PERGAMON

Phytochemistry 51 (1999) 879–881

PHYTOCHEMISTRY

A naphthalene glycoside from callus cultures of *Diospyros kaki*

Miwako Gondo[a], Norie Tanaka[a], Takashi Tanaka[b], Koichiro Shimomura[c], Fumi Nakanishi[d], Kanji Ishimaru[a,*]

[a]*Department of Applied Biological Sciences, Faculty of Agriculture, Saga University, 1 Honjo, Saga 840-8502, Japan*
[b]*Faculty of Pharmaceutical Sciences, Nagasaki University, 1-14 Bunkyo-machi, Nagasaki 852-8521, Japan*
[c]*Tsukuba Medicinal Plant Research Station, National Institute of Health Sciences, 1 Hachimandai, Tsukuba, Ibaraki 305-0843, Japan*
[d]*Department of Biology, Tokyo Gakugei University, Koganei, Tokyo 184-8501, Japan*

Received 27 May 1998; received in revised form 24 September 1998; accepted 24 September 1998

Abstract

Calli of *Diospyros kaki* Thunb. were induced on half-strength Murashige–Skoog solid medium supplemented with 1.0 mg l^{-1} IAA and 0.1 mg l^{-1} BA in the dark and successfully subcultured on the same medium. A new phenolic metabolite, 7-methyl-1, 4, 5-trihydroxy-naphthalene 4-*O*-(6′-*O*-β-xylopyranosyl)-β-glucopyranoside, was isolated from MeOH extract of the callus cultures and its chemical structure was elucidated by NMR spectroscopic analysis.

Keywords: *Diospyros kaki*; Ebenaceae; Callus; Naphthalene; Hydroxynaphthalene; Glycoside; Primeverose

1. Introduction

Japanese persimmon, *Diospyros kaki*, is well-known to bear two types of tannin-rich fruits; one is a non-astringent type and the other is an astringent type which is edible after reduction of its astringency. These fruits are used in the food industry as market products and food ingredients and also for scientific (phytochemical) research. Recently, biochemical studies on the high molecular tannins (proanthocyanidin polymers) have been done, for example, on the degradation (Matsuo & Itoo, 1978), bioactivity (Matsuo et al., 1991b), mechanism of deastringency (Matsuo, Shinohara & Ito, 1976; Matsuo & Itoo, 1982; Matsuo, Itoo & Ben-Arie, 1991a; Pesis, Levi & Ben-Arie, 1988; Tanaka, Takahashi, Kouno & Nonaka, 1994) etc. Plant tissue culture work with this plant have also been done mainly on micropropagation (regeneration from callus (Tamura, Tao & Sugiura, 1992; Tao & Sugiura, 1992) and protoplasts (Tamura, Tao & Sugiura, 1995), but not on its secondary metabolism. In the present study, callus cultures of the plant were established and the secondary metabolites of the cultures were studied. A new naphthalene glycoside was isolated from the cultures and the chemical structure elucidated by spectroscopic evidences. The naphthalene glycoside was a biosynthetically related metabolite to naphthoquinones, which were identified in intact plants of *D. kaki* (Tezuka, Kuroyanagi, Yoshihira & Natori, 1972; Tezuka et al., 1973).

2. Results and discussion

Calli of an astringent persimmon *D. kaki* Thunb. were derived from leaf segments of the in vivo plant. The calli were subcultured on half-strength Murashige–Skoog (1/2 MS) solid medium (Murashige & Skoog, 1962) supplemented with 1.0 mg l^{-1} IAA and 0.1 mg l^{-1} BA in the dark for over 8 months. The

* Corresponding author.

0031-9422/99/$ - see front matter
PII: S0031-9422(99)00131-4

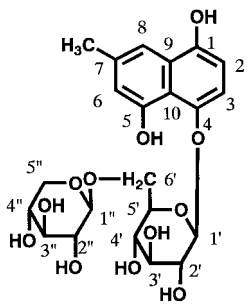
Table 1
^{1}H and ^{13}C NMR spectral data of **1** (in DMSO-d_6, δ values). Coupling constants (J in Hz) in parentheses

	C	H
1	148.13	–
2	107.76	6.68 d (8.3)
3	110.82	7.16 d (8.3)
4	146.68	–
5	153.03	–
6	112.54	6.62 br.d (1.6)
7	135.32	–
8	112.41	7.35 dq (1.6 / −0.9)
9	127.01	–
10	114.22	–
7-Me	21.33	2.36 s
1′	103.40	4.81 d (7.6)
2′	73.41	3.30 m
3′	76.41	3.27 m
4′	69.97	3.14 m
5′	76.14	3.54 m
6′	68.49	3.61 dd (11.2, 6.9)
		4.02 dd (11.2, 1.4)
1″	104.05	4.23 d (7.3)
2″	73.36	3.02 m
3″	76.24	3.06 m
4″	69.59	3.26 m
5″	65.54	3.01 m
		3.70 dd (11.4, 5.5)
1-OH	–	9.61 s
5-OH	–	9.25 s
2′-OH	–	5.65 d (5.0)
3′-OH	–	5.17 d (4.3)
4′-OH	–	5.13 d (5.7)
2″-OH	–	4.91 d (10.5)
3″-OH	–	4.85 d (4.6)
4″-OH	–	4.88 d (5.0)

MeOH extract of the lyophilized calli was separated by Sephadex LH-20 and Preparative C18 125 Å column chromatography to afford compound **1**.

Compound **1** was obtained as an off-white amorphous powder exhibiting $[M–H]^-$ peak at m/z 483 ($C_{22}H_{28}O_{12}$) in the negative FABMS. The ^{1}H NMR spectrum (Table 1) of **1** showed *ortho* (δ 6.68 and 7.16) and *meta* (δ 6.62 and 7.35) coupled aromatic resonances as well as a methyl (δ 2.36) proton signal. These signals, in conjunction with 11 carbon signals (C-1–10 and a methyl) in the ^{13}C NMR spectrum (Table 1) of **1**, had chemical shifts which were identical with those of the aglycone of rossoliside (Budzianowski, 1995), i.e. it clearly indicated the presence of 7-methyl1-1, 4, 5-trihydroxy-naphthalene structure. The assignment of the aglycone (C-1–10) was supported by the data of its HMBC correlation spectrum. The ^{13}C NMR spectrum of **1** exhibited signals of hexose (δ 68.49, 69.97, 73.41, 76.14, 76.41 and 103.40) and pentose (δ 65.54, 69.59, 73.36, 76.24 and 104.05) moieties, each of which were characterized as glucopyranose and xylopyranose, respectively, by their chemical shifts of the carbon signals. The correlations observed between C-4 and H-1′ signals in HMBC of **1** indicated the glucose anomeric carbon to be connected at C-4. The low-field (chemical) shift of C6′ (δ 68.49), accompanied with the signal of C-1″ (δ 104.05), also showed the xylose anomeric carbon to be connected to glucose C-6′. The interglycosidic linkage between glucose C-6′ and xylose C-1″ was also supported by HMBC spectrum. The configuration of the anomeric carbons of glucose and xylose moieties were concluded to be β from the J values (7.6 Hz of H-1′ and 7.3 Hz of H-1″) in ^{1}H NMR spectrum. From these spectroscopic data, **1** was characterized as 7-methyl-1, 4, 5-trihydroxy-naphthalene 4-*O*-(6′-*O*-β-xylopyranosyl)-β-glucopyranoside.

Naphthalene and naphthoquinone glycosides, sometimes observed in *Drosera* (Budzianowski, 1995, 1996) and *Dionaea* (Kreher, Neszmelyi & Wagner, 1990) genera, are well known to have interesting pharmaceutical activities such as immunomodulation, phagocytosis, antimicrobial, antifungal and antitumor. In the aspect of plant chemotaxonomy, it is noteworthy that a new naphthalene diglycoside, whose chemical structure resembles to those in Droseraceae, was produced in this species *(Diospyros kaki)* Although the data are not shown, the MeOH extract of *D. kaki* callus cultures, established in this study, contained small amount of flavonoid such as (+)-gallocatechin (0.064%, dry weight).



1

3. Experimental

^{1}H and ^{13}C NMR: 500 and 125 MHz, respectively, references against the major deuterium signal of the solvents (DMSO-d_6). TLC was conducted on silica gel and spots were detected by UV illumination and visualized by spraying with 5% H_2SO_4. MS solid medium used for experiments contained 30 g l^{-1} sucrose. All media were adjusted to pH 5.7 before autoclaving at 121° for 15 min. Cultures were placed in the dark at

25°C. Pieces of calli were cultured in sterile petri dish (9 cm in the diameter, a piece of the callus per one petri dish) containing 25 ml medium.

3.1. Plant material and induction of the callus

Leaf segments of *Diospyros kaki* Thunb. (Japanese name; Kakinoki) collected in August 1996 at Saga city, Japan, were surface sterilized and placed aseptically on 1/2 MS solid medium (solidified with 2.5 g l^{-1} gelrite) supplemented with 1.0 mg l^{-1} IAA and 0.1 mg l^{-1} BA. After 2 months of culturing, the calli derived on the segments were cut off, transferred to the same medium and subcultured at 20 days intervals. The calli subcultured for over 8 months were used for the extraction. Voucher specimens are deposited at Faculty of Agriculture, Saga University.

3.2. Extraction and isolation of naphthalene glycoside

Lyophilized calli (17 g) were mashed and extracted at room temp. with MeOH (150 ml × 5). The extract, after concn under red. pres., was subjected to Sephadex LH-20 (3.0 × 21 cm) CC and eluted with H_2O containing increasing amount of MeOH (60–100%) to afford 6 frs (Frs 1–6). Fr. 2 was applied on Sephadex LH-20 (2.5 × 18.5 cm) (EtOH) CC and next to preparative C18 125 Å (2.0 × 15 cm) (H_2O–MeOH) CC to afford **1** (21.6 mg).

3.3. 7-Methyl-1, 4, 5-trihydroxynaphthalene 4-O-(6′-O-β-xylopyranosyl)-β-glucopyranoside (*1*)

An off-white amorphous powder, $[\alpha]_D^{17}$ -128.5° (DMSO; c 0.4); ^1H and ^{13}C NMR: see Table 1 (assignments were aided with HMBC); Negative FABMS *m*/*z* (rel. int): 483 $[M–H]^-$ (22).

Acknowledgements

This work was supported in part by a subsidy from The San-Ei Gen Foundation for Food Chemical Research and by the Ministry of Health and Welfare, Science Research Fund Subsidy granted to the Japan Health Science Foundation.

References

Budzianowski, J. (1995). *Phytochemistry*, *40*, 1145.
Budzianowski, J. (1996). *Phytochemistry*, *42*, 1145.
Kreher, B., Neszmelyi, A., & Wagner, H. (1990). *Phytochemistry*, *29*, 605.
Matsuo, T., Shinohara, J., & Itoo, S. (1976). *Agric. Biol. Chem.*, *40*, 215.
Matsuo, T., & Itoo, S. (1978). *Agric. Biol Chem.*, *42*, 1637.
Matsuo, T., & Itoo, S. (1982). *Agric. Biol. Chem.*, *46*, 683.
Matsuo, T., Itoo, S., & Ben-Arie, R. (1991a). *Jpn. Soc. Hort. Sci.*, *60*, 437.
Matsuo, T., Kinoshita, M., Itoo, S., Shimoi, K., Okada, Y., & Tomita, I. (1991b). *J. Jpn. Soc. Hort. Sci.*, *60*, 429.
Murashige, T., & Skoog, F. (1962). *Physiol. Plant.*, *15*, 473.
Pesis, E., Levi, A., & Ben-Arie, R. (1988). *J. Food Sci.*, *53*, 153.
Tamura, M., Tao, R., & Sugiura, A. (1992). *Hort. Science*, *27*, 1048.
Tamura, M., Tao, R., & Sugiura, A. (1995). *Plant Science*, *108*, 101.
Tanaka, T., Takahashi, R., Kouno, I., & Nonaka, G. (1994). *J. Chem. Soc. Perkin Trans.*, *I*, 3013.
Tao, R., & Sugiura, A. (1992). *Hort Science*, *7*, 259.
Tezuka, M., Kuroyanagi, M., Yoshihira, K., & Natori, S. (1972). *Chem. Pharm. Bull.*, *20*, 2029.
Tezuka, M., Takahashi, C., Kuroyanagi, M., Satake, M., Yoshihira, K., & Natori, S. (1973). *Phytochemistry*, *12*, 175.