Ring-A Oxygenated Derivatives of 5α- and 5β-Cardenolides

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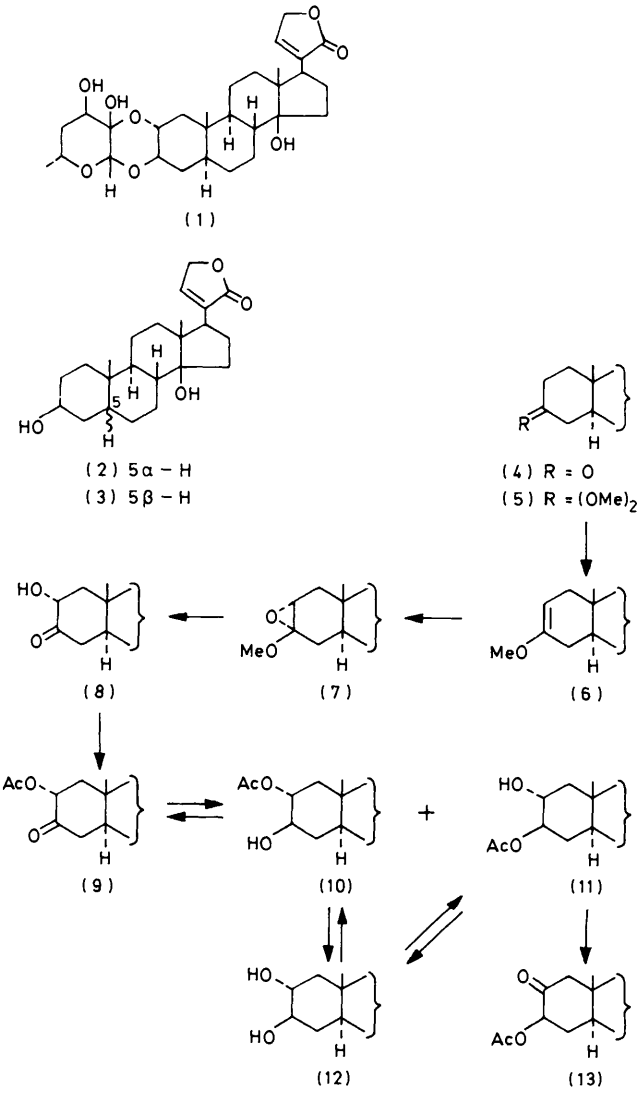
The introduction of an hydroxy-group α to the 3-keto-function of the 5α- and the 5β-cardenolides uzarigenone (4) and digitoxigenone (15) was carried out in three steps in good overall yield. The dimethyl acetals (5) and (16) of uzarigenone and digitoxigenone were pyrolysed to the respective Δ^2- and Δ^3-enol ethers (6) and (17). Epoxidation followed by spontaneous rearrangement led to respectively 2α-hydroxyuzarigenone (8) and 4β-hydroxydigitoxigenone (18). Sodium borohydride reduction of 2α-acetoxyuzarigenone (9) gave the 2- and 3-monoacetates (10) and (11) of gomphogenin (12) (2α-hydroxyuzarigenin), the latter monoacetate by acyl migration. On hydrolysis each formed gomphogenin (12). Reduction of the keto-function of 4β-acetoxydigitoxigenone (19) by lithium tri-t-butoxyaluminium hydride proceeded without rearrangement to yield the corresponding 3α-alcohol (20). The ^{13}C n.m.r. data of 18 members of the title compounds are analysed.

Naturally occurring cardenolide glycosides related to gomphoside (1) [1] and characterised by a carbohydrate moiety rigidly attached to the 2- and 3-positions of the steroid aglycone have shown interesting cardiotonic activities.[2] As part of a programme to study the pharmacological properties of synthetic analogues of such a class of cardiac glycosides, we have developed convenient routes to analogues of the cardenolides uzarigenin (2; 5α-H) and digitoxigenin (3; 5β-H), having additional oxygen functions at positions 2 and 4 respectively.

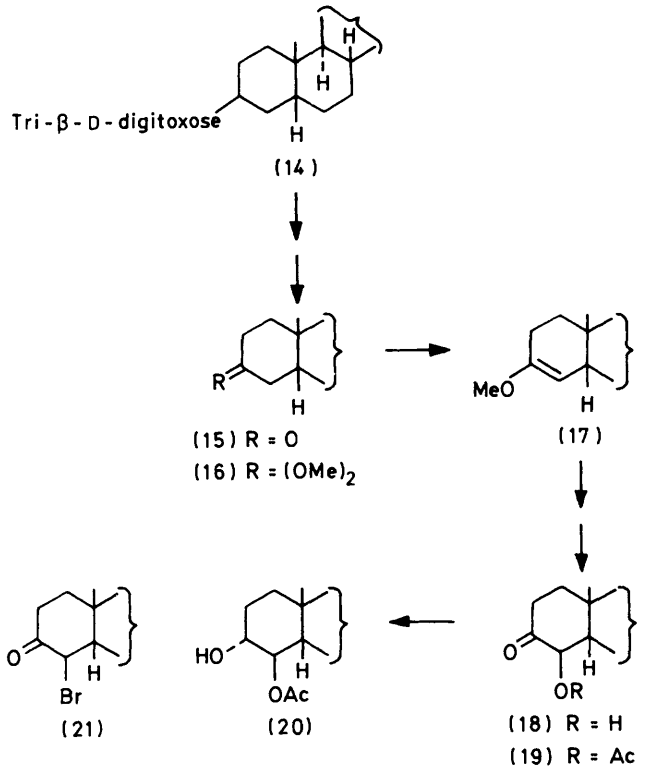
In 1969 Lardon, Stöckel, and Reichstein [3] established the structure proposed [4] for gomphogenin, *viz.* 2α-hydroxyuzarigenin (12), by a partial synthesis from uzarigenin (2). The method involved lead tetra-acetate acetoxylation of uzarigenone (4) at position 2α, reduction of the acetoxy-ketone (9) with lithium tri-t-butyloxyaluminium hydride to yield 2α-acetoxyuzarigenin (10), and finally alkaline hydrolysis. The yield from uzarigenin was no more than 11%.‡

In our work, 2-oxygenated derivatives of uzarigenin are to be attached to a variety of carbohydrates. Since uzarigenin is not a readily available starting material (see below), a more efficient method of introducing a 2α-acetoxy-group is required. The method adopted, which is described below, besides being compatible with the sensitivity to acids and bases of 14β-hydroxycard-20(22)-enolides, is suitable for the preparation of two series of suitably protected derivatives of gomphogenin (12), *viz.*, the 2- and 3-monoacetates.

Uzarigenone (4) was converted in 85% yield into its methyl enol ether (6) by pyrolysis of the dimethyl acetal (5).[5] Reaction of the enol ether (6) with *m*-chloroperbenzoic acid in dichloromethane resulted in spontaneous conversion of the intermediate epoxide (7) into 2α-hydroxyuzarigenone (8) § in 57% overall yield from uzarigenone (4). Mild acetylation gave the known 2α-acetoxyuzarigenone (9) [3] which was then reduced with sodium borohydride in dioxan–water to give quantitatively (total yield) and after chromatography, pure samples of the 2- and 3-monoacetates of gomphogenin in roughly equal amounts. Both the ' normal ' 2α-acetoxy-3β-hydroxy-product (10) and the ' rearranged ' 2α-hydroxy-3β-acetoxy-product (11) were shown by ^1H and ^{13}C n.m.r. spectroscopy (see below) to have 2α, 3β- or di-equatorial oxygen functions, and each was saponified to gomphogenin (12). The above work thus constitutes a second partial synthesis of gomphogenin from uzarigenin.



† On leave of absence from the Institute of Zoology, Academia Sinica, China.
‡ Yield of one of the steps was not recorded.[3]
§ For earlier examples of the enol ether to α-hydroxy-ketone transformation see ref. 6.

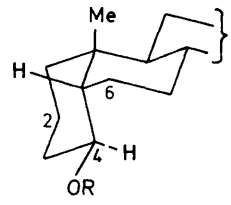


To distinguish between the two monoacetates, each was subjected to Jones oxidation. The 2-monoacetate (10) reverted to 2α-acetoxyuzarigenone (9) while the 3-monoacetate (11) formed the new 3β-acetoxy-2-ketone (13).

Formation of a rearranged product in the sodium borohydride reduction of 2α-acetoxyuzarigenone (9) may result either from an acyloin rearrangement [7] prior to reduction, or from an acyl group migration [8] after reduction, both of which may occur in basic medium. Unless reduction is slow, the latter process, known to take place *via* a cyclic ortho-acid intermediate,[8] appears more likely. It is of interest that when gomphogenin was treated with trimethyl orthoacetate in the presence of toluene-*p*-sulphonic acid,[9] pure 2- and 3-monoacetates (10) and (11) were isolated, again in roughly equal amounts.

The 5α-cardenolide uzarigenin (2) needed in the above-described transformation is not readily available in abundant amounts from natural sources. We have prepared uzarigenin from its 5β-epimer digitoxigenin (3) [in turn obtained from the commercially available glycoside digitoxin (14)] using a variation of the method of Kamano and Pettit.[10] In our transformation, the bromination of digitoxigenone (15) at carbon 4 by bromine–dioxan complex [11a] replaced the literature method of chlorination with t-butyl hypochlorite.[10]

To test the generality of the α-acetoxylation procedure as described earlier, digitoxigenone (15) was subjected to the same sequence of reactions. Digitoxigenone dimethyl acetal (16) was smoothly pyrolysed to the methyl Δ^3-enol ether (17). Treatment of the latter with *m*-chloroperbenzoic acid produced 4β-hydroxydigitoxigenone (18) which was then converted into 4β-acetoxydigitoxigenone (19). The equatorial position of the 4 oxygen function in both compounds (18) and (19) was shown by the 11 Hz coupling between the axial protons at 4 and 5. Reduction of the acetoxy-ketone (19) by lithium tri-t-butoxyaluminium hydride gave the 4β-acetoxy-3α-alcohol (20). The diequatorial orientation of the 3,4-substituents is shown by an observed 9 Hz coupling between the protons at 3 and 4 (see Table 1), and by ^{13}C n.m.r. spectroscopy (see below).



Figure

^{13}C *N.m.r.*—In Tables 2 and 3 are shown the ^{13}C n.m.r. data of the cardenolides prepared in this work, and of some other cardenolides for comparison. The assignments shown are based on multiplicities, chemical-shift theory, internal consistency, and comparison with our assignments of the corresponding glycoside derivatives, the derivation of which had been fully discussed.[1,12] As a minor departure from the earlier assignments,[12] C-7 is designated the more upfield of two signals near 21 p.p.m. for the 5β-cardenolides [except the enol ether(17)] in Table 2, and C-11 the other. The former carbon signal is perturbed somewhat by structural changes at positions 3 and 4, but the latter is not. The biggest perturbation at C-7 is observed for the enol ether (17); the conversion of C-4 into an sp^2 carbon results in loss of a γ-gauche interaction, and an observed slight deshielding for C-7.

The β-configuration of the oxygen functions at position 4 of the 5β-cardenolides (18)—(20) as arrived at (above) by ^1H n.m.r. spectroscopy is well supported by the ^{13}C n.m.r. shift differences. Thus comparing the 4-hydroxy- and 4-acetoxy-compounds (18) and (19) with the parent A/B-*cis* 3-ketone (15), the 4β-oxygen exerts a γ-gauche effect on C-6 (−4·5 p.p.m.), and a periplanar heteroatom effect [13] on C-2 (*ca.* −2 p.p.m.) (see Figure). Likewise for compound (20), the γ-gauche effect of the 4β-acetoxy-group on C-6 (−5 p.p.m.) is discernible. The 3α-configuration of the hydroxy-group in the same compound is evident from the lack of a γ-gauche effect on C-1; C-1 in the 3β-alcohol (3), for example, is 4·5 p.p.m. upfield.

Turning to the 5α-cardenolides, the 2α-configuration of the oxygen function in the monoacetates (10) and (11) [as well as in gomphogenin (12)] is supported by the observed periplanar heteroatom effect [13] on C-4 of OH or AOc (−1 to −3 p.p.m.) when (10) and (12) are compared with uzarigenin (2), and when (11) is compared with 3-acetyluzarigenin. Acetylation of a hydroxy-group is normally accompanied by downfield shift of *ca.* 3 p.p.m. at the α carbon, and upfield shifts of roughly the same magnitude at the two β carbons. These shifts are indeed observed when the 2,3-diol (12) is compared with the 2-monoacetate (10) (Δδ: C-2, +3·5; C-1, −2·5; C-4, −3·5 p.p.m.), and with the 3-monoacetate (11) (Δδ: C-3, +3; C-2, −3·5; C-4, −3 p.p.m.), all in the same solvent ($CDCl_3$–CD_3OD, 3 : 1).

Experimental

The ^1H and ^{13}C n.m.r. data of the compounds described below are given in Tables 1 to 3 and were determined on a JEOL FX-90Q spectrometer operating at 89.6 MHz and 22.5 MHz respectively in the Fourier-transform mode. Acquisition time, pulse delay, and pulse width were, for ^1H: 4.57 s, *ca.* 1 s, and 43 μs; for ^{13}C: 0.37 or 0.73 s, *ca.* 0.5 s, and *ca.* 7 μs respectively.

Uzarigenone Dimethyl Acetal (5).—To uzarigenone (4) (386 mg) in dry methanol (125 ml) was added toluene-*p*-

Table 1. ^{1}H Chemical shifts in δ (*J* or half-height width in Hz in parentheses) [a]

Compounds	Functional group at position 2	3	4	2-H	3-H	4α-H	21-H [c]	22-H [d]	17-H [e]	18-H	19-H	OAc	OMe
5α-series													
(5)		$(OMe)_2$					4.85, 5.05	5.9	2.8	0.90	0.81		3.5
(6)	Δ^2	OMe		4.5 (*J* 5, *ca.* 0)			4.8, 5.0	5.9	2.8	0.90	0.76		3.15, 3.2
(8)	α-OH	O		4.25 (*J* 7, 12) [f]			4.8, 5.0	5.9	2.75	0.89	1.08		
(9)	α-OAc	O		5.3 (*J* 7, 14)			4.85, 5.0	5.9	2.8	0.90	1.12	2.14	
(13)	O	β-OAc			5.2 (*J* 6, 11)		4.8, 4.95	5.9	2.75	0.88	0.77	2.15	
(10)	α-OAc	β-OH		*ca.* 4.8 m [g]	3.6 ($W_{h/2}$22)		4.8, 5.0	5.9	2.75	0.88	0.90	2.08	
(12) [b]	α-OH	β-OH		3.2—3.6 [g]	3.2—3.6 [g]		4.9, 5.0	5.9	2.8	0.90	0.85		
(11)	α-OH	β-OAc		3.75 ($W_{h/2}$22)	*ca.* 4.6m [g]		4.8, 5.0	5.9	2.75	0.88	0.84	2.08	
5β-series													
(16)		$(OMe)_2$					4.8, 5.05	5.9	2.8	0.88 [h]	0.95 [h]		3.15, 3.2
(17)		OMe	Δ^3		4.25 (*J ca.* 0)		4.8, 5.05	5.85	2.8	0.88 [h]	0.94 [h]		3.5
(18)		O	β-OH			4.4 (*J* 11) [f]	4.85, 5.05	5.9	2.8	0.92	1.03		
(19)		O	β-OAc			5.5 (*J* 11.5)	4.85, 5.05	5.9	2.8	0.92	1.07	2.12	
(20)		α-OH	β-OAc		*ca.* 3.6m	5.1 X of ABX (J_{AX} + J_{BX} 20.5)	4.85 5.05	5.9	2.8	0.92	0.99	2.12	

[a] Measured at 89.6 MHz in $CDCl_3$ except for gomphogenin (12); chemical shifts quoted to nearest 0.05 p.p.m. except for methyl signals (to 0.01 p.p.m.). [b] In $CDCl_3$–CD_3OD (3 : 1). [c] AB quartet (J_{AB} 8.7 Hz) further split by 22-H. [d] Triplet, $J_{21\alpha,22} \approx J_{21B,22}$ = 1.5 Hz. [e] Multiplet, $W_{h/2}$ 12 Hz. [f] J_{CH-OH} 3—3.5 Hz. [g] Masked by other signals. [h] Assignments of 18-H and 19-H may require reversal.

sulphonic acid monohydrate (625 mg). After 16 h the solution was poured into aqueous sodium hydrogen carbonate and extracted with chloroform. On removal of chloroform and crystallisation from dichloromethane–methanol (containing traces of pyridine) 3,3-dimethoxy-14β-hydroxy-5α-card-20(22)-enolide (5) was obtained as needles, m.p. 192—196 °C (Found: C, 71.95; H, 9.05. $C_{25}H_{38}O_5$ requires C, 71.75; H, 9.15%).

Uzarigenone Methyl Enol Ether (6).—The dimethyl acetal (5) (above), maintained under nitrogen and at water-pump pressure, was heated at 210 °C for 3—5 min whereupon the evolution of methanol accompanying melting ceased, and the material solidified. Upon crystallisation from dichloromethane–methanol, 3-methoxy-14β-hydroxy-5α-card-2,20(22)-dienolide (6) (341 mg) [85% overall yield from uzarigenone (4)] was obtained as needles, m.p. 211—216 °C (Found: C, 74.55; H, 8.85. $C_{24}H_{34}O_4$ requires C, 74.6; H, 8.85%).

2α-Hydroxyuzarigenone (8) *and 2α-Acetoxyuzarigenone* (9).—The crude dimethyl acetal (5) from 0.37 g of uzarigenone (4) was pyrolysed as described above to the enol ether (6) which, without purification was treated with *m*-chloroperbenzoic acid (0.22 g) in dichloromethane (30 ml) at 0 °C. The conversion into the intermediate α-methoxy-epoxide (7) and thence to the final product over a period of several minutes was followed by t.l.c. After being washed with aqueous sodium carbonate, the dichloromethane solution was evaporated to give 0.37 g of crude product which upon crystallisation from dichloromethane–methanol, yielded 2α,14β-dihydroxy-3-oxo-5α-card-20(22)-enolide (8) as needles (0.22 g) (57% overall yield from uzarigenone), m.p. 235—242 °C (Found: C, 71.3; H, 8.35. $C_{23}H_{32}O_5$ requires C, 71.1; H, 8.3%).

Acetylation of 2α-hydroxyuzarigenone (8) (0.22 g) using acetic anhydride in pyridine at room temperature yielded 2α-acetoxy-14β-hydroxy-3-oxo-5α-card-20(22)-enolide (9) as needles from dichloro-methane–methanol (0.17 g), m.p. 255—256 °C (lit.,[3] m.p. 255—261 °C).

2α-Acetoxyuzarigenin (10) *and 3-Acetyl-2α-hydroxyuzarigenin* (11).—(a) *From 2α-acetoxyuzarigenone* (9). To a stirred solution of 2α-acetoxyuzarigenone (9) (173 mg) in dioxan (15 ml) was added water (0.3 ml) followed by sodium borohydride (150 mg). After 30 min, the solution was poured into water and extracted with chloroform. The residue obtained on removal of chloroform was chromatographed over silica gel (Merck Kieselgel H, thin-layer grade) and eluted with benzene–ethyl acetate to give in order of elution, (i) 3β-acetoxy-2α,14β-dihydroxy-5α-card-20(22)-enolide (11) (78 mg) as needles from dichloromethane–methanol, m.p. 262—267 °C (Found: C, 69.6; H, 8.5. $C_{25}H_{36}O_6$ requires C, 69.4; H,8.4%); (ii) 2α-acetoxy-3β,14β-dihydroxy-5α-card-2υ(22)-enolide

Table 2. ^{13}C Chemical shifts of 5α-cardenolides (p.p.m. downfield from $SiMe_4$)

Carbon atom	(2) [b,12]	3-Acetate of (2) [a,*]	(4) [a]	(5) [a]	(6) [a]	(9) [a]	(10) [a]	(10) [c]	(12) [c]	(11) [c]	(11) [a]
1	37.0	36.8	38.0 †	35.2 †	39.8	44.7	42.3	42.5	45.2	45.6	45.5
2	31.2	27.3	38.6 †	28.2	91.4	74.1	76.3	76.1	72.5	69.2	70.0
3	69.7	73.5	211.6	99.2	153.8	203.8	73.5	72.6	75.9	78.7	79.0
4	38.0	33.8	44.4	35.0 †	32.1	43.3	35.8	35.9	35.5	32.7	33.2
5	44.3	44.2	46.2	41.8	41.7	47.3	44.1	44.3	44.6	44.4	44.1
6	28.6	28.4	28.8	28.2	28.5	28.2	27.7	27.9	28.0	27.8	27.7
7	27.3	27.3	27.1	27.2	27.1	27.0	27.3	27.4	27.4	27.4	27.3
8	41.0	41.5	41.5	41.5	41.4	40.7	41.1	40.7	40.8	40.7	41.1
9	49.5	49.6	49.3	49.4	49.4	49.2	49.7	49.7	49.9	49.7	49.6
10	35.5	35.7	35.9	35.8	35.2	37.3	37.6	37.6	37.6	37.2	37.2
11	20.9	21.1	21.4	21.0	21.1	21.3	21.5	21.5	21.5	21.4	21.4
12	39.3	39.7	39.7	39.7	39.8	39.4	39.8	39.7	39.9	39.8	39.3
13	49.5	49.6	49.6	49.5	49.5	49.5	49.5	49.7	49.9	49.9	49.6
14	84.0	85.1	85.2	85.2	85.3	84.9	85.4	85.0	85.1	84.9	85.4
15	32.3	33.0	33.1	32.9	33.1	32.9	33.1	32.6	32.6	32.7	32.5
16	26.6	26.9	26.9	26.8	26.9	26.8	27.0	27.0	27.1	27.0	27.0
17	50.6	50.9	50.8	50.8	50.9	50.7	50.9	51.0	51.1	51.1	50.9
18	15.7	15.8	15.8	15.6	15.8	15.7	15.8	15.8	15.8	15.8	15.9
19	12.1	12.1	11.4	11.4	11.5	12.6	13.1	12.8	13.2	12.9	13.3
20	175.6	175.1	174.8	175.0	175.0	174.9	174.4	176.6	176.7	176.5	174.7
21	73.2	73.5	73.5	73.5	73.6	73.5	73.5	74.1	74.1	74.1	73.6
22	116.7	117.4	117.6	117.3	117.5	117.5	117.9	117.2	117.1	117.2	117.8
23	173.8	174.7	174.8	174.6	174.6	174.6	174.4	176.0	176.1	176.0	174.7
-OCO*Me*		21.4				20.6	21.5	21.1		21.1	21.4
-O*C*OMe		170.6				170.0	171.5	172.0		172.0	171.6
-OMe				47.3	53.8						
				47.3							

[a] In $CDCl_3$, δ($CDCl_3$) 77.1 p.p.m. [b] In 1 : 2 $CDCl_3$–CD_3SOCD_3, δ($CDCl_3$) 78.8 p.p.m. [c] In 3 : 1 $CDCl_3$–CD_3OD, δ($CDCl_3$) 77.6 p.p.m.

* H. T. A. Cheung, T. R. Watson, J. N. Seiber, and C. Nelson, *J. Chem. Soc., Perkin Trans. 1*, 1980, 2169. † Signals within a vertical column may be interchanged.

Table 3. ^{13}C Chemical shifts of 5β-cardenolides [a]

Carbon atom	(3) [12]	3-Acetate of (3) *	(15)	(16)	(17)	(21) [b]	(18)	(19)	(20)
1	29.6	30.5	36.7 †	27.4	28.4	36.4	37.4 †	36.9 †	34.2
2	27.8	25.0	37.1 †	32.7	33.8	37.5	34.9 †	35.7 †	28.3
3	66.7	70.5	212.9	100.7	154.7	202.3	212.1	204.9	74.5
4	33.1	30.5	42.2	33.2	97.9	59.5	72.2	73.9	75.4
5	35.9	36.8	43.8	39.2	41.5	53.6	53.3	49.2	46.5
6	26.4	26.4	26.6	26.6	24.4	25.6	22.0	22.2	21.5
7	21.2	21.2	21.0	21.1	22.2	20.4	20.8	20.8	21.0
8	41.6	41.7	41.4	41.7	41.8	41.5	41.5	41.4	41.6
9	35.3	35.6	36.5	35.7	36.2	36.4	38.0	37.8	37.9
10	35.3	35.2	35.2	35.0	34.2	38.5	37.1	37.3	37.4
11	21.2	21.2	21.2	21.4	21.3	21.4	21.5	21.3	21.5
12	39.9	39.9	39.6	39.9	39.9	39.6	39.7	39.5	39.8
13	49.6	49.6	49.8	49.6	49.6	49.7	49.7	49.6	49.7
14	85.4	85.3	84.9	85.3	85.3	84.9	85.2	84.9	85.2
15	32.9	33.1	32.9	33.1	33.0	32.9	33.1	33.1	33.4
16	26.9	26.9	26.9	26.8	26.8	27.0	26.9	26.8	26.9
17	50.9	51.0	50.9	50.9	50.9	50.8	50.8	50.8	51.0
18	15.7	15.8	15.8	15.7	15.8	15.8	15.8	15.7	15.8
19	23.7	23.7	22.5	23.1	22.3	23.1	22.6	22.7	23.5
10	175.3	175.1	175.4	175.0	175.1	174.9	174.7	174.7	174.7
21	73.6	73.6	73.7	73.5	73.6	73.7	73.6	73.5	73.5
22	117.3	117.5	117.4	117.4	117.4	117.6	117.7	117.6	117.7
23	174.9	174.7	174.8	174.6	174.8	174.9	174.7	174.6	174.7
-OCO*Me*		21.2						20.6	21.0
-O*C*OMe		170.8						170.4	174.7
-OMe				47.4	54.1				
					54.1				

[a] In p.p.m. downfield from $SiMe_4$ in $CDCl_3$, δ($CDCl_3$) 77.1 p.p.m. unless otherwise stated. [b] In 10 :1 $CDCl_3$–CD_3OD, δ($CDCl_3$) 77.1 p.p.m.

* See the footnote * in Table 2. Earlier data adjusted to give δ($CDCl_3$) 77.1 p.p.m. † Signals within a vertical column may be interchanged.

(10) (92 mg) crystallising as needles from dichloromethane–ethyl acetate, m.p. 235—240 °C * (Found: C, 69.7; H, 8.55 $C_{25}H_{36}O_6$ requires C, 69.4; H, 8.4%); and (iii) gomphogenin (12) (traces).

(b) *From gomphogenin.* A solution of gomphogenin (12) (0.77 g) in a mixture of 1,1,1-trimethoxyethane (25 ml) and dry tetrahydrofuran (25 ml) containing toluene-*p*-sulphonic acid monohydrate (0.25 g) was stood at room temperature for 1 h, and then poured into an excess of aqueous sodium hydrogen carbonate. Extraction with dichloromethane gave a mixture of gomphogenin monoacetates which was separated by chromatography over silica to give in order of elution 3-acetyl-2α-hydroxyuzarigenin (11) (0.20 g) and 2α-acetoxyuzarigenin (10) (0.24 g), having the same m.p. and ^{13}C n.m.r. spectra as the respective samples in part (a) above.

Gomphogenin (12) *from Hydrolysis of* 2α-*Acetoxyuzarigenin* (10) *and of* 3-*Acetyl*-2α-*hydroxyuzarigenin* (11).—2α-Acetoxyuzarigenin (10) (19 mg) from reduction of 2α-acetoxyuzarigenone (9) (see above) was hydrolysed by aqueous methanolic potassium hydrogen carbonate [3] to give gomphogenin (12) (12 mg) as needles from dichloromethane–ethyl acetate, m.p. 232—235 °C (lit.,[3] m.p. 234—236 °C) having a ^{13}C n.m.r. spectrum (Table 2) identical with that of an authentic sample derived from *Asclepias fruticosa*.[4] 3-Acetyl-2α-hydroxyuzarigenin (11) (10 mg) also from borohydride reduction of 2α-acetoxyuzarigenone was similarly hydrolysed to give gomphogenin (12) (6 mg), m.p. 234—238 °C, again with a ^{13}C n.m.r. spectrum identical with that of an authentic sample.

3-*Acetyl*-2-*oxouzarigenin* (13).—3-Acetyl-2α-hydroxyuzarigenin (11) (60 mg) in acetone (30 ml) was treated with an excess of Jones reagent [11b] at 0 °C for 20 min. The product obtained by addition of an excess of methanol and aqueous sodium hydrogen carbonate, and extraction with chloroform was crystallised from dichloromethane–ethyl acetate to give 3β-acetoxy-14β-hydroxy-2-oxo-5α-card-20(22)-enolide (13) (33 mg) as needles from dichloromethane–ethyl acetate, m.p. 250—251 °C (Found: C, 69.95; H, 8.1. $C_{25}H_{34}O_6$ requires C, 69.75; H, 7.95%).

Reconversion of 2α-*Acetoxyuzarigenin* (10) *into* 2α-*Acetoxyuzarigenone* (9).—2α-Acetoxyuzarigenin (10) was oxidised by Jones reagent as described immediately above to give 2α-acetoxyuzarigenone (9) with a ^1H n.m.r. spectrum identical with that of a sample obtained from uzarigenone (see above).

Digitoxigenone Dimethyl Acetal (16).—This was prepared as described above for its 5α-epimer (5); 3,3-dimethoxy-14β-hydroxy-5β-card-20(22)-enolide (16) was obtained as needles from ethyl acetate, m.p. 162—165 °C (Found: C, 72.05; H, 9.05. $C_{25}H_{38}O_5$ requires C, 71.75; H, 9.15%).

Digitoxigenone Methyl Enol Ether (17).—This was prepared by pyrolysis of the acetal (16) as described above for the enol ether (6); 3-methoxy-14β-hydroxy-5β-card-3,20(22)-dienolide (17) was obtained as needles from dichloromethane–ethyl acetate, m.p. 168—172 °C (Found: C, 74.55; H, 9.0. $C_{24}H_{34}O_4$ requires C, 74.6; H, 8.85%).

4β-*Hydroxydigitoxigenone* (18).—The enol ether (17) (1.19 g) was converted *via* epoxidation as described above for enol ether (6) into 4β,14β-dihydroxy-3-oxo-5β-card-20(22)-enolide (18), which was purified by chromatography over silica and crystallisation from ethyl acetate to yield needles (0.50 g), m.p. 217—218 °C (Found: C, 71.1; H, 8.3. $C_{23}H_{32}O_5$ requires C, 71.1; H, 8.3%). Some digitoxigenone (15) (0.30 g) was recovered.

4β-*Acetoxydigitoxigenone* (19) *and* 4β-*Acetoxydigitoxigenin* (20).—4β-Hydroxydigitoxigenone (18) was acetylated by acetic anhydride in pyridine at room temperature to give, after work-up and chromatography over silica, 4β-acetoxydigitoxigenone (19) as a colourless gum (63%) with ^1H and ^{13}C n.m.r. data as shown in Tables 1 and 3, *m/z* 430 (33%, M^+), 388 (100, $M - CH_2CO$), 370 (81, M — HOAc), and 217 (78, 370 — $C_8H_9O_3$) [Found: M by (mass spec.) 430.236. $C_{25}H_{34}O_6$ requires M, 430.236]. A solution of the acetate (19) (45 mg) in anhydrous dioxan (10 ml) was treated overnight with lithium tri-t-butoxyaluminium hydride (107 mg). Chloroform (60 ml) was added, and the solution was washed with 5% aqueous sodium hydrogen carbonate and water, and evaporated to give 4β-acetoxy-3α,14β-dihydroxy-5β-card-20(22)-enolide (20) as a gum (42 mg), with ^1H and ^{13}C n.m.r. as shown in Tables 1 and 3, *m/z* 432 (3%, M^+), 372 (95, M — HOAc), 219 (25, 372 — $C_8H_9O_3$), and 201 (100, 219 — H_2O) [Found: M by (mass spec.) 432.251. $C_{25}H_{36}O_6$ requires M 432.252].

Bromination of Digitoxigenone (15).—To a solution of digitoxigenone (298 mg) in a mixture of dioxan–ether (1 : 1) (8 ml) at 0 °C was added in several portions dioxan dibromide [11a] (224 mg) in the same solvent (4 ml). The mixture was stirred at 0 °C until t.l.c. showed the reaction was completed (4—5 h) whereupon it was poured into water, and extracted with ethyl acetate (3 × 40 ml). The combined extracts were washed with aqueous sodium hydrogen carbonate and water, dried, and evaporated. The residue crystallised from ethyl acetate–ether (5 : 1) as needles of 4β-bromo-14β-hydroxy-3-oxo-5β-card-20(22)-enolide (21) (295 mg), m.p. 159—161 °C (Found: C, 59.7; H, 6.9. $C_{23}H_{31}O_4Br \cdot \frac{1}{2}H_2O$ requires C, 60.0; H, 7.0%) giving the expected MH^+ (451 and 453) in the methane chemical-ionisation mass spectrum.

* Lardon *et al.*[3] did not record the m.p.

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References

1 H. T. A. Cheung and T. R. Watson, *J. Chem. Soc., Perkin Trans. 1*, 1980, 2162 and references cited therein.
2 L. Brown, H. T. A. Cheung, R. E. Thomas, and T. R. Watson, unpublished results.
3 A. Lardon, K. Stöckel, and T. Reichstein, *Helv. Chim. Acta*, 1969, **52**, 1940.
4 R. G. Coombe and T. R. Watson, *Aust. J. Chem.*, 1964, **17**, 92.
5 J. T. Templeton, V. G. Paslat, and C. W. Wie, *Can. J. Chem.*, 1978, **56**, 2058.
6 C. L. Stevens and A. J. Weinheimer, *J. Am. Chem. Soc.*, 1958, **80**, 4072; R. E. Lyle, S. A. Leone, H. Troscianiec, and G. H. Warner, *J. Org. Chem.*, 1959, **24**, 330.
7 See *e.g.* L. F. Fieser and R. Stevenson, *J. Am. Chem. Soc.*, 1954, **76**, 1728; K. L. Williamson and W. S. Johnson, *J. Org. Chem.*, 1961, **26**, 4563; H. B. Henbest, D. N. Jones, and G. P. Slater, *J. Chem. Soc.*, 1961, 4472.
8 See *e.g.* R. U. Lemieux, ' Molecular Rearrangements,' ed. P. de Mayo, Interscience, New York, 1963, Vol. II, p. 763—765.
9 S. Hanessian and R. Roy, *J. Am. Chem. Soc.*, 1979, **101**, 5839.
10 Y. Kamano and G. R. Pettit, *J. Org. Chem.*, 1974, **39**, 2319.
11 (*a*) L. F. Fieser and M. Fieser, ' Reagents for Organic Synthesis,' Wiley, New York, 1967, vol. I, pp. 333—334; (*b*) p. 142.
12 H. T. A. Cheung, L. Brown, J. Boutagy, and R. Thomas, *J. Chem. Soc., Perkin Trans. 1*, 1981, 1773.

13 E. L. Eliel, W. F. Bailey, L. D. Kopp, R. L. Willer, D. M. Grant, R. Bertrand, K. A. Christensen, D. K. Dalling, M. W. Duck, E. Wenkert, F. M. Schell, and D. W. Cochran, *J. Am. Chem. Soc.*, 1975, **97**, 322; E. L. Eliel and K. M. Pietrusiewicz, in 'Topics in Carbon-13 NMR Spectroscopy' ed. G. C. Levy, Wiley-Interscience, New York, Vol. III, pp. 203—205.

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