# Significance of infrared spectroscopic branching factor for investigation of structural characteristics of alkanes, geochemical properties and viscosity of oils

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*Abstract*: A detailed investigation of significance of the infrared (IR) spectroscopic branching factor ($CH_2/CH_3$; the ratio of methylene and methyl group peak heights at 2917–2921 and 2951–2954 cm$^{-1}$, respectively in the IR spectra) for characterization of alkane structure, geochemical properties and viscosity of 76 oil samples was performed. These oils, originating from 13 Serbian oil fields in SE Pannonian Basin, differ according to source and depositional environment of organic matter (OM), as well as by thermal maturity and biodegradation stage. Methylene and methyl asymmetric stretching peak absorbances were used for the branching factor calculation. $CH_2$ peak positions exhibited 3–4 cm$^{-1}$ red shift with increasing the $CH_2/CH_3$ ratio, due to a greater contribution of *trans vs. gauche* rotamers in aliphatic chains. Comparing IR spectra of the oils and model *n*-alkanes, it was established that the average $(CH_2)_n$ methylene chain length per $CH_3$ group varied from $n = 3.5$ to 6.5. The $CH_2/CH_3$ ratio showed significant concordance with geochemical parameters, enabling clear distinction of the oils according to source and depositional environment of OM. At the same time, dependence of the $CH_2/CH_3$ ratio on oil maturity in the range from immature to mature was not observed, allowing for an accurate determination of oil genetic types irrespective of maturity. The $CH_2/CH_3$ ratio showed good accordance with oil biodegradation scale and oil viscosity.

*Keywords*: infrared spectroscopy; alkane branching; oil genetic types; viscosity; Serbian oil fields; Pannonian Basin.

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## INTRODUCTION

Normal and branched alkanes, as the most thoroughly studied components of crude oil, play a crucial role in organic geochemistry, helping to genetically relate oils and source rocks.[1,2] Normal to branched alkane ratio is also an important characteristic of crude oil feedstock, determining detonability and performance characteristics of gasoline, kerosene pour point and diesel fuel flammability.[3]

Infrared spectroscopy is a rapid and robust analytical technique, which avoids the use of hazardous organic solvents. The method has a long and fruitful application history with group structure analysis of oil, allowing to collect information on all of its components regardless of class and molecular weight.[4–6] Methyl and methylene absorption bands are unhindered by aromatics and other functionalities peaks, thus offering several approaches to quantify a degree of alkane branching.[7]

For oil hydrocarbons, the stretching vibrations of CH bonds in the 2800 to 3000 cm$^{-1}$ region are the strongest. Zenker's early work on long-chain aliphatics[8] demonstrated linear dependence of the $CH_2/CH_3$ asymmetric stretching peak ratio on $(CH_2)_n$ methylene chain length for $n = 5$–18. This ratio was subsequently widely applied to characterize aliphatics branching in saturated fraction, polar fraction and asphatlenes in crude oils,[9–11] bitumens,[12] coals[13,14] and shales,[15,16] due to its convenient greater-than-unity scale and clear physical meaning as a measure of the average length of unsubstituted $(CH_2)_n$ methylene chain per methyl group.

Considering the importance of alkane branching for organic geochemistry and oil refining, the authors believe that potential of the spectroscopic $CH_2/CH_3$ branching factor (representing the ratio of methylene and methyl group peak heights at 2917–2921 cm$^{-1}$ and 2951–2954 cm$^{-1}$, respectively in the IR spectra) has so far been largely underutilized. For the most part, the $CH_2/CH_3$ ratio from IR experiments has been used solely as an indicator of the relative abundance of methylated saturated hydrocarbons.[17–19] Therefore, the paper is aimed at a more detailed investigation of the significance of this parameter for the characterization of alkane structure, geochemical properties and viscosity of oils.

## EXPERIMENTAL

*Samples*

A set of 76 oil samples originating from 13 Serbian oil fields in SE Pannonian Basin was studied. The oils in this set differ according to source and depositional environment of precursor OM, as well as thermal maturity and biodegradation degree.

Experiments were performed on water-free crude oils, obtained by refluxing of well fluid at 60 °C for 48 h, and on individual model *n*-alkanes: *n*-octane (Alfa Aesar, Germany, > 98 % pure); *n*-nonane and *n*-dodecane (Fluka, Germany, > 99 % pure); *n*-tetradecane, *n*-pentdecane, *n*-hexadecane and *n*-heptadecane (Chem-Lab NV, Belgium, > 99 % pure).

*Methods*

*Infrared spectroscopy (IR)*. Absorbance IR spectra were recorded on a Thermo Nicolet 380 FTIR spectrometer, equipped with a DTGS detector and a Smart Orbit Diamond ATR accessory, by averaging over 1024 scans in the 500–4000 cm$^{-1}$ interval at 4 cm$^{-1}$ resolutions using OMNIC software, without additional corrections or processing. Background single--beam spectrum of air was collected prior to each single-beam sample spectrum. High viscosity and strong absorption of the oils precluded spectra collection using cells for liquid samples and KBr tablets. These difficulties were alleviated by the attenuated total reflectance (ATR) technique, providing measurement convenience and respectable signal-to-noise ratio. Repeatability and accuracy were confirmed by comparing spectra obtained from triplicate measurements on the same sample, the root-mean-square coordinate difference (*RSMD*) of peak positions being less than 0.5 cm$^{-1}$.

*Gas chromatography (GC)*. GC analysis of whole oils was performed using a Chromatec Chrystal 9000 gas chromatograph (non-polar capillary CP-Sil 5 CB column, 30 m×0.53 mm, 1.5 μm film thickness, He carrier gas at 17.2 cm$^3$ min$^{-1}$ flow rate) with a flame ionization detector (FID). On-column injection was performed using an autosampler. Prior to injection, the samples were diluted with carbon disulfide in 1:10 ratio. The inlet was heated from 40 °C to 310 °C at a rate of 50 °C min$^{-1}$. The following oven temperature program was used: heating from 0 °C (with 2 min initial hold) to 305 °C, at a rate of 15 °C min$^{-1}$, and then isothermal at 305 °C for 57 min. FID temperature was 350 °C. Individual peaks were identified by comparison of their retention times with those of a standard mixture of hydrocarbons. Quantification of the compounds used for calculation of geochemical molecular parameters was performed by integrating peak areas using Chromatec Analytic software.

*Viscosity measurements*. Dynamic viscosity of oils was measured using an Anton Paar MCR 302 rheometer in a rotational mode, at a shear rate of 10 s$^{-1}$ and following a temperature program from 50–60 °C to 10–15 °C with a rate of cooling of 1 °C min$^{-1}$.

## RESULTS AND DISCUSSION

*Significance of the branching factor for structural characterization of oils*

Typical IR spectra of the samples are shown in Fig. 1. Assignment of the principal absorption bands is straightforward: 2951–2954/2917–2921 cm$^{-1}$ and 2868–2871/2849–2852 cm$^{-1}$ are CH$_3$/CH$_2$ asymmetric and symmetric stretching; 1462–1465 cm$^{-1}$ is CH$_2$ symmetric bending; 1456–1457 cm$^{-1}$ and 1376–1377 cm$^{-1}$ are CH$_3$ asymmetric and symmetric bending; 740–744 cm$^{-1}$ and 719–729 cm$^{-1}$ are (CH$_2$)$_3$ and (CH$_2$)$_{4+}$ rocking vibrations, respectively.[7] As opposed to other well-resolved CH$_2$ and CH$_3$ features, methyl symmetric stretching mode is evident in most spectra only in the form of a plateau. Tertiary CH vibrations absorb around 2890–2900 cm$^{-1}$ and are present as a weak shoulder on the low wavenumber slope of CH$_2$ asymmetric stretching peak.[7]

Classification of the oils into the groups is given in Tables I–III.

Near-baseline absorbance values in the 3000–3100 cm$^{-1}$ region, together with weak peaks around 1600 and 810 cm$^{-1}$ due to C=C and out-of-plane aromatic CH bending, respectively, point out to a low concentration of highly substituted aromatics in the oils studied, which is in agreement with a general trend of

alkylated aromatic rings prevalence over non-substituted ones in crude oils worldwide. Very weak absorbance around 1700 cm$^{-1}$ is registered in some samples, attesting to the presence of C=O stretching, that is in accordance with low acid numbers, not exceeding 0.4 mg KOH/g for non-biodegraded oils.

CH$_2$/CH$_3$ branching factor was calculated as the ratio of methylene and methyl group peak heights at 2917–2921 cm$^{-1}$ and 2951–2954 cm$^{-1}$, respectively. Peak heights were measured from a flat baseline drawn at 3100 cm$^{-1}$ absorbance value. From Fig. 1B it is evident that with increasing CH$_2$/CH$_3$ branching factor, both methylene peak positions shift to lower wavenumbers. According to Fig. S-1A and B of the Supplementary material to this paper, when the CH$_2$/CH$_3$ ratio increases from 2.0–2.4 to 3.0–3.4, the corresponding shift comprises 3–4 cm$^{-1}$, while methyl peak position essentially fluctuates between 2952 and 2954 cm$^{-1}$ without a pronounced trend (Fig. S-1C).
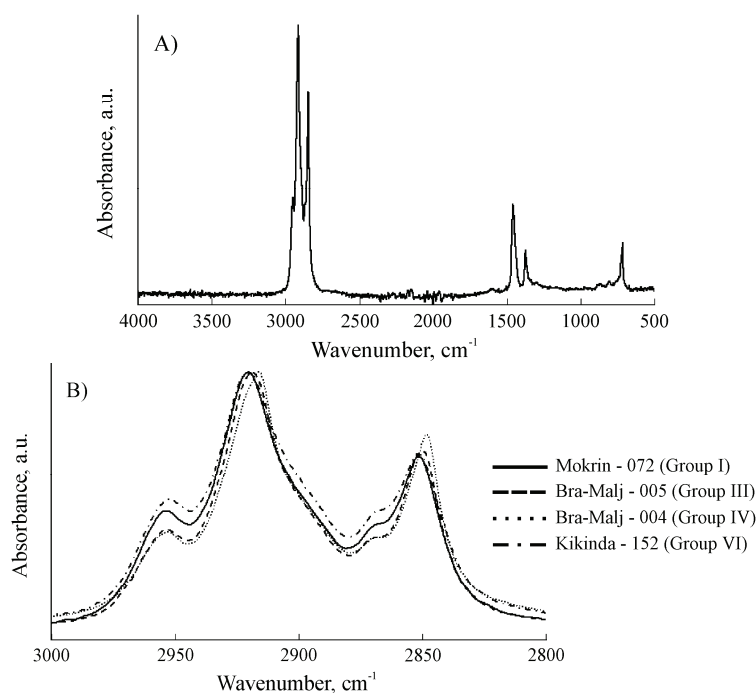


Fig. 1. A) Full IR spectrum of Maljurevac-Bubušinac-004 oil sample; B) stretching region of IR spectra of Mokrin-072 (group I), Bradarac-Maljurevac-005 (group III), Bradarac-Maljurevac-004 (group IV) and Kikinda-152 (group VI) samples.

From infrared studies of phospholipid cell membranes,[20] it is well known that CH$_2$ stretching vibrations are sensitive to the *trans*/*gauche* ratio in lipids' hydrophobic tales. Gel to liquid crystal phase transition upon temperature increase is accompanied by a 3–4 cm$^{-1}$ methylene peak shift to higher wavenumbers simi-

TABLE I. Geochemical data, the IR branching factor ($CH_2/CH_3$), geochemical parameters and the methylene to methyl group number ratio ($N(CH_2)/N(CH_3)$) for group 1 (aquatic origin, reducing environment, non-biodegraded) and group II (aquatic origin, oxic environment, non-biodegraded) oils; parameter average value and standard deviation (in parentheses)

| Group | Oil field | No. of samples | Oil maturity | $CH_2/CH_3$ | $LHCPI^a$ | $TAR^b$ | $Pr/Ph^c$ | $Pr/n\text{-}C_{17}^d$ | $Ph/n\text{-}C_{18}^e$ | $ACL$ $(n\text{-}C_1\text{-}n\text{-}C_{40})^f$ | $n$-Alkane maximum(s)$^f$ | $\Sigma(n\text{-}C_{10}\text{-}n\text{-}C_{40})/\Sigma(i\text{-}C_{13}\text{-}i\text{-}C_{20})^g$ | $N(CH_2)/N(CH_3)^h$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| I | Elemir | 1 | $M^i$ | 2.18 | 2.23 | 0.42 | 0.96 | 0.78 | 0.86 | 16.80 | $C_9$ | 5.09 | 4.02 |
| I | Kikinda-Varoš | 1 | M | 2.20 | 2.01 | 0.42 | 0.67 | 0.67 | 0.85 | 17.43 | $C_{14}$ | 5.94 | 4.05 |
| I | Mokrin (western part) | 2 | $MM^j$ | 2.22 (0.07) | 1.91 (0.01) | 0.49 (0.00) | 0.76 (0.01) | 0.77 (0.01) | 1.04 (0.02) | 17.63 (0.38) | $C_{14}$–$C_{15}$ | 4.89 (0.01) | 4.09 (0.13) |
| I | Palić | 13 | MM | 2.18 (0.03) | 2.36 (0.11) | 0.38 (0.03) | 0.74 (0.06) | 0.70 (0.05) | 1.00 (0.11) | 18.05 (0.77) | $C_{13}$–$C_{16}$ | 5.01 (0.42) | 4.01 (0.05) |
| I | Turija-sever (western part) | 7 | $IM^k$ | 2.22 (0.03) | 1.41 (0.09) | 0.38 (0.07) | 0.86 (0.02) | 0.98 (0.06) | 1.14 (0.07) | 19.66 (0.96) | $C_{15}$, $C_{19}$ | 5.26 (0.42) | 4.13 (0.05) |
| I | All group I oils | 24 | IM-M | 2.19 (0.04) | 2.03 (0.45) | 0.51 (0.19) | 0.79 (0.08) | 0.79 (0.14) | 1.04 (0.12) | 18.45 (1.15) | $C_9$, $C_{13}$–$C_{16}$, $C_{19}$ | 5.08 (0.37) | 4.04 (0.07) |
| II | Kikinda, Kikinda-Polje | 8 | M | 2.26 (0.03) | 2.49 (0.01) | 0.32 (0.02) | 1.14 (0.10) | 0.48 (0.04) | 0.48 (0.04) | 15.55 (0.47) | $C_8$, $C_9$, $C_{11}$, $C_{14}$ | 6.01 (1.26) | 4.17 (0.06) |
| II | Mokrin (central part) | 2 | M | 2.43 (0.01) | 2.78 (0.04) | 0.29 (0.01) | 1.14 (0.09) | 0.37 (0.07) | 0.38 (0.00) | 16.61 (0.32) | $C_{13}$ | 8.98 (0.03) | 4.50 (0.01) |
| II | Idoš (central part) | 3 | MM | 2.34 (0.08) | 1.54 (0.03) | 0.56 (0.03) | 1.38 (0.14) | 0.70 (0.03) | 0.59 (0.06) | 18.14 (0.54) | $C_{14}$–$C_{15}$ | 7.06 (0.40) | 4.32 (0.15) |
| II | All group II oils | 13 | MM-M | 2.31 (0.08) | 2.31 (0.46) | 0.37 (0.11) | 1.19 (0.13) | 0.51 (0.12) | 0.49 (0.08) | 16.31 (1.19) | $C_8$, $C_9$, $C_{11}$, $C_{13}$–$C_{15}$ | 6.71 (1.47) | 4.26 (0.14) |

$^a$Low vs. high carbon preference index of $n$-alkanes, calculated according to the following formula: $LHCPI = (n\text{-}C_{17}+n\text{-}C_{18}+n\text{-}C_{19})/(n\text{-}C_{27}+n\text{-}C_{28}+n\text{-}C_{29})$; $^b$terrigenous/aquatic ratio of $n$-alkanes, calculated according to the following formula: $TAR = (n\text{-}C_{27}+n\text{-}C_{29}+n\text{-}C_{31})/(n\text{-}C_{15}+n\text{-}C_{17}+n\text{-}C_{19})$; $^c$pristane (2,6,10,14-tetramethylpentadecane)/phytane (2,6,10,14-tetramethylhexadecane) ratio calculated from peak areas of these compounds in gas chromatograms; $^d$pristane (2,6,10,14-tetramethylpentadecane)/$n$-heptadecane ratio, calculated from peak areas of these compounds in gas chromatograms; $^e$phytane (2,6,10,14-tetramethylhexadecane)/$n$-octadecane, calculated from peak areas of these compounds in gas chromatograms; $^f$average chain length of $n$-alkanes; $^g\Sigma(n\text{-}C_{10}\text{-}n\text{-}C_{40})/\Sigma(i\text{-}C_{13}\text{-}i\text{-}C_{20}) = $ sum of $C_{10}$ to $C_{40}$ $n$-alkanes/sum of $C_{13}$ to $C_{20}$ regular isoprenoids; $^h$methylene to methyl group number ratio; $^i$mature; $^j$moderately mature; $^k$immature

TABLE II. Geochemical data, the IR branching factor ($CH_2/CH_3$), geochemical parameters and the methylene to methyl group number ratio ($N(CH_2)/N(CH_3)$) for group III (terrestrial origin, oxic environment, non-biodegraded), group IV (terrestrial origin, reducing environment, non-biodegraded) and group V (prevailing terrestrial origin, oxic environment, non-biodegraded) oils; parameter average value and standard deviation (in parentheses); for the abbreviations, see Table I

| Group | Oil field | No. of samples | Oil maturity | $CH_2/CH_3$ | LHCPI | TAR | Pr/Ph | $Pr/n\text{-}C_{17}$ | $Ph/n\text{-}C_{18}$ | ACL ($n\text{-}C_1$–$n\text{-}C_{40}$) | $n$-Alkane maximum(s) | $\Sigma(n\text{-}C_{10}\text{–}n\text{-}C_{40})/\Sigma(i\text{-}C_{13}\text{–}i\text{-}C_{20})$ | $N(CH_2)/N(CH_3)$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| III | Iđoš (western part) | 2 | IM | 3.03 (0.11) | 0.80 (0.04) | 1.07 (0.04) | 1.77 (0.36) | 0.65 (0.08) | 0.41 (0.12) | 21.33 (0.03) | $C_{27}$ | 11.91 (1.43) | 5.64 (0.22) |
| III | Sirakovo | 3 | MM | 3.14 (0.09) | 1.33 (0.07) | 0.61 (0.04) | 1.05 (0.06) | 0.29 (0.01) | 0.30 (0.03) | 20.60 (0.39) | $C_{15}$ | 12.59 (0.63) | 5.85 (0.18) |
| III | Bradarac-Maljurevac | 2 | IM-MM | 3.35 (0.13) | 1.07 (0.01) | 0.75 (0.01) | 1.30 (0.40) | 0.35 (0.03) | 0.31 (0.08) | 21.32 (0.86) | $C_{15}$ | 12.80 (0.24) | 6.25 (0.25) |
| III | All group III oils | 7 | IM-MM | 3.17 (0.16) | 1.10 (0.24) | 0.78 (0.21) | 1.33 (0.39) | 0.41 (0.17) | 0.33 (0.08) | 21.01 (0.57) | $C_{15}$, $C_{27}$ | 12.46 (0.80) | 5.90 (0.31) |
| IV | Maljurevac-Bubušinac All group IV oils | 5 | IM | 2.78 (0.12) | 0.92 (0.09) | 0.95 (0.11) | 0.60 (0.20) | 0.46 (0.07) | 0.65 (0.09) | 21.11 (0.87) | $C_{23}$, $C_{25}$, $C_{27}$ | 10.62 (1.65) | 5.15 (0.24) |
| V | Turija-sever (eastern part) All group V oils | 7 | IM | 2.60 (0.03) | 0.93 (0.06) | 1.00 (0.09) | 1.08 (0.10) | 0.63 (0.05) | 0.63 (0.09) | 20.50 (0.50) | $C_{14}$, $C_{15}$, $C_{29}$, $C_{31}$ | 9.36 (0.56) | 4.82 (0.06) |

lar in magnitude to the one observed in the current study. The shift reflects higher tale mobility and disorder, as well as appearance of *gauche* conformations in the initially all-*trans* polymethylene fragments. In turn, quantum mechanical calculations predict significant lowering of *trans* to *gauche* transition energy barrier of linear alkanes upon methylation,[21] which is in line with methylene peak behavior from our IR spectra. dos Santos *et al.*[22] similarly concluded that the 2–3 cm–1 shift of methylene peaks to lower wavenumbers upon rising bitumen crystallinity is due to an increase of the *trans/gauche* ratio in long-chain alkanes comprising the bulk of this material.

Spectra of individual $C_8$–$C_{17}$ model *n*-alkanes were used to establish linear fit parameters for the dependence of methylene to methyl group number ratio, $N(CH_2)/N(CH_3)$ on the branching factor from IR measurements (Fig. S-1D). Correlation factor $r = 0.999$ is on par with fit quality for other model alkane series.[10,12] According to Fig. S-1D, $CH_2/CH_3$ branching factor values between 2 and 3.5, established for the studied set of oils, correspond to $N(CH_2)/N(CH_3)$ variation from 3.5 to 6.5.

*Significance of the $CH_2/CH_3$ branching factor for characterization of geochemical properties and viscosity of oils*

Typical gas chromatograms of studied oils are shown in Fig. S-2 of the Supplementary material. In the majority of the samples (Fig. S-2A and B), *n*-alkanes are predominant compounds, indicating that these oils are not biodegraded. Microbially altered oils of 2nd–4th stage of biodegradation[23] were also analyzed (Fig. S-2C and D), in order to determine the influence of biodegradation on the $CH_2/CH_3$ factor. Geochemical parameters[24–27] calculated from distributions of *n*-alkanes and regular isoprenoids, obtained by GC analysis of whole oil, are listed in Tables I–III.

Their values, in combination with our previous detailed investigations of other wells in the corresponding oil fields,[28-32] allowed a classification of the studied oils into 8 groups. All investigated oils are of mixed aquatic-terrestrial origin, but considerably differ according to the contribution of aquatic- *vs*. land--plants biomass, depositional environment of precursor OM, thermal maturity and biodegradation degree (Tables I–III).

Since the oils of the 2nd stage of biodegradation (group VI; Fig. S-2C) still contain *n*-alkanes and isoprenoids, geochemical parameters were calculated (Table III) as for non-biodegraded counterparts (Tables I and II). However, these values should be considered with great caution due to the destruction of original alkanes' distributions by microbial activity. Groups VII and VIII comprise the oils of the 3rd–4th and 4th degree of biodegradation, in which *n*-alkanes are almost absent and isoprenoids are either absent or their distributions are remarkably altered (Fig. S-2D), thus disabling any calculation of the parameters (Table III).

TABLE III. Geochemical data, the IR branching factor ($CH_2/CH_3$), geochemical parameters and the methylene to methyl group number ratio ($N(CH_2)/N(CH_3)$) for group VI (aquatic origin, reducing environment, $2^{nd}$ stage of biodegradation), group VII (aquatic origin, reducing and oxic environments, $3$–$4^{th}$ and $4^{th}$ stage of biodegradation) and group VIII (terrestrial origin, reducing environment, $4^{th}$ stage of biodegradation) oils; parameter average value and standard deviation (in parentheses); N.C. – not calculated due to the absence of components, HM – highly mature; for other abbreviations, see Table I

| Group | Oil field | No. of samples | Oil maturity | $CH_2/CH_3$ | LHCPI | TAR | Pr/Ph | Pr/$n$-C$_{17}$ | Ph/$n$-C$_{18}$ | ACL ($n$-C$_1$–$n$-C$_{40}$) | $n$-Alkane maximum(s) | $\Sigma(n$-C$_{10}$–$n$-C$_{40})/\Sigma(i$-C$_{13}$–$i$-C$_{20})$ | $N(CH_2)/N(CH_3)$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| VI | Kikinda All group VI oils | 3 | M | 2.05 (0.05) | 1.90 (0.53) | 0.59 (0.07) | 0.94 (0.04) | 2.29 (0.85) | 2.63 (0.91) | 18.44 (0.07) | C$_5$-C$_6$ | 2.00 (0.53) | 3.77 (0.03) |
| VII | Kikinda, Kikinda-Polje | 2 | M | 1.97 (0.05) | N.C. | N.C. | N.C. | N.C. | N.C. | N.C. | N.C. | N.C. | 3.62 (0.09) |
| VII | Velebit | 13 | HM | 1.94 (0.01) | N.C. | N.C. | N.C. | N.C. | N.C. | N.C. | N.C. | N.C. | 3.57 (0.02) |
| VII | All group VII oils | 15 | M-HM | 1.95 (0.02) | N.C. | N.C. | N.C. | N.C. | N.C. | N.C. | N.C. | N.C. | 3.57 (0.04) |
| VIII | Jermenovci All group VIII oils | 2 | IM | 2.55 (0.08) | N.C | N.C. | N.C. | N.C. | N.C. | N.C. | N.C. | N.C. | 4.71 (0.15) |

The data from Tables I and II show that the oil groups clearly differ according to the IR $CH_2/CH_3$ branching factor, which continuously increases upon addition of terrigenous precursor material, enriched in long-chain $n$-alkanes and derived from epicuticular waxes of land plants. Lower values of the $CH_2/CH_3$ ratio for group I and II oils are consistent with prevalence of short- and mid-chain $n$-alkanes, as well as greater content of methyl groups originating from methyl- and dimethylalkanes, typical for aquatic OM sources (*e.g.* cyanobacteria and *Botryococcus braunii* race A).[2]

In order to compare the results derived from IR and GC, a correlation diagram of the $n$-alkanes to regular isoprenoids ratio *vs.* the $CH_2/CH_3$ branching factor was designed for 56 non-biodegraded oils belonging to groups I–V (Fig. 2A). To avoid the influence of evaporation and water washing on light hydrocarbons, $n$-alkanes containing less than 10 carbon atoms were excluded.

Consequently, the $n$-alkanes to isoprenoids ratio (as a "branching factor" derived from GC) represents the ratio between the sum of $C_{10}$ to $C_{40}$ $n$-alkanes and the sum of $C_{13}$ to $C_{20}$ acyclic regular isoprenoids: $\Sigma(n\text{-}C_{10}\text{–}n\text{-}C_{40})/\Sigma(i\text{-}C_{13}\text{–}i\text{-}C_{20})$, assigned as "$n$-C/$i$-C" in the further text. Significant correlation between the two methods (IR and GC) is evident from Fig. 2A. The oils of predominantly aquatic origin (groups I and II) have values of $CH_2/CH_3 < 2.5$ and $n$-C/$i$-C $< 8$, with the exception of two marginal samples of group II (Mokrin oil field; $CH_2/CH_3 = 2.43$ and $2.44$; $n$-C/$i$-C $= 8.96$ and $9.00$, respectively). On the other hand, the oils which prevalently originate from terrestrial biomass show values of the above-mentioned parameters higher than 2.50 and 8.00, respectively (Tables I and II).

Besides that, the data from Fig. 2A indicate poorer correlation between IR and GC branching factors for the oils of predominantly aquatic origin (groups I and II, with a correlation coefficient $r = 0.76$, at a significance level $p = 99.9$ %), comparing to the oils of prevalent terrestrial source (groups III-V: $r = 0.86$, $p = 99.9$ %). It is in accordance with the fact that terrestrial OM is enriched in long-chain $n$-alkanes and contains very low amounts of methyl- and dimethylalkanes. Consequently, in the oils from groups III–V, methyl-substituents mainly originate from isoprenoids.[2] Therefore, the obtained result indicates that the correlation of $n$-C/$i$-C and $CH_2/CH_3$ factors can be used for an assessment of contribution of aquatic and terrestrial biomass in the precursor OM, in parallel with the numerical values of these parameters.

Furthermore, the data from Fig. 2A show that significant correlation between $n$-C/$i$-C and $CH_2/CH_3$ factors exists up to $CH_2/CH_3 \approx 3$ for dominantly terrestrially sourced oils, *i.e.*, for the oils in groups IV and V (Table II). With a further increase of content of methylene *vs.* methyl groups, the branching factor derived from GC ($n$-C/$i$-C) attains a plateau for the oils with the highest contribution of terrigenous OM deposited in the oxic environment (group III oils). This can be

attributed to the fact that routine GC analysis allows the identification of hydrocarbons up to $C_{40}$, whereas IR considers higher molecular weight *n*-alkanes as well, which were observed in group III oils during a determination of paraffin content using a distillation method (BS EN 12606-1).[33]
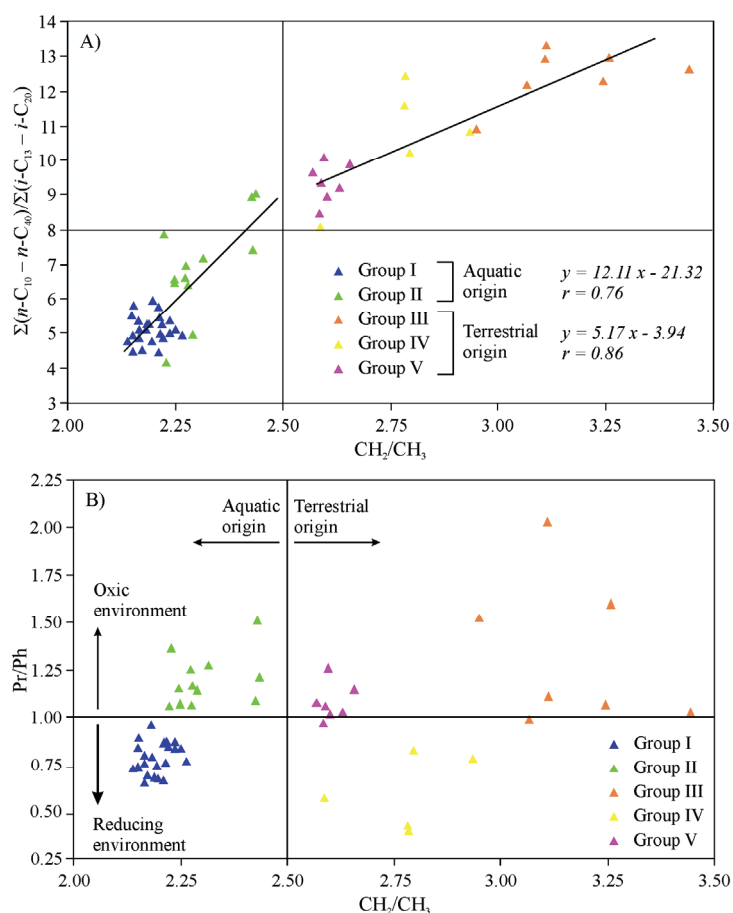


Fig. 2. A) Dependence of the *n*-C/*i*-C on the $CH_2/CH_3$ branching factor and B) the Pr/Ph *vs.* $CH_2/CH_3$ diagram, which indicates redox conditions of OM depositional environment. $CH_2/CH_3$ – the branching factor (the ratio of methylene and methyl group peak heights at 2917–2921 cm⁻¹ and 2951–2954 cm⁻¹, respectively in the IR spectra); Pr/Ph – pristane/phytane ratio, calculated from peak areas of these compounds in gas chromatograms.

The IR branching factor also demonstrated sensitivity to a depositional environment of oil precursor OM. Namely, a clear increase of the $CH_2/CH_3$ factor is associated with the rising oxidizing properties of depositional environment for all the investigated oils, independently on their prevalent origin (aquatic or terrestrial; Fig. 2B).

The $CH_2/CH_3$ branching factor is not substantially affected by thermal maturity in the range from immature to mature oils. This is reflected through almost identical average values of the $CH_2/CH_3$ branching factor for immature (Turija--sever), moderately mature (Palić, Mokrin) and mature (Elemir) oils belonging to group I; very similar average values for moderately mature (Iđoš) and mature (Kikinda) group II oils; and very similar average values for immature (Iđoš) and moderately mature (Sirakovo) group III oils (Tables I and II). This can be explained by the fact that epimerization at chiral C-atoms, isomerization and rearrangement of methyl groups in the rings all together have an impact on molecules of biomarkers (resulting in formation of thermodynamically more stable isomers) during transformations from immature to mature oils,[2] but these processes do not affect the $CH_2/CH_3$ ratio. A cracking of the long-chain alkanes producing shorter homologues can result in insignificant lowering of the $CH_2/CH_3$ ratio; however, simultaneous reactions of degradation of side chains of cyclic and polycyclic hydrocarbons and/or their successive aromatization followed by demethylation[2] contribute to certain increasing of the ratio, resulting in the observed constancy of the $CH_2/CH_3$ branching factor. Therefore, in a maturity range from immature to mature oils (corresponding to vitrinite reflectance of source rocks, *Rr* in range 0.60–0.80 %), the $CH_2/CH_3$ branching factor can be considered as an effective indicator of the origin and depositional environment of oils' precursor OM.

Biodegradation also affects the $CH_2/CH_3$ factor independently on origin and maturity of oils. Predominantly aquatically sourced, mature oils of the 2nd stage of biodegradation from the Kikinda oil field (group VI) have lower values by *ca.* 0.20 of the $CH_2/CH_3$ branching factor comparing to non-biodegraded oils from the same oil field (group II; Tables I and III). Prevalently aquatically sourced group VII oils, *i.e.*, mature Kikinda oil of the 3rd–4th stage of biodegradation and highly mature Velebit oil attaining the 4th stage of biodegradation, as well as immature terrigenous oils of the 4th stage of biodegradation (Jermenovci oil field, group VIII), have values of the $CH_2/CH_3$ branching factor 0.25–0.30 lower than non-biodegraded oils of the same genetic type (groups I and IV, respectively; Tables I–III). The obtained results are in concordance with the biodegradation scale,[23] according to which normal alkanes are most prone to microbial alteration among all aliphatic hydrocarbons present in oils, being followed by branched methylated alkanes, including isoprenoids. Having the similar origin, oils of the VI and VII groups exhibit negligible decrease of the $CH_2/CH_3$ branching factor from the 2nd to the 4th biodegradation stage (Table III), which can be explained by the fact that microbes primarily attack alkylnaphthalenes and alkylphenanthrenes in this range of biodegradation,[23] which does not influence infrared aliphatic stretching region.

Oils from groups III and IV, having the highest $CH_2/CH_3$ branching factor, showed a notable increase of viscosity at temperatures below 40 °C. Group V oils

exhibited analogous viscosity growth at temperatures below 35 °C, whereas for group II and particularly for group I oils no intensive viscosity increase was observed even at lower temperatures (Fig. 3).
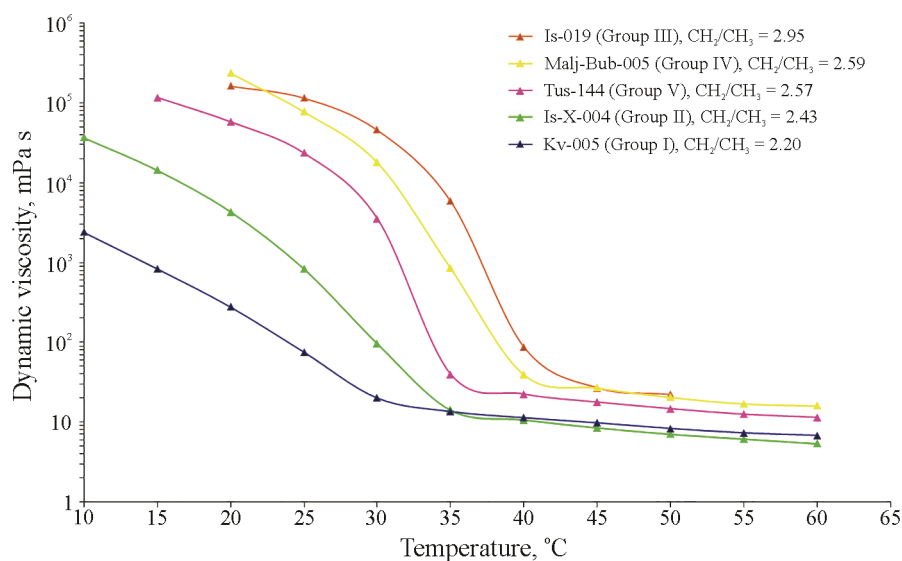


Fig. 3. Temperature dependence of dynamic viscosity for representative oil samples from groups I–V, reflecting variations of rheological behavior with the $CH_2/CH_3$ branching factor; Is-019 – Iđoš-019; Malj-Bub-005 – Maljurevac-Bubušinac-005; Tus-144–Turija-sever-144; Is-X-004–Iđoš X-004; Kv-005–Kikinda-Varoš-005. The lowest temperature of measurements differs, because the measurement is possible down to temperature 15 °C lower than the oil pour point.

This indicates that high viscosity is primarily related to a substantial content of high molecular weight *n*-alkanes in oils. It is typical for immature terrigenous oils having $CH_2/CH_3 > 2.75$, enriched in solid paraffins, which have been insignificantly affected by cracking due to their high thermal stability and low oil maturity.

CONCLUSION

Increase of the $CH_2/CH_3$ branching factor determined from IR measurements on studied oil samples set resulted in a 3–4 cm$^{-1}$ shift of methylene stretching peaks to lower wavenumbers for alkanes containing on average from 3.5 to 6.5 methylenes per methyl group as established by comparison with IR spectra of $C_8$–$C_{17}$ model *n*-alkanes. This effect may be explained by a corresponding increase of the *trans*/*gauche* conformer ratio of aliphatic chains.

The $CH_2/CH_3$ branching factor showed significant concordance with geochemical parameters, enabling clear distinction of oils according to the source

and depositional environment of precursor OM. On the other hand, dependence of the $CH_2/CH_3$ branching factor on oil maturity in the range from immature to mature (corresponding to vitrinite reflectance of source rocks, *Rr* in range 0.60––0.80 %) was not observed, thus allowing the accurate determination of the oil genetic type irrespective of maturity. Values of the IR $CH_2/CH_3$ branching factor and the ratio between sum of *n*-alkanes and sum of acyclic regular isoprenoids, $\Sigma(n\text{-}C_{10}\text{–}n\text{-}C_{40})/\Sigma(i\text{-}C_{13}\text{–}i\text{-}C_{20})$, derived from GC, as well as their mutual correlation are useful for the assessment of contribution of aquatic *vs.* terrestrial land plant biomass in the oil precursor OM.

Good accordance between the $CH_2/CH_3$ branching factor and the oil biodegradation level up to the 4th stage of biodegradation was confirmed. Also, it was established that a notable increase of viscosity at temperatures below 40 °C is typical for immature oils of predominantly terrestrial origin, having values of the $CH_2/CH_3$ branching factor above 2.75.

Therefore, using a relatively large oil sample set in the current study, quantitative data relating the IR $CH_2/CH_3$ branching factor with structural characteristics of alkanes, geochemical properties and viscosity of oils were constituted.

## SUPPLEMENTARY MATERIAL

Additional data and information are available electronically at the pages of journal website: https://www.shd-pub.org.rs/index.php/JSCS/article/view/11114, or from the corresponding author on request.

ИЗВОД
### ЗНАЧАЈ ИНФРАЦРВЕНО-СПЕКТРОСКОПСКОГ ФАКТОРА РАЧВАЊА ЗА ИСТРАЖИВАЊЕ СТРУКТУРНИХ КАРАКТЕРИСТИКА АЛКАНА, ГЕОХЕМИЈСКИХ СВОЈСТАВА И ВИСКОЗИТЕТА НАФТИ

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Урађено је детаљно испитивање значаја инфрацрвено (IC)-спектроскопског фактора рачвања, $CH_2/CH_3$ (однос висина пикова метиленских и метил група на 2917–2921 cm^{-1} и 2951–2954 cm^{-1}, редом, у IC спектрима) за карактеризацију структуре алкана, геохемијских својстава и вискозитета нафти. Испитивано је 76 узорака нафти из 13 нафтних поља у Србији у југоисточном делу Панонског басена. Нафте се разликују према пореклу и средини таложења прекурсорске органске супстанце (OS), као и према степену термичке матурисаности и степену биодеградације. За израчунавање фактора рачвања коришћене су апсорбанције пикова који одговарају антисиметричном истезању метиленских и метил група. Код положаја $CH_2$ пикова запажено је померање за 3–4 cm^{-1}

ка већим таласним дужинама са порастом односа CH₂/CH₃, које потиче од већег допри-
носа *trans* у односу на *gaushe* изомере у алифатичним ланцима. Поређењем IC спектара
нафти и стандардних *n*-алкана, утврђено је да средња дужина метиленског низа, $(CH_2)_n$
по једној CH₃ групи варира за *n* од 3,5 до 6,5. Однос CH₂/CH₃ је показао значајну
сагласност са геохемијским параметрима и омогућио прецизно разликовање нафти
према пореклу и средини таложења прекурсорске OS. Истовремено, зависност односа
CH₂/CH₃ од матурисаности нафти у опсегу од незрелих до зрелих није запажена, што
омогућава тачно одређивање генетског типа нафте, невезано од степена зрелости. Однос
CH₂/CH₃ је показао добру сагласност са скалом биодеградације и вискозитетом нафте.

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