permanganateperiodate reagent we tested its effect on several types of steroidal olefins available to us. Oxidation of 12-methylene tigogenin[7] proceeded smoothly to give hecogenin in excellent yield. Similar treatment of 3β-hydroxy-16-pregnen-20-one converted this steroid completely to acidic products which were not further characterized. An enol-acetate, 3β,20-diacetoxy-17-pregnene, and stigmasterol, a steroid with both a tri- and a disubstituted olefinic linkage, were recovered unchanged. From our brief experiences with a limited variety of steroid olefins we conclude that only highly reactive steroid double bonds are attacked by the permanganate-periodate reagent. This is not a general observation because as previously shown by Lemieux and von Rudloff the disubstituted olefinic bond in oleic acid is easily oxidized. Whether this is due to the greater solubility of sodium oleate in the aqueous medium or to steric factors which may be encountered with steroids cannot be decided by evidence on hand.

EXPERIMENTAL

All pseudosapogenins were prepared in a manner described previously.[4d] The following oxidation procedure is typical. A solution of 1.2 g. of potassium permanganate (3.2 Mm.), 10.0 g. of anhydrous potassium carbonate (71.4 Mm.), and 41.0 g. of sodium metaperiodate (190.4 Mm.) in 1.0 l. of water was mixed with a solution of 10.0 g. (23.8 Mm.) of pseudosarsasapogenin in 500 ml. of purified dioxane and 500 ml. of benzene. The mixture was vigorously shaken for 1 hr. Probe tests on smaller quantities indicated the reaction was substantially complete in 5 min. The benzene layer was separated from the aqueous fraction, the latter extracted several times with benzene, and all the benzene extracted united and washed with distilled water. The benzene was concentrated to dryness *in vacuo* leaving a pale yellow glass which was undoubtedly 3β-hydroxy-16β-(γ-methyl-3-hydroxy)-valeroxy-pregnan-20-one. The compound had negligible ultraviolet absorption at 215 mμ in contrast to the starting pseudosarsasapogenin. The infrared spectrum of I showed two strong carbonyl bands at 1720 and 1707 cm.$^{-1}$ and a strong band at 1250 cm.$^{-1}$ attributable to the C—O—C bond of the 16β-ester. Because of the well known difficulty in crystallizing compounds with structures similar to IV and because of the general instability of 16β-ester steroids no further attempts were made to characterize it. The viscous product was taken up in 200 ml. of *t*-butyl alcohol by warming on the steam bath. To the solution was added 5.0 g. of potassium hydroxide in 4 ml. of water. Nitrogen was passed through the *t*-butyl alcohol solution. The flask was stoppered and vigorously stirred with a magnetic stirrer for 3 hr. The initial temperature was 40° which fell to 30° at the expiration of 3 hr. To the hydrolyzed solution was added 400 ml. of water with vigorous stirring. The aqueous solution was seeded with authentic 3β-hydroxy-16-pregnen-20-one and another 400 ml. of water added with stirring. Crystalline plates began to form and the stirring was continued for 1 hr. The crystalline product was filtered, washed with water, and dried, yield 6.91 g., m.p. 170–180°, 85% purity based on ultraviolet absorption at 239 mμ, equivalent to 77% of theory. Crystallization from methanol gave 5.0 g. of plates, m.p. 185–187°[4c] with infrared spectrum identical to an authentic specimen.

In exactly the same manner described above pseudodiosgenin, pseudotigogenin, and pseudohecogenin were oxidized to their corresponding 16β-acyl esters and hydrolytically cleaved to give respectively 3β-hydroxy-5,16-pregnadiene-20-one, 3β-hydroxy-5α-pregn-16-en-20-one, and 3β-hydroxy-5α-pregn-16-en-12,20-dione, all characterized by melting point and identity with known samples.[4c] The yields were not so high as in the pseudosarsasapogenin oxidation ranging from 30–50% of theory by ultraviolet absorption analysis. It was found that *t*-butyl alcohol was as effective a solvent as dioxane for this purpose and since it was much easier to purify, the former solvent was adopted for all subsequent work. It was also found that the use of benzene during oxidation was unnecessary. However no reaction occurred if an aqueous solution of the inorganic oxidant was

shaken with a benzene solution of steroid in the *absence* of dioxane or *t*-butyl alcohol.

Oxidation of 12-methylene-tigogenin to hecogenin. 0.4 g. of 12-methylene tigogenin[7] in 100 ml. of *t*-butyl alcohol was oxidized with a solution of 1.7 g. of sodium metaperiodate, 0.42 g. of potassium carbonate, and 0.05 g. of potassium permanganate in 100 ml. of water, shaking the mixture in a 500-ml. bottle. Infrared analysis for carbonyl indicated maximum formation in 5 hr. The solution was extracted with benzene, yielding after the usual work-up 0.3 g. of hecogenin, m.p. 250–253°, infrared spectrum identical with that of an authentic specimen.

Oxidation of 3β-acetoxy-16-pregnen-20-one. A solution of 0.42 g. of 3β-acetoxy-16-pregnen-20-one in 100 ml. of *t*-butyl alcohol was shaken overnight with the aqueous oxidation solution used above. The aqueous mixture was further diluted with water and extracted with ether. The ether solution contained a negligible weight of steroid. The aqueous layer was acidified with hydrochloric acid and the resultant precipitate extracted with ether to yield 0.4 g. of amorphous glass. The infrared absorption spectrum showed, as might be expected, a strong carboxyl carbonyl band at 1700 cm.$^{-1}$.

Under the above reaction conditions stigmasterol and 3β,20-diacetoxy-17-pregnen-20-one were recovered unchanged.

(7) F. Sondheimer and R. Mechoulam, *J. Am. Chem. Soc.*, **79**, 5029 (1957).

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Steroidal Aldosterone Blockers. I

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The synthesis and specific biological activities of a variety of C-17 steroidal 5 and 6 membered spirolactones are presented. The 19-nor compound with a 5 membered lactone (Xa) is the most potent aldosterone blocker.

Since the first reports of the antialdosterone activity of several steroidal 17-spirolactones[1(a),(b)] we have prepared a number of new spirolactones in order to test the effect on blocking activity of changes in both the lactone and steroid portions of the molecule. It is our purpose in this article to record the experimental details of synthesizing the drugs reported in earlier communications[1(a),2] and to report on some of the new compounds in this series.

The first member of this series to show aldosterone blocking activity was 3-(3-keto-17β-hydroxy-4-androsten-17α-yl)propanoic acid lactone (VIa). This was prepared by the sequence shown on Chart 1. The Grignard reagent of 17α-ethynyl-5-androstene-3β,17β-diol (Ia)[3] was carbonated in good yield to give an acetylenic acid (IIa). The acetylenic bond was selectively reduced to an olefin by catalytic hydrogenation over palladium on calcium carbonate using dioxane and pyridine as solvents. The resulting product on treatment with mineral acid yielded an unsaturated lactone (IIIa) which could be readily reduced to a saturated lactone (Va) by hydrogen over palladium on charcoal. Oxidation of IIIa and Va by the Oppenauer method produced the corresponding 3-oxo-4-ene compounds IV and VIa.

Because of the interesting antialdosterone activity of VIa we decided to make the correponding 19-nor compound. To this end the spirolactone side chain was built onto a steroid nucleus containing an aromatic A ring by the same series of reactions used in the androstane series (Chart I). 17α-Ethynyl-3-methoxy-1,3,5(10)-estratrien-17β-ol[4] (Ic) was carbonated to give an acetylenic acid (IIc) which could be hydrogenated partially or completely to give an unsaturated (IIIc) or saturated (Vc) lactone. As shown on Chart 2, the A ring of this could be most effectively reduced to the dihydroaromatic system (VIIIa) by preparing the sodium salt (VIIa) of the saturated lactone (Vc) and reducing this with lithium in ammonia and *t*-butyl alcohol.[5] Hydrolysis of the enol ether (VIIIa) with dilute acetic acid afforded a compound (IXa) in which simultaneous lactonization of the liberated hydroxy acid had occurred. On the other hand, hydrolysis with mineral acid gave the compound (Xa) containing not only a lactone, but also a conjugated ketone. Xa was also prepared by treating IXa with strong acid. Lactonization of the hydroxy acids was best accomplished by treating them with strong acid in solution; stirring the precipitated hydroxy acid with aqueous acid was usually ineffective.

In the hope of reducing the triple bond and aromatic A ring simultaneously the acetylenic acid (IIc) was subjected directly to reduction by lithium in ethanol and ammonia. After hydrolysis of the uncharacterized intermediate enol ether with strong acid, there was obtained not only the satu-

(1) (a) J. A. Cella and C. M. Kagawa, *J. Am. Chem. Soc.*, **79**, 4808 (1957). (b) C. M. Kagawa, J. A. Cella, and C. G. Van Arman, *Science*, **126**, 1015 (1957).

(2) J. A. Cella, U. S. Patent **2,705,712**, April 5, 1955.

(3) H. E. Stavely, *J. Am. Chem. Soc.*, **61**, 79 (1939).

(4) F. B. Colton, U. S. Patent **2,666,769**, June 19, 1954.

(5) This is a modification of the Birch reduction described by A. L. Wilds and N. A. Nelson, *J. Am. Chem. Soc.*, **75**, 5360 (1953) which was developed by Dr. H. Dryden of these laboratories.