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Sodium Borohydride Reduction of 15-Oxocardenolides

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From the stereochemical point of view, sodium borohydide reduction of the four possible 14-deoxy-15-oxocardenolides (14β, 17β; 14β, 17α; 14α, 17β; 14α, 17α) was investigated. The 15-oxo group in the 14β-cardenolides (C/D *cis*) was reduced to give 15α-hydroxy epimer as the main product, while predominant formation of 15β-hydroxy epimer was observed with the 14α-cardenolides (C/D *trans*), irrespective of the configuration of the butenolide at C-17.

It was found that the introduction of an oxygen function into C-15 considerably affected the cardiotonic activity of digitoxigenin (I) determined by using the Straub's frog heart preparation.[2-4] Thus, 15α-hydroxydigitoxigenin (III)[5,6] was unexpectedly inactive, while 15-oxodigitoxigenin (II)[7] and 15β-hydroxydigitoxigenin (IV) possessed a definite cardiotonic activity in the following order: I>IV>II>III. This suggested that the essential steric features in the vicinity of the C and D rings, or combination of the molecule with its site of action, may somehow be interfered by a group attached at C–15. It was demonstrated, on the other hand, that the presence of 14β-hydroxyl group is not indispensable for the cardiotonic activity of the cardenolide, since 3β-hydroxy-5α-card-20(22)-enolide(14-deoxy-14βH-uzarigenin)[8] was about one third as active as uzarigenin.[4] In connection with and extending these findings concerning the structure-activity relationship of the cardenolide, the preparation of epimeric 15-hydroxy-14-deoxy-14β-cardenolides was carried out for pharmacological examinations by treatment of the corresponding 15-oxocardenolide (14β,17β) with sodium borohydride. Furthermore, sodium borohydride reduction of other 15-oxocardenolides (14α,17β; 14β,17α; 14α,17α) was examined, since stereochemistry of this reduction of the 15-oxo group seemed to be affected by the steric configuration of the butenolide at C–17 as well as substituent at C–14.[9]

Treatment of 3β-acetoxy-15-oxo-5β, 14β-card-20(22)-enolide (VIb)[6] with sodium borohydride gave a mixture, whose thin–layer chromatography (TLC) revealed the formation of two epimeric 15-hydroxycardenolides, VIIb and VIIIb, as shown in Fig. 1a. On column chromatography of aluminum oxide VIIb and VIIIb were obtained in the ratio of approximately 4: 3. Hydrolysis of VIIb and VIIIb in aqueous methanol with hydrochloric acid afforded the desired cardenolides, 3β,15α-dihydroxy-5β,14β-card-20(22)-enolide (VIIa) and 3β,15β-dihydroxy-5β,14β-card-20(22)-enolide (VIIIa), respectively. Acetylation of VIIb and VIIIb with acetic anhydride-pyridine in the way described in the Experimental gave the corresponding acetates, VIIc and VIIIc.

1) Location: *Takada 3-Chome, Toshima-ku, Tokyo.*
2) T. Shigei, M. Katori, H. Murase, and S. Imai, *Experientia*, **20**, 572 (1964).
3) S. Imai, H. Murase, M. Katori, M. Okada, and T. Shigei, *Jap. J. Pharmac.*, **15**, 62 (1965).
4) T. Shigei and S. Mineshita, *Experientia*, **24**, 466 (1968).
5) M. Okada and M. Hasunuma, *Yakugaku Zasshi*, **85**, 822 (1965).
6) H. Ishii, T. Tozyo, and D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **11**, 576 (1963).
7) M. Okada and Y. Saito, *Chem. Pharm. Bull.* (Tokyo), **15**, 352 (1967); *idem*, *ibid.*, **17**, 515 (1969).
8) M. Okada and Y. Saito, *Chem. Pharm. Bull.* (Tokyo), **16**, 2223 (1968).
9) *a*) Y. Saito, Y. Kanemasa, and M. Okada, *Chem. Pharm. Bull.* (Tokyo), **19**, 1461 (1971); *b*) *Idem*, *ibid.*, **19**, 1363 (1971); *c*) M. Okada, K. Kimura, and Y. Saito, *ibid.*, **20**, 2729 (1972).

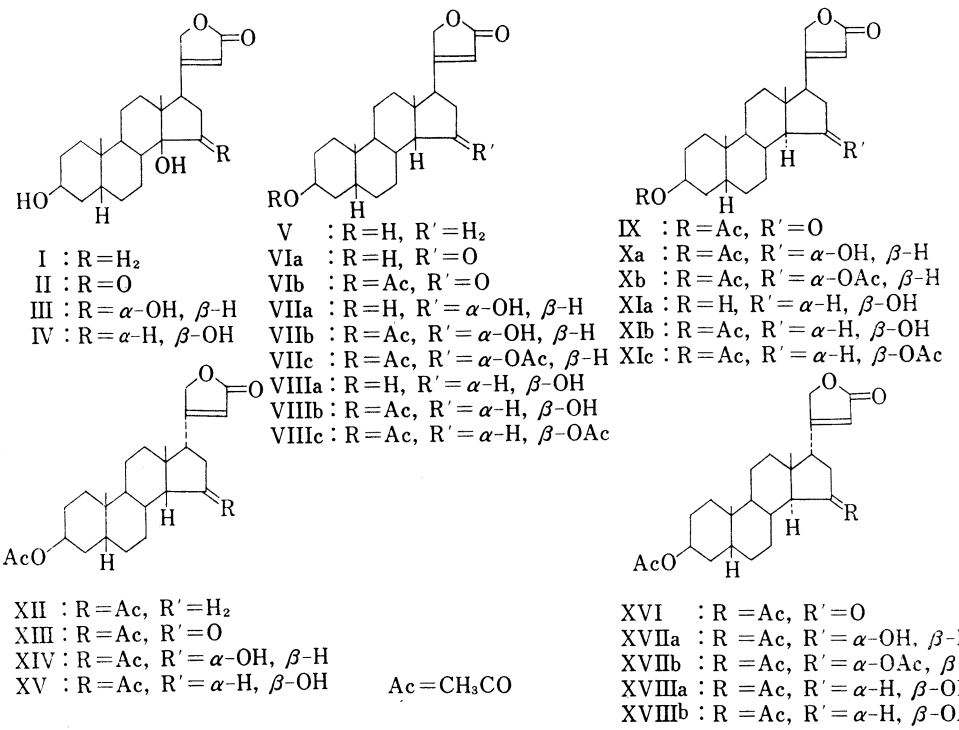

Chart 1

3β-Acetoxy-15-oxo-5β,14α-card-20(22)-enolide (IX)[6] was similarly treated with sodium borohydride to give a mixture of two 15-hydroxy epimers, Xa and XIb, whose TLC indicated the predominant formation of the latter (Fig. 1b). Actually, fractional crystallization of the mixture afforded Xa and XIb in the ratio of about 1: 15. Both epimers were acetylated with acetic anhydride-pyridine in the way described below to give the corresponding acetates, Xb and XIc, while acid hydrolysis was carried out only with XIb, owing to shortage of the materials, in the same way as described above affording 3β,15β-dihydroxy-5β,14α-card-20(22)-enolide (XIa).

Reduction of 3β-acetoxy-15-oxo-5β,14β,17α-card-20(22)-enolide (XIII)[10] and 3β-acetoxy-15-oxo-5β,14α,17α-card-20(22)-enolide (XVI)[10] with sodium borohydride resulted in the formation of two 15-hydroxy epimers, XIV and XV with the former, XVIIa and XVIIIa with the latter, respectively (Fig. 1c and 1d). In the case of XIII, both epimers were actually isolated in crystalline in the ratio of about 9: 1, the 15α-epimer (XIV) being predominant. The formation of the 15α-epimer (XVIIa), on the other hand, could be demonstrated only by

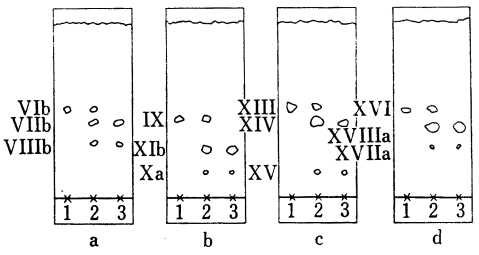

Fig. 1. Thin-Layer Chromatography

a: reaction product obtained from VIb after treatment with $NaBH_4$: 1) VIb, 2) 1+3, 3) reaction product
b: reaction product obtained from IX after treatment with $NaBH_4$: 1) IX, 2) 1+3, 3) reaction product
c: reaction product obtained from XIII after treatment with $NaBH_4$: 1) XIII, 2) 1+3, 3) reaction product
d: reaction product obtained from XVI after treatment with $NaBH_4$: 1) XVI, 2) 1+3, 3) reaction product

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

TLC in the case of XVI, whose principal reduction product, the 15β-epimer (XVIIIa), was obtainable in crystalline only as the acetate (XVIIIb).

The configuration of the 15-hydroxyl groups in the new cardenolides was assigned as indicated above on the basis of the following observations on 1) acetylation, 2) optical rotation, and 3) nuclear magnetic resonance (NMR) spectrum.

1) Acetylation The 15-hydroxycardenolides were acetylated with acetic anhydride-pyridine and completion of the reaction was followed by TLC. Among the cardenolides having 14α-configuration, Xa and XVIIa[11] were easily acetylated at room temperature for 5 hours giving Xb and XVIIb,[12] while XIb and XVIIIa required standing for several days at room temperature or heating to 70° for complete acetylation to afford XIc and XVIIIb, respectively. Among the cardenolides holding 14β-configuration, on the other hand, VIIb and XIV[12] required standing for several days at room temperature or heating to 70° for complete acetylation, while VIIIb and XV[12] were acetylated without difficulty at room temperature for 6 hours. As is repeatedly observed, 15α-hydroxyl group of the C/D *trans* steroid readily undergoes acetylation with acetic anhydride-pyridine at room temperature,[13] while 15β-hydroxyl group is more hindered for acetylation reaction than 15α-hydroxyl group.[14] On the contrary, 15α-hydroxyl group is more hindered sterically in the C/D *cis* steroid for acetylation reaction than 15β-hydroxyl group.[5,9c,15-17]

2) Optical Rotation Molecular ratation (M_D) of the 15-hydroxycardenolides (VIIa, VIIIa, XIV, XV, Xa, XIb) and their molecular rotation differences (ΔM_D) calculated from reference cardenolides are listed in Table I, together with those of related compounds. The above assignment of the configuration of the 15-hydroxyl group is reasonably supported by the ΔM_D values indicated.

3) NMR Spectrum[18] The NMR spectra of an epimeric pair, Xa and XIb, exhibited signals at 0.67 and 1.00 ppm with the former and 0.88 and 1.03 ppm with the latter ascribable to the protons of the angular C–18 methyl and C–19 methyl groups respectively, thus indicating the 15β-configuration for the hydroxyl group in XIb and its derivatives (XIa, XIc). The NMR spectrum of XVIIIb exhibited signals at 1.18 and 1.03 ppm ascribable to the protons of the angular C–18 methyl and C–19 methyl groups respectively. The large downfield shift

11) Since XVIIa was not obtained in pure state as described in the Experimental, a mixture of XVIIa and XVIIIa was used as such for acetylation.

12) XVIIb as well as acetates of XIV and XV were not prepared actually, but the formation of these acetates could be demonstrated by TLC.

13) A. Gubler and Ch. Tamm, *Helv. Chim. Acta*, **41**, 301 (1958); Ch. Tamm, A. Gubler, G. Juhasz, E. Weiss-Berg, and W. Zürcher, *ibid.*, **46**, 889 (1963); M. Shirasaka and M. Tsuruta, *Chem. Pharm. Bull.* (Tokyo), **9**, 238 (1961); M. Okada, A. Yamada, and M. Ishidate, *Yakugaku Zasshi*, **85**, 816 (1965); H. Rönsch and K. Schreiber, *Ann. Chem.*, **694**, 169 (1966).

14) C. Djerassi, T.T. Grossnickel, and L.B. High, *J. Am. Chem. Soc.*, **78**, 3166 (1956); S. Bernstein, M. Heller, L.I. Feldman, W.S. Allen, R.H. Blank, and C.E. Linden, *ibid.*, **82**, 3685 (1960); K. Tsuda, T. Asai, Y. Sato, T. Tanaka, T. Matsuhisa, and H. Hasegawa, *Chem. Pharm. Bull.* (Tokyo), **8**, 626 (1960); K. Tsuda, T. Asai, Y. Sato, T. Tanaka, and H. Hasegawa, *ibid.*, **9**, 737 (1961); M. Shirasaka, *ibid.*, **9**, 54 (1961); R. Tschesche and G. Wulff, *Chem. Ber.*, **94**, 2019 (1961); C. Djerassi, G. von Mutzenbecher, J. Fajkos, D.H. Williams, and H. Budzikiewicz, *J. Am. Chem. Soc.*, **87**, 817 (1965).

15) R. Brandt, W. Stöcklin, and T. Reichstein, *Helv. Chim. Acta*, **49**, 1662 (1966).

16) A. Lardon, H.P. Sigg, and T. Reichstein, *Helv. Chim. Acta*, **42**, 1457 (1959); A. Lardon and T. Reichstein, *ibid.*, **45**, 943 (1962).

17) In connection with this finding, 3-monoacetates of the following 15-hydroxycardenolides reported earlier were acetylated again with acetic anhydride-pyridine at room temperature and the reaction was followed by TLC: 15β-hydroxydigitoxigenin,[7] 15β-hydroxy-17α-digitoxgenin,[7] 15β-hydroxy-14α-digitoxigenin,[9c] 15α-hydroxy-14α-digitoxigenin.[7] The results also confirmed the finding described here.

18) The NMR spectra were measured at room temperature in deuteriochloroform at 60 MHz, using Hitachi Model R-20A 60-MHz spectrometer. Chemical shifts are expressed in δ (ppm) with tetramethylsilane as internal standard.

TABLE I. Molecular Rotations (M_D) and Molecular Rotation Differences (ΔM_D) of 15-Hydroxycardenolides and Related Compounds

Compound	M_D	ΔM_D
Digitoxygenin (I)	+72°	—
15α-Hydroxydigitoxigenin (III)	+141°	+69°
15β-Hydroxydigitoxigenin (IV)	0°	−72°
14β-Hydroxyprogesterone[a]	+446°	—
14β,15α-Dihydroxyprogesterone[b]	+564°	+118°
14β,15β-Dihydroxyprogesterone[b]	+367°	−79°
3β-Hydroxy-5β,14β-card-20(22)-enolide (V)[21]	(+112°)[c]	—
3β,15α-Dihydroxy-5β,14β-card-20(22)-enolide (VIIa)	+4°	(−108°)
3β,15β-Dihydroxy-5β,14β-card-20(22)-enolide (VIIIa)	−118°	(−230°)
3β-Acetoxy-5β,14β,17α-card-20(22)-enolide (XII)[d]	+302°	—
3β-Acetoxy-15α-hydroxy-5β,14β,17α-card-20(22)-enolide (XIV)	+346°	+44°
3β-Acetoxy-15β-hydroxy-5β,14β,17α-card-20(22)-enolide (XV)	+137°	−165°
3β-Acetoxy-14-hydroxy-5β,14α-card-20(22)-enolide[e]	+90°	—
3β-Acetoxy-14,15α-dihydroxy-5β,14α-card-20(22)-enolide[7]	+152°	+62°
3β-Acetoxy-14,15β-dihydroxy-5β,14α-card-20(22)-enolide[9a]	+13°	−77°
3β-Acetoxy-5β,14α-card-20(22)-enolide[f]	+48°	—
3β-Acetoxy-15α-hydroxy-5β,14α-card-20(22)-enolide (Xa)	+75°	+27°
3β-Acetoxy-15β-hydroxy-5β,14α-card-20(22)-enolide (XIb)	0°	−48°

a) A. Lardon, *Helv. Chim. Acta*, **32**, 1517 (1949)
b) H. Hasegawa, Y. Sato, and K. Tsuda, *Chem. Pharm. Bull.* (Tokyo), **11**, 1275 (1963)
c) Since V was not available,[18] the values were calculated from those of 3β-hydroxy-5α,14β-card-20(22)-enolide (14-deoxy-14βH-uzarigenin),[8] I, and uzarigenin, and are indicated in parentheses.
d) T. Wada, *Chem. Pharm. Bull.* (Tokyo), **13**, 312 (1965)
e) W. Zürcher, E. Weiss-Berg, and Ch. Tamm, *Helv. Chim. Acta*, **52**, 2449 (1969)
f) E. Hauser, H. Linde, and K. Meyer, *Helv. Chim. Acta*, **49**, 1212 (1966)

of the chemical shift of the C–18 methyl protons is consistent with a 15β-acetoxyl group.[20] In contrast to the 15-hydroxy-14α-cardenolides, the configuration of the 15-hydroxyl groups in the 14β-cardenolides described in this paper could not be assigned on the basis of their NMR spectra. We are working on this problem with other 15-hydroxy-14β-cardenolides and by using paramagnetic shift reagent $Eu(FOD)_3$.

From the results presented in this paper, coupled with the previous findings,[9a] it could be concluded that sodium borohydride reduction of 15-oxocardenolides affords 15α-hydroxy epimer as the main product in the 14β-cardenolide, while giving 15β-hydroxy epimer predominantly in the 14α-cardenolide, irrespective of the configuration of the butenolide at C–17. Substitution of the hydrogen atom at C–14 by a hydroxyl group does not seem to affect this reduction.[9a] It is noteworthy, however, that the introduction of chlorine into 14β-position does exert a great influence on the reduction.[9b,c]

Pharmacological examinations[21] using the isolated frog's heart (Straub's preparation) disclosed that VIa[9a] and VIIIa possessed a definite cardiotonic activity while VIIa was inactive, thus demonstrating a similar structure-activity relationship to that on the 14β-hydroxy series.[2–4,19]

19) Preparation of 3β-hydroxy-5β,14β-card-20(22)-enolide (14-deoxy-14βH-digitoxigenin) (V) as reference cardenolide was attempted by converting VIb to thioketal followed by desulfurization with Raney nickel and by deacetylation. Owing to partial epimerization at C-14 occurred on thioketalization, V could not be obtained in pure state but as a mixture with its 14-epimer, which possessed a much less cardiotonic activity than expected.[21]

20) A.I. Cohen and S. Rock, Jr., *Steroids*, **3**, 243 (1964); L.L. Smith, *ibid.*, **4**, 395 (1964); N. Bhacca and D.A. Williams, "Applications of NMR Spectroscopy in Organic Chemistry," Holden-Day, Inc., San Francisco, 1964, p. 22.

21) T. Shigei, T. Tsuru, Y. Saito, and M. Okada, *Experientia*, **29**, (1973), in press.

Experimental[22]

Reduction of 3β-Acetoxy-15-oxo-5β,14β-card-20(22)-enolide (VIb) with $NaBH_4$——To a solution of VIb[6] (92 mg) in MeOH (7 ml) was added $NaBH_4$ (90 mg) at 0° while stirring. The reaction mixture was allowed to stand for 30 min at the same temperature. After addition of AcOH (0.5 ml) and H_2O (30 ml) the solution was concentrated *in vacuo* to a small volume, and the product was extracted with $CHCl_3$. The organic layer was washed with H_2O and dried over anhyd. Na_2SO_4. Evaporation of the solvent gave an oily residue (90 mg), whose TLC is shown in Fig. 1a. The residue was chromatographed on a column of Al_2O_3 (5 g) by successive elution with hexane, hexane–benzene mixture, benzene, benzene–EtOAc (10:1, 5:1), and EtOAc. The fraction (49 mg) eluted with benzene–EtOAc (10:1) gave VIIb (40 mg) after recrystallization from acetone–petroleum ether. mp 212—213°. $[\alpha]_D^{26}$ +24.9° (c=1.41, $CHCl_3$). UV λ_{max} mμ (log ε): 218 (4.21). IR ν_{max} cm^{-1}: 3525 (OH), 1795 (sh), 1780 (sh), 1740, 1730 (sh), 1629 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 72.16; H, 8.90.

The fraction (38 mg) eluted with benzene–EtOAc (5:1) afforded VIIIb (30 mg) after recrystallization from acetone–petroleum ether. mp 209—211°. $[\alpha]_D^{26}$ +2.6° (c=0.67, $CHCl_3$). UV λ_{max} mμ (log ε): 216.5 (4.22). IR ν_{max} cm^{-1}: 3500 (OH), 1800 (sh), 1780 (sh), 1730, 1630 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 72.38; H, 8.99.

3β,15α-Dihydroxy-5β,14β-card-20(22)-enolide (VIIa)——A solution of VIIb (30 mg) in a mixture of MeOH (10 ml) and 10% HCl (10 ml) was allowed to stand for 18 hr at room temperature. After neutralization with 5% Na_2CO_3, H_2O (20 ml) was added and the solution was concentrated *in vacuo* to yield a crystalline precipitate which was recrystallized from MeOH affording VIIa (25 mg). mp 209—214°. $[\alpha]_D^{26}$ +0.94 (c=0.21, MeOH). UV λ_{max} mμ (log ε): 218.5 (4.16). IR ν_{max} cm^{-1}: 3480, 3375 (OH), 1800 (sh), 1784 (sh), 1750, 1629 (butenolide). *Anal.* Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.63; H, 9.30.

3β,15α-Diacetoxy-5β,14β-card-20(22)-enolide (VIIc)——A solution of VIIb (60 mg) in a mixture of acetic anhydride (0.8 ml) and pyridine (1 ml) was allowed to stand for 6 days at room temperature.[24] Working up in the usual way gave VIIc (50 mg) after recrystallization from acetone–ether–petroleum ether. mp 200.5—202°. $[\alpha]_D^{26}$ +60° (c=0.67, $CHCl_3$). UV λ_{max} mμ (log ε): 215.5 (4.23). IR ν_{max} cm^{-1}: 1777, 1745, 1730, 1630 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{27}H_{38}O_6$: C, 70.71; H, 8.35. Found: C, 70.91; H, 8.39.

3β,15β-Dihydroxy-5β,14β-card-20(22)-enolide (VIIIa)——VIIIb (30 mg) was hydrolyzed in the same way as described above to give VIIIa (24 mg) after recrystallization from MeOH. mp 218—220°. $[\alpha]_D^{26}$ −31.6° (c=0.77, MeOH). UV λ_{max} mμ (log ε): 217 (4.21). IR ν_{max} cm^{-1}: 3550 (sh), 3505 (OH), 1780 (sh), 1741 (sh), 1718, 1618 (butenolide). *Anal.* Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.61; H, 9.30.

3β,15β-Diacetoxy-5β,14β-card-20(22)-enolide (VIIIc)——A solution of VIIIb (30 mg) in a mixture of acetic anhydride (0.5 ml) and pyridine (0.5 ml) was allowed to stand at 20° for 6 hr.[24] Working up in the usual way yielded VIIIc (21 mg) after recrystallization from acetone–ether–petroleum ether. mp 203—204°. $[\alpha]_D^{26}$ −22.1° (c=0.91, $CHCl_3$). UV λ_{max} mμ (log ε): 216 (4.22). IR ν_{max} cm^{-1}: 1783, 1750, 1633 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{27}H_{38}O_6$: C, 70.71; H, 8.35. Found: C, 70.40; H, 8.37.

Reduction of 3β-Acetoxy-15-oxo-5β,14α-card-20(22)-enolide (IX) with $NaBH_4$——IX[6] (500 mg) was treated with $NaBH_4$ (500 mg) in the same way as described above. TLC of the reduction product is shown in Fig. 1b. Repeated recrystallizations of the reduction product from MeOH afforded XIb (400 mg). mp 212—213°. $[\alpha]_D^{26}$ 0° (c=0.73, $CHCl_3$). UV λ_{max} mμ (log ε): 217 (4.20). IR ν_{max} cm^{-1}: 3550 (OH), 1781, 1739, 1630 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 72.09; H, 8.97.

The mother liquor from recrystallization of XIb was concentrated under reduced pressure to dryness. The residue (90 mg) was chromatographed on a column of Al_2O_3 (5 g) by successive elution with hexane–benzene (1:1) and benzene–EtOAc (10:1, 7:1, 5:1). The fraction eluted with benzene–EtOAc (10:1, 7:1) gave XIb (51 mg). The fraction (34 mg) eluted with benzene–EtOAc (5:1) afforded Xa (17 mg) after recrystallization from MeOH. mp 189—191°. $[\alpha]_D^{26}$ +18° (c=0.67, $CHCl_3$). UV λ_{max} mμ (log ε): 216.5 (4.25). IR ν_{max} cm^{-1}: 3580 (OH), 1797, 1758, 1717, 1637 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{25}H_{36}O_5 \cdot 1/4\ H_2O$: C, 71.31; H, 8.74. Found: C, 71.37; H, 8.90.

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22) Melting points were determined on a Kofler block and are uncorrected. Ultraviolet (UV) spectra were measured in 99% EtOH solution. Infrared (IR) spectra were determined in KBr disks on Hitachi EPI-S2 spectrophotometer; sh=shoulder. TLC plates were prepared according to the Stahl's procedure using silica gel H (E. Merck AG) as adsorbent. The solvent system used was methyl ethyl ketone–heptane (1:1), and the cardenolide spots were revealed by heating plates at 110° for 10 min after spraying 95% H_2SO_4 or by Kedde reagent. Aluminum oxide used for column chromatography was acid-washed and the activity grade III—IV.[23]

23) S. Heřmánek, V. Schwarz, and Z. Čekan, *Collection Czech. Chem. Commun.*, **26**, 3170 (1961).

24) Completion of the acetylation was followed by TLC.

3β,15α-Diacetoxy-5β,14α-card-20(22)-enolide (Xb)——A solution of Xa (15 mg) in a mixture of acetic anhydride (0.5 ml) and pyridine (0.5 ml) was allowed to stand at 20° for 5 hr.[24] Working up in the usual way gave Xb (10 mg) after recrystallizatiom from acetone–petroleum ether. mp 189—191°. $[\alpha]_D^{26}$ +85.2° (c=0.29, $CHCl_3$). UV λ_{max} mμ (log ε): 215 (4.14). IR ν_{max} cm^{-1}: 1794, 1762, 1744, 1640 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{27}H_{38}O_6$: C, 70.71; H, 8.35. Found: C, 70.53; H, 8.41.

3β,15β-Dihydroxy-5β,14α-card-20(22)-enolide (XIa)——Acid hydrolysis of XIb (59 mg) was done in the same way as described above to give XIa (23 mg) after recrystallization from MeOH. mp 199—200°. $[\alpha]_D^{26}$ −31.1° (c=0.35, MeOH). UV λ_{max} mμ (log ε): 217.5 (4.21). IR ν_{max} cm^{-1}: 3495 (OH), 1784 (sh), 1764 (sh), 1749, 1629 (butenolide). *Anal.* Calcd. for $C_{23}H_{34}O_4$: C, 73.76; H, 9.15. Found: C, 73.77; H, 9.31.

3β,15β-Diacetoxy-5β,14α-card-20(22)-enolide (XIc)——Acetylation of XIb (90 mg) with a mixture of acetic anhydide (1 ml) and pyridine (1 ml) was carried out at room temperature for 7 days.[24] Working up in the usual way yielded XIc (66 mg) after recrystallization from acetone–ether–petroleum ether. mp 200—203°. $[\alpha]_D^{26}$ −35.7° (c=0.81, $CHCl_3$). UV λ_{max} mμ (log ε): 215 (4.24). IR ν_{max} cm^{-1}: 1790, 1755, 1739, 1629 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{27}H_{38}O_6$: C, 70.71; H, 8.35. Found: C, 70.79; H, 7.98.

Reduction of 3β-Acetoxy-15-oxo-5β,14β,17α-card-20(22)-enolide (XIII) with $NaBH_4$——Reduction of XIII[10] (160 mg) by $NaBH_4$ (150 mg) was performed in the same way as described above to give an oily product (165 mg), whose TLC is shown in Fig. 1c. The oily reduction product was chromatographed on a column of Al_2O_3 (6.5 g) by eluting successively with hexane–benzene (1:1), benzene, and benzene–EtOAc (4:1). The fraction eluted with benzene and benzene–EtOAc (4:1) afforded XIV (100 mg) after recrystallization from acetone–petroleum ether. mp 189°. $[\alpha]_D^{24}$ +82.9° (c=1.21, $CHCl_3$). UV λ_{max} mμ (log ε): 218 (4.18). IR ν_{max} cm^{-1}: 3510, 3410 (sh) (OH), 1786, 1745, 1705, 1629 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 71.98; H, 8.63.

Further elution with benzene–EtOAc (4:1) gave XV (12 mg) after recrystallization from MeOH–ether. mp 196—198°. $[\alpha]_D^{24}$ +33.1° (c=0.73, $CHCl_3$). UV λ_{max} mμ (log ε): 217 (4.25). IR ν_{max} cm^{-1}: 3495, 3450 (sh) (OH), 1780 (sh), 1740, 1705, 1625 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{25}H_{36}O_5$: C, 72.08; H, 8.71. Found: C, 72.34; H, 8.95.

Reduction of 3β-Acetoxy-15-oxo-5β,14α,17α-card-20(22)-enolide (XVI) with $NaBH_4$——Treatment of XVI[10] (36 mg) with $NaBH_4$ (36 mg) was performed in the same way as described above. TLC of the oily reduction product is shown in Fig. 1d. It was chromatographed on a column of Al_2O_3 (1.5 g) by successive elution with hexane, hexane–benzene (1:1), benzene, and benzene–EtOAc (10:1, 5:1). Elution with benzene, and benzene–EtOAc (10:1) gave XVIIIa (30 mg) which could not be crystallized. Elution with benzene–EtOAc (5:1) afforded an oily mixture (2 mg) of XVIIa and XVIIIa.

3β,15β-Diacetoxy-5β,14α,17α-card-20(22)-enolide (XVIIIb)——The above XVIIIa (30 mg) was acetylated with a mixture of acetic anhydride (0.5 ml) and pyridine (0.5 ml) at 20° for 5 days.[24] Working up in the usual way yielded XVIIIb (20 mg) after recrystallization from MeOH–ether. mp 214—216°. $[\alpha]_D^{24}$ −30.8° (c=1.20, $CHCl_3$). UV λ_{max} mμ (log ε): 215.5 (4.14). IR ν_{max} cm^{-1}: 1780, 1740, 1725 (sh), 1623 (butenolide and acetyl C=O). *Anal.* Calcd. for $C_{27}H_{38}O_6$: C, 70.71; H, 8.35. Found: C, 70.63; H, 8.47.