Isotopic Criteria in the Characterization of Aromatic Molecules. 2. Influence of the Chemical Elaboration Process

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Most valued natural aromatic molecules can be substituted by their low-cost chemical counterparts. Isotopic methods, which offer the most powerful tool to infer the origin of a molecule, are applied to the characterization of a large number of chemical aromatic species. Isotopic affiliation between precursors and products is investigated in several types of reactions: oxidation of benzyl chloride and benzyl alcohol and hydrolysis of benzylidene chloride and cinnamaldehyde. The isotopic parameters strongly depend not only on the type of process but, for a given process, on the experimental conditions of the reaction. Kinetic isotope effects occurring in several formylation reactions are estimated. It is shown that, in the drastic experimental conditions of many industrial processes, the benzenic hydrogen atoms may be affected by exchange phenomena. Consequently, the site-specific isotopic parameters of the ring fragment of chemical species are usually much less stable than those of the corresponding natural molecules biosynthesized in mild environments. The isotope ratios of substituents such as CH_3, CH_2Cl, and CHO are more resistant to exchange and provide useful criteria for characterizing both the raw materials and the process. It is shown in particular that radical hydrogen abstraction in toluene to produce benzyl chloride induces relatively moderate fractionation effects. In contrast, oxidation reactions frequently produce strong fractionation effects. In particular, industrial direct oxidation of toluene into benzaldehyde is characterized by deuterium enrichments at the formyl site, which may exceed 900 ppm. Taking into account the large magnitude and high variability of many fractionation effects occurring in chemical reactions, the isotopic fingerprint may provide unambiguous criteria, not only for excluding a natural origin and characterizing the type of process, but also for differentiating molecules synthesized by a given process in different industrial contexts. The isotopic fingerprint may therefore be used by manufacturers as a powerful label for characterizing their production batches.

KEYWORDS: Aromatic molecules; hydrogen isotope ratios; kinetic isotope effects; SNIF-NMR; authentication

INTRODUCTION

Among the most important components of natural aromas, many pertain to the benzenoid/phenylpropanoid family. These aromatic molecules can also be produced by chemical synthesis, and the organic chemical industry has exploited this opportunity to confer added value to basic intermediates of petrochemistry. Oxygenated aromatic molecules, for instance, are frequently used to prepare custom and commodity chemicals for the food industry. Such synthetic molecules, defined in Europe as "nature-identical", compete with their natural analogues. To benefit from the "natural" status, a product not only must originate from natural raw materials but also must have been preserved from any chemical transformation. Natural raw materials chemically transformed are considered as semisynthetic and are not allowed to display the "natural" label. Differentiating natural molecules from their synthetic or semi-synthetic analogues is a great challenge for the food industry and especially for flavor and aroma companies. Isotopic methods certainly provide the most powerful tools for characterizing both the origin of the raw materials and the transformation process. In particular, site-specific hydrogen isotope ratios measured by NMR (SNIF-NMR), possibly combined with overall carbon or oxygen isotope parameters determined by isotope ratio mass spectrometry (IRMS), are now currently used to authenticate a number of natural aroma components (*1*). In this context, we have investigated, in parallel, the isotopic affiliation in benzenoid/phenylpropanoid molecules biosynthesized by plants (*2*). Here we analyze isotopic properties of various non-natural aromatic molecules in terms of affiliation to fossil raw materials and of isotope effects associated with different synthetic

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processes. Specific criteria are defined for unambiguously characterizing several kinds of synthetic molecules. More generally, the results illustrate the aptitude of site-specific isotopic parameters, not only for identifying the type of raw material utilized but also for characterizing the industrial process and therefore for protecting patented methods.

MATERIALS AND METHODS

Origin and Preparation of Samples. The investigated samples were obtained directly from manufacturers or purchased from suppliers of laboratory grade chemicals, or they were synthesized from commercial raw materials. To appraise the diversity of the production processes, a maximum number of commercial sources was considered: Acros, Adrian, Aldrich, BASF, Ega-Chemie, Extrasynthese, Fluka, IFF, Interchim, Janssen, Kodak, Lancaster, LRFC, Merck, Prolabo, Rectapur, Rhône-Poulenc, Rhodia, SDS, Serva, and Sigma. Most samples were studied without further purification.

Isotopic Methodologies. *NMR.* The site-specific hydrogen isotope ratios were determined by using the SNIF-NMR method (*3*) on AM400 or AM500 Bruker spectrometers at a temperature of 308 K. The spectra were recorded at either 61.42 or 76.77 MHz using a dedicated 10 mm (o.d.) probe equipped with a ^{19}F locking device. Broadband ^{1}H decoupling was applied continuously. Quantitative conditions were ensured by the introduction of relaxation delays higher than $5T_1$ after the 90° pulses. The spectra were analyzed by a global least-squares curve-fitting procedure (*4*) managed through EUROSPEC software. The D/H values, expressed in parts per million, were determined either by an internal referencing procedure involving a suitable working standard or by a combined use of SNIF-NMR and isotope ratio mass spectrometry (IRMS) (*3*). In the first method the signal area of the investigated molecule is referred to that of the added referencing material, *N,N*-tetramethylurea (TMU), delivered by the European Union Institute for Reference Materials and Measurements. In the second method the molar fractions of the monodeuterated isotopomers are computed from the ^{2}H NMR spectrum, and the overall D/H isotope content of the molecule is measured by IRMS.

IRMS. The mass spectrometry determinations of the molecular isotope ratios were carried out by means of a Carlo-Erba NA 1500 II elemental analyzer fitted to a Finnigan MAT Delta E mass spectrometer. The carbon isotopic results are expressed on the δ‰ scale with respect to the international standard V.PDB. The precision of the method may be estimated at 0.2‰. To measure the overall D/H isotope ratios, the sample was first combusted into water, which was subsequently reduced into hydrogen gas before introduction into the mass spectrometer.

Chemical Syntheses. In all reactions, the conversion rates, expressed with respect to the starting aromatic material, were determined by gas chromatography using an internal standard and checked by integration of the ^{1}H NMR signals. By investigating samples of benzaldehyde obtained at different rates of distillation, it was checked that both carbon and hydrogen isotope ratios are not significantly influenced by isotope effects associated with phase transitions (*5*).

Monochlorination of Toluene. Radical chlorination of toluene was carried out with SO_2Cl_2 at 80 °C without solvent. The mixture of 9.21 g (0.10 mol) of toluene and 15.88 g (0.12 mol) of SO_2Cl_2 was first degassed, and 0.04 g (0.2 mmol) of 2,2′-azobis(2-methylpropionitrile) (AIBN) was added to initiate the radical reaction. After extraction by dichloromethane, benzyl chloride was separated by distillation. Three experiments, A, were carried out with different concentrations of SO_2-Cl_2 associated with different yields. Similar experiments, B, associated with two different yields, were carried out in CCl_4.

Another radical chlorination of toluene (experiments C) made use of *tert*-butylhypochlorite, which was prepared from *tert*-butanol and sodium hypochlorite according to the Mintz and Walling reaction (*6*). Two experiments at different concentrations ($tBuOCl/PhCH_3$ molar ratios of 0.43 and 0.68) leading to different yields were carried out.

Oxidation of Benzyl Chloride. Oxidation of benzyl chloride by hexamethylenetetramine involves a hexaminium salt intermediate, which undergoes a hydride transfer. A mixture of water (0.325 mol), 12.67 g of benzyl chloride (0.100 mol), and hexamethylenetetramine at three $C_6H_{12}N_4/PhCH_2Cl$ molar ratios (0.15, 0.25, and 1.08) was heated in a

reflux during 2 h. After the addition of HCl, filtration, three ether extractions, and evaporation of the solvent, the product was purified through the addition of a saturated aqueous solution of sodium hydrogenosulfite. Benzaldehyde was finally separated using an aqueous solution of sodium carbonate.

Oxidation of Benzyl Alcohol. Benzyl alcohol was oxidized either by sodium dichromate in aqueous solution or by sodium hypochlorite in aqueous solution and in the presence of a phase transfer catalyst. Typically in a first experiment, 24.59 g (0.08 mol) of sodium dichromate dihydrate were added to 150 mL of water; 16.22 g (0.15 mol) of benzyl alcohol was added, and the mixture was heated in a reflux during 3 h. After filtration and extraction with dichloromethane, benzaldehyde was separated from residual alcohol through the formation of adduct with hydrogenosulfite. In a second case, 250 mL of a 12% sodium hypochlorite solution was added to 16.22 g (0.15 mol) of benzyl alcohol and 150 mL of dichloromethane. After the addition of 0.5 g (1.5 mmol) of tetrabutylammonium hydrogenosulfate, the biphasic mixture was stirred during 3 h at room temperature. The aqueous phase was extracted three times with dichloromethane. After drying and solvent evaporation, benzaldehyde was purified by formation of the adduct with $NaHSO_3$. Several experiments were conducted in basic (E), neutral (F, G), or acid (H, I) media. These experiments either involved different concentrations of reactant (three concentrations of dichromate in experiment E and two concentrations of NaOCl in experiment I) or were carried out with a fixed oxidant/alcohol ratio but were stopped at different times of reaction (experiments F and H).

Hydrolysis of Benzylidene Chloride. Conversion of benzylidene chloride (15.20 g/0.10 mol) into benzaldehyde was carried out in water (9.01 g/0.5 mol) at 100 °C. The mixture was heated in a reflux during 2 h and extracted with ether. Benzaldehyde was separated from residual benzylidene chloride through transformation into the addition product with $NaHSO_3$. Experiments with different benzylidene/water molar ratios, corresponding to different yields, were carried out.

Hydrolysis of Cinnamaldehyde. Hydrolysis of *trans*-cinnamaldehyde was performed in a slightly basic aqueous medium (retroaldolization). This reaction is reversible, and cinnamaldehyde can be obtained from condensation of benzaldehyde with acetaldehyde. Ten grams (0.076 mol) of cinnamaldehyde was added to an aqueous solution of sodium carbonate (3%). The mixture was brought to the boil and maintained in a reflux during 7 h. The organic products were extracted with ether. Benzaldehyde and cinnamaldehyde were separated by distillation. A conversion rate of 0.68 was obtained. Unreacted cinnamaldehyde is a mixture of cis and trans isomers as a result of the reversibility of the reaction.

RESULTS AND DISCUSSION

Overall Isotope Ratios of Fossil Raw Materials. Commercial aromatic products produced by industrial methods are usually derived from fossil raw materials. Because final products are expected to retain, at least partially, the isotopic characteristics of their precursors, it is worth examining the isotopic fingerprints of feedstock. Presently, coal chemistry is nearly abandoned and fossil materials used in aromatic chemistry are mainly obtained from oil and natural gases. Many studies have been devoted to the isotopic characterization of various components of fossil resources (*7*). Thus, it has been observed that the transformation of organic deposits in sedimentary basins into fulvic and humic acids, which in turn lead to kerogen, oil, and gas, is accompanied by overall impoverishment in the ^{13}C isotope. The isotopic results provide helpful information about the genesis, age, maturation, and paleogeographic origin of these products. To characterize, on a statistical basis, the isotopic composition of precursors of the investigated molecules described in **Table 1**, we have collected typical values of carbon and hydrogen isotopic parameters of kerogen, coal, fossil oils, and natural gases (*7−10*). In addition, because benzene, toluene, and xylenes are the most common starting chemicals of petrochemistry, we have investigated several small molecules

Table 1. CAS Registry Numbers and Formulas of the Investigated Molecules[a]

compound	formula	CAS Registry No.	1 C substituent	OH	OCH$_3$
phenol	C$_6$H$_6$O	108-95-2		1	
pyrocatechol	C$_6$H$_6$O$_2$	120-80-9		1, 2	
benzonitrile	C$_7$H$_5$N	100-47-0	CN		
benzaldehyde	C$_7$H$_6$O	100-52-7	CHO		
p-hydroxybenzaldehyde	C$_7$H$_6$O$_2$	123-08-0	CHO	4	
salicylaldehyde	C$_7$H$_6$O$_2$	90-02-8	CHO	2	
benzylidene chloride	C$_7$H$_6$Cl$_2$	98-87-3	CHCl$_2$		
benzyl chloride	C$_7$H$_7$Cl	100-44-7	CH$_2$Cl		
toluene	C$_7$H$_8$	108-88-3	CH$_3$		
anisole	C$_7$H$_8$O	100-66-3			1
benzyl alcohol	C$_7$H$_8$O	100-51-6	CH$_2$OH		
cresol (m)	C$_7$H$_8$O	108-39-4	CH$_3$	3	
guaiacol	C$_7$H$_8$O$_2$	90-05-1		1	2
xylene[b]	C$_8$H$_{10}$	1330-20-7	2(CH$_3$) (a)		
2-phenylethanol	C$_8$H$_{10}$O	60-12-8	CH$_2$CH$_2$OH		
p-anisaldehyde	C$_8$H$_8$O$_2$	123-11-5	CHO		4
vanillin	C$_8$H$_8$O$_3$	121-33-5	CHO	4	3
cinnamaldehyde	C$_9$H$_8$O	104-55-2	CH=CH−CHO		
benzyl acetate	C$_9$H$_{10}$O$_2$	140-11-4	CH$_2$OCOCH$_3$		

[a] The hydroxyl and methoxy groups on the benzene ring are numbered from the position of the alkyl substituent, which is given number 1. [b] Xylene contained in feedstock is a mixture of three isomers: o-, meta-, and p-dimethylbenzene.

from different commercial origins (**Table 2**). These molecules come, for a small part, from coal coking and subsequent distillation of coal tar. However, the main production is due to cracking and re-forming of naphtha (the so-called BTX fraction). **Figure 1** represents the 95% confidence existence domains, in the ^{2}H, ^{13}C plane, of the considered feedstock from oil and from natural gases. Considering the diversity and complexity of the petrochemistry supply, and in the absence of precise information on the industrial resource, the starting materials used in aromatic chemistry must therefore be assigned the high level of carbon isotopic variability described in **Figure 1**. Whereas the range of D/H values of the usual petroleum resources is relatively restricted, strong deuterium and ^{13}C depletions have been measured in gases. For instance, δ^{13}C values of methane from biogenic or thermogenic origin stretch over a range from −90 to −20‰. In particular, methanogen bacteria are responsible for strong fractionation effects. This behavior may provide helpful criteria for interpreting chemical processes. Thus, when the ^{13}C/^{12}C ratio of a molecular fragment is smaller than about −50‰, there is a high probability that this fragment has been introduced in the molecule via a C1 process. It is also noted that, when compared to feedstock, simple aromatic hydrocarbons usually exhibit a significant enrichment in deuterium (∼10 ppm).

Influence of Exchange Phenomena on Site-Specific Isotopic Parameters. The hydrogen isotope contents measured at the different ring positions of substituted benzenes from various commercial sources exhibit significant deviations with respect to a statistical distribution (**Table 2**). In the drastic conditions of industrial processes, the aromatic hydrogen atoms may be involved in exchange phenomena at rates that depend on the nature of the substituent, the position of the hydrogen atom with respect to the substituent, and the pH of the medium. For instance, an investigation of isotopic exchange in 2-methoxyphenol using an experimental design has emphasized the high sensitivity of position ortho (and to a lesser extent para) to pH and temperature factors (*11*). When guaiacol is maintained at 80 °C during 24 h in a basic solution, variations of several tens of parts per million may be observed. Such exchange phenomena may be invoked to partly explain the variability of the isotopic distribution in aromatic precursors. In toluene, which mainly results from pyrolysis or re-forming of gasoline, the para position is systematically enriched in deuterium with respect to the ortho

and meta positions, and it exhibits a high level of dispersion. In contrast, in phenol, which is mainly obtained from benzene or toluene, the ortho position exhibits higher deuterium enrichment and higher isotopic variability than the para position. More generally the exchange aptitude is enhanced by electrodonor groups such as CH$_3$, OH, OCH$_3$, whereas it is reduced by the electron-withdrawing CHO substituent in particular. However, in experimental conditions that affect the ring hydrogen atoms of benzyl chloride, for instance, the chloromethyl group remains relatively stable toward exchange (*11*). Similarly, limited isotopic variability is exhibited by the hydrogen atoms of methyl substituents, which are hardly engaged in exchange phenomena.

The great sensitivity of the aromatic hydrogen atoms to exchange phenomena is also illustrated by the results collected in **Table 3** and discussed below in light of the reaction mechanism. Large variations of the ring (D/H)$_i$ ratios occur in formylation reactions even though the ring hydrogen atoms are expected to be only slightly affected by secondary kinetic isotope effects. The (D/H)$_4$ ratio in particular exhibits very large and apparently erratic variations.

Influence of the Reaction Mechanism: Isotopic Fractionation Effects in Aromatic Formylation. The SNIF-NMR method, which avoids the need for isotope labeling of starting materials, is particularly efficient for investigating isotopic fractionation due to kinetic isotope effects (*5, 12*). To further appraise the fractionation phenomena occurring in the formation of aromatic molecules, we have considered the example of several reactions involved in the synthesis of benzaldehyde, such as chlorination of toluene followed by oxidation of the benzyl chloride intermediate, oxidation of benzyl alcohol, and hydrolysis of benzylidene chloride or cinnamaldehyde. In each case, we have carried out several series of experiments with different reactants, different experimental conditions, and different yields.

Chlorination of Toluene. Experiments characterized by different yields of reaction were carried out either with SO$_2$Cl$_2$ or with *tert*-butyl hypochlorite as reagent. The moderate isotopic fractionation effects occurring in the radical chlorination of the methyl group are illustrated in **Table 3-1**. An increase in the δ^{13}C value of residual toluene is observed due to a smaller reaction rate of ^{13}CH− as compared to ^{12}CH− isotopomers. In parallel, initial ^{13}C impoverishment with respect to toluene is measured in benzyl chloride. This behavior is typical of a direct

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Table 2. Statistical Values of the Isotopic Parameters of Aromatic Molecules[a]

molecule	δ 13C	(D/H)A	ortho (2)	ortho' (6)	meta (3)	meta' (5)	para (4)	O-alk	C 1'	2'	3'
phenol	−26.7	154.1	166.0		155.7		128.5				
n = 4	2.8	10.0	18.4		8.7		4.0				
pyrocatechol	−28.1	140.3	/	125.1		155.5					
n = 3	0.4	2.7	/	3.3		2.2					
benzonitrile	−26.8	138.6	139.3		134.9		144.5				
n = 1											
benzaldehyde[b]	−29.2	155.0	139.5		148.6		149.8		166.7		
ex benzal, n = 16	0.5	5.6	2.8		7.2		11.1		6.0		
	−26.0	266.5	134.9		146.2		144.4		892.4		
ex-toluene, n = 13	1.3	33.5	5.7		9.4		4.0		65.9		
p-hydroxybenzaldehyde	−27.5	181.5	151.5		150.6				303.0		
n = 4	0.3	21.0	8.1		10.3				79.9		
salicylaldehyde	−31.5	177.4	/	168.7	179.2	180.2	172.8		222.0		
n = 2	0.3	8.1	/	2.8	14.9	26.1	0.4		8.1		
benzylidene chloride[c]	−28.9	149.2	143.9		151.8		151.8		165.7		
n = 4	1.2	6.0	3.7		3.4		3.4		3.9		
benzyl chloride	−27.9	147.8	143.0		140.2		135.1		166.5		
n = 4	0.2	3.4	5.8		11.7		12.5		2.6		
toluene	−28.0	145.8	153.4		158.8		178.1		121.0		
n = 6	2.0	7.5	7.1		9.4		16.4		4.4		
anisole	−30.5	156.0	153.7		166.1		194.1	138.1			
n = 2	1.7	5.9	8.6		10.8		11.0	1.1			
benzyl alcohol	−27.6	162.2	162.2		149.3		153.5		179.1		
n = 3	0.4	13.6	20.9		4.4		26.2		10.0		
	−25.9	257.5	159.0		164.4		148.9		503.5		
n = 2	0.5	13.3	7.0		3.8		17.7		34.7		
cresol (m)[c]	−28.0	148.0	170.6	170.6		174.6	88.4		143.9		
n = 4	1.6	6.1	11.8	11.8		10.9	4.3		7.7		
guaiacol[c]	−30.6	143.0	/	136.9	122.0	144.4	144.4	140.4			
n = 4	3.1	4.0	/	5.7	6.8	9.6	9.6	2.9			
xylene (m)[d]	−26.3	142.6	138.5	140.3		143.8	140.3		143.9		
n = 2	0.5	1.1	5.9	3.8		3.0	3.8		1.0		
2-phenylethanol[c,e]	−27.7	140.1	149.1		135.2		135.2		120.2	140.6	
n = 5	1.8	6.9	4.3		6.2		6.2		9.2	5.4	
	−28.1	166.0	136.9		143.8		143.8		283.5	100.3	
n = 2	1.6	3.3	1.8		25.9		25.9		68.6	15.0	
p-anisaldehyde	−31.5	152.8	143.2		135.3			126.1	287.7		
n = 2	1.2	1.2	6.9		1.1			1.2	5.7		
vanillin[c]	−30.1	163.0	127.6	127.6		138.5		142.3	326.3		
n = 6	1.4	4.5	3.9	3.9		11.0		3.7	9.9		
cinnamaldehyde[c]	−28.4	150.7	145.8		144.9		144.9		163.8	144.9	151.4
n = 1	−25.5	229.7	140.4		137.0		137.0		864.7	141.7	138.6
n = 3	1.4	15.7	10.7		6.3		6.3		75.5	12.8	12.8
benzyl acetate[c]	−29.2	152.9	171.9		137.3		137.3		178.3	139.0	
n = 4	0.1	1.6	4.5		3.2		3.2		1.9	1.0	
	−27.4	221.4	173.6		140.2		140.2		542.9	120.3	
n = 4	0.0	1.8	9.5		7.6		7.6		2.5	0.8	

[a] The mean values and standard deviations are computed from results obtained on n samples from different commercial origins. The ring carbon given the number 1 is usually C-substituted. In the absence of a C-substituent, the number 1 is given to the carbon substituted by the −OH group (or OCH3 in anisole). In vanillin, the carbon substituted by the aldehyde group is given the number 1. The lateral chain is numbered 1′, 2′, ..., starting from the ring. The carbon isotopic deviations are expressed in ‰ and the hydrogen isotope parameters in ppm. (D/H)A is the overall isotope ratio of the molecule. [b] These statistical data include samples investigated by Remaud et al. (*21*). [c] When equal values are given to both the mean and the standard deviation, the 2H NMR signals are too close to be estimated separately. [d] Sites 4 and 6 are magnetically equivalent. [e] Different ranges of values have been measured in several commercial samples (*32, 33*).

kinetic isotope effect, $k(^{12}C)/k(^{13}C) > 1$. Deuterium enrichment at the methyl site of toluene also characterizes a direct primary kinetic hydrogen isotope effect. Although this reaction is expected to produce only small secondary isotope effects at the benzenic hydrogen positions, the isotopic parameters measured on both residual toluene and benzyl chloride exhibit significant and largely irregular variations, especially at position para. As discussed above, this behavior illustrates the role of hydrogen exchanges, which are highly dependent on the experimental conditions and are oriented by electronic effects of the substituent.

Oxidation of Benzyl Chloride and Benzyl Alcohol into Benzaldehyde. At natural isotopic abundance, oxidation of −CH2X (or −CH3) fragments involves intramolecular competition (**Scheme 1**).

The equations describing isotopic fractionation as a function of the fraction of reaction are governed by both primary and secondary isotope effects (*12*). The isotope ratio of the remaining reactant, S, at time t, $(D/H)_t^S$, referred to the initial value, $(D/H)_{t=0}^S$, is given by eq 1 (*12*)

$$(D/H)_t^S/(D/H)_{t=0}^S = (1 - \rho)^{[(1/2)(k_D/k_H + k_{H\alpha}/k_H)-1]} \quad (1)$$

where ρ denotes the fraction of reaction.

For the product of the reaction, P, the corresponding ratio is

$$(D/H)_t^P/(D/H)_{t=0}^S = (2/\rho)[(k_H/k_D)/(k_H/k_D + k_H/k_H^\alpha)] \times$$
$$[1 - (1 - \rho)^{[(1/2)(k_D/k_H + k_{H\alpha}/k_H)]}] \quad (2)$$

*Oxidation of Benzyl Chloride (**Table 3-2**).* We have carried out several experiments (experiments D) in which benzyl chloride was oxidized into benzaldehyde by using hexamethylenetetramine at different molar ratios. An initial 13C impov-
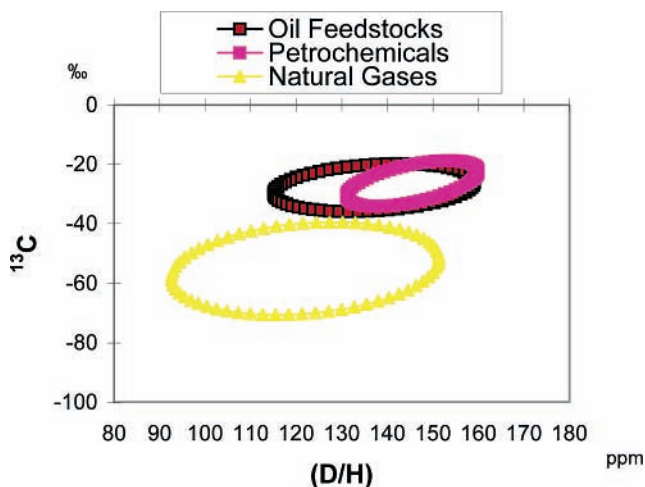
Figure 1. Representation of different kinds of fossil raw materials in the plane of the overall carbon, δ^{13}C (‰), and hydrogen, D/H (ppm), isotope ratios: the bivariate ellipsoids are computed from values published in the literature (*8–10, 26–31*). In the case of fossil oils, 90 samples of marine oils and 30 samples from terrestrial origin have been considered. The mean D/H and δ^{13}C values of kerogen and fossil oils are not very different, in ranges from 132 to 144 ppm and from −27 to −28‰, with standard deviations on the order of 6 ppm and 6‰, respectively. Natural gases, either biogenic or thermogenic, are characterized by smaller D/H values in a range of 119–128 ppm with relatively high standard deviations (≈14 ppm). Similarly, natural gases are depleted in ^{13}C with respect to oils. Biogenic gases in particular exhibit values between −73 and −55‰ (standard deviations of ≈20 ‰). Higher values are measured in thermogenic gases.

erishment of the product reaching about 3‰ is indicative of a relatively high $k(^{12}$C)/$k(^{13}$C) isotope effect. An important deuterium enrichment is observed at the formyl position. Thus, at 91% conversion, hydrogen fractionation with respect to benzyl chloride reaches 66%. This behavior is mainly due to a relatively high value of the direct primary kinetic hydrogen isotope effect. From eqs 1 and 2, the ratio $(D/H)_t^{CHO}/(D/H)_{t=0}^{CH_2Cl}$ at $\rho = 1$ can be expressed (eq 3) as a function of the kinetic isotope effects:

$$(D/H)_t^{CHO}/(D/H)_{t=0}^{CH_2Cl} = 2(k_H/k_D)/(k_H/k_D + k_H/k_H^{\alpha}) \quad (3)$$

In the approximation of a secondary kinetic isotope effect close to 1, a value of k_H/k_D on the order of 2.6 is estimated from the experimental results of **Table 3-2**.

It should be emphasized that the benzenic position para to the substituent is also noticeably affected in the reaction, possibly as a result of exchange phenomena. The isotopic values determined in this laboratory process, in particular those of the formyl group, are not fully consistent with those of the commercial benzaldehyde considered in **Table 2**. This behavior illustrates the strong dependence of isotopic fractionation on the conversion rate and experimental conditions in these reactions.

*Oxidation of Benzyl Alcohol (**Table 3-3**).* From kinetic experiments carried out on benzyl alcohol samples deuterated at the methylene position it has been shown (*13–16*) that kinetic isotope effects, and therefore mechanistic features of the reaction, largely depend on the nature of the reactant: bromine, potassium permanganate, sodium bromate, pyridinium fluorochromate, ... We have investigated the oxidation of benzyl alcohol by sodium dichromate either at different oxidant/alcohol ratios in basic, neutral, and acid media or as a function of time

for a given oxidant/alcohol ratio. The results of experiments performed in identical conditions, but stopped at different amounts of reaction (experiments F and H), may be analyzed in terms of overall $k(^{12}$C)/$k(^{13}$C), primary k_H/k_D, and secondary k_H/k_H^{α}, kinetic isotope effects. In all experiments an initial ^{13}C impoverishment of the product as compared to the starting material is indicative of a significant direct $k(^{12}$C)/$k(^{13}$C) isotope effect. As expected, a continuous increase in the carbon isotope parameter of the reactant is observed in parallel. The specific hydrogen isotope ratios of the reacting site are governed by eqs 1−3. As predicted for such competitive reactions (*12*), deuterium impoverishments of the product initially observed at low conversion rates are indicative of secondary KIE values, k_H/k_H^{α}, higher than unity (see, for example, experiments F−H in **Table 3-3**: impoverishments on the order of 40 and 30 ppm at fractions of reaction 0.10 and 0.12). On the other hand, the relatively fast increase of the methylene isotope ratio, of residual alcohol as a function of the fraction of reaction, is typical of primary KIE values largely higher than unity (see, for example, experiments F in **Table 3-3**: enrichment reaching 40 ppm at a fraction of reaction 0.19 in neutral medium). In parallel, the combination of primary and secondary direct isotope effects is responsible for the continuous increase of the formyl isotope ratio toward a limit value defined by eq 3. From experiments performed in neutral medium (experiments F), values of k_H/k_D and k_H/k_H^{α} can be estimated by fitting the experimental curves $(D/H)_t^{CH_2OH}/(D/H)_{t=0}^{CH_2OH} = f(\rho)$ and $(D/H)_t^{CHO}/(D/H)_{t=0}^{CH_2OH} = f(\rho)$ to eqs 1 and 2. For a given value of ρ, the kinetic isotope effects can be explicitly expressed as a function of the fractionation ratios of both the reactant and the product. Moreover, k_H/k_H^{α} can be directly determined from the value of $(D/H)_{t=0}^{CHO}/(D/H)_{t=0}^{CH_2OH}$ measured at $\rho \rightarrow 0$. The computed average values are $k_H/k_D = 6.6$ and $k_H/k_H^{\alpha} = 1.1$. A similar treatment of the results obtained in acid medium (experiment H) gives $k_H/k_D = 3.4$ and $k_H/k_H^{\alpha} = 1.08$.

The isotopic properties of products resulting from such oxidation reactions can therefore be reliably interpreted and, conversely, they can be helpful for characterizing a given process.

Hydrolysis of Benzylidene Chloride and of Cinnamaldehyde into Benzaldehyde. As expected from the preservation of the CH bond in these kinds of reaction, only small isotope fractionation effects are observed in the two series of experiments described in **Table 3-4,-5**. Thus, in contrast to oxidation reactions, hydrolysis of benzylidene chloride leaves the carbon isotope ratio nearly unchanged. Similarly, all of the hydrogen isotope parameters exhibit only small variations. In particular, the isotope ratio of site 1′, in position α to the ring of cinnamaldehyde, is nearly preserved in the transformation into benzaldehyde. Moreover, by repeating experiments of **Table 3-5** in the same experimental conditions except for a slight ^{2}H enrichment of water, we have shown that no substantial hydrogen exchange is involved. Consequently, when these types of reaction are concerned, the isotopic data measured on products may be considered as directly representative of the origin of the starting material.

Origin Inference of Aromatic Molecules. In a context where the natural status of products is considered as an important quality criterion, it is of prime importance to be able to ascertain, for a given molecule, whether it has been extracted from plants or has been obtained by chemical synthesis using either fossil or natural raw materials. We have shown, in parallel (*2*), that isotopic distributions are a rich source of information on the biogenesis of natural aromatic products. In comparison, **Table**

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Table 3. Variations of the Isotopic Parameters in 11 Series of Experiments (A–K)[a]

Molecule	Yield	$\delta^{13}C$	(D/H)$_A$	2 , 6	3 , 5	4	C-subst
(A) Toluene ⟶ Benzyl chloride *(sulfuryl chloride, neat)*							
toluene	neat	-31.2	136.2	144.6	147.8	150.3	118.0
res. toluene	0.21	-31.0	138.7	143.2	147.1	180.3	116.1
benzyl chloride.		-32.9	144.9	142.0	169.7	123.9	133.6
res. toluene	0.38	-30.5	140.7	144.0	148.7	174.0	122.0
benzyl chloride.		-32.7	144.5	135.0	183.0	121.6	127.0
res. toluene	0.67	-29.9	144.8	145.0	147.2	170.3	134.6
benzyl chloride..		-32.2	144.8	130.9	175.3	132.4	134.4
(B) Toluene ⟶ Benzyl chloride *(sulfuryl chloride in carbon tetrachloride solution)*							
res. Toluene	0.25	-30.6	135.0	136.9	140.1	191.3	111.5
benzyl chloride.		-33.0	140.4	135.6	175.0	102.4	129.7
res. toluene	0.62	-29.6	132.5	131.2	135.6	221.4	101.7
benzyl chloride.		-32.5	141.8	130.9	146.0	160.3	139.2
(C) Toluene ⟶ Benzyl chloride *(tert-butylhypochlorite)*							
benzyl chloride.	0.24	n.d.	138.9	122.0	171.2	133.6	126.1
benzyl chloride.	0.49	-33.2	138.2	107.4	146.0	202.7	128.8

Table 3-1. Chlorination of toluene into benzyl chloride[b]

Molecule	Yield	$\delta^{13}C$	(D/H)$_A$	2 , 6	3 , 5	4	C-subst
(D) Benzyl chloride ⟶ Benzaldehyde *(hexamethylenetetramine)*							
benzyl chloride	neat	-28.0	143.8	135.4	123.4	149.9	169.4
res.benzyl chloride	0.25	-25.6	144.1	139.0	142.3	128.7	158.7[d]
benzaldehyde		-31.4	154	137.0	137.6	176.3	198.6
benzaldehyde	0.43	-31.0	157.9	140.8	140.2	171.0	214.3
benzaldehyde	0.91	-29.2	158.8	137.4	137.4	177.1	225.7

Table 3-2. Oxidation of benzyl chloride into benzaldehyde[c]

Molecule	Yield	$\delta^{13}C$	(D/H)$_A$	2 , 6	3 , 5	4	C-subst
(E) Benzyl alcohol ⟶ Benzaldehyde *(sodium dichromate in basic media)*							
benzyl alcohol	neat	-26.2	248.1	154.0	167.1	136.3	478.9
benzaldehyde	0.20	-28.9	203.6	135.4	146.2	178.8	479.7
benzaldehyde	0.36	-29.0	206.9	140.2	149.8	176.3	485.2
res.benzyl alcohol	0.54	-23.3	302.5	168.8	156.6	136.8	714.7
benzaldehyde		n.d.	212.6	139.4	151.4	154.9	539.0
(F) Benzyl alcohol ⟶ Benzaldehyde *(sodium dichromate in neutral media)*							
res.benzyl alcohol	0.10	-26.0	251.8	154.0	168.9	108.3	504.1
benzaldehyde		-29.9	191.3	140.4	142.0	146.9	435.8
res.benzyl alcohol	0.19	-25.6	257.7	159.8	165.2	95.2	529.3
benzaldehyde		-30.0	194.6	143.1	141.9	143.3	454.7
res.benzyl alcohol	0.28	-25.1	265.7	151.6	165.1	115.1	555.8
benzaldehyde		-29.6	203.8	139.1	145.2	184.6	469.3
benzaldehyde	0.36	-29.5	203.9	140.4	148.6	146.4	499.3
res.benzyl alcohol	0.42[f]	-24.4	287.8	164.4	171.1	72.1	635.9
(G) Benzyl alcohol ⟶ Benzaldehyde *(sodium dichromate in neutral media)*							
benzaldehyde	0.37	-29.3	198.9	136.5	143.6		457.0
benzaldehyde	0.54	-28.7	201.7	135.1	142.4	161.9	493.4
(H) Benzyl alcohol ⟶ Benzaldehyde *(sodium dichromate in acid media)*							
res.benzyl alcohol	0.12	-25.9	250.5	158.1	163.4	105.0	503.0
benzaldehyde		-29.0	197.4	135.3	140.3	183.6	449.8
res.benzyl alcohol	0.15	-25.8	253.6	158.7	167.2	102.3	510.4
benzaldehyde		-29.1	199.2	138.2	141.8	178.3	456.9
res.benzyl alcohol	0.22	-25.6	258.7	160.2	164.0	99.3	531.6
benzaldehyde		-29.4	202.0	138.3	143.7	180.7	467.4
(I) Benzyl alcohol ⟶ Benzaldehyde *(NaOCl in acid media)*							
benzaldehyde	0.58	-27.4	231.8	145.6	147.6	177.4	627.1
benzaldehyde	0.86	-26.2	241.3	147.7	148.9	175.6	679.0

Tableau 3-3   Oxidation of benzyl alcohol into benzaldehyde[e]

Table 3 (Continued)(Continued)

Molecule	Yield	$\delta^{13}C$	$(D/H)_A$	2 , 6	3 , 5	4	C-subst
(J) Benzylidene chloride			Benzaldehyde	*(aqueous media)*			
benzylidene chloride	neat	-29.6	153.6	145.4	153.9		169.0
benzaldehyde	0.28	-29.9	143.3	133.1	141.4	167.7	158.4
res.benzylidene chl.	0.49	-29.2	152.1	146.2	150.2		169.8
benzaldehyde		-29.9	144.1	138.9	141.5	159.8	160.5
res.benzylidene chl.	0.52	-29.3	149.1	141.8	148.1		166.5
benzaldehyde		-30.0	141.4	136.9	140.4	152.2	160.4

Tableau 3-4. Hydrolysis of benzylidene chloride into benzaldehyde[g]

Molecule	Yield	$\delta^{13}C$	$(D/H)_A$	2 , 6	3 , 5	4	C-subst
(K) Cinnamaldehyde			Benzaldehyde	*(aqueous media)*			
cinnamaldehyde	neat	-28.0	150.7	145.8	144.9		163.8[h]
benzaldehyde	0.68	-27.6	147.2	137.6	142.5	158.7	164.2

Tableau 3-5. Retroaldolisation reaction of cinnamaldehyde into benzaldehyde

[a] Within every series of experiments the results are given, for both residual substrate and product, in increasing order of reaction yield. The isotope ratios are expressed in ‰ for carbon and in ppm for hydrogen. $(D/H)_A$ is the overall isotope ratio of the molecule; n.d. is for not determined and res. for residual. [b] The chlorination was performed either with sulfuryl chloride, neat (A) or in carbon tetrachloride solution (B), or with *tert*-butyl hypochlorite at two molar ratios, 0.43 and 0.68 (C). [c] The formylation reactions have been carried out with three different concentrations (molar ratios 0.15, 0.25, and 1.08) of hexamethylenetetramine (D). [d] Dubious value. [e] The commercial sample of benzyl alcohol used in these experiments is from Aldrich. The oxidation reactions E were performed in basic medium with three different concentrations of sodium dichromate, corresponding to three different yields. The results of experiments F were obtained by stopping, at five different times, a reaction performed with a given concentration of dichromate in neutral medium (oxidant/alcohol 0.33). Experiments G, carried out with dichromate in neutral medium, correspond to two different molar ratios of $Na_2Cr_2O_7/PhCH_2OH$ (1.81 and 0.55). [f] At reaction yield 0.42 only residual benzyl alcohol was measured. [g] The three experiments correspond to different concentrations of water, associated with different yields. [h] This value is that of position 1′ (α to the ring). The other data are $(D/H)_{2'}$ = 164.2 and $(D/H)_{CHO}$ = 151.4 ppm.

Scheme 1

$$Ar-CH_2X + Oxid \quad ---- \quad 2k_H \rightarrow \quad Ar-CHO + Red\ H$$
$$Ar-CHDX + Oxid \quad ---- \quad k^{\alpha}_H \rightarrow \quad Ar-CDO + Red\ H$$
$$---- \quad k_D \rightarrow \quad Ar-CHO + Red\ D$$
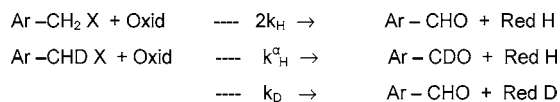
2 gives the basis for easily differentiating plant molecules from their synthetic counterpart and illustrates the aptitude of isotopic parameters for providing further information on the chemical history of the molecule. Thus, it is noted, for instance, that the ^{13}C contents of methoxy phenols are smaller than those of phenols. This behavior may be explained by the use of depleted raw materials derived from gas in the methylation process. We may also observe that similarities in the isotopic values are compatible with a synthesis of guaiacol through methylation of catechol.

In the case of benzyl chloride, the dispersion of the isotopic values measured on samples from different commercial sources is relatively limited (**Table 2**). In particular, the D/H value of the chloromethyl fragment remains close to 166 ppm. Exchanges are probably minimized in industrial syntheses of benzyl chloride involving thermal or photochemical chlorination with molecular chlorine at moderate temperature. Moreover, it should be noted that, in these industrial syntheses, the deuterium enrichment of the chloromethyl group is higher than in our laboratory experiments, as expected for more advanced reactions with significant primary kinetic isotope effects.

Frequently a given chemical species can be produced not only from different raw materials but also through different chemical processes (*17*). Thus, many formylation methods can be used for synthesizing aromatic aldehydes: direct formylation, oxidation of a saturated or unsaturated lateral chain, hydrolysis of functional derivatives, retrogradation of α-hydroxyacid, ...

However, in practice, the number of processes used in the industrial production of commercial products is relatively restricted. In the case of benzaldehyde, for instance, two main groups are distinguished, at first approximation, according to the isotope ratio of the formyl group (**Table 2**). A first group of commercial benzaldehyde characterized by relatively low values of $(D/H)_{CHO}$ and moderate values of the standard deviations is from benzal origin. As shown above, oxidation of benzyl chloride or hydrolysis of benzylidene chloride is accompanied by relatively small isotope effects. Moreover, it may be noted that hydrogen isotopic distributions in benzylidene chloride and benzyl chloride are very similar. Concerning this resource, it should be noted that residual benzal chloride was recently found to be carcinogenic, leading major manufacturers to abandon the production of benzaldehyde from chlorinated feedstock (*18*). As already observed (*19*, *20*), direct catalytic oxidation of toluene is associated with higher values of the carbon isotope ratio. Moreover, it is known to be accompanied by strong deuterium enrichment of the −CHO fragment (*21*). Very high fractionation effects are therefore indicative of this type of process. However, it should be noted that this group of samples ex-toluene is characterized by a high value of the standard deviation (**Table 2**). Indeed, the experiments described above have brought to light the great sensitivity of the site-specific hydrogen isotope ratios, not only to the mechanism of the synthetic process but also to many parameters of the experiment, reagents, pH, temperature, yield, etc. In particular, for a given reaction, a large dispersion of the isotopic ratios may be induced by kinetic fractionation effects conditioned by the rate of conversion. In practice, the great sensitivity of the $(D/H)_{CHO}$ parameter to the experimental conditions of the process can be exploited to characterize a given industrial source or even a given batch of production.

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Whatever the synthetic process, the ring fragment is expected to be preserved in the considered transformations, and only small secondary isotope effects should affect the aromatic hydrogen atoms. In fact, relatively high standard deviations are determined, in particular at position 4. As illustrated by the results concerning toluene, a common raw material, this dispersion partly reflects differences in the fingerprint of the fossil precursor. In addition, exchange perturbations may occur in the course of the transformations, as observed above in several experiments.

An important process for the industrial synthesis of hydroxybenzaldehyde is the condensation of the aromatic substrate with a salt of glyoxylic acid, followed by oxidative retrogradation of the α-hydroxyacid obtained. In the case of vanillin, the aromatic substrate is guaiacol and the precursor material of glyoxylic acid is either naphtha or biomass. The results measured in six samples of commercial chemical vanillin illustrate the strong deuterium enrichment of the formyl site occurring in these reactions. The influence of the production process of glyoxylic acid has already been considered, and $(D/H)_{CHO}$ values in the range of 180−230, 220−260, or 330−400 ppm have been distinguished in industrial vanillin (*22*, *23*). High values of $(D/H)_{CHO}$ are also observed in *p*-hydroxybenzaldehyde, and the dispersion also characterizes differences in the production process. More generally, oxidation reactions, in which electrophilic hydrogen eliminations are accompanied by high KIE values, are the source of significant deuterium enrichments at the formyl site. Aldehydes such as *p*-anisaldehyde and salicylaldehyde, for instance, which are mainly obtained from *p*-methoxytoluene and hydroxybenzyl alcohol, respectively, exhibit $(D/H)_{CHO}$ values in the range of 200−300 ppm.

Two types of synthetic benzyl alcohol are also clearly distinguished. On the basis of the previous experiments it may be concluded that samples characterized, in particular, by high values of $(D/H)_{1'}$ are obtained by reduction of benzaldehyde that results itself from the catalytic oxidation of toluene. In contrast, more uniform isotopic distributions are expected when other sources of benzaldehyde are used or when the industrial process involves hydrolysis of a benzyl chloride precursor. Similarly, at least two sources of phenylethanol may be distinguished, one of them being characterized by high values of the isotope ratio at position α to the ring. It should be noted that, according to results concerning natural aromatic molecules (*2*), moderate values of $(D/H)_{1'}$ are expected for non-natural molecules obtained by chemical reduction of phenylacetaldehyde, itself obtained from L-phenylalanine.

Benzyl acetate may be obtained, in particular, from benzyl chloride and sodium acetate and from benzyl alcohol and acetic acid or acetic anhydride. As in benzyl alcohol, two ranges of $(D/H)_{1'}$ values, situated around 180 and 540 ppm, are distinguished. In addition, because the esterification step is accompanied by negligible kinetic isotope effects, the D/H value of the methyl site is a source of information on the origin of the acetic precursor (*24*).

Two types of isotopic profiles are distinguished in cinnamaldehyde. As already mentioned, at least two different ranges of carbon isotope ratios are distinguished (*25*). Condensation of benzaldehyde with acetaldehyde is the usual synthetic route to commercial cinnamaldehyde. This reaction is reversible (*3*, *20*, *21*). In light of the results, described above, concerning the hydrolysis of cinnamaldehyde it may be concluded that the isotopic parameters measured on commercial samples are typical of a reverse transformation of benzaldehyde into cinnamaldehyde. The measured values of $(D/H)_{1'}$ in a range of 800−900

or 160−170 ppm reflect two different origins of benzaldehyde. The use of benzaldehyde precursors derived from direct catalytic oxidation of toluene may explain the drastic isotopic enrichment at the 1′ position.

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