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Comparison of the Acids determined in Biodegraded and Non-degraded Oils

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Summary

In this project the main emphasis has been placed on comparing the acids contained in the non-degraded Tyrihans oil with that of a biodegraded Heidrun oil. These two oils are believed to be sourced from the same Spekk source rock. Before one can start comparing oils from different areas and with widely differing source rocks, it seemed necessary to look for evidence as to whether the source rock does affect the suite of acids occurring in oils. The acids determined in the Heidrun and Tyrihans oils are compared with the acids reported to be in naphthenic acid fractions obtained from refineries which had processed a different set of oils.

The Heidrun and Tyrihans oils were distilled and separated into the C_{5-9}, C_{10-11}, C_{12-13}, C_{14-15}, C_{16-17}, C_{18-19}, C_{20-21}, C_{22-23}, C_{24-25} and C_{26+} distillation cuts. The C_{22-23} distillation cut of the Heidrun crude oil contained the highest concentration of acids. The acids extracted from the C_{22-23} cut of the Tyrihans oil and those of the Heidrun oil were examined using Negative Ion Fast Atom Bombardment – Mass Spectrometry (FAB-MS). The Total Acid Number (TAN) of the Heidrun C_{22-23} was measured to be 4.42 mg KOH/g whereas that of Tyrihans was 0.04 mg KOH/g. The absolute concentration of acids is thus high in Heidrun oil, whereas there is only a very small amount of acids in the Tyrihans oil. However, the FAB-MS analysis revealed that the C_{22-23} fraction of the two oils contained a similar range in cyclic carboxylic acids. This spread of C_{20} cyclic carboxylic acids in Heidrun and Tyrihans oils was compared with that of two commercial samples of Naphthenic Acid obtained from refinery operations in the USA (literature data). The spread of the acids for the Spekk sourced oils was different from those of the commercial Naphthenic acids. The commercial samples contained a relatively small amount of 5-ring carboxylic acid and no 6-ring carboxylic acid. This observation suggests that the presence of acyclic, 1-, 2-, 3-, 4-, 5-, and 6-ring carboxylic acids in Heidrun and Tyrihans oils (despite the fact that Tyrihans oil is not biodegraded), may be because both oils inherited similar compounds from their Spekk source rock. An alternative explanation for this similarity of acids could be that the same variety of bacteria are present in the Tyrihans and Heidrun oils/formation water. If the latter explanation is correct, then the only difference was that conditions for oxidation and replication were favourable for the Heidrun oil, but not for the Tyrihans oil.

Biodegradation has altered the suite of acids in Heidrun by decreasing the straight – chain or branched alkanes (Z = 2) and monocyclic saturates (z = 0). It appears that the relative contents of the compounds which are more recalcitrant to biodegradation (3-, 4-, 5- and 6-ring carboxylic acids with z = -4, -6, -8 and –10) progressively accumulate relative to the other acids as the degree of biodegradtion increases.

The $C_{20:5}$ acid was found at significant levels in the biodegraded Heidrun oil, but not in the Tyrihans oil. This acid is reported in the literature to be indicative of an oxygen – dependant metabolic pathway.

1   Introduction

The ability to predict whether the oil in a prospect is biodegraded is important seeing that costs of production and the price fetched for biodegraded oil is lower. This is especially true for small marginal fields. We feel that a better understanding of the chemical products (organic acids) of bacterial action on oils could give clues which will eventually help geochemists to better interpret available data regarding prospects. Refineries could in future become aware in advance what the prominent naphthenic acids will be from a knowledge of the oil purchased. This would have consequences on the corrosion and life time of equipment and the range of products for which acids are used to manufacture (corrosion inhibitors, fuel additives, tyres, paint dryers etc.).

In this project the main emphasis has been placed on comparing the acids contained in the non-degraded Tyrihans oil with that of a biodegraded Heidrun oil. These two oils are believed to be sourced from the same Spekk source rock (Karlsen et al. 1995). Before one can start comparing oils from different areas and with widely differing source rocks, it seemed necessary to look for evidence as to whether the source rock does affect the suite of acids occurring in oils. The acids determined in the Heidrun and Tyrihans oils are compared with the acids reported to be in the two sets of naphthenic acid samples obtained from refineries.

In order to assist Eric Michael in the data bank he is building up, Total Acid Numbers (TAN) were collected for about 60 oils. Sulphur contents were also reported for about 40 of these oils. A preliminary comparison of the relationship between TAN and API gravity and between TAN and wt % S is also made in this report.

2   Experimental arrangement

The total acid number (TAN) of various oils was determined using ASTM method D-664 on a 682 Titroprocessor (Metrohm). TAN is expressed as the mg KOH used in the titration of the acids per gram of oil (mg KOH/g). Metals were determined using a VG PlasmaQuad PQ2+ Inductively Coupled Plasma-Mass Spectrometer (ICP-MS). Instrumental conditions are given in Olsen et al. 1997. A Fischer 500AC distillation system using a 95 plates column was used to distill the biodegraded Heidrun oil and non-degraded Tyrihans oil respectively.

Acids were extracted from the C_{22-23} cuts of the Tyrihans and Heidrun oils. 188 mg of oil was dissolved in 30 ml of pentane. This was then extracted with 2 x 50 ml portions of aqueous KOH (pH 12). The KOH extract was backwashed with 50 ml pentane. The pH of the KOH extract was then altered to pH 2 with HCl and extracted with 2 x 50 ml dichloromethane (DCM). The extracts were reduced under vacuum to ca. 1.5 ml, and then blown down to ca. 0.2 ml under oxygen and organics-free nitrogen. Negative ion Fast Atom Bombardment – Mass Spectrometry (FAB-MS) analyses were carried out on these extracts. The FAB-MS method is described in Large et al. 1989.

3   Results

3.1   Properties measured in a biodegraded oil was compared with those of a non-degraded oil thought to be derived from the same source rock

3.1.1   TAN of the crude oil compared with that of the distillation fractions

The TAN and metals determined in the distillation cuts and crude oils of Heidrun and Tyrihans oils are given in the table in Appendix 1. Figure 1 compares the TAN determined in the biodegraded Heidrun oil 6507/7-2 DST 2 and its distillation cuts with that of the Tyrihans oil 6407/1-3 DST 1. The Heidrun distillation cut samples have a range of TAN from 0.04 to 4.42 mg KOH/g. The corresponding range for the Tyrihans distillation cut samples is 0.01 to 0.14 mg KOH/g.
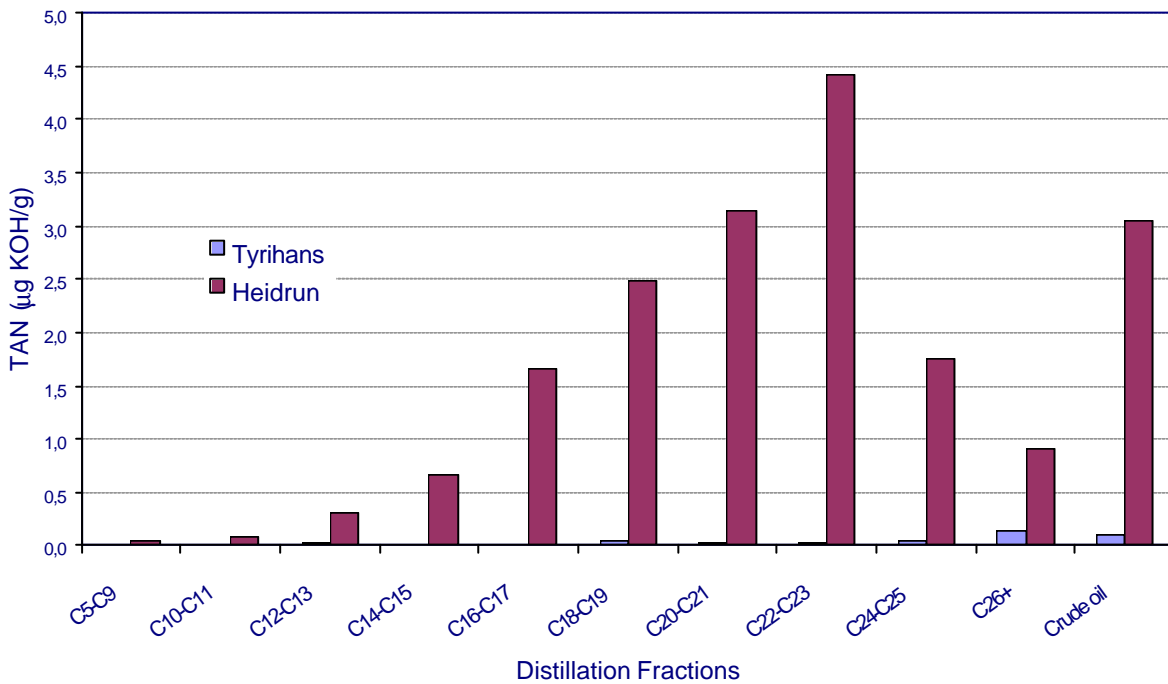

Figure 1   Comparison of the total acid number (TAN) determined in the Heidrun oil 6507/7-2 DST 2, the Tyrihans oil 6407/1-3 DST 1 and their distillation cuts.
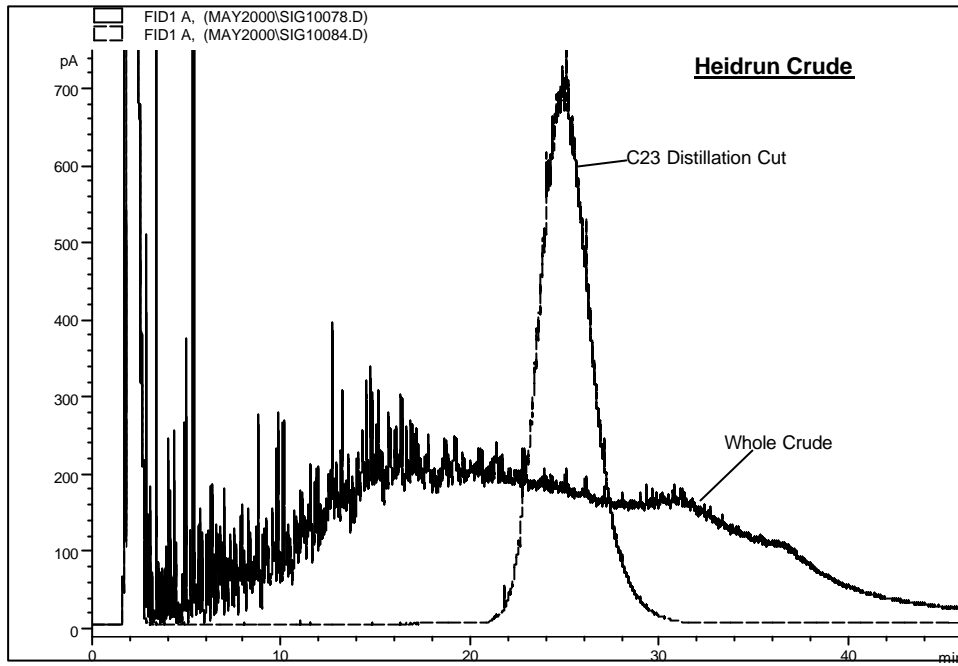
Figure 2: Superimposed GC traces of Heidrun 6507/7-2 DST 2 crude oil and its C_{22-23} distillation cut
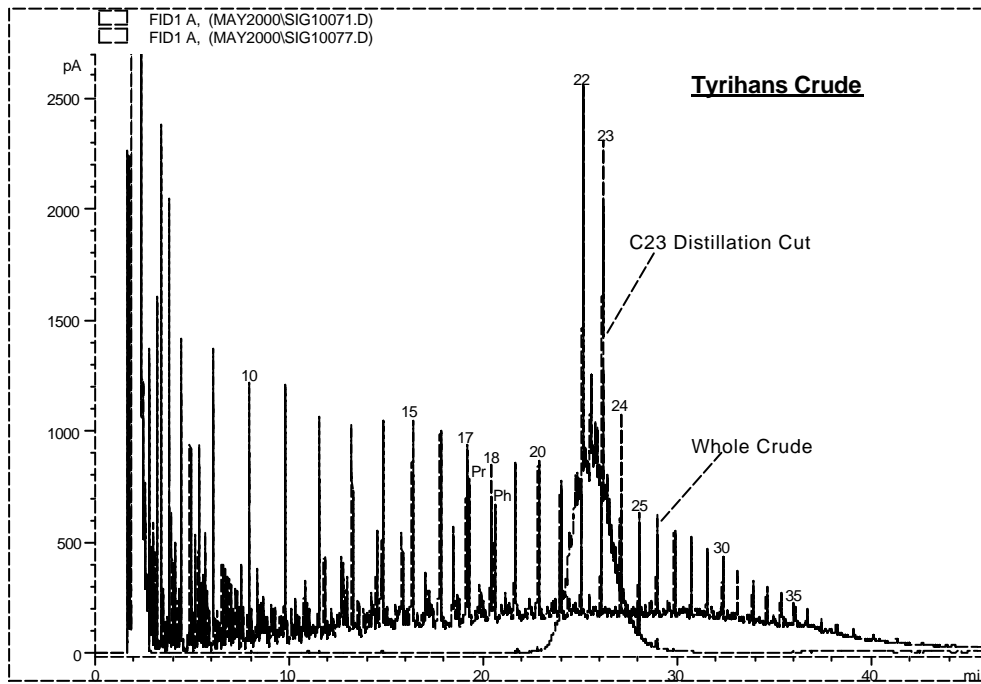

Figure 3: Superimposed GC traces of Tyrihans 6407/1-3 DST 1 crude oil and its C_{22-23} distillation cut

3.1.2   GC analysis of saturated hydrocarbon fractions and the C_{22-23} distillation cuts prior to KOH extraction of the acids

Gas chromatograms of the saturated hydrocarbon fraction from the biodegraded Heidrun oil and that of the non-degraded Tyrihans oil are shown in Figures 2 and 3 respectively. In these figures, the GC traces of the C_{22-23} distillation cuts (prior to extraction with KOH) of each of the oils is superimposed on that of the saturated hydrocarbon fraction. The strong C_{22} and C_{23} n-alkane peaks are seen in the superimposed trace of the Tyrihans oil in Figure 3. The C_{22-23} distillation cut of the strongly biodegraded Heidrun oil showed only a big hump of unresolved components in Figure 2.

3.1.3   Negative ion FAB-MS analysis of the acid fractions of the C_{22-23} distillation cuts of Heidrun and Tyrihans oils.

The spectra obtained when using negative ion fast atom bombardment mass spectrometry (FAB-MS) to analyse the acids in the distillation cuts of the oils are shown in Figures 4 and 5.

The results of the normalisation of the quantified FAB-MS spectra are given in Tables 1 and 2.

Table 1   Normalised Concentrations of Carboxylic Acids, determined by FAB-MS, showing the distribution of the C_{15} to C_{22} Carboxylic Acids in the C_{22-23} distillation cut of Tyrihans and Heidrun crude oils.

Acid Carbon No.	15	16	17	18	19	20	21	22
% Rel. Abundance **Tyrihans**	13.4	16.3	11.8	15.5	10.7	14.5	9.5	8.2
% Rel. Abundance **Heidrun**	3.6	5.5	11.8	19.2	26.2	18.7	8.9	6.0

Table 2   Normalised Concentrations of Carboxylic Acids, determined by FAB-MS, showing the relative abundance of the 0 to 6 Ring Carboxylic Acids in the C_{22-23} distillation cut of Tyrihans and Heidrun crude oils

Number of rings	0	1	2	3	4	5	6
% Rel. Abundance **Tyrihans**	20.5	16.5	19.5	12.5	10.4	8.8	11.7
% Rel. Abundance **Heidrun**	7.2	5.8	16.1	19.4	18.0	16.2	17.3

3.2 Relationship of degree of biodegradation of oils from the same source rock and TAN

The relationship between the phytane/n-C18 ratio and TAN for the Heidrun oils is shown in Figure 6. Values of pristane/n-C17 and phytane/n-C18 are shown for a selection of degraded and non-degraded oils in Table 5.
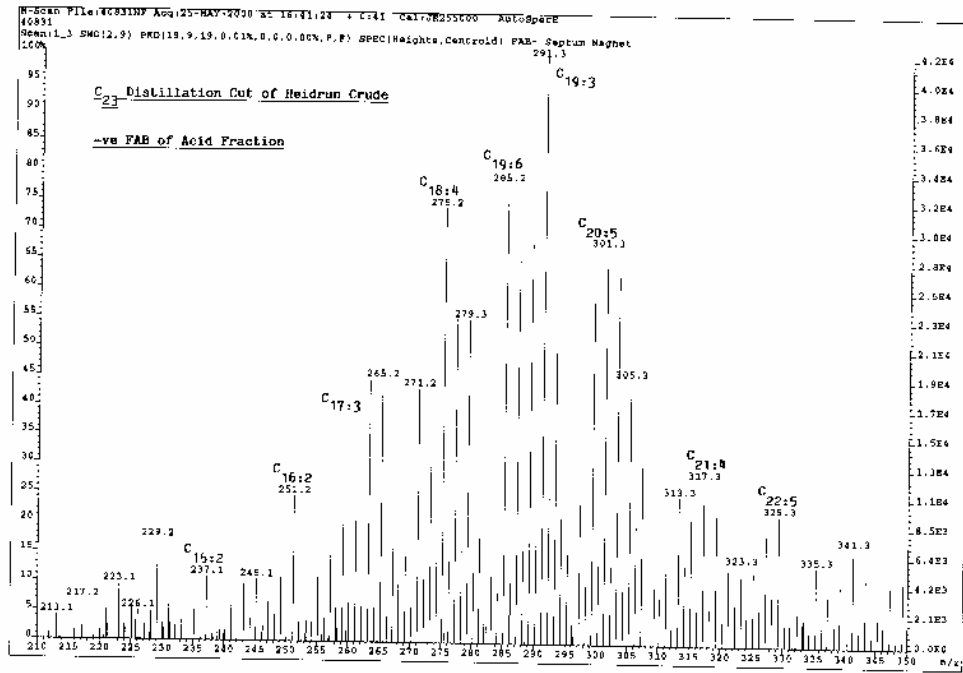
Figure 4. Negative ion FAB-MS spectrum of the acid fractions of the C_{22-23} distillation cut of Heidrun oil.
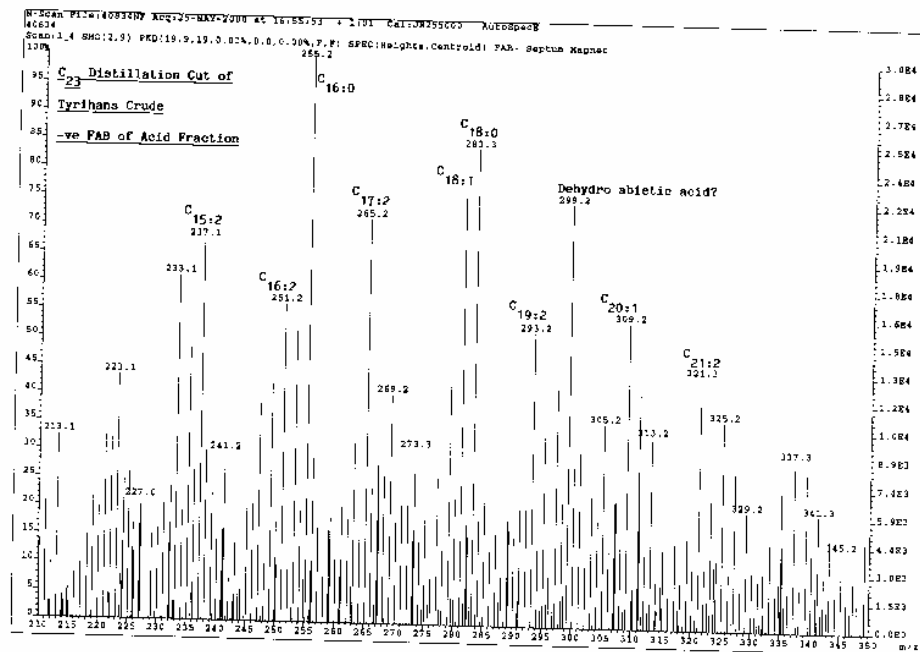
Figure 5. Negative ion FAB-MS spectrum of the acid fractions of the C_{22-23} distillation cut of Tyrihans oil.
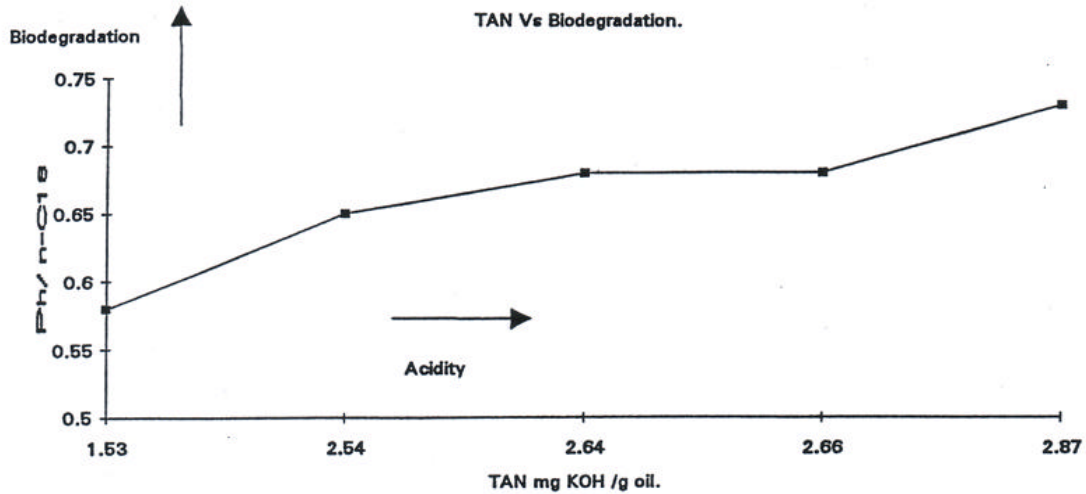
Figure 6. Five Heidrun oils from the same source rock and same degree of maturity, showing a relationship of increase in acidity (TAN) with increasing biodegradation (Ph/n-C18).

3.3. Observations regarding metals and biodegradation

As illustrated in Table 5, the concentrations of V and Ni in crude oils is somewhat higher in biodegraded oil than in non-degraded oils (Heidrun-Tyrihans, Balder–Frøy). Some biodegraded oils such as those of Balder and Heidrun 6507/7-2 DST 2 contain exceptionally high concentrations of Fe. Note also the relatively high concentrations of Co in the biodegraded Balder and Alba oils. The reproducibility of the Fe measurements are very poor compared to the reproducibility of the measurements of metals such as V, Ni and Co. This is illustrated using the example of the Heidrun 6507/7-2 DST 2 oil in Table 4.

Table 4   Showing the long term repeatability when analysing different batches of Heidrun 6507/7-2 DST 2 oil (API Gravity 22) between 1992 and 1999. Concentrations in μg/g (ppm).

	23.4.99	18.1.96	9.8.95	1994	11.4.92	Average	Std Dev.	Rel. Std
V/(V+Ni)	0.763	0.806	0.806	0.796	0.799	0.794	0.016	2 %
^{51}V	11.36	11.65	13.51	11.94	10.07	11.70	1.23	10 %
^{58}Ni	3.53	2.80	3.25	3.06	2.53	3.03	0.39	12 %
^{59}Co	0.082	0.066	0.065	0.067	0.042	0.064	0.012	20 %
^{57}Fe	120.7	19.6	15.5	124.8	66.7	69.5	52.6	76 %
^{138}Ba	9.27	0.023	0.002	0.057	3.71	2.61	4.05	155 %

Table 5. Showing relationship of total acid number (TAN), degree of biodegradation and metals
Concentations in µg/g (ppm).

Oil sample	Field	Depth (m)	TAN mg KOH pr. g	Degra-dation Pr/n-C_{17}	Degra-dation Ph/n-C_{18}	Fe	V	Ni	Co conc ng/g
25/5-1 DST0	Frøy	3254	0.19			0.9	5.4	0.7	3
25/5-2 DST0	Frøy	3200	0.36			1.3	2.3	0.3	3
25/8-1 DST0	Hermod	1753	1.82			2.9	17.4	5.0	257
25/10-5 DST1	Balder	1740	1.4			74.1	12.3	4.1	403
25/11-5 DST2	Balder	1765	1.42			111.3	14.7	4.6	224
25/11-6 DST0	Balder	1740	1.61			121.1	12.8	4.1	300
25/11-8 DST0	Balder	1735	1.52			90.0	15.3	4.7	220
Balder blend	Assay		1.46				12.3	3.8	
34/7-5 DST1A	Statfjord E	~2500	0.05	0.71	0.54	2.5	1.5	0.6	3
34/7-6 DST3B		2522	0.04			33.7	2.9	2.0	32
34/7-12 DST2	Tordis	2235	0.03	0.74	0.58	2.1	2.6	1.1	17
34/7-17A DST1		2491	0.03			3.1	2.2	1.0	6
34/10-1 DST1	Gullfaks	1935	0.22			6.2	2.9	1.5	34
34/10-1 DST2	Gullfaks	1844	0.22	0.95	0.73	4.0	2.8	1.6	20
Gullfaks blend	Assay		0.12				1.8	1.2	
6407/7-3 DST 1	Tyrihans	3700	0,11	0.59	0.51	1.07	8.10	1.21	12
6507/7-5 DST2A	Heidrun	2375	1.53	0.78	0.52	6.1	4.6	1.1	25
6507/7-4 DST1	Heidrun	2499	2.54	0.75	0.58	3.4	8.6	1.4	20
6507/7-4 DST2	Heidrun	2476	2.66	0.77	0.59	2.9	8.5	1.4	18
6507/7-4 DST3	Heidrun	2465	2.64	0.75	0.63	1.5	8.1	1.3	19
6507/7-2 DST2	Heidrun	2439	2.87	0.79	0.74	124.5	11.8	2.9	58
Heidrun blend	Assay		2.41				8.2	1.7	
	Brent		0.12	0.65	0.5	3.2	4.3	1.1	4
Alba	Alba		1.82	0.89	0.76	8.8	36.8	8.2	600

Table 5 gives the TAN and the Fe, Ni and Co for various oils selected. Where available, the Pr/n-C_{17} and Ph/n-C_{18} data is also shown in this table. If we examine the oils keeping in mind common geographic locations and similar source and reservoir rocks, we get group 1 from the Ekofisk (oils not biodegraded) area, group 2 from block 25, group 3 from block 34/Tampen Spur, group 4 from Heidrun and a series of single oils.

4   Discussion

4.1. Suggested explanation for the acid composition found in crude oil

4.1.1. Acids expected to accumulate in oils when considering microbial synthesis

Figure 8 illustrates the steps in the oxidation of an aliphatic hydrocarbon as taken from Madigan et al. 2000. The end product is acetyl-CoA and eventually acetic acid. The benzene molecule, C_6H_6, will for example result in the production of three molecules of acetic acid, CH_3COOH (Madigan et al. 2000). More detail on microbial degradation schemes of hydrocarbons for the different compound classes found in oils, is given in Higgins and Gilbert 1978. Figure 7 shows, for example, the C_{18} acids synthesised in the bacteria in order to build the lipids in their cell walls. Relatively large concentrations of acetic acid, C_{16} and C_{18} acids would be expected with lesser amounts of incompletely oxidised compounds shown in Higgins and Gilbert 1978. Microbial fatty acids are usually of a narrow range of chain lengths, C_{16} and C_{18}, with relatively small amounts of shorter (C_{12} and C_{14}) and longer (C_{20} and C_{22}) acids. Romero et al 1966, Erwin and Bloch 1964 and Bloch et al 1961 studied how aerobic and anaerobic bacteria used hydrocarbons to synthesise cellular lipids. The results of Mackenzie et al. 1981, where the most prominent acids were those of C_{16} and C_{18} best agree with the situation I would expect regarding the relative contents of acids in oils.
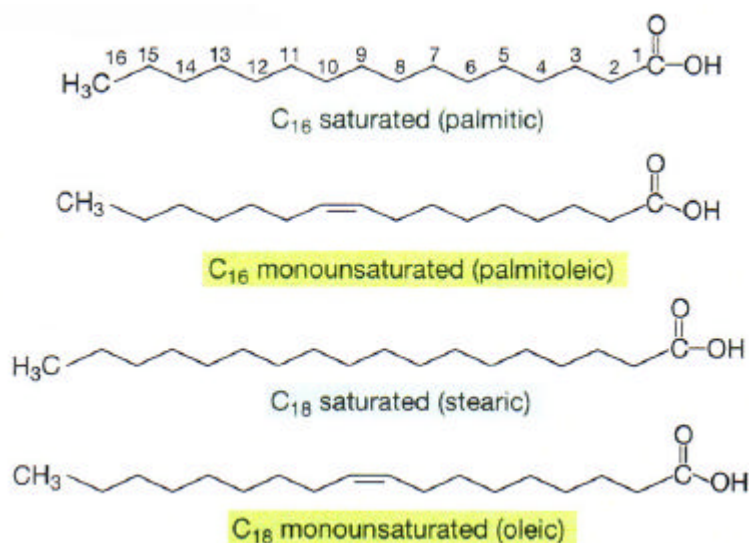

Figure 7   Example showing the C_{18} saturated and unsaturated fatty acids synthesised during degradation of hydrocarbons for inclusion in the lipids of the cell walls of bacteria. (From Madigen, M.T., J.M. Martinko and J. Parker. 2000. Brock Biology of Microorganisms, 9th Edition, Prentice-Hall, Upper Saddle River, New Jersey. 991p.)
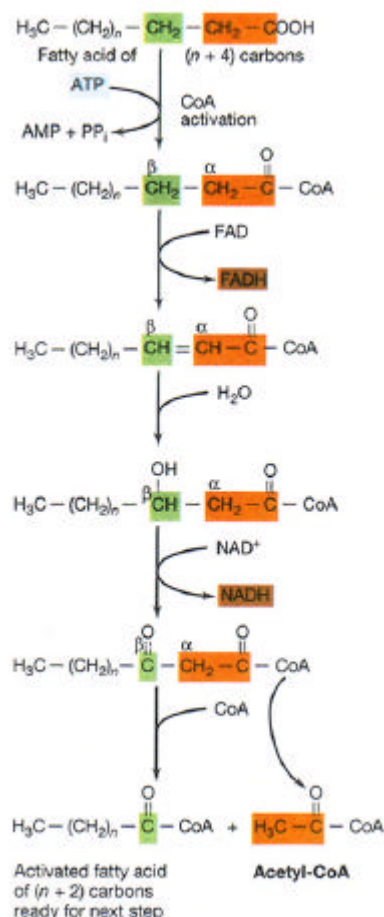
Figure 8   Mechanism of beta oxidation of a fatty acid which leads to successive formation of two-carbon fragments of acetyl-CoA. (From Madigen, M.T., J.M. Martinko and J. Parker. 2000. Brock Biology of Microorganisms, 9th Edition, Prentice-Hall, Upper Saddle River, New Jersey. 991p.)

4.1.2.   Acids actually found in the Tyrihans and Heidrun oils

Figure 1 gives an indication of the relative quantities of acids found in the C_{22-23} fractions. The amount of acids in Tyrihans oil can be seen in this figure to be very small (TAN = 0.04) compared to that in Heidrun (TAN = 4.42). An indication of the types of compounds and the relative amounts of each is obtained from an examination of Tables 1 and 2 and Figures 4 and 5.

The C_{23} distillation cut appears to be the correct boiling range to include the C_{20} acids. A C_{20} acid has 20 carbon atoms; add one carbon number for each of the two oxygens in the acid group and add an additional carbon number for the high polarity of the acid group (making a total theoretical carbon number of 23).

The results of the normalisation of the quantified FAB spectra are given in Tables 1 and 2. The acid carbon number will be a reflection of the distillation cut. The cut of the Tyrihans crude therefore appears to be slightly lower, but wider than that of the Heidrun crude. The great contrast between the two oils, however, comes comes from a comparison of the relative amounts of the ring compounds. The acids of the Tyrihans crude are dominated by acyclic and one – and two-ring acids (56.5 %),

whereas these acids in the Heidrun crude represent only 29.1 %. In contrast the five- and six-ring acids in the Heidrun crude represent 33.5 % of the acids, as opposed to 20.5 % in the Tyrihans crude. Of course, we are assuming that each degree of 'unsaturation' represents an additional ring rather than a double bond. The prominent acyclic $C_{16:0}$ and $C_{18:0}$ in Figure 5 for Tyrihans are closer to what would be expected as discussed in 4.1.1. Considering that there is such a vast store of easily digestible n-alkanes in the Tyrihans oil, it was surprising that 20.5 % of all the acids in this C_{22-23} fraction of the Tyrihans oil were five- and six-ring acids. The possible reason for this is discussed further in 4.1.4

Figure 9   Typical structures of naphthenic acids in commercial samples obtained from refineries (From Brient, J.A. 1998. Commercial utility of naphthenic acids recovered from petroleum distillates. Preprints of the American Chemical Society, Division of Petroleum Chemistry 3, 131 - 133).

4.1.3   Comparison of the Heidrun acid composition with that of naphthenic acids obtained from refinery operations separating acids from other oils

The commercial use of naphthenic acids was discussed by Brient 1998. Typical structures of the acids in commercial samples are shown in Figure 9. The relative distribution of acyclic, monocyclic, bicyclic, tricyclic, tetracyclic and pentacyclic acids in two types of commercial samples are shown in Figures 10 and 11. These commercial acids are recovered from kerosene and diesel refinery fractions and typically contain C_{9-25+} acids with up to 5 rings. According to Brient 1998, mono- and bicyclic rings and C_{13-19} acids predominate. This predominance is probably due to the large proportion of non degraded oil which is blended in with lesser amounts of biodegraded oils.

In this work, the relative composition of the C_{20} acids of Heidrun and Tyrihans were compared with those of the CG Naphthenic acid sample of Brient. For purposes of comparison, it was assumed that the C_{20} acids were also 10 % of the total acids of Tyrihans and Heidrun as is the case for the CG sample in Figure 10. The values in Table 2 (normalised to 10 % in Figure 12) are used for this plot against the C_{20} fraction of Brient's CG sample of Figure 10. The Relative distribution of the acyclic, monocyclic, bicyclic, tricyclic, tetracyclic, pentacyclic and hexacyclic naphthenic acids in the C_{20} acids is compared for these three oils in Figure 12.

Figure 10   Commercial sample of naphthenic acids with a relatively large proportion of acyclic acids at the same time as having quite a high proportion of tetracyclic and pentacyclic acids. (From Brient, J.A. 1998. Commercial utility of naphthenic acids recovered from petroleum distillates. Preprints of the American Chemical Society, Division of Petroleum Chemistry 3, 131 - 133)

Though the Tyrihans oil is not biodegraded, it nevertheless contains a substantial proportion of tetracyclic, pentacyclic and hexacyclic acids. This range of cyclic alkanoic acids are also found in the biodegraded Heidrun oil. (See Table 2 and Figure 12.) The CG naphthenic acid sample contains substantially smaller relative proportions of tetra and pentacyclic acids and no hexacyclic acids. This similarity in the suite of ring carboxylic acids for the Heidrun and Tyrihans oils suggests that the two oils inherited similar compounds from their Spekk source rock. An alternative explanation for this similarity of acids could be that the same variety of bacteria are present in the Tyrihans and Heidrun oils/formation water. If the latter explanation is correct, then the only difference was that conditions for oxidation and replication were favourable for the Heidrun oil, but not for the Tyrihans oil.
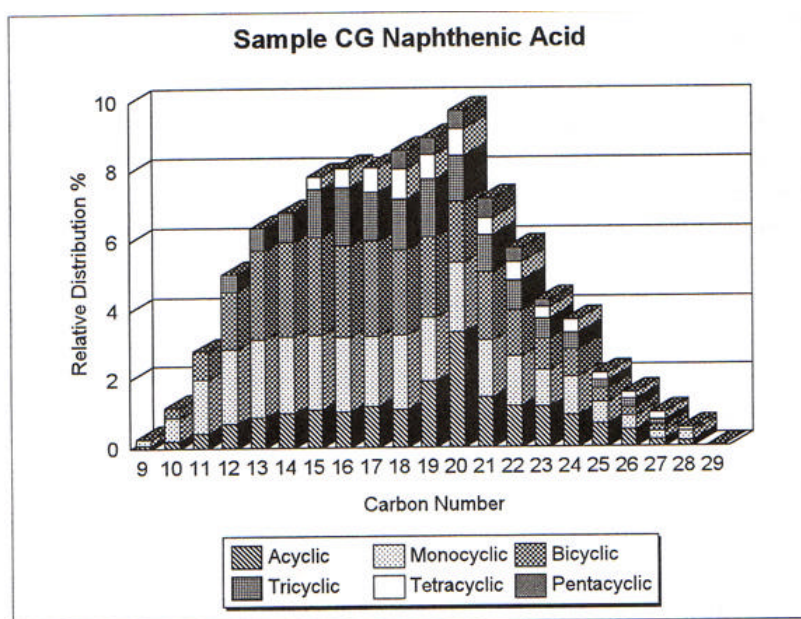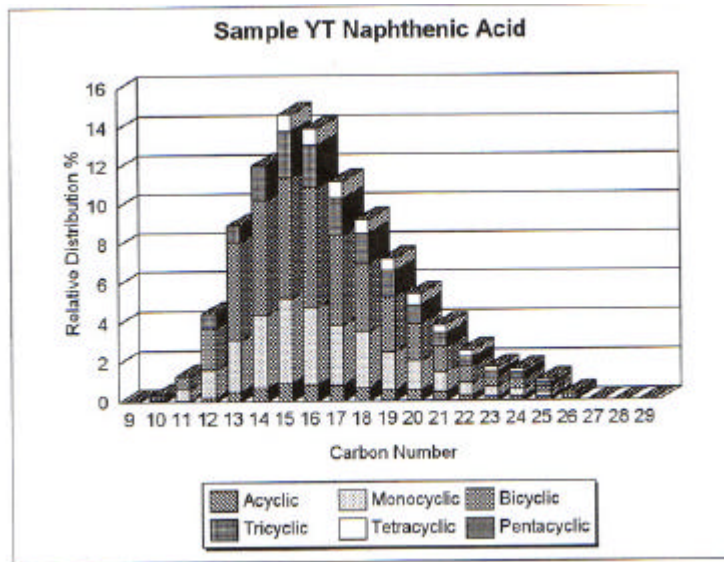
Figure 11   Commercial sample of naphthenic acids with a relatively small proportion of acyclic acids at the same time as having a very small proportion of tetracyclic and pentacyclic acids. (From Brient, J.A. 1998. Coomercial utility of naphthenic acids recovered from petroleum distillates. Preprints of the American Chemical Society, Division of Petroleum Chemistry 3, 131 - 133)
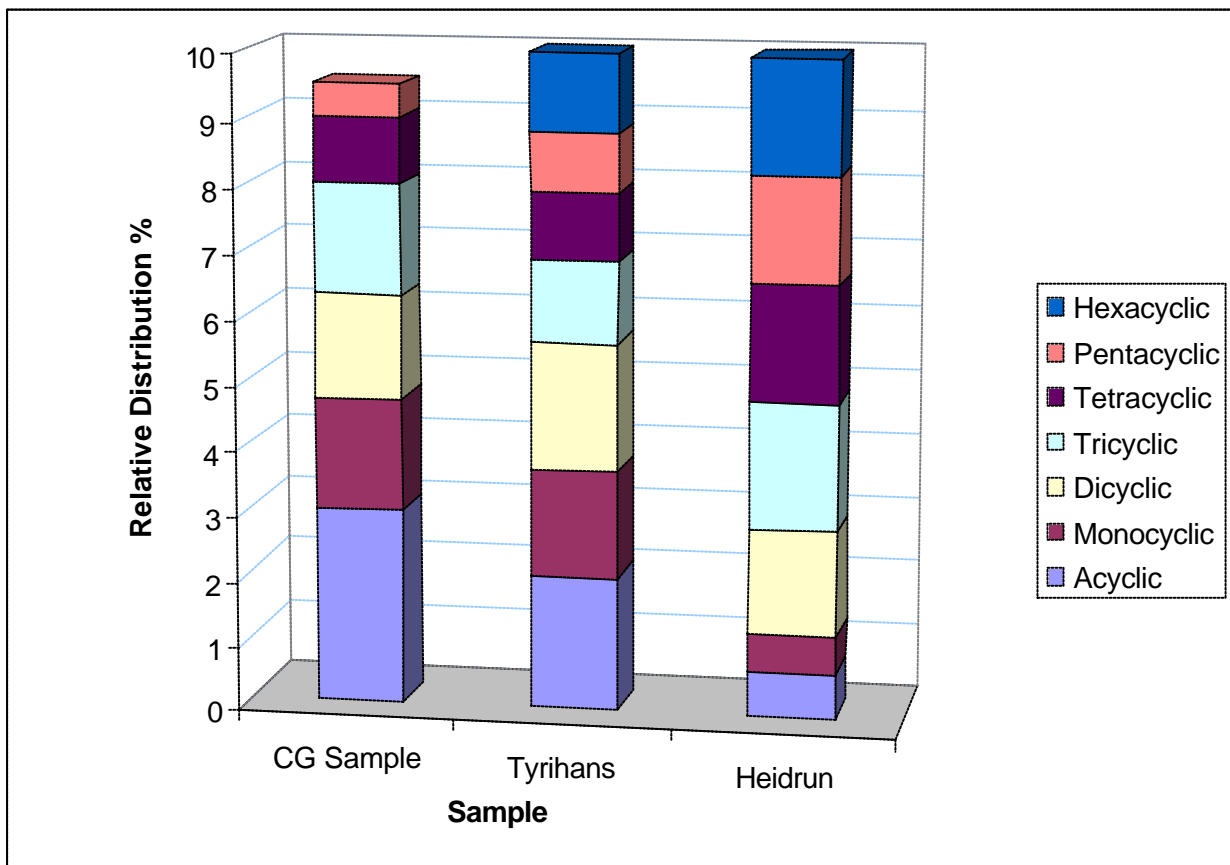
Figure 12   Comparison of the Relative Distribution % of the C_{20} acids in the CG refinery sample (Figure 10) with acids extracted from the Tyrihans and Heidrun crude oils (Table 2).

4.1.4.   Cycloalkanes are less vulnerable to microbial attack and therefore their acids accumulate relative to other acids as the extent of biodegradation increases

Reports of work done by Higgins and Gilbert 1978, Atlas 1981, Leahy and Colwell 1990, Huesemann 1995, Thorn and Aiken 1998 provide insight into the reason why the composition of acids in biodegraded oils contains such a large proportion of cycloalkanes.

Hydrocarbons differ in their susceptibility to microbial attack. From the work of the above authors, an order of decreasing susceptibility can be given:

n-alkanes > branched alkanes > low molecular weight aromatics > cyclic alkanes

The above work of microbiologists has shown that as biodegradation proceeds, the straight chain, monocyclic saturates will be decomposed and be removed. As the extent of degradation increases, the relative amount of tetracyclic and pentacyclic saturates will increase because bacteria struggle the most to biodegrade these compounds.
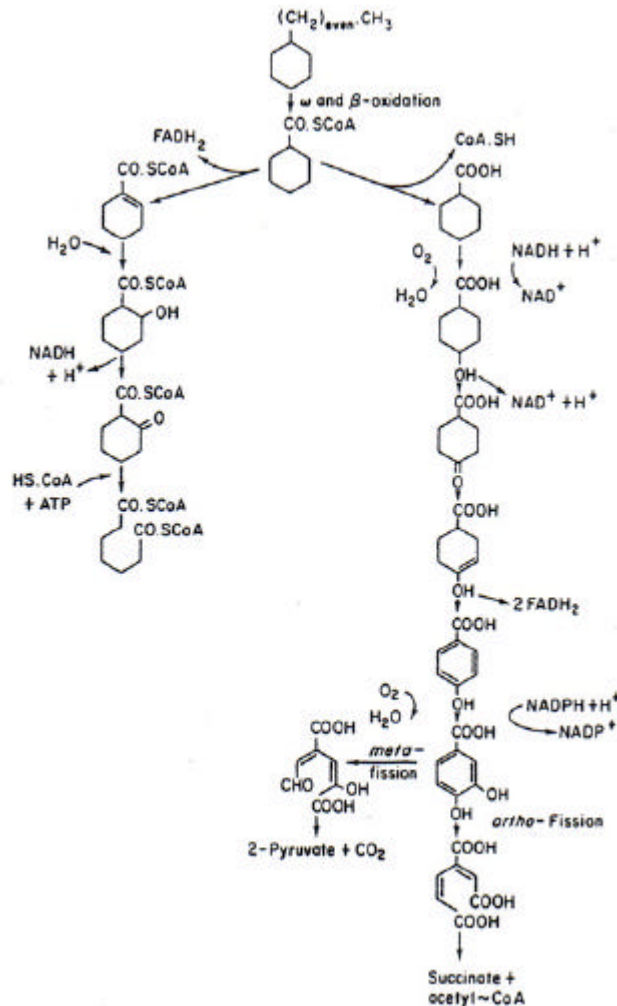
Figure 13   Pathway of microbial degradation of cyclohexanes (from Higgins, I.J. and P.D. Gilbert. 1978 The biodegradation of hydrocarbons in "The oil industry and microbial ecosystems", Ed. By Chater, K.W.A. and H.J. Somerville, Heyden and Son, London, pp. 80 – 117.)

It would thus appear that the acetic acid (see 4.1.1 and Figure 8) is either removed by water washing or by complete mineralisation to CO_2 and H_2O. The demand for easily digestable hydrocarbons must

be the reason why the C_{16}, C_{18} acids, although always present where there are bacteria, do not accumulate in the same way that the more recalcitrant cyclic alkanes do.

The biodegradation pathway of cycloalkanes is illustrated using hexane in Figure 13. Alkyl-substituted alicyclic hydrocarbons are more susceptible to microbial attack than the unsubstituted parent compounds. One route which can operate anaerobically involves non-oxygenative cleavage of the saturated ring, whilst the other involves the aromatization of the ring. This pathway illustrates the fact that some aromatics are more readily degraded tha cyclic alkanes.

Future work should examine how the relative proportions of ring carboxylic acids fit into the Biodegradation Ranking of Peters and Moldowan 1991.

4.1.5. Explanation for the increase in relative contents of 4-, 5-, and 6- ring carboxylic acids in Heidrun oil compared to acid distribution in Tyrihans oil

Figure 14 compares the relative contents (% Distillate) of the different Acid Ring Types found in the Tyrihans and Heidrun C_{22-23} cut. The degree of biodegradation has proceeded much further in the Heidrun oil (Figure 2 and 3). Similarly to what was observed by microbiologists who observed laboratory or field biodegradation of hydrocarbons (Atlas 1981, Leahy and Colwell 1990, Huesemann 1995, Thorn and Aiken 1998), the acyclic and monocyclic alkanes were more readily removed from the Heidrun oil as they were more susceptible to biodegradation. Following a bioremediation study, Huesemann 1995 stated: "Overall, the removal of hydrocarbons by biodegradation correlates strongly with the molecular ring structure (i.e., z-number) independent of the contaminant source (crude oil or refined product types), treatment conditions, or carbon number (molecular weight). More than 90 % of straight –chain or branched alkanes ($z = 2$) and monocyclic saturates ($z = 0$) were biodegraded in each of five treatments. The extent of biodegradation for bicyclic ($z = -2$) and tricyclic ($z = -4$) saturates was around 75 % and 50 %, respectively. Tetracyclics ($z = -6$) and pentacyclics ($z = -8$) appeared to be recalcitrant to bioremediation treatment. The mass concentrations of hexacyclics ($z = -10$) were not sufficiently large enough in any treatment to reliably compute the extent of biodegradation for these compounds." From Figure 14, it can be seen that the acyclic acids decreased from what probably was a relative content of 20.5 % as for Tyrihans acids to 7.2 % in the Heidrun C_{20} acids component. On the other hand, the relative proportion of tricyclic, tetracyclic, pentacyclic and hexacyclic acids in Heidrun have increased as a result of the difficulty microbes experienced in degrading these cyclic alkanes. As the more biodegradable components are depleted, the more recalcitrant ones remain and progressively accumulate as illustrated in Figure 12.

Cyclopentane, cyclohexane and cycloheptane have a strong solvent effect on lipid membranes and are toxic to the majority of hydrocarbon utilising microorganisms. On the other hand, fatty acids enhance biodegradation as follows. Microorganisms utilising hydrocarbon substrates typically position themselves at the hydrocarbon-water interface, assuring maximal access to both the oil substrate and the water soluble mineral nutrients. The interfacial tension tends to minimise the oil-water interface and thus restrict the space available for the microbial population. The spatial constraint is however counteracted by production of surface active emulsifying agents such as fatty acids by the microorganisms (Bartha et al 1987). These bioemulsifiers will both increase dispersion, providing additional contact area for the microbes on the substrate and promote solubilisation of the oil. According to Bartha, leakage of fatty acids and other metabolites from the microbial cell is an essential way to increase substrate availability and some of them excrete copious amounts of microbial dispersants.

Seifert and Teeter 1969, suggested that the 2-, 3-, 4-, and 5-ring carboxylic acids were possibly derived from steroids or polycyclic terpanes. (A summary of the compound classes of carboxylic acids determined in a California oil is given by Seifert et al. 1970). Thorn and Aiken 1998, claimed to have evidence that these nonvolatile organic acids originated from the C_{18} or greater alkylaromatic, naphthene noaromatic and sulphur-containing constituents of the crude oil, including possibly the resins and asphaltenes. Behar and Albrecht 1984, linked the presence of some of the acids, or the alkanes that produced them, to diagenesis and maturation products. These authors also mention the presence of pentacyclic triterpane acids occurring in Boscan oil before complete removal of the n-alkanes.

Note the presence of the prominent $C_{20:5}$ in the Negative FAB Mass Spectogram of the Heidrun oil in Figure 4. This species is absent in the mass spectrogram of the Tyrihans oil in Figure 5. Johns and Perry 1977, stated that biosynthetic precursors of $C_{20:5}$ acid were characteristic of an oxygen-dependant metabolic pathway.
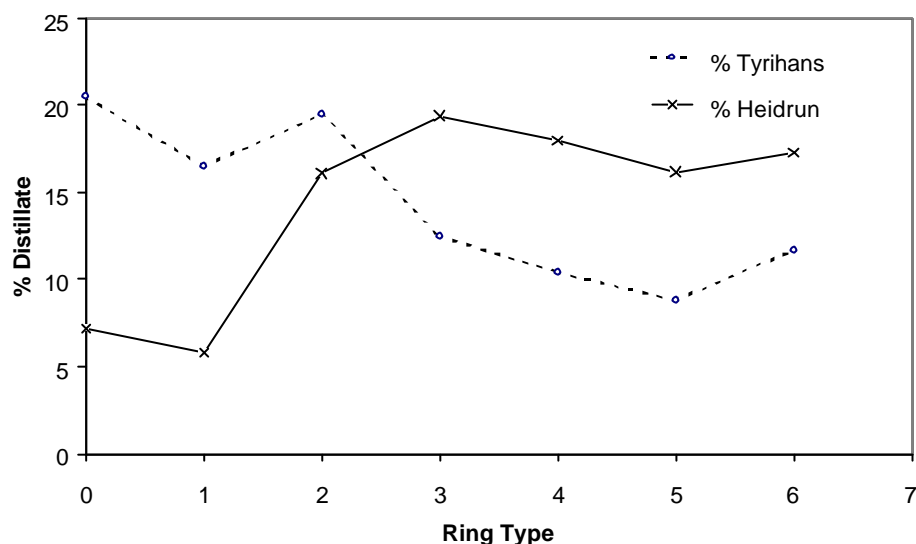
Figure 14   Showing a plot of the relative abundance (a percentage in the distillate) of the 0 to 6 Ring Carboxylic Acids in the C_{22-23} distillation cut of Tyrihans and Heidrun oils.

4.2 Identification of Abietic and Dehydroabietic acid in Tyrihans oil

The identification of dehydroabietic acid in the Tyrihans crude is entirely speculative at present. However, it would be extremely unlikely that the C_{20} acids would be the only carbon number group to be dominated by the six-ring isomers, when the other carbon numbers are dominated by the 0-2 ring compounds. Abietic acid is a common natural product and is found with resin acids in rosin. It is not inconceivable that this could have been contributed to the original palaeoenvironment when the source rock was laid down and then aromatized during diagenesis to convert it to the more stable compound dehydroabietic acid. We do not know whether this has been reported previously in crude oils. Alternatively, it could be a contaminant, as it is the basis for modern thermoplastic resins.
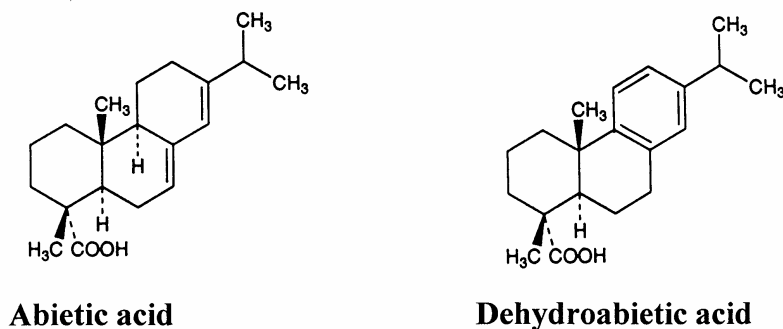
Figure 15   Structures of Abietic acid and dehydrobietic acid. The fragment at m/z = 299.2 in the negative FAB-MS spectrum of Figure 4 is interpreted as dehydroabietic acid.

4.3.   Fe and Co and biodegradation

It can be seen in Tables 5 that biodegraded oils such as those from Heidrun and Balder contain higher levels of Fe and Co than non degraded oils such as Tyrihans and Frøy. Chapelle and Lovley 1992, for example, found that Fe(III) - reducing bacteria in high-iron zones were responsible for oxidising organic substrates. Albrechtsen 1994, studied an aquifer contaminated with landfill leachate. He found that the iron reducing bacteria were able to use the organic matter in the leachate as a carbon source and the iron oxides present in the aquifer as an electron acceptor to degrade toluene. Vitamin B_{12}, which contains Co, is necessary so that methanogenic bacteria can degrade hydrocarbons (Madigen et al. 2000).

The low reproducibility of the Fe compared to the reproducibility of V and Ni in Table 4 could be an indication that Fe is associated with the water or particulates in the oil.

More work is needed in order in order to understand the relationship between the fact that iron minerals occur in both Heidrun and Balder and to what extent the presence of iron will affect the vulnerability of reservoired oil to biodegradation. Similarly, the availability and influence of Co needs further study.

4.4 Relationship between TAN and S and between TAN and API gravity

The data obtained from oils analysed during my own studies and that obtained from Statoil (www.statoil.com) and Esso (www.exxonmobil.com) is tabulated in Appendix 2. Figure 16 shows a plot of the TAN versus the % S, and that of TAN versus API for the oils in Appendix 2. There is quite a good relationship between TAN and API for this oil set. The scatter is much greater for % S than for the API. Meredith et al. 2000, showed that the trend between S and TAN could be seen only

with the UK oils separately, and not when their California and Italy samples were included. There are apparently other factors such as source type which contribute to the % S in the oil and which are superimposed on the influence of the degree of biodegradation.
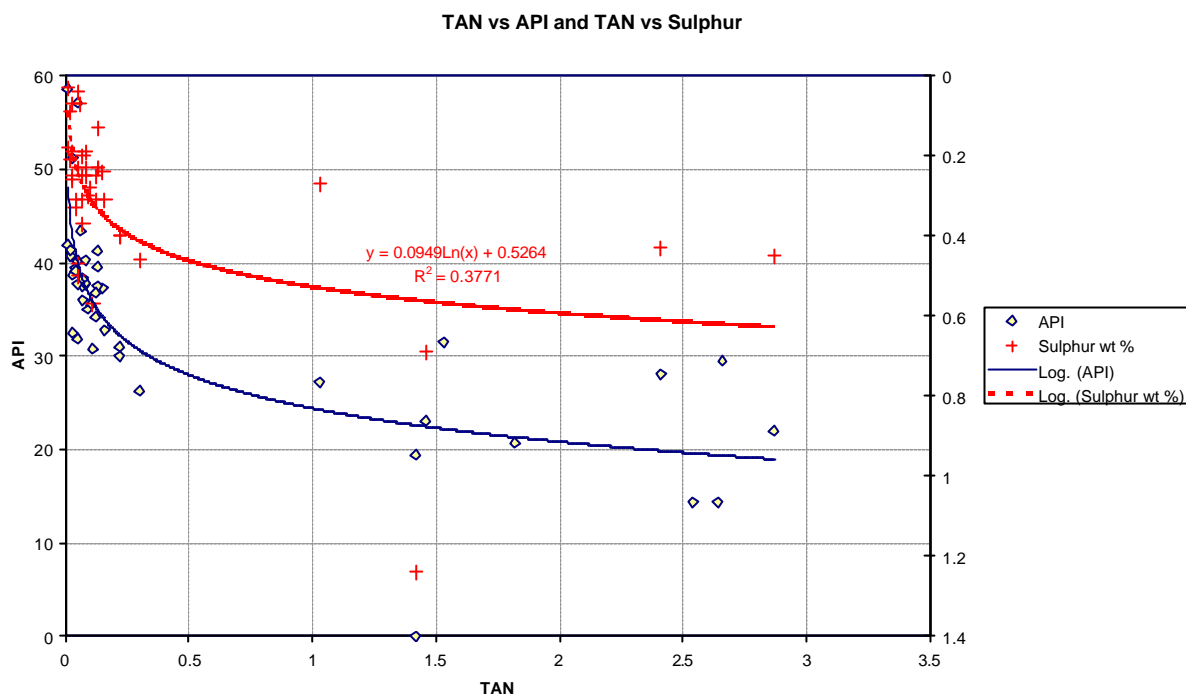
Figure 16   Sulphur content versus TAN, and API gravity versus TAN respectively, for the oils in Appendix 2.

5   Conclusion

The comparison of the suite of acids in biodegraded and non degraded oils from the same source rock and the use of distillation cuts has simplified a very complex comparison of chemical compositions. This work suggests that the contents of acyclic, 1-, 2-, 3-, 4-, 5- and 6-ring carboxylic acids in Heidrun and Tyrihans oils could be because both oils inherited similar compounds from their Spekk source rock. The suite of acids is different from those of two commercial Naphthenic Acid samples.

Biodegradation has altered the suite of acids in Heidrun by decreasing the straight – chain or branched alkanes (Z = 2) and monocyclic saturates (z = 0). It appears that the compounds which are more recalcitrant to biodegradation (3-, 4-, 5- and 6-ring carboxylic acids with z = -4, -6, -8 and –10) progressively accumulate relative to the other acids as the degree of biodegradation increases.

The $C_{20:5}$ acid was found at significant levels in the biodegraded Heidrun oil, but not in the Tyrihans oil. Further work needs to be done to find out how reliable this degradation product is to indicate that an oxygen dependant degradation path has been followed. The link between the presence of Fe at elevated levels in this and other biodegraded oils also needs further investigation. The relationship between the elevated levels of Co in Balder and Alba and the relationship of this to the amount of anaerobic biodegradation also needs further work.

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Appendix 1

TAN and metals determined in distillation cuts of Tyrihans oil 6407/71-3 DST 1 and Heidrun oil 6507/-2 DST 2																
	TAN		V		Ni		Co		Fe		As		Hg		Pb	
Dist. cut	Tyrihans	Heidrun	Tyrihans	Heidrun	Tyrihans	Heidrun	Tyrihans	Heidrun	Tyrihans	Heidrun	Tyrihans	Heidrun	Tyrihans	Heidrun	Tyrihans	Heidrun
C_5-C_9	0,01	0,04	0,8	0,4	0,1	0,1	0,1	0,2	8	10	0,1	0,5	9	8	4	5
C_{10}-C_{11}	0,01	0,08	0,3	0,4	0,1	0,1	0,1	0,2	4	9	0,3	0,3	7	7,6	3	5
C_{12}-C_{13}	0,03	0,30	0,3	0,4	0,1	0,1	<0,1	0,3	6	8	0,4	0,4	5	8	5	5
C_{14}-C_{15}	0,01	0,67	0,3	1	0,2	1	<0,1	0,5	7	24	0,5	17	6	32	5	13
C_{16}-C_{17}	0,01	1,66	0,7	0,6	0,3	0,1	<0,1	0,2	13	10	2	33	13	11	8	7
C_{18}-C_{19}	0,04	2,48	0,5	0,6	<0,1	<0,1	<0,1	<0,1	9	8	5	5	14	14	6	7
C_{20}-C_{21}	0,03	3,14	0,7	0,6	0,2	0,1	<0,1	<0,1	11	10	2	45	81	24	5	10
C_{22}-C_{23}	0,02	4,42	1,3	1,4	0,1	0,1	<0,1	<0,1	18	23	3,6	55	104	59	12	12
C_{24}-C_{25}	0,05	1,75	2,4	20	0,2	6	<0,1		24	90	6		73		16	
C_{26+}	0,14	0,90	14678	14262	1349	2902	98	127	1626	32752	52	nd	254	263	97	1164
Crude oil	0,11	3,05	8102	11794	1208	2909	12	58	1073	124500	11	113	67	70	48	1711

Appendix 2   Data on TAN, % S and metals in oils from different parts of the world

Sample	Field	Formation	Depth (m)	TAN mg KOH/g oil	API	Sulphur wt %	Mercaptan S ppm	Nitrogen ppm	Redox environ V/(V+Ni)	Concentration in ng/g(V	Ni
	NORTH SEA										
Siri-Stine blend	www.statoil.com			0,07	37,5	0,20	<3	1530,00	0,227	1000	3400
Ek2/5-7	Ekofisk		m3275	0,07					0,485	717	760
Ekofisk AR 2071			~4000	0,15					0,408	3027	4385
Eldfisk B9				0,62					0,288	1750	4323
Ekofisk blend	www.statoil.com	Auk, Clyde, Ekofisk,		0,08	37,7	0,25			0,395	1500	2300
Ekofisk blend	www.exxonmobil.com	Embla, Fulmar,Gyda, Judy, Ula, Janice		0,08	37,9	0,23	2,8	1100	0,328	1900	3900
Schiehallion	www.statoil.com			0,30	26,3	0,46			0,615	8000	5000
Varg	www.statoil.com			0,07	36,0	0,25			0,500	2000	2000
Sleipner E+W	www.exxonmobil.com			0,05	57,2	0,04	0	13		<100	<100
Sleipner mix	www.statoil.com			0,01	58,7	0,03		9		<100	<100
Glitne mix	www.statoil.com			0,05	31,9	0,5		1430	0,805	14000	3400
F25/2-13 DST 5	Rind	Brent	3360	0,18					0,802	607	150
25/5-1 DST 0	Froy		3254	0,19					0,880	3863	527
25/5-2 DST 0	Froy		3200	0,36					0,874	2323	335
Jotun blend	www.exxonmobil.com	J+Elli, Elli S+Tau W		0,05	37,7	0,23	3	760	0,698	3000	1300
25/8-1 DST 0	Hermod		1750	1,82	20,7				0,777	17440	4997
25/10-5 DST 1	Balder		1736	1,40					0,749	12300	4132
25/11-5 DST 2	Balder		1757	1,42	23,3?				0,761	14761	4642
25/11-6 DST 0	Balder		1732	1,61					0,756	12800	4121
25/11-8 DST 0	Balder		1727	1,52					0,763	15276	4732
Balder mix	www.exxonmobil.com			1,46	23,1	0,69	0	1672	0,764	12300	3800

Sample	Field	Formation	Depth (m)	TAN mg KOH/g oil	API	Sulphur wt %	Mercaptan S ppm	Nitrogen ppm	Redox environ V/(V+Ni)	Concentration of metal V	Ni
Troll B+C mix 40	www.statoil.com			1.03	27.2	0.27		1050	0.450	900	1100
Oseberg blend	www.exxonmobil.com			0.15	37.3	0.24	2.6	1000	0.481	1300	1400
Oseberg mix	www.statoil.com			0.13	37.5	0.23		900	0.500	1000	1000
Oseberg NSO1	NPD standard	B-18 Test separator	~2500	0.16	32.8	0.31			0.554	2560	2058
Statfjord blend	www.exxonmobil.com			0.07	38.4	0.31	5	700	0.690	2000	900
Statfjord blend	www.statoil.com	Stfj, N, E+Snorre		0.03	38.7	0.25		870	0.677	2100	1000
34/7-5 DST 1A	Statfjord East	Brent 1	2507	0.05					0.695	1480	650
34/7-6 DST 3B	Snorre		2520	0.04					0.591	2950	2040
34/7-12 DST 2	Tordis	Brent 3	2232	0.03		0.26			0.695	2610	1146
34/7-17A DST 1	?	Brent 2	2491	0.03					0.689	2240	1009
	NB. Brent 1,2,3,4 follow the migration sequence of Horstad and Larter										
34/10-1 DST 1	Gullfaks A	Brent	1932	0.22	30.0	0.40			0.659	2956	1530
34/10-1 DST 2	Gullfaks A	Brent 4	1844	0.22	31.0	0.40			0.628	2790	1654
Gullfaks blend	www.statoil.com	A,B, Vigdis, Visund		0.12	36.8	0.25		785	0.583	1400	1000
Gullfaks A/B	www.exxonmobil.com	A,B, Vigdis, Visund		0.12	34.2	0.31	6.3	800	0.600	1800	1200
Gullfaks C mix	www.statoil.com	C and Tordis		0.10	35.5	0.28		890	0.560	1400	1100
Gullfaks C mix	www.exxonmobil.com	C and Tordis		0.09	35.0	0.3	6.2	900	0.556	1500	1200
Njord	www.exxonmobil.com			0.06	43.4	0.07	2	275	0.200	100	400
Njord B mix	www.statoil.com			0.02	40.8	0.09				<100	310
Draugen mix	www.statoil.com			0.13	39.6	0.13		520	0.526	1000	900
Aasgard mix	www.statoil.com			0.01	41.9	0.18		260		100	<100
Aasgard	www.exxonmobil.com			0.02	41.4	0.21	1	327	0.500	100	100

Sample	Field	Formation	Depth (m)	TAN mg KOH/g oil	API	Sulphur wt %	Mercaptan S ppm	Nitrogen ppm	Redox environ V/(V+Ni)	Concentration of metal V	Ni
6407/1-3 DST 1	Tyrihans Nord		3700	0.11	30.8	0.57		1800	0.939	17789	1162
6507/7-2 DST 2	Heidrun		2428	2.87	22.0	0.45		1300	0.806	13500	3245
6507/7-4 DST 1	Heidrun		2497	2.54	14.4				0.866	14286	2210
6507/7-4 DST 2	Heidrun		2473	2.66	29.5				0.866	15550	2404
6507/7-4 DST 3	Heidrun		2421	2.64	14.4				0.853	13300	2289
6507/7-5 DST 2A	Heidrun		2365	1.53	31.5				0.816	8366	1883
Heidrun blend	www.statoil.com			2.41	28.1	0.43		820	0.828	8200	1700
Norne mix	www.statoil.com			0.03	32.5	0.19		460	0.231	300	1000
Alba				1.82					0.817	36800	8225
Alba mix	www.statoil.com			1.42	19.4	1.24	25	2240	0.820	42000	9200
Anasuria blend	www.exxonmobil.com	Teal, Teal S+Guillemot		0.04	39.5	0.33	4.1	750	0.795	3500	900
Beryl blend	www.exxonmobil.com	B+Bucland, Nevis S+N		0.04	39.1	0.31	3	852	0.784	2900	800
Brent blend	www.exxonmobil.com	Brent+Ninian systems		0.07	38.3	0.37	4.2	1000	0.833	5500	1100
Brent	Brent			0.12					0.797	4290	1094
Curlew blend	www.exxonmobil.com			0.03	51.3	0.07	1	122		<100	<100
Forties blend	www.exxonmobil.com	Scott, Nelson,Everest,Brae,Tiffa		0.13	41.3	0.23	0	606	0.700	1400	600
Kittiwake	Kittiwake	Fulmar	3400		37.4	0.6			0.909	13535	1350
Kittiwake blend	www.exxonmobil.com	Kittiwake+Mallard		0.05	40.2	0.47	3.1	310	0.898	4400	500
Triton blend	www.exxonmobil.com	T, Bitern,NW+W Guillemot		0.08	40.3	0.19	1	634	0.652	3000	1600
BH9	Martin ?			1.27					0.276	4600	12040
A1	?			1.79					0.832	45600	9200
A29	?			0.25					0.347	2600	4900