Biochemical Systematics and Ecology 83 (2019) 4–6

Contents lists available at ScienceDirect

Biochemical Systematics and Ecology

journal homepage: www.elsevier.com/locate/biochemsyseco

Investigation of chemical compounds from *Chlamydomonas* sp. KSF108 (Chlamydomonadaceae)

Huynh Nguyen Khanh Tran[a], Jeong Ah Kim[b], Ui Joung Youn[c], Sanghee Kim[c], Mi Hee Woo[a], Byung Sun Min[a,*]

[a] College of Pharmacy, Drug Research and Development Center, Daegu Catholic University, Gyeongbuk 38430, Republic of Korea
[b] College of Pharmacy, Research Institute of Pharmaceutical Sciences, Kyungpook National University, Daegu 41566, Republic of Korea
[c] Division of Life Sciences, Korea Polar Research Institute, Incheon 21990, Republic of Korea

ARTICLE INFO

Keywords:
Chlamydomonadaceae
Chlamydomonas
Norisoprenoid
Chemotaxonomy

ABSTRACT

Nine compounds (**1–9**) including one norisoprenoid (**1**), one polyol-glycoside (**2**), three sterols (**3–5**), three phenols (**6**, **8**, and **9**), and one fatty acid (**7**) were isolated from *Chlamydomonas* sp. KSF108. Their chemical structures were established using NMR spectroscopic techniques and compared with published data. None of the compounds have been previously reported from the genus *Chlamydomonas* and they may therefore serve as chemotaxonomic markers for *Chlamydomonas* sp. KSF108 within the genus.

1. Subject and source

Microalgae are a polyphyletic group of unicellular phytosynthetic eukaryotes comprising at least 40,000–70,000 species from various phyla such as Cyanophyta (blue algae), Rhodophyta (red algae), Chlorophyta (green algae), Pyrrophyta, Cryptophyta, Haptophyta, Heterokontophyta, and Streptophyta (Gimpel et al., 2015; Suh et al., 2017).

Chlamydomonas is a genus containing many species of green algae in the globally-distributed family Chlamydomonadaceae. Previous chemical investigation of the algae revealed the presence of carotenoids, fatty acids, sterols, mycosporine-like amino acids, phycobilins, polyketides, pectins, halogenated compounds, and toxins (Borowitzka, 2013; Leu et al., 2014; Cardozo et al., 2007; Hallmann, 2007; Milledge, 2011; Pulz et al., 2004).

The Antarctic freshwater microalga *Chlamydomonas* sp. KSF108 was collected from freshwater near King Sejong Antarctic Station (62° 13′ S, 58° 47′ W) in 2014 and maintained in Bold's basal medium (BBM) with incubation at 2–3 °C under continuous lighting from light-emitting diodes at an intensity of 35 μmol photons m^{-2} s^{-1}.

Botanical identification was performed by Professor Sanghee Kim and the voucher specimen, *Chlamydomonas* sp. KSF108 (KPRI-KSF108), was deposited at the herbarium of the Division of Polar Life Sciences, Korea Polar Research Institute, Korea.

2. Previous work

Chemical studies have revealed that algae contain a variety of constituents, including fatty acids, sterols, carotenoids, mycosporine-like amino acids, phycobilins, polyketides, pectins, halogenated compounds, and toxins (Borowitzka, 2013; Leu et al., 2014; Cardozo et al., 2007; Hallmann, 2007; Milledge, 2011; Pulz et al., 2004). However, investigation into the chemical composition of extracts from the genus *Chlamydomonas* has been limited. Hence, we carried out a chemical investigation of *Chlamydomonas* sp. KSF108 in the present study.

3. Present study

3.1. General experiment procedures

The 1D and 2D NMR spectra were obtained using Varian Unity Inova 400 MHz and a Bruker Ascend™ 500 MHz spectrometer with tetramethylsilane (TMS) as an internal standard and the chemical shifts were recorded in δ values (ppm). Mass spectrum was recorded using a JEOL JMS-AX 300L spectrometer. Genomic DNA extraction of microalga *Chlamydomonas* sp. KSF0108 was performed using a DNeasy Plant Mini Kit. PCR amplification of nuclear SSU rDNA was carried out using ExTaq polymerase and specific primers G01 and G07. Sequencing was analyzed by Macrogen, Inc. using an ABI 3730xl DNA Sequencer and the sequence was aligned using the Genetic Data Environment (GDE 2.2) program (Smith et al., 1994). The optimal model was determined

* Corresponding author.
E-mail address: bsmin@cu.ac.kr (B.S. Min).

https://doi.org/10.1016/j.bse.2018.12.009
Received 21 September 2018; Received in revised form 23 November 2018; Accepted 22 December 2018
Available online 27 December 2018
0305-1978/

using MODELTEST ver. 3.7 (Posada et al., 1998) and the molecular identification was analyzed using Bayesian (Huelsenbeck et al., 2001) and Randomized Axelerated Maximum Likelihood (RAxML) methods (Stamatakis, 2006).

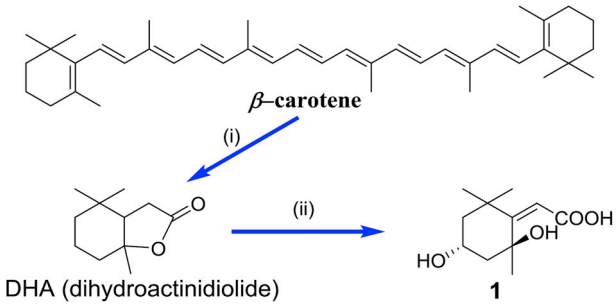
3.2. Extraction and isolation

Freeze dried *Chlamydomonas* sp. KSF108 (110.0 g) was extracted three times (3 h × 500 mL) with methanol. After removing the solvent under reduced pressure, the residue was suspended in H_2O and then successively partitioned with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH. The *n*-hexane-soluble fraction (7.0 g) was separated chromatographically on a silica gel column (60 × 3.5 cm, 63–200 μm particle size, Merck) using a stepwise gradient of *n*-hexane-EtOAc (40:1 to 0:1, each 500 mL) to yield eight fractions (HFr.1-HFr.8), distinguishable by their TLC profiles. HFr.3 (2.5 g) was subsequently subjected to silica gel column chromatography (60 × 3.5 cm) and eluted with *n*-hexane-EtOAc (30:1) to yield 4 sub-fractions (HFr.3-1 to 3–4). HFr.3.3 (1.5 g) was chromatographed on a silica gel column (60 × 3.5 cm) using a gradient solvent system of *n*-hexane-acetone (15:1 to 0:1) to yield compounds **3** (10.0 mg), **4** (2.0 mg), **5** (2.0 mg), **6** (3.0 mg), and **7** (50.0 mg). The fractions of CH_2Cl_2 and EtOAc were combined (1.7 g) and subjected to silica gel column chromatography (60 × 3.5 cm) with *n*-hexane-EtOAc (2:1) to yield 6 sub-fractions (HFr.9 to 14). HFr. 12 (280.0 mg) was further purified using a YMC RP-18 column with an MeOH-H_2O mobile phase (4:1 to 6:1), and a semi-preparative Waters HPLC systems with an isocratic solvent system of 80% MeOH in H_2O + 0.1% trifluoroacetic acid (flow rate 5 mL/min) over 90 min as an eluent, UV detection at 254 and 210 nm to yield **8** (2.1 mg) and **9** (6.1 mg). The *n*-BuOH and H_2O fractions (5.4 g) were combined and subjected to separation on a silica gel column (60 × 3.5 cm) using a gradient solvent system of CH_2Cl_2-MeOH-H_2O (60:1:0.1 to 0:0:1). Subsequently, the resultant eluents were separated using a semi-preparative Waters HPLC systems with an isocratic solvent system of 90% MeOH in H_2O + 0.1% trifluoroacetic acid (flow rate 5 mL/min) as an eluent over 90 min, with UV detection at 254 and 210 nm. This resulted in the isolation of compounds **1** (10.5 mg) and **2** (3.8 mg).

By comparison of their physical and spectroscopic data with those of compounds reported in the literature, the chemical structures of the compounds were identified as (*Z*)-2-(2,4-dihydroxy-2,6,6-trimethylcyclohexylidene)acetic acid (**1**) (Wu et al., 2014), lilioside D (**2**) (Kaneda et al., 1984), cholesterol (**3**) (Popjak et al., 1976), β-sitosterol (**4**) (Salleh et al., 2016), hurgadacin (**5**) (Shaaban et al., 2013), α-tocopherol (**6**) (Cohen et al., 1976), 9*Z*,12*Z*-nonadecadienoic acid (**7**) (Fang et al., 2012), *m*-hydroxyphenol (**8**) (Landy et al., 1999), and *p*-methoxybenzaldehyde (**9**) (Tran et al., 2018) (Fig. 1).

4. Chemotaxonomic significance

Here, nine compounds (**1–9**) including one norisoprenoid (**1**), one polyol-glycoside (**2**), three sterols (**3–5**), three phenols (**6**, **8**, and **9**), and one fatty acid compound (**7**) are reported for the first time from *Chlamydomonas* sp. KSF108.



Scheme 1. Biosynthetic Pathway of **1** from β-carotene.

In this study, a norisoprenoid is reported for the first time from the genus *Chlamydomonas* (Cardozo et al., 2007). Its precursor may be β-carotene, modified sequentially by phyto-oxygenation (i) and hydration (ii), to produce (*Z*)-2-(2,4-dihydroxy-2,6,6-trimethylcyclohexylidene) acetic acid (**1**). Among the products formed under phyto-oxygenation conditions, DHA was the first compound produced during heat treatment of β-carotene at 30 °C (Kanasawud et al., 1990; Manuela et al., 2009; Isoe et al., 1969). The ring-opening hydration of lactone was described and made possible through a variety of mechanisms for hydrolyzed esters (Bombarelli et al., 2013). A putative biosynthesis transformation mechanism from β-carotene to **1** is presented in Scheme 1. **1** might represent useful chemotaxonomic marker for *Chlamydomonas* sp. KSF108.

A polyol-glycoside was expected to be produced from polysaccharides, which are the main structural components of algae, such as *Chromochloris zofingiensis*, *Acutodesmus obliguus*, and *Chlorella sorokiniana* species (Potin et al., 1999; Trincone et al., 1995; Kaneda et al., 1984; Bartosova et al., 2015). In this study, compound **2**, a polyol-glycoside, was found in the genus *Chlamydomonas* for the first time, which could confirm the chemotaxonomic relationship between *Chlamydomonas* sp. KSF108 and the other species of *Chlamydomonas*.

In this study, three sterols (**3–5**) were isolated for the first time from the genus *Chlamydomonas*. Sterols are one of the most important chemical constituents of microalgae such as *Pyramimonas cf. cordata* and *Atteya ussurensis* sp. nov. and a major component in the diet of aquacultured organisms (Ponomarenko et al., 2004). In particular, phytosterols are reported to be found in a variety of substituted groups, added to carbon-24 of the C27 cholestance side chain. They are an important chemotaxonomic biomarker for microalgae (Nes, 2000; Leblond and Chapman, 2002).

Phenols are classes of primary metabolites and mono-phenyl compounds, α-tocopherol (**6**), *m*-hydroxyphenol (**8**), and *p*-methoxybenzaldehyde (**9**) have been found in most algae: *Anabaena doliolum*, *Spongiochloris spongiosa, Porphyra tenera* and *Undaria pinnatifida* (Onofrejová et al., 2010), but were first isolated from the genus *Chlamydomonas*. They may serve as precursors in the formation of secondary

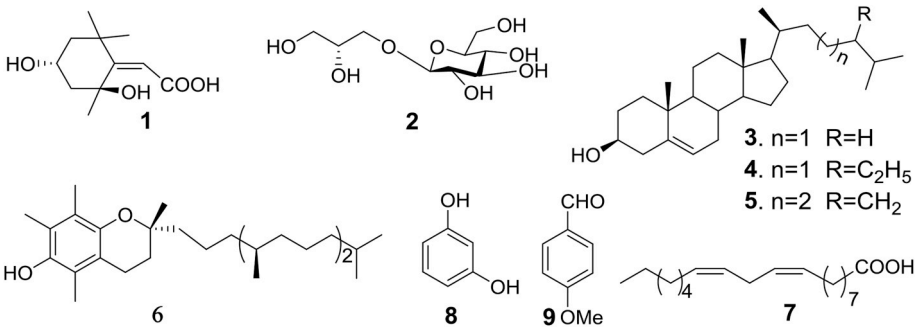


Fig. 1. The structure of compounds (**1–9**) isolated from *Chlamydomonas* sp. KSF108.

microalgal metabolites, such as halogenated and polyphenolic compounds (Butler and Franklin, 2004) and another chemical markers to differentiate *Chlamydomonas* sp. KSF108 from other species of *Chlamydomonas*.

Fatty acids with two methylene-interrupted double bonds as 9*Z*,12*Z*-nonadecadienoic acid (**7**) confirming by mass spectrum analysis with EIMS at *m/z* 294 $[M]^+$ were commonly found in a variety of microalgae (Ginneken et al., 2011). Most species from the classes Cyanophyceae, Prymnesiophyceae, Bacillariophyceae, Rhodophyceae, Cryptophyceae, Chlorophyceae, Xantophyceae, and Eustigmatophyceae have been described in terms of their fatty acid profiles (Cardozo et al., 2007; Patil et al., 2007; Otles et al., 2001), and fatty acid was described as major compound in the genus *Chlamydomonas* during its first isolation. Therefore, the isolation of **7** from *Chlamydomonas* sp. KSF108 could be of chemotaxonomic significance and serve as valuable chemotaxonomic markers for *Chlamydomonas* sp. KSF108.

In conclusion, the structural skeleton of **1** − **9** were firstly isolated from species of *Chlamydomonas*. Especially, polyol-glycoside (**2**), sterols (**3**–**5**), phenols (**6**, **8** and **9**) and fatty acid (**7**) exist widely in microalgae and could potentially be used to describe the chemotaxonomic relationships between *Chlamydomonas* species and other lineages. Ours is the first report to describe the chemical composition of a *Chlamydomonas* species, which may serve as chemotaxonomic markers for the identification of genus. However, further studies into the chemical makeup of other *Chlamydomonas* species are required.

Acknowledgement

This research was supported by a grant to the Korea Polar Research Institute, KOPRI, under project PE18180. We are grateful to the Korea Basic Science Institute (KBSI) for mass spectrometric measurements.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bse.2018.12.009.

References

Bartosova, A., Blinova, L., Gerrulova, K., 2015. Characterisation of polysaccharides and lipids from selected green algae species by FTIR-ATR spectroscopy. J. Slovak Univ. Technol. 23, 97–102. https://doi.org/10.1515/rput-2015-0011.

Butler, A., Franklin, J.N.C., 2004. The role of vanadium bromoperoxidase in the biosynthesis of halogenated marine natural products. Nat. Prod. Rep. 21, 180–188. https://doi.org/10.1039/B302337K.

Bombarelli, R.G., Calle, E., Casado, J., 2013. Mechanisms of lactone hydrolysis in neutral and alkaline conditions. J. Org. Chem. 78, 6868–6879. https://doi.org/10.1021/jo400258w.

Borowitzka, M.A., 2013. High-value products from microalgae and their development and commercialisation. J. Appl. Phycol. 25, 743–756. https://doi.org/10.1007/s10811-013-9983-9.

Cardozo, K.H.M., Guaratini, T., Barros, M.P., Falcao, V.R., Tonon, A.P., Lopes, N.P., Campos, S., Torres, M.A., Souza, A.O., Colepicolo, P., Pinto, E., 2007. Metabolites from algae with economical impact. Comp. Biochem. Physiol. 146, 60–78. https://doi.org/10.1016/j.cbpc.2006.05.007.

Cohen, N., Eichel, W.F., Lopresti, R.J., Neukom, C., Saucy, G., 1976. Synthetic studies on (2*R*,4′*R*,8′*R*)-α-tocopherol. An approach utilizing side chain synthons of microbiological origin. J. Org. Chem. 42, 3505–3511. https://doi.org/10.1021/jo00884a002.

Fang, M.J., Fang, H., Li, W.J., Huang, D.M., W, Z., Zhao, Y.F., 2012. A new diphenyl ether from *Phoma sp*. Strain, SHZK-2. Nat. Prod. Res. 26, 1224–1228. https://doi.org/10.1080/14786419.2011.559947.

Gimpel, J.A., Henriquez, V., Mayfield, S.P., 2015. Metabolic Engineering of Eukaryotic Microalgae: In metabolic engineering of eukaryotic microalgae: potential and challenges come with great diversity. Front. Microbiol. 6, 1–14. https://doi.org/10.3389/fmicb.2015.01376.

Ginneken, V.J.T.V., Helsper, J.P.F.G., Visser, W.D., Keulen, H.V., Brandenburg, W.A., 2011. Polyunsaturated fatty acids in various macroalgal species from north atlantic and tropical seas. Lipids Health Dis. 10, 2–8. https://doi.org/10.1186/1476-511X-10-104.

Hallmann, A., 2007. Algal Transgenics and Biotechnology. Transgenic Plant Journal © Global Science Books, pp. 81–98.

Huelsenbeck, J.P., Ronquist, F., 2001. MRBAYES: bayesian inference of phylogenetic trees. Bioinformatics Oxford University Press, pp. 754–755.

Isoe, S., Hyeon, S.B., Sakan, T., 1969. Photo-oxygenation of carotenoids. The formation of hydroactinnidiolide and α-ionone from α-catotene. Tetrahedron Lett. 4, 279–281. https://doi.org/10.1016/S0040-4039(01)87530-4.

Kanasawud, P., Crouzet, J.C., 1990. Mechanism of formation of volatile compounds by thermal degradation of carotenoids in aqueous medium α-carotene degradation. J. Agric. Food Chem. 38, 1237–1244. https://doi.org/10.1021/jf00091a052.

Kaneda, M., Kobayashi, K., Nishida, K., Katsuta, S., 1984. Lilioside D and E, two glycerol glucoside from *lilium japonicum*. Phytochemistry 23, 795–798. https://doi.org/10.1016/S0031-9422(00)85029-3.

Landy, D., Fourmentin, S., Elhoujhaji, F., Surpateanu, G., 1999. ^{1}H NMR, circular dichoism and UV-VIS spectroscopic study of inclution complexes formation between *o*-, *m*-, *p*-hydroxyphenol and β-cyclodextrin. In: Proceedings of the Ninth International Symposium on Cyclodextrins, pp. 663–667. https://doi.org/10.1007/978-94-011-4681-4.

Leblond, J.D., Chapman, P.J., 2002. A survey of the sterol composition of the marine dinoflagellates *Karenia brevis, Karena mikimotoi*, and *Karlodinium micrum*: distribution of sterols within other members of the class Dinophyceae. J. Phycol. 38, 670–682. https://doi.org/10.1046/j.1529-8817.2002.01181.x.

Leu, S., Boussiba, S., 2014. Advances in the production of high-value products by microalgae. Ind. Biotechnol. 10, 169–183. https://doi.org/10.1089/ind.2013.0039.

Manuela, M., Pinto, M., 2009. Carotenoid breakdown products the norisoprenoids in wine aroma. Arch. Biochem. Biophys. 483, 236–245. https://doi.org/10.1016/j.abb.2009.01.008.

Milledge, J.J., 2011. Commercial application of microalgae other than as biofuels: a brief review. Rev. Environ. Sci. Biotechnol. 10, 31–41. https://doi.org/10.1007/s11157-010-9214-7.

Nes, W.D., 2000. Sterol methyl transferase: enzymology and inhibition. BBA-Mol. Cell Biol. L. 1529, 63–88. https://doi.org/10.1016/S1388-1981(00)00138-4.

Onofrejova, L., Vasickova, J., Klejdus, B., Stratil, P., Misurcova, L., Kracmas, S., Kopecky, J., Vacek, J., 2010. Bioactive phenols in algae: The application of pressurized-liquid and solid-phase extraction techniques. J. Pharmaceut. Biomed. Anal. 51, 464–470. https://doi.org/10.1016/j.jpba.2009.03.027.

Otles, S., Pire, R., 2001. Fatty acid composition of Chlorella and Spirulina microalgae species. J. AOAC Int. 84, 1708–1714. https://doi.org/10.5897/AJB11.1602.

Patil, V., Kallqvist, T., Olsen, E., Vogt, G., Gislerod, H.R., 2007. Fatty acid composition of 12 microalgae for possible use in aquaculture feed. Aquacult. Int. 15, 1–9. https://doi.org/10.1007/s10499-006-9060-3.

Ponomarenko, L.P., Stonik, I.V., Aizdaicher, N.A., Orlova, T.Y., Popvskaya, G.I., Pomazkina, G.V., Stonik, V.A., 2004. Sterols of marine microalgae *Pyramimonas cf. cordata* (prasinophyta), *Atteya ussurensis* sp. nov. (Bacollariophyta) and a spring diatom bloom form Lake Baikal. Comp. Biochem. Physiol., B 138, 65–70. https://doi.org/10.1016/j.cbpc.2004.02.007.

Popjak, G., Edmond, J., Anet, F.A.L., Easton, N.R., 1976. Carbon-13 NMR studies on cholesterol biosynthesized from [^{13}C] mevalonates. J. Am. Chem. Soc. 99, 931–935. https://doi.org/10.1021/ja00445a041.

Posada, D., Crandall, K.A., 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14, 817–818. https://doi.org/10.1093/bioinformatics/14.9.817.

Potin, P., Bouarab, K., Kupper, F., Kloareg, B., 1999. Oligosaccharide recognition signals and defence reactions in marine plant-microbe interactions. Curr. Opin. Microbiol. 2, 276–283. https://doi.org/10.1016/S1369-5274(99)80048-4.

Pulz, O., Gross, W., 2004. Valuable products from biotechnology of microalgae. Appl. Microbiol. Biotechnol. 65, 635–648. https://doi.org/10.1007/s00253-004-1647-x.

Salled, W.M.N.H.W., Ahmad, F., Yen, K.H., Zulkifli, R.M., 2016. Anticholinesterase and anti-inflammatory constituents from *Beilschmiedia pulverulenta* kostern. Nat. Prod. Sci. 22, 225–230. https://doi.org/10.20307/nps.2016.22.4.225.

Shaaban, M., Shaaban, K.A., Ghani, M.A., 2013. Hurgadacin: a new steroid from *Sinularia polydactyla*. Steroids 78, 866–873. https://doi.org/10.1016/j.steroids.2013.05.006.

Smith, S.W., Overbeek, R., Woese, C.R., Gilbert, W., Gillevet, P.M., 1994. The genetic data environment an expandable GUI for multiple sequence analysis. Comput. Appl. Biosci. 10, 671–675. https://doi.org/10.10 93/bioinformatics/10.6.671.

Stamatakis, 2006. RAxML-VI-HPC: maximum likelihood-based hylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22, 2688–2690. https://doi.org/10.1093/bioinformatics/btl446.

Suh, S., Yang, E.J., Lee, S.G., Youn, U.J., Han, S.H., Kim, I., Kim, S., 2017. Bioactivities of ethanol extract from the antartic freshwater microalga, *Chloromonas* sp. J. Med. Sci. 14, 560–569. https://doi.org/10.7150/ijms.18702.

Tran, H.N.K., Cao, T.Q., Kim, J.A., Youn, U.J., Kim, S., Woo, M.H., Min, B.S., 2018. Anti-inflammatory activities of compounds from the rhizome of *Cnidium officinale*. Arch Pharm. Res. 41, 977–985. https://doi.org/10.1007/s12272-018-1048-9.

Trincone, A., Improta, R., Gambacorta, A., 1995. Enzymatic synthesis of polyol- and mashed polyol-glycosides using β-glycosidase of *Sulfolobus solfataricus*. Biocatal. Biotransform. 12, 77–88. https://doi.org/10.31 09/10242429508998153.

Wu, Y., Su, J., Guo, R., Ren, T., Zhang, M., Dong, M., Sauriol, F., Shi, Q., Gu, Y., Huo, C., 2014. Two new non-taxoids from leaves of *Taxus cuspidata*. Chem. Nat. Comp. 50, 603–605. https://doi.org/10.1007/s10600-014-1033-6.