and centrifuged at 30,000 G for 15 min. The clear supernatant solution was deionized by passing it through a mixed resin bed composed of amberlite IR-120 and amberlite IR-4B. The deionized filtrate was reduced under vacuum to 5 ml. A 2-ml. portion of this concentrate was tested by a standard orcinol method for hexose.[11] Although the test was not quantitative, it was clearly evident that the sugar was a hexose.

As noted in the literature[12] pure β-D-glucosidase is generally considered very specific for hydrolyzing normal β-linked D-aglucones. Therefore, the confirmation of a hexose by the orcinol test coupled with the fact that β-D-glucosidase will not hydrolyze β-linked D-fructose or D-mannose might be considered sufficient to indicate the presence of β-D-glucose. However, as some doubt exists as to whether this enzyme may also hydrolyze β-D-galactose under favorable conditions, the following confirmatory experiment to identify the sugar was completed:

To the remaining 3 ml. of filtrate were added 0.4 g. of phenylhydrazine, 0.6 g. of sodium acetate and 0.5 ml. of a saturated bisulfite solution. The volume was made up to 5 ml. and the solution immersed in a boiling water bath. An osazone of the sugar formed between 4 and 5 min. The time for osazone formation coincided exactly with that established for glucosazone,[13] as well as with time of osazone formation in a known glucose solution treated simultaneously in an identical manner. A galactose solution run under similar conditions did not form osazone crystals until 19 min. had elapsed.

Microscopic examination of the osazone crystals indicated that the unknown gave crystals that were identical with glucosazone and differed significantly from those formed for galactose.[14] As a result of these experimentations, the presence of glucose was confirmed in the saponin.

CONCLUSIONS

On the basis of the results recorded, it is expected that the pure root saponin isolated was a β-linked glucoside of 2β-hydroxy-Δ^{12}-oleanene-23,28-dioic acid (Medicagenic acid). Because of the difficulty encountered in the acid hydrolysis of glucose it is predicted that glucose is initially attached to the triterpene nucleus in a majority of alfalfa root saponins of this type regardless of the sugar chain complexity. This conclusion was suggested in that several different impure water soluble saponins isolated in initiating this research were all reduced by controlled hydrolysis to the same saponin nucleus reported in this paper.

Surface contact enzyme hydrolysis using pure β-D-glucosidase not only proved that a β-linkage for D-glucose was involved, but that the glucosidic attachment was probably free of further substitution or unusual binding to the aglycone.[12] This latter evidence was strengthened when the hydroxyl group determination presented evidence of five free hydroxyl groups in the saponin which is expected when a normal glucoside linkage exists.

Alfalfa roots have been found to be an excellent source for the isolation of saponins of this type or for isolating pure medicagenic acid. Should these compounds prove valuable as intermediates, alfalfa root powder would be a practical source material.

Acknowledgment. We wish to acknowledge the assistance of Dr. C. Blincoe for his professional advice on biochemical methods and R. M. Maffi for laboratory assistance. The authors are also indebted to Mr. C. R. Van Atta of Western Regional Laboratories for supplying infrared spectrograms of medicagenic acid and its diacetate for comparison studies and to Dr. LeRoy Johnson of Varian Associates for the NMR spectrogram and its interpretation.

RENO, NEV.

(11) R. J. Winzler *et al.* Editor D. Glick, *Methods of Biochemical Analysis*, Vol. II, p. 291, Interscience, New York.

(12) M. Dixon and C. E. Webb, *Enzymes*, Academic Press, New York (1958).

(13) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, *The Systematic Identification of Organic Compounds*, 4th Ed., Wiley, New York (1956).

(14) W. Z. Hassid and R. M. McCready, *Ind. & Eng. Chem., Anal. Edition*, **14**, 683–6 (1942).

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

Steroidal Sapogenins. L.[2,3] Conversion of 12-Ketosapogenins to 11β,12β-Epoxypregnanes

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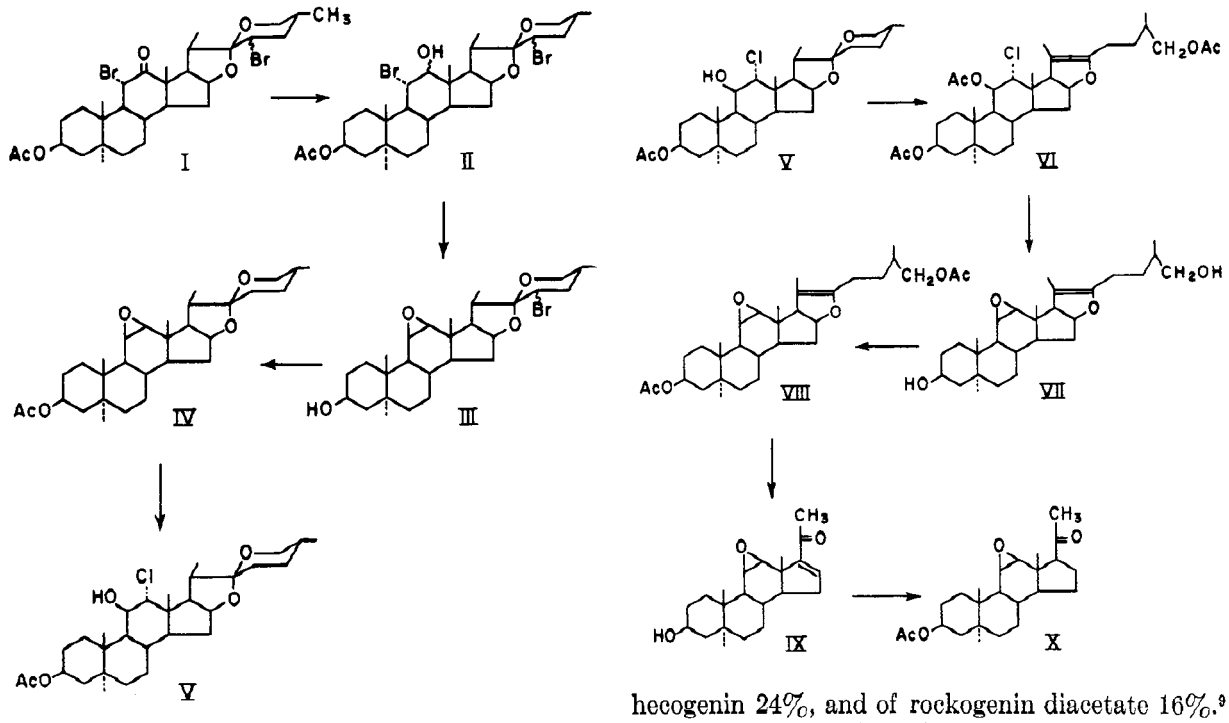
Hecogenin was converted to 11β,12β-epoxytigogenin (IV), and then to 3β-acetoxy-11β,12β-epoxy-5α-pregnane-20-one (X). Gentrogenin or gentrogenin-correllogenin mixtures were converted to 11β,12β-epoxydiosgenin (XV) and then to 11β,-12β-epoxy-3β-acetoxy-5-pregnene-20-one (XXI).

The elegant researches of Fried and his associates have demonstrated that 12-halosteroids have physiological activities comparable to those of corresponding 9-halosteroids.[4] Probably the most available route to such compounds are *via* the

(1) Eastern Utilization Research and Development Division, Agricultural Division, Agricultural Research Service, U. S. Department of Agriculture, Philadelphia 18, Pennsylvania. Article not copyrighted.

(2) Previous paper in this series, Steroidal Sapogenins. XLIX, *J. Org. Chem.*, **23**, 1741 (1958).



11β,12β-epoxides.[6] We wish to report at this time on the preparation of 3β-acetoxy-11β,12β-epoxy-5α-pregnan-20-one, X, derived from hecogenin, and the corresponding Δ^5-analogue, XXI, derived from gentrogenin.

11α,23ξ-Dibromohecogenin acetate[6] was conveniently reduced with sodium borohydride in a refluxing mixture of methylene chloride and methanol. The crude bromohydrin, a mixture of II and its 12α epimer, was obtained quantitatively and was treated with refluxing methanolic potassium hydroxide. The crude product, of which the chief constituent was the 23-bromo-11β,12β-epoxide, III, was treated with a zinc-copper couple in refluxing ethanol.[6,7] As was anticipated from the previous work of Cornforth, Osbond, and Phillipps,[8] the debrominated epoxide, IV, was not the sole product. Hecogenin, derived from the 12α-hydroxy-11α-bromo epimer,[8] was separated from the mixture by use of Girard's Reagent T. On acetylation of the nonketonic fraction, the insoluble 3β-acetoxy-11α,12β-epoxytigogenin, IV, was easily separated from the soluble rockogenin diacetate by crystallization. Based on the dibromide I, the yield of the desired epoxide IV was 53%, of hecogenin 24%, and of rockogenin diacetate 16%.[9] Attempts to pseudomerize the epoxide IV with refluxing acetic anhydride in the presence of pyridine hydrochloride[10] or acetic anhydride–acetic acid at 180°[11] were unsuccessful. As long as the 11β,12β-oxide ring was intact, there was no attack on the side chain.[12] After prolonged heating, cleavage of the oxide ensued and then side chain attack took place in the usual manner. On treatment of IV with hydrochloric acid in dioxane, the 11β,12β-oxide reacted smoothly to give the known 11β-hydroxy-12α-chloro-derivative, V.[13] On treating V with acetic anhydride containing 0.1% acetic

(3) Presented in part at the 134th National Meeting of the American Chemical Society, Chicago, Ill., September 1958.

(4) J. Fried and A. Borman, *Vitamins and Hormones*, **16**, 303 (1958).

(5) L. Fieser and M. Fieser, *Steroids*, Reinhold, New York, 1959, pp. 684–5.

(6) J. Elks, G. H. Phillipps, T. Walker, and L. J. Wyman, *J. Chem. Soc.*, 4330 (1956).

(7) Use of zinc–acetic acid resulted in opening of the epoxide.

(8) J. W. Cornforth, J. M. Osbond, and G. H. Phillipps, *J. Chem. Soc.*, 907 (1954).

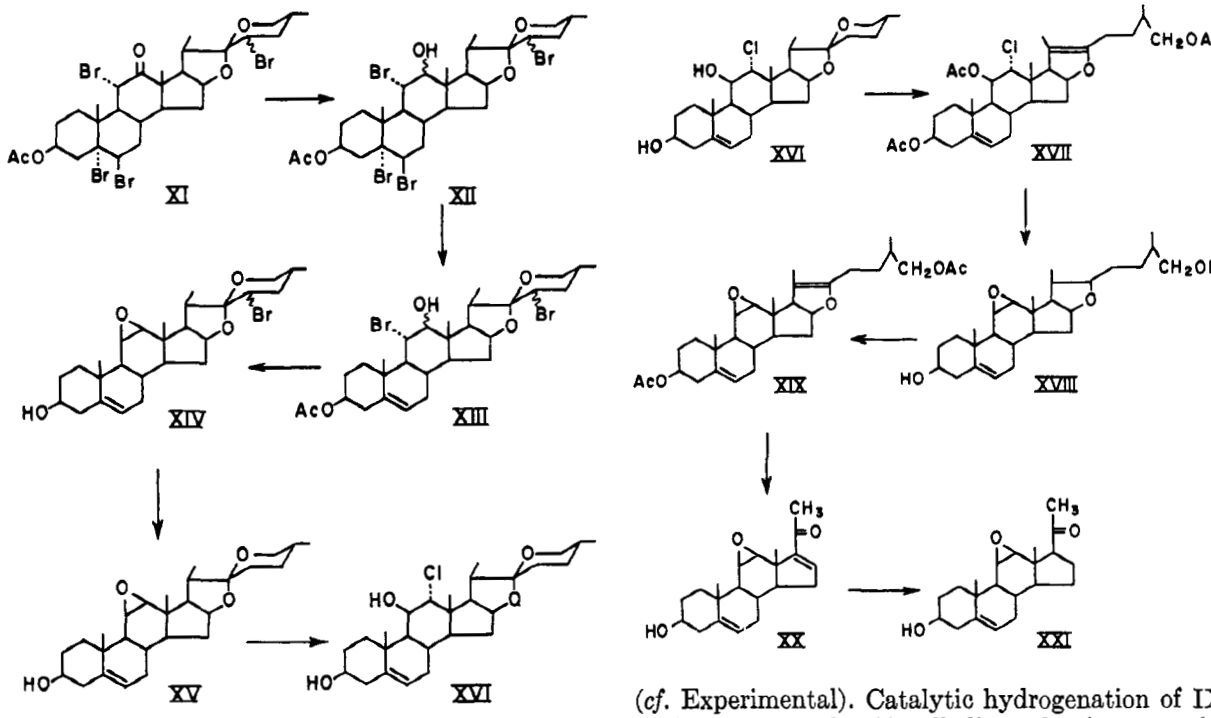
(9) During the sodium borohydride reduction of I, a substantial, although minor, fraction of the *cis*-12α-hydroxy-11α-bromo epimer is formed, *cf. J. Am. Chem. Soc.*, **78**, 3752 (1956). The *cis* epimer cannot form an epoxide[8] [*cf.* also *J. Am. Chem. Soc.*, **57**, 224 (1935)]. The rockogenin diacetate may originate as a result of reduction of the 11α-bromo moiety prior to reduction of the ketone.

(10) W. G. Dauben and G. J. Fonken, *J. Am. Chem. Soc.*, **76**, 4618 (1954).

(11) M. E. Wall and S. Serota, *J. Am. Chem. Soc.*, **79**, 6481 (1957).

(12) It seems well established that coordination of the ring F oxygen with a Lewis acid, thus yielding a positively charged intermediate, is a requisite for pseudomerization. The resistance of IV to pseudomerization could be explained by assuming that the oxide preferentially coordinates with a Lewis acid to give a positively charged species which would inhibit formation of a similar charge on the F ring oxygen. Recently we have shown that amines can inhibit catalytic hydrogenation of ring F, *J. Am. Chem. Soc.*, **82**, 1444 (1960), a reaction which also requires coordination of the F ring oxygen with an acid. However, the 12-ketosapogenins, wherein the 12-ketone would presumably coordinate more readily with a Lewis acid than the epoxide oxygen, show no inhibition of pseudomerization, thus weakening the above rationalization.

(13) J. Schmidlin and A. Wettstein, *Helv. Chim. Acta*, **36**, 1241 (1953).



acid at 170°,[14] V was converted to 3β,11β,26-triacetoxy-12α-chloropseudotigogenin, VI, with surprising rapidity. The pseudotriacetate, VI, was not isolated in crystalline form but was characterized by infrared spectroscopy which indicated absence of characteristic spiroketal bands,[15a,b] presence of a band at 1685 cm.$^{-1}$ characteristic of pseudosapogenins[16] and absence of hydroxyl bands. Attempts to convert VI to the desired epoxypregnene, IX, *via* standard oxidation and alkaline hydrolysis techniques[17] were unsuccessful.[18] Treatment of VI with refluxing methanolic potassium hydroxide gave 11β,12β-epoxy-3β,26-dihydroxypseudotigogenin, VII. Compound VII was not crystalline and was characterized by its infrared spectrum and subsequent reactions. Acetylation of VII followed by standard chromium trioxide oxidation and hydrolysis with potassium hydroxide in *t*-butyl alcohol gave 3β-acetoxy-11β,12β-epoxy-5α-pregn-16-ene-20-one, IX, in 50% yield based on VII and 25% over-all yield based on I. The epoxypregnene IX was characterized by its carbon and hydrogen analysis, and the infrared and ultraviolet spectra are in agreement with the assigned structure (*cf.* Experimental). Catalytic hydrogenation of IX in the presence of 10% palladium-alumina gave the saturated pregnane, X.

A similar series of reactions were successfully conducted with gentrogenin[19] (12-ketodiosgenin). Gentrogenin tetrabromide,[20] XI, was reduced with sodium borohydride to give the bromohydrin XII which on treatment with sodium iodide in ethanol regenerated the Δ^5-double bond, giving XIII. Alkaline treatment followed by use of the zinc-copper couple gave a mixture of 11β,12β-epoxy diosgenin, XV, and gentrogenin, in a ratio of approximately 4 to 1 respectively. We were unable to find significant quantities of any 12-hydroxy compound. The yield of XV from XI was 78%. Treatment of the epoxide XV with hydrochloric acid in dioxane gave the chlorohydrin XVI as a gummy, noncrystalline compound. Without isolation, it was pseudomerized to give XVII which was then hydrolyzed and converted to 3β-acetoxy-11β,12β-epoxy-5,16-pregnadiene-20-one, XX, by the same route used in the hecogenin series. The crystalline pregnadiene XX was characterized by correct analytical values and infrared and ultraviolet spectra in agreement with the assigned structure (*cf.* Experimental). Catalytic hydrogenation of XX under mild conditions gave the saturated pregnene XXI. The conversion of the 11β,12β-epoxy diosgenin XV to the pregnadiene XX took place in 65% yield. The above reactions were conducted with the pure 25D-isomer, gentrogenin, as the starting material. It is more convenient to work with the naturally occurring 25D and 25L mix-

(14) The reaction required only 2.5 hr. for completion, in contrast to the 10–17 hr. normally required for complete pseudomerization.[11]

(15)(a) C. R. Eddy, M. E. Wall, and M. K. Scott, *Anal. Chem.*, **25**, 266 (1953); (b) R. N. Jones, E. Katzenellenbogen, and K. Dobriner, *J. Am. Chem. Soc.*, **75**, 158 (1953).

(16) A. Hayden, P. Smeltzer, and I. Scheer, *Anal. Chem.*, **26**, 550 (1954).

(17) M. E. Wall, H. E. Kenney, and E. S. Rothman, *J. Am. Chem. Soc.*, **77**, 5665 (1955).

(18) Among other reasons, treatment of the product of chromium trioxide oxidation of VI with potassium hydroxide in *t*-butyl alcohol failed to regenerate the 11β,12β-epoxide.

(19) H. A. Walens, S. Serota, and M. E. Wall, *J. Org. Chem.*, **22**, 182 (1957).

(20) E. S. Rothman and M. E. Wall, *J. Am. Chem. Soc.*, **79**, 3228 (1957).

tures,[19,21a,b] which on side chain cleavage give the same 16-dehydropregnene. The series XI to XX conducted with the 25D and 25L mixtures gave yields of the same order as those in which the pure 25D isomer was used. By means of available procedures, particularly the elegant Syntex method for 21-acetoxylation,[22] compounds IX, X, XX, and XXI should be excellent points of departure for elaboration of a variety of active steroid hormones.[23]

EXPERIMENTAL

11β,12β-Epoxytigogenin acetate (IV). 11α,23ξ-Dibromohecogenin acetate I was prepared in the usual manner.[6] Fifty grams of I was dissolved in 600 ml. of methanol and 200 ml. of methylene chloride and the solution heated to reflux. A refluxing solution of 23 g. of sodium borohydride in 200 ml. of methanol was added to this as quickly as possible (about 10–15 min.). The solution was refluxed an additional 15 min. and then the methylene chloride was distilled. The remaining solution was cooled to room temperature by the addition of 1.4 l. of ethanol. Twenty-five grams of potassium hydroxide was added and the mixture was stirred for 3 hr. and then allowed to stand overnight at room temperature. The solution was concentrated to about 600 ml. and then poured into 1500 ml. of water. The product was extracted with ether, three portions of 350 ml., and the ether layers combined. The ether was washed with water, dried over sodium sulfate, and evaporated *in vacuo* at room temperature. The residue was dissolved in 1 l. of ethanol and treated with a zinc-copper couple[6] (200 g. of zinc and 1350 ml. of 15% w./v. copper sulfate) at reflux for 3 hr. The reaction mixture was filtered while hot and the filter cake washed with methylene chloride, two 100-ml. portions. The combined filtrates were then evaporated to dryness *in vacuo*, giving 34 g. of product. Infrared analysis showed that this product contained some ketone, some dihydroxy, and some epoxy material. The mixture (8.4 g.) was dissolved in 175 ml. ethanol containing 5% acetic acid, 4 g. Girard's Reagent T was added, and the solution was refluxed for 1.5 hr. The solution was cooled to room temperature and then poured into a separatory funnel containing 400 ml. of ice and water and 5 g. of sodium carbonate. The aqueous layer was extracted with three 300-ml. portions of ether and the ether layers were combined. The ether was then washed with five 400-ml. portions of water, the last two washes giving no precipitate when acidified with hydrochloric acid. The ether was then dried over sodium sulfate and evaporated to dryness, giving 6.5 g. of crystalline material. This was acetylated in pyridine–acetic anhydride at room temperature for 16 hr. The product was isolated in the usual manner and infrared analysis showed the presence of an epoxide (875 cm.$^{-1}$ as well as some diacetoxy material. The mixture was crystallized from methanol–methylene chloride, giving 4.9 g. of 11β,12β-epoxytigogenin acetate, IV. Concentration of the mother liquors did not yield any further crystalline material; however, 1.5 g. of rockogenin diacetate was obtained upon evaporating the solvent. The aqueous layers from the Girard Reagent T separation were acidified and the ketonic material was recovered, giving 2.0 g. of hecogenin. IV was identified by infrared analysis (1735, 1248 cm.$^{-1}$ acetate, normal F-ring bands, and 875 cm.$^{-1}$ epoxide, no hydroxy or ketone bands) and melting point 203–206° (lit.,[13] m.p. 205–207°).

3β-Acetoxy-11β,12β-epoxy-16-pregnen-20-one (IX). One gram of IV was dissolved in 100 ml. of dioxane, 20 ml. of 3*N* hydrochloric acid and 5 ml. of water were added, and the resulting one phase solution was stirred for 1 hr. Seventy milliliters of water was added with stirring over a 10-min. period. Material started to precipitate and the mixture was allowed to stand for an additional hour. The precipitate was filtered off, the mother liquor was diluted with additional water and refiltered. The combined filter cakes were air dried, giving 1.1 g. of 12α-chloro-11β-hydroxytigogenin acetate (V). Infrared analysis showed bands at 3650 (hydroxyl), 1735, 1245 (acetate), normal F-ring bands, and absence of the 875 cm.$^{-1}$ (epoxide) band.

Nine grams of V was placed in a flask, 23 ml. of acetic anhydride containing 0.1% acetic acid was added, the flask sealed and then heated in a bath at 170° for 2.5 hr. The flask was cooled and the acetic anhydride was evaporated off *in vacuo*. Attempts to crystallize the product were fruitless. Infrared analysis agreed with VI, showing no F-ring bands, strong acetate bands (1736, 1250 cm.$^{-1}$), and double bond (1685 cm.$^{-1}$). VI was dissolved in 500 ml. of methanol, 5 g. of potassium hydroxide was added, and the solution was allowed to stand at room temperature overnight. The solution was poured into 1 l. of water and extracted with three 500-ml. portions of ether. The ether layers were combined and washed with water, dried over sodium sulfate, and evaporated to dryness. The residue was dissolved in 20 ml. of pyridine and 15 ml. of acetic anhydride, and allowed to stand overnight at room temperature. The product was isolated in the usual manner, giving 9.2 g. of 11β,12β-epoxypseudotigogenin diacetate (VIII), identified by infrared analysis.

Two grams of VIII was dissolved in 30 ml. of acetic acid and cooled to 15° in an ice bath. Fifteen milliliters of 50% acetic acid–water containing 0.8 g. of chromium trioxide was cooled to 10° and then added dropwise with stirring to the steroid solution, the addition taking 10 min. The reaction mixture was allowed to come to room temperature and then stirred for an additional 1 hr. The mixture was drowned in three volumes of water and the steroid was isolated by ether extraction. The residue was dissolved in 50 ml. of *t*-butyl alcohol, 1 g. of potassium hydroxide and 2 ml. of water were added and the mixture was shaken at room temperature for 3 hr. The reaction mixture was drowned in 100 ml. of water and product was isolated by ether extraction. The ether was washed with water, dried over sodium sulfate, and evaporated to dryness. The residue was acetylated in 5 ml. of pyridine and 3 ml. of acetic anhydride overnight at room temperature. The product (IX) was isolated by drowning in water and extraction with ether, giving 1 g. of colored resin. The resin was chromatographed on Florisil (20 g.), the desired product being eluted over a wide range, starting with benzene and ending with chloroform, giving 0.7 g. of acetoxy-11β,12β-epoxy-5α-pregn-16-en-20-one (IX). The analytical sample was crystallized from heptane, then from hexane, m.p. 182–184°, $[\alpha]_D^{25}$ 103.5, log ε 3.98.

Anal. Calcd. for $C_{23}H_{32}O_4$: C, 74.18; H, 8.66. Found: C, 73.96; H, 8.51.

The infrared spectrum showed bands at 1735 and 1248 (acetate), 1668 (conj. ketone), and 875 cm.$^{-1}$ (epoxide).

Hydrogenation of 3β-acetoxy-11β,12β-epoxy-5α-pregn-16-en-20-one (IX). Three grams of IX and 1 g. of 10% palladium on alumina were added to 200 ml. of ether, and hydrogenated for 3 hr. at 50 p.s.i.g. of hydrogen. The solution was filtered to remove the catalyst and the product isolated by evaporating the ether. The residue was crystallized from methanol and then from petroleum ether (b.p. 35–60°), giving 3β-acetoxy-11β,12β-epoxy-5α-pregnene-20-one (X), m.p. 113.5–114.5°, no absorption in the ultraviolet, bands at 1735 and 1248 (acetate), 1710 (20-ketone), 875 cm.$^{-1}$ (epoxide), and no band for conjugated carbonyl.

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(21)(a) E. S. Rothman and M. E. Wall, *J. Am. Chem. Soc.*, 81, 411 (1959); (b) O. Halpern and C. Djerassi, *J. Am. Chem. Soc.*, 81, 439 (1959).

(22) H. J. Ringold and G. Stork, *J. Am. Chem. Soc.*, 80, 250 (1958).

(23) Because of scarcity of starting materials and a shifting in research orientation, our laboratory is not contemplating further work in this area.

Preparation of 12ξ-hydroxy-5α,6β,11α,23-tetrabromodiosgenin 3β-acetate (XI). Twenty grams of 5α,6β,11α,23ξ-tetrabromogentrogenin 3-acetate in 600 ml. of methanol and 250 ml. of methylene chloride was heated at reflux temperature. Ten grams of sodium borohydride in 100 ml. methanol was also heated to reflux temperature and was then added to the steroid solution portionwise over a time interval of about 4 min. Heating at reflux was continued for 15 min. The reaction mixture was then poured into 2 l. of water and the steroid recovered by three extractions with ether (500 ml.). The ether solution was washed with 3*N* hydrochloric acid, sodium bicarbonate, and distilled water, and dried over sodium sulfate. The ether was evaporated *in vacuo* at room temperature to give 20 g. of 12ξ-hydroxy,-5α,6β,11α,23ξ-tetrabromodiosgenin 3β-acetate (XII). Infrared showed no 12-ketone band, presence of hydroxyl (3420) and acetate (1735 and 1248 cm.$^{-1}$). The product was not purified at this stage.

12ξ-Hydroxy-11α,23ξ-dibromodiosgenin 3β-acetate (XIII). Twenty grams of XII was dissolved in 500 ml. ethanol, 25 g. of sodium iodide was added, and the solution was refluxed for 30 min. The ethanol solution was poured into 1500 ml. of water. Ethyl ether (600 ml.) was added and the two phase system was washed with just enough sodium thiosulfate to decolorize the system. The layers were separated and the aqueous layer was reextracted with ether. The ether portions were combined, washed with water, dried over sodium sulfate, and the dry ether solution evaporated *in vacuo* at room temperature, giving 16 g. of 12ξ-hydroxy-11α,23ξ-dibromodiosgenin 3β-acetate (XIII). The infrared spectrum had the characteristic 23-bromosapogenin fingerprint[24] and hydroxyl (3600) and acetate (1734 and 1248 cm.$^{-1}$) peaks.

Preparation of 11β,12β-epoxydiosgenin (XV). Sixteen grams of XIII was dissolved in 600 ml. ethanol and 8 g. potassium hydroxide was added. The solution was stirred for 3 hr. and then allowed to stand for 20 hr. The product was isolated by pouring the solution into 1500 ml. of water and then extracting with three 600-ml. portions of ether. The ether layers were combined and washed with water to remove any residual base, then the ether was dried over sodium sulfate, and evaporated to dryness *in vacuo*. The residue was then dissolved in 500 ml. ethanol and treated with a zinc-copper couple prepared from 60 g. of zinc and 450 ml. of 15% aqueous copper sulfate solution. The mixture was refluxed with stirring for 3 hr. and then filtered while hot. The filter cake was washed with additional hot ethanol. The alcoholic solution was diluted with two volumes of water and then extracted with ether (3 × 500 ml.). The ether portions were combined, washed with water, dried over sodium sulfate, and evaporated to dryness; yield 11 g. Infrared showed this to be a mixture of ketone and epoxide. The mixture was separated by two methods.

(a) *Girard T Reagent.* Eleven grams of the mixture was dissolved in 250 ml. ethanol containing 12.5 ml. acetic acid. Six grams of Girard T Reagent was added and the solution was refluxed for 1 hr. The solution was poured into 750 ml. of ice and water which was saturated with sodium carbonate. Two 800-ml. portions of ether were used to extract the aqueous mixture. The ether fractions were combined and washed with three portions of water, the last wash giving no precipitate upon being acidified with hydrochloric acid. The ether was then taken to dryness, giving 8.5 g. of crude 11β,12β-epoxydiosgenin (XV). The aqueous washes were acidified, allowed to sit overnight, and the precipitate filtered off, giving 1.9 g. of gentrogenin.

(b) *Partition chromatography.* A partition column, 2 inches in diameter and 36 inches long, was prepared by the slurry technique using 250 g. of Celite and 100 ml. of phenyl cellosolve. The steroid mixture, as the acetate, 2.2 g., was dissolved in 250 ml. of heptane and placed on the column. All the heptane used was saturated with phenyl cellosolve. The column hold-up was approximately 500 ml. Steroid appeared after an additional 250 ml., and all the nonketonic steroid was off after another 300 ml. The column was clean after another 500 ml. of solvent was passed through it. The ketonic fraction amounted to 0.3 g. and the nonketonic fraction amounted to 1.87 g., giving a total of 2.17 g. recovered.

The analytical sample of XV acetate was crystallized from methanol, acetone, and finally hexane, giving long rods, m.p. 186–189°, $[\alpha]_D^{25}$ −83.7 (dioxane). Infrared analysis showed a shoulder at 3030 (Δ^5), 1738, 1245 (acetate), normal F-ring bands, 875 cm.$^{-1}$ (oxide).

Anal. Calcd. for $C_{29}H_{42}O_5$: C, 74.01; H, 9.00. Found: C, 73.72; H, 8.85.

Preparation of 11β,12β-epoxy-3β-hydroxy-5,16-pregnadiene-20-one (XXI). Two grams of XV was dissolved in 100 ml. of dioxane, containing 20 ml. of 3*N* hydrochloric acid and 4 ml. of water. The solution was stirred for 1 hr., then 200 ml. of water was added, and stirring continued for 1 hr. A viscous gummy residue resulted. The product (XVI) was isolated by ether extraction in the usual manner and the ether was evaporated *in vacuo*, giving a resin. Without further purification, the resin was placed in a 50-ml. flask, 5 ml. of acetic anhydride containing 0.1% acetic acid was added, the flask sealed, and heated at 170° for 2 hr. The reaction mixture was cooled to room temperature and the acetic anhydride–acetic acid solvent was evaporated *in vacuo*, giving a glassy residue (XVII). The residue was dissolved in 40 ml. of methanol, 2 g. of potassium hydroxide was added, and the solution sat overnight at room temperature. The reaction mixture was poured into 120 ml. of water and then extracted with ether. The ether was washed with water, dried over sodium sulfate, and evaporated to dryness *in vacuo* at room temperature.

The residue (XVIII) was dissolved in 10 ml. of pyridine and acetylated with 2.5 ml. of acetic anhydride at room temperature overnight. The diacetate XIX was isolated in the usual manner. Attempts to crystallize the compound were fruitless. Infrared showed peaks at 875 (oxide), 1735 and 1250 (diacetate), 1685 cm.$^{-1}$ (pseudosapogenin), and lack of F-ring bands.

The steroid (XIX) was dissolved in 50 ml. of acetic acid and the solution was cooled to 15°. Eight-tenths of a gram of chromium trioxide in 30 ml. of 50% acetic acid–water was cooled to 10° and then added to the steroid solution dropwise, with stirring, over a period of 15 min. The temperature of the reaction was maintained at 15° or less during the addition. The mixture was allowed to come to room temperature and stirred for 1 hr. The reaction mixture was drowned in 100 ml. water and extracted with ether (3 × 60 ml.). The ether was neutralized with sodium carbonate solution, washed with water, dried over sodium sulfate, and evaporated *in vacuo* at room temperature. The residue was dissolved in 50 ml. of *t*-butyl alcohol, 1 g. of potassium hydroxide in 2 ml. of water was added, and the mixture was stirred vigorously for 3 hr. The reaction mixture was poured into 200 ml. of water and extracted with ether, using the usual work-up. Evaporation to dryness *in vacuo* gave 1 g. of semicrystalline 11β,12β-epoxy-3-hydroxy-5,16-pregnadiene-20-one (XX). The analytical sample was recrystallized from acetone, hexane, and then methanol; m.p. 240–250° (sublimes off slide), $[\alpha]_D^{25}$ −29.0 (dioxane), log ε 3.92.

Anal. Calcd. for $C_{23}H_{30}O_4$: C, 76.79; H, 8.59. Found: C, 76.11; H, 8.96.

Infrared analysis (potassium bromide disk) showed bands at 3460 (hydroxyl), 1655 (conj. ketone), 875 (epoxide), and 808 cm.$^{-1}$ (Δ)^5.

Hydrogenation of 11β,12β-epoxy-3β-hydroxy-5,16-pregnadiene-20-one (XX). Three tenths of a gram of a 2% palladium–carbon catalyst was placed in 25 ml. of ethanol and allowed to absorb hydrogen at atmospheric pressure. When the hydrogen uptake ceased, 0.320 g. of XX was added and the hydrogenation proceeded at atmospheric pressure and

(24) M. E. Wall and H. W. Jones, *J. Am. Chem. Soc.*, 79, 3222 (1957).

room temperature. The hydrogenation was stopped after 15 min., at which time the hydrogen uptake was 1.1 moles. The solution was filtered to remove the catalyst and the ethanol was evaporated. The product, 11β,12β-epoxy-3β-hydroxy-5-pregnen-20-one (XXI) was crystallized from hexane, then methanol, m.p. 174–179°, no ultraviolet absorption. Infrared analysis (potassium bromide disk) showed bands at 3400 (hydroxy), 1697 (20-ketone), 875 (epoxy), and 808 cm.$^{-1}$ (Δ^5).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, SYRACUSE UNIVERSITY]

Monosaccharide Sulfates. I. Glucose 6-Sulfate. Preparations, Characterization of the Crystalline Potassium Salt, and Kinetic Studies[1]

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Speed of preparation and yield and purity of product are improved when glucose is sulfated directly using pyridine–sulfur trioxide in *N,N*-dimethylformamide. Purification of a directly sulfated glucose mixture ultimately leads to the crystalline, nonhygroscopic potassium salt of the 6-sulfate.

Two unequivocal syntheses of glucose 6-sulfate are described involving the removal of the protecting groups from 1) barium 1,2,3,4-tetra-*O*-acetyl-β-D-glucopyranose 6-sulfate and 2) barium 1,2;3,5-di-*O*-benzylidine-α-D-glucofuranose 6-sulfate.

Kinetic studies on the hydrolysis of the pure potassium salts of glucose 6-sulfate and ethyl sulfate were made, adapting the recently reported colorimetric determination of sulfate ions with barium chloranilate. The energy of activation for acid hydrolysis was determined for each compound. The effect of nitrogenous bases upon the hydrolysis of sulfate from unpurified glucose sulfate in buffered, slightly acid, or basic solution was determined.

Although glucose 6-sulfate has been known in varying degrees of purity for about forty years,[2–8] no one has succeeded in preparing a crystalline metallic salt. Soda and Egami[9] investigated the products of the direct sulfation of glucose and found, in addition to the 6-sulfate, a disulfate, which they concluded was the 1,6-sulfate. More recently, Dodgson and Spencer[10] reported a means of purifying the product of directly sulfated glucose by repeated recrystallization of the brucinium salt. Lloyd[11] has described a definitive synthesis for glucose 6-sulfate.

This paper describes first, some modifications of the direct method of synthesis and two indirect syntheses for glucose 6-sulfate, and, subsequently, characterization of the crystalline potassium salt and kinetic studies on the hydrolysis of the ester sulfate.

The most common direct sulfation procedure employs chlorosulfonic acid in chloroform-pyridine as the sulfating agent,[5] and results in a product which contains about 15% of glucose disulfate,[12] unless the purification technique of Dodgson and Spencer[10] is employed, in which case most of the disulfate is removed.

A more recent method[8] uses pyridine-sulfur trioxide in pyridine but gives a product containing up to 30% disulfate.[13] Furthermore, the use of barium carbonate does not insure complete removal of the pyridinium ion during the neutralization step.

The method finally worked out[14] for the direct sulfation of glucose employed pyridine–sulfur trioxide, with *N,N*-dimethylformamide as a solvent. The method minimizes polysulfation produced to a large extent by the heterogeneity of

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(14) Previously sulfamic acid was tried as a sulfating agent since it was known [M. E. Cupery, *Ind. Eng. Chem.*, **30**, 627 (1938)] to sulfate primary alcohols preferentially unless a suitable base was present [R. L. Burwell, Jr., *J. Am. Chem. Soc.*, **71**, 1769 (1949)]. However, as we later realized, the solvent, *N,N*-dimethylformamide, was basic enough to catalyze the sulfation of secondary alcohols, with the result that the products contained glucose disulfate. In addition, it was difficult to remove ammonium ion during neutralization and barium sulfamate after precipitation. Because of these factors, the method was not pursued further.