been made that the cooperative phenomena found in the reactions of carboxypeptidase could be associated with the monomeric state of the enzyme.[4,5] The findings reported here demonstrate that CBZ-Gly can act as an inhibitor as well as an activator in carboxypeptidase-catalyzed hydrolyses and pose an intriguing new problem. What is the relationship between the sites on the carboxypeptidase molecule which CBZ-Gly occupies when it acts as an activator or as an inhibitor? Also, what is the relationship between the sites for substrate inhibition and substrate activation? In this connection it is important to mention that the value of K_1 which we have found for the action of CBZ-Gly as an inhibitor is very close to the enzyme–CBZ-Gly dissociation constant determined for this compound as an activator,[5,15,27] and this suggests that the inhibitor and activator sites on carboxypeptidase to which CBZ-Gly binds may be closely related, if not the same.[32]

Acknowledgments. This work was supported by a grant from the National Institute of Arthritis and Metabolic Diseases, U. S. Public Health Service.

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(32) There has been a report that 9-aminoacridine can act as an activator in the chymotrypsin-catalyzed hydrolysis of methyl hippurate whereas other related compounds such as acridine itself and 2-aminoacridine act as competitive inhibitors. See: R. A. Wallace, R. L. Peterson, C. Niemann, and G. E. Hein, *Biochem. Biophys. Res. Commun.* 23, 243 (1966).

Mass Spectrometry of Porphyrins. II.[1] Characterization of Petroporphyrins

Earl W. Baker, Teh Fu Yen, John P. Dickie, Robert E. Rhodes, and Leslie F. Clark

Contribution from the Mellon Institute, Pittsburgh, Pennsylvania 15213.
Received December 10, 1966

Abstract: The nonhomogeneity of petroporphyrins extracted from petroleum sources has been previously established by low-voltage mass spectrometry. To extend these findings, the asphaltenes of Agha Jari, Baxterville, Belridge, Boscan, Burgan, Mara, Melones, Rozel Point, Santiago, and Wilmington petroleums, as well as of a gilsonite, Athabasca tar sand, and Colorado oil shale (Green River), were selected for study. The results of this survey confirm the presence of two major and one minor homologous series of petroporphyrins: the major series are a monocycloalkano series (DPEP) with visible spectra similar to that of deoxophylloerythroetioporphyrin, and a series of alkylporphyrins (etio) with visible spectra indicative of either incomplete β substitution or of bridge substitution. The ratios of the two series as well as the width of the envelope of molecular weights present were found to vary rather widely with the source. A high DPEP/etio series ratio coupled with a narrow envelope of molecular weights is suggested as indicative of a nonmarine origin of the petroporphyrins. An alkylbenzporphyrin structure is suggested for the minor series on the basis of the mass and electronic spectral evidence. The possibility is pointed out that petroporphyrins present in petroleum of marine origin with carbon skeletons of greater than 34 carbons may be derived from photosynthetic bacteria which contain chlorophylls with carbon skeletons of up to 38 carbons.

In an earlier paper, we showed that petroporphyrins obtained from certain asphaltenes were not homogeneous.[1] This interesting observation prompted a further investigation to determine if the distribution and types of porphyrins in a wider variety of asphaltenes and other bituminous materials varied significantly. We report here the results of such a survey on ten petroleum asphaltenes and three other bituminous materials selected to cover a range of geologic types and ages.

Results

Table I shows the sources, geologic ages, and yields of the petroporphyrins selected for study. The petroporphyrins were obtained by extraction of asphaltenes with methanesulfonic acid (MSA) in the manner described previously.[1,2] In cases where the starting material was solid (*i.e.*, Athabasca tar sand, gilsonite, and Green River Colorado oil shale) direct extractions were performed. The petroporphyrins were readied for spectroscopic analysis by a two-step purification: (1) transfer to an organic phase (methylene chloride) without neutralization, and (2) conversion to the free base and column chromatography over silica gel. That these purification steps were adequate is demonstrated by the quality of the mass spectra; extraneous peaks not due to porphyrins are, generally, less than 5% of the main porphyrin peaks.

In Figure 1a, b, and c are shown portions of the low-voltage (12 ev) mass spectra of the porphyrins extracted from the Baxterville, Rozel Point, and Melones petroleums. These were selected to demonstrate the differences in type and distribution of porphyrins present in asphaltenes. These spectra are typical of those obtained on other petroporphyrin samples.

In Table II are listed the normalized peak intensities of the major porphyrin peaks in the low-voltage mass spectra of the petroporphyrins from ten asphaltenes and three other bituminous materials. In obtaining the mass spectra an ionizing voltage of 12 ev was used so that essentially only parent molecular ions appear in these spectra (see Experimental Section). Two homologous series of porphyrins, the etioporphyrin (etio) series corresponding to a molecular weight of 310

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[1] For the first paper of the series see: E. W. Baker, *J. Am. Chem. Soc.*, 88, 2311 (1966). This work was presented in part at the 153rd National Meeting of the American Chemical Society, Division of Petroleum Chemistry, Symposium on Asphalts and Metals in Petroleum, Miami Beach, Fla., April 1967.

[2] J. G. Erdman, U. S. Patent No. 3,190,829 (June 22, 1965).

Table I. Sources and Yields of Petroporphyrins

Asphaltene or bitumen	Country, state	Geologic age[j]	Yield, ppm[f]
Agha Jari	Iran	Miocene, lower	120
Athabasca tar sands[a]	Canada, Alberta	Cretaceous	145[g]
Baxterville	U.S.A., Miss.	Cretaceous, upper	28
Belridge	U.S.A., Calif.	Pliocene	3100
Boscan	Venezuela, Zulia	Cretaceous	1800
Burgan	Kuwait	Cretaceous	220
Colorado oil shale[b]	U.S.A., Colorado	Eocene	55[h]
Gilsonite[c]	U.S.A., Utah	Eocene	40[i]
Mara	Venezuela, Zulia	Cretaceous	300
Melones	Venezuela, Anzoategui	Cretaceous	300
Rozel Point[d]	U.S.A., Utah	. . .	17
Santiago[e]	U.S.A., Calif.	Pleistocene	38
Wilmington	U.S.A., Calif.	Miocene	570

[a] For geology and geochemistry of this formation, see B. Nagy and G. C. Gagnon, *Geochim. Cosmochim. Acta*, **23**, 155 (1961). For details on types of porphyrin present, see M. F. Millson, D. S. Montgomery, and S. R. Brown, *ibid.*, **30**, 207 (1966). [b] From Mahogany Ledge, Green River Formation, Rifle, Colo. For geology of this area, see J. M. Hunt, F. Stewart, and P. A. Dickey, *Bull. Am. Assoc. Petrol. Geologists*, **38**, 1671 (1954). For a study of mass spectra of porphyrins extracted from oil shale, see J. R. Morandi and H. B. Jensen, *J. Chem. Eng. Data*, **11**, 81 (1966). [c] From Cowboy Vein, Uinta Basin, Utah. For geology of area, see Hunt, *et al.*, footnote *b*, this table. For a previous study of porphyrins in gilsonite, see J. M. Sugihara and L. R. McGee, *J. Org. Chem.*, **22**, 795 (1957). [d] From Box Elder County, Utah, shallow production. [e] Shallow production. [f] Yields are based on the asphaltenes except where noted below and were assayed by measurement of the OD of the 546-mμ dication peak after volumetric dilution of the acid extract. Background corrections were made by interpolation between 490 and 700 mμ; in calculating the yields, an ϵ value of 17×10^3 was used. This value was determined for etioporphyrin I in 33% aqueous MSA. The value is somewhat higher than the 15×10^3 given for *meso*-IX in 0.1 *N* HCl (J. E. Falk, "Porphyrins and Metalloporphyrins," Elsevier Publishing Co., New York, N. Y., 1964, p 236). [g] Based on an organic content of 16%. On the total tar sands, the yield was 24 ppm. [h] Corrected to a yield based on organic content of 36%. The extraction was done directly on powdered shale, and the yield on the total sample was 20 ppm. [i] Based on an organic content of 26%. On the total gilsonite, the yield was 10 ppm. [j] Age of source bed based on best available information.

$+ 14n$ and the deoxophylloerythroetioporphyrin series (DPEP) corresponding to a molecular weight of 308 $+ 14n$, where n is an integer, 2 or greater, comprise the major portion of the petroporphyrins. In Figure 1a the peaks corresponding to the etio series from $n = 5$ to $n = 15$, and in Figure 1b the peaks corresponding to the DPEP series from $n = 9$ to $n = 14$ are shown in terms of the corresponding mass numbers.

Qualitatively it can be seen from the raw spectra in Figure 1a, b, and c, and the data in Table II that in some petroporphyrins one homologous series predominates while in others nearly equal amounts are present. Also represented are fairly wide differences in the molecular weight ranges.

To place these qualitative ideas of series ratios and average molecular weights and ranges on a firmer basis, straightforward statistical analysis was applied. The results are presented in Table III. In columns 1–3 are shown the weighted average mass (arithmetic mean) for each of the two series and for the total petroporphyrin sample. In the samples studied the average mass of the petroporphyrins varies over a range of 448–476 corresponding to a difference of two methylene groups.

The band widths of the porphyrin envelope for both the DPEP and etio series are indicated in columns 4 and 5. The band width is given as the standard deviation (σ) in mass units and was computed in the standard way. One σ above and below the mean mass encompasses *ca.* 68% of the porphyrins while two σ take in *ca.* 95%. For the etio series of Baxterville petroporphyrins, in order to include 95% of the porphyrins, it would be necessary to take in those with molecular weights as low as 384 to as high as 504 (444 ± 60). It will be noted that the porphyrins in Colorado oil shale, gilsonite, and Rozel Point crude oil have unusually narrow band widths in the DPEP series corresponding to 13 and 14 mass units or about one methylene group.

If the envelope of mass peaks corresponding to each of the series is sketched (for such a sketch see dashed lines in Figure 1c) it is apparent that the distribution of intensities is not symmetric about the mean mass. One measure of such asymmetry is the skewness (Sk) which is calculated by the formula $Sk = (\bar{X} - Mo)/\sigma$, where $\bar{X}$ is the mean mass, Mo the mode, and σ the band width.[3] The skewness may be either positive or negative; it will be positive when the mean exceeds the mode, as in the etio series (Figure 1c), and negative when the mean is smaller than the mode, as in the DPEP series (Figure 1c). The skewness of the envelopes is shown in columns 6 and 7, Table III.

Treibs[4] observed that the relative amounts of DPEP and etio varied from petroleum to petroleum and reported qualitative results on a number of samples. His observations were based on the visible spectrum and were given simply in terms of the predominance of one series or the other or equal amounts. We have extended and refined this idea by defining a quantitative parameter called the series ratio (ΣDPEP/Σetio) based on mass spectral measurements.

The series ratio (ΣDPEP/Σetio) is shown in column 8, Table III, and for the petroporphyrins investigated the series ratio varied from 0.27 for Baxterville (Figure 1a) to 5.8 for Rozel Point (Figure 1b).

The visible spectra of the petroporphyrins were recorded and in a qualitative way were in accord with the conclusions reached from the mass spectra. From the mass spectral series ratio of 0.27 (Table III, sample 3, column 8), it would be expected that Baxterville porphyrin would exhibit essentially an etio-type visible spectrum, and this expectation is confirmed (Figure 2a). Conversely, the petroporphyrins of Rozel Point with series ratio of 5.8 (Table III, sample 11, column 8) should have a DPEP-type spectrum, and this is what is observed (Figure 2b). For other petroporphyrins with *ca.* equal content of DPEP and etio, mixed type spectra would be expected, and qualitative agreement is obtained. In column 9, Table III, the visible spectral type is given a qualitative designation of etio if the peak intensities correspond generally to those for this series (IV > III > II > I); the Baxterville spectrum (Figure 2a) illustrates this type. Similarly, a spectrum is referred to as DPEP if the intensities fall generally in the order IV > I > II > III; this is illustrated in the case of Rozel Point (Figure 2b). Other spectra which are intermediate between these two types are referred to

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(3) A. E. Waugh, "Elements of Statistical Methods," McGraw-Hill Book Co., Inc., New York, N. Y., 1939, p 121.

(4) A. Treibs, *Ann. Chem.*, **510**, 42 (1934).

Table II. Low-Voltage Mass Spectra of Petroporphyrins[a]

Asphaltene or bitumen	Peak mass																									
	392	394	406	408	420	422	434	436	448	450	462	464	476	478	490	492	504	506	518	520	532	534	546	548	560	562
Agha Jari	3		12		27		62		*91*		88		70		25		10		4							
		12		44		80		*100*		78		50		24		11		11		4						
Athabasca tar sand			7		16		36		72		*100*		96		55		29		19							
				17		36		62		*67*		55		38		24		19		12						
Baxterville	2		5		11		18		*22*		19		14		10		7		5		4		3			
		16		49		86		*100*		79		50		26		17		11		8		5		3		
Belridge			5		16		40		64		*100*		64		15		8									
				11		26		32		34		*40*		12		5		3								
Boscan					6		16		49		74		*100*		82		54		30		16		10		6	
						20		50		76		*79*		60		37		22		12		9		6		4
Burgan			6		18		39		60		*70*		60		42		30		21		15		12		9	
		11		18		48		83		*100*		92		70		45		30		21		18		12		9
Colorado oil shale			1		2		7		23		*100*		73		9		5									
				2		4		5		8		*12*		9		2		1								
Gilsonite							2		6		26		*100*		73		12									
								10		31		*57*		45		16		4								
Mara			7		18		39		71		93		*100*		82		46		25		14		5			
		4		21		50		78		*100*		80		50		28		16		11		7		2		
Melones			3		10		31		63		88		*100*		54		29		12							
				11		38		79		*100*		92		60		33		15		7						
Rozel Point							4		10		28		*100*		33		4									
								3		3		4		*9*		6		5								
Santiago			3		9		30		59		*100*		73		6											
				5		7		*25*		*25*		16		7												
Wilmington	4		16		36		56		*92*		72		68		28		20									
		20		52		76		*100*		96		64		36		20		12								

[a] Spectra obtained using a solid sample inlet on an AEI MS-9 using an ionizing voltage of 12 ev; essentially only parent peaks are observed. Peak intensities are normalized to 100 for the most intense peak. Intensities given in the first line for each sample are for the DPEP series; those in the second line, for the etio series.

Table III. Molecular Weight Distribution of Petroporphyrins
(average mass, mass distribution, band widths, skewness, homologous series ratios, and visible spectral type)

Sample no.	Asphaltene or bitumen	Weighted av mass[a]			Band width,[e] σ		Skewness[f]			
		1 DPEP[b] series	2 Etio[c] series	3 Total petroporphyrin	4 DPEP[b] series	5 Etio[c] series	6 DPEP[b] series	7 Etio[c] series	8 $\frac{\Sigma\text{DPEP}^d}{\Sigma\text{etio}}$	9 Visible[h] spectral type
1	Agha Jari	455	442	448	23	25	0.30	0.24	0.96	Mixed
2	Athabasca tar sands	467	457	463	24	25	0.21	0.28	1.3	Mixed
3	Baxterville	461	444	447	34	30	0.38	0.27	0.27	Etio
4	Belridge	458	448	454	20	22	−0.20	−0.73	1.9	Mixed
5	Boscan	482	469	476	27	29	0.22	0.17	1.2	Mixed
6	Burgan	473	466	469	34	34	0.32	0.47	0.70	Etio
7	Colorado oil shale	466	457	464	14	21	0.29	−0.33	5.0	DPEP
8	Gilsonite	479	469	476	13	16	0.23	0.31	1.5	Mixed
9	Mara	472	456	464	28	29	−0.14	0.21	1.1	Etio
10	Melones	468	457	462	23	32	−0.35	0.22	0.89	Mixed
11	Rozel Point	475	477	475	13	21	−0.77	*g*	5.8	DPEP
12	Santiago	458	446	455	16	18	−0.25	0.17	2.9	Mixed
13	Wilmington	454	443	448	25	26	0.24	0.27	0.82	Mixed

[a] Obtained by $\Sigma IM/\Sigma I$ from Table II, where M is the mass and I is the intensity. [b] Corresponds to the series having mol wt 308 + 14*n* where *n* is an integer, 2 or greater. Porphyrins of this series must contain an isocyclic ring. See E. W. Baker, *J. Am. Chem. Soc.*, **88**, 2311 (1966). [c] Corresponds to the series having mol wt 310 + 14*n*. Porphyrins of this series are referred to as belonging to the etio series but obviously also include phyllo- and deuterioporphyrins. [d] Obtained from Table II by Σ of intensities of each series. [e] The band width is given as the standard deviation (σ) in mass units, computed according to the formula $\sigma = \sqrt{\Sigma(IM^2)/\Sigma I - (\Sigma IM/\Sigma I)^2}$ where I is the intensity and M the mass, both taken from Table II. [f] The skewness is a measure of the asymmetry of the envelope and is calculated from the formula $Sk = (\bar{X} - Mo)/\sigma$ where $\bar{X}$ is the arithmetic mean from columns 1 and 2 of this table and Mo is the mode from Table II. The skewness value obtained in this way is valid only for distributions which are moderately asymmetrical, *i.e.*, the difference between the mean and the mode is a small part of one standard deviation. For the basis of the formula, see A. E. Waugh, "Elements of Statistical Methods," McGraw-Hill Book Co., Inc., New York, N. Y., 1938, p 121. [g] Amount of material of this series insufficient to make meaningful calculations. [h] Qualitative designation of visible spectral types. For example, see Figure 2a for etio type where the order of intensities of the visible bands is that of etioporphyrin, IV > III > II > I; Figure 2b for DPEP type where the order of intensities is IV > I > II > III; and Figure 2c is typical of a "mixed" type.

as mixed; generally the intensity of peak IV is greater than that of peak II while peaks III and I are approximately equal. In Baxterville petroporphyrin, even when allowance is made for the presence of material of the DPEP series, the ratio of peak III to IV is still lower than would be expected for a fully β-alkylated porphyrin (see Table V for peak ratios of etioporphyrin I). The visible spectrum is compatible with the presence of either incomplete β substitution (*i.e.*, pyrro- or deuterioporphyrins) or bridge substitution (*i.e.*, phylloporphy-

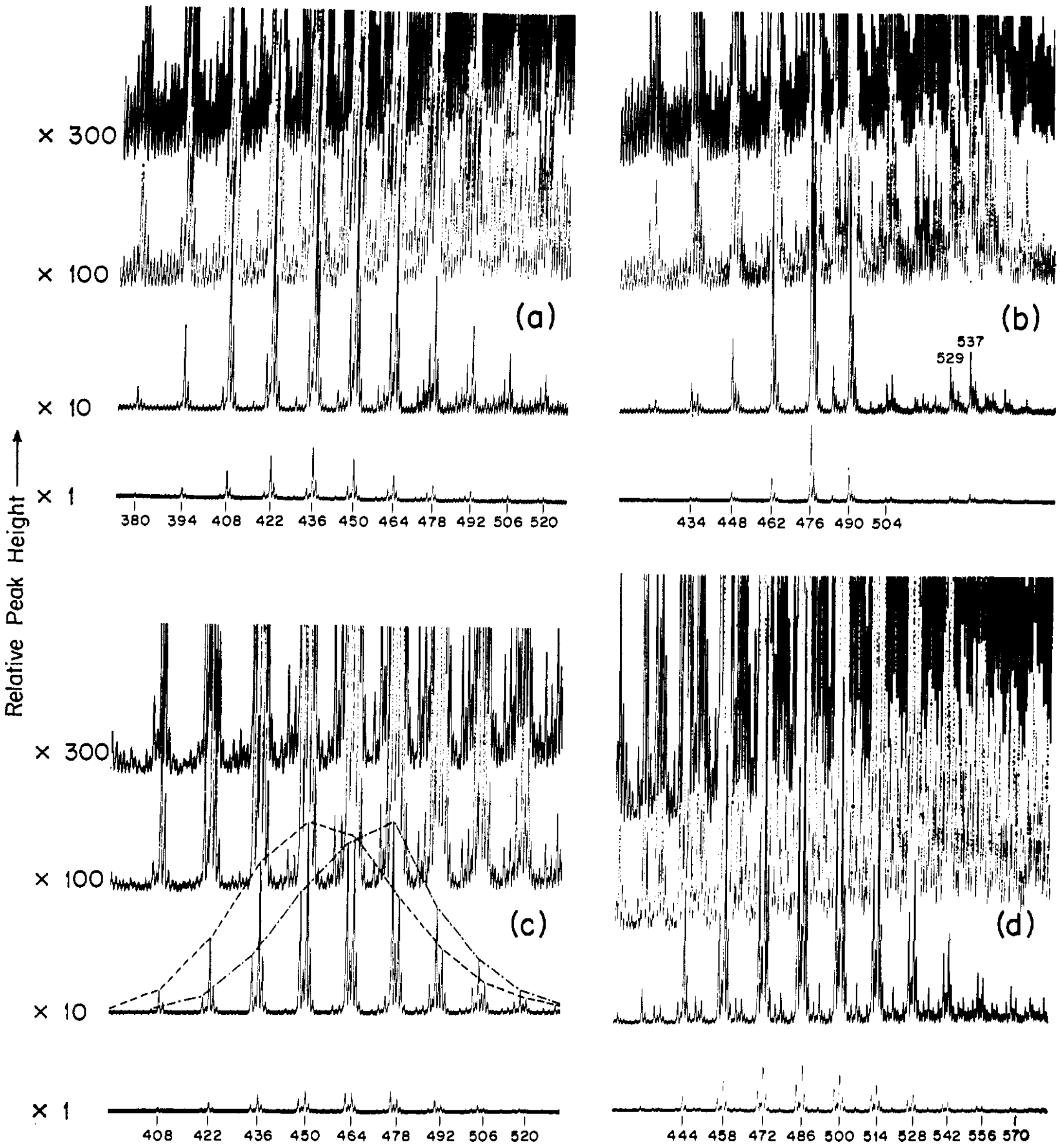

Figure 1. Low-voltage mass spectra of petroporphyrins: a, Baxterville; b, Rozel Point; c, Melones; d, rhodo type from Melones. See text for details.

rins), both of which cause some weakening of the intensity of peak III. It seems likely that both types of porphyrins are present, with incomplete β substitution occurring in the porphyrins of lower molecular weight (5–7 methylene groups) and bridge substitution in those of higher molecular weight (13–17 methylene groups).

Mass Spectrum of the Rhodo-Type Petroporphyrin. Mention has been made in the previous paper[1] of the chromatographic separation of a minor porphyrin component with a rhodo-type visible spectrum. A number of other investigators have separated from various petroleums and bitumens a porphyrin fraction with a similar type visible spectrum.[5-11] Because Melones petroporphyrin is relatively rich in this component, a quantity was separated and its mass spectrum obtained (Figure 1d). These porphyrins represent a third series, and it is apparent that the minor peaks in the mass spectra of the petroporphyrins at 4 mass units lower than the DPEP series are of this type (the small peaks falling between the regular porphyrin bands in Figures 1a and 1c are typical). Accurate mass determinations were made on a number of selected peaks in the spectrum (Table IV). These mass measurements are in excellent agreement with formulas containing only C, H, and N, but no O or S. The masses computed for formulas which contain one and two oxygens differ from the observed masses (column 1, Table IV) by *ca.* 35 and 23 mmass units, respectively. Thus, the rhodo-type visible spectrum must have some other basis than the usual conjugated carbonyl function. This point is considered in a later section of this paper.

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(5) W. W. Howe, *Anal. Chem.*, 33, 255 (1961).

(6) M. F. Millson, D. S. Montgomery, and S. R. Brown, *Geochim. Cosmochim. Acta*, 30, 207 (1966).

(7) G. W. Hodgson, E. Peake, and B. L. Baker in "Volume of Papers on the Athabasca Oil Sands," K. A. Clark, Ed., Information Series No. 45, Research Council of Alberta, Oct 1963, p 75.

(8) G. Costantinides and G. Arich, paper V-11, 6th World Petroleum Congress, Frankfurt, Germany, 1963.

(9) R. A. Dean and E. V. Whitehead, ref 8, paper V-9.

(10) L. R. Fisher and H. N. Dunning, Bureau of Mines Report of Investigation No. 5844, 1961.

(11) D. W. Thomas and M. Blumer, *Geochim. Cosmochim. Acta*, 28, 1147 (1964).

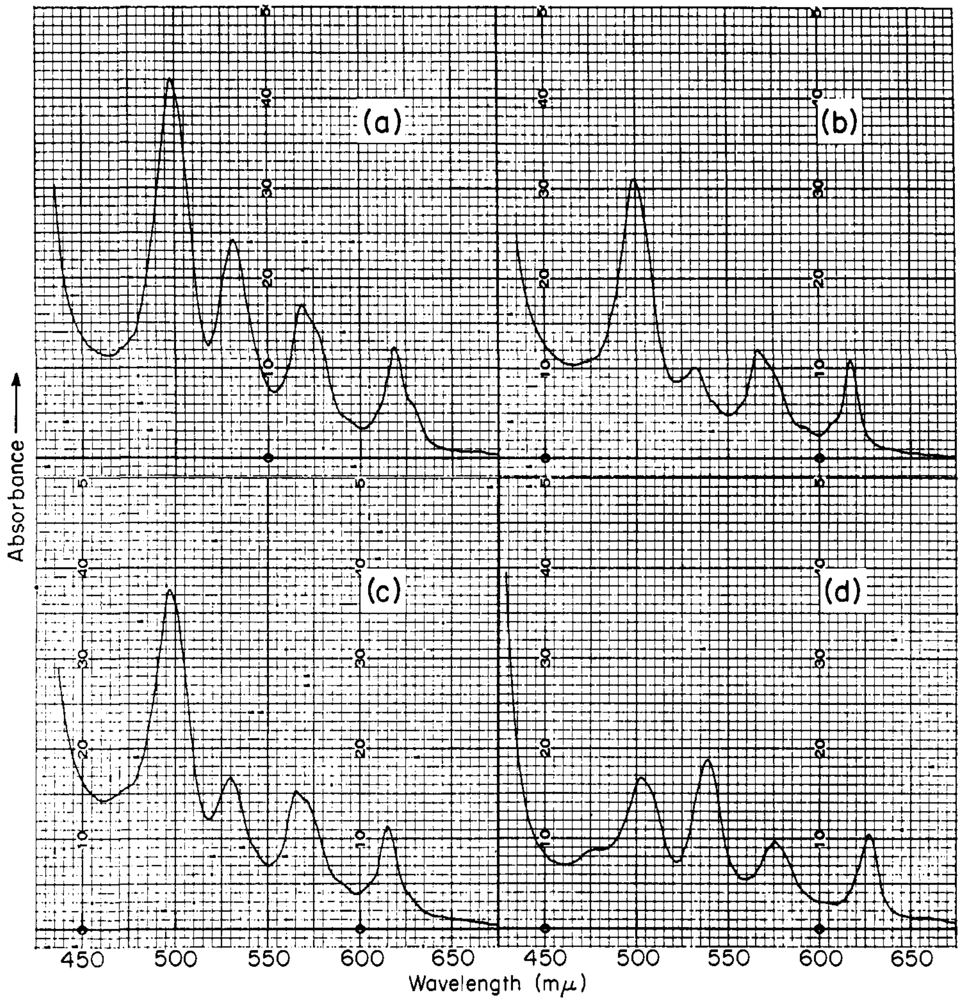

Figure 2. Visible spectra of petroporphyrins: a, Baxterville; b, Rozel Point; c, Melones; d, rhodo type from Melones. See text for details.

The Composition of the Gilsonite Porphyrin. In the earlier paper[1] we had indicated that the most abundant gilsonite porphyrin had a molecular weight of 476 and also that no envelope was present. Closer examination of the mass spectrum (Table II) shows that this is an oversimplification. Actually, a narrow envelope is present with a half-band width of *ca.* one methylene unit with an appreciable amount of material (*ca.* 30%) of the etio series also present. This conclusion is supported by detailed examination of the visible spectrum of the gilsonite porphyrins. Values for the peak ratios and extinction coefficients for the gilsonite porphyrins and model compounds are given in Table V.

Table IV. Accurate Mass Determination of Selected Peaks of Rhodo-Type Petroporphyrins

Obsd mass[a]	Computed mass	Δm*M*[b]	Formula
470.2453	470.2470	1.7	$C_{32}H_{30}N_4$
472.2610	472.2627	1.7	$C_{32}H_{32}N_4$
484.2610	484.2627	1.7	$C_{33}H_{32}N_4$
486.2766	486.2783	1.7	$C_{33}H_{34}N_4$
498.2774	498.2783	0.9	$C_{34}H_{34}N_4$
500.2931	500.2940	0.9	$C_{34}H_{36}N_4$
512.2934	512.2940	0.6	$C_{35}H_{36}N_4$
514.3095	514.3097	0.2	$C_{35}H_{38}N_4$

[a] Resolution *ca.* 1 in 15,000, mass measured by peak matching against perfluoro-*t*-butylamine. [b] Difference between observed and calculated mass in millimass units.

Table V. Absorption Spectra of Gilsonite Porphyrins and Model Compounds

	Solvent		Peak			
			IV	III	II	I
Gilsonite petroporphyrin[a]	Chloroform	ϵ_{mM}[e] Ratios[f]	11.2 1.0	5.1 0.46	5.22 0.47	3.53 0.31
Gilsonite petroporphyrin[b]	Benzene	Ratios	1.0	0.54	0.40	0.31
Deoxophylloerythrin monomethyl ester[c]	Dioxane	ϵ_{mM} Ratios	16.7 1.0	3.6 0.21	6.3 0.38	6.5 0.39
Deoxophylloerythroetioporphyrin[d]	Benzene	ϵ_{mM} Ratios	19.2 1.0	3.9 0.20	6.7 0.35	7.3 0.39
Etioporphyrin I	Benzene	ϵ_{mM} Ratios	15.8 1.0	11.2 0.71	7.4 0.47	6.3 0.40

[a] J. M. Sugihara and L. R. McGee, *J. Org. Chem.*, **22**, 795 (1957). [b] This work. For source of sample, see Table I. For mass spectrum of this sample, see Table II. [c] A. Stern and H. Wenderlein, *Z. Physik. Chem.* (Leipzig), **174**, 81 (1935). For structure of this compound, see footnote *a*. The visible spectrum of this compound should be essentially identical with that of DPEP. [d] E. W. Baker and A. H. Corwin, to be published. [e] Millimolar extinction coefficient. [f] Ratios normalized to peak IV.

The data show that in pure DPEP, peak III is smallest. Mixture with etio-type porphyrins raises the intensity of this band markedly because band III in etio is quite strong. The visible spectrum observed by us is in complete accord with the mass spectral findings and is the expected resultant of a mixture of DPEP and etio types. Likewise, it seems likely that the spectrum reported by Sugihara and McGee[12] is of a similar mixture but with a slightly higher content of the DPEP series. Thus, we conclude that, although the largest component of the gilsonite porphyrins is nickel DPEP (comprising about 26% of the total) as reported by Sugihara and McGee, the porphyrins in gilsonite do have a typical distribution of types and molecular weights.

Discussion

It is apparent that notable differences in both type and distribution of the petroporphyrins are represented in the asphaltenes and bitumens studied. Study of the porphyrin types then suggests itself as an additional means of characterizing petroleum and perhaps as a geochemical tool. Before such information can be exploited it is necessary to understand its basis and limitations. It is recognized that the spectra are not taken of the totality of the porphyrins present in the crude petroleum or bituminous substance because of failure to obtain quantitative extraction and unavoidable losses during purification. This in itself is not important, but what is important is whether the spectra are sufficiently representative of the totality of the porphyrins present so that the results are meaningful and reproducible. In the course of the separation and analysis, there exist a great many opportunities for fractionation. Some specific examples of where fractionation could occur can be mentioned. In the initial pentane precipitation a separation into asphaltic-associated and soluble porphyrins might occur. In the acid demetallation and extraction, possibilities for selection are obvious; in the solvent extraction from the acid layer and again in the chromatographic purification selection is possible. Finally, in the mass spectroscopy of the mixture, selective volatilization into the ion beam does occur under some conditions. Two main lines of evidence may be cited to indicate that significant fractionation of the alkylporphyrins can be avoided by proper experimental technique. The first is the identity of the Wilmington petroporphyrins obtained by MSA demetallation and extraction and those obtained by chromatography without demetallation.[1] The second is the demonstrated reproducibility of the method (see Experimental Section).

Taken together these observations indicate that by proper selection of the experimental conditions, real differences in molecular weights and distributions of the alkylporphyrins are observed.

However if quantities of porphyrin other than alkylporphyrins were present in the asphalts or bitumens, these materials might not be recovered by this method. In fact, the method of concentration and separation certainly discriminates against carboxylated porphyrins. Such polar porphyrins would probably be too strongly absorbed on the asphaltene to be extracted.

Classes of Petroporphyrins. Broadly viewed, the mass spectra of the petroporphyrins in this study fall into two classes: (1) those having a narrow band of molecular weights, and (2) those with a much broader band. To the first class belong the petroporphyrins from Colorado oil shale, from gilsonite, and from the asphaltenes of Rozel Point and Santiago crudes. In these samples, the preponderance of the porphyrin is accounted for by the major peak plus one methylene group above and below it. This group of petroporphyrins also tends to a high DPEP/etio ratio and generally low yields. In contrast, the remainder of the petroporphyrins has a much broader band, so that two to three methylene units above and below the major peak are required to account for most of the porphyrin. In further contrast to the first group, in many of the samples well-defined porphyrin peaks could be observed at a molecular weight of 560 and possibly even higher, and the DPEP/etio ratio is lower, falling in the range of 1.

We suggest that the common factor for petroleum and bituminous materials from which the narrow band petroporphyrins are derived may be a nonmarine origin and conversely the unifying factor for the broad band petroporphyrins is a marine origin. The nonmarine origin of the organic deposits in the Uinta Basin which include gilsonite and oil shale has been well established by Hunt, Stewart, and Dickey.[13] The nonmarine origin of Rozel Point and Santiago is less certain but appears as a reasonable possibility. The marine origin of the remainder of the samples is reasonably certain.

The Nature of the Rhodo-Type Porphyrin. The first report of a rhodo-type petroporphyrin was by Howe,[5] who chromatographically separated it as a minor fraction from the total porphyrin obtained by the Groennings[14] procedure from a Wyoming crude petroleum. The answer to the question of whether this minor porphyrin component was an artifact of the demetallation procedure was provided by an extensive investigation of the minor vanadyl porphyrin component in Athabasca oil sands which has an abnormal spectrum (long wavelength band at 590 mμ) by Millson, Montgomery, and Brown.[6] By demonstrating the spectral identity of the native vanadyl chelate and a reconstituted chelate prepared by demetallation and remetallation, these workers established Howe's claim to the existence of a rhodo-type porphyrin component. A number of workers reported that a minor vanadyl porphyrin component with a long wavelength absorption at 590 mμ was present in a large number of crude petroleums of different origins.[7-9] Interest in the nature of this minor, but widely distributed, component increased when Fisher and Dunning chromatographically separated and studied a rhodo-type porphyrin from Rhodes (Kansas) crude.[10] They pointed out that the most obvious explanation for the rhodo-type visible spectrum is a carbonyl group in conjugation with the porphyrin ring. They took the absence of a carbonyl absorption at 5.8 μ as evidence that the rhodo-type material was not a standard rhodoporphyrin and suggested that some other rhodofying group was present.[15] They then considered and rejected the possibility that the rhodofying

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[12] J. M. Sugihara and L. R. McGee, *J. Org. Chem.*, 22, 795 (1957).

[13] J. M. Hunt, F. Stewart, and P. A. Dickey, *Bull. Am. Assoc. Petrol. Geologists*, 38, 1671 (1954).

[14] S. Groennings, *Anal. Chem.*, 25, 938 (1953).

[15] A rhodofying group is a strong electron-withdrawing group; see J. E. Falk, "Porphyrins and Metalloporphyrins," Elsevier Publishing Co., New York, N. Y., 1964, p 79.

group was bromine resulting from free bromine in the hydrobromic–acetic acid reagent.

From the uncomplexed porphyrin in Swiss shale, Thomas and Blumer[11] separated by thin layer chromatography a group of porphyrins having a long-wavelength absorption maximum near 630 mμ and an absorption spectrum which could be described as the rhodo type.[16] On the basis of high-voltage mass spectra these workers deduced that unsaturation corresponding to six hydrogens was present and with some reservations suggested that the unknown materials might be trivinylporphyrins or might produce them under conditions in the inlet of the mass spectrometer. This suggestion seems unacceptable to us since either trivinylporphyrins or their precursors, tris(α-hydroxyethyl)porphyrins, would be predicted to have an etio-, not a rhodo-type, visible spectrum as is observed.[17]

We have noted that the porphyrins possessing the rhodo-type visible spectrum have nominal masses of an homologous series, 4 and 6 mass units lower than the DPEP series (Figure 1d). On the basis of the high-resolution accurate mass measurements, it is quite certain that only carbon, hydrogen, and nitrogen are present and that no oxygen is present. The empirical formulas (Table IV) require a high degree of unsaturation in the substituents. For example, a porphyrin with a formula $C_{32}H_{32}N_4$ would have side chains corresponding to $C_{12}H_{18}$ after subtracting out $C_{20}H_{14}N_4$ for the porphyrin nucleus and including an end group (hydrogen) for each side chain.

A summary of the data concerning rhodo-type petroporphyrins shows: (1) their occurrence as minor components of the petroporphyrins is common; (2) two series of porphyrins appear to be present, the first series being the 456 + 14*n*, and the second, the 458 + 14*n*; (3) only C, H, and N are present and a side-chain unsaturation corresponding to six hydrogens in the first series and eight hydrogens in the second is present; (4) based on chromatographic behavior, the solubility in aromatic hydrocarbons is considerably greater than etioporphyrin;[1] (5) the visible spectral type (Figure 1d) and shift (long-wavelength peak at 632 mμ *vs.* 620 mμ for etioporphyrin) indicate the presence of an electron-withdrawing group(s); (6) the absence of a strong 5.8-μ band indicates the absence of a carbonyl group;[10] and (7) the materials appear to resist both bromination[10] and catalytic hydrogenation[11] indicating considerable chemical inertness.

Based on these data, we propose that the rhodo-type petroporphyrins have the structure shown in Figure 3. Such an alkylbenzporphyrin structure accounts for the observed six-hydrogen deficiency of the 458 + 14*n* series. In an analogous way the 456 + 14*n* series are proposed as monocyclanoalkylbenzporphyrins. Alkylbenzporphyrins would be expected to be quite inert to either substitution or reduction, as is observed.

If one takes tetrabenzporphyrin[18,19] and porphin[20,21] as model compounds in which the red absorption peaks are, respectively, 666 and 616 mμ, and divides the shift (50 mμ) linearly, the expected shift per benz substituent is *ca.* 12 mμ. Such additivity of spectral shifts is shown in the monovinyl- and divinylporphyrins. Thus, the position of the long-wavelength peak at 632 mμ in the rhodo-type petroporphyrin, a red shift of 12 mμ compared to etioporphyrin, seems reasonable.

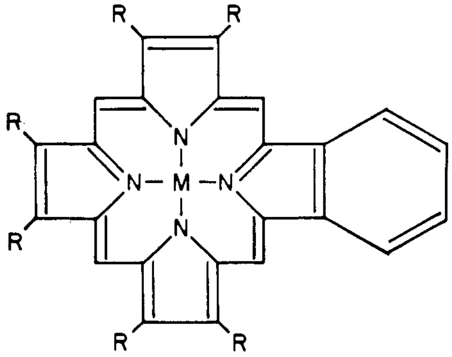

Figure 3. Proposed structure for rhodo-type petroporphyrin.

The rhodo-type spectrum of these materials also requires explanation since this spectral type is generally associated with the presence of strong electron-withdrawing substituents (so-called rhodofying groups) such as carboxyl or acetyl. That the benz substituent does function in a similar way can be shown by consideration of pyrrole and its analog with a fused benzene ring, the hypothetical isoindole. Longuet-Higgins and Coulson give values of 1.692 and 1.651 for the calculated π-electron densities on the nitrogen of pyrrole and isoindole.[22] The lower value for isoindole indicates that we can reasonably consider the fused benzene ring as an electron-withdrawing substituent. β-Alkyl substituents would be expected to raise the π-electron density of pyrrole even higher, thus making methylethylpyrrole a considerably stronger base than isoindole. Implicit in this argument is the concept that immersion of either isoindole or β-alkyl-substituted pyrrole in the superaromatic porphyrin system preserves the *relative* difference in basicity mentioned above and leads to a rhodo-type spectrum.

Finally, the observation of the rhodo-type component from such a variety of sources suggests ease of formation, and the survival through geologic time indicates a high degree of thermodynamic stability. The proposed structure meets these criteria also. We cannot propose a precursor for these materials except to point out that there is no known chlorophyll structure with a fused benzene ring. Therefore, we would tentatively suggest a diagenetic ring closure and aromatization as the probable source.

Concerning the Origin of Petroporphyrin with Carbon Numbers Greater Than 32. The petroporphyrins with carbon skeletons of 32 carbons are accounted for by the mechanism proposed by Treibs[23] and reviewed in the earlier paper.[1] In his scheme, the starting material is chlorophyll a and the carboxyl carbons are lost; by invoking a reduction rather than a decarboxylation

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(16) In the original paper, these porphyrins were called "unknowns" and a spectral type was not designated; however, in a private communication Dr. Blumer has indicated that he regards these porphyrins as having a rhodo-type spectrum.

(17) Reference 15, p 233.

(18) J. H. Helberger, A. V. Rebay, and D. B. Hever, *Ann. Chem.*, **533**, 206 (1938).

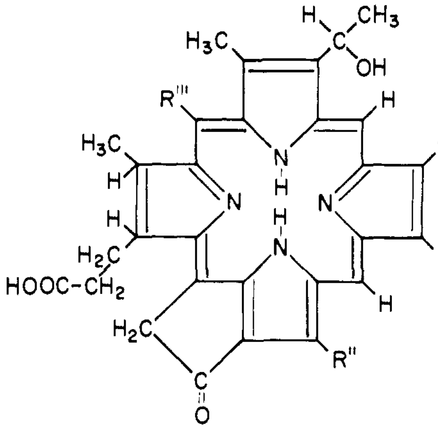
(19) P. A. Barrett, R. P. Linstead, F. G. Rundall, and G. A. P. Tuly, *J. Chem. Soc.*, 1079 (1940).

(20) H. Fischer and W. Gliem, *Ann. Chem.*, **521**, 157 (1935).

(21) C. Rimington, S. F. Mason, and O. Kennard, *Spectrochim. Acta*, **12**, 65 (1958).

(22) H. C. Longuet-Higgins and C. A. Coulson, *Trans. Faraday Soc.*, **43**, 87 (1947).

(23) A. Treibs, *Angew. Chem.*, **49**, 682 (1936).



Fraction	R′	R′′	R′′′
1	isobutyl	Et	Et
2	isobutyl	Et	Me
3	*n*-propyl	Et	Et
4	*n*-propyl	Et	Me
5	Et	Et	Me
6	Et	Me	Me

Figure 4. Proposed structures of the pheophorbides from *Chlorobium* chlorophylls (660); see ref 25.

Table VI. Distribution of *Chlorobium* Chlorophylls[a]

Fraction	Derived porphyrin,[b] mol wt	CH_2 groups[c]	Normalized yield
1	546	17	0.006
2	532	16	0.025
3	532	16	
4	518	15	0.16
5	504	14	0.70
6	490	13	0.11

[a] Taken from Holt, *et al.*[25] [b] Assuming reduction, aromatization, and decarboxylation according to Treibs' scheme.[23] [c] Number of methylene groups attached to porphyrin nucleus.

one could account for carbon skeletons of 33 and possibly 34 carbons.[24] Carbon skeletons of less than 32 carbons can also be accounted for by slight modification of the original Treibs' mechanism which might include devinylation, decarboxylation, etc. In fact, it is rather easy to imagine a variety of mechanisms which could operate to degrade the carbon skeleton.

However, the proof, as we have given here, that a considerable portion of the petroporphyrins have carbon numbers in excess of 34 demands an explanation. We had earlier invoked a diagenetic transalkylation to account for these higher molecular weight petroporphyrins.[1] An alternate pathway which should be considered is that these petroporphyrins arose through a more or less standard Treibs' scheme from aberrant chlorophylls such as those found in the *Chlorobium* series of photosynthetic bacteria. Recently, Holt, Purdie, and Wasley[25] reported the separation and identification of six chlorophylls from *Chlorobium thiosulfatophilium* strain VN. Their proposed structures are shown in Figure 4.

In Table VI are given the molecular weights of the porphyrins which would be obtained from each of the six chlorophyll fractions, assuming that reduction, aromatization, and decarboxylation occur according to Treibs' scheme.[23] Interestingly, the normalized yield for the fractions falls off at the higher molecular weights in a manner suggestive of the petroporphyrins.

It is recognized that the chlorobia are of very limited distribution at present, and therefore in themselves must be considered as unlikely sources of the widely distributed petroporphyrins. However, their present existence does perhaps suggest that not all past chlorophylls, even widely distributed ones, possessed the unique C-32 chlorophyll a skeleton. In this way, it is necessary to consider biogenesis as a possible alternate or supplement to the previously discussed diagenetic routes to the higher molecular weight petroporphyrins.

At this stage, no clear choice between the two alternates is possible although intuitively we would favor the diagenetic path as the major contributor.

Experimental Section

Preparation of Petroporphyrins. The asphaltenes were obtained in the standard way from liquid petroleum by precipitation with ten volumes of *n*-pentane at room temperature. The asphaltenes were recovered by filtration after settling and decantation of the liquid, and washed with pentane until no further discoloration of the pentane was observed. The asphaltenes were then stored under nitrogen and used as soon as practicable since it was found that storage even under nonoxidizing conditions in some cases reduced the yield of porphyrins noticeably.

The porphyrins were extracted from the solid asphaltenes, using methanesulfonic acid (MSA) as in our earlier study, by the method of Erdman.[2] A typical extraction was performed in the following way. Boscan asphaltene, obtained as above (40.0 g), was treated with MSA (200 ml) in a ball mill (0.3 gallon) containing ceramic balls (usually 10 balls, 1 in. diameter). After stirring to break up lumps, the mill was closed and placed on rollers at slow speed. Heat was supplied with a bank of infrared lamps or a heat gun. The heat input was adjusted by trial and error to give a temperature at the end of a 4-hr run of 105 ± 5°. After 4 hr, the mill was opened, and 200 g of ice with 200 ml of water was added with stirring. The solution (*ca.* 50°) was filtered with suction through an 11-cm filter funnel.

The volume of the wine-colored extract was measured, the yield was assayed by volumetric dilution, and the optical density of the 546-mμ dication peak was determined. An ϵ value of 17×10^3 was used to calculate the yield after subtraction of a small background correction.

The porphyrins were then transferred from the aqueous acid to methylene chloride (*ca.* 250 ml) in a liquid-liquid extractor. The methylene chloride layer was then withdrawn from the extractor and the porphyrin converted from the dication to the free base by treatment with sodium acetate solution (100 ml, 10% w/v). The methylene chloride layer was transferred to a rotary evaporator and the solvent removed. The oily black residue was taken up with a minimum of benzene and applied to a silica gel chromatographic column (40 mm diameter; 12 cm of silica gel) which had been prepared with cyclohexane. The column was developed with cyclohexane-benzene, benzene, and finally with benzene containing 1-5% ether. The purified porphyrin was finally recovered by evaporation of the solvent.

In cases where the starting material was a solid (gilsonite, Colorado oil shale), the finely powdered solid (100 mesh) was treated directly in the mill. All other steps were identical except that in some cases excessive amounts of oil were obtained. In those cases, an additional purification step prior to chromatography was used. The oily residue was taken up with a small volume of N,N-dimethylformamide (DMF) and extracted in a separatory funnel three times with an equal volume of cyclohexane. The porphyrin was then transferred to benzene in a separatory funnel by dilution of the

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(24) The 33-carbon skeleton could arise by reduction of the carboxyl group of the propionic acid side chain and lead to 7-desethyl-7-propyl-DPEP (mol wt 490). The 34th carbon would then have to arise by reduction of the carboxyl group on the isocyclic ring. That such a reduction could be a pathway to a detectable amount of petroporphyrins seems highly unlikely since the β-keto acid formed on hydrolysis of the 10 carbomethoxyl group decarboxylates in the laboratory in a few hours at 60°.

(25) A. S. Holt, J. W. Purdie, and J. W. F. Wasley, *Can. J. Chem.*, **44**, 88 (1966).

DMF with three volumes of water. Most of the benzene was removed and the residue placed on the chromatography column as described.

Great care was necessary to prevent conversion of the free base porphyrins to their metallochelates, particularly in those cases where very small amounts of petroporphyrin were available. The chromatographic reagents were of the highest purity available; all glassware and equipment were acid washed, rinsed with deionized water, and dried in a dust-free area prior to use. Even with these precautions, occasional contamination was noted, as in the case of Rozel Point petroporphyrin (Figure 1b). The peaks at masses 529 and 537 are almost certainly the result of some contamination by copper.

Mass Spectrometry of Petroporphyrins. The mass spectra were obtained on an AEI MS-9 mass spectrometer equipped with a solid sample probe. The indicated temperature of the probe for a typical run was 260° and the indicated beam energy was 12 ev. These parameters were selected as being the most suitable for the particular kinds of mixtures under investigation on the following basis.

A suitable temperature range for obtaining mass spectra of these mixtures was arrived at in the following way. A series of five samples of Boscan petroporphyrin was introduced to the spectrometer at temperatures ranging from 195 to 280°. In Table VII are shown the mean mass of the Boscan petroporphyrin as a function of the indicated temperature of the probe. It is seen that the center of the porphyrin envelope moves up as a function of temperature until about 230° and then remains relatively constant, thus setting a lower level on the probe temperature. It was also known that at temperatures somewhat above 300° pure alkylporphyrin begins to produce thermal fragment peaks. Thus, a probe temperature of *ca.* 260° was selected.

Since in analysis of mixtures one wishes to observe insofar as possible only parent molecular ions without the complications of fragment ions, it is necessary to select an appropriate beam energy. To be certain that only parent ions were observed, the energy of the beam would have to be somewhat above the ionization potential of the molecule and below the appearance potential of the first fragment ion. Practically, such a selection is not possible because the appearance potentials of the parent ions and fragments for the components of the mixture are not known and indeed may overlap. Furthermore, the spectrometer tends to lose regulation at very low beam energies, and the over-all intensity of the spectrum suffers. Taking all of these factors into consideration, a compromise value of 12 ev was selected.

Table VII. Temperature Dependence of Mass Spectra of Boscan Petroporphyrin

Temp, °C	Mean mass of etio series of Boscan petroporphyrin[a]	Height of largest peaks in the spectrum[b]
195	459	8.5
213	463	28
233	468	34.5
259	468	81
265	468	107

[a] Calculated according to $\Sigma IM/\Sigma I$. [b] Peak heights measured in mm on the X10 scale. The noise level was roughly constant at 0.75 mm.

At this beam energy, the greatest fragment peak is probably not greater than 1% of the parent, less than the noise and impurity level. Using these selected instrument parameters, quadruplicate analyses of petroporphyrin from Boscan asphaltene gave an average standard deviation (σ) of 5.7% on the 15 largest peaks in the spectrum. The accuracy of the mass spectra was further checked in the case of Wilmington petroporphyrin by comparison with the data of Morandi and Jensen.[26] Very good agreement was found. The average difference was 5.8% in the normalized intensities of 14 major peaks.

Acknowledgment. This work was sponsored by the Gulf Research & Development Company as part of the research program of the Multiple Fellowship on Petroleum.

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[26] J. R. Morandi and H. B. Jensen, *J. Chem. Eng. Data*, **11**, 81 (1966).

Communications to the Editor

Reactive Tetravalent Sulfur Intermediates. A Nonclassical Thiophthene

Sir:

We have previously reported a convenient synthesis of benzo[*c*]thiophenes by dehydration of the sulfoxides of the corresponding dihydrothiophenes.[1] The utilization of this method in the synthesis of thieno[3,4-*b*]thiophene (I), the last of the three classical thiophthene isomers (I–III), was described recently.[2]

We now report evidence for the transient existence of a derivative of thieno[3,4-*c*]thiophene (IV), a fourth thiophthene for which the only uncharged resonance contributors are structures containing tetravalent sulfur. An analogous structure (V) has been long suggested as an appreciable contributor to the thiophene molecule.[3]

The reaction of 3,4-bis(chloromethyl)-2,5-dimethylthiophene[4] with aqueous ethanolic sodium sulfide afforded, in 35% yield, 1H,3H-dihydro-4,6-dimethylthieno[3,4-*c*]thiophene (VI),[5] mp 65–71°. Periodate oxidation[6] of VI in aqueous methanol gave the corresponding sulfoxide, VII, mp 115–119°, in 91% yield. Attempted pyrolysis of VII in the presence of neutral alumina[1] afforded only polymeric material. On the other hand, when a solution of VII and N-phenylmaleimide was refluxed for 4 hr in acetic anhydride,

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[1] M. P. Cava and N. M. Pollack, *J. Am. Chem. Soc.*, **88**, 4112 (1966).

[2] (a) H. Wynberg and D. J. Zwanenburg, *Tetrahedron Letters*, 761 (1967); (b) for the first syntheses of the 1H,3H-thienothiophenes, see: H. Wynberg and D. J. Zwanenburg, *J. Org. Chem.*, **29**, 1919 (1964).

[3] V. Schomaker and L. Pauling, *J. Am. Chem. Soc.*, **61**, 1769 (1939); C. C. Price and S. Oae, "Sulfur Bonding," The Ronald Press, New York, N. Y., 1962, p 29; S. Gronowitz, *Advan. Heterocyclic Chem.* **1**, 3 (1963).

[4] K. Dimroth, G. Pohl, and H. Follmann, *Chem. Ber.*, **99**, 634 (1966).

[5] All melting points are uncorrected. Satisfactory analyses were obtained for all new compounds reported. Nmr spectra were determined in $CDCl_3$.

[6] N. J. Leonard and C. R. Johnson, *J. Org. Chem.*, **27**, 282 (1962).