Determination of the Configuration of Diastereoisomers of 2-Alkyl-3-hydroxybutanoates with Gas Chromatography

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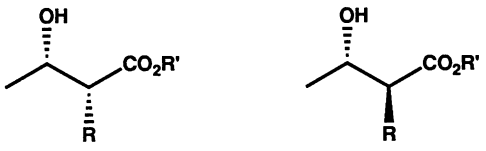
Synopsis. Diastereoiscomers of 2-alkyl-3-hydroxybutanoates are separated by gas chromatography. The threo (anti)-isomers have shorter retention times than those of the corresponding erythro (syn)-isomers.

An ester of 2-alkyl-3-hydroxybutanoic acid (**1**) is an important chiral building block in organic syntheses, particularly in the syntheses of natural products and antibiotics.[1] To synthesize these valuable compounds,



syn-1 *anti-1*

Structures of *syn*- and *anti*-alkyl 2-alkyl-3-hydroxybutanoate

1 must be prepared in diastereselective manner, and the development of methodology to distinguish syn/anti-diastereoisomers of **1**[2] has also become important. However, ^{1}H NMR spectroscopy is only one device so far reported, to the authors' best knowledge, to analyze the diastereoisomers,[3,4] where the difference in coupling constant between the methine protons on C_2 (–CHRCO$_2$R′) and C_3 (–CHCOH–) is employed. However, since these methine protons also couple with many other protons in the molecule, the signals from these protons become broad and the coupling constant becomes obscure. Furthermore, it is impossible to determine relative amounts of the syn- and anti-isomers by ^{1}H NMR spectroscopy when they are mixed in a solution because their key-signals overlap each other. We report here that gas chromatography (GC) is much superior to the ^{1}H NMR spectroscopy in determining the configuration of diastereisomers of **1** and to analyze their amounts in a mixture quantitatively.

Results and Discussion

Various esters of 2-alkyl-3-hydroxybutanoic acid were prepared by reduction of the corresponding 3-oxo-derivatives with sodium borohydride. Methl, ethyl, propyl, propargyl, and allyl were employed as the 2-alkyl groups. The reduction gave a nearly 1:1 mixture of the syn- and anti-isomers in every cases. GC analysis of the product with polyethylene glycol (PEG) column exerted two peaks that correspond to two diastereoisomers of **1**. To confirm the relationship between the configuration and the retention time on GC, the authentic anti-isomers of **1** were prepared by alkylation of the dianion of 3-hydroxybutanoate with alkyl halides.[5] The anti-isomers so far prepard were subjected to the GC analysis and their retention times were compared with those of the products from the borohydride reduction. Results are listed in Table 1. In each case, it appears that the anti-isomer is retained less in the PEG column than the corresponding syn-isomer.

The relationship between the retention time and the configuration of the molecule seems to be accounted for by the stability of the conformations of syn- and anti-isomers that are shown in Fig. 1. As will be discussed later, the molecule is stabilized by intramolecular hydrogen bond. Therefore, it is resonable to expect that A_1 is the most abundant conformation among three major conformations of *anti*-**1**. The importance of intramolecular hydrogen bond also suggests the most stable conformation for the *syn*-**1** to be either S_1 or S_2. However, at the same time, these conformations seem to be unfavorable from the viewpoint of steric effect, and the contribution of the S_3-conformation for this molecule in certain extent cannot be denied.

Similar conclusion has been reported by House and his co-workers.[4] That is, ^{1}H NMR and IR spectroscopies reveal that the intramolecular hydrogen bonding is more important for the anti-isomer of a

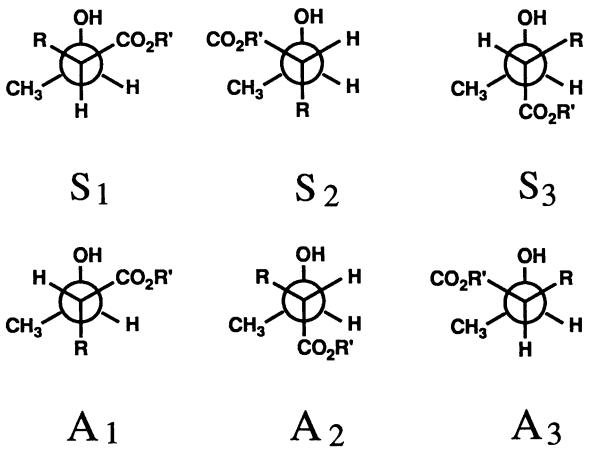


Fig. 1. Possible conformations for *syn*- and *anti*-2-alkyl-3-hydroxybutanoates.

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Table 1. Retention Times of 2-Alkyl-3-hydroxybutanoates on Gas Chromatograph[a]

Alkoxyl group (R′)	Alkyl group at the 2-position (R) Methyl syn	anti (α)	Ethyl syn	anti (α)	Propyl syn	anti (α)	Propargyl syn	anti (α)	Allyl syn	anti (α)
C_2H_5O	15.0	14.2 (1.06)[b]	22.6	18.2 (1.24)[b]	31.2	25.3 (1.23)[b]	27.1	22.3 (1.21)[c]	39.2	30.9 (1.27)[b]
C_3H_7O	—		30.3	24.2 (1.24)[b]	38.8	31.5 (1.23)[b]	37.4	30.7 (1.22)[c]	55.7	43.7 (1.27)[b]
C_4H_9O	32.2	30.0 (1.07)[b]	49.5	39.1 (1.27)[b]	—		19.0	16.5 (1.15)[d]	25.6	21.4 (1.20)[c]
$C_5H_{11}O$	46.6	43.4 (1.07)[c]	—		—		—		38.9	30.6 (1.27)[c]
$C_6H_{13}O$	23.0	22.3 (1.03)[c]	13.5	11.9 (1.13)[d]	—		—		19.8	17.3 (1.14)[d]
$C_8H_{17}O$	50.0	48.2 (1.04)[c]	25.3	22.1 (1.14)[c]	—		67.9	58.3 (1.16)[d]	36.9	32.5 (1.14)[d]
Me_3CO	13.3	12.3 (1.08)[b]	20.3	15.7 (1.29)[b]	26.9	21.2 (1.27)[b]	23.4	18.8 (1.24)[c]	33.4	25.4 (1.31)[b]
Me_3CCH_2O	26.4	24.9 (1.06)[b]	39.7	31.3 (1.27)[b]	53.6	43.0 (1.25)[b]	42.4	35.0 (1.21)[c]	19.9	16.7 (1.19)[c]
$MeO(CH_2)_2O$	69.5	64.5 (1.08)[b]	—		—		36.8	33.7 (1.09)[d]	17.2	16.3 (1.06)[d]
$EtO(CH_2)_2O$	69.1	64.0 (1.08)[b]	—		—		—		19.7	17.3 (1.14)[d]
$BuO(CH_2)_2O$	—		—		—		61.4	52.7 (1.17)[d]	33.5	29.0 (1.16)[d]
$MeS(CH_2)_2O$	70.4	65.0 (1.08)[b]	—		—		—		49.0	43.3 (1.13)[d]

a) Retention time in min. α: Separation factor. b) Column temp=120 °C. c) Column temp=150 °C. d) Column temp=180 °C.

β-keto alcohol than for the corresponding syn-isomer. The difference in the importance of intramolecular hydrogen bonding diminishes when the hydroxyl group is acylated or when the spectra are recorded in a protic solvent.

Because of the contribution of the polar conformation $\mathbf{S_3}$, the syn-isomer is more polar than the anti-isomer, and, consequently, the former interacts with polar PEG stronger than the latter resulting in longer retention time for the former than for the latter when they are subjected to GC packed with PEG. The idea of the dipolar interaction between the molecule and the stationary phase of GC was further tested by the use of GC with less polar stationary phase (OV-330), where the separation between the diastereoisomers was much worse[6] than that with PEG.

The satisfactory separation of the diastereoisomers was observed not only with alkyl esters but also with oxygen- or sulfur-substitued alkyl ester. Thus, the diastereoisomers of 2-methoxyethyl, 2-ethoxyethyl, 2-butoxyethyl, and 2-methylthioethyl esters were separated on GC satisfactorily for quantitative analyses, and the order of retention times of the syn- and anti-isomers were found to be the same as those for the alkyl esters.

2-Methylacetoacetate reductase from *Ascaris lumbricoides* has been reported to reduce ethyl 2-methyl-3-oxobutanoate to afford only syn-isomer as the major product along with the formation of another diastereoisomer in minor extent.[7] The assignment was based on the coupling constant of methine protons which are 7.2 Hz and 4.8 Hz respectively. Fortunately, the literature shows the gas chromatograms of the isomers obtained from GC with a polar (20% diethylene glycol adipate–3% phosphoric acid) column, and it is comfirmed that the major product retains shorter than the minor one. Since the polarity of their and our PEG columns do not differ extensively, we believe that the major isomer obtained by the enzymic reduction is the anti-isomer and vice versa in contrast to the stereochemistry reported. The large contribution of the conformation $\mathbf{A_1}$ to the anti-isomer accounts for the large ^1H NMR coupling constant for this isomer, and the importance of the conformations $\mathbf{S_1}$ and $\mathbf{S_2}$ explains why the syn-isomer excerts such a small coupling constant as reported.

Experimental

Materials. Various esters of 2-alkyl-3-hydroxybutanoates were prepared according to the literature method.[5] Other chemicals were purchased from Nakalai Tesque Co. and used without further purification.

Analysis. Gas chromatography was carried out with a Yanaco G-1800 Gas Chromatograph equipped with FID and packed with 5% polyethylene glycol 20 M (PEG) or OV-330 supported on Chromosorb W AW-DMCS (3 mm×1 m). The injection and detecter temperatures were set at 180 °C and 200 °C, respectively. GC was done at carrier (N_2) flow rate of 29 ml min^{-1}.

References

1) T. Oishi and T. Nakata, *Acc. Chem. Res.*, **17**, 338 (1984): S. Masamune and W. Choy, *Aldrichimica*, **15**, 47

(1982).

2) For simplicity, we define that the (2*R*,3*R*)- and (2*S*, 3*S*)-isomers as the anti-isomer and the (2*R*,3*S*)- and (2*S*, 3*R*)-isomers as the syn-isomer, respectively. We prefer, in this article, the syn/anti expression rather than the threo/erythro notation because the latter is a subject of substituent-dependent priority.

3) K. Maskens and N. Polgar, *J. Chem. Soc., Parkin Trans. 1*, **1973**, 109.

4) H. O. House, D. S. Crumrine, A. Y. Teranishi, and H. G. Olmstead, *J. Am. Chem. Soc.*, **95**, 3310 (1973).

5) G. Fráter, U. Müller, and W. Günther, *Tetrahedron*, **40**, 1269 (1984).

6) The separation factor, α, for ethyl 2-allyl-3-hydroxybutanoate on PEG and OV are 1.27 and 1.16, respectively. α for ethyl 2-methyl-3-hydroxybutanoate is 1.06 on PEG, whereas the isomers of this ester are not separated each other on OV.

7) Z. Suarez de Mata, H. J. Saz, and D. J. Pasto, *J. Biol. Chem.*, **252**, 4215 (1977).