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REVIEW

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PRECONCENTRATION TECHNIQUES FOR TRACE ELEMENTS ANALYSIS IN WATER

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Summary

A study of the preconcentration techniques used in multielement trace analysis of water is presented, based mainly on bibliographic research. The preconcentration methods which are evaluated are evaporation, surface adsorption, liquid-liquid extraction, precipitation, ion exchange, ion exchange with impregnated materials and electrodeposition. For each method a brief description of the procedure is given, as well as the cases where the method is applicable with its advantages and limitations. The purpose of the paper is to give the reader a simple and concentrated reference for the selection of the most suitable preconcentration technique in each case of water analysis.

Key words: Water analysis, preconcentration, trace elements.

1. Introduction

Water analysis is a complicated procedure with many problems waiting to be solved yet. The difficulties start from the very beginning, namely in choosing the sites for sample collection, the depth of sampling, the weather conditions and other time dependent factors which may affect the results. Among the latter the most serious problem, which is directly related to the nature of fresh water, is the thermodynamic equilibrium. As mentioned in the report of Pytkowicz et al.[1] "the physical chemistry of sea water", transport and mixing rates in the oceans are much faster than molecular diffusion so that thermodynamic equilibrium is not generally reached. Problems also arise during the storage and transport of the water samples, such as the adsorption of constituent on the walls of the vessel, volatilization etc.

Besides the above mentioned general problems, one faces difficulties related specifically to trace elements analysis. The most serious among them are the low concentrations of the elements which are often below the minimum detection limits (MDL) of the analytical techniques for the non-treated samples and the interference of the major elements. The latter may affect the detection and the quantitative determination of the trace elements even in cases with concentrations much higher than the MDL. These problems necessitate a procedure of preconcentration and separation before the analysis. In fact these are two different preliminary procedures, which however are mentioned in bibliography under the general title of

"preconcentration", since they are usually performed simultaneously. In addition one should mention the general problems of accuracy, precision, reproducibility, etc., which are also serious as deduced for example from the "Intercomparison Test of the International Atomic Energy Agency".[2]

Several methods of preconcentration are described in bibliography. The most commonly used, which are presented in this study, are: evaporation, surface adsorption, extraction, precipitation, ion exchange, ion exchange with impregnated materials and electrodeposition. None of the above methods has prevailed since the choice depends on various factors such as the type of water to be analyzed, the elements of interest, the analytical technique to be used etc. As it is expected the greater difficulties arise in the case of sea water.

The purpose of this study is to contribute in the evaluation of the different techniques of preconcentration, indicating the advantages, the problems and the limitations of each method, in order to help the experimenter in choosing one of them according to the circumstances. The abbreviations used for the various reagents and analytical techniques are summarized in Tables I and II respectively. The study

TABLE I: Abbreviations of reagents.

A.C. Active Carbon. APDC Ammonium Pyrrolidine-Dithio-Carbamate. DDTC Dyethylammonium DiThio-Carbamate. DEN 2,2'-diamino-diethylamine. DIBK Di-Iso-Butyl Ketone. EDTA Ethylen-Diamine- Tetracetic Acid. HD dionyl-naphthalene-sulphonic acid. HQ 8-Hydroxy-Quinoline. MIBK Methyl-Iso-Butyl Ketone. NaDDTC Sodium Diethyl-DiThio-Carbamate. PAN 1-(2-PyridAzo) -2-Naphthol. TEPA TetraEthylene-PentAmine.

TABLE II: Abbreviations of analytical techniques.

AAS Atomic Absorption Spectroscopy. ICP-AES Inductively Coupled Plasma Atomic Emission Spectrometry. INAA Instrumental Neutron Activation Analysis. NAA Neutron Activation Analysis. PIXE Proton Induced X-ray Emission. XRF X-Ray Fluorescence spectrometry.

was based on the bibliography mentioned in the text and to some extent on our experience from water analysis in our Laboratory using the PIXE technique. One may also find useful the recent review articles by Ellis et al.,[3, 4] Van Grieken[5], Fishman et al.,[6] Leyden et al.,[7] and Zolotov.[8]

2. Solvent Evaporation

2.1. Large volume samples.

The evaporation of the water, so that one can obtain for analysis the solids contained, is probably the most "straight-forward" preconcentration method, and one of the most commonly used for fresh water.

First we shall consider a case where one is faced with the evaporation to dryness of a large volume of liquid, usually of the order of a few hundreds of ml. The evaporation is usually performed by heating the solution at atmospheric pressure. Details for this method are not given in bibliography, except that the evaporation is

usually performed by heating the solution on a heating plate[9-11] or by an infra-red (I.R.) lamp.[12] The evaporation by an I.R. lamp appears to give better results (tests in our laboratory) because it is a faster process and produces smooth evaporation without the danger of boiling. The most important advantage therefore is that it needs no attendance. One may use a porcelain dish or a large beaker heated by a 250 or 350 W I.R. lamp. The time required for the evaporation is about 3 to 4 hours for 250 ml of water and it depends on such factors as shape of the vessel, distance of lamp etc. If necessary the evaporation can take place in an isolated space (for example under a glass bell) which is continuously supplied by filtered air.

The evaporation in most cases is continued to dryness as the precipitation starts very soon at the surface, and it would not produce a uniform small volume of liquid, if it is so desired. Exceptions to this rule are refered by Tanaka et al..[13] Thompson et al.,[11] and Hashimoto et al.[10] where the evaporation continues down to 1 ml of liquid sample. The recovery of the salts can be achieved by scraping them from the inner side of the vessel, and it must be as complete as possible in order to get a more representative sample; and this, because the less soluble salts precipitate earlier than the more soluble ones. The evaporation of 250 ml of tap water usually yields 50-100 mg of precipitate. For the case of the river water the residue is usually less, but there is the example of Thames[14] where 600 mg of precipitate have remained from 1 l of filtered water. The evaporation does not have to be continued to dryness in the case where one intends to redissolve the residue, usually by a small volume of an acid.[15]

Some other techniques, besides heat-evaporation, are the freeze-drying,[14] (which is actually equivalent to sublimation of the solvent) and the vapor filtration.[16, 17] The latter method has been used for volumes up to 30 ml and it is based on the use of a membrane which is permanable by the vapours but not by the liquid matter.

2.2. Small volume samples

This case is applied mainly for the preparation of thin samples, by evaporating a small volume of liquid, of the order of a few micro-litres, onto a suitable backing material such as Mylar, Kapton, etc.[15, 18-21] The samples which are thus prepared are usually used directly as targets for the PIXE or XRF methods. The targets can be prepared by deposition of a large drop of the solution directly on the backing material, or even better the solution may be sprayed in the form of finaly dispersed droplets with various techniques such as air spray or electro-spray on a stationary or a rotating target.[22] The sample is then dried in a dessicator (vacuum or calsium sulfate) or air-dried.

The method is simple and requires only a small sample quantity. Some disadvantages are the uncertainty in the volume of the deposited liquid and the limited use of the method to specific analytical techniques. In addition, in the case of the drop evaporation there is also the problem of homogeneity due to fractional crystallization and different migration speeds of the compounds on the backing material. The suggestion of Robaye et al.[23] for addition of liposomes (synthetic phospholipid bilayer vesicles) to the samples as homogenigers could be of particular interest.

2.3. General remarks

Evaporation is a simple method, and no attendance is required during the process. The recovery is quantitative for all non volatile elements, and independent of their chemical form. An analytical examination of the problem of the loss of the volatile elements during the evaporation process has not been found. Nakahara et al.[24] report that the loss of arsenic during the dry up process is negligible. Goulden et al.[25] use evaporation for the analysis of arsenic and selenium after some chemical pretreatments. Another advantage of the evaporation is that there is no need for any reagents to be used, which may contaminate the sample.

On the other hand evaporation can not be used for sea water samples because of the large content of Sodium Chloride; difficulties may arise during the analysis of volatile elements; and finally, the inhomogeneity of the precipitate due to fractional crystallization is an additional problem which can be partially solved by redissolving the residue with a smaller quantity of a solvent.

In conclusion evaporation is proposed for the analysis of all types of water (tap-water, river water, etc.) except of sea water, and it can not be used in cases of interest in volatile elements.

3. Surface adsorption

This method is based on the same principle as the liquid-liquid extraction. The ditribution of a chemical compound into two non-mixing phases can concentrate the compound into one of them almost quantitatively. Here one of the phases is solid.

The general process for this method is the following: The trace elements are been complexed with an organic reagent, the complexes are adsorbed on a surfactant solid and then the solid is separated from the liquid phase. Attempts for the adsorpion of the dissolved ions directly on Active Carbon (A.C.) surface did not give satisfactory results, as the capacity of the A.C. is too small in this case; this is the result of the organic nature of the A.C. phase.[26] However some authors[28-30] still use simple A.C. adsorption.

Vanderborght et al.,[26, 27] propose the use of 8-hydroxy- quinoline (HQ) (oxine) as complexing reagent of the trace elements, and adsorption of the chelate complexes onto A.C. The reagent HQ forms complexes with most of the elemets of intermediate atomic number, except alkali and alkaline earths. The volume of the water used is 1 l and the time needed for the equilibrium between A.C. and the metal chelates about 1 h. The most suitable quantities of A.C. and HQ in various cases are given by Vanderborght et al.[31] The recoveries of the elements vary between 90 and 100% and the enrichment coefficients are of the order of 10,000 for ground and tap water, 7,000 for surface water, and 20,000 for sea water. The method has been applied in combination with XRF, INAA, PIXE and ICP-AES elemental analysis. Akselsson et al.[32] have applied this method for rain water and sea water analysis.

Other complexing reagents reported are Ammonium Pyrrolidine Dithio-Carbamate (APDC),[33, 34] dithizone, and sodium diethyl dithio-carbamate (NaDDTC).[28, 29] The first of these (APDC) is claimed to be suitable for waters with high contents of calcium and magnesium, e.g. sea water, because it does not form chelates with any of the alkaline earth metals in contrast with oxine which has a

recovery factor of about 10% for magnesium and calcium. For dithizone and NaDDTC a comparative table of recoveries can be found in Lieser's papers.[28, 29]

With regard to the adsorber, some other materials tested besides A.C. are Quartz Glass,[34] Tungsten Wire,[35] cellulosepiperazinedithiocarboxylate,[36] HQ immobilized on Silica Gel,[37] and C-18 bonded Silica Gel in the form of a column.[38] The use of a dithiocarbamatecellulose derivative for the determination of 4 elements has also been recently reported.[39] Heuss et al.[28] in a comparison study of various adsorbents report that silica gel and aluminium oxide were excluded because of contamination with trace elements.

Adsorption is a fast method and the recovery is quantitative for most of the elements of interest (Cr, Mn, Ni, Cd, Hg etc.). It can be used successfully for sea water and hard water and it also suitable for natural water which contains strong organic complexes. This is an advantage compared to ion exchange and coprecipitation which can not concentrate these complexes. However, if the quantities of organic compounds are high they can saturate the capacity of A.C.; this is a real danger only for concentrated solutions (e.g. sewage treatment of plant effluents[26]). With regard to the reagents used, oxine and APDC appear to prevail, although it is our opinion that more research is needed on the subject. On the other hand A.C. seems to have no opponent as adsorber.

Disadvantages of the method are the uncertainty on the recoveries (about 10%) and the problem of contaminated A.C. In our laboratory A.C. was found to contain manganese, iron, nickel and copper. The amounts of the contaminants can be reduced with different pretreatments,[40, 41] but not always to satisfactory levels. Furthermore the addition of A.C. reduces the sensitivity of the method in PIXE and XRF but has no influence for the case of NAA.

4. Liquid-Liquid Extraction

Extraction is a widely used method to preconcentrate trace elements, especially when the detection is to be performed spectro-photometrically. When the distribution of a substance between two or more phases is shifted towards one of them, then the substance may concentrate at this phase almost quantitatively. In this case one of the phases is our sample and the other a suitable non-mixable with water organic solvent. The increase in the concentration of the trace elements is achieved by their transportation to the organic phase whose volume should be evidently smaller than the volume of water. In order to make this transportation possible the metals should be previously complexed with a suitable organic ligand and form non-ionized molecules. Therefore the two basic parameters which define an extraction are the complexing reagent and the organic solvent.

A method in common use is the complexing of metal ions with APDC or Dyethylammonium Dithiocarbamate (DDTC). NaDDTC which has nearly the same chelating properties as the DDTC[42] has also been used. The organic solvent is usually Methyl Iso-Butyl Ketone (MIBK) or Di-Iso-Butyl Ketone (DIBK). The method can be successfully applied even for sea water as it can be deduced from the papers that have been published on the subject.[9, 43] A more extensive study of the extraction method is given by Kinrade et al.[44] where the chelating agents APDC and

DDTC have been chosen, after testing nine different agents from the point of view of their ability to extract most of the metals of interest for a sufficiently wide range of pH. In the same paper MIBK was chosen as the most suitable solvent between eight candidates. The time required for the stirring of the two solvents (water, organic solvent) is 30 sec and the time for equilibrium 5 min. The study of the stability of the metal complexes in MIBK has shown that they all remain stable for close to 9 hours. In the papers of Tsu Kal Jan et al.[45] and Magnusson et al.[46] the extraction is followed by a second stage, called back extraction with nitric acid, in order to stabilize the metal complexes for a longer period. In this way the complexes remain stable for more than one week with the exception of silver. The method was applied for 200 ml spiked sea water and the recoveries for 7 elements were found to be more than 65% with an average value of 82%. However, the recovery of silver was less than 20%. A disadvantage of MIBK which is used as organic solvent is its solubility in water, which is 2.15 ml/100 ml water at 25°C and it is highly altered by temperature changes and by the ionic strength of the liquid phase. The advantage of MIBK is the high sensitivity achieved in AAS.

The same reagents APDC and DDTC are used by Danielson et al.[47] in combination with freon TF as organic solvent. Chloroform and carbon tetrachloride were excluded because of their possible carcogenetic properties. Extraction was again followed by back extraction with nitric acid. Bone et al.[42] describe a method using the same complexing reagents for the analysis of 10 elements found in natural water, and in this case the organic solvent is DIBK which is prefered to MIBK because it is less mixable with water.

Another reagent which can be used for the transportation of the metal ions to organic phase is dinonyl naphthalene sulfonic acid (HD) as well as its salt sodium dinonyl naphthalene sulfonate.[48] HD is known as a liquid cation exchanger and exchanges ions according to the reaction:

$$M^{m+}\ (aq) + (HD)_n\ (org) = MH_{(n-m)}\ D_n\ (org) + mH^+\ (aq)$$

where aq = aqueous phase, and org = organic phase. A thermodynamic study of the extraction of trivalent Am (3+), Cm (3+) and Cf (3+) with HD has been published by Raieh et al.[49] The HD procedure in general is described by Yang et al.[48] In this 50 ml of HD solution in n-hexane are added to 500 ml of the water to be analyzed. The organic phase is then stripped with 5 ml nitric acid which is finally analyzed with NAA. HD was also used by Wans et al.[50] for the determination of zinc.

An interesting way of handling the problem of separating the two phases is presented by Fujinaga et al.[51] The liquid-liquid extraction is performed at a high temperature followed by a solid-liquid separation at room temperature. The complexing reagent used is 8-hydroxyquinoline in diphenyl as extractant. The same problem is similarly handled by Fujinaga et al.[52] (Procedure B) using HQ and o-phenylphenol. Burba[53] suggests the use of 1- (2-hydroxy-5- b-hydroxyethyl- sulfonyl-phenyl- azo) -2-naphthol (Hyphan I) and extraction with MIBK.

A more specialized method for the determination of Be using gas chromatography is the one applied by Ross et al.[54] In this Be is complexed and at the same time extracted with trifluoroacetylacetone. The extraction process is easy

and quantitative, whereas the lowest detection limit is approximately 4×10^{-13} gr Be.

The final remarks for the method of extraction are as follows: It is recommended for those analytical methods which use liquid samples. It is quite fast and may be applied for sea water as well. On the other hand it has low concentration factors (for a single extraction their value can not be higher than 100) and stability problems of the complexes in the organic phase. One way of overcoming these difficulties is to perform the extractions in a series. This however, apart from the fact that it requires more work, increases the problem of losses that take place during the separation of the two phases. Finally there are limitations for the simultaneous determination of many elements.

5. Precipitation

This is one of the oldest methods that have been applied for the preconcentration of elements in solutions. It can be mainly used in combination with a technique which can analyze directly solid samples. The precipitation of the elements of interest may take place either directly using some reagent (organic or inorganic) or with the cooperation of another element which is easily precipitated by the reagent and exists in the solution in a sufficient quantity (coprecipitation). With the method of coprecipitation one may overcome various problems such as the possible oversaturation of the solution, the small size of the particles of the precipitate, etc. Coprecipitation is a fairly complicated process.

The following reagents have been used for precipitation or coprecipitation of trace elements:

a) 8-Hydroxyquinoline (other names: oxine, 8-quinolinol). Silvey et al.[55] use the above reagent in combination with tannic acid thionalide as coprecipitants. Fujinaga et al.[52] examined several organic coprecipitants, and o-phenyl-phenol was found to be the most effective among them; the recoveries under suitable conditions were reported to be quite satisfactory. An advantage of the method is the ability of HQ to form complexes with most of the trace elements, and the lowest detection limit is 1 ppb. A critical point of the method is the possible contamination from the reagents. In the paper of Luke[56] which was published before this of Fujinaga et al.[52] the use of HQ as a precipitant is characterized disappointing, with no further explanation.

b) Carbamates (DDTC, APDC and diethylammonium diethyl-dithiocarbamate). Carbamates have been used in many cases[56-63]. Large quantities of iron (more than 2 ppm) create problems in the determination of Zn. For this reason in the paper of Watanabe et al.[57] tartrate is proposed as a masking agent for Fe. Holynska et al.[64] suggest the determination of Hg by using EDTA as a masking agent for Zn. In the paper of Boyle et al.[58] the precipitate is redissolved with acid-acetone solution and then evaporated. Pik et al.[59] used Mo as carrier, Boyle et al.[58] used Co, and finally Cu or Ni were used by Watanabe et al.[57] Recently, precipitation with a mixture of dibenzylammonium dibenzyl-dithiocarbamate and sodium dibenzyldithiocarbamate has been reported for the separation of 22 trace elements.[65]

c) 1-(2-Pyridazo)-2-napthol (PAN). This reagent was used as a precipitant by Puschel[66] and by Watanabe et al.[57] In the latter PAN is compared with DDTC. The disadvantages of PAN are its low solubility in water and the fact that the

precipitates produced float on the surface of the water and tend to adhere to the walls of the vessel, so that a surfactant solution has to be added. Finally, the time needed for the reaction between metal ions and PAN is rather long. On the other hand the determination with PAN has better precision. In a more recent paper of Vanderstappen et al.[67] seven elements were determined with this method with reported recoveries more than 90% and detection limit approximately 1 ppb. The effect of organic matter on the recoveries are reduced by 15% for a 2 ppm concentration of humic material (typical concentration for several surface waters). This may be the explanation for the comparatively lower values of the various elements in the analysis of tap water by this method.

Finally other reagents reported for this technique are phenylfluorone, cupferron, ammonium hydroxide, iron hydroxide, magnesium hydroxide, and indium hydroxide.[56, 68-70]

To summarize, precipitation is a simple process which can be used even for sea water, since sodium does not precipitate easily. The process is fast, the enrichment factors are high and the precipitates homogeneous. Some problems can be faced, as previously mentioned, with coprecipitation, but in this case the coprecipitant can not be determined. On the other hand problems can arise from several factors such as solubility of the precipitate, pH regulation, the presence of organic matter which may act as a complexing reagent and the presence in excess of certain ions, e.g. iron. Additionally, in several cases in which the solution is heated or even boiled to speed the reaction or remove part (or the whole) of the solvent, errors due to evaporation may be introduced. Finally, an especially important factor is the hazard that contaminations may be introduced from the reagents used.

6. Ion Exchange

One of the most conventional ways of preconcentration is the exchange of the trace elements in a water solution with others (usually Na, H or ammonium ion) which are attached on an inert matrix, named ion exhange resin. The process is reversible, and using elution it is possible to bring the trace elements in a smaller volume of solution.

The basic techniques with which the solution can come in contact with the resin are the batch extraction and the column technique. In the first a quantity of resin is added into the solution and stirred for a period of time. The solution is then filtered and the resin obtained is pelletized[71, 72] and directly analyzed. Batch extraction may be applied even when the amount of the resin used is too small for a column to be made, through which the solution could pass with a satisfactory large flow rate. The technique has also the advantage of the homogeniety of the trace elements on the resin.[73, 74] In the column technique resin is placed into a cylindrical vessel and the solution passes through it. This technique is used more often than the previous one and it is applied combined with elution of trace elements from the resin, without excluding the possibility of a direct analysis of the resin.[75-77] This technique is also applied for the separation of a specific element from the others, and it is described in most of the following references.

Among all resins in use, Chelex 100 prevails. It is a purified form of the Dowex

A-I resin. It is a resin based on iminodiacetic acid substituted with cross linked polystyrene, and its chemical behavior is similar to the one of EDTA. It is highly selective for bivalent and multivalent metal ions. Riley et al.[78] were the first who studied extensively the use of this resin for the concentration of trace elements. Their method was then used by several laboratories. The recoveries of 16 different elements were found to be perfect (99-100%). However problems arise for some elements, among which are As, in the form of As_2O_3, Cr and Hg. For the Cr (III) and Cr (VI) a method of determination using Chelex 100 is described by Leyden et al.[79] In the paper by Riley et al.[78] the amount of sample used is 1 l and the flow rate about 5 ml/min. The range of pH used varies from 5.0 to 9.1. Below pH 5 the uptake of trace elements is drastically reduced, while above 9.1 Ca and Mg start precipitating. Most elements are taken up at the natural pH 7.7-8.2, although there are many exceptions. The resin remains stable in acid as well as in alkaline conditions. However, the change of the central ion creates serious problems to the monitoring of the flow rate, because of the simultaneous change of the size of the resin particles. Florence et al.[80] express some objections for the use of the Riley and Taylor's method on the quantitative concentration of Cu, Pb, Cd and Zn. The recoveries described by them are based on spiking of the samples with radiotracers, which is the most widely used method. This method can determine the recoveries of the metals in the solution having the form of free ions, but not of the complexed ions. In order to destroy the organic complexes, Florence et al.[80] suggest a method for the irradiation of water using an ultra violet (U.V.) lamp in the presence of hydrogen hyperoxide. Furthermore, Kingston et al.[81] suggest the use of a mixture of the resin with either Pyrex glass powder or another inert material (Silica gel etc.) of the same mesh size, in order to get a more constant flow rate. Kingston et al.[81] study more specifically the problem of interferring elements and use ammonium acetate for the selective elution of Na, K, Ca and Mg. In the paper of Hirose et al.[82] the resin containing the trace elements is first activated with neutrons and then the trace elements are eluted. Greenberg et al.[77] analysed 12 elements directly on Chelex 100 with NAA without elution. One of the disadvantages of Chelex 100 is the small selectivity towards transition elements. Charachteristically, it is mentioned by Leyden et al.[83] that for Chelex 100 the distribution factor for Ca is approximately the same as the one for Zn. Leyden et al.[75] suggest the use of resin prepared by tetraethylenepentamine (TEPA) and toluene diisocyanate. This resin is more selective for the ions of the transition elements. The recoveries are found to be constant in the range of normal ion concentrations in natural waters. Lenvik et al.[84] uses the anion exchange resin Dowex 1-X8 followed, after the elution, by concentration and precipitation processes. In this way six elements have been detected. In the paper of Mlyazaki et al.[85] Cr (VI) and Cr (III) are determined using polydithiocarbamate chelating resin.

Burba et al.[86] use cellulose exchanger, called Hyphan, which contains Hyphan I as anchor group. Seven elements have been determined with concentrations in the range of ppb. The same exchanger is also used by Lieser et al.[29]

Other resins have also been used but to a lesser degree. Corsini et al.[87] have used the macromolecular acrylic ester resin SM-7 for the determination of 8 elements. Van Niekerk[73] determined U using the anion exchange resin AG-1X with an

enrichment factor of 250. Collin[88] determined Sr in solutions of calcium acetate using the cation exchange resin Dowex 50W-X8. Both papers are quite old.

In conclusion the ion exchange technique using resins was found to be a handly method with many capabilities. It is possible to analyse directly the trace elements on solid resin, thus taking bigger enrichment factors, or take them in the form of a concentrated liquid solution. It can be successfully applied for most of the ions and for sea water analysis as well. From the point of view of the time needed, there are usually several hours needed as shown from the flow rates and the volumes of the samples mentioned. The main problems are: a) the monitoring of the pH while eluting different elements; however several elements are eluted at the noraml pH values, b) the presence of the interferring elements which occupy the sites of the resin as the case of Ca which has already been mentioned, c) the fact that the recoveries of the elements are not quantitative, especially when complexing reagents or non-ionized molecules are present, and d) the possible contamination which may be caused by the reagents used.

7. Ion Exchange Impregnated Materials

7.1. General description

The creation of the ion exchange impregnated materials (IEIM) was the result of an effort to modify the ion exchange techniques in order to enable their wider and more convenient use. The IEIM may be divided in two groups: the ion exchange impregnated papers and the ion exchange membranes which have much more limited applications. The immobilization of a resin onto an inert material and the retention of the trace elements by the resin can produce a target suitable for the x-ray methods. Elution of the trace elements from the IEIM has not been found in the bibliography. The biggest problem of IEIM is the restricted capacity of these materials for ion retention, as it will be mentioned below.

7.2. Ion exchange resin loaded papers (RP)

Several extraction techniques can be used[89] which are all based on the filtration of the liquid sample through RP. The filtration should be repeated many times in order to achieve a quantitative recovery. The bathing technique requires a quite long equilibration time. The filtration through the RP may take place a) with the use of a pump resulting in high flow rates, b) with an "hour-glass" system which allows the use of both sides of the filter and leads to better homogeneity, and c) with the use of a dropping procedure which makes easier the monitoring of the flow rate. Hooton et al.[90] suggest a filter unit for the "hour-glass" technique and they also mention other systems that have been used. In most cases 7 filtrations are mentioned to be satisfactory for a quantitative recovery with SA-2 RP. The types of RP used more frequently are SA-2 RP (Strong Acid 2) for cation exchange SB-2 RP (Strong Base 2) for anion exchange. The types WA-2 and WB-2 (Weak Acid and Weak Base) as well as Chelex 100 Impregnated Papers are also in use. They contain approximately 50% cellulose and 50% ion exchange chelating resin. Stephen et al.[91] mention also the use of SRXL RP which was prepared using a Srafion NMRR type chelating

resin selective for Au, Pt, Hg and methyl mercuric forms of Hg. The NMRR is a commercial resin of the general structure $[R\text{-}C\ (NH)\ (NH_3)^+]_n\ Cl^-_n$. An extensive study of the characteristics of the first four types of RP is given by Campbell et al.[92] Regarding pH, the study for SA-2 has shown that a pH of about 2 is satisfactory for the concentration of most of the elements. The characteristics of Chelex 100 RP have been studied by Van Grieken et al.[93] where the dependence of the recovery on pH is shown graphically for 3 elements. A similar study was reported by Stephan et al.[91] The best pH value required is different for the various elements. The presence of the alkali metals and alkaline earths creates a problem which, as mentioned by Van Grieken et al.,[93] makes possible the use of Chelex 100 for rain water only.

Becknell et al.[94] use SB-2 RP for the detection of mercury with NAA after complexing Hg with chlorine. In the papers of Cesareo et al.[89] and Holynska et al.[95] SA-2 have been used for the analysis of trace elements in water. In the second paper strong acid ion exchange foils are used and the phenomenon is studied from the kinetics point of view. The maximum allowable limit of Ca concentration is reported to be 50 ppm. The RP SA-2 has also been used for the detection of trace elements in Mo[96] as well as for the detection of rare earths in perchloric, nitric, and hydrochloric acid solutions.[97] Of interest here is the use of the novel chelating cellulose filter with 2,2'-diamino- diethylamine (DEN).[98] The advantages are that it is insensitive to practically all naturally occuring abundant substances, and no pH adjustment is required. In the paper of Smits et al.[99] DEN filters are used for trace anion preconcentration. The efficiency of the method is strongly depressed with salt concentrations above 0,01 M.

As previously mentioned, the most serious disadvantage of RP is their limited capacity for ions. This prevents them to be used for the analysis of hard water and sea water. For these reasons Kingston et al.[100] suggest the use of SA-2 for sea water after using the Chelex 100 method[80] for the removal of the major elements.

7.3. Ion exchange membranes

The use of the ion exchange membranes is especially limited because of the adsorption of the major elements, and there are few bibliographic references. Lohmuller et al.[101] suggest a semiquantitative method for the detection of the changes of metal distributions in a water system.

7.4. Remarks

In conclusion the study of the ion exchange impregnated materials has shown that the problem of their small capacity constitutes a highly restrictive factor. Due to this and in spite of the fact that they offer an easy technique they can be used directly only to fresh water with a low Ca concentration.

8. Electrodeposition

Electrodeposition is a method of preconcentration with restricted applications in the analysis of natural water samples. The relevant papers refer mostly to the

analysis of standard solutions of several ions and to the determination of the most suitable conditions for this.

The principle of the method is the deposition of dissolved metal ions onto a suitable electrode using electric current. In bibliography[102-105] several apparatus are suggested for this purpose. The deposition usually takes place at the cathode of the cell used. However Wundt et al.[104] have achieved anodic electrodeposition of 12 elements after transforming them to their cyanometalic complexes, with the use of an ion exchange resin. The choice of the electrode material should be made so that it does not increase the background during detection. Graphite is most frequently used either in the form of graphite cloth electrodes[103] or graphite rods.[105] Mercury can also be used as a cathode. In this case there is a possibility of mercury distillation after the deposition.

Electrodeposition, as a method of preconcentration of water solutions for the multielement analysis, presents serious problems. The main one is the dependence of the quantity of the metals deposed on the composition of the solution. As mentioned by Marshall et al.[103] it is not possible to predict the relation between the composition of the deposit and relative concentrations of the metals in the solution. Another disadvantage in the case of water samples with low conductivity is that another electrolyte must be added with all the contamination hazards involved. In XRF the use of graphite as a backing material creates high background problems. Finally, there are time limitations for the case of the deposition of the whole load of the metals.

A relatively recent review on the subject electrochemical preconcentration methods has been published by Krasilshchik et al.[106] which, however, does not only refer to water samples.

9. Discussion

For the detection and determination of intermediate and heavy trace elements in water the most frequently used methods on analysis are Atomic Absorption Spectroscopy (AAS), Neutron Activation Analysis (NAA), X-Ray Fluorescence (XRF), and Proton Induced X-ray Emission (PIXE). A large number of elements may be detected with the above methods, but the main interest is on the toxic elements As, Cd, Hg, Ni, Pb and V.

From the bibliography mentioned and from the conclusions in each of the previous chapters we may assume that the use of some preconcentration method is always necessary before the analysis of a water sample. The elements that can be analyzed directly in the original sample are very few (Ca, Na and Cl). Specific problem in the case of sea water is the separation of NaCl which prevents the detection of the trace elements.

As shown in this study the method of preconcentration used in each case is a function of several factors, as it has already been noted in the introduction. However, in a statistical manner one may say that (Van Grieken,[5] table-1) precipitation is the method used more frequently, followed by ion exchange, evaporation and ion exchange impregnated materials. At any rate even this way of evaluation is of limited importance.

On the other hand we may devide the problem of water analysis in sea water analysis and analysis of other types of water. From this study it is obvious that for the latter type of analysis the quiet evaporation technique is suggested with the hazard of losing the volatile elements such as As, Se and Hg (the case of Hg will be mentioned below). For sea water analysis the evaporation technique can not be used as an independent method. In this case the problem is quite complicated in order to suggest a universal solution.

The rest of the techniques should be examined from the point of view of the elements of interest, the final form of the sample received (solid in the surface adsorption, precipitation, ion exchange impregnated materials and electrodeposition, liquid in extraction, solid or liquid in ion exchange), the convenience in use, the contamination dangers and the time required. For mercury, which presents a special interest as well as special difficulties in the analysis, details are discussed in the review article of Chilov[107] entitled "Determination of small amounts of mercury".

Περίληψη

Τεχνικές προσυγκέντρωσης για την ανάλυση ιχνοστοιχείων σε νερά

Η απ' ευθείας ανάλυση ιχνοστοιχείων σε νερά είναι συχνά αδύνατη αφού πολλά από αυτά βρίσκονται σε συγκεντρώσεις κάτω από τα ελάχιστα όρια ανίχνευσης των χρησιμοποιούμενων μεθόδων (φασματοφωτομετρία ατομικής απορρόφησης, νετρονική ενεργοποίηση, μέθοδοι εκπομπής ακτίνων Χ κλπ.). Προβλήματα δημιουργούνται επίσης μερικές φορές από την παρουσία στοιχείων σε περίσσεια, όπως στην περίπτωση του Θαλάσσιου νερού (NaCl). Γι αυτό είναι απαραίτητη πριν από την ανάλυση μια μέθοδος προσυγκέντρωσης (preconcentration) και ταυτόχρονα απομάκρυνσης των στοιχείων σε περίσσεια που παρεμποδίζουν.

Οι τεχνικές προσυγκέντρωσης που χρησιμοποιούνται περισσότερο είναι η εξάτμιση, η προσρόφηση, η εκχύλιση, η καθίζηση, η ιονταναλλαγή με ρητίνες ή με υλικά εμποτισμένα με ρητίνες, και η ηλεκτροεναπόθεση. Για κάθε τεχνική δίνεται μια συνοπτική παρουσίαση της χρησιμοποιούμενης διαδικασίας, οι περιπτώσεις στις οποίες η μέθοδος είναι εφαρμόσιμη, τα πλεονεκτήματα, τα μειονεκτήματα και οι δυσκολίες που παρουσιάζει.

Σκοπός της εργασίας είναι να δώσει ένα απλό και συγκεντρωμένο βοήθημα για την πρώτη επιλογή της πιο κατάλληλης μεθόδου προσυγκέντρωσης ιχνοστοιχείων κυρίως σε φυσικά νερά πριν από την ανάλυση και μια εκτενή βιβλιογραφία για την περαιτέρω αναφορά του ερευνητή.

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SYNTHÈSE DES PHÈNYLALKYLAMINES ENCOMBRÉS

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Résumé

L'interêt du présent mémoire se porte à la synthèse des diméthyl-4,4 aryl-3 pentylamines I, qui résultent théoriquement des diaryl-3,3 propylamines II, pharmacologiquement actives, en remplaçant l'un des aryles avec un radical tert-butyle. La préparation des amines I est effectuée à partir des acides β-tert-butylcinnamiques XI, qui par hydrogénation sont transformés aux acides β-tert-butyl-β-arylpropioniques XII; par l'intermediaire des chlorures XIII de ces derniers on obtient les β-tert-butyl-β-arylpropionamides XIV, qui par réduction avec $LiAlH_4$ fournissent les amines I.

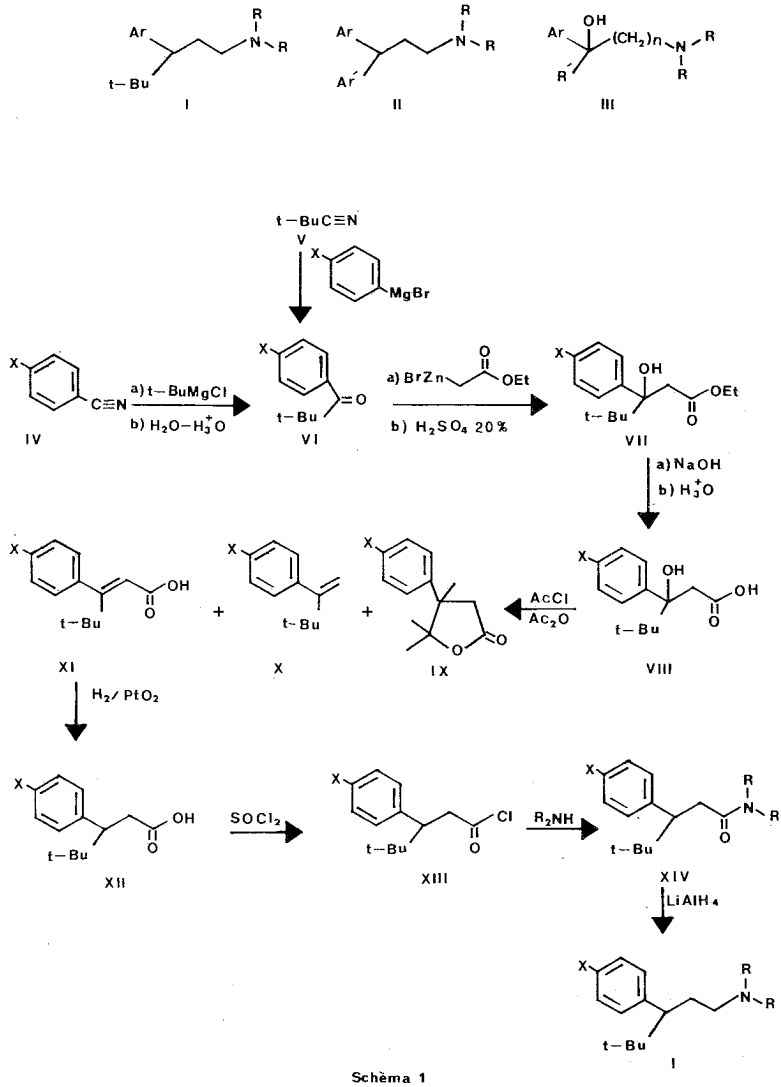
Partie théorique

Dans le cadre d'une étude plus générale sur les modifications de l'action pharmacologique que provoque, sur les molécules pharmaceutiques, le remplacement d'un radical aryle ou cycloalkyle par un volumineu groupement aliphatique, nous avons préparé les diméthyl-4,4 aryl-3 pentylamines I qui proviennent théoriquement des diaryl-3,3 propylamines II antihistaminiques et parasympatholytiques par substitution d'un des deux aryles par le radical butyle tertiaire.

Par ailleurs, comme il est rapporté dans une récente publication des résultats pharmacologiques intéressants ont été obtenus par la substitution analogue du radical cycloalcyle R′ par le butyle tertiaire dans les molécules des aminoalcools III antiparkinsoniens.[1]

La préparation des diméthyl-4,4 aryl-3 propylamines I N, N′-disubstituées, a été réalisée suivant le schéma 1. Les matières premières utilisées sont soit les benzonitriles IV, soit le pivalonitrile V qui donnent respectivement avec le chlorure de tert-butylmagnésium ou le bromure d'arylmagnésium, les pivalophénones VI. Avec la réaction de Reformatsky[2, 3] sur les cétones VI, on obtient les β-tert -butyl-β-hydroxy-hydrocinnamates d'ethyle VII qui, par saponification donnent les acides alcools VIII correspondants. L'action du mélange déshydratant: chlorure d'acétyle, anhydride acétique sur les acides β-tert-butyl- β-hydroxyhydrocinnamiques VIII conduit à la formation d'un mélange de triméthyl-4, 5, 5 aryl-4 dihydrofurannone-2 (3H) IX, α-tert-butyl-styrène X et d'acide β-tert-butyl-cinnamique XI.[4, 5] La lactone IX provient du déplacement intramoléculaire du méthyle sur le carbocation intermédiaire qui se forme dans le milieu acide de la réaction,[6-9] tandis que le produit oléfinique X provient de la décarboxylation de l'acide cinnamique XI.

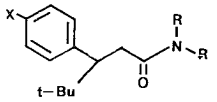
Par hydrogénation catalytique des acides cinnamiques XI on obtient les acides β-tert-butyl- β-arylpropioniques XII qui par l'intermédiaire des chlorures XIII donnent les amides XIV correspondants. Enfin par réduction des amides XIV avec $LiAlH_4$ on aboutit aux amines I correspondantes que l'on transforme en chlorhydrates. La



Schèma 1

vérification de la structure des produits intermédiaires et finaux a été fait par les analyses élémentaires et par analyse spectroscopique IR et RMN. En particulier, on trouvera les constantes physiques des amides XIV des sels des amines I dans les tableaux I et II, et les paramètres spectroscopiques dans les tableaux III et IV.

TABLEAU I: Constantes physiques des N,N-dialkyl-β-tert-butyl-β-arylpropionamides XIV.

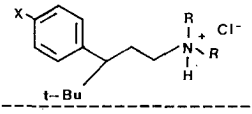


X-	$\begin{matrix}R\\R\end{matrix}\!\!>\!N-$	Edt	Formule brute	F (°C)
H-	$\begin{matrix}Et\\Et\end{matrix}\!\!>\!N-$	84%	$C_{17}H_{27}NO$	38-40 (b)
H-		86%	$C_{18}H_{27}NO$	70-72 (b)
CH_3O-	$\begin{matrix}Et\\Et\end{matrix}\!\!>\!N-$	84%	$C_{18}H_{29}NO_2$	huileux
CH_3O		86%	$C_{19}H_{29}NO_2$	104°(a)
CH_3-		99,6%	$C_{19}H_{29}NO$	103-104 (a)
CH_3-		95%	$C_{18}H_{27}NO_2$	92-93 (a)

(a) Solvant de recrystallisation: Et_2O-pentane.

(b) Solvant de recrystalisation: n-pentane

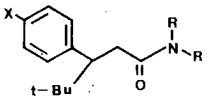
TABLEAU II: Constantes physiques des chlorhydrates des N,N-Dialkyl-diméthyl-4.4 aryl-3 pentylamines I.



X-	$\begin{matrix}R\\R\end{matrix}\!\!>\!N-$	Rdt	Formule brute	F (°C) [a]
H-	$\begin{matrix}Et\\Et\end{matrix}\!\!>\!N-$	91%	$C_{17}H_{30}ClN$	142-143
H-		90%	$C_{18}H_{30}ClN$	235-287
CH_3O-	$\begin{matrix}Et\\Et\end{matrix}\!\!>\!N-$	84%	$C_{18}H_{32}ClNO$	121-122
CH_3O-		85%	$C_{19}H_{32}ClNO$	236-237
CH_3-		99%	$C_{19}H_{32}ClN$	244-245
CH_3-		63%	$C_{18}H_{30}ClNO$	264-265

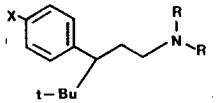
(a) Solvant de recristallisation : EtOH-Et_2O.

TABLEAU III: Constantes spectroscopiques des amides XIV.



X	R_2N-	I.R. (Nujol, cm^{-1})	R.M.N. (δ ppm, $CDCl_3$)
H		ν(C=O) 1630 (Amide I) δ(C-H) 1382, 1368 (t-Bu) δ(C-H) 765,708 (aromatiques)	0,93 (s, 9H, t-Bu) 0,8-1,25 (dt, 6H, $2\underline{CH_3}$ Et_2N) 2,4-2,8 (m, 2H, $\underline{CH_2}CO$) 2,8-3,45 (m,5H, $N(\underline{CH_2})_2$ Et_2N, Ar$\underline{CH}$) 6,8-6,98 (m, 5H, $C_6\underline{H}_5$).
H		ν(C=O) 1630 (Amide I) δ(C-H) 1381 1362 (t-Bu) δ(C-H) 760,705 (aromatiques)	0,9 (s, 9H, t-Bu) 1,2-1,5 (m, 6H, 3,4,5-pipéridiniques H) 2,5-2,9 (m, 2H, $\underline{CH_2}CO$) 2,9-3,1 (m, 1H, Ar$\underline{CH}$) 3,1-3,55 (m, 4H, 2,6-pipéridiniques H) 6,9-7,25(m,5H,$C_6\underline{H}_5$).
CH_3		ν(C=O) 1630 (Amide I) δ(C-H) 1380, 1360 (t-Bu) δ(C-H) 832 (aromatiques)	0,9 (s, 9H ,t-Bu) 1,1-1,8 (m,6H, 3,4,5-pipéridiniques H) 2,3 (s,3H, p-$\underline{CH_3}$) 2,6-2,88 (m,2H, $\underline{CH_2}CO$) 2,93-3,16 (m,1H, Ar$\underline{CH}$) 3,25-3,5 (m,4H, 2,6-pipéridiniques H) 7,05-7,1 (m, 4H, $C_6\underline{H}_4$).
CH_3		ν(C=O) 1625 (Amide I) δ(C-H) 1383, 1370 (t-Bu) δ(C-M) 838 (aromatiques)	0,91 (s, 9H, t-Bu), 2,31 (s,3H, p-$\underline{CH_3}$) 2,5-2,83 (m, 2H, $\underline{CH_2}CO$) 2,83-3,2 (m,1H, Ar$\underline{CH}$) 3,2-3,65 (m, 8H, morpholiniques H) 7,08-7,22 (m, 4H, $C_6\underline{H}_4$.
CH_3O		ν(C=O) 1635 (Amide I) δ(C-H) 1390, 1368 (t-Bu) δ(C-H) 848 (aromatiques)	0,9 (s, 9H, t-Bu) 0,76-1,0 (t,3H,$\underline{CH_3}$ Et_2N A_3X_2, J_{AX}=7Hz) 1-2,23 (t, 3H, $\underline{CH_3}$ Et_2N A_3X_2, $J_{AX}\simeq$7Hz) 2,55-2,76 (m, 2H,$\underline{CH_2}CO$) 2,95-3,45 (m, 5H, $N(\underline{CH_2})_2$ Et_2N, Ar$\underline{CH}$) 6,72-7,22 (≈q 4H, $\underline{C_6H_4}$, AA'BB', $J_{AB}=J_{A'B'}$ = 9Hz, $J_{AA'}=J_{BB'}$=1Hz.)
CH_3O		ν(C=O) 1625 (Amide I) δ(C-H) 1382, 1370 (t-Bu) δ(C-H) 840 (aromatiques)	0,9 (s,9H, t-Bu) 1,2-1,78 (m, 6H, 3,4,5-pipéridiniques H) 2,45-2,9 (m, 2H, $\underline{CH_2}CO$) 2,9-3,15 (m, 1H, Ar$\underline{CH}$, 3,18-3,55 (m, 4H, 2,6-pipéridiniques H) 3,8(s,3H,p-$\underline{CH_3}O$) 6,65-7,31(≈q 4H,$C_6\underline{H}_4$,AA'BB',$J_{AB}=J_{A'B'}$=9Hz, $J_{AA'}=J_{BB'}$= 1Hz

TABLEAU IV: Constantes spectroscopiques des alkylamines I.



X	R_2N-	I.R. des chlorhydrates (KBr, cm^{-1})	N.M.R. des bases ($CDCl_3$, δ ppm)
H	Et_2N-	ν(C=C) 1610, 1510 (aromatiques) δ(C-H) 1395, 1390 (t-Bu) δ(C-H) 742, 720 (aromatiques)	0,85 (s, 9H, t-Bu) 0,7-1,2 (t, 6H, $\underline{CH_3}$ Et_2N A_3X_2, J_{AX}= 7Hz) 1,75-2,12 (m, 3H $ArCHCH_2$) 2,15-2,65 (m, 6H, $\underline{CH_2}N$ $(\underline{CH_2})_2$) 6,9-7,2 (m, 5H, $\underline{C_6H_5}$).
H	N-	ν(C=C) 1600, 1585 (aromatiques) δ(C-H) 1380, 1365 (t-Bu), δ(C-H) 732, 708 (aromatiques)	0,9 (s, 9H, t-Bu) 1,22-1,8 (m, 6H, 3,4,5-piperidiniques H) 1,8-2,5 (m, 9H, $ArCHCH_2CH_2N$ 2,6-piperidiniques H) 6,85-7,23 (m, 5H, C_6H_5).
CH_3	N-	ν(C=C) 1615 (aromatiques) δ(C-H) 1390, 1380 (t-Bu) δ(C-H) 838 (aromatiques)	0,85 (s, 9H, t-Bu) 1,3-1,75 (m, 6H, 3,4,5-piperidiniques H) 1,9-2,45 (dm, 9H, $ArCHCH_2CH_2N$, 2,6 pipèridiniques H) 2,3 (, 3H, p-$\underline{CH_3}$) 7,02) 7,02-7,1 (m, 4H, C_6H_4).
CH_3	O N-	δ(C-H) 1385 (t-Bu) δ(C-H) 832 (aromatiques)	0,88 (s, 9H, t-Bu) 1,9-2,45 (dm, 9H, $ArCHCH_2CH_2N$, 3,5-morpholiniques H) 2,33 (s, 3H, p-$\underline{CH_3}$ 3,62-3,78 (t, 4H, $O(\underline{CH_2})_2$ morpholiniques) 7,03-7,1 (m, 4H, C_6H_4).
CH_3O	Et_2N-	ν(C=C) 1615, 1590 (aromatiques) δ(C-H) 1385, 1375 (t-Bu) δ(C-H) 845 (aromatiques)	0,85 (s, 9H, t-Bu) 0,7-1,45 (t, 6H, Et_2N A_3X_2, J_{AX}=7Hz) 1,45-2,62 (m, 9H, Ar $\underline{CHCH_2CH_2N}$ $(\underline{CH_2})_2$ 3,78 (s, 3H, p-$\underline{CH_3O}$) 6,52-7,3 (≈q, 4H, $\underline{C_6H_4}$ AA'BB' J_{AB}= $J_{A'B'}$= 9Hz, $J_{AA'}$=1Hz)
CH_3O	N-	ν(C=C) 1615 (aromatiques) δ(C-H) 1385, 1377 (t-Bu) δ(C-H) 845 (aromatiques)	0,86 (s, 9H, t-Bu) 1,28-1,7 (m,6H, 3,4,5,-pipéridiniques H) 1,83-2,38 (dm, 9H $ArCHCH_2CH_2N$, 2,6 pipéridiniques H) 3,76 (s, 3H, p-$\underline{CH_3O}$), 6,52-7,3 (q, 4H, C_6H_4, AA'BB', J_{AB}=$J_{A'B'}$ = 9Hz, $J_{AA'}$= $J_{BB'}$=1 Hz).

Bien que la synthèse des produits I comprenne un nombre de stades relativement important il semble que ce soit la meilleure méthode de préparation pour les amines I avec un rendement global d'environ 25% par rapport aux nitriles IV ou V. Au contraire, la méthode de Casy et Ison qui a été employée seulement pour la préparation des tétraméthyl-4,4 N, N phényl-3 pentylamine I ($R_2N = Me_2N$, X=H) et qui consiste à l'action du phényllithium sur la diméthylamino-1 dimethyl-4,4 pentanone-3, suivie de déshydratation et d'hydrogénation donne un rendement très faible (1,26%)[10]; cela est dû à la énolisation importante et à l'encombrement stérique de la matière du départ l'aminocétone.

Partie experimentale

Les points de fusion ont été déterminés dans les tubes capillaires de l'appareil de Büchi et n'ont pas été corrigés.

Les analyses élémentaires ont été réalisées par le Centre de Microanalyse du C.N.R.S. (France). Les spectres IR ont été obtenus avec le spectrophotomètre Perkin-Elmer-177 avec pour référence le polystyrène, et les spectres RMN avec le spectrophotomètre Bruker HX-90, dans $CDCl_3$ en utilisant comme référence interne le TMS.

Pivalophénone VI (X=H)[4]

Méthode A: Dans une solution du chlorure de tert-butylmagnésium préparé à partir de 12g (0,5 gratom) de magnésium en tournures, 56g (0,6 mole) de chlorure de tert-butyle et 250 ml d'éther anhydre on ajoute goutte à goutte sous agitation et sous atmosphère d'azote 31 g (0,3 mole) de benzonitrile dans 300 ml de toluène anhydre. L'éther est éliminé par distillation avec réfrigérant latéral jusqu'à ce que la température d'ébullition du liquide que l'on distille atteigne 105°C. On ajoute du toluène anhydre pour remplacer la quantité qui a été éliminée azéotropiquement et le mélange est porté à ébullition dans une atmosphère d'azote pendant 6 heures. Après refroidissement on hydrolyse le complexe avec de l'eau et HCl 18% jusqu' à pH fortement acide et formation d'un système à 2 phases. Le mélange est remué au bain-marie pendant deux heures de façon à compléter l'hydrolyse de la cétimine et après refroidissement on receuille la phase organique et la phase aqueuse est extraite par l'éther. On lave les extraits organiques réunis avec de l'eau, avec NaOH 10% et de nouveau avec de l'eau, on les séche avec Na_2SO_4 et on les évapore sous vide. On distille de résidu. Rendement 29,2g (60%). Eb 100°C/12mm *I.R.* (film) ν(C=O) 1680 cm^{-1} ν(C=C) 1605 cm^{-1} (aromatique) δ(C—H) 1392, 1365 cm^{-1} (t—Bu).

Méthode B. Dans une solution agitée de bromure de phénylmagnésium préparé à partir de 12g (0,5 gratome) de magnésium en tournures 86,9 g (0,55 mole) de bromobenzène et 250 ml d'éther anhydre, on ajoute goutte à goutte et dans une atmosphère d'azote 25g (0,3 mole) de pivalonitrile dans 150 ml d'éther anhydre. Le mélange est porté à ébullition légère pendant 2 heures et après refroidissement on hydrolyse le compexe avec de l'eau et HCl 18%. On laisse le système diphasique au repos pendant une nuit de façon à compléter l'hydrolyse de la cétimine, ensuite on procède comme il a été décrit dans la méthode A. Rendement (44,7 g (92%).

p-Méthoxypivalophénone VI[5] (X=CH_3O)

Préparé suivant la méthode A par action du chlorure de tert-butyl magnésium sur le p-anisonitrile. Rendement 61%. Eb. 142-144°C/17mm *I.R.* (film), ν (C=O) 1665 cm^{-1}, ν (C=C) 1615 cm^{-1} (aromatiques), δ (C-H) 1394, 1368 cm^{-1} (t-Bu).

p-Méthylpivalophénone VI[5] (X=CH_3): Préparé soit suivant la méthode A par action du chlorure de tert-butyl magnésium sur le p-toluonitrile (rendement 65%), soit suivant la méthode B par action du bromure de p-tolylmagnésium sur le pivalonitrile (rendement 92%). Eb. 114-116°C/ 14mm. *I.R.* (film) ν (C=O) 1678cm^{-1}, ν (C=C) 1695 cm^{-1} (aromatiques), δ (C-H) 1392, 1362 cm^{-1} (t-Bu).

β-tert-butyl-β-hydroxyhydrocinnamate d'éthyle VII[5] (X=H): Préparé par action du bromozinc acétate d'éthyle sur la pivalophénone conformémenet à la réaction classique de Reformatsky. Rendement 58%. Eb. 152-156°C/10mm *I.R.* (film) ν (OH) 3498 cm^{-1}, ν (C=0) 1720 cm^{-1}, δ (C-H) 1398 1363 cm^{-1} (t-Bu).

On prépare de la meme façon les 2 esters suivants:

β-tert-butyl-β-hydroxy-p-méthoxy-hydrocinnamate d'éthyle VII[5] (X=CH_3O) Rendement 62%. Eb. 114°C/0,01 mm *I.R.* (film) ν (OH) 3510-3490 cm^{-1}, ν (C=O) 1718 cm^{-1}, δ (C-H) 1396, 1362 cm^{-1} (t-Bu).

β-tert-butyl-β-hydroxy-p-méthyl-hydrocinnamate d'éthyle VII[5] (X=CH_3). Rendement 61% Eb. 104-106 °C/0,01 mm *I.R.* (film) ν (OH) 3495 cm^{-1}, ν (C=0) 1725 cm^{-1} δ (C-H) 1398, 1365 cm^{-1} (t-Bu).

Par saponification des esters-alcools précédents dans une solution hydralcoolique de NaOH on prépare les acides alcools suivants:

Acide β-tert-butyl-β-hydroxy-hydrocinnamique VIII[5] (X=H). Rendement 87% F 136-137°C (éther de pétrole).

Acide β-tert-butyl-β-hydroxy-p-méthoxy-hydrocinnamique VIII[5] (X=CH_3O). Rendement 95% F: 141-143°C (éther de pétrole).

Acide β-tert-butyl- β-hydroxy-p-méthyl-hydrocinnamique VIII[5] (X=CH_3). Rendement 98% F: 147-149°C (benzène-n-pentane).

Acide β-tert-butyl-cinnamique XI[4] (X=H)

On porte à ébullition au bain-marie pendant 2 heures un mélange de 11,1 g (0,05 moles) d'acide *β-tert-butyl-β-hydroxy-hydrocinnamique, de* 25,5g (0,25 mole) d'anhydride acétique et de 13,4 g (0,17 mole) de chlorure d'acétyle. Le chlorure et l'anhydride sont eliminés sous vide et on ajoute avec précaution de l'eau au résidu. On alcalinise avec précaution le produit obtenu avec NaOH 20% et sous refroidissement, puis on l'extrait par l'éther afin d'éloigner la lactone IX et le tert-butylstyrène X. On acidifie la phase aqueuse, sous refroidissement, avec HCl 10% et on laisse au refrigérateur une nuit. L'acide cinnamique précipité est filtré, séché et recristallisé dans l'éther de pétrole. F: 125-126°C. Rendement 6,2 g (60%). *I.R.* ($CHCl_3$) ν (C=O) 1720 cm^{-1}, ν (C=C) 1640 cm^{-1} *RMN* ($CDCl_3$) δ: 1,08 ppm (s, 9H, t-Bu) 5,96 ppm (s, 1H, *CH* =) 6,79 – 7,01 ppm (m, 5H, C_6H_5).

Après élimination de l'acide cinnamique XI, on évapore à sec la solution éthérée puis on la soumet à un entrainement à la vapeur d'eau afin d'éliminer le tert-butyl

styrène X. Dans le résidu de distillation on obtient la lactone IX (X=H) par extraction par l'éther. On séche la couche éthérée, on évapore sous vide et on recristallise le résidu dans le solvant: éther-éther de pétrole. F: 92°C *IR* (Nujol) ν (C=O) 1763 cm^{-1} *RMN* ($CDCl_3$) δ: 1,01 ppm (s, 3H, 4-CH_3) 1,42 ppm (s, 3H, 5-CH_3), 1,53 ppm (s, 3H, 5-CH_3) 2,32-3,53 ppm (q, 2H, CH_2, AB, $J_{AB} \simeq 15Hz$) 6,92-7,22 ppm (m, 5H, C_6H_5).

On prépare de la meme façon les 2 acides cinnamiques suivants:

Acide β-tert-butyl-p-méthoxycinnamique XI[5]. (X=CH_3O).
Rendement 59% F: 153-155°C (Et_2O-éther de pétrole). *IR* (KBr) ν (C=O) 1715 cm^{-1}, ν (C=C) 1642 cm^{-1}. *RMN* ($CDCl_3$) δ: 1,1 ppm (s, 9H, t-Bu) 3,82 ppm (s, 3H, CH_3O) 5,98 ppm (s, 1H, CH=) 6,89 ppm (m, 4H, C_6H_4).

Acide β-tert-butyl-p-méthylcinnamique XI[5]. (X=CH_3).
Rendement 51% F: 146-147°C (Et_2O-éther de pétrole) *I.R.* (KBr) ν (C=O) 1705-1690 cm^{-1}, ν (C=C) 1639 cm^{-1} *RMN* ($CDCl_3$) δ: 1,08 ppm (s, 9H, t-Bu) 2,3 ppm (s 3H, CH_3) 5,97 ppm (s, 1H, CH=) 6,82-6,89 ppm (m, 4H, C_6H_4).

Acide β-tert-butyl-β-phénylpropionique XII[4]. (X=H).
On dissout 13g (0,06 moles) d'acide β-tert-butylcinnamique dans 50 ml d'alcool éthylique absolu, on ajoute 0,4g PtO_2 selon Adams et le mélange est hydrogéné sous une pression de 50 Lb/in^2-. A la fin de l'hydrogénation on élimine le catalyseur par filtration et on chasse l'alcool éthylique sous vide. On recrystallise le résidu dans le solvant benzène-éther de pétrole. F: 113-114°C. Rendement presque quantitative *I.R.* (KBr) ν (C=O) 1695 cm^{-1} δ (C-H) 1368 cm^{-1} (t-Bu), *RMN* ($CDCl_3$) δ: 0,9 ppm (s, 9H, t-Bu) 2,62-3,91 ppm (m, 3H, $CHCH_2CO$) 6,98-7,22 ppm (m, 5H, C_6H_5), 10,02 ppm (br. s. 1H, CO_2H).

On prépare de la meme façon les 2 acides suivants:

Acide β-tert-butyl-β-(p-tolyl)-propionique XII. (X=CH_3).
Rendement presque quantitative F: 134-135°C (benzène-éther de pétrole) *IR* (KBr) ν (C=O) 1695 cm^{-1} *RMN* ($CDCl_3$) δ: 0,92 ppm (s, 9H, t-Bu) 2,32 ppm (s, 3H, CH_3) 2,58-3,18 ppm (m, 3H, $CHCH_2CO$) 7,05 ppm (m, 4H, C_6H_4) 9,25 ppm (br.s. 1H, CO_2H). Analyse ($C_{14}H_{20}O_2$) % Calc. C: 76,32, H: 9,15, % Trouv. C: 76,57, H: 9,02.

Acide β-tert-butyl-β-(p-méthoxyphényl)-propionique XII. (X= CH_3O).
Rendement presque quantitatif F: 131-132°C (benzène - éther de pétrole) *IR* (KBr) ν (C=O) 1705 cm^{-1} *RMN* ($CDCl_3$) δ: 0,85 ppm (s, 9H, t-Bu) 2,61-2,90 ppm (m, 3H, $CHCH_2CO$) 3,78 ppm (s, 3H, CH_3O) 6,71-7,15 ppm (q, 4H, AA'BB', $J_{AB} = J_{A'B'} \simeq$ 10-11 H_Z, $J_{AA'} = J_{BB'} \simeq 1H_{Z'}$, C_6H_4) 10,25 ppm (br.s. 1H, CO_2H) Analyse ($C_{14}H_{20}O_3$) % Calc. C: 71,16, H: 8,53, % Trouv. C: 71,41, H: 8,41.

Chlorure de β-tert-butyl-β-phénylpropionyl XIII. (X=H).
On chauffe à 50-60°C pendant 2 heures, un mélange de 20,5 g (0,105 moles) d'acide β-tert-butyl-β-phénylpropionique et de 20g de chlorure de thionyle. On élimine le chlorure de thionyle sous vide à l'aide de benzène anhydre et on utilise le résidu sans autre purification pour le stade suivant. On prépare de la même façon les chlorures XIII avec X=CH_3 et X= CH_3O.

N-(γγ-diméthyl-β-phénylpentanoyl) pipéridine XIV (X=H, R_2N = Pipéridyl
Dans une solution de 11,2g (0,112 moles) de pipéridine dans 100 ml de benzène anhydre, on ajoute sous agitation et goutte à goutte une solution de 6,4 g (0,028 moles) de chlorure de β-tert-butyl-β-phénylpropionyle dans 25 ml de benzène anhydre. On porte le mélange à ébullition pendant 4 heures puis on le verse dans de l'eau. On prélève la phase benzènique et on extrait la phase aqueuse par le benzène, on lave les couches benzèniques réunies avec HCl 10% et de l'eau, on séche avec Na_2SO_4 et on évapore sous vide. Le résidu est recristallisé dans le n-pentane. Rendement 6,6g (86%). F: 70-72°C - *I.R.* (Nujol) ν (C=O) 1630 cm^{-1} δ (C-H) 1381, 1362 cm^{-1} (t-Bu), δ (C-H) 760,705 cm^{-1} (aromatiques) *RMN* ($CDCl_3$) δ 0,9 ppm (s, 9H, t-Bu) 1,2-1,5 ppm (m, 6H, 3, 4, 5- H pipéridiniques) 2,5-2,9 ppm (m, 2H, CH_2CO) 2,9-3,1 ppm (m, 1H, *ArCH*) 3,1-3,55 ppm (m, 4H, 2, 6-H pipéridiniques) 6,9-7,25 ppm (m, 5H, C_6H_5).
Analyse ($C_{18}H_{27}NO$) % Calc. C: 79,07, H. 9,96 % Trouv. C 78,80, H. 9,99.
On prépare de la meme façon les autres amides XIV (tableaux I et III).

N-(diméthyl-4,4 phényl-3 pentyl)-pipéridine I. (X=H, R_2N = Pipéridyl
Dans une suspension de 4,26 g (0,112 moles) de $LiAlH_4$ dans 200 ml de THF anhydre on ajoute goutte à goutte et sous agitation 8,2g (0,028 Moles) de N- (γ,γ-diméthyl- β-phénylpentanoyl) pipéridine dans 100 ml de THF anhydre. On porte le mélange à ébullition pendant 7 heures et après refroidissement, on hydrolyse avec EtOH, H_2O et NaOH 10%. Les hydroxides minéraux formés sont éliminés par filtration et lavés soigneusement à l'éther. Le filtrat et le liquide de lavage réunis sont evaporés à sec. On récupère le résidu avec HCl 10%. On lave soigneusement la phase acide à l'éther, on alcalinise sous refroidissement, avec NaOH 20%. Il se dépose un produit solide qui après filtration et séchage est transformé en chlorhydrate F: 235-237°C (EtOH - Et_2O). Rendement 7,5 g (90%) *IR* (KBr) ν (C=C) 1600, 1585 cm^{-1} (aromatique), δ (C-H) 1380, 1365 cm^{-1} (t-Bu), δ (C-H) 732, 708 cm^{-1} (aromatiques) *RMN* ($CDCl_3$) δ: 0,9 (ppm, s, 9H, t-Bu) 1,22-1,8 ppm (m, 6H, 3, 4, 5- H pipéridiniques) 1,8-2,5 ppm (m, 9H, Ar*CH*,*CH*$_2$*CH*$_2$N, 2,6-H pipéridiniques) 6,85-7.23 ppm (m, 5H, C_6H_5) Analyse ($C_{18}H_{30}$ CIN) % Calc. C: 73,06, H: 10,22% Trouv. C: 73,16, H: 10,15. On prépare de la meme façon les autres amines I.

Summary

Synthesis of 4,4-dimethyl-3-arylpentylamines

This paper deals with the synthesis of 4,4-dimethyl-3- arylpentylamines I, which theoretically result from molecules of the pharmacologically active 3,3-diarylpropylamines II, by replacing one of two aryls with the tert-butyl group. The amines I are prepared from the corresponding β-tert-butyl- cinnamic acids XI, which are hydrogenated to β-tert-butyl- β-arylpropionic acids XII and the latter through the corresponding chlorides XIII lead to the β-tert-butyl-β-arylpropionamides. XIV. Finally, the amines I are prepared by reduction of propionamides XIV with $LiAlH_4$.

Key words: N,N-dialkyl-β-tert-butyl-β-arylpopianamines. N,N-dialkyl-4,4-dimethyl-3-arylpentylamines.

Περίληψη

Σύνθεση 4,4-διμεθυλο-3-αρυλοπεντυλαμινών

Στην παρούσα εργασία περιγράφεται η σύνθεση 4,4-διμεθυλο-3-αρυλοπεντυλαμινών I, που προκύπτουν θεωρητικά από τα μόρια των φαρμακολογικώς ενεργών 3,3-διαρυλοπροπυλαμινών II, με αντικατάσταση του ενός από τα δύο αρύλια με την ρίζα του tert-βουτυλίου. Η παρασκευή των αμινών I πραγματοποιείται από τα αντίστοιχα β- tert-βουτυλοκινναμωμικά οξέα XI, τα οποία με υδρογόνωση μετατρέπονται στα β- tert -βουτυλο-β-αρυλοπροπιονικά οξέα XII και τα οποία, μέσω των αντιστοίχων χλωριδίων XIII, οδηγούν προς τα β- tert-βουτυλο-β-αρυλοπροπιοναμίδια XIV.

Τελικά οι αμίνες I λαμβάνονται με αναγωγή των προπιοναμιδίων XIV με $LiAlH_4$.

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THE EFFECT OF FUNCTIONALIZATION ON THE MESOMORPHIC-LIKE CHARACTER OF SOME QUATERNARY AMMONIUM SALTS*

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Summary

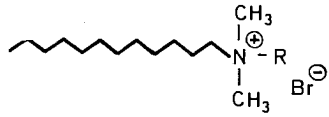
In the present study the synthesis and the mesomorphic-like character of a series of functionalized aliphatic quaternary ammonium salts is reported. The mesomorphic character of these compounds which was established by optical studies and differential scanning calorimetry was correlated with their molecular structure and found to be crucially dependent on the nature of the functional group.

Key words: Mesomorphic-like, liquid crystal, Differential Scanning Calorimetry, optical microscopy studies

Introduction

Recently some publications appeared in the literature reporting that thermotropic liquid crystallinity is not an exclusive property of long, planar and rigid molecules, i.e. compounds bearing the typical mesogenic moieties coupled with appropriate substituents. Thus phospholipids[1], with two long alkyl chains exhibit liquid crystalline character in the melt in addition to their property to organize in aqueous solutions forming bilayers and/or liposomes. Furthermore, Vacatello and his coworkers[2-5] investigated the thermal behavior of certain primary alkylammonium salts and established the existence of a lamellar smectic mesophase. Recently also Iwamoto et al[6] investigating the thermal transitions of some long-chain n-alkyltrimethylammonium halides showed that they exhibit a solid-solid transition leading to «mesophases» or mesomorphic-like phases since completely free motion of the quaternary salts is not allowed because of the ionic bonding between the molecules. On the other hand certain conventional mesogenic moieties[7-9] when functionalized with strong polar heads, i.e. quaternary ammonium group, phosphate, in addition to their thermotropic liquid crystalline nature they form bilayers in water as well. It is evident, therefore, that certain surfactant molecules, which organize in water, can also form thermotropic liquid crystals. According to Kunitake[9] who investigated this type of compounds «liquid-crystalline nature of bilayers (both natural and synthetic) is the cause and not the result of bilayer formation». Following our preliminary work[10] on the liquid crystalline character of long-chain quaternary ammonium salts bearing the dimethyl

dodecyl amine moiety as basic group and in conjunction with our studies on the micellar properties of functionalized quaternary ammonium surfactants[11] we now examine the organizational properties of compounds of formula A in the bulk (Scheme).



A

I

1. $R=CH_2CH_3$
2. $R=CH_2CH_2CH_3$
7. $CH_2CH_2CH_2CN$

II

3. $R=CH_2CH_2OH$
4. $R=CH_2CH_2CH_2OH$

V

8. $R=CH_2COCH_3$
9. $R=CH_2COOCH_2CH_2OH$

III

5. $R=CH_2COCH$
6. $R=CH_2CH_2COOH$

Scheme

Experimental

Synthesis of quaternary ammonium salts.

To 0.01 mole of dimethyl dodecyl amine dissolved in ethyl acetate 0.0125 mole of the appropriate bromide also dissolved in the same solvent, were added. The mixture was stirred at room temperature for periods of time ranging from few minutes to several hours. The precipitated salts were filtered and recrystallized from ethyl acetate with the exception of compound 3 which was recrystallized from a mixture of ethanol: ethylacetate (1:10). They were dried over phosphorous pentoxide. The elemental analysis results are shown below:

Optical microscopy was performed with a Reichert «Thermopan» polarizing microscope. For the thermal studies a DuPont 910 Differential Scanning Calorimeter at a scanning rate of 10°C/min was used. Cooling was module's natural rate.

Results and discussion

Quaternization was performed in ethylacetate where due to the medium polarity of the solvent the reaction proceeded relatively fast[12] and the salts precipitated easily

Compound	Formula	Calculated			Found		
		C	H	N	C	H	N
1	$C_{16}H_{36}BrN$	59.61	11.26	4.34	59.55	12.02	4.35
2	$C_{17}H_{38}BrN$	60.69	11.39	4.16	61.14	11.34	4.29
3	$C_{16}H_{36}BrNO$	56.79	10.72	4.14	57.15	10.92	4.19
4	$C_{17}H_{38}BrNO$	57.94	10.87	3.98	58.04	11.30	4.03

Compound	Formula	Calculated			Found		
		C	H	N	C	H	N
5*	$C_{16}H_{34}BrNO_2$	54.54	9.73	3.98	57.60	11.62	4.77
6*	$C_{17}H_{36}BrNO_2$	55.73	9.90	3.82	57.92	11.49	4.76
7	$C_{18}H_{37}BrN_2$	59.82	10.32	7.75	60.30	10.72	7.49
8	$C_{17}H_{36}BrNO_2$	55.73	9.90	3.82	55.59	10.25	3.68
9	$C_{18}H_{38}BrNO_3$	54.53	9.66	3.53	55.12	9.94	3.68

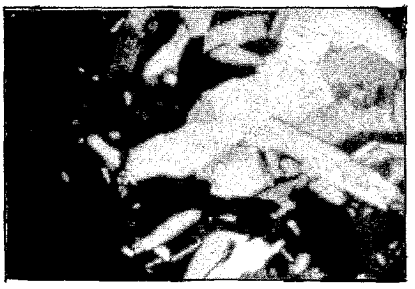
* The experimental C,H,N elemental analysis results of carboxylic group functionalized quaternary ammonium salts were systematically found higher than calculated. This is apparently due to the fact that the charge on the quaternary nitrogen is not neutralized by bromide counterions only but also to a certain degree by carboxylates resulting from the weak ionization of the -COOH group. Therefore COO^- acts partially as an internal counterion for the quaternary nitrogen (partial zwitterionic character) and consequently the overall percentage of the heavy bromide ions is reduced resulting to enhanced, C,H,N elemental analysis data.

as polycrystalline materials. The salts were more or less hygroscopic and they were handled with care.

In optical microscopy experiments mesomorphic-like textures were not observed at the first transition temperatures as determined by DSC. It was, however, possible to observe smectic-like textures when the material was deformed by exercising pressure on the cover slip at temperatures slightly exceeding the first thermal transition found by DSC. Similar deformation behavior has also been observed for alkyltrimethylammonium halides[6], which also showed a first solid-solid transition as established by X-ray studies. Similarly, in quaternaries prepared in this study and resulting from the replacement of one methyl by the appropriate functional group it may be assumed that the first transitions shown by DSC and also observed by optical microscopy when the materials were by pressure deformed are also solid-solid transitions giving rise to mesomorphic-like phases (for simplicity mesophases or liquid crystalline phases). The main optical and thermal analysis data for compounds 1 to 9 are summarized below:

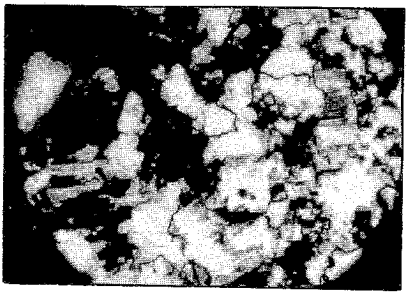
Compound 1 ($R=CH_2CH_3$). This compound melts by pressing the cover slip at about 80°C to a blurred primarily homoetropic texture which from about 170°C it is transformed to a fan texture, Fig. 1, which on 190°C becomes isotropic. On cooling

Compound 1

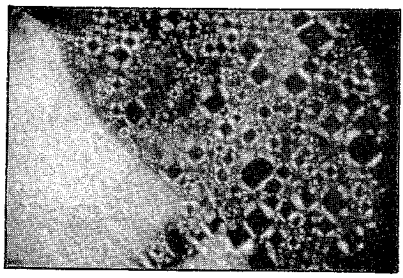


Compound 2

Compound 3



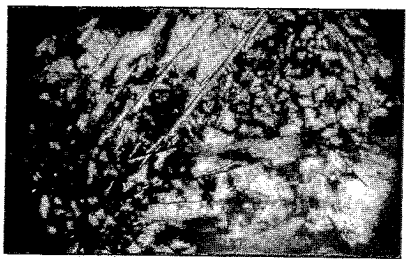
Compound 4



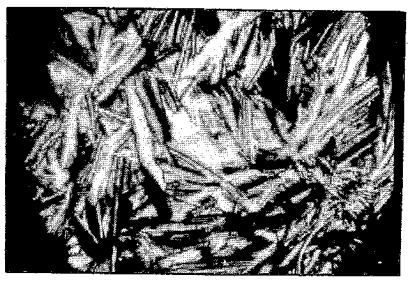
Compound 5

Compound 6

Compound 7



Compound 8



Compound 9

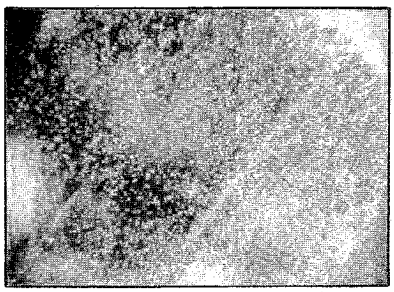
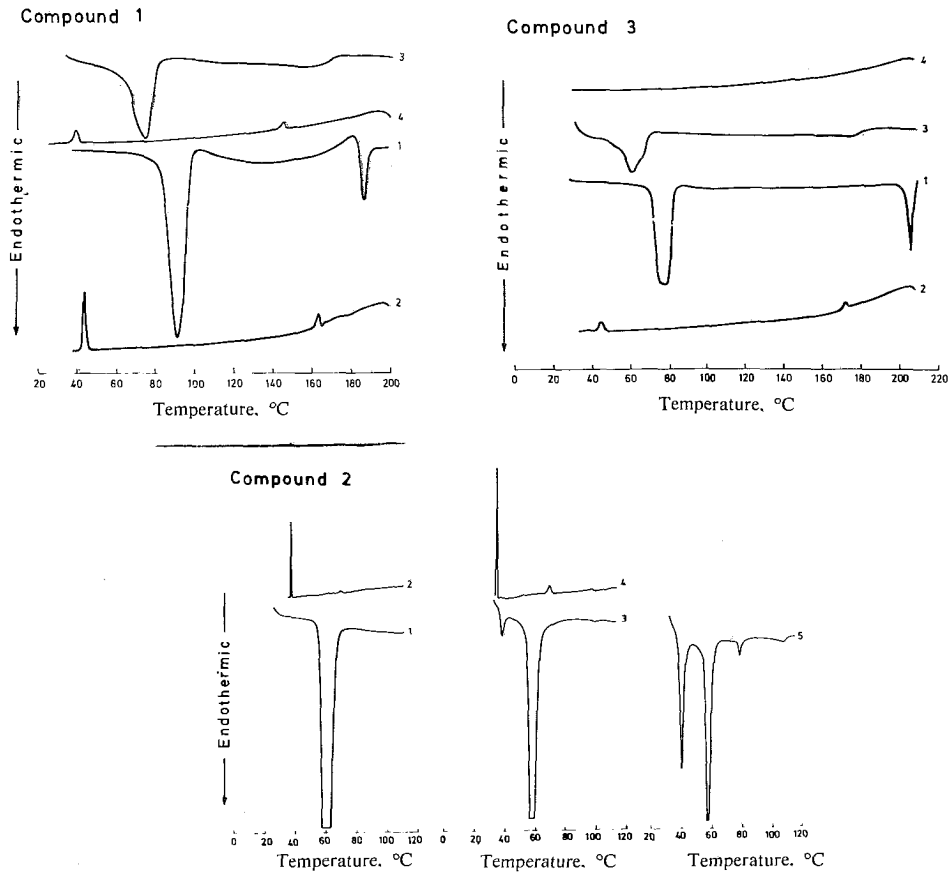


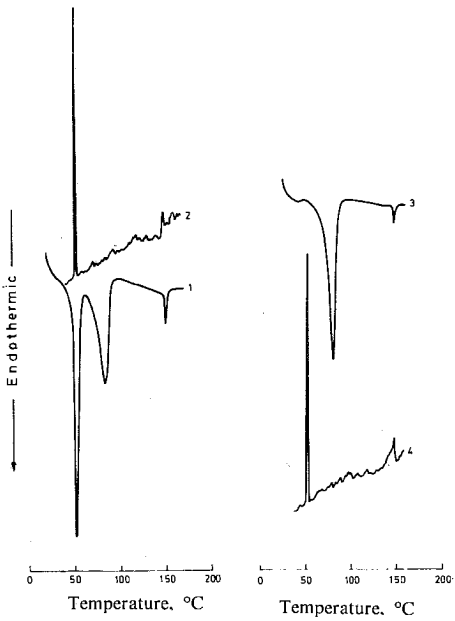
Fig. 1: *Liquid crystalline structures of compounds 1-9.*

from the isotropic melt the fan-texture does not appear and the isotropic phase crystallizes without the intermediate appearance of a mesomorphic-like phase apparently attributed to a decomposition of the compound. A partial decomposition of this salt during the heating runs is also shown by thermal analysis (Fig. 2). Thus, a modification of specific heat is observed during the heating-cooling runs and the transitions are shifted to lower temperatures in the second heating-cooling run.

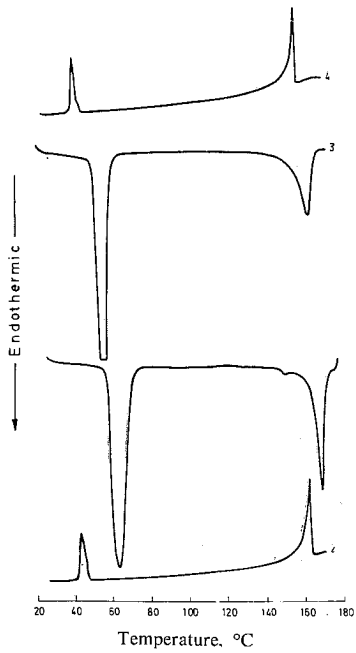
Compound 2 (R=$CH_2CH_2CH_3$). Upon melting of this compound[1], at 57°C, a primarily homoetropic texture appears, which is transformed to isotropic at 107°C. On cooling a fan texture is observed, a typical microphotograph of which is shown in Fig. 1. Analogous was the behavior of the compound during the second and third heating runs indicating the thermal stability of this compound. The thermal behavior of this compound, as shown by DSC diagram, Fig. 2, seems peculiar. This is the reason that three heating curves are included for this compound. Thus on first heating only one endothermic peak clearly appears, at 56°C, corresponding to solid-mesomorphic transition while on cooling two exothermic peaks are shown i.e. at 71°C and 39°C. During the second heating an additional peak appears at 38°C while on the third heating four endothermic peaks are observed. It should be mentioned, however, that it was not always possible to detect the last two transitions by DSC at high temperature. This is also in agreement with the difficulty experienced to observe



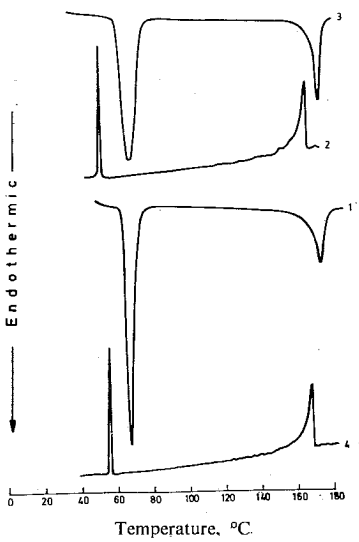
Compound 4



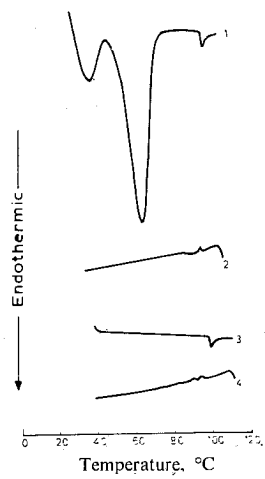
Compound 5



Compound 6



Compound 7



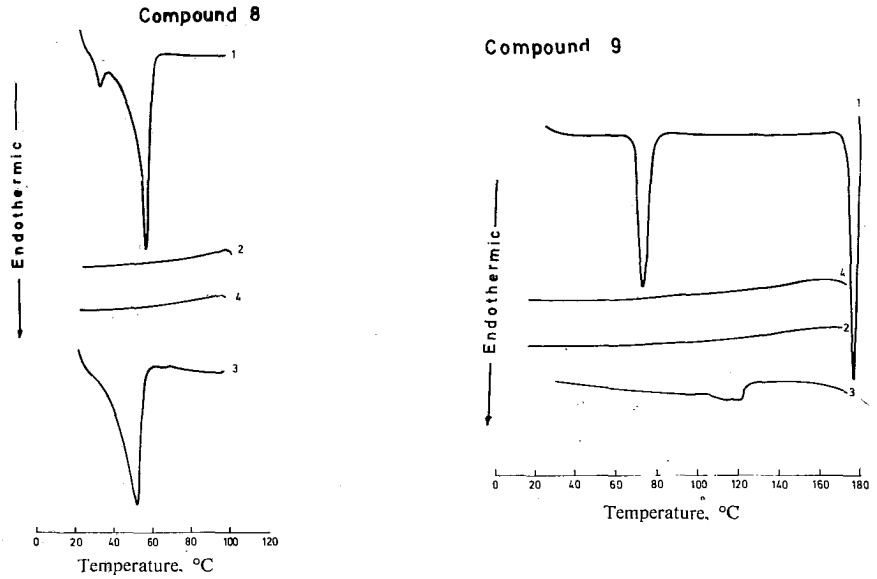


Fig. 2: *DSC curves of compounds 1-9.*

mesomorphic-isotropic transition during the optical microscopy experiments (rubbing seems to induce the appearance of the mesophase).

Compound 3 (R=CH_2CH_2OH). Two distinct textures appear on melting of this salt: The first is blurred, primarily homoetropic texture, appearing immediately after pressing the cover slip at about 70°C, which is transformed to mosaic, Fig. 1, at 190°C and which is finally transformed to isotropic at about 202°C. This compound does not exhibit satisfactory thermal stability as judged by the second heating run in which the clearing point occurs at a lower temperature. The thermal behavior of this compound is shown in Fig. 2, showing a broad endothermic solid to mesomorphic-like transition apparently attributed to overlapping of two peaks. The diagrams also confirm the thermal instability of this salt as exhibited by the lowering of the transitions of the second cooling curve.

Compound 4 (R=$CH_2CH_2CH_2OH$). When this compound was heated it melted at 45°C to a blurred, partially homoetropic texture, which at about 76°C it was transformed to a spherulitic texture (Fig. 1) and at 154°C to isotropic. Homoetropy was induced by pressing the cover slip. The same mesomophic-like textures were also observed on second and third heating runs a fact attributed to the thermal stability of the compound. On cooling, the mesophase appears at the clearing point temperature. The liquid crystalline character of this compound has also been confirmed by DSC. Thus, on heating, the first transition corresponds to a solid-mesomorphic transition, the second to a mesomorphic-mesomorphic transition, whereas the third to a mesomorphic-isotropic. On the second heating the first transition almost disappears which, however, reappeared if the sample were subjected to the second heating after about 40 hours. The other two transitions appear practically at the same temperature. The same behavior was also observed during the third heating (not included in Fig. 2). These results coupled by the almost constancy of the transitions during cooling indicate the thermal stability of this compound.

Compound 5 (R=CH_2COOH). This carboxylic compound melts with pressing the cover slip at about 58°C to a blurred, partially homoetropic texture which it is transformed to a fan texture at about 155°C and finally to isotropic at 174°C. On cooling a fan texture appears again as shown in Fig. 1. It is interesting to notice that this compound although bearing the carboxylic group which is susceptible to decarboxylation is thermally stable as exemplified the almost constancy of the transitions in the two heating-cooling cycles (Fig. 2).

Compound 6 (R=CH_2CH_2COOH). A predominately homoetropic texture appears by pressing the cover slip at about 65°C which, starting from 140°C, is transformed to a fan texture and finally to isotropic at 175°C. On cooling from the isotropic melt it is transformed again to a fan texture (Fig. 1) at the clearing point temperature and crystallizes at room temperature by inducing crystallization on touching the cover glass. Similar behavior is shown during the second heating-cooling run which is indicative of the thermal stability of this compound. The DSC diagrams (Fig. 2) also confirm the thermal stability of this compound.

Compound 7 (R=$CH_2CH_2CH_2CN$). There is some difficulty in handling this compound because of its hygroscopicity. This salt melts at about 38°C to a blurred, partially homoetropic, texture which becomes completely homoetropic at 51°C. Subsequently at 88°C it is transformed to a fan texture which becomes isotropic at 96°C. On first and second cooling from the isotropic melt a fan texture (Fig. 1) appears at the clearing point temperature which is indicative of the thermal stability of the compound. The liquid crystalline character of this compound is also confirmed by DSC diagrams (Fig. 2). The liquid crystalline phase is preserved in the solid phase as shown by the DSC traces of the first cooling run and the second heating as well.

Compound 8 (R=CH_2COOCH_3). This salt melts to a blurred, partially homoetropic texture which becomes isotropic at 56°C. On cooling a fan texture appears which is shown in Fig. 1. It exhibits only a relatively good thermal stability as judged by the small lowering of the clearing point temperature on the second and third heating run. Concerning DSC diagrams (Fig. 2) it is interesting to notice that during the two cooling runs no exothermic peaks are observed except of a modification of the specific heat. An explanation about this phenomenon cannot now be provided.

Compound 9 (R=$CH_2COOCH_2CH_2OH$). This compound melts by pressing the cover slip at about 73°C to a blurred, predominately homoetropic, texture which becomes isotropic at 172°C. On cooling there is substantial supercooling and some spherulites are observed at 140°C (Fig. 1). According to thermal analysis diagrams (Fig. 2) the second heating curve is drastically modified compared to the first one which is an indication of the thermal instability of this compound.

From these results it becomes evident that the functionalized long chain (C_{12}) quaternary ammonium salts studied here form more or less thermally stable mesomorphic-like textures in the temperature range of 33°-202°C. In view of X-ray studies of similar molecules[8], i.e. of n-alkyltrimethylammonium halides, containing long aliphatic chains it is reasonable to assume that the crystal structure of these functionalized quaternary salts consists primarily of polar layers within which ionic

type bonding between the quaternary centers and bromide anions occurs, and nonpolar layers built of the C_{12} alkyl chain and held together by van der Walls forces. In this context the formation of the smectic-like phase can be visualized as involving the breakage of the terminal van der Walls bonding between neighboring nonpolar layers forming structures (Fig. 3) (consisting of ionic and nonpolar layers) that can now slide with respect to each other while still retaining internal structure due to the rigidness of the polar layer. At higher temperatures when the thermal

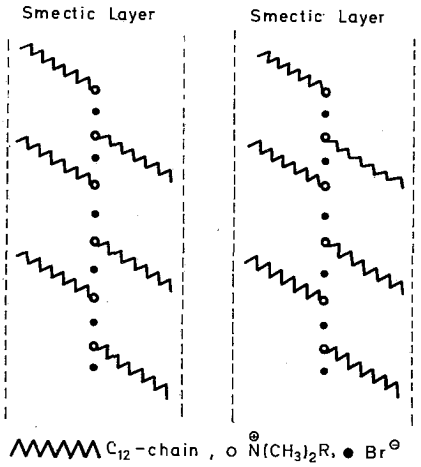


Fig. 3: *Smectic liquid crystalline structure of some long-chain quaternary ammonium salts.*

energy is sufficient to break the ionic bonds, the liquid crystalline structure collapses forming the isotropic melt at the characteristic for each compound clearing point temperature.

In analogy with conventional liquid crystals where their thermodynamic stability is characterized in terms of the clearing point, the stability of the smectic-like textures of these quaternary salts is also correlated with their clearing points which are tabulated in the Table as a function of the respective group. Although a systematic dependence of the clearing point as a function of R is not probably possible from the data in the Table, some trends are quite obvious.(i) The introduction of an extra methylene group in the R groups results in a decrease of the clearing point as exemplified by the compounds for which R=CH_2CH_3(C.P.=190°C), $CH_2CH_2CH_3$, (C.P.=107°C) and CH_2CH_2OH (C.P.=202°C). $CH_2CH_2CH_2OH$ (C.P.=154°C). The compound in which R is methyl is not included in the Table since it decomposes before reaching the clearing point. It has, however, to be noticed that in N_2 atmosphere the clearing point of this compound is 250°C, i.e. in line with our previous observation. When a carboxylic group is incorporated in the R group the clearing points are practically the same, i.e. R=CH_2COOH (C.P.=174°C), CH_2CH_2COOH (C.P.=175°C). In this case the presence of the bulky carboxylic group overides apparently the effect created by the introduction of the methylene group. (ii) Substitution of the apolar CH_3 group in R by polar hydroxylic groups such as OH,COOH enhances in most cases the thermodynamic stability of the smectic-like phase, for instance, R=$CH_2CH_2CH_3$ (C.P.=107°C), CH_2CH_2OH

TABLE: Clearing Points of Type A Liquid Crystals.

R	Clearing Point, °C	R	Clearing Point, °C	R	Clearing Point, °C	R	Clearing Point, °C	R	Clearing Point, °C
CH_2CH_3	190			CH_2COOH	174			CH_2COOCH_3	56
$CH_2CH_2CH_3$	107	CH_2CH_2OH	202	CH_2CH_2COOH	175			$CH_2COOCH_2CH_2OH$	172
		$CH_2CH_2CH_2OH$	154			$CH_2CH_2CH_2CN$	96		

(C.P.=202°C), and CH_2CH_2COOH (C.P.=175°C). These groups also stabilize more effectively the mesophase as compared to non-hydroxylic polar groups such as cyano, i.e. R=$CH_2CH_2CH_2CN$ (C.P.=96°C), and $CH_2CH_2CH_2OH$ (C.P.=154°C). The same effect on mesophase stability, in a negative manner, however, is also shown by the esterification of CH_2COOH to CH_2COOCH_3, i.e. R=CH_2COOH (C.P.=174°C) and CH_2COOCH_3 (C.P.=56°C). The lowering of the clearing point is such that the corresponding peak in the DSC trace almost overlaps with the solid - mesomorphic - like transition (broad assymetric peak). Moreover, when R is $CH_2COOCH_2CH_2OH$, i.e. a group which in addition to the ester group it also bears a hydroxy group, the clearing point occurs at relatively high temperature (172°C). From these results it is clear that there is a complicated relationship between the thermodynamic stability of the smectic phases and the R group of the functionalized quaternary salts. Polar groups in general seem to enhance stability while nonpolar decrease it. Furthermore quantitative discussion concerning the effect of R group on the ionic bonding and consequently the clearing point and thermodynamic stability of the mesophase would be highly conjectural since the detailed crystal structure of these compounds is not known yet.

In conclusion, our results indicate that quaternary ammonium salts of the type A organize in the melt to form more or less thermally stable smectic - like phases in the temperature range of 33-202°C, whose stability depends on the nature (size and polarity) of R group.

Περίληψη

Η επίδραση χαρακτηριστικών ομάδων στον προσομοιάζοντα με υγρή κρυσταλλική φάση χαρακτήρα μερικών τεταρτοταγών αμμωνιακών αλάτων

Στην παρούσα μελέτη ανακοινώνεται η σύνθεση μιας σειράς τεταρτοταγών αμμωνιακών αλάτων, υποκατεστημένων με χαρακτηριστικάς ομάδας, που το τήγμα τους προσομοιάζει την υγρή κρυσταλλική φάση. Ο ιδιάζων χαρακτήρας αυτών των ενώσεων, που αποδείχθηκε με μικροσκοπικές οπτικές μελέτες καθώς και με διαφορική θερμιδομετρική ανάλυση, συσχετίζεται με τη μοριακή τους δομή και βρέθηκε οτι εξαρτάται αποφασιστικά από τη φύση των χαρακτηριστικών ομάδων.

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SHORT PAPER

Chimika Chronika, New Series, 14, 101-105 (1985)

SILICIC ACID COLUMN CHROMATOGRAPHY OF PHOSPHONOLIPIDS: VI. SEPARATION OF N-PALMITOYL AND N-LIGNOCERYL DL-DIHYDROCERAMIDE-N, N, N-TRIMETHYLAMINOETHYL PHOSPHONATES FROM THEIR PHOSPHORYL ANALOGS AND OTHER RELATED PHOSPHOLIPIDS

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Introduction

In a previous paper[1] the unusual chromatographic behaviour of phosphono-sphingomyelin was noted and an explanation of this experimental observation was provided. In an attempt to determine whether other related ceramide phosphonates possessed similar properties, N-palmitoyl and N-lignoceryl DL-dihydroceramide phosphonates, together with their phosphoryl analogs, have been synthesized and their silicic acid column chromatographic behaviour investigated. For comparison purposes, cardiolipin, phosphatidylethanolamine, phosphatidylcholine, lysolecithin, sphingomyelin, phosphono-sphingomyelin, N-palmitoyl and N-lignoceryl ceramides were included in this elution experiment.

Collected fractions were analysed by thin layer chromatography (TLC) and IR spectroscopy to confirm species identification.

Under the specific experimental conditions, complete separation of the closely related phosphonolipids and phospholipids is effected.

Experimental

Instrumentation

IR spectra were recorded on a Perkin-Elmer 197 grating IR spectrophotometer.

A glass column was employed for the separation with length 40 cm and I.D. 2.4 cm.

Reagents

Solvents for column chromatography and TLC were analytical reagent grade (Merck) and were distilled before use. TLC was conducted on 20×20 cm chromatoplates of 0.25 mm thick silica gel G or 60 F_{254} (Merck) and visualisation was effected with molybdenum blue, iodine vapour, UV. irradiation and ninhydrin spray.

Standards

N-palmitoyl and N-lignoceryl ceramides were purchased from Serdary Research Laboratories, Ontario, Canada. The phosphoryl and phosphono analogs of the ceramides were synthesized in this laboratory.[2]

Cardiolipin, phosphatidylethanolamine and sphingomyelin were purchased from Koch-Light, Colnbrook, England. Phosphatidylcholine and lysolecithin were purchased from Merck, Darmstadt, W. Germany.

Silicic acid for column chromatography was purchased from SIGMA Chemical Company, St. Louis, Missouri, U.S.A.

Procedure

The chromatographic column, which was fitted at the bottom with a glass-wool plug, was loaded with a slurry of 10.00 g of silicic acid in 50 ml of chloroform, to a column height of 5.5 cm and a column volume of 26 ml. The column was washed with two column volumes of chloroform and the flow rate maintained in the elution was 1.7-1.9 ml per minute. The volume of the eluate collected by fraction collector was about 5.0 ml. When total weight was desired, a total of 15-25 ml of the eluates was obtained by pooling of fractions. Evaporation of the solvents was accomplished under vacuum and a bath temperature of 35° C or under nitrogen. Column elution was effected with combinations of methanol in chloroform mixtures as indicated in Table I.

TABLE I. Elution of the chromatographic column with dimensions 40 cm and I.D. 2.4 cm, packed with 10 g of silicic acid (SIGMA) to a height of 5.5 cm and total column volume of 26 ml. Flow rate: 1.7 - 1.9 ml per minute. Fractions of approximately 5.0 ml were collected.

% methanol in chloroform	column volumes	total ml of solvent	fractions collected
5	3	75	1- 14
20	5	125	15-35
40	7	175	36-64
80	5	150	65-91

IR spectra of the various pilot fractions were run as chloroform solutions or KBr discs. TLC chromatograms were run on silica gel G F_{254}. Merck plates and also on plates coated in this laboratory to a thickness of 0.30 mm. Development of the chromatograms was effected in two chambers of dimensions 8×20.5 cm (DESAGA) and the run normally took about 45 minutes. The plates were developed in chloroform: methanol: water (65:25:4) (system A), methanol: water (2:1) (system B) and chloroform: methanol: acetic acid: water (25:15:4:2) (system C).

Visualisation was effected with molybdenum blue, iodine vapour, ninhydrin spray and U.V. irradiation.

Standards were also spotted on the plates to ease in the detection of the developed spots.

Results

Column elution was effected with combinations of methanol in chloroform as indicated on Table I.

Fractions were identified by TLC and IR spectroscopy (Table II) and the nature

TABLE II. Composition of fractions obtained from chromatography of lipids on silicic acid. 53 mg of phosphono and phospholipids were applied to the column. Total recovery was 52,95 mg (99.90 per cent).
a. For IR spectral frequencies see the Results section.[3-7]

solvent	fractions colected	TLC R_F system A	TLC system B	TLC system C	IR spectral data component identified
5% methanol in chloroform	2-7	0.96	0.98	---	phosphono-sphingomyelin[a]
20% methanol in chloroform	I9-27	0.68	0.00	---	cardiolipin[a]
40% methanol in chloroform	39-46	0.00	----	---	dihydroceramide[a]
	42-5I	0.74	0.00		phosphatidyl ethanolamine[a]
	55-63	---	0.69		dihydroceramide phosphonate[a]
	60-68	0.30	---		phosphatidyl choline[a]
80% methanol in chloroform	7I-80	0.00	0.00	0.I0	lyso-lecithin[a]
	79-9I	0.I7/0.I5			dihydroceramide phosphoryl choline/sphingomyelin[a]

of the fractionation pattern of the phosphonolipids under examination is graphically depicted in Fig. 1. With the solvents used 99.90% per cent of the lipids applied to the column could be recovered.

The following IR frequencies were observed for the various lipids under examination:[3-7]

phosphono-sphingomyelin: amide I and II, 1690, 1640 and 1540 cm^{-1} ; choline and trans, 955 cm^{-1} ; (P=0) v1260 cm^{-1} ; $(P-C)_v$, 1005, 1050, 1180 cm^{-1} and (C—P) stretch, 730 cm^{-1} ;

cardiolipin: ester carbonyl, 1738 cm^{-1} ; $(P=0)_v$, 1230 cm^{-1} ; P—0 (C), 970 cm^{-1} ; (P)—0—C, 1070 cm^{-1} ;

phosphatidyl ethanolamine: ester carbonyl, 1741 cm^{-1} ; P—0—H stretch, 2631 cm^{-1} ; C—N stretch and N—H in plane bend, 1554 cm^{-1} ; $(P=0)_v$, 1265 cm^{-1} ;
(P)—0—C, 1075 cm^{-1} ; N—H stretch, 3480 cm^{-1} ; N—H deformation, 1658 cm^{-1} ;

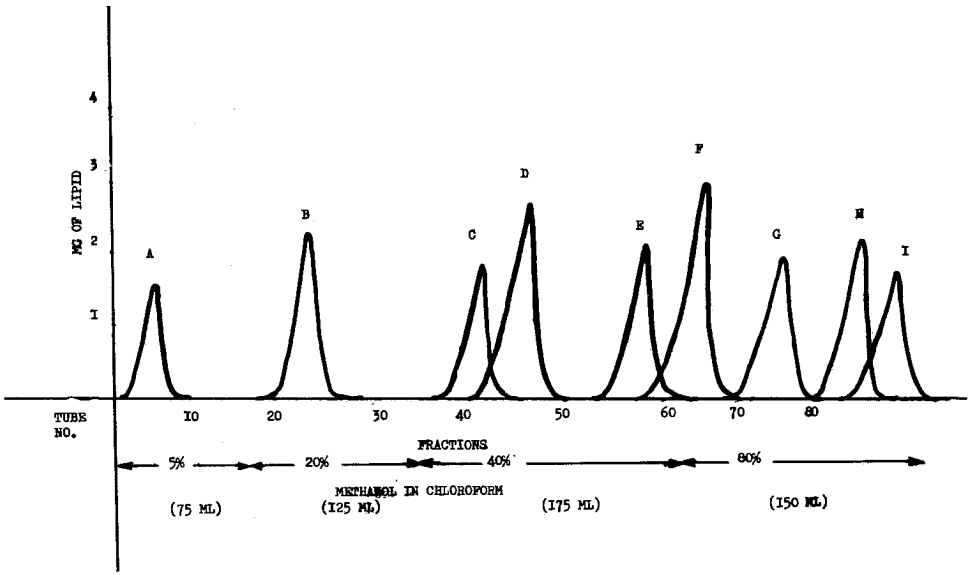

FIG. 1: *Chromatography of the various indicated phosphono and phospholipids on silicic acid. Solvents were methanol in chloroform (a) 5% (b) 20% (c) 40% and (d) 80%. The composition of the various fractions was: A. phosphono-sphingomyelin, 3 mg; B. cardiolipin, 6 mg; C. dihydroceramides, 5 mg; D. Phosphatidylethanolamine, 7 mg; E. dihydroceramide phosphonate, 6 mg; F. phosphatidyl choline, 8 mg; G. lyso-lecithin, 7 mg; H. dihydroceramide phosphorylcholine, 6 mg; I. sphingomyelin, 5 mg. The lipids were applied to the column in 5.0 ml of chloroform.*

dihydroceramide phesphonate: frequencies observed were similar to those for phosphono-sphingomyelin;
phosphatidyl choline: frequencies were similar to those of cephalin; amide I and II, 1685, 1550 cm^{-1};
lyso-lecithin: $(0-H)_v$, 3578 cm^{-1}; other frequencies similar to those of lecithin;
dihydroceramide phosphorylcholine and sphingomyelin:
amide I and II, 1640, 1550 cm^{-1}; choline, 980 cm^{-1}; $(P=0)_v$, 1240 cm^{-1}; P–0–(C), 965 cm^{-1}; (P)–0–C, 1080 cm^{-1}.

Discussion

From the experimental results is seen that the N-palmitoyl and N-lignoceryl ceramides are eluted together with phosphatidyl ethanolamine and the phosphorylated ceramides are eluted with sphingomyelin in the respective fraction, with 40 and 80 per cent methanol in chloroform respectively. The phosphono-ceramides are eluted prior to phosphatidyl choline, and is thus seen that phosphono-sphingomyelin and the phosphono-ceramides do not exhibit similar chromatographic properties.

The chromatographic properties of phosphono-sphingomyelin remain thus unique in this class of compounds.

Summary

The silicic acid column chromatographic behaviour of dihydroceramide-N, N, N-trimethylaminoethyl phosponates is reported.

The above named compounds have been separated from phosphono-sphingomyelin and their phosphoryl analogs. The elution pattern of the dihydroceramide phosphonates is quite different from that of phosphono-sphingomyelin and this difference has been noted.

Key Words: Silicic Acid, Column Chromatography, Phosphonolipids Phosponodihydroceramides.

Περίληψη

Χρωματογραφία στήλης των φωσφόνο-λιποειδών

Στη παρούσα εργασία περιγράφεται η χρωματογραφική συμπεριφορά σε στήλη πυριτικού οξέως, των DL-διϋδρο-φωσφόνοκηραμιδίων. Παράλληλα γίνεται σύγκριση με τις αντίστοιχες ιδιότητες της φωσφόνο-σφιγγομυελίνης. Τα αποτελέσματα δείχνουν ότι οι ιδιότητες των παραπάνω ενώσεων είναι κατά πολύ διάφορες από αυτές της φωσφόνο-σφιγγομυελίνης. Τα διϋδρο-φωσφόνο-κηραμίδια εκλούωνται με 40 τα εκατό μεθανόλη σε χλωροφόρμιο ενώ η φωσφόνο-σφιγγομυελίνη εκλούεται με 5 τα εκατό μεθανόλη σε χλωροφόρμιο. Από τα παραπάνω συνάγεται ότι οι ιδιότητες της φωσφόνο-σφιγγομυελίνης παραμένουν ιδιάζουσες.[1]

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SHORT PAPER

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SOLVENT EFFECT ON COMPLEX FORMATION BETWEEN 1,2-DIMETHYLIMIDAZOLE AND IODINE

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Introduction

It is well known that the solvent may influence the donor-acceptor (DA) equilibria by nonspecific physical effects which depend on solvent polarity and molecular structure as well as by specific interactions. Numerous authors have studied the influence of solvent on such equilibria.[1-7]

In continuing our studies on imidazole-iodine charge-transfer complexes[8] we communicate the results of a spectrophotometric investigation on 1,2-dimethylimidazole-iodine charge-transfer complex in solvents of different polarities. The organic solvents used are cyclohexane, carbon tetrachloride, benzene and chloroform. The equilibrium constants, K_{ct}, and the molar extinction coefficients, ε_{ct}, of the formed 1:1 complexes are computed employing the rearranged equations of the Benesi-Hildebrand.[9] These equations enable independent evaluation of K_{ct} and ε_{ct} graphically.

Experimental

Organic solvents used, B.D.H. AR grade, were purified using the standard methods[10] and stored under nitrogen atmosphere over 4A Linde molecular sieves. Fisher certified-iodine was sublemed under reduced pressure. 1,2-dimethylimidazole was supplied by Aldrich Co.

Stock solutions were prepared by dissolving an accurately weighted amount of the donor and/or iodine in the appropriate volume of solvent. Solutions were made up by mixing appropriate amount of stock donor, stock iodine and pure solvent immediately before running the spectra and kept from moisture as far as possible. Each set contained eight to ten solutions with the same iodine concentration and varying amounts of the donor.

Measurements of absorption were carried out on a Unicam SP 8-100 recording spectrophotometer. The apparatus was equiped with a temperature controlled cell holder and matched 1 cm stoppered silica cells were used. The temperature in the cell holder was controlled by means of a Haake water circulator.

The equilibrium constants, K_{ct}, and the molar extinction coefficients, ε_{ct}, for the complexes formed were determined graphically making use of the following equations[9] (c.f. Fig. 1).

$$\left(\frac{C_A^o + C_D^o}{C_D^o} \right) d = C_A^o \varepsilon_{ct} l - d/K_{ct} C_D^o \qquad (1)$$

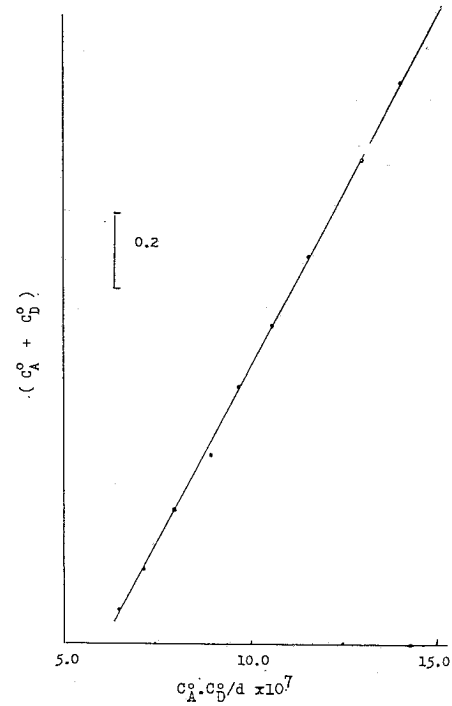

FIG. 1: Plot of $(C_A^o + C_D^o)$ vs $C_A^o \cdot C_D^o/d$ for 1,2-dimethylimidazole- iodine system in cyclohexane.

$$(C_A^o + C_D^o) = \varepsilon_{ct}\ 1\ C_A^o\ C_D^o / d - 1/K_{ct} \qquad (2)$$

where C_A^o, C_D^o are the total concentrations of iodine and 1,2-dimethylimidazole respectively, in mole L^{-1}, $1 = 1$ and d is the absorbance at the wavelength of the charge-transfer band.

Calculations were made using a BASIC computer program for the unweighted linear least-square fit method on a NEWBRAIN computer.

Results and Discussion

The measured absorption spectra of a set of solutions containing a constant concentration, 5.0×10^{-4}M of iodine and varying concentrations of the donor-solvent systems at 298°K, listed in Table I, were typical of all systems previously reported.[8] They showed sharp isosbestic points and did not exhibit any variations in intensity with time. The data fitted the 1:1 equilibrium

$$D + I_2 \rightleftharpoons DI_2$$

satisfactorily, the consistence of the K_{ct} values obtained from different wavelengths (λ_{ct}, $\lambda_{ct} \pm 5$ nm) usually being within 2%.

The experimental results (Table I) of the 1,2-dimethylimidazole-iodine complex in the different solvents used show that the values of the equilibrium constant and the extinction coefficient as well as the spectrophotometric properties are markedly affected by variation of the solvent in which the measurements are carried out. The effect of changing the medium on the K_{ct} values runs in the following order:

cyclohexane > carbon tetrachloride > benzene > chloroform

TABLE I: Equilibrium constants, K_{ct}, extinction coefficients, ε_{ct}, and spectral characteristics of the 1,2-dimethylimidazole-iodine complexes in different solvents at 298°K.

Solvent	diel. const.	1		2		λ_{ct} (nm)
		K_{ct} ($M^{-1}L$)	ε_{ct} ($M^{-1}L\ cm^{-1}$)	K_{ct} ($M^{-1}L$)	ε_{ct} ($M^{-1}cm^{-1}L$)	
Cyclohexane	2.02	2840	1947	2933	1920	400
Carbon tetrachloride	2.24	1191	1670	1215	1675	390
Benzene	2.28	1023	1401	1109	1438	382
Chloroform	4.81	977	1910	1010	1898	360

1,2 refer to the values obtained using equations 1,2 respectively; standard deviations of K_{ct} ± 2%; ε_{ct} ± 3%.

The same order was found for the solvent dependence of K_{ct} for iodine complexes with various donors of the pyridine family.[7] We can expect that the increase in solvent polarity would result in increased K_{ct} values because the complex should be better solvated than the donor and the acceptor. The revers order is observed for the sequence cyclohexane to chloroform. This can be explained by a decrease in the number of solvent molecules in the solvation spheres of the donor and the acceptor on complex formation.

The solvent effect on the donor-acceptor equilibria should be referring to the data of the gas phase reaction. Unfortunately, these data for the present systems are not available. Thus, we have to relate the observed data to a medium which can be arbitrarily assumed to be a non-solvating one. A non-polar and non-reacting aliphatic hydrocarbon solvent, cyclohexane, is used as a reference.

The decrease in K_{ct} values must be attributed to the solute-solvent and solute-solute competing equilibria. In carbon tetrachloride, the competing interactions would mainly involve the n-donor action of 1,2-dimethylimidazole, the pyridine-nitrogen atom[8] towards iodine and the solvent. Interactions between carbon tetrachloride, weak acceptor, and 1,2-dimethylimidazole will be weak. The iodine-CCl_4 specific interaction with the equilibrium constant as great as 1.45 - 0.15 $M^{-1}L$ was reported.[11] The interactions between the complex and carbon tetrachloride should be of non-specific character.

The competing interactions in benzene taking into account are the well-known classical iodine-benzene interactions, the interactions between the donor and benzene and the solvation of the donor-acceptor complex.

Lower values of K_{ct} are found for chloroform solutions. This is mainly ascribed to the relatively strong competing amine-solvent, N lone pair ... H interaction, because the iodine-chloroform specific interactions seem to be negligible. The last conclusion stems from the fact that I_2-$CHCl_3$ mixtures behave like the I_2-CCl_4 system as regular solutions.[12] However, the decrease in the K_{ct} value in chloroform with respect to carbon tetrachloride as solvent seems to be smaller than one could expect from allowance for competing donor-acceptor-solvent equilibria. This suggests that the complex is better solvated with chloroform than with carbon tetrachloride. The reason may here be the polarity of the medium and the possibility of a weak I...H hydrogen bonding between the complex and chloroform. The hydrogen bonding in-

teraction being favored by the negative charge aquired by the terminal iodine as a sequence of complex formation.[13, 14] The specific solvation of the imidazole ring with chloroform through π ... H hydrogen bonding seems to be of minor importance. This is due to the relatively weak π -site interactions of the free 1,2-dimethylimidazole may be expected to be further weakened after complexation due to the transfer of the charge from the imidazole ring to iodine.

Summary

The absorption spectra of 1,2-dimethylimidazole-iodine solutions in organic solvents viz cyclohexane, carbon tetrachloride, benzene and chloroform have been measured and interpreted in terms of the $D + I_2 \rightleftharpoons DI_2$ equilibrium. The values of K_{ct} and ε_{ct} for the reaction were calculated. It was found that K_{ct} values run in the following sequence:

cyclohexane > carbon tetrachloride > benzene > chloroform.

This behaviour was attributed to solute-solute and solute-solvent competing equilibria.

Key Words: Complexes, 1,2-Dimethylimidazole, Iodine, Chemical equilibrium, Absorption spectra, Equilibrium constant.

Περίληψη

Σύμπλοκα του 1,2-διμεθυλοϊμιδαζολίου-ιωδίου

Μετρήθηκαν τα φάσματα απορρόφησης σε οργανικούς διαλύτες (κυκλοεξάνιο, τετραχλωράνθρακα, βενζόλιο και χλωροφόρμιο) συστημάτων 1,2-διμεθυλοϊμιδαζολίου-ιωδίου και ερμηνεύτηκαν σε σχέση με την ισορροπία $D + I_2 \rightleftharpoons DI_2$. Υπολογίστηκαν οι τιμές K_{ct} και ε_{ct} της αντιδράσεως. Βρέθηκε ότι οι τιμές των K_{ct} ακολουθούν τη σειρά:

κυκλοεξάνιο > τετραχλωράνθρακας > βενζόλιο > χλωροφόρμιο.

Η συμπεριφορά αυτή αποδόθηκε σε ανταγωνιστική ισορροπία μεταξύ διαλυμένης ουσίας - διαλυμένης ουσίας και διαλυμένης ουσίας - διαλύτη.

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SHORT PAPER

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ZINC CONCENTRATION IN PLASMA, SERUM AND HAIR OF HEALTHY PERSONS

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Abbreviations

A.A.S.: Atomic absorption spectrophotometry. T.C.A.: Trichloracetic acid.

Introduction

Zinc has proven to be as an essential nutrient for plants and lower animals. Raulin, more than a century ago, has demonstrated, that Aspergillus niger requires zinc for growth[1,2]. Since then accumulated evidence show that zinc may be important for the activity of at least seventy metalloenzymes[3].

Chronic zinc deficiency is associated with various diseases in man. Abnormaly low zinc values have been obtained in cases of alcoholic cirrhosis, other types of liver diseases, active tuberculosis, indolent ulcers, uremia, myocardial infarct e.t.c. There is also evidence that certain cases of nutritional dwarfism in man, are associated with zinc deficiency[4], as well as, hypogeusia[6], e.t.c. some cases of zinc intoxication in humans have also been reported[7].

In order to estimate zinc deficiency or excess in humans, it is important to define its normal levels in various biological specimens.

According to literature data, plasma, serum and hair zinc concentrations are indicative of acute[8] and chronic zinc status disorders respectively[9].

In the present study zinc levels in serum, plasma and hair were determined.

Materials and Methods

Twenty five males, 26-76 years old and thirty seven females 17-80 years old, all healthy, inhabitants of the Ioannina district, were studied.

This population was distributed according to age as following. < 20 years old: 5females, 20-40 years old: 11males and 12females and 40-60 years old: 7males and 9females and 60-80 years old: 7males and 11females.

Blood samples were taken from vena cubutalis and plasma was separated and placed in sterilized tubes which had been rinsed with acidified distilled water and dried, twice. Free zinc heparin (heparin-Li) was used for plasma collection.

Plasma and serum samples were diluted (1+4) with distilled water[10].

Hair samples of approximately 100mg, 2-3cm long, were removed from the occipital region of each subject's head. The samples were placed in a stoppered polyethylene tube and washed and treated twice successively for 30min with each of hexane, ethanol and distilled water. Samples were then dried for 8 hours at 90°C and kept in a desiccator. 25mg from each sample were placed in a 10ml glass volumetric flask and 5ml of 20% w/v T.C.A. solution were added. The flasks were then placed in an oven at 90°C for 8-9 hours. The volume was restored to 10ml with distilled water[8].

All samples (plasma, serum, hair) were measured for zinc by A.A.S. with a Perkin Elmer 560 Spectrophotometer.

Results were evaluated statistically according to age and sex by computer model SINCLAIR SX81 and the t-test was applied in order to calculate the statistical significance of the results.

Results and Discussion

Detailed results concerning serum, plasma and hair zinc concentrations according to age and sex are shown in Table I, II and III. Mean plasma, serum and hair zinc

TABLE I. Plasma, serum and hair zinc concentration values of male subjects.

R.G.	Name (abr.)	Age (years)	*Zn mg/l*		*Zn μg/g*
			Plasma	serum	Hair
1	A.T	26	1.154	1.178	234.8
2	N.Ch	29	1.090	1.066	—
3	S.M.	25	1.133	1.189	203.6
4.	G.K	25	1.220	1.044	199.2
5.	Ch.N	24	1.233	1.291	—
6.	D.P	32	0.968	1.082	—
7.	E.A	36	1.145	1.245	195.6
8.	K.S	35	0.993	1.008	237.6
9	G.B	37	0.902	0.899	231.2
10.	D.Th	37	0.989	1.237	246.8
11.	Ch.Th	33	0.899	1.320	218.4
12.	Ch.D	43	0.966	1.299	232.4
13.	C.Ch	43	0.695	0.705	—
14.	K.N	49	0.940	0.835	220.4
15.	Ch.E	41	0.955	1.075	225.6
16.	A.G	43	1.372	1.243	227.6
17.	G.Th	54	0.697	1.160	198.4
18.	K.G	58	0.497	0.614	—
19.	K.D	68	0.518	0.918	241.2
20.	I.S	65	0.886	1.230	230.0
21.	D.I	68	0.569	0.737	244.8
22.	Z.E	61	0.829	0.958	243.2

23.	G.Ch	70	1.061	1.146	236.4
24.	P.N	73	0.712	0.734	192.8
25.	K.Th	76	1.032	0.708	—

TABLE II. Plasma, serum and hair zinc concentration values of female subjects.

R.G.	Name (abr.)	Age (Years)	*Zn mg/l*		*Zn* µg/g
			Plasma	Serum	Hair
1	B.A	17	0.669	0.714	236.4
2.	G.Th	13	0.951	0.927	212.8
3.	P.E	18	1.022	0.914	218.0
4.	P.V	18	0.887	1.072	239.6
5.	M.N	18	0.744	0.909	—
6.	G.S	29	0.981	0.972	228.4
7.	M.F	22	0.933	0.854	240.4
8.	J.P	22	0.710	0.907	243.6
9.	Ch.E	22	0.957	0.962	245.6
10.	V.E	21	0.663	1.079	—
11.	A.D	32	0.830	1.002	204.8
12.	V.V	33	0.923	1.040	198.4
13.	M.L	31	0.957	0.962	237.2
14.	A.P	37	0.657	0.622	—
15.	B.E	37	0.997	0.952	192.4
16.	Ch.M.	36	1.087	1.020	215.6
17.	B.P	39	0.812	1.118	224.4
18.	H.S	41	0.744	0.801	187.2
19.	P.S	49	0.865	0.802	248.8
20.	K.D	45	1.255	1.175	252.4
21.	K.K	48	0.616	1.077	—
22.	V.E	44	0.683	1.070	—
23.	M.E	52	1.066	1.380	238.4
24.	J.R	58	0.682	0.766	—
25.	J.V	59	1.962	1.296	—
26.	G.V	53	1.043	0.960	—
27.	T.M	64	0.844	0.952	—
28.	P.V	68	0.590	0.634	192.8
29	Z.S	60	0.797	1.055	216.4
30.	T.S	68	0.804	1.145	—
31.	G.U	68	1.040	0.843	204.8
32.	G.U	73	0.553	0.637	227.2
33.	R.L	77	0.539	0.797	243.6
34.	K.A	75	0.988	0.864	—
35	A.A	80	0.851	0.934	—
36.	L.A	80	0.606	0.553	235.6
37.	G.V	80	0.611	0.752	244.4

TABLE III. Mean plasma, serum and hair zinc concentration values in males and females according to age.

Males	*Age:<20*	20-40	40-60	60-80	Total
Plasma Zn mg/l	–	N:11 $\bar{X}$:1.060 SD:0.125	N:7 $\bar{X}$:0.874 SD:0.280	N:7 $\bar{X}$:0.798 SD:0.211	N:25 $\bar{X}$:0.934 SD:0.226
Serum Zn mg/l	–	N:11 $\bar{X}$:1.148 SD:0.125	N:7 $\bar{X}$:0.990 SD:0.271	N:7 $\bar{X}$:0.918 SD:0.208	N:25 $\bar{X}$:1.108 SD:0.447
Hair Zn µg/g	–	N:8 $\bar{X}$:220.9 SD:19.5	N:5 $\bar{X}$:220.9 SD:13.3	N:6 $\bar{X}$:231.4 SD:19.7	N:19 $\bar{X}$:224.2 SD:17.8
Females					
Plasma Zn mg/l	N:5 $\bar{X}$:0.854 SD:0.145	N:12 $\bar{X}$:0.839 SD:0.112	N:9 $\bar{X}$:0.879 SD:0.176	N:11 $\bar{X}$:0.760 SD:0.185	N:37 $\bar{X}$:0.823 SD:0.157
SerumZn mg/l	N:5 $\bar{X}$:0.906 SD:0.127	N:12 $\bar{X}$:0.957 SD:0.127	N:9 $\bar{X}$:1.036 SD:0.222	N:11 $\bar{X}$:0.814 SD:0.121	N:37 $\bar{X}$:0.932 SD:0.182
Hair Zn µg/g	N:4 $\bar{X}$:226.7 SD:13.3	N:10 $\bar{X}$:223,1 SD:19.4	N:4 $\bar{X}$:231.7 SD:30.2	N:7 $\bar{X}$:223.5 SD:19.8	N:25 $\bar{X}$:225.7 SD:19.6

concentrations for men were: $\bar{X}$:0.934±0.226mg/l, 1.108±0.447mg/l and 224.2±17.8µg/g respectively. Mean plasma, serum and hair zinc concentrations for women were $\bar{X}$:0.823±0.157mg/l, 0.932±0.157mg/l, and 225.7±19.6µg/g respectively. Serum zinc concentrations were found higher than those for plasma the difference being statistically insignificant, presumably because of contamination from disintegrated platelets[11,12]. It seems more appropriate to measure plasma rather serum zinc concentrations.

A slight decrease in serum and plasma zinc concentrations was observed in subjects over 60 years old in both groups (figure 1), compared to those of younger subjects, the difference being statistically insignificant. This slight decrease could be due to restricted food intake and some malabsorption problems[13] and hormonal changes related to age[14,15]. Lower values of zinc concentration in all investigated specimens but differences from the respective male's samples were of no statistical significance. No linear correlation between plasma and serum zinc values in male (r:0.639) of female (r:0.481) was found.

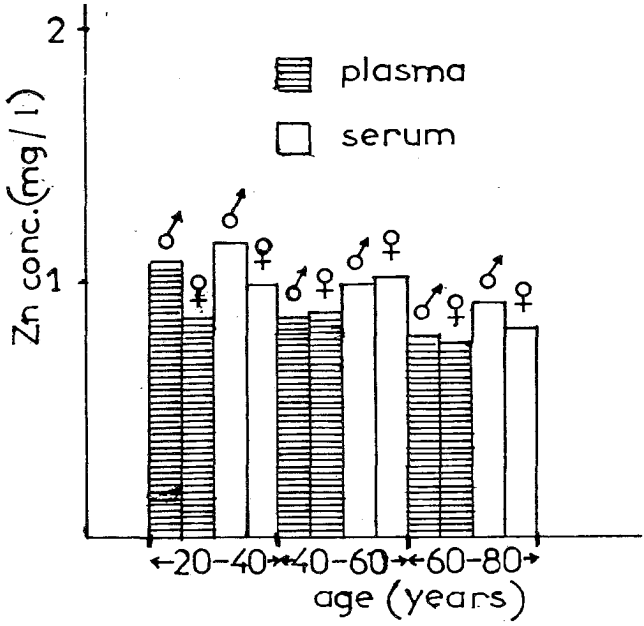

FIG. 1: *Plasma and serum zinc concentration distribution according to age and sex.*

No statistically significant difference was found, between hair zinc concentrations of males and females. Hair zinc concentrations were found slightly higher (≃8.5%) compared to literature data, probably due to regional nutrition factors.

The results indicate that zinc concentrations in plasma and serum of the Ioannina population are within normal limits, as they are defined by literature data[2,11,13,16]

Summary

Plasma, serum and hair zinc concentration were determined by A.A.S. in the Ioannina district population (25males and 37females).

Mean values for the above mentioned biological specimens were 0.934±0.226, 1.108±0.447 mg/l, 224.2±17.8μg/g for males and 0.823±0.157, 0.932±0.182mg/l, 225.7±19.6μg/g for females, respectively.

Plasma zinc evaluation seems to be the more reliable method for the determination of zinc status in humans.

Key words: Zinc concentration plasma, serum, hair, healthy persons.

Περίληψη

Συγκεντρώσεις ψευδαργύρου στο πλάσμα, στον ορό και στις τρίχες κεφαλής σε υγιή άτομα.

Στην εργασία αυτή προσδιορίσθηκαν οι συγκεντρώσεις ψευδαργύρου στο πλάσμα, στον ορό και τις τρίχες κεφαλής με φασματοφωτομετρία ατομικής απορρόφησης, σε ενήλικες κάτοικους της περιοχής Ιωαννίνων (25 άνδρες και 37 γυναίκες).

Οι μέσες τιμές ψευδαργύρου που βρέθηκαν στο πλάσμα, τον ορό και τις τρίχες των ατόμων που μελετήθηκαν ήταν:
0.934±0.226, 1.108±0.447 mg/l και 224.2±17.8 μg/g για τους άνδρες και 0.823±0.157, 0.932±0.182 mg/l και 225±19.6 μg/g για τις γυναίκες αντίστοιχα.

Δεν βρέθηκαν στατιστικά σημαντικές διαφορές στις τιμές των συγκεντρώσεων ψευδαργύρου στα δείγματα που εξετάστηκαν, τόσο σε σχέση με το φύλο όσο και με την ηλικία, των ατόμων που μελετήθηκαν.

Ο προσδιορισμός του ψευδαργύρου στο πλάσμα φαίνεται να είναι περισσότερο αξιόπιστη μέθοδος για την εκτίμηση των επιπέδων ψευδαργύρου στον άνθρωπο.

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NOTE

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COMPLEX GLYCO AND PHOSPONO LIPIDS IN A TERRESTRIAL MOLLUSC

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Abbreviations

AEP_n: 2 aminoethylphosphonic acid. $CAEP_n$: ceramide aminoethylphosphonate. $CMAEP_n$: ceramide-N-methylaminoethyl phosphonate. TLC: thin layer chromatography. MAS: mild alkaline stable.

Introduction

The isolation of 2-aminoethylphosphonic acid (ciliatine, AEP_n) in 1959 by Horiguchi[1a] from ciliated protozoa of the rumen initiated extensive research on the Biochemistry of lipids containing a C-P bond.[1b, c, d, 30]

In 1963 Rouser[2] isolated $CAEP_n$ from a sea anemone. This phosphonolipid together with $CMAEP_n$ has been found to be widely distributed in molluscs[3-5] especially in a variety of species of fresh-water and marine bivalves,[6] snails,[4, 7] cephalopods,[4] oysters,[8, 9] also in protozoa,[10-17] cnidaria,[18, 31, 32] ox,[19] and in *"Bdellovibrio bacteriovorus"*[20] and *"Pythium prolatum"*.[21, 22]

Spingophosphonolipids of a new type, aminoalkylphosphonyl cerebrosides, were isolated from the total lipids of the viscera, of the marine gastropod, *"Turbo cornutus"*. they have been characterized as 1-0-(6′-0-(N-methylaminoethyl phosphonyl) galactopyranosyl) ceramide and 1-0-[6′-0-(aminoethylphosphonyl) galactopyranosyl] ceramide by Hayashi and Matsuura.[23]

More recently 2 kinds of sphingophosphonoglycolipids have been isolated from the water/methanol soluble lipids of the marine gastropod *"Aplysia Kurodai"*[24, 25] which were shown to have 2 mol of 2-AEP and an oligosacharide chain in their molecule.

Interestingly, the above ceramide phosphonolipids and glycophosphonolipids seem to replace sphingomyelin and gangliosides respectively in these organisms.

Material - Methods

A. Materials and reagents: The terrestrial snails *"Eobania vermiculata"* were collected in the area of East Crete, Greece, early in the spring. They were kept in a perforated box at room temperature for approximately one month before lipid extrac-

tion. Silica gel G type 60 was purchased from MERK, Silica gel from Mallinckrodt; silicic acid 100 Mesh for column chromatography, and cardiolipin, sphingosine, cerebrosides, ceramides from SERVA, phosphatidyl ethanolamine and phosphatidyl choline were extracted from egg yolk and sphingomyelin from bovine brain in our laboratory.

B. Extraction of lipids: The total lipids were extracted by a modification of Bligh-Dyer method.[26] The final proportion of the solvent system volumes was C-M-W 1.5:2:0.8, instead of 1.5:1:0.9 (v/v/v). The lipids of the water/methanol phase were extracted with 1 vol. chloroform and $^1/_2$ vol. ethanol. The lower phase was evaporated to dryness, redissolved in chloroform/ethanol (9:1) and analyzed for the "acidic lipids".

C. Analytical Methods: Phosphorus was determined by the Long-Staples method,[27] phosphonolipids by a modification of Aalbers-Bieber method[28] also with a method devised in our laboratory[33] and carbohydrate by the phenol-sulfuric acid colorimetric method.

D. Separation and purification of the acidic lipids: Acidic lipids dissolved in a minimal amount of chloroform/methanol (98:2, v/v) were applied to a silicic acid column (13cm × 0.5cm i.d.) packed with 2.5g of silicic acid activated at 110°C for 15 h and 2.5 g Hyflo Super Cel and eluted stepwisely as shown in table I. Elution was monitored by TLC with chloroform/ methanol/ water (65:25:1 v/v/v).

E. T.L.C. was performed on glass chromatoplates coated with silica gel G (thickness 0.25 mm) and activated 1h at 120°C. Solvent systems: chloroform/ methanol/ water (65:25:4 v/v/v), chloroform/ methanol (95:5) or n-propanol/ water (7:3) or n-propanol/28% NH_4OH/water (6:2:1) or chloroform/ methanol/ acetic acid/ water (50:25:6:2).

F. Mild Alkaline Hydrolysis was carried out with 0.1N NaOH in 50% MeOH for 20 min at 45°C.[29]

Results and Discussion

Extraction of the lipids of *"E. vermiculata"* was carried out by the Bligh-Dyer method.[26] The yield of total lipid-P in the chloroform layer was 15±3mg P/100g wet tissue (4 experiments). The "acidic lipids", including the recently discovered glycophosphonolipids[23-25] may be recovered from the aqueous-methanolic layer of the above extraction method by extraction with chloroform-ethanol (V.M. Kapoulas and E. Tsamberis: Unpublished data). By this procedure another 3.5±0.5% of total lipid-P along with carbohydrate (0.022 g per 100 g wet tissue) were recovered in the new chloroform layer. The total "acidic lipids" were examined by T.L.C. (see fig. 1).

The components of this lipid extract were fractionated into 9 fractions on a silicic acid column eluted with acetone-methanol and chloroform-methanol mixtures of increasing polarity, as indicated in Table I.

Each fraction was examined by TLC combined with phosphorus and carbohydrate assays on the intact and alkali-stable (MAS) lipids.

As shown in Table I, the bulk of carbohydrate (69.2%) accompanied by 3.1% of the total lipid-P were eluted, as fraction Ia, with the first 2 bed volumes of acetone/

TABLE I: Elution of snail "acidic" lipids from silicic acid column.

Fraction No.	Void	I		II	III		IV		
		a	b		a	b	a	b	c
bed volumes	2	2	4	4	2	1	1	1	30
Solvent system	C/M 98:2	Ac/M 4:1	Ac/M 4:1	C/M 3:2	C/M 1:1	C/M 1:1	CH_3OH	CH_3OH	CH_3OH
A. Phosphorus content									
1. Intact column fractions									
–P% of total lipid-P	0.45	3.1	8.3	54.0	15.4	3	3	9	3.5
–P (mol/column fraction)	0.15	1.05	2.82	18.36	5.24	1.02	1.02	3.06	1.19
2. After mild alkaline hydrolysis column fractions (MAS)									
–P% of column fraction		60	57.4	47	35				
–P (mol/column fraction)		0.63	1.62	8.63	1.83				
–% total P		2	4.7	25.3					
–Phosphonate % of MAS		99		37	0				
–Phosphonate % of total-P		2		8.3	0				
–Main components		Glyco-phospho-nate		phospho-nate					
B. Sugar content									
1. Intact column fractions									
–% of total Hexose (Sugar)	2.9	69.2	8.7	6.1	3				
–Hexose (mol/column fraction)	1.63	32.0	4.90	3.44	1.69				
2. After mild alkaline hydrolysis column fractions (MAS)									
–Hexose % of column fraction		30	27	33.3	15.9				
–Hexose (mol/column fraction)		11.7	1.32	1.15	0.27				
–% of total Hexose		20.8	2.4	2	0.5				
C. Molar ratio: Hexose/P									
1. Intact column fraction	10.9	37.1	1.74	0.19	0.32	0.83	1.22	0.83	0.9
2. MAS		18.6	0.82	0.13	0.15				

methanol (4:1). About 60% of this P-content belongs to phosphonolipids. After mild alkaline hydrolysis about 30% of its carbohydrate (20.8% of total carbohydrate) were partitioned in the chloroform layer. The next 4 bed volumes of the same solvent (fraction Ib) eluted 8.3% of the total lipid-P and 8.7% of total carbohydrate. Approx. 50% of this lipid-P corresponds to a M.A.S., ninhydrin positive phosphono-sphingolipid. Ceramides were also eluted mainly in this fraction, while 27% of its sugar corresponds to MAS glycosphingolipids. The bulk of lipid-P (54%) were eluted with 4 bed volumes of chloroform/ methanol 3:2 (fraction II). About 47% of it belongs to MAS components and represents 37% of P-content of this fraction, according to phosphonate-P analysis. Another 15% of lipid-P were eluted next with 2

bed volumes of chloroform/ methanol (1:1). Approx. 35% of it corresponds to a MAS phospholipid moving on T.L.C. slightly lower than sphingomyelin.

As shown in Fig. 2, fraction Ia contains 3 phospholipid spots and several spots positive to α-naphthol. After mild alkaline hydrolysis the spots between phosphatidylcholine and sphigosine remain unchanged suggesting that in this area there is a P-free glycolipid plus a glyco-phosphonolipid (because, according to Table I, the MAS fraction contains 99% phosphonate-P). This lipid is suspected to be similar to the glycophosphonolipid reported by Araki and Satake,[24] on the basis of

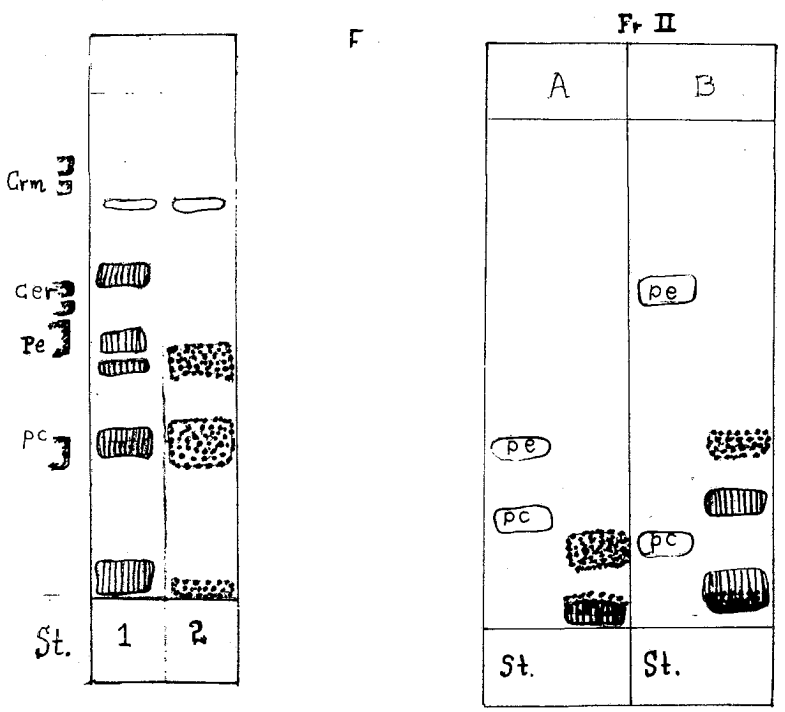

FIG. 1: *TLC of total "acidic lipids" before column separation along with standards. The chromatograms were developed with chloroform/MeOH/Water (65:25:4, v/v/v). The spots were detected by 1) spraying with naphtol reagent 2) spraying with molybdenum blue reagent.*
Abbreviations: see fig. 2.

FIG. 3: *TLC of the MAS components of the column fraction No. II along with standards (st). The chromatograms were developed with*
A: Chloroform/MeOH/conc. ammonia (60:35:5, v/v/v)
B: Chloroform/MeOH/A.A./ Water (50:25:6:2, v/v/v/v)
The standards and the visualization as shown in fig. 2.

their chromatographic behavior. The MAS fraction contains also another intact spot moving close to cerebroside standards. In addition, this fraction contains another two unknown phospholipids plus another P-free, stable to alkali, spot moving on TLC close to ceramide standars (see fig. 2).

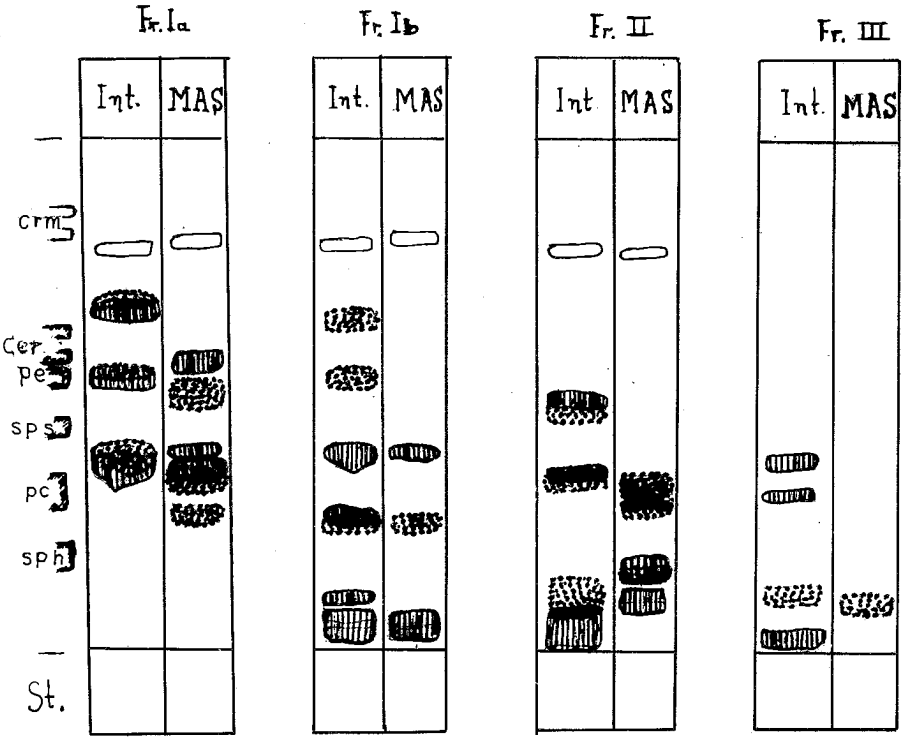

FIG. 2: *TLC of the intact (Int) and MAS lipids of column fractions No: Ia, Ib, II and III along with standards.*
Pe: Phosphatidyl ethanolamine, pc: phosphatidyl choline, Crm: ceramides, Sps: sphingosine, Sph: sphingomyelin, Cer: cerebrosides. The chromatograms were developed with chloroform/MeOH/Water (65:25:4, v/v/v).
The spots were detected by
i) iodine vapor
ii) spraying with molybdenum bleue reagent
iii) spraying with napthol reagent.

Fraction Ib contains three phospholipid spots (fig. 2) of which only one remains in MAS fraction, moving between phosphatidylcholine and sphingomyelin. In addition, there are three P-free glycolipids (possibly ceramide-oligosaccharides) some of which are modified by the mild alkali treatment. Finally, this fraction contains also the ceramide-like, alkali stable lipid of the previous fraction.

In fraction II, one of the MAS components corresponds to CMAEP according to chromatographic behavior (see Figs. 2 and 3). There is also another MAS phospholipid plus two MAS glycolipids, one of which seems to contain also -P. This spot possibly is a glyco-phosphonoplasmalogen (see fig. 3b).

Fraction III contains also at least three minor glycolipids (see fig. 2) but due to limited quantity, it was not possible to obtain a TLC picture of a-napthol positive spots of the MAS components of this fraction.

Summary

Complex glyco and phosphono lipids in a terrestrial mollusc

The total lipids of the gastropod mollusc *"E. vermiculata"* were isolated by the Bligh-Dyer method, and 15±3 mg lipid- P/100 g wet tissue were partitioned in the chloroform layer; another 3.5±0.5% of total lipid-P were partitioned in the water methanol layer along with sugar (0.0229% sugar) containing lipids ("acidic lipids"). The latter were extracted with chloroform/ ethanol (2:1, v/v) and fractionated by column chromatography on silicic acid.

A glycophosphonolipid was eluted with 2 bed volumes of acetone/ methanol, (4:1, v/v) and an alkali stable phosphonolipid (possibly CMAEP) was eluted with another 4 bed volumes of the same solvents) Next another molecular species of CMAEP was eluted with chloroform/ methanol (3:2, v/v). Several alkali stable cerebrosides, ceramides and ceramide-oligosaccharides were also identified in the eluates.

Key words: Invertebrate lipids, column chromatography, phosphonolipids, glycolipids, sphingolipids, ceramide aminoethyl phosphonate.

Περίληψη

Σύμπλοκα γλύκο και φωσφόνο λιποειδή σε χερσαία μαλάκια

Τα ολικά λιποειδή του γαστρόποδου μαλάκιου *"E. Vermiculata"* απομονώθηκαν με την μέθοδο Bligh-Dyer. Απ' αυτά 15±3 mg λιποειδικού -P/100 γρ νωπού ιστού εκχυλίσθηκαν στη χλωροφορμική φάση ενώ ποσοστό 3,5 ± 0,5% του ολικού λιποειδικού-P πέρασε στην υδατομεθανολική φάση μαζί με λιποειδή περιέχοντα σάκχαρα (σάκχαρο ≃ 0,022 gr/100 gr νωπού ιστού) («όξινα λιποειδή»). Τα τελευταία μετά την εκχύλισή τους με χλωροφόρμιο/ αιθανόλη (2:1, v/v) κλασματώθηκαν με χρωματογραφία στήλης πυριτικού οξέος.

Τα κλάσματα της στήλης μελετήθηκαν με T.L.C. σε συνδυασμό με την ποσοτική ανάλυση, ολικού και σταθερού σε αλκαλική υδρόλυση φωσφόρου, επίσης φωσφόνο-φωσφόρου, καθώς και υδατανθράκων. Το μεγαλύτερο μέρος των υδατανθράκων ≃ 70% και 3,1% του λιποειδικού-P εκλούστηκαν με τους 2 πρώτους όγκους ακετόνης/μεθανόλης (4:1 v/v). 60% του λιποειδικού αυτού P αντιστοιχεί σε ένα σταθερό σε αλκαλική υδρόλυση (θετικό σε νινυδρίνη) φωσφόνοσφίγγολιποειδές, ενώ 30% του σακχάρου του ίδιου κλάσματος αντιστοιχεί σε ένα σταθερό στην ήπια αλκαλική υδρόλυση γλυκοσφιγγολιποειδές. Σ' αυτό το κλάσμα εκλούστηκαν επίσης κηραμίδια.

Με τους επόμενους 4 όγκους του ίδιου διαλύτη κλάσμα I_b εκλούστηκαν φωσφόνοενώσεις και 27% του σακχάρου αυτού του κλάσματος αντιστοιχεί σε M.A.S. γλυκο-σφίγγο-λιποειδές.

Το μεγαλύτερο μέρος του λιποειδικού P (54%) εκλούστηκε με τους 4 επόμενους όγκους χλωροφορμίου/ μεθανόλης (3:2, v/v) και 37% αυτού αντιστοιχεί σε σφίγγοφωσφόνο ενώσεις διαφορετικού μοριακού είδους (δι-, τρι- και τέτρα- υδρόξυ παράγωγα).

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