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ORGANIC AMMONIUM SALTS OF LIGNIN ACIDS

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The present invention relates (1) to a new type of compound comprising certain high molecular weight organic ammonium salts of woody plant lignin-like acids, useful as antimicrobial agents, (2) to a method of making and using these compounds and (3) to new compositions of matter and products including these compounds as antimicrobial agents.

There are certain organic nitrogen containing compounds in the form of quaternary ammonium and amine salts having the following type formula:



where R_1 is an alkyl, alkenyl or alkaryl group containing from 8 to 20 carbon atoms in straight, branched or homocyclic arrangement and X is an anion rendering the compound water-soluble, such as a halide, sulfate or acetate radical. These water-soluble compounds have high antimicrobial properties. We have produced derivatives of these nitrogen compounds, (1) which contain the active antimicrobial group,



(2) which have high molecular weight anions, (3) which are extremely insoluble in water, oils and most organic solvents, and (4) which, in spite of characteristics (2) and (3) have high antimicrobial properties. It is surprising and quite unexpected to find derivatives of the nitrogen compounds described retaining their strong antimicrobial properties in the face of the accepted views and theories that the replacement of the water-solubilizing anion of such nitrogen compounds by anions of high molecular weight, with the consequent conversion to insolubility, is attended by substantial or total loss of bactericidal, bacteriostatic, fungistatic and algistatic properties.

Our new compounds are organic ammonium salts of anions which are present as major constituents in alkaline solutions of lignin and lignin-like bodies obtained from wood or bark by alkaline extraction processes. These lignin constituents are acidic in nature, non-fibrous, non-cellulosic and of relatively high molecular weight, being in the range of about 2000 to 8000. In the acidic form, they are insoluble in water and organic solvents. The alkali metal salts are water-soluble.

One type of lignin acids comprises the so-called "alkali lignin" obtained from alkaline processes for pulping wood and available commercially, for instance, as various "Indulin" products from the West Virginia Pulp and Paper Company. In the Kraft process for alkali pulping, where sulfides are used, the "alkali lignin" produced contains sulfur, probably in the form of —SH groups.

Another type of alkali extractable lignin-like acid which can be used for the purpose of the present invention, comprises redwood acids. These are available commercially in crude form under the trade name "Palconate" from the Pacific Lumber Company. These redwood acids

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are obtained from the bark dust of the redwood tree by extraction with alkali. Similar products can be obtained from redwood itself. These products from redwood have been referred to by authorities in the field as "acid lignin" (see H. F. Lewis, Institute of Paper Chemistry, article entitled, "The chemical nature of redwood," published in the Vortex publication of the California Section of the American Chemical Society, vol. VII, May 1946). One distinction between redwood acids and other lignin-like acids is that the former contain carboxyl groups, whereas the latter do not.

The above-described lignin acids contain a variety of functional groups which are capable of reacting with high molecular weight quaternary ammonium and primary, secondary or tertiary amine salts to form compounds which are extremely insoluble in water, oils and most organic solvents and which have high antimicrobial activity in spite of their insolubility.

Sulfonated derivatives of lignin and of redwood acids, such as result from the sulfite process of wood pulping and the treatment of redwood bark with sodium sulfite, respectively, react similarly to the alkali lignin and redwood acids with high molecular weight quaternary ammonium and amine salts. The reaction products are likewise insoluble in water and most organic solvents and display antimicrobial properties.

Hereinafter, by "lignin acid" is intended to include alkali lignin, redwood acid and lignin sulfonic acid. This is according to chemical usage.

The ammonium derivative component of the lignin acid salt of the present invention may have the following formula:



(to lignin acid radical)

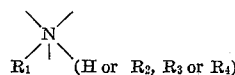
in which R_2 , R_3 and R_4 are either (a) hydrogen, (b) alkyl, aryl, alkaryl, aralkyl or alkoxy (OCH_3) groups or (c) combination of (a) and (b).

The nitrogen compound residue in the lignin acid salt of the present invention may more specifically have the formula



(to lignin acid radical)

or



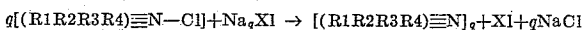
(to lignin acid radical)

in which two or three nitrogen valences, shown partially attached, form part of a heterocyclic ring structure, such as in derivatives of pyridine, quinoline, morpholine and imidazoline. In such cases, R_1 will be a high molecular weight alkyl or equivalent group, as described above, and R_2 , R_3 and R_4 will be replaced by one or the other of these ring structures or by condensed heterocyclic ring structures.

Our insoluble quaternary ammonium and amine salts of lignin acids are prepared by reacting in aqueous solution a water-soluble quaternary ammonium or an amine salt of the character described with a water-soluble salt of the lignin acid. The reaction between these two water-soluble reactants in water solution produces a salt of the lignin acid, which is insoluble in water, in oils and in most organic solvents, and which therefore precipitates out as the result of the reaction. The reactants are preferably taken in chemically equivalent

proportions, so that no substantial excess of either remains after the reaction. This, in general, avoids undesirable dispersion effects, which may cause filtration difficulties.

The reaction may be represented by the following general equation:



where q represents the number of reacting groups per unit molecular weight of the lignin acids, and where R_1, R_2, R_3, R_4 have the meanings given above and Xl indicates the lignin acid residue. Analogous equations may be written for quaternary ammonium and amine salts in which N is part of a heterocyclic ring. The formation of such compounds by direct reaction between amines and lignin acids (in acidic form) is obviously possible.

While the chloride is the more common anion used in the soluble quaternary ammonium and amine salts, other anions, such as bromide, iodide, sulfate, acetate, etc., may be employed. As equivalents to sodium, the lignin acid salts, known as lignates, may be used in other water-soluble forms, such as the potassium or ammonium salts.

Our quaternary ammonium and amine salts of lignin acids represent a class of compounds which exhibit marked insolubility in water, oils and most organic solvents, and which therefore can be employed as antimicrobial agents in cases where the original quaternary ammonium or primary, secondary and tertiary amine salts, although possessing marked antimicrobial properties, cannot be used. A slight solubility is shown in certain polar organic solvents, more particularly methanol. In extreme cases with alkali lignin when prepared at very high alkalinity the solubility in methanol can be as high as ten percent. In spite of the general insolubility, the new compounds surprisingly show distinct and useful antimicrobial properties.

Examples of organic ammonium salts of lignin acids of the present invention, which are insoluble in water, oils and most organic solvents, and which possess antimicrobial properties are as follows:

Dodecyl dimethyl benzyl ammonium salt of redwood acids (commercially dodecyl in the reactant employed in making the compound of the present invention is also referred to as "alkyl ($C_8H_{17}-C_{18}H_{37}$)").

Lauryl isoquinolinium salt of red wood acids.

Dodecyl dimethyl benzyl ammonium salt of alkali lignin.

N-alkylbenzyl trimethyl ammonium salt of alkali lignin.

Dodecyl amine salt of redwood acids.

Tertiary alkyl ($C_{18}-C_{24}$) primary amine salt of alkali lignin.

Alkenyl ($C_{16}-C_{20}$) dimethyl ethyl ammonium salt of alkali lignin.

Alkyl (C_8-C_{18}) dimethyl 3,4-dichloro benzyl ammonium salt of redwood acids.

Di-isobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium salt of alkali lignin.

Cetyl pyridinium salt of redwood acids.

N-ethyl N-"soya" morpholinium salt of redwood acids (derived from alkyl found in soya bean oil).

Alkyl benzyl hydroxyethyl imidazolinium salt of alkali lignin.

Rosin amine (D) salt of redwood acids.

Dimethyl "Coco" amine salt of alkali lignin (derived from coconut oil).

Dodecyl amine salt of alkali lignin.

Secondary di-"Coco" amine salt of redwood acids.

Tertiary alkyl ($C_{18}-C_{24}$) primary amine salt of redwood acids.

2-heptadecyl glyoxalidine salt of redwood acids.

Stearamido methyl pyridinium salt of alkali lignin.

N-alkyltolyl methyl trimethyl ammonium salt of redwood acids (alkyl= C_9-C_{15}).

Di-quaternary ammonium compounds, as well as diamines, containing high molecular weight alkyl groups, form corresponding insoluble quaternary ammonium and

amine salts of lignin acids. Such nitrogen derivatives are included within the scope of this invention. In these cases, both nitrogens are capable of combining with the lignin acid anions. Examples of such combinations are:

N-(2-dimethyl dodecyl ammonium ethyl) pyridinium dichloride (U. S. Patent 2,617,806) reacted with sodium salt of redwood acids.

Dodecyl-imino-propylene amine acetate (from Armour and Company's "Duomeen 12") reacted with sodium salt of redwood acids.

Since the composition and structure of lignin acids are not completely established, it is not possible to describe the anion forming groups in terms of exact chemical structure. We have found that the equivalent weight of the lignin acids for reaction with high molecular weight quaternary ammonium salts is a function of the alkalinity of the solution from which the precipitation constituting the product of this reaction is made. In general, the higher the alkalinity, the lower the equivalent weights of the lignin acids (i. e., the higher the combining power). This, as well as evidence from titration curves, indicates a multiplicity of acidic groups of different strengths.

We have also found that the antimicrobial activity and physical properties of our compounds vary with the pH at which the precipitation is made.

To correlate functional groups with acidic radicals in the lignin acids, an electrometric titration curve was determined for redwood acids. The titration was carried out with purified redwood acids prepared as to be described in Example 1. The redwood acids had been dried to constant weight at 103° C. One gram of the purified acid was dissolved in an excess of carbonate-free sodium hydroxide and then an exactly equivalent amount of hydrochloric acid, corresponding to the sodium hydroxide used, was added. Titration was carried out with one normal sodium hydroxide (carbonate-free) and pH values determined with a glass electrode and corrected for sodium ion and electrode asymmetry effects.

The results indicated a variety of acidic groups with considerable overlapping. For the purpose of determining the major acidic groups, the quantity Δ pH per ml. was plotted against equivalents of sodium hydroxide used. This curve showed well defined maxima and minima. The minima correspond to the pK values of the various acidic groups and the maxima to the amount of sodium hydroxide required to neutralize a particular group and all other groups of greater acidity (lower pK values). The results are given in Table I.

TABLE I.—ACIDIC GROUPS IN REDWOOD ACIDS

Probable Chemical Nature	pK Value	Milli-equivs. NaOH per gram Redwood Acids ¹	Equivalent Weight ²	Number of Groups in Molecule ³
Carboxyls.....	2.6	0.73	1,370	2
Do.....	4.1	3.19	253	9
Do.....	8.6	0.72	215	2
				13
Phenolic.....	10.4	1.08	175	3
Do.....	11.4	1.43	140	4
				7
Very weakly Acidic groups.....	>11.4	4.65	85	11

¹ This group only.

² Includes all acidic groups of greater strength.

³ Of molecular weight equal to 2750.

The data in Table I indicate well defined carboxyl and phenolic groups and some not so well defined very weakly acidic groups. The equivalent weight of the redwood acids, taking the carboxyls alone as functional, is 215. This value is decreased to 175 or 140 when one or both sets of phenolic groups, respectively, are taken as functional along with the carboxyls. Values of the

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equivalent weight of as low as 85 are indicated when the very weakly acidic groups are included.

From the titration curve, a minimum molecular weight of 2750 for the redwood acids was estimated. As shown in column 5, this is the minimum which allows each of the identified acidic groups to have integral values.

No corresponding titration data are available on alkali lignin. Literature references indicate one phenolic group per molecular weight unit of 840 but no mention has been found of other more weakly acidic groups, except the probably presence of —SH groups in alkali lignin from the Kraft process. As will be shown subsequently, equivalent weights for reaction with high molecular weight quaternary ammonium compounds of as low as 235 have been observed when high alkalinity is used. This indicates the presence of additional very weakly acidic groups of unknown nature. It will be shown that the reaction with these groups is of particular significance with respect to compounds of high bactericidal value.

As already described, sulfonated derivatives of lignin, such as result from the sulfite process of wood pulping, also react with high molecular weight quaternary ammonium and amine salts. The data in Table II illustrate the effect of pH on the reacting ratios of lignin sulfonate to dodecyl dimethyl benzyl ammonium chloride.

TABLE II

pH	Reacting Ratio ¹	Equivalent Weight Lignin Sulfonate	Equivalents of Quaternary per Gram of Lignin Sulfonate	Number of Quaternary Groups Combined Per Lignin Residue ²
2.3.....	2.3/1.0	610	.00164	1.65
7.6.....	1.7/1.0	451	.00221	2.22
10.0.....	1.47/1.0	391	.00256	2.59
13.0.....	0.95/1.0	252	.00398	4.01

¹ Ratio by weight of sodium lignin sulfonate (Marasperse N, Marathon Paper Co.) to dodecyl dimethyl benzyl ammonium chloride.

² Based on an equivalent weight of 1010 for the sodium lignin sulfonate. This value was derived by the assumption that the incremental reaction between pH 2.3 and 7.6 is due largely to the combination of quaternary ammonium groups with a strong phenolic group corresponding to the one already identified in alkali lignin. The reaction at pH 2.3 is assumed to be entirely with the sulfonic groups. These assumptions are consistent in that the increase from 840 to 1010 for the unit molecular weight of lignin sulfonate is approximately what would be expected for the addition of 1.65 sulfonic groups per lignin residue.

The incremental reactions above pH 7.6 are with more weakly acidic groups, whose existence in the lignin sulfonate has not been previously definitely established. As in the case of alkali lignin, the reaction with these groups is necessary to form compounds of high bactericidal value.

Summarizing the effect of pH in the preparation of quaternary ammonium salts of lignin acid, it has been found that the operable pH is from 8 upwards and the preferred range is from 10 upwards. Quaternary ammonium salts of lignin acid prepared below a pH of 8 show minor antimicrobial activity in terms of phenol coefficient for bactericidal power, although good useful activity in terms of other antimicrobial properties is apparent in these compounds when prepared at a lower pH, as will be shown hereinafter. These compounds prepared from pH of 10 upwards, show high bactericidal activity and computations based on the quaternary ammonium salt added above pH 10 give results corresponding approximately to those which would be expected from the simple or unbound quaternary ammonium ion.

Example 1

Since the high molecular weight organic ammonium radical attached to the weakly acidic groups is the more effective antimicrobial agent, this suggests blocking the stronger acidic groups so that they will no longer accept organic ammonium radical. We have accomplished this

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by forming insoluble metallic compounds of the lignin acids and treating these compounds at a pH above 8 with a quaternary ammonium salt, thereby forming a compound containing metallic atoms attached to the stronger acidic groups and quaternary ammonium radicals attached to the weaker acidic groups. It is possible, for example, to precipitate from a solution of the sodium salt of redwood acid at pH 7 an insoluble copper salt by addition of copper sulfate solution equivalent to the stronger acidic groups. This requires approximately 0.175 gram of cupric copper per gram of redwood acid radical and indicates an equivalent weight of about 170 for the redwood acid radical reacting with copper at pH 7. The copper then has combined with approximately half the functional groups while the other half remains available for reaction at high pH. When the precipitate formed is suspended in an alkaline solution at a pH of about 11 and there is added a soluble quaternary ammonium salt, reaction takes place between the weakly acidic groups and the quaternary ammonium radicals forming water insoluble compounds containing both copper and quaternary ammonium radicals.

Since copper salts are far less expensive than quaternary ammonium salts and since the latter attached only to the weakly acidic groups are the more effective, a considerable economy is thus achieved. Zinc sulfate, aluminum sulfate, etc., can be used in place of the copper sulfate, and other lignin acids can be used in place of the redwood acid.

The following additional examples illustrate certain ways in which the principle of the invention has been applied, but are not to be construed as limiting the invention.

35 Example 2.—Preparation of purified redwood acids from caustic extract of redwood bark dust

This example illustrates the preparation of redwood acid in purified form from its crude sodium salt.

Forty grams of "Sodium Falconate" (Pacific Lumber Company), dried at 103° C., were dissolved in 1200 ml. distilled water. The solution was filtered to remove suspended matter. Frequent changes of filter paper were made and the volume of the filtered solution was increased to 2 liters through washing of the filters with water. To the solution was added 50 ml. of concentrated hydrochloric acid and the temperature brought to 90° C. This excess of acid operated to coagulate the precipitated redwood acids. After allowing to cool to room temperature the mixture was filtered with suction and the filter cake washed three times with distilled water. The cake was oven dried at 55° C. Yield of purified redwood acids was 18.6 grams. The purified redwood acid is a reddish brown solid, insoluble in water and organic solvents. It can be ground without difficulty. It dissolves in sodium hydroxide and other alkalies to form a solution of its salts.

Example 3.—Preparation of dodecyl dimethyl benzyl ammonium salt of redwood acids

This example describes the preparation of the above compound at (a) neutrality and (b) high alkalinity.

(a) A solution of the sodium salt of purified redwood acids (prepared as in Example 2) was prepared by dissolving 8.0 grams of the dry redwood acids in an excess of sodium hydroxide solution. Sufficient ammonium chloride was added to reduce the pH to about 7.5 and the volume adjusted to about 250 ml. The quaternary ammonium chloride reactant was prepared by diluting 30.0 grams of dodecyl dimethyl benzyl ammonium chloride (50%) to 750 ml. with water. The redwood acids solution was added to the quaternary ammonium salt solution with good agitation. Tests on the filtrate showed a very slight excess of quaternary ammonium salt. The voluminous dark-brown precipitate filtered readily and, after washing, was dried at 55° C. A yield of 19.3 grams of

dodecyl dimethyl benzyl ammonium salt of redwood acids was obtained. It was a dark brown solid readily ground to a powder for antimicrobial tests and suitable for agricultural dusting. It was insoluble in water and non-polar organic solvents and slightly soluble in methanol.

In separate experiments, following the method described in Example 5 below, the reacting ratio of these reactants was found to be 0.64 part by weight of purified redwood acids to 1.0 part of dodecyl dimethyl benzyl ammonium chloride. This corresponds to an equivalent weight of 217 for the redwood acids under these conditions and indicates (see Table I) that the quaternary ammonium ion reacted only with the carboxyl groups of the redwood acid.

A duplicate preparation was made using the reactants in exactly equivalent amounts and the product retained as a wet cake for use in the preparation of dispersions for antimicrobial tests.

(b) In separate experiments, it was found that a 1% solution of the sodium salt of redwood acids in 0.1 normal sodium hydroxide reacting with 1% dodecyl dimethyl benzyl ammonium chloride in 0.1 normal sodium hydroxide gave a reacting ratio by weight of 0.33 part to 1.0 part, respectively, corresponding to an equivalent weight of 113.5 for the purified redwood acids under these conditions. This lower equivalent weight means that additional acid functional groups have reacted.

Using this reacting ratio, 125 ml. of the 1% alkaline solution of sodium salt of redwood acids mentioned above was reacted with 375 ml. of the 1% quaternary ammonium chloride solution. The voluminous precipitate which was formed filtered readily by suction. It was washed with three portions of water and retained in the wet cake form for use in the preparation of dispersions of dodecyl dimethyl benzyl ammonium salt of redwood acids for antimicrobial tests. The yield was 44.1 grams of a cake containing 10.3% solids.

Example 4.—Preparation of lauryl isoquinolinium salt of redwood acids

From the reacting ratios separately determined, the equivalent weight of redwood acids for lauryl isoquinolinium bromide was found to be 172 under approximately neutral conditions. This indicates that this quaternary ammonium salt is capable of reacting with part of the phenolic, as well as the carboxyl groups.

A dilute aqueous solution of lauryl isoquinolinium bromide was made by diluting 75 grams of 20% Isothan Q-15 (Onyx Oil and Chemical Co.) to 750 ml. with distilled water. To this was added with mechanical agitation 200 ml. of a solution of the sodium salt of redwood acids containing 6.75 grams of purified redwood acids (prepared as in Example 1) and 4 grams of saturated sodium hydroxide solution. When one-fourth of the redwood acids solution had been added, 3 grams of ammonium chloride were dissolved in the mixture and the addition of redwood acids completed.

The brown precipitate was filtered and washed. Tests on the filtrate showed a pH of 6.5 and the presence of a trace excess of lauryl isoquinolinium ion. The product was dried at room temperature and gave a yield of 18.5 grams of the lauryl isoquinolinium salt of redwood acids. This was a dark brown solid insoluble in water and non-polar organic solvents, but slightly soluble in isopropanol.

A similar preparation was made by reacting 8.0 grams of redwood acids in the form of a solution of its sodium salt (volume 635 ml.) with 17.2 grams of lauryl isoquinolinium bromide (95% of the calculated equivalent amount) in 800 ml. volume. The filtrate in this case showed excess of redwood acids. The washed filter cake (116.1 grams) gave a yield on the dry basis of 19.0 grams of the lauryl isoquinolinium salt of redwood acids. The wet cake was used in the preparation of dispersions for antimicrobial tests.

Example 5.—Preparation of dodecyl dimethyl benzyl ammonium salt of alkali lignin

This example describes the preparation of the above compound at (a) neutrality and (b) high alkalinity. Preliminary experiments were carried out to determine the equivalent weight of the alkali lignin (from the combining weight ratio). This involved a series of reactions between dilute solutions of known concentrations of the two reactants, filtration of the reaction product and tests of portions of the filtrate by addition of a few drops of a solution of each reactant, corresponding to the solutions used for the main reaction. The presence of either reactant in excess as shown by turbidity was readily determined in this manner. By starting the series with a volume ratio giving a definite excess of one reactant and varying the ratio to an excess of the other reactant, the equivalent point (where no precipitation was given by either reactant in the filtrate tests) was found with a satisfactory degree of precision. The equivalent weight was calculated from this reacting weight ratio.

(a) A dilute solution of the sodium salt of alkali lignin was prepared by dissolving 10.5 grams (5% moisture content) of Indulin A (West Virginia Pulp and Paper Company's purified pine wood lignin) in 35 ml. of 0.726 normal NaOH diluted to 200 ml. with water. This solution was then diluted to 500 ml. and adjusted to pH 9.0 by a few drops of dilute HCl. A solution (approximately 1% concentration) was prepared containing 3.85 grams of dodecyl dimethyl benzyl ammonium chloride (Onyx Oil and Chemical Company's "BTC"). The pH of this solution was 6.5–7.0. The quantities of reactants taken in these solutions corresponded with the reacting ratio of 2.45 parts by weight of alkali lignin to 1.0 part of the quaternary ammonium chloride (determined in separate experiments at pH 7.4 by the method described above), except that only 95% of the quaternary ammonium salt was used in order to insure that no excess of this reactant was present. The equivalent weight of alkali lignin under the conditions used was 833 (calculated from 2.45×340 , where 340 is the molecular weight of the quaternary ammonium chloride). Note that this corresponds to reaction with the phenolic hydroxyls attributed to alkali lignin (Indulin A) and referred to earlier.

The dodecyl dimethyl benzyl ammonium chloride was added slowly with good agitation to the solution of the sodium salt of alkali lignin. After the precipitation was complete the suspension was adjusted to pH 7.4. This seemed to improve coagulation of the precipitate. The precipitate was then filtered and washed on the funnel with distilled water. A test of the filtrate showed the presence of a slight excess of alkali lignin. The washed filter cake was reserved as a supply of the dodecyl dimethyl benzyl ammonium salt of alkali lignin for the preparation of dispersions for the study of antimicrobial properties.

(b) A dilute solution of alkali lignin in 0.1 normal NaOH was prepared by dissolving 10.5 grams of Indulin A, plus 27.4 ml. of 0.900 normal NaOH, plus 4.1 grams solid NaOH in water and made up to a volume of one liter. A 1% solution of dodecyl dimethyl benzyl ammonium chloride in 0.1 normal NaOH was made by dissolving 20.0 grams of "BTC" (50%) and 4.1 grams solid NaOH in water and making up to one liter.

Using these solutions by the above described method, the reacting ratio was found to be 0.69 part by weight of alkali lignin to 1.0 part of dodecyl dimethyl benzyl ammonium chloride. This leads to an equivalent weight of 235 for the alkali lignin under conditions of this high alkalinity. Note that this value is less than one-third of the value obtained in (a) above at lower alkalinity, indicating that the quaternary ammonium ion is reacting with very weakly acidic groups in the alkali lignin.

Using this reacting ratio, 900 ml. of the above quaternary ammonium chloride solution was reacted with 644 ml. of the above solution of the sodium salt of alkali

lignin. The precipitate of the dodecyl dimethyl benzyl ammonium salt of alkali lignin formed as a tarry solid which adhered strongly to the sides of the beaker. It was recovered, washed with water and oven dried at 60° C. The yield was 11.5 grams of a dark brown tarry semi-solid, which was found to be quite soluble in methanol, but insoluble in water and non-polar organic solvents.

Example 6.—Preparation of dodecyl dimethyl benzyl ammonium salt of lignin sulfonic acid

This example describes the preparation of the above compound at (a) low pH and (b) high pH. A series of experiments was first carried out to determine the equivalent weight of sodium lignin sulfonate from the reacting ratios of sodium lignin sulfonate to dodecyl dimethyl benzyl ammonium chloride at several pH's, including pH 2.3 and 13.0, following the method described under Example 4.

(a) Preparation at pH 2.3—equivalent weight 610: A dilute acid solution of sodium lignin sulfonate was prepared by dissolving 18.4 grams (6% moisture content) of Marasperse N (Marathon Paper Co.) in 910 ml. of approximately 0.01 normal HCl. This solution showed a pH of about 2.5. A solution containing 15.0 grams of 50% dodecyl dimethyl benzyl ammonium chloride (onyx) in about 350 ml. dilute HCl was prepared, this solution showing a pH of 2.5–3.0. The reacting proportions in these solutions were 17.25 grams sodium lignin sulfonate to 7.5 grams of the quaternary ammonium salt, or 2.3 to 1.0 (see Table II).

The solution of quaternary ammonium salt was added slowly to the lignin sulfonate solution with good agitation. The light brown precipitate which formed settled well. The supernatant liquid had a clear, faintly yellow color and showed a pH of 2.5. Tests showed that neither reactant was present in excess. The precipitate was filtered and washed with water in the usual manner. After drying to constant weight at 50–55° C., the yield was found to be 18.45 grams. The product was in the form of black brittle lumps. It was readily ground to a medium brown colored powder, which was insoluble in water and non-polar solvents but slightly soluble in methanol.

(b) Preparation at pH 13.0—equivalent weight 252: A dilute alkaline solution of sodium lignin sulfonate was prepared by dissolving 10.1 grams (6% moisture content) of Marasperse N in 495 ml. of 0.1 normal NaOH. A second solution was prepared containing 20.0 grams of 50% dodecyl dimethyl benzyl ammonium chloride in 480 ml. of 0.1 normal NaOH. The reacting proportions were therefore 9.5 grams of the lignin sulfonate to 10.0 grams of the quaternary ammonium salt, corresponding to the 0.95 to 1.0 ratio in Table II.

The solution of quaternary ammonium salt was added to the solution of lignin sulfonate following the usual procedure. The precipitate was a chocolate brown semitar like material. The supernatant liquid had a clear, pale yellow color and tests on it showed no excess of either reactant. It also showed by indicator test papers a pH above 11.

The precipitate was washed several times by decantation with water until the washings were neutral to litmus. The black tar-like precipitate was dried at 60–65° C. to constant weight. The yield of brownish-black brittle product was 14.4 grams. It was readily ground to a chocolate brown powder, which was insoluble in water and non-polar solvents but slightly soluble in methanol.

Example 7.—Preparation of N-alkylbenzyl trimethyl ammonium salt of alkali lignin

This example covers the preparation of the above indicated compound at substantial neutrality from Indulin C (a commercial crude sodium salt of alkali lignin in powder form). Preliminary experiments showed the re-

acting ratio as 2.0–2.1 parts by weight of Indulin C to 1.0 part of the quaternary ammonium chloride.

An aqueous solution was prepared containing 11.0 grams of Indulin C in one liter. The quaternary ammonium salt solution was prepared by diluting 10.0 grams of ATM-50 (Oronite Chemical Co.), a 50% solution of N-alkylbenzyl trimethyl ammonium chloride, to 300 ml. with water. This gives a ratio of 2.2 parts by weight Indulin C to 1.0 part of the quaternary ammonium chloride, representing a slight deficiency of the latter in comparison with the experimentally determined reacting ratio. The sodium lignin solution had a pH of 8 and the quaternary salt solution a pH of 6.5.

The quaternary ammonium chloride solution was run into the solution of the sodium salt of alkali lignin with good agitation. The precipitate, which was very voluminous, was filtered after washing three times by decantation. It was dried at 50° C. to constant weight, the yield being 12.0 grams. The dry N-alkylbenzyl trimethyl ammonium salt of alkali lignin was readily ground to a dark brown powder, which was insoluble in water and non-polar solvents and slightly soluble in methanol.

Example 8.—Preparation of the dodecyl amine salt of redwood acids

A solution of dodecyl amine acetate was prepared by dissolving 15.0 grams of "Armec 12" (Armour and Company) in distilled water and making up to a total volume of 750 ml. To this was added with mechanical agitation 350 ml. of a solution of the sodium salt of redwood acids containing 7.3 grams of purified redwood acids (prepared as in Example 1), 4 grams of saturated sodium hydroxide solution and 3 grams of ammonium chloride. This solution had a pH of 7–8. The precipitate was filtered, the filtrate showing a very slight excess of the amine reactant. After washing and drying at 55° C. the product, which was brown in color, gave a yield of 17.0 grams. It was insoluble in water and non-polar solvents.

From this yield of the product, it was calculated that the equivalent weight of the redwood acid for reaction with this amine at pH 7 is 140. Referring to Table 1, this demonstrates that the dodecyl amine reacts with all acidic groups of pk 11.4 or less under these conditions.

Example 9.—Preparation of tertiary alkyl (C₁₈–C₂₄) primary amine salt of alkali lignin

Preliminary experiments were carried out to determine the combining weight ratio of alkali lignin to the tertiary alkyl primary amine by the method described in Example 4. The results showed that 1.35 parts by weight of alkali lignin (as sodium salt) react with 1.0 part of this amine (as acetate).

A solution of the sodium salt of alkali lignin was prepared by dissolving 10.5 grams of Indulin C (crude sodium salt of pine wood lignin, West Virginia Pulp and Paper Co.) in 990 ml. of water. This solution had a pH of 8.0–8.5. The amine acetate solution was prepared by dissolving 7.5 grams of "Primac JMA-T" (Rohm and Haas Chemical Company, tertiary alkyl (C₁₈–C₂₄) primary amine acetate) in 490 ml. of water (solution pH 6.5–7.0).

The amine salt solution was run into the sodium alkali lignin solution with good agitation. After completion of the precipitation the filtrate showed a slight excess of the alkali lignin reactant. The precipitate was filtered, washed and dried at 50° C. The dry tertiary alkyl primary amine salt of alkali lignin was obtained in 13.25 grams yield. It was ground readily to a medium brown colored powder, which was insoluble in water and non-polar solvents.

The high molecular weight organic ammonium salts of lignin acids of the present invention may be produced in the form of aqueous colloidal dispersions. This can be accomplished by vigorously stirring the material in the

form of wet filter cake with additional water containing non-ionic dispersing agents. Alternatively, a water soluble salt of a lignin acid (redwood acids or alkali lignin) in low concentration may be used as the dispersing agent. The use of non-ionic agents, however, is preferable since in some cases the presence of sodium salts of redwood acids and alkali lignin has a tendency to suppress the antimicrobial action of the dispersed compound.

The slight solubility in methanol of certain of the compounds of this invention may be utilized in the preparation of colloidal dispersions by dissolving the compound in methanol and adding this solution to water containing the dispersing agent under conditions of vigorous agitation. Alternatively, the dispersing agent may be contained partially in the methanol solution and partially in the added water.

These colloidal dispersions have varying bactericidal power as shown by the data in Table III.

TABLE III

Compound	pH in Preparation of Compound	Concentration of Dispersion Tested, Percent	Phenol Coefficient (100% basis) <i>S. aureus</i>
1. Dodecyl dimethyl benzyl ammonium salt of redwood acids	7.0	0.1	25
2. Dodecyl dimethyl benzyl ammonium salt of redwood acids	12.5	1.0	75
3. Dodecyl dimethyl benzyl ammonium salt of redwood acids	Above 13	10.1	100
4. Lauryl isoquinolinium salt of redwood acids	7.0	0.1	75
5. Dodecyl dimethyl benzyl ammonium salt of alkali lignin	Above 13	10.1	100
6. Tertiary alkyl (C ₁₅ -C ₂₄) primary amine salt of alkali lignin	7.0	10.1	25
7. Dodecyl dimethyl benzyl ammonium lignin sulfonate	2.3	10.1	No activity
8. Dodecyl dimethyl benzyl ammonium lignin sulfonate	13.0	10.1	80-100

¹ These dispersions were prepared by the methanol method described above.

The dispersions in Table III were prepared using Tween 80 (polyoxyethylene sorbitan mono-oleate) as dispersing agent in a concentration equal to one-half of the indicated concentration of the dispersion.

The foregoing tests for phenol coefficient against *Staphylococcus aureus* were carried out by the official method set up by the Food and Drug Administration, U. S. Department of Agriculture, as described in A. O. A. C., Methods of Analysis, 7th edition, page 88. An accepted modification of this method was followed in which "Lethen" broth was used for the subculture.

It is to be noted that when the quaternary ammonium salt of redwood acids is prepared at high alkalinity, the antimicrobial power, as indicated by values of phenol coefficient, is greatly increased over the value for the compound prepared at neutrality. This is a clear indication that at the higher alkalinity, the more weakly acidic groups in redwood acids react with additional quaternary ammonium groups with the result that these additional groups are less firmly bound and therefore biologically more active. Similarly, it has been found that complete reaction of all acidic groups in alkali lignin with high molecular weight organic ammonium cations is obtained at high alkalinity, giving higher values of phenol coefficient as compared with the values from the compound prepared at neutrality.

In Table III, item 7 is a compound containing a considerable amount of quaternary ammonium groups and shows no phenol coefficient at all. Item 8 is a compound made from the same reactants at high pH and shows a phenol coefficient of 80-100. Obviously, it is the incremental quaternary which has imparted the bactericidal property.

Colloidal dispersions of these high molecular weight organic ammonium salts of lignin acids also show bacteristatic action. Table IV summarizes for several of these compounds the dilutions at which growth of *S. aureus* and *S. typhosa* are inhibited.

TABLE IV

Compound	pH in preparation of compound	Dilutions of Dispersions Inhibiting Growth of	
		<i>S. aureus</i>	<i>S. typhosa</i>
Dodecyl dimethyl benzyl ammonium salt of redwood acids	13.0	1:50,000	1:30,000
Dodecyl dimethyl benzyl ammonium salt of alkali lignin	>13.0	1:50,000	1:30,000
Lauryl isoquinolinium salt of redwood acids	7.0	1:40,000	1:25,000
Tertiary alkyl (C ₁₅ -C ₂₄) primary amine salt of alkali lignin	7.0	1:20,000	1:10,000

By virtue of the unique properties of these high molecular weight quaternary ammonium and amine salts of lignin acids, they have wide fields of utility. For example, cellulosic surfaces in the form of sheets or filaments may be protected from the action of mildew or other fungi. Practical forms of such surfaces are fabrics, yarns, cordage, paper, cardboard, etc. The insoluble fungistatic agents of this invention may be applied in the form of colloidal dispersions or by other suitable methods.

Fungistatic tests of dry powders comprising the dodecyl dimethyl benzyl ammonium salt of redwood acids and the N-alkylbenzyl trimethyl ammonium salt of alkali lignin prepared at a pH below 8 were carried out by seeding the entire surface of Sabaroud's agar plates with spores of *Penicillium citrinum* (A. T. T. C. 9840). In the center of each plate was placed 0.1 gram of the dry powder. The plate was then incubated for one week at 25° C., at the end of which time a zone 12 mm. in diameter surrounding the powder of each of the above compounds showed an inhibition of the growth of the organism used. Examination of the same plates one month later showed that the zone of inhibition was persistent. A comparative test of copper 8-quinolinolate, a well-known fungistat, gave only a 2-4 mm. diameter zone of inhibition. The two insoluble quaternary ammonium salts used in these tests were prepared at pH 7.

The insoluble organic ammonium salts of lignin acids of the present invention show resinous characteristics with a pronounced tendency to form firmly adhering films on drying. We have found that these films, when produced on cellulosic and other surfaces, show marked fungistatic properties. The fungistatic action is illustrated by the results of tests on cellulosic fabrics treated by the following four methods:

(a) The fabric was immersed in a colloidal dispersion (generally 1% concentration) of the compound for about ten minutes. After rinsing off the excess of dispersion with water, the treated cloth was allowed to drain and dry. It was then tested for growth of *Chaetomium globosum*. This test and those described in the paragraphs below were made following the U. S. Army, Ordnance Department, Specification AXS-1244 (March 1944).

(b) This method makes use of the slight solubility of our new compounds in methanol. The fabric to be used in the test was first soaked in methanol and drained. It was then soaked in a 1% solution in methanol of the compound being tested for 5-10 minutes. After draining off the excess of solution, the treated fabric was dried in the air. The dried fabric was then tested for growth of *Chaetomium globosum*. (Very uniform adsorption of the quaternary ammonium and amine salts of redwood acids and alkali lignin was indicated by the coloring of the fabric.)

(c) In this method the fabric or other form of cellulosic

material is treated successively with solutions of the soluble reactants in separate baths, whereby the insoluble fungistatic compound is formed in situ in intimate contact with the surface. For example, the cellulosic material, whatever the form may be, is first dipped into or run through a dilute solution of the soluble quaternary ammonium or amine salt. After a short contact, the excess of solution is squeezed out, and the material is then dipped into or run through a dilute solution of the salt of the lignin acid. After squeezing out most of the excess of unreacted second reactant, the coated material is preferably soaked in water for a period of time to remove any of this reactant still adhering to the surface of the material. The treated fabric is then dried for use.

The order of treatment indicated above is preferable to the reverse order of applying the two reactants, since high molecular weight quaternary ammonium and amine salts in solution show substantive properties toward cellulose, and a better penetration is insured for the reaction product than when the salt of the lignin acid is applied first.

(d) We have found a still more effective method of forming the antimicrobial compounds of the present invention in situ on cellulosic fabric. This comprises a three-step process in which the first two steps are similar to those in method (c) above, being carried out at substantial neutrality. After the excess of unreacted second reactant has been squeezed out and before soaking in water, the fabric is dipped into or run through a dilute solution of the soluble quaternary ammonium or amine salt (first reactant) which has been made strongly alkaline by sodium hydroxide. The fabric after this third step is squeezed to remove most of the retained solution and then soaked in water and finished as in method (c).

This three-step process is superior to the two-step method (c) since in the latter a considerable proportion of the weakly acidic groups in the lignin acids remain unreacted. In accordance with the principle described above, the third step under highly alkaline conditions then results in the maximum number of biologically active cations combining with these unreacted weakly acidic groups.

The results of some fungistatic tests carried out on cotton cloth (unbleached muslin) treated with various compounds according to the above methods are shown in Table V.

TABLE V

Compound ¹	pH of Formation	Method of Fabric Treatment	Growth of <i>Chaetomium globosum</i> After 3 Weeks' Incubation
1.-----	12.5	(a) above---	Negative.
1.-----	>13	(b)-----	Do.
2.-----	>13	(b)-----	Do.
3.-----	>13	(b)-----	Do.
1.-----	7	(c)-----	Slight.
4.-----	7	(c)-----	Very Slight.
1.-----	>12	(d)-----	Negative.

¹ 1=Decyl dimethyl benzyl ammonium salt of redwood acids; 2=dodecyl dimethyl benzyl ammonium salt of alkali lignin; 3=tertiary alkyl (C₁₅-C₂₀) primary amine salt of alkali lignin; 4=N-alkylbenzyl trimethyl ammonium salt of redwood acids.

(Control tests with untreated cloth in these experiments gave the usual heavy growth of *Chaetomium globosum*.)

Coating of cellulosic materials with insoluble films of our organic ammonium salts of lignin acids gives physical properties in the finished goods superior to those in the case of cellulosic materials impregnated with soluble high molecular weight quaternary ammonium salts. This superiority lies principally in the fact that the treated surface is dry but still flexible and, in particular, it does not show the softening and moisture absorbing properties induced by treatment with soluble quaternaries and amine salts.

Tests for algistatic properties showed that the dodecyl dimethyl benzyl ammonium salt of redwood acids in colloidally dispersed form is effective against a pure culture of chlorella at a concentration of 10 parts per million.

The properties of these new quaternary ammonium and amine salts of lignin acids indicate a wide range of usefulness for them, as for example in the following fields:

- (1) Mildew-proofing and prevention of the growth of other fungi on cellulosic fabrics.
- (2) Prevention of mildewing and other fungus growths on paper and cellulosic structures of various types, including cardboard, fibre board and wall board.
- (3) Preservation of cellulosic felts.
- (4) Wood preservation.
- (5) Prevention of mildewing and rotting of cordage, rope, fish nets and similar materials.
- (6) Similar preservation of canvas, awnings, sails, etc.
- (7) Slime control in the manufacture of paper and similar cellulosic products.
- (8) Use in air filters to catch and inactivate bacteria and the spores of fungi.
- (9) Use in agricultural dusts and sprays, with or without other antifungal agents, such as sulfur, for control of plant mildew and fungi of various types.
- (10) Prevention of mold growth in latex and resin emulsion paints, as well as use in oil paints as fungicides.
- (11) Prevention of mold growth in adhesives.
- (12) Uses in the pharmaceutical field in bacteristatic and fungistatic ointments, salves, lotions, dusting powders and similar products.
- (13) Uses as algistatic agents.
- (14) Additives to lubricating oils and greases used in food machinery to prevent growth of bacteria and fungi.
- (15) Use as germicidal additives in non-ionic detergents, particularly those detergents for use in solid form.
- (16) Preservative coatings on food products, such as fresh fruits, eggs, etc., whereby molds and bacterial growth are inhibited.

Although the invention has been described in connection with lignin acid, and specifically alkali lignin, redwood acid and lignin sulfonic acid, as far as certain aspects of the invention are concerned, other acid derivatives of woody plants may be employed. It has been found, for example, that the alkali metal salts of tannin, tannic acid (digallic acid), gallic acid and in general the acid components in tan liquor from wood sources form water insoluble compounds with high molecular weight quaternary ammonium and amine salts. In contrast to the compounds formed with the lignin acids, however, these compounds are soluble in a wide range of organic solvents. Also, while the products formed from lignin acids are solids ranging in character from hard brittle materials to soft semi-solids or tars, the products from the tannins are oily liquids. These compounds also exhibit anti-microbial properties.

While the invention has been described with particular reference to specified embodiments, it is to be understood that it is not to be limited thereto but is to be construed broadly and restricted solely by the scope of the appended claims.

What is claimed is:

1. The water insoluble, oil insoluble, anti-microbial compounds formed by the reaction between substantially equivalent quantities of aqueous solutions of soluble salts of organic nitrogen bases having anti-microbial properties and aqueous solutions of soluble salts of lignin-acids; said salts of the organic nitrogen bases being selected from the group consisting of:

- N-alkyl benzyl trimethyl ammonium salt
- Alkenyl (C₁₆-C₂₀) dimethyl ethyl ammonium salt
- 70 Alkyl (C₈H₁₇-C₁₈H₃₇) dimethyl 3-4 dichlorobenzyl ammonium salt
- Di-isobutyl phenoxy ethoxy ethyl-dimethyl-benzyl ammonium salt
- Cetyl pyridinium salt
- 75 N-ethyl "Soya" morpholinium salt

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Alkyl benzyl hydroxy-ethyl imidazolinium salt
 Stearamido methyl pyridinium salt
 N-alkyl tolyl (C_9-C_{15}) methyl trimethyl ammonium salt
 N-(2-dimethyl dodecyl ammonium ethyl) pyridinium dichloride
 Dodecyl amine salt
 Tertiary alkyl ($C_{18}-C_{24}$) primary amine salt
 Rosin amine (D) salt
 Dimethyl "coco" amine salt
 Secondary di-"coco" amine salt
 2-heptadecyl glyoxalidine salt
 Lauryl isoquinolinium salts
 Dodecyl-imino propylene amine acetate;

said salts of lignin-acids being selected from the group consisting of soluble salts of redwood acids, alkali lignin, soluble salts of ligno-sulfonic acids; the quantities of said reactants being such that neither is in substantial excess; the pH of said aqueous solutions being adjusted to the alkalinity whereby the weakly acidic groups having a pk value more than 10 of said salts of lignin acids react with said solutions of salts of said organic-nitrogen bases to form said water insoluble, oil insoluble, anti-microbial compounds.

2. The water-insoluble, oil-insoluble, salts of claim 1 wherein the lignin-acid is alkali lignin.

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3. The water-insoluble, oil-insoluble, salts of claim 1 wherein the lignin-acid is redwood acid.

4. The water-insoluble, oil-insoluble, salts of claim 1 wherein the lignin-acid is lignin sulfonic acid.

5. For use as an anti-microbial agent an article of manufacture containing one of the compounds of claim 1 as an active ingredient.

6. For use as a bactericidal agent an article of manufacture containing one of the salts of claim 1 as an active ingredient.

7. The compounds of claim 1 produced as in claim 1 wherein the soluble salts of said lignin-acids are reacted with a blocking agent selected from the group consisting of copper ion, zinc ion and aluminum ion, before reacting with the aqueous solution of salts of the organic nitrogen bases of claim 1.

References Cited in the file of this patent

UNITED STATES PATENTS

2,503,297	Pierce	Apr. 11, 1950
2,530,770	Hopperstead	Nov. 21, 1950
2,594,302	Ehrensperger	Apr. 29, 1952