0.5 mg	MnO ₂	produced	0.65 mg	purpurogallin
5.0 mg	„	„	1.15 mg	„
50.0 mg	„	„	4.65 mg	„

MnO₂ produces purpurogallin from pyrogalllic acid without the co-operation of H₂O₂ but only in very small amounts. For example, 50 mg MnO₂ produced in 10 minutes 4.6 mg purpurogallin in the presence of H₂O₂, and only 0.6 mg without the aid of H₂O₂. The addition of H₂O₂ alone to pyrogalllic acid solution gave rise to no appreciable amount of purpurogallin within the same duration of time. As well known a certain small amount of purpurogallin is produced when pyrogalllic acid solution is left exposed to the air. Apparently MnO₂ accelerates to some extent this simple oxidative process, in addition to possessing the marked peroxidase action, which is revealed in the presence of H₂O₂.

We made no attempt to work out the mode of the peroxidase action of MnO₂. It may be mentioned in passing, without giving experimental details, that unlike the action of the true peroxidase, the peroxidase activity of MnO₂ is not influenced by hydrogen ion concentration within the range of pH 1.8–9.4. On the other hand certain known inhibitors of the true peroxidase inhibit the action of MnO₂ also. These inhibitors are KCN, HgCl₂, NH₂(OH)HCl, and phenylglyoxime. KClO fails to produce any inhibition.

SUMMARY

MnO₂ was placed on record as an “Inorganic peroxidase” per excellence, and some aspects of its activity were discussed. MnO₂ has long been known to possess a strong “catalase activity”, so that one and the same substance has now been demonstrated to exhibit the actions of two different enzymes. PbO₂, another example of “inorganic catalase”, is devoid of peroxidase action.

The author wishes to express her appreciation to Dr. Waro Nakahara, under whose guidance the present experiments were carried out.

ON THE CHEMISTRY OF VITAMIN B₆*

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Within the last few years we have seen a revival of interest in the study of vitamin B₆ from biological rather than chemical standpoint; much work is now being done on the metabolite of this vitamin and its relation to the metabolism of protein and to enzyme systems. Of particular interest are the recent works of Snell et al. and Gunsalus, Bellamy and Umbereit (1).

*Dedicated to the memory of the late Prof. U. Suzuki

With respect to the chemical nature of this vitamin, however, there remain no details yet to be cleared up. It may not be without significance, therefore, to look upon the progress of investigations made during the last few years.

It is especially desired to stress the remarkable progress in this field during the years from 1938 to 1940, during which period, the isolation, the structural elucidation, and the synthesis of this vitamin were accomplished in quick succession. Our present knowledge of the chemical nature of this vitamin we owe mainly to Kuhn et al. (2) and American workers (3), especially Harris and Folkers. The contributions of major significance by them must be praised. We also made independently attempts to gain proper understanding of the chemical aspect and made some valuable contributions along this line.

It is not our purpose here to give a full description of all the worthy works that have appeared and to assess the relative merits of so many contributions. In this short review we consider it advisable to mention our results so far obtained in their relation to the work of others.

Almost simultaneously and independently crystalline vitamin B₆ was obtained in a number of laboratories (2, 3, 5, 6) in the year 1938 and, moreover, there was a good agreement concerning the chemical composition and biological assay of the product isolated. Rats were cured of dermatitis and resumed growth when fed 5γ to 10γ of the hydrochloride per day. As far back as 1931 Ohdake, (7) working with U. Suzuki, had isolated a crude preparation of this substance from which pure vitamin B₆ was readily obtained. Ohdake was aheads of his time since the technique for the biological recognition of vitamin B₆ had not yet been developed.

Our method of isolation (5) deviated somewhat from those applied by others inasmuch as an adsorption on charcoal and elution with alcohol and then with ammoniacal aqueous acetone was introduced prior to the final precipitation with mercuric chloride or phosphotungstic acid.

The accepted formula for the hydrochloride was C₈H₁₀O₃N.HCl mp. 209–210° C (dec.).

The free base mp. 154–5° C was optically inactive, contained neither -OCH₃ nor :NCH₃ residues and gave a deep red color with aqueous ferric chloride, indicating the presence of a phenolic hydroxyl group. And our crystalline hydrochloride showed defined absorption bands at 327, 293 and 246 m in 10⁻³ mol aqueous solution (5b).

To secure some knowledge on the function of oxygen atoms in molecule, we prepared triacetyl, tribenzoyl, and monomethyl derivatives; C₈H₈NO₃(COCH₃)₃ viscous oil, C₈H₈NO₃(COC₆H₅)₃ mp. 121–122° C and C₈H₁₀NO₂(OCH₃) mp. 103–105° C respectively (5c).

Special attention was called to the fact that by the methylation with diazomethan we obtained, in addition to monomethyl ether a reaction product which was N-methyl derivative, isomeric to the C-methyl derivative (5d).

On the basis of this fact and on other observations on the zinc dust distillation, we were inclined to make a hasty conclusion, without full consideration of phenol betain as described by R. R. Williams (8), that the enolic hydroxyl in the vitamin molecule might be of 2- or 4-position of pyridine.

Just as we made these observations we received the valuable paper by R. Kuhn and G. Wendt, dealing with the functional groups in the vitamin molecule (2c). They skillfully demonstrated that all three oxygen atoms in the molecule exist in the form of hydroxyl and the nitrogen atom is evidently "ringtertiar" on the basis of the formation of the monomethyl and diacetylmonomethyl derivatives and of no evolution of methane even at higher temperature at the determination of active hydrogen in the latter. Furthermore the fact that the base was of tertiary cyclic nature and gave a deep blue color with Folin-Denis reagent, similar to that produced by β -hydroxypyridine, led them to a comparison of the vitamin with β -hydroxypyridine and its derivatives. Shortly afterwards Kerestztesy and his co-workers published the same observations.

In fact, with the isolation of vitamin B₆ there followed almost immediately communications on the chemical structure of this vitamin. Kuhn et al., in a series of publications (2d,e,f) showed that the formula for vitamin B₆ is

Independent confirmation for this structure was quickly forthcoming both from us and from the American workers. Our attempt to clear up this subject had been made by a careful oxidative degradation (5d,e); Two products, one neutral, mp. 105-107° of presumable formula C₈H₉NO₃, and the other, dicarboxylic, mp. 217-219°C (dec.) of C₈H₉NO₃, we obtained so far in the oxidation of monomethyl ether of vitamin with potassium permanganate in alkaline medium.

Kuhn and his co-workers obtained by oxidation substances having formula, C₈H₉NO₃, C₈H₉NO₃ and C₈H₉NO₃, first two of which we had already obtained. Furthermore, they obtained, by the anhydride formation of the latter two, C₈H₉NO₄ and C₈H₉NO₄ respectively, and established the constitution of these anhydrides to be 2-methyl-3-methoxypyridine dicarboxylic (4, 5) acid anhydride and 3-methoxypyridine dicarboxylic (4, 5) acid anhydride. Thus the chemical constitution of the vitamin was elucidated by them to be 3-hydroxy-4, 5-bis(hydroxymethyl)-2-methylpyridine above cited. (2f)

One of the grounds, upon which their work was based, was the synthetical elucidation that the dicarboxylic substance C₈H₉NO₃ was 2-methyl-3-methoxypyridine dicarboxylic acid (4, 5) but so far they gave no detailed information on its synthesis.

For the purpose of the identification of the compound C₈H₉NO₃, we made attempts at the synthesis of 2-methyl-3-methoxypyridine dicarboxylic acid (4, 5). (5e)

The method we used was first to prepare 2-methyl-3-methoxy-1-chlorisoquinoline according to Gabriel and Colman, then to reduce it to 2-methyl-3-methoxyisoquinoline with tin and hydrochloric acid, and finally to obtain the desired substance by oxidation with potassium permanganate. (5e)

The mixed melting point of this compound with the substance C₈H₉NO₃ derived from

As the most promising route for the vitamin synthesis, 2-methyl pyridine substituted with suitable precursor of hydroxymethyl group in the position 4 and 5 seemed to be available. In this connection, the feasible methods fall into two groups; one, 2-methyl-3-methoxy-pyridine dicarboxylic acid by way of isoquinoline or quinoline derivatives and the other, suitably substituted 2-methyl pyridine through condensing α - γ -diketon with cyanoacetic ester.

Our first attempt to synthesize vitamin B₆ was to obtain it reductively from 2-methyl-3-methoxy pyridine dicarboxylic acid (4, 5), the oxidative degradation product of B₆-methylether (above cited). This had been successfully accomplished by R. Kuhn and his co-workers, when our work was still in progress and there were several details yet to be cleared up. (2i, 5g)

In the course of our study in this line, we met with a difficulty in the preparation of the corresponding acid amide of 2-methyl-3-methoxy cinchomeronic acid by the usual established method, which involved esterification with alcoholic hydrochloric acid and subsequent conversion to acid amide with alcoholic ammonia.

We therefore fell back on two other methods for the same purpose. One of these methods consisted of the preparation of the desired di-ester with diazomethan from 2-methyl-3-methoxy pyridine dicarboxylic acid (4, 5). The other process involved first the

production of acid anhydride of dicarboxylic acid which was transformed to imide and then to acid amide. Both of these methods yielded identical amide mp. 209–210° C. with decomposition.

Formerly Bardham (9) and also Simonsen and Nyak (10) described the synthesis of 3-cyano-4, 6-dimethyl-2-pyridone by condensation of acetylacetone and cyanoacetic amide.

The chemistry of 2-pyridone has long been a subject of great interest in the preparation of pharmaceuticals and it has gained still greater prominence since the advent of vitamin B₆. In the synthesis of 2-pyridone, the pyridine ring system is frequently synthesized from suitable aliphatic compounds.

Harris and Folkers adopted this principle and accomplished their meritorious synthesis of the vitamin (4C). Their procedure consisted in the reaction of cyanoacetamide and ethoxyacetylacetone leading to the formation of 3-cyano-4-ethoxymethyl-6-methyl-2-pyridone.

Theoretically the condensation can also occur in an alternative way illustrated by the equation.

The mechanism of the reaction has been studied by Bardham with cyanoacetamide and several 1, 3 diketones. He produced evidence that formation of the pyridine derivatives proceeds through unsaturated intermediates as illustrated by the scheme.

He postulated that the relative reactivity of the respective carbonyl group will determine the proportion of I and II in the condensation product. The predominant isomer will have I, if the carbonyl group adjacent to group X is more reactive and formula II if the more reactive carbonyl group is adjacent to Y.

In the reaction of ethoxyacetone with cyanoacetamide both possible compounds I' and II are formed. If instead of ethoxyacetylacetone the acetopyruvic acid amide $\text{CH}_3\text{COCH}_2\text{COCONH}_2$ is used, the isomer I'' is predominant.

The process of Harris and Folkers was employed which involved condensing ethoxyacetylacetone with cyanoacetamide in the presence of piperidine in 95 % ethylalcohol to form 3-cyano-4-ethoxymethyl-6-methyl-2-pyridone, treating the latter compound with nitric acid to form 3-cyano-4-ethoxymethyl-5-nitro-6-methyl-2-pyridone, chlorinating the latter to 2-chlopyridine, reducing the latter to 5-amino-3, 4-bisaminomethyl compound, diazotizing the latter to the vitamin.

In view of our first attempt being through the vicinal dicyano compound as an intermediate and, moreover, the latter being easily synthesized as compared with the corresponding alkoxyethyl cyano compound which was used by Harris and Folkers, our second method of synthesis (5h) consisted of a series of the processes described below:—

We employed the process which involved condensing acetopyruvic amide with cyanoacetamide in the presence of diethylamine to form the amide of 3-cyano-4-carboxy-6-methyl-2-pyridone, which was dehydrated to 3, 4-bis (dicyano)-6-methyl-2-pyridine. This product was nitrated to form 5-nitrocompound which, by halogenation with phosphorpentachloride and phosphoroxychloride, was converted to 2-chlor-3, 4-bis (cyano)-5-nitro-6-methylpyridine. The latter compound was partially reduced with titantrichloride to give 2-methyl-3-amino-4, 5, bis cyano pyridine and by the subsequent reduction with palladium bariumcarbonate, dehalogenated to form 4, 5 bis cyano 3-amino-2-methylpyridine. This compound was subjected to a more complete reduction to give 2-methyl-3-amino-4, 5 bis (aminomethyl)-pyridine, which, on diazotizing, gave vitamin B hydrochloride.

In the recent publication on the vitamin synthesis almost similar procedures to ours was reported by J. H. Mowat, F. J. Pilgrim and G. H. Carlson (11). It is especially desired, however, to stress the more profitable procedures adopted by us in the dehydration of acid amide to nitrile ($-\text{CONH}_2 \rightarrow \text{CN}$) and the reduction of nitro group to amino group ($-\text{NO}_2 \rightarrow \text{NH}_2$) in the course of synthesis.

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STUDIES ON DENGUE FEVER I. STUDIES ON DENGUE VIRUS AND IMMUNITY. (2)

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(This report is a summary of our studies during the period of 1942-1944)

E. IMMUNOLOGIC TESTS

1. Neutralization test

The neutralization tests of the convalescent sera of patients, infected with mouse virus, against mouse virus or human dengue virus, and the neutralization tests of the rabbit sera, immunized with mouse virus, against mouse virus, were carried out.

a. Patient serum, infected with mouse virus, against mouse virus

The convalescent sera of three persons infected with mouse virus of about twenty serial passages, and of four persons infected with rabbit eye fluid and mouse brain of the primary infection tests, were used for the neutralization tests against mouse virus.

Equal doses of the convalescent serum and the emulsion of infected mouse brain were mixed and kept at room temperature for thirty minutes. Thirty cubic millimeter of this mixture were injected intracerebrally into mice. As shown in table 5, the neutralizing substance was produced in human body by the animal virus, and both K-strain and M-strain were demonstrated to exhibit the same behavior.

Table 5

Serum Virus	Convalescent serum caused by mouse virus			Convalescent serum caused by mouse and rabbit virus				Control
	INOUE (K-st)	SHIMA BUKURO (K-st)	SHIRAIISHI (M-st)	YAJIMA (K-st)	MITOMA (K-st)	ISHII (M-st)	ICHO (M-st)	
K-st.	++		++	++	++	++	++	—
M-st.		++		++		++	++	—

+.....neutralized

—.....not neutralized

K-strain.....virus passed through rabbits

M-strain.....virus passed through mice