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

# Steroidal Sapogenins. LXV. Hydrogenation Products of 3β,20-Diacetoxy-5α,Δ$^{16,20(21)}$-pregnadien-12-one[2]

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Reaction of the 12-keto-16-dehydropregnene, I, with isopropenyl acetate gave the conjugated 12-keto-20-enol acetate, II. Hydrogenation of II in the presence of a palladium catalyst yielded the monounsaturated steroid, III. Hydrogenation of III in the presence of a platinum oxide catalyst under acid conditions gave a mixture which was shown to consist of the 12,20-desoxypregnane, IV, and 12-keto-20-desoxypregnane, V, and the 12-keto-20-acetate, VI.

During studies on reactions of 16-dehydro-12,20-diketopregnenes, we found it necessary to investigate the hydrogenation of 3β,20-diacetoxy-5α,Δ$^{16,20(21)}$ pregnadiene-12-one, II. The 12-keto-enol acetate, II, could be prepared in 75% yield by refluxing 3β-acetoxy-5α,16-pregnene-12,20-dione,[3] I, with isopropenyl acetate in the presence of catalytic quantities of concentrated sulfuric acid.[4a,b] Under our experimental conditions, the 12-ketone did not form an enol acetate. The inertness of the 12-ketone toward enolization has been noted in the bile acid series.[5] The structure proof of the enol acetate, II, was obtained from the following data. The carbon and hydrogen analysis was in agreement with the indicated structure. The ultraviolet spectrum of II showed a maximum at 237 mμ, ε = 12,680 and a general shape similar to that observed by Moffett and Weisblat[4a] for 3β,20-diacetoxy-5α,Δ$^{16,20(21)}$-pregnadiene. The infrared spectrum of II (*cf.* Experimental) exhibited three strong bands in the carbonyl region which were observed at the correct wave numbers for enol acetate, 3β-acetate, and 12-ketone, respectively. Catalytic hydrogenation of II with 5% palladium-carbon catalyst indicated uptake of only one mole of hydrogen to give 3β-20-diacetoxy-5α.Δ$^{16}$-pregnene-12-one, III. The fact that the Δ$^{20(21)}$ double bond had been preferentially hydrogenated rather than the Δ$^{16}$-group was proved by the disappearance of the characteristic enol acetate band[4b] and bands attributable to C = C stretching vibrations of an enol acetate with a terminal ethylenic group.[4b] Although the Δ$^{16}$-double bond in III was resistant toward hydrogenation under neutral conditions, it could be reduced by use of platinum oxide in the presence of 5% acetic acid. Under these conditions the 12-ketone was also reduced. Treatment of the noncrystalline reduction product with chromium trioxide in acetic acid oxidized the 12-hydroxyl group formed during the hydrogenation of III. The infrared spectrum of the glassy oxidation product showed absence of hydroxyl. However, the intensity of the band at 1710 cm.$^{-1}$ attributed to the 12-carbonyl group was considerably weaker than anticipated, thus indicating that we might be dealing with a mixture of ketonic and nonketonic steroids. This hypothesis was confirmed by a Girard T separation which gave ketonic and nonketonic fractions in a 2:1 ratio, respectively. After acetylation the nonketonic fraction was identified as 3β-acetoxy-5α-pregnane,[6a,b] IV. The infrared spectrum of IV was identical with that published by R. N. Jones and coworkers.[7]

Alkaline hydrolysis of the ketonic fraction followed by chromatography on Florisil[8] separated this fraction into mono- and dihydroxy components in 1:1 ratio. Wolff-Kishner reduction of the monohydroxy compound followed by acetylation gave the known 3β-acetoxy-5α-pregnane,[6] IV. Hence, the structure of the ketone from which IV was derived must be 3β-acetoxy-5α-pregnan-12-one, V. The carbon and hydrogen analysis, infrared spectrum, and optical rotation data[9] observed for V were all in accord with the assigned structure.[10]

Wolff-Kishner reduction of the dihydroxy component of the ketonic fraction followed by acetylation gave the known 3β,20α-diacetoxy-5α-preg-

(1) Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture. Article not copyrighted.

(2) Previous paper in this series, Steroidal Sapogenins. LXIV. ....., Edward S. Rothman, Theodore Perlstein, and Monroe E. Wall, *J. Org. Chem.*, in press.

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

(4) (a) R. B. Moffett and D. I. Weisblat, *J. Am. Chem. Soc.*, **74**, 2183 (1952). (b) H. Vanderhaeghe, E. R. Katzenellenbogen, K. Dobriner, and T. F. Gallagher, *J. Am. Chem. Soc.*, **74**, 2810 (1952).

(5) R. Hirschmann, M. Brown, and N. L. Wendler, *J. Am. Chem. Soc.*, **73**, 5373 (1951).

(6) (a) Huang-Minlon, *J. Am. Chem. Soc.*, **71**, 3301 (1949). (b) A. Petit and J. P. Mathieu, *Constantes Selectionées Pouvoir Rotatoire Naturel I. Steroides*, Masson et cie, Paris, 1956.

(7) G. Roberts, B. S. Gallagher, and R. N. Jones, *Infrared Absorption Spectra of Steroids. An Atlas.* Vol. II, chart 337, 338. Interscience Publishers, Inc., New York, 1958.

(8) Mention of trade names does not imply preference over any equivalent product.

(9) According to Barton and Klyne, *Chem. and Ind.*, 755 (1948), the average molecular rotation contribution of a 12 ketone is +270. $M_D$ of V − $M_D$ IV = +321.

(10) The remote possibility that V might have the structure 3β-acetoxy-5α-pregnane-20-one, a known compound, was ruled out by direct comparison of the infrared spectra which showed nonidentity, particularly throughout the "fingerprint" region.

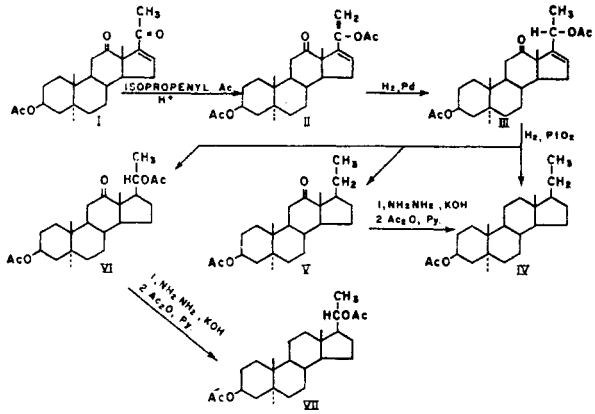

Figure 1

nane, VII,[11] with an infrared spectrum identical with that of a published spectrum.[12] Hence, the ketone from which VII was derived must be 3β,20α-diacetoxy-5α-pregnan-12-one, VI. The corresponding 20β-isomer was not isolated. However, the yield of crystalline VII from the sequence II → III → VI → VII was low. Thus the possibility that the 20β-isomer was also formed during the reduction of II to III cannot be rigidly excluded.

The saturated 12-keto diacetate, VI, was the expected reduction product. However, the $\Delta^{16}$-double bond in III exhibited sluggish hydrogenation properties. For example, hydrogenation of III with platinum oxide as a catalyst and in the presence of 0.2% acetic acid resulted in reduction of the 12-ketone to hydroxyl with no attack on the double bond.[13] Reduction of the double bond occurred only after prolonged hydrogenation in the presence of platinum oxide and with use of 5% acetic acid. The hydrogenolysis of the allylic 20-acetate group resulting in formation of the 12-keto monoacetate, V, is analogous to the hydrogenolysis of 12-substituents (hydroxyl, methoxyl, acetate) allylic to the sluggishly reducible $\Delta^{9(11)}$ double bond.[14] We have been unable to find any precedent for the hydrogenolysis of the 12-ketone resulting in formation of IV during the reduction of III.[15]

## EXPERIMENTAL[16]

*3β,20-Diacetoxy-5α,$\Delta^{16,20(21)}$-pregnadiene-12-one*, II. A solution of 3.43 g. of 3β-acetoxy-5α,$\Delta^{16}$-pregnene-12,20-dione, I, (m.p. 177–180°) in 20 ml. of isopropenyl acetate containing 0.02 ml. of concd. sulfuric acid was heated under reflux for 3 hr. The solution was cooled, diluted with 30 ml. of toluene, and washed with dilute aqueous sodium bicarbonate solution followed by washing with a saturated sodium chloride solution and then dried over anhydrous sodium sulfate. On concentration of the solvent, 3.8 g. of a crude, solid residue was obtained. This was taken up in 25 ml. of benzene, filtered through 4 g. of Florisil,[8] and the concentrated eluate crystallized from freshly distilled petroleum ether (b.p. 88–98°) giving 2.92 g. of II, m.p. 166–170° (yield 75%). The analytical sample was recrystallized from methanol, needles m.p. 167.5–168.5°, $[\alpha]_D^{25}$ +201°, $\lambda_{max}$ (methanol) 237 mμ, $\epsilon$ = 12,680 log $\epsilon$ = 4.1, infrared spectrum shows three strong bands of approximately equal intensity at 1758, 1733, and 1712 cm.$^{-1}$ attributed to the enol acetate,[4b] 3β-acetate, and 12-ketone, respectively, and strong bands at 1240–1245 and 1205 cm.$^{-1}$ due to C—O stretching vibrations of the 3β- and 20-acetoxy moieties respectively.[17] In addition bands were noted at 3030 and 1640 cm.$^{-1}$ due respectively to CH stretching and C=C stretching vibrations.[17]

*Anal.* Calcd. for $C_{25}H_{34}O_5$: C, 72.43; H, 8.27. Found: C, 72.64; H, 8.23.

*3β,20-Diacetoxy-5α,$\Delta^{16}$-pregnen-12-one*, III. A solution of 0.212 g. of the enol acetate II in 10 ml. of ethyl acetate was hydrogenated at 1 atm. in the presence of 0.05 g. of 5% palladium on carbon. The sample took up 14.4 ml. of hydrogen in 16 min. (114%). The sample was filtered and the filtrate concentrated. The residue was crystallized from methanol to give 0.067 g. of needles, m.p. 184–188°. The analytical sample melted at 195–196°, $[\alpha]_D^{25}$ +120°, ultraviolet spectrum showed only end absorption; the infrared absorption spectrum shows a strong broad band at 1740–1735 and a somewhat weaker band at 1712 cm.$^{-1}$, the former attributed to the 3β- and the 20-acetoxy groups and the latter to the 12-ketone; and three strong bands at 1245, 1220, and 1165 cm.$^{-1}$, the first being attributed to the C—O stretching vibration of the 3β-acetate and the last two bands to C—O stretching vibrations associated with the $\Delta^{16}$-20-acetate moiety.

*Anal.* Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 72.04; H, 8.64.

(11) R. B. Turner and D. M. Voitle, *J. Am. Chem. Soc.*, **73**, 2283 (1951).

(12) K. Dobriner, E. R. Katzenellenbogen, and R. N. Jones, *Infrared Absorption Spectra of Steroids. An Atlas.* Vol. 1, chart 75. Interscience Publishers, Inc., New York, 1953.

(13) The presence of the $\Delta^{16}$-20-acetoxy moiety in the 12-keto diacetate, III and its 12-hydroxy analogue can be readily determined by examination of the infrared spectra in the region 1250–1150 cm.$^{-1}$ In this region steroids with the $\Delta^{16}$-20-acetate grouping show three strong bands at 1245, 1220, and 1165 cm.$^{-1}$, the band at 1245 being attributable to the C—O—C stretching vibrations of the 3β-acetate. Saturation of the $\Delta^{16}$-double bond of III or the 12-hydroxy analogue results in disappearance of the 1220 and 1165 cm.$^{-1}$ bands, and one strong broad band between 1250–1240 cm.$^{-1}$ is observed.

(14) B. McKenzie, V. Mattox, and E. Kendall, *J. Biol. Chem.*, **175**, 249 (1948).

(15) The intermediate in the hydrogenolysis of the 12-ketone may be the 12β-hydroxyl group as the ketone is reduced much more rapidly than the $\Delta^{16}$-double bond (*cf.* experimental on hydrogenation of III, part (a)). Because of the unprecedented nature of the hydrogenolysis at $C_{12}$ and because of the fact that of necessity "working grade" products rather than analytically pure substances were used for the reaction sequences, the 16-dehydro pregnene, I, the enol acetate II, and the partially hydrogenated steroid III were carefully examined for 12-desoxy-contaminants which could account for the 12-desoxy pregnane, IV. In all cases the infrared spectra of the working grade products I, II and III showed the same ratio of the intensities of the 12-carbonyl to the acetate band as did the analytical samples. Only after catalytic reduction of III was there observed a marked decrease in the intensity of the 12-carbonyl band of the crude reduction product.

(16) All infrared spectra were obtained in carbon bisulfide solution, concentration 10.0 g./l.; ultraviolet spectra in methanol, 0.04 g./l., and optical rotation data in chloroform. We wish to thank S. Serota for the optical rotation data and R. Kelley for the carbon and hydrogen determinations.

(17) L. J. Bellamy, *The Infrared Spectra of Complex Molecules*, John Wiley and Sons, New York (1954), pp. 31 et seq.

*Catalytic hydrogenation of* III. (a) *With platinum oxide in 0.2% acetic acid.* A solution of 0.5 g. of III in 25 ml. of ethyl acetate containing 0.05 ml. of acetic acid was hydrogenated at 3 atm. in presence of 0.5 g. of platinum oxide for 3 hr. After the usual workup the crude product was isolated. The infrared spectrum showed presence of hydroxyl (3600 cm.$^{-1}$); acetate (1735 cm.$^{-1}$); absence of 12-ketone and presence of the same three strong bands in the 1250–1165 cm.$^{-1}$ discussed under III.

(b) *With platinum oxide in the presence of 5% acetic acid.* A solution of 5.0 g. of III in 240 ml. of ethyl acetate containing 5% acetic acid was hydrogenated at 3 atm. for 18 hr. in the presence of 5.0 g. of platinum oxide. The solvent was evaporated and the glassy product was oxidized with chromium trioxide in acetic acid at room temperature in the usual manner. The product, weighing 4.77 g., was examined by infrared spectroscopy and showed absence of hydroxyl, a strong band at 1735 cm.$^{-1}$ and a considerably weaker band at 1710 cm.$^{-1}$ Only one strong band at 1245 cm.$^{-1}$ was present in the 1250–1165 region.

*3β-Acetoxy-5α-pregnane,* IV. The total crude product obtained by method (b) above (4.77 g.) was treated with Girard T reagent. The noncarbonyl fraction, 1.5 g., was crystallized from acetone to give 0.8 g. of IV, m.p. 114–116°, $[\alpha]_D^{25}$ +3.9° (lit.,[6a,b] gives m.p. 115–116°, $[\alpha]_D^{19}$ +6°), infrared spectrum shows 1735 cm.$^{-1}$ and 1245 cm.$^{-1}$ bands.

*3β-Acetoxy-5α-pregnan-12-one,* V. The carbonyl fraction from the Girard T separation (3.0 g.) was deacetylated by hydrolysis with refluxing 5% potassium hydroxide in methanol. The residue after standard work-up was dissolved in benzene and chromatographed on Florisil. Elution with 20% methylene chloride in benzene and 100% methylene chloride gave 1.2 g. of a monohydroxy product. Acetylation of this compound followed by crystallization from ethanol-water gave V, m.p. 139–140°, $[\alpha]_D^{25}$ +95°; infrared spectrum shows two strong bands at 1735 and 1706 cm.$^{-1}$, one band at 1243 cm.$^{-1}$

*Anal.* Calcd. for $C_{23}H_{36}O_3$: C, 76.62; H, 10.07. Found: C, 76.84; H, 10.33.

*Conversion of* V *to* IV. Wolff-Kishner reduction of 0.3 g. of V by the Huang-Minlon procedure[6] yielded, after workup and acetylation, 0.15 g. IV, m.p. 113–115°, infrared spectrum identical with that of IV isolated from hydrogenation of III.

*3β,20α-Diacetoxy-5α-pregnan-12-one,* VI. After removal of the monohydroxy fraction described under V, elution with 5% ethanol in benzene gave 1.3 g. of a dihydroxy compound. Acetylation yielded 0.9 g. of VI, m.p. 181–183°, $[\alpha]_D^{25}$ +69°; infrared spectrum shows strong broad band at 1735–1730 cm.$^{-1}$ and a strong but less broad band at 1706 cm.$^{-1}$, and one strong broad band at 1250–1240 cm.$^{-1}$

*Anal.* Calcd. for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15. Found: C, 71.54; H, 9.04.

*3β,20α-Diacetoxy-5α-pregnane,* VII. A 0.36-g. sample of the 12-ketone VI was reduced by the Huang-Minlon modification[6] of the Wolff-Kishner procedure. After acetylation, 0.2 g. of VII was obtained, m.p. 163–165° (lit.,[11] gives m.p. 165–167°), with an infrared spectrum showing one strong band at 1735 cm.$^{-1}$ and one broad band at 1250–1240 cm.$^{-1}$ The entire infrared spectrum was identical with that of VII given in reference 12.

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[CONTRIBUTION FROM THE UNIT OF NATURAL PRODUCTS, NATIONAL RESEARCH CENTRE]

# Natural Coumarins. I. Marmesin and Marmesinin, Further Products from the Fruits of *Ammi majus* L.

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The isolation of marmesinin and its synthesis are described. Several reactions with marmesin and its derivatives establish that bromination and nitration occur at the six position and the same is suggested to happen with nodakenetin and analogous dihydrofurocoumarins. A correlation has been made between marmesin and products derived from peucedanin.

In a preliminary communication,[1] we reported the isolation of marmesin[2] (I, R = H), in 0.25% yield, from the alcoholic extract of defatted *A. majus* fruits, after mineral acid hydrolysis, and its presence in the fruits as a glycoside has been alluded to. In a more recent publication[3] the isolation of this glycoside, in fact a glucoside, from the same source has been described and the name "ammajin" was given to it. With the unfortunate[4] names ammoidin, ammidin, and majudin given,[5] on the basis of the botanical name of the source, to the constituents of *Ammi majus* L. before they were realized[6] to be the already well known xanthotoxin, imperatorin, and bergapten respectively having led to nomenclatural confusion—the choice of "ammajin" to denote the natural glycoside of marmesin is in our view an unjustifiable carry on of an erroneous system of names for the products of one plant source. The apparently relevant "marmesinin" would conform more closely to the conventional system of generic derivation of aglycone-glycoside names. The name is therefore proposed by the present authors to denote the natural marmesin glucoside.

(1) E. A. Abu-Mustafa, N. Badran, M. B. E. Fayez, and N. A. Starkowsky, *Nature*, **182**, 54 (1958).

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(3) N. A. Starkowsky and N. Badran, *J. Org. Chem.*, **23**, 1818 (1958).

(4) T. B. Fitzpatrick and M. A. Pathak, *J. Invest. Dermat.*, **32**, 229 (1959).

(5) I. R. Fahmy, H. Abu-Shady, A. Schönberg, and A. Sina, *Nature*, **160**, 468 (1947); I. R. Fahmy and H. Abu-Shady, *Quart. J. Pharm. and Pharmacol.*, **20**, 281 (1947).

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