HYDROGENATION OF DIGITALIS GENINS AND ANHYDROGENINS

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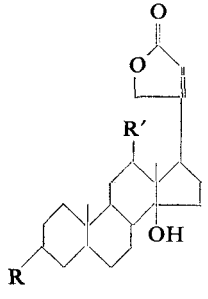
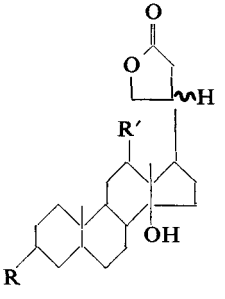
Hydrogenation of the 14,15-unsaturated linkage in the digitalis β-anhydrogenins without simultaneous reduction of the cyclobutenolide group is described. The reduced compounds have an α configuration for the hydrogen at C(14). Hydrogenation of the cyclobutenolide ring produces two isomeric compounds which have been separated and assigned to the 20α and 20β steroid series respectively.

To investigate the influence of the 14β-hydroxyl group characteristic of cardiac glycosides on the pharmacological action of these compounds we have eliminated this group from digoxigenin (Ia) and digitoxigenin acetate (Ic) by dehydration followed by hydrogenation of the anhydrogenin so produced without at the same time reducing the unsaturated 20,22 linkage of the cyclobutenolide ring. It was necessary to avoid reduction of this ring as Jacobs and Hoffman (1927), and Chen and Elderfield (1940) have shown that 20,22-dihydroglycosides have much reduced activity. The C/D ring juncture of the 14-deoxygenins so produced was however *trans* instead of *cis* in the original genins.

The reduction of the unsaturated 20,22 linkage of the cyclobutenolide ring has also been investigated. Hydrogenation of this linkage introduces a centre of asymmetry at C(20) and the isomers produced may be related



Ia. R = R′ = OH
Ib. R = OH, R′ = H
Ic. R = OAc, R′ = H

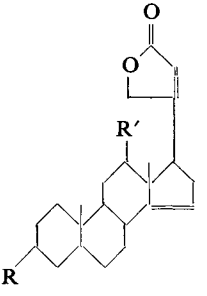


IIa. R = R′ = OH
IIb. R = OH, R′ = H

to the 20α or 20β series of steroids as typified by the 20-isonorallocholanic acids (20α) and the slightly more dextrorotatory norallocholanic acids (20β) (Plattner, 1951). We have hydrogenated digoxigenin (Ia) and 3β-acetoxy-14α-card-20(22)-enolide (IVb) (14-deoxydigitoxigenin acetate) with platinum catalyst, separated the isomers by crystallisation and related each isomer to the 20α or 20β series on the basis of optical rotation and melting points.

14-*Deoxygenins*

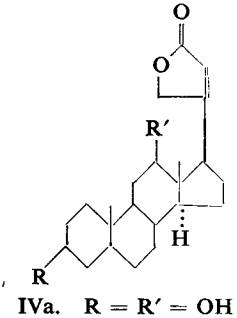
Preferential reduction of the 14,15-unsaturated linkage in β-anhydrodigoxigenin (IIIa) and β-anhydrodigitoxigenin acetate (IIIb) without the simultaneous reduction of the cyclobutenolide ring was achieved by hydrogenation in ethanolic solution with palladium catalyst at room temperature. Under these conditions one molecular equivalent of hydrogen was absorbed and the reduced products showed absorption



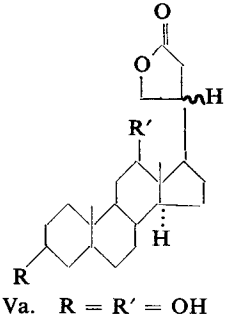
IIIa. R = R′ = OH
IIIb. R = OAc, R′ = H

spectra in the ultra-violet similar to the parent genins (14-deoxydigitoxigenin acetate max. Log $\epsilon = 4{\cdot}236$ at 216 mμ and 14-deoxydigoxigenin max. Log $\epsilon = 4{\cdot}195$ at 217 mμ). Colour reactions with alkaline *m*-dinitrobenzene and tetranitromethane confirmed the presence of the cyclobutenolide ring and the absence of the 14,15-unsaturated linkage.

The α configuration of the hydrogen atom at C(14) (C/D ring *cis*) of the 14-deoxygenins has been assigned on the following evidence.



IVa. R = R′ = OH
IVb. R = OAc, R′ = H



Va. R = R′ = OH
Vb. R = OAc, R′ = H

Complete reduction of 14-deoxydigoxigenin (IVa) by catalytic hydrogenation with platinum produced a substance with no absorption at 217 mμ and which gave no colour with alkaline *m*-dinitrobenzene. The melting point and optical rotation were identical with that of tetrahydro-β-anhydrodigoxigenin (Va) prepared by Plattner, Ruzicka and Pataki (1945) by complete reduction of β-anhydrodigoxigenin (IIIa). Chromic acid oxidation of tetrahydro-β-anhydrodigoxigenin (Va) by these workers gave the diketone 3,12-dioxo-14α-cardanolide which was identical with the diketone

obtained by oxidation of 3α,12β-dihydroxy-14α-cardanolide prepared by synthesis from 3α,12β-dihydroxypregnan-20-one which has a 14α (C/D *trans*) configuration (Ruzicka, Plattner and Pataki, 1944). By analogy, 14-deoxydigitoxigenin acetate (IVb) would have a 14α (C/D *trans*) configuration.

20,22-*Dihydrogenins*

The cyclobutenolide double bond of digoxigenin (Ia) was hydrogenated in ethanolic solution with platinum catalyst. By repeated crystallisation of the 20,22-dihydrodigoxigenin (IIa) from ethyl acetate two crystalline isomerides which differed in melting point and optical rotation were separated. The more soluble material, which had the higher dextrorotation, was designated the 20β isomer and the isomeride with lower dextrorotation, 20α. Constants for both isomers are recorded in Table I.

TABLE I

Compound	α Isomer		β Isomer		Reference
	m.p.	$[\alpha]_D$	m.p.	$[\alpha]_D$	
3α,12β-Dihydroxy-14α-cardanolide	243°	+56·8°	225°	+62·8°	Plattner and others (1945)
3α,12β-Diacetoxy-14α-cardanolide	209°	+116°	188°	+122°	*Ibid.*
3,12-Dioxo-14α-cardanolide ..	312°	+123°	297°	+132°	*Ibid.*
3β-Acetoxy-21-oxo-nor*allo*cholanic acid lactone (23,21)	243°	+5·9°	204°	+19°	Ruzicka and others (1941)
21-Oxonorallocholanic acid lactone (23,21)	178°	+11·3°	180°	+24·3°	Tschesche (1933)
20,22-Dihydrodigitoxigenin acetate	186°	+8°	178°	+14°	Meyer (1946)
20,22-Dihydrodigoxigenin (IIa) ..	225°	+11°	209°	+15°	This paper
3β-Acetoxy-14α-cardanolide (Vb)	139°	+24°	128°	+51°	This paper

3β-Acetoxy-14α-card-20(22)-enolide (IVb) was similarly hydrogenated using platinum oxide catalyst. The dihydro-compound (3β-acetoxy-14α-cardanolide (Vb) was repeatedly crystallised from aqueous ethanol and gave two crystalline isomers. The more soluble material, which also had a higher dextrorotation, was designed 20β, and the isomeride with lower dextrorotation, 20α. Melting points and rotations for both isomers are shown in Table I. None of the 20,22-dihydro-compounds prepared showed absorption at 218 mμ in ethanol, nor at 235 mμ in sulphuric acid. This is the absorption maxima of an unsaturated cyclobutenolide in sulphuric acid (Repke, 1960; Brown and Wright, 1960).

Plattner and others (1945) have hydrogenated the 20,22-double bond of synthetically prepared 3α,12β-dihydroxy-14α-card-20(22)-enolide and after acetylation and oxidation have, by crystallisation, obtained the C(20) isomers of 3α,12β-dihydroxy-14α-cardanolide, the 3,12-diacetate and the 3,12-diketone. These workers have related the higher dextrorotatory isomer of these isomeric pairs to the 20β series of steroids, and the lower dextrorotatory to the 20α series. In Table I the melting points and rotations of these isomers are recorded, together with values for a number of other 20α- and 20β-saturated 23,21 steroid lactones.

In each of the C(20) isomeric pairs listed in Table I, with the possible exception of 21-oxo-norallocholanic acid lactone (23,21), the melting point

of the β isomer (higher dextrorotation) is lower than the melting point of the corresponding α isomer. Both the isomeric pairs prepared in the present study have a similar relationship of melting point and optical rotation, that is, the β isomer has a lower melting point and a higher dextrorotation than the corresponding α isomer.

Smith (1930) prepared 20,22-dihydrodigoxigenin which melted at 170°, resolidified and remelted at 215°, $[\alpha]_D + 19°$. From the melting point and rotation it would appear that this compound is predominately the 20β isomer.

We have also prepared 20,22-dihydrodigitoxigenin (IIb) from digitoxigenin (Ib) by hydrogenation with platinum catalyst. We could obtain only one 20,22-dihydro product, which was similar in melting point and rotation to the 20,22-dihydrodigitoxigenin obtained by Cardwell and Smith (1954) (m.p. 226°, $[\alpha]_D + 17°$). These workers assigned a 20β configuration to this compound, as its rotation agreed more with that of the 20β-dihydrodigitoxigenin acetate ($[\alpha]_D + 14°$) than with that of the 20α isomeride ($[\alpha]_D + 8°$). (Meyer, 1946).

We have also hydrogenated the glycoside digoxin. Using palladium-carbon catalyst in ethanol, reduction occurred only when the material was heated to 50°, while hydrogenation using platinum oxide catalyst proceeded quite rapidly at room temperature. In each instance, reduction was complete as judged by the absence of an absorption maxima at 218 mμ in ethanol. The major portion of the material was amorphous, though a small number of crystals were obtained from methanol-water. Attempts to separate 20α and 20β isomers were unsuccessful.

EXPERIMENTAL

Effect of hydrogenation of digoxigenin using palladium catalyst. Digoxigenin (103 mg.) was dissolved in ethanol (10 ml.) and palladium-carbon catalyst (5 per cent) (190 mg.) added. At room temperature and atmospheric pressure there was no absorption of hydrogen over 8 hr., and the material was recovered unchanged.

14-*Deoxydigoxigenin (IVa).* β-Anhydrodigoxigenin (m.p. 180°, 259 mg., prepared by treatment of digoxigenin with sulphuric acid (Smith, 1930) was hydrogenated with palladium-carbon catalyst (5 per cent) (208 mg.) in 50 ml. ethanol. Hydrogenation proceeded rapidly for the first 6 hr. and was complete after 16 hr., absorbing 17·7 ml. hydrogen (1 mol. requires 16·6 ml.). Recrystallisation from ethyl acetate gave 102 mg. material, m.p. 114°, which on further recrystallisation was raised to m.p. 117–118°, $[\alpha]_D + 20°$ ($c = 0{\cdot}97$ in MeOH). Found: C, 72·65, H, 9·00. $C_{23}H_{34}O_4$ requires C, 73·76; H, 9·15 per cent. Absorption maximum in ethanol, Log ϵ 4·197 (217 mμ); in sulphuric acid at 235 mμ. A tetranitromethane test was negative, and with alkaline *m*-dinitrobenzene the blue colour characteristic of the unsaturated cyclobutenolide ring was observed.

3α,12β-*Dihydroxy*-14α-*cardanolide* (*Va*) (*Tetrahydro-*β*-anhydrodigoxigenin*). 14-Deoxydigoxigenin (30 mg.) was dissolved in ethanol (10 ml.)

and platinum oxide (8 mg.) added. Complete hydrogenation required 2 hr. After recrystallisation from ethyl acetate, the material melted 116°, $[\alpha]_D + 20°$ ($c = 0{\cdot}88$ in $CHCl_3$). The material showed no absorption maxima at 217 mμ in ethanol and no maxima at 235 mμ in sulphuric acid and gave no colour with alkaline *m*-dinitrobenzene.

14-*Deoxydigitoxigenin acetate* (*IVb*). β-Anhydrodigitoxigenin acetate (m.p. 185°, 279 mg. prepared from digitoxigenin acetate by dehydration) (Hunziker and Reichstein, 1945) was hydrogenated with palladium-carbon catalyst (5 per cent) (307 mg.) in 50 ml. ethanol. Complete reduction required 7 hr. and 20·4 ml. were absorbed (1 mol. requires 19·7 ml.). On recrystallisation from ethyl acetate and then methanol-water, the material melted 176–178°,$[\alpha]_D + 18{\cdot}4°$ ($c = 0{\cdot}49$ in $CHCl_3$). Found: C, 74·93; H, 9·14. $C_{25}H_{36}O_4$ requires C, 74·96; H, 9·06 per cent.

20,22-*Dihydrodigoxigenin* (*IIa*). Digoxigenin (412 mg.) was dissolved in ethanol (20 ml.) and hydrogenated with platinum oxide (67 mg.) at room temperature. Complete reduction required 6 hr. and 31·0 ml. of hydrogen were absorbed (1 mol. requires 29·8 ml.). After removal of the catalyst, the solution was evaporated to dryness and the solid (405 mg.) dissolved in ethyl acetate. The first crop of crystals were large and granular and melted 220°. On further recrystallisation from ethyl acetate the melting point was raised to 223–225°, $[\alpha]_D + 11°$ ($c = 0{\cdot}99$ in $CHCl_3$). Found: C, 70·78; H, 9·13. $C_{23}H_{36}O_5$ requires C, 70·37; H, 9·25 per cent. This material was designated the 20α isomer.

The more soluble material crystallised from ethyl acetate in white, feathery crystals. On recrystallisation from ethanol-water, the material melted 145°, resolidified about 160°, and remelted 209–211°, $[\alpha]_D + 15°$ ($c = 0{\cdot}82$ in $CHCl_3$). (Found C, 70·30; H, 9·00, $C_{23}H_{36}O_5$ requires C,70·37; H, 9·25 per cent). This material was designated the 20β isomer.

20,22-*Dihydrodigitoxigenin*. Digitoxigenin (408 mg.) was hydrogenated with platinum oxide (50 g.) in ethanol (25 ml.). Reduction required 7 hr. and 31·3 ml. hydrogen were absorbed (1 mol. requires 29·0 ml.). After recrystallisation from ethanol-water the material melted 222–224°, $[\alpha]_D + 16{\cdot}5$ ($c = 1{\cdot}0$ in MeOH). Found: C, 74·68; H, 9·78. $C_{23}H_{36}O_4$ requires C, 73·36; H, 9·64 per cent.

3β-*Acetoxy*-14α-*cardanolide* (*Vb*). 14-Deoxydigitoxigenin acetate (85 mg.) was dissolved in ethanol (40 ml.) and hydrogenated at room temperature and atmospheric pressure with platinum oxide (79 mg.). Reduction required 1 hr. and 4 ml. hydrogen were absorbed (1 mol. requires 4·8 ml.). The first crop of crystals from ethanol were large and needle-like, melted 103–105°, resolidified and finally melted 115–116°. Further recrystallisation raised the melting point to 137–139° with no lower melting point, $[\alpha]_D + 24°$ ($c = 0{\cdot}8$ in $CHCl_3$). This isomer was designated 20α. Found: C, 73·62; H, 9·69. $C_{25}H_{38}O_4$ requires C, 74·59; H, 9·52 per cent. The more soluble material crystallised on concentration with m.p. 120–126° which was raised to 126–128° on further crystallisation. This was designated the 20β isomer. $[\alpha]_D + 51°$ ($c = 0{\cdot}6$ in $CHCl_3$). Neither of the above compounds gave a colour reaction with alkaline *m*-dinitrobenzene.

20,22-*Dihydrodigoxin*

Palladium Reduction. Digoxin (248 mg.) in ethanol (25 ml.) was hydrogenated with palladium-carbon (5 per cent) (535 mg.) at room temperature. Over a period of 8 hr. no absorption of hydrogen was observed. The solution was then heated to 50° and complete hydrogenation as judged by the absence of a colour reaction with *m*-dinitrobenzene required 8 hr. The major portion of the material would not crystallise, but from methanol-water some crystals were obtained m.p. 148°, which on further recrystallisation melted 160–166°, $[\alpha]_D$ + 12° (c = 1·28 in MeOH). The material showed no absorption at 217 mμ in ethanol and no absorption at 235 mμ in sulphuric acid.

Platinum reduction. Digoxin (251 mg.) in ethanol (25 ml.) was hydrogenated with platinum oxide (25 mg.) at room temperature. Reduction required 8 hr. and 8·2 ml. hydrogen were absorbed (1 mol. requires 8·0 ml.). After recrystallisation from ethanol-water the material melted 155–162°, resolidified 200°, and finally melted 252–254°. Found: C, 61·03; H, 8·52. $C_{41}H_{66}O_{14}.H_2O$ requires C, 61·50; H, 8·54 per cent.

A further sample of digoxin (273 mg.) was reduced with platinum oxide (28 mg.) and 10·8 ml. of hydrogen were absorbed (1 mol. requires 9·0 ml.). After recrystallisation from ethyl acetate the material melted 268–270°, with no lower melting point. $[\alpha]_D$ + 13° (c = 1·4 in MeOH). Found: C, 61·39; H, 8·54. $C_{41}H_{66}O_{14}.H_2O$ requires C, 61·50; H, 8·54 per cent. Neither of the above compounds showed absorption in ethanol at 217 mμ. Both samples showed absorption in sulphuric acid at 320 mμ, 390 mμ and 490 mμ but not at 235 mμ. Attempts to separate possible 20α and 20β isomers were not successful.

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