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Tetracycline-7-boronic acid : a thesis...

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TETRACYCLINE-7-BORONIC ACID

A THESIS
PRESENTED TO
THE FACULTY OF THE DEPARTMENT OF CHEMISTRY
COLLEGE OF THE PACIFIC

IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE
MASTER OF SCIENCE

by
WILLIAM DENHAM RHOADS
JUNE 1960
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INTRODUCTION

The possibility of destroying or weakening cancer cells by the general or selective absorption of neutrons by cells was first pointed out by Locher in 1939 (1). Boron or lithium atoms were suggested as the absorbing agent since they are good acceptors of neutrons. Neutron capture by either boron or lithium atoms produces high energy alpha radiation. The radiation is localized and confined to the area of boron or lithium concentration because the mean free path of the alpha particles produced is approximately equal to the diameter of a single cell.

The nuclear reactions for boron and lithium are as follows:

For boron:
$$ ^{10}\text{B} + ^{1}\text{N} \rightarrow ^{7}\text{Li} + ^{4}\text{He} + 2.5 \text{ MeV} $$

For lithium:
$$ ^{6}\text{Li} + ^{1}\text{N} \rightarrow ^{4}\text{He} + ^{1}\text{H} + 4.6 \text{ MeV} $$

Locher also pointed out that neutrons themselves cause very little damage to tissue, and thus they are an improvement over X-ray therapy in the treatment of tumors. He emphasized that X-rays cause equal damage to both healthy and cancerous tissue. Thus the upper limit of
the X-ray treatment is directly dependent upon the skin damage done.

In 1940 the first successful use of boron as a chemotherapeutic agent was reported by Kruger (2). He reported that neoplastic cells can be destroyed in vivo if sufficient boron in some suitable form can be applied to the tumor in vivo. Zahl, Cooper and Dunning (3) in 1940 also reported significant increase in tumor regression following neutron-beam exposure of transplantable mouse sarcomas injected with boron and lithium compounds. These workers pointed out at that time that in order to be effective the boron or lithium must be localized in the tumor only and that the surrounding normal tissue must be free of the materials which would absorb neutrons. At that time such a localization was improbable.

Localization of suitable neutron absorbers in cancerous tissue was begun as early as 1929 when Ludford (4), Duran-Reynals (5) and others reported that certain dyes, when injected intravenously, would concentrate to a greater degree in tumor tissue than in normal tissue. In 1941, Zahl and Cooper (6, 7) substituted lithium for sodium in various azo dyes (e.g. pontamine sky blue, trypan blue, carminic acid) and administered these compounds intravenously both into mice having spontaneous mammary tumors and mice bearing implanted tumors of sarcoma 180. Deposition of twice as much lithium in the
tumor as compared to the normal surrounding tissue resulted.

Extensive neutron-beam therapy with cancer patients was carried out in 1948 by Stone (8), who published data on neutron therapy with two hundred and fifty patients. In his studies he found that the survival rate of patients treated with fast neutrons was very discouraging. Stone pointed out that slow or thermal neutrons would be the only probable neutron therapy which could be applied to humans. By use of boron-10, which has a greater capture potential for slow neutrons, the effects of fast neutrons can be overcome.

Javid, Sweet and co-workers (9,10) in 1952 reported that boron, administered intravenously as borax solution, concentrated in many rapidly growing brain tumors to more than three times the extent that it does in normal brain tissue. Subsequent studies (11) of the concentration ratios between neoplasms and surrounding normal tissue indicated, however, that boron-neutron therapy offered little promise for treatment of malignant tumors other than those of the brain.

The use of the neutron-boron-10 reaction in the treatment of cancer was studied in 1954 by Farr, et al (12). They made their studies on ten patients with brain tumors (glioblastoma), some of whom received a total of four irradiation treatments given at five to six weeks intervals. Depressed tumor growth was noted following
eight out of twenty one capture therapy trials, questionable improvement being noted in six of the remaining thirteen trials, and no discernible change following seven of the treatments. Only one out of five patients having multiple treatments demonstrated no improvement at any time.

The search for a molecule which was preferentially absorbed by cancerous tissue and to which boron could be attached was implemented in 1957 when Hall, et al (13) reported the appearance and persistence of fluorescent material in tumor tissue following administration of the tetracycline compounds. Since the tetracycline compounds were found to be present in tumor tissue for as long as twenty one days following injection it was apparent that tetracycline had the potential of acting as the vehicle which would carry boron-10 to the cancerous tissue. Of even greater importance than this discovery was the fact that tetracycline could not be detected in normal tissue forty eight hours after the tetracycline administration. The absorption of tetracycline has been noted in carcinomas, sarcomas, and some lymphomas, but it is apparently not absorbed by melanomas.

The objective of this study is the preparation of a tetracycline-boron-10 derivative which is stable under biological conditions. The boron-10 should then provide a concentration of material capable of accepting
thermal neutrons, thus producing localized high-energy alpha radiation. The fact that the tetracyclines are not retained by normal tissue should remove the danger of destruction of this tissue.
CHAPTER II

EXPERIMENTAL

Metallic lithium will replace aromatic halogens under proper conditions (14).

\[
\text{Cl} + 2 \text{Li} \rightarrow \text{Li} + \text{LiCl}
\]

7-chlorotetracycline was selected as the most suitable starting material for the preparation of a boron-tetracycline compound through the aryl lithium intermediate.

\[
(15) + 7 \text{Li} \rightarrow \text{LiCl}
\]

\[
+ 2\frac{1}{2} \text{H}_2
\]
Aureomycin neutral base, rather than Aureomycin hydrochloride, was selected as the starting material because of the reactivity of the chloride ion with lithium.

Solubility studies of 7-chlorotetracycline showed maximum solubilities of .15 mg/ml, or less, in benzene, nitrobenzene, ethyl acetate, toluene, chloroform, cyclohexene and diethyl ether at 25°C and 14.9 mg/ml in tetrahydrofuran and dioxane at 25°C. Reddish gum-like materials, indicating chemical change, were obtained on evaporation of the dioxane solution; thus tetrahydrofuran was chosen as the most promising solvent for the study of the replacement of the halogen by lithium.

The first step in the synthesis of tetracycline-7-boronic acid was the preparation of 7-lithium tetracycline. 7.45 grams of Aureomycin, Neutral, 4115B16-1, lot number 7-9105, supplied by Lederle Laboratories Division, American Cyanamid Company, was dissolved in five hundred milliliters of anhydrous tetrahydrofuran and passed over pea sized pieces of metallic lithium in a glass reaction vessel thirty centimeters by one centimeter. The lithium metal was held in place by a constriction at the lower end of the tube and a cotton plug at the upper end of the column containing the metallic lithium. Calcium chloride tubes were built into the apparatus to prevent atmospheric moisture hydrolyzing the 7-lithium tetracycline. The solution was passed through the lithium
column by gravity flow. Adjustment of the rate of flow of the solution was made by a stopcock and adjusted to five hundred milliliters of solution per hour. A sintered glass filter was installed below the reaction vessel to remove insoluble materials, chiefly lithium chloride, formed during the reaction. A diagram of the apparatus used appears in figure 1.

One hundred milliliters of an anhydrous saturated solution of boric acid in tetrahydrofuran (12.3 mg/ml) was placed in a one liter round bottom flask fitted with a reflux condenser and protected from moisture by a calcium chloride tube. The tetrahydrofuran solution of 7-lithium tetracycline was added and the resulting solution refluxed for three hours.

\[
\text{Li} \quad \text{CH}_3 \quad \text{OLi} \quad \text{CH}_3 \quad \text{N} \quad \text{OLi} \quad \text{CH}_3 \quad \text{OLi} \quad \text{C} = \text{O} \quad \text{NH}_2
\]

\[+ \text{H}_3\text{BO}_3 \rightarrow\]

\[
\text{HO} \quad \text{B} \quad \text{CH}_3 \quad \text{OLi} \quad \text{CH}_2 \quad \text{N} \quad \text{CH}_3 \quad \text{OLi} \quad \text{OLi} \quad \text{C} = \text{O} \quad \text{NH}_2
\]

\[+ \text{LiOH}\]
Precipitation of tetracycline-7-boronic acid occurred, presumably due to differences in the solubility of 7-chlorotetracycline and tetracycline-7-boronic acid in tetrahydrofuran. 7-chlorotetracycline has a maximum solubility of 14.9 mg/ml in tetrahydrofuran at 25°C, whereas tetracycline-7-boronic acid has a maximum solubility of only 7.6 mg/ml in tetrahydrofuran at 25°C and 6.9 mg/ml in boiling tetrahydrofuran (66°C).

The mixture of solids containing boric acid, lithium borate and tetracycline-7-boronic acid was filtered by suction at 66°C. The substances on the filter were washed with cold absolute ethanol to remove boric acid and lithium borate. The remaining crude tetracycline-7-boronic acid was dried in vacuo (yield 48.4% theoretical).

The crude tetracycline-7-boronic acid was a yellow material which analyzed a maximum of 1.84% boron. Calculated for tetracycline-7-boronic acid is 2.21%. The method of boron analysis is discussed in a later section of this chapter.

Alkali fusion of the tetracyclines has been shown by Pasternack (16) to yield both derivatives of salicylic acid and m-hydroxybenzoic acid from the left hand ring of the tetracycline molecule. Thus by analogy with Pasternack's result, the degradation of tetracycline-7-boronic acid by alkali fusion may be expected to proceed
as follows:

\[
\begin{align*}
\text{Steric hindrance should prevent the formation of 4-hydroxy-1-boro-benzoic acid.} \\
\text{Following closely the method used by Pasternack to isolate salicylic acid, an attempt was made to isolate 5-borosalicylic acid. 12.5 grams of tetracycline-7-boronic acid were added portionwise over one half hour to a melt of sixty grams of potassium hydroxide and thirty grams of sodium hydroxide at 200° to 210°C. After one hour of further heating the melt was cooled, dissolved in two hundred milliliters of water, acidified with 50% hydrochloric acid and extracted with three seventy five milliliter portions of ethyl acetate. The ethyl acetate}
\end{align*}
\]
extract was evaporated to dryness and the black tarry product sublimed at 200°C (1mm) to yield 1.08 grams of brownish oily crystals.

Treatment of an aqueous solution of the sublimed material with activated charcoal did not remove all the colored impurities present in a single treatment. A second activated charcoal treatment and fractional crystallization of the material from water yielded approximately twenty milligrams of white crystals with a melting point of 158° to 159°C and .0624 grams of a brownish crystalline material with a melting point of 169° to 180°C. The crystalline material which showed a melting point of 158° to 159°C, when mixed with an authentic sample of salicylic acid, did not depress the melting point. The brownish crystalline material could not be purified further due to the small amount present.

The brownish crystalline material has been studied and the following evidence indicates the presence of a carbon-boron bond. Under the conditions of the sublimation of the fusion products, as outlined by Pasternack (16), boric acid will not sublime. Alkali fusion and acidification of the tetracycline-7-boronic acid would likely produce boric acid unless a carbon-boron bond were present. The sublimed material when subjected to flame photometric analysis shows a strong boron emission band between 548 μm and 566 μm.
Methyl borate has been extensively used in the
detection and determination of boron as boric acid. Many
methods have been proposed in which the boric acid is
separated from accompanying substances by converting to
the volatile methyl borate and distilling (17-23). The
distillate can be subjected to flame photometric methods
of analysis (24).

The samples of tetracycline-7-boronic acid were
analysed for boron content by the following procedure.
The samples were digested with four milliliters of boron
free concentrated sulfuric acid in a two hundred and fifty
milliliter flask modified for either reflux or distillation.
Ten milliliters of boron free methanol was added and the
mixture refluxed for fifteen minutes, pivoted to the
distillation position and the solution distilled to a
temperature of 70°C. Ten milliliters of methanol was
added to the distillation flask and the solution again
distilled to 70°C. A final ten milliliters of methanol
was added to the distillation flask and the solution
distilled to a volume of twenty five milliliters. The
distillate obtained was subjected to flame photometric
analysis and compared to a standard which was prepared by
the same method. The standard was prepared by plotting
the log of the optical density versus the concentration
of boron in micrograms per milliliter. A flame attachment
with a Beckman model B spectrophotometer was used in the
Presence of sodium in the distillate of methyl borate results in higher optical density readings of the boron emission spectrum (24). It was found that a concentration of one microgram, or less, of sodium per milliliter did not cause error in the boron analysis. Baffle plates built into the distillation flask prevented spray, containing sodium, being carried over into the distillate. A diagram of the apparatus used appears in figure 2.
FIGURE 1

DIAGRAM OF THE APPARATUS USED FOR THE PREPARATION OF
7-LITHIUM TETRACYCLINE

A) Drying tube
B) Inlet for the 7-chlorotetracycline-tetrahydrofuran solution
C) Stopcock
D) Metallic lithium reaction vessel
E) Sintered glass filter
F) Collection flask
FIGURE 2

DIAGRAM OF THE APPARATUS USED FOR BORON DETERMINATION

A) Reaction and distillation flask
B) Baffle plates
C) Inlet for acid and methanol
D) Thermometer
E) Water cooled condenser
F) Ice cooled trap
G) Volumetric flask
H) Pivot for setting the apparatus in either reflux or distillation position
CHAPTER III

DISCUSSION

The presence of chloride ion in the insoluble residue from the lithium metal-7-chlorotetracycline reaction has shown the successful removal of the chlorine. Replacement of the lithium by boric acid is not described in the literature; however, it was indicated by a boron emission band present in flame photometric analysis of the alkali fusion products of tetracycline-7-boronic acid.

5-borosalicylic acid, which may be one of the degradation products of alkali fusion of tetracycline-7-boronic acid is not described in the literature; however, the synthesis of the compound is being attempted at College of the Pacific.

Further studies are suggested in the areas of better methods of synthesis of tetracycline-7-boronic acid, preventing gum-like material forming and studies of the aryl-lithium-boric acid reaction. It is evident that the above problems which exist must be completed before an absolute preparation of tetracycline-7-boronic acid may be claimed.
BIBLIOGRAPHY


