

CHAPTER XVII

VITAMINS

§1. Introduction. In addition to oxygen, water, proteins, fats, carbohydrates and certain inorganic salts, a number of organic compounds are also necessary for the life, growth and health of animals (including man). These compounds are known as the "accessory dietary factors" or *vitamins*, and are only necessary in very small amounts. Vitamins cannot be produced by the body and hence must be supplied. Vitamin D, however, may be supplied in food or may be produced in the skin by irradiation (ultraviolet) of sterols.

Many vitamins have now been isolated and their structures elucidated. As each vitamin was isolated, it was named by a letter of the alphabet, but once its structure had been established (or almost established), the vitamin has generally been renamed (see text).

The vitamins have been arbitrarily classified into the "fat-soluble group" (vitamins A, D, E and K), and the "water-soluble group" (the remainder of the vitamins).

A number of vitamins have already been dealt with in various chapters dealing with natural products with which these particular vitamins are closely associated chemically, *viz.* vitamins A₁ and A₂ (§7. IX), vitamin C (§11. VII) and the vitamin D group (§§6, 6a, 6b. XI). This chapter is devoted to a number of other vitamins (see the reading references for further information).

From the point of view of chemical structure, there is very little common to the various vitamins, but from the point of view of chemical reactions, many of the water-soluble vitamins have one feature in common, and that is their ability to take part in reversible oxidation-reduction processes. Thus they form a part of various co-enzymes (see §17. XIII), *e.g.*, nicotinamide is present in co-enzyme I (diphosphopyridine nucleotide; DPN), and in co-enzyme II (triphosphopyridine nucleotide; TPN); phosphorylated pyridoxal is the co-enzyme of transaminases; riboflavin in flavin adenine nucleotide (FAD); pantothenic acid in co-enzyme A; etc.

VITAMIN B COMPLEX

§2. Introduction. Eijkman (1897) found that birds developed polyneuritis when fed with polished rice, and were cured when they were given rice polishings. Then Grijns (1901) found that rice polishings cured beriberi in man (beriberi in man corresponds to polyneuritis in birds; it is a form of paralysis). Grijns suggested that the cause of this paralysis was due to some "deficiency" in the diet, and this was confirmed by Funk (1911, 1912), who prepared a concentrate of the active substance from rice polishings. Funk believed that this active substance was a definite chemical compound, and since he separated organic bases when he prepared his concentrate, he named his "deficiency compound" a *vitamine*. It was then found that "vitamine B" was a complex mixture, and when a number of "vitamines" were obtained that contained no nitrogen, the name *vitamin* was retained for them. The name vitamin B is now reserved for the complex mixture of vitamins in this group.

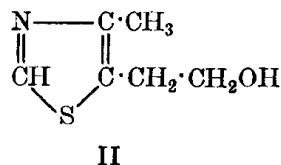
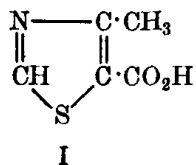
§3. **Vitamin B₁, thiamine (aneurin).** Thiamine is one member of the water-soluble vitamin B complex, and is in the thermolabile fraction; it is the absence of thiamine which is the cause of beriberi in man; thus this vitamin is the antineuritic factor (hence the name *aneurin*). Rice polishings and yeast have been the usual sources of thiamine; eggs are also a rich source.

Thiamine is obtained crystalline in the form of its salts; the chloride hydrochloride has been shown to have the molecular formula $C_{12}H_{18}ON_4Cl_2S$ (Windaus *et al.*, 1932); this salt is isolated in the form of its hemihydrate, d. 248–250°. When treated with a sodium sulphite solution saturated with sulphur dioxide at room temperature, thiamine is decomposed quantitatively into two compounds which, for convenience, we shall label A and B (R. R. Williams *et al.*, 1935).

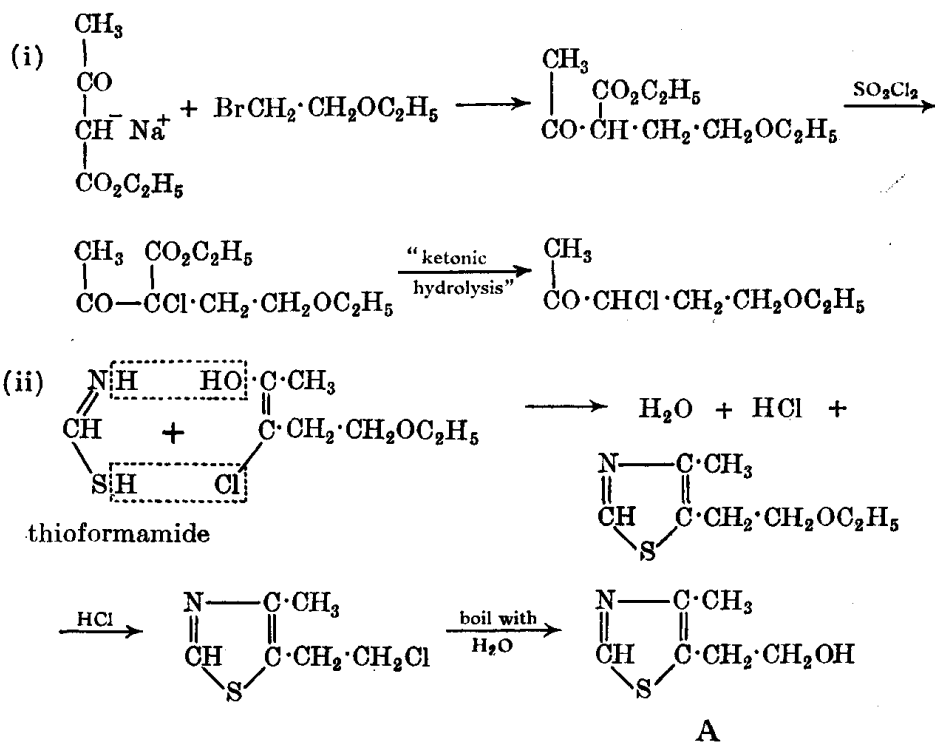


Compound A, C_6H_9ONS . This compound shows basic properties, and since it does not react with nitrous acid, it was inferred that the nitrogen atom is in the tertiary state. The functional nature of the oxygen atom was shown to be alcoholic, *e.g.*, when A is treated with hydrochloric acid, a hydroxyl group (one oxygen atom and one hydrogen atom) is replaced by a chlorine atom. Furthermore, since the absorption spectrum of the chloro derivative is almost the same as that of the parent (hydroxy) compound, this suggests that the hydroxyl group is in a side-chain. The sulphur did not give the reactions of a mercapto compound nor of a sulphide; in fact, the stability (*i.e.*, unreactivity) of this sulphur atom led to the suggestion that it was in a heterocyclic ring. This conclusion was confirmed by the fact that A has an absorption spectrum characteristic of a thiazole (§5. XII).

R. R. Williams *et al.* (1935) found that oxidation of A with nitric acid gives the compound $C_5H_7O_2NS$, which can also be obtained by the direct oxidation of thiamine with nitric acid. This latter reaction had actually been carried out by Windaus *et al.* (1934), but these workers had not recognised the presence of the thiazole nucleus. Williams *et al.* showed that this oxidation product was a monocarboxylic acid, and found that it was identical with 4-methylthiazole-5-carboxylic acid, I, a compound already described in the literature (Wöhmann, 1890). From this it follows that A has a side-chain of two carbon atoms in place of the carboxyl group in I

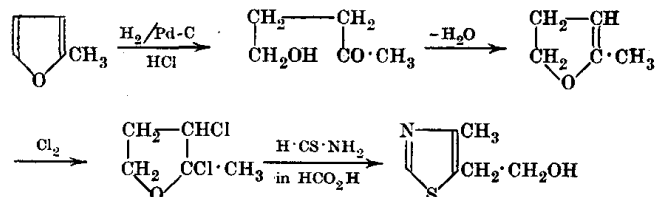


(one carbon atom is lost when A is oxidised to I). Since it is this side-chain which must contain the alcoholic group, the side-chain could be either $-CH_2 \cdot CH_2OH$ or $-CHOH \cdot CH_3$. Either of these could lose a carbon atom to form a carboxyl group directly attached to the thiazole nucleus. The second alternative, $-CHOH \cdot CH_3$, was excluded by the fact that A does not give the iodoform test, and that A is not optically active (the second alternative contains an asymmetric carbon atom). Thus A was given structure II, and this has been confirmed by synthesis (Clarke *et al.*, 1935).

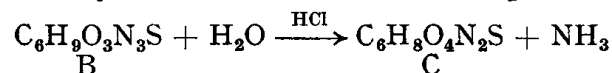


The hydrochloride of this compound is identical with that of the product obtained from thiamine (by fission), and also gives I on oxidation with nitric acid.

Londergan *et al.* (1953) have synthesised A from 2-methylfuran as follows:

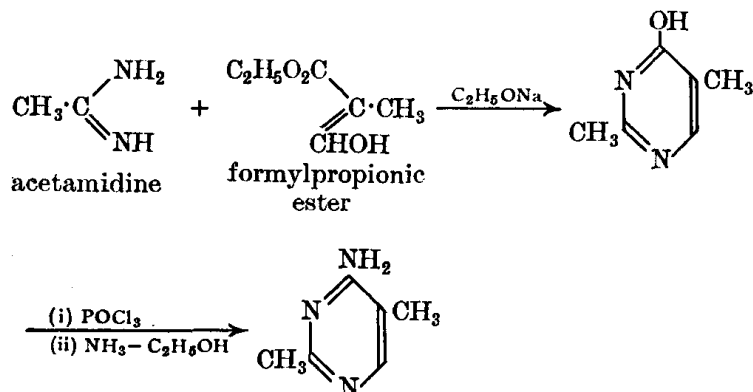


Compound B, $\text{C}_6\text{H}_9\text{O}_3\text{N}_3\text{S}$. This was shown to be a sulphonic acid, *e.g.*, when heated with water under pressure at 200° , B gives sulphuric acid; it also forms sodium sulphite when heated with concentrated sodium hydroxide solution. On treatment with nitrous acid, B evolves nitrogen; thus B contains one or more amino-groups. Analysis of the product showed that one amino-group is present in B (the product contained only one hydroxyl group). Furthermore, since the evolution of nitrogen was slow, and the reaction of B with benzoyl chloride was also slow, this suggests that B contains an amidine structure (Williams *et al.*, 1935). Williams *et al.* (1935) then heated B with hydrochloric acid at 150° under pressure, and obtained

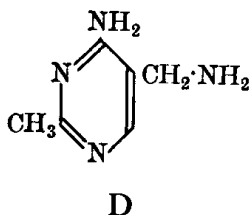


compound C and ammonia. The formation of ammonia indicates the replacement of an amino-group by a hydroxyl group. This type of reaction is characteristic of 2- and 6-aminopyrimidines; it was therefore inferred that B is a pyrimidine derivative (*cf.* §14. XII). This is supported by the fact that the ultraviolet absorption spectrum of compound C was similar to that of synthetic 6-hydroxypyrimidines; thus B is probably a 6-aminopyrimidine.

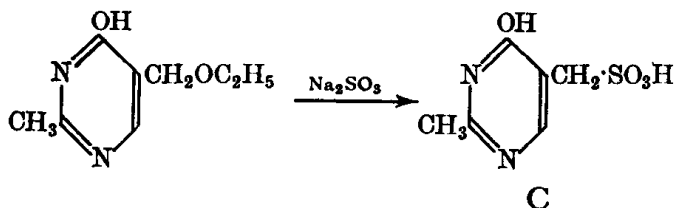
When B is reduced with sodium in liquid ammonia, a sulphonic acid group is eliminated with the formation of an aminodimethylpyrimidine (Williams, 1936). Comparison of the ultraviolet absorption spectrum of this product with various synthetic compounds showed that it was 6-amino-2:5-dimethylpyrimidine, and this was confirmed by synthesis (Williams *et al.*, 1937).



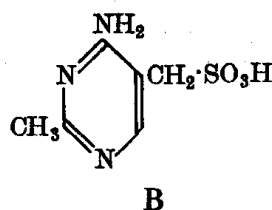
Thus B is 6-amino-2:5-dimethylpyrimidine with one hydrogen atom (other than one of the amino-group) replaced by a sulphonic acid group. When thiamine is treated with sodium in liquid ammonia, one of the products is the diamino derivative D, $\text{C}_6\text{H}_{10}\text{N}_4$ (Williams *et al.*, 1937). Compound D was identified as 6-amino-5-aminomethyl-2-methylpyrimidine by comparison



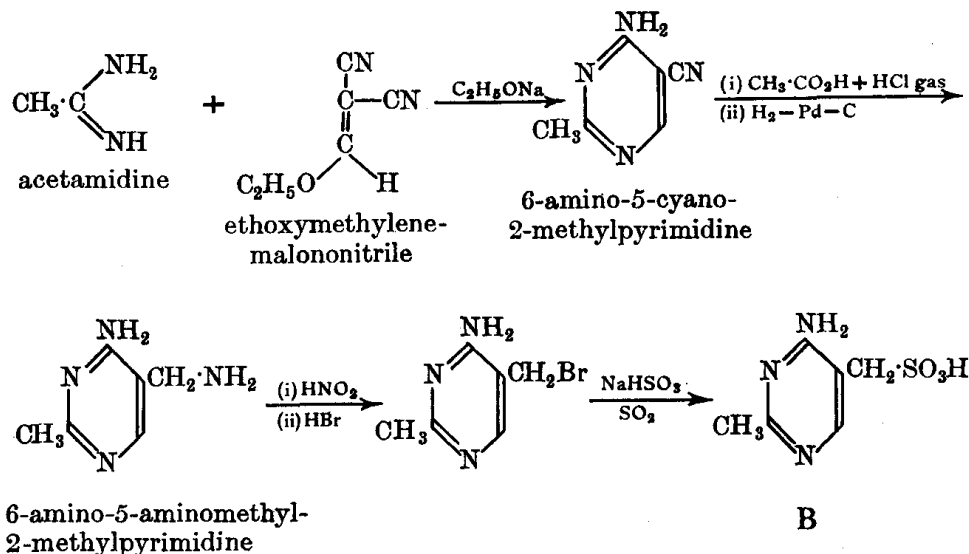
with the absorption spectra of methylated aminopyrimidines of known structure (Williams *et al.*, 1937). This is confirmed by the synthesis of Grewe (1936); Williams *et al.* had arrived at their conclusion independently of Grewe's work (see below for this synthesis). Thus, in compound D, there is an amino-group instead of the sulphonic acid group in B. Williams therefore concluded that the sulphonic acid group (in B) is joined to the methyl group at position 5. This was confirmed (in 1937) by treating 5-ethoxymethyl-6-hydroxy-2-methylpyrimidine (see the synthesis described for thiamine) with sodium sulphite, whereby 6-hydroxy-2-methylpyrimidyl-5-methanesulphonic acid was obtained, and this was shown to be identical with compound C.



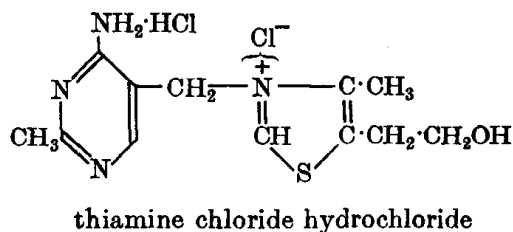
Thus B has the following structure:



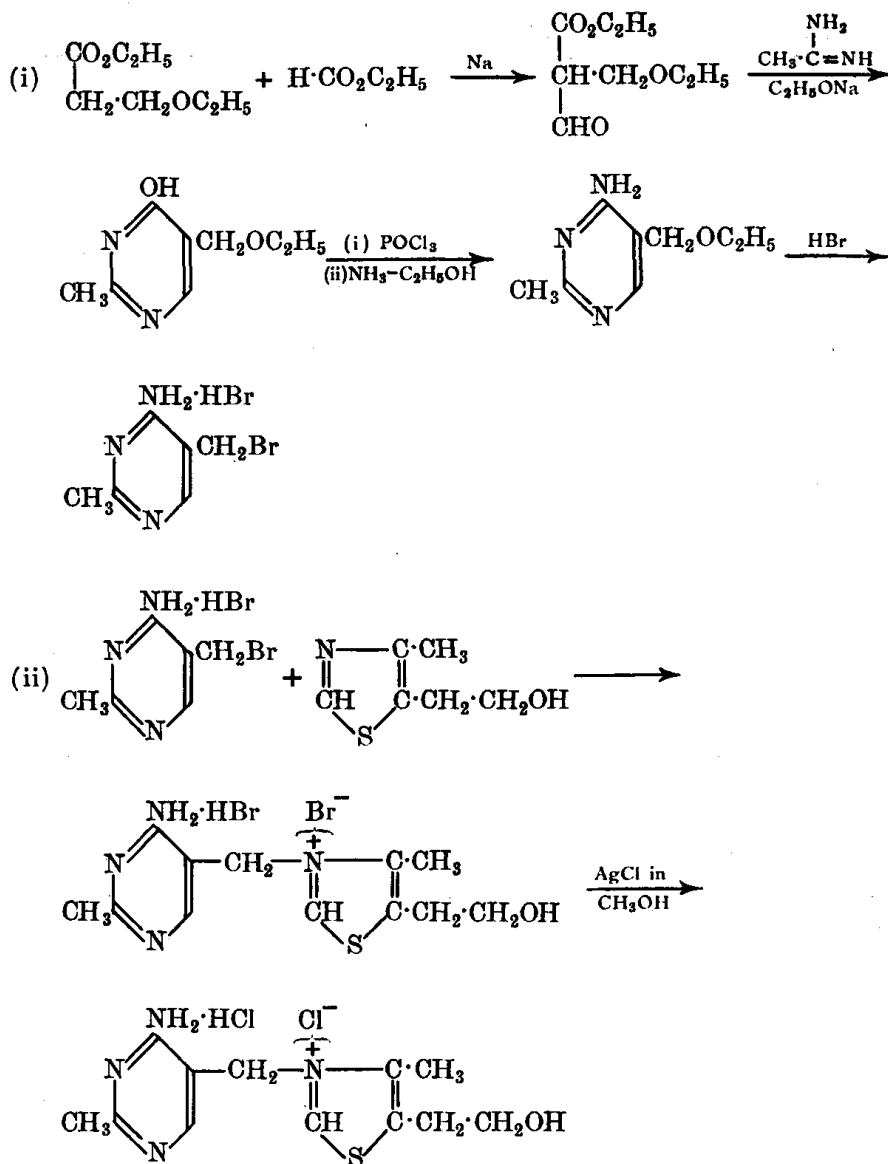
This structure is confirmed by synthesis (Grewe, 1936; Andersag *et al.*, 1937).



The final problem is: How are fragments A and B united in thiamine? As we have seen, the sulphonic acid group in B is introduced during the fission of thiamine with sodium sulphite; thus the point of attachment of fragment B is at the CH_2 group at position 5. To account for the formation of compound D, fragment B must be linked to the nitrogen atom of fragment A; in this position, the nitrogen atom of the thiazole ring is in a quaternary state, and so accounts for the chloride hydrochloride of thiamine. Had B been connected to A through a carbon atom of the latter, it would not be easy to account for the ready fission of this carbon-carbon bond by means of sodium and liquid ammonia, nor for the fact that thiamine does not form a *dihydrochloride*. Thus the chloride hydrochloride of thiamine is



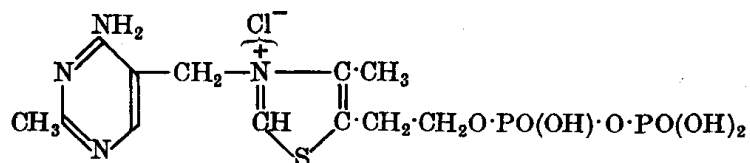
This structure has been confirmed by synthesis, *e.g.*, that of Williams *et al.* (1936, 1937).



§4. **Co-carboxylase.** This is the co-enzyme of *carboxylase*, and has been shown to be the pyrophosphate of thiamine (Lohmann *et al.*, 1937). Carboxylase, which requires the co-enzyme for action (see §15. XIII), breaks down pyruvic acid, formed in alcoholic fermentation, to acetaldehyde and carbon dioxide.



Co-carboxylase is



§5. **Thiochrome** was isolated from yeast by Kuhn *et al.* (1935); it is a yellow basic solid and its solutions show a blue fluorescence. Thiochrome