Fluorescent Product from Vitamin B_1 and Cytidine. A Thiochrome Mimic

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Vitamin B_1 (**1**) and cytidine (**2**) in methanol give a fused tricyclic product (**3**) whose excitation and fluorescence emission spectra are remarkably similar to those of thiochrome (**5**), a derivative into which the vitamin is routinely converted before quantitative analysis by fluorometry.

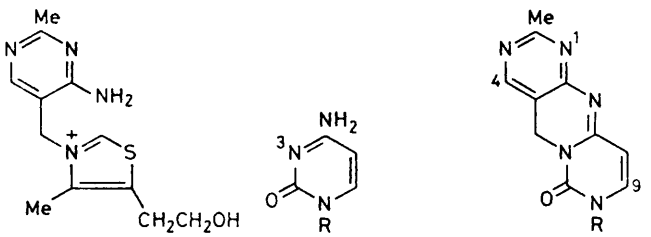
Nucleophilic substitution of vitamin B_1 (thiamin) (**1**) at the methylene position to displace the thiazole leaving group has been observed rarely. Exceptions include the classic reaction with sulphite ion in water[1,2] and more recently oligomerization by reaction with itself in methanol.[3,5] The quaternized derivative 1′-methylthiaminium ion, however, readily undergoes such substitutions with a wide variety of nucleophiles.[4]

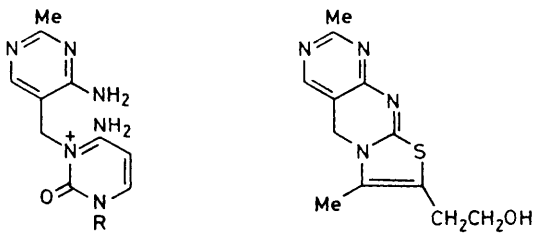
We now report that (**1**) reacts with the nucleoside cytidine (**2**) in methanol. Thus, a mixture of (**1**) (8.96 mmol) as its commercially available mononitrate free base† and (**2**) (20 mmol) in methanol (135 ml) was heated on a steam bath for 3 h. After filtration the volume was reduced by one-half and the mixture was left overnight; the product (**3**) was collected. Recrystallizations from water gave the pure sample (3.6 mmol, 40%), m.p. 224—226 °C decomp. (monohydrate).

The product, given the trivial name 2-methyl-8-ribosylcytosichrome,‡ is assigned structure (**3**) by considering its mode of formation and by analogy with similar products,[5] including those synthesized using quaternized thiamin and bidentate heterocycles.[6,7] Thus, nucleophilic substitution at the CH_2 group of (**1**) by N-3 of (**2**) is expected to form intermediate (**4**) which then cyclizes intramolecularly with loss of ammonia to give (**3**). Substitution may well take place on the conjugate acid of (**1**) by a multi-step route similar to those for sulphite ions[2] and for oligomerization.[3] A related pathway is also observed for quaternized thiamin.[8] N.m.r. and mass spectral data are consistent with (**3**).§

A 10^{-6} M solution of (**3**) in water (pH 6.5), methanol, or acetonitrile shows an excitation maximum at 358 nm and emissions at 407, 428, and *ca.* 454 nm (corrected). Emission of the 'triplet' is so intense that an undegassed 1×10^{-8} M sample in water can be detected when excited at 340 nm. The solution was stable over several days.

The spectra and the melting point of (**3**) are similar to those for thiochrome (**5**), an oxidative addition product of (**1**) formed in its standard quantitative analysis.[9] Figure 1 compares the emission spectra of (**3**) (B and C) and (**5**)[10] (A) in isobutyl alcohol. This solvent is commonly employed to extract (**5**) from the aqueous solutions used to generate it.[9]





R = Ribosyl

† More soluble salts tend to oligomerize.[2]

‡ λ_{max} (3.67×10^{-5} M; MeOH)(log ϵ): 356 (4.31), 2.75 (3.54, sh), 250 (3.78, sh), and 235 mm (3.93).

§ δ[^{1}H, $(CD_3)_2SO$, D_2O, Me_4Si, 60 MHz], 8.30 (H-4), 7.86 (H-9, *J* 8 Hz), 5.83 (H-10, *J* 8 Hz), 5.80 (1′-H), 5.03 (5-CH_2), 4.3—3.8 (m, ribose), and 2.43 (Me); δ [^{13}C, $(CD_3)_2SO$, Me_4Si, 40 °C, 75 MHz], 167.1 (s), 155.5 (s), 153.5 (d), 148.9 (s), 137.6 (d), 110.1 (s, C-4a), 102.2 (d, C-10–H), 89.1, 84.8, 73.6, 69.5 (4d, ribose), 60.5 (t, CH_2O), 41.6 (t, CH_2N), and 25.3 p.p.m. (q, Me); *m/z* (70 eV) (relative intensity): calc. for M^+ $C_{15}H_{17}N_5O_5$ 347.1230, found 347.1249 (0.5), $C_{10}H_9N_5O$ (84.62), $C_{10}H_8N_5O$ (100), $C_5H_9O_4$ (3.32).

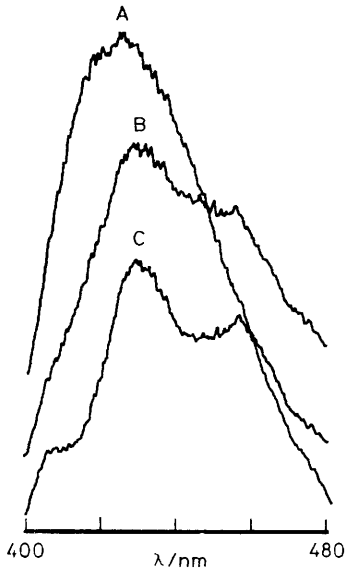


Figure 1. Emission spectra of thiochrome (**5**) (A) and the ribocytosichrome (**3**) (B and C). Emission slit widths are 20 or 10 nm for A, 20 nm for B, and 10 nm for C. Intensities are selected arbitrarily. The excitation wavelength is 365 nm.

Spectra from the two are quite similar, especially with wide (20 nm) emission slit settings (see A and B). With a narrower slit width (10 nm) the spectrum of (**3**) contains the characteristic 'triplet' and becomes dissimilar to that of (**5**). The main excitation bands of (**3**) (363 nm) and (**5**) (370 nm) are also close. Spectra of aqueous solutions of (**3**) and (**5**) may be confused owing to these similarities.

Emission spectra of (**3**) and (**5**) may be distinguished (a) by using narrow emission slits and (b) by acidifying aqueous samples. Addition to (**3**) of concentrated perchloric acid (pH 1) leads to a substantial enhancement in intensity with a shift to 410 nm but similar treatment of (**5**) produces strong quenching of its spectrum.[11] Such acidification also extends the detection limits of (**3**).

The similar luminescence properties of (**3**) and (**5**) raise the question of whether the hitherto unreported (**3**) may have been mistakenly identified as (**5**) and therefore may have gone undetected. Moreover, quantitative analysis of (**1**) as its derivative (**5**) may be in error because (**3**) is present.

Perhaps (**1**) and (**2**) in biological samples give (**3**), especially when thiaminase I is present. This enzyme which catalyses nucleophilic substitution reactions of (**1**)[12] is expected to give (**4**), as in our synthesis. Subsequent intramolecular cyclization of this intermediate to (**3**) is likely to be rapid.

Our demonstration that nucleophilic substitution of vitamin B_1 by (**2**) in methanol is facile suggests it is worthwhile to examine other nucleophiles, especially those present in biological media. Moreover, a new method using (**2**) or some other nucleophile to derivatize and estimate (**1**) may emerge. The current thiochrome scheme has limitations.[9,13]

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