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Structural identification of petroleum acids by conversion to hydrocarbons and multi-dimensional gas chromatography-mass spectrometry

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ABSTRACT: Identification of individual petroleum acids ('naphthenic' acids, NA) has proved challenging for decades, due to the extreme complexity of many petroleum acid mixtures. This has hindered detailed understanding of the role of NA in petroleum generation and oil production processes, refinery corrosion, as wood preservatives, and as environmental toxicants. Some recent advances have been made due to improved chromatographic separation of esters of the acids by multi-dimensional gas chromatography-mass spectrometry (GC×GC-MS), but relatively few reference spectra of esters are available for comparison. Here we report a complementary method based on a combination of a modified historical approach of converting NA to the corresponding hydrocarbons, followed by analysis by GC×GC-MS. Many published spectra exist for reference hydrocarbons making comparisons of reference spectra with those of the unknowns, much more feasible. As an example, we report identification of over 30 individual bicyclic naphthenic acids as the bicyclane hydrocarbons. These include both fused and bridged acids possessing methyl, dimethyl and ethyl alkyl substituents, as well as some terpenoid-derived acids. The study provides the most comprehensive analysis of one of the major classes of NA (the bicyclic acids) to date. There is now clear potential for this method to be used for the structural elucidation of other unknown acids (e.g. oil sands acids) and functionalised biomarkers in complex mixtures.

The structural elucidation of petroleum acids has been a challenge facing chemists for over 140 years due to the extreme complexity of the mixtures.[1] Better known as 'naphthenic' acids (NA), these compounds are found in both crude oils and bitumens. Early research into the structures of individual NA was driven by interests in petroleum formation processes and later by requirements in maintaining crude oil quality and flow during production, by the realization that NA were also involved in corrosion processes, and in the commercial uses of isolated naphthenate salts as biocides and wood preservatives.[2, 3] More recent interest has also arisen from concerns over the presence of naphthenic acids in oilfield produced waters and oil sands process-affected waters (OSPW) and their associated toxicity.[4]

Some attempts to advance knowledge of the nature of the individual acids were made in the 1970s, particularly by Seifert and co-workers, who used an approach based on conversion of the acids to the respective hydrocarbons, which were deemed likely to be more amenable for study than the acids.[5] However, in fact, only a few individual hydrocarbons were identifiable due to the lack of sufficient chromatographic separation, which prevented assignable mass spectra from being obtained routinely.[5, 6] Recently, improved gas chromatographic methods, such as multi-dimensional gas chromatography-mass spectrometry (GC×GC-MS) allowed some NA in complex mixtures to be identified as methyl esters.[7] However, such identifications were also limited, mainly by the lack of reference mass spectra of the esters needed for comparison with those of the esters of the unknown NA.

Numerous studies have shown that the most abundant class of NA in petroleum NA, in commercial acids and in NA extracted from oil sands, is the non-aromatic bicyclics.[8-10] Until recently, none had been identified; even now, few have been.[11]

Here, we report an alternative method for the identification of bicyclic NA, based on converting the methyl esters separated by argentation chromatography, to the corresponding hydrocarbons, followed by GC×GC-MS. The individual mass spectra of the corresponding hydrocarbons were comparable with the numerous reference mass spectra available for petroleum hydrocarbons. We report mass spectral identification of more than thirty individual compounds, by comparison with reference spectra and reference compounds when available, including bicyclanes with fused and bridged structures possessing methyl, dimethyl and ethyl alkyl substituents and terpenoid-derived acids.

EXPERIMENTAL DETAILS

4-methylbicyclo[3.3.0]octane-2-carboxylic acid, 4-pentylbicyclo[2.2.2]octane-1-carboxylic acid, 2,6,6-trimethylbicyclo[3.1.1]heptane-3-carboxylic acid, bicyclo[3.3.1]nonane-1-carboxylic acid and bicyclo[3.3.0]octane-2-carboxylic acid (endo-/exo- mixture) were purchased from Sigma Aldrich (Poole, UK). Bicyclo[4.4.0]decane-1- and 2-carboxylic acid and bicyclo[4.4.0]decane-1- and 2-ethanoic

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acid were synthesised from (5,6,7,8)-tetrahydro-1 and 2-naphthoic acid and naphthalene-1 and 2-ethanoic acids purchased from Sigma, by hydrogenation over 20% $Pd(OH)_2/C$ using a H-Cube® (ThalesNano Nanotechnology Inc., Budapest), fitted with a HPLC Pump and Rheodyne injector, in cyclohexane at 0.2 mL min^{-1} at 100 bar and 100°C.

Instrumentation

Fourier transform infrared (FT-IR) spectroscopy was performed using a Bruker Alpha FT-IR spectrometer with a Platinum ATR module. The spectra were attained at 4 cm^{-1} resolution using a DTGC detector, running 16 scans per spectrum. ^{1}H-NMR and ^{13}C-NMR spectra were obtained in $CDCl_3$ using a JEOL ECP-400 NMR Spectrometer (Experimental Details in Supporting Information for further details).

Gas chromatography-mass spectrometry (GC-MS) was conducted using an Agilent GC-MSD; 7890A Gas Chromatograph fitted with a 7683B Autosampler and a 5975A quadrupole mass selective detector. The column was a 30 m × 0.25 mm × 0.25 µm Rxi®-1ms (Restek, Bellefonte, USA). The helium carrier gas was kept at a constant flow rate of 1.0 mL min^{-1} and a 1.0 µL sample was injected at 300 °C splitless. The oven programme was 40 – 300 °C at 10 °C min^{-1} and held at 300 °C for 10 min. The ion source temperature in the quadrupole detector was 280 °C, and an ionisation energy of 70 eV was used. The chromatograms were recorded using Chemstation™ and the instrument operated in full scan mode, with a scan range of m/z 50 – 550.

Comprehensive multi-dimensional gas chromatography-mass spectrometry (GC×GC-MS) analyses were conducted as described previously,[11] using an Agilent 7890A gas chromatograph (Agilent Technologies, Wilmington, DE) fitted with a Zoex ZX2 GC×GC cryogenic modulator and secondary oven (Houston, TX, USA) interfaced with an Almsco BenchTOFdx™ time-of-flight mass spectrometer (Almsco International, Llantrisant, Wales, UK). The first-dimension column was a 100% dimethyl polysiloxane 60 m × 0.25 mm × 0.25 µm Rxi®-1ms (Restek, Bellefonte, USA), followed by a 1 m x 0.1 mm deactivated fused silica modulation loop. The second-dimension column was a 50% phenyl polysilphenylene siloxane 2.5 m × 0.1 mm × 0.1 µm BPX50 (SGE, Melbourne, Australia). Helium was used as carrier gas and the flow was kept constant at 1.0 mL min^{-1}. 1 µL samples were injected at 300°C splitless. The primary oven was programmed from 30°C, held for 1 min, then heated to 120°C at 5°C min^{-1}, to 220°C at 0.8°C min^{-1}, to 280°C at 5°C min^{-1} and to 320°C at 10°C min^{-1} and then held for 10 min. The secondary oven was programmed to track the primary oven at 40°C above. The hot jet was programmed to start 30°C above the primary oven temperature until 150°C, it was then ramped to 260°C at 1.3°C min^{-1} and then to 400°C at 4°C min^{-1}. Optimum separation of the hydrocarbons was achieved with a slower temperature gradient and shorter modulation but did not lead to further identifications (Figures S-1 and S-2). Modulation periods of 2, 4 and 6 s were used. The MS transfer line temperature was 290°C and ion source 300°C.

Data processing was conducted using GC Image™ v2.3 (Zoex, Houston, TX, USA). The CLIC expression tool (Computer Language for Identifying Chemicals) within GC Image™ v2.3 was used to aid location of compounds and for determination of the presence or absence of compounds. The CLIC expression tool is similar to the LECO ChromaTOF® mass spectral filters which use Microsoft Visual Basic Scripting. CLIC expressions are a powerful tool for producing advanced extracted ion chromatograms with additional constraints and have been described previously.[12]

Some mass spectral matches were made using NIST Search MS 2.0. Reference mass spectra obtained from the literature were input into a local NIST library using the NIST librarian tool so mass spectral match quality values could be determined; mass spectral match quality was typically >85%.

Derivatisation and Fractionation of NA

A commercial petroleum-derived NA mixture was derivatised and fractionated using a scaled up procedure previously described by Scarlett et al.[13] based on the method reported by Jones et al.[14]. Briefly, the acids were derivatised with BF_3-methanol at 70°C and 310 mg of the acid methyl esters were passed through 37.5 g of Discovery® Ag-Ion phase. Two fractions eluted with hexane (F2 and F3) were examined by GC-MS and GCxGC-MS and shown to contain (molecular ions) predominantly alicyclic, particularly bicyclic, acid methyl esters, as determined previously.[14]

Reduction of Methyl Esters to Alcohols

The acid methyl esters (F2; 50 mg and F3; 9 mg) were dissolved in anhydrous diethyl ether (inhibitor-free, Sigma Aldrich) before lithium aluminum hydride (LAH) solution (1.0 M in diethyl ether, Sigma Aldrich) was added in excess (5 molar equivalents). The reaction mixtures were stirred for 30 mins at room temperature and then completed with a microscale acid work-up with H_2SO_4 (10%), before extraction, washing with $NaHCO_3$ (5%) and brine (6%) and drying over anhydrous $MgSO_4$. Products were filtered and evaporated to dryness. The average yield for the reduction based on the model acids was 91% (Table S-1).

Formation of Tosyl Esters

Alcohol products (F2; 43.5 mg and F3; 8.7 mg) were dissolved in DCM in 5 mL reaction vials (HPLC Grade; Rathburns Chemical Ltd.). Reaction vials were placed in an ice bath, then 4-(dimethylamino)pyridine (DMAP) (1 molar equivalent), followed by tosyl chloride (TsCl) in slight excess (1.2 molar equivalent) and finally an aliquot of triethylamine (TEA) (1 molar equivalent) was added and the solution left to react for 12 hours. Diethyl ether and water was added, stirred and the product extracted and washed with HCl (2.0 M), $NaHCO_3$ solution (5%) and finally brine (6%) before being dried over anhydrous $MgSO_4$. Tosylate products were filtered before evaporation to dryness. The average yield based on the tosylation of the reduced model compounds was 86% (Table S-1).

Reduction of Tosyl Esters

Tosylate products (F2; 63.8 mg and F3; 11.3 mg) were dissolved in anhydrous tetrahydrofuran (THF) (≥99.9%; Sigma Aldrich) under a stream of N_2, then excess lithium triethylborohydride ($LiEt_3BH$) solution (1.0 M in THF; Sigma Aldrich) was added (15 molar equivalents) and the reaction left

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stirring at room temperature for 2 hours. The excess hydride was decomposed with the dropwise addition of NaOH (20%). The product was extracted with diethyl ether and washed with HCl (2.0 M) and brine (6 %) and dried over $MgSO_4$. To retain volatile, low molecular weight compounds, the product was filtered and then concentrated to ~1.0 mL using a micro-Kuderna-Danish apparatus; therefore no gravimetric weight of the final products were recorded.

Hydrocarbon Concentration and Clean-up

The hydrocarbon product underwent a simple silica chromatography clean-up step. However careful concentration of the product using a Kuderna-Danish apparatus meant the clean-up step had to involve the least possible addition of solvent. Therefore a cut glass Pasteur pipette was plugged with a small quantity of defatted cotton wool. Next 300 µL of concentrated product solution was applied dropwise onto ~1.2 g of activated silica (600 A mesh, activated at temp 200°C for 12 hours) in an aluminum weighing boat, allowing the excess solvent to absorb or evaporate between applying each drop. The loaded silica was then transferred into the shortened column and the hydrocarbons eluted through with hexane until 100 µL was collected in an insert vial ready for analysis.

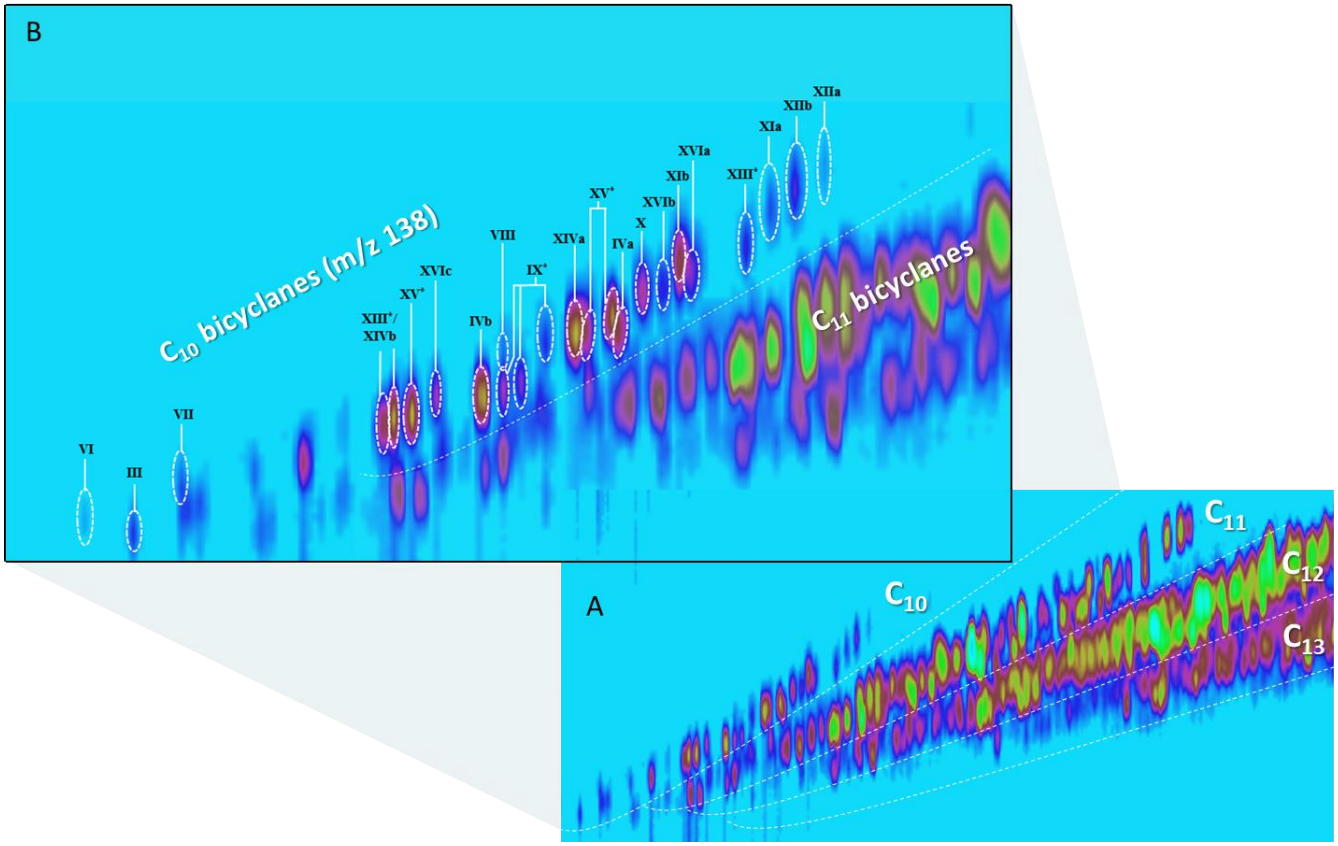

Figure 1. (A) GC×GC extracted ion chromatogram (EIC) of $C_{10\text{-}13}$ bicyclanes (m/z 138, 152, 166, 180) in the F3 reduced acid hydrocarbon product showing clear separation of homologous series by carbon number. (B) Zoomed insert showing sufficient separation of individual components between C_{10} homologues for identification by comparison with literature reference mass spectra. Labels correspond with structures in Chart 1.

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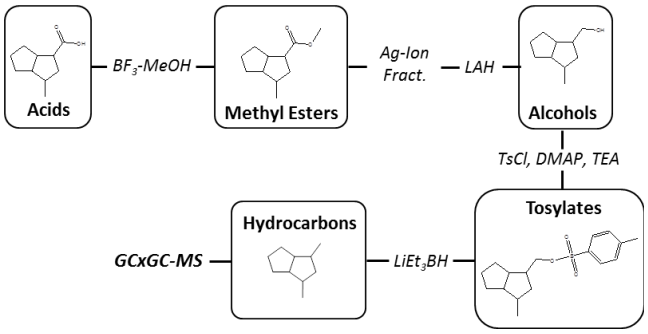
RESULTS AND DISUCSSION

Conversion of the acid methyl esters to the hydrocarbons was first optimised on known 'model' bicyclic acids before being performed on commercial NA fractions in an attempt to identify unknown individual acids structures.

Synthetic routes adopted previously for conversion of NA to their hydrocarbon counterparts consisted of three-step transformations: reduction of the carboxylic acids,[5] or their methyl[15-17] or ethyl[18] esters, to the corresponding primary alcohols, followed by formation of tosyl or other derivatives and reduction of the tosyl,[5] mesyl[19, 20]or iodide[15-18] intermediates to the hydrocarbons. An alternate, direct deoxygenation of petroleum acids was attempted by hydrogenolysis of the methyl esters over a nickel catalyst.[21] The initial reduction is usually carried out with LAH. The deoxygenation of primary alcohols is the most versatile step of the conversion, with a variety of derivative options available. Tosylation reactions are usually carried out in the presence of an amine base, classically pyridine with either chloroform or pyridine as the solvent.[5, 22] The reduction via the formation of an iodide has been achieved by heating the alcohols with hydroiodic acid.[16, 17] However, studies investigating the ring structure of such acids, concerned about possible isomerisation, used iodine and red phosphorus as an alternative.[15, 23] Reduction of the iodides is usually carried out over zinc dust, in the presence of hydrochloric acid.[17, 18] The final reduction of other intermediates is often a repetition of the first step using LAH.

Since these earlier studies, the use of "Superhydride" i.e. $LiEt_3BH$ has been advocated for the more efficient reduction of tosylate derivatives.[24-26] Also, formation of tosyl and mesyl esters has been shown to produce the corresponding chlorides and alkenes.[27, 28] Catalytic tosylation in the presence of DMAP using TEA as a base was used herein, as opposed to previous methods of refluxing in pyridine. The catalytic tosylation allowed for milder conditions and gave better yields for the model tosylates (Table S-1). No alkene or chloride by-products were observed in the NMR and GC-MS analyses of the tosylate products.

Overall therefore, the scheme utilised herein was a further development of the methods reported by Seifert et al.[5]. Production of the hydrocarbons from the NA methyl esters, involved reduction of the fractionated esters with LAH, derivatisation to the tosylates in the presence of DMAP and TEA and then a "Superhydride" reduction to the hydrocarbons (Scheme 1).



Scheme 1. Route of conversion of the acids, via derivatisation and fractionation of the acid methyl esters, to the corresponding hydrocarbons. Model compound 4-methylbicyclo[3.3.0]octane-2-carboxylic acid is shown as example.

Chart 1. Bicyclic hydrocarbons identified by conversion of petroleum acids to alkanes and comparison of mass spectra with those of known hydrocarbons. The identification of the alkanes allows inference of the structures of the corresponding bicyclic acids, which were previously unknown for decades (* presence of acid methyl ester confirmed with reference compound). For mono-substituted bicyclics R = CH_3 in bicyclanes; CO_2H in acids. For di-substituted bicyclics R,R' = CH_3 in bicyclanes; R = CO_2H, R' = CH_3 or R = CH_3, R' = CO_2H in acids.

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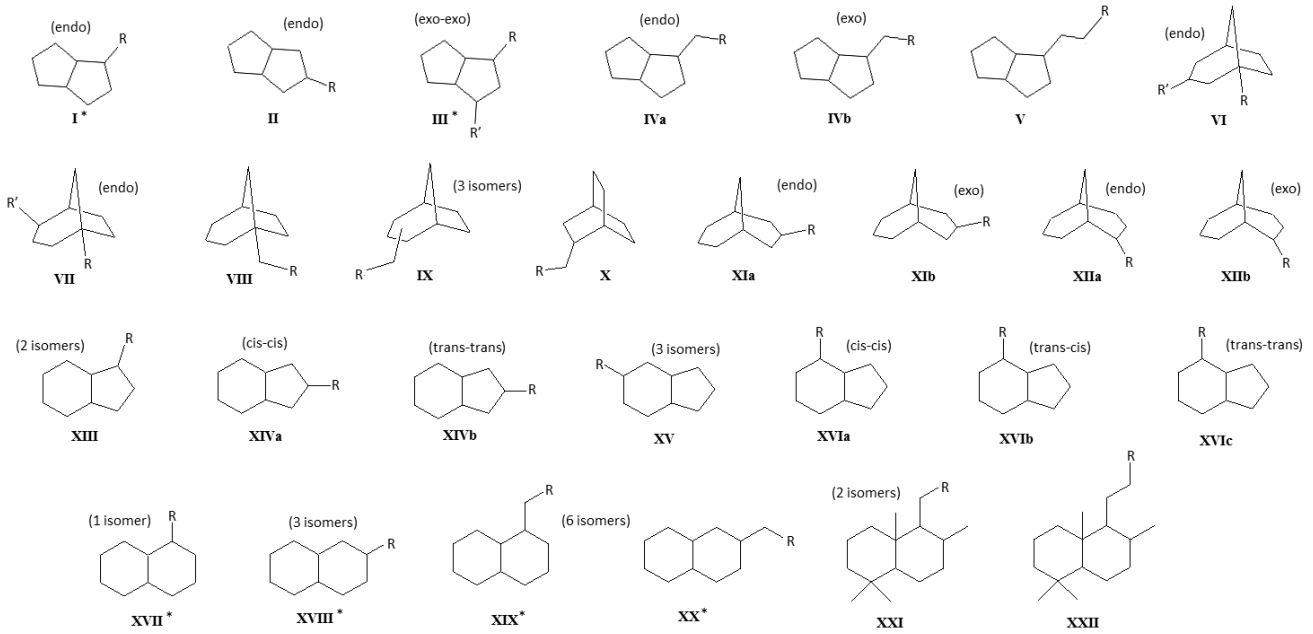

Conversion of known bicyclic ('model') acids

The reduction was first performed on known individual 'model' acids, to optimise yields and to ensure that the structure of the acids was maintained without extensive rearrangement. The model acids were (i) 4-methylbicyclo[3.3.0]octane-2-carboxylic acid, (ii) 2,6,6-trimethylbicyclo[3.1.1]heptane-3-carboxylic acid and (iii) 4-pentylbicyclo[2.2.2]octane-1-carboxylic acid. These contain fused (i), bridged (ii, iii), stable (i, iii) and strained (ii, containing a cyclobutane ring within the bicyclic core) structures, with multiple methyl or alkyl substitutions as postulated in the literature for bicyclic NA [29, 30] and with the carboxyl groups substituted at both tertiary (i, ii) and potentially more hindered quaternary (iii) positions.

The alcohol, tosylate and hydrocarbon products were each characterised by infrared spectroscopy (IR), gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance spectroscopy (NMR). These results are summarised in the Supporting Information Experimental Details and the yields are given in Table S-1.

The mass spectra of the hydrocarbon products from (i)- (iii) (Figure S-1) all displayed molecular ions corresponding with the expected carbon numbers and were all comparable with literature spectra.[31, 32]

Conversion of unknown commercial NA

A commercial petroleum-derived NA mixture was derivatised to the acid methyl esters and fractionated by argentation chromatography as reported previously.[11] Fractionation reduced the overall complexity and allowed separation of broadly 'alicyclic' (eluting with hexane; F2 and F3) ester fractions. Previously, GC×GC-MS of two other commercial NA samples showed that over 100 bicyclic acids were typically present[33] and a subsequent study of the F2 fraction showed that amongst the NA present were the esters of bicyclic acids.[11] Most investigations reporting mass spectral data for commercial NA only report group classification by hydrogen deficiency ('z' values), with bicyclic or 'z = -4' acids frequently observed as one of the most abundant classes.[33-36] Identification of individual acid components as the methyl esters has only been achieved recently but is still limited.[11, 37]

Reduction of the 'alicyclic' (F2 and F3) ester fractions by the methods developed herein based on those reported by Seifert et al.[5] (Scheme 1), as expected, produced mixtures of the corresponding bicyclic hydrocarbons (bicyclanes). Examination of these by GC×GC-MS resulted in excellent separation and extracted ion monitoring of the expected molecular ions (e.g. m/z 138, 152, 166) revealed highly resolved homologous series (Figure 1; A). The excellent GC×GC separation meant clear, distinguishable mass spectra could be obtained for individual components (Figure 1; B), in contrast to those obtained in some earlier GC-MS studies.[5, 38] This was crucial for identification of individual compounds by MS.

The hydrocarbon products obtained from transformation of NA ester fractions F2 and F3 contained bicyclanes assigned from molecular ions as $C_{9\text{-}16}$ (F2) and $C_{10\text{-}17}$ (F3). Recovery of the volatile, lower carbon number (e.g. $C_{9\text{-}11}$) bicyclanes was attributed to the mild reaction conditions employed and effective use of the micro-Kuderna-Danish apparatus to reduce evaporative losses.[39] Retaining these low molecular weight bicyclics was important for the subsequent identifications because the majority of published studies only report the mass spectra of low molecular weight alkanes.[31, 32, 40, 41] A mass spectral database of bicyclic hydrocarbons was collated herein and spectra plotted from tabulated data in the literature, along with published retention position and elution order data, where

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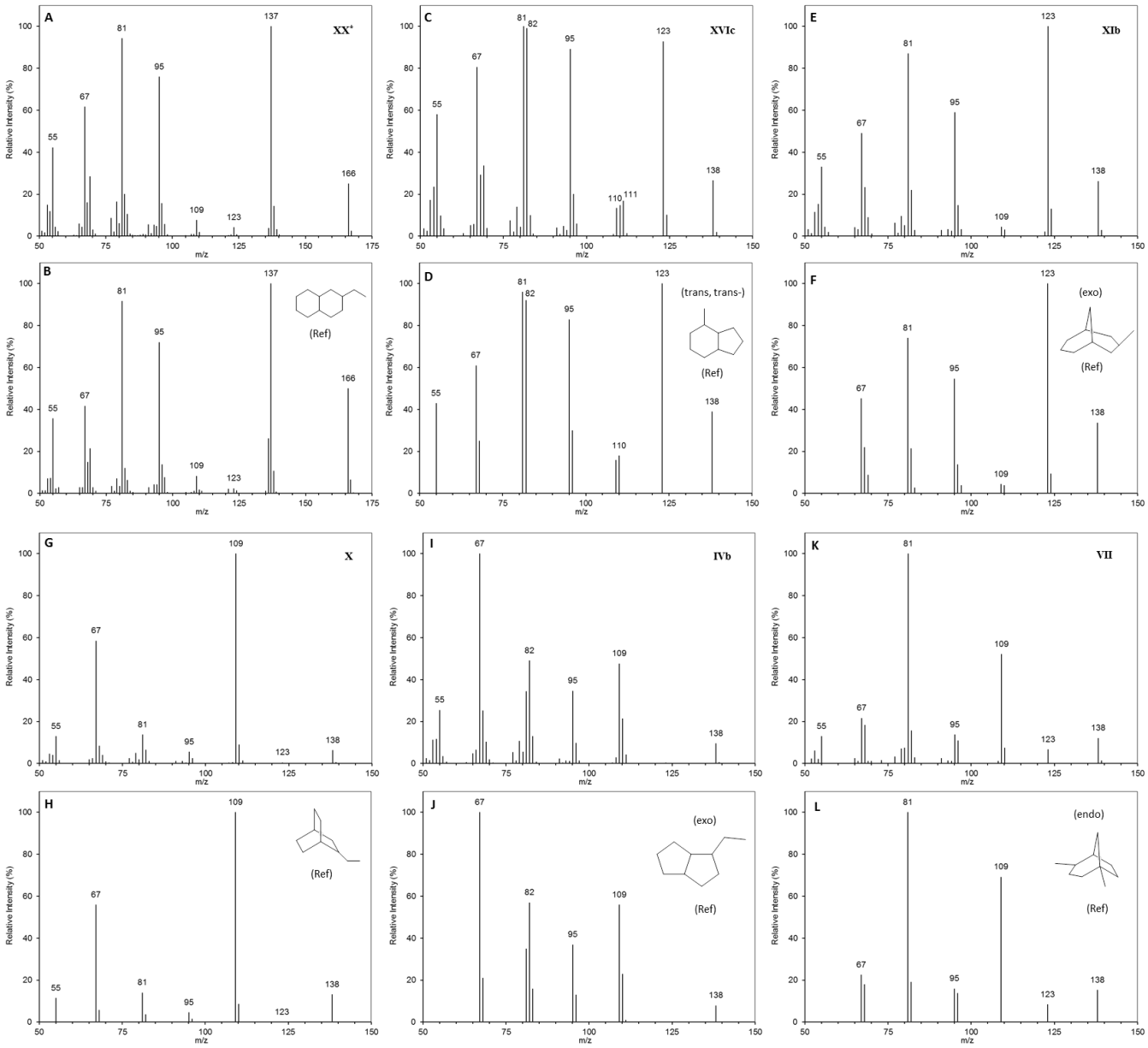

Figure 2. Examples of mass spectral identification of bicyclic hydrocarbons from the reduced acid methyl esters by comparison with literature mass spectra including (A and B) bicyclo[4.4.0]decanes, (C and D) bicyclo[4.3.0]nonanes, (E and F) bicyclo[3.3.1]nonanes, (G and H) bicyclo[2.2.2]octanes, (I and J) bicyclo[3.3.0]octanes and (K and L) bicyclo[3.2.1]octanes. Reference mass spectra plotted from tabulated values previously reported.[31, 32, 40-43]

available. Much of this mass spectral information was obtained from older literature, particularly from Russia; early Russian investigations involved extensive research into the isolation and identification of individual petroleum hydrocarbons, which rivaled that of the famous API Project 6.[2] However, none of these studies used the approach herein to infer the structures of the corresponding bicyclic acids.

Fractionation by argentation chromatography of the methyl esters prior to the reduction step herein, also lowered the complexity of the final hydrocarbon products; analysis of hydrocarbons resulting from transformation of each of the alicyclic NA ester fractions F2 and F3, further reduced co-elution. For example, some bicyclic hydrocarbons, which co-eluted with monocyclic hydrocarbons in F2, were identifiable in F3, where the monocyclics were absent. The combination of the separation power of GC×GC-MS coupled with the older approach of chemical transformation of the acids to hydrocarbon resulted in the mass spectral identification of over 30 individual bicyclic NA as their corresponding hydrocarbons by comparison with published mass spectra of known alkanes (Chart 1 and Figures 1 and 2). These included bicyclo[4.4.0]decanes, bicy-

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clo[4.3.0]nonanes, bicyclo[3.3.1]nonanes, bicyclo[2.2.2]octanes, bicyclo[3.3.0]octanes and bicyclo[3.2.1]octanes, as well as some more highly substituted terpenoid bicyclanes.

For example, comparison of mass spectra obtained herein with those reported by Denisov et al.[41] gave good matches with two dimethyl- and one ethyl-substituted bicyclo[3.2.1]octane (Figure S-2), all three isomers substituted at the quaternary, bridgehead 1-position (Chart 1; VI-VIII). The 1,3- and 1,4-dimethyl- isomers were two of the earliest eluting peaks within the homologous series and the 1-ethyl- isomer eluted later, as expected (Figure 1).

One of two peaks with similar mass spectra was identified as 2-ethylbicyclo[2.2.2]octane (Chart 1; X), the second peak resembled that of the 1-isomer but co-eluted with another unknown making the assignment speculative. The mass spectra matched those reported by Denisov et al.[32] (Figure 2; G and H), easily distinguished from the C_{10} dimethylbicyclo[2.2.2]octane isomers by their intense base peak (m/z 109), with the fragmentation dominated by the strong loss of an ethyl group (M-29) (Figure S-3). The two peaks had similar mass spectra but could be differentiated by the intensities of the m/z 81 and 82 ions; the loss of $C_4H_9^+$ (m/z 81) being greater for 2-ethylbicyclo[2.2.2]octane and loss of C_4H_8 (m/z 82) being greater for the 1-ethyl isomer.

Three peaks, eluting the latest of the C_{10} hydrocarbons had mass spectra comparable with those of three methylbicyclo[3.3.1]nonane isomers (Chart 1; XI-XII) reported by Golovkina et al.[43] (Figures 1 and S-4). This corresponds with previous observations of bicyclo[3.3.1]nonane-1- and 3-carboxylic acids identified previously in commercial NA as the methyl esters.[11] Herein, two isomers of 3-methylbicyclo[3.3.1]nonane were identified (Figure 2; E and F and Figure S-4), but 1-methylbicyclo[3.3.1]nonane, which has a mass spectrum distinguished by a base peak ion at m/z 95, due to the loss of a propyl group (M-43), was not, despite the presence of the acid methyl ester being confirmed with a reference standard. A pair of resolved peaks eluting later than the 3-methyl isomer had mass spectra matching two isomers of 2-methylbicyclo[3.3.1]nonane (Figure S-4).

Wilde et al.[11] reported the presence of a few bicyclo[2.2.1]heptane acids in an OSPW acid extract. The corresponding hydrocarbons would be expected to have the earliest retention times of the C_{10} hydrocarbons based on the relative positions of the acid methyl esters; bicyclo[2.2.1]heptane-2-ethanoic acid eluted earlier than bicyclo[3.2.1]octane-2-carboxylic acid and bicyclo[2.2.2]octane-2-carboxylic acid.[11] Interestingly, no $C_{10\text{-}11}$ bicyclo[2.2.1]heptanes were observed in the reduced commercial NA fractions when compared with the mass spectra of the trimethyl-/methyl-ethyl- or tetramethyl/-dimethyl-ethyl- bicyclo[2.2.1]heptane isomers.[44]

Previously Rowland et al.[37] reported the presence of 4-methylbicyclo[3.3.0]octane-2-carboxylic acid (also called 3-methyloctahydropentalene-1-carboxylic acid) in commercial NA, but they did not report the mass spectrum. Here, we identified the corresponding 2,4-dimethylbicyclo[3.3.0]octane by comparison with a series of bicyclo[3.3.0]octane mass spectra (Figure S-5; A and B).[31] Such assignments were made possible by the pre-fractionation of the alicyclic acid methyl esters by argentation chromatography, prior to reduction to the alkanes. Thus, 2,4-dimethylbicyclo[3.3.0]octane in the reduced hydrocarbons of F2 could not be initially firmly identified, due to co-elution with an unknown C_{10} monocyclic hydrocarbon, which made the mass spectrum less clear. However, in the reduced fraction F3, co-elution with the monocyclic was no longer observed and the mass spectrum of the unknown was clearly similar to that of the authentic bicyclooctane, which was then assigned in both F2 and F3.

We were then able to confirm the corresponding acid methyl ester in the esterified NA by comparison of the mass spectrum with that of a reference compound (Figure S-5; C and D). This shows the complementary approach of analysis of both ester and alkane fractions by GCxGC-MS. In the alkanes, two later eluting peaks were also identified as both 2-ethylbicyclo[3.3.0]octane isomers (Figure S-6; A and C), the exo- isomer eluting before the endo- isomer; an observation made by Jørgensen et al.[45] as well as Bagrii et al.[46] for the 2-methyl isomers.

Concentration of the final hydrocarbon products with the micro-Kuderna-Danish apparatus proved extremely efficient at reducing evaporative losses of the $<C_{10}$ bicyclanes. The mass spectra of two C_9 components were similar to those of 2-methyl and 3-methylbicyclo[3.3.0]octane and comparison of the F2 acid methyl esters with that of an esterified reference compound showed bicyclo[3.3.0]octane-2-carboxylic acid methyl ester was indeed present in this NA mixture, as it was previously in another.[37]

Amongst the C_{11} bicyclic hydrocarbons (m/z 152) was a peak with a matching mass spectrum to that of 2-propylbicyclo[3.3.0]octane (Figure S-6; E). This series of bicyclo[3.3.0]octanes, with increasing alkyl chain length (from methyl to propyl) reaffirms the belief that the higher carbon number homologues are more alkylated equivalents of the lower carbon structures identified herein.[11] It is also strong evidence for the current understanding of biodegradation of hydrocarbons along alkyl side chains and the occurrence of NA.

Rowland et al.[37] tentatively identified a series of bicyclo[4.3.0]nonane carboxylic acids based on mass spectral interpretation and comparison with one available mass spectrum of the 2-carboxylic acid isomer (perhydroindane-1-carboxylic acid). They proposed the presence of several isomers. However, the lack of mass spectra available meant they were unable to confirm this or to identify the position of the carboxyl group. Reduction of the commercial NA herein resulted in the identification of several methylbicyclo[4.3.0]nonane isomers (Chart 1; XIII-XVI) identified by comparison with reference hydrocarbon mass spectra[40] (Figure S-7 and S-8). The isomers with matching mass spectra included structures with the methyl group substituted on the cyclohexyl and cyclopentyl ring, but not on a bridgehead carbon. Methylbicyclo[4.3.0]nonanes substituted at the 7- or 8- position (on the cyclopentyl ring) showed a prominent loss of C_3H_6 (m/z 96) (Figure S-8) compared to those substituted at the 2- and 3- positions (on the cyclohexyl ring) which showed a prominent loss of $\cdot C_3H_7$ (m/z 95) (Figure S-7).

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Bicyclo[4.4.0]decane acids (decalin acids) are probably the most studied or identifiable acids within the few studies which report EI mass spectra for individual NAs. Aitken et al.[47] identified two isomers of bicyclo[4.4.0]decane-3-carboxylic acid (decahydro-2-naphthoic acid) within several biodegraded oils from deep surface reservoirs and proposed they were the reduced product of the anaerobic biodegradation of naphthalene. Rowland et al.[37] also identified two isomers of bicyclo[4.4.0]decane-3-carboxylic acid and suggested that their presence could be evidence that at least some of the acids present within their commercial NA were a result of anaerobic biodegradation. They also reported not detecting any bicyclo[4.4.0]decane-2-carboxylic acid (decalin-1-carboxylic acid) isomers.[37] Careful examination of the C_{11} hydrocarbon mass spectra herein and comparison with the reported mass spectra of methylbicyclo[4.4.0]decanes (decalins)[48] allowed the identification of three isomers of bicyclo[4.4.0]decane-3-carboxylic acid and one isomer of bicyclo[4.4.0]decane-2-carboxylic acid (decahydro-1- and 2-naphthoic acid) (Figure S-9). Subsequent comparison of the corresponding acid methyl ester fractions with synthesised reference compounds confirmed the presence of the bicyclo[4.4.0]decane-2- and 3-carboxylic acid methyl ester isomers.

The mass spectra of methylbicyclo[4.4.0]decanes are distinguishable by their strong molecular ions (m/z 152). Using the CLIC expression tool to return data points where the m/z 152 ion had a relative intensity greater than 50% severely reduced the complexity of the image down to only five peaks, making it easier to identify the methylbicyclo[4.4.0]decanes present (Figure S-9; A).

Examination of the C_{12} bicyclane mass spectra and comparison with the plotted mass spectra of the tabulated values reported by Brodskii et al.[49] led to the tentative identification of two dimethylbicyclo[4.4.0]decane isomers (Figure S-10). Furthermore, two major peaks and four later eluting minor peaks had NIST mass spectral matches with 1- and 2-ethylbicyclo[4.4.0]decane isomers (Figure S-11; A-F). Subsequent analysis of the acid methyl esters and synthesis of the corresponding eight possible ethanoic acid isomers confirmed the two major peaks were isomers of bicyclo[4.4.0]decane-1- and 2-ethanoic acid (methyl esters) with matching mass spectra and retention positions of the reference compounds (Figure S-11; G-L).

As well as mono- and di-substituted bicyclo[4.4.0]decanes, a few peaks were observed, which eluted late in chromatograms of both F2 and F3, with mass spectra matching those of bicyclic sesquiterpanes possessing drimane structures.[28, 50, 51] Bicyclic sesquiterpane hydrocarbons such as drimanes, rearranged drimanes and eudesmanes have been studied and used as biomarkers and their mass spectra are well documented.[51-53] To the best of our knowledge, the corresponding drimane acids have not been reported in commercial NA mixtures. Cyr and Strausz[54] reported the mass spectrum of one C_{16} bicyclic acid (methyl ester) present in the mineral-bound organic extract of the Alberta oil sands which possessed a similar mass spectrum to that of a C_{16} drimane hydrocarbon identified in Athabasca bitumen.[28] Nascimento et al.[20] also identified a C_{16} bicyclic drimane acid in a heavily biodegraded oil from the Albacora oil field, Brazil, along with higher $C_{19\text{-}20}$ labdanic acid homologues. Koike et al.[19] reported the presence of one C_{16} drimane peak present in the reduction product of the acids from Albacora crude oil. However, synthesis of the corresponding acid methyl ester, based on the structure previously reported [54] showed that it was absent. The mass spectrum of the synthetic C_{16} acid methyl ester was slightly different to that previously reported by Cyr and Strausz[54], with a distinct M-89 (m/z 177), possibly the mass spectrum of a different diastereoisomer.

Here we noted the presence of two C_{16} bicyclic hydrocarbons (M^+ = m/z 222) eluting 3 mins apart and one C_{17} bicyclic hydrocarbon eluting 6 mins later, in the F3 hydrocarbon product. Mass spectra matched those of C_{16} homodrimane and higher homologues (Figure S-12; A, C and E). [28, 50] The mass spectra of the drimane hydrocarbons show very characteristic fragmentation patterns, with a common loss of M-15 and dominated by a strong base peak at m/z 123, corresponding to fragmentation of the gem-dimethyl substituted ring (Figure S-12). Complementary analysis of the original F3 acid methyl esters by GC×GC-MS herein, prompted by the identification of the bicyclanes, confirmed the presence of the corresponding C_{16} and C_{17} drimane acids in the same elution order (3 and 6 mins apart) (Figure S-12; B, D and F). Interestingly, the mass spectrum of the earlier eluting C_{16} acid methyl ester matched the mass spectrum reported by Cyr and Strausz[54], whereas the mass spectrum of the later eluting C_{16} acid methyl ester matched the mass spectrum reported by Koike et al.[19] with a more abundant m/z 177 ion. Using the CLIC expression tool to return data points with a base peak of m/z 123 only (CLIC expression: Ordinal(123)=1), revealed a further two later eluting peaks with mass spectra and molecular ions matching those of C_{19} and C_{20} homologues (m/z 264 and 278) (Figure S-13; A and B).[28]

The identifications presented herein of individual hydrocarbons and thus acids, represent the most comprehensive study of bicyclic petroleum NA to date, we believe. The assignments are supported by the identification of multiple isomers of each acid type and are consistent with the (albeit sparse) previous evidence of such acids in other matrices, identified as the esters.[11] Where possible the assignments were supported by close matches with the GC×GC retention positions and mass spectra of reference compounds and, for various isomers, the GC×GC elution order of the hydrocarbons additionally matched those reported for other complex mixtures e.g. from catalytic conversion.[46, 55-57] The retention indices of the peaks in Figure 1 assigned as the structures in Chart 1 are reported in Table S-1.

CONCLUSIONS

Limited identification of individual petroleum acids has hindered detailed understanding of their role in petroleum generation and oil production processes, refinery corrosion, as wood preservatives and as environmental toxicants, for decades. Here we showed that a method based on a combination of an historical approach of converting acids to the corresponding hydrocarbons, followed by analysis by GC×GC-MS, resulted in identification of over 30 individual bicyclic acids as the bicyclane hydrocarbons. The study provides the most comprehensive analysis of one of the major classes of petroleum acids to date. There is now clear potential for this method to be used

for the structural elucidation of other unknown acids (e.g. oil sands acids) and functionalised biomarkers in complex mixtures.

ASSOCIATED CONTENT

Supporting Information

Mass spectra of all identified bicyclic hydrocarbons are provided in Supporting Information (Figures S-2-13). This material is available free of charge via the Internet at http://pubs.acs.org.

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