

[Chem. Pharm. Bull.]
36(1) 48—59 (1988)

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

JUN MORI,^a SHIN-ICHI NAGAI,^a JINSAKU SAKAKIBARA,^{*,a}
KAZUMI TAKEYA,^b and YOSHIHIRO HOTTA^b

*Faculty of Pharmaceutical Sciences, Nagoya City University,^a Tanabe-dori,
Mizuho-ku, Nagoya 467, Japan and Department of Pharmacology,
Aichi Medical University,^b Nagakute,
Aichi 480-11, Japan*

(Received June 22, 1987)

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)-H₂O₂ have been undertaken. Further, alkoxyalkylation of the tertiary C_{14β}-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 pIC₅₀ 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 C₂₂-C₂₃ 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-H₂O₂) system, as well as alkoxyalkylation of the tertiary C_{14β}-hydroxy group located near the lactone ring. The cardiotonic activities (pIC₅₀ and pD₂ 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 $1650\text{--}1660\text{ cm}^{-1}$. 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).

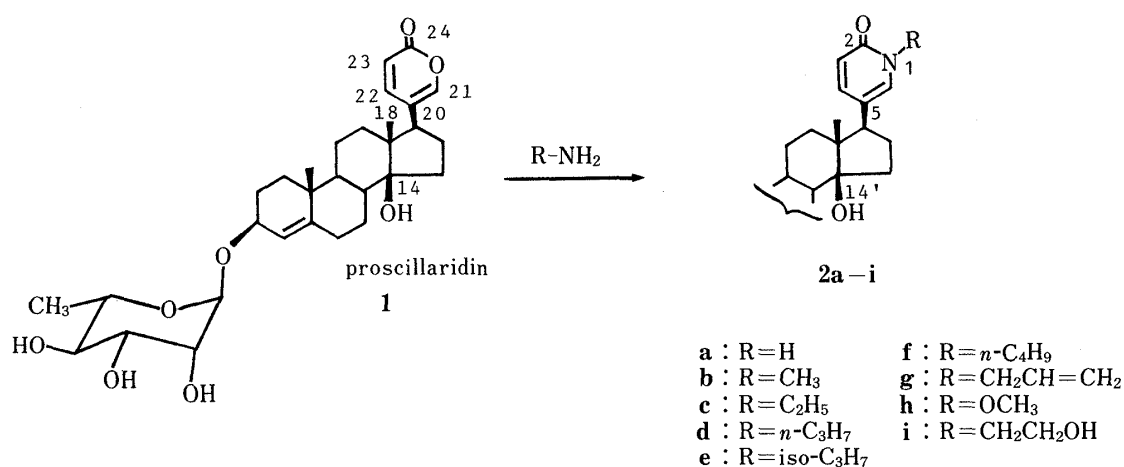


Chart 1

The IR spectra of **2d–i** exhibited absorptions at $1650\text{--}1660\text{ cm}^{-1}$ 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 (^1H -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 $138\text{--}140^\circ\text{C}$ in 98% yield. The IR spectrum showed an ester carbonyl absorption at 1720 cm^{-1} . The ^1H -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, $J=10\text{ Hz}$) and 7.61 (1H, d, $J=10\text{ Hz}$), and two methyl signals at δ 3.94 (3H $\times$ 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 ^1H -NMR spectrum of **4** showed methylene signals at δ 4.56 and 4.58 and no methyl ester signals. The IR spectrum showed disappearance 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-[3 β -[(6-Deoxy- α -L-mannopyranosyl)oxy]-14 β -hydroxyandrost-4-ene-17 β -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 · 1/2 H ₂ O	365	66.78	8.17	2.45
2b	CH ₃	41	5	300—303 ^{b)}	Colorless prisms (MeOH—Me ₂ CO)	C ₃₁ H ₄₅ NO ₇	379	(66.89)	8.23	(2.60)
2c	C ₂ H ₅	42	3	267—274 ^{c)}	Colorless prisms (MeOH—Me ₂ CO)	C ₃₂ H ₄₇ NO ₇	393	68.48	8.34	2.58
2d	<i>n</i> -C ₃ H ₇	20	3	285—287	Colorless prisms (MeOH—Me ₂ CO)	C ₃₃ H ₄₉ NO ₇ · H ₂ O	407	(68.16)	8.00	(2.51)
2e	iso-C ₃ H ₇	7	3	271—273	Crystalline powder (Me ₂ CO—Et ₂ O)	C ₃₃ H ₄₉ NO ₇ · 1/2 H ₂ O	407	68.91	8.49	2.51
2f	<i>n</i> -C ₄ H ₉	28	3	249—254	Crystalline powder (Me ₂ CO—Et ₂ O)	C ₃₄ H ₅₁ NO ₇ · 1/2 H ₂ O	421	(68.97)	8.96	(2.39)
2g	CH ₂ CH=CH ₂	22	3	260—272	Crystalline powder (Me ₂ CO—Et ₂ O)	C ₃₃ H ₄₉ NO ₇ · 1/2 H ₂ O	405	67.20	8.72	2.38
2h	OCH ₃	60	3	280—283	Colorless prisms (MeOH—Me ₂ CO)	C ₃₁ H ₄₅ NO ₈ · 1/2 H ₂ O	395	(67.24)	8.53	(2.49)
2i	CH ₂ CH ₂ OH	28	3	276—277	Crystalline powder (Me ₂ CO—Et ₂ O)	C ₃₂ H ₄₇ NO ₈ · 1/2 H ₂ O	409	68.25	8.68	2.41

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

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

Compd. No.	R	¹ H-NMR (δ)				R	¹³ C-NMR (δ)					IR (cm ⁻¹) (CON) (C=C)	
		1''-H (s)	3-H (d)	4-H (dd)	6-H (d)		C-2 (s)	C-3 (d)	C-4 (d)	C-5 (s)	C-6 (d)		
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) (CH ₃)	162.5	119.5	138.0	123.2	143.0	36.9 (q) (CH ₃)	1658 1575
2c	C ₂ H ₅	5.55	6.72 (J=10)	8.05 (J=3, 10)	7.44 (J=3)	1.27 (t, J=7) (CH ₂ CH ₃) 3.98 (q, J=7) (CH ₂ CH ₃)	161.9	119.7	136.8	123.3	142.9	14.9 (q) (CH ₂ CH ₃) 44.4 (t) (CH ₂ CH ₃)	1652 1570
2d	n-C ₃ H ₇	5.54	6.70 (J=10)	7.98 (J=3, 10)	7.45 (J=3)	0.82 (t, J=7) (CH ₂ CH ₂ CH ₃) 3.93 (t, J=7) (CH ₂ CH ₂ CH ₃)	162.1	119.7	137.5	123.4	143.0	11.2 (q) (CH ₂ CH ₂ CH ₃) 22.9 (t) (CH ₂ CH ₂ CH ₃) 51.0 (t) (CH ₂ CH ₂ CH ₃)	1660 1570
2e	iso-C ₃ H ₇	5.56	6.72 (J=10)	7.90 (J=3, 10)	7.63 (J=3)	1.27 (d, J=7) (CH(CH ₃) ₂)	161.7	119.4	132.9	—	142.3	21.7 (q) (CH(CH ₃) ₂) 46.2 (d) (CH(CH ₃) ₂)	1652 1568
2f	n-C ₄ H 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) ₂ CH ₃) 3.98 (m) (CH ₂ (CH ₂) ₂ CH ₃)	162.0	119.7	137.4	123.3	142.9	13.8 (q) (CH ₂ (CH ₂) ₂ CH ₃) 20.1 (t) (CH ₂) ₂ CH ₂ CH ₃) 31.8 (t) (CH ₂ CH ₂ CH ₂ CH ₃) 49.2 (t)	1660 1575
2g	CH ₂ CH=CH ₂	5.54	6.70 (J=10)	8.04 (J=3, 10)	7.41 (J=3)	4.68 (d, J=6) (CH ₂ CH=CH ₂) 5.14 (d, J=15) (CH ₂ CH=CH ₂) 5.8–6.3 (m) (CH ₂ CH=CH ₂) 4.04 (s) (OCH ₃)	161.8	119.7	134.0	—	143.2	50.8 (t) (CH ₂ CH=CH ₂) 117.4 (d) (CH ₂ CH=CH ₂) 134.0 (t) (CH ₂ CH=CH ₂) 64.3 (q) (OCH ₃)	1665 1585
2h	OCH ₃	5.52	6.72 (J=10)	7.92 (J=3, 10)	7.78 (J=3)	4.2–4.23 (m) (CH ₂ CH ₂ OH)	157.8	121.4	134.9	123.8	142.6	52.9 (t) (CH ₂ CH ₂ OH) 60.4 (t) (CH ₂ CH ₂ OH)	1655 1580
2i	CH ₂ CH ₂ OH	5.54	6.69 (J=10)	8.04 (J=3, 10)	7.64 (J=3)	—	162.4	119.5	138.9	—	143.1	—	1655 1570

^1H - and ^{13}C -NMR spectra were measured in $\text{C}_5\text{D}_5\text{N}$. IR spectra were measured by the KBr disc method. Coupling constants are given in $J = \text{Hz}$. Signals of C-5 of **2e**, **2g** and **2i** were overlapped with $\text{C}_5\text{D}_5\text{N}$ solvent signals.

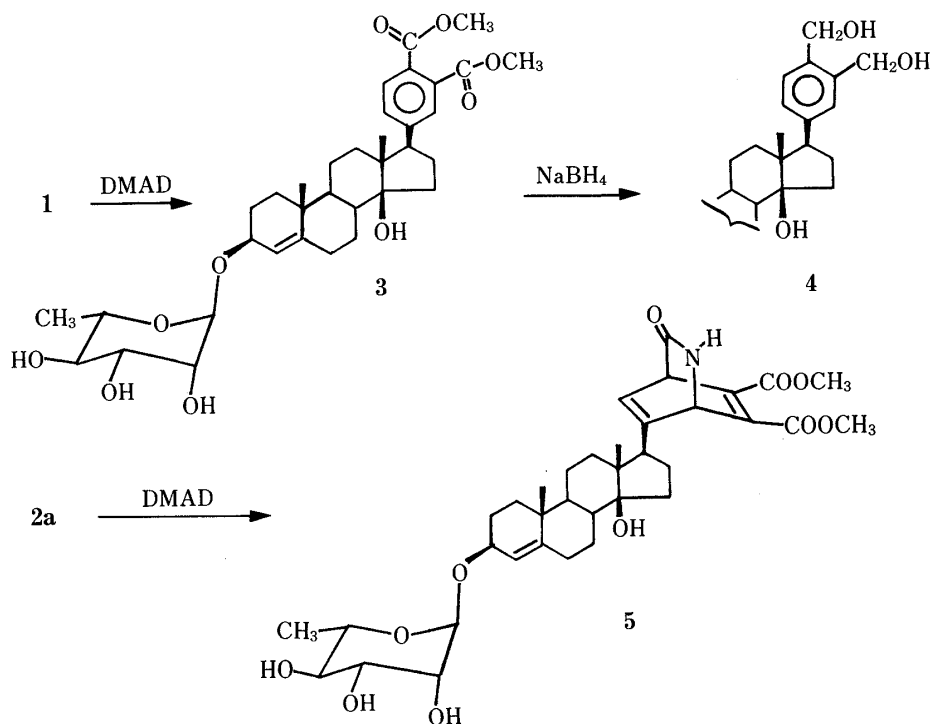


Chart 2

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-[3 β -[(6-deoxy- α -L-mannopyranosyl)oxy]-14 β -hydroxyandrost-4-en-17 β -yl]-3-oxo-2-aza-bicyclo[2.2.2]oct-5,7-diene-5,6-dicarboxylate (5).

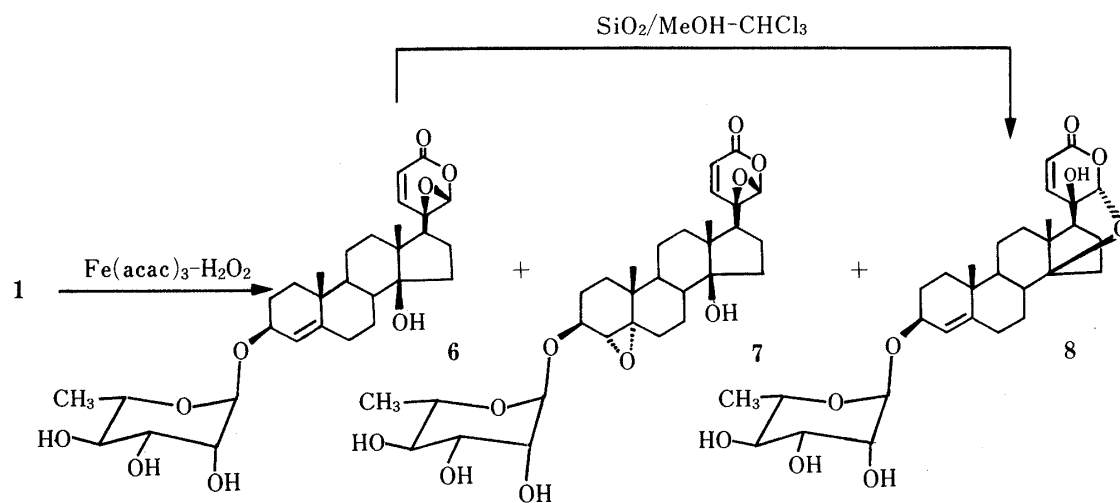
It is well known that steroidal epoxy groups contained in natural bufadienolides are related to the development of cardiotoxic activity.⁶⁾ Thus, we carried out the epoxidation with $\text{Fe}(\text{acac})_3\text{-H}_2\text{O}_2$, developed by Yamamoto and Kimura.⁷⁾

When 1 was treated with $\text{Fe}(\text{acac})_3\text{-H}_2\text{O}_2$ 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 3 β -[(6-deoxy- α -L-mannopyranosyl)oxy]-20 β ,21 β -epoxy-14 β -hydroxybufa-4,22-dienolide (6), 3 β -[(6-deoxy- α -L-mannopyranosyl)oxy]-4 α ,5 α ;20 β ,21 β -diepoxy-14 β -hydroxybuf-22-enolide (7) and 3 β -[(6-deoxy- α -L-mannopyranosyl)oxy]-14 β ,21 α -epoxy-20 β -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 $\text{C}_{20}\text{-C}_{21}$ 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 $\text{C}_4\text{-C}_5$ linkage since the 4-



H signal in the ^1H -NMR spectrum appeared at δ 2.90 as a singlet, which is in accord with that of authentic 3 β -[(6-deoxy- α -L-mannopyranosyl)oxy]-4 α ,5 α -epoxy-14 β -hydroxybufa-20,22-dienolide prepared by epoxidation with *m*-ClC₆H₄CO₃H in dioxane-CHCl₃.⁸⁾ The ^{13}C -NMR spectrum of **7** showed C₄ and C₅ 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 C₁₄, C₂₀ and C₂₁ 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 β} -hydroxy group nucleophilically attacked the C₂₁-carbon of **6** followed by ring-opening of the C₂₀-C₂₁ epoxide ring to yield **8**.

Kamano and Komatsu⁹⁾ 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 14 β -oxygen and C₂₁-carbon. According to the described method, compound **1** was readily converted into *trans*-methyl 3 β -[(6-deoxy- α -L-mannopyranosyl)oxy]-14 β ,21-epoxychole-4,20(21),22-trienoate (**9**) which, on treatment with sodium hydroxide in dioxane, yielded *trans*-3 β -[(6-deoxy- α -L-mannopyranosyl)oxy]-14 β ,21-epoxychole-4,20(21),22-trienoic acid (**10**). The ^1H -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-H₂O₂ system in CH₃CN unexpectedly resulted in the formation of *trans*-methyl 3 β -[(6-deoxy- α -L-mannopyranosyl)oxy]-20 ξ -formyl-14 β ,20 ξ -dihydroxychole-4,22-dienoate (**11**) and *trans*-methyl-3 β -[(6-deoxy- α -L-mannopyranosyl)oxy]-21-nor-20-oxo-14 β -hydroxychole-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 ^1H -NMR spectrum showed the appearance 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 C₁₄ 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 β} -C₂₁ 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

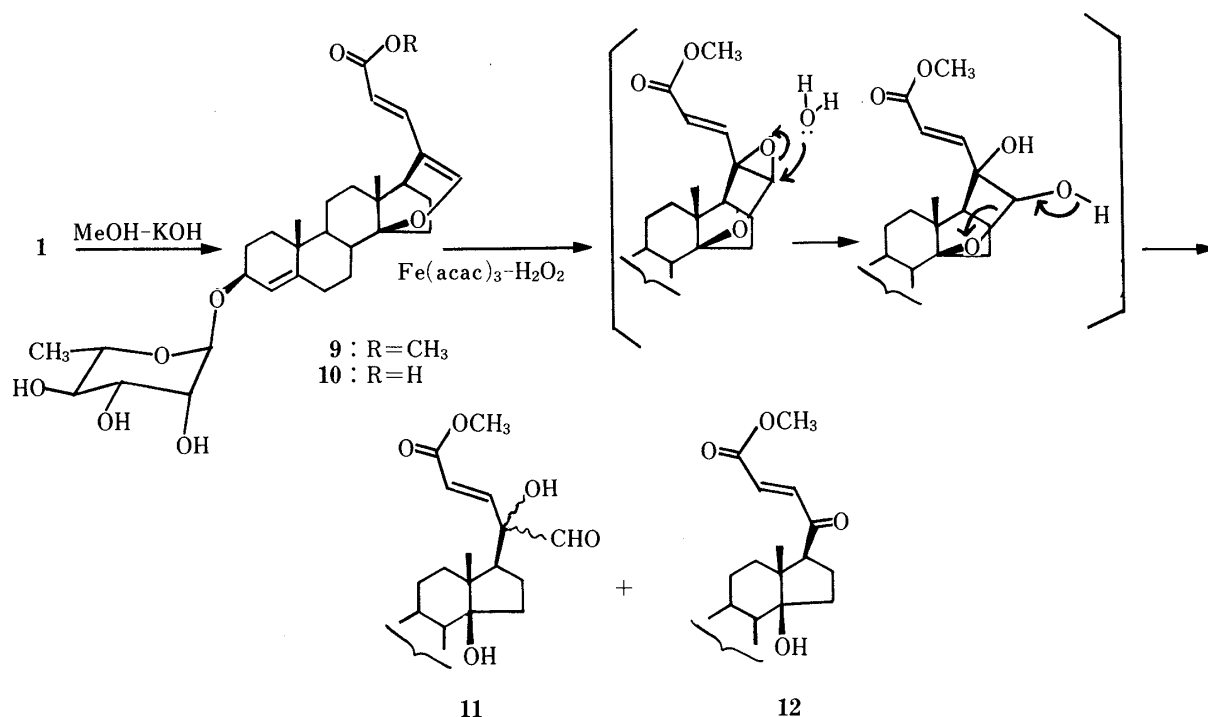


Chart 4

(s), and the C_{14 β} signal at δ 85.0 (s), while the carbon signal attributable to C₂₁ 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 β} -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)₂NEt) to yield 3 β -[(2,3,4-tri-*O*-acetyl-6-deoxy- α -L-mannopyranosyl)oxy]-14 β -*O*-methoxymethylbufa-4,20,22-trienolide (**14a**) and 3 β -[(2,3,4-tri-*O*-acetyl-6-deoxy- α -L-mannopyranosyl)oxy]-14 β -*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 ¹³C-NMR spectrum, the C_{14 β} signals of **14a** and **15a** were observed at δ 92.2 (s). Such a downfield shift is consistent with the displacement of C_{14 β} -OH seen in the ¹³C-NMR spectra of **8** and **9**.

Deacetylation of **14a** and **15a** with 5% K₂CO₃ in MeOH proceeded quantitatively to provide the corresponding 3 β -[(6-deoxy- α -L-mannopyranosyl)oxy]-14 β -*O*-methoxymethylbufa-4,20,22-trienolide (**14b**) and 3 β -[(6-deoxy- α -L-mannopyranosyl)oxy]-14 β -*O*-ethoxy-

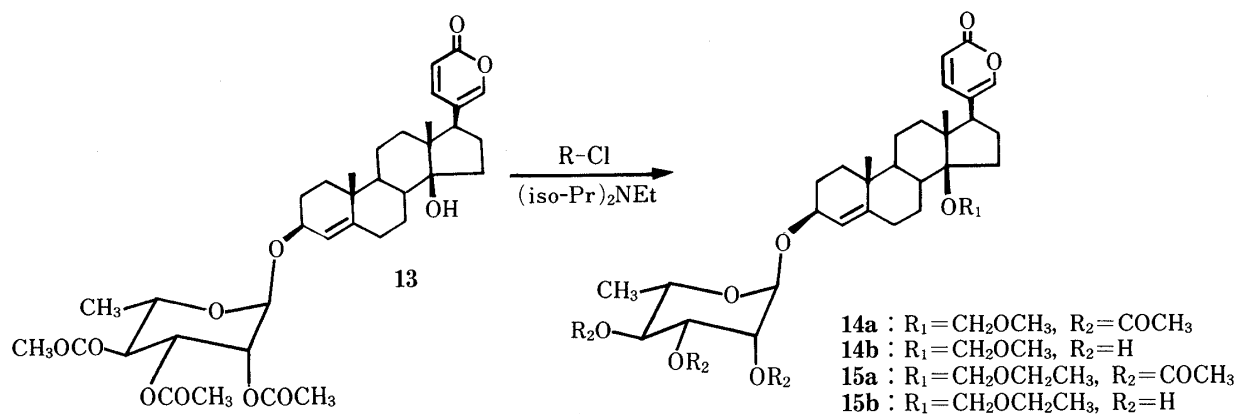


Chart 5

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 $\pm$ 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 $\pm$ S.E.). c) Compound **2i** was not tested.

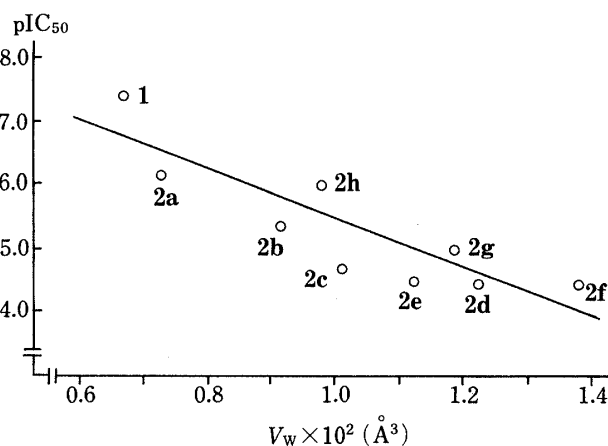


Fig. 1. Correlation between pIC_{50} Values and van der Waals Volumes (V_w) Values of **1** and the Lactams (**2a**—**h**)

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 ^1H -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 $\text{C}_5\text{D}_5\text{N}$ or CDCl_3 - $\text{DMSO}-d_6$ (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 60F₂₅₄ plates. Column chromatography was carried out on Silica gel BW-200 (Fuji Davison Chemicals, Ltd.).

General Procedure for Preparation of 1-Substituted 5-[3 β -[(6-Deoxy- α -L-mannopyranosyl)oxy]-14 β -hydroxyandrost-4-en-17 β -yl]-2(1H)-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 $\text{MeOH}-\text{CHCl}_3$ (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 $\text{MeOH}-\text{CHCl}_3$ (1:20) as the eluent to afford **2a-i**. The spectral data of **2a-i** are shown in Tables I and II.

Dimethyl 4-[3 β -[(6-Deoxy- α -L-mannopyranosyl)oxy]-14 β -hydroxyandrost-4-en-17 β -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 $\text{Me}_2\text{CO}-\text{Et}_2\text{O}$ (1:1) to give a crystalline powder of mp 138–140 °C. Yield 232 mg (98%). IR $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 1720 (C=O). ^1H -NMR (CDCl_3 - $\text{DMSO}-d_6$ (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 $\times$ 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 $\times$ 2, d, $J=10$ Hz, 5- and 6-H). ^{13}C -NMR (CDCl_3 - $\text{DMSO}-d_6$ (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 $\text{C}_{35}\text{H}_{48}\text{O}_{10}$: C, 66.86; H, 7.70. Found: C, 66.50; H, 7.58.

4-[3 β -[(6-Deoxy- α -L-mannopyranosyl)oxy]-14 β -hydroxyandrost-4-en-17 β -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 $\text{MeOH}-\text{Me}_2\text{CO}$ (1:1) to give colorless prisms of mp 271–272 °C. Yield 157 mg (86%). IR $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 3450 (OH). ^1H -NMR (CDCl_3 - $\text{DMSO}-d_6$ (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 $\times$ 2, s, $-\text{CH}_2\text{OH}$), 4.79 (1H, s, 1''-H), 4.9–5.1 (2H, m, $-\text{CH}_2\text{OH}$), 5.28 (1H, s, 4'-H), 7.22 (1H, s, 3-H), 7.27, 7.28 (1H $\times$ 2, d, $J=10$ Hz, 5- and 6-H). ^{13}C -NMR (CDCl_3 - $\text{DMSO}-d_6$ (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, $-\text{CH}_2\text{OH}$), 57.5 (d, C-17'), 18.8 (q, C-19'), 17.6 (q, C-6''), 17.3 (q, C-18'). *Anal.* Calcd for $\text{C}_{33}\text{H}_{48}\text{O}_8 \cdot \text{H}_2\text{O}$: C, 67.09; H, 8.53. Found: C, 66.78; H, 8.24.

Dimethyl 7-[3 β -[(6-Deoxy- α -L-mannopyranosyl)oxy]-14 β -hydroxyandrost-4-en-17 β -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 ($\text{Me}_2\text{CO}-\text{Et}_2\text{O}$ (1:1)). IR $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 1735 (COOCH_3), 1665 (CONH). FAB-MS m/z : 672 ($\text{M}^+ + 1$). ^1H -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 $\times$ 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 $\text{C}_{36}\text{H}_{49}\text{NO}_{11} \cdot \text{H}_2\text{O}$: C, 62.68; H, 7.45; N, 2.03. Found: C, 62.57; H, 7.15; N, 2.10.

Reaction of Proscillaridin (1) with $\text{Fe}(\text{acac})_3 \cdot \text{H}_2\text{O}_2$ in CH_3CN —A solution of **1** (300 mg) and $\text{Fe}(\text{acac})_3$ (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 $\text{MeOH}-\text{CHCl}_3$ (1:9) to give **6** (139 mg, 45%), **7** (41 mg, 13%) and **8** (80 mg, 26%).

3 β -[(6-Deoxy- α -L-mannopyranosyl)oxy]-20 β ,21 β -epoxy-14 β -hydroxybufa-4,22-dienolide (6)—Colorless needles from $\text{Me}_2\text{CO}-\text{Et}_2\text{O}$ (1:1), mp 182–184 °C. IR $\nu_{\text{max}}^{\text{KBr}} \text{cm}^{-1}$: 1736 (C=O). EI-MS m/z : 382 ($\text{M}^+ - \text{C}_6\text{H}_{12}\text{O}_5$). ^1H -NMR (CDCl_3 - $\text{DMSO}-d_6$ (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 - $\text{DMSO}-d_6$ (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.

3 β -[(6-Deoxy- α -L-mannopyranosyl)oxy]-4 α ,5 α ;20 β ,21 β -diepoxy-14 β -hydroxybuf-22-enolide (7)—Crystalline powder from Me_2CO-Et_2O (1:2), mp 179–183 °C. IR $\nu_{max}^{KBr} cm^{-1}$: 1735 (C=O). EI-MS m/z : 398 ($M^+ - C_6H_{12}O_5$). 1H -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.

3 β -[(6-Deoxy- α -L-mannopyranosyl)oxy]-14 β ,21 α -epoxy-20 β -hydroxybufa-4,22-dienolide (8)—Colorless needles from Me_2CO-Et_2O (1:1), mp 169–172 °C. IR $\nu_{max}^{KBr} cm^{-1}$: 1712 (C=O). EI-MS m/z : 382 ($M^+ - C_6H_{12}O_5$). 1H -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-3 β -[(6-deoxy- α -L-mannopyranosyl)-oxy]-14 β ,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 $\nu_{max}^{KBr} cm^{-1}$: 1710 (C=O). EI-MS m/z : 544 (M^+). 1H -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*-3 β -[(6-Deoxy- α -L-mannopyranosyl)oxy]-14 β ,21-epoxychola-4,20(21),22-trienolide (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 $\nu_{max}^{KBr} cm^{-1}$: 1670 (C=O). EI-MS m/z : 486 ($M^+ + 1 - CO_2H$). 1H -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-3 β -[(6-deoxy- α -L-mannopyranosyl)oxy]-20 ξ -formyl-14 β ,20 ξ -dihydroxychola-4,22-dienoate (11)**—Colorless needles from Me_2CO-Et_2O (1:1), mp 147–150 °C. IR $\nu_{max}^{KBr} cm^{-1}$: 1705, 1720 (C=O). EI-MS m/z : 414 ($M^+ - C_6H_{12}O_5$). 1H -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-3 β -[(6-deoxy- α -L-mannopyranosyl)oxy]-21-nor-20-oxo-14 β -hydroxychola-4,22-dienoate (12)**—Crystalline powder from Me_2CO-Et_2O (1:2), mp 128–130 °C. IR $\nu_{max}^{KBr} cm^{-1}$: 1710, 1720 (C=O). EI-MS m/z : 384 ($M^+ - C_6H_{12}O_5$). 1H -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⁾

3 β -[(2,3,4-Tri-*O*-acetyl-6-deoxy- α -L-mannopyranosyl)oxy]-14 β -*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-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 Et₂O-*n*-hexane (1:1), mp 103–108 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1720 (C=O), 1740 (COCH₃). ¹H-NMR (C₅D₅N) 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 $\times$ 3, s, COCH₃), 3.40 (3H, s, -OCH₂OCH₃), 4.62, 4.96 (1H $\times$ 2, d, *J*=6 Hz, -OCH₂OCH₃), 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). ¹³C-NMR (C₅D₅N) δ : 170.3, 170.2, 170.2 (s, COCH₃), 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, -OCH₂OCH₃), 55.5 (q, -OCH₂OCH₃), 51.2 (d, C-17), 20.6 (s, COCH₃ $\times$ 3), 18.8 (q, C-19), 18.6 (q, C-18), 17.7 (q, C-6'). *Anal.* Calcd for C₃₈H₅₂O₁₂: C, 65.12; H, 7.48. Found: C, 65.34; H, 7.30.

3 β -[(6-Deoxy- α -L-mannopyranosyl)oxy]-14 β -O-methoxymethylbufa-4,20,22-trienolide (14b)—A 5% K₂CO₃ 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 CHCl₃. Removal of CHCl₃ gave a solid which was purified by chromatography on silica gel with MeOH-CHCl₃ (1:20) as a solvent to give **15** (40 mg, 98%). Crystalline powder from Me₂CO-Et₂O (1:1), mp 126–131 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1720 (C=O). FAB-MS *m/z*: 575 (M⁺ + 1). ¹H-NMR (C₅D₅N) 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, -OCH₂OCH₃), 4.68, 4.99 (1H $\times$ 2, d, *J*=6 Hz, -OCH₂OCH₃), 5.54 (1H $\times$ 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). ¹³C-NMR (C₅D₅N) 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, -OCH₂OCH₃), 55.5 (q, -OCH₂OCH₃), 51.2 (d, C-17), 18.8 (q, C-19), 18.6 (q, C-6' and -18). *Anal.* Calcd for C₃₂H₄₆O₉ · 1/2H₂O: C, 65.85; H, 8.12. Found: C, 65.51; H, 7.86.

3 β -[(2,3,4-Tri-O-acetyl-6-deoxy- α -L-mannopyranosyl)oxy]-14 β -O-ethoxymethylbufa-4,20,22-trienolide (15a)—A mixture of **13** (100 mg) and CH₃CH₂OCH₂Cl (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 Et₂O-*n*-hexane (1:1), mp 105–111 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1720 (C=O), 1740 (COCH₃). ¹H-NMR (C₅D₅N) δ : 0.81 (3H, s, 18-H), 0.99 (3H, s, 19-H), 1.22 (3H, t, *J*=7 Hz, -OCH₂OCH₂CH₃), 1.40 (3H, d, *J*=6 Hz, 6'-H), 2.04, 2.06, 2.12 (3H, s, COCH₃), 3.70 (2H, q, *J*=7 Hz, -OCH₂OCH₂CH₃), 4.73, 4.95 (1H $\times$ 2, d, *J*=6 Hz, -OCH₂OCH₂CH₃), 5.54 (1H $\times$ 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). ¹³C-NMR (C₅D₅N) δ : 170.2 (s, COCH₃ $\times$ 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, -OCH₂OCH₂CH₃), 63.8 (t, -OCH₂OCH₂CH₃), 51.2 (d, C-17), 20.6 (s, COCH₃ $\times$ 3), 18.8 (q, C-19), 18.6 (q, C-18), 17.6 (q, C-6'), 15.4 (q, -OCH₂OCH₂CH₃). *Anal.* Calcd for C₃₉H₅₄O₁₂: C, 65.53; H, 7.61. Found: C, 65.74; H, 7.85.

3 β -[(6-Deoxy- α -L-mannopyranosyl)oxy]-14 β -O-ethoxymethylbufa-4,20,22-trienolide (15b)—**15a** (50 mg) was hydrolyzed with 5% K₂CO₃ in MeOH in the same manner as described for **14b** to give **15b** (41 mg, 99.6%). Crystalline powder from Me₂CO-Et₂O (1:1), mp 115–118 °C. IR $\nu_{\text{max}}^{\text{KBr}}$ cm⁻¹: 1720 (C=O). FAB-MS *m/z*: 589 (M⁺ + 1). ¹H-NMR (C₅D₅N) δ : 0.80 (3H, s, 18-H), 0.96 (3H, s, 19-H), 1.20 (3H, t, *J*=7 Hz, -OCH₂OCH₂CH₃), 1.68 (3H, d, *J*=6 Hz, 6'-H), 3.69 (2H, q, *J*=7 Hz, -OCH₂OCH₂CH₃), 4.71, 4.96 (1H $\times$ 2, d, *J*=6 Hz, -OCH₂OCH₂CH₃), 5.54 (1H $\times$ 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). ¹³C-NMR (C₅D₅N) 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, -OCH₂OCH₂CH₃), 63.7 (t, -OCH₂OCH₂CH₃), 51.2 (d, C-17), 18.8 (q, C-19), 18.7 (q, C-6' and -18), 15.5 (q, -OCH₂OCH₂CH₃). *Anal.* Calcd for C₃₃H₄₈O₉ · 1/2H₂O: C, 66.31; H, 8.26. Found: C, 66.56; H, 8.32.

Biological Activity—PIE (pD₂ and pIC₅₀ 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⁾

Acknowledgement We are grateful to Mr. H. Ando and Mrs. E. Kuroiwa of this division for their technical assistance in the biological experiments at Aichi Medical University. We thank Mr. Y. Sato of this faculty for the syntheses of some related compounds. We are also indebted to Miss S. Kato and Miss T. Naito of the Laboratory of Instrumental Analysis of our university for NMR spectral measurements and elemental analyses. This work was supported in part by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science and Culture of Japan.

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**, 147194n (1978)].
- 6) For example, marinobufagin, cinobufagin, resibufogenin, orbicusine, 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**, 97895s (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).