

Absolute Stereochemistry and Enantiomeric Excess of 2-Butanol in Distilled Spirits of Different Origin

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A great number of distillates of different origin were analyzed to determine the concentration of 2-butanol as well as the predominant enantiomeric form of this secondary alcohol and its excess. Chirality determinations were carried out using bidimensional gas chromatography ("heart cut" technique coupled to GC analysis with methylated β -cyclodextrin capillary column). Enantiomeric excesses of the *R*-form, ranging from 50 to 80%, were observed in all cases. This finding can be regarded as a further support to the general assumption that 2-butanol present in distillates is formed by the action of lactic acid bacteria on the mashes.

INTRODUCTION

2-Butanol is a typical constituent of distillates obtained from the fermented residues of the grape-pressing procedure in the wine-making process (Postel and Adam, 1989). Its concentration appears to be largely variable in pomace brandies ranging, when expressed in milligrams per 100 mL of pure ethanol, from 20 to 300 in Italian grappa (Tandoi Scopigno et al., 1982), from 1 to 100 in French marc, and from 30 to 50 in German tresterbraunwein (Postel and Adam, 1989).

The average content of this alcohol is also very high in fruit brandies, particularly in Calvados, pome, and Williams pear distillates (20–100 mg/100 mL of pure ethanol) (Postel and Adam, 1989; Postel, 1982, 1984). By contrast, 2-butanol has been reported to be present only in minor amounts in wine brandies (0–60 mg/L) (Nykänen and Suomalainen, 1983; Reinhard, 1968, 1969; Tandoi et al., 1984) and in wine (<6 mg/L) (Hieke, 1972; Versini et al., 1983).

It is generally assumed that 2-butanol is indicative of bacterial spoilage in the mashes or wines used for distillation (Postel and Adam, 1985, 1989). This is supported by a number of experimental studies (Postel, 1982, 1984; Usseglio-Tomasset, 1971) showing that 2-butanol is formed by bacteria during storage of the grape marc after fermentation, and its production depends on the activity of these bacteria inside the ensilaged pomace, especially near the bottom of the tanks where living conditions are more satisfactory (e.g., higher pHs).

It has been found that certain strains of *Lactobacillus brevis* are able to form 2-butanol by reduction of meso-2,3-butanediol (Hieke and Vollbrecht, 1980; Radler and Zorg, 1986). However, conclusive proof that 2-butanol present in distillates is produced by lactic acid bacteria in mashes or wines is still lacking; the enzymatic reactions involved in the metabolic route to this alcohol remain to be clarified, too.

Bearing such a question in mind, we undertook a gas chromatographic (GC) analysis of spirits and alcoholic beverages of different origin. The aim was to determine the predominant enantiomeric form of 2-butanol and its

excess. To our knowledge, this aspect concerning the enzymatic formation of butanol had not been explored before.

MATERIALS AND METHODS

Samples of wine distillates and other distilled beverages were from industrial or commercial sources. (\pm)-Butanol and 2-butanone were purchased from Merck (Darmstadt, Germany) and (+)-(*S*)- and (-)-(*R*)-2-butanol from Aldrich Chemie (Steinheim, Germany); bakers' yeast was from the Distillerie Italiana (San Quirico-Trecasali, Parma, Italy) and *Saccharomyces bayanus* from the Institut Oenologique de Champagne, Epernay, France.

GC analyses reported in Table 1 were carried out on a Perkin-Elmer Sigma 1B gas chromatograph (Perkin-Elmer Co., Norwalk, CT) equipped with a flame ionization detector (FID). A glass column (2 m \times 2 mm i.d.) packed with Supelco 0.2% Carbowax 1500 on Carbowax C 80–100 mesh (Supelco Inc., Bellefonte, PA) was used. The GC parameters were as follows: injector, 170 °C; oven, from 55 to 135 °C at 2 °C/min and kept at 135 °C for 10 min; detector, 250 °C; carrier gas, He at 35 psi; injection volume, 3 μ L. Data were processed by a Perkin-Elmer Sigma 15 chromatography data station, and 2-butanol is given in milligrams per 100 mL of pure ethanol. Multidimensional GC (Perkin-Elmer 8700 gas chromatograph, dual FID, multidimensional configuration) was used to determine the enantiomeric excess of 2-butanol as reported in Table 2. This alcohol was separated from all other volatile compounds by means of the "heart cut" and the "on-column cryofocusing" techniques (Bertsch et al., 1981); it was then analyzed by chiral GC (König, 1987). Experimental conditions were as follows.

Isolation of 2-Butanol. The GC column was a 25-m Chrompack CP Sil 19 CB wide-bore capillary column (0.53 mm i.d., 2.13- μ m film thickness) (Chrompack International B.V., Middelburg, The Netherlands). The column temperature was held at 50 °C for 5 min and then increased to 90 °C at 3 °C/min and maintained for 2 min (*R_t* of 2-butanol, ca. 16.1 min). Injector and FID temperatures were 200 and 250 °C, respectively. Spirits were injected directly without any preparation, except for samples containing 2-butanol in concentration lower than 10 mg/100 mL of pure ethanol. In such cases a preliminary distillation was performed to obtain distillates enriched in butanol content. Injection volumes varied from 0.5 to 5 μ L, and 1:50 effluent splitter was used. The oven temperature was programmed to rise from 40 to 200 °C at a rate of 10 °C/min and kept at 200 °C for 24 min for "purge".

Enantiomer Separation. The column was a MEGA 2,3,6-trimethyl- β -cyclodextrin 5% OV 1701-OH capillary column (25 m \times 0.25 mm i.d., 0.3- μ m film thickness) (Mega, Legnano, Italy) (Bicchi et al., 1992). Operating conditions: isothermal analysis at 10 °C for 35 min; injector, FID, and purge conditions as above.

Microbial Reduction of Butanone. (A) Fresh bakers' yeast (100 g) was dispersed in distilled water (1 L). After 2 h at 37 °C,

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Table 1. 2-Butanol Content in Different Spirits and Distilled Beverages^a

type	no. of samples examined	ranges of 2-butanol concn (mg/100 mL of pure ethanol)				
		<1 ^b	1-5	5-10	10-50	50-200
raw spirits						
wine distillate	57		46	8	2	1
grape pomace distillate	16		4	8	4	
lees distillate	2			1	1	
commercial beverages						
brandy	8		8			
grappa	25		7	4	13	1
Calvados	1					1
Armagnac	1	1				
cognac	1	1				
whiskey	1	1				
rum	1	1				
cherry brandy	1				1	
bilberry brandy	1		1			

^a Each column reports the number of samples whose 2-butanol concentrations fall in the range specified. ^b GC absence or presence in trace amounts.

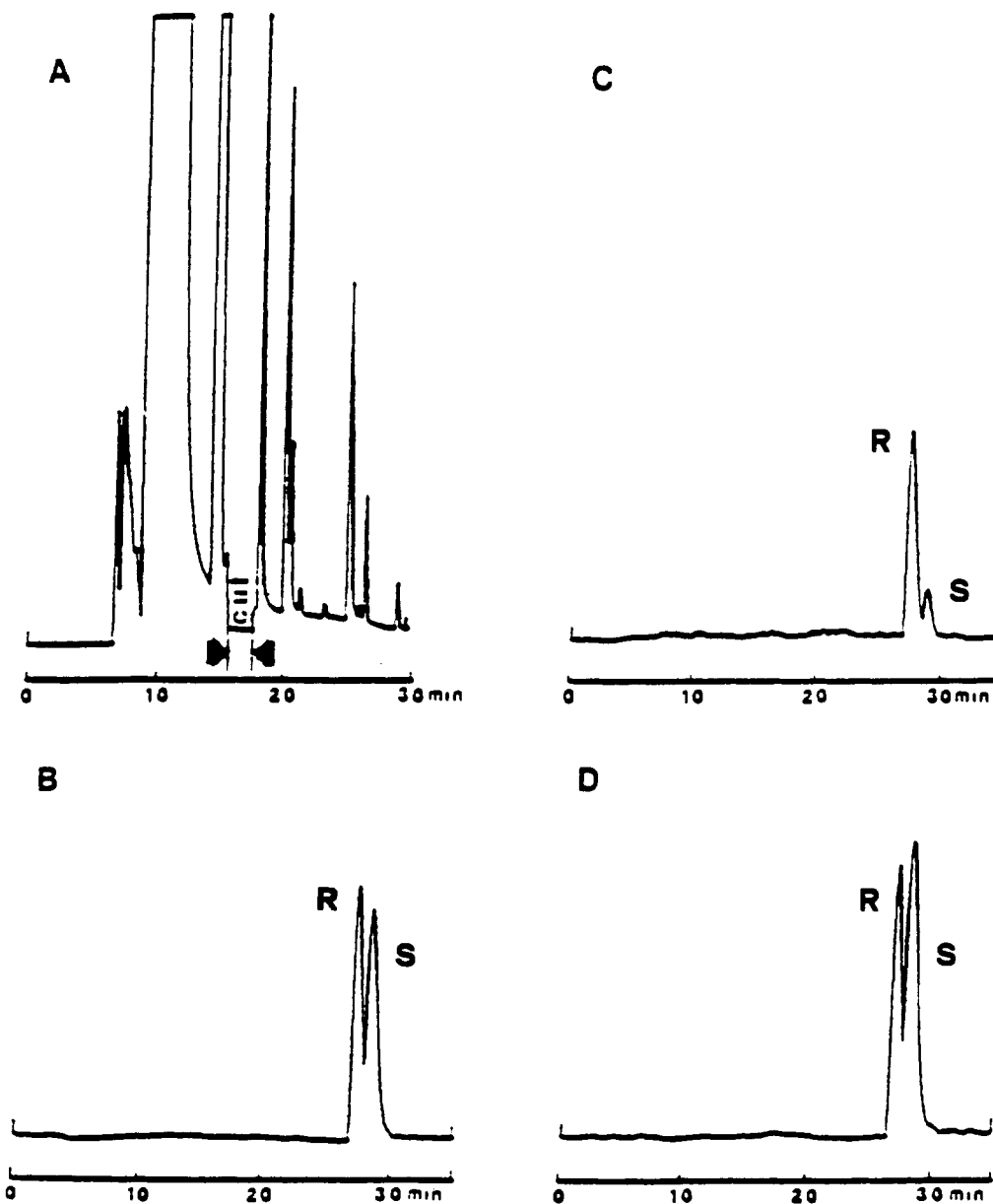


Figure 1. Multidimensional GC analysis of a pomace brandy (Italian grappa) for determining the enantiomeric form and purity of 2-butanol. See Materials and Methods for conditions. (A) GC showing the heart cut; (B) GC of (*R,S*)-2-butanol; (C) GC of the sample arising from the heart cut zone; (D) GC of the sample shown in (C) to which authentic (+)-(*S*)-2-butanol was added.

2-butanone (1 g) was added and the mixture kept under stirring at 37 °C for 70 h. Addition of ethanol (100 mL) followed by distillation of the azeotropic mixture gave a distillate which was

shown to contain enantiomerically pure (*S*)-2-butanol by the GC procedure described above. (B) Red grape must was prepared and inoculated with *S. bayanus* (0.5 g/100 L). Just after this

Table 2. Abundance of the *R*-Isomer of 2-Butanol in the Enantiomer Mixture

sample	origin	2-butanol (mg/100 mL of ethanol)	(-)- <i>R</i> -isomer ee (%)
1	wine distillate	11.9	60.0
2		8.8	63.8
3		9.9	75.8
4		60.3	63.8
5		10.7	70.0
6		9.3	77.0
7	grape pomace distillate	30.0	62.2
8		36.0	55.8
9		30.4	60.6
10		46.0	55.6
11		14.2	54.8
12	wine lees distillate	10.2	47.6
13		57.3	60.2
14	Italian grappa	35.8	63.6
15		43.6	69.4
16		28.8	62.4
17		31.9	67.0
18		152.9	55.0
19		28.9	57.2
20		32.0	62.4
21	Calvados	68.6	81.6
22	cherry brandy	26.3	70.2

inoculation, 2-butanone (1 g) was added to the must (2 L), allowing the fermentation to continue for 2 weeks. Distillation afforded an aqueous ethanol solution which was examined by GC in the usual manner to determine the 2-butanol content. Pure *S*-form (3) was shown to have formed (at the conversion of ca. 20% of 2-butanone).

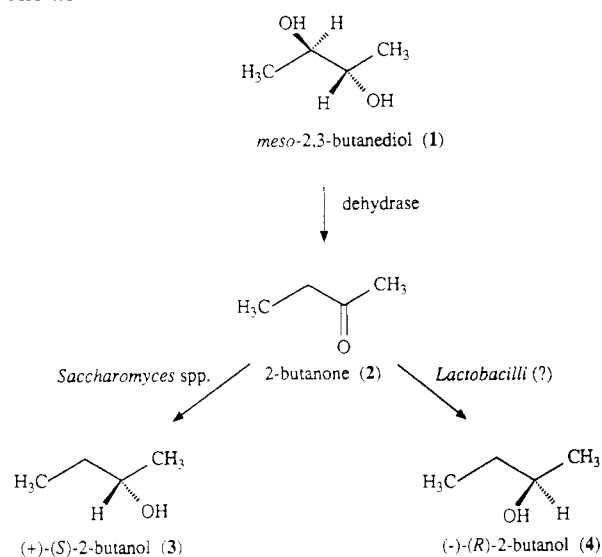
RESULTS AND DISCUSSION

More than a hundred samples of distillates of different origin, including raw spirits used for the preparation of bottled alcoholic beverages, were analyzed for their 2-butanol content. The distribution of this alcohol, summarized in Table 1, was in good agreement with that reported by other authors concerning the same type of distillates (Postel and Adam, 1989; Nykänen and Soumalainen, 1983).

The determination of the prevalent enantiomer as well as its excess was performed by coupling a bidimensional GC with a chiral capillary GC. The former allowed the "pure" alcohol to be isolated from the complex mixture of volatiles, while the enantiomeric resolution was achieved by the latter. Chromatograms resulting from a typical experiment are shown in Figure 1. In all samples examined, a marked predominance of the *R*-form of 2-butanol (4) was found (Table 2). In addition, no evident relationship could be observed between the enantiomeric excess (ee), falling in each case between 50 and 80%, and the concentration of this alcohol or the source of the sample.

Concerning the origin of 2-butanol, one can presume that the alcohol results from enzymatic reduction of the corresponding ketone, i.e., 2-butanone (2), which in turn could derive from 2,3-butanediol through dehydration (see Scheme 1). Such a route was suggested to explain the occurrence of 2-butanol in Cheddar cheese and was supported by the isolation of two strains of *Lactobacillus plantarum* and *L. brevis*, each showing a specific capacity: the former converts the diol to the ketone, and the latter reduces the ketone to the secondary alcohol (Keen et al., 1974).

2-Butanone (2) was reported to be present in wine in trace amounts (Versini et al., 1984). 2,3-Butanediol is well

Scheme 1

recognized to be a metabolite of both yeasts (Neish, 1950; Tittel and Radler, 1979; Maiorella et al., 1983; Ciolfi and Di Stefano, 1983; Heidlas and Tressl, 1990a,b; Delteil and Jarry, 1991) and heterofermentative lactic acid bacteria (Cantoni et al., 1965; Radler and Gerwarth, 1971; Chrow, 1990). Recently, it has been shown that 2,3-butanediol occurring in wine consists of all three stereoisomers (*R,S* or *meso*, 1, 70–80%; *R,R*, 20–30%; *S,S*, less than 1%) (Hagenauer-Heuer, 1990).

It must be pointed out that a diol dehydrase catalyzing the conversion of *meso*-2,3-butanediol (1) into 2-butanone (2) was isolated and characterized from a strain of *L. brevis* (Radler and Zorg, 1986). Such a strain, as well as others of the same species and one of *Lactobacillus bucheri*, have previously been shown to produce 2-butanol in a medium containing *meso*-2,3-butanediol (1) (Hiege and Vollbrecht, 1980; Radler and Zorg, 1986). However, the absolute configuration of 2-butanol was not determined either in this case or in the microbial reduction of 2-butanone quoted above (Keen et al., 1974).

On the other hand, it is well-known that yeasts are also capable of hydrogenating ketones stereoselectively (Faber, 1992). Generally, when *Saccharomyces* spp. are acting, the stereochemistry of the ketone reduction is in agreement with Prelog's rule (Prelog, 1984); this predicts the prevalent configuration of the secondary alcohol on the basis of the steric bulk difference between the two groups linked to the carbonyl function. If the larger group has a higher preference order according to the "sequence rule", then the *S*-configuration is expected. The ee is usually higher the larger the steric difference, but it appears also to be markedly affected by the fermentation conditions (Nakamura et al., 1991; Faber, 1992). Thus, reduction of 2-butanone by bakers' yeast has been reported to give (*S*)-2-butanol (3) having 67% ee when reduction was carried out in water containing 20% (w/v) glucose (MacLeod et al., 1964).

We were able to further confirm the prediction for a preferred *S*-configuration of 2-butanol arising from hydrogenation of 2-butanone (2) by *Saccharomyces* spp.: ee > 99% were obtained using both *S. cerevisiae* in pure water and *S. bayanus* in freshly prepared must.

In conclusion, our finding that the *R*-form of 2-butanol is largely predominant in distilled beverages, when judged in the light of the stereochemical preference shown by yeast in reducing 2-butanone, can be regarded as a strong support to the current assumption that lactic acid bacteria

are responsible for the formation of most (if not all) of the secondary alcohol.

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