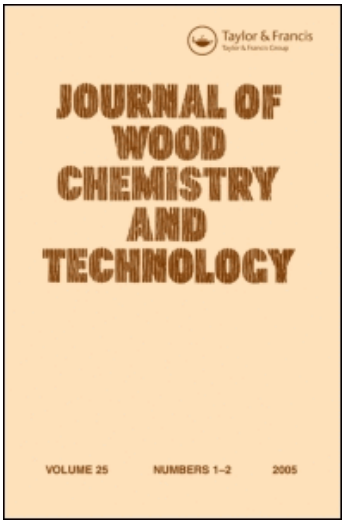

Journal of Wood Chemistry and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597282

Carboxylic Acids Produced Through Oxidative Cleavage of Aromatic Rings During Degradation of Lignin in Spruce Wood by Phanerochaete Chrysosporium

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To cite this Article Chen, Chen-Loung , Chang, Hou-Min and Kirk, T. Kent(1983) 'Carboxylic Acids Produced Through Oxidative Cleavage of Aromatic Rings During Degradation of Lignin in Spruce Wood by Phanerochaete Chrysosporium', Journal of Wood Chemistry and Technology, 3: 1, 35 — 57
To link to this Article: DOI: 10.1080/02773818308085150
URL: http://dx.doi.org/10.1080/02773818308085150

JOURNAL OF WOOD CHEMISTRY AND TECHNOLOGY, 3(1), 35-57 (1983)

CARBOXYLIC ACIDS PRODUCED THROUGH OXIDATIVE CLEAVAGE OF AROMATIC RINGS DURING DEGRADATION OF LIGNIN IN SPRUCE WOOD BY PHANEROCHAETE CHRYSOSPORIUM[1,2]

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ABSTRACT

Pre-extracted spruce wood chips were decayed by Phanerochaete chrysosporium to a 20% loss in lignin (8% loss in total weight), and extracted successively with petroleum ether, chloroform, acetone, methanol, and aqueous dioxane. The low molecular weight fraction of the methanol extract was analyzed by gas chromatography/high resolution mass spectrometry after acetylation and methylation. Examination of the spectra resulted in structural assignments for 28 compounds, 10 of which were aromatic acids identified and reported previously (Holzforschung 36,3 (1982)). At least 13 of the remaining compounds were formed via aromatic ring cleavage. In addition to ring cleavages, the new structures revealed oxidation of α- and γ-hydroxyl groups, oxidative cleavage of C_α-C_β and

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C_β-C_γ bonds, and 3-O-demethylation. It is postulated that oxidative cleavage of aromatic rings in the lignin units with an ether linkage at C-4 of the guaiacyl group involves 3-O-demethylation, hydroxylation at C-2 and subsequent o-cleavage of the resulting catechol structures, whereas for lignin units with a phenolic hydroxyl group at C-4 of the guaiacyl group only 3-O-demethylation produces the catechol substrate for subsequent cleavage. In both cases, the resulting products are 2,4-hexadiene-1,6-dioic acid intermediates, differing merely in the nature, number, and position of substituent groups. These intermediates undergo further degradation via various pathways, depending on the nature of substituent groups, to produce the observed ring cleavage products. It is considered probable that the ring cleavages and subsequent reactions occurred at the macromolecular level.

INTRODUCTION

This paper is part of a series describing the chemical changes that occur in the lignin of spruce wood during its degradation by the white-rot fungus Phanerochaete chrysosporium Burds. Our purpose is to describe the chemistry of the fungal degradation of lignin, thus providing insight into the underlying biochemistry of this important process. Previous papers described the fractionation and gross characterization of extracts of spruce wood, pre-extracted and then decayed by P. chrysosporium,[4] characterization of the high molecular weight polar components (i.e. of the degraded lignin polymer),[5] and the identification of some of the low molecular weight acidic components of the extracts.[6]

The low molecular weight acidic components of the methanol extract (M-LMWA) were first analyzed directly by high-performance liquid chromatography (HPLC), then by gas chromatography/mass spectrometry (GC/MS) after acetylation and methylation. The analyses resulted in identification of 10 aromatic acids.[3]

The objective of the present investigation was to assign structures to the more complex components of fraction M-LMWA. Gas chromatography/high resolution mass spectrometry was employed, again using the O-acetyl methyl esters. The 16 new degradation products that are reported here provide considerable insight into the chemistry of lignin biodegradation.

EXPERIMENTAL

Wood Decay and Extraction of the Decayed Wood

Pre-extracted spruce (Picea glauca L.) wood chips were decayed by the white-rot fungus Phanerochaete chrysosporium Burds. ME 446 (ATCC 35541). The decayed chips (20% loss in lignin; total weight loss 8%) were air-dried, ground to pass a 40-mesh screen, then extracted successively with ligroin, chloroform, acetone, methanol, and aqueous dioxane (4% H_2O). Details of the procedure have been described.[5]

Fractionation of the Methanol Extract

The methanol extract was divided into acidic and phenolic fractions by successive treatment with $NaHCO_3$ and NaOH solutions. The acidic fraction was further divided into high and low molecular weight acidic fractions (M-HMWA and M-LMWA) by means of extraction with chloroform.[5]

GC/High Resolution Mass Spectroscopic (MS) Analysis of the M-LMWA Fraction

Sample preparation.--The M-LMWA fraction (100 mg) was treated with pyridine-acetic anhydride, then with etheral diazomethane. Details of the procedure have been described.[6] The resulting reaction mixture was dissolved in 5 ml of tetrahydrofuran (THF) and passed through a Sep-Pak Silica Cartridge (Waters Associates, Inc.) to remove any residual high molecular weight substances. The cartridge was washed with 5 ml of THF, and the total THF solution was concentrated to 2 ml, then added dropwise to 5 ml of diethyl ether (Et_2O) with gentle stirring. The precipitate was centrifuged off and washed thoroughly with cold Et_2O. The Et_2O/THF-soluble part consisted mostly of "monomeric" and "dimeric" degradation products of lignin.

GC/MS analysis.--Preliminary GC analysis of the Et_2O/THF-soluble part was conducted with a Hewlett-Packard Model 5750 gas

chromatograph with 2-m stainless steel columns packed with 3% OV 101.[6]

GC/high resolution MS analysis was conducted with a Perkin-Elmer Sigma II GC/MS spectrometer with a 1.8 m x 2 mm i.d. glass column packed with 3% OV-3 on Chromosorb G. The temperature was programmed from 150° to 270°C at 4°C/min using He (20 ml/min) as carrier gas. The eluent was monitored with successive mass spectral scans, with an ionization energy of 70 eV. The mass spectra were acquired under high resolution conditions with R ≅ 10,000. The MS fragmentation pattern, mass number, and composition of the important ions for each compound are listed in the Appendix.

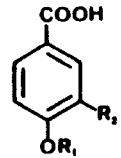
Synthesis

The O-acetyl methyl ester of compound 11 was prepared from α-bromo-4-O-acetylacetovanillone and vanillic acid methyl ester through a Williamson ether synthesis.[6]

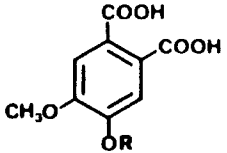
RESULTS

Figure 1 shows the total ion chromatogram of the Et_2O/THF-soluble portion of the M-LMWA fraction, obtained from the GC/high resolution MS analysis. Examination of the mass spectra resulted in elucidation of the structures of a total of 28 compounds. These compounds included aromatic and aliphatic carboxylic acid derivatives, and were identified as the corresponding methyl esters or O-acetyl methyl ester derivatives.

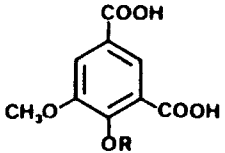
Compounds 1-10, identified and reported previously,[6] were as follows: 4-hydroxybenzoic acid (1), vanillic acid (2), isovanillic acid (3), veratric acid (4), 4-hydroxy-5-methoxyphthalic acid (5), m-hemipinic acid (6), 4-hydroxy-5-methoxyisophthalic acid (7), isohemipinic acid (8), dehydro-divanillic acid (9), and 2'-hydroxy-2,3'-dimethoxydiphenylether-4,5'-dicarboxylic acid (10). Compounds 2, 3, 4, and 6 were the major components of the M-LMWA fraction.



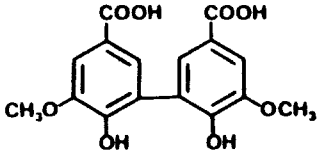
1. $R_1 = R_2 = H$
2. $R_1 = H$; $R_2 = OCH_3$
3. $R_1 = CH_3$; $R_2 = OH$
4. $R_1 = CH_3$; $R_2 = OCH_3$



5. $R = H$
6. $R = CH_3$



7. $R = H$
8. $R = CH_3$



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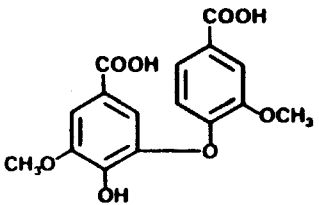

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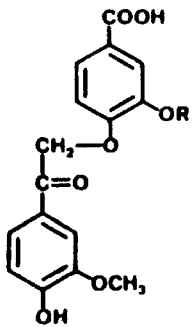
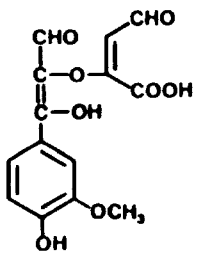


11. $R = CH_3$
12. $R = H$



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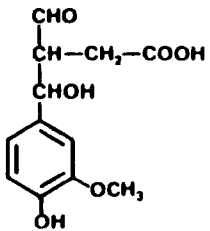

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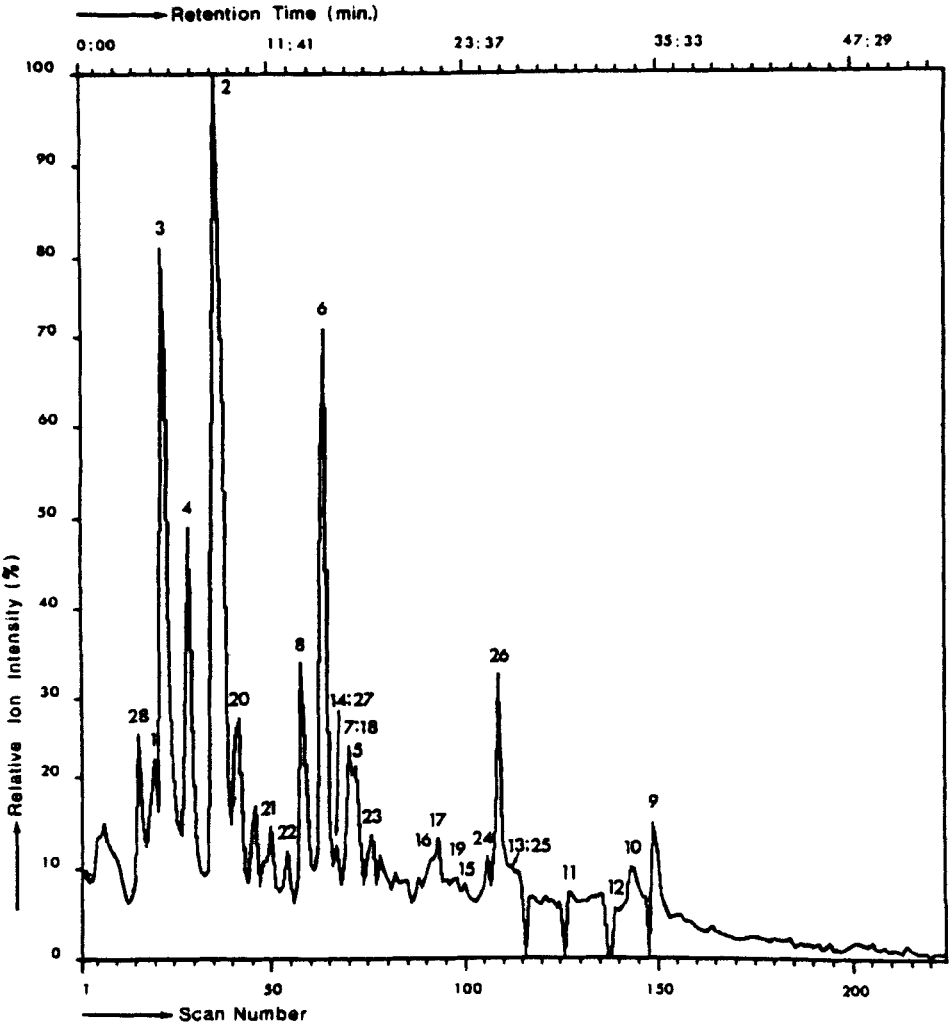
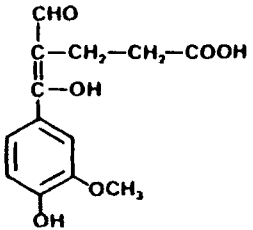


FIGURE 1 Total ion chromatogram obtained by gas chromatography/ high resolution mass spectrometry of the M-LMWA fraction after acetylation and methylation (100% corresponds to an ion count of 155,359).

The following new components were characterized in the present work: (a) three carboxylic acids derived from the fungal degradation of guaiacylglycerol-β-aryl ether (β-O-4) units--3-methoxy-4-(4-hydroxy-3-methoxy-β-oxophenethoxy)benzoic acid (11), 3-hydroxy-4-(4-hydroxy-3-methoxy-β-oxophenethoxy)benzoic acid (12), and 2-O-(α-formyl-β,4-dihydroxy-3-methoxystyryl)-2-hydroxy-4-oxo-2-butenoic acid (13); (b) six carboxylic acids derived from degradation of 2,3-bisguaiacyl-1,3-propanediol (β-1) units--3-formyl-4-hydroxy-4-(4-hydroxy-3-methoxyphenyl)butanoic acid (14), 4-formyl-5-hydroxy-5-(4-hydroxy-3-methoxyphenyl)-4-pentenoic acid (15), α-hydroxymethyl-β-hydroxyferulic acid (16), 4-carboxy-5-hydroxy-5-(4-hydroxy-3-methoxyphenyl)-2-oxo-4-pentenoic acid (17), α-(2-hydroxyethyl)-β-hydroxyferulic acid (18), and 2-(α,4-dihydroxy-3-methoxybenzylidene)-4-carboxy-β-butenolide (19); (c) seven carboxylic acids derived from degradation of biphenyl (5-5) units--5-(2-oxoethyl)vanillic acid (20), 5-(3-oxopropyl)vanillic acid (21), 5-(1-hydroxy-3-oxo-1-propenyl)vanillic acid (22), β,2-dihydroxy-3-methoxy-5-carboxycinnamic acid (23), 2-(2-hydroxy-3-methoxy-5-carboxyphenyl)-4-formyl-4-methyl-α-butenolide (24), 2-(2-hydroxy-3-methoxy-5-carboxyphenyl)-4-acetyl-4-methyl-α-butenolide (25), 2-(2-hydroxy-3-methoxy-5-carboxyphenyl)-4-carboxy-β-butenolide (26); and (d) two carboxylic acids of unknown origin--ferulic acid (27), and 2-(2-methylpropyl)-3-phenylpropanoic acid (28). Compounds 20 and 26 were moderately abundant, whereas most of the other compounds were minor components.

Structures for the compounds 11-28, as the corresponding methyl esters or O-acetyl methyl esters, were elucidated from their high resolution MS fragmentation patterns. The MS fragmentation patterns, mass numbers, and composition of important ions are given in the Appendix. The identity of compounds 11 and 27 as the corresponding O-acetyl methyl esters was further confirmed by HPLC analysis[3] of the Et_2O/THF-soluble part with spiking of authentic samples; identities were confirmed by comparison of the mass spectra with those of authentic samples.



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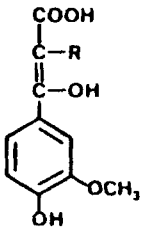
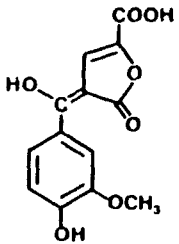


16. R = CH_2—OH
17. R = CH_2—CO—COOH
18. R = CH_2—CH_2—OH



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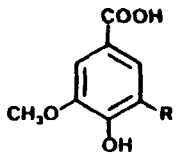
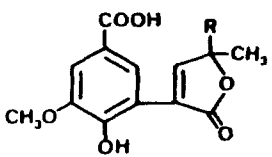
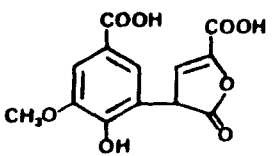


20. R = CH_2—CHO
21. R = CH_2—CH_2—CHO
22. R = C(OH)=CH—CHO
23. R = C(OH)=CH—COOH



24. R = CHO
25. R = CO—CH_3



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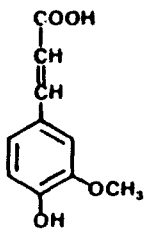

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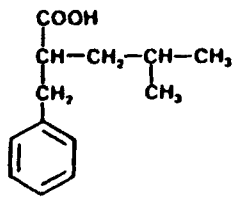

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DISCUSSION

Elucidation of Structures (12-26)

The structures of compounds 12-26 were elucidated solely by analysis of the high resolution mass spectra of O-acetyl methyl ester derivatives obtained by GC/high resolution mass spectrometry. Although most of the spectra are straightforward, the structures should still be regarded as tentative. Even so, it is clear that oxidative cleavage of aromatic rings occurred, together with C_α- and C_γ-oxidations, oxidative cleavages of side chains, and 3-O-demethylations. Absolute certainty about the structures 12-26 is not as important as the principles involved in the formation of such products and the insights gained into the mechanism of lignin metabolism by white-rot fungi.

Aromatic Ring- Versus Side Chain Cleavages

Cleavage between C_α and C_β in the propyl side chains is an important reaction in the fungal degradation of lignin. Aromatic acid residues in the partially degraded lignin polymer show that this is the case.[5,7,8] Studies of the metabolism in ligninolytic cultures of Phanerochaete chrysosporium of nonphenolic substructure model compounds of the β-O-4,[9] β-5,[10] and β-1,[11,12] types have also shown that C_α-C_β cleavage is a prominent reaction. This cleavage obviously produced the aromatic acid moieties in products 11 and 12, and in 20-26 here, and in all 10 of those reported earlier (compounds 1-10).[6]

Many of the products in the present study, however, contained intact C_α-C_β linkages, in addition to ring cleavage fragments. With the exception of compound 14, which was a very minor product, all of those products contained α- and γ-carbonyl groups (seen in the enol form). Thus we suspect that concomitant C_α- and C_γ-oxidation reduces the susceptibility of the side chains to C_α-C_β cleavage, and in effect directs the degradative attack to other parts of the substructures. In accord with this interpretation,

polymeric spruce lignin degraded by white-rot fungi has been shown to have an increased content of α-carbonyl structures.[5,7,8] Model compound studies with P. chrysosporium are also fully in agreement with this interpretation; nonphenolic models of both the β-O-4 and β-1 types containing α-carbonyl groups exhibit much greater resistance to degradation than the corresponding α-carbinol structures (unpublished data).

Apparent Resistance of Phenolic Units in Wood

A noticeable inconsistency between studies of the degradation of model compounds in ligninolytic cultures and studies of the lignin in decayed spruce wood is the extreme lability of phenolics in the former, and the survival of phenolics in the latter. This inconsistency was addressed briefly in our first report describing several phenolic acid degradation products from decayed spruce wood,[6] the major one being vanillic acid (2). Vanillic acid and other low molecular weight phenolics are very rapidly metabolized by ligninolytic cultures of P. chrysosporium, in large part via phenol-oxidative coupling. The accumulation of such phenolics during decay of wood by P. chrysosporium, therefore, points to the existence of a protective mechanism or mechanisms for the labile products. Methylation is one such mechanism,[6] but this was clearly not what protected the phenolics 11-26 here. Another biochemical protective mechanism might be back-reduction of oxidized phenolics by the enzyme cellobiose-quinone oxidoreductase.[13] Still another protective mechanism might be simple diffusion of the phenolics into the woody matrix where they cannot be reached by the oxidative enzymes.[6]

Because phenolic structures are so readily degraded by P. chrysosporium, it seems likely that the free phenolic hydroxyl groups seen in products 11-26 were not present in the substructures during their degradation. That is, degradations occurred while the substructures were still ether-linked through C_4 to the lignin matrix, and the reactions that released the phenolic groups also

released the products from the polymer. That the degradations might have occurred in the polymer is in accord with evidence that aromatic rings are cleaved and further degraded in the polymer during fungal metabolism.[5,8,14]

Origins of Products 11-26

Compounds 11 and 12 were formed from β-O-4 substructures of the type 29 (Figure 2), via oxidative cleavage of the $C_{\alpha'}$-$C_{\beta'}$ bond

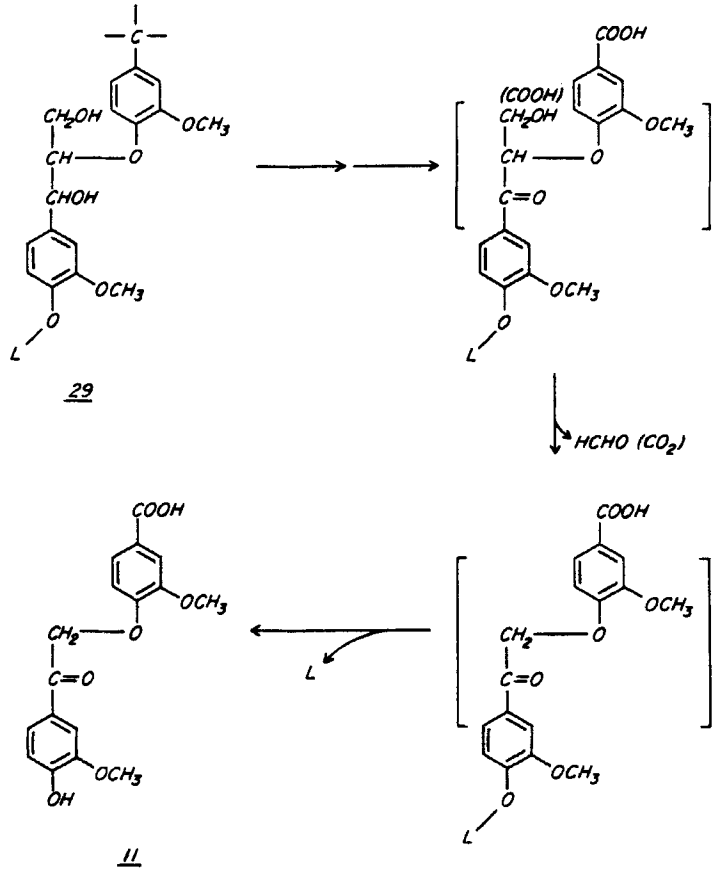

FIGURE 2 Possible sequence of biodegradative reactions in β-O-4 substructures 29 leading to compound 11. (L = lignin polymer.)

to form a 4-O-alkylated vanillic acid intermediate. Such vanillic acid moieties are prominent in lignin after partial degradation by white-rot fungi.[5,7,8] Formation of 11 and 12 also involved oxidation of the C_α-hydroxyl group. Elimination of C_γ as formaldehyde through a reversed aldol addition (or, following oxidation to a carboxyl group, as CO_2) would be facile after C_α-oxidation. Formation of compound 12 involved demethylation of 11 or a precursor structure. Presumably, 11 and 12 were released from lignin or an oligomer by cleavage of the ether linkage at C_4 (Figure 2).

The identification of compounds 11 and 12 provides the first direct evidence for cleavage of the C_β-C_γ bond, and for 3-O-demethylation during the degradation of lignin by white-rot fungi. The low methoxyl content of white-rotted lignins[7,15,16] established some years ago that methoxyl loss occurs during lignin degradation. However, methoxyl-deficient moieties, such as seen prominently in brown-rotted lignins,[17] are not present.[8] (Brown-rot fungi are taxonomically very closely related to white-rot fungi, and their effects on lignin may well represent "partial white rot."[18]) In an examination of white-rotted lignin, Kirk and Chang[8] concluded that 3-O-demethylation had occurred, but had been followed immediately by oxidative cleavage of the aromatic rings.

Compound 13 resulted from cleavage of an aromatic ring linked 4-O-β (structure 29, Figure 3). Presumably, this ring cleavage was preceded by 3-O-demethylation (as in compound 12) and C_2-hydroxylation. Products containing C_2-hydroxyl groups were not found in the present study, nor were compounds containing o-dihydroxyaryl (catechol) moieties. Catechol structures, C_2-hydroxyl groups, and 3-O-demethylated structures are all seen in lignin degraded by brown-rot fungi.[17,19] We surmise that 3-O-demethylation preceded C_2-hydroxylation here, and that ring cleavage of the resulting catechol moiety ensued rapidly. Further degradation of the cleaved ring fragment produced structure 13, which presumably was then released from the lignin by cleavage of the ether linkage at C_4 (Figure 3).

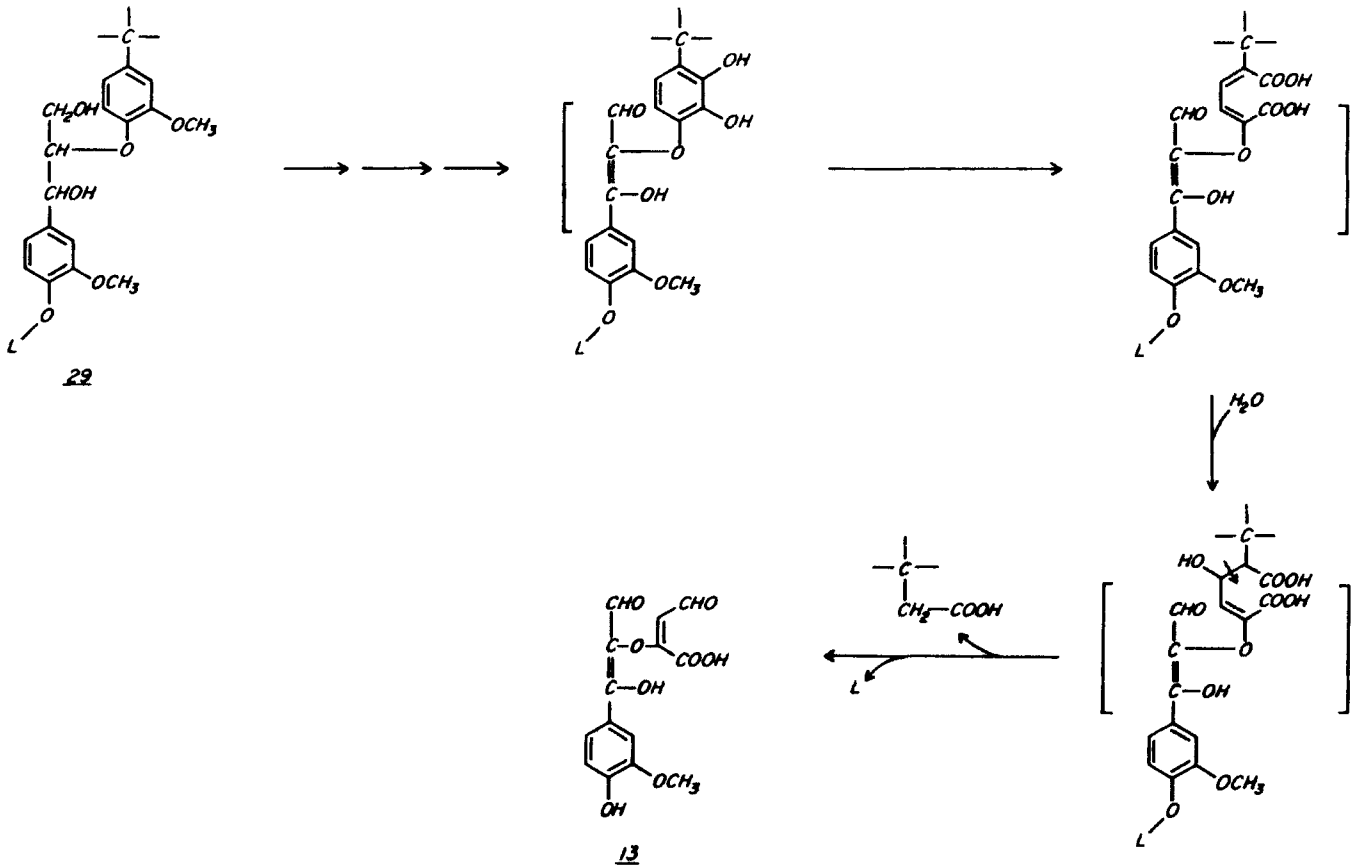

FIGURE 3 Hypothetical biodegradative pathway for the formation of compound 13 from β-O-4 substructures 29. (L = lignin polymer.)

Compounds 14-19 were derived from either β-1 or β-5 substructures. Our inability to formulate probable degradative pathways from β-5 structures leads us to suspect that the products were primarily from β-1 structures 30 (Figure 4), but this is only speculation. As in the case of the β-O-4-derived products, the β-1- (or β-5-) derived products were formed via cleavage of the β-linked aromatic ring, followed by further degradations. Ring cleavage was presumably preceded by methoxyl demethylation, and, if the 4-hydroxyl group were etherified, by C_2-hydroxylation. By way of illustration, Figure 4 depicts a possible origin of product 14. Again it is assumed that the final step was release of the product 14 by ether cleavage at C_4.

Compounds 20-26 were formed from 5-5' or β-5 substructures. The structure of compound 20 suggests an origin from β-5 substructures via C_α-C_β cleavages in both phenylpropanoid units, followed by, or preceded by α-O-4 cleavage. Degradation of nonphenolic β-5 model compounds in ligninolytic cultures of P. chrysosporium involved C_α-C_β cleavages in both units, although a product analogous to 20 was not detected,[10] perhaps due to the extreme lability of low molecular weight phenols in such cultures, as discussed above. It is also possible that 20 arose from 5-5', instead of β-5, substructures. If compounds 21-26 arose from β-5 substructures, C_γ elimination had to occur in the "β" side of the structure (as in compounds 11 and 12). C_β-C_γ cleavage was not observed during degradation of the β-5 models mentioned above in ligninolytic cultures.[10] It is probable, therefore, that compounds 21-26 were derived from 5-5' structures.

As with compounds 13-19, one of the aromatic rings in the 5,5' (or β-5) substructure was cleaved, and the ring cleavage fragments degraded further to various extents. Again we assume that ring cleavage was preceded by methoxyl demethylation, and, if the 4-hydroxyl group were etherified, by C_2-hydroxylation, to yield catechol structures. By way of illustration, Figure 5 depicts a possible origin of compound 26 from a 5,5' substructure 31;

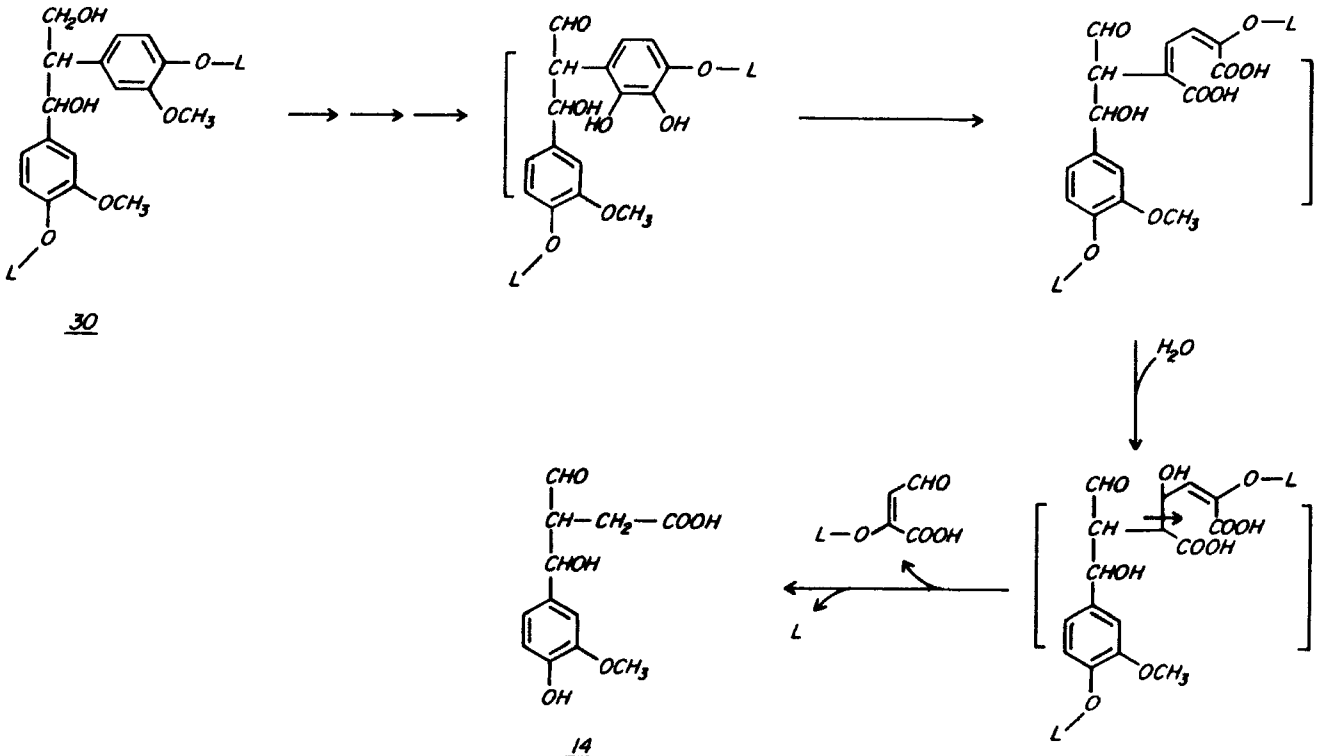

FIGURE 4 Hypothetical biodegradative pathway leading to product 14 from β-1 substructures 30. (L = lignin polymer.)

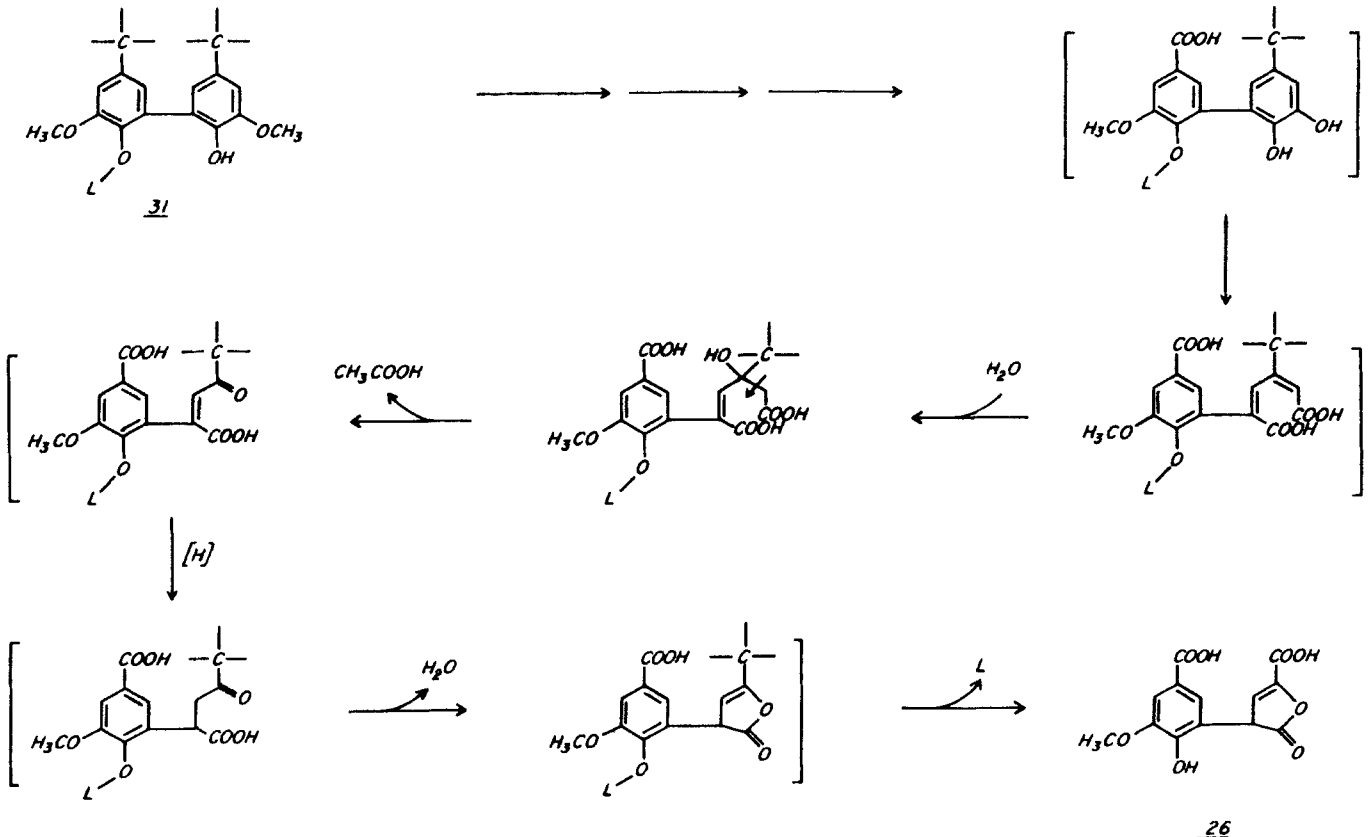

FIGURE 5 Hypothetical biodegradative pathway leading to product 26 from 5-5' substructures 31. (L = lignin polymer.)

product 26 was the most abundant ring cleavage product in the M-LMWA extract.

Origins of Compounds 27 and 28

Ferulic acid (27), present as a trace component, could have arisen by C_γ-oxidation of terminal 4-O-ether-linked coniferyl alcohol- or aldehyde moieties in lignin, or as a fungal metabolite derived from phenylalanine. Studies have recently shown that the secondary metabolite veratryl alcohol, synthesized de novo by P. chrysosporium,[20] is derived from phenylalanine, probably via ferulic acid.[21]

Compound 28 is presumably of fungal origin. It is unlikely that it was produced from lignin, which would have required reductive 4-O-dehydroxylation, reductive 3-O-demethoxylation, and C_α-reduction.

Nature of Reactions Involved in Formation of Products 13-26

It is probable that key reactions in the formation of ring cleavage products 13-26 were 3-O-demethylation and C_2-hydroxylation to produce the catechol moieties that were cleaved, as pointed out above. Where the aromatic rings contained free phenolic hydroxyl groups at C_4, C_2-hydroxylation would not have been necessary. Intradiol cleavage of the catechol structures between C_3 and C_4, or between C_2 and C_3, would produce substituted 2,4-hexadiene-1,6-dioic acids. These would differ merely in the nature, numbers, and positions of substituent groups. The 2,4-hexadiene-1,6-dioic acid intermediates were probably further degraded mainly by hydration of one of the double bonds and subsequent reversed aldol addition of the resulting β-hydroxy group as illustrated in Figures 3-5. Further degradative reactions produced the products 13-26, as well as some of the structures in the partially degraded lignin polymer.[5] In formulating possible pathways for the formation of products 13-26, we have had to invoke not only hydrations and eliminations, but also oxidations and even reductions. Which of these various kinds of

reactions occurred in the polymer can only be determined through further investigations.

ACKNOWLEDGMENTS

The GC/high resolution mass spectra were obtained at the Midwest Center for Mass Spectrometry at the University of Nebraska, supported under the National Science Foundation Regional Instrumentation Facilities Program; we are grateful to Dr. Michael Gross and his staff for their key role in this research.

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APPENDIX

Fragmentation patterns, mass numbers, and compositions of important ions for compounds 11-28.

Composition	m/z	Dev.[a]	RI[b]	Fragmentation
	11 (Scan 127)			
$C_{20}H_{20}O_8$	388.1158			M^+, missing
$C_{18}H_{18}O_7$	346.1039	-1.4	9	$M^+ - CH_2{=}C{=}O$
$C_{17}H_{15}O_6$	315.0876	0.7	1	m/z 346 - $\dot{O}CH_3$
$C_{10}H_{11}O_4$	195.0673	1.6	3	m/z 346 - $\dot{C}_8H_7O_3$
$C_9H_9O_3$	165.0546	-0.5	8	m/z 346 - $\dot{C}_9H_9O_4$
$C_8H_7O_3$	151.0389	-0.7	100	m/z 346 - $\dot{C}_{10}H_{11}O_4$
$C_8H_5O_3$	149.0242	0.4	28	m/z 151 - 2 H·
$C_7H_7O_2$	123.0442	-0.4	13	m/z 151 - CO
	12 (Scan 136)			
$C_{21}H_{20}O_9$	416.1106			M^+, missing
$C_{17}H_{16}O_7$	332.0889	-0.7	11	$M^+ - 2\ CH_2{=}C{=}O$
$C_{16}H_{13}O_6$	301.0722	1.0	3	m/z 332 - $\dot{O}CH_3$
$C_8H_7O_4$	167.0327	-1.7	5	m/z 332 - $\dot{C}_9H_9O_3$
$C_9H_9O_3$	165.0569	1.7	6	m/z 332 - $\dot{C}_8H_7O_4$
$C_8H_7O_3$	151.0395	0.0	100	m/z 332 - $\dot{C}_9H_9O_4$
$C_8H_5O_3$	149.0242	0.3	29	m/z 151 - 2 H·
$C_7H_7O_2$	123.0437	-0.9	14	m/z 151 - CO
	15 (Scan 100)			
$C_{18}H_{20}O_8$	364.1159			M^+, missing
$C_{14}H_{16}O_6$	280.0929	-1.8	1	$M^+ - 2\ CH_2{=}C{=}O$
$C_{14}H_{15}O_6$	279.0842	-2.7	1	m/z 280 - H·
$C_{13}H_{13}O_5$	249.0733	-3.0	1	m/z 280 - $\dot{O}CH_3$
$C_{11}H_{10}O_4$	206.0607	2.8	24	m/z 280 - $CH_2{=}C(OH){-}OCH_3$
$C_8H_7O_3$	151.0390	-0.5	34	m/z 280 - $\dot{C}H{-}CHO$ / $CH_2{-}CH_2{-}COOCH_3$
$C_8H_5O_3$	149.0235	-0.4	11	m/z 151 - 2 H·
$C_7H_7O_2$	123.0446	0.0	5	m/z 151 - CO
$C_4H_7O_2$	87.0437	-0.9	100	$^+CH_2{-}CH_2{-}COOCH_3$
	16 (Scan 91)			
$C_{18}H_{20}O_9$	380.1108			M^+, missing
$C_{14}H_{16}O_7$	296.0927	3.1	1	$M^+ - 2\ CH_2{=}C{=}O$
$C_{13}H_{13}O_6$	265.0793	-1.1	1	m/z 296 - $\dot{O}CH_3$
$C_{12}H_{12}O_5$	236.0690	0.5	9	m/z 296 - CH_3COOH
$C_8H_7O_3$	151.0389	-0.6	100	m/z 296 - $CH_2O{-}COCH_3$ / $\cdot CH^2COOCH_3$
$C_8H_5O_3$	149.0241	0.2	22	m/z 151 - 2 H·
$C_7H_7O_2$	123.0444	-0.2	7	m/z 151 - CO

13 (Scan 112)

$C_{19}H_{18}O_{10}$	406.0896			M^+, missing
$C_{15}H_{14}O_8$	322.0676	-1.3	50	$M^+ - 2\ CH_2{=}C{=}O$
$C_{14}H_{11}O_7$	291.0487	-1.8	100	m/z 322 - $\dot{O}CH_3$
$C_{13}H_9O_6$	261.0406	0.7	12	m/z 291 - CH_2O
$C_{10}H_9O_4$	193.0499	-0.1	7	m/z 322 - $\dot{O}$-C=CH-CHO, COOCH$_3$
$C_8H_7O_3$	151.0397	0.2	71	m/z 322 - $\dot{C}_7H_7O_5$
$C_8H_5O_3$	149.0229	-1.0	21	m/z 151 - 2 H·
$C_7H_7O_2$	123.0446	0.0	10	m/z 151 - CO

14 (Scan 67)

$C_{17}H_{20}O_8$	352.1158			M^+, missing
$C_{15}H_{18}O_7$	310.1052			$M^+ - CH_2{=}C{=}O$, missing
$C_{13}H_{16}O_6$	268.0949	0.2	1	m/z 310 - $CH_2{=}C{=}O$
$C_{12}H_{12}O_5$	236.0678	-0.7	15	m/z 310 - $CH_2{=}C(OH)\text{-}OCH_3$
$C_{10}H_{11}O_4$	195.0659	0.2	4	m/z 268 - $\dot{C}H_2\text{-}COOCH_3$
$C_{10}H_{10}O_4$	194.0566	-1.3	11	m/z 236 - $CH_2{=}C{=}O$
$C_{10}H_9O_4$	193.0501	0.0	11	m/z 194 - H·
$C_9H_9O_3$	165.0540	-1.1	17	m/z 193 - CO
$C_8H_8O_3$	153.0531	-2.0	15	m/z 268 - CHO, $\cdot$CH-CH_2-$COOCH_3$
$C_8H_7O_3$	151.0388	-0.7	100	m/z 193 - $CH_2{=}C{=}O$
$C_8H_5O_3$	149.0225	-1.3	8	m/z 151 - 2 H·
$C_7H_7O_2$	123.0455	0.9	12	m/z 151 - CO

17 (Scan 93)

$C_{19}H_{20}O_{10}$	408.1057			M^+, missing
$C_{15}H_{16}O_8$	324.0845	0.0	16	$M^+ - 2\ CH_2{=}C{=}O$
$C_{14}H_{13}O_7$	293.0644	-1.7	18	m/z 324 - $\dot{O}CH_3$
$C_{13}H_{13}O_6$	265.0712	0.0	100	m/z 324 - $\dot{C}OOCH_3$
$C_{11}H_{11}O_5$	223.0600	-0.7	12	m/z 265 - $CH_2{=}C{=}O$
$C_8H_7O_3$	151.0394	-0.1	45	m/z 324 - $\dot{C}H\text{-}COOCH_3$, $CH_2\text{-}CO\text{-}COOCH_3$
$C_8H_5O_3$	149.0226	-1.3	12	m/z 151 - 2 H

18 (Scan 70)

$C_{19}H_{22}O_9$	394.1258			M^+, missing
$C_{13}H_{16}O_6$	268.0928	-1.9	55	$M^+ - 3\ CH_2{=}C{=}O$
$C_{12}H_{13}O_5$	237.0752	-1.0	6	m/z 268 - $\dot{O}CH_3$
$C_{12}H_{12}O_5$	236.0694	0.9	4	m/z 237 - H·
$C_{11}H_{11}O_5$	223.0604	-0.2	100	m/z 268 - $\dot{C}H_2\text{-}CH_2OH$
$C_8H_7O_3$	151.0395	0.0	89	m/z 268 - COOCH$_3$, $\cdot$CH-CH_2-CH_2OH
$C_7H_7O_2$	123.0443	-0.3	12	m/z 151 - 2 H·

[a] Δ m/z x 10^3.
[b] RI = Relative Intensity.

Composition	m/z	Dev.[a]	RI[b]	Fragmentation
	19 (Scan 97)			
$C_{18}H_{16}O_9$	376.0795			M^+, missing
$C_{14}H_{12}O_7$	292.0589	0.6	11	$M^+ - 2\ CH_2{=}C{=}O$
$C_{13}H_9O_6$	261.0393	-0.6	11	m/z 292 - $\dot{O}CH_3$
$C_{12}H_9O_5$	233.0432	-1.8	8	m/z 261 - CO
$C_8H_7O_3$	151.0388	-0.8	100	m/z 292 - $C_6H_5O_4$
$C_8H_5O_3$	149.0241	0.2	33	m/z 151 - 2H·
	20 (Scan 42)			
$C_{13}H_{14}O_6$	266.0794			M^+, missing
$C_{11}H_{12}O_5$	224.0681	-0.3	18	$M^+ - CH_2{=}C{=}O$
$C_{10}H_9O_4$	193.0474	-2.7	100	m/z 224 - $\dot{O}CH_3$
$C_{10}H_7O_4$	191.0337	-0.7	44	m/z 193 - 2 H·
$C_9H_9O_3$	165.0558	0.6	6	m/z 224 - $\dot{C}OOCH_3$
$C_9H_7O_3$	163.0401	0.6	9	m/z 191 - CO
$C_8H_7O_2$	135.0444	-0.2	7	m/z 163 - CO
$C_8H_5O_2$	133.0291	-0.1	10	m/z 163 - CH_2O
	21 (Scan 50)			
$C_{14}H_{16}O_6$	280.0947			M^+, missing
$C_{12}H_{14}O_5$	238.0841	0.0	16	$M^+ - CH_2{=}C{=}O$
$C_{11}H_{11}O_4$	207.0666	0.9	18	m/z 238 - $\dot{O}CH_3$
	24 (Scan 106)			
$C_{17}H_{16}O_8$	348.0845			M^+, missing
$C_{15}H_{14}O_7$	306.0727	-1.3	41	$M^+ - CH_2{=}C{=}O$
$C_{14}H_{11}O_7$	291.0522	1.7	100	m/z 306 - $\dot{C}H_3$
$C_{14}H_{13}O_6$	277.0703	-0.9	5	m/z 306 - $\dot{C}HO$
$C_{14}H_{11}O_6$	275.0560	0.5	20	m/z 306 - $\dot{O}CH_3$
	25 (Scan 112)			
$C_{18}H_{18}O_8$	362.1002			M^+, missing
$C_{16}H_{16}O_7$	320.0915	-1.9	31	$M^+ - CH_2{=}C{=}O$
$C_{15}H_{13}O_7$	305.0657	-0.4	6	m/z 320 - $\dot{C}H_3$
$C_{15}H_{13}O_6$	289.0698	-1.4	20	m/z 320 - $\dot{O}CH_3$
$C_{14}H_{13}O_6$	277.0691	-2.1	100	m/z 320 - $\dot{C}OCH_3$
$C_{13}H_{10}O_5$	246.0546	1.6	30	m/z 277 - $\dot{O}CH_3$
	26 (Scan 109)			
$C_{17}H_{16}O_9$	364.0794			M^+, missing
$C_{15}H_{14}O_8$	322.0649	-3.9	95	$M^+ - CH_2{=}C{=}O$
$C_{14}H_{11}O_7$	291.0500	-0.5	100	m/z 322 - $\dot{O}CH_3$

$C_{10}H_{11}O_4$	195.0640	1.9	100	m/z 238 - $\dot{C}H_2$-CHO
$C_{10}H_{11}O_3$	179.0707	-0.2	13	m/z 238 - $\dot{C}OOCH_3$
$C_9H_9O_3$	165.0548	-0.3	13	m/z 195 - CH_2O
$C_9H_9O_2$	149.0235	-1.7	13	m/z 179 - CH_2O
$C_9H_7O_2$	147.0454	0.8	26	m/z 149 - 2 H·
$C_8H_9O_2$	137.0604	0.2	16	m/z 165 - CO
C_8H_7O	119.0504	0.7	16	m/z 149 - CH_2O

22 (Scan 54)

$C_{16}H_{16}O_8$	336.0740			M^+, missing
$C_{12}H_{12}O_6$	252.0634	0.0	1	M^+ - 2 CH_2=C=O
$C_{11}H_{11}O_5$	223.0567	3.9	10	m/z 252 - $\dot{C}HO$
$C_{11}H_9O_5$	221.0437	-1.3	100	m/z 252 - $\dot{O}CH_3$

23 (Scan 76)

$C_{17}H_{18}O_9$	366.0951			M^+, missing
$C_{13}H_{14}O_7$	282.0741	0.1	9	M^+ - 2 CH_2=C=O
$C_{12}H_{11}O_6$	251.0533	-2.2	100	m/z 282 - $\dot{O}CH_3$
$C_{11}H_{11}O_5$	223.0603	-0.4	5	m/z 282 - $\dot{C}OOCH_3$
$C_{11}H_7O_5$	219.0290	-0.3	14	m/z 251 - CH_3OH

27 (Scan 67)

$C_{13}H_{14}O_5$	250.0854	1.3	1	M^+
$C_{11}H_{12}O_4$	208.0737	0.1	100	M^+ - CH_2=C=O
$C_{10}H_9O_3$	177.0563	1.2	33	m/z 208 - $\dot{O}CH_3$
$C_9H_9O_2$	149.0587	-1.6	25	m/z 177 - CO

28 (Scan 16)

$C_{14}H_{20}O_2$	220.1476	1.2	32	M^+
$C_{13}H_{17}O_2$	205.1227	-0.1	17	M^+ - $\dot{C}H_3$
$C_{13}H_{17}O$	189.1269	-1.1	7	M^+ - $\dot{O}CH_3$
$C_{11}H_{13}O_2$	177.0922	0.6	9	M^+ - $\dot{C}H(CH_3)$-CH_3
$C_{10}H_{12}O_2$	164.0425	-2.1	6	M^+ - CH_2=C(CH_3)-CH_3
$C_{10}H_{11}O_2$	163.0740	-1.9	24	M^+ - $\dot{C}H_2$-CH(CH_3)-CH_3
C_7H_7	91.0546	-0.2	40	
C_6H_5	77.0395	0.3	43	
C_5H_5	65.0380	-1.1	21	
C_4H_9	57.0636	-6.8	100	M^+ - $\dot{C}H(CH_2$-Ph)-$COOCH_3$

[a] Δ m/z x 10^3.
[b] RI = Relative Intensity.