Strategies for Reducing the Mutagen Content of Chlorinated Aqueous Media

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(ABSTRACT)

In anticipation of government mandates regulating the quantity of the toxic mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone, which can be released into potable waters or from industrial facilities, technologies for monitoring the concentration, reducing the quantity, and inhibiting the formation of this compound were investigated. Adsorption of the chlorinated hydroxyfuranone from laboratory systems (pH 7.2, 22°) utilizing the non-polar, polymeric resin, Amberlite XAD-4, was successful. As these system conditions are comparable to those present in drinking water treatment facilities, application of this resin to such systems should be investigated. However, typical concentrations of the chlorinated mutagen released from such facilities are some 100 times less then those employed in laboratory experiments. As such, highly sensitive analytical technology, such as fluorescent

spectroscopy, would be required for direct detection of the mutagen in these aqueous systems. Since development of a fluorescent adduct of the mutagen, through either Schiff's base or halogen replacement reactions, failed, application of the adsorption methodology was not investigated. Approaching the problem in a different manner lead to investigations aimed at reducing the formation of the mutagenic compound. Since the mutagen is formed upon chlorination of waters containing lignin derived materials, lignification in intact growing plants, and the effects of modified substrates on this process, were investigated. All modified substrates tested increased the uptake of a C^{14} labelled lignin precursor into the lignin containing fraction of a living poplar stem. Research continues in this area to determine through what mechanism these compounds are affecting lignification.

Acknowledgements

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Introduction

Chlorine has been used to disinfect and deodorize drinking water and wastewater since the mid 1800's (1). Its current industrial use spans sterilization and purification, prevention of biofouling in water transport lines and filter media, and bleaching of wood pulp for papermaking. In all cases, this practice causes the formation of chlorinated organics, of which several are known toxins (2).

In 1974, it was discovered that chlorination of water resulted in the formation of chloroform, a known carcinogen (3,4). Subsequent studies, utilizing short-term bioassays, also revealed that chlorinated potable water and bleach plant effluent were mutagenic (2000-3000 revertant counts/ml of bleach effluent (5) and 20-2000 revertant counts/ml of drinking water (6)). This discovery was disconcerting for two reasons: (i) less than 40 percent of this mutagenicity (<20 percent for bleaching effluents) was due to volatile compounds such as chloroform (6,7) and (ii) more disturbingly, by testing known carcinogens, Ames showed a 90

percent correlation between carcinogenicity and mutagenicity (8). Consequently, a concerted effort was initiated to identify the chemical nature of the unknown mutagens.

These studies resulted in the identification of a potent mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone 3, that was produced upon chlorination of waters containing humic acids, fulvic acids, or lignin-derived materials (5,9,10,11,12). This furanone 3 has a mutagenic activity of 2,800-10,000 revertant counts/nanomole (Ames test, Salmonella typhimurium TA100; no S-9 addition) placing it on par with aflatoxin in toxicity (5,12,13). Though present only in low concentrations (0.04-10.0 ug/l (5,8)), it has been found in bleach plant effluents (5), and drinking and swimming pool waters (9). It is now conservatively estimated to be responsible for about 30 percent of the mutagenic activity of chlorinated drinking water (8) and at least 30-50 percent of pulp chlorination effluent mutagenicity (5). Unfortunately, its long term toxicity, bioaccumulation potential, chemical stability, and biodegradability are presently unknown (12).

As its carcinogenic properties have not yet been determined, the release of this compound into the environment is not subject to control by government regulations. EPA researchers, however, are currently

evaluating its potential as a carcinogen. In expectation of future regulatory mandates concerning the release of this potent mutagen 3, the development of methodologies for its detection and removal are timely.

In the past, chlorinated organic contaminants have been successfully adsorbed from aqueous streams by adsorbents such as activated carbon and polymeric resins (14,15). Polymeric resins offer several advantages over carbon, including ease of regeneration and the ability to "tailor-make" a resin for a particular situation. By conducting batch adsorption capacity tests under various conditions (ie. time, temperature, pH, and resin concentration), the feasibility of using a specific polymeric resin for mutagen 3 removal can be determined.

Due to the low concentration of this trichlorinated hydroxyfuranone 3 in chlorinated aqueous systems, lengthy preconcentration and isolation procedures are necessary for facile detection by current analytical techniques, eg., HPLC (5,16). Detection is also hindered by the presence of other, more strongly, UV-absorbing organics which mask the mutagen's 3 absorbance (13). However, if the hydroxyfuranone 3 could be selectively derivatized with reagents containing intense chromophores or fluorophores, then its direct detection in effluents and potable waters

would be possible. This could be accomplished by selectively `tagging' the mutagen 3 with an intense fluorophore, such as a porphyrin macromolecule. HPLC separation of the adduct followed by fluorescence spectroscopic detection would then permit a direct, quantitative measurement of the furanone 3. Methodology for direct detection of the mutagen 3 in aqueous media would also aid in the evaluation of strategies for its removal and destruction.

Additionally, since the mutagen 3 is apparently formed via chlorination of lignin or lignin derived material, any process which reduces the quantity or intractability of lignin in plants may also diminish the potential for its formation. Selective reduction or modulation of lignin in plants would also provide additional benefits of increased ruminant digestibility of plant material, along with reduced chemical requirements and processing times for wood pulping operations. As such, it could revolutionize the process of paper production through the utilization of less chemically intensive processing schemes, thereby producing less toxic effluents.

Lignin, a network polymer comprised of phenylpropanoid units, is found only in vascular plants. In woody plants, its formation occurs following free-radical induced

polymerization of the monolignols: p-coumaryl, coniferyl, and sinapyl alcohols, with the free radicals being generated by H_2O_2 and peroxidase (17,18). Lignification apparently then proceeds without further enzymatic control to produce a highly cross-linked, random polymer.

Depending upon the species in question, the lignin content of woody plants normally ranges from 20-40 percent (19). Interestingly, lignin distribution is also tissue specific, with the middle lamella exhibiting a higher lignin concentration then the secondary walls (19). Additional variations in lignin concentration also occur for different types of wood, eg., reaction and normal wood.

The monolignol composition of the lignin polymer is also species specific. For example, in hardwoods, lignin is mainly derived from coniferyl and sinapyl alcohols, whereas softwoods are predominantly formed from coniferyl alcohol (19). In all woody species, p-coumaryl alcohol is a minor constituent. Furthermore, a higher content of syringyl units is present in the secondary walls of hardwoods than in the middle lamella, which contains larger amounts of guaiacyl units (19).

As expected, the higher the lignin content in the plant, the more difficult it is to biodegrade, digest by ruminants, or chemically pulp. To some extent, lignin

deposition in plant tissue can be reduced chemically, eg., through administration of inhibitors of monolignol synthesis to growing plants (20,21,22,23). These inhibitors affect lignification by disrupting specific enzymes along the biosynthetic pathway thereby reducing the quantity of monolignols available for lignin formation. At this time it is unknown to what extent lignin deposition can be reduced in plants without adversely affecting structural and defensive functions.

In a related manner, it may be possible to modify lignin's structure <u>in situ</u> without affecting the plant's normal physiological functions. For example, if pseudosubstrates could be introduced into the plant that would compete with the natural monolignol substrates and thereby be incorporated into the lignification pathway, it would be possible to introduce different monomeric units into lignin. In this manner, the bonding patterns within lignin, or the degree of polymerization, could be altered to make the tissue more susceptible to chemical or biochemical conversion. For woody plants, enhanced biodegradability of plant material by organisms and facilitated removal of lignin during pulping could result. Plants containing such modified lignin might reduce bleaching chemical requirements, thereby diminishing the number of chlorinated

toxins released into the environment.

As has been stated, the overall objective of this study was to examine ways to eliminate the mutagen, 3-chloro-4-(dichloromethyl-5-hydroxy-2(5H)-furanone 3 from aqueous media. Specifically, the research was aimed at development of methodologies for the:

1) Physical removal of the mutagen 3 from chlorinated aqueous solutions,

2) Direct detection of the mutagen 3 in chlorinated aqueous solution,

and

3) Limitation of mutagen 3 formation through modulating *in situ* lignin.

LITERATURE REVIEW

CHLORINATION

Disinfection and deodorization of drinking and wastewater systems has been accomplished with chlorine for over a century. Although recognized mainly for its purification properties, chlorine also aids in taste and odor control and the removal of iron, manganese, and hydrogen sulfide (1,24). It is added to potable and cooling water transport lines to control biofouling within systems (1) and enhances flocculation and sedimentation when applied during prechlorination of drinking waters (1,24). Its extended use by industry arises from ease of handling and application, low cost, and ready availability. While efficiently controlling and destroying bacteria and other disease-producing organisms, chlorine additionally oxidizes some organic and inorganic materials to more readily biodegradable or terminal end-products, respectively (25). Unfortunately, the environmental impact of water chlorination is the annual introduction of several thousand

tons of chloro-organic compounds into American waterways (26).

Currently, the pulp and paper industry consumes 4 to 5 times as much chlorine as is required for sanitary purposes (1). During processing operations, chlorine is utilized to bleach the wood pulp white for papermaking. This is required because the cellulosic fibers are darker in color than the original wood from which they were derived even though much of the lignin is removed during pulping operations. This is a consequence of the generation of new, strongly absorbing chromophoric groups within the residual lignin in the pulp. Removal of this residual lignin is accomplished through a succession of oxidation and extraction stages, with chlorine bleaching usually initiating the sequence.

Chlorination Chemistry

Research into bleaching chemistry has shown that delignification proceeds through a variety of reactions including aromatic and olefinic substitutions and oxidations, aromatic ring cleavages, cleavage of aryl-alkyl ether bonds, and aliphatic oxidations (17,18). The first stage of pulp bleaching operations usually consists of the

addition of a high concentration of chlorine to the strongly acidic aqueous solution of pulp. Under these highly acidic conditions, the reacting species is the chloronium ion, Cl^+, produced via either heterolytic cleavage of elemental chlorine, Cl-Cl, or cleavage of the Cl-O bond in hypochlorous acid (27). The chloronium ion then undergoes electrophilic attack on high electron density areas within lignin (27). Aromatic or olefinic structures within lignin containing methoxyl and etherified substituents are sites of initial reaction.

In water treatment systems, chlorination of organic materials involves a variety of similar reactions including oxidation, addition, and substitution, although the mechanisms are not identical (26,28). Addition of chlorine to dilute acidic aqueous systems, results in the hydrolysis of elemental chlorine to give chloride and hypochlorite,

$$Cl_2 + H_2O \longrightarrow HOCl + Cl^- + H^+$$

with an equilibrium constant of 4×10^{-4} (1,24,25). The hypochlorous acid, HOCl, thus formed is a weak acid that dissociates in alkaline systems according to the equation:

$$HOCl \longrightarrow H^+ + OCl^-$$

with a dissociation constant of 3.2×10^{-8} (1,24,25). The above equilibrium reactions are strongly pH dependent, with hypochlorite, HOCl, being the dominant oxidizing species

between pH 5 - 7.5 (1,25). Consequently, either the chloronium ion, or the hydroxide ion of hypochlorite, can initiate reactions through electrophilic or nucleophilic attack, respectively. A more detailed review of this chemistry is described by Morris (29). When the species HOCl or OCl^- oxidizes substrates, the chlorine moieties are chemically reduced to the chloride ion by the reaction:

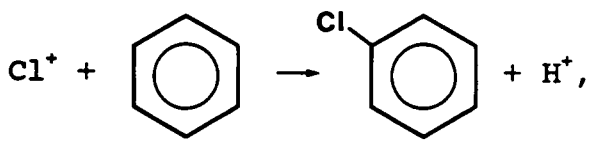
$$HOCl \text{ (or } OCl^-) + SS \longrightarrow SS_{oxid} + Cl^-$$

where SS is the soluble substrate and SS_{oxid} is the oxidized soluble substrate (29).

Hypochlorous acid can also react with unsaturated double bonds to form chlorohydrins by the reaction:

$$R\text{-}(H)C{=}C(H)\text{-}R' + HOCl \longrightarrow R\text{-}(H)C(OH)\text{-}(Cl)C(H)\text{-}R' \quad (28).$$

The chloronium ion can also be substituted into aromatic rings by the reaction:



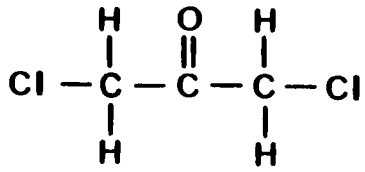
where X denotes an electron donating group (29). Additionally, replacement of hydrogens on the carbon adjacent to a carbonyl group in ketones occurs via the haloform reaction; this reaction terminates with hydrolysis and the formation of chloroform and a carboxylate ion (29).

Chlorination Toxicity

In the early 1970's, it first became known that chlorination of aqueous solutions such as drinking water, produced trihalomethanes which were suspected carcinogenic compounds (4,30,31). These findings triggered interest concerning the risks associated with water chlorination. Subsequent investigations revealed that numerous organochlorine compounds were produced via chlorination of both natural and synthetic organics present in water (32). This initiated further research into the area of toxic substances produced upon aqueous chlorination and revealed the mutagenicity of chlorinated waters through implementation of the Salmonella-microsome assay (9,11,12,13). The importance of this assay for screening suspected toxins had been previously demonstrated by Ames and co-workers when they established a 90 percent correlation between carcinogenicity and mutagenicity (7).

Mutagenic activity of heavily chlorinated aqueous solutions such as bleach plant effluents was first documented in 1977 (9). Extensive research was then initiated to isolate the compound, or compounds, responsible for this mutagenicity. Identified mutagens included halogenated methanes, ethanes, ethenes, propenes, and

acetones. However, the major contributors to the overall mutagenicity of these effluents were 1,3-dichloroacetone 1, 2-chloropropenal 2, and 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone 3 (Figure 1) with the latter predominating (5,10). Of the three major compounds contributing to the mutagenicity of bleach plant effluents, the chlorinated hydroxyfuranone 3 was reported to have the highest mutagenic activity. At 2,800-10,000 revertant counts/nanomole (TA100; no S-9 addition), the compound was comparable to aflatoxin in its toxicity (5,12,13). Subsequent studies showed that the chlorinated hydroxyfuranone 3 was also present in finished potable waters (9) and that it was formed upon chlorination of humic material or waters containing humic material such as ground or surface waters (33). Knowing that the toxicity of chlorinated aqueous systems is due in a great part to the high mutagenicity of one compound, the development of strategies for its removal from aqueous media are urgently needed.



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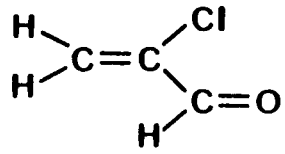

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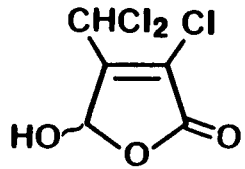

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Figure 1. Major Mutagens in Pulp and Paper Effluents.

ADSORPTION

Activated carbon has long been used to remove unwanted materials from aqueous streams. However, modern use of the adsorbent probably dates back only to the mid 1960's when it was used to remove petrochemicals from drinking water (14). Adsorption of chemicals from aqueous streams has since changed emphasis from blanket removal of chemicals which cause taste and odor problems, to removal of specific organic contaminants. Organic pollutants came under government regulation in 1976 when the EPA established that 129 Priority Pollutants, 114 of which were organic, presented probable hazards to water resources (15). Maximum concentration levels of four trihalomethane compounds in drinking water and effluents were established in 1979 to protect public health and the quality of our water resources (3). This regulation of the release of organic contaminants prompted the significant increase in use of adsorbents for the removal of trace contaminants from aqueous systems.

Adsorption Theory

Adsorption is a surface phenomenon characterized by material (adsorbate) being extracted from one phase and

concentrated at the surface of another (adsorbent). Detailed reviews of the subject have been presented by Weber (34) and Faust and Aly (14). In general, any solute which lowers the surface tension of a liquid in which it is dissolved is capable of adsorbing at the interface between two phases (34). Organic compounds containing both a polar hydrophilic group and a nonpolar hydrophobic group can lower the surface tension of aqueous solutions. These solute molecules tend to accumulate at the surface of the liquid phase due to the hydrocarbon residue's low affinity for water. Such migrations to the liquid phase boundary reduce the work required to enlarge the surface area, thereby reducing the surface tension. Thus, concentration of solute at the interface is termed positive adsorption and is one of the primary driving forces for adsorption.

Besides the lyophobic (solvent-disliking) character of the solute towards a particular solvent, a second driving force exists, namely the specific affinity of the solute for the solid. The attractive forces between a molecule in solution (adsorbate) and a solid surface (adsorbent) interact so that the adsorbate binds to the adsorbent. Three different types of adsorption exist depending on the type of attractive forces between adsorbate and adsorbent. Ion exchange involves an electrostatic attachment of an

ionic species in solution to a surface site of opposite charge on the adsorbent. In chemical adsorption, or chemisorption, the adsorbate undergoes a chemical reaction with the surface of the adsorbent which results in a change in the chemical form of adsorbate and adsorbent. Physical adsorption is characterized by the binding of the adsorbate to the solid surface by relatively weak intermolecular interactions known as Van der Waal's forces.

Positive adsorption occurs in a liquid-solid system until the concentration of the residual solute in solution is in dynamic equilibrium with that at the surface (34). At this equilibrium there is a defined distribution of solute between liquid and solid phases (34). The position of equilibrium is designated by a number of parameters specific to a given system including the adsorbate, adsorbent, pH, and temperature. With respect to the adsorbate, concentration, molecular weight, molecular size, molecular polarity, steric configuration, and nature of competitive adsorbates all influence adsorption (35). The physical size and form of the adsorbent particles, along with the size, nature, and availability of its surface, determines the adsorptive capacity of the adsorbent (35). And finally, the temperature and pH of the system can effect the equilibrium capacity by altering any one of the above parameters.

In general, the greater a compound's solvophobicity with respect to a given solvent, the more likely it is to be adsorbed at the interface between a liquid and solid phase. Any change in a parameter which decreases the solubility of the compound in the solvent or increases the affinity of the solute for the adsorbent will increase the adsorption equilibrium of the system.

Adsorption Isotherms

Adsorption equilibrium data for a given system at constant temperature is depicted by an adsorption isotherm. Such isotherms express the amount of solute adsorbed per unit weight of solid adsorbent, q or x/m, as a function of residual concentration, C_e, remaining in solution at equilibrium. Usually, the quantity of adsorbed material per unit weight of adsorbent increases with increasing residual concentration, but not in direct proportion (34,35). Several types of relationships have been developed to describe adsorption isotherms and these equilibrium models include both single and multimolecular layering of solute on the adsorbent surface. Langmuir's, BET's and Freundlich's adsoption isotherms are most commonly used.

The Langmuir adsorption model is valid for monolayer adsorption and incorporates these basic assumptions:

1) Adsorbent surface adsorbs solute molecules onto definite sites
2) Only one adsorbate molecule can occupy an adsorbent site
3) Adsorption site area is fixed and determined by the geometry of the adsorbent surface
4) Adsorption energy is equivalent for all sites
5) Adsorbed molecules can not migrate along the adsorbent surface or interact with other solute molecules (35)

The Langmuir model has been deduced from both kinetic considerations and thermodynamics of adsorption (34). For adsorption from solution by solid adsorbents, the model can be expressed as:

$$q = q_m b C_e / (1+bC_e),$$

where q=x/m, the amount of solute adsorbed, x, per unit weight of adsorbent, m; C_e=equilibrium concentration of solute; q_m=amount of solute adsorbed per unit weight of adsorbent required for monolayer coverage of the surface (monolayer capacity); and b=a constant related to the heat of adsorption (14). Linearization of this equation gives:

$$(1/q) = (1/q_m) + (1/C_e)(1/bq_m),$$

where the monolayer capacity, q_m, determined from the equation, defines the total capacity of the adsorbent for a specific adsorbate.

The BET, Brunauer, Emmett, and Teller, model extends the Langmuir model to include multilayer adsorption, that

is, it assumes that a number of adsorbate layers form on the surface of the adsorbent and that the Langmuir model applies to each layer (34). Furthermore, the BET model assumes that any given layer need not be complete before a subsequent layer starts (35). The linearized form of the BET model is:

$$C_e/q(C_s-C_e) = 1/q_m b + (C_e/C_s)(B-1)/q_m B,$$

where q, q_m, and C_e have the same meaning as for the Langmuir isotherm, C_s=solubility of the solute in water at a specific temperature, and B=a constant expressive of the energy of adsorption (14,35).

It should be noted that while experimental data may follow either the Langmuir or BET isotherms, this does not insure the formation of ideal mono- or multilayers on the surface of the adsorbent. Furthermore, both equations have proven useful for modeling physical systems despite the unrealistic assumptions made in their derivation.

Unlike the Langmuir or BET isotherms, the Freundlich model is an empirical expression that allows for heterogeneous surfaces. In this equation the energy term, b, from the Langmuir equation, is a variable dependent on surface coverage, q, and incorporates an exponential distribution of sites and their energies (14,34,35). The linearized form of the Freundlich equation is:

$$\log q = \log K + (1/n)\log C_e,$$

where q and C_e have been defined for the Langmuir and BET equations and K and n are constants characteristic of the adsorption system (14,34,35). This equation has proven very useful for modeling water and wastewater systems.

Adsorption Rates

Adsorption of solutes onto porous particles is a time-dependent process. Essentially there are four consecutive steps involved in adsorption, only two of which are important as rate-determining steps. These four steps include bulk diffusion, film diffusion, intraparticle diffusion, and adsorption (14,34,35). Bulk transport of a solute through a solution phase is usually rapid due to mixing and convective flow (35). It can therefore be neglected as a rate-controlling step. Additionally, adsorption of the solute on the interior surface sites of the adsorbent is considered rapid, equivalent to an equilibrium reaction, and can also be neglected (34,35). Film and intraparticle diffusion are therefore the major factors influencing adsorption rates.

Film transport involves the diffusion of a solute molecule from bulk solution through a film or boundary layer. In reality there is no film surrounding the

adsorbent, but the term is used to describe the resistance to mass transfer at the surface of the particle (14,35). The concentration gradient in the boundary layer surrounding the adsorbent particle maintains the driving force for film diffusion (35). Diffusion through this surface film will be of importance in determining the rate-controlling step unless sufficient turbulence is present within the system to diminish its significance.

Intraparticle diffusion involves the transport of adsorbate from the particle surface through pores into the interior of the particle (pore diffusion) and the migration of the adsorbate along the solid surface of the pore (surface diffusion) (14,35). Because pore and surface diffusion act in parallel, the more rapid one will control the overall rate on intraparticle transport. Investigation of typical aqueous adsorption systems has shown that the rate of surface diffusion typically predominates and pore diffusion can usually be neglected (35).

Film and intraparticle diffusion act in series, therefore the slower of the two processes will be rate-determining. However, control may be distributed between intraparticle and external mechanisms in some systems (14,35). In general, while adsorption in reactors which provide adequate mixing or agitation will typically be

limited by pore diffusion, adsorption in a continuous flow system will usually be controlled by film diffusion (14,35).

Polymeric Resins

In recent years, the use of polymeric resins to adsorb organic compounds from aqueous solution has risen significantly (36). Reasons for this rise include the ability to manufacture resins for a particular situation and the ease of resin regeneration (35,36). Characterized by being highly porous, these insoluble, durable beads exhibit a large surface area for their size. During manufacture, the pore size distribution can be controlled by regulating the amount of extender used during polymerization, thereby varying the level of cross-linking within the polymer matrix (35,36). The size of pores within the adsorbate determines both the size of molecules which can be adsorbed and the capacity of the adsorbent due to the inverse relationship between pore size and surface area. A further feature of the non-polar polymeric resins is the lack of ionic functional groups in their structure (35). Therefore, these hydrophobic adsorbents are capable of adsorbing hydrophobic solutes from aqueous solutions via Van der Waal's interactions (36).

In order to assess the feasibility of resin adsorption for a specific application, adsorption isotherms must be developed and then analyzed. Laboratory data for these isotherms are obtained from treating an aqueous solution containing the organic substance with a succession of known amounts of adsorbent (15). These batch resin-water systems are agitated at a fixed temperature until equilibrium is attained. The residual contamination is separated from the resin and analyzed by an appropriate analytical technique such as high performance liquid chromatography (HPLC). Adsorption isotherms obtained in this manner can provide the following information:

1) Degree of contaminant removal attainable
2) Equilibrium adsorption capacity of the adsorbent
3) Optimum pH and temperature for adsorption
4) Effect of concentration on the adsorption capacity
5) Relative affinity of adsorbate for adsorbent
6) Effect of competitive adsorption on capacity
7) Presence of single, competing, or non-adsorbing compounds (15)

The information gained from these preliminary laboratory tests are valuable for making an initial assessment of the feasibility and economic viability of the adsorbent. And while direct correlation between batch equilibrium and dynamic performance of a resin is both difficult and unreliable, the isotherms are useful for determining what additional testing is necessary.

Amberlite XAD-4

Amberlite XAD-4 resin is manufactured by Rohm and Haas, Philadelphia, Pennsylvania. The synthetic resin is a non-polar styrene-divinylbenzene copolymer of hydrophobic character. The resin exhibits a specific surface area of 750 m^2/g and an average pore diameter of 40 Å (37). The adsorbent particles have a grain size of 20-60 mesh, a porosity of 0.45, and a dipole moment of 0.3 (37).

Commercially bought resins are packaged with sodium chloride and sodium carbonate to control bacteria and mold growth during storage (37). Additionally, residual monomeric materials from manufacture may contaminate the resin. These preservative agents and organic impurities present in the resin need to be removed before resin use.

Several cleaning procedures for polymeric resins are documented in the scientific literature (38,39,40). Recommendations basically include decantation of the resin with water to remove the preservatives and smallest resin particles, followed by Soxhlet extraction with polar organic solvents to remove the residual organics. The cleaned resin is stored until use under distilled methanol in a dark sealed container to prevent further biological or chemical contamination.

Resin Study

Adsorption by polymeric resins can be accomplished using either batch or column techniques. For column techniques, the polymeric resin is loaded into a vertical column and the solution to be treated is passed through the column. In batch methods, the adsorbent is added to the contaminated solution and the slurry is gently agitated. Choice of a particular method depends on application and economic feasibility in addition to adsorption capacity data and bench and pilot plant studies.

Adsorption capacity studies are conducted utilizing batch methodologies. The adsorbent is added to a solution containing the contaminant and the resulting slurry is agitated until equilibrium is reached. At this time, the residual concentration of the contaminant is measured. To ensure correlation of experimental data to actual applications, the variables for these experiments, eg., pH, temperature, and concentration, should be chosen as close to the typical values of the aqueous stream as possible.

MUTAGEN DETECTION

Today it is known that chlorination of phenol, lignin, lignin fragments, humic and fulvic acids in aqueous media contributes to the increase in the carcinogenicity and mutagenicity of aqueous solutions. Furthermore, chlorination of these solutions changes the mutagenic activity from metabolic to non-metabolic activity (28). However, due to the low concentration of toxins in chlorinated aqueous media, identification of the constituents responsible for the toxicity has been most difficult.

For example, the mutagenic chlorinated hydroxyfuranone **3** is only present in trace quantities in chlorination effluents (<5-10 ug/l (5)) containing many other organic substances. Consequently, lengthy isolation and/or concentration procedures have been necessary to establish its presence in aqueous media. Undoubtedly, severe losses were accrued during such isolations. Nevertheless, even without taking these losses into account, the chlorinated hydroxyfuranone **3** was responsible for at least 30-50 percent of the mutagenicity of bleach plant effluents (5) and 30 percent of the mutagenic activity of chlorinated drinking water (9).

Direct Detection

Since the fate and concentration of this highly mutagenic compound in chlorinated aqueous media is unknown, the development of methodology for its direct detection is necessary. Such technology must also be suitable for industrial application. In the past few years, fluorescence spectroscopy has received a great deal of attention from industry due to its speed and sensitivity. If selective derivatization of the hydroxyfuranone with intense chromophores or fluorophores was possible, then direct detection techniques could be devised using HPLC coupled with fluorescence spectroscopy.

Fluorescence

Fluorescence is a photochemical process whereby a molecule absorbs and then re-emits energy. The absorbed energy causes a transition of electrons from the ground state to an excited singlet state. From this excited state, a variety of mechanistic steps exist for its return to ground state. If the molecule returns to the ground singlet state by emission of radiation from the excited electrons, fluorescence is observed. Fluorescence can therefore be

defined as the emission of energy in all directions from electrons returning to a singlet ground state from an excited singlet state (41). Compounds that contain aromatic rings, aliphatic or alicyclic carbonyl structures, or highly conjugated double-bond system may exhibit fluorescence.

Fluorescence Spectroscopy

Fluorescence spectroscopy offers two important advantages over other analytical techniques: sensitivity and speed. Under optimal conditions, fluorescence spectroscopy can detect nanomolar concentrations in milliseconds (42). Because of these properties, fluorescence spectroscopic techniques have been applied to on-line monitoring for quality control, detection of low concentration of compounds in analytical laboratories, and real-time analysis of (bio)chemical reactions (42). The excellent sensitivity offered by fluorescence spectroscopy could therefore aid in monitoring trace contaminants in aqueous systems upon development of appropriate procedures.

Porphyrins

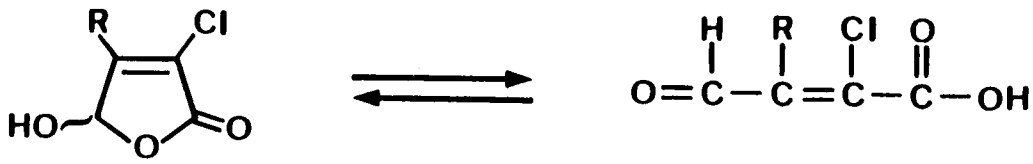
Porphyrins, derivatives of porphine, are among the most highly fluorescent compounds known. The ring system of porphine consists of four pyrazole rings joined by four methane bridges allowing for a highly conjugated system (41). When excited by visible light, porphyrins re-emit this adsorbed energy in the red or infrared region (41). The sensitivity achievable when using fluorescence spectroscopic techniques to detect porphyrins exceeds all other analytical techniques with detection limits at nanomolar concentrations and below (42).

Schiff's Base Reaction

Aldehydes and ketones can react with primary amines to form imines or Schiff's bases by the reaction:

$$R\text{-}C(R')=O + H_2N\text{-}R'' \longrightarrow R\text{-}C(R')=N\text{-}R'' + H_2O$$

which usually takes place in organic solutions (43). Some cyclic ring structures, such as substituted furanone rings, can exist in both a closed ring and an open chain form, with the latter containing a free aldehyde group (Figure 2) (44). Consequently, they can react to form Schiff's bases (45). As an example, the hydroxyfuranone, mucochloric acid **4**, is

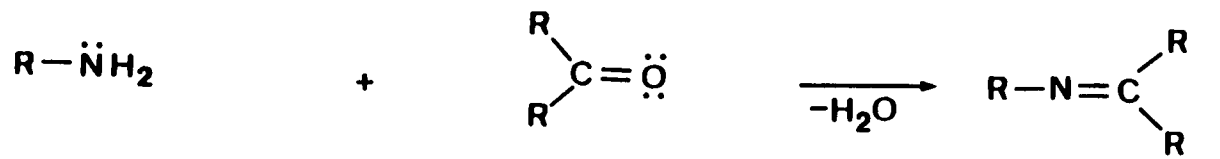


3 R= $CHCl_2$

4 R= Cl

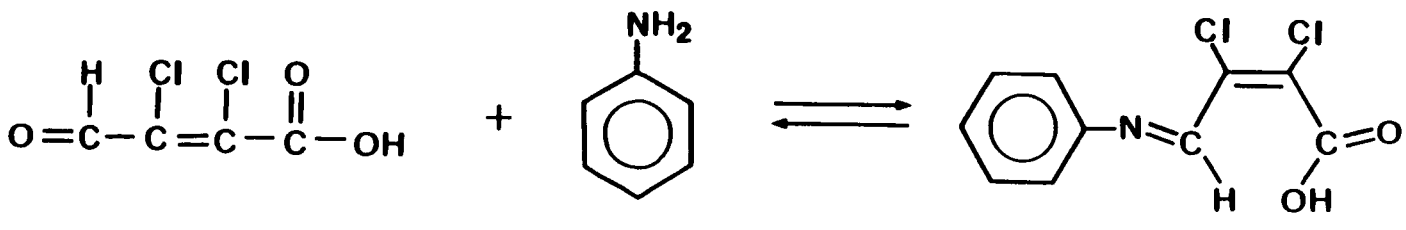
Figure 2. Resonance Structures of Chlorinated Hydroxyfuranones.

present mainly in its open chain form in alkaline media as shown by infrared and Raman spectroscopy (44). The free aldehydic group of mucochloric acid 4 is able to react with primary amines thereby forming imines (Figure 3). For example, the Schiff's base, anilmucochloric acid 5, is formed by reaction of the open chain form of mucochloric acid 4 with aniline 6 (Figure 4) (45). Taking into consideration the similarities between mucochloric acid 4 and the mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone 3, it should also be possible to react the mutagen 3 with aniline 6, or an aminoporphyrin, eg., 7, by the same reaction (Figure 5). Derivatization of the mutagen 3 in this manner would permit direct detection using a combination of HPLC and fluorescence spectroscopy.



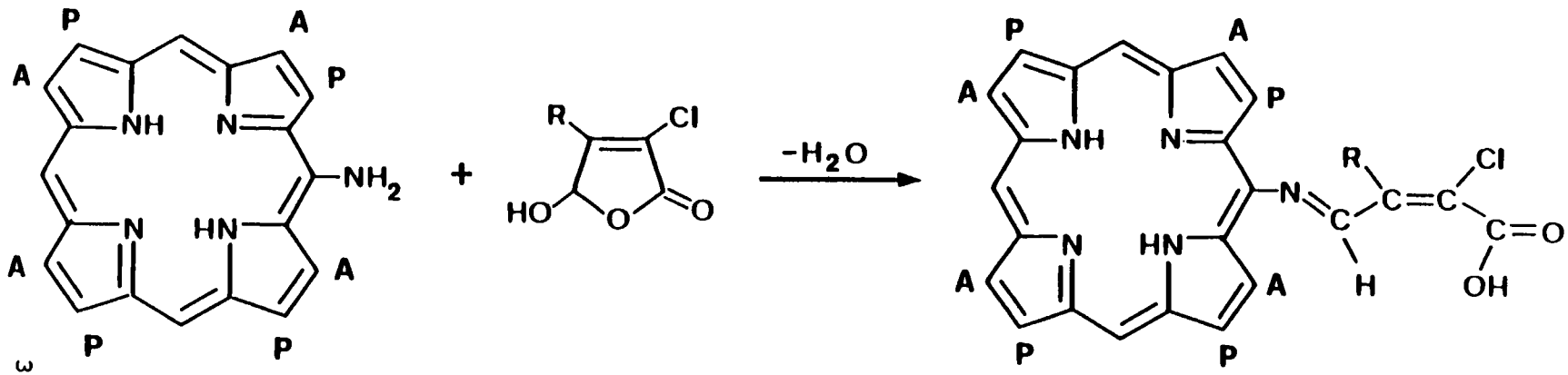
Primary Amine Aldehyde or Ketone Schiff Base

Figure 3. Formation of a Schiff's Base.



4 6 5

Figure 4. Formation of the Schiff's Base-Anilmucochloric Acid 5.



a, A = $CH_2CO_2CH_3$ P = $CH_2CH_2CO_2CH_3$

b, A = CH_2CO_2H P = $CH_2CH_2CO_2H$

Figure 5. Formation of the Schiff's Base-Amino-porphryin Adducts 7a,b.

LIGNIN

A constituent of all vascular plants, lignin is a complex, network polymer that provides structural and defense roles (17). The polymer is formed through random dehydrogenative polymerization of phenylpropanoid units. In woody plants, lignin content normally ranges from 20 to 40 percent of dry plant material (47). Due to this variation of lignin content within plants, the possibility exists for reduction of lignin in plants without deleterious effects on its physiological functions.

Benefits of Reduction/Modification of Lignin

Since prior research has indicated that the mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone **3**, is produced from chlorination of lignin or lignin-derived materials (9,11,13), it follows that any reduction in lignin content within plants may cause a corresponding reduction in the formation of the mutagen **3**. Additional advantages obtained from a reduction of lignin content within plants include i) an increase in digestibility of plant material by ruminants and ii) a decrease in the chemical requirements of, and processing times for, chemical pulping and bleaching

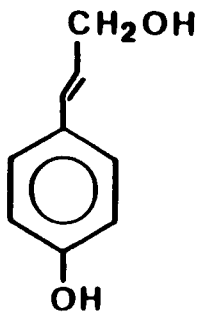
operations. Additionally, if lignin's structure within the plant were selectively modified to promote easier chemical and biochemical removal, without actually affecting the quantity of lignin depostion or its physiological functions, then similar benefits might be achievable. Such modifications might include i) a reduction in the degree of polymerization, ii) a lowering of the lignin-polysaccharide interlinkages, or iii) an increase in the number of chemically labile linkages. Altering the structure of lignin by introducing modified substrates which produce any of the above affects might improve the chemical and/or biochemical delignification of plant material, thereby reducing the quantity of mutagen precursors present in aqueous systems receiving chlorine treatment.

Lignification

Overall, the pathways leading to, and the enzymes involved in, monolignol formation are fairly well known and understood. However, the actual process of lignin deposition including its initiation, regulation, and final structure *in situ*, is not firmly established.

Monolignol Biosynthesis

In woody plants, the monolignols, p-coumaryl **8**, coniferyl **9** and sinapyl alcohols **10**, are the immediate precursors for lignification (Figure 6). The p-hydroxy-cinnamyl alcohols in woody plants are derived from L-phenylalanine **11** through the following scheme. Deamination first converts phenylalanine **11** to cinnamic acid **12** by the action of phenylalanine ammonia lyase, (PAL) (Figure 7) (19). A series of hydroxylations and methoxylations then affords the p-hydroxy cinnamic acids: p-coumaric **13**, ferulic **14**, and sinapic acid **15** (19) (Figure 8). These acids are then converted into the corresponding alcohols via esterification with coenzyme A, followed by a two step reduction to first give the corresponding aldehyde and then the monolignol (19) (Figure 9). The enzymes involved in these transformations are listed in Table 1.



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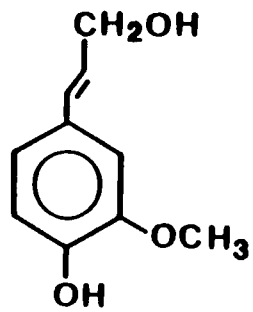

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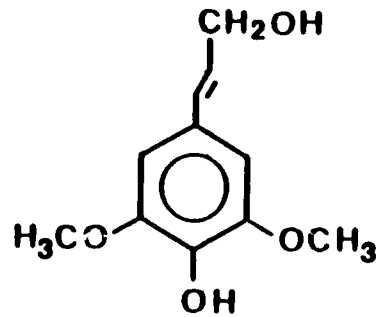

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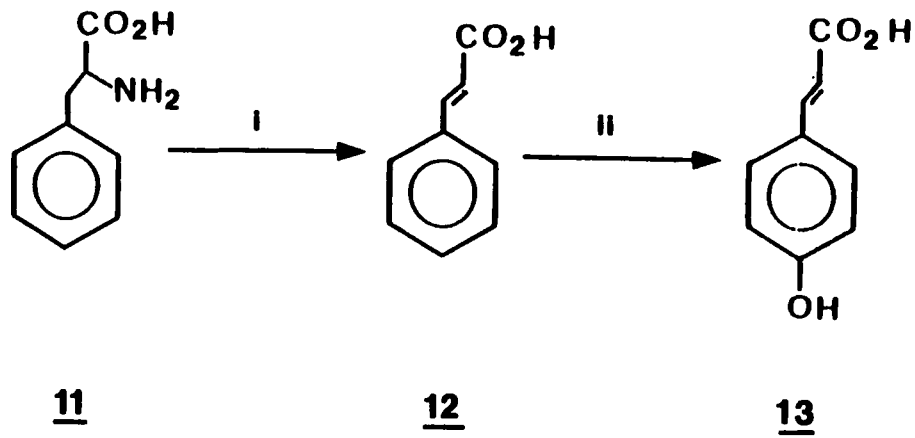
Figure 6. Monolignols Involved in Lignification.



11 12 13

Figure 7. Phenylpropanoid Metabolic Pathway: Phenylalanine 11 to p-Coumaric Acid 13.

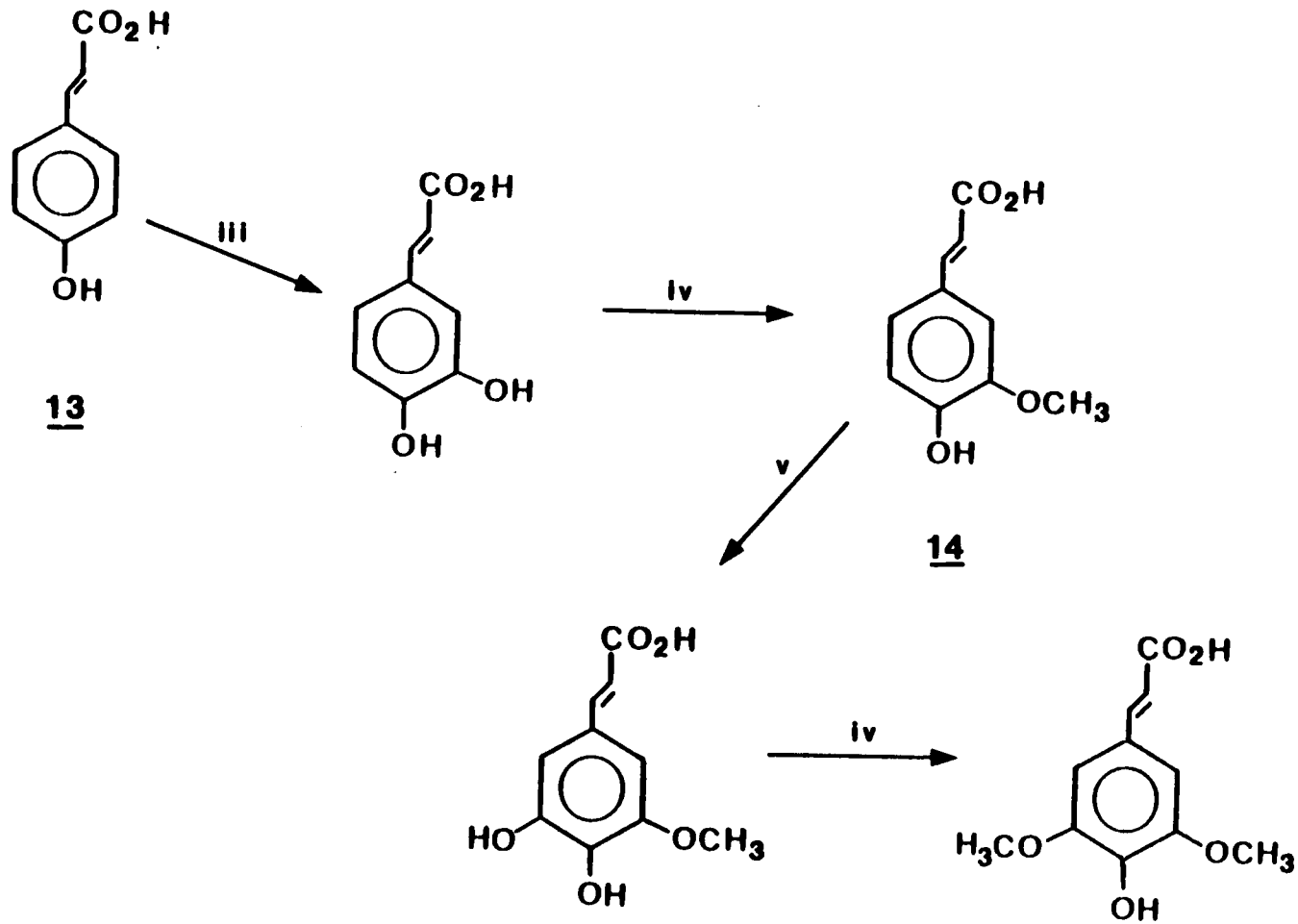
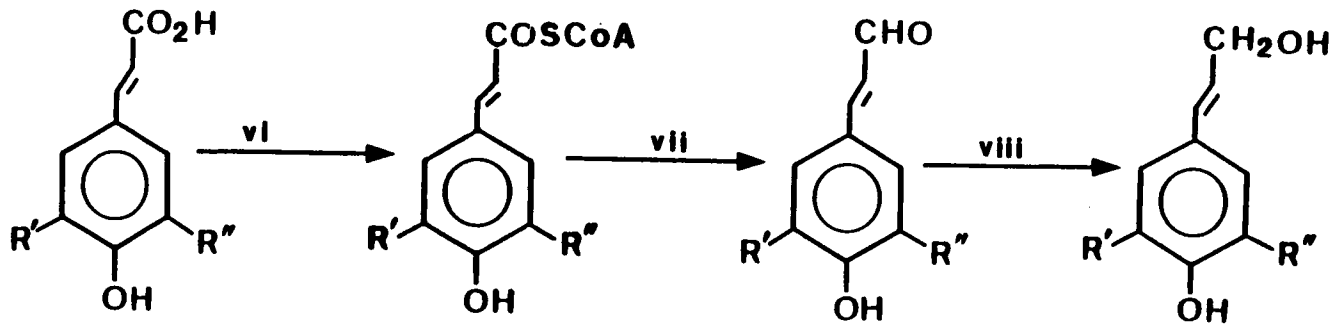


Figure 8. Conversion of p-Coumaric Acid **13** to Ferulic **14** and Sinapic Acid **15**.



8, 9 or 10

8 R′ = R″ = H

9 R′ = H R″ = OCH_3

10 R′ = R″ = OCH_3

Figure 9. Reduction of the Cinnamic Acids to the Corresponding Monolignols.

Table 1. Enzymes Involved in Monolignol Formation

i. phenylalanine ammonia lyase (PAL)

ii. cinnamate-4-hydroxylase

iii. p-coumarate-3-hydroxylase

iv. O-methyltransferase

v. ferulate-5-hydroxylase

vi. hydroxycinnamate:CoA ligase

vii. cinnamoyl:CoA reductase

viii. cinnamyl alcohol dehydrogenase (CAD)

Inhibition of Lignin

Manipulation of lignin deposition in vascular plants has intrigued researchers for years. Recent studies demonstrated that lignification can be repressed via chemical means (20,21,22,23). For example, compounds such as L-α-aminooxy-β-phenylpropionic acid, (AOPP), and (1-amino-2-phenylethyl)propionic acid, (APEP), affect lignin deposition by competitively inhibiting L-phenylalanine ammonia lyase activity (20,21,22). However, inhibition of this enzyme not only alters the deposition of lignin, but also the formation of other secondary metabolites derived from phenylalanine **11** including simple phenols, condensed tannins, flavanoids, etc. (20,21,22). Other research has lead to the development of compounds which inhibit enzymes specifically involved in the latter stages of monolignol biosynthesis. N-(O-hydroxyphenyl)- and N-(O-aminophenyl)-sulfinamoyltertiobutyl acetate, OHPAS and NH_2PAS, respectively, have been shown to be effective inhibitors of cinnamyl alcohol dehydrogenase, CAD (23). These compounds were designed to simulate the natural substrates and yet competitively bind to the enzyme. The pseudoirreversible binding of OHPAS and NH_2PAS to CAD has been reported to cause a 45 percent reduction in the uptake of cinnamic acid

into the lignin fraction of poplar stems (23). To test the specificity of these inhibitors, the effect of these compounds, NH_2PAS and OHPAS, on other enzymes involved in the phenylpropanoid pathway was examined. Of the enzymes tested, phenylalanine ammonia lyase, catechol O-methyl-transferase, and hydroxycinnamate:CoA ligase were not affected by either compound, but cinnamoyl:CoA reductase was slightly inhibited by NH_2PAS (23). Development of compounds capable of specifically inhibiting key enzymes involved in lignification offers the potential for regulating the extent of lignin deposition within vascular plants.

Lignin Deposition

Monolignols are incorporated into lignin by dehydrogenative polymerization in a H_2O_2 requiring reaction initiated by peroxidases which abstract the phenolic protons to create phenoxy radicals (19). Phenoxy radicals are capable of existing in several mesomeric forms (Figure 10) (17). Coupling of the various forms of phenoxy radicals then ensues randomly, thereby accounting for the complexity of the bonding patterns within lignin (17). Lignin bonding patterns are dependent on by electron spin densities with phenoxy radicals having the highest electron density at the

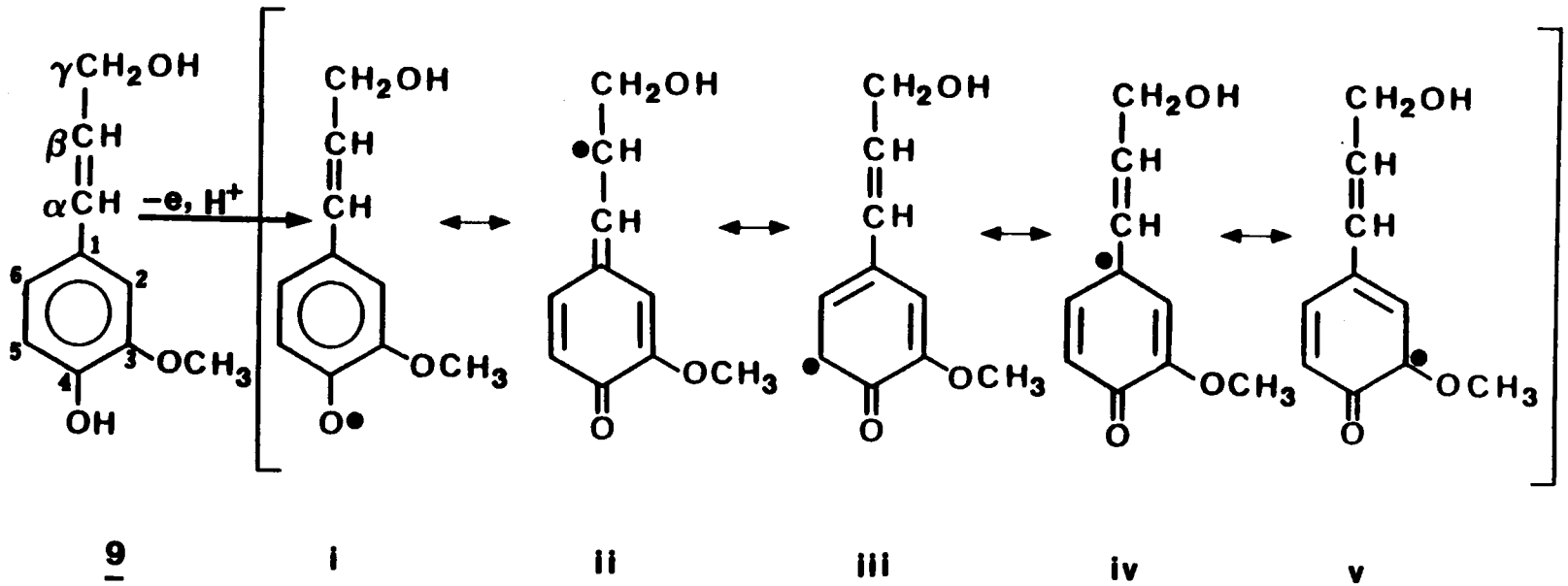


Figure 10. Resonance Structures of the Monolignol Phenoxy Radicals as Exemplified by Coniferyl Alcohol 9.

phenolic oxygen (17). However, coupling of two phenoxy radicals at the phenoxy oxygen end to produce the unstable peroxide has never been observed. Consequently, formation of arylether bonds should dominate over other interunit linkages, and as such, has been demonstrated by the predominance of β-O-4 linkages in lignin (48). A variety of other ether and carbonyl linkages are formed with linkages involving the bonding of the resonance structures i, ii, and iii (Figure 10) being favored over the sterically hindered structures iv and v (Figure 10). Three of the most common interunit linkages in lignin are shown in Figures 11, 12, and 13. Upon coupling of two phenoxy radicals, the dimer exists as a reactive quinonemethide species (17). The quinonemethide intermediate then reacts through intramolecular or external addition of hydroxy containing compounds. These moieties include phenolic hydroxyls (preferred), hydroxy containing aliphatics, and water (19). Cellulose and hemicellulose can also add to the quinonemethide, thereby becoming covalently linked with the developing polymer (17). Through continued reaction, a three-dimensional network polymer with a high degree of polymerization is achieved.

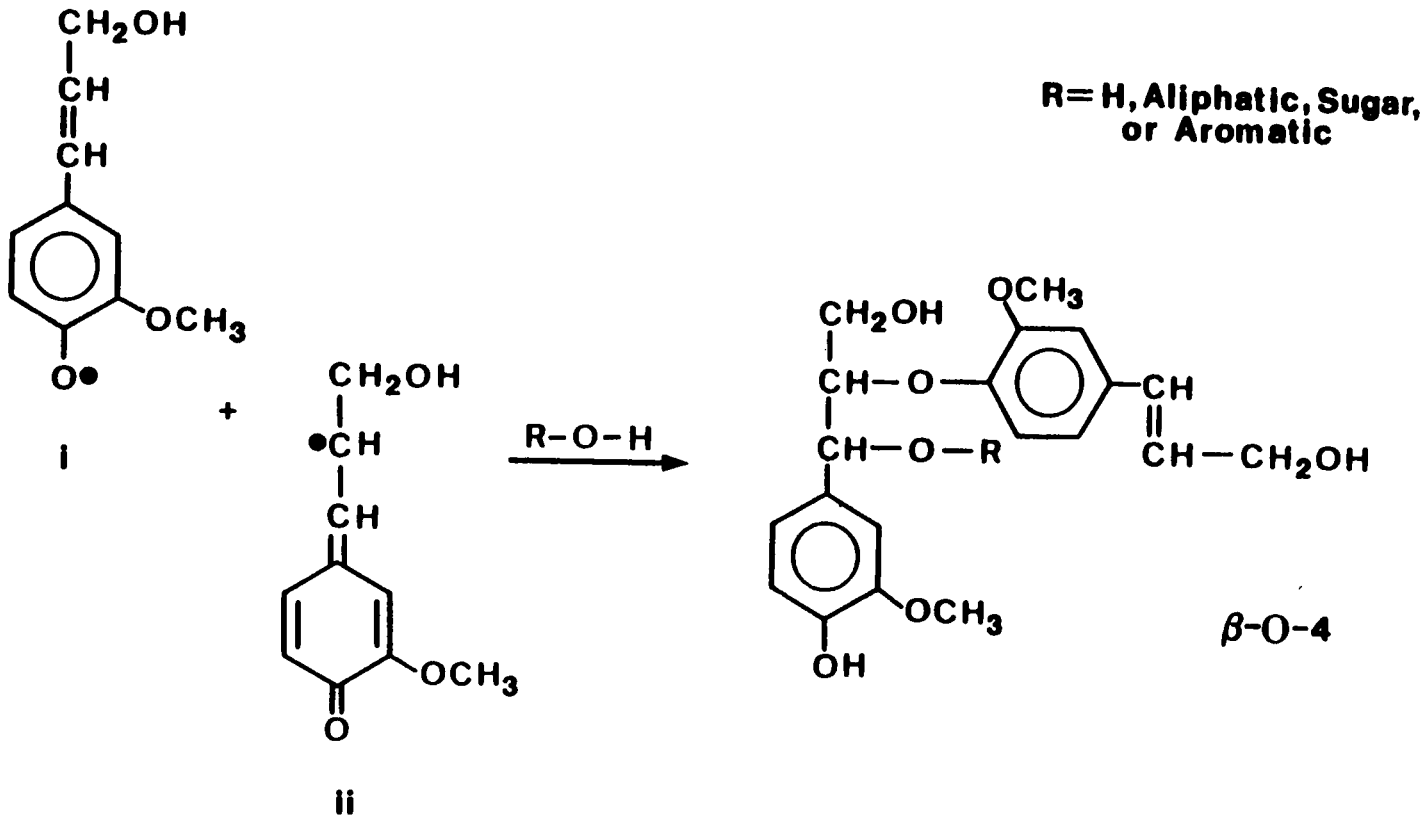


Figure 11. Formation of the β-O-4 structure in Lignin (i and ii refer to corresponding structures in Figure 10).

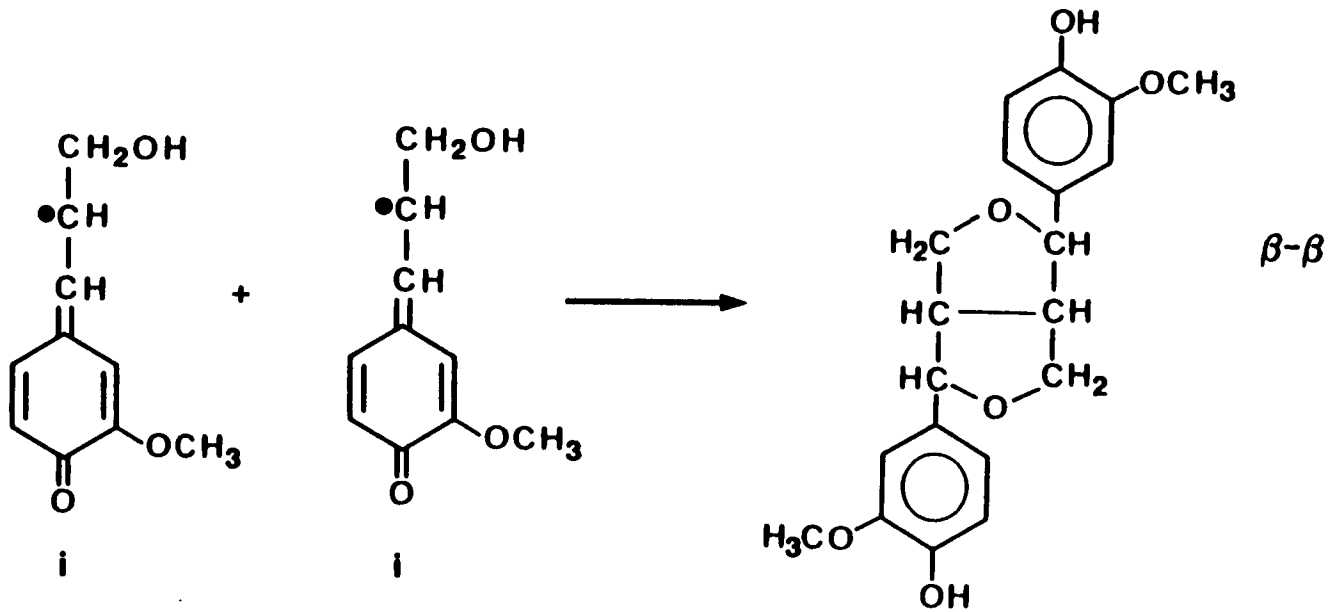


Figure 12. Formation of the β-β structure in Lignin (i refers to corresponding structure in Figure 10).

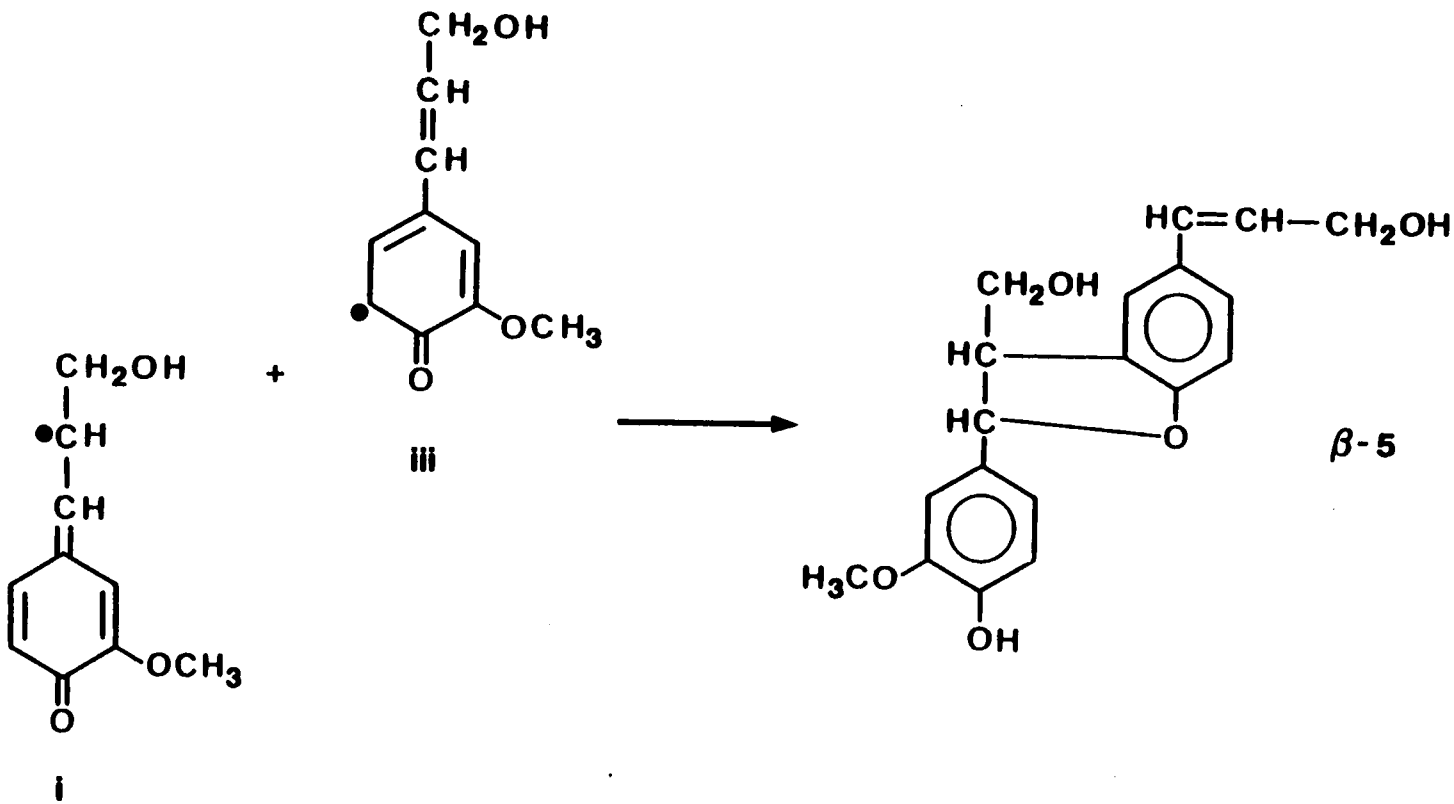


Figure 13. Formation of the β-5 structure in Lignin (i and iii refer to corresponding structures in Figure 10).

Modification of Lignin

While researchers have reported partial inhibition of lignin in plants via chemical means, there have been no reports of incorporation of synthetic pseudosubstrates into lignin. Incorporation of modified natural substrates into lignin affords the chance to vary the structure of lignin without affecting the quantity of lignin within plants. For example, if psuedosubstrates could be introduced into lignin with sterically hindered carbons alpha to the aromatic ring, then the transient β-O-4 quinonemethide would be less likely to add large aromatic or polysaccharide groups with respect to hydroxy addition. Such a reduction in the number of intimate linkages between lignin and cellulose would provide for easier removal of the lignin from wood during pulping and bleaching operations. Furthermore, a reduction in the internal linkages of lignin (crosslinking) would reduce its resistance to both chemical and biochemical degradation, thereby decreasing the energy and chemical requirements of delignification.

If specifically designed pseudosubstrates could be introduced into lignin, the bonding patterns and degree of polymerization could be altered in a manner which lessened the intractable nature of lignin. Ultimately, manipulation

of lignin deposition would succeed in forming a more (bio)degradable structure which still provided the plant all the functional properties of natural lignin. In so improving the degradability of lignin, the potential for formation of the chlorinated hydroxyfuranone 3 may be diminished through reduction of its precursors in waters to be chlorinated.

EXPERIMENTAL

GENERAL METHODS

Melting points were obtained on a 200 watt Mel-temp apparatus at settings between 40 to 50 volts and are uncorrected. Infrared spectra (KBr, neat) were recorded on a Nicolet 5SXC Fourier Transform Spectrphotometer. Ultraviolet-visible spectra were recorder on a Varian Cary 219 Spectrophotometer with HPLC grade methanol as the solvent. Proton nuclear magnetic resonance spectra were recorded on a Brüker NR-80 or Brüker WP 270 SV Spectrometer. Samples were dissolved in deuterated acetone (CD_3COCD_3) or chloroform ($CDCl_3$) with tetramethylsilane (TMS) added as the internal standard. High and low resolution mass spectra were recorded on a VG Analytical 7070 E-HF mass spectrometer.

High Performance (Pressure) Liquid Chromatography was performed on a Water's Tri-Module system consisting of two model 510 pumps, a 721 programmable system controller, a 730 data module, a 710B Wisp injection system, and a 481

variable wavelength UV spectrophotometer. Water's Novapak C_{18} reverse phase column (3.9 mm x 15 cm) with an apparent efficiency of 3 micron particles was used with an eluent system of 25:75 acetonitrile:deionized, distilled water. The water was adjusted to pH 3 with glacial acetic acid for the mutagen adsorption study. A Beckman Altex Zeromatic IV pH meter, standardized with room temperature buffer solutions at pH 4, 7, and 10, was used to read pH. Solvents were vacuum filtered through a Water's Durapore filter to remove dissolved gases and particulate matter above 0.45 microns.

Prior to use in a synthesis procedure, solvents and reagents were redistilled or recrystallized. Mucochloric acid **4** and N,N dimethylaniline **26** were obtained from the Aldrich Chemical Company, Inc., Milwaukee, Wisconsin, 53233. Ethyl acetoacetate **28** and vanillin **20** were purchased from Fisher Scientific Company, Fairlawn, NJ, 07410 while J.T. Baker Chemical Company, Phillipsburg, NJ supplied cyanoacetic acid **19**. Qualitative thin layer chromatography utilized either Merck DC-alufolien, or plastikfolien, Kieselgel 60 F_{254} plates. Preparatory thin layer chromatography was done using Analtech silica gel GF 500 micron plates.

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EXPERIMENTAL PROCEDURES

Adsorption Capacity Study

XAD-4 Preparation

The Amberlite XAD-4 resin, was cleaned by decantation with 3 x 1 bed volumes of distilled, deionized water followed by Soxhlet extraction for 24 hr with i) methanol, ii) acetonitrile, and iii) diethyl ether. Purified resin was stored under methanol in a dark, sealed jar until use. At that time, the resin was filtered to remove methanol and washed three times with distilled, deionized water to remove organics. This resin was vacuum dried for 10 minutes, referred to as 'vacuum dried resin', before weighing and addition to vials.

Mucochloric Acid Standardization Curve

Mucochloric Acid 4 solutions ranging in concentration from 1.0 mg to 0.10 g/l were prepared and aliquots (10 ul)

were analyzed by HPLC. Computer integrated peak areas were obtained from the chromatograms for each concentration. These areas were plotted versus concentration to obtain the standardization curve subsequently used to determine residual contamination in the adsorption experiments.

Rate of Adsorption

Mucochloric Acid **4** (5 ml @ 100 mg/l) was added to each of ten tubes. Clean, 'vacuum-dried resin' (100 mg) was added to each of nine tubes, no resin was added to the control. The tubes were sealed and placed in a shaker bath (Model 25, Precision Scientific Company, Chicago, IL, 60647) at room temperature (21°). The tubes were removed at intervals of 15, 30, 45, 60, 90, 120, 180, 240, and 300 min. The control tube was also removed after 300 min. Upon removal, the aqueous media was immediately separated from the resin and the residual concentration of Mucochloric Acid **4** was analyzed using HPLC with UV detection at 235 nm.

XAD-4 Resin Isotherms

Mucochloric Acid **4** (10.0 mg) was dissolved in HPLC grade acetonitrile (1 ml) and diluted with deionized,

distilled water in a 100 ml volumetric flask to form the standard solution. To each of four 50 ml volumetric flasks was transferred 1 ml of the standard solution via an Eppendorf 1000 ul pipette. Employing dilute solutions of either glacial acetic acid or sodium hydroxide, the pH of each of the flasks was adjusted to 3.1, 5.1, 7.0, or 9.2 (Beckman Altex Zeromatic IV) while simultaneoulsy diluting to 50 ml. With a mucochloric acid 4 concentration of 2 mg/l, these solutions were deemed the pH variables in the resin adsorption capacity study.

Five 2 ml crimp-top vials (Kimble) were alloted to each pH. Into each vial was transferred 1 ml of the corresponding test pH solution via the 1000 ul Eppendorf pipette. To each of these five vials was added either 0, 5, 10, 50, or 100 mg of 'vacuum-dried resin'. Vials were then capped with silicone lined PTFE seal aluminum caps (Kimble) and placed in a shaker bath (Precision Scientific Co.) at 20°C for two hours.

Meanwhile, 4 ml HPLC vials (Water's) were equipped with 300 ul inserts (Water's) in order to receive samples at the end of equilibration. Disposable 3 ml hypodermic syringes (Becton Dickinson) were assembled to transfer residual contaminated solution from the crimp-top vials to the HPLC analysis vials.

At the completion of the reaction (2 hr), the crimptop vials were removed from the shaker bath. The aqueous media containing any residual, unadsorbed mucochloric acid was immediately separated from the resin by inserting the hypodermic through the rubber seal and transferring part of the solution to the HPLC vials. These samples were then analyzed using the Nova-pak C_{18} column with a system of 25/75 acetonitrile/water (pH 3) to obtain the residual concentration utilizing a variable wavelength UV detector set at 235 nm with absorbance units full scale set at 0.005.

This procedure was repeated at temperatures of 50 and 70 degrees centigrade.

Degradation of Mucochloric Acid 4, contamination from the resin, and interferences from acid or base were ruled out by appropriate control.

Mutagen/Resin Study

In order to approximate actual adsorption conditions for drinking water applications, the adsorption capacity tests for the XAD-4/mutagen 3 system were conducted at ambient pH and temperature using ordinary tap water.

The mutagen 3, (29.8 mg, 0.138 mmol), was dissolved in 5 ml acetonitrile (5.97 mg/ml). 10 ul of this solution was

diluted to 1000 ul with tap water (pH 7.2) using Eppendorf pipettes (0.597 mg/ml). To each of five 2 ml crimp-top vials (Kimble) was added 10 ul of this second solution and 990 ul of tap water (pH 7.2) using the Eppendorf pipettes (0.000597 mg/ml). To each of these five vials was added either 0, 5, 10, 50, or 100 mg of 'vacuum-dried resin'. Vials were then capped with silicone lined PTFE seal aluminum caps (Kimble) and placed in a shaker bath (Precision Scientific Co.) at ambient temperature (22°C).

At the completion of the reaction (2 hrs) the crimp-top vials were removed from the shaker bath. The aqueous media containing the unadsorbed mutagen 3 was immediately removed from the vial by inserting a 1 ml disposable hypodermic needle (Becton Dickinson) through the rubber seal. The solution was transferred to 300 ul HPLC insert vials in preparation for analysis. The samples were separated using the Nova-pak C_{18} column and eluting with acetonitrile/water (75:25,pH 3) to obtain the residual concentration utilizing a variable wavelength UV detector set at 235 nm with absorbance units full scale set at 0.002.

Synthesis Procedures

3,4-dichloro-5-hydroxy-2(5H)-furanone (4) (Mucochloric Acid)

Mucochloric Acid 4 (1.5 g, 8.93 mmol) was dissolved in 10 ml warm diethyl ether. A small quantity of petroleum ether (boiling range 38.2-57.3) was added with warming as the diethyl ether evaporated. At the saturation point, the mixture was then allowed to stand at room temperature. The crystals were filtered, washed with petroleum ether, and dried in vacuo (1.34 g, 89% recovery). mp. 125°, lit. mp. 125-128°; **IR** ν_{max}^{KBr} cm^{-1}: 3368, 1773, 1761, 1643, 1236, 1153, 1118, 1026, 949, 896, 889, 777, 746; **UV** λ_{max}^{MeOH} nm(log ϵ): 227(3.40); ^{1}**H NMR** [$(CD_3)_2CO$] δ: 3.31 (1H,s,OH), 6.29 (1H,s,CH); **EIMS** m/z (%): 168 [M^+] (18), 133 [M^+-Cl] (81), 124 [M^+-COO] (38), 105 [M^+-COCl] (16), 94 [M^+-$C_2O_3H_2$] (44), 87 [M^+-CO_2ClH_2] (100).

2,3-dichloro-4-(N-phenylimino)-but-2-enoic acid (5) (Anilmucochloric Acid)

Basis for procedure from H. Simonis, Ueber die Einwirkung von primaren Aminen auf Mucobrom- und Mucochlorsaure und deren Ester, Berichte, Vol. 34, pp. 509-519, (1901).

Mucochloric Acid **4** (1.31 g, 7.8 mmol) was dissolved in methanol (4 ml). Aniline **6** (0.76 g, 8.5 mmol) was diluted with methanol (2 ml). Both solutions were chilled in a rock salt ice bath for 10 minutes and then combined. The reaction mixture turned bright yellow and crystallized. The crude product was removed by filtration, washed with 100 ml of 10% ethanol solution, and dried in vauco (1.16 g). Recrystallization from diethyl ether and petroleum ether gave Anilmucochloric Acid **5** (1.14g, 60% yield). mp. 129°C^{dec}, lit. mp. 132°; **IR** ν_{max}^{KBr}cm^{-1}: 3367, 1757, 1636, 1605, 1527, 1240, 1043, 838, 753, 418; **UV** λ_{max}^{MeOH}nm(log ϵ): 233(4.05); ^{1}**H NMR** [$(CD_3)_2CO$] δ: 6.76 (1H,s,-N=C-H), 6.87-7.30 (5H,m,C_6H_5); **EIMS** m/z (%): 243 [M^+] (20), 208 [M^+-Cl] (5), 199 [M^+-COO] (7), 180 [M^+-COCl] (11), 163 [M^+-CO_2ClH] (75), 149 [M^+-OH, -C_6H_5] (23), 136 [M^+-COH_2, -C_6H_5] (14), 128 [M^+-CO_2Cl_2H] (79).

3,4-dichloro-5-methoxy-2(5H)-furanone (16)
(Mucochloric `pseudo' Ester)

Basis for procedure from Harry H. Wasserman and Frank M. Precopio, Studies on the Mucohalic Acids. IV. Replacement of Halogen in the Pseudo Ester Series, JACS, 76, pp. 1242-43, (1954).

Mucochloric Acid **4** (9.5 g, 56.5 mmol) was introduced into a 100 ml round bottom flask containing a stir bar and anhydrous methanol (50 ml). Ten drops of concentrated sulfuric acid were added to the reaction mixture and the flask connected to a condenser equipped with a dry nitrogen inlet. The reaction solution was heated until reflux began and maintained for 13 h. The mixture was then allowed to cool to room temperature, transferred to a 500 ml separatory funnel containing distilled water (200 ml), and extracted with benzene (100 ml). The organic layer was dried over anhydrous sodium sulfate and the solvent removed in vacuo. Vacuum distillation of this lachrymatory oil at 125 torr gave the product at 58.5-59° (7.9 g, 77% yield).
IR ν_{max}^{neat} cm^{-1}: 2944, 1787, 1641, 1369, 1328, 1235, 1206, 1143, 1022, 964, 901, 748; **UV** λ_{max}^{MeOH} nm(log ϵ): 227(3.53); **^{1}H NMR** [(CD_2Cl_2] δ: 3.59 (3H,s,OCH_3), 5.85 (1H,s,CH); **EIMS** m/z (%): 182 [M^+] (17), 151 [M^+-OCH_3] (83), 147 [M^+-Cl] (90), 103 [M^+-CO_2Cl] (100), 95 [M^+-$C_3O_3H_3$] (51), 87 [M^+-$C_2O_2ClH_4$] (43).

3-chloro-4-(N-phenylamino)-5-methoxy-2(5H)-furanone (17) (Anilmucochloric 'pseudo' Ester)

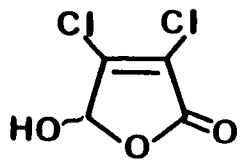
Mucochloric 'pseudo' Ester **16** (2.0 g, 11.0 mmol) was dissolved in methanol (4 ml). Aniline **6** (1.74 g, 19.0 mmol) was diluted with methanol (4 ml). Both solutions were chilled in a rock salt ice bath and then combined. The resulting light yellow solution was refrigerated overnight. Distilled water (2 ml) was added dropwise with swirling to the chilled reaction mixture. The crystals that formed were filtered and allowed to air dry, mp. 111-113°. The crude product was recrystallized from methanol twice to give a purefied product, Anilmucochloric 'pseudo' ester **17**. mp. 122-24°, lit. mp. 123-25°; **IR** ν_{max}^{KBr} cm^{-1}: 3237, 2961, 1756, 1739, 1647, 1596, 1327, 1195, 976, 713; **UV** λ_{max}^{MeOH} nm (log ϵ): 288(4.08); ^{1}**H NMR** [$(CD_3)_2CO$] δ: 3.29 (1H,s,NH), 3.41 (3H,s,OCH_3), 6.14 (1H,s,CH), 7.20-7.42 (5H,m,C_6H_5); **EIMS** m/z (%): 239 [M^+] (43), 207 [M^+-COH_4] (69), 178 [M^+-$C_2O_2H_5$] (100), 144 [M^+-$C_2O_2ClH_4$] (15), 124 [M^+-CCN-C_6H_5] (20), 116 [M^+-$C_3O_3ClH_4$] (16).

3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (3) (Mutagen MX, chlorinated hydroxyfuranone)

Aged, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone 3 was purified as follows:

The mutagen 3, (235.24 mg, 1.09 mmol) was applied to a 500 micron thick silica gel plate (Analtech). Mucochloric acid 4 was spotted on the edge of the plate and used as a reference. The plate was developed with methylene chloride:methanol (9:1,v/v). The band corresponding to the mutagen 3 was removed, eluted with methanol, and dried in vacuo (37.03 mg, 15.7 % recovery).

IR $\nu_{max}^{neat}cm^{-1}$: 3403, 1790, 1660, 1334, 1142, 1028, 963, 755, 692; **UV** $\lambda_{max}^{MeOH}nm(\log\epsilon)$: 224(3.83); ^{1}**H NMR** [($CDCl_3$] δ: 4.35 (1H,bs,OH), 6.45 (1H,s,C<u>H</u>OH), 6.84 (1H,s,$CHCl_2$); **EIMS** m/z (%): 216 [M^+] (1), 187 [M^+-OH] (23), 181 [M^+-Cl] (13), 172 [M^+-CO_2] (5), 153 [M^+-COCl] (1), 133 [M^+-CHCl_2] (100), 107 [$M^+-C_2O_3H_2Cl$] (83), [$M^+-C_4O_3H_2Cl$] (7). Exact mass calcd for $C_5H_2O_2Cl_3$: 198.9120, found: 198.9132. Exact mass calcd for $(C_5O_3H_3Cl_3)H^+$: 216.9226, found: 216.9227.



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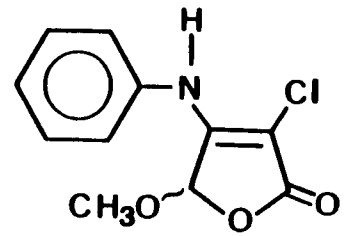

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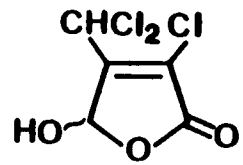

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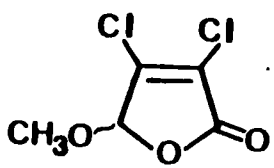

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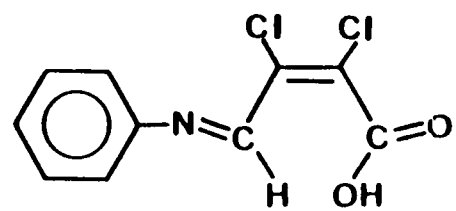

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Figure 14. Compounds Involved in the Development of the Mutagen Detection Technique.

2-cyano-3-(4-hydroxy-3-methoxyphenyl)-prop-2-enoic acid (β-cyanoferulic acid) (18)

Cyanoacetic acid **19** (7.2 g, 85 mmol) and vanillin **20** (12.0 g, 79 mmol) were introduced into a 250 ml round bottom flask containing 80 ml dry pyridine **21** and a magnetic stirrer. The flask was connected to a reflux condenser equipped with a dry nitrogen inlet. Piperidine **22**, aniline **6**, and glacial acetic acid **23** (40 drops each) were added to the flask and the temperature raised to 50°. Progression of the reaction was monitored by silica gel TLC using petroleum ether:ethyl acetate (2:1, v/v) as solvent system. At the completion of the reaction (36 h), the mixture was transferred to a 1 l Erlenmeyer flask and acidified to pH 1.3 with 50 ml additions of 2N H_2SO_4 (800 ml). The precipitate that formed during the acidification was filtered and redissolved in 10% $NaHCO_3$ (300 ml). This solution was again acidified to pH 1.3 with 2N H_2SO_4. The bright yellow purified product was filtered, washed with water, and dried in vacuo (16.6 g, 96% yield). mp. 210-214°; **IR** $\nu_{max}^{KBr}cm^{-1}$: 3475, 1685, 1578, 1520, 1317, 1271, 1211; **UV** $\lambda_{max}^{MeOH}nm(\log \epsilon)$: 346(4.27); ^{1}**H NMR** [$(CD_3)_2CO$] δ: 3.94 (3H,s,OCH_3), 7.03 (1H,d,J=8.3 Hz, C'_5H), 7.65 (1H,dd, J_1=2.1 Hz,J_2=8.3 Hz,C'_6H), 7.88 (1H,d,J=2.1 Hz,C'_2H), 8.22 (1H,s,C_3H); **EIMS** m/z (%): 219 [M^+] (100), 175 [M^+-CO_2] (22), 158 [M^+-CO_3H] (15), 132 [$M^+-C_2O_3NH$] (19), 104 [$M^+-C_4O_3NH_5$]

(14). Exact mass calcd. for $C_{11}H_9O_4N$: 219.0531579; found: 219.052765.

3-(4-hydroxy-3-methoxyphenyl)-prop-2-enenitrile (24) (ferulonitrile)

β-cyanoferulic acid **18** (5.5 g, 25 mmol) and copper powder (1.6 g) were introduced into a 250 ml round bottom flask containing dry cyclohexanol **25** (110 ml) and a magnetic stirrer. The flask was connected to a reflux condenser equipped with a dry nitrogen inlet. N,N dimethlyaniline **26** (27.5 ml, 26.3 g, 217 mmol) was added to the flask and the temperature raised until reflux began. Progression of the reaction was monitored by silica gel TLC with hexane:acetone (7:3, v/v) as solvent system. At the completion of the reaction (3.5 h), the reaction mixture was cooled to room temperature and filtered to remove the copper powder. Cyclohexanol **25** was removed *in vacuo* (55°C, 0.6 mm Hg) leaving a dark, viscous oil. This oil was diluted with chloroform and transferred to a 250 ml separatory funnel. N,N dimethylaniline **26** was neutralized with 1N HCl (4 x 50 ml) and the organic layer was washed with distilled water (2 x 75 ml). The organic layer was concentrated *in vacuo* and the resulting oil applied to a silica gel column and eluted with methylene chloride: petroleum ether (8:2, v/v). Extracts containing the product, as shown by silica gel TLC

using methylene chloride:methanol (99:1, v/v), were combined, concentrated <u>in vacuo</u>, and recrystallized twice from chloroform and petroleum ether (1.53 g, 35% yield). mp. 95-98°; **IR** $\nu_{max}^{KBr}cm^{-1}$: 3392, 2214, 1601, 1509, 1463, 1427, 1282, 1273, 1238, 1205, 1188, 1031; **UV** $\lambda_{max}^{MeOH}nm$ (log ϵ): 323(4.29); ^{1}**H NMR** [$(CD_3)_2CO$] δ: 3.89 (3H,s,OCH_3), 6.11 (1H,d,J=16.6 Hz, C_2H), 6.88 (1H,d,J=8.3 Hz, C'_5H), 7.14 (1H,dd,J_1=2.0 Hz,J_2=8.3 Hz,C'_6H), 7.32 (1H,d,J=2.1 Hz,C'_2H), 7.45 (1H,d,J=16.6,C_3H); **EIMS** m/z (%): 175 [M^+] (100), 160 [M^+-CH_3] (22), 132 [M^+-CONH] (91), 104 [$M^+-C_3H_5NO$] (36). Exact mass calcd. for $C_{10}H_9O_2N$: 175.0633286; found: 175.063370.

Ethyl-2-[1'-(4-hydroxy-3-methoxyphenyl)methylenyl]-2-onebutanoate (27) (ethyl-2-aceto ferulate)

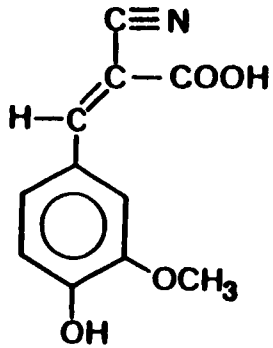
Ethyl acetoacetate **28** (17.0 g, 131 mmol) and vanillin **20** (15.0 g, 99 mmol) were introduced into a 250 ml round bottom flask containing 100 ml dry pyridine **21** and a magnetic stirrer. The flask was connected to a reflux condenser equipped with a dry nitrogen inlet. Piperidine **22**, aniline **6**, and glacial acetic acid **23** (50 drops each) were added to the flask and the temperature raised to 50°. Progression of the reaction was monitored by silica gel TLC using a solvent system of hexane:acetone (8:3, v/v). At the reaction equilibrium as shown by silica gel TLC (64 h), the solution was transferred to a 1 l beaker and acidified to pH 1.3 with 50 ml additions of 2N H_2SO_4 resulting in the formation of an oil. This oil was extracted from the aqueous solution with chloroform (3 x 100 ml). The organic extracts were combined, backwashed with distilled water (4 x 75 ml), and concentrated *in vacuo*. The resulting viscous oil was applied to a silica gel column and eluted with petroleum ether:ethyl acetate (3:1, v/v). Fractions containing the product as shown by silica gel TLC were combined, concentrated *in vacuo*, and recrystallized from chloroform and petroleum ether (7.3 g, 28% yield). mp. 111-115°; IR ν_{max}^{KBr} cm^{-1}: 3406, 1728, 1653, 1580, 1520, 1291, 1269, 1216, 1175, 1159; UV λ_{max}^{MeOH} nm(log ϵ): 336(4.24); ^1H

NMR [$(CD_3)_2CO$] δ: 1.30 (3H,t,J=7.1 Hz,$CH_2C\underline{H}_3$), 2.40 (3H,s,O=C-CH_3), 3.85 (3H,s,OCH_3), 4.33 (2H,m,J=7.1 Hz,$C\underline{H}_2CH_3$), 6.91 (1H,d,J=8.3 Hz, C'_5H), 7.12 (1H,dd,J_1=2.3 Hz,J_2=8.1 Hz,C'_6H), 7.17 (1H,d,J=2.0 Hz,C'_2H), 7.60 (1H,s,C_3H); **EIMS** m/z (%): 264 [M^+] (100), 249 [M^+-CH_3] (46), 235 [$M^+-CH_2CH_3$] (14), 219 [$M^+-OCH_2CH_3$] (31), 190 [M+-$CO_2CH_2CH_3$] (26). Exact mass calcd.: 264.0997738, found: 264.091446.

4-(4-hydroxy-3-methoxyphenyl)-but-3-en-2-one (29)

Vanillin **20** (18.0 g, 118 mmol) and dry acetone **30** (9.9 g, 171 mmol) were introduced into a 250 ml round bottom flask containing dry pyridine **21** (120 ml) and a magnetic stirrer. The flask was connected to a reflux condenser equipped with a dry nitrogen inlet. Piperidine **22**, aniline **6**, and glacial acetic acid **23** (60 drops each) were added to the flask and the temperature raised to 50°. Progression of the reaction was monitored by silica gel TLC using petroleum ether:ethyl acetate (9:5, v/v) as the solvent system. After 36 h, the mixture was transferred to a 1.5 l beaker, placed in an ice bath, and acidified to pH 1.3 with 50 ml additions of 2N H_2SO_4. This resulted in the formation of an oil. This oil was extracted from the aqueous solution with chloroform (3 x 100 ml). The organic extracts were combined, backwashed with distilled water (4 x 100 ml), and concentrated *in vacuo*. The vicsous oil obtained was applied to a silica gel column and eluted with a gradient solvent system of petroleum ether:methylene chloride (300 ml, 2:3, v/v), methylene chloride (500 ml), methylene chloride: chloroform (1500 ml, 1:1 v/v), and chloroform:methylene chloride (1000 ml, 3:2, v/v). Appropriate fractions containing the product, as shown by silica gel TLC using petroleum ether:ethyl acetate (4:3, v/v), were combined,

concentrated in vacuo, and recrystallized twice from chloroform and petroleum ether (4.2 g, 18.6% yield). mp. 126-128°; **IR** $\nu_{max}^{KBr}cm^{-1}$: 3303, 1638, 1584, 1519, 1298, 1269, 1188; **UV** $\lambda_{max}^{MeOH}nm(\log \epsilon)$: 347(4.23); ^{1}**H NMR** [$(CD_3)_2CO$] δ: 2.28 (3H,s,O=C-CH_3), 3.92 (3H,s,OCH_3), 6.62 (1H,d,J=16.2 Hz, C_2H), 6.86 (1H,d, J=8.1 Hz, C'_5H), 7.14 (1H,dd,J_1=1.9 Hz,J_2=8.1 Hz,C'_6H), 7.32 (1H,d,J=2.0 Hz,C'_2H), 7.53 (1H,d,J=16.2 Hz, C_3H); **EIMS** m/z (%): 192 [M+] (95), 177 [M^+-CH_3] (100), 161 [M^+-OCH_3] (9), 145 [M^+-OC_2H_7] (55), 134 [M^+-OC_3H_6] (17), 117 [M+-$C_3O_2H_7$] (31), 105 [M+-$C_4O_2H_7$] (12). Exact mass calcd.: 192.0786444, found: 192.077087.



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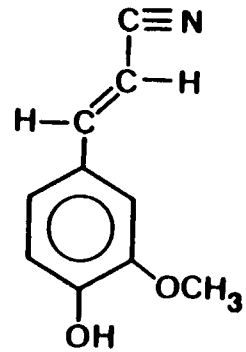

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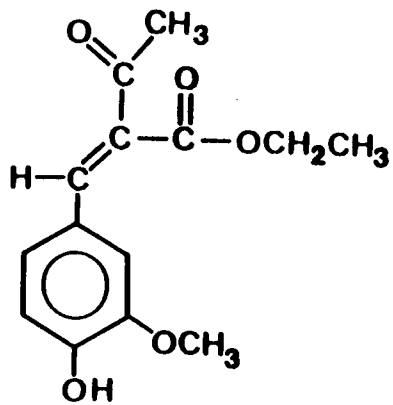

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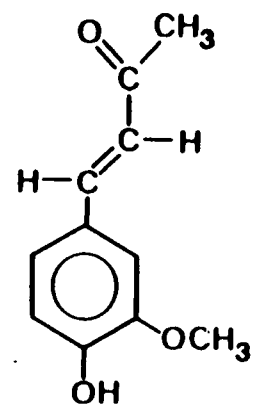

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Figure 15. Modified Substrates Synthesized for the Lignin Modification Study.

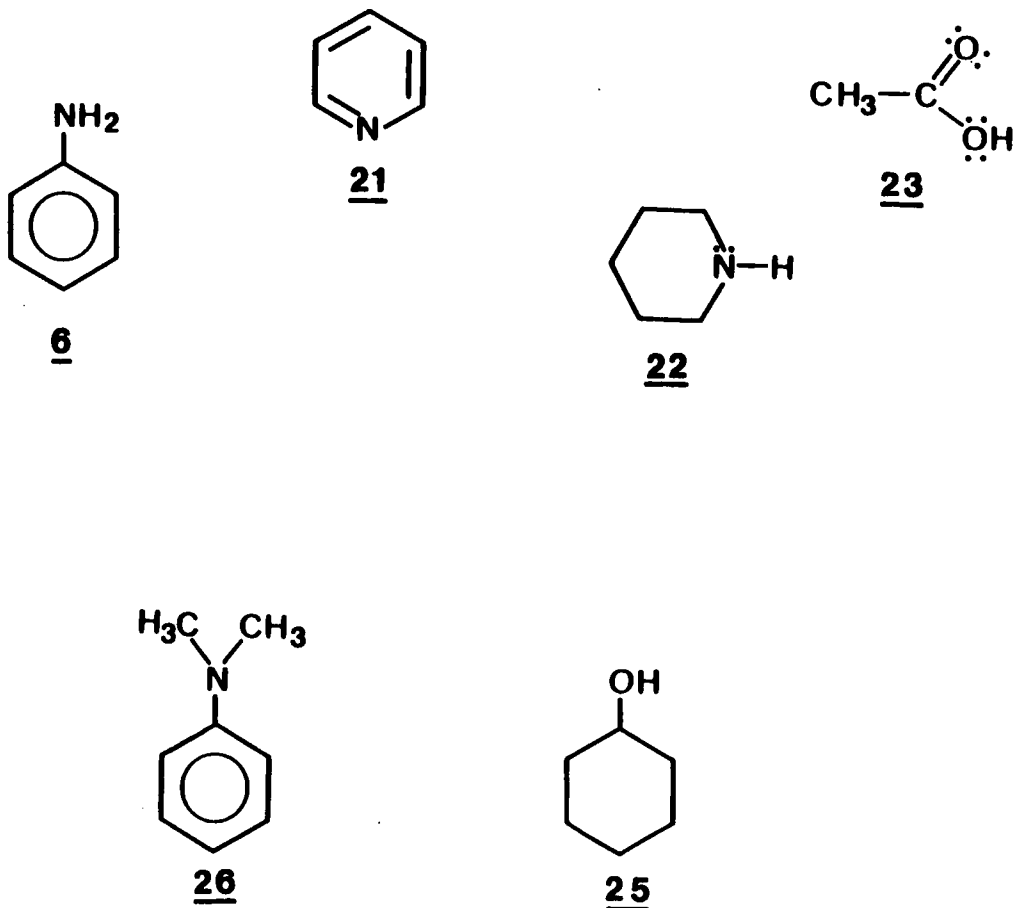


Figure 16. Compounds Utilized in the Synthesis of Modified Lignin Substrates.

RESULTS

POLYMERIC RESIN ADSORPTION STUDY

Resin Selection

Previous studies utilizing polymeric resins have demonstrated their ability to selectively adsorb organic compounds from aqueous media. Such studies indicate that treatment of aqueous solutions with appropriate resins can result in nearly complete removal of desired compounds (36). Selection of the appropriate resin is dependent on the material to be adsorbed and the solute from which it is to be removed. For example, removal of non-polar materials from polar solutions, eg., removal of chlorobenzene from water, can be accomplished using a non-polar resin such as Amberlite XAD-4. These adsorptions are favorable due to the organic material's lyophobic nature and its attraction to a material of similar properties. Weakly ionic organic molecules, eg., chlorinated phenols, can also be adsorbed by non-polar resins, but adsorption will be most efficient at a

pH where ionization of the molecules is suppressed.

The contaminant studied in this investigation was 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone **3**, an ionic compound possessing strong mutagenic properties. The compound is known to be produced upon chlorination of aqueous media containing lignin or lignin derived materials including humic acids (11). As the solutions from which the mutagen **3** would be adsorbed (eg. drinking water, swimming pool water, and first stage bleach plant effluent) are acidic or only slightly basic, the compound would not be expected to be highly ionized. Therefore, adsorption of the mutagen **3** from these solutions by a non-polar resin should be possible.

A literature review of possible resin candidates revealed that the Rohm and Haas resin, Amberlite XAD-4, could provide appropriate adsorbent characteristics. The resin is a non-polar, styrene-divinylbenzene copolymer with an average pore size of 40 Å and a surface area of 750 m^2/g. Previous studies employing XAD-4 have shown it capable of removing a variety of chlorinated organics from aqueous solutions (36). Furthermore, Amberlite XAD-4 can be quickly purified before use, works well in either batch or packed bed applications, and can be easily regenerated after utilization. For these reasons, Amberlite XAD-4 was the

adsorbent chosen for our study.

Model Compound Selection

Due to the high mutagenicity of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone 3, it was suggested that a model compound be utilized for conducting the initial adsorption tests. Similar characteristics, including lyophobicity, size, and structure, were matched with the easily accessible and less toxic compound mucochloric acid 4, thereby making it the compound of choice for the model. Conditions under which mucochloric acid 4 was preferentially adsorbed from aqueous media by Amberlite XAD-4 could be assumed to affect the mutagen 3 in a similar manner. In order to determine if Amberlite XAD-4 was suitable for removing the mutagen 3 from aqueous solutions, adsorption isotherm data was required.

Adsorption Conditions

To simulate the variety of conditions under which adsorption of the mutagen 3 might occur, isotherm data was required over a range of pH's and temperatures. The chlorinated hydroxyfuranone 3 has been isolated from highly

acidic bleaching effluents (80°C), neutral potable waters (20°C), and slightly basic swimming pool waters (25°C). The effect of temperature on the adsorption capacity of XAD-4 for the mutagen 3 (or the model 4) was determined by conducting the isotherms at 20, 50, and 70°C. Acquisition of this information could be used in evaluating the feasibility of applying a certain resin to a specic application. The isotherms were also conducted over a range of pH, (ie. 3, 5, 7, and 9) to determine the influence of pH on adsorption capacity. Reasoning behind the requirement for testing the adsorption of this compound at various pH stems from the fact that the model 4 (and presumably the mutagen 3 also) can exist in either a cyclic or open chain form (Figure 2), with the latter predominating at higher pH. Adsorption of an ionized compound by the non-polar XAD-4 resin may be reduced as compared to the adsorption of the non-ionized species present at lower pH. Therefore, the study was conducted to ascertain the degree to which this ionization would affect adsorption. Batch adsorption techniques were utilized to determine the adsorptive capacity of the resin for the model compound, mucochloric acid 4, with respect to these conditions.

Standardization Curve

Analysis of the residual (model) contaminant 4 remaining in solution after equilibration was performed by high performance (pressure) liquid chromatography (HPLC) followed by ultra-violet (UV) detection of the separated components. To ensure the accurateness of this methodology at the concentrations examined during this study, various amounts of mucochloric acid 4 were loaded onto a Nova-pak C_{18} reverse phase column and the linearity of the response determined by HPLC-UV. Computer integrated areas of these chromatograms were then plotted against the corresponding amount of the acid injected (10 ul injections) and the linear standardization curve shown in Figure 17 was obtained. Integrated chromatogram areas obtained from injection of equilibrated resin/water solutions could therefore be used to determine residual contaminant 4 concentrations provided that signals from other materials in the system did not interfere with the adsorbate signal.

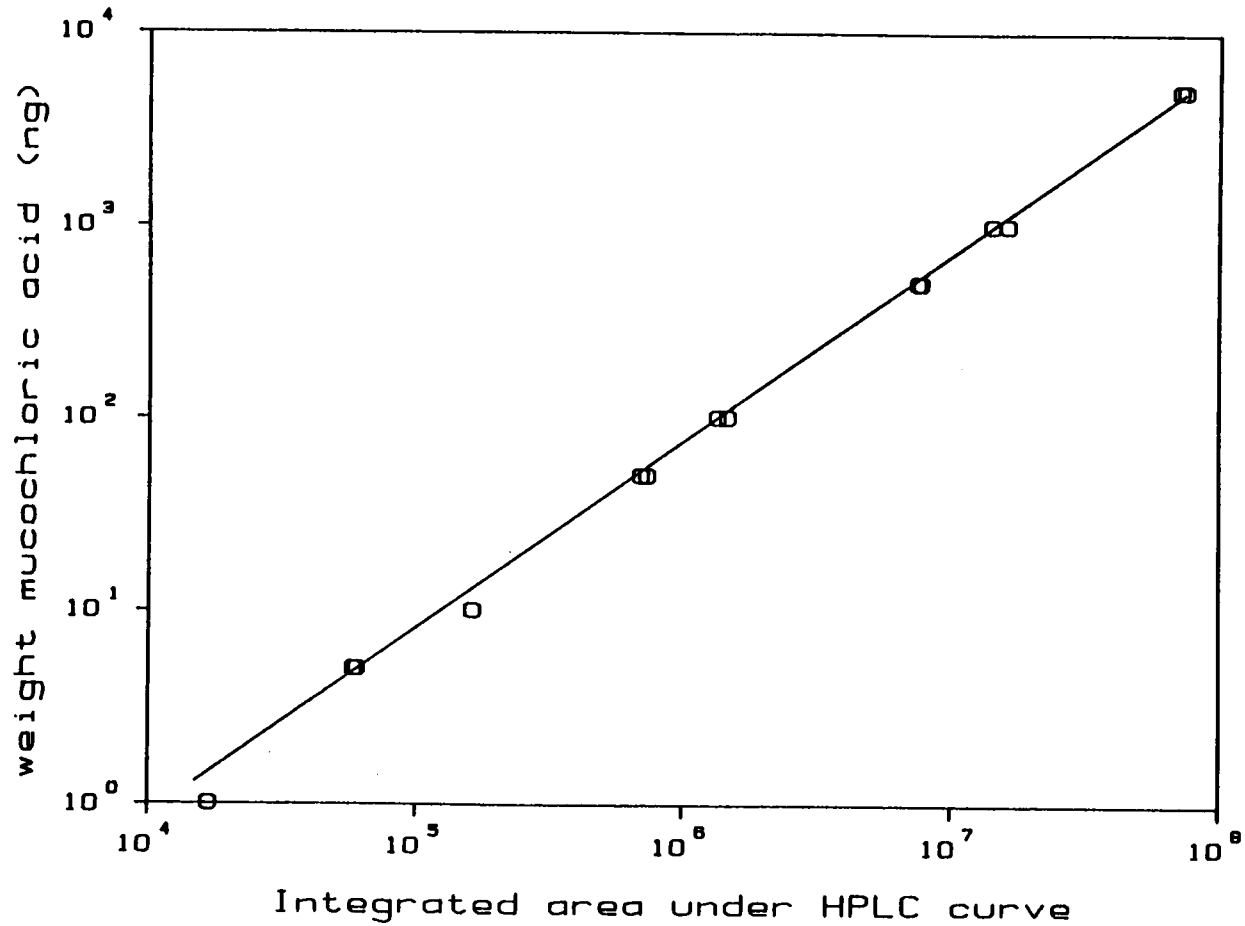


Figure 17. Linear Standarization Curve for Mucochloric Acid **4**.

Rate of Adsorption

Since adsorption of a solute from solution is time dependent, a curve was constructed to determine the time necessary to attain equilibrium between adsorbed and unadsorbed mucochloric acid **4** at pH 4.6 and ambient temperature (21°C). The curve (Figure 18) shows that the rate of adsorption of mucochloric acid **4** onto the XAD-4 resin is rapid with equilibrium being reached after approximately 30 minutes. Thus to ensure that maximum adsorption was obtained during the adsorption capacity experiments, a reaction time of two hours was adopted.

Adsorption Isotherm Experiments

The pH of the solutions used in the adsorption isotherm experiments was adjusted by the addition of either aqueous glacial acetic acid or sodium hydroxide solution. Control experiments ensured that such adjustments did not result in any absorbances which might interfere with residual adsorbate analysis. Equilibration of the resin with the acidic and basic solutions (no adsorbate) during isotherm experiments further confirmed that contamination from the resin, acid, or base would not interfere with the residual adsorbate

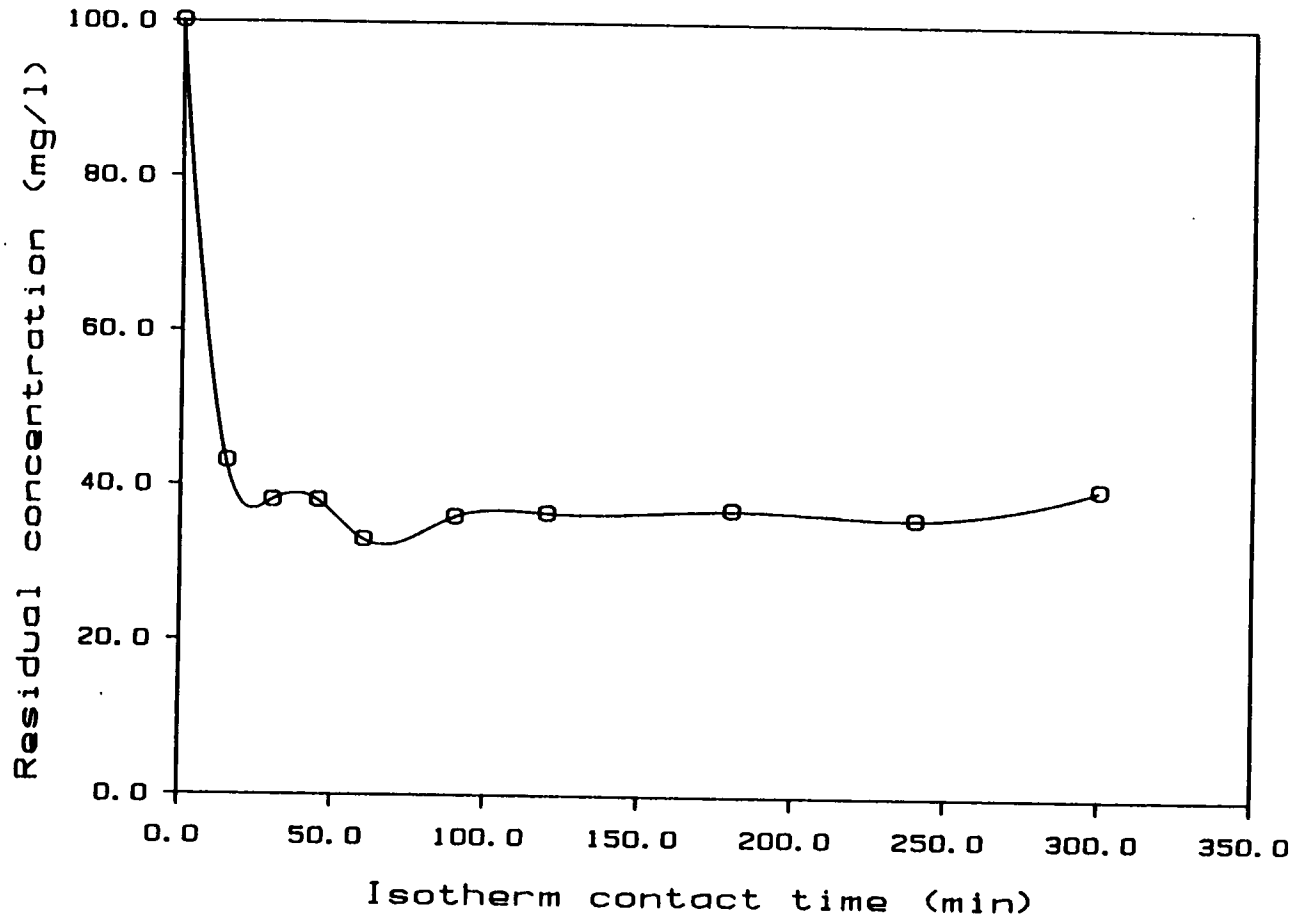


Figure 18. Rate of Adsorption of Mucochloric Acid 4 by Amberlite XAD-4.

chromatogram.

Adsorption isotherm experiments were repeated at each pH (3, 5, 7, and 9) value chosen to be studied. An aqueous solution of mucochloric acid **4** (1 ml x 2.0 mg/l) at a specified pH was added to each of five (2 ml) vials, which were then individually dosed with 0, 5, 10, 50, or 100 mg of XAD-4 resin and equilibrated at the appropriate temperature (20, 50, or 70°). The vial containing no resin was used as a control to ensure that no degradation of the mucochloric acid **4** occurred during equilibration.

Analyzing the data from the initial isotherm experiments showed the development of certain trends. Lower pH values, for instance, produced a higher adsorption of the model compound **4**. The highest level of contaminant removal was observed at pH 3, where a 96 percent removal of mucochloric acid **4** was obtained (20°). Lower removal levels of 68, 56, and 50 percent were observed for pH 5, 7, and 9, respectively (20°) (Table 2). Similar trends were seen for the isotherms at 50° (Table 3) where removals of 89, 53, 42, and 45 percent were obtained for pH 3, 5, 7, and 9, respectively. However, the significance of pH on adsorption capacity at higher temperatures was decreased. The 70° isotherm study (Table 4) showed that pH 3 still produced the highest removal, 86 percent, but that

Table 2. Percent Removal of Mucochloric Acid 4 by XAD-4 Resin
Temperature of Study 20 degrees

Resin	Acid Removed (mg/l)				Percent Removal			
(mg/ml)	pH 3	5	7	9	pH 3	5	7	9
5	1.10	0.20	0.20	0.10	58	10	10	5
10	1.39	0.30	0.25	0.20	73	16	13	10
50	1.81	1.00	0.80	0.60	95	53	42	30
100	1.82	1.30	1.06	1.00	96	68	56	50

Table 3. Percent Removal of Mucochloric Acid 4 by XAD-4 Resin
Temperature of Study 50 degrees

Resin	Acid Removed (mg/l)				Percent Removal			
(mg/ml)	pH 3	5	7	9	pH 3	5	7	9
5	0.80	0.20	0.10	0.10	42	10	5	5
10	1.10	0.35	0.15	0.15	58	18	8	8
50	1.66	0.80	0.60	0.50	87	40	31	25
100	1.70	1.06	0.80	0.90	89	53	42	45

Table 4. Percent Removal of Mucochloric Acid 4 by XAD-4 Resin
Temperature of Study 70 degrees

Resin	Acid Removed (mg/l)				Percent Removal			
(mg/ml)	pH 3	5	7	9	pH 3	5	7	9
5	0.50	0.20	0.20	0.20	25	10	11	10
10	0.80	0.30	0.40	0.40	40	16	22	21
50	1.55	1.00	0.94	1.00	78	53	52	53
100	1.72	1.22	1.22	1.36	86	64	68	72

differences between adsorbtion capabilities at various pH was significantly reduced with removals of 64, 68, and 72 percent being obtained for pH 5, 7, and 9, respectively.

Adsorption isotherm diagrams were generated by plotting the adsorptive capacity (x/m) of the resin for mucochloric acid **4** versus the residual concentration of the acid remaining after equilibration (Figures 19-22). Due to the limited number of data points gathered during this study, correlation of the isotherms to a specific adsorption model eg., Langmuir or Freundlich was not undertaken. Instead a linear relationship was assumed with the equations being generated by the method of least squares.

Analysis of this data shows that while percentage removal reached an acceptable level, the capacity of the resin for mucochloric acid **4** was low. For example, the highest removal (96%) occurred during the 20° isotherm experiment at pH 3; however, the adsorptive capacity of the resin was only 18.2 ug of mucochloric acid **4** per gram of adsorbent (100 mg resin dose) (Table 5). Lower adsorptive capacities of 12.4, 10.6, and 10.0 mg/g were observed as the pH increased (5, 7, and 9, respectively) through this isotherm study. The trend of reduced adsorptive capacity at increased pH was also observed at the higher temperatures of 50 and 70° (Tables 6 and 7).

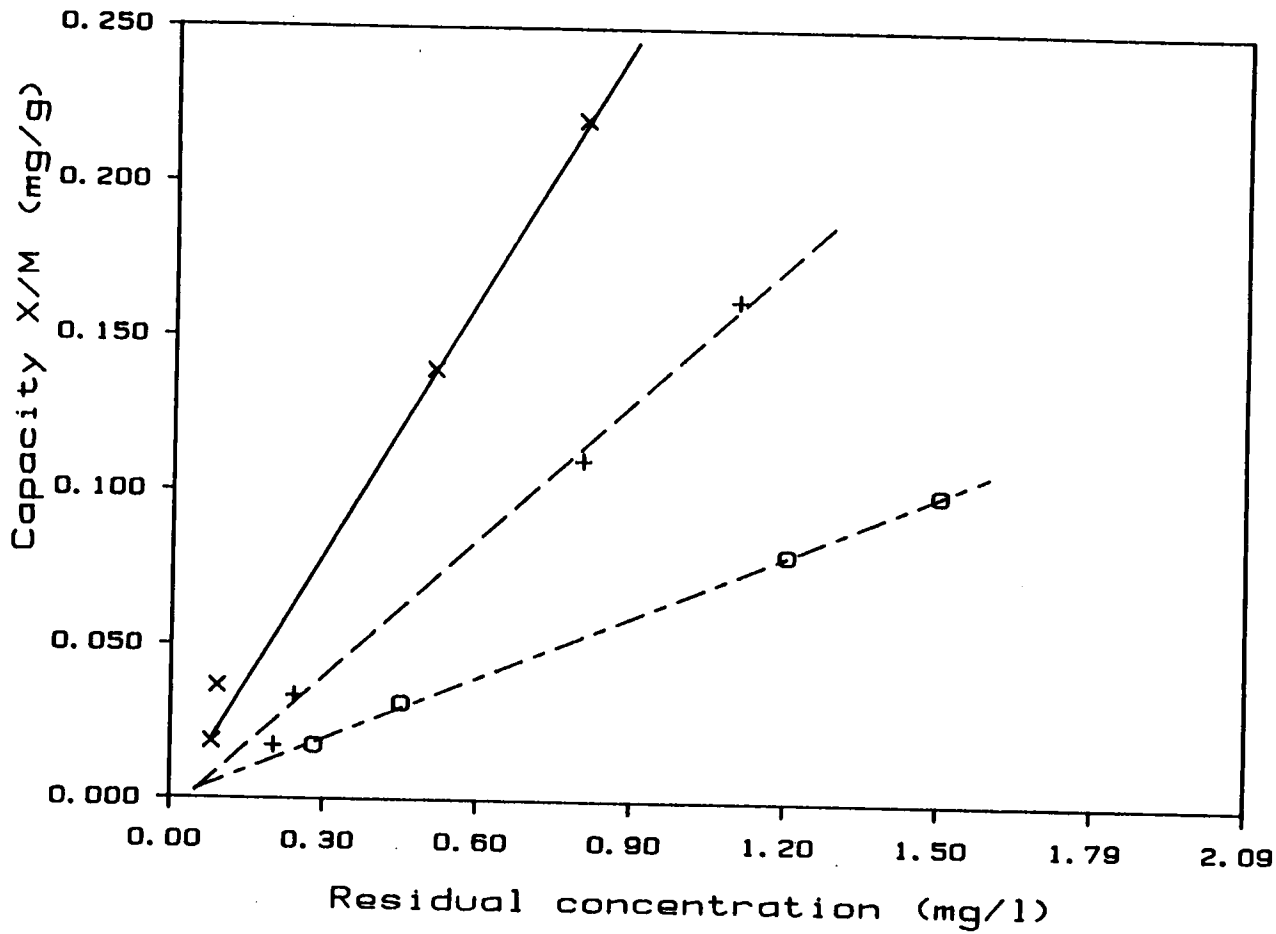


Figure 19. Adsorption Capacity of Amberlite XAD-4 for Mucochloric Acid 4 at pH 3.

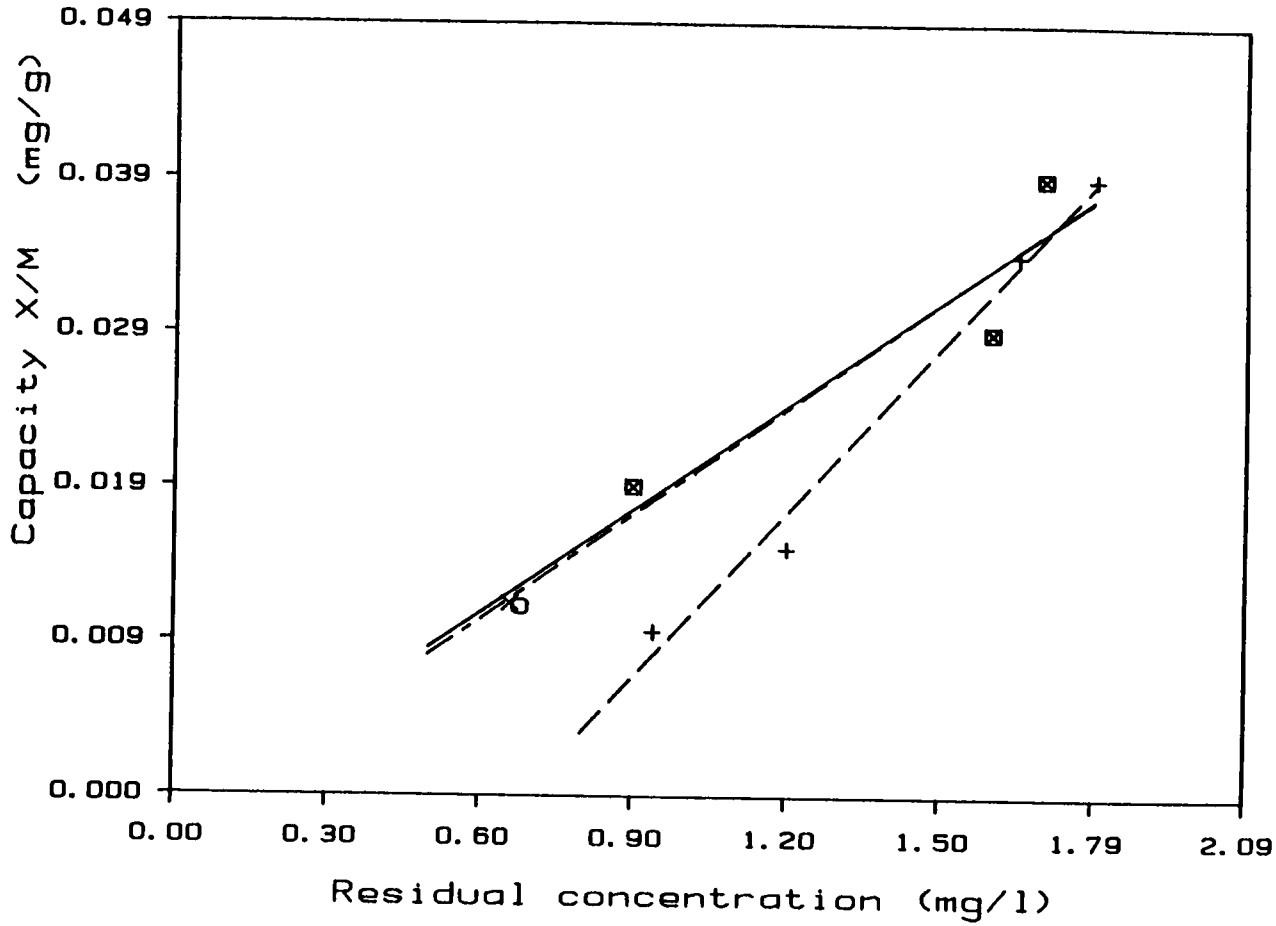


Figure 20. Adsorption Capacity of Amberlite XAD-4 for Mucochloric Acid 4 at pH 5.

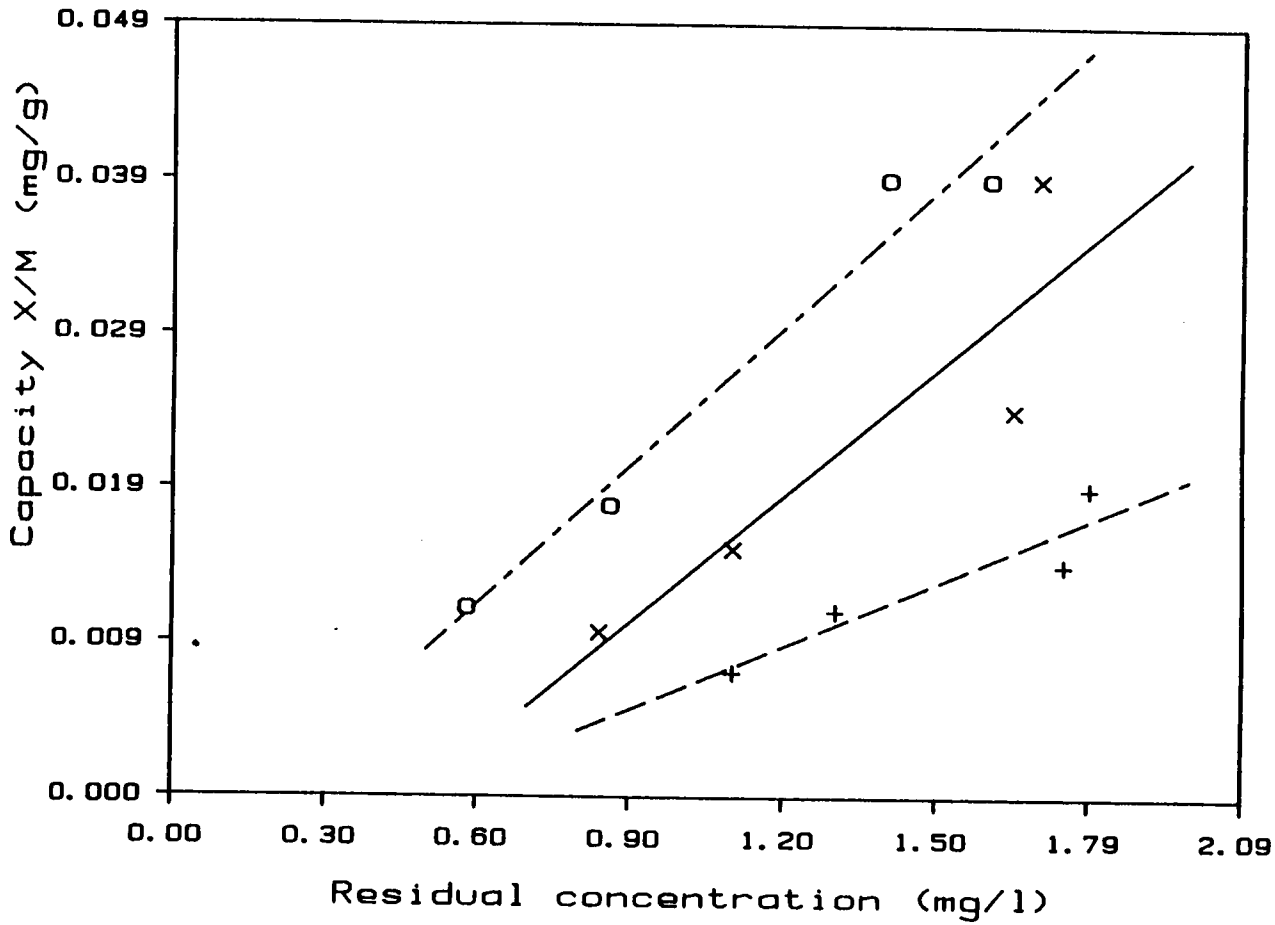


Figure 21. Adsorption Capacity of Amberlite XAD-4 for Mucochloric Acid 4 at pH 7.

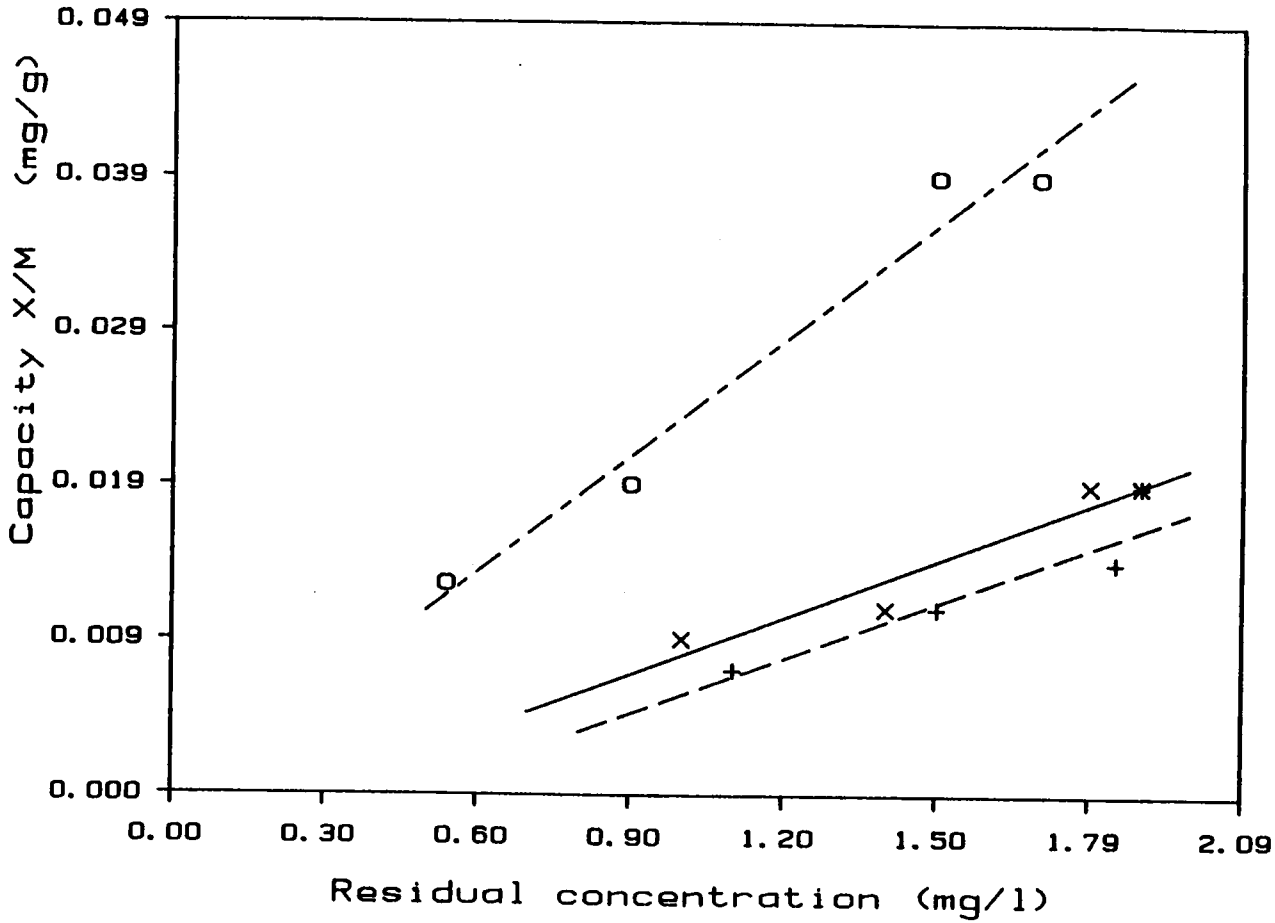


Figure 22. Adsorption Capacity of Amberlite XAD-4 for Mucochloric Acid 4 at pH 9.

Table 5. Adsorption of Mucochloric Acid 4 by XAD-4 Resin
Temperature of Study 20 degrees

Resin	Residual Concentration(ug/l)				X/M Capacity (ug/g)			
(mg/ml)	pH 3	5	7	9	pH 3	5	7	9
0	1900	1900	1900	2000	-	-	-	-
5	800	1700	1700	1900	220	40	40	20
10	510	1600	1650	1800	139	30	25	20
50	90	900	1100	1400	36.2	20	16	12
100	80	600	840	1000	18.2	12.4	10.6	10

Table 6. Adsorption of Mucochloric Acid 4 by XAD-4 Resin
Temperature of Study 50 degrees

Resin	Residual Concentration(ug/l)				X/M Capacity (ug/g)			
(mg/ml)	pH 3	5	7	9	pH 3	5	7	9
0	1900	2000	1900	2000	-	-	-	-
5	1100	1800	1800	1900	160	40	20	20
10	800	1650	1750	1850	110	35	15	15
50	240	1200	1300	1500	33.2	16	12	10
100	200	940	1100	1100	17	10.6	8	9

Table 7. Adsorption of Mucochloric Acid 4 by XAD-4 Resin
Temperature of Study 70 degrees

Resin	Residual Concentration(ug/l)				X/M Capacity (ug/g)			
(mg/ml)	pH 3	5	7	9	pH 3	5	7	9
0	2000	1900	1800	1900	-	-	-	-
5	1500	1700	1600	1700	100	40	40	40
10	1200	1600	1400	1500	80	30	40	40
50	450	900	860	900	31	20	18.8	20
100	280	680	580	540	17.2	12.2	12.2	13.6

Though not nearly as noticeable as the reduction of adsorptive capacity at increased pH, variances in adsorptive capacity with respect to temperature were also observed. At pH 3 a reduction in adsorptive capacity was noted for increases in temperature, 18.2 ug/g at 20° versus 17.0 and 17.2 ug/g at 50° and 70°, respectively (Tables 5-7). However, at other pH's, and most notably at pH 9, the effect of temperature on adsorptive capacity was not as pronounced, with slightly better adsorption capacities noted for the higher temperatures in the study, eg., 13.6 ug/g at 70° versus 10 ug/g at 20° and 9 ug/g at 50° (Tables 5-7). Similar results were noted for pH 5 and 7 (Tables 5-7), although the development of a specific trend in the data could not be discerned.

Mutagen Removal Experiments

In order to simulate adsorption conditions likely to occur if XAD-4 was applied to treatment of drinking water, the mutagen 3 adsorption study was carried out in ordinary tap water at ambient pH (7.2) and temperature (22°). As with the initial investigation, five vials (2 ml) containing an aqueous solution of the mutagen 3 (1 ml x 0.597 mg/l) were dosed with either 0, 5, 10, 50, or 100 mg of resin. Again, the vial containing no resin was used to ensure that no degradation had occurred during equilibration. The residual contaminant was analyzed twice to check for chromatogram integration error due to the low concentrations involved. Utilizing the integrated area of the chromatogram from the initial sample (before resin introduction) and the integrated areas of the residual concentration chromatograms, percentage removal and residual contaminant concentration could be obtained.

Analysis of the data from this isotherm showed that an average of 93 percent removal could be obtained under these conditions (Table 8). Graphical representation of this adsorption isotherm signifies favorable adsorption of the mutagen 3 (Figure 23). However, as with the mucochloric acid 4 study, adsorptive capacity was low, averaging 4.8 ug of mutagen per gram of resin (100 mg resin dose) (Table 9).

Table 8. Percent Removal of Mutagen 3 by XAD-4 Resin
Temperature of Study 22 degrees

Resin (mg/ml)	Contaminant Removed (mg/l) Trial	1	2	3	Percent Removal Trial	1	2	3
5	a)	0.259	0.198	0.201		43	33	34
	b)	0.217	0.196	0.279		36	33	47
10	a)	0.321	0.260	0.344		54	44	58
	b)	0.271	0.282	0.356		45	47	60
50	a)	0.505	0.597	0.522		85	83	88
	b)	0.482	0.477	0.510		81	80	85
100	a)	0.554	0.561	0.557		93	94	93
	b)	0.543	0.559	0.563		91	94	94

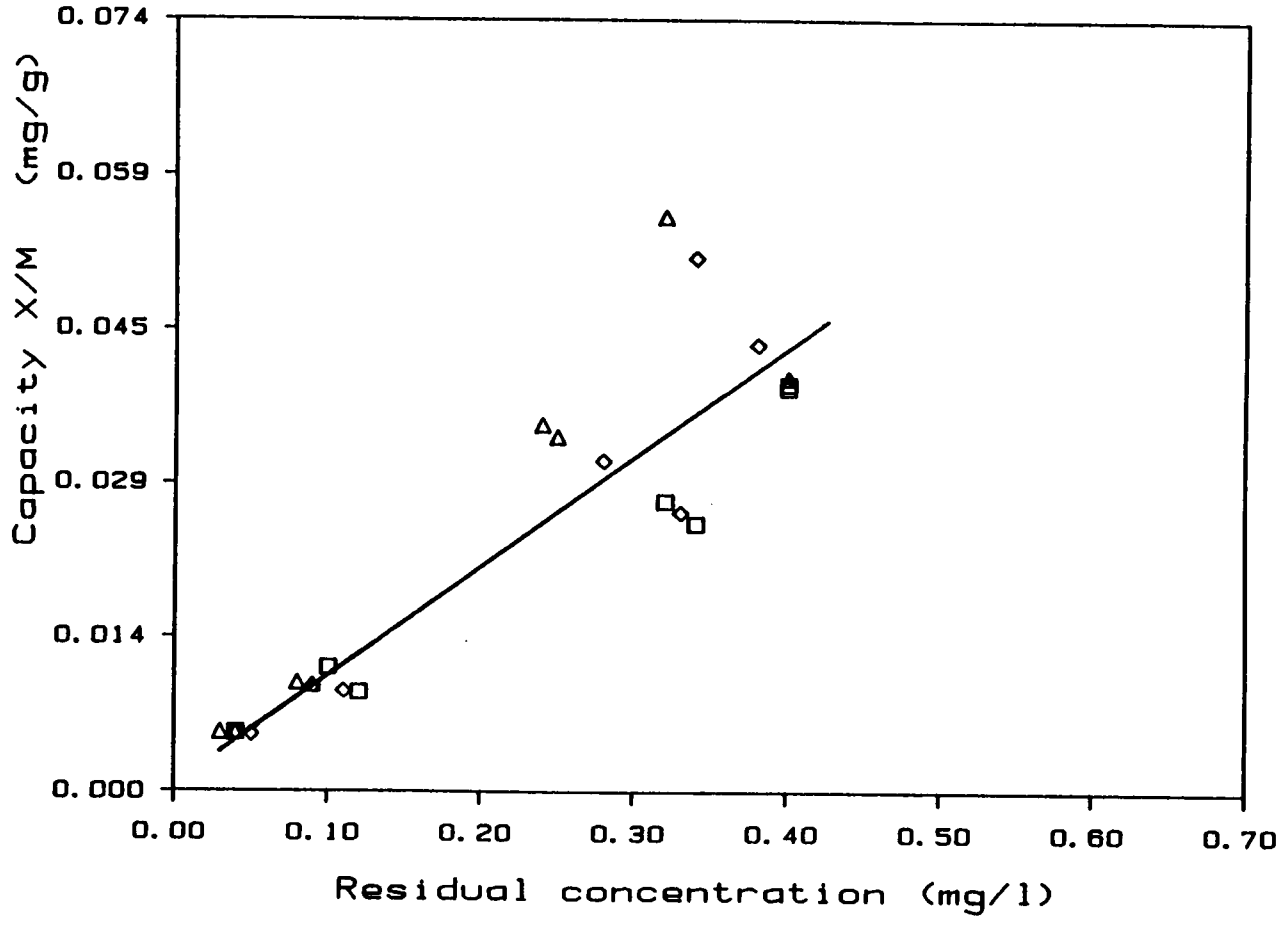


Figure 23. Adsorption Capacity of Amberlite XAD-4 for the mutagen 3 at pH 7.2 and 22°C.

Table 9. Adsorption of Mutagen 3 by XAD-4 Resin
Temperature of Study 22 degrees

Resin	Residual Contaminant (ug/l)			X/M Capacity (ug/g)		
mg/ml)	Trial 1	2	3	Trial 1	2	3
5	a) 338	399	396	51.8	39.6	40.2
	b) 380	401	318	43.4	39.2	55.8
10	a) 276	337	252	32.1	26.0	34.4
	b) 326	315	241	27.1	28.2	35.6
50	a) 92	100	75	10.1	11.9	10.4
	b) 115	119	87	9.6	9.5	10.2
100	a) 43	36	40	5.5	5.6	5.6
	b) 54	38	34	5.4	5.6	5.6

DIRECT DETECTION STUDY

Technique Development

As previously mentioned, the concentration of the mutagen 3 in chlorinated aqueous media ranges from 0.04-10 ug/l (5,9). However, current limitations in UV detection capabilities forced the aforementioned adsorption study to be carried out at higher concentration levels than would be observed for chlorinated aqueous media. As a result of the HPLC-UV limitations, a solution concentration of approximately 0.6 mg/l was required for facile detection of the mutagen 3. Consequently, the ability to directly monitor the presence or concentration of the mutagen 3 in effluents or drinking water would require the development of a more sensitive analytical technique.

Detection by adduct formation of the mutagen 3 with an intensely fluorescing chromophore could potentially circumvent such difficulties. One of the most strongly fluorescent classes of compounds are porphyrins. Detection of these compounds at nanomole concentrations and less has been reported (42). It was envisioned that an porphyrin adduct could be formed by taking advantage of the open chain (aldehyde) form of the hydroxyfuranone. In this form, the

hyroxyfuranone (mucochloric acid 4 or the mutagen 3) would be capable of reacting with a primary amine to form the corresponding Schiff's base. Reaction of the mutagen 3, in its aldehyde form, with a porphyrin macromolecule containing a primary amine, eg., 7, would yield an adduct detectable by fluorescence spectroscopy (Figure 5).

The derivatization procedures for the formation of the porphyrin adduct were initially conducted with model systems. Mucochloric acid 4 was chosen as the model for the mutagen 3, and aniline 6, as the model for an aminoporphyrin 7. Upon determination of optimal synthesis and detection techniques, derivatization was then to be performed with an aminoporphyrin 7 and mucochloric acid 4, and then finally with the mutagen 3.

Schiff's Base Technique

Anilmucochloric acid 5 was prepared via the Schiff's base reaction previously described (45). The free aldehydic group on the substrate necessary for this reaction is present due to an equilibrium reaction between the open chain and cyclic forms of mucochloric acid 4 (Figure 24). The first part of the reaction proceeds through nucleophilic addition of aniline 6 to the aldehyde carbonyl group of

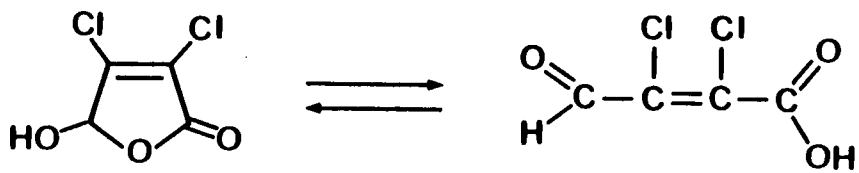


Figure 24. Resonance Structures of the Hydroxyfuranone Mucochloric Acid 4.

mucochloric acid **4** to form a carbinolamine (Figures 25 and 26). Formation of this intermediate is then followed by elimination of water to produce the Schiff's base, anilmucochloric acid **5**. Typically, dehydration of the intermediate carbinolamine is the rate limiting step for product formation (47).

Under the conditions employed, the crude product **5** was obtained in 60 percent yield with unreacted starting reagents and the dianilino-compound **31** being the primary contaminants (Figure 27). By slowing the rate of addition of aniline **6** to mucochloric acid **4** and chilling the reaction mixture during this addition, the formation of the dianilino-compound **31** could be reduced.

After purifying the product through recrystallization, the compound was analyzed by HPLC-UV. The eluent system developed for analyzing this compound which gave the best product resolution was an isocratic system of acetonitrile and water (25:75,v/v) and employed a Nova-pak C_{18} reverse phase column. Chromatograms of mucochloric acid **4** and anilmucochloric acid **5** utlizing this system gave retention volumes of 5.87 and 12.53 ml, respectively. However, analysis of the recrystallized adduct always showed the presence of two signals, the larger of which corresponded to anilmucochloric acid **5**, the lesser one corresponding to the

Mechanism:

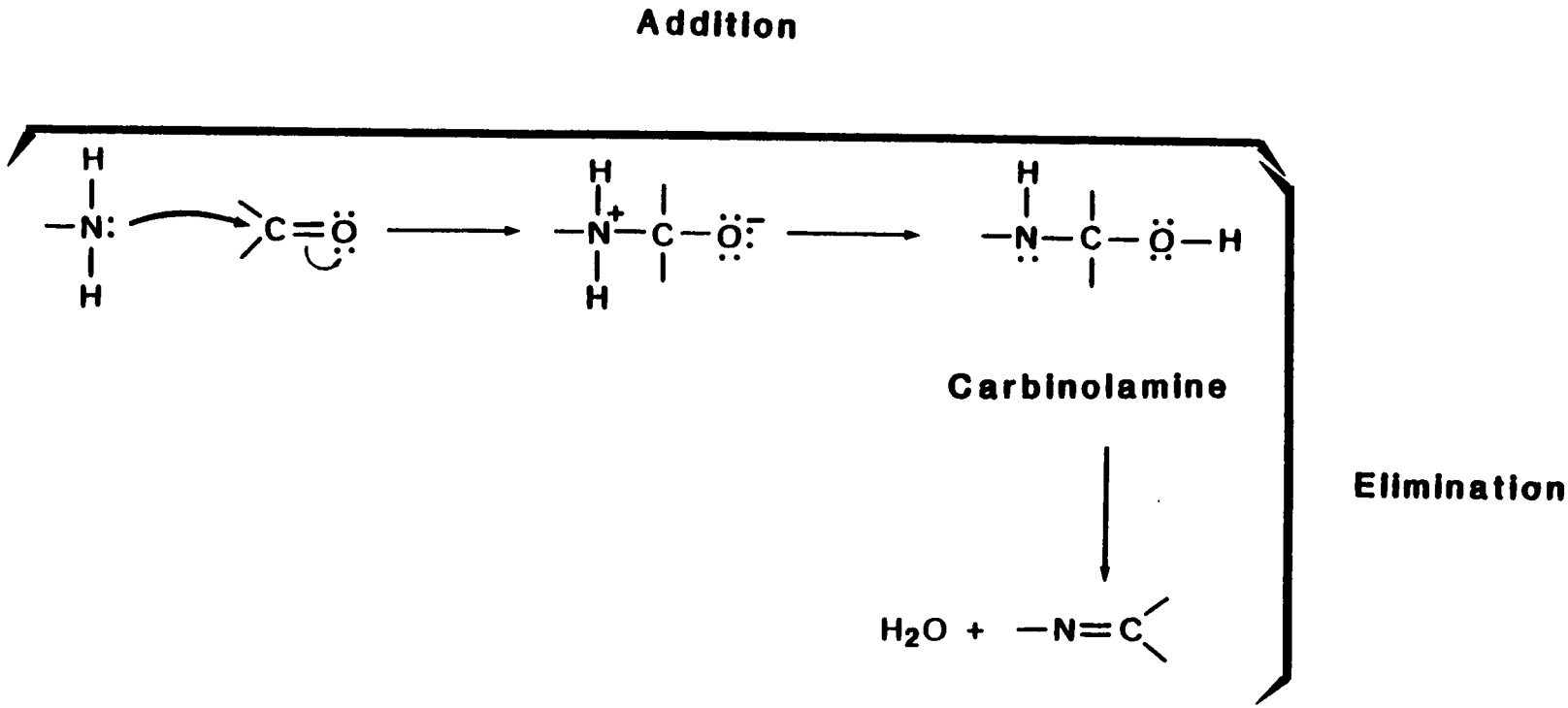


Figure 25. Mechanism for the Schiff's Base Reaction.

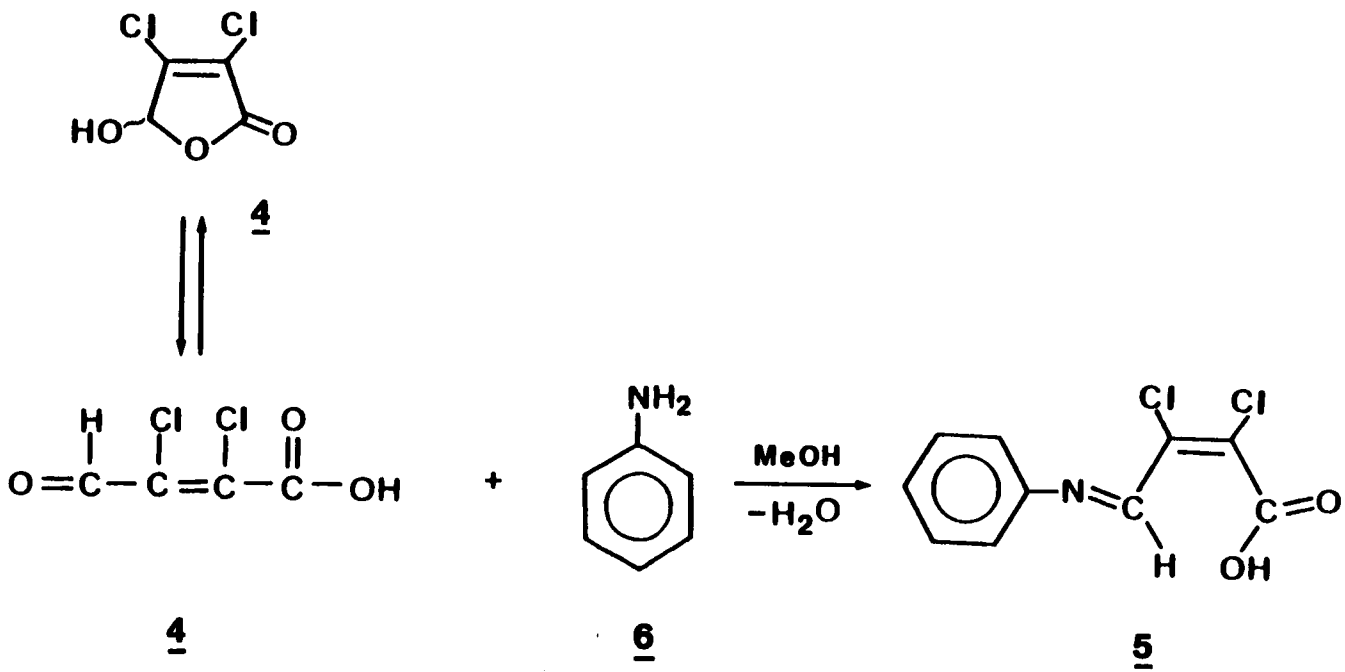


Figure 26. Synthesis of Anilmucochloric Acid 5.

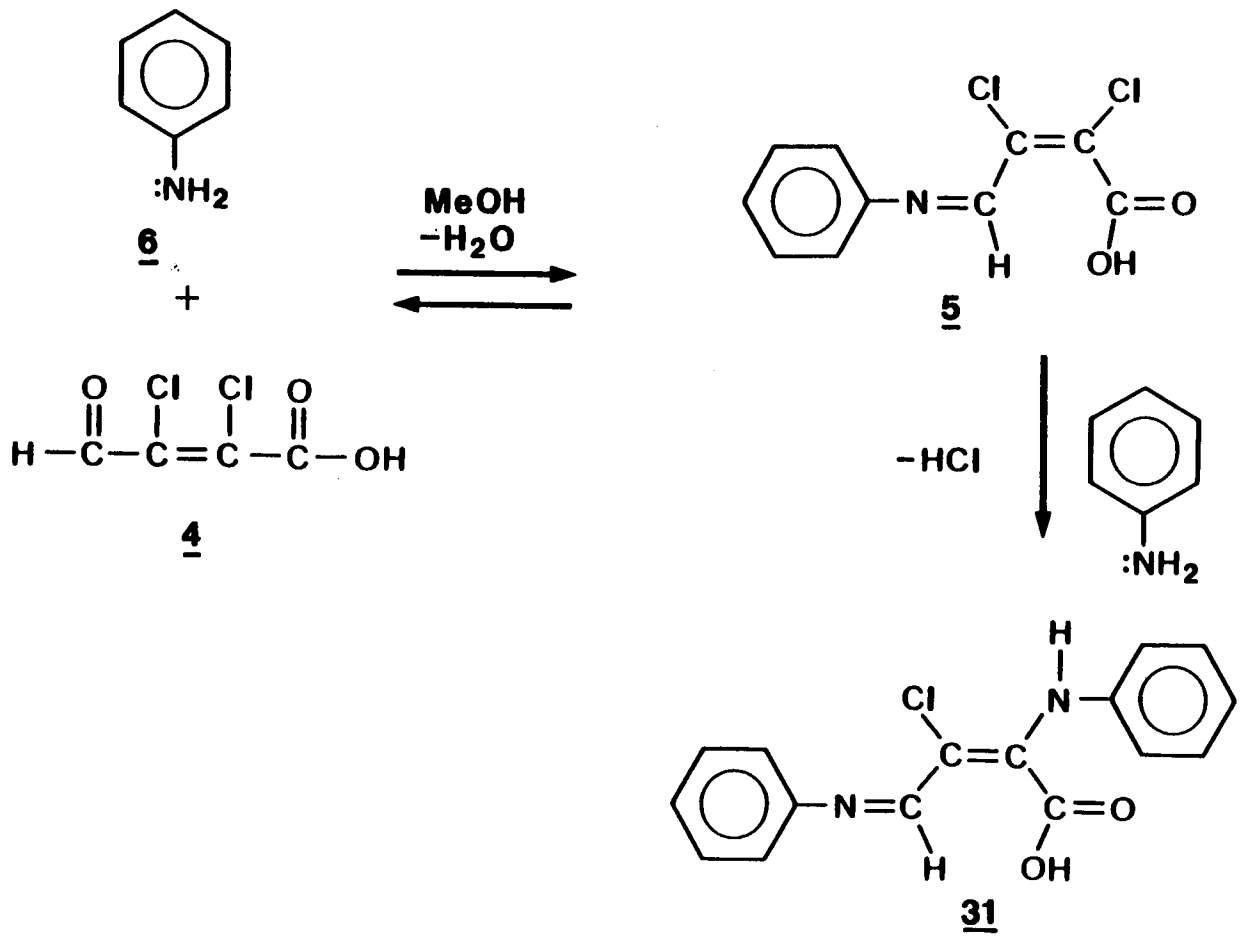


Figure 27. Dianilino-compound 31 Produced During the Synthesis of Anilmucochloric Acid 5.

starting material, mucochloric acid **4**. The presence of the mucochloric acid **4** signal was attributed to partial hydrolysis of the adduct during elution. Thus, due to the inability to quantitatively produce and maintain the simple model adduct, an alternate approach to forming the mutagen-porphyrin adduct was taken.

Derivatization of Mucochloric 'pseudo' Ester

Avoidance of the formation of the dianilino-compound **31** was accomplished through hemiacetal formation as shown in Figure 28. Though the hemiacetal **16** thus formed would be unable to form a Schiff's base, a substitution reaction would still be possible.

Formation of the 'pseudo' ester **16** was achieved by protonation of the hydroxyl group on the solvent, methanol, by acid catalysis (Figure 28). Electrophilic attack by this protonated alcohol, followed by elimination of water, produces the desired methylated product. The product thus formed was purified through vacuum distillation to give the clear lachrymatory oil, mucochloric 'pseudo' ester **16** (77 percent yield).

The 'pseudo' ester **16** was then reacted with aniline **6** to produce the substitution product, anilmucochloric

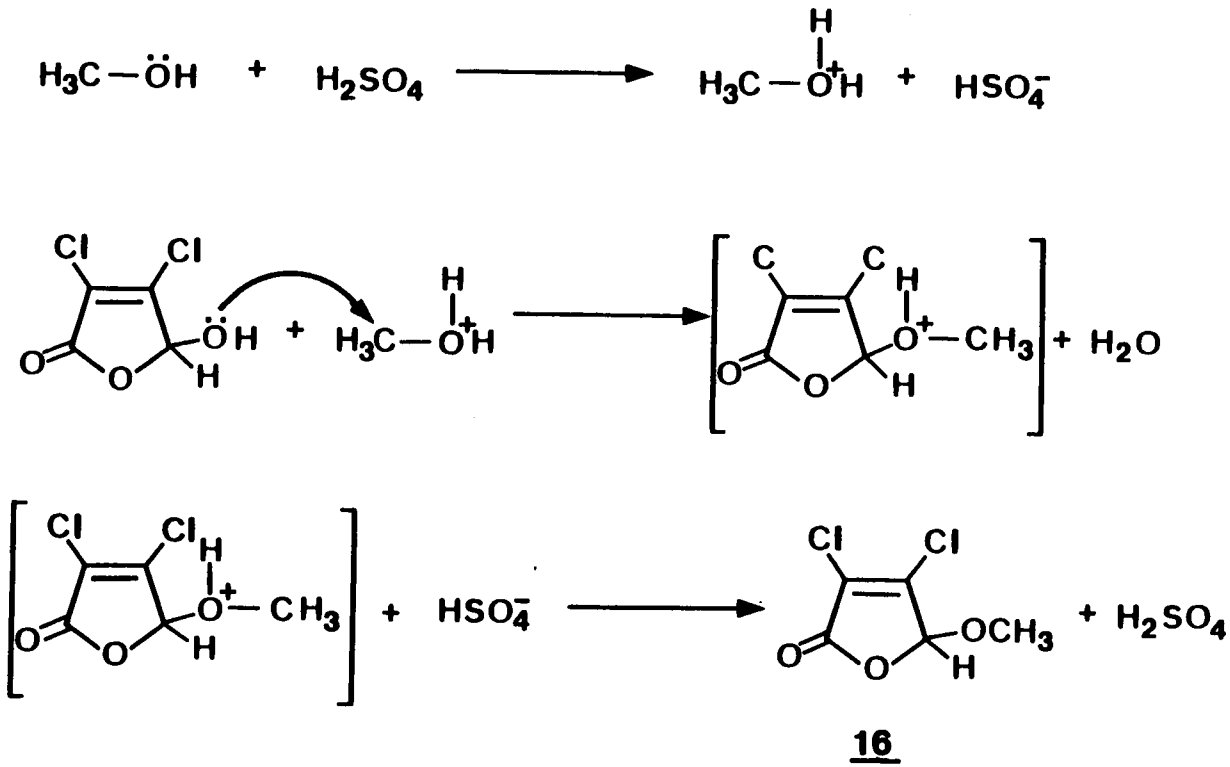
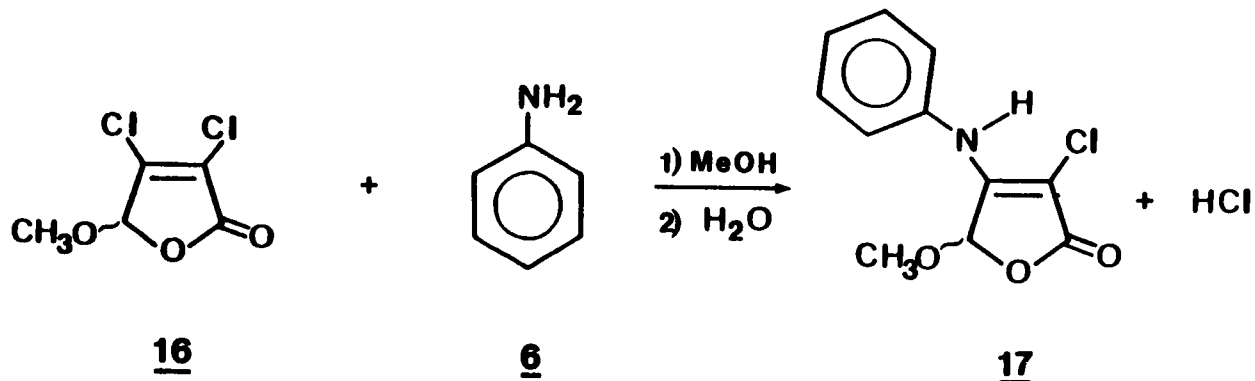


Figure 28. Synthesis of Mucochloric 'pseudo' Ester **16**.

'pseudo' ester 17, in an overall yield of 43 percent (Figure 29). HPLC-UV analysis utilizing the acetonitrile/water (25:75,v/v) eluent system and the Nova-pak C_{18} reverse phase column showed that the adduct was stable since the chromatogram produced only one signal. However, due to the unquantitative nature of the mechanism and the expected formation of additional products when applied to the mutagen 3, derivitization of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone 3 was not undertaken.



16 6 17

Figure 29. Synthesis of Anilmucochloric 'pseudo' Ester 17.

LIGNIN MODIFICATION STUDY

As long as chlorine is utilized to purify drinking waters, or bleach chemical pulps, the formation of chlorinated toxic compounds will continue. However, it has been shown that a reduction in the toxicity of drinking water can be achieved through removal of carcinogen precursors prior to chlorination (50). This is usually accomplished through a reduction in the total organic carbon (TOC) level of waters to be chlorinated by processes such as adsorption. Such adsorption processes could similarly be applied to the removal of mutagen precursors before chlorination of potable waters. Adsorption techniques can also be applied following chlorination, and as such, have proven successful at reducing the level of toxic contaminants present in drinking waters (36). While either scenario would be suitable for treating the relatively low levels of organics (TOC's) present in drinking water, such processes would not be applicable to the pulp and paper industry.

Bleaching processes, the final step in chemically delignifying pulp, produce effluents containing large quantities of chlorinated organics. As such, application of adsorption methodologies to the aqueous streams would not be

economically viable. Research was therefore initiated to further investigate the process of lignification, specifically, those factors relating to lignin deposition. Expansion of the knowledge base in this area could eventually lead to modulation of the quantity, or intractable nature, of lignin in terrestrial plants.

Modification Technique

Studies concerned with the formation of the mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone 3, have shown it to be produced upon chlorination of lignin or lignin-derived materials, eg., humic acids (33). One mechanism for the formation of the mutagen 3 has been proposed by Lindstrom and Osterberg (51). They suggest that the mutagen 3 is derived from chlorinated ortho-benzoquinones, which are, in turn, proposed to be formed from chlorinated residual lignins (51). As such, it could be assumed that a reduction in the lignin content of the wood to be pulped should cause a corresponding decrease in the amount of mutagen 3 produced during chlorine bleaching. Moreover, any increase in the susceptibility of the lignin to chemical degradation, eg., pulping, should also produce an increase in the efficacy of chemical bleaching. If this

could be achieved, the delignification of wood would therefore be expected to be less energy and chemical intensive. As such, the necessity of using chlorine to obtain a desired level of pulp brightness might be lessened, or even negated, thereby reducing, or eliminating, the potential for formation of the mutagen 3.

A study was therefore undertaken to investigate the possibility of altering lignin deposition in terms of both structure and quantity. A reduction in the quantity or intractability of lignin would presumably improve both the chemical and biochemical degradability of lignin. Such a modification of lignin could be accomplished through introducing into the lignification process compounds capable of blocking specific enzymatic pathways, or through incorporating into the lignin polymer i) more chemically-labile sub-structures, ii) sub-structures incapable of crosslinking, or iii) sub-structures incapable of bonding with polysaccharides. Judicious uptake of modified natural substrates by intact growing plants could achieve such a modulated lignin. Modified substrates were therefore designed for this investigation to act as potential inhibitors to, or pseudosubstrates for, lignification. The synthesized modified substrates were then tested to determine their effect on the lignification process.

Inhibitors, or pseudoirreversible inhibitors, can reduce the formation of lignin within the plant by binding to enzymes involved in lignification, thereby reducing the formation of lignin precursors, monolignols **8**, **9**, and **10**. Studies by Amrheim, et. al. (20, 21, and 22) and Grand, Sarni, and Boudet (23) have shown that uptake of such inhibitors by plants offers the potential for reducing lignin deposition. However, the extent to which lignin content can be reduced without causing detrimental effects in the physiological function of lignin is currently unknown.

Pseudosubstrates would not necessarily reduce the lignin content, but rather, affect lignification through their incorporation into the polymer. As such, they would offer the ability to alter the structure of lignin without reducing its quantity. A reduction in crosslinking, cell wall binding, or carbon-carbon linkages could result from the addition of designed substrates into the lignin polymer. Such an introduction of specifically designed natural substrates into the lignification pathway of growing plants could result in the formation of a less intractible lignin.

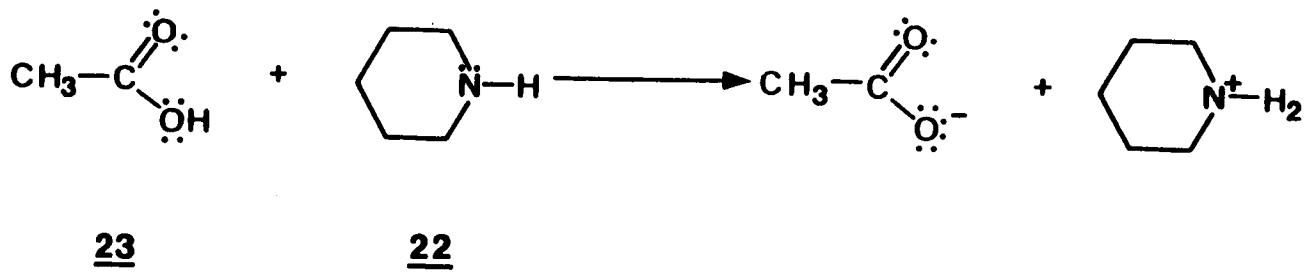
Modified substrates synthesized for the initial phase of this investigation were designed to enter the ligninification process along the phenylpropanoid pathway.

For example, a cyano group was added to the beta carbon of ferulic acid through reaction of vanillin and cyanoacetic acid. If successfully incorporated into the lignification pathway and reduced to the alcohol, this structure might alter the bonding patterns within lignin through its ability to extend the phenoxy radical through the cyano group. Introduction of a modified aldehyde, specifically a methyl ketone produced by reacting vanillin with acetone, into the pathway may result in the incorporation of a less reactive secondary alcohol into the lignin polymer. Inhibition of crosslinking within the growing polymer could thereby be achieved. Furthermore, the addition of a sterically hindered group to this ketone, eg., an ethyl aceto group, at the beta carbon may further inhibit its potential for internal bonding within the developing lignin structure. Additionally, incorporation of such a compound into the lignin polymer would alter the main bonding patterns of lignin through the compound's inability to bond at the beta carbon (refer to Figures 11-13).

Modified Substrate Synthesis

β-cyanoferulic acid **18**, ethyl-2-aceto-ferulate **27**, and (4-hydroxy-3-methoxyphenyl)-but-3-en-2-one **29**, were all synthesized by base catalyzed condensation in yields of 96, 28, and 18.6 percent, respectively. Vanillin **20** and an appropriate reactant eg., cyanoacetic acid **19**, ethyl acetoacetate **28**, or acetone **30**, were added to a solution of pyridine **21**, containing piperidine **22**, and aniline **6**. Glacial acetic acid **23** was then added to initiate the reaction. Piperidine **22** abstracts a proton from glacial acetic acid **23** to form the strong base, CH_3COO^- (Figure 30). This compound then abstracts an acidic proton from the reactant **19**, **28**, or **30** (Figures 30 and 31). Thus formed, these anionic compounds react with vanillin **20** through nucleophilic addition (Figures 32 - 34). Acid catalyzed elimination of water is the final step in product formation.

Ferulonitrile **24** was formed by a copper catalyzed decarboxylation of β-cyanoferulic acid **18** in 35 percent yield. The exact mechanism for this reaction is as yet unspecified.



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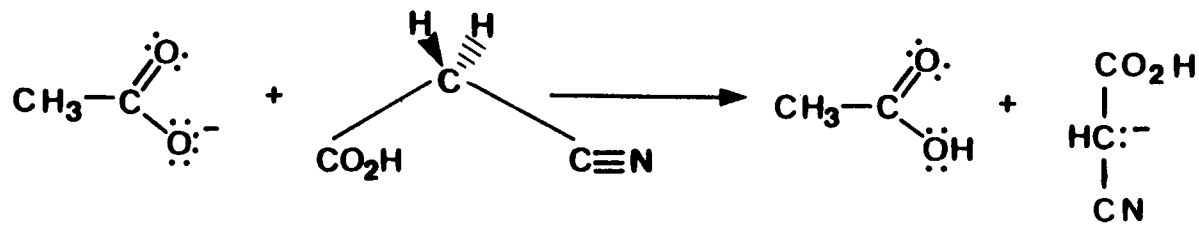

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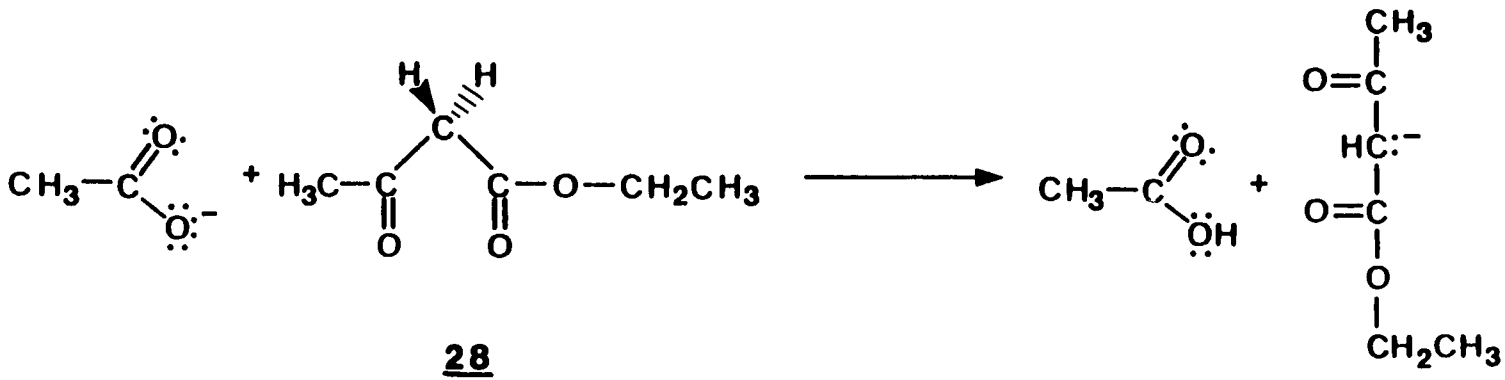
Figure 30. Proposed Mechanism for the Formation of the Cyanoacetic Acid 19 Anion.



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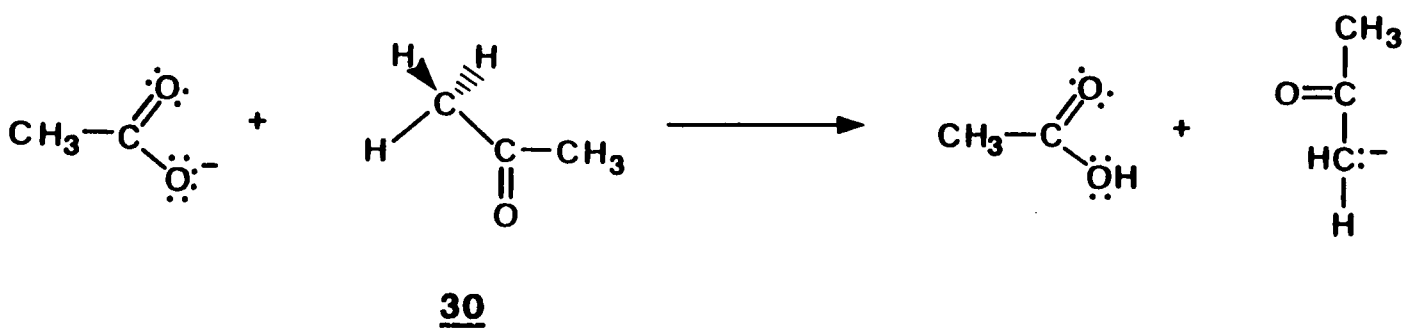

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Figure 31. Proposed Mechanism for the Formation of the Ethyl Acetoacetate **28** and Acetone **30** Anions.

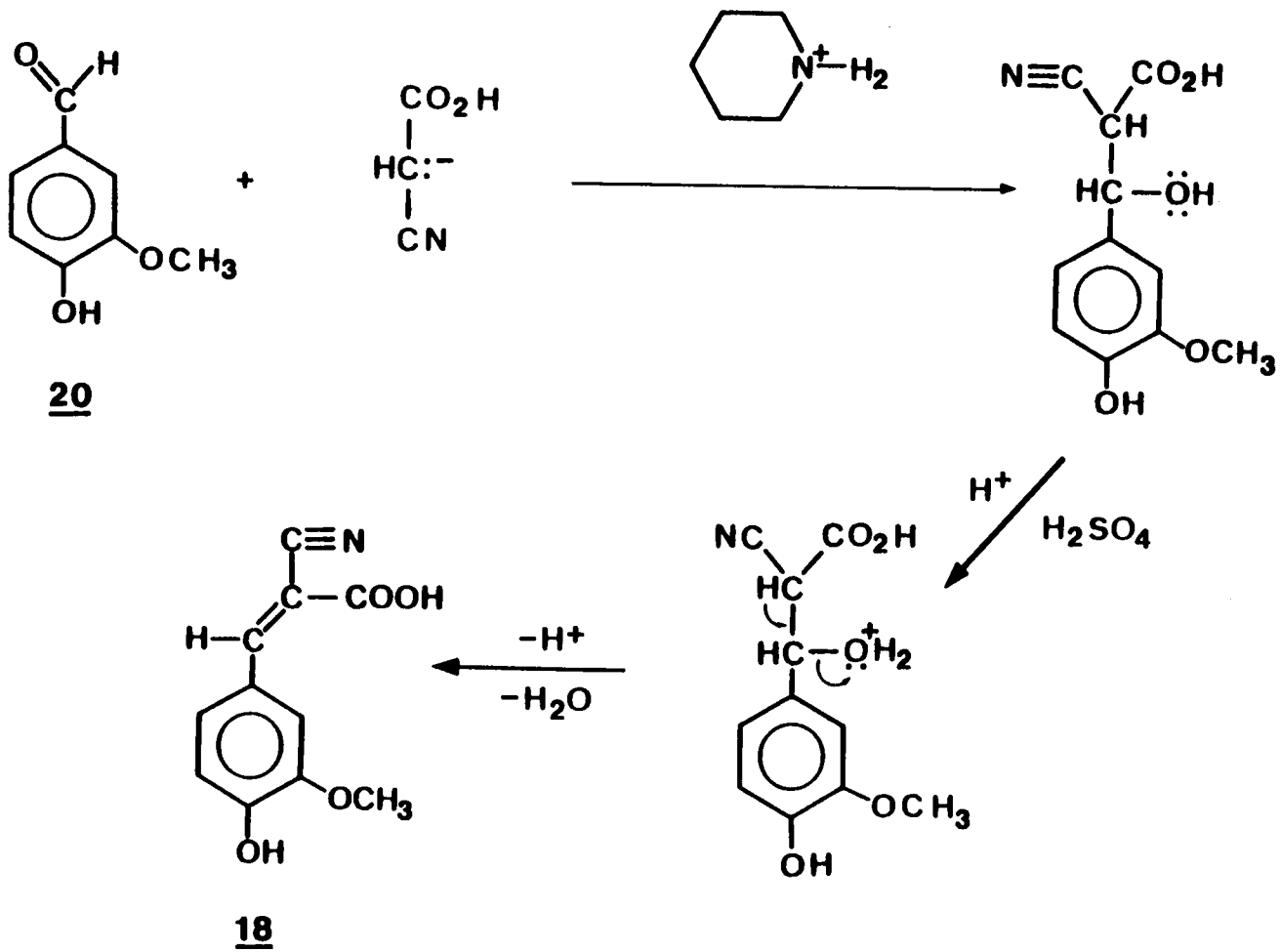

Figure 32. Synthesis of β-cyanoferulic Acid **18**.

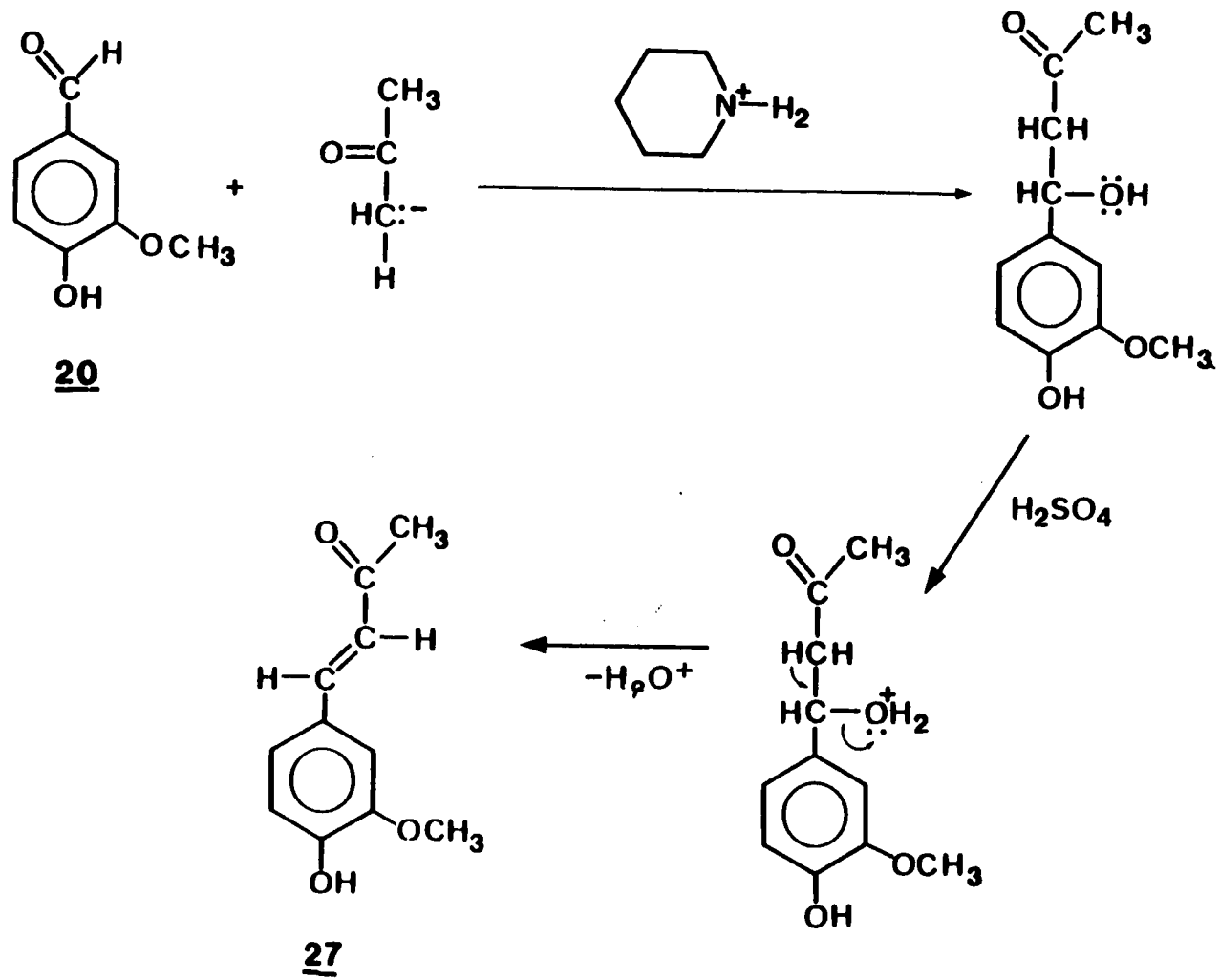

Figure 33. Synthesis of Ethyl-2-aceto Ferulate 27.

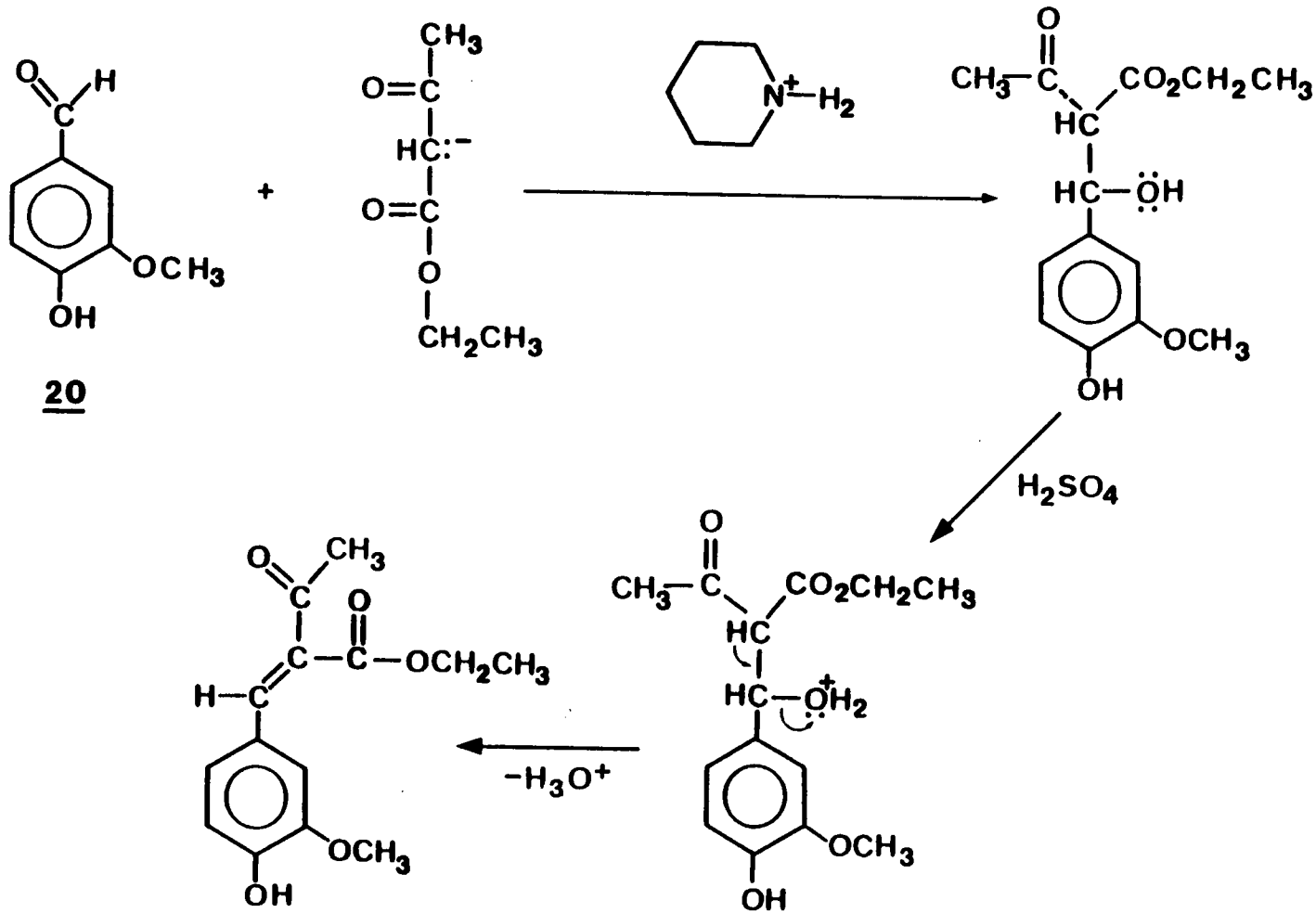

Figure 34. Synthesis of (4-hydroxy-3-methoxyphenyl)-but-3-en-2-one **29**.

Modification of Lignin in situ

Investigation of the effect of these synthesized modified substrates **18**, **24**, **27**, and **29** on lignification of three month old poplar stems was conducted by fellow researcher, T. R. Lantzy utilizing the established procedure of Grand, Sarni, and Boudet without modification (23).

In general, feeding experiments were conducted by incubating poplar (Populus X euramericana) cuttings individually with C^{14} labelled cinnamic acid solutions until adsorption of the solution was complete. Following uptake, the cutting was transferred to a solution containing a modified lignin precursor and allowed to adsorbed this solution over a 96 hour period. The poplar stem was then harvested, freeze-dried, and extracted with benzene:ethanol (2.5:1, v/v, 12 hr) to remove any soluble extractives. Comparison of the radioactivity in this insoluble fraction allows us to determine the effect of uptake on each monolignol substrate and the metabolism of C^{14} labelled cinnamic acid into lignin.

The results obtained are shown in Tables 10 and 11. Interestingly, each of the compounds **18**, **24**, **27**, and **29**,

resulted in an increase, and not a decrease, in the uptake of C^{14} labelled cinnamic acid into the insoluble fraction as compared to the control, ie., ligninification was apparently stimulated significantly. Amrheim, et. al. (20, 21, and 23), along with Grand, Sarni, and Boudet (23), have previously synthesized substrates which inhibited the plant's incorporation of C^{14} labelled cinnamic acid. As such, compounds are now known which can positively and negatively affect the process of lignification.

Of the modified substrates tested during this study, β-cyanoferulic acid **18** had the largest effect on the incorporation of C^{14} labelled cinnamic acid into the insoluble fraction. The modified substrate **18** produced an average activity of 1,638,777 disintegrations per minute, (DPM's), 1,436,389 DPM's over the control. Overall, the plant utilized 97 percent of the fed C^{14} labelled cinnamic acid, 59 percent of which was incorporated into the insoluble fraction of the stem.

(4-hydroxy-3-methoxyphenyl)-but-3-en-2-one's **29** and ethyl-2-aceto-ferulate's **27** affect on the uptake of the labelled acid was comparable to that of β-cyanoferulic acid **18** (98 and 99 percent, respectively). However, a slightly reduced incorporation of the label into the stem fraction

Table 10. Incorporation Results of Cinnamic Acid Uptake into Poplar Lignin As Affected by Modified Natural Substrates*

Compound		DPM Fed	Freeze-dried Plant Weight		DPM of Sample
Control	a)	6,329,700	188.24	mg	209,347
	b)	3,910,101	160.41		245,595
	c)	3,840,991	192.00		152,222
I.	a)	4,409,925	77.41	mg	1,564,252
	b)	3,733,899	119.55		2,016,154
	c)	3,765,216	149.75		1,335,925
II.	a)	3,984,123	195.14	mg	351,271
	b)	3,808,467	145.69		1,252,532
	c)	3,760,068	136.15		1,436,378
III.	a)	3,912,168	138.15	mg	827,231
	b)	3,650,556	101.35		623,243
	c)	3,770,754	125.96		674,436
IV.	a)	3,654,409	130.51	mg	1,246,666
	b)	3,749,733	113.73		1,358,337
	c)	unreported	98.00		1,027,182

Compound:

I. β-cyanoferulic acid **18**
II. Ethyl-2-acetyl ferulate **27**
III. Ferulonitrile **24**
IV. (4-hydroxy-3-methyoxyphenyl)-But-3-en-2-one **29**

*Effects of Modified Lignin Precursors, using the Methods of Grand, Sarni, and Boudet (23), were obtained by Mr. T. R. Lantzy

Table 11. Percent Incorporation of Cinnamic Acid into Poplar Lignin As Affected by Modified Natural Substrates*

Compound	% of the Fed Activity that was Taken up by the Plant	% of the Activity in the Insoluble Stem Fraction
Control	83.4	9.7
I.	96.9	59.3
II.	99.0	36.2
III.	84.0	29.6
IV.	98.2	45.2

Compound:

I. β-cyanoferulic acid **18**
II. Ethyl-2-acetyl ferulate **27**
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*Effects of Modified Lignin Precursors, using the Methods of Grand, Sarni, and Boudet (23), were obtained by Mr. T. R. Lantzy

was noticed (45 and 36 percent, respectively). The but-3-en-2-one **29** produced an average of 1,008,340 DPM's over the control, while Ethyl-2-aceto-ferulate **27** followed with an average of 811,006 DPM's more than the control.

Uptake of the C^{14} labelled cinnamic acid was diminished for the studies involving Ferulonitrile **24** with only 84 percent of the fed activity being incorporated into the poplar cutting. Of this activity, only 30 percent showed up in the insoluble fraction of the stem. However, this fraction still contained an average of 505,915 DPM over the control sample.

DISCUSSION

Halogenated organics have received a great deal of attention in recent years as several of these compounds have been shown to cause cancer in research animals. But due to the difficulty in extrapolating the research data to causes of human cancer, formulation of regulatory legislation has been difficult. The problems are compounded for mutagens whose effects may only be manifested in subsequent generations.

Through the utilization of the Ames Salmonella-microsome assay, a 90 percent correlation between carcinogens and mutagens has been achieved (6). And though the reverse has yet to be proven, it is strongly suspected that many mutagens are also carcinogens. Therefore, it is expected that mandates regulating the release of strongly mutagenic compounds into the environment will be legislated as the knowledge base concerning these compounds improves.

The mutagenicity of chlorinated aqueous solutions has been shown to be due mainly to the potent mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone 3 (8,9). In

anticipation of regulations pertaining to its discharge from drinking water treatment facilities and pulp and paper bleach plants, research was initiated to determine the possibility of removing the compound from, or preventing its formation in, chlorinated aqueous media.

Adsorption Study

Previous studies have demonstrated that halogenated neutral organics can be removed from aqueous media via non-polar, polymeric resins (36). As such, the removal of the chlorinated hydroxyfuranone should be feasible upon determination of an appropriate resin adsorbent and optimal adsorption conditions.

The use of polymeric resin adsorbents offers two main advantages over the use of activated carbon. The first being that polymeric resins can be 'tailor-made' to selectively adsorb a certain compound, or class of compounds, from an aqueous solution. Activated carbon, on the other hand, is considered more of a general adsorbent of organic compounds. The second advantage of polymeric resins is that regeneration of the resin is inherently easier. Adsorption onto activated carbon may involve both physical and chemical interactions. Desorption of adsorbates from

carbon is therefore usually accomplished through either steam or thermal desorption. While steam regeneration can be used to remove volatile organics, thermal regeneration of the resin is required to remove the higher weight organics. Regeneration of activated carbon through thermal desorption requires the removal of the carbon from the bed and transport to an appropriate facility for regeneration. Therefore, removal of adsorbed compounds by this method is both time consuming and expensive. Furthermore, regeneration losses for activated carbon may be as high as 10 percent per cycle (35).

However, since adsorption onto non-polar polymeric resin is predominately controlled by physical interactions, regeneration of these adsorbents simply requires overcoming the Van der Waal's attractive forces between the adsorbate and the adsorbent. For such resin systems, removal of the adsorbate is accomplished via organic solvents without necessitating the removal of the column or the resin from the system. Efficiency of the regeneration process is due to the fact that i) solvating forces are greater than the physical forces holding the adsorbate on the resin and ii) the solvent may itself be adsorbed onto the resin, thereby displacing the adsorbate (35).

For our purposes, the decision to utilize a polymeric adsorbent was based on this regeneration capability since the study was specifically aimed at removal of a mutagen. Expected government regulations concerning 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone 3 will undoubtably place the compound under hazardous waste mandates. Similarly, any transport or disposal of used adsorbent containing the mutagen 3 would also be regulated. Therefore, the ability to regenerate the adsorbent on site was of primary importance. Destruction of the mutagen 3 present in the regenerant can be accomplished through application of strong base or sulfur dioxide without transport of the material from the site (5).

The resin of choice for this investigation was Amberlite XAD-4 (Rohm and Haas, Philadelphia, PA) due to i) its physical characteristics, ii) its known ability to adsorb chlorinated organics, and iii) the ease of regenerating the resin. In order to determine the feasibility of using XAD-4 for removal of the mutagen 3 from aqueous systems, data on the capacity of the resin for the mutagen 3 was required. This adsorptive capacity information was obtained through development of adsorption isotherms.

The initial isotherm data was gathered by using a model compound for the highly toxic mutagen 3. The model compound selected for the study was mucochloric acid 4, another chlorinated hydroxyfuranone quite similar to mutagen 3. The difference between the two compounds being that the 4-chloro functionality on mucochloric acid 4 is replaced by a dichloromethyl group on the mutagen 3. The similarity between the compounds should make it possible to correlate the conditions under which mucochloric acid 4 is preferentially adsorbed from aqueous solution to adsorption of the mutagen 3.

The adsorption isotherm data was gathered over a range of pH's and temperatures in order to not only determine optimal conditions for adsorption, but also simulate the variety of conditions under which adsorption might be required. Data was therefore collected from pH 2 (bleaching effluents) to pH 9 (above swimming pool water) and over the range of 20° (drinking water) to 70° (effluents, cooling waters).

Initial adsorption isotherm experiments showed that mucochloric acid 4 was preferentially removed at lower pH and temperature (96 percent removal at pH 3, 20°, 100 mg/ml resin dose). This result is supported by the fact that the polarity of the furanone rises with pH. At higher pH, the

open chain resonance structure of mucochloric acid 4 is capable of undergoing ionization (Figure 2). As Amberlite XAD-4 is a non-polar resin, the formation of such an ionized species would be expected to cause a reduction in the adsorptive capacity of the resin (36).

The formation of the ionized species of mucochloric acid 4 is sustained by the data which reflects a decrease in adsorptive capacity as the pH rises. However, the prescence of such an ionized species could not be confirmed from HPLC-UV analysis since reformation of the non-ionized, cyclic structure would have occurred during elution in the acetonitrile/water (25/75, v/v) system which was at pH 3. The heightened adsorption of mucochloric acid 4 at the lower pH's indicates that application of the XAD-4 resin in an adsorption process would be more benefical at the lower pH's present in bleach plant effluents and drinking waters then at the higher pH's of swimming pool waters.

The effect of temperature on adsorption capacity, though not as pronounced, is more difficult to explain. A slight reduction in the adsorptive capacity of the resin was noticed for increases in temperature at the lower pH's. This could be due to the exothermic nature of the adsorption process, an increase in the desorption rate of the adsorbed material, or an increase in the solubility of the furanone 4

in solution. As such, removal of contaminants should be greater at lower temperatures present in drinking waters.

However, the increases in adsorptive capacity which were noted for the higher temperatures under alkaline conditions may be due to better attainment of equilibrium. Determination of the equilibrium time for the XAD-4/ mucochloric acid 4 system was conducted under acidic, low temperature conditions (pH 4.6, 21°). Attainment of equilibrium between XAD-4 and mucochloric acid 4 under alkaline conditions may require a longer equilibration time. The higher temperatures may have reduced this equilibration time. However, to be economically viable for application purposes an adsorbate/absorbent system must reach equilibrium rapidly. As the contact time allowed for these experiments was 2 hours, attainment of equilibrium should have been achieved, regardless of the temperature, if the adsorbent was to be considered for industrial application. As such, the results suggest that the best applications of the resin to adsorption systems would occur in the low temperature, lower pH conditions present in drinking waters.

Removal of the mutagen 3 from aqueous solution by XAD-4 was feasible for the laboratory conditions tested (22°, pH 7.2). This result suggests that the mutagen 3 may not be as susceptible to ring opening and ionization as the

model compound 4. As such, the adsorption technique may be directly applicable to treatment of chlorinated drinking water without alteration of pH or temperature. However, further experimentation would be required before application to industrial processes could be undertaken.

Differences in concentration of the adsorbate in solution, 0.6 mg/l for the mutagen 3 versus 2.0 mg/l for mucochloric acid 4, may have affected the adsorptive capacity of XAD-4 for these compounds. However, the effect of concentration on adsorption could not be adequately ascertained due to the limitations of UV-detection. At the concentrations utilized, detection of these species by UV analysis mandated the maximum sensitivity of the detector. Therefore, continuation of application studies with less concentrated solutions would have required the concentration of the residual contaminant after adsorption or the development of a more sensitive detection technique.

Direct Detection

Due to the low concentration of the mutagen 3 in chlorinated aqueous media (<0.04-10 ug/l (5,9)) the ability to directly detect this compound by current analytical techniques is not possible. Furthermore, losses associated

with the preconcentration and isolation techniques necessary to achieve detectability leave us with only an estimate of the mutagen 3 concentration. Therefore, research was undertaken to determine if a derivatization technique could be employed to improve detectability of the mutagen 3.

Since fluorescence spectroscopy offers one of the highest detection sensitivities currently available, investigation into the possibility of derivatizing the mutagen 3 with a highly fluorescent compound was initiated. However, the technique needed to be relatively selective so that derivatization of the numerous organic compounds present in chlorinated aqueous media would not occur. It was further envisioned that those compounds undergoing derivatization would be separable by HPLC techniques. By taking advantage of the fact that hydroxyfuranones resonate into open chain forms containing a free aldehyde group (Figure 2), judicious formation of a fluorescent adduct could be accomplished through a Schiff's base reaction (Figure 3). Only compounds containing an aldehyde or ketone group would be derivatized. The fluorescent compound selected for the derivatization was a porphyrin macromolecule which contained a primary amine (Figure 5).

Derivatization procedures were initially performed with the model compound, mucochloric acid 4, and a model for the

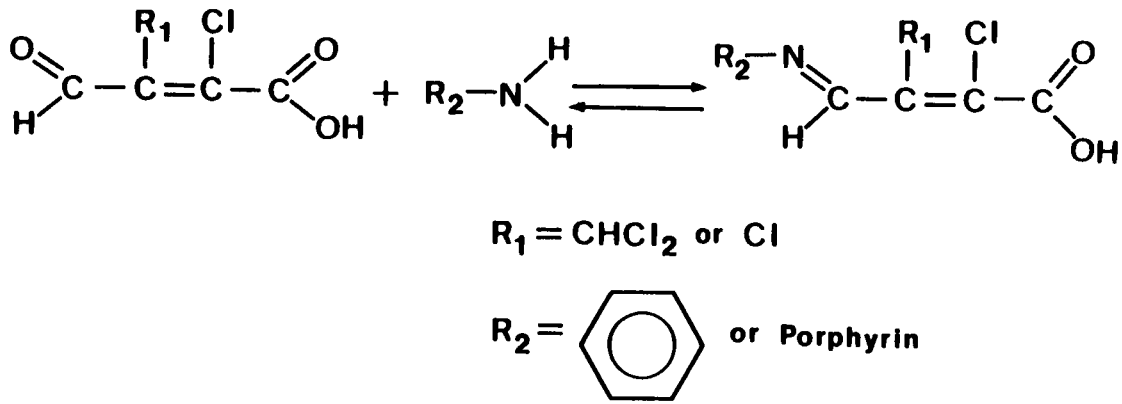

Figure 35. Formation of the Schiff's Base Adduct.

aminoporphyrin, aniline 6 (Figure 35). Though production of the Schiff's base was successful, competing reactions, including the formation of the dianilino-compound 31, occurred (Figure 27). Furthermore, the adduct, anilmucochloric acid 5, was not stable during HPLC analysis, undergoing partial hydrolysis to reform the parent compound, mucochloric acid 4. Due to the inability to eliminate the side reactions, or maintain the Schiff's base, a modified approach to formation of a fluorescent adduct was developed.

To avoid the production of the dianilino-compound 31, methylation of the hydroxyl on mucochloric acid 4 was conducted (Figure 28). This left only the halogen replacement reaction between aniline 6 and mucochloric acid 4 for formation of the adduct (Figure 29). HPLC analysis of the reaction mixture indicated that the adduct was the only product and that this adduct was stable. However, it was realized that a similar result could not be expected with the mutagen 3. The halogen replacement reaction occurred at the carbon beta to the carbonyl group on the hydroxyfuranone ring due to the more favorable transition state stabilized by resonance of the anion through the carbonyl group (Figure 36). However, the presence of the dichloromethyl group on the beta carbon of the mutagen 3 would prevent the elimination reaction from producing the corresponding adduct

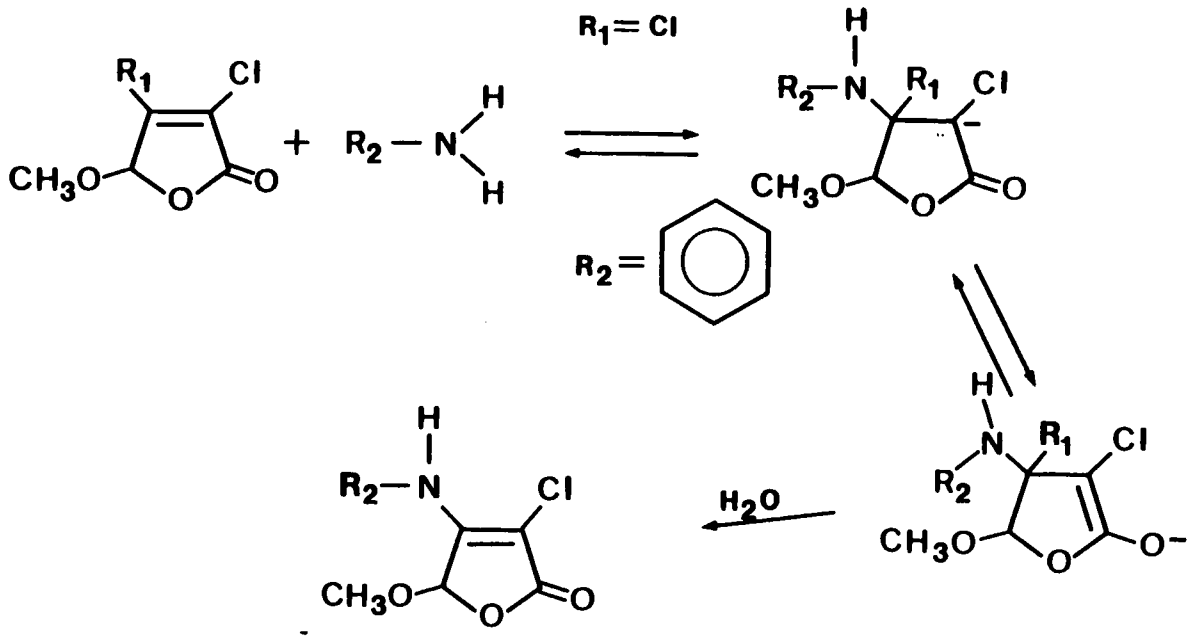

Figure 36. Halogen Replacement Mechanism for the 'pseudo' Ester 17.

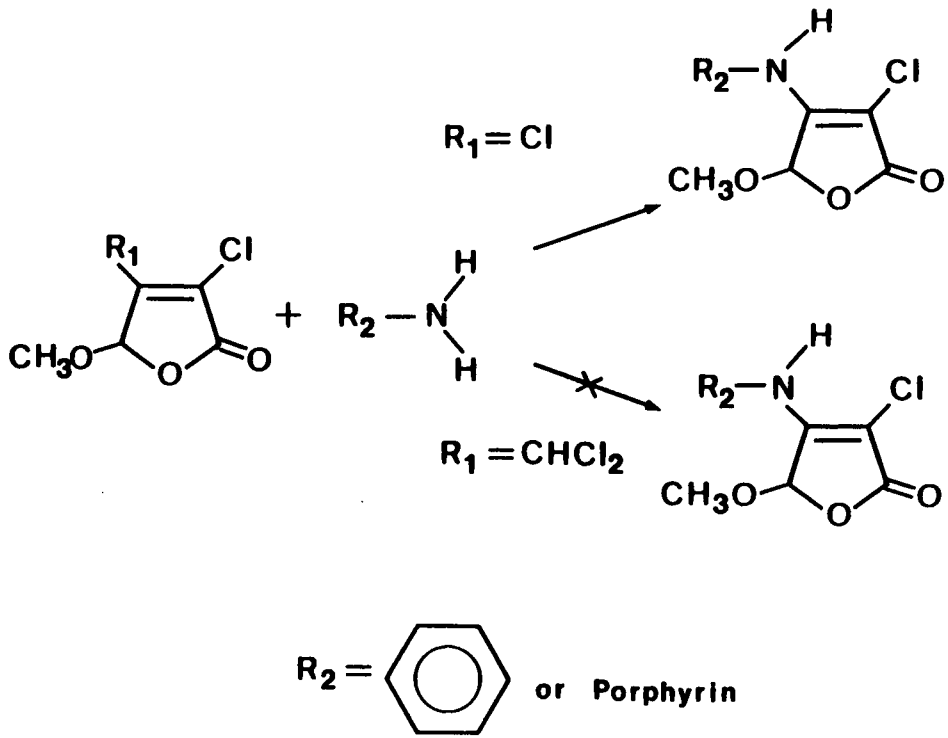

Figure 37. Prevention of Similar Halogen Reaction on Mutagen 3 due to the Dichloromethyl Group.

(Figure 17). Furthermore, competing side reactions involving the dichloromethyl group would be expected. Due to this anticipated difficulty in obtaining a single, or major, adduct from reacting the mutagen 3 with analine 6, further investigation of this technique was therefore abandoned.

Adsorption Application

The inability to develop a technique for detecting the mutagen 3 at concentrations typically associated with chlorinated effluents (0.04-10 ug/l (5,9)) constrained the adsorption study to concentrations detectable by UV analysis. As such, solutions 100 times as concentrated (0.6 mg/l) were necessary for development of the adsorption isotherms. At these concentrations, the resin, Amberlite XAD-4 was capable of a 93 percent removal of the mutagen 3 from ambient aqueous solutions at a resin dose level of 100 mg/ml. However, whether comparable results would be obtained at the lower concentrations present in chlorinated effluents has yet to be determined. If methodology had been available, or devised, for the detection of the mutagen 3 at these low concentrations, then further development of a conceptual design leading to commercialization of the

adsorption process would have been undertaken.

Data necessary for the development of batch adsorption designs can be extrapolated directly from adsorption isotherm data obtained in the laboratory. The mode of application depends on the degree of removal of the contaminant necessary, the presence of other contaminants, and the optimization of the economics of the system. If a single application of the resin to the contaminanted solution provides adequate adsorption, then a single stage batch reactor of appropriate capacity may be all that is needed. Quantity of effluent achievable and resin usage rates are determined directly from batch adsorption isotherms.

However, if further treatment of the solution is necessary to obtain a desired quality of effluent, multistage adsorption may be required. In a two stage system, the contaminated solution from the first stage is filtered and contacted again with fresh adsorbent. The process may be repeated as often as necessary to achieve the desired quality of effluent. Carbon usage rates and effluent concentration are determined from simulated adsorption isotherm experiments conducted in the laboratory.

Cost effectiveness of batch systems may be improved through the utilization of countercurrent systems. For

example, in a two stage system, the once used resin from the second stage is contacted with fresh effluent. The resin is then filtered and regenerated while the partially treated effluent is contacted with fresh resin in the second stage. The process can be expanded as required to achieve the desired effluent concentration. Again, the simulation of the stages in the laboratory is done to obtain adsorption isotherm data necessary to determine adsorbent dosage and the number of stages required.

Actual application of batch adsorption techniques to industry is infrequent because of intensive capital and operating costs. However, use of the technique may be required in certain situations such as excessive suspended solids concentration. When possible, the use of column adsorption techniques are preferred because of these economic considerations and their effectiveness.

Conceptual design of a column treatment scheme is more complicated than batch design due to the variety of data required for, and the variety of possibilities in, design of the system. Data necessary for a conceptual design include: i) adsorption isotherms, ii) loading, or breakthrough curves, iii) regeneration curves, and iv) other miscellaneous information. Information obtained from these areas is used to evaluate design options and criteria in

order to develop the optimal system, ie., minimization of cost to obtain specified effluent quality.

For column design, the adsorption isotherm data depicts the concentration of the solute adsorbed as a function of the influent concentration. This data can be generated on the basis of equilibrium, eg., capacity of adsorbent, or rate of adsorption. Selection of an appropriate resin may therefore depend on the application of the process such as the importance of speed of adsorption as opposed to quantity adsorbed.

Loading, or breakthrough curves show the concentration of the effluent from the column as a function of throughput. As a result, they represent a good model of expected column performance. Such curves are generated to select adsorbents, optimize resident time values, define adsorbent capacities, etc..

Regeneration curves are developed to establish the parameters involved in the cycling of the adsorbent. Cycling of the adsorbent is usually performed in order to reduce the costs of adsorption operations. The variables defined by these studies include the best method of regeneration, the optimum regenerate, the length of the cycling time, etc..

The final area of information necessary for assessment of design options is the aquisition of miscellaneous data. Such data should include the hydraulic expansion of, and pressure drop through, the adsorbent. Furthermore, any information about the influent to the adsorption system, including pH, temperature, suspended solids, and feed concentration, would also be included.

From this data, development of a conceptual design can be initiated based on minimization of the capital and operational costs required to obtain the desired effluent quality. However, development of an adsorption system should be considered an iterative process between the conceptual design and experimental programs. Experimental data may indicate the use of a certain option in the initial conceptual design which in turn suggest further experimentation necessary to better define and optimize that option. The interaction process continues until uncertainty in design parameters is minimized. The completion of the optimal conceptual design leads to pilot plant studies.

The results of our initial studies indicated that the most efficient application of the Amberlite XAD-4 resin for the removal of the mutagen 3 occurs in low temperature, acidic, aqueous solutions. Since such conditions exist in drinking water treatment facilities, direct application of

the resin without extensive pretreatment process schemes may be possible. As such, application of the resin in a column configuration which incorporates on site regeneration capabilities should be investigated for removing the mutagen 3 from processed drinking waters.

Lignin Alteration

While adsorption techniques are suitable for the removal of the mutagen 3 from chlorinated aqueous solutions such as drinking waters which contain low levels of organic materials, the process is not applicable to pulp and paper bleach plant effluents. The large quantities of soluble organic material produced during delignification would rapidly overload the adsorbent. As such, adsorption technology would not be an economically viable method for removal of the mutagen 3. An alternate method for eliminating the mutagen 3 from bleach plant effluents was therefore necessary.

Since it is known that the mutagen 3 is produced upon chlorination of lignin or lignin derived materials (33), it follows that any reduction in the quantity of lignin to be removed during chlorine bleaching, or increase in the efficacy of lignin removal during pulp bleaching operations,

should generate a corresponding reduction in the formation of the mutagen 3. Such reductions in the residual lignin to be removed after pulping processes could be achieved through i) more efficient pulping techniques, ii) increases in the succeptibility of native lignin to pulping techniques, or iii) a reduction in the quantity, or type, of native lignin to be removed.

Current chemical pulping techniques are halted when a specified residual lignin concentration is reached. This is done to protect the cellulosic fibers from chemical degradation. However, much of the remaining lignin is ionically and/or covalently linked to these fibers. Removal of this lignin is accomplished through the use of more specific degradation chemicals, eg., chlorine bleaching.

Selective chemical alteration of the content, or the structure, of lignin in wood could potentially increase the efficiency of pulping and/or bleaching operations. Furthermore, if the chemical linkages between lignin and cellulosic fibers could be decreased, the use of chlorine to remove residual lignin might be unnecessary. Previous research has led to the development of certain compounds capable of inhibiting certain key enzymes involved in the lignification process (20-23). In continuation of these earlier efforts, the effect of selectively modified natural

substrates on *in situ* lignification were studied.

The modified substrates to be tested were synthesized to enter the phenylpropanoid pathway and alter lignin through inhibition of, or incorporation into, the lignification pathway. The compounds synthesized for the initial stage of this investigation included β-cyanoferulic acid **18**, ferulonitrile **24**, ethyl-2-aceto ferulate **27**, and (4-hydroxy-3-methoxyphenyl)-but-3-en-2-one **29**, all of which were modified versions of precursors involved in the formation of the monolignol, coniferyl alcohol. Therefore, results from this study may be correlated to lignin alteration in both hardwoods and softwoods.

The investigation concerning the effect these compounds have on the lignification process was performed by fellow researcher, T. R. Lantzy utilizing the procedure of Grand, Sarni, and Boudet without modification (23).

The results indicate that all four modified substrates synthesized for these initial experiments were capable of increasing the uptake of the C^{14} labelled cinnamic acid into the lignin containing fraction of the stem. These results differ from previous research findings (20-23) where CAD inhibiting compounds decreased the incorporation of the labelled acid into lignin. As such, compounds are now known

which can either increase or decrease the flux of precursors into the lignin (insoluble) fraction of poplar stem cuttings.

A reduction in lignin deposition in either woody or grassy species could potentially increase the efficacy of pulping operations and their digestibility by ruminants. However, the extent to which the lignin content of such species could be reduced without causing detrimental effects is not known.

The increase in incorporation of the labelled cinnamic acid may have resulted from a heightened enzymatic activity effected by the modified substrates. If the modified substrates were, in turn, also incorporated into the lignin polymer, then the potential exists for incorporation of judiciously modified substrates which could enhance the (bio)degradability of lignin and this needs to be investigated. However, whether these compounds were actually incorporated into the polymer has yet to be determined. Research aimed at answering this question is currently being conducted. Knowledge of how, and through what enzymes, these modified substrates are affecting lignification could lead to control of <u>in situ</u> lignin depostion and structure.

Attention Patron:

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SUMMARY AND CONCLUSIONS

The objective of this study was focused on the development of technologies aimed at determining and reducing the concentration of the mutagen, 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone 3, in chlorinated aqueous solutions. As such, the research dealt with the physical removal of the mutagen 3 from, and the reduction of its potential for formation in, chlorinated aqueous media.

Removal of the mutagen 3 from aqueous solutions utilized the adsorbent, Amberlite XAD-4, a non-polar, hydrophobic polymeric resin. Adsorption isotherm data was generated to determine the adsorptive capacity of this resin for the contaminant. Under the experimental laboratory conditions employed, an adsorptive capacity of 5.6 ug of mutagen 3 per gram of resin was obtained at pH 7.2 and 22°. As these conditions simulate those found in drinking water treatment facilities, the results indicate that the adsorption methodology could potentially be directly applied to such facilities. However, it is emphasized that these preliminary laboratory experiments were conducted on

solutions 100 times more concentrated then those characteristically noted for chlorination effluents due to the limits of detectability of current analytical techniques.

Research was initiated to develop a derivatization procedure to alleviate this situation. The derivatization of mutagen 3 with a highly fluorescent compound, eg., a porphyrin macromolecule, would have achieved this goal. However, the instability of the Schiff's base, anilmucochloric acid 5, a model for the aminoporphyrin-mutagen adduct, and the inability to correlate the halogen replacement procedure for anilmucochloric 'pseudo' ester 17 to the mutagen 3, forced the abandonment of this direction of the research.

Since direct detection of the mutagen 3 at concentrations typically present in chlorinated aqueous media was not possible, research concerned with the development of a commercially applicable adsorption procedure was not undertaken. Furthermore, any application of an adsorption process to commercial operations would be limited to effluents containing low concentrations of organic compounds. Suitable effluents might include drinking waters, swimming pool waters, and cooling waters. However, effluents from pulp and paper bleaching operations

would not be applicable to an adsorption process due to the high levels of organics present. Therefore, an alternate method for reducing the concentration of the mutagen **3** in these effluents was necessary.

If a reduction in the intractable nature of lignin in wood could be achieved through the introduction of more chemically labile groups into the lignin polymer, then an increase in the efficiency of pulping operations would ensue. Furthermore, residual lignin remaining after pulping operations could potentially be more suceptible to bleaching techniques if lignin-polysaccharides linkages were reduced through incorporation of modified substrates unable to form such covalent bonds. As such, the use of chlorine to obtain a desired level of brightness in the wood pulp may be unnecessary, thereby reducing the potential for formation of the mutagen **3**.

Research was therefore initiated to investigate the possibility of incorporating modified natural substrates into lignin. These substrates, modifications of cinnamyl alcohol precursors, were synthesized and tested for their ability to affect the incorporation of C^{14} labelled cinnamic acid. Following the procedure established by Grand, Sarni, and Boudet (23), fellow researcher, T. R. Lantzy, determined that all four modified substrates, β-cyanoferulic acid **18**,

ferulonitrile **24**, ethyl-2-aceto-ferulate **27**, and (4-hydroxy-3-methoxyphenyl)-but-3-en-2-one **29**, affected an increase in the uptake of the labelled precursor into the insoluble fraction of a poplar stem cutting. It is presumed that the increase in the C^{14} labelled cinnamic acid incorporation was effected by the presence of the modified substrates on the phenylpropanoid enzymatic process and that the substrates (to some extent) were also incorporated into the lignin (insoluble) fraction. As such, the potential exists for incorporation of specifically designed substrates into the lignin polymer which would alter the bonding patterns thereby producing a less intractable lignin. Research is currently continuing to determine i) if the substrates were actually incorporated into the lignin polymer and ii) which enzymes are being affected by the substrates.

The significant conclusions obtained from this research are:

1) Adsorption of the mutagen **3** from aqueous media by Amberlite XAD-4 is possible at the concentration (0.6 mg/l) and conditions (pH 7.2, 22°) investigated.

2) Since the laboratory adsorption conditions simulate those present in drinking water treatment facilities, the methodology may be directly applicable.

3) Derivatizing the mutagen 3 with an aminoporphyrin 7 through either a Schiff's base or halogen replacement reaction was not an acceptable method for developing a direct detection technique to measure the concentration of the mutagen in chlorinated aqueous media.

4) The modified substrates, β-cyanoferulic acid 18, ferulonitrile 24, ethyl-2-aceto ferulate 27, and (4-hydroxy-3-methoxyphenyl)-but-3-en-2-one 29, increased the incorporation of C^{14} labelled cinnamic acid into the lignin fraction of poplar stems, presumably through enhancement of enzymatic activity.

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