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NOVEL AMINO ACIDS SYNTHESIS USING NH_3 THROUGH BIOGENETIC-TYPE CO_2 FIXATION

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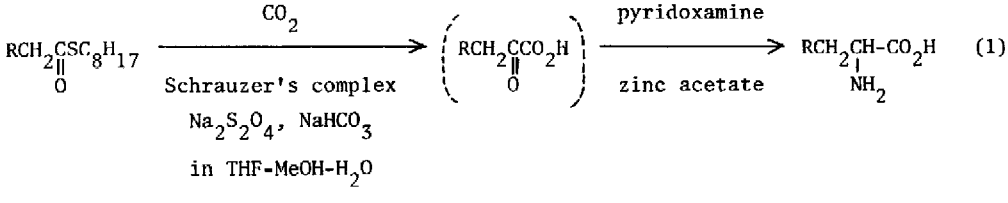
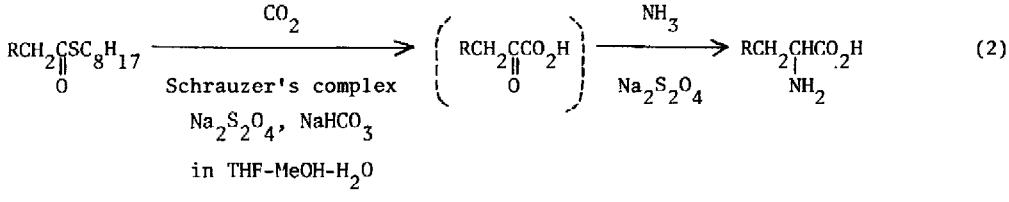
Recently, we have discovered the biogenetic-type reductive CO_2 fixation modelling ferredoxin by use of a simple iron sulfur complex where α-amino acids were successfully obtained in one step as shown in eq 1 in which an α-keto acid is considered as a plausible intermediate[1].



(1)

In this communication, we wish to report the convenient α-amino acid preparation from corresponding ketoacids or thiolesters by use of the simplest nitrogen source, ammonia, instead of pyridoxamine. The reaction is shown in eq 2. A solution of ammonia (0.3 ml of 28% aqueous solution, diluted with



(2)

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2.2 ml water, 4.5 mmol) in tetrahydrofuran-methanol (10 ml and 5 ml) was added dropwise to a mixture which consisted of Schrauzer's iron-sulfur complex I, $(FeS_4C_4Ph_4)_2$ (500 mg, 0.92 mmol), sodium hydrosulfite (5 g, 30 mmol), sodium bicarbonate (500 mg, 6 mmol), and n-octyl thiolphenylacetate $PhCH_2COSC_8H_{17}$ (264 mg, 1 mmol) dissolved in a mixture of 30 ml of tetrahydrofuran, 15 ml of methanol, and 7.5 ml of water under CO_2 bubbling at room temperature. After 8 hrs of CO_2 bubbling, the reaction mixture was extracted three times with 70 ml ether. The resulting aqueous solution was subjected to anion exchange resin chromatography using quarternary ammonium resin (IRA-400).

An amino acid absorbed on the resin was eluted with aqueous HCl. Chromatographic behavior (R_f values for several eluents, color developed by ninhydrin) of the product was the same as that of the authentic phenylalanine and the fraction appeared in the first 1N-HCl elute was identical as phenylalanine. Moreover, the product was subjected to high-speed liquid chromatography[2], which showed a formation of phenylalanine in 0.08% yield (0.13 mg) based on thiolester used (using pyridoxine as an internal standard). The yield was also determined by gas chromatography.

Alanine and leucine were similarly synthesized from thiolesters of corresponding carboxylic acids(eq 2).

Amino acid formation[3] was also investigated, by use of ammonia and an organic reducing agent, starting from α-keto acid which is the expected intermediate of the one-step amino acid synthesis. Thus, 1.0 ml of 28% aqueous ammonia (15 mmol) was added to a mixture of 164 mg of phenylpyruvic acid (1 mmol), 10 ml of tetrahydrofuran, 5 ml of methanol and 1.5 ml of water. After 1 hr stirring, 1.0 g of sodium hydrosulfite (6 mmol) was added to the solution, then stirring was continued for further 168 hrs at room temperature. Formation of phenylalanine was monitored by high speed liquid chromatography and thin layer chromatography. After usual work-up, solvent was distilled off under reduced pressure. Pyridoxin was added to the resulting aqueous solution,

then the solution was subjected to high speed liquid chromatography. The phenylalanine was identified by use of a rapid scan UV monitor. Phenylpyruvic acid was recovered as the first fraction. Phenylalanine was obtained in 73% yield, when phenylpyruvic acid completely consumed. Among products, 11% of benzaldehyde and 4% of unknown material were also detected by GLPC analysis. The result is summerized in Table 1.

TABLE 1

Conversion of α-keto acid into α-amino acids using NH_3 and $Na_2S_2O_4$

α-keto acid RCH_2COCO_2H R	mmol	NH_3 mmol	$Na_2S_2O_4$ mmol	reaction time hr	α-amino acid	yield* (%)
Ph	1	15	6	148	phenylalanine	73
H	1	15	6	24	alanine	70
HO_2CCH_2	1	15	6	40	glutamic acid	51

* based on α-keto acid consumed

In the early stages of the reaction, a probable intermediate, the Schiff base, was detected on the basis of UV spectrum (306 nm), and IR spectrum (intense absorption at 1595 cm^{-1}).

Similarly, the reduction of pyruvic acid and ammonia using sodium hydrosulphite in the same solvent mentioned above at room temperature gave alanine in 70% yield based on pyruvic acid consumed. Similarly, glutamic acid was obtained from α-ketoglutaric acid in 51% yield. The results are summerized in Table 1. Very small amounts of aldehydes and amines were also detected.

REFERENCES AND NOTES

1) T. Nakajima, Y. Yabushita and I. Tabushi, Nature 256, 60 (1975)

2) Shimazu-DuPont, type 830 ; analytical conditions : column, cation exchange 1 m ; mobile phase, 0.2 M acetic acid buffer, pH 3.8 ; column pressure, 150 Kg/cm^2 : detector, UV photometer. UV monitor was connected at the column outlet.

3) Important synthetic methods of α-amino acid reported are : Strecker synthesis, aminolysis of α-halo acid, acylaminomalonate method, and reduction of azomethine. By catalytic hydrogenation of Schiff-base of α-keto acid and ammonia, α-amino acid was prepared, where phenylalanine and glutamic acid were reported to be obtained from corresponding α-keto acid in 64% and 23% yield, respectively[4].

4) F. Knoop and H. Oesterlin, Z Physiol. Chem., 148, 294 (1925) ; 170, 186 (1927)