Synthesis and Structure Revision of Intensely Sweet Saponin Osladin

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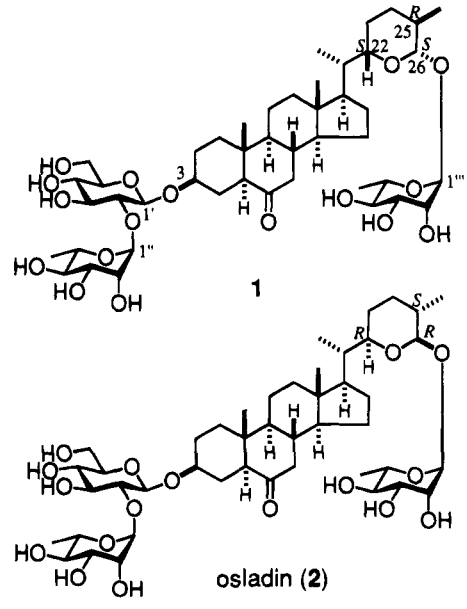
Received April 8, 1994 (Revised Manuscript Received October 17, 1994®)

The synthesis of compound **1**, which is the reported structure of the intensely sweet saponin osladin, has been completed. However, it is not sweet at all. Extraction of the rhizomal sweet principle of the fern *Polypodium vulgare* (Polypodiaceae) and a single-crystal X-ray diffraction study revealed its real structure to be **2**. The synthesis of osladin was achieved from steroidal aldehyde **6** by using a newly developed β-selective and 2′-hydroxyl group discriminating glucosylation procedure and an α-selective thermal rhamnosylation reaction. Synthetic osladin was very sweet, thus proving that osladin is the real sweet principle of the fern.

Introduction

A large number of studies have been carried out on sweet tasting compounds in order to develop additional noncaloric dietary sweeteners.[1] It is well known that the rhizome of the European fern *Polypodium vulgare* (Polypodiaceae) is intensely sweet. In 1967, Jizba and Herout reported the isolation of a structurally new saponin as the sweet tasting principle and named it osladin based on the Czech name of this fern, osladic.[2,3] In 1971, they reported its structure.[4] Shortly thereafter, Havel and Cerny achieved a partial synthesis of the aglycon from solasodine and established the absolute stereochemistry.[5] While the stereochemistry of the glucosidic bond was determined to be β on the basis of an enzymatic hydrolysis using β-glucosidase, the stereochemistry of two rhamnosidic bonds as well as the stereochemistry at C-26 was not determined. Although the structure of **1** was not completely assigned, this compound became well known due to its intense sweetness. Farnsworth described that **1** is 3000 times sweeter than glucose in a review article.[6] During the course of our synthetic studies of baiyunoside and related sweet glycosides,[7] we developed a thermal glycosylation protocol[8] that we thought might be applicable to the total synthesis of osladin. If realized, it would be the first synthesis of a saponin. Herein, we would like to report the following results: (1) We achieved the total synthesis of saponin **1**, which was found not be be sweet. This suggests that **1** is not the structure of the natural saponin osladin (Scheme 1). (2) We isolated natural osladin from the rhizomes of the fern *P. vulgare*. A single-crystal X-ray diffraction study of natural osladin shows that it is the stereoisomeric compound **2**.[9a] Furthermore, osladin is only 500 times sweeter than sucrose. (3) A total synthesis of **2** was achieved, and thus the sweet principle of this fern was proved to be osladin.[9b]

Scheme 1



Synthesis of Compound 1

A retrosynthetic analysis suggests **3** as an advance intermediate toward the synthesis of **1** (Scheme 2). Intermediate **3** will be accessible from disaccharide lactone **4**. Modification of steroidal aldehyde **6**[10] into **5** and subsequent stereoselective glycosylation of **5** are anticipated to produce lactone **4**. Thus by using **6** as the

® Abstract published in *Advance ACS Abstracts*, December 15, 1994.

(1) (a) Ariyoshi, Y. *Kagakusousetsu* **1976**, *14*, 85. (b) Kanda, H.; Tanaka, O. *Yakugakuzasshi* **1975**, *95*, 246. (c) Kinghorn, A. D.; Soejarto, D. D. *Med. Res. Rev.* **1989**, *9*, 91.

(2) Jizba, J.; Herout, V. *Collect. Czech. Chem. Commun.* **1967**, *32*, 2867.

(3) Jizba, J.; Dolejs, L.; Herout, V.; Sorm, F.; Fehlhaber, H. W.; Snatzke, G.; Tschesche, R.; Wulff, G. *Chem. Ber.* **1971**, *104*, 837.

(4) Jizba, J.; Dolejs, L.; Herout, V.; Sorm, F. *Tetrahedron Lett.* **1971**, 1329.

(5) Havel, M.; Cerny, V. *Collect. Czech. Chem. Commun.* **1975**, *40*, 1579.

(6) The sweetness intensity was reported in a private communication from Herout. See: Farnsworth, N. R. *Cosmetics Perfumery* **1973**, *88*, 27.

(7) (a) Nishizawa, M.; Yamada, H.; Hayashi, Y. *Tetrahedron Lett.* **1986**, *27*, 3255. (b) Yamada, H.; Nishizawa, M. *Tetrahedron Lett.* **1987**, *28*, 4315. (c) Nishizawa, M.; Yamada, H.; Hayashi, Y. *J. Org. Chem.* **1987**, *52*, 4878. (d) Yamada, H.; Nishizawa, M. *Tetrahedron* **1992**, *48*, 3021.

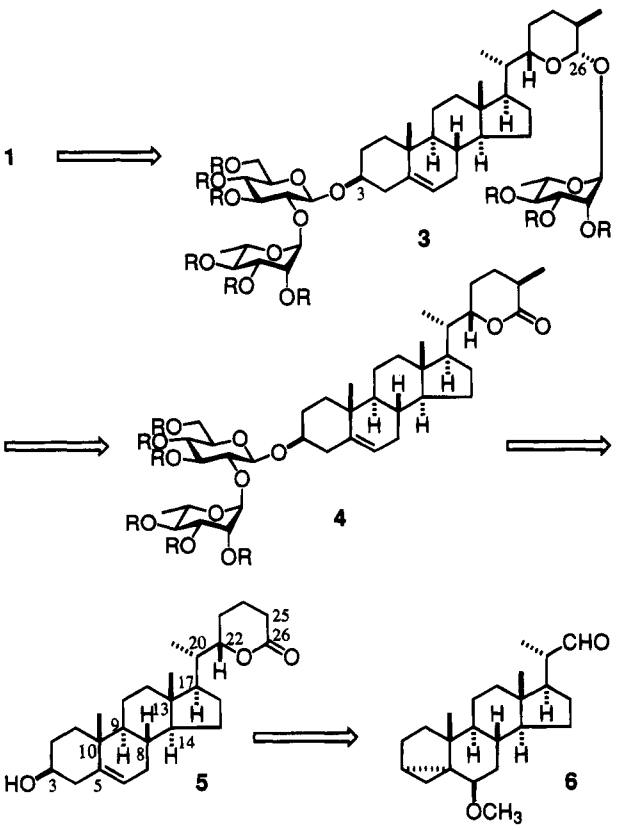
(8) (a) Nishizawa, M.; Kan, Y.; Yamada, H. *Tetrahedron Lett.* **1988**, *29*, 4597. (b) Nishizawa, M.; Kan, Y.; Yamada, H. *Chem. Pharm. Bull.* **1989**, *37*, 565. (c) Nishizawa, M.; Kan, Y.; Simomoto, W.; Yamada, H. *Tetrahedron Lett.* **1990**, *31*, 2431. (d) Nishizawa, M.; Simomoto, W.; Momii, F.; Yamada, H. *Tetrahedron Lett.* **1992**, *33*, 1907. (e) Nishizawa, M.; Imagawa, H.; Kan, Y.; Yamada, H. *Tetrahedron Lett.* **1991**, *32*, 5551. (f) Nishizawa, M.; Imagawa, H.; Kubo, K.; Kan, Y.; Yamada, H. *Synlett* **1992**, 447.

(9) (a) Yamada, H.; Nishizawa, M. *Tetrahedron Lett.* **1992**, *33*, 4009. (b) Yamada, H.; Nishizawa, M. *Synlett* **1993**, 54.

(10) Compound **6** is easily prepared from commercially available natural stigmasterol. See: Steele, J. A.; Mosettig, E. *J. Org. Chem.* **1963**, *28*, 571.

0022-3263/95/1960-0386$09.00/0

Scheme 2



starting material, the stereochemistry at C-3, 8, 9, 10, 13, 14, 17, and 20 can be established automatically. The stereochemistry at C-5, 22, 25, and 26 must be controlled by stereoselective reactions. Sugar residues are used as protecting groups for the C-3 and 26 oxygen functionalities and are introduced during the early stages of the synthesis. Glycosylation of the hemiacetalic hydroxyl group at C-26 must be stereoselective.

Grignard reaction of 4-pentenylmagnesium bromide with aldehyde **6** afforded alcohols **7** and **8** in 89% yield in a 97:3 ratio (Scheme 3). At this point the stereochemistry of the C-22 center of **7** and **8** was not clear. Compound **7** was easily transformed into lactone **9** by Sudan III directed ozonolysis[11] in ethanol/water (10:1) at −78 °C,[12] followed by an oxidation of the resulting hemiacetal with PDC. Triflic acid (0.005 equiv) catalyzed solvolysis of the three-membered ring of **9** in dioxane/water (9:1) afforded lactone **5** in 97% yield.[13] In order to determine the stereochemistry at C-22 of **7**, we attempted an X-ray analysis of lactone **11** which was prepared from **9**. Methylation of **9** afforded two isomers **10a** and **10b** (1:1) in 69% yield. After the isomers were separated by HPLC, isomer **10a** was converted into **11** by triflic acid catalyzed hydrolysis in 98% yield. Lactone **11** was easily crystallized from $CHCl_3$/hexane to provide single crystals (mp 236.5−237.5 °C). The stereochemistry at C-22 was determined to be *S* by an X-ray analysis of lactone **11** as seen in the ORTEP drawing.[14] The X-ray study also showed that the γ-lactone ring of **11** has a twist-boat conformation. This result confirmed that the Grignard reaction of **6** follows Cram's rule to provide C-22 *S* alcohol **7** as the major product.[15]

Since compound **1** has C-25 *R* configuration, we attempted to isomerize the C-25 methyl group of **10a,b**. Under kinetic conditions (LDA, −78 °C, then MeOH), the 1:1 ratio of **10a,b** did not change. This result showed that the intermediate enolate anion does not favor the C-25 *R* configuration. Attempted methylation of **9** also gave a 1:1 mixture of **10a,b**. Further, under thermodynamic conditions (catalytic *t*-BuOK, *t*-BuOH, 25 °C), the ratio did not change. This result showed that the C-25 *R* configuration is not thermodynamically more stable than the C-25 *S* compound due to a twist-boat conformation of the lactone ring. However, the DIBALH reduction product **12b** was isomerized into a 10:1 mixture of **12a** and **12b** under thermodynamic conditions (catalytic NaOMe in MeOH) (Scheme 4).[16] The ratio was determined by comparing ^1H NMR spectra of isolated compounds with those of pure **12a**. **12a** was independently prepared by reduction of pure **10a**.

In this synthetic study we intended to use glycosyl moieties as protecting groups for the C-3 and C-26 hydroxyl functionalities and employed glucosyl chloride **13**[17] and rhamnosyl chloride **14** as glycosylation donors. A 2′-discriminating and β-selective glucosylation of **5**[18] was achieved by using glucosyl chloride **13**, a catalytic amount of triflic acid, and tetramethylurea (abbreviated TMU hereafter) as an acid scavenger to give β-glucoside **15** in 59% yield. Further glucosylation of the C-2′ hydroxyl group to give a disaccharide was observed in less than 10% yield. The increased steric hindrance around C-2′ limits the second glucosylation.[19]

(11) Veysoglu, T.; Mitscher, L. A.; Swayze, J. K. *Synthesis* **1980**, 807.

(12) The presence of water is essential to obtain the hemiacetal in high yield.

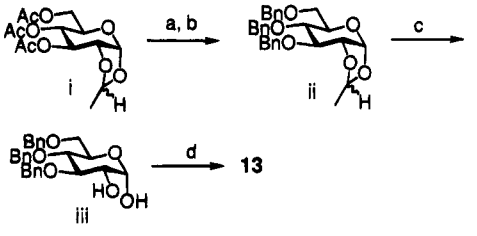
(13) A *p*-toluenesulfonic acid catalyzed solvolysis in aqueous dioxane was reported. See: Giner, J. L.; Margot, C.; Djerassi, C. *J. Org. Chem.* **1989**, *54*, 369. An original method using zinc(II) acetate afforded 3-*O*-acetate. See: Steele, J. A.; Mosettig, E. *J. Org. Chem.* **1963**, *28*, 571.

(14) Orthorhombic colorless crystals; space group $P2_12_12_1$; a = 17.658(2) Å, b = 21.060(1) Å, c = 6.439(1) Å, V = 2394.6 Å^3; Z = 4; D(calc) 1.33 g/cm^3; Cu Kα radiation 23 °C; maximum 2θ 126.1°; 1521 reflections collected, of which 394 were used in the solution of structure; R index = 0.049; diffractometer Rigaku AFC5R. This experiment was carried out in collaboration with Mr. S. Takaoka of Ono Pharmaceutical Co. Ltd.

(15) Cram, D. J.; Abd Elhafez, F. A. *J. Am. Chem. Soc.* **1952**, *74*, 2910. Anh, N. T. *Top. Curr. Chem.* **1980**, *88*, 145. The same stereoselection was observed by Grignard reactions of C-22 ketones. Barton, D. H. R.; Poyser, J. P.; Sammers, P. G. *J. Chem. Soc., Perkin Trans. 1* **1972**, 53. Poyser, J. P.; Ourisson, G. *Ibid.* **1974**, 2061.

(16) Both **12a** and **12b** were obtained as a mixture at C-26 (7:3 and 3:2, respectively).

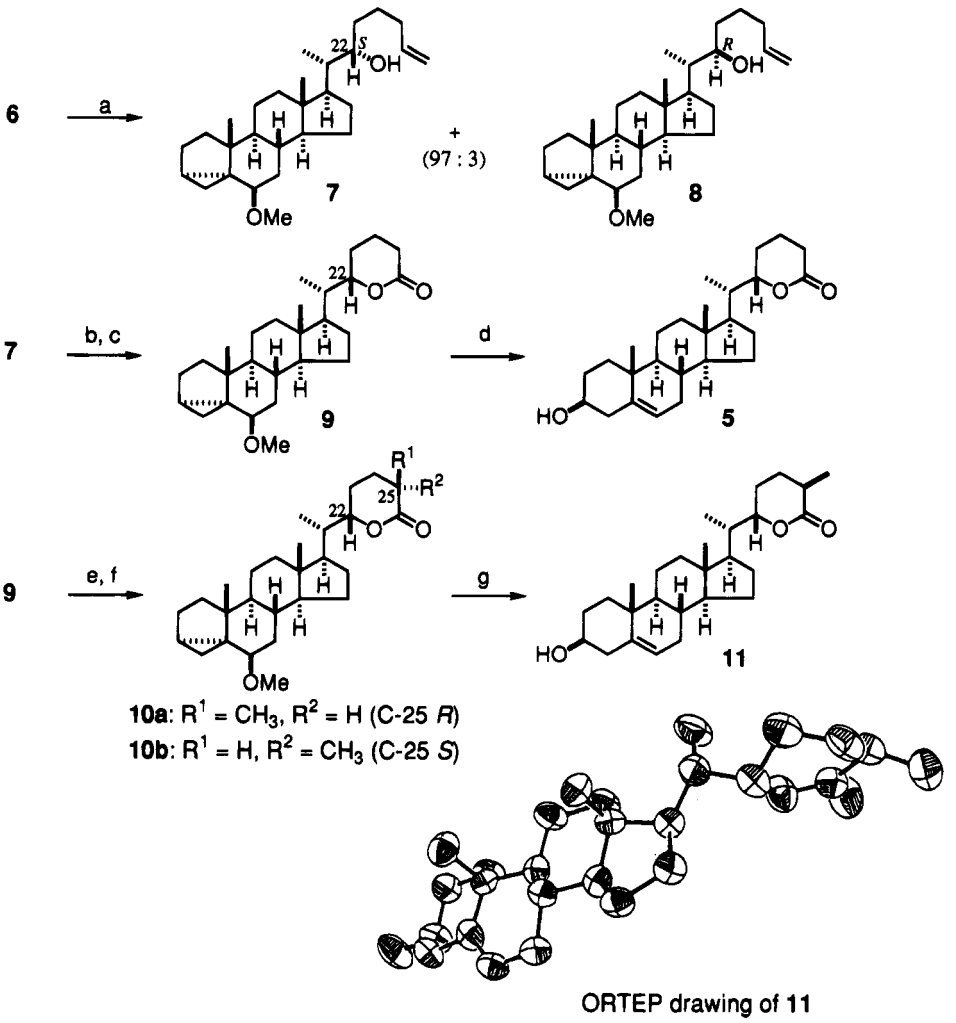
(17) Glucosyl chloride **13** was efficiently prepared in four steps (overall 55% yield) from known acetal **i** (Betaneli, V. I.; Ovchinnikov, M. V.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1982**, *107*, 285) as seen in the following scheme. The diol **iii** was previously prepared by different routes. See: Gent, P. A.; Gigg, R. *Carbohydr. Res.* **1976**, *49*, 325. Schmidt, R. R.; Effenberger, G. *Carbohydr. Res.* **1987**, *171*, 59. Charette, A. B.; Marcoux, J. F.; Cote, B. *Tetrahedron Lett.* **1991**, *32*, 7215.



(a) MeONa, MeOH, rt, 12 h; (b) BnCl, KOH, dioxane, reflux, 5 min; (c) 80% AcOH, 90°C, 36 h; (d) $SOCl_2$, DMF, CH_2Cl_2, 0°, 3 h.

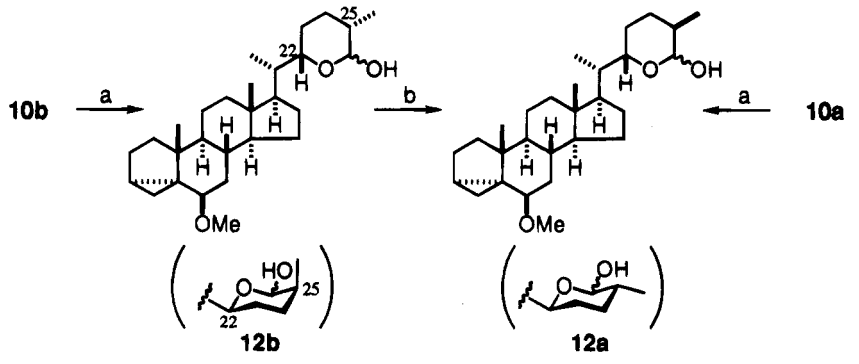
(18) When using pure C-25 *R* methyl compound **11** for a synthesis of **1**, isomerization of the methyl group was observed during the thermal rhamnosylation. Using a mixture of **11** and its C-25 isomer made assignment of the NMR spectra following the glycosilation reactions difficult. Thus, for a synthesis of **1**, C-25 unmethylated **5** was used.

Scheme 3[a]



[a] (a) CH_2=$CH(CH_2)_3MgBr$, ether, −78 to −20 °C; 89%; (b) O_3, Sudan III, $C_2H_5OH-H_2O$ (10:1), −78 °C, then $(CH_3)_2S$, 25 °C, 6 h; (c) PDC, CH_2Cl_2, 25 °C, 7 h then reflux, 5 h; 87% (2 steps); (d) TfOH (0.005 equiv), dioxane−H_2O (9:1), reflux, 80 min; 97%; (e) LDA, HMPA, THF, −78 °C, then CH_3I, −78 to 25 °C; 69%; (f) HPLC; (g) TfOH (0.005 equiv), dioxane−H_2O (9:1), reflux, 70 min; 98%.

Scheme 4[a]



[a] (a) DIBALH, PhMe, −55 °C; (b) NaOMe (0.1 equiv), MeOH/THF (1:1), reflux, 3 h.

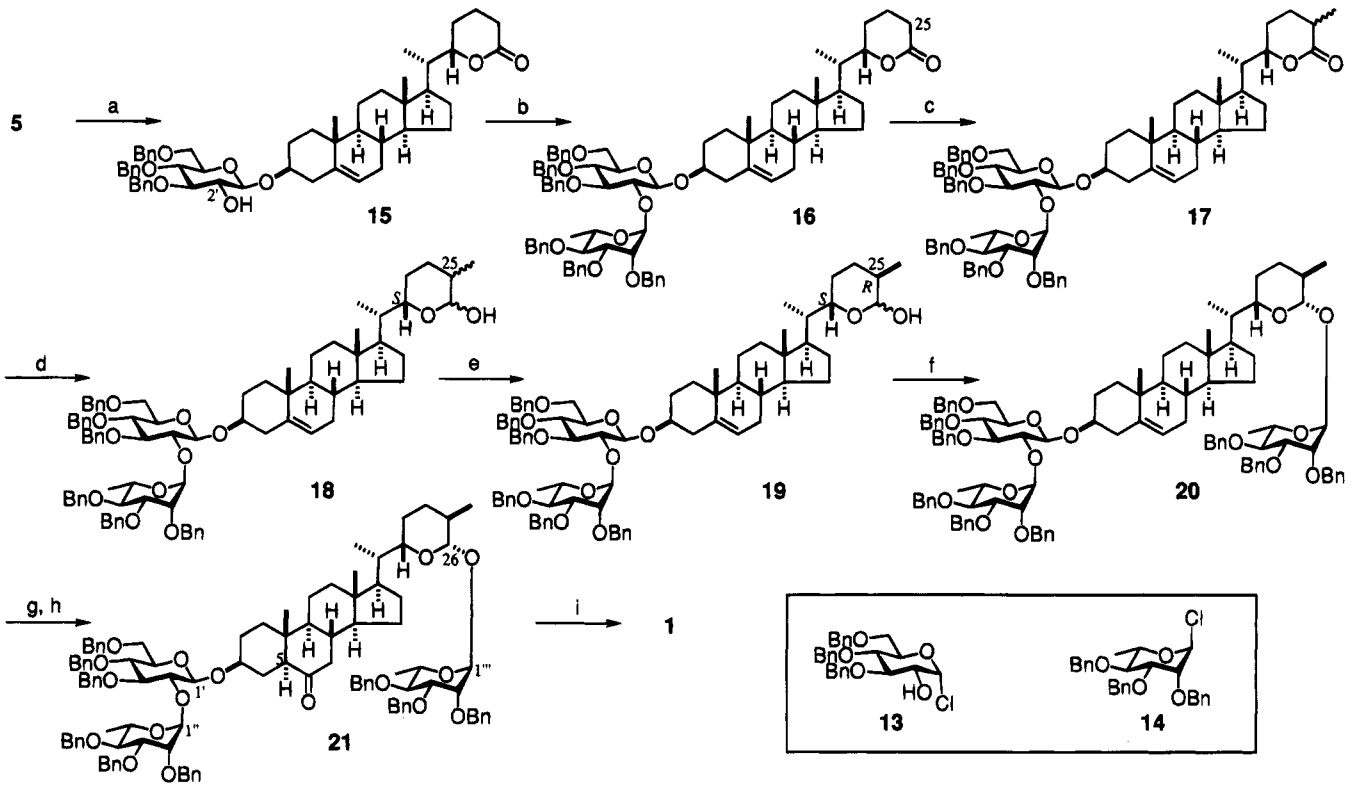
The α-rhamnoside linkage was introduced with the highly α-selective thermal glycosylation reaction that we recently developed.[8c-f] A mixture of **15**, 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranosyl chloride (**14**) (3.1 equiv), and α-methylstyrene (9 equiv, acid scavenger) was heated at 80 °C for 60 h without solvent to give disaccharide **16** in 59% yield (Scheme 5). The newly formed rhamnoside bond was found to be completely α by NMR analysis.[20] The stereochemistry of the original β-glucosidic linkage of **15** was retained during thermal glycosylation.

A methyl group was introduced at C-25 of **16** by sequential treatment with LDA and methyl iodide to give **17** in 39% yield as a 1:1 mixture of stereoisomers. After

(19) Ogawa, T. *Kagakutoseibutsu* **1981**, *20*, 789. Halcomb, R. L.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6661.

(20) Stereochemical assignment of anomeric center was carried out by determining the J_{C1-H1} value of each anomeric carbon. Kasai, R.; Okihara, M.; Asakawa, J.; Mizutani, K.; Tanaka, O. *Tetrahedron* **1979**, *35*, 1427.

Scheme 5[a]



[a] (a) **13**, TMU, TfOH (0.05 equiv), CH_2Cl_2, 25 °C, 7 days; 59%; (b) **14**, α-methylstylene, neat, 80 °C, 60 h; 59%; (c) LDA, HMPA, THF, −78 °C then MeI, −78 to 25 °C; 39%; (d) DIBAH, ether, −50 °C, 6 h; (e) MeONa, MeOH−THF (1:2), 25 °C, 8 h; 79% (2 steps); (f) **14**, AgOTf, TMU, CH_2Cl_2, rt, 25 °C; 55%; (g) BH_3·THF, 25 °C, 8 h, then H_2O_2, NaOH, 25 °C, 1.5 h; (h) PDC, CH_2Cl_2, reflux, 1 h; 70% (2 steps); (i) H_2 (1 atm), $Pd(OH)_2$, MeOH−EtOAc−H_2O (12:2:1), 25 °C, 24 h; 98%.

DIBALH reduction of **17**, the product **18** was smoothly isomerized into the C-25 *R* compound **19** by treatment with sodium methoxide (79% yield over 2 steps).[21]

Glycosylation at the C-26 hemiacetal of **19** was achieved by using the classical Koenigs−Knorr glycosylation.[22] Treatment of **19** with rhamnosyl chloride **14** and silver triflate in the presence of TMU provided trisaccharide **20** in 55% yield.[23] Hydroboration, followed by PDC oxidation, afforded desired ketone **21**. A doublet of doublets at δ 2.25 (H-5) with coupling constants of 13.2 and 4.4 Hz indicates that the A/B ring system is *trans* fused. The stereochemistry at C-26 was established to be *S* (equatorial) on the basis of a doublet proton NMR signal at δ 4.23 with J = 8.3 Hz and a doublet carbon signal at δ 102.8 with J = 157 Hz.[20,24] Stereochemistries of the three glycosidal linkages were determined to be 1′β, 1″α, and 1‴α, based on ^{13}C NMR [doublets at δ 98.3 (J = 157 Hz), δ 98.1 (J = 172 Hz), and δ 93.8 (J = 169 Hz), respectively].[25] A palladium(II) hydroxide catalyzed debenzylation of **21** under a hydrogen atmosphere gave **1** in 98% yield. This completes the first total synthesis of a saponin; however, aqueous solutions of **1** were not sweet at all.[26]

Isolation and Structure Determination of The Real Osladin (2).

Since the original samples of osladin and spectral data were not available, we isolated the rhizomal sweet principle of *P. vulgare* from plants collected in the southern part of Germany by Professors Y. Asakawa and H. Becker. Dried and crushed rhizomes were successively extracted with hexane, dichloromethane, ethyl acetate, and ethanol. Only the ethanol extract was sweet. Successive chromatography on silica gel, sephadex, silica gel, and ODS (octadecyl silica gel), HPLC with an ODS column, and finally recrystallizations afforded a pure sweet compound as colorless crystals (0.02% isolated yield).[27] Since the sweet compound has a mp of 202−204 °C (lit.[2] mp 201−203 °C) and a molecular formula of $C_{45}H_{74}O_{17}$ suggested from HRMS (FAB), this is the same compound, osladin, that was isolated by Jizba and Herout. The ^1H and ^{13}C NMR spectra of the natural osladin were not identical with those of synthetic **1**. Thus, the structure of real osladin differs from that of **1**.

(21) Because of the complex ^1H NMR of **19**, we could not determine the ratio at C-25. The ^{13}C NMR of **19** shows several relatively small peak sets of anomeric carbons. This suggests that **19** is a mixture of isomers at the C-26 hydroxyl group (similar to **12a**), and very little of **18** was remaining. Considering the isomerization of **12b**, the ratio of **19** and **18** is possibly around 10:1.

(22) Richmyer, N. K. *Method Carbohydr. Chem.* **1962**, *1*, 370.

(23) Hanessian, S.; Banoub, J. *Carbohydr. Res.* **1977**, *53*, C13.

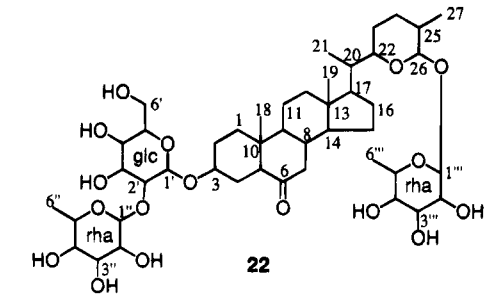
(24) Assignment of this position was supported by correlation of the H-27−H-25−H-26 of ^1H−^1H COSY and C-26−H-26 correlation of ^{13}C−^1H COSY spectra.

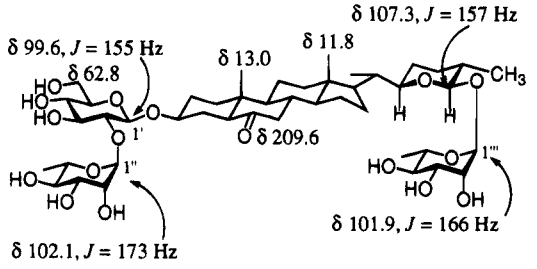
(25) Stereochemistry of 1′β was also assigned by ^1H NMR (δ 4.48, J = 7.7 Hz for **21** and δ 4.39, J = 7.8 Hz for **36**).

(26) We tasted 100 ppm and 1% (w/w) aqueous solutions of **1**.

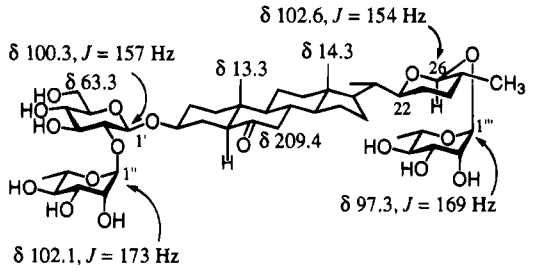
(27) The isolated compound, osladin, was not the only sweet component of this fern. From the mother liquid of the recrystallization, $\Delta^{7,8}$-osladin (polypodoside A) was isolated. Polypodoside A was first isolated and characterized by Kinghorn and co-workers from the rhizomes of *P. glycyrrhiza* as a sweet component of this fern. The structure which they determined was also incorrect, and it has been revised. Isolation and characterizing of polypodoside A: Kim, J.; Pezzuto, M.; Soejarto, D. D.; Lang, F. A.; Kinghorn, A. D. *J. Nat. Prod.* **1988**, *51*, 1166. Structure revision of polypodoside A: Nishizawa, M.; Yamada, H.; Yamaguchi, Y.; Hatakeyama, S.; Kennelly, E. J.; Lee, I-S.; Kim, J.; Kinghorn, A. D. *Chem. Lett.* **1994**, 1555, 1979.

Scheme 6





^{13}C NMR data of sweet natural osladin (**2**) in C_5D_5N



^{13}C NMR data of synthetic **1** inC_5D_5N

After assuming that this compound has planar structure **22** as determined by Jizba and co-workers,[4] ^{1}H and ^{13}C NMR spectra were fully assigned by considering ^{1}H–^{1}H COSY, NOESY, HMQC, HMBC, and HOHAHA spectra (Scheme 6 and Table 1).[28] A NOESY correlation (H-3–H-1′) suggests that glucose connects to the C-3 hydroxyl group of the aglycon. The coupling constants (6.6 Hz at H-1′ in ^{1}H NMR and 155 Hz at C-1′–H-1′ in ^{13}C NMR) show that this glucosidic bond is β.[20] HMBC (C-2′–H-1″) and NOESY (H-1″–H-2′) correlations establish that rhamnose is linked to the C-2′ hydroxyl group of the glucose moiety; the coupling constant (173 Hz at C-1″–H-1″ in ^{13}C NMR) shows that the rhamnosidic bond is α. Connection of C-26–O–C-1‴ is established by HMBC (C-26–H-1‴) and NOESY (H-26–H-1‴) correlations. The C-1‴–H-1‴ coupling constant in the ^{13}C NMR spectrum (166 Hz) shows that this is an α rhamnoside. Utilization of the glycosylation shift method (δ 101.9 at C-1‴) suggests that the stereochemistry of C-26 is *R*;[29] the C-26–H-26 coupling constant in the ^{13}C NMR spectrum (157 Hz) shows that this oxygen functionality is equatorial.[20] A coupling constant of H-26 (8.8 Hz) in the ^{1}H NMR spectrum suggests a *trans* relationship between the C-26 oxygen functionality and the C-25 methyl group. An NOE cross peak between H-26 and H-22 suggest a *cis* relationship between these protons. Comparison of the ^{13}C NMR data of **1** and real osladin shows that the steroidal moieties and the left disaccharide parts are very similar. Remarkable differences were observed at the C-26 and C-1‴ positions. From the results described above, the structure of real osladin was determined to

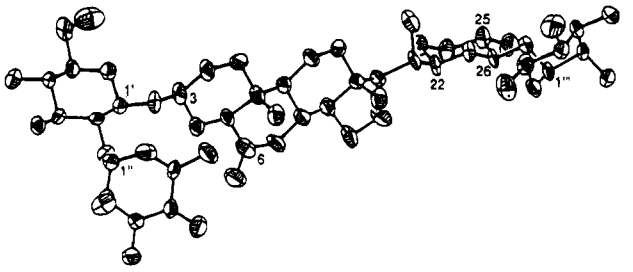
Table 1. Full Assignment of ^{1}H and ^{13}C NMR Spectra and Direction of NOE and HMBC of Osladin (2) (600 MHz in C_5D_5N)

position	^{1}H	^{13}C	NOE	HMBC
1	α 1.02	36.8	11α	H18
	β 1.59			
2	α 2.09	29.4		
	β 1.80		18	
3	4.00	76.2	1α, 5, 1′	
4	α 2.49	26.5	18	
	β 1.94			
5	2.01	56.3	9	H18
6		209.6		
7	α 2.00	46.8		
	β 2.37		5	
8	1.66	36.5	18, 19	
9	1.10	53.8	7α, 12α	H18
10		40.9		H18
11	α 1.57	27.3	1α	
	β 1.20		19	
12	α 1.89	39.7	17	H19
	β 1.08			
13		43.2		H19
14	1.10	56.4		
15	α 0.94	24.1		
	β 1.41		7β	
16	α 1.20	21.6		
	β 1.46		20	
17	1.10	52.8	15α	H19, H21
18	0.78	13.1	2β, 4β, 8, 11β	
19	0.56	11.9	8, 16α, 18, 20	H13, H14, H17
20	1.87	40.1	16β	H21
21	1.03	13.7	12α, 19	
22	3.46	78.3	16α, 17, 26	H21
23	α 1.32	23.9		
	β 1.32			
24	α 1.76	31.5		
	β 1.13			
25	1.65	37.8		
26	4.48	107.3	22, 1‴	H1‴
27	0.92	16.7	24α	H22
1′	5.08	99.6	3, 4α, 3′, 5′	
2′	4.27	78.2		H3′, H1″
3′	4.27	79.5	1′, 5′	H2′, H4′
4′	4.14	72.0	2′	
5′	3.92	78.3	1′, 3′	H6′ (δ 4.36)
6′	4.36	62.8		
	4.57		4′	
1″	6.36	102.1	2′	
2″	4.80	72.5		
3″	4.66	72.8		H1″, H2″, H5″
4″	4.35	74.1	6″	H2″, H3″, H5″, H6″
5″	4.98	69.5		H1″, H4″, H6″
6″	1.80	18.7		H4″
1‴	5.67	101.9	26	H26
2‴	4.63	72.2		
3‴	4.56	72.8	5‴	H1‴, H4‴, H5‴
4‴	4.32	74.0	6‴	H3‴, H6‴
5‴	4.67	70.5		H1‴, H4‴, H6‴
6‴	1.71	18.4		

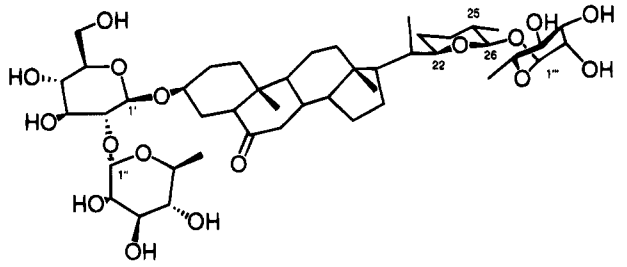
(28) We thank Miss Yukiko Kan of our institute for carrying out the 2D experiments on the isolated osladin (**2**). NOESY: nuclear Overhauser and exchange spectroscopy. HMQC: ^{1}H-detected multiple quantum coherence spectrum, see Bodenhausen, G.; Ruben, D. J. *Chem. Phys. Lett.* **1980**, *69*, 185. HMBC: ^{1}H-detected multiple-bond heteronuclear multiple quantum coherence spectra, see Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093. HOHAHA: homonuclear Hartmann–Hahn spectrum, see Bax, A.; Davis, D. G. *J. Magn. Reson.* **1985**, *65*, 355.

(29) The ^{13}C glycosylation shifts of a large number of 1,1′-disaccharides have been evaluated. The characteristic low-field shifts observed for C-26 of **2** as well as **36** are due to a combination of *S*-hemiacetal and *R*-hemiacetal. Nishizawa, M.; Kodama, S.; Yamane, Y.; Kayano, K.; Hatakeyama, S.; Yamada, H. *Chem. Pharm. Bull.* **1994**, *42*, 982.

Scheme 7



ORTEP drawing of osladin (**2**)



Stereostructure of osladin (**2**)

be **2**. However, the NMR results were not sufficient to establish the stereochemistry of real osladin completely.

We then decided to prepare a single crystal for X-ray diffraction study. Repeated recrystallizations from a mixture of acetone and water afforded a monoclinic single crystal of space group $P2_1$. Even though the R value of this X-ray analysis is 0.11 due to rather large thermal vibration, sweet osladin should be represented by structure **2** as seen in the ORTEP drawing as well as the stereostructure (Scheme 7).[30] The X-ray structure of **2** corresponds to previous NMR studies. The structure was also confirmed by the total synthesis of osladin (see below). Thus, the C-22*S*, 25*R*, 26*S* stereochemistry assigned by Havel and Cerny[5] needs to be revised to C-22*R*, 25*S*, 26*R*, respectively (see Scheme 1). In addition, it is important to note that **2** is intensively sweet while **1** is totally free from any taste even though the configurational change is minor.

In the original paper on the structure determination of osladin, Jizba, Dolejs, Herout, and Sorm did not mention the intensity of the sweetness. Although osladin has been claimed to be 3000 times sweeter than sucrose by Farnsworth,[6] further examination by Ajinomoto Co. suggests that it is only 500 times sweeter.[31]

In 1975, Havel and Cerny reported the chemical correlation of osladin aglycon with solasodine (**23**).[5] They prepared **25a** and **25b** from solasodine through sodium borohydride or lithium tri-*tert*-butoxyalumimum hydride reduction of ketone **24** followed by nitration to retain the C-25 R stereochemistry (Scheme 8). They assigned C-22 S for minor isomer **25a** and C-22 R for major isomer **25b**. Their criterion for the assignment of stereochemistry at C-22 was optical rotation. According to Burrows and coworkers,[32] they assigned the C-22 R configuration to the more dextrorotatory isomer **25b**. Today, it is clear that the reduction of C-22 steroidal keto compounds like **24** provides the C-22 S alcohol predominantly.[33] Thus the structures **25a** and **25b** should be revised to **25a'** and **25b'**, respectively. From these nitrates, they derived **26a** and **26b**. Thus, **26a** and **26b** must be revised into **26a'** and **26b'**. Upon finding that the natural product derivative was identical with **26a**, Havel and Cerny gave the C-22 S, C-25 R stereostructure for the osladin aglycon. We found that the C-25 axial methyl group easily epimerizes when C-26 is a hemiacetal. Thus, we suppose that Havel and Cerny isolated **27** (C-22 R, C-25 S) after isomerization of **26a'**; they identified **27** with the natural product derivative.[34]

Synthesis of The Real Osladin (2)

To complete the synthesis of sweet osladin (**2**), we needed to modify the synthesis of **1**. As already discussed, Grignard reaction of the aldehyde **6** generates the C-22 S product **7** predominantly. Since the C-22 stereochemistry of real osladin (**2**) is R, we needed to invert the stereochemistry at C-22 of **7**. Although a variety of Mitsunobu reaction conditions failed to give any inversion product,[35] Corey's S_N2 reaction of mesylate **28** with KO_2 achieved clean inversion at C-22 to give C-22 R carbinol **8** in 88% yield.[36] The alcohol **8** was transformed into lactone **29** by ozonolysis in ethanol/water (10:1) and subsequent PDC oxidation (Scheme 9). Methylation at the α-position of the lactone afforded monomethylated product **30** as a 1:1 mixture of stereoisomers. Since it is possible to isomerize the configuration of the C-25 methyl group at a later stage, this mixture was employed for the solvolysis and glycosylation reactions.[37] Treatment of **30** with 0.005 equiv of triflic acid in dioxane/water (9:1) afforded homoallylic alcohol **31** in 81% yield. Condensation of **31** and glucosyl chloride **13** catalyzed by triflic acid in the presence of TMU took place in a β-selective manner to give 2'-hydroxyl group discriminated glucoside **32** in 57% yield. By means of an α-selective thermal rhamnosylation reaction, the L-rham-

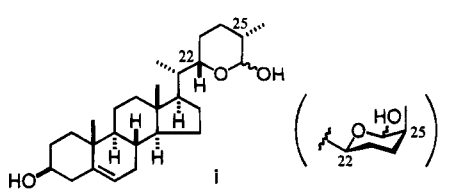
(30) Colorless crystal; crystal system monoclinic; space group $P2_1$; a = 24.670(3) Å, b = 7.189(1) Å, c = 15.512(2) Å, β = 90.54°, V = 2751.1(6) Å^3; Z = 2; D(calc) 1.09 g/cm^3; Cu Kα radiation; maximum sin (θ) 0.584; 5352 reflections collected, of which 4538 were used in the solution of the structure; R index = 0.117; diffractometer Mac Science MXC18. Atomic coordinates, bond lengths and angles, and thermal parameters are deposited in the Cambridge Crystallographic Data Centre. We thank Dr. Chuji Katayama of Mac Science Co. Ltd. for carrying out this X-ray crystallographic study.

(31) Sweetness intensity relative to sucrose is evaluated by diluting the aqueous solution of osladin until it exhibits an equivalent sweetness intensity to that of a 2% w/w sucrose solution. Thus the sweetness intensity is a simple ratio of the two solution concentrations.

(32) Burrows assigned C-22 S configurations for major isomers which were derived from several kinds of C-22 ketones. In their paper, each C-22 R alcohol is more dextrorotatory than the C-22 S isomer. Burrows, E. P.; Horney, G. M.; Caspi, E. *J. Org. Chem.* **1969**, *34*, 103.

(33) Piatak, D. M.; Wicha, J. *Chem. Rev.* **1978**, *78*, 199.

(34) Havel and Cerny's last step of the synthesis of **26a** (**26a'**) was removal of the THP groups at C-3 and C-26 under acidic conditions (80% AcOH, 100 °C, 5 min). We thought these conditions led to isomerization at C-25. Thus we attempted to isomerize **i**. Although the C-25 axial methyl group of compound **i** was easily isomerized under basic conditions (0.1 equiv of NaOMe in MeOH/THF, reflux, 3 h), no isomerization was observed under acidic conditions [80% AcOH/THF (2:1), reflux, 30 min]. Thus the reason of why **26a'** isomerized is unclear.



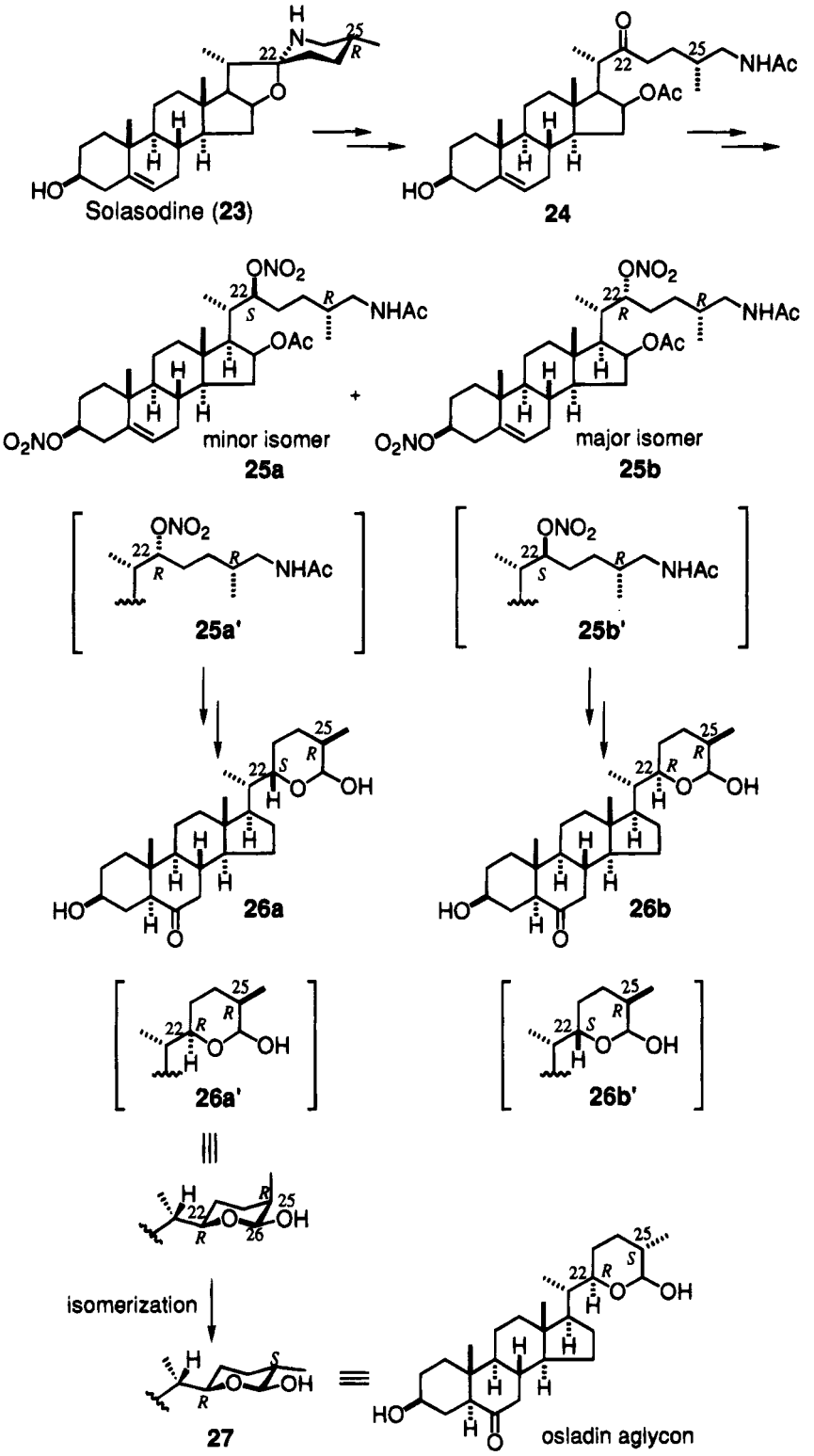
i

(35) Mitsunobu, O. *Synthesis* **1981**, 1.

(36) Corey, E. J.; Nicolaou, K. C.; Shibasaki, M.; Machida, Y.; Shiner, C. S. *Tetrahedron Lett.* **1975**, 3183.

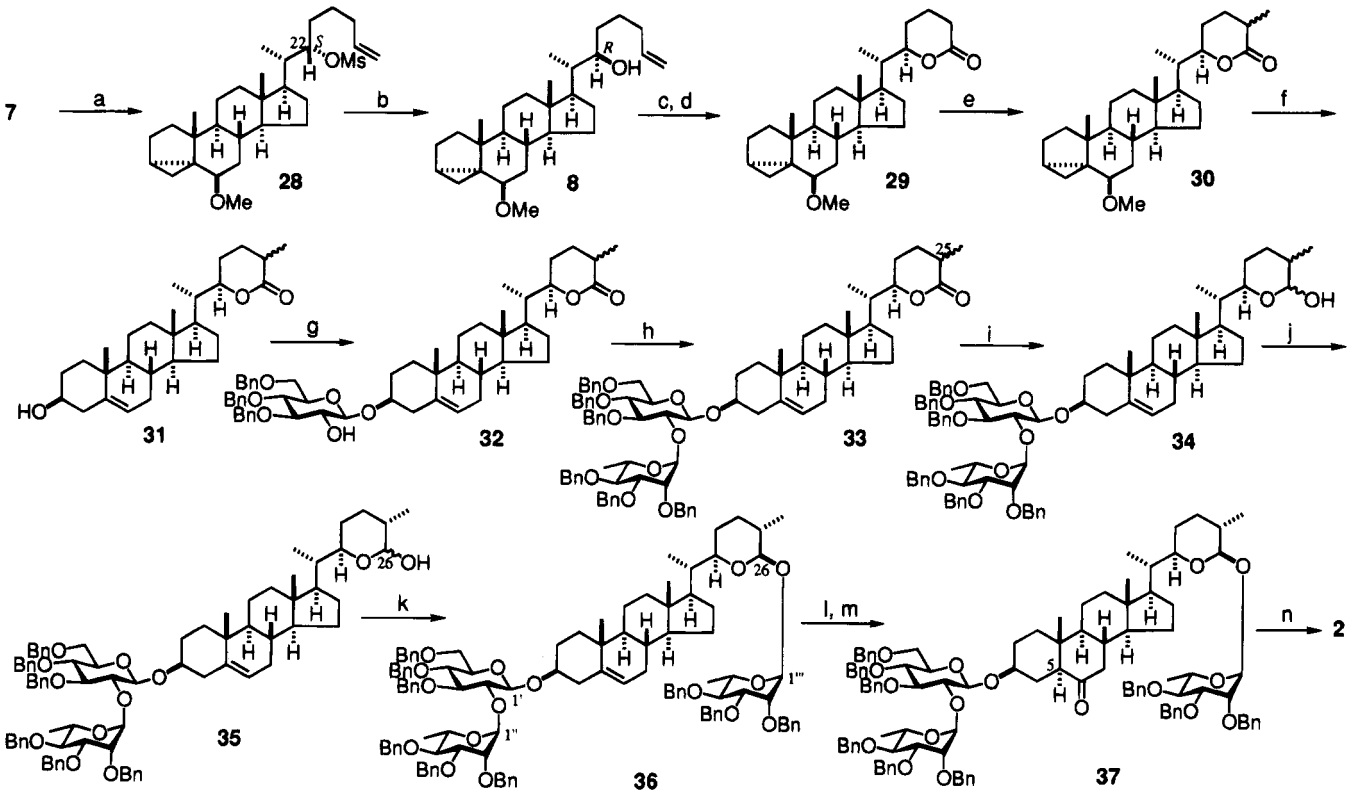
(37) In order to confirm the structures of **31** and **32** from NMR spectra, pure C-25 S and C-25 R of these samples were prepared from the C-25 S and C-25 R isomers of **30**, respectively.

Scheme 8



nosyl residue was coupled to **32** with **14** and TMU (neat, 80 °C, 56 h) to give disaccharide **33** in 81% yield.[8] The hemiacetals **34** obtained by DIBALH reduction of **33** were treated with base to give the more stable equatorial methyl product **35**. Glycosylation of the hemiacetal hydroxyl group at C-26 was achieved by using rhamnosyl chloride **14**, AgOTf, and TMU to yield trisaccharide **36** stereoselectively. The characteristic low-field shift (δ 106.7) of the C-26 hemiacetal carbon was observed in the ^{13}C NMR of **36**. A similar low-field shift was observed in osladin itself (δ 107.3) (see Scheme 6).[29] A doublet at δ 4.08 with a coupling constant of 8.6 Hz (H-26) indicates a *trans* relationship between the C-25 methyl group and the *O*-rhamnosyl functionality at C-26.[24] A coupling constant C-26–H-26 (153 Hz) in the ^{13}C NMR spectrum shows that the *O*-functional group at C-26 is equatorial,[20] and the chemical shift at C-1‴ (δ 97.1) shows that the stereochemistry at C-26 is *S*.[29] The stereochemistry of the newly introduced *O*-rhamnoside linkage (C-1‴) was assigned α due to a coupling constant of 166 Hz in the ^{13}C NMR.[20] The stereochemistries of the left disaccharide were confirmed to be 1′β and 1″α on the basis of ^{13}C NMR

Scheme 9[a]



[a] (a) MsCl, pyridine, 0 °C, 0.5 h, then 25 °C, 1 h; 100%; (b) KO_2, 18-crown-6, DMSO–DME (1:1), 0 °C, 30 min; 88%; (c) O_3, Sudan III, EtOH–H_2O (10:1), −78 °C, then Me_2S, 25 °C, 5 h; 89%; (d) PDC, CH_2Cl_2, reflux, 4 h; 91%; (e) LDA, HMPA, THF, −78 °C, then MeI, −78 to 0 °C; 90%; (f) TfOH (0.005 equiv), dioxane–H_2O (9:1), reflux, 2 h; 81%; (g) **13**, TfOH (0.1 equiv), TMU, $(CH_2Cl)_2$, reflux, 4 h; 57%; (h) **14**, TMU, neat, 80 °C, 56 h; 81%; (i) DIBAH, ether, −60 °C, 10 h; (j) MeONa, MeOH, reflux, 6 h; 81% (2 steps); (k) **14**, AgOTf, TMU, CH_2Cl_2, 25 °C, 10 min; 61%; (l) BH_3·THF, 25 °C, 8 h, then H_2O_2, NaOH, 25 °C, 1.5 h; (m) PDC, CH_2Cl_2, reflux, 1 h; 61% (2 steps); (n) H_2 (1 atm), $Pd(OH)_2$, MeOH–EtOAc–H_2O (12:2:1), 25 °C, 36 h; 78%.

doublets at δ 99.8 (J = 156 Hz) and δ 98.1 (J = 174 Hz), respectively.[26] The trisaccharide **36** was subjected to hydroboration and subsequent PDC oxidation to give ketone **37**. A double doublet at δ 2.32 (J = 13.2 and 4.4 Hz) due to H-5 clearly showed the A/B *trans* relationship. The benzyl groups of **37** were cleaved by $Pd(OH)_2$-catalyzed hydrogenolysis to give osladin **2**. This product was very sweet and showed indistinguishable spectral properties (^1H and ^{13}C NMR, optical rotation, IR, and high-resolution FAB mass) with those of natural osladin. Thus we have shown that the structure **2** represents the real sweet principle of *P. vulgare*.

Experimental Section

General. Reactions were run under a positive pressure of Ar unless otherwise noted. Solvents were distilled before use. After workup by extraction, the organic layer was dried over $MgSO_4$, filtered through a cotton–Celite pad, and concentrated. Purifications were carried out by method A, column chromatography on silica gel; method B, column chromatography on ODS; method C, normal phase HPLC; and method D, reverse phase HPLC. Eluents used are indicated by parentheses. HPLC was performed with a refractive index (RI) detector. Columns used are indicated in the supplementary material. Optical rotations were observed in $CHCl_3$, unless otherwise noted. IR spectra were observed in film and reported in cm^{-1}. NMR spectra were observed in $CDCl_3$, unless otherwise noted. Data of ^1H NMR are reported only for characteristic protons: in particular, only anomeric protons are listed for saccharides. Coupling constants (J) are reported in hertz.

Alcohols 7 and 8. To a Grignard reagent prepared from finely cut Mg (2.07 g, 85.4 mmol) and 5-bromopentene (11.6 g, 77.6 mmol) in ether (50 mL) was dropwise added a solution of aldehyde **6** (12.77 g, 344.5 mmol) in ether (100 mL) at −78 °C. The mixture was stirred for 30 min at −78 °C and slowly (1.5 h) warmed to −20 °C. A saturated aqueous NH_4Cl solution was added to the mixture, and it was extracted with ether. The mixture was purified by method A (hexane/AcOEt 20:1 → 9:1) to give 13.2 g (86%) of **7** ($[\alpha]^{22}_D$ +28° (c 1.9); IR 3410, 3061, 1100; ^1H NMR 0.43 (1H, dd, J = 8.0, 4.8), 0.65 (1H, dd, J = 4.6, 4.2), 0.73 (3H, s), 0.90 (3H, d, J = 6.3), 1.02 (3H, s), 2.77 (1H, dd, J = 2.9, 2.7), 3.33 (3H, s), 3.67 (1H, dd, J = 7.3, 4.6), 4.95 (1H, dddd, J = 10.0, 3.1, 1.2, 1.0), 5.01 (1H, ddd, J = 17.1, 3.6, 1.7), 5.81 (1H, ddt, J = 17.1, 10.3, 6.6); ^{13}C NMR 11.5 q, 12.2 q, 13.1 t, 19.3 q, 21.4 d, 22.8 t, 24.1 t, 24.9 t, 25.8 t, 27.8 t, 30.5 d, 33.3 t, 33.8 t, 34.9 t, 35.0 t, 35.2 s, 40.2 t, 40.3 d, 42.7 s, 43.4 s, 47.9 d, 52.7 d, 56.4 d, 56.6 q, 73.5 d, 82.4 d, 114.6 t, 138.7 d; HRMS (EI) m/z calcd for $C_{28}H_{46}O_2$ (M^+) 414.3498, found 414.3489) and 382 mg (2.6%) of **8** ($[\alpha]^{21}_D$ +41° (c 1.9); IR 3441, 3061, 1100; ^1H NMR 0.43 (1H, dd, J = 7.7, 6.0), 0.65 (1H, t, J = 4.4), 0.74 (3H, s), 0.91 (3H, d, J = 6.6), 1.02 (3H, s), 2.77 (1H, dd, J = 3.8, 2.2), 3.32 (3H, s), 3.66 (1H, br d, J = 10.4), 4.96 (1H, ddd, J = 9.4, 2.4, 1.3), 5.02 (1H, ddd, J = 17.1, 3.4, 1.3), 5.83 (1H, ddt, J = 17.0, 9.9, 6.6); ^{13}C NMR 12.2 q, 12.3 q, 13.1 t, 19.3 q, 21.5 d, 22.8 t, 24.2 t, 24.9 t, 26.0 t, 27.5 t, 29.3 t, 30.5 d, 33.4 t, 33.8 t, 35.1 t, 35.2 s, 40.3 t, 42.4 d, 43.1 s, 43.4 s, 48.1 d, 53.3 d, 56.1 d, 56.5 q, 73.4 d, 82.4 d, 114.5 t, 138.8 d; HRMS (EI) m/z calcd for $C_{28}H_{46}O_2$ (M^+) 414.3498, found 414.3483).

Lactone 9. O_3 gas was passed through a solution of **7** (13.17 g, 31.76 mmol) and Sudan III (0.1% CH_2Cl_2 solution, 1 mL) in EtOH/H_2O (10:1) (275 mL) at −78 °C until the red color of Sudan III disappeared (1.5 h). Excess O_3 was evacuated by bubbling of Ar gas; then Me_2S (50 mL) was added. The mixture was stirred for 6 h at 25 °C. The concentrated mixture was purified by method A (hexane/AcOEt 15:1 → 9:1) to give 12.46 g (94%) of hemiacetal. To a solution of the hemiacetal

(12.42 g, 29.8 mmol) in CH_2Cl_2 (150 mL) was added PDC (16.8 g, 44.7 mmol), and the mixture was stirred for 7 h at 25 °C and then refluxed for 5 h. The reaction mixture was diluted with ether (1 L) to precipitate the inorganic material. After filtration through a cotton–Celite pad and concentration, the mixture was purified by method A (hexane/AcOEt 4:1 → 3:1) to give 11.4 g (92%) of lactone **9**: mp 137.0–137.5 °C; $[\alpha]^{21}_D$ +56° (*c* 1.0); IR 1740, 1244, 1098, 1018, 735; ^{1}H NMR 0.43 (1H, dd, *J* = 7.7, 5.0), 0.65 (1H, dd, *J* = 5.0, 3.9), 0.72 (3H, s), 1.00 (3H, d, *J* = 6.6), 1.02 (3H, s), 2.41 (1H, ddd, *J* = 17.6, 9.4, 7.2), 2.60 (1H, ddd, *J* = 18.7, 6.6, 5.0), 2.78 (1H, dd, *J* = 2.8, 2.7), 3.32 (3H, s), 4.38 (1H, br dd, *J* = 9.7, 3.4); ^{13}C NMR 11.6 q, 12.1 q, 12.6 t, 18.4 t, 18.7 q, 20.9 t, 22.2 t, 23.5 t, 24.4 t, 25.2 t, 27.1 t, 29.0 t, 30.0 d, 32.8 t, 34.7 s, 34.7 t, 39.6 d, 39.6 t, 42.0 s, 42.8 s, 47.3 d, 51.2 d, 55.6 q, 55.9 d, 81.7 d, 82.0 d, 171.3 s; HRMS (EI) *m/z* calcd for $C_{27}H_{42}O_3$ (M^+) 414.3126, found 414.3130. Anal. Calcd for $C_{27}H_{42}O_3$: C, 78.21; H, 10.21. Found: C, 77.84; H, 10.33.

Lactone Alcohol 5. A solution of lactone **9** (11.27 g, 27.2 mmol) and TfOH (20 mg, 0.135 mmol) in dioxane/H_2O (9:1) (120 mL) was refluxed for 80 min. The cooled mixture was concentrated. After addition of dioxane (200 mL), azeotropic concentration was carried out two times in order to complete lactonization of contaminated carboxylic acid. The precipitates obtained after the addition of H_2O (300 mL) were filtered, washed with H_2O and dried over P_2O_3 under reduced pressure to give 10.62 g (97%) of lactone alcohol **5**: mp 238–240 °C; $[\alpha]^{21}_D$ −39° (*c* 1.1); IR 3515, 1723, 1254, 1086; ^{1}H NMR 0.69 (3H, s), 1.00 (3H, d, *J* = 6.6), 1.01 (3H, s), 2.60 (1H, ddd, *J* = 17.6, 6.6, 4.4), 3.53 (1H, dddd, *J* = 11.5, 11.5, 6.2, 4.4), 4.38 (1H, dd, *J* = 10.1, 2.7), 5.35 (1H, br d, *J* = 5.6); ^{13}C NMR 11.7 q, 12.6 q, 19.0 t, 19.4 q, 21.0 t, 24.1 t, 25.8 t, 27.6 t, 29.6 t, 31.5 t, 31.8 t, 31.9 d, 36.4 s, 37.2 t, 39.5 t, 40.3 d, 42.2 t, 42.2 s, 49.9 d, 51.4 d, 56.4 d, 71.7 d, 82.8 d, 121.5 d, 140.8 s, 172.5 s; HRMS (EI) *m/z* calcd for $C_{26}H_{40}O_3$ (M^+) 400.2977, found 400.2967.

Lactones 10a,b. To a solution of LDA (1.5 M cyclohexane solution, 6.59 mL, 9.89 mmol) in THF (10 mL) was dropwise added a solution of lactone **9** (4.10 g, 9.89 mmol) in THF (20 mL) at −78 °C. Three minutes later, HMPA (2.13 g, 11.9 mmol) was added to the reaction mixture, and it was stirred for an additional 15 min at −78 °C. After addition of MeI (1.48 g, 10.4 mmol) in one portion at −78 °C, the reaction mixture was slowly (2 h) warmed to 25 °C. A saturated aqueous NH_4Cl solution was added to the mixture, and it was extracted with ether. The mixture was purified by method A (hexane/AcOEt: 6:1) to give a mixture of **10a** and **10b** (2.94 g, 69%). This mixture was separated by method C (hexane/AcOEt 12:1) to give **10a** ($[\alpha]^{25}_D$ +35° (*c* 1.9); IR 1705, 1473, 1087; ^{1}H NMR 0.42 (1H, dd, *J* = 8.2, 4.9), 0.64 (1H, dd, *J* = 4.9, 3.8), 0.70 (3H, s), 0.97 (3H, d, *J* = 6.6), 1.01 (3H, s), 1.29 (3H, d, *J* = 6.6), 2.39 (1H, ddd, *J* = 12.1, 6.7, 6.0), 2.76 (1H, br s), 3.31 (3H, s), 4.39 (1H, dd, *J* = 11.5, 1.9); ^{13}C NMR 12.1 q, 12.7 q, 13.1 t, 17.4 q, 19.3 q, 21.5 d, 22.8 t, 24.1 t, 24.9 t, 27.0 t, 27.7 t, 28.8 t, 30.6 d, 33.4 t, 35.0 t, 35.3 s, 36.4 d, 40.4 t, 40.5 d, 42.6 s, 43.4 s, 47.9 d, 51.7 d, 56.1 d, 56.6 q, 82.4 d, 83.9 d, 174.9 s; HRMS (EI) *m/z* calcd for $C_{28}H_{44}O_3$ (M^+) 428.3290, found 428.3272) and **10b** ($[\alpha]^{25}_D$ +61° (*c* 2.4); IR 1730, 1452, 1081; ^{1}H NMR 0.42 (1H, dd, *J* = 7.7, 4.9), 0.63 (1H, dd, *J* = 5.0, 3.9), 0.71 (3H, s), 0.99 (3H, d, *J* = 6.2), 1.00 (3H, s), 1.21 (3H, d, *J* = 6.6), 2.59 (1H, td, *J* = 8.8, 6.6), 2.76 (1H, t, *J* = 2.8), 3.31 (3H, s), 4.37 (1H, br d, *J* = 9.3); ^{13}C NMR 12.1 q, 12.5 q, 13.1 t, 16.4 q, 19.3 q, 21.5 d, 22.8 t, 24.1 t, 24.2 t, 24.9 t, 25.9 t, 27.8 t, 30.6 d, 33.0 d, 33.4 t, 35.1 t, 35.3 s, 39.8 d, 40.1 t, 42.7 s, 43.4 s, 47.9 d, 51.9 d, 56.2 d, 56.6 q, 79.9 d, 82.4 d, 176.8 s; HRMS (EI) *m/z* calcd for $C_{28}H_{44}O_3$ (M^+) 428.3290, found 428.3251).

Lactone Alcohol 11. Following the procedure used for the preparation of lactone alcohol **5**, 988 mg of **10a** (2.30 mmol) was converted to 935 mg of **11** (98%) using dioxane/H_2O (9:1) (12 mL) and TfOH (1.7 mg, 0.012 mmol) by refluxing for 70 min. Single crystals were obtained by recrystallization from $CHCl_3$ and hexane. Data of **11**: mp 236.5–237.5 °C; $[\alpha]^{25}_D$ −41° (*c* 1.4); IR 1712, 1374, 1077; ^{1}H NMR 0.68 (3H, s), 0.99 (3H, d, *J* = 7.1), 1.00 (3H, s), 1.29 (3H, d, *J* = 7.1), 2.23 (1H, ddd, *J* = 12.8, 11.0, 2.4), 2.30 (1H, dd, *J* = 12.8, 5.5), 2.39 (1H, m), 3.53 (1H, dddd, *J* = 10.7, 10.7, 4.4, 3.8), 4.40 (1H, dd, *J* = 12.6, 2.5), 5.34 (1H, br d, *J* = 5.7); ^{13}C NMR 11.8 q, 12.7 q, 17.4 q, 19.4 q, 21.1 t, 24.2 t, 27.0 t, 27.6 t, 28.7 t, 31.6 t, 31.8 t, 32.0 d, 36.4 d, 36.5 s, 37.2 s, 39.6 t, 40.5 d, 42.2 t, 42.3 t, 50.0 d, 51.5 d, 56.4 d, 71.7 d, 83.9 d, 121.5 d, 140.8 s, 174.9 s; HRMS (EI) *m/z* calcd for $C_{27}H_{42}O_3$ (M^+) 414.3134, found 414.3134.

Hemiacetal 12a. To a stirred solution of **10a** (50 mg, 0.117 mmol) in toluene (2 mL) was added DIBALH (1 N solution in hexanes, 0.233 mL) at −55 °C, and the mixture was stirred for 2 h at −55 °C. The mixture was diluted with ether (10 mL), and MeOH (0.5 mL) was added at −55 °C. After being stirred for 5 min at −55 °C, the mixture was warmed to 25 °C. After addition of H_2O (0.5 mL), the mixture was stirred for 20 min to give a white suspension. After addition of $MgSO_4$ and Celite, the mixture was filtered and concentrated to give 51 mg (100%) of hemiacetal **12a** as a mixture (7:3) of diastereomers at C-26. Data of **12a**: $[\alpha]^{25}_D$ +28° (*c* 2.5); IR 3414, 1460, 1085, 1047; ^{1}H NMR 0.42 (dd, *J* = 8.0, 5.0), 0.64 (dd, *J* = 5.0, 4.0), 0.70 (s), 0.92 (d, *J* = 6.6), 0.96 (d, *J* = 6.6), 1.01 (s), 2.76 (s), 3.32 (s), 3.45 (d, *J* = 11.3), 3.96 (d, *J* = 9.9 hz), 4.23 (m), 5.04 (s); ^{13}C NMR 12.1, 13.0, 13.3, 16.8, 17.1, 19.3, 21.5, 22.8, 24.1, 24.9, 26.1, 27.8, 28.5, 28.9, 30.5, 31.4, 33.3, 34.8, 35.0, 35.2, 35.3, 37.8, 39.9, 40.1, 42.5, 43.3, 47.9, 52.3, 52.5, 56.3, 56.5, 78.4, 82.4, 95.2, 101.8.

Hemiacetal 12b. Following the procedure used for the preparation of **12a**, **10b** (47 mg, 0.110 mmol) was reduced to 47 mg of **12b** (99%) as a mixture (3:2) of diastereomers at C-26 by using DIBALH (1 N solution in hexanes, 0.22 mL). Data of **12b**: $[\alpha]^{25}_D$ +30° (*c* 2.2); IR 3431, 1456, 1086; ^{1}H NMR 0.42 (dd, *J* = 8.0, 5.0), 0.64 (dd, *J* = 5.0, 4.0), 0.70 (s), 0.95 (d, *J* = 6.6), 0.96 (d, *J* = 6.6), 1.01 (s), 1.03 (d, *J* = 6.6), 2.67 (s), 3.31 (s), 3.47 (d, *J* = 9.7), 4.01 (d, *J* = 11.5), 4.72 (s), 4.92 (s); ^{13}C NMR 9.8, 12.1, 13.0, 13.1, 13.2, 16.4, 19.3, 21.5, 22.3, 22.8, 23.0, 24.0, 24.1, 25.0, 27.8, 27.9, 29.2, 29.9, 30.5, 31.3, 32.4, 33.3, 35.0, 35.3, 40.2, 42.5, 43.4, 48.0, 52.3, 52.4, 56.4, 56.6, 70.1, 78.7, 82.4, 96.7, 98.0.

Isomerization of 12b. A mixture of **12b** (33 mg, 0.0766 mmol) and NaOMe (0.024 M in MeOH, 0.32 mL, 0.0077 mmol) in MeOH/THF (1:1) (1 mL) was refluxed for 3 h. After being cooled to 25 °C, the mixture was diluted with ether (30 mL). This was washed with H_2O (30 mL × 2), dried, filtered, and concentrated to give a mixture of **12a** and **12b** (10:1) (33 mg, 100%).

β-Glucoside 15. To a mixture of **5** (6.1 g, 15.2 mmol), TMU (4.83 g, 41.9 mmol), and glucosyl chloride **13** (18.16 g, 38.7 mmol) in CH_2Cl_2 (150 mL) was added TfOH (262 mg, 1.75 mmol). The mixture was stirred for 7 days at 25 °C, poured into saturated aqueous $NaHCO_3$, and extracted with ether. The mixture was purified by method B (acetone/H_2O: 4:1, MeOH, then $CHCl_3$) to give 7.43 g (59%) of β-glucoside **15**: $[\alpha]^{21}_D$ −12° (*c* 1.0); IR 3434, 3063, 3030, 1715, 1127, 741, 700; ^{1}H NMR 0.69 (3H, s), 1.00 (3H, d, *J* = 6.6), 1.01 (3H, s), 3.48 (1H, m; H3), 4.36 (1H, d, *J* = 7.7; H1′); ^{13}C NMR 11.7 q, 12.7 q, 19.0 t, 19.4 q, 21.1 t, 24.2 t, 25.8 t, 27.6 t, 29.6 t, 29.7 t, 31.8 t, 31.9 d, 36.7 s, 37.2 t, 38.9 t, 39.5 t, 40.3 d, 42.2 s, 50.0 d, 51.5 d, 56.4 d, 69.0 t, 73.4 t, 74.7 d, 75.0 t, 75.1 d, 75.1 t, 77.7 d, 79.1 d, 82.8 d, 84.6 d, 101.3 d (J_{C1-H1} = 160), 121.9 d, 127.6–128.4 many d, 138.1 s, 138.2 s, 138.7 s, 140.4 s, 172.3 s; HRMS (FAB) *m/z* calcd for $C_{53}H_{68}O_{13}Na$ (M^+) 855.4824, found 855.4818.

Disaccharide 16. A solution of β-glucoside **15** (1.0 g, 1.20 mmol), rhamnosyl chloride **14** (1.68 g, 3.71 mmol), and α-methylstyrene (1.28 g, 10.8 mmol) in CH_2Cl_2 (20 mL) was refluxed through 4A molecular sieves for 1.5 h to remove moisture. The concentrated mixture was heated at 80 °C for 60 h. The crude product was purified by method B (MeOH, then $CHCl_3$) to give 884 mg (59%) of disaccharide **16** ($[\alpha]^{20}_D$ −4.1° (*c* 1.0); IR 3063, 3030, 1732, 1065, 737, 698; ^{1}H NMR 0.69 (3H, s), 0.89 (3H, s), 1.01 (3H, d, *J* = 6.6), 1.30 (3H, d, *J* = 7.0), 3.44 (1H, m; H3), 5.32 (1H, d, *J* = 1.7; H1″); ^{13}C NMR 11.4 q, 12.3 q, 18.5 q, 18.8 t, 18.9 q, 20.7 t, 23.8 t, 25.3 t, 27.2 t, 29.2 t, 29.2 t, 29.4 t, 31.5 d, 36.3 s, 36.8 t, 38.1 t, 29.2 t, 39.8 d, 41.8 s, 49.6 d, 51.1 d, 56.0 d, 67.5 d, 68.5 t, 71.6 t, 71.8 t, 73.0 t, 74.4 t, 74.4 t, 74.4 t, 74.4 d, 75.1 d, 75.6 d, 78.0 d, 78.3 d, 79.6 d, 80.3 d, 82.3 d, 85.5 d, 97.6 d (J_{C1-H1} = 172), 99.4 d

(J_{C1-H1} = 155), 121.3 d, 126.2–128.2 many d, 137.6 s, 137.8 s, 138.0 s, 138.0 s, 138.3 s, 138.7 s, 140.1 s, 171.7 s; HRMS (FAB) *m/z* calcd for $C_{80}H_{96}O_{12}Na$ (M^+) 1271.6819, found 1271.6810) along with 233 mg (23%) of starting material **15**.

Lactone 17. Following the procedure used for the methylation of **9**, 829 mg of **16** (0.663 mmol) was treated with LDA (0.696 mmol), HMPA (143 mg, 0.796 mmol), THF (6.5 mL), and MeI (99 mg, 0.696 mmol). Crude product was purified by method A (hexane/AcOEt 4:1 → 1:1) to give 325.2 mg (39%) of **17** as a 1:1 mixture of diastereomers along with 221 mg (27%) of starting material **16**. Data of **17**: mp 142–146 °C; IR 3063, 3030, 1736, 1061, 737, 698. Anal. Calcd for $C_{81}H_{98}O_{12}$: C, 76.99; H, 7.82. Found: C, 76.67; H, 8.11.

Hemiacetal 19. Following the procedure used for the preparation of **12a**, lactone **17** (305 mg, 0.242 mmol) was reduced using DIBALH (0.481 mmol) in ether (5 mL) by stirring at −50 °C for 6 h to give crude hemiacetal **18** as a mixture of stereoisomers. A solution of crude product **18** and NaOMe (28% MeOH solution, 0.07 mL) in THF (5 mL) and MeOH (3 mL) was stirred at 25 °C for 8 h. The mixture was neutralized with Amberlite IR-120B, filtered, and concentrated. The residue was purified by method A (hexane/AcOEt: 9:1) to give 241 mg (79%) of hemiacetal **19**: $[\alpha]^{23}_D$ +1.5° (*c* 1.8); IR 3449, 3063, 3030, 2938, 1061, 737, 698; ^{1}H NMR 0.61 (3H, s), 0.82 (3H, s), 0.82 (3H, d, *J* = 6.6), 0.87 (3H, d, *J* = 6.6), 0.92 (3H, d, *J* = 7.2), 1.23 (3H, d, *J* = 6.1); ^{13}C NMR 11.7 q, 13.1 q, 13.3 q, 16.8 q, 17.1 q, 17.8 q, 19.1 q, 21.0 t, 24.2 t, 26.1 t, 27.8 t, 28.5 t, 28.9 t, 29.6 t, 31.4 t, 31.9 d, 31.9 t, 34.9 d, 36.7 s, 37.2 t, 37.8 d, 38.4 t, 39.6 t, 40.0 d, 40.2 d, 42.1 s, 50.1 d, 52.2 d, 52.3 d, 56.6 d, 68.0 d, 68.9 t, 69.6 d, 72.0 t, 72.2 t, 73.4 t, 74.8 t, 74.9 t, 75.4 d, 76.0 d, 78.3 d, 78.8 d, 80.0 d, 80.6 d, 85.9 d, 95.2 d, 98.0 d, 99.8 d, 101.8 d, 121.7 d, 126.5 d, 126.9–128.4 many d, 137.7 s, 138.1 s, 138.4 s, 138.4 s, 138.7 s, 139.1 s, 140.5 s.

Trisaccharide 20. A solution of **19** (140 mg, 0.111 mmol), rhamnosyl chloride **14** (149 mg, 0.33 mmol), and TMU (71 mg, 0.61 mmol) in CH_2Cl_2 (10 mL) was refluxed through 4A molecular sieves for 1 h to remove moisture. To the cooled mixture was added AgOTf (141 mg, 0.55 mmol), and this was stirred for 3 h at 25 °C. One drop of H_2O was added and stirred for an additional 30 min at 25 °C to hydrolyze excess rhamnosyl chloride. The mixture was filtered, poured into saturated aqueous $NaHCO_3$ solution, and extracted with CH_2Cl_2. The extract was purified by method B (MeOH/acetone 10:1 → 5:1, then $CHCl_3$), followed by method A (hexane/AcOEt 10:1 → 4:1) to give 101.2 mg (55%) of trisaccharide **20**: mp 71–73 °C; $[\alpha]_D^{19}$ −18° (*c* 2.5); IR 3063, 3030, 1454, 1059, 735, 698; ^{1}H NMR 0.69 (3H, s), 0.87 (3H, d, *J* = 6.6), 0.89 (3H, s), 0.99 (3H, d, *J* = 6.6), 1.30 (3H, d, *J* = 6.6), 1.33 (3H, d, *J* = 6.1), 4.23 (1H, d, *J* = 8.6; H26), 4.39 (1H, d, *J* = 8.0; H1′), 5.26 (1H, br s; H6), 5.32 (1H, d, *J* = 2.5; 1″), 5.34 (1H, d, *J* = 2.7; 1‴); ^{13}C NMR 11.7 q, 13.3 q, 16.7 q, 17.8 q, 18.0 q, 19.1 q, 21.0 t, 24.2 t, 27.8 t, 28.3 t, 29.6 t, 31.5 t, 31.9 t, 31.9 d, 35.5 d, 36.6 s, 37.2 t, 38.4 t, 39.9 t, 40.1 d, 42.1 s, 50.1 d, 52.8 d, 56.8 d, 67.9 d, 68.6 d, 68.9 t, 71.8 t, 72.0 t, 72.1 t, 72.3 t, 73.4 t, 74.5 d, 74.8 t, 74.8 d, 74.9 t, 74.9 t, 75.3 d, 75.5 t, 75.9 d, 78.0 d, 78.3 d, 78.7 d, 80.0 d, 80.0 d, 80.3 d, 80.6 d, 85.9 d, 93.7 d (J_{C1-H1} = 170), 98.0 d (J_{C1-H1} = 171), 99.8 d (J_{C1-H1} = 158), 102.8 d (J_{C1-H1} = 157), 121.8 d, 126.5–128.4 many d, 137.7 s, 138.1 s, 138.2 s, 138.4 s, 138.4 s, 138.5 s, 138.5 s, 138.7 s, 139.1 s, 140.3 s. Anal. Calcd for $C_{108}H_{128}O_{16}$: C, 77.11; H, 7.67. Found: C, 76.78; H, 7.69.

6-Keto Trisaccharide 21. To a stirred solution of **20** (100 mg, 0.059 mmol) in THF (2 mL) was added a solution of BH_3 in THF (1 N, 0.6 mL), and the resulting solution was stirred for 8 h at 25 °C. H_2O (0.4 mL), 30% aqueous NaOH (1.4 mL), and 30% aqueous H_2O_2 (1.4 mL) were added successively, and the mixture was stirred for an additional 1.5 h at 25 °C. After addition of brine, the mixture was extracted with ether. The concentrated extract was dissolved in CH_2Cl_2 (5 mL) and PDC (110 mg) was added. After being refluxed for 1 h, the cooled mixture was diluted with ether (80 mL). Celite was added to the mixture, and this was stirred for an additional 10 min. The mixture was filtered, concentrated, and purified by method A (hexane/AcOEt 6:1 → 4:1) to give 70.4 mg (70%) of ketone **21**: mp 68–71 °C; $[\alpha]^{19}_D$ −14° (*c* 3.1); IR 3063, 3030, 1709, 1454, 1061, 736, 698; ^{1}H NMR 0.48 (3H, s), 0.65 (3H, s), 0.87 (3H, d, *J* = 6.6), 0.99 (3H, d, *J* = 6.6), 1.29 (3H, d, *J* = 6.0), 1.32 (3H, d, *J* = 6.1), 2.25 (1H, dd, *J* = 13.2, 4.4; H5), 4.23 (1H, d, *J* = 8.3; H26), 4.48 (1H, d, *J* = 7.7, H1′), 5.29 (1H, d, *J* = 1.7; 1″), 5.34 (1H, d, *J* = 1.7, 1‴); ^{13}C NMR 11.8 q, 12.7 q, 13.3 q, 16.6 q, 17.9 q, 18.1 q, 21.4 t, 23.9 t, 25.5 t, 27.6 t, 28.3 t, 28.4 t, 31.5 t, 35.5 d, 36.6 t, 37.6 d, 39.7 t, 40.1 d, 40.9 s, 42.6 s, 46.5 t, 52.8 d, 53.7 d, 56.3 d, 56.7 d, 68.0 d, 68.7 d, 69.0 t, 71.9 t, 72.3 t, 72.5 t, 72.6 t, 73.4 t, 74.8 t, 74.9 t, 74.9 d, 74.9 t, 75.2 d, 75.4 t, 75.7 d, 75.9 d, 75.9 d, 77.9 d, 78.3 d, 80.0 d, 80.3 d, 80.4 d, 80.6 d, 86.0 d, 93.8 d (J_{C1-H1} = 169), 98.1 d (J_{C1-H1} = 172), 98.3 d (J_{C1-H1} = 157), 102.8 d (J_{C1-H1} = 157), 126.6–128.4 many d, 137.8 s, 138.1 s, 138.4 s, 138.5 s, 138.6 s, 138.6 s, 139.0 s, 139.2 s, 210.7 s. Anal. Calcd for $C_{108}H_{128}O_{17}$·1H_2O: C, 75.58; H, 7.63. Found: C, 75.58; H, 7.79.

Compound 1. A mixture of **22** (26.5 mg, 0.0156 mmol) and 20% $Pd(OH)_2$ on C (2.4 mg, 0.48 mg as $Pd(OH)_2$, 0.00312 mmol) in MeOH/AcOEt/H_2O (12:2:1) (2 mL) was stirred for 24 h at 25 °C under H_2 (1 atm). The mixture was filtered and concentrated to give 13.6 mg (98%) of **1**: $[\alpha]^{20}_D$ −44° (*c* 0.3, MeOH); ^{1}H NMR (C_5D_5N) 0.31 (3H, s), 0.58 (3H, s), 0.77 (3H, d, *J* = 6.1), 0.83 (3H, d, *J* = 6.6), 1.49 (3H, d, *J* = 6.5), 1.58 (3H, d, *J* = 6.1), 5.75 (1H, s; 1″), 6.14 (1H, s; 1‴); ^{13}C NMR (C_5D_5N) 12.3 q, 13.3 q, 13.9 q, 16.9 q, 18.6 q, 18.6 q, 21.9 t, 24.3 t, 27.0 t, 27.8 t, 29.0 t, 29.7 t, 32.1 t, 36.3 d, 37.2 t, 38.2 d, 40.1 s, 40.4 d, 41.2 s, 43.2 t, 46.9 t, 53.5 d, 54.1 d, 56.7 d, 56.9 d, 63.3 t, 69.5 d, 70.4 d, 72.5 d, 72.5 d, 72.7 d, 72.9 d, 73.0 d, 74.1 d, 74.5 d, 77.1 d, 78.0 d, 78.4 d, 78.6 d, 79.5 d, 97.3 d (J_{C1-H1} = 169), 100.3 d (J_{C1-H1} = 157), 102.1 d (J_{C1-H1} = 173), 102.6 d (J_{C1-H1} = 154), 209.4 s; HRMS (FAB) *m/z* calcd for $C_{45}H_{74}O_{17}Na$ (M^+) 909.4824, found 909.4808.

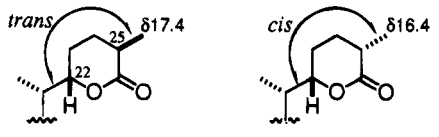
Isolation of Natural Osladin (2). Dried and powdered rhizomes of *P. vulgare* L. (1 kg) were successively extracted with hexane, CH_2Cl_2, AcOEt, and EtOH to give extracts of 77.7 g, 15.8 g, 9.61 g, and 36.3 g, respectively. The EtOH extract (30 g) was separated by method A ($CHCl_3$/MeOH 6:1 → 4:1 → 3:1 → 2:1 → 0:1) to give a sweet fraction (4.99 g). The sweet fraction was successively purified by column chromatography on sephadex LH-20 (2 × 30 cm), eluting with MeOH/$CHCl_3$ (4:1), to give 2.84 g of the sweet fraction, by method A ($CHCl_3$/MeOH: 4:1) to give 1.95 g of the sweet fraction, and by method B (MeOH/H_2O 1:1 → 2:1) to give 253 mg of the sweet fraction. The sweet fraction was purified by method D (MeOH/H_2O 20:7) to give crude osladin. Repeated recrystallization from aqueous acetone afforded 211 mg of pure osladin (**2**) as colorless needles: mp 202–204 °C; $[\alpha]^{19}_D$ −36° (*c* 2.0, EtOH); IR 3381, 1709, 1068; ^{1}H NMR (C_5D_5N) 0.56 (3H, s), 0.78 (3H, s), 0.92 (3H, d, *J* = 6.6), 1.03 (3H, d, *J* = 6.6), 1.71 (3H, d, *J* = 6.3), 1.80 (3H, d, *J* = 6.1), 2.37 (1H, dd, *J* = 12.7, 4.4), 2.49 (1H, br d, *J* = 13.2), 3.46 (1H, m), 3.92 (1H, ddd, *J* = 8.9, 5.9, 1.7), 4.00 (1H, ddd, *J* = 18.6, 10.8, 4.2), 4.14 (1H, m), 4.25–4.29 (2H), 4.31–4.39 (3H), 4.48 (1H, d, *J* = 8.4), 4.55–4.58 (2H), 4.63 (1H, br s), 4.65–4.69 (2H), 4.80 (1H, br s), 4.98 (1H, qd, *J* = 6.3, 3.2), 5.08 (1H, d, *J* = 6.6), 5.67 (1H, s), 6.35 (1H, s); ^{13}C NMR (C_5D_5N) 11.9 q, 13.1 q, 13.7 q, 16.7 q, 18.4 q, 18.7 q, 21.6 t, 23.9 t, 24.1 t, 26.5 t, 27.3 t, 29.4 t, 31.5 t, 36.5 d, 36.8 t, 37.8 d, 39.7 t, 40.1 d, 40.9 s, 43.2 s, 46.8 t, 52.8 d, 53.8 d, 56.3 d, 56.4 d, 62.8 t, 69.5 d, 70.5 d, 72.0 d, 72.2 d, 72.5 d, 72.8 d, 72.8 d, 74.0 d, 74.1 d, 76.2 d, 78.2 d, 78.3 d, 78.3 d, 79.5 d, 99.6 d (*J* = 155), 101.9 d (*J* = 166), 102.1 d (*J* = 173), 107.3 d (*J* = 157), 209.6 s; HRMS (FAB) *m/z* calcd for $C_{45}H_{74}O_{17}Na$ (M^+) 909.4823, found 909.4829.

C-22 *R* Alcohol 8. To a stirred solution of **7** (158 mg, 0.381 mmol) in pyridine (1.5 mL) was dropwise added CH_3SO_2Cl (87.3 mg, 0.762 mmol) at 0 °C. The mixture was stirred for 30 min at 0 °C and an additional 1 h at 25 °C. This reaction mixture was poured into 2 N HCl and extracted with ether. The organic layer was successively washed with 2 N HCl and saturated aqueous $NaHCO_3$ and then concentrated to give 193.8 mg (100%) of crude mesylate **28**. To a solution of **28** (80 mg, 0.162 mmol) and 18-crown-6 (129 mg, 0.487 mmol) in DMSO/DME (1:1) (2 mL) was added KO_2 (34.6 mg, 0.487 mmol) in one portion at 0 °C, and the mixture was stirred for 30 min at 0 °C. The reaction mixture was poured into brine and extracted with CH_2Cl_2. The mixture was purified by method A (hexane/AcOEt 10:1) to give 59 mg (88%) of C-22 *R*

alcohol **8**. Every spectral feature (^{1}H and ^{13}C NMR, high- and low-resolution EI mass spectra, IR, optical rotation) was identical with the minor product of the Grignard reaction of aldehyde **6**.

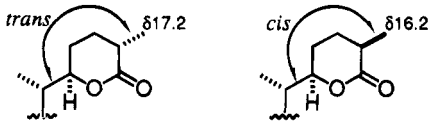
Lactone 29. Following the procedure used for the preparation of **9**, 841 mg of **8** (2.03 mmol) was converted to 680 mg of **29** (81%) using Sudan III (0.1% CH_2Cl_2 solution, 0.1 mL), EtOH/H_2O (10:1) (11 mL), and Me_2S (1.5 mL) for ozone oxidation and PDC (1.02 g, 2.71 mmol) and CH_2Cl_2 (20 mL) by refluxing for 4 h for oxidation of hemiacetal. Data of **29**: $[\alpha]^{21}_D$ +42° (*c* 3.2); IR 1736, 1240, 1098, 1024, 754; ^{1}H NMR 0.44 (1H, dd, *J* = 8.3, 5.5), 0.66 (1H, t, *J* = 3.4), 0.75 (3H, s), 0.96 (3H, d, *J* = 6.6), 1.03 (3H, s), 2.40 (1H, ddd, *J* = 17.6, 9.4, 7.2), 2.61 (1H, ddd, *J* = 18.2, 7.2, 3.9), 2.78 (1H, br s), 3.33 (3H, s), 4.36 (1H, dt, *J* = 11.5, 3.6); ^{13}C NMR 12.1 q, 12.8 q, 13.0 t, 18.9 t, 19.2 q, 21.0 t, 21.4 d, 22.7 t, 24.2 t, 24.9 t, 27.3 t, 29.7 t, 30.5 d, 33.3 t, 35.0 t, 35.2 s, 39.4 d, 40.1 t, 43.2 s, 43.3 s, 47.9 d, 52.3 d, 56.1 d, 56.5 q, 82.2 d, 83.1 d, 172.2 s; HRMS (EI) *m/z* calcd for $C_{27}H_{42}O_2$ (M^+) 414.3134, found 414.3121.

C-25 Methyl Lactone 30. Following the procedure used for the preparation of **10a,b**, lactone **29** (672 mg, 1.50 mmol) was methylated using LDA (1.5 M cyclohexane solution, 1.0 mL), HMPA (323 mg, 1.8 mmol), and MeI (224 mg, 1.58 mmol) in THF (8 mL). The crude product was purified by method A (hexane/AcOEt 6:1 → 3:1) to give 578 mg (90%) of lactone **30** as a 1:1 mixture of diastereomers. Analytical samples were obtained by method C (hexane/AcOEt 12:1) to give C-25(*S*)-**30** ($[\alpha]^{26}_D$ +41° (*c* 1.1); IR 1732, 1186, 754; ^{1}H NMR 0.43 (1H, dd, *J* = 8.1, 5.2), 0.65 (1H, dd, *J* = 5.2, 4.1), 0.74 (3H, s), 0.94 (3H, d, *J* = 6.8), 1.02 (3H, s), 1.29 (3H, d, *J* = 6.7), 2.39 (1H, m), 2.77 (1H, t, *J* = 2.7), 3.32 (3H, s), 4.38 (1H, dt, *J* = 10.8, 3.7); ^{13}C NMR 12.0 q, 12.8 q, 13.0 t, 17.2 q, 19.2 q, 21.4 d, 22.2 t, 22.7 t, 24.2 t, 24.9 t, 27.3 t, 28.5 t, 30.4 d, 33.3 t, 35.0 t, 35.2 s, 36.4 d, 39.5 d, 40.1 t, 43.1 s, 43.3 s, 47.9 d, 52.2 d, 56.0 d, 56.5 q, 82.2 d, 84.2 d, 174.8 s; HRMS (EI) *m/z* calcd for $C_{28}H_{44}O_3$ (M^+) 428.3290, found 428.3284) and C-25(*R*)-**30** ($[\alpha]^{26}_D$ +23° (*c* 1.0); IR 1742, 1098, 1082, 754; ^{1}H NMR 0.44 (1H, dd, *J* = 8.1, 5.4), 0.66 (1H, dd, *J* = 5.4, 3.7), 0.75 (3H, s), 0.97 (3H, d, *J* = 6.6), 1.03 (3H, s), 1.22 (3H, d, *J* = 6.8), 2.61 (1H, m), 2.77 (1H, t, *J* = 2.9), 3.32 (3H, s), 4.34 (1H, ddd, *J* = 9.0, 5.7, 3.3); ^{13}C NMR 12.0 q, 12.7 q, 13.0 t, 16.2 q, 19.2 q, 19.2 t, 21.4 d, 22.6 t, 24.1 t, 24.9 t, 25.6 t, 27.4 t, 30.4 d, 33.0 d, 33.3 t, 35.0 t, 35.1 s, 38.9 d, 40.1 t, 43.1 s, 43.3 s, 47.9 d, 52.2 d, 56.0 d, 56.5 q, 80.0 d, 82.2 d, 176.5 s; HRMS (EI) *m/z* calcd for $C_{28}H_{44}O_3$ (M^+) 428.3291, found 428.3293). The configuration at C-25 of **30** was tentatively assigned by comparing the ^{13}C NMR chemical shifts of **30** with those of **10a** and **10b**. The structures of **10a** and **10b** were established by X-ray analysis of **11**.



10a

10b



C-25 (*S*)-**30**

C-25 (*R*)-**30**

Lactone Alcohol 31. Following the procedure used for the preparation of lactone alcohol **5**, 530 mg of **30** (1.24 mmol) was converted to 414.8 mg of **31** (81%) using dioxane/H_2O (9:1) (6 mL) and TfOH (0.93 mg, 0.012 mmol) by refluxing for 2 h. Analytical samples were obtained by the same operation of pure C-25(*S*)-**30** and C-25(*R*)-**30**, respectively. C-25(*S*)-**31**: $[\alpha]^{23}_D$ −30° (*c* 0.9); FT IR 3412, 1726, 756; ^{1}H NMR 0.70 (3H, s), 0.95 (3H, d, *J* = 6.6), 1.01 (3H, s), 1.29 (3H, d, *J* = 7.2), 2.24 (1H, dddd, *J* = 13.0, 10.9, 5.2, 2.6), 2.30 (1H, ddd, *J* = 13.5, 5.0, 2.4), 2.39 (1H, dqd, *J* = 6.9, 6.9, 5.8), 3.52 (1H, dddd, *J* = 11.1, 11.1, 4.5, 4.5), 4.38 (1H, dt, *J* = 11.6, 3.3), 5.35 (1H, br d, *J* = 5.0); ^{13}C NMR 11.7 q, 12.9 q, 17.3 q, 19.4 q, 21.0 t, 22.3 t, 24.3 t, 27.3 t, 28.6 t, 31.6 t, 31.9 t, 31.9 d, 36.5 s, 36.5 d, 37.3 t, 39.6 d, 39.7 t, 42.3 t, 42.8 s, 50.1 d, 52.1 d, 56.4 d, 71.7 d, 84.3 d, 121.4 d, 140.9 s, 174.9 s; HRMS (EI) *m/z* calcd for $C_{27}H_{42}O_3$ (M^+) 414.3134, found 414.3130. C-25(*R*)-**31**: $[\alpha]^{23}_D$ −48° (*c* 1.3); IR 3382, 1742, 756; ^{1}H NMR 0.71 (3H, s), 0.98 (3H, d, *J* = 6.6), 1.01 (3H, s), 1.22 (3H, d, *J* = 6.6), 2.24 (1H, dddd, *J* = 13.6, 11.6, 5.1, 2.7), 2.30 (1H, ddd, *J* = 12.8, 5.3, 1.9), 2.61 (1H, ddq, *J* = 9.7, 7.5, 7.5), 3.52 (1H, dddd, *J* = 11.4, 11.4, 4.6, 4.6), 3.52 (1H, dt, *J* = 11.0, 4.6), 5.35 (1H, br d, *J* = 5.5); ^{13}C NMR 11.7 q, 12.7 q, 16.3 q, 19.2 t, 19.3 q, 21.0 t, 24.3 t, 25.6 t, 27.3 t, 31.5 t, 31.8 t, 31.8 d, 33.0 d, 36.4 s, 37.2 t, 38.9 d, 39.6 t, 42.1 t, 42.7 s, 50.0 d, 52.1 d, 56.3 d, 71.6 d, 80.1 d, 121.3 d, 140.8 s, 176.7 s; HRMS (EI) *m/z* calcd for $C_{27}H_{42}O_3$ (M^+) 414.3134, found 414.3128.

β-Glucoside 32. A solution of **31** (666.3 mg, 1.607 mmol), glucosyl chloride **13** (1.13 g, 2.41 mmol), and TMU (299 mg, 2.57 mmol) in 1,2-dichloroethane (20 mL) was refluxed through 4 A molecular sieves to remove moisture in the reaction mixture. To the cooled mixture was added TfOH (24.2 mg, 0.161 mmol), and the mixture was refluxed for 4 h. To this was added H_2O (0.2 mL) and THF (5 mL), and the mixture was refluxed for an additional 15 min. The concentrated mixture was purified by method A (hexane/AcOEt 4:1) to give a mixture of α- and β-glucosides. Method C (hexane/AcOEt 4:1) afforded 779 mg (57%) of β-glucoside **32** and 139 mg (10%) of α-glucoside. Analytical samples were prepared from pure C-25 *S* and C-25 *R* isomers of **31**, respectively. C-25(*S*)-**32**: $[\alpha]^{22}_D$ −17° (*c* 0.7); IR 3461, 3063, 3030, 1732, 1090, 1067, 737, 698; ^{1}H NMR 0.70 (3H, s), 0.95 (3H, d, *J* = 6.6), 1.01 (3H, s), 1.30 (3H, d, *J* = 7.0), 4.35 (1H, d, *J* = 7.2; H1′), 5.35 (1H, br d, *J* = 5.5; H6); ^{13}C NMR 11.7 q, 12.9 q, 17.3 q, 19.4 q, 21.0 t, 22.3 t, 24.3 t, 27.3 t, 28.6 t, 29.7 t, 31.9 d, 31.9 t, 36.5 d, 36.7 s, 37.3 t, 38.9 t, 39.6 d, 39.7 t, 42.8 s, 50.1 d, 52.1 d, 56.4 d, 69.1 t, 73.4 t, 74.7 d, 75.0 t, 75.1 t, 75.1 d, 77.7 d, 79.2 d, 84.3 d, 84.6 d, 101.3 d (J_{C1-H1} = 158), 121.8 d, 127.6−128.4 many d, 138.1 s, 138.2 s, 138.7 s, 140.5 s, 174.9 s; HRMS (FAB) *m/z* calcd for $C_{54}H_{70}O_8Na$ (M^+) 869.4968, found 869.4926. C-25-(*R*)-**32**: $[\alpha]^{21}_D$ −26° (*c* 1.2); IR 3443, 3063, 3030, 1742, 1067, 752, 698; ^{1}H NMR 0.71 (3H, s), 0.98 (3H, d, *J* = 6.6), 1.01 (3H, s), 1.22 (3H, d, *J* = 6.6), 4.35 (1H, d, *J* = 7.7; H1′), 5.35 (1H, br d, *J* = 5.0; H6); ^{13}C NMR 11.7 q, 12.8 q, 16.4 q, 19.3 t, 19.4 q, 21.0 t, 24.3 t, 25.7 t, 27.4 t, 29.7 t, 31.9 t, 31.9 d, 33.1 d, 36.7 s, 37.3 t, 38.9 t, 39.0 d, 39.7 t, 42.8 s, 50.1 d, 52.2 d, 56.4 d, 69.1 t, 73.4 t, 74.9 d, 75.0 t, 75.1 t, 75.2 d, 77.7 d, 79.1 d, 80.1 d, 84.6 d, 101.3 d (J_{C1-H1} = 158), 121.8 d, 127.6−128.4 many d, 138.1 s, 138.2 s, 138.7 s, 140.5 s, 176.6 s.

Disaccharide 33. A solution of β-glucoside **32** (109.3 mg, 0.129 mmol), rhamnosyl chloride **14** (116.9 mg, 0.258 mmol), and TMU (33 mg, 0.284 mmol) in CH_2Cl_2 (10 mL) was refluxed through 4A molecular sieves for 1 h to remove moisture of the reaction mixture. After removal of the solvent, the residue was heated at 80 °C for 40 h. **14** (50 mg, 0.11 mmol) and TMU (16.5 mg, 0.14 mmol) were added to the reaction mixture and it was heated for an additional 16 h at 80 °C. The mixture was purified by method A (hexane/AcOEt: 6:1), followed by method B (MeOH), to give 131.9 mg (81%) of disaccharide **33** as a mixture of diastereomers at C-25: IR 3063, 3030, 1738, 1061, 750, 698; ^{1}H NMR 0.71 (s), 0.72 (s), 0.89 (s), 0.95 (d, *J* = 6.6), 0.99 (d, *J* = 6.6), 1.22 (d, *J* = 6.6), 1.30 (*J* = 6.6), 1.31 (d, *J* = 6.6); ^{13}C NMR 11.6 q, 12.7 q, 12.8 q, 16.3 q, 17.2 q, 17.8 q, 19.1 q, 19.2 t, 20.9 t, 22.2 t, 24.3 t, 25.5 t, 27.3 t, 28.6 t, 29.5 t, 31.8 d, 31.8 t, 33.0 d, 36.4 d, 36.6 s, 37.2 t, 38.3 t, 38.9 d, 39.6 t, 42.7 s, 50.0 d, 52.0 d, 56.3 d, 67.9 d, 68.9 t, 71.9 t, 72.1 t, 73.4 t, 74.7 d, 74.7 t, 74.9 t, 74.9 t, 75.2 d, 75.9 d, 78.3 d, 78.6 d, 80.0 d, 80.0 d, 80.5 d, 84.2 d, 85.9 d, 98.0 d, 99.7 d, 121.4 d, 126.5 d, 126.8−128.4 many d, 137.7 s, 138.1 s, 138.3 s, 138.3 s, 138.6 s, 139.0 s, 140.5 s, 174.9 s, 176.6 s; HRMS (FAB) *m/z* calcd for $C_{81}H_{98}O_{12}Na$ (M^+) 1285.6880, found 1285.6920.

Hemiacetal 35. To a solution of **33** (117 mg, 0.095 mmol) in ether (3 mL) was added DIBALH (1.0 M hexane solution, 189 μL, 0.189 mmol) at −60 °C, and the mixture was stirred for 10 h at −60 °C. The reaction was quenched by addition of

2 N HCl. AcOEt extract was washed with saturated aqueous $KHCO_3$, dried, and concentrated to give crude **34**: ^{13}C NMR 96.7, 97.4, 98.0, 99.7 for anomeric carbon. A solution of the crude **34** and NaOMe (0.51 mg, 0.0095 mmol) in THF (2 mL) and MeOH (4 mL) was refluxed for 6 h. The cooled mixture was acidified by the addition of 2 N HCl and extracted with AcOEt. The mixture was purified by method A (hexane/AcOEt 5:1) to give 95.5 mg (81%) of hemiacetal **35**: $[\alpha]^{22}_D$ −4.8° (*c* 2.8); IR 3443, 3063, 3030, 1061, 735, 698; ^1H NMR 0.71 (3H, s), 0.89 (3H, s), 0.96 (3H, d, *J* = 6.1), 0.99 (3H, d, *J* = 7.2), 1.31 (3H, d, *J* = 6.1), 4.37 (1H, d, *J* = 7.3; H26), 4.38 (1H, d, *J* = 7.8; H1), 5.26 (1H, br d, *J* = 4.8; H6), 5.31 (1H, d, *J* = 2.6; H1″); ^{13}C NMR 11.7, 13.5, 16.7, 17.8, 19.1, 20.9, 23.5, 27.5, 29.5, 31.1, 31.8, 31.8, 36.6, 37.2, 37.7, 38.3, 39.6, 39.8, 42.5, 50.1, 52.6, 56.4, 67.9, 68.9, 71.9, 72.1, 73.4, 74.7, 74.7, 74.8, 74.8, 75.3, 75.9, 77.2, 78.3, 78.4, 78.6, 79.9, 80.5, 85.9, 98.8, 99.7, 101.3, 121.6, 126.5−128.4 many peaks, 137.6, 138.0, 138.3, 138.3, 138.7, 139.0, 140.5; HRMS (FAB) *m/z* calcd for $C_{81}H_{100}O_{12}Na$ (M^+) 1287.7029, found 1287.7070.

Trisaccharide 36. A solution of hemiacetal **35** (87.7 mg, 0.0693 mmol), rhamnosyl chloride **14** (161 mg, 0.355 mmol), and TMU (48 mg, 0.416 mmol) in CH_2Cl_2 (6 mL) was refluxed through 4A molecular sieves for 1 h. The cooled mixture was added to a suspension of AgOTf (89 mg, 0.347 mmol) in CH_2Cl_2 (1 mL), and the resulting mixture was stirred for 10 min at 25 °C under dark. The mixture was filtered, poured into a saturated aqueous $NaHCO_3$ solution, and extracted with $CHCl_3$. The mixture was purified by method B (MeOH/acetone 10:1 → 3:1), followed by method A (hexane/AcOEt 8:1 → 3:1), to give 70.7 mg (61%) of trisaccharide **36**: $[\alpha]^{21}_D$ −4.9° (*c* 3.5); IR 3063, 3030, 1497, 1059, 737, 698; ^1H NMR 0.70 (3H, s), 0.76 (3H, d, *J* = 6.1), 0.89 (3H, s), 0.95 (3H, d, *J* = 6.6), 1.30 (3H, d, *J* = 6.1), 1.31 (3H, d, *J* = 6.1), 4.08 (1H, d, *J* = 8.6; H26), 4.39 (1H, d, *J* = 7.8; H1′), 5.05 (1H, d, *J* = 2.6; H1‴), 5.27 (1H, br d, *J* = 5.4; H6), 5.32 (1H, d, *J* = 2.9; H1″); ^{13}C NMR 11.7 q, 13.5 q, 16.4 q, 17.7 q, 17.9 q, 19.1 q, 21.0 t, 23.4 t, 24.4 t, 27.4 t, 29.6 t, 31.2 t, 31.9 d, 31.9 t, 35.6 d, 36.7 s, 37.2 t, 38.4 t, 39.7 t, 39.9 d, 42.5 s, 50.2 d, 52.7 d, 56.4 d, 68.0 d, 68.5 d, 69.0 t, 72.0 t, 72.2 t, 72.2 t, 72.6 t, 73.4 t, 73.4 t, 74.8 d, 74.8 t, 74.9 t, 75.0 d, 75.3 t, 75.4 d, 76.0 d, 78.3 d, 78.4 d, 78.7 d, 80.0 d, 80.0 d, 80.6 d, 80.6 d, 85.9 d, 97.1 d (J_{C1-H1} = 166), 98.1 d (J_{C1-H1} = 174), 99.8 d (J_{C1-H1} = 156), 106.7 d (J_{C1-H1} = 153), 121.6 d, 126.5−128.4 many d, 137.8 s, 138.1 s, 138.3 s, 138.4 s, 138.4 s, 138.7 s, 138.7 s, 138.7 s, 139.1 s, 140.6 s; MS (FAB) *m/z* 1705 (M^+ + Na).

Nonabenzylosladin (37). To a solution of **36** (66 mg, 0.0392 mmol) in THF (2 mL) was added a BH_3·THF solution (1 N, 0.6 mL), and the mixture was stirred for 8 h at 25 °C. To this were successively added H_2O (0.4 mL), 30% aqueous NaOH (1.5 mL), and 30% H_2O_2 (1.5 mL). The mixture was stirred for an additional 1.5 h at 25 °C, poured into brine, and extracted with ether to give crude alcohol. A mixture of crude alcohol and PDC (74 mg, 0.196 mmol) in CH_2Cl_2 (4 mL) was refluxed for 1 h. After dilution with ether (30 mL), the mixture was stirred with Celite (2 g) for 5 min. This mixture was filtered, concentrated, and purified by method A (hexane/AcOEt 6:1 → 4:1) to give 40.7 mg (61%) of ketone **37**: $[\alpha]^{21}_D$ −6.4° (*c* 2.0); IR 3063, 3030, 1711, 1454, 1059, 737, 698; ^1H NMR 0.50 (3H, s), 0.70 (3H, s), 0.79 (3H, d, *J* = 6.6), 0.97 (3H, d, *J* = 6.6), 1.32 (3H, d, *J* = 6.1), 1.33 (3H, d, *J* = 6.1), 2.32 (1H, dd, *J* = 13.2, 4.4; H5), 4.10 (1H, d, *J* = 8.7; H26), 4.51 (1H, d, *J* = 7.9; H1′), 5.06 (1H, d, *J* = 3.0; H1‴), 5.32 (1H, d, *J* = 2.9; H1″); ^{13}C NMR 11.8 q, 12.7 q, 13.5 q, 16.4 q, 17.7 q, 17.9 q, 21.4 t, 23.5 t, 24.1 t, 25.5 t, 27.2 t, 28.4 t, 29.7 d, 31.2 t, 35.7 d, 36.6 t, 37.6 d, 39.5 t, 39.8 d, 40.9 s, 43.2 s, 46.6 t, 52.7 d, 53.8 d, 56.4 d, 68.0 d, 68.6 d, 69.0 t, 72.2 t, 72.3 t, 72.5 t, 72.6 t, 73.4 t, 73.4 t, 74.9 t, 74.9 d, 75.0 t, 75.0 d, 75.4 t, 75.6 d, 75.7 d, 75.8 d, 78.2 d, 78.3 d, 80.0 d, 80.4 d, 80.6 d, 80.6 d, 86.0 d, 97.3 d (J_{C1-H1} = 169), 98.1 d (J_{C1-H1} = 173), 98.2 d (J_{C1-H1} = 158), 106.8 d (J_{C1-H1} = 156), 126.7−128.4 many d, 137.7 s, 138.1 s, 138.3 s, 138.4 s, 138.5 s, 138.7 s, 138.7 s, 139.0 s, 139.2 s, 211.0 s.

Synthetic Osladin (2). A mixture of nonabenzylosladin (**37**) (35 mg, 0.0206 mmol) and 20% $Pd(OH)_2$ on C (29 mg, 5.9 mg as $Pd(OH)_2$, 0.042 mmol) in MeOH/AcOEt/H_2O (12:2:1, 2 mL) was stirred under H_2 (1 atm) for 36 h at 25 °C. The mixture was filtered, concentrated, and purified by method A ($CHCl_3$/MeOH 4:1) to give 14 mg (78%) of osladin (**2**). Every spectral feature (^1H and ^{13}C NMR, high- and low-resolution FAB mass spectra, IR, optical rotation) was identical with those of natural osladin.

Acknowledgment. We are very grateful to Professor Y. Asakawa of Tokushima Bunri University and Professor H. Becker of Universität des Saarlandes for collecting the fern *P. vulgare*. We also thank Dr. C. Katayama for carrying out the X-ray diffraction study of osladin (**2**), Mr. S. Takaoka of our Institute for X-ray analysis of compound **11**, Dr. U. Diederichsen and Mr. D. Christen of the University of Pittsburgh for suggestions regarding presentation, and Dr. K. Izawa of Ajinomoto Co. Ltd for the determination of the sweetness intensity of **2**. This investigation was supported by financial aid from Ono Pharmaceutical Co. Ltd.

Supplementary Material Available: ^1H and ^{13}C NMR spectra of compounds **1**, **2**, **5**, **7**, **8**, **9**, **10a**, **10b**, **11**, **12a**, **12b**, **15**, **16**, **17**, **19**, **20**, **21**, **29**, C-25(*S*)-**30**, C-25(*R*)-**30**, C-25(*S*)-**31**, C-25(*R*)-**31**, C-25(*S*)-**32**, C-25(*R*)-**32**, **33**, **35**, **36**, and **37** (56 pages). This material is contained in libraries on microfilm, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

JO9405720