SYNTHESIS OF $^{14}\underline{C}$-CONIFERYL ALCOHOL (4-HYDROXY-3-METHOXYCINNAMYL ALCOHOL)[1]

H. M. Balba and Gerald G. Still[2]
Department of Chemistry, North Dakota State University, and
Metabolism and Radiation Research Laboratory, Agricultural
Research Service, U. S. Department of Agriculture, Fargo, ND 58102
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SUMMARY

Coniferyl alcohol-β-^{14}C or -γ-^{14}C (IVa or IVb) was prepared by the reduction of methyl 4-acetylferulate-β-^{14}C or γ-^{14}C (IIIa or IIIb) with Vitride-T reagent [70% (w/v) solution of sodium bis(2-methoxyethoxy) aluminium hydride in benzene]. Several synthetic methods were compared in the preparation of 4-acetylferulic acid β-^{14}C or γ-^{14}C (IIa or IIb). The synthetic reactions used to optimize the yield of (II) are shown in Fig. 1. These reactions were:

(1) A Doebner reaction of malonic acid-2-^{14}C with acetylvanillin, followed by acetylation of the ferulic acid.

(2) A Perkin reaction of acetylvanillin with acetic anhydride-1-^{14}C. The Doebner reaction of acetylvanillin with malonic acid 2-^{14}C was superior to the other reactions for the preparation of 4-acetylferulic acid-β-^{14}C (IIa) and resulted in a 75% yield. The overall yield of coniferyl alcohol-β-^{14}C (IVa) from malonic acid-2-^{14}C was 20.6% (Note: When coniferyl alcohol-γ-^{14}C (IVb) was the desired product, malonic acid-1-^{14}C was used).

Key Words: Coniferyl alcohol, 4-hydroxy-3-methoxycinnamyl alcohol, Vitride, Carbon-14.

INTRODUCTION

Coniferyl alcohol (IV) is an important compound in lignin studies. It is

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[2] Present address: National Program Staff, Agricultural Research Service, U. S. Department of Agriculture, Beltsville, Maryland 20705.

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a common monomer in lignin formation either synthetically or biochemically in higher plants(1). The preparation of side chain ^{14}C-coniferyl alcohol was necessary for studies of the incorporation of chloroaniline compounds into synthetic lignin(2).

Coniferyl alcohol was first isolated by the enzymatic hydrolysis of coniferin (4-D-glucoside of coniferyl alcohol) with emulsin(3). Allen and Byers(4) reported the first synthesis of coniferyl alcohol by the $LiAlH_4$ reduction of ethyl acetylferulate. However, when the same conditions were used for the reduction of methyl acetylferulate, coniferyl alcohol was not produced in a reasonable yield (<5%). Instead, the main product was methyl ferulate. It was isolated by HPLC and identified by mass spectrometry [for $C_{11}H_{12}O_4$:$M^{\dot{+}}$ = (cal'd. 208)].

Sodium bis (2-methoxyethoxy) aluminium hydride is a mild reducing agent for carboxylic acids and derivatives containing double bonds(5). It is not as active as lithium aluminium hydride, and reductions are carried out at elevated temperatures. Cerny *et al.*(5) reported that 3-phenyl-2-propen-1-ol was produced in 75% yield from 3-phenyl-2-propenoic acid when this reagent was used at 80°. When the same reaction was carried out at lower temperatures (15-20°), 3-phenyl-1-propanol was the principal product. Vitride-T reagent has not been applied to reduce *p*-hydroxycinnamic acid derivatives. These compounds have always been difficult to reduce by metal hydrides because of the insolubility of the metal complex of the phenolic intermediate(6).

Several reactions have been described in the literature for the preparation of ferulic acid or 4-acetylferulic acid from vanillin or acetylvanillin. The reactions that are commonly used are the Perkin, Doebner, Knoevenagel, Claisen, and Reformatsky reactions. These reactions have been reviewed by Johnson(7). This report describes the preparation of coniferyl alcohol-β-^{14}C and -γ-^{14}C via several synthetic routes (Fig. 1).

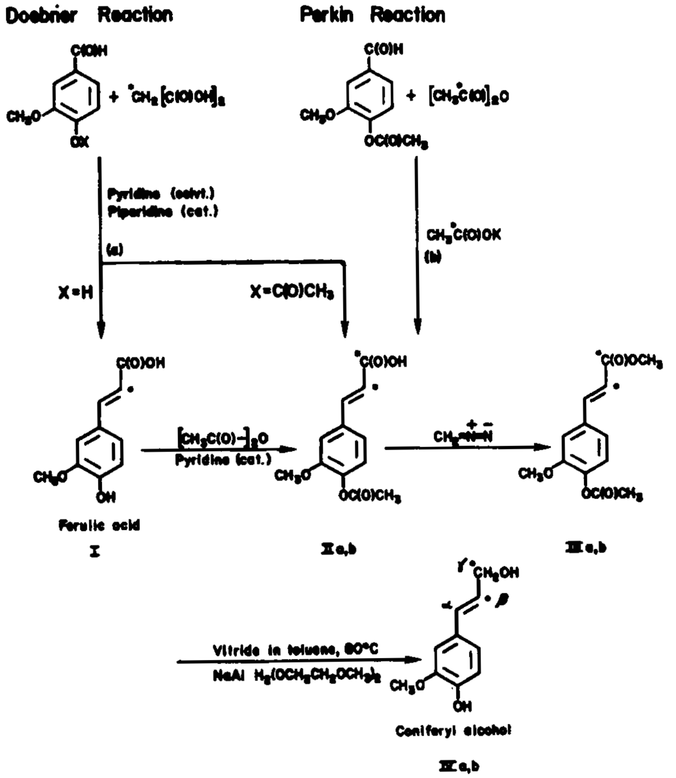

Fig. 1. Reactions used for the synthesis of coniferyl alcohol-β-^{14}C or -γ-^{14}C (IVa or IVb). [Subscripts a and b indicate that the labeled carbon is at β and γ positions, respectively].

EXPERIMENTAL

Materials and Methods

Radioactive starting materials, malonic acid-2-^{14}C (1 mCi, 9.05 mCi/mmole), acetic anhydride-1-^{14}C (0.5 mCi, 8.3 mCi/mmole), and sodium acetate-1-^{14}C (0.25 mCi, 2.55 mCi/mmole), were purchased from New England Nuclear, Boston, MA. Diazald (N-methyl-N-nitroso-p-toluenesulfonamide), vanillin 99% pure, and ferulic acid (4-hydroxy-3-methoxycinnamic acid) (II) 99% pure, were obtained from Aldrich Chemicals, Milwaukee, WI. Malonic acid and Vitride-T [70% (w/v) solution of sodium bis (2-methoxyethoxy) aluminium hydride in toluene or benzene] were purchased from Eastman Kodak, Rochester, NY. Vanillin was recrystallized from benzene (m.p. 81-82°). Piperidine (Fisher Scientific, Fair Lawn, NY) was redistilled under nitrogen, and only the distillate at 106° was used.

Radioactivity measurements were made with a Packard 3375 liquid scintillation spectrometer. Reaction yields were estimated by determining the radioactivity in the isolated pure products.

Reaction products were purified by TLC or dry column (5 x 50 cm) chromatography on silica gel(8). In some cases, liquid chromatography was used for product isolation. A liquid chromatograph (Waters Associates) equipped with a UV detector was used, and separation was achieved on a 30-cm microbondapak C_{18} column eluted with a linear water-acetonitrile gradient. The identity of synthesized products was verified by mass spectrometry (Varian CH-5 DF) and, in some cases, by mixed melting points with authentic nonradioactive samples.

Preparation of 4-acetylvanillin. Vanillin, 30.43 g (0.2 mole), was dissolved in 100 cc acetic anhydride in a 500-ml round-bottomed flask, and 0.5 ml of pyridine was added. The mixture was refluxed for 1 hr; 100 cc of water was added, and the reaction mixture was allowed to cool. The crystalline product was filtered, washed several times with hot water, and recrystallized from ethanol. The 31.8 g of 4-acetylvanillin (82%) was again recrystallized from ethanol to yield 21.85 g of 4-acetylvanillin (m.p. 75-77°, Handbook of Chemistry and Physics). The mass spectrum of the product agreed with the proposed structure [for $C_{10}H_{10}O_4$:$M^{+\cdot}$ = 194 (calc'd. 194)].

Synthesis of 4-acetylferulic acid-β-^{14}C (IIa) via the Doebner reaction. The reaction was carried out as described in the literature(9) but with a slight modification in the ratio of the reactants. One mCi of malonic acid-2-^{14}C was mixed with 0.5 g nonradioactive malonic acid (5 mmole) and placed into a 25-ml round-bottomed flask. Acetylvanillin (2.0 g, 10 mmole) was added, and the mixture was dissolved in 3 ml of glass-distilled pyridine. Six drops of piperidine were added to the solution. The reaction flask was connected to a small condenser with a drying tube. The reaction mixture was stirred and heated in an oil bath at 60 ± 2° for 8 hr.

After 8 hr, the solvent was removed under vacuum with a rotary evaporator at 40°. Three ml of pyridine and 3 drops of piperidine were added to the residue. The solution was heated at 60 ± 2° for another 8 hr. The solvent was again removed under vacuum at 40°. One ml of 40% sodium bisulfite solution and 10 glass beads (2 mm dia.) were added to the residue. The reaction flask was covered by a glass stopper, and the contents were stirred for 20 min. The reaction mixture was filtered, washed 3 times with 5 ml ice-cold water, pressed, and dried in a vacuum dessicator overnight. The solid material was dissolved in 5 ml boiling glacial acetic acid. Water was then added to the clear solution until it became turbid. The solution was cooled in an ice bath for 2 hr, and more water was added until precipitation ceased (about 15 ml water). The mixture was allowed to stand overnight at 0°. The crystallized product was obtained by filtration and washed with 5 ml portions of ice-cold water until no acetic acid odor could be detected. The crystals were pressed and dried under vacuum (m.p. 198-201°). The yield was 0.7675 g and contained 650 μCi (65% yield).

The filtrates of the reaction mixture and the recrystallization were combined and extracted 10 times with 25-ml portions of ether. The combined ether extract was dried with anhydrous sodium sulfate and concentrated to about 20 ml. The ether solution was shaken twice with 1-ml portions of 40% (w/v) sodium bisulfite solution, dried with anhydrous sodium sulfate, and the ether evaporated. The residue was recrystallized from glacial acetic acid-water as described. An additional 0.12 g of product (m.p. 198-201°) contained 101 μCi and resulted in a total yield of 75%. A mixed m.p. of the product and nonradioactive 4-acetylferulic acid (II) gave the same m.p.

Preparation of methyl 4-acetylferulate-β-^{14}C (IIIa). The combined product (IIa) was dissolved in 50 ml warm dioxane and cooled quickly in an ice bath to about 10°. Excess diazomethane in ether was added slowly to the dioxane solution until a permanent yellow color formed. The reaction was allowed to stand for 10 min in the ice bath. Solvents were removed at reduced pressure with a rotary evaporator, and the residue was recrystallized from methanol after the addition of 0.5 g of nonradioactive methyl 4-acetylferulate. The first crystallization yielded 1.05 g (m.p. 122-123°) and contained 514 μCi. The product was identified as methyl 4-acetylferulate from mixed m.p. and mass spectrum. A second crystallization from the mother liquor was obtained after the addition of another quantity of nonradioactive methyl 4-acetylferulate. The second crystallization yielded 0.5 g (m.p. 120-123°) and contained 150 μCi. The total yield was 66.4% (664 μCi), starting from malonic acid-2-^{14}C.

Reduction of methyl 4-acetylferulate-β-^{14}C (IIIa) to coniferyl alcohol- -^{14}C (IVa) with Vitride-T reagent. Methyl 4-acetylferulate-β-^{14}C (1.05 g, 514 μCi) was dissolved in 80 ml toluene in a 500-ml three-necked round-bottomed flask. The flask was equipped with a thermometer, a condenser connected to a drying tube, and a 50-ml dropping funnel. The contents of the flask were stirred with a large teflon-coated magnetic rod and magnetic stirrer. The flask was kept in a water bath at 80 ± 2°. A solution of 3.5 ml Vitride-T reagent (12 mmole) in 25 ml toluene was placed in the dropping funnel. The reagent was added slowly over a 20-min period with vigorous stirring.

After Vitride-T addition, 100 ml of ice-cold water was added, and CO_2 gas was bubbled through the reaction mixture until the color changed from yellow to white, indicating pH 8.5. The mixture was transferred to a separatory funnel and the toluene layer removed. The pH of the aqueous layer was ajusted to 6-7 with 1 N HCl and extracted 5 times with 100-ml portions of ethyl acetate. The combined ethyl acetate extracts were dried with anhydrous sodium sulfate and concentrated to about 10 ml. Nonradioactive coniferyl alcohol (0.5 g) was added to the yellow oily concentrate, and the mixture was chromatographed on a silica gel dry column developed with ether:hexane (4:1)(8). Samples of silica gel were taken with the tip of a spatula through holes about 5 cm apart along the length of the developed column, placed in test tubes, and shaken with about 0.5 ml acetone. Acetone-soluble products were chromatographed on silica gel G thin-layer plates with ether:hexane (4:1) to locate the coniferyl alcohol zone by comparison with a standard. The product was recovered by washing the silica gel of the separated column zone with methanol. The product contained 260 µCi of coniferyl alcohol-β-^{14}C (IVa) (50.6% yield). The methanol was removed in vacuo by a rotary evaporator, and the residue was recrystallized from 1,2-dichloroethane. The first crystallization yielded 0.84 g (m.p. 73-74°) and contained 130 µCi. Mass spectra and TLC R_f values (ether:hexane, 4:1) proved that the product was coniferyl alcohol-β-^{14}C (IVa). The mother liquor was fortified with additional nonradioactive coniferyl alcohol (about 0.2 g) and purified again on a dry column. A second crystallization of coniferyl alcohol yielded 0.41 g (m.p. 70-73°) and contained 23 µCi. A third crystallization contained 20 µCi and was obtained after additional nonradioactive coniferyl alcohol (about 0.1 g) was added. The third crystallization yielded 0.4 g (m.p. 59-73°); the mother liquor contained 62 µCi. Thin-layer chromatography showed that the mother liquor was composed of 53% coniferyl alcohol (33 µCi) and 47% by-products. The overall yield of coniferyl alcohol-β-^{14}C (IVa) was 206 µCi or 20.6% of the theoretical yield starting from malonic acid-2-^{14}C.

Synthesis of 4-acetylferulic acid-γ-^{14}C (IIb) via the Perkin reaction. Initial attempts to prepare ^{14}C-coniferyl alcohol were made by reducing the methyl ester of 4-acetylferulic acid-γ-^{14}C prepared by Perkin condensation of acetylvanillin with acetic anhydride-1-^{14}C(10). Acetic anhydride-1-^{14}C

(500 μCi) was diluted to 3.8 cc with nonradioactive acetic anhydride (4.08 g, 40 mmole), in a cooled 20-ml round-bottomed flask. Sodium acetate-1-^{14}C (250 μCi) was mixed with 1.96 g of freshly fused anhydrous potassium acetate (20 mmole) and added to the reaction flask. 4-Acetylvanillin (3.88 g, 20 mmole) was also added, and the mixture was refluxed with continuous stirring at 155-165° for 5.5 hr. The reaction was quenched by the addition of 25 ml of water. The product crystallized overnight at 0°, was filtered, washed several times with cold water until no acetic acid was detected, and dried in vacuo. A total of 3.71 g of the product was recovered.

Liquid chromatographic analysis of the reaction mixture indicated 3 major peaks. The product was purified in the next synthetic step.

Methylation of 4-acetylferulic acid-γ-^{14}C (IIb). The [^{14}C] product was dissolved in acetone and methylated with excess diazomethane. The solvents were removed, and the methylated product was assayed with liquid chromatography (HPLC). Several contaminating materials were found in the preparation. Methyl 4-acetylferulate-γ-^{14}C (IIIb) was purified by TLC on silica gel G (1 mm) with ether:hexane (4:1). The methyl 4-acetylferulate-γ-^{14}C zone was located under UV and extracted with methanol. The methanol solution was concentrated, 0.1 g of methyl 4-acetylferulate was added, and the material was crystallized from methanol and water. The following fractions of methyl 4-acetylferulate-γ-^{14}C were obtained: (1) 0.1292 g contained 2.3 μCi; (2) 0.03 g fraction was recovered after the addition of nonradioactive (III) to the mother liquor (0.12 μCi). Both fractions melted at 122-123° and co-chromatographed with a nonradioactive (III). The overall yield of (III) via the Perkin reaction was very low (1%) compared with the Doebner reaction (66.4%).

DISCUSSION

In the condensation step, it was clear that the yield of the Doebner reaction (66%) was considerably higher than the yield from the Perkin reaction (1%) [as methyl 4-acetylferulic acid (III)]. In the Perkin reaction, half of the radioactivity of the acetic anhydride was lost as acetic acid. It is possible that the ^{14}C-acetate moiety exchanged with the nonradioactive acetate in the 4-position of acetylvanillin. The high temperature required for this reaction may have produced some thermal degradation of the products or the reactants, as indicated from the number of peaks in the liquid chromatogram of the reaction mixture. In addition, 20% of the total radioactivity in the reaction mixture was lost after the refluxing period.

The ratio of acetylvanillin to malonic acid played an important role in the yield of 4-acetylferulic acid. A ratio of 1.2:1 gave a 65% yield [Freudenberg et al.(9) obtained a 60% yield with the same ratio]. A ratio of 1.5:1 gave a 70% yield, and 2:1 gave a 75% yield. Reversing this latter molar ratio [i.e., acetylvanillin to malonic acid (1:2)] gave a very poor yield in the case of acetylvanillin but excellent yield in the case of vanillin with the Vorsatz method(11). It was possible to condense vanillin with malonic acid to produce ferulic acid in a 75% yield by the method of Vorsatz(11). However, subsequent acetylation to the 4-acetylfuralic acid (85%) resulted in an overall yield of only 64%. The reaction of acetylvanillin with malonic acid to produce 4-acetylferulic acid in 75% yield was preferred.

The reaction of vanillin with dimethyl malonate under the conditions described by Allen and Spangler(12) for the reaction of benzaldehyde and diethyl malonate, produced an 85% yield of diethyl 4-hydroxy-3-methoxytolylidenemalonate (m.p. 104-105°), as identified by mass spectrometry [for $C_{15}H_{18}O_6$:$M^{\cdot +}$ 294 (calc'd. 294)]. Alkaline hydrolysis of this product produced almost quantitatively 4-hydroxy-3-methoxytolylidenemalonic acid (m.p. 205-209°), as identified by mass spectrometry [for $C_{11}H_{10}O_6$:$M^{\cdot +}$ 238 (calc'd. 238)].

Several unsuccessful attempts were made to decarboxylate the dicarboxylic acid and produce ferulic acid. Mild conditions, such as warming the solution of 4-hydroxy-3-methoxytolylidenemalonic acid in 2 H HCl, were not effective, and the dicarboxylic acid was removed intact. Vigorous conditions (i.e., boiling the dicarboxylic acid in 50% HCl) resulted in polymerization.

Several problems were encountered in the reduction of methyl acetylferulate with $LiALH_4$. When the temperature was low (-10 to -15°), the ester crystallized out of the ether solution. Diluting the solution with ether [as much as 5 times the amount used by Allen and Byers(4)] did not solve this problem. The acetate group of methyl acetylferulate was reduced faster than the methyl ester group, as indicated by the high yield of methyl ferulate. The salt complex that was formed in this reaction forced the methyl ester intermediate out of solution before the reduction of the methyl ester group was completed. Similar results are reported in the literature(6).

The Vitride-T reagent was not as active as $LiAlH_4$; consequently, the reaction was carried out at an elevated temperature of 80°. At elevated temperatures, the complex formed by the reduction of the acetate group of methyl acetylferulate was more soluble and facilitated the reduction of the methyl ester.

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