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Short communication

Vanadium(V)-catalyzed epimerization of isoleucine

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ABSTRACT

All stereoisomers of isoleucine were transformed to the mixtures of the corresponding epimers by epimerization in alkaline aqueous solution. The catalyst was formed *in situ* by condensation of salicylaldehyde and isoleucine followed by complexation with vanadate. No derivatization of the amino acid was necessary. The tetrabutylammonium salts of $[VO_2(N\text{-salicylidene-isoleucinato})]^-$ can be used for diastereomeric separation of the epimers providing low yields and moderate diastereoselectivities.

1. Introduction

Isoleucine is a branch-chained amino acid carrying two chiral carbon atoms and thus exists as four stereoisomers (Scheme 1). L-Ile dominates in biological systems and it is essential in humans. The other stereoisomers were found in animals and in humans in trace amounts [1]. D-Ile was found in rat urine [2]. L-*allo*-Ile was found in rats [2] as well as in human plasma [3] and the level of L-*allo*-Ile in blood plasma is a sensitive diagnostic marker for Maple Syrup Urine Disease (MSUD) – a recessive deficiency of the branched-chain-ketoacids dehydrogenase complex [4]. The origin of L-*allo*-Ile in human plasma is not yet clear: an *in vivo* study with L-[^{13}C]-Ile proposed that L-*allo*-Ile is formed *via* retransamination of the corresponding α-keto acid; [5] however, different study with L-[^{15}N, ^{13}C]-Ile indicates that the C–N bond is preserved in an aldimine Schiff base formed with the aldehyde moiety of pyridoxyl phosphate-aminotransferase enzyme complex which is responsible for the isomerization [6]. Nevertheless, both mechanisms indicate that the carbon skeleton of L-*allo*-Ile is derived from L-Ile and that racemization of some of its derivative must be involved in the process. D-*allo*-Ile is probably more encountered than L-*allo*-Ile. It was found in human urine [3], in other mammals [2] and it is incorporated in some biologically active peptides [7,8,9], *e.g.* in antibiotic peptides isolated from amphibian skin [10,11]. Recently, isoleucine-2-epimerase was isolated from *Lactobacillus otakiensis* JCM 15040 bacteria [12]. The enzyme is the first racemase known to act significantly on L-Ile. In addition, amino acids often serve as a cheap source of chirality in asymmetric catalysis. The availability of all stereoisomers of isoleucine is therefore important not only to understand their role in organisms and for potential medicinal use (*e.g.* D-*allo*-Ile is a precursor to isostatine exhibiting antitumor activity) [13], but also asymmetric catalysis involving different isoleucine stereoisomers could lead to chiral products so far inaccessible. Thus, several procedures involving multi-step synthesis of D-*allo*-Ile from L-Ile and subsequent separation of the epimers exist [14].

Herein is reported a simple and cheap method for epimerization of isoleucine under alkaline condition catalyzed by the vanadium(V) complex with a Schiff base prepared by condensation of salicylaldehyde and isoleucine *in situ*. For the first time, no derivatization of isoleucine is required for the transformation and the method was successfully used for all stereoisomers of isoleucine. In addition, a new method for separation of isoleucine epimers is presented.

2. Experimental

All reagents and solvents were obtained from commercial sources and used as received. The NMR spectra were recorded with a Varian VNMRS 600 MHz instrument (600 MHz for ^{1}H, 157.88 MHz for ^{51}V). Chemical shifts (δ) are given in ppm relative to tetramethylsilane (^{1}H) and $VOCl_3$ (^{51}V). Samples of isoleucine were dissolved in 0.5 M solution of KOH in D_2O. The diastereomeric ratio was calculated from peak intensities attributed to chemical shifts of hydrogen atoms at α – carbon atoms of isoleucine.

2.1. Epimerization of isoleucine

A round-bottom flask was loaded with isoleucine (1.31 g, 10 mmol), NaOH (0.4 g, 10 mmol) and water (5 mL). The mixture was gently heated until dissolution. Salicylaldehyde (0.011 mL, 0.1 mmol) and NH_4VO_3

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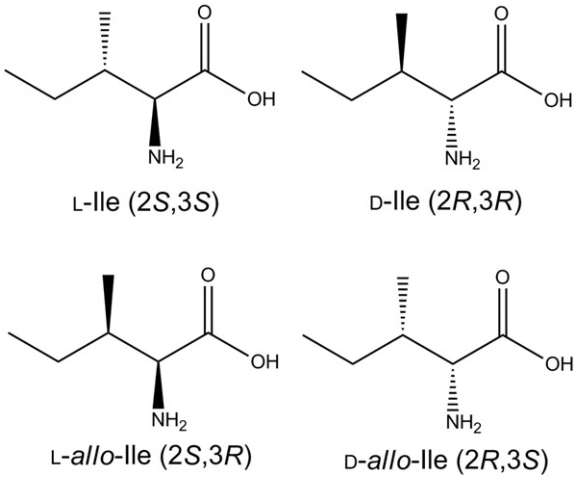


Scheme 1. Stereoisomers of isoleucine.

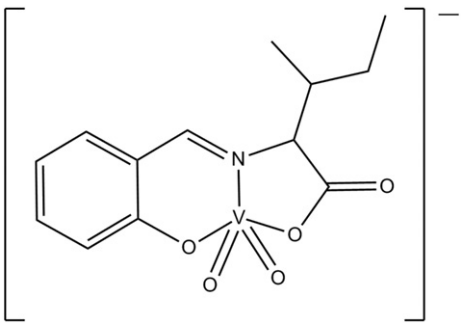
(0.0112 g, 0.1 mmol) were added and the yellow solution (pH = 11) was heated under reflux 6 h. After this period the heating was carefully continued without reflux to evaporate approx. 1/2 of the solvent. Then the solution was cooled to room temperature, 20 mL of EtOH and 0.7 mL of acetic acid were added. The mixture was cooled in a freezer below 0 °C, white precipitate of isoleucine was filtered off, washed with EtOH and dried in air.

3. Results and discussion

3.1. Epimerization of isoleucine

Isoleucine itself undergoes epimerization with the reaction half–life 10^5–10^7 years (D-allo-Ile/L-Ile ≈ 0.4) [15,16] what has found application in dating of biological samples in geochronology [17]. Treatment of L-Ile in strong alkaline solution (6 M NaOH) 6 h at 100 °C results in formation of D-*allo*-Ile in ≈0.7% yield, while in 6 M HCl no conversion is observed [18]. Thus, the conversion of L-Ile to D-*allo*-Ile occurs to small extend only in the alkaline regime. Recently, in the basic crystallization solutions of stereoisomers of $NBu_4[VO_2$(*N*-salicylidene-isoleucinato)] an unprecedented epimerization of the isoleucine residue was observed by ^{1}H NMR spectroscopy [19]. The formation of the complex anion (Scheme 2) *in situ* can be readily used for catalytic acceleration of isoleucine epimerization in alkaline solution.

The epimerization reaction (Table 1) was carried out upon dissolution of equimolar amounts of isoleucine (**A**) and NaOH in H_2O and addition of catalytic amounts of salicylaldehyde and NH_4VO_3 (1 mol%). After 6 h of heating under reflux the equilibrium of epimers (**B**) was achieved and isoleucine was recovered in high yields by the addition of EtOH, acidification with concentrated acetic acid and cooling below 0 °C (the solubility of isoleucine in the mixed EtOH/H_2O solvent decreases markedly in



Scheme 2. Structural formula of $[VO_2$(*N*-salicylidene-isoleucinato)$]^-$ as revealed by X-ray crystallography [19].

Table 1
Vanadium(V)-catalyzed epimerization of isoleucine under alkaline condition.

Entry	**A**	Yield, %	*de*, %	**B**, %			
				L-Ile	D-Ile	L-*allo*-Ile	D-*allo*-Ile
1	L-Ile	99	0	50	x	x	50
2	D-Ile	99	2	x	51	49	x
3	L-*allo*-Ile	89	4	x	48	52	x
4	D-*allo*-Ile	87	12	44	x	x	56

de = diastereomeric excess.

conjunction with rising proportion of EtOH and decreasing temperature) [20]. If equimolar amounts of salicylaldehyde and vanadate are used, the equilibrium is reached within 1 h. It should be noted here, that it is not expected that the epimerization shows up at the β – carbon position under the conditions presented here or in the earlier works. This process is much slower in Nature than α – epimerization [15] and so far can be achieved exclusively by enzymes [5,6].

NH_4VO_3 can be replaced by another common vanadate (*e.g.* KVO_3, Na_3VO_4) or even by a vanadium(IV) compound ($VOSO_4 \cdot nH_2O$) because under the reflux in air atmosphere V^{IV} is readily oxidized to V^V and the epimerization proceeds as with NH_4VO_3. A control reaction without a vanadium compound resulted in no epimerization. In order to inspect the effect of transition metal ions on the epimerization under alkaline condition the reaction was performed with salts of Mn^{II}, $Fe^{II/III}$, Co^{II}, Ni^{II}, Cu^{II}, Zn^{II}, $Mo^{VI}O_4^{2-}$ and $W^{VI}O_4^{2-}$. Indeed, there is a general discussion that metal ions, especially coordinated by Schiff base ligands, can catalyze racemization of amino acids [21]. However, replacement of vanadium(V) by other metal ions resulted in no epimerization.

The reaction was performed not only with L-Ile, but also using other stereoisomers of isoleucine. Thus, D-*allo*-Ile was transformed to L-Ile, D-

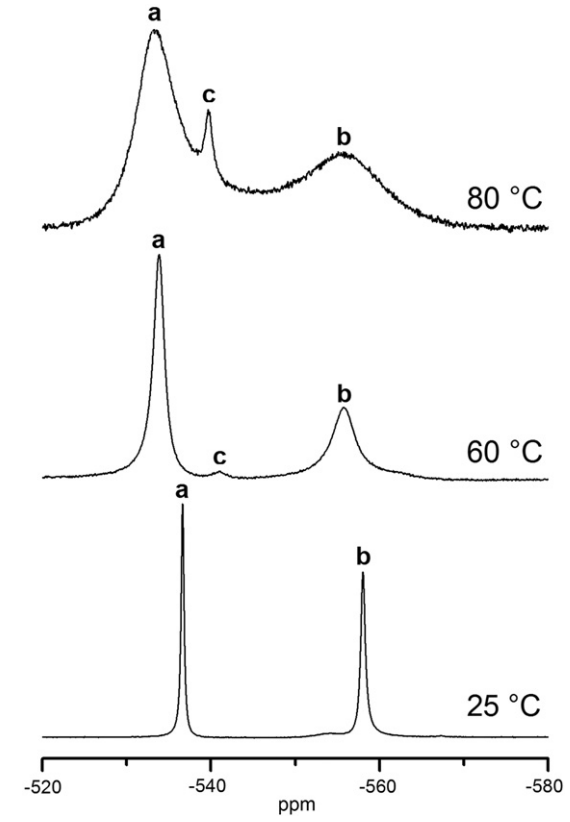


Fig. 1. ^{51}V NMR spectra of the reaction mixture at different temperatures; a: HVO_4^{2-}, b: $H_xV_2O_7^{(4-x)-}$, c: $[VO_2$(*N*-salicylidene-isoleucinato)$]^-$. Slight systematic movement of the chemical shifts and peak broadening are due to elevated temperature.