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Comparison of the composition of some petroleum samples which may be applied for skin and mucous membrane treatment

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Abstract

A particular Croatian petroleum (sample P1) and its three derivatives (samples P2, P3 and P4), potentially applied as healing preparations for skin and mucous membrane treatment, were studied in order to learn their composition and to discriminate them according to two criteria: composition of natural petroleum compounds, and lacking aromatics. Elemental (C, H, N and S) and group composition (by LC, UV/VIS, IR and ^{1}H NMR) were determined and the single component distributions were analyzed (by GC) and identified (by GC-MS). Focussed saturated compounds (n-alkanes, pristane and phytane, drimanes/eudesmanes, steranes and hopanes) were studied in order to emphasize the preservation or destruction of genuine petroleum structures in derivatives. Samples P2 (petroleum-brownish color, petroleum like smell) and P3 (colorless, transparent, slight pine-like odor), were found, now constituting petroleum, to still be composed of the components of their native structure. Compared to sample P1, they were missing light and heavy compounds. While sample P2 contained different compound classes, sample P3 comprised exclusively saturated hydrocarbons, satisfying pharmacopoeia's requirement regarding the low aromatics content. Almost a half of sample P3 was composed of cyclic moieties, including terpenoids, possibly responsible for the odor. Samples P1, P2 and P3 were found rather rich in steranes. Sample P4 (colorless, transparent, no smell) was found denaturalized. In spite of high similarity in bulk properties to sample P3, it comprised no detectable amount of n-alkanes, pristane and phytane, or drimanes/eudesmanes, steranes and hopanes (although found rich in oligocycles).

Keywords: Biological markers; Croatian petroleum composition; Naftalan; Naphthalane; Petroleum healing preparations; Preparations for skin and mucous membrane treatment

1. Introduction

Petroleum (oil) and its derivatives are known [1,2] to be rather complex mixtures of organic

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compounds, predominantly composed of carbon and hydrogen, and some hetero-atoms (most frequently nitrogen, sulphur and oxygen). Petroleum constituents belong to different compound classes: to saturates (composed of acyclic and cyclic, naphthene, moieties), to aromatics (compounds containing at least one aromatic ring) and to polars (besides carbon and hydrogen they constitute at least one hetero-atom). Petroleum components with high molecular mass, which precipitate in n-alkanes, are classified as asphaltenes.

Today, there are plenty of published data [1–3] on the composition of petroleum. As analytical tools improve regarding separation and sensitivity, the number of components thought to compose petroleum increases. Some classes of oligocyclics which belong to the so-called biological markers, are extensively reported [4–7]. Those are complex organic compounds which, during the process of petroleum generation in nature, have retained the chemical skeleton characteristics of the parent organic molecules (of biological origin). They often occur among the variety of stereoisomers which are described as changing sensitively indicating the different stresses which they had suffered [7].

For centuries, petroleum has been considered to possess curative properties, being thought to accelerate skin and mucous membrane cell regeneration. Baths and preparations as ointments, pastes, liniments and creams have been applied. Azerbaijani petroleum, called naphthalane, was reported [8] to be used for antimicrobial and healing preparations for burns and injuries, for treatment of eczema, arthritis and neurodermitis, and against skin fungi and parasites. Naphthalane baths are used for rheumatism and gout treatment and were also reported to be effectively applied for some gynecological diseases related to vagina, uterine wall and tubes. Saturated petroleum oligocycles (naphthenes) were considered to be the main active ingredients in disease treatment. Therapeutical effects of naphthalane petroleum and its preparations were intensively studied [9–13].

Some encouraging results were reported [11] in the treatment of psoriasis vulgaris and neurodermitis with preparations derived from a Croatian naphthalane petroleum. The necessity of precaution in health service makes intensive research obligatory in order to enjoy the natural advantages and to minimize predictive hazards. In order to evaluate the Croatian naphthalane petroleum realistically, an interdisciplinary study has been started, hoping to bring some elucidation to the complexity of the subject.

To the authors' knowledge, there are no published details on structure and composition of that particular Croatian petroleum nor on its derivatives which are supposed to be applied for medical preparations (for skin and mucous membrane treatment). For that reason the research started with sample composition, in order to learn and compare.

Comparison among the derivatives was focused on two criteria: to be low in aromatics and to be composed of natural petroleum compounds. As evidence for conservation of the native structures, some selected compound classes served.

Gas chromatography (GC) and gas chromatography-mass spectrometry coupled system (GC-MS) are the methods of choice for identification and comparison of the compounds in complex mixtures (composed of the constituents which can elute through GC column). Resolving the samples into components by GC and taking MS data for identification/characterization of the separated compounds, GC-MS offered, without any preseparation, insight into the structure of single components.

Beside GC and GC-MS no analytical tool (familiar and available to the authors) was found to be as appropriate for single component study of the petroleum derivatives. The applied IR and NMR spectrometries were found adequate for structural group analysis. In spite of the advanced spectroscopy approaches available today, spectroscopy methods were not found to be suitable for single compound identification even if sophisticated instrumentation were applied, because of the sample complexity (in addition to the presence of stereoisomers and homologues). Huge isolation procedures would be necessary, but hardly efficient enough for reliable results; it would be difficult to make it completely clear whether the potentially elucidated structure belonged to a single compound or to a mixture.

UV/VIS spectroscopy is an appropriate method for determination of low aromatics content.

In this paper structure and composition were investigated and compared prior to pharmacological and clinical research.

2. Experimental

2.1. Samples

A sample of genuine North Croatian petroleum (drilled from the Križ well in the Sava depression) (sample P1), which is used for manufacturing healing preparations, as well as three related derivatives (samples P2, P3 and P4), were studied (preparation procedures were not available). The samples were declared by density (ρ) at 15°C (ASTM D 1298) and by refraction index (n_D^{20}) (ASTM D 1218); sample P1: $\rho = (0.9583 \pm 0.0002)$ g/cm^3, n_D^{20}: 'no light transmission'; sample P2: $\rho = (0.9414 \pm 0.0002)$ g/cm^3, $n_D^{20} = 1.5215 \pm 0.0002$; sample P3: $\rho = (0.8819 \pm 0.0002)$ g/cm^3; $n_D^{20} = 1.4811 \pm 0.0002$; and sample P4: $\rho = (0.8807 \pm 0.0002)$ g/cm^3, $n_D^{20} = 1.4807 \pm 0.0002$.

Samples P1 and P2 had a dark petroleum-brownish color and smell typical for petroleum. Samples P3 and P4 were transparent and colorless. While sample P3 had a slight odor of pine resin, sample P4 was odorless.

2.2. Analytical procedures

Elemental analysis of carbon (C), hydrogen (H), nitrogen (N) and sulphur (S) in the samples was performed by Leco CHNS 932 Analyzer. Oxygen (O) was determined as the mass difference. Asphaltenes were checked by precipitation in n-heptane (IP 143/90). The deasphalted samples underwent separation into saturates (SAT), aromatics (ARO) and polars (POL) on the liquid chromatography column (LC) by means of solvents in the range of increasing polarity [14]. IR spectrometry (Perkin-Elmer 783) was used for functional group analysis (measured as a thin sample layer between the plates). The (low) aromatics content (expressed as absorption at 275 nm, scanning range: 260–420 nm) [15] was confirmed by UV/VIS spectrophotometry (Varian, Cary 1E). ^{1}H NMR (Varian EM-390) served for determination of the ratios: cyclic versus non-cyclic and straight versus branched moieties (solvent: $CDCl_3$, standard: tetramethylsilane) [16].

High resolution gas chromatography (GC) working conditions were as follows: GC (Pye Unicam 304); column: fused-silica, non-polar, DB-1, L 60 m; 50°C, 10°C/min to 300°C; injection: splitless mode 30 s for sample P1 ($\sim 10\%$ solution in n-hexane), and splitting mode for samples P2, P3 and P4; carrier gas: hydrogen; detection: flame ionization.

Gas chromatography-mass spectrometry (GC-MS) analysis was performed under the following conditions: GC (Varian 3700) column: fused-silica, non-polar, DB-1, L 60 m; 50°C, 6°C/min to 280°C; carrier gas: helium; GC-MS coupling: direct inlet; MS (Varian MAT 112S): scanning rate: 1 s/decade; interscan time: 0.2 s; ionization: electron impact; electron energy: 70 eV; emission current: 0.7 mA; resolution: 1:600; m/z range: 50–500; ion source temperature: 260°C; ion source pressure: 10^{-5} Pa.

In GC-MS analysis, full mass spectra and/or specific mass fragmentograms (m/z 57 for alkanes, 123 for drimanes, 217 and 218 for steranes and 191 for hopanoids) were used (Table 1). Published mass spectra and fragmentogram fingerprints [7,17] served in the component identification.

3. Results and discussion

Petroleum healing potential is ascribed to natural saturated oligocycles (of geogenic origin) [8], any derivation of which must not threaten the native structure which they had while they were composing genuine petroleum. At the same time, paraffinic preparations should be rather low in aromatics content [15].

Table 2 shows the bulk properties of the studied samples. Regarding elemental analysis, the uncolored samples P3 and P4 might be considered as fully composed of carbon and hydrogen. IR spectrometry confirmed the absence of the functional groups containing hetero-atoms. From these two samples neither aromatics nor polars

were eluted by LC. UV/VIS spectrometry found that absorption at 275 nm was $(0.043 \pm 0.003)\%$ and $(0.032 \pm 0.003)\%$ for P3 and P4, respectively, both satisfying the pharmacopoeia requirements (the absorption at 275 nm must not exceed 0.1%) [15] regarding the aromatics content. ^{1}H NMR analysis determined that roughly half of sample P3 (48.3%) and sample P4 (47.1%) was made of the cyclic moieties while the remainder consisted of the acyclic counterparts, found to be predominately branched (66.1% of the non-cycled moieties).

Table 1
Components used for comparison among the samples

Related to Fig. 2; mass fragmentogram m/z 57
n_{10}, n-decane
n_{11}, n-undecane
n_{12}, n-dodecane
n_{13}, n-tridecane
n_{14}, n-tetradecane
n_{15}, n-pentadecane
n_{16}, n-hexadecane
pr, pristane
ph, phytane
pp, unknown monocyclic compound with molecular mass 350

Related to Fig. 3; mass fragmentogram m/z 123
d_1, drimane/eudesmane
d_2, drimane/eudesmane
d_3, drimane/eudesmane
d_4, homodrimane/homoeudesmane
d_5, homodrimane/homoeudesmane
d_6, homodrimane/homoeudesmane
d_7, homodrimane/homoeudesmane

Related to Fig. 4; mass fragmentograms m/z 217 and 218 and to Fig. 5; mass fragmentogram m/z 217
ch_1, 14α(H),17α(H)-cholestane 20S
ch_2, 14β(H),17β(H)-cholestane 20R
ch_3, 14β(H),17β(H)-cholestane 20S
ch_4, 14α(H),17α(H)-cholestane 20R
er_1, 14α(H),17α(H)-ergostane 20S
er_2, 14β(H),17β(H)-ergostane 20R
er_3, 14β(H),17β(H)-ergostane 20S
er_4, 14α(H),17α(H)-ergostane 20R
st_1, 14α(H),17α(H)-stigmastane 20S
st_2, 14β(H),17β(H)-stigmastane 20R
st_3, 14β(H),17β(H)-stigmastane 20S
st_4, 14α(H),17α(H)-stigmastane 20R

Related to Fig. 6; mass fragmentogram m/z 191
nH, 17α(H)21β(H)-30-norhopane
H, 17α(H)21β(H)-hopane
hHS, 17α(H)21β(H)-29-homohopane 22S
hHR, 17α(H)21β(H)-29-homohopane 22R

In samples P1 and P2 sulphur, nitrogen and oxygen were present, while only sample P1 contained ash ($(2.1 \pm 0.1)\%$) and asphaltenes ($(3.4 \pm 0.2)\%$). By LC the colored samples produced fractions of saturates, aromatics and polars, all in significant proportions.

GC chromatograms (Fig. 1a–d) showed that all the studied samples presented unresolvable mixtures with highly overlapped peaks due to the composition complexity. Sample P1 was found comparatively rich in peaks at the light part of GC chromatogram (Fig. 1a). The basic shape characteristics of the medium part were preserved in GC chromatograms of samples P2 and P3 (Fig. 1b and c), indicating that the components which remained in samples P2 and P3 conserved their native geogenic structures. In spite of the differences in bulk composition, both samples P2 and P3 showed the GC chromatograms in bimodal shape and with the peak groupings such that the prominent peaks could be compared.

GC chromatogram of sample P4 (Fig. 1d) was not comparable to the other chromatograms (nor to that related to P3 which was found greatly similar in bulk properties), indicating that the components in P4 dramatically differ in structure; a denaturalization of the native geogenic petroleum compounds occurred.

The indications offered by the GC analysis were confirmed by GC-MS results. Because of the complexity of the sample compositions, the GC-MS study was focused on some specific (non-aromatic) compounds, positively known [1,2,7] to be of petroleum origin. The obtained data, in spite of being taken sporadically, were considered to reflect a general rule. The results served to emphasize the preservation or change of the structure of the compounds composing samples P2, P3 and P4.

Relying on the mass fragmentogram m/z 57 (Fig. 2), the homologous range of normal alkanes (commonly present in petroleum unless the biodegradation exceeds the rank classified as light [7]), was traceable in samples P1, P2 and P3. Pristane (pr) and phytane (ph) (petroleum acyclic

Table 2
Bulk composition of the samples[a]

Composition samples	Elemental composition					LC fractions		
	%C	%H	%N	%S	%O	%SAT	%ARO	%POL
P1[b]	85.1 ± 0.3	11.0 ± 0.1	0.6	0.7	0.5	44.6 ± 1.1	29.2 ± 0.7	22.8 ± 0.4
P2	86.2 ± 0.3	12.5 ± 0.1	0.4	0.5	0.4	53.6 ± 1.1	41.0 ± 0.7	5.4 ± 0.2
P3	86.5 ± 0.3	13.5 ± 0.1	ND	ND	NC	≅ 100	ND	ND
P4	86.3 ± 0.3	13.7 ± 0.1	ND	ND	NC	≅ 100	ND	ND

[a] NC, not calculated; ND, not detected.
[b] For sample P1: the difference in 100% in elemental composition was made of ash ((2.1 ± 0.1)%) and in LC fractions of asphaltenes ((3.4 ± 0.2)%).

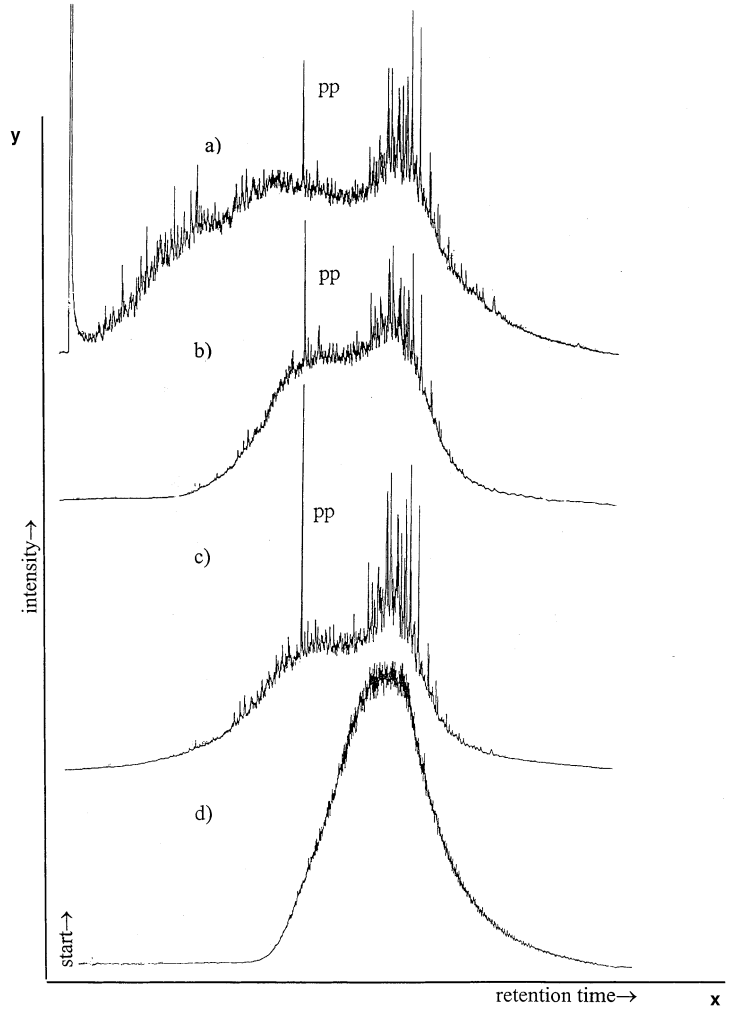

Fig. 1. GC chromatograms (working conditions as in Section 2; pp, prominent peak): (a) sample P1; (b) sample P2; (c) sample P3; (d) sample P4.

isoprenoid tetramers, present in any petroleum [1,2,7] if not highly biodegraded) were also found.

In the mass fragmentogram m/z 123 (Fig. 3), acluster of bicyclic sesquiterpenoids was identified in samples P1, P2 and P3. Four of them were found with molecular mass 208 (drimanes and/or eudesmanes, labeled d_1–d_4) and three with molecular mass 220 (homodrimanes and/or homoeudes

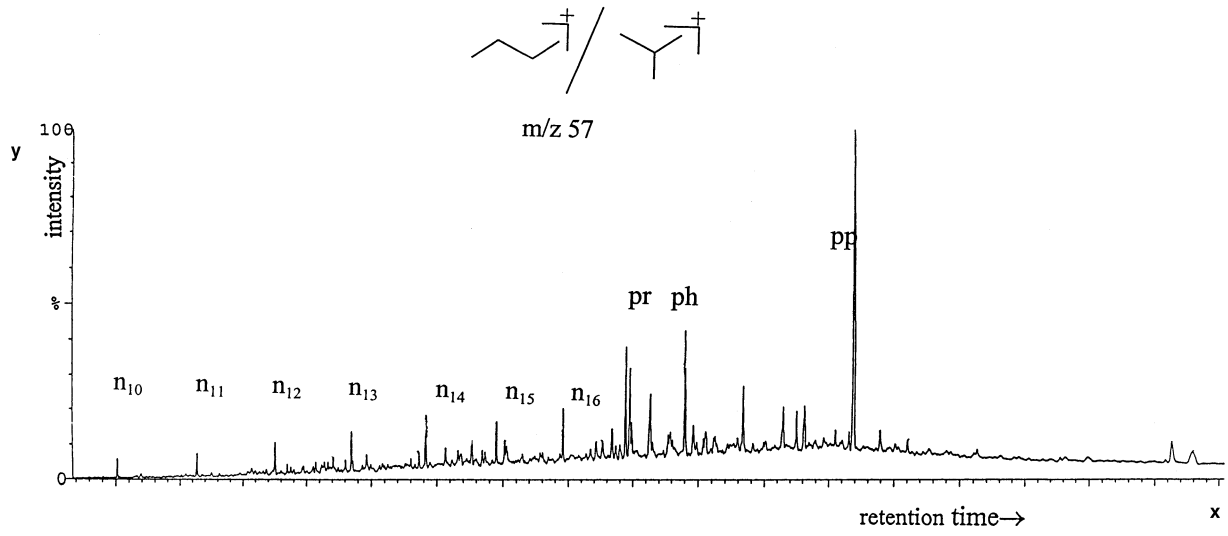

Fig. 2. Sample P3: mass fragmentogram m/z 57 for detection of alkanes (n, normal alkanes (the number in the subscript corresponds to the number of carbon atoms in molecule); ph, phytane; pp, prominent peak; pr, pristane).

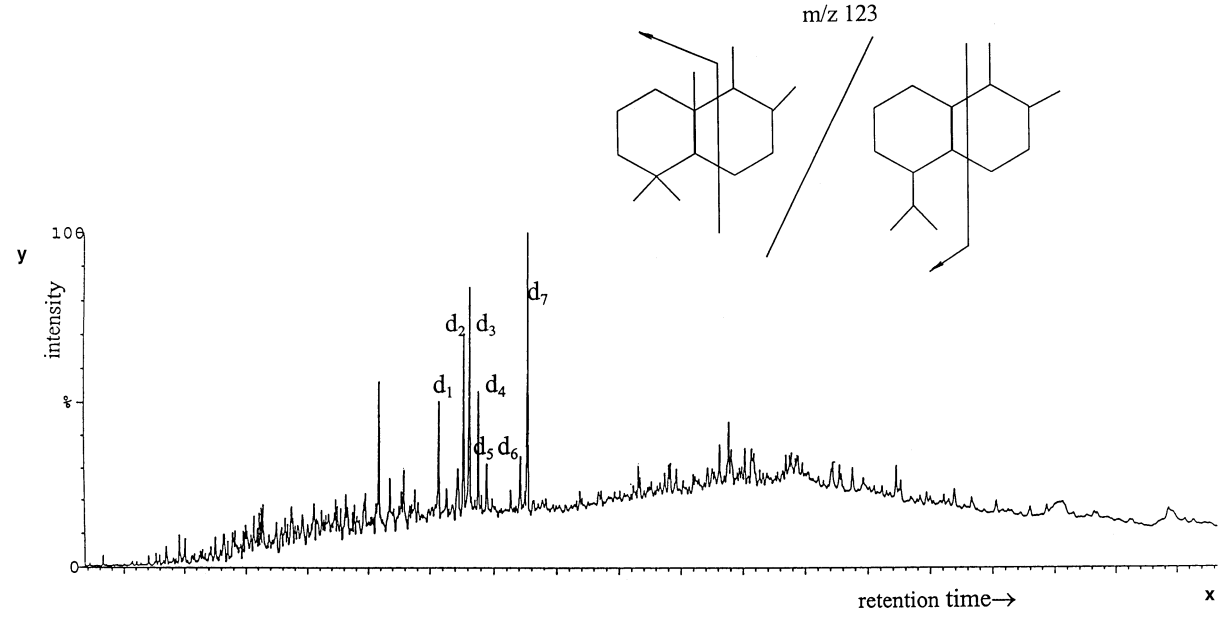

Fig. 3. Sample P3: mass fragmentogram m/z 123 for detection of drimane and/or eudesmane cluster (d_{1-3}, drimanes/eudesmanes; d_{4-7}, homodrimanes/homoeudesmanes).

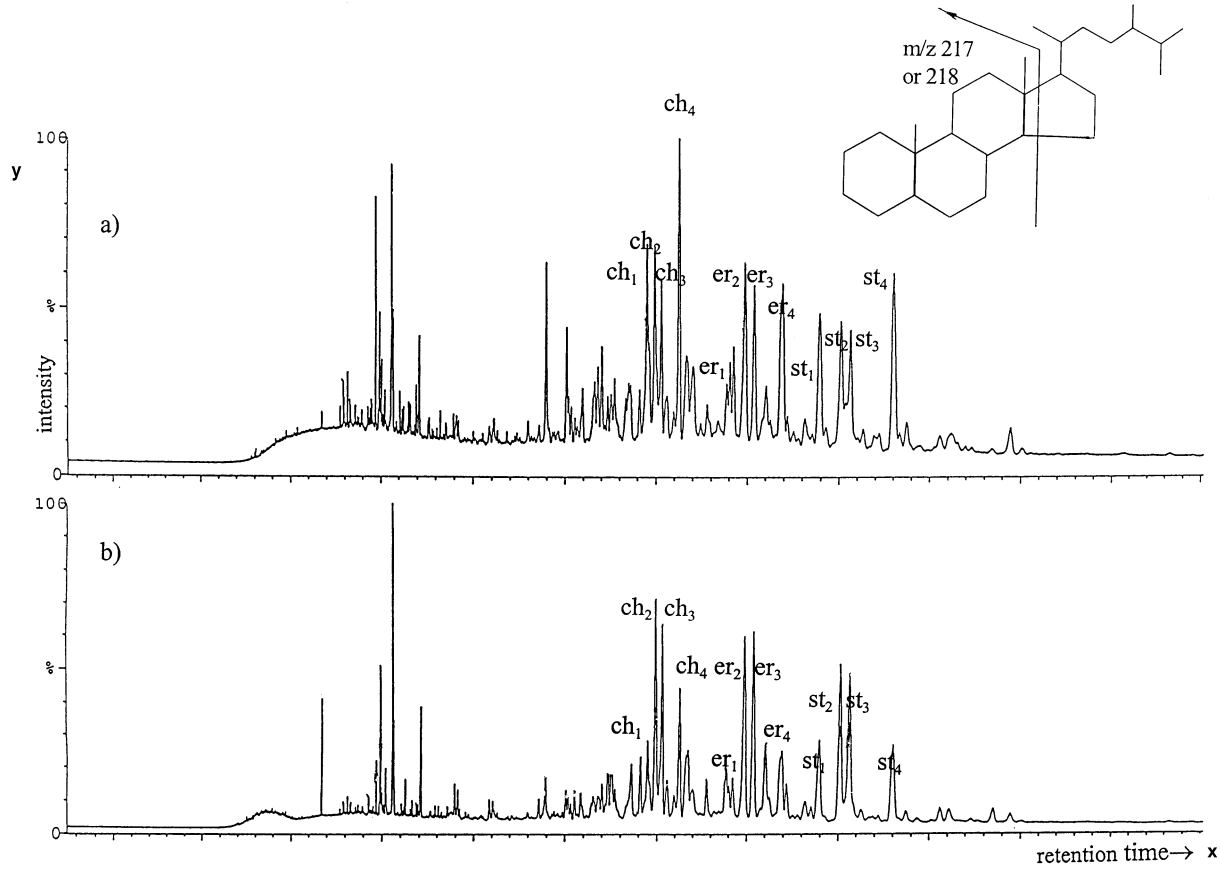

Fig. 4. Sample P3: (a) mass fragmentogram m/z 217 for detection of 14α(H) steranes (ch, cholestanes; er, ergostanes; st, stigmastanes; $_1$ = 14α(H),17α(H) 20S, $_2$ = 14β(H),17β(H) 20R, $_3$ = 14β(H),17β(H) 20S, $_4$ = 14α(H),17α(H) 20R); (b) mass fragmentogram m/z 218 for detection of 14β(H) steranes (labels as in Fig. 4a).

manes: d_5–d_7), corresponding to the data published on natural petroleum drimanes [7].

The remarkable group of peaks in the heavy part of GC chromatograms in Fig. 1a–c, was confirmed to belong predominantly to saturated steranes (Fig. 4a for 14α(H) and Fig. 4b for 14β(H)). All three typical groups of petroleum sterane homologs (cholestanes, $C_{27}H_{48}$ (ch_{1-4}); ergostanes, $C_{28}H_{50}$ (er_{1-4}); and stigmastanes, $C_{29}H_{52}$ (st_{1-4})), were identified in samples P1, P2 and P3.

A high similarity between the sterane fragmentogram in Fig. 4a and the sterane fragmentogram in Fig. 5 related to the original petroleum sample P1, offering a nice example of preservation of the genuine composition and structure of the compounds that remained in sample P3. The comparison showed that the native variety of isomers ($_1$14α(H),17α(H) 20S, $_2$14β(H),17β(H) 20R, $_3$14β(H),17β(H) 20S, $_4$14α(H),17α(H) 20R) was saved in the natural relative distribution of peak intensities (one sterane to other steranes). In the comparison of GC chromatograms (Fig. 1a–c), the sterane group of peaks seemed to be best presented in sample P3, most possibly due to the absence of aromatics and polars. Compared to the data published on the steranes content in different petroleums [2,7], samples P1, P2 and P3 seemed to be rather high in steranes. This fact might be interesting since saturated oligocycles were published [8] as the main healing ingredients in petroleum. Petroleum steranes saved in their skeleton the structure similarity to the related

bio-precursors, sterols (originating from the cell- and nucleus-membrane bilayer in eukaryota [7]), but they had lost (during the geological past) the hydroxyl group and the unsaturation.

Pentacyclic triterpenoids of petroleum origin were also confirmed in samples P1, P2 and P3 (Fig. 6). Hopane (H), nor-hopane (nH), *S*- and *R*-homohopanes C_{31} (ShH and RhH) were iden-

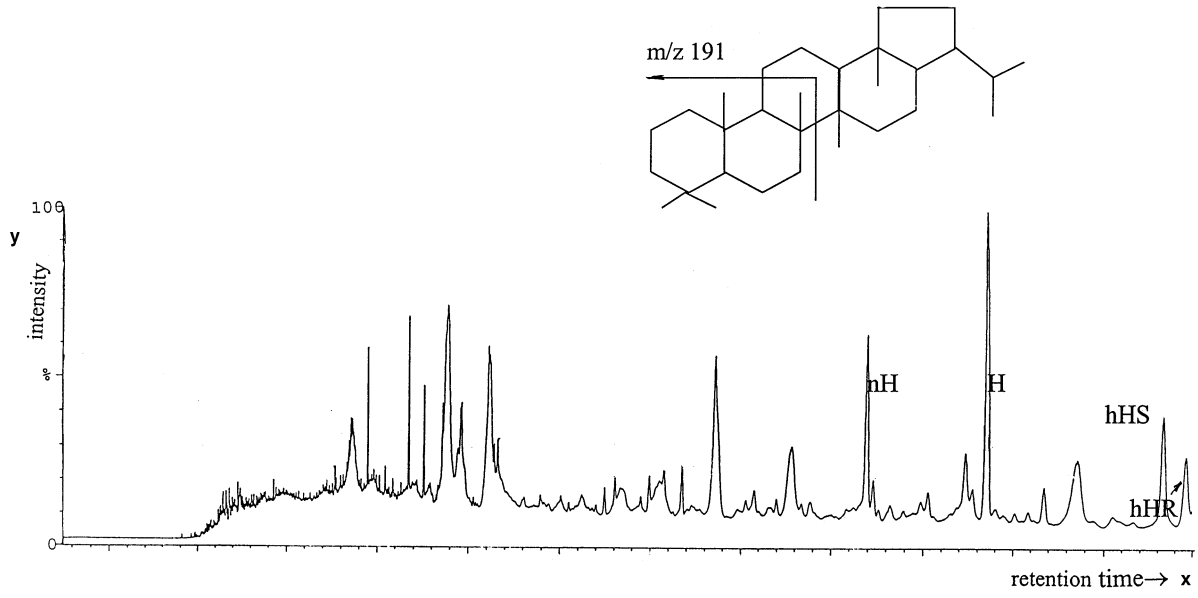

Fig. 5. Sample P1: mass fragmentogram m/z 217 for detection of 14α(H) steranes (labels as in Fig. 4a).

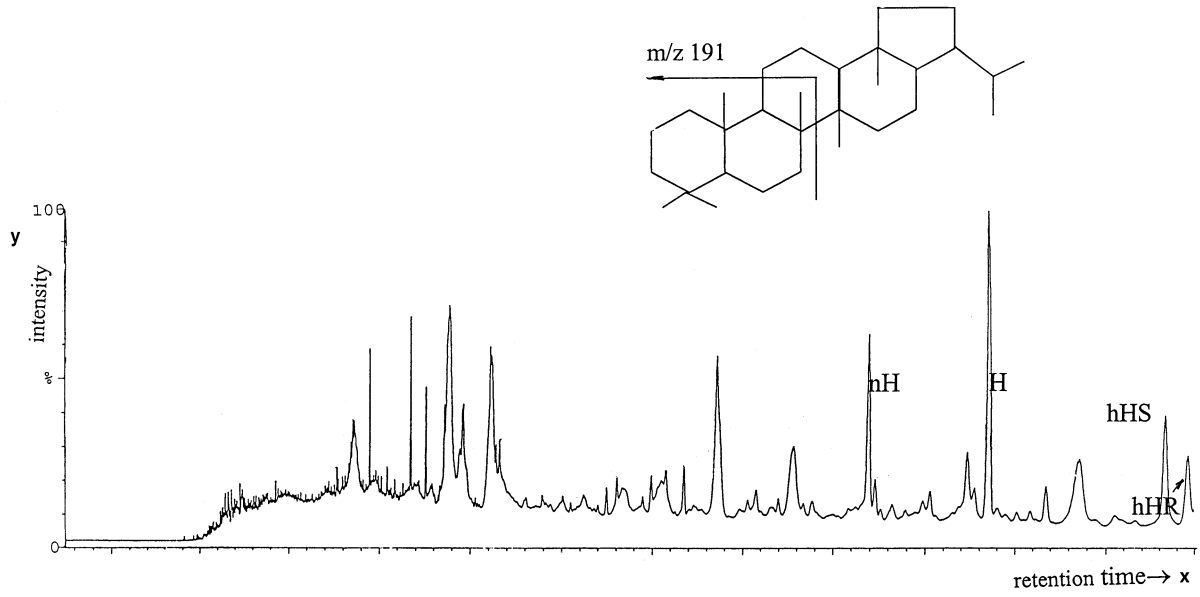

Fig. 6. Sample P3: mass fragmentogram m/z 191 for detection of hopanes (H, 17α(H),21β(H)-hopane; hHR, 17α(H),21β(H)-29-homohopane 22R; hHS, 17α(H),21β(H)-29-homohopane 22S; nH, 17α(H),21β(H)-30-norhopane).

tified (the higher homologs were not followed).

The prominent peak labeled as pp in Figs. 1 and 2, was not fully identified. Regarding the GC-MS spectrum of the pp peak, it might be a saturated monocyclic hydrocarbon with molecular mass of 350. According to the related GC retention and to the mass spectrum, it rather seemed to belong to the same compound present in the genuine petroleum sample P1 (in concentration $(1.3 \pm 0.1)\%$) as well as in samples P2 ($(2.9 \pm 0.1)\%$) and P3 ($(5.6 \pm 0.1)\%$).

Sample P4, in spite of being abundant in cyclic moieties, showed no recognizable finger-print pattern which might be ascribed to steranes, drimanes, or hopanoids. It showed neither n-alkane range nor isoprene tetramers pristane and phytane. No pp peak was indicated.

4. Conclusion

A selected Croatian petroleum thought to possess curative properties, and three related preparations, potentially used for skin and mucous membrane treatment, were studied in order to compare their compositions. The (non-colored) samples P3 and P4 were found to be composed of saturated hydrocarbons. Their aromatic compounds contents were low enough to satisfy the pharmacopoeia requirements and no compounds containing functional groups (comprising nitrogen, sulphur and oxygen) were detected. The (colored) sample P2 contained saturated and aromatic compounds, as well as the compounds with functional groups, while the genuine petroleum sample P1 additionally contained asphaltenes.

Some specific petroleum compounds (including biological markers), which were taken as evidence for the conservation of the native structures and of the natural relative intensity ratios among the components, occurred in samples P2 and P3 (just as in P1) in recognizable clusters of homologs and stereoisomers. For example, sample P3 conserved the saturated steranes in a variety of stereo-isomers and in relative intensities as they had had while they had been composing petroleum. The slight odor of sample P3 might be ascribed to the spared terpenoid constituents.

Comparated to the published data, the genuine Croatian petroleum and two of the related derivatives (samples P2 and P3) were found unusually rich in steranes which made unexpectedly intensive peak clusters in GC chromatograms (and in the related CG-MS fragmentograms). It should be studied more in detail whether that fact makes the particular Croatian petroleum possess curative properties as reported.

Sample P3 might be expected to be in application comparatively safer than the genuine petroleum, regarding the lack of easily flammable light components and regarding the lack of aromatics, possibly rich in the molecules with fused aromatic rings.

In spite of a great similarity in bulk properties to sample P3, sample P4 did not conserve the specific petroleum constituents in the variety and intensity offered by nature. This fact emphasized the necessity that, beside the bulk properties, the petroleum related preparations, for comparison, should undergo a detailed procedure of structure investigation.

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