Chromatographic and Spectroscopic Properties of Regioisomers of Some 1*H*-1,5-Benzodiazepines

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The separation of the two regioisomeric derivatives of 1*H*-1,5-benzodiazepine yielded from the reaction of 1,2-diamino-4-methylbenzene with 4-substituted acetophenones was performed by reversed phase high performance liquid chromatography, and the absorption spectra of the separated isomers have been determined for three isomer pairs which have been obtained starting from acetophenones with substituents of different electronegativity. The isomer ratio then could be estimated as well from the ratio of the peak areas as from the absorption spectra. They agree well with the known ratio determined from nmr intensities.

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Introduction.

The regiospecific cyclocondensation reaction in methanol between 1,2-diamino-4-methylbenzene and five different acetophenones substituted in the 4-position by substituents that exhibit different electronegativity has been studied [1]. The resulting new derivatives of 1*H*-1,5-benzodiazepine shown in Figure 1 have been characterized [1] by ^{1}H- and by ^{13}C-nmr by ir and by their isomer ratio which was estimated from the nmr intensities, only, since the separation of the isomers was not possible.

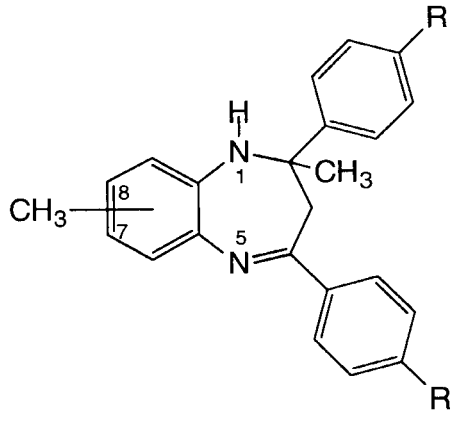

Figure 1. The investigated two regioisomers (the methyl group in the 7 or 8-position):
I: R = NO_2; II: R = Cl; III: R = Br.

These new 1*H*-1,5-benzodiazepines are assumed to have interesting pharmacological properties [2] and therefore will be investigated in more detail here. In the present communication the analytical scale separation of the isomers and their chromatographic characterization is dealt with, and their uv/visible spectra are determined and compared.

Preparation and Separation of the Isomer Pairs.

The isomer pairs shown in Figure 1 have been prepared closely following the procedure described [1]. A stoichiometric mixture of 1,2-diamino-4-methylbenzene and the respective 4-substituted acetophenone has been refluxed for 18 hours in methanol in the presence of catalytic amounts of sulfuric acid. The isomer mixture which precipitated by cooling was then recrystallized from methanol.

Since the 7- or 8-position of the methyl group does not make much difference in the physico-chemical properties of the respective isomeric derivatives of 1*H*-1,5-benzodiazepine, the separation of these isomers by hplc turned out to be at least time consuming. The best system was a Bischoff (Leonberg, Germany) Ultrasep 6 μ spherical particle stationary phase, packed into a Bischoff 250 mm x 4 mm diameter analytical scale column, on which the separation of each isomer pair was achieved using acetonitrile (ACN)/water mixtures of adequate water content, at flow rates of 0.8 or 0.9 ml/minute. A modular hplc equipment has been used consisting of a Bischoff pump model 2200, a Bischoff variable wavelength detector Lambda 1000, a

Rheodyne injection valve 8125 with 5 μl loop, and a Shimadzu Integrator SP3A. Table 1 shows the retention times τ_{R1} and τ_{R2} and the capacity ratios k'_1 and k'_2 of the first and second eluting isomer, respectively.

Table 1

Results from the Chromatographic Separation (ACN/H_2O = 72:28, v/v) of Three Derivatives of 1*H*-1,5-Benzodiazepine (R = NO_2, Cl, and Br) [a]

substituent R	NO_2	Cl	Br
τ_{R1}/min	9.03	21.1	25.6
τ_{R2}/min	9.38	22.6	27.4
k'_1	3.78	10.18	12.57
k'_2	3.97	10.98	13.52
$\omega_{245\ nm}$	0.29	0.56	0.36
$\omega_{340\ nm}$	0.29	0.45	0.29
$\omega_{425\ nm}$	0.29	0.64	0.40
ω_{nmr}	0.30	0.61	0.39
ω_{spec}	0.32	0.56	0.395

[a] Abbreviations and experimental conditions see text.

Varying the chromatographic conditions revealed that the investigated samples obviously did not contain any interferring impurities. Figure 2 shows the separation of the nitro isomers by the system outlined above, using a 55/45 (v/v) ACN/water mixture as mobile phase. The nitro isomers have been the most difficult isomers to separate, obviously since there is a considerable charge transfer over the whole skeleton which makes the difference introduced into the chromatographic interactions by the different positions of the methyl groups in this case even smaller than with the bromo- or chlorobenzodiazepine derivatives.

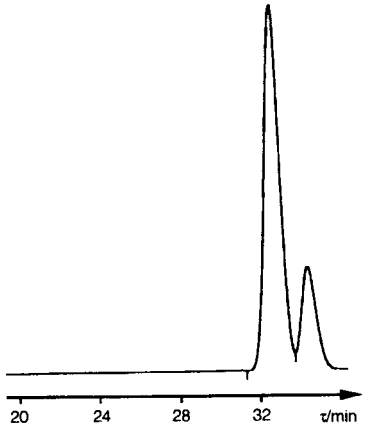

Figure 2. Highly symmetric peaks with the separation of the nitro isomer pair by a 55/45 (v/v) acetonitrile/water mixture on a 250 mm x 4 mm Bischoff Ultrasep 6 μm column, detected at 400 nm.

For all three isomer pairs, the peak area was recorded at λ = 245 nm, 340 nm, and 425 nm and the ratio $\omega\lambda$ defined as the peak area of the second eluting isomer divided by that of the first one has been calculated for each isomer pair. It was found to be highly reproducible. The values for $\omega\lambda$ are recorded in Table 1.

Since the position of the methyl group in the isomers considered is not expected to have a strong influence on the uv absorption of these compounds, the ratio $\omega\lambda$ may be considered as a first rough estimation of the isomer ratio and then may be compared to the ratio ω_{nmr} which is defined as the quotient of the nmr intensities of the 7-methyl by the 8-methyl isomer. This ratio has been dealt with [1] and is again recorded in Table 1, for comparison. It can be seen that the agreement between $\omega\lambda$ and ω_{nmr} is reasonably good. It cannot be perfect since there is of course, the minor effect of the position of the methyl group in the benzene ring of the benzodiazepine subunit on the absorption coefficient within the range of those transitions that involve the aromatic ring of the benzodiazepine subunit to a larger extent. This will be discussed in more detail in the next section. The comparison also shows immediately that the first eluting isomer contains the methyl group in the 8-position. The lower retention time of the 8-methyl isomer indicates that this in all cases behaves as the more polar one. Although this hint should not be stressed too much it makes sense since it fits well into the model of an although not too large long range charge transfer from the chloro, bromo or nitro substituted phenyl rings as the electron acceptor end to the 8-position methyl group as the electron donor end of the molecules. In such a case a simple model of pure electronic interaction of the solute with the mobile phase (assuming constant interaction with the stationary phase) should be able to describe the solvent dependent k' values. Then, using Onsager's model [3] of a reaction field E_R around a polar solute with dipole moment μ, it is

$$E_R = f\,\mu \qquad (1)$$

with

$$f = (\epsilon - 1)/(2\epsilon + 1) \qquad (2)$$

where ϵ is the relative permittivity of the solvent (mobile phase). Then, the interaction energy W_μ due to solely di-

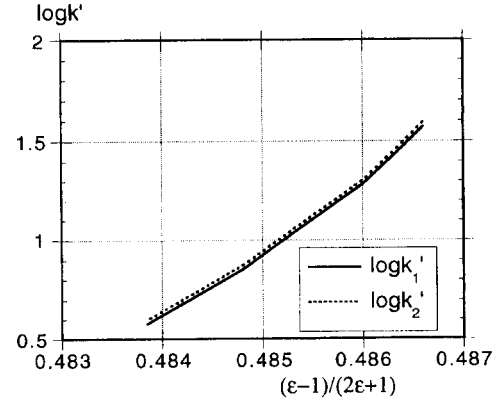

Figure 3. Logarithmic plot of the k'-values for both nitro regioisomers against $(\epsilon - 1)/(2\epsilon + 1)$ in four different acetonitrile/water mixtures. Acetonitrile content: 50, 55, 65, and 72% (v/v).

electric interactions is

$$W_\mu = -\mu E_R \quad (3)$$

and with equation (1)

$$W_\mu = -f\mu^2 \approx (\epsilon-1)/(2\epsilon+1) \quad (4)$$

Hence, including basic theromdynamics, a plot of log k' against $(\epsilon-1)/(2\epsilon+1)$ should yield a straight line. ϵ-values for the acetonitrile/water mixtures have been taken as experimental values [4]. Figure 3 shows as an example the respective plot for the nitro isomer pair.

The deviation from a straight line is not large thus indicating that the predominant interaction is dielectric. The conclusion then may be drawn that there is not great chance to considerably improve the separation conditions for these pairs with other stationary/mobile phase systems, since being the dielectric interactions the predominant ones.

UV/Visible Spectra of the Isomers and their Native Mixture.

Regrettably, no weighable amounts of the isolated isomers could be prepared since no semi-preparative conditions for their separation could be worked out - see above. Even chromatography on a 20 mm diameter column packed with the same stationary phase did not give a baseline separation in a reasonable time. Hence, lacking a photodiode array detector, 5 μl of a fairly concentrated solution (~1 mg/ml) has been injected, the whole peak volume V_1 and V_2 of each isomer was collected, the masses m_1 and m_2 of these volumes have been determined and their absorption spectra A_1 and A_2 have then been recorded against the respective eluant, the density ρ of which has been determined independently, in order to determine V_1 and V_2 with better accuracy than were possible by measuring the volumes with a syringe. The absorption spectrum A_{mix} of a solution with concentration c_o of the respective original isomer mixture has been separately determined in the same solvent mixture. Absorption measurements have been performed at room temperature using standard suprasil cuvettes with optical path d = 1 cm, in a Perkin-Elmer Lambda 2 spectrophotometer, optionally equipped with a 1 nm optical bandwidth. Reference was in all cases the respective eluant. If the recrystallized original isomer mixture contains only the two regioisomers, a plot of A_{mix} against $A_1 + A_2(V_2/V_1)$ should give a straight line over the whole spectrum. As an example, such a plot is given as Figure 4 for the chloro isomer pair. It shows a perfect straight line with R = 0.99998 (R = 0.99997 for the bromo isomer pair and R = 0.99961 for the nitro isomer pair).

On this basis, the absorption spectra $\epsilon_1(\lambda)$ and $\epsilon_2(\lambda)$ of each isomer can be determined using the following equations:

$$A_{mix} = (A_1 + A_2 V_2/V_1)\, c_d \quad (5)$$

where c_d is a dilution factor which follows immediately with high precision from a plot according to Figure 4.

$$c_o = c_1 + c_2 \quad (6)$$

where c_1 and c_2 are the unknown concentrations of the isomers in the peak volumes V_1 and V_2.

$$c_2 = c_1\, \omega_{nmr} \quad (7)$$

where the isomer ratio is taken from the nmr intensities [1].

Then

$$c_1 = c_o/(1 + \omega_{nmr}) \quad (8)$$

and

$$c_2 = c_o\, \omega_{nmr}/(1 + \omega_{nmr}) \quad (9)$$

It is

$$\epsilon_1 = A_1\, c_d/(c_1 d) \quad (10)$$

and

$$\epsilon_2 = A_2(V_2/V_1)c_d/(c_2 d). \quad (11)$$

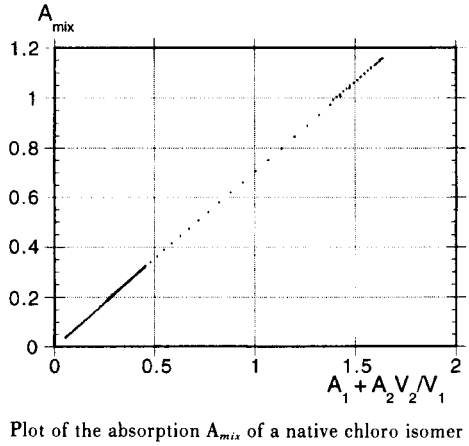

Figure 4. Plot of the absorption A_{mix} of a native chloro isomer mixture against the volume corrected sum of the absorption spectra A_1 and $A_2(V_2/V_1)$ of the isolated chloro isomers in acetonitrile/water, 72/28 (v/v).

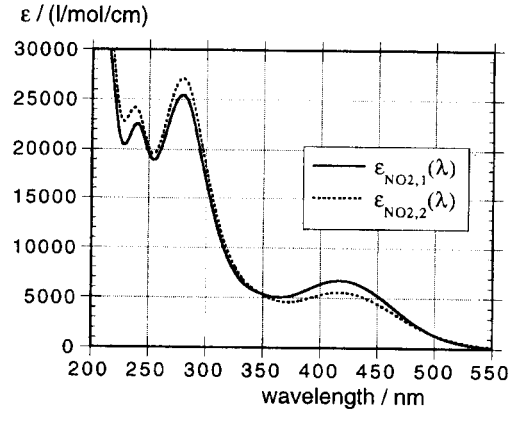

Figure 5. The absorption coefficient $\epsilon_{NO_2}(\lambda)$ of the isolated nitro isomers in acetonitrile/water, 55/45 (v/v), making use of the isomer ratio ω_{nmr} determined from nmr intensities.

Putting equations (8) and (9) into (10) and (11), all data on the right side of the latter two are known and thus the absorption spectra $\epsilon(\lambda)$ can readily be achieved with good accuracy. Figures 5 to 7 show the absorption spectra determined in this way.

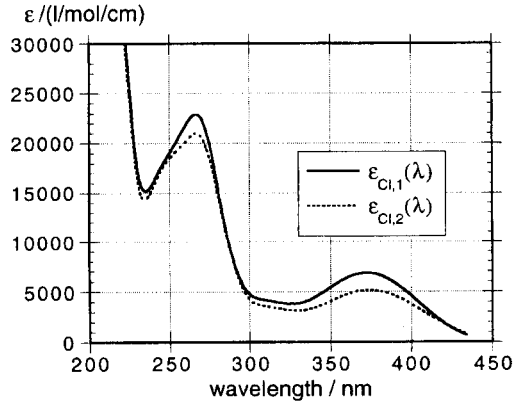

Figure 6. The absorption coefficient $\epsilon_{Cl}(\lambda)$ of the isolated chloro isomers in acetonitrile/water, 72/28 (v/v), making use of the isomer ratio ω_{nmr} determined from nmr intensities.

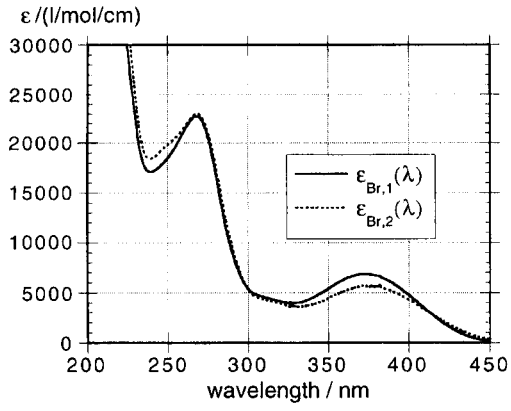

Figure 7. The absorption coefficient $\epsilon_{Br}(\lambda)$ of the isolated bromo isomers in acetonitrile/water, 72/28 (v/v), making use of the isomer ratio ω_{nmr} determined from nmr intensities.

As was to be expected, the absorption spectra of the three investigated isomer pairs are basically similar and agree well with the results given [5] for the non-methylated compounds. There are two intense bands, the shorter wavelength one at about 270 nm for the chloro and the bromo pair and at 280 nm for the nitro isomer pair, the long wave length band at roughly 375 nm for the chloro and bromo pairs but distinctly shifted by about 45 nm to 420 nm with the nitro isomer pair. There is another small less distinct peak around 240 nm in the uv spectrum of the nitro pair which is only indicated as a shoulder in the case of the other pairs. Another even minor indication of a lower intensity transition peaks at about 315 nm in the chloro and bromo pairs but appears only as a slight indication at about 350 nm in the nitro pair. The overall structure of the spectrum is very similar to that of benzylideneaniline [6] as was pointed out already in the case of the parent compound 1*H*-2,4-diphenyl-1,5-benzodiazepine [7].

The uv spectra of both isomers of each pair are very similar the largest effect being observed in all cases in the long wave length band which is more intense for isomer **1** (the 8-methyl isomer) and where the peaks of isomer **2** are slightly red shifted compared to those of isomer **1**.

It must be pointed out that the small difference in the absorption coefficient of the isomer pair at almost all wave lengths now makes understandable why the estimation of the isomer ratio *via* the chromatographic peak area ratio $\omega\lambda$ at the reported wave lengths yields results in good agreement with the nmr estimated value ω_{nmr}.

Final Discussion.

Of course, the absolute values of the absorption coefficients of the isolated isomers reported in the previous section depend on the absolute accuracy of the values for ω_{nmr}. Looking at the 270 nm band one is inclined to assume that this band is not affected by the isomeric position of the methyl group but that instead the difference is to be attributed to slightly erroneous values of ω_{nmr}. Hence in opposition to the procedure outlined above, the absorption coefficients at the maximum of this band have been assumed to be the same for both isomers and on this basis then the isomer ratio ω_{spec} (7-methyl isomer:8-methyl isomer) has been determined by uv spectroscopy. The resulting spectra are shown in Figures 8 to 10.

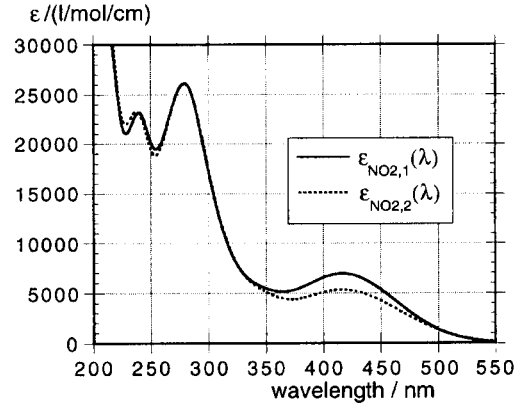

Figure 8. The absorption coefficient $\epsilon_{NO_2}(\lambda)$ of the isolated nitro isomers in acetonitrile/water, 55/45 (v/v), assuming no difference of the band around 280 nm of the isomers.

Obviously, the 270 nm bands of both regioisomers of each pair coincide perfectly over the whole band which therefore is a strong argument for the assumption of equal intensities which lead to ω_{spec} and to the spectra Figures 8 to 10. The resulting values for ω_{spec} are included in Table 1. They do not differ much from the values for ω_{nmr} nor from those for $\omega\lambda$.

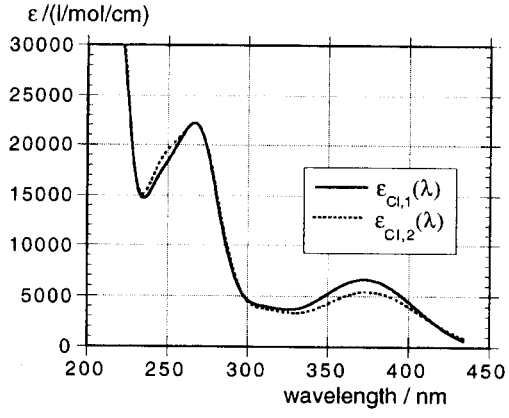

Figure 9. The absorption coefficient $\epsilon_{Cl}(\lambda)$ of the isolated chloro isomers in acetonitrile/water, 72/28 (v/v), assuming no difference of the band around 270 nm of the isomers.

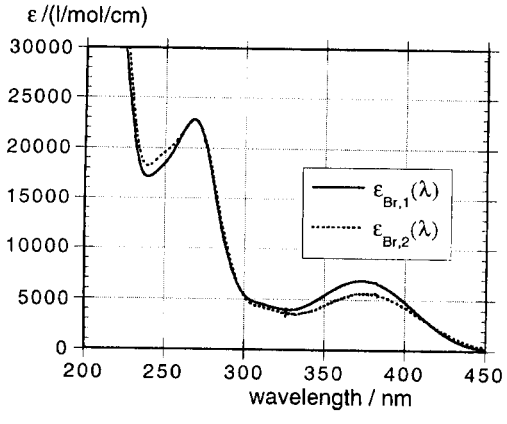

Figure 10. The absorption coefficient $\epsilon_{Br}(\lambda)$ of the isolated bromo isomers in acetonitrile/water, 72/28 (v/v), assuming no difference of the band around 270 nm of the isomers.

A more precise absolute determination of the absorption coefficients of the isomers is not assumed to be possible with a reasonable effort, since this would include weighable amounts of each isomer.

Whether one believes in ω_{nmr} or in ω_{spec} does not alter very much the intensity of the uv/visible absorption bands of the three isomer pairs and of course does not influence the spectral position at all. The difference of the intensities in the long wave length bands shows up in all cases with both outlined procedures thus indicating that this band is due to a transition between molecular orbitals which comprise the whole molecular skeleton from the electronegative group on one end to the 7- or 8-methyl group on the other end, similar to the planar conjugated benzylideneaniline [7]. The strong effect of the nitro group on the position of this band also points to this interpretation. In addition, it was observed that the position of this band in *n*-hexane for the nitro isomer mixture is 20 nm shifted to the blue side thus indicating a considerable difference between the ground and excited state dipole moment - in agreement with a light induced charge transfer over a larger distance. The determination of the ground and excited state dipole moment from the effect of an external electric field on the absorption bands of the isomers may reveal the spectroscopic nature of the involved states [8,9].

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