A Novel Metabolic Pathway for Benzothiadiazoles; X-Ray Molecular Structure of 5-Chloro-4-(4,5-dihydroimidazol-2-ylamino)-1,3-dimethyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide

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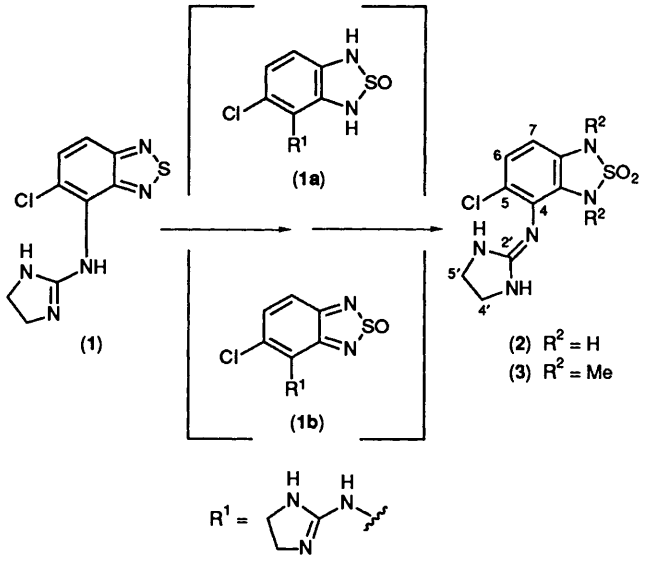
The methylated derivative of the major metabolite of sirdalud [5-chloro-4-(4,5-dihydroimidazol-2-ylamino)-2,1,3-benzothiadiazole hydrochloride] has been examined by X-ray crystallography. The structure confirms the existence of a new metabolic pathway. A comparison of the structure with that of a number of α2-adrenergic drugs is made.

Despite their wide pharmaceutical and agricultural applications, especially as fungicides, herbicides, and plant growth regulators,[1] very little is known about the metabolism of 2,1,3-benzothiadiazoles. A recent investigation of the biotransformation of ^{14}C-labelled sirdalud®, 5-chloro-4-(4,5-dihydro-[2-^{14}C]imidazol-2-ylamino)-2,1,3-benzothiadiazole hydrochloride (**1**), a novel myotonolytic agent,[2] revealed that one of the major oxidative pathways in four animal species and in man[3] led to a new type of metabolite, the dihydro-2,1,3-benzothiadiazole 2,2-dioxide (**2**). The present work describes the X-ray structure of its corresponding methylated derivative (**3**), and its structure is compared with that of a number of α-adrenergic agonists of the clonidine family.



Experimental

General.—360 MHz ^{1}H NMR spectra were recorded on an AM-360 Bruker spectrometer. Electron-impact (EI) and fast-atom bombardment (FAB) mass spectra were obtained with AEI MS 30 and MAT 212 instruments, respectively. A Perkin-Elmer Mod 21 spectrophotometer was used for IR spectra.

Preparation of 5-Chloro-4-(4,5-dihydroimidazol-2-ylamino)-1,3-dimethyl-1,3-dihydro-2,1,3-benzothiadiazole 2,2-Dioxide (**3**).—The water-soluble metabolite (**2**) was methylated at pH 6 with diazomethane according to the method of Eisenbraun.[4] The title compound (**3**) was isolated by preparative TLC (EtOH–toluene–conc. ammonia, 60:40:5) and was crystallised from methanol (51%, determined by radioactivity measurements); ν_{max}(KBr) 3 400 (NH), 2 910, 1 655 (C=N), 1 580 and 1 440 (C=C arom), and 1 315 and 1 150 cm^{-1} (SO_2); δ_H($CDCl_3$; Me_4Si) 3.23 (3 H, s, NMe), 3.37 (3 H, s, NMe), 3.57 (4 H, s, CH_2CH), 6.34 (1 H, d, J_{arom} 9 Hz, ArH), and 7.07 (1 H, d, J_{arom} 9 Hz, ArH); *m/z* (EI–MS) 317 (M^+ + 2, 12%), 315 (M^+, 31), 252 (M + 2 − SO_2, 36), 250 (M − SO_2, 100), 209 (10), 207 (23), 149 (8), 134 (10), and 105 (10), *m/z* (FAB–MS) 318 (MH^+ + 2, Cl isotope, 39%), 316 (MH^+, 100), 252 (M + 2 − SO_2, 15), and 250 (M − SO_2, 22).

X-Ray Structure Analysis.—Crystal data. $C_{11}H_{14}ClN_5O_2S{\cdot}0.5H_2O$, M = 324.78. Triclinic, a = 9.791(2), b = 13.306(3), c = 13.456(3) Å, α = 60.55(2)°, β = 72.14(2)°, γ = 75.82(2)°. V = 1443.44 Å^3 (by least-squares refinement on diffractometer angles for 16 automatically centred reflections, λ = 1.5418 Å), space group $P\bar{1}$, No. 2, Z = 4, D_c = 1.494 g cm^{-3}, colourless plates, crystal dimensions 0.23 × 0.30 × 0.08 mm. μ(Cu-Kα) = 38.4 cm^{-1}.

Data collection and processing. CAD4 diffractometer ω/2θ mode. Graphite-monochromated Cu-Kα radiation. 4 336 Reflections measured [$I > 3.0\sigma(I)$]. Monitoring of check intensity reflections showed no crystal decay. No absorption correction was applied. A direct method (SHELX-86[5]) was followed by blocked full-matrix least-squares refinement (SHELX-76[6]) with all non-hydrogen atoms anisotropic and most hydrogen atoms in ideal positions. Hydrogen atoms attached to N(11′), N(13′), N(21′), N(23′) were determined from difference Fourier maps and were included in fixed positions. The weighting scheme $w = 1/[\sigma^2(F_o) + 0.0007\ F_o^2]$ gave a satisfactory analysis of ΔF as a function of both sinθ and $|F|$. Final R and R_w values are 0.057 and 0.08. Bond lengths, bond angles, and torsion angles are given in Tables 1–3 and fractional co-ordinates are given in Table 4.

Results and Discussion

The crystal structure showed that the two independent molecules in the asymmetric unit have similar bond lengths, bond angles, and torsion angles, though the packing and hydrogen-bonding arrangements are quite different. A labelled drawing of both molecules is given in Figure 1. In the subsequent discussion we refer to chemically equivalent atoms in the two molecules by the postscript '*n*'. For example, C(*n*4) means C(14) and C(24).

The principal interest of this structure is to provide proof of the oxidative metabolic pathway converting compound (**1**) into

Table 1. Bond lengths (Å), with standard deviations in parentheses, for compound (**3**).

N(11)–C(111)	1.447(6)	N(21)–C(211)	1.428(8)
N(11)–S(12)	1.626(4)	N(21)–S(22)	1.617(4)
N(11)–C(18)	1.388(5)	N(21)–C(28)	1.396(5)
S(12)–O(11)	1.429(4)	S(22)–O(21)	1.415(4)
S(12)–O(12)	1.411(3)	S(22)–O(22)	1.426(3)
S(12)–N(13)	1.629(3)	S(22)–N(23)	1.642(4)
N(13)–C(112)	1.459(5)	N(23)–C(212)	1.414(7)
N(13)–C(19)	1.406(5)	N(23)–C(29)	1.411(5)
C(19)–C(18)	1.399(5)	C(29)–C(28)	1.407(5)
C(19)–C(14)	1.379(5)	C(29)–C(24)	1.381(5)
N(110)–C(12′)	1.305(4)	N(210)–C(22′)	1.279(5)
N(110)–C(14)	1.411(4)	N(210)–C(24)	1.417(5)
C(12′)–N(11′)	1.351(5)	C(22′)–N(21′)	1.361(6)
C(12′)–N(13′)	1.346(4)	C(22′)–N(23′)	1.345(5)
N(11′)–C(15′)	1.469(6)	N(21′)–C(25′)	1.428(7)
C(15′)–C(14′)	1.509(6)	C(25′)–C(24′)	1.509(8)
C(14′)–N(13′)	1.470(5)	C(24′)–N(23′)	1.436(7)
C(15)–Cl(15)	1.739(4)	C(25)–Cl(25)	1.748(4)
C(15)–C(16)	1.375(5)	C(25)–C(26)	1.366(6)
C(15)–C(14)	1.406(5)	C(25)–C(24)	1.399(5)
C(16)–C(17)	1.383(5)	C(26)–C(27)	1.381(6)
C(17)–C(18)	1.385(5)	C(27)–C(28)	1.361(6)

Table 2. Bond angles (°), with standard deviations in parentheses, for compound (**3**).

C(111)–N(11)–S(12)	121.9(3)	C(211)–N(21)–S(22)	122.3(4)
C(111)–N(11)–C(18)	125.2(4)	C(211)–N(21)–C(28)	124.4(4)
S(12)–N(11)–C(18)	112.1(3)	S(22)–N(21)–C(28)	112.9(3)
N(11)–S(12)–O(11)	111.83(21)	N(21)–S(22)–O(21)	112.48(20)
N(11)–S(12)–O(12)	111.74(20)	N(21)–S(22)–O(22)	109.99(19)
N(11)–S(12)–N(13)	94.05(19)	N(21)–S(22)–N(23)	94.23(19)
O(11)–S(12)–O(12)	114.63(21)	O(21)–S(22)–O(22)	115.65(20)
O(11)–S(12)–N(13)	111.04(20)	O(21)–S(22)–N(23)	111.75(20)
O(12)–S(12)–N(13)	111.80(18)	O(22)–S(22)–N(23)	110.75(19)
S(12)–N(13)–C(112)	118.0(3)	S(22)–N(23)–C(212)	117.5(3)
S(12)–N(13)–C(19)	112.41(25)	S(22)–N(23)–C(29)	111.9(3)
C(112)–N(13)–C(19)	129.4(3)	C(212)–N(23)–C(29)	130.6(4)
N(13)–C(19)–C(18)	109.6(3)	N(23)–C(29)–C(28)	110.1(3)
N(13)–C(19)–C(14)	128.2(3)	N(23)–C(29)–C(24)	128.8(3)
C(18)–C(19)–C(14)	122.2(3)	C(28)–C(29)–C(24)	121.1(3)
C(12′)–N(110)–C(14)	117.2(3)	C(22′)–N(210)–C(24)	118.3(3)
N(110)–C(12′)–N(11′)	128.2(3)	N(210)–C(22′)–N(21′)	127.3(4)
N(110)–C(12′)–N(13′)	122.3(3)	N(210)–C(22′)–N(23′)	124.3(4)
N(11′)–C(12′)–N(13′)	109.5(3)	N(21′)–C(22′)–N(23′)	108.4(4)
C(12′)–N(11′)–C(15′)	109.4(3)	C(22′)–N(21′)–C(25′)	111.7(4)
N(11′)–C(15′)–C(14′)	102.0(4)	N(21′)–C(25′)–C(24′)	102.6(5)
C(15′)–C(14′)–N(13′)	101.6(3)	C(25′)–C(24′)–N(23′)	103.5(4)
C(12′)–N(13′)–C(14′)	110.3(3)	C(22′)–N(23′)–C(24′)	110.8(4)
Cl(15)–C(15)–C(16)	118.6(3)	Cl(25)–C(25)–C(26)	118.4(3)
Cl(15)–C(15)–C(14)	118.6(3)	Cl(25)–C(25)–C(24)	118.4(3)
C(16)–C(15)–C(14)	122.8(3)	C(26)–C(25)–C(24)	123.2(4)
C(15)–C(16)–C(17)	121.0(3)	C(25)–C(26)–C(27)	120.2(4)
C(16)–C(17)–C(18)	117.4(3)	C(26)–C(27)–C(28)	118.4(4)
N(11)–C(18)–C(19)	111.6(3)	N(21)–C(28)–C(29)	110.8(3)
N(11)–C(18)–C(17)	127.3(3)	N(21)–C(21)–C(27)	127.9(4)
C(19)–C(18)–C(17)	121.1(3)	C(29)–C(28)–C(27)	121.3(4)
C(19)–C(14)–N(110)	122.0(3)	C(29)–C(24)–N(210)	122.3(3)
C(19)–C(14)–C(15)	115.4(3)	C(29)–C(24)–C(25)	115.8(3)
N(110)–C(14)–C(15)	122.3(3)	N(210)–C(24)–C(25)	121.9(3)

Table 3. Torsion angles (°), with standard deviations in parentheses, for compound (**3**).

C(14)–N(110)–C(12′)–N(11′)	1.2(5)
C(14)–N(110)–C(12′)–N(13′)	−176.7(3)
C(12′)–N(110)–C(14)–C(15)	−95.9(4)
N(110)–C(12′)–N(13′)–C(14′)	171.7(3)
C(12′)–N(11′)–C(15′)–C(14′)	23.8(4)
C(15′)–C(14′)–N(13′)–C(12′)	20.8(4)
C(16)–C(15)–C(14)–N(110)	−175.7(3)
C(24)–N(210)–C(22′)–N(21′)1.6(6)	
C(24)–N(210)–C(22′)–N(23′)	−179.3(3)
C(22′)–N(210)–C(24)–C(25)	95.9(4)
N(210)–C(22′)–N(23′)–C(24′)	172.1(4)
C(22′)–N(21′)–C(25′)–C(24′)	12.9(6)
C(25′)–C(24′)–N(23′)–C(22′)	16.2(5)
C(26)–C(25)–C(24)–N(210)	177.2(4)

Table 4. Fractional atomic co-ordinates for non-H atoms, with standard deviations in parentheses, for compound (**3**).

	x	*y*	*z*
N(11)	0.471 6(3)	0.148 7(3)	0.735 4(3)
C(111)	0.340 2(4)	0.097 4(4)	0.769 3(5)
S(12)	0.499 36(10)	0.274 95(7)	0.623 65(8)
O(11)	0.497 6(3)	0.276 2(3)	0.517 3(3)
O(12)	0.408 4(3)	0.365 75(22)	0.644 8(3)
N(13)	0.663 9(3)	0.267 64(23)	0.632 7(3)
C(112)	0.746 3(4)	0.364 9(3)	0.546 9(3)
C(19)	0.708 6(3)	0.160 52(24)	0.720 6(3)
N(110)	0.958 1(3)	0.190 43(20)	0.687 03(21)
C(12′)	0.971 6(3)	0.262 58(25)	0.721 92(25)
N(11′)	0.888 3(3)	0.280 61(25)	0.813 67(25)
C(15′)	0.957 1(5)	0.354 7(4)	0.832 3(4)
C(14′)	1.051 1(4)	0.417 4(3)	0.712 9(3)
N(13′)	1.074 7(3)	0.334 98(23)	0.664 62(24)
C(15)	0.856 1(4)	0.009 4(3)	0.840 4(3)
Cl(15)	1.021 55(10)	−0.046 65(8)	0.880 56(8)
C(16)	0.745 3(4)	−0.059 5(3)	0.897 7(3)
C(17)	0.611 8(4)	−0.019 8(3)	0.868 6(3)
C(18)	0.594 8(3)	0.091 6(3)	0.779 3(3)
C(14)	0.841 2(3)	0.123 21(24)	0.750 45(25)
N(21)	1.466 9(4)	0.354 7(3)	−0.035 0(3)
C(211)	1.510 1(7)	0.409 1(6)	−0.159 7(4)
S(22)	1.581 26(10)	0.306 25(8)	0.047 72(8)
O(22)	1.660 8(3)	0.200 44(23)	0.049 21(24)
O(21)	1.665 0(3)	0.392 45(24)	0.021 9(3)
N(23)	1.456 2(3)	0.280 5(3)	0.168 50(25)
C(212)	1.502 1(5)	0.241 0(6)	0.273 0(4)
C(29)	1.316 0(4)	0.306 1(3)	0.147 7(3)
N(210)	1.173 3(3)	0.256 86(23)	0.347 18(22)
C(22′)	1.182 6(4)	0.147 8(3)	0.415 9(3)
N(23′)	1.172 2(4)	0.101 6(3)	0.531 72(24)
C(24′)	1.167 3(8)	−0.021 6(3)	0.586 5(3)
C(25′)	1.220 0(8)	−0.051 3(4)	0.485 2(3)
N(21′)	1.206 0(6)	0.059 5(3)	0.386 8(3)
C(25)	1.062 6(4)	0.325 1(3)	0.180 1(3)
Cl(25)	0.892 34(10)	0.315 99(9)	0.274 47(9)
C(26)	1.070 0(5)	0.365 3(3)	0.063 9(3)
C(27)	1.202 2(4)	0.378 1(3)	−0.014 3(3)
C(28)	1.323 6(4)	0.349 2(3)	0.027 7(3)
C(24)	1.184 6(4)	0.293 41(25)	0.227 0(3)
O(99)	0.232 4(3)	0.401 07(24)	0.422 78(24)

its dioxide (**2**). There are 31 crystal structures in the Cambridge Crystallographic Database (CCDB)[7] which contain the C–N–SO_2–N–C fragment, and which give average bond lengths S–N = 1.63(4) Å, S=O = 1.425(12) Å, values similar to those found in this structure. As expected the thiadiazole ring of the title compound (**3**) is planar and the methyl groups deviate by a maximum of 0.2 Å from the plane. There is only one example in the Database in which the C–N–SO_2–N–C fragment forms a five-membered ring, but this is a dihydrothiadiazole.[8]

The metabolic mechanism for compound (**1**) is quite unique in that a sulphur with two bonded nitrogen atoms can be enzymatically converted into a dioxide; a reaction which has until now defied all attempts at simulation using conventional

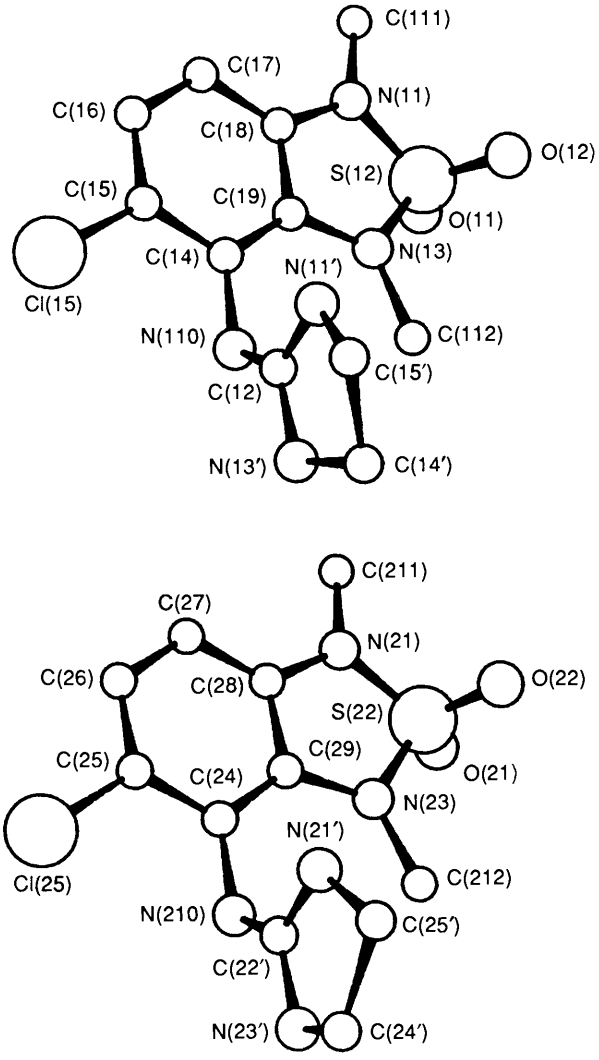
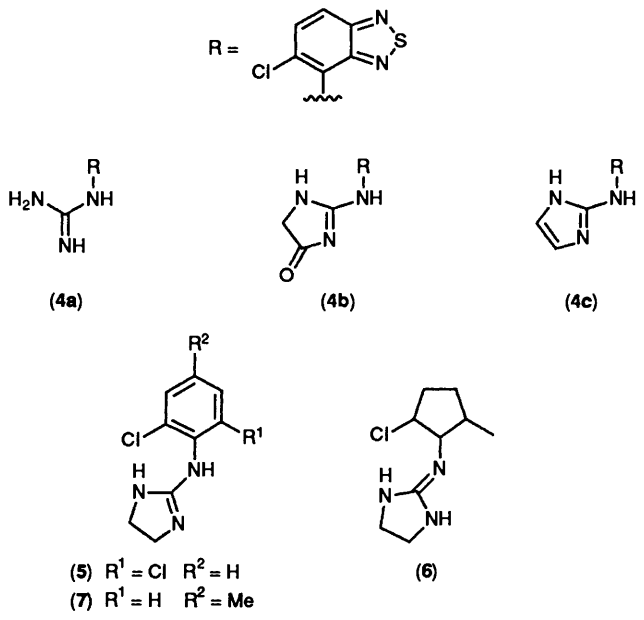


Figure 1. Atom-numbering scheme of the two crystallographically independent molecules of compound (**3**).



chemical methods. As a first step in the enzymatic conversion of compound (**1**) into its dioxide (**2**) it is reasonable to assume an intermediate sulphoxide either in the benzenoid (**1a**) or quinonoid (**1b**) form. Preliminary work in which the formation of the metabolite appears to be inhibited by carbon monoxide further suggests that the step converting the sulphoxide intermediate into the sulphone is controlled by cytochrome P450.

A number of other major metabolites of sirdalud are known, compounds (**4a–c**), but all are devoid of biological activity. Only metabolite (**2**) is known to retain some adrenergic activity, which seems to be strongly dependent on the imidazoline group.

The parent molecule sirdalud (**1**) (also known as tizanidine®) has been shown to act as an α-adrenergic agonist[9,10] and has a number of chemical and pharmacological similarities to the well known α-adrenergic agonist clonidine (**5**). The crystal structure of sirdalud is not known; however, the structure of its methylated metabolite (**3**) provides a good model for the parent compound and can be used to examine those molecular features which are common to the clonidine family[11] of adrenergic agonists.

Only twelve crystal structures have been published for compounds containing an aminoimidazoline group.[7] There are significant differences in bond lengths in this group depending on protonation and conjugation. The aromatic C(*n*4)–N(*n*10) bond at 1.411 and 1.417 Å is shorter than the expected C(sp²)–N bond length of 1.426 Å and indicates conjugation with the aromatic benzothiazole group. All four hydrogen atoms on the nitrogens of both imidazole rings were located from difference Fourier maps. N(*n*10) is, however, unprotonated and forms a double bond to C(*n*2′) (bond lengths 1.305 and 1.279 Å). Of the three other solved free-base structures, only tiamenidine (**6**)[12] has the same protonation pattern with an exocyclic C=N bond length of 1.29 Å. In the other two structures, the exocyclic nitrogen is protonated and bonded to an acetyl and methyl group, respectively. Clearly, the effect of the aromatic group in the title compound and in tiamenidine reduces the basicity of this nitrogen.

The other guanidinium C–N bonds have an average value of 1.354 Å, compared with an average of 1.34 Å calculated from the protonated N–C bond lengths of the twelve known (normally charged) imidazoline structures. There may be an energetically favourable interaction between the π-electrons of the aromatic group with H(*n*1′) lying only 2.55 and 2.40 Å above the plane of the aromatic ring. This H···C interaction is further helped by the rather narrow C–N(*n*10)–C bond angles of 117.2 and 118.3°. This angle is similar to that in tiamenidine (117.7°), but considerably narrower than the average of 122° found in the 11 related structures which have this exocyclic nitrogen protonated.

Theoretical studies of clonidine and analogues[13] have shown that the molecular conformation (rotation of the torsion angle [C(*n*5)–C(*n*4)–N(*n*10)–C(*n*2′)] is surprisingly relaxed and can vary between 60 and 120° with almost no change in energy. Another molecular mechanics study[11] showed a small energy-minimum value at 74°. The conformation found in structure (**3**) (+95.9° or −95.9°) is essentially orthogonal despite the fact that the imidazole ring has unsymmetrical neighbouring groups of Cl and NMe. This torsion angle ranges between 55° in the crystal structure of tolonidine[14] (**7**) and 86° in clonidine phosphate.[15]

The crystal structure of compound (**3**) is composed of dimers of the two crystallographically independent molecules (in mirror-image conformations). Three of the four unique hydrogen bonds serve to tie the molecules together (Figure 2), either directly [N(23′)···N(110) = 2.947 Å] or *via* a water bridge [N(11′)···W = 2.918, W···N(210) = 2.807 Å]. The single water molecule of crystallisation is involved in a third hydrogen

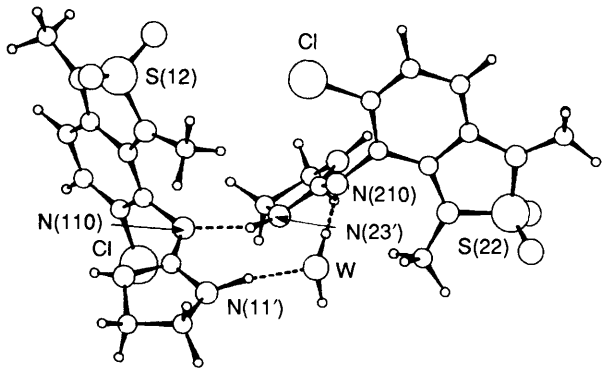


Figure 2. Intermolecular hydrogen bonds between the two independent molecules and a water of crystallisation.

bond to one of the sulphone oxygen atoms [W ··· O(11) = 2.992 Å].

The chlorine atoms are also involved in a number of interactions, notably Cl(15) ··· Cl(15) = 3.858 Å and Cl(15) ··· O(22) = 3.396 Å. Short Cl ··· Cl contacts ranging between 3.3 and 4.1 Å have been observed in various crystal structures[16] and can be regarded as an electrophile–nucleophile pairing interaction.[17] Additional stabilisation may also come from C–H ··· Cl 'hydrogen bonds'[16] which have been observed in the range 2.8–3.3 Å [H(122) ··· Cl(25) = 2.98, H(251) ··· Cl(25) = 2.97 Å]. It seems reasonable to assume that such interactions will be important in the recognition and binding of clonidine-like drugs to the adrenergic receptor.

References

1 L. M. Weinstock and I. Shinkai, in 'Comprehensive Heterocyclic Chemistry,' ed. K. T. Potts, Pergamon, Oxford, 1984, vol. 6, p. 513.
2 D. M. Coward, *Triangle*, 1981, **20**, 151; A. C. Sayers, H. R. Buerki, and E. Eichenberger, *Arzneim.-Forsch.*, 1980, **30**, 793.
3 P. Koch, D. R. Hirst, and B. R. von Wartburg, submitted for publication in *Xenobiotica*.
4 E. J. Eisenbraun, R. N. Morris, and G. Adolphen, *J. Chem. Educ.*, 1970, **47**, 710.
5 G. M. Sheldrick, SHELX-86, University of Göttingen, 1984.
6 G. M. Sheldrick, SHELX-76, University of Cambridge, 1976.
7 F. H. Allen, S. Bellard, M. D. Brice, B. A. Cartwright, A. Doubleday, H. Higgs, T. Hummelink, B. J. Hummelink-Peters, O. Kennard, W. D. S. Motherwell, J. R. Rodgers, and D. G. Watson, *Acta Crystallogr., Sect. B*, 1979, **35**, 2331.
8 R. J. Baker, S. Chiu, C. Klein, J. W. Timberlake, and L. M. Trefonas, *J. Org. Chem.*, 1980, **45**, 482.
9 L. Turski, M. Schwarz, T. Klockgether, and K. H. Sontag, *Brain Res.*, 1986, **379**, 367.
10 T. Kameyama, T. Nabeshima, K. Matsuno, and A. Sugimoto, *Eur. J. Pharmacol.*, 1986, **125**, 257.
11 A. Carpy, J. M. Leger, G. Leclerc, N. Decker, B. Rouot, and C. G. Wermuth, *Mol. Pharmacol.*, 1982, **21**, 400.
12 J. M. Leger, D. Hickel, and A. Carpy, *C.R. Acad. Sci., Ser. C*, 1979, **282**, 93.
13 F. Avbelj and D. Hadzi, *Mol. Pharmacol.*, 1985, **27**, 466.
14 A. Carpy, D. Hickel, and J. M. Leger, *Cryst. Struct. Commun.*, 1980, **9**, 731.
15 A. Carpy, D. Hickel, and J. M. Leger, *Cryst. Struct. Commun.*, 1979, **8**, 433.
16 J. A. R. P. Sarma and G. R. Desiraju, *Acc. Chem. Res.*, 1986, **19**, 222.
17 N. Ramasubbu, R. Parthasarathy, and P. Murray-Rust, *J. Am. Chem. Soc.*, 1986, **108**, 4308.

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