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^{1}H-NMR INVESTIGATIONS OF A HAMMET-TYPE SUBSTITUENT EFFECT ON THE HYDROGEN BOND INTERACTION OF SOME 1,4-BENZODIAZEPINES*

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Abstract—The hydrogen bond formation between some 1,4-benzodiazepines, differently substituted in positions 1 and 7, and the nucleobases 1-ethyl-2,4-dihydroxy-5-methylpyrimidine (e^1Thy) and 2,4-dihydroxy-1,3-dimethylpyrimidine (m^1m^3Ura) has been investigated by means of proton magnetic resonance technique in chloroform solution. It has been shown that the substituents influence the thermodynamic parameters ΔH^0, ΔS^0, and ΔG^0_{25} of this interaction. The standard free energy ΔG^0_{25} correlates linearly with the Hammet substituent constant σ^n of the substituent in position 7 of the benzodiazepine molecule. Moreover, a correlation between the free energies of the hydrogen bond interaction and the pharmacological activities of the drugs not methylated at N-1 was found. This may indicate a possible contribution of hydrogen bonds to the molecular interaction of the 1,4-benzodiazepines at their endogenous receptor(s).

Since brain specific benzodiazepine receptors have been identified and characterized recently [1–6] it seems to be possible to elucidate the molecular mechanism of the interaction of these drugs. Recently the hydrogen bond formation between the two benzodiazepines diazepam and nitrazepam and nucleobases has been reported [7]. The results obtained support the assumption that hydrogen bond interactions are not responsible for the chromosomal anomalies obtained with diazepam [8,9]. Nevertheless, this non-covalent interaction might be part of the binding of the drugs to their receptor(s) or to carrier molecules.

Various binding mechanisms of the 1,4-benzodiazepines have been discussed: Mueller and Wollert [10] studied their binding to human serum albumin and suggested that the A-benzene ring is the main binding group. Lucek and Coutinho [11] concluded from their results on the binding of the 1,4-benzodiazepines to plasma protein that the strength of the interaction can be correlated to the Hansch lipophilic constant and to the Hammet substituent constant. They observed that the plasma protein binding varied considerably with regard to the nature of the substituent in position 7 and concluded that the binding was mainly lipophilic. They pointed out, that an increase in the electrophilic character of the substituent might cause a reduced availability for hydrogen bonding of the lone pair electrons in positions 1 and 2 of the benzodiazepine molecule. Blair and Webb [12] computed the charge densities and dipole moments of 59 differently substituted benzodiazepines on the basis of CNDO/2 calculations and obtained direct correlations between the known pharmacological activities of the drugs [13] and their carbonyl oxygen charge density and their dipole moments.

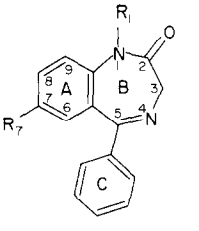
In order to elucidate the influence of the substituents in positions 1 and 7 of the benzodiazepine molecule on the hydrogen bond formation between 1,4-benzodiazepines and the nucleobase derivatives e^1Thy and m^1m^3Ura, this interaction has been investigated by means of proton magnetic resonance (^{1}H-NMR) spectroscopy in chloroform solution.

MATERIALS AND METHODS

The 1,3-dihydro-5-phenyl-2H-1,4-benzodiazepin-2-ones (I–VII) shown in Table 1 were kindly donated by Hoffmann-La Roche, Grenzach Whylen. 2,4-Dihydroxy-1-ethyl-5-methylpyrimidine (e^1Thy) and 2,4-dihydroxy1,3-dimethylpyrimidine (m^1m^3Ura)

Table 1. Chemical formulae of the 1,4-benzodiazepines used

No.	Substance	R_1	R_7
I	Ro 05-2921	H	H
II	Ro 05-2180 Desmethyldiazepam	H	Cl
III	Ro 05-2904	H	CF_3
IV	Nitrazepam	H	NO_2
V	Diazepam	CH_3	Cl
VI	Ro 05-4528	CH_3	CN
VII	Ro 05-3453	CH_3	NO_2



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were purchased from Cyclo Biochemicals, Tucson, U.S.A. Deuterated chloroform (Sharp & Dohme, Muenchen) was kept on molecular sieves (4Å Riedel-de Haën, Seelze-Hannover). All substances were used without further purification.

The proton magnetic resonance spectra were recorded on a Varian HA-100 spectrometer, locked on an internal reference of 3 per cent tetramethylsilan. Chemical shifts were determined to an accuracy of ± 0.002 p.p.m. The sample temperature was regulated with an accuracy of ± 1° by a Varian variable temperature system and calculated from the methanol chemical shift of a separate sample [14].

The spectroscopic changes due to molecular associations were analyzed by means of the dimer model of self- and mixed associations, in which case the latter one also considers the self-association of both interacting molecules [7]. All calculations were carried out on the Cyber 174 computer of the Hochschulrechenzentrum der Universitaet Giessen.

RESULTS AND DISCUSSION

The ^{1}H-NMR resonance lines of the (N-1)-H proton of the drugs I–IV shifted downfield with increasing concentration. This can be attributed to the formation of dimeric self-associates of the 1,4-benzodiazepines. The thermodynamic (standard entropy ΔS°, standard enthalpy ΔH°, and standard free energy ΔG°_{25}*) and spectroscopic parameters (monomer shift δ_m and association shift of self-association Δ_2*) of the association were obtained from the concentration- and temperature- dependence of the (N-1)-H signal by means of the methods described previously [7]. The results are shown in Table 2. For the 1-methyl-1,4-benzodiazepines used, no concentration dependent shifts of resonance signals could be observed. As in the case of self-association, the (N-1)-H signals of the compounds I-IV shifted downfield after addition of e^{1}Thy and, vice versa, the (N-3)-H resonance line of e^{1}Thy shifted downfield after addition of the drugs.

The addition of m^{1}m^{3}Ura to compounds I–IV results in downfield shifts of the (N-1)-H resonance of the drugs at high temperatures and low benzodiazepine concentrations and, furthermore, to upfield shifts at lower temperatures and high drug concentrations, as has been reported previously for the interaction of nitrazepam with m^{1}m^{3}Ura [7]. This behaviour can be explained by the small value of the complex shift Δ_c of mixed association as compared with the complex shift Δ_2 of self-association.

Assuming that the drugs form dimers with e^{1}Thy and m^{1}m^{3}Ura, the data of Tables III and IV have been obtained by applying the dimer model of mixed association [7]. For these calculations the self-association data of the pyrimidines [7] and of the drugs of Table 2 have been used.

The (N-3)-H resonance of e^{1}Thy exhibited only small downfield shifts when the *N*-methylated drugs V–VII were added. Therefore, the thermodynamic and spectroscopic parameters of the interaction could not be calculated separately from a least square fit. The complex shift of mixed association was estimated to be 3.5 p.p.m., which is in good agreement with the complex shifts of e^{1}Thy in the case of mixed association with compounds I–IV. With this assumption the data included in Table 3 have been calculated. In all cases, the spectroscopic parameters showed a temperature dependence of 10^{-3} to 10^{-2} p.p.m./deg. The values of δ_m, Δ_2, and Δ_c reported in Tables 2–4 correspond to a temperature of 0°.

The observation that the standard enthalpy ΔH° of the self-association of the 1,4-benzodiazepines I–IV and of their interaction with e^{1}Thy is about twice as large as that one obtained for their complexation with m^{1}m^{3}Ura or for the interaction between the N-1-methylated compounds V–VII with e^{1}Thy, supports our earlier suggestion that cyclic dimers are formed in the case of self-association of the non-methylated 1,4-benzodiazepines and of their associates with e^{1}Thy. Moreover, the amide group (N-1)-H, (C-2)=O seems to be the binding site of the 1,4-benzodiazepines for the hydrogen bonds [7]. From the data in Tables 2–4 it can be seen that the substituent in position 7 seems to have a profound influence on the thermodynamic parameters of the interactions of the non-methylated benzodiazepines. A systematic effect on the spectroscopic parameters does not seem to exist. They seem to be rather distributed around a mean value.

In Fig. 1 a plot of ΔG°_{25} of the different interactions investigated vs the Hammet substituent constant σ^n [15] of the para-substituent is shown. As can be seen, the interaction is getting stronger with increasing electronegativity of the 7-substituent in the case of the non-methylated drugs. A methylation at position 1 results in an independence of the free energy on electronegativity. Furthermore, in this case, the ΔG°_{25}-values are positive. The parameters of a linear regression were calculated on the basis of the equation $\Delta G^\circ_{25} = \Delta G^{\circ\circ}_{25} + \rho_I \cdot \sigma^n$ where ρ_I is the Hammet reaction constant and $\Delta G^{\circ\circ}_{25}$ is the

*For a definition of the parameters see ref. [7].

Table 2. Data of self-association of non-methylated 1,4-benzodiazepines obtained from the concentration and temperature dependent shift of the (N−1)-H proton

Compound	R_7	$-\Delta S^\circ$ [J/mol/deg]	$-\Delta H^\circ$ [kJ/mol]	$-\Delta G^\circ_{25}$ [J/mol/deg] [KJ/mol]	δ_m [ppm]	Δ_2 [ppm]
Nitrazepam	NO_2	35.7 ± 4.7	20.4 ± 1.3	9.74 ± 0.28	8.09 ± 0.10	3.16 ± 0.08
Ro 05-2904	CF_3	30.8 ± 8.7	18.4 ± 2.5	9.17 ± 0.49	7.79 ± 0.21	3.36 ± 0.18
Ro 05-2180	Cl	32.7 ± 3.6	18.0 ± 1.8	8.27 ± 0.34	7.95 ± 0.14	3.09 ± 0.12
Ro 05-2921	H	31.5 ± 5.4	16.1 ± 1.5	6.74 ± 0.31	7.86 ± 0.10	3.35 ± 0.08

Table 3. Data of mixed association of the 1,4-benzodiazepines with e¹Thy

Compound	R7	Proton observed	$-\Delta S^{\circ}$ [J/mol/deg]	$-\Delta H^{\circ}$ [kJ/mol]	$-\Delta G^{\circ}_{25}$ [kJ/mol]	Δ_c [ppm]
Nitrazepam	NO_2	(N−3)-H e¹Thy	38.0 ± 2.5	18.6 ± 0.1	7.11 ± 0.68	3.20 ± 0.36
		(N−1)-H drug	36.9 ± 1.7	18.9 ± 0.5	7.96 ± 0.18	3.14 ± 0.03
Ro 05-2904	CF_3	(N−3)-H e¹Thy	30.0 ± 4.8	16.1 ± 1.3	7.12 ± 0.25	3.36 ± 0.11
		(N−1)-H drug	3.14 ± 2.5	16.5 ± 0.8	7.11 ± 0.19	3.33 ± 0.06
Ro 05-2180	Cl	(N−3)-H e¹Thy	24.5 ± 4.5	13.4 ± 1.2	6.13 ± 0.25	3.49 ± 0.12
		(N−1)-H drug	33.3 ± 3.5	15.8 ± 1.0	5.90 ± 0.20	2.93 ± 0.06
Ro 05-2921	H	(N−3)-H e¹Thy	24.8 ± 14.2	13.0 ± 3.6	5.59 ± 0.71	3.53 ± 0.35
		(N−1)-H drug	26.6 ± 11.6	13.1 ± 3.1	5.19 ± 0.57	3.03 ± 0.15
Ro 05-3453	NO_2	(N−3)-H e¹Thy	36.3 ± 6.3	8.6 ± 1.6	−2.21 ± 0.32	3.5★
Ro 05-4528	CN	(N−3)-H e¹Thy	34.9 ± 3.2	8.3 ± 0.8	−2.10 ± 0.14	3.5★
Diazepam	Cl	(N−3)-H e¹Thy	38.4 ± 2.4	9.4 ± 0.6	−2.00 ± 0.11	3.5★

★ Estimated value (see text).

standard free energy of the unsubstituted molecule. The Hammet reaction constant ρ_i is very similar (−3.0 to −3.8) for the different interactions of the non-methylated benzodiazepines I–IV and is about 0.3 in the case of the (N-1)-methylated drugs V–VII. On the other hand, the $\Delta G^{oo}{}_{25}$-values seem to be characteristic for the interacting molecule, indicating differences in electronic and sterical properties of the complexes.

From the results obtained the following conclusions might be drawn: With increasing electronegativity of the substituent in position 7 of the benzodiazepine molecule the hydrogen bond involving (N-1)-H becomes stronger ($-\Delta G^{\circ}_{25}$ increases) whereas that one involving (C−2)=0 remains almost unchanged, as can be seen from the interaction of the drugs V-VII with e¹Thy. If both binding sites are involved, the effect of the (N−1)-H group dominates. This reflects a diminished electron density in the amide group as caused by an enhanced electronegativity of the para-substituent. The effect is the stronger, the nearer the atoms are located to the aromatic A-ring, i.e. it is more efficient for N−1 than for (C−2)=0. This agrees well with the assumption [11] that the electron density at positions 1 and 2 is reduced with an enhanced electrophilic character of the substituent in position 7. In accordance with the Hammet theory [16], the results obtained might be interpreted more generally: the observation, that ρ_j is similar for self-associates of the benzodiazepines I–IV as well as for their mixed complexes with e¹Thy and m¹m³Ura, suggests that ρ_j will be about the same as obtained in the present investigation for other molecules interacting at (N−1)-H and (C−2)=0 via hydrogen bonds. A comparison of the strength of the hydrogen bond interactions investigated with the pharmacological activities of the drugs as have been reported by Sternbach *et al.* [13], reveals that the free energy of the interaction increases with increasing pharmacological activity of the substances I–IV. This is demonstrated in Fig. 2. The free energy of the mixed association of the drugs I–IV with e¹Thy is plotted vs the dose applied in the footschock test. This test determines the taming activity of the drugs

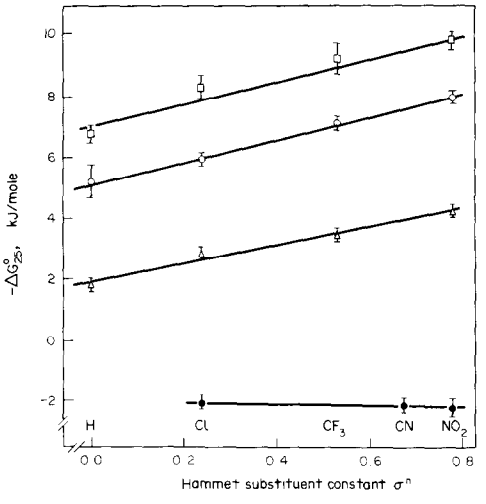

Fig. 1. Correlation of the free energies of the interactions investigated with the Hammet substituent constant σ^n. (□-self-association of cpds. I–IV, ○-mixed association of cpds. I–IV with e¹Thy, △-mixed association of cpds. I–IV with m¹m³Ura, ●-mixed association of cpds. V–VII with e¹Thy)

Table 4. Data of mixed association of the non-methylated 1,4-benzodiazepines with m¹m³Ura

drug	R_7	$-\Delta S^{\circ}$ [J/mol/deg]	$-\Delta H^{\circ}$ [kJ/mol]	$-\Delta G^{\circ}_{25}$ [kJ/mol]	Δc [ppm]
Nitrazepam	NO_2	20.7 ± 3.7	10.4 ± 0.9	4.23 ± 0.18	2.21 ± 0.01
Ro 05-2904	CF_3	12.2 ± 3.6	7.0 ± 1.1	3.42 ± 0.19	2.52 ± 0.02
Ro 05-2180	Cl	8.2 ± 4.5	5.3 ± 1.3	2.83 ± 0.22	2.02 ± 0.02
Ro 05-2921	H	19.0 ± 6.4	7.5 ± 2.7	1.83 ± 0.40	2.15 ± 0.03

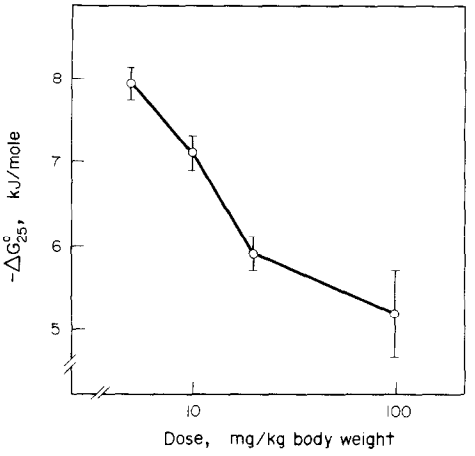

Fig. 2. Correlation of dose applied in the footschock test [13] with the free energy of mixed association of the non-methylated 1,4-benzodiazepines I–IV with e^{1}Thy.

[13]. Similar correlations were obtained with three other pharmacological tests: inclined screen test, metrazol test, and behaviour of cats. In the case of the (N−1)-methylated 1,4-benzodiazepines V–VII, no such correlation could be established, although these drugs are slightly more active than their 1-desmethyl-derivatives [13].

From these findings one might conclude that the interactions involving the amide group of the 1,4-benzodiazepines might not be important for the *in vivo* activity of the drugs. However, it has been demonstrated that most of the benzodiazepines of current interest are metabolized to their desmethyl-derivatives shortly after administration [17]. In this way, drug and metabolite, containing the amide structure, can equally be responsible for their *in vivo* activity. In this context it seems to be of interest that hypoxanthine and inosine have been shown to function as competitive inhibitors for the [^{3}H]diazepam binding in isolated bovine brain. [18,19]. These two substances, which are supposed to be endogenous neuromodulators, also possess an amide group in their purine ring system. Thus, one might conclude that this group might play an important role for the binding of the benzodiazepines at their receptor(s).

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