Digitalis Glucosides. Part VII. The Structure of the Digitalis Anhydrogenins and the Orientation of the Hydroxy-groups in Digoxigenin and Gitoxigenin.*

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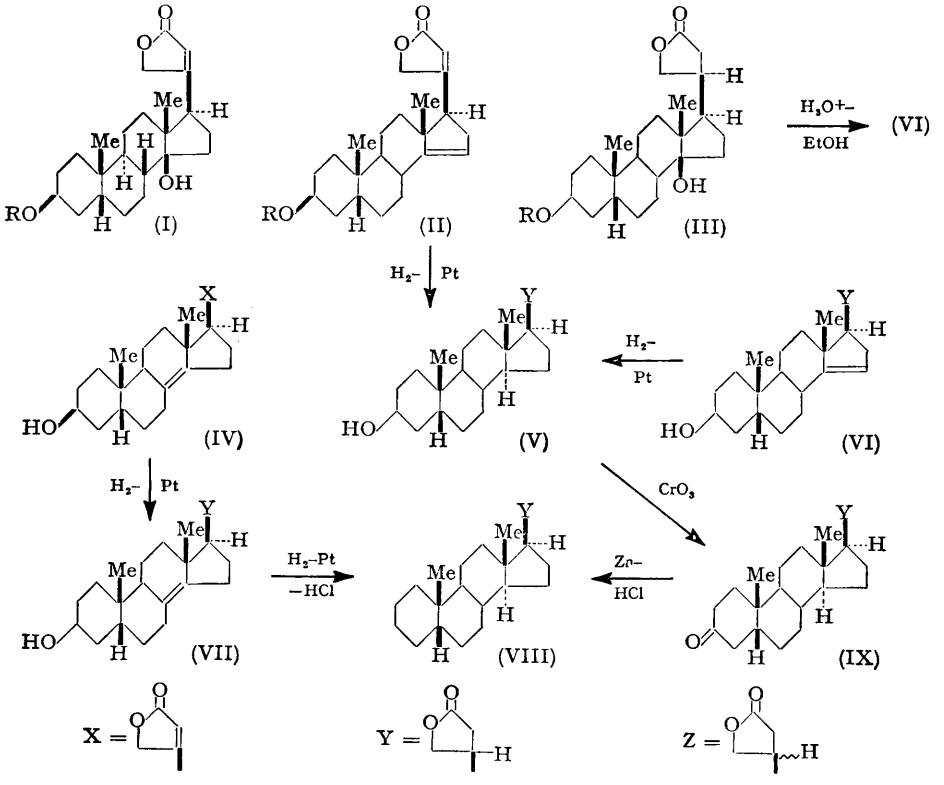
Hydrogenation experiments and spectral studies on the anhydrodigitoxigenins and anhydrodigoxigenins have shown that the dextrorotatory " α "-anhydrogenins are unsaturated at the 8 : 14-position and that the lævorotatory " β "-anhydrogenins are the 14 : 15-unsaturated isomerides. Adynerigenin is shown to be " α "-anhydrogitoxigenin. Evidence is cited for the α-orientation of the 16-hydroxy-group in gitoxigenin and for the β-orientation of the 3-hydroxy-group in digoxigenin. Structures are proposed for the anhydro-*iso*gitoxigenic acids. Four cardanolides differing in configuration at $C_{(14)}$, $C_{(17)}$, and $C_{(20)}$ are described. The mechanism of the formation of the anhydrogenins, which is relevant to the controversy on the mechanism of unimolecular reactions (Dewar, *Ann. Reports*, 1951, **48**, 121), is discussed.

A general mechanism for the chromic acid oxidation of olefins is outlined.

In Parts II, IV, and VI (refs. *c*, *e*, *f*; for references cited thus, see p. 2013) the preparation of pairs of isomeric anhydrogenins from digitoxigenin (I*a*) and digoxigenin (X*a*) was described. It was suggested that the isomers differed in the location of the nuclear double bond which was introduced on removal of the 14-hydroxy-group and could therefore be at position 8 : 14 or 14 : 15 (steroid numbering). The proof (ref. *o*) that the lævorotatory " β "-anhydrodigoxigenin had the 14 : 15-unsaturated structure (XI) therefore established the alternative 8 : 14-unsaturated structure for the dextrorotatory " α "-anhydrogenins. A double bond position 14 : 15 is readily hydrogenated, whilst an 8 : 14-double bond is inert to hydrogenation under neutral conditions; in the presence of mineral acid partial migration of double bonds takes place with consequent hydrogenation. Tschesche's report (*Z. physiol. Chem.*, 1933, **222**, 50) that 3-β-acetoxy-5α-card-8(14) : 20(22)-dienolide (then called " β "-dianhydrouzarigenin acetate) was hydrogenated under neutral conditions with the uptake of three molecules of hydrogen therefore led Fieser and Fieser (" Natural Products related to Phenanthrene," 3rd Edn., Reinhold Publ. Corpn., 1949, p. 534) to suggest that the dextrorotatory " α "-anhydrogenins contained a nuclear 14 : 15-double bond, the difference from the lævorotatory " β "-anhydrogenins then being ascribed to inversion at a nearby centre. Tschesche's experimental work does not, in our opinion, warrant this conclusion as no hydrogenation product was isolated. The report by Shah, Meyer, and Reichstein (*Pharm. Acta Helv.*, 1949, **24**, 113) that Tschesche's acetate absorbs only one mol. of hydrogen, although doubtless correct, suffers from the same objection.

* Part VI, *J.*, 1936, 354. The present paper is also regarded as " Elimination Reactions. Part III " (Part II, Cardwell, *J.*, 1951, 2442).



	M. p.	$[\alpha]_{Hg}$	$[\alpha]_D$	Refs.		M. p.	$[\alpha]_{Hg}$	$[\alpha]_D$	Refs.
I*a*; R = H	252°	+21° M	+17° M	*h*, *r*	IV	234°	+44° M	+39° M	*e*, *i*
I*b*; R = Ac	217	+19 C	+21 C	*h*, *r*	V	167	+26 M	+21 M	*a*, *g*
II*a*; R = H	202	−17 M	−13 M	*e*, *q*	VI	185°	+49 M	+42 M	*a*, *h*
II*b*; R = Ac	185	—	−18 C	*p*, *s*	VII(*a*)	168	+47 M	+40 M	*a*
			−12 M		VII(*b*)	170	—	+32 C	*k*
III*a*; R = H	226	+20 M	+17 M	*a*, *h*	VIII	195	+38 C	+34 C	*a*, *h*, *j*, *l*
III*b*; R = Ac	178	—	+14 C	*n*	IX	240	+44 C	+38 C	*a*, *g*, *k*, *l*

C = chloroform; M = methanol.

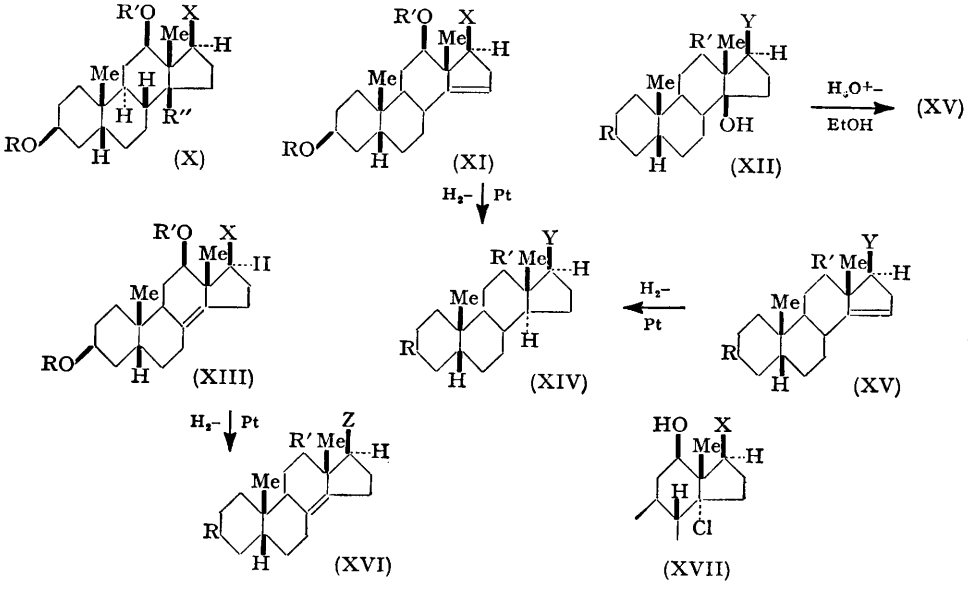
a, This work. *b*, Smith, *J.*, 1930, 508. *c*, *Idem*, *J.*, 1930, 2478. *d*, *Idem*, *J.*, 1931, **23**. *e*, *Idem*, *J.*, 1935, 1050, 1305. *f*, *Idem*, *J.*, 1936, 354. *g*, Windaus and Freese, *Ber.*, 1925, **58**, 2503. *h*, Windaus and Stein, *Ber.*, 1928, **61**, 2436. *i*, Jacobs and Elderfield, *J. Biol. Chem.*, 1933, **100**, 671. *j*, Tschesche and Bohle, *Ber.*, 1936, **69**, 793. *k*, *Idem*, *Ber.*, 1938, **71**, 654. *l*, Neumann, *Ber.*, 1937, **70**, 1547; Fleury and Neumann, *Klin. Woch.*, 1935, **14**, 562. *m*, Tschesche, Bohle, and Neumann, *Ber.*, 1938, **71**, 1927. *n*, Meyer, *Helv. Chim. Acta*, 1946, **29**, 718. *o*, Plattner and Heusser, *Helv. Chim. Acta*, 1946, **29**, 727. *p*, Hunziker and Reichstein, *ibid.*, 1945, **28**, 1472. *q*, Helfenberger and Reichstein, *ibid.*, 1948, **31**, 1470. *r*, Rangaswami and Reichstein, *ibid.*, 1949, **32**, 939. *s*, Rheiner, Hunger, and Reichstein, *ibid.*, 1952, **35**, 687. *t*, Plattner, Ruzicka, and Pataki, *ibid.*, 1945, **28**, 389. *u*, Plattner, Ruzicka, Heusser, and Meier, *ibid.*, 1946, **29**, 2023. *v*, Schindler and Reichstein, *ibid.*, 1952, **35**, 442. *w*, Meyer, *ibid.*, 1946, **29**, 1580. *x*, Tamura, Kobayashi, and Tokita, *Japan. Med. J.*, 1948, **1**, 206.

To remove this confusion we now describe hydrogenation experiments on the anhydrodigitoxigenins and digoxigenins carried out by the senior author in 1934, which, with later spectral evidence, conclusively establish the 8 : 14- and the 14 : 15-structure for the " α "- and " β "-anhydrogenins respectively.

The hydrogenation experiments (see Charts) show that the " α "-anhydrogenins absorb only one mol. of hydrogen, to give dihydro-" α "-anhydrogenins. Dihydro-" α "-anhydrodigitoxigenin (VII*a*) is clearly identical with tetrahydroanhydroadynerigenin (VII*b*) which by hydrogenation under acid conditions has been converted into derivatives of tetrahydro-β-anhydrodigitoxigenin (VIII and IX) (refs. *k*, *m*) (we discuss the structure of

adynerigenin below). This establishes that the isomeric anhydrogenins have the same nuclear structure and the same configuration at $C_{(17)}$. They must therefore be 8 : 14- and 14 : 15-unsaturated isomerides.

Ultra-violet absorption measurements in methanol (2060—2200 Å; Unicam spectrophotometer, model S.P. 500) show that dihydro-" α "-anhydrodigitoxigenin has the absorption characteristics of a tetrasubstituted double bond (see Experimental section) (Bladon, Henbest, and Wood, *J.*, 1952, 2737). As *apo*cholic acid shows lower absorption than cholest-8(14)-en-3β-ol, dihydro-" α "-anhydrodigoxigenin (XVI*a*) might be expected to show lower absorption than the digitoxigenin derivative. The measured absorption however was even lower than expected and was similar to that shown by cholest-5-ene. This suggestive but ambiguous result made it necessary to test the nature of the unsaturated systems by other methods. Colour reactions (Légal, and tetranitromethane) confirmed



	M. p.	$[\alpha]_{Hg}$	$[\alpha]_D$	Refs.
X*a*; R = R′ = H, R″ = OH	222°	+27° M	+20° E +18 A	*a*, *b*
X*b*; R = R′ = Ac, R″ = OH	221	+61 M	+49 C	*c*
XI*a*; R = R′ = H	182	−16 M	−13 M	*c*
XI*b*; R = H; R′ = Ac.....	199	+32 C	+26 C	*a*
XI*c*; R = R′ = Ac	199	+39 M	+30 C	*c*, *o*
XII*a*; R = R′ = β-OH	215	+23 M	+19 M	*a*, *c*
XII*b*; R = R′ = β-OAc	224	+30 M	+24 C	*b*, *l*
XII*c*; R = R′ = O:	243	+120 C	+98 C	*a*, *e*
XIII*a*; R = R′ = H	192	+46 M	+38 M	*a*, *f*
XIII*b*; R = Ac, R′ = H	218	—	+31 C	*a*
XIII*c*; R = R′ = Ac	155	+68 M	—	*f*
XIV*a*; R = R′ = β-OH	118	—	+20 C	*t*
XIV*b*; R = R′ = O:	290	+150 C	+132 C	*a*, *j*, *t*
XV*a*; R = R′ = β-OAc	204	—	+52 C	*u*
XV*b*; R = R′ = O:	288	+172 C	+144 C	*a*
XVI*a*; R = R′ = β-OH	218	+81 M	...	*a*
XVI*b*; R = R′ = β-OAc	165	+67 M	—	*a*
XVI*c*; R = R′ = O:	105	+103 M	—	*a*

A = acetone; C = chloroform; E = ethanol; M = methanol.

the absence of the butenolide ring in the dihydro- and tetrahydro-derivatives and the presence of a double bond in the dihydro-" α "-anhydrogenins. Similarly, infra-red spectroscopy (these measurements form part of a larger investigation by Dr. F. B. Strauss and the junior author which will be reported later) has shown replacement of the butenolide doublet at 5·72 μ (1750 cm.$^{-1}$) and 6·13 μ (1630 cm.$^{-1}$) by the butanolide peak at 5·64 μ

(1770 cm.$^{-1}$) in the reduced anhydrogenins. In addition the " β "-anhydrogenins showed absorption at 12·0 μ (835 cm.$^{-1}$) to 12·6 μ (795 cm.$^{-1}$) due to the system $>C_{(14)}:C_{(15)}H-$ (Bladon, Fabian, Henbest, Koch, and Wood, *J.*, 1951, 2402) which was absent in the parent genins, " α "-anhydrogenins, dihydro-" α "-anhydrogenins, and tetrahydro-" β "-anhydrogenins.

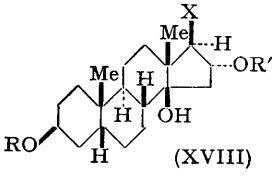
A further objection to the 8 : 14-unsaturated structure for the dextrorotatory " α "-anhydrogenins has been raised by Fieser and Fieser (*op. cit.*). They point out that *apo*cholic acid [3α : 12α-dihydroxychol-8(14)-enoic acid], $[\alpha]_D$ +50°, is less dextrorotatory than its 14 : 15-unsaturated isomeride, $[\alpha]_D$ +60°, in contrast to the 8 : 14-unsaturated anhydrodigoxigenin (XIII*a*) and its 14 : 15-unsaturated isomeride (XI*a*). This divergence is due to the proximity of the polar butenolide ring. When this is reduced to the butanolide ring the optical relation of the " α "- and the " β "-anhydro-isomers is reversed, *viz.*, 3 : 12-dioxo-20β-card-8(14)-enolide * (XVI*c*) and 3 : 12-dioxo-20β-card-14-enolide (XV*b*). Comparison with 3 : 12-dioxo-14α : 20β-cardanolide (XIV*b*) shows that introduction of an 8 : 14-double bond produces a negative change, and a 14 : 15-double bond a slight positive change, as in the similarly substituted *apo*cholic acid series (Barton and Klyne, *Chem. and Ind.*, 1948, 755). In the digitoxigenin series, 3β-hydroxy-20β-card-8(14)-enolide (VII*a*) is similarly less dextrorotatory than 3β-hydroxy-20β-card-14-enolide (VI), but now the saturated compound (V) is less dextrorotatory than either unsaturated isomer. These figures agree (Professor D. H. R. Barton, personal communication) with the observation that the rotation of coprosteryl acetate, $[\alpha]_D$ +27° (calculated from the observed value for coprosterol), is slightly less positive than that of coprost-8(14)-enyl acetate, $[\alpha]_D$ +31° (Windaus and Zühlsdorff, *Annalen*, 1938, **536**, 204). Clearly the 8 : 14-double bond is too near substituents in rings B, C, and D for there to be any standard value for the molecular increment of rotation on introduction of this double bond. The fact that there is agreement both in the dihydrodigitoxigenin and in the digoxigenin series with values obtained from similarly nuclear-substituted steroids is even more convincing evidence for the location of the double bond than if a standard value independent of substitution applied.

Anhydrogitoxigenins and Adynerigenin.—Owing to the lability of the 16-hydroxy-group, " α "-anhydrogitoxigenin has not yet been prepared by conventional chemical procedures. Dehydration of gitoxigenin derivatives catalysed by dilute acid gives mixtures of the " β "- or 14-anhydrogenin † (XIX) and the 14 : 16-dianhydrogenin † (XXIV) (ref. *l*; Windaus and Westphal, *Nachr. Ges. Wiss. Göttingen*, 1925, 78) which may contain small quantities of " α "-anhydrogenins, whilst concentrated hydrochloric acid or dry hydrogen chloride in acetone at low temperatures gives only the dianhydrogenin (Windaus and Schwarte, *Ber.*, 1925, **58**, 1515; Cloetta, *Arch. expt. Path. Pharmak.*, 1926, **112**, 261; see also p. 2022 below). However an enzyme in the leaves of *Nerium oleander* can apparently form " α "-anhydrogitoxigenin derivatives, for adynerin (XXI*a*) (a physiologically inactive glycoside) is an " α "-anhydro-derivative of oleandrin (XVIII; R = Oleandrose; R′ =

* The 20β-structure is assigned to the reduced products on the following grounds. *X*-Ray crystallographic studies (personal communication from Mrs. D. Crowfoot Hodgkin) have shown that cholesterol, lumisterol, and calciferol have the same configuration at $C_{(20)}$ which by the Plattner convention (*J.*, 1951, 3536) is designated 20β. The natural nor*allo*cholanoic acids are therefore 20β, whilst the slightly less dextrorotatory 20-*iso*nor*allo*cholanoic acids are 20α (Plattner and Pataki, *Helv. Chim. Acta*, 1943, **26**, 1241; Bergmann and Low, *J. Org. Chem.*, 1947, **12**, 67). We therefore assign the 20β-configuration to the more dextrorotatory of the isomeric dihydrogenins. In the digitoxigenin series transformations lead, as shown in the Chart, to the ketone (IX). This was prepared by Windaus and Stein (*Ber.*, 1928, **61**, 2436) by dehydration of dihydrodigitoxigenin, m. p. 200°, to " β "-anhydrodihydrodigitoxigenin, m. p. 181°, followed by hydrogenation and oxidation. Our crude dihydrodigitoxigenin (III*a*), prepared similarly, had m. p. 203°, $[\alpha]_D$ +18°, and gave an anhydro-compound (VI). Our dihydrogenin must be 20β, for its rotation agrees with that of the more dextrorotatory dihydrodigitoxigenin acetate (III*b*) and not with that of the isomeric 20α-acetate, $[\alpha]_D$ +8° (ref. *n*). In the digoxigenin series, 20β-tetrahydroanhydrodigoxigenone (XIV*b*) and the 20α-isomeride, $[\alpha]_D$ +123°, have been prepared from synthetic 3α : 12α-dihydroxy-14α-card-20(22)-enolide (ref. *t*). The former is identical with the product from " β "-anhydrodigoxigenin. By analogy, dihydro-" α "-anhydrodigoxigenin should also have the 20β-configuration but this has not been proved.

† In this series, by custom, the prefix " anhydro " refers to loss of water with formation of a double bond, and not to presence of an oxide ring as in general nomenclature. Numeral(s) attached to this " anhydro "-prefix refer to the position of the double bond thus introduced.

Ac) (ref. *l*). On hydrolysis it gives adynerigenin (XXI*b*) (ref. *k*). Tschesche and Bohle (ref. *k*) were led, by their conviction that this genin formed only a monoacetate (undescribed), to formulate it as 3β : 14-dihydroxycard-8 : 20(22)-dienolide (XXV). This structure does not explain the chemistry of adynerigenin (cf. Fieser and Fieser, *op. cit.*,



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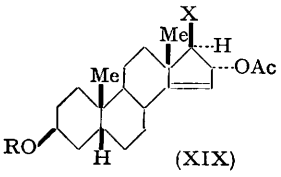

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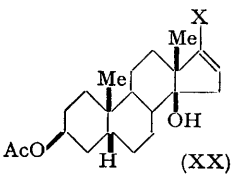

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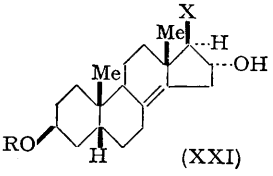
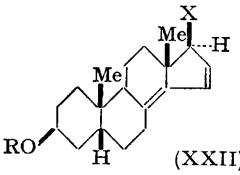


(XXI)



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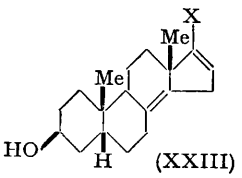

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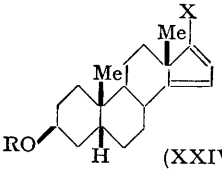

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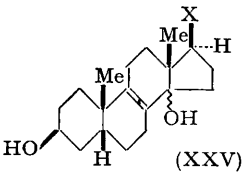

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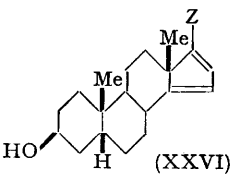

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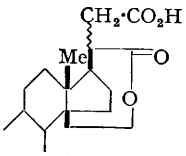

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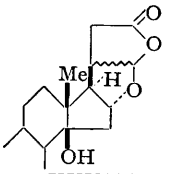

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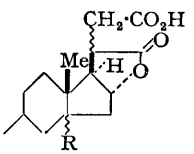

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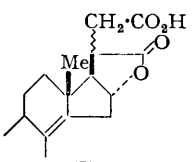

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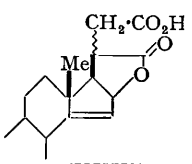


(XXXI)

	M. p.	$[\alpha]_{Hg}$	$[\alpha]_D$	Refs.
XVIII*a*; R = R′ = H	228°	+39° M	+33° M	*d*, *v*
XVIII*b*; R = R′ = Ac	252	−3 M	−8 C	*v*
XVIII*c*; R = Ac; R′ = H	232	—	+33 C	*a*, *l*, *w*, *x*
XVIII*d*; R = H; R′ = Ac	223	—	−9 M	*l*
XIX*a*; R = Glucose	208	—	±0 P	*m*
XIX*b*; R = H	259	—	—	*m*
XIX*c*; R = H	262	—	—	*l*
XIX*d*; R = Ac	184	—	—	*m*
XIX*e*; R = Ac	168	—	—	*m*
XX	207	—	+81 C	*a*, *n*
XXI*a*; R = Oleandrose	234	—	+8 P	*l*
XXI*b*; R = H	238	—	+18 P	*l*
XXII*a*; R = H	176	—	−109 C	*k*
XXII*b*; R = Ac	152	—	−106	*k*, *l*
XXIII	235	—	+147 C	*k*
XXIV*a*; R = H	212	+737 M	+580 M	*a*, *v*
XXIV*b*; R = Ac	208	+574 M	+502 C	*a*
XXVI	210	+276 M	+227 M	*a*

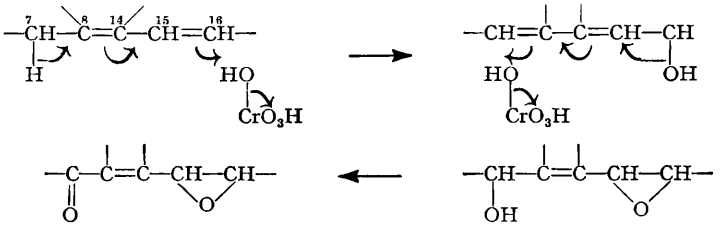
C = Chloroform; M = methanol; P = pyridine.

p. 541; Turner, *Chem. Reviews*, 1948, **43**, 1) or its physiological inactivity. Its formulation as 8(14)-anhydrogitoxigenin removes these difficulties. Thus the anhydroadynerigenin ($\lambda_{max.}$ 2470 Å) formed on dehydration catalysed by dilute acid becomes 8(14) : 15-dianhydrogitoxigenin (XXII*a*) (calc. $\lambda_{max.}$ 2440 Å), isomerised by cold concentrated hydrochloric acid to 8(14) : 16-dianhydrogitoxigenin (XXIII) ($\lambda_{max.}$ 2800 Å; cf. 16-anhydrogitoxigenin acetate (XX), $\lambda_{max.}$ 2730 Å, ref. *w*). Hydrogenation of anhydroadynerigenin gave tetra-anhydrohydroadynerigenin (VII*b*), which is clearly identical with dihydro-" α "-anhydro-

digitoxigenin (VII*a*), this identity being confirmed by its hydrogenation under acid conditions and oxidation to tetrahydro-" β "-anhydrodigitoxigenone (IX) and to 14α : 20β-cardanolide (VIII). In addition these experiments suggest an α-orientation of the 16-hydroxy-group in adynerigenin, for although the 8(14) : 16-dianhydrogen in (XXIII) is more stable than the 8(14) : 15-unsaturated isomeride, the latter is first formed on mild dehydration. There must therefore be a mechanistic barrier to dehydration towards $C_{(17)}$. This may be, in an ionic mechanism favouring *trans*-elimination, the *cis*-arrangement of the 17α-hydrogen atom and the 16-hydroxyl group.* Reichstein (*Angew. Chem.*, 1951, **63**, 412; and personal communication) depicted the 16-hydroxy-group in gitoxigenin as α-orientated, the ready loss of acetic acid from gitoxigenin diacetate on alumina to give 16-anhydrogitoxigenin acetate (XX) (ref. *w*) suggesting a *cis*-relation of the eliminated groups in this heterogeneous reaction. The preparation of 15-anhydrogitoxigenin by solvolysis of a 16-gitoxigenin toluene-*p*-sulphonate would have completed the proof of the α-orientation at $C_{(16)}$. In fact, however, the sole product isolated (see p. 2022) was the 16 : 17-unsaturated isomer; this cannot, nevertheless, be cited as evidence for β-orientation at $C_{(16)}$ as we are uncertain whether the elimination occurred before or during chromatographic purification. We think it very unlikely that the glycosides of *Nerium oleander* are derived from genins isomeric at $C_{(16)}$ (the main glycoside, oleandrin, is a gitoxigenin derivative), and thus depict gitoxigenin as 3β : 16α : 14-trihydroxycard-20(22)-enolide (XVIII*a*).†

The above proof of structure of the anhydrogenins allows an interpretation of certain aspects of their chemistry.

Chromic Acid Oxidation of the " α "-Anhydrogenins.—Steroids containing a ditertiary double bond, *e.g.*, cholest-8(14)-enyl acetate, are readily oxidised by chromic acid to 7- or 15-oxo-8(14)-enyl oxides (Wintersteiner and Moore, *J. Amer. Chem. Soc.*, 1943, **65**, 1513). A similar by-product was isolated (ref. *e*) on oxidation of " α "-anhydrodigitoxigenin and is now shown to be $C_{23}H_{28}O_5$.‡ In Part VI (ref. *f*) isolation of a by-product from the oxidation of " α "-anhydrodigoxigenin was also recorded and this was probably a 7- or 15-oxo-8(14)-enyl oxide. The same type of structure may also be assigned to the substance $C_{23}H_{30-32}O_5$ isolated in low yield (ref. *k*) on oxidation of tetrahydroanhydroadynerigenin. The compound $C_{23}H_{26-28}O_5$ ($\lambda_{max.}$ 2520 Å) isolated on oxidation of anhydroadynerigenin is of more interest. If it is assumed by analogy with action of chromyl chloride



on olefins (Cristol and Eilar, *J. Amer. Chem. Soc.*, 1950, **72**, 4353) that the elements of OH^+ are added to the negative end of the conjugated system, an expected product would be the 7-oxo-8(14)-enyl 15 : 16-oxide [(calc. $\lambda_{max.}$ 2590 Å; cf. 5-hydroxy-ergost-8(14)-ene-3 : 7-dione, $\lambda_{max.}$, 2540 Å; Dannenberg, *Abhandl. preuss. Akad. Wiss.*, 1939, **21**, 3). [Other possible structures by this mechanism would be 16-oxo-14-enyl 7 : 8(or 8 : 9)-oxides

* This argument would be invalidated if (XXIII) were not the major product of dehydration, but the experimental details allow no decision on this point. The strained nature of the fused ring system in *iso*gitoxigenin as depicted in (XXVIII) makes such a reservation necessary (Professor C. W. Shoppee, personal communication).

† For the same reason we formulate neriantogenin (XIX*b*) as " β "-anhydro-oleandrigenin (XIX*c*), the melting points of the genins and acetates (XIX*d* and *e*) suggesting identity. Tschesche, Bohle, and Neumann (ref. *m*) give no experimental evidence to support their statement that the acetates are different. Unfortunately no measurements of optical rotation were made.

‡ Kiliani's anhydrodigitoxigenin, m. p. 251—220° (*Ber.*, 1920, **53**, 246), called " β " (ref. *g*), was clearly substantially our " α "(8 : 14-unsaturated)-isomer. The derived toxigenone, m. p. 260° (C, 75·8; H, 8·0%), was probably a mixture of " α "- and " β "-anhydrodigitoxigenone (C, 77·9; H, 8·5%), and the keto-oxide, m. p. 273° (C, 71·8; H, 7·3%).

(cf. Barton, Holness, Overton, and Rosenfelder, *J.*, 1952, 3751).] This type of mechanism may be general for the chromic acid oxidation of olefins and explains the various products obtained in the oxidation of 7 : 8-, 8 : 9-, and 8 : 14-unsaturated steroids (Fieser and Fieser, *op. cit.*, p. 227 *et seq.*) and removes the unlikely reactions (dehydration of α-hydroxy-ketones, and formation of keto-oxides from αβ-unsaturated ketones) postulated in other mechanisms.

Mechanism of Dehydration.—The precise mechanism of, and degree of stereochemical control in, substitution and elimination reactions, assumed to be unimolecular by close analogies, has been the subject of much discussion (cf. Dewar, *Ann. Reports*, 1951, **48**, 121). The reactions of the 14β-hydroxy-group in these genins indicate that the stereochemical control normally associated with bimolecular reactions (replacement with inversion and *trans*-elimination) (Cowdrey, Hughes, Ingold, Masterman, and Scott, *J.*, 1937, 1252; Hückel, Tappe, and Legutke, *Annalen*, 1940, **543**, 191) is largely operative. Thus "β"-anhydrogenins are the sole product of dehydration with phosphorus oxychloride and pyridine (ref. *p*), the intermediate phosphorus ester (X; R = R = Ac, R'' = O·$POCl_2$) (cf. Gerrard, *J.*, 1950, 2088) undergoing *trans*-elimination. Dehydration of the genins with dilute acids gives mixtures in which the "β"-anhydrogenins predominate, despite the fact that the "α"-anhydrogenins are more stable (Jacobs and Elderfield, *J. Biol. Chem.*, 1936, **113**, 611). On treatment with concentrated hydrochloric acid, digoxigenin gives, by substitution with inversion, the 14α-chloro-compound (XVII), which on dissolution in hot methanol undergoes *trans*-elimination to give a quantitative yield of "α"-anhydrodigoxigenin (XIII*a*) (ref. *f*). All these reactions show a degree of stereochemical control which indicates that the ionising group has not fully separated before elimination or substitution takes place.

14α-Chlorogitoxigenin cannot be prepared from gitoxigenin as the 16-hydroxy-group is very labile; when this lability is reduced by saturation of the butenolide ring, as in *iso*gitoxigenin (XXVIII) and *iso*gitoxigenic acid (XXIX; R = β-OH), 14α-chloro-compounds can be formed (Jacobs and Gustus, *J. Biol. Chem.*, 1929, **82**, 403; 1930, **86**, 199). The 14α-orientation in chloro*iso*gitoxigenin is proved by its ready elimination, giving 8(14)-anhydro*iso*gitoxigenin, whose resistance to catalytic hydrogenation is now explained. Similarly, 14α-chloro*iso*gitoxigenic acid (XXIX; R = *a*-Cl), on dissolution in dilute aqueous ammonia (unimolecular conditions), gave the hydrate of 8(14)-anhydro-*iso*gitoxigenic acid, $[\alpha]_D$ +27° (XXX).* In contrast, on dissolution in aqueous sodium hydroxide (bimolecular elimination) the chloro-acid gave 14-anhydro*iso*gitoxigenic acid (XXXI), $[\alpha]_D$ +61°. The control by the inductive effect of the 16-oxygen atom in the bimolecular elimination is not unexpected. The great difference in rotation between the 8 : 14- and the 14 : 15-unsaturated acids shows how sensitive the contribution of these nuclear double bonds is to substitution at $C_{(16)}$ (cf. Mancera, Barton, Rosenkranz, and Djerassi, *J.*, 1952, 1021).

Structure of Digoxin and Digoxigenin.—Although crystalline digoxin (the tridigitoxoside of digoxigenin) was isolated in 1930 (Smith, *J.*, 1930, 508) and has been in clinical use in this country since 1933 † (*Brit. Med. J.*, 1933, 295, 364) the generally accepted proof of its structure is unsatisfactory. It has been degraded to a methyl 3 : 12-dihydroxyetianate (A), $[\alpha]_{Hg}$ +46°, $[\alpha]_D$ +39° (Steiger and Reichstein, *Helv. Chim. Acta*, 1938, **21**, 828). This substance did not give a melting-point depression with material (B), $[\alpha]_{Hg}$ +49°, obtained by hydrogenation of methyl 3 : 12-dioxoetianate and was oxidised to this diketone (Mason and Hoehn, *J. Amer. Chem. Soc.*, 1938, **60**, 2824; 1939, **61**, 1614). Nor did it

* Jacobs and Gustus called this acid γ-*iso*gitoxigenic acid and assumed that it was related to *iso*-gitoxigenic acid, $[\alpha]_D$ −50°, in the same way as γ-*iso*digitoxigenic acid, $[\alpha]_D$ +60°, is to α-*iso*digitoxigenic acid (XXVII), $[\alpha]_D$ −31°. This cannot be the case. The latter change does not involve any rearrangement of the nuclear skeleton, as shown by the identity of the derived digitoxanol diacid with a product resulting from reduction of 14-anhydro*iso*gitoxigenic acid; it must therefore involve movement of the lactone ring from $C_{(14)}$ to $C_{(15)}$ or $C_{(16)}$. As *iso*gitoxigenic acid does not have a lactone ring at $C_{(14)}$ it cannot undergo this change. The difference in the rotational changes in the two series supports this interpretation.

† Reichstein (*Angew. Chem.*, 1951, **63**, 412) (see also Fieser and Fieser, *op cit.*, p. 573) is incorrect in describing the use of digitoxin in America in 1945 as the first departure from digitalis galenical preparations.

depress the melting point of (C), $[\alpha]_D$ +52°, obtained from the reduction of authentic 3α-hydroxy-12-oxoetianate (Wenner and Reichstein, *Helv. Chim. Acta*, 1944, **27**, 965). Substance (C) is certainly the 3α : 12β-dihydroxy-compound as it differed from the known 3α : 12α-compound. In this field, optical rotations are probably of more significance than melting points and we note that the rotations of (A) and (B) are significantly different from that of (C) and in the direction to be expected if (A) and (B) are the 3β : 12β-dihydroxyetianates. Professor Reichstein (*loc. cit.*, and personal communication) has reached the same conclusion and has kindly informed us that he is now investigating the isomeric 3 : 12-dihydroxyetianates. We have not therefore probed this question by repetition of the degradation to an etianate but have carried out partial acetylation and hydrolysis experiments. Treatment of digoxigenin with one equivalent of acetic anhydride in cold pyridine or boiling benzene gave digoxigenin and digoxigenin diacetate with no trace of monoacetate. With β-anhydrodigoxigenin however we were able to isolate the 12-monoacetate (XI*b*) with ease. With α-anhydrodigoxigenin a complex mixture was obtained from which, after chromatography, the 3-monoacetate (XIII*b*) was isolated by virtue of its insolubility but we have no doubt that some of the 12-monoacetate was also formed. Hydrolysis experiments were confined to digoxigenin diacetate. Hydrazine hydrate in methanol at room temperature (24 hours) did not affect the diacetate. Slow addition of 0·1N-sodium hydroxide to a hot aqueous-ethanolic solution of the diacetate gave a large acidic fraction from which some diacetate could be recovered, thus confirming that this reagent opens the butenolide ring, at least in part, without isomerisation to the aldehyde (cf. Paist, Blout, Uhle, and Elderfield, *J. Org. Chem.*, 1941, **6**, 373). The neutral fraction gave some unchanged diacetate, but the mother-liquors contained the 3-monoacetate as oxidation with chromic acid gave the 3-acetoxy-12-oxo-compound, $[\alpha]_D$ +113°. The orientation of these new products was determined by rotational analysis, the rotation change on acetylation or oxidation of the 12-hydroxy-group being so large that it could not be confused with reactions involving a 3α- or 3β-hydroxy-group. The derived molecular-rotation increments for acetylation of the 3-hydroxy-group were +28° and −15°. Comparison with the standard values for 3β (+17° ± 17°) and 3α (+83° ± 30°) (Barton, *J.*, 1946, 1116) strongly suggests a 3β-orientation for the hydroxy-group in digoxigenin. In addition, we noted that the 12-ketone 3-acetate from digoxigenin and 3-monoacetate of the "α"-anhydrogenin showed, in paraffin mulls, a complex acetate band at 8 μ. A complex band in carbon disulphide is characteristic of 3β-acetoxycholane derivatives (Jones, Humphries, Herling, and Dobriner, *J. Amer. Chem. Soc.*, 1951, **73**, 3215); in paraffin mull a 3β-acetoxycholane derivative may show a simple band but the inverse, *i.e.*, a complex band with a 3α-acetoxycholane derivative, has not yet been observed (Schindler and Reichstein, *Helv. Chim. Acta*, 1952, **35**, 730). A simple band would therefore not have been informative, but the complex bands observed are, we think, significant. The conclusion that digoxigenin is 3β : 12β : 14-trihydroxycard-20(22)-enolide and not the 3α : 12β-compound as usually depicted is strengthened by the realisation that the former orientation alone allows a satisfactory explanation of the partial acetylation and hydrolysis experiments. Thus the combination of a non-reactive 3β("polar")-hydroxy-group with a reactive 12β("equatorial")-hydroxy-group, the latter being hindered by the 14β-hydroxy-group and ring D in digoxigenin but not in the "β"-anhydrogenin, explains the results. In the "α"-anhydrogenin the 8 : 14-double bond modifies the conformation of ring C in a manner whose consequences have not yet been evaluated. Rigid proof of the structure of digoxigenin must now await Professor Reichstein's current experiments, the weight of the existing evidence strongly favouring the new 3β : 12β-hydroxy-structure and thus bringing digoxigenin into line with all the heart poisons of proved structure. [The day after submission of this manuscript, Professor Reichstein informed us that the 3β : 12β-configuration had been established (see Pataki, Meyer, and Reichstein, *Experientia*, 1953, **9**, 253; *Helv. Chim. Acta*, 1953, **36**, 1295; Taylor, *J.*, 1953, 3325).]

The Experimental section also includes the proof of the common nuclear structure of digitoxigenin and digoxigenin by their conversion into deoxycardanolides (VIII). In this we were anticipated, but as our products were crystallised to constant rotation

experimental details are given. In the gitoxigenin series two further isomeric 3β-hydroxycardanolides were isolated. By analogy with the reduction of 16-anhydrogitoxigenin (XX) (ref. *w*) the three isomers obtained are all probably 17α-cardanolides.

Experimental

Hydrogenations were carried out at atmospheric pressure in ethanol with previously reduced platinum oxide. In oxidations Kiliani's chromic acid solution (water, 400 g.; concentrated sulphuric acid, 80 g.; chromic oxide, 53 g.) was used except where stated. Products were isolated by pouring into water and filtration if crystalline. If oily, the product was extracted with chloroform and washed with dilute sulphuric acid and/or aqueous sodium hydrogen carbonate as appropriate and dried ($MgSO_4$). In the determination of optical rotations, *c* ranged from 0·1 to 5. In the earlier work, where crystallisation was frequently to constant rotation, estimated errors have not been given. Except where stated, rotations were determined in chloroform. In ultra-violet measurements the substance in methanol in a 1-mm. cell was examined in a Unicam model S.P. 500. In chromatography, columns of acid-washed alumina were prepared in benzene and eluted with benzene–chloroform.

Dihydro-"α"-anhydrodigitoxigenin (VII).—"α"-Anhydrodigitoxigenin (0·1 g.) absorbed hydrogen (7·3 c.c.; 1 mol., 6·3 c.c.) in 25 min. The *dihydrogenin* crystallised from aqueous methanol in needles, m. p. 172°, $[\alpha]_{Hg}^{20}$ +46·5°, $[\alpha]_{D}^{20}$ +39·7° in MeOH (Found: C, 76·7; H, 9·6. $C_{23}H_{34}O_3$ requires C, 76·6; H, 10·1%). It gave a negative Légal reaction and a yellow colour with tetranitromethane and had absorption max. at 2060 (ε 10,500), 2100, (ε 8800), 2140 (ε 6100), and 2180 Å (ε 4500). A mixture with tetrahydro-"β"-anhydrodigitoxigenin (m. p. 167°) melted at 155°.

8 : 14-*Epoxy*-3 : 7(or 15)-*dioxocard*-20(22)-*enolide*.—The *by-product* (ref. *e*) from the chromic acid oxidation of "α"-anhydrodigitoxigenin was crystallized to constant m. p. 273° (Found: C, 71·4; H, 7·5. $C_{23}H_{30}O_5$ requires C, 71·5; H, 7·8%).

Tetrahydro-"β"-anhydrodigitoxigenin (V).—"β"-Anhydrodigitoxigenin (0·179 g.) absorbed hydrogen (24·2 c.c.; 2 mol., 22·6 c.c.) in 1½ hr. The product crystallised from methanol in needles, m. p. 167°, $[\alpha]_{Hg}^{20}$ +26·3°, $[\alpha]_{D}^{20}$ +21·3° in MeOH (ref. *g*). It gave negative Légal and tetranitromethane reactions.

Tetrahydro-"β"-anhydrodigitoxigenone (IX).—"β"-Anhydrodigitoxigenin (1·0 g.) absorbed hydrogen (116·8 c.c.; 2 mol., 126·6 c.c.) in 1 hr. The product, in aqueous acetic acid (80%; 20 c.c.), was treated with chromic acid solution (5 c.c.) for 20 min. The ketone crystallized readily from methanol and from ethyl acetate and had m. p. 235°, $[\alpha]_{Hg}^{20}$ +43·9°, $[\alpha]_{D}^{20}$ +38·0° (ref. *g*, *k*, *l*).

14α : 20β-*Cardanolide* (VIII).—The foregoing ketone (0·3 g.), acetic acid (15 c.c.), concentrated hydrochloric acid (10 c.c.), and amalgamated zinc (4 g.) were heated on a water-bath for 6 hr. The oily product slowly crystallised. After two crystallisations from methanol and one each from ethanol and acetone it melted at 188—190° (Found: C, 80·3; H, 10·3. Calc. for $C_{23}H_{36}O_2$: C, 80·2; H, 10·5%) (refs. *h*, *j*, *l*).

Dihydrodigitoxigenin (III*a*).—Digitoxigenin (1·0 g.) absorbed hydrogen (65 c.c.; 1 mol., 60 c.c.) in 1 hr. On concentration the solution was acid to litmus, suggesting that some βγ-isomeride had been hydrogenated with ring opening. The product after one crystallisation from methanol had m. p. 203°, $[\alpha]_{D}^{20}$ +18·6° in MeOH, raised by crystallisation from benzene and then ethyl acetate to m. p. 226°, $[\alpha]_{Hg}^{20}$ +19·9°, $[\alpha]_{D}^{20}$ +17·1° in MeOH (ref. *h*). The more soluble crops had lower rotations.

"β"-Anhydrodihydrodigitoxigenin (VI).—The preceding dihydrogenin (0·85 g.) in ethanol (12 c.c.) was refluxed with 10% sulphuric acid (12 c.c.) for 0·5 hr. The anhydrodihydrogenin crystallised from ethyl acetate with a molecule of solvent (Found: Loss at 100° *in vacuo*, 19·0. Calc. for $C_{23}H_{32}O_3,C_4H_8O_2$: 19·8%), m. p. 185°, $[\alpha]_{Hg}^{20}$ +49·0°, $[\alpha]_{D}^{20}$ +41·5° in MeOH (an anhydrous specimen had m. p. 187°) (ref. *h*).

Dihydro-"α"-anhydrodigoxigenin (XVI*a*).—"α"-Anhydrodigoxigenin dihydrate (2 g.; 1·1 g.; 0·22 g.) absorbed hydrogen (111 c.c.; 67·9 c.c.; 16·6 c.c.: 1 mol., 110 c.c.; 60·4 c.c.; 12·1 c.c. respectively). *Dihydro-"α"-anhydrodigoxigenin* crystallised from methanol in needles, m. p. 218°, $[\alpha]_{Hg}^{16}$ +81·4° in MeOH (Found: C, 73·9; H, 9·2. $C_{23}H_{34}O_4$ requires C, 73·8; H, 9·2. $C_{23}H_{36}O_4$ requires C, 73·4; H, 9·6%). It gave a negative Légal reaction, a yellow colour with tetranitromethane, and absorption max. at 2060 (ε 4600), 2100 (ε 3400), 2140 (ε 2420), and 2180 Å (ε 1270). The *diacetate* (XVI*b*) crystallised from aqueous methanol in needles,

m. p. 165°, $[\alpha]_{Hg}^{20}$ +66·7° in MeOH (Found: C, 70·7; H, 8·5; Ac, 19·5. $C_{27}H_{38}O_6$ requires C, 70·7; H, 8·4; Ac, 18·6%).

Dihydro-"α"-anhydrodigoxigenone (XVI*c*).—The above dihydrogenin (0·6 g.) in aqueous acetic acid (90%; 10 c.c.) was treated with chromic acid solution (3 c.c.) with ice-cooling. After 0·5 hr. the solution was diluted with water. The ketone separated as a *monohydrate*, in needles, m. p. 105° (Found: C, 71·1; H, 8·4; loss, 4·1. $C_{23}H_{30}O_4,H_2O$ requires C, 71·1; H, 8·3; H_2O, 4·6%). The anhydrous *form* had m. p. 105°, $[\alpha]_{Hg}^{20}$ +103° in MeOH (Found: C, 74·3; H, 8·4. $C_{23}H_{30}O_4$ requires C, 74·6; H, 8·2%). A *monosemicarbazone* (presumably 3) separated overnight, on treatment of the ketone with aqueous-methanolic semicarbazide acetate, in needles, m.p. 238° (decomp.) (Found: N, 10·2. $C_{24}H_{33}O_4N_3$ requires N, 9·8%).

Tetrahydro-"β"-anhydrodigoxigenone (XIV*b*).—"β"-Anhydrodigoxigenin (1·35 g.) absorbed hydrogen (161 c.c.; 2 mol., 163 c.c.) in 5 hr. The product in aqueous acetic acid (80%; 15 c.c.) was treated with chromic acid solution (6·7 c.c.). After 0·5 hr. at room temperature the mixture was diluted with water. The ketone crystallised from aqueous ethanol in needles, m. p. 288°, $[\alpha]_{Hg}^{20}$ +150°, $[\alpha]_D^{20}$ +140° (Found: C, 74·2; H, 8·5. Calc. for $C_{23}H_{32}O_4$: C, 74·2; H, 8·7%) (ref. *j*, *t*).

12-*Oxo*-14α : 20β-*cardanolide*.—The preceding diketone (0·25 g.), concentrated hydrochloric acid (5 c.c.), and amalgamated zinc (3 g.) were heated under reflux for 5 hr. Ethanol (5 c.c.), hydrochloric acid (5 c.c.), and zinc (3 g.) were added and refluxing was continued for 2 hr. The product crystallised from aqueous acetone in rods, m. p. 217—218°, $[\alpha]_{Hg}^{20}$ +134° (Found: C, 77·5; H, 9·8. Calc. for $C_{23}H_{34}O_3$: C, 77·1; H, 9·6%) (cf. Tschesche and Bohle, *Ber.*, 1936, **69**, 2497).

14α : 20β-*Cardanolide* (VIII).—The diketone (1·02 g.), acetic acid (24 c.c.), concentrated hydrochloric acid (12 c.c.), and amalgamated zinc (10 g.) were heated under reflux for 1·5 hr. Further additions of acetic acid (46 c.c.), hydrochloric acid (20 c.c.), and zinc (20 g.) were made during 5 hr. After a total of 8·5 hours' refluxing the product was isolated in the usual manner. The first crystalline crop (0·5 g.) had $[\alpha]_{Hg}$ +33° and the second $[\alpha]_{Hg}$ +75°, indicating that, even after the vigorous treatment, reduction was not complete. The first crop, after four recrystallisations from ethyl acetate, gave 51 mg. of the pure saturated lactone, m. p. 195°, $[\alpha]_{Hg}^{20}$ +38·0°, $[\alpha]_D^{20}$ +33·0° (Found: C, 80·0; H, 10·1. Calc. for $C_{23}H_{36}O_2$: C, 80·2; H, 10·5%).

"β"-*Anhydrodihydrodigoxigenone* (XV*b*).—Dihydrodigoxigenone (XII*c*), $[\alpha]_D^{20}$ +98° (0·95 g.) (from dihydrodigoxigenin, $[\alpha]_D^{20}$ = +19° in MeOH), in ethanol (20 c.c.) and aqueous 10% sulphuric acid (20 c.c.) were heated on a water-bath for 2·5 hr. The *product* crystallised from aqueous ethanol in needles, m. p. 288°, $[\alpha]_{Hg}^{20}$ +172°, $[\alpha]_D^{20}$ +144° (Found: C, 74·3; H, 8·3. $C_{23}H_{30}O_4$ requires C, 74·6; H, 8·2%). Further crops from the mother-liquors had much lower rotations, *viz.*, $[\alpha]_{Hg}$ +105°, $[\alpha]_D$ +86°, suggesting the presence of the dihydro-"α"-anhydro-isomer (XVI*c*).

12β-*Acetoxy*-3β-*hydroxycard*-14 : 20(22)-*dienolide* (XI*b*).—β-Anhydrodigoxigenin (2·287 g.) in pyridine (10 c.c.) was treated with acetic anhydride (0·68 c.c.). The product was chromatographed and the following fractions were collected: (i) 157 mg., $[\alpha]_D$ +10·2°, (ii) 325 mg., (iii) 654 mg., $[\alpha]_D$ +1·4°, (iv) 390 mg., (v) 118 mg., $[\alpha]_D$ −14·4°, (vi) 105 mg., (vii) 197 mg., $[\alpha]_D$ −17·2°, (viii) 261 mg., (ix) 390 mg., $[\alpha]_D$ −12·5°, (x) 278 mg., (xi) 155 mg., $[\alpha]_D$ −11·0°, (xii) 55 mg., $[\alpha]_D$ 0°. Fractions (i) and (ii) on crystallisation from methanol gave the 12-*monoacetate*, m. p. 199—200°, $[\alpha]_D^{20}$ +25·3° ± 1° (Found: C, 72·7; H, 8·5. $C_{25}H_{34}O_5$ requires C, 72·4; H, 8·3%). The infra-red spectrum showed a hydroxyl and an acetate band. When mixed with "β"-anhydrodigoxigenin diacetate (XI*c*) it melted at 160—170°. Fraction (viii) on crystallisation from methanol gave rectangular prisms of "β"-anhydrodigoxigenin, m. p. and mixed m. p. 177—178°. The mother-liquors from fractions (i) and (ii) were combined with (iii) and (iv) and rechromatographed, the following fractions being collected: (*a*) 144 mg., $[\alpha]_D$ +20·8°, (*b*) 136 mg., $[\alpha]_D$ +22·8°, (*c*) 86 mg., $[\alpha]_D$ +26·8°, (*d*) 224 mg., $[\alpha]_D$ −4°, (*e*) 54 mg., $[\alpha]_D$ −24°. Fractions (*a*), (*b*), and (*c*) on crystallisation gave further crops of the 12-monoacetate, m. p. 185—190°, $[\alpha]_D^{16}$ +26·8° ± 1°. Fraction (*e*) gave a further crop of "β"-anhydrodigoxigenin.

3β-*Acetoxy*-12β : 14-*dihydroxycard*-8(14) : 20(22)-*dienolide* (XIII*b*).—"α"-Anhydrodigoxigenin (0·459 g.; $[\alpha]_D^{20}$ +39·3° in MeOH) was acetylated as for the "β"-isomer. Chromatography gave a little diacetate and then a very small fraction (50 mg.) of the 3-*monoacetate*, m. p. 218—225° (from aqueous methanol), $[\alpha]_D^{20}$ +31·3° ± 3° (Found, on a sample dried at 100° *in vacuo*: C, 72·4; H, 8·3. $C_{25}H_{34}O_5$ requires C, 72·4; H, 8·3%). The infra-red spectrum showed a hydroxyl and an acetate band. After the 3-monoacetate mixed fractions were obtained before pure "α"-anhydrogenin was eluted.

3β-*Acetoxy*-14-*hydroxy*-12-*oxocard*-20(22)-*enolide*.—Digoxigenin diacetate (2·4 g.) in aqueous ethanol (50%; 300 c.c.) was slowly treated on a water-bath with aqueous sodium hydroxide (0·1N; 50·5 c.c.). Initial addition of the alkali was at such a rate that the solution was kept near neutral to phenolphthalein. The hydrolysis under such conditions was extremely slow. After concentration under reduced pressure the mixture was extracted with chloroform. The neutral fraction on crystallisation from ethyl acetate gave unchanged digoxigenin diacetate (700 mg.), m. p. and mixed m. p. 232°. The mother-liquors from the diacetate on oxidation with chromic acid and crystallisation from aqueous methanol gave fine needles, m. p. 228—229°, of the *acetoxy-ketone*, $[\alpha]_D^{16}$ +113° ± 1° (Found: C, 69·2; H, 7·9. $C_{25}H_{34}O_6$ requires C, 69·7; H, 8·0%). The infra-red spectrum showed a hydroxyl and butenolide, ketone, and acetate bands; the substance gave a red Légal reaction. The water-soluble fraction on acidification gave, after extraction with chloroform and storage for 24 hr. to complete relactonisation, much digoxigenin diacetate, m. p. and mixed m. p. 230° (positive Légal reaction). Examination of these fractions has not been completed.

Reduction of Anhydrogitoxigenins.—(a) *Dianhydrogitoxigenin*, m. p. 212—213°, $[\alpha]_{Hg}$ +737°, $[\alpha]_D^{21}$ +583° *in* MeOH (XXIV*a*). The combined products from the several hydrogenations of dianhydrogitoxigenin (in all, 5·25 g.) were fractionally crystallised from aqueous methanol. The least-soluble portion, crystallised to constant m. p. and rotation, furnished "β"-3β-*hydroxy*-14ξ:17ξ:20ξ-*cardanolide*, m. p. 203°, $[\alpha]_{Hg}^{20}$ +19·0°, $[\alpha]_D^{20}$ +16·3° in MeOH (0·25 g.) (Found: C, 76·5; H, 10·1. $C_{23}H_{36}O_3$ requires C, 76·6; H, 10·1%). Other crops, m. p. 165° to 188°, $[\alpha]_{Hg}$ +53° to +87°, may have contained the "α"-isomer as reported by Windaus and Schwarte (*Ber.*, 1925, **58**, 1515). The alkaline washings from these neutral products gave, on acidification and three crystallisations from aqueous methanol, an isomer or mixture of isomers of 3β-*hydroxy*-14ξ:17ξ:20ξ-*norcholanoic acid*, m. p. 212° (Found: C, 76·5; H, 10·5. $C_{23}H_{38}O_3$ requires C, 76·2; H, 10·5%). (b) *Dianhydrodihydrogitoxigenin*, $[\alpha]_{Hg}^{20}$ +276°, $[\alpha]_D^{20}$ +227° *in* MeOH (XXVI). The product from the hydrogenation of dianhydrodihydrogitoxigenin (3·87 g.) was fractionally crystallised from aqueous methanol. The more insoluble portions gave "α"-3β-hydroxy-14ξ:17ξ:20ξ-cardanolide, m. p. 217—218°, $[\alpha]_{Hg}^{20}$ +85·7°, $[\alpha]_D^{20}$ +72·9° in EtOH (Found: C, 76·7; H, 10·1. Calc. for $C_{23}H_{36}O_3$: C, 76·6; H, 10·1%). Later fractions gave "γ"-3β-*hydroxy*-14ξ:17ξ:20ξ-*cardanolide*, m. p. 160°, $[\alpha]_D^{20}$ +52·8° in MeOH (Found: C, 76·8; H, 10·1. $C_{23}H_{36}O_3$ requires C, 76·6; H, 10·1%).

Attempted Preparation of "α"-Anhydrogitoxigenin.—(*a*) Gitoxigenin diacetate (2 g.) was dissolved in ice-cold concentrated hydrochloric acid (40 c.c.) by shaking. After 10 hr. at −5° the solution was poured on ice. The derived product was reacetylated with acetic anhydride and pyridine. Crystallisation from methanol gave dianhydrogitoxigenin acetate, m. p. 207—208°, $[\alpha]_D^{20}$ +502° ± 5°, $[\alpha]_D^{20}$ +574° ± 8° in MeOH. (*b*) Gitoxigenin diacetate (372 mg.) in acetone (10 c.c.) was saturated with hydrogen chloride at −76°. After this mixture had been in a Dewar flask overnight its temperature had reached 0°. After working up as above, crystallisation from methanol gave dianhydrogitoxigenin diacetate, m. p. 206—207°, $[\alpha]_D^{20}$ +500° ± 4°. The mother-liquors from (*a*) and (*b*) were then chromatographed twice; apart from substantial further quantities of the dianhydro-acetate only a few mg. of oil of lower rotation were obtained.

3β-*Acetoxy*-14:16α-*dihydroxycard*-20(22)-*enolide* (XVIII*c*).—Gitoxigenin diacetate (10 g.) in ethanol (750 c.c.) and water (500 c.c.) was slowly treated on a water-bath with aqueous 0·1N-sodium hydroxide (220 c.c.). Hydrolysis was much faster than with digoxigenin diacetate and was complete in 10 min. The mixture was concentrated under reduced pressure until copious crystallisation occurred. The product was filtered off and extracted with hot methanol (25 c.c.) (when hydrolysis was incomplete, filtration at this point left solid unchanged diacetate). Evaporation of the methanol and crystallisation of the residue from ethyl acetate gave the 3-monoacetate, m. p. 227—232°, $[\alpha]_D^{15}$ +33·1° ± 1·3°, $\lambda_{max.}$ 2170 Å (ε 16,000); the presence of butenolide and acetate absorption in the infra-red, coupled with a positive Légal reaction, showed that this substance was not a derivative of *iso*gitoxigenin. The above procedure (ref. *l*) was preferable to hydrolysis with potassium carbonate in aqueous dioxan (ref. *w*) whence much unchanged diacetate was recovered.

3β-*Acetoxy*-14-*hydroxycard*-20(22)-*enolide* 16α-*Toluene*-p-*sulphonate*.—The foregoing monoacetate was treated in pyridine with toluene-*p*-sulphonyl chloride at room temperature for 18 hr. The resulting waxy solid, on extraction with hot benzene, left much insoluble unchanged starting material, m. p. 212—220°, $[\alpha]_D^{17}$ +39° ± 2°. The benzene solution deposited a small crop of plates, m. p. 150—155°. Recrystallisation from aqueous methanol gave the *toluene*-p-*sulphonate* as flat prisms, m. p. 155—157°, $[\alpha]_D^{18}$ +39·4° ± 0·5° (Found: C, 67·8; H, 7·8. $C_{32}H_{42}O_7S$ requires C, 67·4; H, 7·4%). In a second experiment the monoacetate was warmed with pyridine and

toluene-*p*-sulphonyl chloride on a water-bath for 20 min. The dark gummy product could not be crystallised then or after solvolysis with hot ethanolic potassium acetate for 4 hr. Chromatography finally gave 16-anhydrogitoxigenin acetate (XX), m. p. 190—192°, $[\alpha]_D^{20}$ +79·5° ± 2° (Found: C, 72·3; H, 8·3. Calc. for $C_{25}H_{34}O_5$: C, 72·4; H, 8·3%).

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