Crystal Structure and Quantum Electronic Analyses of Pitrazepin, a γ-Aminobutyric Acid (GABA) Receptor Antagonist

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The crystal structure of pitrazepin has been solved by direct methods from single-crystal *X*-ray diffraction data and refined by full-matrix least squares: monoclinic, space group $P2_1/c$, a = 18.845(2), b = 15.424(5), c = 16.950(4) Å, β = 104.51(2) °, Z = 4, two pitrazepin and three water molecules per asymmetric unit, final R factor is 0.042. This compound binds with high affinity to pharmacological GABA-A receptors; it acts as an antagonist. *Ab initio* molecular orbital calculations for pitrazepin and GABA show that the piperazine and triazole rings of pitrazepin might be considered as the GABA-mimetic moieties.

The study of new ligands for the γ-aminobutyric acid (GABA) receptor has led to the synthesis of pitrazepin (Figure 1) (all non-stereoscopic figures have been drawn using ChemDraw™ software [1]). This compound is specific to the GABA-A receptor (bicuculline sensitive) though it can also interact with the benzodiazepine receptor sites, being able to displace flunitrazepam.[2]

The crystal structure of pitrazepin is needed to obtain better understanding of its mode of action and, thus, to give better knowledge of the GABA-A receptor. However, a structural study is insufficient to explain how pitrazepin can bind to the GABA receptor when it does not possess a carboxy function or even an oxygen atom. Consequently, an analysis of the electronic properties (charge distributions, delocalization effects, *etc.*) computed by *ab initio* molecular orbital calculations is also required to develop the similarities between pitrazepin and GABA. Results from the Mulliken population analyses (net atomic charges, total and partial overlap populations) can be compared with those of GABA. They help us to find, on the one hand, which function has electronic delocalization interacting with the receptor in the same way as carboxylate, and, on the other, which nitrogen atom fulfils the role of the ammonium group in GABA.

Experimental

X-*Ray Diffraction*.—Pitrazepin was crystallized from acetonitrile solution at room temperature. The crystals were colourless and prismatic. 25 Medium-angle reflections provided the cell parameters by least-squares refinement. The intensities, collected on an Enraf–Nonius CAD-4 diffractometer, were corrected for Lorentz and polarization effects. The structure was solved by direct methods using the MULTAN 80 program.[3] Crystallographic data are reported in Table 1. All heavy atoms of both pitrazepin molecules were directly found in the best E map. Three cocrystallized water molecules in the asymmetric unit became apparent in a difference Fourier map. The structure was refined by full-matrix least squares using the SHELX 76 program.[4] Although many hydrogen atoms were localized in a difference Fourier map, their positions have been calculated, except for the amine hydrogen atom. For the water molecules, three hydrogen atoms have been localized experimentally; the positions of the three others were easily calculated. All hydrogen atom co-ordinates were then fixed for the next refinements. The last least square cycle gave a final R = 0.044. Anisotropic temperature factors were used for all non-hydrogen atoms and

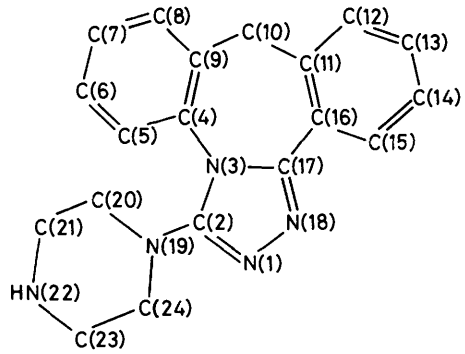


Figure 1. Planar structure and atomic numbering of pitrazepin. In later text, molecule (**1**) is numbered from N(101) to C(124), molecule (**2**) from N(201) to C(224)

Table 1. Crystallographic and refinement data for pitrazepin

Molecular formula	$C_{19}H_{19}N_5$
Molecular weight	317
Crystal system	Monoclinic
Space group	$P2_1/c$
a (Å)	13.845(2)
b (Å)	15.424(5)
c (Å)	16.950(4)
β (°)	104.51(2)
V (Å^3)	3 504.06
Z	8
D_c (g cm^{-3})	1.20
Crystal dimension (mm)	0.31 × 0.34 × 0.22
Radiation	Graphite-monochromated Mo-K_α (λ = 0.710 73 Å)
Diffractometer	Enraf–Nonius CAD-4
Absorption coeff. (cm^{-1})	0.42
F(000)	1 344
θ range (°)	2—25
Unique data	6 148 ($-16 \leq h \leq 16$) ($0 \leq k \leq 18$) ($0 \leq l \leq 20$)
Unique data with $I \geq 2.5\,\sigma(I)$	2 601
Final R factor	0.044
Max. and min. heights in final Δ-Fourier (e Å^{-3})	0.23 and −0.33

Table 2. Final atomic co-ordinates for pitrazepin

	x	*y*	*z*
N(101)	9 596(2)	337(2)	8 212(1)
C(102)	9 459(2)	981(2)	8 671(2)
N(103)	11(1)	1 696(1)	8 568(1)
C(104)	215(2)	2 473(2)	9 059(2)
C(105)	385(2)	2 408(2)	9 897(2)
C(106)	597(2)	3 152(4)	355(2)
C(107)	642(4)	3 942(4)	9 993(4)
C(108)	473(2)	3 995(2)	9 147(2)
C(109)	256(2)	3 259(2)	8 665(2)
C(110)	123(2)	3 289(2)	7 756(2)
C(111)	1 013(2)	2 860(2)	7 540(2)
C(112)	1 672(4)	3 355(2)	7 225(2)
C(113)	2 493(4)	2 965(4)	7 039(2)
C(114)	2 663(2)	2 098(4)	7 164(2)
C(115)	2 018(2)	1 585(2)	7 480(2)
C(116)	1 187(2)	1 977(2)	7 671(1)
C(117)	509(2)	1 431(2)	7 993(2)
N(118)	276(2)	630(2)	7 779(1)
N(119)	8 874(2)	958(1)	9 225(1)
C(120)	8 526(2)	84(2)	9 356(2)
C(121)	8 098(4)	108(2)	101(2)
N(122)	7 268(2)	734(2)	9 961(1)
C(123)	7 638(2)	1 600(2)	9 837(2)
C(124)	8 062(2)	1 603(2)	9 096(2)
N(201)	5 605(2)	−1 046(2)	6 811(1)
C(202)	5 958(2)	−1 024(2)	6 162(2)
N(203)	6 633(1)	−207(1)	6 068(1)
C(204)	6 697(2)	119(2)	5 394(2)
C(205)	6 252(2)	−157(2)	4 610(2)
C(206)	6 631(4)	139(4)	3 970(2)
C(207)	7 418(4)	713(4)	4 110(2)
C(208)	7 835(2)	1 000(2)	4 897(2)
C(209)	7 487(2)	710(2)	5 548(2)
C(210)	7 935(2)	1 042(2)	6 399(2)
C(211)	7 193(2)	1 593(2)	6 704(2)
C(212)	7 314(4)	2 483(4)	6 840(2)
C(213)	6 617(5)	2 950(4)	7 141(2)
C(214)	5 803(4)	2 541(4)	7 311(2)
C(215)	5 665(4)	1 668(4)	7 167(2)
C(216)	6 356(2)	1 191(2)	6 856(2)
C(217)	6 161(2)	274(2)	6 714(2)
N(218)	5 731(2)	−221(2)	7 153(1)
N(219)	5 910(1)	−1 696(1)	5 623(1)
C(220)	5 252(2)	−2 401(2)	5 756(2)
C(221)	5 081(4)	−3 015(2)	5 041(2)
N(222)	6 050(2)	−3 373(2)	4 968(1)
C(223)	6 692(2)	−2 656(2)	4 839(2)
C(224)	6 886(2)	−2 038(2)	5 543(2)
O(001)	4 381(1)	122(1)	1 202(1)
O(002)	3 622(2)	4 793(2)	3 805(1)
O(003)	8 065(5)	575(5)	2 128(5)
H(11)	5 655	−87	8 198
H(12)	6 244	254	9 179
H(21)	3 557	792	9 291
H(22)	4 323	16	8 795
H(31)	2 544	−285	8 203
H(32)	1 360	−157	7 838
H(105)	347	1 789	186
H(106)	733	3 119	1 009
H(107)	802	4 526	355
H(108)	515	4 617	8 863
H(110)	9 452	2 947	7 459
H(111)	71	3 956	7 555
H(112)	1 540	4 042	7 127
H(113)	3 002	3 355	6 800
H(114)	3 298	1 801	7 010
H(115)	2 153	899	7 577
H(121)	7 967	−122	8 827
H(122)	9 151	−364	9 466
H(123)	8 665	302	631
H(124)	7 821	−527	204
H(125)	6 912	643	488
H(126)	7 028	2 059	9 745
H(127)	8 210	1 783	368
H(128)	8 353	2 236	9 020
H(129)	7 488	1 431	8 561
H(205)	5 623	−593	4 494
H(206)	6 301	−84	3 360
H(207)	7 707	935	3 610
H(208)	8 450	1 455	5 006
H(210)	8 159	494	6 804
H(211)	8 586	1 427	6 395
H(212)	7 950	2 814	6 711
H(213)	6 712	3 643	7 239
H(214)	5 278	2 907	7 551
H(215)	5 030	1 344	7 293
H(221)	5 594	−2 742	6 312
H(222)	4 545	−2 134	5 802
H(223)	4 735	−2 671	4 488
H(224)	4 606	−3 539	5 131
H(225)	5 845	−3 795	4 401
H(226)	7 394	−2 914	4 775
H(227)	6 327	−2 315	4 287
H(228)	7 344	−1 506	5 430
H(229)	7 261	−2 368	6 094

isotropic ones for hydrogens (isotropic temperature factors U_{eq} of the carrier atoms were incremented by 0.02).

The *X*-RAY 76 program[5] was used for molecular geometry and crystal-packing analyses. Final atomic parameters are listed in Table 2. Bond lengths and valence angles for both molecules are given in Figures 2 and 3, respectively. Figure 4 shows crystal packing. Figure 5 presents a stereoscopic view of the molecular conformation with ellipsoids of vibration (both Figures 4 and 5 were drawn using the ORTEP program[6]).

ab initio *Calculations.*—Calculations were made at the MO RHF level of the electronic theory. Within this framework, calculations have been performed at the STO-3G degree of sophistication in LCAO expansion of the molecular orbitals, introduced by Pople.[7] Its parameters (coefficients and exponents of the Gaussian expansion) were carefully optimized to reproduce selected molecular properties such as molecular geometries. The advantages of this basis set are summarized in a review.[8] Moreover, for qualitative interpretations, minimal basis sets have the great advantage of relating molecular properties to simple atomic parameters and allow for a conceptual approach common to both theoreticians and experimentalists.

Net atomic charges and overlap populations were calculated by Mulliken charge population analysis.[9] This analysis has a good relation with very common chemical concepts such as bond properties, polarization, mesomeric, and inductive effects. All computations were performed with the GAUSSIAN 82 program[10] adapted to an IBM 4341-2 (running under the VM/CMS operating system) computer.

For pitrazepin, the experimental geometry was used for the *ab initio* calculations. Optimizing the geometry of pitrazepin would have been time consuming and, furthermore, the crystal structure is reliable enough for such rigid compounds. The triazole ring has been placed in the *xz* plane (Figure 1) in order to differentiate the π electron contribution (*i.e.*, the $2p_y$ component) from the total contribution. Partial geometry optimization, starting from the crystallographic data, and using the *ab initio* force method[11,12] was performed on GABA, a flexible molecule.[13] All parameters (bond lengths, valence, and

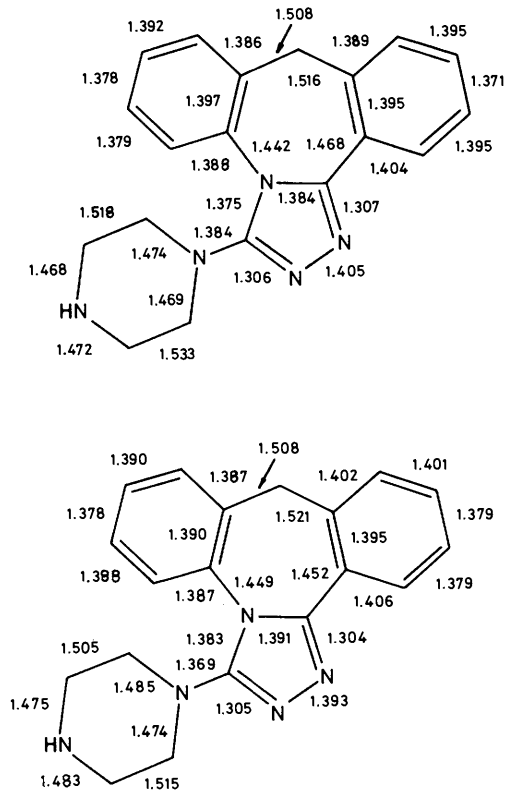


Figure 2. Bond lengths (Å) of the pitrazepin structures: (a) molecule (1) and (b) molecule (**2**) (e.s.d. max. 0.007 Å)

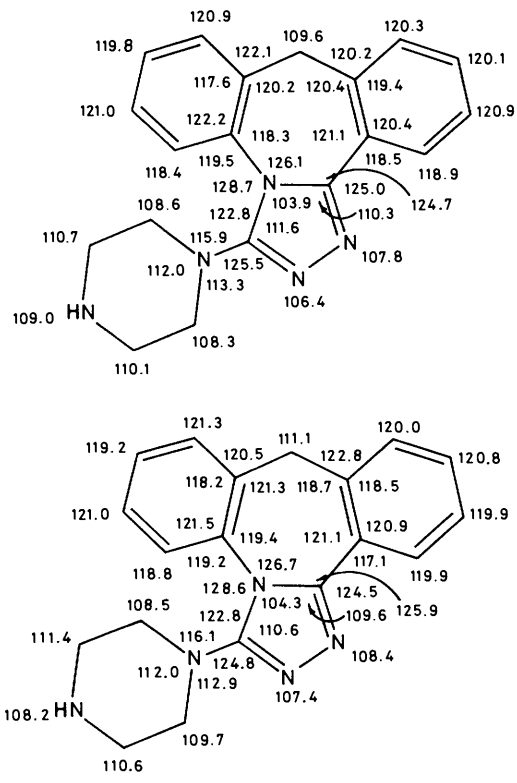


Figure 3. Valence angles (°) of the pitrazepin structures: (a) molecule (**1**) and (b) molecule (**2**) (e.s.d. max. 0.5°)

torsion angles) involving heavy atoms were optimized; parameters referring to hydrogen atoms were also optimized but kept equal for each carrier atom separately in order to decrease the number of variables. The carboxylate function lies in the *xz* plane too. The various conformations of GABA, semi-extended as observed by *X*-ray diffraction or with an intramolecular hydrogen-bond as determined by the geometry optimization, are compared with other rigid, active GABA-ergic compounds such as THIP (4,5,6,7-tetrahydroisoxazolo[5,4-*c*]-pyridin-3-ol).[13]

The bielectronic integral cutoff and convergence on the density matrix thresholds have been fixed at 10^{-8} a.u. and 10^{-7}, respectively. Both thresholds have been lowered to 10^{-10} a.u. and 10^{-9}, respectively, for the geometry optimization computations. Our experience has shown that, within the STO-3G basis used, the computed total energy has at least seven significant digits; it corresponds in our calculations to a numerical error of *ca.* 1 kcal mol^{-1}. The atomic charges and overlap populations (in electrons) have four significant digits.

A single geometry calculation for pitrazepin with 139 basis functions needs 10 h 30 min on the IBM 4341-2 computer.

Results and Discussion

X-Ray Diffraction.—The crystal structure of pitrazepin gives us two important pieces of information not predictable from a standard Dreiding model, the conformation of the piperazine ring and the electronic delocalization pattern within the triazole ring.

The observed molecular conformations are the same for both molecules in the unit cell. The orientation of the chair piperazine ring is indicated by the N(1)–C(2)–N(19)–C(20) torsion angle, 9.3(6) and 10.8(5)° for molecules (**1**) and (**2**), respectively. This is in agreement with the planar conformation of the THIP isoxazole ring, fused with a piperidine ring. Moreover, this piperidine ring forces the butyric chain to be in a semi-extended conformation, as in the piperazine ring of pitrazepin. It can also be noted that both aromatic rings are pointing like two 'ears', in such a way that they lower steric hindrance with the triazole and piperazine rings. This is indicated by the following torsion angle values: C(5)–C(4)–N(3)–C(2) = 37.9(6) or 34.2(6)° and C(15)–C(16)–C(17)–N(18) = −35.2(6) or −33.2(6)°. The global conformation of molecule (**1**) is shown in Figure 5.

The analysis of the bond lengths (Figure 2) in the triazole ring confirms the planar structure. There is a real distinction between single and double bonds. The N(1)–C(2) (double), C(2)–N(3) (single), N(3)–C(17) (single), C(17)–N(18) (double), and N(18)–N(1) (single) bond lengths are respectively 1.306(7), 1.375(6), 1.384(6), 1.307(6), and 1.405(6) Å for molecule (**1**) and 1.305(7), 1.383(6), 1.391(6), 1.304(7), and 1.393(6) Å for molecule (**2**). Consequently, simple comparison of the bond lengths does not allow us to propose a delocalization scheme and a complementary *ab initio* theoretical study is required.

Another point of interest is to describe the crystal packing. It implicates two molecules of pitrazepin and three molecules of water in the asymmetric unit; all molecules are linked by a dense network of hydrogen bonds. The atoms involved in intermolecular bonding are two nitrogen atoms per molecule, the unsaturated N(18) belonging to the triazole ring and the secondary amine N(22) of the piperazine ring. A first molecule of water [O(1), H(11), H(12)] shares one hydrogen atom with N(122) and the other with N(218), thereby linking the two pitrazepin molecules. The second molecule of water binds H(22) to O(1) and the third one, H(31), to O(2), forming a chain of three molecules. O(3) shares its second hydrogen atom H(32) with N(118). Moreover, H(21) of the second water molecule is bound to N(222) of a fourth pitrazepin molecule. The cycle is thus completed. This network is shown in Figure 4.

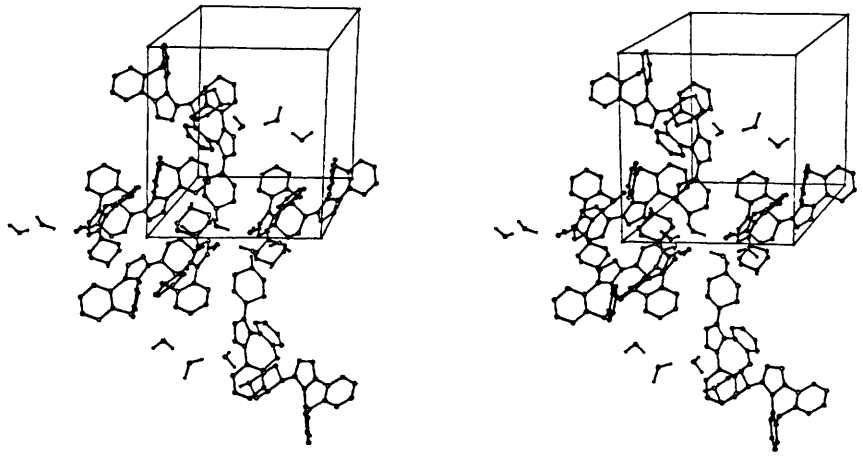


Figure 4. Stereoview of molecular conformation and crystal packing of pitrazepin

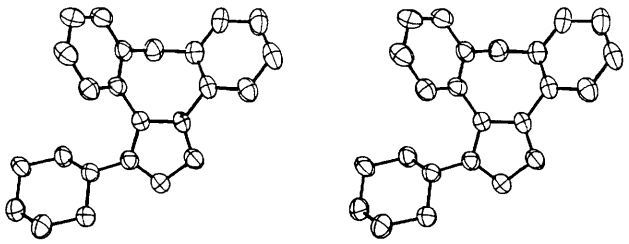


Figure 5. Stereoview of pitrazepin molecular conformation [molecule (**1**)] with vibration ellipsoids (probability 50%)

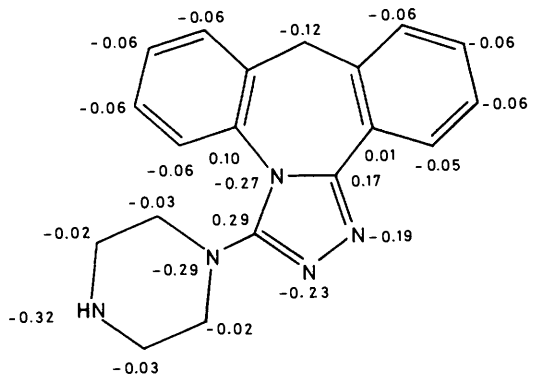
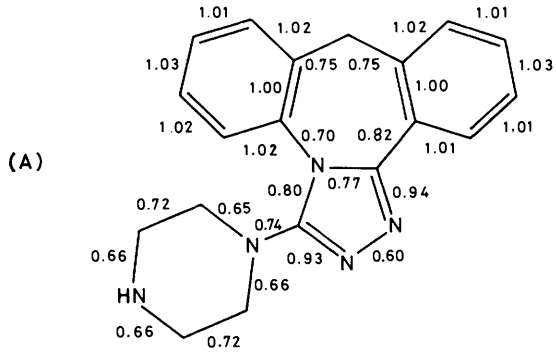


Figure 6. STO-3G net atomic charges (e) calculated for the pitrazepin molecule (**1**)



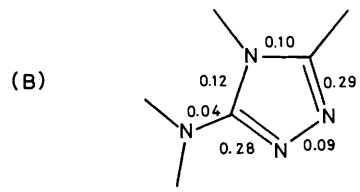


Figure 7. STO-3G (A) total and (B) π overlap populations (e) for the first pitrazepin molecule (**1**)

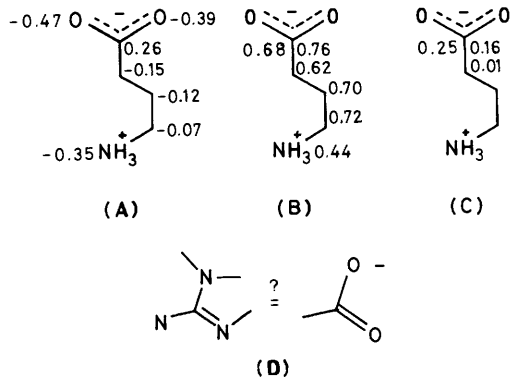


Figure 8. STO-3G (A) net atomic charges, (B) total, and (C) π overlap populations (e) for the GABA molecule

Theoretical Calculations.—To improve the description of electronic delocalization in the molecular framework, *ab initio* calculations have been performed. Only the first molecule (**1**) was considered. Differences between distance and angle values for molecules (**1**) and (**2**) are not sufficiently significant to produce changes among the theoretical results. For example, C(20)–C(21) and C(23)–C(24), in the piperazine ring, are 1.518(8) and 1.533(8) Å, respectively, but the corresponding computed overlap population values are 0.72 e in both cases.

Our principal aim is to characterize the GABA-mimetic fragment in pitrazepin. Apparently, both phenyl rings would only be additional sites to bind to the receptor leading therefore to antagonist character. This is the case for other GABA antagonists like bicuculline or tubocurarine. Our discussion is mainly oriented towards the electronic structure of the piperazine and triazole rings. N(22), which forms a secondary amine, probably plays the same role as the nitrogen atom of the GABA ammonium function; its computed charge is −0.32 e

versus −0.35 e for GABA. We, thus, try to see if the triazole ring has some similarity with the GABA carboxylate function. We compare atomic charges, total, and π overlap populations around the triazole ring in pitrazepin (Figures 6 and 7) and over the carboxylate in GABA (Figure 8).

Looking at the net atomic charges of N(1), C(2), and N(3) (Figure 6), we observe a similarity with the bidentate carboxylate anion, a positive charge on a carbon atom (0.29 e) and two negatively charged heteroatoms [−0.27 and −0.23 e for N(1) and N(3)] compared with 0.26, −0.47, and −0.39 e for the GABA carboxylate atoms (Figure 8A). Evidently, the charges carried by the nitrogen atoms of the triazole ring are weaker than those of the oxygen atoms in GABA, but it is probable that antagonists need not possess the exact charge pattern of GABA or agonists.[13] Contrary to the weaker net atomic charges, the total overlap populations between corresponding atoms are stronger for pitrazepin, 0.80 and 0.93 e (Figure 7A) *versus* 0.68 and 0.76 e for GABA (Figure 8B), but still indicate quasi-symmetry between both carbon–nitrogen bonds. We may conclude that the N(1), C(2), N(3) three-atom unit accurately mimics the carboxylate by taking into account the π overlap populations (Figure 7B). Indeed, the N(1)–C(2) carbon–nitrogen double bond is characterized by a value of 0.28 e; the intracyclic C(2)–N(3) single bond has a value of 0.12 e whereas the exocyclic single C(2)–N(19) bond is characterized by 0.04 e, compared with 0.25, 0.16, and 0.01 for GABA (Figure 8C). These results indicate that the C(2)–N(3) bond shows partial double-bond character, unlike the single C(2)–N(19) bond. This underlines the delocalization from N(1) to N(3) (see also Figure 8D). The absence of conjugation between C(2) and N(19) can be explained by the orientation of the piperazine ring which prevents the N(19) lone pair from being in the *xz* plane.

Conclusions.—The molecular structure of pitrazepin has been solved by *X*-ray diffraction. The co-ordinates have been introduced into *ab initio* computations in order to obtain electronic information to be compared with that for GABA. On the one hand, the triazole ring, rich in π electrons, has a specific local delocalization and might be related to the receptor in the same way as a carboxylate function. On the other, the secondary amine function of the piperazine ring, at a good distance from the triazole ring, plays the same role as the ammonium group in GABA.

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