705. *Steroids. Part XVII.* The Structure of Diginin and Diginigenin.*

By C. W. Shoppee, Ruth E. Lack, and A. V. Robertson.

The digitenolides diginin and digitalonin are the 3-D(+)-diginoside and the 3-D(+)-digitaloside of the digitenol diginigenin, which is shown to be 3β-hydroxy-12α,20α-epoxy-14β-pregn-5-ene-11,15-dione. Digifolein and lanafolein are the 3-D(+)-diginoside and 3-D(−)-oleandroside of 2β-hydroxydiginigenin (digifologenin).

The digitenolides are physiologically inactive glycosides of pregnane derivatives and occur, together with cardioactive glycosides (cardenolides) of 5α- or 5β-norcholane derivatives, in plants, especially in *Digitalis* species. The digitenolides contain the 2,6-deoxyhexose sugars characteristic of the cardenolides, give the Keller–Kiliani reaction, and are readily hydrolysed by acid to aglycones termed digitenols. The digitenolides and derived digitenols give the Legal test, but contain no unsaturated γ-lactonic ring and do not exhibit the ultraviolet absorption ($\lambda_{max.}$ 216—218 mμ, log ε 4·0—4·3) characteristic of the cardenolides.

Diginin, the first and simplest digitenolide to be discovered and for 20 years the sole

* For a preliminary and partial presentation of this work see *Proc. Chem. Soc.*, 1962, 65. Part XVI, preceding paper.

representative of the class, was isolated by Walter Karrer [1] from the leaves of *D. purpurea*. It was shown by Shoppee and Reichstein [2, 3] to be the D(+)-diginoside of the digitenol diginigenin, $C_{21}H_{28}O_4$. Extensive chemical investigation of diginigenin by Shoppee [4, 5] characterised the four oxygen atoms and furnished the parent hydrocarbon " diginane," $C_{21}H_{36}$, but led only to tentative structural conclusions. Some years later, Press and Reichstein [6] identified " diginane " as 5α,14β,17α-pregnane, and so proved that diginigenin belongs to the pregnane series.

Diginin has subsequently been isolated in small quantities from *D. lanata* seeds,[7] *D. purpurea* seeds [8] and leaves,[9–12] and from a drug preparation [" Verodigen " (Boehringer)] derived from *D. purpurea*,[13, 14] and has been detected by paper chromatography.[15] The related glycoside digitalonin, isolated from *D. purpurea* leaves, has been shown by Satoh *et al.*[16, 17] to be diginigenin D(+)-digitaloside.

Numerous digitenolides have been isolated during the last five years from *Digitalis* species by the use of chromatographic techniques; these digitenolides, the derived digitenols, and the constituent sugars are listed in Table 1.

TABLE 1.

Digitenolides, digitenols, and sugars.

Digitenolide		Aglycone		Sugar		Source
Diginin	$C_{28}H_{40}O_7$	Diginigenin	$C_{21}H_{28}O_4$	D-Diginose	$C_7H_{14}O_4$	*D. purpurea*
Digitalonin	$C_{28}H_{40}O_8$			D-Digitalose	$C_7H_{14}O_5$	*D. lanata*
Digifolein	$C_{28}H_{40}O_8$	Digifologenin	$C_{21}H_{28}O_5$	D-Diginose	$C_7H_{14}O_4$	*D. purpurea*
Lanafolein	$C_{28}H_{40}O_8$			D-Oleandrose	$C_7H_{14}O_4$	*D. lanata*
14α-Digipronin	$C_{28}H_{40}O_9$	Digipronogenin	$C_{21}H_{28}O_5$	D-Digitalose	$C_7H_{14}O_5$	*D. purpurea* / *D. lanata*
Digipurpurin	$C_{39}H_{64}O_{14}$	Anhydrodigi-purpurogenin	$C_{21}H_{32}O_4$	3 D-Digitoxose	$C_6H_{12}O_4$	*D. purpurea*
Purpnin	$C_{39}H_{62}O_{13}$	Purpnigenin	$C_{21}H_{32}O_4$	3 D-Digitoxose	$C_6H_{12}O_4$	*D. purpurea*
Purpronin	$C_{39}H_{60}O_{14}$	Purprogenin	$C_{21}H_{30}O_5$	3 D-Digitoxose	$C_6H_{12}O_4$	*D. purpurea*
Digacetinin *	$C_{43}H_{64}O_{16}$	Deacetyldig-acetigenin	$C_{21}H_{30}O_5$	3 D-Digitoxose (+2AcOH)	$C_6H_{12}O_4$	*D. purpurea*
		Genin B	$C_{21}H_{30}O_4$			*D. grandiflora* Mill. (*D. ambigua* Murr.)
		Genin D	$C_{21}H_{30}O_5$			*D. grandiflora* Mill. (*D. ambigua* Murr.)
		Genin E	$C_{21}H_{32}O_5$			*D. grandiflora* Mill. (*D. ambigua* Murr.)
		Genin F	$C_{21}H_{34}O_5$			*D. grandiflora* Mill. (*D. ambigua* Murr.)

* Diacetate of glycoside $C_{39}H_{60}O_{14}$.

Digifolein, described as " Crystal A," [8, 9] was isolated by Satoh *et al.*[9–11] and by Tschesche and Grimmer [13] from *D. purpurea*; we have obtained digifolein from extracts of leaves of *D. lanata*. Lanafolein was obtained by Tschesche *et al.*,[13, 14] and Tschesche and Lipp [18] showed that digifolein is digifologenin D(+)-diginoside [13] whilst lanafolein is digifologenin D(−)-oleandroside.[14, 18]

[1] Walter Karrer, " Festschrift für Emil Barell," Birkhäuser, Basle, 1936, p. 228.
[2] Shoppee and Reichstein, *Helv. Chim. Acta*, 1940, **23**, 975.
[3] Shoppee and Reichstein, *Helv. Chim. Acta*, 1942, **25**, 1611.
[4] Shoppee, *Helv. Chim. Acta*, 1944, **27**, 246.
[5] Shoppee, *Helv. Chim. Acta*, 1944, **27**, 426.
[6] Press and Reichstein, *Helv. Chim. Acta*, 1947, **30**, 2127.
[7] Mohr and Reichstein, *Pharm. Acta Helv.*, 1949, **24**, 246.
[8] Okada and Yamada, *J. Pharm. Soc. Japan*, 1953, **73**, 525.
[9] Satoh, Yoshito, Ishii, and Nishimura, *Chem. and Pharm. Bull.* (*Japan*), 1953, **1**, 305, 396.
[10] Satoh, Ishii, and Oyama, *J. Pharm. Soc. Japan*, 1955, **75**, 1025.
[11] Satoh, Ishii, and Oyama, *J. Pharm. Soc. Japan*, 1955, **75**, 1173.
[12] Kaiser, Haack, and Spingler, *Annalen*, 1957, **603**, 75.
[13] Tschesche and Grimmer, *Chem. Ber.*, 1955, **88**, 1569.
[14] Tschesche and Buschauer, *Annalen*, 1957, **603**, 59.
[15] Gunzel and Weiss, *Z. analyt. Chem.*, 1955, **148**, 250; *Pharmazie*, 1955, **10**, 725.
[16] Satoh, Ishii, Oyama, Wada, and Okumura, *Chem. and Pharm. Bull.* (*Japan*), 1956, **4**, 284.
[17] Satoh, Wada, Ishii, Oyama, and Okumura, *Chem. and Pharm. Bull.* (*Japan*), 1957, **5**, 253.
[18] Tschesche and Lipp, *Annalen*, 1958, **615**, 210.

Digipronin, first isolated by Satoh *et al.*[10] from *D. purpurea* and *lanata* and described as " Crystal C," and subsequently investigated by Satoh *et al.*[16, 19, 20] and by Tschesche, Lipp, and Grimmer,[18, 21] is 14α-digipronogenin D(+)-digitaloside; the structures of 14α- and 14β-digipronogenin [(VIII) and (IX), see below] have recently been established by Satoh [22] but we were unaware of this until our work on diginigenin, now reported, had been completed. Digipurpurin, described as " Crystal D," and isolated by Tschesche and Grimmer [13, 21] and by Satoh,[16] purpnin,[10, 19, 21, 23] and purpronin [23] appear to be closely related tri-D(+)-digitoxosides. Digipurpurin, on acid hydrolysis, gives anhydrodigipurpurogenin [13] and is rapidly altered by water at 100° to give purpnin; [21] purpnin, on acid hydrolysis, yields purpnigenin [19] [$\nu_{max.}$ (in Nujol) 1680 cm.$^{-1}$], regarded by Satoh *et al.*[23] as 20-oxopregn-5-ene-3β,14α,15α-triol, whilst purpronin [$\nu_{max.}$ (in Nujol) 1712, 1689 cm.$^{-1}$] similarly yields purprogenin regarded by Satoh *et al.*[23] as 3,14α,15α-trihydroxypregn-5-ene-1,20- or 12,20-dione, so that digipurpurogenin [$\nu_{max.}$ (in potassium bromide) 1700 cm.$^{-1}$] is probably 1ξ,3β,14α- or -3β,12ξ,14α-trihydroxypregn-5-en-20-one.

Digacetinin, isolated by Tschesche, Hammerschmidt, and Grimmer,[24] gives, on acid hydrolysis, acetic acid, three mols. of D(+)-digitoxose, and digacetigenin, which is hydrolysed by potassium hydrogen carbonate to deacetyldigacetigenin; these compounds show ultraviolet absorption in the range 275—288 mμ (log ε 2·15—1·67), but resemble diginin and diginigenin in possessing two infrared carbonyl maxima (in potassium bromide, 1750, 1703 cm.$^{-1}$).

A biologically inactive but chemically uncharacterised digitenolide, " DA7," m. p. 287°, $[\alpha]_D$ +31°, has been isolated by Stoll and Kreis [25] from the leaves of *D. grandiflora* (*D. ambigua*), from which source Repic and Tamm [26] obtained a mixture of non-crystalline digitenolides giving on mild acidic hydrolysis four crystalline digitenols, " Genins B, D, E, and F," in quantities too small for structural investigation. Stoll and Renz [27, 28] have isolated two biologically inactive digitenolides " DF1 " and " DF11 " from *D. ferruginea* and *D. mariana*, respectively; " DF1," m. p. 158—160°, $[\alpha]_D$ −182°, and " DF11," m. p. 199—208°, $[\alpha]_D$ −181°, $\lambda_{max.}$ 285 mμ (log ε 2·15), give the Legal test and appear to be α-ketols since they reduce triphenyltetrazolium chloride,[29] whilst " DF11 " (in potassium bromide) exhibits $\nu_{max.}$ 3500 (OH), 1735, 1710 (2CO), 1635 (C=C), 1090, 1070, 1030 (C·O·C), 900, 840, 820 cm.$^{-1}$ (CO·C·O·C) and therein resembles diginin.

Other pregnane compounds have been isolated from plants; 3β-hydroxy-pregn-5-en-20-one and -5α-pregnan-20-one occur as D(+)-glucosides in uzara root and in *Xysmalobium undulatum*,[30] whilst a wide variety of C_{21}-aza-steroids occur in *Holarrhena* and *Fontumia* species.

Diginin,[2, 13, 14] $C_{28}H_{40}O_7$, $[\alpha]_D$ −176°, digifolein,[13-15] $C_{28}H_{40}O_8$, $[\alpha]$ −189°, and lanafolein,[14, 15] $C_{28}H_{40}O_8$, $[\alpha]_D$ −204°, possess closely similar chemical properties. All give intense violet colours with 3,5-dinitrobenzoic acid and alkali (Kedde reaction), reduce ammoniacal silver solution (Tollens's reagent) immediately at 20°, and react with triphenyltetrazolium chloride. All give yellow colours with tetranitromethane in chloroform solution and are unsaturated; all possess two carbonyl groups. One carbonyl group is reduced by brief treatment with sodium borohydride or catalytically with platinum in methanol, to give an acetylatable secondary hydroxyl group; the other carbonyl group is

[19] Satoh, Ishii, Oyama, and Okumura, *J. Pharm. Soc. Japan*, 1955, **75**, 1573.
[20] Satoh, *J. Pharm. Soc. Japan*, 1959, **79**, 1474.
[21] Tschesche, Lipp, and Grimmer, *Annalen*, 1957, **606**, 160.
[22] Satoh, *Chem. and Pharm. Bull.* (*Japan*), 1960, **8**, 270.
[23] Satoh, Ishii, and Oyama, *Chem. and Pharm. Bull.* (*Japan*), 1960, **8**, 657.
[24] Tschesche, Hammerschmidt, and Grimmer, *Annalen*, 1958, **614**, 136.
[25] Stoll and Kreis, *Helv. Chim. Acta*, 1951, **34**, 1431.
[26] Repic and Tamm, *Helv. Chim. Acta*, 1957, **40**, 689.
[27] Stoll and Renz, *Helv. Chim. Acta*, 1952, **35**, 1310.
[28] Stoll and Renz, *Verhandl. Naturforsch. Ges. Basel*, 1956, **67**, 392.
[29] Kiesewalter, *Pharmazie*, 1952, **7**, 580.
[30] Tschesche and Snatzke, *Annalen*, 1960, **636**, 105.

TABLE 2.

Digitenolides, dihydrodigitenolides, tetrahydrodigitenolides: spectroscopic properties.

Compound	U.V. ($m\mu$) $\lambda_{max.}$	U.V. log ε	Infrared ($cm.^{-1}$) OH	C=O *	C=O †	C=C	C·O·C	CO·C·O·C	Medium	Ref.
Diginin	310	2·00	3585	1735	1712	1655	1095, 1060, 1032	891, 872, 853	$CHCl_3$	‡
	309	1·94	3500	1755	1726	1640			KBr	13, 14
			3500	1740	1710	1635	1090, 1065, 1030	880, 850, 810	KBr	28
Digitalonin	310	1·97								16, 17
Digifolein	309	1·94	3500	1743	1723	1655	1092, 1083, 1070, 1032	898, 870, 845	KBr	13, 14
	310	2·01					1083, 947, 927	907, 877, 849	$CHCl_3$	10
Lanafolein	310	1·91	3480	1743	1715	1653			KBr	13
Dihydrodiginin **	306	1·60	3500	—	1723, 1715				KBr	14
Dihydrodigifolein **	306	1·59	3480	—	1710	1638			KBr	13
Dihydrolanafolein **	303	1·55	3500	—	1712	1648			KBr	13
Tetrahydrodiginin ††	—	—	3500	—	—				KBr	14
Tetrahydrodigifolein ¶ ††	—	—	3500	—	1723	—			KBr	13
Tetrahydrodigifolein § ††	—	—	3400	—	—	1643			KBr	13
Tetrahydrolanafolein § ††	—	—	3420	—	—	1640			KBr	13
Tetrahydrolanafolein C ¶ ††	—	—	3400	—	—				KBr	18

*† Two carbonyl groups. ‡ This paper. ¶ Reducing agent platinum–acetic acid. § Shown to be a mixture of 2 isomers.[18] ** These " dihydro "-compounds are actually 15β-alcohols. †† These " tetrahydro "-compounds are actually 5,6-dihydro-15β-alcohols or 11α,15β-diols diols, as indicated by the spectra.

TABLE 3.

Digitenols and derivatives: spectroscopic properties.

Aglycone	U.V. ($m\mu$) $\lambda_{max.}$	U.V. log ε	Infrared ($cm.^{-1}$) OH	C=O *	C=O †	C=C	C·O·C	CO·C·O·C	Medium	Ref.
Diginigenin, m. p. 108—112°	310	1·93	3585	1735	1712	1655	1065, 1040	—	$CHCl_3$	‡‡
	309	1·95	3585	1736	1712	1660	1070, 1042	870, 850, 825	CH_2Cl_2	26
Diginigenin, m. p. 150—153°	310	1·93	3610	1733	1710	1660	1093, 1070, 1042	890, 870	$CHCl_3$	‡‡
Diginigenin acetate	302	2·19	—	1735 ‡§	1716 ‡	1660 ‡			$CHCl_3$	
Dihydrodiginigenin §§	304	1·54	3610, 3520	—	1710	1655	1085, 1062, 1037	890, 870	$CHCl_3$	
Tetrahydrodiginigenin §§	305	1·55	3680, 3610	—	1712	—			$CHCl_3$	
Digifologenin	311	1·89	3475	1740	1710	1663			KBr	13, 14, 18
Digifologenin >CMe_2 deriv.	311	1·94	—	1745	1724	1663			KBr	18
Dihydrodigifologenin §§	305	1·60	3440	—	1716	1652			KBr	14
Tetrahydrodigifologenin >CMe_2 deriv. A §§	—	—	3410	—	—				KBr	18
Tetrahydrodigifologenin >CMe_2 deriv. B §§	—	—	3370	—	—				KBr	18
Genin B ¶	307	1·42	3450	1754	—		1058, 1045	886, 845, 823	KBr	26
Genin D **	305	1·84	3390	1742	1712		1035	—	KBr	
Genin E	—	—	3425	1748	—		1060, 1045	887, 845, 825	KBr	
Genin F ††	282	1·62	3380	—	1692		1075, 1040	—	KBr	

*† Two CO groups. ‡ $\nu_{max.}$ 1745, 1720, and 1666 $cm.^{-1}$ in CS_2. § CO of acetyl group coincides with reactive CO group; intensity double that of $\nu_{max.}$ 1735 $cm.^{-1}$ in diginigenin. ¶ Monoacetate has no band at 3450 $cm.^{-1}$. ** Diacetate still shows band at 3450 $cm.^{-1}$. †† Diacetate still shows band at 3367 $cm.^{-1}$. ‡‡ This paper. §§ See footnotes on " dihydro " and " tetrahydro " in Table 2.

relatively unreactive and resists hydrogenation with platinum in acetic acid, but is reduced by extended treatment with sodium borohydride again to give an acetylatable secondary hydroxyl group. All possess an inert oxygen atom regarded as oxidic. The spectroscopic properties of diginin, digifolein, lanafolein, and their dihydro- and tetrahydro-derivatives, collected in Table 2, confirm the presence of the foregoing molecular features, and the successive disappearance of the two carbonyl groups by reduction.

The ultraviolet absorption maximum at 300—310 mμ remains visible in the dihydrodigitenolides with a decreased extinction coefficient, but disappears in the tetrahydrodigitenolides, and so appears to result from several chromophores, which must include both carbonyl groups.

The infrared peaks in the 950—800 cm.$^{-1}$ region, regarded by Repic and Tamm [26] as characteristic of α-epoxy-ketones, are present but with low intensities.

Diginigenin, like digifologenin,[18] is difficult to obtain crystalline. Diginin is usually contaminated [13, 28] with digifolein; thus, the original specimen of diginin, isolated by Walter Karrer in 1936 [1] and made available by Hofmann–LaRoche Ltd., Basle, to one of us in 1939, has very kindly been examined by Professor Tschesche by partition chromatography on silica gel in the system formamide–benzene–chloroform and found to consist of diginin (75%), digifolein (10%), and lanafolein (5%). Further, diginigenin, m. p. 115°, $[\alpha]_D$ −226°,[2] m. p. 108—112,[26] is probably a hydrate, whilst the crude product obtained by brief hydrolysis of diginin with 0·05N-acid contains a quantity of an αβ-unsaturated ketone formed by aerial oxidation. We have been able to obtain the hydrated form, m. p. 108°, but by hydrolysis under nitrogen, repeated azeotropic distillation of the hydrolysis product with benzene, and crystallisation from dry ether, we have obtained the anhydrous form, m. p. 150—153°, $[\alpha]_D$ −227°.

Diginigenin, $C_{21}H_{28}O_4$, and digifologenin, $C_{21}H_{28}O_5$, by mass spectrometry give the appropriate molecular weights of 344 and 360, but fragmentation of the highly oxygenated molecule is so extensive that no useful structural information can be derived from the mass spectrum.

Diginigenin,[2, 4, 5] $[\alpha]_D$ −226°, and digifologenin,[13, 14, 18] $[\alpha]_D$ −269°, show closely similar chemical properties. Both contain one double bond and give yellow colours with tetranitromethane; both reduce ammoniacal silver solution at once at 20°, and give positive reactions with triphenyltetrazolium chloride and in the Kedde test; both contain two carbonyl groups. One carbonyl group is reactive and reduced (*a*) by sodium borohydride rapidly to yield, respectively, dihydrodiginigenin (giving a diacetate) and dihydrodigifologenin (giving a triacetate [14]), which give negative reactions with ammoniacal silver solution at 20° and in the Kedde test, and a very weak positive reaction with triphenyltetrazolium chloride, (*b*) catalytically by platinum in methanol or by platinum in acetic acid (which also saturates the double bond). The second carbonyl group is unreactive and not detected by use of a Grignard reagent; [14] it resists hydrogenation with platinum in acetic acid, but is reduced by extended treatment with sodium borohydride. Both compounds contain an inert oxygen atom regarded as oxidic. Whilst diginigenin contains a single secondary hydroxyl group characterised in a monoacetate,[2] digifologenin contains two secondary hydroxyl groups, present as a *cis*-α-glycol grouping, readily cleaved by periodic acid, and characterised in an isopropylidene derivative.[18]

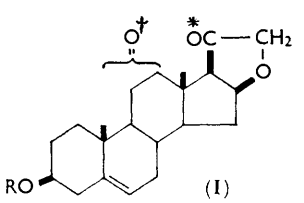
The spectroscopic properties of diginigenin, digifologenin, and their reduction products, collected in Table 3, confirm the presence of the various structural features outlined above, and the successive disappearance of the two carbonyl groups on reduction. The " genins B, D, E, and F " of Repic and Tamm [26] exhibit similar properties and probably possess structures analogous to those of diginigenin and digifologenin.

Shoppee and Reichstein [2] originally considered that the reactive carbonyl group of diginin and diginigenin was aldehydic, but numerous unsuccessful attempts to obtain homogeneous acids by oxidation, *e.g.*, of diginigenin acetate, with chromium trioxide in acetic acid led Shoppee [4, 5] to conclude that this carbonyl group (CO*) is ketonic, and to

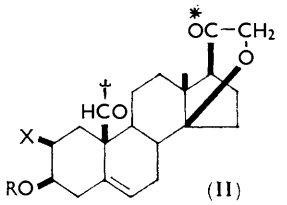
locate the unreactive carbonyl group (CO†) at position 11 or 12, and on the basis of an assumed analogy with the *Digitalis* sapogenins to propose a 16β,21-epoxypregnene structure for diginin (I; R = $C_7H_{13}O_3$) and diginigenin (I; R = H).

Tschesche and Grimmer [13] suggested that digifolein and digifologenin contain an aldehyde group, but clearly also included by implication diginin and diginigenin, since Tschesche and Buschauer [14] say: " Für das Carbonyl 2 [marked † below] war schon von Tschesche und Grimmer auch im Diginin eine Aldehydfunktion angenommen worden." This suggestion was made on account of the observed ultraviolet maxima (see Tables 2 and 3), which are consistent with the maxima found for strophanthidin [31] (5β-series; $\lambda_{max.}$ 303 mμ, log ε 1·45), for corotoxigenin [32] (5α-series; $\lambda_{max.}$ 310 mμ, log ε 1·5), and for other 19-aldehydic cardenolides and (boistroside, christyoside, gofruside, milloside, paulioside, stroboside; adonitoxigenin, antiarigenin, cannogenin, carpogenin, pachygenin), and for bufadienolides (bovogenin A, hellibrigenin, bufotalinin). The suggestion can also be supported by the observed infrared maxima of the unreactive carbonyl (CO†) in diginin, digifolein, and lanafolein (see Table 2) and in diginigenin and digifologenin (see Table 3), which can be reconciled with those observed for the 19-aldehyde group in strophanthidin [33] [$\nu_{max.}$ (in $CHCl_3$) 1718 cm.$^{-1}$] and in 19-oxoprogesterone [33] [$\nu_{max.}$ (in $CHCl_3$) 1717 cm.$^{-1}$]. Tschesche and Buschauer [14] noted that digifolein and dihydrodigifologenin triacetate, like diginigenin acetate, are only oxidised very slowly by chromium trioxide in acetic acid and fail to yield carboxylic acids; however, they concluded that a 19-aldehyde group is present, and by analogy with the 14β-cardenolides suggested the 14β,21-epoxy-19-oxopregn-5-ene formulæ for diginigenin (II; X = H) and digifologenin (lanafologenin [18]) (II; X = OH). Tschesche and Lipp,[18] although observing that tetrahydrodigifolein-A triacetate was effectively stable to chromium trioxide in acetic acid, continued to consider digifologenin and its parent glycosides digifolein and lanofolein as possessing a 19-aldehyde group.

The nuclear magnetic resonance spectrum of diginin shows that it does not contain an angular aldehyde group. The spectrum discloses the presence of five methyl groups:* $C_{(18)}$, singlet at τ 8·45 for methyl on quaternary $C_{(13)}$; $C_{(19)}$, singlet at τ 8·99 for methyl on quaternary $C_{(10)}$ allylic to the 5,6-double bond; $C_{(21)}$, doublet at τ 8·72 for methyl on tertiary $C_{(20)}$ with coupling constant J = 6·5 c./sec., typical of a freely rotating methyl group; $C_{(6')}$, doublet at τ 8·67 (J = 6·5 c./sec.) for methyl on tertiary $C_{(5')}$ in the diginose residue; $C_{(3')}$, singlet at τ 6·60 for methyl in the methoxyl group in the diginose residue.



(I)



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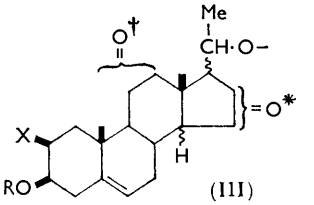


(III)

The nuclear magnetic resonance spectrum of digifolein likewise contains signals for five methyl groups and shows that this compound does not contain an angular aldehyde group.

The nuclear magnetic resonance spectrum of diginigenin acetate discloses the presence of four methyl groups:* $C_{(18)}$, singlet at τ 8·46 for methyl on quaternary $C_{(13)}$; $C_{(19)}$, singlet

* The assignment of peaks for the angular methyl groups is now the reverse of that given in our preliminary communication (*Proc. Chem. Soc.*, 1962, 65), for reasons to be discussed in a forthcoming paper on digifologenin.

[31] Paist, Blout, Uhle, and Elderfield, *J. Org. Chem.*, 1941, **6**, 273; Fried, Linville, and Elderfield, *ibid.*, 1942, **7**, 362.

[32] Schindler and Reichstein, *Helv. Chim. Acta*, 1952, **35**, 673, 730; 1953, **36**, 370.

[33] Nowacynski, Steyermark, Koiw, Genest, and R. N. Jones, *Canad. J. Biochem. & Physiol.*, 1956, **34**, 1023.

at τ 8·99 for methyl on quaternary $C_{(10)}$ allylic to the 5,6-double bond; $C_{(21)}$ doublet at τ 8·73 (J = 6·5 c./sec.) for methyl on tertiary $C_{(20)}$; and singlet at τ 8·02 for methyl of the acetoxyl group.

A 19-aldehydic proton should appear as a sharp singlet between τ 0 and τ 1, and this region is completely bare in all three nuclear magnetic resonance spectra. The nuclear skeleton of diginin and diginigenin and of digifolein, lanafolein, and digifologenin thus contains two angular methyl groups.

The oxidic oxygen in diginin and diginigenin must be bound to $C_{(20)}$ to account for the chemical shift and splitting pattern of the 21-methyl group. These facts are expressed in the partial formulæ for diginin (III; R = $C_7H_{13}O_3$, X = H) and diginigenin (III; R = X = H), whilst digifolein and lanafolein are represented by (III; R = $C_7H_{13}O_3$, X = OH), and digifologenin by (III; R = H, X = OH).

The infrared spectra (Tables 2, 3) of diginin and diginigenin, and of digifolein, lanafolein, and digifologenin, suggest that the reactive carbonyl group (CO*) is attached to the five-membered ring and that the unreactive carbonyl group (CO†) is attached to a six-membered ring. Since diginigenin is not an αβ-unsaturated ketone,[2] positions 4 and 7 are excluded.

The optical rotatory dispersion curves of diginin, diginigenin, " dihydro "diginigenin, and " tetrahydro "diginigenin, indicate the positions of the two carbonyl groups. The sign and molecular amplitude of the Cotton effects for these four compounds are collected in Table 4, the lower section of which gives the sign and magnitude of the Cotton

TABLE 4.

Optical rotatory dispersion (in methanol).

Compound	Peak and trough: ϕ	Peak and trough: λ (mμ)	Peak and trough: Δλ	Molecular amplitude ($10^{-2}a$)
Diginin	−13,650°	335	42	−247
	+11,100	293		
Diginigenin	−12,300	338	46	−226
	+10,300	292		
Dihydrodiginigenin **	−2500	330	45	−30
	+350	285		
Tetrahydrodiginigenin **	−1600	326	43	−34
	+1780	283		

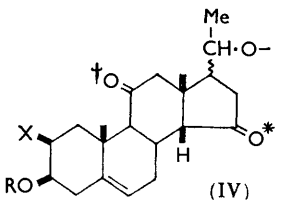
Type	$10^{-2}a$	Type	$10^{-2}a$	Type	$10^{-2}a$
15-Oxo-14α-steroid ...	+120 *	16-Oxo-14α-steroid ...	−279 ‡	17-Oxo-14α-steroid ...	+140 ‡
15-Oxo-14β-steroid ...	−125 †	16-Oxo-14β-steroid ...	+150 §	17-Oxo-14β-steroid ...	+34 ‡

* Djerassi, Closson, and Lippman, *J. Amer. Chem. Soc.*, 1956, **78**, 3163. † Lardon, Sigg, and Reichstein, *Helv. Chim. Acta*, 1959, **42**, 1457. ‡ Djerassi, Riniker, and Riniker, *J. Amer. Chem. Soc.*, 1956, **78**, 6362. § Djerassi, personal communication. ** See footnotes in Table 2.

effect for 15-oxo-, 16-oxo-, and 17-oxo-steroids of the 14α- and 14β-series. In contrast to substituted cyclohexanones, where the ring structure is symmetrical about the carbonyl group and asymmetry is due to the second-order effects of substituents, the intrinsically skewed character of the five-membered ring in substituted cyclopentanones exerts a powerful influence on the symmetry of the carbonyl group. The very strong negative Cotton curves indicate the presence of a skewed cyclopentanone system [34] and are consistent only with a 15-oxo-14β-structure [$10^{-2}a$ −125] or a 16-oxo-14α-structure [$10^{-2}a$ −279]. Because of the established 14β-orientation of diginane (5α,14β,17α-pregnane),[6] the ring D carbonyl group is placed at position 15. The small negative Cotton curves given by " dihydro "diginigenin and " tetrahydro "diginigenin show that the main " Cottonogenic " 15-carbonyl group has been reduced to CH·OH. The small amplitudes observed ($10^{-2}a$ −30, −34) are compatible with values found [35] for 11-oxo-14β-steroids ($10^{-2}a$ −48, −36) but not with those found [35] for 12-oxo-14β-steroids ($10^{-2}a$ +130, +135);

[34] Klyne, *Tetrahedron*, 1961, **13**, 29.
[35] Djerassi, Halpern, Halpern, Schindler, and Tamm, *Helv. Chim. Acta*, 1958, **41**, 250.

the six-membered-ring carbonyl group is therefore placed at position 11. The partial structure (III) can thus be replaced by (IV; R = $C_7H_{13}O_3$ or H, X = H) for diginin and diginigenin and probably by (IV; R = $C_7H_{13}O_3$ or H, X = OH) for digifolein, lanafolein, and digifologenin.



(IV)

It may be noted that 11-oxo-12β-hydroxy-14β-steroids (*e.g.*, sarmutogenin and its 3β,12β-diacetate, leptogenin and its 3β,12β-diacetate, methyl 3β,12β-diacetoxy-14-hydroxy-5β,14β-etianate) give positive Cotton curves ($10^{-2}a$ +76, +117, +117),[35] as do other 11-oxo-14α-steroids, whereas 11-oxo-12α-hydroxy-14β-steroids (*e.g.*, caudogenin 3β,12α-diacetate, inertogenin and its 3β,12α-diacetate, inertogenin-etianic acid methyl ester 3β,12α-diacetate) give negative Cotton curves ($10^{-2}a$ −187, −92, −135, −137) which have been attributed to conformational alteration affecting the asymmetry centres adjacent to the 11-carbonyl chromophore.[35]

The very high Δλ values observed suggest [34, 35] a group of the form OR adjacent to a carbonyl group; since these high values are shown, not only by diginin and diginigenin, but also by the Δ^5-derivative 15-deoxy-15-hydroxydiginigenin ‡ and by the saturated 15-deoxy-5α,6-dihydro-15-hydroxydiginigenin, the carbonyl group in question must be that at position 11 and a 12,*x*-oxidic structure is indicated. Such an 11-oxo-12,*x*-oxidic structure might be in part responsible for the ultraviolet absorption ($\lambda_{max.}$ 303—311 mμ, log ε 1·55—2·0) shown by diginin, digifolein, lanafolein, and their 15-deoxy-derivatives but not by their 11,15-dideoxy-11,15-dihydroxy-derivatives (see Tables 2 and 3 where they are listed as dihydro- or tetrahydro-derivatives). Tamm and his colleagues [36] have found that the ultraviolet absorption maximum of a carbonyl group is increased by up to 18 mμ by an adjacent oxidic ring (1α,2α-epoxy-5α-cholestan-3-one, $\lambda_{max.}$ 302 mμ, log ε 1·43; 1β,2β-epoxy-5α-cholestan-3-one, $\lambda_{max.}$ 295 mμ, log ε 1·44; methyl 1α,2α-epoxy-3-oxo-5α-etianate, $\lambda_{max.}$ 300 mμ, log ε 1·36; methyl 1ε,2ε-epoxy-3-oxo-5β-etianate, $\lambda_{max.}$ 295 mμ, log ε 1·33); in the infrared spectrum, although the absorption maximum of the carbonyl group is unaffected, an adjacent oxidic ring leads to the appearance of characteristic frequencies in the 950—800 cm.$^{-1}$ region (cf. Tables 2 and 3).

We now propose formula (V) for diginigenin, with formula (VI) for the 15-deoxy-15-hydroxy-derivatives (" dihydro "), and formula (VII) for the 11,15-dideoxy-11,15-dihydroxy-derivatives (" tetrahydro "). The quasi-equatorial 15β-configuration is provisionally assigned because (*a*) the 15-carbonyl group is not hindered [2, 14] and reduction of unhindered carbonyl groups by sodium borohydride gives largely the equatorial epimers,[37] and (*b*) the 15β-hydroxyl group has been found to be acetylated readily by acetic anhydride–pyridine at 20°; [2, 4, 5, 14, 18] the assignment is being further investigated. The axial 11β-configuration is assigned because (*a*) the 11-carbonyl group is hindered [2, 14] and reduction of hindered carbonyl groups by sodium borohydride gives preferentially the axial epimers,[37] (*b*) reduction of 11-ketones by sodium borohydride gives exclusively 11β-alcohols,[38] and (*c*) the resulting 11β-hydroxyl group resists acetylation with acetic anhydride in pyridine at 100°.[2, 5, 14, 18]

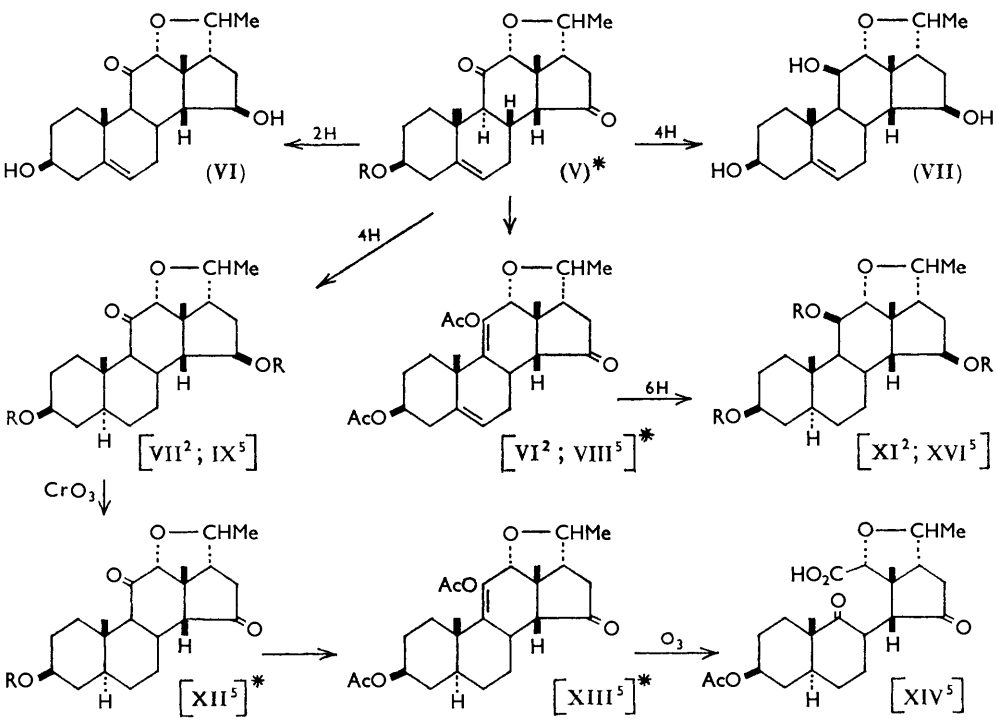
The reactions of diginigenin and its derivatives are readily interpreted on the basis of formula (V); to facilitate reference to the derivatives, the roman numeral used in earlier papers [2, 4, 5] is given in square brackets with the appropriate reference as a superscript, whilst an asterisk indicates that the compound reduced Tollens's reagent at once at 20°. Diginigenin (V) has an activated methylene group (positive Legal and Zimmerman tests)

‡ With the information now available it is possible to describe the " dihydro " and " tetrahydro " derivatives accurately.

[36] Striebel and Tamm, *Helv. Chim. Acta*, 1954, **37**, 1094; Schlegel, Tamm, and Reichstein, *ibid.*, 1955, **38**, 1013; Sallmann and Tamm, *ibid.*, 1956, **39**, 1340; Albrecht and Tamm, *ibid.*, 1957, **40**, 2216.
[37] Barton, *J.*, 1953, 1027.
[38] Fieser and Haymann, *J. Amer. Chem. Soc.*, 1951, **73**, 5252; Wendler, Graber, Jones, and Tishler, *ibid.*, 1952, **74**, 3630; Oliveto, Clayton, and Hershberg, *ibid.*, 1953, **75**, 486, 488.

and yields a 16-piperonylidene derivative.[5] 15-Deoxy-5α,6-dihydro-15β-hydroxydiginigenin [VII[2]; IX[5]], on brief heating with acetic anhydride, gives a 3β-monoacetate, and with acetic anhydride–pyridine at 20° yields a 3β,15β-diacetate; both the monoacetate and the diacetate are readily saponified, to regenerate the dialcohol. Diginigenin diacetate readily gives a semicarbazone;[2] it must therefore be, not an 11-oxo-14-enol acetate, but a 15-oxo-9(11)-enol acetate [VI[2]; VIII[5]] for which analogies exist.[39] By implication, the diacetate [XIII[5]] of 5α,6-dihydrodiginigenin [XII[5]] is also a 15-oxo-9(11)-enol acetate, cleaved by ozonolysis to the 9,15-dioxo-9,11-seco-11-oic acid [XIV[5]].[40] Hydrogenation of the 9(11)-double bond[41] in [VI[2]; VIII[5]] by α-*cis*-addition gives the 3β,11β-diacetate of the triol [XI[2]; XVI[5]] (hexahydrodiginigenin), which affords a 3β-monoacetate on brief heating with acetic anhydride and a 3β,15β-diacetate with acetic anhydride–pyridine at 100°; the latter is readily saponified, regenerating the triol.

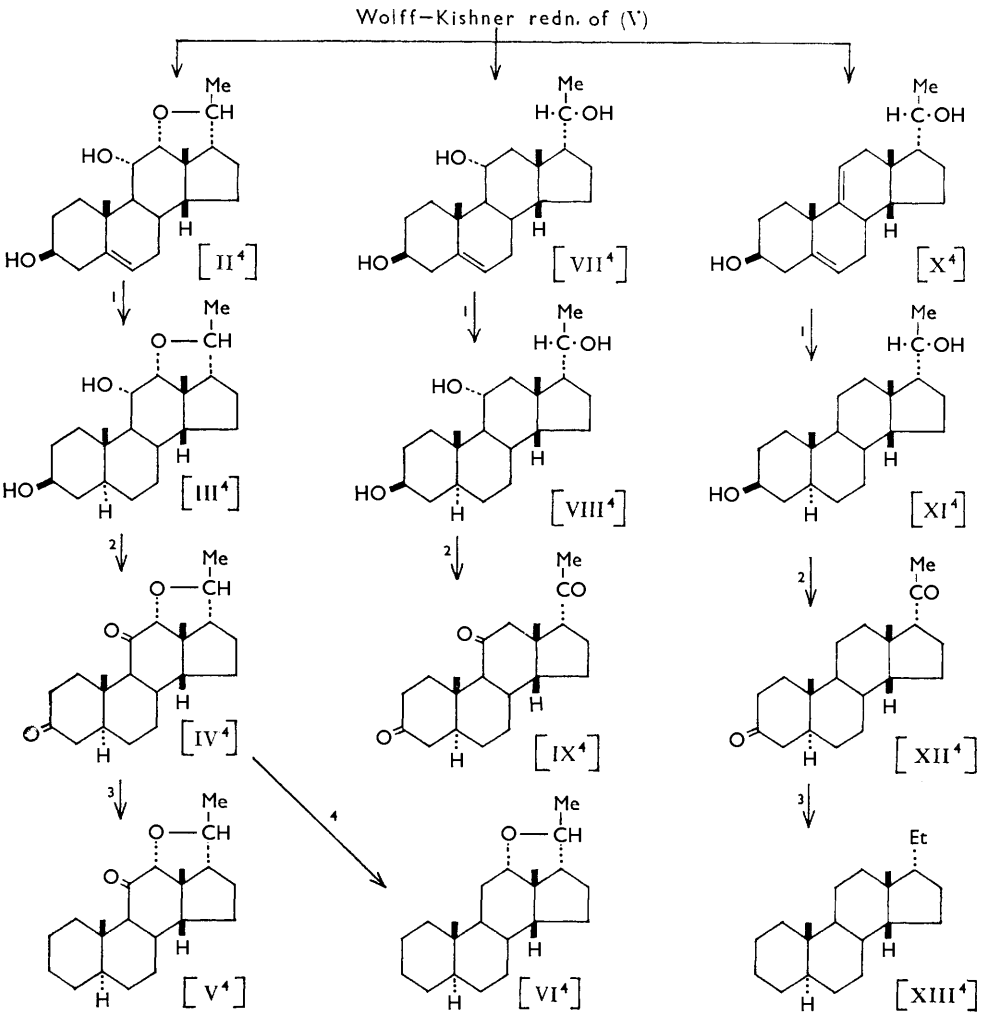
Wolff–Kishner reduction of diginigenin under pressure at 180° gives three series of well-defined products. The first product [II[4]], as originally surmised,[4] results by normal reduction of the reactive 15-carbonyl group, and it does not reduce Tollens's reagent at



20°. It gives no oxime under forcing conditions, and was regarded as still possessing the unreactive 11-carbonyl group; its infrared spectrum, however, shows no carbonyl absorption in the range 1780—1650 cm.$^{-1}$. We believe that the 11-carbonyl group is reduced to an 11α-hydroxyl group by alkali alkoxide;[42–44] thus Wolff–Kishner reduction of 3α,12β-dihydroxy-11-oxo-5β-cholanic acid gives mainly 3α,11α,12β-trihydroxy-5β-cholanic

[39] Kritchevsky, Garmaise, and Gallagher, *J. Amer. Chem. Soc.*, 1952, **74**, 483; Barton, Evans, Hamlet, Jones, and Walker, *J.*, 1954, 747; Crawshaw, Henbest, and E. R. H. Jones, *ibid.*, 731.
[40] Cf. Elks, *J.*, 1960, 3333.
[41] Shoppee, *Helv. Chim. Acta*, 1940, **23**, 740.
[42] Sondheimer, Yashin, Rosenkranz, and Djerassi, *J. Amer. Chem. Soc.*, 1952, **74**, 2696; 1953, **75**, 1282.
[43] Heusser, Anliker, and Jeger, *Helv. Chim. Acta*, 1952, **35**, 1537.
[44] Hershberg *et al.*, *J. Amer. Chem. Soc.*, 1952, **74**, 4470; 1953, **75**, 269, 1505.

acid and its three 3α,11,12-diastereoisomers.[45, 46] Compound [II[4]] and its 5α,6-dihydro-derivative [III[4]] readily give 3β-monoacetates with hot acetic anhydride; unfortunately more vigorous conditions of acetylation, possibly leading to 3β,11α-diacetates, were not investigated. We have attempted to obtain the 11-keto-analogue of [II[4]], 15-deoxodi-ginigen, by the thioketal–nickel procedure but, like 14β-digitogenone,[47] diginigenin fails to form a thioketal. Compound [III[4]], originally thought to possess an 11-carbonyl group, survives attempted Wolff–Kishner reduction; oxidation regenerates the 11-carbonyl group to give the 3,11-diketone [IV[4]], from which Wolff–Kishner reduction removes the



3-carbonyl group to furnish the 11-ketone [V[4]] (this exhibits no carbonyl reactivity), and Clemmensen reduction removes both carbonyl groups [48] to give the 12α,20α-epoxide [VI[4]].

[45] Gallagher, *J. Biol. Chem.*, 1946, **162**, 539.
[46] Wintersteiner, Moore, and Reinhardt, *J. Biol. Chem.*, 1946, **162**, 707.
[47] Klass, Fieser, and Fieser, *J. Amer. Chem. Soc.*, 1955, **77**, 3829; cf. Lardon, Sigg, and Reichstein, *Helv. Chim. Acta*, 1959, **42**, 1957.
[48] Steiger and Reichstein, *Helv. Chim. Acta*, 1938, **21**, 161.

The second product [VII[4]] gives a 3β,20α-diacetate with acetic anhydride and shows no carbonyl reactivity; its infrared spectrum exhibits no carbonyl absorption in the range 1780—1650 cm.$^{-1}$. The Wolff–Kishner procedure apparently cleaves the 12α,20α-epoxide ring and reduces the 11-carbonyl group to afford a 3β,11α,20α-triol [VII[4]]; accordingly we regard its 5α,6-dihydro-derivative, which gives a 3β,20α-diacetate with acetic anhydride, as 5α,14β,17α-pregnane-3β,11α,20α-triol [VIII[4]], oxidised with regeneration of the 11-carbonyl group to furnish 5α,14β,17α-pregnane-3,11,20-trione [IX[4]], which yields both mono- and di-ketonic derivatives.

The third product [X[4]] arises by removal of the 11-carbonyl group, accompanied by fission of the 12α,20α-epoxide ring; Wolff–Kishner reduction of 3α,12β-dihydroxy-11-oxo-5β-cholanic acid gives analogously small quantities of 3α-hydroxy-5β-chol-11-enic acid and 3α-hydroxy-5β-cholanic acid (lithocholic acid).[45, 46] The product [X[4]] could not be obtained crystalline, but by catalytic hydrogenation gave 5α,14β,17α-pregnane-3β,20α-diol [XI[4]], which readily gave a diacetate and was oxidised by chromium trioxide in acetic acid at 20° to 5α,14β,17α-pregnane-3,20-dione [XII[4]], m. p. 138—141°, $[\alpha]_D$ +40°, identical with a synthetic specimen, m. p. 140—141°, $[\alpha]_D$ +40°, prepared by Press and Reichstein [6] from 3β-hydroxy-5α,14β,17α-etianic acid. Wolff–Kishner reduction of the 3,20-diketone [XII[4]] gave 5α,14β,17α-pregnane (diginane) [XIII[4]], m. p. 75—77°, $[\alpha]_D$ +24°, identical with the synthetic hydrocarbon, m. p. 74—77°, $[\alpha]_D$ +25°, prepared by Press and Reichstein.[6]

The formation of the 5α,14β,17α-pregnane derivatives [X[4], XI[4], XII[4], XIII[4]] is explained simply and naturally by the new formula (V) for diginigenin and furnishes direct evidence for its 14β,17α-configuration.

The integrated nuclear magnetic resonance spectrum of diginigenin acetate provides complete and convincing proof of the new formula (V) for diginigenin. It shows four protons at low field which are assigned as follows: (i) $H_{(6)}$, complex multiplet at τ 4·55 for one olefinic proton; (ii) $H_{(3)}$; and (iii) $H_{(20)}$, overlapping complex multiplets centred at τ 5·40 and corresponding to two protons; and (iv) $H_{(12)}$, sharp singlet at τ 6·08 for one proton on carbon attached to oxidic oxygen.

The 5,6-double bond disclosed by signal (i) is confirmed chemically for diginigenin by Oppenauer oxidation of the 3β-hydroxy-Δ^5-system to a Δ^4-3-ketone ($\lambda_{max.}$ 240 mμ, log ε 4·0), and physically by molecular-rotation differences (see below).

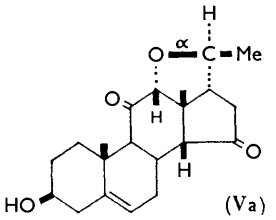
Signals (ii) and (iii) are consistent with the presence of a 3β-hydroxyl group and the tertiary 21-methyl group already disclosed, and the former is again confirmed by molecular-rotation differences (see below). The overlapping signals prevent assignment of an exact chemical shift to the centre of the multiplet from each proton. $H_{(3)}$ absorbs in the expected region. The remainder of the two-proton band must represent $H_{(20)}$, because the signal from this proton has to be extensively split by the 21-methyl group and $H_{(17)}$; it therefore cannot be the singlet at τ 6·08. The low chemical shift shows that $H_{(20)}$ must be subjected to a deshielding influence in addition to that of the adjacent oxygen atom.

Signal (iv) determines the point of attachment of the other end of the oxide bridge as $C_{(12)}$. Models indicate that the oxide bridge from $C_{(20)}$ could only be joined to $C_{(12)}$, $C_{(14)}$, or $C_{(16)}$: a sharp singlet would be expected from $H_{(12)}$ of a $C_{(12)}$ structure. The second possibility is excluded, since there would be no proton on $C_{(14)}$ to absorb at τ 6·08. The third possibility contains a four-membered oxide ring *cis*-united to ring D; the dihedral angle between $H_{(16)}$ and $H_{(17)}$ is close to 0° and splitting of *ca.* 8 c./sec.[49] should be observed for $H_{(16)}$. The appearance of a one-proton singlet therefore proves conclusively that the oxide bridge is joined to $C_{(12)}$. The atom $H_{(12)}$ is adjacent to both the 11-carbonyl group and the ether-oxygen atom and yet its signal is upfield from that of $H_{(20)}$, which is adjacent only to the ether-oxygen atom. The final structure must account for this fact.

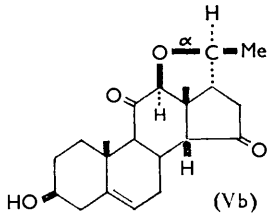
[49] Conroy, "Advances in Organic Chemistry," Interscience Publ. Inc., New York, 1960, Vol. II, p. 310.

The chemical evidence, namely, that diginigenin easily forms a piperonylidene derivative,[5] is consistent with a 12,20-oxide bridge but not with a 16,20-oxide bridge, since a 12-piperonylidene derivative, required by a 16,20-oxide structure, would be expected to be formed with great difficulty if at all.

It is only possible to construct models with the 12,20-epoxide bridge if $H_{(14)}$ is β-orientated and the side chain at $C_{(17)}$ is α-orientated. This stereochemistry is in striking agreement with that disclosed by the optical rotatory dispersion measurements and with that of the known degradation products 5α,14β,17α-pregnane-3,20-dione [XII[4]] and 5α,14β,17α-pregnane [XIII[4]]. On the models the oxide bridge can be closed easily if the oxygen atom has the 12α-configuration (Va), although the more strained 12β-configuration (Vb)



(Va)



(Vb)

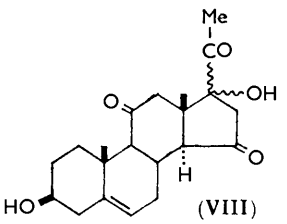
appears capable of existence. No rationalisation of the relative chemical shifts of $H_{(12)}$ and $H_{(20)}$ seems possible for structure (Vb). On the other hand, the observed spectrum is explicable in terms of structure (Va) as follows. The model, which is essentially strain-free, can have two conformations of ring C: a chair form in which $H_{(12)}$ is quasi-equatorial or a boat form in which $H_{(8)}$ and $H_{(12)}$ form " flagpole " bonds. In the chair conformation, $H_{(12)}$ lies almost in the plane of the 11-carbonyl group and would certainly be deshielded to a greater extent than $H_{(20)}$. In the boat conformation, the dihedral angle between $H_{(12)}$ and the $C_{(11)}$–oxygen bond is about 110° and, because of the shielding anisotropy of carbonyl functions,[50] $H_{(12)}$ would certainly not be deshielded and may even be in the region of slight positive shielding. Furthermore, if $H_{(20)}$ has the β-configuration, it is actually closer in space to the 11-carbonyl-oxygen atom than is $H_{(12)}$ and in this situation it might be expected that the 11-carbonyl group would deshield $H_{(20)}$ more than $H_{(12)}$. Deshielding of $H_{(12)}$ and $H_{(20)}$ due to the oxidic-oxygen atom would not be expected to be the same because the hydrogen atoms are not symmetric about the oxygen orbitals. If $H_{(20)}$ had the α-configuration, it would be too remote from any additional deshielding influence to account for its chemical shift. Other factors may also be operative; it is becoming recognised that tertiary hydrogen atoms in caged skeletons have abnormally low chemical shifts, but there are as yet no analogues available for comparison with diginigenin. Thus it is possible to rationalise the spectrum in terms of only one structure (Va) for diginigenin, provided that the oxide link has the 12α,20α-configuration and ring C possesses a boat conformation. The proposed configuration of the 20-methyl group in diginigenin (Va) corresponds with that of the 20β-methyl group in cholesterol, because the latter has been related configurationally to the 20-methyl group in pregn-5-ene-3β,20α-diol.[51]

14α-Digipronogenin has recently been shown by Satoh [22] to be 3β,17ξ-dihydroxypregn-5-ene-11,15,20-trione (VIII); it is converted by 0·02N-potassium hydroxide at 20° with inversion [52] at $C_{(14)}$ into 14β,17ξ-digipronogenin (IX), which by acid-catalysed dehydration, selective hydrogenation of the resulting 16,17-double bond, and Oppenauer oxidation gave 14β,17α-pregn-4-ene-3,11,15,20-tetraone identical with synthetic material.[22]

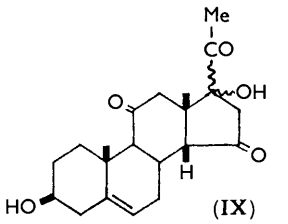
Digacetigenin may have the structure (X) and could be regarded as the 14β-product of

[50] Jackman, " Application of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry," Pergamon Press, London, 1959, p. 124.
[51] Wieland and Miescher, *Helv. Chim. Acta*, 1949, **32**, 1922.
[52] Cf. Elderfield, *J. Biol. Chem.*, 1936, **113**, 631; Meyer, *Helv. Chim. Acta*, 1947, **30**, 1976; Plattner, Heusser, and Segré, *ibid.*, 1948, **31**, 249.

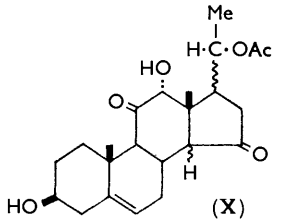
acetolysis of diginigenin. The small positive rotations [24] of digacetinin, digacetigenin, and related compounds indicate the 14α-configuration for (X) and suggest a closer relationship to 14α-digipronin (VIII).



(VIII)



(IX)



(X)

Diginin, digifolein, lanafolein, digipronin, and possibly digacetinin are 11,15-diketones of the pregnane series; they represent a new and characteristic type of glycoside occurring in *Digitalis* species.

APPENDIX: Molecular rotation differences.

		Δ Value found	Standard Δ value
$C_{(3)}$:	3-H[V^4] ⟶ 3β-OH[III^4]	−4°	−2°
	3β-OH-Δ^5(V) ⟶ 3β-OAc-Δ^5	−34	−34
	3,3-H_2[V^4] ⟶ 3-CO[IV^4]	+42	+71
$C_{(5,6)}$:	3β-OH-5α[XII^5] ⟶ 3β-OH-Δ^5(V)	−335	−298
	3β-OH-5α[III^4] ⟶ 3β-OH-Δ^5[II^4]	−263	−298
$C_{(15)}$:	15-H[IV^4] * ⟶ 15β-OH[VII^2]	+130	
	15β-OH[VII^2] ⟶ 15β-OAc[VII^2]	−78	
	15β-OH(VI) ⟶ 15-CO(V)	−574	
	Dihydrodigifolein ⟶ digifolein (15β-OH ⟶ 15-CO)	−523	
	Dihydrolanafolein ⟶ lanafolein (15β-OH ⟶ 15-CO)	−442	
	Dihydrodigifologenin ⟶ digifologenin (15β-OH ⟶ 15-CO)	−516	

* $[M]_D$ computed from the 3β-hydroxy-analogue of [IV^4] by subtraction of the standard Δ value +73°.

EXPERIMENTAL

For general directions see *J.*, 1959, 345; $[\alpha]_D$ are for acetone solutions; ultraviolet absorption spectra were measured for EtOH solutions in a Perkin–Elmer 4000A spectrophotometer; infrared absorption spectra were determined for $CHCl_3$ solutions in a Perkin–Elmer model 221 double-beam instrument. Analytical samples were dried at 70°/0·5 mm. for 5 hr. Nuclear magnetic resonance spectra were determined on a Varian DP 60 instrument at 60 Mc./sec., with deuterochloroform as solvent and tetramethylsilane as internal reference; the charts were calibrated by the audio side-band technique.

Diginin.—The original specimen,[2] m. p. 155—183°, had $\lambda_{max.}$ 310 mμ (log ε 2·0) and $\nu_{max.}$ 3585, 1735, 1712, 1655, 1095, 1060, 1032, 891, 872, 853 cm.$^{-1}$ when measured in 1956; an earlier spectrum taken in 1949 showed $\nu_{max.}$ 3595, 1737, 1715, 1650 cm.$^{-1}$; optical rotatory dispersion: in methanol, $[M]$ −13,650° (335 mμ, trough), +11,100° (292·5 mμ, peak).

Diginigenin.—(*a*) Diginin (250 mg.) in methanol (7·5 ml.) was treated with 36N-sulphuric acid (0·4 ml.) in water (7·5 ml.) on the steam-bath for 0·5 hr. The solution was concentrated to ~7 ml. at 35°/10 mm., diluted with water, and extracted with chloroform. After being washed with ice-cold 0·5N-sodium hydroxide and with water, the solution was rapidly dried (Na_2SO_4) and then evaporated. The product did not crystallise during several days in methanol at 0° and, after removal of the solvent, was chromatographed on a column of neutral aluminium oxide (5 g.; Woelm) prepared in benzene. Elution with ether (6 × 20 ml.) and with chloroform (3 × 20 ml.) gave diginigenin (180 mg.), m. p. 104—108° (from ether–pentane), $\nu_{max.}$ 3610, 1733, 1710, 1680w, $\lambda_{max.}$ 238 mμ (log ε 3·05). Optical rotatory dispersion: in MeOH, $[M]$ −12,300° (337·5 mμ, trough), +10,300° (292·5—290 mμ, peak).

The original specimen of diginigenin,[2] m. p. 115° (prepared in 1940 and sealed in a high vacuum), had $\lambda_{max.}$ 310 mμ (log ε 1·93), $\nu_{max.}$ 3585, 1735, 1712, and 1655 cm.$^{-1}$, when measured by Dr. R. Norman Jones in 1949.

(*b*) Diginin (300 mg.) in methanol (7 ml.) was treated under nitrogen with 0·5N-sulphuric acid (7 ml.) on the steam-bath for 0·5 hr. Concentration of the solution to 7 ml. under reduced pressure, followed by chloroform extraction, gave a light brown oil, which was dried by repeated azeotropic distillation with benzene (5 × 20 ml.) at 10 mm., to give diginigenin (220 mg.) as prisms (from anhydrous ether), m. p. 151—153°, giving a positive test with tetranitromethane in chloroform and reducing Tollens's reagent at once at 20° [*M* (mass spectrometry) = 344], $[\alpha]_D$ −227° (*c* 1·0), $\nu_{max.}$ 3609, 1733, 1709, 1656, 1140, 1080, 1070, 1040, 890, 870 cm.$^{-1}$, $\lambda_{max.}$ 310 mμ (log ε 1·93) (Found: C, 72·85; H, 8·2. Calc. for $C_{21}H_{28}O_4$: C 73·2; H, 8·2%).

(*c*) The residues (200 mg.) from a previous hydrolysis of diginin, performed in 1940 by Shoppee,[2] were purified by chromatography on neutral aluminium oxide (5 g.) in benzene. Elution with chloroform–ether (1 : 20; 10 × 20 ml.), followed by azeotropic distillation of the eluted material with benzene at 10 mm., gave diginigenin (120 mg.), m. p. and mixed m. p. 151—153° [from anhydrous ether after seeding with the sample obtained as in (*b*)]. Further elution with chloroform (5 × 20 ml.) gave crystalline fractions, m. p. 160—172°, thought to be mainly digifologenin (lit.,[13] 176°) since digifolein has been shown to be present in the original glycoside.

Diginigenin Monoacetate.—(*a*) Diginigenin (m. p. 102—108°) (25 mg.) in pyridine (0·5 ml.) was treated with acetic anhydride (0·5 ml.) at 20° for 1 hr. After complete evaporation of the reagents at 10 mm. and then at 0·5 mm., diginigenin monoacetate (16 mg.) crystallised from acetone–pentane and had m. p. 165—170°, $\nu_{max.}$ 1735, 1708 cm.$^{-1}$; the 1735 cm.$^{-1}$ peak (5-ring CO + COMe) had double the intensity of the 1708 cm.$^{-1}$ peak (6-ring CO).

(*b*) Diginigenin, m. p. 151—153° (25 mg.), was treated as in (*a*) with acetic anhydride in pyridine. Complete evaporation of the reagents, followed by recrystallisation of the solid residue from acetone–pentane, gave diginigenin monoacetate (19 mg.), m. p. 175—178° (lit.,[2] 178°). The infrared absorption spectrum was identical with that of the sample prepared as in (*a*). The original specimen of diginigenin acetate,[2] m. p. 178°, had $\nu_{max.}$ (in $CHCl_2$) 1735, 1716, 1660 cm.$^{-1}$ (in CS_2), 1745, 1720, 1666 cm.$^{-1}$ when measured by Dr. R. Norman Jones in 1949 using a calcium fluoride prism.

Diginigenone.—Diginigenin (20 mg. of residues) and aluminium isopropoxide (50 mg.) in dry acetone (5 c.c.) and benzene (5 c.c.) were heated under reflux for 18 hr. The cooled solution was poured into 2N-sulphuric acid and extracted with benzene, and the extract dried and evaporated to an oil (10 mg.), $\lambda_{max.}$ 240 mμ (log ε 4·0).

15-*Deoxy*-15-*hydroxydiginigenin* (*Dihydrodiginigenin*).—Diginigenin, m. p. 151—153° (50 mg.), in methanol (1 ml.) was treated with sodium borohydride (14 mg.) in methanol (2 ml.) at 20° for 1 hr. Acidification with 2N-sulphuric acid and extraction with chloroform gave 15-*deoxy*-15-*hydroxydiginigenin* (45 mg.), m. p. 95°, crystallising from ether with 0·5 mol. of water, as needles, m. p. 175—179°, $[\alpha]_D$ −60° (*c* 0·9 in acetone), and giving a positive test with nitromethane in chloroform and a negative test with Tollens's reagent (Found: C, 70·9; H, 8·7. $C_{21}H_{30}O_4,\frac{1}{2}H_2O$ requires C, 71·0; H, 8·75%); it had $\nu_{max.}$ 3610, 3520, 1710, 1670, 1092, 1085, 1062, 1037, 890 cm.$^{-1}$, $\lambda_{max.}$ 302 mμ (log ε 1·54). Optical rotatory dispersion: in MeOH, [*M*] −1600° (327·5—325 mμ, trough), +1780° (285—282·5 mμ, peak).

15-*Deoxy*-5α,6-*dihydro*-15-*hydroxydiginigenin* (*Tetrahydrodiginigenin*).—Diginigenin, m. p. 102—108° (50 mg.), in acetic acid (5 ml.) was added to a suspension of pre-reduced platinum oxide (25 mg.) in acetic acid (5 ml.) and shaken with hydrogen for 4 hr. Filtration and evaporation of the filtrate gave the product as needles (from methanol), m. p. 229—231°, $[\alpha]_D$ +39° (*c* 0·9) (lit.,[2, 5] 229—231°, +37°), $\nu_{max.}$ 3680, 3609, 1708, 1065, 1040, 1020 cm.$^{-1}$. Optical rotatory dispersion: in MeOH, [*M*] −2495° (330 mμ, trough), +342° (290 mμ, shortest wavelength reached). The substance gave no colour with tetranitromethane in chloroform; it was too insoluble to permit determination of the infrared spectrum in $CHCl_3$ or CS_2.

Digifolein.—Isolated from extracts of leaves of *D. lanata* by chromatography on aluminium oxide in benzene and elution with chloroform, digifolein had m. p. 198—202°, $[\alpha]_D$ −220° (*c* 1·0 in $CHCl_3$). This material (Found: C, 66·3; H, 8·05. Calc. for $C_{28}H_{40}O_8$: C, 66·6; H, 8·0%) was homogeneous on paper chromatography with the system isobutyl methyl ketone–isopropyl ether saturated with formamide, development being with antimony trichloride in formamide.

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