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DARWINENE: A BRANCHED HYDROCARBON FROM A GREEN FORM OF *BOTRYOCOCCUS BRAUNII*

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Key Word Index—*Botryococcus braunii*; Chlorophyceae; alga; GC/MS; darwinene; branched hydrocarbon.

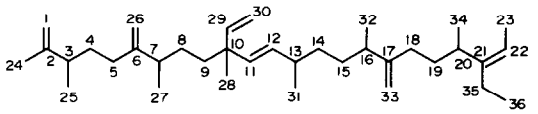
Abstract—A branched chain hydrocarbon ($C_{36}H_{62}$, darwinene) of the botryococcene type has been isolated from a green form of the alga, *Botryococcus braunii*. The compound was the major hydrocarbon component from colonies collected in the Darwin River Reservoir. A structure is proposed for the compound based on the ^{13}C and ^{1}H NMR spectra and mass spectrometry.

INTRODUCTION

The alga *Botryococcus braunii* Kutzing is known to produce highly branched hydrocarbons [1]; 13 of these have been observed by GC/MS each having the general formula C_nH_{2n-10}, where n equals 34–37 [2, 3]. The structure of one member of the series, botryococcene, from a red form of the alga, has previously been identified [4]. The alga has been of interest as the biological precursor of the kerosene shales and as a possible energy-crop, yielding oil fuels [5, 6]. More recently the fully saturated derivative of botryococcene, botryococcane, was identified in a Sumatran crude oil [7]. In this paper a structure is proposed for another hydrocarbon, darwinene, based on evidence drawn from the ^{13}C and ^{1}H NMR spectra and the mass spectrum of the compound. The nature of the algal bloom from which darwinene was isolated has been described elsewhere [8].

RESULTS

The molecular mass of darwinene (1; 494; $C_{36}H_{62}$), was obtained from the mass spectrum. The fragmentation pattern, resulting from the use of a chemical ionization technique employing methane as the reagent gas, displayed summation peaks at m/z 495 $[M+1]^+$ and 523 $[M+29]^+$ which established the MW. The mass spectrum resembles that of botryococcene [8] suggesting close structural similarities.



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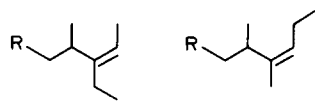

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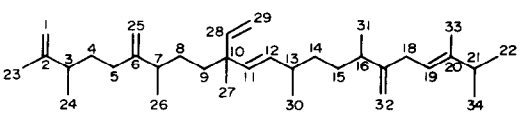

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It has been shown that the size of alkyl groups attached to the quaternary carbon atom of the hydrogenated form of botryococcene, viz. botryococcane, can be inferred from ion doublets present in the mass spectrum of the compound [1]. These ion doublets at m/z values of 238, 239 $[M-C_{17}H_{35}]^+$, 294, 295 $[M-C_{13}H_{27}]^+$ and 448, 449 $[M-C_2H_5]^+$ were used to identify botryococcane in a crude oil sample [7]. In the fully hydrogenated form of darwinene (darwinane,) the doublet at m/z 238, 239 $[M-C_{19}H_{39}]^+$ is retained but the other low mass doublet appears at 322, 323 $[M-C_{13}H_{27}]^+$. It is, therefore, inferred that the longest alkyl chain attached to the quaternary carbon of botryococcene has been increased by the addition of two methylene units in darwinene.

Unlike botryococcane, which shows no M^+ [1, 7], darwinane yields an ion at m/z 505 $[M-H]^+$ that is typical for saturated hydrocarbons. Thus, the ion at m/z 476 $[(M-C_2H_5)-H]^+$ confirms the retention of a C-2 side chain attached to the quaternary carbon, as is found in botryococcane. The ^{1}H NMR spectrum of darwinene integrates for one less exomethylene group at δ 4.7 than botryococcene. Inspection of the peak at δ 1.65 in the α-methyl region confirms that darwinene retains only one of the two terminal exomethylene groups of botryococcene.

A number of observations, made by Stehling and Bartz [9] in a comprehensive study of ^{1}H NMR spectra of olefinic hydrocarbons, were of assistance in the interpretation of the spectrum of the $C_{36}H_{62}$ compound. The chemical shift at δ 1.55 in darwinene is indicative of a methyl group forming part of a trisubstituted double bond system. The chemical shift for a methyl substituted α-methine group (in a non-*cis* position) is observed in botryococcene between δ 2.0 and 2.3. While this feature is retained with reduced intensity in the ^{1}H NMR spectrum of darwinene, an absorption at δ 2.5–2.8 consistent with a *cis*-α-methine is also observed.

In the α-methylene shift region of the ^{1}H NMR spec-

trum, i.e. δ 1.75–2.00, the spectrum of darwinene is consistent, with an increase in the number of methylene units compared to botryococcene. Darwinene also shows further peaks around δ 0.9–1.1 indicative of the presence of at least one additional methyl group. Thus, the ^{1}H NMR spectrum of darwinene suggests the partial structures **2** or **3**.

There is a broad baseline band in the olefinic region of the ^{1}H NMR spectrum of darwinene between δ 5.0 and 5.5. This spectral characteristic is also displayed by (*E*)-3-methyl-2-pentene, a compound resembling the terminal moiety of the proposed structure for darwinene, in which the olefinic hydrogen yields a very broad quartet with a low peak height due to coupling with the geminal methyl group [10]. The ^{13}C NMR chemical shifts and assignments for darwinene are given in Table 1 (which has been arranged to facilitate comparison of the chemical shifts of the corresponding carbon atoms of darwinene and botryococcene.) The assignments are based on those for botryococcene and for the model compounds (*Z*)-3-ethyl-4-methyl-2-pentene [11] and 2,3-dimethyl-1-butene [12]. On the basis of the chemical shift assignments for the methyl substituents of 2,3-dimethyl-1-butene, 2,3-dimethyl-1-pentene and 2,3-dimethyl-1-hexene [12] the earlier assignments in botryococcene [4] for the carbon atom pairs 23, 34 and 23, 33 have been interchanged. (It should be noted that the botryococcene molecule is numbered in the reverse direction to that of Cox *et al.* [4] in order to conform with IUPAC nomenclature.) On the basis of the above information the structure **1** is proposed for darwinene.

Table 1. Chemical shifts (from TMS) and assignments for the ^{13}C NMR spectra of darwinene and botyryococcene

Darwinene C		Botryococcene	
Carbon No.	Chemical shift	Carbon No.	Chemical shift
1	109.5	1	109.6
2	149.8	2	149.8
3	41.0	3	41.0
4	33.4	4	33.4
5	31.6	5	31.6
6*	154.7	6	154.6
7†	40.0	7†	40.1
8	30.1	8	30.1
9	39.0	9	39.1
10	41.8	10	41.8
11	135.8	11	135.9
12	133.8	12	133.8
13	37.3	13	37.3
14	35.0	14	35.0
15	33.4	15	33.4
16†	40.6	16†	40.6
17*	155.1	17	154.8
18	32.0	18	31.6
19‡	33.8	19	33.4
20‡	33.6	20	41.0
21	145.1	21	149.8
22	116.6	22	109.6
23§	13.0	—	—
24	18.9	23	18.9
25	19.3	24	19.5
26	107.8	25	107.4
27	20.2	26	20.2
28	23.5	27	23.6
29	146.9	28	146.8
30	111.0	29	111.0
31	21.2	30	21.2
32	20.4	31	20.4
33	107.1	32	107.4
34	19.7	33	19.5
35	23.0	34	18.9
36§	13.2	—	—

*, †, ‡, §Assignments with the same sign could be interchanged.

DISCUSSION

The ^{1}H NMR spectrum of darwinene [1] has a number of features in common with those of isobotryococcene. In the unsaturated region between δ 4.5 and 5.5 the two spectra are virtually identical. Isobotryococcene also appears to share the following characteristics: (a) the gain of a *cis*-α-methine group at the expense of a non-*cis*-α-methine; (b) the loss of an α-methyl substituted exomethylene group; and (c) the gain of an α-methyl group as part of a trisubstituted double bond system. Isobotryococcene shows reduced intensity in the α-methylene region at δ 1.7–2.0 and in the β-methylene region at 1.2 compared to both darwinene and botryococcene.

It is already known that isobotryococcene has the same carbon skeleton as botryococcene [1] implying the occurrence of either *cis–trans* isomerism or double bond migration. The loss of a terminal exomethylene group suggests the latter as does the appearance of a new double bond structure. The appearance of a *cis*-α-methine group and a methyl group substituted at the new double bond position infers two possible structures for the isobotryococcene molecule, namely (*Z*)-3-botryococcene or (*Z*)-19-botryococcene (**4**), depending on which terminal exomethylene group is lost.

EXPERIMENTAL

Hydrocarbons extracted [1] from freeze-dried algal colonies were fractionated by HPLC using heptane as solvent. The solvent was previously dried by molecular sieves and repeated passage through a Si gel column [13] packed with 'Spherisorb' SGP. The concn of the algal hydrocarbons was 40 mg/ml using a UV detector at 205 nm. 30 chromatographic separations were carried out to obtain material for analysis, corresponding peaks from each run being pooled. The separated hydrocarbons were examined by GC/MS prior to NMR spectral analyses. Hydrogenation of darwinene and botryococcene for GC/MS studies was carried out using a Pb catalyst at 25° and atm. pres. A CI technique, utilizing methane as the reagent gas, was employed to obtain MS. The operating conditions have been described elsewhere [2]. Retention indices for the algal hydrocarbons were obtained by coinjection with $nC_{24}C_{28}$ and C_{32} hydrocarbons on a 50 m SCOT column coated with SE-30 and temp. programmed from 220° to 280° at 4°/min. They were: (a) botryococcene, 2730; (b) darwinene, 2920; (c) botryococcane, 2885; and (d) darwinane, 3020.

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