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# OXIDATIVE DIMERIZATION OF FERULIC ACID BY EXTRACTS FROM *SORGHUM*

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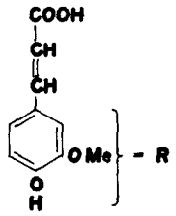
**Key Word Index**—*Sorghum bicolor*; Gramineae; ferulic acid; oxidative phenol coupling; ferulic-β-β-dimerase; dilactone of ferulic acid.

**Abstract**—Extracts from leaves and first internodes of *Sorghum bicolor* catalyze the conversion of ferulic acid to a β-β-coupled dimer, the dilactone dimer of ferulic acid. The dilactone is then hydrolyzed, probably non-enzymatically, to a blue fluorescing compound, tentatively identified as a β-β-coupled dimer with at least one lactone ring opened to form a carboxylic acid. Both the initial enzymatic and the subsequent non-enzymatic steps are greater at pH 8 than pH 6. The most active preparation is a crude particulate fraction from leaves obtained by centrifugation at 37000 *g*; white light increases the amount of dimer formed.

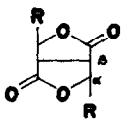
## INTRODUCTION

Ferulic acid, 4-hydroxy-3-methoxycinnamate (**1**) is a central metabolite in the highly branched pathway of phenolic metabolism in higher plants [1]. Besides being oxidatively polymerized by peroxidase into core lignin after conversion to coniferyl and syringyl alcohols, it may be directly incorporated peroxidatically into lignin-like products or converted into esters found either in the cell walls or in vacuoles [1,2]. Both laccases and peroxidases oxidize ferulic acid [3–6].

This paper describes an enzyme, called ferulic acid dimerase, which catalyzes the dehydrogenative coupling of two ferulic acid molecules (**1**) in the presence of $O_2$ to the β-β-linked dilactone dimer of ferulic acid, (**2**) a compound previously synthesized non-enzymatically [7]. At pH 8, the dilactone hydrolyses to a blue fluorescing product called "X", which is isolated and analyzed fluorometrically in the assay for dimerase activity.




(1)

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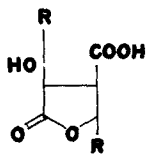


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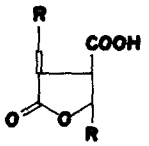


(4)

## RESULTS

### *Basic requirements for the production of "X"*

Most of the work reported here was done with a crude particulate fraction $P_1$ obtained by centrifugation of a pH 8 homogenate of green leaves between 500 and 37000 g. In the presence of light, ferulic acid, enzyme extract, air and pH 8 buffer, 1.63 μmol of "X"/hr/g fresh wt was produced, calculated as hydrolyzed dilactone, using method 1, representing 12% of the ferulic acid consumed. The production of "X" was prevented by boiling the enzyme, or by the elimination of ferulic acid or of enzyme, and was dependent on the presence of air, presumably supplying $O_2$. Although some "X" was produced in assays performed in laboratory light or in the dark, additional white light treatment had a significant activating effect. The reaction was linear with time up to 1–2 hr, and leveled off when approximately 20–25% of the substrate had disappeared. The addition of NADPH sometimes increased the production of "X", but this effect was variable and needs further study with purified preparations. Equimolar concentrations of ascorbate almost completely inhibited the reaction.

### *Relationship of the dimerase to peroxidase and phenoloxidase activity*

Since peroxidase, acting peroxidatically via an endogenous supply of $H_2O_2$ or oxidatively via $O_2$, might be responsible for this oxidation of ferulic acid [3–6], the effect of added $H_2O_2$ and the distribution of dimerase relative to peroxidase activity was studied. The addition of 0.06% $H_2O_2$ to a mixture containing ferulic acid, sorghum $P_1$ extract and pH 8 buffer produced amounts of "X" greater than that formed in the dark, and similar to that in the light. But several other reaction products were produced, and a greater proportion (38%) of the ferulic acid disappeared. $H_2O_2$ alone had no detectable effect on ferulic acid in the absence of enzyme.

When horseradish peroxidase was substituted for the sorghum extract, no "X" was produced and ferulic acid

Table 1. Distribution of ferulic dimerase and peroxidase activities in various crude fractions isolated from sorghum

| | $P_1$ | $P_2$ (% Total) | $P_3$ | Total activity (ΔA/min/g fr. wt) |
|---|---|---|---|---|
| Peroxidase activity | | | | |
| pH 8 ground, pH 6 assay | | | | |
| Green leaves – whole tissue (Mat + mesophyll) | 4 | 5 | 91 | 70 |
| Ferulic dimerase activity | | | | "X" as hydrolyzed dilactone (μmol/hr/g fr. wt) |
| First internodes | | | | |
| pH 8 ground, pH 8 assay* | | | | |
| Whole – mat + cortex* | 39 | 25 | 35 | 0.1 |
| Mat only* | 40 | 35 | 25 | 0.1 |
| Green leaves – mesophyll + bundle sheath | | | | |
| pH 8 ground, pH 8 assay* | 43 | 27 | 30 | 1.8 |
| ph 6 ground, pH 8 assay* | 59 | 14 | 27 | 0.5 |
| Green leaves – (Mat only) | | | | |
| pH 8 ground, pH 8 assay* | 56 | 21 | 23 | 1.1 |
| pH 8 ground, pH 8 assay†‡ | 88 | 7 | 7 | 1.9 |

* "X" only was analyzed by method 1. † "X" plus dilactone analyzed by method 2. ‡ No NADPH system, all others contained 1 mg $NADP^+$, 2 μmol glucose-6-phosphate, and 8 units glucose-6-P-dehydrogenase.

did not disappear in the absence of added $H_2O_2$. Considerable "X" was accumulated when $H_2O_2$ was added but several other products were also formed, and only traces of ferulic acid remained even after a 10 min incubation.

The distribution of the dimerase and peroxidase activities in the three major fractions of sorghum extracts (Table 1) would also argue against peroxidase activity alone accounting for the dimerase activity. About 40–80% of the total recoverable activity of ferulic dimerase was present in the crude 37000 *g* pellet ($P_1$) when tissues were ground either at pH 6 or 8, while only 4% of the total peroxidase activity was detected in $P_1$. The presence of NADPH in the system may increase the % found in $P_2$ and $P_3$. The amount of dimerase activity in $P_1$ did not differ significantly if the tissues were ground in a high osmotic medium containing 600 mM sorbitol in 100 ml Tricine at pH 8.

The distribution of the dimerase activity also differs from that of the phenoloxidase complex reported previously [8]. In extracts ground at pH 6, the monophenol hydroxylase activity was found mainly in the low ammonium sulfate fraction, $P_2$, while the diphenol oxidase (chlorogenic acid oxidase) was evenly distributed between this and the higher ammonium sulfate fraction, $P_3$. In pH 8 extracts, however, much of both activities of the phenoloxidase activity shifted to the particulate $P_1$ fraction [8].

The ferulic dimerase activity may be linked to vascular tissue. All of the detectable activity in excised internodes, incubated for 3 days in moist filter paper, was associated with a fibrous mat tissue obtained by differential grinding. This mat contained only tissue of the vascular stele. Approximately 50–70% of the total activity was associated with a fibrous mat obtained from one week old green shoots. The significant amount of activity sometimes found in the mesophyll tissue fraction could be due to the breaking of the small veins in young leaf tissues rather than to a valid different tissue localization, but it also is possible that the presence of NADPH may affect the enzyme distribution. But the mat preparation would also contain chloroplast containing bundle sheath cells, since sorghum is a $C_4$ plant with "Kranz" anatomy.

Preliminary inhibitor data would also argue against a peroxidase involvement. Ferulic acid dimerase was not inhibited significantly by KCN at the 5 mM level, while DIECA (diethyldithiocarbamate) gave a 30–40% inhibition at the 1 mM level and 75–88% at the 10 mM level.

*Evidence that the dilactone of ferulic acid is an intermediate in the production of "X"*

UV analysis of the dilactone of ferulic acid (10 μg/ml) showed $\lambda_{max}^{MeOH}$ (log $\epsilon_{max}$) 282 nm (3.82); $\lambda_{max}^{0.1\,N\,KOH\,in\,MeOH}$ (log $\epsilon_{max}$) 386 nm (4.38) and 298 nm (3.88) and 255 nm (4.30); $\lambda_{max}^{5\%\,bicarbonate}$ (log $\epsilon_{max}$) 386 (4.40) and 255 (4.09). When the alkaline MeOH solution of the dilactone with a long wavelength peak at 386 was acidified, the peak shifted to 336, indicating that a new compound had been formed in alkali. Upon the addition of alkali to the acidified mixture, the peak at 386 nm reappeared.

UV analysis of the alkaline hydrolysis product of the dilactone (synthetic "X"), isolated chromatographically and equivalent to 10 μg dilactone/ml, showed $\lambda_{max}^{MeOH}$ (log $\epsilon_{max}$) 336 nm (4.21), 290 *sh* nm (3.96); $\lambda_{max}^{0.1\,N\,KOH\,in\,MeOH}$ (log $\epsilon_{max}$) 386 nm (4.43) and 298 nm (3.85) and 255 nm (3.93). Upon reacidification to pH 2 the 336 and 290 nm peaks reappeared. Both the neutral, alkaline, and reacidified solutions of the hydrolyzed dilactone had UV spectra identical to those obtained from the enzymatically produced "X". The spectrum of the dilactone in Tricine buffer at pH 8 indicated a mixture of neutral and alkaline forms of "X".

PC also indicated that the alkaline hydrolysis product of the synthetic dilactone was identical to the enzymatic product "X" at pH 8. While the dilactone can only be seen as a dark spot under a 260 nm lamp when present in high concentrations, it can be easily seen under a 360-nm lamp at 1 μg levels as a blue fluorescing compound after fuming with $NH_3$. $R_f$ values after paper chromatography of the synthetic "X" and that formed by sorghum preparations at pH 8 were identical in several solvents, i.e. 0.30 in BeAW, 0.80 in BAW (16:1:2) and 0.0 in *n*-BuOH–$NH_4OH$–$H_2O$ (6:1:2). While the initial spectral and fluorometric analyses of "X" were done in 0.05 N NaOH, subsequent work indicated that direct elution of the spot in 5% $NaHCO_3$ not only con-

Table 2. Analysis of the relative amounts of the dilactone and of "X" and total "X" at pH 6 and pH 8 after incubation with sorghum $P_1$ extracts from green leaves. Each value is an average of 2 analyses of aliquots from the same incubation mixture

| | pH 6 (μmol/hr/g fr. wt) | pH 8 |
|---|---|---|
| Dilactone* | 0.23 | 0.18 |
| "X"* | 0.29 | 1.43 |
| Σ dilactone "X" | 0.52 | 1.61 |
| "X" + dilactone† | 0.6 | 1.3 |

*Method 3. †Method 2.

verted the dilactone completely to "X", but that the fluorescence of "X" was more stable and gave higher fluorescence values.

In order to determine whether the dilactone could be detected in the incubation mixture, it was necessary to separate this product from both the ferulic acid and "X". No solvent mixture was found that separated the dilactone from ferulic acid, but the two-dimensional method 3 (see Experimental) separated the dilactone and "X" present in incubation mixtures. It was important to chromatograph the aliquots of the incubation mixture immediately upon acidification, and to examine the chromatograms under UV as briefly as possible.

Comparisons are made in Table 2 between the relative amounts of the dilactone and "X" present after a 1 hr incubation period at pH's 6 and 8, using method 3. The amount of "X" present and the total summed product including that due to the residual dilactone, were greater at the higher pH. At pH 6, a larger % of the reaction was still present as the dilactone. These results indicate that both the enzymatic and non-enzymatic steps are greater at pH 8. The relative amount of the dilactone detected by method 3 varied, and was sometimes as high as 3 times that of "X". The simplest interpretation of the data is that the sorghum extracts oxidized ferulic acid to the β-β-linked dilactone, which was then non-enzymatically converted to "X".

Values are also shown in Table 4 for total "X" obtained by method 2, in which any dilactone remaining upon acidification of the incubation mixture was converted to "X" prior to chromatography. In method 2, the total value tended to be smaller, unless the spots at the origin were sprayed twice with $NH_4OH$, with drying in between.

*Tentative identification of "X"*

It has been shown above that the alkaline hydrolysis product derived from the synthetic dilactone and the enzymatically derived "X" at pH 8 are identical chromatographically and spectrally in the UV region. "X" has been difficult to identify because of its instability in air under certain conditions. "X" was relatively stable under the incubation conditions of the enzyme assay, the 386 nm peak was stable in methanolic-KOH for at least 1 hr, and chromatography was reproducible at the acid pH values. On the other hand, the area on a dried chromatogram turns visibly yellow within a few days when the papers are stored in air, and only a brown amorphous powder with a wide mp range of 70–90° was obtained in attempts to recrystallize after a large scale preparation. But this synthetic material gave only a single UV visible spot. Unsuccessful attempts were made to obtain a TMSi or a methylated derivative.

Although "X" migrates as a single spot in most chromatograms, it may exist as a mixture of easily interconvertible forms in solution, such as shown in structural formulas (3) and (4). The following characteristics of "X" form the basis for this statement. "X" gives a stable blue color when a chromatogram was sprayed with a solution of 0.1% N-2,6-dichlorobenzoquinoneimine in EtOH followed by 0.5 M Tris buffer at pH 9. This blue color, which also formed in a test tube assay and was extractable into *n*-BuOH, may indicate the presence of a benzyl alcohol group as in (3) [9]. A pH titration curve with KOH, the lack of migration of "X" chromatographically in a *n*-BuOH–$NH_4OH$–$H_2O$, and the quantitative extractibility from a $CH_2Cl_2$ phase with 5% $NaHCO_3$, indicates that at least one free carboxyl was present in "X". Manometric measurements indicated no detectable loss of carboxyl groups as $CO_2$ when about 2 mg solid dilactone was dissolved in 0.1 N KOH and then acidified with $H_2SO_4$.

The IR spectra of synthetic "X" in a KBr pellet and in $CHCl_3$ were not identical. The KBr pellet spectrum might indicate the presence of a benzyl hydroxyl group, in support of the blue color test with the quinoneimine reagent. There was no sharp peak for the carboxyl hydroxyl, but instead there was a broad rise from 3400 to 2000 $cm^{-1}$, with a small peak at 2950 $cm^{-1}$. This is more typical of a hydroxyl group *beta* to a carboxyl as in 3-hydroxypropionic acid, than to free carboxyl groups as in ferulic acid [10]. The IR spectrum in $CHCl_3$, however, was more consistent with the presence of at least one free carboxylic acid, with carboxyl hydroxyl peaks at 2940 and 2900 $cm^{-1}$, similar to that for ferulic acid.

Neither of the IR spectra for "X" showed resolution of two carbonyl stretch peaks, one for the lactone at 1750 $cm^{-1}$ and the other for the carboxylic acid at 1690 $cm^{-1}$. Instead, the spectra contained only a single broad peak at 1720 $cm^{-1}$. But structure (3), or its dicarboxylic acid form, would not show the necessary conjugated double bond system necessary to account for the UV spectra. Dehydration to structure (4), however, could account for the peaks in neutral and alkaline methanol at 336 and 386 respectively, due to the double bond system in conjugation with a phenolic aromatic ring. At high pH in concentrated aqueous solutions, further changes involving quinones and polymerization might be expected.

## DISCUSSION

The product of ferulic dimerase activity in sorghum preparations was a β-β-coupled dimer. This conversion has been postulated to go through a racemoid β-β-coupling and a diquinonemethide intermediate in the $FeCl_3$ oxidation [11]. Only one major product was detected in the enzymatic system, although the presence of non-UV visible products would not have been detected. Such a single type of dimer differs from the varied coupled dimers found in laccase or peroxidase catalyzed peroxidations of phenols, such as the aldehyde and alcohol precursors to lignin [11,12]. Ferulic acid radicals can cross-couple with the coniferyl alcohol radicals to form

β-β-dilignols in cell free preparations [12,13]. "X" was also shown to be one of several visible products in the peroxidatic oxidation of ferulic acid catalyzed by the sorghum preparations described in this paper. Ferulic acid is not only a substrate for peroxidase acting peroxidatically [2,3,6], but also oxidatively in the presence of IAA [4]. It also catalyzes the consumption of $O_2$ by laccase, but not by polyphenol oxidase [5].

Ferulic dimerase activity, however, does not appear to be due to either a laccase or a peroxidase in sorghum preparations acting oxidatively with $O_2$ or peroxidatively with an endogenous source of $H_2O_2$. Besides producing only one major product, the intracellular distribution of the activity of this dimerase differed from that of both peroxidase and polyphenolase. The lack of cyanide inhibition would also rule out a peroxidase, unless it is a non-limiting component of a system coupled with a flavoprotein. The mechanism of the activation by light might involve a flavin moiety or be related to the presence of chloroplast fragments. The inhibition by DIECA indicated that the dimerase might be a copper enzyme, but a laccase activity was not demonstrated in sorghum tissue and polyphenoloxidase would be ruled out because of the above cited distribution data.

From an enzymatic and a physiological point of view, the dilactone may be of greater interest than its hydrolysis product "X". The latter compound may never accumulate *in vivo*, since the dilactone might become linked to other compounds directly from the surface of the enzyme. For instance, if both lactone groups were converted to carboxyls, ester linkages to a carbohydrate of the wall on the one side and to the lignin core on the other could be made. Thus, this dimer of β-β-coupled ferulic acid could be an important link in cell wall structures, and such ester linkages could be responsible for the solubility of grass lignins in 0.5 N NaOH [14].

## EXPERIMENTAL

*Plant material and preparation of extracts at 4°.* Seeds of *Sorghum bicolor*, variety wheatland milo were surface sterilized and grown for 1 week in a commercial bedding mixture (Grotron) at 30° in a 16-hr photo-period under VHO cool white fluorescent light of approximately 20000 lx. Green leaves (lamina plus sheath) were ground in a mortar in a vol. (100 mM) tricine buffer at pH 8, equal to 2× fr. wt of tissue (in g) in the presence of sand and hydrated Dowex I (equal to 20% fr. wt of shoots). Vascular mat preparations were made by grinding shoots for 15 sec in an Omnimixer in a vol. of 0.1 M Pi buffer, pH 6, equal to about 5× its fr. wt. The mixture of broken mesophyll cells was separated from the vascular mat plus bundle sheath cells in a basket centrifuge. The mat was washed thoroughly with pH 6 Pi buffer followed by a rinse with a pH 8 buffer. This washed mat was then ground as above in a mortar with sand and Dowex I in 100 mM tricine buffer at pH 8. A pellet sedimented between 500 and 37000 *g* ($P_1$), and three $(NH_4)_2SO_4$ fractions of the 37000 *g* supernatant were precipitated between 0–200 g/l. ($P_2$) and 200–500 g/l. ($P_3$). Excised first internodes incubated for 3 days [8] were ground in a similar fashion.

*Assay for ferulic dimerase activity.* The incubation mixture contained in a 1-ml vol. 100 mM tricine buffer, pH 8, or of MES buffer, pH 6, 0.1% bovine serum albumin (w/v), 4 μmol of ferulic acid plus Sorghum extract in a 1 ml vol. This mixture was stirred for 1–3 hr at 30° in approximately 10000 lx white light. The reaction was stopped with the addition of 50 μl 6 N HCl. After isolation and fluorometric analysis of the products as described below, activity per hr/g fr. wt of original tissue was based on μmol dilactone hydrolyzed to "X", using 0.5–10 μg samples of the dilactone as standards isolated chromatographically. Standard stock solns of 1 mg dilactone/ml were made in spectrally pure $Me_2CO$.

*Chromatographic separation of substrate and products.* While 3 methods were used to analyze the products, all involved PC of 0.1 ml aliquots of the acidified reaction mixture on Whatman No. 1 in a BeAW solvent mixture ($C_6H_2$–HOAc–$H_2O$, 40:15:1). Approximate $R_f$ values were: ferulic acid and the dilactone of ferulic acid 0.9; "X" 0.3; 4-hydroxycinnamic acid 0.62; 5-hydroxyferulic acid 0.26; caffeic acid 0.2. Fluorescing spots were identified under a UV lamp (360 nm), with and without fuming with $NH_3$.

*Method* 1. Only "X" was analyzed fluorometrically after elution of the spot in 2 ml MeOH, isolated by 1-D PC. Any residual dilactone in the ferulic acid area would not be included, but this was minimal at pH 8. Aliquots of MeOH extract were analyzed fluorometrically after 30 min in 0.05 N NaOH.

*Method* 2. Any residual dilactone in the acidified incubation mixture was converted to "X" by spraying the origin area with concentrated $NH_4OH$, 20–30% w/w, prior to 1-D PC, so that the "X" area included the original "X" plus that converted from the dilactone present in the incubation mixture. The single "X" area was analyzed fluorometrically 30 min after elution of the spot with 2.5 ml 5% $NaCHO_3$.

*Method* 3. The dilactone and "X" in the incubation mixture were separated chromatographically and analyzed separately. After chromatography of an enzyme mixture in the first dimension in $C_6H_6$–AcOH–$H_2O$ in which "X" migrates to an $R_f$ of 0.3, the ferulic-dilactone area at $R_f$ 0.9 was sprayed with 30% $NH_4OH$ (w/w) converting the dilactone to "X". Subsequently the dried paper was re-chromatographed in the same solvent in the second dimension. The blue fluorescing area due to ferulic acid was unaltered by the treatment, and moves again to the high $R_f$ area of 0.9. The "X" derived from the dilactone migrates to the 0.3 area. The two blue fluorescing areas due to the original "X" and that produced from the dilactone in the incubation medium by the alkaline spray were eluted in 2.5 ml 5% $NaHCO_3$ and were analyzed fluorometrically. Synthetic dilactone was added to separate chromatograms as a standard.

*Fluorometric analysis of "X".* The above eluted areas of "X" were analyzed fluorometrically 30 min after the addition of alkali, using Wratten filter 7–60 as the excitation and 47B + 2B as emission filters.

*Fluorometric analysis of ferulic acid.* See Ref. [9].

*Peroxidatic assay of the production of "X".* The incubation mixture contained in a 1-ml vol. 50 mM MES, pH 6, or tricine, pH 8, 4 μmol of ferulic acid, 0.06% $H_2O_2$ (w/v), 2 μg horseradish peroxidase (HRP). After an incubation period of 10 or 60 min at 30°, the reaction was stopped with HCl and analyzed fluorometrically after chromatography using method 1 of the dimerase assay. Activity was expressed as Δ *A*/min/g fr. wt.

*Peroxidase activity assay.* The *o*-dianisidine assay was used [15].

*Synthesis of the dilactone of ferulic acid.* The dilactone was synthesized from ferulic acid via oxidation by $FeCl_3$ in air, and recrystallized from glacial HOAc [7]. Yield was 18%, (reported 22%), mp 207.5–209.5 with decomposition (reported 208–209 with decomposition) [7]. The dilactone was insoluble in 5% $NaHCO_3$ and only slightly soluble in MeOH. It was soluble in EtOAc acetate or $Me_2CO$ at least up to 1 mg/ml. The spectral characteristics of the dilactone, mp, MS and IR data of the dilactone and its methyl ether are consistent with its identification as the dilactone of ferulic acid (**2**), with a MW of 386 and of 414 for its methyl ether.

*Synthesis of gram quantities of "X".* To a soln containing 1.5 g dilactone in 50 ml $Me_2CO$, 80 ml of 100 mM Tricine, pH 8, was introduced over a 1.5-hr period, in $N_2$. After acidification with HCl, using congo red paper, the soln was quantitatively extracted with $CH_2Cl_2$. After drying and filtering with charcoal, the soln was evaporated to dryness. The residue was

dissolved in minimum $Et_2O$ and precipitated with petrol (bp 60–90). After drying under vacuum, the amorphous residue was stored under vacuum to prevent oxidation. Yield was about 50%, with a mp of 70–90°.

*Synthesis of methyl derivatives.* The dimethyl ether, dimethyl ester of the dilactone of ferulic acid was synthesized with $CH_2N_2$. Yield from 1 g dilactone was 0.9 g, mp 207–208 (reported as 209 in Ref. [4]).

*IR spectral data.* (1) $\lambda_{max}^{KBr}$ dilactone of ferulic acid, 3450 ($\phi$-OH), 1765 (>C=O), 1600, 1520, 1222, 1145, 1025 $cm^{-1}$. (2) Methylether of the dilactone, $\lambda_{max}^{KBr}$ 2941 *w*, 1760 (>C=O), 1590, 1520, 1450, 1260, 1180, 1160, 1135, 1015, 990, 855, 815, 755, 712 $cm^{-1}$. (3) Hydrolysis product of the dilactone or synthetic "X", $\lambda_{max}^{KBr}$ 3400 vs, 2950 *w*, 1725 (>C=O), 1590, 1500, 1260, 1170, 1020, 850, 812, 760 $cm^{-1}$. (4) Hydrolysis product of the dilactone, $\lambda_{max}^{CHCl}$, 3450, 2940, 2900, 1740, 1635, 1600, 1515, 1460, 1430, 1380, 1260, 1180, 1120, 1035, 877, 847 $cm^{-1}$.

*MS data.* Methyl ether of the dilactone. MS: $M^+$ 414. *m/e* (relative abundance) 414 (84), 220 (9), 204 (24), 196 (17), 167 (9), 166 (53), 165 (100), 151 (38), 138 (9).

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