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## [8] Synthetic <sup>14</sup>C-Labeled Lignins

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One of the keys to progress in lignin biodegradation research has been the development of unequivocal quantitative assays for degradation based on <sup>14</sup>C-labeled lignins. The radioactive lignins can be prepared either by labeling specifically the lignin in plant materials (see chapter [3] in this volume), or as described here, by *in vitro* synthesis.<sup>1</sup>

Lignin is synthesized in plant cell walls by the polymerization of radicals generated by the one-electron oxidation of the lignin precursors, which are three *p*-hydroxycinnamyl alcohols: *p*-coumaryl alcohol, coniferyl alcohol, and sinapyl alcohol (see Scheme 1 in chapter [6] of this volume for structures). For a description of the principles of this polymerization, see Adler<sup>2</sup> and Sarkanen<sup>3</sup> (see also chapter [12] in this volume).

Most gymnosperm lignins are essentially homopolymers derived from coniferyl alcohol, with minor proportions of the units derived from *p*-coumaryl and sinapyl alcohols. Angiosperm lignins are largely copolymers of coniferyl and sinapyl alcohols, but they also contain a small proportion of units derived from *p*-coumaryl alcohol. Polymerization takes place primarily by the addition of incoming “monomer” radicals to radicals in the growing polymer within and between the plant cell walls. The *in vivo*

<sup>1</sup> T. K. Kirk, W. J. Connors, R. D. Bleam, W. F. Hackett, and J. G. Zeikus, *Proc. Nat. Acad. Sci. U.S.A.* **72**, 2515 (1975).

<sup>2</sup> E. Alder, *Wood Sci. Technol.* **11**, 169 (1977).

<sup>3</sup> K. V. Saanen, *in* “Lignins” (K. V. Sarkanen and C. H. Ludwig, eds.), p. 95. Wiley (Interscience), New York, 1971.

oxidant that generates the radicals is thought to be a phenol-oxidizing peroxidase.<sup>4</sup>

Similarly, synthetic lignins can be prepared in the laboratory by the peroxidase-catalyzed polymerization of the precursor alcohols. The latter are synthesized using organic chemical techniques with commercially available starting materials. For most studies, it is sufficient to use only coniferyl alcohol, which is the most common natural lignin precursor. Synthetic lignin is often referred to in the literature as “dehydrogenative polymerizate,” or simply “DHP.”

Polymerization of the *p*-hydroxycinnamyl alcohols, alone or in mixtures, is accomplished by the separate and simultaneous addition over a period of several hours of two aqueous solutions (precursor and hydrogen peroxide) to a buffered solution of peroxidase. The insoluble lignin polymer is recovered by centrifugation, is washed, is fractionated by molecular size if desired, and is stored as a frozen aqueous suspension.

### Preparation of the Precursors

In the following, we describe the synthesis of <sup>14</sup>C-labeled coniferyl alcohol, with the label in the  $\beta$ - and  $\gamma$ -carbons of the side chain, in the methoxyl carbon, or uniformly in the aromatic ring carbons. These coniferyl alcohols permit the synthesis of synthetic gymnosperm-type lignins. As mentioned, these lignins should suffice for most investigations. However, at the end of this section, we briefly summarize methods that have been, or could be, used for synthesizing labeled sinapyl and *p*-coumaryl alcohols, if it is desired to prepare homopolymers from these other precursors or copolymers derived from mixtures. In addition, we briefly reference methods that can be used to label specific carbon atoms in the side chains.

#### *[b, $\gamma$ -<sup>14</sup>C]Coniferyl Alcohol*

Vanillin methoxymethyl ether is synthesized by condensing the sodium salt of vanillin with chloromethylmethyl ether. The sodium salt of vanillin is prepared by reacting sodium ethoxide with vanillin in toluene.<sup>5,6</sup> Sodium vanillate (16.4 g, 94 mmol) is powdered in a mortar and added to a three-necked 500-ml round bottom flask fitted with a mechanical stirrer and containing 100 ml of benzene. Chloromethylmethyl ether (10.0 g, 125 mmol) is added with stirring. As the reaction takes place, the mixture warms. Stirring is continued for 5 hr. The mixture is then extracted with

<sup>4</sup> J. M. Harkin and J. R. Obst, *Science* **180**, 296 (1973).

<sup>5</sup> H. Pauly and K. Wäscher, *Chem. Ber.* **56**, 603 (1923).

<sup>6</sup> K. Freudenberg and T. Kempermann, *Annalen* **602**, 184 (1957).

1 M NaOH until the aqueous layer is colorless and then with water until neutral. Evaporation of the benzene and recrystallization from ether gives a product in excess of 90% (mp 40°). Alternatively, the product can be purified by distillation (bp 135 - 138°/1.5 mm Hg).

Coniferaldehyde methoxymethyl ether is synthesized by condensing vanillin methoxymethyl ether with [<sup>14</sup>C]acetaldehyde. Vanillin methoxymethyl ether (5.88 g, 30 mmol) is dissolved in 35 ml of methanol/(65 ml of H<sub>2</sub>O in a 500-ml three-necked round bottom flask fitted with a mechanical stirrer, addition funnel, and water-cooled condenser. The flask is maintained at 75° in an oil bath. Acetaldehyde (1.45 g, 33 mmol, containing [1,2-<sup>14</sup>C]acetaldehyde) in 30 ml of methanol/ 10 ml of H<sub>2</sub>O is added dropwise from the addition funnel to the stirred solution over 11 hr. The reaction mixture is maintained at pH 9–9.5 by adding 5 M NaOH periodically. The coded reaction mixture is then extracted with benzene; this solution is washed with water, is dried over Na<sub>2</sub>SO<sub>4</sub>, and most of the benzene is removed by vacuum evaporation. The product is purified by silica gel column chromatography, with benzene, then with 2% (v:v) ethyl ether in benzene as eluting solvent; vanillin methoxymethyl ether is eluted by the benzene. A suitable silica gel for the column chromatography is Sil-A-200 (Sigma); approximately 500 g (1.25 liter) suffices for this separation. The final yield of coniferaldehyde methoxymethyl ether after recovery from the column and recrystallization from methanol is approximately 3.0 g (45% based on vanillin methoxymethyl ether).

The methoxymethyl group is hydrolyzed to yield coniferaldehyde.<sup>7</sup> For this, the above product is heated for 30 min on a steam bath in 35 ml of 50% aqueous acetic acid containing one drop of concentrated H<sub>2</sub>SO<sub>4</sub>. The mixture is poured over ice, and the (yellow) coniferaldehyde precipitate is recovered by filtration and is dried. Recrystallization from ethyl ether gives [ $\beta,\gamma$ -<sup>14</sup>C]coniferaldehyde (mp 82 - 83 °) in approximately 95% yield.

The coniferaldehyde is reduced to coniferyl alcohol with NaBH<sub>4</sub>. Coniferaldehyde (1.8 g, 10 mmol), is dissolved in 100 ml of 95% ethanol in a 1-liter Erlenmeyer flask to give a yellow solution, and a solution of NaBH<sub>4</sub> (200 mg, 5.4 mmol) in water is added in portions at room temperature. The solution becomes colorless when the reduction is complete. The solution is *carefully*<sup>8</sup> adjusted to pH 6.5–7.0 with 1 M H<sub>2</sub>SO<sub>4</sub>; it is then saturated with NaCl, and is extracted with ethyl ether. The ether solution is dried over Na<sub>2</sub>SO<sub>4</sub>, and the ether is evaporated; the coniferyl alcohol

<sup>7</sup> H. Pauly and K. Feuerstem, *Chem. Ber.* **62**, 797 (1929).

<sup>8</sup> Coniferyl alcohol is readily polymerized in the presence of acid (to a nonligninlike material), and care must be taken to avoid overacidification, or the use of acid-containing solvents; we have found that chloroform sometimes contains too much acid and have avoided its use.

crystallizes and is obtained in essentially quantitative yield. It can be recrystallized from 1,2-dichloroethane. Coniferyl alcohol is stable for prolonged periods if stored dry, under inert gas, in the dark, and at low temperature.

Because acetaldehyde labeled only in C-1 or C-2 is apparently not available commercially, coniferyl alcohol labeled only in C- $\beta$  or C- $\gamma$  cannot be prepared by this synthesis unless one first synthesizes the appropriate [ $^{14}\text{C}$ ]acetaldehyde. However, syntheses of specifically C- $\beta$ - and C- $\gamma$ -labeled coniferyl alcohols can be accomplished with [1- $^{14}\text{C}$ ]- or [2- $^{14}\text{C}$ ]malonate in a preparation that is essentially that described below for ring-labeled coniferyl alcohol. Coniferyl alcohol labeled in c-a, can be made from [ $^{14}\text{CHO}$ ]-vanillin, which is prepared according to Kratzl and Billek.<sup>9</sup>

#### [ring- $^{14}\text{C}$ ]Coniferyl Alcohol

[ring- $^{14}\text{C}$ ]Guaiacol is prepared from uniformly labeled phenol using published procedures.<sup>10-14</sup> In the first step, phenol (940 mg, = 10 mmol, containing [ $^{14}\text{C}$ ]phenol), 2-chloro-5-nitrobenzophenone (2.62 g, 10 mmol), and powdered KOH (680 mg, 12 mmol; powdered by grinding in a dry, hot mortar) are mixed well in 2 ml of *N,N*-dimethylformamide (DMF) and are heated under reflux ( $\sim 150^\circ$ ) for 45 min, and then are allowed to cool; the reaction mixture solidifies. The product is broken up with a spatula and is washed with a few milliliters of 0.1 M NaOH into a coarse porosity fritted glass funnel. The product is washed with approximately 80 ml of 0.1 M NaOH and then with cold distilled water until the filtrate is approximately pH 6. The bright yellow product, 2-[ $^{14}\text{C}$ ]phenoxy-5-nitrobenzophenone, is then dried *in vacuo* over  $\text{P}_2\text{O}_5$ . The yield is approximately 3 g (95%).

In the second step, the product from above is dissolved in 6 ml of concentrated  $\text{H}_2\text{SO}_4$  with efficient stirring (a large magnetic stirring bar is convenient) in a 125-ml Erlenmeyer flask; dissolution takes approximately 30 min. Glacial acetic acid (39 ml) is added, followed by 8.5 ml of 30%  $\text{H}_2\text{O}_2$ , which is added dropwise with stirring. During addition of  $\text{H}_2\text{O}_2$ , a tan solid begins to separate while the liquid is still very dark; the reaction mixture finally becomes buff colored. The mixture is allowed to stand for

<sup>9</sup> K. Kratzl and G. Billek, *Monatsh. Chem.* **85**, 845 (1954).

<sup>10</sup> K. Freudenberg and H. Hübner, *Chem. Ber.* **85**, 1181 (1952).

<sup>11</sup> D. Gagnaire, C. Lacoste, and D. Robert, *Bull. Soc. Chim. Fr.* **1970**, 1067 (1970).

<sup>12</sup> K. Kratzl and F. Vierhapper, *Monatsh. Chem.* **102**, 224 (1971).

<sup>13</sup> K. Kratzl and F. Vierhapper, *Monatsh. Chem.* **102**, 425 (1971).

<sup>14</sup> J. Okabe and K. Kratzl, *Tappi* **48**, 347 (1965).

3 hr and then is poured over 200 g of ice, and the mixture is held until the ice melts. The bright yellow product, 2-(2-hydroxy[<sup>14</sup>C]phenoxy)-5-nitrobenzophenone, is recovered by filtration, is washed until the wash water is pH 5 - 7, and is dried *in vacuo* over P<sub>2</sub>O<sub>5</sub>. The yield is approximately 2.9 g (93%).

The third step is methylation of the phenolic hydroxyl group with methyl iodide. The above product is dissolved in 40 ml of DMF in a 250-ml glass-stoppered flask equipped with a magnetic stirring bar. Powdered K<sub>2</sub>CO<sub>3</sub> (1.4 g) and 1100 μl of CH<sub>3</sub>I are added, the flask is stoppered, and the mixture is stirred overnight at room temperature. The mixture is filtered through glasswool and the DMF removed by vacuum evaporation. The residue is dissolved in a few milliliters of dry acetone and is filtered to remove insolubles. Removal of the acetone yields 2-(2-methoxy[<sup>14</sup>C]phenoxy)-5-nitrobenzophenone as a pale yellow syrup which crystallizes. The yield is essentially quantitative. Alternatively, the methylation can be performed with diazomethane in ether/methanol, which also gives an essentially quantitative yield.<sup>1</sup> (Caution must be used with diazomethane which is explosive.)

In the fourth step, [<sup>14</sup>C]guaiacol is cleaved from the above product. For this, the product is dissolved in 5 ml of piperidine by stirring overnight, and the solution is sealed in a Pyrex glass tube. This tube in turn is sealed under piperidine in a stainless-steel tube bomb and heated in an oil bath at 150° for 90 min, followed by cooling in cold water. The tube is opened and the contents are transferred to a 125-ml separatory funnel with 25 ml of benzene. The piperidine is removed by three extractions with 1 M H<sub>2</sub>SO<sub>4</sub> (3 × 25 ml). The aqueous solution is back-extracted with ether and the ether is added to the benzene solution. This organic solution is extracted with three portions (total 100 ml) of 2 M NaOH. The alkaline extract is carefully acidified with 5 M H<sub>2</sub>SO<sub>4</sub> (~20 ml), and the guaiacol is extracted out with chloroform-acetone, 1 : 1 by volume (3 × 100 ml), followed by chloroform (100 ml). The organic phase is washed with approximately 5 ml of saturated NaCl, is dried over Na<sub>2</sub>SO<sub>4</sub>, and solvents are removed under reduced pressure at < 30°. (Care must be taken to avoid distilling off the guaiacol.) The yield of [ring-U-<sup>14</sup>C]guaiacol is approximately 850 mg (75%); the product is slightly impure.

Vanillin is synthesized by formylating the impure [<sup>14</sup>C]guaiacol with dichloromethylthiomethyl ether.<sup>13</sup> Guaiacol (~850 mg, 6.85 mmol) is transferred with and is dissolved in 60 ml of dry CH<sub>2</sub>Cl<sub>2</sub> in a 125-ml Erlenmeyer flask, and this is cooled on an ice bath. With stirring, 1.66 ml (2.25 g; 17.2 mmol) of dichloromethylthiomethyl ether<sup>15</sup> is added, fol-

<sup>15</sup> F. Boberg, G. Winter, and J. Moos, *Annalen* **616**, 1 (1958).

lowed by the dropwise addition, still with stirring, of 2.1 ml (17.8 mmol) of SnCl<sub>4</sub>. Five min after the final addition, the mixture is transferred into a vigorously stirring solution of 16.3 g of HgCl<sub>2</sub> in 100 ml of 2 M HCl in a 250-ml beaker at 5°. Stirring is continued for an additional 10 min. The black precipitate is removed by centrifugation (in Teflon tubes) and is washed with CH<sub>2</sub>Cl<sub>2</sub>. The combined (orange) organic phase is separated from the aqueous phase, which is washed several times with CH<sub>2</sub>Cl<sub>2</sub>. The combined organic phase is washed with a few milliliters of water, is dried over MgSO<sub>4</sub>, and solvents are removed by vacuum evaporation.

[ring-U-<sup>14</sup>C]Vanillin is isolated from the reaction mixture by silica gel column chromatography. For this, a 500-ml column containing approximately 220 g of silica gel (Sil-A-200, Sigma) is packed in ethyl acetate: hexanes (2 : 5, v: v). The column is run with the same solvent at approximately 2 ml/min *o*-Vanillin elutes first, followed by vanillin, and then isovanillin; separation of these isomers is essentially complete. The yield of recovered vanillin is approximately 700 mg (57% based on guaiacol).

An alternate procedure for formylating guaiacol involves the use of bis(1,3-diphenylimidazolidinylidene-2).<sup>16</sup> The guaiacol is condensed with an equimolar amount of this reagent in refluxing DMF solution. We have found that the product, without purification, can be hydrolyzed directly to yield vanillin, which is extracted with ether and recrystallized from water. The yield after recrystallization is 38%.

Vanillin is condensed with monoethyl malonate to give ethyl ferulate. The [<sup>14</sup>C]vanillin from above is combined with 0.75 ml of pyridine, 0.77 ml of monoethyl malonate,<sup>17</sup> one drop of aniline, and one drop of piperidine in a 25-ml boiling flask and is held at 55° for 24 hr (the flask is stoppered after reaching 55°). The cooled reaction mixture is dissolved in 25 ml of ethyl ether; this solution is extracted with 50 mM H<sub>2</sub>SO<sub>4</sub> (5 × 20 ml), is washed with 1 ml of saturated NaCl, is dried over MgSO<sub>4</sub>, and solvents are removed *in vacuo*. The product, ethyl [ring-U-<sup>14</sup>C]ferulate, is purified by column chromatography. A column similar to that used for vanillin above is used with the solvent mixture ethyl acetate: hexanes (3 : 2, v: v). The yield of recovered product is approximately 0.9 g (90%).

The ethyl ferulate is reduced to coniferyl alcohol with bis(2-methoxyethoxy)aluminum hydride (Red-Al, Aldrich Chemical Co., Milwaukee, Wisconsin). Red-Al (4.05 g) is mixed with 40 ml of dry benzene in a dry 250-ml three-necked flask fitted with a condenser with a drying tube. Ethyl ferulate from above in 20 ml of dry benzene is added dropwise from an addition funnel over 20 min with stirring. The reaction mixture is refluxed for 30 min and is cooled to room temperature. Water (20 ml) is then added

<sup>16</sup> H. Giesecke and J. Hocker, *Liebigs Ann. Chem.* **1978**, 345 (1978).

<sup>17</sup> R. E. Strube, *Org. Synth. Coll.* **4**, 417 (1963).

carefully with stirring (dropwise at first), followed by 25 ml of saturated sodium potassium tartrate. With continued stirring, the reaction mixture is adjusted with approximately 1.75 ml of acetic acid to pH 6.5. The organic layer is washed with saturated NaHCO<sub>3</sub>, and the combined aqueous layers are extracted exhaustively with ethyl ether. The combined organic layers are washed with a few milliliters of water, are dried over MgSO<sub>4</sub>, and solvents are removed in vacuo. The yield of coniferyl alcohol is approximately 600 mg (78%). The coniferyl alcohol can be recrystallized from 1,2-dichloroethane.

#### [methoxyl-<sup>14</sup>C]Coniferyl Alcohol

Methoxyl-labeled coniferyl alcohol is prepared by methylating the (unlabeled) intermediate 2-(2-hydroxyphenoxy)-5-nitrobenzophenone in the above synthesis with <sup>14</sup>CH<sub>3</sub>I, and then by following the same procedure as above to prepare coniferyl alcohol. The methylation is done in two steps. the first with <sup>14</sup>CH<sub>3</sub>I, and the second with excess unlabeled CH<sub>3</sub>I to complete the methylation. Alternatively, [<sup>14</sup>C]diazomethane can be used for this methylation.<sup>1</sup>

#### Polymerization of Coniferyl Alcohol<sup>18</sup>

Polymerization is done with a total of 1.7 g of coniferyl alcohol (9.45 mmol). Consequently, the total coniferyl alcohol from one of the above syntheses is diluted with unlabeled coniferyl alcohol to give 1.7 g. This sample is dissolved in about 20 ml of acetone and is added with stirring and under N<sub>2</sub> to 400 ml of degassed sodium phosphate buffer (0.01 M, pH 6.5) to give a clear solution. To this is added 1200 purpurogallin units of horseradish peroxidase (we have used Sigma Type II, 200 U/mg). A second 400 ml of the degassed buffer contains 9.45 mmol H<sub>2</sub>O<sub>2</sub> (the concentration must be accurate; 9.45 mmol is approximately 1.1 ml of 30% H<sub>2</sub>O<sub>2</sub>). Both solutions are maintained under a stream of N<sub>2</sub>. The two solutions are added simultaneously over approximately 20 hr<sup>19</sup> to a stirring solution of approximately 30 mg of vanillyl alcohol or guaiacylglycerol<sup>20,21</sup> in 200 ml of the same degassed phosphate buffer as above; this

<sup>18</sup> The polymerization procedure should be practiced with unlabeled coniferyl alcohol before it is attempted with the labeled material.

<sup>19</sup> We have used peristaltic pumps for this addition. The rates of delivery by each pump (or by each tube) are determined, and the volume of one of the solutions is adjusted if necessary to compensate for the difference; this assures simultaneous delivery of the two solutions.

<sup>20</sup> The vanillyl alcohol or guaiacylglycerol serves as a water-soluble initiator (site of polymerization) and retards precipitation of the growing polymer. Guaiacylglycerol, because it has four hydroxyl groups, is more water-soluble and, therefore, better than vanillyl alcohol (two hydroxyl groups), but it must be synthesized.<sup>21</sup>

<sup>21</sup> E. Adler and S. Yllner, *Acta Chem. Scand.* 7, 570 (1953)

reaction mixture. in a 2-liter Erlenmeyer flask, also is maintained under a stream of N<sub>2</sub>, and it is kept dark by covering the flask with aluminum foil. After final addition of reactants, stirring is continued in the dark under N<sub>2</sub> for 10 hr. The synthetic [<sup>14</sup>C]lignin is recovered by centrifugation and is washed twice with a total of 150 ml of water. The yield is approximately 1.5g.

The lignin is stored as a suspension in 100 ml of water at -20° in the dark; it is stable for several years under these conditions. Solid lignin can be recovered by carefully evaporating the water from some of the suspension using a rotary evaporator; the temperature should be maintained below 30°. The dry lignin can be stored in the dark at -20° also, but may not be stable for more than a few months.

The synthetic lignin, recovered by evaporating the water, should be soluble in DMF (a 20% solution is readily achieved); the lignin is conveniently added as a DMF solution to water to give a fine suspension for addition to microbial cultures or to enzyme reaction mixtures.<sup>22</sup> If the sample is not soluble in DMF, it has probably condensed due to the use of too much H<sub>2</sub>O<sub>2</sub>. The polymerization procedure should then be repeated with special care being taken in making up the H<sub>2</sub>O<sub>2</sub>. Alternatively, the DMF-insoluble portion of the preparation can be separated by centrifugation from the soluble portion, and the lignin can be recovered from the latter by solvent evaporation. The lignin can be stored as a DMF solution in the dark at -20° for several months.

The synthetic lignin is best characterized by <sup>13</sup>C NMR spectroscopy.<sup>23-25</sup> It should be similar but not identical to milled wood lignin or Brauns' native lignin isolated from conifer wood (see chapter [1] in this volume).

The synthetic lignins prepared by this procedure should be essentially free of oligomers smaller than 1000 MW i.e., the lignins should be excluded from Sephadex LH-20 (DMF as solvent).<sup>26</sup> If a preparation contains low-molecular-weight components, these can be removed by preparative gel permeation chromatography,<sup>27</sup> or perhaps by precipitating into ethyl ether from 1,2-dichloroethane-ethanol as described for milled wood lignin (see chapter [1] in this volume).

<sup>22</sup> T. K. Kirk, E. Schultz, W. J. Connors, L. F. Lorenz, and J. G. Zeikus, *Arch. Microbiol.* **117**, 277 (1978).

<sup>23</sup> H. Nimz, J. Mogharab, and H.-D. Lüdemann, *Makromol. Chem.* **175**, 2563 (1974).

<sup>24</sup> D. Gagnaire and D. Robert, *Makromol. Chem.* **178**, 1477 (1977).

<sup>25</sup> H. Nimz, U. Tschirner, M. Stähle, R. Lehmann, and M. Schlosser, *J. Wood Chem. Technol.* **4**, 265 (1984).

<sup>26</sup> W. J. Connors, L. F. Lorenz, and T. K. Kirk, *Holzforschung.* **32**, 106 (1978).

<sup>27</sup> O. Faix, M. D. Mozuch, and T. K. Kirk, *Holzforschung.* **39**, 203 (1985).

### Preparation of *p*-Coumaryl and Sinapyl Alcohols

Both *p*-coumaryl and sinapyl alcohols have been prepared using the same methods as for coniferyl alcohol. For preparation of the former,<sup>28</sup> *p*-hydroxybenzaldehyde (12.2 g) is reacted with monoethyl malonate (19.8 g, 1 ml pyridine and 0.2 ml piperidine; 60°; 24 hr) to yield *p*-coumaric acid ethyl ester (mp 137°, 87% yield), after a similar workup as for ethyl ferulate. The product is reduced with Red-A1 in the same manner as for coniferyl alcohol. *p*-Coumaryl alcohol crystallizes from dichloromethane (mp 124°, - 80% yield). <sup>14</sup>C Labeling of the alcohol in the aromatic nucleus is accomplished by formylating labeled phenol in the same manner as described above for guaiacol or by the newer procedure for Giesecke and Hocker,<sup>16</sup> which employs bis(1,3-diphenylimidazolidinylidene-2) as the formylating reagent. By using labeled malonic acid, the alcohol can be labeled in the propyl side chain.

For preparation of sinapyl alcohol,<sup>29</sup> acetylsyringaldehyde (5 g, prepared by acetylation of syringaldehyde) is condensed with monoethyl malonate (7 g, 15 ml of pyridine and three drops each of piperidine and aniline; 50°; 24 hr); the reaction mixture is diluted with 25 ml of ethanol and is poured into a mixture of 70 ml of 4 *M* HCl and 50 ml of ethanol. After cooling to 0°, the crystalline product is filtered and washed with water. Yield is approximately 5 g (80%, mp 120–121 °). The product is reduced with Red-A1 in the same manner as for coniferyl alcohol. The yield, however, is lower, and it is often difficult to obtain the alcohol in crystalline form (mp 66–67 °), even when it is chromatographically pure. It should be noted that sinapyl alcohol is extremely sensitive to air oxidation. Ring-labeled sinapyl alcohol can be prepared from ring-labeled vanillin via 5-iodovanillin according to Haider,<sup>30</sup> and side-chain-labeled sinapyl alcohol can be prepared by the use of labeled malonic acid.

<sup>28</sup> K. Freudenberg and G. Gehrke, *Berichte* **84**, 443 (1951).

<sup>29</sup> K. Freudenberg and H. Hübner, *Berichte* **85**, 1181 (1952).

<sup>30</sup> K. Haider, *J. Labelled Compd.* **2**, 174 (1966).