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ARTICLE *in* ENERGY & FUELS · FEBRUARY 2011

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Identification of Methylhopane and Methylmoretane Series in Ceará Basin Oils, Brazil, Using Comprehensive Two-Dimensional Gas Chromatography Coupled to Time-of-Flight Mass Spectrometry

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ABSTRACT: Comprehensive two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GC × GC-TOFMS) was used to identify methylhopanes in oils. 2α-Methyl-17α(H),21β(H)-hopane and 2α-methyl-17β(H),21α(H)-hopane (2α-methylhopane) series were found in oils from Ceará Basin, Brazil. In the 3β-methylhopane series, only C_{31} 3β-methyl-17α(H),21β(H)-hopane was detected. In addition, a novel terpane series (C_{30}−C_{34}), which eluted after the regular hopanes (comparing compounds of both families with the same carbon number) either in 1D (first dimension) or 2D (second dimension), was identified. A compound likely to be bisnorgammacerane was separated from C_{29} 25-norhopane in the 2D, and consequently identified. An unusual compound pair eluting after the C_{35}R moretane was tentatively identified as C_{36} 17α(H),21β(H)-homohopanes (22S, 22R). The presence of 2α-methylhopanes in oils from Ceará Basin may indicate oxygen-producing cyanobacteria were important biological inputs in the deposition environment. This is the first study that shows the separation between hopane and 2α-methylhopane series and their tentative spectral identification using GC × GC-TOFMS, as they are usually not well separated in one-dimensional gas chromatography.

1. INTRODUCTION

Biomarkers are organic compounds that can be used as tracers for geological and environmental processes.[1,2] Regarding petroleum exploration problems, one of the major applications of organic geochemistry, chromatography and mass spectrometry, is routinely used in determining the distribution of various classes of biomarkers in oils and source rocks and the identification of these individual compounds. Applications of biomarker determination include identification of the sources of petroleum, oil−oil, and oil−source rock correlations,[1−3] and for use in describing the thermal evolution of the sample, biodegradation in the reservoir, and migration aspects, which are all essential to the study of petroleum systems.[4]

Until now, hopanes, tricyclic terpanes, steranes, aromatic steroids, and specific or unique biomarkers, such as gammacerane, have been the primary biomarker series of interest in petroleum geochemistry.[1,3] Gas chromatography coupled to mass spectrometry (GC-MS) has been used for determination of these compounds, but it has many limitations due to the complexity of petrochemical samples and coelution problems that interfere in the mass spectrum identification. In addition, gas chromatography coupled to tandem mass spectrometry (GC-MS/MS) has been frequently used.[2,5,6]

Comprehensive two-dimensional gas chromatography (GC × GC) has become of increasing interest for the analysis of highly complex mixtures, such as petrochemical, environmental, and biological samples.[7−10] The principle of GC × GC is based on the hyphenation of two capillary columns, which contain different stationary phases. The interface between the columns is called the modulator. The first separation usually takes place on a conventional nonpolar GC column, where the analytes are separated mainly according to their vapor pressure in a one-dimensional GC system (1D-GC). The modulator enables sampling, focusing by trapping successive fractions of the first-column eluate into a large number of narrow bands sequentially transferred onto the second (medium) polar or shape-selective GC column. The separation on the second column is very much faster and is performed under essentially isothermal conditions. GC × GC has three main advantages compared to 1D-GC: (i) the peak capacity is much higher; (ii) sensitivity is better due to the peak narrowing in the modulator; and (iii) structured chromatograms are obtained, thereby facilitating recognition of compound families and unknowns.[7−10] Applications of GC × GC for petrochemical samples are reported in the literature.[7,8,11−19] GC × GC can provide group-type separations; however, individual identification of substances in oils can be achieved when coupled to time-of-flight mass spectrometry (TOFMS), because narrow peaks require detectors with fast data acquisition rate.[11,17−19]

Recently, a systematic biomarker search in crude oils and extra heavy gas oils by GC × GC-TOFMS revealed several compounds for the first time for geochemical oil prospecting, which were identified individually and collectively as families having a similar structure.[18,19] In these previous works, hopane, 25-

Received: October 7, 2010
Revised: January 19, 2011
Published: February 17, 2011

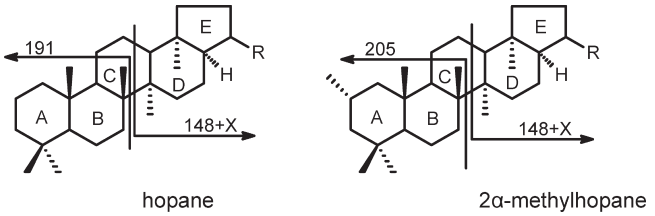

Figure 1. Mass spectrometric fragmentation of hopanes and 2α-methylhopanes; X = C_nH_{2n+1}.

norhopane, tricyclic terpane, and sterane series were tentatively identified, as well as biomarkers hardly detected in 1D-GC, such as C_{29} 28-norspergulane, dinosteranes, and the demethylated tricyclic terpane series. These biomarkers are seldom detected because of their low concentrations in the oils and because they are prone to coelute with major compounds. In addition, coelutions between tricyclic terpanes/hopanes, 25-norhopanes/hopanes, and steranes/hopanes, which hampered better identification and quantification of these compounds, were resolved.

The identification of other important biomarkers, such as methylhopanes, can be exploited by this technique. Addition of a methyl group to the A-ring of the hopane series transfers its characteristic fragment mass from *m/z* 191 to *m/z* 205 (Figure 1). A-Ring methylated hopanes can originate from various eubacteria: 2α-methylhopanes appear to be specific for oxygen-producing cyanobacteria, while various microorganisms, including oxygen-respiring methanotrophic bacteria, synthesize 3-methylhopanoids, which are precursors to 3β-methylhopanes.[20] The 2α-methylhopanes (geological), which are common in some mature sediments and oils, probably originate from the biogenic, less stable 2β-methylhopanes.[20] Ratios of methylhopanes to hopanes are useful as source input parameters, thus reflecting bacterial populations at the time of sedimentation.[5,6,21,22]

2α-Methylhopanes elute very close to the regular hopane series on apolar methylsilicone columns.[6,22,23] Moreover, C_{31} 3β-methylhopane, the most usual 3-methylhopane, coelutes with gammacerane.[24] These compounds usually are identified by GC-MS/MS transitions[5,6] or GC-MS selective-ion monitoring (SIM);[22] unfortunately, these two procedures do not provide mass spectra, thus hindering analyte identification. Due to the coelution and identification problems, the aim of the present study was to explore the application of GC × GC-TOFMS to unravel methylhopane and methylmoretane occurrence in oils.

2. EXPERIMENTAL SECTION

2.1. Samples. Ceará Basin is located on the continental shelf of the Brazilian equatorial margin, covering an area of approximately 34,000 km^2. We analyzed two samples from this Basin (#1 and #2) in the present work, both from Trairi Member, Paracuru Formation, Xareu Field, and Cretaceous Period. Another sample (#3), from Potiguar Basin (Brazil), was also analyzed.

2.2. Sample Fractionation. Saturated hydrocarbons, aromatic hydrocarbons, and NSO (nitrogen-, sulfur-, and oxygen-) compound fractions of the oils were obtained using liquid chromatography (LC) with previous asphaltene separation.[4] Next, the isolation of cyclic and branched saturated hydrocarbons from the saturated hydrocarbon fraction was realized, using column chromatography with preactivated silicalite as the stationary phase and hexane as the eluent.[25] The samples were dissolved in dichloromethane and injected in the gas chromatograph.

2.3. Instrumentation and Chromatographic Conditions. The GC × GC-TOFMS system used in this study was a Pegasus 4D instrument (Leco, St. Joseph, MI, USA), composed of an Agilent 6890 GC (Palo Alto, CA, USA) equipped with a secondary oven and a nonmoving quad-jet, dual-stage modulator. Data acquisition and processing were performed using ChromaTOF software version 4.0 (Leco, St. Joseph, MI, USA). The GC column set consisted of a HP-5 ms (Hewlett-Packard, Palo Alto, CA, USA), 5%-phenyl-95%-methylsiloxane (30 m, 0.25 mm i.d., 0.25 μm d_f) as the first dimension (1D) column and a BPX50 (Austin, TX, USA), 50%-phenyl-50%-methylsiloxane (1.5 m, 0.1 mm i.d., 0.1 μm d_f) as the second dimension (2D) column. The second column was connected to the TOFMS by means of an empty deactivated capillary (0.5 m × 0.25 mm i.d.). The columns and the empty deactivated capillary were connected by SGE unions (Austin, TX, USA) using SilTite metal ferrules (Austin, TX, USA) for 0.10−0.25 mm i.d. GC columns. The GC and MS conditions followed published experimental settings.[19]

To calculate hopane/sterane ratio, the oil samples were injected in a GC-MS system, according to previous work.[4]

Biomarkers were monitored in the extracted ion chromatograms (EIC) using *m/z* 191 (tri-, tetra- and pentacyclic terpanes), *m/z* 177 (C-10 demethylated terpanes), *m/z* 205 (methylhopanes, C_{30} 25-norhopanes, and C_{31} homohopanes), *m/z* 369 (28-norspergulanes), *m/z* 217 and 218 (ααα- and αββ-steranes, respectively), *m/z* 231 and 232 (methylsteranes), *m/z* 259 (diasteranes and tetracyclic polyprenoids), and *m/z* 558 (β-carotane). The identification was performed by examination and comparison with literature mass spectra, retention times, and elution orders.[1−4,18−21,26]

3. RESULTS AND DISCUSSION

The present work evaluated the biomarker separation and identification, mainly methylhopanes, using GC × GC-TOFMS. Although some studies in the literature have analyzed petrochemical samples without fractionation,[8,12−14] Mao and coworkers[15] showed that a separation of saturated and aromatic hydrocarbons by HPLC (high-performance liquid chromatography) was essential for elucidating the composition of motor oil by GC × GC. Other works in the literature also analyze petrochemical samples using GC × GC hyphenated with LC prefractionation.[16,17] In the present work, saturated hydrocarbons were also separated from crude oils by LC. After that and with the use of silicalite in a second LC column, it was possible to isolate branched and cyclic compounds free from *n*-paraffins, which interfere in biomarker analyses.[1,2,25]

3.1. Identification of Terpanes. C_{19} to C_{26} Tricyclic, C_{24} tetracyclic, and C_{27} to C_{35} pentacyclic terpanes, including hopanes and gammacerane (Gam), were detected in the samples. According to the analysis conditions adopted, it could be possible to identify up to the C_{40} tricyclic terpane (Tr_{40}), but only C_{19}−C_{26} tricyclic terpanes were found in reasonable amounts in these oils.

Among C_{19}−C_{23} tricyclic terpanes, some stereoisomers were found. Demethylated tricyclic terpanes were not detected, while some demethylated hopanes were found (C_{27}−C_{32} 25-norhopanes).

Among the regular hopanes, the identified structures included the C_{27} to C_{35} 17α(H),21β(H)-hopane series (αβ-hopanes, H_{27}−H_{35}) as well as C_{29} to C_{35} 17β(H),21α(H)-hopanes (βα-hopanes or moretanes, M_{29}-M_{35}). The early eluting homohopane and homomoretane are the 22S epimers.[27] Changes in stereochemistry of the hopanes resulting from maturity differences can be used in petroleum exploration to provide information on the maturity of potential source rocks and oils.[3] The presence of appreciable amounts of extended moretanes indicates the low maturity of the studied oils.

The hopane configurations can be distinguished by their fragmentation patterns in GC-MS. Similarly to literature,[1,2] the αβ-hopane

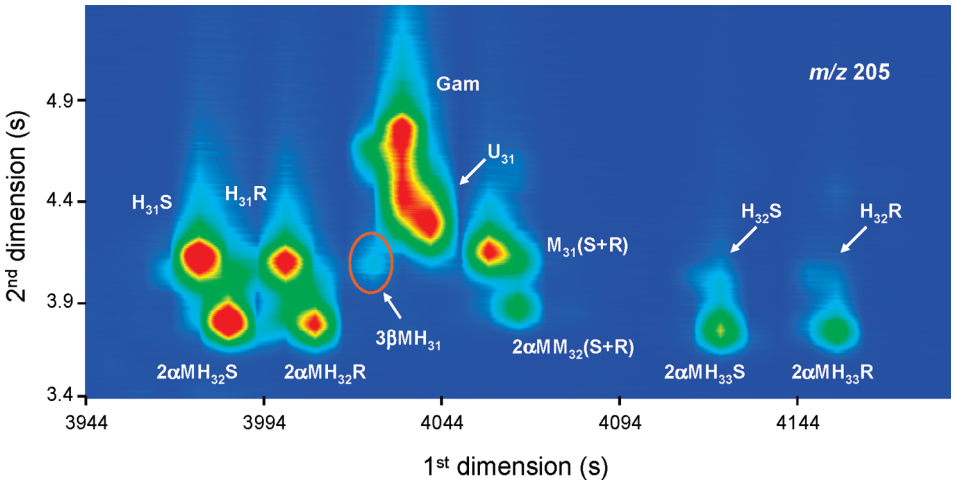

Figure 2. GC × GC-TOFMS m/z 205 EIC of sample #1. $H_{31}S$: C_{31} 22S 17α(H),21β(H)-homohopane; $H_{31}R$: C_{31} 22R 17α(H),21β(H)-homohopane; $2\alpha MH_{32}S$: C_{32} 22S 2α-methyl,17α(H),21β(H)-homohopane; $2\alpha MH_{32}R$: C_{32} 22R 2α-methyl-17α(H),21β(H)-homohopane; $3\beta MH_{31}$: C_{31} 3β-methyl-17α(H),21β(H)-hopane; Gam: gammacerane; U_{31}: C_{31} unknown terpane; M_{31}(S + R): C_{31} (22S+22R) 17β(H),21α(H)-homohopanes (C_{31} homomoretanes); $2\alpha MM_{32}$(S + R): C_{32} (22S + 22R) 2α-methyl-17β(H),21α(H)-homohopanes (C_{32} methylhomomoretanes); $H_{32}S$: C_{32} 22S 17α(H),21β(H)-bishomohopane; $H_{32}R$: C_{32} 22R 17α(H),21β(H)-bishomohopane; $2\alpha MH_{33}S$: C_{33} 22S 2α-methyl-17α(H),21β(H)-bishomohopane; $2\alpha MH_{33}R$: C_{33} 22R 2α-methyl-17α(H),21β(H)-bishomohopane. Although the 2α-methylhopanes occur as $C_{28}-C_{36}$ and 2α-methylmoretanes occur as $C_{30}-C_{35}$, only the distributions of the C_{32} and C_{33} isomers are presented.

isomers showed a stronger m/z 191 response for the A + B ring portion of the molecule than that for the m/z 148 + X (side-chain) fragment ion for the D + E ring portion; on the other hand, moretanes presented a more intense 148 + X fragment ion (D + E ring portion) compared to the hopanes. This difference was useful to distinguish hopane from moretane series.

The 22R and 22S diastereoisomers of the homohopanes ($H_{31}-H_{35}$) and homomoretanes ($M_{32}-M_{35}$) were well resolved in 1D; however, C_{31} homomoretanes (M_{31}S and M_{31}R) coeluted in 1D and were not resolved in 2D under the analytical conditions (Figure 2). Co-elution of this moretane pair is common on apolar columns.[23,27]

Among rearranged hopanes, 18α(H)-neohopanes (Ts, C_{29}Ts, C_{30}Ts), 17α(H)-diahopanes ($C_{29}-C_{31}$), C_{29} 28-norspergulane, and C_{24} des-A-hopane were found.

3.2. Identification of Methylhopanes. The C-2 methylated hopane series with an intense m/z 205 fragment ion was found in the Ceará Basin oils (Figure 2), presenting less abundance than the hopanes. These compounds eluted earlier in the second column and were almost totally separated from the regular hopanes in the m/z 205 EIC. The 2α-methylhopanes occurred as a series extending from C_{28} to C_{36} with carbon number distributions comparable with those of the regular hopanes (hopanes from C_{27} to C_{35}; $H_{27}-H_{35}$) plus one carbon. This last carbon is referring to the C-2 methyl group. The 2α-methylmoretanes series extended from C_{30} to C_{35}. The methylhopane contents (based on the peak intensities) decreased with increasing carbon number. Eigenbrode and co-workers[5] also observed this behavior in rock sample extracts from Hamersley Province on Pilbara Craton, Western Australia.

Besides 2α-methylhopanes, C_{30} 25-norhopanes (not shown), C_{31} hopanes (H_{31}), and moretanes (M_{31}) were also detected at m/z 205 (EIC) because of the D + E ring portion of these compounds. In GC-MS analysis, it would be impossible to detect C_{32} methylhopanes ($2\alpha MH_{32}$) and methylmoretanes ($2\alpha MM_{32}$) due to interference with H_{31} and M_{31} in 1D, respectively; while using GC × GC, these compounds were separated in 2D (Figure 2).

Similarly, the methylated analogues of C_{31} moretanes ($2\alpha MM_{32}$S and $2\alpha MM_{32}$R) were not resolved even in 2D under the conditions used herein (Figure 2). On the other hand, resolution between $C_{33}-C_{35}$ 2α-methylmoretanes and all of the 2α-methylhopane pairs (22R, 22S) was observed.

Linear relationships from 1D GC × GC-TOFMS were obtained between the retention times (s) and carbon numbers of members of the homohopane (diastereoisomers 22S: $y = 161.6x - 1042$; 22R: $y = 171.4x - 1322$) and homomoretane series (diastereoisomers 22S: $y = 162.2x - 1010$; 22R: $y = 172.8x - 1304$) and also 2α-methylhomohopane (diastereoisomers 22S: $y = 157.6x - 1067$; 22R: $y = 168x - 1376$) and 2α-methylhomomoretane series (diastereoisomers 22S: $y = 158.4x - 1014$; 22R: $y = 168.8x - 1345$). $C_{27}-C_{30}$ Hopanes and $C_{29}-C_{30}$ moretanes showed a uniform retention time shift compared to their 2α-methylated analogues. Linear relationships for homohopanes/2α-methylhomohopanes have already been reported by Summons and Jahnke,[20] but not for homomoretanes/2α-methylhomomoretanes.

The same procedure for differentiating regular hopanes and moretanes by mass spectral analysis can also be applied to their methylated analogues. 2α-Methylmoretanes also presented more intense 148 + X (D + E ring portion) fragment ions compared to their 2α-methylhopane counterparts. Figure 3 shows mass spectra of C_{34}S ($2\alpha MH_{34}$S) and C_{34}R 2α-methylhopanes ($2\alpha MH_{34}$R), and C_{34}R 2α-methylmoretane ($2\alpha MM_{34}$R). No significant differences were observed between the spectra of the 2α-methylhopanes or between other methylhopane diastereoisomer pairs. However, it is important to note the higher intensities of the m/z 233 fragment ion for C_{34}R 2α-methylmoretane. Both 2α-methylhopanes and 2α-methylmoretanes have a characteristic m/z 383 fragment ion, corresponding to an alkyl side-chain loss. Similarly, between regular hopanes/moretanes, the greater abundance of methylhopanes was observed, although the significant presence of methylmoretanes can also indicate a low maturity of the samples.

Methylhopane composition varies widely due to variable bacterial inputs to the original depositional environments. Higher

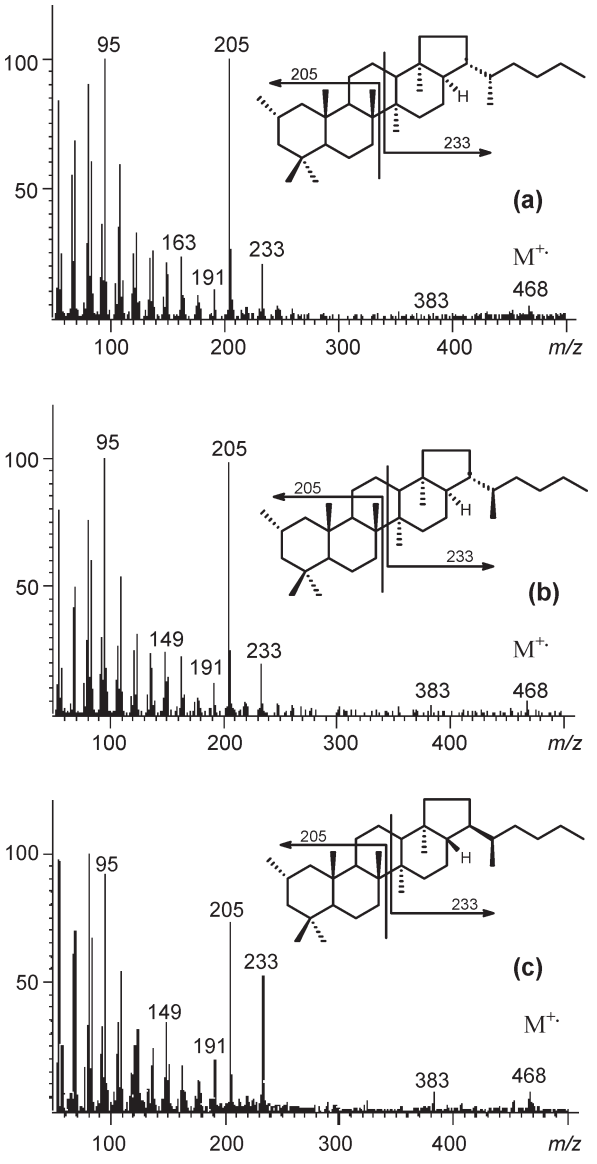

Figure 3. Mass spectra of $C_{34}S$ 2α-methylhopane (a), $C_{34}R$ 2α-methylhopane (b), and $C_{34}R$ 2α-methylmoretane (c).

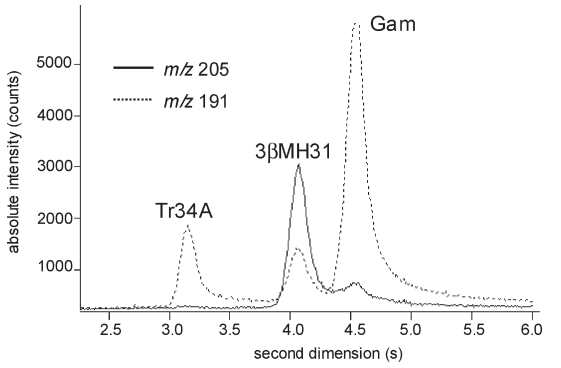

Figure 4. Chromatogram expansion of sample #3 showing separation in 2D between a C_{34} tricyclic terpane ($Tr_{34}A$), C_{31} 3β-methylhopane ($3\beta MH_{31}$), and gammacerane (Gam) in the m/z 191 and 205 (EIC).

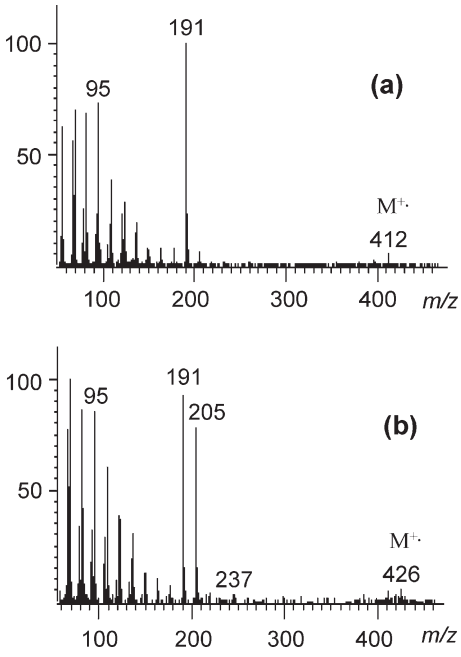

Figure 5. Mass spectra of C_{30} (a) and C_{31} (b) late-eluting terpanes.

proportions of 2α-methylhopanes, which originate from cyanobacteria, generally characterize marine samples.[6] Lacustrine oils and source rocks exhibit a diverse methylhopane composition, but some, which represent a distinct source facies, are enriched in 3β-methylhopanes.[6] In the present work, the 2α-methylhopane and 2α-methylmoretane series were predominantly found. Based on GC-MS analysis, these samples presented a hopane/sterane ratio lower than 1.6, corroborating that they are of marine origin.

Regarding the 3β-methylhopane series, only C_{31} 3β-methyl-17α(H),21β(H)-hopane ($3\beta MH_{31}$), which was less abundant than its isomer, 2α-methylhopane ($2\alpha MH_{31}$), was detected. C_{31} 3β-Methylhopane ($3\beta MH_{31}$) eluted between $C_{31}R$ homohopane ($H_{31}R$) and C_{31} homomoretanes ($M_{31}R$ + S) in 1D (Figure 2), being less volatile that its 2α-methylhopane isomer ($2\alpha MH_{31}$). In addition, C_{31} 3β-methylhopane had a similar interaction to the regular hopanes in 2D. In 1D, this compound coeluted with gammacerane (Gam) and a C_{34} tricyclic terpane ($Tr_{34}A$), but the three compounds were satisfactorily separated and well resolved in 2D (Figure 4). This coelution between the three terpanes was observed for a lacustrine oil sample (#3) from another Brazilian Basin (Potiguar), which is more abundant in 3β-methylhopanes. For the marine samples used in the present work (Ceará Basin), tricyclic terpanes were detected up to C_{26} (Tr_{26}). This result is significant for unequivocal characterization of oils based on gammacerane content, because GC × GC eliminated the presence of interfering compounds.

3.3. Terpane Novel Series?. Some unknown terpanes, probably a homologous series (C_{30}−C_{34}), were found in the chromatographic plane with higher retention times than those of hopanes (comparing compounds of both families with the same carbon number) either in 1D or 2D. They presented a more intense m/z 191 fragment ion, but their tentative identification as terpanes was achieved by monitoring the 148 + X fragment ion. As for the moretanes and methylmoretanes, the D + E ring portion was very useful for detection of these novel extended chain terpanes. Only one peak and not a pair was observed for these compounds, as is the case for hopanes and moretanes. A linear

dx.doi.org/10.1021/ef1013659 |*Energy Fuels* 2011, 25, 1060–1065

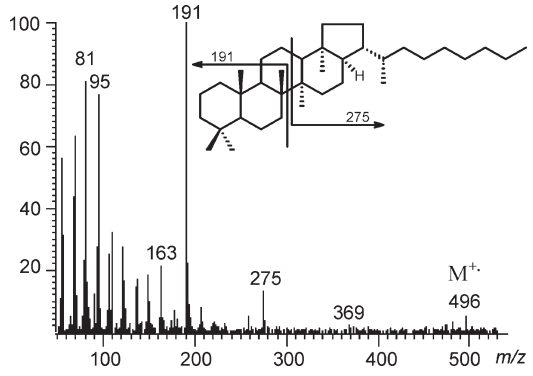

Figure 6. Mass spectrum of a proposed C_{36}S homohopane.

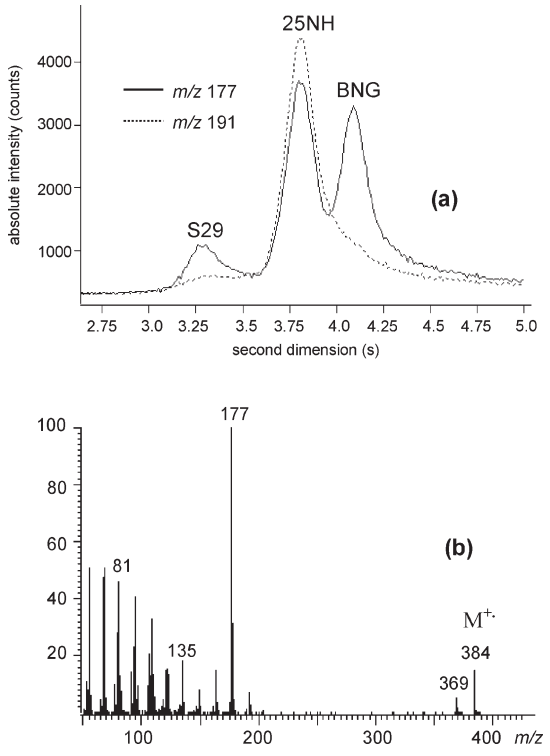

Figure 7. Chromatogram expansion of sample #2 showing separation between a C_{29} sterane (S_{29}), C_{29} 25-norhopane (25NH), and the possible bisnorgammacerane (BNG) in the *m/z* 177 and 191 (EIC) (a); mass spectrum of the proposed bisnorgammacerane (b).

relationship from 1D GC × GC-TOFMS was obtained between the retention times (s) and carbon numbers of the members of this series ($y = 170.4x - 1034$). The C_{30} late-eluting terpane coeluted with C_{31} homomoretanes ($M_{31}S + R$) in 1D (not shown); however, it was separated in 2D. The mass spectra of the C_{30} and C_{31} late-eluting terpanes are shown in the Figure 5.

3.4. Other Biomarkers. In the analysis of $C_{27}-C_{29}$ regular steranes, the four stereoisomers (ααα*S*, αββ*R*, αββ*S*, and ααα*R*), C_{30} and C_{31} methylsteranes, and C_{27} diasteranes (βα*S*, βα*R*, αβ*S*, and αβ*R*) were identified. One C_{26} sterane (norcholestane), C_{30} tetracyclic polyprenoids, and β-carotane were also identified.

A compound pair eluting after C_{35}R homomoretane ($M_{35}R$), probably C_{36} homohopanes (H_{36}), showed typical fragmentation of hopanes (more intense *m/z* 191 fragment ion) and has an *m/z* 275 fragment ion, which may correspond to the 148 + X fragment ion. Figure 6 shows the mass spectrum of a compound proposed as being the C_{36}S homohopane ($H_{36}S$). The diastereoisomers ($H_{36}S$ and $H_{36}R$) presented similar mass spectra. $C_{27}-C_{35}$ Hopanes are derived from the conversion of bacteriohopanetetrol present in bacteria during catagenesis.[3] The presence of a C_{36} hopane is not common; however other unknown precursors could exist. Extended hopanes beyond C_{40} have been reported in Liaohe Basin, China.[28]

An important separation between a C_{29} sterane, the C_{29} 25-norhopane (25NH), and a C_{28} bisnorterpane at *m/z* 177 (EIC) was observed (Figure 7a). The mass spectrum of the latter (Figure 7b) is similar to 25,30-bisnorgammacerane, which was previously found eluting next to C_{29} 25-norhopane during 1D-GC analysis of a nonbiodegraded Chinese oil.[29] In the *m/z* 191 (EIC), the interference of the sterane and bisnorgammacerane (BNG) over 25-norhopane was eliminated. The late-elution in 2D suggests that this C_{28} terpane may be a bisnorgammacerane (suggested as being the 25,28-bisnorgammacerane) because its probable precursor, gammacerane, also presents a late-elution in the second column than that of regular hopanes.

4. CONCLUSIONS

GC × GC-TOFMS for biomarker analysis in petroleum promoted the separation of hopanes and methylhopanes that are poorly separated using a GC-MS system. In the Brazilian oils under investigation, methylhopane and methylmoretane series were tentatively identified. The presence of 2α-methylhopanes in oils from Ceará Basin indicates oxygen-producing cyanobacteria were important biological inputs in the deposition environment. The combination of improved separation and enhanced response with GC × GC technology allowed for tentative identification of these compounds. With the discovery of novel biomarkers and their series, it is possible to build new parameters for unequivocal geochemical characterization of oil.

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ACKNOWLEDGMENT

We thank CNPq, the Brazilian National Research Council for fellowships. This study was supported by Cenpes/Petrobras (contract 0050.0043105.08.4), FAPERJ, and FUJB. We thank Energy & Fuels reviewers for comments, which improved greatly the manuscript.

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