Research Report

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for Dr. Linus Pauling
in fulfillment of the
Ch 80 requirement

Abstract

The purpose of this investigation was to show interaction between iodoform and other small inert molecules as evidence supporting Dr. Linus Pauling's General Theory of Anesthesia. Such interaction was taken to be indicated by the increased solubilization of iodoform in the presence of other small inert molecules in aqueous solution. Such increased solubilization was not observed with nitrogen, carbon dioxide, xenon, and the magnesium ion. It was observed with hydrogen sulfide and cyclopropane. However, the possibility of iodoform - hydrogen sulfide hydrogen-bonding and the possibility of the existence of an iodoform - cyclopropane clathrate solid phase in suspension prevented these observed interactions from being the conclusive evidence desired.

Research Report

This research report is executed in fulfillment of the Ch80 requirement and covers work done during the summer of 1963 for Dr. Linus Pauling. The work was grounded on Dr. Pauling's Theory of General Anesthesia as explicated in Science, July 7, 1961, Vol. 134, No. 3471, pages 15-21.

This theory points out that many anesthetic agents are known to be relatively non-hydrogen bonding, and some such as xenon are known to be almost completely chemically unreactive. These compounds, however, are known to form a class of inclusion compounds known as the water hydrates. In compounds of this class water holds often stoichiometrically small inert molecules in cages. Examples of such molecules are xenon, ethylene, carbon dioxide, hydrogen sulfide, and chloroform. In the water hydrates such molecules are held in structures of water molecules arranged in interconnecting pentagonal dodecahedra and tetra and hexakaidecahedra. There are two main lattice types each with distinct stoichiometry and unit cells. Usually water hydrates are stable only at low temperatures or high pressures of the included molecule. An example is xenon, which forms a hydrate stable at $0^{\circ}C$. at xenon pressures of 1.15 atmospheres and above. Another example is argon, which requires an argon pressure of at least 95.5 atmospheres for hydrate formation. More stable are some of the double water hydrates, which form a water clathrate structure having cages of two sizes. The larger included molecules are held in the larger cages and the smaller one in the smaller cages. An example of such a structure is the xenon-carbon tetrachloride double hydrate, which at one atomosphere is stable to $+13.7^{\circ}C$. Dr. Pauling postulates that although these compounds as such could not exist in biological systems, their

formation stabilized by some component of the biological system could explain anesthesia. The intent of the 1963 Anesthesia Research Project was to find experimental evidence corroborating Dr. Pauling's theory.

If it could be shown that under certain conditions non-hydrogen bonding clathrate-forming small inert molecules solubilized each other, such an interaction might be considered to be such experimental evidence. The chemical iodoform was assigned for investigation as it is only slightly soluble, possesses a yellow color indicating the possibility of spectrophotometric detection, and has a relatively large molecule indicating an increased probability of interaction with water clathrate frameworks. In the summer of 1962 examination of the iodoform spectrum had shown that it has an absorption peak at 3360 Å. in water, 3400 Å. in ethyl alcohol, and 3500 Å. in decalin. The extinction coefficient was determined to be 1.88×10^3 O.D. l./mole cm. or 4.77 O.D. l./gm. cm. It was also found that iodoform in toluene or benzene quickly changed from the yellow color characteristic of iodoform to a dark purple. The conversion was much slower in ethyl alcohol and very slow in water. During the second summer it was shown that this conversion was a photo-conversion and was particularly sensitive to ultra-violet light. It was shown that iodine released from the iodoform was probably the cause of the dark purple coloration observed when iodofrom was dissolved in organic solvents. Iodoform was also found to interact with BSA in that in the presence of 10% BSA iodofrom was solubilized to the extent of two iodoform molecules per BSA molecule independent of temperature. Experimental evidence indicated also that iodoform was solubilized in a saturated hydrogen sulfide solution. However, oxidation of the hydrogen sulfide probably to colloidal sulfur introduced light scattering making quantitization of the effect difficult. Iodoform was also shown to be

solubilized by lysozyme, ribonuclease, and micelle-formers such as n-decylammonium chloride and sodium caprate.

The research work of the second summer was a continuation of that of the first. The first project was to see if certain gases could solubilize iodoform. Carbon dioxide, nitrogen, and xenon were investigated. For both carbon dioxide and nitrogen the gases, contained under pressure in the usual commercially available cylinders, were forced through gas wash bottles. Each of the two gas wash bottles used was shaped like an elongated cylinder and was topped by a ground glass joint allowing material to be place inside. The two pieces were held together by springs, which permitted pressures in excess of atmospheric to be used. The top piece contained two tubes. One was for entrance of gas and was attached to a sintered glass disc, which was located below solution level during operation. The other was for exit of gas. All parts with the exception of the springs were of glass. In operation a magnetic stirring bar was placed in each wash bottle and an excess of solid iodoform in one. While stirring with a magnetic stirrer, the gas under investigation was forced through both bottles at low flow rates for about thirty minutes. After that the gas pressure was maintained at a slight positive value with the aid of a manometer attached to the system. Both gas wash bottles and their contents were stirred for five hours, the period previously found necessary to approach complete iodoform saturation. After this period the gas-iodoform solution was passed through a sintered-glass filter into a 10 cm. spectrophotometric cell containing 35 cc. The solution of the gas alone was used as a blank in a similar cell. Then the spectrum of each of these samples was taken in the visible and near ultra-violet using a recording Cary spectrophotometer. The iodoform content was computed from the differential absorption of the two cells at 3360 Å. relative to the

baseline figure taken at 6000 Å. The nitrogen-iodoform sample was at a temperature of 24.40°C., and the carbon dioxide-iodoform sample was at a temperature of 29.89°C. and a pH of 4.23. No increased solubilization of iodoform with either nitrogen or carbon dioxide was observed.

The procedure used with xenon was the same except a special apparatus was used to reduce free gas volume. The samples were saturated in 60 cm. bottle with hypodermic needles through syringe caps used to provide an entrance and exit for the xenon. Instead of a continuous flow of gas, the xenon was held under pressure over the solutions until decreased pressure indicated that xenon had been dissolved in the water. Then more xenon was added to the system increasing the pressure. It was found necessary to repeat this procedure only a few times. Runs were made at 29.20°C. and 6.10°C. No increased solubilization of iodoform by xenon was observed.

It is known that the magnesium ion can act as an anesthetic agent. For this reason it was decided to investigate the magnesium ion - iodoform system. Iodoform was stirred in excess in a solution of 1 F. magnesium sulfate for five hours. The resultant solution was filtered through a sintered glass filter into a 10 cm. spectrophotometric cell. Then a spectrum was taken with the recording Cary spectrophotometer. No increased solubilization of iodoform by magnesium sulfate was observed upon comparing this spectrum with that of a 1 F. aqueous magnesium sulfate solution.

An investigation of the iodoform - hydrogen sulfide system was also undertaken. Using the previous procedure, it was found that if the solutions were filtered and placed in the spectrophotometric cell under a flow of nitrogen, the difficulties from air oxidation of hydrogen sulfide producing colloidal sulfur could be avoided. The following data were obtained:

T	pH	O.D. Change	% Increase	(H_2S)	O.D./(H_2S)
4.62 °C.	3.80	0.035	7.09%	0.374	0.0936
5.00 °C.	3.47	0.245	49.4%	1.69	0.145
27.66 °C.	3.11	0.530	50.7%	3.98	0.133

After washing air out of the system and solution with nitrogen, saturation was performed at one atmosphere of hydrogen sulfide. Hydrogen sulfide concentrations were calculated from pH values. The column labeled "% Increase" gives the increased solubilization of iodoform showing that an interaction does occur. However, iodoform - organic solvent hydrogen - bonding reported in the literature suggests hydrogen bonding as a possible explaination along with clathrate structure formation.

The anesthetic potency of cyclopropane as well as its availability in cylinders suggested the investigation of the cyclopropane - iodoform system. Using standard procedure, the follwing data were obtained.

T	% Increased Iodoform Solubilization
1.50 °C.	+67.4%
2.80 °C.	+15.0%
27.90 °C.	-2.62%

At the lower temperatures formation in solution of platelets with an icelike appearance were observed. It is believed that these platelets were an iodoform - cyclopropane water clathrate. It is not known whether the increased iodoform solubility observed in the filtered solution was the result of the failure of filtration to remove completely the solid phase clathrate or whether clathrate structures were stabilized in solution. Furthermore, due to lack of facilities it was found impossible to further investigate the temperature dependence of the effect.

An attempt was made to redevelop for the use of others in the group a determination for chloroform originally developed during the previous summer by Peter Hall. The determination involved taking chloroform in water to chloride ion with saturated potassium hydroxide and then using the standard chloride ion determination. Due to the sparcity of details in Peter Hall's notebook and lack of time, the determination was not successfully redeveloped.

In summary no conclusive evidence for the existence of iodoform water clathrate structures in solution was found.