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Synthesis and structure revision of intensely sweet saponin, osladin

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Abstract

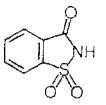
The total synthesis of compound **10**, which is the reported structure of intensely sweet saponin osladin, has been completed. However, it is not as sweet as suggested. Re-extraction of the sweet principle of rhizomes of the fern *Polypodium vulgare* (Polypodiaceae) and single crystal X-ray diffraction study revealed its real structure to be **27**. We also found it to be only 500 times sweeter than sucrose as against 3,000 times suggested elsewhere. Therefore, the total synthesis of the real osladin was achieved from steroidal aldehyde **20** by using newly developed β-selective and 2' hydroxyl group-discriminated glucosylation procedure and our original α-selective thermal rhamnosylation reaction. Synthetic osladin was also very sweet and thus we prove that osladin is the real sweet principle of the fern.

Key words: Saponin, osladin, sweet taste, structure, synthesis, glycosylation.

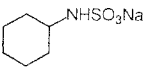
Sugarcane has been widely cultivated worldwide for the production of sucrose (**1**). A variety of intensely sweet-tasting artificial sweeteners such as saccharin (**2**), cyclamate (**3**), dulcin (**4**), aspartame (**5**), or acesulfame K (**6**) have also been developed and employed for sugar substitutes. Each of these artificial sweeteners was accidentally discovered during some other experiment on synthetic organic chemistry.

Nature also produces a variety of intensely sweet-tasting compounds[1]. These are excellent research themes for natural product chemists. A sweet principle of the Chinese drug *Glycyrrhiza glabra* (Fabaceae) is the well-known glycyrrhizin (**7**). A saponin mogroside V (**8**) is found in the fruit of a Cucurbitaceae plant *Momordica grosvenorii*[2]. The triterpene glycoside abrusoside A (**9**) is the sweet principle of *Abrus precatorius* (Leguminosae). A fern metabolite osladin (**10**) is the intensely sweet glycoside of a steroid. The sweet diterpene glycoside stevioside (**11**) is isolated from Paraguayan Compositae plant *Stevia rebaudiana*, and baiyunoside (**12**) is the sweet principle of a Chinese drug *Phlomis betonicoides* (Labiatae).

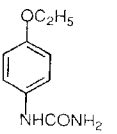
Since such sweet-tasting natural glycosides have not been a subject of organic synthesis, we were interested in the total synthesis of the intensely sweet natural glycosides. Our first synthetic target was baiyunoside (**12**)[2,3]. Baiyunoside as well as structurally related



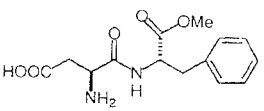
Saccharin (**1**)



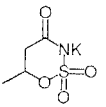
Cyclamate (**2**)



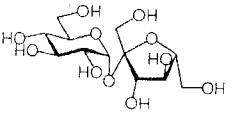
Dulcin (**3**)



Aspartame (**4**)

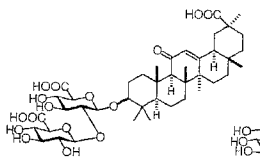


Acesulfame K (**5**)

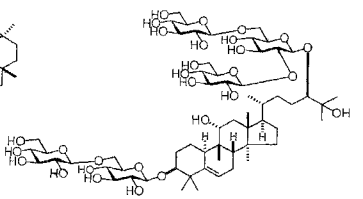


Sucrose (**6**)

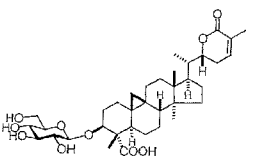
SCHEME 1



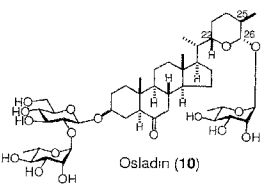
Glycyrrhizin (**7**)



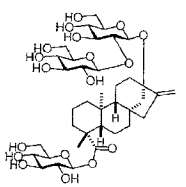
Mogroside V (**8**)



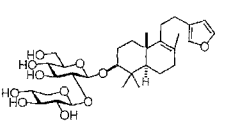
Abrusoside A (**9**)



Osladin (**10**)



Stevioside (**11**)



Baiyunoside (**12**)

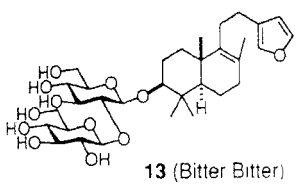
SCHEME 2.

compounds including **13–16** have been synthesized[4–7] using the biomimetic olefin cyclization chemistry involving an original reagent, mercury triflate/amin complex[8–11], developed by us. Through our investigation we observed that changes in taste depend dramatically upon a minor structural modification. For example, compounds **12, 14, 15,** and **16** were very sweet, while **13** was extremely bitter[7]. Among various kinds of baiyunoside analogs, the glycoside **14** with (+)-baiyunol, β-glucose, and α-glucose showed much stronger sweet taste than the natural baiyunoside itself. Therefore, osladin (**10**) was selected as our next synthetic target since this compound is known to be one of the sweetest natural glycosides[12].

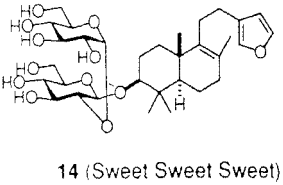
The rhizome of a fern *Polypodium vulgare* (Polyopodiaceae) is widely known in Europe to be intensely sweet. In 1967, the Czechoslovakian scientists, Jizba and Herout, reported the isolation of a structurally new saponin as the sweet-tasting principle, and named it osladin based on the Czech name of this fern, osladic[13]. Its planar structure[14] was reported in 1971. Shortly thereafter, Havel and Cerny achieved a partial synthesis of the aglycone from solasodine, and established its absolute stereochemistry[15]. While the stereochemistry of the glucosidic bond was determined to be β based on the enzymatic hydrolysis using β-glucosidase, two rhamnosidic bonds and the stereochemistry at C-26 have not been determined. Thus, though the structural study of this compound was not completed, it became quite well known by its structure **10** due to its exceptionally intensive sweetness among natural sweet glycosides, which is 3,000 times sweeter than sucrose[12].

A retrosynthetically expected precursor **17** of **10** will be accessible from disaccharide lactone **18**. Successive and stereoselective glycosylations of a key intermediate **19** will lead to **18**, and the lactone **19** could be obtained from steroidal aldehyde **20**. Thus, by using **20** as the 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 needs to be controlled by some stereoselective reactions. Sugar residues are regarded as protecting groups of C-3 and C-26 oxygen functionalities and are introduced at a relatively earlier stage of the synthesis. Glycosylation of hemiacetalic hydroxyl group at C-26 has to be overcome stereoselectively. Therefore, if this total synthesis were to be achieved, it could be the first total synthesis of saponin[16].

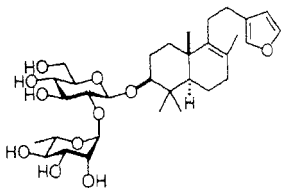
Our starting material is steroidal aldehyde **20**, which is easily derived from commercially available stigmasterol. Grignard reaction of **20** with 4-pentenylmagnesium bromide provided desired 22*S* alcohol **21** selectively (22*S* *vs* 22 *R* 97:3). Ozonolysis and oxidation followed by solvolysis, afforded lactone alcohol **19**. Glucose derivative **22** was introduced into C-3 hydroxyl group of **19** in β-selective manner, and then rhamnosyl chloride **23** was introduced to the 2' hydroxy group leading to a disaccharide **24**. Methyl group was introduced to α position of carbonyl group as a 1:1 stereoisomeric mixture. Although the mixture was not converted into single isomer by basic treatment, hemiacetal obtained by diisobutylaluminium hydride (DIBAH) reduction was smoothly isomerized into equatorial β-methyl compound **25** exclusively. Rhamnosyl chloride **23** was again condensed with **25** to give **26**, and the double bond of **26** was converted into ketone by hydroboration/oxidation sequence. Finally, benzyl group was cleaved by catalytic hydrogenolysis to give saponin **10**. However, the aqueous solution of this product was not sweet at all! As neither the samples of osladin nor the spectra



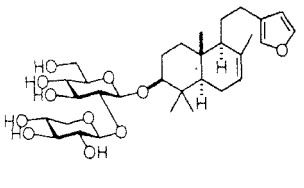
13 (Bitter Bitter)



14 (Sweet Sweet Sweet)



15 (Sweet Sweet)



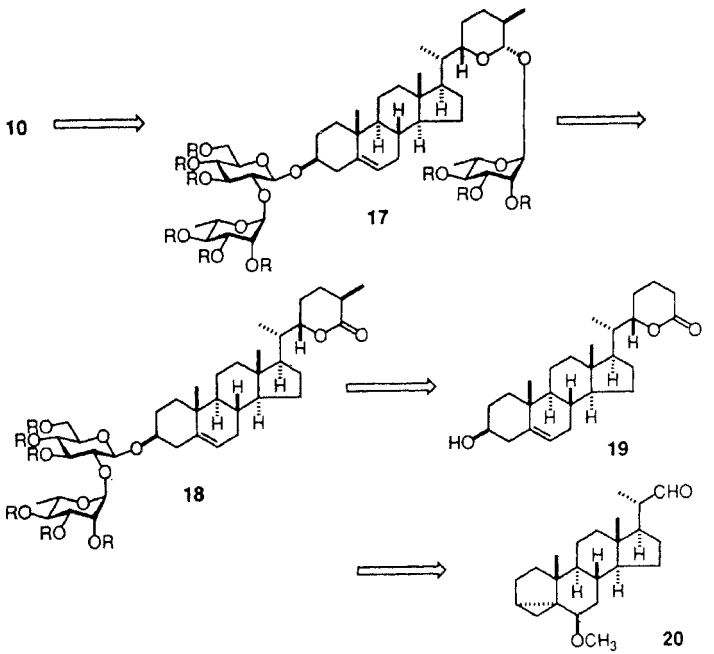
16 (Sweet Sweet)

SCHEME 3

were available from original investigators, we could not compare the synthetic material with the natural product. There are three possibilities for this: (i) Stereochemical misassignments during the synthetic study of **10**, (ii) The reported structure **10** of osladin may not be correct, (iii) Instead of osladin some minor contamination could be the sweet taste principle of the fern.

Therefore, we decided to isolate the sweet principle from the rhizome of *P.vulgare*. Fern was collected from the southern part of Germany through the kind help of Professors Y. Asakawa and H. Becker. Dried and crushed rhizomes were successively extracted with dichloromethane, ethyl acetate, and ethanol. The extract of ethanol alone was sweet: Successive chromatography on silica gel, sephadex, silica gel, ODS, HPLC with ODS column, and finally recrystallizations afforded pure osladin as colorless crystals of mp202–204°C. The NMR spectra of the sweet osladin was not identical with synthetic **10**. While the steroidal moiety and the disaccharide part were almost superimposable, remarkable distinctions were observed at C-26 and C-1‴ positions. However, the NMR results were not sufficient enough to establish the whole structure of osladin with complete stereochemistry.

We then decided to prepare a single crystal for X-ray diffraction study. Repeated recrystallizations from a mixture of acetone and water afforded the monoclinic single crystal of space group $P2_1$. As seen in the ORTEP drawing as well as stereostructure, sweet osladin

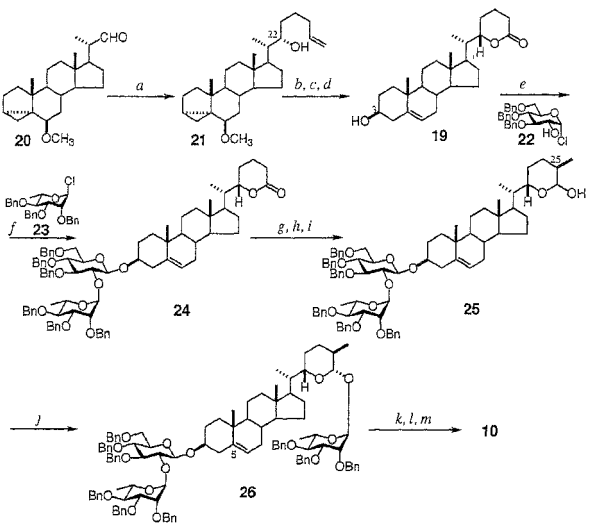


SCHEME 4.

must be represented by structure **27**[17]. Thus, the 22*S*, 25*R*, 26*S* stereochemistries assigned by Havel and Cerny need to be revised to 22*R*, 25*S*, 26*R*, respectively. It is important to note that **27** is intensively sweet while **10** is totally free from any taste even though the structural change is minor.

Jizba *et al* have not reported in their original paper the intensity of sweetness of osladin. It has been claimed to be 3000 times sweeter than sucrose[12] but it turned out to be only 500 times sweeter when the compound was examined by Ajinomoto company.

In 1975, Havel and Cerny reported the chemical correlation of osladin aglycone with solasodine (**28**)[15]. They derived compound **31a** and **31b** from solasodine by maintaining the 25*R* stereochemistry by the way of **29** and **30a** and **30b**, and found that the natural product

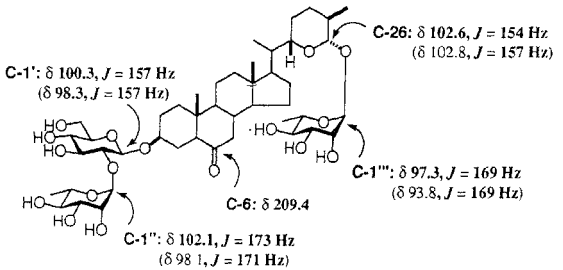


a $CH_2{=}CH(CH_2)_3MgBr$/ether. *b* O_3/C_2H_5OH-H_2O (9:1), then $(CH_3)_2S$. *c* PDC/CH_2Cl_2. *d* CF_3SO_3H/dioxane-H_2O (9:1). *e* **22**/TMU/CF_3SO_3H/$(ClCH_2)_2$. *f* **23**/TMU, neat, 80°C, 56 h. *g* LDA/ HMPA/THF, then CH_3I. *h* DIBAH/ether. *i* CH_3ONa/CH_3OH. *j* **23**AgOTf/ TMU/4A MS/CH_2Cl_2. *k* BH_3/THF, then 30% H_2O_2/30% NaOH. *l* PDC/CH_2Cl_2. *m* H_2/Pd$(OH)_2$/CH_3OH-EtOAc-H_2O (12:2:1).

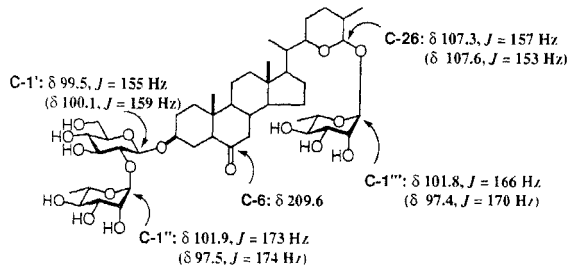
SCHEME 5

derivative was identical with **31a**. Since the stereochemistry of sweet osladin at C-25 is *S*, it could not happen. We found that C-25 axial methyl group is easily isomerized into the equatorial when C-26 is hemiacetal. Since they did not have this information, they simply assigned *R* configuration for C-25. Thus we believe that they prepared **32** from **30b** as a result of auto-isomerization and identified the natural product derivative. Thus, the assignment of 22*S* stereochemistry to **30a** and **31a** is also questionable.

To achieve total synthesis of sweet osladin (**27**), we needed to modify the stereochemistry at C-22. As already discussed, Grignard reaction of the aldehyde **6** generates 22*S* product **21** predominantly. Now, we need to invert this stereochemistry. Although a variety of Mitsunobu reaction conditions did not afford any inversion product, Corey's S_N2 reaction of the mesylate **33** with KO_2 achieved clean inversion at C-22 affording 22*R* carbinol **34** in 88% yield[18]. The alcohol **34** was transformed into lactone **35** by ozonolysis in ethanol–water (10:1), and subsequent PDC oxidation. Methylation at the α-position of lactone afforded monomethylated product **36** as a mixture of stereoisomers. Since it is possible to isomerize the configuration



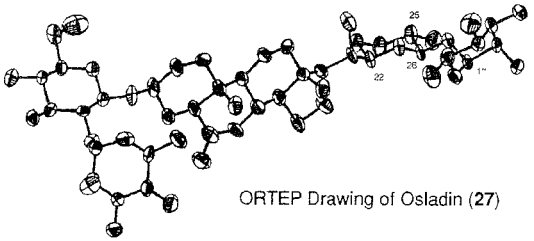
^{13}C NMR of Glycoside **10** in Py-d_5 (Benzyl Ether in $CDCl_3$)



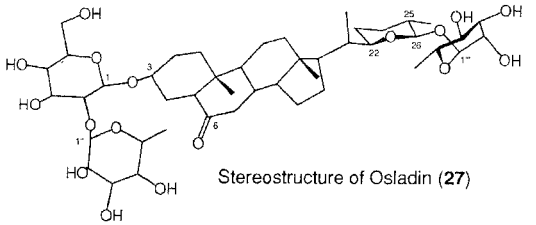
^{13}C NMR of Natural Sweet Osladin (**27**) in Py-d_5 (Acetate in $CDCl_3$)

SCHEME 6.

of the methyl group at C-25 into *S* at a later stage, this mixture was employed for solvolysis and glycosylation reaction. Treatment of **36** with 0.005 equiv. of trifluoromethanesulfonic acid in dioxane–water (9:1) afforded homoallyl alcohol **37** in 81% yield. Condensation of **37** and glucosyl chloride **22** catalyzed by triflic acid in the presence of TMU took place in β-selective manner to give 2' hydroxyl group-discriminated glucoside **38** in 57% yield along with 10% of α-glucoside. L-Rhamnosyl residue was introduced to **38** with **23** and TMU by means of α-selective thermal rhamnosylation conditions (neat, 80°C, 56 h) to give disaccharide **39** in 81% yield[19,20]. Hemiacetals **40** obtained by DIBAH reduction of **39** were treated with base providing more stable equatorial methyl product **41**. Glycosylation of the hemiacetal hydroxyl group at C-26 was achieved by using rhamnosyl chloride **23**, AgOTf, and TMU to yield trisaccharide **42** stereoselectively[21]. Characteristic low field shift (δ 106.7) of the C-26 hemiacetal carbon was observed in the ^{13}C-NMR of **42**. Similar low field shift was observed in osladin itself (δ 107.3). A doublet at δ 4.08 with coupling constant 8.6 Hz, assigned to C-26 proton, indicates *trans* relationship between C-25 methyl group and *O*-rhamnosyl function at C-26. The stereochemistry of the newly introduced *O*-ramnoside

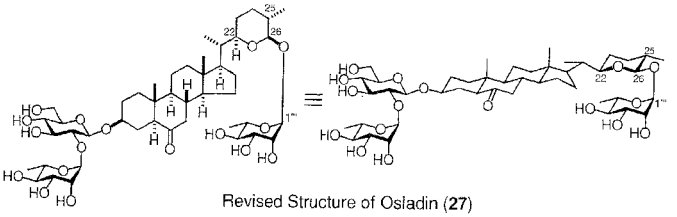


ORTEP Drawing of Osladin (**27**)

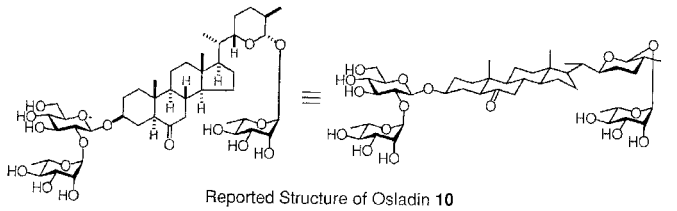


Stereostructure of Osladin (**27**)

SCHEME 7

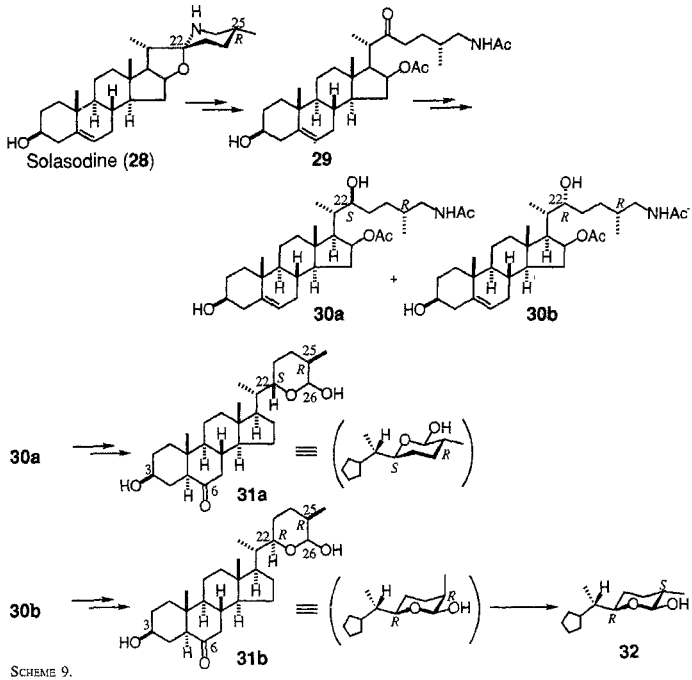


Revised Structure of Osladin (**27**)



Reported Structure of Osladin **10**

SCHEME 8.

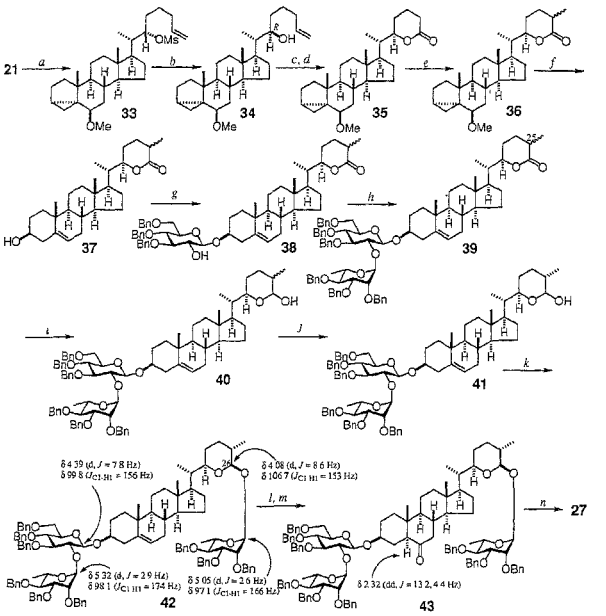


SCHEME 9.

linkage was α due to a coupling constant (J_{CH}) of 166 Hz in ^{13}C-NMR. The trisaccharide **42** was subjected to hydroboration, and subsequent PDC oxidation to give a ketone **43**. A double doublet at δ 2.32 (J= 13.2 and 4.4 Hz) due to the C-5 proton clearly showed the A/B *trans* relationship. Benzyl groups of **43** were cleaved by $Pd(OH)_2$-catalyzed hydrogenolysis to give osladin **27**. This product was very sweet and showed indistinguishable spectral properties (^{1}H and ^{13}C-NMR, optical rotation, IR, and high- resolution FAB mass) with those of natural osladin[22]. Thus we have proved beyond doubt that osladin **27** is the real sweet principle of *P. vulgare*.

Acknowledgements

We are deeply indebted to Professor Y. Asakawa of Tokushima Bunri University and Professor H. Becker of Universität des Saarland for kind cooperation in collecting the fern, *P.vulgare*. We also thank Dr C. Katayama for the X-ray diffraction study of osladin.



a MsCl/Py *b* KO_2/18-Crown-6/DMSO-DME (1:1), 0°C *c* O_3/Sudan III/EtOH-H_2O, then Me_2S *d* PDC/CH_2Cl_2 *e* LDA/HMPA/THF, -78°C then MeI *f* TfOH (0.005 equiv)/dioxane-H_2O (9:1) *g* **22**/TfOH/TMU/CH_2Cl_2 *h* **23**/TMU, neat, 80°, 56 h *i* DIBAH/ether *j* MeONa/MeOH *k* **23**/AgOTf/TMU/CH_2Cl_2 *l* BH_3·THF then H_2O_2/NaOH *m* PDC/CH_2Cl_2 *n* H_2/$Pd(OH)_2$/MeOH-EtOAc-H_2O (12:2:1)

SCHEME 10.

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8 HINGDON, A	*Engineering mechanics*, 1968, Vol. 1, Ch. 3, pp 79–104, Prentice-Hall
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