TABLE I

MICROBIOLOGICAL ACTIVITY OF PYRIDOXYLAMINES

Product,[a] hydrochloride of pyridoxyl-	*Neurospora sitophila* 299 Activity[b] Auto-claved[c]	Fil-tered[d]	*Sac-charo-myces carls-bergen-sis*[e]	*Strepto-coccus faecalis*[e]	*Lacto-bacillus casei*[e]
Benzylamine	0.65		0.0008	0.003	<0.0002
Ethylamine	.91	0.80	.018	.0001	.0002
Methylamine		.70			
Ethanolamine	.44	.001	.0008	.001	.001
Isopropanolamine	.030		.001	.001	.0006
Histamine	.058		.0035	.006	.0008
β-Phenylethylamine	.37		< .0005	.002	
3-Phenylpropyl-amine	.24	.062			
3,4-Dihydroxy-β-phenylethylamine	.005				
Arterenol	.050				
Tryptamine	.034		.0009	< .0009	< .0002
Tyramine	.082		.0007	.001	

[a] We are indebted to Dr. Karl Folkers for samples of these products. [b] The growth-promoting activity is compared on a molar basis with that of pyridoxal hydrochloride, assigned an activity of 1.0. [c] Ten minutes at 15 lb. pressure. [d] Sterilized by filtration and added aseptically to the sterile medium. [e] Compounds dissolved in sterile water and added aseptically to the sterile medium.

doxylethylamine being the only one to show activity greater than 1.8% that of an equimolar amount of pyridoxal. The pyridoxylamino acids were similarly inactive for these organisms.[5] For *Neurospora sitophila*, many of these compounds are of low activity, but others show activity approaching that of pyridoxal. In some cases, but not all, this activity appears due to breakdown during autoclaving with the medium.[5] The pyridoxylamino acids also showed higher activity for *Neurospora* than for other vitamin B_6-requiring organisms.[5]

The ease with which decomposition (*via* dehydrogenation and hydrolysis) of such compounds to pyridoxal or pyridoxamine can occur has been discussed,[5] and the high activity of certain of them for *Neurospora* (and rats[3]) suggests that this decomposition may occur enzymatically, and thus explain their activity in replacing vitamin B_6. The most active compounds are of the general type oxidized by monoamine oxidase, which occurs widely distributed in mammalian tissues[6] and probably in molds.[7] The view that the pyridoxylamines act *via* pyridoxal (or pyridoxamine) is also consistent with the fact that pyridoxylethylamine was less than $1/20$ as active as pyridoxamine, and less than $1/500$ as active as pyridoxal or pyridoxine in counteracting the inhibitory effects of 4-desoxypyridoxine for *Neurospora sitophila* (Table II). The inactivity of the pyridoxylamines for most of the organisms tested indicates that they have no general utility as sources of vitamin B_6 in living systems, and there is no evidence as yet to indicate their natural occurrence.

TABLE II

INHIBITION OF GROWTH OF *Neurospora sitophila* 299 BY 4-DESOXYPYRIDOXINE AND ITS COUNTERACTION BY VARIOUS COMPOUNDS WITH VITAMIN B_6 ACTIVITY

Reversing agent	Level of reversing agent 1γ	5γ	10γ
	Inhibition index[a]		
Pyridoxine·HCl	630		1000
Pyridoxal·HCl	500		1000
Pyridoxamine·2HCl	17	19	23
Pyridoxylethylamine·HCl	0.73	0.73	1.1

[a] The inhibition index is the molar ratio of 4-desoxypyridoxine to reversing agent at which growth of the organism is 50% of that obtained in the absence of the inhibitor.

(5) E. E. Snell and J. C. Rabinowitz, THIS JOURNAL, **70**, 3432 (1948).

(6) H. A. Lardy, "Respiratory Enzymes," Burgess Publ. Co., 1949, p. 237.

(7) J. W. Foster, "Chemical Activities of Fungi," Academic Press, Inc., New York, N. Y., 1949, p. 516.

Experimental

Test organisms, procedures and basal media employed were those described[5] in a similar study of the pyridoxylamino acids.

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Concerning the Absorption Spectrum of Bacteriochlorophyll

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In the course of a survey of the vibrational spectra of chlorophyll and related compounds,[1] the author had occasion several times to measure the electronic spectra of bacteriochlorophyll and bacteriopheophytin. This work has revealed that the main near-ultraviolet peak of both compounds has a prominent violet shoulder, that the "orange" peak of bacteriochlorophyll is strongly shifted by polar solvents, and that the spectrum of the pheophytin is surprisingly independent of *p*H.

Bacteriochlorophyll was prepared from *Rhodospirillum rubrum* by the method of French,[2] slightly modified. Because of the reported photo-lability of this compound, it was not purified chromatographically. Bacteriopheophytin was produced by treating the chlorophyll with an excess of 4×10^{-2} *N* sulfuric acid in ether for about one hour. The solution was neutralized with an equivalent amount of ammonia, or with excess basic magnesium carbonate, then extracted with water. Solvent transfers were carried out by evaporating the solution to near dryness under a stream of inert gas, and then rediluting with the desired solvent. The pigments were kept cold and in dim light during extraction and all subsequent experiments. All spectra were run within 24–48 hours of initial extraction in a Beckman quartz spectrophotometer which had been calibrated against Hg, H, Na, K and Cs emission lines. Pigment concentrations were adjusted to about 10^{-5} *M*, to permit the use of 10-mm. silica cells.

In one set of experiments, a measured volume of bacteriochlorophyll solution was converted to the pheophytin, and the latter was dissolved in a known volume of chloroform. The optical density of this solution was compared to the absolute extinction coefficients determined by French[2] for a sample of bacteriopheophytin prepared by van Niel. This permitted calculation of absolute extinction coefficients for both pigments in several

(1) R. Livingston and J. W. Weigl, to be published.

(2) C. S. French, *J. Gen. Physiol.*, **23**, 483 (1940).

TABLE I

ABSORPTION PEAKS

Solvent	Peak 1 λ, mμ	Peak 1 $\alpha_{rel.}$	Peak 2 λ, mμ	Peak 2 $\alpha_{rel.}$	Peak 3 λ, mμ	Peak 3 $\alpha_{rel.}$	Peak 4 λ, mμ	Peak 4 $\alpha_{rel.}$	Violet shoulder λ, mμ	Violet shoulder $\alpha_{rel.}$	Violet peak λ, mμ	Violet peak $\alpha_{rel.}$
Bacteriochlorophyll												
In vivo[b]	881 ± 1	1.00	805 ± 2	0.13[d]	762 ± 5	0.06	588 ± 2	0.18	(Obscured by carotenoids?)		378 ± 1	0.81
Ether	772 ± 0.5	1.00[a]	697 ± 2	.12[d]	575 ± 2	.23	526 ± 2	.13[c]	391 ± 2	0.55	358 ± 0.5	.89
Benzene	782 ± 0.5	1.00	705 ± 5	.12[d]	581 ± 1	.25	543 ± 2	.17[c]	396 ± 0.5	0.55	362.5 ± 1	.85
Acetone	771 ± 2	1.00	698 ± 5	.14[d]	580 ± 2	.31	528 ± 5	.15[c]	Not resolved		358 ± 1	1.19
Methanol	771 ± 1	1.00	695 ± 5	.27[d]	609 ± 1	.32	530 ± 3	.16[c]	Not resolved		364 ± 1	1.26
Bacteriopheophytin												
Ether[f]	750 ± 0.5	1.00[a]	680 ± 2	0.15	620 ± 5	0.05	525 ± 1	0.43[e]	384.5 ± 1	0.97	357 ± 0.5	1.72
Chloroform[g]	758 ± 1	1.00[a]	686 ± 3	.20	625 ± 5	0.07	531.5 ± 1	.43[e]	389 ± 1	0.89	362 ± .5	1.66
Neutral MeOH	752 ± 2	1.00	680 ± 5	.28	*c* 625	*c* 0.12	528 ± 1	.56[e]	Not resolved		358 ± .5	2.26
Basic MeOH	757 ± 2	1.00	683 ± 5	.22	*c* 625	*c* 0.11	526 ± 1	.46[e]	Not resolved		358 ± .5	2.11

[a] Absolute extinction coefficients (log 10, in liters/mole cm.); bacteriochlorophyll in ether 9.6×10^4; bacteriopheophytin in ether 6.3×10^4, in chloroform 5.8×10^4. [b] Suspension of *R. rubrum* in 90% glycerol; approximately corrected for residual scattering. [c] May be due largely to carotenoid impurities. [d] Shoulder, resolved approximately by subtraction. [e] Probably includes a little absorption due to carotenoids. [f] Acidic, neutral or basic ether. [g] Chloroform containing 0.5% ethanol as preservative.

solutions which had been prepared by quantitative volumetric transfers.

Table I summarizes the absorption bands of bacteriochlorophyll and bacteriopheophytin between 330 and 1000 mμ, in several solvents. The infrared peak positions of the chlorophyll *in vivo* and in solution are in agreement with those reported by Katz and Wassink[3] while the spectrum of bacteriopheophytin in chloroform matches that of French[2] very closely. The spectrum found for bacteriochlorophyll in methanol differs from that reported by Manten[4] in revealing an unresolved but definite shoulder to the main near-ultraviolet peak at about 390 mμ, and two minor bands at about 525 and 500 mμ. While the former is certainly significant, the latter may be due to traces of carotenoids.

The near-ultraviolet double band of these pigments resembles that of chlorophyll and pheophytin *a*, with the difference that the relative intensities of the peaks are reversed.[5] The two peaks are clearly resolved in solvents of low dielectric constant, such as ether, benzene and chloroform.

In all solvents tried, as well as *in vivo*, the spacings (on an energy scale) of bands 1, 2 and 4 of both pigments were found so nearly constant that the corresponding energy levels appear to be closely related. (Band 2 very probably represents a vibrational excited state of level 1.) All three of these peaks shift slightly with changes in the polarizability of the solvent, in the manner shown by Katz and Wassink[3] for peak 1. In bacteriochlorophyll, band 3 (the "orange" band) is, however, drastically shifted toward the red by polar solvents, such as ethanol and methanol; for this reason it appears to pertain to a different electronic transition.

By contrast, the spectrum of bacteriopheophytin is quite constant in a variety of solvents and over a wide *p*H range (4×10^{-2} *N* H_2SO_4 to 0.3 *N* NH_3 in ether; neutral to 1.5 *N* NH_3 in methanol[6]). In this respect it differs sharply from pheophytin-*a*, which is a *p*H indicator, having reversible acid, neutral and basic forms.[7,8]

The band pattern of bacteriochlorophyll and bacteriopheophytin closely resembles that of chlorophyll and pheophytin-*a*. Our evidence does not support Rabinowitch's suggestion[9] that reduction of dihydroporphin to tetrahydroporphin derivatives produces a new, low-energy electronic transition; rather, there appears to be a general spreading of energy levels.

The observations are more nearly in agreement with the theoretical predictions of Platt and coworkers,[10,11] according to which bands 1 and 3 and the two violet peaks correspond to four electronic levels, differing in angular momentum and polarization, and bands 2 and 4 are vibrational satellites of bands 1 and 3. However, the theory so far developed predicts an intensity ratio of the two violet peaks which is the reverse of that observed; it also does not account for the relatively great intensity of band 4 in bacteriopheophytin or for the selective effect of polar solvents on the position of the "orange" band of bacteriochlorophyll.

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(3) E. Katz and E. C. Wassink, *Enzymologia*, **7**, 97 (1939).

(4) A. Manten, Thesis, University of Utrecht, 1948.

(5) A similar reversal of the corresponding peaks of bacteriomethyl-pheophorbide was noted by A. Stern and F. Pruckner, *Z. physik. Chem.*, **A185**, 140 (1939).

(6) The pheophytin was found to decompose in 0.4 *N* acidic acetone and methanol.

(7) R. Pariser, Ph.D. Thesis, University of Minnesota, 1950.

(8) Compare E. I. Rabinowitch, "Photosynthesis," Vol. II, Interscience Publishers, Inc., New York, N. Y., 1951, p. 624.

(9) Reference 8, pp. 619–624, 630–633; *Rev. Mod. Phys.*, **16**, 226 (1944).

(10) H. C. Longuet-Higgins, C. W. Rector and J. H. Platt, *J. Chem. Phys.*, **18**, 1174 (1950).

(11) J. H. Platt in "Radiation Biology, Vol. III. Biological Effects of Visible Radiation," McGraw-Hill Book Co., Inc., New York, N. Y., in press.