^{13}C-Pattern of Natural Glycerol: Origin and Practical Importance

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The average δ^{13}C-value of glycerol from plant origin is 4−5‰ more negative than that of carbohydrates from the same source. This depletion is exclusively dealing with position C-1 of the molecule. This is also observed for glycerol from other natural sources, although to a smaller extent, while synthetic glycerol shows a statistical ^{13}C-pattern. On the basis of these data an illegal addition of glycerol to wine should be detectable. In fermentations of glucose with yeast the extent of the depletion in position C-1 of glycerol proved to be reciprocal to its yield. Furthermore a total carbon and isotope balance showed that the depletion of glycerol is a compensation for small ^{13}C-enrichments in the corresponding positions of main products. An isotope effect on the aldolase reaction and a noncomplete equilibration of the triose phosphates are discussed as main reasons for these findings.

Keywords: *Authenticity proof; carbon-13; glycerol; isotopic pattern; stable isotopes; wine*

INTRODUCTION

Glycerol is an important and quality-determining ingredient of wine, being mainly responsible for its full-bodiedness, and therefore sometimes the compound must be the subject of adulteration investigations. The proof of the illegal addition of glycerol from foreign sources should only be possible on the basis of isotopic correlations, either of global δ^{13}C-values or of isotopic patterns. Already S. Epstein postulated in 1968 the existence of intermolecular isotopic balances, and this demand must—after our experience—be extended to complementary positions in natural products originating from the same source. As a matter of fact, fixed isotopic correlations and complementary patterns have been found (Weilacher et al., 1996; Weber et al. 1997).

Some years ago we have found that glucose from C_3- and C_4-plants shows a reproducible nonstatistical ^{13}C-distribution, mainly characterized by a relative ^{13}C-enrichment in positions 3 and 4 and a depletion in positions 1 and 6 (Rossmann et al., 1991). Relative values of natural isotope abundances are expressed as δ^{13}C-values relative to the PDB (Pee Dee Belemnite limestone, South Carolina) international standard, where

$$\delta^{13}\mathrm{C} = \frac{(^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{sample}} - (^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{standard}}}{(^{13}\mathrm{C}/^{12}\mathrm{C})_{\mathrm{standard}}} \times 1000‰$$

The reasons discussed were isotope effects on different reactions of the Calvin cycle, especially on the aldolase reaction. As a matter of fact, we have recently found a kinetic and an equilibrium isotope effect on this reaction, which will probably be responsible for the ^{13}C-enrichment in positions 3 and 4 of glucose (Gleixner and Schmidt, 1997). Since then, we have detected that this pattern of glucose is partially transferred to descendents, even to secondary products, where it is, however, overlapped by effects on reactions in the secondary metabolism.

Assuming that, due to the triose phosphate isomerase reaction, a scrambling between positions 3 and 4, 2 and 5, 1 and 6 of glucose occurs in trioses (see Table 3 and Scheme 2), one should expect for all descendents of trioses a relative enrichment in ^{13}C by approximately 4‰ in position 1 (originating from positions 3 and 4 of glucose), a depletion of ∼1‰ in position 2 (from positions 2 and 5 of glucose) and a depletion of ∼3‰ in position 3 (from positions 1 and 6 of glucose) relative to the molecular average δ^{13}C-value. This has really been found in some amino acids and their descendents and in the corresponding positions of C_4 dicarboxylic acids deriving from pyruvate (Gensler and Schmidt, 1994; Schmidt et al., 1994). Furthermore, all these compounds did show a quite close average δ^{13}C-value to that of glucose from the same origin (Schmidt et al., 1993).

The same should be expected for glycerol, an immediate descendent of dihydroxyacetone phosphate. However, indications from literature (Bianchi et al., 1993) and our own observations (Schwarz, 1991) indicated a remarkable ^{13}C-depletion of glycerol relative to carbohydrates from the same source. As this was unexpected in regard to the generally accepted metabolic correlations, we decided to study the pattern of this compound relative to that of glucose as a possible base for explanation.

MATERIALS AND METHODS

(1) Chemicals and Research Material. All current chemicals were purchased in the highest purity available. Glycerokinase [EC 2.7.1.30] from *Candida mycoderma* was obtained from SERVA Feinbiochemica GmbH & Co KG, Heidelberg, Germany. The oil and sugar samples were bought in food stores. Glycerol from white and red wine was provided by F. Reniero from the Istituto Agrario Provinciale, San Michele, Trento, Italy. The δ^{13}C-values of carbohydrates (polysaccharides from white and red wine; cellulose from mustard seed, orange peel, and olive fruit) as well as of glycerol from black mustard oil were provided from this department. The sugars with known ^{13}C-patterns used for the incubations were from our laboratory (Rossmann et al., 1991).

(2) Isolation of Glycerol from Different Sources. The fats were hydrolyzed by heating to 60 °C in 2 N NaOH for 1 h (Pardun, 1969). After acidification the fatty acids were extracted with diethyl ether/petroleum ether (1:1). The remainder was concentrated and the glycerol was extracted with a small amount of ethanol, which was subsequently removed by distillation under reduced pressure. The glycerol was purified by distillation (200 °C/0.1 Torr). "Synthetic" glycerol was obtained by alkaline hydrolysis of commercial epichlorhydrine at 60 °C and distillation of the reaction product.

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(3) Yeast Incubation of Glucose and Isolation of Products. For a standard yeast fermentation 50 g of glucose with known ^{13}C-pattern (from corn starch hydrolysate as well as from beet sugar) (Rossmann et al., 1991) in 1000 mL of water, containing 10 mg of KCl, 10 mg of $MgCl_2$, 10 mg of $CaCl_2$, and 0.8 g of $(NH_4)_2$ HPO_4, was incubated with 42 g of fresh baker's yeast at pH 6.5 for 3 days at 35 °C in a closed reaction vessel under N_2-atmosphere (Neuberg und Reinfurth, 1918; Connstein und Lüdecke, 1919). The CO_2 produced was flushed with N_2 through two absorption vessels filled with 800 mL of 3 N NaOH each, and the CO_3^{2-} was precipitated as $BaCO_3$ (Simon and Floss, 1967). The incubation medium was centrifuged, and from an aliquot of the supernatant, ethanol was isolated by fractionated distillation (Rossmann and Schmidt, 1989). In order to isolate the glycerol, another aliquot of the incubation medium was concentrated by vacuum distillation, and the glycerol was extracted from the remainder and purified as before. Acetaldehyde and ethanol were separated from the medium by distillation (Simon and Floss, 1967) and collected in a flask at −115 °C (ethanol/liquid N_2). In an aliquot of this distillate acetaldehyde was oxidized at 60 °C with silver oxide (Ag_2O) (Houben-Weyl, 1975), and the acetic acid formed was separated by distillation, and finally isolated as sodium acetate. In another aliquot of the distillate the acetaldehyde was precipitated with 2,4-dinitrophenylhydrazine (*Organikum*, 1986), and the ethanol was purified by distillation from the filtrate. The yields of the different products (glycerol, ethanol, acetaldehyde, and glucose) were determined by means of enzymatic test kits.

For a yeast fermentation with Na_2SO_3, 25 g of glucose in 1500 mL of medium as above was used, 42 g of fresh baker's yeast and 25 g of Na_2SO_3 were added, and the fermentation was run for 1 week at 35 °C at pH 6 (Neuberg und Reinfurth, 1918; Connstein und Lüdecke, 1919). After that, an additional 20 g of fresh yeast in 200 mL of water was added, and the fermentation was continued for another week. The products were isolated as before.

(4) Degradation of Glycerol. To 100 μmol of glycerol in 2 mL water were added 100 μL of 10% H_3PO_4 and 250 μmol of periodic acid. After 5 min at 20 °C the glycerol was quantitatively cleaved into formaldehyde (C-1 and C-3) and formic acid (C-2). The solution was directly used for isotope analysis by GC−C−IRMS (see below).

Glycerol 3-phosphate was prepared by enzymatic phosphorylation of glycerol with glycerokinase [EC 2.7.1.30] (Rauschenbach und Lamprecht, 1969). The product was precipitated as a barium salt by addition of an excess of $Ba(NO_3)_2$ solution (2 mol/mol) and ethanol. From the precipitate the free acid was obtained by the addition of an equivalent amount 2 N H_2SO_4. The $BaSO_4$ was eliminated by centrifugation, and to the clear supernatant an excess of periodic acid (120%) was added. After 5 min incubation at 20 °C formaldehyde (C-1) and glycolaldehyde phosphate (C-2 + C-3) were formed. For an isotopic analysis of formaldehyde, see below.

Each step of the method was adapted for quantitative turnover and controlled in regard to isotope discriminations by determination of its isotope balance.

(5) Carbon Isotope Analysis. The combustion and measurement of the different isolated compounds were performed according to Winkler and Schmidt, 1980. The carbon isotope ratio was measured in ‰ relative to the PDB standard.

The simultaneous separation and isotopic analysis of the volatile products from the periodic acid oxidation were carried out in a GC−combustion−IRMS on-line coupled system (SIRA 24, consisting of Isochrome 1, GC−C−IRMS of VG, Middlewich, GB; GC, Pora Plot U column, length 5 m; injector temp 180 °C; split 20 mL/min; temperature program 3 min at 120 °C, increasing to 160 °C at 15 °C/min).

Calculation for isotope balance and δ-value increment determinations were as given under Results and Discussion.

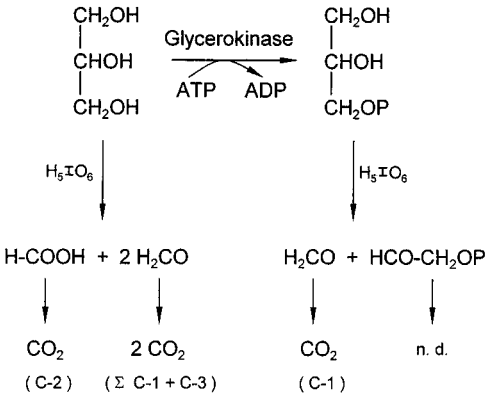
RESULTS AND DISCUSSION

(1) Average δ^{13}C-Value of Glycerol Relative to Carbohydrates from the Same Origin. As already pointed out from earlier results (Bianchi et al., 1993; Schwarz, 1991), we expected that glycerol was depleted relative to the carbohydrates from the same origin. This could be confirmed (Table 1) and the mean depletion attained was −4.2‰ relative to that of carbohydrates from the same source. In the case of glycerol from animal fats, no standard carbohydrate reference is available.

Table 1. Average Mean δ^{13}C-Values of Glycerol and Reference from the Same Origin (White and Red Wine: Polysaccharides; Mustard Seed, Orange Peel, and Olive Fruit: Cellulose; Goat Fat/Cod-Liver Oil: Total Fat); The Experimental Error of the δ^{13}C-Values Is about 0.1‰

origin of compounds	δ^{13}C [‰]$_{PDB}$ (experimental error < 0.1‰)		
	glycerol	reference	difference
white wine	−28.6	−26.0	−2.6
red wine	−31.6	−28.2	−3.4
mustard seed	−30.5	−24.7	−5.8
orange peel	−29.5	−25.1	−4.4
olive oil	−31.3	−26.0	−4.5
goat fat	−26.2	−26.9	+0.7
cod-liver oil	−21.4	−25.4	+4.0

Scheme 1. Degradation of Glycerol for Positional ^{13}C-Analysis[a]



[a] It has to be pointed out that the phosphorylation takes place only in position 3 of the molecule and that this position is identical to the originally phosphorylated position 1 of dihydroxyacetone phosphate.

While the generally observed relative depletion of fatty acids can be attributed to the loss of the enriched atom C-1 of pyruvate and to the isotope effect on the pyruvate dehydrogenase reaction (Melzer and Schmidt, 1987), this depletion of glycerol relative to carbohydrates cannot be understood, and therefore we decided to study the ^{13}C-pattern of this compound in comparison to that of its immediate precursor.

(2) ^{13}C-Pattern of Natural and Synthetic Glycerol. Glycerol has a prochiral center in position 2 and is therefore stereospecifically phosphorylated by glycerokinase in position 3 (Scheme 1). This is the base for the defined degradation of the compound.

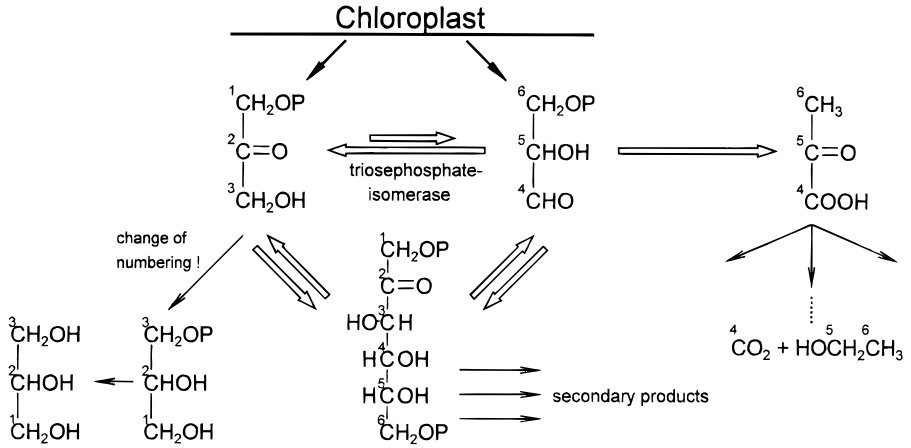
The oxidation of glycerol itself by periodic acid yields 2 mol of formaldehyde (C-1 and C-3) and 1 mol of formic acid (C-2). From here the δ^{13}C-values of position 2 and that of the mean of positions 1 + 3 are available by GC coupled to combustion and IRMS. By the same reaction glycerol 3-phosphate yields 1 mol of formaldehyde (C-1) and phosphoglycolate (C-2 + C-3). This degradation provides the isotope abundance of position 1; that of position 3 is obtained by difference. For control the mean of the individual positional δ-values is compared and must be identical to the independently obtained average δ^{13}C-value of the glycerol.

Table 2. ^{13}C-Pattern of Glycerol from Different Origin and Difference between Total δ^{13}C-Values and Values of Atom C-1, with Respect to C-1 and C-2. According to Scheme 1, the Compound Was Phosphorylated; Glycerol and Glycerol 3-Phosphate Were Submitted to a Degradation with Periodic Acid, Yielding the δ^{13}C-Values of C-1 and C-2 Directly, of C-3 by Difference

	total δ^{13}C [‰]$_{PDB}$		individual δ^{13}C [‰]$_{PDB}$			difference $\Delta\delta^{13}$C [‰]$_{PDB}$	
glycerol from	measured	calculated[a]	C-1	C-2	C-3[d]	C_{total} − C-1	C-1 − C-2
olive oil (A)	−31.3 ± 0.1	−31.1	−44.6 ± 0.6	−22.2 ± 0.3	−26.6	+13.3	−22.2
mustard oil (B)	−30.5 ± 0.1	−30.6	−40.7 ± 0.5	−23.1 ± 0.3	−28.0	+10.2	−17.3
red wine (C)	−31.6 ± 0.1	−31.1	−41.8 ± 0.5	−23.5 ± 0.4	−28.0	+10.2	−18.3
white wine (D)	−28.6 ± 0.1	−29.0	−40.6 ± 0.7	−21.3 ± 0.3	−25.2	+12.0	−19.3
mean A to D	−30.5	−30.5	−41.9	−22.5	−27.0	+11.4	−19.4
expected from glucose pattern[b]	−25.0		−20.9	−26.0	−28.1	−4.1	+1.0
difference glucose − glycerol (mean A to D)	−5.5		−21.0	+3.5	+1.1		
goat fat	−26.2 ± 0.1		−33.3 ± 0.7	n.d.[e]	n.d.	+7.1	
cod-liver oil	−21.4 ± 0.1		−26.9 ± 0.8	n.d.	n.d.	+5.5	
commercial (Aldrich)	−27.0 ± 0.1	−27.9	−32.7 ± 0.7	−26.8 ± 0.3	−24.1	+5.7	−5.7
commercial (Fluka)	−23.7 ± 0.1	−24.3	−33.6 ± 0.9	−16.6 ± 0.5	−22.8	+9.9	−17.0
synthesis[c]	−25.5 ± 0.1	−25.4	−23.9 ± 0.9	−25.9 ± 0.4	−26.5	−1.6	+2.0

[a] Mean of individual values (C-1 + C-2 + C-3)/3. [b] "Standard glucose", Rossmann A., et al., 1991. [c] Alkaline hydrolysis from epichlorhydrine. [d] Calculated values as described in the text. [e] n.d. = not determined.

Scheme 2. Metabolic Fluxes from Dihydroxyacetone Phosphate Demonstrating the Formation of Glycerol as a Byproduct[a]



[a] Hexoses and pyruvate derivatives are enriched with ^{13}C in positions 3 and 4: hence for compensation glycerol must be depleted in position 3 (⇒ position 1 of glycerol!).

The results thus obtained for glycerol from various natural origins (Table 2) indicate a dramatic relative ^{13}C-depletion in position 1, while the δ-values in positions 2 and 3 and even their differences are, at least in the case of plant products, only slightly changed as compared to the corresponding values of glucose. Hence the observed global depletion of natural glycerol relative to its precursor is obviously solely due to a depletion in position 1, attaining −21‰ relative to the original δ^{13}C-value.

What can be the reason for this discrepancy? Dihydroxyacetone phosphate is the primary precursor of any other compound outside the chloroplast of plants (Scheme 2). The main products from here are hexoses and—via glyceraldehyde 3-phosphate—intermediates and descendents of the glycolysis or, in the case of isolated glucose, those of the alcoholic fermentation, while glycerol is normally only an unimportant by-product. Any of the above mentioned main descendents of trioses or pyruvate show—as far as we know—^{13}C-enrichments in positions corresponding to atoms C-3 and C-4 of hexoses (Abelson and Hoering, 1961; Gensler and Schmidt, 1994; Schmidt et al., 1994). Therefore an isotope balance demands a corresponding depletion in some other product, and when this is a by-product such as glycerol, its depletion must be very large.

A distinct relative depletion was also found in two commercial glycerol samples. One of them is originating from cattle fat (information Fluka); the origin of the other one could not be obtained. The positional depletion was also found in samples of glycerol that we had obtained from animal fat (goat fat and cod-liver oil) by hydrolysis, however, it was not as distinct as in the case of the plant material (see Table 2). Up to now the amount of samples analyzed is not sufficient to establish criteria—on the basis of either the global δ^{13}C-value or the pattern—for a distinction between plant or animal originating glycerol. A definitely synthetic glycerol was obtained by alkaline hydrolysis of epichlorhydrine, and this product showed a more or less statistical ^{13}C-pattern. We are therefore convinced that it will be easy to distinguish synthetic and natural glycerol, and maybe in the future even products from plant and animal origin, respectively.

(3) Cause for the Origin of the Pattern. *(a) Dependence of Glycerol Pattern on Relative Yield.* Provided the pattern found is a consequence of an isotopic balance between DHAP descendents, in a system with glucose as a sole carbon source and a metabolic branching after the aldolase reaction, the extent of the depletion in position 1 of glycerol should become reciprocally dependent on the yield of this compound. In a fermentation in the presence of Na_2SO_3, glycerol will become a main product, because acetaldehyde is bound to HSO_3^- and not available for reduction, which is now orientated toward dihydroxyacetone phosphate.

Table 3. ^{13}C-Pattern of Glycerol Obtained by Fermentation of Beet Glucose and Corn Glucose under Different Conditions; The Patterns of the Glucose Samples (Rossmann et al., 1991) Are Given in the Lower Part; Equilibrated = 50% from C-1 to C-3 and 50% from C-4 to C-6 of Glucose; Nonequilibrated = 100% from C-1 to C-3 of Glucose

fermentation conditions	glycerol yield [%]	glycerol, total values δ^{13}C [‰]$_{PDB}$ measured	glycerol, total values δ^{13}C [‰]$_{PDB}$ calculated	glycerol, pattern δ^{13}C [‰]$_{PDB}$ in position C-1	C-2	C-3	difference δ^{13}C [‰]$_{PDB}$ C-1 − C-2
normal, C_3-plant (beet sugar)	4.2 ± 0.2	−30.2 ± 0.1	−30.6	−39.9 ± 0.7	−26.0 ± 0.3	−25.9	−13.9
0.7 mol Na_2SO_3 per mol glucose	22.6 ± 0.2	−31.1 ± 0.1	−30.8	−35.5 ± 0.6	−28.9 ± 0.4	−28.1	−6.6
1.4 mol Na_2SO_3 per mol glucose	22.9 ± 0.2	−31.3 ± 0.1	−31.4	−36.4 ± 0.8	−29.5 ± 0.3	−28.4	−6.9
expected from glucose (C_3), nonequilibrated				−23.1	−25.9	−26.3	+ 2.8
expected from glucose (C_3), equilibrated		−25.0		−20.9	−26.0	−28.1	+ 5.1
normal, $C_{4-plant}$ (corn starch hydrolysate)	2.5 ± 0.2	−16.5 ± 0.1	−17.5	−31.4 ± 0.8	−10.2 ± 0.4	−10.9	−21.2
expected from glucose (C_4), nonequilibrated				−11.0	−10.4	−9.4	−0.6
expected from glucose (C_4) − equilibrated		−10.3		−8.1	−10.4	−12.3	+ 2.3

sugar	δ^{13}C [‰]$_{PDB}$ in position C-1	C-2	C-3	C-4	C-5	C-6	δ^{13}C [‰]$_{PDB}$ total value
beet glucose (C_3)	−26.3	−25.9	−23.1	−18.7	−26.1	−29.9	−25.0
corn glucose (C_4)	−9.4	−10.4	−11.0	−5.1	−10.4	−15.1	−10.3

In order to verify this expectation, C-3- and C-4-plant glucose of known ^{13}C-pattern (Rossmann et al., 1991) were incubated with yeast under normal conditions and in the presence of Na_2SO_3. The glycerol yield increased from 4% to 23% (Table 3). Unexpectedly, the average δ^{13}C-value of this glycerol did not change (a possible explanation will be given later); however, the depletion in position 1 relative to position 2 became smaller with the increase of the glycerol yield. In addition, we compared the pattern in position C-2 and C-3 to that of the fermented glucose. In the case of the incubation with low glycerol yield this pattern fitted much better to that of C-2 and C-1 of glucose and not to that after scrambling of the corresponding positions in the trioses. This indicates that the immediate glycerol precursor dihydroxyacetone phosphate has probably been reduced before its equilibration with glyceraldehyde 3-phosphate. This result is in line with the findings of Kikuta and Erikson, 1969, who detected that in avocado fruits a ^{14}C-label of glucose was transferred into glycerol to a much higher extent when it was in position C-1 than a label of position C-6, obviously due to a nonequilibration of the two triose phosphates. In the experiment with high glycerol yield only atom C-3, the only C atom not touched by the conversion of glucose to glycerol, corresponded to the scrambled δ^{13}C-values (mean of C-1 and C-6 of glucose). This could be the consequence of a scrambling of the assumed triose phosphate pools possibly caused by a damage of the yeast cells, after addition of Na_2SO_3.

(b) Total Metabolic and Isotopic Balance of Glucose Fermentation. For final confirmation of our results we incubated glucose in order to establish a total metabolite and isotope balance (Table 4). In both experiments with and without Na_2SO_3 the molar yields of glycerol and ethanol (+ acetaldehyde) were close to 90%, and the amounts of ethanol (+ acetaldehyde) were, as expected, equivalent to the yield of CO_2. Moreover, in the incubation in the presence of Na_2SO_3, the amounts of glycerol and acetaldehyde were equivalent. All these findings are in line with the stoichiometry of a fermentation of glucose by "resting" yeast cells and in agreement with corresponding data from literature (Bruchmann, 1976). Only about 10% or 2%, respectively, of the carbon from glucose was not identified.

For the calculation of the isotope balance, the δ^{13}C-value of the original glucose was attributed to the unidentified part in the carbon balance. For each identified compound a δ^{13}C-value increment was calculated from its individual yield fraction (% yield × 0.01),

Table 4. Total Metabolic and Isotopic Balance of a Yeast Fermentation of Glucose (δ^{13}C = −25.0 [‰]$_{PDB}$) under Different Conditions. Upper Part: Normal Conditions. Lower Part: Inhibition with Na_2SO_3. δ^{13}C-Increment: Product of Measured δ^{13}C-Value, Yield, and Number of C Atoms Represented by the Compound (Calculation, See Main Text)

product	yield [%]	δ^{13}C-value [‰]$_{PDB}$	δ^{13}C-increment
glycerol	7.2 ± 0.2	−29.3 ± 0.1	−2.110
ethanol	78.0 ± 0.2	−27.0 ± 0.2	−14.040
acetaldehyde	n.d.[a]	n.d.	
carbon dioxide	82.0 ± 1.0	−20.4 ± 0.1	−5.576
unidentified[b]	10.8	−25.0	−2.700
		sum of increments	−24.426
glycerol	36.0 ± 0.2	−32.4 ± 0.2	−11.664
ethanol	24.0 ± 0.7	−26.3 ± 0.1	−4.208
acetaldehyde	34.0 ± 0.8	−21.2 ± 0.2	−4.805
carbon dioxide	62.0 ± 1.0	−20.6 ± 0.1	−4.257
unidentified[b]	2.0	−25.0	−0.500
		sum of increments	−25.434

[a] Expected to be close to zero (n.d. = not determined). [b] Balance of yield (glycerol + CO_2) to 100%, calculated as glucose.

the number of the C-atoms, and the number of molecules obtained per molecule glucose and its measured δ^{13}C-value (e.g. for ethanol $0.78 \times {}^2/_6 \times 2 \times (-27.0) = -14.04$). The sum of the calculated δ-value increments thus obtained agreed excellently with that of the glucose used.

Without any doubt the glucose fermentation under the conditions applied (large excess of resting cells of yeast) proceeded fast and irreversibly. This implies two consequences.

(1) In the normal incubation with low glycerol yield this product seems to originate, as already outlined, merely exclusively from the upper part (atoms C-1, C-2, and C-3) of the glucose. In the "inhibited" fermentation, a scrambling of both parts of the trioses is more likely, which would lead to glycerol with the average value of C-1 and C-6 of glucose. Exactly this was found comparing the δ^{13}C-values of C-3 (glycerol), and therefore we can postulate, at least under certain conditions, the existence of two different pools of trioses. As an explanation for the unexpected relative depletion in position C-2 of glycerol, we propose that it could be caused by an isotope effect on the reversible bisulfite adduct formation of dihydroxyacetone phosphate, favoring the reduction of the "light molecules". Correspondingly the relative enrichment found for acetaldehyde as compared to ethanol could be the consequence of an

isotope effect on the bisulfite adduct formation of acetaldehyde, preferring the "heavy" isotopomer.

(2) The kinetic isotope effect on the aldolase reaction, not the equilibrium isotope effect, must dominate these conversions. The kinetic isotope effect, as determined by us (Gleixner and Schmidt, 1997), was $k_{12}/k_{13} = 1.0159 \pm 0.007$ on C-3 (C-1 of glycerol) and $k_{12}/k_{13} = 0.9968 \pm 0.009$ on C-4 . This again supports the origin of glycerol from the upper part of glucose and means that the depletion on C-1 may be mainly due to this kinetic isotope effect.

CONCLUSIONS

The global δ^{13}C-value of glycerol from plant material is always correlated to that of the corresponding carbohydrates. The observed global depletion is restricted to a relative depletion in position 1 and is a compensation for small enrichments of other glucose descendents in the corresponding position.

This seems to be a further example of a general phenomenon, in that isotopic patterns of different molecules from the same precursor are correlated to each other, but they must not be congruent; they can also be complementary. Behind a metabolic branching point, an isotope effect in one direction may lead to a depletion in a given position of one product, and this has as a consequence a corresponding enrichment in the other product. The extent of the discrimination in either direction will be reciprocal to the turnover rate in this direction.

It will certainly be possible on this basis to prove the addition of synthetic glycerol to wine taking into account the large δ^{13}C-value difference between the global value and position 1 or between the values for position 1 and 2. The proof of an addition of glycerol from animal sources will not be so easy, because only smaller differences exist. Further studies will therefore include the corresponding ^{2}H-pattern of glycerol from different sources.

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