suddenly at 83–85° yielding an orange smoke and a maroon-colored residue. Chemical analysis indicated that neither of these were pure substances.

The compound is practically insoluble in bromoform, symmetrical tetrachloroethane, hexane, heptane and carbon tetrachloride. It is slightly soluble in diethyl ether, benzene, carbon disulfide and liquid sulfur dioxide, and appreciably soluble in the following solvents: anhydrous dioxane, anhydrous acetone, acetophenone, vinyl trichloride and glacial acetic acid. If small amounts of water are added, decomposition takes place. However, the solutions are reasonably stable if kept in tightly stoppered containers and stored in a dark place. Attempts to obtain accurate solubility data have not been successful, but are being continued.

Selenium dithiocyanate is best recrystallized by carefully adding anhydrous ether to a saturated solution of selenium dithiocyanate in anhydrous dioxane until a faint turbidity persists and then keeping the mixture at 0° for eight hours.

The density of crystalline selenium dithiocyanate was determined by preparing a mixture of bromoform and symmetrical tetrachloroethane having the same density as the crystals. The density found in this manner was 2.265 ± 0.005.

The molecular weight of the compound was determined by cryoscopic method using acetophenone in one case, and dioxane in another, as the solvents. The molecular weight found in acetophenone was 206, and in dioxane 204. Although the experimentally determined values are all higher than the calculated value (195) based on the simple formula, the existence of selenium dithiocyanate in the monomeric form is definitely established.

Selenium dithiocyanate decomposes more or less rapidly at room temperature in the following liquids: water, dilute hydrochloric acid solution, dilute sodium hydroxide solution, methyl-, ethyl-, propyl-, *t*-butyl alcohol and pyridine. It also decomposes violently in liquid ammonia.

While it was not found possible accurately to represent the reaction between selenious acid and thiocyanic acid by a single equation, the following appears to be the main reaction

$$3H_2SeO_3 + 7HSCN = 3Se(SCN)_2 + 5H_2O + HCN + H_2SO_4$$

To ascertain whether this equation correctly represents the main reaction, the preparation of selenium dithiocyanate from selenious acid and thiocyanic acid, as described above, was carried out in a closed vessel attached to a suction flask through a tube provided with a sintered glass disc. The suction flask contained sodium hydroxide in excess of that required to neutralize all of the acid.

When the reaction was completed the liquid part of the contents of the reaction vessel were passed through the sintered disc into the sodium hydroxide solution, while the selenium dithiocyanate was retained on the disc. Air was aspirated through the apparatus to complete the transfer of hydrogen cyanide to the caustic soda solution, which contained, in addition to the hydrogen cyanide also the sulfuric acid formed in the reaction. The tube with the sintered disc was then disconnected from the reaction vessel and washed with small portions of nitric acid. Selenium and sulfur were determined in the nitric acid solution as described above.

The potassium hydroxide solution to which the liquid part of the reaction mixture had been added was analyzed for cyanide and sulfate. Cyanide was determined by the Liebig[12] method. An aliquot was acidified slightly with nitric acid, the thiocyanate precipitated with silver nitrate, and the filtrate was used for the sulfate determination. The results are given in Table I.

TABLE I

FORMATION OF SELENIUM DITHIOCYANATE, HYDROGEN CYANIDE AND SULFURIC ACID FROM SELENIOUS AND THIOCYANIC ACID.

Millimoles formed	Millimoles HCN Theory	Millimoles HCN Found	Millimoles H_2SO_4 Theory	Millimoles H_2SO_4 Found
6.83	2.28	1.61	2.28	2.12
15.37	5.12	3.84	5.12	4.93
16.41	5.47	3.93	5.47	5.15

(12) W. W. Scott, "Standard Methods of Chemical Analysis," D. Van Nostrand Co., Inc., New York, N. Y., 1939, p. 661.

It is apparent, that while the equation as written does not describe quantitatively the over-all reaction involved in the formation of selenium dithiocyanate, the results indicate that this equation is in agreement with the main reaction.

The fact that less than the theoretical amounts of hydrogen cyanide and sulfuric acid were found is in agreement with the work of other investigators[13] who studied the hydrolysis of thiocyanogen.

Reaction of Selenium Dithiocyanate with Water.—The decomposition of selenium dithiocyanate by water yields, as the major products, elemental selenium, selenious acid and thiocyanate ion. As a result of the interaction of selenious acid with thiocyanic acid there is also formed a considerable amount of sulfate ion. This result is in agreement with an observation of Hall.[3]

Synthetic Applications.—Selenium dithiocyanate can be used as a reagent for introducing the thiocyanate group into organic compounds. For example, the treatment of dimethylaniline with selenium dithiocyanate in glacial acetic acid yields *p*-thiocyanodimethylaniline, with a yield of 55%.

In the same manner, the reaction between 1-naphthol and selenium dithiocyanate in glacial acetic acid results in the formation of 4-thiocyano-1-naphthol, with a yield of 60%.

The compound will also react with an olefinic type of double bond. Attempts to thiocyanate cyclohexene yielded a crystalline addition product whose empirical formula corresponded to one molecule of selenium dithiocyanate and two molecules of cyclohexene.

Finally, the intermediate oxidation state of the selenium suggests that selenium dithiocyanate might find further application as either an oxidizing agent or a reducing agent for synthetic purposes.

(13) H. Lecher, M. Wittmer and W. Speer, *Ber.*, **56**, 1104 (1923).

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The Microbiological Activity of Pyridoxylamines

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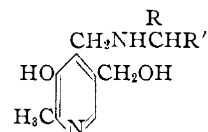
Several pyridoxylamines (I), recently described by Heyl, *et al.*,[3] were reported to possess vitamin B_6 activity for rats 50 to 100% that of pyridoxine, although the corresponding pyridoxylamino acids[4] (II) possessed relatively low activity. Because of



I, R = H, R′ = H or organic radical
II, R = -COOH, R′ as in I

this high activity, and because comparable structural modifications of other vitamins with similar maintenance of activity has not proved possible, they suggested that "compounds of this type may occur in living systems as members of the vitamin B_6 group or as intermediates in their function."

In view of these suggestions, it appears worthwhile to present results of assays of these compounds with other organisms that require vitamin B_6 (Table I). For *Saccharomyces carlsbergensis*, *Streptococcus faecalis* and *Lactobacillus casei*, most of the compounds have negligible activity, pyri-

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(3) D. Heyl, E. Luz, S. A. Harris and K. Folkers, THIS JOURNAL, **74**, 414 (1952).

(4) D. Heyl, S. A. Harris and K. Folkers, *ibid.*, **70**, 3429 (1948).

TABLE I

MICROBIOLOGICAL ACTIVITY OF PYRIDOXYLAMINES

Product,[a] hydrochloride of pyridoxyl-	*Neurospora sitophila* 299 Auto-claved[c]	*Neurospora sitophila* 299 Fil-tered[d]	Activity[b] for *Saccharomyces carlsbergensis*[e]	*Streptococcus faecalis*[e]	*Lactobacillus 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).