Identification of Nitrogen Bases in Heavy Gas Oil; Chromatographic Methods of Separation

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Petroleum-type basic nitrogen compounds were isolated from a heavy gas oil by extraction with aqueous HCl. The resulting complex mixtures of bases was separated by fractional elution of an alumina column; the various eluates were subsequently separated by a two-step paper chromatographic technique employing toluene: methanol:water (1:10:1) and heptane saturated with methanol as developing solvents; paper electrophoresis was also utilized as a separation technique. The following compound-types were identified or their presence was indicated in the resulting fractions by various spectral techniques: quinolines, benzoquinolines, indolo- and carbazoloquinolines, hydroxybenzoquinolines, 1,10-phenanthroline, and tetrahydrocarbazolenines.

THE PETROLEUM INDUSTRY has a continuing interest in the nature of the nitrogen-containing constituents of crude oils and distillates in various stages of processing. A better knowledge of these nitrogen compounds is imperative for the development of improved catalysts and processing techniques for their subsequent removal.

It is well known that both basic and nonbasic nitrogen compounds are present in petroleum distillates. This paper presents evidence for new types of basic nitrogen compounds recently isolated from a nonhydrogenated, straight-run, heavy gas oil. Earlier studies in this Laboratory (*10*) have shown that dilute mineral acids extract the basic nitrogen compounds from petroleum distillates. However, methods for the separation and characterization of these isolated bases are still being sought. Since no single technique has been found completely adequate for the purpose, the present paper describes a sequence of methods that have been developed to separate a mixture of basic nitrogen compounds. This separation incorporates adsorption and partition chromatography and paper electrophoresis.

The separation of nitrogen bases isolated from the heavy gas oil resulted in the enrichment of minor compound-types to a level making their detection possible. Evidence for the presence of quinolines, benzoquinolines, indolo- and carbazoloquinolines, hydroxybenzoquinolines, 1,10-phenanthroline, and alkyl-tetrahydrocarbazolenines in the gas oil are presented. Characterization of the isolated compound-types was made by mass spectrometry, infrared and ultraviolet spectroscopy, color reactions, and picrate derivatives.

EXPERIMENTAL

Instrumental. A Consolidated Electrodynamics Corporation mass spectrometer, Model 21-103, modified in this laboratory with a high temperature inlet system, was used for mass spectrometric analyses of isolated nitrogen compounds. Only low voltage mass spectra were considered in this investigation. Since fragmentation is greatly reduced by operating with a very low ionizing voltage (8 ev.), molecular weights are easily determined for aromatic bases.

A Cary recording spectrophotometer, Model 11, fitted with quartz cuvettes with a one cm. light path, was used to obtain the ultraviolet spectra of the isolated substances. Spectrograde methanol, methanolic-HCl or 1*N* HCl were used as solvents.

A Perkin Elmer Model 21 spectrophotometer was used to obtain the infrared spectra of selected fractions.

An Elphor Va continuous flow paper electrophoresis apparatus was used for separating selected fractions. A solvent consisting of a 1:1 mixture of 1*N* HCl and 1*N* KCl, diluted to a concentration of 1×10^{-4}*N* HCl, was found most suitable for subsequent separations if a constant voltage (800 v.) and low current (10–20 ma.) was maintained throughout.

Standard techniques of adsorption chromatography and of ascending paper chromatography were used for separating the nitrogen compounds.

Analysis. A straight-run heavy gas oil containing 53% aromatics, 46% saturates, and 1% olefinic compounds and having a boiling range of 600–1000° F. was used for this investigation. The aromatic, saturated hydrocarbons, and olefin contents were determined by the F.I.A. method (ASTM D1319). Total nitrogen content was determined by the Kjeldahl method and basic nitrogen by potentiometric titration with acetic acid-perchloric acid. Pyrroles plus indoles were determined colorimetrically with *p*-dimethylaminobenzaldehyde (*23, 28*) and carbazoles (*11*) with 2-bromo-2-nitroindanodione-1,3. The various nitrogen data of the oil are shown in Figure 1.

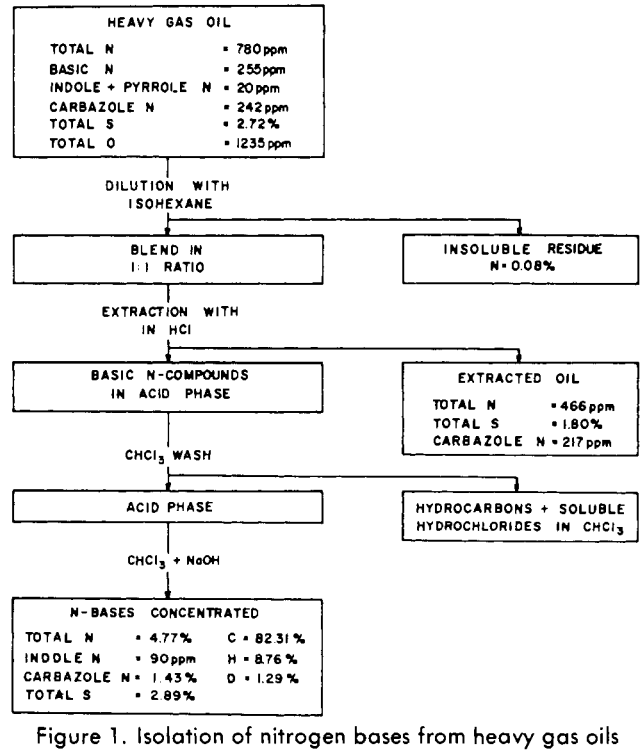


Figure 1. Isolation of nitrogen bases from heavy gas oils

Isolation of Basic Nitrogen Compounds. The preparation of a basic nitrogen concentrate is illustrated schematically in Figure 1. Three liters of the heavy gas oil were initially diluted with isohexane in a 1:1 ratio to lower the viscosity of the oil and permit better contact during the ensuing acid extraction. This dilution also precipitated a large quantity (58 grams) of insoluble compounds having a total nitrogen content of 0.08 weight per cent. Because of the low nitrogen level, this residue was reserved for a later investigation. The filtered blend of isohexane and gas oil was mixed with 1*N* HCl in a 3:1 ratio and shaken for three hours. This was repeated with successive portions of acid until the aqueous phase remained colorless. It was observed that a precipitate formed during the acid extraction and remained suspended at the aqueous-oil interface. This precipitate was of minor quantity and was easily removed by filtration. Based on previous work (*10*), it is believed that this precipitate contained polymerized indoles. After separation of the layers, the combined aqueous acidic phases were extracted three times with chloroform (3:1) to remove entrained oil; this extraction also removed a moderate quantity (1.148 grams) of soluble hydrochlorides of nitrogen bases (*3*). Finally, the acid solution of the nitrogen bases was made basic with sodium hydroxide and the liberated nitrogen compounds were extracted with four portions of chloroform (3:1). The combined organic extracts were dried over anhydrous sodium sulfate prior to their concentration. This extraction yielded 0.5455 grams of nitrogen bases containing 82.31% carbon, 8.76% hydrogen, 4.77% nitrogen, 1.29% oxygen, and 2.98% sulfur.

Since the original oil contained 255 p.p.m. basic nitrogen, a concentration factor of 187 was obtained with the procedure described. It is interesting that 40.3% of the total nitrogen was removed from the oil, rather than 32.7% attributed to the basic portion. This is partially due to the formation of soluble hydrochlorides of indole polymers (*10*). The concentrate was found to contain 90 p.p.m. pyrrole + indole nitrogen and 1.43% carbazole nitrogen which suggests that some basic nitrogen compounds contain two or more nitrogen atoms per molecule, one of which may be non-basic in character. 43.7% of the total sulfur was removed by this acid extraction which resulted from the entrainment of organic sulfides in the acid layer (*2*), or the actual presence of basic nitrogen-sulfur compounds in the gas oil.

A low voltage mass spectrometric examination of the total concentrate of bases yielded the spectrum shown in Figure 2 in which the mass to charge ratios, *m/e*, are plotted against the peak intensities. The vast number of compounds present is obvious; in fact, thirteen different homologous series are indicated. The graph has subdivided the spectrum into three groups: Group 1 contains homologous series of highest intensities while Group III contains those series of lowest intensities. To ease differentiation of series within each group, various symbols have been used; e.g., *m/e* 221 in Group I, is marked with a dot (·) as well as the remaining homologues beginning with 207 and continuing through *m/e* 305. Series *B* has the highest peak intensities, and its *m/e* ratios can be attributed to the presence of benzoquinolines or acridines. This series has a molecular *Z* number (*16*) or −17. Series *A* in Group 1, having *m/e* 185 through 311, would be expected to have a *Z* value of −11 by the same reasoning, indicative of alkylquinolines. Further evidence for these compounds, as well as for compounds having the same molecular weight but different structures, will be shown later. The qualitative aspects of the above mass spectrum are important and useful since it provided a means of certifying the true complexity of the mixture of nitrogen bases.

Table I is a compilation of all the series together with representative basic nitrogen compound-types. The molecular weight of the parent compounds illustrated, frequently does not appear in the mass spectrum, Figure 2. However, higher homologues of all these types could conceivably be present. This table can, therefore, be used as a useful guide for interpreting the above spectrum and will be referred to frequently as further evidence for particular compounds is shown.

Separation and Characterization of Nitrogen Bases. Since the mass spectrum of the basic nitrogen concentrate showed a vast number of compounds, methods of separation which would allow the characterization of compound-types and individual compounds were necessary. Adsorption chromatography on Al_2O_3 was used as an initial step for this separation which is shown schematically in Figure 3. To

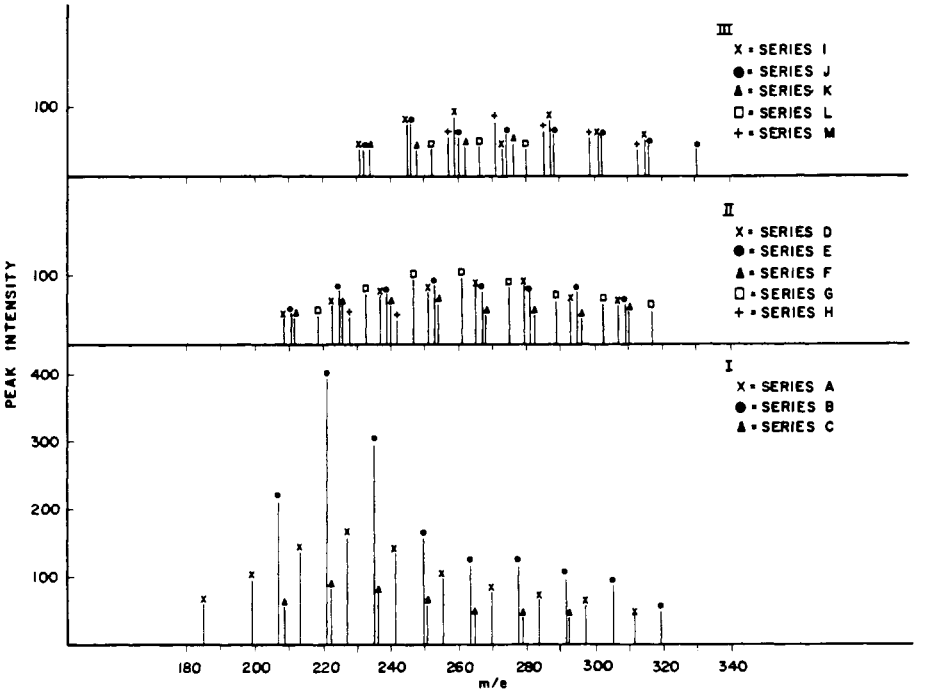
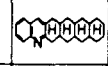


Figure 2. Low voltage mass spectrum of total basic nitrogen concentrate

Table I. Correlation of Various Structures with Molecular Weights of Basic Nitrogen Compounds

SERIES	COMPOUND TYPE	STRUCTURE	M.W.	SERIES	COMPOUND TYPE	STRUCTURE	M.W.
A	QUINOLINE		129	G	CYCLOPENTYL - BENZO [h] QUINOLINE(A)		219
A	1,2,3,4-TETRAHYDRO-CARBAZOLENINE		171	G	TETRANAPHTHENO-QUINOLINE		345
A	THIONAPHTHOPYRIDINE		185	G	α - HYDROXY-2-AZA-PYRENE		219
A	TRINAPHTHENOPYRIDINE		241	G	CYCLOPENTYL-BENZO [h] QUINOLINE(B)		247
B	ACRIDINE		179	H	QUINAZOLINE		130
B	3,4-BENZO [c] QUINOLINE		179	H	1,5-NAPHTHYRIDINE		130
B	5,6-BENZO [f] QUINOLINE		179	I	1-AZAPYRENE		203
B	7,8-BENZO [h] QUINOLINE		179	I	2-AZAFLUORANTHENE		203
B	2,3-THIONAPHTHO-[2', 3']-QUINOLINE		235	I	11 H-INDENO [1,2-b] QUINOLINE		217
B	TRINAPHTHENOQUINOLINE		291	I	6 H-INDENO [2,1-b] QUINOLINE		217
C	PHENAZONE		180	I	11 H-INDENO [2,1-f] QUINOLINE		217
C	PHENANTHROLINE		180	I	INDOLINE		119
C	p-PHENANTHROLINE		180	J	INDOLO [3':2'-2:3] - QUINOLINE		218
C	m-PHENANTHROLINE		180	J	INDOLO [2':3'-2:3] - QUINOLINE		218
D	4-AZAFLUORENE		167	J	11 H-PYRIDO [3,2-a] CARBAZOLE		218
D	DINAPHTHENOQUINOLINE		237	J	7 H-PYRIDO [3,4-c] CARBAZOLE		218
D	PENTANAPHTHENO-PYRIDINE		349	K	DIINDOLE		234
D	2-HYDROXYBENZO [c] QUINOLINE		195	K	CYCLOPENTYL-PHENAZONE		220
E	NAPHTHENOQUINOLINE		183	L	β-CARBOLINE		168
E	2-PHENYLPYRIDINE		155	L	1-AZACARBAZOLE		168
E	1,2,3,4-TETRAHYDRO-ACRIDINE		183	L	PYRROLO [2':3'-5,6] QUINOLINE		168
E	CYCLOPENTYLQUINOLINE		197	M	2-HYDROXYQUINOLINE		145
E	CYCLOHEXYLQUINOLINE		211	M	INDOLENINE		117
E	TETRANAPHTHENO-PYRIDINE		295	M	NAPHTHO [1,2-f] - QUINOLINE		229
F	CARBAZOLO [2':3'-6,7] QUINOLINE		268	M	DINAPHTHENOPYRIDINE		187

ADSORPTION CHROMATOGRAPHY Al_2O_3			PAPER CHROMATOGRAPHY	COMPOUND TYPES IDENTIFIED
ELUATE NO.	ELUTING SOLVENT	WEIGHT % OF TOTAL N CONCENTRATE	SOLVENT NO. 1 — SOLVENT NO. 2	
I	ISOHEXANE	34.7	1S → 1SS, 1SF 1F → 1FS, 1FF	SERIES: F, J CONTAIN THE INDOLE-MOIETY *
II	CARBONTETRA-CHLORIDE ISOHEXANE	15.4	2S → 2SS, 2SF 2C → 2CS, 2CF 2F → 2FS, 2FF	SERIES: A, B, C, D CONTAIN 1, 10-PHENANTHROLINE **
III	BENZENE ISOHEXANE	19.9	3S → 3SS, 3SF 3C → 3CS, 3CF 3F → 3FS, 3FF	SERIES: A, B, I, M CONTAIN HYDROXY GROUPS ***
IV	CHLOROFORM ISOHEXANE	11.4	4S → 4SS, 4SF 4C → 4CS, 4CF 4F → 4FS, 4FF	SERIES: D, G, I CONTAIN HYDROXY GROUPS ***
V	METHYLENE-CHLORIDE ISOHEXANE	1.1	NOT INVESTIGATED	-------
VI	ACETONE ISOHEXANE	16.0	6S → 6SS, 6SF 6FF → 6FS, 6FF	SULFUR-NITROGEN BASE
VII	METHANOL ISOHEXANE	1.2	7S → 7SS, 7SF 7F → 7FS, 7FF	II-ALKYLTETRAHYDRO-CARBAZOLENINE ****

* INFRARED + COLOR REACTION
** PICRATE + COLOR REACTION
*** INFRARED + COLOR REACTION
**** ULTRAVIOLET & INFRARED ONLY

S = START ZONE = R_f 0.0 - 0.33
C = CENTER ZONE = R_f 0.33 - 0.66
F = FRONT ZONE = R_f 0.66 - 1.00

EXAMPLES: 1SS MEANS: FRACTION ELUTED FROM Al_2O_3 WITH ISOHEXANE, REMAINED IN START ZONES WITH BOTH DEVELOPING SOLVENTS

7FS MEANS: CH_3OH ELUATE OF Al_2O_3 FRONT ZONE WITH SOLVENT NO.1 START ZONE WITH SOLVENT NO.2

Figure 3. Scheme for separating basic nitrogen compounds

conduct this separation, 500 mg. of the concentrate were mixed with 2 grams of Al_2O_3 and the mixture transferred to a column (120 cm. × 1 cm.) containing 50 grams Al_2O_3 (Alcoa F-20 as received). This, in turn, was covered with another two gram portion of Al_2O_3. A standard elution sequence was employed, using solvents of increasing polarity. Each solvent was followed by 200 ml. of isohexane to remove that portion of the eluting solvent which remained on the column. In each case, the isohexane extract was combined with the preceeding solvent. The distribution of the bases in each eluate is shown in column 3 as weight per cent of the total concentrate. Investigation of each eluate by low voltage mass spectrometry revealed that a concentration of minor constituents in certain fractions had been achieved. However, it seemed desirable to separate each fraction further before attempting characterization.

Portions of each eluate from the Al_2O_3 column were transferred to the starting lines of individual paper chromatograms. This separation was then conducted in two different steps. Both steps used an ascending development on sheets of Whatman No. 1 paper having the dimensions 18 cm. × 42 cm. All chromatograms in step one were developed in a closed glass jar with a mixture of toluene: methanol:water (1:10:1) (*17*). Each developed chromatogram contained two or three zones which were visible as fluorescent areas under an ultraviolet lamp. Each zone was then eluted from the chromatograms with methanol and methylene chloride and used as a new sample for further separation in the second step. Heptane saturated with methanol was employed as the second developing solvent and methanol saturated with heptane for saturation of the atmosphere in the glass jar (*6*). Two or three zones again resulted from each sample. Column 4 in Figure 3 illustrates the separation pattern with the paper chromatograms. The fraction-numbering code is explained by the use of two examples at the bottom of the figure. This separation scheme provided thirty fractions of petroleum bases for characterization by low voltage mass spectrometry, ultraviolet and infrared spectroscopy and color reactions with specific reagents. Column 5 illustrates the most important compound types identified in these various fractions.

QUINOLINES

An early investigation of petroleum bases showed the presence of alkylquinolines in California crude oil (*26*). The present work verifies the presence of these compounds in a heavy gas oil. The mass spectrum of the total concentrate of bases (Figure 2) shows high peak intensities for series *A* which may be partially attributed to the quinoline family (Table I). Figure 4 shows the ultraviolet spectra of quinolines concentrated in fraction 2*FS*; the spectra obtained in both methanol and hydrochloric acid are compared with the spectra of pure 2,6-dimethylquinoline. The absorption maximum in the 322 mμ region in CH_3OH is indicative of substitution on the benzene ring. The shift of the absorption maxima from 230 to 237 mμ can be attributed to differences in the number of alkyl groups in the isolated and the authentic compounds. La Lau (*16*) and Dineen, Cook, and Jensen (*7*) presented some mass spectral evidence for the occurrence of naphthenoquinolines in certain petroleum distillates and shale oils. Since cycloalkylation makes no appreciable contribution to the UV absorption of the quinoline ring system, the spectra in Figure 4 may be affected by trace amounts of these types. Mono-, di-, tri- and tetranaphthenoquinolines are represented by the homologous series *E*, *D*, *B*, and *G*, respectively, illustrated in Table I. It is also shown that each of these series of homologues may be interpreted by the presence of other types of nitrogen bases.

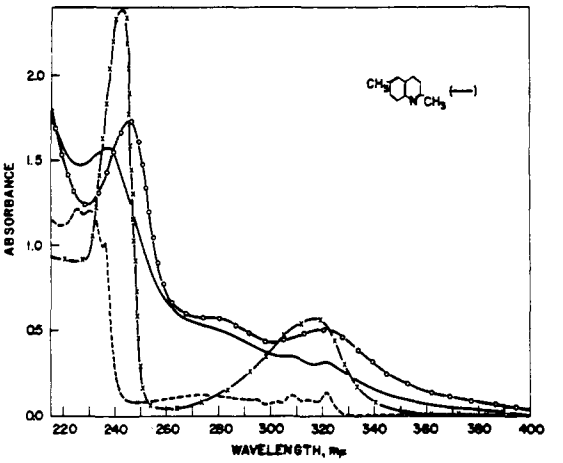


Figure 4. UV spectra of fraction 2*FS* in CH_3OH (———) and HCl-CH_3OH (—o—o—) and of pure 2,6-dimethylquinoline in CH_3OH (------) and HCl-CH_3OH (--x--x)

BENZOQUINOLINES

Schenck and Bailey presented some evidence for the presence of dimethybenzo [h] quinolines, substituted in the 2, 3, and 4 positions in California crudes (*27*). Figure 2 indicates that series *B* contains the highest peak intensities of all bases in the concentrate. Investigation of fractions 2*FF* and 3*FF* revealed the presence of this family of compounds. However, it is obvious from the UV spectra of each fraction that different isomers were present. Figure 5 shows the ultraviolet spectra of the isolated benzo [h] quinoline-type in fraction 3*FF* and its similarity to the spectra of the pure parent compound. The alkyl substit-

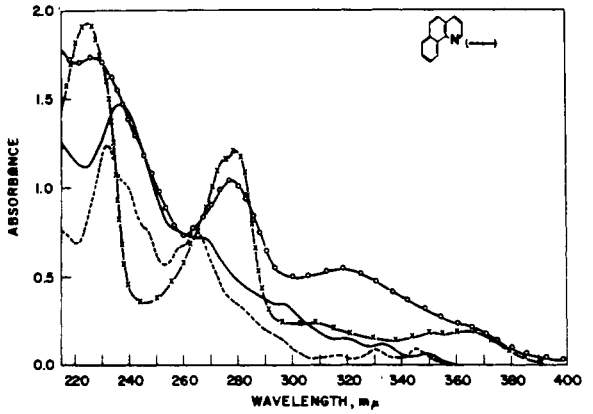


Figure 5. UV spectra of fraction 3*FF* in CH_3OH (———) and HCl-CH_3OH (—o—o) and of 7,8-benzo[h]quinoline in CH_3OH (- - - -) and HCl-CH_3OH (—x—x)

uents on the isolated compound exhibit a slight bathochromic effect on its absorption spectra in both methanol and hydrochloric acid. Figure 6 shows the ultraviolet spectra of the isolated benzo [c] quinoline-type in fraction 2*FF* and its similarity to the spectra of the pure parent compound. The two types of compounds have several marked differences in their UV spectra: the [c] form exhibits strong absorption at 245–247 mμ and moderate absorption in the 265–75 mμ region in both neutral and acidic solvents; the [h] form absorbs strongly at 232–5 and 265–70 mμ in CH_3OH and at 225 and 278 mμ in acid. Although both the [c] and [h] forms are isolated by this technique, further separations are needed before individual members of these families can be identified; low voltage mass spectra of fractions 2*FF* and 3*FF* indicate the presence of several homologues. The relative ease of isolating these two types of benzoquinolines in large quantities indicates that they are the major components in this concentrate of bases.

INDOLO- AND CARBAZOLOQUINOLINES

Since benzoquinolines are the major components of the nitrogen bases, their separation was considered essential for the characterization of remaining types. This was achieved by the initial separation on Al_2O_3. The isohexane eluate constituting 34.7 wt. per cent of the concentrate was of prime interest since it did not contain any series *B* bases but showed a high concentration of series *F* and *J* by low voltage mass spectrometry. Further separation of this eluate concentrated series *F* in fraction 1*FF* and series *J* in fraction 1*SF*. The ultraviolet spectra of both 1*SF* and 1*FF* were nearly identical having peaks at 233, 260, and 270 mμ with shoulders at 305, 330, and 347 mμ—differing only in intensity. This indicated that the compounds in these two fractions were structurally similar. The ultraviolet spectrum of fraction 1*S* exhibited strong absorption in the 267–75 mμ region which led to the consideration of an indole-type of nitrogen base. Figure 7 shows the UV spectra of 1*SF* and pure indole. The infrared spectra indicated a weak, broad adsorption band in the O–H or N–H region (2.9μ) for both 1*SF* and 1*FF*; however, 1*FF* also showed moderate absorption in the carbonyl region (5.8μ). Each of these fractions gave positive, reddish-brown, color reactions with *p*-dimethylaminobenzaldehyde spray reagent which was further indication for the pyrrole- or indole-type structure. Investigating the total concentrate of bases for these types revealed the presence of 90 p.p.m. indole plus pyrrole nitrogen; furthermore, this concentrate contained 1.43% carbazole nitrogen. With these data the presence of indoloquinolines in series *J* and carbazoloquinolines in series *F* are considered very probable. Fraction 1*FF* may contain traces of α-hydroxypyrroloquinolines, having the same molecular weight as series *F*, which would absorb in the carbonyl region of the infrared as indicated above. Although no further attempt was made to characterize these isolated types, the UV data reported above do show some correlation with that of certain derivatives of indolo- and carbazoloquinolines given earlier (*4, 5, 9*).

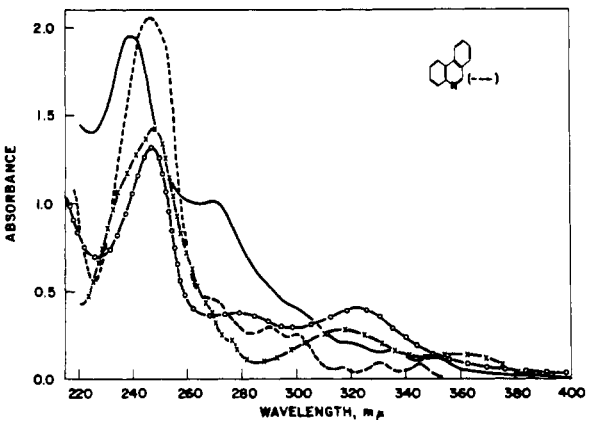


Figure 6. UV spectra of fraction 2*FF* in CH_3OH (- - - - -) and HCl-CH_3OH (—o—o) and of pure 3,4-benzo[c]quinoline in CH_3OH (- - - -) and HCl-CH_3OH (—x—x)

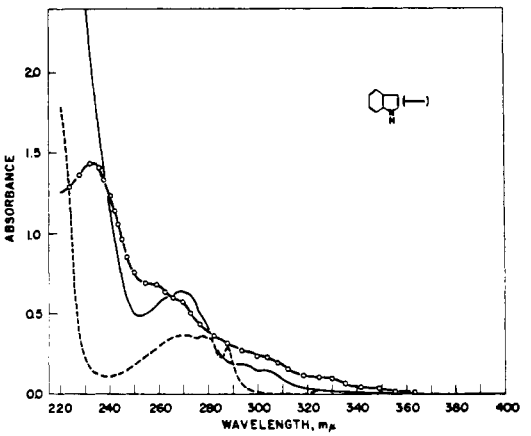


Figure 7. Comparison of the UV absorption spectra of fraction 1*SF* (-----), 1*SF* (—o—o) and indole (·---·) in CH_3OH

o-PHENANTHROLINE

The low voltage mass spectra of the carbon tetrachloride and benzene eluates from the Al_2O_3 column indicated the predominance of series *A* and *B*. However, further separation of each eluate by paper chromatography enriched other series. Fraction 2*SF* was given particular attention since its low voltage mass spectrum indicated the presence of only one compound having the *m/e* ratio 180 (series *C*). This *m/e* ratio has a molecular *Z* number of −16 when assuming only one nitrogen atom per molecule; structures for nitrogen bases having the empirical formula $C_nH_{2n-16}N$ are highly improbable. However, when assuming the presence of two nitrogen atoms per molecule, $Z = -16$ again; this yields the empirical formula $C_{12}H_8N_2$. The phenanthroline and phenazone types of bases can be deduced from this formula. The ultraviolet spectrum of the isolated compound had maxima at 232, 261, and 304 mμ. Figure 8 illustrates the similarity of this spectrum to that of 1,10-phenanthroline when using methanol as solvent. The small peak at 304 mμ may be due to the presence of phenazone [also named dibenzopyridazine (*1*)] which absorbs in this region (*31*); this latter compound also

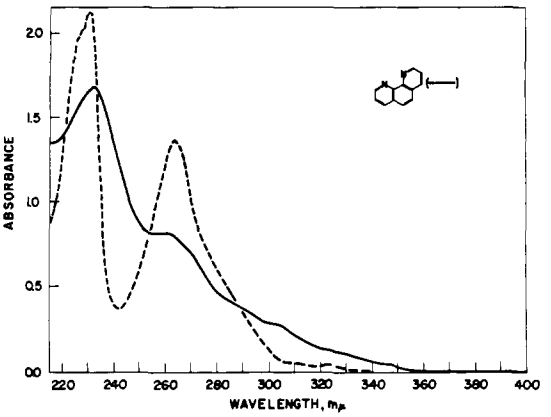


Figure 8. UV absorption spectra of fraction 2SF and of pure 1,10 phenanthroline in CH_3OH (- - - - -)

type of linkage (*8, 13, 14*). The total fraction readily yielded a red coloration with this reagent. Finally, the picrate of this compound was prepared by mixing with methanolic picric acid on a cover glass placed on a Kofler Hot Stage; this derivative sublimated at 177–8° C.; the picrate of pure 1,10-phenanthroline, when prepared in a similar manner, sublimated at 178–9° C.

HYDROXYBENZOQUINOLINES

The low voltage mass spectrum of fraction 3*CF* indicated the several-fold enrichment of series *M* and *D* (Table I); although series *B* were still present, it was possible to detect a compound-type differing from the benzoquinolines by other techniques. The ultraviolet spectrum of the isolated fraction, in CH_3OH, had peaks at 260, 343, 360, and 376 mμ, with shoulders at 242 and 326 mμ, while the spectrum in methanolic -HCl exhibited broad absorption peaks at 235, 280, and 365 mμ. The general pattern of these spectra are similar to that of benzo [h] quinoline shown in Figure 5; however, the additional absorption bands of the isolated fraction at 360 and 376 mμ indicated the presence of another chromophoric group, such as the hydroxyl (*21*) or a four-ring condensed quinoline (Naphthoquinoline Series) (*15*).

The infrared spectrum of this fraction showed a weak band at 2.9 (N–H or O–H) but a very intense band in the absorbs at 251 mμ which would cause the disagreement in the λ minima values between the two curves illustrated.

In order to ascertain the presence of the 1,10-phenanthroline isomer in fraction 2*SF*, the well known reaction of ferrous ions with these types of nitrogen bases was employed; the ferrous ions form stable, water soluble, red complexes with compounds having an α,α′-phenanthroline carbonyl region (5.8μ); this indicated that the hydroxyl groups are either in an α or γ position to the ring nitrogen since these compounds tend to exist mainly in the amide form (*20*). If the hydroxyl groups are neither α nor γ to the ring nitrogen, a sharp bond for free O-H would be expected. Also, this latter type is essentially phenolic in character and would exhibit a shift in its ultraviolet spectrum when using solvents with a pH > 7.0; the isolated fraction did not undergo any spectral change in this respect. Because of the lack of authentic reference compounds in the hydroxybenzoquinoline families, a complete identification of these types cannot be given at this time.

Since spectral evidence for hydroxylated nitrogen compounds was frequently found throughout these studies, it was decided to obtain semi-quantitative chemical evidence for these types. A modified method of Moss, Eliot, and Hall (*22*) was adopted for this purpose. Using anhydrous sodium aminoethoxide in ethanolamine as titrant, several representative nitrogen compounds containing an active hydrogen were found sufficiently acidic to yield sharp equivalence points. The equivalence points for 2, 4, 5, 7, and 8-hydroxyquinolines, indole, and carbazole are very nearly the same, 1.9–1.7 mv., and illustrate that the dissociation constants of these compounds are of the same magnitude in ethylenediamine. The fact that the hydrogen on the pyrrole-type nitrogen compounds can be titrated under these conditions indicates the necessity for knowing their content in an unknown sample of bases prior to tiration. Using a 20 mg. of the total concentrate of bases for this titration, the presence of 0.11 meq. of active hydrogen, was determined after correction for the known amounts of the indole and carbazole types. This is confirming evidence for the presence of phenolic groups in the original concentrate.

SULFUR-CONTAINING NITROGEN COMPOUNDS

While investigating the bases concentrated in the acetone eluate from the Al_2O_3 column, series *A* and *B* were found in fraction 6*SS* by low voltage mass spectrometry. However, the UV spectrum of this fraction, in CH_3OH, revealed the absence of either simple quinolines or benzoquinolines; this fraction exhibited absorption maxima at 236 and 294 mμ, with shoulders at 252, 262, and 320 mμ. It was evident that other isomeric compound-types had been isolated.

Reference to Table I will show that series *A* can also be attributed to thionaphthopyridines and series *B* to thionaphthoquinolines. A micro-determination of sulfur by the sodium fusion technique readily affirmed the presence of this heteroatom in fraction 6*SS*. Although many possible structures for biheteroaromatic bases can be deduced for series *A* and *B*, one can assume the presence of the above-mentioned types since La Lau had conclusively illustrated their occurrence in petroleum distillates (*16*). Although the above UV data are somewhat similar to that reported for thianaphtheno[3-2-c]pyridine (λ max. = 230, 252, 305, and 316 mμ) (*12*), further attempts to isolate individual homologues were not deemed desirable in the present investigation. However, it is evident that the scheme illustrated in Figure 3 could successfully separate distinctly different compounds having the same molecular weight.

11-ETHYL 1,2,3,4-TETRAHYDROCARBAZOLENINE

Figure 3 shows that the methylene chloride and methanol eluates from Al_2O_3 constitute a minor portion of the total bases. Since methanol represents the most polar eluting solvent used, one would expect only the most strongly adsorbed bases to be present in this fraction. In addition to the paper chromatographic separation of this fraction, paper electrophoresis was also utilized. It is conceivable that the hydrochlorides of strong bases would differentially migrate towards the negative electrode under optimum current and voltage conditions. The system used to conduct such separations was described earlier. Approximately three hours are required to saturate the curtain with the buffer and stabilize the enclosed system to a constant 800 v. and 10–15 ma. The nitrogen compounds are dissolved in 5 ml. 1*N* HCl and continuously fed onto the curtain at a rate of 1 ml. per hour. The syringe containing the sample is operated automatically by a variable speed motor. Eight to 12 hours are frequently required for the complete elution of the curtain. At the completion of each separation, the resulting pattern is observed with an ultraviolet lamp.

When the methanol eluate is separated in this system, a distinct pattern occurs. The compound which migrates the least towards the negative electrode is distinctly different from the remaining fractions. The isolated compound has only one intense ultraviolet absorption maximum at 255 mμ for both the free neutral base and its hydrochloride. These

spectra suggest the identity, or close similarity, of the light absorbing system. A basic nitrogen compound-type which would be expected to have this property is indolenine or tetrahydrocarbazolenine (*29*). Figure 9 illustrates the ultraviolet spectra of the isolated compound and show their similarity to the spectra of 11-ethyl 1,2,3,4-tetrahydrocarbazolenine; the pure reference compound was synthesized according to Lions (*18*). If this type of compound is present in the isolated fraction, its infrared spectrum should not show a N-H band but should have a band at 6.10-6.25 μ, which is characteristic for the indolenines-C=N group (*30*). The infrared spectrum of this fraction, using $CHCl_3$ as solvent, has these characteristics with a band at 6.20 μ.

Due to the small amount of this fraction from the paper electrophoresis, a low voltage mass spectrum could not be obtained. It is interesting that alkyltetrahydrocarbazolenines have the same molecular weights as alkylquinolines; therefore, differentiation cannot be made by low voltage mass spectrometry alone. Due to the preponderance of series *A*, Figure 2, in the total concentrate and in many of the eluates from Al_2O_3, the actual presence of alkyltetrahydrocarbazolenines in this heavy gas oil seems very probable.

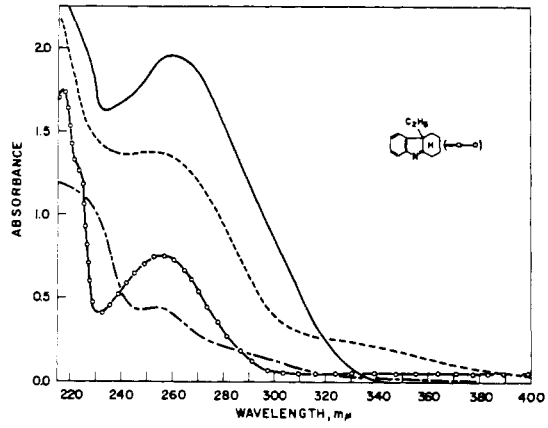


Figure 9. UV spectra of compound isolated by paperelectrophoresis in CH_3OH (——), NHCl (----) and of 11-ethyltetrahydrocarbazolenine in CH_3OH (—o—o) and NHCl (——)

DISCUSSION

Chromatographic techniques for separating petroleum-type nitrogen bases have been illustrated. Compound-types which were positively identified in various fractions include: alkylquinolines, alkylbenzo [c] quinolines, alkylbenzo [h] quinolines, 1,10-phenanthroline, and alkyl-1,2,3,4-tetrahydrocarbazolenines. Strong evidence is also given for the following compound-types: indoloquinolines, carbazoloquinolines, α-hydroxypyrroloquinolines and α-hydroxybenzoquinolines. This study also confirmed the presence of sulfur-containing bases in heavy gas oil distillates; these biheteroaromatics are assumed to contain the thianaphthenopyridine structures as revealed earlier by La Lau. The evidence given for the above compounds prevents any absolute structural assignments such as the exact position of alkyl substituents. This is apparent from the low voltage mass spectra of isolated fractions which generally indicated several homologues of any given compound. Absolute identification was beyond the scope of the present work.

Two examples of basic nitrogen compounds which contain an acidic phenolic group have been illustrated. Although they were not discussed, several other types of these "mixed systems" are present in the heavy gas oil and are believed to be of prime importance. This may be indicative of the role played by molecular oxygen dissolved in the oil; this heavy gas oil distillate was found to contain 6 p.p.m. O_2 by gas chromatography (*24*). Since several pure compounds from both the quinoline and benzoquinoline families were found to proceed through the separation sequence outlined without any appreciable hydroxylation, we believe that the phenolic bases were originally present in the oil and do not represent artifacts. Although other investigators (*25*) were unable to find evidence for hydroxyl groups in bases from kerosene petroleum fractions, the present studies confirm their presence in a heavy gas oil.

Evidence has also been presented for compound-types having a fused system such as carbolines, pyrroloquinolines, and indoloquinolines. It is apparent, therefore, that a portion of the non-basic nitrogen in straight-run petroleum distillates will be found in isolated bases. Since many of the above types have been found or postulated in alkaloids or alkaloid degradation products, it is not unreasonable to consider their presence in petroleum when assuming that "the nitrogen compounds are derived from animal or vegetable substances." The same alkaloids may have exhibited optical activity as reported earlier (*19*). The isolation of individual members of the above types may be of utmost importance in the development of any solid theory explaining the origin of petroleum nitrogen compounds.

Although the proposed method of separating nitrogen bases by adsorption and paper chromatography is somewhat lengthy, it does partially resolve the complex mixtures isolated from petroleum distillates. Obviously, further separations are desirable and necessary before pure, individual compounds are obtained. This study has illustrated that several isomeric structures generally can be attributed to any given molecular weight nitrogen compound; the proposed scheme was able to separate compound-types of different and of similar molecular weights.

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LITERATURE CITED

(1) *Beilstein Handbuch des Organischer Chemie* **23**, 222 (1936).
(2) Bezinger, N.N., Abdurakhmanov, M.A., Gal'pern, G.D., *Petrol. Chem. USSR* **1**, No. 1, 80 (1962).
(3) Cabaness, W.R., *Dissertation Abstr.* **18**, 1977 (1958).
(4) Clemo, G.R., Felton, D.G.I., *J. Chem. Soc. (London)* **1951**, p. 671.
(5) *Ibid.* **1952**, p. 1658.
(6) Dietrichs, H.H., *Angew. Chem.* **70**, 141 (1958).
(7) Dinneen, G.V., Cook, G.L., Jensen, H.B., *Anal. Chem.* **30**, 2026 (1958).
(8) Feigl, F., Hamburg, H., *Z. Anal. Chem.* **86**, 1 (1931).
(9) Govindachari, T.R., Rajappa, S., Sudarsanam, V., *Tetrahedron* **16**, 1 (1961).
(10) Hartung, G.K., Jewell, D.M., Larson, O.A., Flinn, R.A., J. CHEM. ENG. DATA **6**, No. 3, 477 (1961).
(11) Hartung, G.K., Jewell, D.M., *Anal. Chim. Acta.* **26**, 514 (1962).
(12) Herz, W., Tsai, L., *J. Am. Chem. Soc.* **75**, 5122 (1953).
(13) Hill, R., *Proc. Roy. Soc. (London) B* **107**, 205 (1930).
(14) Irving, H., Mellor, D.H., *J. Chem. Soc.* **1962**, p. 5222.
(15) Johnson, W.S., *J. Am. Chem. Soc.* **69**, 566 (1947).
(16) La Lau, G., *Anal. Chim. Acta.* **22**, 239 (1960).
(17) Lijinsky, W., *Anal. Chem.* **32**, 684 (1960).
(18) Lions, F., *J. Proc. Roy. Soc. N. S. Wales* **71**, 192 (1937).
(19) Lochte, H.L., Littmann, E.R., "The Petroleum Acids and Bases," p. 348 Chemical Publishing Company, (1955).
(20) Mason, S.F., *J. Chem. Soc.* **1957**, p. 4874.
(21) *Ibid.*, p. 5010.

(22) Moss, M.L., Elliot, J.H., Hall, R.T., *Anal. Chem.* **20**, 784 (1948).
(23) Muhs, M.A., Weiss, F.T., *Ibid.*, **30**, 259 (1952).
(24) Petorocelli, J.A., Lichtenfels, D.H., *Anal. Chem.* **31**, 2017 (1959).
(25) Savinov, B.G., Chertkov, Ya. B., Klimenkov, P.L., *J. Appl. Chem. U.S.S.R.* **35**, No. 2, 374 (1962).
(26) Schenck, L.M., Bailey, J.R., *J. Am. Chem. Soc.* **63**, 1365 (1941).
(27) *Ibid.*, p. 2331.
(28) Thompson, R.B., Symon, T., Wankat, C., *Anal. Chem.* **24**, 1465 (1952).
(29) Witkop, B., Partrick, J.B., *J. Am. Chem. Soc.* **73**, 713 (1951).
(30) *Ibid.*, p. 1558.
(31) Wittig, G., Zimmerman, H., *Ber.* **86**, 636 (1953).

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Normal and Isoprenoid Hydrocarbons Isolated from Oil-Shale Bitumen

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Normal and isopreniod paraffins were identified in a bitumen obtained from Colorado oil shale. The identified *n*-paraffins represented 1.0 precent of the total bitumen and ranged from C_{13} to C_{33} compounds. Odd carbon numbered *n*-paraffins were present in greater quantity than even carbon numbered *n*-paraffins by a ratio of 3.6 to 1 in the range of C_{25} to C_{33} compounds. Five isoprenoid compounds represented 3.4 percent of the total bitumens and were identified as 2,6,10,14-tetramethylhexadecane, 2,6,10,14-tetramethylpentadecane, 2,6,10-trimethylpentadecane, 2,6,10-trimethyltridecane, and 2,6,10-trimethyldodecane. Identification of isoprenoid compounds in an oil-shale bitumen provides information for the presence of biological remains in oil shale and may serve as a means to the better understanding of the overall structure of kerogen.

VARIOUS COMPOUNDS of biological origin have been isolated and identified from petroleum, kerogen (insoluble carbonaceous material), and bitumen (soluble carbonaceous material). One of the first evidences of the presence of these compounds in petroleum was obtained when Treibs (*14*) discovered prophyrins in crude oils. Meinschein (*9*), working with sediment extracts and crude oils, isolated polycyclic hydrocarbons that appeared to be related to steroids. Phytane (2,6,10,14-tetramethylhexadecane) was isolated from petroleum by Dean (*6*). Seven isoprenoid compounds ranging from C_{14} (2,6,10-trimethylundecane) to C_{21} (2,6,10,14-tetramethylheptadecane) were isolated from petroleum by Bendoraitis (*1, 2*). Mair (7) recently identified 2,6,10-trimethylundecane and 2,6,10-trimethyldodecane in petroleum. Moore and Dunning (*10*) identified prophyrins in oil shale.

Meinschein (*8*) found that the soluble extracts from soil deposits contained more odd than even carbon numbered *n*-paraffins. Normal paraffin distributions in a wide variety of recent sediments, ancient sediments, and crude oils were determined by Bray (*3*). He described the relative abundance of odd over even carbon numbered *n*-paraffins in terms or carbon preference index (C.P.I.). C.P.I. values were approximately 1.0 for crude oils, 1.0 to 2.4 for ancient sediments, and 2.4 to 5.5 for recent sediments. More recently, Cooper and Bray (5) postulated that fatty acids were precursors of the *n*-paraffins in petroleum and proposed a mechanism to account for the differences in the C.P.I. values of the *n*-paraffins found in crude oil and those found in recent sediments. Normal paraffins were identified previously in this laboratory in room temperature tetralin extracts of kerogen (*12*) and from reduced kerogen oxidation products (*13*).

This report describes the identification and determination of the carbon preference index of C_{13} to C_{33} *n*-paraffins and the identification of a series of five isoprenoid compounds in a benzene-soluble extract from Colorado oil shale. This appears to be the first report of the identification of isoprenoid compounds from oil-shale extracts.

EXPERIMENTAL

Reagents. Alumina (Alcoa, F20, SO-200 mesh) was extracted with pentane, dried, and activated at 700° C. for two hours.

Silica gel (Davison Chemical Company, 200 mesh) was extracted with pentane and dried at 100° C.

Molecular sieves (Linde Air Products, 5A) were extracted with pentane, dried, and activated at 240° C. under vacuum overnight.

Benzene, ACS grade, was distilled prior to use.

Isooctane, knock engine grade, was passed through a column of 5A molecular sieves before use.

All other solvents were of ACS or equivalent grade and were shown to be of high purity by GLC prior to use.

Reference Standards. Phytane (2,6,10,14-tetramethylhexadecane) was prepared by hydrogenating phytyl alcohol (2,6,10,14-tetramethyl-2-hexadecane-1-OL) using chloroplatinic acid and sodium borohydride (*4*). The resulting dihydrophytol was converted to the *p*-tolulenesulphonate, then was reduced to phytane using lithium aluminum hydride. The crude hydrocarbon was purified by GLC, and a trapped portion was used to obtain mass, infrared, and NMR spectra.

A C_{18}-isoprenoid hydrocarbon (2,6,10-trimethylpentadecane) was prepared from phytyl alcohol. Ozonization of