# SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME CARDIOTONIC COMPOUNDS RELATED TO GITOXIGENIN

David C. Humber[a*], Paul S. Jones[b] and Gordon H. Phillipps[b]

Departments of Microbiological Chemistry[a] and Medicinal Chemistry[b], Glaxo Group Research Ltd., Greenford, Middlesex. UB6 OHE, England.

Received 4-24-85

## ABSTRACT

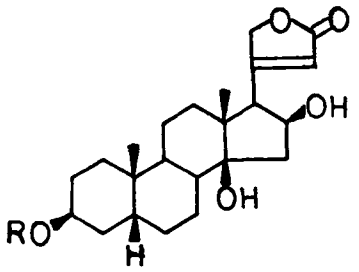
The four diastereomeric 3,16-diacetates 6, 9, 10 and 11 were prepared from gitoxin 1 by the routes shown in Schemes 1 and 2 and tested for inotropic activity in the isolated guinea-pig atrial preparation. In line with earlier findings in the digitoxigenin series, derivatives with a 3α-acetoxy function, viz 9 and 10, possessed high biological activity.

## INTRODUCTION

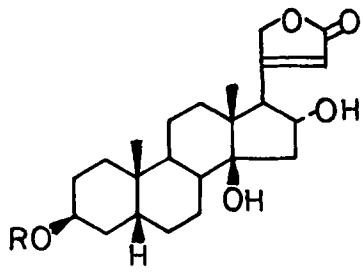
Derivatives of gitoxin [3β,14,16β-trihydroxy-5β,14β-card-20(22)-enolide, 3-O-(D-digitoxyl-D-digitoxyl-D-digitoxoside)] 1 have attracted considerable interest in recent years because of reports that with certain of them a partial separation of arrhythmogenic and positive inotropic effects can be achieved [1-3].

Epimerisation of the 16-hydroxyl group of the glycoside 1 or its genin 2 leads in both cases to a decrease in inotropic activity [4,5]. However, Repke [6] found that with the epimeric glycoside 3 there is a lower frequency of arrhythmias at maximum inotropic concentrations than with the natural isomer 1. Also, 16-epigitoxin 3 showed a wider dose range than gitoxin 1 between minimum and maximum inotropic effects [7]. Acetylation of the 16-hydroxyl group of either the natural, 1 and 2, or epimeric series, 3 and 4, resulted in a uniform increase in inotropic potency [8, 9]. More important, the 16-epi derivative 5 showed a substantial dissociation between the doses giving rise to toxic and to maximum therapeutic effects [9].

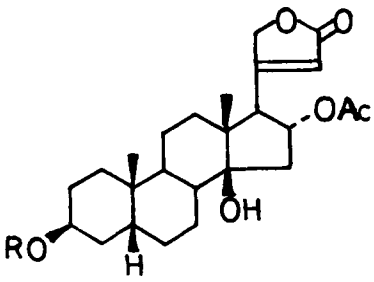
In a recent paper [10] we have observed that acetylation of



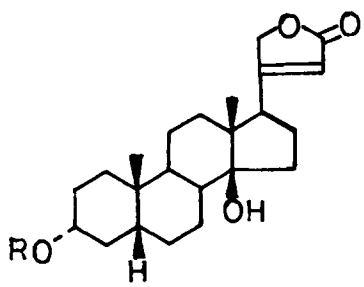

1 R=(digitoxose)$_3$
2 R=H



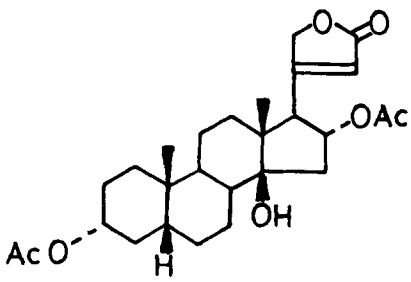

3 R=(digitoxose)$_3$
4 R=H



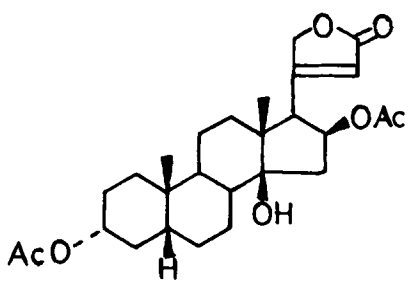

5 R=(digitoxose)$_3$
6 R=Ac



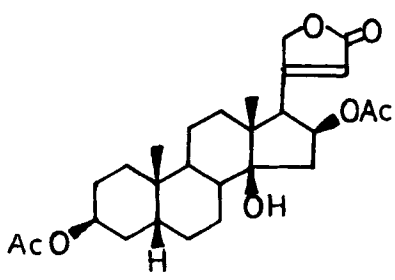

7 R=H
8 R=Ac



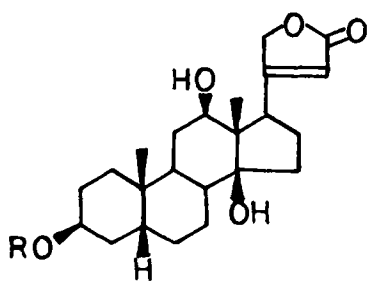

9




10




11




Digoxin:R=(digitoxose)$_3$

weakly active 3-epidigitoxigenin [3α,14-dihydroxy-5β,14β-card-20(22)-enolide] 7 gave the corresponding 3α-acetate 8 which possessed high inotropic activity. This result was not expected on the basis of established structure-activity relationships [1-3] but was found also to extend to related derivatives with modified 17β-ring systems [10]. As a continuation of these studies [10], this paper describes the preparation and pharmacological activity of the novel diacetates 9 and 10 derived from gitoxin 1. Also included for comparison purposes are biological results obtained for the known isomeric diacetates 6 [11] and 11 [12] and for a number of their synthetic precursors.

## CHEMISTRY

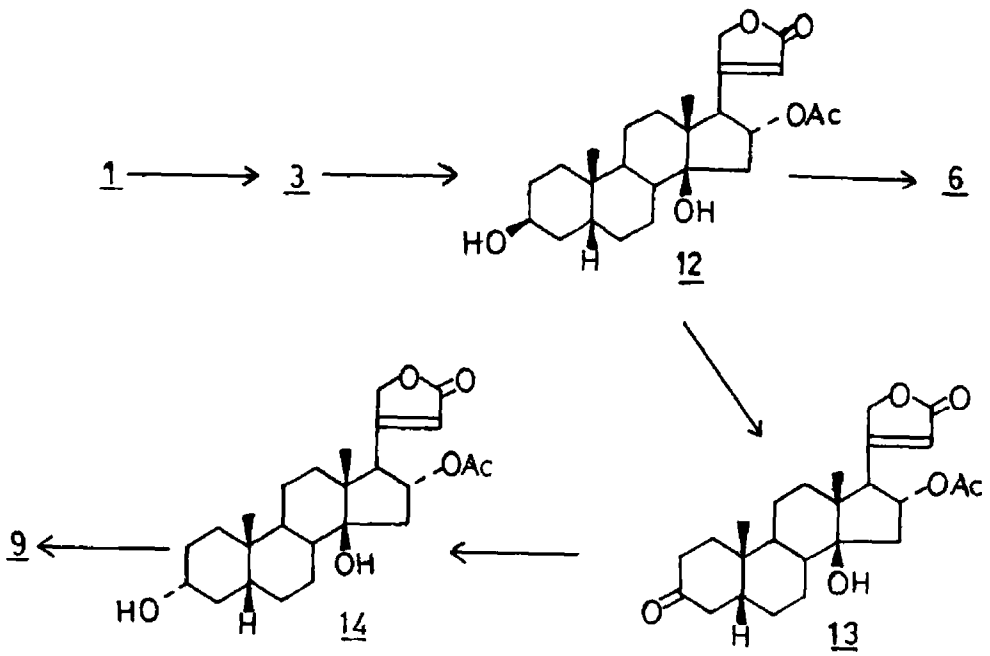
The 3α,16α-diacetate 9 and its 3β,16α-isomer 6 were prepared as outlined in Scheme 1.

Epimerisation of gitoxin 1 to its 16α-isomer 3 [11] was effected by treatment with hot aqueous base [13] (21% yield). Acetylation of 16-epigitoxin 3 with acetic anhydride/pyridine followed by acid hydrolysis of the glycoside linkage gave the monoacetate 12 in 50% yield. Further acetylation of the monoacetate 12 gave the known 3β,16α-diacetate 6 [11] (87% yield).

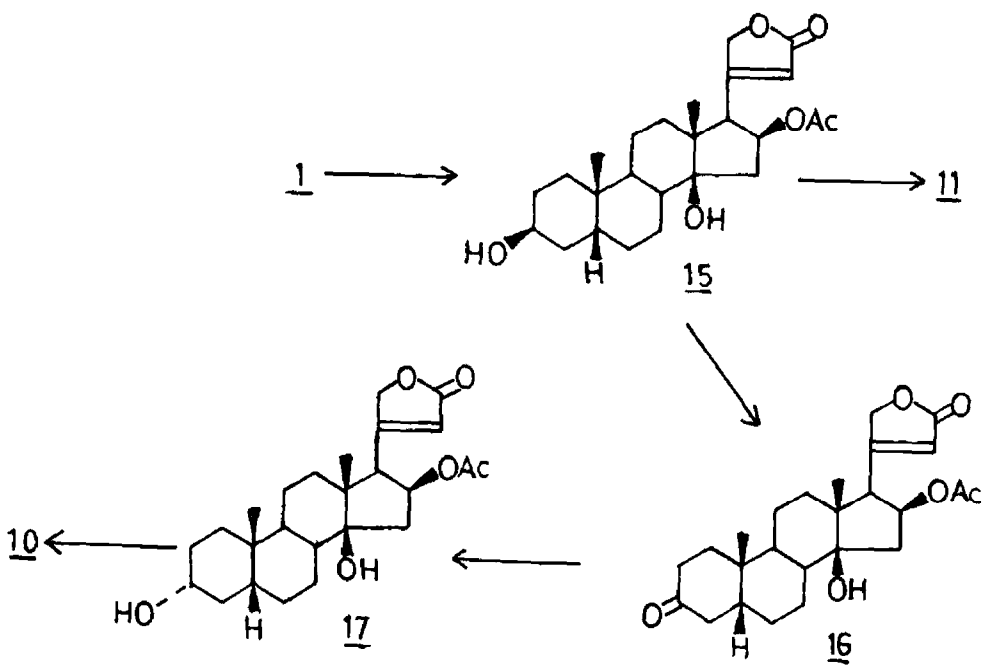
Oxidation of the monoacetate 12 with Jones' reagent [14] gave a 53% yield (after chromatography) of the ketone 13, which was then reduced with sodium borohydride to give a 66% yield of the 3α-hydroxy-16α-acetate 14. Acetylation of the monoacetate 14 by the usual procedure gave the 3α,16α-diacetate 9 in 70% yield.

The two remaining 3,16-diacetates, 10 and 11, were prepared as

SCHEME 1




SCHEME 2



shown in Scheme 2.

Gitoxin 1 was converted to oleandrigenin [16β-acetoxy-3β,14-dihydroxy-5β,14β-card-20(22)-enolide] 15 [12] in 65% yield by the procedure described above for its 16α-isomer 3 (viz acetylation followed by acid hydrolysis). Further acetylation of oleandrigenin 15 with acetic anhydride/pyridine in the presence of a catalytic amount of 4-dimethylaminopyridine provided the known 3β,16β-diacetate 11 [12] (82% yield).

Oxidation of oleandrigenin 15 with Jones' reagent [14] gave the ketone 16 [15] in 67% yield, reduction of which with sodium borohydride gave the 3α-hydroxy-16β-acetate 17 [15] (59% yield). Acetylation of the monoacetate 17 with acetic anhydride/pyridine furnished the 3α,16β-diacetate 10 in 64% yield.

## PHARMACOLOGICAL METHODS

The inotropic and chronotropic activities of the target compounds were determined using guinea-pig left and right atrial preparations as detailed previously [17].

Compounds were formulated as 1% solutions in dimethyl sulphoxide (DMSO) with subsequent dilutions being made with Krebs-Henseleit solution. DMSO has been shown to exert no inotropic effect at the concentrations used [18], which did not exceed 0.1% of the total organ bath volume.

In Table 1, 'inotropic effect' refers to the cumulative molar concentration required to increase left atrial force by 25%. 'Toxic effect' refers to the cumulative molar concentration producing a characteristic right atrial tachyarrhythmia in which the normal resting

rate of about 160 beats $min^{-1}$ increased to over 200 beats $min^{-1}$. 'Potency' is the inotropic effect for the test compound divided by that for digoxin [3β,12β,14-trihydroxy-5β,14β-card-20(22)-enolide, 3-O-(D-digitoxyl-D-digitoxyl-D-digitoxoside] which was used as standard. All the concentration values quoted are geometric means.

## PHARMACOLOGICAL RESULTS

Results obtained for the test compounds are given in Table 1.

TABLE 1 - Biological activity on isolated guinea-pig atria

| COMPOUND | INOTROPIC EFFECT[a] | TOXIC EFFECT[b] | POTENCY[c] |
|---|---|---|---|
| Digoxin[d] | $3.0 \times 10^{-7}$ M | $1.0 \times 10^{-6}$ M | 1.000[e] |
| 2 (gitoxigenin) [16] | $4.0 \times 10^{-6}$ M | $1.6 \times 10^{-5}$ M | 0.075 |
| 4 [11] | $1.3 \times 10^{-4}$ M | $3.8 \times 10^{-4}$ M | 0.002 |
| 6 [11] | $3.2 \times 10^{-5}$ M[f] | - | - |
| 9 | $4.0 \times 10^{-7}$ M | $5.5 \times 10^{-6}$ M | 0.750 |
| 10 | $4.0 \times 10^{-8}$ M | $6.7 \times 10^{-8}$ M | 7.500 |
| 11 [12] | $5.9 \times 10^{-7}$ M | $1.1 \times 10^{-6}$ M | 0.508 |
| 12 | $3.5 \times 10^{-6}$ M | $1.9 \times 10^{-5}$ M | 0.086 |
| 13 | $9.5 \times 10^{-5}$ M | $2.0 \times 10^{-4}$ M | 0.003 |
| 14 | $4.8 \times 10^{-5}$ M | $3.0 \times 10^{-4}$ M | 0.006 |
| 15 [12] | $1.9 \times 10^{-7}$ M | $5.6 \times 10^{-7}$ M | 1.579 |
| 16 [15] | $5.9 \times 10^{-7}$ M | $2.7 \times 10^{-6}$ M | 0.508 |
| 17 [15] | $5.2 \times 10^{-7}$ M | $1.0 \times 10^{-5}$ M | 0.577 |

a/Cumulative concentration required to produce a 25% increase in left atrial force; b/cumulative concentration required to cause right atrial tachyarrhythmia; c/inotropic effect for the test compound divided by that for digoxin; d/Lanoxin (250 μg/ml)-Burroughs Wellcome; e/by definition; f/maximum effect of 17% only.

In agreement with the findings of earlier workers [4, 5], 16-epigitoxigenin 4 was less active than its natural counterpart gitoxigenin 2. Also as expected [8, 9], acetylation of the 16-hydroxyl function of gitoxigenin 2 and 16-epigitoxigenin 4, to give oleandrigenin 15 and its

16-epi counterpart 12 respectively, led in both cases to an increase in activity.

Also anticipated from our earlier results in the digitoxigenin [3β,14-dihydroxy-5β,14β-card-20(22)-enolide]series [10] and from long-standing structure-activity data [1-3], inversion of configuration at $C_3$ resulted in a drop in potency (compare activities of the 3-epi derivative 17 and oleandrigenin 15 and the alcohol 14 and its 3β-counterpart 12). The corresponding 3-ketones 16 and 13 showed the same order of activities as their 3α-hydroxy analogues 17 and 14 respectively.

Again in agreement with previous findings [1-3], acetylation of the 3β-hydroxyl groups of oleandrigenin 15 and 16-epioleandrigenin 12, to give the 3β,16β-diacetate 11 and 3β,16α-diacetate 6 respectively, resulted in loss of inotropic activity. However, in agreement with our unexpected findings in the digitoxigenin series [10], derivatives with a 3α-acetoxy function were found to have higher than expected activities. Thus the 3α,16β-diacetate 10 was approximately an order of magnitude more active than both its 3β,16β-counterpart 11 and the digoxin employed as a standard. The 3α,16α-diacetate 9 also showed a high activity which was substantially greater than its 3β,16α-epimer 6.

All of the active compounds described in Table 1 gave rise to typical digitalis-like toxic effects at higher doses. Further studies would be necessary to ascertain if any of them showed any therapeutic advantage over existing steroidal cardiotonic agents.

## CONCLUSION

The pharmacological results are largely in good agreement with previous data and with established structure-activity relationships.

The high inotropic potencies of the 3α-acetoxy compounds 9 and 10 support our earlier results with 3α-acetoxy compounds related to digitoxigenin [10] and are not readily accommodated by current theories for the digitalis receptor [19-22]. They also complement recent findings by the groups of Thomas [23] and Fullerton [24] with regard to stereochemical and conformational requirements of the sugar moiety of digitoxigenin D-glycosides.

## EXPERIMENTAL SECTION

Melting points were determined in unsealed capillaries using an Electrothermal apparatus and are corrected. IR spectra were recorded on a Perkin Elmer Model 21 spectrophotometer. PMR spectra were determined on Perkin Elmer R32 (90 MHz) and Jeol MH 100 (100 MHz) spectrometers. UV spectra were run on a Pye Unicam SP8-200 UV/VIS spectrophotometer. Reaction extracts were dried over anhydrous $MgSO_4$ and evaporated on a rotary evaporator. Petrol refers to petroleum ether (b.p. 60-80°). Column chromatography was performed on Merck Kieselgel 60 and preparative TLC on 2 mm layers of Merck Kieselgel $F_{254 + 366}$.

Physical constants and spectral data (UV and IR) are given in Table 2 and PMR values in Table 3.

16α-Acetoxy-3β,14-dihydroxy-5β,14β-card-20(22)-enolide 12

$Ac_2O$ (5 mL) was added to a solution of 3 [11, 13] (1.0 g, 1.3 mmol) in pyridine (10 mL). The mixture was stirred for 20 h, diluted with ice-cold $H_2O$ (100 mL) and extracted with $CHCl_3$ (3 x 100 mL). The combined extracts were washed with 2M HCl (2 x 100 mL) and $H_2O$ (100 mL) then dried and evaporated to give a white foam (1.17 g). This was dissolved in EtOH (200 mL)-$H_2O$ (55 mL) and the solution brought to reflux and treated with hot 1M HCl (65 mL). The mixture was heated at reflux for 15 min, cooled to 20° and neutralised with 2M NaOH. The solution was concentrated to ca. 25 mL, diluted with $H_2O$ (25 mL) and extracted with $CHCl_3$-EtOH (4:1, 100 mL and 2 x 50 mL). The combined extracts were washed with $H_2O$ (100 mL), and evaporated to give a white foam which was purified by preparative TLC ($CHCl_3$-MeOH; 19:1) to afford 12 as a white foam (380 mg) which crystallised from EtOAc-petrol as colourless prisms (277 mg, 50.0%).

16α-Acetoxy-14-hydroxy-3-oxo-5β,14β-card-20(22)-enolide 13

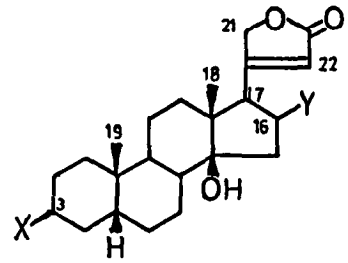
Jones' reagent [14] (0.3 mL) was added over 5 min to a stirred solution of 12 (320 mg, 0.7 mmol) in dioxane (10 mL)-$Me_2CO$ (7 mL) at 0 to 5°. The mixture was stirred for 20 min, treated with i-PrOH (1 mL), diluted with water (100 mL) and extracted with EtOAc (2 x 100 mL). The combined extracts were washed with water (100 mL), dried and evaporated

TABLE 2 - Physical constants and spectral data

| No. | m.p. $^0$C | Cryst$^n$ solvent | $[\alpha]_D^a$ | $\lambda_{max}^{MeOH}$ nm (ε) | $\nu_{max}^{CHBr_3}$ cm$^{-1}$ | Lit. constants/analysis$^b$ |
|---|---|---|---|---|---|---|
| 2 | 219-221 | EtOH-$H_2O$ | +32$^c$ | 220 (14,630) | 3550, 1788, 1760$^d$ | m.p. 234$^0$, $[\alpha]_D$+38.5 [16] |
| 4 | 204-206 | MeOH-$Et_2O$ | -29$^c$ | 221 (14,035) | 3610, 1778, 1740 | m.p. 212-214$^0$, $[\alpha]_D$-25 [11] |
| 6 | 289-294 | EtOAc-petrol | -11 | 218.5 (14,710) | 3590, 1780, 1742, | m.p. 295-298$^0$, $[\alpha]_D$-9 [11] |
| 9 | 185-187 | EtOAc-petrol | +15 | 218 (15,165) | 3590, 1784, 1742 | $C_{27}H_{38}O_7$ |
| 10 | 222-224 | EtOAc-petrol | +13 | 217 (14,610) | 3590, 1780, 1740 | $C_{27}H_{38}O_7$ |
| 11 | 250-253 | EtOAc-petrol | -8.5 | 217 (14,610) | 3580, 1780, 1740 | m.p. 249$^0$, $[\alpha]_D$-8 [12] |
| 12 | 145-152$^e$ | EtOAc-petrol | -15 | 218.5 (14,290) | 3620, 1782, 1745 | $C_{25}H_{36}O_6.H_2O$ |
| 13 | 279-282 | MeOH | -4.5 | 218 (14,960) | 3600, 1780, 1740, 1710 | $C_{25}H_{34}O_6$ |
| 14 | 245-246 | MeOH-$Et_2O$ | -7.5 | 218.5 (14,655) | 3620, 1780, 1742 | $C_{25}H_{36}O_6$ |
| 15 | 220-221 | $CHCl_3$-$Et_2O$ | -9.5 | 217.5 (13,810) | 3630, 1770, 1735 | m.p. 225-228$^0$, $[\alpha]_D$-10 [12] |
| 16 | 260-261 | MeOH | -4 | 217 (14,120) | 3630, 1780, 1740, 1710 | m.p. 250-252$^0$ [15] |
| 17 | 208-210 | MeOH-$Et_2O$ | +3$^f$ | 217 (14,190) | 3600, 1780, 1740 | m.p. 206-212$^0$, $[\alpha]_D$-8 [15] |

a/ c1.0, $CHCl_3$ at +21$^0$; b/ elemental analyses for C and H were within ± 0.4% of theoretical values; c/ in MeOH; d/ in nujol; e/ and 240-243$^0$(double m.p.); f/ in EtOH.

TABLE 3 - PMR spectra (δ values; coupling constants in Hz in parentheses)




| Cpd. | Solvent | 18-$H_3$ | 19-$H_3$ | 17-H | 3-H | 16-H | 21-H | 22-H | Other |
|---|---|---|---|---|---|---|---|---|---|
| 2 | $Me_2SO-d_6$ | 0.84s | 0.89s | 3.05d(8) | 3.94m($w_{1/2}$ 8) | 4.60m($w_{1/2}$ 16) | 5.10 broad s | 5.94 broad s | |
| 4 | $Me_2SO-d_6$ | 0.75s | 0.89s | 2.52m* | 3.94m($w_{1/2}$ 8) | 4.37* | 4.90 broad s | 5.94 broad s | |
| 6 | $CDCl_3$ | 0.87s | 0.97s | 2.68d(4) | 5.15m($w_{1/2}$ 8) | 5.32t,d(8,4) | 4.94 broad s | 5.96 broad s | 2.07s=16α-OAc, 3β-OAc |
| 9 | $CDCl_3$ | 0.87s | 0.94s | 2.68d(4) | 4.76m* | 5.31t,d(8,4) | 4.93 broad s | 5.96 broad s | 2.05s, 2.08s=16α-OAc, 3α-OAc |
| 10 | $CDCl_3$ | 0.94s | 0.94s | 3.22d(8) | 4.75m* | 5.52t,d(8,4) | 4.96 broad s | 6.00 broad s | 1.98s, 2.05s=16β-OAc, 3α-OAc |
| 11 | $CDCl_3$ | 0.96s | 0.98s | 3.22d(8) | 5.15m($w_{1/2}$ 8) | 5.53t,d(8,3) | 4.98 fine m | 6.00 fine m | 1.98s, 2.07s=16β-OAc, 3β-OAc |
| 12 | $CDCl_3$ | 0.88s | 0.97s | 2.67d(4) | 4.15m($w_{1/2}$ 8) | 5.28t,d(8,4) | 4.92 broad s | 5.98 broad s | 2.06s=16α-OAc |
| 13 | $CDCl_3$ | 0.91s | 1.04s | 2.68d(4) | - | 5.31t,d(8,4) | 4.93 broad s | 5.97 broad s | 2.06s=16α-OAc |
| 14 | $CDCl_3$ | 0.87s | 0.93s | 2.68d(4) | 3.65m($w_{1/2}$ 22) | 5.30t,d(8,4) | 4.93 broad s | 5.97 broad s | 2.07s=16α-OAc |
| 15 | $CDCl_3$ | 0.94s | 0.94s | 3.22d(8) | 4.18m($w_{1/2}$ 8) | 5.52t,d(9,2) | 4.98 fine m | 6.00 fine m | 1.97s=16β-OAc |
| 16 | $CDCl_3$ | 0.98s | 1.06s | 3.21d(8) | - | 5.52t,d(9,2) | 4.96 fine m | 6.00 fine m | 1.97s=16β-OAc |
| 17 | $CDCl_3$ | 0.94s | 0.94s | 3.21d(8) | 3.67m($w_{1/2}$ 22) | 5.52t,d(9,2) | 4.96 fine m | 6.00 fine m | 1.98s=16β-OAc |

*=obscured or partly obscured signal; s=singlet; d=doublet; t=triplet; m=multiplet

to give a white solid which was purified by preparative TLC ($CHCl_3$-$Me_2CO$; 4:1) to give 13 as a white solid (170 mg, 53.0%). Crystallisation from MeOH afforded colourless prisms (105 mg, 33.0%).

16α-Acetoxy-3α,14-dihydroxy-5β,14β-card-20(22)-enolide 14

A solution of $NaBH_4$ (31 mg, 0.8 mmol) in dioxane-$H_2O$ (4:1, 2 mL) was added to a stirred solution of 13 (350 mg, 0.8 mmol) in the same solvent mixture (15 mL). The mixture was stirred for 15 min, and glacial HOAc (2 mL) and $H_2O$ (50 mL) were added. The solution was extracted with $CHCl_3$ (3 x 100 mL) and the combined extracts were washed with 5% aqueous $NaHCO_3$ (50 mL) and $H_2O$ (50 mL), dried and evaporated to give a white foam (363 mg). This was purified by chromatography on a column of silica gel (20 g) using $CHCl_3$-MeOH (19:1) to give 14 as a white foam (351 mg) which crystallised from MeOH-$Et_2O$ as colourless prisms (231 mg, 65.7%).

3α,16α-Diacetoxy-14-hydroxy-5β,14β-card-20(22)-enolide 9

$Ac_2O$ (1 mL) was added to a solution of 14 (443 mg, 1.0 mmol) in pyridine (10 mL). The mixture was stirred for 17 h, diluted with ice-cold $H_2O$ (100 mL) and extracted with EtOAc (100 mL). The organic extract was washed successively with 2M HCl, 5% aqueous $NaHCO_3$ and $H_2O$ (50 mL each), dried and evaporated to give a white solid (477 mg). This was purified by chromatography on a column of silica gel (25 g) using $CHCl_3$-MeOH (39:1) to give 9 as a white solid (453 mg) which crystallised from EtOAc-petrol as colourless prisms (339 mg, 69.8%).

3α,16β-Diacetoxy-14-hydroxy-5β,14β-card-20(22)-enolide 10

The reaction of 17 (433 mg, 1.0 mmol) with $Ac_2O$ (1 mL) in pyridine (10 mL) as described above for the corresponding 16α-isomer 14 gave a crude product (431 mg) which was similarly purified by chromatography to give 10 as a white solid (430 mg). Crystallisation from EtOAc-petrol gave colourless prisms (305 mg, 64.2%).

ACKNOWLEDGEMENTS

We are indebted to Mr. I Machin and Dr. A. Sullivan (Pharmacology Departments, Glaxo Group Research Ltd., Greenford, Middlesex and Ware, Hertfordshire) for the biological results displayed in Table 1.

REFERENCES

1. Thomas, R., Boutagy, J., and Gelbart, A., J.PHARM.SCI. 33, 1649 (1974).
2. Güntert, T.W., and Linde, H.H.A., EXPERIENTIA 33, 697 (1977).
3. Güntert, T.W., and Linde, H.H.A., Chemistry and structure-activity relationships of cardioactive steroids, Chapter 2, in Cardiac Glycosides, Part 1, Experimental Pharmacology (Greef, K., Editor), Springer-Verlag, Berlin (1981).
4. Kovarikova, A., Kolarova, H., and Pitra, J., EXPERIENTIA 20, 263 (1964).
5. Haustein, K.-O., Megges, R., and Hauptmann, J., PHARMACOLOGY 10, 65 (1973).
6. Repke, K.R.H., PHARMAZIE 27, 693 (1972).
7. Haustein, K.-O., Markwardt, F., and Repke, K.R.H., EUR. J. PHARMACOL. 10, 1 (1970).
8. Haustein, K.-O., PHARMACOLOGY 11, 117 (1974).
9. Haustein, K.-O., and Hauptmann, J., ibid. 11, 129 (1974).

10. Humber, D.C., Phillipps, G.H., Dodds, M.G., Dolamore, P.G., and Machin, I., STEROIDS 42, 189 (1983).
11. Ragab, M.S., Linde, H., and Meyer, K., HELV. CHIM. ACTA 57, 474 (1962).
12. Hunger, A., and Reichstein, T., ibid. 33, 76 (1950).
13. Lindig, C., and Repke, K., BRITISH PATENT 1,153,698 (1969).
14. Bowers, A., Halsall, T.G., Jones, E.R.H., and Lemin, A.J., J. CHEM. SOC., 2555 (1953).
15. Hauser, E., Boffo, U., Meister, L., Sawlewicz, L., Linde, H.H.A., and Meyer, K., HELV. CHIM. ACTA 56, 2782 (1973).
16. Smith, S., J. CHEM. SOC. 23 (1931).
17. Humber, D.C., Jones, P.S., Phillipps, G.H., Dodds, M.G., and Dolamore, P.G., STEROIDS 42, 171 (1983).
18. Burges, R.A., Blackburn, K.J., and Spilker, B.A., LIFE SCI. 8, 1325 (1969).
19. Fullerton, D.S., Yoshioka, K., Rohrer, D.C., From, A.H.L., and Ahmed, K., MOL. PHARMACOL. 17, 43 (1980); SCIENCE 205, 917 (1979).
20. Thomas, R., Allen, J., Pitts, B.J.R., and Schwartz, A., EUR. J. PHARMACOL. 53, 227 (1979).
21. Rohrer, D.C., Fullerton, D.S., Yoshioka, K., From, A.H.L., and Ahmed, K., in: Advances in Chemistry: Computer Assisted Drug Design (Christoffersen, R.E., and Olsen, E.C., Editors), American Chemical Society, Symposium Series 112 (1979) p. 259.
22. Thomas, R., Brown, L., Boutagy, J., and Gelbart, A., CIRC. RES. SUPP. I 46 167 (1980).
23. Brown, L., Boutagy, J., and Thomas, R., ARZNEIM. FORSCH. 31, 1059 (1981).
24. Fullerton, D.S., Kihara, M., Deffo, T., Kitatsuji, E., Ahmed, K., Sumat, B., From, A.H.L., and Rohrer, D.C., J. MED. CHEM. 27, 256 (1984).