Bufadienolides and a New Lignan from the Bulbs of *Urginea maritima*

Masaru IIZUKA, Tsutomu WARASHINA,* and Tadataka NORO

Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52–1 Yada, Shizuoka 422–8526, Japan. Received August 28, 2000; accepted November 20, 2000

Thirty three compounds were obtained from the bulbs of *Urginea maritima* (Liliaceae). The compounds were identified by means of fast atom bombardment mass spectrometry (FAB-MS), nuclear magnetic resonance (^{1}H-NMR), (^{13}C-NMR), nuclear overhauser effects (NOE) and two dimensional (2D) NMR. Ten of them were new natural compounds. Nine were bufadienolides and only one was lignan in these compounds.

Key words *Urginea maritima*; Liliaceae; bufadienolide; lignan

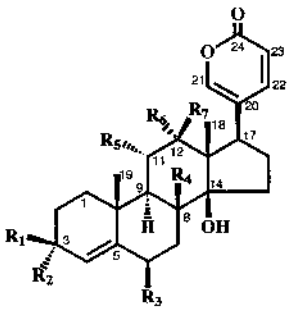
Urginea maritima (Liliaceae) is cultivated in the Mediterranean area.[1] It is used as a cardiotonic diuretic in Europe for the treatment of cardiac marasmus and edema.[2] From these facts, it was expected that this plant inhibited Na^+, K^+-adenosine triphosphatase (Na^+, K^+-ATPase: EC 3.6.1.3).[3] On the basis of this expectation, we started this study. In this paper, we report on the structure of 10 new compounds, and in the following paper, we will report on the inhibitory effect on enzymic activity. We have found 23 known compounds and 10 new compounds through extraction and isolation using the bulbs of *Urginea maritima*. Scillaren A (**1**),[4] scillirubroside (**15**),[5] scilliroside (**16**),[5] scillarenin 3-*O*-β-D-glucopyranoside (**18**),[6] 6-desacetyl-scilliroside (**20**),[1] scillarenin bis-rhamnopyranoside (**21**),[7] 5-α-4,5-dihydro-scillaren A (**27**),[4] proscillaridin A (**28**),[8] 5-α-4,5-dihydro-scillirosidin 3-*O*-β-D-glucopyranoside (**29**),[1] scillirosidin 3-*O*-α-L-rhamnopyranoside (**30**),[6] and 5-α-4,5-dihydro-scillirosidin 3-*O*-α-L-quinovopyranoside (**33**)[1] were known as bufadienolide glycosides; especially **1**, **16** and **28** were major components. Scillarenin (**2**),[9] scillirosidin (**3**),[10] scilliglaucosidin (**4**),[11] 3-methoxy-scilliphaeosidin (**5**), 5-β-14-dihydroxy-bufa-3,20,22-trienolide (**7**), scilliglaucogenin (**8**),[6] scilliglaucosidone (**9**),[11] 3-oxo-scilliphaeosidin (**10**),[12] 3-*epi*-scilliglaucosidin (**11**),[13] 3-*epi*-scillarenin (**12**),[14] 12-*epi*-scilliphaeosidin (**13**),[6] and 14,19-dihydroxy-3-oxo-bufa-4,20,22-trienolide (**31**)[15] were known bufadienolides which had the absence of sugars. On the other hand, compounds **6**, **14**, **17**, **19**, **22**—**26** and **32** were new compounds. Compounds **6**, **14** and **17** were bufadienolides which had the absence of sugars and **32** was lignan glycoside, which is rare in *Urginea maritima* (L). Compounds **19** and **22**—**26** were unknown bufadienolide glycosides. The structures of these new compounds were determined by means of FAB-MS, high resolution (HR)-FAB-MS, $[\alpha]_D$, circular dichroism (CD), ultraviolet (UV), infrared (IR), ^{1}H-NMR, ^{13}C-NMR, nuclear Overhauser effects (NOE) and two dimensional (2D) NMR spectra.

For the determination of the component sugars, a mixture of bufadienolide glycosides was subjected to acid hydrolysis. The sugars to be obtained by the reaction were identified as glucose and/or rhamnose for comparison with authentic samples by analysis of gas chromatography(GC).

All these compounds, except for compound **32**, were suggested to have unsaturated lactone rings by indicating good agreement with the data of scillaren A(**1**), which had signals defined at δ 123.3 (C-20), δ 149.5 (C-21), δ 147.5 (C-22), δ 115.3 (C-23), δ 162.1 (C-24), δ 7.46 (1H, d, J=2.5 Hz, H-21), δ 8.20 (1H, dd, J=10, 2.5 Hz, H-22) and δ 6.35 (1H, d, J=10 Hz, H-23).[4] And this lactone ring has a characteristic maximum absorption around 298 nm by UV spectrum.[2]

Compound **6** was suggested to have the molecular formula $C_{25}H_{34}O_5$, based on FAB-MS [positive FAB-MS ion at m/z 415 $[M+H]^+$]. In the ^{13}C-NMR spectrum of **6**, the signals due to the aglycone moiety were very similar to that of the known compound, scilliphaeosidin 3-*O*-β-D-glucoside.[6] The methoxy group was deduced from ^{1}H- and ^{13}C-NMR spectra data observed at δ 3.34 (3H, s) and δ 55.8. The linkage position at C-3 was confirmed by 2D-NMR, the heteronuclear multiple quantum coherence (HMQC) spectrum between δ 73.2 (C-3)/δ 3.60 (H-3) and the heteronuclear multiple bond connectivity (HMBC) spectrum between δ 73.2 (C-3)/δ 3.34 (methoxy proton); δ 55.8 (methoxy carbon)/δ 3.60 (H-3). The configuration of the methoxy group was determined to be α based on proton signals correlation between δ 3.60 (1H, br s, H-3) and δ 5.65 (1H, d, J=4.5 Hz, H-4).[14,16] Thus, the structure of **6** was determined to be 3-*epi*-*O*-methyl-scilliphaeosidin.

Compound **14** would be expected to have the molecular



	R_1 (β)	R_2 (α)	R_3	R_4	R_5	R_6	R_7
1	O-α-L-Rha$^{4\to1}$$\beta$-D-Glc	H	H	H	H	H	H
2	OH	H	H	H	H	H	H
6	H	OCH_3	H	H	H	H	OH
14	=O		H	H	OH	H	H
17	H	OH	H	H	H	OH	H
19	O-β-D-Glc	H	OAc	H	H	H	H
22	O-α-L-Rha$^{4\to1}$$\beta$-D-Glc	H	OAc	H	H	H	H
23	O-α-L-Rha$^{4\to1}$$\alpha$-L-Rha	H	H	OH	H	H	H
24	O-α-L-Rha$^{4\to1}$$\beta$-D-Glc	H	OAc	OH	H	H	H
25	O-3'-O-Ac-α-L-Rha$^{4\to1}$$\beta$-D-Glc	H	H	H	H	H	H
26	O-2'-O-Ac-α-L-Rha$^{4\to1}$$\beta$-D-Glc	H	H	H	H	H	H

Chart 1

* To whom correspondence should be addressed. e-mail: warashin@sea.u-shizuoka-ken.ac.jp

formula $C_{24}H_{30}O_5$, based on FAB-MS. As compared to the C-3 hydroxyl compound, scillarenin(**2**),[9] the ^{1}H- and ^{13}C-NMR signals of H-3 (δ 4.53) and C-3 (δ 67.4) were not observed in **14**, although the signal (δ 199.0) derived from ketone was observed. On the basis of these facts, it was assumed that C-3 was ketone which was also confirmed by the 2D-NMR, HMQC and HMBC spectra between δ 199.0 (C-3)/δ 3.27, 2.24 (H-1); δ 199.0 (C-3)/δ 2.62, 2.54 (H-2). Since both δ 1.63 (1H, t, J=11.5 Hz) and δ 4.16 (1H, td, J=11.5, 4.0 Hz) were observed by ^{1}H-NMR, and δ 68.2 was observed by ^{13}C-NMR, these were assigned as H-9, H-11 and C-11, respectively. So, it would seem to have the hydroxyl group at C-11. These facts were confirmed by the HMQC and HMBC spectra between δ 68.2 (C-11)/δ 1.63 (H-9); δ 68.2 (C-11)/δ 2.01, 1.84 (H-12). With respect to H-11, β-configuration was determined by strong NOE between H-11 and H-18, and H-19. As a result, these facts proved to be α-configuration with the hydroxyl group at C-11. Thus, the structure of **14** was elucidated.

The molecular formula of compound **17** was $C_{24}H_{32}O_5$, based on FAB-MS. Compared to 12-*epi*-scilliphaeosidin 3-*O*-β-D-glucoside,[6] the carbon signals of **17** were similar, with a range of glycosylation shifts at C-2 (+1.3 ppm), C-3 (−11.9 ppm), and C-4 (+1.9 ppm).[6] With respect to the configurations of the two hydroxyl groups at C-3 and C-12, α-configuration was determined at C-3 by the proton signals correlation between δ 4.38 (1H, br s, H-3) and δ 5.79 (1H, d, J=5.0 Hz, H-4),[16] and also at C-12 by NOE betweenδ 3.92 (H-12) and δ 1.00 (H-18). Hence, these facts suggested that the structure of **17** is epimer of scilliphaeosidin.[8] Finally, we determined the present compound to be 3-α, 12-α-scilliphaeosidin.

Compounds **19** and **22**—**24** had the molecular formulae $C_{32}H_{44}O_{11}$, $C_{38}H_{54}O_{15}$, $C_{36}H_{52}O_{13}$ and $C_{38}H_{54}O_{16}$, respectively, based on FAB-MS. In **19** and **22** the acetyl group was explained by ^{1}H-NMR, δ 1.76 (s); δ 1.75 (s) and ^{13}C-NMR, δ 170.0, 21.3; δ 170.0, 21.2, respectively. Also, linkage position and β-configuration of the acetyl group were established by HMQC, HMBC between acetyl carbon and H-6, and NOE between the 6-*O*-acetyl proton and H-19/H-8. In the ^{1}H-NMR and ^{13}C-NMR spectra of **19** and **22**, one anomeric proton and carbon signals and two anomeric protons and carbon signals, respectively, were observed, so these compounds were thought to be monoglycoside and diglycosides. The sugar moiety of each compound was determined by acid hydrolysis as described in the experimental section. The acid hydrolysis of **19** afforded glucose as the component sugar, so the sugar moiety of **19** was composed of glucose alone, and the coupling constant of anomeric proton δ 5.04 (1H, d, J=8.0 Hz) and the chemical shift of anomeric carbon δ 104.0 were indicative of one glucose. The sugar linkage was determined by the consequence of the HMQC and HMBC spectra between δ 5.04 (anomeric proton of β-D-glucopyranose)/δ 75.6 (C-3 of the aglycone); δ 104.0 (anomeric carbon of β-D-glucopyranose)/δ 4.57 (H-3 of the aglycone). In conclusion, we found that the structure of **19** was 6-β-acetoxy scillarenin 3-*O*-β-D-glucopyranoside. The component sugars of **22** were one rhamnose and glucose which were composed of one α-L-rhamnopyranose and β-D-glucopyranose from the results of acid hydrolysis and the ^{1}H- and ^{13}C-NMR spectra. The sugar sequence was determined by the NOE difference spectrum.

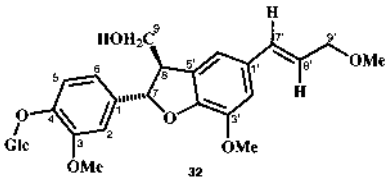


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Chart 2

The NOEs were observed between δ 5.48 (H-1′ of α-L-rhamnopyranose)/4.38 (H-3 of the aglycone); δ 5.22 (H-1″ of β-D-glucopyranose)/4.39 (H-4′ of α-L-rhamnopyranose). Based on the above evidence, the structure of **22** was elucidated to be 6-β-acetoxy scillarenin 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside. In compounds **23** and **24**, the aglycones showed good agreement with the ^{13}C-NMR spectra data of scillirubrosidin[17] and scillirosidin(**3**),[10] respectively, as compared to the published report.[1] On acid hydrolysis of **23**, rhamnose was obtained as the component sugar. The sugar was identified as two α-L-rhamnose based on the ^{13}C-NMR spectral data and the chemical shift, and the coupling constant of each anomeric proton signal H-1′ δ 5.50 (1H, d, J=1.5 Hz) and H-1″ δ 6.27 (1H, d, J=1.5 Hz).[6] This sugar linkage was confirmed based on the NOEs between δ 5.50 (H-1′ of α-L-rhamnopyranose)/4.45 (H-3 of the aglycone); δ 6.27 (H-1″ of α-L-rhamnopyranose)/4.40 (H-4′ of α-L-rhamnopyranose). From these results, the structure of **23** proved to be scillirubrosidin 3-*O*-α-L-rhamnopyranosyl-(1→4)-α-L-rhamnopyranoside. Rhamnose and glucose were obtained by acid hydrolysis of **24**. It was expected that the sequence of the sugar moiety was the same as that of compound **22** because the ^{1}H- and ^{13}C-NMR spectra data were very similar to those of **22**. Experimental evidence for the above expectation was obtained by examining the NOE of **24**. Thus, the structure of **24** was determined to be scillirosidin 3-*O*-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside.

The molecular formulae of compounds **25** and **26** were suggested to be $C_{38}H_{54}O_{14}$ by the observation of $[M+Na]^+$ ion peaks in FAB-MS. From the ^{1}H- and ^{13}C-NMR spectral data, the aglycones of **25** and **26** were determined to be scillarenin(**2**),[9] which was the same as that of scillaren A(**1**).[4] Compound **25**, as well as **26**, had one acetyl rhamnose and one glucose with respect to the component sugars from the results of the ^{1}H-, ^{13}C-NMR, ^{1}H–^{1}H correlation spectroscopy (^{1}H–^{1}H COSY), HMQC, HMBC spectra and acid hydrolysis. The acetylated position of **25** was determined by the H-3′ proton coupling constant δ 5.88 (1H, dd, J=9.5, 3.5 Hz), the range of acylation shift at C-2′ (−1.7 ppm), C-3′ (+3.1 ppm) and C-4′ (−6.7 ppm) when compared to compound **22** and the HMBC, HMQC and ^{1}H–^{1}H COSY spectra; while that of **26** was determined by the H-2′ proton coupling constant δ 5.71 (1H, br d, J=2.5 Hz), the range of acylation shift at C-2′ (+1.7 ppm), C-3′ (−2.3 ppm) and C-1′ (−3.5 ppm) when compared to compound **22**, HMBC and ^{1}H–^{1}H COSY. The sugar sequence of **25** and **26** were established on the basis of the NOE between δ 5.47 (H-1′ of α-L-rhamnopyranose)/4.36 (H-3 of the aglycone), δ 5.16 (H-1″ of β-D-glucopyranose)/4.62 (H-4′ of α-L-rhamnopyranose), δ 5.38 (H-1′ of α-L-rhamnopyranose)/4.35 (H-3 of the aglycone), δ 5.34 (H-1″ of β-D-glucopyranose)/4.27 (H-4′ of α-L-rhamnopyranose), respectively. Thus, the structures of **25** and **26** were

Table 1. ^{1}H-NMR Spectral Data

	6[a]	**14**[a]	**17**[a]	**19**[a]	**22**[a]
Aglycone moiety					
H- 3	3.60 (br s)	—	4.38 (br s)	4.57 (m)	4.38 (m)
4	5.65 (d, 4.5)	5.90 (s)	5.79 (d, 5.0)	6.17 (br s)	5.97 (br s)
6	2.14[c]	2.62[c]	2.23 (m)	5.54 (t, 3.0)	5.57 (br t, 3.0)
	1.76[c]	2.22[c]	2.15 (m)		
8	1.91 (td, 12.0, 3.0)	1.97 (td, 11.5, 3.0)	1.95[c]	2.23 (td, 12.0, 2.5)	2.24 (td, 12.0, 2.5)
9	1.28 (td, 12.0, 3.0)	1.63 (t, 11.5)	1.97[c]	1.12 (td, 12.0, 2.5)	1.14 (td, 12.0, 2.5)
11	1.98[c]	4.16 (td, 11.5, 4.0)	1.93[c]	1.45[c]	1.46[c]
	1.68[c]		1.72[c]	1.35[c]	1.37[c]
12	3.63 (dd, 12.0, 4.0)	2.01 (dd, 11.5, 4.0)	3.92 (br s)	1.49[c]	1.52[c]
		1.84[c]		1.33[c]	1.38[c]
17	3.45 (dd, 10.0, 7.0)	2.61 (dd, 10.5, 6.0)	3.60 (dd, 9.5, 6.0)	2.50 (dd, 9.5, 6.0)	2.51 (dd, 10.0, 6.5)
18	1.16 (s)	1.04 (s)	1.00 (s)	0.94 (s)	0.95 (s)
19	0.94 (s)	1.34 (s)	1.01 (s)	1.18 (s)	1.25 (s)
21	7.59 (d, 2.5)	7.44 (d, 2.5)	7.44 (d, 2.5)	7.47 (d, 2.5)	7.47 (d, 2.5)
22	8.21 (dd, 10.0, 2.5)	8.15 (dd, 10.0, 2.5)	8.31 (dd, 10.0, 2.5)	8.20 (dd, 10.0, 2.5)	8.20 (dd, 10.0, 2.5)
23	6.35 (d, 10.0)	6.33 (d, 10.0)	6.37 (d, 10.0)	6.35 (d, 10.0)	6.35 (d, 10.0)
Ac	—	—	—	1.76 (s)	1.75 (s)
OMe	3.34 (s)	—	—	—	—
Sugar moiety					
				Glc	Rha
H-1′	—	—	—	5.04 (d, 8.0)	5.48 (br s)
2′	—	—	—	4.02[c]	4.49[c]
3′	—	—	—	4.28[c]	4.54 (dd, 9.0, 2.5)
4′	—	—	—	4.26[c]	4.39 (t, 9.0)
5′	—	—	—	4.01[c]	4.24 (dq, 9.0, 6.0)
6′	—	—	—	4.58 (dd, 12.0, 2.5)	1.72 (d, 6.0)
				4.40 (dd, 12.0, 5.0)	
					Glc
H-1″	—	—	—	—	5.22 (d, 8.0)
2″	—	—	—	—	4.10 (t, 8.0)
3″	—	—	—	—	4.19 (t, 8.0)
4″	—	—	—	—	4.24[c]
5″	—	—	—	—	3.81 (m)
6″	—	—	—	—	4.46 (dd, 12.0, 2.5)
					4.36 (dd, 12.0, 5.0)

	23[a]	**24**[a]	**25**[a]	**26**[a]		**32**[b]
Aglycone moiety						
H- 3	4.45 (m)	4.37 (m)	4.36 (m)	4.35 (m)	H-2	7.03 (d, 2.0)
4	5.58 (br s)	6.01 (br s)	5.50 (s)	5.48 (s)	5	7.15 (d, 8.0)
6	2.84 (br t, 12.0)	5.62 (t, 3.0)	1.69[c]	1.69[c]	6	6.93 (dd, 8.0, 2.0)
	1.95[c]		1.26[c]	1.26[c]	7	5.58 (d, 7.0)
8	—	—	1.79[c]	1.79[c]	8	3.48 (m)
9	1.37[c]	1.32[c]	1.06 (td, 12.0, 3.5)	1.06 (td, 12.0, 3.5)	9	3.85[c]
12	1.54[c]	1.49[c]	1.45[c]	1.45[c]		3.78 (dd, 11.0, 7.0)
	1.39[c]	1.38[c]	1.28[c]	1.28[c]	2′	6.95 (s)
17	2.48 (dd, 10.0, 6.5)	2.47 (dd, 10.0, 6.0)	2.48 (dd, 10.0, 7.0)	2.47 (dd, 10.0, 6.5)	6′	6.97 (s)
18	1.11 (s)	1.09 (s)	0.93 (s)	0.93 (s)	7′	6.56 (d, 16.0)
19	1.42 (s)	1.55 (s)	0.96 (s)	0.96 (s)	8′	6.16 (dt, 16.0, 6.0)
21	7.43 (d, 2.5)	7.42 (d, 2.5)	7.46 (d, 2.5)	7.46 (d, 2.5)	9′	4.05 (dd, 6.0, 1.5)
22	8.09 (dd, 10.0, 2.5)	8.07 (dd, 10.0, 2.5)	8.20 (dd, 10.0, 2.5)	8.20 (dd, 10.0, 2.5)		4.05 (dd, 6.0, 1.5)
23	6.35 (d, 10.0)	6.34 (d, 10.0)	6.35 (d, 10.0)	6.35 (d, 10.0)	OMes	3.89 (s)
Ac	—	1.90 (s)	—	—		3.83 (s)
						3.36 (s)
Sugar moiety						
	Rha	Rha	3′-O-Ac-Rha	2′-O-Ac-Rha		Glc
H-1′	5.50 (d, 1.5)	5.48 (d, 1.5)	5.47 (d, 1.5)	5.38 (br s)	G-1	4.88 (d, 7.5)
2′	4.45[c]	4.49[c]	4.68 (br s)	5.71 (br d, 2.5)	2	3.48[c]
3′	4.55[c]	4.54 (dd, 9.0, 2.5)	5.88 (dd, 9.5, 3.5)	4.71 (dd, 9.0, 2.5)	3	3.47[c]
4′	4.40 (t, 9.5)	4.39 (t, 9.0)	4.62 (t, 9.5)	4.27 (t, 9.0)	4	3.39[c]
5′	4.28 (dq, 9.5, 6.0)	4.24 (dq, 9.0, 6.0)	4.27 (dq, 9.5, 6.0)	4.26 (dq, 9.0, 6.0)	5	3.38[c]
6′	1.64 (d, 6.0)	1.72 (d, 6.0)	1.72 (d, 6.0)	1.73 (d, 6.0)	6	3.87[c]
						3.68 (m)
Ac	—	—	2.17 (s)	2.09 (s)		—
	Rha	Glc	Glc	Glc		
H-1″	6.27 (d, 1.5)	5.23 (d, 8.0)	5.16 (d, 8.0)	5.34 (d, 8.0)		—
2″	4.86[c]	4.10 (t, 8.0)	3.97 (t, 8.0)	4.11 (t, 8.0)		—
3″	4.54[c]	4.19 (t, 8.0)	4.17 (t, 8.0)	4.18 (t, 8.0)		—
4″	4.30 (t, 9.5)	4.23[c]	4.24[c]	4.24[c]		—
5″	4.39 (dq, 9.5, 6.0)	3.81 (m)	3.83 (m)	3.88 (m)		—
6″	1.64 (d, 6.0)	4.45 (dd, 12.0, 2.5)	4.45 (dd, 12.0, 2.5)	4.51 (dd, 12.0, 2.5)		—
		4.36 (dd, 12.0, 5.0)	4.36 (dd, 12.0, 5.0)	4.37 (dd, 12.0, 5.0)		

Measured at 400 MHz, δ, ppm in *a*) pyridine-d_5. *b*) CD_3OD solution at 35 °C. *c*): Overlapping with other signals. Rha: α-L-rhamnopyranosyl. Glc: β-D-glucopyranosyl.

Table 2. ^{13}C-NMR Spectral Data (Measured at 100.40 MHz, δ, ppm 35 °C, in Pyridine-d_5 Except for **32** (CD_3OD) as Solvent)

	6	**14**	**17**	**19**	**22**	**23**	**24**	**25**	**26**		**32**
Aglycon moiety											
C- 1	32.6	38.5	32.4	37.3[a]	37.2[a]	37.1	37.9	33.0[a]	33.0[a]	C-1	138.1
2	24.4	34.8	29.2	27.7	27.4	27.4	27.0	27.6	27.5	2	111.4
3	73.2	199.0	64.1	75.6	73.7	74.0[b]	73.6	73.8	74.6	3	151.1
4	119.8	124.6	123.4	130.2	129.5	121.4	130.3	121.4	121.1	4	147.8
5	149.1	170.9	147.5	142.3	142.6	147.8	142.3	147.0	147.4	5	118.3
6	33.0	34.2	33.2	76.1	76.1	28.8	76.5[a]	35.8	35.7	6	119.5
7	29.3[a]	28.3	29.3	33.4	33.4	36.0	38.3	29.3[b]	29.3[b]	7	88.8
8	42.3	42.5	43.2	37.7[a]	37.6	76.3	76.5[a]	42.6	42.6	8	55.3
9	46.9[b]	54.9	43.3	50.2	50.2	51.9[a]	52.0	50.5	50.4	9	65.0
10	37.8	40.4	37.6	37.3[a]	37.3[a]	38.3	37.3	37.8	37.7	1′	132.4
11	31.4	68.2	30.8	21.5	21.5	18.7	18.7	21.7	21.6	2′	112.5
12	75.2	51.8	75.4	40.6	40.6	41.5	41.1	40.7	40.6	3′	145.6
13	55.3	49.4	52.6	48.9	48.9	49.4	49.5	48.8	48.8	4′	149.5
14	84.8	83.8	84.5	83.9	83.9	85.4	85.4	84.2	84.2	5′	130.1
15	33.3	33.2	34.8	32.8	32.8	34.7	34.6	32.8[a]	32.7[a]	6′	116.7
16	29.4[a]	29.5	31.6	29.3	29.3	29.8	29.6	29.4[b]	29.4[b]	7′	134.3
17	46.9[b]	51.3	45.7	51.5	51.5	52.0[a]	51.2	51.4	51.4	8′	124.4
18	10.9	18.2	18.7	17.3	17.3	19.5	19.5	17.1	17.1	9′	74.3
19	18.5	18.4	19.0	21.1[b]	21.1[b]	20.9	22.1	19.0	19.0		—
20	123.4	122.8	124.2	123.5	123.4	123.4	124.0	123.3	123.3		—
21	150.0	149.8	149.3	149.5	149.5	149.4	149.4	149.4	149.4		—
22	147.8	147.2	148.2	147.5	147.4	147.1	147.1	147.5	147.5		—
23	115.2	115.4	115.4	115.3	115.3	115.4	115.4	115.3	115.3		—
24	162.1	162.0	162.2	162.1	162.1	162.0	162.0	162.1	162.1		—
Ac	—	—	—	170.0	170.0	—	169.7	—	—		—
				21.3[b]	21.2[b]		21.4				
OMe	55.8	—	—	—	—	—	—	—	—	OMes	58.0
											56.9
											56.8
Sugar moiety											
				Glc	Rha	Rha	Rha	3′-*O*-Ac-Rha	2′-*O*-Ac-Rha		Glc
C-1′	—	—	—	104.0	100.9	100.6	100.8	100.4	97.4	G-1	102.9
2′	—	—	—	75.3	72.1	73.3[c]	72.1	70.4	73.8	2	74.9
3′	—	—	—	78.6[c]	72.5	73.4[c]	72.5	75.6	70.2	3	77.9
4′	—	—	—	71.9	85.1	80.3	85.0	78.4	84.8	4	71.4
5′	—	—	—	78.6[c]	68.4	68.2	68.4	68.5	68.2	5	78.2
6′	—	—	—	63.0	18.4	19.0	18.4	18.7	18.3	6	62.6
Ac	—	—	—	—	—	—	—	170.9	170.7		—
								21.5	21.0		
					Glc	Rha	Glc	Glc	Glc		
C-1″	—	—	—	—	106.7	103.3	106.7	105.5	106.5		—
2″	—	—	—	—	76.5	72.6	76.4	75.1	76.4		—
3″	—	—	—	—	78.6	72.9	78.6	78.6	78.5[c]		—
4″	—	—	—	—	71.7	74.1[b]	71.6	71.8	71.6		—
5″	—	—	—	—	78.5	70.4	78.5	78.0	78.5[c]		—
6″	—	—	—	—	62.8	18.5	62.8	63.0	62.8		—

a—c): Assignments may be interchangeable in each column. Rha: α-L-rhamnopyranosyl. Glc: β-D-glucopyranosyl.

determined to be scillarenin 3-*O*-β-D-glucopyranosyl-(1→4)-3′-*O*-acetyl-α-L-rhamnopyranoside and scillarenin 3-*O*-β-D-glucopyranosyl-(1→4)-2′-*O*-acetyl-α-L-rhamnopyranoside, respectively.

Compound **32** was found to have the formula $C_{27}H_{34}O_{11}$ based on the FAB-MS spectra and confirmed by its ^{1}H- and ^{13}C-NMR data. The ^{1}H-NMR spectrum showed the presence of five aromatic proton signals at δ 7.15 (1H, d, J=8.0 Hz, H-5), δ 7.03 (1H, d, J=2.0 Hz, H-2), δ 6.97 (1H, s, H-6′), δ 6.95 (1H, s, H-2′) and δ 6.93 (1H, dd, J=8.0, 2.0 Hz, H-6); the signals three methoxy groups at δ 3.89, 3.83 and 3.36 (each 3H, s, C-3′, C-3, C-9′-OMe, respectively); two trans olefinic proton signals at δ 6.56 (1H, d, J=16.0 Hz, H-7′) and δ 6.16 (1H, dt, J=16.0, 6.0 Hz, H-8′); two methine proton signals at δ 5.58 (1H, d, J=7.0 Hz, H-7) and δ 3.48 (1H, d, J=7.0 Hz, H-8) and an anomeric proton signal at δ 4.88 (1H, d, J=7.5Hz, H-1″). The sugar moiety was identified by acid hydrolysis to glucose. These results showed that compound **32** is a dihydro-benzofuran-type neolignan glucoside in combination with its ^{13}C-NMR spectrum data. The sugar linkage was confirmed by the NOE between δ 4.88 (H-1″ of β-D-glucopyranose)/7.15 (H-5 of aromatic proton). The HMQC and HMBC spectra provided strong support for the structure of **32**. The CD spectrum showed negative cotton effects ($[\theta]_{270}$ −8117 and $[\theta]_{284}$ −12175), so it was found that compound **32** had the 7*S*,8*R*-configuration.[18]

Experimental

Optical rotation was determined by measurement with a JASCO-DIP 1000 digital polarimeter. CD spectra were determined by a JASCO J-20A. FAB-MS and HR-FAB-MS spectra were taken on a JEOL JMS-SX 120 and

JEOL JMS-700 spectrometer in *m*-nitrobenzyl alcohol, respectively. IR spectra were recorded on a JASCO FT/IR-230 spectrometer. UV spectra were obtained by BECKMAN DU 640 spectrophotometer. ^{1}H- and ^{13}C-NMR were recorded on a JEOL JNM A-400 (400 and 100.40 MHz, respectively) spectrometer. Chemical shifts were given on the δ (ppm) scale with tetramethylsilane (TMS) as an internal standard. GC was run on a Hitachi G-3000 gas chromatograph, and HPLC was run on a JASCO 980 system instrument.

Plant Material *Urginea maritima* (L.) were collected from the plants garden, University of Shizuoka in Japan.

Extraction and Isolation The dried bulbs of *Urginea maritima* (2.5 kg) were extracted three times with MeOH under reflux. The methanol extract (315 g) was partitioned between diethyl ether and water. The water layer was passed through a Diaion HP-20 column and then after being passed MeOH, the MeOH elute was concentrated under reduced pressure. The residue (9.7 g) was chromatographed on a silica gel column with $CHCl_3$–MeOH (98 : 2→8 : 2) to give thirteen fractions.

Fraction 1 (193 mg) was subjected to HPLC [YMC ODS-AQ (20× 250 mm), CH_3CN–H_2O (25—75→40—60), MeOH–H_2O (40—60→67.5—32.5), 205 nm] to give **2** (11 mg), **3** (8 mg), **9** (7.5 mg) and **12** (36 mg). Fraction 2 (311 mg) to give **5** (6 mg), **6** (6.6 mg), **7** (17 mg) and **11** (29 mg). Fraction 3 (663 mg) to give **4** (20 mg) and **8** (41 mg). Fraction 4 (211 mg) to give **10** (24 mg), **13** (4 mg) and **14** (2.8 mg). Fraction 5 (130 mg) to give **17** (11 mg), **31** (3 mg) and **33** (3 mg). Fraction 8 (1.15 g) to give **28** (22 mg), **29** (9 mg), and **30** (5 mg). Fraction 9 (660 mg) to give **15** (13 mg), **16** (59 mg), **18** (66 mg), **19** (80 mg) and **32** (6.7 mg). Fraction 10 (167 mg) to give **20** (5 mg), **21** (7 mg), **25** (5.7 mg) and **26** (5 mg). Fraction 11 (817 mg) to give **1** (35 mg), **22** (6 mg), **23** (25 mg), **24** (9 mg) and **27** (5 mg).

Compound **6**: Amorphous powder. $[\alpha]_D^{28}$ +49.9° (*c*=0.66, MeOH). UV λ_{max}^{MeOH} nm (log ε): 299 (3.54), 216 (*sh*). IR (KBr) cm^{-1}: 1717. FAB-MS *m*/*z*: 415 $[M+H]^+$. HR-FAB-MS *m*/*z*: 415.2507 (Calcd for $C_{25}H_{35}O_5$: 415.2484). ^{1}H- and ^{13}C- NMR: Tables 1 and 2.

Compound **14**: Amorphous powder. $[\alpha]_D^{24}$ +32.0° (*c*=0.28, $CHCl_3$–MeOH 1 : 1). UV $\lambda_{max}^{CHCl3-MeOH\ 1:1}$ nm (log ε): 298 (3.43), 243 (3.90). IR (KBr) cm^{-1}: 1721,1655. FAB-MS *m*/*z*: 399 $[M+H]^+$. HR-FAB-MS *m*/*z*: 399.2197 (Calcd for $C_{24}H_{31}O_5$: 399.2171). ^{1}H-NMR (pyridine-d_5 at 35 °C): δ 2.24 (m, H-1), 2.54 (m, H-2), 2.62 (m, H-2), 3.27 (dt, H-1). Other data of ^{1}H- and ^{13}C-NMR are in Tables 1 and 2.

Compound **17**: Amorphous powder. $[\alpha]_D^{26}$ +110.3° (*c*=1.07, MeOH). UV λ_{max}^{MeOH} nm (log ε): 299 (3.66), 215 (*sh*). IR (KBr) cm^{-1}: 1715. FAB-MS *m*/*z*: 401 $[M+H]^+$. HR-FAB-MS *m*/*z*: 401.2354 (Calcd for $C_{24}H_{33}O_5$: 401.2328). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Compound **19**: Amorphous powder. $[\alpha]_D^{28}$ −52.4° (*c*=8.04, MeOH). UV λ_{max}^{MeOH} nm (log ε): 298 (3.60). IR (KBr) cm^{-1}: 1735, 1717. FAB-MS *m*/*z*: 605 $[M+H]^+$. HR-FAB-MS *m*/*z*: 605.2944 (Calcd for $C_{32}H_{45}O_{11}$: 605.2962). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Compound **22**: Amorphous powder. $[\alpha]_D^{26}$ −83.4° (*c*=0.6, MeOH). UV λ_{max}^{MeOH} nm (log ε): 298 (4.17), 204 (4.96). IR (KBr) cm^{-1}: 1735, 1718. FAB-MS *m*/*z*: 773 $[M+Na]^+$. HR-FAB-MS *m*/*z*: 773.3346 (Calcd for $C_{38}H_{54}O_{15}$+Na: 773.3360). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Compound **23**: Amorphous powder. $[\alpha]_D^{26}$ −94.5° (*c*=2.5, MeOH). UV λ_{max}^{MeOH} nm (log ε): 299 (3.67). IR (KBr) cm^{-1}: 1718. FAB-MS *m*/*z*: 715 $[M+Na]^+$. HR-FAB-MS *m*/*z*: 715.3334 (Calcd for $C_{36}H_{52}O_{13}$+Na: 715.3306). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Compound **24**: Amorphous powder. $[\alpha]_D^{26}$ −71.1° (*c*=1.0, MeOH). UV λ_{max}^{MeOH} nm (log ε): 298 (3.71). IR (KBr) cm^{-1}: 1735, 1718. FAB-MS *m*/*z*: 789 $[M+Na]^+$. HR-FAB-MS *m*/*z*: 789.3325 (Calcd for $C_{38}H_{54}O_{16}$+Na: 789.3310). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Compound **25**: Amorphous powder. $[\alpha]_D^{26}$ −60.6° (*c*=0.57, MeOH). UV λ_{max}^{MeOH} nm (log ε): 298 (3.71). IR (KBr) cm^{-1}: 1735, 1718. FAB-MS *m*/*z*: 757 $[M+Na]^+$. HR-FAB-MS *m*/*z*: 757.3414 (Calcd for $C_{38}H_{54}O_{14}$+Na: 757.3411). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Compound **26**: Amorphous powder. $[\alpha]_D^{26}$ −67.3° (*c*=0.50, MeOH). UV λ_{max}^{MeOH} nm (log ε): 298 (3.70). IR (KBr) cm^{-1}: 1735, 1718. FAB-MS *m*/*z*: 757 $[M+Na]^+$. HR-FAB-MS *m*/*z*: 757.3401 (Calcd for $C_{38}H_{54}O_{14}$+Na: 757.3411). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Compound **32**: Amorphous powder. $[\alpha]_D^{28}$ −54.8° (*c*=0.67, MeOH). UV λ_{max}^{MeOH} nm (log ε): 292 (*sh*), 278 (4.26), 219 (4.51), 204 (4.84). IR (KBr) cm^{-1}: 1655, 1600, 1510, 1458. CD [θ] (nm): −12175 (284), −8117 (270) (*c*=0.5×10^{-3} g/ml). FAB-MS *m*/*z*: 557 $[M+Na]^+$. HR-FAB-MS *m*/*z*: 557.1996 (Calcd for $C_{27}H_{34}O_{11}$+Na: 557.1999). ^{1}H- and ^{13}C-NMR: Tables 1 and 2.

Acid Hydrolysis of Compounds 19, 22—26 and 32 A solution of each compound (*ca*. 0.1 mg) in dioxane (4 drops) and 1% H_2SO_4 (4 drops) was heated at 100 °C for 1 h. After hydrolysis, this solution was passed through an Amberlite IRA-60E column and the elute was concentrated to give a residue. For sugar analysis the residue was reduced with $NaBH_4$ (*ca*. 1 mg) for 1 h at room temperature. The reaction mixture was passed through an Amberlite IR-120B column and the elute was concentrated to dryness. Boric acid was removed by co-distillation with MeOH, and the residue was acetylated with acetic anhydride and pyridine (4 drops each) at room temperature for one night. The reagents were evaporated off *in vacuo*. From each glycoside, glucitol acetate and/or rhamnitol acetate were detected by GC [conditions: column, Supelco SP-2380 capillary column 0.25 mm×30 m, column temperature 250 °C, carrier gas N_2, t_R (min); glucitol acetate 12.32, rhamnitol acetate 5.36].

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References and Notes

1) Kopp B., Krenn L., Draxler M., Hoyer A., Terkola R., Vallaster P., Robien W., *Phytochemistry*, **42**, 513—522 (1996).
2) Mitsuhashi H., Tanaka O., Nozoe S., Nagai M. (ed.), "Chemistry of Organic Natural Products," 4th ed., Nankoudou Press, Tokyo, 1994, pp. 168—174.
3) Schoenfeld W., Weiland J., Lindig C., Masnyk M., Kabat M. M., Kurek A., Wicha J., Repke K. R. H., *Naunyn Schmiedeberg's Arch. Pharmacol*., **329**, 414—426 (1985).
4) Majinda R. R. T., Waigh R. D., Waterman P. G., *Planta Med*., **63**, 188—190 (1997).
5) Kopp B., Unterluggauer M., Robien W., Kubelka W., *Planta Med*., **56**, 193—197 (1990).
6) Krenn L., Kopp B., Deim A., Robien W., Kubelka W., *Planta Med*., **60**, 63—69 (1994).
7) Krishna Rao R. V., Rangaswami S., *Tetrahedron Lett*., **1967**, 4563—4565.
8) Krenn L., Jambrits M., Kopp B., *Planta Med*., **54**, 227—232 (1988).
9) Tanase T., Nagatsu A., Murakami N., Nagai S., Ueda T., Sakakibara J., Ando H., Hotta Y., Takeya K., Asano M., *Chem. Pharm. Bull*., **42**, 2256—2262 (1994).
10) Verbiscar A. J., Patel J., Banigan T. F., Schatz R. A., *J. Agric. Food Chem*., **34**, 973—979 (1986).
11) Shimada K., Umezawa E., Nambara T., Kupchan S. M., *Chem. Pharm. Bull*., **27**, 3111—3114 (1979).
12) Jha S., Sen S., *Phytochemistry*, **20**, 524—526 (1981).
13) Lichti H., Niklaus P., von Wartburg A., *Helv. Chim. Acta*, **56**, 2083—2087 (1973).
14) Gorlich B., Durr F. H., Wolter J., *Justus Liebigs Ann. Chem*., **753**, 116—134 (1971).
15) Goerlich B., *Planta Med*., **23**, 39—63 (1973).
16) Kupchan S. M., Moniot J. L., Sigel C. W., Hemingway R. J., *J. Org. Chem*., **36**, 2611—2616 (1971).
17) von Gsell L., Tamm Ch., *Helv. Chim. Acta*, **52**, 551—568 (1969).
18) Uchiyama T., Miyase T., Ueno A., Usmanghani K., *Phytochemistry*, **30**, 655—657 (1991).