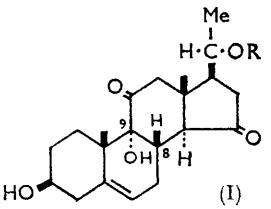
698. *Steroids. Part XXI.* The Structure of Digacetigenin.*

By C. W. Shoppee and Ruth E. Lack.

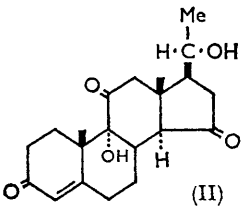
The digitenolide digacetinin is the 3-tris-D(+)-digitoxoside acetate of the acetyldigitenol digacetigenin, for which the structure 1β-acetoxy-3β,17β-dihydroxy-14α,17α-pregn-5-ene-15,20-dione is proposed.

The glycoside digacetinin was isolated by Tschesche, Hammerschmidt, and Grimmer [1] from a drug preparation derived from a Portuguese specimen of *Digitalis purpurea*, which may have also contained *D. thapsi* and possibly other varieties of *Digitalis*. It was also isolated from technical digitoxin by Reichstein and Schindler,[2] and this was the source of our material.

Tschesche *et al.*[1] showed that the digitenolide digacetinin, $C_{43}H_{64}O_{16}$, consists of an acetylated aglycone digacetigenin, $C_{23}H_{32}O_6$, united to three molecules of D(+)-digitoxose, one of which contains an acetyl group. Deacetyldigacetigenin, $C_{21}H_{30}O_5$, contains two secondary hydroxyl groups and two carbonyl groups. One secondary hydroxyl group is acetylated in digacetinin and digacetigenin; the other secondary hydroxyl group carries the sugar residue in digacetinin and deacetyldigacetinin, and forms part of a 5-en-3β-ol system since digacetigenin by Oppenauer oxidation affords a 4-en-3-one system, $\lambda_{max.}$ 240 mμ (log ε 4·0). One of the two carbonyl groups, $\nu_{max.}$ (KBr) 1750 cm.$^{-1}$, is located in ring D, whilst the other, $\nu_{max.}$ (KBr) 1703 cm.$^{-1}$, was suggested [1] to be present as a CO·Me



(I)



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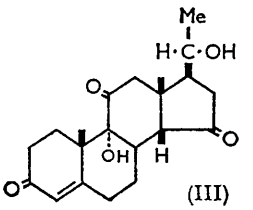


(III)

group at C-17. The fifth oxygen atom was later [3] recognised to be present as a tertiary hydroxyl group and placed at C-9; thus, digacetigenin by treatment with acetic anhydride–potassium hydrogen sulphate at 100° furnished a diacetate, and, although resistant to thionyl chloride–pyridine at 50°, underwent dehydration with concentrated hydrochloric acid in refluxing methanol.

Tschesche, Hammerschmidt, and Snatzke [3] suggested the structure (I; R = Ac) for digacetigenin, and (I; R = H) for deacetyldigacetigenin, and confirmed the presence of a 5-en-3β-ol system. Hydrogenation of the 3-monoacetate of digacetigenin with palladium–methanol gave the 5α,6-dihydro-compound, whilst Oppenauer oxidation of digacetigenin gave a mixture of seven compounds, from which two isomeric triketones, $C_{21}H_{28}O_5$, were isolated, whose relationship as a 14α-4-ene-3,15-dione (II), $[\alpha]_D$ +154°, and a 14β-4-ene-3,15-dione (III), $[\alpha]_D$ +1°, was correctly interpreted. Deacetyldigacetinin and deacetyldigacetigenin (I; R = H) by treatment with alkali likewise underwent inversion of configuration at C-14, to furnish 14β-isomers with the characteristic large decrease in specific rotation.[4]

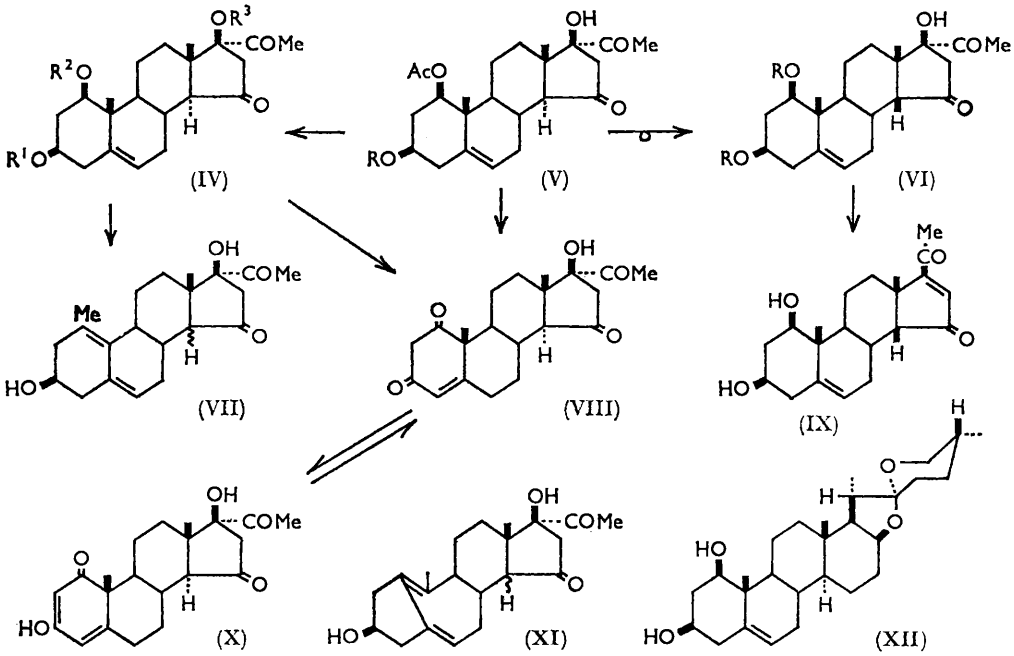
The assignment [3] of the residual functional groups to rings C and D in structure (I) was largely based on the apparent absence † from the infrared spectra of digacetigenin and deacetyldigacetigenin of the " O "-band at 1356 cm.$^{-1}$ associated with the bending vibrations

* Part XX, *J.*, 1964, 877.

† Tschesche *et al.*,[3] nevertheless, record a peak at 1367 cm.$^{-1}$ in the infrared spectrum of digacetigenin diacetate.

[1] Tschesche, Hammerschmidt, and Grimmer, *Annalen*, 1958, **614**, 136.
[2] Reichstein and Schindler, personal communication.
[3] Tschesche, Hammerschmidt, and Snatzke, *Annalen*, 1961, **642**, 199.
[4] Lardon, Sigg, and Reichstein, *Helv. Chim. Acta*, 1959, **42**, 1457.

of the 20-methyl group in the side-chains COMe and >C(OH)·COMe.[5] Whilst digacetigenin was stable to chromium trioxide–pyridine at 20°, deacetyldigacetigenin was oxidised to two isomeric triketones, $C_{21}H_{28}O_5$, both of which showed a strong " O "-band at 1361—1364 cm.$^{-1}$. It was concluded that the second carbonyl group in digacetigenin could not be at C-20; instead, the acetoxyl group was placed at this position in digacetigenin, giving rise to a 20α-hydroxyl group in deacetyldigacetigenin, which by oxidation with chromium trioxide–pyridine furnished the third carbonyl group in the above triketones. The ultraviolet maximum of one triketone at 291 mμ was unchanged by addition of alkali; it could not, therefore, possess a 16,20-dione system with an enolisable 17-hydrogen atom, whilst the presence of a 17-hydroxyl group was excluded since deacetyldigacetigenin would be a 17,20-diol but was stable to lead tetra-acetate. The ring-D carbonyl group was therefore located at C-15. The second carbonyl group, which generally appears at 1706—1702 cm.$^{-1}$, but is found at 1682 cm.$^{-1}$ in deacetyldigacetigenin, was placed at C-11 [although



it readily gave oximes (cf. ref. 6) in which the 5-ring-carbonyl group was still present, $\nu_{max.}$ (KBr) 1733 or 1722 cm.$^{-1}$], on the grounds that vigorous treatment of deacetyldigacetigenin with concentrated hydrochloric acid in refluxing methanol gave a low yield of an amorphous but homogeneous product, $\lambda_{max.}$ 252 mμ (log ε 4·07), regarded as an αβ-unsaturated ketone, and, by exclusion, as an 8-ene-11,15-dione formed from a 9α-hydroxy-11,15-dione (cf. I).

Our findings are incompatible with structure (I; R = Ac) for digacetigenin, and we propose that 14α-digacetigenin is 1β-acetoxy-3β,17β-dihydroxy-14α,17α-pregn-5-ene-15,20-dione (V; R = H). One carbonyl group is located at C-20 in a 17-COMe group, because (a) 14α-digacetigenin (V; R = H) gives a good yield of iodoform [steroids containing the 17-CH(OH)·Me group do not yield iodoform], and (b) the infrared spectra of 14α-digacetigenin (V; R = H), deacetyl-14α-digacetigenin (IV; $R^1 = R^2 = R^3 = H$), and deacetyl-14β-digacetigenin (VI; R = H) all exhibit the " O "-band [5] at 1356 cm.$^{-1}$ * associated with the

* The " O "-band at 1356 cm.$^{-1}$ appears as a shoulder for a Nujol mull, but as a sharp peak for carbon tetrachloride solution.

[5] Jones, R. N., and Cole, *J. Amer. Chem. Soc.*, 1952, **74**, 5648.

[6] Jones, W. H., Tristram, and Benning, *J. Amer. Chem. Soc.*, 1959, **81**, 2151; cf. Hershberg, Oliveto, and Benning, *Chem. and Ind.*, 1958, 1477.

bending vibrations of the 20-methyl group in the combinations COMe and >C(OH)·COMe. The other carbonyl group, $\nu_{max.}$ 1742 cm.$^{-1}$, must be in ring D, and is located at C-15 because of the facile inversions from the 14α-series to the 14β-series reported by Tschesche *et al.*[1,3] and observed by us.

14α-Digacetinin (V; R = $C_{20}H_{32}O_{10}$) and 14α-digacetigenin (V; R = H) reduce triphenyltetrazolium chloride; thus, they are α-ketols.[7] The tertiary hydroxyl group appears, therefore, to be associated with the 20-carbonyl group, and to be attached to C-17 in a >C(OH)·COMe grouping.

The presence of a 17-COMe grouping is supported by the nuclear magnetic resonance spectrum of 14α-digacetigenin (Table 1) which discloses the presence of four methyl groups. The signals for the 10- and 13-angular methyl groups occur as sharp singlets, each for three protons, at τ 9·03 and 8·92, respectively. The signal for the methyl in the acetoxyl group occurs as a singlet at τ 7·87, whilst the signal for the 20-methyl group appears as a sharp singlet for three protons at τ 7·68; this chemical shift is characteristic for a methyl adjacent to a carbonyl group.

The 17α-configuration of the COMe group is indicated by the position of the 13-methyl peak at τ 9·03. In pregnenolone this peak appears at τ 9·33, so that inversion of configuration from 17β to 17α is associated with a paramagnetic shift of 0·30 p.p.m.; similarly, a

TABLE 1.

Nuclear magnetic resonance spectra (τ values).

Compound	1-Me	10-Me	13-Me	20-Me	1-COMe	3-COMe	1-H	3-H	4-H
14α-Digacetigenin (V; R = H).....	—	8·92	9·03	7·68	7·87	—	5·62	6·5	4·56
14α-Digacetigenin 3-acetate	—	8·93	9·03	7·68	7·88	8·01	5·62	5·34	4·56
14β-Digacetigenin 3-acetate (VI; R = Ac)	—	8·89	8·34	7·74	7·87	7·97	5·3	5·3	4·48
3β,17β-Dihydroxy-1-methyl-19-norpregna-1(10),5-diene-15,20-dione (VII)	7·71	—	{8·98 / 8·47}	7·68	—	—	5·8	6·5	4·7
17β-Pregn-4-ene-3,11,15,20-tetraone	—	—	9·32	—	—	—	—	—	—
17α-Pregn-4-ene-3,11,15,20-tetraone	—	—	9·04	—	—	—	—	—	—
11α-Acetoxypregn-4-ene-3,20-dione	—	8·75	9·30	7·95	—	—	—	4·8	—
3β,11α-Diacetoxy-17α-hydroxy-5β-pregnan-20-one	—	9·0	9·28	7·78	—	—	—	5·1	—
3α,11α,17α-Trihydroxy-5β-pregnan-20-one	—	8·85	9·30	7·70	—	—	—	—	—
12α-Acetoxy-5β-pregnane-3,20-dione	—	8·97	9·31	7·91	—	—	—	4·9	—
3α,12α-Diacetoxy-5β-pregnan-20-one	—	9·11	9·35	7·90	—	—	—	4·94	—

paramagnetic shift of 0·28 p.p.m. occurs when pregn-4-ene-3,11,15,20-tetraone, τ 9·32, undergoes inversion at C-17 to 17α-pregn-4-ene-3,11,15-20-tetraone,[8] τ 9·04 (cf. Table 1).

The signal for the 20-methyl group at τ 7·68, which is lower than the normal figure (7·9—7·95) for the 20-methyl group in the pregnan-20-one series (Table 1), is in good agreement with the value (7·7—7·78) found in the 17α-hydroxypregnan-20-one series (Table 1), and occurs at the same position as in ("γ")-14α-digiprogenin (see following Paper).

The optical rotatory dispersion curves of 14α-digacetigenin (V; R = H) and deacetyl-14α-digacetigenin (IV; $R^1 = R^2 = R^3 = H$) suggest that, in both of these compounds, the side-chain has the 17α-orientation. The sign and molecular amplitude of the Cotton effects for these two compounds are given in Table 2, the lower section of which records the sign and magnitude of the Cotton effect for 15-oxo-14α-steroids and 20-oxo-17α- and -17β-steroids. The small molecular amplitudes observed for 14α-digacetigenin ($10^{-2}a$ +40)

[7] Kiesewalter, *Pharmazie*, 1952, **7**, 580.
[8] Slomp, personal communication.

and deacetyl-14α-digacetigenin ($10^{-2}a$ +25) are consistent with a 15,20-dioxo-14α,17α-structure, for which the calculated molecular amplitude [9] is $10^{-2}a$ +12, but not with a 15,20-dioxo-14α,17β-structure, for which the calculated value is $10^{-2}a$ +285. Some vicinal interaction between the 15- and 20-carbonyl groups may occur as in 3,6-dioxo-5α-steroids.[9]

14α-Digacetigenin (V; R = H) by treatment with acetic anhydride–pyridine at 20° readily gives the 3β-monoacetate (V; R = Ac),[1] and at 100° affords the 3β,17α-diacetate (IV; $R^1 = R^2 = R^3$ = Ac) (cf. ref. 3). The n.m.r. spectrum of the monoacetate reveals signals for five methyl groups (Table 1), each of which appears as a sharp singlet for three protons; four signals are in the same positions as those for 14α-digacetigenin, whilst the fifth signal for the methyl group in the acetoxyl group is at τ 8·01.

14α-Digacetigenin (V; R = H) by mild hydrolysis with potassium hydrogen carbonate in methanol at 20° gives deacetyl-14α-digacetigenin (IV; $R^1 = R^2 = R^3$ = H); use of methanolic sodium hydroxide at 20° also causes inversion of configuration at C-14, to yield deacetyl-14β-digacetigenin (VI; R = H). This 1β,3β,17β-triol was too insoluble in the usual solvents to furnish a satisfactory n.m.r. spectrum, but by acetylation with acetic anhydride–pyridine at 20° was readily converted into the 1β,3β-diacetate (VI; R = Ac). The n.m.r. spectrum of this diacetate shows the presence of five methyl groups, each appearing as a sharp singlet for three protons. The 14β,17α-configuration is confirmed by the position of the signal for the 13-methyl group at τ 8·34 as in diginin [10] and diginigenin acetate [10] and in digifolein [11] and digifologenin; [11] the signals for the other four methyl groups appear at positions closely similar to those in 14α-digacetigenin 3-monoacetate (V; R = Ac).

TABLE 2.

Optical rotatory dispersion (in methanol).

Compound	Peak and trough ϕ	Peak and trough λ (mμ)	Peak and trough Δλ	Molecular amplitude ($10^{-2}a$)
14α-Digacetigenin	+2550	312		
	−1420	270	42	+40
Deacetyl-14α-Digacetigenin	+1920	315		
	−0600	265	50	+25

Type	$10^{-2}a$	Type	$10^{-2}a$
15-Oxo-14α-steroid *	+120	20-Oxo-17β-steroid †	+165
20-Oxo-17α-steroid †	−108	17α-Hydroxy-20-oxo-17β-steroid ‡	+124

* Djerassi, Closson, and Lippman, *J. Amer. Chem. Soc.*, 1956, **78**, 3163. † Djerassi, *Bull. Soc. chim. France*, 1957, 741. ‡ Klyne, personal communication.

Further proof of the presence of a tertiary 17β-hydroxyl group in 14α-digacetigenin (V; R = H) was obtained by treatment with methanolic sodium hydroxide at 70° for 6 hours. The product consisted of a mixture of compounds, from which there could be isolated in fair yield the enedione 14β-anhydrodigacetigenin (IX), $\lambda_{max.}$ 242 mμ (log ε 4·1), $\nu_{max.}$ 1706 (16-en-15-one) and 1685 cm.$^{-1}$ (20-one). 14α-Digiprogenin similarly affords 14β-anhydrodigiprogenin, $\lambda_{max.}$ 239 mμ (log ε 4·02), $\nu_{max.}$ 1709 (16-en-15-one) and 1678 cm.$^{-1}$ (20-one) (see following Paper).

The positions of the two carbonyl groups, the position and configuration of the tertiary hydroxyl group, and the character of the C/D ring junction in 14α-digacetigenin (V; R = H) being established, we directed our attention to the remaining features of the molecule.

The presence of the fourth oxygen atom as a secondary hydroxyl group in a 5-en-3β-ol system, established by Tschesche *et al.*,[1,3] was confirmed by the n.m.r. spectra of 14α-digacetigenin (V; R = H) and its 3-monoacetate (V; R = Ac), in which the vinylic 6-proton appears at τ 4·56 as in cholesterol and cholesteryl acetate. In addition, the axial 3-proton

[9] Djerassi, "Optical Rotatory Dispersion," McGraw-Hill, London, 1960, pp. 71, 72.
[10] Shoppee, Lack, and Robertson, *J.*, 1962, 3610.
[11] Shoppee, Lack, and Sternhell, *J.*, 1963, 3281.

appeared at τ 6·5 in 14α-digacetigenin and was shifted to 5·33 in its 3-monoacetate; a precisely similar relationship exists in cholesterol and cholesteryl acetate.

The fifth oxygen atom is present as a secondary acetoxyl group; it remains to determine its position and configuration. The equatorial conformation is suggested by its ready hydrolysis and re-esterification. The proton attached to the carbon atom bearing the acetoxyl group appears in the nuclear magnetic resonance spectra of 14α-digacetigenin and its 3-monoacetate as an approximate quartet at τ 5·62; the profile of the signal is similar to those given by the axial 12α-proton in 12β-acetoxy-5α-androstane [12] and the axial 1α-proton in a 1β-acetoxy-5α-cholestane,[13] but different in both shape and position from those given by the axial 11β-protons and the equatorial 12β-protons, respectively, in a series of 11α- and 12α-acetoxypregnane derivatives (Table 1).

We assign the secondary acetoxyl group to the equatorial 1β-position in 14α-digacetigenin (V; R = H), and support this assignment by the following evidence.

Whilst brief treatment of 14α-digacetigenin with methanolic hydrochloric acid at 20° hydrolyses the 1β-acetoxyl group without concomitant inversion at C-14, to afford deacetyl-14α-digacetigenin (IV; $R^1 = R^2 = R^3 = H$), extended treatment for 6 hours gave a mixture of products including deacetyl-14α-digacetigenin, deacetyl-14β-digacetigenin (VI; R = H), 14β-anhydrodigacetigenin (IX), and a reasonable yield of the 1-methyl-19-nor-pregna-1(10),5-diene (VII), $\lambda_{max.}$ 252 mμ (log ε 4·05), $\nu_{max.}$ 1745, 1688, 1610, and 1356 cm.$^{-1}$. The rearrangement product (VII) could not be induced to crystallise; it was also obtained by Tschesche *et al.*,[3] amorphous but homogeneous, $\lambda_{max.}$ 252 mμ (log ε 4·07), $\nu_{max.}$ 1742, 1687, 1600 cm.$^{-1}$, but was regarded as 3β,20α-dihydroxypregna-5,8-diene-11,15-dione formed by forced dehydration of the 9α-hydroxy-11,15-dione system present in Tschesche's formula (I; R = H) for deacetyldigacetigenin. The infrared bands at 1745 and 1688 cm.$^{-1}$ show that the 15- and 20-carbonyl groups are present unchanged, whilst the " O "-band at 1356 cm.$^{-1}$ confirms the presence of the 20-carbonyl and the adjacent 20-methyl group; in addition, the very intense band at 1610 cm.$^{-1}$ indicates the presence of a diene chromophore. In the n.m.r. spectrum of the diene (VII), the signal for a 10-methyl group is missing, whilst a new signal appears as a singlet of area for three protons at τ 7·71 corresponding to a methyl group attached to a conjugated diene system CMe:C·C:C; the signal for the 13-methyl group occurs with partial intensity at τ 8·98 and also at 8·47, indicating that the product (VII) is a mixture * of 14α- and 14β-isomers. Further column chromatography on alumina or on silica gel did not separate the isomers, and shortage of digacetigenin prevented further investigation. The configuration assigned to the 1-acetoxyl group appears to exclude a *trans*-diaxial *E*2 elimination mechanism and suggests that the rearrangement (V; R = H) ⟶ (VII) involves consecutive production of a 1-carbonium ion, migration of the 10-methyl group, and ejection of a proton. Concerted elimination of the equatorial 1β-acetoxyl with migration of the 5,10-bond would lead to a cisoid homoannular A-nor-B-homo-diene (XI), which according to the Woodward [14] rules should possess $\lambda_{max.}$ ~288 mμ (log ε <4·0).

Deacetyl-14α-digacetigenin (IV; $R^1 = R^2 = R^3 = H$) by mild oxidation with chromic acid in acetone [15,16] at 0° gave 17β-hydroxy-17α-pregn-4-ene-1,3,15,20-tetraone (VIII), also obtained, but in lower yield, by similar oxidation of 14α-digacetigenin (V; R = H). The infrared spectrum of the tetraketone (VIII) showed ring D to be intact ($\nu_{max.}$ 1742 cm.$^{-1}$, 15-carbonyl group), and the side-chain to be unaltered ($\nu_{max.}$ 1693 cm.$^{-1}$, 20-carbonyl group) with the " O "-band at 1365 cm.$^{-1}$ [20-methyl group in the system >C(OH)·COMe]; the

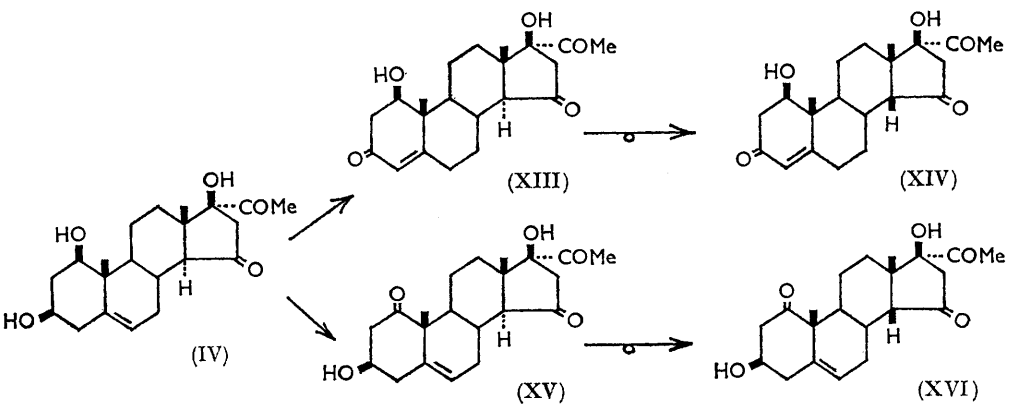
* A subsidiary signal at τ 8·63 may be due to the 13-methyl group of the 16,17-anhydro-derivative of (VII), present in traces.

[12] Shoppee and Bellas, unpublished results.
[13] Shoppee, Lack, and Havlecek, unpublished results.
[14] Woodward, *J. Amer. Chem. Soc.*, 1942, **64**, 72.
[15] Bowden, Heilbron, Jones, E. R. H., and Weedon, *J.*, 1946, 39.
[16] Djerassi, Engle, and Bowers, *J. Org. Chem.*, 1956, **21**, 1547.

spectrum also exhibited marked shoulders at 1720 and 1690 cm.$^{-1}$ (1-carbonyl group and 4-en-3-one system, respectively], and a band at 1613 cm.$^{-1}$ (4-ene). The tetraketone (VIII) exhibited properties typical of a β-diketone; it coupled with benzenediazonium chloride solution, and behaved as a quasi-acid. The ketonic form (VIII) ($\lambda_{max.}$ 242, 310 mμ; log ε 3·8, 3·15) was readily soluble in cold 2N-sodium hydroxide, to give the yellow enolic form (X), $\lambda_{max.}$ 256, 368 mμ (log ε 3·78, 4·03), from which the ketonic form was regenerated by treatment with ice-cold 2N-hydrochloric acid. The maximum observed at 368 mμ is in good agreement with the value of 362 mμ calculated for the enolic form (X) by the Woodward [14] rules.

Oppenauer oxidation of deacetyl-14α-digacetigenin (IV) (cf. ref. 3) gives 1β,17β-dihydroxy-14α,17α-pregn-4-ene-3,15,20-trione (XIII) ($\nu_{max.}$ 1748, 1692, 1667, 1616 cm.$^{-1}$; 15-one, 20-one, 4-en-3-one, and 4-ene), and its 14β-isomer (XIV) ($\nu_{max.}$ 1744, 1704, 1656, 1618 cm.$^{-1}$), previously formulated by Tschesche *et al.*[3] as (II) and (III), respectively. One non-crystalline fraction, which appeared to be homogeneous, had $\lambda_{max.}$ 242 mμ (log ε 4·1), $\nu_{max.}$ 1742, 1695, 1667, 1600 cm.$^{-1}$, and may consist of the 14α- and/or the 14β-1,4-diene-3,15,20-triones arising from dehydration of (XIII) and/or (XIV). The spirost-5-ene-1β,3β-diol ruscogenin [17-19] (XII) by Oppenauer oxidation gives a 4-en-1β-ol-3-one, converted by treatment with base,[17] or furnishing directly, after chromatography on silica,[18] a 1,4-diene-3-one, $\lambda_{max.}$ 245 mμ (log ε 4·18), $\nu_{max.}$ 1658, 1624, 1605 cm.$^{-1}$, whereas ruscogenin 1β-monoacetate yields [17] the 4-en-3-one 1β-monoacetate, $\nu_{max.}$ 1740, 1663, 1629 cm.$^{-1}$.

Oxidation of deacetyl-14α-digacetigenin (IV) with chromium trioxide in pyridine at 20° (cf. ref. 3) gives 3β,17β-dihydroxy-14α,17α-pregn-5-ene-1,15,20-trione (XV), $[\alpha]_D$ +143°, $\lambda_{max.}$ 283 mμ (log ε 1·7), $\nu_{max.}$ 1745, 1710, 1685, 1365 cm.$^{-1}$ [15-one, 1-one, 20-one, and " O "-



band], and its 14β-isomer (XVI), $[\alpha]_D$ +75°, $\lambda_{max.}$ 291 mμ (log ε 2·0), $\nu_{max.}$ 1743, 1718, 1696, 1361 cm.$^{-1}$. Tschesche *et al.*[3] obtained mainly the 14β-isomer (XVI), but we were able to isolate crystalline only the 14α-isomer (XV).

The n.m.r. spectra of 14α-digacetigenin (V; R = H) and its 3-monoacetate (V; R = Ac) show one further discernible signal for one proton appearing as a quartet at τ 6·85 (J_1 = 5, J_2 = 9 c./sec.) which is unchanged by increase of temperature from 40° to 60° and to 80°. Since this signal is not present in the spectrum of the 14β-isomer (VI; R = Ac), it appears to arise from the 14α-proton by long-range coupling with another proton in addition to coupling with the 8β-proton. Alternatively, it may be the signal for the allylic equatorial 7β-proton deshielded by the 15-carbonyl group; recently Djerassi and his co-workers [20] by

[17] Lapin and Sannie, *Bull. Soc. chim. France*, 1955, 1552, 1556; Lapin, *Compt. rend.*, 1957, **244**, 3065.
[18] Burn, Ellis, and Petrow, *Proc. Chem. Soc.*, 1957, 119; *J.*, 1958, 795.
[19] Benn, Colton, and Pappo, *J. Amer. Chem. Soc.*, 1957, **79**, 3920.
[20] Williams, Bhacca, and Djerassi, *J. Amer. Chem. Soc.*, 1963, **85**, 2810.

a study of 7-keto-steroids showed that a 7-carbonyl group induces a considerable paramagnetic shift in the position of the equatorial 15-proton.

Experimental

For general experimental directions, see *J.*, 1958; $[\alpha]_D$'s are for chloroform solutions. Ultraviolet absorption spectra were measured for ethanol solutions in a Perkin-Elmer model 4000A spectrophotometer, and infrared absorption spectra were determined on a Perkin-Elmer model 221 spectrophotometer. Nuclear magnetic resonance spectra were determined on a Varian D.P. 60 instrument at 60 Mc./sec. with deuterochloroform as solvent and tetramethylsilane as internal reference.

14α-*Digacetigenin* 3-*Acetate* (V; R = Ac).—Digacetigenin (m. p. 166—170°; 200 mg.) in pyridine (6 ml.) was treated with acetic anhydride (4 ml.) at 20° for 14 hr. Solvents were removed at reduced pressure, to give 14α-digacetigenin 3-acetate (205 mg.), m. p. 174° (from acetone), $\lambda_{max.}$ 288 mμ (log ε 1·7), $\nu_{max.}$ 3320, 1743, 1730—1735, 1692, 1365, and 1250 cm.$^{-1}$ (Found: C, 67·0; H, 7·7. Calc. for $C_{25}H_{34}O_7$: C, 67·25; H, 7·7%). Attempts to dehydrate 14α-digacetigenin 3-acetate with thionyl chloride in pyridine were unsuccessful.

Deacetyl-**14α-*digacetigenin*** (IV; $R^1 = R^2 = R^3 = H$).—14α-Digacetigenin (1 g.) in methanol (300 ml.) was treated with potassium hydrogen carbonate (1 g.) in water (50 ml.) at 20° for 18 hr. Evaporation of the solvent gave deacetyl-14α-digacetigenin (795 mg.), m. p. 278—281° (from acetone), $[\alpha]_D$ +63° (*c* 0·5), $\lambda_{max.}$ 285 mμ (log ε 1·71), $\nu_{max.}$ (Nujol) 1745, 1680, 1360 cm.$^{-1}$ (Found: C, 69·65; H, 8·3. Calc. for $C_{21}H_{30}O_5$: C, 69·6; H, 8·3%). Optical rotatory dispersion (in MeOH); [*M*] +1920 (315 mμ, peak), −600 (265 mμ, shortest wavelength measured); $10^{-2}a$ +25.

Deacetyl-**14β-*digacetigenin*** (VI; R = H).—14α-Digacetigenin (200 mg.) was treated with 0·05N-methanolic potassium hydroxide (4 ml.) at 20° for 12 hr. Removal of the methanol at reduced pressure, extraction with chloroform, evaporation, and recrystallisation from acetone gave deacetyl-14β-digacetigenin (170 mg.), m. p. 247—252°, $[\alpha]_D$ −45° (*c* 1·05), $\lambda_{max.}$ 280 mμ (log ε 1·85), $\nu_{max.}$ (Nujol) 1738, 1695, 1360 cm.$^{-1}$ (Found: C, 69·3; H, 8·5. Calc. for $C_{21}H_{30}O_5$: C, 69·6; H, 8·3%). This material was too insoluble in the usual solvents to furnish a satisfactory n.m.r. spectrum.

14β-*Digacetigenin* 3-*Acetate* (VI; R = Ac).—Deacetyl-14β-digacetigenin (100 mg.) in pyridine (6 ml.) was treated with acetic anhydride (4 ml.) at 20° for 14 hr. Evaporation in a vacuum gave 14β-digacetigenin 3-acetate (85 mg.), m. p. 239—242° (from ether–pentane), $\lambda_{max.}$ 282 mμ (log ε 1·7), $\nu_{max.}$ 1742, 1735—1728, 1695, and 1365 cm.$^{-1}$. The n.m.r. spectrum is recorded in Table 1.

Anhydro-**14β-*digacetigenin*** (IX).—14α-Digacetigenin (100 mg.) was treated with 0·5N-methanolic potassium hydroxide (20 ml.) at 65° for 6 hr. Evaporation of the methanol and extraction with chloroform gave an oil (90 mg.), which crystallised on addition of ether, to afford *anhydro*-14β-*digacetigenin*, m. p. 220—223°, $\lambda_{max.}$ 242 mμ (log ε 4·1), $\nu_{max.}$ 3610, 3510, 1706, 1685, 1618, and 1365 cm.$^{-1}$ (Found: C, 73·2; H, 8·1. $C_{21}H_{28}O_4$ requires C, 73·2; H, 8·2%). This material was too insoluble in the usual solvents to give a satisfactory n.m.r. spectrum.

The mother-liquors gave an oil, which could not be purified by chromatography on alumina or silica gel, and which was shown by thin-layer chromatography to be a mixture of several compounds.

Treatment of **14α-*Digacetigenin* with *Methanolic Hydrogen Chloride***.—(*a*) 14α-Digacetigenin (100 mg.) in methanol (50 ml.) was treated with 16N-hydrochloric acid (8 ml.) at 65° for 30 min. The usual working up gave deacetyl-14α-digacetigenin, m. p. and mixed m. p. 278—280° (from acetone–ether).

(*b*) 14α-Digacetigenin (200 mg.) in methanol (100 ml.) was treated with 16N-hydrochloric acid (16 ml.) at 65° for 6 hr. The usual working up gave an oil (185 mg.) which was chromatographed on neutral alumina (4 g.) prepared in benzene. Elution with ether–benzene gave **3β,17β-*dihydroxy*-1-*methyl*-19-*norpregna*-1(10),5-*diene*-15,20-*dione*** (VII) (65 mg.), m. p. 125—135°, $\lambda_{max.}$ 252 mμ (log ε 4·05), $\nu_{max.}$ 1745, 1688, 1610, 1356, with shoulders at 1780 and 1710 cm.$^{-1}$. This product (VII) could not be induced to crystallise, and was unaltered by re-chromatography on either silica gel or alumina. The n.m.r. spectrum (Table 1) suggests that it is a mixture of

the 14α- and 14β-isomers of (VII) (Found: C, 73·4; H, 8·3. $C_{21}H_{28}O_4$ requires C, 73·2; H, 8·2%).

Further elution with chloroform–ether gave oils, which crystallised on addition of ether, to give anhydro-14β-digacetigenin (IX), m. p. and mixed m. p. 220—223°, $\lambda_{max.}$ 242 mμ (log ε 4·0). Further elution with chloroform gave an oil, $\lambda_{max.}$ 285 mμ (log ε 1·85), $\nu_{max.}$ 1745 and 1695 cm.$^{-1}$, which is thought to be a mixture of (IV; $R^1 = R^2 = R^3 = H$) and (VI; R = H).

17β-*Hydroxy*-17α-*pregn*-4-*ene*-1,3,15,20-*tetraone* (VIII).—(*a*) Deacetyl-14α-digacetigenin (IV; $R^1 = R^2 = R^3 = H$) (50 mg.) in acetone (3 ml.) was titrated with Jones's reagent [15,16] [a solution of chromium trioxide (26·7 g.) in concentrated sulphuric acid (23 ml.) diluted with water to 100 ml.] under nitrogen at 0° during 10 min. The product was extracted with chloroform, and, in order to convert 5-en-3-one into 4-en-3-one, the extract was not washed with sodium hydrogen carbonate, but was evaporated, to give 17β-*hydroxy*-17α-*pregn*-4-*ene*-1,3,15,20-*tetraone* (VIII) (42 mg.), m. p. 190—193° (from ether–acetone), $\lambda_{max.}$ 243, 310 mμ (log ε 3·8, 3·15) [after the addition of 2 drops of 2N-sodium hydroxide, $\lambda_{max.}$ 256, 368 mμ (log ε 3·78, 4·03)], $\nu_{max.}$ 1742, 1693, 1365, 1613, with shoulders at 1720 and 1690 cm.$^{-1}$ (Found: C, 70·2; H, 7·2. $C_{21}H_{26}O_5$ requires C, 70·35; H, 7·3%). This triketone (VIII) dissolved in alkali to give the yellow enol form (X), which gave a red dye when coupled with diazotized *p*-nitroaniline.

(*b*) Digacetigenin (V; R = H) (50 mg.) in acetone (3 ml.) was titrated with Jones's reagent [15,16] under nitrogen during 15 min. The crude product, isolated as in (*a*), was dissolved in a minimum of chloroform and the solution poured on to an alumina column. A yellow band formed at the top of the column. Elution with ether, chloroform, and methanol gave only traces of unidentified oils, but elution with acetic acid gave a crude solid (35 mg.). This was an inorganic salt of the enol (X), and by acidification with 2N-hydrochloric acid and extraction with chloroform gave 17β-hydroxy-17α-pregn-4-ene-1,3,15,20-tetraone (VIII) identical with the sample obtained in (*a*).

Oxidation of Deacetyl-14α-*digacetigenin* (IV; $R^1 = R^2 = R^3 = H$).—(*a*) *With aluminium isopropoxide–acetone.* Deacetyl-14α-digacetigenin (63 mg.) in acetone–benzene (1 : 1; 20 ml.) was treated with aluminium isopropoxide (300 mg.) in benzene (10 ml.) at 85° for 22 hr. The product was chromatographed on alumina (2 g.); elution with ether and chloroform–ether gave fractions which had spectral characteristics corresponding to the triketones (XIII) and (XIV). Material eluted with chloroform–ether (1 : 1) (9 mg.) appeared to be homogeneous and had spectral properties consistent with 17β-hydroxy-17α-pregna-1,4-diene-3,15,20-trione, $\lambda_{max.}$ 242 mμ (log ε 4·1), $\nu_{max.}$ 1742, 1695, 1667, and 1600 cm.$^{-1}$.

(*b*) *With chromium trioxide–pyridine.* Deacetyl-14α-digacetigenin (150 mg.) in pyridine (6 ml.) was added to a suspension of chromium trioxide (300 mg.) in pyridine (12 ml.) and left at 20° for 18 hr. The usual isolation procedure gave a solid, which was chromatographed on neutral alumina (4 g.) prepared in benzene. Elution with ether–benzene (1 : 1) gave 3β,17β-dihydroxy-14α,17α-pregn-5-ene-1,15,20-trione (XV) (30 mg.), m. p. 265—270° (lit.,[3] 259—264°), $[\alpha]_D$ +150° (*c* 0·9), $\lambda_{max.}$ 283 mμ (log ε 1·7), $\nu_{max.}$ 1745, 1710, 1685, and 1365 cm.$^{-1}$ (Found: C, 69·9; H, 8·0. Calc. for $C_{21}H_{28}O_5$: C, 70·0; H, 7·8%). Further elution with chloroform gave unchanged deacetyl-14α-digacetigenin (100 mg.), m. p. and mixed m. p. 180—181°.

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Department of Organic Chemistry, The University of Sydney,
Australia. [*Received, November 5th*, 1963.]