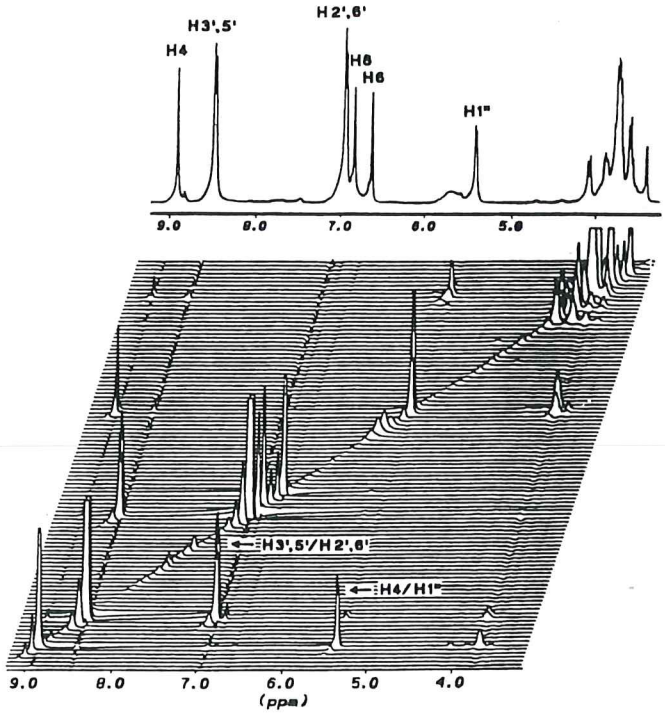
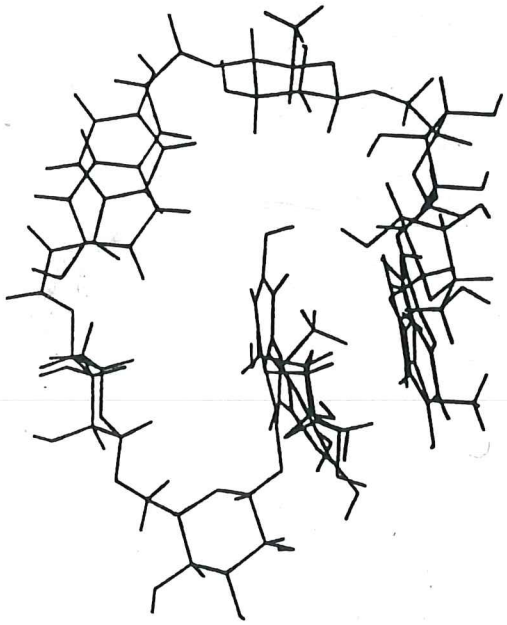
Nasjonalt NMR-møte





Fefor Best Western Hotell, Vinstra 7.-8. januar 1998

NASJONALT NMR-MØTE

FEFOR BEST WESTERN HOTEL, VINSTRA

7 - 8 JANUAR 1998

Støttet av:
Norges Forskningsråd
(via Samarbeidsutvalget for NMR)
Bruker Spectrospin AB

Organisasjonskomite:
Dagfinn W. Aksnes
Einar Sletten
Nina Berg-Johannesen
Kjemisk institutt,
Universitetet i Bergen

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14.20- 14.50	**Ingrid Gribbestad**, MR-senteret, Sintef Unimed *Comparison of High Resolution Magic-Angle Spinning of ex vivo Human Breast Tissue with Conventional NMR Spectroscopy of ex vivo Intact Static Samples and Extracts.*
14.50 -15.10	**Tone Frost Bathen**, NMR-senteret, Sintef Unimed *Quantitative Study of Blood Plasma Lipids Combining NMR Spectoscopy, Multivariate Calibration and Neural Network Analysis*
15.10 - 15.30	**Hege Widerøe**, Kjemisk inst., AVH, NTNU *^{13}P NMR spektroskopi av viable perfuserte leukemiceller*
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17.40 - 18.00	**Torgils Fossen**, Kjemisk inst., UiB *Strukturoppklaring av antocyaner og andre flavonoider i vannliljer*
18.00 - 18.20	**Odd Inge Optun,** Kjemisk inst. NTNU *Konformasjonsanalyse av alkyl 3-hydrosyalkanoater ved bruk av variabel temperatur NMR*
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10.00 - 10.20	**Willy Nerdal**, Kjemisk inst., UiB *Fast-fase NMR studier av lipidmembraner*
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10.40 - 11.40	Tema: *Fysikalsk-organisk NMR* Ordstyrer: **Nils Åge Frøystein**, Kjemisk inst. UiB
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11.20 - 11.40	**Finn Drabløs,** NMR-senteret, Sintef Unimed *Zinc binding in Bacitracin A - NMR and computational studies*
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Pulsed field gradient MNR investigations of n-hexane diffusion in MCM-41.

Hans Christian Gran and Eddy W. Hansen:
Characterization of Pores in Cement Paste.

Eddy W. Hansen, Jan Arve Haaland, Per Olav Kvernberg and Bjørn Pedersen:
Water dynamics and pore distribution in microporous zeolite HY.

Dagfinn W. Aksnes og Lars Gjerdåker:
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Per Eugen Kristiansen, Eddy W. Hansen and Bjørn Pedersen:
Crystallinity of polyethylene determined by NMR.

Biomolekylær -NMR

B. Sitter, I. S. Gribbestad, M. Aursand and J. Krane:
High Resolution ^{1}H NMR spectroscopy of PCA extracts from muscle tissue of stored halibut. NMR as a quality control method?

Halvard Hårklau og Lars Skjeldal:
Paramagnetisk NMR av ferredoxiner.

Håvard H. Hauge, Jon Nissen Meyer og Lars Skeldal:
Induksjon av sekundærstruktur i Plantaricin A alph (Pln A-alpha) ved hjelp av løsemidler.

Erlend Moldrheim and Einar Sletten:
The ability of zinc (II) to stabilize triplex DNA. A high resolution NMR study.

Martin Hartman, Hans Grasdalen and Gudmund Sjåk-Bræk:
Enzyme Kinetics of the Recombinant Mannuronan-C-5 Epimerase AlgE 4, studied by NMR spectroscopy (presentert av Are Kristiansen).

Jamil Saad Shehadeh and Einar Sletten:
The Interaction Between Antitumor Derived Cis-platin with Mono, Di and Oligonucleotides Studied by NMR-Spectroscopy.

Degefa Arasho Wondwossen, Willy Nerdal, Mohamad Osman and Harald Høiland:
Solid State NMR Study of the Interaction of Surfactin (a Lipopeptide with a Model Lipid Membrane.

Signe Steinkopf, Willy Nerdal and Holm Holmsen:
Solid State Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy Study of Glycerophospholipid Liposomes.

Muhammad-Emwas, A. H. and Sletten E.
Copper Complexation of Adenosine monohosphate Nucleotides.

H. Grasdalen, M. Aursand and L. Jørgensen:
NMR Study of Lipid Fluidity in Frozen Red Muscle of Atlantic salmon (Salmo salar): Relation to Autoxidation of Lipids?

Høyoppløsende NMR

Dagfinn W. Aksnes. Paul Juvvik og Knut Skogmo:
Syntese og NMR studier av dioxathianer og dioxarsenaner.

Per Olav Kvernberg and Bjørn Pedersen:
Oxidation of D-Isoascorbic Acid with Hydrogen Peroxide in Aqueous Solution.

Hijazi Abu Ali and Songstad Jon:
Restabilization of the Activated (CH) Group in Phosphorus Ylids Through Coordination to Pt(II) and Ag(I) Transition Metals.

Sammendrag av foredrag

Abstracts of Lectures

Comparison of High-Resolution, Magic-Angle Spinning NMR Spectroscopy of *ex vivo* Human Breast Tissue with Conventional NMR Spectroscopy of *ex vivo* Intact Static Samples and Extracts

I. S. Gribbestad[1], B. Sitter[1] and J. Krane[2]

[1] *SINTEF Unimed MR-Center, N-7034 Trondheim, Norway and* [2] *Norwegian University of Science and Technology, N-7034 Trondheim, Norway*

Introduction

^{1}H NMR spectroscopy has recently proven to give unique information about tumors *in vivo* (1) and *ex vivo* (2). Perchloric acid (PCA) extracts of breast tumors have demonstrated large variations in contents of water-soluble phospholipid metabolites and amino acids, sugar and lactate in malignant versus non-involved breast tissue from the same patient (3). High contents of choline compounds in breast tumors have also been detected *in vivo* (4). In this study we have evaluated the quality of high-resolution MAS spectra of *ex vivo* breast cancer tissue and non-involved tissue and compared the findings of NMR spectra from *ex vivo* static tissue samples and perchloric acid extracts and lipid extracts.

Material and Methods

Human breast tissue specimen were obtained from patients undergoing scheduled surgical procedures. The dissected breast tissues were immediately frozen and stored in liquid nitrogen. 0.1 g of thawed tissue was placed into a 4-mm outer diameter zirconium rotor for use in the MAS experiment. For the static experiments, 0.4 g of the thawed tissue was placed in a 5-mm NMR tube filled with 0.5 ml of PBS/D_2O. All spectra were acquired on BRUKER DRX 400, DRX 500 and DRX 600 instruments. Static samples and liquid extracts samples were analyzed using a 5-mm ^{1}H/^{13}C/^{15}N triple resonance probe, equipped with a Z-gradient (TXI-Z probe, BRUKER Analytik) for the 500 MHz instrument and a 5-mm ^{1}H/^{13}C/^{15}N/^{31}P resonance probe equipped with gradients (QXI-XYZ probe, BRUKER Analytik) for the 600 MHz instrument. Spun samples were analyzed at 400 MHz using a 4-mm high-resolution ^{1}H/^{13}C MAS probe. Spin rates of 4.5 - 7.0 kHz were used for all MAS studies.

Results

In a ^{1}H MAS T_2-filtered NMR spectrum obtained from a breast carcinoma, well resolved resonances from a number of metabolites can be identified. The signal centered at 3.2 ppm is dominating. From studies of PCA extracts and of tissue samples using different 2D NMR techniques, resonances from *myo*-inositol, taurine, glycerophospho-ethanolamine, glycero-phosphocholine, phosphocholine, choline and phosphoethanolamine have been found to contribute to the total signal intensity. The concentrations of the various metabolites differ in tumors and non-involved tissue in such a way that multivariate analysis and neural network analysis render classification possibilities (5).

Discussion and Conclusion

Signal broadening due to restricted molecular mobility and local field inhomogeneity in tissue can be reduced by MAS and T_2-filtering. High-resolution magic-angle spinning ^{1}H NMR is rapid and requires only small amounts of intact tissue. With the added capability of obtaining 2D homonuclear and heteronuclear correlation spectra (TOCSY, 2D J-resolved and ^{1}H/^{13}C) combined with gradient experiment the results suggest that the high-resolution MAS spectroscopy will be a potential important tool in tissue characterization with minimum manipulation of the intact tissue.

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Qantitative Study of Blood Plasma Lipids Combining NMR Spectroscopy, Multivariate Calibration and Neural Network Analysis

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*Norwegian University of Science and Technology, Trondheim, Norway. **Queens University, Kingston, Ontario, Canada.
*** University Hospital, Trondheim, Norway

Introduction

Recently a great deal of efforts have been made in using ^{1}H-NMR spectroscopy as an analytical tool for quantifying lipoprotein lipids directly from plasma samples. Curvefitting procedures [1], lineshape analysis [2] and neural network analysis (NNA) [3] have been introduced. The purpose of our study was to compare methods for quantification and classification of plasma lipids based on high field ^{1}H-NMR spectra, multivariate calibration and neural network analysis.

Clinical interest in the study of plasma lipids has increased with many recent studies that emphasise the variations that occur in the plasma lipid metabolism with different diseases [4]. The variations in the concentrations of the different plasma lipoproteins are reflected in the modification of the shape and area of the methylene and methyl resonances in the NMR spectra arising from lipids in the lipoproteins.

Subjects and Methods

A total of 51 blood samples were collected from 11 healthy non-medicated subjects, 23 patients with malignant disease treated or non-treated, 13 with coronary heart disease (CHD), 2 with renal failure, 1 with diabetes mellitus and 1 with hypercholesterolemia.

The plasma was immediately separated and stored at 4 °C until NMR-analysis and ultra centrifugation could commence. Aliquots were frozen at -80 °C for the determination of triglycerides, cholesterol and apolipoprotein A1 and B content.

The fractionation of the lipoproteins into very low density lipoprotein (VLDL), low density lipoprotein (LDL), intermediate lipoprotein (IDL) and high density lipoprotein (HDL) was done by serial ultra centrifugation [5].

Cholesterol and triglyceride in whole plasma and fractions were determined by enzymatic colorimetric methods. The apolipoproteins A1 and B were analysed by immunoturbidimetry.

The ^{1}H-NMR spectra were collected on a BRUKER DRX600 Fourier Transform spectrometer at 43 °C.

The samples were characterised by Principal Component Analysis (PCA) and calibration models for quantification were constructed by use of Partial Least Squares Regression (PLS). Several NNA approaches were also used for lipid quantification.

Results

PCA: Samples with a high score on PC1 are characterised by a high methylene/methyl signal intensity ratio. Samples with a low score on PC1 have a lower content of triglyceride than samples with a high score. Samples with a high score on PC2 have a higher signal intensity on the high field side of the methyl and methylene signals. This might be due to increased concentration of LDL- and HDL- cholesterol.

PLS1: Full cross validation was used for testing of the models predictive ability. Correlation coefficients for predicted versus observed lipid values are given in Table 1.

NNA: Table 2 summarises the data for the training and test data sets. Correlation coefficients for all variables within the training set are uniformly excellent. For the test data the correlation coefficients are acceptable. Additional testing using Group Method of Data Handling (GMDH, polynomial nets analysis) were chosen for two variables (Table 2). The results confirm that this method is an alternative to backpropagation when the latter is inadequate.

Variable	CORRELATION Calibration	Validation
VLDL-cholesterol	0,84519	0,80978
IDL-cholesterol	0,67581	0,50529
LDL-cholesterol	---------	----------
HDL-cholesterol	0,82028	0,61068
VLDL-triglyceride	0,94116	0,87969
IDL- triglyceride	0,63596	0,42769
LDL- triglyceride	0,92115	0,72509
HDL- triglyceride	0,34141	0,13721
HDL-ApoA1	0,90811	0,74921
LDL-ApoB	0,87277	0,76858
Plasma-ApoA1	0,67709	0,41217
Plasma-ApoB	0,89550	0,75480

Table 1: Correlation Coefficients for Data Predicted by PLS

Variable	CORRELATION Training Data	Test Data	Correlation GMDH
VLDL-cholesterol	0,99232	0,97113	
IDL-cholesterol	0,98577	0,93241	
LDL-cholesterol	0,97994	0,58730	0,8502
HDL-cholesterol	0,98842	0,75919	
VLDL-triglyceride	0,98795	0,96139	
IDL- triglyceride	0,93624	0,80756	
LDL- triglyceride	0,98809	0,86708	
HDL- triglyceride	0,98614	0,16212	0,8746
HDL-ApoA1	0,99433	0,79423	
LDL-ApoB	0,97342	0,62689	
Plasma-ApoA1	0,98783	0,59322	
Plasma-ApoB	0,98603	0,84390	

Table 2: Training and Test Data Summary for Backpropagation NNA of Lipoproteins and for GMDH Analysis of selected variables

Discussion

The neural network method handles extreme values better than PLS. The correlation coefficients observed with this method are also generally better. This can be explained as the neural networks capability of processing noisy, incomplete and inconsistent data. The limitations of neural networks are that they require a large amount of training data and long training times. But once they are established, implementation is extremely fast.

Conclusion

In this study, both multivariate modelling and neural networks form adequate basis for prediction of selected lipoprotein lipids from ^{1}H-NMR spectra of plasma. However, neural network seems to be a better choice. NNA offers a complete lipoprotein lipid status from a spectrum of human plasma.

A direct method for determination of triglycerides and LDL- and HDL-cholesterol without fractionation, as demonstrated here, might have important implications in clinical chemistry.

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^{31}P NMR spektroskopi av viable perfuserte leukemiceller

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Med ^{31}P NMR spektroskopi studeres metabolismen i levende hvite blodceller kontinuerlig over et tidsrom på 3-4 dager. Cellene holdes i live i NMR røret ved et perfusjonssystem som kontinuerlig pumper inn friskt medium i bunnen av røret og fjerner brukt medium fra toppen av røret. For å hindre at cellene føres med strømmen av medium støpes de inn i kuler av strontium-alginat. Strontium-alginatens molekylkjeder danner et nettverk som holder cellene på plass og som samtidig tillater diffusjon av stoffer til og fra cellene.

For å oppnå gode/rimelige signal-til-støy forhold av hvite blodceller er det nødvendig å oppkonsentrere ~10^9 celler i det sensitive området av spolen. Det høye kravet til antall celler begrenser tilgangen på primærceller. Pasienter med kronisk lymfatisk leukemi (KLL) har et høyt antall maligne celler i blodet over lengre tid slik at én blodprøve gir nok celler til å gjøre NMR spektroskopi, og det gjør det også mulig å bruke celler fra samme pasient flere ganger.

Under perfusjonen er det ikke tilstrekkelig å bruke spektrometerets egen temperaturenhet for regulering og måling av temperaturen. Ekstra reguleringsutstyr benyttes for å holde en stabil og jevn temperatur over hele prøven. Temperaturmålinger i perfusjonskammeret gjøres ved to ^{1}H NMR metoder; måling av forskjell i kjemisk skift mellom toppene til metanol, i et kapillærrør sentrert i NMR røret, og måling av kjemisk skift til vanntoppen, med aceton som referanse på 2.225 ppm. Temperaturavhengigheten til vannets kjemiske skift i det aktuelle mediet er kalibrert mot målinger av metanol i et eksperiment uten perfusjon.

Opptakene er gjort med et BRUKER DRX500 spektrometer. De NMR-synlige fosformetabolittene i KLL-cellene er fosfoetanolamin (PEth), fosfocholin (PCho), uorganisk fosfat (P_i), glycero fosfoetanolamin (GPE), glycero fosfocholin (GPC), adenosin trifosfat (ATP) og uridin difosfat glukose (UDPG). Med perfusjonssystemet kan cellenes respons på ulike stimuli studeres direkte ved tilsetning av stoffene i perfusjonsmediet. Cellene kan frigjøres fra alginatkulene for videre analyser etter endt perfusjon ved å løse strontium-alginaten i en Hepes-buffret Na-citrat løsning.

The conformation of pterins bound to tyrosine hydroxylase

Aurora Martínez[a], Knut Teigen[a] and Nils Åge Frøystein[b]
Departments of [a]Biochemistry and Molecular Biology and [b]Chemistry, University of Bergen, Norway

In order to get further insights on the catalytic mechanism of the aromatic amino acid hydroxylases, we are involved in the determination of the conformation of their amino acid substrates and pterin cofactor when bound to the enzymes. In the case of human tyrosine hydroxylase, we have obtained preliminary information on the binding of L-*erythro*-7,8-dihydrobiopterin (BH_2), an inactive analogue binding competitively to the natural pterin cofactor (6*R*)-L-*erythro*-5,6,7,8-tetrahydrobiopterin (BH_4), in the presence of L-Phe by ^{1}H-NMR spectroscopy. As apoenzyme, TH1 is activated by the binding of one Fe(II) per subunit and inhibited by Co(II) and Zn(II) which bind at the Fe(II) site. The paramagnetic effect of enzyme-bound Co(II) on the longitudinal relaxation rates ($1/T_1$) of proton resonances of the ligands was measured, and the distances (± 1.3 Å) from the metal were estimated to be in the range of 5.3 to 6.4 Å for the observable protons of BH_2 and from 7.0 to 7.9 Å for protons of L-Phe. The interproton distances in the enzyme-bound pterin were determined by transferred NOESY spectra of the Zn(II)-reconstituted TH1. Due to the lack of constraints from the pyrimidine ring in BH_2, two families of conformers were computed, with an average distance from the C4a in the pterin ring to the iron of 3.7 Å (family A) and 8.8 Å (family B). The conformers for 6-methyl-tetrahydropterin bound to the enzyme at near anaerobic conditions in the absence of L-Phe were also grouped into two families, with C4a-metal distances of 3.0-4.0 Å (A) and 7.0-7.5 Å (B). Based on the estimated conformation of the enzyme bound 5,6,7,8-tetrahydro-3-methylpterin, the family A of conformers was selected for both oxidized and reduced pterins. Our results indicate that the pterin does not coordinate to the iron. Moreover, the conformations of the enzyme-bound pterin and substrate are in agreement with a role of the Fe(II) both in the binding and activation of O_2 and in the C-O bond formation in the aromatic ring of the substrate.

Copper Complexation of Anti-Viral Nucleotide Analogues

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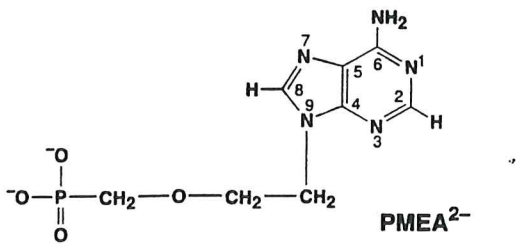
PMEA "9-[2-(phosphonomethoxy)ethyl]adenine" which is considered as an analogue to adenosine 5´- monophosphate [5´-AMP],[1] is active against various kind of viruses, including human immunodeficiency viruses [HIV-1 and HIV-2][2,3], but the mechanism of PMEA as antiviral is still unclear. The function of the most relevant enzyme like DNA and RNA ploymerases and ATP synthases are metal ion dependent. Beside that, they use nucleotides as substrates only as metal ion[4]. Therefore, we are interested to study the metal ion-binding properties of PMEA and it's base-deficient analogue (phosphonomethoxy) ethane(PME). Several transition metal ions like Zn^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+} can be used for this purpose. Among many transition metal ions, potentiometric pH titration shows that Cu(II) ion form the most stable complexes with 1-,3-,7-, deaza analogues of PMEA. Thus, we concentrated our efforts on the complexes formed with Cu(II) because the results from this ion should allow some generalisation. Moreover, Cu(II) itself is a highly biologically active metal ion. Also potentiometric pH titration has shown that the stability of Cu(II) with PMEA and other analogue derivatives is higher than the stability with phosphate group without the nitrogen base. In addition to that, the ^1H NMR data shows that Cu(II) is more stable with the adenine base in the case of AMP than the stability of Cu(II) with the free purine base. These two observations led us to conclude that there is a cooperative stability effect between the purine base and the phosphate group.

Also copper(II) shows special stability of Cu(PMEA) compared to Cu(PME). Thus, one can conclude that this increase in stability may be caused by two isomeric complexes involving, macrochelation between phosphate group and, adenine ring.So the macrochelation where Cu(II) coordinates to the phosphate group and N7 or N3 is the reason behind the extra stability of PMEA compared to PME.



PMEA^{2-}

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Enzymatic generation of block-copolymeric structures: Mathematical analysis of enzyme mechanisms based on triad frequencies evaluated by NMR.

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A mathematical model is derived for describing a multiple-attack pathway for enzymatic generation of block structure in binary linear copolymers having initially a randomised sequential structure. The model is based on sequential information in terms of copolymeric monads, diads and triads estimated by NMR spectroscopy. In case of preferred enzymatic attacks next to reacted units in the polymer chains, the block distribution of unreacted units remains constant and explicit relationships are provided. The probabilities of triad frequencies as a function of monads, *i.e.* progress curve of enzyme copolymeric sequential structure, allow to characterise the enzymatic mode of attack independently of enzyme kinetics. The produced fractions of heterogeneous triads centred by reacted units are shown to be affected, to a large extent, by the degree of multiple attack (d, number of units reacted per enzymatic attack) entering the for! mulas as a variable parameter. The single-chain, d=∞, and multiple-chain mechanisms, d=1, representing the two extremes of the treated mechanism, are very clearly discriminated.

PROTEIN NMR

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There does not exist many specific drugs for woman in labor, and the funding for this science is poor. This plant protein has the potential to be used in drug design due to its uterotonic activity. It has already been used for decades by native Africans in accelerating contractions and childbirth, but very little is known about this peptide. Protein chemistry is a growing science, mostly due to the success of DNA expressions, and it is possible to do structure elucidation's of most proteins in solution. NMR is the only tool for studying proteins in solutions at the atomic level, and both hardware and software have improved vastly the last years. There is no need to incorporate NMR active nucleus like C13 and N15 nowadays by use of the late models of NMR spectrometers if the protein has a molecular weight less than 50.000. The need of a expression system is then no point in this connection, and e.g. in this study, the protein was purified from a plant. The 1H-N15 data proved to be very informativ, because it resolved well all the amid protons. The amid protons are those who are most sensitive to pH. From these spectra it was easy to follow each amid proton during titration, which was impossible in 1D due to overlap. The 1H-C13 data were helpful in the assignment work, especially with amino acids that was difficult to trace their spin systems in TOCSY.

The interaction between transition metal ions and DNA oligomers studied by ^{1}H NMR spectroscopy.

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Metal ions play an important role in the natural processes involving nucleic acids and their constituents. The metal ions may interact noncovalently as counterions to balance the negative charge of the backbone and/or establish strong covalent bonds with specific ligands sites on the nucleobases. It has been demonstrated that competition between quadruplex self-association and triplex assembly is altered in the presence of Mn^{2+}, Co^{2+} or Ni^{2+} ions [1].

In the present work we have investigated the interaction between Mn^{2+}, Ni^{2+}, Co^{2+} and Cu^{2+} ions and double helical duplexes by means of one and two dimensional ^{1}H NMR spectroscopy.

Three different sequences have been studied:

5′-A_1-T_2-G_3-G_4-G_5-T_6-A_7-C_8-C_9-C_{10}-A_{11}-T_{12} (I)
5′-C_1-G_2-C_3-G_4-A_5-A_6-T_7-T_8-C_9-G_{10}-C_{11}-G_{12} (II)
5′-G_1-A_2-C_3-G_4-G_5-C_6-C_7-G_8-T_9-C_{10} (III)

We found that the metal ions followed the selectivity pattern proposed by Frøystein and Sletten [2]. Results will be discussed and compared with recent ab initio calculations done on comparable systems [3].

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Strukturoppklaring av antocyaner og andre flavonoider i vannlilje

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Antocyaner, som tilhører stoffgruppen flavonoider, er de viktigste og mest utbredte rød/blå fargestoffene i planteriket. Flavonoider ser ut til å være sterke antioksidanter og inneha høy biologisk aktivitet. Denne aktiviteten ser ut til i stor grad å avhenge av individuelle strukturelle trekk, som bindingspunkter eller type acylering.

Antocyaner og andre flavonoider blir isolerte fra planter, som er levende organismer med kompleks kjemisk sammensetning. De rød/blå fargestoffene er relativt ustabile overfor både varme, lys og ekstreme pH-verdier. Det er derfor vanskelig å isolere større mengder av rene antocyaner. Forbindelsene består av en aromatkjerne, som er glykosylert med ett eller flere sukkere, som igjen kan være acylert med en rekke alifatiske og aromatiske organiske syrer.

Dette arbeidet presenterer strukturoppklaring av antocyaner og andre flavonoider, isolert fra vannliljer (*Nymphaéa* spp.) ved hjelp av 2-dimensjonale NMR-teknikker. Følgende teknikker blir vektlagt: TOCSY, ROESY, COSY, HMBC, HSC og HSQC. Fargestoffene som ble isolert omfatter antocyanene delfinidin-3-(2``-galloyl-6``-acetylgalaktosid), cyanidin-3-(2``-galloyl-6``-acetylgalaktosid) og delfinidin-3-(6``-acetylgalaktosid), og flavonolen myricetin-3-(6``-rhamnosylglalaktosid). Disse forbindelsene er tidligere ikke funnet i naturen.

"Interactions between chitosans and lysozyme studied by ^{1}H NMR spectroscopy".

Are Kristiansen, Kjell M. Vårum and Hans Grasdalen, Dept. of Biotechnology,
Norwegian University of Science and Technology, 7034 TRONDHEIM.

Chitosans are a family of linear polysaccharides which contain 2-acetamido-2-deoxy-β-D-glucose (GlcNAc; A-unit) and 2-amino-2-deoxy-β-D-glucose (GlcN; D-unit) residues in various proportions, connected through (1→4) glycosidic linkages. Chitosans are water-soluble polycations which have potential applications in pharmacy and biomedicine due to e.g. their degradability by human lysozyme. Polysaccharide degradation, including lysozyme degradation of chitosans, have been studied at the Department of Biotechnology at the Norwegian University of Science and Technology, using both experimental and theoretical approaches. The rate of lysozyme degradation of chitosans increases dramatically with increasing fraction of A-units (F_A), and the kinetic data suggest extensive non-productive (inhibitory) binding of certain chitosan sequences. Here, we report on ^{1}H NMR spectroscopic studies of the binding interactions between highly de-*N*-acetylated chitosans ($0<F_A<0.05$) and lysozyme. The picture that emerged was that of a strong, pH-dependent, strikingly stable and non-productive binding of a specific chitosan sequence to lysozyme, containing an A-unit primarily surrounded by D-units. The results contribute to the understanding of lysozyme degradation kinetics of chitosans, and suggest that certain purified chitosan sequences may be used as substrate analogues of lysozyme that bind with high affinity but without subsequent degradation.

What do extremely high magnetic fields gain in MNR.

Hans Förster, Bruker, Karlsruhe, Tyskland

NMR at high magnetic fields

1. Sales of high field (750, 800 MHz) instrumensts
2. What can we expect from high magnetic fields
3. Practical examples, mostly from solids NMR

Invited Lecture, Norwegian NMR Symposium 1998, Vinstra, January 07, 1998

Characterization of Porous Materials by Solid-State NMR Spectroscopy

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Abstract:

Solid-state NMR spectroscopy is nowadays a well established technique for characterization of zeolites and other porous materials with respect to structure elucidation, pore architecture, catalytic behaviour and mobility properties like diffusion. The objective of this presentation is to highlight recent solid-state NMR results of zeolitic materials, based on new techniques, methods and pulse sequences.

The application of double oriented rotation (DOR) and two-dimensional NMR spectroscopy to assist structure elucidation of microporous materials will be presented besides quadrupole nutation NMR spectroscopy. Recent highlights related to the framework characterization of zeolites and other microporous materials will be shown, concentrating on 29-silicon (ZSM-5, mordenite), 27-aluminium and 31-phosphorous NMR spectroscopy (VPI-5 and $AlPO_4$-8).

A new family of silicate/aluminosilicate mesoporous molecular sieves designated as M41S were introduced a few years ago, and solid-state NMR investigations have been done to characterize those materials. The 29-Si MAS NMR spectra of the hexagonal MCM-41 (one-dimensional framework structure) and the cubic MCM-48 (three-dimensional framework structure) are presented and discussed as well as the spectra of ETS-10, a representative of another new family of porous materials, the so-called octahedral molecular sieves.

One of the main advantages of application of zeolitic or other porous materials is the shape-selectivity of this type of material, which arises due to the differential diffusion of molecules with different sizes and shapes in the porous materials. Therefore, it is very instructive to monitor the pore architecture directly, with a molecule that "observes" the porous structure. 129-Xe was shown to be a very suitable and sensitive nucleous for this purpose, and the chemical shift of adsorbed xenon depends on the pore architecture and can by monitored by NMR spectroscopy. Examples of 129-Xe NMR on zeolites will be demonstrated. On the other hand, water and organic molecules were used to investigate the pore architecture of mesoporous materials by following the 1-H NMR signal intensity of water confined in the pores of those materials when the freezing point depression was measured vs. temperature. The results depend on the pore dimensions of the studied material.

Finally, in-situ NMR and acidity measurements of zeolitic materials will be presented.

Korttidsdiffusjon i heterogene system

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Diffusjonsmålingar i heterogene system ved hjelp av kjernemagnetisk resonans er påverka av interne magnetiske feltgradientar som oppstår på grunn av susceptibilitetsendringar gjennom prøvematrialet. Dersom ein ikkje kompenserer for denne påverknaden, vil den målte diffusjon bli skifta mot ein lågare verdi enn den reelle.
For å kompensere for eksistensen av interne feltgradientar, kan ein introdusere meir enn 3 RF-pulsar i NMR-eksperimentet og bipolare magnetiske feltgradient pulsar. Eit bieffekt av dette er uønska NMR ekko-signal som igjen kan gjere diffusjonsmålingane usikre.

Ein relativt ny teknikk for å bli kvitt desse uønska ekkoa er den såkalla "unequal bipolar gradient method" (1) . Ved å gje dei bipolare gradient pulspara ulik styrke, så vil ein svært effektivt kvitte seg med dei uøsnka signala samtidig som ein fortsatt kompenserer for interne magnetiske feltgradientar.

Då dei interne magnetiske feltgradientane berre har samme polaritet over små områder, så vil ikkje denne kompenserande teknikken vere effektiv når ein lar diffusjonstida vere så lang at ein lar spinna diffundere over større områder. Det er difor fokusert på studia av diffusjon ved korte observasjonstider (2). Desse målingane verifiserer den nye teknikken samt viser at ein ikkje utan vidare kan anta ein gaussisk diffusjonsprogator når spinna vert påverka av dei magnetiske feltgradient pulsane.

1) Sørland et.al. : Journal of Mag. Res., 124, p. 172-176 (1997)
2) Sørland: Journal of Mag. Res., 126, p. 146-148 (1997)

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Fast-fase kjernemagnetisk resonans studier av lipidmembraner

SAMMENDRAG
Fosfatidylserin (PS) ekstrahert fra grisehjerne og syntetisk 1,2 dipalmitoylfosfatidylcholin (DPPC) og dimyristoylfosfatidylcholin (DMPC) ble brukt til å lage DPPC/DMPC og DPPC/DPPS liposomer med diameter på 1000 nm. Effektene av å tilsette klorpromazinHCl (CPZ) (et psykotropisk medikament i fenothiazin gruppen) til disse unilamilære membranene ble studert med fast-fase kjernemagnetisk resonans spektroskopi (MAS-^{13}C-NMR).
MAS-^{13}C-NMR spektra av PC(60%)/PS(40) og PC(54%)/PS(36)/CPZ(10%) tørkede liposomer viser at klorpromazin inkluderes i membranstrukturen og at en del av klorpromazin er i nær kontakt med membranoverflaten (metyl grupper i lipidene som er bundet til nitrogen). Klorpromazin er også posisjonert slik at det er i nær kontakt med lipidene's hydrokarbonkjeder. MAS-^{13}C-NMR spektra av DPPC(60%)/DMPC(40) og DPPC(54%)/DMPC(36)/CPZ(10%) viser derimot at CPZ ikke inkluderes i denne lipidmembranen.

Dette arbeidet er del av et EU BIOMED 2 forsknings-program:
EC BMH4-97-2609.

Static and Dynamic Behaviour of the Tetraspiro Nonacyclic Compound: [5,5][11,11][17,17][23,23]-tetrakis[pentane-1,5-diyl] 1,3,7,9,13,15,19,21-octaazaquinquecyclo [19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]octacosane.

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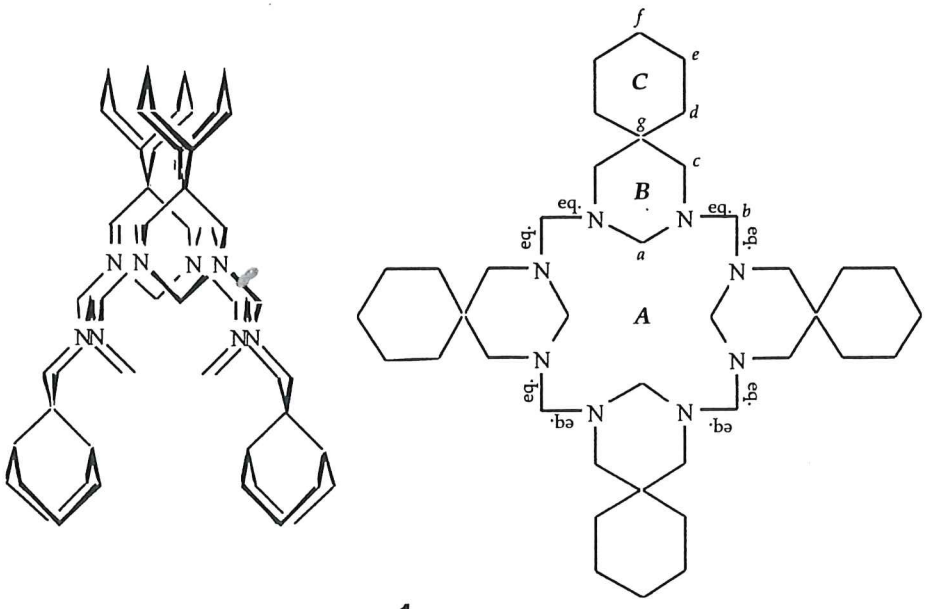
We have recently prepared compounds **1** in a very high yield by condensation of 1,1-bis-(aminomethyl)-cyclohexane with formaldehyde. Compound **1** was desgined so that the core of the molecule, the centeral 16-membered ring (*A*) and the four 6-membered rings directly attached to it (*B*) can only adopt one conformation (D_{2d}), while the external 4 cyclohexane ring (*C*) are free to each choose between two possible chair conformations. A very special NMR-spectroscopic situation can be predicted from the symmetry properties of the expected diamond-lattice conformation of **1**.



1

The central part (*A* + 4*B*) has two "vertical" planes of symmetry passing through atoms *b* and *d*, and two "horizontal" two-fold axes passing through atoms *a*. At room temperature the terminal rings *C* will flip rapidly between the two possible chair forms to produce by

averaging the NMR-spectrum of the apparent "planar" rings, and the molecular D_{2d}-symmetry is not destroyed. When the flipping becomes slow at low temperature, the ^{13}C signal from carbons *c* will split into two, having very different shifts due to the upfield gauche interaction when carbons *f* are on the same side of the symmetry plane, and not when on the other side. The ^{13}C signals of atoms *f* themselves will however remain unchanged since they will get the same interaction with carbons *c* on either side. Interestingly then, what is going on in ring *C* is not observed in its own spectrum but by "remote sensing" in the spectrum of ring *B*.

A modest signal splitting should be expected also for the more distant "corner" atoms *a* on the two-fold axes, each interacting weakly with two *C*-rings. Assuming that the choice of frozen conformation is independent in each *C*-ring, three situations are possible for each *a*-carbon: the two neighbouring *C* -chairs can either both be in the closest position, or one in the closest and one in the distant position (and vice versa), or both can be in their distant position. Statistically, this results in the splitting of the ^{13}C signal of carbon *a* into a triplet with intensity of 1:2:1.

By ^{1}H NMR spectroscopy the flipping process (or geminal CH_2 exchange) could in principle be determined directly on the signals of ring *C* itself, but, is prevented by complex coupling. Full geminal site exchange of the remaining CH_2 groups in ring *A* and *B* would require the passage of much higher barriers, and at 110 °C clear exchange broadening has started but T_c could not be reached.

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Fysikalsk NMR i Oslo

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I Oslo er NMR-spektrometerene fordelt over to seksjoner: organisk NMR og fysikalsk NMR. I fysikalsk NMR har vi et DMX-200 Bruker NMR-spektrometer. Konsollet er fra 1994 og magneten er fra 1981. Vi har nye prøveholdere for ^{13}C-MAS, ^{1}H-CRAMPS og diffusjon og en rekke prøveholdere fra 1981 for faststoff- og væske-NMR.

Arbeidet er konsentrert om heterogene materialer som det fremgår av 4 postere fra gruppen på denne konferansen. Vi forsøker også å avrunne arbeidet med oksidasjon av askorbater som ble startet for tyve år siden i et samarbeid med Jan Hvoslef - en autoritet på askorbater som døde så altfor tidlig[1].

Vi har også studert faseomvandling i 1,4-diklorsykloheksan[2] og et pyridin-glukose kompleks[3] og jeg vil gi noen resultater fra disse undersøkelsene.

Posters:

F. Courivaud, E. W. Hansen, A. Karlson, S. Kolboe and M. Stöcker: **Pulsed Field Gradient NMR Investigations of n-Hexane Diffusion in MCM-41**

H. C. Gran, E. W. Hansen: **Characterization of Pores in Cement Paste**

E. W. Hansen, J. A. Haaland, P. O. Kvernberg* and B. Pedersen: **Water dynamics and pore distribution in microporous zeolite HY**

P. E. Kristiansen, E. W. Hansen, and B. Pedersen***Crystallinity of polyethylene determined by NMR**

P. O. Kvernberg and B. Pedersen:**Oxidation of D-Isoascorbic acid with Hydrogen Peroxide in Aqueous Solution**

1. Jan Hvoslef: Crystallography of the Ascorbates Adv. Chem. **200**(1982)37-79

2. B. Pedersen to be published in J. Mol. Spec.

3. H. Hope and B. Pedersen unpublished

Zinc binding in Bacitracin A - NMR and computational studies

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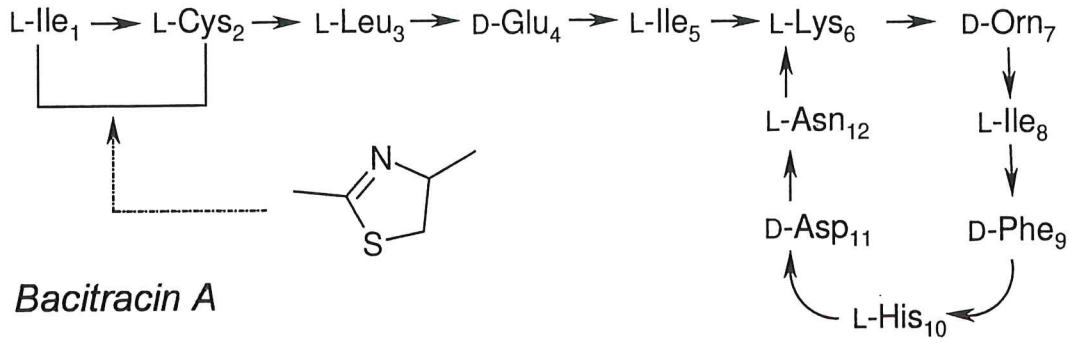
Bacitracin consists of a group of closely related dodecapeptides produced by certain strains of *Bacillus licheniformis* and *Bacillus subtilis*. Bacitracin shows antibiotic activity towards gram-positive bacteria, and it is believed that this activity is caused by inhibition of the peptidoclycan synthesis, as Bacitracin may form a complex with an essential carrier lipid involved in this synthesis. Crude Bacitracin is a mixture of several components, where the major component is Bacitracin A.



Bacitracin A

The C-terminal end of the linear peptide forms a bond to the side chain of Lys-6, as shown. This gives a macrocyclic ring with 7 residues, linked to the 5 residue "tail" region. The first two residues at the N-terminal end form at thiazoline ring through a condensation reaction.

It is known[1] that a metal ion (most likely Zn^{++}) is important for the antibiotic activity of Bacitracin A. It has also been shown[2] that the thiazoline ring is involved in metal binding. However, it is still unclear whether this binding takes place at nitrogen or sulphur, and to what extent the N-terminal end of Bacitracin A is involved in metal binding.

We have previously shown[3] that ^{1}H-NMR of Bacitracin A titrated with a solution of $ZnCl_2$ gives both broadening and shift effects on selected signals in the spectrum, and that semi-empirical simulations of charge distributions seem to indicate a possible explanation for these effects. We have now repeated these computations using *ab initio* theory, including theoretical estimation of chemical shifts. The computations were done using the CADPAC[4] and DALTON[5] programs on several simplified model systems. The simulations do not give a clear answer with respect to how the zinc ion interacts with the thiazoline ring, although they seem to give some support to a binding mode involving the nitrogen. However, the simulations show very clearly that an intuitive interpretation of the NMR data, assuming that we have the largest effects on the atoms close to the binding site, may be wrong. The data also indicate some possible experiments for resolving this question.

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Sammendrag av plakater

Abstracts of posters

PULSED FIELD GRADIENT NMR INVESTIGATIONS OF n-HEXANE DIFFUSION IN MCM-41

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The mesoporous materials MCM-41 are well known as model systems for fundamental adsorption studies. Their regular intracrystalline channels can be shrunk in a controlled way by treating them with methyltrichlorosilane or tetrachlorosilane followed by water vapour exposure. MCM-41 materials (crystals size of 10 μm) was synthesised with a pore diameter of 27 Å, and used as a reference. Silanation with methyltrichlorosilane and tetrachlorosilane were performed giving respectively hydrophobic and hydrophilic channels of less diameter. Pulsed Gradient Field (PFG) NMR was used to investigate the effect of decreasing the pore volume of MCM-41, and changing the surface properties (done by silanation) on the diffusion coefficient of n-hexane. Mitra et al.[1] have reported that in case of restricted diffusion behaviour in mesoporous materials, the measured diffusion coefficient (i.e. the apparent diffusion coefficient D(t)) versus the diffusion time leads to the surface to volume ratio. A model that considers the cylindrical internal geometry of MCM-41 will be used to analyse the PFG NMR results. The relative differences in S/V ratio derived from PFG NMR analysis are in agreement with data obtained from adsorption isotherms of n-hexane. A recently published pulse sequence[2] was employed to cancel the effect of internal gradients in the samples and remove unwanted echoes.

1. P.P. Mitra, P.N. Sen, and L.M. Schwartz, *Phys. Rev.* B **47**, 8565 (1993)

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Characterization of Pores in Cement Paste

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The ^{1}H-NMR peak intensity, I(t), of water confined in the pore system of white hydrated cement pastes has been monitored as a function of temperature. The experiments are carried out on saturated samples by cooling and heating in the temperature range - 80 °C and 0 °C. A hysteresis effect is observed between cooling and heating data at w/c (mix ratio of dry cement and water) = 0.60 which corresponds to a coarse structure dominated by capillary pores. At w/c = 0.40, which contains pores in the micro and meso porous range, hysteresis is not observed. Combining NMR measurements and mercury intrusion porosimetry, a linear relation between freezing point and inverse pore radius is found which is in agreement with the Kelvin's rule.

Water dynamics and pore distribution in microporous zeolite HY

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The ^{1}H-NMR signal intensity and spin-lattice relaxation (T_1) time of water confined in a microporous zeolite HY have been measured vs temperature below 273 K. The sample was cooled to 178 K and then the ^{1}H-NMR spectrum and T_1 were recorded at different temperatures from 178 K to 268 K in steps of 10 K. The experimental parameters were chosen such that only the liquid part of the proton spin system was detected.

The observed intensity increased smoothly from near zero at 178 K suggesting that most of the water was frozen at 178 K. A progressively larger part of the ice melted as the temperature increased. The measured T_1 showed a "two-phase" behavior. At low temperature most of the protons where in one phase and with increasing temperature the amount in each phase got more equal. Possible reasons for this behavior will be discussed.

Relaksasjons- og diffusjonsmålingar for plastiske krystallar inneslutta i mesoporøst materiale

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Når væske er inneslutta i porar vil frysepunktet verta senka med minkande porestorleik[1]. I sykloheksan, til dømes, vert frysepunktet redusert frå 280K i bulk til omlag 213K i 40Å silicaporar. I dette arbeidet er T_1 og T_2 relaksasjonstider og diffusjon koeffisientar for sykloheksan inneslutta i silica porar med diameter 40Å, 60Å, 200Å og 500Å målt som ein funksjon av temperatur mellom 295K og 160K. Resultata er samanlikna med verdiar gjort for bulk sykloheksan.

^{1}H NMR målingane vart utførd på eit Bruker DMX 400 spektrometer ved 400,13 MHz. T_1 og T_2 relaksasjonstider vart målt ved å nytta inversion-recovery og CPMG spinn-ekkosekvensane. For dei kortaste T_2-verdiane vart ein enkel ein-puls-sekvens nytta. Dette avdi ventetida mellom π-pulsane i CPMG-sekvensen måtte haldast ≥300μs for å unngå spinn-låsing om B_{1y} og såleis $T_{1\rho}$-effekt. Diffusjonsmålingane vart gjort ved å nytta stimulert-ekko med pulsa felt-gradientar opp til 10 T/m. 13-sekvens programmet til Sørland et al.[2] har óg vorte nytta for å stadfesta at interne gradientar bidreg lite i dette systemet.

I væskefase over normalt frysepunkt for bulk sykloheksan (280K) vert både relaksasjonshastigheit og diffusjon koeffisient redusert med minkande porestorleik. Under frysepunktet er eit tynt lag nær overflata funne til å ha ein svært høg mobilitet samanlikna med den plastiske fasen i sentrum av poren. Dersom hastigheita for utbyting mellom det væske-liknande overflatelaget og den plastiske fasen er sein, vil eit to-komponent system gje opphav til eit smalt og eit breidt NMR-signal. Grunna transversal magnetisering i periodane (τ_1) etter fyrste og tredje π-puls, vil det ikkje vera mogleg å observera komponentar med T_2 kortare enn omlag τ_1. Signalet vil i denne perioden avta med ein faktor $e^{-2\tau_1/T_2}$ Dette gjer at me berre observerer signal frå den smale komponenten (med lang T_2). Diffusjon koeffisient resultata frå inneslutta sykloheksan syner eit væske-liknande signal for temperaturar godt under senka smeltepunkt og, i motsetnad til resultat frå Kimmich et al[3], er resultata kontinuerlege over heile temperaturintervallet. Relaksasjons-målingar byggjer opp om desse resultata, der T_2-verdiar (≥0,7 ms) for den smale komponenten indikerer ein fase med høg mobilitet. I dei største porane minkar T_2 for den breie komponenten hurtig (frå 0,3ms) for temperaturar under 250K ned til eit platå med T_2 omlag lik 17 μs. For dei minste porane vert denne komponenten observert i eit avgrensa temperaturintervall under omlag 215K.

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Crystallinity of polyethylene determined by NMR

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Polyethylene (PE) consists of amorphous and crystalline regions. The polymer chains have a much higher mobility in the amorphous regions than in the crystalline regions. The ^{1}H-NMR-spectrum therefore consists of at least two peaks - one broad peak from the H-atoms in the crystalline regions and a narrow peak from the H-atoms in the amorphous regions. From the relative areas of the two peaks the crystallinity can be determined. However, because the broad signal decays fast (≈ 15 ms) and the initial part of the fid is lost due to receiver dead time (≈ 4 μs) the spectrum will be distorted. We have therefore analyzed the fid directly and not its fourier transform (the spectrum). As PE can be seen as a collection of proton pairs (CH_2-groups) we have analyzed the contribution from the crystalline regions as the fourier transform of a Pake curve earlier calculated by Lowe et al. (1966). The contribution from the H atoms in the amorphous regions are simulated as an exponential curve (the fourier transform of a lorentzian peak). The results will be compared to the crystallinity determined by calorimetry and from a NMR model developed by Dadayli and Harris (1994).

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High-Resolution ^{1}H NMR Spectroscopy of PCA Extracts from Muscle Tissue of Stored Halibut. NMR as a Quality Control Method?

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Introduction

Various methods are used to preserve freshness of fish during storage. Degree of freshness is often expressed in terms of the value K (1). The equation describing the K value constitutes of ATP and its degradation products (equation 1). The phosphocreatine/inorganic phosphate ratio has also been found to be a sensitive index of early metabolic hypofunction (2).

Equation 1 $$K = \frac{[\text{Inosine}]+[\text{ Hypoxanthine }]}{[\text{ATP}] + [\text{ADP}]+[\text{AMP}]+[\text{IMP}]+[\text{Inosine}]+[\text{ Hypoxanthine }]}$$

So called "strips" for freshness measurements are commercially available which are easy to use. Unfortunately they are highly inaccurate in determination of low K values, and in addition small amounts of fat in the sample introduces large errors.

Our aim with this study was to examine the potential of high-resolution ^{1}H NMR spectroscopy as a method for evaluation of freshness indicators on stored halibut.

Materials and methods

Farmed halibuts (5 kg) were stored for 20 days. Samples were taken at days 0, 1, 2, 6, 14 and 20 from three halibuts and at days 0, 3, 5, 10 and 14 from three others. The samples were taken using a "freeze-clamping" technique. Perchloric acid extracts were prepared (3) and freeze-dried. They were redissolved in 0.6 ml phosphate buffer in D_2O (pH 7.5±0.1) with TSPA-d_4 as reference. The compounds contributing to the K value were also analysed in phosphate buffer. The chemical shifts of the pure compounds in buffer differ from those in the PCA extracts due to differences in dielectricity constant of the solutions, and therefor two of the PCA extracts were spiked with small amounts of the reference model compounds for proper identification.

^{1}H NMR spectroscopy was performed on SPECTROSPIN DRX500 and DRX600 instruments, operating at 500.130 and 600.130 MHz for protons, respectively. The spectra were recorded using 10 seconds water presaturation followed by a 90° excitation pulse. 128 free induction decays of a spectral width of 12 kHz were collected into 64 K data points, giving an acquisition time of 2.7 seconds. The free induction decay was multiplied with a matched exponential filter before zero filling to 128 K and Fourier transformation. The spectra were analysed both by integration and non-linear curve fitting program using least square minimisation method (PeakFit from Jandel Scientific).

Results and discussion

The ^{1}H NMR spectra of extracts from halibut show numerous metabolites. The spectra are dominated of signals from lactate and creatine/phosphocreatine. Assignment of the signals from ATP degradation was done on basis of ^{1}H NMR spectra of pure compounds and spiking of two samples.

The results show a change in K value as a function of storage time. Phosphocreatine is only detected in the baseline samples (day 0).

Conclusion

High-resolution ^{1}H NMR spectroscopy can be used to monitor changes in a number of metabolites simultaneously. K values determined by ^{1}H NMR spectroscopy of PCA extracts seem to be an interesting method for freshness evaluation.

References

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PARAMAGNETISK NMR AV FERREDOXINER

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Ferredoxiner er små (55-75 aminosyrer) jern-svovel proteiner som er involvert i elektronoverføringer, nitrogenfiksering, fotosyntese, og steroid metabolisme. De inneholder komplekse jern-svovel grupper som gir opphav til paramagnetisme. Dette medfører at atomer som ligger nær det paramagnetiske senteret vil oppføre seg annerledes enn andre atomer, og kan detekteres for seg selv via paramagnetisk NMR.

Induksjon av sekundærstruktur i Plantaricin A alph (Pln A-alpha) ved hjelp av løsemidler.

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Plantaricin A (22 amino syrer) ble syntetisert, og renset via HPLC. Ved tilsats av metanol ble det indusert en helix struktur, men løseligheten av peptidet var like lav som i vann. Derimot var peptidet lett løselig i trifluoretanol (TFE). TFE er kjent for å indusere alfa helix til "unfolded peptides", hvilket er også tilfelle med plantaricin A. Metanol og TFE induserte forskjellig sekundær struktur i peptidet.
Peptidet er karakterisert som en induksjonsfaktor (kalles også et feromon).

The ability of zinc(II) to stabilize triplex DNA. A high resolution NMR study.

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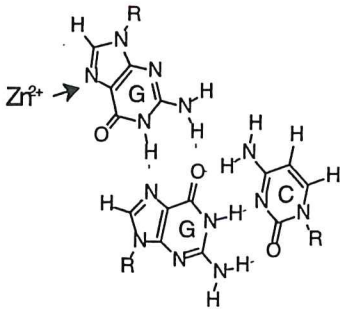
During the last 7 to 8 years the research on multistranded nucleic acids has been focused on triplex DNA. The big goal for the ongoing research is to produce an irreversible interaction between a double- and singlestranded DNA [1]. If this can be done, we will see a revolution in the treatment of genetic based diseases. This treatment is called the «antigene strategy».



This figure shows the triplex which consists of a central dodecamer with to single stranded hexamers attached forming the triplex. Possible binding-sites for Zn^{2+} are indicated by arrows. The figure next to it shows the binding-site of Zn^{2+} in the guanine-guanine-cytosine triplet. The triplex is designed so that we have bindingsites of the guanines «faced» outward.
We will report how the stability of the triplex is increased with increasing concentration of Zn^{2+}. We will also discuss the mechanism behind this stabilization.

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Enzyme Kinetics of the Recombinant Mannuronan-C-5 Epimerase AlgE 4, studied by n.m.r. spectroscopy

Martin Hartmann, Hans Grasdalen, and Gudmund Skjåk-Bræk

The epimerase AlgE 4 belongs to a family of epimerases found in *Azotobacter vinelandii*. These enzymes are active in the biosynthesis of alginate. In the polymer they epimerize (β)-D-mannuronic acid residues into (α)-L-guluronic acid residues. The different enzymes produce alginates that differ widely in proportion and distribution of the residues along the polymer chain. AlgE 4 specifically creates a block pattern of alternating mannuronic- and guluronic acid. It is a recombinant mannuronan-C-5-epimerase from *A. vinelandii*, that was expressed in *E. coli*.

Alginate with a mannuronic acid content of 93%, and pure poly-mannuronic acid, produced by a epimerase-deficient strain of *Pseudomonas aeruginosa*, were epimerized with AlgE 4.
The epimerisation was continuously observed by ^{1}H-n.m.r. spectroscopy. The monomeric composition and sequence were determined, and the enzyme-kinetics and mechanism were investigated for various reaction conditions.

The Interaction Between Antitumor Derived Cis-platin With Mono, Di, and Oligonucleotides Studied By NMR Spectroscopy

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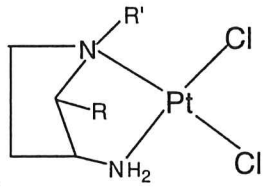
The mode of action of the anticancer drug cisplatin [$PtCl_2(NH_3)_2$], which is called cis-DDP, has been under intensive studies since its discovery 30 years ago. This compound is remarkable and the most widely used anticancer drugs. Its effectiveness against a number of cancers, particularly testicular cancer is quite notable.[1] A key reaction step in the mechanism of action appears to be the binding of the cis-$Pt(NH_3)_2$ unit to cellular DNA at two neighboring guanine bases, and more specifically at their N7 atoms.[2] A new group of platinum (II) compounds of both cis and trans isomers has been developed during the last decade.

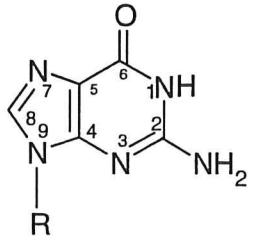
Recently, V. Moreno and her group in Spain have synthesized a new cis-paltinum complex, cis-(3-amino,1,2-diethyl)pyrrolidinedichloroplatinum(II) that has steric ligands and typed as EEpyrr-Pt (scheme 1.a). The interaction of this new active platinum compound with 5´GMP, 5´AMP, 5´ApG, 5´GpA and 5´d(CCTGGTCC) has been studied to establish whether or not this compound can bind to DNA in analogous manner to cis-platin. 1D and 2D NMR spectroscopy was mainly used to investigate the reactions.

From the data obtained, it is concluded that the EEpyrr-Pt complex binds to the N7 position of the G base (scheme 1.b) in an analog manner to cis-platin with the chlorines acting as leaving groups. In addition to that, N7 and N1 in AMP were found to be very week targets for the platinum. In the single strand 5´d($C_1C_2T_3G_4G_5T_6C_7C_8$), the platinum complex binds the adjacent G_4(N7) and G_5(N7) to form a platinated intrastrand structure. According to the NMR measurements for the mentioned interactions monitored at certain time periods, the platinum complex was found to proceed according to the order: 5´GMP >> 5´AMP and 5´ApG > 5´GpA.



R and R' = C_2H_5

(a)



R = Sugar+phosphate

(b)

Scheme 1: Molecular structures of Pt-EEpyrr (a) and guanine (b).

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Solid-State NMR Study of the Interaction of Surfactin(a Lipopeptide) with a Model Lipid Membrane.

Abstract : surfactin, an acidic cyclic lipopeptide produced by strains of *Basillus subtilis* is a powerful bio-surfactant possessing important biological activities. The interaction of surfactin with a mixture of dipalmitoylphosphatidylcholine(DPPC) and cholesterol as model membrane systems was studied by Solid-State CP-MAS ^{13}C NMR spectroscopy. The CP-MAS ^{13}C NMR of DPPC(53mol%) and cholesterol(47mol%) mixtures was studied at different temperatures. The CP-MAS ^{13}C NMR was also studied on the DPPC(50mol%), cholesterol(42mol%), and surfactin(8mol%) mixtures at different temperatures. CP-MAS with high power proton decoupling was used for high temperature studies. The 13C NMR data clearly indicate that the lipopeptide surfactin is present in the acyl chain region of the DPPC/ cholesterol membrane, and the data also indicate that the packing of the lipid membrane, especially in the acyl chain region is affected by interaction with the surfactin.

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Poster presentation:

Solid State Magic Angle Spinning Nuclear Magnetic Resonance Spectroscopy Study of Glycerophospholipid Liposomes.

ABSTRACT

Synthetic 1,2-dipalmitoylphosphatidylcholine (DPPC) was used to make liposomes with a diameter of 1000 nm. A temperature study on these model membranes was carried out by solid state magic angle spinning nuclear magnetic resonance spectroscopy (MAS-^{13}C-NMR).

This work is part of a EU BIOMED 2 research programme: EC BMH4-97-2609.

Copper Complexation of Adenosine monophosphate Nucleotides

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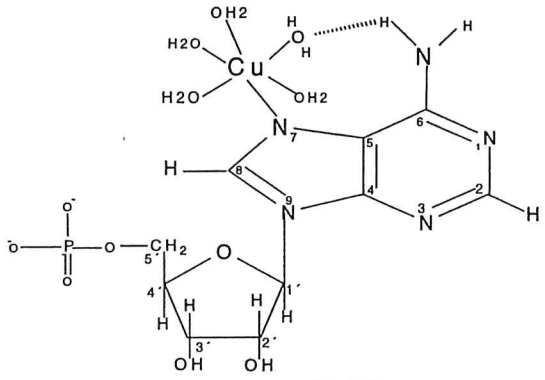
The nucleic acids, DNA and RNA are composed of monomer units called nucleotides. In addition to their importance as components of DNA and RNA, nucleotides play a key role in a wide range of biological significant reactions.[1] In order to get an idea about it's biological function, the interaction between transition metal ions and nucleotides have been extensively studied.[2-4] The purpose of the present work is to give a picture of the possible metal ion binding sites with nucleotides, and help us to understand the interaction between transition metal ions and DNA.

In the previous work we studied the effect of the phosphate group on the interaction between Cu(II) and (5´-, 3´- and 2´-)AMPs. The proton NMR results show that at 310 K H8 of 5´-AMP is more affected than H2. On the other hand, the interaction of Cu(II) ion with 2´-AMP reveal that H2 is the most affected one. This different behaviour is due to copper macrochelation between the phosphate group and N7 of 5´-AMP and N3 of 2´-AMP.[5] In contrast of this result, the proton NMR data at (298, 288 and 280) K show that H8 is more affected compared to H2 for both 2´-AMP and 5´-AMP. This result suggests that monodentate Cu(II) coordination of N7 of 2´-AMP at low temperature is stabilized by hydrogen bond between a copper coordinated water molecule and the hydrogen atom on 6-NH2 group, figure(1).



Fig(1)

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NMR Study of Lipid Fluidity in Frozen Red Muscle of Atlantic salmon (*Salmo salar*): Relation to Autoxidation of Lipids?

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[b]SINTEF, Division of Applied Chemistry, Group of Aquaculture, 7034 Trondheim.

100MHz ^{13}C NMR spectra of lipids in intact red muscle of Atlantic salmon (*Salmo salar*) have been measured in the temperature range from 60°C to –80°C. NMR-visible contents and composition of lipids, as monitored by line intensities, indicated a gradual freezing out of long chained monounsaturated fatty acids like 20:1 and 22:1 below 20°C, whereas all omega-3 (n-3) fatty acids remained almost unaffected down to –40°C. At lower temperatures the total lipid phase became largely immobile yielding completely unresolved ^{13}C NMR spectra at ≈ -70°C.

Segmental motions, monitored by relaxation time measurements, indicated a substantial motional restriction in the glycerolmoiety with C_α in the region of motion, showing an increase in T_1 from 0.17s to 0.4s by cooling from 20°C to –20°C. The ω3 carbons in n-3 fatty acids and the CH_3 carbons stayed in the region of fast motion with T_1 values decreasing from 2.5s to 0.4s and from 2.5s to 0.7s, respectively, by cooling from 20°C to –40°C.

The remarkable fluid lipid phase in the frozen tissue is compatible with a distribution of lipids consisting of droplets both inside the cells and in the connective tissue.

Lipid fluidity may play a role for lipid autoxidation as far as diffusive communication is concerned.

Syntese og NMR studier av dioxathianer og dioxarsenaner.

DAGFINN W. AKSNES, PAUL JUVVIK OG KNUT SKOGMO
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I dette arbeidet er 2-oxo-4,5,6-trimetyl-1,3,2-dioxathian og 2-klor-4,5,6-trimetyl-1,3,2-dioxarsenan fremstilt og de forskjellige steriske isomerene er separert ved hjelp av destillasjon av 2-oxo-4,5,6-trimetyl-1,3,2-dioxathian slik at strukturen til isomerene kunne bestemmes.
Strukturen til isomerene er bestemt ved hjelp av kjernemagnetisk resonans-spektroskopi (NMR). Tilordningen er gjort ved hjelp av vanlige ^{1}H og ^{13}C 1D-spektere samt COSY og HXCOOR 2D-spektere. Det er også tatt spektere ved lav temperatur for å løse strukturen til den ene fraksjonen.

2-Oxo-4,5,6-trimetyl-1,3,2-dioxathian viste seg å bestå av 4 isomerer, det er tidligere hevdet at det er 6 ulike isomerer og enkelte overgangsformer utenom disse 6 igjen[1]. I dette arbeidet ble det samlet opp 3 fraksjoner ved destillasjon og den ene av disse fraksjonene viste seg å inneholde to isomerer. Spekterne i dette arbeidet viser ingen tegn til isomerer med ekvatorial substituent i 2 posisjon, slik som beskrevet tidligere. De fire isomerene er:

1 : 2a-Oxo-4e,5e,6e-trimetyl-1,3,2-dioxathian

2 : 2a-Oxo-4e,5a,6e-trimetyl-1,3,2-dioxathian

3 : 2a-Oxo-4a,5e,6e-trimetyl-1,3,2- dioxathian og 2a-oxo-4a,5a,6e-trimetyl-1,3,2- dioxathian.

2-Klor-4,5,6-trimetyl-1,3,2-dioxarsenan ble dannet fra 3-metyl-2,4-pentandiol gjenvunnet fra 2-oxo-4,5,6-trimetyl-1,3,2-dioxathian fraksjonene, fraksjon 1 var imidlertid så liten at det ikke var mulig å rense stoffet og det ble ikke tatt noen spektere for denne. Fraksjon 2 viste seg å inneholde en isomer, fraksjon 3 hadde 1 tydelig isomer ved romtemperatur utenom en del brede topper; ved nedkjøling viste det seg at denne fraksjonen inneholdt 3 isomerer, en stabil og en som forelå i en likevekt mellom 2 former. Forholdet mellom de to isomerene i likevekt er ut i fra karbonspekteret ca 1 : 2. De 4 identifiserte isomerene er :

2 : 2a-Klor-4e,5a,6e-trimetyl-1,3,2-dioxarsenan

3 : 2a-Klor-4a,5a,6e-trimetyl-1,3,2-dioxarsenan, 2a-Klor-4a,5e,6e-trimetyl-1,3,2-dioxarsenan og 2e-Klor-4e,5e,6a-trimetyl-1,3,2-dioxarsenan

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Oxidation of D-Isoascorbic acid with Hydrogen Peroxide in Aqueous Solution

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The oxidation of D-isoascorbic acid with aqueous H_2O_2 gives CO_2, D-erythronic acid and its γ-lactone as the final products in solution. The reaction has been followed quantitatively using ^{1}H and ^{13}C NMR spectroscopy and several intermediates have been detected. The concentration of the intermediates depends on the relative concentration of acid and H_2O_2. Some of the species observed have been formed by breaking bonds and some by carbonyl groups reacting with H_2O_2 and H_2O to give diols or C(OOH)OH groups. These groups react further forming intra- and intermolecular -O- or -O-O- bridges giving rings and dimers. Experimentally determined rate constants will be given.

We published in 1994 a similar study of L-ascorbic acid where the final products are L-threonic acid and its γ-lactone[1]. Compared to that study we observe much less of the peroxy form of the dihydro oxidation product. Furthermore the fraction [erythronic acid]/[erythronic acid lactone] decreases during reaction while the fraction [threonic acid]/[threonic acid lactone] increases.

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Restabilization of The Activated (CH) Group in Phosphorus Ylids Through Coordination to Pt(II) and Ag(I) Transition Metals

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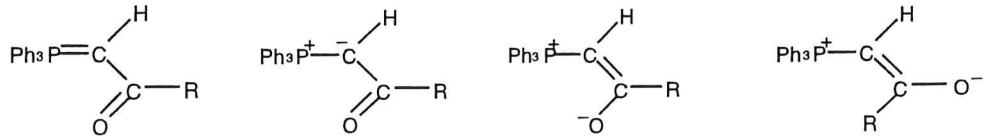
The activated (CH) group in the phosphorous ylids $Ph_3P{=}C(H)COR$, where (R = OCH_3, OC_2H_5) stabilized by their tendency to have the following resonance forms:



These stabilized ylids have shown useful applications in the organometallic chemistry, due to their ambidentate character as ligands in which they can coordinate to metals through O or C atoms. While a large number of compounds containing C-coordinated ylids are known,[1] very few examples of O-bonded have been reported.[2]

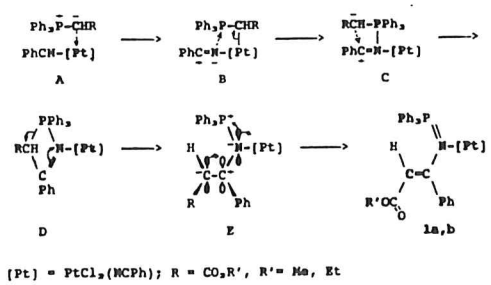
In our present work, we have investigated cis-$[PtCl_2(NCPh)_2]$ and $AgNO_3$ reactions with $Ph_3PC(H)COR$, [R = OCH_3, OC_2H_5]. The reaction of the parent phosphonium salt $(Ph_3P^+CH_2COR)Br^-$ as 1:1 ratio with diluted NaOH or K_2CO_3 in water, gives the ylid as a pure white powder, which was washed carefully with deionized water and recrystalized from hot ethylacetate/n-pentane. Investigation of the ylids by ^1H, ^{13}C and ^{31}P NMR in dried pure $CDCl_3$, IR spectroscopy and conductometry confirms their structures. Unexpected exchange broadening process for the activated CH group, with extremely upfield broad singlet compared with downfield doublet in the initial bromide salt was recorded. This exchange broadening was not notable after the following complexation reactions were performed [3]

$$AgNO_3 + 2Ph_3PCHCOR \longrightarrow [Ag(CHPPh_3COR)_2]\,NO_3$$

The reaction of the same ylids with cis-$[PtCl_2(NCPh)_2]$ complex [4] gave the unexpected product of trans-platinum iminophosphorane complex:

$$cis\text{-}[PtCl_2(NCPh)_2] + Ph_3PCHCOR \rightarrow trans\text{-}[PtCl_2\{E\text{-}N(=PPh_3)C(Ph)=CHCOR\}(NCPh)] \cdot Me_2CO$$

The coordination to the silver in these ligands went through the activated CH group, where to platinum the reaction was found to follow the suggested pathway:



[Pt] = $PtCl_2(NCPh)$; R = CO_2R', R'= Me, Et

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Tidligere Nasjonale NMR-møter:

14 - 17 mars 1983	Oppdal — Jostein Krane, AVH, UNIT
10 - 12 mai 1989	Geiranger — Jostein Krane, AVH, UNIT
	(Norsk - svensk NMR diskusjonsgruppe)
07 - 08 oktober 1993	Ustaoset — Tore Skjetne, MR-senteret, Sintef Unimed
16 - 17 oktober 1995	Trondheim — Tore Skjetne, MR-senteret, Sintef Unimed

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