Archaeal C_{80} isoprenoid tetraacids responsible for naphthenate deposition in crude oil processing†

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The structure of a novel class of octaterpene tetracarboxylic acids which is responsible for naphthenate deposition in crude oil processing has been determined by NMR and mass spectroscopy.

Naphthenate deposition in crude oil processing has been a recognised problem over the last decade.[1–3] Worst case scenarios lead to production irregularities accompanied by unplanned and expensive production shutdowns. The result of naphthenate deposition in an oil–water separator is shown in Fig. 1.

Fig. 1 Naphthenate deposits in crude oil processing equipment, by courtesy of Oil Plus Ltd., UK.

Naphthenic acids are single and multiple fused cyclopentane and cyclohexane rings where the carboxylic acid group is attached to the aliphatic side chain or to the cycloaliphatic ring. Naphthenic acids are predominantly found in immature heavy crude, and they are assumed to be generated from in-reservoir biodegradation of petroleum hydrocarbons.[4,5] Traditionally, total acid number (TAN) has been correlated with the severity of biodegradation[6–8] and naphthenate deposition. Recently the correlation between TAN value and naphthenate deposition has been questioned.[9] The scientific fundamentals governing these formations are therefore important to elucidate.

In pioneering works by Baugh *et al.*[9,10] it was demonstrated that the molecular-weight distribution of naphthenic acids in calcium naphthenate deposits at the Heidrun oilfield on the Norwegian continental shelf was different from that in the crude oil. A family of tetrameric acids, referred to by the generic name ARN acids, in the molecular weight range of 1227 to 1235 Da was the main contributor to the organic residue in the precipitated calcium salt. Similar observations were made by analysing deposits from other oilfields offshore Great Britain, China and West Africa. The homologous series corresponds to empirical formula of $C_{80}H_{138}O_8$, $C_{80}H_{140}O_8$, $C_{80}H_{142}O_8$, $C_{80}H_{144}O_8$ and $C_{80}H_{146}O_8$ with double bond equivalences (DBE) ranging from 12 to 8, indicating 8 to 4 rings in the hydrocarbon skeleton, respectively. Whereas these tetraacids only constitute a few ppm of the crude oil, the total amount of naphthenic acids may be up to several wt%.

Naphthenic tetraacids isolated from a calcium naphthenate deposit sample acquired from an oilfield offshore West Africa was recently shown to be much more interfacially active than the majority of the naphthenic acids existing in crude oil. A concentration of only 0.005 mM tetraacid is sufficient to lower the interfacial tension (IFT) between water (pH 9.0) and *n*-hexadecane–toluene (9 : 1 *v*/*v*) by about 45 units, whereas naphthenic monoacids lowered the IFT less than 30 units at 2000 times higher concentration. Hence the tetraacids are more prone than the monoprotic acids to react with inorganic cations across the interface to form naphthenates.

The calcium salt of the tetraacids was also shown to be sticky towards solid surfaces. This behaviour was ascribed to their ability to cross-link with Ca^{2+} to form extended network layers. Monoprotic acids, on the other hand, may only form dimeric complexes in reaction with divalent cations. These are easily dispersed into the bulk oil solution. The sticky behaviour and the high interfacial activity are likely reasons why the tetraacids are found to dominate in naphthenate deposition despite of their low concentration in the crude oil.

The same naphthenate sample was further characterised by electrospray ionisation Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) in the negative mode.[11] The main peak located at m/z 1230.0627 was consistent with the ion $[C_{80}H_{141}O_8]^-$, *i.e.* the parent compound being $C_{80}H_{142}O_8$.

In this study, the naphthenic tetraacids from the same oilfield have been subjected to an extensive nuclear magnetic resonance (NMR) spectroscopy investigation. At first inspection, the 1D ^{13}C-NMR spectrum comprised 38 resonances (3 primary, 23 secondary and 11 tertiary sp^3 hybridised carbons and 1 sp^2 carboxylic carbon) with two signals (carboxylic carbon, 174.40 ppm and methyl carbon, 18.22 ppm) of approximately twice the integrated areas relative to each of the other signals. This effectively pointed to a C_{80} compound of dimeric nature. However, closer scrutiny of the spectrum after line-narrowing resolution enhancement revealed that most of the resonances are split up in additional peaks with different intensities. The ^1H spectrum was very complex with

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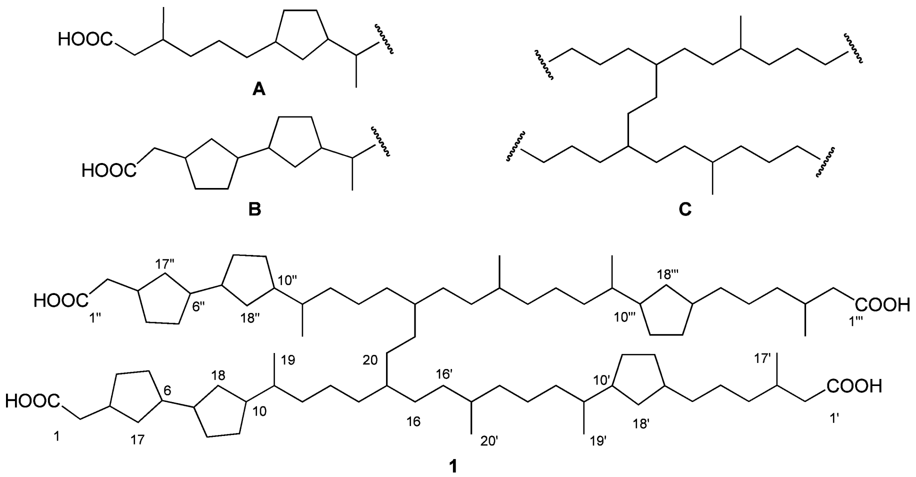

Fig. 2 Structures of molecular fragments **A**, **B** and **C**, and (6:17,10:18,10′:18′,6″:17″,10″:18″,10‴:18‴)-hexacyclo-20-bis-16,16′-biphytane-1,1′,1″,1‴-tetracarboxylic acid (**1**), one of four possible regioisomers of the tetraacid with six rings, as determined by NMR spectroscopy.

resonances in the region of 0.75–2.35 ppm with only a few isolated signals. In addition, one resonance comprised labile protons, the chemical shift of which varied with the polarity of the solvent used.

A series of edited 1D and correlation NMR spectra, including COSY, ROESY, HSQC, HMSC, 1,1-ADEQUATE, and 2D and 3D HSQC-TOCSY, was recorded on a 14.6 Tesla NMR instrumentation equipped with a cryogenic probe. The obtained correlations between proton and carbon resonances allowed the determination of the C_{14} structural units **A** and **B**, and the central C_{24} unit **C**, as shown in Fig. 2. Chemical shifts are given in Table 1 in the ESI. The 1,1-ADEQUATE experiment turned out to be crucial for the structure assignment, as the 2D HSQC-TOCSY spectrum was too crowded, and the 3D spectra had too low resolution to allow identification of all resonances.

The two end-units **A** and **B**, carrying the carboxylic acid moieties, were present in a 1.2 : 0.8 ratio relative to the **C**-unit, based on NMR integrals. In addition, a set of signals in the ^{13}C NMR spectrum ($<10\%$ relative intensity) were present, which might be due to minor carboxylic acid moieties. Due to the overlapping signals of the terminal methylene groups in the structural unit **C**, the positions of the end groups could not be determined. Thus four regioisomers are possible for the tetraacid with six five-membered rings. However, these overlapping signals were in fact an advantage in the structure determination, as they simplified the spectra of the mixture of octaterpenes into a mixture of the three subunits **A**, **B** and **C**.

The tetraacids were permethylated by treatment with BF_3 in methanol. The NMR spectrum of the tetramethyl esters was in agreement with that published previously.[10] Electrospray ionisation MS data showed major peaks for *m*/*z* 1310, 1312 and 1314 ($M + Na^+$) and 1326, 1328 and 1330 ($M + K^+$), for molecular formulas $C_{84}H_{150}O_8$, $C_{84}H_{152}O_8$ and $C_{84}H_{154}O_8$ with 10, 9 and 8 DBE, respectively. The intensities of the peaks corresponded to a composition of tetramethyl esters with 6, 5 and 4 rings in a 70 : 16 : 14 ratio, giving a composition of 1.22 : 0.78 for moieties **A** and **B**, in agreement with the NMR data.

One of the regioisomeric structures, **1**, for the major tetraacid with six five-membered rings is shown in Fig. 2. The structure is consistent with the isoprene rule, and can be considered as two head-to-head coupled phytanes, 16,16′-biphytane (**2**, Fig. 3), covalently bridged by a central methyl-to-methyl bridge (also head-to-head). This novel class of octaterpenes may therefore be regarded as derivatives of 20-bis-16,16′-biphytane (**3**, Fig. 3), and is most conveniently named as such. The tetraacid shown in Fig. 2 with six five-membered rings would thus be named (6:17,10:18,10′:18′,6″:17″,10″:18″,10‴:18‴)-hexacyclo-20-bis-16,16′-biphytane-1,1′,1″,1‴-tetracarboxylic acid (**1**).[12]

The relative stereochemistry of the five-membered ring systems was determined from chemical shift values, spin–spin J-couplings and nuclear Overhauser effects (NOE). For the structural unit **A**, with the 3-methylhexanoic acid moiety, a *trans* 1,3-substitution pattern could be assigned from the ROESY data for the monocyclic ring. This conclusion was supported by the ^{1}H- and ^{13}C chemical shifts, which are similar to those reported previously for this moiety.[13]

The bicyclopentane moiety (unit **B**) has previously only been identified from MS data,[14] and the stereochemistry of this moiety in biphytanes has therefore not been determined. The geminal hydrogen atoms on C-17 are 1.2 ppm apart (0.77 and 1.95 ppm) in the ^{1}H spectrum, in contrast to the corresponding protons on C-18 whose chemical shifts are identical (1.40 ppm). The protons on C-4, C-5, C-8 and C-9 all have pairwise axial–equatorial relationship with approximately 0.6 ppm difference in chemical shift. The protons on C-17 proved valuable handles in establishing the spatial arrangement in the bicyclopentane moiety, and the coupling constants of these protons[15] combined with the ROESY spectra established a *cis* substitution pattern for this ring, and *trans* for the next ring counting from the carboxymethyl substituent. If we assume that the stereocenters in the 16,16′-biphytane precursor have the same absolute stereochemistry as determined for acyclic glycerol dibiphytanyl glycerol tetraether (GDGT-0),[16,17] the stereochemistry of each individual phytane moiety will be as shown for (10:18)-cyclophytanic acid (**4**) and (7:17,10:18)-dicyclo-phytanic acid (**5**) in Fig. 3.

An energy minimised force field (MM2)[18] calculated structure of the bicyclopentyl moiety **5** is given in Fig. 4. It is interesting to note that in the flattened structural element, the five-membered

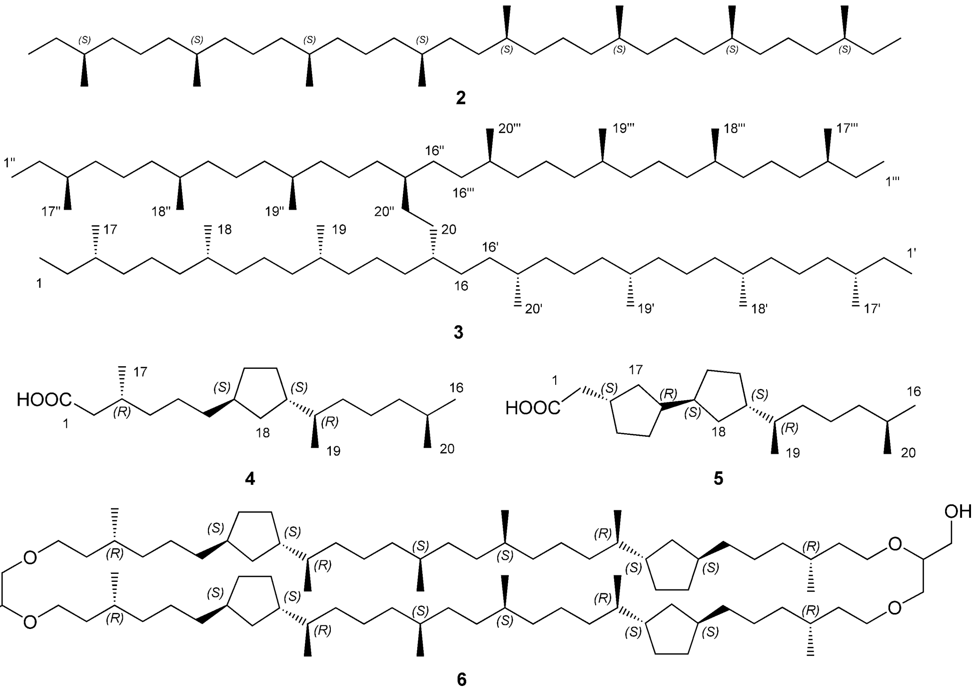

Fig. 3 Structures of 16,16′-biphytane (**2**), 20-bis-16,16′-biphytane (**3**), phytanic acid moieties **4** and **5** with stereochemistry according to the Cahn–Ingold–Prelog sequence rules indicated, and tetracyclic glycerol dibiphytanyl glycerol tetraether (GDGT-4, **6**).

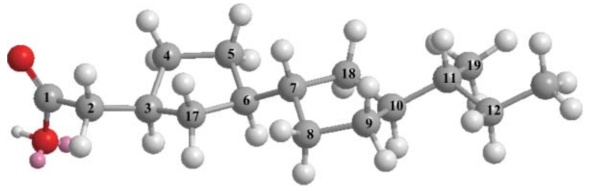

Fig. 4 3D structure of the bicyclopentyl moiety with *cis–trans* relationship in the two cyclopentane rings.

rings appear to exist in two different conformations. The ring comprised of carbons C-7, C-8, C-9, C-10 and C-18 is in a half-chair form with a local C_2-axis, rendering the protons on C-18 isochronous, whereas the C-3, C-4, C-5, C-6, C-17 ring is in an envelope conformation with a local C_s symmetry element where the protons on C-17 are in an axial-equatorial orientation. Although cyclopentane rings are conformationally very flexible, the NMR data support that our assignments are in agreement with the MM2 generated structure.

However, it is not possible to determine the absolute stereochemistry of the entire molecule as the selectivity in the head-to-head coupling of the phytane moieties is unknown. It seems plausible that four phytanes are coupled to form two molecules of 16,16′-biphytane (**2**) initially, and that these subsequently are symmetrically coupled to form the 20-bis-16,16′-biphytane. If this is the case, one diastereoisomer (**3a**, see Fig. 1 in the ESI) will result regardless of whether the coupling occurs between the methyl groups on the same, or opposite, side of the C-16-C-16′ coupling. The MM2 calculations of the structure of the parent GDGT shows that the distance between the two methyl groups on the same side of the central C-16-C-16′ coupling is much shorter than the distance between methyl group on opposite sides of this central bond, thus making the former the most likely coupling pattern.

An alternate route to the formation of the 20-bis-16,16′-biphytanes is the coupling of two phytane moieties to the central methyl groups of a preformed 16,16′-biphytane (**2**) either in a free form, or bound to glycerol as a glycerol–phytanyl diether. This reaction pathway may provide a stereoisomeric mixture, see Fig. 2 in the ESI.

The structures determined for the naphthenic tetraacids suggests an archaeal origin.[19–21] The Archaea are one of the three main domains of life on Earth, along with Bacteria and Eucarya, and are generally known to inhabit extreme environments where other organisms cannot exist, *e.g.* volcanic vents, strongly acidic or basic springs, evaporitic settings and deep-subsurface sediments.[22] However, recent findings indicate that large amounts of archaea are also present as picoplankton in the open ocean.[13,23] The basic skeleton found for the tetraacids in naphtenate deposits, 20-bis-16,16′-biphytane (**3**), shows resemblance to the GDGTs, which structure is represented with the tetracyclic GDGT-4 **6** in Fig. 3. GDGTs are core lipids in many archaea, and are mainly found in methanogens and Crenarchaeota. GDGTs with between zero (acyclic) and eight five-membered rings have been identified in the lipid membranes of archaea.[14]

A novel ether core lipid (designated as FU) with H-shaped C_{80} isoprenoid hydrocarbon chain from the hyperthermophilic methanogen *Methanothermus fervidus* was reported in 1998.[24] The structure was found to be a modified caldarchaeol in which two hydrocarbon chains are bridged with a covalent bond, but the location of this bond could not be determined from the available data. The presence of a C_{80} core lipid was also suggested in another hyperthermophilic archaea, *Pyrococcus horikosii* OT3.[25] Additional structural details of these C_{80} isoprenoids have not appeared in the literature.

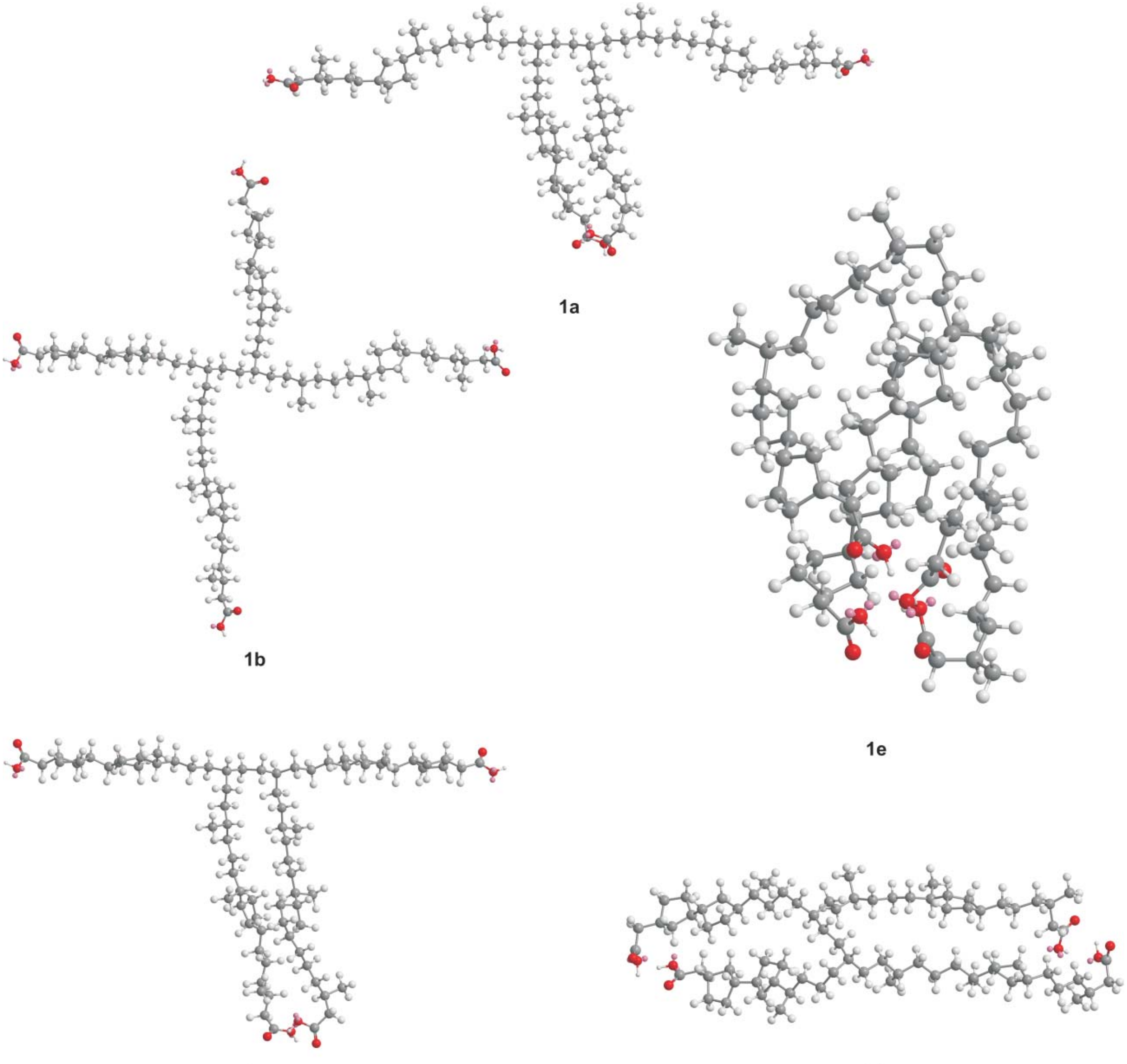

Fig. 5 Conformations calculated for (6:17,10:18,10′:18′,6″:17″,10″:18″,10‴:18‴)-hexacyclo-20-bis-16,16′-biphytane-1,1′,1″,1‴-tetracarboxylic acid (**1**), with stereochemistry preserved from 20-bis-16,16′-biphytane (**3**). Free energies of the conformations are given in Table 2 in the ESI.

Thermophilic archaea form cyclopentane rings by internal cyclisation in the biphytane moieties, and the number of rings seems to increase with increasing growth temperature.[26,27] The same structural elements have subsequently also been found in non-thermophilic archaea, which, based on phylogenic studies, is ascribed to a thermophilic ancestry in these species.[28]

So far, however, a covalent bond between the two 16,16′-biphytane related chains as elucidated here, have only been reported from two hyperthermophilic species.[24,25] Whether this is a further adaptation to extreme environment, and thus an indication that the C_{80} tetraacids responsible for naphthenate deposition in oil processing originates from species of thermophilic archaea, remains open.

Neither the C_{80} tetraacids characterised here, nor corresponding 16,16′-biphytanyl diacids, have been reported in Nature previously. An obvious reason for this may be the low volatility of these compounds, making the standard procedure of GC-MS analysis inadequate. A renewed search for corresponding high molecular weight biomarkers and molecular fossils in kerogen and bitumen of appropriate sedimentary rocks and oil shales[29–31] by use of a combination of liquid chromatography and MS or NMR spectroscopy might open new possibilities for improved knowledge of probable biomarker sources. Also membrane lipids from thermophilic archaea in culture should be searched for presence of these new C_{80} hydrocarbon skeletons.

The structure of the 20-bis-16,16′-diphytanyl tetraacids may indicate that the compounds are produced by oil degrading archaea that have included a simple oxidation step in the biosyntheses of the core lipid to produce the tetraacids for use as biosurfactants. A large variety of compounds is produced by oil degrading organisms in order to enable the organisms to come in contact with the hydrocarbon substrates.[32] The high interfacial activity of the 20-bis-16,16′-diphytanyl tetraacids should make these compounds ideal for such use.

Alternatively, the tetraacids may originate from oxidative degradation of GDGTs present in the sediments. Phytanic acids are particularly resistant to degradation since the usual β-oxidation pathway is blocked by the methyl group in the 3-position.[33] Phytanic acids are therefore mainly degraded by an α-oxidation pathway through the release of CO_2, or through an ω-oxidation pathway, which is also blocked in the tetraacids due to the four terminal acid groups. It would be of interest to investigate whether crude oil naphthenic acids in general may originate from degradation of archaeal GDGTs.

Conformational studies by MM2 calculations might aid in understanding the chemical properties of these tetraacids. As an example is shown some results for one of the stereoisomers of **1** where the absolute stereochemistry has been kept identical to the corresponding stereocenters in **3**–**5**. Five different conformations are illustrated in Fig. 5. Four of them (**1a**–**d**) can be organised in planar arrangements with increasing van der Waals interactions. One particular conformation, **1b** on the anionic form, is an excellent candidate as a building block for a sheet-like polymer with calcium ions. Conformation **1d**, which resembles the GDGTs found in archaea lipid membranes, might have *trans*-membrane properties as well. Finally conformer **1e**, with the lowest free energy, is internally "solvated" and stabilised through extensive van der Waals interactions and might be a form found in non-polar non-hydrogenbonding solvents (*e.g.* hydrocarbons).

The structure disclosed here for the tetraacids reflects the large surface area determined for monolayers of the tetraacids onto aqueous surfaces by the Langmuir technique.[11] In the non-interacting region, the tetraacids had a molecular area of about 160 Å^2 per molecule in the undissociated state.

Naphthenate deposition is usually fought by injection of various chemical additives, although the inhibition mechanism is not well understood. Structural knowledge about the tetraacids will make it possible to construct more effective and environmentally friendly inhibitors on a rational basis. In addition, the tetrameric acids might have potential technological and industrial applications due to the unique surfactant properties. The structure elucidated here may therefore lay the framework for making rational decisions if waste is to be turned into added value products.

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