**DIAMONDOID DIACIDS (‘O₄’ SPECIES) IN OIL SANDS PROCESS AFFECTED WATER**

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**Abstract**

RATIONALE: As a by-product of oil sands extraction, large volumes of oil sands process water (OSPW) are generated, which are contaminated with a large range of water-soluble organic compounds. The acids are thought to be derived from hydrocarbons via natural biodegradation pathways such as α- and β-oxidation of alkyl substituents, which could produce mono- and diacids, for example. However, while several monoacids (‘O₂’ species) have been identified, the presence of diacids (i.e. ‘O₄’ species) has only been deduced from results obtained via Fourier transform infrared (FTIR) spectroscopy, Fourier transform ion cyclotron resonance high-resolution mass spectrometry (FTICR-HRMS) and nuclear magnetic resonance (1H-NMR) spectroscopy as well as previous studies and observations, including a number of mass configurations.

METHODS: An extract of an OSPW from a Canadian tailings pond was analysed and the retention times and the electron ionization mass spectra of some analytes were compared with those of bis-methyl esters of authentic diacids by gas chromatography × gas chromatography/time-of-flight mass spectrometry (GCxGC/TOFMS) in nominal and accurate mass configurations.

RESULTS: Two diamondoid diacids (3-carboxymethyladamantane-1-carboxylic acid and adamantane-1,3-dicarboxylic acid) were firmly identified as their bis-methyl esters by retention time and mass spectral matching and several other structural isomers were more tentatively assigned. Diacids have substantially increased polarity over the hydrocarbon and monoacid species from which they probably derive: as late members of biodegradation processes they may be useful indicators of weathering and ageing, not only of OSPW, but potentially of crude oil residues more generally.

CONCLUSIONS: Structures of O₄ species in OSPW have been identified. This confirms pathways of microbial biodegradation, which were only postulated previously, and may be a further indication that remediation of OSPW toxicity can occur by natural microbial action. The presence and abundance of these diacids might therefore be useful as a measure of biodegradation and weathering.

**INTRODUCTION**

Exploitation of the oil sands of the Canadian Athabasca region involves using large volumes of water, which becomes contaminated with water-soluble compounds during the extraction process. This oil sands process water (OSPW) is stored in large tailings ponds, due to a non-release policy, since concerns exist about its toxicity to aquatic and terrestrial life. Concerns have been raised over whether the oxidised alkyl chain was of even or odd carbon numbers. When the toxicities of fractions of naphthenic acids of increasing molecular weight (MW) were tested, it appeared that higher MW compounds did not exhibit a higher toxicity, contrary to what is usually observed.[14] The reason for this was not understood, until Fourier transform ion cyclotron resonance high-resolution mass spectrometry (FTICRHRMS),[11–13] as well as Fourier transform infrared (FTIR) spectroscopy and 1H-nuclear magnetic resonance (NMR) spectroscopy analyses of different MW fractions of naphthenic acids from OSPW indicated the presence of some higher oxidized compounds. Compounds with four oxygen atoms (‘O₄’ species) were proposed to be diacids and suggested to be products of the biodegradation of alkylated monoacid (‘O₂’ species). Significantly, such diacids, particularly when bis-derivatised, would have higher MWs than the corresponding monoacids, yet would be more water-soluble and less toxic. This agrees with the observed decrease in toxicity with increasing MW. Suggested biodegradation pathways explaining the origins of the acids included β-oxidation or combined α- and β-oxidation, depending on whether the oxidised alkyl chain was of even or odd carbon...
Adamantane-1,3-dicarboxylic acid, 3-carboxymethyladamantane-1-carboxylic acid and noradamantane carboxylic acid were purchased from Sigma-Aldrich (Gillingham, UK). The OSPW sample was obtained from the West In-Pit tailings pond of Syncrude Ltd, Canada, by Environment Canada on 21-11-2011. Samples were filtered through a 0.2 μm filter cartridge to remove suspended solids, acidified to pH 2 and cleaned using 200 mg ENV+ solid-phase extraction (SPE) cartridges (Biotage, Charlotte, NC, USA), followed by elution with 10 mL of acetonitrile, and an aliquot was used for esterification. The pure acids and the environmental extracts were esterified (converted into methyl esters) by reaction with BF₃-methanol complex (Sigma-Aldrich) at 70°C for 20 min. Deutério-methylated standards were obtained by conducting the same derivatization with BF₃-trideuteriomethanol complex (Sigma-Aldrich). OSPW was also spiked with a deuterated internal standard, noradamantane carboxylic acid d₄ methyl ester, prior to analysis to facilitate retention time comparisons between the OSPW sample mixture and mixtures of reference compounds. Comprehensive gas chromatography/time-of-flight mass spectrometry (GCxGC/TOFMS) with electron ionization (EI) analyses were conducted using a model 7890A gas chromatograph (Agilent Technologies, Wilmington, DE, USA) fitted with a ZZX2 GCxGC cryogenic modulator (Zoex, Houston, TX, USA) interfaced with a BenchTOFdx™ time-of-flight mass spectrometer (Almsco International, Llantrisant, UK) at a scan speed of 50 Hz, i.e. 50 spectra were acquired per second. The first-dimension column was a 5% phenylmethyl polysiloxane HP-5 ms (30 m× 0.25 mm× 0.25 μm; Agilent Technologies, Wilmington, DE, USA) and the second-dimension column was a 50% phenyl polysilphenylene siloxane BPX50 (3 m× 0.10 mm× 0.10 μm; SGE, Melbourne, Australia). Helium was used as the carrier gas and the flow rate was 2 mL/min. Samples (1 μL) were injected at 280 °C splitless. The EI mass spectrum of component I (Fig. 2(a)) showed a molecular ion at m/z 252, which eluted at identical retention times with a run time of 6.6 s. The EI mass spectrum of component II showed a molecular ion at m/z 236, which eluted at identical retention times with a run time of 6.7 s. The EI mass spectrum of component III showed a molecular ion at m/z 266, which eluted at identical retention times with a run time of 6.8 s.
Figure 1. (a) Whole EI total ion current (TIC) chromatogram and (b) partial EI TIC chromatogram (retention time, Rt, 61–74 min, Rt2 5.5–6.5 s). Diacids are shown labelled with I ([M]+ • m/z 252) and II ([M]+ • m/z 266), where structures I and II were confirmed by comparison with spectra and Rt of authentic acids and Ia–e and IIa–f are tentatively assigned as other isomers (for which spectra are shown in Figs. 2 and 3). (c) Extracted ion current (EIC) 2D and 3D chromatogram representations for ions m/z 191.5–193.5 showing I and (d) isomers and (d) EIC 2D and 3D chromatogram representations for ions m/z 205.5–207.5 showing II and isomers.
adamantane-1,3-dicarboxylic acid and (d) 3-carboxymethyladamantane-1-carboxylic acid.

The presence of compound Ia showed that the ions of H-transfer from the charge-retaining fragment, as shown by Walt, the base peak of Id and Ie was at 191, indicating a loss of COOCH₃.

Ic showed a base peak at 192 or 193 (which might not be favoured for steric reasons).

Thus, peaks Ia–Ie were tentatively assigned as isomers of I due to apparent molecular ions at m/z 252. In theory, a total of eight structural and steric isomers of such a diacid are possible (excluding enantiomers), i.e. in addition to the 1,3-disubstituted adamantane, the 1,2-, 2,4-isomers, two isomers of the 1,4-, 2,6-disubstituted adamantane and maybe the dimethyl adamantane-2,2-dicarboxylate (which might not be favoured for steric reasons). While Ib and Ie showed a base peak at m/z 193, indicating a loss of COOH, the base peak of Id and Ie was at m/z 192, suggesting the loss of the methyl ester molecule via a concerted process involving H-transfer from the charge-retaining fragment, as shown by Waltman and Ling, who described such fragmentations in detail.

This has also been reported previously for the EI mass spectrum of the methyl ester of adamantane-2-carboxylic acid. The spectrum of compound Ia showed that the ions of m/z 192 and 193 had similar intensities. Favoring of a hydrogen transfer strongly suggests that at least one of the substituents is placed on a distal carbon atom, i.e. neighbouring a bridgehead, where removal of H will result in the formation of a tertiary carbocation. Another feature of these isomers is the higher intensity of m/z 220, corresponding to a loss of 32 Da (i.e. CH₂OH) from the molecular ion, also indicating that at least one of the carbonyl groups is placed on a distal carbon, since with substitution on a bridgehead (or ‘apical’ carbon), the loss of the complete carbonyl group would be favoured in order to produce a tertiary carbocation.

We can thus speculate that these spectra correspond to the 1,2-, 1,4-, 2,4-, 2,6-diacid isomers, and perhaps also the dimethyl adamantane-2,2-dicarboxylate. For diacids such as II, there are 14 possible isomers excluding enantiomers. Six peaks in the chromatogram eluting in the same region as II (Fig. 1) showed EI mass spectra that were similar to that of II. The mass spectra of the peaks IIa–IIc showed a weak molecular ion at m/z 266 (0.3–13%; Fig. 3), thus allowing tentative identification of these compounds as isomers of II.

As discussed for I, the main differences in the mass spectra were the loss of the carboxy methyl group as such or plus a hydrogen (59 or 60 Da), resulting in the formation of fragment ions at m/z 207 or 206. In addition, the loss of CH₂OH (32 Da) resulted in the formation of a fragment ion at m/z 234 of varying intensity. All these isomers were also present in other OSPW extracts from Canadian tailings ponds that were analysed.

Interestingly, in samples of fresh OSPW supplied by Environment Canada in 2009, which were taken from the inlet pipe to the West In-Pit tailings pond, rather than from the pond itself, Rowland et al. could not detect any O₂ species by GCxGC/TOFMS. Re-examination of that sample by higher accuracy
Figure 3. EI mass spectra of unknowns tentatively assigned as isomers of I and II.
GCxGC/TOFMS herein confirmed that the diacids were absent. However, since the relative ratio of $O_4$ species to $O_2$ species has been shown to increase with progressing biodegradation,[12] it is possible that the absence of diacids in the inlet pipe OSPW, and their presence in stored OSPW in the pond, reflects further in situ environmental biodegradation of the monoacids. $O_2$ species are presumably first generated in geological time by biodegradation of the corresponding hydrocarbons in the oil sands deposits. $O_4$ species, including the adamantane diacids detected here, are thus probably further degradation products of diamondoid hydrocarbons, which have been shown to be present in oil with up to five alkyl substituents of varying chain lengths.[18] This is in line with the biological oxidation of petroleum hydrocarbons to acids and monoacids to diacids (Fig. 4). This pathway has been hypothesised previously[19–21] to account for observed reductions in the toxicity of some OSPW with time. The proposed route entails carboxylation of a terminal carbon (Fig. 4), followed by β-oxidation of the chain if longer than two carbon atoms. An even carbon number alkyl chain attached to cyclic structures would leave a -CH$_2$COOH substituent attached to the adamantane core, odd chains a -COOH. The -CH$_2$COOH group can then be converted via α-oxidation into a -COOH, and potentially further degraded via β-oxidation resulting in ring opening and producing, perhaps, bicyclic or monocyclic intermediates.

The cage-like structures of the adamantanes could, however, impede ring opening and might explain why numerous diacids appear to be somewhat recalcitrant and present in several OSPW. Diacids exhibit a higher polarity than hydrocarbons or monoacids, which have been shown to be present in oil with up to five alkyl substituents of varying chain lengths. Addition of a further carboxylic acid group increases hydrophilicity and thus seems to be a natural mechanism to further decrease the toxicity of the monoacids.

**CONCLUSIONS**

Two naphthenic acid 'O$_4$' compounds in OSPW from a Canadian tailings pond have been identified and confirmed for comparison with authentic compounds, using GCxGC/TOFMS. Several others have been more tentatively assigned. The presence of these diacids in an OSPW sample from a tailings pond supports previously postulated biodegradation pathways for diamondoid hydrocarbons and monocarboxylic acids. Addition of a further carboxylic acid group increases hydrophilicity and thus seems to be a natural mechanism to further decrease the toxicity of the monoacids.

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