THE STEREOCHEMISTRY OF VINYLACETYL-CoA-ISOMERASE OF *CLOSTRIDIUM KLUYVERI*

H. HASHIMOTO, H. GÜNTHER and H. SIMON

Lehrstuhl für Organische Chemie und Biochemie des Organisch-Chemischen Laboratoriums der T.U. München, D 8 München 2, Arcisstr. 21, Germany

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1. Introduction

Vinylacetyl-CoA isomerases [EC 5.3.3.3], catalyzing the reaction **1a** → **2a** have been found in mammals [1] and microorganisms, such as *Clostridi* [2]. Little about the mechanism, and nothing about the stereochemistry of this 1,3-proton shift is known. An internal hydrogen transfer appears to be involved because tritium was not incorporated into the acyl portion of β-methyl-crotonyl-CoA when β-methylvinyl-acetyl-CoA was isomerized in H_2O/HTO by the enzyme from ox liver [1]. From the inhibitory action of *p*-chloromercuribenzoate Bartsch and Barker [2] inferred a functional thiol group. In the isomerization **1** → **2** the carbon atoms 2 and 4 are involved. Whether the pro R or pro S hydrogen atom at C-2 is eliminated and from which side the hydrogen atom at carbon atom 4 enters, was here determined by the fermentation of (R,S)-α-methylvinylacetate (**1b**) and tiglate (**2b**) with whole cells in H_2O or with Δ^3-pentenate (**1c**) in D_2O, respectively.

2. Materials and methods

C. kluyveri cultures were originally revived from dried crotonate cells (C.F. Boehringer u. Soehne, Biochemica, Tutzing) and then grown as described [3] without changing the crotonate medium to ethanol acetate. α-Methylvinyl-acetate [4] and Δ^3-pentenate [5] were synthesized. The purity was checked by GLC and NMR-spectroscopy.

For GLC aliquots were acidified with 0.2 N HCl and the organic acids separated on a column (69 in. × 1/12 in.) filled with 20% neopentylglycol succinate and 2% phosphoric acid on Chromosorb P.

Compounds **1b** and **3a** were separated by countercurrent distribution with n-hexane and water.

Other preparative separations of acids were carried out by the method of Marvel and Rands [6].

For polarometric measurements a Jasco Model ORD/UV-5 (Japan Spectroscopic) was used.

3. Results and discussion

In the presence of crotonate or butyrate, *C. kluyveri* converted (R,S)-α-methylvinylacetate (R,S **1b**) only partially to α-methylbutyrate (**3a**) (table 1). The remaining α-methylvinylacetate was isolated and was found to be the (R)(−)-enantiomer (R, **1b**) with a specific optical rotation (table 1) nearly identical to the highest reported value [7]. Obviously, reaction occurred only with the (S)(+)-enantiomer of **1b**, and its hydrogen atom (R^1 = H) had migrated. Therefore one has to assume that the pro S hydrogen atom vinylacetyl-CoA migrates to form crotonyl-CoA.

The fermentation product, [S] (+)-α-methylbutyrate (**3a**), had the optical rotation (table 1) reported before [8], in accordance with the absolute stereochemistry of the butyryl-CoA dehydrogenase recently determined by us [3]. Then it was assumed that **2b** was the intermediate. Substrate **2b** led also to **3a**, with the same specific rotation (table 1).

In the fermentation of Δ^3-pentenate (**1c**) in D_2O, **1c** is hydrogen donor and acceptor. Obviously, one equivalent of **1c** was hydrogenated to **3b** and another was degraded via **1c** → **2c** → **4** → **5** → **6** + **7**.

Table 1
Fermentation[a] of (R,S) α-methylvinylacetate (R,S**1b**) 2-methylcrotonate (**2b**) in H_2O and Δ^3-pentenate (**1c**) in D_2O.

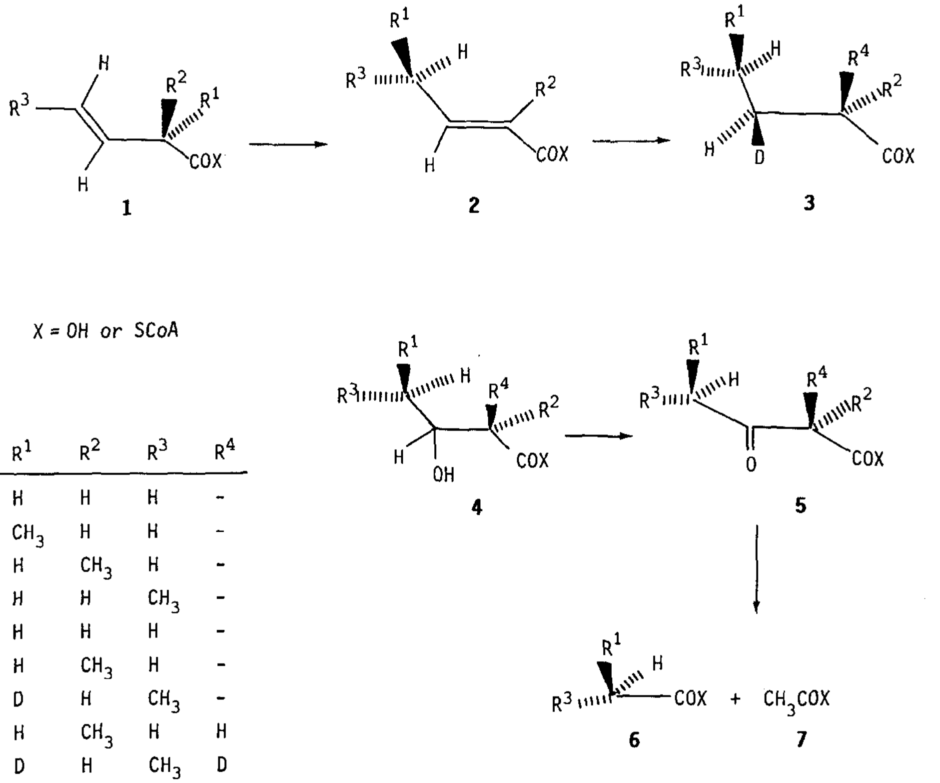
Substrate and cosubstrate (mMol)	*C. kluyveri* (g wet packed cells/ mMol substrate)	Products[b] (mMol)	Specific optical rotation
(R,S) α-methylvinylacetate (20) and butyrate (10)	1.5	(S) α-Methylbutyrate (9.6) and	$[\alpha]^D = +21.0°$
		(R) α-methylvinylacetate (7.0)	$[\alpha]^D = -39.7°$
2-Methylcrotonate (tiglinate) (1.0) and crotonate (1.0)	0.75	(S) α-Methylbutyrate (0.54)	$[\alpha]^D = +21.0°$
Δ^3-Pentenate (5.0)	1.5	(R) [2-^{2}H]propionate[c] (1.33)	$[\alpha]^{365} = -2.0°$
Δ^3-Pentenate (2.0)	3.6	(R) [2-^{2}H]propionate[d] (0.42)	$[\alpha]^{365} = -1.3°$

[a] The cells were harvested by centrifugation (12 000*g*), washed twice with 0.1 M phosphate buffer pH 7.0 and incubated (40 hr) with substrates at 37°C. All manipulations were done under nitrogen.

[b] Only products of interest from a stereochemical point of view are given.

[c] 0.64 deuterium atoms/mole.

[d] 0.50 deuterium atoms/mole.



X = OH or SCoA

	R^1	R^2	R^3	R^4
1a	H	H	H	-
(S) **1b**	CH_3	H	H	-
(R) **1b**	H	CH_3	H	-
1c	H	H	CH_3	-
2a	H	H	H	-
2b	H	CH_3	H	-
2c	D	H	CH_3	-
3a	H	CH_3	H	H
3b	D	H	CH_3	D
4	D	H	CH_3	D
5	D	H	CH_3	D
6	D	-	CH_3	-

Scheme 1

For this reason, crotonate or butyrate was not added during the incubations. The table shows the deuterium amounts and optical rotations of propionate **6**, isolated from the experiment in D_2O. According to the determination of the absolute stereochemistry [9, 10] *it had the* R *configuration. The conversion* **2c** → **6** should not change the configuration of the original carbon atom 4 of **2c**. Consequently, the hydrogen atom entered from the re-side. The deuterium content of the medium was 90% D_2O, but only 0.6 deuterium atoms were incorporated. Thus, the 1.3 proton shift appears to be only partially intramolecular, in contrast to the results of Rilling and Coon [1].

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