

## A CONCISE REVIEW OF THE NATURAL EXISTANCE, SYNTHESIS, PROPERTIES, AND APPLICATIONS OF SYRINGALDEHYDE

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Syringaldehyde is a promising aromatic aldehyde that no longer deserves to remain in obscurity. It possesses worthy bioactive properties and is, therefore, used in pharmaceuticals, food, cosmetics, textiles, pulp and paper industries, and even in biological control applications. Mostly, the synthetic form of syringaldehyde is being used. This review serves as an appraisal of potential research and commercialization of naturally occurring syringaldehyde beyond the scope of the food and cosmetic industries. This article also provides a comprehensive account of the various conventional extraction and chromatographic techniques used in the separation, isolation, and quantification of syringaldehyde. Further, to understand this unique compound, a brief outline on the natural formation of syringaldehyde in lignin is accentuated in this article.

*Keywords:* Syringaldehyde; Bioactive properties; Separation and extraction; Lignin

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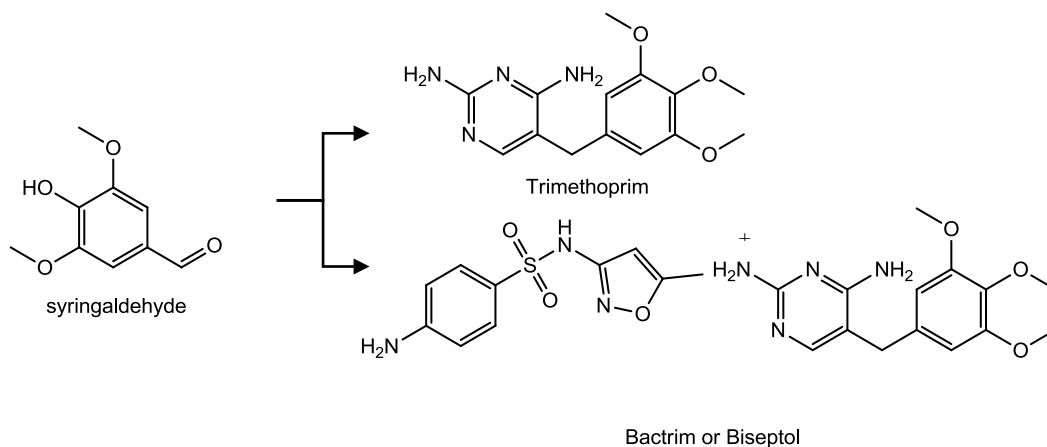
### INTRODUCTION

The ever-increasing safety concerns over synthetic antioxidants and the harmful side effects of chemo-therapeutic drugs, coupled with their high costs (Goodwin and Shepherd 1998; Sacristán *et al.* 2000; Vergnenegre 2001), have created a new path for the development of cheaper, sustainable, and most crucially, natural anti-oxidants, drugs, and food additives (Garrote *et al.* 2004). Syringaldehyde, a compound found only in a minute quantity in nature, is believed to be a promising source that matches the above-mentioned requisites.

Syringaldehyde, or 3,5-dimethoxy-4-hydroxybenzaldehyde, is a naturally occurring unique compound with assorted bioactive characteristics that belongs to the phenolic aldehyde family. Some of the physico-chemical properties of syringaldehyde are given in Table 1, which is based on data from the Material Safety Datasheet provided by Sigma Aldrich. Syringaldehyde is very similar in structure to its infamous counterpart, vanillin (Fig. 1(b)), and it has comparable applications (Bortolomeazzi *et al.* 2007). Though not as well-commercialized as vanillin, syringaldehyde chemistry and its manipulation are emerging rather rapidly, especially after the discovery of its role as an essential intermediate of the antibacterial drugs Trimethoprim, Bactrim, and Biseptol (Rouche 1978). Bactrim or Biseptol are combinations of Trimethoprim with sulfamethoxazole.

Their structures are as shown in Fig. 1. These drugs are common bactericides (Erofeev *et al.* 1990).

Over the years, however, a meager number of articles dedicated to syringaldehyde have been published. This can probably be attributed to its simple synthesizability and the lack of understanding of its true capacities. Accordingly, there arose a motivation to write this review paper.



**Fig. 1.** The structures of syringaldehyde, as well as Trimethoprim and Bactrim or Biseptol

## NATURAL EXISTENCE OF SYRINGALDEHYDE

An excellent natural source of syringaldehyde lies within the cell walls of plants. Being the second most copious biopolymer only to cellulose, lignin offers a continuous, renewable, and cheap supply of syringaldehyde. This is promising, since lignin is discarded as waste by the pulping industry (Wallberg *et al.* 2006) and is also a major by-product from the biomass-to-ethanol conversion process (Xiang and Lee 2001). Despite the fact that the fate of lignin ends at a bio-fuel refinery (Kleinert and Barth 2008), its hidden wealth can be extracted prior to its conversion into biomass feedstock. Although this practice is not common for the recovery of syringaldehyde, it is slowly emerging, since value-added products from wastes offer a promising future.

Years of tedious research have led to the current development and understanding of the synthesis of the syringyl unit in plants. Lignin being an amorphous heteropolymer, the elucidation of its biosynthetic pathway is not an easy task. In order to appreciate the complexity and diversity of nature and her unique attributes, it is vital to know how the syringyl unit comes into existence in lignin. Moreover, the bio-origin of this compound has not been adequately reviewed. Protolignin (naturally occurring lignin) varies in molecular make-up from plant to plant and even from cell to cell (Christiernin *et al.* 2005). Research demonstrated that *Arabidopsis* mutants were no longer upright since they lacked lignified interfascicular fibers, providing evidence that macro-metabolite lignin is responsible for the structural integrity of plants (Zhong *et al.* 1997). Lignin also provides plants with a vascular system for the conveyance of water and solutes (Hacke and Sperry 2001).

The biosynthetic pathway of protolignin comes primarily from the breakthrough discovery and characterization of the enzymes that lead to monolignols syntheses of *p*-coumaryl, coniferyl, and sinapyl alcohols, whereby they form the hydroxyphenyl (H), guaiacyl (G), and syringyl (S) units in lignin, respectively. These units vary structurally due to different degrees of methoxy substituents (Christiernin *et al.* 2005). The xylem vessels in plants are known to provide both mechanical support and water conduction. These vessels are mainly composed of G-lignin and do not contain S-lignin since the enzymatic genes that encode for sinapyl alcohol are lacking in gymnosperms (Boerjan *et al.* 2003).

Because G-lignin is lacking in angiosperms, additional specialized cells referred to as fiber cells provide much needed mechanical support (Fergus and Goring 1970). Fascinatingly, in angiosperms, these fiber cells are mainly composed of S-lignin. The genes involved in S-lignin synthesis developed much later than G-lignin, rendering evidence of evolution from softwood plants (gymnosperms) to hardwood plants (angiosperms) (Li *et al.* 2001). Additionally, a list of various plants commonly used as wood sources and crops with their lignin content is represented in Table 1. These S-lignins are the source from which syringaldehyde can be obtained when lignocellulosic materials undergo certain oxidation reactions.

In addition to furnishing mechanical support and vascular elements, lignin is also indirectly involved in a plant's defense mechanism (Menden *et al.* 2007). In conformity with this finding, when the biotrophic pathogen *Puccinia graminis* attacked wheat cells, this attack was retaliated by a sequence of hypersensitive responses, which triggered an augmentation of lignin content. Spectroscopic and biochemical methods of analysis concurred that the syringyl unit was entirely responsible for the accretion of lignin mass in the fungus-penetrated wheat plant cells. This finding supports a notion that syringaldehyde has reasonable bioactive characteristics.

In another instance, Creighton *et al.* (1994) reported that different types of gymnosperms only yield vanillin, while angiosperms yield both vanillin and syringaldehyde. The same study reported that yields of vanillin in gymnosperms vary between 15% and 24% based on the Klason lignin content. The combined yield of vanillin and syringaldehyde in angiosperms is between 35% and 51% with a ratio of 1:3 (vanillin to syringaldehyde).

## EXTRACTION AND ISOLATION OF SYRINGALDEHYDE

The available percentage of precursors in the lignin structure strictly determines the formation of phenolic compounds such as vanillin or syringaldehyde. It becomes more useful in producing phenolic aldehydes when the lignin is subjected to fewer transformations or chemical treatments. Tarabanko *et al.* (1995) presented a study using lignin oxidation, in which the influence of lignin origin, condition of production, and type of pre-treatment on obtained yields of vanillin and syringaldehyde was inspected (Rodrigues Pinto *et al.* 2010). The results indicated a competition between lignin fragments (syringyl fragments and guaiacyl fragments) condensation and lignin oxidation into aldehydes. Villar *et al.* (2001) obtained a maximum yield of 14% for the total

phenolic aldehydes (syringaldehyde + vanillin), based on nitrobenzene oxidation using lignin precipitated from kraft black liquor with the addition of a calcium salt dissolved in water soluble alcohol. In another study, a yield of about 50 to 59.7% syringaldehyde and vanillin in equal proportions of the total phenolic aldehydes was obtained via nitrobenzene oxidation from the lignin extracted from rice straw (Sun *et al.* 2000).

**Table 1.** Lignin Content in Various Plants

Plant Group	Plant Scientific/ Common Name	Lignin Content (%)	Reference
<b>Gymnosperm</b>	<i>Picea abies</i> , Norway spruce	28.0	(Önnerud, Gellerstedt 2003)
	<i>Picea abies</i> , Norway spruce (compression wood)	39.0	(Önnerud 2003)
	<i>Pinus radiata</i> , Monterey pine	27.0	(Sjostrom 1993)
	<i>Pinus sylvestris</i> , Scots pine	28.0	(Sjostrom 1993)
	<i>Pseudotsuga menziesii</i> , Douglas fir	29.0	(Sjostrom 1993)
	<i>Tsuga canadensis</i> , Eastern hemlock	31.0	(Sjostrom 1993)
<b>Angiosperm</b>	<u>Eudicotyledons</u>		
	<i>Acacia mollissima</i> , Black wattle	21.0	(Sjostrom 1993)
	<i>Betula verrucosa</i> , Silver birch	19.0	(Seppänen <i>et al.</i> 2007)
	<i>Eucalyptus globulus</i> , Blue gum eucalyptus	22.0	(Sjostrom 1993)
	<i>Eucalyptus grandis</i> , Rose gum eucalyptus	25.77	(Emmel <i>et al.</i> 2003)
	<i>Corchorus capsularis</i> , Jute	13.0	(Rio <i>et al.</i> 2006)
	<i>Hibiscus cannabinus</i> , Kenaf	12.0	(Rio <i>et al.</i> 2006)
	<i>Linum usitatissimum</i> , Flax-shives	34.2	(Buranov and Mazza 2007)
	<i>Linum usitatissimum</i> , Flax-fibre	5.0	(Sain and Fortier 2002)
	<i>Vitis vinifera</i> , Grape – Stalks	39.6	(Ping <i>et al.</i> 2011)
	<u>Monocotyledons</u>		
	<i>Oryza sativa</i> , Rice straw	19.0	(Deng <i>et al.</i> 2007)
	<i>Saccharum</i> species, Bagasse	25.3	(Hoareau <i>et al.</i> 2004)
	<i>Triticum aestivum</i> , Wheat straw	17.0	(Xu <i>et al.</i> 2006)
	<i>Zea mays</i> , Corn – stover	21.2	(Öhgren <i>et al.</i> 2007; Thammasouk <i>et al.</i> 1997)
	<i>Elaeis guineensis</i> , Oil palm – empty fruit bunches	14.2	(Sun <i>et al.</i> 1999)
	<i>Elaeis guineensis</i> , Oil palm trunk	34.4	(Sun <i>et al.</i> 1998)
	<i>Musaceae</i> family, Banana	9.0	(Jústiz-Smith <i>et al.</i> 2008)
	<i>Cocos nucifera</i> , Coconut coir	59.4	(Jústiz-Smith <i>et al.</i> 2008)

Syringaldehyde has been reported to be separated and analyzed via a recrystallization process. A study carried out by Creighton and Hibbert (1944) utilized the recrystallization process on the oxidation products of corn stems on one of the fractions using water and obtained syringaldehyde with a reported melting point of 110 to 112 °C. It was also reported that the oxidation of corn stems produced 3.2% crude yields and 2.6% pure syringaldehyde product. In a study of syringaldehyde composition in angiosperm monocotyledons and dicotyledons (Creighton *et al.* 1941b), the recrystallization process was used in purifying the syringaldehyde sublimate. This study reported a yield of total phenolic aldehydes (vanillin and syringaldehyde) in monocotyledons between 21 to 30%, and dicotyledons between 39 and 48%. The precipitation and recrystallization of syringaldehyde from maple wood was also reported (Creighton *et al.* 1941a). In this procedure, syringaldehyde was isolated from the sodium bisulfite fraction and recrystallized to get a pure compound with a melting point of 110.5 to 112 °C. Based on the Klason lignin content of maple wood, the yield of syringaldehyde isolated was 31.8%.

## INSTRUMENTAL ANALYSIS IN THE SEPARATION OF SYRINGALDEHYDE

### Capillary Electrophoresis

Capillary electrophoresis can be used for the separation and analysis of syringaldehyde. Priego-Capote *et al.* (2004) utilized capillary electrophoresis in a study to extract 20 phenolic compounds from alperujo (a semisolid waste from the olive oil industry), which is considered to have a complex matrix. Syringaldehyde was among the phenolic compounds separated. The study consisted of an ultrasound-assisted extraction carried out on the alperujo sample before running a capillary electrophoresis-diode array detection system to separate and determine the compounds. The detection was carried out at a wavelength of 350 nm. It was reported that the syringaldehyde concentration obtained from the method was  $365 \pm 25$  µg/g, with a limit of detection (LOD) of 3.27 µg/g and limit of quantification (LOQ) of 10.80 µg/g.

In a study on brandy and wine (Panossian *et al.* 2001), high-performance capillary electrophoresis was used to analyze vanillin, syringaldehyde, coniferaldehyde, and sinapaldehyde. Borate buffer at pH 9.3 was used to evaluate the electrophoretic mobility of the target compounds. It was also observed that the UV detection sensitivity of the phenolic aldehydes increased at pH 9.3. Syringaldehyde was analyzed and monitored directly at 362 nm from the samples without concentration, extraction, or any other preliminary treatment. The limit of detection for syringaldehyde was found to be 0.275 ppm.

### High Performance Liquid Chromatography (HPLC)

High-performance liquid chromatography (HPLC) is the most versatile technique used for the separation of syringaldehyde. A study conducted by Hartley on graminaceous cell walls demonstrated the basics of using HPLC in the separation of syringaldehyde. Separation was carried out with PRP-1 resin, and the detection was achieved by UV absorption at 320 nm. An HPLC study on alkaline pre-treatment of

wheat straw was carried out by (Sun *et al.* 1996) using a linear gradient of two solvent systems and UV detection at 280 and 320 nm. It was reported that 2.22% of syringaldehyde was separated from the wheat straw and 1.89% from the pre-treated wheat straw.

The HPLC method developed by Sun was later used by Lawther *et al.* (1995) in the study of free phenolic monomers, loosely bound phenolic acids and aldehydes, and tightly bound phenolics in wheat straw lignin. It was reported that syringaldehyde was a major component in the determination of free phenolic and tightly bound phenolic monomers, but only traces of syringaldehyde were detected as loosely bound phenolic monomers.

A study by (Galletti *et al.* 1989) on phenolics obtained from alkaline nitrobenzene hydrolysis of wheat straw used HPLC with electrochemical and UV detectors. Hydrolysates were injected both by a direct procedure and after a classical analytical procedure involving solvent partitioning. The electrochemical detector was not affected by nitrobenzene interference and allowed the direct injection of nitrobenzene hydrolysates into HPLC. Direct analysis of syringaldehyde separation was reported (as a percentage of the original material) at  $1.466 \pm 0.1016\%$  with electrochemical detection, while with UV detection at  $1.932 \pm 0.0963\%$ . Classical analysis of the separation was reported at  $1.268 \pm 0.0479\%$  with electrochemical detection, while with UV it was reported at  $1.373 \pm 0.0649\%$ . It was argued in the study that the UV provided better results even in the classical method of sample preparation because of the effects of the co-elution of incompletely removed nitrobenzene. It was reported that electrochemical detection is selective to phenolics since they are electrochemically active compounds (Chiavari *et al.* 1987).

### Gas Chromatography-Mass Spectrometry (GC-MS)

Gas chromatography (GC) and GC with mass spectrometry (GC-MS) have been used for the separation for syringaldehyde. Syringaldehyde was one of the main phenolic monomers reported in a study carried out by Klinke *et al.* (2002) using an alkaline wet air oxidation pre-treatment (water, sodium carbonate, oxygen, high temperature, and pressure) on wheat straw. The GC retention time (RT) for syringaldehyde was 17.16 min, and its major fragments in the MS occurred at  $m/z$  111, 181, and 182. Revel *et al.* (2008) identified syringaldehyde by using GC-MS analysis in a study on malolactic fermentation (MLF) by lactic acid bacteria (LAB) which has a significant influence on the stability and organoleptic quality of wine. In that study, syringaldehyde was separated and reported in all of the fractions of MeOH/H<sub>2</sub>O and was considered as other sources of aroma.

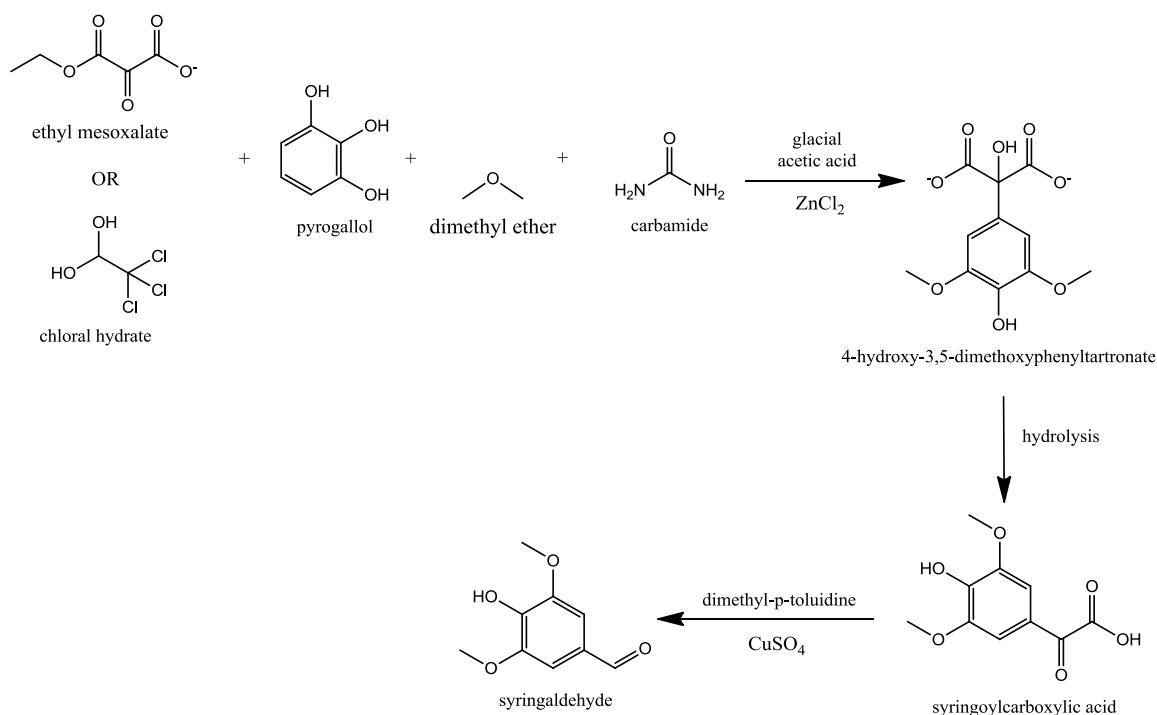
A study on volatile compounds in Changyu XO brandy was carried out by Zhao *et al.* (2008) with GC-MS. Syringaldehyde was identified as one of the compounds in the neutral/basic fraction eluted with *n*-pentane/diethyl ether (90/10, v/v). The relative content of syringaldehyde in the fraction was 0.11%. Viriot *et al.* (1993) studied the solubility and evolution of high molecular weight polyphenols, ellagitannins, and lignins by selective degradation methods and by gel-permeation chromatography in cognacs and brandies of different ages. GC was used to analyze low molecular weight phenolic compounds that contain syringaldehyde as one of their components. It was reported that

syringaldehyde together with other low molecular weight phenols account for only a minor proportion of the total phenols.

Wines treated with oak chips were analyzed for its syringaldehyde content and other components in a study conducted by Arapitsas *et al.* (2004). The content of syringaldehyde was measured by gas chromatography using a cross-linked, fused silica capillary column of 100% methyl siloxane (30 m length, 0.25  $\mu\text{m}$  film thickness, 0.32 mm i.d.). Nitrogen was used as a carrier gas fixed with a flow rate of 2.84  $\mu\text{L}/\text{min}$ , and the injection temperature was set at 220  $^{\circ}\text{C}$ . Detection was carried out using flame ionization maintained at 280  $^{\circ}\text{C}$ . The concentration of syringaldehyde obtained from different wine varieties treated for about 14 days lies within the range of 0.0116 to 0.488 mg/L.

## SYNTHESIS OF SYRINGALDEHYDE

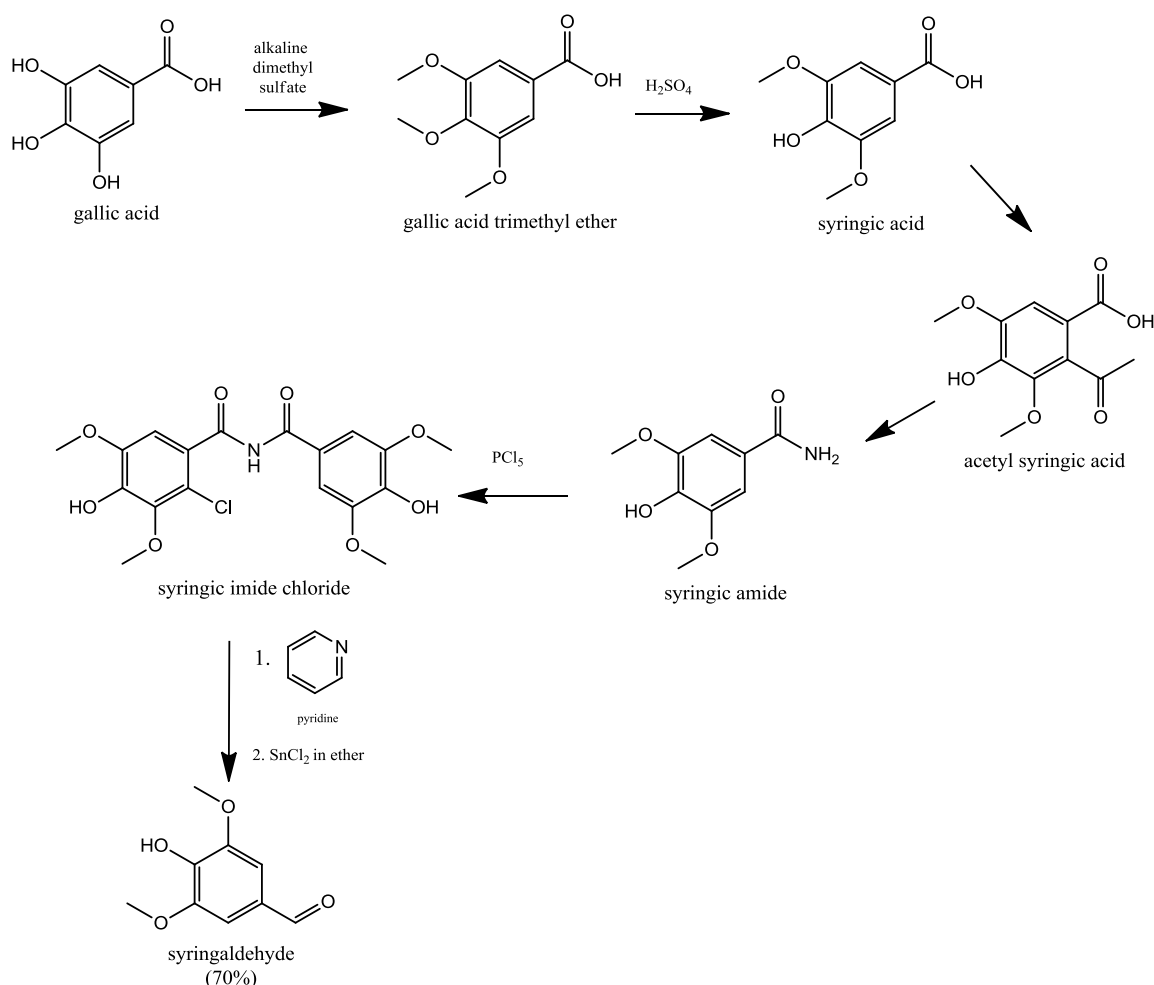
The earliest method of synthesizing syringaldehyde was based on the condensation of 2,6-dimethoxyphenol with chloroform in an alkaline solution (Graebe and Martz 1903). However, this procedure gave a poor yield of syringaldehyde. In another synthesis, chloroform was replaced with ethyl mesoxalate, and it was added to a mixture of anhydrous zinc chloride, one part pyragallol, and three parts dimethyl ether with a little carbamide and then aged in glacial acetic acid for a fortnight (Mauthner



**Fig. 2.** The synthesis of syringaldehyde using ethyl mesoxalate or chloral hydrate as proposed by Mauthner and Pauly, respectively

1913). The ester, ethyl-4-hydroxy-3,5-dimethoxyphenyltartronate was obtained and was hydrolyzed by boiling with aqueous potassium hydroxide and subsequently acidified below 10°C. This process yields syringoylcarboxylic acid, which was then treated with copper(II) sulphate at its boiling point. The product was later treated with boiling dimethyl-*p*-toluidine to yield syringaldehyde. The synthesis was improved by substituting ethyl mesoxalate with chloral hydrate (Pauly and Strassberger 1929), and their reaction is outlined in Fig. 2. However, these techniques have major drawbacks, since raw materials like 2,6-dimethoxyphenol cannot be easily prepared using ordinary lab equipment, and the process is uneconomical due to a poor yield.

A revolutionary way of synthesizing syringaldehyde was proposed by McCord (1931) using gallic acid as the starting reagent. In this process, the methylation of gallic acid was done by treating it with dimethyl sulfate in an alkaline solution, which produces trimethyl ether. Upon reaction with sulfuric acid, the ether was converted into syringic acid, and upon methylation, acetyl syringic acid was obtained. This product was converted first into acid chloride and then into its acid amide. The acid amide was then

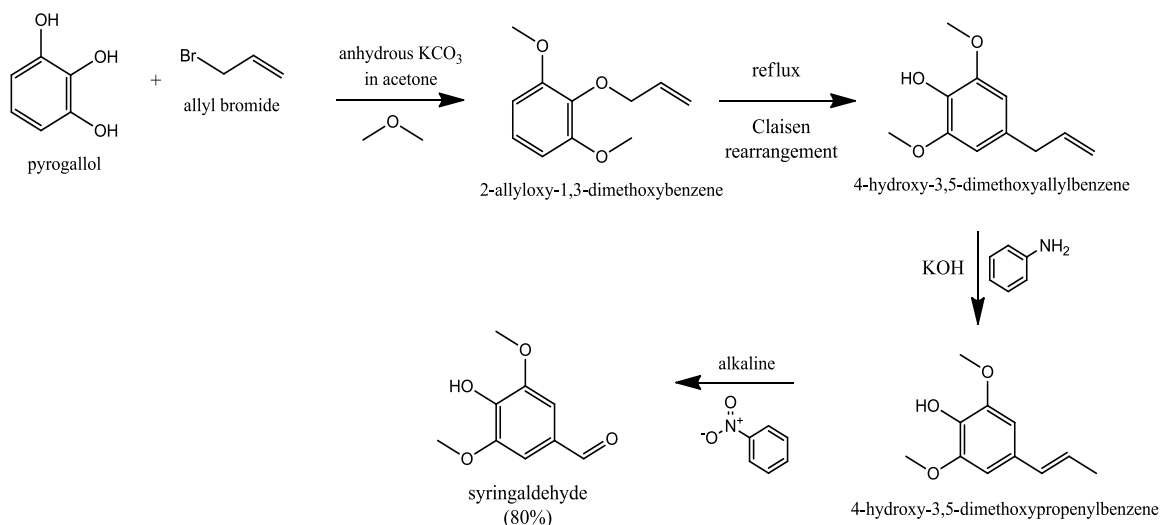


**Fig. 3.** The synthesis outline of syringaldehyde using gallic acid as the starting material



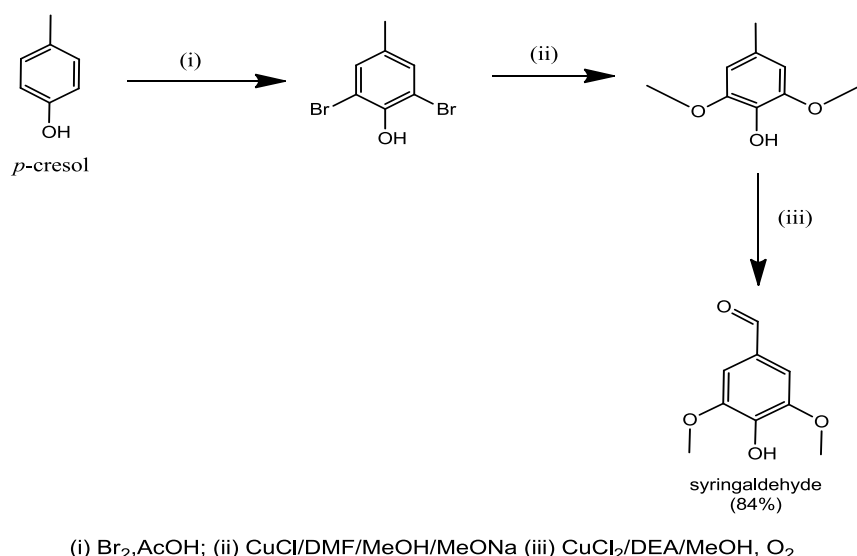
added to phosphorous pentachloride to form imide chloride. The imide chloride was converted into its corresponding nitrile of the acetyl syringic acid by warming it in pyridine solution. This was then reduced with stannous chloride in ether solution, yielding up to 70% of syringaldehyde. The production of syringaldehyde via the McCord method is shown in Fig. 3.

Later initiatives in synthesizing syringaldehyde include simplified methods, one of which includes pyragallol as the starting material (Pearl 1948); this approach is outlined in Fig. 4. According to the study, pyragallol and dimethyl ether were reacted in a 1 to 3 ratio with allyl bromide in anhydrous acetone in the presence of anhydrous potassium carbonate, forming 2-allyloxy-1,3-dimethoxy benzene. When refluxed, this product undergoes a Claisen rearrangement to form 4-hydroxy-3,5-dimethoxyallyl benzene. The 4-hydroxy-3,5-dimethoxyallyl benzene was then boiled with potassium hydroxide in aniline. Upon boiling, it isomerized into 4-hydroxy-3,5-dimethoxypropenyl benzene. The propenyl derivative was later reacted with nitrobenzene and alkali to produce syringaldehyde with a good yield of 80%.



**Fig. 4.** A simplified method for producing syringaldehyde from pyragallol

Over the years, syringaldehyde has been manufactured from vanillin (Pepper and McDonald 1953; Rao and Stuber 1983). However, the method adopted can be conceived as economically unviable for the production of syringaldehyde. Interestingly, a current study has successfully circumvented this problem by considering 4-methyl phenol as a starting material (Ji *et al.* 2002; Tripathi *et al.* 2010). This starting material is a by-product of the petroleum industry, which can be obtained easily as well as cheaply. This novel approach was reported by Tripathi *et al.* and consists of an elaborate three-step synthesis sequence. In the methoxylation process, attempts were made to avoid the use of dimethylformamide by substituting it with other easily recoverable solvents such as methanol. Up to 84% yield of syringaldehyde was reported. The reaction scheme as outlined in literature by Tripathi *et al.* is shown in Fig. 5.



**Fig. 5.** The synthesis of syringaldehyde as proposed by Tripathi *et al.* (2010)

## PROPERTIES OF SYRINGALDEHYDE

### Bioactive Properties

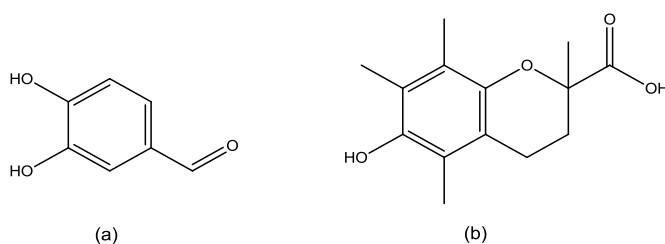
Advancements in analytical instruments coupled with breakthroughs in chemistry and pharmacology have allowed for the identification, quantification, and isolation of phenolic aldehydes for the diverse applications such as antioxidants, antifungal or antimicrobial, and anti-tumorigenesis agents in pharmaceuticals. In the food industry there is also a tendency to utilize naturally occurring flavor compounds that exhibit antioxidant and antimicrobial properties, hence providing a potential source of non-synthetic preservatives and additives. Only preliminary in vitro tests have been reported in most cases, but a new potential research area and application of syringaldehyde has been identified. Keeping this in mind, some of the reported bioactive properties of syringaldehyde are exemplified here.

#### *Antioxidant capacity*

Wines that have been aged in chestnut and oak barrels have long been recognized for elevated levels of antioxidants (Canas *et al.* 2008), primarily contributed by phenolic compounds originating from the wood itself. Syringaldehyde was detected in wines aged in toasted wooden barrels (Matejíček *et al.* 2005). Phenolic extracts from wild rice hulls (Asamarai *et al.* 1996) and phenolic compounds released from hydrothermal treatments of olive tree pruning with significant antioxidant capacity were also detected. Bortolomeazzi *et al.* (2007) determined the antioxidant capacity of syringaldehyde using the Trolox C method and the oxidation potential method using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The results showed that syringaldehyde did indeed have a good antioxidant property.

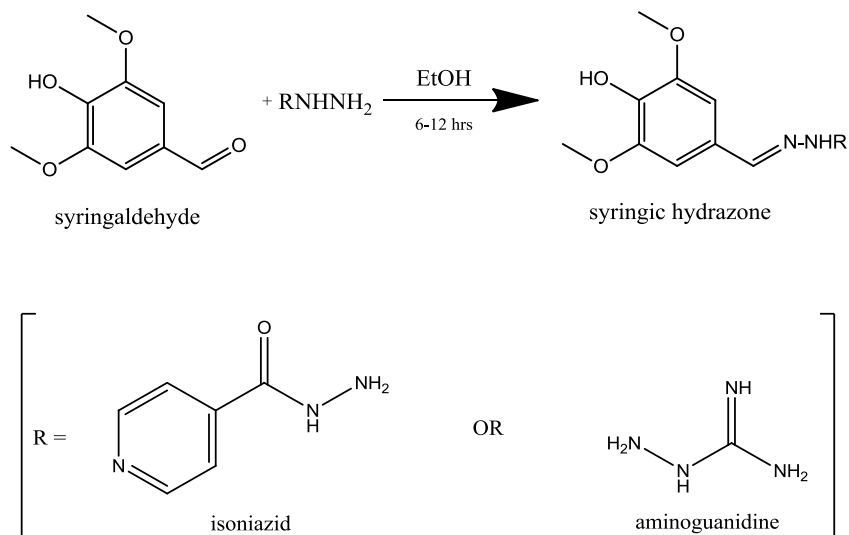
A study related to the structural motifs of syringaldehyde and other benzaldehydes for their antioxidant capabilities was approached by (Boundagidou *et al.* 2010). In

that study the presence of syringaldehyde in low quantities exhibited impressive results in peroxy scavenging activity, based on the CB assay. Its antioxidant activity was recorded to be six times higher than that of protocatechuic aldehyde. The higher the Trolox equivalent value (TEV), the more antioxidant property a molecule will have. This value decreased in the order from syringaldehyde > protocatechuic aldehyde > vanillin. This method measures the ability of molecules with antioxidant properties to suppress ABTS, which is a blue-green chromophore exhibiting characteristic absorption at 734 nm. The suppression ability of the molecule is compared with that of Trolox, a vitamin-E analog. Figure 6 shows the structures of (a) protocatechuic aldehyde and (b) Trolox. According to their study, the dimethoxy substitution in syringaldehyde as well as its syringol moiety was acknowledged for exhibiting enhanced antioxidant properties (Boundagidou *et al.* 2010).



**Fig. 6.** The chemical structures of (a) protocatechuic aldehyde (b) Trolox

Flavonoids function as chemo-preventive agents, but they tend to show pro-oxidant effects under the influence of transition metals. This is especially true for flavonoids with pyrogallyl and catecholic structures. Such tendency can cause reversed effects like mutagenicity and carcinogenicity on healthy cells (Cao *et al.* 1997). Thus, it was claimed that syringaldehyde-based dendrimers exerted strong antioxidant characteristics but reduced pro-oxidant effects. This characteristic of syringaldehyde can circumvent the possible threats of some flavonoids that are pro-oxidant (Lee *et al.* 2009).



**Fig. 7.** The production of syringic hydrazone from syringaldehyde as outlined by Belkheiri *et al.* (2010)

Likewise, neither syringaldehyde, aminoguanidine, nor isoniazid were capable of preventing cell-mediated LDL (low density lipoprotein) oxidation effectively when interacting individually, but syringic isoniazid hydrazone and syringic aminoguanidine hydrazone (syringaldehyde based hydrazones) were very capable (Belkheiri *et al.* 2010). Figure 7 shows the production of syringaldehyde-based hydrazones.

#### *Antimicrobial/antifungal activity*

The role of syringaldehyde as an antifungal agent against the medicinally important yeast *Candida guilliermondii* seems to be promising (Gurpilhares *et al.* 2006; Kelly *et al.* 2008). It was reported that syringaldehyde successfully inhibited the *C. guilliermondii* growth rate and reduced xylitol production effectively. The fungicidal effect is most likely due to the aldehyde moiety. The hydroxyl substituent in syringaldehyde is suspected to play a key role in enhancing this fungicidal effect.

Syringaldehyde extracted from the roots of a Rutaceae, *Vepris uguenensis* was tested against a protozoan parasite *Plasmodium falciparum* (Cheplogoi *et al.* 2008). This parasite causes malaria and is the most lethal in humans. In this study, syringaldehyde was tested against 3D7 (chloroquine susceptible, CQS) and FCM29 (chloroquine resistant, CQR) strains of *Plasmodium falciparum* and showed a mild anti-malarial activity with an  $IC_{50}$  value of  $21.4 \pm 8.2 \mu\text{g mL}^{-1}$ .

In a search for new high quality value-added bioactive papers, Fillat *et al.* (2012) studied the effects of non-leachable low molecular weight phenols with laccase on unbleached flax fibers in producing bio-modified pulp and paper. The researchers successfully added anti-bacterial characteristic value to pulp and paper generated from flax fibers by testing monophenols such as syringaldehyde and acetosyringone (a derivative of syringaldehyde) in reducing the population of *Staphylococcus aureus* (Gram +), *Klebsiella pneumonia* (Gram -), and *Pseudomonas aeruginosa* (Gram -), which are known widely to cause diseases in humans.

The antimicrobial activity tests on the paper and pulp were carried out in adherence to ASTM Standard Test Method E2149-01. The population of *Klebsiella pneumonia* was reduced to 61% by syringaldehyde, whereas acetosyringone gave a major reduction up to 99%. In the case of *Staphylococcus aureus*, its reduction in population by syringaldehyde was 55%, which was 15% higher than acetosyringone. Another bacterium, *Pseudomonas aeruginosa*, was reduced by 71% using syringaldehyde and to a staggering 97% level by acetosyringone.

#### *Anti-oncogenic property*

A tobacco-specific nitrosamine ketone known as NNK or 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone is a potent pro-carcinogen when exposed to humans (El-Bayoumy *et al.* 2006). Being a pro-carcinogen, it requires metabolic activation to exert its oncogenic effects. NNK metabolites cause pulmonary carcinogenicity in humans and rats, leading to lung oncogenesis. Two of the major metabolites formed by NNK murine microsomes are 4-oxo-4-(3-pyridyl)butyric acid (OPBA) and 4-(methylnitrosamino)-1-(3-pyridyl-N-oxide) or NNAL (Schrader *et al.* 1999).

Research done by Morse *et al.* (1995) has illuminated the inhibitory action on NNK metabolism by benzaldehydes, which includes syringaldehyde. The murine hepatic and pulmonary microsomes were prepared by adding the desired volume of 0 to 500  $\mu\text{M}$

inhibitor into 2.5  $\mu$ L of methanol and diluting to a volume of 225  $\mu$ L. These microsomal samples were incubated, centrifuged, and characterized according to procedures outlined in the study. Syringaldehyde was noted to show potency in inhibiting the formation of OPBA and NNAL in hepatic microsomes.

In another study, 51 phenolic compounds were isolated from maple syrup extract and were tested for anti-proliferation of colon cancer cells (González-Sarriás *et al.* 2012). According to this research, all of the compounds demonstrated anticancer activity especially against HCT-116 cell lines. However, syringaldehyde along with seven other compounds showed a higher potential in inhibiting the proliferation of these cancer cells. Since the data was obtained collectively involving a combination of these phenolic compounds, the researchers believe that the bioactivity observed could be initiated through synergy between these compounds.

## Other Properties

### Mediator

Syringaldehyde was one of the first natural laccase mediators discovered (Kawai *et al.* 1989). It has been reported to be used as a mediator in the degradation of indigo carmine by bacterial laccase (benzenediol oxygen oxidoreducase) obtained from the organism  *$\gamma$ -Proteobacterium JB* (Singh *et al.* 2007). The study ascertained that syringaldehyde was able to increase the degradation of indigo carmine by 57%. The enhanced degradation was made possible by the electron-donating methyl and methoxy substituents.

Notable decolorization rate enhancement of reactive dyes (Reactive Red 120 and Reactive Blue 171) by white radish peroxidase occurs with the use of syringaldehyde as a redox mediator (Satar and Husain 2009). In the treatment of simulated reactive dye effluent using laccase, the redox mediator system is necessary for effluent decolorization. A study was conducted by comparing a common synthetic mediator 1-hydroxybenzotriazole (HBT) with syringaldehyde (natural redox mediator) (Murugesan *et al.* 2009), and it was observed that syringaldehyde was more effective than the synthetic mediator with an initial rate of effluent decolorization 5.6 times higher than HBT. In the presence of mixed metal ions in the effluent, syringaldehyde showed significant color removal compared to HBT, which did not effectively remove the effluent color. Camarero *et al.* (2005) reported that phenolic aldehydes were among the best mediators for decolorizing recalcitrant dyes. Their work has shown that laccase activity increases when used with syringaldehyde, compared to other mediators.

Syringaldehyde is also used as a mediator in laccase-assisted biobleaching processes. In these processes, synthetic mediators such as HBT, violuric acid, and promazine were used. Another research focused on potentially cost-effective lignin-derived natural mediators, including syringaldehyde obtained from spent pulping liquors and plant materials used in the paper pulp laccase-mediator delignification process in combination with peroxide bleaching (Camarero *et al.* 2007). Syringaldehyde exhibited significant results in terms of pulp brightness and kappa number, but the results were not as good as those achieved with HBT. This is the first study that provides evidence of natural phenols being laccase mediators acting on paper pulp lignin.

A study carried out by Moldes *et al.* (2008) compared synthetic mediators with natural phenolic compounds such as syringaldehyde and vanillin in laccase-assisted biobleaching of eucalyptus kraft pulp. That report suggests that the synthetic mediators were the most effective mediators, but the laccase-syringaldehyde system also improved final pulp properties (28% delignification and 63.5% ISO brightness) compared to the process without mediator (23% and 61.5%, respectively). Additionally, there was an insignificant denaturation effect over laccase. An advantage of using syringaldehyde as a mediator compared to its synthetic counterpart is the low extent of laccase inactivation, which allows the enzyme to be reutilized. It also permits longer and safer processing of the material, including biocatalyst treatments. A laccase-mediator system employing natural phenolic compounds, such as syringaldehyde, was also reported being used in the transformation of fungicides like cyprodinil (Kang *et al.* 2002). Inorganic catalysts such as birnessite have been studied in combination with natural phenols (including syringaldehyde) in the transformation of the fungicide cyprodinil (Kang *et al.* 2004). Using birnessite alone, only 0.6% conversion of cyprodinil was possible. However, when birnessite was reacted with 1 mM of syringaldehyde, a conversion of about 31.8% was achieved. This shows that syringaldehyde is able to act as a mediator with both oxidoreductive enzymes and inorganic catalysts.

#### *Inhibitor of enzymatic hydrolysis*

It has been reported that phenolic compounds (such as syringaldehyde) can affect the ethanol fermentations of glucose and xylose by microorganisms (Delgenes *et al.* 1996). The inhibitory effect of vanillin and syringaldehyde during xylose fermentation by recombinant *Escherichia coli* LY01 was studied by Zaldivar *et al.* (1999). The inhibition of the growth of organisms was observed for both compounds, but the study found that vanillin exhibited a more intense inhibitory effect compared to syringaldehyde on *E. coli*. The inhibitory effects of syringaldehyde on *Gluconobacter oxydans* (a Gram-negative bacteria) as a model organism were also evaluated by Buchert and Niemela (1990). The inhibition effect of syringaldehyde in the bioconversion of xylose-to-xylitol by *Candida guilliermondii* FTI 20037 was studied by Cortez and Roberto (2010). It was reported that the xylitol yield was significantly reduced by syringaldehyde, which showed a more pronounced inhibitory effect at pH 7.0.

In lignocellulosic biomass conversion to produce H<sub>2</sub> through dilute-acid pre-treatment and subsequent enzymatic hydrolysis, it has been reported that phenolic compounds produced as byproducts negatively affected the cell membrane functions, the growth, and the glycolysis of yeast and bacteria (Delgenes *et al.* 1996; Klinke *et al.* 2001, 2004; Taherzadeh *et al.* 1999). A study was carried out on the inhibitory effects of added representative inhibitors sodium acetate, furfural, HMF, vanillin, and syringaldehyde individually or in combination with fermentative hydrogen production by *Thermoanaerobacterium thermosaccharolyticum* W16 (Cao *et al.* 2010). It was reported that, depending on its concentration, syringaldehyde was one of the most potent inhibitors of growth and hydrogen production by *T. thermosaccharolyticum* W16.

Another investigation on the effect of individual sugars and various inhibitors potentially present in dried distillers grain and soluble (DDGS) hydrolysates on the growth and acetone-butanol-ethanol (ABE) production by representative solvent-

producing clostridia was conducted (Ezeji and Balschek 2008). It was reported that syringaldehyde was among the most potent inhibitors of ABE produced by the solventogenic clostridia. Fermentation of non-detoxified dilute acid-pretreated DDGS was unsuccessful because of the presence of syringaldehyde and the other inhibitors. Enzymatic hydrolysis of pretreated cellulosic materials during ethanol production was also affected by the presence of syringaldehyde. Ximenes *et al.* (2010) confirmed that phenols, including syringaldehyde, inhibit cellulose hydrolysis in wet cake by endo- and exo-cellulases and cellobiose hydrolysis by  $\beta$ -glucosidase.

Urease catalyzes the hydrolysis of urea to ammonia and carbon dioxide, which allows the survival of *Helicobacter pylori*, leading to gastric and peptic ulcer besides contributing to the pathogenesis of a wide range of diseases such as urolithiasis, pyelonephritis, and others (Abid *et al.* 2010). In order to heal peptic ulcer, inhibition of urease is as vital as non-inhibition of  $\alpha$ -chymotrypsin. According to the study conducted by Ramsay *et al.* (2012), syringaldehyde displayed effective inhibition of urease and did not inhibit the  $\alpha$ -chymotrypsin enzyme.

#### *Organic markers in wood smoke*

For confirmation of carbon-based fractions in smoke emissions, biomarkers or molecular tracers are employed as indicators to detect the origins from natural products of vegetation and their post-combustion residuals (Simoneit *et al.* 1995). Phenolic compounds (like syringaldehyde), which are obtained from lignin pyrolysis in vegetation, have been proposed as tracers specific for plant taxonomy. Syringaldehyde is widely used as a molecular marker for biomass smoke from aerosol particulate matter, namely to monitor pollution sources and detect the extent of combustion (Robinson *et al.* 2006). Since global climate change is affecting the occurrence of wildfires, a need to quantitatively identify atmospheric particulate matter from smoke appears to be of grave importance (Simoneit 2002). Syringaldehyde seems to play a key role in the detection of hardwood smoke.

#### *Biological control activity*

Syringaldehyde has been reported as an *Agrobacterium tumefaciens* virulence gene inducer (Delmotte *et al.* 1991; Lee *et al.* 1996). A study on the insecticidal properties of syringaldehyde was carried out on *Acanthoscelides obtectus* beetles (Regnault-Roger *et al.* 2004). Syringaldehyde showed a significant decrease in natural mobility by the 4<sup>th</sup> day and caused significant mortality on the 8<sup>th</sup> day. An investigation utilizing spectrophotometric analysis to determine amino acids using syringaldehyde was also reported (Medien 1998). A simple and sensitive spectrophotometric method was developed for kinetic determination of amino acids through their condensation with syringaldehyde. This provides an additional option in the analysis of amino acids with advantages of reagent availability, reagent stability, and less time consumption.

#### *Quantitative analysis of “free” chlorine in water*

An accurate measurement of “free” chlorines such as hypochlorous acid or hypochlorite ion without the interference from “bound” chlorines is crucial in determining the potability of water. A big difference between “bound” chlorines and

“free” ones is in terms of bactericidal capability. Combined chlorines such as chloramines that are less reactive may interfere with “free” chlorine readings. An investigation on the use of syringaldazine (a derivative of syringaldehyde) as a chromophoric reagent to measure “free” chlorine levels in water samples was carried out by (Bauer and Rupe 1971). Syringaldazine was synthesized from syringaldehyde and hydrazine, which react with chlorine in a 1:1 molar ratio. The molar absorptivity of this reaction is 65000 at  $\lambda=530$  nm. Syringaldazine, as shown in Fig. 8, is a chromophore that turns violet upon encountering chlorine, and it was tested against chlorine with concentrations ranging from 0 to 1 ppm. It was concluded that syringaldehyde was acknowledged to be more selective towards “unbound” chlorines by an amperometric titration procedure in comparison to the standard water and wastewater examination methods using *o*-tolidine arsenite. According to the test, at increasing levels of chloramine, the *o*-tolidine method recorded higher levels of “free” chlorine, unlike syringaldazine, due to its inability to discriminate chloramine from “free” chlorine at room temperature. It is therefore less accurate in measuring chlorine concentration actually present in the solution. Syringaldazine also eliminates the requirement of using a blocking agent like arsenite in the chromophoric reagent.

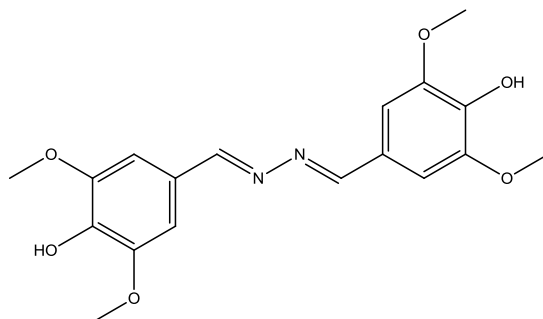


Fig. 8. The structure of syringaldazine

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