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Studies on Digitalis Glycosides. XXVIII.[1] The Structure of Digiprogenin. (3)[2]

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From the results of lead tetraacetate oxidation of γ-digiprogenin 3-acetate as well as sodium metaperiodate oxidation of tetrahydro-γ-digiprogenin 3-acetate, the position of the tertiary hydroxyl group was revised to C-14 from C-17, and the new structure, 3β, 14-dihydroxy-14β,17β-pregn-5-ene-11,15,20-trione (VIIIa) was assigned to γ-digiprogenin. α-Digiprogenin is its 17-epimer (IXa).

In the previous paper,[4] Satoh and Horie reported that γ-digiprogenin 3-acetate and its 17-epimer, α-digiprogenin 3-acetate, were considered to have 14β,17β- and 14β,17α-configuration formulated as I and II, respectively, based on the data of nuclear magnetic resonance (NMR) spectra and of the optical rotatory dispersions (ORD) of their 20-thioketals (IV, VI) and 20-deoxo derivatives (V, VII). The tertiary hydroxyl groups of γ- and α-digiprogenin 3-acetates were presumed to locate at C-17 as indicated in the formula Ia and IIa from the fact that refluxing of these compounds with 5% hydrochloric acid gave the same 16-dehydro compound, β-digiprogenin (III).[5]

However, the results of the later studies were inconsistent with this position as follows:

i) An intensive red shift ($\Delta\nu$=285 cm^{-1}, in CCl_4) of the stretching frequency of the tertiary hydroxyl group in the infrared (IR) spectrum of γ-digiprogenin 3-acetate (I) comparing with the corresponding red shift ($\Delta\nu$=20 cm^{-1}, in CCl_4) of α-digiprogenin 3-acetate (II) showed a strong intramolecular hydrogen bonding between the tertiary hydroxyl group and a carbonyl group in I. This fact could not be explained by the formulas Ia and IIa having the tertiary hydroxyl groups at C-17.

ii) The Cotton effect of 20-carbonyl group of I calculated by substracting the circular dichroism (CD) curve of 20-deoxo-γ-digiprogenin 3-acetate (V) from that of I was positive ($[\theta]_{295}$ +4954, in MeOH). On the contrary, the Cotton effect of 20-carbonyl group of II calculated analogously was negative ($[\theta]_{290}$ −3879, in MeOH). The wave lengths of the peak and trough of the CD curves (295 mμ and 290 mμ) corresponded to those of 20-carbonyl groups which do not have hydroxyl groups at C-17.[6]

iii) Though the 17-hydroxy-20-oxosteroids give the D-homosteroids on treatment with boron trifluoride in general, I and II did not respond to this reagent.

On the other hand, when the tertiary hydroxyl group is located at C-14 in place of C-17 as in formula Ib and IIb, the above mentioned data can be interpreted favorably. So we studied the cleavage reaction of D-ring of I to elucidate this problem, and the results led us

1) Part XXVII. D. Satoh, S. Kobayashi, and J. Morita, *Chem. Pharm. Bull.* (Tokyo), **17**, 682 (1969).
2) Preliminary communication was reported in *Chem. Pharm. Bull.* (Tokyo), **14**, 552 (1966).
3) Location: *Sagisu, Fukushima-ku, Osaka.*
4) D. Satoh and M. Horie, *Chem. Pharm. Bull.* (Tokyo), **12**, 979 (1964). This paper constitutes Part 2 of the series entitled "The Structure of Digiprogenin."
5) D. Satoh, *Chem. Pharm. Bull.* (Tokyo), **10**, 43 (1962). This paper constitutes Part 1 of the series entitled "The Structure of Digiprogenin".
6) In the 17α-hydroxy-20-oxosteroid, the peak of CD curve should be over 300 mμ.

to conclude that C-14 position as in formulas Ib as well as VIII is most reasonable. This paper deals with these studies.

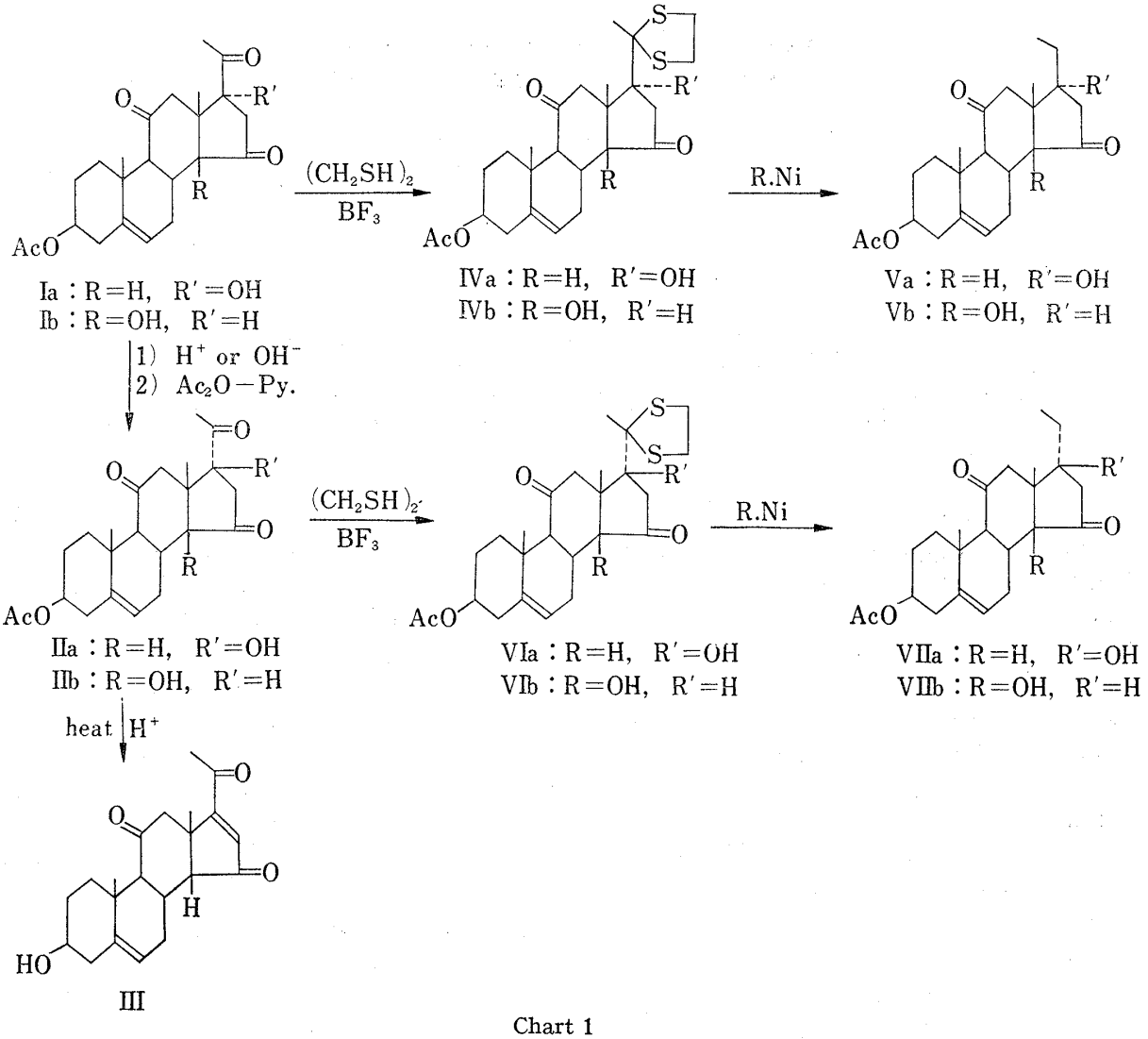

Chart 1

γ-Digiprogenin 3-acetate (VIIIb) was inert to sodium metaperiodate but it was oxidized gradually with lead tetraacetate to give an alkali soluble amorphous product (XI). The IR spectrum of XI exhibited absorptions of a γ-lactone at 1784 cm^{-1} and a hydroxyl group at 3583 cm^{-1} besides those of an acetyl group at 1726 cm^{-1} and a six-membered ring ketone at 1717 cm^{-1}. NMR spectrum exhibited a signal of a tertiary methyl group as a singlet at 8.34τ which corresponds to that connects to a carbon atom bearing the oxygen linkage. Methylation of XI with diazomethane gave a methyl ester (XII). In the IR spectrum of XII, four absorptions due to carbonyl groups were observed at 1739, 1728 cm^{-1} (acetyl and ester groups), 1712 and 1704 cm^{-1} (six-membered ring ketone and aliphatic ketone). NMR spectrum of XII exhibited four signals corresponding to five methyl groups as singlet which were respectively assigned as follow: 8.82 τ (6H, C-18 and C-19 methyl groups), 7.98 τ (3H, acetyl group), 7.83 τ (3H, methylketone side chain) and 6.28 τ (3H, methyl ester). The appearance of a methyl signal at 7.83 τ due to a methyl ketone showed that the side chain at C-17 of VIIIb was not eliminated in the lead tetraacetate oxidation, and this observation indicated that the location of the tertiary hydroxyl group of γ-digiprogenin 3-acetate at C-14 is most reasonable, because, in the case of C-17 position, the side chain should be splitted off in this oxidation. XI was thought to be a hemiacetal formed from a lactol of the keto acid (X), a fission product of D-ring between C-14 and C-15.

The C-14 position of the tertiary hydroxyl group was further supported by the metaperiodate oxidation of a tetrahydro derivative of VIIIb as described below. Partial reduction of VIIIb with sodium borohydride gave three tetrahydro derivatives A, B and C (XIIIa, XIIIb, XIIIc), and their characteristics are as follows:

Tetrahydro derivative	mp	$[\alpha]_D$	IR ν_{max}^{Nujol} cm^{-1} (11-CO)	NMR ($CDCl_3$) (21-CH_3)
A(XIIIa)	272—275°	+ 13.7°	1712	8.84 (d, J=6.0 cps)
B(XIIIb)	200—201°	−153.9°	1703	8.82 (d, J=6.0 cps)
C(XIIIc)	210—213°	− 83.1°	1681	8.75 (d, J=6.0 cps)

The facts that, in the NMR spectra of these tetrahydro derivatives, signals of 21-methyl groups appeared as doublet, and in the IR spectra, absorptions of carbonyl groups were observed at about 1700 cm^{-1}, and the oxime formations[7] were unsuccessful, indicated that the three tetrahydro derivatives are isomers produced by the reduction of both of the 15- and 20-carbonyl groups leaving the 11-carbonyl group intact.

When the main product B (XIIIb) was oxidized with sodium metaperiodate at room temperature, there was obtained an aldehyde (XV). In the NMR spectrum of XV, a signal of the aldehyde group appeared at 0.22 τ as a triplet (J=2.0 cps) and a signal of a methyl group was observed at 8.70 τ as a doublet(J=6.0 cps) beside three singlets of acetyl, 18- and 19-methyl groups at 7.98, 8.83 and 8.91 τ. The IR spectrum of this product exhibited an

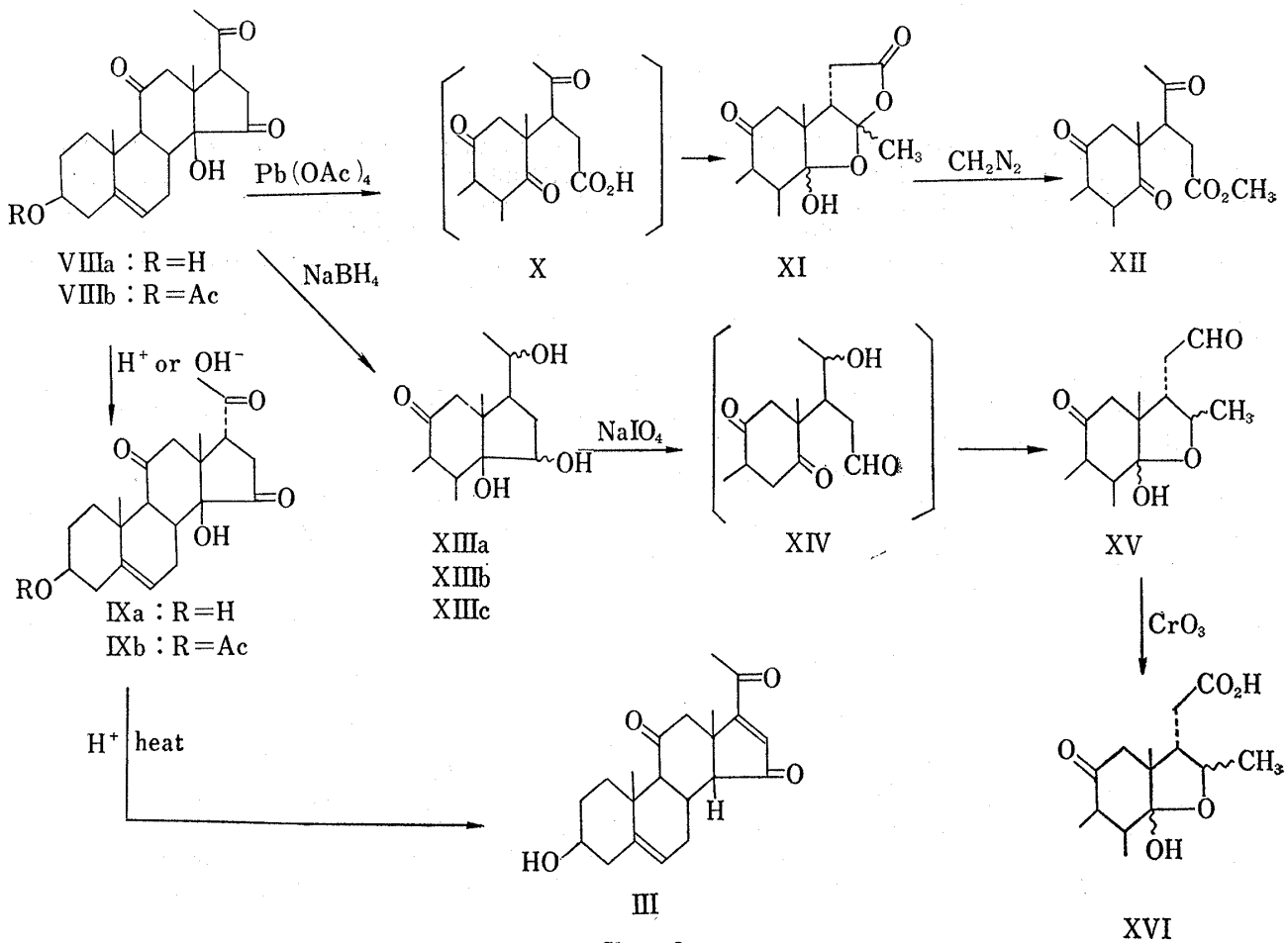

Chart 2

7) γ-Digiprogenin (the parent compound) gave a dioxime.[5]

absorption of a hydroxyl group at 3453 cm^{-1} and three absorptions of carbonyl groups at 1731, 1720 (aldehyde and acetyl groups) and 1708 cm^{-1} (six-membered ring ketone). Oxidation of XV with chromium trioxide gave an acid (XVI). This acid did not form oxime and its IR spectrum showed absorption at 3593 cm^{-1} due to a tertiary hydroxyl group and at 3502, 1748, 1730 and 1719 cm^{-1} corresponding to a carboxyl, an acetyl and a six-membered ring ketone groups, respectively. From these results, it was found that, on the metaperiodate oxidation of XIIIb, the side chain at C-17 was retained intact and a fission of D-ring took place between C-14 and C-15 to produce a ketoaldehyde (XIV) which formed a intramolecular hemiacetal (XV). These facts provided a further proof of 14-position of the tertiary hydroxyl group in γ-digiprogenin 3-acetate.

Since 14β,17β- and 14β,17α-configurations were given to γ- and α-digiprogenin 3-acetate as described earlier, it is most reasonable to assign 3β,14-dihydroxy-14β,17β-pregn-5-ene-11,15,20-trione (VIIIa) to γ-digiprogenin and 3β,14-dihydroxy-14β,17α-pregn-5-ene-11,15,20-trione (IXa) to α-digiprogenin.

β-Digiprogenin was considered to have the formula (III) as previously reported,[5] and its NMR spectrum, in which a signal of 16-vinyl proton appeared as a singlet at 3.48 τ and that of 18-methyl group shifted to the lower field at 8.43 τ, supported this formula. Accordingly, the formation of β-digiprogenin (III) by refluxing γ- and α-digiprogenin (VIIIa and IXa) having a hydroxyl group at C-14 with acid should occur through an unusual dehydration process.

Further studies to confirm the C-14 position of the tertiary hydroxyl group in digiprogenin by partial synthesis will be described in the forthcoming paper.

Experimental[8]

20-Thioketal (IVb) of γ-Digiprogenin 3-Acetate (Ib)——To a mixture of a solution of Ib (250 mg) in AcOH (20 ml) and ethane dithiol (1.25 ml) was added BF_3–ether (1.25 ml) dropwise under stirring at room temperature. The mixed solution was allowed to stand at the same temperature for 2 hr and then in a refrigerator overnight. Thin-layer chromatography (TLC) (SiO_2, AcOEt: benzene=1:4) showed the formation of two products. The reaction mixture was concentrated *in vacuo* under 50°, diluted with H_2O and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with 5% $NaHCO_3$ and H_2O, dried over Na_2SO_4, and evaporated *in vacuo* to dryness. The crude product was separated into two fractions by preparative TLC (SiO_2, AcOEt: benzene=1:4).

i) The less polar fraction (81 mg) was recrystallized from a mixture of $CHCl_3$: MeOH=1:1 to give 15,20-dithioketal (55 mg) as colorless needles, mp 231—233°. *Anal.* Calcd. for $C_{27}H_{38}O_4S_4 \cdot H_2O$: C, 56.60; H, 7.04; S, 22.39. Found: C, 56.27; H, 6.84; S, 22.01.

ii) The more polar fraction (198 mg) was recrystallized from MeOH to give IVb (158 mg) as colorless needles, mp 252—255°. *Anal.* Calcd. for $C_{25}H_{34}O_5S_2$: C, 62.73; H, 7.16; S, 13.39. Found: C, 62.64; H, 7.19; S, 13.68. IR ν_{max}^{Nujol} cm^{-1}: 3410 (OH), 1738 (five-membered ring ketone), 1710 (Ac), 1698 (six-membered ring ketone). NMR ($CDCl_3$) τ: 7.99 (Ac), 8.09 (21-CH_3), 8.87 (19-CH_3), 8.95 (18-CH_3).

20-Deoxo-γ-digiprogenin 3-Acetate (Vb)——To a solution of IVb (100 mg) in dioxane (10 ml) was added Raney Ni (2 g, inactivated by refluxing in acetone for 2 hr) and the mixture was refluxed at 100—110° for 5 hr. After Raney Ni was filtered off, the filtrate was evaporated *in vacuo* to dryness. The residue (92 mg) was recrystallized from MeOH to afford Vb (57 mg) as colorless needles, mp 191—194°. *Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 70.76; H, 8.28. IR ν_{max}^{Nujol} cm^{-1}: 3410 (OH), 1745 (five-membered ring ketone), 1711 (Ac), 1705 (six-membered ring ketone).

20-Thioketal (VIb) of α-Digiprogenin 3-Acetate (IIb)——To a mixture of a solution of IIb (300 mg) in AcOH (25 ml) and ethane dithiol (1.6 ml) was added BF_3–ether (1.6 ml) dropwise under stirring at room temperature and the mixed solution was allowed to stand at the same temperature for 2 hr and then in a refrigerator overnight. The crystalline product (270 mg) was collected and recrystallized from a mixture of $CHCl_3$: MeOH=1:1 to give VIb (240 mg) as colorless needles, mp 263—267°. *Anal.* Calcd. for $C_{25}H_{34}$-

8) All melting points are uncorrected, and all NMR spectra were measured at 60 Mc in $CDCl_3$.
9) Without binder. If SiO_2 contains $CaSO_4$ as a binder, the formation of Ca-salt of acidic product was observed in preparative TLC.

O_5S_2: C, 62.73; H, 7.16; S, 13.39. Found: C, 62.60; H, 7.25; S, 13.02. IR ν_{max}^{Nujol} cm^{-1}: 3415 (OH), 1745 (five-membered ring ketone), 1726 (Ac), 1714 (six-membered ring ketone). NMR ($CDCl_3$) τ: 7.98 (Ac), 8.05 (21-CH_3), 8.83 (19-CH_3), 8.71 (18-CH_3). TLC of the mother liquor of the crude product showed the formation of a small amount of probable dithioketal.

20-Deoxo-α-Digiprogenin 3-Acetate (VIIb)——A mixture of a solution of VIb (100 mg) in dioxane (10 ml) and inactivated Raney Ni (2 g) was refluxed for 5 hr at 100—110°. Raney Ni was filtered off and the filtrate was evaporated *in vacuo* to dryness. The residue (90 mg) was recrystallized from MeOH to afford VIIb (55 mg) as colorless needles, mp 229—231°. *Anal.* Calcd. for $C_{23}H_{32}O_5$: C, 71.10; H, 8.30. Found: C, 71.35; H, 8.04. IR ν_{max}^{Nujol} cm^{-1}: 3410 (OH), 1749 (five-membered ring ketone), 1712 (Ac), 1705 (six-membered ring ketone).

Oxidative Cleavage of D-Ring of γ-Digiprogenin 3-Acetate (VIIIb) with Lead Tetraacetate——To a solution of VIIIb (240 mg) in AcOH (20 ml) was added $Pb(OAc)_4$ (1 g) under stirring at room temperature and the stirring was continued for 22 hr at the same temperature to complete oxidation which was followed by TLC (SiO_2, AcOEt: benzene=2:1). The reaction mixture was saturated with H_2S to remove Pb as PbS by filtration. The filtrate was evaporated *in vacuo* to dryness and a crude product (243 mg) was separated into the following two fractions by preparative TLC (SiO_2,[9] AcOEt: benzene=2:1).

i) The less polar fraction (59 mg) was not homogeneous and unidentified.

ii) The more polar fraction (131 mg) was purified with acetone–*n*-hexane to give intramolecular hemiacetal (XI, 98 mg) as a homogeneous colorless powder. IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 3583, 1784, 1726, 1717. NMR

($CDCl_3$) τ: 8.85 (6H, s, 18- and 19-CH_3), 8.34 (3H, s, C–C–CH_3), 7.98 (3H, s, Ac).

Methyl Ester (XII) of XI——To a solution of XI (73 mg) in $CHCl_3$ (15 ml) was added an excess of ether solution of CH_2N_2 (8 ml) and the mixed solution was set aside overnight at room temperature. After removing the solvent, the residue was recrystallized from acetone–*n*-hexane to give XII (48 mg) as colorless prisms, mp 125—135°. *Anal.* Calcd. for $C_{24}H_{32}O_7$: C, 66.65; H, 7.46. Found: C, 66.42; H, 7.72. IR and NMR spectra were described in the main text.

Tetrahydro-γ-digiprogenin 3-Acetate A, B and C (XIIIa, XIIIb and XIIIc) from VIIIb——To a solution of VIIIb (500 mg) in 80% dioxane (25 ml) was added $NaBH_4$ (50 mg) under stirring and the mixed solution was allowed to stand at room temperature for 1 hr. Disappearance of VIIIb and formation of three products were detected by TLC (SiO_2, AcOEt: benzene=2:1). The reaction mixture was neutralized with 5% AcOH, concentrated under reduced pressure and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo*. The residue (550 mg) was separated into three fractions by preparative TLC (SiO_2, AcOEt: benzene=2:1).

i) The least polar fraction (114 mg) was recrystallized from acetone–*n*-hexane to give tetrahydro derivative A (XIIIa, 92 mg) as colorless needles, mp 272—275°, $[\alpha]_D^{23}$ +13.7° (c=1.061, MeOH). *Anal.* Calcd. for $C_{23}H_{34}O_6$: C, 67.95; H, 8.43. Found: C, 67.77; H, 8.58. IR ν_{max}^{Nujol} cm^{-1}: 3505, 3260, 3130 (OH), 1712, 1265 (Ac), 1712 (11-CO). NMR ($CDCl_3$) τ: 7.99 (Ac), 8.79, 8.95 (18- and 19-CH_3), 8.84 (d, J=6.0 cps, 21-CH_3).

ii) The less polar fraction (232 mg) was recrystallized from acetone–*n*-hexane to give tetrahydro derivative B (XIIIb, 173 mg) as colorless needles, mp 200—201°, $[\alpha]_D^{23}$ −153.9° (c=1.075, MeOH). *Anal.* Calcd. for $C_{23}H_{34}O_6$: C, 67.95; H, 8.43. Found: C, 67.74; H, 8.47. IR ν_{max}^{Nujol} cm^{-1}: 3540, 3485, 3425 (OH), 1703, 1268 (Ac), 1703 (11-CO). NMR ($CDCl_3$) τ: 8.00 (Ac), 8.76, 8.93 (18- and 19-CH_3), 8.82 (d, J=6.0 cps, 21-CH_3).

iii) The more polar fraction (49 mg) was recrystallized from acetone–*n*-hexane to give tetrahydro derivative C (XIIIc, 35 mg) as colorless needles, mp 210—213°, $[\alpha]_D^{23}$ −83.1° (c=1.082, MeOH). *Anal.* Calcd. for $C_{23}H_{34}O_6$: C, 67.95; H, 8.43. Found: C, 67.98; H, 8.53. IR ν_{max}^{Nujol} cm^{-1}: 3445, 3365 (OH), 1708, 1270 (Ac), 1681 (11-CO). NMR ($CDCl_3$) τ: 7.98 (Ac), 8.80, 8.86 (18- and 19-CH_3), 8.75 (d, J=6.0 cps, 21-CH_3).

Oxidative Cleavage of D-Ring of Tetrahydro-γ-digiprogenin 3-Acetate B (XIIIb)——To a solution of XIIIb (85 mg) in MeOH (14 ml) was added a solution of $NaIO_4$ (145 mg) in H_2O (5 ml), and the mixed solution was allowed to stand at room temperature for 2 hr. Completion of the reaction was checked by TLC (SiO_2, AcOEt:benzene=2:1). The reaction mixture was concentrated *in vacuo* to remove MeOH and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to dryness. The residue (65 mg) was recrystallized from acetone to give aldehyde (XV, 35 mg) as colorless needles, mp 206—210°, $[\alpha]_D^{22}$ −91.5° (c=1.028, MeOH). *Anal.* Calcd. for $C_{23}H_{32}O_6$: C, 68.29; H, 7.97. Found: C, 68.41; H, 8.22. IR and NMR spectra were described in the main text.

Oxidation of Aldehyde (XV) to Carboxylic Acid (XVI)——To a solution of XV (105 mg) in acetone (5 ml) was added 6 N CrO_3 (0.2 ml) and the mixed solution was allowed to stand at room temperature overnight. After the excess of CrO_3 was reduced with MeOH (6 ml), the reaction mixture was concentrated *in vacuo*, extracted with $CHCl_3$ and the $CHCl_3$ solution was extracted with 5% $NaHCO_3$. The alkaline solution was acidified and extracted with $CHCl_3$. The $CHCl_3$ solution was washed with H_2O, dried over Na_2SO_4 and evaporated *in vacuo* to obtain an acidic product (57 mg) which was recrystallized from acetone–*n*-hexane to afford

XVI (49 mg) as colorless crystals, mp 169—172° (decomp.). *Anal.* Calcd. for $C_{23}H_{32}O_7$: C, 65.69: H, 7.67. Found: C, 65.37; H, 7.71. IR spectrum was described in the main text.

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