[CONTRIBUTION FROM THE RESEARCH LABORATORIES OF SYNTEX, S.A.]

Steroids. CXXX.[1] Synthesis of 12β-Hydroxyprednisolone 11β,12β-Acetonide 21-Acetate

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The synthesis of 12β-hydroxyprednisolone 11β,12β-acetonide 21-acetate is described by a multiple stage sequence based on hecogenin.

An important advance in the field of cortical hormone chemistry occurred when the Lederle group announced[2] that the introduction of a 16α-hydroxyl group in 9α-fluoroprednisolone eliminated the salt-retaining properties of the parent compound while still maintaining reasonable glucocorticoid and anti-inflammatory activities. A further increment in activity of this new class of compounds was more recently reported when Fried and co-workers[3] described the preparation of a number of 16α,17α-cyclic ketals and acetals in the 9α-fluoro-16α-hydroxyprednisolone acetate series. Indeed this enhancement of activity over that of the parent 16α-hydroxy compounds appears to be a general phenomenon of 9α-fluorinated corticoids since a similar increase in activity has now been observed in these laboratories following 16α-17α-acetonide formation in 6α-chloro-9α-fluoro-16α-hydroxyprednisolone 21-acetate.[4]

In the original paper[3] describing the cyclic ketals and acetals the authors noted their surprising resistance to acid hydrolysis as well as their altered physiological properties and suggested that the observed potentiations of activity were due to the cyclic ketal or acetal structures *per se* rather than to a slow *in vivo* hydrolysis to the parent compounds.

With these points in mind we were led[5] to investigate the activity of 1,2- and 6,7-glycols as well as the 6α,7α-acetonide in the prednisone acetate series and as a continuance of this project we now report the preparation of 12β-hydroxyprednisolone 21-acetate (Xb) and its corresponding 11β,12β-acetonide Xa.

Previous reports of structural modification at C-11 and C-12 in ring C of the steroid hormones have appeared. Amongst the first were the syntheses of 12α-hydroxy-compound S-acetate,[6] 12α-chloro-cortisone[7] and 12α-fluoro-11β-hydroxyprogesterone.[8] There then followed a paper describing[9] the preparation of 12α-fluoro, 12α-chloro, 12α-hydroxyl and 12β-methoxyl derivatives in the corticosterone series. In addition, substitution of an 11α-methyl group in both 11β-hydroxytestosterone[10a] and hydrocortisone[10b] has been recorded in the literature. More recently the ability of *Calonectria decora* to effect 12β-hydroxylation in addition to 15α-hydroxylation has been reported and it is in this work that the only account of a ring-C acetonide, namely 11β,12β,15α-trihydroxyprogesterone 11β,12β-acetonide, appears.[11]

As a synthetic entry to the desired 12β-oxygenated compounds the degradation of the spiroketal side chain of 11β,12β-dihydroxytigogenin 3,12-diacetate (Ic)[12] was investigated first. The yields encountered were fair, but the product obtained after the alkaline treatment stage (0.5) hr. reflux in acetone–potassium hydroxide proved to be a mixture of the diacetate IIc and the triacetate IId. The structures of these acetates followed from their analytical data as well as the fact that IIc could be converted to IId under mild acetylating conditions. Since these compounds proved extremely resistant to complete saponification (overnight reflux in potassium hydroxide–*t*-butyl alcohol), this approach was abandoned.

An improved route was afforded by the conversion of hecogenin ketol diacetate (Ia)[13] to allopregnane-3β,12β,17α-triol-11,20-dione (IVc), a sequence which had been previously described[14] from these laboratories. A more detailed study of this earlier degradation has now shown that the time required for the formation of the furostene may be lowered to 50 minutes at 200° instead of 14 hours.[14] In the original publication[14] Δ[16]-allopregnene-3β,12β-diol-11,20-dione diacetate (IIa) was epoxidized to provide IIIa which was then treated with hydrogen bromide to yield the bromohydrin IVa. Upon catalytic debromination the bromohydrin IVa was transformed to allopregnane-3β,12β,17α-triol-11,20-dione (IVc). In our hands the insolubility of the bromohydrin IVa complicated its reductive debromination on a large scale and for this reason we chose instead to work with the epoxide diacetate IIIb which is formed in quantitative yield on acetylation of the parent epoxide IIIa. Upon treatment with hydrogen bromide in acetic acid, IIIb smoothly provided the bromo-

(1) Paper CXXIX, A. Bowers, THIS JOURNAL, **81**, 4107 (1959).

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(3) J. Fried, A. Borman, W. B. Kessler, P. Grabowich and E. F. Sabo, *ibid.*, **80**, 2338 (1958).

(4) H. J. Ringold, O. Mancera, C. Djerassi, A. Bowers, E. Batres, H. Martínez, E. Necoechea, J. Edwards, M. Velasco, C. Casas Campillo and R. I. Dorfman, *ibid.*, **80**, 6464 (1958).

(5) J. A. Zderic, H. Carpio and C. Djerassi, *J. Org. Chem.*, **24**, 909 (1959).

(6) W. J. Adams, D. K. Patel, V. Petrow and I. A. Stuart-Webb, *J. Chem. Soc.*, 1825 (1954).

(7) J. Fried, J. E. Herz, E. F. Sabo and M. H. Morrison, *Chemistry & Industry*, 1232 (1956).

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(9) D. Taub, R. D. Hoffsommer and N. L. Wendler, *ibid.*, **79**, 452 (1957).

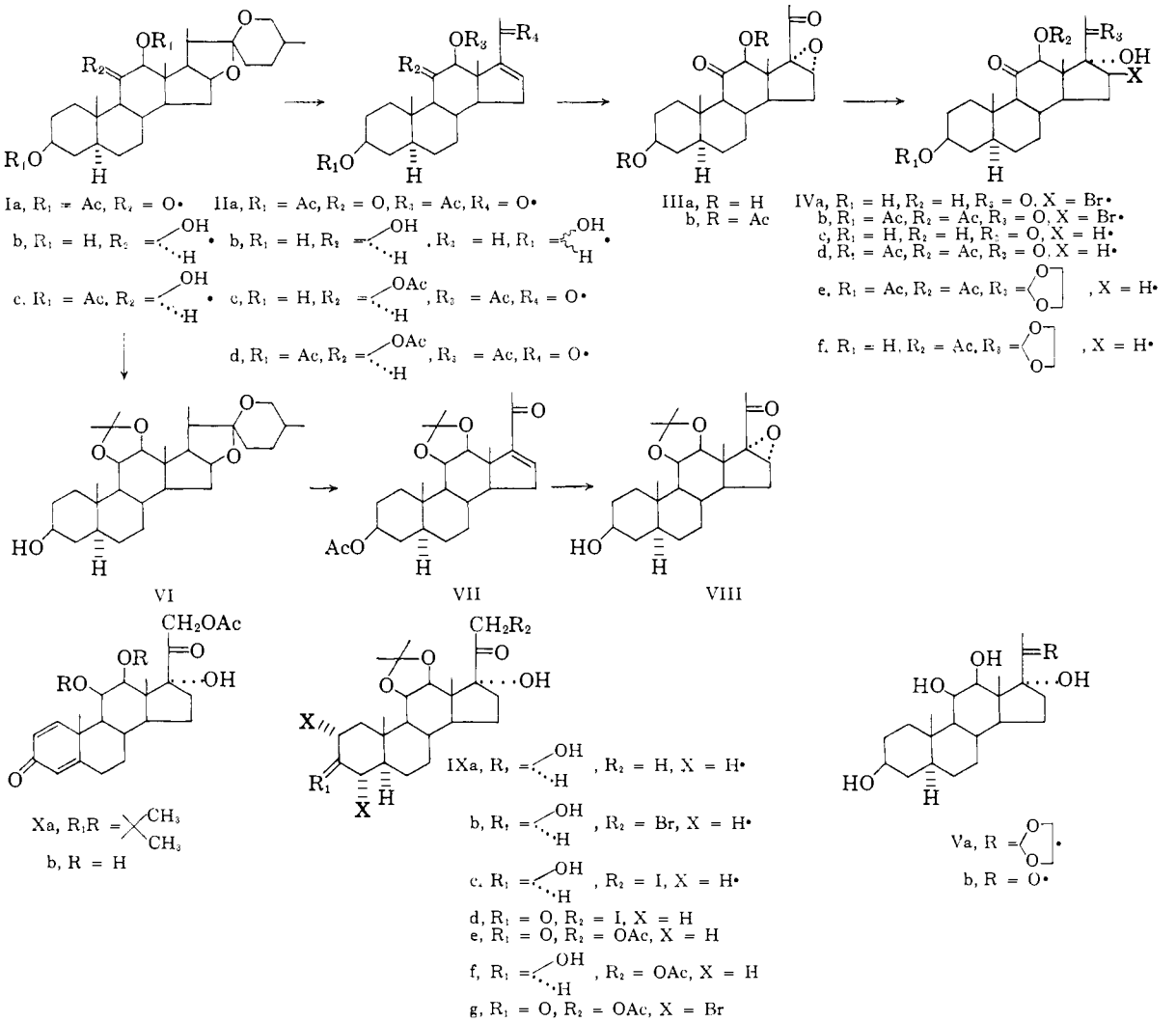
(10) (a) H. J. Ringold, E. Batres and J. A. Zderic, *Tetrahedron*, **2**, 164 (1958); (b) G. S. Fonken and J. A. Hogg, *ibid.*, **2**, 365 (1958).

(11) A. Schubert, R. Siebert and L. Koppe, *Angew.*, **70**, 742 (1958); no physical constants are recorded for the acetonide.

(12) C. Djerassi, H. Martínez and G. Rosenkranz, *J. Org. Chem.*, **16**, 1278 (1951).

(13) (a) C. Djerassi, H. Martínez and G. Rosenkranz, *ibid.*, **16**, 303 (1951); (b) J. Elks, G. H. Phillips, T. Walker and L. J. Wyman, *J. Chem. Soc.*, 4330 (1956).

(14) H. Martínez, H. J. Ringold, G. Rosenkranz and C. Djerassi, THIS JOURNAL, **75**, 239 (1953).



hydrin diacetate IVb, which could then be further converted in high yield to the triol diacetate IVd by the use of Raney nickel in boiling methanol.

When allopregnane-3β,12β,17α-triol-11,20-dione 3,12-diacetate (IVd) was submitted to the ethylene glycol ketalization procedure[15] the 20-ethylene ketal IVe could be prepared in excellent yield during small scale (0.5 g.) experiments. In larger preparations the course of the reaction was complicated due to partial hydrolysis of one of the acetate groups of the ketal IVe thus providing IVf. Following lithium aluminum hydride reduction of IVe or IVf the resulting tetrol ketal Va was hydrolyzed to allopregnane-3β,11β,12β,17α-tetrol-20-one (Vb) by the action of 3 *N* perchloric acid in tetrahydrofuran.[16]

An alternate synthesis of the tetrol-one Vb was eventually formulated when it was observed that the 11β,12β-acetonide of 11β,12β-dihydroxytigogenin (VI) was stable to the conditions of the sapogenin side chain degradation and thus led to Δ[16] - allopregnene - 3β,11β,12β - triol - 20 - one 11β,12β-acetonide (VII) in 40% yield. Following oxidation with alkaline hydrogen peroxide this substance was converted to its corresponding 16α,17α-epoxide VIII and then further transformed to a bromohydrin which was not characterized. Since upon treatment of this bromohydrin with Raney nickel the same tetrol-one Vb was obtained as previously described, it was obvious that during the epoxide-opening step with hydrogen bromide in acetic acid concomitant hydrolysis of the acetonide function had occurred.

Upon treatment of allopregnane-3β,11β,12β,17α-tetrol-20-one (Vb) in acetone with a few drops of 70% perchloric acid an almost quantitative yield of the corresponding acetonide IXa was obtained. It is interesting to note that virtually quantitative yields also occur in the conversion of Ib to VI and of 11β,12β-dihydroxydiosgenin to its corresponding 11β,12β-acetonide.[17] An exception to this facile acetonide formation appears in the case of Δ[16]-allopregnene-3β,11β,12β,20-tetrol (IIb) obtained by the lithium aluminum hydride reduction of IIa. With this compound under the above conditions only non-crystallizable gums were encountered.

(15) W. S. Allen, S. Bernstein and R. Littell, THIS JOURNAL, **76**, 6118 (1954).

(16) G. I. Poos, G. E. Arth, R. E. Beyler and L. H. Sarett, *ibid.*, **75**, 422 (1953).

(17) Unpublished observation.

Evidence that this reaction was not being complicated by dehydration of the 20-hydroxyl group was provided by the fact that the reaction product was free of any characteristic ultraviolet absorption such as might be expected from the resulting $\Delta^{16,20}$-diene.

In order to avoid hydrolysis of the acetonide moiety, allopregnane-3β,11β,12β,17α-tetrol-20-one 11β,12β-acetonide (IXa) was brominated in dry chloroform and after an initial induction period the bromination proceeded smoothly to provide the 21-bromo compound IXb. Upon treatment with sodium iodide in refluxing acetone[18] IXb provided fair yields of the corresponding 21-iodo compound IXc. While this compound was somewhat unstable when dry it could be oxidized with 8 *N* chromium trioxide in acetone[19] to provide the 3-keto-21-iodo compound IXd which was then transformed to the 21-acetate IXe by prolonged (48–65 hours) heating with anhydrous potassium acetate in acetone.

When the 3β-hydroxy-21-iodide IXc was treated directly with potassium acetate in refluxing acetone the reaction usually required nearly 6 days for completion. The resulting allopregnane-3β,11β,12β,17α,21-pentol-20-one 11β,12β-acetonide 21-acetate (IXf) could then be oxidized without purification to afford in 50% yield (based on IXb) the same 3-ketone IXe as that obtained from the acetoxylation of the 3-keto-21-iodo compound IXd. The very slow rate at which the 21-iodo compounds undergo displacement by acetate ions is noteworthy and is perhaps a reflection of new steric factors created by the presence of the acetonide moiety in ring C.

For the introduction of the $\Delta^{1,4}$-system two different methods were applied. Selenium dioxide oxidation of the tetrahydroprednisolone acetate analog IXe in boiling *t*-butyl alcohol[20] provided after 48 hours a 20% yield of the desired 12β-hydroxyprednisolone 11β,12β-acetonide 21-acetate (Xa). Alternatively, IXe was dibrominated[18,21] in tetrahydrofuran solution to yield 2α,4α-dibromoallopregnane-11β,12β,17α,21-tetrol-3,20-dione 11β,12β-acetonide 21-acetate (IXg) as a pure crystalline compound in *ca.* 40% yield. Dehydrobromination was then effected with collidine[18] to provide Xa which was identical in all respects with that obtained from the selenium dioxide oxidation.

Upon being submitted to a 15–30 minute hydrolysis in refluxing 60% aqueous formic acid the acetonide moiety of Xa was hydrolyzed to provide in low yield an amorphous product to which the structure of 12β-hydroxyprednisolone acetate (Xb) was assigned. This assignment followed not only from the infrared spectrum which indicated the presence of an acetate band as well as a very strong hydroxyl absorption but also from the fact that under the usual conditions of acetonide formation Xb could be reconverted to the crystalline and pure 11β,12β-acetonide of 12β-hydroxyprednisolone acetate.

Bioassays[22] of the acetonide Xa indicate that it possesses a low order of activity when compared to hydrocortisone acetate in terms of thymolytic and anti-inflammatory activity.

Experimental[23]

Side Chain Degradation of 11β,12β-Dihydroxytigogenin 3,12-Diacetate (Ic).—Three grams of 11β,12β-dihydroxytigogenin 3,12-diacetate (Ic)[12] and 15 ml. of acetic anhydride were placed in a sealed tube and heated for 9 hours at 200°. By following the general procedures for the oxidation and elimination reaction (*vide infra*) approximately 2.5 g. of gum was obtained which was directly chromatographed on 60 g. of neutral alumina without prior acetylation.

Elution with benzene–hexane (3:1) and pure benzene provided 0.58 g. of crystals, m.p. 195–200°, which were recrystallized twice from hexane to provide pure Δ^{16}-allopregnene-3β,11β,12β-triol-20-one triacetate (IId), m.p. 203–204°, $[\alpha]_D$ +39°; λ_{max} 234–236 mμ, log ϵ 3.92.

Anal. Calcd. for $C_{27}H_{38}O_7$: C, 68.33; H, 8.07; O, 23.60. Found: C, 68.06; H, 7.98; O, 23.73.

Further elution of the column with benzene–ether (4:1) gave an additional 0.28 g. of crystals, m.p. 175–185°. By recrystallization from acetone–ether there was obtained the analytical sample of Δ^{16}-allopregnene-3β,11β,12β-triol-20-one 11β,12β-diacetate (IIc),[24] m.p. 216–218°, $[\alpha]_D$ +24°; λ_{max} 234 mμ, log ϵ 3.92.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39; O, 22.19. Found: C, 69.12; H, 8.23; O, 22.38.

Fifty mg. of diacetate IIc was allowed to remain overnight in a solution containing 0.5 ml. each of pyridine and acetic anhydride. The solution was then diluted with water and the resultant crystals, 35 mg., m.p. 192–197°, were collected. After one recrystallization this substance proved to be identical in all respects with the triacetate IId described above.

Δ^{16}-Allopregnene-3β,12β-diol-11,20-dione Diacetate (IIa).—Hecogenin ketol diacetate (Ia)[13] (66 g.) was divided among 22 sealed tubes each containing 22.5 ml. of acetic anhydride and heated at 200° for 50 minutes. Following this, the contents of the tubes were combined and the excess acetic anhydride was hydrolyzed with 1.5 liters of water. The solution was then extracted with methylene chloride and the organic extracts were washed to neutrality first with aqueous bicarbonate solution and then water.

The residue obtained upon evaporation was dissolved in 1030 ml. of acetic acid and to it was added 830 ml. of dichloroethane and 388 ml. of water. After being cooled to 0° the stirred mixture was treated over 1.5 hours with a solution of 31.77 g. of chromium trioxide in 565 ml. of 90% acetic acid. The resulting solution was then maintained at room temperature for 2.25 hours after which time it was diluted with 3 l. of water. Following extraction with methylene chloride (2 × 3 l.) the organic extracts were washed with water, dried over sodium sulfate and evaporated to dryness. The non-crystalline residue was then dissolved in 660 ml. of acetone containing 33 g. of potassium hydroxide and 132 ml. of water, and heated on a steam-bath for 0.5 hr. At the end of this time most of the acetone was distilled, water was added and the mixture was extracted with ethyl acetate. The organic extract was washed neutral with water, dried and evaporated. The residue was then allowed to stand at room temperature for 21 hours, with 80 ml. of pyridine and 80 ml. of acetic anhydride.

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(21) R. M. Evans, J. C. Hamlet, J. S. Hunt, P. G. Jones, A. G. Long, J. F. Oughton, L. Stephenson, T. Walker and B. M. Wilson, *J. Chem. Soc.*, 4356 (1956).

(22) We wish to thank Dr. R. I. Dorfman of the Worcester Foundation, Shrewsbury, Mass., for these assays.

(23) All melting points are uncorrected and the rotations and ultraviolet spectra were determined in chloroform and 95% ethanol, respectively. The infrared spectra of all compounds have been recorded and are in agreement with the proposed structures. We are indebted to Dr. Lewis J. Throop and staff for the determination of the rotations and spectra.

(24) The assignment of the acetate groups at the 11- and 12-positions is arbitrary although these are the more likely positions to be hydrolysis resistant.

After dilution with water the mixture was ether extracted and the combined extracts were washed first with dilute acid and then with aqueous sodium bicarbonate and finally water. After drying and evaporation the residue was passed through 1.3 kg. of neutral alumina in benzene. Elution was continued with the same solvent until 22 l. had been collected. After evaporation, the residue was crystallized from ether–pentane to provide 23.7 g. of IIa, m.p. 223–225°, $[\alpha]_D \pm 0°$; λ_{max} 232 mμ, log ε 3.92; lit.[14] m.p. 214–216°, $[\alpha]_D$ +22°; λ_{max} 230 mμ, log ε 4.08.

Δ16-Allopregnene-3β,11β,12β,20-tetrol (IIb).—To 1 liter of anhydrous tetrahydrofuran containing 3 g. of lithium aluminum hydride was added 1.5 g. of IIa. The resulting mixture was allowed to reflux overnight whereafter the excess hydride was decomposed with ethyl acetate and saturated aqueous sodium sulfate. After evaporation the residue was chromatographed on 30 g. of neutral alumina. Elution with benzene–ether then provided 1.1 g. of IIb, m.p. *ca.* 170°, raised by recrystallization from ether–hexane to m.p. 186–187°, $[\alpha]_D$ +9°, no high selective ultraviolet absorption.

Anal. Calcd. for $C_{21}H_{34}O_4$: C, 71.96; H, 9.78; O, 18.26. Found: C, 71.74; H, 9.95; O, 17.75.

16α,17α-Oxidoallopregnane-3β,12β-diol-11,20-dione Diacetate (IIIb).—To 7 ml. of pyridine containing 3.5 ml. of acetic anhydride was added 2.55 g. of IIIa (obtained from IIa by the method reported in the literature[14]). After being kept at room temperature for 20 hours the solution was poured into cold water and the resultant crystals were collected. Upon recrystallization once from methanol 2.76 g. of solid, m.p. 192–195°, was obtained; upon further recrystallization from ether–hexane this material provided the analytical sample of IIIb, m.p. 196–197°, $[\alpha]_D$ +51°.

Anal. Calcd. for $C_{25}H_{34}O_7$: C, 67.24; H, 7.67; O, 25.09. Found: C, 66.85; H, 7.60; O, 25.31.

16β-Bromoallopregnane-3β,12β,17α-triol-11,20-dione 3,12-Diacetate (IVb).—A solution of 0.60 g. of IIIb in 6 ml. of glacial acetic acid was allowed to stand at room temperature for 75 minutes with 1.2 ml. of a solution of saturated hydrogen bromide in acetic acid. The solution was then diluted with water and extracted with methylene chloride. After washing first with aqueous sodium bicarbonate, then water, evaporation of the organic solvent gave a frothy residue which was easily crystallized from ether–hexane to provide 0.60 g. of the bromohydrin IVb, m.p. 178–182°. One further recrystallization then led to the pure sample, m.p. 180–182°, $[\alpha]_D$ +64°.

Anal. Calcd. for $C_{25}H_{35}O_7Br$: C, 56.93; H, 6.68; O, 21.23. Found: C, 56.90; H, 6.80; O, 21.22.

Allopregnane-3β,12β,17α-triol-11,20-dione 3,12-Diacetate (IVd).—A sample of the 16α,17α-epoxide diacetate IIIb (30 g.) was treated exactly as was described in the previous experiment. The resulting crude non-crystalline bromohydrin IVb was dissolved in 300 ml. of methanol and added all at once to 165 g. of W-4 Raney nickel[25] in 4.5 l. of refluxing methanol. After being stirred vigorously at reflux temperature for 2 hours, the mixture was cooled and filtered. The filter cake was then copiously washed with several liters of hot methanol. After combining the filtrates, they were evaporated to dryness and the residue was crystallized from ether–hexane to give 23 g. of crystals, m.p. 118–122°, which after one further recrystallization provided 19 g., m.p. 155–158°. Several more recrystallizations then gave the pure sample of IVd, m.p. 162–163°, $[\alpha]_D$ −23°.

Anal. Calcd. for $C_{25}H_{36}O_7$: C, 66.94; H, 8.09; O, 24.97. Found: C, 66.69; H, 8.05; O, 25.46.

Allopregnane-3β,12β,17α-triol-11,20-dione 3,12-Diacetate 20-Cycloethylene Ketal (IVe).—To 60 ml. of ethylene glycol containing 40 mg. of *p*-toluenesulfonic acid monohydrate was added 0.50 g. of IVd. The resulting mixture was then slowly distilled at 60–75° under reduced pressure for 1.5 hours.[15] At the end of this time the solution was made alkaline by the addition of methanolic potassium hydroxide and 150 ml. of water was added. The resulting crystals (0.47 g., m.p. 226–236°) were collected and recrystallized three times from acetone–hexane containing a drop of pyridine to yield IVe, m.p. 245–246°, $[\alpha]_D$ +15°.

(25) (a) H. Adkins and A. A. Pavlic, THIS JOURNAL, **69**, 3039 (1947); (b) A. A. Pavlic and H. Adkins, *ibid.*, **68**, 1471 (1946).

Anal. Calcd. for $C_{27}H_{40}O_8$: C, 65.83; H, 8.19; O, 25.98. Found: C, 65.46; H, 8.12; O, 26.11.

Allopregnane-3β,22β,17α-triol-11,20-dione 12-Acetate 20-Cycloethylene Ketal (IVf).[26]—Ethylene glycol (1275 ml.) containing 0.72 g. of *p*-toluenesulfonic acid monohydrate and 9.5 g. of IVd was treated as described in the preceding experiment. By dilution with water, 5.75 g. of IVe was obtained, m.p. 235–239°. Upon extraction of the mother liquors with methylene chloride and then washing the extract with water, a solution was obtained which upon drying and evaporation provided 2.25 g. of crystals, m.p. 209–212°. By repeated recrystallization from acetone–hexane the analytical sample was obtained, m.p. 233–234°, $[\alpha]_D$ +19°, strong hydroxyl absorption at 2.92 μ in the infrared.

Anal. Calcd. for $C_{25}H_{38}O_7$: C, 66.64; H, 8.50; O, 24.86. Found: C, 66.49; H, 8.25; O, 25.10.

Allopregnane-3β,11β,12β,17α-tetrol-20-one 20-Cycloethylene Ketal (Va).—A mixture of 150 ml. of tetrahydrofuran, 0.50 g. of IVe and 0.60 g. of lithium aluminum hydride was allowed to reflux for 4 hours. At the end of this time the excess reagent was destroyed by the addition of ethyl acetate and saturated aqueous sodium sulfate was added to the mixture. Upon evaporation and three recrystallizations from ethyl acetate–hexane there was obtained 0.22 g. of Va, m.p. 195–196°, $[\alpha]_D \pm 0°$, no carbonyl absorption in the infrared.

Anal. Calcd. for $C_{23}H_{38}O_6$: C, 67.29; H, 9.33; O, 23.38. Found: C, 67.00; H, 9.14; O, 23.55.

Allopregnane-3β,11β,12β,17α-tetrol-20-one (Vb). (A) From **Allopregnane-3β,12β,17α-triol-11,20-dione 3,12-Diacetate 20-Cycloethylene Ketal (IVe).**—In an experiment similar to that described above 5.75 g. of IVe was reduced in 500 ml. of tetrahydrofuran with 6.0 g. of lithium aluminum hydride. The resulting tetrol was then added, without purification, to 115 ml. of tetrahydrofuran containing 64 ml. of 3 *N* aqueous perchloric acid.[16] After 3 hours at room temperature the solution was neutralized by the addition of solid sodium bicarbonate and concentrated to *ca.* 25 ml. Following dilution with water the resultant crystals were collected to provide 4.0 g., m.p. 270–273°. Recrystallization from methanol then led to the analytical sample of Vb, m.p. 284–286°, $[\alpha]_D \pm 0°$ (dioxane).

Anal. Calcd. for $C_{21}H_{34}O_5$: C, 68.82; H, 9.35; O, 21.83. Found: C, 68.94; H, 8.94; O, 21.98.

(B) From **16α,17α-Oxidoallopregnane-3β,11β,12β-triol-20-one 11β,12β-Acetonide (VIII).**—To 2 ml. of acetic acid containing 0.20 g. of VIII was added 0.4 ml. of a saturated solution of hydrogen bromide in acetic acid. After 15 minutes at room temperature water was added and the solution was extracted several times with ethyl acetate. The extract was washed with aqueous sodium bicarbonate and finally to neutrality with water. After evaporation, the residue of oily crystals was dissolved in 30 ml. of methanol containing 3 g. of W-4 Raney nickel.[24] The mixture was then allowed to reflux for 2 hours whereafter filtration and evaporation yielded 60 mg. of a semi-solid. Upon crystallization from methanol this provided 30 mg. of material (m.p. 280–283°) identical in all respects with that prepared above in A.

11β,12β-Dihydroxytigogenin 11β,12β-Acetonide (VI).—A 4-g. sample of 11β,12β-dihydroxytigogenin (Ib)[12] was stirred at room temperature in 150 ml. of acetone containing 4.9 ml. of 70% aqueous perchloric acid. The solution was then made alkaline by the addition of 5 g. of solid sodium bicarbonate and 50 ml. of water. After concentration to *ca.* 60 ml. the mixture was filtered to provide 4.2 g. of slightly damp crystals, m.p. 205–209°. After two recrystallizations from acetone the analytical sample was obtained, m.p. 214–216°, $[\alpha]_D$ −73°.[27]

Anal. Calcd. for $C_{30}H_{48}O_5 \cdot {}^1/_2C_3H_6O$: C, 73.07; H, 9.93; O, 17.00. Found: C, 72.70; H, 9.89; O, 17.77.

Δ16-Allopregnene-3β,11β,12β-triol-3-one 3-Acetate 11β,12β-Acetonide (VII).—Three grams of the acetonide VI was

(26) Placement of the acetate function at C-12 rather than C-3 is predicated upon the known ease with which 3-acetates undergo hydrolysis.

(27) The infrared spectrum of this compound and all others containing the 11β,12β-acetonide grouping were characterized by a sharp strong band in the 11.4 μ region.

heated with 23 ml. of acetic anhydride in a sealed tube at 200° for 45 minutes. After this time, the tube was cooled and the excess anhydride was hydrolyzed with 100 ml. of water. After extraction with methylene chloride the extracts were washed with water, dried and evaporated. The residue was then dissolved in 46 ml. of acetic acid containing 38 ml. of dichloroethane and 18 ml. of water and the mixture was cooled to 0°. To it was then added slowly 1.36 g. of chromium trioxide in 25 ml. of 90% acetic acid and upon completion of the addition, the solution was allowed to stir at room temperature for 2 hours. Following dilution with an equal volume of water, the solution was extracted with methylene chloride and the extracts were washed neutral with water, dried and evaporated. The residue was then heated at reflux temperature for 30 minutes with 1.5 g. of potassium hydroxide, 6 ml. of water and 30 ml. of acetone. The solution was concentrated, diluted with more water and extracted several times with ethyl acetate. The combined extracts were then washed with water, dried and evaporated to provide a non-crystalline substance which was left at room temperature for 15 hours, with 5 ml. of pyridine and 5 ml. of acetic anhydride.

After dilution with water the solution was extracted with ether and the extracts, following several water washes, were dried and evaporated to leave 2.4 g. of non-crystalline material, λ_{max} 236 mm., log ϵ 3.72. This substance was adsorbed on 60 g. of neutral alumina from which it was eluted by benzene–hexane (25:75). By this means there was obtained 0.90 g. of crystals, m.p. 143–146°, which were repeatedly recrystallized from acetone–hexane to provide the analytical sample, m.p. 150–151°, $[\alpha]_D$ ±0°, λ_{max} 238 mμ, log ϵ 3.89.

Anal. Calcd. for $C_{26}H_{38}O_5$: C, 72.52; H, 8.90; O, 18.58. Found: C, 72.36; H, 8.79; O, 19.15.

16α,17α-Oxidoallopregnane-3β,11β,12β-triol-20-one 11β,12β-Acetonide (VIII).—Methanol (70 ml.) containing 0.68 g. of VII, 1.7 ml. of 30% hydrogen peroxide, 0.7 g. of sodium hydroxide and 0.7 ml. of water was maintained at 0° for 96 hours. The solution was then diluted with 350 ml. of methylene chloride and was washed to neutrality with water. After drying and evaporation there remained 0.65 g. of oil which was chromatographed on 13 g. of neutral alumina. Following elution with benzene–hexane (1:1) to benzene–ether (1:1) there was obtained 0.40 g. of crystals, m.p. 160–165°. Repeated recrystallization from acetone–hexane provided VIII, m.p. 178–179°, $[\alpha]_D$ +56°.

Anal. Calcd. for $C_{24}H_{36}O_5$: C, 71.25; H, 8.97; O, 19.78. Found: C, 71.08; H, 9.09; O, 19.72.

Allopregnane-3β,11β,12β,17α-tetrol-20-one 11β,12β-Acetonide (IXa).—To 400 ml. of acetone was added 4.0 g. of Vb and 4.9 ml. of 70% aqueous perchloric acid. After 20 minutes of stirring at room temperature the solution was completely homogeneous. Following a further 1.5 hours at room temperature 5 g. of solid sodium bicarbonate was added and the mixture was concentrated to *ca.* 50 ml. To it was then added 100 ml. of water whereafter filtration provided 3.87 g., m.p. 262–264°. After several recrystallizations from acetone the pure acetonide IXa was obtained, m.p. 272–274°, $[\alpha]_D$ −19°.

Anal. Calcd. for $C_{24}H_{38}O_5$: C, 70.90; H, 9.42; O, 19.68. Found: C, 71.10; H, 9.35; O, 19.64.

21-Bromoallopregnane-3β,11β,12β,17α-tetrol-20-one 11β,12β-Acetonide (IXb).—Chloroform (270 ml.) containing 2.72 g. of acetonide IXa was treated dropwise over a period of one hour with 1.17 g. of bromine in 30 ml. of chloroform. After an initial induction period of 10–15 minutes the bromination was fairly rapid. When the addition was complete the solution was poured into a saturated aqueous solution of sodium bicarbonate and then washed neutral with water. After drying and evaporation at reduced pressure there remained 3.15 g. of non-crystalline material. An aliquot of this substance (0.32 g.) was used to prepare the analytical sample. Upon crystallization from ether–hexane this provided 0.18 g., m.p. 238–240°, which then led to the analytical sample after only one recrystallization from the same solvent pair, m.p. 243–245°, $[\alpha]_D$ +38°.

Anal. Calcd. for $C_{24}H_{37}O_5Br$: Br, 16.47. Found: Br, 17.00.

Allopregnane-11β,12β,17α,21-tetrol-3,20-dione 11β,12β-Acetonide 21-Acetate (IXe). (A) *Via* **21-Iodoallopregnane-3β,11β,12β,17α-tetrol-20-one 11β,12β-Acetonide (IXc).**—To 900 ml. of acetone containing 7 g. of sodium iodide was added 10.0 g. of the 21-bromo compound IXb. After being heated at reflux temperature for 1.5 hours the solution was concentrated to 300 ml. and diluted with water. Following extraction with methylene chloride the extracts were washed several times with water, dried over sodium sulfate and evaporated to dryness leaving a semi-crystalline residue. An aliquot of the residue after seven recrystallizations from acetone provided a sample of **21-iodoallopregnane-3β,11β,12β,17α-tetrol-20-one 11β,12β-acetonide (IXc)**, m.p. 186–188° dec., $[\alpha]_D$ +41°.

Anal. Calcd. for $C_{24}H_{37}O_5I$: I, 23.84. Found: I, 15.45.[28]

The semi-crystalline residue from above was then taken up in 3 liters of acetone and to the resulting solution was added 100 g. of anhydrous potassium acetate. This mixture was then allowed to reflux for six days whereafter the excess potassium acetate was removed by filtration and the filtrate was evaporated to dryness leaving a mixture of oily crystals. At this stage by repeated recrystallization of a small aliquot from acetone–hexane pure **allopregnane-3β,11β,12β,17α,-21-pentol-20-one 11β,12β-acetonide 21-acetate (IXf)** could be obtained, m.p. 236–238°, $[\alpha]_D$ +36°.

Anal. Calcd. for $C_{26}H_{40}O_7$: C, 67.21; H, 8.68; O, 24.11. Found: C, 67.80; H, 8.39; O, 23.62.

The residual oily crystals described above were then dissolved in 300 ml. of pure acetone (distilled from potassium permanganate and cooled to 0°. Several ml. of 8 *N* chromium trioxide solution[19] was then added with constant stirring. After 8 minutes 1 l. of water was added and the solution was thoroughly extracted with five 500-ml. portions of methylene chloride. The combined extracts were washed 3 times with 200 ml. of water, dried over sodium sulfate and evaporated to dryness. By crystallization from acetone–hexane 3.55 g. of the 3-one 21-acetate IXe, m.p. 210–212°, was obtained followed by a second crop of 1.45 g., m.p. 205–207°. This material was identical in all respects with authentic IXe obtained below.

(B) **From 21-Iodoallopregnane-11β,12β,17α-triol-3,20-dione 11β,12β-Acetonide (IXd).**—Acetone (100 ml.) containing 1.0 g. of anhydrous potassium acetate and 100 mg. of IXd was allowed to reflux with vigorous stirring for 72 hours. The mixture was then filtered and the filtrate was evaporated to dryness. The crystalline residue, 85 mg., m.p. 201–203°, was then recrystallized twice from acetone–hexane to yield pure IXe, m.p. 212–214°, $[\alpha]_D$ +86°.

Anal. Calcd. for $C_{26}H_{38}O_7$: C, 67.51; H, 8.28; O, 24.21. Found: C, 67.58; H, 8.30; O, 23.59.

21-Iodoallopregnane-11β,12β,17α-triol-3,20-dione 11β,12β-Acetonide (IXd).—To 50 ml. of acetone containing 2.1 g. of IXc was added 1 ml. of 8 *N* chromium trioxide solution.[19] The solution was then vigorously stirred for 2 minutes at 0° whereafter an additional 1 ml. of reagent was added. After 2 more min. the solution was diluted with water (100 ml.) and extracted with methylene chloride. Following washings first with aqueous sodium bicarbonate and then water, the solvent was dried and evaporated to leave 1.9 g. of a colored froth. After treatment with carbon in acetone the material was crystallized from acetone–hexane to provide 1.1 g., m.p. 167–170° dec. Repeated recrystallization from the same solvent pair provided the final sample, m.p. 176–178° dec., $[\alpha]_D$ −70°.

Anal. Calcd. for $C_{24}H_{35}O_5I$: I, 23.93. Found: I, 17.44.[27]

2α,4α-Dibromoallopregnane-11β,12β,17α,21-tetrol-3,20-dione 11β,12β-Acetonide 21-Acetate (IXg).—A solution of 200 mg. of IXe in 2 ml. of anhydrous tetrahydrofuran containing 0.2 ml. of glacial acetic acid and 1 drop of acetic acid saturated with hydrogen bromide was treated slowly with 145 mg. of bromine. After 10 min. the bromine color was completely discharged and the solution was diluted with 5 ml. of water. Following extraction with methylene chloride the combined extracts were washed with aqueous sodium bicarbonate and water and then dried over sodium sulfate and evaporated to leave 240 mg. of non-crystalline material. By one crystallization from acetone–hexane there was obtained 100 mg. of pure dibromo compound IXg, m.p. 200–202°, $[\alpha]_D$ +67°.

(28) Presumably due to the rapid decompositions of IXc and IXd, satisfactory analyses could not be obtained.

Anal. Calcd. for $C_{26}H_{36}O_7Br_2$: C, 50.34; H, 5.85; O, 18.05. Found: C, 50.12; H, 5.62; O, 18.39.

12β-Hydroxyprednisolone 11β,12β-Acetonide 21-Acetate (Xa). (A) From the Dibromo Compound IXg.—To 2 ml. of collidine was added 250 mg. of IXg and the resulting solution was allowed to reflux for 1.25 hours under an atmosphere of nitrogen. At the end of this time the total mixture was taken up in 50 ml. of ethyl acetate and the solution was then washed several times with 2% hydrochloric acid. After washing with aqueous sodium bicarbonate solution followed by water, the solvent was dried and evaporated to leave 200 mg. of gum. Following adsorption on 4 g. of silica gel and elution with benzene–acetone (19:1) there was obtained 75 mg. of crystals, m.p. 238–250°. Repeated recrystallization from acetone–ether then provided 25 mg. of Xa, m.p. 273–276°, identical in all respects with that obtained below in B.

(B) By Selenium Dioxide Oxidation of IXe.—Three hundred mg. of IXe was allowed to reflux for 48 hours in 20 ml. of *t*-butyl alcohol containing 0.05 ml. of pyridine and 0.2 g. of selenium dioxide. At the end of this time an additional 0.2 g. of selenium dioxide was added and the heating was continued for another 48 hours. Following evaporation, the residue was triturated with water and then dissolved in benzene. After being chromatographed twice over silica gel, elution with benzene–acetone (19:1) provided 70 mg. of oily crystals. Three recrystallizations from acetone–ether then led to 50 mg. of crystals, m.p. 274–276°, $[\alpha]_D$ +131°, λ_{max} 242 mμ, log ε 4.24; λ_{max}^{KBr} 3.02(s), 5.71(vs), 5.79(vs), 6.01(vs), 6.17(vs), 11.38(s) μ.[26]

Anal. Calcd. for $C_{26}H_{34}O_7$: C, 68.10; H, 7.47; O, 24.43. Found: C, 67.75; H, 7.55; O, 24.52.

12β-Hydroxyprednisolone 21-Acetate (Xb).—A solution of 60% aqueous formic acid (9.2 ml.) and 370 mg. of Xa was allowed to reflux for 20 min., whereafter it was evaporated almost to dryness under reduced pressure and the residue was dissolved in 50 ml. of methylene chloride. The resulting solution was washed several times with aqueous sodium bicarbonate solution and finally with water. After drying and evaporation there remained 270 mg. of a colorless froth which could not be obtained crystalline from any of the ordinary solvent systems even after careful chromatography. Using methylene chloride–ether the material could be obtained as a gel which upon drying exhibited melting points varying from m.p. 150–159° to m.p. 170–177°, $[\alpha]_D$ +78°, λ_{max} 242 mμ, log ε 4.14; λ_{max}^{KBr} 2.92(vs, broad), 5.80(vs, broad), 6.02(vs), 6.20(s) and 8.00(s) μ. The characteristic band at 11.4 μ associated with the 11β,12β-acetonide moiety[26] was no longer present. Paper chromatography indicated that the substance was considerably more polar than a standard of prednisolone acetate and that it contained a small amount of still more polar material, presumably the 21-acetate free tetrol.

Proof that no major structural change had occurred was forthcoming when it was observed that 50 mg. of the amorphous material upon treatment with acetone (0.5 ml.) containing 70% perchloric acid (2 drops) for 2 hours at room temperature provided after chromatography over silica gel *ca.* 10 mg. of Xa, m.p. 250–258°. A single recrystallization from acetone–ether gave m.p. 271–274°. The infrared spectrum of this compound was identical with that of authentic Xa and a mixture melting point was not depressed.

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[CONTRIBUTION FROM THE OLYMPIC RESEARCH DIVISION, RAYONIER, INC.]

The Nature of a Galactoglucomannan Associated with Wood Cellulose from Southern Pine[1a,b]

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A mixture of galactoglucomannans and araboxylans was extracted with 5% sodium hydroxide from wood cellulose produced from a 1:1 combination of slash pine (*Pinus elliottii* var. elliottii) and longleaf pine (*Pinus palustris* Mill) by the conventional kraft process. These polysaccharides were acetylated and readily separated into an acetone-insoluble araboxylan acetate and an acetone-soluble galactoglucomannan acetate in which the ratio of D-galactose:D-glucose:D-mannose was approximately 1:1:3. Methylation of the galactoglucomannan acetate yielded a product (44.3% OMe, $[\alpha]^{25}_D$ +9.5°, *c* 3, $CHCl_3$) that was essentially homogeneous as shown by fractional precipitation studies. Hydrolysis of the methylated polymer gave 2,3-di-*O*-methyl-D-mannose (10.0%), 2,3-di-*O*-methyl-D-glucose (3.2%), 2,3,6-tri-*O*-methyl-D-mannose (55.0%), 2,3,6-tri-*O*-methyl-D-glucose (13.2%), 2,3,4,6-tetra-*O*-methyl-D-mannose (3.2%) and 2,3,4,6-tetra-*O*-methyl-D-galactose (15.3%). Periodate oxidation and formic acid production studies were in good agreement with the branched structure indicated by methylation. Qualitative graded acid hydrolysis studies are also presented. The structural significance of these findings and the relationship of this family of polysaccharides to the coniferous wood cellulose system in general is discussed.

Coniferous and decidous woods have both been shown to contain true glucomannans.[2] In the case of the deciduous woods, the glucomannan, although representing what is probably the predominant mannose-containing polymer, constitutes only a minor portion of the total hemicellulosic material.[3] The mannose-containing polysaccharides of conifers, however, represent the major hemicellulosic component of these woods. About half of the anhydromannose units of a number of conifers has been shown to occur as an essentially linear polymer composed of glucose and mannose in which the ratio of these sugars is about 1 to 3.[2–6] Investigations of other conifers have shown that glucomannan polymers may be isolated which contain a very high percentage of anhydromannose units joined to only a few glucose units.[2,6,7] However, glucomannans are not the only mannose-containing polysaccharides present in coniferous woods since mannose-containing polymers also have been shown to be associated with galactose.[2,6]

(1) (a) Contribution No. 47 from the Olympic Research Division, Rayonier Incorporated, Shelton, Wash. (b) Presented at the Northwest Regional Meeting of the American Chemical Society, Seattle, Wash., June, 1959.

(2) J. K. Hamilton and N. S. Thompson, *Pulp and Paper Mag. Can.*, **59**, (10) 233 (1958). [A review of the hemicelluloses of hardwoods and softwoods is included in this paper.]

(3) The alder glucomannan described in ref. 2 was erroneously given a specific rotation of −51.5°; however, it has been found that this polymer from both alder holocellulose and semichemical wood cellulose has a rotation very close to −32°.

(4) J. K. Hamilton, H. W. Kircher and N. S. Thompson, THIS JOURNAL, **78**, 2508 (1956).

(5) J. K. Hamilton and H. W. Kircher, *ibid.*, **80**, 4703 (1958).

(6) J. K. Hamilton and E. V. Partlow, *ibid.*, **80**, 4880 (1958).

(7) G. G. S. Dutton and K. Hunt, *ibid.*, **80**, 5697 (1958).