3. Yields of α-ketoglutarate approaching the theoretical are obtained from citrate in the dialysed extract. The further breakdown of α-ketoglutarate to oxaloacetate and pyruvate is not inhibited by arsenicals.

4. As similar results could be obtained with a pure sample of *Saccharomyces cerevisiae* grown in the laboratory it is concluded that the tricarboxylic acid cycle represents a significant pathway of substrate oxidation in yeast.

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Note on an Improved Method for the Preparation of Oxythiamine

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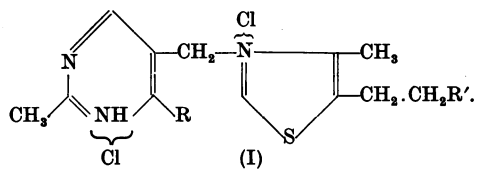
Oxythiamine (I; R=R′=OH) is of some interest as an antagonist of vitamin B_1 (Soodak & Cerecedo, 1944; Eusebi & Cerecedo, 1949; Daniel & Norris, 1949), more especially since it has been reported to confer significant protection in mice against the Lansing strain of the poliomyelitis virus (Jones, Foster & Henle, 1948).



(I)

It was first prepared synthetically by Bergel & Todd (1937), but this procedure is laborious and a simple method of preparation from commercially available aneurin (thiamine, I; R=NH_2; R′=OH) is clearly desirable. Soodak & Cerecedo (1944) converted aneurin into oxythiamine in 50–70% yield by treatment with nitrous acid, but this method did not prove very satisfactory in our hands. 3-Aminopyrimidines are generally readily converted into the 3-hydroxy compounds by boiling with dilute acids and this seemed to offer an attractive simple route to oxythiamine; it was already known (Buchman & Williams, 1935; cf. Barger, Bergel & Todd, 1935) that treatment of aneurin with concentrated hydrochloric acid in a sealed tube at 150° gave chlorooxythiamine (I; R=OH; R′=Cl), the side-chain hydroxyl having been replaced by chlorine and the 3-amino group by hydroxyl. Milder conditions were

accordingly tried, the reaction being followed qualitatively by the thiochrome test, and it was found that consistently good yields of oxythiamine, substantially free from aneurin, could be obtained by simply refluxing aneurin with 5N-hydrochloric acid for 6 hr.; shorter periods of refluxing result in incomplete conversion and the use of stronger acid in the formation of some chloro-oxythiamine.

EXPERIMENTAL

Aneurin chloride hydrochloride (2 g.) was refluxed for 6 hr. with conc. HCl (50 ml.) and water (50 ml.). The product was evaporated to dryness under reduced pressure and the residue redissolved and re-evaporated with successive 50 ml. batches of ethanol and methanol. The final residue was dissolved in warm methanol (50 ml.); dry ether (75 ml.) was added and the mixture set aside to crystallize, finally at 0°. Oxythiamine chloride hydrochloride (I; R=R'=OH) (1·60 g.; 80%) separated in rosettes of flattened needles, m.p. 195° (decomp.), which were filtered off, washed with ether and dried in a vacuum desiccator. (Found: N, 12·0, 12·3; Cl, 21·3, 21·1, 20·8%; equiv., by electrometric titration, 332. Calc. for $C_{12}H_{17}O_2N_3SCl_2$: N, 12·4; Cl, 21·0%); oxythiamine chloride is a very weak base (pK_a less than 1) and the hydrochloride slowly loses HCl on drying *in vacuo*. The light absorption curve shows maxima at 2680, 2600, 2280 and 2210A. (ϵ=9100, 9100, 10,100, 10,100) in alkaline (M/15-Na_2HPO_4) and at 2650, 2580, 2280 and 2230A. (ϵ=10,800, 9300, 9500, 7900) in acid (0·1N-HCl) solution. Dr H. E. Cox kindly assayed two preparations for aneurin and found 0·00015% and less than 0·0001% respectively (Soodak & Cerecedo, 1944, report 0·1–0·4% for material prepared by their method). The dipicrate crystallizes from water in thin prismatic needles, m.p. 111° (Soodak & Cerecedo, 1949, report m.p. 102–108°). (Found: C, 39·5, 39·5; H, 3·6, 3·6. Calc. for $C_{24}H_{21}O_{16}N_9S$: C, 39·9; H, 2·9%.

SUMMARY

Oxythiamine, essentially free from aneurin, can be prepared in 80% yield by refluxing aneurin with 5N-hydrochloric acid for 6 hr.

I am much indebted to Dr H. E. Cox for the aneurin assays and to Dr E. A. Braude for the light-absorption data.

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