Pergamon

0031-9422(95)00204-9

Phytochemistry, Vol. 40, No. 1, pp. 345-347, 1995

0031-9422/95 \$9.50 + 0.00

TAXIFOLIN 3-ARABINOSIDE FROM *FRAGARIA X ANANASSA*

KANJI ISHIMARU,* TOSHIO OMOTO,† IWAO ASAI,‡ KATSUSHI EZAKI,§ and KOICHIRO SHIMOMURA§

Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, 1 Honjo, Saga 840, Japan; †San-Ei Gen F·F·I. Inc., 1-1-11 Sanwa, Toyonaka, Osaka 561, Japan; ‡Division of Applied Sciences, Graduate School of Integrated Science and Art, University of East Asia, 2-1 Ichinomiya-Gakuen, Shimonoseki 751, Japan; §Tsukuba Medicinal Plant Research Station, National Institute of Health Sciences, 1 Hachimandai, Tsukuba, Ibaraki 305, Japan

(*Received* 14 *November* 1994)

Key Word Index—*Fragaria x ananassa*; Rosaceae; flavonoid; tannin; taxifolin; arabinoside; catechin; procyanidin; pedunculagin.

Abstract—From the roots of *Fragaria x ananassa*, a new flavonoid, (+)-taxifolin 3-*O*-α-L-arabinofuranoside was isolated and its structure was established on the basis of chemical and spectroscopic evidence. A hydrolysable tannin, pedunculagin, and condensed tannins, (+)-catechin, (+)-afzelechin-(4α-8)-(+)-catechin, procyanidin B-3 and procyanidin B-6 were also isolated.

INTRODUCTION

Fragaria plants (strawberry; Rosaceae) have been examined mainly for the anthocyanins in the fruits [1]; some proanthocyanidins in the roots of *F. vesca* L. were analysed by HPLC [2]. *Fragaria x ananassa* Duch. cv. Reikov (hybrid between *F. x ananassa* Duch. cv. Harunoka and *F. x ananassa* Duch. cv. Hukuba), established in 1975 in Japan, is one of the most important varieties in this country. In the present chemical studies, we isolated a new flavonoid, (+)-taxifolin 3-*O*-α-L-arabinofuranoside, from the roots together with some hydrolysable and condensed tannins.

RESULTS AND DISCUSSION

The aqueous acetone extract of the roots of *F. x ananassa* Duch. cv. Reikov was separated on Sephadex LH-20, MCI-gel CHP 20P and Bondapak C18 Porasil B column chromatography to give six phenolics, **1**, (+)-catechin (**2**), (+)-afzelechin-(4α-8)-(+)-catechin (**3**) [3], procyanidin B-3 (**4**) [4], procyanidin B-6 (**5**) [5] and pedunculagin (**6**) [6].

Compound **1** was obtained as an off-white, amorphous powder exhibiting $[M-H]^-$ peak at *m/z* 435.0925 ($C_{20}H_{19}O_{11}$) in the high-resolution negative, secondary ion mass spectrum (SIMS). The ^{1}H NMR spectrum (Table 1) of **1** showed two coupled ($J = 11.3$ Hz) methine (δ4.91 and 5.09), *meta*-coupled aromatic (δ5.91 and 5.95) and ABX-type aromatic (δ6.86, 6.92 and 7.08) proton signals. These signals, in addition to the 15 carbon signals in the ^{13}C NMR spectrum (Table 2) of **1**, suggested the presence of the 5,7,3′,4′-tetrahydroxyflavanonol structure. The ^{13}C NMR spectrum of **1** also exhibited signals of a pentose moiety at δ63.2, 79.4, 80.8, 90.1 and 108.0.

Both enzymatic (with hesperidinase) and acid (with 1N HCl) hydrolysis of **1** gave L-arabinose ($[\alpha]_D + 72.0$) as the sugar, together with an aglycone, **1a**, which was identified as (+)-taxifolin by its spectroscopic (^{1}H NMR, see Table 1) and physical ($[\alpha]_D + 17.0$) data [7]. Therefore, based on the chemical shifts of the carbon signals of the arabinose moiety (^{13}C NMR, see Table 2), Compound **1** was presumed to be the arabinofuranoside of **1a**. Acetylation of **1** gave a heptaacetate, **1b**, FAB-mass spectrum *m/z*: 731 $[M+H]^+$, $C_{34}H_{34}O_{18}$. In the ^{1}H NMR spectrum (Table 1) of **1b**, the signal assignable to H-3 (δ4.48) was not shifted downfield compared with that of **1a** (δ4.55), which clearly indicated the location of the arabinofuranose unit in **1** to be at this position (C-3). The configuration of the anomeric carbon of **1** was determined to be α on the basis of the small *J* value (s) of the anomeric proton signal (δ4.12) in the ^{1}H NMR spectrum of **1** [8]. Thus **1** is (+)-taxifolin 3-*O*-α-L-arabinofuranoside. This is its first report in nature.

EXPERIMENTAL

^{1}H and ^{13}C NMR: 270 and 67.5 MHz, respectively, locked to the major deuterium resonance of the solvents (acetone-d_6 and $CDCl_3$). TLC: precoated Kieselgel 60 F254 plates, spots detected by UV illumination and visualized by spraying with 10% H_2SO_4, 2% $FeCl_3$ and/or anisaldehyde–H_2SO_4 reagents.

---

*Author to whom correspondence should be addressed.

Table 1. ^1H NMR spectral data for **1**, **1a** and **1b** (270 MHz, δ values)

H	**1***	**1a***	**1b**†
Flavononol			
2	5.09 1H, *d* (11.3)	4.98 1H, *d* (11.3)	5.35 1H, *d* (10.1)
3	4.91 1H, *d* (11.3)	4.55 1H, *d* (11.3)	4.38 1H, *d* (10.1)
6	5.95 1H, *d* (1.4)	5.96 1H, *d* (1.4)	6.79 1H, *d* (1.5)
8	5.91 1H, *d* (1.4)	5.92 1H, *d* (1.4)	6.60 1H, *d* (1.5)
2′	7.08 1H, *d* (1.6)	7.05 1H, *d* (1.6)	7.38 1H, *d* (1.6)
5′	6.86 1H, *d* (5.1)	6.83 1H, *d* (5.1)	7.25 1H, *d* (7.6)
6′	6.92 1H, *dd* (5.1, 1.6)	6.87 1H, *dd* (5.1, 1.6)	7.42 1H, *dd* (1.6, 7.6)
Arabinofuranose			
1	4.12 1H, *s*		4.29 1H, *s*
2	3.78 1H, br s		5.09 1H, *d* (0.7)
3	3.78 1H, *br s*		4.88 1H, *dd* (0.7, 6.8)
4	4.20 1H, *m*		4.69 1H, *m*
5	3.62 2H, *br d* (2.6)		4.17 1H, *dd* (3.2, 12.6) 4.43 1H, *dd* (1.6, 12.6)
Ac			2.02, 2.08, 2.15, 2.30, 2.31, 2.32, 2.39 each 3H, *s*

*In acetone-d_6 + D_2O.

†In $CDCl_3$.

Coupling constants (*J* in Hz) in parentheses.

Table 2. ^{13}C NMR spectral data for **1**, **1a** and **1b** (67.5 MHz, δ values)

C	**1***	**1a***	**1b**†
Flavanonol			
2	83.9 (83.3)	84.4	81.2
3	74.2 (73.6)	73.1	77.1
4	198.2 (196.7)	198.1	187.3
4a	102.6 (101.9)	101.4	111.0
5	165.5 (165.0)	164.9	156.2
6	97.7 (97.5)	97.0	109.0
7	168.8 (169.3)	167.7	162.3
8	96.8 (96.4)	96.6	111.3
8a	164.8 (163.8)	164.0	151.4
1′	129.6 (128.3)	129.6	134.5
2′	116.2 (116.2)	115.6	122.2
3′	146.5 (147.5)	145.6	142.3
4′	147.5 (148.4)	146.4	142.8
5′	116.6 (116.5)	115.7	123.9
6′	121.3 (120.2)	120.7	125.5
Arabinofuranose			
1	108.0 (107.9)		105.9
2	80.8 (81.2)		80.5
3	79.4 (79.3)		77.2
4	90.1 (89.5)		80.9
5	63.2 (63.0)		63.0
Ac-Me			20.5, 20.6, 20.7 (× 2), 20.8, 21.0, 21.2
Ac-CO			167.8, 167.9, 168.0, 169.0, 169.4, 170.5, 170.7

*In acetone-d_6 + D_2O. Values in parentheses for **1** are in pyridine-d_5.

†In $CDCl_3$.

Plant material. Plants of *Fragaria x ananassa* Duch. cv. Reikov were propagated and cultivated in Tsukuba, Japan. Voucher specimens are deposited at Faculty of Agriculture, Saga University.

Extraction and isolation. Lyophilized roots (200 g) of *F. x ananassa* Duch. cv. Reikov were mashed and extracted at room temp. with 80% aq. Me_2CO (1.5 l and 1 l × 2). The extract, after concn. under red. pres., was subjected to Sephadex LH-20 (3.1 cm × 36 cm) CC and eluted by 60% MeOH to afford three frs (Frs 1–3). Fr. 2 was sepd by MCl-gel CHP 20P (3.2 cm × 23 cm, H_2O–MeOH) to give frs 21, 22 and 23. These three frs were purified by Bondapak C18 Porasil B (H_2O–MeOH) and Sephadex LH-20 (EtOH) CC to give **2** (3.6 g), **3** (330 mg), **4** (2.3 g), **6** (73 mg) (from fr. 21), **5** (86 mg) (from fr. 22) and **1** (661 mg) (from fr. 23). Compounds **2**–**6** were identified by the comparison of ^1H NMR spectral data with literature values.

(+)-taxifolin 3-O-α-L-arabinofuranoside (**1**). Amorphous powder, $[\alpha]_D^{25}$ −32.0° (MeOH; *c* 0.4); ^1H NMR: see Table 1; ^{13}C NMR: see Table 2; high-resolution negative SI-MS *m/z* (rel. int.): 435.0925 $[M - H]^-$ (97).

Partial hydrolysis of **1** *with hesperidinase.* A soln of **1** (113 mg) in H_2O (2 ml) was incubated with hesperidinase (90 mg) (Sigma H-8137) for 19 days at 37°. Compound **1a**, crystallized in the reaction mixt. was sepd by filtration. Compound **1a** (48 mg), needles, was identified as (+)-taxifolin; $[\alpha]_D^{25}$ + 17.0° (Me_2CO; *c* 0.3); ^1H NMR: see Table 1. The filtrate, treated with MeOH, was sepd by Sephadex LH-20 (2.3 cm × 23 cm, MeOH) and Bondapak C18 Porasil (2.6 cm × 18 cm, H_2O–MeOH) CC to afford **1** (20 mg, recovered) and L-(+)-arabinose (23 mg); $[\alpha]_D^{25}$ + 72.0° (H_2O; *c* 0.5); TLC in $CHCl_3$-MeOH-H_2O (13:10:2), R_f 0.56, in C_6H_6-$EtCO_2H$-HCO_2H (1:7:1), R_f 0.14. The L-(+)-

arabinose was also identified by direct comparison of its ^{1}H and ^{13}C NMR (in pyridine-d_5) spectral data with those of an authentic sample.

Partial hydrolysis of **1** *with* 1N HCl. A soln of **1** (61 mg) in 1N HCl (1 ml) was incubated for 16 hr at 50°. The reaction mixt. after neutralization with $Ba(OH)_2$, was subjected to Sephadex LH-20 (2.3 cm × 23 cm) and eluted with H_2O to afford **1a** (32 mg) and L-(+)-arabinose (15 mg).

Heptaacetate (**1b**). Amorphous powder, $[\alpha]_D^{25} + 6.1°$ ($CHCl_3$; *c* 0.5); ^{1}H NMR: see Table 1; ^{13}C NMR: see Table 2; FAB-MS *m/z* (rel. int): 731 $[M + H]^+$ (0.76), 753 $[M + Na]^+$ (2.49), 769 $[M + K]^+$ (0.23).

Acknowledgements—The authors are grateful to Dr T. Tanaka (Faculty of Pharmaceutical Sciences, Nagasaki University) for measurements of optical rotation and MS. This work was supported in part by Grants-in-Aid for Science Research (06760036) from the Ministry of Education, Science and Culture of Japan to K. Ishimaru and by a Ministry of Health and Welfare, Science Research Fund Subsidy granted to the Japan Health Science Foundation.

REFERENCES

1. Sondheimer, E. and Kertesz, Z. I. (1948) *J. Am. Chem. Soc.* **70**, 3476.
2. Vennat, B., Pourrat, A., Texier, O., Pourrat H. and Gaillard, J. (1987) *Phytochemistry* **26**, 261.
3. Hsu, F.-L., Nonaka, G. and Nishioka, I. (1985) *Chem. Pharm. Bull.* **33**, 3142.
4. Thompson, R. S., Jacques, D., Haslam, E. and Tanner, R. J. N. (1972) *J. Chem. Soc. Perkin Trans. I* 1387.
5. Fletcher A. C., Porter, L. J., Haslam, E. and Gupta, R. K. (1977) *J. Chem. Soc. Perkin Trans. I* 1628.
6. Tanaka, T., Nonaka, G. and Nishioka, I. (1985) *J. Chem. Res.* (*M*) 2001.
7. Nonaka, G., Goto Y., Kinjo, J., Nohara, T. and Nishioka, I. (1987) *Chem. Pharm. Bull.* **35**, 1105.
8. Higuchi, R. and Komori, T. (1987) *Phytochemistry* **26**, 2357.