Thesises of Ph.D. dissertation of

STRUCTURE ELUCIDATION OF NATURAL COMPOUNDS, INVESTIGATION OF SPECIAL HOST-GUEST INTERACTIONS

Author:

Tamás Gáti

Supervisor:

Prof. Dr. Gábor Tóth

Budapest University of Technology and Economics

Institute for General and Analytical Chemistry

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Introduction

My Ph.D. dissertation was made in the NMR Research Group of the Institute for General and Analytical of the Budapest University of Technology and Economics, under the supervision of Prof. Dr. habil. Gábor Tóth. During my work I have investigated novel natural compounds as wall as synthetised substances possessing biological activity. I have also studied special host - guest interactions (Rh_2[(*R*)-MTPA]$_4$ – alkyl-aryl-selenoothers).

The structure elucidation of the natural compounds was in corporation with three international research group. The structural and stereochemical investigation were carried out in our Institute. M. Hani A. Elgamal and his co-workers isolated the cardenolides from *Calotropis procera*. The plant *Achillea holosericea* and *Achillea ligustica* which grow in North-Africa and Eurasia was collected by M. Couladis. The isolation and purification was made by A. Ahmed. The investigation of ester-disaccharides isolated from green coffee beans was in corporation with the University of Würzburg. In some cases mass-spectra were also necessary, which were provided by Tom J. Mabry (The University of Texas at Austin) and Gyula Horváth (Drug Research Institute).

In corporation with the Organic Chemistry Institute of the University of Hannover we have developed a new method for determining enantiomeric ratio of organoseleno compounds. Applying chiral Rh_2[(*R*)-MTPA]$_4$-t (MTPA = **M**ethoxy-**T**rifluoromethyl-**P**henyl-**A**cetic-acid) as an auxiliary („*dirhodium-method*") soft-basis (e.g. nitrils, olefines, selenides) can be investigated. I have studied the stochiometry, thermodynamics and kinetics of the equilibrium describing this adduct formation.

My Ph.D. studies were financed by Gedeon Richter Co. Ltd. In the Spectroscopic Division of this company I had the opportunity to learn how to operate on a VARIAN-INOVA NMR instrument. Beside the everyday routine activities (measuring, spectra interpretation) I joined the antithrombotic research project of the Pharmacutical Company.

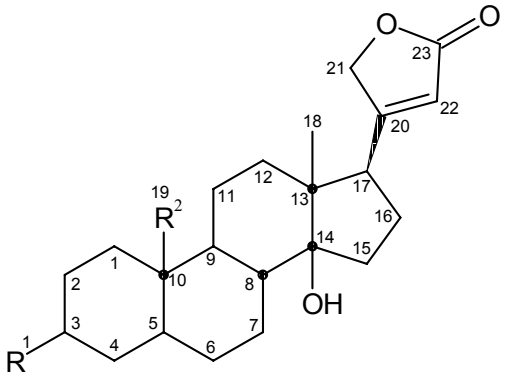
Summary of my results

Structure elucidation of natural compounds

The backbone of my Ph.D. work was the natural compounds investigation. Studying four plants in corporation with three different international research group I have determined many structures such as steroids, sesquiterpenes, lignanes, monoterpenes, sugar derivatives, which were not yet publicated. The purification and isolation were made in all cases by our colleges. My task was the determination of the unknown substances with the help of ^{1}H, ^{13}C, DEPT and 2D NMR spectra (^{1}H,^{1}H COSY, HSQC, HMBC, NOESY).

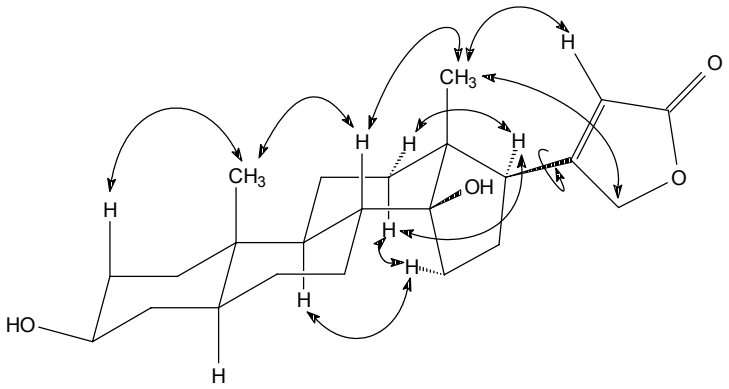
1. Complete ^{1}H and ^{13}C signal assignments of 5α-cardenolides isolated from Calotropis *procera*

I determined the two and three dimensional structure and I gave the complete ^{1}H and ^{13}C signal assignments for four cardenolides isolated from *Calotropis procera*.



	R^1	R^2	name
1	=O	CH_3	uzarigenone
2	OH	CH_3	uzarigenine
3	OGlc	CH_3	deglucouzarin
4	O- (6-desoxyallosyl)	CH_2OH	frugoside

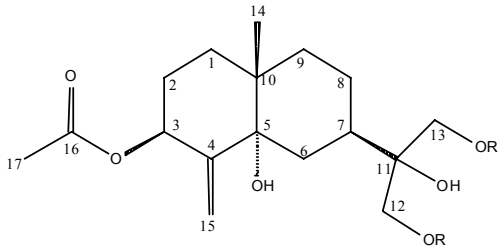
Based on the vicinal coupling constants and on the steric proximities obtained from the ROESY spectrum concluded that A/B, B/C ring junction are *trans*, C/S ring junction is *cis*. In compounds **1-4** the γ-lactone ring, and in compounds **2-4** R^1 group are in β position. In compounds **3**, **4** a sugar moiety attached to C-3. Steric proximities obtained from ROESY are depicted by double ended arrows.



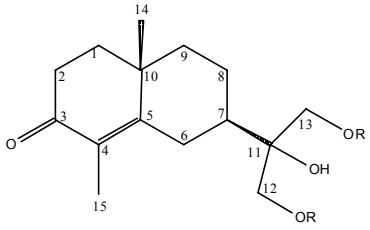
3D structure of **2**

2. Structure elicidation of lignanes sesquiterpenes isolated from *Achillea holosericea*

I determined two novel, highly oxygenated sesquiterpenes (**5** and **7**) and their acetylated derivatives (**6** and **8**) from *Achillea holosericea*. The molecules have a so-called eudesmane skeleton. The ringjunction is *trans*, the group at C-7 is in β position.

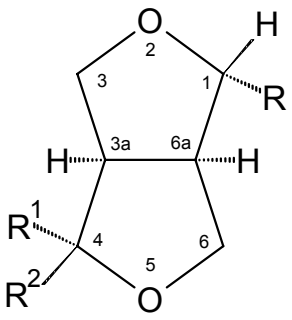


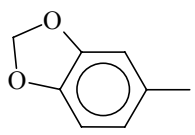
5: R=H
6: R=Ac



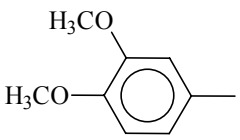
7: R=H
8: R=Ac

From the same plant I identified ten so-called lignanes, 1,4-diaryl-tetrahidrofurofurane-derivatives.

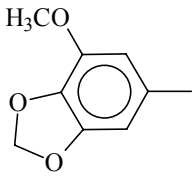




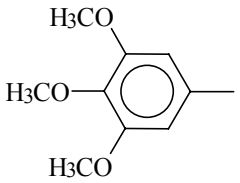
Ar^1



Ar^2

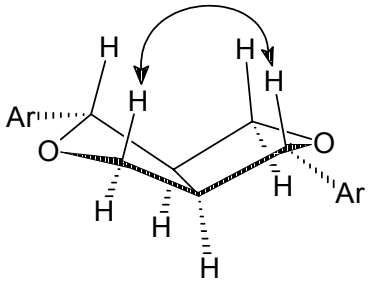


Ar^3

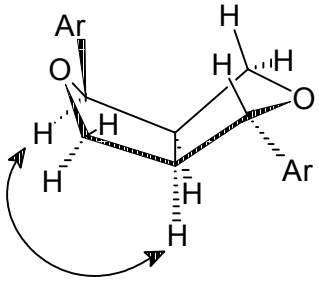


Ar^4

Depending on the position of the aromatic substituent *cis* and *trans* lignanes can be differentiated. According to the vicinal coupling constants and to the steric proximities obtained from NOESY in both type of compounds the big aromatic groups try to possess an equatorial position, and actually they are in pseudo equatorial position. As a result the tetrahidro-furofurane skeleton of the *cis* compounds has a so-called „endo-endo" structure, i.e. the oxygen is below of the plane determined by the carbon atoms. In case of the *trans* molecules the dominant conformer is „exo-endo".



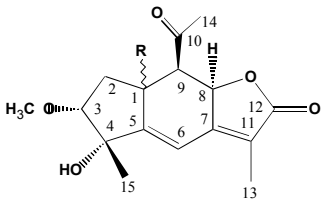
„endo-endo"



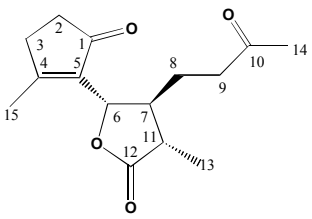
„exo-endo"

3. Monoterpenes, sesquiterpenes and *seco*-sesquiterpenes isolated from *Achillea ligustica*

I identified six novel compounds (**9-14**) and six known molecules (**15-20**) from *Achillea ligustica*.



9 R =β OH
10 R =α OH



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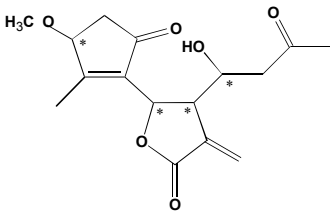

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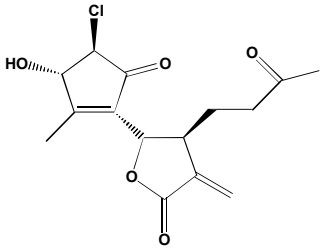

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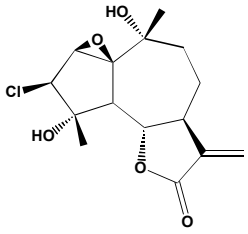
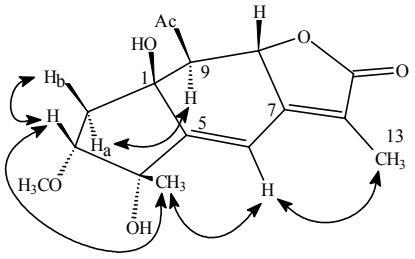


14

Ligustolide A (**9**) and B (**10**) are sesquiterpenes and have a rare 5/6/5 membered skeleton. According to the characteristic steric proximities and to the chemical shift differences the compounds differ only in the configuration of C-1.



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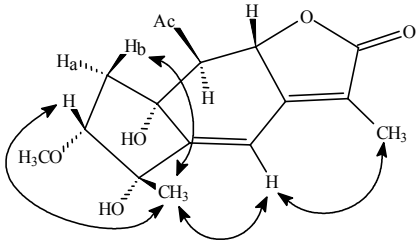
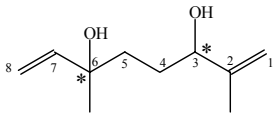


10

Molecules **11-13** are 1,10-*seco*-guainolides. The substances were obtained by the oxidative cleavage of the corresponding guainolide. The relative configuration was based on the vicinal coupling constants. Compound **14** is a sesquiterpen-lactone having guainolide skeleton. The chlorine atom at position C-3 was proved by high resolution mass data. The coupling constant $^{1}J_{C\text{-}2,H\text{-}2}$=195 Hz is characteristic for epoxides.



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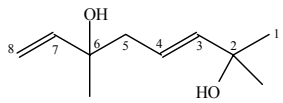

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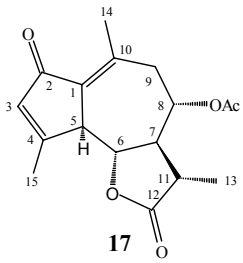

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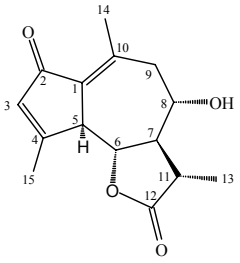

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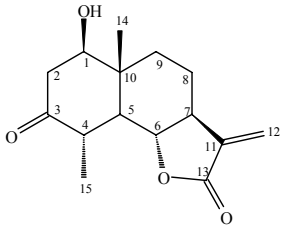

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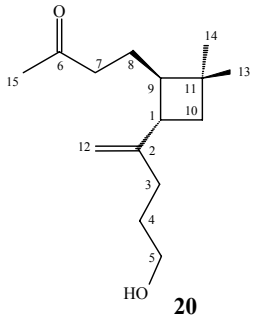
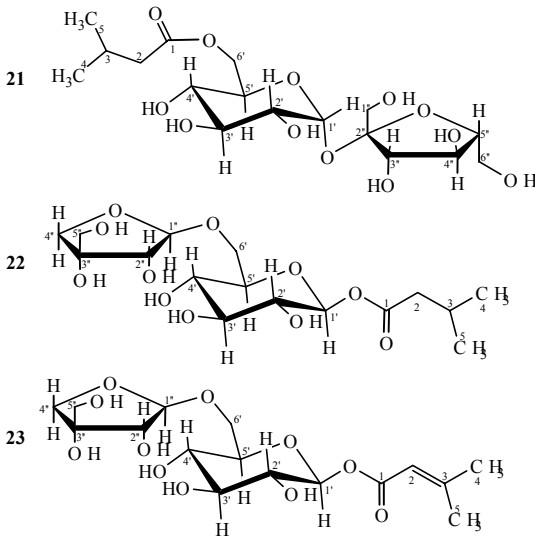


20

Molecules **15**, **16** are monoterpenes. Compound **17** - in the literature called matricarin - is a sesquiterpene with a guainolide skeleton. The relative configuration of the carbon atoms was determined on the basis of vicinal coupling constants obtained from 1D selective TOCSY experiment. Compound **18** is the desacetyl derivative of **17**. Subtance **19** is a sesquiterpene-lactone with an eudesmane skeleton, molecule **20** is a four substituted cyclobutane derivative.

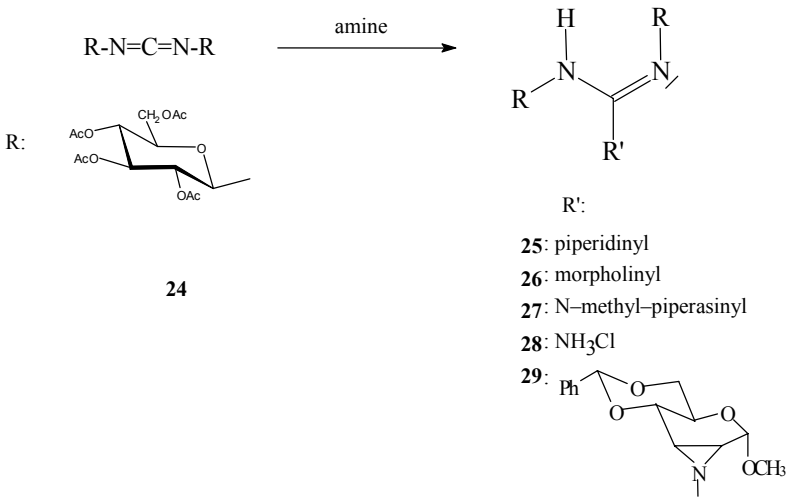
4. Ester disaccharides isolated from *Coffea arabica*

I identified three ester-disaccharides from *Coffea Arabica*. The ^{1}H assignment and the determination of the vicinal coupling constants were achieved by the help of 1D selective TOCSY experiments. The sugar moieties were elucidated on the basis of coupling constants and carbon chemical shift analogy.



5. Investigation of *bis*- and *tris*- saccharido guanidines.

The reaction of symmetrically substituted carbodiimides [N,N'-bis(tetra-O-acetyl-β-D-glucopyranosil)-carbodiimide] (**24**) with secondary amines (piperidine, morpholin, N-methyl-piperazine) yield the corresponding three substituted guanidine derivatives (**25**-**29**).



24

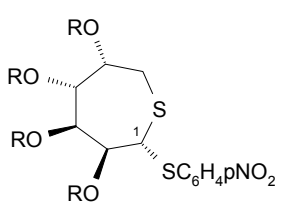
R':

25: piperidinyl
26: morpholinyl
27: N–methyl–piperasinyl
28: NH_3Cl
29:

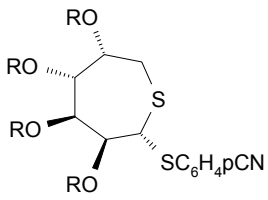
For the characterization of the guanidine moiety the propensity for tautemerism and isomerism should be considered. I achieved a complete ^{1}H and ^{13}C signal assignment utilizing HMQC, HSQC and TOCSY experiments. The steric proximities obtained from ROESY indicated an E configuration of the imine group, furthermore a threefold equilibrium was established. The EXSY spectrum proved the existence of tautomerism. I determined the exchange rates from the intensity of exchange ROESY cross-peaks obtained by volume integration of a series of ROESY spectra measured with different mixing times.

6. NMR investigation of 1,6-dithio-D-manno, L-ido and D-gluco-septanosides possessing antithrombotic activity.

I determined the structures and configurations of 1,6-dithio-D-manno- (**30**-**33**), L-ido- (**34**-**36**) and D-gluco-septanosides (**37**-**40**) based on standard 2D NMR experiments.

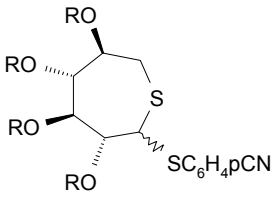


30 R = Ac
31 R = H

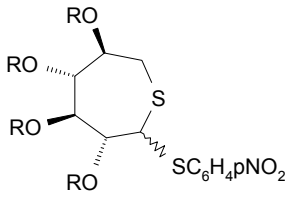


32 R = Ac
33 R = H

D-manno

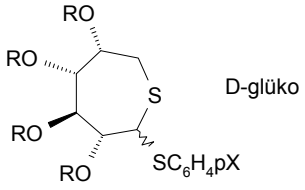


34 R = H



35 R = Ac
36 R = H

L-ido

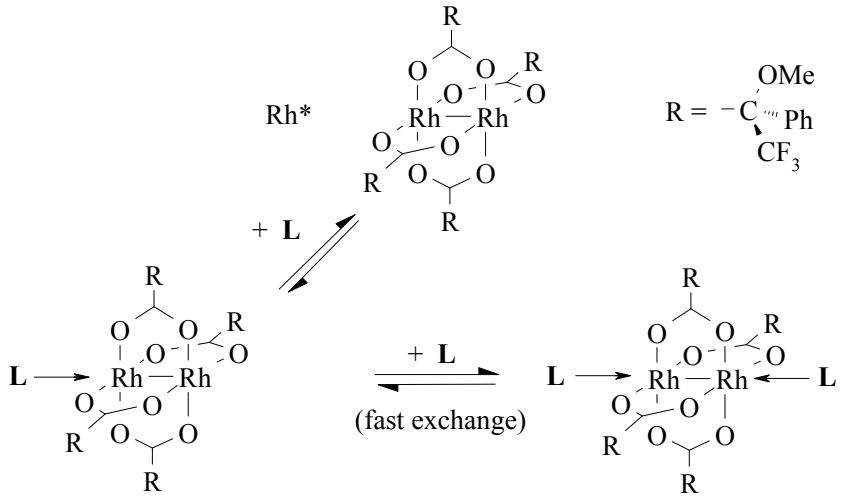


D-glüko

37 R = Ac, X = CN
38 R = H
39 R = Ac, X = NO_2
40 R = H

7. Study of host-guest interactions. Application of $Rh_2[(R)\text{-MTPA}]_4$ as a new NMR shift-reagent

In the past two decades the organoselenium compounds play an increasing role in modern stereo- and regioselective organic chemistry. In parallel, ^{77}Se NMR spectroscopy became more and more popular because this nucleus is easy to monitor and its chemical shifts and coupling constants are often very sensitive to structural properties in the molecule. Our goal was to develop a method for determining enatiomeric ratio of selenoethers. We applied the recently introduced "dirhodium method", using $Rh_2[(R)\text{-MTPA}]_4$ (**Rh***) which is working excellently particular with soft-base functionalities such as diorganyl selenides. The auxiliary (**Rh***) forms an adduct with the ligand (**L**), where the process can be characterized with the following equilibrium.



NMR investigation of selenium adducts: thermodynamics and stoichiometry

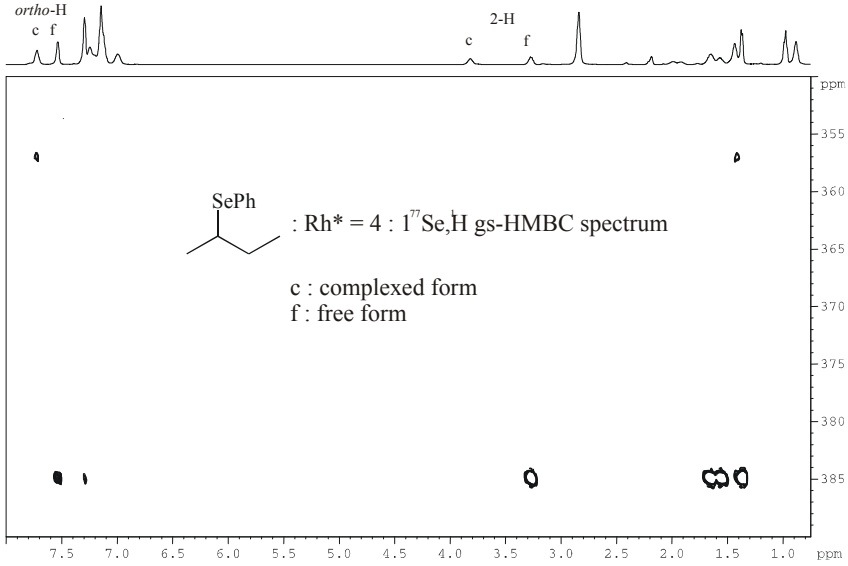
I studied the adduct formation on a selenoether model compound (Ph-CH=CH-CH_2-Se-Ph). According to the ^1H, ^{13}C and ^{77}Se spectra recorded at different temperatures (213 K - 330 K) with different compound-ratio (1:1, 2:1, 5:1) I found:

In case of **Rh*** excess there is no free selenides in the equilibrium, i.e. the equilibrium is shifted toward the adduct formation.

If less than two selenide molecules per **Rh*** molecule exist, a dynamic mixture of free **Rh***, 2:1 and 1:1 adducts will be formed.

With an excess of selenide molecules, only 2:1 adducts, plus remaining free selenide molecules can be observed. Here an intermolecular selenide exchange exists.

Due to the rather low sensitivity of selenium, the detection of the ^{77}Se NMR signal could take a long time. (2-3 hours). Utilizing indirect detection the problem can be remedied. I applied an indirect detected ^{77}Se,^{1}H-gs-HMBC experiment to our spectrometer, and as a result the measuring time could reduced dramatically (5 min).



8. Investigation of alkyl aryl-selenides.

I achieved a complete ^{1}H ^{13}C and ^{77}Se signal assignment for compounds **41**-**52**, as well as I investigated their conformational behavior. Based on the spectra obtained by temperature dependent NMR spectroscopy and by varying the molar ratio of host-guest molecules I set a model for the kinetics of the adduct formation.

(a)

$$Se \rightarrow [Rh\text{-}Rh] \leftarrow Se^{a} + Se^{b} \rightleftharpoons Se \rightarrow [Rh\text{-}Rh] \leftarrow Se^{b} + Se^{a}$$

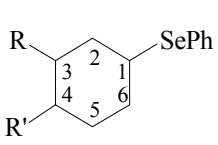
(b)

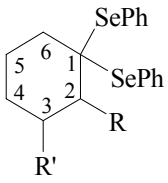
$$[Rh\text{-}Rh]^{A} + [Rh\text{-}Rh]^{B} \leftarrow Se \qquad Se^{a} \rightarrow [Rh\text{-}Rh]^{C} \leftarrow Se^{b} + [Rh\text{-}Rh]^{D} \leftarrow Se^{c}$$

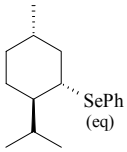
$$\downarrow\uparrow \qquad\qquad\qquad\qquad \downarrow\uparrow$$

$$Se \rightarrow [Rh\text{-}Rh]^{A} + [Rh\text{-}Rh]^{B} \qquad Se^{a} \rightarrow [Rh\text{-}Rh]^{C} + Se^{b} \rightarrow [Rh\text{-}Rh]^{D} \leftarrow Se^{c}$$

„Replacement” (a) and *„Switch”* (b) equilibrium







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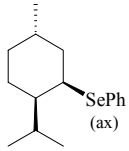


50

41 : R/R' = H

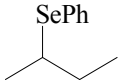
42 : R = H ; R' = *cis*-*t*-Bu

43 : R = H ; R' = *trans*-*t*-Bu

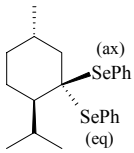
44/45: R = *cis/trans*-CH_3; R' = H

46 : R = CH_3 ; R' = H

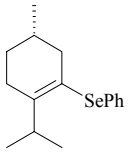
47 : R = H ; R' = CH_3



48

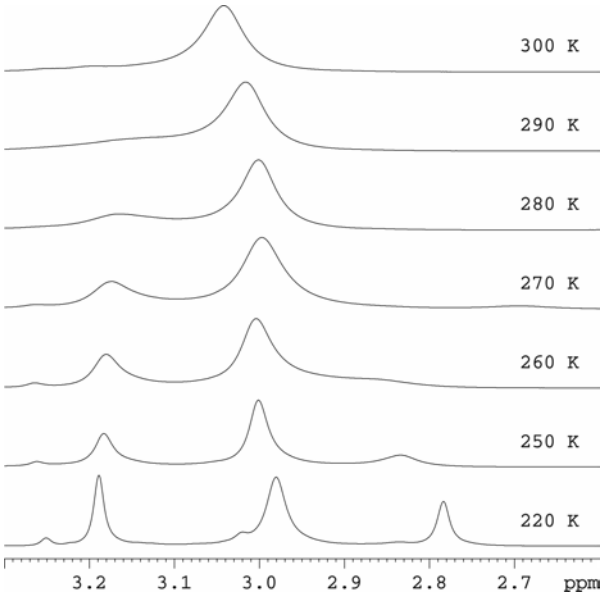


51



52

At a molar ratio 1:1, by varying the temperature two coalescences appear in the ^{1}H spectrum ("*Switch*-equilibrium"). At low temperature (220 K) free **Rh*** (3.19 ppm), 1:1 (2.97 ppm) and 2:1 adducts (2.78 ppm) are in the solution, respectively.



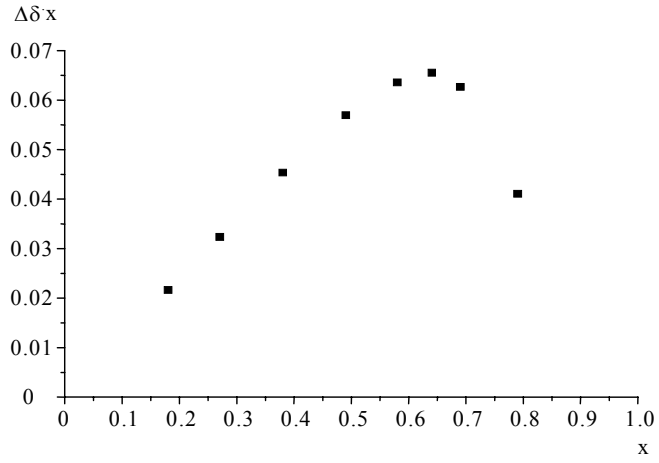
Methoxy signal of **Rh*** in the presence of **2** at different temperature; **42** : **Rh*** = 1:1

At 4:1 molar ration an intermolecular exchange process appears („*Replacement*"-equilibrium) where the free ligands are in exchange with the attached ligands.

An approximate estimation of the energy barrier, for each equilibrium at its coalescence temperature leads to a surprisingly high value of 54-55 kJ/mol for these exchange processes.

9. Dirhodium Tetraacylate Complexes and Monovalent Ligands. Adduct Formation in Solution as Monitored by NMR Spectroscopy

Applying Job's method the stoichiometry of an adduct (or complex) can be determined. Representing the product of the chemical shift difference (Δδ) and molar ratio in the function of molar ratio the peak of the curve gives the composition of the adduct.



Job plot of a Rh* : selenide adduct

I established that in case of selenides a 2:1 adduct, in case of nitriles a congloremate **L→Rh*←L→Rh*←L** (3:2 adduct), in case of olefins - depending on the quality of the double bond (endocycle or exocycle) - 1:1 or 3:2 adduct will be formed, respectively.

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   József Kovács, András Simon**, Tamás Gáti**, Helmut Duddeck, Gábor Tóth
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    Tamás Gáti, Gábor Tóth, Helmut Duddeck and Shahid Malik
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    Shahid Malik, Helmut Duddeck**, Tamás Gáti**, Gábor Tóth
    2nd German-Polish Workshop, Chemistry of Natural Products, Synthesis, Chirality, Diversity; Hannover, 13-15. 06. 2002.
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    Tamás Gáti, Gábor Tóth, Ahmed A. Ahmed, Taha A. Hussein, Aptehal T. Ali, Olga A. Tzakou, Maria A. Couladis and Tom J. Mabry
    4th Central European NMR Symposium, Budapest, 2-3. 09. 2002.