schen Ln^{3+}-Emission vollständig verschwindet [4f→4f-Übergänge innerhalb der Elektronenkonfiguration $4f^2$ (Pr^{3+}) bzw. $4f^8$ (Tb^{3+}); Fig. 1 für CAGG : $Tb_{0,01}$]. Alle Tb^{3+}-aktivierten Verbindungen phosphoreszieren bei Raumtemperatur (RT) sowohl nach CR- als auch UV-Anregung minutenlang mit grüner Farbe; bei Pr^{3+}-Aktivierung tritt ebenfalls Phosphoreszenz auf, wobei sich die Emissionsfarbe mit sinkendem Pr^{3+}-Gehalt von rötlich weiß nach orange-rot verschiebt (PL-Emissionsspektren von CAGG : $Pr_{0,1}$ und CAGG : $Pr_{0,001}$ in Fig. 2).

Die Phosphoreszenz ist auf eine Beteiligung von Gitterdefekten (traps) am Lumineszenzprozeß zurückzuführen: Beim Abkühlen auf 77 K verschwindet beispielsweise das Nachleuchten vollständig, da bei dieser Temperatur die Ladungsträger an die traps gebunden sind. Durch erneute Zuführung von thermischer Energie (Erwärmen) werden die traps entleert und die freigesetzten Ladungsträger auf den Aktivator übertragen, der anschließend strahlend relaxiert.

Für kleinere Aktivatorkonzentrationen ($x \approx 0{,}001$) ist die Wirtsgitter-Emission nicht mehr gelöscht. Nach CR-Anregung tritt sofort dessen auf einem raschen Lumineszenzprozeß beruhende blaue Lumineszenz auf, gefolgt von der langsamer an- und abklingenden orange-roten (Pr^{3+}) bzw. grünen (Tb^{3+}) Aktivator-Emission. Die Lumineszenzfarbe ändert sich dabei von Blau nach Orange-rot bzw. von Blau nach Grün.

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Solid-State ^{13}C NMR of Middle Precambrian Anthracite and Related Anthraxolite

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Some of the oldest, if not the oldest coal in the world is that found in the Michigamme slate Iron County (the Lake Superior Region), Michigan, USA. According to [1], this coal, in excess of 1.7 Ga in age (Middle Precambrian), is anthracite and occurs as lenticular bodies concordantly bedded with Michigamme black shale. It also exhibits numerous graphitic bodies which is believed to be of organic origin. The lithologic, paleontologic, petrographic, and biochemical evidence obtained on the origin and composition of the Michigamme coal suggest probable derivation from algal mats.

According to [2] anthraxolite is a black combustible coal-like material found in Precambrian rock. Anthraxolite resembles anthracite coal in many ways and is regarded as having been formed by very low grade metamorphism of liquid bituminous materials that were probably derived from living organisms [3]. Beds and lenses of coals and veins of anthraxolite are common occurrence in relatively non-metamorphosed Precambrian deposits of the Lake Superior Region [4]. In Ontario, veins of anthraxolite occur in rocks of the Middle Precambrian Onwatin Formation in the Sudbury basin. The Onwatin Formation consists of dark gray to black well-bedded slate with veins of anthraxolite. Coleman [2] concluded that the Onwatin slate was a carbonaceous shale and that heat and pressure drove the bituminous materials into fissures to form anthraxolite.

To our knowledge the Michigamme anthracite and related anthraxolites have never been studied using modern methods or organic structural analysis. Such an experimental approach is the ^{13}C nuclear magnetic resonance (NMR) for solids which provides a convenient means of analysis of the carbon-skeleton structures of coals and related materials [5]. We report here ^{13}C NMR spectra of the Michigamme anthracite-like material, anthraxolite from Sudbury (ca. 1.8 Ga old), and the insoluble carbon of the Onwatin slate (ca. 1.7 Ga old). For comparison, we also recorded the ^{13}C NMR spectrum of the Vrška Čuka anthracite (Jurassic, ca. 0.15 Ga, Serbia) [6]. These spectra were obtained with magic angle spinning (MAS), high-power decoupling, and with use of cross polarization (CP) for signal enhancement.

Demineralization procedure was similar to that used in [7, 8]. Powdered geological material (50 g) was treated with boiling HCl (4 *M*) to remove most carbonates. The insoluble residue was, then, demineralized further by repeated

treatment with boiling HF/HCl (22 *M* and 0,25 *M*, respectively). This acid mixture removes most silicates. The remaining material was then extracted with 6:1 (v:v) benzene/methanol in a Soxhlet apparatus for 24 h. Analytical data for the dried solvent-extracted samples are: C 93.7%, H 1.0%, and ash 4.3% (the Michigamme coal); C 92.1%, H 3.4%, O 2.9%, and ash 8.0% (the Vrška Čuka coal); C 44.7%, H 2.2%, and ash 44.3% (the Sudbury anthraxolite); C 40.7%, H 2.1%, and ash 46.7% (the Onwatin insoluble material). The Michigamme coal is classified as anthracite according to the ASTM classification of coals [9].

All ^{13}C NMR spectra were recorded at 25.15 MHz on a Bruker CXP 100 spectrometer as previously described [5, 10]. Figure 1 shows high-power CP-MAS ^{13}C NMR spectra of the Michigamme (a) and Vrška Čuka (b) anthracites, the Sudbury anthraxolite (c), and the Onwatin insoluble carbon (d).

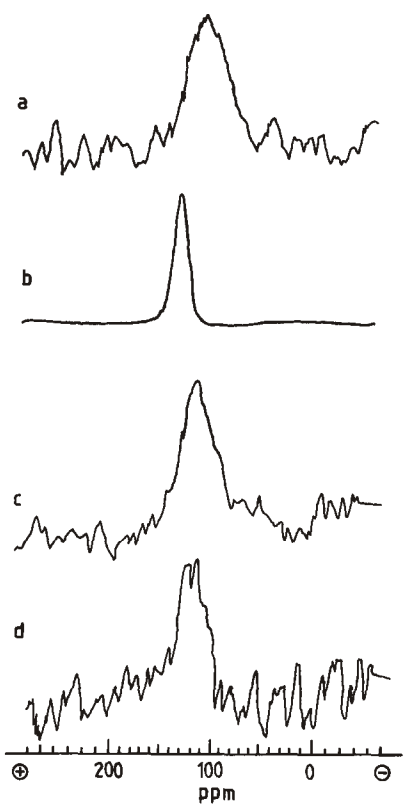

Fig. 1. ^{13}C CP-MAS NMR spectra of carbonaceous materials. a) The Michigamme anthracite (422954) and the Vrška Čuka anthracite (7500 scans), b) the Sudbury anthraxolite (129947 scans), and c) Onwatin material (207380 scans)

The spectrum of the Michigamme material shows a peak centered at ca. 110 ppm (all chemical shifts are given relative to TMS) with half-height width of 55 ppm. The ^{13}C NMR signals appearing between 100 and 160 ppm have been interpreted as arising from sp^2-hybridized carbon of olefinic and/or aromatic groups. Thus, the insoluble macromolecular carbon of the Michigamme anthracite is probably of aromatic nature. Broad band-width indicates an inhomogeneity of the aromatic species consistent with relatively small polycyclic aromatic ring structures. This can be contrasted to the Vrška Čuka anthracite derived from higher plants [6]. The ^{13}C NMR spectrum of this coal shows a strong peak located at 130 ppm with a shoulder at ca. 140 ppm. The narrow-band width of 15 ppm indicates a homogeneity consistent with extensive polycyclic aromatic structures. The NMR and other studies [11] imply that these aromatic sheets are probably the predominant structural feature of the high-plant coals. Moreover, our electron spin resonance (ESR) and ^{13}C dynamic nuclear polarization (DNP)-CP studies of the Vrška Čuka anthracite indicates a high unpaired electron spin concentration of 4.6×10^{19} spins g^{-1} associated with extensive polyaromatic ring structures [12]. In contrast, polyaromatic paramagnetic species could not be detected in the Michigamme coal. The limit of detection of the ESR spectrometer employed was about 10^{14} spins g^{-1}.

Elemental analysis shows that the Michigamme coal-like material has comparable carbon content (C 93.7%) with the Vrška Čuka anthracite (C 92.1%). However, the signal-to-noise ratio of the Michigamme spectrum is much lower than of the Vrška Čuka spectrum despite the accumulation of over 5600 times the number of scans (Fig. 1) reflecting a much lower concentration of the corresponding polycyclic aromatic species of the sample. The CP-MAS employed to obtain the spectra of Fig. 1 relies on the presence of a system abundant in protons in order to observe the ^{13}C NMR signal. Consequently, such spectra do not exhibit resonance from carbon atoms in structural domains lacking protons (such as graphite). For comparison, the Michigamme material contains 1.0% hydrogen but the Vrška Čuka anthracite contains 3.4% hydrogen.

Though we detected strong ^1H NMR MAS resonance of the Vrška Čuka anthracite (not shown), it was not possible to obtain a detectable NMR signal for protons of the Michigamme material [13]. The limit of detection of the ^1H NMR spectrometer employed was about 0.3% proton. Thus, our results suggest that the Michigamme anthracite contains mainly the NMR-silent protons.

The ^{13}C NMR spectra of the Sudbury and Onwatin materials are qualitatively similar to that of the Michigamme coal and afford little basis for differentiating between these three types of Precambrian insoluble carbons. The peaks are centered at 110 ppm (Sudbury) and 115 ppm (Onwatin) with half-height widths, respectively, of 40 and 50 ppm. Despite the accumulation of over three (or two) times the number of scans represented by the Sudbury anthraxolite (or the Onwatin material) the signal-to-noise ratio in the Michigamme spectrum is lower, though the Michigamme carbon content (C 93.7%) is about two times higher than that of the Sudbury (C 44.7%) and Onwatin (C 46.7%) samples. This reflects the lower concentration of polyaromatic ring systems in the Michigamme sample than that of the Sudbury and Onwatin materials. By comparison, the Sudbury and the Onwatin samples contain 2.2 and 2.1% hydrogen, respectively. However, the percentage of hydrogen for these materials is meaningless because experimental error is expected to be high since the percentage of hydrogen is low and ash content high. The ESR and ^1H NMR spectra of the Sudbury and Onwatin samples did not reveal the presence of either polyaromatic paramagnetic ring structures (ESR) or NMR-active protons. These close similarities between three Precambrian insoluble carbons studied would suggest a common or similar (biological or abiological) paleosource.

In summary, these results suggest that relatively small polycyclic aromatic structures are an important feature of Precambrian Michigamme anthracite (and related Sudbury anthraxolite), indicating that this material is structurally quite different from anthracites of higher-plant origin derived from lignin.

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In vitro Reconstitution of Nail Intermediate Filaments

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Intermediate filaments (IF), microfilaments, and microtubules form the cytoskeleton of epithelial cells (for reviews see [1, 2]). Keratins, a multigene family, comprise the most complex group of IF proteins. Up to 19 different epithelial keratins have been identified [3] and, recently, several other keratins that are typical for hair and nails have been characterized [4 – 6]. Microfibrillar (low-sulphur) wool keratins were included in the IF family [7] on the basis of their sequence and structural homology with other IF proteins. Meanwhile, the in vitro reconstitution of wool keratin IF has been recorded which is more arduous compared with the reconstitution of other IFs [8]. It was shown that the formation of wool keratin IF in vitro does not require the presence of IF-associated proteins (IFAPs, formerly called "matrix" proteins). Encouraged by our wool experiments, the in vitro reconstitution of nail low-sulphur keratins, another class of hard α-keratins, has been attempted in order to classify nail microfibrillar proteins as members of the IF family on the basis of the electron microscopy criterion which, until the present, was not possible.

Human fingernails of several subjects were ground in a ball mill at the temperature of liquid nitrogen. Keratins were extracted with 8 *M* urea, 50 m*M* Tris-HCl (pH 8.5), 19 m*M* DTE (1 : 200 w/v, 6 °C, 18 h). Thiol groups were then converted into S-sulphonates (Bunte salts) by the addition of 0.13 *M* Na_2SO_3 and 0.13 *M* $Na_2S_4O_6$ into the same solution and reaction time of 24 h. The S-sulpho keratins were separated from the IF-associated ("matrix") proteins by precipitation with zinc acetate according to [9, 10]. Next, they reacted with dithiothreitol to convert them into the thiol form, then suitable for assembly experiments. In vitro reconstitution and the preparation of the specimens for electron microscopy was performed in principle according to the wool experiments [8] but dithiothreitol was used instead of 2-mercaptoethanol and 1 mmol EDTA per l was used in the dialysis buffers throughout. Control experiments with human heel callus keratins were performed to check the experimental conditions. Nail intermediate filaments were observed when two-step dialysis (details in legend to Fig. 1) was performed at room temperature (20 – 25 °C, Fig. 1), but not at 6 °C (results not shown). Heel callus keratin IF, however, were reconstituted in vitro after dialysis steps were carried out at 20 – 25 °C as well as at 6 °C.

Nail filaments are 7 – 15 nm in diameter and show a rather rough surface structure like the wool keratin IF and in contrast to the reconstituted callus keratin filaments. Beside the nail intermediate filaments, some macrofibril formation was observed (Fig. 1, arrow). Macrofibrils are 110 to 150 nm in diameter and a few micrometers in length. The diameter range corresponds to the diameter of hair and wool macrofribrils in vivo [11, 12]. The bundles seem to consist of laterally aggregated IF, several of which were monitored as they protrude from one macrofibril and end in another one. Dark and bright stain intensity was observed within the macrofibrillar structures suggestive of a penetration of the negative stain.

Work is now in progress to characterize the two nail protein fractions (low-sulphur IF proteins and the high-sulphur "matrix" proteins). It has been proved already by gel electrophoresis that the nail keratin fraction does not contain any substantial amounts of IFAPs which might lead to the macrofibril formation. Furthermore, special investigations will be carried out to induce macrofibril formation by mixing the proteins from the two separated fractions. One- and two-dimensional gel electrophoresis of the isolated S-sulpho nail keratin fraction showed the typical nail keratins in agreement with

