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NEW DIAZOETHERS WITH POTENTIAL APPLICATIONS IN MEDICAL LASER DOMAIN

Teodor Octavian Nicolescu[1], Florica Nicolescu[1], Aurel Ardelean[2], Dorina Ardelean[2], Corina Dalia Toderescu[2], Aurelia Hulbar[2]
[1]University of Medicine and Pharmacy "Carol Davila" Bucharest, Romania
[2]"Vasile Goldis" Western University, Arad, Romania

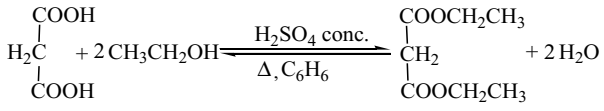
Introduction
Laser therapy includes today various types of lasers. They are classified by composition and experimental parameters: the ruby laser, semiconductor laser, Nd: YAG laser, Ar^+ laser, excimer laser, dye laser, CO_2 laser, Ne-He laser, free electron laser.
The present study is focused on the laser dyes synthesis because of the advantages it brings this experimental parameter. Dye laser is an acordable laser, the wavelength depends on the energetic level of the transition.
Diversity of active medium is determined by a relatively large number of organic structures: xanthine dyes, coumarin, pyrazoles, salts etc. (Tărăbăşanu C., *et al*, 2000).
An important application is tumor laser therapy; it uses a dye absorber, this allows the selective destruction of tumor cells. Another benefit of dye laser is microsurgery, which provide localized damage of a substrate and selective absorption of monochromatic radiation (Dumitraş D.C., 1999).
Key words: diazoethers, barbiturates sodium salt, diazo compounds dyes, laser medical applications

MATERIALS AND METHODS

1.Intermediates synthesis

a. Malonic ester synthesis

To obtain diethyl malonate we used a known method: malonic acid esterification with absolute alcohol, in strongly acidic medium (H_2SO_4 conc.):

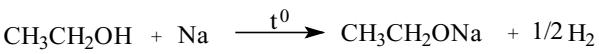


The reaction occurs in the presence of 2,2-dimethoxypropane which reacts easily with water (secondary product of the esterification reaction), resulting acetone and methanol, easily removed by distillation, due to low boiling temperatures.

The reaction yield was about 35%.

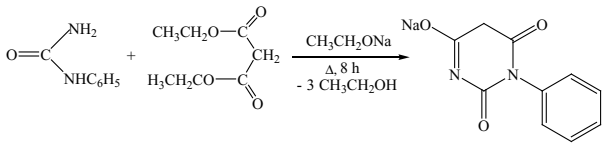
b. Synthesis of sodium salts of 1-N-methyl, 1-N-ethyl and 1-N-phenylbarbituric acids

1-N-alkyl or 1-N-aryl sodium barbiturate was obtained by condensation of N-alkyl- and N-aryl urea with diethyl malonate. The reaction takes place in the presence of sodium ethoxid obtained from absolute ethanol and metallic sodium.



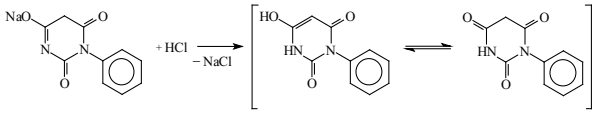
Practically, to obtain sodium etoxid we worked with a large excess of absolute ethylic alcohol.

The second step, condensation of diethyl malonate with urea derivatives, was performed at a temperature about 60 ^{0}C, for 6-8 hours, on electric bath.



The condensation reaction yields was between 61.5 % and 80.5 %.

The melting temperatures could not be determined for salts. Therefore, in order to be able to verify to obtain compounds based on these chemical reactions, we turned the sodium barbiturate salts in the corresponding acids by treatment with cold hydrochloric acid 10 %.



1-N-methyl-, 1-N-ethyl- and 1-N-phenylbarbituric acids are insoluble in water, are crystallized, colorless. Melting points are between 119 ^{0}C and 262 ^{0}C. Melting points values are according with literature.

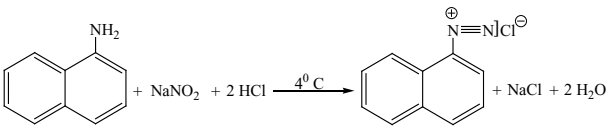
For purification, the acids were recrystallised from absolute ethanol.

c. Synthesis of the α- -naphthylamine diazonium salt

For 1-naphthylamine diazoting we chose a classic method that we adapted to a semimicro working conditions. Therefore 0.36 g (0.0025 moles) 1-naphthylamine was treated with an equivalent quantity (1:1 molar) hydrochloric acid 30 % (0.0025 moles = 0.3

ml) and 10 ml distilled water. The reaction mixture was continuous stirred and was boiled for 3 minutes. After cooling to about 30 ^{0}C, a part of amine hydrochloride precipitated. Then we added the remaining hydrochloric acid 30 % (0.0025 moles = 0.3 ml) and 10 ml distilled water. After stirring 5 minutes at room temperature we added another 0.3 ml HCl 30 % and we cooled the mixture on ice at about 4 ^{0}C. The result was a fine precipitate of 1-naphthylamine hydrochloride, slightly soluble in water.

Aside from this we prepared sodium nitrite solution in water, from 0.0025 moles $NaNO_2$ (0.18 g) and 10 ml distilled water, also cooled on ice bath. Sodium nitrite solution was added little by little, continuously stirring on ice bath, over the solution of 1-naphthylamine hydrochloride. The result was a slightly brown solution with suspended particles. Insoluble impurities were cold filtered at low pressure. The clear solution was further used in the condensation reaction of barbiturate derivatives.



The diazonium salt is extremely reactive and must be kept on ice bath until condensation reaction with barbiturate derivative happens.

Because there were solid impurities from the amine in solution, we made a rapid low pressure filtration, maintaining the solution temperature at 4-5 ^{0}C.

2. Diazoethers synthesis

Condensation of α-naphthylamine diazonium salt with N-substituted sodium barbiturates

Barbiturate derivative solution was prepared by dissolving the sodium salt in distilled water and by adding an stoichiometric quantity (compared with hydrochloric acid) sodium carbonate. We checked the pH solution with an pH indicator paper.

Therefore, for N- phenyl sodium barbiturate we have worked with these quantities: 0.5 g N-phenyl sodium barbiturate (0.0025 moles), 10 ml distilled water and 0.005 moles anhydrous sodium carbonate.

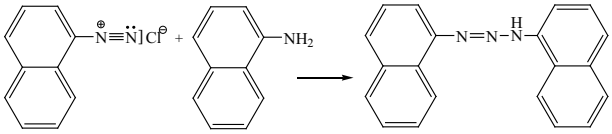
For the condensation with the others two sodium N-alkyl barbiturates, we proceeded similarly.

According to literature data, the coupling reaction of diazonium salts with phenols is carried out at an alkaline pH (8-9), and coupling reaction with aromatic amines is carried out in acid or slightly acid pH.

In the light of previous research in diazonium salts class and the results of recent research in diazoethers class, according to which a coupling reaction in an acid medium is usually accompanied by numerous side effects (ionic and radical decomposition), we tried to get the optimum pH value at which the main reaction (obtaining diazoethers) has an optimum yield.

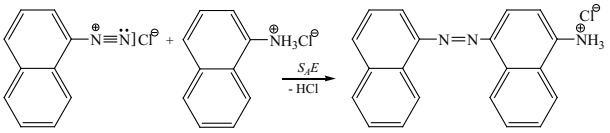
Generally speaking, in the absence of an reactive molecules with OH groups or a coupling component which would allow electrophyle aromatic substitution (S_AE) in the reaction medium, the diazonium salt can evolve either to an triazene, if the pH value turns to neutral, or it can quite easily decompose to radicals and / or cations that can be reduced even where they formed.

For example, arendiazonium salt derived from 1-naphthylamine is coupled with amine which has not reacted at a weakly acid to neutral pH value, resulting the corresponding triazene:



Triazene

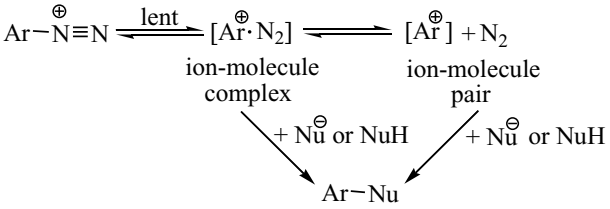
In strongly acidic medium the coupling reaction of arendiazonium salt with unreacted amine hydrochloride occurs normal. Basically is obtained a mixture of isomers, all α reactive positions being electrophylic attacked:



Azoderivate

These reactions are accompanied by side processes with loss of nitrogen, no matter of the reaction medium, as we demonstrated in our study by high-performance liquid chromatography (HPLC).

Studying the literature we could conclude that even in weak acid or strong acid medium the arendiazonium salt spontaneously decomposes in a secondary reaction, even in the dark, with intermediate formation of an "ion-molecule complex". This step is critical for speed. Then this complex evolving to an "ion–molecule pair" generating a highly reactive species, an aryl cation.



Aryl cations easily react with nucleophiles (Nu-) in the reaction medium, without showing a high selectivity to them. Basically we are talking about a reduction reaction in which the aryl cation is oxidant, or a condensation reaction, where the aryl cation as a Lewis acid and the nucleophile as a Lewis base.

In alkaline medium (pH 8-9) the arendiazonium salt has more evolution possibilities dependent on the pH value and composition of the reaction mass, that means

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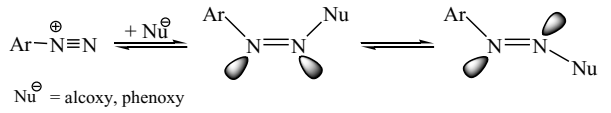
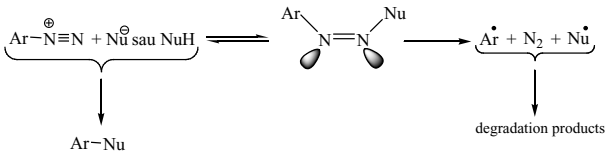
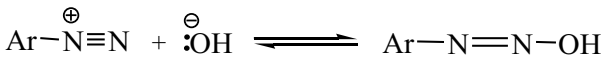
we find in solution alkoxyde nucleophiles groups or phenoxyde reactive groups. It can react with an alkoxyde anion nucleophile or phenoxyde, resulting an diazoether with reduced stability:



On the other hand, the arendiazonium salt may suffer a desintegration (nucleophilic elimination reaction) followed by a nucleophile reduction (may be an alkoxide or phenoxyde).



In strongly basic medium (pH> 11) it occurs the HO-reaction and obtain an unstable arendiazonium hydroxide.



The purpose of our work was to obtain stabilized diazoethers by electronic effects of steric prevent, using the diazonium salt of 1-naphthylamine and sodium barbiturates derivates salt where the tautomeric phenomenon was partially blocked by substitution at the nitrogen atom with an alkyl or aryl group. We also wanted to find out the optimal working conditions to avoid competing reactions with O-coupling reaction.

As previously specified, 1-naphthylamine arendiazonium salt was obtained with good yields using a classic method, but adapted to our conditions of semimicro synthesis scale. Coupling component solution (barbiturate derivative) was obtained by dissolving it in distilled water to which we added a stoichiometric quantity of sodium carbonate (reported to the quantity of hydrochloric acid from).

Barbiturate derivative alkaline solution was added gradually under stirring, at room temperature, over the arendiazonium salt solution. After we added the entire quantity of coupling component, it was left to rest at room temperature for 30 minutes, stirring occasionally. For sodium N-phenylbarbiturate it was obtained an red-orange precipitate. It was low pressure filtered and washed repeatedly with distilled water on filter paper. A combustion flame test was performed to check for a salt or sodium carbonate adsorbed in the organic molecule. Because of the positive sample, we concluded that the reaction product is must be a salt, which later we proved by FT-IR spectrometry. To remove all of the quantity of sodium carbonate, the precipitate was boiled in distilled water for 15 minutes. Then we made a low pressure filtration and dried in a vacuum desiccator.

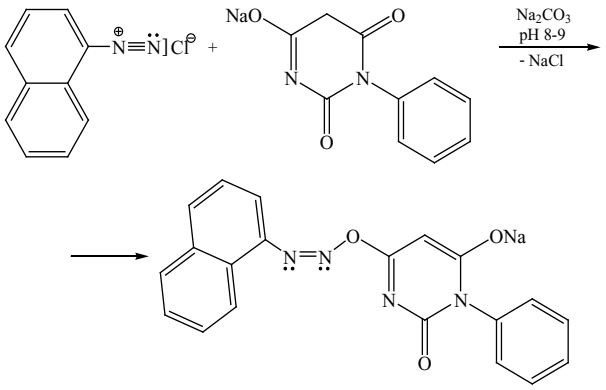
We tried to find out the melting temperature, although preliminary tests indicate the presence of a salt. Of course, the precipitate did not melts in the normal range of temperatures of Boetius device that we uses.

In order to remove impurities soluble in organic solvents, we put the precipitated in anhydrous toluene and we heated to boiling. The toluene soluble fraction was removed, the precipitate is separated by cold low pressure filtering and then dried in the vacuum desiccator. On this precipitated we made UV-Vis spectrometry and FT-IR spectrometry measurements.

It should be noted that is although a sodium salt, the diazoether that we obtained, it still has a very low water solubility and a low polar organic solvents solubility too.

Being a salt with very low solubility in organic solvents (chloroform, dimethylsulfoxyde) it was not possible to determine magnetic resonance (^{1}H-NMR).

Similarly we did at the arendiazonium salt condensation with N-methyl-and N-ethyl sodium barbiturate, with the remark that in these cases the reaction yields were lower and the O-coupling reaction was not competitive with the electrophyle aromatic substitution reaction (S_AE), the normal coupling on aromatic nucleus, which was not present.



In the toluene soluble fraction detected by HPLC, there was secondary reaction products between which it coluld also be the azoderivate - normal coupling product of electrophyle aromatic substitution.

RESULTS AND DISCUSSIONS

a. Checking the purity and spectral characterization of intermediate products and final products reaction

Chromatography

Checking the intermediates and reaction products purity was done by high performance liquid chromatography (HPLC) using the a UV-Vis spectrometer detector. Sodium N-phenylbarbiturate was analyzed by liquid chromatography (HPLC) with UV-Vis detector system methanol: water (80:20). We used an ODS column, 25 cm x 4 mm, 5 mm diameter, detection was made at 258-259 nm. Because the other

two barbiturates had no chromophore, they couldn't be verified by this method, the tautomeric phenomenon, have hipsocrom moved the characteristic bands. In aqueous or hydroalcoholic solutions, the tautomeric phenomenon occurs, resulting in C=O bond an order decreases (λ <210 nm), uncharacteristic bands.

The O-coupling products were also analyzed by HPLC using a INERTSIL column, 25 cm x 4 mm, 5 mm diameter, detection was made between 258-300 nm, using methanol: water 50:50 and 40:60 and a flow rate of 1 ml / min.

Electronic excitation spectra (UV-Vis)

Electronic excitation spectra were taken in methanol solutions with a UV-Vis Analytik-Jena spectrometer, and with Cecil HPLC chromatograph detector. Electronic excitation spectrum of N-phenyl sodium barbiturate was obtained in aqueous solution. At absorbance 0.9483 was obtained the 259.20 nm value, corresponding to the benzene ring. For N-ethyl sodium barbiturate at 1.459 absorbance we obtained an maximum absorption at 238 nm. The new diazoethers had batocrome and hyperchrome intense displacements which supports our hypothesis on the formation of an extended molecular orbital. The three diazoethers synthesized had maximum absorption at 380-410 nm.

Infrared spectroscopy (FT-IR)

The vibration-rotation spectra were recorded with two Fourier transform FT-IR devices, an Biorad FTS-135 spectrometer and an Bruker Vertex 70 spectrometer in KBr pellets. Spectra were made at the corresponding acids and salts which allowed the structural correlations. Determinations were made on raw materials and intermediaries in order to make correlations.

The barbiturates derivates FT-IR spectra are very characteristic and relatively easy to interpret. Therefore, in N-phenyl sodium barbiturate spectrum the coresponding bands to valence vibrations of O-H bonds (present in the molecule due to the tautomeric phenomenon) are distinguish, at 3205 cm^{-1} and 3165 cm^{-1}. At 3057 cm^{-1} there is the ν_{C-H} vibration valence band (sp^2 aromatic carbon). The methylene groups determined bands corresponding to C-H asymmetric valence vibration at 2894 cm^{-1} and symetric valence vibration at 2844 cm^{-1}. Carbonyl groups C=O determined very intense characteristic bands at 1690 and at 1670 cm^{-1}. The C=N bond is very polar and has a high force value, determined an intense band at 1597 cm^{-1}. The C=C bond appeared because a tautomeric phenomenon between carbon atoms from 5 and 6 positions, gave an intense band at 1580 cm^{-1}. Intense bands due the δ deformation vibration, are observed at 706 cm^{-1}, 563 $cm^{-1}$and 543 cm^{-1}. The C-O bonds determine ν_{C-O} valence vibrations at 1357 cm^{-1} and 1319 cm^{-1}, these values are explained by the presence of an extended molecular orbital.

At 1597 cm^{-1} is the corresponding vibration valence band of the $C_{Ar}=C_{Ar}$ bond from the benzene ring.

In the N-methyl sodium barbiturate spectrum we can see the corresponding vibration valence bands for methyl and methylene groups. Due to the presence of the water (not enough removed in the precipitate processing), we can attributed only the bands from 2983.4 cm^{-1} for antisymmetric ν_{CH3} and 2840.6 cm^{-1} for symmetric ν_{CH2} . The hydroxyl group from the molecule because the tautomeric phenomenon presents a wide band at 3431,1 cm^{-1}. This band also appears in the other barbitals, for example, in N-phenyl sodium barbiturate at 3421 cm^{-1}.

The carbonyl groups determined an intense characteristic bands at 1690.46 cm^{-1} is 1659.59 cm^{-1}, and the C=N bond leads to a vibration band at 1613.27 cm^{-1}. Methyl group is characterized by the δ_{C-H} deformation vibration at 1370.12 cm^{-1}. The C-O bond presents a medium intensity band at 1308.37 cm^{-1}. We can also interpret the C-H deformation vibration bands from 791.15 cm^{-1} and 756.46 cm^{-1}, but they are less important.

In N-ethyl sodium barbiturate spectrum the OH group resuled from tautomery and the water not enough removed from the compound, gives band at 3438.82 cm^{-1} and 3218.83 cm^{-1}. The C-H valence vibration bonds bands of methyl and methylene groups are easy to attribute, and there are at 2883.05 cm^{-1} antisymmetric vibration for CH_2 group, 2829.02 cm^{-1} symmetric vibration for CH_2 group, 2983.40 cm^{-1} antisymmetric vibration for CH_3 group and 2937.08 cm^{-1} symmetric vibration for CH_3 group.

The carbonyl bonds determined intense bands at 1678.88 cm^{-1} and 1725.2 cm^{-1}. The C=N bond present valence vibration at 1632.5 cm^{-1} and the C=C bond (by tautomery) at 1613.27 cm^{-1}. The C-O bond appears at 1300.65 cm^{-1}. Also in the 1500-500 cm^{-1} region are observed less important bands as δ_{C-H} deformation vibration corresponding to methylene group at 771.901 cm^{-1} and at 798.918 cm^{-1}.

The δ_{CH3} symmetric deformation vibration is observed at 1377.84 cm^{-1}.

In FT-IR spectra of 1-naphthylamine we observed characteristic bands corespondig to ν_{NH} valence vibration at 3343 and 3225 cm^{-1}. The C_{Ar-H} bonds determined medium intensity vibrational bands at 3043 cm^{-1} and at 3016 cm^{-1}. We also observed in 1500-500 cm^{-1} area the valence vibration (ring vibration) of the $C_{Ar}=C_{Ar}$ bonds at 1623 cm^{-1} and 1574 cm^{-1}.

Of course, we can also observe other characteristic intense bands in 1500-500 cm^{-1} area, corresponding to deformation vibrations inside and outside the plan, at 791 cm^{-1} and 771 cm^{-1}. However, in this case, our analysis considers only the above mentioned bands, which would be sufficient to perform spectral correlations.

The diazoether FT-IR spectrum obtained by O-coupling the diazonium salt of 1-naphthylamine with N-phenyl sodium barbiturate have new characteristic bands. We can also easily notice the disappearance

from the spectrum of some characteristic bands of raw materials, which proves the reaction evolution to the desired product.

Therefore, it is noted the disappearance of ν_{N-H} bands from 3343 and 3225 cm^{-1}, present at 1-naphthylamine. In spectrum is present the coresponding C_{Ar-H} valence vibration band at 3055 cm^{-1} and the ν_{C-H} symmetric valence vibration band at 2831 cm^{-1}. Carbonyl groups cause intense bands at 1725 cm^{-1} and 1697 cm^{-1}, different from those of N-phenyl sodium barbituratethe C=C bond derived by tautomery on heterocyclic ring leads to a band at 1644 cm^{-1}. A new band apper in spectrum at 1207 cm^{-1} attributed to ether bond from diazoether group. Disappearance of some very characteristic bands of raw materials and the appearance of new ones, also relatively easy to assign, allows us to say that the O-coupling reaction occurred in the conditions described above.

Similarly, we could demonstrate the appearance of the others two O-coupling products derived from N-methyl and N-ethyl sodium barbiturate.

The appearance of a molecular orbital extended in the double bonds conjugation system and the presence of tautomeric phenomenon, lead to significant changes in the wave numbers values for some bonds. Such connections explain the values obtained for C-O etheric bonds for the barbituric derivatives moved to smaller wave numbers and much higher frequency.

Proton nuclear magnetic resonance spectroscopy (^{1}H-NMR)

^{1}H-NMR spectra have not been made for N-substituted barbituric acid salts because they can not be dissolved in deuterated solvents (Nicolescu T.O., 2009).

Barbituric acids have a relatively low solubility in $CDCl_3$, CCl_4 even in DMSO-d_6, so we added a small quantity of deuterated trifluoroacetic acid in the chloroform solution. Spectra made at 60 MHz with a VARIAN spectrometer not provide clear information on the compounds tautomery, we only found out in spectra the methylene group signal from the pyrimidine ring. The nitrogen atoms substitutes, methyl groups, ethyl and phenyl gave characteristic signals easily assigned.

One problem in interpreting the spectra is related to nitrogen proton bound signal. It has a very low intensity and is difficult to evaluate because of "noise", because it is operating with a high amplification. Solution concentrations were low, due to low solubility in deuterated solvent.

Also, the low solubility of new diazoethers in usual deuterated solvents ($CDCl_3$ or DMSO-d_6) made practically impossible to obtain nuclear magnetic resonance spectra.

CONCLUSIONS

As we specified in the introduction part, dye lasers are monochromatic light sources with acordable wavelength, with photophysical and spectroscopic applications in optoelectronics, molecular energy, biotechnology, medicine, etc. Dye lasers, with spectral range between 340 and 1100 nm, are essential in high resolution spectroscopy, atomic absorption and the photochemical separation of isotopes. Azoderivatives type structures with a residue of naphthalene have proved their ability to change the laser effect to wavelength over 300 nm (380 nm for naphthalene substituted with benzene rings or 370 nm for naphthalene substituted with oxadiazoles heterocyclic ring).

The use of dyes laser gives us the advantage of emitted wavelength batocrome moving over 50 nm, which contributes to increased monochromaticity, consistency and brightness.

Starting from these premises, we synthesized new diazoethers by condensation of 1-N-substituted sodium barbituric acid salt with 1-naphthylamine diazonium salt.

Choosing for the reaction of α-naphthylamine was made considering some conclusion from previous research, related to basic structural model of some azoderivates dyes, with specific properties for the chosen study domain.

Spectral data provided by FT-IR and UV-Vis, confirmed the presence of some extended molecular orbitals and intense batocrome and hyperchrome moves, 380-410 nm.

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