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NOTE

Jomthonic acids B and C, two new modified amino acids from *Streptomyces* sp

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Actinomycetes are recognized as the most prolific secondary metabolite producers and hence of high pharmacological and commercial interest. Until now, *ca.* 40% of antibiotics of natural origin were found from actinomycetes and *ca.* 75% of actinomycete metabolites are produced by *Streptomyces* spp.[1] With these prominent scores, the search of new bioactive secondary metabolites from this species still remains the core of pharmaceutical discovery efforts.[2] In the course of our screening program for structurally unique secondary metabolites from this richest source, we have reported jomthonic acid A (**1**), a new modified amino acid, as an inducer of adipocyte differentiation from the culture broth of *Streptomyces* sp. BB47.[3] Compound **1** is an interesting modified amino acid containing rare structural units, 4-methyl-2,4-hexadienoic acid and *β*-methylphenylalanine, which have been found only in a few microbial metabolites and there are no natural products in which both units are present (Figure 1).

Adipocyte differentiation is the process of cell differentiation by which a preadipocyte changes into an adipocyte.[4] Mature adipocytes secrete adiponectin, a peptide hormone that improves insulin sensitivity in type-II diabetes patients.[5] Inducers of preadipocyte differentiation are thus expected as a promising therapeutic agent for insulin resistance and type-II diabetes.[6] In an effort to obtain more insight into the structure–activity relationship of jomthonic acid A (**1**) as an adipocyte differentiation inducer, HPLC-UV inspection with a new batch of culture of this strain resulted in the discovery of two new minor congeners, jomthonic acids B (**2**) and C (**3**). We herein report the isolation, structure determination and biological activity of these new compounds.

Strain BB47 was cultured in A-3M medium as previously described.[3] HPLC-UV analysis of the culture extract indicated the presence of two minor peaks displaying the UV spectrum similar to **1**. A combination of silica gel and ODS column chromatographies, followed by preparative HPLC purification, gave **2** (10.1 mg) and **3** (9.8 mg) from 5 l of culture.

Jomthonic acid B (**2**) was obtained as a pale yellow oil that gave an $[M+Na]^+$ peak at *m/z* 396.1782 in the positive ion mode HR-ESITOFMS appropriate for a molecular formula of $C_{21}H_{27}NO_5$ ($\Delta +0.1$ mmu, calcd for $C_{21}H_{27}NO_5Na$ 396.1781), corresponding to the loss of a methylene fragment (CH_2) from **1**. ^{13}C NMR and HSQC spectral data were consistent with this observation. Comparing to the spectral data of **1**, the resonances for a singlet methyl group had disappeared and those for an sp^2 methine appeared in the ^1H and ^{13}C NMR spectra of **2** (See Supplementary Information). Sequential ^1H–^1H COSY correlations were observed for four olefinic protons from H-5 to H-2, and the H-2 proton was further correlated to the methyl proton H-1. HMBC correlations were observed from H-4 and H-5 to the carbonyl carbon C-6 (Figure 2). The *E* configuration of the double bond between C-4 and C-5 was deduced by a large coupling constant (15.0 Hz) between the protons bonding to these carbons (Table 1). The *E* geometry for C-2–C-3 was established by the NOESY correlations for H-2/H-4 and H-3/H-5 (Figure 2). The chemical shifts for the remaining parts were almost identical to those for **1**. From these spectroscopic data, **2** was determined as a new congener of **1**, lacking the methyl branching in the hexadienoate moiety.

Jomthonic acid C (**3**) was also isolated as a pale yellow oil. The molecular formula of $C_{21}H_{27}NO_5$, established by the HR-ESITOFMS data (*m/z* 396.1789 for $[M+Na]^+$, $\Delta +0.8$ mmu), again suggested a loss of one methylene from **1**. In the ^1H and ^{13}C spectra of **3**, the resonances for a doublet methyl and an sp^3 methine group present in **1** were missing, while an additional methylene group was detected (See Supplementary Information). Further 2D NMR analysis revealed that the structural difference of **3** was present in the hydroxyacid

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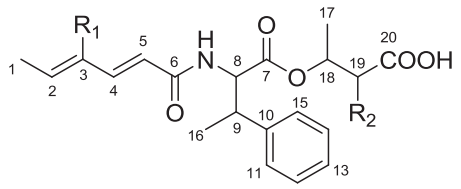

jomthonic acid A (**1**) R_1=CH_3, R_2=CH_3 (8*S*, 9*R*, 18*R*, 19*R*)
B (**2**) R_1=H, R_2=CH_3
C (**3**) R_1=CH_3, R_2=H

Figure 1 Structures of jomthonic acids A (**1**)–C (**3**).

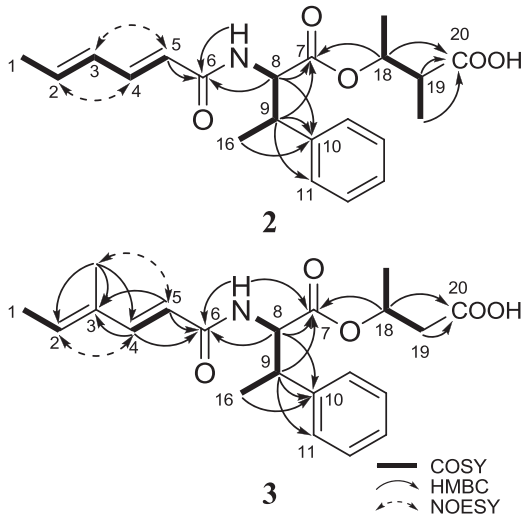

Figure 2 COSY, key HMBC and key NOESY correlations for **2** and **3**.

moiety. The doublet methyl protons H_3-17 showed a COSY correlation to H-18 that was in turn correlated to a methylene H_2-19. HMBC correlations were observed from H-18 and H_2-19 to the carbonyl carbon C-20 (Figure 2). These correlation data allowed the identification of the hydroxyacid unit of **3** as 3-hydroxybutanoic acid. The large coupling constant (15.5 Hz) between H-4 and H-5 established the *E* configuration (Table 1) and NOESY correlations for H-2/H-4 and 3-Me/H-5 confirmed the *E* configuration for C-2–C-3 double bond (Figure 2). On the basis of these results, **3** was determined as a 19-demethyl congener of **1**.

Inducing potential of jomthonic acids B (**2**) and C (**3**) for murine ST-13 preadipocyte differentiation into adipocytes were evaluated at concentrations of 25 and 50 μM (Figure 3). By the treatment with 25 μM of **1** and **2**, 30 and 40% of preadipocytes were differentiated into adipocytes, respectively. At 50 μM, 53 and 60% of preadipocytes were differentiated by the treatment with **1** and **2**, respectively. In contrast, **3** was not active even at 50 μM with $<10\%$ of differentiation induction.

In conclusion, two new modified amino acids, jomthonic acids B (**2**) and C (**3**), were isolated from a soil-derived *Streptomyces* sp. BB47. Comparing to jomthonic acid A (**1**), **2**, which lacks a methyl branch in the unsaturated fatty acid unit, showed slightly better inducing activity for adipocyte differentiation. Meanwhile, **3**, which lost a methyl group from the hydroxyacid part, was inactive, indicating the important role of this part in bioactivity. Further biological and structure–activity relationship analyses are now in progress.

EXPERIMENTAL SECTION

General experimental procedures

Optical rotations were measured using a JASCO DIP-3000 polarimeter. UV spectra were recorded on a Hitachi U-3210 spectrophotometer. IR spectra were measured on a Perkin-Elmer Spectrum 100. NMR spectra were obtained on a Bruker AVANCE 400 or a

Table 1 ^{1}H and ^{13}C NMR Data for 2 and 3 in $CDCl_3$

	2			*3*		
Position	δ_H, *mult (J, Hz)*[a]	δ_C[b]	*HMBC*[a,c]	δ_H, *mult (J, Hz)*[a]	δ_C[b]	*HMBC*[a,c]
1	1.85, d (6.0)	16.9	2, 3, 4, 5	1.81, d (7.0)	14.3	2, 3, 4, 5
2	6.11, m[d]	136.8	1, 4	5.96, q (7.0)	135.5	1, 4, 3-Me
3	6.16, dd (15.0, 10.0)	128.0	1, 4, 5		133.2	
4	7.19, dd (15.0, 10.0)	140.6	2, 3, 5, 6	7.26, m[d]	146.7	2, 5, 6, 3-Me
5	5.80, d (15.0)	119.1	3, 6	5.80, d (15.5)	116.6	3, 4, 6
6		164.7			164.7	
7		169.3			171.0	
8	4.87, dd (8.8, 8.0)	56.0	6, 7, 9, 10, 16	4.86, dd (8.5, 7.8)	57.6	6, 7, 9, 10, 16
9	3.17, dq (7.3, 8.0)	41.5	7, 8, 10, 11, 15, 16	3.17, dq (7.0, 7.8)	43.1	7, 8, 10, 11, 15, 16
10		139.7			141.1	
11	7.23, m[d]	126.2		7.24, m[d]	127.7	
12	7.30, m[d]	126.8		7.31, m[d]	128.2	
13	7.24, m[d]	125.5		7.25, m[d]	126.9	
14	7.30, m[d]	126.8		7.31, m[d]	128.2	
15	7.23, m[d]	126.2		7.24, m[d]	127.7	
16	1.40, d (7.3)	16.0	8, 9, 10	1.41, d (7.0)	17.5	8, 9, 10
17	0.92, d (6.5)	14.5	18, 19	0.98, d (6.0)	18.9	18, 19
18	4.99, dq (7.0, 6.5)	70.8	7, 17, 19, 20	5.14, m[d]	68.0	7, 17, 19, 20
19	2.62, dq (7.0, 7.5)	42.6	17, 18, 20, 19-Me	2.46, dd (15.7, 5.5)	40.1	17, 18, 20
				2.57, dd (15.7, 7.5)		17, 18, 20
20		175.6			173.2	
3-Me				1.77, s	11.7	2, 3, 4
19-Me	1.10, d (7.5)	10.7	18, 19, 20			
8-NH	6.28, d (8.8)		6	6.25, d (8.5)		6, 8

[a]Recorded at 500 MHz.
[b]Recorded at 100 MHz.
[c]HMBC correlations are from proton(s) to the indicated carbon.
[d]Overlapping signals.

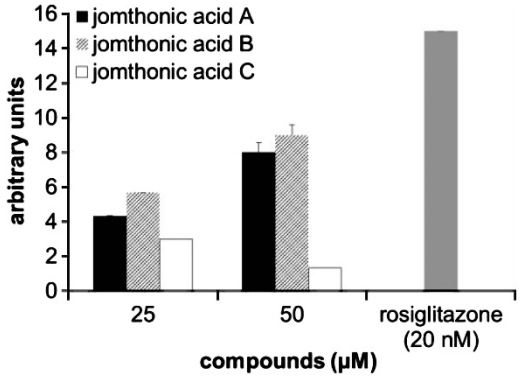

Figure 3 Differentiation-inducing activity of jomthonic acids A (**1**)–C (**3**).

Bruker AVANCE 500 spectrometer. The 7.26 and 77.0 p.p.m. resonances of residual $CHCl_3$ were used as internal references for ^1H and ^{13}C NMR spectra, respectively. HR-ESITOFMS were recorded on a Bruker microTOF focus. Cosmosil 75C18-PREP (Nakalai Tesque Inc., 75 μm) was used for ODS column chromatography. HPLC separation was performed using a Cosmosil 5C18-AR-II (Nacalai Tesque Inc., 20 × 250 mm) with a photodiode array.

Isolation

Streptomyces sp. BB47 was cultured in A-3M medium as described in our previous paper.[3] The crude extract (23.8 g from 5 l broth) was subjected to silica gel column chromatography with a step gradient of $CHCl_3$/MeOH (1:0, 20:1, 10:1, 4:1, 2:1, 1:1 and 0:1 *v*/*v*). The fraction eluted with 4:1 $CHCl_3$-MeOH was concentrated to give 2.8 g of brown oil, which was further purified by reversed phase ODS column chromatography with a gradient of MeCN/0.1% HCO_2H (2:8, 3:7, 4:6, 5:5, 6:4, 7:3 and 8:2 *v*/*v*). The 6:4 fraction was evaporated and the remaining aqueous solution was extracted with EtOAc, and the organic layer was concentrated to give a semi-pure mixture of jomthonic acids. Further purification was performed by repeated C_{18} RP HPLC using a Cosmosil 5C18-AR-II column (Nacalai Tesque Inc., 20 × 250 mm) with MeCN/0.1% HCO_2H (37:63) at a flow rate of 15 ml min^{-1}, followed by evaporation and extraction with EtOAc, yielding jomthonic acid A (**1**, 469.1 mg, t_R 64.4 min), B (**2**, 10.1 mg, t_R 44.0 min) and C (**3**, 9.8 mg, t_R 46.4 min).

*Jomthonic acid B (**2**).* Pale yellow oil; $[\alpha]^{22}_D$ −46 (*c* 0.22, MeOH); UV (MeOH) λ_{max} (log ε) 260 (4.59) nm; IR (ATR) ν_{max} 3291, 2980, 1731, 1656 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESITOFMS $[M+Na]^+$ 396.1782 (calcd for $C_{21}H_{27}NO_5Na$, 396.1781).

*Jomthonic acid C (**3**).* Pale yellow oil; $[\alpha]^{22}_D$ -35 (*c* 0.23, MeOH); UV (MeOH) λ_{max} (log ε) 263 (4.26) nm; IR (ATR) ν_{max} 3300, 2979, 1729, 1657 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HR-ESITOFMS $[M+Na]^+$ 396.1789 (calcd for $C_{21}H_{27}NO_5Na$, 396.1781).

Biological assays

Adipocyte differentiation assay was carried out according to the procedure previously described.[7–10] Rosiglitazone, an antidiabetic drug, was used as a positive control in the adipocyte differentiation assay. It induced differentiation in 80% of murine ST-13 preadipocyte cells at 0.02 μM.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Bérdy, J. Thoughts and facts about antibiotics: where we are now and where we are heading. *J. Antibiot.* **65,** 385–395 (2012).
2 Watve, M. G. *et al.* How many antibiotics are produced by the genus *Streptomyces*? *Arch. Microbiol.* **176,** 386–390 (2001).
3 Igarashi, Y. *et al.* Jomthonic acid A, a modified amino acid from a soil-derived *Streptomyces*. *J. Nat. Prod.* **75,** 986–990 (2012).
4 Cornelius, P. *et al.* Regulation of adipocyte development. *Annu. Rev. Biochem.* **14,** 99–129 (1994).
5 Ukkola, O. *et al.* Adiponectin: a link between excess adiposity and associated comorbidities? *J. Mol. Med.* **80,** 696–702 (2002).
6 Kadowaki, T. *et al.* Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J. Clin. Invest.* **116,** 1784–1792 (2006).
7 Kunimasa, K. *et al.* Identification of nobiletin, a polymethoxyflavonoid, as an enhancer of an adiponectin secretion. *Bioorg. Med. Chem. Lett.* **19,** 2062–2064 (2009).
8 Ikeda, M. *et al.* Norlichexanthone isolated from fungus P16 promotes the secretion and expression of adiponectin cultured in ST-13 adipocytes. *Med. Chem.* **7,** 250–256 (2011).
9 Igarashi, Y. *et al.* Prajiamide, a new modified peptide from a soil-derived *Streptomyces*. *J. Anitibiot.* **65,** 157–159 (2012).
10 Indananda, C. *et al.* Linfuranone A, a new polyketide from plant-derived *Microbispora* sp. GMKU 363. *J. Antibiot.* **66,** 675–677 (2013).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)