THE PREPARATION AND BIOLOGICAL ACTIVITY OF 9α,11β-DICHLORO-21-HYDROXY-16α,17α-ISOPROPYLIDENEDIOXYPREGNA-1,4-DIENE-3,20-DIONE

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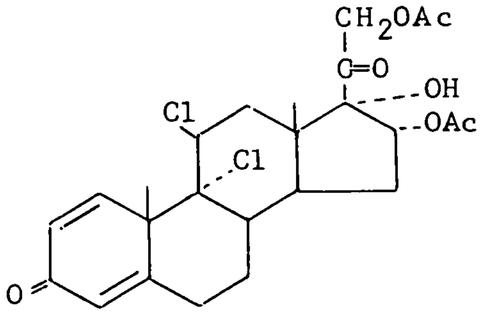
Received February 9, 1963

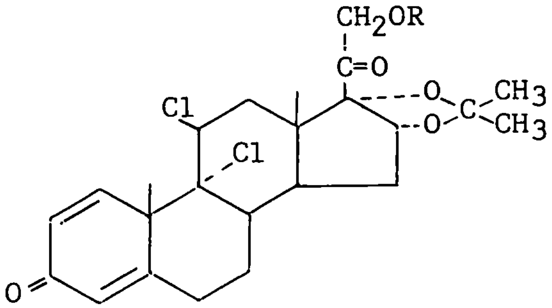
The preparation of 16α,21-diacetoxy-9α,11β-dichloro-17α-hydroxypregna-1,4-diene-3,20-dione (I) and 9α,11β-dichloro-21-hydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione (IIa) is described. The thymolytic activities of these and other 9α,11β-dichlorocorticoids are discussed.

The disclosures of the preparation of an interesting series of 9α,11β-dihalocorticoids[1] and their 17,21-acetonide derivatives[2] with potent anti-inflammatory activity[1,2,3] prompted us to investigate the activities of a 16-hydroxylated-9α,11β-dihalocorticoid.

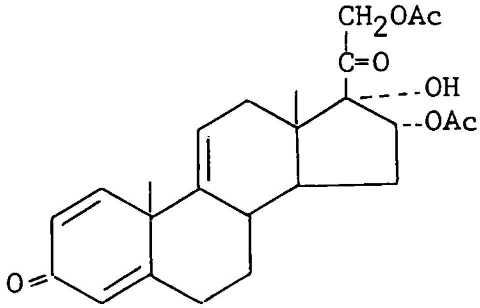
For this purpose, it was decided to prepare 16α,21-diacetoxy-9α,11β-dichloro-17α-hydroxypregna-1,4-diene-3,20-dione (I) and 9α,11β-dichloro-21-hydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione (IIa). The 9α,11β-dichloro-16α,21-diacetate I was prepared by simple chlorination of 16α,21-diacetoxy-17α-hydroxypregna-1,4,9(11)-triene-3,20-dione[4] (III) in carbon tetrachloride-pyridine.[2] The 9α,11β-dichloro-16α,17α-acetonide IIa



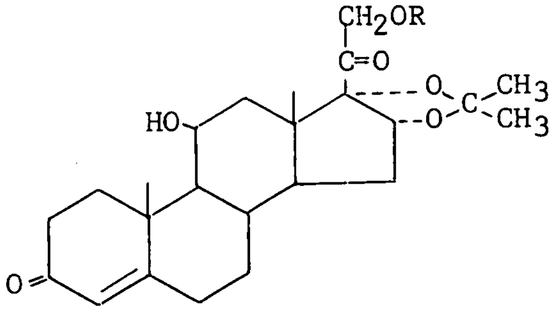
I



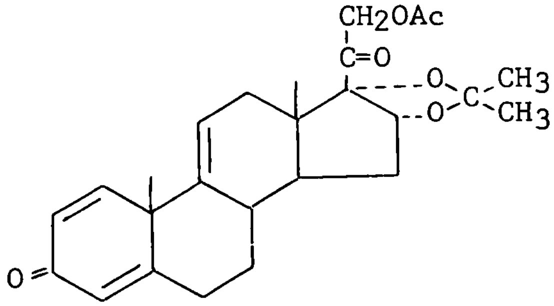
IIa, R = H
IIb, R = Ac



III



IVa, R = H
IVb, R = Ac
IVc, Δ^1; R = Ac



V

was prepared in the following manner. Routine acetylation of the 16α,17α-acetonide (IVa)[5] of 16α-hydroxyhydrocortisone gave IVb which was treated with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone[6] to afford the 1,4-diene IVc.[7] The latter compound was dehydrated with N-bromoacetamide-pyridine-sulfur dioxide[8] to give the 1,4,9(11)-triene V which in turn was chlorinated to yield the 9α,11β-dichloro-compound IIb. Acid hydrolysis afforded 9α,11β-dichloro-21-hydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione (IIa).

The biological activities of compounds I and IIa were determined in a 48 hr. thymolytic assay in the rat.[9] In Table 1 are listed activities of these compounds with those for dichlorisone[1] (9α,11β-dichloro-17α,21-dihydroxypregna-1,4-diene-3,20-dione), dichlorisone acetate[1] and dichlorisone 17,21-acetonide.[2] Also in Table 1 are listed the electrolyte activities of the above compounds. It may be noted that our thymolytic value for dichlorisone acetate approximates that reported by Dorfman and coworkers.[10] These assay results are in contradistinction to the high thymolytic activity (equal to that of prednisolone acetate) cited by Tolksdorf.[3] Furthermore, dichlorisone 17,21-acetonide possessed no significant activity. However, the 9α,11β-dichloro-16,17-acetonide IIa showed considerable thymolytic activity, especially when compared to dichlorisone or the 16α,21-diacetate I. This potentiating effect parallels similar results obtained

Table 1. Biological Activities - Rat

Compound	Thymolytic Activity (Hydrocortisone=1)[a,b]	Electrolyte Activity[a,d]
Dichlorisone	no response at 1000 mcg.	Na-retainer K-no effect
Dichlorisone 21-acetate	0.25 (0.16-0.39)[c]	no effect
Dichlorisone 17,21-acetonide	no response at 1000 mcg.	no effect
9α,11β-Dichloro-16,21-diacetate (I)	0.21 (0.11-0.39)	no effect
9α,11β-Dichloro-16,17-acetonide (IIa)	4.2 (3.2-5.3)[c]	Na-no effect K-excretion

[a] The thymus involution and electrolyte assays were carried out by the Metabolic Chemotherapy Department of these Laboratories.

[b] Activities are on a weight basis. Figures in parentheses represent 95% confidence limits.

[c] N. R. Stephenson (Department of National Health and Welfare, Ottawa, Canada) has found in his thymus involution assay (subcutaneous, corn oil) that dichlorisone acetate possessed an activity of 1.26 (1.14-1.40) (hydrocortisone=1 on a weight basis) whereas the activity of dichlorisone 16,17-acetonide (IIa) was 5.42 (4.93-5.98). We are indebted to Dr. Stephenson for this information.

[d] These excretion patterns were obtained from salt-loaded adrenalectomized male rats in a 5 hr. test following subcutaneous injection of the steroid. The sodium and potassium values were obtained by use of a flame attachment to a Technicon Autoanalyzer.

by 16,17-acetonide[5,11] formation. It is also interesting to note that a carrageenin assay[12] of the 16α,17α-acetonide IIa administered subcutaneously revealed an activity about 9 times that for hydrocortisone.[13]

Experimental[14]

16α,21-Diacetoxy-9α,11β-dichloro-17α-hydroxypregna-1,4-diene-3,20-dione (I). - To a stirred solution of 16α,21-diacetoxy-17α-hydroxypregna-1,4,9(11)-triene-3,20-dione (III, 0.5 g.) in carbon tetrachloride (40 ml.) and pyridine (0.6 ml.) was added a saturated solution of chlorine in tetrahydrofuran (3 ml.). A precipitate soon appeared, and the mixture was stirred for 1 hr. when methylene chloride (100 ml.) was added. The resulting solution was washed successively with sodium thiosulfate solution, dilute sulfuric acid, potassium carbonate solution, and then with water. The dried extract was evaporated to yield a colored solid which was chromatographed on Florisil.[15] The eluate from acetone:petroleum ether (60-70°) (4:21) gave 0.19 g. of I, m.p. 234-234.5° (d.) after crystallization from acetone-petroleum ether (60-70°). Further crystallization from the same solvent pair gave the analytical sample, m.p. 245.5-246° (d.); λ_{max} 238 mμ (ϵ 14,200); ν_{max} 3450, 1752, 1678, 1645, 1625 and 1247 cm.$^{-1}$; $[\alpha]_D^{25}$ + 68° (chloroform).

Anal. Calcd. for $C_{25}H_{30}O_7Cl_2$ (513.40): C, 58.48; H, 5.89; Cl, 13.81. Found: C, 58.95; H, 6.05; Cl, 13.65.

21-Acetoxy-11β-hydroxy-16α,17α-isopropylidenedioxypregn-4-ene-3,20-dione (IVb).[16] - Acetylation of 11β,21-dihydroxy-16α,17α-isopropylidenedioxypregn-4-ene-3,20-dione (IVa, 10.3 g.) in pyridine (100 ml.) and acetic anhydride (10 ml.) at room temperature gave IVb /recrystallized from acetone-petroleum ether (60-70°)/, m.p. 249-252°; λ_{max} 242 mμ (ϵ 15,900); ν_{max} 3360, 1749, 1727, 1660, 1625, 1238 and 1053 cm.$^{-1}$; $[\alpha]_D^{30}$ + 151° (chloroform).

Anal. Calcd. for $C_{26}H_{36}O_7$ (460.55): C, 67.80; H, 7.88. Found: C, 67.60; H, 8.19.

21-Acetoxy-11β-hydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione (IVc). - Compound IVb (1 g.) was dehydrogenated in the usual manner[6] with 2,3-dichloro-5,6-dicyanobenzoquinone (700 mg.) in dioxane (10 ml.) (20 hr. reflux). The resultant material was chromatographed on Florisil[15] and the eluate obtained from acetone:petroleum ether (60-70°) (18:82) gave white crystals of IVc (0.295 g.), m.p. 246-248°. Crystallization from acetone-petroleum ether (60-70°) provided the analytical sample, m.p. 248-250°; λ_{max} 244 mμ (ϵ 13,700); ν_{max} 3380, 1753, 1730, 1660, 1619, 1605, 1229 and 1053 cm.$^{-1}$; $[\alpha]_D^{25}$ + 108° (chloroform).

Anal. Calcd. for $C_{26}H_{34}O_7$ (458.53): C, 68.10; H, 7.47. Found: C, 68.07; H, 7.49.

21-Acetoxy-16α,17α-isopropylidenedioxypregna-1,4,9(11)-triene-3,20-dione (V). - The 1,4-diene IVc (4.065 g.) in dry pyridine (40 ml.) was dehydrated with N-bromoacetamide (1.845 g.) and sulfur dioxide in the manner described.[8] The resultant crude product (3.10 g., m.p. 200.5-201.5°) was recrystallized from acetone-petroleum ether (60-70°) to provide the pure triene V, m.p. 214.5-216°, λ_{max} 239 mμ (ε 15,400); ν_{max} 1757, 1732, 1630, 1610, 1233 and 1049 cm.$^{-1}$; $[\alpha]_D^{25}$ + 42° (chloroform).

Anal. Calcd. for $C_{26}H_{32}O_6$ (440.52): C, 70.89; H, 7.32. Found: C, 70.62; H, 7.29.

21-Acetoxy-9α,11β-dichloro-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione (IIb). - Chlorine was bubbled through a solution of the triene V (1.0 g.) in chloroform (40 ml.) and pyridine (5 ml.) for 30 seconds. The solution was stirred at room temperature for 30 min., diluted with methylene chloride (100 ml.) and worked up as reported above. The extract was evaporated in vacuo to give a glass which was crystallized from acetone-hexane yielding IIb (1.07 g.), m.p. 245-246° (d.). Two additional crystallizations from acetone gave the analytical sample, m.p. 246-251° (d.); λ_{max} 237.5 mμ (ε 15,000); ν_{max} 1759, 1739, 1670, 1637, 1614, 1231 and 1039 cm.$^{-1}$; $[\alpha]_D^{25}$ + 146°

(chloroform).

Anal. Calcd. for $C_{26}H_{32}O_6Cl_2$ (511.43): C, 61.06; H, 6.31; Cl, 13.87. Found: C, 61.16; H, 6.44; Cl, 14.17.

9α,11β-Dichloro-21-hydroxy-16α,17α-isopropylidenedioxypregna-1,4-diene-3,20-dione (IIa). - A suspension of the dichloro-acetonide 21-acetate IIb (1.81 g.) in 0.27 N perchloric acid in methanol (150 ml.) was stirred at room temperature for 22 hr. whereupon complete solution occurred. Dropwise addition of this solution to a large quantity of water (ca. 1.5 l.) with stirring gave IIa (1.6 g.), m.p. 217.5-219° (d.). Several crystallizations from acetone-hexane gave m.p. 230-231° (d.); λ_{max} 237.5 mμ (ϵ 14,100); ν_{max} 3490, 1718, 1670, 1639, 1615 and 1044 cm.$^{-1}$; $[\alpha]_D^{25}$ + 164° (methanol).

Anal. Calcd. for $C_{24}H_{30}O_5Cl_2$ (469.39): C, 61.41; H, 6.44; Cl, 15.11. Found: C, 61.23, 61.56; H, 6.64, 6.70; Cl, 15.33.

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13. We thank Dr. A. Vogel of these Laboratories for this result.

14. All melting points are uncorrected. The ultraviolet spectra were determined in methanol; the infrared spectra were determined in a potassium bromide disk. We wish to thank Louis M. Brancone and associates for the analytical data, and William Fulmor and associates for the spectral and optical rotational data.

15. Florisil, a trademark of the Floridin Company for a synthetic magnesium silicate.

16. This experiment was done by Stephen M. Stolar.