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NADH MODEL REDUCTION
BIOMIMETIC SYNTHESIS OF α-AMINO ACIDS FROM α-KETO ACIDS

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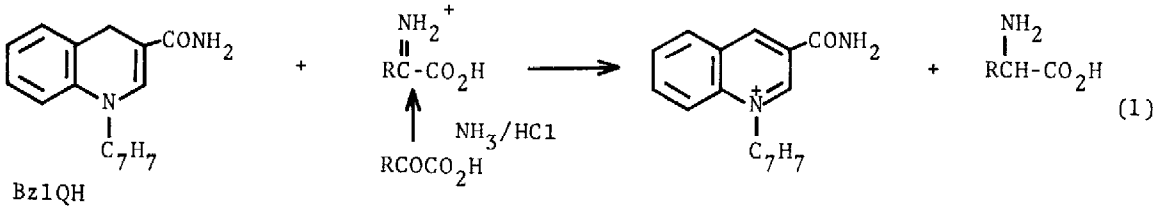
Protonated α-imino acids, $RC(=NH_2^+)COOH$, were easily reduced to α-amino acids by an acid-stable NADH analogue, 1-benzyl-3-carbamoyl-1,4-dihydroquinoline. This is the first model reaction of NADH-mediated α-amino acid synthesis from α-keto acids.

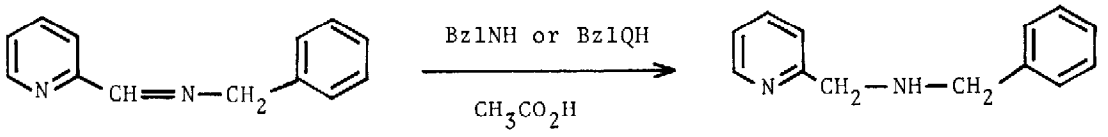
Considerable interest has recently centered around the model reaction of NADH-dependent enzymes, and several double bonds are now reducible nonenzymatically by NADH model compounds.[1] As for the C=N double bond, the reduction has been attained partially in the presence of Mg^{2+} ion or by protonation of the Schiff base.[2-4] It occurred to us that the reaction can be applied to synthesis of α-amino acids from α-keto acids plus ammonia This reaction is of great significance as a model reaction of numerous NAD(P)H-mediated amino acid syntheses in the enzymatic system(e g., L-glutamate dehydrogenase) [5] Ohno and coworkers[6] have reported the synthesis of N-phenyl alanine methyl ester from methyl pyruvate and aniline(52% yield), but no precedent for the direct, reductive amination of α-keto acids exists

The difficulty has been attributed to the strongly acidic nature of intermediary α-imino acids which causes the rapid decompostion of 1,4-dihydropyridines, conventional NADH model compounds. We recently found that 1-benzyl-3-carbamoyl-1,4-dihydroquinoline(BzlQH) is surprisingly stable against proton acids and thus becomes a useful model compound to understand the NADH-mediated reactions in acidic media.[7] Here, we wish to report the first example of biomimetic α-amino acid synthesis from α-keto acids with the aid of the acid-stable BzlQH.



(1)

As a prelude to the α-amino acid synthesis, a Schiff base, N-(2-picolylidene)benzylamine was subjected to the NADH model reduction. Table 1 indicates that in the presence of 1.5 M acetic acid the Schiff base was reduced by both BzlQH and BzlNH(1-benzyl-1,4-dihydronicotinamide acid-sensitive NADH model compound), though BzlQH giving the somewhat higher yield.[8]



(2)

The treatment of benzoylformic acid with ammonia in refluxing anhydrous methanol gave slightly yellow powder(mp 128-135°C). On the basis of IR measurement and elemental analysis, the compound was identified to be the zwitterionic imino acid, $C_6H_5(=NH_2^+)CO_2^-$ (I).[9] Introduction of HCl gas into the methanolic solution of (I) gave the protonated imino acid, $C_6H_5(=NH_2^+)CO_2H$ (II).[10] For the sake of synthetic simplicity, these samples were subjected to the reduction without further purification. Among four reaction runs for (I) and (II), only the BzlQH reduction of (II) afforded the product positive to ninhydrin test. The yields determined by high-pressure liquid chromatography are summarized in Table 1 The result proves that acid-sensitive BzlNH is totally useless to attain the biomimetic reduction of the α-imino acid. On the other hand, acid-stable BzlQH resulted in α-phenylglycine in 55% yield

Similarly, glycine and alanine were synthesized by BzlQH reduction from protonated imino acids of glyoxalic acid and pyruvic acid, respectively As shown in Table 1, however, the yields were extremely low and considerable amounts of gummy products were recovered. Importantly, we found that the formation of the undesired gummy products can be suppressed by introduction of dry

Table 1. Biomimetic reduction of imino acids and related Schiff base by BzlNH and BzlQH[a)]

Substrate	Reaction time (hr)	Yield of amino acids and amine (%)[b)]			
		BzlNH	BzlQH	BzlQH+HCl	BzlQH+HCl
$2\text{-}C_5H_4N\text{-}CH{=}N\text{-}CH_2C_6H_5$[c)]	48	58[d)]	85[d)]		
$C_6H_5C(=NH_2^+)CO_2^-$ (I)	24	0	0		
$C_6H_5C(=NH_2^+)CO_2H$ (II)	24	0	55		
$HC(=NH_2^+)CO_2H$	48		7.6	20	22[e)]
$CH_3C(=NH_2^+)CO_2H$	48		7 8	21	34[e)]

a) [Substrate]=0.0441 M and [BzlNH or BzlQH]=0.0882 M in refluxing methanol.
b) The yield of amino acids was determined as the 2,4-dinitrophenyl derivatives by high-pressure liquid chromatography F. Sanger, Biochem. J., 39, 507(1945).
c) $[2\text{-}C_5H_4N\text{-}CH{=}N\text{-}CH_2C_6H_5]$=[BzlNH or BzlQH]=0 050 M and [acetic acid]=1.5 M.
d) in refluxing ethanol. e) in acetic acid at 100°C.

HCl gas into the reaction media and the yields of the amino acids are significantly improved. The yields were further enhanced by replacing the reaction medium from methanol to acetic acid.

Here we consider the role of introduced HCl gas. In case the reaction is carried out without the feed of HCl gas, the "pH" of the methanolic solutions would not remain constant. As the reaction (1) proceeds, the "free" amino acid is accumulated in the medium. Expectedly, the equilibrium of Eq. (3) should lead to production of the much less reactive zwitterionic imino acid.

$$\underset{\underset{NH_2}{|}}{RCH\text{-}CO_2H} + \underset{\underset{NH_2^+}{\|}}{RC\text{-}CO_2H} \rightleftharpoons \underset{\underset{NH_3^+}{|}}{RCH\text{-}CO_2H} + \underset{\underset{NH_2^+}{\|}}{RC\text{-}CO_2^-} \qquad (3)$$

This would cause the undesired byreactions(e. g., decomposition of imino acids, aldol-type condensation, polymerization, etc.) and would result in the gummy byproducts.

As a summary of the foregoing results, one may conclude that BzlQH acts as

a useful reducing agent for the strongly acidic substrates, whereas the weakly acidic substrate(e.g., protonated Schiff base) can be reduced by both BzlNH and BzlQH. It would require little comment that the biomimetic α-amino acid synthesis is achieved owing to the stability of BzlQH against proton acids. Also suggested from the present results is that the enzymatic NADH reduction of α-imino acids would be markedly facilitated by protonation of the substrate or by its equivalents(e. g., strong hydrogen-bonding). This would provide a key information to consider the reaction mechanism by which NADH-dependent enzymes synthesize α-amino acids

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8) The authentic sample of N-benzyl-2-picolylamine was prepared by the $NaBH_4$ reduction of N-(2-picolylidene)benzylamine in absolute ethanol yield 70%, mp 205-209°C. The structure was confirmed by elemental analysis and NMR.
9) IR(KBr) ν_{COO^-}, 1590 cm^{-1}, $\nu_{C=N^+}$, 1655 cm^{-1}. Elemental analysis C/N=7.97.
10) Hygroscopic solid IR(Nujol), ν_{COOH}, 1760 cm^{-1}

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