ORGANIC LETTERS

2012
Vol. 14, No. 6
1464–1467

# Ieodoglucomides A and B from a Marine-Derived Bacterium *Bacillus licheniformis*

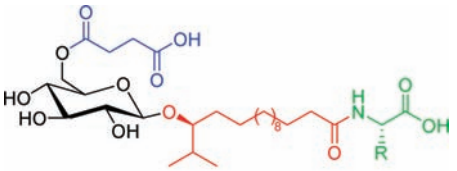
**Fakir Shahidullah Tareq,[†,‡] Ji Hye Kim,[‡] Min Ah Lee,[‡] Hyi-Seung Lee,[‡] Yeon-Ju Lee,[‡] Jong Seok Lee,[‡] and Hee Jae Shin*[,†,‡]**

*Department of Marine Biotechnology, University of Science and Technology, 217 Gajungro, Yuseong-gu, Daejeon, Republic of Korea, and Marine Natural Products Chemistry Laboratory, Korea Ocean Research & Development Institute, Ansan, Republic of Korea*

*shinhj@kordi.re.kr*

**Received January 26, 2012**

## ABSTRACT




**1**: R = $CH_3$
**2**: R = H

**Ieodoglucomides A (1) and B (2), unique glycolipopeptides consisting of an amino acid, a new fatty acid, a succinic acid, and a sugar, were isolated from a Marine-derived bacterium *Bacillus licheniformis*. The absolute stereochemistry of 1 and 2 was determined by deploying coupling constants, Marfey's and Mosher's methods, and literature reviews. Compounds 1 and 2 displayed moderate *in vitro* antimicrobial activity. Furthermore, ieodoglucomide B (2) exhibited cytotoxic activity against lung cancer and stomach cancer cell lines with $GI_{50}$ values of 25.18 and 17.78 $\mu$g/mL, respectively.**

Glycolipids have been identified as membrane receptors of various pathogenic microorganisms, principally bacteria and viruses as well as antineoplastic agents during the past few years.[1,2] Several types of two-chain and geminal glycolipids have been described in the literature.[3,4] We report herein two novel glycolipopeptides, ieodoglucomides A (**1**) and B (**2**), possessing a new skeleton from a marine sediment bacterium *Bacillus licheniformis*.

*B. licheniformis* is a saprophytic Gram-positive bacterium which has been devoted typically in the fermentation industry for the rendering of amylases, proteases, antibiotics, and special chemicals with low risk of adverse effects to humans and the environment.[5] Several groups also accounted for biosurfactants, antibiotics, and antifungal (polypeptide) compounds from this strain originated from terrestrials.[6,7] Marine bacteria have been proven to be potential producers of bioactive compounds with unique structural characteristics, since the biodiversity of the marine environment is higher than that of the terrestrial environment.[8,9] Thus, the strain 09IDYM23, identified as *B. licheniformis* by 16s rRNA sequencing (GenBank accession no. JQ349055) and showing antimicrobial activity, was further advanced to chemical examination.

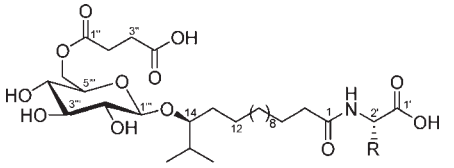
The producing strain 09IDYM23 was isolated from a sediment sample collected from Ieodo, Republic of Korea's southern reef where salt concentration and pH

[†] University of Science and Technology, Republic of Korea.
[‡] Korea Ocean Research & Development Institute.

(1) Karlsson, K. A. *Annu. Rev. Biochem.* **1989**, *58*, 309.
(2) Mammen, M.; Choi, S. K.; Whitesides, G. M. *Angew. Chem., Int. Ed.* **1998**, *37*, 2754and references cited therein.
(3) (a) Lockhoff, O. *Angew. Chem., Int. Ed. Engl.* **1991**, *30*, 1611 and references cited therein. (b) Zhang, Z.; Fukunaga, K.; Shinizu, T.; Nakao, K. *Carbohydr. Res.* **1995**, *277*, C1–C3 and references cited therein.
(4) Castro, M. J. L.; Kovensky, K.; Fernandez Cirelli, A. *Tetrahedron Lett.* **1997**, *38*, 3995.
(5) He, L.; Chen, W.; Liu, Y. *Microbiol. Res.* **2006**, *161*, 321–326.
(6) Sung-Chyr, L.; Mark, A. M.; Sharma, M. M.; Georgioul, G. *Appl. Environ. Microbiol.* **1994**, *60*, 31–38.
(7) Thaniyavarn, J.; Rossngsawang, N.; Kameyama, T.; Haruki, M.; Imanaka, T.; Morikawa, M.; Kanaya, S. *Biosci. Biotechnol. Biochem.* **2003**, *67*, 1239–1244.
(8) Fenical, W. *Chem. Rev.* **1993**, *93*, 1673–1683.
(9) Fenical, W.; Jensen, P. R. In *Marine Biotechnology*; Attaway, D., Zaborsky, O., Ed.; Plenum Press: New York, 1993; Vol. 1, pp 419–457.

10.1021/ol300202z 
**Published on Web 02/24/2012**

of water were 32.3 (g/L) and 8.01, respectively. Thus, the growth conditions of the strain were adjusted by varying pH, temperature, and salt concentration of seawater before proceeding to large-scale cultures. It was found from the findings that the productivity and growth of the strain was significant at salt concentration 18.3 g/L, pH 7.02, and temperature 32 °C. In fact, the physiological process of bacteria altered at diverse conditions.[10] Cultures of the unicellular bacterium were then grown following the above conditions and fermentation broth (50 L) was extracted with ethyl acetate. The chromatographic separation of the ethyl acetate extract using ODS open column in combination with semipreparative MPLC and HPLC resulted in the isolation of two novel glycolipopeptides, ieodoglucomides A and B.




Ieodoglucomide A (**1**) was isolated as a yellowish oil. Its molecular formula was assigned as $C_{30}H_{53}NO_{12}$ with the aid of $^1$H NMR, $^{13}$C NMR data and HRESI-MS at $[M - H]^-$ $m/z$ 618.3497 (calcd 619.3568 for $C_{30}H_{53}NO_{12}$). The IR spectrum showed absorption bands for hydroxyl (3349 $cm^{-1}$) and amide carbonyl (1660 $cm^{-1}$) groups. The $^1$H NMR spectrum of **1** (Table 1) in $CD_3OD$ showed signals for one methyl proton at $\delta$ 1.38 (H-3′) and one methine proton at $\delta$ 4.37 (H-2′) and in DMSO-$d_6$ an additional signal for the amide proton at $\delta$ 8.05 (1H, d, $J$ = 9.0 Hz) was shown. Associated $^{13}$C NMR signals at $\delta$ 176.3 (C-1′), 49.3 (C-2′) and 17.8 (C-3′) corresponding to carboxylated proton, methine proton, and methyl proton demonstrated the presence of Alanine (Ala) in the compound **1**. The $^1$H NMR spectrum also displayed signals for two methyl protons at $\delta$ 0.93 (H-16 and H-17), one methine proton at $\delta$ 1.87 (H-15), an oxygenated methine proton at $\delta$ 3.42 (H-14), and a signal appearing as a singlet at $\delta$ 1.29 for long alkyl chain, revealing the presence of a 14-hydroxy-15-methylhexadecanoic acid (HMA) unit in the molecule. The COSY, HMBC, and ROSEY correlations (Figure 1) also established the structure of the HMA unit. Carbon resonances also supported the existence of the HMA unit in the compound **1**, which was confirmed by ESI-MS analysis [$(M + H)^+$ $m/z$ at 286.19)] after acid hydrolysis (Figure 2) of **1**. Hexadecanoic acid with 14-hydroxyl and 15-methyl groups has not been reported yet in natural products. Thus, the established HMA is a new fatty acid from natural sources. In the $^1$H and $^{13}$C NMR spectra of **1**, an anomeric proton appeared at $\delta$ 4.29 (H-1‴) and carbon signals resonated at $\delta$ 65.2, 71.9, 75.1, 78.1, 75.5, and 104.2 revealing the presence of $\beta$-glucopyranose (Glu) moiety[11] as the sugar residue in **1**.

Succinic acid (SA) moiety attached to the molecule was determined by the presence of two carbonyl carbon signals at $\delta$ 171.8 (C-1″) and $\delta$ 173.2 (C-4″) and two methylene carbon signals at $\delta$ 29.9 (C-3″) and $\delta$ 30.2 (C-2″). HSQC

**Table 1.** $^1$H and $^{13}$C NMR and HMBC Data of **1** in $CD_3OD$

| no. | $\delta_H$, mult ($J$ in Hz) | $\delta_C$ | HMBC |
|---|---|---|---|
| **HMA** | | | |
| 1 | | 176.3 | |
| | | 173.2[a] | |
| 2 | 2.22, t (7.5) | 36.9 | 1, 3 |
| 3 | 1.61, m | 27.0 | |
| 4–11 | 1.29, brs. | 29.9–30.9 | |
| 12 | 1.29, brs. | 26.6 | |
| 13 | 1.46, m | 32.2 | |
| 14 | 3.42, m | 85.6 | 12, 13, 1‴ |
| 15 | 1.87, m | 32.3 | 14 |
| 16 | 0.93, d (6.5) | 18.3 | 14, 15 |
| 17 | 0.93, d (6.5) | 18.7 | 14, 15 |
| **Ala** | | | |
| 1′ | | 176.3 | |
| | | 174.3[a] | |
| 2′ | 4.37, q (7.0, 14.5) | 49.3 | 1, 1′ |
| | | 47.3[a] | |
| 3′ | 1.38, d (7.5) | 17.8 | 1′, 2′ |
| NH | 8.05, d (9.0)[a] | | 1, 2′, 3′ |
| **SA** | | | |
| 1″ | | 174.0 | |
| | | 171.8[a] | |
| 2″ | 2.61, m | 30.2 | 1″ |
| 3″ | 2.61, m | 29.9 | 1″, 4″ |
| 4″ | | 175.9 | |
| | | 173.2[a] | |
| **Glu** | | | |
| 1‴ | 4.29, d (8.0) | 104.2 | 14 |
| 2‴ | 3.18, dd (8.5, 12.7) | 75.5 | 3‴ |
| 3‴ | 3.33, m | 78.1 | 4‴ |
| 4‴ | 3.28, m | 71.9 | 3‴, 5‴ |
| 5‴ | 3.40, m | 75.1 | |
| 6‴ | 4.18, dd (6.0, 12.5) | 65.2 | 4‴, 1″ |
| | 4.44, d (11.5) | | |

[a] Chemical shifts and coupling constant were determined in DMSO-$d_6$.

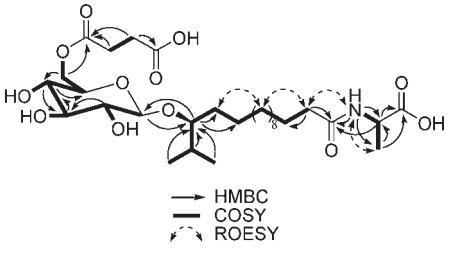



**Figure 1.** Key HMBC, COSY, and ROESY correlations of **1** in $CD_3OD$ and DMSO-$d_6$.

(10) Thomas, D. N.; Dieckmann, G. S. *Science* **2002**, *295*, 641–644.
(11) (a) Bock, K.; Pederson, C. In *Advances in Carbohydrate Chemistry and Biochemistry*; Jipson, R. S., Horfon, D., Eds.; Academic Press: New York, 1983; Vol. 41, pp 27–46. (b) Mansoor, T. A.; Shinde, P. B.; Luo, X.; Hong, J.; Lee, C. O.; Sim, C. J.; Son, B. W.; Jung, J. H. *J. Nat. Prod.* **2007**, *70*, 1481–1486. (c) Pettit, G. R.; Tang, Y.; Knight, J. C. *J. Nat. Prod.* **2005**, *68*, 974–978.

spectra revealed the attachments of these two methylene carbons with protons resonated at $\delta$ 2.61 (m, overlapped). Furthermore, HMBC correlations (Figure 1) between H-2″ and C-1″ and between H-3″ and C-4″ confirmed the existence of SA moiety in the molecule. Moreover, these four distinct units were connected with the help

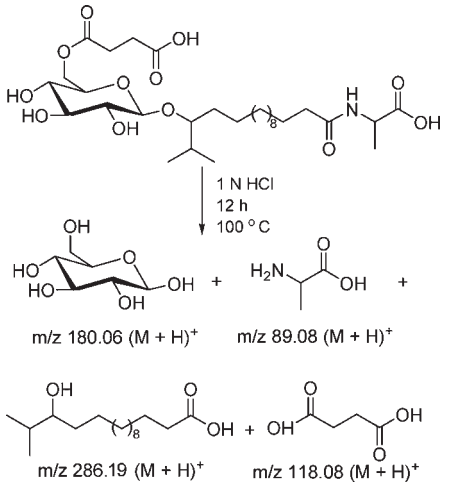



**Figure 2.** Acid hydrolysis of **1**.

of HMBC and ROSEY correlations. The connectivity between HMA and NH of Ala group was confirmed by the ROESY and HMBC correlations between NH proton and H-2 and C-1 of HMA unit, respectively. The oxygenated proton at $\delta$ 3.42 (H-14) of HMA unit showed an HMBC correlation with the anomeric carbon of Glu at $\delta$ 104.2 (C-1‴). On the other hand, methylene proton signals at $\delta$ 4.18 (H-6a‴) and 4.44 (H-6b‴) of Glu unit correlated with a carbon signal at $\delta$ 174.0 (C-1″) of SA unit. From these detailed NMR data analyses, the planar structure of ieodoglucomide A (**1**) was unambiguously determined.

Ieodoglucomide B (**2**) was also isolated as a yellowish oil similar to **1**, and its molecular formula was assigned to be $C_{29}H_{51}NO_{12}$ with the aid of HRESI-MS $[M - H]^-$ at $m/z$ 604.3339 (calcd 605.3411 for $C_{29}H_{51}NO_{12}$) and $^1H$ and $^{13}C$ NMR analysis (Table 1). The IR spectrum showed absorption bands for hydroxyl (3349 $cm^{-1}$) and amide carbonyl (1660 $cm^{-1}$) groups. The NMR results were found to be essentially identical to those of **1** which confirmed that **2** also contained HMA, SA and Glu moiety. The only difference was the observation of glycine (Gly) instead of Ala which was also confirmed by the absence of $^1H$ and $^{13}C$ signals of methyl group and the existence of an amide proton at $\delta$ 8.02 and a methylene proton as singlet at $\delta$ 3.89 (H-2′). The ROESY and HMBC data in DMSO-$d_6$ indicated the correlations between NH proton of Gly with H-2 and C-1 of HMA unit and with methine proton of Gly at $\delta$ 3.89 (H-2′). The oxygenated proton at $\delta$ 3.43 (H-14) of HMA unit showed an HMBC correlation with the anomeric carbon of Glu at $\delta$ 104.2 (C-1‴). Furthermore, methylene protons at $\delta$ 4.17 (H-6a‴) and 4.44 (H-6b‴) of Glu unit showed a correlation with a carbon signal at $\delta$ 174.1 (C-1″) of SA unit. Thus, the structure of ieodoglucomide B (**2**) was determined and the overall assignments of $^1H$ and $^{13}C$ NMR data were unambiguously made on the basis of the $^1H-^1H$ COSY, ROESY, HSQC, and HMBC spectra.

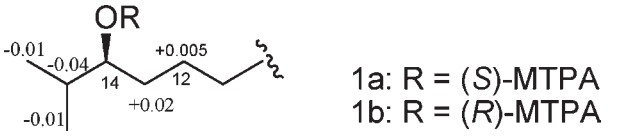



**Figure 3.** $\Delta\delta_H$ values ($\Delta\delta_H = \delta_S - \delta_R$) obtained for (*S*)- and (*R*)-MTPA esters of HMA of **1**.

The absolute stereochemistry of amino acid (AA), C-14 of HMA, and Glu moiety in **1** and **2** was determined by Marfey's method,[12] Mosher's method,[13,14] and comparisons of optical rotation and $R_f$ value with an authentic sample, respectively. After acid hydrolysis, the reaction mixtures were extracted with n-Hexane, ethyl acetate and methanol to obtain HMA, AA, and Glu moiety respectively. Ala of **1** was found to be of the L form. The absolute configuration of the stereocenter at C-14 of HMA unit in **1** and **2** was determined by treating HMA with (*R*)-(−)- and (*S*)-(+)-α-methoxy-α-(trifluoromethyl)phenylacetyl chloride (MTPA-Cl) in dry pyridine separately to yield (*S*)- and (*R*)- MTPA ester derivatives **1a**, **1b**, **2a**, and **2b**, respectively (Figure 3 and Supporting Information). All proton signals of the derivatives were assigned by $^1H-^1H$ COSY experiment and $^1H$ NMR chemical shifts ($\Delta\delta_H = \delta_S - \delta_R$). These data allowed the assignment of the absolute configuration of C-14 of **1** and **2** as *S* (Figure 3). The absolute configuration of the Glu moiety for both compounds was determined to be the $\beta$-D-form by comparing the sign of the optical rotation [0.6 mg, $[\alpha]^{23}_D$ +27 (*c* 0.14, $H_2O$)] and $R_f$ value (0.58) with an authentic sample.

Ieodoglucomides A and B were found to have moderate antimicrobial activity when tested against both Gram-positive and Gram-negative bacteria and fungi following broth dilution assay technique (Table 2).[15] Different growth conditions of bacteria and fungi were maintained while culturing these microorganisms.[16,17]

The cytotoxicity of **1** and **2** was evaluated against six human cancer cell lines (breast cancer: MDA-MB-231, colon cancer: HCT15, prostate cancer: PC-3, lung cancer: NCI-H23, stomach cancer: NUGC-3, and renal cancer:

(12) Marfey, P. *Carlsberg Res. Commun.* **1984**, *49*, 591–596.
(13) Ohtani, I.; Kusumi, T.; Kashman, Y.; Kakisawa, H. *J. Am. Chem. Soc.* **1991**, *113*, 4092–4096.
(14) Tsuda, M.; Toriyabe, Y.; Endo, T.; Kobayashi, J. *Chem. Pharm. Bull.* **2003**, *51*, 448–451.
(15) Appendio, G.; Gibbons, S.; Giana, A.; Pagani, A.; Grassi, G.; Stavri, M.; Smith, E.; Rahman, M. M. *J. Nat. Prod.* **2008**, *71*, 1427–1430.
(16) Oluwatuyi, M.; Kaatz, G. W.; Gibbons, S. *Phytochemistry* **2004**, *65*, 3249–3254.
(17) Yu, J. Q.; Lei, J. C.; Yu, H. D.; Cai, X.; Zou, G. L. *Phytochemistry* **2004**, *65*, 881–884.

**Table 2.** Minimum Inhibitory Concentrations (MICs) of **1** and **2**

| microorganisms | MICs ($\mu$g/mL) **1** | **2** | PC[a] |
|---|---|---|---|
| Gram-positive bacteria | | | |
| *S. aureus* | 8 | 8 | 2 |
| *B. subtilis* | 16 | 16 | 2 |
| *B. cereus* | 16 | 8 | 2 |
| Gram-negative bacteria | | | |
| *S. Typhi* | 16 | 16 | 2 |
| *E. coli* | 8 | 16 | 2 |
| *P. aeruginosa* | 8 | 8 | 2 |
| fungi | | | |
| *C. albicans* | 32 | 32 | 4 |
| *A. niger* | 32 | 16 | 4 |

[a] PC: positive control (azithromycin for bacteria and amphotericin B for fungi).

**Table 3.** Human Cancer Cell Line Inhibition Values ($GI_{50}$ expressed in $\mu$g/mL) of **1** and **2**

| cancer cell lines | **1** | **2** | ADR[a] |
|---|---|---|---|
| breast cancer: MDA-MB-231 | >30 | >30 | 0.38 |
| colon cancer: HCT-15 | >30 | >30 | 0.26 |
| prostate cancer: PC-3 | >30 | >30 | 0.93 |
| lung cancer: NCI-H23 | >30 | 25.18 | 0.93 |
| stomach cancer: NUGC-3 | >30 | 17.78 | 0.28 |
| renal cancer: ACHN | >30 | >30 | 0.22 |

[a] ADR: adriamycin as standard.

ACHN) according to a sulforhodamine B (SBR) assay (Table 3).[18] Compound **2** exhibited cancer cell growth inhibition against lung cancer (NCI-H23) and stomach cancer (NUGC-3) cell lines, with $GI_{50}$ values of 25.18 and 17.78 $\mu$g/mL, respectively.

The most structurally distinct part of ieodoglucomides A and B is the presence of HMA, AA, and SA that possesses a $C_{30}$ and $C_{29}$ skeleton with a glycolipid backbone which has never been described before from natural sources. Ieodoglucomides A and B bear a slight resemblance to the well-known powerful immunostimulant α-galactosyl ceramide (α-GalCer) KRN7000,[19] but the overall structures are completely unprecedented. It is also noteworthy that some glycolipids are known to possess antiviral, antifungal, antimicrobial, COX-2 inhibitory, immunostimulative, and immunesuppessive activities.[20] Some of these activities of glycolipids would appear promising for the treatment of Alzheimer's disease.[21] Accordingly, we intend to advance further biological investigation of **1** and **2** as these novel glycolipopeptides may have a useful role against some diseases.

In conclusion, to the best of our knowledge, ieodoglucomides A and B represent the first examples of novel bioactive metabolites containing a new fatty acid unit (HMA) from a marine-derived bacterium.

**Acknowledgment.** We are grateful to Dr. C. Kun, Korea Basic Science Institute, Ochang, Republic of Korea, for providing mass data and to Dr. Jieun Yun, Bioevaluation Center, KRIBB, Republic of Korea, for carrying out cytotoxicity tests. We also express gratitude to Professor Dr. Choudhury Mahmood Hasan, Department of Pharmaceutical Chemistry, University of Dhaka, Bangladesh, for helping in part with chemical studies. This research was supported in part by the Ministry of Land, Transport and Maritime Affairs, Korea.

**Supporting Information Available.** General procedures, bioassay protocols, chemical derivatization, data tables, and NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.

(18) Skehan, P.; Storeng, R; Scudiero, D.; Monks, A.; McMohan, J.; Vistica, D.; Warren, J. T.; Bokesch, H.; Kenney, S.; Boyed, M. R. *J. Natl. Cancer Inst*. **1990**, *82*, 1107–1112.

(19) Morita, M.; Motoki, K.; Akimoto, K.; Natori, T.; Sakai, T.; Sawa, E.; Yamaji, K.; Kobayashi, E.; Fukushima, H.; Koezuka, Y. *J. Med. Chem*. **1995**, *38*, 2176–2187.

(20) Pettit, G. R.; Tang, Y.; Knight, J. C. *J. Nat. Prod*. **2005**, *68*, 974–978.

(21) Tan, R. X.; Chen, J. H. *Nat. Prod. Rep*. **2003**, *20*, 509–534.