Pergamon

0031-9422(95)00053-4

Phytochemistry, Vol. 40, No. 2, pp. 543–554, 1995

0031-9422/95 $9.50 + 0.00

n-ALKENYLPYROGALLOL DIMETHYL ETHERS, ALIPHATIC DIOL MONOESTERS AND SOME MINOR ETHER LIPIDS FROM *BOTRYOCOCCUS BRAUNII* A RACE

P. METZGER and Y. POUET

Laboratoire de Chimie Bioorganique et Organique Physique. UA CNRS D 1381, Ecole Nationale Supérieure de Chimie de Paris, 11, Rue P. et M. Curie, 75231 Paris Cedex 05, France

(*Received* 28 *November* 1994)

Key Word Index—*Botryococcus braunii*; Chlorophyceae; alga; A race; very long chain *n*-alkenylpyrogallol dimethyl ethers; very long chain *n*-alkenylhydroquinol dimethyl ethers; esters of aliphatic vicinal diols; ether lipids; alkoxy ether lipids; phenoxy ether lipids.

Abstract—From two strains of the green microalga, *Botryococcus braunii* (A race), we have isolated two series of very long chain *n*-alkenylphenols: *n*-alkenylpyrogallol dimethyl ethers along with the already known series of *n*-alkenylhydroquinol dimethyl ethers, two series of monoesters of aliphatic vicinal diols derived from alkadienes and from botryals, two new series of alkoxy ether lipids formed by oxidative coupling of two alkatrienes, and four new series of phenoxy ether lipids structurally related to an homologous series of three long chain *n*-alkenylresorcinols, linked by phenoxy bonds to alkenyl, botryalyl or alkylhydroquinol moieties, in the mid-chain. The structures were elucidated by spectroscopic and chemical methods. Among them, very long chain *n*-alkenylpyrogallol dimethyl ethers are reported for the first time from a natural source, and esters of aliphatic vicinal diols are a new class of lipids in *B. braunii*. By comparison with a recent report about a Bolivian strain, phenoxy ether lipids are only minor constituents.

INTRODUCTION

The green microalga, *Botryococcus braunii*, is a rich source of non-classical lipids. Previous chemical analyses of strains belonging to the A race, which is chemically defined by the production of straight-chain hydrocarbons, have revealed the presence of *n*-alkenylhydroquinol derivatives, C_{52}–C_{64} aldehydes (botryals) synthesized via aldol condensation of fatty aldehydes, epoxides and unusual ether lipids [1]. According to the type of ether linkage, two families of ether lipids, not glycerol derivatives, can be considered in *B. braunii*. In the alkoxy ether lipids, an oxygen bridge binds in the mid-chain two aliphatic moieties derived from hydrocarbons, *n*-alkenylhydroquinols or botryals, hydroxyl groups (esterified or not by fatty acids) being also present α to the ether bridge [2, 3]. In the recently described phenoxy ether lipids, it is a 5-*n*-alkenylresorcinol unit which links aliphatic moieties via phenoxy bonds [4]. Moreover, a strong strain dependence has been noticed regarding the preferential synthesis of alkoxy or of phenoxy ether lipids [5].

Continuing our investigations on *B. braunii* A race, we have now isolated from two strains originating from a culture collection and from a freshwater lake, two series of very long chain *n*-alkenyl-dimethoxyphenols **1** and **2**, two series of monoesters of aliphatic diols **3** and **4**, two series of new alkoxy ether lipids **5** and **6**, and four series of phenoxy ether lipids **7**–**10**. This paper deals with their isolation and structural determination.

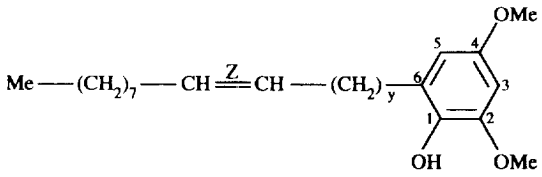
RESULTS AND DISCUSSION

The strains investigated originated from the Culture Center at Austin, Texas, and from a French lake located in Brittany (Coat ar Herno). Hexane extracts of the three-week-old cultures (43% of dry wt for the Austin strain, 60% of dry wt for the Coat ar Herno one) were submitted to CC over silical gel. By elution with hexane and increasing amounts of diethyl ether, five fractions were recovered (labelled I–V in order of elution from the column, see Experimental). From these fractions and by prep. TLC over silica gel and sometimes by additional HPLC separations, were obtained 10 series of compounds besides other previously described lipids [2, 3, 6, 7]; their occurrence and abundance are listed in Table 1.

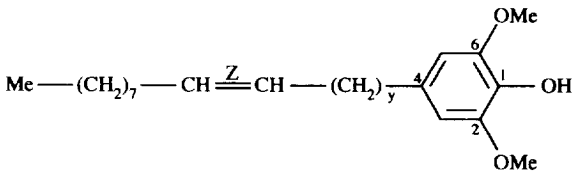
n-*Alkyl- and* n-*alkenyldimethoxyphenols* **1** *and* **2**

Compounds **1** and **2** were isolated from the Coat ar Herno strain and recovered from fractions II and V, respectively, both as solid mixtures. 6-*n*-Alkenyl-2,4-dimethoxyphenols **1** (hydroquinol derivatives) exhibiting C_{27}, C_{29} and C_{31} (minor) hydrocarbon chains were previously isolated from the Austin strain [6].

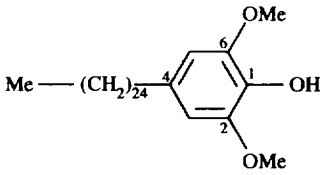
Compound **2** showed an UV maximum at 215 nm. The probe EI mass spectrum established a series of five compounds: $C_{33}H_{58}O_3$, $[M]^+$ *m/z* 502, $C_{33}H_{60}O_3$, $[M]^+$ *m/z* 504, $C_{35}H_{62}O_3$, $[M]^+$ *m/z* 530, $C_{37}H_{66}O_3$,



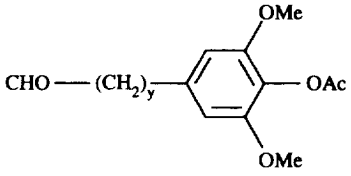
1
y = 17, 19, 21 (minor)



2a
y = 15, 17 (major), 19 (major), 21



2b



11
y = 15, 17, 19

Table 1. Lipid contents of the two strains of *B. braunii*

Strains	Hexane extractable lipids, % of dry wt	Compounds and (% of dry wt)
Coat ar Herno	60	**1** (1.15)
		2 (0.85)
		3 (1.2)
		4 (0.2)
		5 (2.2)
		6 (0.6)
Austin	43	**7** (0.2)
		8 (0.1)
		9 + **10** (0.03)

$[M]^+$ *m/z* 558 and $C_{39}H_{70}O_3$, $[M]^+$ *m/z* 586, in a ratio of *ca* 1.5:1.5:53:42:2. The IR spectrum exhibited absorptions for a free OH vibration at 3530 cm^{-1}, an aromatic ring at 1610 cm^{-1}, a mid-chain unsaturation at 3000 cm^{-1}, C–O bonds at 1240, 1210 and 1115 cm^{-1}, and a polymethylenic chain at 720 cm^{-1}. The ^1H NMR spectrum was dominated by a peak at δ1.34 for polymethylenic protons; it also exhibited signals for mid-chain unsaturation at δ5.51, allylic protons at δ2.11, a terminal methyl at δ0.89 and a 1, 2, 3, 5-tetrasubstituted phenyl group with a singlet (2H) at δ6.35. Moreover, a singlet for six protons at δ3.39 was assigned to two methoxy groups, and two signals at δ2.54 and 1.64 were attributed to two benzylic and two homobenzylic protons, respectively. The ^{13}C NMR spectrum confirmed the existence of a symmetry for the aromatic nucleus, with the presence of four signals at δ147.7 (two C_{ar}-OMe), 134.5 (C_{ar}-OH), 133.6 (C_{ar}-alkyl) and 106.1 (two C_{ar}-H). Moreover, a *cis*-configuration for the mid-chain unsaturation was deduced from the ^{13}C chemical shift of the allylic carbons, δ27.7.

Furthermore, ozonolysis of the acetate derivatives of **2**, followed by reductive cleavage of the ozonides by triphenylphosphine, furnished nonanal, a series of four aldehydes **11**, with C_{16}, C_{18}, C_{20} and C_{22} chains, bearing in the terminal position a 1,3-dimethoxy-2-acetoxy-phenyl group, and unreacted starting compound (EI-mass spectrum: $[M-CH_2CO]^+$ at *m/z* 504 $[C_{34}H_{62}O_4-CH_2O]^+$). This established a $\omega 9'$–$\omega 10'$ position for the mid-chain unsaturation in the C_{25}, C_{27}, C_{29} and C_{31} *n*-alkenyl derivatives **2a** and the presence of a C_{25} saturated counterpart **2b**. From all these results, the major phenolic compounds of **2** were deduced to be 5-[*n*-heptacos-18′(Z)-enyl] pyrogallol 1,3-dimethyl ether and its nonacos-20′(Z)-enyl homologue (53% and 42% of the mixture, respectively). The *n*-C_{25} (saturated and mono-unsaturated) and *n*-C_{31} (mono-unsaturated) derivatives were only minor compounds.

TLC analysis of fraction V from the Austin strain suggested also the presence of **2** in the extract of this strain, however, due to the presence of numerous other lipids in this fraction, its isolation was unsuccessful. To the best of our knowledge, this is the first report of natural very long chain *n*-alkyl or *n*-alkenylpyrogallol derivatives. In an earlier report [6], on the basis of a feeding experiment with [1,2-^{13}C acetate], *n*-alkenyl-

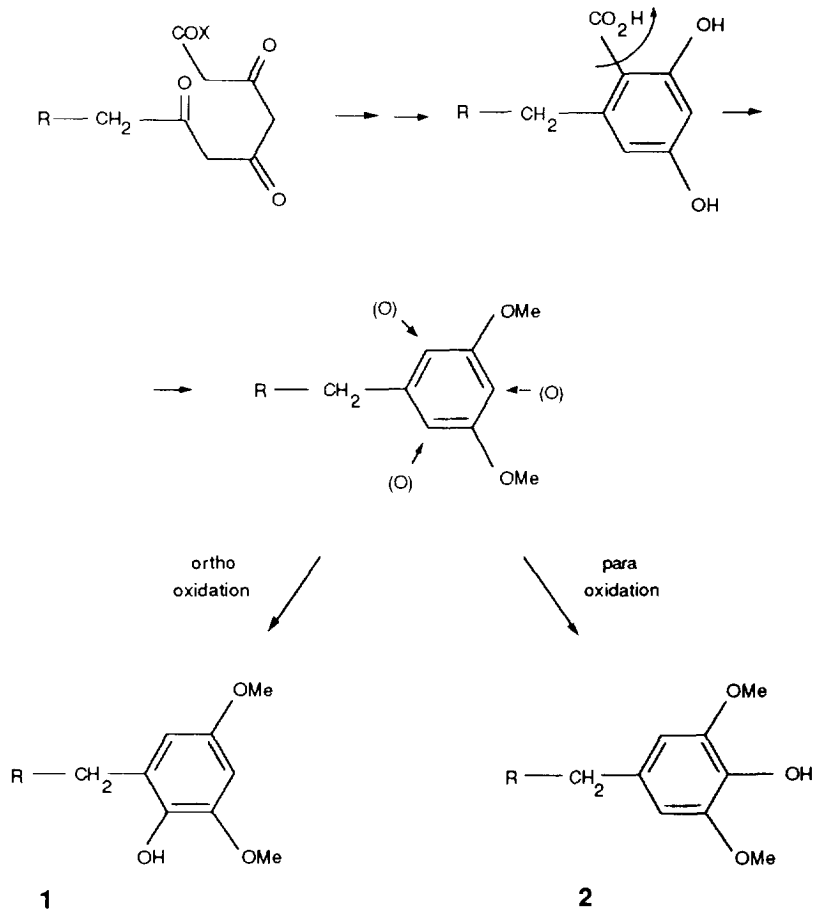


Fig. 1. Proposed biosynthetic pathway for the synthesis of *n*-alkenyldimethoxyphenols in *B. braunii*.

hydroquinols **1** were assumed to have a tetraketide origin in *B. braunii* and to result from the oxidation of a resorcinol derivative at one *ortho*-position relative to the alkenyl chain. Such a biosynthetic pathway can be proposed for the synthesis of **2**, with in this case, however, oxidation at the *para*-position relative to the aliphatic chain (Fig. 1).

Aliphatic diol monoesters, **3** *and* **4**

CI(NH_3) mass spectroscopy showed that **3** was a mixture of two compounds of molecular formulae $C_{47}H_{90}O_3$ and $C_{45}H_{86}O_3$ in a ratio of *ca* 3:2. The IR spectrum indicated the presence of a terminal double bond, with absorptions at 910, 990 and 3080 cm^{-1}, of a hydroxyl group (3600 cm^{-1}) and of an ester function (1730 cm^{-1}). The ^1H NMR spectrum displayed signals of a secondary alcohol group (δ3.58, *m*) adjacent to a secondary carbon bearing an ester function (δ4.84, *dt*, J = 4.1, 6.4 Hz), signals typical for a terminal olefin (δ5.58, 4.98 and 4.92, each 1H) and for a methylene group α to a carbonyl (δ2.33, *t*, J = 6.5 Hz). Its ^{13}C NMR spectrum confirmed the presence of a secondary alcohol (δ72.6) and of an ester (carboxyl at δ173.7) linked to a secondary carbon (δ76.3). Finally, CI(NH_3) mass spectral data established that the components of **3** were mono-oleyl esters (oleyl ion at *m/z* 265) of 9,10-dihydroxyalkenes, with a free hydroxyl at C-10, and that the chain lengths were C_{27} and C_{29} (Fig. 2). The structures of the two compounds constituting **3** were therefore established as 9-oleyloxy-10-hydroxy-heptacos-26-ene and 9-oleyloxy-10-hydroxy-nonacos-28-ene.

The FAB-mass spectrum of **4** showed four ions from *m/z* 1160 to 1076 with a regular increase of 28 mu, each corresponding, as shown from further spectroscopic data, to four $[M-H_2O]^+$ ions. As found in the mixture **3**, series **4** displayed NMR signals for monoesters of vicinal diols. The main differences lay in the replacement of the signals of the terminal double bond by those of an α-unsaturated, α-branched aldehydic function, as in botryals [6]: $-CH_2-CH_a=C_b(CH_cO)-CH_{2d}-CH_2^-$, δ_H 9.36 (H_c, *s*), 6.44 (H_a, *t*), 2.22 (H_d, *t*), δ_C 195.3 (C_c), 155.3 (C_a), 143.9 (C_b) and 24.1 (C_d). Moreover there was an additional terminal methyl and supplementary mid-chain unsaturation. The IR spectrum confirmed the presence of an α-unsaturated aldehyde function (2720 and 1690 cm^{-1}). These results suggested that **4** was a mixture of esters derived from botryal diols. The EI-mass spectrum exhibited four ions of very low intensity at *m/z* 895

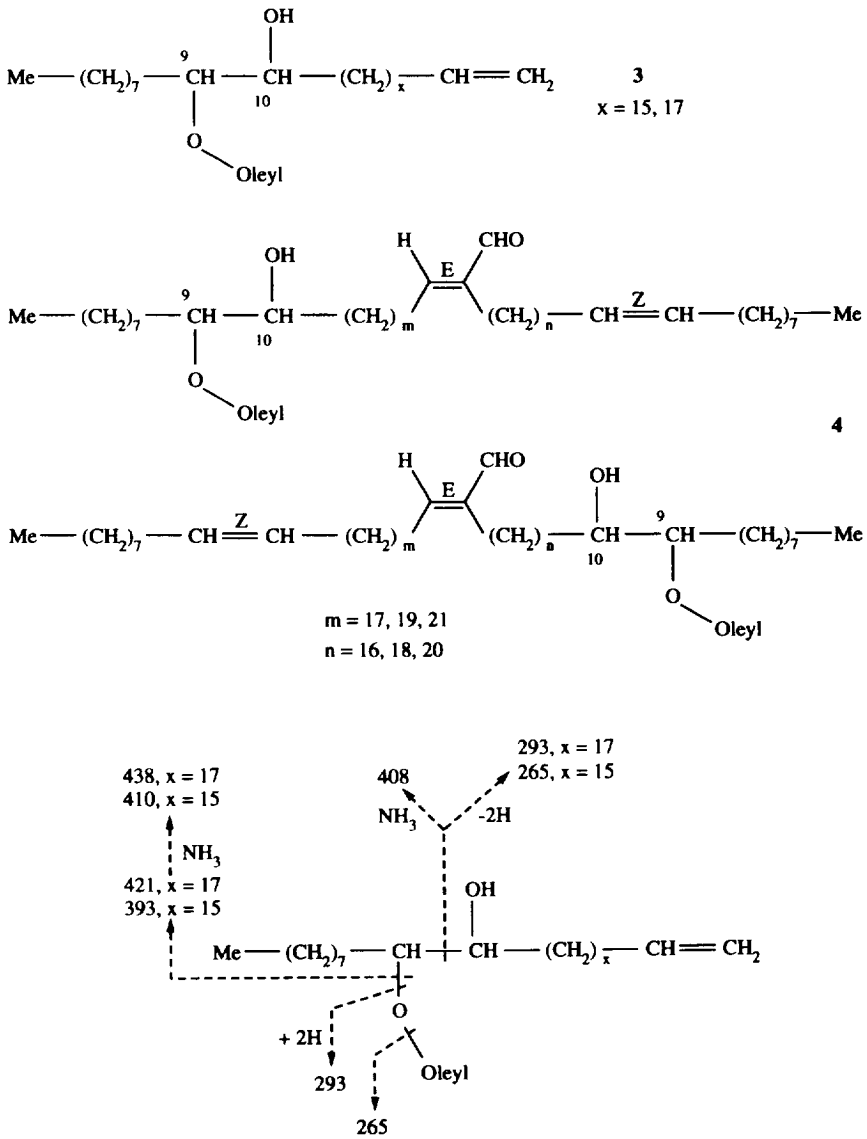


Fig. 2. CI(NH_3) mass spectral fragmentation pattern of **3**.

$[C_{62}H_{119}O_2]^-$, 867 $[C_{60}H_{115}O_2]^+$, 839 $[C_{58}H_{111}O_2]^+$, and 811 $[C_{56}H_{107}O_2]^+$, consistent with four botryalyl ions, each containing an hydroxyl group. Moreover, a peak at *m/z* 265 was assigned to an oleyl ion and the one at *m/z* 391 was attributed to the ion resulting from the cleavage of the CH(ester)–CH(OH) bond; as observed for **3**, this latter ion is indicative of a C-9 position for the ester group. Taken together, these data allowed us to establish that **4** was a mixture of 9-oleyloxy-10-hydroxy-botryals.

Aliphatic vicinal diols are known to occur in plants as dihydroxy fatty acids, the cutin monomers, which are formed by hydration of the corresponding epoxides [8]. On structural grounds, it may be assumed that esters **3** and **4** are also very likely derived from epoxides, via a biosynthetic route close to the one which operates in the formation of alkoxy ether lipids. The occurrence of such a biosynthetic pathway in alkoxy ether lipid synthesis, has been demonstrated by the very high incorporation levels of ^{14}C-labelled 9,10-epoxynonacosene in some of these compounds [3]. In both syntheses, the first step would be ring-opening in a protonated epoxide with the specific cleavage of the C–9–O bond. Then, attack of the resulting carbocation by a second epoxide or by a fatty acid derivative would lead to alkoxy ether lipids or to monoesters of vicinal diols, respectively (Fig. 3).

Ether lipids **5** *and* **6**

Fraction V of the Coat ar Herno extract gave by TLC separation, an oily compound **5** ($C_{54}H_{102}O_3$, $[M]^+$ at *m/z* 798). Its IR spectrum indicated the presence of hydroxyl groups (3580 cm^{-1}), C–O bonds (1065 cm^{-1}), terminal and mid-chain unsaturations (3070, 990, 910

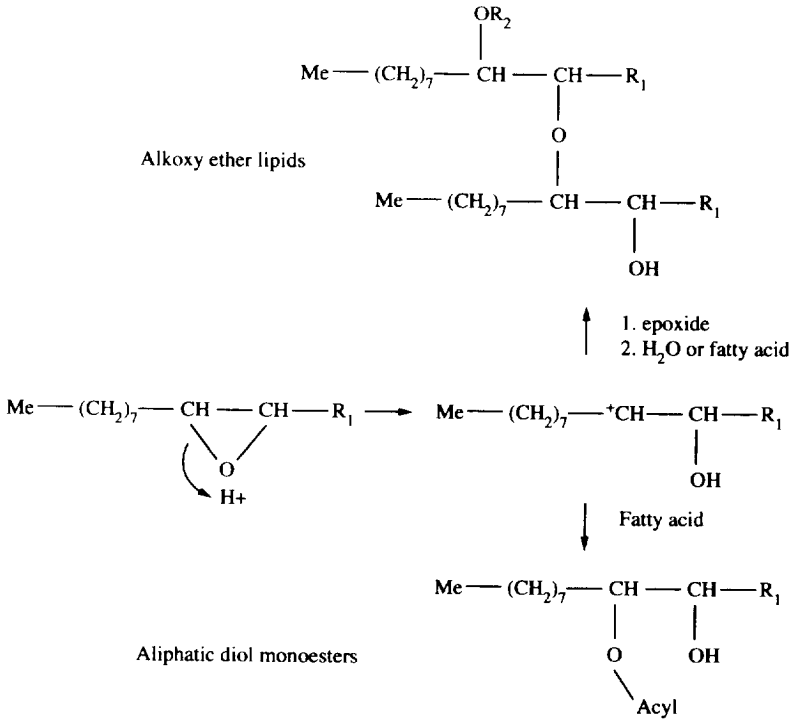
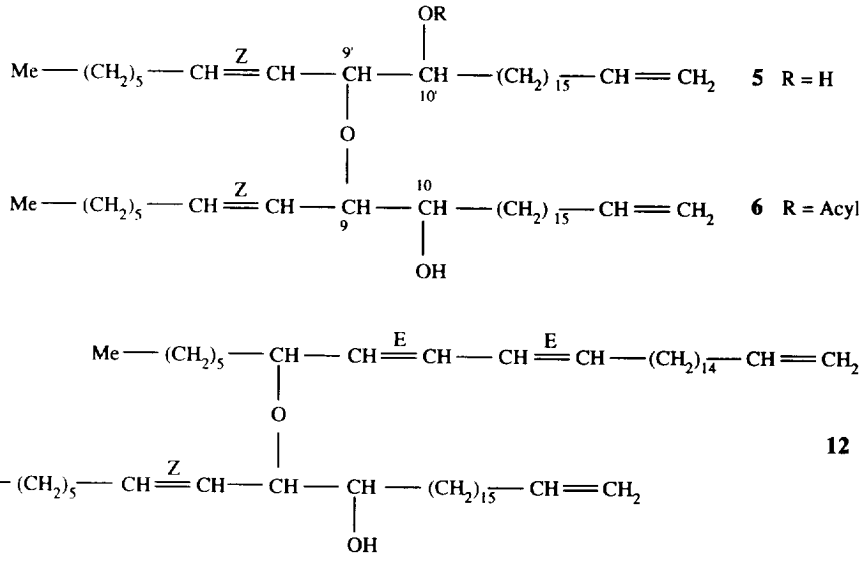


Fig. 3. Proposed biosynthetic mechanisms for the synthesis of alkoxy ether lipids and of monoesters of vicinal diols.

R_1: $-(CH_2)_x-CH=CH_2$ (alkene)

$-(CH_2)_m CH=C(CHO)-(CH_2)_n-CH=CH-(CH_2)_7-Me$(botryal)

R_2: H, acyl



and 3000 cm^{-1}, respectively). The ^1H NMR spectrum confirmed the occurrence of terminal unsaturations with signals at δ5.80, 5.03 and 4.98 and revealed the existence of a $-CH_2-CH_a=CH_b-CH_c$(O-aliph.)$-CH_d(OH)-CH_2-$ pattern, as previously observed in **12**, the first ether lipid isolated from *B. braunii* [2], with signals at δ5.62 (H_a, *dt*), 5.30 (H_b, *dd*), 4.17 (H_c, *dd*) and 3.63 (H_d, *dt*) [Table 2]. The two olefinic proton signals indicated that the double bond was here also of *cis*-geometry (J = 11.1 Hz). The ^{13}C NMR spectral data of this sub-structure for **5** were

Table 2. Selected ^1H and ^{13}C NMR data* of **5** (C_6D_6) and **6** ($CDCl_3$)

	5		6	
C	^1H	^{13}C	^1H	^{13}C
7	5.62 *dt* (11.1, 7.2)	135.8	5.69 *dt* (11.8, 6.8)	136.0[a]
8	5.30 *dd* (11.1, 9.8)	128.4	5.15 *dd* (11.8, 9.5)	127.5[b]
9	4.17 *dd* (9.8, 9.5)	76.7	3.90 *dd* (9.5, 7.5)	75.7[c]
10	3.63 *m*	73.9	3.38 *m*	73.7[d]
7'	5.62	135.8	5.68 *dt* (11.2, 6.8)	135.8[a]
8'	5.30	128.4	5.23 *dd* (11.2, 9.6)	127.0[b]
9'	4.17	76.7	4.18 *dd* (9.6, 5.7)	75.3[c]
10'	3.63	73.9	4.90 *dt* (5.7, 6.3)	72.6[d]

*Chemical shifts; multiplicity and (J in Hz).
[a–d] Interchangeable signals.

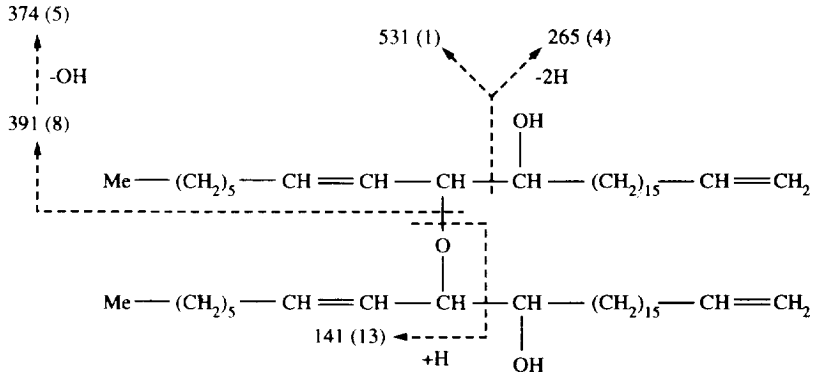


Fig. 4. EI mass spectral fragmentation pattern of **5**: *m/z* and (rel. int.); *m/z* 55 (100).

very similar to those observed for **12** [2], except for the signal of the carbon of the ether function, slightly deshielded in **5**. The absence of other signals in the olefinic and CH–O regions, other than those noticed above, suggested the ether linkage of two similar C_{27} aliphatic chains in **5**. Conclusive evidence for the structure was drawn from the EI mass spectrum. Cleavage of the ether bond furnished a hydroxy-heptacosadienyl ion at *m/z* 391. Moreover, the positions of the hydroxyls and ether function were deduced from ions resulting from the cleavage of CH(OR)–CH(OH) bonds, at *m/z* 265 $[CH_2{=}CH(CH_2)_{15}CHOH-2H]^+$, *m/z* 531 and from the rearranged fragment of this latter ion, *m/z* 141 (Fig. 4). On the basis of these spectral data, **5** was concluded to be 9-*O*-di-[10-hydroxy-heptacosadi-7(Z), 26-enyl] ether.

Series **6** was obtained as an oily mixture from fraction III of the Coat ar Herno extract by prep.TLC and subsequent HPLC purifications. The FAB-mass spectrum exhibited eight $[M + Li]^+$ peaks ranging from 1042 to 1266 (Table 3). The IR spectrum showed absorptions for unsaturations, as observed in **5**, a free hydroxyl vibration at 3580 cm^{-1} and a carbonyl absorption at 1730 cm^{-1}. Evidence for the presence of fatty ester derivatives of **5** was obtained from the hydrolysis of **6** under basic conditions. Thus, in addition to ether lipid **5**, eight fatty acids were identified, three saturated: C_{24} (4%), C_{26} (18%) and C_{28} (18%), and five monounsaturated: C_{16} (3%), C_{18} (9%), C_{28} (6%), C_{30} (36%) and C_{32} (6%). The structure for **6** was further confirmed by its ^1H and ^{13}C NMR spectra (Table 2 and Experimental).

Table 3. Molecular formulae from FAB-MS analysis of **6** and nature of the acyl group

$[M + Li]^+$ found	Molecular formula	Acylating agent
1042.0	$C_{70}H_{130}O_4$	16:1
1070.0	$C_{72}H_{134}O_4$	18:1
1156.1	$C_{78}H_{148}O_4$	24:0
1184.1	$C_{80}H_{152}O_4$	26:0
1210.3	$C_{82}H_{154}O_4$	28:1
1212.2	$C_{82}H_{156}O_4$	28:0
1238.4	$C_{84}H_{158}O_4$	30:1
1266.4	$C_{86}H_{162}O_4$	32:1

Previous biosynthetic studies [9] have suggested that ether lipid **12** would be synthesized via the coupling of two epoxides, coupling initiated by ring-opening of

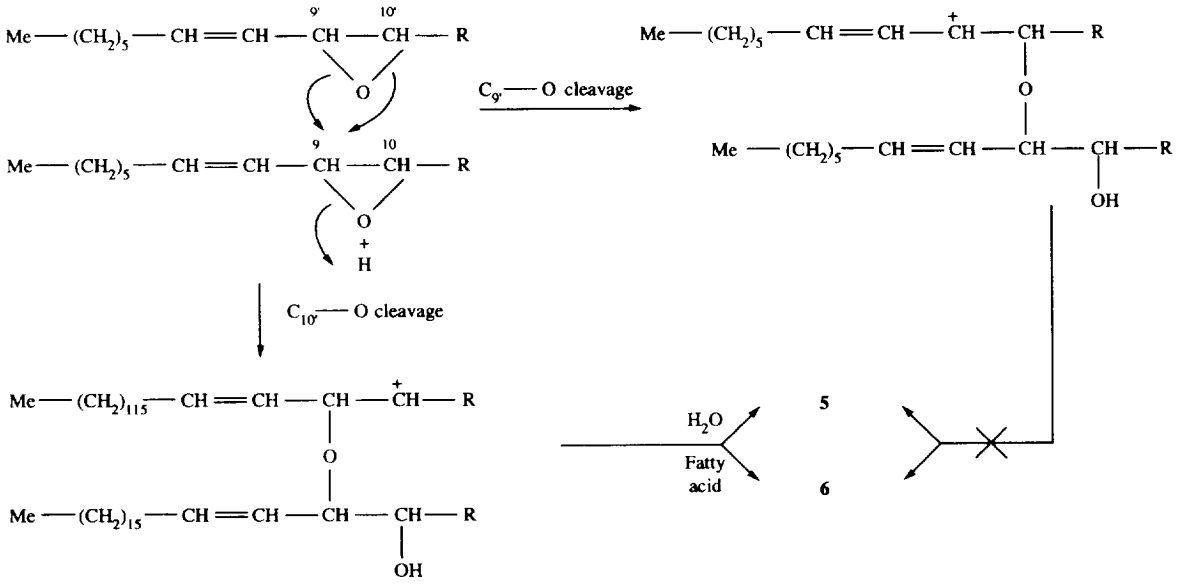


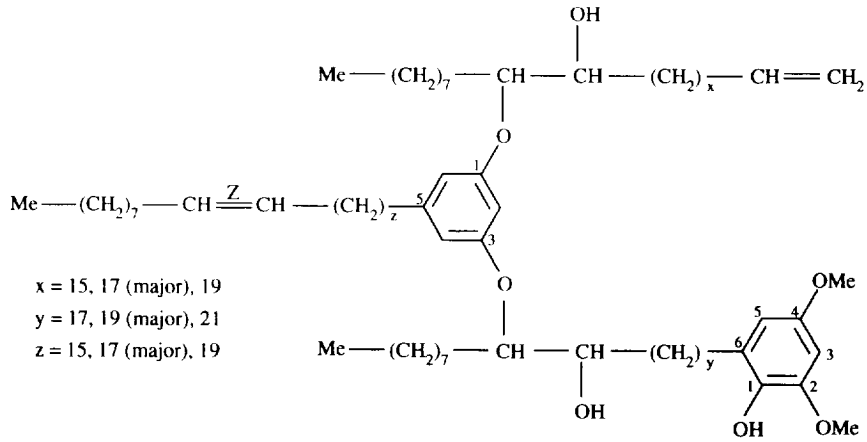
Fig. 5. Hypothetical biogenesis of **5** and **6** via epoxide coupling.
(R: $-(CH_2)_{15}-CH=CH_2$)

a protonated epoxide. Bearing in mind such a biosynthetic pathway, the formation of **5** and **6** is likely to be accounted for by the coupling of two identical C_{27} epoxides derived from a C_{27} triene, exhibiting two unsaturations at positions 7 and 26, and an epoxide ring at positions 9, 10. Therefore, the synthesis of **5** and **6** would require the formation of a carbocation at an unfavourable position. Consequently, this suggests the possible occurrence of another mechanism, such as a direct oxidative coupling of two C_{27} trienes (Fig. 5).

Phenoxy ether lipids **7**–**10**

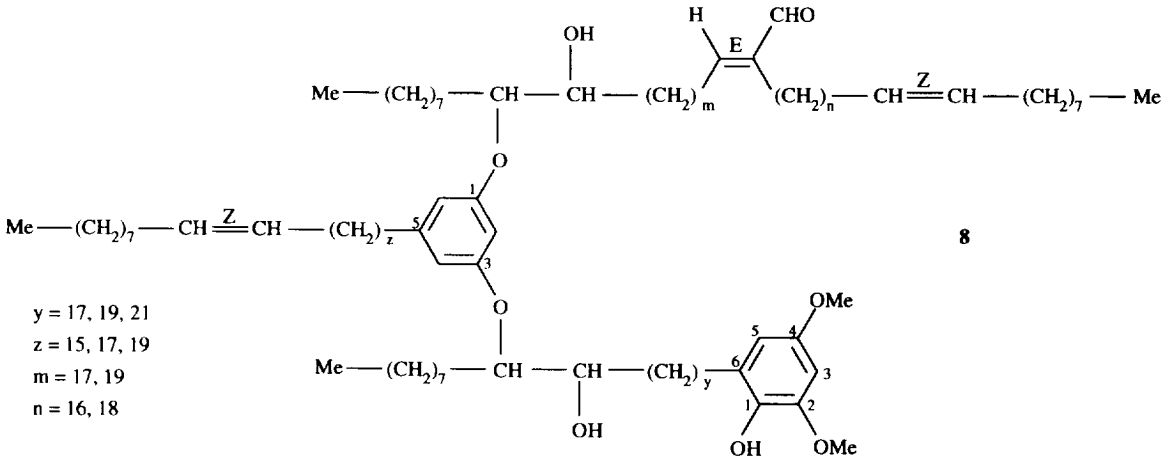
A mixture of compounds **7** and **8** was obtained by TLC separation of fraction V of the Austin strain extract. HPLC on a normal phase separated the two series, both of which exhibited UV absorptions at 203, 222 and 282 nm. The positive FAB mass spectrum of **7** showed a series of six adduct ions $[M + H]^+$ from *m/z* 1427 to *m/z* 1567 with a regular increase of 28 mu (*m/z* 1511, major). They corresponded to the molecular formula $C_{95-x}H_{172+2x}O_7$, with x even from 0 to 10. The IR spectrum showed the presence of hydroxyl absorptions (3600 and 3560 cm^{-1}), of terminal unsaturations (3080, 990 and 910 cm^{-1}), of mid-chain unsaturations (3000 cm^{-1}) and of aromatic absorption (1590 cm^{-1}). Careful analysis of both ^1H and ^{13}C NMR spectra, and comparison of these data with those of alkenylresorcinol-di-*O*-alkenyl diethers [4] and of alkenylhydroquinols **1** [6], revealed the existence of three substructures (Table 4). Thus, the ^1H NMR spectrum dominated by an intense signal at δ1.3, showed the presence of a triplet at δ6.77 (1H, J = 1.6 Hz) and of a doublet at δ6.65 (2H, J = 1.6 Hz) for protons of a resorcinol nucleus, substituted at position 5 by an aliphatic chain (two benzylic protons at δ2.58, *t*, J = 7.2 Hz). Moreover, a 2,4-dimethoxyphenol nucleus, substituted at position 6 by an aliphatic chain gave signals at δ6.42 (1H, *d*, J = 2.7 Hz), 6.32 (1H, *d*, J = 2.7 Hz), 3.45 (OMe, *s*), 3.14 (OMe, *s*) and 2.86 (two benzylic protons, *t*, J = 7.4 Hz). Three signals at δ5.81 (1H), 5.06 (1H) and 5.01 (1H) were attributed to protons of a terminal unsaturation. Moreover, in the CH-O region, two signals at δ4.25 (2H, *dt*, J = 5.2, 4.8 Hz) and 3.77 (2H, *dt*, J = 5.2, 5.0 Hz) were indicative of a $-CH_2-CH(O-Ar)-CH(OH)-CH_2-$ pattern in the structure. ^{13}C NMR data, listed in Table 4, confirmed these findings.

Thus, all these NMR spectral data strongly suggested that **7** contained lipids exhibiting two ether bridges of the phenoxy type, binding on one hand a resorcinol nucleus and on the other an alkenyl chain and an alkyl one bearing in a terminal position a dimethoxyphenol group. In order to locate the ether functions and the hydroxyls in the chains, the tri-TMSi derivatives were analysed by EI-mass spectrometry. The spectrum was dominated by a peak at *m/z* 718 $[C_{29}H_{57}(OSiMe_3)C_6H_2(OMe)_2(OSiMe_3)-H]^+$ associated with two minor homologous ions at *m/z* 690 $[C_{27}H_{53}(OSiMe_3)C_6H_2(OMe)_2(OSiMe_3)-H]^+$ and *m/z* 746 $[C_{31}H_{61}(OSiMe_3)C_6H_2(OMe)_2(OSiMe_3)-H]^+$, indicating that the alkyl chains of the hydroquinol moieties were C_{27}, C_{29} (major) and C_{31}. A second group of ions at *m/z* 465 $[C_{27}H_{52}OSiMe_3]^+$, 493 $[C_{29}H_{56}OSiMe_3]^+$ (major) and 521 $[C_{31}H_{60}OSiMe_3]^+$ were indicative of the length of the alkenyl chains. Moreover, ions at *m/z* 603 $[C_{25}H_{49}C_6H_3(OSiMe_3)_2+H]^+$, 631 $[C_{27}H_{53}C_6H_3(OSiMe_3)_2+H]^+$ (major) and 659 $[C_{29}H_{57}C_6H_3(OSiMe_3)_2+H]^+$, resulting from the cleavage of ether



x = 15, 17 (major), 19
y = 17, 19 (major), 21
z = 15, 17 (major), 19

7



y = 17, 19, 21
z = 15, 17, 19
m = 17, 19
n = 16, 18

8

bonds and concomitant rearrangement of $SiMe_3$ groups, established that the alkenyl chains of the resorcinol moieties were C_{25}, C_{27} (major) and C_{29}. Furthermore, ions at *m/z* 593 and 367 resulting from the fragmentation of the tri-TMSi derivative of the ether **7a** very likely predominating in the series (Fig. 6), allowed to assign hydroxyls at C-10 in **7**. Together with the ^1H NMR data, the above observations indicated that the ether functions could only be located at C-9. Finally, an $\omega 9$ position for the unsaturation in the hydrocarbon chain of the resorcinol moiety was deduced from the analysis of the acids obtained by oxidative cleavage of the ozonides derived from **7**; only *n*-nonanoic acid was detected as the monoacid. From all these results, phenoxy ethers **7** were considered to be alkenylresorcinol-*O*-[alkenyl]-*O*-[alkylhydroquinol dimethyl ether] diethers.

For series **8**, FAB mass spectroscopy gave six $[M + H]^+$ peaks from *m/z* 1846 to 1986 with a regular increase of 28 mu (maxima for *m/z* 1874, 1902 and 1958), suggesting the molecular formula $C_{124+x}H_{226+2x}O_8$ with *x* even from 0 to 10. Both ^1H and ^{13}C NMR analyses (Table 4) revealed partial sub-structures identical to those of **7**, viz., the alkenyl resorcinol and alkylhydroquinol moieties and two $-CH_2-CH(O-Ar)-CH(OH)-CH_2-$ patterns. The main spectral differences were the lack of terminal unsaturation, the presence of one additional terminal methyl, one supplementary mid-chain double bond and of a α-branched, α-unsaturated aldehyde function. The latter gave signals very similar or identical to those observed for botryals [6]: ^1H δ at 9.33 (*s*), 5.93 (*t*); ^{13}C δ at 194 (CHO); 144.3 and 153.4. In the IR spectrum, absorptions at 1690 and 2720 cm^{-1} were in agreement with the presence of an α-unsaturated aldehyde.

The data suggested that the alkenyl chain of **7** has been replaced by a botryalyl one in **8**. The EI mass spectrum of the tri-TMSi derivatives of **8** showed fragment ions similar to those observed for the resorcinol and hydroquinol moieties of the tri-TMSi derivatives of **7**; however, no botryalyl fragment bearing an $OSiMe_3$ group could be detected. Nevertheless, two peaks of low intensity at *m/z* 785 and 813 ascribable to two homologous ions comprising the aldehyde function and resulting from the cleavage of the $CH(O-Ar)-CH(OSiMe_3)$ bond, suggested the existence of at least two types of botryalyl moieties of C_{56} and C_{58}. These two chain-lengths were consistent with the botryal distribution noticed for the Austin strain, which ranged from C_{52} to C_{64}, with maxima for C_{56}, C_{58}

Table 4. Selected ^1H and ^{13}C NMR data of **7** and **8** (C_6D_6)

C	δ^1H, multiplicity (J in Hz)*	$\delta^{13}C$†
Alkenylresorcinol (**7** and **8**)		
1, 3		160.9
2	6.77 *t* (1.6)	102.3
4, 6	6.65 *d* (1.6)	109.4
5		145.8
Benzylic	2.58 *t* (7.2)	36.8
ω8, ω11	2.0–2.14	27.7
ω9, ω10	5.52 *t* (5.0)	130.2
Alkylhydroquinol (**7** and **8**)		
1		138.3
2		147.2
3	6.42 *d* (2.7)	97.3
4		153.5
5	6.32 *d* (2.7)	106.2
6		128.9
Benzylic	2.86 *t* (7.4)	n.d.
ω8	n.d.	31.8
ω9	4.25 *dd* (4.8, 5.2)	81.8
ω10	3.77 *dd* (5.2, 5.0)	72.8
ω11	n.d.	33.8
OH (phenol)	5.30	
OMe	3.45 *s*, 3.14 *s*	55.3
Alkene (**7**)		
1	5.01 *ddt* (1.8, 10.2, 1.5) 5.06 *ddt* (1.8, 17, 1.5)	114.5
2	5.81 *ddt* (10.2, 17, 6.7)	139.2
3	2.0–2.14	34.2
ω8	n.d.	31.8
ω9	4.25 *dt* (5.2, 4.8)	81.8
ω10	3.77 *dt* (5.2, 5.0)	72.8
ω11	n.d.	33.8
Botryal (**8**)		
1	9.34 *s*	194
2		144.3
3	5.94 *t* (7.4)	153.5
4	2.31 *dt* (7.4, 7.0)	n.d.
3′	n.d.	24.4
ω8	n.d.	31.8
ω9	4.25 *dt* (5.2, 4.8)	81.8
ω10	3.77 *dt* (5.2, 5.0)	72.8
ω11	n.d.	33.8
ω8′, ω11′	2.0–2.14	27.7
ω9′, ω10′	5.52 *t* (5.0)	130.2

n.d.: Not determined.

*Other methylenic protons δ at 1.2–1.8, terminal methyls δ at 0.85–0.95.

†Other ^{13}C resonances: 32.3 (Me–CH_2–CH_2–), 30.8, 30.2, 30.0, 29.8, 29.6, 29.4, 29.3, 29.0, 27.7, 26.4, 25.9, 23.1 (Me–CH_2) and 14.4 (Me).

and C_{60} [6]. On the basis of these results, it can be considered that series **8** is a mixture of alkenylresorcinol-*O*-[botryalyl]-*O*-[alkylhydroquinol dimethyl ether] diethers.

Due to difficulties in HPLC separation, only a mixture of **9** and **10** was obtained from fraction IV of the Austin strain extract. It showed UV absorptions at 215 and 220 nm. Both the ^1H and ^{13}C NMR spectra exhibited signals typical of **7** and **8**, those of the hydroquinol nucleus excepted (see Experimental). The relative intensities of the ^1H NMR signals showed (i) a higher contribution of terminal unsaturations than of aldehyde functions (7:3, respectively) and (ii) that one resorcinol nucleus was linked to two aliphatic moieties via phenoxy bonds. The presence of two distinct series of compounds was revealed by the FAB mass spectrum. A first series comprising nine $[M + H]^+$ peaks from *m/z* 1160 to *m/z* 1384 with a regular increase of 28 mu, maximizing at *m/z* 1244, was in agreement with the general formula $C_{79+x}H_{146+2x}O_4$, with x even from 0 to 16. The second series exhibited 10 $[M + H]^+$ peaks from *m/z* 1636 to 1888, also with a regular increase of 28 mu (maximum intensity at *m/z* 1776), suggesting the general formula $C_{112+x}H_{208+2x}O_5$, with x even from 0 to 18. On the basis of these data, we consider that the mixture isolated from fraction IV was made up of a series of alkenylresorcinol-di-*O*-alkenyl diethers **9** and of a series of alkenylresorcinol-*O*-[alkenyl]-*O*-[botryalyl] diethers **10**, in a ratio of *ca* 2:3.

Phenoxy ether lipids **9** were detected in the Coat ar Herno extract, by TLC and IR analyses; however, we were unable to separate them from a very complex lipid mixture obtained in low amounts. As outlined earlier [5], the content of the Austin and Coat ar Herno strains in phenoxy ether lipids are relatively low (*ca* 0.4% of dry wt for the former), when compared to that found for a Bolivian strain of *B. braunii* (35% of dry wt), emphasizing once again, the great chemical variability of this algal species.

EXPERIMENTAL

General. CC: silica gel (70–230 mesh). TLC: silica gel 60 PF. IR: CCl_4. NMR: TMS as int. standard; ^1H: 250 MHz; ^{13}C: 62.5 MHz. Acetylation and trimethylsilylation were carried out according to standard procedures.

Extraction and fractionation of lipids. The strains investigated originated from the Culture Collection of Austin, TX (UTEX 572) and from a French freshwater lake: Coat ar Herno in Brittany [7]. Culture conditions, extraction of dry biomass with hexane and fractionation of extracts (2.5 g for the Austin strain and 1.7 g for the Coat ar Herno one) by silica gel CC are reported in refs [2, 3, 6]. Five frs were obtained by elution with hexane (I), hexane–Et_2O (19:1) (II), hexane–Et_2O (23:2) (III), hexane–Et_2O (17:3) (IV) and Et_2O (V); relative percentages of the fractions were Austin I (20.4%), II (37.5), III (20), IV (10.2) and V (11.8), Coat ar Herno I (2.6), II (75.4), III (5.7), IV (3.2) and V (12.0).

Isolation of **1**–**7** *of Coat ar Herno strain.* Series **1** was isolated by prep. silica gel TLC of fr. II as previously reported [6]. Fraction III was resolved by prep. silica gel TLC; elution with hexane–Et_2O (21:4) gave the impure compounds **3**, R_f 0.30 and **6**, R_f 0.62. These were further purified by normal phase HPLC; **3** was eluted with

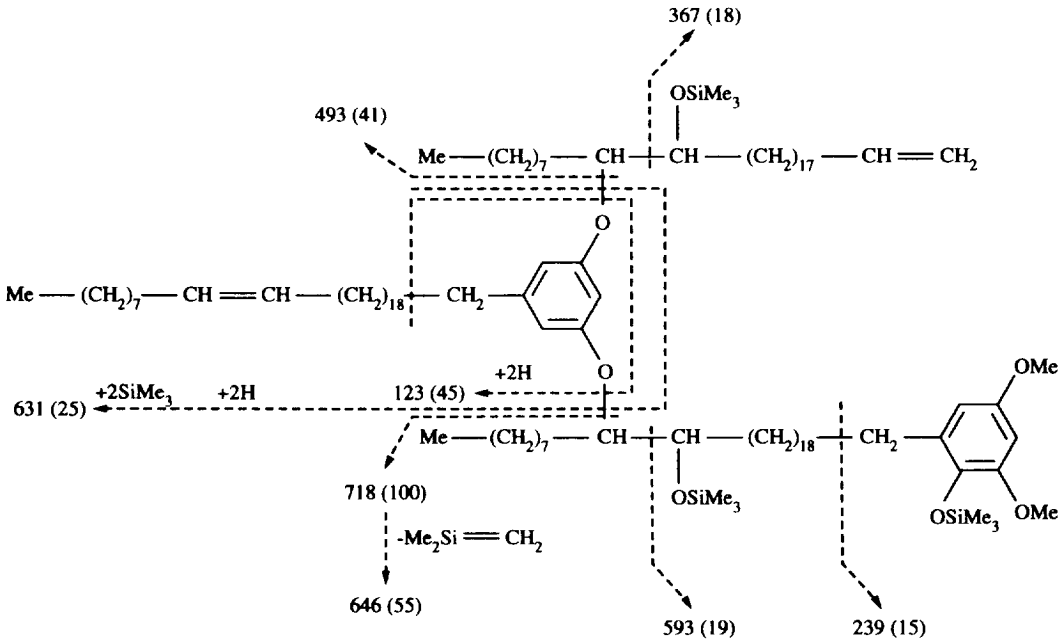
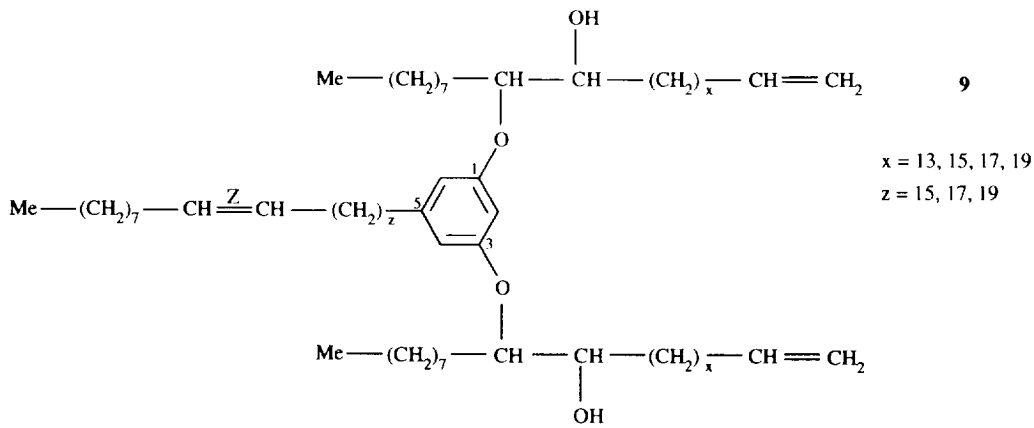
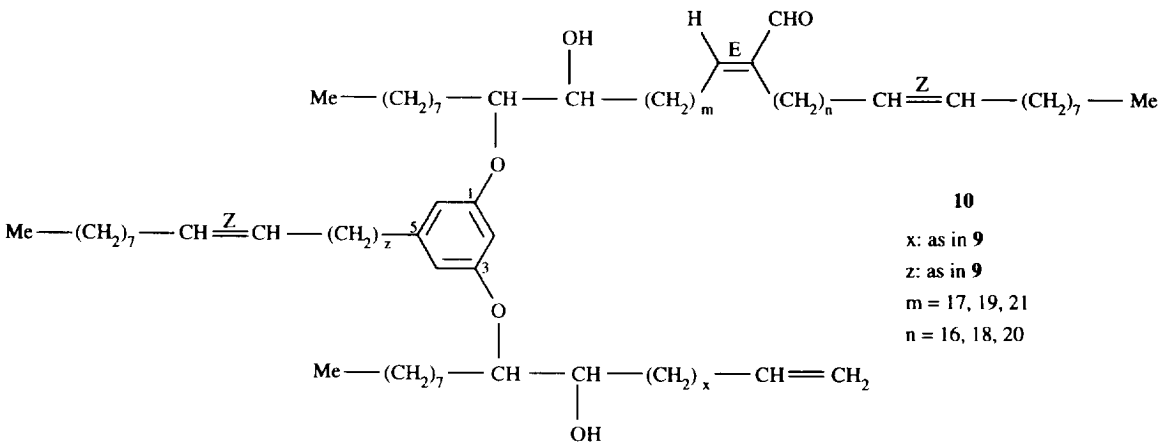


Fig. 6. Structure and EI-MS fragmentation pattern of the tri-TMSi derivative of the predominant phenoxy ether **7a**; *m/z* and (rel. int.).





heptane–Et_2O (10:1), 0.9 ml min^{-1}, R_f: 8 min 45 s, **6** was obtained with heptane–Et_2O (25:1), 1 ml min^{-1}, R_f: 13 min. Purification of fr. IV by TLC and elution with heptane–Et_2O (3:1) gave the impure series **4**, R_f 0.45, further purified by normal phase HPLC; elution with heptane–Et_2O (10:1), 1.5 ml min^{-1}, R_t 8 min. Purification of fr. V by TLC and elution with heptane–Et_2O (11:9) gave **5** R_f 0.38 and **2** R_f 0.31.

Isolation of **7–10** *of the Austin strain.* Elution of fr. IV on TLC with heptane–Et_2O (4:1), gave a mixt. of **9** + **10**, R_f 0.48, unresolved by HPLC. Elution of fr. V on TLC plates with heptane–Et_2O (1:1) afforded a mixt. of **7** and **8**, R_f 0.71. Separation was achieved by repeated normal phase HPLC and elution with hexane–THF (10:1), 1 ml min^{-1}, **7** R_t 9 min, **8** R_t 11 min.

n-*Alkenylhydroquinol dimethyl ethers* **1** [6]. Solid mixt. CI(NH_3)–MS (probe) *m/z* (rel. int. in %) 587 $[C_{39}H_{70}O_3 + H]^+$ (5), 559 $[C_{37}H_{66}O_3 + H]^+$ (100) and 531 $[C_{35}H_{62}O_3 + H]^+$ (72).

n-*Alkyl*-n-*alkenylpyrogallol dimethyl ethers* **2**. Solid mixt. UV $\lambda_{max}^{heptane}$ 215 nm. EI-MS (probe) *m/z* (rel. int.) 167 $[CH_2C_6H_2(OMe)_2OH]^+$ (100), 502 $[C_{33}H_{58}O_3]^+$ (1), 504 $[C_{33}H_{60}O_3]^+$ (1), 530 $[C_{35}H_{62}O_3]^+$ (34), 558 $[C_{37}H_{66}O_3]^+$ (27), 586 $[C_{39}H_{70}O_3]^+$ (1.5). IR ν_{max} 3550, 3000, 2920, 2850, 1610, 1515, 1465, 1365, 1310, 1240, 1210, 1115, 720 cm^{-1}. ^{1}H NMR (C_6D_6): δ 6.35 (2H, *s*), 5.51 (2H, *t*, J = 5.4 Hz), 3.39 (6H, *s*), 2.54 (2H, *t*, J = 7.5 Hz), 2.11 (4H, *t*, J = 5.4 Hz), 1.64 (2H, *m*), 1.34 (*br*), 0.89 (3H, *t*, J = 6.3 Hz). ^{13}C NMR (C_6D_6): δ 147.7 (C-1, C-3), 134.5 (C-2), 133.6 (C-5), 130.2 (olefinic carbons), 106.1 (C-4, C-6), 56.0 (OMe), 36.6 (benzylic carbons), 32.4 (homobenzylic carbons), 32.3 (Me–CH_2–$\underline{C}H_2$–), 30.2, 30.0, 29.8, 29.7, 27.7 (allylic carbons), 23.1 (Me–$\underline{C}H_2$), 14.3 (terminal methyl).

Ozonolysis of acetate derivatives of **2**. Ozonolysis at $-78°$ in CS_2 soln and treatment of the resulting ozonides with triphenylphosphine were as previously reported [10]. After concn under red. pres., the reaction mixt. was directly analysed by EIMS (probe) *m/z* (rel. int.) 167 $[CH_2C_6H_2(OMe)_2OH]^+$ (100), 209 $[CH_2C_6H_2(OMe)_2OAc]^+$ (4), 262 $[(C_6H_5)_3P]^+$, 277 $[(C_6H_5)_2PO - H]^+$, 392 $[C_{26}H_{42}O_5 - CH_2CO]^+$ (7), 420 $[C_{28}H_{46}O_5 - CH_2CO]^+$ (42), 448 $[C_{30}H_{50}O_5 - CH_2CO]^+$ (32), 476 $[C_{32}H_{46}O_5 - CH_2CO]^+$ (3), 504 $[C_{35}H_{62}O_4 - CH_2CO]^+$ (3). Nonanal was detected by GC-EIMS analysis (CPSil-5CB capillary column, 100°). *m/z* (rel. int.) 142 $[M]^+$ (1), 124 $[M-H_2O]^+$ (5), 98 $[M-CH_2CHOH]^+$ (30), 57 (100).

Alkenyl diols, oleyl monoesters **3**. Oily mixt. CI(NH_3)-MS (probe) *m/z* (rel. int.) 703.8 $[C_{47}H_{90}O_3 + H]^+$ (18) (calc. 704.2), 675.8 $[C_{45}H_{86}O_3 + H]^+$ (11) (calc. 676.2), 438 (89), 421 (100), 410 (33), 408 (73), 393 (49), 391 (64), 300 (22), 293 (8), 283 (16), 265 (33). IR ν_{max}: 3600, 3080, 3000, 2920, 2850, 1730, 1640, 1465, 1375, 1170, 990, 910, 720 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ5.81 (1H, *ddt*, J = 17, 10.2, 6.7 Hz), 5.35 (2H, *t*, J = 5.3 Hz), 4.98 (1H, *ddt*, J = 17, 1.7, 1.6 Hz), 4.92 (1H, *dd t*, J = 10.2, 1.7, 1.6 Hz), 4.84 (1H, *dt*, J = 4.1, 6.4 Hz), 3.58 (1H, *m*), 2.33 (2H, *t*, J = 6.5 Hz), 2.02 (6H, *m*), 1.6 (6H, *m*), 1.26 (*br*), 0.88 (6H, *t*, J = 6.2 Hz). ^{13}C NMR ($CDCl_3$): δ173.7, 139.3, 129.9, 129.7, 114.1, 76.3, 72.6, 34.6, 33.9, 31.9, 30.7, 29.7, 29.3, 29.2, 29.0, 27.2, 25.6, 25.4, 25.2, 22.7, 14.1.

Botryalyl diols, oleyl monoesters **4**. Oily mixt. FAB-MS *m/z* (rel. int.) 1160.3 $[C_{80}H_{152}O_4 - H_2O]^+$ (17) (calc. 1161.0), 1132.6 $[C_{78}H_{148}O_4 - H_2O]^+$ (100) (calc. 1133.0), 1104.6 $[C_{76}H_{144}O_4 - H_2O]^+$ (39) (calc. 1105.0) and 1076.5 $[C_{74}H_{140}O_4 - H_2O]^+$ (14) (calc. 1077.0). EI-MS (probe) *m/z* (rel. int.) 895 (<1), 867 (<1), 839 (<1), 811 (<1), 265 (16), 141 (22), 55 (100). IR ν_{max}: 3600, 3000, 2920, 2850, 2720, 1730, 1690, 1640, 1465, 1375, 1170, 720 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ9.36 (1H, *s*), 6.44 (1H, *t*, J = 7.4 Hz), 5.35 (4H, *t*, J = 5.2 Hz), 4.84 (1H, *dt*, J = 4.1, 6.4 Hz), 3.58 (1H, *m*), 2.33 (4H, overlapping), 2.22 (2H, *t*, J = 7.5 Hz), 2.01 (8H, *t*, J = 5.2 Hz), 1.60 (6H overlapping), 1.26 (*br*), 0.85 and 0.88 (terminal methyls). ^{13}C NMR ($CDCl_3$): δ195.3, 173.7, 155.3, 143.9, 130.0, 129.9, 129.7, 76.3, 72.6, 34.6, 33.9, 31.9, 30.8, 29.7, 29.4, 29.2, 29.0, 28.8, 27.2, 25.6, 25.4, 25.2, 24.1, 22.7, 14.1.

Di-O-alkadienyl ether **5**. Oil. EIMS (probe) *m/z* (rel. int.) 798 (0.5), 391 (8), 374 (6), 265 (4), 262 (6), 141 (19), 137 (3), 123 (11), 109 (19), 95 (28), 81 (43), 69 (45), 67 (43), 57 (64), 55 (100). IR ν_{max} 3580, 3070, 3000, 2920, 2850, 1640, 1465, 1370, 1065, 990, 910 and 720 cm^{-1}. ^{1}H NMR (C_6D_6): δ5.80 (2H, *ddt*, J = 17, 10.3, 6.6 Hz), 5.62 (2H, *dt*, J = 11.1, 7.2 Hz), 5.30 (2H, *dd*, J = 9.8, 11.1 Hz), 5.03 (2H, *ddt*, J = 17, 2.2, 1.5 Hz), 4.98 (2H, *ddt*, J = 10.3, 2.2, 1.5 Hz), 4.17 (2H, *dd*, J = 9.5, 9.8 Hz), 3.63 (2H, *m*), 2.90 (hydroxyl protons), 2.06 (4H, *m*), 2.01 (4H, *m*), 1.58 (4H, *m*), 1.32 (*br*), 0.91 (6H, *t*, J = 6.9 Hz). ^{13}C NMR (C_6D_6): δ139.2, 135.8, 128.4, 114.5, 76.7, 73.9, 34.2, 33.2, 32.2, 30.3, 30.2, 30.1, 30.0, 29.6, 29.4, 28.9, 26.4, 23.0, 14.3.

Di-O-alkadienyl ethers **6**. Oily mixt. FAB-MS *m/z* 1266.4 $[C_{86}H_{162}O_4 + Li]^+$ (calc. 1267.1), 1238.4 $[C_{84}H_{158}O_4 + Li]^+$ (calc. 1239.0), 1212.2 $[C_{82}H_{156}O_4 + Li]^+$ (calc. 1213.0), 1210.3 $[C_{82}H_{154}O_4 + Li]^+$ (calc. 1211.0), 1184.1 $[C_{80}H_{152}O_4 + Li]^+$ (calc. 1185.0), 1156.1 $[C_{78}H_{148}O_4 + Li]^+$ (calc. 1156.9), 1070.1 $[C_{72}H_{134}O_4 + Li]^+$ (calc. 1070.7), 1042.0 $[C_{70}H_{130}O_4 + Li]^+$ (calc. 1042.7). IR ν_{max} 3580, 3080, 3000, 2920, 2850, 1730, 1640, 1465, 1375, 1170, 1070, 990, 910, 720 cm^{-1}. ^{1}H NMR ($CDCl_3$): δ5.81 (2H, *ddt*, J = 17.1, 10.1, 6.7 Hz), 5.69 (1H, *dt*, J = 11.8, 6.8 Hz), 5.68 (1H, *dt*, J = 11.2, 6.8 Hz), 5.35 (2H, *t*, J = 4.6 Hz), 5.23 (1H, *dd*, J = 9.6, 11.2 Hz), 5.15 (1H, *dd*, J = 9.5, 11.8 Hz), 4.99 (2H, *ddt*, J = 17.1, 2.2, 1.6 Hz), 4.93 (2H, *ddt*, J = 10.1, 2.2, 1.6 Hz), 4.90 (1H, *m*), 4.18 (1H, *dd*, J = 5.7, 9.6 Hz), 3.90 (1H, *dd*, J = 9.5, 7.5 Hz), 3.38 (1H, *m*), 2.29 (2H, *t*, J = 7.3 Hz), 2.0 (12H), 1.60 (4H), 1.26 (*br*), *ca* 0.88 (overlapping Me). ^{13}C NMR ($CDCl_3$): δ173.5, 139.3, 136.0, 135.8, 129.9, 127.5, 127.0, 114.1, 75.7, 75.3, 73.7, 72.6, 34.6, 33.9, 32.5, 31.9, 29.8, 29.4, 29.3, 29.0, 28.4, 27.3, 25.8, 25.5, 25.2, 22.7, 14.1.

Alkaline hydrolysis of **6**. A portion of **6** (10 mg) was saponified in 15 ml THF with 0.25 g KOH dissolved in 3 ml MeOH for 1 hr under reflux. After cooling, the reaction mixt. was acidified with 10% aq. HCl, extracted with Et_2O and then washed with H_2O. The crude product obtained by evapn of solvent was esterified with CH_2N_2 in Et_2O, and then chromatographed on silica gel CC. Elution with heptane–Et_2O (19:1) yielded the Me

esters; they were identified by GC-EIMS (fused silica CPSil-5CB, temp. prog. from 170° to 260° at 5° min^{-1}). Elution with Et_2O gave an oily product exhibiting similar R_f on TLC and spectroscopic properties to **5**.

Alkenylresorcinol-O-[alkenyl]-O-alkylhydroquinol] diethers **7**. Solid mixt. FAB-MS *m/z* 1567.7 $[C_{105}H_{192}O_7 + H]^+$ (calc. 1567.7), 1538.9 $[C_{103}H_{188}O_7 + H]^+$ (calc. 1539.6), 1511.7 $[C_{101}H_{184}O_7 + H]^+$ (calc. 1511.6), 1483.4 $[C_{99}H_{180}O_7 + H]^+$ (calc. 1483.5), 1455.4 $[C_{97}H_{176}O_7 + H]^+$ (calc. 1455.5), 1427.9 $[C_{95}H_{172}O_7 + H]^+$ (calc. 1427.4). IR ν_{max} 3600, 3560, 3080, 3000, 2920, 2850, 1640, 1590, 1500, 1465, 1375, 1260, 1225, 1200, 1150, 1050, 990, 910, 720 cm^{-1}. ^1H and ^{13}C NMR (C_6D_6): Table 5.

Alkenylresorcinol-O-[botryalyl]-O-[alkylhydroquinol] diethers **8**. Solid mixt. FAB-MS *m/z* 1986.2 $[C_{134}H_{246}O_8 + H]^+$ (calc. 1986.4), 1957.9 $[C_{132}H_{242}O_8 + H]^+$ (calc. 1958.4), 1930.4 $[C_{130}H_{238}O_8 + H]^+$ (calc. 1930.3), 1902.5 $[C_{128}H_{234}O_8 + H]^+$ (calc. 1902.3), 1873.9 $[C_{126}H_{230}O_8 + H]^+$ (calc. 1874.2), 1846.6 $[C_{124}H_{226}O_8 + H]^+$ (calc. 1846.2). IR ν_{max} 3600, 3560, 3000, 2920, 2850, 2720, 1690, 1640, 1590, 1500, 1465, 1375, 1260, 1225, 1200, 1150, 1050, 720 cm^{-1}. ^1H and ^{13}C NMR (C_6D_6): Table 5.

Tri-TMSi derivatives of **7**. EI-MS *m/z* (rel. int.) 746 (17), 718 (100), 690 (5), 659 (19), 646 (55), 631 (25), 603 (4), 593 (19), 521 (16), 493 (41), 465 (10), 167 $[CH_2C_6H_2(OMe)_2OH]^+$ (21), 123 $[CH_2C_6H_3(OH)_2]^+$ (42).

Tri-TMSi derivatives of **8**. EI-MS *m/z* (rel. int.) 813 (7), 785 (16), 746 (15), 718 (100), 690 (6), 659 (17), 646 (50), 631 (26), 603 (4), 593 (17), 167 (20), 123 (40).

Ozonolysis of **7**. Treatment of a CH_2Cl_2 soln of **8** (5 mg), decomposition of the ozonides by refluxing with H_2O_2–HCO_2H, extraction with Et_2O and esterification by CH_2N_2 were performed as previously described [6]. Me nonanoate was identified by CG-EIMS (CPSil-5CB, isoth. 170°) and comparison with an authentic standard.

Alkenylresorcinol diethers **9** *and* **10**. Oily mixt. UV $\lambda_{max}^{heptane}$ 203, 214 and 225 nm. FAB-MS of **9** *m/z* 1384.7 $[C_{95}H_{178}O_4 + H]^+$ (calc. 1385.4), 1356.7 $[C_{93}H_{174}O_4 + H]^+$ (calc. 1357.4), 1328.7 $[C_{91}H_{170}O_4 + H]^+$ (calc. 1329.3), 1300.7 $[C_{89}H_{166}O_4 + H]^+$ (calc. 1301.3), 1272.7 $[C_{87}H_{162}O_4 + H]^+$ (calc. 1273.2), 1244.7 $[C_{85}H_{185}O_4 + H]^+$ (calc. 1245.2), 1216.6 $[C_{83}H_{154}O_4 + H]^+$ (calc. 1217.4), 1188.6 $[C_{81}H_{150}O_4 + H]^+$ (calc. 1189.1), 1160.6 $[C_{79}H_{146}O_4 + H]^+$ (calc. 1161.0), FAB-MS of **10** *m/z* 1887.2 $[C_{130}H_{244}O_5 + H]^+$ (calc. 1888.4), 1859.3 $[C_{128}H_{240}O_5 + H]^+$ (calc. 1860.3), 1831.3 $[C_{126}H_{236}O_5 + H]^+$ (calc. 1832.2), 1803.2 $[C_{124}H_{232}O_5 + H]^+$ (calc. 1604.2), 1775.2 $[C_{122}H_{228}O_5 + H]^+$ (calc. 1775.4), 1747.2 $[C_{120}H_{224}O_5 + H]^+$ (calc. 1748.1), 1720.1 $[C_{118}H_{220}O_5 + H]^+$ (calc. 1720.1), 1692.1 $[C_{116}H_{216}O_5 + H]^+$ (calc. 1692.0), 1665.0 $[C_{114}H_{212}O_5 + H]^+$ (calc. 1663.9), 1636.0 $[C_{112}H_{208}O_5 + H]^+$ (calc. 1635.8). IR ν_{max} of **10** + **11**: 3595, 3080, 3000, 2920, 2850, 2720, 1690, 1640, 1590, 1465, 1375, 1145, 1050, 990, 910, 720 cm^{-1}. ^1H NMR (C_6D_6): δ9.33 (*s*), 6.73 (*t*, J = 1.8 Hz), 6.61 (*d*, J = 1.8 Hz), 5.93 (*t*, J = 7.8 Hz), 5.79 (*ddt*, J = 17, 10.2, 6.7 Hz), 5.48 (*t*, J = 4.6 Hz), 5.06 (*ddt*, J = 17, 1.8, 1.5 Hz), 5.01 (*ddt*, J = 10.2, 1.8, 1.5 Hz), 4.24 (*dt*, J = 5.2, 4.8 Hz), 3.77 (*dt*, J = 5.2, 5.0 Hz), 2.58 (*t*, J = 7.5 Hz), 2.32 (*dt*, J = 7.8, 5.0 Hz), broad peaks from 2.0 to 2.13 and from 1.2 to 1.8, 0.9 (overlapping methyls). ^{13}C NMR (C_6D_6): δ194.0, 160.9, 153.4, 145.8, 144.3, 139.2, 130.2, 114.5, 109.4, 102.3, 81.8, 72.8, 36.8, 34.2, 33.8, 31.8, 30.8, 30.2, 29.8, 29.6, 29.4, 29.3, 29.0, 27.7, 26.4, 25.9, 24.4, 23.1, 14.4.

REFERENCES

1. Metzger, P., Largeau, C. and Casadevall, E. (1991) in *Progress in the Chemistry of Organic Natural Products* (Herz, W., Grisebach, H., Kirby, G. W. and Tann, C., eds), Vol. 57, p. 1. Springer, Wien.
2. Metzger, P. and Casadevall, E. (1991) *Phytochemistry* **30**, 1439.
3. Metzger, P. and Casadevall, E. (1992) *Phytochemistry* **31**, 2341.
4. Metzger, P. (1994) *Phytochemistry* **36**, 195.
5. Metzger, P. and Largeau, C. (1994) *Org. Geochem.* **22**, 801.
6. Metzger, P. and Casadevall, E. (1989) *Phytochemistry* **28**, 2097.
7. Metzger, P., Villarreal-Rosales, E., Casadevall, E. and Couté, A. (1989) *Phytochemistry* **28**, 2349.
8. Croteau, R. and Kolattukudy, P. E. (1974) *Arch. Biochem. Biophys.* **162**, 458.
9. Villarreal-Rosales, E., Metzger, P. and Casadevall, E. (1992) *Phytochemistry* **31**, 3021.
10. Barr, J. R., Scannell, R. T. and Yamaguchi, K. (1989) *J. Org. Chem.* **54**, 494.