

NEW FLAVONOID GLYCOSIDES FROM *LINARIA JAPONICA*

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LINARIA JAPONICA

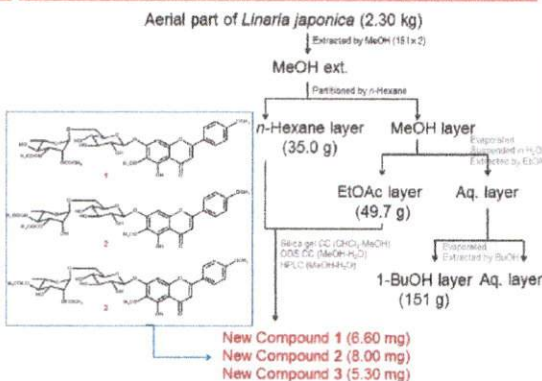
Family : Scrophulariaceae
 Genus : *Linaria*
 Species : *Linaria japonica*
 Japanese : un-ran (ウンラン)
 Chinese : 海蘭
 Korean : 해란초



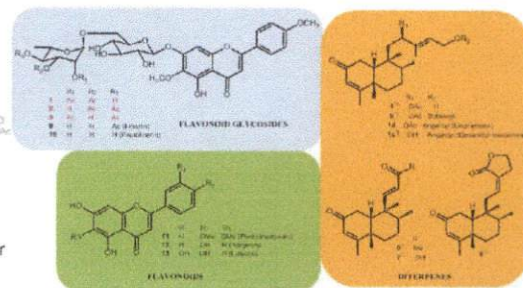
Phytochemical investigations:
 several flavonoid glycosides, iridoid glycosides
 including a chlorine-containing iridoid glycoside,
 linarioside, and phenylethanoids

Traditional used : diuretic and purgative

Extraction and Isolation



Isolated Compounds



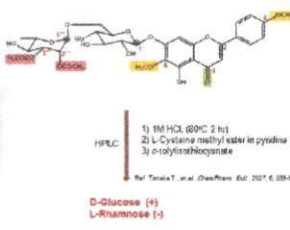
Sugimoto, S. et al. The 12th Annual Meeting of the Pharmaceutical Society of Japan (2014), 225-225E
 Compound 15 is not available in literature.

Compound 1

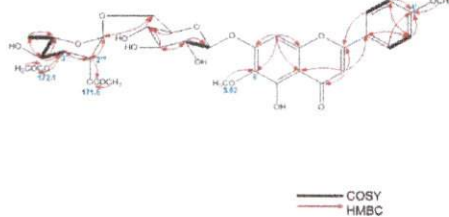
yellow amorphous solid
 HR-ESI-MS (positive) m/z : 729.1980 [M+H]⁺
 (calcd. for C₂₃H₂₃O₇, Na : 729.2001)
 [α]_D²⁰ = +21 (c = 0.77, MeOH)
 UV λ_{max} (MeOH) (log ε) 324 (3.83), 221 (3.80),
 274 (3.84), 222 (3.83)
 IR ν_{max} (film) cm⁻¹: 3437, 2933, 1746, 1659, 1608,
 1589, 1503, 1480, 1381, 1261, 1182, 1064,
 827, 667, 598

¹³C and ¹H NMR data of Compound 1 (150 and 500 MHz, MeOD)

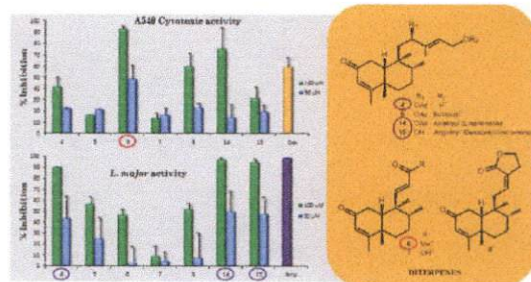
Position	¹³ C	¹ H
2	166.9	-
3	104.6	6.62 (1H, s)
4	158.6	-
5	154.2	-
6	134.0	-
7	157.9	-
8	95.6	6.64 (1H, s)
9	154.2	-
10	108.0	-
1'	124.7	-
2', 6'	129.9	7.98 (2H, d, 8 Hz)
3', 5'	115.3	7.01 (2H, d, 8 Hz)
4'	164.5	-
5-OCH ₃	61.7	3.82 (3H, s)
4-OCH ₃	56.2	3.82 (3H, s)
1"	103.8	6.11 (1H, d, 7 Hz)
2"	74.9	3.50 (1H, s, 9 Hz)
3"	77.9	3.47 (1H, s, 9 Hz)
4"	71.6	3.33 (1H, m)
5"	77.3	3.68 (1H, m)
6"	67.5	3.67 (1H, m)
1"	99.3	4.67 (1H, d, 11 Hz)
2"	71.1	5.09 (1H, dd, 3, 1 Hz)
3"	73.2	4.63 (1H, dd, 3, 9 Hz)
4"	71.3	3.32 (1H, d, 9 Hz)
5"	75.0	3.67 (1H, m)
6"	18.0	1.11 (2H, d, 6 Hz)
2"-OOCCH ₃	205.6	1.87 (3H, s)
1"-OOCCH ₃	171.5	-
3"-OOCCH ₃	20.8	1.85 (3H, s)
4"-OOCCH ₃	172.1	-



HMBC and COSY of Compound 1



% Inhibition of Isolated Compounds (Diterpenes) toward A549 Cytotoxic and *L. major* Activity

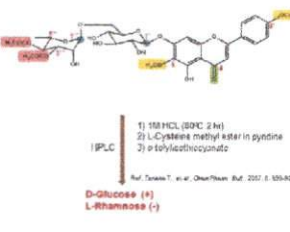


Compound 2

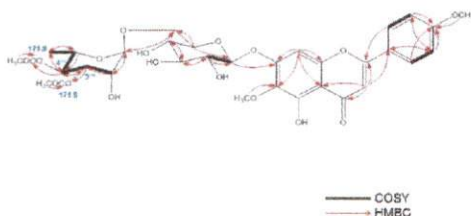
yellow amorphous solid
 HR-ESI-MS (positive) m/z : 728.1987 [M+H]⁺
 (calcd. for C₂₃H₂₃O₇, Na : 729.2001)
 [α]_D²⁰ = -56 (c = 1.20, MeOH)
 UV λ_{max} (EtOH) (log ε) 329 (3.84), 222 (3.80),
 275 (3.85), 220 (3.85)
 IR ν_{max} (film) cm⁻¹: 3443, 2932, 1736, 1465, 1607,
 1562, 1510, 1488, 1360, 1250, 1192, 1044,
 836, 669

¹³C and ¹H NMR data of Compound 2 (150 and 500 MHz, MeOD)

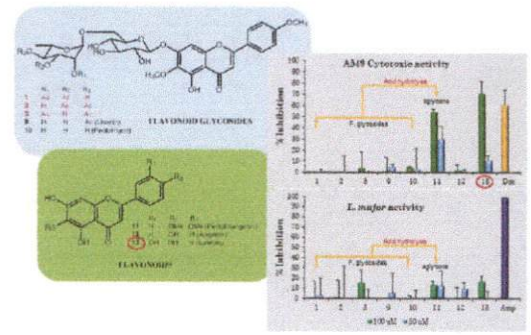
Position	¹³ C	¹ H
2	166.9	-
3	104.6	6.61 (1H, s)
4	158.6	-
5	154.2	-
6	134.0	-
7	157.9	-
8	95.6	6.64 (1H, s)
9	154.2	-
10	107.8	-
1'	124.8	-
2', 6'	129.9	7.87 (2H, d, 9 Hz)
3', 5'	115.9	7.00 (2H, d, 9 Hz)
4'	164.6	-
5-OCH ₃	61.7	3.82 (3H, s)
4-OCH ₃	56.2	3.82 (3H, s)
1"	103.8	6.12 (1H, d, 7 Hz)
2"	74.9	3.52 (1H, m)
3"	75.3	3.49 (1H, m)
4"	71.3	3.41 (1H, m)
5"	77.2	3.67 (1H, m)
6"	67.1	3.67 (1H, m)
1"	99.3	3.97 (1H, d, 9 Hz)
2"	70.5	3.91 (1H, m)
3"	73.4	4.84 (1H, m)
4"	72.5	4.94 (1H, m)
5"	67.7	3.71 (1H, m)
6"	17.7	0.90 (2H, d, 6 Hz)
2"-OOCCH ₃	20.8	1.76 (3H, s)
1"-OOCCH ₃	171.5	-
3"-OOCCH ₃	20.9	1.91 (3H, s)
4"-OOCCH ₃	171.9	-



HMBC and COSY of Compound 2



% Inhibition of Isolated Compounds (flavonoids) toward A549 Cytotoxic and *L. major* Activity

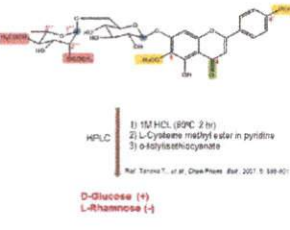


Compound 3

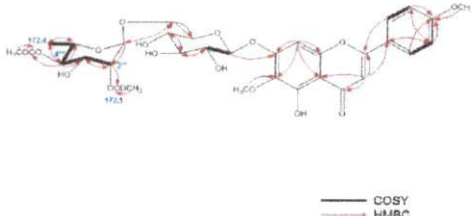
yellow amorphous solid
 HR-ESI-MS (positive) m/z : 729.1997 [M+H]⁺
 (calcd. for C₂₃H₂₃O₇, Na : 729.2001)
 [α]_D²⁰ = -68 (c = 0.35, MeOH)
 UV λ_{max} (EtOH) (log ε) 340 (4.30), 319 (3.78),
 276 (3.78), 229 (3.78)
 IR ν_{max} (film) cm⁻¹: 3381, 2921, 1739, 1692, 1600,
 1580, 1506, 1487, 1360, 1230, 1182, 1051,
 827, 679, 677

¹³C and ¹H NMR data of Compound 3 (150 and 500 MHz, MeOD)

Position	¹³ C	¹ H
2	166.9	-
3	104.6	6.64 (1H, s)
4	158.6	-
5	154.2	-
6	134.7	-
7	157.9	-
8	96.1	6.88 (1H, s)
9	154.2	-
10	107.9	-
1'	124.5	-
2', 6'	129.7	7.90 (2H, d, 8 Hz)
3', 5'	115.9	7.02 (2H, d, 8 Hz)
4'	164.7	-
5-OCH ₃	61.7	3.88 (3H, s)
4-OCH ₃	56.3	3.82 (3H, s)
1"	103.8	6.12 (1H, d, 7 Hz)
2"	75.0	3.60 (1H, m)
3"	77.9	3.46 (1H, m)
4"	70.9	3.46 (1H, m)
5"	78.9	-
6"	66.8	3.05 (1H, m)
1"	98.8	4.67 (1H, d)
2"	73.9	4.59 (1H, dd, 3, 2 Hz)
3"	68.8	-
4"	75.1	4.72 (1H, m)
5"	67.7	3.71 (1H, m)
6"	17.7	0.85 (2H, d, 6 Hz)
2"-OOCCH ₃	20.2	1.56 (3H, s)
1"-OOCCH ₃	172.1	-
3"-OOCCH ₃	21.1	1.56 (3H, s)



HMBC and COSY of Compound 3



Conclusion

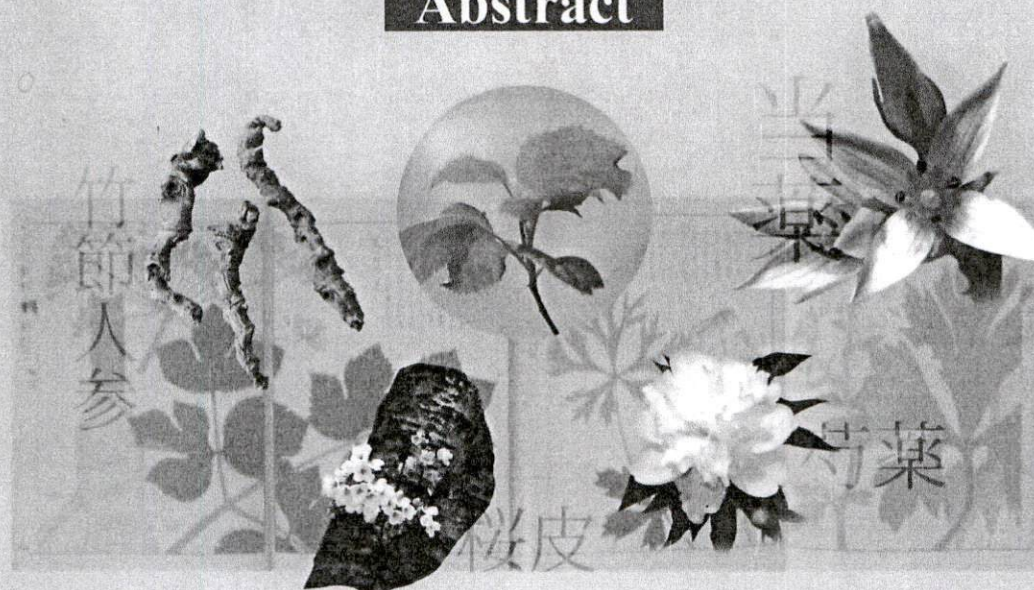
➢ Chemical investigation of the mixture of hexane and ethyl acetate layer of *Linaria japonica* led to the isolation of three new flavonoid glycosides (1-3), five new diterpenoids (4-8), two known flavonoid glycosides (9-10), three known flavonoids (11-13) and two known diterpenes (14-15)

➢ The isolated compounds were evaluated the A549 cytotoxic and *L. major* activity. Among them, new diterpenoid (6) showed the strongest inhibition activity for A549 cytotoxic and new diterpenoid (4), linarienone (14), and desacetyl-linarienone (15) have strong inhibition activity for *L. major*

The 8th JSP-CCTCNM-KSP Joint Symposium on Pharmacognosy

*The Joint Symposium will be held in conjunction with
the 61st Annual Meeting of JSP.

Abstract



Date | September 13 (Sat), 2014

Venue | Faculty of Pharmaceutical Sciences,
Fukuoka University

8-19-1 Nanakuma, Jonan-ku, Fukuoka

Hosted by

The Japanese Society of Pharmacognosy (JSP)

Chinese Committee of Traditional Chinese Natural Medicines (CCTCNM)

The Korean Society of Pharmacognosy (KSP)

The 8th JSP-CCTCNM-KSP Joint Symposium Executive Committee Office:
Division of Pharmacognosy, Institute of Natural Medicine, University of Toyama
Chairperson of the Executive Committee: Prof. Katsuko Komatsu
E-mail: shigen@inm.u-toyama.ac.jp



日本生薬学会

- SP-34 Comparative Analysis of Phenolic Peroxynitrite-Scavengers in Nine Korean Wild Vegetables of Compositae
Agung Nugroho¹, Sang-Cheol Lim², Subash Karki³, Jae Sue Choi³, Hee-Juhn Park⁴
¹Department of Agro-industrial Technology, Lambung Mangkurat University; ²Department of Horticulture and Landscape; ³Department of Pharmaceutical Engineering, Sangji University; ⁴Department of Food and Nutrition, Pukyong University;
- SP-35 Aromatic natural products from Actinomycetes with TRAIL-resistance overcoming activity
Xuefeidan Liu, Kazufumi Toume, Kentaro Tsukahara, Masami Ishibashi
Grad. Sch. Pharm., Chiba Univ.
- SP-36 Elmenol A and B, new benzofuran-naphthalenes from *Streptomyces* sp. IFM11490
Yixizhuoma, Kazufumi Toume, Kentaro Tsukahara, Masami Ishibashi
Grad. Sch. Pharm. Sci., Chiba Univ.
- SP-37 Unique Polyketides from the Jellyfish-derived Fungus *Paecilomyces variotii*
Haibo Wang¹, Eun La Kim¹, Jongki Hong², Kyung Sook Bae³ and Jee H. Jung^{1,*}
¹College of Pharmacy, Pusan National University, ²College of Pharmacy, Kyoung Hee University, ³Korea Research Institute of Bioscience and Biotechnology
- SP-38 Changes in ginsenoside composition of ginseng flower buds extracts after a ultrasonication process
Yun Min Nam¹, Jue Hee Kwon², Hye Jung An², Sung Hyun Yoon², Sung Kwon Ko^{2,*}
¹Research Institute, Koyon, ²The Department of Oriental Medical Food & Nutrition, Semyung University
- SP-39 Phenolic Antioxidants from *Aconogonon molle*
Khem Raj Joshi^{1,2}, Hari Prasad Devkota^{1,2}, Takashi Watanabe³, Shoji Yahara¹
¹Graduate School of Pharmaceutical Sciences, Kumamoto University, ²Program for Leading Graduate Schools "HIGO (Health life science: Interdisciplinary and Global Oriented) Program", Kumamoto University, ³Research Organization for Regional Alliances, Kochi University of Technology
- SP-40 A new polyoxygenated cyclohexane and other constituents from *Kaempferia rotunda* and their cytotoxic activity
Subehan^{1,2}, Hiroyuki Morita¹
¹Institute of Natural Medicine, University of Toyama, ²Hasanuddin University
- SP-41 Secondary metabolites from cultures of marine fungus *Penicillium glabrum*
Jin-Young Min, Sang Hee Shim*
School of Biotechnology, Yeungnam University
- SP-42 Three new neolignans isolated from feces of *Troglodytes xanthipes*
Yuna Kjm,¹ So Yoon Baek,¹ Byung-sun Min,² Sang Hee Shim^{1,*}
¹School of Biotechnology, Yeungnam University, ²College of Pharmacy, Catholic University of Daegu
- SP-43 Flavonoid Glycosides from Leaves of *Crateva unilocularis*
Hari Prasad Devkota^{1,2}, Khem Raj Joshi^{1,2}, Takashi Watanabe³, Shoji Yahara²
¹Program for Leading Graduate Schools, Health life science: Interdisciplinary and Global Oriented (HIGO) Program, Kumamoto University, ²Graduate School of Pharmaceutical Sciences, Kumamoto University, ³Research Organization for Regional Alliances, Kochi University of Technology
- SP-44 Crataegusin A and B, novel flavanocoumarins from the dried fruits of *Crataegus pinnatifida* var. *major*
Kohei Kazuma¹, Yuka Isobe¹, Haruka Asahina², Motoyoshi Satake² and Katsuhiro Kono¹
¹Institute of Natural Medicine, University of Toyama, ²Institute of Environmental Science for Human Life, Ochanomizu University
- SP-45 New Flavonoid Glycosides from *Linaria japonica*
Retno Widjowati^{1,2}, Sachiko Sugimoto¹, Yoshi Yamano¹, Hideaki Otsuka^{1,3}, Katsuyoshi Matsunami¹
¹Graduate School of Biomedical and Health Sciences, Hiroshima University, ²Faculty of Pharmacy, Airlangga University, ³Faculty of Pharmacy, Yasuda Women's University
- SP-46 Barceloneic acid C, a new polyketide from an endophytic fungus *Phoma* sp. JS752 and its antibacterial activities
Sunghee Bang, Changyeol Lee, Xuekui Xia, Sang Hee Shim*
School of Biotechnology, Yeungnam University

Event Outline

Organization & Outline

Symposium Name:

The 8th JSP-CCTCNM-KSP Joint Symposium on Pharmacognosy

Date:

September 13 (Sat), 2014

Venue:

Faculty of Pharmaceutical Sciences, Fukuoka University
(8-19-1 Nanakuma, Jonan-ku, Fukuoka, JAPAN)

Hosted by:

The Japanese Society of Pharmacognosy (JSP),
Chinese Committee of Traditional Chinese Natural Medicines (CCTCNM),
The Korean Society of Pharmacognosy (KSP)

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Assoc. Prof. Yohei Sasaki

(College of Medical, Pharmaceutical and Health Sciences, Kanazawa University)

Assist. Prof. Shu Zhu

(Institute of Natural Medicine, University of Toyama)

For official use

New Flavonoid Glycosides from *Linaria japonica*

Retno Widyowati^{1,2}, Sachiko Sugimoto¹, Yoshi Yamano¹, Hideaki Otsuka^{1,3}, Katsuyoshi Matsunami¹

¹ Graduate School of Biomedical and Health Sciences, Hiroshima University, Japan;

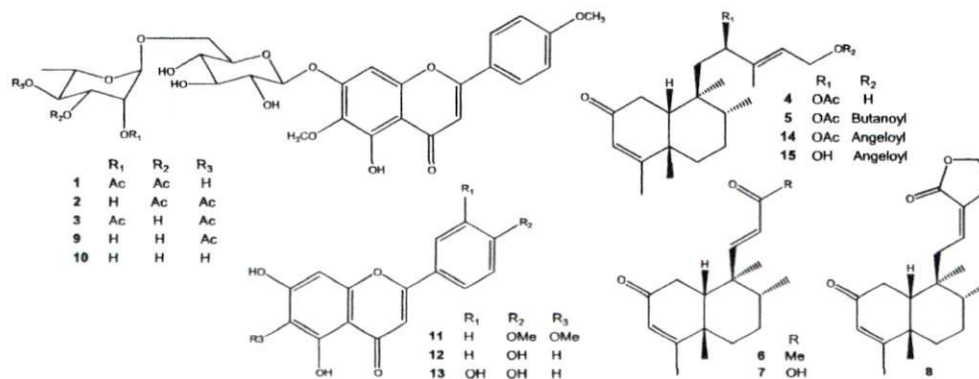
² Faculty of Pharmacy, Airlangga University, Indonesia;

³ Faculty of Pharmacy, Yasuda Women's University, Hiroshima, Japan

[Objective] *Linaria japonica* (ウンラン, 海蘭, 해란초, Scrophulariaceae) is a perennial herb which grows on the sandy place along seashores in Japan with elliptic and fleshy leaves. The whole plant extract is used as a folk medicine due to its diuretic and purgative pharmacological activities. In our previous phytochemical investigations on this plant, several new flavonoid, iridoid and megastigmane glycosides were isolated from 1-butanol fraction¹⁾. In this study, further phytochemical investigation was performed on non-polar fraction, *i.e.* mixture of hexane-ethyl acetate soluble fraction of this plant.

[Methods] Whole plants of *Linaria japonica* were collected in Tottori prefecture. The air-dried plants were extracted with methanol two times. The obtained methanol extract was concentrated and suspended in water, then partitioned with *n*-hexane, ethyl acetate, and 1-butanol.

[Results] On investigation of the mixture of hexane-ethyl acetate layer of the plant, three new flavonoid glycosides (**1-3**) and five new diterpenes (**4-8**)²⁾ along with five known flavonoid glycosides (**9-10**), three known flavonoids (**11-13**) and two known diterpenes (**14, 15**) were isolated by various chromatographic techniques such as silica gel, ODS column chromatography and HPLC. The structures of these compounds were determined as follows by spectrometric analysis (UV, IR, 1D- and 2D-NMR, and HR-ESI MS).



1) Otsuka H, *Phytochemistry*, 39(5), 1111-14(1995) and references cited therein.

2) Retno W, *et al.* The 134th Annual Meeting of the Pharmaceutical Society of Japan (2014), 28L-am05S