Enzymic Dehydrogenation of the Lignin Model Coniferaldehyde

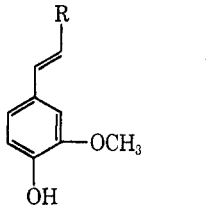
W. J. CONNORS, C.-L. CHEN, AND J. C. PEW

Forest Products Laboratory,[1] U. S. Department of Agriculture, Forest Service, Madison, Wisconsin 53705

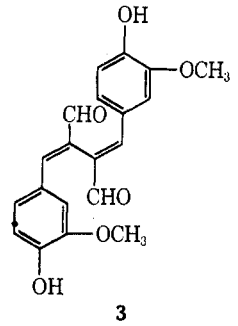
Received October 20, 1969

The lignin model *trans*-coniferaldehyde (**1**) was dehydrogenated in aqueous solution by peroxidase and H_2O_2. Three new dehydro dimers, 2,3-diformyl-1,4-di-5-guaiacylbuta-1,3-diene (**3**), α-(4-β-formylvinyl-2-methoxyphenoxy)coniferaldehyde (**4**), and α-(5-β-formylvinyl-2-hydroxy-3-methoxyphenyl)coniferaldehyde (**5**), were isolated. Their significance is discussed, and their nmr and mass spectra are reported.

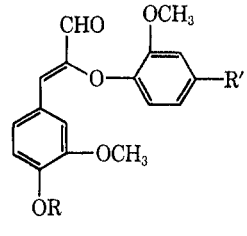
trans-Coniferaldehyde (**1**) is considered one of the genuine monolignol precursors of lignin. It has been found in the cambial sap of various species of trees, and its incorporation into the lignin macromolecule has been demonstrated spectrally.[2,3] It has been shown to be responsible for many color reactions in lignin, and it has often been reported among the monomeric products from lignin hydrolysis. When *trans*-coniferyl alcohol (**2**) was converted to an artificial lignin by the action of air and oxidative enzymes, **1** was among the monolignols formed in the reaction, and its incorporation into dilignols and subsequently into the artificial lignin has been demonstrated.[2-4] The dehydrogenation of **1** by phenol oxidase and air has also been reported, but only the polymer formed was analyzed.[5] For these reasons we decided that it was important to investigate the low-molecular-weight products formed in the dehydrogenation reactions of **1** using peroxidase enzyme and hydrogen peroxide in aqueous medium.



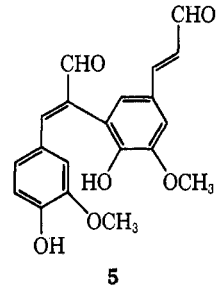
1, R = CHO
2, R = CH_2OH



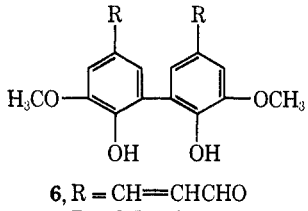
3



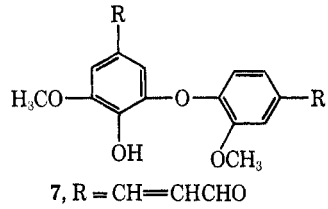
4, R = H, R′ = CH=CHCHO
14, R = OCH_3, R′ = H



5

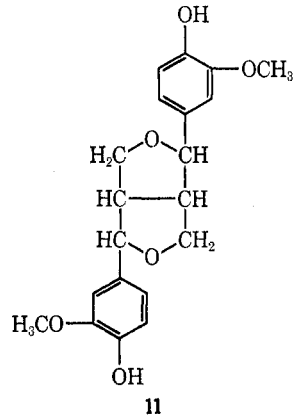


6, R = CH=CHCHO
8, R = CH=CHCH$_2$OH
10, R = $CH_2CH_2CH_2OH$

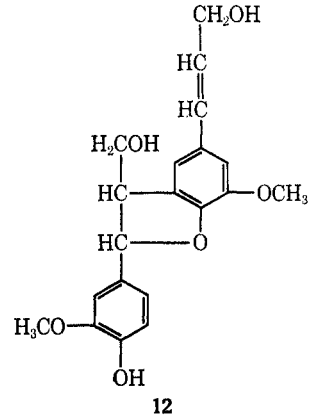


7, R = CH=CHCHO
9, R = CH=CHCH$_2$OH

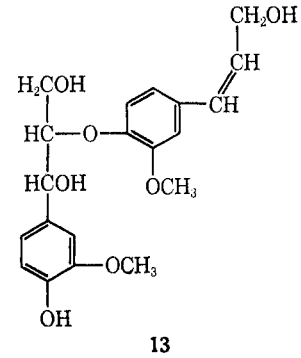
Three dimers, **3**, **4**, and **5**, were isolated from the dehydrogenation reaction mixture. A low level of incorporation of dimers of this type could well take place in natural lignin and thus with **1** contribute to the color of lignin. Tlc analysis of the whole dehydrogenation mixture at any one time showed residual **1** and dehydro dimers **3**, **4**, and **5** to be the major low-molecular-weight compounds, **3** being the predominant dimer. Determination of the yield of dimers cannot be readily undertaken owing to the extreme susceptibility of the compounds to continued oxidative polymerization. Trimers and higher oligomers were undoubtedly formed in the dehydrogenation reaction but have not been isolated. Neither the *o*,*o*′-dihydroxybiphenyl (**6**) nor the *o*-hydroxy diphenyl ether (**7**) was detected in the reaction mixture, although analogous compounds have been prominent products formed in the enzymic dehydrogenation reactions of lignin model phenols with



11



12



13

(1) Maintained at Madison, Wis., in cooperation with the University of Wisconsin.

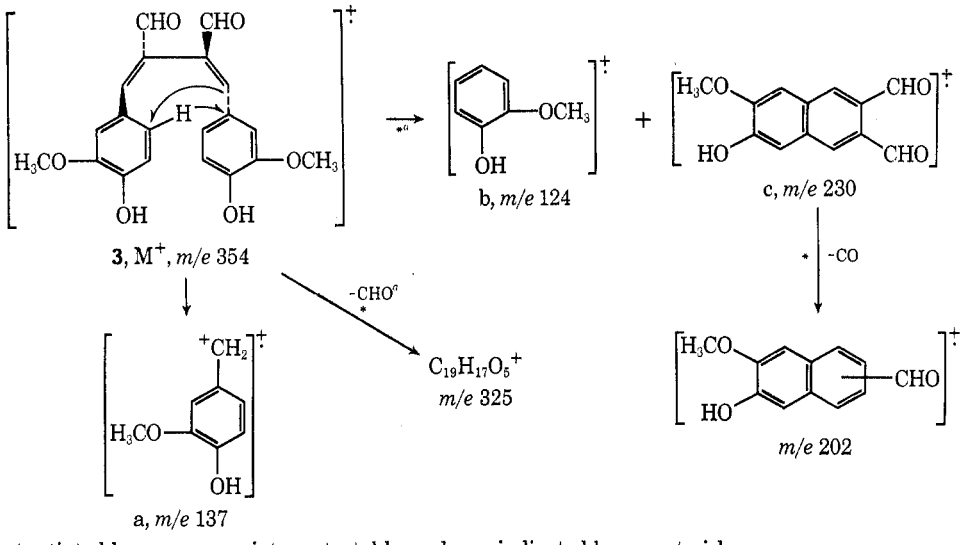
(2) K. Freudenberg and A. C. Neish, "Constitution and Biosynthesis of Lignin," Springer-Verlag, New York, N. Y., 1968.

(3) J. M. Harkin in "Oxidative Coupling of Phenols," W. I. Taylor and A. R. Battersby, Ed., Marcel Decker, Inc., New York, N. Y., 1967, Chapter 6.

(4) F. E. and D. A. Brauns, "The Chemistry of Lignin," Suppl. Vol., Academic Press Inc., New York, N. Y., 1960.

(5) K. Freudenberg and W. Heimberger, *Chem. Ber.*, **83**, 519 (1950).

SCHEME I[a]



[a] Transitions substantiated by an appropriate metastable peak are indicated by an asterisk.

saturated side chains.[6-9] Dimers with biphenyl (**8**) and diphenyl ether (**9**) bonds were not isolated from the enzymic dehydrogenation mixture of coniferyl alcohol **2**, but the tetrahydrobiphenyl compound **10** was isolated after hydrogenation of the reaction mixture, and carboxylic acids indicating the presence of biphenyl and diphenyl ether bonds have been isolated from the degradation of methylated spruce lignin.[2,3]

Pinoresinol (**11**), dehydrodiconiferyl alcohol (**12**), and guaiacylglycerol-β-coniferyl ether (**13**) were among the major dehydro dimers formed from **2**; they have also been found in the cambial sap of spruce and other trees as well as appearing among lignin hydrolysis products.[2,3] It has been postulated that these come about first through the coupling of mesomeric forms of the phenoxyl radical, followed by the addition of a nucleophile onto the intermediate quinone methide.[2] With **13**, the nucleophile is water, and an arylglycerol-β-aryl ether is formed; with **12** and **13**, intramolecular nucleophilic addition takes place to give, respectively, the ditetrahydrofuran ring and the phenylcoumaran ring.

With compounds **3**, **4**, and **5**, however, the aldehydic side chains comprise a conjugated α,β-enone system, and the intermediate quinone methides must rearrange to stable phenol forms through loss of the acidic proton from the carbon α to the carbonyl, followed by rearomatization and protonation of the phenoxyl anion to give the dimeric compounds.

Compounds **3**, **4**, and **5** were all shown by mass spectrometry to have the molecular formula $C_{20}H_{18}O_6$. The uv and ir spectra of these indicated that each had phenolic hydroxyl and conjugated carbonyl groups.

The nmr spectrum of **3** showed two two-proton singlets at δ 7.77 and 9.66 indicating two CH=C—CHO groupings, as well as the six-proton methoxyl peak at δ 3.68 and the aromatic protons with resonance centers at δ 6.81 (d, 2, J = 8.2 Hz), 7.21 (m, 2, J = 8.2 Hz, J = 2 Hz), and 7.27 (d, 2, J = 2 Hz).

This was indicative of the presence of a β,β linkage and compatible with the structure proposed for this compound. The mass spectrum (Figure 1, Scheme I) exhibited a very prominent ion peak at m/e 137 (a),[10,11] which is ascribed to the highly characteristic benzyl ion radical. The m/e 137 (a) peak is also prominent in the mass spectra of **4** and **5**, and the analogous peak at m/e 151 is prominent in the spectra of **14**. As indicated by a metastable peak at 149.5, the molecular ion lost a neutral fragment corresponding to b to give the ion at m/e 230 (c).

Compound **4** gave a rather complex nmr spectrum. However, application of double-irradiation technique resulted in decoupling the one-proton quartet with resonance center at δ 6.60, the one-proton doublet at δ 7.37, and the one-proton doublet at δ 9.65. This revealed the presence of an ABX system on one ring (Ar—CH_A=CH_B—CH_XO) with coupling constants J_{AB} = 15.8 Hz and J_{BX} = 7.8 Hz. A one-proton singlet at δ 9.44 therefore indicated substitution on the β carbon of the formylvinyl side chain on the second ring. The singlet vinyl proton was not detectable in **4** nor in compound **14**, and was thus apparently shifted upfield into the very complex aromatic region in the spectrum of both compounds. After deuterium oxide treatment, the m/e of the molecular ion and ions d and g were increased by 1 mass unit, and the deuterium exchange ratio was calculated to be 45% for the three ions. The compound, therefore, had only one hydroxyl group. The uv spectrum in ethanol showed maxima at 243, 312, and 345 nm. When sodium hydroxide solution was added, it produced a bathochromic shift to give maxima at 309, 334, and 421 nm. The coniferaldehyde ether chromophore had a maximum *ca.* 340 nm. All of these data are compatible with structure **4**. The mass spectrum (Figure 1, Scheme II) showed two fragmentation patterns. The ether cleavages of the molecular ion produced the ions at m/e 177 (d) and 161 (e) in addition to the familiar ion at m/e 137 (a). The molecular ion also lost 57 mass units (C_2HO_2) to

(6) J. C. Pew, *J. Org. Chem.*, **28**, 1048 (1963).
(7) J. C. Pew, W. J. Connors, and A. Kunishi, *Chim. Biochim. Lignine, Cellul., Hemicellul., Actes Symp. Int., Grenoble, Fr.*, **1964**, 229 (1965).
(8) J. C. Pew and W. J. Connors, *J. Org. Chem.*, **34**, 580 (1969).
(9) J. C. Pew and W. J. Connors, *ibid.*, **34**, 585 (1969).
(10) V. Kovacik, J. Skamla, D. Dusan, and B. Kosikova, *Chem. Ber.*, **102**, 1513 (1969).
(11) A. Pelter, *J. Chem. Soc., C*, 1376 (1967).

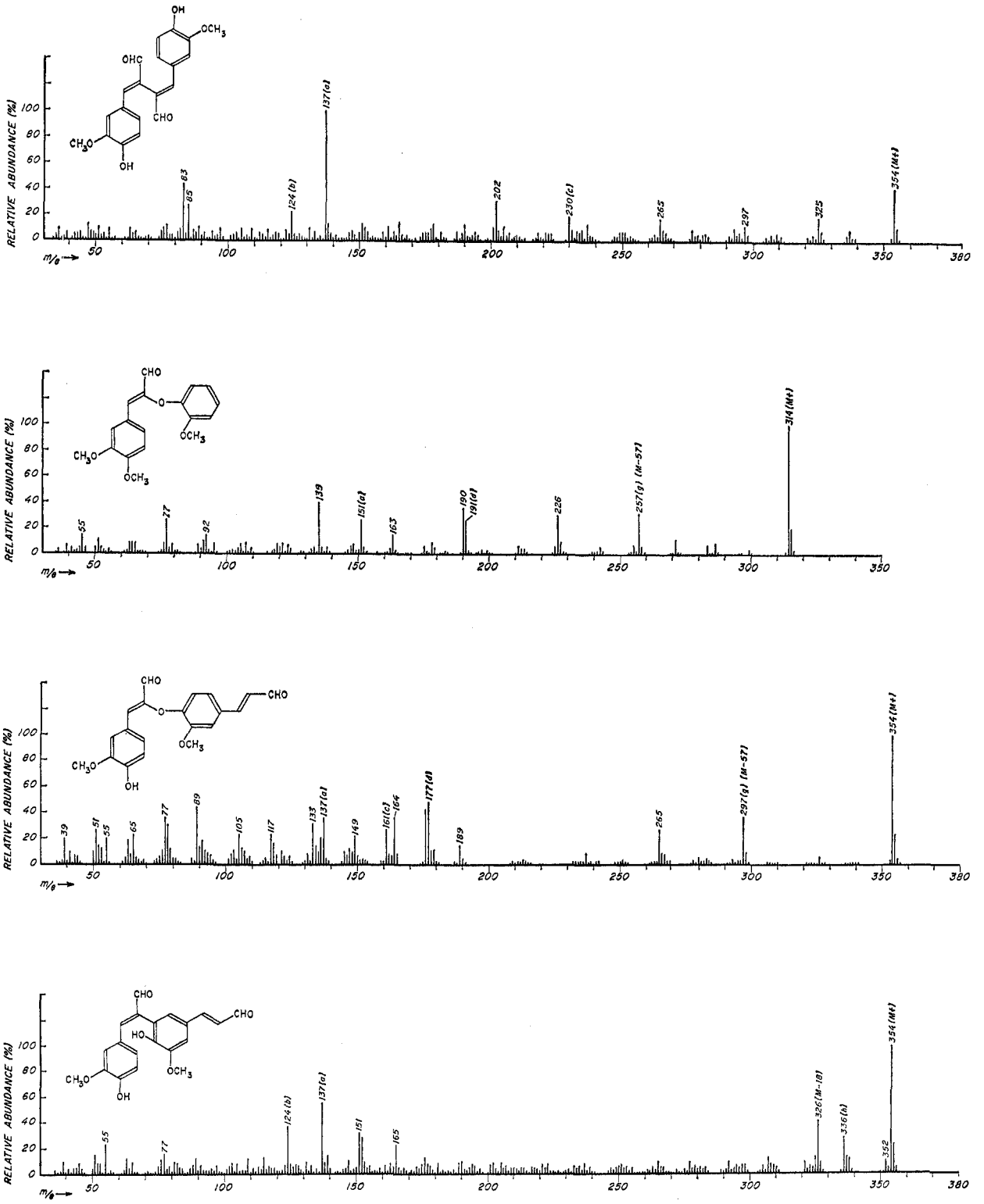
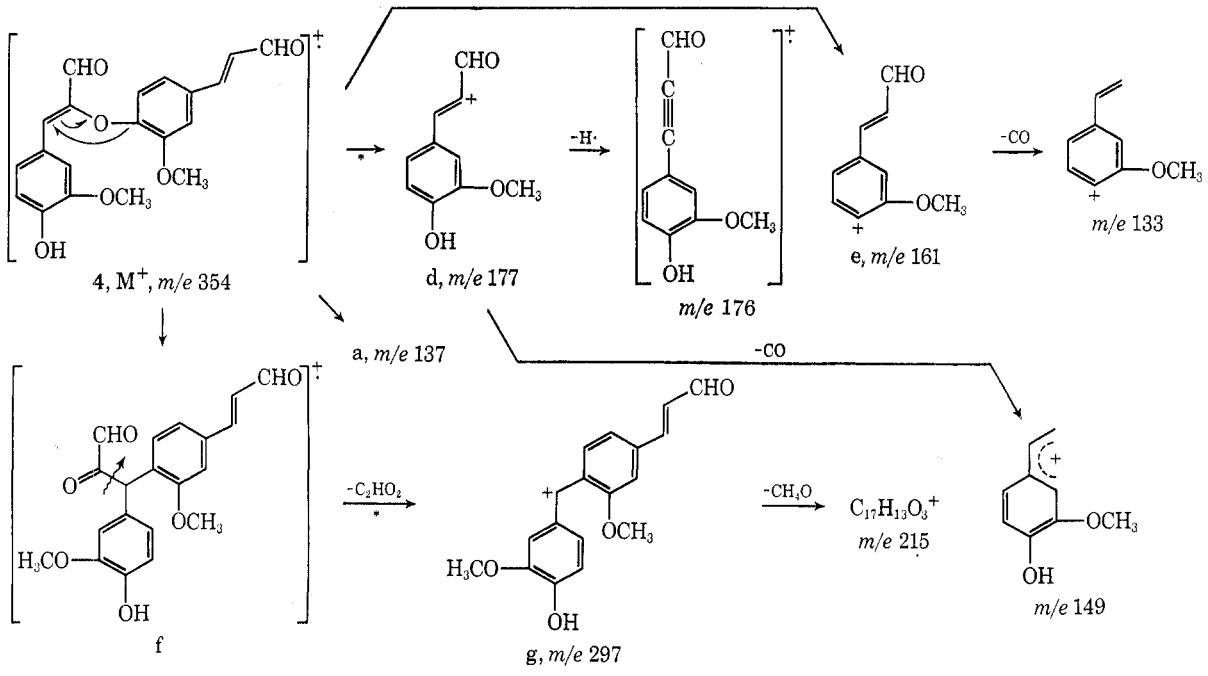


Figure 1.—Mass spectra (from top to bottom) of compounds **3**, **14**, **4**, and **5**.

give the ion at *m/e* 297 (g), as indicated by a metastable peak at *m/e* 249.2. This could be rationalized by assuming 1,3 rearrangement of aryl group to form intermediate f which would give the ion g by the expulsion of HCO–C(O)–. To ensure this, the mass spectrum of α-(2-methoxyphenoxy)coniferaldehyde methyl ether (**14**) was examined (Figure 1). It also exhibited the M − 57 ion peak, which followed the same fragmentation patterns as **4**. The M − 57 ion had thus furnished a further proof of the 2-aroxy-3-arylpropenal skeleton of **4**.

The nmr spectrum of **5** also showed the presence of ArCH=$\overset{|}{\text{C}}$CHO and ArCH=CHCHO. With **5** as with **3**, the vinyl proton singlet was distinguishable and was at δ 7.56. After D_2O exchange, the molecular ion increased by 2 mass units, and there was also an equivalent increase in the M + 1 ion which was attributed to the two possifle monodeuterated forms of the molecular ion. The M + 2:M + 1:M ratio was 44:42:14, and the overall D_2O exchange ratio was calculated to be 45%. The ion akin to h after D_2O treatment showed

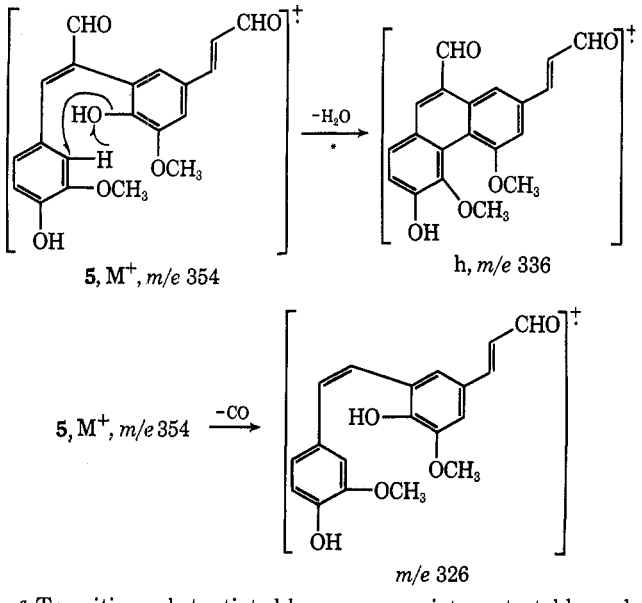
SCHEME II[a]



[a] Transitions substantiated by an appropriate metastable peak are indicated by an asterisk.

prominent peaks at 336 and 337 due to loss of H_2O and HDO from the hydroxyl groups of the molecular ions. The presence of a prominent 338 peak indicates that the water could also be lost from the carbonyl.[12] These were indicative of β-(C-5) linkage, and the structure **5** for the compound was apparent. The mass spectrum (Figure 1, Scheme III) exhibited ion peaks corresponding to M − 18 and M − 28. 1,6 elimination of water from the molecular ion to form the stable anthracene ion (h) was compatible with the structure proposed for the compound.

SCHEME III[a]



[a] Transition substantiated by an appropriate metastable peak is indicated by an asterisk.

Experimental Section

trans-**Coniferaldehyde** (**1**) was prepared by the method of Pauly[13] and recrystallized from aqueous alcohol: mp 80–82° (lit.[13] mp 82.5°); nmr $CDCl_3$ δ 3.8 (s, 3, OCH_3), 6.55 (m, 1, J = 15.8 Hz, J = 7.8 Hz, CH=CHCHO), 7.0 (m, 3, aromatic), 7.39 (d, 1, J = 15.8 Hz, CH=CHCHO), 9.25 (d, 1, J = 7.8 Hz, CHO).

Dehydrogenation of 1 and Isolation of Dehydro Dimers.—**1** (1 g, 0.0056 mol) was dissolved in 1 l. of H_2O with warming, and the solution was cooled to 3° in an ice bath. Horseradish peroxidase[14] (5 mg) was dissolved in a few milliliters of H_2O and added to the solution; then 9.5 ml (0.0056 equiv) of 1% H_2O_2 was added over 30 min while the solution was stirred. Stirring was continued for an additional hour; the mixture was then extracted with EtOAc and residual **1**; and the dimeric compounds **3**, **4**, and **5** were isolated by column chromatography on silicic acid with benzene and benzene–EtOH 100:1 solvents. The compounds were purified by preparative tlc on silica gel with benzene–EtOH 100:5 solvent.

2,3-Diformyl-1,4-di-5-guaiacylbuta-1,3-diene (**3**) was crystallized from aqueous EtOH: mp 178–180°; uv max 343 mμ (ε 3.88 × 10⁴), showed a characteristic bathochromic shift to 396 mμ on the addition of 1 drop of 50% NaOH; ir (KBr) 3400 (OH), 1680 (conjugated C=O), 1640 sh, 1595 (C=C), 1520 cm^{-1} (aromatic); nmr (d_6-acetone) δ 3.68 (s, 6, OCH_3), 6.81 (d, 2, J = 8.2 Hz, aromatic), 7.21 (m, 2, J = 8.2 Hz, J = 2 Hz, aromatic), 7.27 (d, 2, J = 2 Hz, aromatic), 7.77 (s, 2, olefinic), 9.66 (s, 2, –CHO); mol wt 354 (mass spectrum).

Anal. Calcd for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12. Found: C, 67.91; H, 4.99.

α-(4-β-Formylvinyl-2-methoxyphenoxy)coniferaldehyde (**4**) was crystallized from aqueous alcohol: mp 87–89°; uv max 345 mμ (ε 3.8 × 10⁴), showed a characteristic bathochromic shift to uv max 421 mμ on addition of 1 drop of 50% NaOH (a second uv max at 334 nm indicates etherified coniferaldehyde moiety); ir (KBr) 3410 (OH), 1675–1655 (conjugated C=O), 1620, 1600 (C=C), 1515 cm^{-1} (aromatic); nmr $(CDCl)_3$ δ 3.78 (s, 3, OCH_3), 3.95 (s, 3, OCH_3), 6.60 (m, 1, J = 15.8 Hz, J = 7.8 Hz, CH=CH=CHO), 7.37 (d, 1, J = 15.8 Hz, CH=CHCHO), 9.65 (d, 1, J = 7.8 Hz, CHO), 9.44 (s, 1, CHO) (the remaining portion of the spectrum could not be interpreted); mol wt 354 (mass spectrum).

Anal. Calcd for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12. Found: C, 67.65; H, 5.25.

α-(5-β-Formylvinyl-2-hydroxy-3-methoxyphenyl)coniferaldehyde (**5**) was crystallized from aqueous EtOH: mp 191–193°;

(12) J. H. Bowie and P. Y. White, *J. Chem. Soc., B*, 89 (1969).
(13) H. Pauly and K. Feuerstein, *Ber.*, **62B**, 297 (1929).
(14) Peroxidase (horseradish), type I activity approximately 72 purpurogallin units/mg. Purchased from Sigma Chemical Co., St. Louis, Mo.

uv max 339 mμ (ε 3.8 × 10⁴), which showed a characteristic bathochromic shift to uv max 404 mμ on the addition of 1 drop of 50% NaOH; ir (KBr) 3410 (OH), 1670 (conjugated, C=O), 1610, 1600 (C=C), 1520 cm⁻¹ (aromatic); nmr (d_6-acetone) δ 3.52 (s, 3, OCH_3), 4.01 (s, 3, OCH_3), 6.69 (m, 1, J = 7.8 Hz, J = 15.4 Hz, CH=CHCHO), 7.56 (s, 1, CH=CCHO), 7.58 (d, 1, J = 15.4 Hz, CH=CHCHO), 9.57 (d, 1, J = 7.8 Hz, CHO), 9.65 (s, 1, CHO); mol wt 354 (mass spectrum).

Anal. Calcd for $C_{20}H_{18}O_6$: C, 67.79; H, 5.12. Found: C, 68.02; H, 5.08.

α-(2-Methoxyphenoxy)coniferaldehyde Methyl Ether (14).—**14** was synthesized by the condensation of veratraldehyde, which is commercially available, and 2-methoxyphenoxyacetaldehyde (**15**) using the method described[13] for the synthesis of **1**: uv max 339 mμ (ε 2.36 × 10⁴), unchanged by the addition of 1 drop of 50% sodium hydroxide; ir (KBr) 1685 (conjugated C=O), 1625, 1600 (C=C), 1515, 1505 cm⁻¹ (aromatic); nmr ($CDCl_3$), δ 3.75 (s, 1, OCH_3), 3.81 (s, 1, OCH_3), 3.91 (s, 1, OCH_3), 9.43 (s, 1, CHO), and a complex aromatic region which was not interpreted; mol wt 314 (mass spectrum).

Anal. Calcd for $C_{18}H_{18}O_5$: C, 68.77; H, 5.77. Found: C, 68.75; H, 5.57.

2-Methoxyphenoxyacetaldehyde (15).—This compound was synthesized by the $Pb(OAc)_4$ oxidation of guaiacol glyceryl ether, which is available commercially, following a previously reported procedure,[15] and the compound was crystallized from benzene, mp 72–74°.

Anal. Calcd for $C_9H_{10}O_3$: C, 65.05; H, 6.02. Found: C, 65.16; H, 6.18.

Registry No.—**1**, 20649-42-7; **3**, 24058-19-3; **4**, 24058-20-6; **5**, 24058-21-7; **14**, 24058-22-8.

(15) R. J. Speer and H. R. Mahler, *J. Amer. Chem. Soc.*, **71**, 1133 (1949).

Synthesis of *cis*- and *trans*-4-Mercapto-L-proline Derivatives

ANTHONY J. VERBISCAR AND BERNHARD WITKOP

Institute of Drug Design, Inc., Sierra Madre, California 91024, and the National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Maryland 20014

Received October 16, 1969

Both *cis*- and *trans*-N,O-ditosyl-4-hydroxy-L-proline methyl esters under special precautions underwent almost complete SN2 displacements by potassium thiobenzoate to 4-benzoylmercaptoprolines, which were cleaved by dilute methoxide to the autoxidizable (and in the *cis* series lactonizable) N-tosyl-4-mercaptoprolines, easily alkylatable to the N-tosyl-*p*-methoxybenzylmercaptoprolines. These were electrolytically detosylated and converted to N-*t*-butylcarbonyl-*cis*- and -*trans*-4-*p*-methoxybenzylmercapto-L-prolines suitable for incorporation into oligopeptides by the conventional or the solid-phase method.

Although many analogs and homologs of proline and hydroxyproline have been reported,[1,2] the sulfur-substituted prolines[3,4] have not received much attention. For the synthesis of inhibitors of proline hydroxylase,[5] we needed sulfur analogs of natural and *allo*-4-hydroxy-L-proline suitably protected for incorporation into synthetic polypeptides.

The synthesis of such mercaptoprolines becomes an exercise in the proper sequence of putting on and taking off protecting groups with sufficient lability and differential activity to permit these steps to be selective.

The requirement for the S-protecting group of the resulting *cis*- and *trans*-4-mercapto-L-proline peptides was easy removal to liberate sulfhydryl without cleavage of peptide bonds. We chose *p*-methoxybenzyl, which is easily removed from S-protected cysteine peptides by anhydrous hydrogen fluoride.[6,7]

The starting material for the *cis*-mercapto series was N,O-ditosylhydroxy-L-proline methyl ester (I) (Scheme I).[8,9] Analogously the *trans*-mercapto series started with N,O-ditosyl-*allo*-hydroxy-L-proline methyl ester (II) (Scheme II).[2,8]

N-Tosyl-*cis*-4-benzoylmercapto-L-proline methyl ester (III) was prepared from I and potassium thiobenzoate following procedures similar to those developed for conversion of serines to cysteines.[10] The proline reactions are slower and proceed with inversion of configuration requiring additional precautions. When the *trans* → *cis* inversion reaction is run for 6–10 days at a temperature not exceeding 35–40°, the conversion is essentially stereoselective, in contrast to the reaction in refluxing methanol for 20 hr which gave a poor yield of III along with a substantial quantity of N-tosyl-*trans*-4-benzoylmercapto-L-proline methyl ester (IV). The presence of *trans* products in similar displacements on *trans*-4-tosylhydroxy-L-proline methyl ester has been explained by intramolecular participation of the ester carbonyl to form a cyclic carbonium intermediate which favors SN1 substitutions.[11] However, the corresponding *cis* → *trans* inversion to N-tosyl-*trans*-4-benzoylmercapto-L-proline methyl ester (IV) at 35–40° for 6 days also gave a small quantity of the *cis* epimer. The *cis* product probably arose by solvolysis of the *trans*-tosylate with subsequent attack by S-benzoyl anion in a normal SN1 reaction. Thin layer chromatography showed the product resulting from inversion to be major and retention to be the minor pathway.

In analogy to S-benzoyl derivatives of cysteine, sodium alkoxides[12,13] easily cleaved the benzoylthioprolines. With 0.5 *M* methanolic sodium hy-

(1) A. B. Mauger and B. Witkop, *Chem. Rev.*, **66**, 47 (1966).
(2) R. H. Andreatta, V. Nair, A. V. Robertson, and W. R. J. Simpson, *Aust. J. Chem.*, **20**, 1493 (1967).
(3) A. A. Patchett and B. Witkop, *J. Amer. Chem. Soc.*, **79**, 185 (1957).
(4) S.-I. Yamada, Y. Murakami, and K. Koga, *Tetrahedron Lett.*, 1501 (1968).
(5) J. J. Hutton, Jr., A. Marglin, B. Witkop, J. Kurtz, A. Berger, and S. Udenfriend, *Arch. Biochem. Biophys.*, **125**, 779 (1968).
(6) S. Akabori, S. Salikibara, Y. Shimonishi, and Y. Nabuhara, *Bull. Chem. Soc. Jap.*, **37**, 433 (1964).
(7) S. Sakikibara, Y. Shimonishi, M. Okada, and Y. Kishida, "Peptides," North Holland Publishing Co., Amsterdam, 1967, pp 44–49.
(8) Y. Fujita, A. Gottlieb, B. Peterkofsky, S. Udenfriend, and B. Witkop, *J. Amer. Chem. Soc.*, **86**, 4709 (1964).
(9) P. S. Portoghese and A. A. Mikhail, *J. Org. Chem.*, **31**, 1059 (1966).
(10) I. Photaki and V. Bardakos, *J. Amer. Chem. Soc.*, **87**, 3489 (1965).
(11) A. A. Gottlieb, Y. Fujita, S. Udenfriend, and B. Witkop, *Biochemistry*, **4**, 2509 (1965).
(12) L. Zervas, I. Photaki, and N. Ghelis, *J. Amer. Chem. Soc.*, **85**, 1337 (1963).
(13) R. G. Hiskey, T. Mizoguchi, and H. Igeta, *J. Org. Chem.*, **31**, 1188 (1966).