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Research Article

Synthesis of [uniformly ring-^{14}C]-labelled 4-hydroxybenzaldehyde, vanillin, and protocatechualdehyde

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Summary

[Uniformly ring-^{14}C]-labelled 4-hydroxybenzaldehyde, vanillin, and protocatechualdehyde were synthesized from [^{14}C]-labelled phenol, guaiacol, and catechol with methyl dichloromethyl sulfide (CH_3SCHCl_2) under *Friedel–Crafts* alkylation conditions in dichloromethane at $-78^{\circ}C$ for 5 min (in the case of phenol and guaiacol) or at $-20^{\circ}C$ for 1 min (in the case of catechol), by rapid addition of $SnCl_4$ to mixtures of the phenolic compound and CH_3SCHCl_2, followed by hydrolysis with HCl. Regioselective formylation (*para* to the –OH group) was achieved. The conversion rates were 96, 81, and 88% for 4-hydroxybenzaldehyde, vanillin, and protocatechualdehyde, respectively, and the yields of the recovered products after work-up amounted to 88, 75, and 83%, respectively. In the case of guaiacol, 17% of isovanillin was obtained as by-product. It was found that the presence of water or ethyl acetate in the reaction mixture, at a molar ratio of 60:1 (water:guaiacol) or 120:1 (ethyl acetate:guaiacol), had little influence on the yields under the reaction conditions. Factors influencing the yields are discussed in the study. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: isotope labelling; *Friedel–Crafts* alkylation; formylation; ^{14}C-*p*-hydroxybenzaldehyde; ^{14}C-vanillin; ^{14}C-protocatechualdehyde

Introduction

Monomeric phenols, stemming from degradation of plant and animal residues, microbial synthesis, and rood exudation, are ubiquitous in soil.[1,2] In addition to being recognized as allelophathic compounds,[3] these naturally occurring monomeric phenols can be mineralized and stabilized in soil by both

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abiotic and biotic soil processes, and regarded as precursors of soil organic matter.[4–8] In order to elucidate these processes, radioactively labelled phenols are needed to trace the fate of phenols in soil and to identify the binding sites of the residues in soil matrices. Since the aldehydes 4-hydroxybenzaldehyde, vanillin, and protocatechualdehyde are intermediates for the chemical synthesis of isotope-labelled lignin monomers and oligomers, and their transformation products,[9–12] the successful synthesis of these aldehydes is the prerequisite for obtaining isotope-labelled phenolic compounds, e.g. *p*-coumaric acid, *p*-coumaryl alcohol, ferulic acid, coniferyl alcohol, caffeic acid, etc.[10]

The ring-labelling of these aldehydes can be realized by formylation of commercially available starting compounds (e.g. phenol and catechol). The chemical introduction of an aldehyde group on phenols can be performed by many methods,[13,14] such as condensation reaction (with glyoxylic acid or *Reimer–Tiemann* reaction) and *Friedel–Crafts* reaction (*Vilsmeier* reaction or *Gattermann* reaction). Condensation of glyoxylic acid with phenol, guaiacol, or catechol followed by an oxidation process can lead to 4-hydroxybenzaldehyde, vanillin, or protocatechualdehyde in relatively good yields (e.g. Kalikar *et al*., 1986; Bjorsvik *et al*., 2000)[15,16] and is currently the main route for vanillin manufacture (see Li and Frost, 1998).[17] Bjorsvik *et al*.[16] applied a statistical experimental design and multivariate modelling to the synthesis of protocatechualdehyde from catechol leading to 90.5% of the regioselectivity under the optimized experiment conditions, with a conversion of 78.4%.

The introduction of an aldehyde group using methyl dichloromethyl sulfide by *Friedel–Crafts* reaction[18] was many times applied in the literature for the synthesis of radioactively labelled 4-hydroxybenzaldehyde and vanillin[9,11,19] for the reason that this method was superior to other methods (e.g. *Vilsmeier*, *Gattermann*, or *Sandmeyer* reaction) in terms of yield and simplicity.[9] However, under the reaction conditions used in the literature this synthesis gave 50% yield for the 4-hydroxybenzaldehyde synthesis,[9] and a maximal yield of 63% for vanillin,[11] because the formylation is not strictly regioselective under the reaction conditions and other formylation by-products were formed. In addition, vanillin formed in the reaction mixture can react with CH_3SH to give dimethyl mercaptan and other by-products, e.g. di- and triphenyl methane.[11]

In this study, we optimized the reaction conditions for the *Gross–Matthey* method[18] for the synthesis of [uniformly ring-^{14}C]-labelled 4-hydroxybenzaldehyde, vanillin, and protocatechualdehyde to give yields of 88, 75, and 83%, respectively. Except for the formylated products at the *para*-position to the –OH group (in the case of guaiacol, also to the $-OCH_3$ group), hardly any by-products were formed under the modified reaction conditions.

Results and discussion

[Uniformly ring-^{14}C]-labelled 4-hydroxybenzaldehyde (**2**), vanillin (**4**), and protocatechualdehyde (**6**) were synthesized by *Friedel–Crafts* alkylation in solvent CH_2Cl_2 from [^{14}C]-labelled phenol (**1**), guaiacol (**3**), and catechol (**5**) with CH_3SCHCl_2 and catalyzed by $SnCl_4$ at low temperatures, followed by hydrolysis with HCl (Figure 1). The synthesis of 4-hydroxybenzaldehyde and vanillin were conducted at -78°C and protocatechualdehyde at -20°C. The conversion rates, yields, and recoveries of radioactivity are summarized in Table 1.

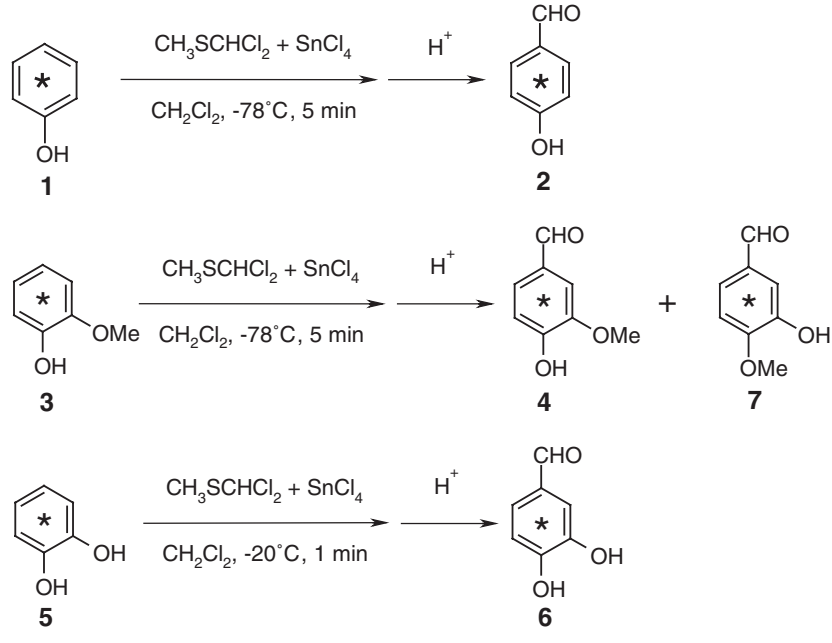

Figure 1. Synthesis of [uniformly ring-^{14}C]-labelled 4-hydroxybenzaldehyde, vanillin, and protocatechualdehyde by *Friedel–Crafts* alkylation

Table 1. Analytical data for the synthesis of [uniformly ring-^{14}C]-labelled 4-hydroxybenzaldehyde, vanillin, and protocatechualdehyde

Synthesis		Conversion rate[a] (%)	Recovered yield[b] (%)	Radiochemical purity (%)
1 → 2		96	88	97
3 → 4 + 7	**4**	81	75	99
	7	17	17	96
5 → 6		88	83	99

[a] Percentage of ^{14}C in product relative to the amount of ^{14}C compound used (as determined from autoradiography).
[b] Percentage of ^{14}C in the purified product.

Compared to the reported yields in the literature, our modified procedure gives higher yields for all three aldehydes. The formylation is almost quantitatively regiospecific at the *para*-positions to the active groups. In the case of phenol (with only one –OH group) and catechol (with two chemically identical –OH groups), the *para*-products accounted for nearly 100% of the products. In the case of guaiacol, two *para*-products (vanillin and isovanillin) were obtained, and the *para*-position ratio of the –OH group to the $-OCH_3$ group is about 81:17 (Table 1). *Ortho*-products of phenol and guaiacol were not observed and therefore the purification procedures were quite simple. In our experiments, we used a ten-fold excess of CH_3SCHCl_2 or $SnCl_4$, due to the volatility of the chemicals and the low volume applied (μl range), even though the preliminary experiments showed that 5% more than the stoichiometric amount is actually enough.

One of the modifications in our method is the lower reaction temperatures. At low temperature, the formation of dimethylmercaptal or di- and triphenylmethane, which was observed at 0°C by Kratzl and Vierhapper,[11] is suppressed. It was shown that with decreasing reaction temperature, the conversion rate of vanillin increases and that of isovanillin decreases slightly, e.g. at 0°C the conversion rates of vanillin and isovanillin amounted to $73.2 \pm 0.2\%$, and $21.5 \pm 1.0\%$ ($n = 5$), respectively, while at −20°C, the figures were $75.7 \pm 0.6\%$ and $20.3 \pm 1.0\%$ ($n = 6$), respectively. At $T < -60$°C, the conversion rates of vanillin and isovanillin were 81.0 ± 0.7 and $17.2 \pm 0.7\%$ ($n = 6$), respectively.

Besides the reaction temperature, the rate of addition of the catalyst $SnCl_4$ to the mixture of phenol and guaiacol is another influential factor in determining the product yields. The addition of $SnCl_4$ should be rapid. Experiments showed that at −18°C, the yields of vanillin and isovanillin amounted to 54 and 27%, respectively, when $SnCl_4$ was added to guaiacol (0.4 mmol) within 20 s, and the yield of 4-hydroxybenzaldeyhde amounted to 30%, when $SnCl_4$ was added to phenol (0.4 mmol) within 4 min. However, the yield of protocatechualdehyde seemed to be independent of the addition rate. As an alternative to the addition of catalyst to the reactants, the phenols can be added dropwise to the mixture of CH_3SCHCl_2 and $SnCl_4$, and the yields remained almost the same.

This *Friedel–Crafts* reaction is very fast and even at −78°C phenol and guaiacol can be completely converted within 5 min. Longer reaction time leads to lower yields. In the case of guaiacol, it was shown that the yields of vanillin and isovanillin decreased from 80.7 to 69.0% and from 17.7 to 14.5%, respectively, when the reaction time was prolonged from 5 to 60 min. In the case of catechol, 1 min reaction time was suitable, even though the conversion of catechol was not complete (about 5% catechol remained). When the reaction time was prolonged to 5 min, the yield of

protocatechualdehyde did not increase and high amounts of by-products were formed (ca. 10%).

As an alternative to CH_2Cl_2 as solvent, petrol ether can be used for synthesis of 4-hydroxybenzaldehyde and vanillin. It was also shown that acetonitrile can be used as a solvent for the synthesis of protocatechualdehyde; however, the yield was not determined. We found that the presence of water and ethyl acetate (EtOAc) in the reaction mixture had little influence on the yield of vanillin. When H_2O was added to the reaction mixture at a molar ratio of 60:1 (H_2O:guaiacol) before reaction at $-78^{\circ}C$ for 5 min, the yields of vanillin and isovanillin amounted to 74.5 and 14.8%, respectively. In the case of EtOAc, with a molar ratio of 120:1 (EtOAc:guaiacol), the yields of vanillin and isovanillin amounted to 73.0 and 14.8%, respectively. The molar ratio of guaiacol:CH_3SCHCl_2:$SnCl_4$ in these experiments was 1:10:10.

Purification of products can be performed by preparative thin-layer chromatography (TLC) or column chromatography. During the work-up, especially, the reduction of mixture volume by rotary evaporation, some compounds may be formed, which, however, can be divided again into products by boiling the mixture in water for 30–60 min. Pure 4-hydroxybenzaldehyde can also be obtained by sublimating the raw product under 0.04 mbar at 105°C for 3 h. Good separation of vanillin and isovanillin by preparative TLC needs several times development of the plate in the eluent (*n*-pentane:EtOAc = 3:1/v:v, containing 0.5% HCOOH).

Experimental

TLC was performed on silica gel 60 with fluorescence indicator (Sil G-25 UV254, 0.25 mm; Macherey-Nagel, Düren, Germany) and viewed under UV light (254 nm). Preparative TLC was performed on plates with 2 mm of silica gel 60 layer, which were pre-treated by eluting the plates in EtOAc. Column chromatography was conducted with silica gel 60 (particle diameter 0.064–0.200 mm, Merck, Germany). For autoradiography of the TLC plate, a bioimaging analyzer (Fujifilm BAS-1000; Tokyo, Japan) was used. Quantitative determination of radioactivity was performed with a liquid scintillation counter LS6500 (Beckman Coulter; CA, USA) using the cocktail Lumasafe Plus (Lumac LSC; Groningen, The Netherlands). Product identification was conducted by TLC and gas chromatography coupled to a mass spectrometer.

[U-^{14}C]-phenol (**1**), with a specific activity of 2.70×10^9 Bq mmol^{-1} and 97% radiochemical purity, was supplied as a solution in petrol ether by Hartmann Analytic (Braunschweig, Germany), its radiochemical purity decreased to 95% during storage. [U-^{14}C]-phenol, without any purification, was diluted with non-labelled phenol, resulting in a specific radioactivity of 4.11×10^8 Bq mmol^{-1}. [U-ring-^{14}C]-guaiacol (**3**, 4.11×10^8 Bq mmol^{-1}, 98.1% radiochemical purity) and [U-^{14}C]-catechol (**5**, 7.28×10^7 Bq mmol^{-1}, 99.7% of

radiochemical purity) were synthesized in our laboratory from [U-^{14}C]-phenol according to Kratzl and Vierhapper[20] and Ji and Schäffer.[21] The remaining chemicals were purchased from commercial sources.

[Uniformly ring-^{14}C]-4-hydroxybenzaldehyde (**2**)

To a 5-ml flask containing 1 ml of CH_2Cl_2, five pieces of molecular sieves 4 Å (diameter 2 mm), and a magnetic stirring bar were added 50 µl of [U-^{14}C]-phenol solution (**1**) (2.60×10^6 Bq) and 7 µl of CH_3SCHCl_2 at room temperature. The mixture was cooled to −78°C in a dry ice-acetone bath and 7 µl of $SnCl_4$–CH_2Cl_2 solution (1:1/v:v, −78°C) were added within 1 s with vigorous stirring. The color of the mixture immediately became yellow. After stirring for 5 min, 2 ml of 2 M HCl were added to the mixture. The reaction flask was heated to room temperature and stirred for 20 min. The mixture was extracted with EtOAc (3 times, each 10 ml). The EtOAc extract was dried over anhydrous Na_2SO_4, rotary evaporated at 30°C to ca. 2 ml, further reduced to 0.5 ml by N_2 passage, and transferred onto a silica gel plate (20 cm × 20 cm × 2.0 mm). The plate was developed in *n*-hexane:EtOAc (3:1/v:v) containing 0.5% HCOOH. Autoradiography showed that 90.8% of the total radioactivity was located on the spot corresponding to 4-hydroxybenzaldehyde (**2**) (R_f of phenol, *para*-, *meta*-, and *ortho*-hydroxybenzaldehyde: 0.48, 0.23, 0.28, and 0.58, respectively). The product (**2**) band was separated and extracted with EtOAc (6 times, each 15 ml). Rotary evaporation of solvent resulted in the yellow product (**2**) with 97% radiochemical purity (2.16×10^6 Bq, yield 87.6% with respect to phenol).

[Uniformly ring-^{14}C]-vanillin (**4**)

To mixture of [U-ring-^{14}C]-guaiacol (**3**) (3.74×10^7 Bq) and CH_3SCHCl_2 (131 µl) in a 25-ml round bottom flask containing 4 ml petrol ether, 5 ml of CH_2Cl_2, 10 pieces of molecular sieves 4 Å (diameter 2 mm), and a magnetic stirring bar were added 308 µl of $SnCl_4$–CH_2Cl_2 solution (1:1/v:v, −78°C) within 1 s with vigorous stirring at −78°C. The color of the mixture turned from yellow–orange to red–brown within several seconds. After a further 5 min stirring, 2 ml of 2 M HCl (room temperature) were added and ice and red solid substances were immediately formed in the mixture. The bath was removed and the slightly orange mixture was transformed to give two phases. The organic phase was carefully separated. The aqueous phase was mixed with 5 ml of H_2O and extracted with CH_2Cl_2 (5 times, each 4 ml). The organic phase and CH_2Cl_2 extracts were combined and dried over anhydrous Na_2SO_4. Autoradiography, which was conducted by developing the plate firstly in *n*-pentane:EtOAc (3:1/v:v) once, then in $CHCl_3$ twice, showed that 79 and 17% of the radioactivity was located on the spots corresponding to vanillin

(**4**) and isovanillin (**7**). The extract was rotary evaporated to a volume of ca. 10 ml and transferred onto a silica gel column (350 ml, diameter 4 cm). The products (**4** and **7**) were sequentially eluted using a mixture of *n*-pentane and EtOAc with the following ratios (*n*-pentane:EtOAc/v:v): 1:0 (400 ml), 9:1 (100 ml), 8:1 (100 ml), 7:1 (100 ml), 6:1 (600 ml), 5:1 (200 ml), 4:1 (200 ml), 3:1 (200 ml), 2:1 (100 ml) and 1:1 (100 ml). Every 20 ml of eluate were collected as one fraction. Fractions of vanillin and isovanillin were identified by TLC, separately combined, and rotary evaporated to dryness, resulting in 2.75×10^7 Bq of vanillin (**4**) (yield 75.1% with respect to guaiacol) and 6.11×10^6 Bq of isovanillin (**7**) (yield 16.7% with respect to guaiacol). Autoradiography (*n*-pentane:EtOAc = 3:1/v:v, containing 0.5% of HCOOH) showed that the vanillin had a radiochemical purity of 99.2% and the isovanillin 96%, containing 2.4% vanillin (R_f of **4** and **7**: 0.32 and 0.25, respectively).

[Uniformly ring-^{14}C]-protocatechualdehyde ***(6)***

To mixture of [U-^{14}C]-catechol (**5**) (6.22×10^6 Bq) and CH_3SCHCl_2 (0.10 ml) in a 25-ml round bottom flask containing 4 ml of CH_2Cl_2 and 10 pieces of molecular sieves (diameter 2 mm, 4 Å) were added 50 µl of $SnCl_4$–CH_2Cl_2 solution (1:1/v:v) within 1 s with strong stirring at −20°C. The color of the mixture became immediately red–orange and a precipitate formed. After 1 min stirring, 2 ml of 2 M HCl was added to terminate the reaction. The mixture was warmed to room temperature and stirred for a further 30 min. After addition of 10 ml of *n*-pentane, the mixture was extracted with 2 M HCl (4 times, each 5 ml). The product in the aqueous extracts was separated by extraction with EtOAc (3 times, each 8 ml). The EtOAc extracts were combined, dried over anhydrous Na_2SO_4, and rotary evaporated at 40°C to ca. 1 ml. The product in the EtOAc solution was separated by preparative TLC using *n*-hexane:EtOAc (2:1/v:v) containing 0.5% HCOOH as eluent. Autoradiography showed that 91 and 4.7% of the radioactivity was located on the spots corresponding to protocatechualdehyde (**6**) and catechol (**5**) (R_f of **6** and **5**: 0.22 and 0.37, respectively). The protocatechualdehyde band was separated and extracted with mixture of EtOAc and *n*-pentane (1:1/v:v). After rotary evaporation of the organic solvents, a brown–red solid was obtained (5.2×10^6 Bq) in 83% yield (with respect to catechol). Autoradiography (*n*-hexane:EtOAc = 2:1/v:v, containing 0.5% HCOOH) showed the radiochemical purity of the product to be 99.2%.

Conclusion

Friedel–Crafts formylation of guaiacol, phenol, and catechol using CH_3SCHCl_2 at low temperature and rapid addition of the catalyst gives the required products in good yields and high regiospecificity (*para* to –OH

group). The reported yields are consistently higher than those reported previously.

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