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Studies on Cardiac Ingredients of Plants. III.[1] Structural Confirmation and Biological Activity of Reduced Proscillaridins

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Catalytic hydrogenation of proscillaridin (I) gave five compounds (II—VI) which were successfully separated by reversed-phase high performance liquid chromatography. The structures of II—VI were determined on the basis of the proton, carbon-13 and two dimensional nuclear magnetic resonance (^{1}H-, ^{13}C- and 2D-NMR) spectra. The configurations of IV and V were determined by circular dichroism spectral analyses.

Relationships between the chemical structures and biological activities of I—VI were studied by the use of isolated guinea-pig papillary muscle and an Na^+, K^+-adenosine triphosphatase preparation from dog kidney. The biological activity of each compound was evaluated in terms of pD_2 and pIC_{50} values, while the inotropic speed of each compound was evaluated in terms of T_{50} value. The activities of the reduced compounds (II—VI) were weaker than that of proscillaridin (I), but the inotropic speeds were faster.

A significant correlation was obtained between the ^{13}C-NMR chemical shifts of C-24 and the pD_2 values of I—VI ($r=0.95$, $p<0.01$). The T_{50} values and ^{13}C-NMR chemical shifts of C-18 also showed a remarkable correlation ($r=0.96$, $p<0.01$).

Keywords——proscillaridin; proscillaridin catalytic hydrogenation; 20*R*-, 20*S*-tetrahydroproscillaridin; reversed-phase HPLC; ^{13}C-NMR; 2D-NMR; CD; Na^+, K^+-ATPase; positive inotropic effect; guinea-pig papillary muscle

The cardiac glycosides digitoxin, digoxin and ouabain have been widely used in the therapy of congestive heart failure in spite of their toxicity and the narrow safety margin.[2] In 1957, Vick *et al.*[3] indicated that hydrogenation of the lactone rings of digitoxin, digoxin and ouabain increased the safety margin of the parent glycosides without a corresponding loss of positive inotropic effect (PIE) on the dog heart–lung preparation. Since then, a number of studies[4] have been undertaken to examine the difference in action between the natural glycosides and their hydrogenated derivatives. The reduction of the unsaturated lactone rings of cardiac glycosides introduces a center of asymmetry at C-20.[5] However, most of the pharmacological investigations[6] on dihydroglycosides have been carried out with mixtures of the 20*R*- and 20*S*-epimers because of the lack of methodology for effective separation of the two epimers. We have, therefore, been interested in separation of the individual 20*R*- and 20*S*-dihydroepimers and subsequent evaluation of their pharmacological differences.

In a previous paper,[1] we reported the synthesis and isolation of 20*R*- and 20*S*-dihydroouabain, and clarified the differences in PIE between the two epimers. As an extension of our program directed towards the development of new cardiac glycosides with a lower risk of toxicity due to an improved ratio of therapeutic and toxic doses, we wish to report in this paper our results concerning the hydrogenation of proscillaridin (I), one of the most potent

cardiac glycosides, and the positive inotropic activities of the resulting reduced proscillaridins (II—VI). In addition, we will describe the relationship between the PIE and chemical shifts in the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra.

Chemistry

Catalytic hydrogenation of proscillaridin (I) over 5% Pd–C gave five compounds (II—VI), which were separated and purified by high performance liquid chromatography (HPLC) with a reversed-phase column (Chart 1).

Compound II was obtained as colorless needles, mp 216—221 °C, $[\alpha]_D^{22}$ −60.7°, $C_{30}H_{44}O_8$ (*m/z* 532, M^+) and confirmed to be 3*β*-[(6-deoxy-α-L-mannopyranosyl)oxy]-14*β*-hydroxybufa-4,20(22)-dienolide by a comparison of the mass spectrum (MS), and infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra with those of an authentic sample prepared according to Repke and Berlin[7] by Al–Hg reduction of I.

Compound III was obtained as a crystalline powder, mp 187—188 °C, $[\alpha]_D^{22}$ −23.2°, $C_{30}H_{44}O_8$ (*m/z* 532, M^+). The IR spectrum showed a carbonyl group absorption at 1745 cm^{-1}. In the two-dimensional ^{1}H shift-correlated (2D-COSY) spectrum of III (Fig. 1), the C-21 olefinic proton signal was observed at *δ* 6.54 as a singlet and the signals of the 22-H_2 and 23-H_2 protons of the lactone ring at *δ* 2.46 (1H, m), 2.93 (1H, m) and 2.50—2.64 (2H, m), respectively. The ^{13}C-NMR spectrum showed the signals of five carbons of the lactone ring at *δ* 21.0 (t), 29.0 (t), 121.6 (s), 138.4 (d) and 169.4 (s). From these results, compound III was confirmed to be 3*β*-[(6-deoxy-α-L-mannopyranosyl)oxy]-14*β*-hydroxybufa-4,20-dienolide.

Compound IV (colorless needles, mp 245—249 °C, $[\alpha]_D^{22}$ −41.1°) and compound V (colorless needles, mp 236—238 °C, $[\alpha]_D^{22}$ −56.8°) were found to have the same molecular formula, $C_{30}H_{46}O_8$, by elementary analyses. The MS of IV and V showed the same fragment ion at *m/z* 388 ($M^+ - C_6H_{10}O_4$). The IR spectra of IV and V showed carbonyl absorptions due to a saturated *δ*-lactone at 1720 and 1725 cm^{-1}, respectively. The ^{1}H- and ^{13}C-NMR spectra showed no signals in the olefinic lactone region. In the ^{1}H-NMR spectrum (Table I), the signals of protons of the lacton ring in IV were observed at *δ* 1.95 (1H, m, 22-H), 2.15 (1H,

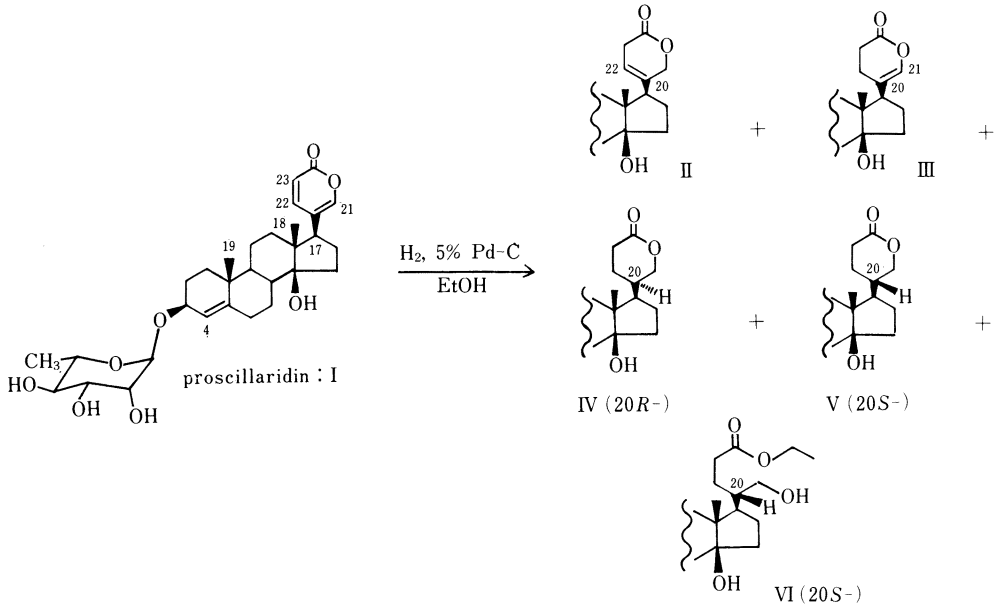

Chart 1

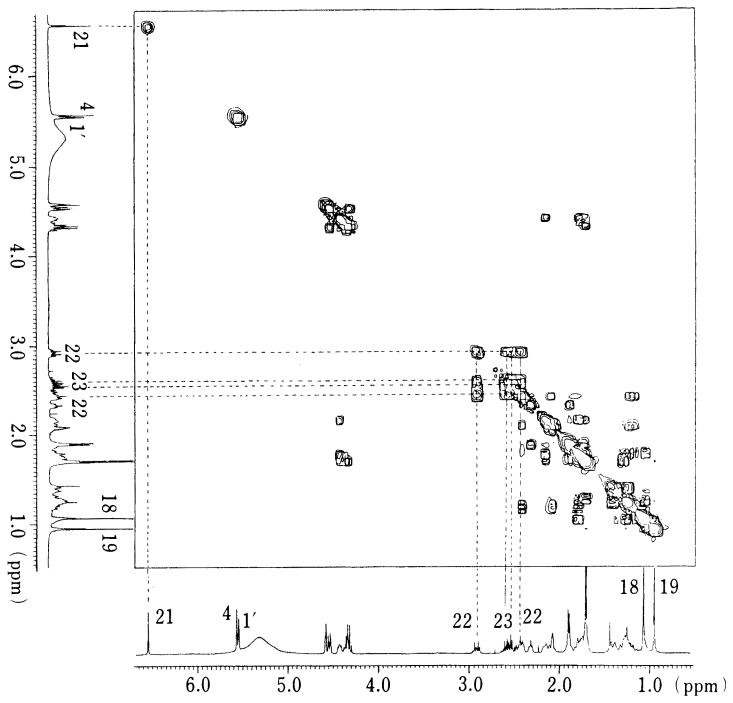

Fig. 1. Contour Plot of the COSY Map of Compound III

m, 22-H), 2.20—2.24 (1H, m, 20-H), 2.45 (1H, ddd, 23-H), 2.56 (1H, ddd, 23-H), 3.98 (1H, dd, 21-H) and 4.26 (1H, dd, 21-H). The signals of V were similarly observed at δ 1.58 (1H, m, 22-H), 1.82 (1H, m, 22-H), 2.16—2.25 (1H, m, 20-H), 2.54 (1H, ddd, 23-H), 2.64 (1H, ddd, 23-H), 4.38 (1H, dd, 21-H) and 4.69 (1H, dd, 21-H). The above signal assignments of IV and V were supported by 2D-COSY. These observations suggested that compounds IV and V are 3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxybufa-4-enolides, and they are clearly the epimers of the chiral center at C-20.

The C-20 configuration of each epimer was deduced by circular dichroism (CD) spectroscopy. Beecham[8] reported that the sign of CD curve for δ-lactones is associated with the location of the β-carbon relative to the plane formed by the lactone moiety and the α-carbon (C_α–CO–O). The CD curve is positive when the conformation of the δ-lactone has the β-carbon above the plane and negative when the β-carbon is below the plane (Fig. 2). The CD spectra of compounds IV and V revealed that IV had a positive CD curve and V had a negative CD curve (Fig. 3). These results indicate that the β-carbon of the lactone is located as in Fig. 2A or 2B for compound IV and as in Fig. 2C or 2D for compound V. Molecular model examination of IV and V suggested that the energetically preferred position for the steroid substituent on C-20 is quasi-equatorial as shown in Fig. 2. We concluded that compound IV has the *R*-configuration at C-20 and compound V has the *S*-configuration.

Compound VI was obtained as a crystalline powder, mp 189—191 °C, $[\alpha]_D^{22} -29.4°$, $C_{32}H_{52}O_9$. The IR spectrum showed a carbonyl group absorption at 1725 cm^{-1}. The ^{1}H-NMR spectrum showed the appearance of new signals attributed to an ethoxy group at δ 1.20 (3H, t) and 4.18 (2H, q). In the ^{13}C-NMR spectrum, ethoxy carbons were also observed at δ 14.4 (q) and 60.2 (t), respectively, and no signals of olefinic lactone ring carbons were observed. The signal of C-21 was observed at δ 63.5 (t), whereas the corresponding signals of IV and V were observed at δ 73.5 (t) and 71.6 (t) Such a high-field shift of C-21 suggests

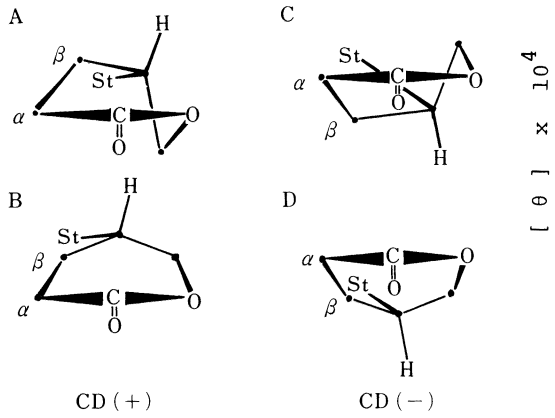

Fig. 2. Perspective Representation of the Four Stable Conformations of the δ-Lactone

A positive CD spectrum is associated with conformation A or B, and a negative CD spectrum with C or D. St represents the steroid nucleus.

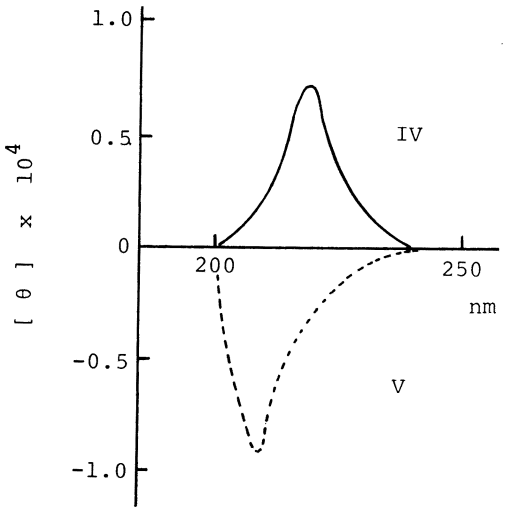

Fig. 3. CD Spectra of IV and V

——, IV (20*R*-tetrahydroproscillaridin); ------, V (20*S*-tetrahydroproscillaridin).

the cleavage of the lactone ring followed by the formation of the ester. Moreover, when IV and V were allowed to stand in EtOH under the same reductive conditions, V was readily converted to VI while IV remained unchanged. On the basis of these results, compound VI was concluded to be 20*S*-ethyl-3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β,21-dihydroxychola-4-enoate.

The spatial structures of I—VI were examined by one-dimensional nuclear Overhauser effect (NOE) and two-dimensional nuclear Overhauser enhancement spectroscopy (NOESY). NOE enhancements common in I—VI were observed among (18-H and 19-H), (19-H and 6-H), (4′-H and 5′-H), (5′-H and 6′-H) and (6′-H and 19-H). Further NOE examinations were undertaken in order to reveal the stereochemical features around the lactone ring, which is well known to be closely related to PIE development. Irradiation of the C-18 methyl signal in I caused 2.9% and 4.4% enhancements of the integral intensities of H-21 and H-22 signals, 4.1% and 1.4% in II, and 2.0% and 4.3% in III, respectively. NOE enhancements between the C-18 methyl and H-21 or H-22 signals were not observed in other compounds (IV—VI). These NOE examinations suggest that the rotation of the C-17 and C-20 linkage in compounds having a double bond in the lactone ring is restricted on account of the steric interaction between the C-18 methyl group and the C-21 or C-22 ring proton, while the corresponding rotation in IV—VI appears to be relatively free due to absence of the steric interaction.

Biological Results and Discussion

The biological activities (pD_2, pIC_{50} and T_{50} values) of I—VI were examined by means of measurements of PIE in isolated guinea-pig papillary muscle and enzyme activity of an Na^+, K^+-adenosine triphosphatase (ATPase) preparation from dog kidney. The results are summarized in Table III. Although the biological activities (pD_2 and pIC_{50}) of II—VI were less potent than that of the parent compound (I), compound III showed the most expanded concentration range in PIE development using guinea-pig papillary muscles, and reduced occurrence of the arrhythmia as judged from recordings of irregular contraction in guinea-pig

TABLE I. ^{1}H-NMR Chemical Shifts of I—VI[a]

Compd.	4-H	18-H	19-H	20-H	21-H	22-H	23-H	1′-H
I	5.58 (1H, s)	0.93 (3H, s)	0.94 (3H, s)		7.49 (1H, d, J=2.7)	8.24 (1H, dd, J=2.7, 9.8)	6.37 (1H, d, J=9.8)	5.55 (1H, s)
II	5.57 (1H, s)	1.07 (3H, s)	0.94 (3H, s)		5.12 (1H, d, J=15.1) 5.42 (1H, d, J=15.1)	5.68 (1H, t, J=3.9)	3.14 (1H, dd, J=3.9, 15.5) 3.19 (1H, dd, J=3.9, 15.5)	5.55 (1H, s)
III	5.56 (1H, s)	1.07 (3H, s)	0.95 (3H, s)		6.54 (1H, s)	2.46 (1H, m) 2.93 (1H, m)	2.50—2.64 (2H, m)	5.54 (1H, s)
IV	5.58 (1H, s)	1.16 (3H, s)	0.95 (3H, s)	2.20—2.24 (1H, m)	3.98 (1H, dd, J=10.6, 10.9) 4.26 (1H, dd, J=5.3, 10.9)	1.95 (1H, m) 2.15 (1H, m)	2.45 (1H, ddd, J=6.8, 7.2, 16.9) 2.56 (1H, ddd, J=7.2, 7.2, 16.9)	5.55 (1H, s)
V	5.57 (1H, s)	1.18 (3H, s)	0.95 (3H, s)	2.16—2.25 (1H, m)	4.38 (1H, dd, J=9.5, 11.5) 4.69 (1H, dd, J=4.8, 11.5)	1.58 (1H, m) 1.82 (1H, m)	2.54 (1H, ddd, J=8.2, 8.3, 16.6) 2.64 (1H, ddd, J=6.4, 8.2, 16.6)	5.54 (1H, s)
VI[b]	5.57 (1H, s)	1.36 (3H, s)	0.96 (3H, s)	1.75—1.84 (1H, m)	3.88 (1H, dd, J=6.8, 10.8) 4.25 (1H, dd, J=5.6, 10.8)	2.06—2.17 (2H, m)	2.59—2.63 (2H, m)	5.55 (1H, s)

a) Measured in C_5D_5N and coupling constants in Hz. *b*) Ethoxy signal: 1.20 (3H, t, J=7.1, $-OCH_2\underline{CH_3}$), 4.18 (2H, q, J=7.1, $-O\underline{CH_2}CH_3$).

heart–lung preparation (data not shown). The speed (T_{50}) of PIE with II—VI was clearly faster than that of the parent compound (I), and IV was found to show the fastest speed of PIE, being superior to ouabain (8.8 min).[9] More detailed biological studies on I—VI will be reported elsewhere.

An excellent correlation was obtained between the ^{13}C-NMR (C_5D_5N) chemical shifts of C-24 and the pD_2 values of I—VI, as shown in Fig. 4. The regression line was expressed by the following equation (r, correlation coefficient); $\delta_{C\text{-}24} = -4.0\ pD_2 + 193.4$ ($r = -0.95$, $p < 0.01$). Similarly, Fig. 5 shows a significant correlation between the ^{13}C-NMR (C_5D_5N) chemical shifts of C-18 and the T_{50} values of compounds (I—VI); $\delta_{C\text{-}18} = 0.03\ T_{50} + 16.09$ ($r = 0.96$, $p < 0.01$).

Na^+, K^+-ATPase is generally recognized to be a receptor of cardiac glycosides,[10] and this has prompted many molecular-level studies on the relationship between chemical structure and biological activity of cardiac glycosides. Fullerton *et al.*[11] synthesized nine digitoxigenin analogues in order to examine the relationship between genin structure and Na^+, K^+-ATPase inhibitory activity. With the use of a multidisciplinary approach including X-ray crystallography and conformational energy calculations, they found a striking correlation between the position of the C-17 side group carbonyl oxygen and biological activity.

TABLE II. ^{13}C-NMR Chemical Shifts of I—VI

Carbon	I	II	III	IV	V	VI
C-4	121.6 (d)	121.6 (d)	121.6 (d)	121.6 (d)	121.6 (d)	121.5 (d)
C-5	146.8 (s)	146.9 (s)	146.9 (s)	146.9 (s)	147.0 (s)	147.1 (s)
C-13	48.7 (s)	49.1 (s)	48.9 (s)	47.5 (s)	47.4 (s)	47.4 (s)
C-14	84.2 (s)	84.0 (s)	83.8 (s)	83.9 (s)	84.1 (s)	84.7 (s)
C-15	32.8 (t)	32.8 (t)	32.9 (t)	32.4 (t)	32.3 (t)	32.7 (t)
C-16	29.4 (t)	26.0 (t)	26.0 (t)	29.9 (t)	29.7 (t)	29.7 (t)
C-17	51.3 (d)	56.5 (d)	53.6 (d)	51.3 (d)	53.6 (d)	52.9 (d)
C-18	17.2 (q)	16.4 (q)	16.3 (q)	16.2 (q)	16.4 (q)	16.7 (q)
C-19	19.0 (q)	19.0 (q)	19.0 (q)	19.0 (q)	19.0 (q)	19.0 (q)
C-20	123.4 (s)	140.6 (s)	121.6 (s)	34.9 (d)	35.3 (d)	43.1 (d)
C-21	149.4 (d)	69.9 (t)	138.4 (d)	73.5 (t)	71.6 (t)	63.5 (t)
C-22	147.5 (d)	120.1 (d)	21.0 (t)	21.5 (t)	24.3 (t)	23.2 (t)
C-23	115.3 (d)	31.8 (t)	29.0 (t)	24.1 (t)	26.6 (t)	33.3 (t)
C-24	162.2 (s)	171.2 (s)	169.4 (s)	172.4 (s)	172.6 (s)	173.8 (s)
$OCH_2\underline{C}H_3$						14.4 (q)
$O\underline{C}H_2CH_3$						60.2 (t)

Measured in C_5D_5N.

TABLE III. Biological Activities of I—VI

Compd.	pD_2[a]	pIC_{50}[b]	T_{50}[c]
I	7.61±0.14	7.44±0.02	32.99±2.19
II	6.18±0.04	7.02±0.04	15.21±2.08
III	5.81±0.12	7.26±0.04	7.13±1.51
IV	5.34±0.07	6.96±0.04	2.31±0.19
V	4.91±0.05	6.47±0.02	6.69±0.38
VI	5.02±0.06	5.54±0.03	22.42±1.96

a) pD_2 is the concentration of test compounds required for 50% of the maximum PIE in guinea-pig papillary muscles ($n=5$—6, mean±S.E.). *b*) pIC_{50} is the concentration of test compounds required for 50% of the maximum inhibition of the activity of an Na^+, K^+-ATPase preparation from dog kidney ($n=3$, mean±S.E.). *c*) T_{50} is the time (min) to the point of half-maximum PIE after addition of test compounds at the pD_2 concentration in guinea-pig papillary muscles ($n=5$, mean±S.E.).

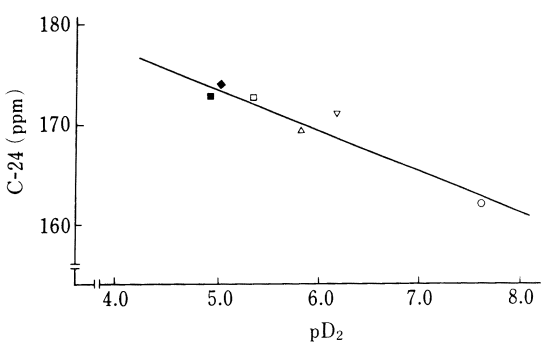

Fig. 4. Correlation between pD_2 Values and Chemical Shifts of Carbon-24 (C-24) in I—VI

○, I; ▽, II; △, III; □, IV; ■, V; ◆, VI. The pD_2 value of each compound is the mean value. The chemical shifts (C-24) of I—VI are those in the ^{13}C-NMR (C_5D_5N) spectra.

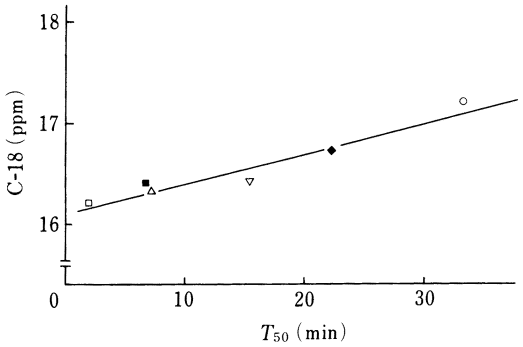

Fig. 5. Correlation between T_{50} Values and Chemical Shifts of Carbon-18 (C-18) in I—VI

○, I; ▽, II; △, III; □, IV; ■, V; ◆, VI. The T_{50} value of each compound is the mean value. The chemical shifts (C-18) of I—VI are those in the ^{13}C-NMR (C_5D_5N) spectra.

On the other hand, we employed the readily obtainable ^{13}C-NMR chemical shift value as a novel parameter for the elucidation of structure–activity relationships, since this method is applicable to non-crystalline compounds such as I—VI, which are not suitable for X-ray analysis, and further, the *in vivo* molecular conformation of the compound is more likely to be duplicated in the liquid medium than in the solid state.

First of all, we investigated the relationship between the cardiotonic activity (pD_2 value) on isolated myocardium in the guinea pig and the ^{13}C-NMR chemical shifts of carbonyl carbons in the lactone. There was an excellent correlation between PIE and the C-24 chemical shifts. This method could therefore be a convenient and useful means to predict the PIE potency of new compounds. Further application to other analogues of cardiac glycosides is under investigation.

A significant correlation was also obtained between the T_{50} values and the C-18 methyl chemical shifts. The steric interactions between the C-18 methyl group and lactone protons are presumed to be closely related to the differences in C-18 chemical shift and T_{50} value, but the chemical shift differences and NOE enhancements of the compounds are too small for us to conclude that the observed significant correlation resulted almost exclusively from the steric interactions.

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The MS were measured with a JEOL JMS DX-300 mass spectrometer. The ultraviolet (UV) spectra were recorded with a Shimadzu UV-210A spectrometer, the IR spectra with a JASCO IRA-2 spectrometer, and the CD spectra with a JASCO J-40S spectrometer. The optical rotations were taken with a JASCO DIP-140 automatic polarimeter. The ^{1}H- and 2D-NMR spectra were recorded with JEOL JNM-MH-100 and JEOL GX-400 spectrometers and the ^{13}C-NMR spectra with a JEOL JNM-FX 100 spectrometer in C_5D_5N using tetramethylsilane as an internal standard. The following abbreviations are used; s, singlet; d, doublet; dd, double doublet; ddd, double double-doublet; t, triplet; q, quartet; m, multiplet. The apparatus used for HPLC was a JASCO TWINCL high-performance liquid chromatograph equipped with a JASCO UVIDEC 100-III detector, and the eluate was monitored at 210 nm. HPLC was carried out on a Develosil ODS-10/20 column (0.76 cm i.d. × 25 cm, Nomura Chem. Co., Seto) at a flow rate of 2.0 ml/min.

Hydrogenation of Proscillaridin (I)——Proscillaridin (1.0 g) was added to a hydrogenation bottle containing 50 ml of EtOH and 1.5 g of 5% Pd–C catalyst. Proscillaridin was hydrogenated for 1.5 h at room temperature under atmospheric pressure. The catalyst was removed and the EtOH was evaporated off *in vacuo* to afford a residue (1.1 g).

Separation by HPLC——The above residue was dissolved in EtOH and subjected to HPLC. Elution with MeCN–H_2O (2 : 3) gave three fractions, 1 (675 mg), 2 (194 mg) and 3 (compound III, 86 mg). Fraction 1 was rechromatographed with MeCN–MeOH–H_2O (3 : 2 : 6) to give compound IV (277 mg) and compound V (331 mg). Similarly, fraction 2 was rechromatographed with MeCN–H_2O (1 : 2) to give compound II (97 mg) and compound VI (68 mg).

3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxybufa-4,20,22-trienolide; Proscillaridin (I)——Colorless needles, mp 221—223 °C (MeOH) (lit.[12] mp 220—222 °C). $[\alpha]_D^{22}$ −65.6° (c=0.1, EtOH). UV λ_{max}^{EtOH} nm (log ε): 211 (3.88), 299 (3.75). IR ν_{max}^{KBr} cm^{-1}: 3480 (OH), 1725 (C=O). *Anal.* Calcd for $C_{30}H_{42}O_8$: C, 67.90; H, 7.98. Found: C, 67.78; H, 8.08. ^{1}H-NMR: Table I. ^{13}C-NMR Table II.

3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxybufa-4,20(22)-dienolide (II)——Colorless needles, mp 216—221 °C (Me_2CO–Et_2O (1 : 1)). $[\alpha]_D^{22}$ −60.7° (c=0.1, EtOH). UV λ_{max}^{EtOH} nm (log ε): 212 (3.54), 300 (1.51). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1725 (C=O). MS *m/z*: 532 (M^+), 386 ($M^+ - C_6H_{10}O_4$), 368 ($M^+ - C_6H_{10}O_4 - H_2O$). *Anal.* Calcd for $C_{30}H_{44}O_8 \cdot 1/2H_2O$: C, 66.52; H, 8.37. Found: C, 66.42; H, 8.21. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxybufa-4,20-dienolide (III)——Crystalline powder, mp 187—188 °C (Me_2CO–isopropyl ether (1 : 1)). $[\alpha]_D^{22}$ −23.2° (c=0.1, EtOH). UV λ_{max}^{EtOH} nm (log ε): 224 (3.72), 293 (2.42). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1745 (C=O). MS *m/z*: 532 (M^+), 386 ($M^+ - C_6H_{10}O_4$), 368 ($M^+ - C_6H_{10}O_4 - H_2O$). *Anal.* Calcd for $C_{30}H_{44}O_8 \cdot H_2O$: C, 65.43; H, 8.42. Found: C, 65.16; H, 8.47. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

20*R*-3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxybufa-4-enolide (IV)——Colorless needles, mp 245—249 °C (Me_2CO–Et_2O (1 : 1)). $[\alpha]_D^{22}$ −41.1° (c=0.1, EtOH). CD (c=0.02, EtOH) $[\theta]^{20}$ (nm): +1.9 × 10^3 (221) (positive maximum). UV λ_{max}^{EtOH} nm (log ε): 213 (2.94), 298 (1.84). IR ν_{max}^{KBr} cm^{-1}: 3400 (OH), 1720 (C=O). MS *m/z*: 388 ($M^+ - C_6H_{10}O_4$), 370 ($M^+ - C_6H_{10}O_4 - H_2O$). *Anal.* Calcd for $C_{30}H_{46}O_8 \cdot 1/2H_2O$: C, 66.27; H, 8.71. Found: C, 66.22; H, 8.44. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

20*S*-3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxybufa-4-enolide (V)——Colorless needles, (mp 236—238 °C (Me_2CO). $[\alpha]_D^{22}$ −56.8° (c=0.1, EtOH). CD (c=0.02, EtOH) $[\theta]^{20}$ (nm): -1.0×10^4 (208) (negative maximum). UV λ_{max}^{EtOH} nm (log ε): 214 (2.96), 297 (2.46). IR ν_{max}^{KBr} cm^{-1}: 3380 (OH), 1725 (C=O). MS m/z: 388 ($M^+ - C_6H_{10}O_4$), 370 ($M^+ - C_6H_{10}O_4 - H_2O$). *Anal.* Calcd for $C_{30}H_{46}O_8 \cdot 1/2H_2O$: C, 66.27; H, 8.71. Found: C, 66.04; H, 8.51. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

20*S*-Ethyl-3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β,21-dihydroxychola-4-enoate (VI)——Crystalline powder, mp 189—191 °C (Me_2CO–isopropyl ether (1 : 1)). $[\alpha]_D^{22}$ −29.4° (c=0.1, EtOH). UV λ_{max}^{EtOH} nm (log ε): 210 (3.41), 295 (1.51). IR ν_{max}^{KBr} cm^{-1}: 3360 (OH), 1725 (C=O). MS m/z: 388 ($M^+ - C_6H_{10}O_4 - C_2H_5OH$), 370 ($M^+ - C_6H_{10}O_4 - C_2H_5OH - H_2O$). *Anal.* Calcd for $C_{32}H_{52}O_9 \cdot 1/2H_2O$: C, 65.17; H, 9.06. Found: C, 65.26; H, 8.75. ^{1}H-NMR: Table I. ^{13}C-NMR: Table II.

Biological Activity——PIE (pD_2, T_{50}, and pIC_{50} values) of test compounds were examined by the use of isolated guinea-pig papillary muscle preparations and Na^+, K^+-ATPase preparation from dog kidney, respectively. The measurements were performed according to the methods described in out previous paper.[1)]

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