J. inorg. nucl. Chem. Vol. 41, pp. 1087–1088
Pergamon Press Ltd., 1979. Printed in Great Britain

SYNTHESIS OF THREONINE AND SEPARATION FROM ITS COPPER COMPLEX ON AN ION EXCHANGE RESIN

PATRICK SHARROCK and CLAUDE H. EON
Département de chimie, Université de Sherbrooke, Sherbrooke, Québec J1K 2R1, Canada

(*Received* 22 *September* 1978; *received for publication* 30 *November* 1978)

Abstract—Threonine can be synthesised in better than 95% yields using the base catalysed condensation of acetaldehyde on a stoechiometric amount of the copper complex of glycine. A convenient removal of the metal ions *via* a cation exchange resin in the NH_4^+ form gives a mixture of threonine and allothreonine (threo to erythro ratio 1.7:1.8). Electrodecomposition of the intermediate oxazolidine complex yields pure threonine.

INTRODUCTION

The Akabori reaction of aldehydes on a variety of metallic coordination compounds is well documented[1–4]. Recently, this condensation reaction was applied in the convenient synthesis of antibiotics *via* a copper amino acid complex[5]. One effective method for liberating the organic end products is to precipitate the copper as the sulfide, the drawback being general contamination of the biologically, important products by sulfides which are difficult to remove, even on ion exchange columns. An alternate method used in the synthesis of hydroxyamino acids or peptides calls for the acidification of the reaction mixture with concentrated hydrochloric acid[6,7]. The copper ions and amino acids are then settled on an H-form cation exchange resin. After washing the column with water, the amino acids (and peptides) can be eluted with aqueous ammonia. We have modified this time consuming process to a simple one step procedure which allows the recovery of the amino acids in pure form and in high yields.

EXPERIMENTAL

(1) *Synthesis of threonine by ordinary methods.* 58 g of copper glycinate monohydrate (0.25 mole) are mixed with 15 g anhydrous sodium carbonate in 180 ml water. 56.5 ml (one mole) acetaldehyde is added and the erlenmeyer flack stoppered. The temperature rises to 42°C and the solution turns dark blue. After three hours agitation, the solution is extracted with 100 ml ether, then acidified with 100 ml HNO_3 3*M*, H_2S is bubbled into the solution until it becomes colorless after decantation (1.5 hr). The yellowish solution is triturated with activated charcoal for one hour, followed by a second treatment with heating to the boiling point. Despite these procedures, the solution remains yellow. Some ethanol is added and the entire solution dried under vacuum. The viscous mass is recrystallized by dissolving in 100 ML H_2O, adding 150 ml ethanol, heating the solution with charcoal, filtering and letting stand overnight below 0°C. The solid product is washed with 2 × 15 ml absolute alcohol. The yield of threonine is 45.6 g (66%).

(2) *Isolation of threonine* via *classical ion exchange resins.* As starting material, we used copper *bis* (2,5-dimethyl oxazolidine, 4-carboxylate), prepared as previously described[8] and recrystalized from methanol. 3.94 g of this complex in 500 ml water is acidified with 3 ml HCl 3*M*, and introduced on 40 ml Amberlite IR 120 (H form) resin. The first third of the column turns green. After washing with 500 ml water, 1*M* NH_4OH is percolated through and the eluate recovered as 100 ml fractions. Evaporation of these fractions gives 0.946, 0.738, 0.339 and 0.05 g of product, with a total yield of 2.382 g threonine (87%).

(3) *Synthesis of threonine and separation under basic conditions.* (A) 2.3 g copper glycinate monohydrate (19 mmole), 0.6 g NaOH, 1.2 ml acetaldehyde (20 mmole) and 10 ml water are shaken together during 5.5 hr. 10 ml concentrated NH_4OH are added and the resulting dark blue solution placed on 25 ml Dowex 50W-8X (NH_4^+ form) resin. Once the blue solution has penetrated the column, it is eluted with 1*M* NH_4OH. The eluate is recovered until it shows a negative ninhydrin test, concentrated under vacuum at 30°C, to yield 2.118 g amino acids consisting of 80.5% threonine and 19.5% glycine (NMR integration of corresponding absorbtions). Recovery of amino acids is 96.5% (19.3 mmole). The final yield of threonine is 72%.

(B) 6 g NaOH dissolved in 100 ml water are mixed with 23 g copper glycinate monohydrate in a 125 ml erlenmeyer. 20 ml acetaldehyde is added to the suspension and the mixture tightly stoppered and shaken overnight. 20 ml concentrated NH_4OH are added and the copper ions trapped on an ion exchange resin (20 cm × 3.6 cm, Dowex 50W-8X, NH_4^+ form). The eluate is concentrated down to 50 ml, and 500 ml absolute alcohol added. 20.45 g of threonine crystallize out (86.2% yield). A similar 24 hr reaction gave 22.748 g threonine (95.5% yield). The *threo* to *erythro* ratios varied from 1.7 to 1.8 in various runs.

(4) *Electrodecomposition studies.* 1.18 g copper glycinate monohydrate is dissolved in 125 ml water containing 1 ml acetic acid (pH = 6). Current is passed between two large platinum electrodes at 8 V, 0.25 A for 0.5 hr, then at 5 V, 0.20 A. The solution warms up and becomes colorless after 1.5 hr. The filtered solution is evaporated and 0.70 g glycine obtained (92.2%). A sample of 0.852 g of Copper *bis* (2,5-dimethyl oxazolidine, 4-carboxylate) was electrolyses 2 hr at 8 V, 0.2 A. After concentrating the solution, adding 5 ml of methanol (no precipitation) concentrating the solution, adding 5 ml of methanol (no precipitation) concentrating again under vacuum at 30°C, only a solid, semi viscous mass of product was obtained. The NMR spectrum showed it to be the pure *threo* isomer of threonine, but contaminated with the acetic acid. The yield was not calculated.

DISCUSSION OF THE METHOD

Fixation of copper by ion exchange resins has been explored previously[9, 10] the main concern usually being separation of various metals[11]. Comparing the usual ion exchange method[6] for separating copper with the other common method which involves precipitating copper as the sulfide, shows the ion exchange resin to be more convenient, cleaner and producing an amino acid in purer form and in high recovery yields. Using sodium sulfide instead of H_2S hardly improves the situation. However, better yields could be obtained for our large scale preparation by using an ion exchange procedure instead of the activated charcoal purifications, as a small scale reaction showed.

Our separation of copper from threonine as the copper-amine cation appears to be even more effective a route, as the entire decomposition-separation procedure

is reduced to a single step. Instead of hydrolysing the complex with HCl, we add ammonia to the reaction mixture. Any solid complex can also be taken up (separately if desired) into 1*M* NH_4OH. Using Dowex 50W-8X instead of Amberlite 120 has the advantage that fixation of copper on the column can be followed easily by the intense blue coloration. Occasionaly, the amino acids have to be redissolved in a minimum of distilled water and lyophilized anew in order to secure a product free of ammonia, a minor inconvenience.

The total recovery of amino acids reaches 96.5%, and the yield of threonine can be as high as 95.5% under optimum conditions. We collected all fractions eluting out with positive ninhydrin tests. This is accomplished rapidly by heating a filter paper on a hot plate to 80°C, adding a drop of eluate and allowing to dry, followed by a drop of ninhydrin solution and checking for any coloration. Examination of the *threo* to *erythro* ratio shows only slight variations from one preparation or method to the other. This can be attributed to epimerisation at the α-carbon under the basic condition of the reaction or of the decomplexation[8]. We also noted that copper can be precipitated as the free metal by heating the basic solutions to 60°C for 2–5 min. Unfortunately, this is accompanied by extensive degradation of the amino acids. Sodium borohydride has been used, the end products being oxazolidines[12].

From the X-ray crystal structure determination of the solid intermediate complex[13] one expects only one isomer from its decomposition, namely the *threo* isomer. In fact, threonine containing barely detectable allothreonine amounts was isolated from the electrodecomposition reactions which avoided basic conditions. This result shows the Akabori reaction to be highly stereoselective, even through the ion exchange work up cannot take advantage of this fact under the present conditions. The high current densities and large overvoltages needed in plating out the copper in the presence of large amounts of complexing amino acids still make the ion exchange procedure most attractive. Extraction, with a small amount of ether, of the excess aldehydes from the reaction solutions can be performed if necessary; however, we would like to point out that we only used stoechiometric amounts of acetaldehyde in our experiments (there are two moles of amino acid per mole of copper). The results confirm that the Akabori reaction is a viable route to the synthesis of the essential amino-acid threonine[14].

REFERENCES

1. A. Pasini and L. Casella, *J. Inorg. Nucl. Chem.* **36**, 2133 (1974).
2. Q. Fernando, *Inorg. Chem. Radiochem.* **7**, 185 (1965).
3. M. M. Jones and J. E. Hix, *Inorg. Biochem.* **1**, 370 (1973).
4. T. T. Otani and M. Winitz, *Arch. Biochem. Biophys.* **90**, 254 (1960).
5. T. Wakamiya, K. Mizuno, T. Ukita, T. Teshima and T. Shiba, *Bull. Chem. Soc. Jap.* **51**, 850 (1978).
6. S. Ohdan, T. Ichikawa, Y. Ishido and Y. Araki, *Bull. Chem. Soc. Jap.* **46**, 1019 (1973).
7. M. Fujidka, Y. Nakao and A. Nakahara, *J. Inorg. Nucl. Chem.* **39**, 1885 (1977).
8. P. Maldonado, C. Richaud, J. P. Aune and J. Metzger, *Bull. Soc. Chim. Fr.* 2933 (1971).
9. D. Delmas, M. Caude and R. Rosset, *Analusis* **3**, 39(1975).
10. P. S. Bull and J. V. Evans, *J. Appl. Chem. Biotechnol.* **23**, 919 (1973).
11. F. Vernon and K. N. Nyo, *J. Inorg. Nucl. Chem.* **40**, 887 (1978).
12. J. Brush, R. Magee, M. O'Connor, S. Teo, R. Geue and M. Snow, *JACS* **95** 2034 (1973).
13. G. Larchères and M. Pierrot, *Acta Cryst.* **B27**, 442 (1971).
14. A. Inuzuka and K. Akubo, *Japan Kokai* **74**, 30, 317 (18 Mar. 1974) (see also *Chem. Abstr.* **81**, P 50038m).