Synthesis of 3β,14-Dihydroxy-5β,14β-pregnan-20-one C-3 Derivatives: Ozonolysis of Digitoxin and Digitoxigenin and their Derivatives followed by Zinc–Acetic Acid Reduction to the C-21 Methyl Ketone

John F. Templeton,*[a] Yangzhi Ling,[a] Jichun Jin,[a] Mark A. Boehmer,[a] Talal H. Zeglam[a] and Frank S. LaBella[b]

[a] *Faculty of Pharmacy, University of Manitoba, Winnipeg, Manitoba, Canada R3T 2N2*

[b] *Department of Pharmacology and Therapeutics, Faculty of Medicine, Winnipeg, Manitoba, Canada R3E 0W3*

Ozonolysis of digitoxin, digitoxin tetraacetate, digitoxin tetrakis-(2,2,2-trichloroethyl carbonate), digitoxigenin, digitoxigenin acetate, digitoxigenin hemisuccinate and digitoxigenin 2,2,2-trichloroethyl carbonate followed by treatment with excess of zinc in acetic acid gave either the corresponding 21-hydroxy ester after 5 min or the 21-methyl ketone after 20 h. This procedure is more efficient than methods previously reported for the conversion of the α,β-unsaturated γ-lactone into an acetyl group and is applicable to the cardiac glycosides directly to give 14β-hydroxypregnan-20-one derivatives. Acidic hydrolysis of 14,21-dihydroxy- and 14-hydroxy-3β-tris(digitoxosyloxy)-5β,14β-pregnan-20-one is reported. Structures are based on ^1H and ^{13}C NMR assignments.

Conversion of the α,β-unsaturated γ-lactone in the cardiac glycosides to the pregnan-20-one was required as part of a study[1–3] on the interaction of pregnanes and related steroids with the digitalis receptor of heart muscle. In particular, synthesis of 3β,14-dihydroxy-5β,14β-pregnan-20-one **14** was necessary for 3β-glycosidation and derivatization at C-20. Previous preparation of compound **14** from digitoxigenin acetate **5** was unsatisfactory because of the difficulty of hydrolysis of the 3β-acetate without incurring concomitant epimerization at C-17 in alkaline media,[1] or dehydration of the C-14 hydroxy function in acidic media (see below). Ozonolysis of the unsaturated lactone moiety in cardiac glycosides and conversion into the 21-hydroxy ketone is well established,[4–6] as is removal of the 21-hydroxy group through the multistep sequence of tosylation, iodine substitution and reduction with zinc–acetic acid[6] or sodium hydrogen sulphite.[7] This procedure to prepare the 21-methyl ketone requires protection of the glycoside alcohols, which is not necessary when using the zinc–acetic acid treatment of the ozonide.

We report here on the ozonolysis and zinc–acetic acid treatment of digitoxigenin (Scheme 1) and digitoxin (Scheme 2) and their derivatives. Acidic hydrolysis of the C-3 trisdigitoxosides **23** and **26** which results in concomitant dehydration of the 14-alcohol is also described (Scheme 2).

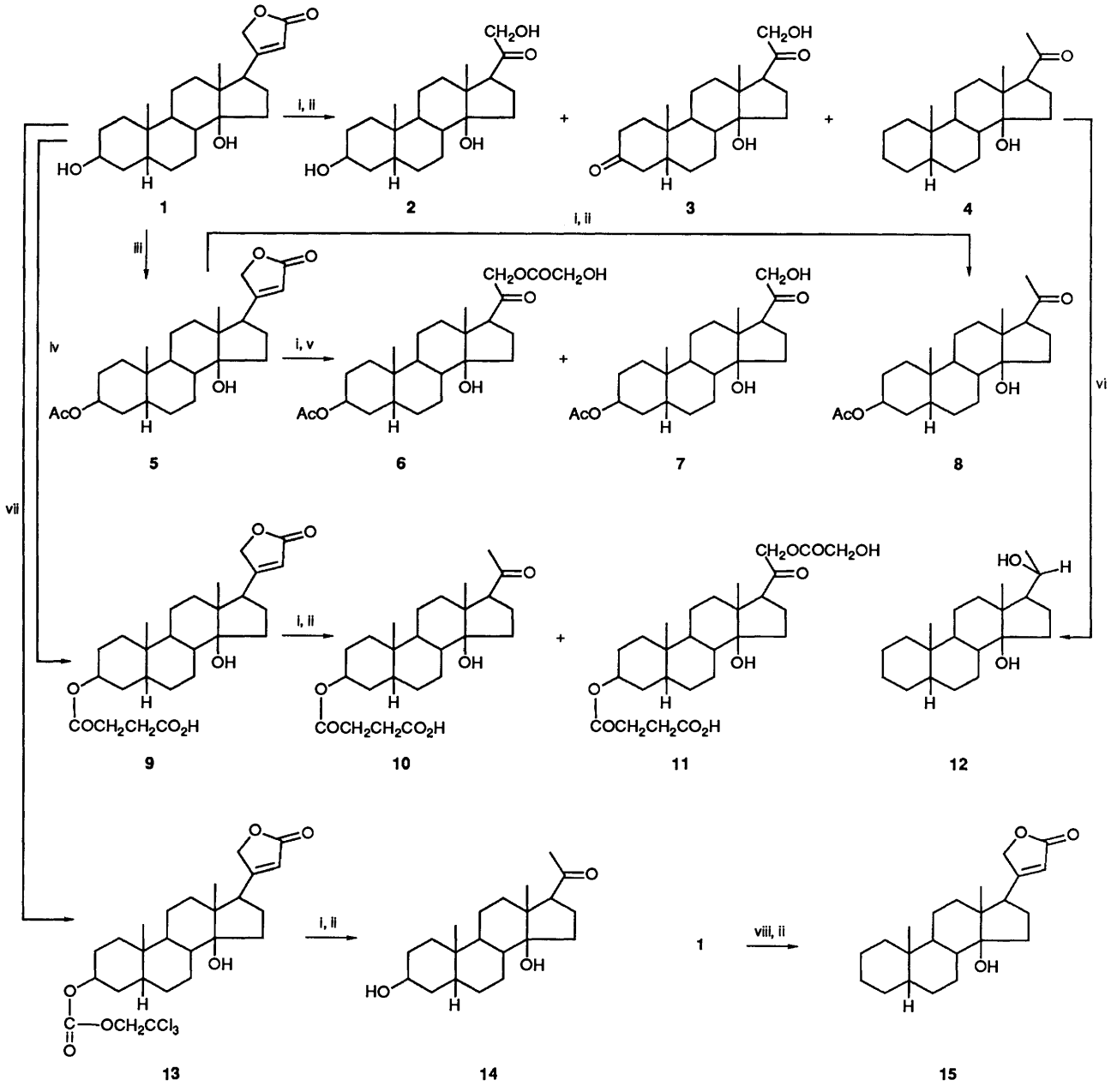
Results and Discussion

When digitoxigenin **1** was treated with ozone and zinc–acetic acid, three products were produced; namely, the 3β,21-dihydroxy ketone **2**, 21-hydroxy diketone **3** and 3-deoxy methyl ketone **4**. A low yield of compound **2** has been reported from this reaction when one mol equiv. of ozone was used.[8] Ozone can oxidize secondary alcohols[9] thereby accounting for the presence of product **3** which, after Clemmenson-type reduction with zinc–acetic acid, loses the 3-keto group together with reductive removal of the C-21 oxygen function (see below) to afford ketone **4**. Lithium tri-t-butoxyaluminium hydride (LTBAH) reduction of compound **4** gave the 20β-alcohol **12**. 3-Deoxydigitoxigenin **15** was prepared by Jones oxidation of digitoxigenin **1** followed by treatment with zinc–acetic acid.

Three products were obtained from ozonolysis and zinc–acetic acid treatment of digitoxigenin acetate **5**. When zinc–acetic acid treatment was carried out for 5–15 min the 21-hydroxy ester **6** was obtained as the major product and the 21-hydroxy ketone **7** as a minor product, whereas treatment for 20 h yielded mainly the methyl ketone **8**. Two products were obtained from ozonolysis of digitoxigenin hemisuccinate **9** followed by treatment with zinc–acetic acid. The major product was the methyl ketone hemisuccinate **10** and the minor product was the 21-hydroxy ester hemisuccinate **11**. Incomplete reduction of the C-21 oxygen function may have resulted from precipitation of the zinc succinate salt. The most efficient procedure to prepare ketone **14** was from ozonolysis of digitoxigenin 2,2,2-trichloroethyl carbonate **13**, since treatment with zinc–acetic acid, besides causing reduction of the oxygen function at C-21, also removed the trichloroethyl carbonate group in good yield.

Ozonolysis of digitoxin **20** in methanol followed by treatment with excess of zinc and acetic acid for 5 min gave the 21-hydroxy ester **25** whereas longer treatment (20 h) resulted in further reduction to the ketone **23**. Mild alkaline hydrolysis of ester **25** gave the 21-hydroxy ketone **26** and acetylation gave compound **28**. Similar ozonolysis and zinc–acetic acid treatment (for 20 h) of digitoxin tetraacetate **16** and digitoxin tetrakis-(2,2,2-trichloroethyl carbonate) **22** gave the corresponding methyl ketones **21** and **23**, respectively, as the major products. Ozonolysis of compound **16** followed by shorter treatment with zinc–acetic acid gave the 21-hydroxy ester **17**, which was selectively hydrolysed at C-21 with $KHCO_3$ to give the 21-hydroxy ketone **18**, which in turn on acetylation gave the 21-acetoxy ketone **19**; compound **19** was also prepared by acetylation of the acyloin **26**. When the 21-hydroxy ester **25** was treated with zinc–acetic acid for 20 h it was converted into the methyl ketone **23** whereas similar treatment of the 21-hydroxy ketone **26** did not yield the methyl ketone **23**. The 21-acetoxy ketone **19** was unchanged after treatment with zinc–acetic acid for 20 h.

Acidic hydrolysis of the methyl ketone **23** and the 21-hydroxy ketone **26** under the conditions used for the hydrolysis of digitoxin resulted in extensive dehydration of the C-14 alcohol to give 1,4-enones **24** and **27** as well as the expected aglycones **14** and **2**. No dehydration was observed in the ^1H NMR spectrum of the crude product obtained after similar treatment of digitoxin. This difference may result from conformational changes to ring D and electronic assistance induced by



Scheme 1 *Reagents and conditions:* i, O_3–MeOH or –CH_2Cl_2–EtOAc, −50 to −70 °C; ii, Zn–HOAc, 20 h; iii, Ac_2O–pyridine; iv, succinic anhydride–DMAP–dioxane; v, Zn–HOAc, 5–15 min; vi, LTBAH; vii, CCl_3CH_2OCOCl–pyridine; viii, Jones oxidation

intramolecular hydrogen-bonding between the C-14 hydroxy group and the C-20 carbonyl. Dehydration accompanying hydrolysis makes this route unsatisfactory for the preparation of compound **14**.

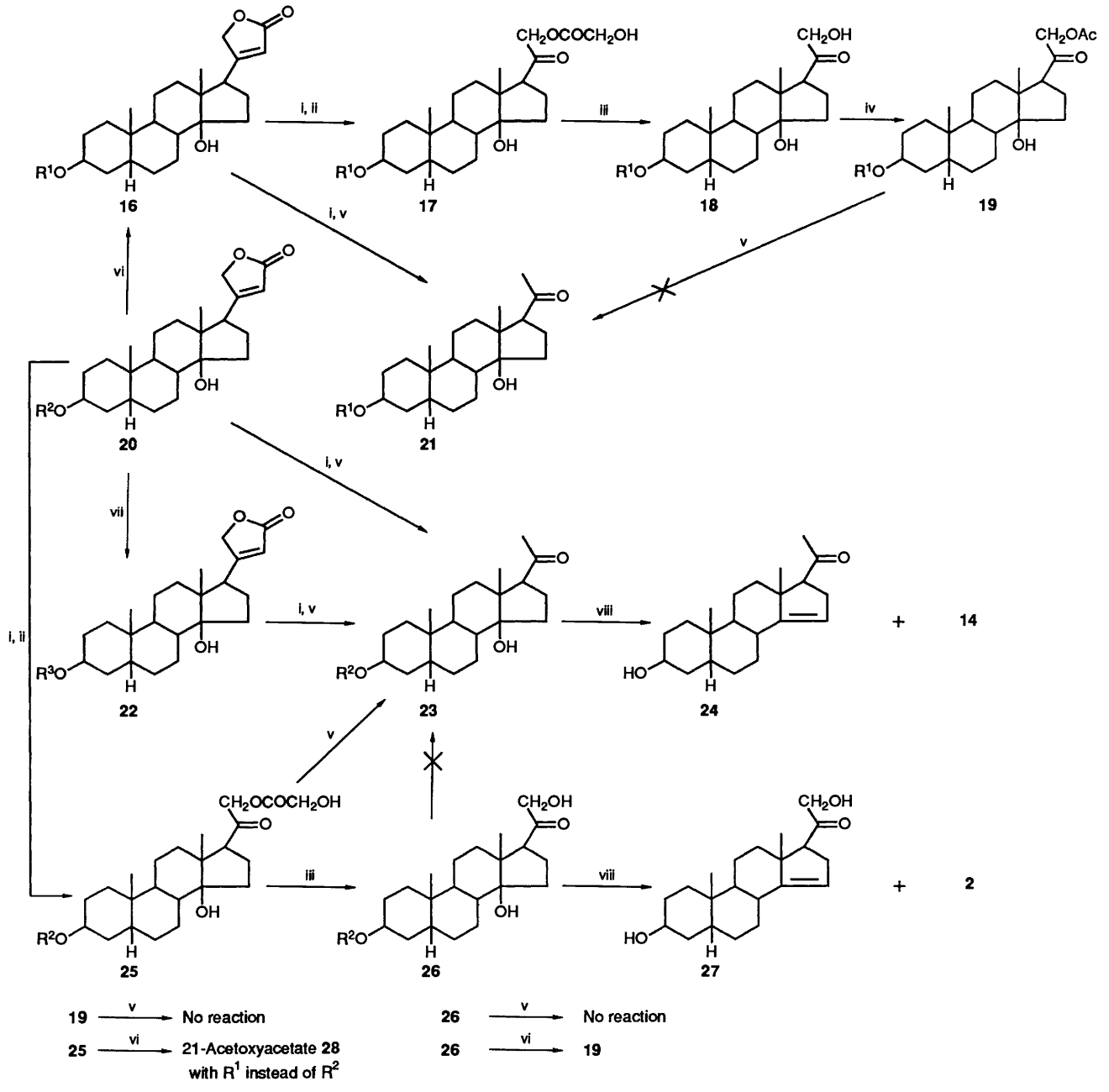
Ozonolysis of the unsaturated lactone, after initial formation of the ozonide and reductive work-up with zinc–acetic acid, affords the 21-hydroxy ester[4] from the intermediate glyoxaldehyde[5,8] and the 21-hydroxy ketone (see Scheme 3) is also formed. Ozonolysis in methanol may involve a hydroperoxide intermediate[10] not formed when dichloromethane and/or ethyl acetate is used as the solvent but gives the same products after zinc treatment. Longer treatment with zinc–acetic acid eliminates the 21-hydroxyacetoxy group to yield the methyl ketone. The 21-hydroxy ester appears to be the principal intermediate in the formation of the methyl ketone, whereas the 21-hydroxy ketone is not significantly converted into the methyl ketone.

Structures were established by ^1H NMR (Table 1) and ^{13}C NMR (Table 2) spectral analysis. ^{13}C NMR assignments are based on published data,[11] polarization transfer[12] and internal consistency.

Experimental

^1H and ^{13}C NMR spectra are reported in Tables 1 and 2. Preparations were monitored by TLC on silica gel (Merck type 60 H) in 25–75% ethyl acetate–hexane (genins) or 10% methanol–dichloromethane (glycosides) and spots were visualized with a UV lamp where appropriate and by dipping in 4% aq. sulphuric acid followed by heating. Zinc powder (Baker or Mallinckrodt) was used. Column chromatography was carried out on silica gel (Merck type 60 for column chromatography) by flash chromatography unless otherwise stated. M.p.s were measured on a Kofler hot-stage apparatus and are uncorrected. Elemental analysis were performed by Mr. W. Baldeo, School of Pharmacy, University of London, England.

Digitoxigenin **1**.—Digitoxin **20** (10 g) dissolved in 0.05 mol



Scheme 2 *Reagents and conditions:* i, O_3–MeOH or –CH_2Cl_2–EtOAc, −50 to −70 °C; ii, Zn–HOAc, 5 min; iii, $KHCO_3$–MeOH, 70 min, 5 °C; iv, Ac_2O–pyridine; v, Zn–HOAc, 20 h; vi, Ac_2O, reflux 1.5 h; vii, CCl_3CH_2OCOCl–pyridine; viii, 0.05 mol dm^{-3} H_2SO_4–MeOH, 4 h

dm^{-3} methanolic sulphuric acid (1 dm^3) at 20 °C for 4 h gave, after adjusting to pH 5–6 with saturated aq. $NaHCO_3$ (*ca.* 80 cm^3), concentration, filtration and recyrstallization from 50% aq. ethanol, digitoxigenin **1** (4 g), m.p. 246–251 °C, (lit.,[13] 253 °C).

Ozonolysis and Zinc–Acetic Acid Treatment of Digitoxigenin **1**.—A solution of digitoxigenin **1** (400 mg) in 20% ethyl acetate–dichloromethane (125 cm^3) was treated with ozone, zinc (15 g) and acetic acid (10 cm^3) as described for digitoxin **20** to give, after elution (10% ethyl acetate–hexane), the least polar fractions (116 mg) of the 20-*ketone* **4** (36 mg), m.p. 153–154 °C (from acetone–hexane) (Found: C, 78.8; H, 11.0. $C_{21}H_{34}O_2$ requires C, 79.2; H, 10.8%). The two more polar components were isolated from a separate reaction with digitoxigenin **1** (100 mg) in which zinc (0.5 g) and acetic acid (5 cm^3) were used. Elution with 1% methanol–ethyl acetate gave the 21-hydroxy ketone **3** (29 mg), m.p. 170–187 °C (from acetone–hexane) (lit.,[8] 170–194 °C). Elution with 2.5% methanol–ethyl acetate yielded fractions of the triol **2** (18 mg), which on recrystallization gave pure compound **2** (9 mg), m.p. 98–100 °C resolidifies and melts 148–154 °C (from acetone–hexane) (lit.,[8] 104–110 and 148–154 °C).

Ozonolysis and Zinc–Acetic Acid Treatment of Digitoxigenin Acetate **5**.—(a) *With zinc–acetic acid for* 5 *min.* Treatment of digitoxigenin acetate **5**[14] (0.5 g) in methanol (100 cm^3) with ozone followed by zinc–acetic acid as described for digitoxin **20** gave on elution (35% ethyl acetate–hexane) the 21-*hydroxy ester* **6** (366 mg), m.p. 137–138 °C (from MeOH) (Found: C, 66.4; H, 8.5. $C_{25}H_{38}O_7$ requires C, 66.6; H, 8.5%) and the 3β-acetoxy-21-hydroxy ketone **7** (43 mg), m.p. 147–149 °C (from ethyl acetate–cyclohexane) (lit.,[8] 146–148 °C).

(b) *With zinc–acetic acid for* 20 *h.* Ozone was passed through a solution of digitoxigenin acetate **5** (5.2 g) in dichloromethane

Table 1 Chemical shifts (J in Hz)[a]

Compound	10-Me	13-Me	3-H[b]	17-H	Other
2	0.97	0.92	4.12	2.73dd (J 4.2, 9.6)	3.04t (J 4.8) (21-OH), 4.08s (14-OH), 4.24t, 4.33t (J_{AX} 4.8) (J_{AB} 20) (21-H_2)
3	1.01	0.93		2.75dd (J 3.9, 9.4)	2.33ddd (J 5.4, 14.5, 20.0), (2α-H); 2.64dd (J 13.9, 14.6) (4α-H), 4.25d, 4.33d (J_{AB} 20.2) (21-H_2)
4	0.92	0.97		2.90dd (J 4.4, 9.3)	2.23s (20-Me), 4.31s (14-OH)
6	0.94	0.94	5.04	2.77dd (J 4.4, 9.4)	2.02s (3-OAc), 2.88t (J 5.9) (CH_2OH), 3.91s (14-OH), 4.29d, (J 5.7) (CH_2OH), 4.76d, 4.86d (J_{AB} 17.5) (21-H_2)
7	0.96	0.92	5.07	2.72dd (J 4.1, 9.4)	2.04s (3-OAc), 3.10s (21-OH), 4.10s, (14-OH), 4.24d, 4.32d, (J_{AB} 21) (21-H_2)
8	0.97	0.97	5.08	2.90dd (J 4.3, 9.2)	2.05s (3-OAc), 2.23s (20-Me), 4.35d (14-OH)
9	0.97	0.88	5.13	2.79m	2.67m ($COCH_2CH_2CO$), 4.80d, 4.98d, (J_{AB} 18.0)[c] (21-H_2), 5.88s (22-H)
10	0.97[d]	0.96[d]	5.11	2.90dd (J 4.1, 9.1)	2.23s (20-Me), 2.65m ($COCH_2CH_2CO$)
11	0.97[d]	0.96[d]	5.12	2.79dd (J 4.4, 9.5)	2.66m ($COCH_2CH_2CO$), 4.32s (CH_2OH), 4.79d, 4.89d (J_{AB} 17.5) ($COCH_2O$)
12	0.92	1.18			1.26dd (J 6.6) (20-Me), 3.84m, (20-H)
13	0.97	0.87	5.02	2.77dd (J 4.0, 9.4)	4.70d, 4.78d (J_{AB} 11.9) (CCl_3CH_2O), 4.79d, 4.98d (J_{AB} 18.6) (21-H_2) 5.86s (22-H)
15	0.91	0.86		2.77dd (J 5.8, 9.0)	4.83d, 5.00d (J_{AB} 18.1)[c] (21-H_2), 5.86s (22-H)
16[e]	0.92	0.86	3.99	2.76m	4.79d, 4.98d (J_{AB} 18.1)[c] (21-H_2), 5.86s (22-H)
17[e]	0.92	0.96	4.00	2.78dd (J 4.4, 9.4)	4.32s ($OCOCH_2OH$), 4.77d, 4.88d (J_{AB} 17.5) (21-H_2)
18[e]	0.90[d]	0.92[d]	4.00	2.72dd (J 3.8, 9.5)	3.07s (21-OH), 4.06s (14-OH), 4.28m (21-H_2)
19[e]	0.92	0.95	4.00	2.78dd (J 4.2, 9.3)	2.17s (21-OAc), 4.03 (14-OH), 4.64d, 4.76d (J_{AB} 17.6) (21-H_2)
21[e]	0.92	0.95	4.00	2.89dd (J 4.3, 9.1)	2.22s (20-Me)
22	0.91	0.87		2.78m	1.24m (6′-, 6″-, 6‴-H), 3.34dd (J 3.0, 9.5) (4′-, 4″-H), 3.88m, 4.00m (5′, 5″, 5″-H, 3-H), 4.44dd (J 3.0, 9.9) (4‴-H), 4.78m (3′-, 3″-, 3‴-H, 21-H_2, CCl_3CH_2O), 5.38m, 5.44m (1′-, 1″-, 1‴-H), 5.87m (22-H)
23[f]	0.95[d]	0.96[d]	4.03	2.95dd (J 4.3, 9.4)	2.26s (20-Me)
24	0.98	0.86	4.11	2.94dd (J 8.0, 9.9)	2.16s (20-Me), 2.78m, 5.15m (15-H)
25[f]	0.95[d]	0.96[d]		2.87dd (J 4.4, 9.4)	4.26m (3′-, 3″-H, CH_2OH), 4.91m (1′-, 1″-, 1‴-H, 21-H_2)
26[f]	0.93[d]	0.95[d]	4.04	2.85dd (J 3.9, 9.8)	4.27d, 4.34d (J_{AB} 19.6) (21-H_2)
27	0.96	0.83	4.09	2.87t (J 10)	3.30m (21-OH), 2.81m (3-OH), 4.16d, 4.25d (J_{AB} 20) (21-H_2), 5.15m (15-H)
28	0.92	0.94	4.00	2.79dd (J 4.3, 9.3)	2.16s (21-CH_2OCOCH_2OAc), 4.73s (21-CH_2OCOCH_2OAc), 4.73d, 4.83d (J_{AB} 17.4) (21-H_2)

[a] For solutions in $CDCl_3$, except **20**, **23**, **24** and **26**, which are in $CDCl_3$–CD_3OD (1:1) ($SiMe_4$ internal standard) on a Bruker AM300 instrument. [b] s,$w_{\frac{1}{2}}$ 8 Hz. [c] Allylic coupling J 1.5. [d] Signals are interchangeable within the line. [e] Trisdigitoxoside tetraacetate spectra (**16**–**19**, **21**) are in agreement with digitoxin tetraacetate **16**, 1.98s, 2.09s (3′-, 3″-, 3‴-, 4‴-OAc), 1.13d (J 6.2), 1.20d (J 6.4), 1.23d (J 6.5, 6′-, 6″-, 6‴-Me), 3.27dd (J 3.1), 9.8, 3.30dd (J 3.1, 9.8, (4′-, 4″-H), 3.83m (5′-, 5″-, 5‴-H), 4.51dd (J 2.9, 9.9) (4‴-H), 4.69dd (J 1.8, 11.0), 4.72dd (J 1.8, 1.09), 4.78dd (J 2.1, 9.4 (3′-, 3″-, 3‴-H), 5.38m (1′-, 1″-, 1‴-H). [f] Trisdigitoxoside spectra **23**, **25**, **26** are in agreement with digitoxin **20**; 1.23d, 1.24d, 1.27d (J 6.2, 6′-, 6″-, 6‴-Me), 3.22m (4′-, 4″-, 4‴-H), 3.83m (5′-, 5″-, 5‴-H), 4.05m (3‴-H), 4.26m (3′-, 3″-H), 4.91m (1′-, 1″-, 1‴-H).

(750 cm^3), zinc (400 g) and acetic acid (500 cm^3) were added, and the mixture was concentrated to remove dichloromethane, shaken for 20 h, and worked up as described for compound **20** to give the methyl ketone **8** (2.6 g), m.p. 145–148 °C (from acetone–hexane) (lit.,[15] 148–152 °C).

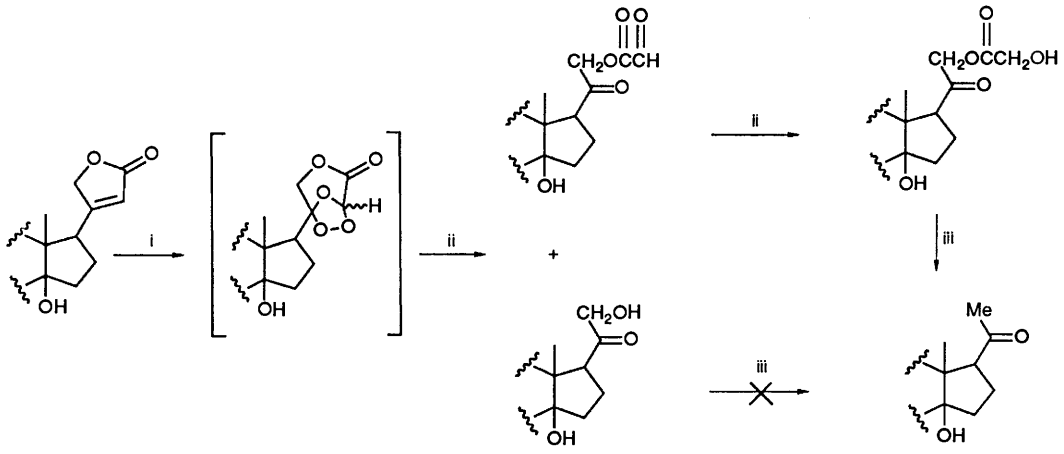
Digitoxigenin Hemisuccinate **9**.—A solution of digitoxigenin **1** (93.6 mg) in benzene (20 cm^3) was evaporated to dryness and the residue was heated to reflux with succinic anhydride (207 mg) and 4-(dimethylamino)pyridine (DMAP) (253 mg) in 1,4-dioxane (3 cm^3) for 1.5 h. The reaction mixture was diluted with water and filtered. Recrystallization gave the hemisuccinate **9** (95.8 mg), m.p. 224–230 °C (from EtOAc) (lit.,[16] 233–236 °C).

Ozonolysis and Zinc–Acetic Acid Treatment of Digitoxigenin Hemisuccinate **9**.—A solution of the hemisuccinate **9** (2.8 g) in a mixture of acetone–dichloromethane–ethyl acetate (1:1:1; 240 cm^3) was treated with ozone as described for compound **20**, zinc (10 g) and acetic acid (25 cm^3) were added, and the mixture was stirred for 1.5 h at −60 °C. After filtration and evaporation, further zinc (150 g) and acetic acid (150 cm^3) were added and the mixture was shaken for 48 h to give two major components on TLC [ethyl acetate–hexane–acetic acid (9:9:1)]. After filtration, dry column chromatography gave on elution (15–25% ethyl acetate–hexane) 3β,14-*dihydroxy*-5β,14β-*pregnan*-20-*one* 3-(*hydrogen succinate*) **10** (1.2 g), m.p. 156–160 °C (from EtOAc) (Found: C, 68.9; H, 8.8. $C_{25}H_{38}O_6$ requires C, 69.1; H, 8.8%). Further elution (EtOAc) gave 3β,14,21-*trihydroxy*-5β,14β-*pregnan*-20-*one* 3-(*hydrogen succinate*) 21-(*hydroxyacetate*) **11** (265 mg), m.p. 176–180 °C (from acetone) (Found: C, 63.5; H, 7.9. $C_{27}H_{40}O_9$ requires C, 63.8; H, 7.9%).

5β,14β-*Pregnane*-14,20β-*diol* **12**.—The 20-ketone **4** (78 mg) and LTBAH (254 mg) were dissolved in dry diethyl ether and the solution was stirred at room temperature for 1 h. Acetone was added followed by acidification with dil. hydrochloric acid and extraction (Et_2O) to give the 14,20β-*diol* **12** (43 mg), m.p. 174–175 °C (from dichloromethane–acetone) (Found: C, 78.9; H, 11.4. $C_{21}H_{36}O_2$ requires C, 78.7; H, 11.3%).

Digitoxigenin 2,2,2-*Trichloroethyl Carbonate* **13**.—To a stirred, cooled mixture of digitoxigenin **1** (75 mg) in pyridine (1 cm^3) was added dropwise 2,2,2-trichloroethyl chloroformate (150 mg). After 18 h at room temperature the mixture was poured into ice–water and extracted with dichloromethane; the extract was washed successively with water, 1 mol dm^{-3} HCl, and aq. $NaHCO_3$, to give, on elution with 2% methanol–dichloromethane, the *carbonate* (63 mg), m.p. 211–213 °C (from aq. MeOH) (Found: C, 57.05; H, 6.2; Cl, 19.2. $C_{26}H_{35}Cl_3O_6$ requires C, 57.1; H, 5.9; Cl, 19.45%).

Ozonolysis and Zinc–Acetic Acid Treatment of Digitoxigenin 2,2,2-*Trichloroethyl Carbonate* **13**.—Digitoxigenin **1** (150 mg) was treated with 2,2,2-trichloroethyl chloroformate as for digitoxin **20** and the product, compound **13**, was dissolved in dichloromethane (30 cm^3) and treated with ozone as described for compound **20**. Zinc (15 g) and acetic acid (15 cm^3) were added and the mixture was shaken for 18 h. The filtrate was



Scheme 3 *Reagents and conditions:* i, O_3, −50 to −70 °C; ii, Zn–HOAc, 5 min; iii, Zn–HOAc, 20 h

worked up as described for digitoxin **20** and the residue gave, on elution (40% ethyl acetate–hexane), the 3β-hydroxy methyl ketone **14** (78 mg), m.p. 95–100 °C resolidifies and melts 151–153 °C (lit.,[1] 154–156 °C).

3-*Deoxydigitoxigenin* **15**.—A solution of digitoxigenin **1** (472 mg) in acetone (94 cm^3) was treated with excess of Jones reagent for 5 min to give the crude ketone, which was dissolved in acetic acid (10 cm^3) and zinc (7.5 g) was added. After the mixture had been stirred vigorously overnight, diethyl ether was added, the mixture was filtered and the filtrate was thoroughly washed with aq. $NaHCO_3$ to give the product (451 mg), which was chromatographed over silica in dichloromethane to yield fractions (275 mg) which, on recrystallization, gave the title compound **15** (56 mg), m.p. 183–185 °C (from dichloromethane–hexane) (lit.,[17] 165–172 °C;[18] 178.5–180.5 °C).

Ozonolysis and Zinc–Acetic Acid Treatment of Digitoxin Tetraacetate **16**.—(a) *With zinc–acetic acid for* 15 *min*. Digitoxin **20** was acetylated by reflux in acetic anhydride for 1.5 h as described by Rabitzch.[5] The tetraacetate product **16** (1.4 g) was taken up in methanol (70 cm^3) and treated with ozone and zinc–acetic acid as described for digitoxin **20** but by use of only one-tenth the quantity of zinc for 15 min. The product after flash chromatography in 2% methanol–dichloromethane gave the 21-*hydroxy ester* **17** (423 mg), m.p. 133–135 °C (from diethyl ether–hexane) (Found: C, 60.7; H, 7.7. $C_{49}H_{74}O_{19}$ requires C, 60.9; H, 7.7%).

(b) *With zinc–acetic acid for* 20 *h*. Digitoxin **20** (500 mg) was acetylated[5] and the tetraacetate product **16** was treated with ozone and zinc–acetic acid for 20 h as described for digitoxin **20** to give 14-*hydroxy*-3β-(*tetraacetoxytrisdigitoxosyloxy*)-5β,14β-*pregnan*-20-*one* **21** (541 mg), m.p. 155–159 °C (from diethyl ether–acetone) (Found: C, 62.5; H, 8.3. $C_{47}H_{54}O_{16}\cdot\frac{1}{2}H_2O$ requires C, 62.6; H, 8.2%).

14,21-*Dihydroxy*-3β-[*tetraacetoxytris*(*digitoxosyloxy*)]-5β,14β-*pregnan*-20-*one* **18** *from Compound* **17**.—To a solution of the 21-hydroxy ester **17** (100 mg) in methanol (10 cm^3) under nitrogen was added 6.75% aq. $KHCO_3$ (0.2 cm^3). After 125 min the mixture was neutralized with acetic acid and extracted with dichloromethane to give the 21-hydroxy ketone **18** (78 mg), m.p. 131–133 °C (from CH_2Cl_2–MeOH) (lit.,[5] 137–140 °C).

21-*Acetoxy*-14-*hydroxy*-3β-[*tetraacetoxytris*(*digitoxosyloxy*)]-5β,14β-*pregnan*-20-*one* **19**.—(a) *From compound* **18**. The 21-hydroxy ketone **18** (100 mg), on treatment with acetic anhydride (0.5 cm^3) and pyridine (1 cm^3), gave the 21-acetoxy ketone **19** (42 mg), m.p. 152–154 and 187–190 °C (lit.,[5] 154–157 resolidifies and melts 189–189.5 °C).

(b) *From compound* **26**. The 21-hydroxy ketone **26** (300 mg) was acetylated[5] to give the 21-acetoxy ketone **19** (180 mg), m.p. 154–158 °C.

Treatment of the 21-*Acetoxy Ketone* **19** *with Zinc–Acetic Acid for* 20 *h*.—To a solution of the 21-acetoxy ketone (100 mg) in acetic acid (30 cm^3) was added zinc (24 g) and the mixture was stirred vigorously for 20 h. After filtration and work-up as described for digitoxin **20** the residue gave the starting material **19** (60 mg recovery), m.p. 152–154 °C. ^1H NMR spectroscopy of the reaction product showed the presence of only compound **19**.

Ozonolysis and Zinc–Acetic Acid Treatment of Digitoxin **20**.—(a) *With zinc–acetic acid for* 5 *min*. A solution of digitoxin (1 g) in methanol (100 cm^3) was cooled to −60 °C in a solid CO_2–acetone-bath. A stream of ozone was passed into the solution until reaction was complete by TLC (*ca.* 45 min) and excess of ozone was removed by a stream of nitrogen. The solvent was removed under reduced pressure at 40 °C and the residue was dissolved in acetic acid (300 cm^3), zinc powder (72 g) was added, and the mixture was stirred vigorously for 5 min. The reaction mixture was filtered and the zinc was washed thoroughly and successively with cold methanol and dichloromethane and the filtrate was washed successively with water and aq. $NaHCO_3$ to give a residue, which on flash chromatography over silica and elution (5% MeOH–CH_2Cl_2) gave the 21-*hydroxy ester* **25** (644 mg), m.p. 217–219 °C (from chloroform–acetone). Further recrystallization gave m.p. 223–225 °C (Found: C, 60.6; H, 8.3. $C_{41}H_{66}O_{15}\cdot\frac{1}{2}H_2O$ requires C, 60.3; H, 8.4%).

(b) *With zinc–acetic acid for* 20 *h*. Digitoxin **20** (1 g) was treated as above with ozone and the mixture was stirred with zinc–acetic acid for 20 h to give the *methyl ketone* **23** (750 mg), m.p. 225–228 °C (from chloroform–acetone). Further recrystallization gave m.p. 233–235 °C (Found: C, 64.6; H, 8.9. $C_{39}H_{64}O_{12}$ requires C, 64.4; H, 9.0%).

Digitoxin Tetrakis-(2,2,2-*trichloroethyl Carbonate*) **22**.—To a solution of digitoxin **20** (0.5 g) in pyridine (10 cm^3), stirred and cooled in an ice-bath, was added dropwise 2,2,2-trichloroethyl chloroformate (0.9 cm^3). After storage at room temperature for 1 h the reaction mixture was diluted with ice–water and extracted with dichloromethane. The extract was washed successively with water, 0.5 mol dm^{-3} hydrochloric acid and aq. $NaHCO_3$ and evaporated to give a product, which was chromatographed on silica. Elution with hexane removed a by-product, and elution with acetone then gave the *tetrakis*(*trichloroethyl carbonate*) **22** (857 mg), m.p. 155–159 °C (from

Table 2 ^{13}C Chemical shifts[a]

Carbon	Compound 2	3	4	6	7	8	9[b]	10	11	12	13	15	16[c]	17[c]	18[c]	19[c]	21[c]	22[d]	23[e,f]	25[e,f]	26[e,f]	27
1	29.73	37.11	37.32	30.37[g]	30.51	30.33	31.47[g]	30.49[g]	30.49[g]	37.30	30.05[g]	37.17	30.15	30.13[g]	30.14[g]	30.07[g]	30.05[g]	29.99[g]	30.34[g]	30.31[g]	30.33[g]	29.70
2	27.92	36.72	20.69[g]	24.92[h]	25.05[g]	24.85[g]	27.65[h]	24.95[h]	24.96[h]	20.35	24.85	20.95[g]	26.63[g]	26.72[h]	26.73	26.67[h]	26.69[h]	26.61[h]	27.02[h]	26.98[h]	26.98[h]	27.90
3	66.93	212.78	26.96[h]	70.37	70.44	70.30	72.49	71.18	71.14	27.18[g]	76.26	27.06[h]	73.00	73.00	73.02	72.96	72.97	73.02	73.61	73.57	73.49	66.86
4	33.46[g]	42.16	27.19[h]	30.39[g]	30.51	30.33	31.61[g]	30.54[g]	30.54[g]	27.45[g]	30.23[g]	27.28[h]	30.15	30.31[g]	30.30[g]	30.25[g]	30.25[g]	30.14[g]	30.76[g]	30.72[g]	30.74[g]	33.27
5	36.13	43.75	43.43	36.77	36.91	36.75	38.39	37.01	36.97	43.38	35.65[h]	43.18	36.25	36.43	36.44	36.39	36.41	36.29	37.03	36.98[i]	37.01	36.26
6	26.53	26.52	27.40[h]	26.24	26.36	26.22	25.91	26.37	26.35	26.95	26.14[i]	26.86	26.90[g]	26.78[h]	26.73	26.71[h]	26.69[h]	26.89[h]	27.13[h]	27.09[h]	27.11[h]	26.18
7	20.89	20.89	21.50[g]	20.71	20.84	20.65	22.34[i]	20.86	20.88	21.46	21.11[j]	21.40[g]	21.15[h]	20.93	20.93[h]	20.88[i]	21.56	21.15	21.32	21.30	21.31	24.05
8	40.08	39.87	40.23	39.94	40.05	39.77	42.68	39.96	40.08	40.96	41.69	42.02	41.85	40.16	40.14	40.07	39.97	41.85	40.59	40.69	40.60	34.95
9	35.01	36.09	35.78	35.04	35.16[h]	34.93	36.75	35.16	35.19	36.21	36.41[h]	36.24	35.75	35.34	35.27	35.26	35.19	35.75	35.86	35.86[i]	35.88	39.74
10	35.45	35.20	35.68	35.04	35.16[h]	34.97	36.29	35.16	35.19	35.68	35.07	35.61	35.16	35.26	35.27	35.19	35.20	35.16	35.70	35.66	35.64	35.33
11	21.60	21.15	21.84[g]	21.33	21.47	21.24	22.44[i]	21.45	21.48	21.70	21.15[j]	21.60[d]	21.36[h]	21.65	21.66[h]	21.59[i]	20.86	21.39	22.06	22.05	22.07	21.85
12	39.26	38.90	39.47	39.09	39.15	39.00	40.89	39.25	39.29	41.72	39.88	40.11	40.05	39.45	39.34	39.35	39.35	40.05	39.75	39.65	39.52	42.12
13	49.83	49.70	49.36	49.72	49.78	49.06	51.05	49.30	49.92	47.66	49.57	49.60	49.60	50.00	49.89	49.76	49.32	49.58	~50	50.32	49.98	49.16
14	84.99	84.70	85.08	84.81	84.92	84.71	86.37	84.99	84.91	85.72	85.36	85.62	85.56	84.99	85.05	84.86	84.97	85.57	86.22	86.03	86.12	152.39
15	33.99[g]	33.98	33.97	33.85	33.98	33.76	33.39	33.97	34.02	32.13	33.08	33.17	33.10	34.03	34.02	34.00	33.92	33.16	34.29	34.25	34.33	116.33
16	24.94	24.89	24.97	24.78[h]	24.91[g]	24.72[g]	28.07[h]	25.05[h]	25.06[h]	26.29	26.85[i]	26.86	26.62[g]	25.00	24.99	25.01	24.92	26.61[h]	25.39	25.21	25.23	31.09
17	57.91	57.71	62.47	57.71	57.84	62.13	52.12	62.37	57.90	56.72	50.84	50.98	50.90	57.97	57.95	57.82	62.38	50.91	63.00	58.49	57.91	60.84
18	15.32	15.24	15.39	15.14	15.29	15.13	16.40	15.36	15.33	16.34	15.74	15.78	15.77	15.37	15.35	15.26	15.34	15.77	15.55	15.42	15.28	18.76
19	23.82	22.58	24.26	23.64	23.75	23.58	24.17	23.78	23.80	24.17	23.46	24.07	23.61	23.78	23.77	23.72	23.72	23.64	24.03	23.98	23.98	23.51
20	217.16	217.19	217.28	210.48	217.16	217.80	178.36	217.78	210.23	71.91	174.47	174.49[i]	174.47	210.29	217.20	217.27	217.75	174.42	219.43	212.20	218.88	210.08
21	70.17	70.18	33.36	69.33	70.15	33.12	75.32	33.37	69.59	23.33	73.42	73.45	73.41	69.71	70.21	69.20	33.34	73.41	33.16	69.69	70.24	69.27
22							117.76				117.59	117.56	117.63					117.65				
23							177.17				174.50	174.67[i]	174.47					174.42				
OCO*Me*				21.37	21.47	21.28										20.44						
O*C*OMe				170.61	170.66	170.39										169.98						
OCOCH_2OH				60.27					60.42					62.45						60.29		
O$COCH_2$OH				172.17					172.44					172.43						172.68		
OCO$CH_2CH_2CO_2H$							30.64[j]	29.96[i]	29.97[i]													
OCO$CH_2CH_2CO_2H$							29.92[j]	28.94[i]	28.84[i]													
O$COCH_2CH_2CO_2H$							173.59	171.44	171.48													
OCO$CH_2CH_2CO_2H$							175.95	171.44	171.48													
OCOCH_2CCl_3											76.51											
O$COCH_2CCl_3$											153.47											
OCOCH_2CCl_3											94.62											

[a] For solutions in $CDCl_3$ unless indicated otherwise ($SiMe_4$ internal standard) on a Bruker AM300 instrument. [b] In CD_3OD. [c] Trisdigitoxoside tetraacetate spectra (**17**, **18**, **19**, **21**) are in agreement with digitoxin tetraacetate **16**: 95.85 (1′), 98.72, 98.83 (1″, 1‴), 35.75, 35.75, 36.31 (2′, 2″, 2‴), 67.38, 67.86 (3′, 3‴), 68.94 (3‴), 79.50, 79.54 (4′, 4‴), 72.32 (4‴), 69.01, 69.62 (5′, 5‴), 70.03 (5‴), 17.65, 17.99, 18.21 (6′, 6″, 6‴). [d] 95.28 (1′), 98.59 (1″, 1‴), 35.57, 35.70, 36.19 (2′, 2″, 2‴), 67.35, 68.70, 68.76 (3′, 3″, 3‴), 79.22, 79.28 (4′, 4‴), 72.16 (4‴), 75.24, 75.74, 76.84 (5′, 5″, 5‴), 17.72, 18.04, 18.09 (6′, 6″, 6‴), 76.57, 76.70, 77.00, 77.06 (OCOCH_2CCl_3), 153.03, 153.20, 153.27, 153.59 (O$COCH_2CCl_3$), 94.05, 94.20, 94.59, 94.72 (OCOCH_2CCl_3). [e] In $CDCl_3$–CD_3OD (1:1). [f] Trisdigitoxoside spectra (**23**, **25**, **26**) are in agreement with digitoxin **20** ($CDCl_3$–CD_3OD, 1:1): 95.91 (1′), 99.39, 99.56 (1″, 1‴), 37.46, 37.81, 38.53 (2′, 2″, 2‴), 67.07, 67.27, 68.22 (3′, 3″, 3‴), 82.82, 83.12 (4′, 4‴), 73.52 (4‴), 68.70, 68.83 (5′, 5‴), 70.20 (5‴), 18.23, 18.30, 18.34 (6′, 6″, 6‴). [g–j] Chemical shifts are interchangeable within a column.

CH_2Cl_2–MeOH) (Found: C, 43.6; H, 4.8; Cl, 28.8. $C_{53}H_{68}Cl_{12}O_{21}$ requires C, 43.3; H, 4.9; Cl, 29.0%).

Ozonolysis and Zinc–Acetic Acid Treatment of Digitoxin Tetrakis-(2,2,2-trichloroethyl Carbonate) **22**.—A mixture of the trichloroethyl ester (747 mg), dichloromethane (10 cm^3) and methanol (60 cm^3) was treated with ozone followed by zinc–acetic acid for 20 h as described for digitoxin **20**, to give the methyl ketone **23** (240 mg), m.p. 224–227 °C (from chloroform–acetone). ^1H and ^{13}C NMR spectra were identical with those from the product similarly obtained from digitoxin **20**.

14-*Hydroxy*-3β-*tris*(*digitoxosyloxy*)-5β,14β-*pregnan*-20-*one* **23** *from Compound* **25**.—A solution of hydroxy ester **25** (168 mg) in acetic acid (150 cm^3) was stirred for 20 h with zinc (15 g), followed by filtration and dilution with dichloromethane. The filtrate was washed successively and thoroughly with water and aq. $NaHCO_3$ to give a residue, which on flash chromatography gave, on elution (3% MeOH–CH_2Cl_2), fractions of the methyl ketone **23** (111 mg); further recrystallization gave pure ketone **23** (76 mg), m.p. 234–235.5 °C (from chloroform–acetone) as shown by ^1H and ^{13}C NMR spectroscopy.

14,21-*Dihydroxy*-3β-*tris*(*digitoxosyloxy*)-5β,14β-*pregnan*-20-*one* **26** *from Compound* **25**.—To a solution of the 21-hydroxy ester **25** (100 mg) in methanol (12 cm^3) under argon was added aq. 5% $KHCO_3$ (4 cm^3). After being stirred at room temperature for 16 h, the solution was neutralized with acetic acid and extracted with dichloromethane give, on work-up, 21-hydroxy ketone **26**[5] (68 mg), m.p. 220–223 °C (from chloroform–acetone). Further recrystallization gave product with m.p. 225–228 °C (Found: C, 63.3; H, 8.7. $C_{39}H_{64}O_{13}$ requires C, 63.2; H, 8.7%.

Acidic Hydrolysis of 14-*Hydroxy*-3β-*tris*(*digitoxosyloxy*)-5β,14β-*pregnan*-20-*one* **23**.—The trisdigitoxide **23** (35 mg) was dissolved in 0.05 mol dm^{-3} sulphuric acid in methanol (5 cm^3) and the solution was treated as described for the hydrolysis of digitoxin **20**. The two major products (TLC) were separated by elution with 35% ethyl acetate–hexane to give fractions (8.5 mg) of the C-14 olefin **24**, m.p. 164–165 °C (from acetone–hexane) (lit.,[19] 164–165 °C) and the dihydroxy ketone **14** (4 mg), m.p. 95–100 resolidifies and melts 151–153 °C (from dichloromethane–hexane) (lit.,[1] 154–156 °C). When digitoxin **20** was treated under identical conditions for 20 h the ^1H NMR spectrum of the crude product showed no absorption corresponding to the 15-H.

Acidic Hydrolysis of 14,21-*Dihydroxy*-3β-*tris*(*digitoxosyloxy*)-5β,14β-*pregnan*-20-*one* **26**.—A solution of the trisdigitoxoside **26** (250 mg) in 0.05 mol dm^{-3} sulphuric acid in methanol (25 cm^3) was treated as described for the hydrolysis of digitoxin **20**. The two major products (TLC) were separated by elution (40% ethyl acetate–hexane) to give the unsaturated diol **27**[20] (28 mg), m.p. 116–117 °C (from acetone–hexane) (Found: C, 75.8; H, 9.9. $C_{21}H_{32}O_3$ requires C, 75.9; H, 9.7%) and the 21-hydroxy ketone **2** (47 mg), m.p. 148–154 °C (from acetone–hexane) (lit.,[8] 104–110 resolidifies and melts 148–154 °C).

Zinc–Acetic Acid Treatment of 14,21-*Dihydroxy*-3β-*tris*(*digitoxosyloxy*)-5β,14β-*pregnan*-20-*one* **26**.—The 21-hydroxy ketone **26** (43 mg) was treated with zinc (3.2 g) in stirred acetic acid (15 cm^3) for 20 h. TLC showed mainly starting material **26** and no component corresponding to the methyl ketone **23**. The ^1H and ^{13}C NMR spectra showed that the crude product was principally starting material.

21-*Acetoxyacetoxy*-14-*hydroxy*-3β-[*tetraacetoxytris*(*digitoxosyloxy*)]-5β,14β-*pregnan*-20-*one* **28**.—The 21-hydroxy ester **25** (255 mg) was acetylated (Ac_2O–pyridine) to give the 21-*acetoxyacetate* **28** (190 mg), m.p. 147–151 °C (Found: C, 60.5; H, 7.5. $C_{51}H_{76}O_{20}$ requires C, 60.7; H, 7.6%).

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