Research Article
Communications in Mass Spectrometry

Received: 2 July 2014 Revised: 21 August 2014 Accepted: 21 August 2014

Rapid Commun. Mass Spectrom. **2014**, *28*, 2352–2362
(wileyonlinelibrary.com) DOI: 10.1002/rcm.7024

Mass spectral characterisation of a polar, esterified fraction of an organic extract of an oil sands process water

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RATIONALE: Characterising complex mixtures of organic compounds in polar fractions of heavy petroleum is challenging, but is important for pollution studies and for exploration and production geochemistry. Oil sands process-affected water (OSPW) stored in large tailings ponds by Canadian oil sands industries contains such mixtures.
METHODS: A polar OSPW fraction was obtained by silver ion solid-phase extraction with methanol elution. This was examined by numerous methods, including electrospray ionisation (ESI) Fourier transform ion cyclotron resonance mass spectrometry (FTICRMS) and ultra-high-pressure liquid chromatography (uHPLC)/Orbitrap MS, in multiple ionisation and MS/MS modes. Compounds were also synthesised for comparison.
RESULTS: The major ESI ionisable compounds detected (+ion mode) were $C_{15\text{-}28}$ SO_3 species with 3–7 double bond equivalents (DBE) and $C_{27\text{-}28}$ SO_5 species with 5 DBE. ESI-MS/MS collision-induced losses were due to water, methanol, water plus methanol and water plus methyl formate, typical of methyl esters of hydroxy acids. Once the fraction was re-saponified, species originally detected by positive ion MS, could be detected only by negative ion MS, consistent with their assignment as sulphur-containing hydroxy carboxylic acids. The free acid of a keto dibenzothiophene alkanoic acid was added to an unesterified acid extract of OSPW in known concentrations as a putative internal standard, but attempted quantification in this way proved unreliable.
CONCLUSIONS: The results suggest the more polar acidic organic SO_3 constituents of OSPW include $C_{15\text{-}28}$ S-containing, alicyclic and aromatic hydroxy carboxylic acids. SO_5 species are possibly sulphone analogues of these. The origin of such compounds is probably via further biotransformation (hydroxylation) of the related S-containing carboxylic acids identified previously in a less polar OSPW fraction. The environmental risks, corrosivity and oil flow assurance effects should be easier to assess, given that partial structures are now known, although further identification is still needed.

Characterisation of the polar constituents of petroleum, particularly of partially degraded crude oils in which the hydrocarbons have been diminished, has long proved to be an important, but challenging goal (reviewed in[1,2]). One of the largest and best known examples of partially degraded petroleum is the Alberta oil sands of Canada, the chemical composition of which has long been studied (e.g.[3–5]). However, the composition of the polar organic compounds in the large volumes (≥840 million m^3 in 2011[6]) of oil sands process water (OSPW) presently stored in ponds is less well known (reviewed in Headley *et al.*[7]). Such knowledge is important because the mixtures of organic and inorganic chemicals in undiluted OSPW are associated with a variety of toxic responses in many higher organisms. Thus, reclamation of some storage lagoons is difficult and can take many years unless treated (e.g.[8,9] and references cited therein). An improved knowledge of the compounds present might aid efforts to remove or reduce the concentrations of the toxic components (e.g. reviewed in Headley *et al.*[7]). In a wider context, improved knowledge of the composition of degraded polar fractions of petroleum generally, including the more mobile, water-soluble constituents such as bacterial metabolites, would be valuable for an improved understanding of pollution problems,[10–13] for geochemistry (e.g.[2,14]) and to industry for understanding acid-based corrosion (e.g.[15]).

The extraordinary complexity of the mixtures of organic compounds in polar petroleum and petroleum-related fractions, stated in some cases to comprise more than a million-but at least tens of thousands, of compounds,[12,16,17] has hindered attempts to identify the individual constituents. This applies even to the aqueous-soluble fractions, where *only* a few thousand components are present (e.g.[9,18,19]).

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Recently, use of modern coupled chromatographic and mass spectrometric techniques has allowed some components to be assigned, or at least delimited (e.g.[20–32]). This is necessary, since compound structure often influences, not only toxicity (e.g.[33]), but also the reactivity of the compounds to remediation (e.g.[34–36]).

In this context, the concentrations of compounds are also important, but again most methods cannot be adequately calibrated until at least some unknowns are identified (e.g.[11,25,37]) since mass spectral ionisation responses often vary with the structures of compounds, as well as with solvent type and salt content.[12,16,24,27,28,38,39]

Preparative-scale fractionations of acid extracts of OSPW, in attempts to reduce the complexity, by techniques such as solid-phase extraction (SPE),[30] silver ion SPE (Ag^+ SPE; e.g.[28,33]), offline reversed-phase ultra-high-performance liquid chromatography (uHPLC)[9] and distillation (e.g.[40]) have provided fractions which are somewhat more amenable to analysis by coupled chromatography/mass spectrometry or direct infusion mass spectrometry techniques.

In the absence of the availability of a reference OSPW (discussed and reviewed in[7]), a sample isolated from a large volume (3000 L) of OSPW, which had already been studied by a variety of analytical and toxicological methods, was again chosen for study herein (e.g.[40] and references cited therein).[20–22,28,33] Since no single reference method of analysis is ideal for the characterisation of such complex mixtures, a multi-method approach (reviewed in[7]) was taken. Jones *et al*.[28] had already examined hexane, and hexane/ether, and West *et al*.,[29] ether and Ag^+ SPE eluates, of this OSPW extract (methylated) and they identified alicyclic, naphthenoaromatic and heteroaromatic carboxylic acids in the methylated fractions. Minor aromatic sulphur-containing acids were also present as methyl esters.[28,29] However, a remaining methanol eluate, probably containing more polar compounds, was not examined as these compounds were probably less amenable to GC-based methods. As more knowledge has at last been obtained about some of the so-called ’classical’ naphthenic acids in OSPW and related samples, (e.g.[21–23,41,42]), interest has now grown in the unknown, more polar, constituents, including sulphur-containing species (reviewed in[7,42]). Without knowledge of the identities of such compounds, any toxic or other effects (e.g. reviewed for some sulphur compounds in Kropp and Fedorak[43]) will be more difficult to predict.

Here the results of an examination of the more polar methanol SPE fraction of the methylated OSPW are reported in order to better address the paucity of structural information regarding polar organics in partially degraded petroleum mixtures.

EXPERIMENTAL

Process water sample

The OSPW was a subsample of ca 3000 L of oil sands tailings pond water collected from Syncrude Canada Ltd, West Inpit settling basin in Fort McMurray, Alberta Canada, in June 2005.[40] The subsequent treatment of this to isolate a concentrated NA (sodium salts) mixture has been described fully.[40,44]

Argentation solid-phase extraction

A subsample (~30 mL) of this concentrate as received (pH 11–12) was acidified with hydrochloric acid to pH <2 and extracted with ethyl acetate. The remaining ethyl acetate extract was dried (nitrogen stream), esterified by heating with fresh BF_3/methanol complex (70 °C, >30 min), back-extracted into hexane, dried and weighed. The resulting esterified fraction was sub-fractionated by argentation SPE as described previously.[28,33] The methanol eluate was collected and reduced to dryness under a steady stream of nitrogen at 40 °C.

Synthetic sulphur-containing acids

A C_{16} SO_3 keto acid (4-dibenzothiophen-2′-yl-4-ketobutanoic acid; Sigma, Poole, UK) was reduced to the corresponding hydroxy acid (4-dibenzothiophen-2′-yl-4-hydroxybutanoic acid) with sodium borohydride, by standard methods (Fig. 1). The resulting free hydroxy acid was examined by FTICRMS by ESI in negative ion mode, by infrared (IR) spectroscopy and by GC/MS of the trimethylsilyl ester, trimethylsilyl ether. Attempts to produce the methyl ester instead unexpectedly generated the methyl ester, methoxy ether (diazomethane or boron trifluoride/methanol).

Infrared spectroscopy

IR spectroscopy was performed on dried solids with an Alpha FTIR spectrometer (Bruker Optics, Coventry, UK).

Ultraviolet absorption spectophotometry

UV spectra of solutions of the esters in dichloromethane were recorded on an Agilent/Hewlett Packard model 8453 (Agilent Technologies, Waldbronn, Germany), wavelength range 190–1100 nm, slit width 1 nm.

GC/MS and GCxGC/MS

The esterified OSPW SPE fraction methanol eluate was reconstituted in dichloromethane prior to analysis by GC/MS and GCxGC/MS with nominal mass resolution, as described previously.[32]

Fourier transform ion cyclotron resonance mass spectrometry (FTICRMS)

The methylated SPE methanol eluate and also the non-esterified OSPW acid extract were analysed with a Solarix FTICRMS instrument (Bruker Daltonic GmbH, Bremen, Germany) equipped with an Infinity ICR cell and a 12 T magnet. Samples were diluted in pure methanol (Merck, Haar, Germany, GC-grade). No ammonia was added.

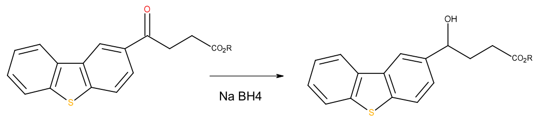

Figure 1. Outline of synthetic scheme for internal standard SO_3 compound (4-dibenzothiophen-2′-yl-4-hydroxybutanoic acid) from 4-dibenzothiophen-2′-yl-4-ketobutanoic acid by reduction with sodium borohydride.

Ionisation was by electrospray ionisation (ESI) in negative or positive ion mode. Nitrogen was used as a drying gas at a flow rate of 4 L min^{-1} and a temperature of 200 °C. The nebuliser gas (nitrogen) pressure was set at 0.7 bar. The capillary voltage was set at 4 kV and the sample was introduced at a flow rate of 200 µL h^{-1} by means of a syringe pump. Data were acquired in broadband 4 M mode and 100 scans were accumulated to produce each final spectrum. The ion accumulation time was set at 0.05 s. To suppress ion cluster formation, an in-source collision voltage of 40 V was applied. CID data were acquired under similar conditions, but the ion accumulation time was then set at 2 s and the CID voltage at 16 V.

uHPLC/orbitrap MS

HPLC separation of the methylated SPE methanol eluate was performed using an ARIA MX transcend system (Thermo Fisher Scientific, San Jose, CA, USA) on a Hypersil Gold column (50 × 2.1 mm, 1.9 µm particle size; Thermo Fisher Scientific) at 40 °C. The flow rate was 0.3 mL min^{-1} and an injection volume of 5 µL was used. The mobile phases consisted of (A) 0.1% acetic acid in water and (B) 100% methanol. The mobile phase composition was 5% B for 1 min, followed by a linear gradient ramp to 90% B at 10 min, to 99% over 5 min, and returning to 5% B in 1 min, followed by a 4 min hold prior to the next injection. An Orbitrap Elite mass spectrometer (Thermo Fisher Scientific) was operated with an ESI, atmospheric pressure chemical ionisation (APCI) or AP photoionisation (APPI) source operating at 350 °C in either positive or negative mode. For APCI and APPI the discharge current was set at ±5 µA and for ESI the spray voltage was set to ±5 kV. The sheath, auxiliary and sweep gas (nitrogen) flow rates were 30, 10 and 5 (arbitrary units), respectively, capillary temperature 300 °C, and S-Lens RF level 65%. The resolving power was set to a nominal value of 240 000 at full width half-maximum at *m/z* 400, and using a minimum injection time (i.e. maximum time that the ion was allowed to accumulate in the analyser) of 200 ms. MS/MS experiments were performed by collision-induced dissociation (CID) with a normalised collision energy of 35 eV or by higher-energy collisional dissociation (HCD) with a normalised collision energy of 35 or 80 eV. Mass calibration and tuning were performed externally by direct infusion of a standard mixture of caffeine, the peptide MRFA (sequence, Met-Arg-Phe-Ala) and Ultramark 1600 in H_2O/acetonitrile 50:50 (v/v), covering a range from *m/z* 138 to 1722. Mass spectral data were collected at 2 full scans per second between *m/z* 100 and 1000 using automatic gain control. Data acquisition and analysis were performed with Thermo Xcalibur 2.0 software.

RESULTS AND DISCUSSION

Methanol Ag$^+$ SPE fraction

Gravimetry

Jones *et al.*[28] reported that apparently about 40% by weight of the acidic methylated fraction of this OSPW eluted from Ag$^+$ SPE with polar solvents such as methanol. These were thought not to be simple naphthenic acids (e.g.[46–50]) since they were more polar than esterified acids. However, these authors did note that this fraction should be studied further in future, probably by HPLC-based methods, given the polarity.

On further, repeat and blank, SPE fractionations of this methylated OSPW extract herein, it was noted that substantial contributions to the apparent 40% by weight of the material in the methanol eluate were in fact due to elution of some of the Ag$^+$ SPE stationary phase (the exact nature of the phase is proprietary and confidential to the manufacturer). This cast some doubt on the weight obtained previously and also prevented elemental analysis of the fraction (cf.[28]). A more reliable estimate of the proportion of this fraction in the acid OSPW extract was therefore obtained by weight difference (i.e. by subtracting the weight contributions of the sum of the other recovered hexane to ether eluates from the total transferred to the SPE columns). This gave a reasonably reproducible value of 14% (95% confidence interval 11–17%, n = 4). About half of the total sulphur content of the OSPW was unaccounted for in these other fractions, suggesting up to about 1% sulphur might be present in this methanol fraction. (It is also possible that some sulphur compounds exist in even more polar fractions, which were not eluted by methanol.)

IR-, UV- and GC-based methods

Examination of the IR spectrum of the blank-subtracted dried methanol eluate of the methylated OSPW showed that a number of components not in the blank were present, or were elevated, in the OSPW fraction. The presence of components with hydroxy, carbonyl, and perhaps sulphoxide or sulphone, groups was suggested.

UV spectrophotometry revealed that at least some aromatic compounds were soluble in dichloromethane, with a distinct maximum absorbance observed at ~245 nm, somewhat higher than the absorbance maxima in the unfractionated methylated OSPW (cf.[28]). This is probably unrepresentative of the whole fraction, since most did not dissolve in dichloromethane.

GCxGC/high-resolution MS of methylated and methylated plus trimethylsilylated extracts of SPE fractions of OSPW re-dissolved in dichloromethane has shown previously that the combination of the high chromatographic and mass spectral resolutions possible is very useful for identifying individual constituents of acidic OSPW (e.g.[29,32]). Indeed, the chromatographic resolution achieved with ionic liquid GC stationary phases is unparalleled to date, certainly for aromatic ester fractions.[51] However, the use of such techniques (and indeed of most techniques) is predicated on efficient solubilisation of samples.[27] Of the present methanol eluate, very little material was apparently sufficiently soluble in GC-compatible solvents (e.g. dichloromethane) to be detectable by GC/MS or GCxGC/MS.

We therefore used direct-infusion and HPLC-based ultra-high-resolution mass spectral methods (Fourier transform ion cyclotron resonance (FTICRMS) and Orbitrap; see reviews[16,52]), but with collision-induced dissociations,[31] to facilitate study of the structures of these more polar unknowns.

ESI-FTICRMS

The dried methanol fraction was re-dissolved in methanol/acetone, in which it was also completely soluble. With an imposed mass error of $\le$0.05 ppm and allowed elemental compositions of up to three S atoms and infinite C, H O contents, the major ions detected by FTICRMS with positive ion ESI were assignable to ~ C_{15-28} SO_3 species with 1 to 8 double-bond equivalents (DBE) and ~ C_{20-40} SO_5 species with 3 to 5 DBE, when four separate repeat aliquots of the fraction were examined (e.g. Fig. 2(a)). Possibly, the latter SO_5 species were sulphone analogues of the former SO_3 species. Minor ionised ~ C_{20-30} NO_3 species were also observed. Negative ion ESI produced no significant response.

This contrasts with the composition of the whole unfractionated OSPW acid extract studied by FTICRMS in negative ion ESI mode, in which ions attributed to 'classical' O_2 naphthenic acids dominated and in which ions due to SO_3 and SO_5 components were only minor (Fig. 2(b)). This suggests that Ag^+ SPE has led to concentration of the SO_x species in the methanol eluate. (Indeed, the more abundant 'classical' O_2 naphthenic acids appeared as expected in the hexane SPE eluate.[33])

Numerous SO_x and NO_x compounds have been detected previously in unfractionated, non-esterified OSPW, by similar ultra-high-resolution mass spectrometry methods, usually in negative ion mode, but occasionally in positive ion mode. Thus, Grewer *et al.*[19] reported an unknown C_{14} SO_5 compound (error ~2 ppm), with no unsaturation, in OSPW from Albian Sands Energy Inc., by negative ion ESI. Barrow *et al.*[24] reported the detection of unknown C_{10-34} SO_x (2–12 DBE; C_{16-17}, 4 DBE maximum) including SO_3 species and several NO_x compounds, by positive and negative ion ESI, with typical mass errors < <1 ppm, but noted that many of the ions observed in positive ion mode were, in fact, sodiated, due to the salt content. Headley *et al.*[53] showed that C_{14-20} SO_3 compounds with 1 and 5–7 DBE could only be detected by negative ion ESI in the absence of salt when a concentrated OSPW sample was examined and Headley *et al.*[27] considered that the distributions of SO_x compounds in OSPW might allow different industrial sources to be distinguished. Nyakas *et al.*[9] investigated the effects of several pre-fractionation steps on monitoring of SO_x (3–9 DBE) and NO_x distributions in OSPW samples by negative ion ESI-FTICRMS.

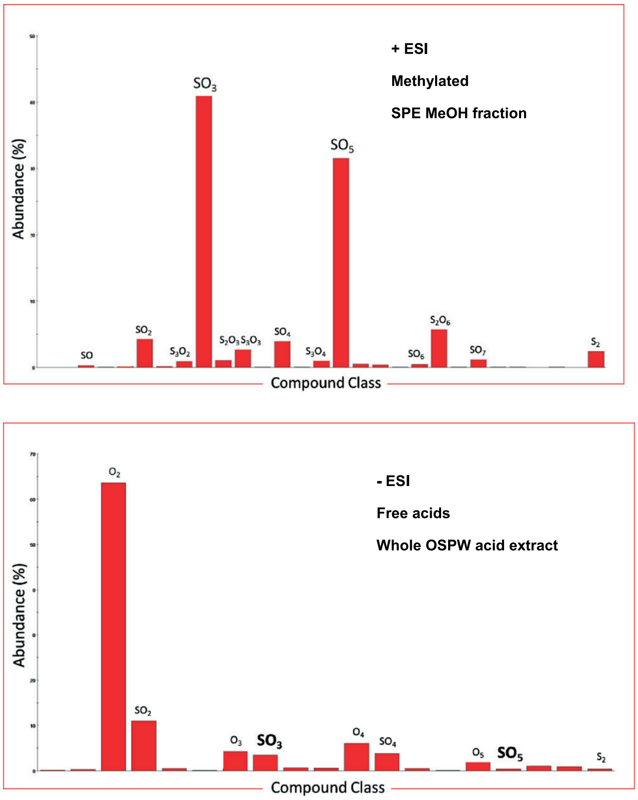

Figure 2. Bar charts showing uncalibrated major ion compositional class responses to (top) C_{10-30} ionisable components of a methanol SPE eluate of OSPW in ESI positive ion mode and (bottom) whole acidic extract of OSPW in ESI negative ion mode. Imposed mass error $\le$0.05 ppm, allowed elemental compositions of S_3, and infinite C, H. O contents.

In only one study was the detailed nature or structures of the species investigated,[42] although Stanford *et al.*[18] speculated that SO_x compounds in the water-soluble fraction of crude oils might be carboxythiophenes (cf.[29]).

In order to investigate further the SO_x and NO_x compounds detected in the present study by positive ion ESI-FTICRMS, and since in any case MS/MS of underivatised acids in negative ion mode suffers from low fragmentation (e.g.[30]), a series of positive ion ESI MS/MS CID experiments was conducted on the major ions attributed to the C_{21} SO_3 compounds with 3, 4 and 5 DBE in the methylated fraction. (It is acknowledged that although FTICRMS is an ultra-high-resolution detector, in MS/MS mode the selection of precursor ions is a lower resolution process. Thus, it is possible that interfering ions may contaminate the product ion MS/MS spectra. Therefore, not all the product ions in the MS/MS spectra may originate from the target precursor ions. Nonetheless, with care, considerable useful information can be derived.)

The compounds investigated by MS/MS exhibited major $[M+H]^+$ ions at *m/z* 371.2614 (theoretical $C_{21}H_{39}SO_3$ 371.2620, within 0.14 ppm), *m/z* 369.2458 (theoretical $C_{21}H_{37}SO_3$ 369.2463, within ~0 ppm), and *m/z* 367.2301 (theoretical $C_{21}H_{35}SO_3$ 367.2307, within 0.12 ppm). MS/MS CID experiments were also conducted on the major ion attributed to the C_{28} SO_5 compound with 5 DBE in the methylated fraction, which exhibited a major $[M+H]^+$ ion at *m/z* 497.3295 (theoretical $C_{28}H_{49}SO_5$ 497.3300, within ~0 ppm) and on the C_{25} NO_3 (6 DBE) species ($[M+H]^+$ *m/z* 404.3159; theoretical $C_{25}H_{41}NO_3$ 404.3165, within 1.4 ppm).

CID mass spectra of each of the above $[M+H]^+$ ions contained the corresponding product ions due to neutral losses of H_2O, methanol, methyl formate or combinations of these, as shown by the accurate masses of the product ions (Table 1). The losses of methanol and methyl formate (cf.[54]) may be good indications that the unknown SO_3 compounds are methyl esters of carboxylic acids (cf.[42]). This is also consistent with their presence in an acidic extract of OSPW following methylation and their detection only by positive ion ESI-MS. The loss of water suggests that the remaining oxygen may be present as a hydroxyl group (*viz* that the unknowns are methyl esters of sulphur- or nitrogen-containing hydroxy acids). Losses of water from other compounds are possible (e.g.[31]), so this is not entirely diagnostic. No losses of carbon monoxide were observed (cf.[31,54]), suggesting that the unknowns were not keto acids. Sulfoxides tend to lose –OH

Table 1. Accurate masses ($[M+H]^+$) of selected SO_3 and SO_5 components of a polar methylated OSPW fraction and masses and compositions of respective product ions created by CID of the protonated molecules

m/z $[M+H]^+$ (error, ppm)	DBE of $[M+H]^+$	*m/z* $[M+H]^+-H_2O$ (error, ppm)	*m/z* $[M+H]^+-CH_3OH$ (error, ppm)	*m/z* $[M+H]^+-CH_3OCOH$ (error, ppm)
367.2301 (0.12) $C_{21}H_{35}O_3S$	4.5	349.2196 (–0.06) $C_{21}H_{33}O_2S$	335.2040 (–0.15) $C_{20}H_{31}O_2S$	307.2091 (–0.39) $C_{19}H_{31}OS$
369.2458 (0) $C_{21}H_{37}O_3S$	3.5	351.2353 (–0.17) $C_{21}H_{35}O_2S$	337.2197 (–0.26) $C_{20}H_{33}O_2S$	309.2248 (–0.47) $C_{19}H_{33}OS$
371.2614 (0.14) $C_{21}H_{39}O_3S$	2.5	353.2509 (–0.04) $C_{21}H_{37}O_2S$	339.2353 (–0.12) $C_{20}H_{35}O_2S$	311.2404 (–0.36) $C_{19}H_{35}OS$
497.3295 (0) $C_{28}H_{49}O_5S$	4.5	479.3190 (–1.06) $C_{28}H_{47}O_4S$	465.3034 (–0.17) $C_{27}H_{45}O_4S$	437.3085 (–0.33) $C_{26}H_{45}O_3S$

and/or SO or O in ESI (reviewed in[55]) and ESI-CID MS/MS,[56] rather than water (H_2O): this was not observed for the above series of compounds. Unknown SO_3 compounds with 4 DBE may include methylated alkylbenzene sulphonates.

uHPLC/Orbitrap MS

Next, we examined the same methanol SPE fraction by UHPLC/orbitrap MS using not only positive and negative ion ESI (cf.[31]), but also atmospheric photo- and chemical ionisation (APPI and APCI) in positive and negative ion modes (cf.[24]). The use of UHPLC has the advantage of possibly providing additional chromatographic resolution of isobaric compounds.[31] Consistent with the FTICRMS results (*vide infra*), in ESI negative ion mode, nothing was detected above blank levels (Supplementary Fig. S1(a), see Supporting Information) in the total ion current chromatogram. This was also the case with negative ion APPI (Supplementary Fig. S1(b), see Supporting Information). However, with negative ion APCI, a minor response for a partially resolved complex mixture, eluting with maxima at retention times of about 11 and 12 min, was observed (Fig. 3(d)). These mass spectra showed the presence of (presumably) unesterified O_2 and O_4 compounds in the approximate range $C_{16\text{-}26}$. In the O_2 species, ions due to $C_{16\text{-}18}$ species with six degrees of unsaturation (possibly partly due to tricyclic monoaromatic monoacids: cf.[33]) dominated, while the major ions corresponding to O_4 compounds ranged from C_{18} to C_{24} and comprised bicyclic and tricyclic non-aromatic species, including possibly, diacids. $C_{20\text{-}26}$ tricyclic terpenoid monoacids have been identified previously in oil sands bitumen (e.g.[57,58]) and the unknowns may well be diacid analogues of compounds similar to the latter. Such non-heteroatom-containing acids have been widely reported in OSPW and petroleum (e.g.[20–23,29,30,32,41]) and are probably unmethylated residues of the acids reported previously, so these will not be

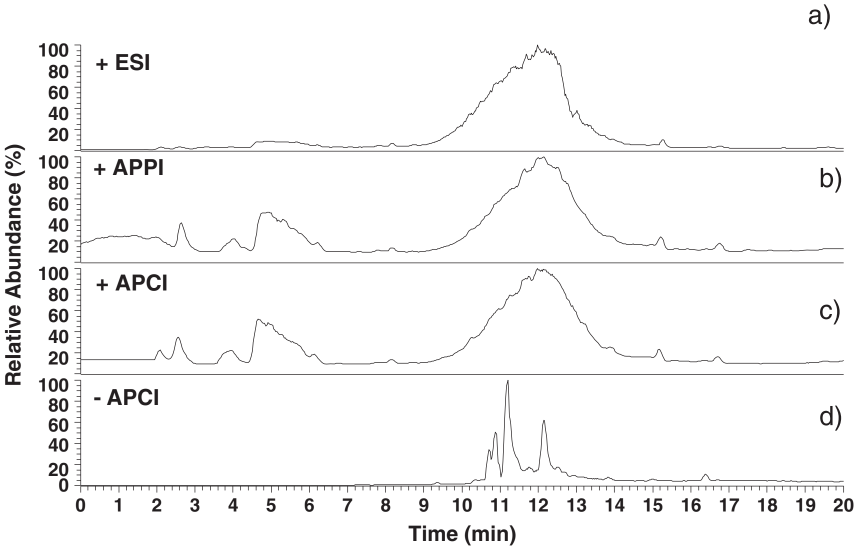

Figure 3. UHPLC/orbitrap MS total ion current chromatograms of methanol SPE eluate of methylated OSPW under (a) positive ion ESI conditions; (b) positive ion APPI conditions; (c) positive ion APCI conditions; and (d) negative ion APCI conditions.

discussed further herein. It appears that, for these particular compounds, under these conditions, negative ion APCI was slightly more sensitive than negative ion ESI-MS.

In positive ion mode, poorly resolved complex mixtures eluting with maxima at retention times at about 12 min were observed in the total ion current chromatograms (Figs. 3(a)–3(c)). In each, the series of SO_3 compounds was detected as the major $[M+H]^+$ ions, with minor ions attributable to unknown O_3 species (cf.[31]). The former were mainly in the range $C_{17\text{-}22}$ and contained from 3–5 or 7–8 DBE. The chromatographic separation of the C_{18} SO_3 series with 3–8 DBE, based on the accurate mass-selected ion mass chromatograms ($[M+H]^+$), was, as expected, better than exhibited by the whole mixture and is shown in Fig. 4. The carbon number maximum and DBE varied somewhat with the ionisation mode (e.g. ESI $C_{18:4}$; APCI $C_{18:7}$; APPI $C_{20:4}$) as has been observed previously by direct-infusion methods (cf.[24]). Although these compounds did not ionise under negative ion conditions, suggesting they were not carboxylic acids, when the methylated fraction was saponified and re-examined by negative ion ESI-MS, the de-methylated compounds with one carbon atom less were now detectable as $[M–H]^-$ ions (e.g. Supplementary Fig. S3, see Supporting Information) and were no longer detectable by positive ion ESI-MS (Supplementary Fig. S3, see Supporting Information). This strongly suggests that the initial esterified fraction contained compounds which are indeed methyl esters of carboxylic acids containing one further oxygen atom and a sulphur atom. Interestingly, a C_{17} SO_3 compound(s) (4 DBE), which was detected herein as the $[M–H]^-$ ion following saponification, was also reported previously by direct-infusion negative ion ESI-FTICRMS in Athabasca River water in the proximity of oil sands operations.[53] It seems likely that the SO_3 compounds detected previously by negative ion ESI (e.g.[24,27,53]) were unesterified hydroxy acids of the compounds reported here; those with 4 DBE may also include methylated alkylbenzene sulphonates.

In order to investigate further the hypothesis that the SO_3 unknowns were indeed esters of acids and since MS/MS of underivatised acids in negative ion mode suffers from low fragmentation (e.g.[30]), a series of positive ion ESI-MS/MS CID experiments was conducted on the major ions attributed to the C_{18} SO_3 compounds with 8, 7 and 5–3 DBE in the methylated fraction. These compounds exhibited major $[M+H]^+$ ions at *m/z* 319.1357 (theoretical $C_{18}H_{23}SO_3$ 319.1368, within 3.4 ppm), *m/z* 321.1505 (theoretical $C_{18}H_{25}SO_3$ 321.1524, within 6.4 ppm), *m/z* 325.1828 (theoretical $C_{18}H_{29}SO_3$ 325.1837, within 2.9 ppm), *m/z* 327.1986 (theoretical $C_{18}H_{31}SO_3$ 327.1994, within 2.4 ppm), and *m/z* 329.2141 (theoretical $C_{18}H_{33}SO_3$ 329.2150, within 2.9 ppm).

CID mass spectra of each of the above $[M+H]^+$ ions contained the corresponding product ions due to losses of water, methanol, methyl formate or combinations of these, as shown by the accurate masses of the product ions (e.g. Fig. 5). In addition to these common losses from the CID of the $[M+H]^+$ ions of the C_{18} SO_3 compounds with 8, 7 and 5–3 DBE, there were a number of product ions which distinguished individual members of the series, suggesting that the compounds were not simple homologues of one another. Thus the $C_{18}H_{23}SO_3$ and $C_{18}H_{25}SO_3$ compounds with 7 and 8 DBE exhibited MS/MS product ions corresponding to $C_{16}H_{17}S^+$, whereas this was not observed in the spectra of the other C_{18} compounds, suggesting different structure(s) for the latter. In the spectra of some unknowns, sulphur was retained in ions devoid of oxygen, in others, not. In the $C_{18}H_{23}SO_3$ and $C_{18}H_{25}SO_3$ compounds, since 1 DBE is due to the (esterified) carboxylic acid group and it is perhaps unlikely that the remaining 7 and 6 DBE are due to hexacyclic or heptacyclic structures, aromaticity is suggested. This is also consistent with the UV spectrophotometry data. One possibility therefore is that these unknowns are methyl esters of hexahydrodibenzothiophene hydroxy acids. Interestingly, a series of alkyl hexahydrodibenzothiophenes, from which such

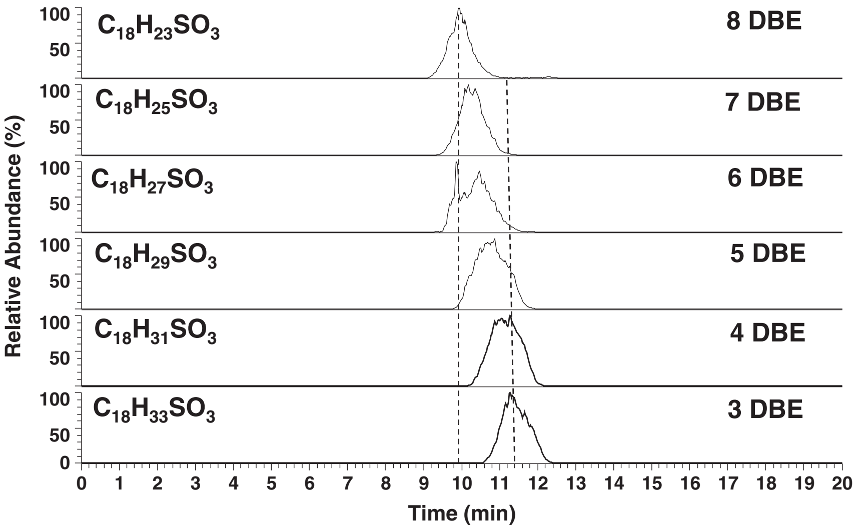

Figure 4. UHPLC/orbitrap MS extracted accurate mass $[M+H]^+$ chromatograms of C_{18} SO_3 compounds (3–8 DBE) in methanol SPE eluate of methylated OSPW under positive ion ESI conditions. Each chromatographic peak may represent co-elution of many isobaric isomers and ESI responses of different components may vary. Dotted lines emphasise ca 1 min separation of 8 DBE and 3 DBE components.

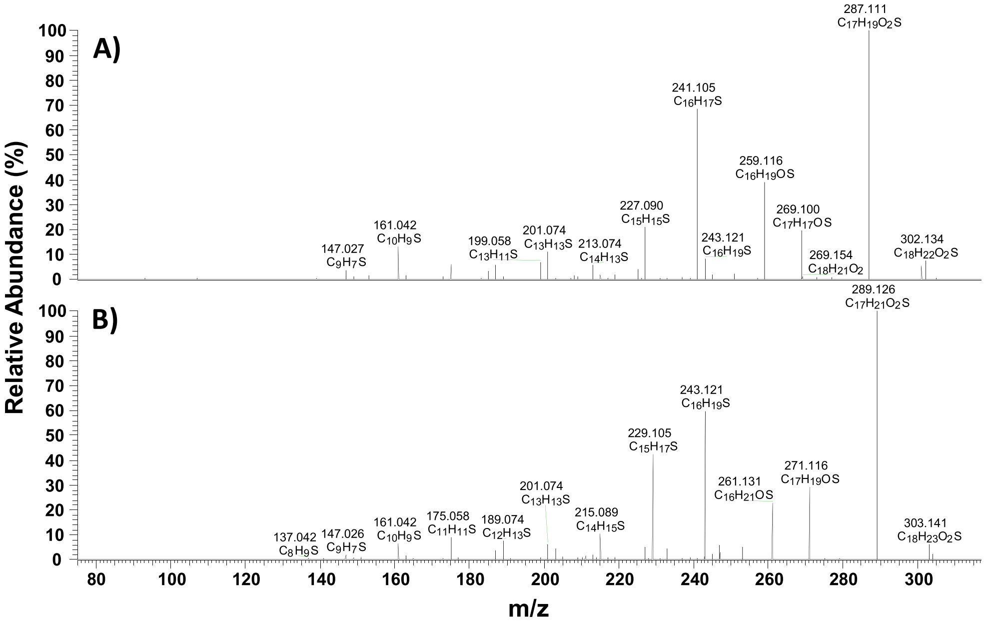

Figure 5. Selected ESI positive ion, CID MS/MS mass spectra of C_{18} SO_3 components of methanol SPE eluate of methylated OSPW: (a) $C_{18}H_{23}SO_3$ and (b) $C_{18}H_{25}SO_3$. Further examples are given in the Supporting Information.

acids could conceivably be produced by microbial action (cf.[59]; for review, see Kropp and Fedorak[43]), was found in a sulphur-rich Egyptian crude oil previously.[60]

To further investigate differences in the structures of the unknowns, a series of high-energy collision-dissociation (HCD) experiments was conducted on the above $[M+H]^+$ ions (Fig. 6). The MS/MS spectra obtained revealed more clearly the differences in the structures of the series of C_{18} unknowns. Thus, the spectra of the $C_{18}H_{23}SO_3$ ($[M+H]^+$) and $C_{18}H_{25}SO_3$ compounds did not exhibit the low-mass C_xH_y ions which were abundant in the spectra of the $C_{18}H_{29}SO_3$, $C_{18}H_{31}SO_3$ and $C_{18}H_{33}SO_3$ compounds (3–5 DBE). The spectra of the latter also included ions due to loss of $C_2H_6O_3S$ (*viz* $C_{16}H_{21}^+$, $C_{16}H_{23}^+$, $C_{16}H_{25}^+$), suggesting that the S atom in these was proximal to the oxygen atoms of the carboxylic group. Bi- to tetracyclic sulfides and

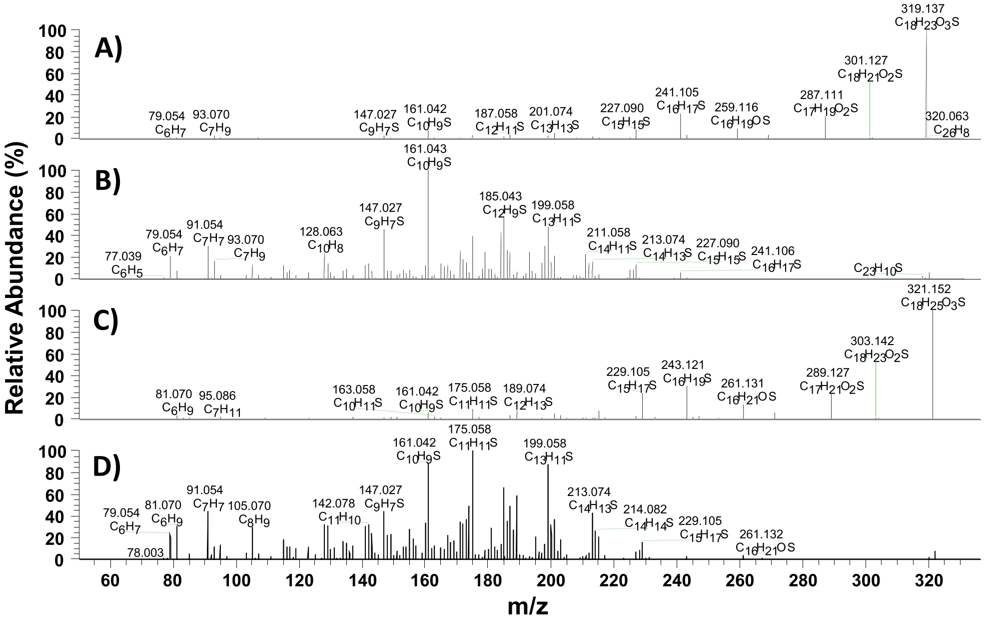

Figure 6. Selected ESI positive ion, high-energy dissociation collision (HCD) MS/MS mass spectra of C_{18} SO_3 components of methanol SPE eluate of methylated OSPW: (A, B) $C_{18}H_{23}SO_3$ and (C, D) $C_{18}H_{25}SO_3$. Further examples are given in the Supporting Information. (A) HCD produced at 35 eV; (B) HCD produced at 80 eV for $C_{18}H_{23}O_3S$; (C) HCD produced at 35 eV; and (D) HCD produced at 80 eV for $C_{18}H_{25}O_3S$

sulphoxides in the C_{13-23} range have been detected in oil sands bitumen previously (e.g.[61–63]) and these unknowns with 3–5 DBE (*viz* two to four rings plus the carboxyl group) may be more oxidised analogues of these. Those with 4 DBE (e.g. $C_{18}H_{31}SO_3$, $[M+H]^+$) may also include methylated alkylbenzene sulphonates.

Possible origins of S-containing hydroxy acids

It is well known that the composition of petroleum can be influenced by the action of microbes (biotransformation or biodegradation) and this can occur *in situ* in oil reservoirs and when oil is spilled in the environment. Often the overall effect is addition of oxygen to the original hydrocarbons; carboxylic acids are common products of these processes (e.g. see review[14]). Thus, the polar components of biodegraded oils are often increased relative to the fresh oils (e.g.[64]). Although the influence of bacterial transformation on the origins of oil sands of Canada has long been debated (e.g.[65,66]), most authors agree that mature oils migrated and *were* altered by biodegradation to varying degrees (e.g.[66]). Thus, it is generally accepted that the naphthenic acids of oil sands originated from bacterial alteration[58] and those of OSPW probably likewise, followed, perhaps, by further alteration in tailings ponds or in the wider environment (e.g.[11,13]).

The structures of organic chemicals can have a profound effect on susceptibility to microbial biotransformation. Thus, the sulphur-containing acids with a dibenzothiophene nucleus and a branched alkanoate side chain, identified previously in the present OSPW,[29] might reflect bacterial oxidation of a branched chain alkyl dibenzothiophene (Fig. 7); bacterial oxidation by carboxylation is often hindered at such a branch point. It is known that at such positions, hydroxylation may then occur (e.g. reviewed by Pirnik and McKenna[67]). Thus, a logical scheme can be proposed for the formation of some of the polar sulphur SO_3 species observed herein by continued bacterial alteration of the original sulphur-containing hydrocarbons, through the corresponding carboxylic acid to the hydroxy acid (Fig. 7). Hence detailed studies of the structures of the present polar constituents might improve understanding of the origins of the polar fractions of crude oils more generally (cf.[2]).

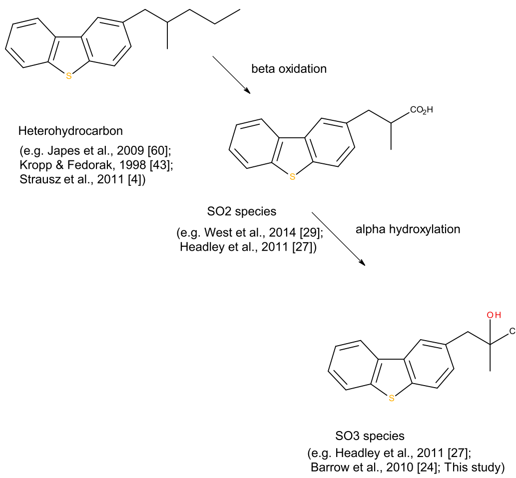

Figure 7. Hypothetical scheme for origin of some SO_3 species in polar petroleum fractions via biotransformation.

Attempted quantification of a SO_3 species in OSPW

The importance and difficulties of quantifying the proportions of the various components of polar fractions, such as naphthenic acids, in petroleum-related samples have been described previously (e.g.[25,37]).

Most attempts to date have been confined to measurement of totals or sub-fractions of whole mixtures of polar compounds such as naphthenic acids (e.g.[37]). This is because it is logistically demanding to measure thousands of individual components (cf.[12]). However, it may be *necessary* to measure individual, discrete compounds where they are deemed to be of particular interest, due to toxicological effects, for example. In such cases it should be relatively straightforward to measure those individual compounds which have now been separated and identified by comparisons with authentic compounds by GCxGC/MS and GCxGC/HRMS.[21–23,29,32,41] Internal standards, such as deuterated esters of identified acids, can be synthesised fairly readily for this purpose (cf.[32]).

Quantification of toxicological effects from mixtures can also be made by comparisons with appropriate commercially available reference compounds (e.g. dehydroabietic acid) once such relevant active compounds have been identified (e.g.[33,45]).

The same *may* be true of ionisation methods such as ESI in cases where individual compounds have been identified by MS and where the reference acids are now available for calibration. For example, Jones *et al.*[28] showed the variability in negative ion ESI response of adamantyl mono- and diacids identified in OSPW (the former identified by Rowland *et al.*,[22] the latter by Lengger *et al.*[23]) and many of these acids are available commercially, so it is at least theoretically possible to use appropriate calibrants.

However, where individual components have not yet been completely identified, as in the present SO_3 species, the variable responses of ionisation methods such as ESI, APPI and APCI are not so easily calibrated.

As an attempt towards this goal, herein, having delimited the structures of some of the SO_3 compounds to C_{17-22} sulphur-containing hydroxy acids, a C_{16} SO_3 hydroxy acid with 10 DBE ($C_{16}H_{14}SO_3$) was synthesised from a commercially available C_{16} SO_3 keto acid, by sodium borohydride reduction (Fig. 1). The compound was characterised by IR spectroscopy (O-H stretch, 3353 cm^{-1}, broad H-bonded; $C=O$ stretch 1708 cm^{-1}), by GC/MS of the bis-trimethylsilyl ester, ether ($M^{+\cdot}$ *m/z* 430, 285 (B+), 73, 75) and by FTICRMS ($[M–H]^-$ *m/z* 285.05909; $C_{16}H_{13}SO_3$ requires 285.05854, 1.93 ppm). CID MS/MS of the free acid produced ions due to losses of water, water plus carbon monoxide and water plus carbon dioxide. Attempts to make the methyl ester (BF_3/methanol or diazomethane) resulted either in no esterification or, on longer reaction times, produced the methyl ester, methoxy ether (characterised by GC/MS, FTICRMS and HPLC/MS. The HPLC retention time of the ether ester was nonetheless very similar to that of the unknown methyl esters (ca 11 min; cf. Fig. 2)).

Examination of the free acids of the OSPW by FTICRMS and MS/MS did reveal the presence of a compound $C_{16}H_{14}SO_3$ with an $[M–H]^-$ and product ions due to losses of water, water plus carbon monoxide, and water plus carbon dioxide. Increasing known amounts of the synthesised free acid (standard addition method, 10–50 ng) were therefore added to the unesterified free acid extract of the OSPW. The negative ion ESI response of known concentrations of the free acid spiked into the OSPW gave a linear response over this range when measured via the $[M–H]^-$ ion at *m/z* 285.05909, but the calculated concentration of the $C_{16}H_{14}SO_3$ compound based on this calibration was ~3% weight/weight in the OSPW, which is transparently not the case for such a minor compound. Since, in ESI, ions are in competition, the responses of analytes and calibrants at different concentrations are often non-linear[68] and clearly the amounts added in the present experiments were not comparable with those present in the OSPW extract which led to an overestimate.

CONCLUSIONS

Minor components detected by negative ion atmospheric pressure chemical ionisation (APCI) in a polar fraction of methylated OSPW were assigned to unesterified, partly aromatic, O_2 and O_4 acids and diacids in the range C_{15-29}. For the O_2 species, ions representing C_{16-18} species with six degrees of unsaturation (possibly due to tricyclic monoaromatic monoacids) dominated, while the major ions corresponding to O_4 compounds ranged from C_{18} to C_{24} and comprised bicyclic and tricyclic non-aromatic species, probably comprising mainly unesterified diacids. C_{20-26} tricyclic terpenoid monoacids have been identified previously in oil sands bitumen and the unknowns may be in part diacid analogues of the latter. Tricyclic C_{12-13} bis-esterified diacids have also been identified in other fractions of this OSPW.

The major ions detected by FTICRMS and UHPLC/orbitrap MS with positive ion electrospray (ESI) and atmospheric pressure chemical and photoionisation (APCI and APPI) were assigned to SO_3 and SO_5 compounds. Since these were only ionised under positive ion ESI, APCI and APPI conditions, they were not free carboxylic acids. MS/MS indicated that common collision-induced losses from these were typical losses of methyl esters of hydroxy acids, but higher energy MS/MS showed that several different species of sulphur-containing hydroxy acids were present. In some, sulphur was retained in ions devoid of oxygen, in others not. Once the fraction was re-saponified, the SO_3 species originally detected by positive ion MS could be detected only by negative ion MS, consistent with identification of several as sulphur-containing hydroxy carboxylic acids. Others may be alkylbenzene sulphonates. Attempts at quantification of the free acids by FTICRMS with a synthesised SO_3 keto acid by standard addition were unsuccessful, but environmental, corrosive or oil flow assurance effects should be easier to assess now that the partial structures are known. The origin of the hydroxy compounds is possibly via further biotransformation (hydroxylation) of the related S-containing carboxylic acids, identified previously in a less polar fraction.[29], cf. [42]

Acknowledgements

We are grateful to Mr Andrew Tonkin (Plymouth University) for technical assistance. Funding for this study was provided by the European Research Council via an Advanced Investigators Award to SJR for project OUTREACH (agreement no. 228149). UHPLC/orbitrap MS analysis was supported at the University of Alberta by grants to JWM (NSERC CRD and Discovery), where daily laboratory operations were also supported by Alberta Health.

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