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3-amino-2,5-dihydroxy-5-sulfo-benzoic acid

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3-AMINO-2,5-DIHYDROXY-5-SULFO-BENZOIC ACID

A THESIS

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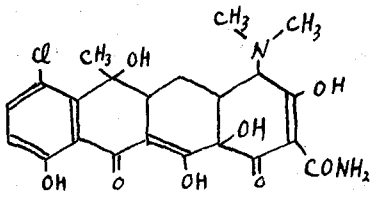
CHAPTER I

INTRODUCTION

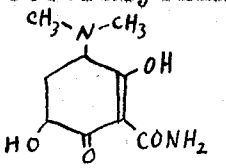
It seems possible that efforts to discover essential chemical differences which might exist between normal and malignant cells will eventually reveal the secret of cancer and thereby lead to new approaches to safer and more selective chemotherapy.

From this standpoint, one may consider the observation in 1957 by Rall et al (1),(2) that after injection of tetracycline the compound appeared in tumor tissue, and persisted in it for as long as twenty one days following injection, while it could not be detected in normal tissue after twenty four hours.

One objective of this study is to find that part of this molecule that causes it to concentrate in the tumor and thence make a smaller size sompound which has similar concentration characteristics to tumor as tetracycline. For this purpose, an attempt was made in this study to synthesize the right side ring of Chlorotetracycline, the structure of which is shown below.



Chlorotetracycline

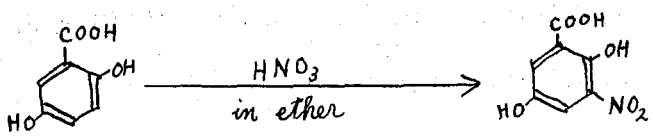


right side ring

After a comprehensive literature survey, the following synthetic procedure was devised to synthesize the right side ring compound.

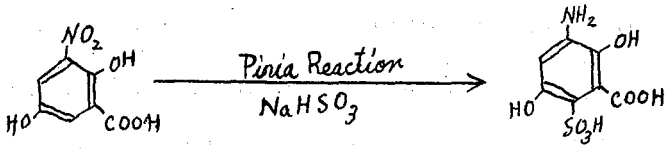
First Step

As a starting material, gentisic acid (3),(4),(5),(6) was selected, which according to the literature (7), by low temperature nitration in absolute ether may be converted to 3-nitro-gentisic acid (7),(8),(9), as is shown in the following formula.



Second Step

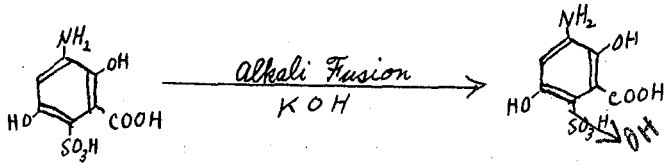
3-Nitrogentisic acid was scheduled to be reduced by the Piria reaction (10),(11),(12) as follows:



It may be well to mention here that when this process was chosen, attention was paid to the fact that an aromatic nitro compound which has a hydroxy or a carboxy group usually gives good results in the Piria reaction (11).

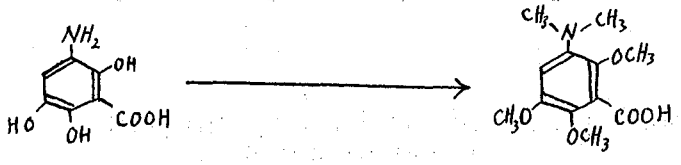
Third Step

The Sulfonic group was scheduled to be replaced by a hydroxy group by means of alkali fusion:



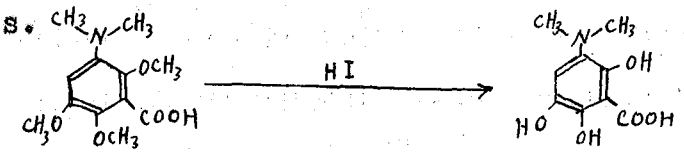
Fourth Step

Methylation by dimethyl sulfate was planned to follow:



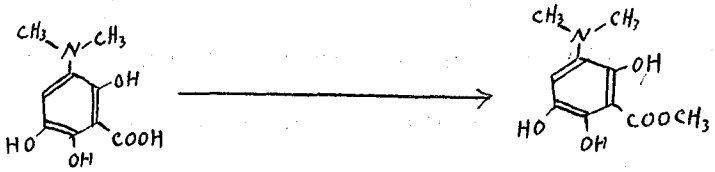
Fifth Step

The Zeisel reaction was to be used to eliminate the ether bondings.



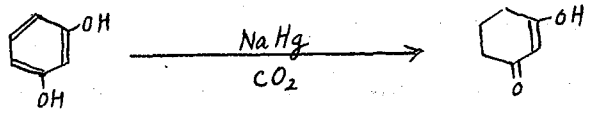
Sixth Step

The methyl or ethyl ester might then be formed as follows:

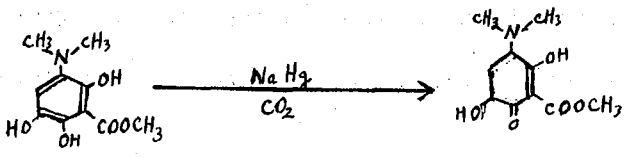


Seventh Step

According to Richter (13), resorcinol may be thus reduced.

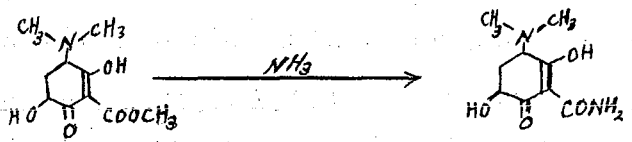


With this as precedent, the same condition was expected to give the following result:



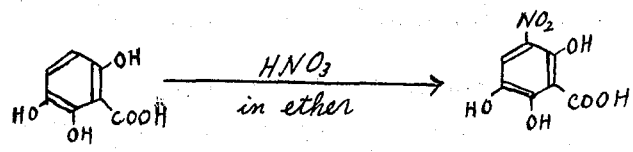
Eighth Step

As the final step, ammonolysis was planned as follows:

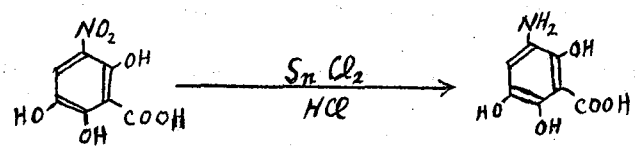


Though it was not experimentally pursued in this study, it may be worthwhile to describe here another possible way to reach the product of the third step.

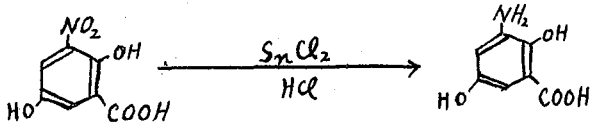
Starting from 2,3,6-trihydroxy benzoic acid (14),(15),(16),(17), we nitrate as follows:



This reaction may then be followed by a reduction reaction:



For this reduction there are literature precedents (18),(19) as is shown by the following reaction:



CHAPTER II

EXPERIMENTAL

First Step:

Synthesis of 3-Nitrogentisic acid from Gentisic acid.

This reaction is based on the procedure of Klemenc (9), however, it must be noted here that as the exact method described in the literature (9) did not give a sufficient yield of the 3-nitrogentisic acid, and so a considerably modified process, as described below, was finally employed.

2.0 grams of gentisic acid (purchased from Eastman Kodak Chemical Company, observed m.p. 196-197°C) and 25 ml. of absolute ether were placed in a 100 ml. four necked reaction flask equipped with a mechanical stirrer, 100°C thermometer, a reflux condenser fitted with a calcium chloride tube and also a 100 ml. dropping funnel similarly fitted with a drying tube. After the mixture formed a clear solution, 1.2 grams of nitric acid (sp.g. 1.42) was added dropwise with vigorous stirring at 25°C. After about 2/3 of the nitric acid was added, the temperature rose to about 30°C, then the dropping was momentarily stopped and the reaction vessle was cooled by ice water to 25°C and the addition of the acid completed under 30°C. The total addition of the nitric acid required some four minutes. After the addition of the nitric acid, the vigorous stirring was continued a further four minutes until some tar-like brown material began to adhere to the wall of the reaction

and after half a minute further stirring, the mixture was transferred to a 250 ml. separatory funnel. The separation of the ether phase from the mixture was helped greatly by locating the few small bubbles which occur between the water and ether phases.

The ether solution was transferred to a 50 ml. round bottom flask, and by washing with 40°C water followed by suction, it was dryed to a brown material admixed with a yellow component.

This powder was thoroughly triturated with 20 ml. of water while held at 0°C. This treatment was followed by suction filteration using a small Hirsh funnel, and the residue was washed twice by 0.5 ml. of cold water and dried in a vaccum desiccator.

The product was a fine yellow powder of m.p. 228.5230°C (literature (9) value:230°C). The yield was 0.57 gram, or 21.5% theoretical.

Second Step:

Synthesis of 3-Amino-2,5-dihydroxy-6-sulfo benzoic acid from 3-Nitrogentisic acid

This reaction is principally founded upon the general procedure for the Piria reactions described by Hunter (11).

1.5 grams of 3-nitrogentisic acid was placed in a 50 ml. beaker and 15.5 ml. of 2.5 M $NaHSO_3$ in aqueous solution was added and, while stirring with a glass rod, 1.14 ml. of 5 N NaOH aqueous solution was added and, also with stirring, a further addition of 9.5 ml. of distilled water followed.

The mixture was heated in a boiling water bath for one and three quarters hours, at which stage, the mixture became a transparent red solution and many small bubbles appeared; the solution was then boiled by direct flame for a further fifty minutes, and the solution became a transparent brown in color. During these heating processes care was paid to keep the volume constant by addition of distilled water. By evaporation on a water bath the solution was reduced to about half volume, at which stage a yellow precipitate appeared. By adding concentrated hydrocholric acid, the pH was brought down to 1.0 and the solution was further heated in a boiling water bath for two hours, again keeping the volume constant by adding distilled water. At this stage the yellow material changed color gradually to a greyish white powder.

After cooling this reaction mixture was filtered by suction and washed twice with 0.4 ml. of cold distilled water and dryed in a vaccum desiccator.

The product was a grayish white powder having the very high decomposition point of over 360°C. The yield was 0.4 grams or 18.5% theoretical.

The recrystallization of this compound was carried out as follows:

0.5 gram of the above compound and 12 grams of distilled water were placed in a 50 ml. round bottom flask and heated to yield a transparent yellow solution. 4 ml. of concentrated HCl solution was then added and after decolorization with active carbon, the clear solution was concentrated under suction

in a 40°C water bath until small amounts of powder-like white crystals appeared in the solution. This solution was left in the refrigerator overnight and filtered by suction followed by washing with about 0.3 ml. of cold distilled water and dried in a vaccum desiccator. Yield was 0.3 gram, 60% theoretical.

This recrystallized product is a white powder-like material whose decomposition point is over 360°C. It is very soluble in alkaline solution, fairly soluble in neutral water, and soluble (but less soluble than in neutral water) in HCl solution. It is almost insoluble in benzene, ether and acetone. An aqueous solution of this compound yields a bright blue color by the ferric chloride test. By ultra violet light, an alkaline solution of this compound shows a strong pale blue fluorescence, a neutral solution shows a pale greenish blue fluorescence but an acidic solution shows no fluorescence.

Third Step:

Alkali Fusion of 3-Amino-2,5-dihydroxy-6-sulfo benzoic acid

0.3 gram of 3-Amino-2,5-dihydroxy-6-sulfo benzoic acid was placed in a nickel crucible equipped with a lid and together with 1.8 grams of KOH and 0.15 ml. of water, it was heated in a sand bath at 260-280C for 1 hour and 25 minutes. After cooling, 10 ml.of distilled water was added to dissolve the mixture by means of a 6 per cent hydrochloric acid solution. The pH was adjusted to 7.0 and the solution filtered with suction to eliminate insoluble nickel compounds. The filtrate was then

further acidified with the hydrochloric acid solution to pH 1.0 and was left in the refrigerator overnight. The crystals which appeared were removed by suction filtration and after washing with a small amount of water, were dryed in a vaccum desiccator. Yield 0.13 gram, 54% theoretical.

By infra-red spectral analysis, this product was proven to be the same as the original 3-amino-2,5-dihydroxy-6-sulfo benzoic acid. It is clear from this result that these conditions for alkali fusion are simply not rigorous enough to accomplish the desired replacement of the sulfonyl by the hydroxyl group.

CHAPTER III

DISCUSSION

Of all the foregoing compounds, only gentisic acid and 3-nitrogentisic acid are reported in the literature. (Literature surveys have been done in Beilsteins Handbuch der Organischen Chemie Hauptwerk, Erstes Erganzungswerk and other periodicals which are not covered by these two were checked through Chemical Abstracts.) Since the expected structure of the product of the Piria reaction is not described in the literature great care must be taken in assigning the precise structure. However, beside the elementary analysis and infra-red spectral analysis, the following considerations may support the presumption that our reaction has proceeded along the classic Piria lines and also the further presumption that the structure of the product is in fact 3-amino-2,5-dihydroxy-6-sulfo benzoic acid.

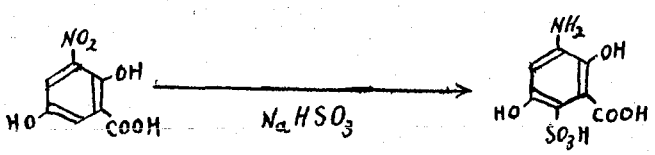
Let us compare the chemical characteristics of a related series of model compounds as in Table 1. This table presents the fact that the product of the Piria reaction is quite different from the other possible related compounds, in other words, it supports the probability of the occurrence of the expected normal Piria reaction.

Secondly, 5-nitro salicylic acid is reported (20),(21), (22) to form 5-amino-2-hydroxy-3-sulfo benzoic acid by the action of sodium bisulfite (Piria reaction) as follows:

Figure I

	Appearance of crystal	m.p.	Color Change by $FeCl_3$ soln.	Color change and fluorescence by NaOH soln.
Salicylic acid	white	157-159°C	purple	brown-yellow
Gentisic acid	white	199-200°C	blue	brown-yellow
3-Nitro gentisic acid	yellow	230°C	brown	purple (non-fluorescence)
3-Amino gentisic acid	white	204°C (decomp.)	not described in literature	not described in literature
Product in Piria Reaction	white	above 360°C (decomp.)	blue	brown (water-blue fluorescence)

In this connection, if we consider the fact that in the Piria reaction if the para-position to the nitro group is not occupied, the sulfon group has a tendency to attack there; we can then presume in this case of 3-nitro-gentisic acid that the position of the sulfon group may be the para-position as follows:



Thirdly, 5-amino-2-hydroxy-x-sulfo benzoic acid, the position of the sulfonyl group being uncertain, which is one of the most closely related compounds to the 3-amino-2,5-dihydroxy-6-sulfo benzoic acid known; it is reported (21) to have a strong green-like fluorescence in alcohol solution.

With regard to the alkali fusion reaction, this study has failed to replace the sulfonyl group with a hydroxy group, and there are indications that more severe conditions, namely higher temperatures and longer reaction times, may be necessary to achieve this reaction. On the other hand, if the replacement of this sulfonyl group continues to be found to be difficult, it may be worthwhile to use the alternative procedure referred to at the end of chapter I, to reach the 3-amino-2,5-dihydroxy-6-sulfo benzoic acid. In this case, the procedure of Thiele (14), (15), (16) in which 2,3,6-trihydroxy benzoic acid was prepared by boiling hydroxy-hydroquinone with a bicarbonate solution, through which carbon dioxide is passed, may be the easiest way to make the trihydroxy benzoic acid.

Finally, though it has no direct relation to this study, perhaps it may be permitted to suggest here that if the 2,3,6-trihydroxy-3-amino benzoic acid was prepared, we might expect anti-fever and analgesic effects in its derivatives such as 2,5,6-trihydroxy-3-acetamino benzoic acid because its structure parallels seven out of eleven previously noted anti-rheumatic chemicals such as acetanilide, p-acetylamino phenol, gamma-resorcilic acid, etc.

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