Steroids and Related Natural Products. Part XCI.[1] Synthesis of the Cardenolides Canarigenin and Uzarigenin[2]

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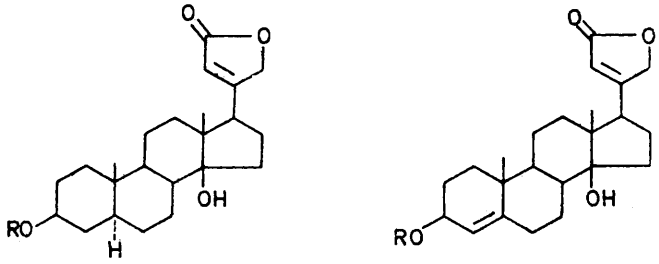
From 3β,14-dihydroxy-5β,14β-card-20(22)-enolide (digitoxigenin) (III), new formal total syntheses of canarigenin [3β,14-dihydroxy-14β-carda-4,20(22)-dienolide] (IIa) and uzarigenin [3β,14-dihydroxy-5α,14β-card-20(22)-enolide] (Ic) have been completed. The route found most convenient involved direct oxidation of digitoxigenin (III) with t-butyl hypochlorite to yield the 4β-chloro-3-ketone (IVb). Dehydrohalogenation to the 4-en-3-one (canarigenone) (V) was followed by selective reduction with lithium hydrido-tri-t-butoxyaluminate to afford canarigenin (IIa) and with lithium borohydride to yield uzarigenin (Ic).

INDIGENOUS use of the South African uzara root for treatment of dysentery attracted early scientific attention and chemical investigation of the uzarigenin glycosides



(I) a; 3-β-D-glucosyl-β-D-glucoside
b; 3-D-diginoside
c; R = H

(II) a; R = H
b; R = Ac

such as uzarin (Ia) was already under way in 1930.[3] Almost 20 years later the Reichstein [4] group found that mild hydrolysis of odoroside B (Ib) would liberate the intact aglycone uzarigenin (Ic). Shortly thereafter enzymic hydrolysis of uzarin (Ia) was used to obtain uzarigenin in good yield.[5] Generally, the uzarigenin glycosides are only weakly cardiac-active and a variety of these cardenolides have been isolated from plants.[6] Unlike cardenolides of the uzarigenin (Ic) type, the closely related canarigenin (IIa) family are more biologically active and their first isolation and structure determination is a fairly recent advance. The first example of a canarigenin glycoside (a D-digitoxoside) was isolated from *Digitalis canariensis* L., var. *isabelliana* and identified by Meyer and his colleagues.[7]

The initial synthetic approach to uzarigenin involved preparation of the isomeric 17α-uzarigenin.[8] More recently uzarigenin (Ic) and canarigenin (IIa) have been synthesized from '15α-hydroxy-5α-dihydrocortexone'[9] (15α,21-dihydroxy-5α-pregnane-3,20-dione). Our interest in further evaluating the anticancer and cardiac activity of natural products related to digitoxigenin (III) led us to undertake new syntheses of both cardenolides. The most direct approach appeared to be use of the readily available digitoxigenin [10,11] (III) as

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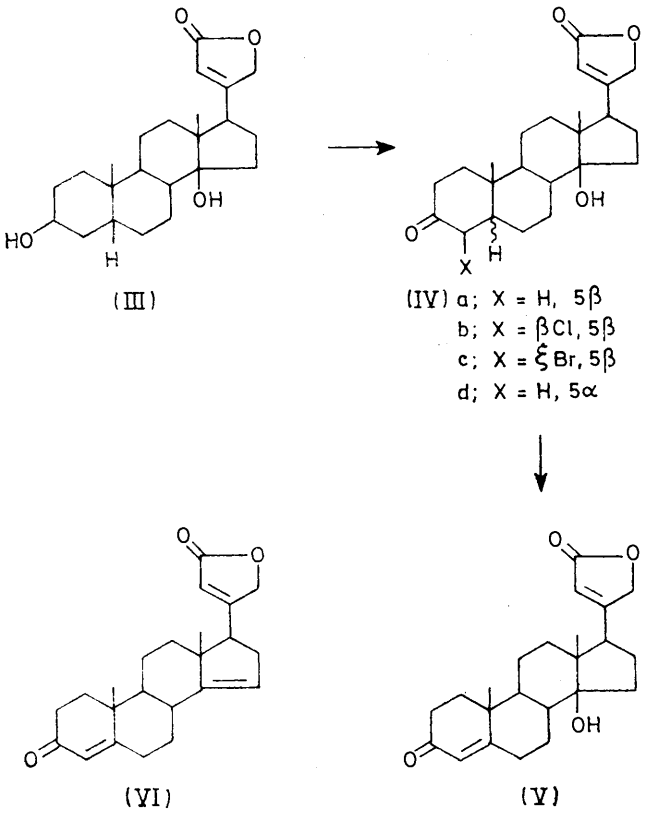
starting material and some of the experimental methods we have developed recently for synthesis of scillarenin.[12] In practice such transformation of digitoxigenin proved



very effective and allowed both uzarigenin and canarigenin to be prepared in satisfactory yields.

Selective oxidation of digitoxigenin (III) to digitoxigenone (IVa) proceeded well with either the chromium trioxide or the N-bromoacetamide procedure. Conversion of the ketone (IVa) into canarigenone (V) (also known as anhydroperiplogenone [7]) by halogenation and dehydrohalogenation [13] was studied in detail. Chlorination of digitoxigenone (IVa) by t-butyl hypochlorite [14] afforded a good yield of the 4β-chloro-ketone (IVb). The stereochemical assignment at position 4 was based on ^{1}H n.m.r. evidence. Appearance of the signal at δ 4.79 as a broad singlet suggested a 4β-orientation for the chlorine atom. The less stable 4-bromo-derivative (IVc) was prepared by using bromine either in acetic acid or in dimethylformamide and was used without purification for the dehydrohalogenation step.

Both halogeno-ketones (IVb and c) were dehydrohalogenated to canarigenone (V) in fair yield by means of lithium chloride in dimethylformamide or dimethylacetamide. Less satisfactory conversion was realized with lithium bromide or hot α-collidine. Attempts to improve the yield of canarigenone (V) by using the semicarbazide or the 2,4-dinitrophenylhydrazine method did in some cases lead to slight increases but overall these procedures were experimentally less convenient than that with lithium chloride–dimethylformamide. Meanwhile, it was found that canarigenone (V) could be obtained in 30—40% overall yields from digitoxigenin by direct oxidation and chlorination of the alcohol (III) followed by elimination of hydrogen chloride with lithium chloride–dimethylformamide. While retention of the 14β-hydroxy-group was essentially compatible with each of the preceding reactions, elimination (V) ⟶ (VI) was readily effected, for example, in hot alcohol with an acidic ion-exchange resin. Also, as expected, treatment with hydrochloric acid or toluene-*p*-sulphonic acid smoothly dehydrated the alcohol (V) to the olefin (VI).

Gentle reduction * of canarigenone (V) with, *e.g.*, lithium hydrido-tri-t-butoxyaluminate [9,12] completed the new route to canarigenin (IIa). Support for the structure was obtained from spectral data, allylic oxidation with active manganese dioxide to give the ketone (V), and comparison with an authentic sample.†

The synthetic route to uzarigenin (Ic) was completed by reducing canarigenone (V) in pyridine with lithium borohydride. The synthetic specimen was identical with the Farbwerken Hoechst sample.* In addition the alcohol (Ic) was further characterized by oxidation to uzarigenone (IVd), previously prepared from periplogenin.[9]

EXPERIMENTAL

Digitoxigenin (III) was prepared by acidic hydrolysis of digitoxin.[10] Otherwise, the general isolation (for example column chromatography on silica gel and t.l.c., with 3 : 3 : 4 acetone–chloroform–hexane as solvent, on silica gel) and characterization techniques have been outlined in Part LXXXVI.[15]

Digitoxigenone (IVa) [14-*Hydroxy*-3-*oxo*-5β,14β-*card*-20(22)-*enolide*].—(A) *By use of chromium trioxide.* A solution of digitoxigenin (III) (0.10 g) in pyridine (2 ml) was added dropwise with stirring to Sarett reagent [chromium trioxide (30 mg) in pyridine (0.4 ml)]. Stirring at room temperature was continued for 10 h, then the mixture was poured into ice–water, acidified with dilute hydrochloric acid, and extracted with chloroform. The combined extract was washed with water and concentrated. The residue (98 mg) was recrystallized from methanol–ether to yield the ketone (IVa) (75 mg), m.p. 197—202° (lit.,[11] 194—197°); δ (10% in $CDCl_3$) 0.95 (18-H_3), 1.20 (19-H_3), 2.83 (1 H, d, *J* 7.5 Hz, 17-H), 4.70

* The terminal reactions were modifications of those already reported by Fritsch and his colleagues for reduction of canarigenone (prepared from periplogenin or from 15α-hydroxycortexalone). We are grateful to Dr. W. Haede for providing us with samples of canarigenin and uzarigenin.

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[15] G. R. Pettit and Y. Kamano, *J. Org. Chem.*, 1974, **39**, 2632.

and 5.05 (2 H, a slightly doubled AB-type q, J 17 and 1.5 Hz, 21-H_2), 5.88 (1 H, t, J 1.5 Hz, 22-H); m/e 372 (M^+) and 354 ($M^+ - H_2O$).

Use of 2% chromium trioxide in acetic acid (12 ml) for oxidation of digitoxigenin (III) (1.0 g) in acetic acid (25 ml) during 4 h at room temperature led to digitoxigenone (0.72 g), m.p. 199—203°.

(B) *Use of N-bromoacetamide.* To a solution at 10 °C of digitoxigenin (III) (0.15 g) in methanol (18 ml), pyridine (6 ml), and water (0.6 ml) was added *N*-bromoacetamide (0.15 g). The mixture was kept in the dark for 18 h at room temperature. The ketone (IVa) (0.145 g), m.p. 198—202°, was isolated as described in (A) and was identical with the specimen obtained by that procedure.

4β-*Chloro*-14-*hydroxy*-3-*oxo*-5β,14β-*card*-20(22)-*enolide* (IVb).—(A) *From digitoxigenin* (III). To a solution prepared from digitoxigenin (III) (0.70 g), t-butyl alcohol (32 ml), and water (1 ml) were added (at 10 °C) t-butyl hypochlorite (0.6 ml) and concentrated hydrochloric acid (0.28 ml). The mixture was allowed to warm to room temperature (25 °C) during 8 h, poured into ice–dilute sodium hydrogen carbonate, and extracted with methylene chloride. The combined extract was washed with water and evaporated to yield crude product (0.65 g), which was recrystallized from methanol–ether to afford the *chloro-ketone* (IVb) as prisms (0.50 g), m.p. 131—133° (Found: C, 67.95; H, 7.65; Cl, 8.8. $C_{23}H_{31}ClO_4$ requires C, 67.85; H, 7.7; Cl, 8.65%); t.l.c. R_F 0.35; $\nu_{max.}$ (KBr) 3 500 (OH), 1 780, 1 735 (butenolide ring), 1 715 (3-one), 1 625, and 1 618 cm^{-1} (C=C); δ (10% in $CDCl_3$) 0.93 (3 H, s, 18-H_3), 1.08 (3 H, s, 19-H_3), 2.77 (1 H, d, J 7 Hz, 17-H), 4.72 and 5.07 (2 H, a slightly doubled AB-type q, J 17 and 1.5 Hz, 21-H_2), 4.79 (1 H, s, 4α-H), and 5.88 (1 H, t, J 1.5 Hz, 22-H); m/e 406 (M^+), 388 ($M^+ - H_2O$), 370 ($M^+ - HCl$), 352 ($M^+ - H_2O - HCl$), 342, 337, 320, and 284.

(B) *From digitoxigenone* (IVa). The ketone (IVa) (0.30 g) in t-butyl alcohol (16 ml), water (0.5 ml), and concentrated hydrochloric acid (0.1 ml) was chlorinated with t-butyl hypochlorite (0.20 ml) during 4 h at room temperature. Otherwise the reaction was performed and the product isolated as described in (A) to give the ketone (IVb) (0.21 g), m.p. 130—133°, identical with that described above.

Bromination of Digitoxigenone (IVa).—A solution prepared from acetic acid (10 ml), anhydrous sodium acetate (90 mg), and bromine (0.175 g) was added dropwise to a solution of the ketone (IVa) (0.35 g) in acetic acid (10 ml) containing 6% hydrogen bromide. The bromination was carried out with stirring at 10—15 °C and when complete sodium acetate (0.90 g) in water (6 ml) was added. The mixture was poured into ice–water and the solid bromo-ketone (IVc) (0.30 g) was collected. The crude product appeared as one spot (R_F 0.45) on t.l.c. and was used without further purification.

The same brominated ketone (IVc) (0.12 g) was obtained by slowly adding bromine (80 mg) in dimethylformamide (2 ml), with stirring at room temperature, to a solution of the ketone (IVa) (0.16 g) in dimethylformamide (4 ml) containing toluene-*p*-sulphonic acid monohydrate (4 mg). After 2 h the mixture was diluted with chloroform and poured into water. The chloroform layer was washed with water, dilute aqueous sodium hydrogen carbonate, dilute hydrochloric acid, and water. Removal of solvent left the 4-bromo-ketone (IVc), which was used without further purification.

14-*Hydroxy*-3-*oxo*-14β-*carda*-4,20(22)-*dienolide* [*Canarigenone*] (V).—(A) *From the chloro-ketone* (IVc). (i) *With lithium chloride or bromide.* A solution prepared from the chloro-ketone (IVc) (0.20 g), lithium chloride (0.20 g), and dimethylformamide (5 ml) was heated at reflux (nitrogen atmosphere) for 6 h. The mixture was poured into ice–water and extracted with chloroform, and the combined extract was washed with water, dilute hydrochloric acid, and water. Removal of solvent left a residue which was separated by preparative t.l.c. with 3 : 3 : 4 acetone–chloroform–hexane as mobile phase. The zone corresponding to R_F 0.23 was eluted with 4 : 1 chloroform–methanol and this fraction was recrystallized from methylene chloride–acetone to yield canarigenone (V) (90 mg, 45%) as prisms, m.p. 257—263° (lit.,[9] 250—264°) (Found: C, 74.6; H, 8.15. Calc. for $C_{23}H_{30}O_4$: C, 74.55; H, 8.15%); δ (10% in $CDCl_3$) 0.95 (3 H, s, 18-H_3), 1.19 (3 H, s, 19-H_3), 2.76 (1 H, d, J 8 Hz, 17-H), 4.73 and 5.07 (2 H, a slightly doubled AB-type q, J 17 and 1.5 Hz, 21-H_2), 5.75 (1 H, d, J 1.5 Hz, 4-H), and 5.89 (1 H, t, J 1.5 Hz, 22-H); m/e 370 (M^+), 352 ($M^+ - H_2O$), 337, 334, 319, 310, 295, 284, and 260.

When lithium bromide (0.10 g) was substituted for lithium chloride, the chloro-ketone (IVb) (0.10 g) gave the αβ-unsaturated ketone (V) (40 mg), m.p. 256—263°. With either lithium chloride or bromide and with dimethylacetamide as solvent the yield was less (38%).

(ii) *By use of α-collidine.* A solution of the chloro-ketone (IVb) (40 mg) in α-collidine (5 ml) was heated (nitrogen atmosphere) at reflux for 10 h. The crude product obtained by removal of solvent was chromatographed and the fraction eluted by hexane–acetone (4 : 1) was recrystallized from methylene chloride–acetone to yield the ketone (V) (4.8 mg), m.p. 253—259°.

(iii) *By use of semicarbazide or 2,4-dinitrophenylhydrazine.* A solution prepared from the chloro-ketone (IVb) (70 mg), semicarbazide hydrochloride (50 mg), sodium acetate (50 mg), and 98% acetic acid (12 ml) was heated (nitrogen atmosphere) at 70 °C for 2 h. Pyruvic acid (0.1 ml) and water (1 ml) were added and heating was continued for an additional 2 h at 70 °C. After cooling, the solution was poured into water and extracted with methylene chloride, and the extract was washed with 0.1% sodium hydroxide, 5% sodium chloride, and water. Removal of solvent left a residue (75 mg), which was subjected to column chromatography. The fraction eluted by 5 : 1 hexane–acetone was recrystallized from methylene chloride–acetone to provide canarigenone (V) (42 mg), m.p. 257—263°.

Repetition of the preceding experiment with the chloro-ketone (IVb) (50 mg) and 2,4-dinitrophenylhydrazine (30 mg) in place of semicarbazide hydrochloride led to the ketone (V) (24 mg, 49%), m.p. 256—261°.

(B) *From the bromo-ketone* (IVc). By means of method (A), procedure (i), the bromo-ketone (IVc) (0.16 g) was converted, with lithium chloride (0.16 g) and dimethylacetamide (4 ml), into canarigenone (V) (52 mg, 38%), m.p. 255—262°. In this experiment the mixture was heated at reflux for 6 h. Substitution of lithium bromide for lithium chloride and use of a 7 h period at reflux provided the ketone (V) (48 mg, 30%), m.p. 253—259°.

Application of method (A), procedure (ii) [bromo-ketone (80 mg), α-collidine (10 ml); 8 h reflux period] gave the ketone (V) (22 mg), m.p. 254—260°.

Modification of method (A), procedure (iii) (as follows) led to the ketone (V) (28 mg, 28%), m.p. 258—263°. Here, dioxan (3 ml) was substituted for acetic acid and the

bromo-ketone (IVc) (0.10 g) was treated with semicarbazide hydrochloride (35 mg). When the 2,4-dinitrophenylhydrazine variation of method (A), procedure (iii) was applied to the bromo-ketone (IVc) (0.10 g), separation of product by preparative t.l.c. afforded the ketone (V) (25 mg, 25%), m.p. 255—261°.

14,15-*Didehydrocanarigenone* (VI) [3-*Oxocarda*-4,14,20(22)-*trienolide*].—A mixture of the ketone (V) (80 mg), ethanol (6 ml), and Amberlite CG-120 resin (H^+ form; 0.40 g) was heated at reflux for 2 h. The solution was filtered and concentrated and the residue was separated by preparative t.l.c. with acetone–chloroform–hexane (3 : 3 : 4) as solvent. The fraction at R_F 0.46 was eluted with chloroform–methanol (4 : 1) and recrystallized from methylene chloride–methanol to yield the *olefin* (VI) as prisms (71 mg), m.p. 288—292° (Found: C, 78.3; H, 8.1. $C_{23}H_{28}O_3$ requires C, 78.34; H, 8.0%); $\nu_{max.}$ (KBr) 1 780, 1 735 (butenolide ring), 1 650 (3-one), 1 620, 1 605, 970, and 785 cm^{-1} (C=C); $\lambda_{max.}$ (MeOH) 227—234 nm (log ε 4.35); δ (10% in $CDCl_3$) 0.89 (3 H, s, 18-H_3), 1.20 (3 H, s, 9-H_3), 2.8 (1 H, d, *J* 7 Hz, 17-H), *ca.* 4.77 (2 H, narrow d, *J* 1.5 Hz, 21-H_2), 5.32 (1 H, d, *J* 1.5 Hz, 15-H), 5.78 (1 H, d, *J* 1.5 Hz, 4-H), and 5.90 (1 H, d, *J* 1.5 Hz, 22-H); *m/e* 352 (M^+), 337, 320, and 284.

A similar yield (69 mg) of ketone (VI) was obtained by employing Dowex 50 W-X80 resin (H^+ form). In another experiment dehydration of canarigenone (V) (40 mg) was performed (90 min at reflux) in methanol (2 ml) containing 35% hydrochloric acid (0.1 ml) to give the olefin (VI) (32 mg), m.p. 287—290°. Similarly, dehydrating the alcohol (V) (20 mg) in ethanol (125 ml) containing toluene-*p*-sulphonic acid (4 mg) at room temperature for 6 h gave the olefin (VI) (17 mg), m.p. 288—291°.

Canarigenin (IIa) [3β,14-*Dihydroxy*-14β-*carda*-4,20(22)-*dienolide*].—Canarigenone (V) (0.10 g) was reduced with lithium hydrido-tri-t-butoxyaluminate (0.60 g) in dry tetrahydrofuran (15 ml) essentially as described by Fritsch and his colleagues.[9] However, in this case the crude product was separated by chromatography on a column of silica gel. The fraction eluted by 3 : 1 hexane–acetone was recrystallized from acetone–hexane to afford canarigenin (71 mg), m.p. 259—261° (lit.,[9] 260—262°) (Found: C, 74.05; H, 8.8. Calc. for $C_{23}H_{32}O_4$: C, 74.15; H, 8.65%); t.l.c. R_F 0.22 (acetone–chloroform–n-hexane, 3 : 3 : 4); δ (10% in $CDCl_3$) 0.89 (3 H, s, 18-H_3), 1.00 (3 H, s, 19-H_3), 2.75 (1 H, d, *J* 7 Hz, 17α-H), 4.12br (1 H, 3α-H), 4.70 (*J* 2 Hz, 21-H_2), 5.32br (1 H, 4-H), and 5.83 (1 H, t, *J* 2 Hz, 22-H); *m/e* 372 (M^+), 354 ($M^+ - H_2O$), 336 ($M^+ - 2\,H_2O$), and 321.

The sodium borohydride (12 mg) procedure of Fritsch and his co-workers [9] was also modified for reduction of canarigenone to canarigenin. Tetrahydrofuran (2.5 ml)–methanol (3 ml) was used as solvent for 20 mg of the ketone (V) and the reaction was allowed to proceed for 3 h at 10 °C. Canarigenin (IIa) (12 mg), m.p. 257—260°, was isolated as just described. The yield of allylic alcohol (IIa) (10 mg), m.p. 256—259°, was less when dioxane–water (4 : 1) (12 ml) was used as solvent at 18 °C.

Each synthetic specimens of canarigenin (IIa) was identical with an authentic specimen.* In addition, the acetate derivative (IIb) (16 mg) was prepared from canarigenin (IIa) (20 mg) and recrystallized from acetone–hexane to provide needles, m.p. 204—206° (lit.,[9] 203—205°), having spectral data in accord with those already reported.[9]

Oxidation of Canarigenin (IIa) *to Canarigenone* (V).—(A) *With manganese dioxide.* A mixture of canarigenin (IIa) (50 mg), chloroform (5 ml), and freshly prepared active manganese dioxide (0.50 g) was shaken at room temperature for 18 h. The solution was filtered and evaporated and the residue subjected to column chromatography. Elution with 5 : 1 hexane–acetone gave a fraction which was recrystallized from methylene chloride–acetone to provide the ketone (V) (36 mg), m.p. 259—262°. When the allylic oxidation was repeated with 20 mg of alcohol (IIa) in benzene (2 ml) and 0.10 g of manganese dioxide, the yield of ketone (V) was 13 mg.

(B) *With chromium trioxide.* The chromium trioxide–pyridine procedure [already described for obtaining the ketone (IVa)] was used for converting 20 mg of the alcohol (IIa) into 15 mg of the ketone (V), m.p. at 258—263°. Similarly, the chromium trioxide–acetic acid method gave 14 mg of the ketone (V), m.p. 251—254°, from 20 mg of the alcohol (IIa). In both cases the product was isolated and purified as summarized in method (A).

Uzarigenin (Ic) [3β,14-*Dihydroxy*-5α,14β-*card*-20(22)-*enolide*].—Lithium borohydride (0.10 g) was added to a solution of canarigenone (V) (0.16 g) in dry pyridine (6 ml) maintained in an ice-bath. Before removal of the excess of reducing agent with dilute sulphuric acid at −5 °C, stirring and cooling were continued for 5 h. The mixture was extracted with chloroform and solvent removed to yield a residue (0.18 g) which was purified by column chromatography. Elution with hexane–acetone (3 : 1) gave a fraction which crystallized from methylene chloride–methanol to yield uzarigenin as prisms (12 mg), m.p. 246—249° (lit.,[9] 247—249°), showing spectral data corresponding with those reported.[9]

Analogous results were obtained by reducing the ketone (V) (20 mg) with sodium borohydride (18 mg) in dioxan–water (4 : 1) (12 ml) for 3 h at 10 °C. Uzarigenin (12 mg), m.p. 246—248° (from chloroform–methanol–hexane), was isolated as described for reduction with lithium borohydride. Both synthetic samples were identical with a sample kindly provided by Dr. Haede.*

Treatment of uzarigenin (Ic) (30 mg) with acetic anhydride (0.45 ml)–pyridine (0.6 ml) for 18 h at room temperature and recrystallization of the crude acetate from methylene chloride–hexane led to uzarigenin 3β-acetate (24 mg), m.p. 265—268° (lit.,[9] 266—267°); *m/e* 416 (M^+), 398 ($M^+ - H_2O$), 356 (M^+ — AcOH), and 338 (M^+ — AcOH — H_2O).

Uzarigenone (IVd) [14-*Hydroxy*-3-*oxo*-5α,14β-*card*-20(22)-*enolide*].—(A) *By use of* N-*bromoacetamide.* Oxidation of uzarigenin (Ic) (20 mg) with *N*-bromoacetamide (25 mg) in methanol (3.5 ml)–acetone (2 ml)–water (0.1 ml) at room temperature during 22 h was carried out as described for the preparation of the ketone (IVa). The product was purified by column chromatography and the fraction eluted with 5 : 1 hexane–acetone was recrystallized from methylene chloride–methanol to yield uzarigenone (IVd) (12 mg), m.p. 269—272° (lit.,[9] 270—273°) (Found: C, 74.25; H, 8.7. Calc. for $C_{23}H_{32}O_4$: C, 74.15; H, 8.65%); t.l.c. R_F 0.18; δ (10% in $CDCl_3$) 0.89 (3 H, s, 18-H_3), 1.01 (3 H, s, 19-H_3), 2.79 (1 H, d, *J* 8 Hz, 17α-H), 4.80 and 5.00 (2 H, slightly doubled AB-type q, *J* 17 and 2 Hz, 21-H_2), and 5.88 (1 H, t, *J* 2 Hz, 22-H); *m/e* 372 (M^+) and 354 ($M^+ - H_2O$).

(B) *By use of chromium trioxide.* The ketone (IVd)

* See footnote p. 1973.

(12 mg) (m.p. 269—273°) was obtained by oxidizing uzarigenin (Ic) (20 mg) with chromium trioxide (15 mg) in acetic acid (3 ml).

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