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Studies on Cardiac Ingredients of Plants. V. Chemical Transformation of Proscillaridin and Biological Activities of Derivatives

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In order to investigate the relationship between the chemical structures and the biological activities of proscillaridin (**1**) and related compounds, transformations of the lactone (**1**) into the lactams (**2a**—**i**) with monoalkylamines, into 1,4-cycloadducts (**3**, **5**) with dimethyl acetylenedicarboxylate, and into epoxides (**6**—**8**) with tris(acetylacetonate)iron(III)–H_2O_2 have been undertaken. Further, alkoxyalkylation of the tertiary $C_{14\beta}$-hydroxy group was carried out with alkoxyalkyl halides. The biological activities of the resulting proscillaridin derivatives were studied by the use of isolated guinea-pig papillary muscle preparations and an Na^+,K^+-adenosine triphosphatase preparation from dog kidney.

Although the activities of proscillaridin derivatives were less potent than that of **1**, compounds **2a**, **2g**, **3** and **9** showed slightly expanded concentration ranges of positive inotropic effect development on guinea-pig papillary muscle preparations.

A significant correlation was obtained between the van der Waals volumes (V_w) and pIC_{50} values of **1** and **2a**—**h** ($r=-0.90$, $p<0.01$).

Keywords——proscillaridin; chemical transformation; lactam; 1,4-cycloaddition; epoxidation; alkoxyalkylation; guinea-pig papillary muscle; Na^+,K^+-ATPase; van der Waals volume

The cardiac glycoside, proscillaridin (**1**) has been widely used in the treatment of congestive heart failure.[1] However, this drug shows very narrow concentration of positive inotropic effect (PIE) development and occasionally causes arrhythmia.[2] Therefore, attempts have been made to develop chemically modified proscillaridins with a higher margin of safety.

We have previously hydrogenated the lactone ring of proscillaridin (**1**) over 5% palladium on charcoal and succeeded in the separation of five reduced proscillaridins by reversed-phase high-performance liquid chromatography (HPLC).[3] We found that the C_{22}–C_{23} hydrogenated proscillaridin, 3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxybufa-4,20-dienolide had the most expanded concentration range of PIE development on guinea-pig papillary muscle preparation and showed a reduced occurrence of arrhythmia.[4]

As an extension of our continuing program directed towards the further development of new proscillaridin analogs with a lower risk of toxicity, we undertook the chemical transformation of the lactone ring, such as lactamization with monoalkylamines, 1,4-cycloaddition with dimethyl acetylenedicarboxylate (DMAD) and epoxidation with the tris(acetylacetonate)iron(III)–aqueous hydrogen peroxide($Fe(acac)_3$–H_2O_2) system, as well as alkoxyalkylation of the tertiary $C_{14\beta}$-hydroxy group located near the lactone ring. The cardiotonic activities (pIC_{50} and pD_2 values) of the resulting proscillaridin derivatives (**2**—**14**) were studied by the use of isolated guinea-pig papillary muscle preparations and an Na^+,K^+-adenosine triphosphatase (ATPase) preparation from dog kidney.

In addition, we investigated the relationship between the PIE (pIC_{50} value) and van der Waals volumes (V_W) of proscillaridin (**1**) and the lactam derivatives (**2a**—**h**). This paper describes our results on chemical transformation of **1** and PIE development of the derivatives obtained.

Chemistry

A few proscillaridin lactam have already been prepared by treatment of **1** with suitable amines by Repke and co-workers.[5] The known lactams (**2a**—**c**) were prepared and their structures were confirmed by comparison of the infrared (IR) spectra with those of authentic samples, showing the lactam carbonyl absorption at 1650—1660 cm^{-1}. Similarly, the reaction of **1** with other monoalkylamines gave the corresponding 1-substituted 5-[3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-en-17β-yl]-2(1*H*)-pyridinones (**2d**—**i**) in 7—60% yields (Chart 1).

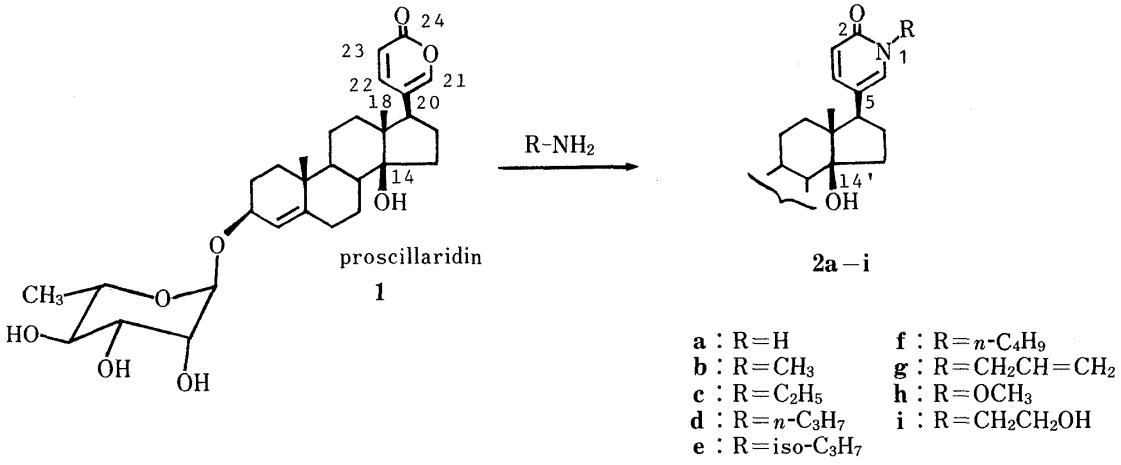

Chart 1

The IR spectra of **2d**—**i** exhibited absorptions at 1650—1660 cm^{-1} due to the lactam carbonyls. The structures of **2d**—**i** were also established by elementary analyses, the electron impact mass spectra (EI-MS), the proton nuclear magnetic resonance (^{1}H-NMR) spectra and the carbon-13 nuclear magnetic resonance (^{13}C-NMR) spectra as shown in Tables I and II.

In contrast, when **1** was treated with aniline or benzylamine, lactams could not be obtained because of steric hindrance around the lactone ring.

The diene fragments in the lactone (**1**) and lactam (**2a**) are expected to undergo 1,4-cycloaddition with DMAD, and so **1** was reacted with DMAD under reflux in dioxane for 48 h to afford a crystalline powder of mp 138—140 °C in 98% yield. The IR spectrum showed an ester carbonyl absorption at 1720 cm^{-1}. The ^{1}H-NMR spectrum showed no olefinic signals of the lactone but showed three aromatic proton signals at δ 7.55 (1H, s), 7.60 (1H, d, J= 10 Hz) and 7.61 (1H, d, J= 10 Hz), and two methyl signals at δ 3.94 (3H × 2, s). In the ^{13}C-NMR spectrum, the signal of lactone carbonyl disappeared and ester carbonyl signals appeared at δ 167.9 (s) and 168.8 (s) as well as the six benzene carbons. From these data, the structure of the adduct was confirmed to be dimethyl 4-[3β-[(6-deoxy-α-L-mannopyranosyl)-oxy]-14β-hydroxyandrost-4-en-17β-yl]phthalate (**3**) as shown in Chart 2.

Compound **3** was reduced with $NaBH_4$ in tetrahydrofuran (THF) to give 4-[3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-en-17β-yl]-1,2-benzenedimethanol (**4**) in 86% yield. The ^{1}H-NMR spectrum of **4** showed methylene signals at δ 4.56 and 4.58 and no methyl ester signals. The IR spectrum showed disappearence of the ester carbonyl absorption.

The similar treatment of **2a** with DMAD yielded **5** in 97% yield. The fast atom

TABLE I. Physicochemical Data for 1-Substituted 5-[3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-ene-17β-yl]-2(1*H*)-pyridinones (**2a—i**)

Compd. No.	R	Yield (%)	Reaction time (h)	mp (°C)	Appearance (Recryst. solv.)	Formula	MS *m/z* ($M^+ - C_6H_{12}O_5$)	Analysis (%) Calcd (Found)		
								C	H	N
2a	H	40	1.5	>300[a]	Colorless prisms (MeOH)	$C_{30}H_{43}NO_7 \cdot 1/2\,H_2O$	365	66.78 (66.89	8.17 8.23	2.45 2.60)
2b	CH_3	41	5	300—303[b]	Colorless prisms ($MeOH–Me_2CO$)	$C_{31}H_{45}NO_7$	379	68.48 (68.16	8.34 8.00	2.58 2.51)
2c	C_2H_5	42	3	267—274[c]	Colorless prisms ($MeOH–Me_2CO$)	$C_{32}H_{47}NO_7$	393	68.91 (68.97	8.49 8.96	2.51 2.39)
2d	*n*-C_3H_7	20	3	285—287	Crystalline powder ($Me_2CO–Et_2O$)	$C_{33}H_{49}NO_7 \cdot H_2O$	407	67.20 (67.24	8.72 8.53	2.38 2.49)
2e	iso-C_3H_7	7	3	271—273	Crystalline powder ($Me_2CO–Et_2O$)	$C_{33}H_{49}NO_7 \cdot 1/2\,H_2O$	407	68.25 (68.24	8.68 8.48	2.41 2.55)
2f	*n*-C_4H_9	28	3	249—254	Crystalline powder ($Me_2CO–Et_2O$)	$C_{34}H_{51}NO_7 \cdot 1/2\,H_2O$	421	68.66 (68.68	8.81 8.53	2.35 2.52)
2g	$CH_2CH=CH_2$	22	3	260—272	Crystalline powder ($Me_2CO–Et_2O$)	$C_{33}H_{47}NO_7 \cdot 1/2\,H_2O$	405	68.49 (68.53	8.36 8.02	2.42 2.63)
2h	OCH_3	60	3	280—283	Colorless prisms ($MeOH–Me_2CO$)	$C_{31}H_{45}NO_8 \cdot 1/2\,H_2O$	395	65.47 (65.10	8.15 7.81	2.46 2.37)
2i	CH_2CH_2OH	28	3	276—277	Crystalline powder ($Me_2CO–Et_2O$)	$C_{32}H_{47}NO_8 \cdot 1/2\,H_2O$	409	65.96 (65.97	8.30 8.12	2.40 2.37)

a) Lit. 5, mp 275—278 °C. *b*) Lit. 5, mp 304—308 °C. *c*) Lit. 5, mp 260—266 °C.

TABLE II. ^{1}H-, ^{13}C-NMR and IR Spectral Data for 1-Substituted 5-[3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-ene-17β-yl]-2(1*H*)-pyridinones (**2a—i**)

Compd. No.	R	^{1}H-NMR (δ)					^{13}C-NMR (δ)						IR (cm^{-1}) (CON) (C=C)
		1''-H (s)	3-H (d)	4-H (dd)	6-H (d)	R	C-2 (s)	C-3 (d)	C-4 (d)	C-5 (s)	C-6 (d)	R	
2a	H	5.56	6.77 (J=10)	8.14 (J=3, 10)	7.48 (J=3)	3.63 (s)	163.6	119.9	136.8	123.8	144.5	—	1650 1608
2b	CH_3	5.56	6.73 (J=10)	8.12 (J=3, 10)	7.38 (J=3)	3.52 (s) ($C\underline{H}_3$)	162.5	119.5	138.0	123.2	143.0	36.9 (q) ($C\underline{H}_3$)	1658 1575
2c	C_2H_5	5.55	6.72 (J=10)	8.05 (J=3, 10)	7.44 (J=3)	1.27 (t, J=7) ($CH_2C\underline{H}_3$) 3.98 (q, J=7) ($C\underline{H}_2CH_3$)	161.9	119.7	136.8	123.3	142.9	14.9 (q) ($CH_2C\underline{H}_3$) 44.4 (t) ($C\underline{H}_2CH_3$)	1652 1570
2d	n-C_3H_7	5.54	6.70 (J=10)	7.98 (J=3, 10)	7.45 (J=3)	0.82 (t, J=7) ($CH_2CH_2C\underline{H}_3$) 3.93 (t, J=7) ($C\underline{H}_2CH_2CH_3$)	162.1	119.7	137.5	123.4	143.0	11.2 (q) ($CH_2CH_2C\underline{H}_3$) 22.9 (t) ($CH_2C\underline{H}_2CH_3$) 51.0 (t) ($C\underline{H}_2CH_2CH_3$)	1660 1570
2e	iso-C_3H_7	5.56	6.72 (J=10)	7.90 (J=3, 10)	7.63 (J=3)	1.27 (d, J=7) ($CH(C\underline{H}_3)_2$)	161.7	119.4	132.9	—	142.3	21.7 (q) ($CH(C\underline{H}_3)_2$) 46.2 (d) ($C\underline{H}(CH_3)_2$)	1652 1568
2f	n-C_4H_9	5.54	6.70 (J=10)	7.99 (J=3, 10)	7.46 (J=3)	0.79 (t, J=7) ($CH_2(CH_2)_2C\underline{H}_3$) 3.98 (m) ($C\underline{H}_2(CH_2)_2CH_3$)	162.0	119.7	137.4	123.3	142.9	13.8 (q) ($CH_2(CH_2)_2C\underline{H}_3$) 20.1 (t) ($CH_2)_2C\underline{H}_2CH_3$) 31.8 (t) ($CH_2C\underline{H}_2CH_2CH_3$) 49.2 (t) ($C\underline{H}_2(CH_2)_2CH_3$)	1660 1575
2g	$CH_2CH=CH_2$	5.54	6.70 (J=10)	8.04 (J=3, 10)	7.41 (J=3)	4.68 (d, J=6) ($C\underline{H}_2CH=CH_2$) 5.14 (d, J=15) ($CH_2CH=C\underline{H}_2$) 5.8—6.3 (m) ($CH_2C\underline{H}=CH_2$)	161.8	119.7	134.0	—	143.2	50.8 (t) ($C\underline{H}_2CH=CH_2$) 117.4 (d) ($CH_2C\underline{H}=CH_2$) 134.0 (t) ($CH_2CH=C\underline{H}_2$)	1665 1585
2h	OCH_3	5.52	6.72 (J=10)	7.92 (J=3, 10)	7.78 (J=3)	4.04 (s) ($OC\underline{H}_3$)	157.8	121.4	134.9	123.8	142.6	64.3 (q) ($OC\underline{H}_3$)	1655 1580
2i	CH_2CH_2OH	5.54	6.69 (J=10)	8.04 (J=3, 10)	7.64 (J=3)	4.2—4.23 (m) ($C\underline{H}_2C\underline{H}_2OH$)	162.4	119.5	138.9	—	143.1	52.9 (t) ($C\underline{H}_2CH_2OH$) 60.4 (t) ($CH_2C\underline{H}_2OH$)	1655 1570

^{1}H- and ^{13}C-NMR spectra were measured in C_5D_5N. IR spectra were measured by the KBr disc method. Coupling constants are given in J=Hz. Signals of C-5 of **2e**, **2g** and **2i** were overlapped with C_5D_5N solvent signals.

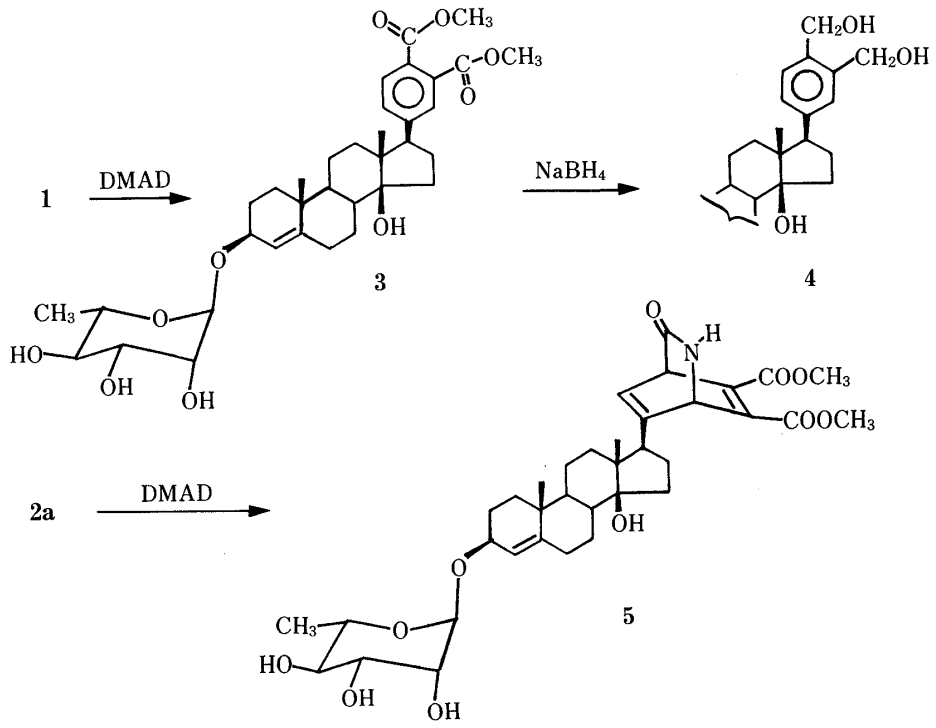

Chart 2

bombardment mass spectrum (FAB-MS) showed the fragment ion at m/z 672 ($M^+ + 1$). The IR spectrum showed ester and lactam carbonyl absorptions at 1735 and 1665 cm^{-1}. The ^1H-NMR spectrum exhibited two methyl ester signals at δ 3.69 (3H, s) and 3.83 (3H, s). In the ^{13}C-NMR spectrum, three carbonyl signals were observed at δ 161.5 (s), 162.6 (s) and 163.0 (s). On the basis of these results, the structure of the adduct was concluded to be dimethyl 7-[3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-en-17β-yl]-3-oxo-2-aza-bicyclo[2.2.2]oct-5,7-diene-5,6-dicarboxylate (**5**).

It is well known that steroidal epoxy groups contained in natural bufadienolides are related to the development of cardiotonic activity.[6] Thus, we carried out the epoxidation with $Fe(acac)_3$–H_2O_2, developed by Yamamoto and Kimura.[7]

When **1** was treated with $Fe(acac)_3$–H_2O_2 in CH_3CN under cooling, three products were detected in thin layer chromatography (TLC), and so the reaction mixture was subjected to column chromatography on silica gel to provide 3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-20β,21β-epoxy-14β-hydroxybufa-4,22-dienolide (**6**), 3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-4α,5α;20β,21β-diepoxy-14β-hydroxybuf-22-enolide (**7**) and 3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β,21α-epoxy-20β-hydroxybufa-4,22-dienolide (**8**) in 45%, 13% and 26% yields, respectively, as indicated in Chart 3.

The IR spectrum of **6** showed a carbonyl absorption at 1736 cm^{-1}. In the ^1H-NMR spectrum, the 21-H observed at δ 7.49 (1H, d, J=3 Hz) in **1** was absent from the olefinic region, and a new signal appeared at δ 5.37 (1H, s). Coupled signals of 22-H and 23-H were still observed at δ 7.94 (1H, d, J=10 Hz) and 6.02 (1H, d, J=10 Hz). The ^{13}C-NMR spectrum showed also signals attributable to the C_{20} and C_{21} carbons at δ 57.3 (s) and 84.0 (d). These spectral data demonstrate the presence of an epoxide ring at the C_{20}–C_{21} linkage, the configuration of which may be β based on molecular model building. Therefore, the structure of **6** is as shown.

It is noteworthy that the epoxidation took place initially on the lactone ring rather than on the steroidal 4-double bond.

Compound **7** was found to have another α-epoxide ring on the C_4–C_5 linkage since the 4-

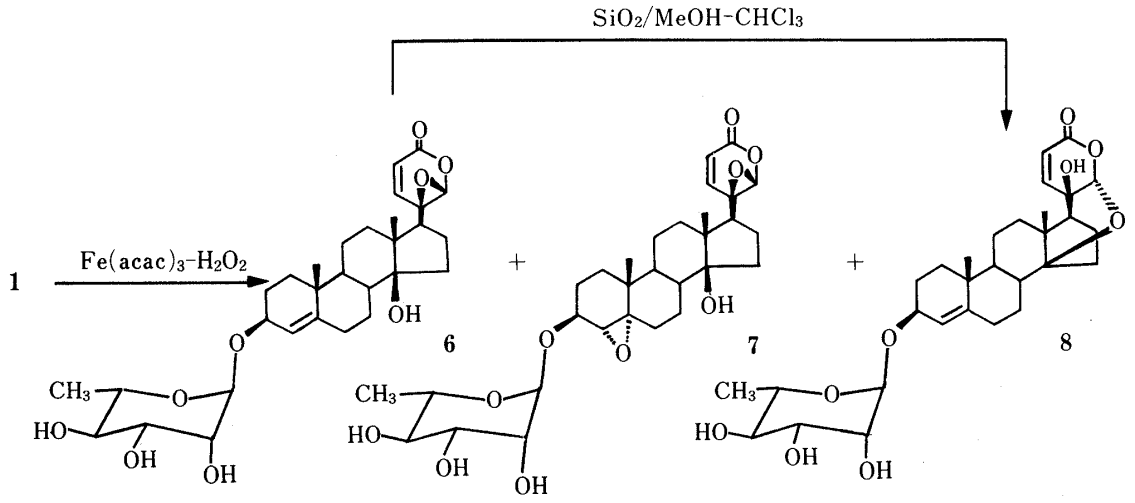

Chart 3

H signal in the ^{1}H-NMR spectrum appeared at δ 2.90 as a singlet, which is in accord with that of authentic 3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-4α,5α-epoxy-14β-hydroxybufa-20,22-dienolide prepared by epoxidation with *m*-$ClC_6H_4CO_3H$ in dioxane–$CHCl_3$.[8] The ^{13}C-NMR spectrum of **7** showed C_4 and C_5 signals at δ 62.5 (d) and 66.4 (s). These spectral data are consistent with the structure **7**.

Compound **8** was identical with the product derived from **6** by treatment with silica gel under the usual column chromatography conditions. In the ^{13}C-NMR spectrum, the signals of C_{14}, C_{20} and C_{21} were observed at δ 90.3 (s), 90.7 (s) and 100.5 (d), whereas the corresponding signals of **6** were observed at δ 84.7 (s), 57.3 (s) and 84.0 (d), respectively. These chemical shifts indicate that the $C_{14\beta}$-hydroxy group nucleophilically attacked the 21-carbon of **6** followed by ring-opening of the C_{20}–C_{21} epoxide ring to yield **8**.

Kamano and Komatsu[9] reported that treatment of bufalin with methanolic potassium hydroxide provides isobufalin, which is formed *via* cleavage of the lactone ring followed by ring closure between the 14β-oxygen and C_{21}-carbon. According to the described method, compound **1** was readily converted into *trans*-methyl 3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β,21-epoxychola-4,20(21),22-trienoate (**9**) which, on treatment with sodium hydroxide in dioxane, yielded *trans*-3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β,21-epoxychola-4,20(21),-22-trienoic acid (**10**). The ^{1}H-NMR spectrum of **9** is consistent with that of isobufalin, that is, the signals of 23-H, 21-H and 22-H appeared at δ 5.62 (1H, d, J = 15 Hz), 6.54 (1H, s) and 7.20 (1H, d, J = 15 Hz), respectively.

The attempted treatment of **9** with $Fe(acac)_3$–H_2O_2 system in CH_3CN unexpectedly resulted in the formation of *trans*-methyl 3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-20ξ-formyl-14β,20ξ-dihydroxychola-4,22-dienoate (**11**) and *trans*-methyl-3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-21-nor-20-oxo-14β-hydroxychola-4,22-dienoate (**12**) in 48% and 26% yields, respectively. The IR spectrum of **11** showed two carbonyl absorptions at 1705 and 1720 cm^{-1}, and the ^{1}H-NMR spectrum showed the appearence of a formyl proton signal at δ 9.72 (1H, s). In the ^{13}C-NMR spectrum, carbonyl signals were observed at δ 204.3 (d) and 166.5 (s), and the C_{14} signal was observed at δ 84.9 (s). These spectral data are in good agreement with the structure **11**.

A possible mechanism for the formation of **11** involves the ring-opening of the initially formed oxirane by nucleophilic attack followed by cleavage of the $C_{14\beta}$–C_{21} epoxy linkage as shown in Chart 4.

Similarly, the IR spectrum of **12** showed two carbonyl absorptions at 1710 and 1720 cm^{-1}. In the ^{13}C-NMR spectrum, carbonyl signals were observed at δ 211.2 (s) and 165.7

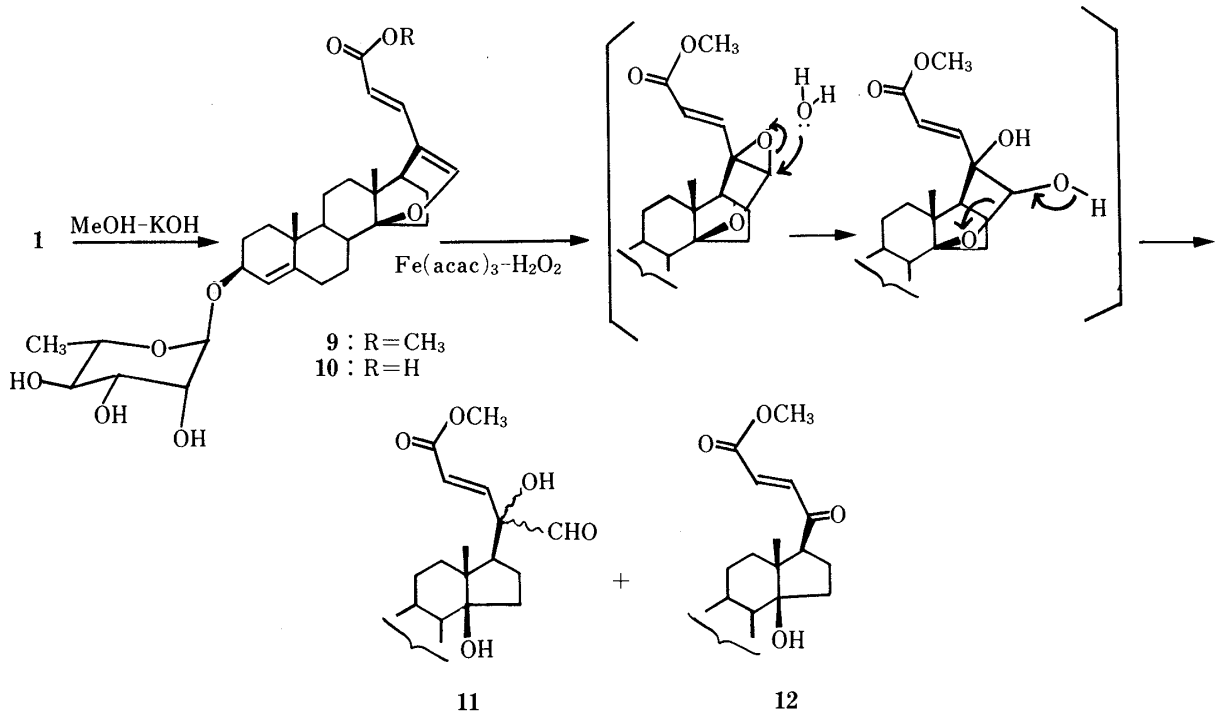

Chart 4

(s), and the $C_{14\beta}$ signal at δ 85.0 (s), while the carbon signal attributable to C_{21} was absent. Accordingly, compound **12** would have arisen through elimination of the formyl group in **11**.

In order to examine the change in cardiotonic activity caused by alkoxyalkylation of the $C_{14\beta}$-hydroxy group, chloromethyl methyl ether and chloromethyl ethyl ether were reacted with tri-*O*-acetyl proscillaridin (**13**)[10] in the presence of *N*,*N*-diisopropylethylamine-((iso-Pr)$_2$NEt) to yield 3β-[(2,3,4-tri-*O*-acetyl-6-deoxy-α-L-mannopyranosyl)oxy]-14β-*O*-methoxymethylbufa-4,20,22-trienolide (**14a**) and 3β-[(2,3,4-tri-*O*-acetyl-6-deoxy-α-L-mannopyranosyl)oxy]-14β-*O*-ethoxymethylbufa-4,20,22-trienolide (**15a**) in high yield, though alkyl halides such as methyl iodide and ethyl iodide failed to react with **13**. In the ^{13}C-NMR spectrum, the $C_{14\beta}$ signals of **14a** and **15a** were observed at δ 92.2 (s). Such a downfield shift is consistent with the displacement of $C_{14\beta}$-OH seen in the ^{13}C-NMR spectra of **8** and **9**.

Deacetylation of **14a** and **15a** with 5% K_2CO_3 in MeOH proceeded quantitatively to provide the corresponding 3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-*O*-methoxymethylbufa-4,20,22-trienolide (**14b**) and 3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-14β-*O*-ethoxy-

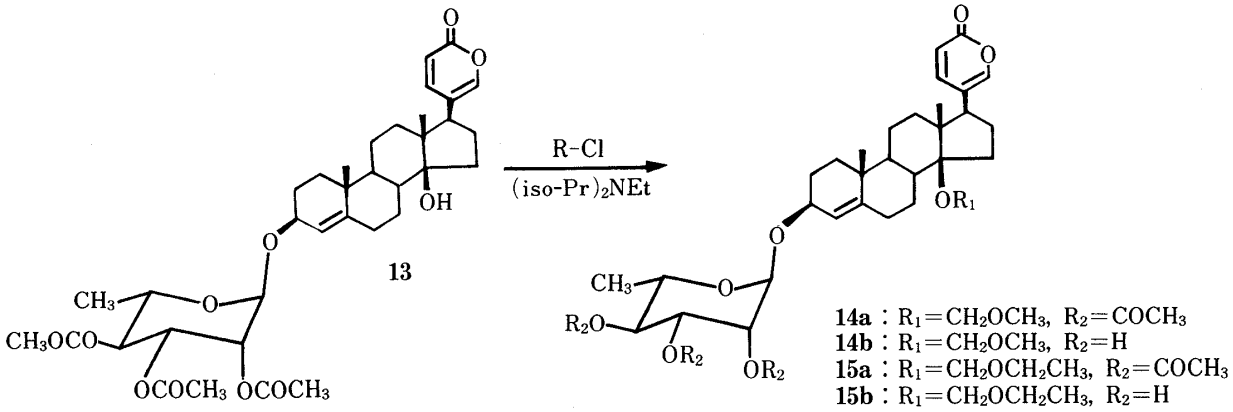

Chart 5

methylbufa-4,20,22-trienolide (**15b**). The structures of **14b** and **15b** were established by FAB-MS (Chart 5).

Biological Results and Discussion

The biological activities (pD_2 and pIC_{50} values) of proscillaridin derivatives (**2**—**15**) were examined by means of measurements of PIE in isolated guinea-pig papillary muscle and of the enzyme activity of an Na^+,K^+-ATPase preparation from dog kidney. The results are summarized in Table III.

Although the biological activities (pD_2 and pIC_{50}) of proscillaridin derivatives were generally less potent than that of the parent compound (**1**), compounds **2a**, **2b**, **2h**, **6**, **7** and **14b** showed moderately potent enzyme-inhibitory activity as compared with the other derivatives, while **2a**, **2g**, **3** and **9** showed slightly expanded concentration ranges of PIE development using guinea-pig papillary muscle, as compared with **1**.

The 1,4-cycloadducts (**3**, **5**) showed remarkably reduced enzyme-inhibitory activity, whereas 1,2-benzenedimethanol (**4**) showed slightly higher activity due to enhancement of the hydrophilicity.

We finally investigated the correlation between the lactam moiety of **2a**—**h** and corresponding pIC_{50} values employing van der Waals volume (V_W) as a parameter, since V_W can be easily calculated for a wide variety of molecules. The V_W values of lactams were calculated on the assumption that the atoms are spherical, with correction for overlap as reported by Moriguchi and co-workers.[11] As shown in Fig. 1, a significant correlation was obtained between pIC_{50} values and V_W values of **1** and **2a**—**h**. The regression line was expressed by the following equation (r, correlation coefficient): $pIC_{50} = -3.8\ V_W + 9.3$

Table III. Biological Activities of Proscillaridin Derivatives

Compd.[c]	pIC_{50}[a]	pD_2[b]
1	7.44±0.02	7.41±0.14
2a	6.17±0.06	5.17±0.02
2b	5.38±0.03	4.47±0.05
2c	4.75±0.04	—
2d	4.5	—
2e	4.5	—
2f	4.5	—
2g	5.01±0.03	4.72±0.24
2h	5.97±0.03	—
3	4.75±0.07	4.95±0.03
4	5.28±0.06	—
5	4.5	—
6	6.11±0.05	6.14±0.06
7	5.89±0.06	6.06±0.01
9	4.90±0.10	—
10	5.64±0.07	5.63±0.09
11	5.19±0.02	—
14b	6.41±0.03	5.63±0.11

a) 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.). *b*) 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.). *c*) Compound **2i** was not tested.

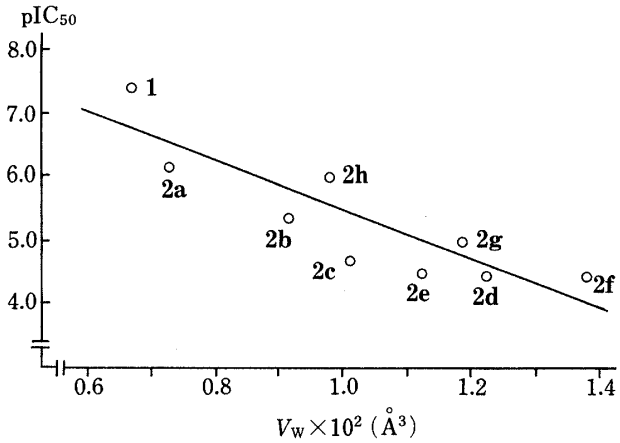

Fig. 1. Correlation between pIC_{50} Values and van der Waals Volumes (V_W) Values of **1** and the Lactams (**2a**—**h**)

The pIC_{50} value of each compound is the mean. The V_W values of **1** and the lactams (**2a**—**h**) were calculated based on the sphere volume of atoms with correction for overlap according to Moriguchi and co-workers.[11]

($r = -0.90$, $p < 0.01$).

Experimental

All melting points were determined on a Yanagimoto micro melting point apparatus and are uncorrected. The FAB-MS and EI-MS were measured with a JEOL JMS DX-300 mass spectrometer, the IR spectra with a JASCO IRA-2 spectrometer. The ^{1}H-NMR spectra were recorded with JEOL JNM-MH-100 and JEOL GSX-400 spectrometers and the ^{13}C-NMR spectra with a JEOL JNM-FX 100 spectrometer in C_5D_5N or $CDCl_3$–DMSO-d_6 (2 : 1) using tetramethylsilane as an internal standard. The following abbreviations are used; s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. TLC was performed on Merck precoated Silica gel $60F_{254}$ plates. Column chromatography was carried out on Silica gel BW-200 (Fuji Davison Chemicals, Ltd.).

General Procedure for Preparation of 1-Substituted 5-[3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-en-17β-yl]-2(1*H*)-pyridinones (2a—i)——A solution of **1** (0.19 mmol), monoalkylamine hydrochloride (3.8 mmol), sodium acetate (3.8 mmol) and glacial acetic acid (0.1 ml) in *N*,*N*-dimethylformamide (DMF) (20 ml) was heated at 160 °C in a sealed tube for 1.5—5 h. After cooling, the reaction mixture was diluted with 50 ml of MeOH–$CHCl_3$ (1 : 2) and the organic layer was washed with 5% HCl, brine and water, and concentrated *in vacuo*. The residue was purified by column chromatography on silica gel using MeOH–$CHCl_3$ (1 : 20) as the eluent to afford **2a—i**. The spectral data of **2a—i** are shown in Tables I and II.

Dimethyl 4-[3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-en-17β-yl]phthalate (3)——A mixture of **1** (200 mg) and DMAD (0.5 ml) in dioxane (10 ml) was refluxed for 48 h. The solvent was removed, and the residue was washed with water and extracted with $CHCl_3$. The extract was concentrated *in vacuo* and the residue was recrystallized from Me_2CO–Et_2O (1 : 1) to give a crystalline powder of mp 138—140 °C. Yield 232 mg (98%). IR ν_{max}^{KBr} cm^{-1}: 1720 (C=O). ^{1}H-NMR ($CDCl_3$–DMSO-d_6 (2 : 1)) δ: 0.58 (3H, s, 18′-H), 1.02 (3H, s, 19′-H), 1.28 (3H, d, J=6 Hz, 6″-H), 3.94 (3H×2, s, $COOCH_3$), 4.88 (1H, s, 1″-H), 5.27 (1H, s, 4′-H), 7.55 (1H, s, 3-H), 7.60, 7.61 (1H×2, d, J=10 Hz, 5- and 6-H). ^{13}C-NMR ($CDCl_3$–DMSO-d_6 (2 : 1)) δ: 168.8, 167.9 (s, $COOCH_3$), 150.3 (s, C-4), 147.1 (s, C-5′), 131.9, 128.4 (s, C-1 and -2), 132.5, 130.1, 128.5 (d, C-3, -5 and -6), 120.7 (d, C-4′), 99.3 (d, C-1″), 85.1 (s, C-14′), 57.8 (d, C-17′), 52.5, 52.4 (q, $COOCH_3$), 19.0 (q, C-19′), 17.6 (q, C-6″), 17.1 (q, C-18′). *Anal.* Calcd for $C_{35}H_{48}O_{10}$: C, 66.86; H, 7.70. Found: C, 66.50; H, 7.58.

4-[3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-en-17β-yl]-1,2-benzenedimethanol (4)——A solution of **3** (200 mg) and $NaBH_4$ (100 mg) in tetrahydrofuran (THF) (10 ml) was refluxed for 10 h. The reaction mixture was poured into water, neutralized with 5% HCl, and extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The resulting precipitate was collected by suction and recrystallized from MeOH–Me_2CO (1 : 1) to give colorless prisms of mp 271—272 °C. Yield 157 mg (86%). IR ν_{max}^{KBr} cm^{-1}: 3450 (OH). ^{1}H-NMR ($CDCl_3$–DMSO-d_6 (1 : 2)) δ: 0.54 (3H, s, 18′-H), 1.01 (3H, s, 19′-H), 1.19 (3H, d, J=6 Hz, 6″-H), 4.56, 4.58 (2H×2, s, $-CH_2OH$), 4.79 (1H, s, 1″-H), 4.9—5.1 (2H, m, $-CH_2OH$), 5.28 (1H, s, 4′-H), 7.22 (1H, s, 3-H), 7.27, 7.28 (1H×2, d, J=10 Hz, 5- and 6-H). ^{13}C-NMR ($CDCl_3$–DMSO-d_6 (1 : 2)) δ: 146.7 (s, C-5′), 144.7 (s, C-4), 138.3, 136.5 (s, C-1 and -2), 129.6, 128.4, 127.1 (d, C-3, -5 and -6), 120.5 (d, C-4′), 99.3 (d, C-1″), 83.8 (s, C-14′), 61.8, 61.3 (t, $-CH_2OH$), 57.5 (d, C-17′), 18.8 (q, C-19′), 17.6 (q, C-6″), 17.3 (q, C-18′). *Anal.* Calcd for $C_{33}H_{48}O_8 \cdot H_2O$: C, 67.09; H, 8.53. Found: C, 66.78; H, 8.24.

Dimethyl 7-[3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β-hydroxyandrost-4-en-17β-yl]-3-oxo-2-azabicyclo-[2.2.2]oct-5,7-diene-5,6-dicarboxylate (5)——A mixture of **2a** (100 mg) and DMAD (0.2 ml) in dioxane (15 ml) was treated under the same conditions as described for **3** to give 124 mg (98%) of **5** mp 155—161 °C, as a pale yellow crystalline powder (Me_2CO–Et_2O (1 : 1)). IR ν_{max}^{KBr} cm^{-1}: 1735 ($COOCH_3$), 1665 (CONH). FAB-MS *m/z*: 672 ($M^+ + 1$). ^{1}H-NMR ($CDCl_3$) δ: 0.75 (3H, s, 18′-H), 1.03 (3H, s, 19′-H), 1.28 (3H, d, J=6 Hz, 6″-H), 3.69, 3.83 (3H×2, s, $COOCH_3$), 4.92 (1H, s, 1″-H), 5.28 (1H, s, 4′-H), 6.46 (1H, d, J=10 Hz, 4-H), 7.00 (1H, d, J=2 Hz, 1-H), 7.59 (1H, dd, J=2, 10 Hz, 8-H). ^{13}C-NMR ($CDCl_3$) δ: 163.0 (s, C-3), 162.6, 161.5 (s, $COOCH_3$), 147.2 (s, C-5′), 144.9 (d, C-1), 141.2 (s, C-7), 136.2 (s, C-8), 125.7 (d, C-5), 123.2 (s, C-6), 120.7 (d, C-4′), 53.5, 53.1 (q, $COOCH_3$), 52.4 (d, C-17′), 18.9 (q, C-19′), 17.5 (q, C-6″), 16.6 (q, C-18′). *Anal.* Calcd for $C_{36}H_{49}NO_{11} \cdot H_2O$: C, 62.68; H, 7.45; N, 2.03. Found: C, 62.57; H, 7.15; N, 2.10.

Reaction of Proscillaridin (1) with Fe(acac)$_3$–H_2O_2 in CH_3CN——A solution of **1** (300 mg) and Fe(acac)$_3$ (500 mg) in CH_3CN (20 ml) was stirred under cooling with ice. A solution of hydrogen peroxide (30%) (2.0 ml) was added dropwise with continuous stirring. The mixture was stirred for 10 h, and then Na_2SO_3 was added. The mixture was extracted with $CHCl_3$, and the organic layer was washed with 5% HCl, and dried over $MgSO_4$. The extract was concentrated *in vacuo*, and the residue was chromatographed on silica gel with MeOH–$CHCl_3$ (1 : 9) to give **6** (139 mg, 45%), **7** (41 mg, 13%) and **8** (80 mg, 26%).

3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-20β,21β-epoxy-14β-hydroxybufa-4,22-dienolide (6)——Colorless needles from Me_2CO–Et_2O (1 : 1), mp 182—184 °C. IR ν_{max}^{KBr} cm^{-1}: 1736 (C=O). EI-MS *m/z*: 382 ($M^+ - C_6H_{12}O_5$). ^{1}H-NMR ($CDCl_3$–DMSO-d_6 (2 : 1)) δ: 0.97 (3H, s, 18-H), 1.04 (3H, s, 19-H), 1.28 (3H, d, J=6 Hz, 6′-H), 4.91 (1H, s, 1′-H), 5.32 (1H, s, 4-H), 5.37 (1H, s, 21-H), 6.02 (1H, d, J=10 Hz, 23-H), 7.94 (1H, d, J=10 Hz, 22-H). ^{13}C-NMR ($CDCl_3$–DMSO-d_6 (2 : 1)) δ: 160.3 (s, C-24), 148.5 (d, C-22), 146.8 (s, C-5), 120.9 (d, C-23), 120.8 (d, C-4), 99.2 (d, C-

1′), 84.7 (s, C-14), 84.0 (d, C-21), 57.3 (s, C-20), 54.0 (d, C-17), 18.9 (q, C-19), 17.6 (q, C-6′), 15.9 (q, C-18). *Anal.* Calcd for $C_{30}H_{42}O \cdot H_2O$: C, 63.81; H, 7.85. Found: C, 63.83; H, 7.41.

3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-4α,5α;20β,21β-diepoxy-14β-hydroxybuf-22-enolide (7)——Crystalline powder from Me_2CO–Et_2O (1 : 2), mp 179—183 °C. IR ν_{max}^{KBr} cm^{-1}: 1735 (C=O). EI-MS *m/z*: 398 ($M^+ - C_6H_{12}O_5$). ^{1}H-NMR ($CDCl_3$–DMSO-d_6 (2 : 1)) δ: 0.98 (3H, s, 18-H), 1.08 (3H, s, 19-H), 1.28 (3H, d, J=6 Hz, 6′-H), 2.90 (1H, s, 4-H), 4.94 (1H, s, 1′-H), 5.37 (1H, s, 21-H), 6.01 (1H, d, J=10 Hz, 23-H), 7.93 (1H, d, J=10 Hz, 22-H). ^{13}C-NMR ($CDCl_3$–DMSO-d_6 (2 : 1)) δ: 160.3 (s, C-24), 148.6 (d, C-22), 120.8 (d, C-23), 99.6 (d, C-1′), 84.6 (s, C-14), 84.1 (d, C-21), 66.4 (s, C-5), 62.5 (d, C-4), 57.3 (s, C-20), 54.0 (d, C-17), 17.6 (q, C-19 and -6′), 16.0 (q, C-18). *Anal.* Calcd for $C_{30}H_{42}O_{10} \cdot H_2O$: C, 62.05; H, 7.64. Found: C, 62.33; H, 7.55.

3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β,21α-epoxy-20β-hydroxybufa-4,22-dienolide (8)——Colorless needles from Me_2CO–Et_2O (1 : 1), mp 169—172 °C. IR ν_{max}^{KBr} cm^{-1}: 1712 (C=O). EI-MS *m/z*: 382 ($M^+ - C_6H_{12}O_5$). ^{1}H-NMR ($CDCl_3$–DMSO-d_6 (2 : 1)) δ: 1.08 (3H, s, 19-H), 1.17 (3H, s, 18-H), 1.28 (3H, d, J=6 Hz, 6′-H), 4.92 (1H, s, 1′-H), 5.32 (1H, s, 4-H), 5.48 (1H, s, 21-H), 5.98 (1H, d, J=10 Hz, 23-H), 7.05 (1H, d, J=10 Hz, 22-H). ^{13}C-NMR ($CDCl_3$–DMSO-d_6 (2 : 1)) δ: 162.4 (s, C-24), 146.5 (s, C-5), 146.2 (d, C-22), 121.2 (d, C-23), 120.9 (d, C-4), 100.5 (d, C-21), 99.3 (d, C-1′), 90.7 (s, C-20), 90.3 (s, C-14), 52.7 (d, C-17), 18.3 (q, C-19), 17.6 (q, C-6′), 17.1 (q, C-18). *Anal.* Calcd for $C_{30}H_{42}O_9 \cdot 1/2H_2O$: C, 64.85; H, 7.80. Found: C, 64.67; H, 7.58.

Preparation of *trans*-Methyl-3β-[(6-deoxy-α-L-mannopyranosyl)-oxy]-14β,21-epoxychola-4,20(21),22-trienolide (9) from 1——A solution of **1** (200 mg) in MeOH (5 ml) was allowed to stand with 5% KOH in MeOH (1 ml) at room temperature for 0.5 h. After acidification with 5% HCl under cooling, and dilution with water, the mixture was extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$, and concentrated *in vacuo*. The residue was recrystallized from Me_2CO–Et_2O (1 : 2) to give a crystalline powder of mp 148—150 °C. Yield 204 mg (99.5%). IR ν_{max}^{KBr} cm^{-1}: 1710 (C=O). EI-MS *m/z*: 544 (M^+). ^{1}H-NMR ($CDCl_3$) δ: 1.00 (3H, s, 19-H), 1.08 (3H, s, 18-H), 1.28 (3H, d, J=6 Hz, 6′-H), 3.71 (3H, s, $COOCH_3$), 4.92 (1H, s, 1′-H), 5.31 (1H, s, 4-H), 5.62 (1H, d, J=15 Hz, 23-H), 6.54 (1H, s, 21-H), 7.20 (1H, d, J=15 Hz, 22-H). ^{13}C-NMR ($CDCl_3$) δ: 168.5 (s, C-24), 150.0 (d, C-21), 147.2 (s, C-5), 143.6 (d, C-22), 120.7 (s, C-20), 120.7 (d, C-4), 109.3 (d, C-23), 99.2 (d, C-1′), 91.7 (s, C-14), 51.2 (q, $COOCH_3$), 19.0 (q, C-19), 17.5 (q, C-6′), 15.4 (q, C-18). *Anal.* Calcd for $C_{31}H_{44}O_8 \cdot H_2O$: C, 66.17; H, 8.24. Found: C, 66.09; H, 8.23.

Hydrolysis of 9 to *trans*-3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β,21-epoxycola-4,20(21),22-trienoic Acid (10)——A solution of **9** (200 mg) in 5% NaOH–dioxane was stirred at room temperature for 1 h. The dioxane was removed *in vacuo*. The aqueous layer was acidified with 5% HCl and extracted with $CHCl_3$. Usual work-up gave **10** as colorless needles of mp 175—178 °C after recrystallization from Me_2CO–Et_2O (1 : 1). Yield 152 mg (78%). IR ν_{max}^{KBr} cm^{-1}: 1670 (C=O). EI-MS *m/z*: 486 ($M^+ + 1 - CO_2H$). ^{1}H-NMR ($CDCl_3$–DMSO-d_6 (2 : 1)) δ: 1.01 (3H, s, 19-H), 1.08 (3H, s, 18-H), 1.28 (3H, d, J=6 Hz, 6′-H), 4.92 (1H, s, 1′-H), 5.31 (1H, s, 4-H), 5.96 (1H, d, J=15 Hz, 23-H), 6.32 (1H, s, 21-H), 7.16 (1H, d, J=15 Hz, 22-H). ^{13}C-NMR ($CDCl_3$–DMSO-d_6 (2 : 1)) δ: 168.0 (s, C-24), 149.0 (d, C-21), 147.1 (s, C-5), 141.6 (d, C-22), 120.7 (s, C-20), 120.7 (d, C-4), 108.3 (d, C-23), 99.2 (d, C-1′), 91.2 (s, C-14), 19.0 (q, C-19), 17.5 (q, C-6′), 15.4 (q, C-18). *Anal.* Calcd for $C_{30}H_{42}O_8 \cdot H_2O$: C, 65.67; H, 8.08. Found: C, 65.66; H, 7.87.

Reaction of 9 with $Fe(acac)_3$–H_2O_2 in CH_3CN——A solution of **9** (200 mg) and $Fe(acac)_3/H_2O_2$ was treated in the same manner as described for the preparation of **6**. The residue was separated by chromatography on silica gel with MeOH–$CHCl_3$ (1 : 20) as a solvent to afford **11** (102 mg, 48%) and **12** (52 mg, 26%).

***trans*-Methyl-3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-20ξ-formyl-14β,20ξ-dihydroxychola-4,22-dienoate (11)**——Colorless needles from Me_2CO–Et_2O (1 : 1), mp 147—150 °C. IR ν_{max}^{KBr} cm^{-1}: 1705, 1720 (C=O). EI-MS *m/z*: 414 ($M^+ - C_6H_{12}O_5$). ^{1}H-NMR ($CDCl_3$) δ: 0.95 (3H, s, 19-H), 1.03 (3H, s, 18-H), 1.30 (3H, d, J=6 Hz, 6′-H), 3.72 (3H, s, $COOCH_3$), 4.92 (1H, s, 1′-H), 5.29 (1H, s, 4-H), 6.22 (1H, d, J=15 Hz, 23-H), 6.64 (1H, d, J=15 Hz, 22-H), 9.72 (1H, s, 21-H). ^{13}C-NMR ($CDCl_3$) δ: 204.3 (d, C-21), 166.5 (s, C-24), 147.1 (d, C-22), 146.6 (s, C-5), 122.6 (d, C-23), 121.0 (d, C-4), 99.4 (d, C-1′), 85.9 (s, C-20), 84.9 (s, C-14), 56.1 (q, $COOCH_3$), 18.8 (q, C-19), 18.1 (q, C-18), 17.5 (q, C-6′). *Anal.* Calcd for $C_{31}H_{46}O_{10} \cdot H_2O$: C, 62.39; H, 8.11. Found: C, 62.43; H, 7.85.

***trans*-Methyl-3β-[(6-deoxy-α-L-mannopyranosyl)oxy]-21-nor-20-oxo-14β-hydroxychola-4,22-dienoate (12)**——Crystalline powder from Me_2CO–Et_2O (1 : 2), mp 128—130 °C. IR ν_{max}^{KBr} cm^{-1}: 1710, 1720 (C=O). EI-MS *m/z*: 384 ($M^+ - C_6H_{12}O_5$). ^{1}H-NMR ($CDCl_3$) δ: 0.94 (3H, s, 19-H), 1.04 (3H, s, 18-H), 1.30 (3H, d, J=6 Hz, 6′-H), 3.76 (3H, s, $COOCH_3$), 4.92 (1H, s, 1′-H), 5.28 (1H, s, 4-H), 6.53 (1H, d, J=15 Hz, 23-H), 7.17 (1H, d, J=15 Hz, 22-H). ^{13}C-NMR ($CDCl_3$) δ: 211.2 (s, C-20), 165.7 (s, C-24), 147.2 (s, C-5), 142.8 (d, C-22), 121.3 (d, C-23), 120.6 (d, C-4), 99.3 (d, C-1′), 85.0 (s, C-14), 52.3 (q, $COOCH_3$), 18.9 (q, C-19), 17.6 (q, C-6′), 15.7 (q, C-18). *Anal.* Calcd for $C_{30}H_{44}O_9$: C, 63.81; H, 7.85. Found: C, 64.15; H, 8.04.

Preparation of 13 from 1——**1** (100 mg) was dissolved in pyridine (1 ml) and acetic anhydride (0.2 ml) and left at room temperature overnight. The reaction mixture was concentrated *in vacuo* and the residue was chromatographed on a silica gel column using $CHCl_3$ to give **13** as an amorphous powder, which was identical with an authentic sample in terms of IR and NMR spectra and TLC behavior.[10]

3β-[(2,3,4-Tri-*O*-acetyl-6-deoxy-α-L-mannopyranosyl)oxy]-14β-*O*-methoxymethylbufa-4,20,22-trienolide (14a)——A solution of CH_3OCH_2Cl (0.2 ml) in CH_2Cl_2 (5 ml) was added slowly to a stirred solution of **13** (100 mg) and $(iso\text{-}Pr)_2NEt$ (1 ml) in CH_2Cl_2 (10 ml) under cooling. The reaction mixture was stirred at room temperature for 10 h, then a saturated solution of citric acid was added and the mixture was extracted with $CHCl_3$. The extract was

concentrated *in vacuo* and chromatographed on a silica gel column to give **14a** (66 mg, 75%) and the starting material (18 mg). Crystalline powder from Et_2O–*n*-hexane (1 : 1), mp 103—108 °C. IR ν_{max}^{KBr} cm^{-1}: 1720 (C=O), 1740 ($COCH_3$). ^{1}H-NMR (C_5D_5N) δ: 0.80 (3H, s, 18-H), 1.00 (3H, s, 19-H), 1.40 (3H, d, J=6 Hz, 6′-H), 2.03, 2.07, 2.14 (3H×3, s, $COCH_3$), 3.40 (3H, s, $-OCH_2OCH_3$), 4.62, 4.96 (1H×2, d, J=6 Hz, $-OCH_2OCH_3$), 5.34 (1H, s, 4-H), 5.42 (1H, s, 1′-H), 6.45 (1H, d, J=10 Hz, 23-H), 7.43 (1H, d, J=3 Hz, 21-H), 7.74 (1H, dd, J=3, 10 Hz, 22-H). ^{13}C-NMR (C_5D_5N) δ: 170.3, 170.2, 170.2 (s, $COCH_3$), 162.0 (s, C-24), 147.3 (s, C-5), 146.7 (d, C-22), 120.7 (d, C-4), 115.5 (d, C-23), 97.3 (d, C-1′), 92.3 (s, C-14), 91.6 (t, $-OCH_2OCH_3$), 55.5 (q, $-OCH_2OCH_3$), 51.2 (d, C-17), 20.6 (s, $COCH_3$×3), 18.8 (q, C-19), 18.6 (q, C-18), 17.7 (q, C-6′). *Anal.* Calcd for $C_{38}H_{52}O_{12}$: C, 65.12; H, 7.48. Found: C, 65.34; H, 7.30.

3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β-*O*-methoxymethylbufa-4,20,22-trienolide (14b)——A 5% K_2CO_3 solution (1 ml) was added dropwise to a solution of **14a** (50 mg) in MeOH (2 ml) with stirring at room temperature. After being stirring for 0.5 h, the mixture was cooled, diluted with water and acidified with 5% HCl. The solution was extracted with $CHCl_3$. Removal of $CHCl_3$ gave a solid which was purified by chromatography on silica gel with MeOH–$CHCl_3$ (1 : 20) as a solvent to give **15** (40 mg, 98%). Crystalline powder from Me_2CO–Et_2O (1 : 1), mp 126—131 °C. IR ν_{max}^{KBr} cm^{-1}: 1720 (C=O). FAB-MS *m/z*: 575 (M^++1). ^{1}H-NMR (C_5D_5N) δ: 0.78 (3H, s, 18-H), 0.95 (3H, s, 19-H), 1.68 (3H, d, J=6 Hz, 6′-H), 3.38 (3H, s, $-OCH_2OCH_3$), 4.68, 4.99 (1H×2, d, J=6 Hz, $-OCH_2OCH_3$), 5.54 (1H×2, s, 4- and 1′-H), 6.46 (1H, d, J=10 Hz, 23-H), 7.46 (1H, d, J=3 Hz, 21-H), 7.78 (1H, dd, J=3, 10 Hz, 22-H). ^{13}C-NMR (C_5D_5N) δ: 162.0 (s, C-24), 149.6 (d, C-21), 146.8 (s, C-5), 146.5 (d, C-22), 122.7 (s, C-20), 121.6 (d, C-4), 115.4 (d, C-23), 100.9 (d, C-1′), 92.2 (s, C-14), 91.6 (t, $-OCH_2OCH_3$), 55.5 (q, $-OCH_2OCH_3$), 51.2 (d, C-17), 18.8 (q, C-19), 18.6 (q, C-6′ and -18). *Anal.* Calcd for $C_{32}H_{46}O_9 \cdot 1/2H_2O$: C, 65.85; H, 8.12. Found: C, 65.51; H, 7.86.

3β-[(2,3,4-Tri-*O*-acetyl-6-deoxy-α-L-mannopyranosyl)oxy]-14β-*O*-ethoxymethylbufa-4,20,22-trienolide (15a)——A mixture of **13** (100 mg) and $CH_3CH_2OCH_2Cl$ (0.2 ml) was treated in the same way as described for the preparation of **14a** to give **15a** (81 mg, 88%) and the starting material (15 mg). Crystalline powder from Et_2O–*n*-hexane (1 : 1), mp 105—111 °C. IR ν_{max}^{KBr} cm^{-1}: 1720 (C=O), 1740 ($COCH_3$). ^{1}H-NMR (C_5D_5N) δ: 0.81 (3H, s, 18-H), 0.99 (3H, s, 19-H), 1.22 (3H, t, J=7 Hz, $-OCH_2OCH_2CH_3$), 1.40 (3H, d, J=6 Hz, 6′-H), 2.04, 2.06, 2.12 (3H, s, $COCH_3$), 3.70 (2H, q, J=7 Hz, $-OCH_2OCH_2CH_3$), 4.73, 4.95 (1H×2, d, J=6 Hz, $-OCH_2OCH_2CH_3$), 5.54 (1H×2, 4- and 1′-H), 6.48 (1H, d, J=10 Hz, 23-H), 7.51 (1H, d, J=3 Hz, 21-H), 7.82 (1H, dd, J=3, 10 Hz, 22-H). ^{13}C-NMR (C_5D_5N) δ: 170.2 (s, $COCH_3$×3), 162.0 (s, C-24), 147.2 (s, C-5), 146.7 (d, C-22), 120.6 (d, C-4), 115.4 (d, C-23), 97.3 (d, C-1′), 92.2 (s, C-14), 90.3 (t, $-OCH_2OCH_2CH_3$), 63.8 (t, $-OCH_2OCH_2CH_3$), 51.2 (d, C-17), 20.6 (s, $COCH_3$×3), 18.8 (q, C-19), 18.6 (q, C-18), 17.6 (q, C-6′), 15.4 (q, $-OCH_2OCH_2CH_3$). *Anal.* Calcd for $C_{39}H_{54}O_{12}$: C, 65.53; H, 7.61. Found: C, 65.74; H, 7.85.

3β-[(6-Deoxy-α-L-mannopyranosyl)oxy]-14β-*O*-ethoxymethylbufa-4,20,22-trienolide (15b)——**15a** (50 mg) was hydrolyzed with 5% K_2CO_3 in MeOH in the same manner as described for **14b** to give **15b** (41 mg, 99.6%). Crystalline powder from Me_2CO–Et_2O (1 : 1), mp 115—118 °C. IR ν_{max}^{KBr} cm^{-1}: 1720 (C=O). FAB-MS *m/z*: 589 (M^++1). ^{1}H-NMR (C_5D_5N) δ: 0.80 (3H, s, 18-H), 0.96 (3H, s, 19-H), 1.20 (3H, t, J=7 Hz, $-OCH_2OCH_2CH_3$), 1.68 (3H, d, J=6 Hz, 6′-H), 3.69 (2H, q, J=7 Hz, $-OCH_2OCH_2CH_3$), 4.71, 4.96 (1H×2, d, J=6 Hz, $-OCH_2OCH_2CH_3$), 5.54 (1H×2, 4- and 1′-H), 6.46 (1H, d, J=10 Hz, 23-H), 7.50 (1H, d, J=3 Hz, 21-H), 7.82 (1H, dd, J=3, 10 Hz, 22-H). ^{13}C-NMR (C_5D_5N) δ: 162.0 (s, C-24), 146.9 (s, C-5), 146.9 (s, C-5), 146.5 (d, C-22), 121.6 (d, C-4), 115.4 (d, C-23), 100.9 (d, C-1′), 92.2 (s, C-14), 90.3 (t, $-OCH_2OCH_2CH_3$), 63.7 (t, $-OCH_2OCH_2CH_3$), 51.2 (d, C-17), 18.8 (q, C-19), 18.7 (q, C-6′ and -18), 15.5 (q, $-OCH_2OCH_2CH_3$). *Anal.* Calcd for $C_{33}H_{48}O_9 \cdot 1/2H_2O$: C, 66.31; H, 8.26. Found: C, 66.56; H, 8.32.

Biological Activity——PIE (pD_2 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 our previous paper.[12]

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References and Notes

1) H. G. Kurbjuweit, *Arzneim.-Forsch.*, **14**, 716 (1964).
2) A. Schwartz, G. E. Lindenmayer, and J. C. Allen, *Pharmacol. Rev.*, **27**, 3 (1975).
3) J. Mori, S. Nagai, J. Sakakibara, K. Takeya, and Y. Hotta, *Chem. Pharm. Bull.*, **35**, 1839 (1987).
4) J. Sakakibara, J. Mori, S. Nagai, Y. Hotta, and K. Takeya, *Folia Pharmacol. Japon*, **90**, 115 (1987).
5) R. Megges, H. Timm, H. J. Hans, E. Glusa, and K. Repke, Ger. (East) Offen 129795 (1976) [*Chem. Abstr.*, **89**, 147194*n* (1978)].
6) For example, marinobufagin, cinobufagin, resibufogenin, orbicuside, cotyledoside, *etc.*

7) T. Yamamoto and M. Kimura, *J. Chem. Soc., Chem. Commun.*, **1977**, 948.
8) W. Steidle and H. Kubinyi, Ger. Offen 2132490 (1971) [*Chem. Abstr.*, **78**, 97895*s* (1973)].
9) Y. Kamano and M. Komatsu, *Chem. Pharm. Bull.*, **17**, 1698 (1969).
10) H. Kubinyi, W. Steidle, and B. Goerlich, *Arch. Pharm.*, **304**, 380 (1971).
11) I. Moriguchi, Y. Kanada, and K. Komatsu, *Chem. Pharm. Bull.*, **24**, 1799 (1976).
12) J. Sakakibara, S. Nagai, J. Mori, K. Takeya, and Y. Hotta, *Shoyakugaku Zasshi*, **40**, 317 (1986).