

STEROIDAL SAPOGENINS—LXII

12-METHYL HEMIKETALS DERIVED FROM 12-KETO-16-DEHYDROPREGNEONES¹

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Abstract—Large-scale degradation of the sidechains of the 12-ketosapogenins, hecogenin and gentrogenin, gave respectively 3 β -acetoxy-5 α ,16-pregnene-12,20-dione and 3 β -acetoxy-5,16-pregnadiene-12,20-dione. Methanol crystallization of the *crude* enones gave in small yield the crystalline hemiketals, 3 β -acetoxy-12 β -hydroxy-12 α -methoxy-5 α ,16-pregnen-20-one and 3 β -acetoxy-12 β -hydroxy-12 α -methoxy-5,16-pregnadiene-20-one. The hemiketals exist largely in a hydrogen-bonded state. Their structure proof and infrared and ultraviolet spectra are discussed in detail.

SEVERAL years ago, Cameron *et al.* briefly described in the experimental section of their paper³ a compound which they thought might be a 12-hemiketal derived from 3 β -acetoxy-5 α ,16-pregnene-12,20-dione. They were unable to repeat their preparation. During the course of the sidechain degradation of hecogenin(I) and gentrogenin(II) to yield 3 β -acetoxy-5 α ,16-pregnene-12,20-dione(III) and 3 β -acetoxy-5,16-pregnadiene-12,20-dione(IV), respectively, we have been able to isolate consistently the corresponding hemiketals, viz. 3 β -acetoxy-12 β -hydroxy-12 α -methoxy-5 α ,16-pregnen-20-one(V) and 3 β -acetoxy-12 β -hydroxy-12 α -methoxy-5,16-pregnadiene-20-one(VI). The hemiketals V and VI have interesting spectral properties. In this paper we wish to report in detail the preparation, structure proof and the ultraviolet and infrared absorption spectra of V and VI.

Degradation of the 12-ketonic sapogenins, I and II was carried out by an improved procedure which gives maximal yields of 16-dehydro-12,20-pregnenes (cf. experimental). The three step sidechain degradation sequence was ordinarily carried out without isolation of intermediates until the final purification of the desired 16-dehydro-12,20-pregnenes, III and IV. The hemiketals V and VI were *obtained only from non-isolation sequences* (cf. Experimental section). The sidechain degradation procedure (described in detail in the experimental section) involved conversion of the sapogenins to pseudosapogenin diacetates with acetic anhydride containing 0.1 per cent acetic acid;⁴ oxidation of the latter with chromium trioxide in acetic acid at low temperatures using dichloroethane as co-solvent to give 16 β -acyloxy-12,20-oxosteroids; and acetic acid cleavage of the 16-acyloxy substituent³ to give the enones III and IV. Each step in this sequence could be interrupted to yield the crystalline intermediate. The crude enones III and IV obtained in non-isolation sequences were purified by chromatography on florisil. On crystallization from methanol the pure

¹ Previous paper in this series: Steroidal Sapogenins—LXI

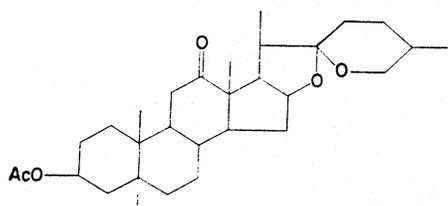
² Eastern Utilization Research and Development Division, Agricultural Research Service, United States Department of Agriculture.

³ 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).

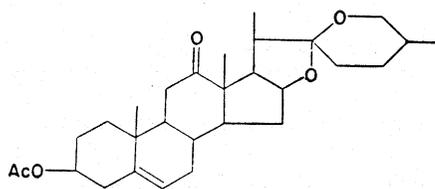
⁴ M. E. Wall and S. Serota, *J. Amer. Chem. Soc.* 79, 6481 (1957).

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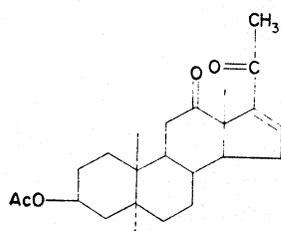
enones III and IV were obtained. Usually several crystalline crops were obtained rapidly. The residual syrupy mother liquors on long standing in the refrigerator slowly deposited further crystalline material. These latter crops contained largely the hemiketals V and VI mixed with the enones III and IV. The hemiketals were easily purified by crystallization.



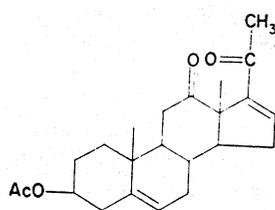
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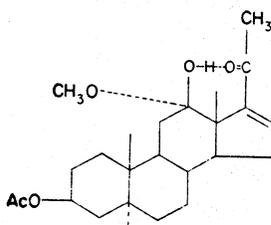
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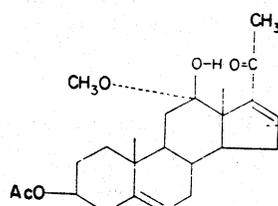
III



IV



V



VI

Structure proof of V and VI was based on the following evidence given in detail for V, that of VI following closely similar lines. Carbon, hydrogen and methoxyl values were in agreement with the assigned structure. The molecular rotation of the hemiketal V exhibited a large negative shift in comparison with that of the corresponding enone, III. Since 12-keto groups make a strong positive contribution to the total molecular rotation,⁵ the large negative shift is consistent with the disappearance of the 12-ketone moiety. Similarly the analysis of the ultraviolet spectrum of the hemiketal leads to the same conclusion. Thus steroids with the Δ^{16-20} -oxo grouping and no substituent at C_{12} generally show a maximum at 239–242 $m\mu$.⁶ The presence of a 12-ketone brings about an hypsochromic shift to 227–229 $m\mu$,^{7,8} which has been

⁵ D. H. R. Barton and W. Klyne, *Chem. & Ind.* 755 (1948).

⁶ L. Dorfman, *Chem. Rev.* 53, 47 (1953).

⁷ G. P. Mueller, R. E. Stobaugh and R. S. Winniford, *J. Amer. Chem. Soc.* 75, 4888 (1953).

⁸ M. E. Wall, H. E. Kenney and E. S. Rothman, *J. Amer. Chem. Soc.* 77, 5665 (1955).

ascribed to the increased energy of excitation in going from the ground state to a state wherein the dipoles of both excited carbon-oxygen systems are opposed.⁷ The hemiketal V exhibits an ultraviolet maximum at 242 m μ again indicating absence of the 12-ketone and presence of the conjugated Δ^{16} -20-ketone moiety. The infrared spectrum of the hemiketal V provided excellent proof of both the overall structure and the stereochemistry at C₁₂. A very intense band was observed in carbon bisulfide solution at 3340 cm⁻¹. Successive dilutions did not change the position of the

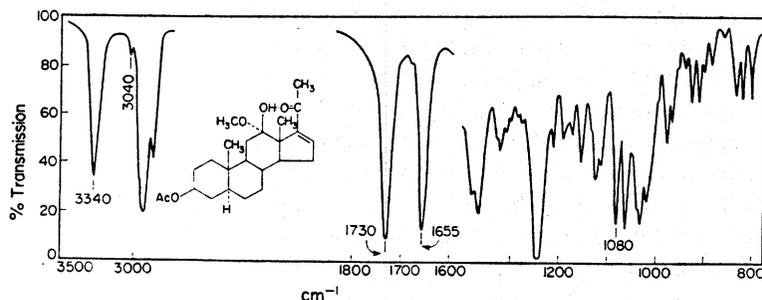


FIG. 1.

absorption band or the relative intensities, indicating that we are dealing with an intramolecular bonded hydroxyl group. The degree of hydrogen bonding exhibited is indeed unique. Thus compound V has an absorption band at 3340 cm⁻¹ with an apparent molecular extinction of 189.5. By comparison the strongly hydrogen bonded 20-hydroxy-tigogenin⁹ acetate has a band at 3520 cm⁻¹, apparent molecular extinction 101.6; and a typical unbonded hydroxyl, as is the 3 β -hydroxyl moiety in sarsasapogenin, exhibits an absorption band at 3620 cm⁻¹, apparent molecular extinction 41.6. In the carbonyl region the infrared spectrum of II shows two strong peaks, 1732 cm⁻¹, acetate, and 1655 cm⁻¹, conjugated 20-ketone. The normal location for the latter band in C-ring unsubstituted steroids is 1666–1670 cm⁻¹¹⁰ whereas in the case of Δ^{16} -12,20-diketones the location is at 1678 cm⁻¹.¹⁰ The direction of the shift observed in the location of the conjugated 20-ketone in V is in accord with previous observations of the effect of hydroxyl groups hydrogen bonded to ketones. Thus Jones *et al.*¹¹ found in the case of 17-hydroxy-20-ketones which exhibit only partial hydrogen bonding that the location of the 20-ketone band shifted in part from 1708 cm⁻¹ which is normal to 1693 cm⁻¹. Rothman and Wall¹² have observed a similar shift with 17 α -hydroxy-12,20-diketones. We conclude therefore from the evidence just presented that practically complete hydrogen bonding of the hydroxyl group in V with the conjugated ketone has occurred. This can take place only if the hydroxyl grouping has the equatorial 12 β -orientation. A strong band is present at 1082 cm⁻¹ and completely absent in the infrared spectrum of the enone III. From the studies of Page¹³ this band should be due to an axial methoxy group, the equatorial methoxy showing a band at 1100 cm⁻¹. According to the classic “rule of the rear,”¹⁴

⁹ M. E. Wall and H. A. Walens, *J. Amer. Chem. Soc.* **80**, 1984 (1958).

¹⁰ R. N. Jones and F. Herling, *J. Org. Chem.* **19**, 1252 (1954).

¹¹ R. N. Jones, P. Humphries, F. Herling and K. Dobriner, *J. Amer. Chem. Soc.* **74**, 2820 (1952).

¹² E. S. Rothman and M. E. Wall, *J. Amer. Chem. Soc.* **77**, 2229 (1955).

¹³ J. E. Page, *J. Chem. Soc.* 2017 (1955).

¹⁴ T. F. Gallagher and T. H. Kritchevsky, *J. Amer. Chem. Soc.* **72**, 882 (1950).

attack by methanol on the C_{12} carbonyl moieties of the enones III or IV should lead to the stereochemistry shown for the products V and VI, i.e. 12 α -methoxy-12 β -hydroxy. Molecular models of the hemiketals V and VI indicate that strong hydrogen bonding should occur between the 12 β -hydroxyl and the 20-ketone moieties. The infrared spectra of V and VI indicate that these compounds exist largely in the hydrogen bonded state. The strong hydrogen bonding may account for the stabilization of the hemiketals, allowing their isolation under appropriate conditions.

The hemiketals V and VI were obtained consistently as by-products from 100 g sapogenin sidechain degradation runs, usually yielding 1.0–2.0 g of crystalline hemiketal. The enones III and IV were obtained in 50–70 per cent yield. The syrupy mother liquors which yield the hemiketals enable one to obtain a much higher concentration of the enones III and IV in methanol at refrigerator temperatures than is possible with the pure enones. This suggested the possibility that the hemiketal could be made from a pure enone and methanol in concentrated solution by use of an appropriate co-solvent.¹⁵ With use of small quantities of methylene chloride, a 10 per cent solution of III in methanol remained in solution at 5°. After 8 days, infrared analysis of a sample indicated that a mixture consisting of 40 per cent III and 60 per cent V had been formed. Another sample taken after 17 days gave the same results. Hence, under experimental conditions which prevent removal of the hemiketal by crystallization, the reaction of the enone, III, with methanol results in an equilibrium mixture of III and V in a 2 : 3 ratio. The mixture could be separated by fractional crystallization. It is of interest to note that methanol is routinely used as the crystallizing agent for the enone III. The combination of low concentrations, short time and increased temperature all tend to shift the equilibrium between enone and hemiketal to the enone.

The hemiketals are very unstable substances. At room temperature they slowly decompose over a period of several weeks to the corresponding enones. Heating at 70° quickly gives quantitative conversion to the enones. Hence, the melting points of the hemiketals are really those of the corresponding $\Delta^{16-12,20}$ -diketones III and IV. Treatment of the hemiketals with base in tertiary butanol gave the enones III and IV. With methanolic hydrochloric acid, the hemiketal V was converted to a mixture of III and the corresponding 16 α -methoxy pregnane with infrared spectrum identical to that obtained by treating III with methanolic potassium hydroxide.¹⁶ Acetylation of the hemiketal V with acetic anhydride in pyridine at room temperature gave starting material.

EXPERIMENTAL

3 β -Acetoxy-5 α ,16-pregnene-12,20-dione (III)

100.0 g hecogenin acetate(I) was heated 17 hr at 180° with 250 ml acetic anhydride to which was added 0.1 % glacial acetic acid. The reaction was conveniently carried out in a 2 l. resin flask made of thick walled pyrex glass with a teflon gasket between the upper and lower sections, the two sections being secured by a heavy duty clamp. The flask was provided with a magnetic stirrer and a thermometer. After cooling, 45 ml water was added to the reaction mixture to convert all the acetic anhydride to acetic acid. Sufficient acetic acid to bring the volume to 1.5 l. was added followed by 1.5 l. of dichloroethane. The single phase solution was cooled in an ice-salt bath to -8° . To the solution containing pseudo-hecogenin diacetate, was added over a period of 15 min a solution of 50.0 g chromium trioxide in 1.5 l. 90% acetic acid, precooled to $+8^\circ$. The oxidation was allowed to proceed for

¹⁵ We wish to thank Professor R. B. Turner of the Rice Institute, Houston, Texas, for this suggestion.

¹⁶ D. K. Fukushima and T. F. Gallagher, *J. Amer. Chem. Soc.* **73**, 196 (1951).

another 60 min during which the temperature was kept at -2 to -5° . At the expiration of this time, excess oxidant was discharged by addition of 50.0 g sodium metabisulfite in 0.4 l. water, precooled to $+2^{\circ}$. To the reaction mixture was added 5 l. 10% sodium chloride solution. The lower phase consisting largely of dichloroethane was withdrawn. The residual aqueous liquors were extracted 6 times with $\frac{1}{2}$ volumes of ether. The ethereal layers were combined with the dichloroethane. The organic layers were washed with sodium bicarbonate solution until neutral. The sodium bicarbonate solution was re-extracted with ether. All the organic layers were combined, dried over anhydrous sodium sulfate and evaporated *in vacuo*. The residual glassy, crude "hecone" was refluxed 2 hr with 1 l. of glacial acetic acid, and then most of the acid was evaporated *in vacuo*. The residue was taken up in heptane, shaken with sodium bicarbonate solution and the heptane layer dried. The heptane solution of crude III was passed through a column containing 200 g of florisil. The column was then eluted with benzene. The solvents were combined, evaporated to dryness and the residue crystallized from methanol. Three crystalline crops, totalling 43.6 g were obtained m.p. $178-180^{\circ}$ infrared spectrum identical to that of an authentic sample.⁸

3 β -Acetoxy-12 β -hydroxy-12 α -methoxy-5 α ,16-pregnen-20-one (V)

The mother liquors from the preparation of the enone, III were concentrated and allowed to stand in the refrigerator. After one week, crystals were noted and after two weeks a crystalline crop of 2.0 g was obtained. The infrared spectrum indicated that a mixture consisting mainly of a new compound but also containing III had been obtained. On taking up the crystalline material in a small volume of dichloromethane and adding low boiling petroleum ether, pure V was obtained, m.p. $158-162^{\circ}$ followed by resolidification and m.p. $180-180.5^{\circ}$, $[\alpha]_D^{25} -15^{\circ}$, (Found C, 71.51; H, 9.02; OCH₃, 8.2. C₂₄H₃₆O₅ requires: C, 71.25; H, 8.97; OCH₃, 7.7%). The ultraviolet spectrum, 0.04 g/liter in methanol shows a maximum band at 242 m μ , $\epsilon = 9,304$. The infrared spectrum was obtained in carbon bisulfide solution, 10.0 g/l. Important bands are at 3340 (bonded hydroxyl) 3040 (C—H stretching due to HC—C=C) 1730 (acetate), 1655 (conjugated carbonyl) and 1082 cm⁻¹ (methoxyl).

Reaction of III with methanol in presence of a co-solvent

A solution of 0.5 g of the enone III was dissolved in 0.8 ml methylene chloride, mixed with 5 ml of methanol and stored in a stoppered flask at 5° . An aliquot was removed after 8 days and the solvent removed *in vacuo* in the absence of heat. The infrared spectrum of the sample indicated that a mixture of the enone III and the hemiketal V was present. In particular the infrared spectrum showed presence of bands at 1685 cm⁻¹, characteristic of the conjugated carbonyl moiety of III and at 1655 cm⁻¹, characteristic of the same grouping in V, the latter band being somewhat more intense than the former. The characteristic hydroxyl band of V at 3340 cm⁻¹ was also present and was used to determine the percentage of V in the mixture. From these calculations it was estimated that the mixture contained 60% V and by difference 40% III. Calculations based on the intensities of the conjugated carbonyl bands were in rough agreement. After several crystallizations from methylene chloride and low boiling petroleum ether, pure V could be separated from III as described previously.

Effect of heat, alkali, and acid on hemiketal (V)

A sample of 100 mg V was allowed to stand at room temperature. Samples were analyzed by means of the infrared spectrum. Conversion of V to III could be estimated by means of the shift in location of the Δ^{16} -20-ketone band from 1655 cm⁻¹ in V to 1680 cm⁻¹ in III. Decomposition of V to III commenced in a few days and was almost complete in 2 weeks. A sample of V was heated 2 hr at 70° , infrared analysis showed complete conversion to III.

A solution of 0.2 g V in 10 ml *t*-butanol was stirred with a solution of 0.2 g potassium hydroxide in 0.24 ml water for 3 hr at 40° . On dilution with water and ether extraction, 3 β -hydroxy-5 α ,16-pregnene-12,20-dione identical to an authentic specimen was the sole reaction product.

A solution of 0.1 g hemiketal V in 10 ml of methanol containing 0.1 ml of conc hydrochloric acid was allowed to stand at room temp for 4.5 hr. After neutralization of the acid with sodium bicarbonate the methanolic solution was diluted with water, extracted with ether and the solvent evaporated. The residual glass, 70 mg, was examined by infrared spectroscopy. The conjugated Δ^{16} -20-ketone had disappeared. The infrared spectrum was similar to that obtained by treating the enone III with

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5% potassium hydroxide or hydrochloric acid in methanol. The product was therefore judged to be a mixture of III and the 3 β -hydroxy-16 α -methoxy-pregnene-12,20-dione obtained by the addition of CH₃OH to the Δ^{16} -double bond in the presence of acid or alkali. Under acidic conditions V \rightarrow III which then forms the 16 α -methoxy derivative.

3 β -Acetoxy-5,16-pregnadiene-12,20-dione (IV)

In the same manner described above for the conversion of I to III, 100 g of gentrogenin acetate gave 35 g of IV, m.p. 172–173° the infrared spectrum was identical to that of an authentic specimen.¹⁷

3 β -Acetoxy-12 β -hydroxy-12 α -methoxy-5,16-pregnadiene-20-one (VI)

The mother liquors remaining from the methanol recrystallization of IV were concentrated. After standing in the refrigerator for one week, 1.2 g of a crystalline product rich in the hemiketal fraction was isolated. On crystallization from dichloroethane-low boiling petroleum ether, the pure hemiketal VI was obtained, m.p. 165–175°, $[\alpha]_D^{25} - 86^\circ$, λ max 241, $\epsilon = 8654$, infrared spectrum similar in all important bands to that of hemiketal V. The infrared spectrum of VI is best distinguished from V by examination in the C—H stretching region, VI shows 5 bands and a shoulder between 3060–2800 cm⁻¹ whereas V shows only 3 bands in this region. (Found C, 71.23; H, 8.50; OCH₃, 8.15; C₂₄H₃₁O₅ requires: C, 71.61; H, 8.51, OCH₃, 7.7).

¹⁷ H. A. Walens, S. Serota and M. E. Wall, *J. Org. Chem.* **22**, 182 (1957).