CROATICA CHEMICA ACTA CCACAA 49 (3) 497—503 (1977)

YU ISSN 0011-1643
CCA-1022 547.46
Original Scientific Paper

Synthesis of Amino Acids by Cathodic Cleavage of Phenylhydrazones of α-Keto Acids

I. Tabaković, M. Trkovnik, and M. Džepina*

Faculty of Technology, University of Banjaluka, 78000 Banjaluka, Bosnia and Hercegovina, Yugoslavia

Received January 24, 1977

Electrochemical synthesis of amino acids by reduction of phenylhydrazones of the folowing α-ketoacids: pyruvic, α-ketovaleric, α-ketoglutaric and α-ketooctane acid was studied. The corresponding α-amino acids were obtained in 44—55% yield using controlled potential electrolysis on mercury pool electrode in 0.5 M HCl aqueous ethanol solution.

A mechanism rationalizing the reduction products of the phenylhydrazone of pyruvic acid as well as polarographic and coulometric data are proposed.

It has been shown in the literature that a number of important amino acids can be synthetised by reduction of esters of substituted α-nitroacrylic acid[1–6]. Other methods such as electroreductive coupling of Schiff bases with alkyl halides[7] and reductive cleavage of the C—S bond[8], have been applied in electrochemical preparations of amino acids. Several authors have used electrochemical cleavage in the peptide chemistry[8,9].

Reductive cleavage of phenylhydrazones of α-keto acids is an important method for the synthesis of α-amino acids, because of the easy availability of those phenylhydrazones by the Japp-Klingemann reaction[10]. V. V. Feofilakov and coworkers[11] have described the reduction of phenylhydrazones of α-ketoacids to the corresponding amino acids by means of zinc dust in 75% alcohol in the presence of mercuric chloride.

In the present work the electrochemical synthesis of α-amino acids by means of reductive cleavage of the corresponding phenylhydrazones was studied. Utilisation of electroanalytical techniques described herein should contribute to the elucidation of the reaction mechanism.

RESULTS AND DISCUSSION

Phenylhydrazones of the following α-ketoacids were included in the investigations: pyruvic, α-ketovaleric α-ketoglutaric and α-ketooctane acid. With all of the compounds studied well defined polarographic waves were observed, the total height of which was approximately the same for all of the substances compared. Comparison of the wave height of selected phenylhydrazones obtained in 0,5 M HCl solution with waves of equimolar concentrations of benzaldehyde and benzophenone semicarbazone, for which the number of electrons was

* The author for correspondence

TABLE I

Electroanalitical and Preparative Data

Phenylhydrazone of	$E_{1/2}$[a] / V sc. S.C.E.	i_d[a] / μA	n-Value	Potential controlled / V vs S.C.E.	Amino acid obtained m.p. (lit. m.p)[b] / °C	Yield / %
Pyruvic acid	— 0.691	8.0	4.0	— 0.9	Alanine 294—296 (295—297)	51
α-Ketovaleric acid	— 0.685	8.1	3.9	— 0.9	α-Aminovaleric acid 298—300 (305)	44
α-Ketoglutaric acid	— 0.666	8.5	3.8	— 0.9	Glutaminic acid 221—224 (224—5)	50
α-Ketooctanoic acid	— 0.655	8.4	3.8	— 0.9	α-Aminooctanoic acid 286—270 (270)	55

[a] 1×10^{-3} M concentration of phenylhydrazones of α-keto acid in 0.5 M HCl aqueous ethanol (50%)
[b] Taken from: R. G. W e a s t (53rd edition) »*Handbook of Chemistry and Physics*«, The Chemical Rubber Co., Ohio (1972).

known[13] is indicating four-electron processes. The results of coulometry at controlled potential, given in Table I, varied between n = 3.8 and 4.0. Logaritmic analysis of polarograms indicated an irreversible nature of the waves. The irreversibility was further confirmed by slow sweep cyclic voltammetry. The linear dependence of limiting currents on (*a*) the square root of the effective height of the mercury reservoir, and (*b*) the concentration of the phenylhydrazons showed that it is diffusion controlled.

Main polarographic and preparative results are shown in Table I. The preparative reduction of phenylhydrazones of α-ketoacids were performed in 0.5 M HCl solution (50% aqueous ethanol) on the mercury pool electrode in a divided cell using electrolysis at controlled potential (— 0.9 V vs. S.C.E.). The corresponding amino acids were isolated in 44—55% yield. All obtained amino acids gave correct elemental analysis and showed identical m.ps. and ir-spectra with authentic samples.

The polarographic behaviour of the phenylhydrazone of pyruvic acid, as well as the products of the preparative reduction, were studied in some details. This compound is reduced in a well-defined wave in the whole pH range examined. Several typical polarograms are shown in the Figure 1.

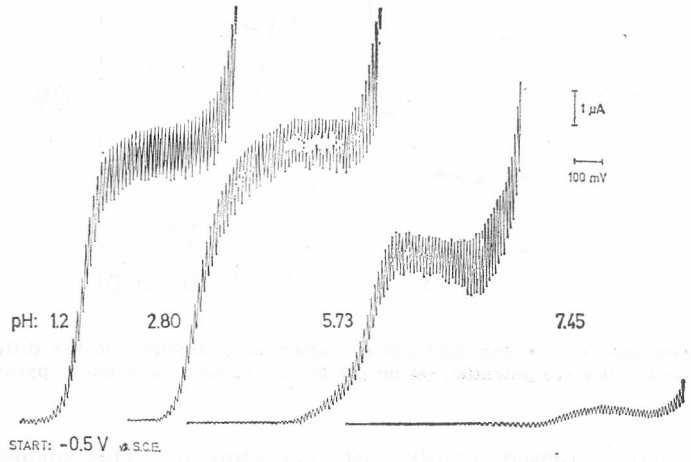


Figure 1. Typical polarograms of the phenylhydrazone of pyruvic acid (1×10^{-3} M)

Below pH = 2.8 the limiting current is controlled by diffusion and practically pH-independent. Above pH = 2.8 wave decreased with the increase of pH in the form of a steep dissociation curve (Figure 2) with pK' equal to 6.2 (pK' is the pH at which $i = i_d/2$). Studies made at pH 6.0 showed that $i_d/Ch^{1/2}$ values were not constant. This fact confirms a kinetically controlled nature of the reduction. A plot of the logarithm of the limiting current against pH gave a curve with a limiting slope of about-1, indicating the participation of one proton in the potential-determining step[14]. Below pH = 2.8 the half-wave potential is practically pH-independent, while above this value the half-wave potential is shifted to more negative values with increasing pH of the solution.

The current is thus an example of the formation of an electroactive species from electroinactive form, diffusing towards the electrode. In pH range 4.2 to 7.2. the

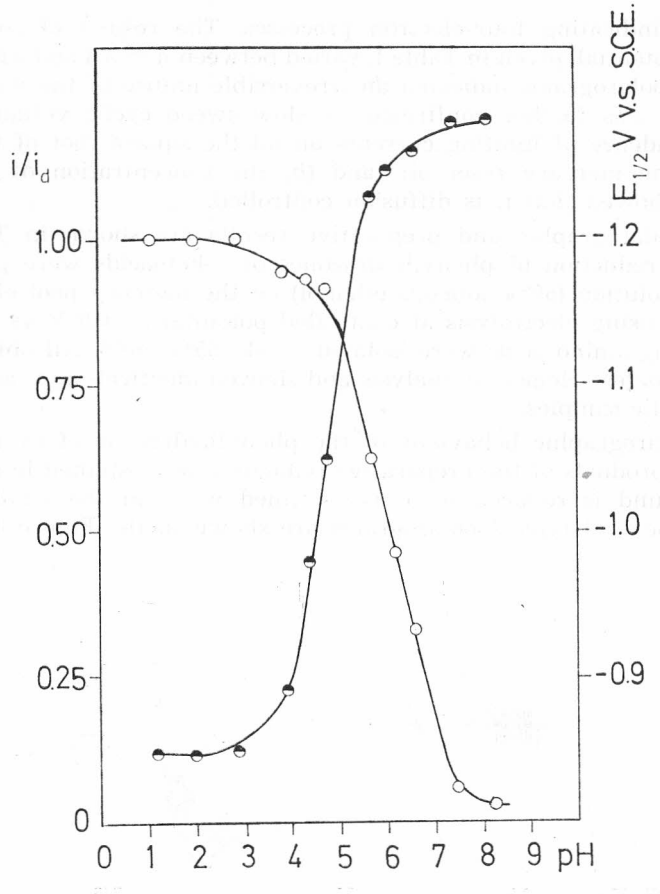


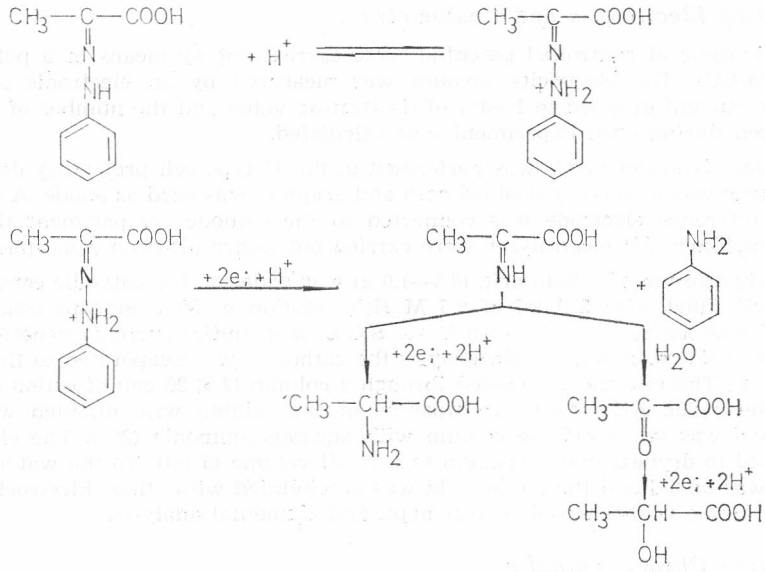
Figure 2. Dependence of limiting currents expressed as a fraction of the diffusion current i/i_d, (o)$_n$ and half-wave potential, (●) on pH for the phenylhydrazone of pyruvic acid.

limiting current decreased rapidly with increasing pH. This suggests that the height of the wave reflects the extent to which a reaction of the following type occurs during the drop life

$$\text{phenylhydrazone} + H^+ \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \text{protonated phenylhydrazone} \xrightarrow{+\,e} \text{product}$$

Using the treatment by Koutecky[15] and the polarographic value of $pK' = 6.2$ at $t_1 = 2.61$s the rate constant for proton recombination reaction was computed as $k_1 = 3 \times 10^5$ l mol^{-1} s^{-1} to deduce that the electrode process is accompanied by an antecedent acid-base reaction.

The reduction of phenylhydrazone of pyruvic acid is in general similar to the reduction of many azomethine derivative types described by H. Lund[15]. The electrode process taking place can be represented by the following scheme:



Scheme

According to the scheme the electroactive form in acidic solution is the protonated phenylhydrazone of pyruvic acid. After two electrons and one proton are accepted by the protonated phenylhydrazone a cleavage of the nitrogen-nitrogen bond occurres giving rise to the α-imino propane acid and aniline, as a good leaving group. α-Imino propane acid may be either further reduced through the transfer of two electrons and two protons leading to alanine, or may be hydrolised to pyruvic acid being a reducible species at applied potential (— 0.9 V) in acidic media and giving rise to lactic acid.

Preparative results and the determination of n-value by coulometry at controlled potential (n = 4) are fitting within the proposed scheme. Namely, besides alanine we have identified by means of TLC only two additional products, e. g. aniline and lactic acid. These results could explain the discrepancy between the chemical yield of alanine (51%) and n-value determined coulometrically (n = 4).

EXPERIMENTAL

Polarography

Polarograms were recorded on a Polariter P04-polarograph (Radiometer Copenhagen). The cell used was a modified H-type of 25 ml capacity with a dropping mercury electrode and saturated calomel electrode connected via a salt bridge of 3% agar in saturated potassium chloride solution. Temperature was kept constant at 25.0 ± 0.1 °C by means of a thermostat. The characteristic of capillary in H_2O open circuit are as follows: $t = 2.61$ s drop^{-1}, $m = 3.10$ mg s^{-1} for $h = 60$ cm. 1.25×10^{-2} M stock solution of phenylhydrazones of α-keto acids were freshly prepared in 50% ethanol. The buffer solution used were: pH = 1—3 hydrochloric acid-sodium acetate; pH = 3—8 Mc Ilvain buffers. Buffer solutions and all other solutions were prepared using Reagent Grade chemicals. Gelatin was added as maximum suppresor at a concentration of 0.01%.

Preparative Electrolysis and Coulometry

Electrolysis at controlled potential were carried out by means of a potentiostat (Amel-555-SU). The electricity amount was measured by an electronic integrator when the current dropped to 1—3% of its starting value and the number of electrons transferred during each experiment was calculated.

Preparative electrolysis was performed in the H-type cell previously described[16]. The cathode was a mercury pool (40 cm^2) and graphite was used as anode. A saturated calomel reference electrode was connected to the cathode compartment through a Luggin capillary. All electrolyses were carried out under nitrogen atmosphere.

The hydrazone of α-keto acid (0.5—1.0 g) was added to the cathodic compartment of the cell filled with 250 ml of 0.5 M HCl solution in 50% aqueous ethanol. The potential was maintained at — 0.9 V vs. S.C.E. with initial currents generally 400—800 mA. After stopping the electrolysis the catholyte was evaporated to the volume of 5—10 ml. The residue was passed through a column (2 × 20 cm) of cation exchange resin (Dower-50). After thorough washing of the column with distilled water, the amino acid was taken off the column with aqueous ammonia (2%). The eluate was evaporated to dryness under vacuum to a small volume (5 ml). To the water solution ethanol was added and the amino acid was precipitated with ether. Electrochemically obtained amino acids showed correct m.ps. and elemental analysis.

Thin Layer Chromatography

The mixture obtained after the reduction of the phenylhydrazone of pyruvic acid was taken from the catholyte and subjected to TLC analysis. Thin layer chromatography was carried out on HF-silicagel plates (250 μm) dried 30 minutes at 110 °C. The developer used was: *n*-butanole : acetic acid : water (7 : 2 : 1). The 0.4% nynhidrine solution in acetone was used for detection of alanine (R_f = 0.23) and aniline (R_f = = 0.86). The detection of lactic acid (R_f = 0.30) was done by means of 0,2% solution of bromophenol blue reagent. The R_f values of the mixture components were also compared with authentic samples.

REFERENCES

1. E. V. Zaporozhets, I. A. Avrutskaya, M. Ya. Fioshin, K. K. Babievskii, and V. M. Belikov, *Elektrokhimiya,* **8** (1972) 1243.
2. E. V. Zaporozhets, I. A. Avrutskaya, M. Ya. Fioshin, K. K. Babievskii, and V. M. Belikov, *Elektrokhimiya,* **9** (1973) 270.
3. E. V. Zaporozhets, I. V. Avrutskaya, M. Ya. Fioshin, K. K. Babievskii, and V. M. Belikov, *Elektrokhimiya,* **8** (1972) 1809.
4. E. V. Zaporozhets. I. A. Avrutskaya, M. Ya. Fioshin, K. K. Babievskii, and V. M. Belikov, *Elektrokhimiya,* **9** (1973) 72.
5. I. A. Avrutskaya, M. Ya. Fioshin, K. K. Babievski, V. M. Bellikov, and E. V. Zaporozhets, *Elektrokhimiya,* **9** (1973) 1127.
6. I. A. Avrutskaya, K. K. Babievskii, V. M. Belikov, E. V. Zaporozhets, I. M. Filatova, and M. Ya. Fioshin, *Elektrokhimiya,* **9** (1973) 1652.
7. T. Iwasaki and K. Harada, *Chem. Comm.* (1974) 338.
8. T. Iwasaki, M. Miyashi, M. Matsuoka, and K. Matsumoto, *Chem. Ind.* (1973) 1163 and references therein.
9. L. Horner and H. Lund in: M. M. Baizer (Ed.). »*Organic Electrochemistry*«, Marcel Dekker, New York, 1973, pp. 729.
10. R. F. Japp and F. Klingemann, *Ber.* **20** (1887) 2942, 3284, 3398.
11. V. V. Feofilaktov and T. N. Ivanova, *Zh. Obsch. Khim.* **21** (1951) 1684 and references therein.
12. N. H. Khan and A. R. Kidwai, *J. Org. Chem.* **38** (1973) 822.

13. B. Fleet and P. Zuman, *Collect. Czech. Chem. Commun.*, **32** (1967) 2066.
14. R. I. Gelb and L. Meites, *J. Phys. Chem.*, **68** (1964) 2599.
15. H. Lund, *Acta Chem. Scand.*, **13** (1959) 249; H. Lund and E. Th. Jensen, *Acta Chem. Scand.*, **25** (1971) 2727 and references therein.
16. H. Lund and P. Iversen in: M. M. Baizer (Ed.) »*Organic Electrochemistry*«, Marcel Dekker, New York, 1973. pp. 177.

SAŽETAK

Sinteza aminokiselina katodnim cijepanjem fenilhidrazona α-ketokiselina

I. Tabaković, M. Trkovnik i M. Džepina

Izučavana je elektrokemijska sinteza aminokiselina redukcijom slijedećih fenilhidrazona α-ketokiselina: pirogrožđane, α-ketovalerijanske, α-ketoglutarne i α-ketooktan kiseline. Elektrolizom kod kontroliranog potencijala živine katode u 0,5 M vodenoetanolnoj otopini HCl dobivene su odgovarajuće α-aminokiseline u 44—55%-tnom iskorištenju. Pretpostavljen je reakcioni mehanizam kojim se objašnjava nastajanje produkata redukcije fenilhidrazona pirogrožđane kiseline, a također su dati rezultati polarografije i kulometrije kod kontroliranog potencijala.

TEHNOLOŠKI FAKULTET
UNIVERZITET U BANJALUCI
78000 BANJALUKA, BOSNA I HERCEGOVINA

Prispjelo 24. siječnja 1977.