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Thiamine-quinone adducts. Colorimetric determination of thiamine

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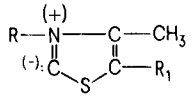
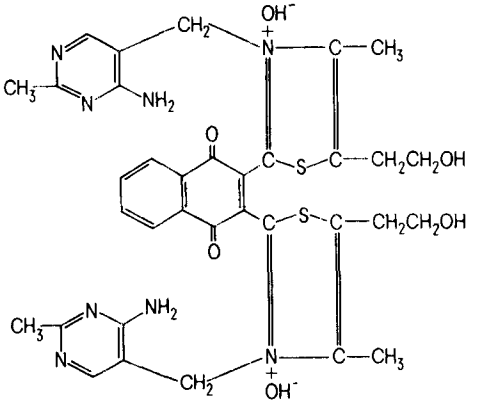
Summary. 2,3-dichloro-1,4-naphtoquinone reacts with thiamine in the presence of NH_3 to give a 1:2 adduct, for which structure **II** is proposed on the basis of analytical and spectroscopic (IR) data. Such a reaction is very useful for thiamine quantitative determination.

Continuing our studies[1,2] on active methylenic compounds, we have investigated the reaction of thiamine (vitamin B_1) with quinones in the presence of ammonia. In thiamine, the hydrogen at C-2 of the thiazolium group is easily deprotonated leading to the anion **I**, which is important



(I)

for the biological activity of thiamine[3,4]. For this reason thiamine can be compared with active methylenic compounds and should be expected to react with quinones (the so-called Craven reaction[5,6]). In fact, it has been observed that thiamine chloride hydrochloride easily reacts with several quinones in presence of ammonium hydroxide giving rise to intensely coloured solutions (table). We have studied the reaction between thiamine and 2,3-dichloro-1,4-naphtoquinone which gives the more sensitive colour reaction for the spectrophotometric determination. From this reaction we have isolated a red product (m.p. 235°C) which contains, by elemental analysis, the 2 components in a molar ratio of 2:1. The molecular adduct does not contain chlorine, proving that the reaction takes place with complete dehalogenation of the quinone; moreover it exhibits properties of quinones[7]. The IR-spectrum (3500–3000 cm^{-1} range, in Nujol mull) shows that the ν(N–H) is lowered by 200 cm^{-1} with respect to that of the NH_2 group in thiamine. This can be explained by hydrogen bonding between NH_2 and the carbonyl groups. The N–H deformation is similarly lowered and 2 bands are present in the 1700–1600 cm^{-1} range, attributable to ν(CO) and assigned respectively to ν(CO) (1668 cm^{-1}; 1670 cm^{-1} in the naphtoquinone alone) and to CO stretching (1650 cm^{-1}) of the carbonyl group hydrogen-bonded to NH_2. In the range 1400–600 cm^{-1}, the spectrum is very similar to that of thiamine except for a band at 732 cm^{-1} attributable to the δ(CH) of bonded naphtoquinone (709 cm^{-1} in the pure quinone). A weak band is still present at 690 cm^{-1} attributable to the ν(C–S) (690 cm^{-1} in thiamine). On the basis of these data, it is possible to ascribe to the molecular adduct the structure **II**.



The calibration curve for the dosage of thiamine has been obtained at 360 nm by measuring the optical density of mixtures containing known amounts of quinone and ammonia and variable amounts of thiamine chloride hydrochloride. The reference for each measurement contained the same amount of quinone and ammonia as the respective sample to be examined, eliminating the absorbance due to the reaction of the quinone with ammonia. The results obtained showed that the optical densities of the red ammonia solutions are a linear function of the thia-

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mine concentration in the range $0.25 \cdot 10^{-4}$ to $3 \cdot 10^{-4}$ mol/l, corresponding to a content of thiamine between 8.4 and 101 μg/ml. Such results suggest that this colour reaction may be used in the spectrophotometric dosage of thiamine, because of the rapidity and simplicity of the procedure with respect to other methods[8–11]. Similar investigations for thiamine pyrophosphate are in progress.

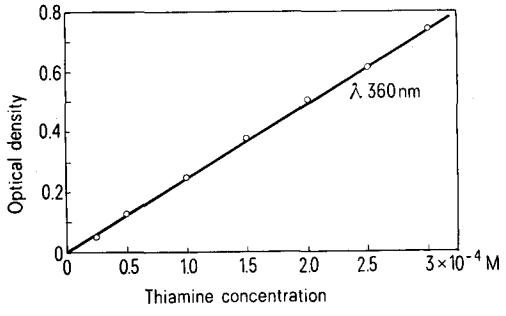
Experimental. Visible spectra were recorded using a Rank Precision Uvichem H 1600 S.T. Spectreophotometer; 1-cm stoppered fused silica cells were used. Thiamine chloride hydrochloride and 2,3-dichloro-1,4-naphtoquinone (Fluka AG, Buchs, Switzerland) were reagent grade for analysis. Dimethylformamide (DMF) was reagent grade for spectrophotometry.

Preparation of adduct. A DFM solution (5 ml) of 2,3-dichloro-1,4-naphtoquinone (0.45 g; 0.02 mol/l was added to the solution of 1.35 g (0.04 mol) of thiamine chloride hydrochloride in 10 ml of methanol. This mixture, treated with 2 ml of ammonium hydroxide, (26°Bé) immediately assumed a deep red colour. The reation product begins to separate at room temperature, and within 2 h the precipitation is almost complete. The collected product, washed with ethanol, was crystallized a few times from warm methanol and small red crystals were obtained (m.p. 235°C). UV λ_{max} at 235, 280, 470 nm (ε = 36200, 33000, 3400 respectively). IR-bands at 1669 cm^{-1} and 1650 cm^{-1} (νCO); 732 cm^{-1} (δC–H); 690 cm^{-1} (νC–S). The presence of the quinone nucleus has been detected as follows: a small amount of the adduct was suspended in methanol and a small quantity of $NaBH_4$ was added. To the decolourized solution, a few drops of H_2SO_4, 0.1N were added to destroy the excess $NaBH_4$. The mixture was gently warmed for a few min and then treated with a benzene solution of dehydroindacum: immediately it assumed the intense blue coloration due to indacum[7].

Calibration curve. Solutions of thiamine chloride hydrochloride and of 2,3-dichloro-1,4-naphtoquinone in DMF, both 10^{-3} M were prepared. The DMF solution of thiamine contained also 12% by volume of water to facilitate dissolution. The absorption spectrum of the DMF solution of each component was transparent, in the range 360–500 nm, whereas the ammonia DMF solution of the quinone showed appreciable absorbance. The wave length of 360 nm was selected as the most appropriate for the calibration curve. To this purpose a series of samples was prepared by adding known volumes (in the range 0.25 to 3 ml) of DMF 10^{-3} M thiamine solution to 4 ml of DMF 10^{-3} M 2,3-dichloro-1,4-naphtoquinone solution. Each of these samples was treated with 0.1 ml of ammonium hydroxide (26°Bé) and diluted to a constant volume of 10 ml with DMF. The samples were allowed to stand for 5 min before reading the optical densities. The reference sample was made adding 0.1 ml of ammonium hydroxide (26°Bé) to 4 ml of DMF 10^{-3} M 2,3-dichloro-1,4-naphtoquinone solution and diluting to 10 ml with DMF. The figure shows the linear correlation between optical density and thiamine concentration in the range $0.25 \cdot 10^{-4}$ to $3 \cdot 10^{-4}$ mol/l, corresponding to a content of thiamine chloride hydrochloride between 8.4 and 101 μg/ml.

Colour reactions of thiamine chloride hydrochloride with several quinones in the presence of ammonium hydroxide

Benzoquinone	red-orange
Toluquinone	brown
1,4-naphtoquinone	red-orange
2-methyl-1,4-naphtoquinone	yellow-orange
2,3-dichloro-1,4-naphtoquinone	red
1,2-naphtoquinone	wine-red
Chloranil	red-orange
Cacotheline	red-brown



Plots of optical density vs. thiamine chloride hydrochloride concentration according to the law of Lambert-Beer.

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Clausarin – a novel coumarin from Clausena pentaphylla (Roxb.) DC.

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Summary. Clausarin (**1**), a novel coumarin, has been isolated along with methyl linolenate, dentatin, clausenidin, β-sitosterol and heptaphylline from the roots of Clausena pentaphylla. Based on spectroscopic evidence, its structure has been established as 3,10-bis (1,1-dimethylallyl)-8,8-dimethyl-5-hydroxy-2H,8H-benzo (1,2-b:5,4-b′) dipyran-2-one.

The ethanolic extract of the roots of Clausena pentaphylla (Roxb.) DC. (Rutaceae) on fractionation and column chromatography over neutral alumina afforded 6 compounds. Of these, 5 were characterized as methyl linolenate (GLC), dentatin[1,2] (major component), clausenidin[2], β-sitosterol and heptaphylline[3]. The sixth one, obtained as a minor constituent is a new coumarin and has been named as clausarin. The present communication is concerned with the elucidation of its structure as 3,10-bis(1,1-dimethylallyl)-8,8-dimethyl-5-hydroxy-2H,8H-benzo (1,2-b:5,4-b′) dipyran-2-one (**1**).

Clausarin, $C_{24}H_{28}O_4$, M^+ 380, m.p. 208 °C (d) showed absorption at ν_{max}^{KBr} 3220 (OH), 1670 (C=O), 1610 and 1590 cm^{-1} (unsaturation); $\lambda_{max}^{CH_3OH}$ 232 (log ε 3.33), 282 (3.44) and 338 nm (3.21) respectively similar to nordentatin[2]. Addi-