ACYL TRANSFER IN 16-MONOESTERS OF 16α-HYDROXYPREGNANES

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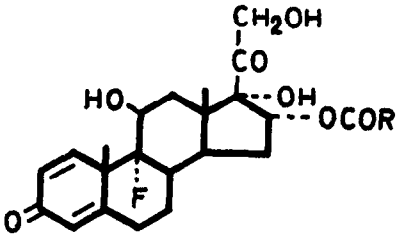
The base catalized acyl migration O-16 ⟶ O-21 in 16α-hydroxy-corticosteroids with dihydroxyacetone side chain is described. The probable mechanism of the rearrangement is proposed. In this connection also the acyl transfer O-16 ⟶ O-20 in 16α,17α,20β-triols is reported and discussed.

Intramolecular transesterifications in corticosteroid side chain have been reported in the last few years. We described the acyl migration O-17 ⟶ O-21 in corticosteroids with dihydroxyacetone side chain [1] and the acyl migration O-20 ⟶ O-21 in 17α,20,21-triols of both 20α and 20β series [2,3]. Other examples of acyl migration had been reported by Fukushima et al. [4] and Taub et al. [5].

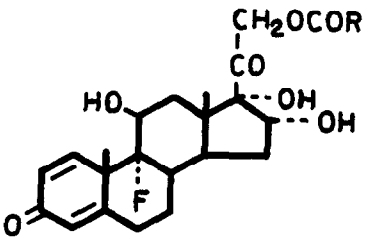
In the present paper we wish to report on the acyl transfer in the esters of 16α-hydroxypregnanes.

By treatment with triethylamine at room temperature, 9α-fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione 16-acetate (Ia), prepared in high yield from the parent tetraol via the 16,17-methylorthoacetate according to the already described procedure [6], underwent an intramolecular transesterification giving a product recognized as the known [7] 9α-fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione 21-acetate (IIa).

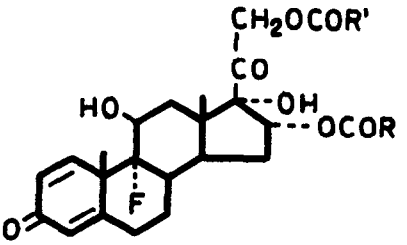
Other 16-monoesters, such as the n-valerate Ib [8] and the cyclopentane-



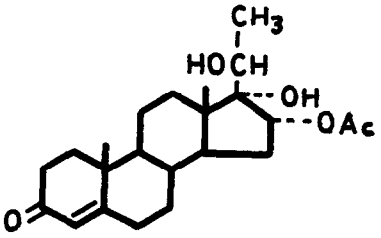
I a) R = CH_3
b) R = C_4H_9
c) R = 



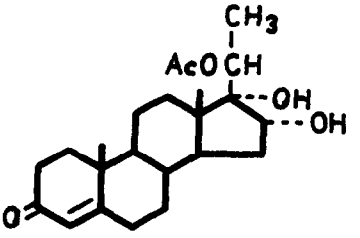
II a) R = CH_3
b) R = C_4H_9
c) R = 



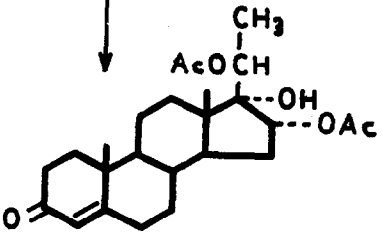
III a) R =  , R' = CH_3
b) R = CH_3 , R' = C_4H_9
c) R = CH_3 , R' = 



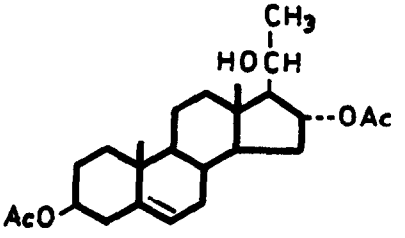
IV



V



VI



VII

carboxylate Ic, prepared by the same procedure, rearranged in similar manner to give the corresponding 21-monoesters IIb and IIc in good yield.

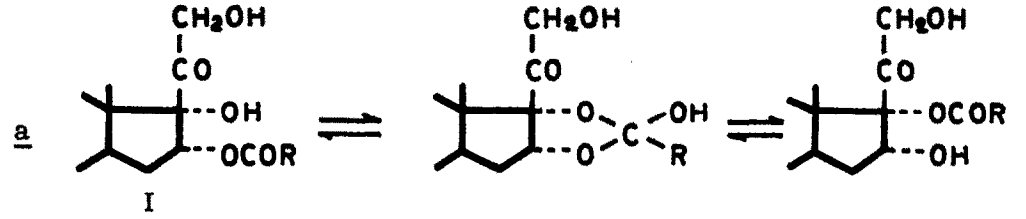
It might be supposed that the acyl transfer O-16 ⟶ O-21 occurred *via* the initial O-16 ⟶ O-17 transfer. Acyl migrations in both directions between 16α and 17α oxygens have been already reported [9]. However, 16α,21-diesters III treated with triethylamine in ethanol did not undergo rearrangement to 17α,21-diesters. Therefore the question might be raised whether the migration of an acyl group from O-16α took place, in these conditions, only in the presence of an additional free hydroxyl group in the side chain.

In this connection we turned our attention to the derivatives of 16, 17,20-triols. By $NaBH_4$ reduction and mild acid hydrolysis, 3-ethoxy-16α,17α-dihydroxypregna-3,5-dien-20-one 16-acetate [10] afforded 16α, 17α,20β-trihydroxypregn-4-en-3-one 16-acetate (IV), whose configuration at C-20 was readily inferred on the basis of the positive increment of molecular rotation after acetylation [11]. As expected, by refluxing in ethanol with triethylamine, IV yielded a new monoacetate recognized as the 20-acetate V, for it gave the same 16,20-diacetate VI obtained from IV.

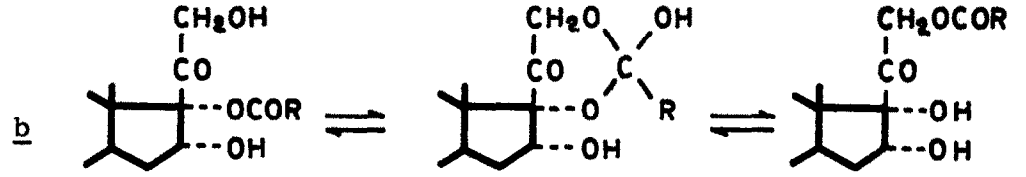
The possibility of a direct O-16 ⟶ O-20 transfer was ruled out on the basis of the behavior of a 17-deoxy analog of IV. $NaBH_4$ reduction of 3β,16α-dihydroxypregn-5-en-20-one diacetate [12] gave pregn-5-en-3β,16α,20β-triol 3,16-diacetate (VII). The latter did not rearrange in the conditions employed for the above described rearrangement.

Therefore, it should be inferred that the migratory ability of the acyl group at the 16α position is closely conditioned by the presence of the 17α-hydroxyl group and of another hydroxyl group in the side chain.

Rearrangement of 16α-monoesters I in derivatives with dihydroxyacetone side chain should involve the equilibria *a* and *b* through the 16α,17α- and 17α,21-ortho-forms.

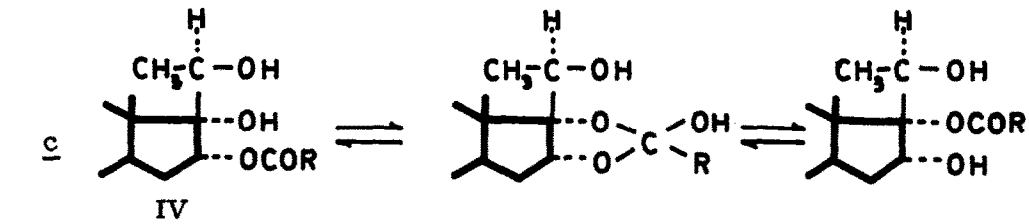


I

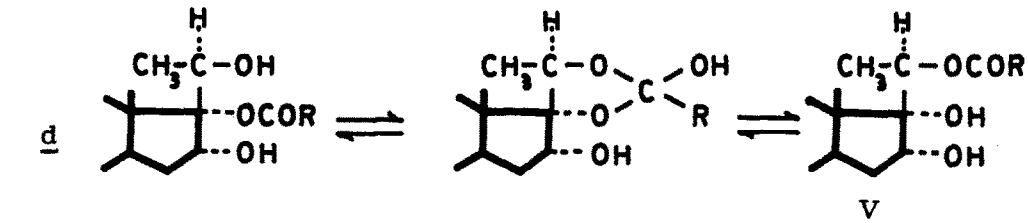


II

Evidently, in 16α,21-diesters III equilibrium a is displaced towards left. The presence of the free hydroxyl group at C_{21} and consequently the establishment of the new equilibrium b, strongly displaced towards right [13], results in the practically complete conversion of I to II. This is the equilibrium relationship in basic medium whereas acidic conditions can give rise to different results [14].



IV



V

A similar pattern can be pictured for the esters of 16α,17α,20β-triol, with the equilibria c and d. Also in this case, very likely, the displacement towards right of equilibrium d is the driving force in conversion of IV to V.

All the rearrangements described above were carried out in ethanol solution, since in methanol and in presence of triethylamine methanolysis occurred to a great extent. The problem was better investigated in some mixed 16,21-diesters of 9α-fluoro-16α-hydroxyprednisolone (III). In our previous paper on methoxide ion catalyzed methanolysis of 21-esters [15] we pointed out that a vicinal hydroxyl group (at C_{20} in that case) was more effective than 20-ketone as assisting group. Accordingly, in compounds III, deacylation of 16α-ester by triethylamine catalyzed methanolysis occurs at a higher rate than that of 21-ester because of the presence of the 17α-hydroxyl group. For example methanolysis of the 16-acetate 21-cyclopentanecarboxylate IIIc afforded the 21-cyclopentanecarboxylate IIc in good yield, while in the same conditions the 16-cyclopentanecarboxylate 21-acetate IIIa was largely recovered unchanged together with small amounts of 21-acetate IIa, 16-cyclopentanecarboxylate Ic and 21-cyclopentanecarboxylate IIc.

EXPERIMENTAL [16]

9α-Fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione 16-acetate (Ia). - To a suspension of 9α-fluoro-16α-hydroxyprednisolone (3 g.) in dioxane (30 ml.) trimethylorthoacetate (3 ml.) and methanol (0.4 ml.) 70% perchloric acid (0.26 ml.) was added under stirring. After 5 min. pyridine (3 ml.) was added to the completely clear reaction mixture which was then diluted with water (50 ml.) and concentrated under reduced pressure. The precipitated product was collected by filtration, redissolved in methanol (15 ml.) and treated with 2N oxalic acid solution (2 ml.) by heating 5 min. on the water bath. Evaporation of the solvent in a stream of nitrogen induced crystallization of the product which, filtered and washed with ether, gave 2.7 g. of Ia, homogeneous on T.L.C. (Rf 0.14); m.p. 229-231°; $[\alpha]_D$ +57.5°; νmax 3450, 1725, 1660, 1615, 1605, 1260 and 1048 cm^{-1}; lit. m.p. 224-228°; $[\alpha]_D$ +49.7° [8]; m.p. 230-231°; $[\alpha]_D$ +63° [6].

According to this procedure were also obtained :

9α-Fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione 16-valerate (Ib) : m.p.213-216°; $[\alpha]_D$ +54°; λ_{max} 239 mμ (ε 14.000); ν_{max} 3430, 1725, 1702, 1672, 1636, 1611, 1178, 1128 and 1043 cm^{-1}; lit.[8] m.p.212-216°; $[\alpha]_D$ +27°.

9α-Fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione 16-cyclopentanecarboxylate (Ic): m.p.241-243°; $[\alpha]_D$ +44°; λ_{max} 239 mμ (ε 15.350); ν_{max} 3480, 3420, 1734, 1715, 1665, 1622, 1606, 1245, 1152 and 1060 cm^{-1}.

Anal. Calcd. for $C_{27}H_{35}O_7F$: C, 66.10 ; H, 7.19.
Found : C, 65.95 ; H, 7.07.

Acetylation of Ic (200 mg.) with acetic anhydride (0.5 ml.) in pyridine (1 ml.) at 0-5° overnight afforded, after crystallization from acetone-ether, 180 mg. of IIIa, m.p.217-220°; $[\alpha]_D$ +47.4°; λ_{max} 239-240 mμ (ε 15.000); ν_{max} 3600, 3400, 1752, 1725, 1663, 1620, 1610, 1238, 1125 and 1061 cm^{-1}.

Anal. Calcd. for $C_{29}H_{37}O_8F$: C, 65.40 ; H, 7.00.
Found : C, 65.54 ; H, 7.30.

9α-Fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione 21-acetate (IIa). - A solution of the 16-acetate Ia (100 mg.) in ethanol (2 ml.) was treated with triethylamine (0.4 ml.). After standing for 3 hours at room temperature, the reaction mixture was evaporated under vacuum. The residue, taken up in water and filtered, gave 84 mg. of crystalline product, R_f 0.34, with only a trace of Ia, R_f 0.14. Crystallization from methanol yielded 74 mg. of pure IIa, m.p.227-230°; $[\alpha]_D$ +72°; λ_{max} 239 mμ (ε 13.500); ν_{max} 3360, 1740, 1712, 1663, 1622, 1607, 1230, 1128 and 1058 cm^{-1}; lit.[7] m.p. 215-217°; $[\alpha]_D$ +74.5°. There was no depression of the melting point upon admixture with an authentic sample of IIa.

9α-Fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione 21-valerate (IIb). - A solution of Ib (100 mg.) in ethanol (2 ml.) was treated with triethylamine (0.4 ml.) and kept overnight at room temperature. After working up the reaction mixture as in the preceding example, crystallization from acetone-ether gave 76 mg. of a product, homogeneous on T.L.C. (R_f 0.40), m.p.227-229°; $[\alpha]_D$ +77°; λ_{max} 239 mμ (ε 14.900); ν_{max} 3550, 3250, 1746, 1715, 1662, 1600, 1247 and 1069 cm^{-1}.

Anal. Calcd. for $C_{26}H_{35}O_7F \cdot 1/2\ H_2O$: C, 64.04 ; H, 7.44.
Found: C, 63.97 ; H, 7.27.

Acetylation of IIb in the usual manner gave the 16-acetate 21-valerate IIIb, m.p. 199-201°; $[\alpha]_D$ +67°; λ_{max} 239 mμ (ε 14.400); ν_{max} 3400, 1727, 1660, 1617, 1606, 1237, 1175, 1125 and 1055 cm^{-1}.

Anal. Calcd. for $C_{28}H_{37}O_8F$: C, 64.60 ; H, 7.16.
Found : C, 64.59 ; H, 6.95.

The same diester IIIb was also obtained from Ia by reaction with pyridine and valeric anhydride.

9α-Fluoro-11β,16α,17α,21-tetrahydroxypregna-1,4-diene-3,20-dione 21-cyclopentanecarboxylate (IIc). - Treatment of Ic (200 mg.) as in the preceding example and recrystallization from methylene chloride-methanol gave 150 mg. of IIc, m.p. 230-233°; $[\alpha]_D$ +80.5°; λ_{max} 239 mμ (ε 14.400); ν_{max} 3450, 1747, 1716, 1663, 1622, 1608, 1244, 1147 and 1063 cm^{-1}.

Anal. Calcd. for $C_{27}H_{35}O_7F$: C, 66.10 ; H, 7.19.
Found : C, 65.86 ; H, 7.38.

Acetylation in the usual manner gave IIIc, m.p. 254-256°; $[\alpha]_D$ +72°; λ_{max} 239 mμ (ε 15.100); ν_{max} 3580, 3400, 1725, 1659, 1617, 1235, 1127 amd 1056 cm^{-1}.

Anal. Calcd. for $C_{29}H_{37}O_8F$: C, 65.40 ; H, 7.00.
Found : C, 65.43 ; H, 7.17.

16α,17α,20β-Trihydroxypregn-4-en-3-one 16-acetate (IV). - To a solution of 3-ethoxy-16α,17α-dihydroxypregna-3,5-dien-20-one 16-acetate [10] (4 g.) in tetrahydrofuran (160 ml.), $NaBH_4$ (0.7 g.) in water (20 ml.) was added while cooling with an ice bath. After standing overnight at room temperature the mixture was concentrated under reduced pressure, then poured into water. The product was filtered, redissolved in methanol (60 ml.) and heated for 10' on the water bath after addition of 2N oxalic acid solution (4 ml.). After removing the solvent in vacuo water was added and the solid collected by filtration. Crystallization from acetone-ether gave 1.5 g. of product, homogeneous on T.L.C. (R_f 0.48), m.p. 209-211°; $[\alpha]_D$ -33.5° (Di); λ_{max} 241-242 mμ (ε 14.800); ν_{max} 3470, 1737, 1654, 1622, 1240 and 1047 cm^{-1}.

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74 ; H, 8.78.
Found : C, 70.44 ; H, 8.62.

Acetylation of IV (0.5 g.) in the usual manner gave 350 mg. of a product, m.p. 167-170°; $[\alpha]_D$ +3.5° (Di). T.L.C. examination revealed the presence of the diacetate VI (see below) as the major component, R_f 0.76, together with a minor component at R_f 0.91, identified as the 16,17,20-triacetate [17].

16α,17α,20β-Trihydroxypregn-4-en-3-one 20-acetate (V). - A solution of IV (0.3 g.) in ethanol (6 ml.) was treated with triethylamine (1.2 ml.). After heating for 3 hours under reflux, the reaction mixture was evaporated to dryness. The residue, taken up in water, filtered and recrystallized from acetone-hexane, gave 180 mg. of a product, m.p. 184-187°, practically homogeneous on T.L.C. (R_f 0.53). Concentration of the mother liquors afforded an additional 100 mg. of V containing about 10% of IV. Recrystallization gave the analytical sample, m.p. 189-191°; $[\alpha]_D$ +76° (Di); λ_{max} 241-242 mμ(ε 15.200); ν_{max} 3525, 3475, 1726, 1670, 1612, 1243, 1080 and 1071 cm^{-1}.

Anal. Calcd. for $C_{23}H_{34}O_5$: C, 70.74; H, 8.78.
Found : C, 70.54; H, 8.70.

Acetylation in the usual manner of V (100 mg.) gave, after recrystallization from acetone-hexane, 85 mg. of the 16,20-diacetate VI, homogeneous on T.L.C.[17] (R_f 0.76), m.p. 174-176°; $[\alpha]_D$ +10° (Di); λ_{max} 241-242 mμ (ε 15.500); ν_{max} 3600, 1730, 1675, 1617, 1240, 1136, 1076 and 1053 cm^{-1}.

Anal. Calcd. for $C_{25}H_{36}O_6$: C, 69.42; H, 8.39.
Found : C, 69.50; H, 8.21.

3β,16α,20β-Trihydroxypregn-5-ene 3,16-diacetate (VII). - Reduction with $NaBH_4$ (90 mg.) of 3β,16α-diacetoxypregn-5-en-20-one (0.5 g.) in tetrahydrofuran (20 ml.) overnight at room temperature gave 350 mg. of VII, m.p. 189-191°. Recrystallization from methanol raised the m.p. to 192-194°; $[\alpha]_D$ -125° (Di); ν_{max} 3570, 3500, 1732, 1708, 1240 and 1057 cm^{-1}.

Anal. Calcd. for $C_{25}H_{38}O_5$: C, 71.74; H, 9.15.
Found : C, 71.44; H, 9.36.

The product was recovered unchanged after refluxing for 6 hours in ethanol-triethylamine solution.

Acetylation gave the 3,16,20-triacetate m.p. 169-171°; $[\alpha]_D$ -98° (Di); ν_{max} 1726, 1242 and 1041 cm^{-1}.

Anal. Calcd. for $C_{27}H_{40}O_6$: C, 70.40; H, 8.75.
Found : C, 70.63; H, 8.88.

Methanolysis of IIIc. - A solution of IIIc (100 mg.) in methanol (4 ml.) was treated with triethylamine (0.4 ml.). After standing at room temperature for 2.5 hours, removal of the solvent under vacuum and crystallization of the residue from methylene chloride-methanol yielded 60 mg. of pure product, m.p. 228-231°, which undepressed the melting point in admixture with the authentic sample of IIc and showed the same mobility on T.L.C. (R_f 0.40) and IR spectrum.

Methanolysis of IIIa. - A similar methanol-triethylamine solution of IIIa was kept at room temperature and examined on T.L.C. at given times. Approximate evaluation of the reaction products was done by comparison with scalar amounts of authentic samples. After 2.5 hours a 70-80% of the diester IIIa (R_f 0.55) was still unchanged, while monoesters Ic (R_f 0.16), IIc (R_f 0.40) and IIa (R_f 0.34) and the free tetraol ($R_f < 0.1$) were present in 5-10% amount each.

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16. Melting points are uncorrected. Optical rotations were taken in methanol unless otherwise indicated. UV spectra were determined in 95% ethanol with an Optica CF_4 spectrophotometer. The IR spectra were measured in Nujol mull on the Perkin-Elmer 21 instrument. Chromatographic analyses were done on thin-layer of silica gel (Carlo Erba) with 1% fluorescence indicator (S 5 grün/1, Leuchtstoffwerk GMBH and Co., Heidelberg) using a solvent system benzene-acetone-methanol 17:2:1. The authors are indebted to Dr. Sergio Cairoli for the mycroanalyses and to Dr. Cesare Pedrali for the IR spectra.

17. Unlike the product obtained from V, the 16,20-diacetate VI prepared from IV contained a little of triacetate. Evidently, the latter arose by acetyl migration O-16 ⟶ O-17, before acetylation of 20-hydroxyl.