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Braunixanthins 1 and 2, New Carotenoids from the Green Microalga *Botryococcus braunii*

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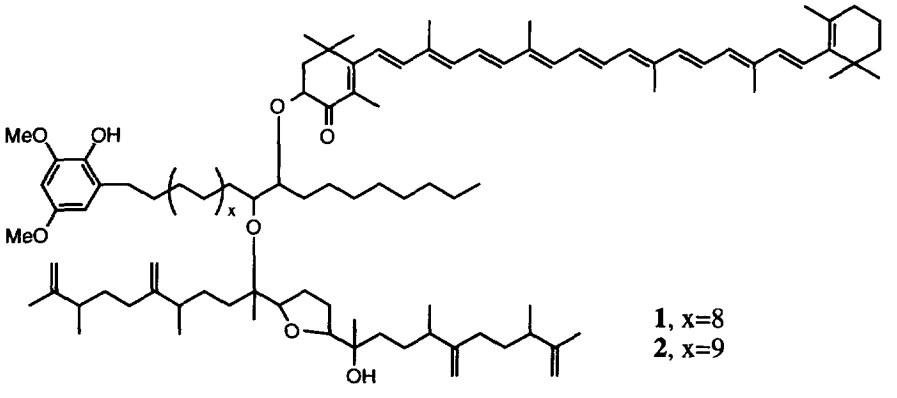
Abstract: Two new carotenoids, braunixanthins 1 (**1**) and 2 (**2**), were isolated from the colonial matrix of the green microalga *Botryococcus braunii* Kawaguchi-1 strain. Their structures were elucidated mainly by NMR and FABMS. Braunixanthins are composed of echinenone, alkylphenol and tetramethylsqualene derivative by ether linkages. This is the first report on the detection of the alkylphenol derivatives in the B race. © 1997 Elsevier Science Ltd.

Botryococcus braunii is a green microalga which produces a large amount of hydrocarbons.[1] This alga can be classified into A, B and L races by the types of hydrocarbons.[2] The A race produces linear hydrocarbons called alkadienes and alkatrienes.[3] The B race produces terpenoid hydrocarbons called botryococcenes[4] and squalene derivatives.[5] The L race produces a tetraterpene called lycopadiene.[6] This alga forms colonies by embedding individual cells into the biopolymers called extracellular matrix and a large part of hydrocarbons is accumulated in the matrix.[7] It is known that constituents of the biopolymers in the A and L races have related structures to the hydrocarbons produced.[8] However, the biopolymers in the B race have not been well characterized.[9] In the extracellular matrix of the B race, botryococcenes, tetramethylsqualene and carotenoids such as echinenone[10] or botryoxanthin A[11] are accumulated. From the extracellular matrix of the Kawaguchi-1 strain belonging to the B race, we have detected new-type carotenoids, braunixanthins 1 (**1**) and 2 (**2**), which seem to contribute to the formation of the biopolymers. In this paper, we report the isolation and structural elucidation of them.



1, x=8
2, x=9

The culture of *B. braunii* Kawaguchi-1 strain was aerated with air containing 2% CO_2 and the algal cells were harvested at the stationary phase (at 40 days). The freeze-dried algal cells (10g) were sonicated in acetone to extract the components in the colonial matrix. The extract was subjected to silica gel column chromatography and the hydrocarbon fraction containing botryococcenes was eluted with *n*-hexane. Remaining matters on the column were eluted with MeOH and then subjected to ODS column chromatography. A fraction containing echinenone was eluted with MeOH/CH_2Cl_2/MeCN (7:2:1) and the fractions containing botryoxanthins A, B and α-botryoxanthin A were eluted with MeOH/CH_2Cl_2/MeCN (7:3:1). The red matters retained on the ODS column were eluted with CH_2Cl_2 and further purified by normal phase and reversed phase HPLC to yield red oily materials, braunixanthins 1 (**1**, 2.4mg) and 2 (**2**, 3.3mg).

The molecular formula of **2** was determined to be $C_{113}H_{182}O_8$ by the HRFABMS [*m/z* 1667.3776 $(M)^+$ Δ -5.9 mmu] and NMR data (Table 1). The UV-VIS absorption of this compound in *n*-hexane showed the presence of an echinenone-type carotenoid moiety [λ_{max} 460 nm (ε 120,000), 295 nm (ε 21,000)]. The ^1H and ^{13}C NMR spectra suggested the presence of three partial structures **A**, **B** and **C**.

Partial structure **A** had an echinenone-like structure. A methylene [H-2' (δ_H 1.49), C-2' (δ_C 39.95)] was coupled to 3'-methylene [H-3' (δ_H 1.60), C-3' (δ_C 19.68)] which was also coupled to 4'-methylene [H-4' (δ_H 1.97), C-4' (δ_C 33.34)] in COSY 45. HMBC correlations between 16', 17'-Me and C-1', C-2', C-6', between H-2' and C-1', between 18'-Me and C-4', C-5', C-6' and between H-4' and C-5' established one β-end group straightforwardly. The conjugated polyene part was also established by combination of the COSY 45 and HMBC. HMBC correlations between H-7' and C-5', between H-8' and C-6' connected the β-ionone ring to the conjugated polyene part. In the COSY45 spectrum, another β-end group showed connectivity between a methylene [H-2 (δ_H 2.03), C-2 (δ_C 45.26)] and an oxymethine [H-3 (δ_H 4.21), C-3 (δ_C 79.44)] . HMBC correlations between 16,17-Me and C-1, C-2, C-6, between H-2 and C-1, C-4 (δ_C 198.52), and between 18-Me and C-4, C-5, C-6 established a 4-keto-β-end group. The 4-keto-β-end group was also connected to the conjugated polyenes by HMBC correlations between H-7 and C-5 and between H-8 and C-6. There was no *cis*-peak in the UV-VIS spectrum, and the PSNOESY spectrum showed correlations between H-7 and 19-Me, H-8 and H-10, H-11 and 20-Me, H-12 and H-14, H-14 and H-15', H-7' and 19'-Me, H-8' and H-10', H-11' and 20'-Me, and H-12' and H-14'. Therefore geometries of the conjugated polyene part are all-*trans*. The partial structure **A** is shown in Fig.1.

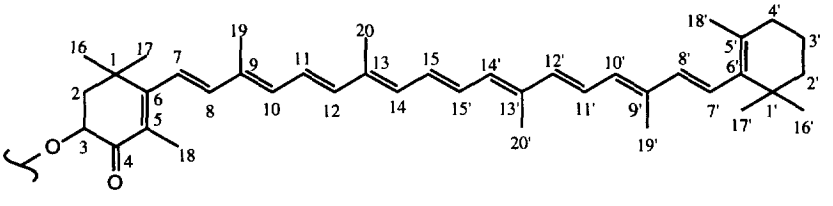


Fig. 1. Partial structure **A**

Table 1. ^{1}H and ^{13}C NMR Data of Braunixanthin 2 (**2**) in C_6D_6

Position	H (mult, J Hz)	C (mult)	HMBC(^{1}H)	Position	H (mult, J Hz)	C (mult)	HMBC(^{1}H)
1		36.80(s)	2, 3, 16, 17	1"	4.79(brs) 4.80(brs)	110.18(t)	3", 25"
2	2.03(m)	45.26(t)	3, 16, 17	2"		149.77(s)	3", 25, 31"
3	4.21(dd, 7.0, 10.0)	79.44(d)	2	3"	2.21(m)	41.37(d)	1", 4", 25", 31"
4		198.52(s)	2, 18	4"	1.48(m) 1.63(m)	33.82(t)	3", 5", 31"
5		128.87(s)	7, 18	5"	2.08(m)	32.25(t)	3", 4", 7", 26"
6		159.39(s)	2, 8, 16, 17, 18	6"		154.52(s)	5", 7", 8", 33"
7	6.12(d, 15.8)	123.96(d)		7"	2.28(m)	40.80(d)	26", 33"
8	6.43(d, 15.8)	141.85(d)	10, 19	8"	1.78(m) 2.20(m)	30.50(t)	7", 33"
9		134.54(s)	7, 11, 19	9"	1.45(m) 1.60(m)	35.99(t)	7", 27"
10	6.29(d, 11.2)	135.25(d)	8, 12, 19	10"		79.60(s)	27", 28"'
11	6.67(dd, 11.2, 15.0)	124.67(d)		11"	4.02(t, 7.5)	84.02(d)	27"
12	6.49(d, 15.0)	140.04(d)	10, 14, 20	12"	1.76(m) 2.21(m)	26.17(t)	
13		136.19(s)	11, 15, 20	13"	1.76(m) 2.28(m)	26.20(t)	
14	6.35(d, 10.0)	134.54(d)	12, 20, 15'	14"	3.84(t, 7.7)	85.48(d)	28"
15	6.65(dd, 10.0, 15.0)	130.27(d)	14'	15"		73.59(s)	28"
16	1.17(s)	27.23(q)		16"	1.46(m) 1.65(m)	36.80(t)	17", 28"
17	1.06(s)	30.50(q)		17"	1.72(m) 2.06(m)	29.65(t)	34"
18	2.17(s)	14.55(q)		18"	2.12(m)	41.28(d)	29", 34"
19	1.80(s)	12.43(q)	8, 10	19"		154.52(s)	18", 20", 34"
20	1.86(s)	12.79(q)	12, 14	20"	2.00(m)	31.89(t)	18", 21", 22", 29"
1'		34.56(s)	2', 3', 16', 17'	21"	1.48(m) 1.58(m)	33.71(t)	20", 22", 32"
2'	1.49(m)	39.95(t)	4', 16', 17'	22"	2.15(m)	41.44(d)	20", 24", 30", 32"
3'	1.60(m)	19.68(t)	2', 4'	23"		149.83(s)	21", 22", 30", 32"
4'	1.97(m)	33.34(t)	2', 3', 18'	24"	4.83(brs) 4.84(brs)	110.08(t)	22", 30"
5'		129.46(s)	3', 4', 7', 18'	25"	1.60(brs)	18.95(q)	1", 3"
6'		138.34(s)	2', 4', 8', 16', 17', 18'	26"	4.93(brs) 4.96(brs)	108.15(t)	5"
7'	6.34(d, 15.9)	127.09(d)		27"	1.63(s)	20.95(q)	
8'	6.36(d, 15.9)	138.58(d)	10',19'	28"	1.52(s)	25.11(q)	
9'		136.30(s)	7', 11', 19'	29"	4.88(brs) 4.89(brs)	108.05(t)	18", 20"
10'	6.32(d, 11.5)	131.78(d)	8', 12', 19'	30"	1.65(brs)	19.01(q)	22", 24"
11'	6.79(dd, 11.5, 14.6)	125.88(d)		31"	1.06(d, 7.5)	20.00(q)	3", 4"
12'	6.45(d, 14.6)	137.81(d)	10', 14', 20'	32"	1.00(d, 6.9)	19.92(q)	21", 22"
13'		137.40(s)	11', 15', 20'	33"	1.22(d, 6.9)	20.15(q)	7", 8"
14'	6.30(d, 10.0)	132.97(d)	15, 12', 20'	34"	1.08(d, 7.3)	20.88(q)	17", 18"
15'	6.71(dd, 10.0, 15.0)	131.51(d)	14	OH-a	3.51		
16'	1.15(s)	29.19(q)	2'				
17'	1.15(s)	29.19(q)	2'				
18'	1.80(s)	21.98(q)	4'				
19'	1.93(s)	12.82(q)	8', 10'				
20'	1.86(s)	12.79(q)	12', 14'				

Table 1 (Continued)

Position	H (mult, J Hz)	C (mult)	HMBC(^{1}H)	Position	H (mult, J Hz)	C (mult)	HMBC(^{1}H)
1'''		138.30(s)	5''', 7''', OH-b	28'''	4.69(m)	72.80(d)	
2'''		147.18(s)	3''', 38''', OH-b	29'''	3.92(brd)	84.92(d)	3
3'''	6.32(d, 2.7)	97.32(d)	5'''	30'''	1.80(m) 2.10(m)	27.65(t)	
4'''		153.51(s)	3''', 5''', 39'''	31'''-34'''	1.30-1.40(m)	30.23(t)	
5'''	6.42(d, 2.7)	106.12(s)	3''', 7'''	35'''	1.30(m)	32.35(t)	
6'''		128.94(s)	7''', OH-b	36'''	1.28(m)	23.09(t)	
7'''	2.85(t, 7.7)	30.71(t)	5'''	37'''	0.90(t, 6.2)	14.35(q)	35''', 36'''
8'''	1.80(m)	30.23(t)	7'''	38'''	3.44(s)	55.28(q)	
9'''-26'''	1.30-1.40(m)	30.23(t)		39'''	3.12(s)	55.28(q)	
27'''	1.80(m) 2.20(m)	27.52(t)		OH-b	5.25		

The ^{1}H, ^{13}C NMR, COSY 45, HMQC, HMQC-HOHAHA and HMBC data indicated the presence of a tetramethylsqualene-related moiety (partial structure **B**) in **2**. The ^{1}H NMR showed the presence of a hydroxy group [OH-a (δ_H 3.51)]. The ^{13}C NMR chemical shifts of C-10", C-11", C-14" and C-15" indicated that each of these carbons attached to an oxygen. The isotope shift was observed at C-15" when D_2O was added, therefore a hydroxy group attached to C-15". The COSY 45 and HMQC-HOHAHA established the connectivities from C-3" to C-5", from C-7" to C-9", from C-16" to C-18", and from C-20" to C22". C-31" (δ_H 1.06) , 32" (δ_H 1.00), 33" (δ_H 1.22), and 34" (δ_H 1.08) methyls also coupled with a methine at C-3" (δ_H 2.21), 22" (δ_H 2.15), 7" (δ_H 2.28) and 18" (δ_H 2.12), respectively. The COSY 45 and HMQC-HOHAHA also established the connectivities from 11"-oxymethine to 14"-oxymethine. HMBC correlations between 25"-Me (δ_H 1.60) and C-1", C-2", C-3", between 33"-Me and C-6", C-7", C-8", between 27"-Me (δ_H 1.63) and C-9", C-10", C-11", between 28"-Me (δ_H 1.52) and C-14", C-15", C-16", between 34"-Me and C-17", C-18", C-19", and between 30"-Me (δ_H 1.65) and C-22", C-23", C-24" established the partial structure **B**.

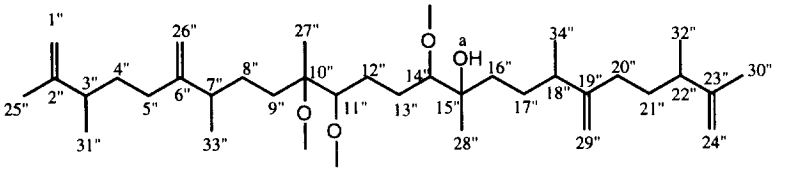


Fig. 2. Partial structure **B**

The ^{1}H and ^{13}C NMR spectra indicated the presence of aliphatic long chains and a 2,4-dimethoxy phenol group as the remaining part (partial structure **C**). An aromatic proton [H-3''' (δ_H 6.32), C-3''' (δ_C 97.32)] coupled to another one [H-5''' (δ_H 6.42), C-5'''(δ_C 106.12)]. HMBC correlations between H-3''' and C-2''', C-4''', C-5''', between H-5''' and C-1''', C-4''', between 38'''-Me [38'''-Me (δ_H 3.44), C-38''' (δ_C 55.28)] and C-2''', between 39'''-Me [39'''-Me (δ_H 3.12), C-39''' (δ_C 55.28)] and C-4''', and between a phenolic hydroxy group [OH-b (δ_H 5.25)] and C-1''', C-2''', C-6''' established a 2,4-dimethoxyphenol group. This phenol group was substituted at C- 6''' by an aliphatic long chain because the HMBC correlation was found

between a benzylic methylene [H-7'''(δH 2.85), C-7''' (δc 30.71)] and C'''-6. There was a terminal methyl [H-37''' (δH 0.90), C-37''' (δc 14.35)] which coupled to a methylene [H-36''' (δH 1.28), C-36''' (δc 23.09)]. Two oxymethines coupling each other [H-28''' (δH 4.69), C-28''' (δc 72.80), H-29''' (δH 3.92), C-29''' (δc 84.92)] were recognized in the COSY 45. These oxymethines were embedded in the long methylene chain because they also coupled to methylenes around δH 1.8 but their positions could not be determined by the NMR data. A series of alkylphenol derivatives in the A race of *B. braunii* commonly have two connected oxymethines at the positions of 9 and 10 relative to the terminal methyl group probably due to their biosynthetic feature. The FABMS spectrum of **2** exhibited an ion peak at *m/z* 476 which was attributed to [$C_{30}H_{52}O_4$] (Scheme 1).

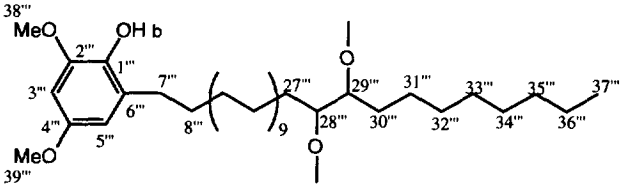
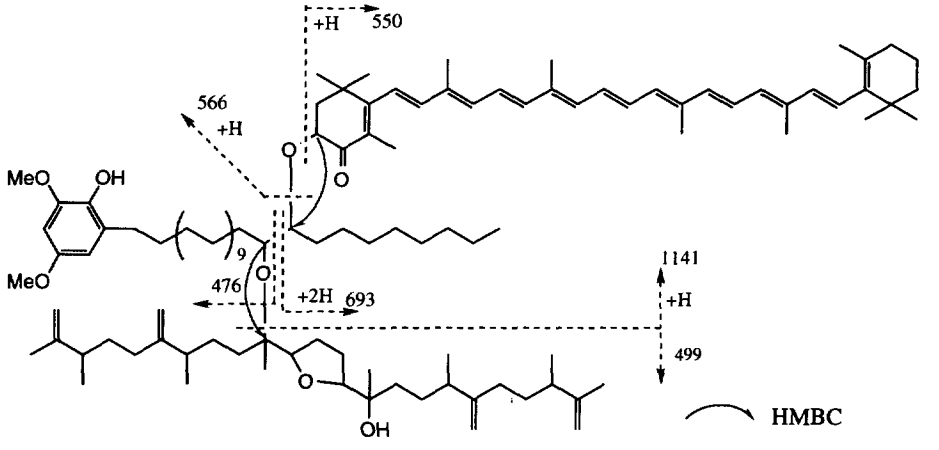


Fig. 3. Partial structure **C**

Therefore the position of two oxymethines in **2** was also deduced to be 9 and 10 relative to the termial methyl. Considering the remaining number of carbons and protons, the partial structure **C** was determined as shown in Fig.3.



Scheme 1. Selected HMBC correlations and FABMS fragmentation pattern of **2**

Gross structure of **2** was determined by HMBC and FABMS data. The HMBC correlation between the down-fielded methine at C-3 [H-3 (δ_H 4.21), C-3 (δ_C 79.44)] of the partial structure **A** and an oxymethine carbon (δ_C 84.92) in **C** was found (Scheme 1). The β-echinenone moiety was connected to a methine of the alkylphenol by an ether bridge. Another HMBC correlation was observed between an oxymethine (δ_H 4.69) in **C** and C-10" (δ_C 79.60) in **B**. The FABMS spectrum of **2** exhibited an ion peak at *m/z* 693 which corresponded to [$C_{49}H_{71}O_2$+2H] (Scheme 1). Therefore the partial structure **A** was connected to an oxymethine of **C** (C-29"') and the **B** was to another oxymethine of **C** (C-28"') by an ether, respectively. From the molecular formula, C-11"[H-11" (δ_H 4.02), C-11" (δ_C 84.02)] and C-14"[H-14" (δ_H 3.84), C-14" (δ_C 85.48)] should be connected by an ether. Then the gross structure of braunixanthin 2 was determined as **2**.

The molecular formula of braunixanthin 1 (**1**) was determined to be $C_{111}H_{178}O_8$ by the HRFABMS [*m/z* 1639.3507 $(M)^+$ Δ -1.5 mmu]. The UV-VIS absorption of this compound in *n*-hexane also showed the presence of an echinenone-type carotenoid moiety [λ_{max} 460 nm (ε 119,000), 295 nm (ε 22,000)]. The ^1H, ^{13}C NMR, HMQC, COSY 45, HMBC and PSNOESY data were almost identical to those of **2** (Table 2). However, its molecular formula was different from that of **2** by 28 which corresponded to two methylene units. The FABMS of **1** exhibited similar ion peaks to those in **2** at *m/z* 499, 550, 566, 693. Ion peaks at *m/z* 448 and 1113 existed in **1** instead of that at 476 and 1141 in **2**. Thus the structure of braunixanthin 1 was determined as **1**.

Braunixanthins 1 and 2 contain a series of alkylphenol moiety. Alkenylphenols and their derivatives have been found in the A race but never in the B or L race.[12] This is the first report on finding alkylphenol derivatives in the B race. There was the report on the distribution of very long fatty acids in the B race as well as in the A race.[13] In respect of biosynthesis, alkylphenol derivatives and very long fatty acids seem to have a close relation to linear hydrocarbons synthesized through elongation of C_{18} fatty acids by the A races. However linear hydrocarbons have never been detected in the B race. On the other hand, braunixanthins also have a moiety related to a tetramethylsqualene derivative which is typically produced by the B race together with botryococcenes. Therefore the Kawaguchi-1 strain have both properties of the A and B races. Further studies on mechanisms of carbon assimilation by the B race of *B. braunii* to hydrocarbons or substances derived from fatty acids would be necessary.

Resistant biopolymers comprising the extracellular matrix of *B. braunii* are said to resemble sporopollenins[14] but it was reported that they did not originate from carotenoids and/or carotenoid esters.[8] The constituents of the resistant biopolymers in the A and L races have similar structures to the hydrocarbons they produce.[8] However those in the B race are thought to be related to linear hydrocarbons rather than botryococcenes.[8] Many kinds of ether lipids exist in the A race and are thought to take part in the formation of the resistant biopolymers.[12,15] Braunixanthins have a common aspect to the ether lipids in the A race because three distinct moieties, a ketocarotenoid, a tetramethylsqualene derivative and a very long alkylphenol are linked by ether each other. Very recently, methylated squalene epoxides were isolated from the B race and their contribution to formation of the resistant biopolymers was suggested.[16] Braunixanthins are specific to the B race because of the presence of tetramethylsqualene moiety but also seem to be close to sporopollenins for the presence of ketocarotenoid moiety . Therefore, it is thought that the braunixanthins comprise some parts of resistant biopolymers in the B race. Considering triterpenoid production by the B race, less attention has been paid for squalene derivatives than botryococcenes because the formers are detected as minor components in extractable fraction. However an actual quantity of squalene derivatives may be larger than ever been expected if braunixanthins comprise the resistant biopolymers which account for 5-10% of the total mass of this alga.

Table 2. ^{1}H and ^{13}C NMR Data of Braunixanthin 1 (1) in C_6D_6

Position	H (mult, J Hz)	C (mult)	HMBC(^{1}H)	Position	H (mult, J Hz)	C (mult)	HMBC(^{1}H)
1		36.80(s)	2, 3, 16, 17	1"	4.80(brs) 4.81(brs)	110.20(t)	3", 25"
2	2.03(m)	45.26(t)	3, 16, 17	2"		149.77(s)	3", 25, 31"
3	4.21(dd, 7.5, 9.7)	79.45(d)	2	3"	2.21(m)	41.37(d)	1", 4", 25", 31"
4		198.52(s)	2, 18	4"	1.48(m) 1.63(m)	33.82(t)	3", 5", 31"
5		128.87(s)	7, 18	5"	2.08(m)	32.25(t)	3", 4", 7", 26"
6		159.39(s)	2, 8, 16, 17, 18	6"		154.90(s)	5", 7", 8", 33"
7	6.12(d, 15.9)	123.97(d)		7"	2.29(m)	40.80(d)	26", 33"
8	6.43(d, 15.9)	141.85(d)	10, 19	8"	1.78(m) 2.20(m)	30.50(t)	7", 33"
9		134.52(s)	7, 11, 19	9"	1.45(m) 1.60(m)	35.99(t)	7", 27"
10	6.30(d, 11.2)	135.27(d)	8, 12, 19	10"		79.60(s)	27", 26"'
11	6.69(dd, 11.2, 15.0)	124.67(d)		11"	4.02(t, 7.5)	84.02(d)	27"
12	6.49(d, 15.0)	140.04(d)	10, 14, 20	12"	1.76(m) 2.21(m)	26.17(t)	
13		136.21(s)	11, 15, 20	13"	1.76(m) 2.28(m)	26.20(t)	
14	6.36(m)	134.52(d)	12, 20, 15'	14"	3.84(t, 7.7)	85.50(d)	28"
15	6.68(m)	130.27(d)	14'	15"		73.59(s)	28"
16	1.17(s)	27.23(q)		16"	1.46(m) 1.65(m)	36.80(t)	17", 28"
17	1.06(s)	30.50(q)		17"	1.72(m) 2.06(m)	29.67(t)	34"
18	2.17(s)	14.55(q)		18"	2.12(m)	41.28(d)	29", 34"
19	1.80(s)	12.43(q)	8, 10	19"		154.52(s)	18", 20", 34"
20	1.86(s)	12.79(q)	12, 14	20"	2.00(m)	31.89(t)	18", 21", 22", 29"
1'		34.56(s)	2', 3', 16', 17'	21"	1.48(m) 1.58(m)	33.71(t)	20", 22", 32"
2'	1.49(m)	39.95(t)	4', 16', 17'	22"	2.15(m)	41.44(d)	20", 24", 30", 32"
3'	1.60(m)	19.70(t)	2', 4'	23"		149.83(s)	21", 22", 30", 32"
4'	1.97(m)	33.34(t)	2', 3', 18'	24"	4.82(brs) 4.85(brs)	110.08(t)	22", 30"
5'		129.46(s)	3', 4', 7', 18'	25"	1.61(d, 0.8)	18.95(q)	1", 3"
6'		138.34(s)	2', 4', 8', 16', 17', 18'	26"	4.96(brs) 4.98(brs)	108.15(t)	5"
7'	6.34(m)	127.09(d)		27"	1.63(s)	20.95(q)	
8'	6.38(d, 16.1)	138.58(d)	10',19'	28"	1.52(s)	25.13(q)	
9'		136.30(s)	7', 11', 19'	29"	4.88(brs) 4.89(brs)	108.05(t)	18", 20"
10'	6.32(m)	131.78(d)	8', 12', 19'	30"	1.65(d, 0.8)	19.01(q)	22", 24"
11'	6.79(dd, 11.4, 15.0)	125.88(d)		31"	1.06(d, 6.9)	20.00(q)	3", 4"
12'	6.46(d, 15.0)	137.82(d)	10', 14', 20'	32"	1.00(d, 6.9)	19.92(q)	21", 22"
13'		137.40(s)	11', 15', 20'	33"	1.22(d, 6.9)	20.15(q)	7", 8"
14'	6.31(m)	132.97(d)	15, 12', 20'	34"	1.08(d, 6.9)	20.88(q)	17", 18"
15'	6.71(m)	131.51(d)	14	OH-a	3.50		
16'	1.15(s)	29.19(q)	2'				
17'	1.15(s)	29.19(q)	2'				
18'	1.80(s)	21.98(q)	4'				
19'	1.93(s)	12.82(q)	8', 10'				
20'	1.86(s)	12.88(q)	12', 14'				

Table 2 (Continued)

Position	H (mult, J Hz)	C (mult)	HMBC(^{1}H)	Position	H (mult, J Hz)	C (mult)	HMBC(^{1}H)
1'''		138.30(s)	5''', 7''', OH-b	26'''	4.69(m)	72.80(d)	
2'''		147.18(s)	3''', 36''', OH-b	27'''	3.92(brd, 8.4)	84.92(d)	3
3'''	6.32(d, 2.7)	97.32(d)	5'''	28'''	1.80(m) 2.10(m)	27.65(t)	
4'''		153.51(s)	3''', 5''', 37'''	29'''-32'''	1.30-1.40(m)	30.23(t)	
5'''	6.42(d, 2.7)	106.12(s)	3''', 7'''	33'''	1.30(m)	32.35(t)	
6'''		128.94(s)	7''', OH-b	34'''	1.28(m)	23.09(t)	
7'''	2.85(t, 7.7)	30.71(t)	5'''	35'''	0.90(t, 6.2)	14.35(q)	33''', 34'''
8'''	1.80(m)	30.23(t)	7'''	36'''	3.44(s)	55.28(q)	
9'''-24'''	1.30-1.40(m)	30.23(t)		37'''	3.12(s)	55.28(q)	
25'''	1.80(m) 2.20(m)	27.52(t)		OH-b	5.25		

EXPERIMENTAL

General methods: ^{1}H and ^{13}C NMR spectra were recorded in C_6D_6 on a JEOL JNM-A600 NMR spectrometer at 600 MHz and 150 MHz, respectively. ^{1}H and ^{13}C NMR chemical shifts were referenced to solvent peaks: δ_H 7.15 and δ_C 128.00 for C_6D_6. All two-dimensional NMR spectra were also measured on the same equipment. COSY 45 spectrum was recorded at 27.0°C according to the protocol of Bax and Freeman.[17] HMQC, HMQC-HOHAHA and HMBC spectra were recorded essentially according to the protocol of Bax and Subramanian,[18] Lermer and Bax[19] and Bax and Summers[20], respectively. PSNOESY spectrum was recorded with a mixing time of 700 ms. UV-VIS spectrum was measured on a Hitachi 330 spectrophotometer. FAB mass spectrum was measured by using *m*-nitrobenzyl alcohol as a matrix on a JEOL SX 102 mass spectrometer.

Culture Conditions: *B. braunii* Kawaguchi-1 strain was isolated from Lake Kawaguchi in Yamanashi Prefecture in Japan and cultured in 1.5 L Roux flasks containing modified Chu 13 medium[2,7] with aeration (filtered air containing 2% CO_2) at 25°C under illumination of 240 μmol photon m^{-2} s^{-1} on a 12L:12D cycle. Cells at the stationary phase (40 days) were harvested by filtration with a 20 μm nylon plankton net, freeze-dried and stored below -20°C until extraction.

Extraction and Isolation: The freeze-dried algal cells (10g) were sonicated in acetone to extract the constituents in the colonial matrix. The extract was centrifuged at 1000 x g at 15°C for 10 min to obtain a supernatant. Extraction was repeated until the supernatants became colorless. The supernatants were combined and evaporated to remove acetone. The residue was dissolved in *n*-hexane, subjected to silica gel column chromatography (Wakogel C-300). At first the hydrocarbon fraction (1720 mg) was eluted with *n*-hexane and remaining components were eluted with MeOH. The fraction (970mg) eluted with MeOH was subjected to ODS column chromatography (YMC-ODS AM) and eluted with MeOH/CH_2Cl_2/MeCN (7:2:1), MeOH/CH_2Cl_2/MeCN (7:3:1) and CH_2Cl_2. The fraction (145.8 mg) eluted with CH_2Cl_2 was subjected to normal-phase HPLC on Develosil 60-3 [*n*-hexane/diethyl ether (9 :1); UV detection at 210 nm] to obtain a red colored fraction. The fraction was finally purified by HPLC on Cosmosil ODS AR with MeCN/CH_2Cl_2 (7:3) to yield braunixanthins 1 (**1**, 2.4mg) and 2 (**2**, 3.3mg).

Braunixanthin 1 (1): red oil; UV (*n*-hexane) λ_{max} 460 nm (ε 119,000), 295 nm (ε 22,000); HRFABMS *m/z* 1639.3507 $(M)^+$ calcd. for $C_{111}H_{178}O_8$ (Δ -1.5 mmu). For ^1H and ^{13}C NMR data, see Table 2.
Braunixanthin 2 (2): red oil; UV (*n*-hexane) λ_{max} 460 nm (ε 120,000), 295 nm (ε 21,000); HRFABMS *m/z* 1667.3776 $(M)^+$ calcd. for $C_{113}H_{182}O_8$ (Δ -5.9 mmu). For ^1H and ^{13}C NMR data, see Table 1.

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REFERENCES

1. Brown, A. C.; Knights, B. A.; Conway, E. *Phytochemistry*, **1969**, 8, 543-547.
2. (a) Metzger, P.; Berkaloff, C.; Casadevall, E.; Couté, A. *Phytochemistry*, **1985**, 24, 2305-2312. (b) Metzger, P.; Allard, B.; Casadevall, E.; Berkaloff, C.; Couté, A. *J. Phycol.*, **1990**, 26, 258-266.
3. (a) Largeau, C.; Casadevall, E.; Berkaloff, C. *Phytochemistry*, **1980**, 19, 1081-1085. (b) Metzger, P.; Templier, J.; Largeau, C.; Casadevall, E. *Phytochemistry*, **1986**, 25, 1869-1872.
4. (a) Maxwell, J. R.; Douglas, A. G.; Eglinton, G.; McCormick, A. *Phytochemistry*, **1968**, 7, 157-171. (b) Cox, R. E.; Burlingame, A. L.; Wilson, D. M. *J. C. S. Chem. Comm.*, **1973**, 284-285. (c) Metzger, P.; Casadevall, E. *Tetrahedron Lett.*, **1983**, 24, 4013-4016. (d) Metzger, P.; Casadevall, E.; Pouet, M. J.; Pouet, Y. *Phytochemistry*, **1985**, 24, 2995-3002.
5. (a) Huang, Z.; Poulter, C. D. *Phytochemistry*, **1989**, 28, 1467-1470. (b) Okada, S.; Murakami, M.; Yamaguchi, K. *J. Appl. Phycol.* **1995**, 7, 555-559.
6. Metzger, P.; Casadevall, E. *Tetrahedron Lett.*, **1987**, 28, 3931-3934.
7. (a) Largeau, C.; Casadevall, E.; Berkaloff, C.; Dhamelincourt, P. *Phytochemistry*, **1980**, 19, 1043-1051. (b) Metzger, P.; David, M.; Casadevall, E. *Phytochemistry*, **1987**, 26, 129-134. (c) Berkaloff, C.; Rousseau, B.; Coute, A.; Casadevall, E.; Metzger, P.; Chirac, C. *J. Phycol*, **1984**, 20, 377-389.
8. (a) Berkaloff, C; Casadevall, E.; Largeau, C.; Metzger, P.; Peracca, S.; Virlet, J. *Phytochemistry*, **1983**, 22, 389-397. (b) Derenne, S.; Largeau, C.; Casadevall, E.; Sellier, N. *Phytochemistry*, **1990**, 29, 2187-2192. (c) Laureillard, J.; Largeau, C.; Casadevall, E. *Phytochemistry*, **1988**, 27, 2095-2098. (d) Templier, J.; Diesendorf, C.; Largeau, C.; Casadevall, E. *Phytochemistry*, **1992**, 31, 113-120.
9. (a) Kadouri, A.; Derenne, S.; Largeau, C.; Casadevall. E; Berkaloff, C. *Phytochemistry*, **1988**, 27, 551-557. (b) Templier, J.; Largeau, C.; Casadevall, E.; Berkaloff, C. *Phytochemistry*, **1992**, 31, 4097-4104.
10. (a) Grung, M.; Metzger, P.; Liaaen-Jensen, S. *Biochem. Syst. Ecol.*, **1989**, 17, 263-269. (b) Grung, M.; Metzger, P.; Berkaloff, C.; Liaaen-Jensen, S. *Comp. Biochem. Physiol.*, **1994**, 107B, 265-272.
11. Okada, S.; Matsuda, H.; Murakami, M.; Yamaguchi, K. *Tetrahedron Lett.* **1996**, 37, 1065-1068.
12. (a) Metzger,P.; Casadevall, E. *Phytochemistry*, **1989**, 28, 2097-2104. (b) Metzger, P. *Phytochemistry*, **1994**, 36, 195-212. (c) Metzger, P.; Pouet, Y. *Phytochemistry*, **1995**, 40, 543-554.
13. Laureillard, J.; Largeau, C.; Waeghemaeker, F.; Casadevall, E. *J. Nat. Prod.*, **1986**, 49, 794-799.

14. (a) Atkinson, A. W.; Gunning, B. E. S.; John, P. C. L. *Planta*, **1972**, 107, 1-32. (b) Burczyk, J.; Dworzanski, J. *Phytochemistry*, **1988**, 27, 2151-2153.
15. (a) Metzger, P; Casadevall, E. *Phytochemistry*, **1991**, 30, 1439-1444. (b) Metzger, P.; Casadevall, E. *Phytochemistry*, **1992**, 31, 2341-2349. (c) Rosales, E. V.; Metzger, P.; Casadevall, E. *Phytochemistry*, **1992**, 31, 3021-3027. (d) Templier, J.; Largeau, C.; Casadevall, E. *Phytochemistry*, **1993**, 33, 1079-1086.
16. Delahais, V.; Metzger, P. *Phytochemistry* **1997**, 44, 671-678.
17. Bax, A.; Freeman, R. *J. Magn. Reson.*, **1981**, 44, 542-561.
18. Bax, A.; Subramanian, S. *J. Magn. Reson.*, **1986**, 67, 565-569.
19. Lerner, L.; Bax, A. *J. Mag. Reson.*, **1986**, 69, 375-380.
20. Bax, A.; Summers, M.F. *J. Am. Chem. Soc.*, **1986**, 108, 2093-2094.

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