STEROIDS

I. 12β-METHYL-12a-HYDROXYPROGESTERONE[1]

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ABSTRACT

Lithium aluminum hydride reduction of 3α,12α-diacetoxypregnan-20-one (I*a*) gives as the major product pregnane-3α,12α,20β-triol (III), whereas the reduction of the corresponding dihydroxypregnanone I affords mainly the epimeric 3α,12α,20α-triol II. Triol III was transformed to 12β-methyl-12α-hydroxyprogesterone (VIII).

As part of a continuing study of the chemistry of steroids bearing functional groups in ring C, and of the interaction between substituents located at positions 12 and in the side-chain (1, 2), the preparation of 12-methyl-12-hydroxypregnane derivatives such as VI and VII was undertaken.

Engel and Huculak (3) have recently described the preparation of 3α,20β-dihydroxypregnan-12-one bis-methyl succinate (V*b*) through the sequence I*a* → III → III*b* → V*b*. This ketone V*b* appeared to us a useful intermediate for the introduction of a 12-methyl substituent.

Lithium aluminum hydride reduction of 3α,12α-diacetoxypregnan-20-one (I*a*) gave pregnane-3α,12α,20β-triol (III) and a small amount of material which Engel and Huculak (3) had tentatively formulated as pregnane-3α,12α,20α-triol (II). Chromic acid oxidation of this material to pregnane-3,12,20-trione (IV) confirmed this assignment of structure. In accordance to the findings of Sarett (4, see also refs. 3 and 5), the difference of increment of molecular rotation due to the acetylation of the 20β- and the 20α-hydroxy group was of the order of 200°.

Surprisingly, lithium aluminum hydride reduction of 3α,12α-dihydroxypregnan-20-one (I) gave as the major product pregnane-3α,12α,20α-triol (II). A more detailed study of the reduction revealed that the yield of the epimeric triols II and III depended on the solvent and on the nature of the functional group attached at position 12. The results are summarized in Table I.

TABLE I

Starting material	Solvent	%II(20α-OH)	%III(20β-OH)
1. Diol I	Tetrahydrofuran	58.5	27.0
2. Diol I	Diethyl ether	65.8	10.2
3. Diacetate I*a*	Tetrahydrofuran	9.2	69.7*
4. Diacetate I*a*	Tetrahydrofuran	5.8	81 (3)
5. Diacetate I*a*	Diethyl ether	37	46.8

*No attempt was made in this case to obtain the maximum yield.

The reduction of the 20-keto diacetate I*a* to the 20β-triol III proceeds according to Cram's rule of steric control of asymmetric induction (6). The stereochemistry of the reduction of the 20-keto diol I to the 20α-triol II can be readily explained, if one assumes

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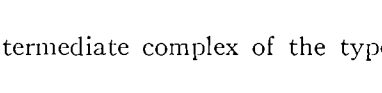
the formation of an intermediate complex of the type  . The carbonyl group is then reduced by another hydride molecule. Sicher *et al.* (7) have postulated a similar complex to explain why dehydrochloramphenicol and its *O*-acetate form threo- and erythro-chloramphenicol, respectively, upon Meerwein-Ponndorf reduction.

The co-ordination of the aluminum hydride alcoholate by the carbonyl group is competing with solvation by ether and tetrahydrofuran (8). As tetrahydrofuran is more basic than ether (9), one would expect less co-ordination of the 20-carbonyl oxygen in the lithium aluminum hydride reduction in tetrahydrofuran, and hence more 20β-alcohol III would be formed in that solvent. This hypothesis is confirmed by experimental evidence (Table I, columns 1 and 2).

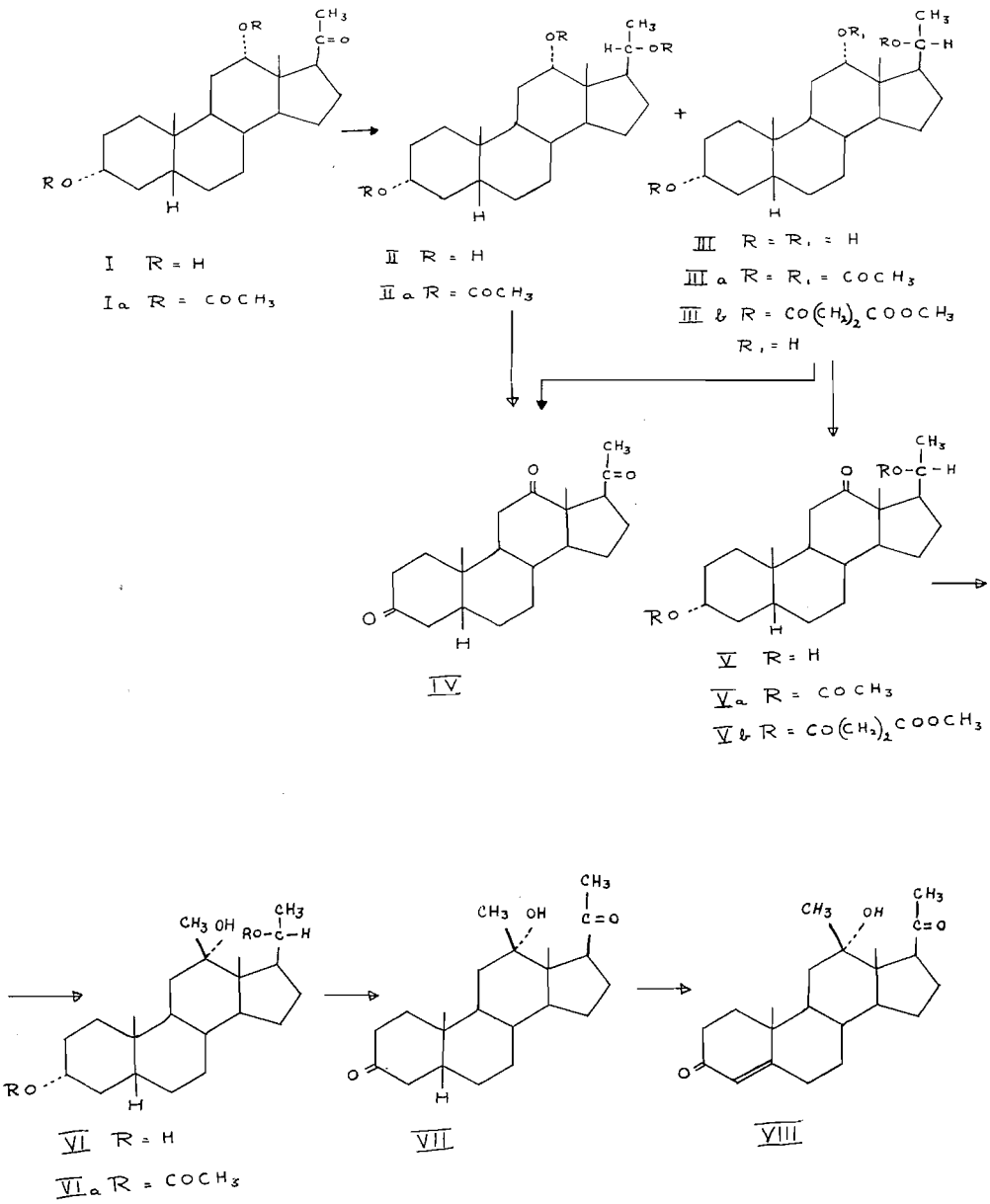
An explanation of the solvent effect in the reduction of the diacetoxy ketone I*a* would follow similar lines. The only assumption which would have to be made is that the 20-keto group is reduced at a similar rate as the 12-acetate group. Reduction of keto diol I with a fivefold excess of sodium borohydride indicated the hindered nature of the 20-carbonyl group, since even after 20 hours it was not complete, as evident from infrared measurements. This experiment does lend some weight to the assumption made, without, however, proving it.

A co-ordination complex involving aluminum hydride would also account for the conversion of 17α-hydroxypregnan-20-ones by the action of lithium aluminum hydride to a mixture of 20β- and 20α-dihydroxypregnane derivatives, in which the latter predominated (5).

Succinylation of triol III to the succinate III*b* (3), followed by oxidation, afforded the 12-keto disuccinate V*b* (3). Prolonged treatment of the ketone V*b* with methyl magnesium iodide in diethyl ether led to a reaction mixture containing sizable amounts of starting material. The keto disuccinate V*b* was therefore hydrolyzed with methanolic potassium hydroxide to the dihydroxy ketone V, which was converted to the corresponding diacetate V*a*. Treatment of the 12-keto diacetate V*a* with excess ethereal methyl magnesium iodide for 20 hours gave a 73% yield of the desired 12β-methylpregnane-3α,12α,20β-triol VI. Triol VI formed a diacetate VI*a*, and was easily oxidized to 12β-methyl-12α-hydroxypregnane-3,20-dione (VII), thus establishing the tertiary nature of the 12-hydroxy group. The assignment of configuration of the 12-methyl group will be substantiated in a following paper. It is in disagreement with the assignment of configuration made by Levine and Wall (10) and Bladon and McMeekin (11), who transformed hecogenin to "12α-methyl-12β-hydroxy tigogenin".

Because of the considerable interest in steroid hormone analogues containing alkyl and other substituents in the steroid nucleus (see, e.g. 10, 11, 12), we decided to transform 12β-methyl-12α-hydroxypregnane-3,20-dione (VII) into the closely related 12β-methyl-12α-hydroxyprogesterone (VIII). This was readily achieved by bromination in position 4, followed by dehydrobromination (13). The progesterone analogue thus obtained did not exhibit any notable progestational, androgenic, diuretic, hypotensive, or anabolic properties.[2]

[2]*Biological tests were carried out by Dr. C. I. Chappel and Dr. Clara Revesz, Ayerst, McKenna and Harrison, Montreal.*



EXPERIMENTAL[3, 4, 5, 6]

Reduction of 3α,12α-Diacetoxypregnan-20-one (Ia) in Ether

A solution of the diacetate I*a* (14) (30.0 g, 0.0717 mole, m.p. 122–123°) in absolute ether (500 ml) was added with stirring to a solution of lithium aluminum hydride (33 g) in absolute ether (1500 ml). The reaction mixture was heated under reflux for 1 hour and

[3]*All the melting points were corrected.*
[4]*Only the best yields were reported.*
[5]*The commercially available aluminum oxide (Woelm) was used.*
[6]*The microanalyses were carried out by Dr. Alfred Bernhardt, Germany.*

left at room temperature overnight. The excess lithium aluminum hydride was destroyed by adding ethyl acetate (175 ml) and then 2 *N* sulphuric acid (450 ml). The solvents were removed by distillation and the reaction mixture extracted with chloroform. The chloroform layer was washed with 5% sodium bicarbonate solution, water, saturated sodium chloride solution, and dried over magnesium sulphate.

Repeated crystallizations from chloroform gave the 20α-alcohol II (8.902 g, 37.0%), m.p. 225–226°, $[\alpha]_D^{29}$ 46° (*c* 0.59 in $CHCl_3$). The substance was slightly hygroscopic. Crystallization from acetone did not change the melting point. Calcd. for $C_{21}H_{36}O_3$: C 74.98%, H 10.78%. Found: C 74.94%, H 10.66%.

Crystallization of the mother liquors from acetone gave the 20β-alcohol III (3) (11.260 g, 46.8%), m.p. 236–238°, $[\alpha]_D^{29}$ 34° (*c* 0.78 in $CHCl_3$).

Calcd. for $C_{21}H_{36}O_3$: C 74.98, H 10.78%. Found: C 74.84, H 10.61%.

Reduction of 3α,12α-Dihydroxypregnan-20-one (I) in Ether

A solution of the diol I (14) (1.414 g, 0.00423 mole, m.p. 168–172°; crude product obtained by alkaline hydrolysis of the diacetate I*a*) in absolute ether (200 ml) was added with stirring to a solution of lithium aluminum hydride (1.5 g) in absolute ether (100 ml). The reaction mixture was worked up as described above. The yields of 20α-alcohol II, m.p. 225–226°, and of 20β-alcohol III, m.p. 236–238°, were 65.5% and 10.5% respectively. The alcohols were identified by mixed melting point and comparison of I.R. spectra.

Reduction of 3α,12α-Dihydroxypregnan-20-one (I) in Tetrahydrofuran

A solution of the diol I (2.635 g, 0.00789 mole, m.p. 168–172°, crude product obtained by alkaline hydrolysis of the diacetate I*a*) in absolute tetrahydrofuran (200 ml) was added dropwise, with stirring, to a solution of lithium aluminum hydride (3.0 g) in absolute tetrahydrofuran (250 ml). The reaction mixture was worked up as described above. The yields of the 20α-alcohol II, m.p. 225–226°, and of 20β-alcohol III, m.p. 236–238°, were 58.5% and 27.2% respectively. The identity of the alcohols was confirmed by mixed melting point and superimposability of I.R. spectra.

Reduction of 3α,12α-Diacetoxypregnan-20-one (Ia) in Tetrahydrofuran (3)

A solution of the diacetate I*a* (30.9 g, 0.0739 mole, m.p. 122–123°) in absolute tetrahydrofuran (200 ml) was reduced with lithium aluminum hydride (36 g) in 1500 ml of absolute tetrahydrofuran (3). Chloroform extraction afforded 21.1 g (85% yield) of the crude product. Separation of the triols, as described above, afforded 2.40 g of the 20α-triol II (m.p. 221–224°, 9.5% yield), and 17.3 g of the 20β-triol III (m.p. 231–233°, 69.7% yield).

3α,12α,20α-Triacetoxypregnane (IIa)

Triol II (130 mg, m.p. 225–226°) was heated under reflux for 1 hour in pyridine (3 ml) and acetic anhydride (1 ml). The reaction mixture was worked up as usual and afforded, on crystallization from ether–hexane, the triacetate II*a*, m.p. 147–148°, $[\alpha]_D^{28}$ 95° (*c* 0.73 in $CHCl_3$).

Calcd. for $C_{27}H_{42}O_6$: C 70.09%, H 9.15%. Found: C 70.27%, H 9.20%.

3α,12α,20β-Triacetoxypregnane (IIIa)

Triol III (130 mg, m.p. 236–38°) was treated as described above. Crystallization of the crude product from ether–hexane yielded the triacetate III*a* (3), m.p. 201–202°, $[\alpha]_D^{28}$ 127° (*c* 0.81 in $CHCl_3$). The melting point was not depressed upon admixture with an authentic sample[7] and the I.R. spectra were identical.

[7]*Kindly supplied by Dr. C. R. Engel, Department of Chemistry, Laval University, Quebec.*

Pregnane-3,12,20-trione (IV)

In parallel runs, a solution of triol II and of triol III (300 mg) in acetic acid (15 ml) was treated with a solution of chromium trioxide (300 mg) in 3 ml of 90% acetic acid at room temperature for 20 hours. Extraction and crystallization gave in each case an 80–90% yield of IV (15), m.p. 203–205°. The identity of the samples in both cases was confirmed by mixed melting point and superimposability of I.R. spectra.

3α,12α,20β-Trihydroxypregnane 3α,20β-bis-Methyl Succinate (IIIb)

To a solution of the triol III (4.38 g) in pyridine (40 ml), succinic anhydride (10.2 g) was added. The reaction mixture was heated at 90° on a water bath for 4 hours, and left overnight at room temperature. It was diluted with ice-cold water (1500 ml) and left for 1 hour. The solution was extracted with ether, washed with 2 *N* sulphuric acid, and twice with water. The ethereal extract was dried over magnesium sulphate. Upon evaporation of the solvent to 250 ml, 4.054 g of the bis-hemisuccinate, m.p. 188–189°, crystallized.

Crystallization of mother liquors from acetone–hexane afforded 2.192 g, m.p. 188–189°.

Recrystallization for analysis raised the m.p. to 190–191° (m.p. 182–184° (3)).

Calcd. for $C_{29}H_{44}O_9$: C 64.91%, H 8.26%. Found: C 64.79%, H 8.35%.

A solution of the bis-hemisuccinate (6.152 g) in ether (1500 ml) was methylated with an excess of diazomethane in ether. One crystallization from ether–hexane afforded 5.855 g of III*b* (3), m.p. 96–97°, $[\alpha]_D^{29}$ 67° (*c* 0.97 in $CHCl_3$). Crystallization of the mother liquors gave 0.257 g of III*b*, m.p. 94–95° (yield 84%).

3α,20β-Dihydroxypregnan-12-one bis-Methyl Succinate (Vb)

Oxidation with chromium trioxide in acetic acid of the bis-methyl succinate III*b* (6.025 g) afforded 5.971 g of the keto bis-methyl succinate V*b* (3), m.p. 112–113°, $[\alpha]_D^{29}$ 110° (*c* 0.91 in $CHCl_3$).

3α,20β-Dihydroxypregnan-12-one (V)

To a solution of the keto bis-methyl succinate V*b* (10.0 g) in methanol (700 ml) and water (100 ml) was added potassium carbonate (12.5 g) and the mixture was refluxed for 17 hours. The major part of the methanol was distilled off *in vacuo*. The reaction mixture was worked up as usual and on crystallization from acetone–hexane, 2.868 g of the hydroxy ketone V, m.p. 221–222°, was obtained. The I.R. spectrum of the mother liquors showed that the hydrolysis was not complete. The mother liquors were further hydrolyzed with 5% methanolic potassium hydroxide for 8 hours at reflux temperature and worked up as usual. Crystallization from acetone–hexane afforded 3.044 g of the hydroxy ketone V, m.p. 220–222° (yield 96%).

A portion of the hydroxy ketone V was crystallized twice from acetone–hexane for analysis, m.p. 222–223°, $[\alpha]_D^{29}$ 131° (*c* 0.95 in $CHCl_3$).

Calcd. for $C_{21}H_{34}O_3$: C 75.41, H 10.24. Found: C 75.20, H 10.06.

3α,20β-Diacetoxypregnan-12-one (Va)

A solution of the hydroxy ketone V (400 mg) in pyridine (10 ml) and acetic anhydride (10 ml) was kept in a water bath at 90° overnight. Ether extraction gave 511 mg of crude product. One crystallization from ether–hexane yielded 416 mg of the ketone V*a*, m.p. 136–137°. Crystallization of mother liquors afforded 53 mg of V*a*, m.p. 135–136° (yield 93%).

A portion of ketone V*a* was crystallized twice from ether–hexane for analysis, m.p. 136–137°, $[\alpha]_D^{29}$ 179° (*c* 1.09 in $CHCl_3$).

Calcd. for $C_{35}H_{28}O_5$: C 71.73, H 9.15. Found: C 71.91, H 9.25.

12β-Methyl-3α,12α,20β-trihydroxypregnane (VI)

To 200 ml of an ethereal solution of methyl magnesium iodide (from 270 mg of magnesium and 0.7 ml of methyl iodide) was added with stirring 575 mg of the ketone V*a*, dissolved in 200 ml of absolute ether. The reaction mixture was refluxed for 20 hours. A solution of ammonium chloride (10 g) in water (200 ml) was added to the reaction mixture. It was then extracted with ether and the organic solution washed with water until neutral. The ethereal extract was dried over magnesium sulphate. On evaporating the major part of the ether, crystals of the triol VI (110 mg, m.p. 213–214°) were obtained. Crystallization of mother liquors afforded 98 mg of the triol VI, m.p. 210–213°.

Recrystallizations from aqueous methanol and methanol raised the m.p. to 213–215°, $[\alpha]_D^{29}$ 39° (*c* 0.88 in $CHCl_3$).

Calcd. for $C_{22}H_{38}O_3$: C, 75.37, H 10.92. Found: C 75.21, H 10.92.

The mother liquors were acetylated and the resulting oil (435 mg) chromatographed on alumina (4.5% water). The hexane–benzene (4:1) fractions consisted mainly of the unreacted ketone. Hexane–benzene (1:1) fractions were collected and on crystallization from hexane gave 73 mg of the diacetate VI*a*, m.p. 119–121°. The mother liquors on crystallization from hexane afforded 15 mg of the diacetate VI*a*, m.p. 119–121°.

A portion of the diacetate VI*a* was crystallized twice from hexane for analysis, m.p. 122–124°, $[\alpha]_D^{29}$ 78° (*c* 1.01 in $CHCl_3$).

Calcd. for $C_{26}H_{42}O_5$: C 71.86, H 9.74. Found: C 71.66, H 9.99.

The mother liquors of the hexane–benzene (1:1) fractions and the remainder of the fractions of the chromatogram afforded upon alkaline hydrolysis and crystallization from ether–hexane 71 mg of the triol VI, m.p. 208–210° (yield 73%).

12β-Methyl-12α-hydroxypregnane-3,20-dione (VII)

Chromium trioxide (490 mg) in 90% acetic acid (5 ml) was added with stirring to a solution of the triol VI (750 mg) in acetic acid (50 ml) and left at room temperature overnight. Ether extraction afforded 723 mg of the crude hydroxy ketone VII, m.p. 184–186°. One crystallization from acetone–hexane gave 663 mg of the hydroxy ketone VII, m.p. 187–188°. Crystallization of mother liquors afforded 15 mg of VII, m.p. 186–187° (yield 92%).

A portion of the hydroxy ketone VII was crystallized twice from acetone–hexane for analysis, m.p. 187–188°, $[\alpha]_D^{29}$ 102°, (*c* 1.05 in $CHCl_3$).

Calcd. for $C_{22}H_{34}O_3$: C 76.25, H 9.89. Found: C 76.10, H 9.87.

12β-Methyl-12α-hydroxyprogesterone (VIII)

A solution of bromine (141 mg) in acetic acid (9.4 ml) was added to a solution of the hydroxy ketone VII (300 mg) in acetic acid (10 ml) with stirring. After 5 minutes, the reaction mixture was poured into ice-water (500 ml), and extracted with ether. The organic layer was washed with water and dried. There was obtained 395 mg of an oil which resisted crystallization.

The crude bromo ketone was dehydrobrominated according to the method of McGuckin and Kendall (13). The crude product (240 mg of oil) on crystallization from ether–hexane afforded 125 mg of the progesterone derivative VIII, m.p. 140–150°. Repeated

crystallizations gave 97 mg of analytically pure VIII, m.p. 152–153°, $[\alpha]_D^{29}$ 182° (*c* 1.01 in $CHCl_3$), λ_{max}^{EtOH} 239 mμ, (ε 17500), $\nu_{max}^{CCl_4}$ 1710 cm^{-1} (20-ketone), 1680 cm^{-1}, 1620 cm^{-1} (Δ^4-3-ketone).

Calcd. for $C_{22}H_{32}O_3$: C 76.68, H 9.37. Found: C 76.40, H 9.40.

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