Phytochemistry, Vol. 27, No. 2, pp. 455–457, 1988.
Printed in Great Britain.

0031-9422/88 \$3.00+0.00

MEIJICOCCENE, A NEW CYCLIC HYDROCARBON FROM *BOTRYOCOCCUS BRAUNII**

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(*Revised received* 15 *May* 1987)

Key Word Index—*Botryococcus braunii*; Chlorophyceae; alga; structural determination; cyclic hydrocarbon; meijicoccene; botryococcenes.

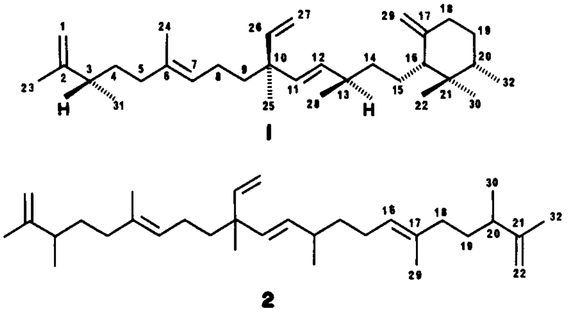
Abstract—A new cyclic hydrocarbon of the botryococcene type, meijicoccene ($C_{32}H_{54}$), has been isolated from the colonial green alga *Botryococcus braunii* strain Berkeley mass-cultured at Meiji University. This compound was a minor hydrocarbon (10% of total hydrocarbons) and was separated from other botryococcenes by reversed-phase HPLC. A structure is proposed for meijicoccene based on ^{13}C NMR, 400 MHz ^{1}H NMR, COSY, NOESY, NOE differential spectra and mass spectrometry.

INTRODUCTION

The colonial green microalga *Botryococcus braunii* is well known to produce unusually high levels of hydrocarbons. The alga has been proposed as a renewable source of fuels from recent concern for future energy requirements. *B. braunii* is classified into two races *A* and *B* according to the differences in composition of hydrocarbons and in the ultrastructure of its cell wall [1, 2]. Cultured strains of race *A* produce linear olefins, odd numbered from C_{23} to C_{31} [3], whereas those of race *B* accumulate isoprenoid polyunsaturated hydrocarbons of general formula C_nH_{2n-10}, $n = 30$–37, termed botryococcenes [4–6]. Especially, *B. braunii* strain Berkeley of the latter race has drawn much attention, because its hydrocarbons closely resemble the constituents of crude oil [7]. Recently, new types of botryococcenes such as a branched hydrocarbon, darwinene ($C_{36}H_{62}$) [8] and a new rearranged cyclic botryococcene ($C_{34}H_{58}$) [6] have been isolated. More recently, the absolute configuration of botryococcene ($C_{34}H_{58}$) has been elucidated [9].

In a previous paper [10], we have reported the lipid composition of *B. braunii* Berkeley mass-cultured at Meiji University. In the course of this study, we found a new type of botryococcene homologue by reversed-phase HPLC. Such a situation promoted us to determine structure of this new hydrocarbon.

In the present paper, a structure is proposed for the new rearranged hydrocarbon, meijicoccene **1** (Scheme 1), based on the data of the ^{13}C NMR 400 MHz, ^{1}H NMR, COSY, NOESY, NOE differential spectra and mass spectrometry along with consideration of a biosynthetic pathway from a homologue of botryococcene (C_{32} botryococcene) **2**.



Scheme 1. Structures of meijicoccene **1** and C_{32} botryococcene **2**.

RESULTS AND DISCUSSION

The new hydrocarbon, meijicoccene, had no UV absorption maximum above 220 nm. The EIMS spectrum showed the $[M]^+$ at m/z 438 (molecular formula: $C_{32}H_{54}$). The fragmentation pattern closely resembled that of C_{32} botryococcene ($C_{32}H_{54}$) **2** [5]. However, the mass spectrum of this compound gave little structural information. ^{13}C NMR (Table 1) revealed 32 carbon signals, which contained three exomethylenes at 111.12 (*t*, C-27), 109.41 (*t*, C-1 or C-29), 109.22 (*t*, C-1 or C-29), 150.25 (*s*, C-2 or C-17), 149.97 (*s*, C-2 or C-17), 146.94 (*d*, C-26), one trisubstituted olefin at 135.05 (*s*, C-6), 124.72 (*d*, C-7), and one disubstituted olefin at δ135.54 (*d*, C-11), 134.37 (*d*, C-12). The spectrum also indicated a structure closely related to **2** [5] except for the existence of signals at δ56.73 (*d*, C-16) and 36.89 (*s*, C-21) instead of signals of one trisubstituted olefin (δ134.91*s* and 124.61*d*) in **2**. According to the degree of unsaturation, meijicoccene must contain one ring system, and these two carbon atoms (C-16, C-21) were thought to be concerned with the formation of the ring system. A detailed analysis of the

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*Part 13 in the series Chemistry and Utilization of Plankton. For Part 12 see ref. [10].

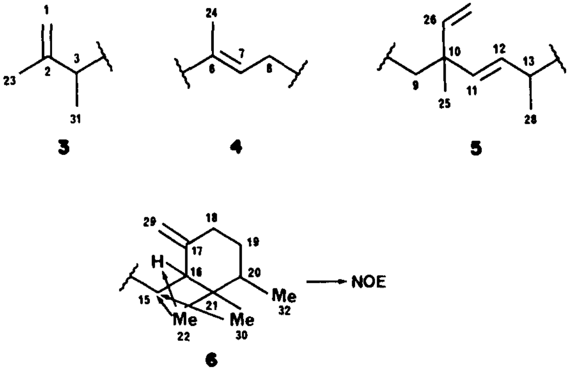
Table 1. ^{1}H and ^{13}C NMR data for meijicoccene and C_{32} botryococcene [5] in $CDCl_3$

	Meijicoccene		C_{32} Botryococcene
Position	δ_H	δ_C	δ_C
1	4.508 (1H, *dd*, 3, 1) 4.67 (3H, *m*)	109.22 *t*[a]	109.35 *t*[a]
2		149.97 *s*[b]	150.01 *s*
3	2.15 (*m*)	40.84 *d*	40.74 *d*
4	0.9–1.1 (*m*), 1.1–1.5 (*m*)	33.48 *t*[c]	33.38 *t*
5	1.8–2.0 (*m*)	37.58 *t*	37.52 *t*
6		135.05 *s*	134.91 *s*
7	5.090 (1H, *tq*, 7, 1)	124.72 *d*	124.61 *d*[a]
8	1.6 (*m*), 1.8–2.0 (*m*)	23.25 *t*	23.13 *t*
9	1.1–1.5 (*m*), 1.8–2.0 (*m*)	41.47 *t*	41.38 *t*
10		42.11 *s*	42.01 *s*
11	5.315 (1H, *dd*, 16, 1)	135.54 *d*	135.81 *d*
12	5.175 (1H, *dd*, 16, 8)	134.37 *d*	133.71 *d*
13	2.0 (*m*)	37.43 *d*	36.77 *d*
14	0.9–1.1 (*m*), 1.1–1.5 (*m*)	35.92 *t*	37.45 *t*
15	1.2 (*m*), 1.4 (*m*)	32.21 *t*[c]	25.84 *t*
16	1.63 (1H, *dd*, 11.5, 4.5)	56.73 *d*	124.49 *d*[a]
17		150.25 *s*[b]	134.91 *s*
18	2.1 (2H, *m*)	24.12 *t*	37.52 *t*
19	0.9–1.5 (*m*), 1.8–2.0 (*m*)	31.05 *t*	33.38 *t*
20	1.6 (*m*)	34.85 *d*	40.74 *d*
21		36.89 *s*	150.01 *s*
22	0.731 (3H, *s*)	26.95 *q*	109.35 *d*
23	1.651 (3H, *s*)	19.06 *q*	18.96 *q*
24	1.559 (3H, *s*)	15.97 *q*	15.97 *q*
25	1.066 (3H, *s*)	23.70 *q*	23.58 *q*
26	5.749 (1H, *dd*, 17, 10.5)	146.94 *d*	146.70 *d*
27	4.936 (1H, *dd*, 17, 1.5) 4.945 (1H, *dd*, 10.5, 1.5)	111.12 *t*	111.09 *t*
28	0.939 (3H, *d*, 7)	21.05 *q*	21.15 *q*
29	4.67 (3H, *m*)	109.41 *t*[a]	15.97 *q*
30	0.860 (3H, *s*)	21.74 *q*	18.96 *q*
31	0.998 (3H, *s*)	19.79 *q*	19.68 *q*
32	0.770 (3H, *d*, 7)	16.03 *q*	19.68 *q*

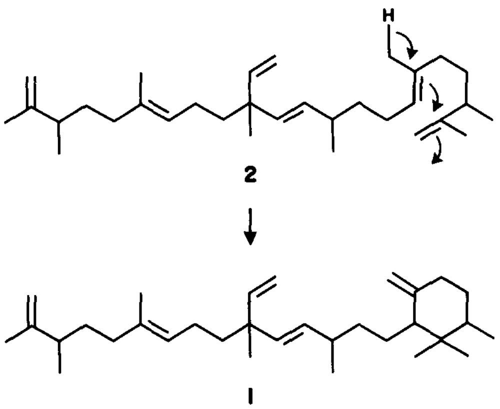
[a–c] Assignments can be interchanged for each compound.

400 MHz ^{1}H NMR (Table 1) and COSY spectra easily defined the presence of the partial structures **3**–**5** (Scheme 2) in consideration of ^{13}C NMR data. But a structure of the ring system remained unknown because of the complication of the methylene region of the 400 MHz ^{1}H NMR spectrum. To solve this problem, NOE differential and 2D-NOESY experiments were performed. Irradiation at C-22 Me δ0.731 (3H, *s*) showed NOE effects to H-16 δ1.63 (1H, *dd*, 11.5, 4.5), which coupled clearly the C-29 exomethylene proton δ4.67 (3H, *m*) in the COSY spectrum, and the C-15 methylene. Furthermore, when C-30 Me δ0.860 (3H, *s*) was irradiated, a NOE effect was observed at the C-15 methylene. These results suggested that meijicoccene should form the ring system between C-16 and C-21 as shown in partial structure **6**. Possibility of the formation of a ring system between C-2 and C-7 was excluded, because of the existence of the two characteristic methylenes at δ23.25 (*t*, C-8) and 41.47 (*t*, C-9) as in **2** [5].

The structure of meijicoccene was also supported by the proposed biosynthetic pathway from C_{32} botryococcene (Scheme 3), which was deduced by referring to the proposed mechanism for the biosynthesis of the cyclic C_{34} botryococcene from botryococcene [6]. However, the absolute configuration of meijicoccene is still unknown.



Scheme 2. Partial structures of **3**–**6**.



Scheme 3. Proposed biosynthetic pathway of meijicoccene.

EXPERIMENTAL

Algal sample and culture conditions. B. braunii strain Berkeley belonging to the *B* race originally isolated to unialgal culture by Dr Arthur M. Nonomura from lily-culturing tanks in the Department of Botany greenhouse of the University of California, Berkeley [11] was mass-cultivated at Meiji University, Japan. Algal culture conditions are described elsewhere [10]. The alga was harvested by filtration. The yield was *ca* 4g/l on a dry wt basis. After lyophilization, the alga was kept below −20° until used.

Extraction and isolation of meijicoccene. Freeze-dried algal samples were extd according to the method of ref. [12]. After evapn of solvent, total lipids dissolved in hexane were applied to a column of silica gel (Wako gel C-300) and hydrocarbons were eluted with hexane. Final purification of the hydrocarbon fraction was performed on a 5 μm reversed-phase HPLC column (YMC-ODS A324, 300 × 10 mm) with Me_2CO–MeOH (3:2) as mobile phase [13] at a flow rate of 2 ml/min using a differential refractometer to give colourless meijicoccene (yield; 2.5% of total lipids). Purity of this compound was checked by FID-GC on a fused silica OV-1 column (25 m) with temp prog from 170 to 190° at 1°/min, inj. temp 220°, using He as carrier gas at 60 ml/min and a split ratio of 100 : 1.

Spectral analysis. UV were measured in $CHCl_3$. EIMS was obtained at 70 eV. ^{13}C NMR (25.15 Hz) was recorded in $CDCl_3$ using TMS as int std. 400 MHz ^1H NMR, COSY, NOESY and NOE differential spectra were obtained also in $CDCl_3$.

Acknowledgments—We are indebted to Drs K. Kyogoku and H. Kondo, Research Center, Taisho Pharmaceutical Co., Ltd, for measurements of NMR spectra. This work was supported in part by a grant from the Ministry of International Trade and Industry, Japan.

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