of the mother liquor. A 180-mg. sample of the recrystallized product was chromatographed and crystallized as described above to give 51 mg. for spectrophotometric study.

Anal. Calcd. for $C_{48}H_{38}N_4O_4$: C, 78.47; H, 5.18; N, 7.63. Found: C, 77.41; H, 4.77; N, 7.95.

Spectrophotometric analysis given in Table I of the various colored impurity bands separated from the chromatographic column gave, in addition to the usual etio-type spectra, a rhodo-type spectrum, which indicated that perhaps a phenyl ring is missing or that a pyrrole ring may be substituted in one of the fractions.

Tetra-(*p*-chlorophenyl)-porphine.—A reaction mixture consisting of 47.7 g. of pyrrole, 100 g. of *p*-chlorobenzaldehyde (m.p. 47.8°) and 98 ml. of pyridine was treated as outlined above for 48 hours at 176°. Direct filtration of the reaction mixture resulted in the isolation of 3.2 g. of purple crystals. Dilution of the mother liquor with ether–acetone resulted in the crystallization of 0.5 g. of additional porphyrin. Further dilution with ether resulted in the isolation of a quantity of *p*-chlorobenzoic acid. The porphyrin was extracted with chloroform; the chloroform solution evaporated to a small volume, and diluted with four times its volume of methanol. The crystalline material thus formed weighed 3.5 g., which represents 2.6% of the theoretical amount. A 174-mg. sample of this material dissolved in a less polar trichloroethylene solvent than previously used was chromatographed as described above and recrystallized from chloroform–methanol to yield 67 mg. of purified porphine.

Anal. Calcd. for $C_{44}H_{28}N_4Cl_4$: C, 70.21; H, 3.46; N, 7.45; Cl, 18.88. Found: C, 70.34; H, 3.38; N, 7.30; Cl, 18.65.

Separation and analysis of the isolated bands gave, in addition to some relatively pure porphine which had been adsorbed on the lower region of the talc, three fractions containing appreciable to large quantities of chlorin. It can be observed in Table I that the presence of a greater amount of chlorin impurity is indicated by an increase of wave length of the first absorption peak near 650 mμ.

Tetra-(*p*-nitrophenyl)-porphine.—A number of small-scale refluxing reactions were run at temperatures between 100 and 130° for lengths of time which varied from 24 to 48 hours. A number of successful reactions are summarized below:

p-Nitrobenzaldehyde, g.	Pyrrole, ml.	Pyridine, ml.	Methanol, ml.	*t*, °C.	Time, hr.	Yield, mg.
2.5	2.0	1.0	3.1	105	24	7
2.5	2.0	1.0	3.0	112	24	30
2.5	2.0	1.0	2.6	120	40	13
5.0	4.0	1.5	4.0	130	40	33
5.0	4.0	2.0	3.3	130	44	50
2.5	1.1	1.0	2.8	120	24	84

The last of the runs listed above represents about 2.6% of the theoretical yield. The combined yields obtained above (~0.20 g.) was extracted with 200 ml. of chloroform in a Soxhlet apparatus. Eight days were necessary to dissolve most of the porphine, which began to crystallize out of the solution before the extraction was complete. The product was recrystallized by the addition of methanol to a chloroform solution. The amount of purified material thus obtained weighed 138 mg.

The compound was chromatographed on an 8-cm. by 3-cm. talc column. A 25-mg. sample was dissolved in 400 ml. of hot chloroform to give a deep orange-red solution which appeared green on the talc. Development of the chromatogram with more chloroform resulted in the recovery of the compound in solution and the formation of a 12-mm. green-brown layer at the top of the column, which was coated with tar impurities. Below this was found a 12-mm. tan-brown-green band. Spectrophotometric analysis of both bands in acetone solution gave spectra very similar to that of tetra-(*p*-nitrophenyl)-porphine itself.

Anal. Calcd. for $C_{44}H_{26}N_4O_8$: C, 71.55; H, 3.52; N, 15.15. Found: C, 67.12; H, 3.63; N, 12.60.

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WORCESTER, MASS.

[CONTRIBUTION OF THE DEPARTMENT OF CHEMISTRY OF CLARK UNIVERSITY]

Absorption Spectra of *para*-Substituted Tetraphenylporphines[1,2]

BY DANIEL W. THOMAS AND ARTHUR E. MARTELL

RECEIVED SEPTEMBER 29, 1955

Absorption spectra of tetraphenylporphine, tetra-(*p*-methoxyphenyl)-porphine, tetra-*p*-tolylporphine, tetra-(*p*-chlorophenyl)-porphine and tetra-(*p*-nitrophenyl)-porphine in the ultraviolet, visible and infrared regions are reported. The visible and ultraviolet spectra indicate that the *para*-substituents exert only a small effect on the electronic transitions of the porphine ring system. Assignments of infrared frequencies are made where possible.

The syntheses of tetraphenylporphine and its *p*-methyl, methoxy, chloro and nitro derivatives have been described in a recent publication.[3] In the present paper are described the ultraviolet, visible and infrared spectra of these compounds, with a view to determining the influence, if any, of the *p*-phenyl substituents on the porphine ring and on the electron-donor properties of the central nitrogen atoms.

A small amount of work has been reported on the infrared spectra of the porphines, but nothing has yet appeared on the tetraphenylporphines. Of the infrared studies made on pyrrole-substituted prophyrins, assignments have been suggested chiefly for the substituent groups,[4,5] and few for the porphine structure itself. However, a number of investigations of N–H bonding have been reported by Falk and Willis[4] and Vestling and Downing.[6] Visible and ultraviolet spectra have been reported for tetraphenylporphine.[7-10] No spectra have

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(1) This research was supported by the National Institutes of Health of the U. S. Public Health Service under Grant No. G-3819(c).

(2) Abstracted from a dissertation submitted by Daniel W. Thomas to the Faculty of Clark University in partial fulfillment of the requirements for the degree of Master of Arts.

(3) D. W. Thomas and A. E. Martell, THIS JOURNAL, **78**, 1335 (1956).

(4) J. B. Falk and J. B. Willis, *Aust. J. Scient. Research*, **4**, No. 4, 579 (1951).

(5) C. W. Craven, K. R. Reissmann and H. I. Chinn, *J. Anal. Chem.*, **24**, No. 7, 1214 (1952).

(6) C. S. Vestling and J. R. Downing, THIS JOURNAL, **61**, 3511 (1939).

(7) V. M. Albers and H. V. Knorr, *J. Chem. Phys.*, **9**, No. 7, 497 (1941).

(8) R. H. Ball, G. D. Dorough and M. Calvin, THIS JOURNAL, **68**^{2}, 2278 (1936).

(9) G. D. Dorough and K. T. Shen, *ibid.*, **72**, 3939 (1950).

(10) G. D. Dorough, J. R. Miller and F. M. Huennekens, *ibid.*, **73**, 4315 (1951).

been described for the remaining *para*-substituted porphines described above; they are new compounds with the exception of the *p*-methoxy derivative.[11]

Experimental

The five tetraphenylporphines used in this investigation were prepared as described previously.[3] All compounds were recrystallized and separated chromatographically from chlorins and porphine-type impurities.

Infrared Spectra.—Infrared absorption measurements were carried out with a Perkin–Elmer model 21 double beam spectrophotometer equipped with sodium chloride optics. All samples were run as both Nujol and hexachlorobutadiene mulls. Since Nujol absorbs strongly at 2915, 1460 and 1375 cm.$^{-1}$, and hexachlorobutadiene does not, the latter mulling agent was used to determine the absorption bands overlapped by Nujol. Potassium bromide pellets were also prepared but the absorption bands were not as sharp or as distinct in this medium as in the mulls.

Visible and Ultraviolet Spectra.—Absorption spectra of benzene solutions of tetraphenylporphine and its p-CH$_3$, p-OCH$_3$, and p-Cl derivatives were measured in the 700–350 mμ region in 1-cm. quartz cells with a Beckman DU quartz spectrophotometer. Tetraphenylporphine and its p-NO$_2$ derivative were measured as pyridine solutions. Readings of the optical densities were taken at 5 mμ intervals and at more frequent intervals near the absorption peaks. The absorption curves represent smooth lines drawn through the plots of the corresponding extinction coefficients, which were calculated from the optical densities and the molar concentration of the solution by Beer–Lambert's law. Measurements of tetraphenylporphine and its derivatives were obtained at concentrations of 10^{-5} *M* by weighing out the appropriate quantity of porphyrin and dissolving to 100 ml. in a volumetric flask. Tetraphenylporphine and its nitro derivative were dissolved by pyridine in 50-ml. volumetric flasks. For regions below 450 mμ, solutions were diluted to 10^{-7} *M*.

TABLE I

ABSORPTION CHARACTERISTICS OF THE PORPHYRINS

	Absorption maxima				
	Benzene		Pyridine		Acetone
Porphine	λ, mμ	$\epsilon \times 10^{-3}$	λ, mμ	$\epsilon \times 10^{-3}$	λ, mμ
Tetraphenyl-	647	3.4	647	3.9	647
	592	5.3	592	5.5	590
	548	8.1	550	8.6	547
	515	18.7	515	18.7	513
	485	3.4	485	3.8	480
	419	478	420	464	
Tetra-(*p*-methoxyphenyl)-	653	4.5			650
	595	5.5			593
	555	11.9			553
	519	17.0			517
	488	4.3			483
	424	485			
Tetra-*p*-tolyl-	650	4.1			647
	592	5.4			590
	550	8.2			547
	516	18.9			513
	485	3.7			480
	420	490			
Tetra-(*p*-chlorophenyl)-	647	3.7			645
	592	6.0			589
	550	9.0			546
	515	21.0			513
	485	4.0			480
	421	515			
Tetra-(*p*-nitrophenyl)-			647	3.4	645
			593	6.5	590
			554	9.6	552
			518	19.3	515
			487	5.0	480
			428	250	

Discussion

Ultraviolet and Visible Regions.—The ultraviolet and visible absorption spectra of tetraphenylporphine and its *p*-methyl, methoxy and chloro derivatives in benzene are given in Fig. 1. The spectrum of the corresponding *p*-nitro compound in pyridine is illustrated in Fig. 2, along with tetraphenylporphine for comparison. Extinction values and wave lengths of the absorption maxima are listed in Table I. The spectra have been divided into two groups, the first in the region of 700 to about 450 mμ, and the second from 450 to 350 mμ. The absorption bands in the 700-450 mμ region can be regarded as vibrational terms of a common electronic transition, while the intense band in the near ultraviolet region, the so-called "soret" band,

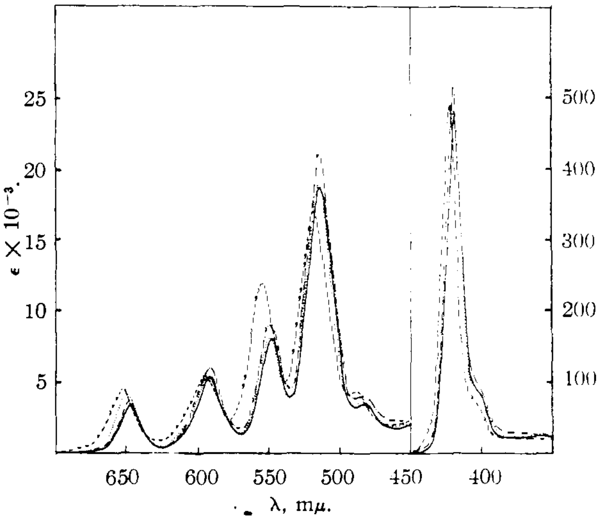

Fig. 1.—Visible and ultraviolet absorption bands in benzene solution of tetraphenylporphine ——, and its *para*-substituted derivatives: – – –, methoxy; –·–·–, chloro; and ....., methyl.

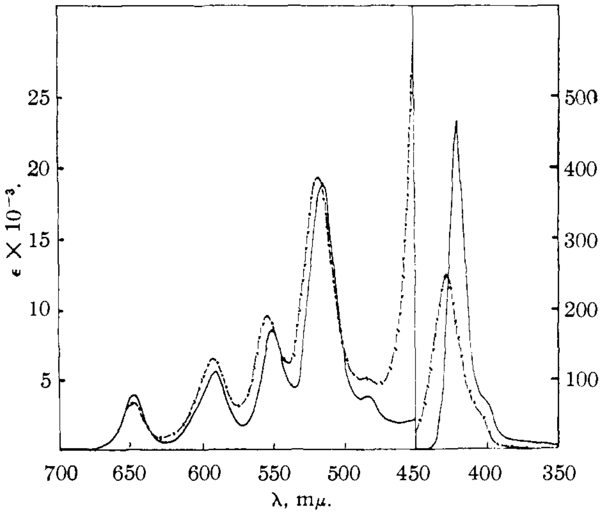

Fig. 2.—Visible and ultraviolet absorption bands in pyridine solution of tetraphenylporphine ——, and its *p*-nitro derivative –·–·–.

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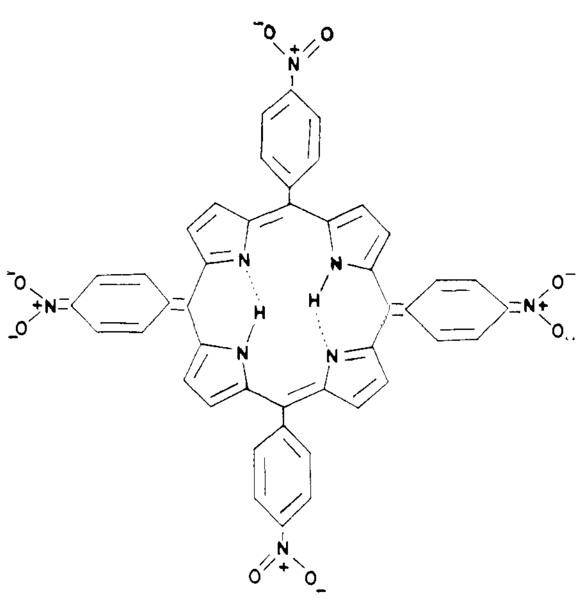
(11) P. Rothemund, THIS JOURNAL, **61**, 2912 (1939).

can correspond to a different electronic excitation.[10,12] The intensities of the four main absorption bands of tetraphenylporphine in the visible region are identical to those previously published by Dorough, *et al.*[10] However, the 419 mμ band was found in the present work to have an extinction coefficient 20% higher than the value previously reported by Dorough and co-workers. This increased intensity was observed for all the derivatives of tetraphenylporphine illustrated in Fig. 1 and is probably due to increased purity of these substances. The *p*-nitro compound, which appears to be unique, does not show this increased absorption.

It is apparent from the absorption bands illustrated in Figs. 1 and 2, and listed in Table I, that small shifts in the absorption maxima occur as the result of substitution of various polar groups in the *para*-positions of tetraphenylporphine. It is well known that the substituent groups used in this investigation increase or decrease the electron density in the ortho and para positions in the benzene ring. For example, the nitro group would be expected to undergo resonance interactions with the porphine ring in the manner indicated by formulas I and II, which involve greater conjugation within the molecule. In addition to the forms shown, other energetically reasonable resonance structures may be shown to contribute toward a hybrid in which the electron densities about the nitrogen atoms are lowered, and the potential energies of the electronic excited states are also reduced. The inductive effect illustrated for the nitro group would be reversed for the other substituents, which usually exert a resonance electron release to an aromatic system in the order $-OCH_3 > -CH_3 > -Cl$. In the latter case the inductive electron attractive effect of the halogen would be expected to more than balance the resonance-type of electron release except under certain conditions favoring such electron release.

Many of the absorption bands of the *para*-substituted derivatives exhibit small shifts to longer wave lengths as compared to the etio-type spectrum of tetraphenylporphine, while other bands showed no change in position. In no case, however, was the maximum of an absorption band displaced to lower wave length than that found in the parent compound.

The most pronounced bathochromic shift occurs in the methoxy derivative. In this case the main absorption peak in the visible region occurs at 519 mμ, and its intensity is decreased somewhat, while the intensities of most of the weaker bands in this group are considerably increased, and are shifted to a greater extent than the main band. Tetra-(*p*-chlorophenyl)-porphine, on the other hand, shows only minor shifts, but the intensities of all the peaks are higher than those of the parent tetraphenylporphine. Like the methoxy derivative in benzene, the spectra of tetra-(*p*-nitrophenyl)-porphine in pyridine also shows a pronounced shift to the red for a majority of bands, including an 8 mμ displacement of the much less intense soret peak.



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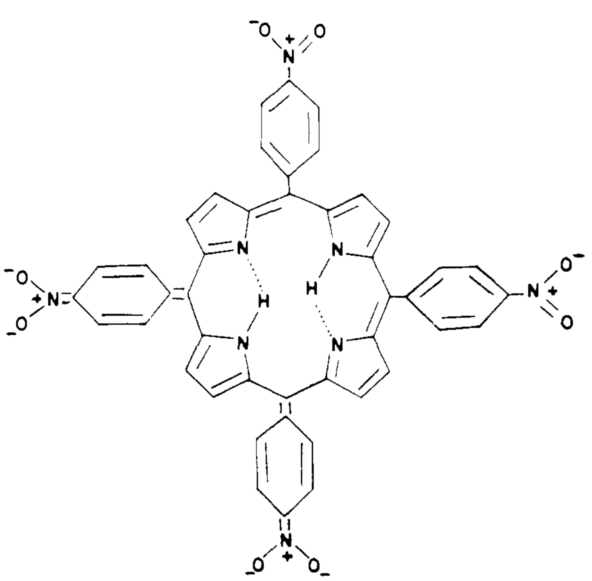
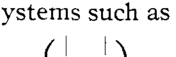


II

In general, it may be concluded that the extent of the influence of substituent groups on the absorption bands of tetraphenylporphine decrease in the order $-OCH_3$, $-NO_2 >> -Cl > -CH_3$. If one neglects the direction of the effects of these groups this is also the relative order of interactions of these groups with aromatic systems, such as the benzene ring. Apparently the intensification and bathochromic shifts in the absorption bands take place regardless of the directional effect of the polar group, and this is in accord with the influence of such groups on the K-type absorption bands of other resonating systems such as



$$X-\left(\overset{|}{C}=\overset{|}{C}\right)-R$$

The introduction of a resonating group X at one end of a general system of this type usually produces a bathochromic shift of the electronic transitions, with a corresponding intensification of the absorption bands.

A factor which must be considered in the interpretation of the spectra in the visible and ultravio-

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(12) F. Haurowitz and W. Klemm, *Ber.*, **68**, 2312 (1935).

let regions is that the phenyl and substituted phenyl rings cannot be coplanar with the porphine nucleus. Examination of molecular models indicates that the four benzene rings have partial rotations which cannot bring them within 60° of being coplanar with the resonating porphine system. Since the average angle of the attached rings is probably considerably greater than this, it is apparent that resonance interactions between the two aromatic systems must be greatly reduced from what would be expected if they were coplanar. Hence, the bathochromic shifts and spectral intensifications observed are considerably less than what would be expected in simpler aromatic systems with greater freedom of rotation.

The small shift in the maxima of all bands to lower wave length when the solvent was changed from benzene to acetone, as indicated in Table I, is a normal occurrence for such a variation of solvent. In the compounds of the present investigation, this shift or solvent effect is not large enough to be considered significant.

Infrared Spectra.—The frequencies, approximate intensities and some assignments of the infrared absorption bands of tetraphenylporphine and its para-substituted products are listed in Table II. In addition to the frequencies given, the spectra of pyrrole, benzaldehyde and its *p*-methyl, methoxy, chloro and nitro derivatives were also determined to aid in the assignment of frequencies.

The N–H . . . . N stretching frequencies were found to be very weak in Nujol mulls, but to be much sharper and about twice as intense when hexachlorobutadiene was used as the mulling agent. Therefore, the frequencies listed for this absorption band in Table II were measured in the latter medium. The N–H stretching frequency, which occurs at 3500 cm.$^{-1}$ in pyrrole, occurs at 3350 cm.$^{-1}$ in tetraphenylporphine, an indication of stronger hydrogen bonding in the condensed ring systems of the porphines. It is to be noted also that replacement of the *p*-hydrogen by an element more electronegative than carbon (*i.e.*, by substitution of methoxyl, chloro and nitro groups) there is a slight increase in the N–H stretching frequency. Thus, it appears that hydrogen bonding is somewhat decreased by the substitution of these electronegative elements. The decrease in hydrogen bond strength indicates that the influence of the substituents is to decrease the electron density about the central nitrogen atoms. Hence, the influence of the *para*-groups is due to their inductive effects rather than their resonance interactions. This conclusion is reasonable in view of the noncoplanarity of the porphine and benzene rings described above. The shift to lower frequency in the *p*-tolyl derivative indicates that hydrogen bonding is perhaps somewhat stronger in this compound.

The frequencies listed from 2850–3140 cm.$^{-1}$ are due to =C—H stretching vibrations of the phenyl and substituted phenyl rings, and of the pyrrole rings. All of these probably occur at the higher part of this frequency range, while the –C–H stretch of the methyl groups would be found in the lower part of this region.

The characteristic skeletal —C=C— in-plane vibrations, which usually occur near 1600 cm.$^{-1}$, have been reported by Bellamy[13] to be frequently shifted to slightly higher frequency by *para*-substitution. The most pronounced shift of this type may be found in the *p*-methoxyphenyl band, which is found at 1610 cm.$^{-1}$ compared to 1597 in tetraphenylporphine. Although listed in Table II as medium, the intensities of these bands in the *p*-nitro-, methyl- and methoxy compounds are considerably enhanced over those of tetraphenylporphine.

The skeletal in-plane conjugated phenyl —C=C— vibrations appear near 1580 cm.$^{-1}$. These absorptions usually appear whenever the phenyl ring is further conjugated in some manner, according to Bellamy[13a] and Fuson.[14] In this case, at least partial conjugation may be stated to take place with the porphine ring.

The weak and medium bands assigned to pyrrole or methine —C=C— stretching vibrations have a somewhat lower frequency (1560–1565 cm.$^{-1}$) than that usually found (~1600 cm.$^{-1}$) for stretching vibrations. The lower frequency in this case is probably due to conjugation with adjacent single bonds. Phenyl rings also exhibit absorption near 1500 cm.$^{-1}$ (1487–1512 cm.$^{-1}$) which is often shifted to higher frequencies with *para*-substitution.

The strong frequencies near 1350 cm.$^{-1}$ are tentatively assigned to the C=N stretching vibration. Observation of the non-ionic resonance forms of porphine indicates two forms with —C=N— and six with —C—N bonds. Hence any C=N vibration would be expected to have considerable single bond character and to occur at a longer wave length (*i.e.*, lower frequency). Thus the assigned frequency is much lower than the value usually found (1600–1650 cm.$^{-1}$) for —C=N groups.

The absorption bands found in all porphyrins near 1440 cm.$^{-1}$ have been assigned to the C–H bending vibration of pyrrole. These absorptions were absent in the aldehydes but were present in pyrrole. A C–H bending vibration in this region (1470 cm.$^{-1}$) also has been assigned to pyrrole in pyrrole-substituted porphyrins by Craven, Reissmann and Chinn.[5]

The C–H bending vibrations of methyl groups give rise to absorptions near 1450 cm.$^{-1}$. Thus frequencies of 1452 and 1457 cm.$^{-1}$ have been given the assignments of antisymmetric –C–H vibrations of the methyl and methoxy groups, respectively. Absorption bands also were found in this region of the spectrum for the corresponding aldehydes. Symmetric deformations of the methyl derivative occur at 1375 cm.$^{-1}$.

A strong absorption at 1247 cm.$^{-1}$ has been identified as due to the –C–O– stretching vibration of the methoxy substituent by comparing the spectrum of the corresponding porphyrin with that of anisaldehyde. Strong absorption at 1173 cm.$^{-1}$ has also been attributed to the methoxy group.

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(13) L. J. Bellamy, "The Infrared Spectra of Complex Molecules," London, Methuen; John Wiley and Sons, Inc., New York, N. Y., 1952, p. 60; (a) p. 60–61; (b) p. 69; (c) p. 271.

(14) N. Fuson, R. G. Fowler, H. M. Randall and J. R. Dangl, "Infrared Determination of Organic Structures," D. Van Nostrand Co., New York, N. Y., 1952, p. 16; (a) p. 58.

TABLE II

INFRARED ABSORPTION SPECTRA OF THE SUBSTITUTED TETRAPHENYLPORPHINES

	p-Substituent (frequencies in cm.$^{-1}$)				
–H	$-OCH_3$	$-CH_3$	–Cl	$-NO_2$	Assignment
3350w[a]	3370w	3340w	3360w	3360w	N–H. . .N stretch
	3140w	3110w	3120wbr	3140wbr	
3060w		3060wsh	3060w		
3040wsh	3040w	3020m			C–H stretch
	2980wbr				
2920w	2910wbr	2920m	2930w	2940w	
2860w	2850wbr	2860w		2860w	
2700vw	2710vw	2700w	2710w	2700w	
2610vw	2610vw	2600w	2610w	2610vw	
2530vw	2540vw	2525w	2540w	2540vw	
				2450vw	
2380vw		2370wbr	2360vw	2350vw	
1955vwbr	1900wbr	1960wbr	1910wbr	1940wbr	
1812wbr		1807wbr	1810wbr		
1800wsh		1795wsh	1780wsh		
1700vw		1700wbr	1685wbr	1700wbr	
1685vw		1680w	1670wsh		
1597m	1610m	1600m	1595m	1596m	
1575m	1575m	1582m	1582msh	1584ms	C=C stretch
1560msh	1563wsh	1560mbr	1565mbr	1564mbr	
1545wsh	1547wsh	1543wsh	1548wsh	1541msh	
				1518s	$-NO_2$
		1510w	1512w	1505msh	
1497m	1512m	1487msh	1490m	1494wsh	C=C phenyl
1477m	1475msh	1470m	1473m	1475m	
	1465m		1462msh	1460wsh	
	1457msh	1452msh			$-CH_3$ def. (anti)
1444m	1442m	1438msh	1443wsh	1440wsh	C–H bend
1403w	1405wbr	1400w	1396m	1402w	
		1375msh			$-CH_3$ def. (symm)
1362msh		1363msh	1367m		
1350m	1350m	1347m	1347m	1345o	–C=N stretch
				1345sbr	$-NO_2$
1325wsh		1320wsh	1332msh		
1310vw	1303msh	1307vw	1300wbr	1309m	
1286vw	1287vw	1288m	1285vw	1285m	
1250w		1270w	1255w	1255w	
	1247s				C–O str.
1222w	1231m	1224msh	1222m	1225w	
1213w	1214wsh	1226msh	1213w	1214w	
		1198w			
1188m	1184msh	1183w	1186w	1186w	
1176m	o	1168wsh	1174m	1175whs	C_6H_5-subst. C_6H_5
	1173s				$-OCH_3$
1155m	1153msh	1150m	1153w	1155w	C_6H_5-subst. C_6H_5
	1105m	1105m	1100msh	1105m	*p*-subst. C_6H_5
1080m		1092m	1088s		
1070m		1070w	1072m		
1055w		1050w	1056w		
1030m	1034m	1036wbr	1013m	1014m	
	1010wsh	1018wbr			
999m	989m	997m	990m	980m	C–H rock (pyrrole)
978m	980m	977msh	980m	978m	C–H rock (pyrrole)
963s	946m	969s	963m	965m	C–H rock (pyrrole)
			941m		C–Cl
		908m			
873m	875vw	883w	872w	878w	
847m			851m	862m	
	837m	838w	837m	844m	
825w		825w			
809m	818wsh	812m	818w	812m	
	803s	799s	800ssh	795s	*p*-disubst. C_6H_5 C–H out of plane def.

TABLE II (*Continued*)

p-Substituent (frequencies in cm.$^{-1}$)					
-H	-OCH$_3$	-CH$_3$	-Cl	-NO$_2$	Assignment
795s		793s	790s		
783m	782m	786s	784ssh	785msh	
		772s			
757s					Monosubst. C$_6$H$_5$
744s				740msh	
727s	734m	723s	725s	725mbr	
720s					Monosubst. C$_6$H$_5$
702s	706wsh	706s	702m	707m	
694s		697m	699m	698m	
654m		655w	665wbr		
637w		633w	635w		

[a] s, strong; m, medium; w, weak; vw, very weak; sh, shoulder; br, broad; o, overlap.

The strong absorption at 1518 cm.$^{-1}$ in the nitroporphine derivative is assigned to the antisymmetric N–O stretching vibration, while the band at 1345 cm.$^{-1}$ is attributed to the symmetric N–O stretching vibration. In *p*-nitrobenzaldehyde these absorptions occur at 1535 and 1345 cm.$^{-1}$, respectively. The shift to a lower frequency in the porphine of the first band is undoubtedly due to greater conjugation in the porphine molecule. Numerous weak and medium phenyl and substituted phenyl vibrations occur in the 1225–950 cm.$^{-1}$ region, in accordance with observations on other compounds by Bellamy,[13b] Colthup[15] and others. A few of these bands have been assigned in Table II. Characteristic *para*-substitution vibrations which usually occur from 1125–1090 cm.$^{-1}$ have been assigned to the bands observed in the frequency range 1100–1105 cm.$^{-1}$. Sharp, strong out-of-plane C–H deformations characteristic of monosubstituted benzene occur within 730–770 cm.$^{-1}$ and also near 700 cm.$^{-1}$. The strong bands of tetraphenylporphine at 757 and 720 cm.$^{-1}$ are assigned to monosubstituted benzene. *para*-Disubstitution is characterized by sharp vibrations in the 800–860 cm.$^{-1}$ range. The sharp bands occurring near 800 cm.$^{-1}$ are assigned to this mode of vibration.

In pyrrole, three sharp bands of increasing intensity occur at 1076, 1046 and 1015 cm.$^{-1}$. The three bands at approximately 965, 980 and 990 cm.$^{-1}$ are common to all porphyrins and are believed to be due to the same mode of vibrations even though they are closer together and at a lower frequency. The influence of the large resonating ring may account for the lower frequency observed in this research. Randall, Fowler, Fuson and Dangl[14a] have assigned the three bands in pyrrole to C–H rocking vibrations. The stretching absorption of the C–Cl bond reportedly occurs at 600–800 cm.$^{-1}$, but interaction with an aromatic ring raises the frequency to 845 cm.$^{-1}$.[13c] If the C–Cl band occurs at the low frequency range, it is most likely obscured by the overlapping of stronger phenyl and other absorptions in the low frequency region. If not, the frequency of 941 cm.$^{-1}$ may possibly be assigned to the C–Cl stretching vibration.

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(15) N. B. Colthup, *J. Opt. Soc. Amer.*, **40**, 397 (1950).

[CONTRIBUTION FROM THE CHEMICAL LABORATORY, SANTA BARBARA COTTAGE HOSPITAL RESEARCH INSTITUTE]

Reaction Products of Insulin in Urea Solution[1]

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On the basis of differences in solubility and bioassay by the hypoglycemic response, five reaction products were isolated when amorphous or crystalline insulin was permitted to react in concentrated aqueous urea solution under various conditions of temperature and time. Insulin OU is formed when the insulin is precipitated immediately, from the urea solution, by the addition of a large volume of water. It is insoluble at *p*H 7.0 and its suspension has a delayed biologic response while its acid solution has a normal response. Insulin IU is formed when the reaction in urea solution is prolonged. It is soluble at *p*H 6.5 and has the same biologic response as regular insulin. Insulin IIU, the next reaction product, is insoluble in 0.03 *N* sulfate at *p*H 3, is soluble at the *p*H of the body fluids and has a delayed biologic response even when given intravenously. Its formation follows first-order kinetics. The energy of activation is about 33,000 cal. per mole, the free energy of activation 26,000 cal. per mole at 38° and the entropy change of activation 18–27 cal. per degree per mole. Insulin IIIU, formed on further prolonging the time in urea solution, differs from insulin IIU in that it is insoluble at *p*H 2.0, dilute HCl, and has half the biologic activity of insulin IIU. It also has a delayed response. Insulin IVU, the next reaction product, is devoid of biologic activity. When heated at 99°, *p*H 2.0, insulin IIU forms both the classic heat precipitate and a precipitate soluble at *p*H 8.4. The heat precipitate of insulin dissolves in concentrated urea solution at 99°, thereby restoring its biologic activity.

The delayed resorption of insulin[2] and certain of the gonadotropins[3] from a subcutaneous depot produces an augmentation effect, and simulates more nearly the physiologic state. Until recently, delayed acting insulins used clinically have depended upon the injection of an insoluble insulin combination or the injection of a solution forming an insoluble insulin complex at the injection site. By intra-

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(2) L. C. Maxwell and F. Bischoff, *Am. J. Physiol.*, **112**, 172 (1935).

(3) F. Bischoff, *ibid.*, **121**, 765 (1938); U. S. Patent 2,121,900 (June 28, 1938).