12. M. Chignard, J. P. LeCouedic, B. B. Vargaftig and J. Benveniste, *Br. J. Haemat.* **46**, 455 (1980).
13. Huzoor-Akbar and D. R. Feller, *Fedn Proc.* **40**, 808 (1981).
14. E. G. Lapetina and P. Cuatrecasas, *Biochim. biophys. Acta* **573**, 374 (1979).
15. Y. Kawahara, Y. Takai, R. Minakuchi, K. Sano and Y. Nishizuka, *Biochem. biophys. Res. Commun.* **97**, 309 (1980).

Biochemical Pharmacology, Vol. 31, No 5, pp 889–891, 1982.
Printed in Great Britain.

0006-2952/82/050889-03 $03.00/0

On the possible role played by hydrogen bonding in benzodiazepine-receptor interactions

(*Received* 5 *June* 1981; *accepted* 8 *September* 1981)

Recent studies [1–4] have singled out high affinity stereospecific binding sites for benzodiazepines (BDZs) in CNS. Several attempts have been carried out to isolate and identify endogenous ligands interacting with the BDZ receptor under physiological conditions and many compounds have been so far proposed as potential candidates, among which are the purines hypoxanthine and its nucleoside inosine, β-carbolines and nicotinamide [5]. The present uncertainties on the nature of the endogenous ligand make very difficult to hypothesize a reasonable scheme of the forces binding BDZs to their receptor. Although these forces can be generally identified in a combination of hydrogen bonding (HB), electrostatic and hydrophobic (or Van der Waals) interactions, only some evidence for a contribution of HB interactions has been so far obtained by Paul, Sapper and Lohmann [6] on the ground of the correlation observed between biologieal activities of BDZs and their free energies of HB interaction with nucleobase derivatives 1-ethyl thymine and 1,3-dimethyl uracil.

In view of the possible role played by HB in the mechanism of action of BDZs, we have thought worthwhile reviewing possible HB types formed by these compounds by an analysis of the crystal packing of all BDZs of known molecular structure. The results of such an analysis are reported in Table 1 and Fig. 1.

Most frequently BDZs appear to be self-associated in dimers, closing an 8- or 12-membered ring by means of two intermolecular HBs (types A, B, C and D of Fig. 1). Sometimes single HBs are observed (types E and F). Of particular interest is HB of type E, which does not correspond to a self-association but to the interaction between BDZ and a molecule of solvent (water or ethanol) and might describe the main interaction of BDZs in water solutions.

As regards the HB donor–acceptor properties of the different atoms or groups, the most frequently observed acceptor is the oxygen of the carbonyl group, while the most common donor is N_1H. The N_4 atom is found to be the acceptor only in two cases and the OH group at carbon atom 3, present in 3-hydroxylated BDZs, is found to act both as donor and acceptor. A particular scheme of HB formation is observed in chlordiazepoxide derivatives (type D), where the acceptor is the oxygen of the N-oxide group and the donor is the N_2H group of methylamine in position 2.

Assuming that HBs reported in Fig. 1 are representative of all possible HB interactions in BDZs, it may be wondered which ones are relevant to the BDZ–receptor interaction. A tentative answer may be sought considering what is known about the metabolic pathways of BDZs [7]. Most of the BDZs in clinical use are derivatives of the 1,3-dihydro-2H-1,4-benzodiazepin-2-one (e.g. nitrazepam; scheme A). A limited number of them are substituted 2,3-dihydro-1H-1,4-benzodiazepines (e.g. medazepam) or 3H-1,4-benzodiazepines (e.g. chlordiazepoxide; scheme D). However it has been proved that both medazepam and chlordiazepoxide are metabolized to the corresponding benzodiazepin-2-one derivatives, that the oxygen of the N — O group is lost and that dealkylation at N_1 is extremely fast in all BDZs tested. In a second time the N_1-dealkylated

Table 1. Hydrogen bond distances ($d_{X\text{-}Y}$) and types (HB type) observed in different crystals of benzodiazepines

	X	H—Y	$d_{X\text{-}Y}$(Å)	HB type	Reference
Nitrazepam	O_2	H—N_1	2.83	A	14
Clonazepam	O_2	H—N_1	2.87, 2.86	A	15
Lorazepam	O_2	H—N_1	2.88	A	16
	O_2	H—O_3	2.73	B	
	O_2	H—O—R	2.83	E	
	N_4	H—N_1	3.01	B	
Oxazepam	N_4	H—O_3	2.82, 2.87	C	17
	O_3	H—N_1	2.94	F	
Clordiazepoxide	O_4	H—N_2	2.85,2.91,2.80,2.79	D	18
Clordiazepoxide HCl	O_4	H—N_2	2.76	D	19
4,5-Dihydro-2'-fluoro-diazepam	O_2	H—O—R	2.81	E	20

X = acceptor and Y = donor in the hydrogen bond X-----H—Y; symbols in the HB type column refer to Fig. 1.

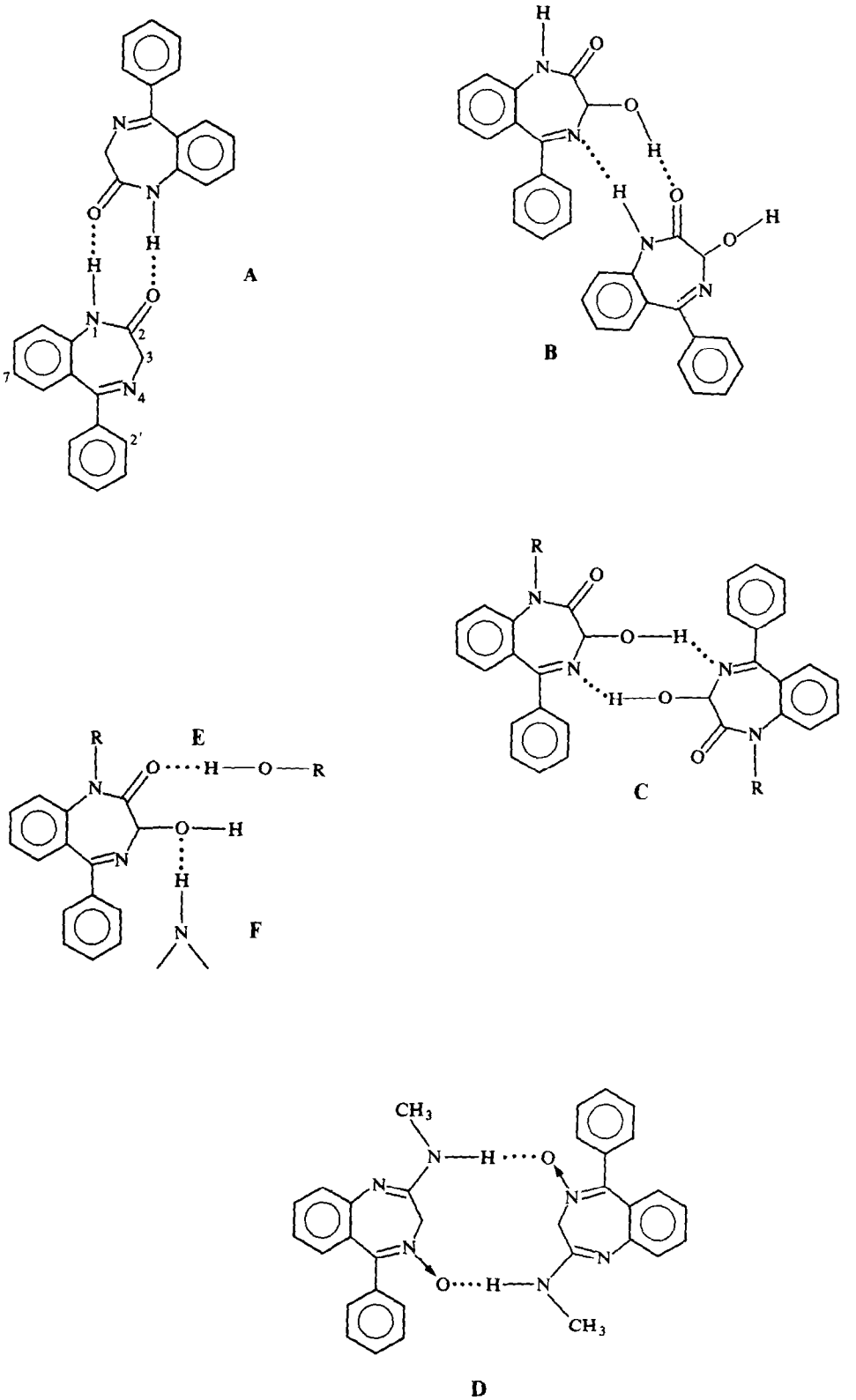

Fig. 1. Different kinds of hydrogen bonds observed in crystals of benzodiazepines.

benzodiazepinones are hydroxylated in position 3. Thus pharmacokinetic data agree in indicating that there is a limited number of HB donor or acceptor groups, which are present in the parent drug or in its metabolites of high biological activity and produced in a reasonably short time. These are the C=O group in position 2 and N_4 (acceptors), N_1H (donor) and the OH group at carbon atom 3 (donor and acceptor).

Another piece of information is given by structure–activity studies on BDZs, in particular by the effect of the substitution in position 7 on their biological activities. It has been reported by different authors [8–10] that substitution in such a position by strong electron withdrawing groups enhances the biological activity by several orders of magnitude: at the same time it causes a general lowering of the energies of both HOMO and LUMO frontier orbitals [11] and a decrease of the electron densities of the hydrogen in position 1 and of the carbonyl oxygen in position 2 [12]. As high activities appear to be associated with low energy values of the frontier orbitals, it may be argued that in the BDZ–receptor interaction the drug plays the role of electron acceptor (Lewis acid) or, which is the same, of HB donor. Similar assertion can be made on the ground of the decreased electron density (increased positive charge) on the hydrogen in position 1.

From these argumentations the following conclusions might be drawn:

1. Assuming that BDZ–receptor interaction is determined by a HB mechanism, the main interaction is to be associated, as far as the BDZ is concerned, with groups which are HB donors, that is the N_1H or OH group at carbon atom 3.
2. This first interaction would be strengthened by the formation of other HBs. Data of Fig. 1 suggest that the closure of a ring by means of two intermolecular HBs correspond to an arrangement of particular stability, in agreement with the spectroscopic and thermodynamic evidence that cooperative HBs are generally stronger than isolated ones [13].
3. Parts of the BDZ molecule suitable for such an interaction are $-N_1H-C_2O-$ (scheme A, Fig. 1) and $-C_3(OH)-N_4=$ (scheme C, Fig. 1) groups. It may be of interest that either or both groups mimic similar groups present in some of the putative endogenous ligands, such as hypoxantine or nicotinamide.
4. As for the relative importance of these two groups, there appear to be some definite indications in favour of the $-N_1H-C_2O-$ group. These are the greatest electron-withdrawing effect caused by electronegative substituents in position 7 on the hydrogen linked to the N_1 atom and the fact that dealkylation at N_1 is far faster than hydroxylation at C_3.
5. A last point supporting the rôle played by the $-N_1H-C_2O-$ group is the already mentioned intercorrelation between biological activities of BDZs and their free energies of HB formation between this group and nucleobases [6].

Acknowledgements—This work was financially supported by the C.N.R. (Rome). The authors thank Mr. G. Bertocchi for technical assistance.

Istituto di Farmacologia
Università di Ferrara
44100 Ferrara, Italy
PIER ANDREA BOREA

Centro di Strutturistica Diffrattometrica
Università di Ferrara
44100 Ferrara, Italy
GASTONE GILLI
VALERIO BERTOLASI
MICHELE SACERDOTI

REFERENCES

1. R. F. Squires and C. Braestrup, *Nature, Lond.* **266**, 732 (1977).
2. C. Braestrup, R. Albrechtsen and R. F. Squires, *Nature, Lond.* **269**, 702 (1977).
3. H. Möhler and T. Okada, *Life Sci.* **20**, 2101 (1977).
4. H. Möhler and T. Okada, *Science* **198**, 849 (1977).
5. H. Möhler, *Trends Pharmac. Sci.* **3**, 116 (1981).
6. H. H. Paul, H. Sapper and W. Lohmann, *Biochem. Pharmac.* **29**, 137 (1980).
7. M. A. Schwartz, in *The Benzodiazepines* (Eds S. Garattini, E. Mussini and L. O. Randall), p. 53. Raven Press, New York (1973).
8. L. H. Sternbach, L. O. Randall, R. Banziger and H. Lehr, in *Drugs Affecting the Central Nervous System* (Ed. A. Burger), p. 237. Marcel Dekker, New York (1968).
9. M. Yoshimoto, T. Kamioka, T. Miyadera, S. Kobayashi, H. Takagi and R. Tachikawa, *Chem. Pharm. Bull.* **25**, 1378 (1977).
10. P. A. Borea, G. Gilli and V. Bertolasi, Il Farmaco, *Ed. Sci.* **12**, 1073 (1979).
11. G. Gilli, P. A. Borea, V. Bertolasi and M. Sacerdoti, *Proceedings of the 4th European Crystallographic Meeting*, p. 38. Oxford (1977).
12. T. Blair and G. A. Webb, *J. med. Chem.* **20**, 1207 (1977).
13. S. N. Vinogradov and R. H. Linnell, *Hydrogen Bonding*, p. 161. Van Nostrand Reinhold, New York (1971).
14. G. Gilli, V. Bertolasi, M. Sacerdoti and P. A. Borea, *Acta Cryst.* **B33**, 2664 (1977).
15. P. Chananont, T. A. Hamor and I. L. Martin, *Cryst. Struct. Commun.* **8**, 393 (1979).
16. G. Bandoli and D. A. Clemente, *J. Chem. Soc. Perkin* **II**, 413 (1976).
17. G. Gilli, V. Bertolasi, M. Sacerdoti and P. A. Borea, *Acta Cryst.* **B34**, 2826 (1978).
18. V. Bertolasi, M. Sacerdoti, G. Gilli and P. A. Borea, *Proceedings of the 5th European Crystallographic Meeting*, p. 51. Copenhagen (1979).
19. C. Herrnstadt, D. Mootz, H. Wunderlich and H. Möhrle, *J. Chem. Soc. Perkin* **II**, 735 (1979).
20. P. Chananont, T. A. Hamor and I. L. Martin, *Acta Cryst.* **B36**, 1690 (1980).