Absolute Configuration and Conformation of 1,3-Dioxanes from Cider

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In extracts obtained by liquid−liquid extraction from French cider (2*S*,4*R*)- and (2*R*,4*R*)-2-methyl-4-pentyl-1,3-dioxane, **1a** and **1b** as well as (2*S*,4*R*)- and (2*R*,4*R*)-2-methyl-4-(2′(*Z*)-pentenyl)-1,3-dioxane, **2a** and **2b**, were identified by capillary gas chromatography (HRGC) and capillary gas chromatography−mass spectrometry (HRGC−MS). Absolute configuration and conformation of the 1,3-dioxanes was determined by nuclear magnetic resonance (NMR) spectrometry techniques [^{13}C, ^{1}H, nuclear Overhauser enhancement (NOE), and H/H homonuclear decoupling], multidimensional gas chromatography (MDGC), and by comparison with synthesized reference compounds. A nonenzymatic formation of **1a** and **1b** and **2a** and **2b** during fermentation of apple juice was proposed leading to 22, 8, 2, and <1 mg/L of **1a**, **2a**, **1b**, and **2b**, respectively in cider.

Keywords: *Cider; enantiodifferentiation; 2-methyl-4-pentyl-1,3-dioxane; 2-methyl-4-(2′(Z)- pentenyl)-1,3-dioxane; conformation*

INTRODUCTION

During fermentation, besides ethanol, a considerable amount of acetaldehyde is formed by yeast (Dittrich, 1987). Due to its high reactivity toward nucleophilic substances it reacts with many alcohols, amines, and thiols present in food (Laws and Peppard 1982; Borea et al., 1980). Acetals, the reaction products with alcohols, have already been detected in many fermented beverages (Williams et al., 1980; Calixto and Bermejo, 1980; Yavas and Rapp, 1992). In wine, e.g., the presence of 1,1-diethoxyethane and 2,4,5-trimethyl-1,3-dioxolane (Brander et al., 1980) has been reported, and the investigators noted that their levels increased during storage (Schreier, 1979; Schreier et al., 1976). To date, only 4-hydroxymethyl-2-methyl-1,3-dioxolane and 2,4,5-trimethyl-1,3-dioxolane have been identified in cider as the first cyclic acetal originating from acetaldehyde and the multifunctional alcohols glycerol and butane-2,3-diol, respectively (Yavas and Rapp 1992). However, there is no information available concerning the configuration of those 1,3-dioxolanes.

Cider or fermented apple juice is well-known and widely produced throughout the world. By tradition, specific cider apple varieties are preferred by the industry. Recently large amounts of octane-1,3-diol and 5(*Z*)-octene-1,3-diol have been detected in some French cider apple varieties used for cider production (Beuerle et al., 1996). Thus, we expected the occurrence of 1,3-dioxanes, derived from 1,3-diols and acetaldehyde formed during fermentation in French cider. Consequently, this paper is concerned with the identification of 1,3-dioxanes in French cider and the determination of the absolute configuration and conformation of the identified products.

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EXPERIMENTAL PROCEDURES

Samples. Apple fruits (*Malus sylvestris* Mill cv. Peau de Chien), apple juice, freshly prepared cider, and one-year-aged cider were kindly provided from Pernod Ricard, France.

General. All chemicals obtained by Aldrich (Steinheim, Germany), Fluka (Neu-Ulm, Germany), Lancaster (Morecambe, England), Merck (Darmstadt, Germany), Roth (Karlsruhe, Germany), and Sigma (Deisenhofen, Germany) were of high purity at purchase, and solvents were redistilled before use. Silica gel 60 (0.032−0.063 mm) (Merck, Darmstadt) was used for flash chromatography.

Isolation of 1,3-Dioxanes. Apple juice (600 mL) or cider (600 mL) was diluted with 200 mL of water and subjected to continuous liquid−liquid extraction using 250 mL of pentane−CH_2Cl_2 2:1. The organic extract was dried (Na_2SO_4), concentrated after addition of 5 mg of the internal standard phenol, and analyzed by HRGC and HRGC−MS. Further purification was achieved by flash chromatograhy on silica gel using pentane−diethyl ether 9:1.

Isolation of (*R*)-Octane-1,3-diol and (*R*)-5(*Z*)-Octene-1,3-diol. Both diols were separated from apple juice according to the procedure of Beuerle et al. (1996).

Synthesis of Reference Compounds. *(a) (R)- and (S)-Octane-1,3-diol*. According to Barchi et al. (1984), 2(*E*)-octenol was epoxidized using the Sharpless method (Katsuki and Sharpless, 1980) employing natural (+)- and (−)-diethyl tartrate for the synthesis of (*S*)- and (*R*)-octane-1,3-diol, respectively.

(b) 5(Z)-Octene-1,3-diol. Racemic 5(*Z*)-octene-1,3-diol was synthesized according to Beuerle et al. (1996).

*(c) 2-Methyl-4-pentyl-1,3-dioxane **1a**−**d** and 2-Methyl-4-(2′(Z)-pentenyl)-1,3-dioxane **2a** and **2b***. Octane-1,3-diol (12 mg) or 5(*Z*)-octene-1,3-diol (12 mg) and 0.1 mg of toluenesulfonic acid were dissolved in 10 mL of absolute diethyl ether and stirred for 24 h after the addition of 5 mg of acetaldehyde and 10 mg of Na_2SO_4. After filtration, the solution was concentrated and analyzed by HRGC and HRGC−MS. Purification was achieved by flash chromatography on silica gel using pentane−diethyl ether (9:1). Quantitative yields were obtained. **1a/1c:** R_i 1418; EI-MS (cf. Figure 2); NMR (cf.

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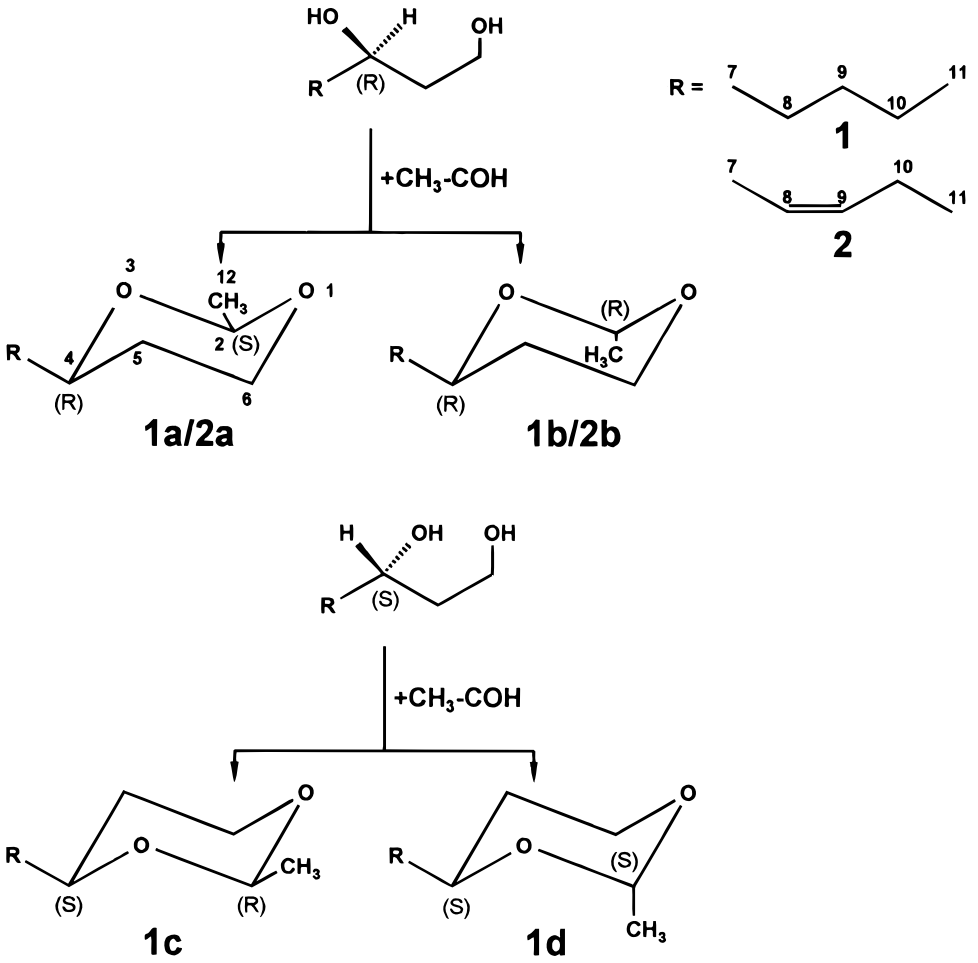

Figure 1. Synthesis of compounds **1a**−**d** and **2a** and **2b**.

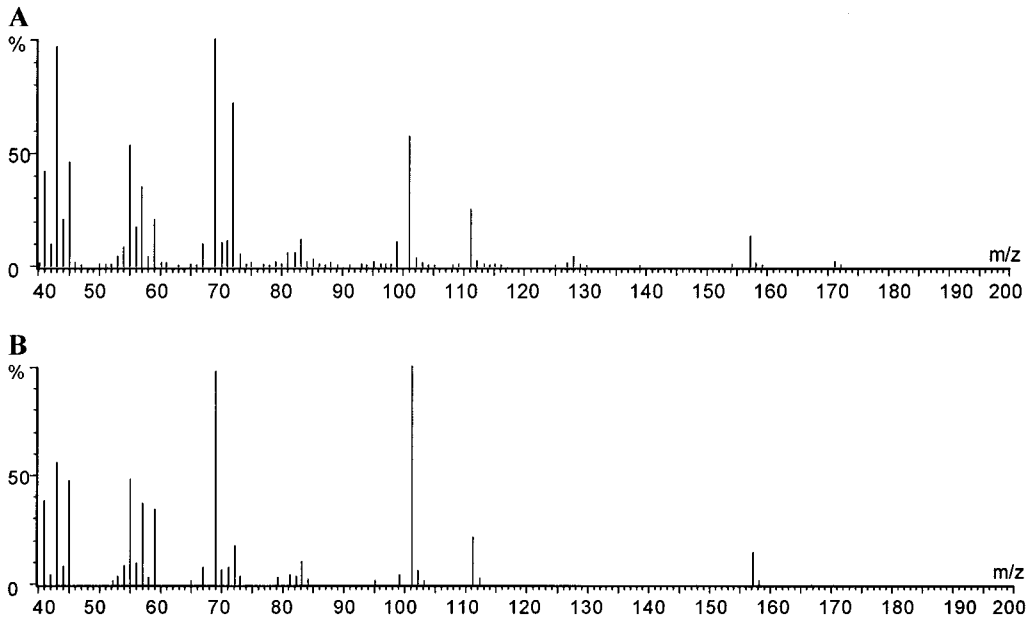

Figure 2. Mass spectral data of **1a/1c** (A) and **1b/1d** (B).

Tables 1 and 2). **1b/1d:** R_i 1485; EI-MS (cf. Figure 2). **2a:** R_i 1475; EI-MS (cf. Figure 3); NMR (cf. Table 1 and 2). **2b:** R_i 1524; EI-MS (cf. Figure 3).

Capillary Gas Chromatography (HRGC). GC analyses were carried out with a Hewlett Packard 5890 Series II instrument fitted with a split injector (1:20) at 250 °C and a FID at 250 °C. A J&W DB-Wax fused silica capillary column (30 m × 0.25 mm i.d.; df = 0.25 μm), which was programmed from 50 °C (for 3 min) to 240 °C at 4 °C/min, was used with 3 mL/min of helium gas. The GC integration of the peaks, as area percent, was performed on a Shimadzu C-R6A Chromatopac data processor. Calculation of retention indices was conducted on the basis of *n*-hydrocarbons with the aid of an additional BASIC program.

Capillary Gas Chromatography−**Mass Spectrometry (HRGC**−**MS).** A Finnigan MAT 44 quadrupol mass spectrometer coupled to a Varian 3300 GC equipped with Finnigan MAT PCDS software (version 4.0) was used. Capillary GC

Table 1. ^{1}H NMR Spectral Data of Compounds 1a and 2a (400 MHz, $CDCl_3$)[a]

δ	signal	J	atom
compound **1a**			
0.90	3H, t	7.0	H11
1.28−1.45	8H, m		H7-H10
1.34	3H, d	5.1	H12
1.40	1H		H5*equatorial*
1.67	1H	12.0/10.3	H5*axial*
3.60	1H, m	12.0/7.0/1.0	H4*axial*
3.75	1H, ddd	12.0/10.0/2.0	H6*axial*
4.08	1H, dd	11.0/5.1	H6*equatorial*
4.70	1H, q	5.1	H2*axial*
compound **2a**			
0.96	3H, t	7.5	H11
1.32	3H, d	5.2	H12
1.44	1H, m		H5*equatorial*
1.61	1H, m	12.0/11.4	H5*axial*
2.05	2H, quintet	7.5	H10
2.24	1H, quintet	7.0	H7b
2.35	1H, quintet	7.0	H7a
3.61	1H, m	12.0/7.0/1.0	H4*axial*
3.72	1H, ddd	12.0/10.0/3.0	H6*axial*
4.09	1H, ddd	10.0/5.0/1.5	H6*equatorial*
4.69	1H, q	5.2	H2*axial*
5.35	1H, m	11.0/7.0	H8
5.50	1H, m	11.0/7.5	H9

[a] δ relative to solvent signal of TMS.

Table 2. ^{13}C NMR Spectral Data of Compounds 1a and 2a (100 MHz, $CDCl_3$)[a]

δ	DEPT	atom
compound **1a**		
13.6	CH_3	C11
20.9	CH_3	C12
22.3	CH_2	C10
24.3	CH_2	C8
30.9	CH_2	C9
31.5	CH_2	C7
35.7	CH_2	C5
66.3	CH_2	C6
76.9	CH	C4
98.7	CH	C2
compound **2a**		
14.1	CH_3	C11
21.3	CH_3	C12
22.6	CH_2	C10
30.8	CH_2	C7
33.8	CH_2	C5
66.6	CH_2	C6
76.9	CH	C4
99.0	CH	C2
123.7	CH	C9
134.1	CH	C8

[a] δ relative to solvent signal of TMS.

conditions as above were employed. Significant MS operating parameters: ionization voltage, 70 eV (electron impact ionization); ion source and interface temperature, 240 °C; scan range, 40−249 u; scan duration, 0.69 s. Constituents were identified by comparison of their mass spectra and retention indices with those of authentic reference compounds.

Multidimensional Capillary Gas Chromatography (MDGC). MDGC analyses of octane-1,3-diol and 5(*Z*)-octene-1,3-diol were carried out according to Beuerle et al. (1996). MDGC analyses of the 1,3-dioxanes **1a** and **1b** and **2a** and **2b** were performed with two Fisons GCs (8160 and 8130) fitted with a split injector (1:10) at 230 °C and two FIDs at 250 °C (Figure 4). A J&W DB-Wax fused silica capillary column (30 m × 0.25 mm i.d.; df = 0.25 μm) was used in the first GC for the preseparation of volatiles. Separation of enantiomers of 1,3-dioxanes was achieved in the second GC using a fused silica capillary column coated with 2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-β-cyclodextrin/OV 1701 (25 m × 0.25 mm i.d.; df = 0.15 μm). The column in GC 1 was connected by a multicolumn switching system (MCSS) (Fisons) to the column in GC 2. The following temperature programs were applied: GC 1, 50 °C, 3 min isothermal, then to 240 °C at 2 °C/min; GC 2, 50 °C, 30 min isothermal, then to 200 °C at 2 °C/min, cut 30.7 min to 31.1 min. Evaluation of the elution order of enantiomers was achieved using synthesized reference compounds with known enantiomeric ratio.

Nuclear Magnetic Resonance. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker WM 400 (400 MHz) spectrometer with $CDCl_3$ (Merck, Darmstadt) as solvent.

RESULTS AND DISCUSSION

Compounds **1a**−**d** and **2a** and **2b** (Figure 1) have been synthesized and characterized by HRGC−MS (Figures 2 and 3) and NMR (cf. Tables 1 and 2). In each case the product ratio of the diastereomeric pairs of **1a/1b, 1c/1d**, and **2a/2b** was approximately 10:1, which corresponded to the results obtained for the synthesis of 1,3-oxathianes (Mosandl and Heusinger 1985). The (*R*)- and (*S*)-enriched octane-1,3-diol was obtained by Sharpless epoxidation of 2(*E*)-octenol using (−)- and (+)-diethyl tartrate as the chiral ligand, respectively followed by reduction with $LiAlH_4$. Pure (*R*)-octane-1,3-diol and (*R*)-5(*Z*)-octene-1,3-diol was isolated from apple juice cv. Peau de Chien (Beuerle et al., 1996), and racemic 5(*Z*)-octene-1,3-diol was synthesized according to Beuerle et al. (1996). Separation of the synthesized enantiomeric pair **1a/1c** was achieved by MDGC using a capillary column coated with 2,3-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-β-cyclodextrin/OV 1701 (Figure 4).

In extracts obtained by liquid−liquid extraction from French cider the previously unknown 1,3-dioxanes **1a** and **1b** and **2a** and **2b** derived from (*R*)-octane-1,3-diol and (*R*)-5(*Z*)-octene-1,3-diol, respectively, have been identified by HRGC−MS and MDGC.

The configuration at carbon 4 carrying the secondary alcohol group of the naturally occurring **1a** and **1b** and **2a** and **2b** was assigned *R* due to the chromatographic behavior of the respective 1,3-dioxanes (cf. Figure 4). As expected, this result agreed very well to the occurrence of enantiomerically pure (*R*)-octane-1,3-diol (Schwab et al., 1989) and (*R*)-5(*Z*)-octene-1,3-diol (Beuerle et al., 1996) in apple fruits. Although 4-hydroxymethyl-2-methyl-1,3-dioxolane and 2,4,5-trimethyl-1,3-dioxolane have already been identified in cider, elucidation of their absolute configuration has not yet been performed.

^{1}H NMR H/H homonuclear decoupling experiments of **1a** and **2a** showed vicinal coupling constants 4-H/5*axial*-H (12 Hz) and 4-H/5*equatorial*-H (1 Hz). These are typical coupling constants for axial protons and incompatible with the presence of equatorial protons in position 4 (Eliel and Knoeber, 1968). Concerning the configuration at carbon 2 originating from the carbonyl carbon of acetaldehyde the equilibrium between equatorial and axial 2-alkyl groups lies very much on the side of the equatorial conformation (Eliel and Knoeber, 1968). NOE experiments of **1a** and **2a** showed positive enhancement of the proton at position 6*axial* and 4 when irradiating the frequency of proton at carbon 2 and *vice versa* (Tables 3 and 4). Consequently, protons at positions 2, 4, and 6*axial* are all axial, confirming the chair conformation of the 1,3-dioxanes and the (*S*)-configuration at position 2 as expected for the major products **1a** and **2b** (Eliel and Knoeber, 1968). In analogy to Mosandl and Heusinger (1985) and Eliel and Knoeber (1968), the minor compounds **1b** and **2b** were assigned as (2*R*,4*R*)-2-methyl-4-pentyl-1,3-dioxane and (2*R*,4*R*)-2-methyl-4-(2′(*Z*)-pentenyl)-1,3-dioxane, respectively, on the basis of their chromatographic behavior

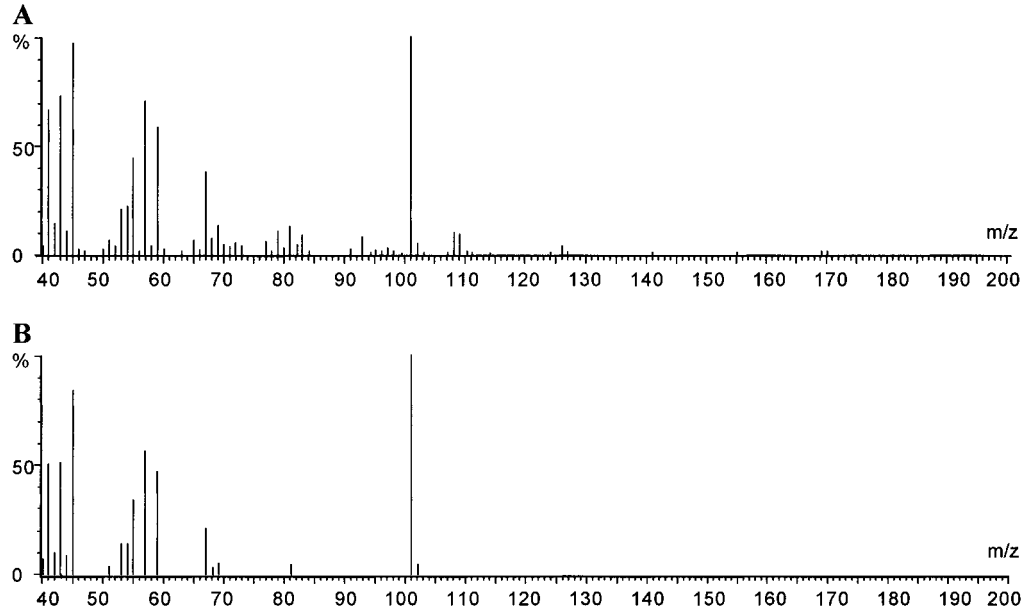

Figure 3. Mass spectral data of **2a** (A) and **2b** (B).

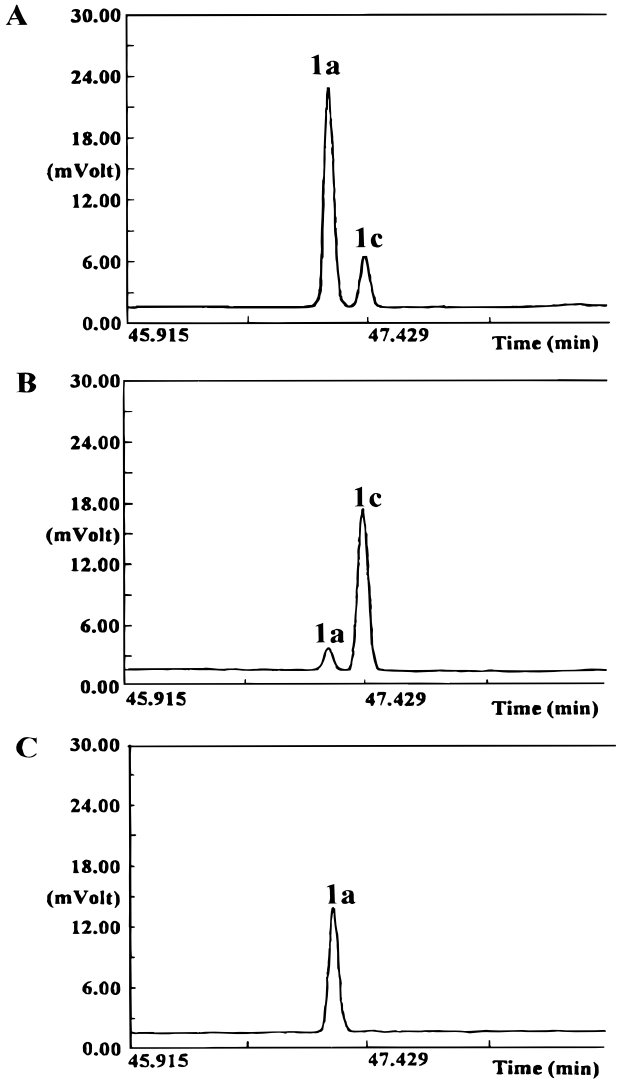

Figure 4. MDGC separation of enantiomeric pair **1a/1c**: enriched **1a** (A), enriched **1c** (B), and compound **1a** isolated from french cider (C). (For MDGC conditions see Experimental Procedures.)

and mass spectra and by comparison with the synthesized reference compounds.

Table 3. NOE Experiment of Compound 1a (400 MHz, $CDCl_3$)[a]

		irradiated				
	proton	2*axial*	4*axial*	6*axial*	6*equatorial*	12*equatorial*
obsd	2*axial* (4.7)		4%	2%		3%
	4*axial* (3.6)	4%		*b*		
	6*axial* (3.7)	3%	*b*		11%	
	6*equatorial* (4.1)			6%		
	12*equatorial* (1.3)	2%				

[a] δ relative to solvent signal of TMS. [b] Evaluation not feasible.

Table 4. NOE Experiment of Compound 2a (400 MHz, $CDCl_3$)[a]

		irradiated				
	proton	2*axial*	4*axial*	5	6*axial*	6*equatorial*
obsd	2*axial* (4.7)		4%		4%	
	4*axial* (3.6)	3%			3%	
	5*axial* (1.6)					2%
	6*axial* (3.7)	3%	2%			10%
	6*equatorial* (4.1)				7%	

[a] δ relative to solvent signal of TMS.

CONCLUSION

Using phenol as internal standard in freshly prepared and one-year-aged cider, 22 mg of **1a**/L and 8 mg of **2a**/L were detected whereas apple juice contained only trace amounts. Concentration of **1b** (2 mg/L) and **2b** (<1 mg/L) corresponded to 10% of the concentration of the thermodynamically more stable **1a** and **2b,** respectively. Thus, we assume a chemical synthesis of **1a** and **1b** and **2a** and **2b** during fermentation of apple juice since a similar ratio of **1a**/**1b** and **2a**/**2b** was obtained during the preparation of the 1,3-dioxanes. As the 1,3-dioxanes exhibit a weak "green note" they might contribute to the flavor of cider.

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