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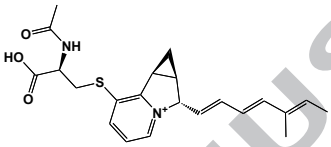
Streptomyces griseus OS-3601



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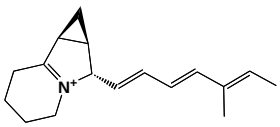
ABSTRACT

Iminimycin B, a novel indolizine alkaloid featuring a rare pyridinium, was isolated from the cultured broth of a streptomycin-producing strain, *Streptomyces griseus* OS-3601, through a physicochemical screening method. Its structure was elucidated on the basis of mass and NMR analyses. Stereochemical assignment of iminimycin B was archived by NMR studies, electronic circular dichroism (ECD) analysis, and advanced Marfey's method.

Natural products produced by microorganisms have been a rich source for drug discovery. Many drugs developed from natural products, such as leucomycin[1] and avermectin[2], have been discovered by bioassay-guided isolation. Our screening program, physicochemical (PC) screening, was allows to detect more different types of natural products in a culture broth than bioassay program.

On our ongoing PC screening program, we has successfully discovered mangromicins[3] and new nanaomycin analogs[4] from actinomycete culture broths. Moreover, this screening program has recently led to discover a new indolizidine alkaloid, iminimycin A, from the culture broth of *Streptomyces griseus* OS-3601. The strain OS-3601 was isolated as a streptomycin-producing strain at Aso, Kumamoto-Prefecture, Japan in 1972, and preserved for over 40 years.[5]

Many indolizine alkaloids with their unique structures and biological activities were mainly isolated from metabolites plants, frogs and ants.[6] The alkaloid compounds have been also found to produced by some actinomycete strains, but a few only compounds are extant. Cyclizidine and its analog were isolated from secondary metabolites of *Streptomyces* sp. and *Saccharopolyspora* sp.,[7] and indolizomycin was isolated from the strain SK2-52 that was formed by protoplast fusion treatment between non-antibiotic-producing mutants of *S. griseus* and *S. tenjimariensis*.[8] Iminimycin A is an indolizidine alkaloid, consisting of an octahydroindolizine skeleton with a triene side chain and a cyclopropane ring, and the most distinctive feature of this compound is to have an unusual iminium group.[5] In PC screening for discovery of new compounds from the culture broth of this strain, an unidentified compound with an absorption maximum at 279 and 394 nm and molecular ion peak at *m/z* 399 was observed in the other ODS fraction without iminimycin A. This compound, designated iminimycin B (**1**), was a new indolizine alkaloid possessing a *N*-acetyl-cysteine and a pyridinium (Figure 1) instead of iminium moiety of iminimycin A.



Iminimycin A

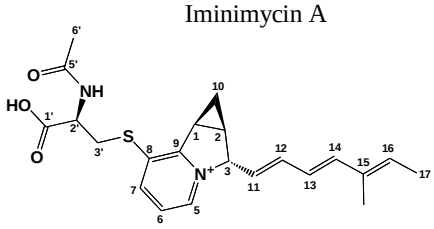


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Figure 1. Structures of iminimycin A and **1**.

Isolation of **1** was guided by physico-chemical properties such as its molecular formula ($C_{22}H_{27}N_2O_3S^+$) and UV spectrum (λ_{max} 279 and 394 nm), using LC/UV and LC/MS equipment. A strain of *S. griseus* OS-3601 was cultured in producing medium (100 mL × 100), for 5 days at 27 °C. The 5-day old culture broth (10 L) was centrifuged to separate cells and supernatant. The

supernatant subjected to Diaion HP-20 column and sequential ODS flash column chromatography. Eventually, **1** was purified by reversed-phase HPLC (Scheme S1).

Iminimycin B (**1**) was isolated as a white powder ($[\alpha]_D^{24.6}$ -13.9 (*c* = 0.1, MeOH). The HR-ESIMS of **1** produced the M^+ ion at *m/z* 399.1736 indicating the molecular formula was $C_{22}H_{27}N_2O_3S^+$ (calculated value for $C_{22}H_{27}N_2O_3S^+$, 399.1737). The ^1H and ^{13}C NMR spectral data of **1** are shown in Table 1.

Table 1. ^1H and ^{13}C NMR Data of Iminimycin B (**1**) in CD_3OD

Position	^{13}C ppm, mult	^1H ppm, mult, Hz
1	24.9, CH	3.23, m
2	23.2, CH	2.46, m
3	78.0, CH	5.59, d (9.5)
5	138.6, CH	8.42, d (6.0)
6	126.1, CH	7.73, dd (6.0, 8.0)
7	145.3, CH	8.53, d (8.0)
8	137.5, qC	
9	159.5, qC	
10a	17.7, CH_2	1.03, m
10b		1.77, m
11	127.4, CH	5.88, dd (9.5, 15.5)
12	139.8, CH	6.73, m
13	124.6, CH	6.21, dd (11.0, 15.5)
14	143.7, CH	6.51, d (15.5)
15	135.8, qC	
15-Me	11.9, CH_3	1.77, s
16	131.4, CH	5.74, q (6.5)
17	14.2, CH_3	1.77, d (6.5)
1'	175.5, qC	
2'	55.0, CH	4.44, dd (4.5, 7.0)
3' a	37.0, CH_2	3.39, dd (7.0, 14.0)
3' b		3.78, dd (4.5, 14.0)
5'	173.0, qC	
6'	22.8, CH_3	1.97 (3H, s)

The ^1H NMR spectrum of **1** in CD_3OD displayed eight sp^2 methine protons from 8.53 to 5.74 ppm, along with two allylic methyl groups (both δ_H 1.77), four sp^3 methines (δ_H 5.59, 4.44, 3.23, and 2.46), two methylenes (δ_H 1.03 and 1.77, 3.39 and 3.78), and one acetyl methyl group (δ_H 1.97). The ^{13}C NMR spectrum showed the resonances of 22 carbons, which were classified into two carbonyl carbons at 175.5 and 173.0 ppm, eleven olefinic and aromatic carbons from 159.5 to 124.6 ppm, two heteroatom bonded methine carbons at 78.0 and 55.0 ppm, two methine carbons (δ_C 24.9 and 23.2), two methylene carbons (δ_C 37.0 and 17.7) and three methyl carbons (δ_C 22.8, 14.2 and 11.9) by HSQC spectra (Figures S4-S6).

Figure 2. Key correlations observed in COSY (bold lines), HMBC (arrows) and ROESY (dotted arrows) spectra of **1**.

As shown in Figure 2, the ^1H-^1H COSY and ^1H-^{13}C HMBC spectra indicated the presence of five partial structures: C-1/C-2/C-10 to form a cyclopropane ring, C-5/C-6, C-3/C-11−C-14, C-16/C-17, C-2'/C-3'. ^1H-^{13}C HMBC Analysis confirmed the presence of a pyrrolidinium ring fused with a cyclopropane, based on the correlations from H_2-10 to C-1, C-2, C-3, and C-9, and from H-2 to C-1, C-3 and C-9. An indolizidinium ring was identified, based on HMBC correlations from H-5 to C-3, C-7 and C-9, from H-6 to C-8, from H-7 to C-5, C-6 and C-9, and from H-3 to C-9 (Figures S7 and S8). A characteristic band at 1388, 1481 and 1605 cm^{-1} in the IR spectra of **1** suggests a pyridine moiety (Figure S1). Moreover, the HMBC correlations from H-11 to C-13, from H-13 to C-11, C-12 and C-15, from H-14 to C-12, C-15 and C-16, from H-16 to C-17 and C-15-Me, from H_3-15-Me to C-14, C-15 and C-16 confirmed the presence of a 5-methyl-hepta-1,3,5-trienyl unit. All geometries of this triene were determined as *E* based on large coupling constants (Table 1) and ROESY correlations (H-13/H_3-15-Me and H-14/H-16) (Figure S10). The HMBC correlations from H-3 to C-11and H-12, from H-11 to C-2 and C-3, from H-12 to C-3 revealed that this triene unit was attached to the C-3 position. Remaining signals were classified into one heteroatom-bound methylene at δ_H 3.39 and 3.78 (dd, *J* = 4.5, 14.0), δ_C 37.0; one heteroatom-bound methine at δ_H 4.44 (dd, *J* = 4.5, 7.0), δ_C 55.0; two carbonyl carbons at δ_C 175.5 and δ_C 173.0; and one methyl group at δ_H 1.97 (3H, s), δ_C 22.8. From these detailed analysis of ^1H-^1H COSY and ^1H-^{13}C HMBC spectra, the presence of *N*-acetyl-cysteine moiety was determined (Figure 1). Since a long-range ^1H-^{13}C heteronuclear correlation was observed from H_2-3' (δ_H 3.39, 3.78) to an aromatic quaternary carbon C-8 (δ_C 137.5), we confirmed that *N*-acetyl-cysteine moiety was connected at C-8 through a sulfur atom, which is also implied by the molecular formula. The relative configuration of **1** was determined as 1*S**,2*R**,3*S** by ROESY spectrum that gave cross peaks for H-3/H-5, H-3/H-10, and H-2/H-12 supported by no coupling between H-2 and H-3 (Figure 3). The NMR, UV and IR spectra of **1** were similar to louludinium chloride, whish is an indolizine alkaloid with pyridinium ion, from a marine bluegreen alga, *Lyngbya gracilis*.[9]

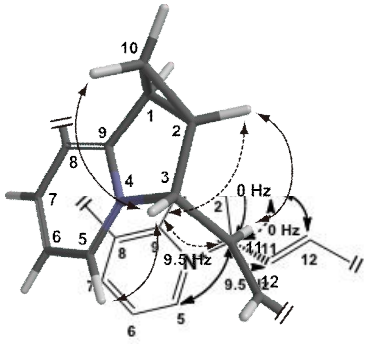

Figure 3. Key ROESY (bold arrows) and coupling constants (dotted arrows) of **1**.

The absolute configuration of **1** was defined by their electronic circular dichroism (ECD) spectra, followed by advanced Marfey's analysis of acid hydrolysate derived from desulfurization of **1** with Raney-nickel. The absolute configuration of **1** at C-1, C-2 and C-3 was deduced by ECD spectra[10] in comparison with their calculated spectra[11] (Figure S2). Conformational searches were performed the Monte Carlo algorithm implemented in Spartan'14 using the Merck molecular force field (MMFF), optimized by semiempirical PM3 calculations in Gaussian 09[12], then further refined by density

functional theory (DFT) at the CAM-B3LYP/TZVP level, which yielded additional relevant conformers. Structures of resulting calculated conformers were also supported by our ROESY analyses and expected NMR calculation (Table S2 & 3). As a result of the comparison between the experimental and calculated ECD spectra, the absolute configuration of **1** was elucidated to be *1S*,*2R*,*3S*. Combination of Raney-nickel desulfurization and advanced Marfey's method were performed in order to determine the absolute configuration of *N*-acetyl-cysteine moiety in **1**. After **1** was subjected to desulfurization with Raney-nickel in ethanol, nickel was removed by filtration through a pad of celite. Following alanine generated from the reacted solution using acid hydrolysis, and the advanced Marfey's procedure led to the assignment of the absolute configuration of the alanine as *S* form (Figure S3). Finally, the absolute configuration of 1 was elucidated to be 1*S*,2*R*,3*S*,2'*S*.

Iminimycin B (**1**) was tested for antibacterial and cytotoxic activities. Compound **1** showed weak antibacterial activity against *Xanthomonas campestris* pv. *oryzae* KB-88 (9 mm, 100 μg/disk). No significant inhibitory activities against HeLa S3 and Jurkat cells were observed at 100 μM under the conditions tested.

To our knowledge, there is no available information regarding actinomycete secondary metabolites with a pyridinium ring except for one compound, 1-(10-Aminodecyl) pyridinium, from a marine actinomycete, *Amycolatopsis alba*.[13] The actinomycete producer of **1**, which is a rare compound with pyridinium ring, was *S. griseus* that isolated in 1972 and preserved for over 40 years. Our PC screening system led to the discovery of new compounds, even from widely studied actinomycete species such as *S. griseus*. Since **1** has a *N*-acetyl-cysteine moiety, which was revealed to originate from mycothiol that involved in the bacterial detoxification system,[14] compound **1** may be derived for inactivation of iminimycin A with antibacterial and cytotoxic activities.

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Supplementary Material

Supplementary material that may be helpful in the review process should be prepared and provided as a separate electronic file. That file can then be transformed into PDF format and submitted along with the manuscript and graphic files to the appropriate editorial office.

References and notes

1. Hata, T.; Sano, Y.; Ohki, N.; Yokoyama, Y.; Matsumae, A., Ito, S. *J. Antibiot*. **1953**, *6*, 87-89.
2. Burg, R. W.; Miller, B. M.; Baker, E. E.; Birnbaum, J.; Currie, S. A.; Hartman, R.; Kong, Y. L.; Monaghan, R. L.; Olson, G.; Putter, I.; Tunac, J. B.; Wallick, H.; Stapley, E. O.; Oiwa, R.; Ōmura, S. *Antimicrob. Agents Chemother*. **1979**, *15*, 361-367.
3. Nakashima, T.: Iwatsuki, M.; Ochiai, J.; Kamiya, Y.; Nagai, K.; Matsumoto, A.; Ishiyama, A.; Otoguro, K.; Shiomi, K.; Takahashi, Y.; Ōmura, S. *J. Antibiot.* **2014**, *67*, 253-260. (b) Nakashima, T.; Kamiya, Y.; Iwatsuki, M.; Takahashi, Y.; Ōmura, S. *J. Antibiot.* **2014**, *67*, 533-539. (c) Nakashima, T.; Kamiya, Y.; Iwatsuki, M.; Sato, N.; Takahashi, Y.; Ōmura, S. *J. Antibiot*. **2015**, *68*, 220-222.
4. Nakashima, T.; Boonsnongcheep, P.; Kimura, T.; Iwatsuki, M.; Sato, N.; Nonaka, K.; Prathanturarug, S.; Takahashi, Y.; Ōmura, S. *J. Biosci. Bioeng*. **2015**, *120*, 596-600.
5. Nakashima, T.; Miyano, R.; Iwatsuki, M.; Shirahata, T.; Kimura, T.; Asami, Y.; Kobayashi, Y.; Shiomi, K.; Petersson, G. A.; Takahashi, Y.; Ōmura, S. *J. Antibiot.* **2016** (*in press*).
6. Carroll, A. R.; Arumugan, G.; Quinn, R. J.; Redburn, J.; Guymer, G.; Grimshaw, P. *J. Org. Chem*. **2005**, *70*, 1889-1892. (b) Daly, J. W.; Brown, G. B.; Mensah-Dwumah, M.; Myers, C. W. *Toxicon* **1978**, *16*, 163-188. (c) Jones, T. H.; Torres, J. A.; Spande, T. F.; Garraffo, H. M.; Blum, M. S.; Snelling, R. R. *J. Chem. Ecol*. **1996**, *22*, 1221-1236.
7. Freer, A. A.; Gardner, D.; Greatbanks, D.; Polyser, J. P.; Sim, G. A. *J. Chem. Soc. Chem. Commun*. **1982**, *20*, 1160–1162. (b) Izumikawa, M.; Hosoya, T.; Takagi, M.; Shin-ya, K. *J. Antibiot*. **2012**, *65*, 41-43.
8. Gomi, S.; Ikeda, D.; Nakamura, H.; Naganawa, H.; Yamashita, F.; Hotta, K.; Kondo, S.; Okami, Y.; Umezawa, H.; Iitaka Y. *J. Antibiot*. **1984**, *37*, 1491-1494.
9. Yoshida, W. Y.; Scheuer P. J. *Heterocycles* **1998**, *47*, 1023– 1027.
10. Stonard, R. J.; Trainor, D. A.; Nakatani, M.; Nakanishi, K. *J. Am. Chem. Soc*. **1983**, *105*, 130-131. (b) Li, X. -C.; Ferreira, D.; Ding, Y. *Curr. Org. Chem*. **2010**, *14*, 1678– 1697.
11. Nugroho, A. E.; Morita, H. *J. Nat. Med*. **2014**, *68*, 1-10.
12. Frisch, M. J. et al. Gaussian 09, Revision D.01 (Gaussian, Wallingford, CT, 2009).
13. Dasari, V. R.; Muthyala, M. K.; Nikku, M. Y.; Donthireddy, S. R. *Microbiol. Res*. **2012**, *167*, 346-351.
14. Ikeda, H.; Shin-Ya, K.; Nagamitsu, T.; Tomoda, H. *J. Ind. Microbiol. Biotechnol*. 2016, *43*, 325-342.

Highlights

・Iminimycin B was discovered from *Streptomyces griseus* OS-3601 by a physicochemical screening.

・Structure of iminimycin B was an unique novel pyridinium alkaloid.

・Absolute configuration of iminimycin B was determined by NMR, eECD analysis and Marfey's method.