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Tetrahedron Letters
Tetrahedron Letters 48 (2007) 2725–2727

Diapolycopenedioic acid xylosyl ester, a novel glyco-C_{30}-carotenoic acid produced by a new marine bacterium *Rubritalea squalenifaciens*

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Received 22 December 2006; revised 6 February 2007; accepted 13 February 2007
Available online 16 February 2007

Abstract—A novel acyl glyco-carotenoic acid, diapolycopenedioic acid xylosyl ester, was isolated from a marine bacterium *Rubritalea squalenifaciens* belonging to subdivision 1 of *Verrucomicrobia* as the major red pigment by using chromatographic methods. The structure of diapolycopenedioic acid xylosyl ester was determined to be 4-[2-*O*-(12-methyltridecanoyl)-β-xylopyranosyl] hydrogen 4,4′-diapo-ψ,ψ-carotene-4,4′-dioate by analysis of the MS and NMR data for this acid and for the diacetyl diapolycopenedioic acid xylosyl ester. The diapolycopenedioic acid xylosyl ester showed potent antioxidative activity against a lipid peroxidation model.

Rubritalea squalenifaciens (MBIC08254^T) is a new marine bacterium belonging to subdivision 1 of *Verrucomicrobia*, a Gram-negative, heterotrophic mesophile which had been isolated from the marine sponge, *Halichondria okdai*.[1] In the course of a screening program for novel carotenoids from marine bacteria, we isolated a novel acyl glyco-carotenoic acid, diapolycopenedioic acid xylosyl ester (**1**), from this bacterium. We describe in this report the fermentation, isolation, structural determination and antioxidative activity of the compound.

The fermentation was carried out in 500-ml Sakaguchi flasks each containing 100 ml of a medium composed of 1% starch, 0.4% yeast extract, and 0.2% peptone in seawater. The seed culture was inoculated into each flask, and the fermentation was carried out at 30 °C for 2 days on a rotary shaker.

The cells of *R. squalenifaciens* were precipitated from the 6-liter culture by centrifugation at 13,000*g*. After removing the supernatant, the red pigment in the cells was extracted 3 times with 50 ml each of CH_2Cl_2:MeOH (1:1). The extracts were combined and concentrated to a small volume in vacuo, and partitioned with EtOAc/H_2O without adjusting the pH. The EtOAc layer was evaporated to dryness and subjected to silica gel chromatography, using CH_2Cl_2–MeOH (20:1). The red-colored fractions were collected and concentrated to dryness to give a red oil (42.6 mg). This red oil was subjected to preparative silica gel HPLC (YMC-Pack SIL column, 20 × 250 mm), and separated with CH_2Cl_2–MeOH (15:1) as a solvent. The red-colored fractions were collected and evaporated to give a crude powder of **1** (5.4 mg). This crude powder of **1** was subjected to preparative ODS HPLC (Senshu Pak PEGASIL ODS column, 20 × 250 mm), and separated with MeOH as a solvent. More than five red components could be separated by this chromatography. The main component was collected and concentrated to give pure **1** (0.9 mg).[2]

Compound **1** was dissolved in CH_2Cl_2–MeOH (1:1) and analysed by APCI-MS. The $(M-H)^-$ peak was intensively apparent at *m*/*z* 801 in the negative-ion mode, and the $(M+H)^+$ peak was weakly apparent at *m*/*z* 803 in the positive-ion mode. These findings enabled the molecular weight of **1** to be deduced as 802, and

Keywords: *Rubritalea squalenifaciens*; Diapolycopenedioic acid xylosyl ester; Antioxidative activity.
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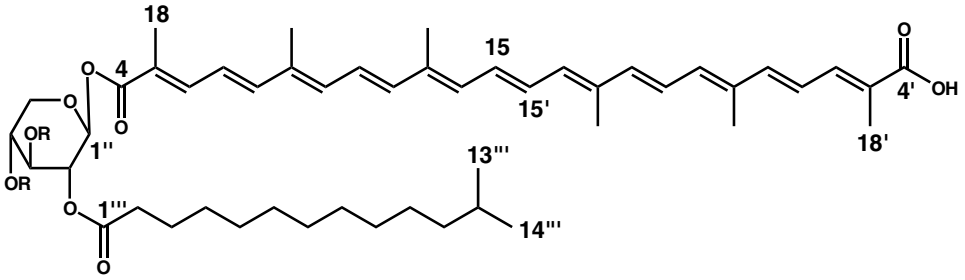
doi:10.1016/j.tetlet.2007.02.065



R=H diapolycopenedioic acid xylosyl ester (**1**)
R=Ac diacetyl diapolycopenedioic acid xylosyl ester (**2**)

Figure 1. Structure of diapolycopenedioic acid xylosyl ester.

the intense $(M-H)^-$ peak suggested the presence of a carboxylic group in **1**. Since **1** was slightly soluble in $CDCl_3$ or CD_3OD, the ^1H NMR and ^1H–^1H DQF COSY spectra of **1** were measured and analysed in $CDCl_3$–CD_3OD (5:1). The analyses clearly showed the presence of β-xylose {H-1″ [δ 5.65 ($J = 8.3$ Hz)], H-2″ [δ 5.01 ($J = 8.3$, 8.9 Hz)], H-3″ [δ 3.62 ($J = 8.2$, 8.9 Hz)], H-4″ [δ 3.73 ($J = 4.7$, 8.2, 9.0 Hz)], H-5″ [δ 3.44 ($J = 9.0$, 11.3 Hz) and δ 4.06 ($J = 4.7$, 11.3 Hz)]} in **1**, and the chemical shifts of H-1″ and H-2″ implied the presence of an ester linkage at C-1″ and C-2″. The analyses also showed the presence of a 12-methyltridecanoyl group in **1** {H-13‴ and H-14‴ [δ 0.85 ($J = 6.3$ Hz), 6H], H-11‴ [δ 1.12 (m), 2H], H-4‴–H-10‴ (δ 1.20–1.30, 14H), H-12‴ [δ 1.50 (m), 1H], H-3‴ [δ 1.57 (m), 2H], H-2‴ [δ 2.32 ($J = 7.3$ Hz), 2H]}.

The acetylation of **1** with Ac_2O in dry pyridine gave diacetyl derivative **2**[3] [FAB-MS *m*/*z* 886.5 (M^+)[4]]. Since **2** was highly soluble in $CDCl_3$, further structural studies were performed on **2**. The molecular formula of **2** was determined to be $C_{53}H_{74}O_{11}$ (M^+, calcd for 886.5239, found 886.5240) by HRFAB-MS. The molecular formula of **1** was therefore determined to be $C_{49}H_{70}O_9$.

The ^1H NMR data for **2** in $CDCl_3$ showed 6 singlet methyls, 2 doublet methyls, 11 sp^3 methylenes, 5 sp^3 methines, and 16 sp^2 methines, apart from the signals derived from the two acetyl groups. The ^{13}C NMR and DEPT experiments revealed 8 methyls, 11 sp^3 methylenes, 5 sp^3 methines, 16 sp^2 methines, and 9 sp^2 quaternary carbons, apart from the signals derived from the acetyl groups. The sp^2 quaternary carbons observed at δ 164.1–172.2 were estimated to be ester or carboxylic acid carbons.

The analyses of the ^1H–^1H COSY and HSQC spectra of **2** reconfirmed the presence of β-xylose in **2**. The downfield shifts of H-3″ (δ 5.26) and H-4″ (δ 5.04) in xylose confirmed that these positions were acetylated in **2**. The ^1H–^{13}C long-range coupling observed from H-2″ (δ 5.17) and H-2‴ (δ 2.28) to C-1‴ (δ 172.2) in the HMBC spectrum (Fig. 2) proved the attachment of the 12-methyltridecanoyl group at C-2″.

At this point, the unassigned ^1H and ^{13}C signals were 6 singlet methyls, 16 sp^2 methines, 6 sp^2 quarternary carbons, and 2 carbonyl carbons. These carbons were proposed to constitute an aglycon. Detailed analyses of the ^1H–^1H COSY spectrum and ^1H–^{13}C long-range couplings from the singlet methyls in the HMBC spectrum (Fig. 2) established the assignment and connectivity of all these unassigned ^1H and ^{13}C signals as shown in Figure 2, and the ^1H–^{13}C long-range couplings from H-18 (δ 1.98) to C-4 (δ 166.3), and from H-18′ (δ 2.02) to C-4′ (δ 164.1) showed the presence of a carbonyl function at both ends of the aglycon (Fig. 2). All double bonds in the aglycon were determined to be E configuration by the *J* values of the sp^2 methines and ^{13}C chemical shifts of the singlet methyls (Fig. 2).[5] These results confirmed the aglycon of **2** to be diapolycopenedioic acid.[6] The UV–vis spectrum of **1** was closely similar to that of diapolycopenedioic acid.

The HMBC experiment showed a long-range coupling from H-1″ (δ 5.78) to C-4 (δ 166.3), and the ester linkage

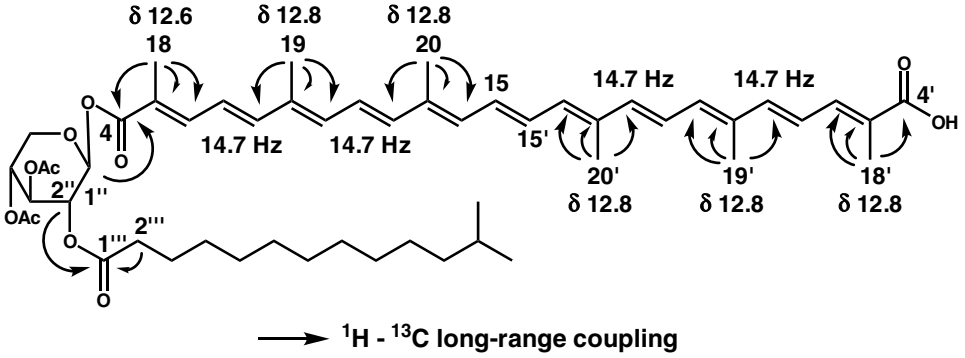


Figure 2. Key ^1H–^{13}C long-range couplings, *J* values, and δ_C values observed in the NMR analyses of **2**.

of β-xylose and the aglycon was proved. Considering the molecular formula of **2**, there was a free carboxylic acid moiety at C-4′. All the foregoing observations allowed the structure of **1** and **2** to be determined as that shown in Figure 1. The IUPAC-IUB semisystematic name of **1** is 4-[2-*O*-(12-methyltridecanoyl)-β-xylopyranosyl] hydrogen 4,4′-diapo-ψ,ψ-carotene-4,4′-dioate.

Since the antioxidative activities of some carotenoids have been reported previously,[7] in vitro inhibitory effects of **1** on lipid peroxidation induced by free radicals in a rat brain homogenate[8] were examined. The IC_{50} value of **1** was 4.6 μM (10.9 μM for β-carotene), indicating that **1** possessed potent antioxidative activity.

The diapolycopenedioic acid glucosyl ester[6] from *Methylobacterium rhodinum* ATCC 14821 (formally *Pseudomonas rhodos*) has previously been reported as a compound related to **1**. The aglycons of **1** and the diapolycopenedioic acid glucosyl ester were identical, while the diapolycopenedioic acid glucosyl ester possessed a β-glucose ester at C-4 of diapolycopenedioic acid. To our knowledge, **1** is the first reported carotenoid containing xylose. The acyl chain attachment at C-2 in xylose is also a structural feature of **1**. Although some carotenoids containing C-6 acyl glucose have previously been reported,[6,9] **1** is the first reported carotenoid containing a sugar acylated at C-2. Studies on the absolute structure of **1** and the minor pigments produced by *R. squalenifaciens* are in progress, and will be reported elsewhere.

Acknowledgments

R. squalenifaciens was kindly provided by Dr. Hiroaki Kasai, Atsuko Katsuta, and Dr. Yoshikazu Shizuri (Marine Biotechnology Institute).

References and notes

1. Kasai, H.; Katsuta, A.; Sekiguchi, H.; Matsuda, S.; Adachi, K.; Shindo, K.; Yoon, J.; Yokota, A.; Shizuri, Y. *Int. J. Syst. Evol. Microbiol.*, submitted for publication.
2. Physical data for compound **1**: $[\alpha]_{633}^{21}$ −20 [*c* 0.007, $CHCl_3$–MeOH (1:1)]; UV–vis (MeOH) λ_{max}: 312, 470, 490, 518 nm, %III/II = 30; ^{1}H NMR [400 MHz, $CDCl_3$–CD_3OD (5:1)] δ: 0.85 (d, 6H, *J* 6.3 Hz, H-13‴, H-14‴), 1.12 (m, 2H, H-11‴), 1.20–1.30 (14H, H-4‴–10‴), 1.50 (m, 1H, H-12‴), 1.57 (m, 2H, H-3‴), 1.98–2.07 (s, 18H, H-18–20, H-18′–20′), 2.32 (t, 2H, *J* 7.3 Hz, H-2‴), 3.44 (dd, 1H, *J* 9.0, 11.3 Hz, H-5″), 3.62 (dd, 1H, *J* 8.2, 8.9 Hz, H-3″), 3.73 (ddd, 1H, *J* 4.7, 8.2, 9.0 Hz, H-4″), 4.06 (dd, 1H, *J* 4.7, 11.3 Hz, H-5″), 5.01 (dd, 1H, *J* 8.3, 8.9 Hz, H-2″), 5.65 (d, 1H, *J* 8.3 Hz, H-1″), 6.30–6.80 (14H, H-7, 8, 10, 11, 12, 14, 15, 7′, 8′, 10′, 12′, 14′, 15′), 7.33 (2H, *J* 11.0 Hz, H-6, H-6′). APCI-MS *m*/*z* 801 $(M-H)^-$.
3. Physical data for compound **2**: ^{1}H NMR (500 MHz, $CDCl_3$) δ: 0.86 (d, 6H, *J* 6.5 Hz, H-13‴, H-14‴), 1.13 (m, 2H, H-11‴), 1.18–1.26 (14H, H-4‴–10‴), 1.55 (m, 2H, H-3‴), 1.98 (s, 3H, H-18), 2.00 (s, 3H, H-19), 2.00 (s, 3H, H-20), 2.00 (s, 3H, H-20′), 2.01 (s, 3H, H-19′), 2.02 (s, 3H, H-18′), 2.07 (s, 3H, 3″-acetyl CH_3), 2.07 (s, 3H, 4″-acetyl CH_3), 2.28 (t, 2H, *J* 7.5 Hz, H-2″), 3.57 (dd, 1H, *J* 8.5, 12.0 Hz, H-5″), 4.17 (dd, 1H, *J* 5.5, 12.0 Hz, H-5″), 5.01 (ddd, 1H, *J* 5.5, 8.5, 8.5 Hz, H-4″), 5.17 (dd, 1H, *J* 7.0, 8.5 Hz, H-2″), 5.26 (dd, 1H, *J* 8.5, 8.5 Hz, H-3″), 5.78 (d, 1H, *J* 7.0 Hz, H-1″), 6.35 (d, 2H, *J* 10.5 Hz, H-14, 14′), 6.39 (d, 1H, *J* 12.0 Hz, H-10), 6.42 (d, 1H, *J* 12.0 Hz, H-10′), 6.49 (d, 2H, *J* 14.7 Hz, H-12, 12′), 6.52 (dd, 2H, *J* 11.5, 14.7 Hz, H-7, 7′), 6.66 (dd, 2H, *J* 12.0, 14.7 Hz, H-11, 11′), 6.70 (dd, 1H, *J* 10.5, 15.0 Hz, H-15, 15′), 6.66 (d, 1H, *J* 14.7 Hz, H-8), 6.73 (d, 1H, *J* 14.7 Hz, H-8′), 7.34 (d, 2H, *J* 11.5 Hz, H-6, 6′); ^{13}C NMR ($CDCl_3$, 125 MHz) δ: 12.6 (C-18), 12.8 (C-19), 12.8 (C-20), 12.8 (C-18′), 12.8 (C-19′), 12.8 (C-20′), 20.7 (3″-acetyl CH_3), 20.7 (4″-acetyl CH_3), 22.7 (C-13‴, 14‴), 24.9 (C-3‴), 28.0 (C-12‴), 27.4*, 29.0*, 29.3 (C-4‴), 29.4*, 29.7*, 29.7*, 29.9*, 34.1 (C-2‴), 39.0 (C-11‴), 62.8 (C-5″), 68.4 (C-4″), 69.1 (C-2″), 70.9 (C-3″), 92.3 (C-1″), 122.6 (C-7)[a], 122.7 (C-7′)[a], 123.9 (C-5), 124.4 (C-5′), 124.8 (C-11)[b], 124.9 (C-11′)[b], 130.8 (C-15)[c], 131.1 (C-15′)[c], 134.3 (C-14)[d], 134.6 (C-14′)[d], 135.2 (C-9′), 135.3 (C-9), 136.9 (C-10), 137.1 (C-13)[e], 137.1 (C-13′)[e], 137.7 (C-10′), 140.3 (C-12)[f], 140.9 (C-12′)[f], 141.5 (C-6′), 143.2 (C-6), 143.6 (C-8′), 146.7 (C-8), 164.1 (C-4′), 166.3 (C-4), 169.8 (C-4″ acetyl C=O), 169.9 (C-3″ acetyl C=O), 172.2 (C-1‴) [*C-5‴–10‴ (unassigned), [a,b,c,d,e,f]interchangeable]; HRFAB-MS *m*/*z* 886.5240 (M^+, $C_{53}H_{74}O_{11}$, calcd for 886.5239).
4. Maoka, T.; Fujiwara, Y.; Hashimoto, K.; Akimoto, N. *Lipids* **2004**, *39*, 179–183.
5. Englert, G. NMR Spectroscopy. In *Carotenoids*; Britton, G., Liaaen-Jensen, S., Pfander, H., Eds.; Birkhäuser: Basel, 1995; Vol. 1B, pp 147–160.
6. Kleinig, H.; Schmitt, R.; Meister, E.; Englert, G.; Thommen, H. *Z. Naturforsch* **1979**, *34c*, 181–185.
7. Woodall, A. A.; Britton, G.; Jackson, J. M. *Biochim. Biophys. Acta* **1997**, *1336*, 575–586.
8. Shindo, K.; Kagiyama, Y.; Nakamura, R.; Hara, A.; Ikenaga, H.; Furukawa, K.; Misawa, N. *J. Mol. Catal. B: Enzymatic* **2003**, *23*, 9–16.
9. Yokoyama, A.; Shizuri, Y.; Hoshino, T.; Sandmann, G. *Arch. Microbiol.* **1996**, *165*, 343–345.