

14,15-*cis* Glycols derived from Digitoxigenin<sup>1)</sup>

MASASHI OKADA and YUKIO SAITO

Tokyo Biochemical Research Institute<sup>2)</sup>

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Treatment of  $\beta$ -anhydrodigitoxigenin acetate (I) with osmium tetroxide gave two *cis*-glycols ( $\beta$ - and  $\alpha$ -), 15 $\beta$ -hydroxydigitoxigenin 3-monoacetate (II) and 15 $\alpha$ -hydroxy-14 $\alpha$ -digitoxigenin 3-monoacetate (IV) in the ratio of approximately 1:9. Acid hydrolysis of II and IV yielded 15 $\beta$ -hydroxydigitoxigenin (III) and 15 $\alpha$ -hydroxy-14 $\alpha$ -digitoxigenin (V). On the other hand, treatment of  $\beta$ -anhydro-17 $\alpha$ -digitoxigenin ( $\beta$ -anhydromenabegenin) (X) as well as its acetate (IX) with the reagent resulted exclusively in the formation of the  $\beta$ -glycol, 15 $\beta$ -hydroxy-17 $\alpha$ -digitoxigenin (15 $\beta$ -hydroxymenabegenin) (XII) and its 3-monoacetate (XI) respectively.

In view of the recent interesting finding<sup>3)</sup> that 15 $\alpha$ -hydroxydigitoxigenin<sup>4,5)</sup> did not exhibit any cardiotonic activity, in spite of possessing all the essential structural requirements for the specific biological activity of the cardenolide,<sup>6)</sup> an attempt was made to prepare 15 $\beta$ -hydroxydigitoxigenin (III) and other related unnatural cardenolides oxygenated at 15-position using digitoxigenin as the starting material, in order to examine further the structure-activity relationship of the cardenolide.

Treatment of  $\beta$ -anhydrodigitoxigenin acetate (I) derivable from digitoxigenin with osmium tetroxide in ether and pyridine<sup>7,8)</sup> gave two *cis*-glycols ( $\beta$ - and  $\alpha$ -), (II) and (IV) (Fig. 1a), in the ratio of approximately 1:9 after repeated fractional crystallizations. The configuration of the minor glycol (II) was definitely established as the  $\beta$ -glycol by oxidizing it with chromic anhydride-pyridine complex to 15-oxodigitoxigenin acetate (VII) reported earlier.<sup>4)</sup> Accordingly, the major glycol (IV) should be the  $\alpha$ -glycol. Predominant formation of the  $\alpha$ -glycol (IV) was reasonably anticipated on the basis of preferred rear-side attack of the reagent. Similar result to that of the present work was reported on another  $\Delta^{14}$ -cardenolide,  $\beta$ -anhydro-uzarigenin acetate, by Brandt, *et al.*,<sup>8)</sup> when this work had been almost accomplished.<sup>4)</sup> In this connection, it is interesting to note that pregna-4,14-diene-3,20-dione afforded 14 $\beta$ ,15 $\beta$ -glycol as the principal product on treatment with osmium tetroxide.<sup>9)</sup>

Hydrolysis of II and IV in methanol with hydrochloric acid gave 15 $\beta$ -hydroxydigitoxigenin (III) and 15 $\alpha$ -hydroxy-14 $\alpha$ -digitoxigenin (V) respectively. Acetylation of IV and V with acetic anhydride in pyridine afforded the identical acetate (VI), while the diacetate of III was not prepared owing to shortage of the materials. On the other hand, 15-oxodigitoxigenin (VIII) was prepared by acid hydrolysis of VII with the object of comparing biological activity.

- 1) The preliminary report of this work appeared in *Chem. Pharm. Bull.* (Tokyo), **15**, 352 (1967).
- 2) Location: *Takada 3-Chome, Toshima-ku, Tokyo*.
- 3) T. Shigei, M. Katori, H. Murase, and S. Imai, *Experientia*, **20**, 572 (1964); S. Imai, H. Murase, M. Katori, M. Okada, and T. Shigei, *Jap. J. Pharmacol.*, **15**, 62 (1965).
- 4) M. Okada and M. Hasunuma, *Yakugaku Zasshi*, **85**, 822 (1965).
- 5) H. Ishii, T. Tozyo, and D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **11**, 576 (1963).
- 6) Ch. Tamm, "Proc. 1st Internat. Pharmacol. Meeting," Vol. 3, ed. by W. Wilbrandt, Pergamon Press, Oxford, 1963, p. 11.
- 7) R. Tschesche, W. Freytag, and G. Snatzke, *Chem. Ber.*, **92**, 3053 (1959).
- 8) R. Brandt, W. Stöcklin, and T. Reichstein, *Helv. Chim. Acta*, **49**, 1662 (1966).
- 9) H. Hasegawa, Y. Sato, and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **11**, 1275 (1963).

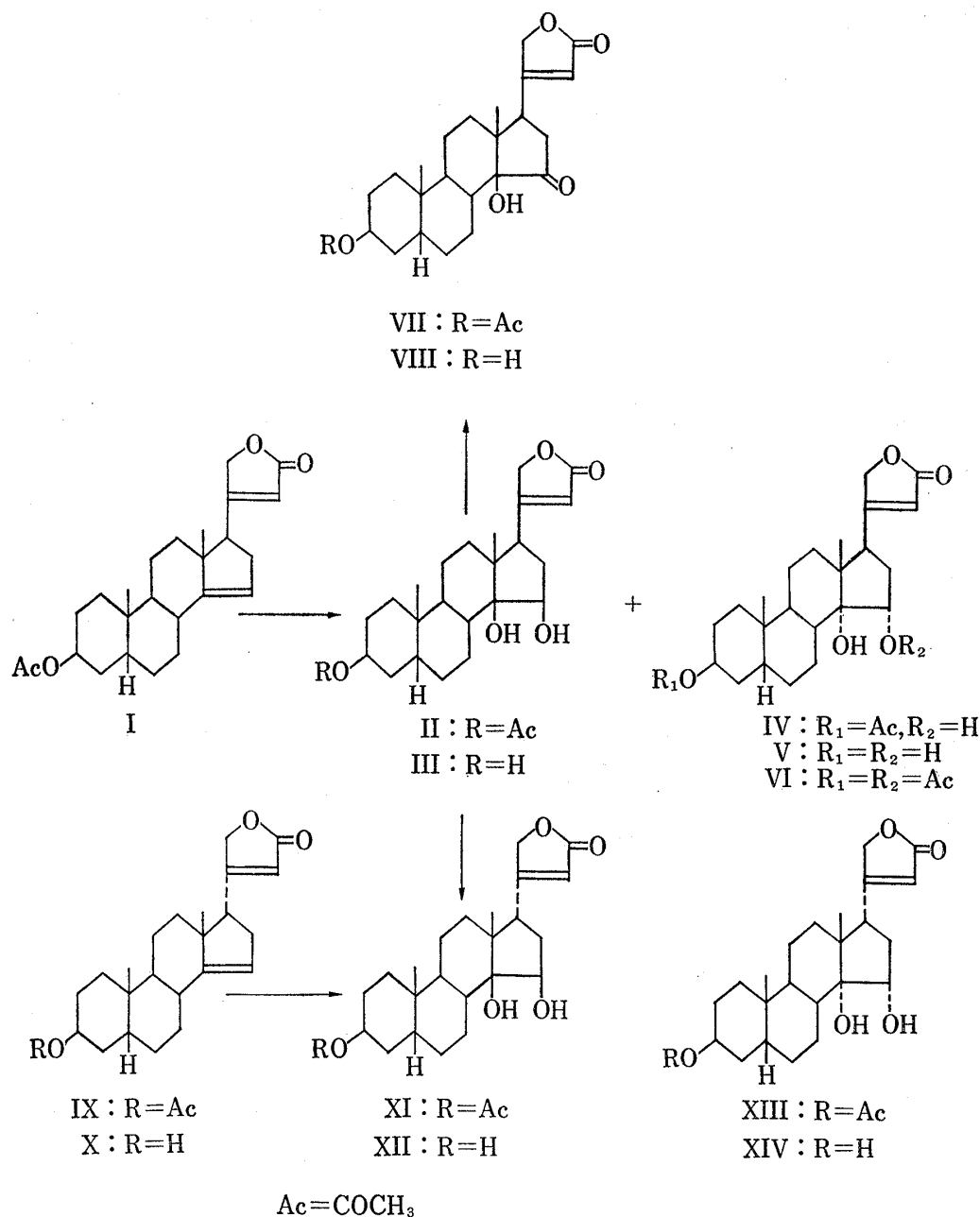


Chart 1

In view of the fact that all the natural potent cardenolides belong to the 14 $\beta$ -hydroxylated 17 $\beta$ -cardenolide (14,17-*cis*), it seemed to be of value from the pharmacological point of view to prepare the 14-hydroxylated 14 $\alpha$ ,17 $\alpha$ -cardenolide, which has also 14,17-*cis*-configuration. Although the first synthesis of a cardenolide, 3 $\beta$ -hydroxy-5 $\alpha$ ,14 $\alpha$ ,17 $\alpha$ -card-20(22)-enolide, was reported earlier<sup>10)</sup> which possesses 14 $\alpha$ ,17 $\alpha$ -configuration but lacks in the hydroxyl group at 14-position, no cardenolide of this unnatural type having 14-hydroxyl group is recorded. So an attempt was made to prepare 3 $\beta$ ,14,15 $\alpha$ -trihydroxy-5 $\beta$ ,14 $\alpha$ ,17 $\alpha$ -card-20(22)-enolide (XIV) in the similar way to that described above on III.

Thus,  $\beta$ -anhydro-17 $\alpha$ -digitoxigenin ( $\beta$ -anhydromenabegenin) acetate (IX)<sup>11,12)</sup> derivable from digitoxigenin was used as the starting material. Treatment of IX in ether as well

10) M. Okada and Y. Saito, *Steroids*, **6**, 357 (1965).

11) M. Frèrejacque, *Compt. Rend.*, **248**, 2382, 3027 (1959).

12) T. Wada and D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **13**, 308 (1965).

as in ether and pyridine with osmium tetroxide<sup>8)</sup> afforded the  $\beta$ -glycol (XI) exclusively, exhibiting a marked contrast to the case of I. Careful thin-layer chromatographic examination of the reaction product did not reveal the presence of another glycol (XIII) (Fig. 1b). Acid hydrolysis of XI gave  $15\beta$ -hydroxy- $17\alpha$ -digitoxigenin ( $15\beta$ -hydroxymenabegenin) (XII), which was also found to be the sole product on treatment of  $\beta$ -anhydro- $17\alpha$ -digitoxigenin (X) with osmium tetroxide under conditions indicated in the Experimental. The configuration of XI and XII was established as such on the basis of the fact that III could be converted into XII under conditions employed for the isomerization of  $17\beta$ -cardenolide to  $17\alpha$ -cardenolide.<sup>13)</sup>

Consequently, the present attempt to prepare XIV in the way described above was foiled by strictly stereospecific front-side attack of the reagent, while predominant formation of the  $\beta$ -glycols (XI, XII) was anticipated because of  $\alpha$ -configuration of the bulky butenolide ring at 17-position of IX and X.

Pharmacological examinations<sup>14)</sup> using the isolated frog's heart (Straub's preparation) disclosed that III and VIII possessed a definite cardiotonic activity, while V and XII did not any cardiotonic activity.

#### Experimental<sup>15)</sup>

**$15\beta$ -Hydroxydigitoxigenin 3-Monoacetate ( $3\beta$ -Acetoxy- $14,15\beta$ -dihydroxy- $5\beta$ -card-20(22)-enolide)(II) and  $15\alpha$ -Hydroxy- $14\alpha$ -digitoxigenin 3-Monoacetate ( $3\beta$ -Acetoxy- $14,15\alpha$ -dihydroxy- $5\beta,14\alpha$ -card-20(22)-enolide)(IV)**—To a solution of  $\beta$ -anhydrodigitoxigenin acetate (I) (1.6 g) in ether (35 ml) and pyridine (10 ml) was added a solution of  $\text{OsO}_4$  (1.3 g) in ether (165 ml). The mixture solution was allowed to stand at  $5^\circ$  for 24 hr. Then the solution was diluted with MeOH (200 ml) and  $\text{H}_2\text{S}$  was passed in to precipitate  $\text{OsO}_2$ , which was removed by filtration. The filtrate was concentrated under reduced pressure to give 1.65 g of a brown crystalline residue, whose thin-layer chromatography (TLC) revealed the presence of two principal reaction products as is shown in Fig. 1a. It was chromatographed on a column of acid-washed alumina (15 g). Elution with benzene- $\text{CHCl}_3$  (1:1) gave a mixture (1.5 g) of II and IV, which was separated by repeated recrystallizations from MeOH to give II (70 mg) and IV (600 mg). II, mp  $254\text{--}257^\circ$ ,  $[\alpha]_D^{20} 0^\circ$  ( $c=1.34$ ,  $\text{CHCl}_3$ ), UV  $\lambda_{\text{max}}$   $m\mu$  (log  $\epsilon$ ) 216 (4.22), IR  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3530 (OH), 1780 (sh), 1735 (br), 1623 (butenolide and acetyl C=O). Anal. Calcd. for  $\text{C}_{25}\text{H}_{36}\text{O}_6$ : C, 69.42; H, 8.39. Found: C, 69.17; H, 8.24. IV, mp  $201\text{--}203^\circ$ ,  $[\alpha]_D^{25} +38.8^\circ$  ( $c=0.747$ ,  $\text{CHCl}_3$ ), UV  $\lambda_{\text{max}}$   $m\mu$  (log  $\epsilon$ ): 217.5 (4.20), IR  $\nu_{\text{max}}$   $\text{cm}^{-1}$ : 3500, 3450 (OH), 1780, 1745, 1630 (butenolide and acetyl C=O). Anal. Calcd. for  $\text{C}_{25}\text{H}_{36}\text{O}_6 \cdot \text{H}_2\text{O}$ : C, 66.64; H, 8.50. Found: C, 66.82; H, 8.44.

**$15\beta$ -Hydroxydigitoxigenin ( $3\beta,14,15\beta$ -Trihydroxy- $5\beta$ -card-20(22)-enolide)(III)**—A solution of II (20 mg) in a mixture of MeOH (10 ml) and 10% HCl (10 ml) was allowed to stand for 18 hr at room temperature. After addition of  $\text{H}_2\text{O}$  (10 ml), MeOH was removed *in vacuo* to yield a crystalline precipitate, which was

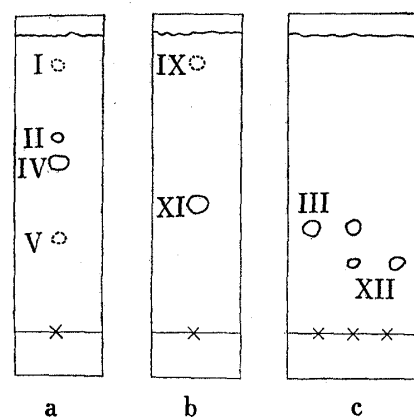


Fig. 1. Thin-Layer Chromatography of *cis*-Glycols

- a: reaction product obtained after treatment of I with  $\text{OsO}_4$   
 b: reaction product obtained after treatment of IX with  $\text{OsO}_4$   
 c: reaction product obtained after treatment of III in dimethylformamide with anhydrous AcONa and sodium tosylate

13) A. Kuritzkes, J. von Euw, and T. Reichstein, *Helv. Chim. Acta*, **42**, 1502 (1959).

14) T. Shigei and S. Mineshita, *Experientia*, **24**, 466 (1968); Private communication from Prof. T. Shigei, Department of Pharmacology, Institute for Cardiovascular Diseases, Tokyo Medical and Dental University.

15) Melting points were determined on a Kofler block and are uncorrected. Ultraviolet (UV) spectra were measured in 99% ethanol solution. Infrared (IR) spectra were determined in potassium bromide disks on Hitachi EPI-S2 spectrophotometer; br=broad, sh=shoulder. TLC plate was prepared according to the Stahl's procedure using aluminum oxide G (E. Merck AG) as adsorbent. The solvent system used was ethyl methyl ketone-heptane (1:1) and the cardenolides spots were revealed by heating plate at  $110^\circ$  for 10 min after spraying it with 95% sulfuric acid or by Kedde reagent.

recrystallized from MeOH to give III (7 mg). mp 245—247°,  $[\alpha]_D^{25}$  0° ( $c=0.32$ , MeOH), UV  $\lambda_{\max}$   $m\mu$  (log  $\epsilon$ ): 217 (4.19), IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 3475 (OH), 1784, 1750, 1625 (butenolide). *Anal.* Calcd. for  $\text{C}_{23}\text{H}_{34}\text{O}_5$ : C, 70.74; H, 8.78. Found: C, 70.48; H, 8.74.

**15 $\alpha$ -Hydroxy-14 $\alpha$ -digitoxigenin (3 $\beta$ ,14,15 $\alpha$ -Trihydroxy-5 $\beta$ ,14 $\alpha$ -card-20(22)-enolide) (V)**—A solution of IV (100 mg) in a mixture of MeOH (50 ml) and 10% HCl (50 ml) was allowed to stand for 20 hr at room temperature. After working up in the same way as described above, the resulting crystalline product was recrystallized from acetone-ether to give V (62 mg). mp 130—140°,  $[\alpha]_D^{25}$  +31.5° ( $c=0.63$ ,  $\text{CHCl}_3$ ), UV  $\lambda_{\max}$   $m\mu$  (log  $\epsilon$ ): 217.5 (4.20), IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 3425 (br) (OH), 1780, 1739, 1620 (butenolide). *Anal.* Calcd. for  $\text{C}_{23}\text{H}_{34}\text{O}_5 \cdot \text{H}_2\text{O}$ : C, 67.62; H, 8.88. Found: C, 67.53; H, 8.55.

**15 $\alpha$ -Hydroxy-14 $\alpha$ -digitoxigenin Diacetate (3 $\beta$ , 15 $\alpha$ -Diacetoxy-14-hydroxy-5 $\beta$ ,14 $\alpha$ -card-20(22)-enolide) (VI)**—Acetylation of IV and V in the usual way with acetic anhydride and pyridine gave the identical acetate (VI), which was recrystallized from acetone-ether-petroleum ether. mp 232—234°,  $[\alpha]_D^{25}$  +38.3° ( $c=1.33$ ,  $\text{CHCl}_3$ ), UV  $\lambda_{\max}$   $m\mu$  (log  $\epsilon$ ): 217.5 (4.20), IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 3540 (OH), 1785, 1745, 1730 (sh), 1630 (butenolide and acetyl C=O). *Anal.* Calcd. for  $\text{C}_{27}\text{H}_{38}\text{O}_7$ : C, 68.33; H, 8.07. Found: C, 68.36; H, 8.08.

**Oxidation of II to 15-Oxidigitoxigenin Acetate (3 $\beta$ -Acetoxy-14-hydroxy-15-oxo-5 $\beta$ -card-20(22)-enolide) (VII)**—A solution of II (20 mg) in pyridine (0.2 ml) was added to a slurry of chromic anhydride (23 mg) and pyridine (0.2 ml). The reaction mixture was allowed to stand at room temperature overnight, poured into ice-water, and extracted with ether. The organic layer was washed with  $\text{H}_2\text{O}$  and dried over anhyd.  $\text{Na}_2\text{SO}_4$ . Evaporation of the solvent afforded 16.4 mg of a crystalline product, which was recrystallized from acetone-ether-petroleum ether to give VII (10 mg), mp 230—238°, identical with the authentic sample prepared earlier<sup>4</sup>) in the mixed melting point and comparison of the IR spectrum.

**15-Oxidigitoxigenin (3 $\beta$ ,14-Dihydroxy-15-oxo-5 $\beta$ -card-20(22)-enolide) (VIII)**—A solution of VII (67 mg) in a mixture of MeOH (40 ml) and 10% HCl (40 ml) was allowed to stand for 30 hr at room temperature. After working up in the way described above, the saponified product was recrystallized from acetone-ether-petroleum ether to give VIII (21 mg). mp 215—217°,  $[\alpha]_D^{25}$  +12.7° ( $c=0.71$ ,  $\text{CHCl}_3$ ), UV  $\lambda_{\max}$   $m\mu$  (log  $\epsilon$ ): 215 (4.19), IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 3550, 3400 (OH), 1775, 1750 (sh), 1735, 1708, 1618 (butenolide and 15 C=O). *Anal.* Calcd. for  $\text{C}_{23}\text{H}_{32}\text{O}_5$ : C, 71.10; H, 8.30. Found: C, 71.45; H, 8.22.

Acetylation of the above (VIII) in the usual way with acetic anhydride and pyridine gave VII, mp 235—240°, which was identical with the authentic specimen of VII in the mixed melting point and comparison of the IR spectrum.

**15 $\beta$ -Hydroxy-17 $\alpha$ -digitoxigenin (15 $\beta$ -Hydroxymenabegenin) 3-Monoacetate (3 $\beta$ -Acetoxy-14,15 $\beta$ -dihydroxy-5 $\beta$ ,17 $\alpha$ -card-20(22)-enolide) (XI)**—a) To a solution of  $\beta$ -anhydro-17 $\alpha$ -digitoxigenin ( $\beta$ -anhydromenabegenin) acetate (3 $\beta$ -acetoxy-5 $\beta$ ,17 $\alpha$ -carda-14,20 (22)-dienolide) (IX)<sup>12</sup>) (58 mg) in absolute ether (10 ml) was added a solution of  $\text{OsO}_4$  (50 mg) in absolute ether (1 ml). The mixture solution was allowed to stand for 2 hr at room temperature. After working up in the same way as described above, a brown crystalline product was obtained, whose TLC revealed the presence of one principal reaction product as is shown in Fig. 1b. Recrystallization of the product from acetone-petroleum ether gave XI (40 mg). mp 110—111.5°/201—207°,  $[\alpha]_D^{25}$  +21.0° ( $c=0.95$ ,  $\text{CHCl}_3$ ), UV  $\lambda_{\max}$  (log  $\epsilon$ ): 217.5 (4.14). IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 3340 (br) (OH), 1780, 1750, 1730, 1630 (butenolide and acetyl C=O). *Anal.* Calcd. for  $\text{C}_{25}\text{H}_{36}\text{O}_6$ : C, 69.42; H, 8.39. Found: C, 69.19; H, 8.63.

b) To a solution of IX (50 mg) in absolute ether (1.2 ml) and pyridine (0.3 ml) was added a solution of  $\text{OsO}_4$  (42 mg) in absolute ether (6 ml). The mixture solution was allowed to stand for 18 hr at room temperature. After working up in the way described above, a brown crystalline product (55 mg) was obtained, whose TLC was entirely similar to that shown in Fig. 1b. The product was chromatographed on a column of acid-washed alumina (1.5 g). Elutions with benzene and benzene-EtOAc mixtures gave a crystalline product, which was recrystallized from acetone-petroleum ether to afford XI (37 mg), mp 109—110°/203—205°.

**15 $\beta$ -Hydroxy-17 $\alpha$ -digitoxigenin (15 $\beta$ -Hydroxymenabegenin; 3 $\beta$ ,14,15 $\beta$ -Trihydroxy-5 $\beta$ ,17 $\alpha$ -card-20(22)-enolide) (XII)**—a) From  $\beta$ -Anhydro-17 $\alpha$ -digitoxigenin ( $\beta$ -Anhydromenabegenin; 3 $\beta$ -Hydroxy-5 $\beta$ ,17 $\alpha$ -carda-14,20(22)-dienolide) (X): To a solution of X (mp 166—169°) (160 mg) in ether (4 ml) and pyridine (1 ml), which was prepared from 17 $\alpha$ -digitoxigenin (menabegenin) acetate by refluxing with 10%  $\text{H}_2\text{SO}_4$  in EtOH,<sup>12</sup>) was added a solution of  $\text{OsO}_4$  (127 mg) in ether (20 ml). The mixture solution was allowed to stand at room temperature for 20 hr. After working up in the way described above, a brown crystalline product (180 mg) was obtained, whose TLC indicated the presence of one principal product corresponding to XII in Fig. 1c. It was chromatographed on a column of acid-washed alumina (6 g). Elutions with benzene-EtOAc mixtures and EtOAc afforded a crystalline product, which was recrystallized from EtOAc-petroleum ether to give XII (100 mg). mp 226—229°,  $[\alpha]_D^{25}$  +47.5° ( $c=0.69$ , MeOH), UV  $\lambda_{\max}$   $m\mu$  (log  $\epsilon$ ): 217 (4.17), IR  $\nu_{\max}$   $\text{cm}^{-1}$ : 3575 (sh), 3500 (br) (OH), 1785, 1733, 1630 (butenolide). *Anal.* Calcd. for  $\text{C}_{23}\text{H}_{34}\text{O}_5$ : C, 70.74; H, 8.78. Found: C, 70.51; H, 8.71.

b) From XI: A solution of XI (50 mg) in a mixture of MeOH (20 ml) and 10% HCl (20 ml) was allowed to stand for 18 hr at room temperature. Working up in the way described above yielded a crystalline precipitate. Recrystallization from EtOAc-petroleum ether gave XII (30 mg), mp 224—228°, identical with the above sample of XII in the mixed melting point, TLC, and comparison of the IR spectrum.

**Isomerization of III to XII**—To a solution of III (80 mg) in dimethylformamide (10 ml, freshly redistilled under reduced pressure) were added anhyd. AcONa (40 mg) and sodium tosylate (80 mg). The mixture was heated for 23 hr at 115°, poured into ice-water. The product was then extracted with a mixture of ether-CHCl<sub>3</sub> (3:1). The organic layer was washed with 2% HCl and H<sub>2</sub>O, and dried over anhyd. Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent yielded a crystalline residue (66 mg), whose TLC (Fig. 1c) revealed two spots corresponding to III and XII. Repeated recrystallizations of the residue afforded III (33.2 mg) and XII (2.3 mg, mp 225—228°). The latter was identical with the above sample of XII in the mixed melting point, TLC, and comparison of the IR spectrum.