Selligueain A, a Novel Highly Sweet Proanthocyanidin from the Rhizomes of Selliguea feei

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SELLIGUEAIN A, A NOVEL HIGHLY SWEET PROANTHOCYANIDIN FROM THE RHIZOMES OF *SELLIGUEA FEEI*[1,2]

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ABSTRACT.—Selligueain A, a novel sweet trimeric proanthocyanidin with a doubly linked A unit, has been isolated from the rhizomes of *Selliguea feei* collected in Indonesia. The structure of this substance was established as epiafzelechin-(4β↦8, 2β↦O↦7)-epiafzelechin-(4β↦8)-afzelechin [**1**], on the basis of a combination of spectral and chemical methods. The compound was not acutely toxic for mice and not mutagenic in a forward mutation assay utilizing *Salmonella typhimurium* strain TM677. Selligueain A [**1**] was rated by a taste panel as exhibiting about 35 times the sweetness intensity of a 2% w/v aqueous sucrose solution, and at a concentration of 0.5% w/v in H_2O was perceived as pleasant-tasting rather than astringent.

In a continuing search for plant-derived sweet compounds, we have investigated the Indonesian medicinal plant *Selliguea feei* Bory [syn. *Polypodium feei* (Bory) Mett.] (Polypodiaceae). Tea made from the sweet-bitter tasting rhizomes of this fern is used traditionally in western Java for the treatment of rheumatism and as a male tonic. Thus far, no phytochemical nor biological work has been conducted on extracts of *S. feei*. In this investigation, selligueain A [**1**], a novel proanthocyanidin constituent of the rhizomes of this plant, was isolated, spectroscopically characterized, and submitted to a preliminary safety evaluation. This compound was shown to be highly sweet by a taste panel. The isolation, structural characterization, and safety and sensory evaluations of selligueain A [**1**] are the subject of the present communication.

RESULTS AND DISCUSSION

Si gel cc of an *n*-BuOH extract of *S. feei* rhizomes afforded a highly sweet proanthocyanidin, selligueain A [**1**], as a major constituent. This substance was obtained as light-brown fine crystals from MeOH and showed uv absorption maxima at 274 nm and 235 nm, as well as a dark-blue color with $FeCl_3$ reagent and an orange-red color with anisaldehyde/H_2SO_4 reagent, all of which are typical of proanthocyanidins (3). In addition, its ir spectrum showed evidence of strong hydroxyl (3387, 1229 cm^{-1}) and aromatic ring absorption (1615, 1518 cm^{-1}). In its ^1H- and ^{13}C-nmr spectra (Tables 1 and 2), **1** exhibited resonances consistent with being a trimeric proanthocyanidin. Furthermore, the coupling patterns of the proton signals at δ 6.75, 6.87, 6.88, 7.15, 7.42, and 7.57 (each 2H, d, J=8.6 Hz) arising from the B rings in each of the upper, middle, and terminal units of **1**, indicated that all three of these moieties were constituted by *p*-hydroxylphenyl groups. The mol wt of **1** was determined by fabms as

[1]Paper no. 29 in the series, "Potential Sweetening Agents of Plant Origin." For part 28, see Suttisri *et al.* (1).

[2]Paper no. 6 in the series, "Studies on Indonesian Medicinal Plants." For Part 5, see Dai *et al.* (2).

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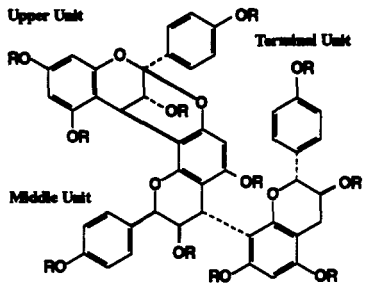
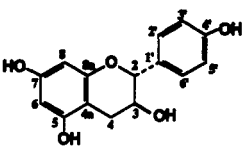
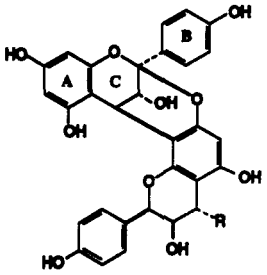


1 R=H
2 R=Ac



3



4 R=−S-CH_2- 
5 R=H

816, which is 18 mass units less than expected had this compound been a singly-linked trimeric proanthocyanidin, and, from the hrfabms, the molecular formula was elucidated as $C_{45}H_{36}O_{15}$ (*m/z* 817.2130). Analysis of all of the foregoing data suggested that **1** was a trimeric compound composed of three 5,7,4′-trihydroxylflavan-3-ol units and possessing a doubly-linked proanthocyanidin-A-type unit (4) in the molecule. Also of importance was the observation in the ^{13}C-nmr spectrum of **1** of the signals at δ 78.00 and δ 82.28 due to the C-2 carbons of the middle and terminal units, respectively, and at δ 99.30 from the C-2 ketal carbon of the A-type proanthocyanidin unit (Table 2).

Acetylation of **1** under standard conditions afforded an undecaacetate compound **2**, which was confirmed by the observation of eleven singlet methyl signals occurring between δ 1.46 and δ 2.26 in its ^{1}H-nmr spectrum (Table 1). The coupling patterns of H-3 (U) [δ 5.04 (d, J=4.0 Hz)], H-2 middle unit (M) [δ 5.51 (d, J=1.4 Hz)] and H-3 (M) [δ 5.16 (dd, J=4.0, 1.4 Hz)] in the ^{1}H-nmr spectrum of **2** suggested that both the upper and middle monomer units of the molecule possessed a 2,3-cis stereostructure (5). In contrast, the coupling pattern of H-2 terminal unit (T) [δ 4.06 (d, J=8.6 Hz)] indicated that the lower monomer unit had the 2,3-trans stereostructure (6). Accordingly, it was inferred that the upper and the middle monomers of **2** were constituted by (−)-epiafzelechin and that the terminal unit was of (+)-afzelechin.

Thiolysis of **1** with α-toluenethiol in the presence of HOAc (7) afforded **3** and **4**, and this latter compound generated **5** by desulfurization with Raney nickel (8). Compound **3** was determined as (+)-afzelechin by comparison with published physical and spectroscopic data (9). Compounds **4** and **5** were assigned as possessing two (−)-epiafzelechin units connected with 2β⟼*O*⟼7 and 4β⟼8 linkages, by comparison of nmr, [α]D, and other spectroscopic data obtained for these known compounds (10–12). All of the ^{1}H and ^{13}C signals of **1** and **2** were assigned unambiguously by ^{1}H-decoupling, nOe, ^{1}H-^{13}C HETCOR, and selective INEPT (13) experiments. As a consequence, the C-2 ketal (U) ^{13}C-nmr assignments for **4** and **5** made by Kashiwada *et al.* (10) and Tanaka *et al.* (11) have been reinterpreted in this study. Thus, on the irradiation of H_2-2′, -6′ (U) (δ 7.60; $^3J_{CH}$=8 Hz) and H-4 (U) (δ 4.08; $^3J_{CH}$=6 Hz), C-2 (U) (δ 99.09) was selectively enhanced in **2**. This rearrangement is consistent with the results of other nmr studies on model compounds (14–17). Also, it may be pointed out that in the ^{1}H-nmr spectrum of **1**, the signals corresponding to H-3 (U) (δ 4.18) of **4** and H-2 (δ 4.58) of **3** appeared at δ 3.29 and 4.06, respectively. These unusual upfield shifts are attributable to the magnetic anisotropic effects of the B (T) and A (M) rings, and are consistent with **1** possessing a 4β(M)⟼8(T) linkage (11). Therefore, the structure of **1** was established as epiafzelechin-(4β⟼8, 2β⟼*O*⟼7)-epiafzelechin-(4β⟼8)-afzelechin. We have adapted the convention of Tanaka (10,18) rather than that of Nishioka (11) in designating the 4(M)⟼8(T) interflavonoid linkage of **1**.

TABLE 1. ^{1}H-nmr Data for Compounds **1–5**.[a]

Proton	Compound				
	1	**2**	**3**	**4**	**5**
Upper unit (U)					
3	3.29 (d, J=3.6)	5.04 (d, J=4.0)		4.18 (d, J=3.5)	4.35 (d, J=3.4)
4	3.93 (d, J=3.6)	4.08 (d, J=4.0)		4.37 (d, J=3.5)	2.94 (d, J=3.4)
6	5.97 (d, J=2.3)	6.42 (d, J=2.2)		6.01 (d, J=2.3)	6.00 (d, J=2.3)
8	6.03 (d, J=2.3)	6.71 (d, J=2.2)		6.12 (d, J=2.3)	6.09 (d, J=2.3)
2′,6′	7.42 (d, J=8.6)	7.60 (d, J=8.6)		7.57 (d, J=8.6)	7.55 (d, J=8.6)
3′,5′	6.87 (d, J=8.6)	7.12 (d, J=8.6)		6.88 (d, J=8.6)	6.86 (d, J=8.6)
Middle unit (M)					
2	5.59 (br s)	5.51 (d, J=1.4)		5.45 (br s)	5.04 (br s)
3	4.03 (br s)	5.16 (dd, J=4.0, 1.4)		4.15 (br s)	4.17 (br s)
4	4.57 (br s)	4.25 (d, J=4.0)		4.04 (br s)	2.84 (br d, J=15.8) 2.85 (br d, J=15.8)
6	5.87 (s)	6.25 (s)		6.19 (s)	6.17 (s)
2′,6′	7.57 (d, J=8.6)	7.20 (d, J=8.6)		7.62 (d, J=8.6)	7.59 (d, J=8.7)
3′,5	6.88 (d, J=8.6)	7.08 (d, J=8.6)		6.92 (d, J=8.6)	6.90 (d, J=8.7)
Terminal Unit (T)					
2	4.06 (d, J=7.6)	4.61 (d, J=8.6)	4.58 (d, J=7.6)	toluene-thiol	
3	3.68 (ddd, J=8.4, 7.6, 5.4)	5.11 (ddd, J=8.6, 8.6, 5.8)	4.00 (ddd, J=8.3, 7.6, 5.4)	H-2, -6,7.43 (d, J=6.9)	
4	2.45 (dd, J=16.0, 8.4) 3.08 (dd, J=16.0, 5.4)	2.56 (dd, J= 16.6, 8.6) 3.09 (dd, J=16.6, 5.8)	2.49 (dd, J=15.9, 8.3) 2.84 (dd, J=15.9, 5.4)	H-3, -5,7.29 (dd, J=7.1, 6.9) H-4,7.21 (dd, J=7.1, 7.1)	
6	6.13 (s)	6.60 (s)	5.84 (s)	-S-CH_2-,4.03 (s)	
2′,6′	7.15 (d, J=8.6)	7.24 (d, J=8.6)	7.17 (d, J=8.1)		
3′,5′	6.75 (d, J=8.6)	6.97 (d, J=8.6)	6.78 (d, J=8.1)		
			H-8, 5.99 (s)		
		-OAc-CH_3, 2.26 2.25 2.25 2.21 2.14 2.06 1.84 1.83 1.80 1.62 1.46			

[a]Measured at 300 MHz in Me_2CO-d_6 (**1**, **3–5**) and $CDCl_3$ (**2**). Chemical shifts (ppm) are expressed relative to TMS.

TABLE 2. ^{13}C-nmr Data for Compounds **1–5**.[a]

Carbon	Compound				
	1	**2**	**3**	**4**	**5**
Upper unit (U)					
2	99.29	99.09		99.79	99.91
3	66.52	67.67		67.11	65.95
4	29.93	27.99		28.56	28.71
4a	103.95	114.65		103.45	103.82
5	155.95	148.83		156.67	156.78
6	95.32	110.03		97.90	97.99
7	157.01	150.08		157.65	157.87
8	95.81	107.41		96.10	96.16
8a	153.31	154.61		153.63	153.86
1′	131.04	134.73		131.17	131.50
2′,6′	128.67	128.81		129.07	129.27
3′,5′	115.22	121.66		115.13	115.17
4′	157.97	151.90		158.12	158.25
Middle unit (M)					
2	78.00	76.14		77.19	81.54
3	71.99	71.36		70.15	67.38
4	37.52	34.20		43.84	30.13
4a	105.78	108.85		102.05	102.19
5	155.06	148.73		156.59	156.07
6	95.81	104.62		96.90	96.38
7	150.30	150.14		153.02	151.73
8	105.39	108.55		106.58	106.68
8a	150.88	153.00		151.37	151.60
1′	130.20	134.67		129.46	130.05
2′,6′	129.63	129.69		129.59	130.22
3′,5′	115.35	122.19		115.66	115.64
4′	157.49	151.41		158.21	158.47
Terminal unit (T)					
2	82.28	78.71	81.71	toluene-thiol	
3	69.37	68.76	67.65	1 139.46	
4	28.08	26.37	28.10	2,6 130.39	
4a	108.06	112.85	100.28	3,5 129.16	
5	154.55	148.78	156.49	4 127.56	
6	97.60	110.82	95.91	-S-CH_2- 37.26	
7	154.79	148.32	156.73		
8	101.02	117.40	95.03		
8a	154.47	153.83	156.09		
1′	131.30	134.55	130.48		
2′,6′	128.67	128.32	129.18		
3′,5′	114.75	121.59	115.57		
4′	157.01	151.28	157.14		
		-OAc			
		170.34 170.29			
		169.78 169.78			
		169.63 169.47			
		169.31 169.31			
		169.28 169.28			
		168.92			
		21.71 21.71			
		21.71 21.57			
		21.52 21.37			
		21.37 21.37			
		21.00 20.62			
		20.27			

[a]Measured at 75.4 MHz in Me_2CO-d_6 (**1**, **3–5**) and $CDCl_3$ (**2**). Chemical shifts (ppm) are expressed relative to TMS.

Prior to being assessed for sweetness, the *n*-BuOH extract of *S. feei* rhizomes and compound **1** were shown to be nontoxic in preliminary acute toxicity tests in mice (19,20) and not mutagenic in forward mutation assays utilizing *Salmonella typhimurium* strain TM677 (21). The sweetness intensity of **1** was evaluated by a small taste panel (19,20) and ranked as about 35 times sweeter than sucrose. However, while much more potent than the monosaccharide, disaccharide, and polyol "bulk" sweeteners, the sweetness intensity of selligueain A [**1**] relative to sucrose is a least an order of magnitude less than that of many other natural sweeteners, such as hernandulcin, rebaudioside A, monatin, and thaumatin, which are, respectively, a sesquiterpene, a diterpene glycoside, an amino acid, and a protein (22,23).

To date, two pairs of trimeric proanthocyanidins, isolated from the fronds of *Arachniodes sporadosora* Nakaike and the root bark of *Cinnamomum sieboldii* Meisner, have been described as sweet-tasting, although these compounds have not been evaluated for their sweetness intensities relative to sucrose (11,24). All four of these compounds contain a doubly linked A unit and a ring-C epicatechin unit. However, the presently described highly sweet substance, selligueain A [**1**], differs structurally from those other sweet compounds in possessing an afzelechin C unit. The isolation and characterization of further proanthocyanidin constituents of *S. feei* rhizomes is ongoing, in order to better understand the relationship between sweetness and structure among this class of plant polyphenols. However, because of the stringent structural requirements necessary to elicit sweetness, it may be anticipated that sweet-tasting proanthocyanidins will prove to be very rare in the plant kingdom.

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURES.—Melting points were determined using a Kofler hot-stage instrument and are uncorrected. Optical rotations were measured with a Perkin-Elmer 241 polarimeter. The uv spectra were obtained on a Beckman DU-7 spectrometer and the ir spectra measured on a Midac Collegian FT-IR spectrophotometer. ^{1}H and ^{13}C-nmr spectra were measured with TMS as internal standard, employing a Varian XL-300 instrument (300 MHz and 75.6 MHz, respectively). Selective INEPT nmr experiments were conducted on a Nicolet NT-360 spectrophotometer operating at 90.8 MHz. Low- and high-resolution mass spectra were obtained with a Finnigan MAT 90 instrument.

PLANT MATERIAL.—The rhizomes of *S. feei* were collected near Lembang, Indonesia in January 1992, by one of us (LBSK) and were taxonomically identified by Dr. Johanis F. Moggea of the Herbarium Bogoriense, Bogor, Indonesia. A voucher specimen representing this collection has been deposited at the Herbarium of the Field Museum of Natural History, Chicago, Illinois.

EXTRACTION AND ISOLATION.—The air-dried and powdered rhizomes of *S. feei* (950 g) were extracted with 80% aqueous MeOH (3 liters×3) at room temperature and filtered. The combined filtrates were evaporated at less than 40° to give a dried MeOH extract (355 g). This residue was suspended in H_2O (1 liter) and extracted with *n*-BuOH (600 ml×3) to afford, on drying, an *n*-BuOH fraction (196 g). A portion of the *n*-BuOH extract (57 g) was fractionated by Si gel cc using $CHCl_3$/MeOH/H_2O mixtures as eluents to give 8 major fractions. A fraction eluting from the column with $CHCl_3$-MeOH-H_2O (65:35:10) was then applied to a Si gel column eluted with EtOAc/*n*-BuOH mixtures. Final purification of a fraction eluting with EtOAc–*n*-BuOH (20:1) was performed on a further Si gel column eluted with $CHCl_3$-MeOH-H_2O (65:35:10) to afford pure **1** (6.54 g; 0.69% w/w yield).

Epiafzelechin-(4β→8, 2β→O→7)-epiafzelechin-(4β→8)-afzelechin [**1**].—Light-brown fine crystals (MeOH): mp ≥300°; $[\alpha]^{25}D$ +103.6° (*c*=1.3, Me_2CO), $[\alpha]^{25}_{365}$ +415.3° (*c*=1.3, Me_2CO); uv λ max (MeOH) (log ε) 274 (3.67), 235 (4.78) nm; ir ν max (film) 3387, 2930, 2359, 1615, 1519, 1454, 1229, 833 cm^{-1}; ^{1}H nmr see Table 1; ^{13}C nmr see Table 2; fabms *m/z* (rel. int.) $[M+H]^+$ 817 (5), 681 (1), 207 (74), 115 (100); hrfabms *m/z* $[M+H]^+$ 817.2130 (calcd for $C_{45}H_{37}O_{15}$, 817.2132).

ACETYLATION OF **1**.—A mixture of **1** (35 mg), pyridine (3 ml), and Ac_2O (3 ml) was stirred for 15 h at room temperature. The reaction mixture was poured into ice-H_2O (50 ml) and extracted wh EtOAc. The organic fraction was washed with 5% aqueous HCl, saturated aqueous $NaHCO_3$, and NaCl, followed by drying over anhydrous $MgSO_4$. The crude acetylated product was purified by Si gel cc, using *n*-hexane–EtOAc (1:2) as eluent, to give **2** (47 mg), the pure undecaacetate of **1**.

Undecaacetylepiafzelechin-(4β↦8, 2β↦O↦7)-epiafzelechin-(4β↦8)-afzelechin [**2**].—White amorphous powder: $[\alpha]^{25}D$ +43.2° (*c*=0.7, $CHCl_3$), $[\alpha]^{25}_{365}$ +232.4° (*c*=0.7, Me_2CO); uv λ max (MeOH) (log ε) 274 (3.59), 227 (4.70) nm; ir ν max (film) 2924, 2363, 1769, 1603, 1198, 907 cm^{-1}; ^1H nmr see Table 1; ^{13}C nmr see Table 2; fabms *m/z* (rel. int.) $[M+H]^+$ 1279 (2), 900 (1), 115 (100).

THIOLYSIS OF **1**.—Compound **1** (1 g) was dissolved in EtOH (50 ml) containing α-toluenethiol (30 ml) and glacial HOAc (30 ml), and the solution was refluxed for 24 h. After evaporation of the reaction mixture, the oily residue was chromatographed over Sephadex LH-20 using $CHCl_3$-MeOH (10:1 and 1:10) for elution. The crude mixture of products obtained was further chromatographed over Si gel [$CHCl_3$-MeOH (9:1)] and Sephadex LH-20 [MeOH-H_2O (8:1↦4:1)], successively, to afford pure (+)-afzelechin [**3**] (154 mg) and a known thioether **4** (427 mg), which were identified by comparison of their spectroscopic characteristics with those of published data (9–12). Compound **3** exhibited $[\alpha]^{25}D$ +19.2° (*c*=1.4, Me_2CO) [lit. (9) $[\alpha]^{20}D$ +20.6° (*c*=5.0, Me_2CO+H_2O). Compound **4** exhibited $[\alpha]^{25}D$ +118.5° (*c*=0.7, Me_2CO) [lit. (10) $[\alpha]^{24}D$ +109.8° (*c*=0.4, Me_2CO), lit. (11) $[\alpha]^{20}D$ +64° (*c*=0.7, Me_2CO)]. The ^1H- and ^{13}C-nmr data are shown in Tables 1 and 2, respectively.

DESULFURIZATION OF **4**.—Compound **4** (70 mg) in 10% HOAc/EtOH (10 ml) was treated with an EtOH slurry of Raney nickel (W-4) at room temperature for 2 h. After removal of the catalyst by filtration, the filtrate was evaporated and chromatographed over Sephadex LH-20 [MeOH-H_2O (4:1)] to afford the known compound **5** (32 mg), which was identified by physical and spectral comparison with published data (10,12). This substance exhibited $[\alpha]^{25}D$ +62.5° (*c*=1.2, Me_2CO) [lit. (10) $[\alpha]^{24}D$ +61.9° (*c*=0.6, Me_2CO], and its ^1H- and ^{13}C-nmr data are shown in Tables 1 and 2, respectively.

PRELIMINARY SAFETY EVALUATION OF **1**.—The *n*-BuOH extract of *S. feei* rhizomes and compound **1** were tested for acute toxicity in male Swiss-Webster mice, administered by oral intubation at dose levels of 1 and 2 g/kg body wt. The procedures and protocols for toxicological testing were followed as published previously (20,21). Administration of both the plant extract and compound **1** did not cause any deaths, and body weights recorded on days 0, 1, 3, 7, and 14 did not differ significantly for treated vs. control animals.

The mutagenic potentials of the *S. feei* rhizome *n*-BuOH extract and compound **1** were evaluated in the dose range of 0.01–10 mg/ml according to established protocols (22). Neither the plant extract nor compound **1** was mutagenic for *Salmonella typhimurium* strain TM677, either in the presence or absence of a metabolic activating system (S9) derived from the livers of Aroclor 1254-pretreated rats.

SENSORY EVALUATION OF COMPOUND **1**.—The sweetness intensity of **1** was compared to that of sucrose by a small taste panel consisting of three persons (19,20). Concentrations of 0.05–0.06% w/v of **1** in H_2O were found to be of equivalent sweetness to a 2% w/v aqueous sucrose solution, and also exhibited no appreciable off-taste or after-taste. At a higher concentration level (0.5% w/v in H_2O), **1** was found to be pleasantly sweet, with only a hint of bitterness and astringency.

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