STUDIES ON THE CONSTITUENTS OF ASCLEPIADACEAE PLANTS XIII.*1

EPIMERIZATION AT C-17 AND OPTICAL ROTATORY DISPERSION STUDY OF C/D CIS PREGNANE-20-ONE DERIVATIVES

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Alkaline treatment of C/D cis-20-ketosteroids produced an equilibrium mixture of 17α- and 17β-H-20-keto compounds. The 17β-H-epimer is more stable and is obtained as the main product. The rotatory dispersion curves of the 17β-H-20-keto compounds show negative and the 17α-H-20-keto compounds show positive Cotton effects, but the amplitude varies with neighbouring groups.

It has been shown already that several Asclepiadaceae plants contain ester glycosides, and the structures of these aglycones have been investigated chiefly by Reichstein's group and by us. The structures of cynanchogenin (I),[1,2] metaplexigenin (II),[3] benzoylramanone (III),[3,4] deacylcynanchogenin = lineolon (Ia),[1,2] isodeacylcynanchogenin (Ib),[2,5] ramanone (= isodigipurpurogenin-II) (IIIa),[3,4,6] isoramanone (= digipurpurogenin-II) (IIIb),[3,4,6] sarcostin,[1,2] tayloron (V),[1] tomentogenin = 5α-dihydroutendin (VI),[7] and dehydrotomentogenin = utendin (VII)[8] have been assigned. All these aglycones possess 12β-OH, 14β-OH and 20-oxygen groups.

This report is an attempt to summarize the relation between the C-17 side chain epimerization of C/D cis 20-ketopregnane and the change of optical rotatory dispersion. Shimizu found that I was easily isomerized by alkaline treatment to give a 7:3 equilibrium mixture of Ia and Ib.[2]

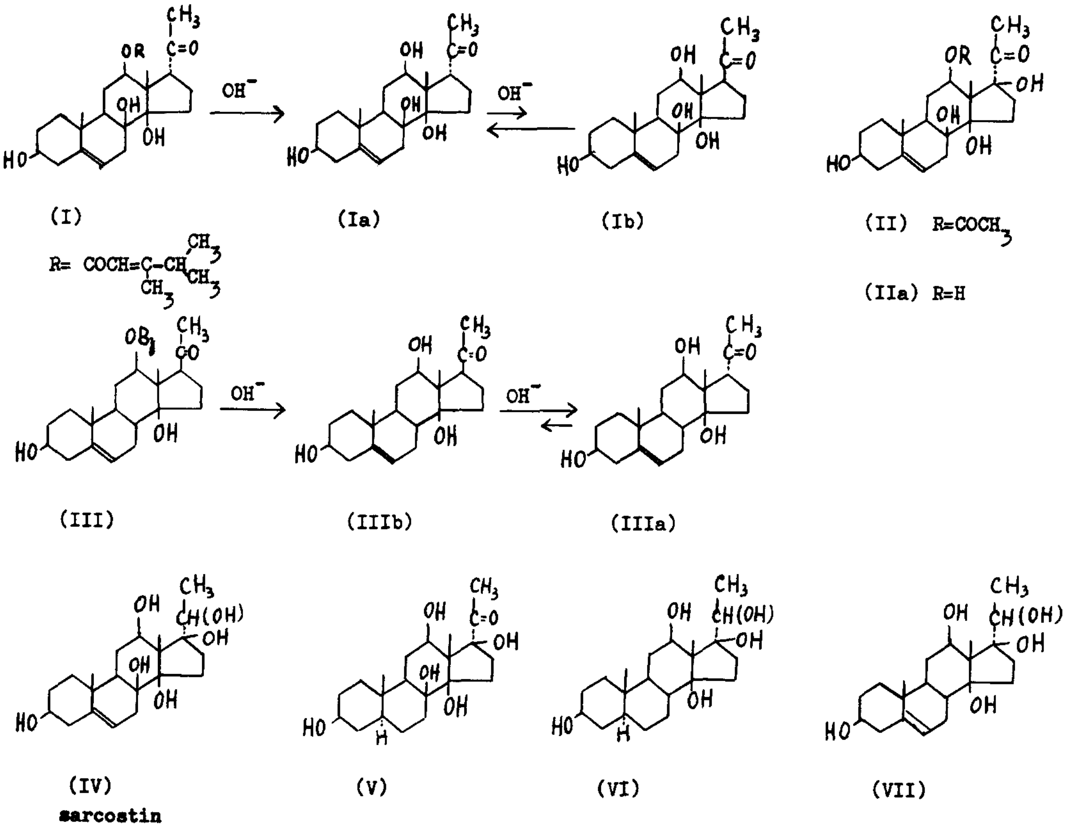
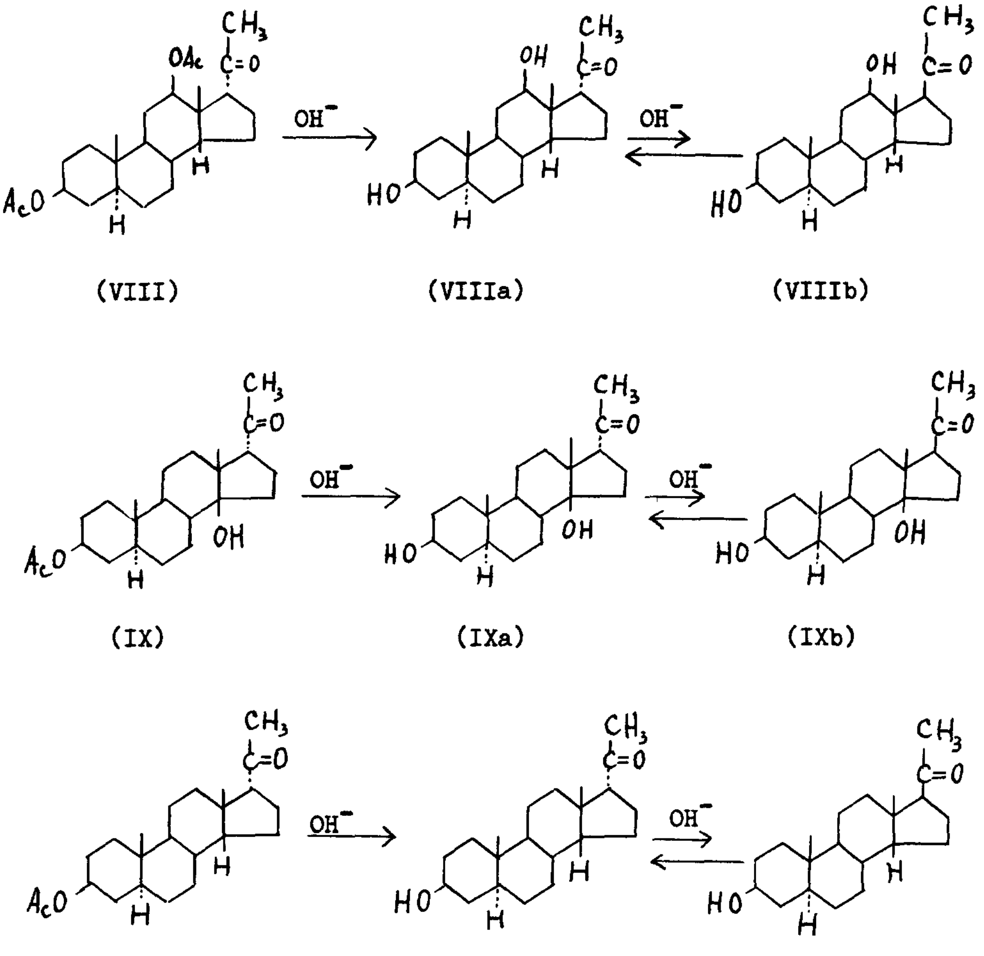


Chart 1

Nomura also reported that by a similar treatment of III, an equilibrium mixture of IIIa and IIIb was obtained and IIIa was the main product.[3,4] It was reported that in acid-, or base-catalysed reactions, C/D trans-20-ketopregnanes were isomerized to give about 7:3 equilibrium mixture of 17α-H-, and 17β-H-20-keto isomers, and the ratio of C-17 epimers varied with different neighbouring group.[9,10] On the other hand, the isomerization of C/D cis 17α-H-20-ketopregnane to 17β-H- by alkaline treatment was reported,[11] but the 17β-H- isomer was isolated as the only product in a poor yield. Meyer found that by alkaline hydrolysis of the 14β,15β-epoxy-17β-carbomethoxy derivative obtained from resibufogenin, two C-17 epimers were formed in about 1:1 ratio.[12] From these reports, it was



(VIII) (VIIIa) (VIIIb)

(IX) (IXa) (IXb)

(X) (Xa) (xb)

Chart 2

obvious that C/D cis 17β-H-20-keto is thermodynamically more stable than the 17α-H-epimer, but equilibration of the C-17 epimers was not mentioned. The equilibration of deacylcynanchogenin and ramanone can be attributed to either the base-catalysed reaction or the effect of neighbouring 12β-OH and 14β-OH in the molecule. Mitsuhashi and co-workers,[2,3,4] employed 5% KOH in MeOH refluxing for 5 hr., followed by fractional crystallization of the resulting mixtures to obtain Ia, Ib and IIIa, IIIb from their naturally occuring ester aglycones. These hydrolysis conditions are rather strong compared to those commonly employed in isomerization. To find the proper conditions for isomerization, Ia was treated with the following concentration of KOH- MeOH; 5,2.5, 1.2, 0.6 and 0.2%. These mixtures were refluxed for 1 and 5 hr., respectively. Compound Ia isomerized to Ib and the ratio of Ia to Ib was about the same in all these experimental conditions. Ia proved to be the main product after paper chromatography.[13]

To investigate the effect of 12ß-OH on C-17 epimerization, 3ß,12ß-diacetoxy-5α,14ß,17α-pregnane-20-one (VIII)[4] was treated with 5% KOH-MeOH, and the resulting mixture was examined by thin-layer chromatography (Al_2O_3, 3.5% MeOH/C_6H_6). Compounds VIIIa, m.p. 80-85°, Rf 0.42 and VIIIb, m.p. 205-206°, Rf 0.33 were obtained by fractional crystallization. After alkaline treatment, VIIIa and VIIIb, each yielded a mixture of VIIIa and VIIIb. The Cotton effect of the rotatory dispersion curves VIII, VIIIa was negative but the curve of VIIIb was positive. These results strongly suggest a 17ß-H-20-keto configuration for VIII, VIIIa and 17α- for VIIIb.

To prove the effect of the 14ß-OH group on C-17 epimerization, 3ß-acetoxy-14ß-hydroxy-5α,14ß,17α-pregnane-20-one (XI)[14] was treated as described above. The resulting mixture was submitted to thin-layer chromatography (Al_2O_3, 2% MeOH/C_6H_6). Two spots, IXa, Rf 0.25 and IXb, Rf 0.42 were located. Compounds IXa, m.p. 215-220°, and IXb, m.p. 238-242° were obtained from the reaction mixture after Al_2O_3 chromatography. When both IXa and IXb were submitted separately to 5% KOH-MeOH treatment, and the resulting mixtures examined by thin-layer chromatography, the C-17 epimers were formed from both compounds but IXa was the main product. The rotatory dispersion curves of IX and IXa showed a negative Cotton effect while the curve of IXb showed a positive effect. 3ß-Acetoxy-5α,14ß,17α-pregnane-

20-one (X)[14] gave exactly the same results as IX after alkaline treatment, Xa, m.p. 180-184°, as the main product, and Xb, m.p. 170-172°, the minor product, were obtained from the reaction mixture. The rotatory dispersion curves of X and Xa showed a negative Cotton effect and the curve of Xb showed a positive one. The optical rotatory dispersion curves of C/D cis 20-keto compounds are shown in Table I.

From these experiments and from previous papers,[1,2,3] one can draw the following conclusions:

1. The Cotton effect and equilibration.- The rotatory dispersion has been employed successfully to determine the configuration of the C-17 side chain of C/D cis 20-keto-pregnanes. The thermodynamically stable 17ß-H-20-keto compound shows a negative Cotton effect whereas a positive Cotton effect is observed with the 17α-isomer. It is notable that in the C/D trans steroids, the more stable 17α-H-20-keto compound shows a positive Cotton effect, and the 17ß-H-isomer a negative effect.[15] These results are described in Table II.

2. Effect of 12ß-acetoxy groups.- The C/D cis 17ß-H-20-keto series, which have 12ß-acetoxy groups show a shift of molecular amplitude to the negative side in the corresponding 12ß-hydroxy compounds (Ia-XI, IIIa-XII, IIa-II) and in the corresponding unsubstituted compounds, (IX-XII, XIII, X-VIII, XVII-XVI). Klyne has also mentioned the effect of the 12ß-acetoxy group.[1]

3. Effect of 12ß-hydroxy groups.- In both 17ß-, and 17α-H-20-keto compounds, the presence of a 12ß-hydroxy group causes a shift of the molecular amplitide to the positive side (IIIa-IXa, VIIIa-Xa, VIIIb-Xb, IIIb-IXb).

4. Effect of 14ß-groups. - The presence of the 14ß-hydroxy group, the 17-isomers of 20-ketopregnanes causes a shift of the molecular amplitude to the

Table I

Compound	[φ] and λ mμ				Sign and amplitude of wave a X 10^{-2}
	first extremum		second extremum		
(Ia)	-2592	(302)	+5270	(255.5)	- 77.8
(Ib)	+3920	(305)	+ 600.6	(270)	+ 33.2
(XI)= 3,12-diacetate of Ia					-112[1]
(II)	-3114.1	(315)	+5869.9	(265)	- 89.8
	-3291.6	(319)	+4135.6	(270)	- 74.2*
(IIa)	-1174.2	(316)	+5506.2	(267)	- 66.8
(V)	-1410	(320)	+4650	(280)	- 61
(III)	+2404.6	(305)	-3073.6	(270)	+ 54.77
(IIIa)	-4542	(301)	+4308	(260)	- 88.5
	-5731	(306)	+4078	(263)	- 98.0*
(IIIb)	+3114.6	(305)	-2283.3	(265)	+ 53.9
	+3824.5	(308)	-3318.4	(269)	+ 71.4*
(XII)=3,12-diacetate of IIIa	-8264	(302)	+3801	(259)	-120.6
(XIII)=3,12-diacetate 5α-dihydroramanone	-7776	(302)	+4968	(259)	-127.4
(VIII)	-4953	(304)	+5421	(256)	-103.7
(VIIIa)	-1870.4	(298)	+1336	(260)	- 32.0
(VIIIb)	+5277.2	(304)	-5911	(260)	+111.9
(IX)	-4286	(302)	+4211	(256)	- 84.9
(IXa)	-5427	(302)	+4328	(262)	- 97.5
(IXb)	+1449	(298)	- 611	(262)	+ 20.6
(XIV)= 14ß,17α-pregnane-3ß,5ß,14ß-triol-20-one 3-acetate					- 86[1]
(XV)= 14ß-pregnane-3ß,5ß,14ß-triol-20-one 3-acetate					+ 25[1]
(X)	-2547	(303)	+4896	(260)	- 74.7
(Xa)	-3377.1	(303)	+4341.8	(256)	- 77.1
(Xb)	+3001.2	(301)	-3889	(260)	+ 68.9
(XVI)= 14ß,15ß epoxide of VIII	-3350	(302)	+4071	(255)	- 74.2[4]
(XVII)=14ß,15ß epoxide of X	-1727	(301)	+3882	(256)	- 56.9[4]

Table II

Cotton effect	Relative stability	C/D ring	C-17-configuration
+	stable	trans	17α-H-
-	stable	cis	17ß-H-
+	unstable	cis	17α-H-
-	unstable	trans	17ß-H-

negative side (Xa-IXa, X-IX, VIIIa-IIIa, VIII-XIII, VIIIb-IIIb, Xb-IXb).

5. Effect of 14ß,15ß-epoxide group.- The 14ß,15ß-epoxide causes the molecular amplitude to move to the positive side (VIII-XVI, X-XVII).

6. Effect of 17ß-hydroxy groups. - The addition of a 17ß-hydroxy group, (II,IIa and Ia,IIIa) results in the trough shifting about 13 mμ to the long wave region. This also occurs with the C/D trans compound.[15,16] In the case of C/D cis 20-ketopregnane, the sign of the Cotton effect is effected by C-17 configuration and the amplitude is effected by the neighbouring substituents.

EXPERIMENTAL

Isomerization of the side chain of Deacylcynanchogenin (Ia). Deacylcynanchogenin (Ia, 1 mg.) and 5% methanol-potassium hydroxide (0.2 ml.) were sealed under nitrogen and heated for 5 hr. on the steam bath. When cool, the reaction mixture was examined by paper chromatography (chloroform/formamide) and the formation of isodeacylcynanchogenin (Ib, R_{dc}=1.9) was proved. The same isomerization was observed with different conditions, such as 0.2% methanol-potassium hydroxide heated 1 hr. Treatment with 0.2% methanol-potassium hydroxide for 3 hr. at room temperature produced a small yield of Ib.

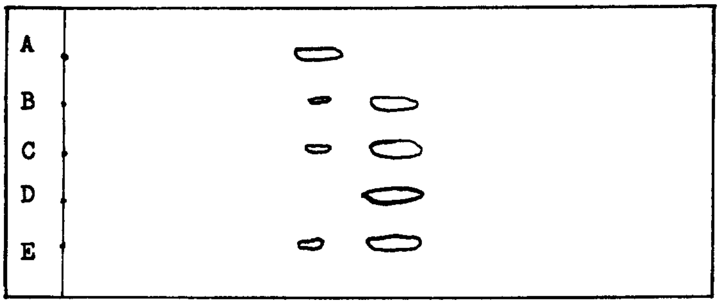
Hydrolysis of 3ß,12ß-diacetoxy-5α,14ß,17α-pregnane-20-one (VIII).

VIII (66 mg.) was hydrolyzed with 5% methanol-potassium hydroxide (3 ml.) in nitrogen for 5 hr. The reaction mixture was extracted continuously with ether. The extract (55 mg.) gave 13 mg. crystals (VIIIb), m.p. 205-209° after recrystallization from benzene. VIIIa, m.p. 80-85° was obtained by recrystallization of the mother liquor with acetone-water. VIIIa and VIIIb were treated with 5% methanol-potassium hydroxide for 5 hr. Thin-layer chromatographic analysis (alumina, 3.5% methanol/benzene) gave the results shown in Fig. 1.

Hydrolysis of 3β-acetoxy-14β-hydroxy-5α,14β,17α-pregnane-20-one (IX). IX (50 mg.) was treated as VIII and after the evaporation of the methanol, 44 mg. of crystals were obtained. These were recrystallized from benzene to colorless needles, IXa (26 mg.), m.p. 215-220°. The mother liquor gave IXb (2 mg.), m.p. 238-240°. IXa and IXb were treated in the same manner as Ia and Ib. Thin-layer chromatographic analyses are shown in Fig. 2.

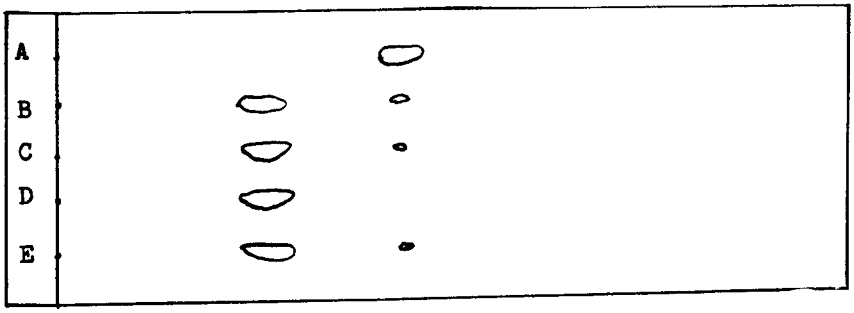
Hydrolysis of 3β-acetoxy-5α,14β,17α-pregnane-20-one(X). X (44 mg.) was treated as VIII, the resulting mixture was extracted with ether. Removal of ether gave 40 mg. crystals. The product was submitted to chromatography. Xb, m.p. 170-174°, 4 mg., was eluted with hexane-benzene (2:1) and Xa, m.p. 180-185°, 10 mg., with benzene. Thin-layer chromatographic analyses of the products of alkaline treatment of Xa and Xb are shown in Fig. 3.

Fig. 1



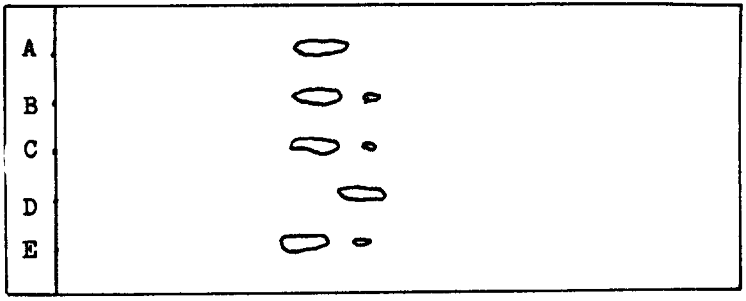
A= VIIIb, B= products of alkaline treatment of VIIIb, C= products of alkaline treatment of VIIIa, D= VIIIa, E= products of alkaline treatment of VIII.

Fig. 2



A= IXb, B= products of alkaline treatment of IXb, C= products of alkaline treatment of IXa, D= IXa, E= products of alkaline treatment of IX.

Fig. 3



A= Xa, B= products of alkaline treatment of Xa, C= products of alkaline treatment of Xb, D= Xb, E= products of alkaline treatment of X.

Optical Rotatory Dispersion. - The rotatory dispersion curves were measured with an automatic recorder Rudolph photoelectric spectropolarimeter and O.R.D.-UV-5-Niphon Bunko. The results are shown in Table I and the measuring conditions are as following;

Ia, Ib, II, IIb, IIIa, IIIb, C= 0.2, l= 0.1 in MeOH, 25°C.

II, IIIa, IIIb *C= 0.2, l= 0.1 in dioxane, 25°C.

III, C= 0.2, 700-290 mμ, l= 0.1, 310-250 mμ, l= 0.01 in MeOH, 14-15°C.

XII, XIII, VIII, VIIIa, VIIIb, IX, IXa, X, Xa, XVI, XVII, C= 0.2, l=0.2, and 0.5 in MeOH, 14-15°C.

IXb, C= 0.08, l= 0.5, in MeOH, 14°C.

[a] = molecular amplitude, and [ϕ] = molecular rotation.

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