SHORT REPORT

A C G publications

Rec. Nat. Prod. 16:6 (2022) 645-650

records of natural products

Degranulation Inhibitors from Petals of *Coreopsis grandiflora*

Takeru Koga [1], Nanako Shiki [2], Hideyuki Ito [3], Yuji Iwaoka [3] and Akihiro Tai [2,4*]

[1] *Graduate School of Advanced Technology and Science, Tokushima University, 2-1 Minamijosanjima-cho, Tokushima 770-8506, Japan*
[2] *Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima, 5562 Nanatsuka-cho, Shobara, Hiroshima 727-0023, Japan*
[3] *Faculty of Health and Welfare Science, Okayama Prefectural University, 111 Kuboki, Soja, Okayama 719-1197, Japan*
[4] *Graduate School of Technology, Industrial and Social Sciences, Tokushima University, 2-1 Minamijosanjima-cho, Tokushima 770-8513, Japan*

(Received February 10, 2022; Revised April 06 2022; Accepted April 09, 2022)

Abstract: The extracts of petals of *Coreopsis grandiflora*, a member of the Asteraceae family, were found to inhibit degranulation in RBL-2H3 cells, and lanceoletin, leptosidin, okanin, and 4-methoxylanceoletin were isolated as degranulation inhibitors by liquid-liquid separation and column chromatography. The intensities of the antigen-stimulated degranulation inhibitory activities of the four flavonoids were in the order of leptosidin, okanin, lanceoletin and 4-methoxylanceoletin. Leptosidin, which inhibited antigen-stimulated degranulation most greatly, inhibited calcium ionophore-stimulated degranulation with the same intensity as that of lanceoletin and okanin. As a result of investigation of the structure-activity relationships of the four isolated active compounds for antigen-stimulated degranulation inhibitory activities, it was found the inhibitory activity of lanceoletin, a chalcone, was enhanced by forming a C ring and becoming leptosidin, an aurone. In addition, the three chalcones tended to have greater degranulation inhibitory activity as their molecule polarities increased.

Keywords: Degranulation inhibitory activity; aurone; chalcone; *Coreopsis grandiflora*; RBL-2H3 cells.

1. Plant Source

Petals from *Coreopsis grandiflora* (1.55 kg, fr. wt) were obtained in Prefectural University of Hiroshima (Shobara, Japan) on May 25, 2018. The plant was identified by Prof. Dr. A.T. and a voucher specimen (number PUHS2018T) was deposited at the Faculty of Life and Environmental Sciences, Prefectural University of Hiroshima.

2. Previous Studies

Secondary metabolites of plants such as flavonoids have been used as folk medicines throughout human history [1]. The flowers of *Chrysanthemum morifolium*, a plant of the Asteraceae

* Corresponding author: E-Mail: atai@tokushima-u.ac.jp

The article was published by ACG Publications
http://www.acgpubs.org/journal/records-of-natural-products November-December 2022 EISSN:1307-6167
DOI: http://doi.org/10.25135/rnp.319.2202.2351
Available online: April 19, 2022

family, are known to have a suppressing effect on the release of *β*-hexosaminidase from antigen-stimulated RBL-2H3 cells [2]. The flowers and leaves of *Cirsium maritimum* Makino of the Asteraceae family were found to have an inhibitory effect on degranulation, and cirsimaritin, which is a flavone, was isolated from the leaves [3]. These plants all belong to the Asteraceae family, and it has been suggested that Asteraceae plants might be effective for suppressing degranulation. *Coreopsis lanceolate*, which belongs to the genus Coreopsis in the family Asteraceae, has been reported to have an antioxidant effect [4] and an anti-leukemia effect [5], and the possibility of an anti-allergic effect has also been reported [6]. However, little is known about the biological activity of *Coreopsis grandiflora* Hogg ex Sweet, which belongs to the same genus Coreopsis that *Coreopsis lanceolate* belongs to. Therefore, this study focused on the petals of *Coreopsis grandiflora* and aimed at the isolation and identification of inhibitors of degranulation of allergic mediators in RBL-2H3 cells. Herein, the purification and identification of active compounds and their structure-activity relationships are reported.

3. Present Study

Petals from *Coreopsis grandiflora* were extracted with 70% MeOH/water, and the extract showed antigen-stimulated degranulation inhibitory activity in RBL-2H3 cells (Figure S1 in the Supporting Information). To investigate degranulation inhibitors from petals of *Coreopsis grandiflora*, bioassay-guided purification was carried out. Then, four degranulation inhibitors, compounds **1**-**4** (Figure 1), were isolated by liquid-liquid separation and various chromatography techniques from the extract and identified as lanceoletin, leptosidin, okanin and 4-methoxylanceoletin, respectively, by NMR and MS analyses (see Supporting Information). The four compounds isolated from petals of *Coreopsis grandiflora* are already known compounds and have been reported to be contained in the flowers of *Coreopsis lanceolate*, which belongs to the same genus Coreopsis in the family Asteraceae [5]. Lanceoletin, leptosidin and 4-methoxylanceoletin have been reported to have a function of inhibiting DPP-4, which is an enzyme that decomposes incretins, and they are expected to be useful for lowering blood glucose levels [7]. The three compounds have also been reported to have strong DPPH radical scavenging activity [6,8]. Okanin has been reported to significantly suppress

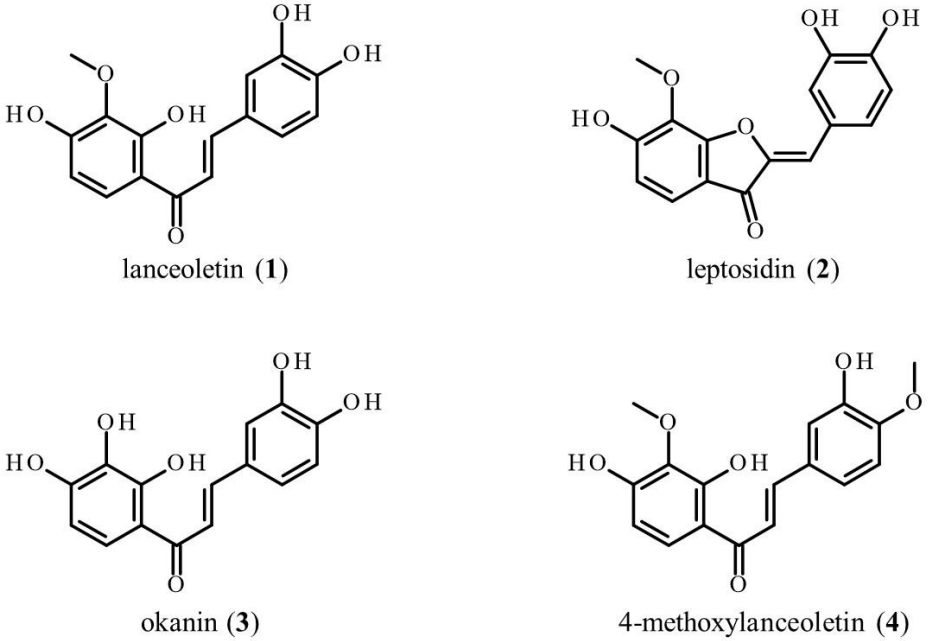

Figure 1. Chemical structures of compounds **1**-**4** isolated from extracts of petals of *Coreopsis grandiflora*.

LPS-induced expression of iNOS and suppress the production of IL-6 and TNF-α in LPS-stimulated BV-2 cells [9]. In another study, 4-methoxylanceoletin showed potent antiproliferative activity against human leukemia HL-60 cells [5]. However, no study has been conducted on the degranulation inhibitory activities of these four compounds. Therefore, the degranulation inhibitory activities of lanceoletin, leptosidin, okanin and 4-methoxylanceoletin are effects discovered for the first time in this study.

In order to investigate the effect of antigen-stimulated degranulation inhibitory activity due to the difference in chemical structure, the inhibitory activities of lanceoletin, leptosidin, okanin and 4-methoxylanceoletin isolated from the extract of petals of *Coreopsis grandiflora* were compared (Figure 2). The degranulation inhibitory activities of these compounds were compared with the inhibitory activity of oxatomide, which is actually used in the treatment of allergies and is known to have inhibitory activity [10,11]. The activities of samples and oxatomide were evaluated at the concentration range for obtaining the IC_{50} values. Then the IC_{50} values were calculated by the results obtained (Table 1). The inhibitory activities of the four isolated compounds were greater than the inhibitory activity of oxatomide. Leptosidin, which is the only aurone among the four isolated compounds, had the greatest activity. A comparison of the activities of the three chalcones showed that the intensity of activity was in the order of okanin, lanceoletin and 4-methoxylanceoletin. Overall, the inhibitory activities in antigen-stimulated degranulation were in the order of leptosidin >> okanin > lanceoletin > 4-methoxylanceoletin. Since leptosidin, an aurone, had a lower IC_{50} value than that of lanceoletin, which is a chalcone-type compound before the aurone is cyclized, it is thought that the formation of the C ring of chalcone enhances the degranulation inhibitory activity. In addition, the structure-activity relationships of the three isolated chalcones showed that the activity of okanin was the greatest, and the activity tended to decrease as the number of methyl groups increased to one and then to two. Some literatures suggest that chalcones increase their degranulation inhibitory activity as increasing of their molecule polarities [12,13]. It was suggested that increasing polarity and

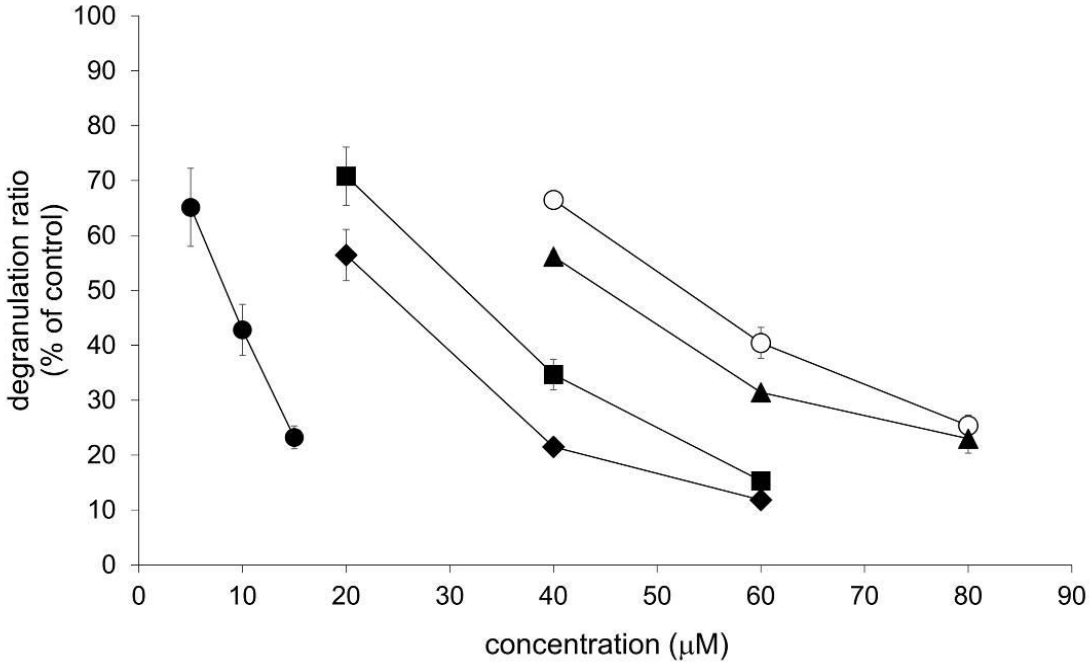

Figure 2. Inhibitory effects of lanceoletin, leptosidin, okanin and 4-methoxylanceoletin on antigen-induced degranulation in RBL-2H3 cells. Oxatomide was used as a positive control (○). Anti-dinitrophenyl (DNP)-immunoglobulin E-sensitized RBL-2H3 cells were incubated with lanceoletin (■), leptosidin (●), okanin (◆) and 4-methoxylanceoletin (▲) and stimulated with DNP-human serum albumin. All data represent means ± SD of three independent cultures.

formation of a C-ring of the molecules were important for the strong antigen-stimulated degranulation inhibitory activity. The degranulation inhibitory activity of leptosidin (IC_{50} value of 8.3 μM) was much stronger than that of cirsimaritin (IC_{50} value of 65.5 μM) shown in the previous study. In addition, leptosidin has inhibitory activity comparable to that of kaempferol, which is reported to have strong inhibitory activity (IC_{50} value of 7.5 μM) [14]. These facts suggest that leptosidin has considerably a strong degranulation inhibitory activity.

The inhibitory activities of the four isolated flavonoids on calcium ionophore A23187-stimulated degranulation were also evaluated in RBL-2H3 cells (Table 1). Calcium signaling is one of the downstream signaling pathways activated by antigen stimulation. The four flavonoids showed greater activity than that of oxatomide for inhibiting calcium ionophore-stimulated degranulation as well as antigen-stimulated degranulation. The inhibitory activities in calcium ionophore-stimulated degranulation were in the order of leptosidin ≈ okanin ≈ lanceoletin > 4-methoxylanceoletin. These results suggest that at least four flavonoids inhibit the later calcium signaling. Leptosidin, an aurone, had the greatest inhibitory activity in antigen-stimulated degranulation among the four isolated flavonoids (Table 1), although leptosidin had the same inhibitory activity as that of okanin and lanceoletin in calcium ionophore-stimulated degranulation. These results suggested that leptosidin not only inhibits points after calcium signaling as well as okanin, lanceoletin and 4-methoxylanceoletin but may also inhibit other points.

Table 1. IC_{50} values of degranulation inhibitory activities of lanceoletin, leptosidin, okanin and 4-methoxylanceoletin

	IC_{50} values of antigen-mediated degranulation (μM)	IC_{50} values of Ca^{2+} ionophore-stimlated degranulation (μM)
lanceoletin	31.4 ± 1.9	19.7 ± 3.9
leptosidin	8.3 ± 1.0	21.3 ± 8.7
okanin	23.4 ± 2.3	19.5 ± 7.3
4-methoxylanceoletin	45.0 ± 0.7	40.6 ± 9.2
oxatomide	52.8 ± 1.5	43.3 ± 6.5

The isolation and identification of four flavonoids, lanceoletin, leptosidin, okanin, and 4-methoxylanceoletin, from petals of *Coreopsis grandiflora* are described in this paper. These compounds are known compounds and have already been reported to be contained in Asteraceae flowers. In this study, degranulation inhibitory activities of these compounds in RBL-2H3 cells were discovered for the first time. To our best knowledge, leptosidin was discovered as the first degranulation inhibitor in aurone. The intensities of antigen-stimulated degranulation inhibitory activities of the four flavonoids were in the order of leptosidin >> okanin > lanceoletin > 4-methoxylanceoletin. The degranulation inhibitory activity of leptosidin seems to be stronger than that of kaempferol, a flavonoid known as a strong degranulation inhibitor [14,15]. In addition, the inhibitory activities in calcium ionophore-stimulated degranulation were in the order of leptosidin ≈ okanin ≈ lanceoletin > 4-methoxylanceoletin. It was considered that leptosidin had a degranulation inhibitory activity with a different mechanism from the other isolated flavonoids. Investigation of the structure-activity relationships of the four isolated flavonoids for antigen-stimulated degranulation revealed that the activity of lanceoletin, a chalcone, is improved by the formation of a C ring to become leptosidin, an aurone. In addition, among the three isolated chalcones, the results suggested that the degranulation inhibitory activity increases as the polarities of the molecules increase. The results of the present study suggested that leptosidin could be an appropriate lead compound for the development of degranulation inhibitors to treat type I allergy.

Acknowledgments

The authors are grateful to the SC-NMR Laboratory of Okayama University for providing the facilities to conduct their experiments.

Supporting Information

Supporting Information accompanies this paper on http://www.acgpubs.org/journal/records-of-natural-products

ORCID
Takeru Koga: 0000-0003-0481-1532
Nanako Shiki: 0000-0003-2742-4279
Hideyuki Ito: 0000-0002-5756-2069
Yuji Iwaoka: 0000-0003-0980-3013
Akihiro Tai: 0000-0003-0285-1469

References

[1] A. Gurib-Fakim (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow, *Mol. Aspects Med.* **27**, 1-93.
[2] Y. Y. Xie, J. L. Qu, Q. L. Wang, Y. Wang, M. Yoshikawa and D. Yuan (2012). Comparative evaluation of cultivars of *Chrysanthemum morifolium* flowers by HPLC-DAD-ESI/MS analysis and antiallergic assay, *J. Agric. Food Chem.* **60**, 12574-12583.
[3] M. Tanaka, M. Suzuki, Y. Takei, T. Okamoto and H. Watanabe (2017). *Cirsium maritimum* Makino inhibits the antigen/immunoglobulin-E-mediated allergic response *in vitro* and *in vivo*, *J. Agric. Food Chem.* **65**, 8386-8391.
[4] H. G. Kim, Y. S. Jung, S. M. Oh, H. J. Oh, J. H. Ko, D. O. Kim, S. C. Kang, Y. G. Lee, D. Y. Lee and N. I. Baek (2020). Coreolanceolins A-E, new flavanones from the flowers of *Coreopsis lanceolate*, and their antioxidant and anti-inflammatory effects, *Antioxidants* **9**, 539.
[5] A. Pardede, K. Mashita, M. Ninomiya, K. Tanaka and M. Koketsu (2016). Flavonoid profile and antileukemic activity of *Coreopsis lanceolata* flowers, *Bioorg. Med. Chem. Lett.* **26**, 2784-2787.
[6] S. Tanimoto, M. Miyazawa, T. Inoue, Y. Okada and M. Nomura (2009). Chemical constituents of *Coreopsis lanceolata* L. and their physiological activities, *J. Oleo. Sci.* **58**, 141-146.
[7] B. R. Kim, S. B. Paudel, J. W. Nam, C. H. Jin, I. S. Lee and A. R. Han (2002). Constituents of *Coreopsis lanceolata* flower and their dipeptidyl peptidase IV inhibitory effects, *Molecules* **25**, 4370.
[8] D. Nakabo, Y. Okano, N. Kandori, T. Satahira, N. Kataoka, J. Akamatsu and Y. Okada (2018). Convenient synthesis and physiological activities of flavonoids in *Coreopsis lanceolata* L. petals and their related compounds, *Molecules* **23**, 1671.
[9] Y. Hou, G. Li, J. Wang, Y. Pan, K. Jiao, J. Du, R. Chen, B. Wang and N. Li (2017). Okanin, effective constituent of the flower tea *Coreopsis tinctoria*, attenuates LPS-induced microglial activation through inhibition of the TLR4/NF-κB signaling pathways, *Sci. Rep.* **7**, 45705.
[10] J. J. Paulussen, M. J. Fischer, R. L. Roozendaal, V. C. van der Heijden, P. van Dijken, N. J. de Mol and L. H. Janssen (1998). Effects of oxatomide and derivatives on high affinity IgE receptor-activated signal transduction pathways in rat basophilic leukemia cells: role of protein tyrosine hyperphosphorylation and inhibition of extracellular calcium influx, *Biochem. Pharmacol*. **56**, 693-701.
[11] M. K. Church and C. F. Gradidge (1980). Oxatomide: inhibition and stimulation of histamine release from human lung and leucocytes *in vitro*, *Agents Actions* **10**, 4-7.
[12] T. Itoh, M. Ninomiya, Y. Nozawa and M. Koketsu (2010). Chalcone glycosides isolated from aerial parts of *Brassica rapa* L. 'hidabeni' suppress antigen-stimulated degranulation in rat basophilic leukemia RBL-2H3 cells, *Bioorg. Med. Chem.* **18**, 7052-7057.
[13] O. Yodsaoue, S. Cheenpracha, C. Karalai, C. Ponglimanont and S. Tewtrakul (2009). Anti-allergic activity of principles from the roots and heartwood of *Caesalpinia sappan* on antigen-induced *β*-hexosaminidase release, *Phytother. Res.* **23**, 1028-1031.

[14] H. Cheong, S. Y. Ryu, M. H. Oak, S. H. Cheon, G. S. Yoo and K. M. Kim (1998). Studies of structure activity relationship of flavonoids for the anti-allergic actions, *Arch. Pharm. Res*. **21**, 478-480.

[15] F. Xu, H. Matsuda, H. Hata, K. Sugawara, S. Nakamura and M. Yoshikawa (2009). Structures of new flavonoids and benzofuran-type stilbene and degranulation inhibitors of rat basophilic leukemia cells from the Brazilian herbal medicine *Cissus sicyoides*, *Chem. Pharm. Bull.* **57**, 1089-1095.