RECENTLY PREPARED DERIVATIVES OF GENTISIC ACID

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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1953

Approved by:

[Signature]
Adviser
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August, 1953

Willis E. Moore
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RECENTLY PREPARED DERIVATIVES OF GENTISIC ACID

INTRODUCTION

Because of the successful use of gentisic acid and its derivatives in the treatment and symptomatic relief of rheumatic fever (1), (2), (3), and rheumatoid arthritis (1), (4), (5), (6), a further study of gentisic acid derivatives was advisable. These reports show that the use of gentisate therapy as an analgetic in rheumatic and arthritic conditions provided symptomatic relief.

The advantages of gentisate therapy over salicylate therapy as well as the present status of gentisate therapy have been reviewed by Nash et al (7). Further elucidation of the human metabolism of gentisic acid, sodium gentisate and gentisylethanolamide has been reported by Clarke and Mosher (8) and Consden and Stanier (9).

This present work is a continuation of the work begun by Nash et al (10), who prepared gentisate derivatives using types of organic radicals which had proven pharmacological activity. For his work, he chose to add the following groups, both singly and in combinations:

a. diethylaminoethyl, as the HCl salt
b. diethylaminopropyl, as the HCl salt
c. 5- methylether
d. 5- acetyl
e. 5- phenylacetyl
f. 5- benzoyl  
g. 5- anisyl  
h. 5- acetylsalicyl  
i. 5- paranetrobenzoyl  
j. 5,5'- succinyl

In order to extend a logical series of compounds that might possess the properties desired, it seemed desirable to prepare new esters of gentisic acid with other carboxylic acids, and with amino alcohols in which the amine portion was both tertiary and secondary in nature.

THE CHEMISTRY OF RECENTLY PREPARED DERIVATIVES OF GENTISIC ACID

The methods of preparing phenolic esters and carboxyl esters have long been established and may be found in many texts devoted to organic synthesis. Basically both processes consist of the removal of the elements of water between a carboxyl group, COOH, and a hydroxyl group, OH. The latter group may occur either as an alcoholic OH or as a phenolic OH.

There are certain cases, however, in which the reactivity of the hydroxyl group is of such low order that either it or the carboxyl group with which it is to be reacted must be modified by the introduction of a halogen. These general reactions may be illustrated by the following equations:
1) R-COOH + HO-R₁ → R-COO-R₁ + H₂O
2) R-OH + HOOC-R₂ → R-OOC-R₂ + H₂O
3) R-COOH + X-R₁ → R-COO-R₁ + HX
4) R-OH + XOC-R₂ → R-COO-R₂ + HX
5) R-COONa + X-R₁ → R-COO-R₁ + NaX
6) R-COO-Me + HO-R₁ → R-COO-R₁ + MeOH

In which R = gentisyl residue, either the 2,5 dihydroxy-phenyl residue or 1-carboxy, 2-hydroxy phenyl residue.

R₁ = aliphatic residue
R₂ = aliphatic or aromatic residue
Na = sodium
Me = methyl radical.

It must be kept in mind that gentisic acid is a tri-functional molecule containing one carboxyl group and two phenolic hydroxyl groups. Its structural formula shows that the 5 hydroxyl is in the meta position to the carboxyl group. However, the experimental work in this laboratory has shown that the 2 hydroxyl group has more influence on the reactivity of the 5 hydroxyl group than does the meta carboxyl group. If the 5 position was dominated by the carboxyl group, esterification through the 5 phenolic hydroxyl would be easier because the hydrogen atom would be more acidic.
In equation 1), esterification of the carboxyl group of gentisic acid with an aliphatic primary alcohol was attempted unsuccessfully by Nash et al (7) using the standard procedure of refluxing the acid in an excess of the amino alcohol using catalytic amounts of hydrogen ion. However, both tertiary and secondary amine esters were prepared, through the primary alcohol group by this procedure using the modification of reacting the products in an inert solvent in the presence of dry hydrogen chloride gas which acted as a dehydrating agent. The procedure was not successful with secondary alcohols however. Dehydration of the alcohol, not esterification, occurred. The relatively low order of the activity of these amino alcohols is illustrated by the length of reaction time required and the relatively low yields obtained. The ease with which methyl and ethyl esters of gentisic acid are made (11) indicates clearly that the carboxyl group of gentisic acid has the usual reactivity of aromatic carboxylic acids.

Equation 2) illustrates the esterification of the 5 hydroxyl group of gentisic acid with some other carboxylic acid. The activity of this hydroxyl is of such low order that it will react only with acid chlorides which in themselves have a high order of reactivity. This reaction is typified by equation 4). In this laboratory, several acid chlorides were reacted with gentisic acid under different
conditions. Nash et al (7) utilized salicyl chloride, diphenylacetyl chloride, phenylacetyl chloride, diethylacetyl chloride, acetyl chloride, benzoyl chloride, paranitrobenzoyl chloride, anisyl chloride, acetylsalicyl chloride and succinyl dichloride as acylating agents. The conditions of reaction usually employed involving acid chlorides and hydroxyl groups are:

a. in an inert solvent
b. in an inert solvent plus pyridine
c. in pyridine as a solvent
d. in an aqueous alkaline media, The Schotten-Bauman reaction.

Nash et al (7) found that the only procedure giving successful esterification was the Schotten-Bauman reaction but this procedure was not successful for the esterification of the acid chlorides of salicylic, diphenylacetic and diethylacetic acids.

Numerous attempts to repeat the esterification of paranitro benzoyl chloride by the Schotten-Bauman reaction were unsuccessful. Likewise, the acid chlorides of fumaric acid, phthalic acid, and dibromosuccinic acid failed to enter the Schotten-Bauman reaction. Furthermore, the latter three acid chlorides formed a complex with pyridine so that methods "b" and "c" were of no value. Method "a" also was of no value.
In the preparation of fumaryl dichloride, zinc chloride was utilized as a catalyst to facilitate the transfer of the two chlorine atoms from phthalyl dichloride to maleic anhydride (12). In this reaction, the zinc chloride acted as a Lewis acid. Therefore zinc chloride was tried as a catalyst to attempt the esterification of acid chlorides which had resisted reaction by the Schotten-Bauman method. Successful esterification occurred with three of six acid chlorides.

The relative electronegative character of the acid chlorides concerned showed immediately that those acid chlorides which did not react were acid chlorides that should have a lower order of activity than those that did react. The low order of activity was probably due to "electron-pull" by the electronegative groups present. In para-nitrobenzoyl chloride, the nitro group is electronegative. In fumaryl dichloride, one chlorine acting through the unsaturated system influences the other. In dibromosuccinyl dichloride, the bromine on the alpha carbon atom is strongly electronegative.

Based upon these considerations, the di-acid chloride of terephthalic acid should not esterify with gentisic acid under the stated conditions. Terephthalyl chloride, in which the acid chloride groups are one-four, should have the same general electronic configuration as p-nitrobenzoyl chloride.
The Structures of Some Selected Acid Chlorides

Acid Chloride

p-nitrobenzoyl chloride

fumaryl dichloride

meso α, α' di bromo-succinyl dichloride

diphenyl acetyl chloride

diethylacetyl chloride

Phthalic dichloride

Structure

\[
\begin{align*}
&O=\text{O} \\
&\text{O} \\
&\text{CH}-\text{C}-\text{Cl} \\
&\text{CH}_3-\text{CH}_2-\text{CH}-\text{C}-\text{Cl} \\
&\text{C}-\text{Cl} \\
&\text{C}-\text{Cl} \\
&\text{Br} \\
&\text{Br}
\end{align*}
\]

Figure 1

Equation 3) was employed successfully for the preparation of several derivatives by Nash et al (10). The general utility of this method was further illustrated by the successful preparation of an ester of 5-ethoxy salicylic acid. Equations 5) and 6) were not employed in this research.
The structures of the seven gentisic acid derivatives prepared in this study were derived from several types of data. Specifically, the structures proposed for these compounds were based upon:

a. The characteristic bluish-purple color produced by the reaction of gentisic acid and its derivatives with ferric chloride. This color was produced by all gentisate compounds prepared in this laboratory. The color is the result of the formation of a complex between the ferric ion and the carbonyl group and ortho hydroxy group of gentisic acid.

b. The neutralization equivalent of the prepared derivative. In each case, the compound prepared was acidic. The esters of gentisic acid with other carboxylic acids contained a free carboxyl group. The amino alcohol esters of gentisic acid contained hydrogen chloride. Both these acidic groups were titrated with 0.01N sodium hydroxide.

c. Elemental analysis. Regardless of the type derivative, the empirical formulæ of the prepared compounds demand a specific carbon and hydrogen content. Since the assays for carbon and hydrogen are performed concurrently, this method was chosen
rather than elemental analysis for nitrogen or chlorine in case of the amine derivatives. The carbon-hydrogen analysis offers two sets of data per compound rather than one set which would result if nitrogen or chlorine were determined.

d. The infrared absorption spectrographs.

e. Other qualitative tests.

The neutralization equivalents were determined by standard procedures utilizing potentiometric titrations (13). The solvents used for the titrations were of hydro-alcoholic character. In the case of the esters of gentisic acid with other carboxylic acids, the presence of ethanol was necessary to solubilize the initial acid. In the case of the esters containing an amine-hydrochloride salt, the ethanol was present to solubilize the liberated amine base. Samples were in the order of 20 mg. to 70 mg. and the sodium hydroxide titrant was approximately 0.01 N. All of the monoesters were monobasic and the neutralization equivalent was equal to the molecular weight. In the case of the diester (5,5'-Dигентисил Фталат), the compound functioned as a monobasic acid, although two carboxyl groups were present in the compound. Only one end point was detected.

This behavior upon titration is exhibited by other dibasic acids in which the pKa values of the two carboxyl groups do not differ by more than a factor of 2. In Figure
In the potentiometric titration curve of tartaric acid compared to that of 5,5'-digentisyl phthalate. The pKₐ values of tartaric acid are 3.01 and 4.55 respectively. It is seen that they differ by a factor of 1.54 and that only one end point is observed. The pKₐ values of 5,5'-digentisyl phthalate were not determined, but they apparently differ by a factor of 2 or less.

In both of these acids, the end point occurred after both carboxyl groups had been neutralized. In each case the neutralization equivalent was one-half of the molecular weight (Table I).

Table I

Neutralization Equivalents for Tartaric Acid and 5,5'-Digentisyl Phthalate

<table>
<thead>
<tr>
<th>Compound</th>
<th>Calculated Mol. wt.</th>
<th>Calculated N.E.</th>
<th>Experimental N.E.</th>
<th>Experimental Mol. Wt.</th>
<th>Absolute Error</th>
</tr>
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<tr>
<td>Tartaric Acid</td>
<td>150</td>
<td>75</td>
<td>76</td>
<td>152</td>
<td>1.3</td>
</tr>
<tr>
<td>5,5'-Digentisyl Phthalate</td>
<td>438</td>
<td>219</td>
<td>224</td>
<td>448</td>
<td>2.2</td>
</tr>
</tbody>
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Infrared Spectroscopy

The degree of molecular motion possessed by a molecule is dependent upon the amount of energy it possesses. If we assume we have a molecule completely devoid of energy, it has no motion. If it is given energy, say in the form of
radiant energy, motion begins and it can take any one of
or a combination of four types of motion depending upon
the amount of energy supplied to it. Mathematical treat-
ment may be given to these four forms of motion for very
simple molecules in which the four forms are assumed to be
absolute, but in more complex molecules mathematical treat-
ment cannot be realized.

These four forms of motion are ranked as follows, ac-
cording to the energy requirements (14). (1) Translation-
al, in which the molecule moves from one point in space to
another. The amount of energy required for this type of
motion is in the order of a few hundred calories per mole,
and in the spectrum scale, radiant energy of a very low
order (twenty microns in wavelength) is sufficient to init-
iate this motion. (2) Rotational, in which the molecule
rotates about some central axis. Molecular motion of this
type is initiated by energies in the order of a thousand
calories per mole and radiation in the order of twenty
microns. (3) Vibrational, in which atoms are displaced
from their normal positions and they oscillate back and
forth or move sidewise with a swinging motion within the
molecule. From a thousand to forty thousand calories per
mole are required for this motion depending, of course, upon
the size and the nature of the molecule. The rotations,
involving small energies, are superimposed on the atomic
displacements, giving rise to absorption bands in the near infrared region of the spectrum which extends from about 2 to 16 microns. (4) Electronic, in which an outer electron is displaced within the molecule. The only difference between ultra-violet and visible absorption spectra is that greater energies and greater displacements are involved in the ultra-violet absorption - thirty-five thousand to seventy-one thousand calories per mole being required for absorption in the visible region of 4000 Å to 8000 Å and seventy-one thousand to several hundred thousand calories per mole in the ultra-violet region of 2000 Å to 4000 Å.

Infrared absorption spectra then are concerned with molecules which are capable of rotation and vibration.

There is an additional requirement, however. A compound, to be active in the infrared region must be sufficiently asymmetrical to possess a dipole moment. Vibration of two similar atoms against each other, as for example in nitrogen or oxygen molecules, will not result in a change in the electrical symmetry of the molecules and therefore, such molecules do not absorb in the infrared region (15).

The most significant portion of the infrared region of the spectrum for structure determination is the region from 2 to 8 microns ($\mu$), for it is in the region that individual bands are more or less characteristic of specific pairs or groups of atoms. Above 8 $\mu$, the bands are due to
vibrations and rotations in which all of the atoms in the molecule take part.

The spectrographs of pure compounds are so highly specific that they have become accepted as the "fingerprints" of those compounds. Very slight traces of impurity will change the fingerprint of a compound. For example, as little as 0.3 per cent of 1,2-dibromopropane may be detected and quantitatively determined in 0.1 ml of 1,3-dibromopropane (15).

When a compound has undergone reaction, the fingerprint changes to that characteristic of the new compound but the groups that are present in the parent compound plus those groups in the compound with which the parent was reacted will quite often show their characteristic absorption bands. Absorption in any particular band, however, is dependent upon the group itself plus neighboring groups. The other structures adjacent to a given group influence its electronic and spatial configuration. According to Brode (16), a homologous series tends to show differences in intensity rather than differences in frequency although some shifting may occur.

A further consideration is the effect of the solvent used upon the spectrograph. The ideal situation is that of the liquid compound in which the absorption observed is due to molecules of the compound only. When the compound is a
solid, it must be dissolved in a solvent or suspended in some liquid that will not harm the container used in the study. The bands due to the presence of the solvent must be considered.

These factors then place the determination of the structure of a new compound somewhat upon an empirical basis. There is some uncertainty present, but when the information from the infrared spectrograph is combined with other analytical data such as the neutralization equivalent, carbon-hydrogen analyses, etc., valid conclusions as to structure may be drawn.

Numerous infrared spectrographs of known compounds have been compiled, such as by Randall et al (17), which show that certain groups absorb regularly in specific wave lengths. Table II presents the absorption bands for specific groups from known compounds studied by the Department of Chemistry, The Ohio State University.

Infrared Spectra and Interpretation for Gentisic Acid and Its Recently Prepared Derivatives

The infrared absorption spectrograph for each of the recently prepared derivatives of gentisic acid was obtained by the use of Nujol as the suspending agent (Figures 3-9). Nujol has only three absorption peaks in the entire infrared spectrum. These three peaks occur at 3.65 μ - 3.70 μ, 6.90 μ - 6.95 μ and 7.20 μ - 7.25 μ, and they were present in
### TABLE II

**ABSORPTION BANDS FOR KNOWN GROUPS COMPILLED BY THE OHIO STATE UNIVERSITY**

<table>
<thead>
<tr>
<th>Functional Group</th>
<th>Wave Length in Microns</th>
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<tbody>
<tr>
<td>Phenolic Hydroxyl</td>
<td>2.95</td>
</tr>
<tr>
<td>Aromatic C-H</td>
<td>3.11 to 3.18</td>
</tr>
<tr>
<td>Amine Hydrochloride</td>
<td>3.62 and 4.2</td>
</tr>
<tr>
<td>Hydrogen Chloride</td>
<td>3.7 and 4.1</td>
</tr>
<tr>
<td>Ester Carbonyl</td>
<td>5.7 to 5.8</td>
</tr>
<tr>
<td>Acid Carbonyl</td>
<td>5.90 to 5.95</td>
</tr>
<tr>
<td>Benzene Overtone</td>
<td>5.00 to 6.0</td>
</tr>
<tr>
<td>Phényl Ring</td>
<td>6.15 and 6.7</td>
</tr>
<tr>
<td>Ether</td>
<td>8.95 to 9.15</td>
</tr>
</tbody>
</table>
all spectrographs in which Nujol was used as the suspending medium.

Figures 10 and 11 show the effect upon the infrared absorption spectrograph of gentisic acid brought about by a change in the solvent.

Tables III to V present the characteristic absorption bands found to be present in gentisic acid and its recently prepared derivatives.

The examination of Figures 3 to 9 and Tables III and IV show clearly that the functional groups expected in the proposed structural formulai of the new derivatives of gentisic acid were present.

In Figures 3 to 6 and in Table III are observed the characteristic bands due to hydrogen chloride, the acid carbonyl and the phenyl ring, which were expected in the esters of gentisic acid with amino alcohols.

In Figures 7 to 9 and in Table IV are observed the characteristic bands due to the ester carbonyl, the acid carbonyl and the phenyl ring, which were expected in the esters of gentisic acid with other carboxylic acids.

In the case of the esters of gentisic acid with amino alcohols, the shift in the acid carbonyl absorption band from 5.90\(\mu\) - 5.95\(\mu\) to the ester carbonyl absorption band of 5.7\(\mu\) - 5.8\(\mu\) did not occur. This same behavior in absorption characteristic was noted by Nash et al (18), who found that the carbonyl of gentisic acid esters with amino
### TABLE III

ABSORPTION BANDS FOUND IN THE ESTERS OF GENTISIC ACID, WITH AMINO ALCOHOLS SUSPENDED IN NUJOL

<table>
<thead>
<tr>
<th>Compound</th>
<th>Figure</th>
<th>Functional Group</th>
<th>Wave Length in Microns</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>3</td>
<td>amine nitrogen</td>
<td>3.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hydrogen chloride</td>
<td>4.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>acid carbonyl</td>
<td>5.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phenyl ring</td>
<td>6.15 and 6.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ether</td>
<td>9.17</td>
</tr>
<tr>
<td>B</td>
<td>4</td>
<td>aromatic C-H</td>
<td>3.17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hydrogen chloride</td>
<td>3.8 and 4.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>acid carbonyl</td>
<td>6.0</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phenyl ring</td>
<td>6.7</td>
</tr>
<tr>
<td>C</td>
<td>5</td>
<td>aromatic C-H</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hydrogen chloride</td>
<td>3.8 and 4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>acid carbonyl</td>
<td>5.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phenyl ring</td>
<td>6.7</td>
</tr>
<tr>
<td>D</td>
<td>6</td>
<td>aromatic C-H</td>
<td>3.15</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hydrogen chloride</td>
<td>3.8 and 4.1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>acid carbonyl</td>
<td>5.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phenyl ring</td>
<td>6.8</td>
</tr>
</tbody>
</table>

A - $\beta$-Diethylaminoethy1-5-ethoxy Salicylate Hydrochloride

B - $\beta$-n-Butylaminoethyl Gentisate Hydrochloride

C - $\beta$-Dimethylaminoethyl Gentisate Hydrochloride

D - 3-(1-Methyl piperidyl)-methyl Gentisate Hydrochloride
Infrared Absorption Spectrograph of β-Diethylaminoethyl-5-ethoxy Salicylate Hydrochloride

Figure 3.
Infrared Absorption Spectrograph of $\beta$-n-Butylaminoethyl Gentiobate Hydrochloride

Figure 4
Infrared Absorption Spectrograph of β-Dimethylaminoethyl Gentisate Hydrochloride

Figure 5
Infrared Absorption Spectrograph of 3- (1- Methylpiperidyl)- Methyl Gentiobiose Hydrochloride

Figure 6
### TABLE IV

**ABSORPTION BANDS FOUND IN THE ESTERS OF GENTISIC ACID WITH OTHER CARBOXYLIC ACIDS SUSPENDED IN NUJOL**

<table>
<thead>
<tr>
<th>Compound</th>
<th>Figure</th>
<th>Functional Group</th>
<th>Wave Length In Microns</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7</td>
<td>phenolic hydroxyl</td>
<td>2.90</td>
</tr>
<tr>
<td></td>
<td></td>
<td>aromatic C-H</td>
<td>3.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>ester carbonyl</td>
<td>5.75</td>
</tr>
<tr>
<td></td>
<td></td>
<td>acid carbonyl</td>
<td>5.93</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phenyl ring</td>
<td>6.15 and 6.72</td>
</tr>
<tr>
<td>B</td>
<td>8</td>
<td>ester carbonyl</td>
<td>5.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>acid carbonyl</td>
<td>5.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phenyl ring</td>
<td>6.15 and 6.70</td>
</tr>
<tr>
<td>C</td>
<td>9</td>
<td>ester carbonyl</td>
<td>5.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td>acid carbonyl</td>
<td>6.00</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phenyl ring</td>
<td>6.15 and 6.75</td>
</tr>
</tbody>
</table>

A - 5,5'-Digentisyl Phthalate

B - 5-Diethylacetyl Gentisic acid

C - 5-Diphenylacetyl Gentisic acid
Infrared Absorption Spectrograph of 5,5'-Digentisyl Pthalate

Figure 7
Infrared Absorption Spectrograph of 5-Diethylacetyl Gentisic Acid

Figure 8
Infrared Absorption Spectrograph of 5- Diphenylacetyl Gentiocic Acid

Figure 9
<table>
<thead>
<tr>
<th>Figure</th>
<th>Solvent</th>
<th>Functional Groups in Microns</th>
<th>Wave Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>10% solution in ether</td>
<td>phenolic hydroxyl</td>
<td>2.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>aromatic C-H</td>
<td>3.10</td>
</tr>
<tr>
<td></td>
<td></td>
<td>acid carbonyl</td>
<td>5.95</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phenyl ring</td>
<td>6.15 and 6.65</td>
</tr>
<tr>
<td>11</td>
<td>suspension in Nujol</td>
<td>aromatic C-H</td>
<td>3.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>acid carbonyl</td>
<td>5.98</td>
</tr>
<tr>
<td></td>
<td></td>
<td>phenyl ring</td>
<td>6.15 and 6.7</td>
</tr>
</tbody>
</table>
Infrared Absorption Spectrograph of Gentiisic Acid in Ether

Figure 10
Infrared Absorption Spectrograph of Gentisic Acid in Nujol

Figure 11
alcohols absorbed in the region of 5.89\(\mu\) - 6.00\(\mu\).

This lack of shift would seem to indicate that esterification did not occur and that the carbonyl group was present as an acid function. However, if this were true, the compound prepared, in order to fit the analytical data would be some isomer of the same empirical formula. The only other conceivable structure would be the formation of an ether between the 5-phenolic hydroxyl and the alcoholic hydroxyl. This type of compound, however, could not have been formed under the conditions of reaction that were employed. Furthermore, if an ether were formed in some completely unexplained manner, the compound that would have resulted would have been a dibasic acid, containing the free carboxyl group and hydrogen chloride. The neutralization equivalent data proved conclusive that these compounds were monobasic. Therefore, it may be concluded that in this type of gentisic acid derivative, the ester carbonyl group shifts its absorption band from the expected 5.7\(\mu\) - 5.8\(\mu\) to 5.9\(\mu\) - 6.0\(\mu\).

Examination of the infrared spectrographs of the compounds prepared in this study as well as examination of the infrared spectrographs of the compounds prepared by Nash et al (18) that were studied with Nujol as the solvent, showed that a triplet was present in the range 5.95 to 6.3 microns. The compounds which show the triplet clearly are:
a. 3-Diethylaminoethyl Gentisate Hydrochloride
b. γ-Diethylaminopropyl Gentisate Hydrochloride
c. 5,5'-Succinyl Digentisic Acid
d. β-n-Butylaminoethyl Gentisate Hydrochloride
e. β-Dimethylaminoethyl Gentisate Hydrochloride
f. 5,5'-Digentisyl Phthalate
g. 5-Diethylacetyl Gentisic Acid

Although Nash employed chloroform as a solvent for some of his derivatives, the triplet was visible in the following compounds:

h. 5-Acetyl Gentisic Acid
i. 5-Methoxy Salicylic Acid
j. Diacetyl Gentisic Acid

k. β-Diethylaminoethyl-5-Acetyl Gentisate Hydrochloride
l. β-Diethylaminoethyl-5-Methoxy Salicylate Hydrochloride
m. β-Diethylaminoethyl-5-Benzoyl Gentisate Hydrochloride
n. γ-Diethylaminopropyl-5-Methoxy Salicylate Hydrochloride

o. 5-Phenylacetyl Gentisic Acid
p. 5-Acetylolsolicyl Gentisic Acid

The triplet was present, but the third peak was weak in the following compounds:

q. 5-Diphenylacetyl Gentisic Acid
r. β-Diethylaminoethyl-5-ethoxy Salicylate Hydrochloride
3-(1-Methylpiperidyl)-methyl Gentiisate Hydrochloride

It is of special interest to note that gentisic acid when studied in ether as a solvent does not show this triplet (Figure 10). The triplet, however, was visible in nineteen compounds prepared in this laboratory whenever the solvent was chloroform or Nujol. It would appear then, that this triplet of absorption bands, absorbing at approximately 5.95 μ, 6.1 μ, and 6.3 μ should be characteristic of gentisic acid as well as of its derivatives whenever the compound is studied in the two solvents mentioned.

When specially purified gentisic acid (re crystallized from hot ethylene dichloride m.p. 204-206°C) was studied in Nujol, the triplet was clearly visible (Figure 11). Therefore, the assumption has been verified.

This difference in absorption characteristics adds further proof to the before-mentioned fact that the solvent used to study the infrared absorption spectra of a compound is an important factor. The solvent can mask or make visible an identifying characteristic absorption band.

Although the color test, the qualitative tests and the infrared spectrograph of the compound designated as β-n-butylaminocethyl gentisate hydrochloride clearly indicate that the compound is a gentisate derivative, the analytical data places the formula and constitution of this compound in doubt.
The neutralization equivalent for this compound was determined by three procedures. Procedure A consisted of the standard procedure used throughout this research in which the carboxyl-ester hydrochloride was titrated in a hydroalcoholic medium. Procedure B involved titration in wholly aqueous media. Procedure C consisted of the titration of the salt in 75 per cent ethanol using a 75 per cent ethanol titrant, as suggested by Saunders and Srivastava (19) for the potentiometric titration of alkaloidal salts. Procedure A gave a pH versus volume curve in which the points were scattered and the end point indefinite. An end point was estimated. Procedure B gave a pH versus volume curve in which the end point was quite definite. However, no precipitation of the free carboxyl-ester amine occurred. Procedure C gave a pH versus volume curve that exhibited a definite end point, but whose accuracy is in doubt because of the long range over which the end point occurred. The results of these titrations are seen in Table VI.

**TABLE VI**

Neutralization Equivalents of β-n-Butylaminoethyl gentisate Hydrochloride

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Experimental N.E.</th>
<th>Calculated N.E.</th>
<th>% Absolute error</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>291</td>
<td>290</td>
<td>0.3</td>
</tr>
<tr>
<td>B</td>
<td>218</td>
<td>290</td>
<td>24.8</td>
</tr>
<tr>
<td>C</td>
<td>144</td>
<td>290</td>
<td>50.4</td>
</tr>
</tbody>
</table>
Procedure C was employed with known non-alkaloidal amine hydrochlorides to test its accuracy and utility. These results are seen in Table VII.

**TABLE VII**
Neutralization Equivalents of Known Amine Hydrochloride Esters in 75% Ethanol Titrant

<table>
<thead>
<tr>
<th>Compound</th>
<th>Experimental N.K.</th>
<th>Calculated N.K.</th>
<th>% Absolute Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>β-Diethylaminopropyl Gentisate Hydrochloride</td>
<td>264</td>
<td>267</td>
<td>1.1</td>
</tr>
<tr>
<td>β-Dimethylnaminoethyl Gentisate Hydrochloride</td>
<td>269</td>
<td>262</td>
<td>2.5</td>
</tr>
</tbody>
</table>

*Prepared by Nash et al (10)*

The compound upon elemental analysis was found to contain 54.02 per cent carbon and 8.10 per cent hydrogen. The calculated values were 53.88 per cent carbon and 6.96 per cent hydrogen. The value for carbon was well within experimental error (0.5% error per 0.2g. sample). The value for the hydrogen content had an absolute error of over 16 per cent (3% error per 0.2g. sample).

The discrepancies in the analytical data for this compound are possibly due to different degrees or kinds of solvation in which water, ethanol or acetone might play a part. The low values for the neutralization equivalent data...
in procedures B and C may have resulted from different degrees of desolvation during the drying process.

Qualitative tests were employed for the esters of gentisic acid with amino alcohols to insure the presence of ionic chlorine, which was present in the form of hydrogen chloride. The two tests that were employed are:

a. The Beilstein test in which the halogen, on a copper wire, imparted a green color to a non-luminous flame (20).

b. The silver nitrate test in which the silver ion precipitated the chloride ion as white silver chloride (21).

The melting points reported herein were determined by use of the Fisher Melting Point Block, whose thermometer calibrations had been checked with the melting points of known pure compounds. No further corrections were applied. The melting points of the purified compounds were determined, after drying each compound in an Abderhalden drying pistol at 100° C and 2 mm. pressure for 2 hours.
EXPERIMENTAL
5,5'-Digentisyl Phthalate

Preparation

5,5'-digentisyl phthalate was prepared by the reaction of phthaloyl dichloride and gentisic acid in the presence of a catalyst, in an inert solvent.

Phthaloyl dichloride was prepared from phthalic anhydride by treatment with thionyl chloride in the presence of a catalyst (12). 250 g. of phthalic anhydride and 1.5 g. of freshly fused zinc chloride were charged into a three-necked flask equipped with an efficient reflux condenser, a dropping funnel and a thermometer almost touching the bottom of the flask. The mixture was heated to 220° C by a glass mantle. 176 ml. of thionyl chloride was added drop-wise over a period of 10 1/2 hours at such a rate that the temperature could be maintained at 220° C. The reaction mixture was heated for 1 hour longer until the evolution of gas ceased and was then distilled under vacuum. A small amount of low-boiling forerun was discarded and the major portion which boiled at 155° C (uncorrected) was collected. The distillate was redistilled and the fraction boiling at

# New Compound
151° C (uncorrected) at 17 mm. pressure was collected. (Reported value 131-133° C at 9 mm pressure). After standing for 24 hours it was filtered from white crystals of unreacted phthalic anhydride. The filtered liquid weighed 277.5 g., which corresponded to a yield of 80.3 per cent.

Fifteen grams of gentisic acid and 10 g. of phthaloyl dichloride were dissolved in 200 ml of anhydrous ether containing 1 g. of freshly fused zinc chloride. The mixture was refluxed for four hours. The ether was removed under reduced pressure and the residue dried to a semi-solid state. 200 ml of anhydrous ether were added to the residue and the mixture heated to reflux. A white amorphous material precipitated. The precipitate was filtered off and the filtrate was refluxed 3 hours. No further product was obtained from this ether solution.

The white amorphous product upon drying weighed 5.8 g., which corresponded to a yield of 31.7 per cent of the theoretical value.

The 5.8 g. of amorphous material was recrystallized from ethylacetate and petroleum ether (1:1) to give 4.9 g. of white crystalline plates, which had a melting range of 247-249.5° C. A second recrystallization from acetone and ethylene dichloride gave almost a quantitative yield of white crystalline plates, m.p. 252-253° C.

The reactions involved in the preparation of this product were:
Properties

5,5'-digentisyl phthalate is a white crystalline solid which appears as transparent plates under low power magnification. It is odorless and tasteless.

This dibasic acid is soluble in ethanol, acetone and ethyl acetate. It is slightly soluble in ether. It is insoluble in cold water, cold ethylene dichloride and petroleum ether.

After standing in air two weeks, the compound showed no visible signs of decomposition.

An alcoholic solution of this compound gave a strongly positive color test with aqueous ferric chloride solution.

5,5'-digentisyl phthalate, upon analysis, was found to contain 60.18 per cent carbon and 3.32 per cent hydrogen. The calculated values are 60.28 per cent carbon and 3.22 per cent hydrogen. Its empirical formula of C_{22}H_{13}O_{12} required a neutralization equivalent of one-half the molecular weight, which is 219. A value of 224 was found by titration of a hydroalcoholic solution of the acid with 0.01N sodium hydroxide (Table I, p. 10).
5-Diphenylacetyl Gentisic Acid

Preparation

5-diphenylacetyl gentisic acid was prepared by reacting equal molar quantities of diphenylacetyl chloride with gentisic acid in the presence of a catalyst, in an inert solvent.

Diphenylacetyl chloride was prepared by the reaction of an excess of thionyl chloride with diphenylacetic acid (E.K.Co.). 50 g. of diphenylacetic acid was placed in a 250 ml round bottomed flask and 41.7 g. (1.5 M) of thionyl chloride added. The flask was heated gently under reflux with a glass heating mantle for seventy-five minutes, at which time solution was complete, and then fifteen minutes longer to insure complete reaction. The flask was allowed to stand at room temperature overnight. After this period, the reaction flask contained large white crystals and a dark red solution. The red solution was decanted from the crystals and discarded. The crystals remaining were re-crystallized from warm petroleum ether to yield 43 g. of

* New Compound *
white rectangular crystals. Their melting range was 54-54.5° C (Reported value 56-57° C). This yield represented 75.7 per cent of the theoretical value.

Repeated recrystallization would not raise the melting range of this compound, but rather resulted in a lowering of the melting range. The product used in the reaction with gentisic acid had a melting range of 50-51° C.

Six grams of gentisic acid and 9.24 g. of diphenyl-acetyl chloride were dissolved in 50 ml. of anhydrous ether containing 1 g. of freshly fused zinc chloride. The mixture was refluxed for one hour. The flask was cooled and the solvent removed under vacuum. The residue was heated gently at reduced pressure (25 mm) for fifteen minutes.

Fifty milliliters of anhydrous ether were added to the flask and refluxing repeated for one hour. The ether was again removed under vacuum. To the residue was added .95 ml. of 60 per cent ethanol and the flask heated to reflux, then allowed to cool. The white amorphous material that precipitated was filtered and upon drying, weighed 9.6 g., representing a yield of 67.7 per cent of the theoretical value. This amorphous product had a melting range of 175-184.5° C.

The amorphous material was recrystallized three times from hot ethylene dichloride. Recovery was almost quantitative from the cold solvent. The melting range of the pure
The compound was 200.5-201.5°C.

The reactions involved in the preparation of this compound were:

1. \[ \text{CH-C-OH} + \text{SOCl}_2 \xrightarrow{\Delta} \text{CH-C-Cl} + \text{HCl} + \text{SO}_2 \]

2. \[ \text{CH-C-Cl} + \text{HO-CH-C-OH} \xrightarrow{\Delta} \text{ZnCl}_2 \xrightarrow{\Delta} \text{CH-C-CH-C-CH-C-OH} + \text{HCl} \]
Properties

5-diphenylacetyl gentisic acid is a white, sparkling, crystalline solid which appears as transparent plates under low power magnification. It is odorless and tasteless. This compound is soluble in ether, acetone, ethanol, glacial acetic acid, isobutanol, hot 60 per cent ethanol, and hot ethylene dichloride. It is very slightly soluble in hot 10 per cent ethanol and is insoluble in water, Skellysolve, chloroform, carbon tetrachloride and cold ethylene dichloride.

This product is stable in air and does not hydrolyze in the presence of boiling water.

An alcoholic solution of 5-diphenylacetyl gentisic acid exhibits the characteristic bluish-purple color of ortho hydroxy acids with aqueous ferric chloride.

Upon analysis, this compound was found to contain 72.66 per cent carbon and 4.32 per cent hydrogen. The calculated values are 72.41 per cent carbon and 4.63 per cent hydrogen. Its empirical formula of \( \text{C}_{21}\text{H}_{16} \text{O}_4 \) has a neutralization equivalent of 348. A value of 347 was found by the titration of a hydroalcoholic solution of the acid with 0.01N sodium hydroxide.
5-Diethylacetyl Gentisic Acid

\[
\begin{align*}
\text{CH}_3\text{-CH}_2 & \quad \text{CH-CH} \\
\text{CH}_3\text{-CH}_2 & \quad \text{H-C-O} \\
\text{C-O-H} & \quad \text{O-C-H}
\end{align*}
\]

Preparation

5-diethylacetyl gentisic acid was prepared by the reaction of diethylacetyl chloride and gentisic acid in the presence of a catalyst, in an inert solvent.

Diethylacetyl chloride was prepared from diethylacetic acid by treatment with phosphorous trichloride. This halogenating agent was used instead of thionyl chloride because the phosphorous acid formed is quite high boiling and no excess reagent is required (22). One hundred grams of diethylacetic acid were placed in a 250 ml round bottomed flask and 56 g. of phosphorous trichloride (Baker and Adamson Co.) were added. The mixture was refluxed gently for thirty minutes. The top of the reflux condenser lead to a water trap. After cooling the mixture the upper layer was decanted into a clean 250 ml round bottomed flask from a thick yellow oil which was discarded. The decanted portion was distilled at atmospheric pressure and the portion boiling between 134-140 °C (uncorrected) was collected. This fraction was then redistilled and the portion boiling between 134-136 °C (uncorrected) was collected. (Reported

* New Compound
The boiling range 134-137°C. The yield of 82 g. represented a yield of 70.5 per cent of the theoretical value.

Ten grams of gentisic acid and 10 g. of diethylacetyl chloride (an excess of 1.3 g. over the theoretical molar equivalent) were dissolved in 150 ml. of anhydrous ether containing 1 g. of freshly fused zinc chloride. The mixture was refluxed for ninety minutes. The ether was removed under vacuum and the crystalline residue was subjected to reduced pressure (27 mm) for thirty minutes. At the end of this period 150 ml. of anhydrous ether were added and refluxing repeated during ninety minutes. Again the ether was removed, leaving a brown crystalline residue.

The residue was washed with 300 ml. of hot water and the water was then decanted from the resulting oil. The oil was dissolved in ethanol and transferred to a 500 ml. beaker. The ethanol was removed by evaporation facilitated by a stream of air blowing over the surface.

The resulting thick oil was dissolved in ether to form a light yellow ethereal solution and a few ml.s. of dark red aqueous solution. The ethereal solution was separated from the aqueous portion and the ether removed by evaporation in a stream of dry air. 8.4 g. of a white crystalline residue were obtained, which melted between 89-101°C.

The 8.4 g. which corresponded to a yield of 50 per cent of the theoretical value were then washed with 400 ml. of
cold water. The washing was done in portions, sucking the compound nearly dry between washings. The melting range after washing was 111-113°C. Recrystallization from hot Skellysolve gave a product that melted between 111.2-113.2°C.

The reactions involved in the preparation of this compound were:

(1) $3 \text{C}_6\text{H}_5\text{CH-C-OH} + \text{PCl}_3 \xrightarrow{\Delta} 3 \text{C}_6\text{H}_5\text{CH-C-Cl} + \text{H}_3\text{PO}_3$

(2) $\text{C}_6\text{H}_5\text{CH-C-OH} + \text{C}_6\text{H}_4\text{CO-CH}_4 \xrightarrow{\text{ZnCl}_2} \text{C}_6\text{H}_5\text{CH-C-O-CH}_4\text{CO-H} + \text{HCl}$
5-diethylacetyl gentisic acid is a gray-white crystal-line solid which appears as transparent needles under low power magnification. It has a sweet aromatic odor and is tasteless.

This compound is soluble in ethanol, acetone ether, ethylene dichloride hot 40% ethanol, hot Skellysolve, and in chloroform. It is insoluble in cold water and in petroleum ether. It is slightly soluble in hot water.

After standing in air for three weeks, the compound showed no visible signs of decomposition.

An alcoholic solution of this compound exhibits the characteristic bluish-purple color of ortho-hydroxy acids with aqueous ferric chloride.

This compound upon analysis was found to contain 62.15 per cent carbon and 6.40 per cent hydrogen. The calculated values were 61.89 per cent carbon and 6.39 per cent hydrogen. Its empirical formula of C₁₃H₁₆O₅ had a neutralization equivalent of 252. A value of 254 was found by titration of a hydroalcoholic solution of the acid with 0.01N sodium hydroxide.
**β-Diethylaminoethyl-5-ethoxy*Salicylate Hydrochloride**

\[
\begin{align*}
\text{CH}_3\text{-CH}_2\text{-O} & \quad \text{CO-CH}_2\text{-CH}_2\text{-N} \quad \text{HCl} \\
\text{C}_6\text{H}_4\text{-OH} & \quad \text{CH}_2\text{-CH}_3 \\
\end{align*}
\]

Preparation

**β-Diethylaminoethyl-5-ethoxy salicylate hydrochloride** was prepared by the reaction of 5-ethoxy salicylic acid with **β-diethylaminoethyl chloride**.

The 5-ethyl ether of gentisic acid was prepared by reacting diethyl sulfate in the presence of an excess of gentisic acid in a strongly alkaline media (11). Twenty-grams of diethyl sulfate were placed in a 500 ml., 2-necked flask equipped with a reflux condenser and a dropping funnel. Twenty grams of gentisic acid were dissolved in water containing two equivalents of potassium hydroxide (30.6 g., 85% pure) so that the total volume of solution was 43 ml.

Ten milliliters of the alkaline solution were added to the diethyl sulfate by means of the dropping funnel. The alkaline solution formed a second phase. The contents of the flask were then heated to reflux. The remainder of the alkaline solution was added at a moderate rate under reflux and the reaction mixture was refluxed until only a single phase was visible. The reaction mixture became quite

* New Compound
thick so 20 ml. of water were added and refluxing was continued for twenty minutes. The total reaction time was less than one hour.

The reaction mixture was cooled and it was strongly alkaline to pHydriion paper. The contents were transferred to a 300 ml. beaker and rendered strongly acid with concentrated hydrochloric acid and heated to boiling. A brown oil separated. The mother liquor was decanted and set aside. Upon standing the brown oil solidified.

The brown solid was recrystallized from 2 l. of hot water. The mother liquor upon cooling yielded brown crystals. The total crude yield from the mother liquor and brown solid was 7 g. The yield was 89 per cent of the theoretical value.

0.1 g. of the brown solid was recrystallized a second time from hot water to give white crystals. M.P. 159.5-160.5° C (Reported value 164° C). The compound was titrated with 0.01N sodium hydroxide in a hydroalcoholic medium, as previously described (13), giving a neutralization equivalent of 189. The calculated neutralization equivalent was 182.

The reactions involved in the preparation of 5-ethoxy salicylic acid were as follows:
β-Diethylaminoethyl chloride was prepared from the corresponding amino alcohol by treatment with thionyl chloride and subsequent neutralization (22).

Seventy-two and five tenths grams of thionyl chloride (K. K. Co.) were placed in a 500 ml. three-necked flask equipped with an air stirrer sealed with mercury, a reflux condenser which lead to a water trap and a dropping funnel. The apparatus was placed in a hood. The thionyl chloride was then thoroughly chilled in a crushed ice bath. Fifty-two grams of β-diethylaminoethanol (E. K. Co.) were added dropwise through the dropping funnel with constant stirring. The reaction was violently exothermic. At the end of the addition of the amino alcohol, 200 ml. of absolute ethanol
were added and the mixture heated to reflux. The hot solution was transferred to an 800 ml. beaker and the solvent was removed by a stream of dry air and gentle heat. The residue was a red-brown crystalline solid. This residue was washed three times with 200 ml. portions of ether. The tan crystalline residue of \( \beta \)-diethy laminoethyl chloride hydrochloride weighed 72 g. The yield was 95.3 per cent of the theoretical value. The reaction was as follows:

\[
(4) \quad (\text{Et})_2 \text{N-EtOH} + \text{SOCl}_2 \rightarrow (\text{Et})_2 \text{N} - \text{Et} - \text{Cl} \cdot \text{HCl} + \text{SO}_2
\]

Seventy grams of crude \( \beta \)-diethy laminoethyl chloride hydrochloride were placed in a 1000 ml. beaker and 30 g. of crushed ice added. Sufficient 40 per cent sodium hydroxide was added to render the mixture distinctly alkaline. The red-brown oil of \( \beta \)-diethyle aminoethyl chloride was removed by a separatory funnel and the aqueous alkaline portion was extracted three times with 20 ml. portions of ether. The combined portions of ether were added to the red-brown oil.

The red-brown ethereal solution was vacuum distilled at 20 mm and the fraction distilling at 48-51\(^\circ\) C (uncorrected) was collected to yield 42 g. of a clear, strongly ammoniacal smelling, frothy liquid, \( \beta \)-diethy laminoethyl chloride. Based upon the starting compound, the over-all yield was 70 per cent of the theoretical value. This procedure, as described, significantly reduces the total reaction time and increases
the yield 3.5 per cent over the procedure described by Schrieber (23). The reaction was as follows:

\[(5) \quad (\text{Et})_2\text{N-Et-Cl} \cdot \text{dCl} + \text{NaOH} \rightarrow (\text{Et})_2\text{N-Et-Cl} + \text{NaCl} + \text{H}_2\text{O}\]

The \(\beta\)-diethylaminoethyl ester of 5-ethoxy salicylic acid was prepared by a method proposed by Horenstein and Pahliche (24). Five and forty-five hundredth grams of crude 5-ethoxy salicylic acid were dissolved in 50 ml. of isopropanal (Petrohal, Sohio). To this solution was added 4.1 g. of \(\beta\)-diethylaminoethyl chloride and the mixture was refluxed four hours. It was filtered while hot to remove a small amount of tarry material. The brown solution was allowed to cool to room temperature and the crystals that precipitated were filtered in a Buchner funnel and washed twice with 10 ml. portions of cold isopropanol. The white crystals weighed 4.92 g. which corresponded to 59.4 per cent of the theoretical yield. This product melted in the range of 173.5-174° C. Recrystallization from hot isobutanol gave a white crystalline product, m.p. 172.5-174.5° C.

The equation for the reaction was:

\[(6) \quad \text{Et}_2\text{O} + \text{Cl-Et-N(Et)_2} \xrightarrow{\Delta} \text{Et}_2\text{O} + \text{Et-N(ET)_2} \cdot \text{HCl}\]
Properties

β-diethylaminoethyl-5-othoxy salicylate hydrochloride is a fluffy almost colorless crystalline solid which appears as colorless needles under low power microscopic examination. It has no detectable odor.

This salt has the usual solubilities of an amine hydrochloride being very soluble in water, ethanol, hot isopropanol and hot isobutanol. It is insoluble in the usual non-polar organic solvents such as benzene, ether, petroleum ether, etc. It is insoluble in acetone.

The solid is stable in air. In aqueous or alcoholic solution it is unstable, developing a brown coloration within four days.

The characteristic bluish-purple color with aqueous ferric chloride solution is produced by this compound. The copper and silver ion tests for halogen were positive.

Upon analysis, this compound was found to contain 56.77 per cent carbon and 7.60 per cent hydrogen. The calculated values were 56.54 per cent carbon and 7.60 per cent hydrogen. The empirical formula of C15H24O4NCl required a neutralization equivalent of 318. A value of 319 was obtained experimentally in the usual manner of titrating amine salts.
3-(1-Methylpiperidyl)-methyl Gentisate Hydrochloride*  

Preparation

3-(1-Methylpiperidyl)-methyl gentisate hydrochloride was prepared by the reaction of 3-(1-methylpiperidyl)-carbinol with gentisic acid in a dehydrating medium (25).

Twenty grams of 3-(1-methylpiperidyl)-carbinol (Winthrop Research Laboratory) were dissolved in 200 ml. of anhydrous benzene. Dry hydrogen chloride gas was passed into the solution until the benzene was saturated. The hydrochloride salt precipitated as an oil. Twenty-four grams of gentisic acid were added and refluxing begun. The benzene condensate returned through a toluene-water refluxing trap. Dry hydrogen chloride gas was passed into the reaction mixture throughout the entire refluxing period of 67 hours. At the end of this period, 2.2 ml. of water had collected in the trap. (Theoretical yield of water = 2.8 ml).

After cooling the reaction mixture to room temperature, the benzene was poured off of the dark brown semi-solid. The benzene portion was set aside.

The brown residue was extracted with 150 ml. of hot acetone and filtered. The gray crystalline residue was
washed twice with 100 ml. portions of acetone. The acetone portions were combined and set aside.

The gray crystalline solid weighed 17.1 g. and had a melting range of 203-223°C.

The acetone portions (350 ml.) were evaporated to a thick oil. This oil was recylized through the refluxing treatment under the influence of dry hydrogen chloride for a period of 44 hours. This reaction mixture was treated as previously described to give 9.2 g. of light tan crystals with a melting range of 215.5-226°C. The total combined crude yield was 26.3 g. which corresponded to 83.6 per cent of the theoretical value.

Recrystallization from ethanol and acetone (2:3) caused a loss in product of 66 per cent but a gave a product which had a melting range of 229-231°C.

The reaction involved in this preparation was:

(13)

\[
\begin{align*}
\text{HO-CH}_2\text{S-NCH}_3 \quad \text{HCl} \quad \text{HCl} \quad \text{HO-CO-CH}_2\text{S-NCH}_3 \cdot \text{HCl} \\
+ \text{H}_2\text{O}
\end{align*}
\]
Properties

3-(1-methylpiperidyl)-methyl gentisate hydrochloride is a very light tan, nearly white crystalline solid. Under low power magnification, it appears as colorless needles. It has no odor.

This amine hydrochloride salt is very soluble in water, ethanol, methanol and hot isopropanol. It is relatively insoluble in hot isobutanol. It is also insoluble in the usual organic solvents.

The aqueous ferric chloride test for ortho-hydroxy-carboxylic acids was positive. The color, however, faded rapidly from bluish-purple to red. When dissolved in alcohol, it gave a permanate dark green color with the aqueous ferric chloride solution. The copper and silver ion tests for halogen were positive.

Aqueous or alcoholic solutions of this compound turned dark red upon standing for four days.

The empirical formula of $\text{C}_14\text{H}_{20}\text{O}_4\text{NCl}$ required a neutralization equivalent of 302. A value of 301 was found experimentally upon titration as previously described. Upon elemental analysis, this compound was found to contain 55.82 per cent carbon and 6.52 per cent hydrogen. The calculated values were 55.72 per cent carbon and 6.68 per cent hydrogen.
\(\beta\)-Dimethylaminoethyl Gentisate Hydrochloride

![Chemical Structure]

**Preparation**

\(\beta\)-Dimethylaminoethyl gentisate hydrochloride was prepared by the reaction of \(\beta\)-dimethylaminoethanol with gentisic acid in a dehydrating medium (24).

Eight and seven tenths gram of \(\beta\)-dimethylaminoethanol (E. K. Co.) were reacted with 15 g. of gentisic acid in the manner described for the preparation of 3-(1-methylperidyl)-methyl gentisate hydrochloride. At the end of 50 hours of reaction time, 1.8 ml. of water had collected in the water trap (theoretical volume 1.75 ml.). The product was isolated in the manner previously described to give 9.3 g. of gray crystals which melted at 194-198° C. This weight corresponded to a yield of 34.8 per cent of the theoretical value. Recrystallization of the acetone soluble products did not yield any additional product.

Recrystallization of the crude product from 500 ml. of hot ethanol gave 5.5 g. of a gray crystalline solid. M.p. 195.5-197.5° C. Recrystallization from ethanol, acetone and benzene (1:1:1) gave 3.6 g. of a gray crystalline solid whose melting range was 198-199° C.

__________

*New Compound*
The reaction involved in this preparation was:

\[
\text{HO-} + \text{HO-}\text{Et-N-(Me)}_2\cdot\text{HCl} \xrightarrow{\text{HCl}} \text{HO-}\text{C-O-Et-N-(Me)}_2\cdot\text{HCl} + \text{H}_2\text{O}
\]
Properties

β-Dimethylymoethyl gentisate hydrochloride is a gray crystalline solid which appears as transparent needles under low power magnification. It is odorless.

This product has the same solubilities as those of 3-(1-methylpiperidyl)-methyl gentisate hydrochloride. However, the ethanol solubility of β-dimethylymoethyl gentisate hydrochloride is somewhat less.

The aqueous ferric chloride test was positive. The copper and silver ion tests for halogen were positive.

Upon elemental analysis, this compound was found to contain 50.55 per cent carbon and 6.43 per cent hydrogen. The calculated values were 50.48 per cent carbon and 6.16 per cent hydrogen. Its empirical formula of \(C_{11}H_{16}O_{4}NCl\) gave an experimental neutralization equivalent of 269. Its calculated value was 262.
\(\beta\)-n-Butylaminoethyl Gentisate Hydrochloride

\[
\text{HO-}
\]
\[
\text{C}=\text{O-CH}_{2}\text{CH}_{2}\text{N-}
\]
\[
\text{H} \cdot \text{HCl}
\]

Preparation

\(\beta\)-n-Butylaminoethyl gentisate hydrochloride was prepared by the reaction of 2 moles of \(\beta\)-n-butylaminoethanol (Sharples Co.) with 1 mole of gentisic acid in a dehydrating medium.

Twenty grams of \(\beta\)-n-butylaminoethanol were reacted with 13.2 g. of gentisic acid in the same manner as described for the preparation of 3-(1-methylpiperidyl)-methyl gentisate hydrochloride. After refluxing for 30 hours, water ceased to be evolved from the reaction. 0.8 ml. of water had been evolved (theoretical volume 1.5 ml.). The product was isolated by cooling the reaction mixture to room temperature, pouring off the benzene from the dark brown semi-solid and extracting the dark brown semi-solid as follows:

One hundred and fifty milliliters of acetone were added to the reaction flask and the mixture heated to boiling. The contents of the flask were transferred while hot to a filter. After filtration was complete, the almost

\# New Compound

\# Doubtful Formula
white crystalline solid was washed twice with 100 ml. portions of cold acetone. The acetone portions were combined and set aside.

Upon drying, the white crystalline solid weighed 9.1 g. The melting range of this product was 198-203°C. This yield corresponded to a yield of 31.5 per cent of the theoretical value.

The acetone soluble products were recylclized as previously described and refluxing was continued until 1.5 ml. of water had collected in the water trap. The acetone in soluble portion from the recylclization weighed only 0.5 g.

The crude product was washed with 300 ml. of anhydrous ether and then extracted with 200 ml. of hot acetone to give 8.5 g. of a white crystalline solid. M.p. 198-203°C.

Repeated recrystallizations from ethanol-acetone and ethanol-ethylacetate mixtures failed to improve the melting range.

After drying the compound in an Abderhalden drying pistol as previously described, the melting range was 198-202°C.

The reaction believed to have occurred is as follows:

\[
\text{HO-CHOH} + \text{HO-} \overset{\text{H}}{\text{Et-N-C}_4\text{H}_4\text{HCl}} \text{HCl} \rightarrow \text{HO-\overset{\text{H}}{C=O-Et-N-C}_4\text{H}_4\text{HCl}} + \text{H}_2\text{O}
\]
Properties

\( \beta \)-n-Butylaminoethyl gentisate hydrochloride is a white, fluffy, crystalline solid which appears as long, transparent needles under low power magnification. It is odorless.

This compound is quite soluble in water, ethanol, methanol, isopropanol and isobutanol. It is very slightly soluble in hot dioxane forming a deep yellow color. It is insoluble in ether, acetone, benzene, ethylene dichloride and petroleum ether.

Its aqueous solution gave a strongly positive color test with aqueous ferric chloride solution. The qualitative tests for halogen were positive.

As previously discussed (p. 33), the calculated neutralization equivalent for this compound was 290. Values of 291, 218, and 144 were obtained. Upon elemental analysis, it was found to contain 54.02 per cent carbon and 8.10 per cent hydrogen. The calculated values were 53.80 per cent carbon and 6.96 per cent hydrogen.
SOME PHARMACOLOGICAL TESTS UPON THE RECENTLY PREPARED DERIVATIVES OF GENTISIC ACID

Toxicity

Although there seems to be a relationship between structure and pharmacological action in drugs such as seen in the \( \beta \)-phenylethylamine series, the sulfonamides and antihistaminics, every compound thought to be of medicinal value because of its structure must be screened pharmacologically to determine what, if any, is its action upon living organisms. One of the first and most important steps in this process is the determination of its toxicity.

The most commonly accepted method of expressing toxicity is in terms of the dose in grams or milligrams per kilogram of body weight that kills 50 per cent of a group of animals. This term is called the \( LD_{50} \) which means, "the lethal dose to 50 per cent." The term applies equally well to acute or chronic toxicity. The acute toxicity, which refers to deaths occurring within 24-48 hours, is usually determined first. If the drug should have medicinal merit, the chronic toxicity is determined, in which sub-lethal doses are administered over a period of weeks followed by autopsy of the animals to determine what physiological changes, if any, have been produced by the drug.

The acute toxicity of recently prepared derivatives of gentisic acid was determined by the use of white Swiss
mice as the test animal. The intraperitoneal route of administration was selected because the LD₅₀ by intraperitoneal injection of sodium gentisate has been reported (26), and because the esters of gentisic acid with other carboxylic acids were water insoluble but were soluble as their sodium salts.

The technique used in the acute toxicity tests was as follows:

a. White Swiss mice from the same source of supply, weighing 12 to 26 g. were used. They were observed for 24 hours prior to use and were apparently in good health. They were housed in standard cages under the prevailing atmospheric conditions. Their diet was an unlimited ration of water and Purina Dog Checkers.

b. The mice were starved for 24 hours before use to standardize their body weight. Their water ration was unlimited.

c. The esters of gentisic acid with amino alcohols were soluble as their hydrochloride salts. They were dissolved in distilled water for use.

d. The esters of gentisic acid with other carboxylic acids, and gentisic acid, were dissolved in an equivalent amount of sodium hydroxide solution. The concentrations of these solutions were expressed in terms of the free acid.
e. All doses were administered by slow intraperitoneal injection from a 1 ml. tuberculin syringe. The maximum volume of solution injected was 1.20 ml.
f. The approximate toxic range was determined for each compound by the use of 3 mice for each dose selected.
g. The LD$_{50}$ for each compound was calculated by Behren's method of statistical evaluation (27) using 12 mice per dose selected.
h. All mice that survived any dose were observed for 24 hours.

It must be kept in mind that Behren's method is an approximation and that more recent and more accurate methods are available for the evaluation of biological data (28). However, due to the fact that there was an error introduced in the measurement of doses (accurate to 0.01 ml.) plus the fact that biological data are not transferable from one animal to another, an estimation of toxicity is acceptable for screening purposes.

The results of the toxicity tests and the calculated LD$_{50}$ values of the recently prepared derivatives of gentisic acid are presented in Tables VIII and IX and in Figures 12 and 13.

The pattern of toxicities of these new derivatives of gentisic acid follows that found by Nash et al (29). The amino alcohol esters were more toxic than the esters with
<table>
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<th>Compound</th>
<th>No. of Animals</th>
<th>Experimental Data</th>
<th>Integrated Data</th>
<th>% Mortality</th>
<th>LD50 in mg/Kg</th>
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A = β-Diethylaminoethyl-5-ethoxy-Salicylate Hydrochloride.
B = β-Dimethylaminoethyl-Gentisate Hydrochloride.
C = 3-(l-methylpiperidyl)-methyl-Gentisate Hydrochloride
D = β-n-Butylaminoethyl-Gentisate Hydrochloride
MORTALITY CURVES - 1

- β-ethylaminomethyl gentisate 801
- 3-(1-Methylpiperidyl)-methyl gentisate 801
- α-dimethylaminoethyl gentisate 801
- β-dimethylaminoethyl-α-ethoxymethylate 801

% Mortality

Dose in mg/kg.

Figure 12
### TABLE IX

The Toxicity of Esters of Gentisic Acid with Other Carboxylic Acids and Gentisic Acid as Their Sodium Salts by Intraperitoneal Injection

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<th>Compound</th>
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<th>Dose mg/Kg of the acid</th>
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<th>Dead</th>
<th>Alive</th>
<th>Dead</th>
<th>Total</th>
<th>Mortality</th>
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<td>100</td>
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<tr>
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<td>2000</td>
<td>12</td>
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<td>36</td>
<td>0</td>
<td>36</td>
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<td>0</td>
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<td>2500</td>
<td>11</td>
<td>1</td>
<td>24</td>
<td>1</td>
<td>25</td>
<td>4</td>
<td></td>
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<tr>
<td></td>
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<td>3000</td>
<td>8</td>
<td>4</td>
<td>13</td>
<td>5</td>
<td>18</td>
<td>23</td>
<td></td>
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<td>5</td>
<td>7</td>
<td>5</td>
<td>12</td>
<td>17</td>
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<td></td>
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<tr>
<td></td>
<td>12</td>
<td>4000</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>24</td>
<td>24</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

**E** = 5-Diphenylacetyl-Gentisic Acid  
**F** = 5-Diethylacetyl-Gentisic Acid  
**G** = 5,5'-Digentisyl-Phthalate  
**H** = Gentisic Acid
$\text{Figure 1}$
other carboxylic acids, being approximately twice as toxic. Nash et al (29) found that their amino alcohol esters were in general approximately one and half times as toxic as the esters with other carboxylic acids, even though the latter were given as the insoluble form.

This research, in which the gentisic acid esters with other carboxylic acids were given as a soluble salt shows clearly that this relationship in toxicity is not a function of solubility but is actually a function of structure.

The most toxic compound was found to be an amino alcohol ester of gentisic acid in which the amino group was secondary. Table VIII shows the supposed \( \text{-n-butylamino-ethyl gentisate hydrochloride} \) with an \( \text{LD}_{50} \) of 193 mg./Kg. to be the most toxic of all the esters prepared. The least toxic compounds were 5,5'-digentisyl phthalate and 5-diethylacetyl gentisic acid as their sodium salts with an \( \text{LD}_{50} \) of 650 mg/Kg. each.

The least toxic of the esters with amino alcohols were \( \text{-diethylaminoethyl-5-ethoxy salicylate hydrochloride} \) and \( \text{3-(1-methylpiperidyl)-methyl gentisate hydrochloride} \) with \( \text{LD}_{50}'s \) of 289 mg./Kg. and 265 mg./Kg. respectively (Table IX). The 4 mg. difference between the two is insignificant at such a high dose level. The most toxic of the gentisic acid esters with other carboxylic acids was 5-diphenylacetyl gentisic acid as its sodium salt with an \( \text{LD}_{50} \) of 520 mg./Kg.
There was a distinct difference in the manner in which death was produced between the two types of esters. All of the esters with amino alcohols in which the amino group was tertiary caused severe clonic convulsions before death. Those animals which received a dose that was sub-lethal to them, but was near the LD$_{50}$, had clonic convulsions lasting upwards to one hour. The compound supposed to be an ester in which the amino group was secondary did not cause convulsions and the animal died of depression. It was not determined whether death was due to cardiac or respiratory depression. The esters with other carboxylic acids as their sodium salts caused death by depression. Nash et al (29) found that this type of ester caused death preceded by convulsions when injected intravenously.

All of the new derivatives of gentisic acid have a greater toxicity than gentisic acid when given in a soluble form by intraperitoneal injection in mice. It is observed that there was a considerable difference in the toxicity of gentisic acid derivatives according to the method of administration; the derivatives of gentisic acid are more toxic when given intravenously than when given intraperitoneally. This relationship has long been established (30). It is also true that oral administration exhibits the lowest toxicity for a compound. Therefore, the therapeutic index (LD$_{50}$/minimum effective dose) of these new derivatives may
be better than these results would indicate. This would be especially true if any of these new derivatives would have an activity greater than gentisic acid.

It was noted further that at least one and in most cases more than one of the survivors that were given the sodium salts, including gentisic acid itself, showed signs of abscess formation at the site of infection. This is undoubtedly due to the alkalinity of the salt when in solution. Table X presents the observed pH at the equivalence point for these compounds.

**Antispasmodic Activity**

Synthetic organic compounds which contain the general type structure of $R'_1 - O - R'_2 - N - R'_3$ have found extensive therapeutic use. The kind of pharmacological response to these compounds is very greatly influenced by the $R'_1$ group. In most cases, the degree of pharmacological response is influenced by groups $R'_2$, $R'_3$ and $R'_4$.

For example, some of the antihistaminics are tertiary amino ethers, such as Bendaryl in which $R'_1$ is a diphenyl methyl radical, $R'_2$ is an ethylene radical and $R'_3$ and $R'_4$ are methyl radicals. In addition, there is a class of compounds which are used as antispasmodics, such as Trasentin, in which $R'_1-O-$ is a diphenyl acetyl radical, $R'_2$ is an ethylene
<table>
<thead>
<tr>
<th>Compound</th>
<th>Wt. of Sample</th>
<th>pH of Neutralized Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.0296 g.</td>
<td>9.45</td>
</tr>
<tr>
<td>B</td>
<td>0.0526 g.</td>
<td>8.38</td>
</tr>
<tr>
<td>C</td>
<td>0.0532 g.</td>
<td>8.37</td>
</tr>
<tr>
<td>D</td>
<td>0.0389 g.</td>
<td>8.38</td>
</tr>
</tbody>
</table>

A = 5-Diphenylacetyl Gentisic Acid  
B = 5-Diethylacetyl Gentisic Acid  
C = 5,5'-Digentisyl Phthalate  
D = Gentisic Acid
radical and $R^3$ and $R^4$ are ethyl radicals.

Tertiary amines of this general type structure have well established pharmacologic activity. Since the four derivatives of gentisic acid with amino alcohols prepared in this research contain the general type structure as found in Trasentin, they were screened for antispasmodic activity. The screening was performed upon the isolated intestinal strip of a rabbit which had been starved for 48 hours.

The procedure followed was that of the standard method of testing drugs upon an isolated muscle strip (31). The tissue was maintained in a constant temperature bath at 38.0–38.5°C in a medium of freshly prepared Locke-Ringer solution through which air was bubbled at a rate of one bubble per second.

The four compounds tested were dissolved in sufficient distilled water so that when 1 ml. of test solution was diluted to 100 ml., the concentration was equal to 1 part per 200,000 parts. The four compounds were: $\alpha$-diethylaminoethyl-5-ethoxy salicylate hydrochloride, designated as A; 3-(1-methylpiperidyl)-methyl gentisate hydrochloride, designated as B; $\alpha$-dimethylaminoethyl gentisate hydrochloride, designated as C; $\alpha$-n-butylaminoethyl gentisate hydrochloride, designated as D.

The standard antispasmodic chosen was Papaverine
Hydrochloride (Merck) which was used in the same concentration as the recently prepared derivatives of gentisic acid. The standard was designated as X.

A, B, C and D were tested in comparison to X during 4.5 hours. The intestinal strip was still responding normally to X at the end of the period.

The manner of testing was as follows: after the intestinal strip was allowed to attain a normal state as indicated by a uniform excursion upon the kymograph, 1 ml. of the test solution was added to 99 ml. of the bath solution. Contact with the tissue was permitted for three minutes. At the end of that time interval, the bath solution was replaced by fresh Locke-Ringer solution. After another three minute interval, it was removed and another fresh portion of Locke-Ringer solution was added. The washing process was designated as Y. The tissue was then allowed to attain a normal state again. For comparison, X was applied to the tissue for the same length of time and removed in the same manner.

In this concentration (1:200,000) very little or no anticonvulsant response was seen with A, B, C, D or X. Unna (32) however, has reported anticonvulsant response to Papaverine Hydrochloride in this concentration. It was determined that a concentration of 1:100,000 (2 ml. of test solution + 98 ml. of bath solution) for Papaverine Hydrochloride
served as a satisfactory concentration for the decrease in amplitude of the intestinal contractions of a rabbit.

The kymograph tracings of the effect of A, B, C, D and X are given in Figures 14 to 17.

Figure 14, which compares \(\beta\)-diethylaminoethyl-5-ethoxy salicylate hydrochloride with Papervine Hydrochloride shows quite clearly that in a 1:100,000 concentration, this derivative of gentisic acid decreased the amplitude of the intestinal contractions. The degree of relaxation, however, was not as strong as the 1:100,000 concentration of Papaverine hydrochloride.

Figure 15, which compares 3-(1-methylpiperidyl)-methyl gentisate hydrochloride with the standard shows that in a 1:100,000 concentration (B) no decrease in amplitude was seen. In a concentration of 1:75,000 (B-3) a slight and fleeting decrease in amplitude was observed. In a concentration of 1:50,000 (B-4), the reduction in amplitude was still slight and fleeting. Therefore, Papaverine Hydrochloride is at least four times as active as an antispasmodic, as this derivative of gentisic acid.

Figures 16 and 17 indicate that in concentrations of 1:100,000 (C) and 1:50,000 (C-4 and D-4) \(\beta\)-dimethylaminoethyl gentisate hydrochloride and \(\beta\)-n-butylaminoethyl gentisate hydrochloride had no effect upon the reduction of the amplitude of the intestinal contraction of a rabbit. These figures also indicate that the intestinal strip was still
The Antispasmodic Activity of $\alpha$-Diethylaminoethyl-5-ethoxy Salicylate Hydrochloride

Figure 14
The Antispasmodic Activity of 3- (1- Methylpiperidyl) - Methyl Gentisate Hydrochloride

Figure 15
The Antispasmodic Activity of \( \beta \)-Dimethylaminoethyl Gentisate Hydrochloride

Figure 16
The Antispasmodic Activity of $\beta$-n-Butylaminoethyl Gentisate Hydrochloride

Figure 17
responding normally and quite well to a 1:100,000 concentration of papaverine hydrochloride.

Local Anesthetic Activity

The same chemical type structure found in compounds possessing antispasmodic activity is present in compounds that are effective as local anesthetic agents, such as Procaine. Procaine is the β-diethylaminoethyl ester of p-amino-benzoic acid. Its type structure is \( R' - \alpha - R^2 - N \). The carboxylic acid esters of gentisic acid with amino alcohols fit this general type structure. In local anesthetics, the \( R' - O \)-group is a carboxyl group; in antispasmodics like Trasentin the \( R' - O \)-group is likewise a carboxyl group. Of the amino alcohol esters of gentisic acid prepared, β-diethylaminoethyl-5-ethoxy salicylate hydrochloride most closely resembles Procaine Hydrochloride and Trasentin Hydrochloride. Their formulae are:

- **Procaine • HCl**
  \[
  (\text{C}_2\text{H}_5)_2\text{N}-\text{CH}_2\text{CH}_2\text{O}-\text{C}-\text{N}\text{H}_2 \cdot \text{HCl}
  \]

- **Trasentin • HCl**
  \[
  (\text{C}_2\text{H}_5)_2\text{N}-\text{CH}_2\text{CH}_2\text{O}-\text{C}-\text{CH} \cdot \text{HCl}
  \]

- **B-Diethylaminomethyl-5-ethoxy salicylate • HCl**
  \[
  (\text{C}_2\text{H}_5)_2\text{NCH}_2\text{CH}_2\text{O}-\text{C}-\text{C}_2\text{H}_5 \cdot \text{HCl}
  \]

- **Salicylate • HCl**
  \[
  (\text{C}_2\text{H}_5)_2\text{N}-\text{CH}_2\text{CH}_2\text{O}-\text{C}-\text{HO} \cdot \text{HCl}
  \]
The four derivatives of gentisic acid with amino alcohols were tested for local anesthetic effect upon the isolated sciatic nerve of a frog. The procedure used was as follows:

Summer frogs were stored at 5° C in 1/2 inch of water containing 0.15% sodium chloride and a trace amount of potassium permanganate. Before testing, the frogs were given a decerebration pithing and allowed to recover during 15 minutes. The frog was then pinned to a frog board and the sciatic nerve was isolated from the thigh. The sciatic nerve was then placed in a paraffin block which contained a well for the introduction of the solution to be tested.

Preliminary testing indicated that the isolation of the nerve and its insertion into the paraffin block caused sufficient trauma that response to sensory stimuli was erratic or absent. The minimum threshold to electric shock to cause flexing of the leg was determined for each leg. In those cases in which the threshold stimulus was less than 0.05 volt, a stimulus of 0.05 V was used. In those cases in which the threshold stimulus was more than 0.05 volt, the threshold stimulus was increased by 0.1 volt.

A 1 per cent solution of Procaine Hydrochloride (Mallinkrodt) was chosen as the standard local anesthetic. The compounds to be tested were used in the same concentration.
After the proper voltage for motor stimulation had been chosen, 5 drops of the test solution was placed into the paraffin well, through which passed the sciatic nerve. The end-point was chosen to be the lack of response to the determined electrical stimulus when the stimulus was applied on the spinal side of the block. The results were reported in terms of the time in minutes required for each compound tested to produce blocking of motor response to the determined electrical stimulus. Both legs of the same frog were used and the data are result of the testing of 6 frogs, or 12 legs.

After anesthesia had been induced, the drug was removed and the ability of the sciatic nerve to recover was determined. If recovery did not occur within 1 1/2 hours, the induced anesthesia was assumed to be irreversible.

The results of these screening tests, which have been evaluated by the method of the "sum of the squares" (33), are presented in Table XI.

Table XI indicates that of the four esters of gentisic acid with amino alcohols, the most active for local anesthetic effect as tested by this method is the compound most closely related to Procaine. The effect of \( \beta \)-diethylaminoethyl-5-ethoxy salicylate hydrochloride (B) is, however, not as great as Procaine Hydrochloride (A) in equal concentrations. The other three esters possess a very slight
### TABLE XI

The Local Anesthetic Effect of the Esters of Gentisic Acid with Amino Alcohols Compared to Procaine Hydrochloride

<table>
<thead>
<tr>
<th>Compound</th>
<th>Time for Anesthesia in Minutes</th>
<th>Standard Deviation in minutes</th>
<th>Fiducial Limits 0.95 Probability Reversibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.1</td>
<td>1.9</td>
<td>3.2 - 11.0 Reversible</td>
</tr>
<tr>
<td>B</td>
<td>9.9</td>
<td>1.7</td>
<td>6.1 - 13.7 Reversible</td>
</tr>
<tr>
<td>C</td>
<td>26.3</td>
<td>6.4</td>
<td>12.2 - 40.4 Reversible</td>
</tr>
<tr>
<td>D</td>
<td>18.5</td>
<td>3.1</td>
<td>11.7 - 25.3 Not reversible</td>
</tr>
<tr>
<td>E</td>
<td>23.2</td>
<td>6.2</td>
<td>9.5 - 36.9 reversible</td>
</tr>
</tbody>
</table>

A = Procaine Hydrochloride

B = \( \beta \)-Diethylaminoethyl-5-ethoxy Salicylate Hydrochloride

C = 3-(1-methylpiperidyl)-methyl Gentisate Hydrochloride

D = \( \beta \)-Dimethylaminoethyl Gentisate Hydrochloride

E = \( \beta \)-n-Butylaminoethyl Gentisate Hydrochloride
anesthetic potency that is somewhat inconsistent as shown by the spread of the fiducial limits of C, D, and E.

$\beta$-Dimethylaminoethyl gentisate hydrochloride (D) is apparently neurotoxic, for the nerve did not recover normal response within 1 1/2 hours.

Anticonvulsant Activity

When the recently prepared derivatives of gentisic acid were tested for acute toxicity, it was found that the esters of gentisic acid with other carboxylic acids, as their sodium salts, and $\beta$-n-butylaminoethyl gentisate hydrochloride did not cause convulsions preceding death. It was therefore assumed that death was due to depression of some sort. If death was due to central nervous system depression, these four esters of gentisic acid might possibly possess anticonvulsant activity. The four esters of gentisic acid that did not cause convulsions were screened for anticonvulsant activity according to the following procedure:

a. White Swiss mice were chosen as the test animals. Mice weighing 15 g. - 25 g. were used. The mice were obtained from the same source of supply and were observed for 24 hours prior to use. They were housed in standard cages at the prevailing conditions of humidity and temperature. They were fed an unlimited amount of Purina Dog Checkers
and water.

b. The mice were starved for 24 hours before testing to standardize body weight. Their water supply was unrationed.

c. The esters of gentisic acid with other carboxylic acids were solubilized as their sodium salts, as described in the section on acute toxicity. \( \beta \)-n-butylaminoethyl gentisate was soluble as the hydrochloride salt.

d. The dosages of the esters of gentisic acid were chosen to be the LD\(_0\) as determined in the section on acute toxicity. This dose will be referred to as the "protective dose." The protective dosages for the four esters of gentisic acid were:

1. 400 mg/Kg of 5,5'-digentisyl phthalate as the disodium salt.

2. 400 mg/Kg of 5-diphenylacetyl gentisic acid as the sodium salt.

3. 350 mg/Kg of 5-diethylacetyl gentisic acid as the sodium salt.

4. 130 mg/Kg of \( \beta \)-n-butylaminoethyl gentisate hydrochloride.

e. Metrazol (Bilhuber Knoll Corp.) was chosen as the convulsant. The LD\(_{99}\) has been reported to be 120 mg/Kg in mice (34). To check the validity of this reported lethal dose, a preliminary testing was carried out using 6 mice.
per dose. To 6 starved mice were given 110 mg/kg, 115/Kg, and 120 mg/kg of Metrazol subcutaneously. The fatalities were as follows: 2, 5 and 6 mice. Therefore, the dose of 120 mg/Kg. of Metrazol was chosen to be the dose against which the esters of gentisic acid should exert their protective action, if any.

f. The protective dose of each of the four esters of gentisic acid was given by intraperitoneal injection to 12 mice. At the end of 10 minutes and 20 minutes, 6 mice received the LD$_{50}$ of Metrazol by subcutaneous injection. Therefore, for each ester of gentisic acid, 12 mice received the protective dose of the ester and the LD$_{50}$ dose of Metrazol.

The results of the screening test for anticonvulsant activity are presented in Table XII.

Table XII shows clearly that the protective dose of the selected esters of gentisic acid exerted no protective action against death induced by Metrazol. All the mice that died, died following the typical "question-mark" convulsion which was induced by Metrazol.
TABLE XII
The Protective Action of Selected Esters of Gentisic Acid Against a Toxic Dose of Metrazol

<table>
<thead>
<tr>
<th>Compound</th>
<th>Protective Dose</th>
<th>No. of mice alive 1 hr. following 10 min. interval before ID₉₉ of Metrazol</th>
<th>No. of mice alive 1 hr. following 20 min. interval before ID₉₉ of Metrazol</th>
</tr>
</thead>
<tbody>
<tr>
<td>5,5'-Digentisyl Phthalate</td>
<td>400 mg/Kg</td>
<td>0 out of 6</td>
<td>0 out of 6</td>
</tr>
<tr>
<td>5-Diphenylacetyl Gentisic Acid</td>
<td>400 mg/Kg</td>
<td>0 out of 6</td>
<td>0 out of 6</td>
</tr>
<tr>
<td>5-Diethylacetyl Gentisic Acid</td>
<td>350 mg/Kg</td>
<td>0 out of 6</td>
<td>0 out of 6</td>
</tr>
<tr>
<td>β-n-Butylamino-ethyl Gentisate Hydrochloride</td>
<td>130 mg/Kg</td>
<td>0 out of 6</td>
<td>0 out of 6</td>
</tr>
</tbody>
</table>

* As the Sodium Salt
SUMMARY

Seven new derivatives of gentisic acid were prepared, their physical properties determined, and their proposed structures verified. In addition, their pharmacological effects were determined in four selected pharmacological screening tests.

Four of the derivatives were esters of gentisic acid with amino alcohols and three were esters of gentisic acid with other carboxylic acids. The seven new derivatives were prepared by three procedures according to the following general type-reactions:

\[
\begin{align*}
a. & \quad R' - \text{OH} + \text{Cl-C-R}^2 \rightarrow R' - \text{O-C-R}^2 + \text{HCl} \\
b. & \quad R^3 - \text{C-OH} + \text{Cl-R}^4 \rightarrow R^3 - \text{C-O-R}^4 + \text{HCl} \\
c. & \quad R^3 - \text{C-OH} + \text{HO-}R^5 \rightarrow \text{HCl} + R^3 - \text{C-O-R}^5 + \text{HCl}
\end{align*}
\]

in which \( R' = 1\text{-carboxy, 2-hydroxy phenyl residue, } R^2 = \text{an aromatic or aliphatic residue, } R^3 = 2,5\text{-dihydroxy phenyl residue, } R^4 = \text{a dialkylamino alkyl residue and } R^5 = \text{a tertiary or secondary amino residue.}

Type-reaction "a" produced 5,5'-digentisyl phthalate, 5-diphenylacetyl gentisic acid and 5-diethylacetyl gentisic acid. Type-reaction "b" produced \( \beta\)-diethylaminoethyl-5-ethoxy salicylate hydrochloride. Type-reaction "c" yielded 3-(1-methylpiperidyl)-methyl gentisate hydrochloride.
\( \beta \)-dimethylaminoethyl gentisate hydrochloride and the compound believed to be \( \beta \)-n-butylaminoethyl gentisate hydrochloride.

The physical properties of the seven recently prepared derivatives of gentisic acid were determined. These properties are summarized in Table XIII.

The proof of structure of the recently prepared esters of gentisic acid was based upon four qualitative tests and three sets of quantitative analytical data.

The first qualitative test was the production of a characteristic bluish-purple color by the reaction of each of these new compounds with aqueous ferric chloride solution. This color is characteristic of ortho hydroxy-carboxylic acids. Metahydroxy-carboxylic acids do not produce this color. Therefore, since all of the new derivatives of gentisic acid produced this color, esterification of the metahydroxyl group occurred and esterification of the ortho hydroxyl group did not occur.

The second and third qualitative tests were performed upon the gentisic acid esters with amino alcohols to insure the presence of halogen in the compound. A white precipitate was produced by each compound when its aqueous solution was mixed with aqueous silver nitrate solution. Each compound imported a green color to a non-luminous flame when in contact with metallic copper.
The results of these three qualitative tests are summarized in Table XIII.

The fourth qualitative test was the examination of the infrared absorption spectrograph of each of the new derivatives and the comparison of each to the infrared absorption spectrograph of gentisic acid. It was found that gentisic acid and its derivatives possess a characteristic absorption triplet absorbing in the region of 5.9 μ, 6.1 μ and 6.3 μ whenever the spectrograph is obtained with Nujol as the suspending solvent. In addition, it was observed that each compound possessed a phenyl ring, that each ester with an amino alcohol possessed one carbonyl function and that each ester with another carboxylic acid possessed two carbonyl functions.

One set of analytical data was the neutralization equivalent for each of the new derivatives. Each derivative prepared was acidic in nature; the esters of gentisic acid with amino alcohols contained hydrogen chloride; the esters of gentisic acid with other carboxylic acids contained one or two free carboxyl groups. These acidic functions were titrated potentiometrically in hydroalcoholic solvents with approximately 0.01 N sodium hydroxide solutions. It was shown that all of these derivatives but one, 5,5′-digentisyl phthalate, were monobasic, 5,5′-digentisyl phthalate however, functioned as a monobasic acid even though
<table>
<thead>
<tr>
<th>R'</th>
<th>R</th>
<th>M.P. °C</th>
<th>Ferric Chloride Test</th>
<th>Silver Nitrate Test</th>
<th>Beilstein Test</th>
<th>W. A. l, E. A c. Me. P. E. I. S. B.</th>
</tr>
</thead>
<tbody>
<tr>
<td>C_{16}H_{9}O_{6}^-</td>
<td>-H</td>
<td>252-253</td>
<td>+^a</td>
<td>-^b</td>
<td>-^c</td>
<td>S, S^e</td>
</tr>
<tr>
<td>(C_{6}H_{5})_{2}CHCO</td>
<td>-H</td>
<td>200.5-201.5</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>I</td>
</tr>
<tr>
<td>(C_{2}H_{5})_{2}CHCO</td>
<td>-H</td>
<td>111.2-113.2</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>I</td>
</tr>
<tr>
<td>C_{2}H_{5}^-</td>
<td>-C_{2}H_{4}N(C_{2}H_{5})_{2}</td>
<td>HCl 172.5-174.5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>S</td>
</tr>
<tr>
<td>H-</td>
<td>CH_{2}</td>
<td>N</td>
<td>CH_{3}</td>
<td>HCl 223-231</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>H-</td>
<td>-C_{2}H_{4}N(CH_{3})_{2}</td>
<td>HCl 133-199</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>S</td>
</tr>
<tr>
<td>H-</td>
<td>-C_{2}H_{4}NHCO_{2}H</td>
<td>HCl 196-202</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>S</td>
</tr>
</tbody>
</table>

1. W-water, A.l.-ethanol, E.-diethyl ether, A.c.-acetone, Me.-methanol, P.E.-petroleum ether, IS,l.-isobutanol
2. C_{15}H_{9}O_{6}^- represents a gentisyl phthaloyl residue
a. positive test
b. value not determined
c. insoluble
d. soluble
e. slightly soluble
two carboxyl groups were present. This behavior was shown to be due to the fact that the $pK_a$ values of the two carboxyl groups of 5,5'-digentisyl phthalate differ by a factor of 2 or less.

The second and third sets of analytical data were the elemental analyses for carbon and hydrogen content. The values found experimentally were compared to the calculated values.

The findings of the quantitative analytical tests are summarized in Table XIV.

The formula $\beta$-n-butylaminoethyl gentisate hydrochloride is in doubt due to the discrepancies in its neutralization equivalent and in its hydrogen content. These discrepancies are believed to be due only to impurities present because of the positive ferric chloride test and because of the presence of the characteristic infrared absorption triplet in its spectrograph.

The activity of the seven recently prepared derivatives of gentisic acid was determined in four selected pharmacological screening tests. The compounds were screened for acute toxicity, antispasmodic activity, local anesthetic activity and anticonvulsant activity. The results of these four screening procedures are summarized in Table XV.

All of the new esters of gentisic were more toxic when

* Clark Microanalytical Laboratory, Urbana, Ill.
### TABLE XIV

**ANALYTICAL DATA FOR THE RECENTLY PREPARED DERIVATIVES OF GENTISIC ACID**

<table>
<thead>
<tr>
<th>R'</th>
<th>R</th>
<th>Calculated Carbon Content</th>
<th>Experimental Carbon Content</th>
<th>Calculated Hydrogen Content</th>
<th>Hydrogen Content</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C_{15}H_{9}O_{6}</td>
<td>-H</td>
<td>219</td>
<td>224</td>
<td>60.23</td>
<td>3.22</td>
</tr>
<tr>
<td>(C_{6}H_{5})_{2}CHCO</td>
<td>-H</td>
<td>348</td>
<td>347</td>
<td>72.41</td>
<td>4.63</td>
</tr>
<tr>
<td>(C_{2}H_{5})_{2}CHCO</td>
<td>-H</td>
<td>252</td>
<td>254</td>
<td>61.99</td>
<td>6.39</td>
</tr>
<tr>
<td>C_{2}H_{5}</td>
<td>-C_{2}H_{4}N(C_{2}H_{5})_{2}·HCl</td>
<td>318</td>
<td>319</td>
<td>56.54</td>
<td>7.60</td>
</tr>
<tr>
<td>H-</td>
<td>-CH_{2}NCH_{3}·HCl</td>
<td>302</td>
<td>301</td>
<td>55.72</td>
<td>6.68</td>
</tr>
<tr>
<td>H-</td>
<td>-C_{2}H_{4}N(CH_{3})_{2}·HCl</td>
<td>262</td>
<td>269</td>
<td>50.48</td>
<td>6.16</td>
</tr>
<tr>
<td>H-</td>
<td>-C_{2}H_{4}NHC_{4}H_{9}·HCl</td>
<td>290</td>
<td>#</td>
<td>54.38</td>
<td>6.96</td>
</tr>
</tbody>
</table>

* C_{15}H_{9}O_{6} - represents a gentisyl phthaloyl residue

# See Table
TABLE XV

The Pharmacological Effects of the Recently Prepared Esters of Gentisic Acid in Four Selected Pharmacological Screening Procedures

<table>
<thead>
<tr>
<th>Compound</th>
<th>$LD_{50}$ in mg/kg</th>
<th>Antispasmodic Effect</th>
<th>Local Anesthetic Effect</th>
<th>Anticonvulsant Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>650</td>
<td>not tested</td>
<td>not tested</td>
<td>none</td>
</tr>
<tr>
<td>B</td>
<td>520</td>
<td>not tested</td>
<td>not tested</td>
<td>none</td>
</tr>
<tr>
<td>C</td>
<td>650</td>
<td>not tested</td>
<td>not tested</td>
<td>none</td>
</tr>
<tr>
<td>D</td>
<td>239</td>
<td>some</td>
<td>some</td>
<td>not tested</td>
</tr>
<tr>
<td>E</td>
<td>235</td>
<td>slight</td>
<td>very slight</td>
<td>not tested</td>
</tr>
<tr>
<td>F</td>
<td>305</td>
<td>none</td>
<td>very slight</td>
<td>not tested</td>
</tr>
<tr>
<td>G</td>
<td>193</td>
<td>none</td>
<td>very slight</td>
<td>none</td>
</tr>
</tbody>
</table>

A = 5,5'-Digentisyl Phthalate  
B = 5-Diphenylacetyl Gentisic Acid  
C = 5-Diethylacetyl Gentisic Acid  
D = 'Diethylaminomethyl-5-ethoxy Salicylate Hydrochloride  
E = 3-(1-methyl piperidyl)-methyl Gentisate Hydrochloride  
F = 1-Dimethylaminomethyl Gentisate Hydrochloride  
G = 1-n-Butylaminomethyl Gentisate Hydrochloride  
1 = as the sodium salt  
2 = isolated rabbit intestine, compared to Papaverine Hydrochloride  
3 = isolated frog sciatic nerve, compared to Procaine Hydrochloride  
4 = protection of mice against Metrazole induced death.
given intraperitoneally into mice than the parent, gentisic acid. In addition, they were all relatively less toxic by this route of administration than related derivatives given intravenously.

Of the four esters of gentisic acid with amino alcohols, only β-diethylaminoethyl-5-ethoxy salicylate hydrochloride possessed any appreciable antispasmodic or local anesthetic activity under the conditions of the described screening procedure.

The three esters of gentisic acid with other carboxylic acids and β-n-butyraminoethyl gentisate hydrochloride did not possess any antispasmodic activity under the conditions of the described screening procedure.
CONCLUSIONS

I. Seven new derivatives of gentisic acid were prepared, three of which were esters with other carboxylic acids and four were esters with amino alcohols.

A. Esters with other carboxylic acids.

1. The esters with other carboxylic acids were prepared in relatively low yields by the interaction of organic acid chlorides with gentisic acid, in the presence of anhydrous zinc chloride.

2. The physical properties were determined.

3. Qualitative and quantitative tests were performed to prove the structures of these esters.

B. Esters with amino alcohols.

1. The esters with amino alcohols were prepared in varying yields by the interaction of an amino alkyl chloride and 5-ethoxy salicylic acid and by the interaction of an amino alcohol and gentisic acid in a dehydrating medium.

2. The physical properties were determined.

3. Qualitative and quantitative tests were performed to prove the structures of these esters.

4. There is doubt as to structure of β-n-butylamino-ethyl gentisate hydrochloride due to the fact that its neutralization equivalent was not con-
stant plus the fact that its hydrogen content was approximately 16 per cent higher than the calculated value.

II Seven new derivatives of gentisic acid were subjected to four selected pharmacological screening procedures.

A. Acute Toxicity as determined by intraperitoneal injection in mice.

1. Esters with other carboxylic acids, as their sodium salts. The most toxic of these esters was 5-diphenylacetyl gentisic acid with an LD₅₀ of 520 mg/Kg expressed as the free acid. The least toxic of these esters were 5-diethylacetyl gentisic acid and 5,5'-digentisyl phthalate with an LD₅₀ of 650 mg/Kg each, expressed as the free acid.

2. Esters with amino alcohols.

The most toxic of these esters was the supposed 3-n-butylaminooethyl gentisate hydrochloride with an LD₅₀ of 193 mg/Kg. The least toxic of these esters was 3-dimethylaminooethyl gentisate hydrochloride with an LD₅₀ of 305 mg/Kg. These esters are most toxic than the esters of gentisic acid with other carboxylic acids.

B. Antispasmodic activity, as determined upon the isolated intestinal strip of a rabbit. Of the four esters of gentisic acid with amino alcohols, only 3-diethylaminooethyl-5-ethoxy salicylate
hydrochloride is worthy of further investigation as an antispasmodic agent.

C. Local Anesthetic activity, as determined upon the isolated sciatic nerve of a rabbit. None of the four esters of gentisic acid with amino alcohols will induce a blocking of motor response to electrical stimulus faster than an equivalent concentration of Procaine Hydrochloride. Since \( \beta \)-diethylaminoethyl-5-ethoxy salicylate hydrochloride induced anesthesia only slightly slower than Procaine, it may possess other properties that make it of value as a local anesthetic. These other properties should be investigated.

D. Anticonvulsant activity, as shown by the protection of mice against death produced by Metrazol. Neither the three esters of gentisic acid with other carboxylic acids nor \( \beta \)-n-butylaminoethyl gentisate hydrochloride possessed sufficient antispasmodic activity to protect mice from death produced by a lethal dose of Metrazol.
BIBLIOGRAPHY

5. Ory, M., Bruxelles-med., 29, 1401 (1949)
14. Daniels, F., Outlines of Physical Chemistry, 8th Revision, John Wiley and Sons, New York, 1948, pp. 593-6


24. Horenstein, H. and Pahliche, M., Ber. 71, 1644 (1933).


32. Unna, K., J. Pharmacol. Exptl. Thera., 70, 179 (1940)


I, Willis E. Moore was born in Plain City, Ohio, December 28, 1924. I received my secondary school education in the public schools of the cities of Plain City and Newark, Ohio. My undergraduate training was obtained at The Ohio State University, from which I received the degree Bachelor of Science, summa cum laude, in Pharmacy in 1949. While in residence at The Ohio State University, I acted as a graduate assistant during the year 1949-1950. In 1950 I received an appointment as a Fellow of the American Foundation for Pharmaceutical Education. I held this position for three years while completing the requirements for the degree Doctor of Philosophy.