

Carnicom Institute Research

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Acknowledgements

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CDB Lipids : An Introductory Analysis

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Clifford E Carnicom Mar 12 2015 Edited May 29 2016

Note: I am not offering any medical advice or diagnosis with the presentation of this information. I am acting solely as an independent researcher providing the results of extended observation and analysis of unusual biological conditions that are evident. Each individual must work with their own health professional to establish any appropriate course of action and any health related comments in this paper are solely for informational purposes and they are from my own perspective.

An introductory qualitative and analytical analysis of certain lipids that have been extracted from the *cross-domain bacteria* (CDB), as they are designated on an interim level by this researcher, has been made. Lipids are a primary biological molecule within any living organism and future studies of this component will be of the greatest importance.

Several major characteristics have been identified using modest means and methods, and the results bring to the forefront additional unusual properties of the organism under study with respect to the so-called "Morgellons" condition. There are, potentially, several important health implications that arise from this recent work; these health factors are in complete accord with the historical record of discovery and examination that is available on this site. This paper will be relatively brief in coverage but it will, hopefully, serve to reiterate certain themes and directions of research that remain to be confronted by society and that are deserving of appropriate support and resources.

The primary characteristics or factors that have been identified in the course of this study are:

- 1. The lipids from the CDB appear to be highly non-polar in nature.
- 2. The lipids have a relatively high index of refraction.
- 3. The lipids appear to be composed, in the main, from long chain poly-unsaturated fatty acids.
- 4. The lipids appear to support combustion (i.e., oxidation) with ease.
- 5. The lipids appear to react readily with the halogens, such as iodine.

6. The visible light spectrum of the lipid – iodine reaction is unique and it serves as an additional means of identification. Peak absorbance of the reaction is at approximately 498 nanometers.

7. A significant portion of the extracted lipids is expected to originate from the membranes of the CDB.

8. Endoxtoxins within the CDB are suspected to exist and this subject remains as a serious prospect for research in the future.

These characteristics will now be discussed in greater detail to formulate a general but composite assessment of the lipid character, as well as a reference to certain health impacts that are necessary to consider.



Variable Solubility of the Lipids as it Relates to Polarity

Polarity is a defining property of a molecular structure, and it is a measure of the distribution of charges within a molecule. Non-polar molecules are generally symmetric in their nature with a tendency toward an equal and symmetric distribution of charges. Polar molecules, in contrast, are usually of an asymmetric nature with the charges on the molecule unevenly distributed. Information on polarity, therefore, provides some generalized nature as to the form or nature of the molecule or substance under study.

In this photo, The lipids are mixed with a mildly polar solvent in the tube to the left in the photo; a clear separation remains after settling. In contrast, the lipids dissolve much more readily in a highly polar solution to the right in the photograph.

The significance of this result is as follows:

Fatty acids are a dominant component of many lipids. They are comprised of a carboxyl group that is attached to a hydrocarbon chain. The length of this chain can vary depending upon the particular fatty acid that is involved. The carboxyl group is polar in nature and therefore the charge distribution on that particular functional group is asymmetric. The carboxyl group is also acidic in nature and this is the origin of the name of fatty acids that is attached to this common lipid structure.

The hydrocarbon chain that is attached to the carboxyl group is generally of a non-polar nature, and it serves to counteract the polar effect from the carboxyl group. Therefore, the more non-polar the lipid is, the more likely it is that the hydrocarbon is of relative greater length. A very long hydrocarbon chain (non-polar) will tend to dominate the character of the molecule in this case and ultimately make the molecule less polar.

This relationship between the polarity of and the length of the attached hydrocarbon chain provides our first useful interpretation as to the structure of the lipid molecule. Some lipids are more or less polar than others; a highly polar lipid is indicative of lengthy hydrocarbon chains within the fatty acid. The longer the fatty acid is, the more complex the lipid structure or interactions with other molecules is likely to be. *The structure of any molecule is of the highest importance, as one of the dogmas of biology is that structure determines function. We are after both, structure and function, and usually in that same order.*

A couple of examples of short vs. long chain fatty acids follows; it can be seen that the differences in form and structure can be substantial:







Acetic acid (acetate)

Propionic acid (propionate)

Butyric acid (butyrate)

Short Chain Fatty Acids Image Source : intechopen.com

The specific conclusion in this case is that we are more likely to be dealing with a lipid form that contains more extensive hydrocarbon chains.

The next topic of interest concerns the index of refraction. The index of refraction is a measure of the ability of a substance to bend a light wave that passes through it. It is also a measure of the speed of light though that same material. It is also an important defining physical property of a substance, and its measurement can be made with relative ease and modest cost. Tables of the index of refraction for a wide variety of substances, including lipids and oils are readily available for comparison purposes.

The index of refraction for the lipids under examination measures at 1.487 as the average between two different samples. The instrument has been calibrated with numerous comparison oil samples and is performing accurately and reliably. The estimated error of the measurement is +/- .001.

The measurement of 1.487 is a relatively high index of refraction, especially as far as oils are concerned. This higher measurement also leads to interpretations of significance as we shall soon discover.

There is a relationship between the index of refraction and the degree of saturation within a fatty acid or lipid. The saturation level (i.e., saturated vs unsaturated) property of a lipid is also a very important characteristic as it expresses itself in terms of the the bond types within the molecule; this is an additional aspect of structure that we have declared as our pursuit.

Let us begin with the definitions for saturated vs. unsaturated fats. A saturated fat is one in which a full complement of attached hydrogen atoms exists. A saturated fat contains only single bonds between the carbon atoms. An unsaturated fat, in contrast, has double (or higher) bonds between

the carbon atoms, and there will be fewer hydrogen atoms attached as a result. Let us present a couple of images to clarify the difference between saturated and unsaturated fats.



An example of a saturated vs. an unsaturated fat image source : staff.jccc.net

In addition, a distinction should be made between mono-unsaturated fats and poly-unsaturated fats. In essence, a mono-saturated fat has a single double carbon bond within the hydrocarbon chain and a poly-unsaturated fat has more than one double carbon bond within the chain. The image below shows this difference



The top image shows another example of a saturated fat.

The lower two images show the distinction between monounsaturated and polysaturated fats. Notice the number of number of double carbon bonds present in the latter examples. image source : 2012books.lardbucket.org As information is gained, let us never lose sight of the end goal: The more that can be understood about the structure of a biological molecule, the closer that we are towards learning about the behavior, interaction and function of that molecular structure. This information is a prerequisite toward the design of effective mitigation strategies. While much of this pursuit remains in our future, we nevertheless can report the modest levels of progress as they occur, albeit under restricted conditions.

Now that we understand the variations of saturation within fats and oils (lipids), let us return to something that can be measured to give us information about the state of saturation within a lipid. Once such measurement is the index of refraction, as has been referred to above.

It will be found in the literature that that there is a 'relationship' between the degree of saturation in a fat and the 'iodine number'. The iodine number is a measure of the level of absorption of iodine by fats, and this number can be used in turn to infer the degree of saturation by that same lipid or fat. The method is commonly used in the food industry to determine the quality of fats. The degree of fat saturation is a variable of high interest within the food industry as it affects the spoilage rate and this in turn affects the economics of the food industry. There are many important reasons to understand the qualitative characteristics of lipids beyond our immediate interest in the 'Morgellons' issue.

Determination of the iodine number is a more demanding laboratory method and it requires additional time, protocols and reagents in comparison to alternative methods that have developed within this study.

There is, however, a more accessible method to fulfill our immediate need, and that is to get some sense of the likely saturation level of this particular lipid. It will be found, with study, that there is also a relationship that can be established between the index of refraction of an oil and the iodine number of that same oil. An increase in the iodine number is indicative of a higher **unsaturation** level and in parallel it will be found that a higher index of refraction is strongly correlated with a higher iodine number. We are able, therefore, to make an equally viable interpretation of the saturation (*i.e., unsaturation as well*) level with the use of the index of refraction as our primary dependent variable. Ultimately, a higher iodine number estimate will indicate a higher level of unsaturation within the lipid. Such a relationship has been researched and established as presented below.





Several different oil types have been investigated and the correlation between the index of refraction is reasonably strong (r = 0.92, n = 13). The accuracy of the refractometer in use has been included as a part of the study. The result of this work is that a viable method to estimate the level of relative saturation from a direct measurement of the index of refraction of the lipid under study now exists.

The application of the linear regression model to the measured index of refraction (1.487) yields an estimate for the iodine value as 218. This magnitude for the estimated iodine value is extremely high and it is significant in its own right.

The conclusion to be reached from this iodine value is meaningful. *This stage of the study indicates that the character of the lipid is more likely to be that of a highly poly-unsaturated lipid.* This result is corroborative with the first interpretation of a relatively lengthy fatty acid chain within the lipid structure. These two interpretations are mutually supportive of one another. This means that the lipid hydrocarbon chains are more likely to be lengthy with several double carbon bonds along the chain. This, in turn, will affect the structure as double bonds cause a bend to take place in the hydrocarbon chain. Several double bonds would only enhance that feature further.

In addition, double bonds within a hydrocarbon chain have another likely and important result. They are much more likely to produce chemical reactions. Two likely candidates for reaction are oxygen and the halogens. Lipids with a high iodine value are more subject to oxidation and therefore have a greater likelihood of becoming rancid (spoiled). High iodine level lipids are also more likely to produce free radicals. Lastly, highly polyunsaturated lipids are more likely to polymerize (i.e, 'plasticize). Each of these impacts offer the prospect of additional harm to the body, and great attention to the effects of oxidation and free radicals has been given in the history of research on this site.

There is a wealth of information that is available on the health risks associated with polyunsaturated fats. The following citations are a couple of representative examples of the issues involved, the first from a lay standpoint and the second from the Commission of European Communities:

Many reviews have appeared on the biological effects of lipid oxidation products and their relevance to cancer. Lipid oxidation products have been implicated in the disruption of biological membranes, the inactivation of enzymes and damage to proteins, the formation of age pigments in damaged cell membranes, oxidative damage to the lung by atmospheric pollutants, and in the causation of cancer.

Source : Reports of the Scientific Committee for Foods, Commission of European Communities

Readers may recall the extensive attention that has given within this site to the role that antioxidants can play in the mitigation of excessive oxidation to the body. Those discussions, once again, appear to be especially relevant in the amelioration of the harmful influences of polyunsaturated fats. The impact of halogens to the thyroid and metabolism have also been extensively discussed on this site and we will return to that topic later in this paper as well.

The issue of oxidation in combination with combustion tests should now be raised. The tests, at this stage of investigation, indicate that this particular species of lipids may be highly subject to the process of oxidation. The purity of the sample can not be quantified at this point since there may be

other compounds present within the lipid samples. However, all indications are that the character of the lipids is somewhat unusual with respect to oxidation and, for that matter, combustion.

The lipids that have been extracted ignite easily, as is shown in the photograph below on the left side:







Lipid Combustion Tests

In this case, the method involves placing a small amount of the lipids into a watchglass with a small piece of paper acting as a wick. The lipids burn easily and steadily under these conditions, and the behavior is somewhat akin to lamp oil. Due to the biological and apparent polyunsaturated nature of the lipids, a comparison might be made with whale oil, which was an important source of fuel in earlier times. There is no suggestion here that the lipids are chemically identical to whale oil by any

means, however, the fish oils and whale oil share many interesting properties of the highly polyunsaturated fats. The photograph on the right shows the wick remaining at the end of combustion; this demonstrates that the oil itself is the primary source of fuel within combustion. The last photograph shows an inclusive example of the failure of any of the other tested lipids or oils to support direct combustion.

Combustion goes hand in hand with oxidation; something that burns oxidizes. It is of interest that of all the other oils tested under similar conditions (approximately 8 varieties of varying degrees of unsaturation), only the lipids under examination here showed any ease of combustion at the level shown within the photographs. Along with the highest index of refraction found within the group that has been examined, the dramatic display of combustion of the sample further reinforces the case for a lipid that is highly unsaturated and thus prone to excessive oxidation. This finding is once again corroborative of the extensive case for excessive oxidation within the body that occurs in association with the 'Morgellons' condition; readers may also recall the lengthy discussions on the apparent marked oxidation of iron within during the examination of blood samples. All signs of the accumulated research indicate that excessive oxidation within the body is one of the most likely outcomes expected to be found within any future studies of the 'Morgellons' condition. Preliminary data from early questionnaires submitted to the public also strongly indicates this same result.

There are at least two primary forms of lipids in the body, one for storage of energy within the cells and another within the membranes of the cell, where they act to to encapsulate and protect the cell. Saturated fats are more likely to be associated with the storage of energy internal to the cell and unsaturated fats are more likely to be associated with the membranes of a cell. Phospholipids are a very important class of lipids that are found within the cell membranes. The degree of unsaturation within phospholipids varies, with one or both tails having double carbon bonds (the site of oxidation). An image of a representative phospholipid follows:



Phospholipid within a Cell Membrane Source : wikipedia.com

The oxidation of lipids is referred to as lipid peroxidation, and it is especially prone to occur with polyunsaturated lipids, as we appear to have in this case. Phospholipids (a bi-layer) are a major constituent of cell membranes, and the oxidation of these lipids subsequently causes damage to the cell. Lipid peroxidation is essentially the theft of electrons from the lipids in the membranes and it occurs as a free radical chain reaction. The oxidation occurs when there is an excess availability of free radicals, or reactive oxygen species. The point of oxidation will be the location of the double bond, which occurs at the bent location within the unsaturated fatty acid tail, as shown in the picture above. An illustration of the lipid peroxidation reaction is shown below; notice the site of activity at the carbon double bond:

One of the best known toxic effects of oxygen radicals is damage to cellular membranes (plasma, mitochondrial and endomembrane systems), which is initiated by a process known as *lipid peroxidation*. A common target for peroxidation is unsaturated fatty acids present in membrane phospholipids. A peroxidation reaction involving a fatty acid is depicted in the figure below.



Source : Colorado State University

It appears to be the case at this point that the CDB contain within them a highly polyunsaturated fat and/or fatty acids, most likely to occur within the membranes of the CDB, and that the CDB may therefore be subject to, or result in, lipid peroxidation in the presence of free radicals. This process, once started, is a chain reaction and is only terminated in the presence of appropriate antioxidants, such as Vitamin E, glutathione peroxidase, transferrin (binding free iron), enzymes (such as catalase), in addition to others[see Robbins above]. As shown within earlier culture trials, Vitamin C and NAC (N-acetyl cysteine acting as a glutathione precursor) may show themselves to be effective antioxidants as well. The issue of oxidants vs. antioxidants has emerged earlier within the research and this information remains available to review. Those seeking therapeutic protocols dependent upon oxidizing protocols vs. antioxidant protocols may wish to examine further the fundamental differences that are apparent within the scientific literature. *Each individual must , of course, seek health consultation that is appropriate to their individual needs*.

Another more complete description of lipid peroxidation comes from Robbins Pathologic Basic of Disease, 4th Edition, where the following sequence is described:

"Lipid peroxidation is one well-studied...mechanism of free radical injury. It it initiated by hydroxyl radicals, which react with unsaturated fatty acids of membrane phospholipids to generate organic acid free radicals, which in turn react quickly with oxygen to form peroxides. Peroxides themselves then act as free radicals, initiating an autocatalytic chain reaction, resulting in further loss of unsaturated fatty acids and in extensive membrane damage"

To reiterate the attention that has been given in the research to the oxidation and antioxidant issues in the case of 'Morgellons', please recall some of the earlier papers (this paper included) that complement this discussion:

Morgellons : A Discovery and a Proposal – February 2010 Morgellons : Growth Inhibition Confirmed – March 2010 Morgellons : The Extent of the Problem – June 2010 Morgellons : In the Laboratory – May 2011 Morgellons : A Thesis – October 2011 Morgellons : The Breaking of Bonds and Reduction of Iron – November 2012 Amino Acids Verified – November 2012 Morgellons : A Working Hypothesis : Part I – December 2013 Morgellons : A Working Hypothesis : Part II – December 2013 Morgellons : A Working Hypothesis : Part II – December 2013 Growth Inhibition Achieved – January 2014 Biofilm, CDB and Vitamin C – April 2014 CDB : General Characteristics (In Progress) – July 2014 CDB Lipids : An Introductory Analysis – March 2015

Lipid peroxidation is a complex area for study, however, the importance of doing so can be understood from the following statement by Marisso Repetto, from the Institute of Biochemistry and Molecular Medicine, Argentina:

"Currently, lipid peroxidation is considered [as one of] the main molecular mechanisms involved in the oxidative damage to cell structures and in the toxicity process that lead[s] to cell death."

The complete paper is detailed but insightful, and it demonstrates the extensive research that is now available on the subject of lipid peroxidation. The paper in its entirety may be accessed <u>here.</u>

Let us introduce an observed reaction with one of the halogens, in this case, iodine. The reaction is shown below on the right hand side, and in comparison to a negative reaction with vegetable oil on the left. Similar to the case of combustion from above, the CBD lipids under study are the only lipids (of approximately eight in comparison) that have displayed this pronounced reaction with iodine. It appears to be a unique, important and characteristic reaction.



It is understood that iodine reacts with lipids; in fact, this is the very basis of the 'iodine number' method and it is used as a measure of the *unsaturation* level of the lipid. The higher the iodine level, the higher the level of unsaturation in the lipid. We have already discussed the relationship between the iodine number and correlation with the index of refraction, and we have very good reason to suspect a very high level of unsaturation within the lipids examined.

What is under discussion here is *the formation of a bright red colored iodine complex* which, thus far, presents itself only within this particular lipid form, at least in relation to numerous sample types that it has been compared with. The colored complex reaction formed is, in itself, worthy of continued chemical analysis and investigation. *This reaction has not occurred in like fashion to any other lipid samples examined thus far*. The nature of the complex is not completely understood at this time; the consideration of an iron-lipid-iodine or transition metal complex, however, is extremely high on the list of possibilities.

What can be concluded from visible light spectroscopy, however, is that the colored complex formed once again assures us that *we are dealing with a structure that contains numerous double carbon bonds*. Visible light spectroscopy is highly dependent upon what is termed conjugation; conjugation is a molecular structure that is based upon alternating single and double carbon bonds. The greater the degree of conjugation, the longer the wavelength of the color that will be absorbed. An example of a highly conjugated form is as follows:



An example of a conjugated structure within a chromophore (portion of a molecule that absorbs color). Source : wikipedia Notice the numerous alternating single and double bonds in the above structure. Chromophores are especially likely to form with compounds that involve the transition metals, such as iron. The color of the complex lends itself well to visual light spectrometry and a spectral plot of the CDB complex formation in the visible light range is shown below:



Visible Light Spectrum of the CDB Lipid-Iodine Complex

The peak absorbance occurs at approximately 498 nanometers. This spectral examination of the lipid-iodine complex is an important identification method to establish the presence or existence of this particular CDB lipid form.

The identification of an iron-lipid-iodine complex is further substantiated with tests for the detection of iron using 1,10 phenanthroline reagent in combination with the lipids in a mildly polar solution. These initial tests are weak in color but nevertheless positive for the presence of the Fe+2 ion within the CDB lipids. This finding is in coincidence with the paramount conclusion of significant Fe+2 iron use and metabolism by the CDB, as it has been discussed extensively within earlier papers.

The impact of halogens upon the body has been discussed extensively in earlier work and it will not be repeated here. Readers are referred to the paper entitled <u>Morgellons : A Working Hypothesis</u> (esp. Parts <u>II</u> & <u>III</u>) for the important effects and toxicity potential discussed therein.

The next topic of importance to discuss is that of polymerization. A polymer is a molecular structure that is composed of many repeating smaller units. They can be either synthetic or natural, and they usually have a large molecular mass compared to that of the basic structural unit. Latex and Styrofoam are examples of both a natural and a synthetic polymer. The architecture and length of the polymer chains strongly affect the physical properties of the polymer, such as elasticity, melting point, and solubility, amongst others. A diagram of various structural forms is shown below:



Source : Wikipedia

The reason that polymerization is relevant here is that unsaturated lipids are prone to polymerization. The higher the degree of unsaturation, the more likely that polymerization will take place. This is due to the oxidation at the double carbon bonds that have been brought to attention repeatedly here. A familiar example of polymerization to many of us is with the use of linseed oil. Linseed oil is a highly unsaturated lipid that is applied to furniture as a protective coating; this is one of the so-called "drying oils". As this type of oil weathers (or oxidizes), it will form a harder and protective coating over the wood surface. This is an excellent example of the oxidation of a highly unsaturated oil, or lipid, that produces a polymer. As mentioned, polymers can vary widely in their physical properties, and the plastics are an excellent additional example of synthetic polymers. Oil paints that artists use are another example of the "drying oils" that share these same characteristics.

It appears that the probability of polymerization for the CDB lipid complex appears to be high at this point, as all of the prerequisite characteristics appear to be in place. It appears to be highly unsaturated and therefore subject to oxidation as has been detailed above. This places us on the alert that the CDB lipids may be a candidate to produce polymers which, in general, would be anticipated to cause harm if internal to the body.

With respect to lipid discovery and extraction, we would be remiss if the subject of endotoxins was not again introduced. Readers may recall that all tests conducted on the CDB to date indicate that they are Gram-negative. A Gram-negative test is important for bacteria as it indicates at least three characteristics of importance:

1. The cell walls are lipid-rich in comparison to Gram-positive bacteria.

2. The negative test indicates the presence of *lipopolysaccharides* (LPS) within the cell wall;

lipopolysaccharides are essentially synonymous with *endotoxins*.

3. Pathogenic bacteria are often associated with endotoxins.

Let us visually compare the cell walls of a Gram-positive bacteria vs. a Gram-negative bacteria:

Gram-positive bacteria vs. a Gram-negative bacteria

Source : microbewiki.kenyon.edu

There are distinctive differences that can be noticed. Starting from the bottom, we can see that both cells contain phospholipids (the lipid bi-layer presented earlier). The Gram-negative cell, however, is lipid rich, while the Gram-positive cells have a much lower lipid content. The lipid content of the Gram-negative cell wall is approximately 20-30%, which is very high compared to the Gram-negative cell wall. The relatively high volume of lipids that have been extracted from the CDB are supportive of the Gram-negative test result.

In the Gram-negative cell, the peptidoglycan layer is about 5-20% by dry weight of the cell wall; in the Gram-positive cell the peptidoglycan layer is about 50-90% of the cell wall by dry weight. Peptidoglycan, also known as murein, is a polymer consisting of amino acids and sugars.

Gram negative bacteria are generally more resistant to antibiotics than Gram-negative bacteria. In consideration of the *cross-domain* terminology currently in use, it is of interest to note that the archaea can be either Gram-negative or Gram-positive; the archaea and the eukaryotes remain under equal consideration within the studies. It is also of interest to know that until relatively recent times that the archaea were classified as bacteria and that the classification systems of biology remain dynamic.

A central difference between the two forms, beyond the relative lipid content and peptidoglycan layer, is the presence of lipopolysaccarides (LPS) on the Gram-negative bacteria. LPS, or endotoxins, elicit a strong immune response in animals.

Aerosolized endotoxins are known to have a significant effect upon the pulmonary system and chronic exposures are known to increase the risk of chronic obstructive pulmonary disease (COPD). COPD is now the third leading cause of death in the United States. Sub-lethal doses cause fluctuations in body temperature (short term increases and longer term decreases), and changes in the blood, immune, endocrine systems and metabolism. They can result in "flu-like" symptoms, cough, headache and respiratory distress. They are linked to increases in asthma and chronic bronchitis. There are no regulatory standards for the levels of endotoxins in the environment (source : National Resources Defense Council).

Endotoxins are associated with increased weight gain, obesity, gum and dental infections and diabetes. A linkage with Chronic Fatigue Syndrome exists, as well as with atherosclerosis, oxidative stress, chronic conditions, cardiovascular disease and Parkinson's Disease. The condition of endotoxins within the blood is referred to as endotoxemia.

There may be a discomforting familiarity with the above symptoms in correlation with the socalled "Morgellons" condition; this familiarity justifies intensive research into the potential linkage between "Morgellons" and endotoxins.

Lastly, let us now review an infrared investigation into the nature of the extracted lipids.



Infrared Spectrum of CDB Lipids

Although a low resolution IR spectrophotometer has been used for this project, a very clear spectrum has been obtained. The spectrum is dominated by peaks at 2900 cm⁻¹ and 1700 cm⁻¹. The 2900 cm⁻¹ peak can be attributed to sp³ single carbon-hydrogen bonds. This functional group is perfectly in accord with the structure that forms the core of a fatty acid, as:



source :http:chemwiki.ucdavis.edu

In addition, the peak at 1700 cm⁻¹ can be attributed to carbon-oxygen double bonding, also in perfect accord with an unsaturated fatty acid, subject to oxidations as extensively described in this report.

A probability model has been developed for the analysis of infrared spectrums, subject to the constraints of the technology available to the Institute. The application of the model to the infrared spectrum above presents the following *relative* probabilities for the existence of the various functional groups:

Functional Group	Relative Probability of Existence
Ketones	90%
Alkanes	70%
Aldehyde	60%
Carboxylic Acid	45%
Phosphonate	45%
Silane	37%
Phosphonic Acid	30%
Ether	30%
Ester	30%
Amide	20%
Phosphine	20%
Sulfate	15%

An analysis of the above probability table will demonstrate that it is highly dominated by the combination and presence of carbon-carbon and carbon-oxygen single and double bonds functional groups. The study and examination of the high probability functional groups and their potential impacts upon health will continue; the strong appearance of the ketone and aldehyde groups with a double carbon-oxygen bond (carbonyl group) is also of high interest here; the aldehydes are very easily subject to oxidation. The potential presence of impurities within the sample will also need to be examined further, including those that might be a part of the extraction process.

All assessments in this report are highly corroborative of one another and they support the assessment of a highly unsaturated lipid, and all that this entails, as comprising a core structure of the CDB extraction that has taken place.

Additional Note:

Some additional analysis of biomolecules with the use of more capable and advanced infrared spectroscopy instrumentation has been completed as of May 2016. The structural information identified continues to support the hypothesis that the *CDB* derive from the bacterial domain and this

remains a primary focal point of research as to its origin. The degree of overlap of genetics, if any, with the remaining archaea or eukaryote domains remains an open topic of research.

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Signs of Hacking

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This pages provides notice that renewed efforts of computer hacking directed toward Carnicom Institute may be in effect. There are strong indications that research data has been selectively retrieved. There appears to be multiple infiltrations of email accounts. Computer failure and software disturbance coincident with these events has occurred. There is a precedent for such actions in the history of research that has been conducted, however, recent years have provided a hiatus in this regard. The security of information acquired through research and its presentation can not be guaranteed. The methods and strategies used appear to be sophisticated. If this statement is found to be in error it will be corrected.

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Preliminary Rainwater Analysis : Aluminum Concentration

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Preliminary Rainwater Analysis : Aluminum Concentration

Clifford E Carnicom Nov 02 2015

ABSTRACT

A method and means to identify the species and concentration of several different trace metals in ionic form has been established. The method employed is that of differential cyclic chronopotentiometry, which is a subset of the science of voltammetry. The brief paper presents a preliminary examination of a rainwater sample for the existence of trace metals. The sample under examination shows the existence of aluminum in a soluble form. An estimate of the concentration level of the aluminum has been made; this level exceeds that of the recommended standards for drinking water. The results indicate that public concerns about the toxicity levels of certain trace metals in the general environment are warranted, and that a more thorough evaluation of the state of atmospheric quality by the responsible agencies is required.



Rainwater Sample of this Study Collected under "Clean" Conditions Note that Visible Pollution is also Evident

The determination of trace metals can be an expensive and sophisticated proposition. One of the more modern methods of detection at trace levels involves the use of Inductively Coupled Plasma (ICP); such means and skill sets are not practiced by the public under normal circumstances. The determination of inorganic compounds at trace levels has always presented a serious challenge to this Institute, and in the past all such efforts have been relegated to that which can be gleaned primarily from qualitative testing methods. One interesting alternative, with a long history and of increasing importance, is the science of voltammetry. Many are familiar with the fact that elements and compounds have unique electromagnetic spectrums, such as those employed in the disciplines of spectroscopy including, for example, infrared spectrometry and atomic absorption. It is valuable to know that many of these same elements also have an 'electrochemical signature', and that they behave in unique and identifiable ways when exposed to variations in voltage and current. It is from this fact that voltammetry was born, and its origin dates back to the the days of Michael Faraday. The basic principle of voltammetry is to examine the relationships of oxidation and reduction within a medium or a reaction; there are numerous variations upon the specifics of this theme. Voltammetry equipment is dramatically more modest in cost than ICP and mass spectrometry, and yet it can still produce usable results that are, on many occasions, commensurate with the more advanced equipment and technology. Such equipment, in is most basic form, is now employed at the Institute and it is yielding promising results in the important domain of inorganic analysis, such as metals and halogens.

The study here refers only to an inorganic analysis that has been made; at a later date a presentation on biological aspects of the rainwater sample will occur as time and circumstances permit.

The rain sample was collected on Oct 30 2015 with new and clean containers with a clear path to the sky above. The sample was then evaporated to 33% of the original volume for the purpose of increasing the concentration level sufficient for testing purposes. The sample was compared to a control volume of distilled water.

The potentiostat used in the voltammetry work is a CV-27 model from Bioanalytical Sciences. The unit has passed all test procedures as described in the manual. The output from the potentiostat is coupled to a Pico 2000 series digital oscilloscope, whereby both voltage input and output can be displayed as a function of time. The basic mode of operation for the testing process is therefore one of chronopotentiometry.

A series of calibration tests were made with a variety of trace metals, including calcium, magnesium, sodium, potassium, iron and aluminum.

The goals of the investigations include both the ability to identify the species as well as concentration; both goals have been achieved with the above elements in an ionic state in sufficient concentration, i.e., on the order of a few parts per million (PPM). The work will extend to other species and combinations thereof in the future.

The particular variation of chronopotentiometry that has been utilized is that of cyclic chronopotentiometry, i.e, the alternating sweep between positive and negative voltages in the effort to identify the peak potential that characterizes the redox reaction of the particular element.

In addition, it has been found that the derivative of the chronopotentiogram is a key and critical factor in the determination of the species. A careful analysis of the derivative of the cyclic chronopotentiogram can be used with favor to identify the peak potential of the element.

When this point is identified and collated with the identifying element, concentration levels can also be established if a set of known standards is available. Concentration determinations on the order of a few parts per million have been achieved on multiple occasions.

Further careful evaluation of the derivative of the cyclic chronopotentiogram in combination with variable voltage sweeps can be used to identify separate components within a mixture of ionic species; this has been accomplished with a combination of three elements in ionic form in aqueous media to date.

The current work, under these preliminary conditions and examinations, leads to an assessment of a concentration level estimate of aluminum (+3, ionic state) within the rain sample at approximately 2.5 PPM. A conservative approach in all manners of examination has been adopted in the preparation of this estimate, and the condensing of the sample is accounted for.

The Environmental Protection Agency in 2012 lists the secondary regulations for aluminum in drinking water as being within the range of 0.05 to 0.2 mg/L. This corresponds to a range of 0.05 to 0.2 PPM for this same standard. It is an interesting observation within the same report that Secondary Drinking Water Regulations exist as non-enforceable federal guidelines. The wisdom of that classification process can be determined by the reader.

Continuing with the most conservative approach possible, one is led to the assessment that this particular rain sample from a rural location in northern Idaho exceeds the EPA drinking water standard and health advisory by roughly a factor of 12.

The following reference statement from the United States Geological Survey (Bulletin 1827-A, 1967) may be of interest in the evaluation of importance that is to take place:

PURPOSE AND SCOPE

Aluminum is an abundant constituent of rock minerals; in fact, only oxygen and silicon exceed aluminum in abundance among the elements present in the outer part of the earth's crust. Aluminum is, however, a very minor dissolved constituent in netural water. In routine water analyses the element is seldom determined, but the available information shows that, except for waters having a pH below 4.0, concentrations rarely exceed a few tenths of a part per million.

It is a point of interest that many individuals have ascribed the detection of aluminum within the atmosphere over a period of many years to my name. Such was never the case. My earlier work did indeed establish the precept that ionizable metallic salts are at the core of atmospheric pollution that we now live under, but the testing of aluminum, specifically, was not a part of that process. The chemistry of aluminum is quite different from that of the alkali earth metals, and the documentation of its existence by others has always raised intriguing questions of physics. Prior to this current work, most of the inorganic analyses that I have made have been restricted to qualitative tests. No means of testing aluminum at the trace levels has existed for the Institute prior to this occasion. Hopefully, this situation is now mildly improved with the current voltammetric studies. This paper adds itself to a long list of documented actions by the citizenry on the consideration that aluminum is certainly, and has been, entitled to.

As a starting point, we might wish to consider the role that aluminum may play within a geoengineered environment, and it may be worthwhile to look at the exothermic energetics of nano-particulates of aluminum under exposure to moisture. It raises some tantalizing prospects for additional capabilities of an induced or artificial plasma state.

It is also an observation that visible pollutants in rainwater may be most pronounced with the advent of a storm. This is logical, and this has certainly been observed in the cases of excessive fires in this region. Time will tell if it is the circumstance of other samples. It remains to be seen how the gradation of pollutants varies with respect to the duration of the rainfall. Nevertheless, this study does exist as a valid data point and the merit of consideration is not weakened by any progression of dilution. The concentration gradient with respect to storm length for invisible pollutants, such as those in ionic form, remains as a topic of equal interest for the future.

There is, of course, considerable debate on the issue of the sources of contamination within our water supplies on this planet. I will not engage in that debate in this paper, as the purpose here is to simply provide another data point of reference that may be of service in helping to establish the accountability that is required. There are arguments by some that wish to frame a state of 'normalcy' for us, regardless of the level of contamination that as a species we now infest ourselves with. Regardless of various machinations that may be in vogue, we may all ask the questions of where standards evolve from, and whether or not we knowingly wish to deny the legacy of health knowledge that has been acquired over decades, if not centuries. We should also be called upon to use our united common sense and intuition, pray coupled with the best scientific information available, to act as stewards for our future, and to be worthy of such a title.

Clifford E Carnicom November 01, 2015.

Born Clifford Bruce Stewart January 19, 1953.

Secondary Rainwater Analysis : Organics & Inorganics

carnicominstitute.org/secondary-rainwater-analysis-organics-inorganics/

Secondary Rainwater Analysis : Organics & Inorganics

Clifford E Carnicom Nov 04 2015

ABSTRACT

A second rainwater sample has been evaluated. On this occasion, both organic and inorganic attributes of the sample have been examined. Although the sample investigated is of much larger volume, the results demonstrate an essentially equivalent level of aluminum present to that defined within the earlier report, i.e., approximately 2 PPM. This magnitude exceeds the US Environmental Protection Agency recommended standards for aluminum in drinking factor by roughly a factor of 10.

In addition, various organic attributes of the sample are introduced within this report.



Concentrated Rain Sample under Study in this Report Distilled Water Reference on Left, Concentrated Rainfall to Right



Residual Solid Materials from the Rainwater Sample of this Study

The volume of the sample collected is approximately 6.5 liters over a three day heavy storm period, collected in clean containers that are were exposed to open sky. The sample was concentrated by evaporation under modest heat to approximately 6% of the original volume. It is apparent from visual inspection and by visible light spectrometry that the concentrated rainfall sample is not transparent and that it does contain materials to some degree.



Visible light spectrum of the concentrated rainfall sample. The increase in absorption in the lower ranges of visible light correspond to the yellow and yellow-green colors that are observed with the sample.

The pH of the concentrated sample is recorded at 8.5; this value is surprisingly alkaline and indicates the presence of substantial hydroxide ions in solution. The pH of the solution prior to concentration measures at 7.5; this also must be registered as highly alkaline under the circumstances.

The pH of 'natural' rain water has been discussed in earlier papers and its relationship to the expected value of 5.7 due to the presence of carbonic acid in the atmosphere (carbon dioxide and water). The departure of natural rainwater from the theoretical neutrality of 7.0 is one aspect of the pH studies that I conducted in conjunction with numerous citizens across

the nation some years ago, and these reports remain available. The current finding is remarkably alkaline and, by itself, is indicative of fundamental acid-base change in the chemistry of the atmosphere.

From those early reports, it may be wise to recall the words of Paul Crutzen, Nobel Prize winner for Chemistry (Atmosphere, Climate and Change, 1995), who stated that *the most important chemical attribute of precipitation is indeed the pH value*. It behooves us, as a species, to act rather quickly on any reasonable claim to a significant change in fundamental atmospheric chemistry that may exist. It must be acknowledged that these same claims now prevail over decades of time, and that any dismissal as an aberration of no consequence is unjustifiably diminutive.

The sample has been examined again for the existence of trace metals using the method of differential cyclic chronopotentiometry, as described in the earlier report. The results are essentially identical to that of the earlier report, and once again the signature of a soluble form of aluminum is detected. The sample in this case, however, is of much larger volume, was collected over a longer duration, and was more highly concentrated that that in the preliminary report.

The concentration level was again determined, and the analysis indicates a level of soluble aluminum within the rainwater sample at 2.0 PPM. This compares quite closely with the earlier sample result of approximately 2.4 PPM. This determination once again takes into account the concentration process that has been applied to the sample for testing sensitivity purposes.

Two facts bear repeating here:

First, this value exceeds the US Environmental Protection Agency (EPA) standards for drinking water by roughly a factor of 10, again using the most conservative approach possible that can be taken.

Second, the previously referenced U.S. Geological Survey statement from the year of 1967 is valuable both in relation to evaluating the EPA standards as well as assessing the expectations of aluminum concentrations in natural waters:

PURPOSE AND SCOPE

Aluminum is an abundant constituent of rock minerals; in fact, only oxygen and silicon exceed aluminum in abundance among the elements present in the outer part of the earth's crust. Aluminum is, however, a very minor dissolved constituent in netural water. In routine water analyses the element is seldom determined, but the available information shows that, except for waters having a pH below 4.0, concentrations rarely exceed a few tenths of a part per million.

There is now a necessity to include an additional aspect of the rainfall analysis that has made its presence known more clearly. This is the case of biologicals. It is a fact, that in addition to the repeated detection of a trace metal at guestionable levels, certain organic constituents are coming to the fore. The test results are repeatable at this point and these organics will eventually require an equal accounting for their existence. I will not enter into an extended discussion of their potential significance at this time, as the first and necessary step is to place on the table that which must be confronted. My introductory suggestion at this point is to become aware of a previous paper on this site, entitled "A New Biology" to gain some familiarity with the scope of the issue. It is fair to say that along with changes of chemistry in this planet, we must also confront certain changes in biology that are in place. The history of this planet, the cosmos, life and our own species is dynamic, and intelligence itself is partially expressed in the ability to adapt to changing circumstances. We are in the process, whether we like it or not, of learning if and how guickly we can adapt to changes that have and are taking place, induced or otherwise. We may also choose whether to participate in the process (hopefully for the betterment of the world, as opposed to its detriment), or if we shall remain ignorant in an effort to ensconce ourselves in a purported comfort zone.

The methods of examination to be presented here are twofold: that of microscopy and that of infrared spectroscopy. Here are some some images that relate to the fact of the matter; they are repeated in both samples that have been examined:





Low Power (~200x) of Biological Filaments Contained in

Residual Materials from Concentrated Rainwater Samples

(The colors of the filaments are a unique characteristic (commonly red and blue) and they exist as an aid to identification with low power microscopy)





High Power (~5000x) of Biological Filaments Contained in Residual Materials from Concentrated Rainwater Samples

These images will not be elaborated on in detail at this time, as it may require a period of time to examine the information that has come forth here. They most certainly indicate a biological nature that shares a common origin with many of the research topics that have evolved on this site over the years. It may be worthwhile to begin by becoming familiar with the 'environmental filament' issue that is so thoroughly examined on this site. Since it seems clear that we are indeed dealing with an 'environmental contaminant' of sorts, the history of communication with the U.S. Environmental Protection Agency may also be worthy of review.

It would also seem to be the case that a significant portion of the residual material is inorganic as well, as in an insoluble metallic form. It may be that the insoluble residual material may be composed in part as an organometallic complex, based upon historical findings.

Regardless of the source or impact of these materials, it does seem to fair to state that an accounting for their existence in the atmosphere and rainfall is deserved. Each of us may wish to play a part in seeking the answers to such issues and questions before us all. I wish for this to happen, as I suspect many of us know that it is the right thing to do.

Clifford E Carnicom November 01, 2015.

Born Clifford Bruce Stewart January 19, 1953.

Tertiary Rainwater Analysis : Questions of Toxicity

Carnicominstitute.org/tertiary-rainwater-analysis-questions-of-toxicity-2/

Clifford E Carnicom Nov 08 2015

ABSTRACT

This paper presents evidence of a chemical signature that exists within an analyzed rain sample that is characteristic of known toxins and pesticides. The method of analysis used is that of mid-infrared spectroscopy. Specifically, certain functional groups involving sulfur, nitrogen, phosphorus, oxygen, and halogens have been identified in the analysis. It is recommended that the investigation be duplicated by independent researchers to determine if an environmental hazard does exist. If these results are verified to be positive, the source of the contaminants is to be identified and eliminated from the environment.



Infrared Spectrum of Concentrated Rain Water Sample (Aqueous Influence Removed)

The original rainwater sample volume for this analysis is approximately 3.25 liters. The sample was evaporated under mild heat to approximately 0.5% of the original volume, or about 15 milliliters. The sample has previously been shown to contain both aluminum, biological components, and a residue that appears to be an insoluble metallic or organometallic complex. The target of this particular study is that of soluble organics.

The organic infrared signal within the solution is weak and difficult to detect with the means available; it is further complicated by being present in aqueous solution. The aqueous influence was minimized by making an evaporated film layer on a KCI cell; the transmission mode was used. The signal is identifiable and repeatable under numerous passes in comparison to the reference background.

The primary conclusion from the infrared analysis is that a core group of elements exists within the solution; these appear to include carbon, hydrogen, nitrogen, sulfur, phosphorus, oxygen and a halogen. The organic footprint appears to be weak but detectable and dominated by the above heteratoms.

As further evidence for the basis of this report, qualitative tests for an amine (nitrogen and hydrogen), sulfates and phosphates (sulfur, oxygen and phosphorus) have each produced a positive test result. A qualitative test for a halogen in the concentrated rainwater sample has also produced a positive result; the most likely candidate at this point is the chloride ion. All elements present have therefore been proven to exist at detectable levels by two independent methods.

This grouping of elements is distinctive; they essentially comprise the core elements of many important, powerful and highly toxic pesticides. For example, three sources directly state the importance of the group above as the very base of most pesticides:

"In pesticides, the most common elements are carbon, hydrogen, oxygen, nitrogen, phosphorus, sulfur and chlorine".

Pesticide Residues in Food and Drinking Water : Human Exposure and Risks, Dennis Hamilton, 2004.

"We can further reduce the list by considering those used most frequently in pesticides: carbon, hydrogen, oxygen, nitrogen, phosphorus, chlorine, and sulfur".

Fundamentals of Pesticides, A Self-Instruction Guide, George Ware, 1982.

"Heteratoms like fluorine, chlorine, bromine, nitrogen, sulfur and phosphorus, which are important elements in pesticide residue analysis, are of major interest".

Analysis of Pesticides in Ground and Surface Water II : Latest Developments, Edited by H.J. Stan, 1995.

It is also true that phosphate diesters are at the core of DNA structure and that many genetic engineering procedures involve the splitting of the phosphate diester complex.

The information provided above is sufficient to justify and invoke further investigation into the matter. The sample size, although it was derived from an extensive storm over several days in the northwest U.S., is nevertheless limited and quite finite after reduction of the sample volume. The residual insoluble components (apparently metallic in nature) are also limited in amount and more materials will be required for further analysis. The signal is weak and difficult to isolate from the background reference; concentration level estimates for elements or compounds (other than that of aluminum which has been assessed earlier) is another entire endeavor. Systematic, wide-area, and long term testing will be required to validate or refute the results. All caveats above aside, it would seem that the duty to address even the prospect of the existence of such toxins in the general rainfall befalls each of us. It would seem wise that this process begins without delay.

There are a few additional comments on this finding that need be mentioned.

The first of these is the issue of local and regional vs. a national and international scope of consideration. It is understood that pesticides or compounds similar in nature are a fact of our environment, and that considerable awareness and effort is in place to mitigate their damage over decades of use. Organic farming and genetically engineered crops are two very divergent approaches to reconciliation with the impact of environmental harm, and they are shaping our society and food supply in the most important ways manageable. Given that the pesticide industry exists, regardless of our varying opinions of merit or harm, I think that it is fair to say that we generally presume that pesticides are under some form of local control. Our general understanding is that pesticides are applied at ground or close to ground level and are intended to be applied to a specific location or, at most, a region within a defined time interval.

The prospect, even I daresay, the hint, of pesticide or pesticide-like compounds in rainfall is more than daunting. It seems immediately necessary to consider what scale of operation would support such toxins finding their way into the expanses of atmosphere and rainfall? For the sake of the general welfare, I think we should all actively wish and seek to disprove the findings within this report. I will not hesitate to amend this report if honest, fair and accurate testing bears out negative reports over an adequate time period, and my motive never includes sensationalizing an issue. This is one test, one time, one place, with limited means and support in the process. I cannot disprove the results at this time and I have an obligation to report on that which seems to be case, uncomfortable as it might be. It is not the first time that I have been in this situation, and judging from the changes in the the health of the planet that have taken place, it is unlikely to be the last. The sooner that the state of truth is reached, the better we shall all be for it in any sense that is real.

The second comment relates to the decline of the bee population. Bees are an indicator species, the canary in the mine, as it were. The bees and the amphibians have both been ringing their alarm for some time now, and we best not remain passive about finding the reasons for decline. A minimum of 1/3 of our agricultural economy, *and that means food*, is dependent upon the bee population for its very existence. This is no trifling matter, and we all need to get up to speed quickly on the importance of this issue, myself included.

Suffice it to say that compounds of this nature, i.e, historical pesticides like organophosphates and the purported safer and more recent alternatives (e.g., the neonicotinoids), have a very close relationship to the ongoing and often ambiguous studies regarding bee Colony Collapse Disorder (CCD). From my perspective, it would seem prudent to eliminate the findings of this report as a contributing cause to the problem as promptly as possible. If that can not be done so readily, then we may have a bigger problem on our hands than is imagined.

One of the interesting side notes is that the elements and groups identified as candidates for investigation actually seem to overlap between the neonicotinioids and the organophosphates. This includes the nitrogen groups that characterize the neonicotinoids and the phosphate esters that characterize the organophosphates. If such a combination were at hand, this would seem especially troublesome as *both* forms remain mired in controversy, let alone any combination thereof.

The third and final comment relates to the toxicity of these compound types in general. It is not just an issue about bees or salamanders. These particular compounds have a history and effects that are not difficult for us to research, and we should become aware of their impacts upon the planet quickly enough. Many of us already are. The fact is that organophosphates have their origins as nerve gas agents in the pre-World War II era, and in theory their use has been reduced but hardly eliminated. Residential use is apparently no longer permissible in the United States, but commercial usage still is. This raises questions on what real effect any such "restrictive" legislation has had.

The neonicotinoids are promoted as a generally safer alternative to the organophosphates, but they are hardly without controversy as well. They too have strong associations with CCD in the research that is ongoing. They also are neuro-active insecticides.

It would seem to me that we all have a job to do in getting up to speed on the source, distribution and levels of exposures to insecticide and insecticide related compounds. A greater awareness of toxins in our environment, in general, also seems in order. If our general environment has been affected to a degree that has avoided confrontation thus far, then we need to face the music as quickly as possible. I trust that we understand the benefits of both rationality and aggressiveness when serious issues face us, and this may be another such time. I hope that I will be able to dismiss this report in due time; at this time, I cannot.

Sincerely,

Clifford E Carnicom Nov 05, 2015

Born Clifford Bruce Stewart Jan 19, 1953

Additional Notes:

The preliminary functional group assignments being made to the absorption peaks at this time are as follows (cm⁻¹):

- ~ 3322 : Amine, Alkynes (R2NH considered)
- ~ 2921 : CH2 (methylene)
- ~ 2854 : CH2 (methylene)
- ~1739 : Ester (RCOOR, 6 ring considered)
- ~1447 : Sulfate (S=O considered)
- ~1149 : Phosphate (Phosphate ester, organophosphate considered)
- ~1072 : Phosphine, amine, ester, thiocarbonyl
- ~677 : Alkenes, aklynes, amine, alkyl halide

The assignments will be revised or refined as circumstances and sample collections permit, however, as a group they appear to provide a distinctive organic signature. A structural model may be developed at a future date.

Some chemical compounds which *may* share some similar properties to that under consideration here include, for example, (not all elements included in any listed compound; only for reference comparison purposes):

p-chlorophenyl (3-phenoxypropyl)carbamate

N-(1-naphthylsulfonyl)-L-phenylalanyl chloride

2,2,2-trichloroethyl 2-(2-benzothiazolyl)dithio-alpha-isopropenyl-4-oxo-3-phenylacetamido-1azetidineacetate

cytidine monophosphate

diiodobis(triphenylphosphine)nickel(II)

per :

SDBSWeb : <u>http://sdbs.db.aist.go.jp</u> (National Institute of Advanced Industrial Science and Technology, Nov 06 2015)