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ALDEHYDES, VERY LONG CHAIN ALKENYLPHENOLS, EPOXIDES AND OTHER LIPIDS FROM AN ALKADIENE-PRODUCING STRAIN OF *BOTRYOCOCCUS BRAUNII*

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Key Word Index—*Botryococcus braunii*; Chlorophyceae; alga; aldehydes; botryals; very long chain alkenyl-phenols; epoxides; free fatty acids; sterols; biosynthesis; cell wall.

Abstract—Analysis of a hexane extract of an alkadiene-producing strain of the green alga *Botryococcus braunii*, led to the isolation of new metabolites. Among these, the structures of even carbon-number C_{52}–C_{64} α-branched, α-unsaturated aldehydes, termed botryals; and very long chain alkenyl-phenols and of their derived epoxides were elucidated. Octacosenoic acid was identified as a free compound besides palmitic and oleic acids. The presence of trace amounts of three sterols, cholest-5-en-3β-ol, 24-methylcholest-5-en-3β-ol and 24-ethylcholest-5-en-3β-ol was also ascertained. Feeding experiments with sodium [1, 2- ^{13}C] acetate provided evidence for an aldol condensation of very long chain aldehydes in botryal biosynthesis and a tetraketide precursor for the origin of the benzene ring in the very long chain alkenyl-phenols. The structures of botryals and their localization in the outer walls of the alga, suggest that they could furnish the basic carbon skeleton of the biopolymer forming the chemically resistant part of the cell walls.

INTRODUCTION

In a previous paper, we reported the major lipid composition for several strains of the green alga *Botryococcus braunii* [1]. These strains belonged to the A race which is characterized by the production of straight-chain hydrocarbons, odd carbon number *n*-alkadienes **1** (major) and trienes (minor), up to 61% of dry weight, depending on the strain. Two other races are known for *B. braunii*, the B race defined by the production of triterpenoid hydrocarbons, the C_{30}–C_{37} botryococcenes (24–43% of dry wt) [2] and the L race synthesizing a single hydrocarbon, a $C_{40}H_{78}$ tetraterpenoid termed lycopadiene (2–8% of dry wt) [3]. These high hydrocarbon contents, unusual in the plant kingdom prompted some authors to investigate the nature of the other components of the lipid extract [4]. When the lipids other than hydrocarbons remained undetermined in the B strain, the authors recognized in an A strain, on the basis of co-thin layer chromatography, a lipid profile similar to that found generally in microalgae, with sterol and wax esters, triacylglycerols, free fatty acids, diacylglycerols, sterols and some other lipids [5–10].

Continuing our screening on *B. braunii* A strains of different geographical origins, the analyses showed the presence in all algae we isolated [1, 2, 11] of even carbon number C_{52}–C_{64} aldehydes, which we termed botryals [12]; these occurred up 18% of the dry wt and 45% of the hexane extractable lipids. Considering this prolific presence of compounds of a peculiar type, in sharp contradiction with the early investigations [4], it was of interest to analyse in more detail the neutral lipids from *B. braunii* A strains. Therefore, the present work deals with the isolation and identification of the components of this lipidic fraction. Using feeding experiments with [1,2-^{13}C] acetate, the biosynthetic process was investigated for two of these components. Their possible implication in the synthesis of the algal cell wall, in which the main part of the lipids are located, is also discussed.

RESULTS AND DISCUSSION

The hexane extract of the dry biomass of the Austin strain of *B. Braunii* (43% of dry wt), afforded, by order of elution on silica gel chromatography (see Experimental): hydrocarbons (20.4% of extract), botryals **2** (2.3%) and **3** (23.2%), epoxyalkenes **4** (5.4%), epoxybotryals **5** (2.0%), very long chain alkenylphenols **6** (3.6%), triacylglycerols (14.6%), epoxyphenols **7** (0.9%), free fatty acids (1.6%) and trace amounts of free sterols, accounting for 74% of the oil. The analyses of hydrocarbons and triacylglycerols have been previously reported [1, 11].

The location of these metabolites in the external walls surrounding the cells which form a dense matrix ensuring the colony cohesion was suggested by their extraction with non polar solvents, such *n*-hexane. This external location has been demonstrated for hydrocarbons [13], *n*-alkdienes and trienes, which form a part of this hexane extract.

Botryals **2** *and* **3**

Botryals **2** or **3**† were not resolved by normal or reversed phase HPLC and did not elute on GC. Probe EI

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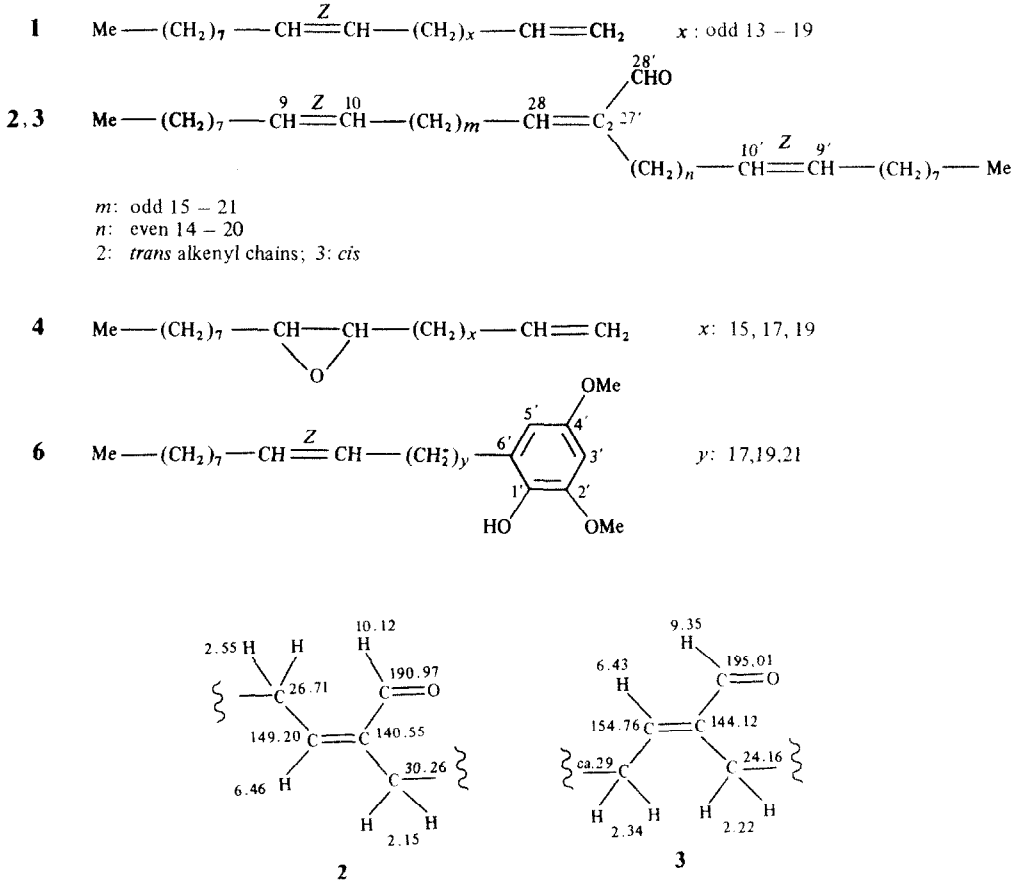
†The carbon numbering on the structures, refers to a C_{56} compound resulting from the condensation of two C_{28} units.

mass spectra established for each purified mixture a series of $[M]^+$ ranging from m/z 738 to 906 by a regular increase of 28 mu. The molecular formula, $C_{52+x}H_{98+2x}O$, with x even from 0 to 12, was confirmed by $CI(NH_3)$ mass spectrometry and was in accord with the spectral and analytical data hereafter mentioned.

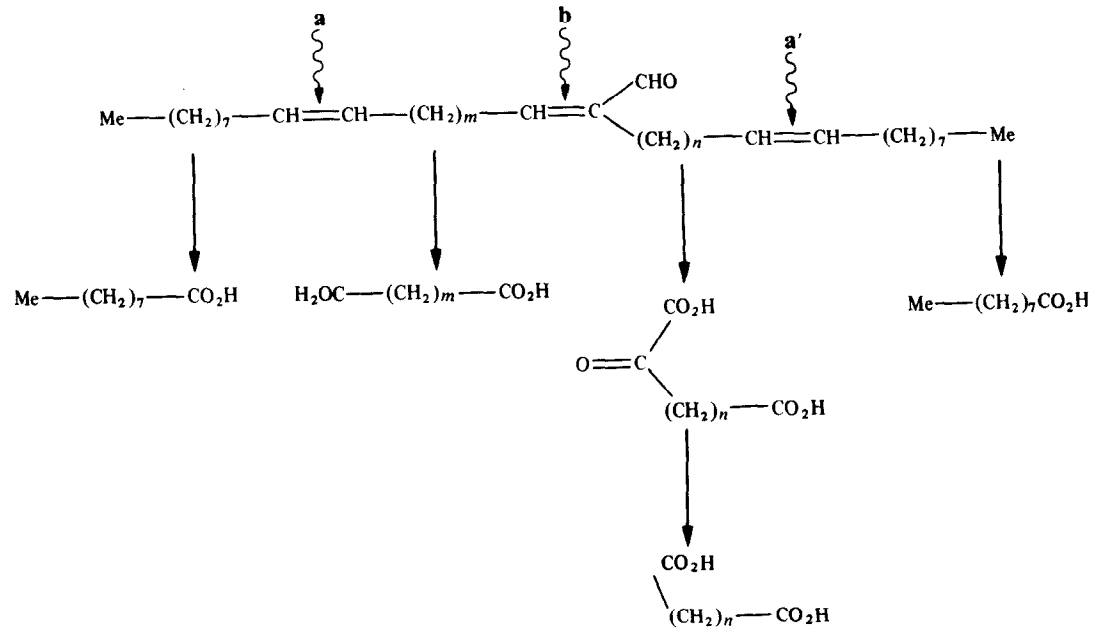
The IR spectra of **2** and **3** showed an absorption for a CH aldehydic bond at 2720 cm^{-1}, associated with a CO absorption affected by α-unsaturation at 1680 cm^{-1} for **2** and 1690 cm^{-1} for **3**; bands for unsaturations at 3000 and 1640 cm^{-1} were also observed. ^1H and ^{13}C NMR spectra agreed with compounds containing one α-unsaturated and α-branched aldehydic function (Scheme 1), two isolated double bonds and two terminal methyls. The *cis* configuration of the isolated double bonds in **2** and **3** was deduced from the ^{13}C chemical shifts of the allylic carbons 8, 11, 8′ and 11′: 27.30 ppm [14]. In the ^{13}C NMR spectra, the allylic carbon 26′ of **2** exhibited a more deshielded signal (δ30.26), than the one of **3** (δ24.16), indicating *trans* alkyl chains on the 28–27′ double bond in **2** and *cis* chains in **3** [14].

Ozonolysis of compounds **2** and **3**, followed by oxidative cleavage of the ozonides and GC-MS of the resulting acids analysed as Me esters verified the double bond positions. The *n*-C_9 monoester indicated double bonds in positions 9–10 and 9′–10′ (**a** and **a′** cleavages, Scheme 2); the odd carbon number *n*-diesters arise from **a** and **b** cleavages, tne even ones from **a′** and **b** cleavages. In this latter case, it may be assumed that the initial odd carbon number ketodiacids gave rise to the even carbon number diacids because of the drastic experimental conditions used.

These structures suggested that the botryals could be derived from a head-to-head condensation of very long chain aldehydes C_{26} to C_{32}, unsaturated in position 9–10 relative to the Me group (investigations failed to detect these possible precursors as free compounds in the extracts). The resulting aldols would then be dehydrated to form botryals. It is interesting to note that, as generally observed for acid or base catalysed dehydration of aldols [15], the most highly favoured products were the isomers for which the formyl and the bulkier group were *trans* (here, H-28 is the smallest group), **2** and **3** were found in a ratio 9/91; ratios 21/79 and 12/88 were observed for two other strains [1]. To prove that this type of compound was not an artefact of extraction, as previously reported for analogous compounds [16], we have recorded the *in vivo* ^{13}C NMR spectrum of a *B. braunii* strain particularly rich in botryals (Fig. 1). This strain originated from Titicaca lake and had a botryal content of 18% of its dry weight [1]. The signals at δ155.3, 146.5 and 195.2 in the *in vivo* spectrum, could be easily attributed in the above order to the central carbons –CH=C(CHO)– of **3**, thus establishing that botryals were not artefacts. The other signals at low field could be associated with compounds also present in the alga: carbons of the terminal double



Scheme 1. Selected ^1H and ^{13}C NMR chemical shifts of botryals **2** and **3**.



Scheme 2. Ozonolysis of botryals **2** and **3**.

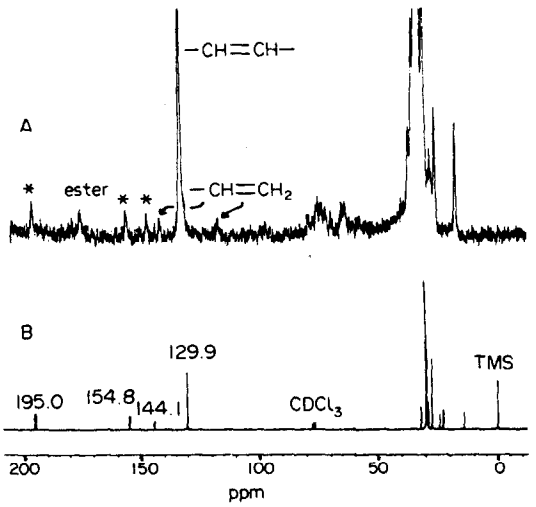


Fig. 1. 62.5 MHz $^{13}C\{^1H\}$ NMR spectra identification of botryals. (A) *In vivo* spectrum (5100 transients) from a *B. braunii* culture (Titicaca strain) [1] in a 10 mm tube; external reference DSS, external lock D_2O; * refers to botryal peaks. (B) Botryals **3** in $CDCl_3$.

bond of the dienes and trienes, the alkenyl unsaturations and $^{13}C{=}O$ of esters.

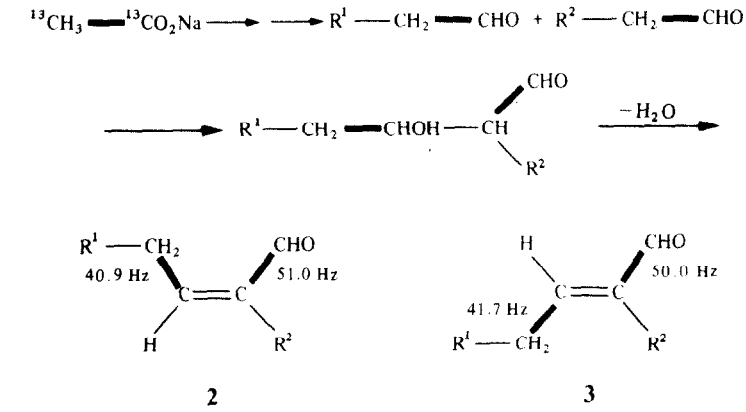
To examine the possibility of a head-to-head condensation mechanism and to discard an eventual formylation process, algae were fed with sodium [1,2-^{13}C] acetate. The incorporation of ^{13}C–^{13}C units observed in the ^{13}C NMR spectra of ^{13}C enriched **2** and **3**, established the repeated condensation of acetate units, leading to the very long chain fatty aldehydes R^1–$^{13}CH_2$–^{13}CHO and R^2–$^{13}CH_2$–^{13}CHO (even C_{26} to C_{32}). These aldehydes would be condensed in a subsequent step, and the resulting aldols dehydrated, as shown in Scheme 3.

Inadvertently kept for a week at room temperature and in air, botryals **3** exhibited a somewhat fast oxidation into acids, as shown by IR analysis. Not further investigated, these acids were associated with a hydrocarbon fraction (8% of starting botryals), differing from the one observed in the living algae. CI(NH_3) mass spectrometry established that this fraction was composed of a series of odd carbon numbered trienes **8** of general formula $C_{51+x}H_{98+2x}$, x even numbers from 0 to 12. Moreover, the IR and ^{13}C NMR spectra supported a *trans* configuration for the central double bond (ν 970 cm^{-1}; δ 32.6 for its allylic carbons). These hydrocarbons presumably arose from a spontaneous decarboxylation of the botryal derived acids, with the co-occurrence of an isomerism of the double bond initially conjugated with the CO.

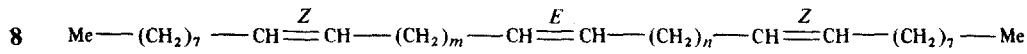
The structures of botryals and their biosynthetic pathway suggest a similarity with mycolic acids. Indeed, these important lipid constituents of the cell wall of some bacteria, are α-branched, β-hydroxy acids occurring as C_{32}–C_{36} compounds in *Corynebacterium* [17], as C_{34}–C_{66} in *Nocardia* [18] and as C_{78}–C_{85} in *Mycobacterium* [19]. They are synthesized by a head-to-head condensation of long chain fatty acids [20, 21]. Moreover, under mild conditions, their derivatives could undergo decarboxylation [21].

Very long chain alkenyl-phenols **6**

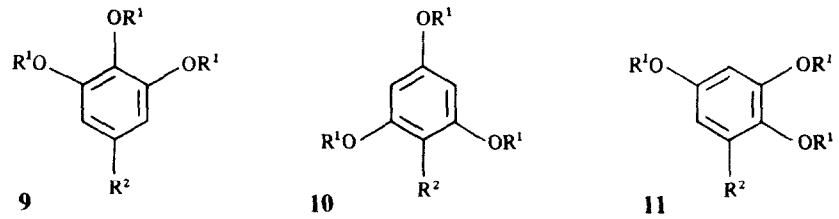
These new metabolites obtained as a solid mixture had λ_{max}^{hexane} nm(ε), 289 (3700), 222 (6800) and 215 (7500). Such absorptions could be indicative of an aromatic type of moiety [22]. The IR spectrum exhibited an OH band at 3560 cm^{-1}, an aromatic absorption at 1620–1600 cm^{-1} and an isolated unsaturation at 1640 cm^{-1}. EI and CI (NH_3) mass spectra (probe) indicated the presence of three compounds $C_{35}H_{62}O_3$ ($[M]^+$ m/z 530), $C_{37}H_{66}O_3$



Scheme 3. Biosynthesis of botryals **2** and **3** from [1,2-^{13}C] acetate, and ^{13}C–^{13}C coupling constants on the enriched species (R^1 and R^2: even C_{24}–C_{30} alkenyl chains).



m: odd 15 – 21
n: even 14 – 20



R^1: H or Me R^2: alkenyl chain

([M]$^+$ *m/z* 558) and $C_{39}H_{70}O_3$ ([M]$^+$ *m/z* 586), in a ratio of *ca* 3:12:5 deduced from the relative intensities of the [M]$^+$ peaks; a benzylic fragmentation was observed in the EI spectrum (*m/z* 167).

The ^{1}H and ^{13}C NMR spectra showed a hydroxy dimethoxy phenyl unit (^{13}C NMR data in Table 1) connected to a mono-unsaturated aliphatic long chain. The ^{1}H spectrum exhibited two doublets at δ6.35 and 6.29 (J = 2.8 Hz) characteristics of a 1,2,3,5-tetrasubstituted phenyl group, a signal for an 'in chain' unsaturation at 5.35, two singlets at 3.85 and 3.76 for two OMe, a triplet for the benzylic protons at 2.61, multiplets for the allylic protons at 2.01 and homobenzylic protons at 1.60, an intense signal for the other methylenic protons and a triplet for the terminal Me.

Methylation of **6** with dimethyl sulphate gave the triMe ethers $C_{36}H_{64}O_3$, $C_{38}H_{68}O_3$ and $C_{40}H_{72}O_3$. The NMR data concerning the OMe groups (^{1}H δ at 3.83, 3.77 and 3.75 ppm; ^{13}C δ at 60.7, 55.8 and 55.4 ppm) showed the absence of symmetry in the molecules and allowed us to establish that they were not pyrogallol **9** or phloroglucinol **10** derivatives.

Thus, all these data indicated that the phenols we isolated from the A strain, were from a structural point of view, hydroquinol derivatives **11**. The relative position of OH and OMe groups on the benzene ring of **6** was deduced from the small chemical shift effect observed on $C_{2'}$ and $C_{6'}$ when we replaced OH ($C_{2'}$: 106, $C_{6'}$: 96.9 ppm) by OMe ($C_{2'}$: 105.4, $C_{6'}$: 98.0). Furthermore ozonolysis of **6** followed by oxidative cleavage of the ozonides furnished the *n*-C_9 acid and *n*-C_{17}, C_{19} and C_{21} diacids, thus establishing the position 9–10 for the double bond, relative to the terminal Me.

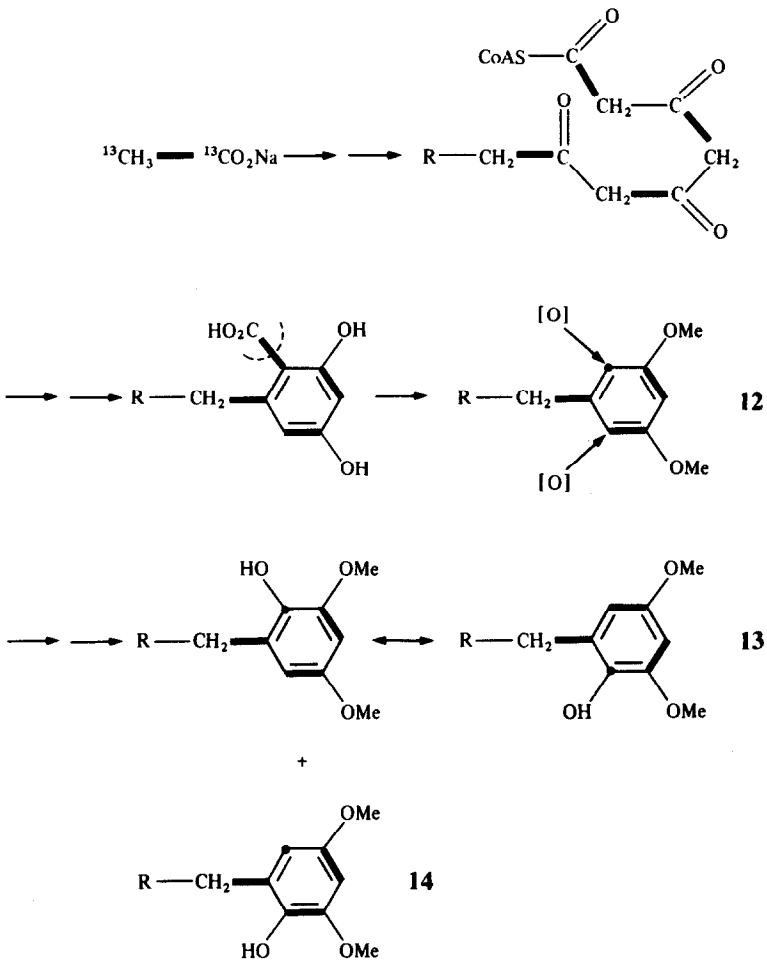
The spectrum of ^{13}C enriched phenols obtained after incubation of the algae with [1,2-^{13}C] acetate exhibited satellites for the six aromatic signals. These satellites indicated an isotopic enrichment of *ca* 2% for $C_{2'}$, $C_{3'}$, $C_{4'}$ and $C_{6'}$ and 1% for $C_{1'}$ and $C_{5'}$; at the same time the central signals of $C_{1'}$ and $C_{5'}$ were doubled in intensity. The satellites of $C_{6'}$ could be related to the ones observed at higher field for the benzylic carbon (Table 1). The satellites of $C_{1'}$ and $C_{5'}$ appeared as single pairs of peaks and the ones of $C_{2'}$ were clearly resolved into a pair of satellites. Finally, the satellites of $C_{3'}$ and $C_{4'}$ were unresolved broad peaks resulting from the overlapping of satellites of similar coupling constants. These results were consistent with the presence of two types of labelled molecules which could be ascribed to the symmetry of a biosynthetic intermediate, and suggested a tetraketide origin for the phenol ring [23]. The symmetrical inter-

Table 1. Assignments of ^{13}C NMR signals and coupling constants for aromatic and benzylic carbons of phenols **6** derived from [1,2-^{13}C] acetate

C	δ(ppm)	J_{CC}, Hz
1′	137.75	$J_{1'-2'}=74$
2′	146.83	$J_{2'-3'}=72$
3′	96.89	$J_{3'-4'}$ *ca* 71
4′	152.92	$J_{4'-5'}$ *ca* 70
5′	106.15	
6′	128.88	$J_{6'-benzyl}=43$
benzylic	30.08	

mediate, a resorcinol derivative **12** (Scheme 4), would be oxidized at one of the two *ortho* positions relative to the alkenyl chain, leading to the trihydroxy derivatives **13** and **14**.

The high concentrations of phenolic compounds in the A race of *B. braunii* (1.6% of dry wt) is comparable to that reported for phenols in some blue green algae (1.8 to 7.1% of dry wt) [24], however in our case, the alkenyl chain (heptacosenyl, nonacosenyl and hentriacontenyl) constitutes *ca* 70% of the M_r. There are some reports on the isolation and identification of long chain alkylphenols in plants, 3-undecyl phenol has been extracted from a briophyte [25], 5-tridecyl and 5-pentadecenyl resorcinols were recently isolated from *Hakea trifurcata* [26], 6-pentadecenylsalicylic acid has been identified in the fruit of *Gingko biloba* [27], 5-heptadecenyl resorcinol from the peel of mango fruit [28], and 5-nonadecyl and 5-heneicosylresorcinol from wheat bran [29]. The major role of the alkyl or alkenyl chain might be to increase the solubility of the phenol moiety in lipidic regions of the plants which require protection against biological degradation or chemical oxidation, as it has been proposed for the phytyl chain of tocopherols [30]. With regard to the localization of phenols **5** in the outer walls of the alga, it may be assumed that they protect lipids, essentially normal aliphatic chains in the A race, against degradation by bacteria and fungi [31]. Furthermore, it is interesting to note that these phenols, present in all A strains we have studied up to date, were not detected in the lipidic extracts of algae of the B and L races (unpublished results); the lipids of these two races are essentially composed of terpenoids, compounds known for their greater resistance towards bacterial oxidation than aliphatic compounds.



Scheme 4. Biosynthesis of phenols **6** from [1,2-^{13}C] acetate.

Epoxides

Epoxides **4**, **5** and **7** derived from *n*-alkadienes **1**, botryals **3** and phenols **6** respectively were isolated by TLC as solid mixtures. They gave rise in NMR spectra to signals characteristics of an epoxide ring (^{1}H δ2.89; ^{13}C δ *ca* 57.2), accompanied by the disappearance of the signals of olefinic protons and carbons 9 and 10 (50% decrease in the case of the epoxy botryals **5**). All exhibited a *cis* epoxide ring, established on the basis of the ^{13}C NMR chemical shift of the α-methylene carbons (δ *ca* 27.9) [32].

GC-EIMS of the epoxy alkenes **4** showed the presence of three compounds: $C_{27}H_{52}O$ (11%), $C_{29}H_{56}O$ (78%) and $C_{31}H_{60}O$ (11%); a α-epoxide fragmentation was observed for each product. It is interesting to note that these compounds arose from the oxidation of the central more reactive, double bond of the dienes **1** as observed for the chemical epoxidation of olefins [33]. Neither the C_{27}, C_{29} or C_{31} diepoxy alkanes resulting from the oxidation of the central and terminal double bonds, easily available as references by the reaction of *m*-chloroperbenzoic acid on **1** (see Experimental), nor the 1,2-epoxy alkenes were detected in the extract.

In the monoepoxy botryals **5**, we could not determine the location of the epoxide ring: 9–10 or 9′–10′. IR and NMR data strongly suggested that they were derived from botryals **3**. With regard to the abundance of botryals **2**, the corresponding epoxy derivatives, if they exist, should be only in traces amounts. Continuing the analogy between botryals and mycolic acids, it is noteworthy that epoxy mycolic acids have been reported to occur in *Mycobacterium* [34]. To the best of our knowledge, the presence in plants of epoxy alkyl phenols has not been previously reported. From a biosynthetic point of view, it can be assumed that the epoxidase implicated in the oxidations of the double bond in position 9–10 on the alkenyl chain has a broad substrate specificity regarding substitutions on the chain beyond 15 carbons.

Free fatty acids

The distribution observed for the free fatty acids of the hexane extract is shown in Table 2. This distribution is very similar to that found for the saponifiables from this same hexane extract with high proportions of oleic acid, followed by octacosenoic and palmitic acids. The predominance of oleic acid in non polar and polar lipids of the A race of *B. braunii* has been previously noted [4, 35]. For example triolein was found to represent 64% of the triacylglycerols extracted from the Austin strain studied in the present work [1]. It can be related to the role played by this acid in the biosynthetic pattern as a direct precursor of the hydrocarbons [36].

Table 2. Distribution of fatty acids from the hexane extract

	16:0	16:1	18:0	18:1	18:2	20:1	22:1
Free components	4.0	0.1	0.4	80.9	0.8	2.1	0.8
Saponifiables	3.0	0.2	0.3	82.9	0.2	1.2	0.5
	24:1	26:1	28:1	28:2	30:1	30:2	
Free components	0.3	0.6	5.4	1.7	0.7	2.2	
Saponifiables	0.4	1.6	4.8	1.5	1.6	1.8	

Sterols

Traces of free sterols were characterized in the hexane extract, whereas more substantial amounts were isolated from the $CHCl_3$–MeOH extract (*ca* 0.1% of dry wt, unpublished results). Cholest-5-en-3β-ol (1%), 24-methylcholest-5-en-3β-ol (41%) and 24-ethylcholest-5-en-3β-ol (58%) were identified; these compounds have been previously reported in many types of algae [7, 9, 10, 37, 38]. No assignment of the C-24 configuration in the C_{28} and C_{29} constituents has been made. In the botryococcene-producing algae, we have observed a similar sterol composition (unpublished results), which deprives these metabolites of any taxonomic significance in the distinction of *B. braunii* races.

CONCLUSION

The presence in the hexane extractable lipids of botryals, very long chain alkenylphenols and epoxides, in some cases in high amount, besides other more common lipids, demonstrates that the lipidic profile of *B. braunii* A race is not typical of other microalgae. The location of botryals in the matrix surrounding the cells, suggests as for mycolic acids from mycobacteria, that they could play an important role in the biosynthesis of the *B. braunii* cell wall, at least in alkadiene-producing algae. From structural studies [39] and analyses of pyrolysis products [40], it has been shown that the structure of this biopolymer is based on long unbranched saturated hydrocarbon chains, cross-linked by ether bridges; fatty acids are bound to these chains through ester functions sterically protected inside the polymethylene network. The occurrence in the IR spectra of biopolymers from A race algae of different origins, of a clear absorption at 1690 cm^{-1} could be consistent with the involvement of botryals in their biosynthesis. Indeed, as the C_{18} family of epoxy acids are implicated in the biosynthesis of cutin [41], a resistant material from higher plants [42], epoxy botryals could participate through hydration and reaction with fatty acids to form the resistant polymer of the alga. The aldehyde groups might be used to anchor the biopolymer to the cell by the formation of a Schiff base with an amino group of a protein.

EXPERIMENTAL

CC: silica gel (70–230 mesh); TLC: silica gel 60PF; IR, CCl_4, MS, 70 eV; ^{1}H NMR: 250 MHz, $CDCl_3$, TMS as int std.; ^{13}C NMR, 62.5 MHz, $CDCl_3$, TMS.

Extraction and isolation of compounds. The strain investigated originated from the culture collection of Austin, U.S.A.; it was cultured under air-lift conditions as described in ref. [2]. Algae were harvested at the end of the linear phase of growth, dried under vacuum at 50° (5.8 g of dry biomasse) and then extracted twice for 1 hr with 500 ml of *n*-hexane. The combined extracts (2.5 g) were sepd by silica gel (168 g) CC into five fractions, I (630 ml *n*-hexane) (20.4%), II (840 ml *n*-hexane–Et_2O, 19:1) (37.5%), III (1050 ml *n*-hexane–Et_2O, 23:2) (20%), IV (1600 ml *n*-hexane–Et_2O, 17:3) (10.2%) and V (630 ml Et_2O) (11.8%).

Fr. I contained pure alkadienes **1** and trienes. Fr. II was resolved by prep. silica gel TLC. Elution with *n*-hexane–Et_2O (23:2) gave botryals **2** (R_f 0.63) and **3** (R_f 0.58), epoxy alkenes **4** (R_f 0.47), epoxy botryals **5** (R_f 0.23) and very long chain alkenylphenols **6** (R_f 0.16). Triacylglycerols were isolated from fr. III by prep. silica gel TLC, using *n*-hexane–Et_2O, 4:1 (R_f 0.45). Epoxy phenols **7** were recovered from for IV by the same TLC

method using *n*-hexane–Et_2O, 13:7 (R_f 0.60). Free fatty acids and free sterols were isolated from fr. V in the same manner using *n*-hexane–Et_2O, 9:11 (R_f: 0.70 and 0.53 respectively).

Botryals **2** were obtained as a pasty mix. MS (probe) of the mixt, *m/z*: $[M]^+$ (int. in % relative to *m/z* 69) 906 (2), 878 (2), 850 (4), 822 (7), 794 (5), 766 (3), 738 (1); IR ν_{max} cm^{-1}: 3000 (=C–H), 2940, 2860, 2720 (aldehydic C-H), 1680 (conjugated CO), 1640 (C=C), 1470, 1370–1380; ^1H NMR: δ10.12 (*s*, H-28′), 6.46 (*t*, H-28, J=6.7 Hz), 5.34 (*m*, H-9, 10, 9′ and 10′), 2.55 (*dt*, H-27, J =7 Hz), 2.15 (*t*, H-26′, J=7.5 Hz), 2.01 (*m*, H-8, 11, 8′ and 11′), 1.49 (*m*, H-26), 1.26 (*m*, other CH_2 protons), 0.88 (*t*, H-1 and 1′, J =6.8 Hz); ^{13}C NMR: δ190.97 (*d*, C-28′), 149.20 (*d*, C-28), 140.55 (*s*, C-27′), 129.95 (*d*, C-9, 9′, 10 and 10′), 31.99 (*t*, C-3 and 3′), 30.26 (*t*, C-26′), 29.84 to 29.38 (C-4 to C-7, C-4′ to C-7′, C-12 to C-26 and C-12′ to C-25′), 27.30 (*t*, C-8, 8′, 11 and 11′), 26.71 (*t*, C-27), 22.71 (*t*, C-2 and 2′), 14.07 (*q*, C-1 and 1′).

Botryals **3** were isolated as an oily mixt. MS *m/z*: $[M]^+$ (int. in % relative to *m/z* 69) 906 (1), 878 (2), 850 (7), 822 (12), 794 (8), 766 (5), 738 (1); IR ν_{max} cm^{-1}: 3000 (=C–H), 2940, 2860, 2720 (aldehydic C-H), 1690 (conjugated C=O), 1640 (C=C), 1470, 1380–1370; ^1H NMR: δ9.35 (*s*, H-28′), 6.43 (*t*, H-28, J=7.4 Hz), 5.34 (*m*, H-9, 10, 9′ and 10′), 2.34 (*dt*, H-27, J=7.4, 7 Hz), 2.22 (*t*, H-26′, J =7.5 Hz), 2.01 (*m*, H-8, 11, 8′ and 11′), 1.49 (*m*, H-26), 1.26 (*m*, other CH_2 protons), 0.88 (*t*, H-1, 1′, J=6.8 Hz); ^{13}C NMR: δ195.01 (*d*, C-28′), 154.76 (*d*, C-28), 144.12 (*s*, C-27′), 129.95 (*d*, C-9, 9′, 10 and 10′), 31.99 (*t*, C-3 and 3′), 29.87 to 28.84 (C-4 to C-7, C-4′ to C-7′, C-12 to C-27 and C-12′ to C-25′), 27.31 (*t*, C-8, 11, 8′ and 11′), 24.16 (*t*, C-26′), 22.73 (*t*, C-2 and 2′), 14.08 (*q*, C-1 and 1′).

Epoxy alkenes **4**, solid mixt. analysed by GC-MS using EI. The apparatus was equipped with a fused silica column 25 m, SE 52, temp 260°; *m/z* (int. in % relative to *m/z* 55) $C_{27}H_{52}O$ 392 $[M]^+$ (1), 374 $[M-H_2O]^+$ (1), 279 $[M-C_8H_{17}]^+$ (8), 261 $[M-C_8H_{17}-H_2O]^+$ (3), 155 $[M-C_{17}H_{33}]^+$ (7), 137 ($[M-C_{17}H_{33}-H_2O]^+$ (4); $C_{29}H_{56}O$ 420 $[M]^+$ (1.5), 402 $[M-H_2O]^+$ (1), 307 $[M-C_8H_{17}]^+$ (7), 289 $[M-C_8H_{17}-H_2O]^+$ (2), 155 $[M-C_{19}H_{37}]^+$ (11), 137 $[M-C_{19}H_{37}-H_2O]^+$ (1), $C_{31}H_{60}O$ 430 $[M-H_2O]^+$ (0.5), 335 $[M-C_8H_{17}]^+$ (6), 317 $[M-C_8H_{17}-H_2O]^+$ (3), 155 $[M-C_{21}H_{41}]^+$ (7.5), 137 $[M-C_{21}H_{41}-H_2O]^+$ (3). ^1H NMR δ5.88 (1H, *ddt*, J=7, 10, 17 Hz, $-C\underline{H}=CH_2$), 5.04 (1H, *m*, J=1.5, 2, 17 Hz, one terminal methylene proton), 4.98 (1H, *m*, J=1.5, 2, 10 Hz, one terminal methylene proton), 2.89 (*br*, epoxide protons), 1.49 (*m*, 4H, α to the epoxide), 2.01 (*m*, 2H α to the terminal double bond), 1.26 (other CH_2 protons), 0.88 (*t*, terminal Me, J=6.8 Hz). ^{13}C NMR: δ139.20 (*d*, –CH=), 114.09 (*t*, $=CH_2$), 57.17 (*d*, epoxide carbons), 33.85 (*t*, $^{13}CH_2$–CH=CH_2), 31.94 (*t*, $^{13}CH_2$–CH_2–Me), 29.75–29.05 (other methylenic carbons), 27.94 (*t*, carbons α to the epoxide ring), 26.69 (*t*, carbons β to the epoxide ring), 22.69 (*t*, $^{13}CH_2$–Me), 14.09 (*q*, Me).

Epoxy botryals **5**, solid mixt analysed by CI(NH_3)MS (probe): *m/z* (rel. int.) $[M+1]^+$ 783 (12), 811 (41), 839 (82), 867 (100), 895 (41), 923 (11), $[M+18]^+$ 800 (5), 828 (18), 856 (36), 884 (35), 912 (19), 940 (4); IR ν_{max} 3000, 2940, 2860, 2720, 1690, 1640, 1470, 1380–1370; NMR spectra exhibited the following differences compared to **3**: ^1H δ5.34 (2H instead of 4), 2.89 (2H, *br*, epoxide H), 2.01 (2H instead of 4), 1.49 (6H: H-26 and protons α to the epoxide); ^{13}C δ at 57.20 ppm (*d*, epoxide carbons), 27.95 (*t*, carbons α to the epoxide), 26.69 (*t*, carbons β to the epoxide).

Very long chain alkenylphenols **6**, solid mixt not eluted on GC. UV (*n*-hexane) λ_{max}215 nm (ε7500), 222 (6800), 289 (3700). EIMS (probe): *m/z* (rel. int.) 41 (37), 43 (59), 55 (100), 57 (61), 69 (73), 79 (16), 83 (30), 95 (9), 139 (21), 153 (16), 167 (46), 530 $[M^+]$ (21), 558 $[M^+]$ (88), 586 $[M^+]$ (34). CI(NH_3) MS: $[M+1]^+$ at 531, 559 and 587; no $[M+18]^+$ peaks; IR ν_{max} 3560, 3000, 2930, 2860, 1600–1620, 1500, 1470, 1430, 1380, 1220, 1200, 1150, 1080, 1050, 920, 820 and 720 cm^{-1}; ^1H NMR: δ6.35 (1H, *d*, J=2.8 Hz, H-3′), 6.29 (1H, *d*, J=2.8 Hz, H-5′), 5.35 (2H, *m*, –CH=CH–), 3.85 (3H, *s*, OMe), 3.76 (3H, *s*, OMe), 2.61 (2H, *t*, J=7.7 Hz, benzylic protons), 2.01 (4H, *m*, allylic protons), 1.60 (2H, *m*, homobenzylic protons), 1.26 (other CH_2 protons), 0.88 (3H, *t*, J=6.8 Hz, terminal CH_3); ^{13}C NMR: δ152.92 (*s*, C-4′), 146.83 (*s*, C-2′), 137.75 (*s*, C-1′), 129.96 (*d*, olefinic carbons), 128.88 (*s*, C-6′), 106.15 (*d*, C-5′), 96.89 (*d*, C-3′), 56.07 (*q*, OMe), 55.83 (*q*, OMe), 31.97 (*t*, $^{13}CH_2$–CH_2–Me), 30.08 (*t*, benzylic carbon), 29.88–29.38 (other $^{13}CH_2$), 27.30 (*t*, $-^{13}CH_2$–CH=CH–$^{13}CH_2$–), 22.73 (*t*, $-^{13}CH_2$–Me), 14.09 (*q*, $-CH_2-^{13}CH_3$).

Epoxy phenols **7**, solid mixt, CI (NH_3) MS (probe) *m/z* (rel. int.) $[M+1]^+$: 547, 575 and 603; $[M+18]^+$: 564, 592 and 620; $[M+1-18]^+$ 529, 557 and 585. The NMR spectra exhibited the following additional signals compared to **6**: ^1H δ2.91 (2H, *br*, epoxide protons), 1.50 (4H, protons α to the epoxide), no signal at 5.35; ^{13}C δ57.23 (*d*, epoxide carbons), 27.93 (*t*, carbons α to the epoxide), 26.68 (*t*, carbons β to the epoxide), no signal at 129.95.

Fatty acids were analysed after esterification with CH_2N_2 in Et_2O, by GC-MS; fused silica SE 52 column, 25 m × 0.25 mm, temp. prog. from 170° to 260° at 3°/mn. The total fatty acids were isolated by saponification of the hexane ext [35].

Sterols were determined by GC-MS, fused silica SE 52 at 260°, and comparison with authentic standards.

Ozonolysis. Compounds **2**, **3** or **6** (30–40 mg) dissolved in 5 ml CH_2Cl_2–MeOH (9:1) were ozonized at −15° for 15 min (30 mg O_3/l air; 40 l/hr). CH_2Cl_2 was then removed at room temp. in a stream of N_2 and the ozonides decomposed by refluxing with H_2O_2 30% (1.5 ml) and HCO_2H (3 ml) for 1 hr. The acid mixts extracted with Et_2O were then esterified with CH_2N_2. The esters were analysed by GC-MS using EI, as described in ref. [11].

Spontaneous degradation of botryals **3**. Dry botryals **3** (150 mg) were kept in air at room temp. After one week IR spectra exhibited OH absorption characteristic of carboxylic acids (3500–3000 cm^{-1}) with an intense band at 1690 cm^{-1}. Silica gel TLC (*n*-hexane–Et_2O, 23:2) gave 9 mg of a hydrocarbon fraction (R_f 0.95) and eight fractions containing aldehydes and acids not further analysed. CI(NH_3)MS of the hydrocarbons **8** (probe): C_nH_{2n-4} $[M+18]^+$, (rel. int.): $C_{51}H_{98}$ [728] (14), $C_{53}H_{102}$ [756] (46), $C_{55}H_{106}$ [784] (73), $C_{57}H_{110}$ [812] (100), $C_{59}H_{114}$ [840] (64), $C_{61}H_{118}$ [868] (44), $C_{63}H_{122}$ [896] (16); IR ν_{max} 3000, 2930, 2860, 1640, 1470, 1380, 970. ^{13}C NMR: δ130.41 (*d*), 129.95 (*d*), 32.64 (*t*), 31.96 (*t*), 29.82–29.22 (numerous peaks), 27.26 (*t*), 22.71 (*t*), 14.10 (*q*).

Epoxidation of n-alkadienes. Pure *n*-alkadienes **1** (145 mg), extd from a Moroccan strain [2], dissolved in 10 ml CH_2Cl_2 were reacted with 200 mg of *m*-chloroperbenzoic acid for 3 hr at room temp. The crude product, extd with CH_2Cl_2 and washed with 10% NaOH, was purified by silica gel (12 g) CC. Elution with *n*-hexane–Et_2O, 19:1 (45 ml) gave 60 mg of epoxy alkenes **4**. Further elution with *n*-hexane–Et_2O, 23:2 furnished the diepoxy alkanes: GC/EI MS (conditions as for **4**) $[M^+]$: 408 $[C_{27}H_{52}O_2]^+$, 436 $[C_{29}H_{56}O_2]^+$, 464 $[C_{31}H_{60}O_2]^+$; ^{13}C NMR: δ57.17 (*d*), 52.29 (*d*) (terminal epoxide), 46.98 (*t*), (terminal epoxide), 32.57 (*t*, carbon α to the terminal epoxide), 31.93 (*t*), 29.73–29.27, 27.94 (*t*), 26.69 (*t*), 26.02 (*t*), 22.69 (*t*), 14.07 (*q*).

Methylation of **6**. **6** (25 mg) dissolved in 5 ml Me_2CO was refluxed for 5 hr with 0.1 ml Me_2SO_4 and 500 mg K_2CO_3. Before the usual extraction with Et_2O, excess of Me_2SO_4 was destroyed by refluxing with aq. NaOH. Purification on silica gel TLC gave 12 mg of **11** (R_f 0.21, *n*-hexane–Et_2O, 23:2). EIMS, (probe), *m/z* (int. % relatively to *m/z* 55): $C_{36}H_{64}O_3$ 544 (9), $C_{38}H_{68}O_3$ 572 (55); $C_{40}H_{72}O_3$ 600 (32), ^{13}C NMR: δ155.96, 153.41, 137.00, 129.96, 105.44, 98.04, 60.77, 55.80, 55.54, 31.96, 30.83, 30.21, 29.84–29.35, 27.28, 22.71, 14.07.

Feeding expts. Algae were fed with [1,2- ^{13}C] NaOAc (isotopic purity 99.8%). To an air-lift culture of 8 days, 25 mg of acetate were added on days 9, 10 and 11, and 50 mg on day 12. The culture was taken for analysis on day 15. Botryals **2** (15 mg) and **3** (190 mg) and phenols **6** (25 mg) were isolated as described above.

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REFERENCES

1. Metzger, P., Villareal-Rosales, E., Casadevall, E. and Coute, A. (1989) *Phytochemistry* (in press).
2. Metzger, P., Berkaloff, C., Casadevall, E. and Couté, A. (1985) *Phytochemistry* **24**, 2305.
3. Metzger, P. and Casadevall, E. (1987) *Tetrahedron Letters*, **28**, 3931.
4. Yamaguchi, K., Nakano, H., Murakami, M., Konosu, S., Nakayama, O., Kanda, M., Nakamura, A. and Iwamoto, H. (1987) *Agric. Biol. Chem.* **51**, 493.
5. Fried, A., Tietz, A., Ben-Amotz, A. and Eichenberger, W. (1982) *Biochim. Biophys. Acta* **713**, 419.
6. Piorreck, M. and Pohl, P. (1984) *Phytochemistry* **23**, 217.
7. Robinson, N., Cranwell, P. A., Eglinton, G. and Jaworski, G. H. N. (1987) *Phytochemistry* **26**, 411.
8. Yu Suen, Hubbard, J. S., Holzer, G. and Tornabene, T. G. (1987) *J. Phycol.* **23**, 289.
9. Cranwell, P. A., Creighton, M. E. and Jaworski, G. H. M. (1988) *Phytochemistry* **27**, 1053.
10. Volkman, J. K. and Hallegraeff, G. M. (1988) *Phytochemistry* **27**, 1389.
11. Metzger, P., Templier, J., Largeau, C. and Casadevall, E. (1986) *Phytochemistry* **25**, 1869.
12. Metzger, P. and Casadevall, E. (1988) *Tetrahedron Letters* **29**, 2831.
13. Largeau, C., Casadevall, E., Berkaloff, C. and Dhamelincourt, P. (1980) *Phytochemistry* **19**, 1043.
14. Pfeffer, R. E., Luddy, F. E. and Unruh, J. (1977) *J. Am. Oil. Chem. Soc.* **54**, 380.
15. Nielsen, A. T. and Houlihan, W. J. (1968) in *Organic Reactions*, Vol. 16. Wiley, New York.
16. Suyama, K., Arakawa, T. and Adachi, S. (1981) *J. Agric. Food Chem.* **29**, 875.
17. Welby-Guisse, M., Laneelle, M. A. and Asselineau, J. (1970) *Eur. J. Biochem.* **13**, 164.
18. Maurice, M. T., Vacheron, M. J. and Michel, G. (1971) *Chem. Phys. Lipids* **7**, 9.
19. Etemadi, A. H. and Lederer, E. (1965) *Bull. Soc. Chim. Fr.* 2640.
20. Gastambide-Odier, M. and Lederer, E. (1959) *Nature* **184**, 1563.
21. Walker, R. W., Prume, J. C. and Lacave, C. S. (1973) *Biochim. Biophys. Acta* **326**, 52.
22. Scott, A. I. (1964) *Interpretation of Ultraviolet Spectra of Natural Products.* Pergamon Press, Oxford.
23. Simpson, T. J. (1987) *Nat. Prod. Rep.* **4**, 339.
24. Barclay, W. R., Kennish, J. M., Goodrich, V. M. and Fall, R. (1987) *Phytochemistry* **26**, 739.
25. Asakawa, Y., Masuya, T., Tori, M. and Campbell, E. O. (1987) *Phytochemistry* **26**, 735.
26. Scannell, R. T., Barr, J. R., Murty, V. S., Reddy, K. S. and Hecht, S. M. (1988) *J. Am. Chem. Soc.* **110**, 3650.
27. Devon, T. K. and Scott, A. I. (1975) in *Handbook of Naturally Occurring Compounds*, Vol. 1. Academic Press, New York.
28. Cojocara, M., Droby, S., Glotter, E., Goldman, A., Gottlieb, H. E., Jacoby, B. and Prusky, D. (1986) *Phytochemistry* **25**, 1093.
29. Wenkert, E., Loeser, E. M., Mahapatra, S. N., Schenker, F. and Wilson, E. M. (1964) *J. Org. Chem.* **29**, 435.
30. Burton, G. W. and Ingold, K. U. (1981) *J. Am. Chem. Soc.* **103**, 6472.
31. Ratledge, C. (1978) in *Developments in Biodegradation of Hydrocarbons* Vol. 1, (Watkinson, R. J., Ed.), p. 3. Applied Science, London.
32. Bascetta, E. and Gunstone, F. D. (1985) *Chem. Phys. Lipids* **36**, 253.
33. Lewis, S. N. (1969) in *Oxidation*, Vol. 1, (Augustin, R. L. ed.), p. 225. Marcel Dekker, New York.
34. Daffe, M., Laneelle, M. A., Puzo, G. and Asselineau, C. (1981) *Tetrahedron Letters* **22**, 4515.
35. Laureillard, J., Largeau, C., Waeghmaeker, F. and Casadevall, E. (1986). *J. Nat. Prod.* **49**, 794.
36. Templier, J., Largeau, C. and Casadevall, E. (1984) *Phytochemistry* **23**, 1017.
37. Patterson, G. W. (1974) *Comp. Biochem. Physiol.* **47B**, 453.
38. Holden, M. J. and Patterson, G. W. (1982) *Lipids*, **17**, 215.
39. Berkaloff, C., Casadevall, E., Largeau, C., Metzger, P., Peracca, S. and Virlet, J. (1983) *Phytochemistry* **22**, 389.
40. Largeau C., Derenne, S., Casadevall, E., Kadouri, A. and Sellier, N. (1986) *Adv. Org. Geochem.* **10**, 1023.
41. Kolattukudy, P. E. (1972) *Biochem. Biophys. Res. Comm.* **49**, 1040.
42. Kolattukudy, P. E. (1981) *Ann. Rev. Plant. Physiol.* **32**, 539.