ESTERS OF GENTISIC ACID AND THEIR TOXICITIES

DISSERTATION

Presented in Partial Fulfillment of the Requirements
for the Degree Doctor of Philosophy in the
Graduate School of The Ohio State
University

By

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The Ohio State University

1952

THE OHIO STATE
UNIVERSITY

Approved by:

Adviser

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ACKNOWLEDGMENTS

I wish to acknowledge with gratitude the advice and suggestions of Dr. Bernard V. Christensen, Dean of the College of Pharmacy, in the development of this thesis.

To Dr. Frank W. Bope I am greatly indebted for the thought, time, and patience while this thesis was developed.

I also wish to acknowledge the advice and suggestions of Dr. John W. Nelson in the development of the toxicity procedure.

I acknowledge the suggestions of Dr. Melvin S. Newman, of the Chemistry Department, in the development of the syntheses.

A special note of appreciation is extended to Dr. Christopher Wilson, of the Chemistry Department, for use of the infrared laboratory.

The financial assistance of the Upjohn Company, in the form of a fellowship, and of the United States Government, in the nature of Public Law 346, is responsible in a large measure for this thesis.

I wish to thank the Eli Lilly Company for the Carbon and Hydrogen analyses.

To all others who have assisted in any way, I wish to express sincere thanks and grateful appreciation.

J. Frank Nash

June, 1952

ESTERS OF GENTISIC ACID AND THEIR TOXICITIES

INTRODUCTION

The preparation of some new esters of 2,5-dihydroxybenzoic acid or gentisic acid which offer possibilities of being of medicinal value as antirheumatics, antiarthritics, or analgesics was suggested by several factors. First, in spite of the wide attention given to adrenal cortical hormones, there is still a great need for good medicinal agents for the treatment and symptomatic relief of rheumatic and arthritic disorders. Since treatment of these disorders is usually of long duration, it is desirable to use medicinal agents of low toxicity, and also agents that will produce a minimum of alteration of normal physiologic functions. Compounds such as the salicylates have been used in this capacity for many years.

A second factor suggesting this work is the increasing use of sodium gentisate in the treatment of rheumatic conditions and as an analgetic.

Still another factor suggesting preparation of some esters of gentisic acid is the apparent need for an efficient non-addicting analgesic stronger than aspirin yet weaker than the opiates.

The groups chosen to be coupled with gentisic acid were groups of established pharmacological activity. Diethylaminoethanol has pronounced analgesic effect without any toxic

side-actions when injected intravenously (1). Also, diethylaminoethanol appears to have analgesic properties when given orally or intramuscularly (2). This grouping is also prominent in local anesthetics, antispasmodics, and antihistaminics. Diethylaminopropanol was used to determine the effect of an additional carbon in the alcohol.

Acetylsalicylic and succinic acids are both analgesics of proven medicinal value.

Phenylacetic acid esters have proven antispasmodic activity and phenylalkylamines are analgesic (3).

Paranitrobenzoic acid and more particularly its reduction product, paraaminobenzoic acid, has a variety of pharmacological properties among which are local anesthetic and anti-infective effects.

Anisic acid or paramethoxybenzoic acid is reported to have antirheumatic and local anesthetic activity (4).

Acetyl, methoxy and benzoyl groups were substituted in the carboxylic acid esters because of the proven value of these functions in enhancing the analgesic value of phenolic compounds.

DISCUSSION OF THE LITERATURE

Although salicylic acid and sodium salicylate have been used in the treatment of rheumatic conditions since 1876 and 1877, respectively (5), salicylic acid's oxidation product, gentisic acid, remained unknown medicinally until September, 1948 when Meyer and Ragen reported the use of sodium gentisate for the relief of rheumatic symptoms (6). Since then, numerous reports from the Western Hemisphere have substantiated the original belief of Meyer and Ragen.

The specific effect of salicylates upon the activity of hyaluronidase and salicylate action in rheumatic conditions date back to the work of Guerra in 1946 (7) (8). Guerra believes rheumatic fever to be a disease of the mesenchyma tissues. During the evolution of the disease, whether of bacterial or endogenous origin, permeability changes take place pointing to interrelationship of several characteristics of rheumatism and the spreading factors of connective tissue.

The importance of the spreading effect of hyaluronidase on connective tissue is based on the observation of Meyer and Palmer (9), who pointed out that hyaluronic acid is the principal substrate of connective tissue and mucoid structures. Connective tissue and mucoid structures compose almost entirely the regions affected by rheumatism, such as articulations and synovial fluid.

Durán-Reynals (10), McClean (11), Kendall and associates (12), and especially Crowley (13), have observed that several microorganisms including more than 200 strains of hemolytic streptococci produce or possess hyaluronidase.

Guerra showed that hyaluronidase of bacterial origin or from testicular extract, in 1% dilutions, increased the spread of Evans Blue in humans and India Ink in rabbits. The spreading resulted from enzymatic activity which hydrolyzed the hyaluronic acid present. The viscosity was decreased and, therefore, the passage of liquids, exudates and pathogenic microorganisms was favored (7).

Sodium salicylate administered orally or intravenously in dosage of 0.079 g./Kg. caused 57% reduction of skin diffusion effect of hyaluronidase in rabbits, compared to saline controls. A dose of 0.10 g./Kg., by the same routes of administration, caused 66% reduction of diffusion effect. Moreover, humans who had contracted rheumatic fever, upon intradermal injection of a mixture of hyaluronidase with Evans Blue gave unique reactions with enormous diffusion of the dye and local edema that sometimes occupied the arm injected. Salicylates in these cases inhibited the activity of the enzyme and reduced its spreading effect in connective tissue. Guerra, therefore believed that the activity of the salicylates was due to the inhibition of the activity of hyaluronidase (8).

It has been demonstrated that salicylates do not inhibit

the activity of hyaluronidase in as low concentrations in vitro as they do in vivo (6), (14), (15), (16), (17). Swyer (17), by using the viscosimetric technique in vitro, recorded no direct inhibitory effect on the activity of hyaluronidase by sodium salicylate or acetylsalicylic acid except at relatively high concentrations, i.e. greater than 3% and 0.33%, respectively. Such inhibition at these concentrations is apparently due to lowering of pH and, in the case of sodium salicylate, to the increased concentration of salt. Swyer believed the results obtained by Guerra were due to the presence of histamine or some substance of similar action, and that sodium salicylate, by inhibiting the activity of the substance reduced the apparent spreading effect caused by hyaluronidase.

Meyer and Ragen (6), (18) claimed that the inhibition of hyaluronidase activity was due to the fact that salicylates are converted to gentisic acid which acts as the true inhibitor. The semi-quinone formed condenses in an irreversible manner with the enzyme. Salicylates are known to yield from 4 to 8% gentisic acid (5), (19), (20). This substantiates the statement that 2,5 benzoquinone carboxylic acid inhibits the activity of hyaluronidase (21).

However, Roseman et al (22) showed pure analytical gentisic acid to be devoid of hyaluronidase inhibitory activity. The crude gentisic acid as well as pure gentisic acid treated with alkali in the presence of air did show

activity. Similar results were obtained with homogentisic acid. Therefore, it was concluded that the activity of gentisic acid was probably due to impurities in the product which have been formed by oxidation. The work of Lowenthal and Gagnon (23) indicated the same lack of inhibitory activity of gentisic acid.

A listing of products that inhibit the activity of hyaluronidase shows the activity of phenol substituted products increases with increase in length of the hydrocarbon chain. The salicylates and gentisates were considered inactive in vitro (24).

Guerra also showed that glucuronic acid excretion increases in the urine, upon injection of salicylates. However, gentisic acid diminishes the excretion of this product. Since hyaluronic acid is composed of D-glucosamine, glucuronic acid and N-acetylglucosamine, the action of gentisic acid appears to be desirable in respect to excretion (25).

Another cause of rheumatoid arthritis has been thought to be hormone imbalance (19). The salicylates may stimulate the adrenal cortex causing liberation of corticoid substance. Adrenal cortical stimulation can be demonstrated in rats and guinea pigs by an increase in excretion of urinary 12 ketosteroids and a decrease in circulating eosinophils (26). However, a decrease in circulating eosinophils could not be found after administration of salicylates or gentisates to

four medical students. Thiebat et al, (27) trying to correlate rheumatic arthritis and permeability of the synovial membrane showed sodium salicylate retards permeability more than sodium gentisate. The retardation was based upon the injection of phenolphthalein into the intrarticle and then recording the time for appearance of urinary coloration. The retardation has a favorable action in rheumatic arthritis. The mechanism of action was concluded to be connected with the function of the suprarenal cortex.

Since 1948 there has been extensive work done in the United States, France, Britian, and Italy in regard to the use of gentisates for rheumatic conditions and analgesia. In rheumatic fever, Meyer and Ragen (6) report uniformly good results using sodium gentisate therapy. The administration of sodium gentisate was followed by disappearance of pain, alleviation of swelling and heat in joints, lowering of temperature to normal and by the falling of the sedimentation rate to normal. In most cases the disease was alleviated for five to ten months after removal of sodium gentisate therapy. However, even in those cases where the disease returned in several days, it could again be relieved by administration of sodium gentisate. Schaefer et al (28) and others (29) also obtained good therapeutic results with sodium gentisate in rheumatic fever.

Meyer and Ragen (6) also used gentisates to treat patients afflicted with rheumatoid arthritis. Results were favorable.

Camelin, et al (5) as well as Ory (30) and others (31) working with large groups of patients obtained encouraging results in rheumatoid arthritis and Bouillard disease. Ory also reported sodium gentisate to be valuable in gout.

Batterman, et al (32) studied the analgesic effects of sodium gentisates in approximately 100 cases. Control of pain regardless of severity, diagnosis, or dosage administration for hospitalized patients was obtained in 71% of the trials, without a single case of toxicity. Satisfactory analgesia occurred in 57% of the ambulatory patients. Un- toward reactions were noted in 11% of the ambulatory patients, but this was within the experimental range of placebos.

Since the activity of sodium gentisate is in the same range as that of salicylates all the reports on activity are relative to that of sodium salicylate. According to the literature the gentisates offer the following advantages over the salicylates in the above diseases. 1. Sodium gentisate removes one step in the metabolic process. Salicylates are oxidized to gentisates before they are effective, so by giving sodium gentisate we give the active drug. 2. Sodium gentisate is not as irritating as the salicylates to the gastric mucosa. The gastric system appears to be able to take in an unlimited amount. 3. Intravenous injection causes no sclerosis; muscular tolerance is good, and the renal functions are not affected. 4. No case has yet been reported showing the development of tinnitus or

signs of salicylism resulting from use of sodium gentisate. No aural symptoms or increased prothrombin time has been reported. 5. There are no indications that sodium gentisate causes methemoglobinemia or liver damage. 6. The increase in urinary glucuronic acid excretion observed with salicylate ingestion does not occur with sodium gentisate.

In spite of numerous reports to the contrary, Capretti and Arduini (33) report gentisate therapy to be less effective than salicylate regime in the treatment of Bouillard disease.

Although recently gentisic acid has been reported to disappear from the body by urinary excretion and metabolism to unknown products (19) (20) as early as 1895 Likhatschaff (34) reported a urinary increase of ethylsulfuric acid after administration of gentisic acid to dogs. The same result was obtained by Staub and associates (35) in 1932.

The only extensive report on the toxicity and pharmacology of gentisic acid was by Gincoulhac and associates (36). The report includes the comparison of sodium gentisate and sodium salicylate. The LD_{50} for rats by intraperitoneal injection was 3.1 g./Kg. and 0.88 g./Kg respectively. Orally, the LD_{50} for sodium gentisate could not be determined because the drug was well tolerated even at the dosage level of 5 g./Kg. However, the oral LD_{50} for sodium salicylate was found to be 2.02 g./Kg.

The two drugs were screened for several activities.

1. Neither drug showed noticeable action on smooth muscle fibers. 2. Sodium gentisate showed little or no hypertensive activity except at high dosage levels, but sodium salicylate gave a response of higher nature and longer duration. 3. Neither drug exhibited notable antihistaminic action. 4. Both drugs showed anti-edema activity but sodium salicylate was the more active. 5. A slight increase of bile flow was produced by both drugs in doses of 100 mg./Kg. 6. The microbiological activity of sodium gentisate against several strains of bacteria and fungi was very moderate or nil and inferior to that of sodium salicylate in equal concentrations of liquid medium.

In spite of the fact that over 100 substituted acids, phenolic esters and carboxylic acid esters have been prepared none of these derivatives have been tested pharmacologically to determine if they have therapeutic value (37). However, in 1951 a U. S. patent was issued to Hoffer in which he prepared complexes of gentisic acid with antipyrine, isopropylantipyrine and 4-dimethylamino antipyrine to yield compounds that possessed antipyretic and antirheumatic activity (38). These products were also patented in Germany (39).

The form of gentisic acid commercially available is the sodium salt. The dosage form is compressed tablets of seven grains. A discussion on tablet preparation of sodium gentisate is given by Lauter and associate (40).

EXPERIMENTAL

Esters of gentisic acid or 2,5 dihydroxy benzoic acid can be prepared either with the carboxylic acid function or with the phenolic function of the compound. Esters of the amino alcohols are of the first type, whereas the esters of acids conform to the latter type.

Carboxylic acid esters may be synthesized as follows:

1. Interaction of an Acid Chloride and Amino Alcohol.

R = Aliphatic or Aromatic

The general procedure is to react equimolar portions of the acid chloride and the amino alcohol in an inert solvent such as benzene. The hydrochloride of the ester usually precipitates from the reaction mixture either as an oil or in crystalline form. If a solid, the product can be purified by recrystallization from a suitable solvent. However, if the product is an oil, the solvent is removed under vacuum and the salt is generally converted into its base by use of alkali. The aqueous solution is then extracted with ether several times. The ether is removed under vacuum, leaving the basic ester. Whenever possible the ester is distilled at reduced pressure. The product is then weighed, placed in a suitable solvent (i.e. ether or benzene) and treated with one equivalent of an anhydrous alcoholic or ethereal solution of hydrogen chloride. The use of excess hydrogen chloride, may delay or inhibit crystallization and result in the

separation of an oil which remains as such indefinitely. Other salts such as the sulfate, hydrobromide and citrate may be prepared in a similar manner (41).

Acids which contain additional functional groups such as alcohols, phenols and/or amines can be made into acid chlorides only when these groups are protected in the usual manner (i.e. formation of acetates or cyclic acetals). After esterification the protective groups can be removed.

2. Interaction of an Acid with a Chloralkamine.

Horenstein and Pahlische (42) proposed this method for syntheses of basic esters. Equimolar quantities of a carboxylic acid and chloralkamine are condensed in one of two ways. The condensation can be carried out by mixing the halide with a isopropanol solution of the acid and refluxing the mixture for several hours. The hydrochloride may precipitate from the solvent and can be purified by recrystallization, many times from the same solvent. However, if the salt is soluble in the solvent, it is advisable to remove the solvent, liberate the ester base with alkali and proceed as under section 1.

An alternate procedure is to add the halide to an alcoholic solution of the acid, remove the alcohol under reduced pressure and heat the residue 10 hours at 90° to 100°. The gummy mass obtained can be extracted with acetone to remove

the unreacted materials and excess solvent. The salt of the amine which is insoluble remains and is filtered off. The salt can be recrystallized from a suitable solvent.

The method is suitable for esterification of acids containing reactive groups or esterification where the acid chlorides are inherently unstable or difficult to prepare (43).

3. Interaction of the Sodium or Silver Salt of a Carboxylic Acid and a Chloralkamine Hydrochloride.

Horenstein and Pahlloke (42) reacted these materials in the absence of a solvent. The reactants were heated generally to 130° or 140° for several hours. The mass was extracted with a suitable solvent while hot and the compound crystallized from the solvent.

4. Reaction of an Alkyl Ester with an Amino Alcohol.

The process consists of heating the methyl or ethyl ester of the acid with an excess of the dialkylaminoalkanol, usually in the presence of a small amount of sodium alkoxide, in such a manner that the lower boiling alcohol distills as it is displaced. Prolonged heating is required, therefore many unstable basic esters can not be prepared in this manner (43).

5. Reaction of a Halogen Derivative with an Amine.

The reaction between chloro- and bromoalkyl esters and amines is of limited value because of low yield and superiority of other methods (44).

6. Reaction of a Carboxylic Acid with an Aminoalcohol in the Presence of a Catalyst.

The alcohol is generally added in excess to dissolve the acid which is generally a solid. As catalyst one can employ either gaseous hydrogen chloride or concentrated sulfuric acid. The excess alcohol is then removed under vacuum and the salt prepared as in section 1 (45).

The experimental work performed by the author demonstrated that esterification of gentisic acid with diethylaminoethanol and diethylaminopropanol took place most successfully and in best yields by following the second procedure. However, the halogen derivative of dimethylaminoethanol could not be found in the literature and the reaction did not yield the desired products. The reaction is good only for tertiary amines, because amides or mixtures of amides and esters are obtained when secondary or primary amino alcohols are used (46).

The first procedure was attempted by utilizing gentisyl

chloride, but no results were obtained. The production of the acid chloride of gentisic acid without protection of the phenolic groups is questionable although salicyl chloride has been reported by Kopetschni and Ladislaus (47), as well as Wolfenstein (48).

The reaction of sodium gentisate with the hydrochloride salts of primary, secondary and tertiary amino halides did not yield esters for any of the amines.

Direct esterification by interaction of gentisic acid and the amino alcohol, dissolved in excess of the amino alcohol, did not yield an ester upon addition of several drops of concentrated sulfuric acid. The other methods of esterification of gentisic acid were not attempted.

Esters of phenolic functions may be prepared in the following manner. In the general case, the reaction is between a phenol and an acid chloride. The variations are in the use of solvent and condensing agent.

R = Aliphatic or Aromatic

1. The Interaction of a Phenol and Acid Chloride in Inert Solvent. Equimolar portions of acid chloride and phenol are dissolved in benzene or other suitable solvent and enough pyridine added to condense with the hydrogen

chloride liberated. The solution, shaken several times with dilute hydrochloric acid, removes any excess pyridine. If the product is a solid, it may be filtered off and recrystallized from a suitable solvent. However, if the product remains in solution the solvent is generally removed under reduced pressure and the mass is shaken well with 5% sodium bicarbonate to remove the unreacted phenol and the excess acid chloride. The residue is filtered and recrystallized from suitable solvent (49).

2. The Interaction of a Phenol and a Acid Chloride in Pyridine. A mixture of equimolar portions of acid chloride and phenol are added to pyridine and the mixture heated on the steam bath from 10 to 30 minutes. When the mixture is cool, it is placed in an ice bath and 5 times its volume of 5% sodium bicarbonate added with constant stirring until the product crystallizes. The solid is filtered, washed with 5% sodium bicarbonate and dried. The crude product may be recrystallized from a suitable solvent (50).

3. The Interaction of an Acid Chloride and a Phenol in Aqueous Alkali. (Schotten-Bauman reaction). Equimolar portions of the phenol and acid chloride are reacted in 10% sodium hydroxide. The phenol is dissolved in the alkaline solution and the acid chloride added with continuous stirring. The mixture is cooled slowly to room temperature and then in an ice bath. Cold dilute hydrochloric acid is added until strongly acid. The precipitate is filtered and dried. The

product can be purified by dissolving it in acetone or alcohol and reprecipitating by adding water. The process is repeated in the warm solvents to give greater purity. However, final purification should be brought about by recrystallization from a suitable solvent(51) .

Although the first two procedures were tried repeatedly with gentisic acid and various acid chlorides, no results could be obtained. In the majority of cases both the gentisic acid and the other acid were recovered. It appears that only hydrolysis of the acid chloride took place.

The third procedure yielded good results and acceptable yields of the phenolic esters. Esters of salicylic acid, 5-acetyl-gentisic acid, diphenylacetic acid and diethyl-acetic acid could not be obtained by this procedure. It is possible that the acid chlorides of these acids were not obtained.

Each ester synthesized was analyzed in the following manner, when applicable, to determine its structure and element content.

1. Physical Properties, i.e. microscopic appearance and solubility.
2. Stability in Air and Aqueous Media.
3. Qualitative Elemental Content.
4. Ferric Chloride Color Test.
5. Kjeldahl Nitrogen.
6. Ionic Chlorine Titration.

7. Carbon and Hydrogen*.

8. Neutral Equivalent.

9. Infrared Spectrum.

The physical properties of each compound were determined by examination of the product under the microscope and determination of its relative solubility in various solvents.

The stability of the compounds in air and aqueous solution was observed during several weeks.

Qualitative elemental content was determined by the procedures of Shriner and Fuson (52).

The phenolic color test, by use of ferric chloride T.S., was determined for each compound.

Kjeldahl nitrogen was determined by the micro technique (53).

Ionic chlorine was determined by titration with standard silver nitrate, the Mohr Method (54).

Carbon and hydrogen analysis was done by Eli Lilly and Co. research laboratories.

Modification of the standard neutral equivalent procedure was necessary to obtain accurate molecular weights of the synthesized carboxylic acid ester hydrochlorides. However, the molecular weights of the phenolic acid esters were determined in the usual manner.

*Only Diethylaminoethyl 5-acetyl-gentisate hydrochloride, diethylaminoethyl 5-benzoyl-gentisate hydrochloride and diethylaminopropyl gentisate hydrochloride, of the carboxylic acid ester hydrochlorides.

The esters formed through the carboxylic acid group were all tertiary amines, in the form of their hydrochlorides. Upon the titration of the compounds with standardized carbonate free base, the basic amines were released and buffer systems were established. Therefore, no sharp break in the titration curves could be obtained. The problem was to remove the basic amines which were partially or completely water soluble.

The first procedure tried was to underlay the aqueous solution of the salts with chloroform. Upon titration of the salts with base the amines passed into the chloroform layer. Only fair results were obtained, due to the fact that the partition coefficient of the amines between water and chloroform was not large enough. The amines therefore, still exerted some effect on the pH readings. The procedure possibly could be improved by use of an immiscible solvent that had a larger partition coefficient for the amines than chloroform with water, or by the determination of the partition coefficient of the amines in water and chloroform and applying a correction to the resulting molecular weight.

A method that was found to give good results was the determination of the neutral equivalent of the base, instead of the acid. In this an excess of standard base was added to 25ml. of a 50% alcoholic solution of the amine hydrochloride. The basic amine was liberated. The excess standard alkali and the amine were then titrated with standard acid to yield

sodium chloride and the salt of the amine, respectively. The entire titration was carried out under nitrogen to prevent air oxidation of the phenol in basic solution and also to prevent contamination by carbon dioxide of the air. A sharp break in the titration curve was obtained at the end point.

Good results can be obtained by use of 0.1 N. alkali and 0.1 N. acid. Microburettes are of value especially when small samples are being titrated.

Care should be exercised to standardize the base and acid in 50% alcohol which is neutral to phenolphthalein. Therefore, although the pH readings may not be accurate, as compared to aqueous solution, the change of pH per volume of acid will be the same. However, a blank should be obtained for 25 ml. of 50% alcohol to determine the amount of acid needed to return the alcohol to a pH equal to the pH at the end point of a titration of a sample.

Saponification of the esters of the carboxylic acid was attempted in alcoholic alkali, but poor results were obtained. More alkali was consumed upon refluxing the ester with alcoholic alkali than by allowing the ingredients to set in the cold, but the consumption did not represent an even number of equivalents of alkali. It is believed that due to the rapid oxidation of phenols, and more particularly quinones, in alkali media, good saponification numbers can be obtained only if both phenolic groups are blocked. The author attempted to make the diacetyl and dimethoxy derivatives of gentisic

acid, and found it very difficult to acetylate or methylate the number 2 position of the acid. Therefore, no saponification values were obtained.

All melting points were obtained by using the melting point bath apparatus. The thermometer was calibrated over the m.p. range of 50° to 285° by means of eight pure compounds. The corrected m.p. refers to correction for emergent stem.

New Esters of Gentistic Acid¹

Diethylaminoethyl Gentisate Hydrochloride*

a. Preparation

Diethylaminoethyl gentisate hydrochloride was prepared by the interaction of the halogen derivative of diethylaminoethanol and gentisic acid; the Horenstein and Pahlische method (42).

Diethylaminoethyl chloride was prepared by two methods. The first procedure was as follows (55):

Diethylaminoethanol, 43 g. (0.37 mole), was added to 50 ml. of benzene contained in a three neck, 250 ml. flask, in the hood. Thionyl chloride, 115.9 g. (0.97 mole), was placed in a separatory funnel and added slowly with constant stirring to the benzene-amino alcohol mixture. The reaction was as follows:

The reaction was violent and strongly exothermic. After addition of all the thionyl chloride the product was refluxed 6 hours. The mixture was dark brown in color and contained

*New Compound.

¹See pages 61-79 for proof of structure.

a voluminous amount of solid. The excess thionyl chloride and the solvent were removed under reduced pressure. The residue was heated to 150°, for 2 hours at the reduced pressure. The residue was then dissolved in water and the solution was filtered. The filtrate was placed in the refrigerator for several hours. The cold solution was made strongly alkaline with 40% sodium hydroxide, until the precipitation of a light brown material subsides. The reaction was as follows:

The mixture was extracted with several portions of ether until only a light yellow aqueous solution remained. After removal of the solvent by evaporation, the remainder was distilled to yield a milky solution of strong ammoniacal odor, b.p. 68-70° at 30 mm.

Redistillation yielded 30.0 g. (60.2%) of a clear, colorless, strongly ammoniacal smelling liquid, b.p. 53-54° at 18 mm.

The second procedure (56) consisted in placing 72.5 g. (0.61 mole) of thionyl chloride in a three neck flask provided with a stirrer, reflux condenser and dropping funnel. The flask was placed in an ice bath in the hood until the liquid was thoroughly cooled. Diethylaminoethanol, 52.2 g. (0.45 mole), was added dropwise with constant stirring to the

cold thionyl chloride. After all the alcohol was added the product was stirred an additional hour at room temperature.

The entire contents of the flask was transferred to a 1000 ml. beaker which contained 250 ml. of absolute alcohol. The solution was heated to a gentle boil for 1 hour or until gases ceased to be evolved. The solution was filtered hot and placed in the refrigerator over night.

The precipitate was filtered and dried. The crude yield of diethylaminoethyl chloride hydrochloride was 52 g. (68.9%) of a light brown solid, m.p. $203.5-206.5^{\circ}$ (Corr. $209-212^{\circ}$).

A less pure fraction was obtained by concentrating the filtrate to $1/3$ the original volume and recovering the precipitate. The yield was 15.72 g. (20.9%) of the less pure, dark brown solid, m.p. $193.6-198.5^{\circ}$ (Corr. $198.5-204^{\circ}$).

The total crude yield was 67.72 g. (89.8%).

Diethylaminoethyl chloride hydrochloride, 30 g. (0.17 mole), was placed in a 1000 ml. beaker and 10 g. of crushed ice added. The beaker was placed in an ice bath and 40 ml. of ether added. When the beaker and contents were cold, the mixture was made strongly alkaline with 40% sodium hydroxide. The extraction was carried out several times. The ethereal solution of the halide was dried over drierite for several hours. After the removal of the solvent by evaporation, the remaining red colored oil was distilled to yield 17.2 g. (74.1%) of a clear, colorless, strongly ammoniacal

smelling liquid, b.p. $44-45^{\circ}$ at 13 mm. (57).

Burtner (58) describes a method for the liberation of the basic halide from the hydrochloride by addition of flaked sodium hydroxide to the salt. The mixture was stirred and the halide distilled under reduced pressure. The halide was dried over potassium carbonate. However, it was found that the yield was poorer and a less pure compound was obtained than the extraction procedure.

Diethylaminoethyl chloride, 6.5 g. (0.05 mole) was added to an alcoholic solution of 7.4 g. (0.05 mole) of gentisic acid (Delta Chemical Corporation). Heat was evolved and the solution was shaken till cool. The alcohol was removed under reduced pressure. The gummy residue was heated 2 hours at 80° and 10 hours longer at 100° .

Acetone was added to the gummy residue and dissolved a portion of it, leaving a flocculant, white precipitate. The precipitate was filtered and the dried product represented a crude yield of 9.6 g. (65.1%), m.p. $140-148.5^{\circ}$ (Corr. $143-151.5^{\circ}$).

The crude ester salt, 9.18 g., was dissolved in water and 3.36 g. of sodium carbonate (67.2 ml. of 5% sodium carbonate) added. A voluminous white, cloudy solution resulted. Three 40 ml. portions of ether were used to extract the basic amine from the aqueous media. To the ethereal solution of the basic amine was added 1.16 g. of hydrogen chloride. (21.8 ml. of 1.4616 N. alcoholic hydrogen chloride solution). The

yield was 6.7 g. (45.3%) of a white, heavy solid, m.p. 149.5-151° (Corr. 152.5-154°).

The diethylaminoethyl gentisate hydrochloride was again made basic, extracted with ether and reprecipitated as the salt, m.p. of 150-151° (Corr. 153-154°).

An alternate procedure described by the same authors was as follows (42):

Gentisic acid, 5 g. (0.03 mole) was dissolved in 50 ml. of isopropanol and to the solution was added 4.1g. (0.03 mole) of diethylaminoethyl chloride. The mixture became slightly warm upon shaking. When the mixture cooled, it was refluxed 2 hours. Upon cooling to room temperature, crystallization occurred. The filtered and dried product represented a crude yield of 2.2 g. (23.5%) of a white, flocculant precipitate, m.p. 140-170° (Corr. 143-173.5°).

The ester upon recrystallization from isobutanol yielded 1.82 g. (19.8%) of a white, crystalline solid, m.p. 151.5-152.5° (Corr. 154-155°).

The product showed no change in melting point range after it was dried in the Abderhalden Drying Pistol for 3 hours at 100° and 1-3 mm.

b. Properties

Diethylaminoethyl gentisate hydrochloride is a white, grainy powder. Colorless plates are seen under microscopic magnification when the compound is purified by the reprecipitation method. However, when recrystallized from isobutanol it appears to be a sparkling, crystalline solid. Microscopic examination revealed large, colorless plates. The compound has a sweet aromatic smell.

The salt is very soluble in water, absolute alcohol, 95% alcohol, dilute alcohol, butanol, hot isobutanol and pyridine. It is partially soluble in isopropanol and insoluble in chloroform, carbon tetrachloride, toluene, benzene, ethyl acetate, ether, acetone, petroleum ether, Skellysolve B & C, dioxane and cold isobutanol.

The solid is stable in air. The compound is unstable in aqueous solution turning from a colorless to a brown solution in several days, and upon adding alkali, the color change takes place in several minutes.

Qualitative element analysis shows nitrogen and chlorine to be present.

The ferric chloride test is positive. (blue-purple in aqueous solution).

Analysis: Calculates for $C_{13}H_{20}O_4HCl$: N, 4.84; Cl, 12.68
 Found: N, 4.73, 4.64; Cl, 12.58, 12.65.

Neutral Equivalent. Calculated for $C_{13}H_{19}O_4N$: 253.30
 Found: 250.92, 253.30.

DIETHYLAMINOETHYL 5-ACETYL-GENTISATE HYDROCHLORIDE*

a. Preparation

Diethylaminoethyl 5-acetyl-gentisate hydrochloride was prepared by the interaction of 5-acetyl-gentisic acid and diethylaminoethyl chloride (42).

5-acetyl-gentisic acid was prepared by two procedures. The first consisted of the interaction of gentisic acid with acetic anhydride, while the second method employed acetyl chloride as the acetylating agent.

The first procedure was carried out as follows (59): To 300 ml. of 2 N. sodium hydroxide, heated to 60°, was added 30 g. (0.20 mole) of gentisic acid. When solution was complete, 39 g. (0.38 mole) of acetic anhydride was added and the solution was stirred till cool. The entire flask and contents were placed in an ice bath till cold. Cold dilute hydrochloric acid was then added and a creamy, white precipitate formed. The precipitate was filtered and dried. The filtrate was made more strongly acid with concentrated hydrochloric acid to insure precipitation of all the 5-acetyl-gentisic acid. The total yield was 35 g. (91.8%) of a light brown solid, m.p. 100-104.5° (Corr. 102-106.5°).

The acetylated compound was recrystallized from toluene

*New Compound.

yielding 24 g. (63.0%) of a white solid, m.p. 131.5-132.2° (Corr. 133.5-134.2°).

The equations for the reactions involved in the above procedure are as follows:

The second method for the preparation of the 5-acetyl derivative used acetyl chloride (60).

Acetyl chloride was prepared by dropwise addition of 80 g. (0.58 mole) of phosphorous trichloride to 100 g. (1.66 mole) of glacial acetic acid.

The reaction flask was placed in an ice bath and was cooled 1/2 hour. After heating at 50° for 1 hour the acetyl chloride was distilled from the mixture yielding 44.2 g. (33.0%) of a colorless, strongly irritant smelling liquid, b.p. 50-55°.

The acetyl chloride was reacted with gentisic acid in basic media, i.e. Schotten-Bauman reaction, but very poor yield of the 5-acetyl-gentisic acid was recovered. The reaction was not investigated further because of the better

yield obtained by the use of acetic anhydride.

Diethylaminoethyl 5-acetyl-gentisic acid hydrochloride was prepared successfully by the same two procedures which were used to prepare diethylaminoethyl gentisic acid.

Equimolar portions of the halide and acid were heated for 2 hours at 80° and then 10 more hours at 100° . A crude product of 49.4% yield, m.p. $108.5-119.5^\circ$ (Corr. $110.5-121.5^\circ$) precipitated when the mass was mixed with acetone.

Purification of the crude product was attempted by dissolving the solid in water, adding an equivalent portion of sodium carbonate, extracting several times with ether and reprecipitating the salt by adding alcoholic hydrogen chloride to the ether. However, an oil separated and could be solidified only by cooling. The yield was about 20% of a white solid, m.p. $118.5-126.5^\circ$ (Corr. $120.5-128.5^\circ$).

Upon repeating the reaction, and recrystallizing the crude compound from isopropanol, the yield was 5.9% of a white, crystalline solid, m.p. $133-134^\circ$ (Corr. $135.3-136.5^\circ$).

Much better success was obtained when equimolar portions of diethylaminoethyl chloride and 5-acetyl-gentisic acid were refluxed in isopropanol for 8 hours. Upon cooling the solu-

tion yielded 82.2% of a white, crystalline solid, m.p. 106.5-130.5° (Corr. 108.5-133.0°).

The ester was recrystallized 3 times from isopropanol yielding 44.2% of a white, needle-like solid, m.p. 133.5-134.5° (Corr. 136-137°).

The compound showed no change in melting point when it was dried in the Abderhalden Drying Pistol as previously described.

b. Properties

Diethylaminoethyl 5-acetyl-gentisate hydrochloride, when recrystallized from isopropanol, appears under the microscope as a colorless, flocculant solid consisting of long, thin, colorless needles. To the naked eye the compound appears as a white, flocculant, feathery powder. The ester has a sweet, aromatic smell.

The salt is very soluble in water, absolute alcohol, 95% alcohol, chloroform and hot isopropanol. It is partially soluble in butanol, isobutanol, ether and acetone, but insoluble in ethyl acetate, cold isopropanol and petroleum ether.

The dry salt is stable in air, however, in aqueous solution it turns brown in several days. The color change takes place in several minutes time in alkaline media.

Qualitative element analysis shows nitrogen and chlorine present.

The ferric chloride test is positive.

Analysis: Calculated for $C_{15}H_{22}O_5NCl$: C, 54.30; H, 6.68; N, 4.22; Cl, 10.69. Found: C, 54.41 ; H, 6.78 ; N, 4.21 and 4.13; Cl, 10.60 and 10.64.

Neutral equivalent: Calculated for $C_{15}H_{21}O_5N$: 295.33. Found: 301.51, 301.79.

DIETHYLAMINOETHYL 5-METHOXY-GENTISATE HYDROCHLORIDE* **a. Preparation**

Diethylaminoethyl 5-methoxy-gentisate hydrochloride was produced by the reaction of diethylaminoethyl chloride with 5-methoxy-gentisic acid (42).

The 5-methoxy derivative of gentisic acid was prepared by dissolving 20 g. (0.13 mole) of gentisic acid in 100 ml. of 10% hydroxide and adding 44.2 g. (0.35 mole) of dimethyl sulfate. The mixture was shaken 30 minutes during which there was a strong evolution of heat. The mixture was refluxed 1 hour. The resulting aqueous mixture was acid and was made strongly alkaline with 20% sodium hydroxide. The heterogenous mixture was refluxed for another hour. At this point the mixture was alkaline or was made alkaline as before and the refluxing repeated. The mixture was acidified with dilute hydrochloric acid and cooled.

A yield of 30 g. (85.6%) of a light brown, crystalline solid, m.p. 133.5-145.5° (Corr. 136-148°) was obtained (61).

The methyl ether was recrystallized from water to yield 15 g. (64.2%) of a colorless, needle-like solid, m.p. 143.5-144.5° (Corr. 146-147°).

*New Compound.

The reactions involved in the preparation of 5-methoxy-gentisic acid were as follows:

Diethylaminoethyl 5-methoxy-gentisate hydrochloride was formed by the reaction of the halogen derivative of the amino alcohol and gentisic acid in absolute alcohol. The alcohol was removed under reduced pressure and the mixture heated for 2 hours at 80° and then 10 additional hours at 100° . Upon crystallization, from isobutanol, a yield of 92.9% of a sweet smelling solid, m.p. $178-183.5^\circ$ (Corr. $182.5-188^\circ$) was obtained.

Recrystallization of the compound from isobutanol, 3 times, yielded 58.0% of a white, crystalline solid, m.p. $182.3-183.0^\circ$ (Corr. $186.8-187.5^\circ$).

The compound was dried in the Abderhalden Drying Pistol as previously described and no change in the melting point was noticed.

b. Properties

Diethylaminoethyl 5-methoxy-gentisate hydrochloride is a light brown, or colorless, crystalline solid. It appears as large, colorless needles under the microscope. The compound has a sweet, aromatic smell.

The salt is soluble in water, absolute alcohol, 95% alcohol, dilute alcohol, chloroform, toluene and hot isobutanol. It is partially soluble in isopropanol and ether, but insoluble in acetone and cold isobutanol.

The amino-ester is stable as a solid. However, an aqueous solution is unstable, turning brown in several days. The color change is greatly hastened in alkaline solution.

Analysis for the element content of the compound shows nitrogen and chlorine to be present.

A positive test is obtained with ferric chloride.

Analysis: Calculated for $C_{14}H_{22}O_4NCl$: N, 4.61; Cl, 11.67. Found: N, 4.46, 4.41; Cl, 11.70, 11.55.

Neutral equivalent: Calculated for $C_{14}H_{21}O_4N$: 267.32. Found: 268.39, 270.31.

DIETHYLAMINOETHYL 5-BENZOYL-GENTISATE HYDROCHLORIDE*

Diethylaminoethyl 5-benzoyl-gentisate hydrochloride was formed by the same reaction as the other carboxylic acid ester hydrochlorides of gentisic acid (42).

5-benzoyl-gentisic acid was prepared by the Schotten-Bauman reaction between benzoyl chloride and gentisic acid (62). The yield was 84.2% of a white, flocculant solid, m.p. 166.5-171.5° (Corr. 170-175°).

Upon recrystallization of the compound from water, a white solid, m.p. 172.5-174.5° (Corr. 176-178°) was obtained.

The solid was recrystallized twice by dissolving it in hot acetone and adding water till the solution was cloudy. Upon cooling, a crystalline solid, m.p. of 178-179° (Corr. 181.5-182.5°), precipitated.

5-benzoyl-gentisic acid and diethylaminoethyl chloride were reacted in equimolar portions in isopropanol. The solution was refluxed 4 hours and was cooled to yield 77.8% of a white solid, m.p. 130.5-146.5° (Corr. 133-149°).

The compound after 3 recrystallizations from isopropanol, yielded 3.1 g. (53.1%) of a white, flocculant solid, m.p. 153.0-154.0° (Corr. 156.5-157.5°).

*New Compound.

The compound was dried in the vacuum oven at 100°, 10-15 mm. for 13 hours. The melting point was the same.

b. Properties

Diethylaminoethyl 5-benzoyl-gentisate hydrochloride is white, flocculant powder. Under the microscope, it appears to be long, thin, colorless needles. The compound is odorless.

The salt is soluble in water, absolute alcohol, 95% alcohol, dilute alcohol, chloroform, hot isobutanol, hot isopropanol and hot toluene. It is partially soluble in ether and insoluble in cold isobutanol, cold isopropanol and cold toluene.

The product is stable in air, when dry. However, it turns brown in aqueous solution within several days. The change of color takes place within several minutes in alkaline solution.

The qualitative element content is found to be nitrogen and chlorine.

The phenolic test with ferric chloride is positive.

Analysis: Calculated for $C_{20}H_{24}O_5NCl$; C, 60.85; H, 6.14; N, 3.56; Cl, 9.00. Found: C. 60.40; H. 6.41; N, 3.45, 3.40; Cl. 8.92, 8.95.

Neutral equivalent: Calculated for $C_{20}H_{23}O_5N$: 357.39
Found: 357.10, 354.39.

DIETHYLAMINOPROPYL GENTISATE HYDROCHLORIDE^{2*} a. Preparation

Diethylaminopropyl gentisate hydrochloride was prepared by the interaction of diethylaminopropyl chloride and gentisic acid (42).

Diethylaminopropyl chloride was prepared from the alcohol. A solution of 52.33 g. (0.43 mole) of 3-diethylamino, 1-propanol in 86.66 ml. of anhydrous chloroform was added, dropwise, to a solution of 96.66 g. (0.81 mole) of thionyl chloride in 400 ml. of anhydrous chloroform. The reaction was controlled by cooling the flask. After the reaction had cooled, the mixture was refluxed 3 hours. The solvent and excess thionyl chloride were then removed under reduced pressure, leaving a brown-black residue.

The solid was placed in a beaker in an ice bath. Enough ether was added to cover the solid and then cold, 40% sodium hydroxide was added. The aqueous solution of basic amine was extracted 3 times with ether. The light-brown ethereal extract was dried over drierite several hours.

After removal of the solvent, the remainder was distilled to yield 47.0 g. (78.0%) of a colorless, strongly ammoniacal smelling liquid, b.p. 93-97° at 25 mm. (63)

An equimolar portion of diethylaminopropyl chloride was

^{2*}New Compound.

added to an isopropanol solution of gentisic acid. The solution was refluxed 6 hours, and cooled.

After evaporation of the isopropanol, the semi-solid was mixed with acetone and a yield of 37.0% of a white solid, m.p. 163.5-169.5° (Corr. 166.0-172°), was obtained.

The compound was recrystallized from isobutanol to yield 24.5% of a white, crystalline solid, m.p. 171.7-172.7° (Corr. 174.2-175.2°).

The melting point remained constant when the ester was dried in the Abderhalden Drying Pistol in the same manner as previously described.

b. Properties

Diethylaminopropyl gentisate hydrochloride is colorless, crystalline needles. Under the microscope it appears to be long, thin, colorless crystals.

The hydrochloride is soluble in water, absolute alcohol, 95% alcohol, dilute alcohol, isopropanol, chloroform and hot isobutanol. It is insoluble in acetone and cold isobutanol.

The dry product is stable in air, but in aqueous solution it darkens in several days; the time is decreased to several minutes in alkaline media.

The analysis of the compound for elements shows nitrogen and chlorine.

The ferric chloride test shows a phenol to be present.

Analysis: Calculated for $C_{14}H_{22}O_4NCl$: C, 55.35; H, 7.30; N, 4.61; Cl, 11.68. Found: C, 55.45 ; H, 7.42 ; N, 4.73, 4.57; Cl, 11.76, 11.84.

Neutral equivalent: Calculated for $C_{14}H_{21}O_4N$: 267.32. Found: 264.98, 266.01.

DIETHYLAMINOPROPYL 5-ACETYL-GENTISATE HYDROCHLORIDE^{*} a. Preparation

Diethylaminopropyl 5-acetyl-gentisate hydrochloride was prepared by the Horenstein and Pahlische reaction (42). Diethylaminopropyl chloride and 5-acetyl-gentisic acid were prepared as previously described. The acid was dissolved in isopropanol and an equimolar portion of halide was added with constant shaking. The mixture was refluxed 6 hours and was cooled to room temperature. No precipitation took place. After evaporation of the isopropanol, the gummy residue yielded 35.5% of white solid, m.p. 152-153.5° (154.5-156°), upon addition of ethyl acetate.

The product was recrystallized 3 times from toluene to yield 20.6% of white, flocculant solid, m.p. 157.0-157.5° (Corr. 160.5-161°).

The melting point remained constant when the compound was dried in the Abderhalden Pistol as outlined for the preceding compounds.

^{*}New Compound.

b. Properties

Diethylaminopropyl 5-acetyl-gentisate is a small white, needle-like solid. It appears to be long, thin, colorless needles under the microscope. The solid has a sweet, aromatic odor.

The compound is soluble in water, absolute alcohol, 95% alcohol, dilute alcohol, ether, chloroform, acetone, and hot toluene. However, it is insoluble in ethyl acetate and cold toluene.

The dry solid is stable upon exposure to air, but unstable in aqueous solution, turning brown in several days. The basic amine is more unstable, turning brown within 1 hour.

The compound gives positive tests for nitrogen and chlorine.

The ferric chloride test is positive.

Analysis: Calculated for $C_{16}H_{24}O_5NCl$: N, 4.05; Cl, 10.25. Found: N, 3.96, 4.11; Cl, 10.44, 10.25.

Neutral equivalent. Calculated for $C_{16}H_{23}O_5$ N: 309.35. Found: 310.36, 308.56.

DIETHYLAMINOPROPYL 5-METHOXY-GENTISATE HYDROCHLORIDE* a. Preparation

Diethylaminopropyl 5-methoxy-gentisate hydrochloride was prepared by the interaction of diethylaminopropyl chloride and 5-methoxy-gentisic acid (42).

Equimolar portions of the ingredients were mixed in isopropanol and refluxed 6 hours. Upon cooling 54.7% of a white, flocculant solid, m.p. 185.0-186.5° (Corr. 189.5-191°), precipitated.

The product was recrystallized from isopropanol twice to yield 48.1% of white, flocculant solid, m.p. 184.5-185.3° (Corr. 189-189.8°).

The compound was dried in the Abderhalden Pistol as previously described. No change in melting point was noticed.

*New Compound

b. Properties

Diethylaminopropyl 5-methoxy-gentisate hydrochloride is a white, flocculant powder. It appears to be short, thin, colorless needles under the microscope. It has a sweet, aromatic aroma.

The compound is soluble in water, 95% alcohol, absolute alcohol, dilute alcohol, chloroform and hot isopropanol. It is insoluble in cold isopropanol.

The salt is stable as a solid, but unstable in aqueous solution, in which it turns brown. The color change is facilitated by alkali.

Qualitative elemental analysis shows nitrogen and chlorine.

The ferric chloride color test is positive.

Analysis: Calculated for $C_{15}H_{24}O_4NCl$: N, 4.41; Cl, 11.16. Found: N, 4.38, 4.32; Cl, 11.25, 11.29.

Neutral equivalent: Calculated for $C_{15}H_{23}O_4N$: 281.34. Found: 275.81, 284.53.

DIETHYLAMINOPROPYL 5-BENZOYL-GENTISATE HYDROCHLORIDE* a. Preparation

Diethylaminopropyl 5-benzoyl-gentisate hydrochloride was prepared by the Horenstein and Pahlische reaction (42).

Diethylaminopropyl chloride was added to a solution of 5-benzoyl-gentisic acid in isopropanol. The mixture was refluxed for 4 hours, was cooled and the solvent was evaporated. The mass yielded 31.6% of a white solid, m.p. 127-135° (Corr. 129.5-137.5°), when mixed with ethyl acetate.

Upon 2 recrystallizations from toluene, the yield was 16.9% of a white, flocculant solid, m.p. 142.7-143.7° (Corr. 145.2-146.2°).

The ester was dried in the Abderhalden Drying Pistol as previously described, and no change in melting point was noticed.

*New Compound.

b. Properties

Diethylaminopropyl 5-benzoyl-gentisate hydrochloride is a white, fluffy solid which appears to be small, colorless needles when viewed with the microscope.

The product is soluble in water, absolute alcohol, 95% alcohol, chloroform, hot toluene and acetone. It is insoluble in cold toluene, ether and ethyl acetate.

The ester is stable when exposed to air, but turns brown in aqueous solution after several days. Basic solution hastens the color change.

Qualitative element analysis shows nitrogen and chlorine to be present.

The ferric chloride test is positive.

Analysis. Calculated for $C_{21}H_{26}O_5NCl$: N, 3.43; Cl, 8.69; Found: N, 3.58, 3.61; Cl, 8.60, 8.64.

Neutral equivalent. Calculated for $C_{21}H_{25}O_5N$: 373.42
Found: 365.12, 367.56.

a. Preparation

5-phenylacetyl-gentisic acid was prepared by the interaction of phenylacetyl chloride and gentisic acid. The Schotten-Bauman type reaction was used (59). The reaction was suitable because of the high reactivity of the number 5 phenolic function of gentisic acid in alkaline media and the inertness of the number 2 phenolic function.

Phenylacetyl chloride was produced in the usual manner, i.e. interaction of phenylacetic acid and thionyl chloride. Upon removal of the excess thionyl chloride, the remainder was distilled to yield 78.0% of red colored, strongly irritant smelling, oily liquid, b.p. 138° at 30 mm.

Gentisic acid, 6 g. (0.04 mole), was dissolved in 30 ml. of 10% sodium hydroxide and 5.4 g. (0.04 mole) of phenylacetyl chloride was added with rapid stirring. A great amount of heat was evolved and when the mixture was cooled only a slight amount of precipitation took place. The mixture, however, yielded 7 g. (65.9%) of a dense, light yellow precipitate, m.p. $126.5-136.5^{\circ}$ (Corr. $129-139^{\circ}$), when made acidic with dilute hydrochloric acid.

*New Compound.

The reactions that took place in the above preparation were as follows:

The product was a mixture of white, fluffy solid and yellow, curdy solid, m.p. 141.5-144.5° (Corr. 144-147°), when reprecipitated from an acetone solution by adding water.

The mixture yielded 3 g. (28.3%) of a white, fluffy solid, m.p. 145.5-146.5° (Corr. 148-149°), when recrystallized from water.

The compound was dried in the drying pistol in the same manner as the other compounds. No change in melting point of compound resulted.

b. Properties

5-phenylacetyl-gentisic acid is a white, cotton-like solid. Under the microscope it appears to be long, thin, colorless fibers. It has no noticeable odor.

The acid is soluble in absolute alcohol, ether, acetone, 95% alcohol and chloroform. It is partially soluble in hot water, but insoluble in cold water.

The product is stable on exposure to air, but will turn brown in water when solubilized by basic sodium salts.

The phenolic ester gives a strong blue color when ferric chloride is added to an alcoholic solution.

Analysis: Calculated for $C_{15}H_{12}O_5$: C, 66.17; H, 4.44.
Found: C, 66.21; H, 4.51.

Neutral equivalent. Calculated for $C_{15}H_{12}O_5$: 272.25.
Found: 272.98, 273.78.

5-PARANITROBENZOYL-GENTISIC ACID* a. Preparation

5-paranitrobenzoyl-gentisic acid was prepared by the Schotten-Bauman reaction between paranitrobenzoyl chloride and gentisic acid (59).

Paranitrobenzoyl chloride was obtained in the usual manner, i.e. interaction of the acid and thionyl chloride (65). The thionyl chloride was evaporated and the residue recrystallized from toluene to yield 66.7% of a yellow, needle-like solid, m.p. 73.0-73.8° (Corr. 74.2-75.1°).

The acid chloride and gentisic acid were reacted as stated to yield 79.9% of a yellow solid, m.p. 210-220.5° (Corr. 215.8-226.3°).

The crude product was recrystallized from dilute alcohol, 5 times, to yield 39.5% of a light, yellow, crystalline solid, m.p. 226-228.5°d. (Corr. 231.8-234.3°d.). Final purification by recrystallization from boiling water yielded 33.0% of a light yellow, fluffy solid, m.p. 229-230.5°d. (Corr. 234.8-236.3°d.).

There was no change in melting point range upon drying the product in the Abderhalden pistol in the manner described for the other compounds.

*New Compound.

b. Properties

5-para-nitrobenzoyl-gentisic acid is a light yellow, flocculant solid. When viewed under the microscope, it appears to be light yellow needles.

The product is soluble in isobutanol, ethyl acetate, hot dilute alcohol, ether and acetone. It is partially soluble in hot water, absolute alcohol and 95% alcohol, but insoluble in water, cold dilute alcohol, chloroform and toluene.

The solid is stable in air, but in alkaline aqueous solution it rapidly turns brown.

Element analysis shows nitrogen.

The acid shows a strong ferric chloride test.

Analysis: Calculated for $C_{14}H_9O_7N$: C, 55.46; H, 2.99.
Found: C, 55.43; H, 2.97.

Neutral equivalent. Calculated for $C_{14}H_9O_7N$; 303.20.
Found: 305.57, 302.80.

5-ANISYL-GENTISIC ACID* a. Preparation

5-anisyl-gentisic acid formed when anisyl chloride was added to an alkaline solution of gentisic acid (59).

The anisic acid was prepared from methyl-parahydroxybenzoate or methyl paraben. The methylation using methyl sulfate has been described previously when the 5-methoxy-carboxylic acid esters were discussed (61).

The yield was 70% of a white, needle-like solid, m.p. 155-175° (Corr. 158.5-179.0°).

The crude product was recrystallized twice from water to yield 50.5% of a white, needle-like solid, m.p. 184.5-186° (Corr. 189-190.5°).

Anisyl chloride was prepared by the interaction of thionyl chloride and anisic acid. Upon removal of the thionyl chloride, the remainder was distilled to yield 74.2% of a colorless, irritant smelling, oily liquid, b.p. 129-131° at 8 mm.

When the Schotten-Bauman type reaction was carried out between anisyl chloride and gentisic acid a yield of 83.7% of light gray solid, m.p. 175-205° (Corr. 178.5-210°), was obtained.

*New Compound.

The product was purified by dissolving it alternately in hot acetone and hot alcohol, then adding hot water till the solution became cloudy. After repeating the purification 3 times a yield of 66.6% of a white, flocculant solid, m.p. 208.4-209.5° (Corr. 213.9-215.0°), was obtained.

When the solid was dried in the Abderhalden Drying Pistol as described previously, no change in melting point took place.

b. Properties

5-anisyl-gentisic acid is a light, white solid. Under the microscope it appears as bundles of dense, fine, colorless needles. It is odorless.

The product is soluble in absolute alcohol, chloroform, ether, 95% alcohol and acetone. It is partially soluble in hot water and insoluble in cold water.

The acid is stable as a solid, but unstable when placed in solution by alkaline solubilizer.

The compound gives a positive ferric chloride test.

Analysis: Calculated for $C_{15}H_{12}O_6$: C, 62.50; H, 4.19
Found: C, 62.43; H, 4.54.

Neutral equivalent. Calculated for $C_{15}H_{12}O_6$: 288.25
Found: 289.23, 291.76.

5-ACETYLSALICYL-GENTISIC ACID* a. Preparation

5-acetylsalicyl-gentisic acid was prepared by the interaction of acetylsalicyl chloride and gentisic acid (59).

Acetylsalicylic acid was treated with thionyl chloride and yielded 62.3% of a colorless, strongly irritating liquid, b.p. 135.5-136.5 at 8 mm. The liquid solidified to a white powder in several days (65).

Gentisic acid was dissolved in 10% aqueous sodium hydroxide and acetylsalicyl chloride added dropwise with constant stirring. Upon cooling, the cloudy solution was made acid with dilute hydrochloric acid. The yield was 73.2% of a light brown solid, m.p. 163.5-168.5° (Corr. 167-172°).

The crude product was recrystallized twice from an acetone-water mixture and once from a alcohol-water mixture to yield 16.1% of a light gray solid, m.p. 203.5-204.7° (Corr. 209°-210.2°).

The m.p. remained constant when the product was dried in the Abderhalden Pistol like the other compounds.

*New Compound.

b. Properties

5-acetylsalicyl-gentisic acid is a white or light gray, fine, flocculant powder. Under the microscope small hair-like sheaves of colorless needles are seen.

The acid is soluble in absolute alcohol, 95% alcohol, ether, isobutanol, acetone, and glacial acetic acid. However, it is insoluble in water, chloroform and dilute acetic acid.

The product is stable in air, but turns dark in aqueous alkaline solution.

A strong positive test for phenols is obtained by use of ferric chloride.

Analysis: Calculated for $C_{16}H_{12}O_7$: C, 60.76; H, 3.83.
Found: C, 61.74, 61.53; H, 3.92, 3.86.

Neutral equivalent, Calculated for $C_{16}H_{12}O_7$: 316.26.
Found: 289.68, 281.11.

5,5'-SUCCINYL-DIGENTISIC ACID* a. Preparation

5,5'-succinyl-digentisic acid was prepared, as were all the phenolic esters, by the Schotten-Bauman reaction (59).

Succinyl chloride was prepared by the following procedure (66).

Succinic acid, 20 g. (0.17 mole), and 35.4 g. (0.17 mole) of phosphorous pentachloride were placed in a flask provided with an air condenser in a well ventilated hood. The mixture was heated, in an oil bath, slowly to 125°. The mixture first liquified and then formed a black mass. The heating was continued 20 hours. Upon cooling, phosphorous oxychloride was removed and the residue distilled to yield 30.0% of a light yellow-green, irritant smelling liquid, b.p. 84° at 8 mm.

The following reaction represents the preparation of succinyl chloride.

Succinyl chloride and gentisic acid when reacted in the ratio of 1 : 2, yielded 27.4% of a light brown solid, m.p.

*New Compound.

210-215°d. (Corr. 215.5-220.5°d).

The crude product was recrystallized, 3 times, from a acetone-water mixture to yield 12.4% of a light brown solid, m.p. 227-229°d. (Corr. 234-236°d.).

Final recrystallization from hot alcohol-hot water yielded 10.1% of a light brown or white solid, m.p. 230-231.5°d. (Corr. 237-238.5°d.).

The solid maintained the same melting point after drying in the Abderhalden Drying Pistol as previously described.

b. Properties

5,5'-succinyl-digentisic acid is a very light brown or white solid. It appears to be small, colorless fibers under microscopic magnification.

The product is soluble in acetone, absolute alcohol, 95% alcohol and pyridine. It is partially soluble in ether, but insoluble in chloroform, water, benzene and toluene.

The acid is stable on exposure to air, but unstable in aqueous alkali, turning brown in a short time.

The ferric chloride test is positive.

Analysis: Calculated for $C_{18}H_{14}O_{10}$: C, 55.39; H, 3.62.
Found: C, 55.20; H, 3.82.

Neutral equivalent: Calculated for $C_{18}H_{14}O_{10}$: 390.29
Found: 387.59, 389.14.

Proof of Structure of the Synthesized Esters by the
Analysis of Their Infrared Spectra.

In any chemical synthesis it is necessary to prove the structure of the compound prepared. Quantitative analysis of the element content of a compound and the determination of other physical constants, i.e. molecular weight, of the product establishes only its empirical formula. The possibility still exists that the compound may be an isomer of the desired synthetic. By use of infrared analysis the functional groups of the compound can be determined. The "finger print" of the compound is said to have been obtained. The infrared spectra of the compounds prepared have been utilized in this way to determine their structure.

An atom may absorb energy in the form of heat or light by converting the incident energy into a form of potential energy. This is accomplished by an electron "jumping" from a low-energy level to a higher energy level. This makes up the absorption spectrum of an atomic vapor which generally consists of a few dark lines.

However, there are three ways in which discrete amounts of energy may be absorbed by molecular vapor. The first is the electron jump. In a molecule the possible electronic energy levels are modified by the presence of several atoms, therefore energy may be absorbed by converting it into energy of vibration or rotation. In general, the energy in-

involved in the change of energy levels of an electron is greater than that involved in vibration or rotation. Therefore, in a molecule, the absorption line corresponding to a single electronic energy change is found to be spread out into a band of many fine lines because of the many possible values of vibrational and rotational energy changes.

This condition holds true in general for a molecular vapor or gas. If this gas is compressed, however, it is observed that the fine lines of the band spectrum coalesce because of the effects of crowding the molecules together. Finally, in a liquid, all the fine structure will become simply a region of absorption. Such an absorption region may represent only a single electron transition, modified by vibrational and rotational effects.

Infrared and Raman spectra usually involve energy transitions due to vibration and rotation alone. It is thus possible to study the dynamics of the molecule itself. (67).

The infrared spectrophotometer is used for quantitative measurements of concentration and even for automatic control and recording. In addition, it is a valuable tool for detecting and identifying small amounts of impurities and for the elucidation of the structure of molecules. (68).

The establishment of molecular structure has an empirical basis and rests on the observation that certain combinations of atoms, frequently in complex molecules, may be associated with certain absorption bands in the

infrared of these substances. If the bands produced by each atomic group were invariant and unique in their position in the spectra of all substances containing the group it would be immediately possible to determine the presence or absence of any group in a given molecule by the measurement of its infrared spectrum. However, each compound possesses its own spectrum. The other structures adjacent to a given group influence its electronic and spatial configurations. In addition, the masses of the other atoms are involved in the vibration change from molecule to molecule. As a result, a given group may show somewhat different values for its absorption bands in the spectra of different molecules which contain it. Further, the bands due to one group in one compound may have the same wave length value as an entirely different structural group in another compound. As a result, some degree of uncertainty may attach itself to the interpretation of bands suspected of emanating from any particular group.

A complete test of a given molecular structure based on infrared data would result only if a precise mathematical computation would predict from the structure the spectrum actually observed. Such proof is possible only in simple molecules. It has been achieved for a number of such molecules where the intramolecular linkages were well known and only the orientation of the bonds and the interatomic distances were unknown.

The above conditions were never fulfilled for complex molecules. The absorption spectrum and limited chemical data are the only sources of information available. The intramolecular distances are unknown, and there is little or no basis for computation. Even when the linkages are known, the complexity of the molecules defies such an approach. Under these conditions it is necessary to resort to empiricism and to draw conclusions about structures by correlations between bands which occur regularly with recurring structural features throughout a series of compounds.

The interpretation of the spectrum, based upon comparison with the spectrum of compounds containing similar known functional groups, in combination with other physical and chemical evidence may furnish a guide sufficient for the identification of the structure of the compound. (69).

Infrared Spectra and Interpretation

The spectra were produced by Model B. Baird Associates Inc. automatic recording infrared spectrophotometer. A standard liquid cell, 0.10 mm. thick, containing sodium chloride windows was used. The solvents used were chloroform, ether and xujol gel. The operator was Robert Lieberman, graduate student, Department of Chemistry, Ohio State University. The interpretations of the spectra were made by the author with the aid of the infrared charts compiled by the Infrared Laboratory, Department of Chemistry, Ohio State University and the Tables compiled by Randall et al (69).

Table I.

Wave length of absorption by functional groups in infrared spectra

Functional Group	Wave Length in Microns (μ)
hydroxy (OH) in phenol.	2.95
phenyl ring/aromatic C-H)	3.11-3.18
hydrogen chloride	3.70-4.10
carbonyl (C=O) in ester	5.70-5.80 (Unconjugated 5.78-6.02)* (Conjugated 5.68-5.81)*
carbonyl (C=O) in acid	5.90-5.95 (Unconjugated 5.70-5.92)* (Conjugated 5.78-6.02)*
carbon double bond (C=C) in benzene	6.04 (6.10-6.62)*
benzene overtone	5.00-6.00
phenyl (C ₆ H ₅)	6.15-6.38 (Unconjugated 6.65-6.70)* 6.20-6.26)*
nitro (O=N=O)	6.48-6.72 (Conjugated 6.30-6.35)* 7.41-7.70 (6.17-6.56)*
acetals or ethers (-O-)	8.95-9.15

The regular numbers are the wave lengths in microns obtained from the charts in the Infrared Laboratory at Ohio State University.

Numbers represent wave length in microns obtained from "Infrared Determination of Organic Molecules" by Randall et al.

Figure 1
Infrared Spectrum of Gentisic Acid

Figure 2
Infrared Spectrum of 5-Acetyl-Gentisic Acid

Figure 3
Infrared Spectrum of 5-Methoxy-Gentisic Acid

Figure 4

Infrared Spectrum of 5-Benzoyl-Gentisic Acid

Figure 5

Infrared Spectrum of Diacetyl-Gentisic Acid

Figure 6

Infrared Spectrum of Diethylaminoethyl-Gentisate Hydrochloride

Table II.

Analyses of Infrared Spectra of Known Compounds

Figure Number	Known Compound	Solvent	% Concentration	Functional Groups	Maximum of Absorption by Functional Groups in μ
1	Gentisic Acid	CH_3OCH_3	10	OH in phenol	2.99
				Aromatic C-H	3.09
				C=O in acid	5.95
				C=C in benzene	6.14
				C_6H_5	6.75
2	5-Acetyl-Gentisic Acid	CHCl_3	5	Aromatic C-H	3.30
				C=O in ester	5.69
				C=O in acid	5.97
				C=C in benzene	6.12
				C_6H_5	6.72
3	5-Methoxy-Gentisic Acid	CHCl_3	2.5	Aromatic C-H	3.34
				C=O in acid	5.98
				C=C in benzene	6.15
				C_6H_5	6.71
4	5-Benzoyl-Gentisic Acid	CHCl_3	5	Aromatic C-H	3.35
				C=O in ester	5.76
				C=O in acid	5.98
				C=C in benzene	6.12
				C_6H_5	6.73
5	Diacetyl-Gentisic Acid	CHCl_3	5	Aromatic C-H	3.32
				C=O in ester	5.67
				C=O in acid	5.87
				C=C in benzene	6.15
				C_6H_5	6.72

Figure 7
Infrared Spectrum of Diethylaminoethyl 5-Acetyl-Gentisate
Hydrochloride

Figure 8
Infrared Spectrum of Diethylaminoethyl 5-Methoxy-Gentisate
Hydrochloride

Figure 9
Infrared Spectrum of Diethylaminoethyl 5-Benzoyl-Gentisate
Hydrochloride

Figure 10
Infrared Spectrum of Diethylaminopropyl Gentisate Hydrochloride

Figure 11
Infrared Spectrum of Diethylaminopropyl 5-Acetyl-Gentisate Hydrochloride

Figure 12
Infrared Spectrum of Diethylaminopropyl 5-Methoxy-Gentisate Hydrochloride

Table III.

Analyses of Infrared Spectra of Diethylaminoethanol Esters of Gentisic Acid

Figure Number	Synthetic Compound	Solvent	% Concentration	Functional Groups	Maximum of Absorption by functional groups in μ
6	Diethyl-aminoethyl Gentisate Hydrochloride	Nujol	---	OH in phenol	3.12
				Aromatic C-H	3.42
				C=O in ester	5.92
				C=C in benzene	6.08
				C ₆ H ₅	6.72
7	Diethyl-aminoethyl 5-Acetyl-Gentisate Hydrochloride	CHCl ₃	10	OH in phenol	3.10
				Aromatic C-H	3.39
				HCl	4.21
				C=O in ester	5.66,
					5.89
8	Diethyl-aminoethyl 5-Methoxy-Gentisate Hydrochloride	CHCl ₃	3	C=C in benzene	6.12
				C ₆ H ₅	6.73
				OH in phenol	3.08
				Aromatic C-H	3.40
				HCl	4.30
9	Diethyl-aminoethyl 5-Benzoyl-Gentisate Hydrochloride	CHCl ₃	10	C=O in ester	5.93
				C=C in benzene	6.15
				C ₆ H ₅	6.69
				OH in phenol	3.08
				Aromatic C-H	3.39
				HCl	4.25
				C=O in ester	5.76,
					5.90
				C=C in benzene	6.11
				C ₆ H ₅	6.70

Figure 13
Infrared Spectrum of Diethylaminopropyl 5-Benzoyl-Gentisate
Hydrochloride

Figure 14
Infrared Spectrum of 5-Phenylacetyl-Gentisic Acid

Figure 15
Infrared Spectrum of 5-Paranitrobenzoyl-Gentisic Acid

Table IV.

Analyses of Infrared Spectra of Diethylaminopropanol Esters of Gentisic Acid

Figure Number	Synthetic Compound	Solvent	% Concentration	Functional Groups	Maximum of Absorption by functional groups in μ
10	Diethyl-aminopropyl Gentisate Hydrochloride	Nujol	---	Aromatic C-H C=O in ester C=C in benzene C_6H_5	3.19 6.00 6.13 6.75
11	Diethyl-aminopropyl 5-Acetyl-Gentisate Hydrochloride	$CHCl_3$	5	Aromatic C-H HCl C=O in ester C=C in benzene C_6H_5	3.35 4.22 5.67, 5.92 6.12 6.72
12	Diethyl-aminopropyl 5-methoxy gentisate Hydrochloride	$CHCl_3$	10	OH in phenol Aromatic-C-H HCl C=O in ester C=C in benzene C_6H_5	3.10 3.40 4.18 5.94 6.15 6.68
13	Diethyl-aminopropyl 5-Benzoyl-Gentisate Hydrochloride	$CHCl_3$	10	OH in phenol Aromatic C-H HCl C=O in ester C=C in benzene C_6H_5	3.08 3.39 4.23 5.76, 5.92 6.12 6.72

Figure 16
Infrared Spectrum of 5-Anisyl-Gentisic Acid

Figure 17
Infrared Spectrum of 5-Acetylsalicyl-Gentisic Acid

Figure 18
Infrared Spectrum of 5,5'-Succinyl-Digentisic Acid

Table V

Analyses of Infrared Spectra of Phenolic Esters of Gentisic Acid

Figure Number	Synthetic Compound	Solvent	% Concentration	Functional Groups	Maximum of Absorption by functional groups in μ
14	5-Phenyl-acetyl-Gentisic Acid	CHCl_3	10	OH in phenol Aromatic C-H C=O in ester C=O in acid C=C in benzene C_6H_5	2.93 3.32 5.67 5.98 6.12 6.71
15	5-Paranitro-benzoyl-Gentisic Acid	CH_3OCH_3	Saturated	Aromatic C-H C=O in ester C=O in acid C=C in benzene Nitro	3.35 5.72 5.92 6.15 6.48
16	5-Anisyl-Gentisic Acid	CHCl_3	5	Aromatic C-H C=O in ester C=O in acid C=C in benzene C_6H_5	3.36 5.79 5.95 6.20 6.62, 6.75
17	5-Acetyl-salicyl-Gentisic Acid	CHCl_3	Saturated	Aromatic C-H C=O in acid C=C in benzene C_6H_5	3.35 5.95 6.15 6.72
18	5,5'-Succinyl-Digentisic Acid	Nujol	---	C=O in ester C=O in acid C=C in benzene	5.65 5.98 6.09

In figures 1 through 5 the infrared spectra for known compounds are shown. The two main features that are outstanding in each spectrum are the low transmissions in the 3.0 to 4.0 micron range and the 5.6 to 6.0 micron range. In figure 1, the strong absorptions in these regions, indicates clearly a phenolic group or groups and one carboxylic acid function, respectively. However, in figures 2 through 5 where the 5-phenolic function has a substituted group on it, the nature of the absorption is markedly changed. Special attention is called to figure 4 because it is a disubstituted phenolic ester. However, here the absorption in the 3.0 to 4.0 micron range appears very similar to that of figures 2,3,and 5. This seems to indicate the absorption pattern change is due to the substitution in the number five position of gentisic acid.

In the region of 5.6 to 6.0 microns of figures 2 and 5 two absorption "humps" are prominent. One represents the ester on the number five phenolic group and the other shows the carboxylic acid function. In figure 4, this region is nearly solid indicating very strong absorption due to the esters and the carboxylic acid groups. Figure 3 shows only the acid function, but the ether does not show clearly because each spectrum has strong absorption in the 8.95 to 9.15 micron region.

Since these two regions, i.e. 3.0 to 4.0 micron and 5.6 to 6.0 micron, are the only ones of value for the interpre-

tation of the known compounds spectra, the unknowns can be evaluated only in these regions.

Figures 6 to 13, inclusive, represent the carboxylic acid ester hydrochlorides synthesized. In each spectrum noticeable change of absorption has taken place in the 3.0 to 4.0 micron region, from that of gentisic acid. It seems to indicate from these tracings that any substitution on the three functional groups greatly decreases the absorption in the 3.0 to 4.0 micron region.

Figure 6 shows strong absorption at 3.40-3.50, 6.78-6.88 and 7.22 microns. The strong absorptions at these wavelengths are due to nujol and are prominent in figures 10 and 18 also.

The "carbonyl" region shows exactly what one would predict from a look at the proposed structure of the synthesized compounds. Figures 6, 8, 10, and 12 show only one absorption maximum in the 5.6 to 6.0 micron region. This represents the carboxylic acid ester group of these compounds. However, diethylaminoethyl 5-acetyl-gentisate hydrochloride, diethylaminoethyl 5-benzoyl-gentisate hydrochloride, diethylaminopropyl 5-acetyl-gentisate hydrochloride and diethylaminopropyl 5-benzoyl-gentisate hydrochloride (figures 7, 9, 11, and 13 respectively) show two absorption maxima in this range. One peak is due to the ester on number five phenolic group and the other is due to the carboxylic acid ester.

Figures 14 to 18, inclusive, represent esters of the number five phenolic hydroxy of gentisic acid.

In each of these compounds the spectrum shows a decreased absorption in the 3.0 to 4.0 micron region, from that of the gentisic acid spectra.

The region of 5.6 to 6.0 micron show two strong absorption bands in each spectrum with the exception of Figure 17. The first "hump" represents the ester linkage at the number five position of gentisic acid and the second represents the carboxylic acid function.

Figure 15 also shows the nitro group peak at a wavelength of 6.48 micron.

Figure 17, 5-acetylsalicyl-gentisic acid shows only one carbonyl group, which is located at 5.95 microns. This indicates no esterification at the number 5 hydroxyl position of gentisic acid. However, the absorption spectrum of the compound is greatly different from that of gentisic acid especially in the 3.0 to 4.0 micron range. Therefore, although the spectrum indicates no phenolic ester, it is believed that Figure 17 represents the spectrum of a new gentisic acid derivative.

From the analyses of the spectra, the facts indicate that the reaction between dialkylaminoalkyl chlorides and gentisic acid yielded dialkylaminoalkyl gentisate hydrochlorides, and the reaction between gentisic acid and the acid chlorides of the specified acids yielded the 5-phenolic esters of gentisic acid.

Toxicity Tests

Every compound thought to be of medicinal value must be screened pharmacologically to determine its activity. One of the first and most important steps in this process is the determination of its toxicity. The usual method of expressing toxicity is in terms of the dose that kills 50% of a large group of animals, or the LD_{50} . The term LD_{50} is the abbreviation for "lethal dose to 50%". The term applies for acute or chronic toxicity tests. The acute toxicity refers generally to deaths that occur within 24-48 hours after administration of the drug. Chronic toxicity studies extend over a period of weeks, followed by autopsy of the animal to determine what tissues and organs, if any, are affected.

The carboxylic acid esters being water soluble were administered intravenously.

The phenolic esters were prepared as follows and administered intravenously. The material was dissolved in alcohol, 3% Tween 80 was added, and the mixture was added to water which contained not more than 5% acacia. The total alcohol content was not more than 5%.

Propylene glycol and polyethylene glycol dissolved the phenolic esters, but both solvents were toxic when administered in doses as low as 0.1 ml. per mouse, hence, could not be used.

The technique used in these tests was as follows:

1. Swiss mice from the same source of supply, weighing from 10 g. to 28 g. were used. They were in good health and had been kept under observation for at least 3 days prior to their use. They were housed in standard cages in a room relatively constant in so far as temperature and humidity are concerned. Their diet consisted of an unlimited ration of water and Purina Dog Checkers.

2. The solutions were injected by means of a 1 ml. tuberculin syringe and a 25 gauge, 1 inch needle.

3. All doses administered were on a mg./Kg. basis.

4. The mice were starved 24 hours to standardize their metabolism.

5. The solutions were given slowly into the tail veins of the mouse.

6. In each group of esters, i.e. carboxylic acid and phenolic, it was necessary to first find the toxicity range. The other compounds in a group were found to fall within this toxic range.

7. The essential requirements for Behren's method of statistical evaluation were met so the LD_{50} is reported and graphs drawn using this integrated data (70).

Toxicity of Carboxylic Acid Esters of Gentisic Acid

Table VI

LD₅₀ of Diethylaminoethyl Gentisate Hydrochloride

<u>Experimental Data</u>					<u>Integrated Data</u>		
<u>No. of</u> <u>animals</u>	<u>Dose</u> <u>mg/Kg</u>	<u>Alive</u>	<u>Dead</u>	<u>%</u> <u>Mortality</u>	<u>Alive</u>	<u>Dead</u>	<u>%</u> <u>Mortality</u>
12	10	12	0	0.00	31	0	0.00
12	30	11	1	8.35	19	1	5.00
12	50	6	6	50.00	8	7	26.67
12	70	2	10	93.45	2	17	89.47
12	90	0	12	100.00	0	20	100.00

LD₅₀ equals 51.2 mg/Kg.

Table VII

LD₅₀ of Diethylaminoethyl 5-Acetyl-Gentisate Hydrochloride

<u>Experimental Data</u>					<u>Integrated Data</u>		
<u>No. of</u> <u>animals</u>	<u>Dose</u> <u>mg/Kg</u>	<u>Alive</u>	<u>Dead</u>	<u>%</u> <u>Mortality</u>	<u>Alive</u>	<u>Dead</u>	<u>%</u> <u>Mortality</u>
12	30	12	0	0.00	31	0	0.00
12	40	10	2	16.67	19	2	9.53
12	50	4	8	66.67	9	10	52.63
12	60	5	7	58.33	5	17	73.92
12	70	0	12	100.00	0	29	100.00

LD₅₀ equals 51.5 mg/Kg.

Table VIII

LD₅₀ of Diethylaminoethyl 5-Methoxy-Gentisate Hydrochloride

<u>Experimental Data</u>					<u>Integrated Data</u>		
<u>No. of</u> <u>animals</u>	<u>Dose</u> <u>mg/Kg</u>	<u>Alive</u>	<u>Dead</u>	<u>%</u> <u>Mortality</u>	<u>Alive</u>	<u>Dead</u>	<u>%</u> <u>Mortality</u>
12	10	12	0	0.00	32	0	0.00
12	20	11	1	8.35	20	1	4.76
12	30	5	7	58.33	9	8	47.06
12	40	4	8	66.67	4	16	80.00
12	50	0	12	100.00	0	28	100.00

LD₅₀ equals 32.2 mg./Kg.

Table IX

LD₅₀ of Diethylaminoethyl 5-Benzoyl-Gentisate Hydrochloride

Experimental Data					Integrated Data		
No. of animals	Dose mg/Kg	Alive	Dead	% Mortality	Alive	Dead	% Mortality
12	10	12	0	0.00	25	0	0.00
12	30	9	3	25.00	13	3	18.75
12	50	4	8	66.67	4	11	73.33
12	70	0	12	100.00	0	23	100.00

LD₅₀ equals 41.3 mg./Kg.

Table X

LD₅₀ of Diethylaminopropyl Gentisate Hydrochloride

Experimental Data					Integrated Data		
No. of animals	Dose mg/Kg	Alive	Dead	% Mortality	Alive	Dead	% Mortality
12	30	12	0	0.00	23	0	0.00
12	50	8	4	33.33	11	4	26.67
12	70	3	9	75.00	3	13	81.25
12	90	0	12	100.00	0	25	100.00

LD₅₀ equals 58.5 mg./Kg.

Table XI

LD₅₀ of Diethylaminopropyl 5-Acetyl-Gentisate Hydrochloride

Experimental Data					Integrated Data		
No. of animals	Dose mg/Kg	Alive	Dead	% Mortality	Alive	Dead	% Mortality
12	30	12	0	0.00	23	0	0.00
12	40	5	7	58.33	11	7	38.88
12	50	4	8	66.67	6	15	71.43
12	60	2	10	83.33	2	25	92.59
12	70	0	12	100.00	0	37	100.00

LD₅₀ equals 43.8 mg./Kg.

Figure 19

Graph of LD₅₀ of Diethylaminoethyl Esters of Gentisic Acid and Substituted Gentisic Acids

- Diethylaminoethyl Gentisate Hydrochloride
- ▲ Diethylaminoethyl 5-Acetyl-Gentisate Hydrochloride
- + Diethylaminoethyl 5-Methoxy-Gentisate Hydrochloride
- × Diethylaminoethyl 5-Benzoyl-Gentisate Hydrochloride

Table XII

LD₅₀ of Diethylaminopropyl 5-Methoxy-Gentisate Hydrochloride

Experimental Data					Integrated Data		
No. of Animals	Dose mg/Kg	Alive	Dead	% Mortality	Alive	Dead	% Mortality
12	10	12	0	0.00	22	0	0.00
12	20	7	5	41.67	10	5	33.33
12	30	2	10	83.33	3	15	83.33
12	40	1	11	91.67	1	26	96.29
12	50	0	12	100.00	0	38	100.00

LD₅₀ equals 22.0 mg./Kg.

Table XIII

LD₅₀ of Diethylaminopropyl 5-Benzoyl-Gentisate Hydrochloride

Experimental Data					Integrated Data		
No. of Animals	Dose mg/Kg	Alive	Dead	% Mortality	Alive	Dead	% Mortality
12	20	12	0	0.00	31	0	0.00
12	25	11	1	8.33	19	1	5.00
12	30	6	6	50.00	8	7	46.67
12	35	2	10	83.33	2	17	89.47
12	40	0	12	100.00	0	29	100.00

LD₅₀ equals 30.2 mg./Kg.

Figure 20

Graph of LD_{50} of Diethylaminopropyl Esters of Gentisic Acid
and Substituted Gentisic Acids

- Diethylaminopropyl Gentisate Hydrochloride.
- ▲ Diethylaminopropyl 5-Acetyl-Gentisate Hydrochloride
- + Diethylaminopropyl 5-Methoxy-Gentisate Hydrochloride
- × Diethylaminopropyl 5-Benzoyl-Gentisate Hydrochloride

The least toxic compound of the carboxylic acid ester hydrochlorides was diethylaminopropyl gentisate hydrochloride with a LD_{50} of 58.5 mg./Kg. and the most toxic compound was diethylaminopropyl 5-methoxy-gentisate hydrochloride with a LD_{50} of 22.0 mg./Kg.

Generally, the diethylaminopropanol ester hydrochlorides are more toxic than the diethylaminoethanol counter-parts. However, the first members of the series are reversed in this respect. These compounds follow the general pattern of increased carbon chain increases toxicity.

The methoxy group shows it's noted toxicity by being the most toxic ester in both the diethylaminoethanol and diethylaminopropanol series.

The 5-benzoyl and 5-acetyl derivatives of the aminoalcohol ester hydrochlorides are less toxic than the 5-methoxy derivative.

Apparently the toxicity of these compounds is due principally to the aminoalcohols because the parent compounds are relatively non-toxic.

The mice, that died, went into convulsions and died within a minute after the compound was injected. No deaths occurred after 5 minutes from the time of injection.

Toxicity of Phenolic Esters of Gentisic Acid

Table XIV
 LD_{50} of 5-Phenylacetic-Gentisic Acid

Experimental Data					Integrated Data		
No. of animals	Dose mg/Kg	Alive	Dead	% Mortality	Alive	Dead	% Mortality
12	160	12	0	0.00	31	0	0.00
12	180	9	3	25.00	19	3	14.29
12	200	6	6	50.00	10	9	47.37
12	220	4	8	66.67	4	17	80.96
12	240	0	12	100.00	0	29	100.00

LD_{50} equals 201.2 mg./Kg.

Table XV
 LD_{50} of 5-Paranitrobenzoyl-Gentisic Acid

Experimental Data					Integrated Data		
No. of animals	Dose mg/Kg	Alive	Dead	% Mortality	Alive	Dead	% Mortality
12	30	12	0	0.00	39	0	0.00
12	50	9	3	25.00	27	3	10.00
12	70	9	3	25.00	18	6	25.00
12	90	7	5	41.67	9	11	55.00
12	110	2	10	83.33	2	21	92.17
12	130	0	12	100.00	0	33	100.00

LD_{50} equals 83.5 mg./Kg.

Table XVI
 LD_{50} of 5-Anisyl-Gentisic Acid

Experimental Data					Integrated Data		
No. of animals	Dose mg/Kg	Alive	Dead	% Mortality	Alive	Dead	% Mortality
12	20	12	0	0.00	36	0	0.00
12	30	10	2	16.67	24	2	7.69
12	40	5	7	58.33	14	9	39.13
12	50	6	6	50.00	9	15	62.50
12	60	3	9	75.00	3	24	88.88
12	70	0	12	100.00	0	36	100.00

LD_{50} equals 44.9 mg./Kg.

TABLE XVII

LD₅₀ of 5-Acetylsalicyl-Gentisic Acid

Experimental Data					Integrated Data		
No. of animals	Dose mg/Kg	Alive	Dead	% Mortality	Alive	Dead	% Mortality
12	10	12	0	0.00	33	0	0.00
12	20	9	3	25.00	21	3	12.50
12	30	7	5	41.67	12	8	40.00
12	40	4	8	66.67	5	16	76.19
12	50	1	11	91.67	1	27	96.43
12	60	0	12	100.00	0	39	100.00

LD₅₀ equals 32.4 mg./Kg.

TABLE XVIII

LD₅₀ of 5,5'-Succinyl-Digentisic Acid

Experimental Data					Integrated Data		
No. of animals	Dose mg/Kg	Alive	Dead	% Mortality	Alive	Dead	% Mortality
12	100	12	0	0.00	28	0	0.00
12	130	8	4	33.33	16	4	20.00
12	160	6	6	50.00	8	10	55.55
12	190	2	10	93.33	2	20	90.91
12	220	0	12	100.00	0	32	100.00

LD₅₀ equals 155.0 mg./Kg.

TABLE XIX

LD₅₀ of Gentisic Acid

Experimental Data					Integrated Data		
No. of animals	Dose mg/Kg	Alive	Dead	% Mortality	Alive	Dead	% Mortality
12	320	12	0	0.00	38	0	0.00
12	340	10	2	16.67	26	2	7.15
12	360	8	4	33.33	16	6	27.27
12	380	6	6	50.00	8	12	60.00
12	400	2	10	83.33	2	22	91.58
12	420	0	12	100.00	0	34	100.00

LD₅₀ equals 374.0 mg./Kg.

Figure 21

Graph of LD₅₀ of Phenolic Esters of Gentisic Acid

- 5-Phenylacetyl-Gentisic Acid
- ▲ 5-Paranitrobenzoyl-Gentisic Acid
- + 5-Anisyl-Gentisic Acid
- × 5-Acetylsalicyl-Gentisic Acid
- ⊙ 5,5'-Succinyl-Digentisic Acid

Figure 22
Graph of LD₅₀ of Gentisic Acid.

The phenolic esters vary in toxicity from 32.4 mg./Kg. for 5-acetylsalicyl gentisic acid to 201.2 mg./Kg. for 5-phenylacetic-gentisic acid.

The increase in toxicity of these compounds over that of gentisic acid may be attributed to the inherent toxicity of substituted functional group, i.e. paranitrobenzoyl and anisyl. However, the increased acidity of the compound may also be partially responsible for its increased toxicity.

Groups on the benzene ring of the substituting function appears to increase the toxicity of the compound.

The phenolic esters caused convulsions before death in the mice killed. However, the action of these compounds was slower, generally requiring several minutes to kill the animal injected.

Since LD_{50} for gentisic acid was found to be 374.0 mg./Kg., intravenously, and the LD_{50} reported for sodium gentisate was 3.1 mg./Kg., intraperitoneal (36), the toxicity of the esters synthesized is considerably greater than gentisic acid or its sodium salt. The toxicity of these compounds is also greater than that reported for sodium salicylate (i.e. 0.88 gm/Kg., intraperitoneal) (36). These reported values were determined on rats and seem high compared to that which was found for gentisic acid intravenously.

Generally, the toxicity of phenolic esters is lower than that of the carboxylic acid esters. This may be due to the

fact that the former types were given in suspensions whereas the latter were aqueous solutions.

Due to the higher toxicity of the prepared products, they will need to have considerably more activity than gentisic acid to be of greater value medicinally.

No chronic toxicity studies of these compounds were made, but from observing the animals injected on a given day, it was noted that no deaths occurred after 5 to 10 minutes from the time of injection.

If these compounds have analgesic value, they would probably be administered orally, i.e. as tablet or powder. Oral toxicity is much lower than intravenous toxicity so the therapeutic index of the compound may be better than the results show.

Summary

Attempts were made to synthesize several amino alcohol ester hydrochlorides of gentisic acid. From the attempts eight new compounds were formed. It was found that the reactions between the chlorides of the alcohols and the free acids gave the best yields and apparently the most nearly pure products. It was also observed that in the case of the successful reactions the intermediate halides had been reported in the literature, whereas in the cases where unreported intermediates had to be used, the yields were poor and the products of doubtful purity.

The five phenolic esters were prepared successfully by the Schotten-Bauman reaction. The yields were good and the products relatively pure. The acid chlorides were prepared by the use of thionyl chloride and the properties of these acid chlorides were found to be of the same nature as literature reported values.

The reaction between the acid chlorides and gentisic acid, using pyridine or dimethylaniline as a condensing medium, was not successful. It is believed that the pyridine or dimethylaniline was not strong enough to remove the hydrogen from the phenolic hydroxide and the chlorine from the acid chloride.

To prove the site at which esterification took place on gentisic acid, when the acid and the halide or acid chloride

were reacted, two qualitative procedures and one quantitative technique were utilized. The first qualitative test was the ferric chloride color test. The test yields a dark blue coloration for orthohydroxyl carboxylic acids, but a negative test with metahydroxyl carboxylic acids and substituted orthohydroxyl carboxylic acid esters. Therefore, since all the esters give a positive test, it is indicative that the number 2 hydroxyl group of gentisic acid was free.

The second qualitative proof of the point of esterification was the determination and analysis of the infrared spectrum of the compound. By comparing the spectrum of the synthesized compound to that of known compounds, it is easy to see whether one or two carbonyl functions are present. Gentisic acid shows one carbonyl and carboxylic acid esters of gentisic acid also show only one carbonyl function when the number 5 hydroxyl is free or is blocked by an ether linkage. However, for those carboxylic acid esters that also have acetyl or benzoyl function in number 5 position of gentisic acid, two carbonyl groups are located on each spectrum. All the spectra of the phenolic esters show two carbonyl functions present, one is due to the carboxylic acid group of gentisic acid and the other is due to the ester at number 5 position of the acid.

The quantitative proof of esterification was determined by the neutral equivalent procedure. The phenolic esters were titrated directly with base and the carboxylic acid

ester hydrochlorides were titrated with base, liberating the amine and the amine was again converted to the salt by acidic titration. The molecular weights are good for the phenolic esters except for 5-acetylsalicyl-gentisic acid. The molecular weight determinations for the carboxylic acid ester hydrochlorides range from good to satisfactory. Better results could have been obtained if a larger sample of synthetic compound and microburettes had been used.

The unreactive nature of the number 2 hydroxyl group of gentisic acid is attributed to strong hydrogen bonding between the hydrogen of the hydroxyl group and the carbonyl of the acid group, and to the electron effect, i.e. the number 5 hydroxyl group supplies electrons to the ring especially ortho and para, thus hindering the removal of the hydrogen of the number 2 hydroxyl. However, the latter effect may be offset by the meta directing carboxylic acid function ortho to the hydroxyl group in question.

The only synthesized compound the formula of which is in doubt is that of 5-acetylsalicyl-gentisic acid. The two outstanding discrepancies between the proposed and the actual structure of the compound are the molecular weight and the infrared spectrum. The carbon and hydrogen analysis also shows the found carbon to be about 1% higher than the calculated amount for 5-acetylsalicyl-gentisic acid. However, the author believes the compound to be a new synthetic derivative of gentisic acid. This is supported by the facts

that the infrared spectrum, neutral equivalent and carbon and hydrogen analysis are all very different from those of the parent compounds. The following may explain the discrepancies: The product was prepared by the interaction of acetylsalicyl chloride and gentisic acid in basic media. The acid chloride was prepared as previously reported in the literature. The acid chloride produced had the accepted physical properties. It is known that acetyl groups are most easily hydrolyzed in basic solution. Therefore, the possibility exists that either before or after the desired esterification took place, hydrolysis of the acetyl group of acetylsalicyl chloride was brought about. The compound produced, therefore, may be 5-salicyl-gentisic acid. The molecular weight of the compound, i.e. 274.22 g., is more nearly equal to the found molecular weight, 285.23g., than is the molecular weight of 5-acetylsalicyl-gentisic acid, i.e. 316.26 g. The carbon and hydrogen analysis is also more nearly correct: Calculated for $C_{14}H_{10}O_6$: C, 61.31; H, 3.63; Found: C, 61.74, 61.53; H, 3.92, 3.86. The assumption that the new compound is actually 5-salicyl-gentisic acid also may explain why only one carbonyl function shows on the infrared spectrum of the compound. It may be that the hydrogen bonding between the hydroxyl function of the salicyl radical and the carbonyl function of the ester group is too strong to be detected. Therefore, only the carboxylic function of gentisic acid shows in the spectrum.

The relative high toxicity of the carboxylic acid ester hydrochlorides appears to be primarily due to the amino alcohol portion of the molecules. However, the groups in number 5 position of gentisic acid do exert some influence in these esters, especially the methoxy group. The methoxy group is noted for its toxic nature and both derivatives containing this grouping are the most toxic of their series.

The increased length of chain, i.e. ethyl to propyl, of the alcohol, also increases toxicity as one would expect.

The toxicity increase of the phenolic esters over that of gentisic acid may be because of the increased acid strength of the compound. The increased acidity is brought about by the replacement of the ortho-para directing hydroxyl group by the meta directing ester function. The functional groups added also play their part because the nitro and methoxy containing compounds are the most toxic of the series. The 5-acetylsalicyl-gentisic acid appeared to be much more toxic than one would predict. This uncertainty again indicates that the proposed structure may be incorrect.

All the esters of gentisic acid were prepared with the hope of producing new and better drugs. The syntheses were planned from the point of view of combining two medicinally active nuclei to form a compound more active than a physical mixture of the compounds. Since the reacting compounds have analgetic and antirheumatic activity, it is believed that

the synthesized compounds should have activity of the same nature. The possibility also exists that the carboxylic acid ester hydrochlorides will have local anesthetic and/or antispasmodic activity. The phenolic esters may also be bacterioid in nature.

The value of these compounds will have to be determined by pharmacological testing before the true medicinal value of the synthetics can be stated. If activity is shown by the carboxylic acid esters, it is suggested that other active compounds may be prepared by altering the amino alcohol chain, as in the case of local anesthetics, and replacing the tertiary amine with primary and secondary amines.

If the phenolic esters show medicinal value, an unlimited number of esters can be prepared by coupling of other medicinally active groups to gentisic acid.

Also, the number 2 hydroxyl position of gentisic acid should be attacked by other procedures to attempt substitution in this position of gentisic acid.

The analyses and toxicity of the synthesized esters of gentisic acid are listed in Table XX. Table XXI includes the physical properties, the stability studies, and the coloration test with ferric chloride for the esters.

TABLE
ANALYSES OF SY

Name of Compound (Empirical Formula)	Structural Formula	Molecular Weight in grams		N
		Calculated	Found	
Diethylaminoethyl Gentisate Hydrochloride ($C_{18}H_{26}O_4NCl$)		253.30	252.11	
Diethylaminoethyl 5-Acetyl-Gentisate Hydrochloride ($C_{18}H_{22}O_5NCl$)		295.33	301.65	
Diethylaminoethyl 5-Methoxy-Gentisate Hydrochloride ($C_{14}H_{22}O_4NCl$)		267.32	269.35	
Diethylaminoethyl 5-Benzoyl-Gentisate Hydrochloride ($C_{20}H_{24}O_5NCl$)		357.39	355.75	
Diethylaminopropyl Gentisate Hydrochloride ($C_{14}H_{22}O_4NCl$)		267.32	265.50	
Diethylaminopropyl 5-Acetyl-Gentisate Hydrochloride ($C_{16}H_{24}O_5NCl$)		309.35	309.46	
Diethylaminopropyl 5-Methoxy-Gentisate Hydrochloride ($C_{15}H_{24}O_5NCl$)		281.34	280.17	

TABLE XX
ANALYSES OF SYNTHESIZED COMPOUNDS

Weight	Nitrogen Content		Chlorine Content		Carbon & Hydrogen		LD ₅₀ mg./kg.
	Found*	Calculated	Found*	Calculated	Found*	Calculated	
52.11	4.84	4.68	12.58	12.61	----	----	51.2
51.65	4.22	4.17	10.69	10.62	C, 54.30 H, 6.68	C, 54.41 H, 6.78	51.5
59.35	4.61	4.70	11.68	11.62	----	----	32.2
55.75	3.56	3.42	9.00	8.94	C, 60.85 H, 6.14	C, 60.40 H, 6.41	41.7
55.50	4.61	4.65	11.68	11.80	C, 55.35 H, 7.30	C, 55.45 H, 7.42	58.5
59.46	4.05	4.04	10.25	10.34	----	----	44.5
50.17	4.41	4.35	11.16	11.27	----	----	22.1

TABLE XX

Diethylaminopropyl 5-Benzoyl-Gentisate Hydrochloride ($C_{21}H_{26}O_5HCl$)		373.42	366.34	3.4
5-Phenylacetyl- Gentisic Acid ($C_{18}H_{12}O_5$)		272.25	273.38	---
5-Paranitrobenzoyl- Gentisic Acid ($C_{14}H_9O_7N$)		303.20	304.19	---
5-Anisyl-Gentisic Acid ($C_{15}H_{12}O_6$)		288.25	290.50	--
5-Acetylsalicyl- Gentisic Acid ($C_{16}H_{12}O_7$)		316.26	285.23	--
5,5' Succinyl- Digentisic Acid ($C_{18}H_{12}O_7$)		390.29	388.37	--
Gentisic Acid ($C_7H_6O_4$)		---	---	--

*Average of two values

#Molecular Weight Calculated for Free Amine.

TABLE XX (CONT'D)

3.34	3.43	3.60	8.69	8.62	----	----	30.3
3.38	---	---	---	---	C, 66.17 H, 4.44	C, 66.21 H, 4.51	201.3
4.19	---	---	---	---	C, 55.46 H, 2.99	C, 55.43 H, 2.97	82.5
0.50	---	---	---	---	C, 62.50 H, 4.19	C, 62.43 H, 4.54	45.0
5.23	---	---	---	---	C, 60.76 H, 3.83	C, 61.63# H, 3.89#	32.4
8.37	---	---	---	---	C, 55.39 H, 3.62	C, 55.20 H, 3.82	155.0
-	---	---	---	---	----	----	374.0

TABLE XXI

Properties of Synthesized Compounds

Name of Compound	m.p. °	Microscopic Appearance	Solubility				
			Water	Alcohol	Ether	Ace-tone	Chloroform
Diethylaminoethyl Gentisate Hydrochloride	150-151°	Colorless Plates	s	s	i	i	i
Diethylaminoethyl 5-Acetyl-Gentisate Hydrochloride	133.5-134.5°	Colorless Needles	s	s	sl s	sl s	s
Diethylaminoethyl 5-Methoxy-Gentisate Hydrochloride	182.3-183.0°	Colorless Needles	s	s	sl s	i	s
Diethylaminoethyl 5-Benzoyl-Gentisate Hydrochloride	153-154°	Colorless Needles	s	s	sl s	s	s
Diethylaminopropyl Gentisate Hydrochloride	171.5-172.5°	Colorless Plates	s	s	i	i	s
Diethylaminopropyl 5-Acetyl-Gentisate Hydrochloride	157-157.5°	Colorless Needles	s	s	s	s	s
Diethylaminopropyl 5-Methoxy-Gentisate Hydrochloride	184.5-185-3°	Colorless Needles	s	s	i	sl s	s
Diethylaminopropyl 5-Benzoyl-Gentisate Hydrochloride	142.7-143.7°	Colorless Needles	s	s	i	s	s
5-Phenylacetyl-Gentisic Acid	145-5-146.5°	Colorless Fibers	i	s	s	s	s

TABLE XXI
of Synthesized Compounds

or	Solubility				Stability#		Ferric#
	Alco- hol	Ether	Ace- tone	Chlo- roform	Solid	Solution	Chloride Test
	s	i	i	i	+	-	+
	s	sl s	sl s	s	+	-	+
	s	sl s	i	s	+	-	+
	s	sl s	s	s	+	-	+
	s	i	i	s	+	-	+
	s	s	s	s	+	-	+
	s	i	sl s	s	+	-	+
	s	i	s	s	+	-	+
i	s	s	s	s	+	-b"	+

TABLE XXI (CONT'D)

5-Paranitrobenzoyl- Gentisic Acid	229- 230.5°d.	Yellow Needles	1	sl	s	s	s	1
5-Anisyl-Gentisic Acid	209.4- 209.5°	Colorless Needles	1	s	s	s	s	s
5-Acetylsalicyl- Gentisic Acid	203.5- 204.7°	Sheaves of Colorless Needles	1	s	s	s	s	1
5,5'-Succinyl- Gentisic Acid	230- 231.5°d.	Colorless Fibers	1	s	sl	s	s	1

* Uncorrected

+ equals positive; - equals negative

" b equals basic.

TABLE XXI (CONT'D)

1	s	l	s	s	s	1	+	-b ⁿ	+
1	s	s	s	s	s	s	+	-b ⁿ	+
1	s	s	s	s	s	1	+	-b ⁿ	+
1	s	s	l	s	s	1	+	-b ⁿ	+

CONCLUSIONS

I. Thirteen new esters of gentisic acid were prepared, eight of which are carboxylic acid esters and five are phenolic esters.

A. The carboxylic acid ester hydrochlorides.

(1) The carboxylic acid esters were prepared in good yield, by the interaction of the chloride of tertiary amino alcohols and gentisic acid or substituted gentisic acids.

(2) The physical properties of the compounds, i.e. melting point, microscopic appearance and solubility, were determined.

(3) The carboxylic acid esters are salts, and therefore, they are readily soluble in water. However, aqueous solutions of the salts must be buffered so the solution is acid to insure stability.

(4) Qualitative and quantitative nitrogen and chlorine content of each carboxylic acid ester salt was determined.

(5) All the carboxylic acid ester hydrochlorides gave a positive ferric chloride test.

(6) Diethylaminoethyl 5-acetyl-gentisate hydrochloride, diethylaminoethyl 5-benzoyl-gentisate hydrochloride and diethylaminopropyl gentisate hydrochloride were analyzed for carbon and hydrogen.

(7) A modification of the usual procedure for determination of neutral equivalents of the carboxylic acid ester hydrochloride was employed. The procedure was to liberate the amine by addition of excess standard base and then titrate the excess base and the amine with standard acid.

B. Phenolic esters.

(1) The number 2 hydroxyl group of gentisic acid was found to be very difficult to react with, if not entirely inert; whereas, the number 5 hydroxyl group was very reactive.

(2) The phenolic esters were prepared by the interaction of the acid chloride and the various acids and the number 5 hydroxyl group of gentisic acid in alkaline media, i.e. Schotten-Bauman reaction.

(3) The physical properties of the compounds, i.e. melting point, microscopic appearance and solubility, were determined.

(4) The phenolic esters can be made partially soluble by making the sodium salt of the gentisic acid portion of the molecule. Although the salts may be unstable in solution, if in tablet form, they would favor absorption of the compound.

(5) All the phenolic esters gave a positive ferric chloride test.

(6) 5-Paranitrobenzoyl-gentisic acid gave a positive test for nitrogen.

(7) The phenolic esters were analyzed for carbon and hydrogen.

(8) The neutral equivalents of the phenolic esters were determined in the usual manner.

(9) The average equivalent of 5-acetylsalicyl-gentisic acid was found to be 30 g. too low. Therefore, doubt exists as to whether this is the correct formula of the compound.

II. The infrared spectra of the new esters of gentisic acid were analyzed to prove the structure of the compounds.

A. The carboxylic acid ester hydrochlorides.

(1) Diethylaminoethyl gentisate hydrochloride, diethylaminoethyl 5-methoxy-gentisate hydrochloride and the diethylaminopropyl counter-parts show only one carbonyl function in the spectra. This indicated esterification through the carboxylic acid of gentisic acid.

(2) Diethylaminoethyl 5-acetyl-gentisate hydrochloride, diethylaminoethyl-5-benzoyl-gentisate hydrochloride and the diethylaminopropyl counter-parts show two carbonyl functions in their spectra. This indicates esterification through the carboxylic acid and the number 5 hydroxyl of gentisic acid.

B. The phenolic esters.

(1) All five phenolic esters show two carbonyl functions in their spectra. This indicates that the carboxylic acid of gentisic acid is free and that the point of esterification is at the number 5 hydroxyl group of gentisic acid.

(2) The spectrum of 5-acetylsalicyl-gentisic acid shows only one carbonyl function. Therefore, doubt exists as to whether the proposed formula is the true nature of the compound.

III. The LD_{50} of each new ester of gentisic acid was determined by intravenous injection of the compounds into white mice.

A. The carboxylic acid ester hydrochlorides.

(1) The most toxic of the carboxylic acid ester hydrochlorides is 58.5 mg./Kg. on mice whereas the least toxic is 22.1 mg./Kg.

(2) Diethylaminoethyl 5-methoxy-gentisate hydrochloride is the most toxic compound of the diethylaminoethanol esters and diethylaminopropyl 5-methoxy-gentisate hydrochloride is the most toxic compound of the diethylaminopropyl esters. The latter is the most toxic compound of all the carboxylic acid ester hydrochlorides.

Diethylaminoethyl 5-acetyl-gentisate hydrochloride is the least toxic compound of the diethylaminoethyl

esters and diethylaminopropyl gentisate hydrochloride is the least toxic compound of the diethylaminopropyl esters. The latter is the least toxic compound of the carboxylic acid ester hydrochlorides.

5-acetyl, 5-methoxy and 5-benzoyl gentisic acid esters of diethylaminopropanol hydrochloride are more toxic compounds than the diethylaminoethanol derivatives of the same acid.

B. The phenolic esters.

(1) The phenolic esters minimum and maximum toxicity varies from 201.3 mg./Kg. to 32.4 mg./Kg., respectively on mice.

5-phenylacetyl-gentisic acid is the least toxic phenolic ester and 5-acetylasalicyl-gentisic acid is the most toxic.

Substitution on the benzene ring of the phenolic esters seems to greatly increase the toxicity of this group of compounds.

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