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A comparative study of macromolecular substances of a Coorongite and cell walls of the extant alga *Botryococcus braunii*

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Abstract—A Coorongite sample of Lake Balkash (Kazakhstan, CIS) was analyzed in detail by ^{13}C-NMR, FTIR, Curie point pyrolysis—gas chromatography—mass spectrometry, and by fractionation and derivatization with dimethyldisulphide of an off-line pyrolysate. Both the spectroscopic and the pyrolysis data indicate that the Coorongite was derived almost entirely of organic matter of the green microalga *Botryococcus braunii* race A. Homologous series of *n*-alkanes and *n*-alk-1-enes in all pyrolysates indicated the presence of algaenan, a highly aliphatic and resistant cell wall biomacromolecule of *B. braunii* race A. Highly specific pyrolysis products, in particular *n*-alkadienes, *n*-alkatrienes, alk-1-en-ω^9-ones, and alk-1-en-ω^{10}-ones with C_{27}, C_{29}, and C_{31} carbon atoms clearly indicated that C_{27}, C_{29}, and C_{31} alkadienes and alkatrienes, originally present in *B. braunii* race A as such, were cross-linked by oxygen during the very early stages of diagenesis under oxic conditions. Furthermore, several types of dialkenylethers, also present as soluble lipids in *B. braunii* race A, had undergone cross-linking by oxygen as well. These cross-linked lipids contribute significantly to the Coorongite and clearly demonstrate that under specific conditions kerogen consists of both preserved biomacromolecules and insoluble, cross-linked, low-molecular-weight lipids.

INTRODUCTION

COORONGITES ARE BLACK ELASTIC organic substances which mainly occur as sheet-like deposits around lagoons or as residues in dried-up basins of ephemeral lakes (WAKE and HILLEN, 1980; DUBREUIL et al., 1989). Upon destructive distillation they yield high amounts of highly aliphatic oils. These deposits are related to blooms of the colonial green alga *Botryococcus braunii* (CANE, 1977; WAKE and HILLEN, 1980; MCKIRDY, 1985; DUBREUIL et al., 1989). The algal biomass produced accumulates at the water surface and is pushed by wind to the shorelines where it deposits and forms a rubbery material. The mechanism of Coorongite formation from the parent alga *B. braunii* has been successively explained by oxidative polymerization of different lipid constituents: polyenic fatty acids (CANE, 1969), botryococcenes (DOUGLAS et al., 1969), and non-isoprenoid unsaturated hydrocarbons (CANE and ALBION, 1973). GLIKSON (1983, 1984) and MCKIRDY (1977) proposed that a large contribution of bacterial products could alternatively lead to the formation of Coorongite. It was also suggested that cell walls of *B. braunii* are major sources of Coorongites (CANE, 1977; WAKE and HILLEN, 1980; MCKIRDY, 1985). Direct evidence of such an origin was obtained from analyses of the Coorongite collected on the shores of the Darwin River Reservoir, Australia. Comparisons with samples of *B. braunii* race B* blooming in this reservoir indicated that ca. 70% of Darwin Coorongite is composed of selectively preserved macromolecular material building up the outer walls of this microalga (DUBREUIL et al., 1989). This macromolecular material of race B outer walls is insoluble and nonhydrolysable (KADOURI et al., 1988) and appears to be highly resistant against bacterial degradation thus surviving diagenesis under suboxic conditions. These kind of similarities between various kerogens and highly resistant biomacromolecules occurring in the outer walls of *B. braunii* (LARGEAU et al., 1984, 1986, 1991; DOUGLAS et al., 1991; DERENNE et al., 1992a), the walls of another green alga *Tetraedron minimum* (GOTH et al., 1988), and several higher plant tissues (DE LEEUW et al., 1991) have been noticed before. These observations led us to propose an alternative pathway of kerogen formation (TEGELAAR et al., 1989; DE LEEUW and LARGEAU, 1993) which differs substantially from the classical degradation-recondensation pathway (DURAND, 1980; TISSOT and WELTE, 1984). According to this newly proposed pathway, highly resistant biomacromolecules present as such in various types of organisms are selectively preserved during sedimentation and diagenesis and thus accumulate in the kerogen fraction. The general nature of this "selective preservation pathway" was recently strengthened by investigations indicating that a number of "amorphous" kerogens appeared to consist of recognizable structures when inspected by transmission electron microscopy (RAYNAUD et al., 1988, 1989) and that the most common of these structures, so called ultralaminae, originate from the selectively preserved very thin outer walls present in a number of microalgae (LARGEAU et al., 1990; DERENNE et al., 1991, 1992a, 1992b, 1992c).

Detailed chemical investigations of *B. braunii* have led to suggestions for the structures of the highly aliphatic biomac-

* Three distinct chemical races of *B. braunii* are so far identified (METZGER et al., 1985, 1990). They exhibit the same general morphology but are characterized by the different nature of their hydrocarbons: C_{25} to C_{31}, odd-carbon-numbered, *n*-alkadienes and -trienes in the A race; C_{30} to C_{37} isoprenoid hydrocarbons (botryococcenes) in the B race; a lycopadiene in the L race.

romolecules, algaenans, present in their cell walls (METZGER et al., 1991). It is suggested that they, depending on the race, consist of long linear (races A and B) or isoprenoid (race L) alkyl chains possibly linked via ether bonds (LARGEAU et al., 1986; KADOURI et al., 1988; DERENNE et al., 1990, 1991; TEMPLIER et al., 1992).

The aim of the present work is to investigate chemical relationships between a Coorongite deposited on the shorelines of Lake Balkash (Kazakhstan) and *B. braunii* derived organic matter and to trace early stage diagenetic changes of the algaenan and lipids of this alga under partly oxic conditions.

EXPERIMENTAL

Sample Description

The Balkashite sample was collected in 1981 on the shores of the southern extensions (Ala Kool), of Lake Balkash (Turkestan, Kazakhstan, CIS) and stored at room temperature until analysis.

Elemental Composition

Elemental analyses were carried out on extracted Balkashite samples by the Service Central de Microanalyse du CNRS, Vernaison, France.

Spectroscopic Methods

FTIR spectra were obtained from KBr pellets using a Nicolet 7000 spectrometer. Solid state ^{13}C NMR spectra of powdered and extracted Balkashite were recorded with a Bruker MSL 400 spectrometer operated at 100 MHz for ^{13}C, using high power dipolar decoupling, cross polarization sequence (contact time 1 ms), and magic angle spinning (spinning rate 4000 Hz). 3000 scans with a cycle time of 5 s were summed.

Extraction and Fractionation

The Balkashite sample (1.4 g) was powdered in a mortar and subsequently extracted ultrasonically using hexane, chloroform, and chloroform/methanol (1/1, v/v; ×3). The total extract (62 mg, ca. 4.4 wt%) was fractionated into an apolar and a polar fraction by column chromatography (Fig. 1). The column (10 cm × 10 mm) was packed with activated (1 h at 150°C) alumina and prewashed with 20 mL MeOH and 20 mL dichloromethane. The apolar fraction (2.8 mg, ca. 4.5% of the total extract) was obtained by elution with 3.5 column volumes of hexane/dichloromethane (9/1, v/v). The far greater part of the extract remained on top of the column, even after successive elutions with chloroform and methanol.

The cultured *B. braunii* samples (Austin strain) were extracted and the residues were saponified and subsequently acid-treated to obtain the resistant cell wall biomacromolecules (LARGEAU et al., 1986).

DMDS Derivatisation

The procedure followed was modified after CARLSON et al. (1989). Aliquots of the appropriate TLC fractions obtained from the off-line pyrolysate (see Fig. 1) were dissolved in 100–200 μL hexane and reacted with 100–200 μL dimethyl disulfide (DMDS) and 20–40 μL iodine solution (60 mg of iodine in 1 mL diethyl ether). Reaction mixtures were stirred at 50°C for 48 h. Then they were diluted with small amounts of hexane. 200 μL of sodium thiosulfate (5% in water) was added and the hexane layer was pipetted off. This procedure was repeated three times after addition of small amounts of hexane.

Authentic Standards

Nonadecan-9-one was synthesized by a Grignard reaction of decane magnesium bromide and nonanal. Subsequent oxidation (pyridine/dichromate) of the secondary alcohol yielded the corresponding ketone. Nonadecan-10-one is commercially available (Aldrich).

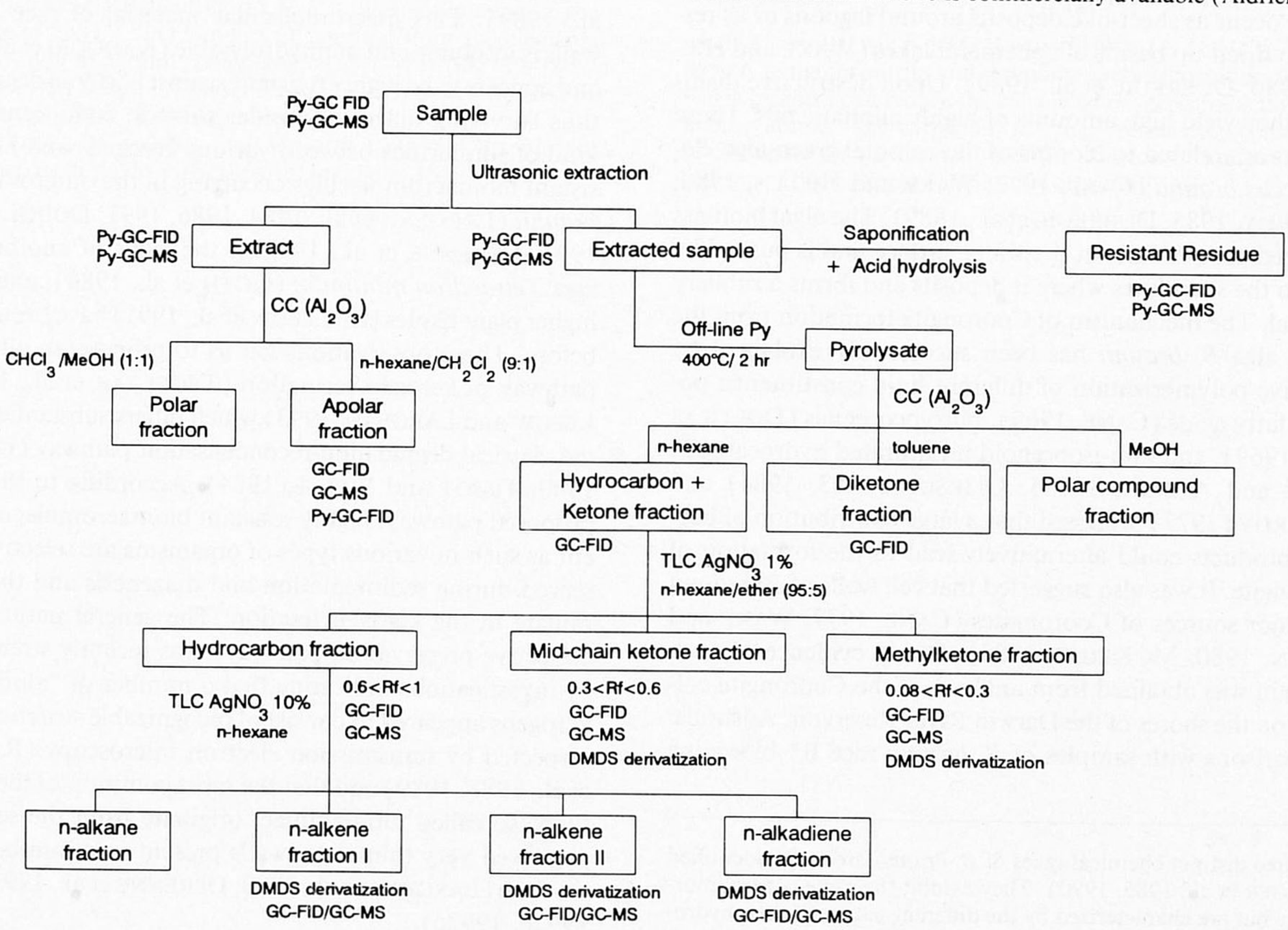


FIG. 1. Scheme of fractionation.

Pyrolysis Methods

Curie point pyrolysis-gas chromatography (Py-GC) as well as Curie point pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) were performed with both the extracted and nonextracted Balkashite, the total extract, and with the residues obtained after base and acid treatments of the cultured algae. The samples were pressed on to flattened ferromagnetic wires with a Curie temperature of 610°C and pyrolysis was performed by inductive heating of the sample-coated wires to their Curie temperatures in 0.15 s. They were held at this temperature for 10 s. A Curie point high frequency generator (Fischer 9425) was used to induce the magnetic field. The gas chromatograph (Hewlett Packard HP-5890) was equipped with a cryogenic unit and programmed from 0°C (5 min) to 300°C (10 min) at a rate of 3°C/min. Separation of the products was achieved by a 25 m fused silica capillary column coated with chemically bound CP-Sil 5 (0.32 mm I.D.; film thickness 0.45 μm). Helium was used as carrier gas. The temperature of the flame ionisation detector was 320°C. Curie point pyrolysis-gas chromatography-mass spectrometry was performed with the same pyrolysis and GC-conditions. The column was directly inserted into the EI ion source of a VG-70SE double focusing mass spectrometer (mass range m/z 40–800; cycle time 1.8 s; ionisation energy 70 eV).

Off-line pyrolysis was performed with 202 mg Balkashite by heating it in a glass tube positioned in a cylindrical oven at a temperature of 400°C under a nitrogen flow for 2 h. The volatile products generated were trapped in two successive cold traps (cooled with ice and solid CO_2/ethanol, respectively) containing pentane/dichloromethane (95/5, v/v). The off-line pyrolysate was fractionated by column chromatography (Al_2O_3). The extract was separated into three fractions by elution with 3.5 column volumes (35 mL of hexane (yield: 69 mg), toluene (yield: 15 mg), and methanol (yield: 33 mg)). An aliquot of the hexane fraction was further separated by thin layer chromatography (TLC) on silica plates impregnated with 1% $AgNO_3$ using hexane/diethylether (95/5, v/v) as developer. Three fractions were thus obtained. The first fraction (R_f = 0.6–1.0) was further separated by TLC on silica plates (10% $AgNO_3$ impregnated) into four subsequent fractions using n-hexane as a developer.

Gas Chromatography

Gas chromatography was performed with a Carlo Erba 4160 instrument equipped with a flame ionization detector (FID) and an on-column injector. A fused silica capillary column (25 m × 0.32 mm) coated with CP Sil-5 (film thickness 0.13 μm) was used with helium as carrier gas. Samples were injected at 70°C. The temperature of the oven was programmed from 70°C to 320°C at 4°C/min.

Gas Chromatography-Mass Spectrometry

GC-MS was performed with the same instrumentation as described for Py-GC-MS. A fused silica capillary column (25 m × 0.32 mm) coated with CP-Sil-5 (film thickness 0.2 μm) was used with helium as carrier gas.

RESULTS

The elemental composition (Table 1) shows that the extracted Balkashite sample almost entirely consists of organic matter (ash content ca. 2%). The high atomic H/C ratio indicates that this sample is highly aliphatic in nature and may be a Type I kerogen precursor. The O/C ratio is relatively high for a Type I kerogen and may be due to the oxic conditions during the deposition of this material. The elemental composition is highly comparable with that of a Darwin Coorongite (DUBREUIL et al., 1989) and with those of other coorongites of various origins (BROOKS and SMITH, 1967; MARCHAND et al., 1969).

The FTIR and solid state ^{13}C NMR spectra obtained from the extracted Balkashite show the same general features as those obtained from the Darwin Coorongite (DUBREUIL et al., 1989): (1) an abundance of long chain aliphatic moieties; (2) a very low content of methyl groups; (3) presence of hydroxyl, ether, carbonyl, and carboxylic functional groups; and (4) a low contribution of aromatic moieties. The spectroscopic data are also similar to those of algaenans derived from cultured *B. braunii* race A and race B (BERKALOFF et al., 1983; KADOURI et al., 1988). From these spectroscopic data it is clear that Balkashite is not derived from *B. braunii* race L algaenan, because the latter is characterized by abundant methyl groups from the lycopadiene building blocks (METZGER and CASADEVALL, 1987; DERENNE et al., 1989, 1990).

Curie point pyrolysis-gas chromatography (Py-GC) and Curie point pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) of both the extracted and nonextracted Balkashite sample (Fig. 2a and b) indicated that their pyrolysates are similar and consist of very complex mixtures of compounds. Relative retention times, mass chromatography, and individual mass spectra revealed the presence of several suites of pyrolysis compounds. Homologous series of n-alkanes and n-alk-1-enes as well as series of n-alkan-2-ones represent a large fraction of both pyrolysates. Series of n-α,ω-alkadienes are also present.

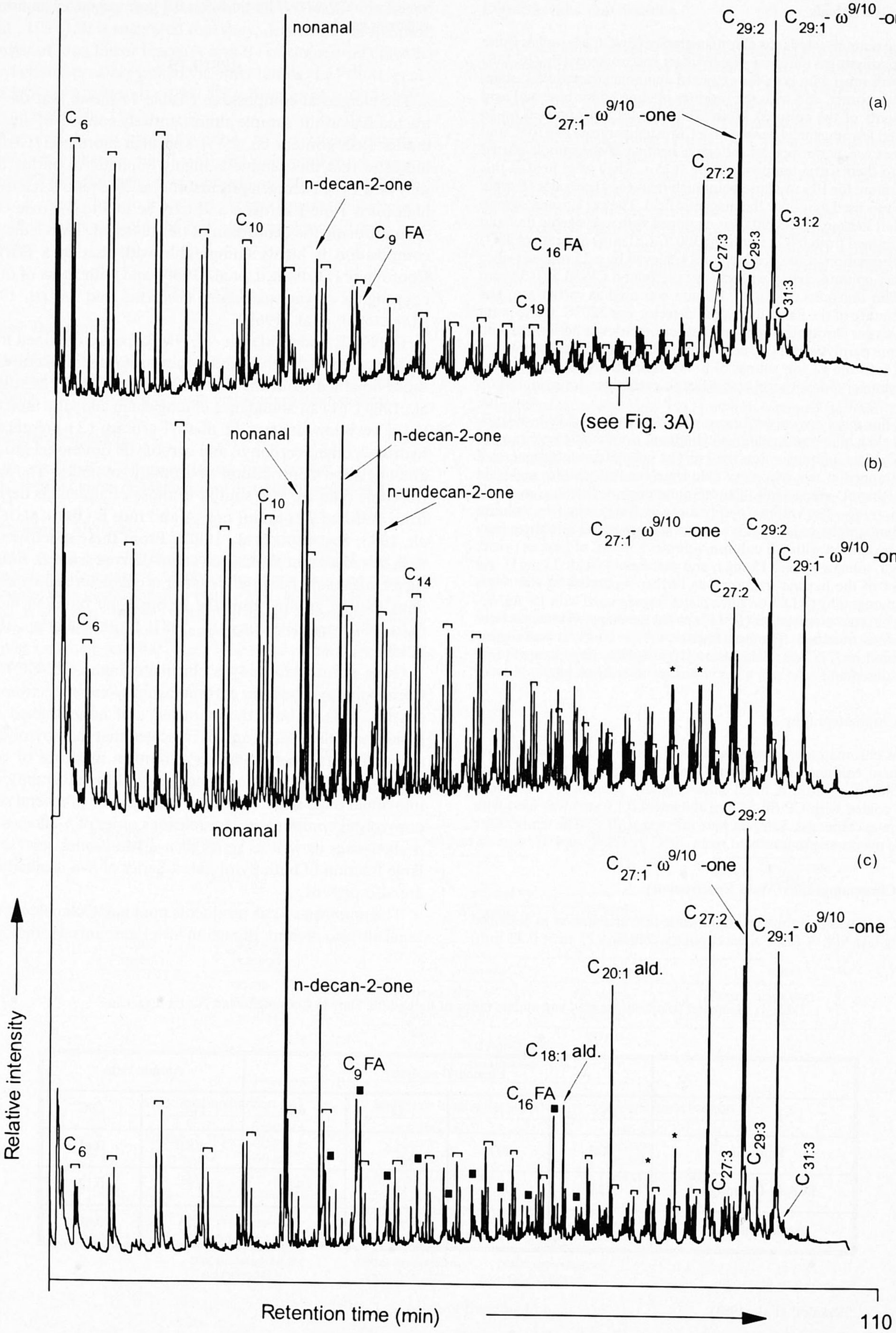
The major pyrolysis product is nonanal. Octanal and heptanal are also present, though in lower amounts. Homologous

Table 1: Elemental compositions (%) and atomic ratios of Balkashite, Darwin Coorongite and Austin algaenan.

	Elemental analysis				Atomic ratio	
	C	H	O	ash	H/C	O/C
Balkashite	70.3	10.8	13.6	2.1	1.84	0.14
Darwin Coorongite[a]	73.2	11.2	11.6	3.5	1.83	0.12
Austin algaenan[b]	71.35	10.3	8.8	8	1.73	0.09

[a]Dubreuil et al. (1989)

[b]Berkaloff et al. (1983)


nonanal
n-decan-2-one
C6
C10
C9 FA
C16 FA
C19
C27:1- ω9/10 -one
C29:2
C29:1- ω9/10 -one
C27:2
C31:2
C27:3
C29:3
C31:3
(see Fig. 3A)
(a)
nonanal
n-decan-2-one
n-undecan-2-one
C10
C14
C6
(b)
C27:1- ω9/10 -one
C29:2
C29:1- ω9/10 -one
C27:2
nonanal
C29:2
C27:1- ω9/10 -one
(c)
C27:2
C29:1- ω9/10 -one
C20:1 ald.
n-decan-2-one
C18:1 ald.
C9 FA
C16 FA
C6
C27:3
C29:3
C31:3
Relative intensity
Retention time (min)
110

series of *n*-alkenes and *n*-alkadienes with a nonterminal double bond position and *n*-alkatrienes as well as linear mid-chain ketones all maximizing at C_{27}, C_{29}, and C_{31} significantly contribute to both pyrolysates, although they are significantly less abundant in the extracted Balkashite (cf. Fig. 2a and b). Alkylbenzenes, alkylcyclohexanes, alkylnaphthalenes, fatty acids, alkylindanes, and alkylindenes are present in lower amounts. A series of alkylphenols was detected in trace amounts.

Gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) of the apolar fraction of the extract representing 0.2 wt% of the Balkashite sample revealed that only a very small part consisted of GC-amenable components (mainly *n*-alkanes). This is attributed to the presence of relatively abundant high-molecular-weight, apolar lipids described to occur in *B. braunii* (for a review, see METZGER et al., 1991). This was confirmed by flash pyrolysis of the apolar fraction of the extract. The composition of this pyrolysate was similar to that of the total extract (Fig. 2c). The pyrolysate consisted of series of pyrolysis compounds probably derived from such high-molecular-weight lipids. Because these high-molecular-weight lipids are considered as precursor molecules of algaenan in *B. braunii* race A (METZGER et al., 1991; TEMPLIER et al., 1992) the similarity of the pyrolysate of the total extract with the pyrolysates of the extracted and nonextracted Balkashite can be rationalized if it is assumed that the Balkashite originates from race A. It is of interest to note that in the pyrolysate of the extract relatively abundant monounsaturated aldehydes are present and that the C_{27} and C_{29} *n*-alkadienes and mid-chain ketones are more abundant than in the pyrolysates of the extracted and nonextracted Balkashite samples (Fig. 2).

The presence of large numbers of homologous series of compounds in the pyrolysates leads to "clusters" in the FID chromatograms and Total Ion Current (TIC) traces. A typical cluster is shown in detail in Fig. 3a and is composed of C_{23} hydrocarbons and C_{21} alkanones. The other clusters are essentially composed of the same suites of compounds although the different distributions of the various homologous series result in differences in the abundances of compound classes relative to each other. This is especially apparent in the clusters of peaks representing the C_{27} hydrocarbons and C_{25} alkanones, the C_{29} hydrocarbons and the C_{27} alkanones, and the C_{31} hydrocarbons and C_{29} alkanones.

A more detailed discussion of the series of compounds present in the pyrolysate, their distribution patterns and the location of functional groups within these compounds is given hereafter.

Aliphatic Hydrocarbons

The mass chromatogram of *m/z* 57 of the pyrolysate of the extracted Balkashite reveals an *n*-alkane distribution

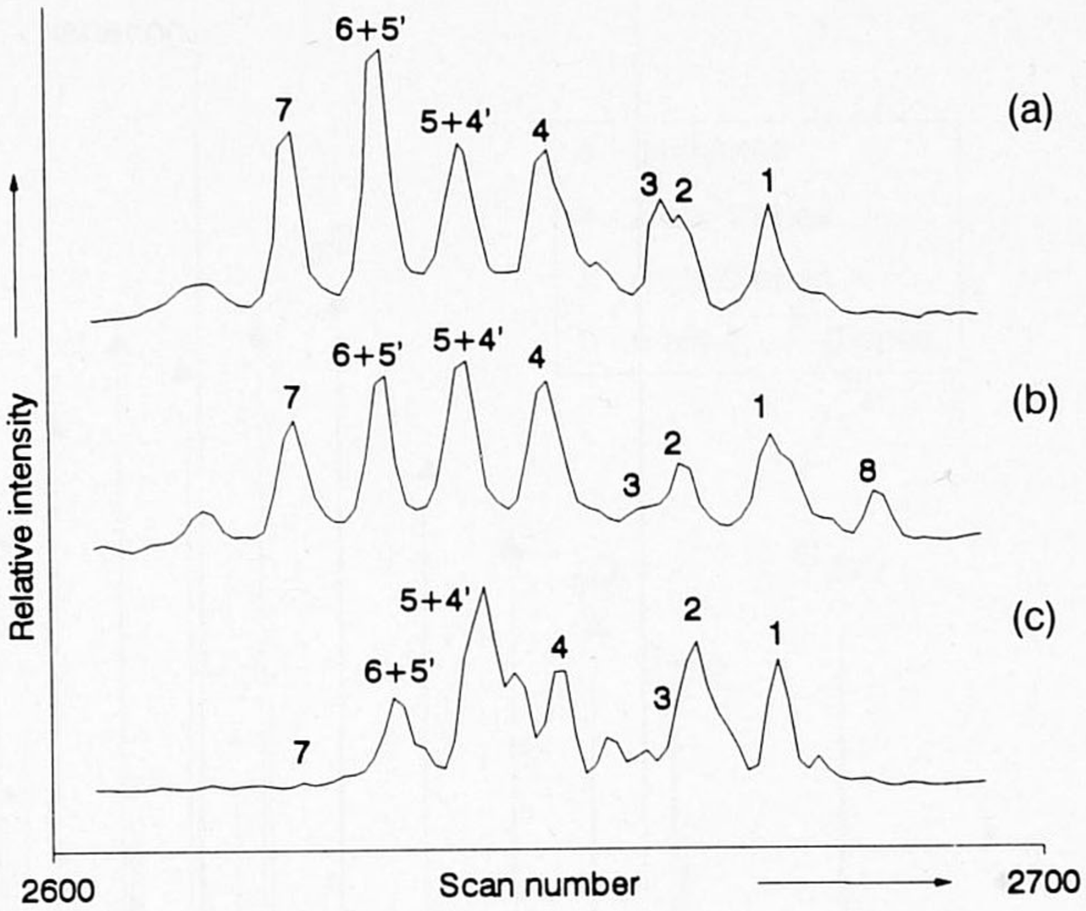


FIG. 3. Partial chromatograms of the flash pyrolysates (Curie temperature 610°C) of the extracted Balkashite (a), the algaenan fraction of Balkashite (b), and algaenan of *B. braunii* race A, Austin strain (c). 1 = *n*-tricosane; 2 = *n*-tricos-1-ene; 3 = *n*-heneicosan-2-one; 4 + 4′ = *n*-tricos-9-ene (cis and trans); 5 + 5′ = *n*-tricos-1,14-diene (*cis* and *trans*); 6 = *n*-heneicosan-9-and-10-ones; 7 = *n*-heneicos-1-en-12- and -13-ones; 8 = methyl-eicosanoate.

ranging from C_6 to C_{30} with a maximum at C_9 (Fig. 4a). The mass chromatogram of *m/z* 55 (Fig. 4b) illustrates the distribution of *n*-alk-1-enes in the C_6 to C_{30} range maximizing at *n*-oct-1-ene and *n*-dec-1-ene. This trace also reveals the distribution of series of other *n*-alkenes and *n*-alkadienes which elute before the corresponding *n*-alk-1-enes and which complicate the *m/z* 55 trace (see Fig. 3). This mass chromatogram further emphasizes the abundance of the C_{27}, C_{29}, and C_{31} *n*-alkadienes. To obtain further information on the identity of these various homologous series of alkenes and alkadienes, the extracted Balkashite sample was also pyrolysed in the off-line mode (see Fig. 1 and the Experimental section). This approach enabled the isolation of these various series of compounds in separate fractions (Fig. 1). The compounds in these fractions were converted with dimethyldisulfide (DMDS) to their corresponding di- and tetramethylthio-derivatives to assess the positions of the double bonds (CARLSON et al., 1989). Figure 5 shows the gas chromatogram of the hydrocarbon fraction isolated from the 400°C off-line pyrolysate. A similar distribution pattern as that in the 610°C flash pyrolysate (Fig. 2b) is observed, although the volatile components are lacking due to selective evaporation of the low-molecular-weight hydrocarbons during the isolation. Argentatious TLC was used to further separate these hydrocarbons into a saturated hydrocarbon fraction, two alkene fractions and an alkadiene fraction.

FIG. 2. Gas chromatograms of the flash pyrolysates (Curie temperature 610°C) of the non-extracted Balkashite (a), the extracted Balkashite (b), and the total extract of Balkashite (c). The square brackets indicate the homologous series of *n*-alk-1-enes and *n*-alkanes, with indication of their number of carbon atoms. C_9 FA and C_{16} FA denote nonanoic and hexadecanoic fatty acids, respectively. $C_{27:2}$, $C_{27:3}$, etc. indicate *n*-alkadienes and *n*-alkatrienes with twenty-seven carbon atoms. $C_{27:1}$-ω^9/ω^{10}-one and $C_{29:1}$-ω^9/ω^{10}-one denote the mixtures of the heptacos-1-en-ω^9-one and heptacos-1-en-ω^{10}-one, nonacos-1-en-ω^9-one, and nonacos-1-en-ω^{10}-one, respectively. Filled squares indicate homologous series of fatty acids. $C_{18:1}$ ald. and $C_{20:1}$ ald. correspond to the octadecenal and eicosenal, respectively. The stars indicate contaminations (dioctyladipate and a phthalate).

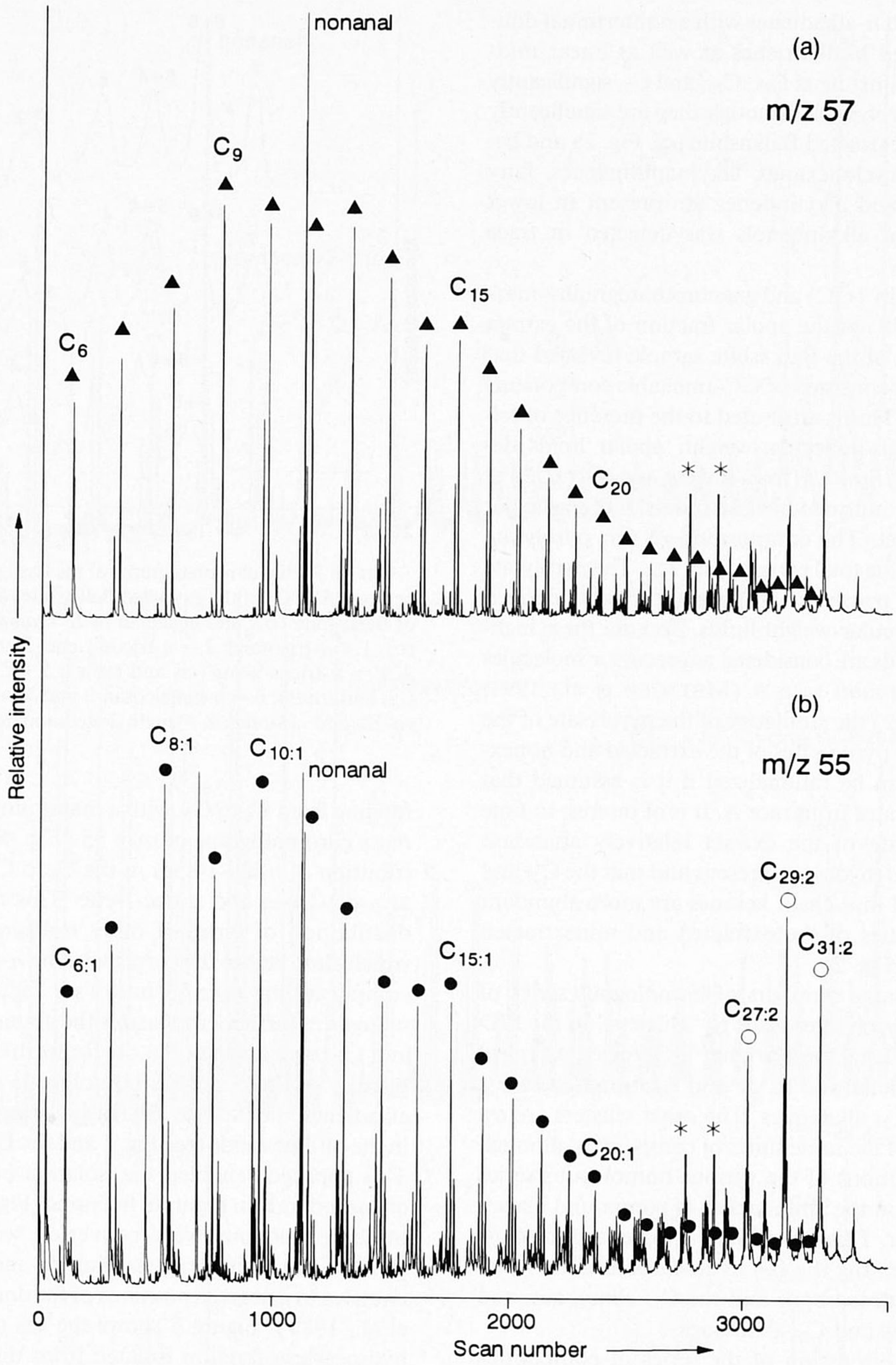


FIG. 4. Mass chromatograms of *m/z* 57 (a) revealing the distribution of the C_6-C_{30} *n*-alkanes (filled triangles) and of *m/z* 55 (b) revealing the distribution of the C_6-C_{30} *n*-alk-1-enes (filled circles) in the flash pyrolysate (Curie temperature 610°C) of the extracted Balkashite. Open circles indicate *n*-alka-1,ω^9-dienes. Stars indicate contaminations (dioctyladipate and a phthalate).

Figure 6a shows the TIC trace of the alkene-I fraction (Fig. 1) treated with DMDS. Three major series of compounds are observed. The most abundant series corresponds to a homologous series of 9,10-bis(methylthio)*n*-alkanes with characteristic fragment ions at *m/z* 173 and M-173 in their mass spectra. Figure 7a shows a typical mass spectrum of a member of this series (the DMDS adduct of nonacos-9-ene). The fragment ions at *m/z* 173 and *m/z* 327 (M-173) clearly indicate the location of the methylthio groups and thus reflect a double bond at the C-9 position in the original alkene. The fragment ions at *m/z* 159 and 187 and at *m/z* 313 and 341 in this spectrum illustrate the presence of traces of DMDS derivatives of nonacos-8-ene and nonacos-10-ene which obviously coelute with the 9,10-bis(methylthio)nonacosane. Mass chromatography of *m/z* 145, 159, and 187 (not shown) revealed the presence of 7,8-bis(methylthio)alkanes, 8,9-bis(methylthio)alkanes, and 10,11-bis(methylthio)alkanes, respectively. These experiments prove that the original double bond was present at C-9 in the *n*-alkene series whilst small contributions of *n*-alk-7-enes, *n*-alk-8-enes, and *n*-alk-10-enes

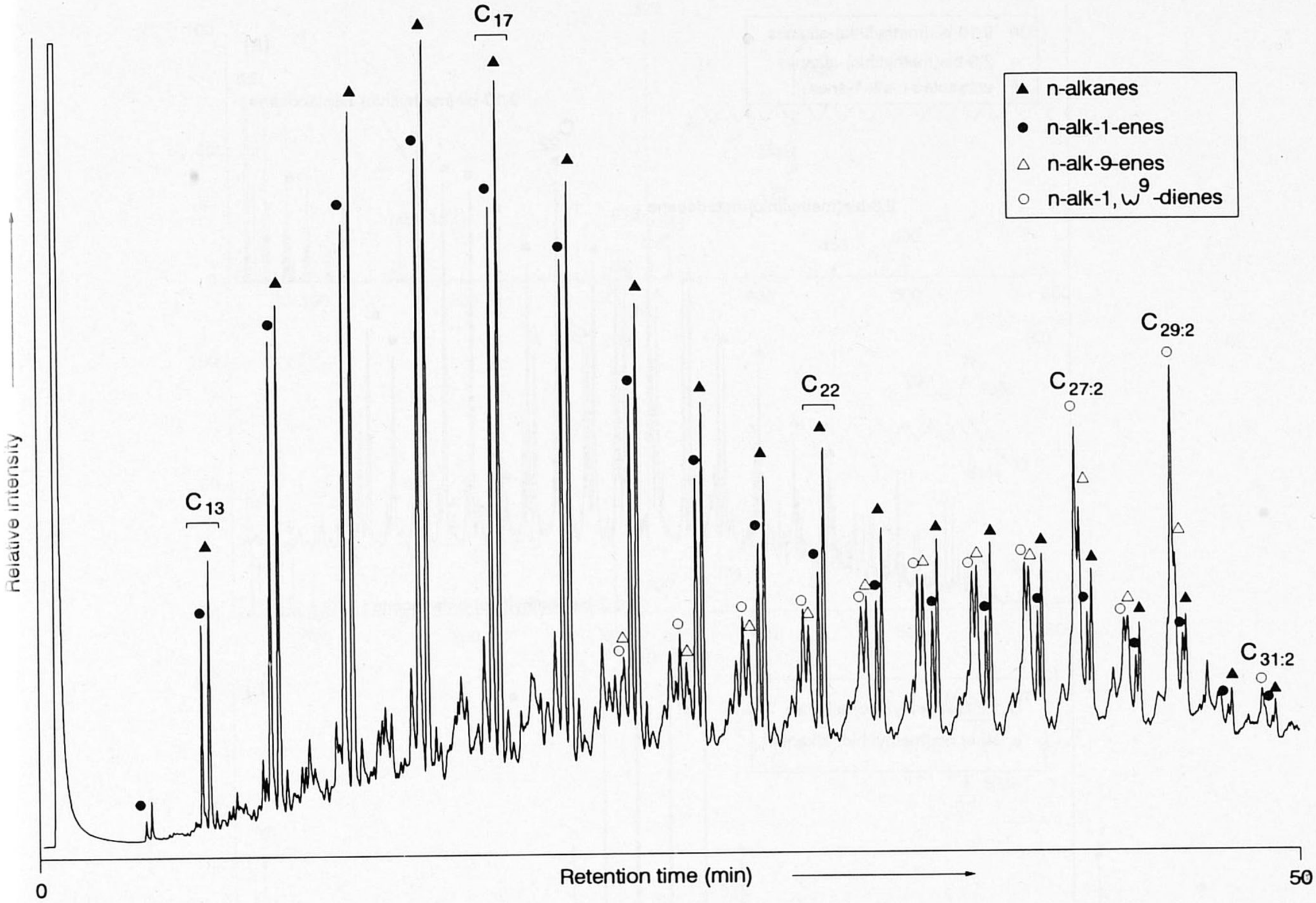


FIG. 5. Gas chromatogram of the total hydrocarbon fraction isolated from the 400°C off-line pyrolysate of the extracted Balkashite. Filled triangles indicate *n*-alkanes, filled circles *n*-alk-1-enes, open triangles *n*-alk-9-enes, and open circles *n*-alk-1,ω^9-dienes.

also occur. The second major series of DMDS adducts present in the alkene-I fraction (Fig. 6a) is identified as an homologous series of 2,3-bis(methylthio)alkanes with characteristic fragment ions at *m/z* 75 and M-75 in their mass spectra. Figure 7b shows a typical mass spectrum of this series of adducts (i.e., that of eicos-2-ene) characterized by a base peak at *m/z* 299 (M-75). The third major series observed in this fraction (Fig. 6a) corresponds to unreacted *n*-alk-1-enes.

Figure 6b shows the TIC trace of the alkene-II fraction treated with DMDS. This fraction is mainly composed of a homologous series of 1,2-bis(methylthio)alkanes with characteristic fragment ions at *m/z* 61 and M-61 in their mass spectra. A typical mass spectrum is shown in Fig. 7c. This series reveals the presence of *n*-alk-1-enes in the original pyrolysate.

The other peaks indicated in the TIC trace (Fig. 6b) correspond to minor homologous series of isomeric bis(methylthio)alkanes. A small contribution of DMDS adducts of *n*-alkylcyclohexenes is also observed in this fraction.

Figure 8 shows partial mass chromatograms of *m/z* 173 and 61 derived from the GC-MS analysis of the *n*-alkadiene fraction treated with DMDS. The mass spectra clearly indicate that the major series of compounds present are stereoisomers of 1,2,ω^9,ω^{10}-tetra(methylthio)alkanes ranging from C_{15} to C_{29} with relatively high abundances of the C_{27} and C_{29} components. A typical mass spectrum is shown in Fig. 7d. The fragment ions at *m/z* 159 and *m/z* 189 in this spectrum reveal the presence of minor amounts of coeluting 1,2,ω^8,ω^9 and 1,2,ω^{10},ω^{11}-tetra(methylthio)alkanes, respectively. These results clearly indicate that the alkadienes generated upon pyrolysis mainly consist of 1,ω^9-*n*-alkadienes with C_{27} and C_{29} homologs as the most abundant ones.

Alkanones

The mass chromatogram of *m/z* 58 of the flash pyrolysate of extracted Balkashite (Fig. 9a) shows the distribution of the *n*-alkan-2-ones. They range from C_6 to C_{28} and maximize at C_9, C_{10}, and C_{11}. Their unsaturated counterparts are also present, though in relatively lower amounts and range from C_8 to C_{21}.

Individual mass spectra and mass chromatography of *m/z* 141 and 155 revealed the presence of two similar series of *n*-alkan-9-ones and *n*-alkan-10-ones and their mono-unsaturated counterparts (Fig. 9b and c). Figure 10a shows a mass spectrum of a mixture of two coeluting mono-unsaturated ketones, nonacos-1-en-21-one, and nonacos-1-en-20-one. This spectrum is characterized by the molecular ions at *m/z* 420 $[C_8H_{17}\text{-}CO\text{-}C_{20}H_{39}]^+$ and $[C_9H_{19}\text{-}CO\text{-}C_{19}H_{37}]^+$, fragments due to loss of water (M-18) at *m/z* 402, and by

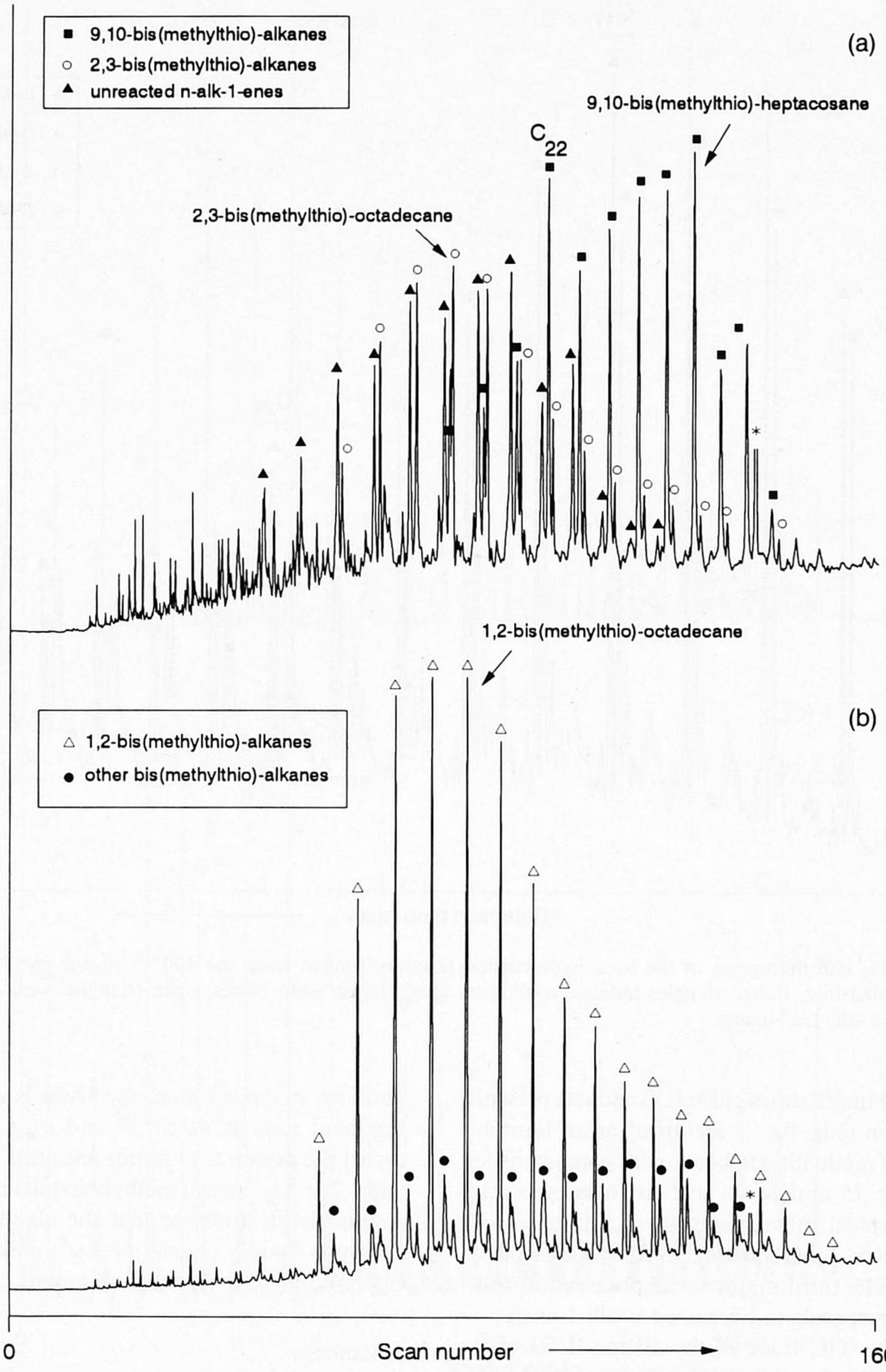


FIG. 6. Total Ion Current (TIC) traces of the DMDS adducts of the *n*-alkene-I (a) and *n*-alkene-II (b) fractions isolated from the total hydrocarbon fraction of the 400°C off-line pyrolysate of the extracted Balkashite. Closed squares indicate 9,10-bis(methylthio)-alkanes, open circles indicate 2,3-bis(methylthio)-alkanes, closed triangles indicate unreacted *n*-alk-1-enes, open triangles indicate 1,2-bis(methylthio)-alkanes, and closed circles indicate other bis(methylthio)-alkanes. The star indicates a contamination.

fragment ions *m/z* 141 $[C_8H_{17}\text{-}CO]^+$, *m/z* 307 $[CO\text{-}C_{20}H_{39}]^+$, *m/z* 155 $[C_9H_{19}\text{-}CO]^+$, and *m/z* 293 $[CO\text{-}C_{19}H_{37}]^+$. Ions due to McLafferty rearrangements associated with the ketone function are observed as well. Although their abundance was difficult to determine because of partial co-elution of these mid-chain ketones with $1,\omega^9$-n-alkadienes (Figs. 2 and 3), mass chromatography showed that they were relatively important pyrolysis products. Three other compounds have been identified on basis of their mass spectra as heptacosen-9,10-dione, nonacosen-9,10-dione, and hentriaconten-9,10-dione. They are present in minor amounts compared to the mid-chain ketones.

Figures 11a and b show the GC traces of the mid-chain and methyl ketone fractions isolated from the off-line pyrolysate (see Fig. 1). The distributions of these ketones are similar to those obtained by on-line flash pyrolysis, although

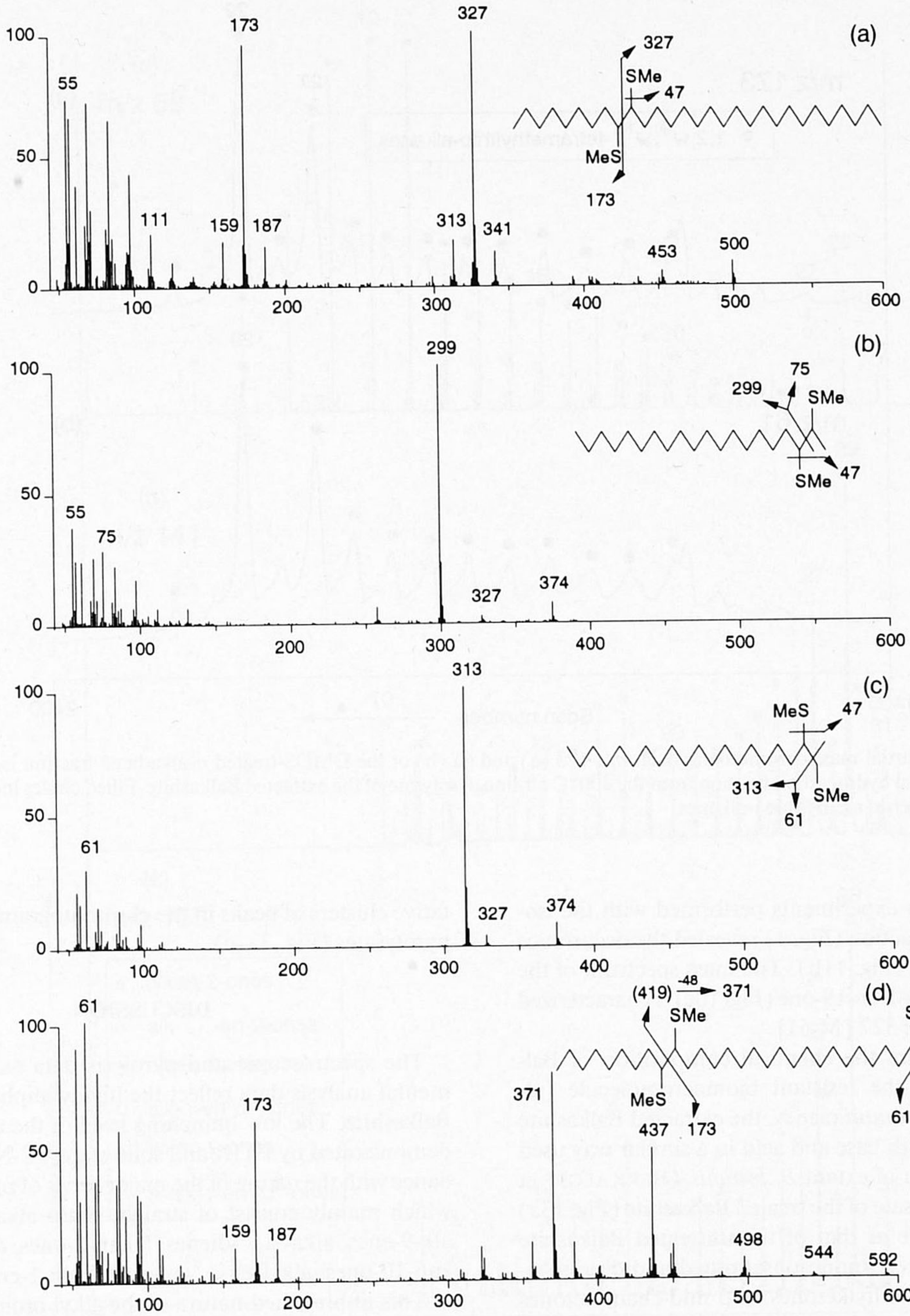


FIG. 7. Mass spectra, corrected for background, of 9,10-bis(methylthio)-nonacosane (a), 2,3-bis(methylthio)-eicosane (b), 1,2-bis(methylthio)-eicosane (c), and 1,2,20,21-tetra(methylthio)-nonacosane (d).

the low-molecular-weight ketones have been preferentially lost during work-up procedures. Coinjections with two authentic standards, nonadecan-9-one and nonadecan-10-one, confirmed the identification of the series of *n*-alkan-9-ones and *n*-alkan-10-ones in the pyrolysate (Fig. 11a).

DMDS derivatization was performed with the ketone fractions of the off-line pyrolysate (Fig. 1). Figure 12 shows the TIC trace of the DMDS adducts of the mid-chain ketones fraction. In addition to the *n*-alkan-9-one and *n*-alkan-10-one series, two series of DMDS adducts of unsaturated *n*-alkan-9-ones and *n*-alken-10-ones were observed. The most important series are the 1,2-bis(methylthio)-alkan-ω^9- and -ω^{10}-ones which emphasize the original presence of a terminal unsaturation generated upon pyrolysis or initially present in the macromolecular structure. A contribution of the 2,3-bis(methylthio)-alkan-ω^9-one and -ω^{10}-one series is also observed and may reflect an isomerization of the terminal double bond to the 2 position. Figure 10b shows the mass spectrum of the DMDS adduct of coeluting nonacos-1-en-21-one and nonacos-1-en-20-one and is characterized by a base peak at *m/z* 453 [M-61] and by fragments at *m/z* 141 and 155 related to the ketone group at C-21 and C-20, respectively.

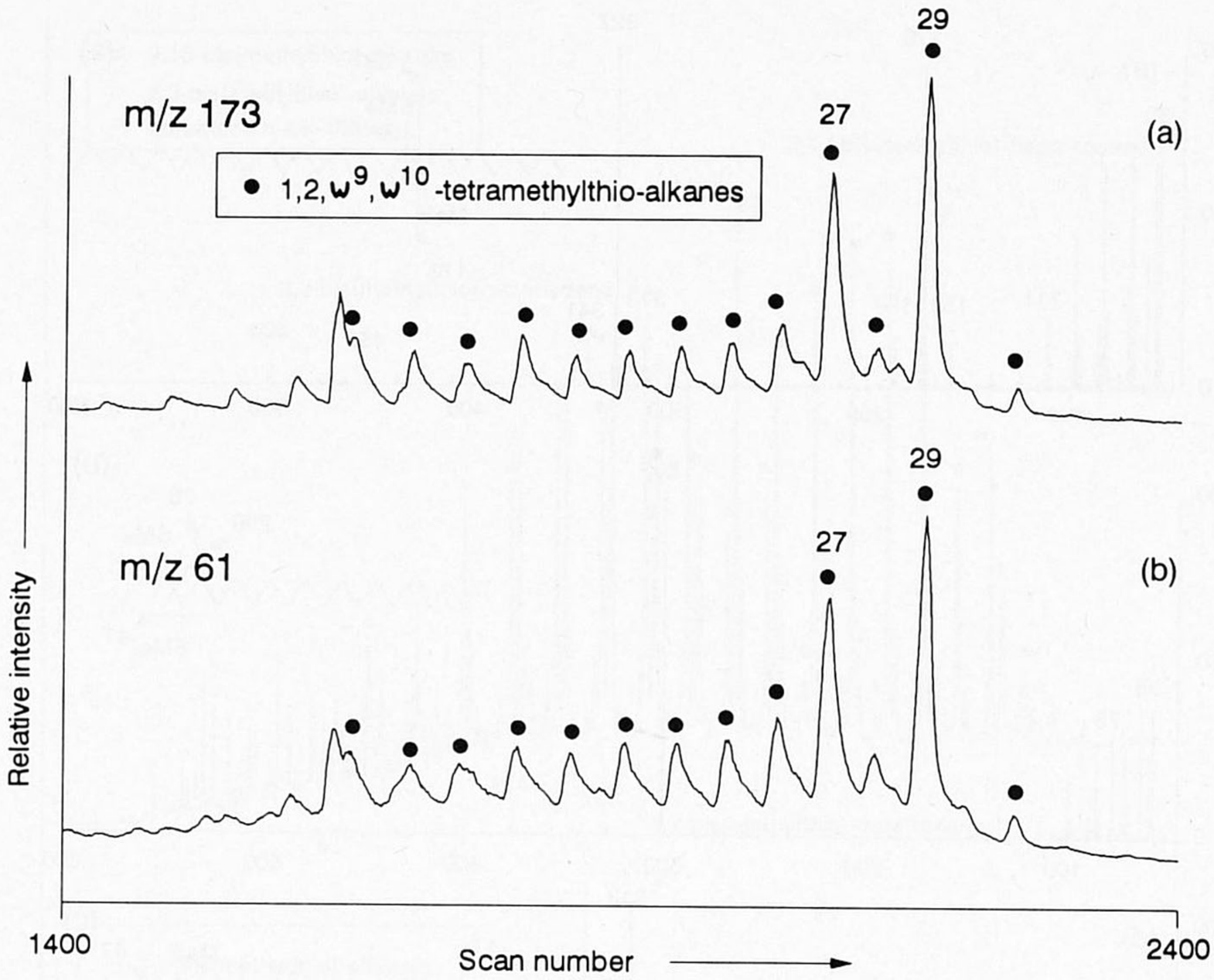


FIG. 8. Partial mass chromatograms of *m/z* 173 (a) and 61 (b) of the DMDS-treated *n*-alkadiene fraction isolated from the total hydrocarbon fraction from the 400°C off-line pyrolysate of the extracted Balkashite. Filled circles indicate 1,2,ω^9,ω^{10}-tetra(methylthio)-alkanes.

DMDS derivatization experiments performed with the isolated methylketone fraction (Fig. 1) revealed the occurrence of *n*-alk-1-en-ω^2-ones (Fig. 11b). The mass spectrum of the DMDS adduct of eicos-1-en-19-one (Fig. 10c) is characterized by a base peak at *m/z* 327 [M-61].

In order to compare the chemical composition of Balkashite with that of the resistant biomacromolecule (algaenan) of extant *B. braunii* race A, the extracted Balkashite sample was treated with base and acid in a similar way used to isolate the algaenan of extant *B. braunii*. (BERKALOFF et al., 1983). The pyrolysate of the treated Balkashite (Fig. 13a) is essentially the same as that of the untreated Balkashite (Fig. 2b). The saturated, mono-unsaturated and di-unsaturated hydrocarbons, methylketones, and mid-chain ketones are also major components in this flash pyrolysate. Detailed comparison of a representative cluster of peaks in the chromatograms of the pyrolysates (cf. Fig. 3a and b) further attests to the similarity of the distribution patterns of the compounds considered.

The pyrolysate of algaenan obtained from *B. braunii* race A (Austin strain; BERKALOFF et al., 1983) is shown in Fig. 13b. The major difference with the other pyrolysates is the considerably decreased abundance of the C_{27}, C_{29}, and C_{31} alkadienes, alkatrienes, and mid-chain ketones. The other pyrolysis compounds showed similar distribution patterns when compared with corresponding distributions in the pyrolysates of the various Balkashite samples including the Balkashite sample obtained after extraction and base and acid treatment. This is emphasized by comparisons of representative clusters of peaks in the chromatograms of the different pyrolysates (Fig. 3a–c).

DISCUSSION

The spectroscopic and pyrolysis data as well as the elemental analysis data reflect the highly aliphatic character of Balkashite. The low branching level of the alkyl moieties as demonstrated by FTIR and solid state ^{13}C-NMR is in accordance with the nature of the major series of pyrolysis products which mainly consist of straight-chain alkanes, alk-1-enes, alk-9-enes, alka-1,ω^9-dienes, alkan-2-ones, alkan-9-ones, alkan-10-ones, alk-1-en-ω^9-ones, and alk-1-en-ω^{10}-ones.

This unbranched nature of the alkyl moieties strongly indicates that the Balkashite must have been derived from algaenan and/or lipids of *B. braunii* race A or B because race L lipids and algaenan mostly consist of isoprenoid units (METZGER and CASADEVALL, 1987; DERENNE et al., 1990). As discussed hereafter, the pyrolysis data also strongly indicate that the Balkashite is derived from race A.

The C_{27}, C_{29}, and C_{31} alkadienes, alkatrienes, and the alk-1-en-ω^9-ones and alk-1-en-ω^{10}-ones are present in the pyrolysates of all Balkashite samples. It should be noted, however, that these components are only minor constituents in the pyrolysate of the algaenan of cultured *B. braunii,* a fraction obtained after extraction with $CHCl_3$ and other organic solvents and subsequent base and acid treatments.

In a separate study (GATELLIER et al., 1993) very similar characteristic distributions of C_{27}, C_{29}, and C_{31} alkadienes,

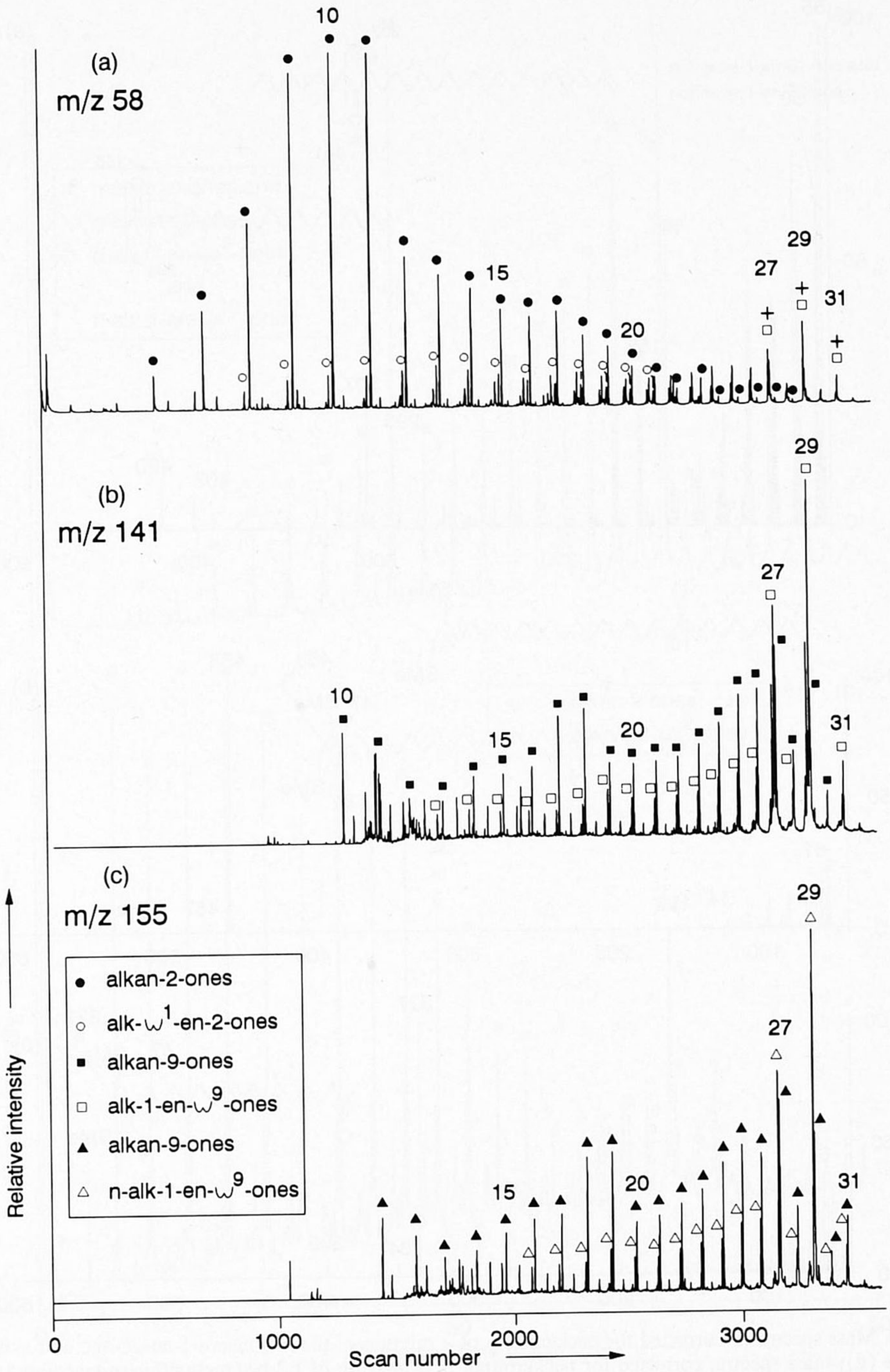


FIG. 9. Mass chromatograms of *m/z* 58 (a), 141 (b), and 155 (c) revealing the distributions of the *n*-alkan-2-ones (filled circles), the *n*-alk-1-en-ω^2-ones (open circles), the *n*-alkan-9-ones (filled squares), the *n*-alk-1-en-ω^9-ones (open squares), the *n*-alkan-10-ones (filled triangles), and the *n*-alk-1-en-ω^{10}-ones (open triangles) in the flash pyrolysate of the extracted Balkashite.

alkatrienes, and monounsaturated alk-1-en-ω^9-ones and alk-1-en-ω^{10}-ones were observed as major pyrolysis products of ether lipids with a general structure as depicted in Fig. 14a. These ether lipids occur in substantial amounts in strains of *B. braunii* race A (METZGER et al., 1991). The distribution of their pyrolysis products can be rationalized by cleavage of the ether bonds between the ω^9- and ω^{10}-carbon atoms as illustrated schematically in Fig. 14b. More detailed mechanisms for these pyrolysis reactions will be described elsewhere (GELIN et al., 1993).

The virtual lack of C_{27}, C_{29}, and C_{31} components in the pyrolysate of algaenan of cultured *B. braunii* (Fig. 13b) obtained after removal of the external and internal lipids, as well as the so-called rubber fraction (METZGER et al., 1991),

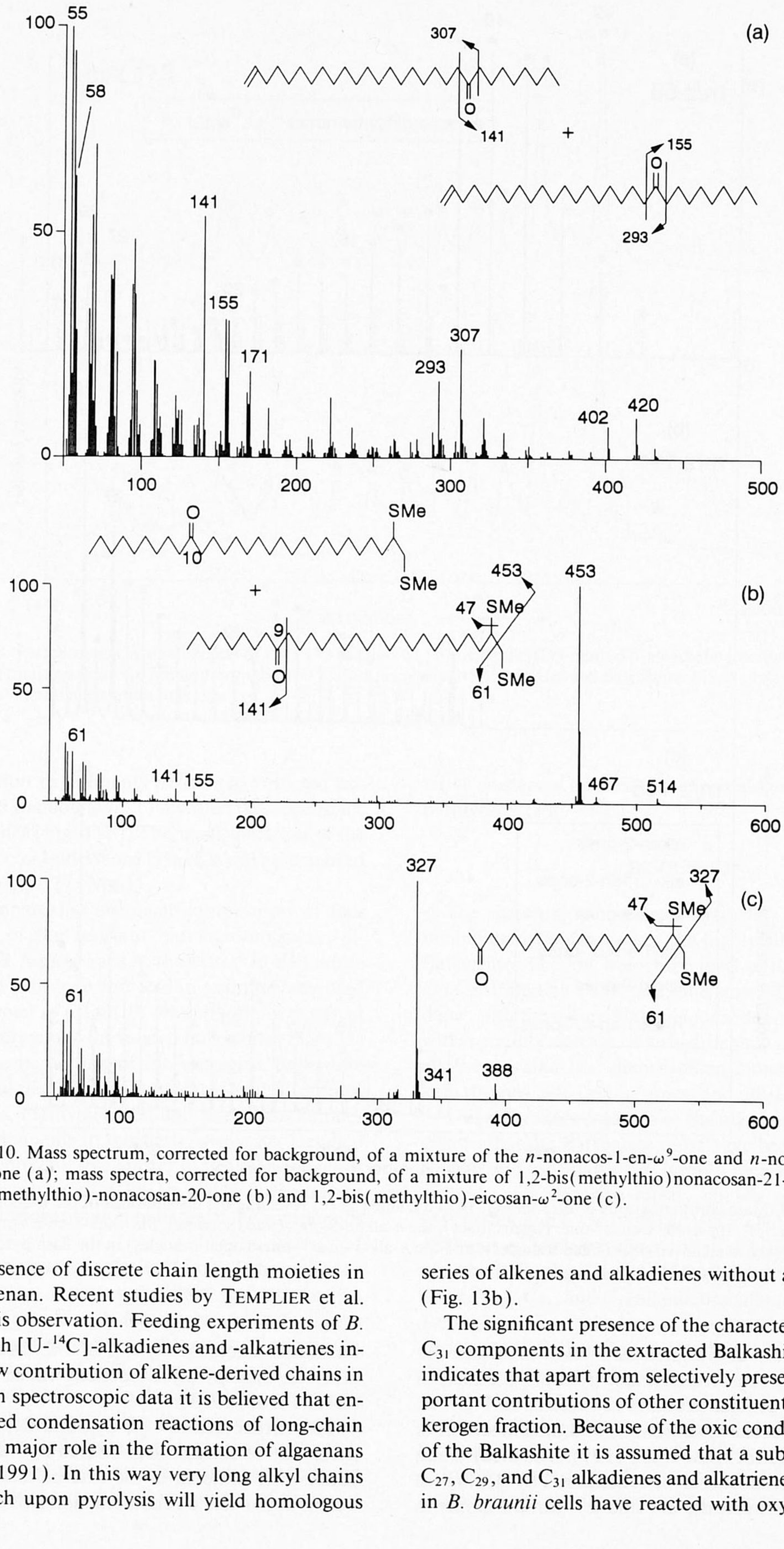


FIG. 10. Mass spectrum, corrected for background, of a mixture of the *n*-nonacos-1-en-ω^9-one and *n*-nonacos-1-en-ω^{10}-one (a); mass spectra, corrected for background, of a mixture of 1,2-bis(methylthio)nonacosan-21-one and 1,2-bis(methylthio)-nonacosan-20-one (b) and 1,2-bis(methylthio)-eicosan-ω^2-one (c).

may reflect the absence of discrete chain length moieties in the insoluble algaenan. Recent studies by TEMPLIER et al. (1992) support this observation. Feeding experiments of *B. braunii* race A. with [U-^{14}C]-alkadienes and -alkatrienes indicated a rather low contribution of alkene-derived chains in algaenan. Based on spectroscopic data it is believed that enzymatically induced condensation reactions of long-chain dialdehydes play a major role in the formation of algaenans (METZGER et al., 1991). In this way very long alkyl chains are produced which upon pyrolysis will yield homologous series of alkenes and alkadienes without any clear optimum (Fig. 13b).

The significant presence of the characteristic C_{27}, C_{29}, and C_{31} components in the extracted Balkashite (Fig. 2b) clearly indicates that apart from selectively preserved algaenan, important contributions of other constituents are present in the kerogen fraction. Because of the oxic conditions of deposition of the Balkashite it is assumed that a substantial part of the C_{27}, C_{29}, and C_{31} alkadienes and alkatrienes originally present in *B. braunii* cells have reacted with oxygen. Cross-linking

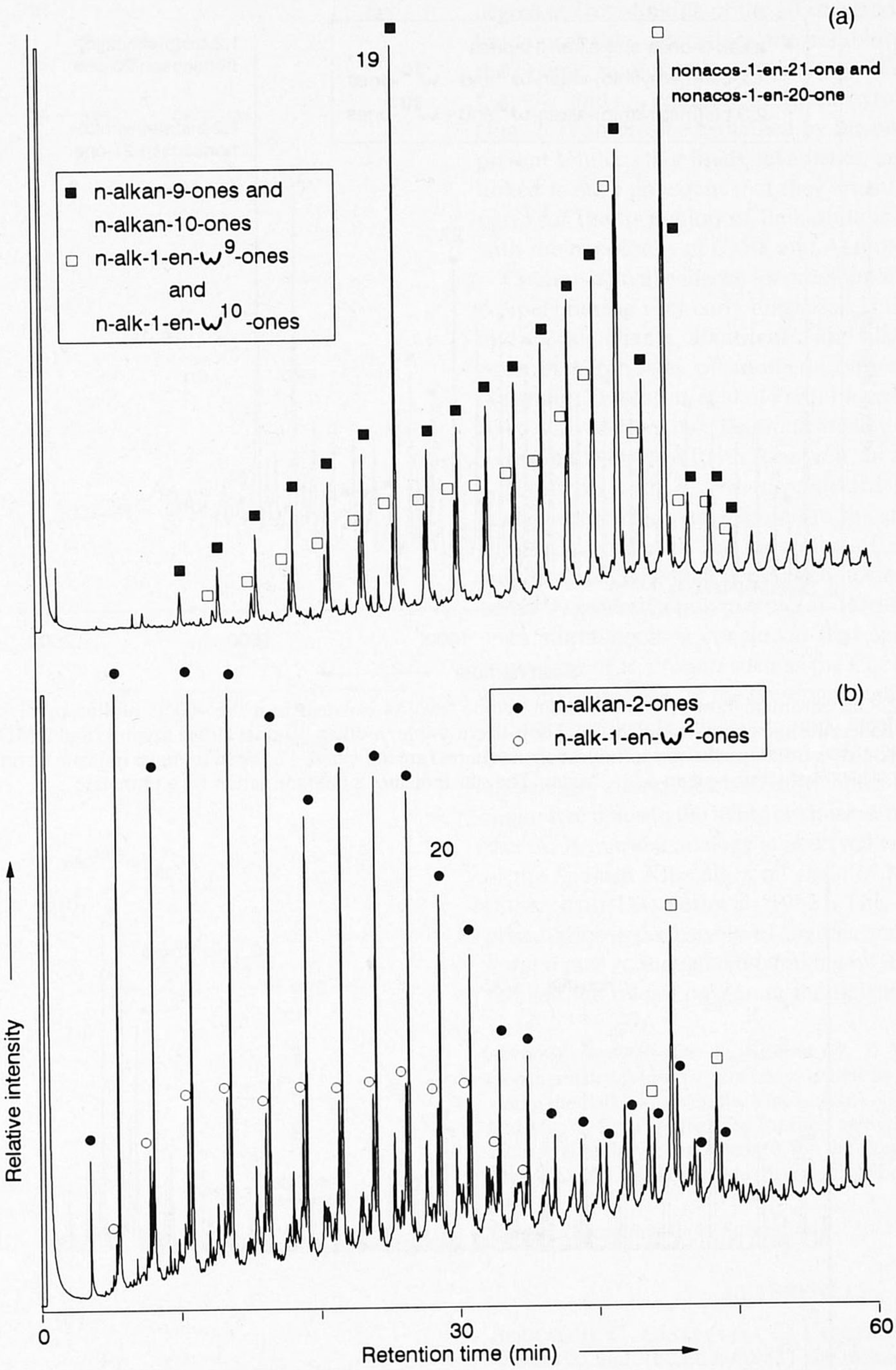


FIG. 11. Gas chromatograms of the mid-chain ketone (a) and the methylketone (b) fractions isolated from the 400°C off-line pyrolysate of the extracted Balkashite. Filled squares indicate *n*-alkan-9-ones and -10-ones. Open squares indicate *n*-alk-1-en-ω^9-ones and -ω^{10}-ones, closed circles indicate *n*-alk-2-ones and open circles indicate *n*-alk-1-en-ω^2-ones. The height of the *n*-nonadecane-9-one peak in Fig. 11a is due to a coelution with and authentic standard of *n*-nonadecan-9-one.

with oxygen at the double bonds in the middle of the chains may have resulted in the diagenetic formation of insoluble macromolecules which upon pyrolysis yield similar suites of pyrolysis compounds as the ether lipids mentioned above. Recent studies have indicated that the internal double bonds are much more reactive towards reaction with oxygen than the terminal ones (P. Metzger, unpub. data). This phenomenon may explain the discrete carbon chain lengths of the characteristic pyrolysis products. The proposed cross-linking of alkadienes and alkatrienes can also explain the total absence of these alkenes in the GC-amenable part of the extract.

It should be noted that the originally present ether lipids

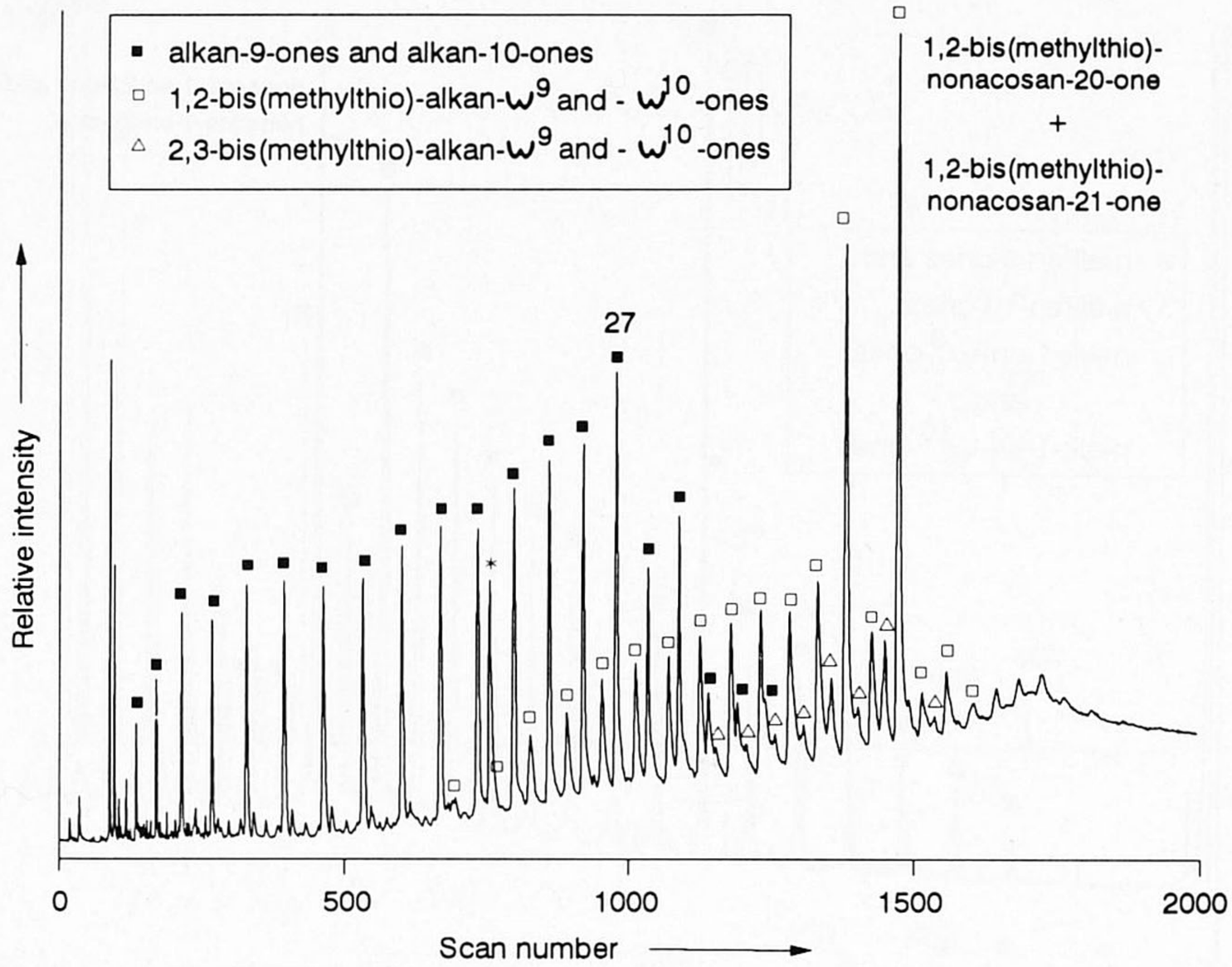


FIG. 12. Gas chromatogram of the mid-chain ketone fraction isolated from the 400°C off-line pyrolysate of the extracted Balkashite and treated with DMDS. The *n*-alkan-9-one/*n*-alkan-10-ones (filled squares) and DMDS adducts of the *n*-alk-1-en-ω^9-one/*n*-alk-1-en-ω^{10}-ones (open squares) are indicated. The open triangles indicate an homologous series of 2,3-bis(methylthio)-alkan-ω^9/ω^{10}-ones. The star indicates a contamination by a phthalate.

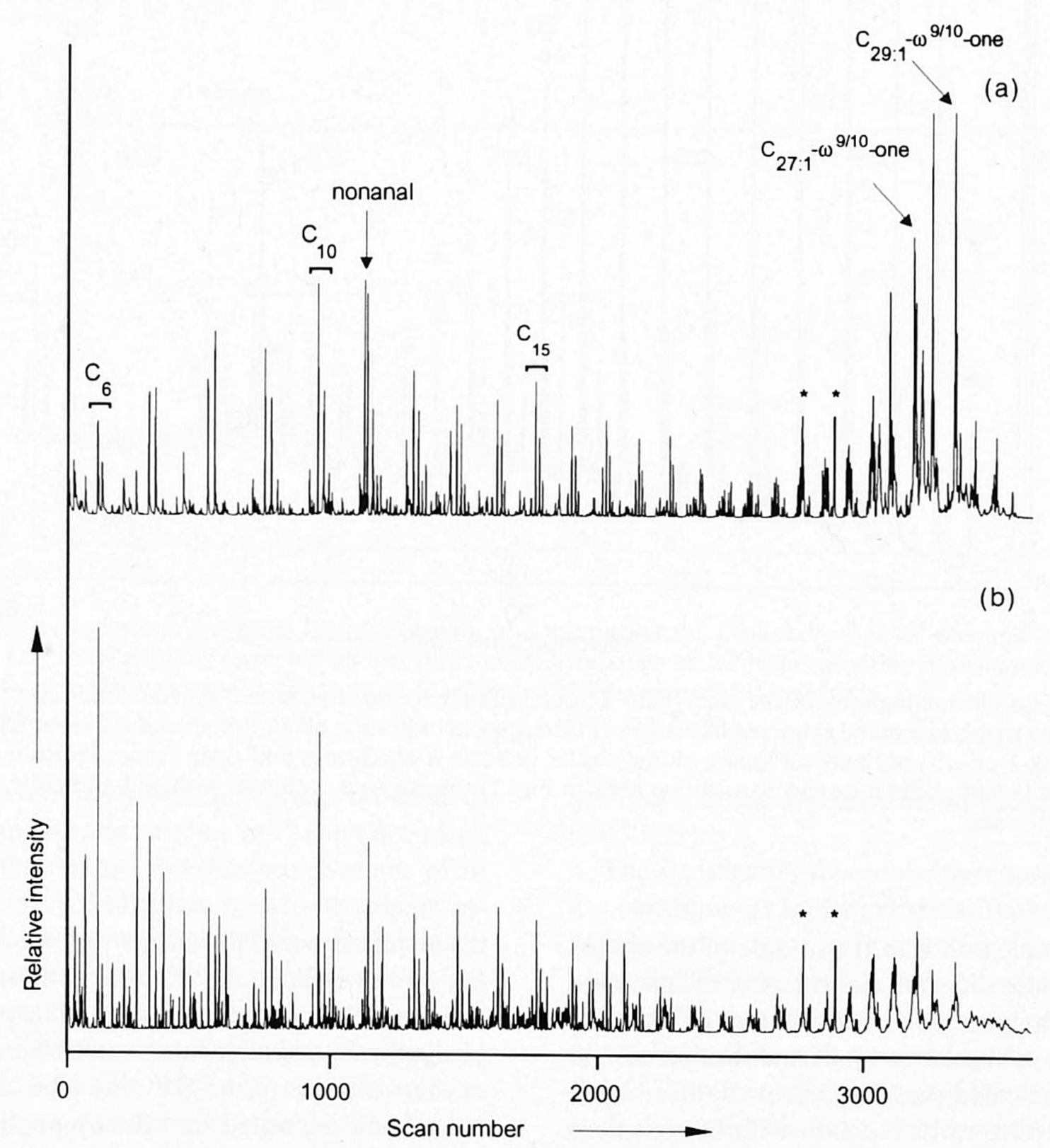


FIG. 13. TIC traces of the flash pyrolysates (Curie temperature 610°C) of the treated Balkashite isolated after base and acid treatments (a) and of the resistant biopolymer of the Austin strain of *B. braunii* race A (b). Homologous series of *n*-alkenes and *n*-alkanes are indicated by C_6, C_{10} and C_{15}.

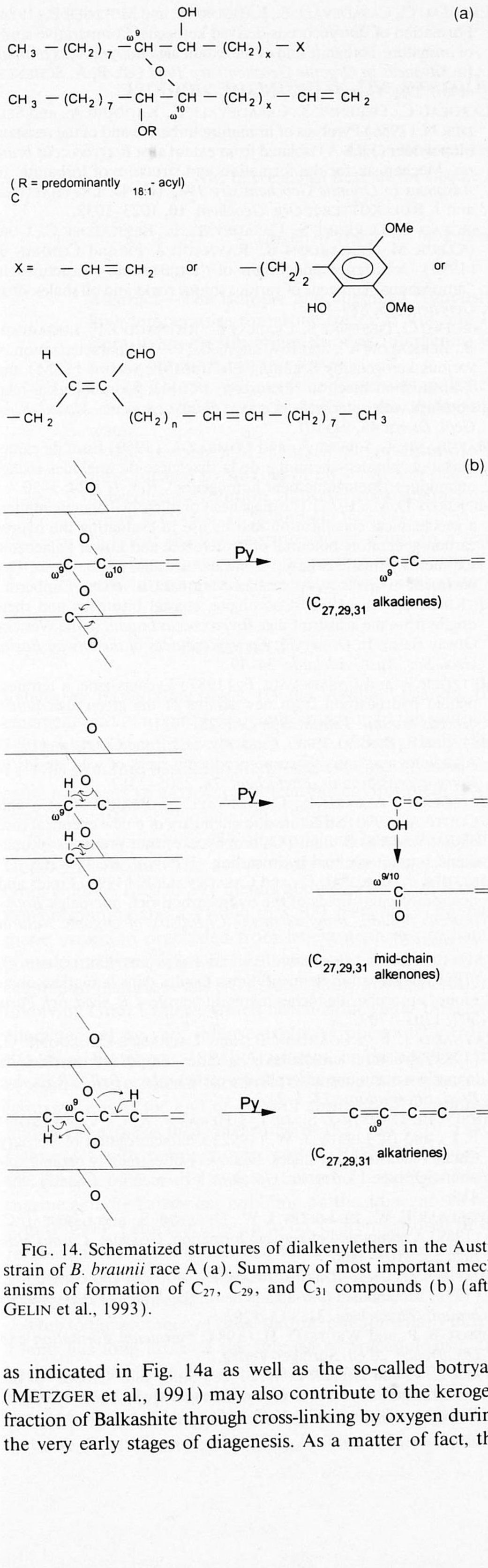


FIG. 14. Schematized structures of dialkenylethers in the Austin strain of *B. braunii* race A (a). Summary of most important mechanisms of formation of C_{27}, C_{29}, and C_{31} compounds (b) (after GELIN et al., 1993).

as indicated in Fig. 14a as well as the so-called botryals (METZGER et al., 1991) may also contribute to the kerogen fraction of Balkashite through cross-linking by oxygen during the very early stages of diagenesis. As a matter of fact, the degree of cross-linking of the alkenes and the more complex lipids probably determines the solubility characteristics of the organic matter in the Balkashite. The abundance of the C_{27}, C_{29}, and C_{31} components in the pyrolysate of the extract (Fig. 2c) can thus be explained by the presence of originally present soluble ether lipids, alkadienes, and alkatrienes cross-linked to such an extent that they are still soluble. This scenario for the formation of Balkashite is in good agreement with the hypothesis of CANE and ALBION (1973).

Circumstantial evidence for cross-linking of *B. braunii* race A lipids during very early diagenesis is the lack of C_{27}, C_{29}, and C_{31} alkadienes, alkatrienes, and alk-1-en-ω^9- and -ω^{10}-ones in pyrolysates of another Coorongite from Darwin, Australia (DUBREUIL et al., 1989). Indeed, the latter is known to be derived from race B as indicated by analysis of the algae living in the Darwin River Reservoir. In addition, laboratory experiments with *B. braunii* collected from Darwin Lake (concentrated biomass exposed to the atmosphere for three months) resulted in the formation of nonhydrolysable insoluble material which is probably formed from botryococcenes by oxidative polymerization (DUBREUIL et al., 1989).

In summary it is concluded that shore-deposited accumulations of *B. braunii* such as the Coorongite of Lake Balkash mainly consist of the preserved highly resistant aliphatic biomacromolecule algaenan and insoluble oxygen cross-linked substances resulting from oxidative polymerization of alkadienes, alkatrienes, and ether lipids originally present as major free lipids in the living organisms. In this case *B. braunii* race A. A marked analogy is observed with the composition of the Spanish Ribesalbes oil shale of Middle Miocene age (SINNINGHE DAMSTÉ et al., 1992). This oil shale is also comprised almost exclusively of organic matter of fossilized *B. braunii* race A, though cross-linking by sulphur seems to have fulfilled the role of oxygen in the Balkashite.

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