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The Conformation of γ-Aminobutyric Acid and Its Analogues

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Theoretical and experimental data on the steric structure of γ-aminobutyric acid and its analogues are presented. The possible relation between the structural flexibility of the γ-aminobutyric acid molecule and its biological activity is examined. The bibliography includes 90 references.

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I. INTRODUCTION

γ-Aminobutyric acid (GABA) was obtained for the first time in 1883.[1] In the middle of the present century it was detected in the nerve tissues of the central nervous system (CNS) of mammals. It has recently come to be realised that GABA is one of the most important natural products from the biological point of view. It is a specific chemical mediator (transferring agent) in the inhibition of nerve pulses in CNS.[2-6] Recently (since 1960) GABA and its derivatives have begun to be used as medicinal preparations in the treatment of nervous and psychiatric conditions[7]. The successful use of GABA and its derivatives in the treatment of various neurotic and psychopathic states led to the possibility of synthesising new synthetic drugs with selective action and with a low toxicity by virtue of their similarity to the metabolic products in the brain. In this connection the problem of the influence of the steric structure of GABA on its biological and pharmacological activity assumes considerable importance. Like many substances with pharmacological activity, GABA has a non-planar structure. Conformational changes in the GABA molecule can lead to its incomplete interaction with its receptor. Probably only a specific conformation of the GABA molecule ensures the optimum disposition of its structural elements relative to the receptor, necessary for the manifestation of the maximum physiological activity. Probably not only the conformation but also the electronic properties of the molecules play a significant role in the pharmacological properties of GABA and its derivatives. Numerous studies, published during the last five years, have been devoted to the relation and between these properties and their mutual influence and to the determination of the active conformations of GABA and its analogues. Both theoretical and experimental methods are widely used in the solution of these problems.

II. THE STERIC STRUCTURE OF γ-AMINOBUTYRIC ACID AND ITS ANALOGUES

1. Quantum-Chemical Modelling of the GABA Molecule

Quantum mechanics permits an approximate calculation of the energy of molecules in terms of the molecular orbital theory by solving the Schrödinger wave equation. Since all the valence electrons can be taken into account in such calculations, the total energy of the molecule becomes sensitive to changes in its conformation. Minimisation of the total energy as a function of specified angles between bonds in the molecule makes it possible to predict its preferred energetically most favourable conformation. Quantum-chemical calculations also permit the determination of the electronic structures of the conformers from the corresponding wave functions, to predict the chemical behaviour of the molecule, to derive parameters associated with the reactivity of a biologically active molecule, etc.

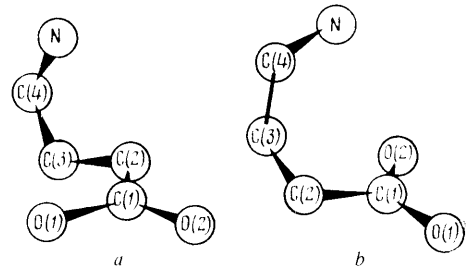


Figure 1. The skeleton of the GABA molecule: *a*) extended structure; *b*) folded structure.

Several semiempirical molecular orbital methods have been used to determine the preferred conformation of GABA.[8-10] The latter was first determined theoretically in 1970;[11] the authors carried out a calculation on this molecule with the aid of the extended Hückel method (EHM).[12] The method takes into account the interactions not only between atoms linked to one another but also between remote atoms, so that the total energy of the molecule is evaluated as a function of the coordinates of all the atoms in the molecule. The calculation showed that in the preferred conformation of GABA the 'onium and α-methylene groups are in the transoid positions and the carboxy-group can rotate freely by 360° (Fig. 1*a*). The GABA molecule is represented as a zwitterion with a distance between the charge centres (N^+ and O^-) of 5.0–6.0 Å, which corresponds to an extended (unfolded) conformation of GABA. However, one should bear in mind that the mutual repulsion of electrons is not taken into account in

EHM, so that the results can sometimes conflict with data derived by other methods, particularly for polar molecules [13].

A more exact quantum-chemical theory—the method of complete neglect of differential overlap (CNDO)—was developed by Pople [14,15]. Calculation for the GABA molecule by this method [10] led to the opposite results—a folded conformation (Fig. 1*b*) is preferred for the isolated GABA molecule in both neutral and zwitterionic forms, which is consistent with data obtained by ^1H nuclear magnetic resonance [16,17]. It has been noted [10] that the influence of the solvent can induce conformational changes and calculations have indeed demonstrated that in the presence of a solvent the extended conformation becomes preferred. However, Pople's theory also suffers from definite limitations [13]. Thus a satisfactory description of the charge distribution is obtained in most cases only for mobile π electrons, the polarisation of single σ bonds being neglected, although the latter may be important for polar molecules, i.e. for the majority of pharmacologically active compounds.

The preferred conformations of GABA and its analogues.

Compound	Method of determination of conformation	Preferred conformation*		References
		coiled	extended	
γ-Aminobutyric acid (GABA)	EHM	—	+ (5.0–6.0)	11
GABA**	CNDO	+	—	10
GABA	LMO	+	—	9
GABA	X-ray diffraction (XRD)	+ (4.5)	—	22, 23
GABA	XRD	—	+	20, 24
Muscimol	EHM	—	+ (5.8)	11
Muscimol	Molecular modelling (MM)	—	+ (5.7)	31
Muscimol	XRD	—	+ (5.77)	32
4-Aminotetrolic acid	MM	—	+ (5.2–6.1)	31, 31, 84
trans-4-aminocrotonic acid	MM	—	+ (5.4–6.3)	84
Hydrazinopropionic acid	EHM	—	+ (5.1–6.1)	38
β-Hydroxy-γ-aminobutyric acid**	EHM	+	+ (5.0–6.0)	51
Homotaurine	EHM	—	+ (5.6–6.6)	38
Homotaurine	XRD	—	+	43
Imidazolylacetic acid	EHM	—	+ (5.5–5.9)	38
Imidazolylpropionic acid	MM	+ (4.3)	+ (5.1–6.3)	53, 55
Imidazolylacrylic acid	MM	—	+ (7.0)	56
α-Guanidinoacetic acid**	MM	+ (3.2–4.8)	+ (5.3–5.7)	54, 56
β-Guanidinopropionic acid**	MM	+ (4.4)	+ (5.7–6.9)	54, 56

*"+" or "–" implies that the given conformation is (or is not) preferred; the distance between N^+ and O^- in Å is indicated in brackets.
**Both conformations are equally probable.

The third quantum-chemical approach to the determination of the GABA conformation employs the method of localised molecular orbitals (LMO), which takes into account configurational and electronic interactions. This method is believed [9,18] to be the most accurate for calculations on highly polarised molecules. The theoretical search for the energetically favourable GABA conformation by the LMO method showed [9] that the folded conformation of the zwitterion is the most stable. The minimum total energy of the extended conformation of an isolated flexible GABA molecule exceeds by 54 kcal mole^{-1} the energy of the folded conformation, which is apparently favoured by the mutual approach of the ionic ends of the molecule in the folded form. The folded conformation is also preferable for the cationic form of GABA (as the hydrogen chloride), although in this case the energy of the extended conformation is higher by only 5 kcal mole^{-1}. Inspection of Dreiding steric models also made it possible to postulate the existence of a folded conformation of the GABA zwitterion with an angle of 40° between the C–N bond and the plane in which the three atoms of the carboxylate group are located, which indicated some interaction between the NH_3^+ and COO^- groups [19].

Conformational analysis of GABA with the aid of X-ray diffraction confirmed that its molecule is fairly flexible and can exist in different conformations [20–24]. In the solid phase GABA forms prismatic crystals with a melting point of 203°C and exists in the zwitterionic form [20–23]. X-Ray diffraction showed that the GABA molecule is to some extent folded with a distance between charge centres of 4.2–5.75 Å [22,23] and that a hydrogen bond with a length of 2.7–3.1 Å can be formed with a deviation from linearity in the N–H...O linkages not greater than by 25°. Nor can one rule out the possibility of the "forking" of the hydrogen bond between adjacent molecules, which is consistent with the melting point [19,22]. The COO^- group is located in the plane passing through the chain of carbon atoms [21,22]. The orientation of this group changes (it becomes almost perpendicular to the given plane) in copper di-γ-aminobutyrate [23]. In the crystalline hydrochloride GABA exists in the cationic form $HOOC(CH_2)_3NH_3^+$. [24] In these crystals the cationic group is always relative to the CH_2COO^- group, while the conformation relative to the $C_{(2)}-C_{(3)}$ bond is either skewed or transoid. Thus both fully extended and partly folded GABA conformations may be found to be preferred in the crystalline state. The zwitterion apparently tends to stabilise the extended conformation, since the oppositely charged atoms of the neighbouring molecules can be located closer to one another than the similar atoms of the same molecule [22].

2. The Conformation of GABA and Its Structural Analogues

Quantum-chemical modelling and X-ray diffraction analysis of GABA make it possible to investigate its static steric structure. However, static stereochemistry does not provide a final answer to the question concerning the conformation of the molecule at the instant when it exhibits its pharmacological activity. Methods for the direct determination of the steric structures of medicinal drugs when the latter act on biological objects have not so far been adequately developed. An indirect approach, which consists in the search for, synthesis, and biological tests of various conformationally constrained analogues of GABA, is therefore extremely fruitful in the solution of stereochemical problems.

Fig. 2 and the Table presents certain fundamental structural GABA analogues exhibiting a GABA-like biological activity. The first of the structural GABA analogues investigated was the natural product muscimol (Fig. 2*b*), which is 5-aminomethyl-3-hydroxyisoxazole, isolated from the fungus *Amanita muscaria* [25]. Johnston et al. [26] suggested that muscimol is an analogue of the fixed extended GABA conformation. Subsequently, in the course of biological tests of this compound, it was found

that, like GABA, it has an inhibiting effect on the central and peripheral neurons[27,28], i.e. it is an agonist of GABA and not its antagonist. The structural analogy between GABA and muscimol is also confirmed by the fact that the latter's bicyclic derivative 4,5,6,7-tetrahydroisoxazolo-[4,5-*c*]pyridine-3-ol proved to be a competitive inhibitor of the absorption of GABA by rat brain sections[29]. In conformity with Dreiding models, the distance between the nitrogen of the tetrahydropyridine ring and the oxygen of the hydroxy-group proved to be 4.4 Å.[30]

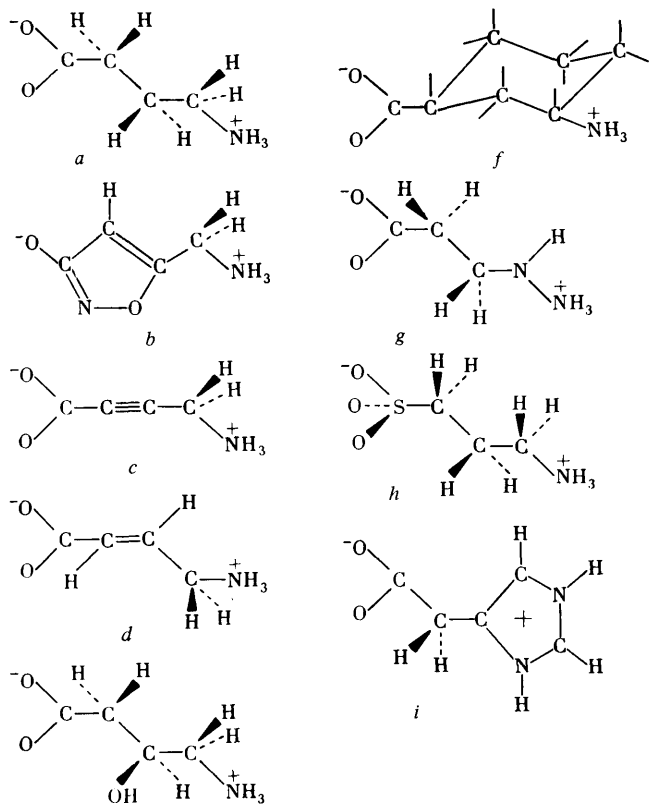


Figure 2. Structural formulae of GABA and some of its agonists: *a*) GABA; *b*) muscimol; *c*) 4-aminotetrolic acid; *d*) 4-amino-*trans*-crotonic acid; *e*) β-hydroxy-GABA; *f*) *cis*-3-aminocyclohexanecarboxylic acid; *g*) hydrazinopropionic acid; *h*) homotaurine; *i*) imidazolylacetic acid.

Theoretical calculations[11] showed that muscimol can assume a conformation analogous to that of GABA, the conformation in which the side chain is coplanar with the five-membered ring and is rotated in the direction of the endocyclic oxygen atom having the minimum total energy. The charged exocyclic oxygen atom is located at a distance of 5.8 Å from the positively charged nitrogen atom. These data suggest, on the one hand, a fully extended chain of carbon atoms in GABA, and, on the other hand, agree with the presence in muscimol of the postulated pharmacophoric GABA group[11]. Steric models showed that the nitrogen atom and the carboxy-group of GABA are isosteric with the exocyclic nitrogen atom and the O–C=N group in the muscimol ring[30]. Such correspondence between the molecular structures of these substances is observed for the extended GABA conformation. X-Ray diffraction studies also indicate the presence of elements of structural analogy between GABA[21] and muscimol[32]. In the crystal state of the latter the side chain is indeed bent in the direction of the oxygen ring atom and makes an angle of 60° with the plane of the ring[32]. The distance between the charged centres in muscimol is then 5.77 Å, which corresponds to the theoretical data[11] for the extended conformation of GABA.

Johnston et al.[30] investigated the relation between the structures of a number of miscimol derivatives and their GABA-like biological activity. An increase in the length of the muscimol side chain by one CH_2 group or substitution of an aliphatic hydrogen atom by the CH_3 group leads to an appreciable lowering of its depressant effect on nerve structures. The substitution of aromatic hydrogen atoms by alkyl groups in muscimol and some of its derivatives led to a sharp decrease of the depressant activity. The authors therefore suggest that the methylidyne group in the 4-position of muscimol is isosteric with the α-methylene group of GABA. The introduction of alkyl groups in the 4-position in the isoxazole ring leads to an increase of its structural rigidity which probably hinders the interaction of substituted muscimol derivatives with the postsynaptic GABA receptors. The study of the biological activities of many muscimol analogues enabled the authors[30] to suggest that different conformers are involved in the absorption of GABA by nerve structures and in its interaction with postsynaptic receptors.

The problem of the preferred GABA conformation was also studied with the aid of its conformationally constrained analogue 4-aminotetrolic acid (4-ATA).[33] In this compound the linearity of the triple bond fixes the positions of the four carbon atoms in such a way that the charged N^+ and O^- centres of the zwitterionic form are located at a distance of 5.2–5.8 Å from one another. The rigid 4-ATA molecule cannot assume a folded conformation and its structure corresponds to the extended GABA conformer. In the study of the action of 4-ATA on the spinal neurons of the cat[34] it was observed that it exhibits between one-half and one-fifth of the activity of GABA on these receptors. Other facts confirming the physiological activity of this compound were also obtained. The GABA-like activity of 4-ATA suggests that GABA exhibits its biological activity in the extended conformation and that the 4-ATA structure apparently includes a GABA-like pharmacophore[11]. This hypothesis received additional experimental confirmation after Johnston et al.[35] synthesised and tested on the same biological objects *cis*- and *trans*-aminocrotonic acids, which are relatively rigid structural analogues of GABA with folded and extended conformations respectively. The *cis*-isomer did not exhibit biological activity, while the *trans*-isomer, which simulates the extended GABA conformer, acted on the central nervous system of mammals just as effectively as GABA. X-Ray diffraction showed that the structure of *trans*-4-aminocrotonic hydrochloride exhibits a definite similarity to that of GABA hydrochloride[20].

The study of other sterically restricted analogues of GABA (see Table) showed[36] that only compounds which simulate the extended GABA conformation and in which the distance between N^+ and O^- differs insignificantly from the corresponding distance in GABA exhibit physiological

activity. Thus, in the series of aminocyclohexanecarboxylic acids, only *cis*-3-aminocyclohexanecarboxylic acid (but not the *trans*-isomer) exhibited a GABA-like activity [36]. The distance between the charges is identical with that in the extended GABA conformation only in the diequatorial conformer.

In the series of piperidinecarboxylic acids, nipecotinic acid (piperidine-3-carboxylic acid), which functions as a strong non-competitive inhibitor of the absorption of GABA by brain sections, is of interest [30]. It possesses conformational rigidity, which can be used in the study of "active" conformations of GABA. In aqueous solution and in the solid state nipecotinic acid exists in the "chair" conformation with the carboxy-group in the equatorial position [30]. The distance between N^+ and O^- in this form of the acid is 4.24–4.76 Å according to NMR and X-ray diffraction data [30].

The discovery of this new inhibitor of the absorption of GABA extended the range of GABA-like compounds. It was found that a series of betel nut (*Areca catechu* L.) alkaloids (arecoline, arecaidine, etc.) combined with nipecotinic acid enhanced the inhibiting effect of GABA on the bioelectrical activity of nerve cells [37].

Kier et al. [38] carried out theoretical calculations on hydrazineopropionic, imidazolylacetic, and 3-aminopropanesulphonic (homotaurine) acids and taurine (Fig. 2 and Table) in order to determine the preferred conformation and the structural elements of similarity with GABA. It has been shown recently [39-43] that these compounds can simulate the specific biological activity of GABA. The results of the calculations [36] demonstrated that the extended conformation is preferred for all the compounds in the zwitterionic form; with the exception of taurine, the extended conformation includes analogous groups, separated from the oxygen atom by an average distance of 5.8 ± 0.2 Å, which are believed [11] to be responsible for the pharmacological activity of GABA. In taurine, where the *gauche*-conformation is energetically more favourable, this distance is somewhat shorter (on average about 5.0 Å). X-Ray diffraction confirmed the existence of the extended conformation in crystalline homotaurine with an analogous N^+-O^- distance [44]. Experimental data for taurine [45] also agree with theoretical calculations [38].

Measurement of the dipole moments of α-, β-, γ-, and ω-aminoacids (up to C_{11}) showed [46,47] that in solution these acids assume mainly the fully extended conformation. Nevertheless, quantum-chemical calculations predict [9,10] that this is not the only possible conformation and there is an extremely high probability that the pharmacological activity is due to the existence of several conformations [48,49]. Indeed, it has been found that in aqueous solutions at pH 7.0 GABA consists of a mixture of both conformers [36,50]. However, it has been suggested [50] that the physiological activity of GABA is associated with the fully extended conformation of its molecule.

Interesting results were obtained [51] in the calculation of the preferred conformation of β-hydroxy-γ-aminobutyric acid (BHGABA), which is an agonist of GABA. [52] Two energetically equally favourable BHGABA conformations have been determined by the extended Hückel method. The first is characterised by an angle of 180° (along the $C_\alpha - C_\beta$ bond), free rotation of the carboxy-group, and a distance of 5.0–6.0 Å between the 'onium group and the oxygen atoms of the carboxy-group. This transoid conformation is consistent with the extended GABA structure predicted theoretically in an earlier investigated [11] and with experimental crystallographic studies [20,21]. An angle of 300° ($C_\alpha - C_\beta$ bond) with a close proximity between hydroxy- and carboxy-groups (apparently by virtue of hydrogen bonding) and with a fixed position of the carboxy-group (an $O=C=C_\alpha$ angle of 120°) is characteristic of the second conformation of BHGABA (the *gauche*-conformation), which corresponds to the folded form of the molecule in crystalline GABA. [19]

Steward and Clarke [53] attempted to establish the relation between the structures of twelve GABA agonists [26,33,53-56] and their activities. Using classical calculations of the potential energy, they determined the probability of the existence of a conformer with minimum energy. Their results are "qualitatively analogous" to data obtained in an NMR study [17] on γ-aminobutyric acid and a number of its agonists. The internuclear distances in various conformational states of the agonists were calculated with the aid of molecular models. The authors [53] took into account the fact that, according to quantum-chemical calculations [57], the charges on the zwitterions are apparently mainly concentrated on the hydrogen atom of the protonated basic group and on the oxygen atoms of the acid groups. On the basis of their calculations, Steward and Clarke [53] concluded that the greatest activity is shown by agonists [22,32,57,58] in which the distance between the zwitterionic charges is approximately 5.4 ± 0.4 Å (see Table). These data support the view that the biological activity of the GABA molecule is associated with its extended conformer.

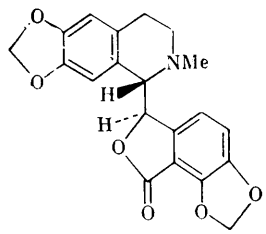


Figure 3. The structural formula of the alkaloid bicuculline.

3. The Conformation of the Alkaloid Bicuculline—a GABA Antagonist

A considerable amount of attention has been devoted recently to the study of the structural characteristics of GABA inhibitors in order to determine the similarity of the agonists and to identify the pharmacophoric group in GABA. The structure of bicuculline—a plant alkaloid of the phthalide–isoquinoline series isolated from *Coridalis species* (Fig. 3), is of greatest interest. Curtis et al. [31] were the first to show that, among the series of alkaloids with convulsant activity which they tested, containing the isoquinoline structure and potentially capable of combining with GABA receptors, bicuculline is the most specific antagonist of the depressant activity of GABA; the antagonism is probably due to the competition for the same receptors on the postsynaptic membrane. The discovery of a selective GABA antagonist stimulated theoretical and experimental studies designed to determine the structural analogy between GABA and its receptor. Attempts were made [19,35] to account for the antagonistic properties of bicuculline in relation to GABA by their stereochemical correspondence, i.e. by the fact that part of the alkaloid

molecule must be isosteric with the biologically active conformation of GABA and certain conformationally constrained GABA analogues [35,60]. This enables the bicuculline molecules to compete with GABA for a proportion of its receptors. Examination of the molecular models of the two compounds showed that the extended (i.e. fully transoid) GABA molecule, containing at its end the nitrogen atom and the carboxylate group O=C−O, can be exactly isosteric with the section of the bicuculline molecule with the nitrogen atom and the O=C−C group [31,35]. The structure of the partly folded GABA conformation is more isosteric with the section of the bicuculline molecule containing the nitrogen atom and the lactone group O=C−O.[19] Subsequent experimental and theoretical studies [61-63] confirmed the correspondence between the steric structures of these sections of the molecules of the two substances and demonstrated the similarity of the charge distribution among the atoms in these groups.

In all probability the early molecular models of bicuculline [19,34] were based on an incorrect configuration of the alkaloid [11] and merely represented its diastereoisomer with a pseudoaxial (and not pseudoequatorial) disposition of the CH_3 group at the nitrogen atom of the piperidine ring [62-64]. However, this should not be reflected in the determination of the isosteric groups of GABA and bicuculline [62]. The absolute configuration of crystalline bicuculline was determined by the analysis of the anomalous dispersion of X-rays [61,65]. Fig. 4*a* illustrates the absolute steric structure of the bicuculline molecule in the crystalline state [61]. The relative configurations of the asymmetric carbon atoms in the molecule are of greatest interest. Fig. 4*b* illustrates schematically the central part of the bicuculline molecule, which is usually compared with the GABA molecule. It has been established that in this conformation of crystalline bicuculline the $N_{(6)}$ and $O_{(3)}$ atoms are antiperiplanar (almost *trans*-) in relation to the $C_{(4)}-C_{(5)}$ bond. The fully extended form of GABA requires only a slight constraint in order to correspond to the bicuculline conformation in the crystalline state. The carboxy-group of GABA is isosteric with the group of $O_{(1)}$, $C_{(2)}$, and $C_{(26)}$ atoms of bicuculline. The conformation of bicuculline corresponding to the extended form of GABA is consistent with data obtained in a study [23] of the steric structure of one of the crystalline forms of the copper(II) complex of GABA.

However, bicuculline hydrochloride and methochloride, which are also biologically active [60], can have other conformations, different from the extended conformation of crystalline bicuculline. Fig. 4*a* shows that the introduction of a proton or a methyl group at the $N_{(6)}$ atom of the alkaloid causes an appreciable steric hindrance between $H_{(19)}$ and $N_{(6)}$, which are separated by a maximum distance of 2.86 Å in the free state. This can lead in its turn to the rotation of the aromatic groups about the $C_{(4)}-C_{(5)}$ bond and to a change in the conformation of the GABA-like fragment of the bicuculline molecule. EHM [51] and LMO [65] calculations as well as ^{1}H NMR data for the protonated form of the alkaloid and its methochloride in aqueous solutions [50,66] were used to determine the preferred conformation of bicuculline. Protonated bicuculline was found to have a preferred conformation with the proton in the *cis*-position relative to the lactone ring [50]. The lowest energy level was observed for the *cis*-position with a dihedral angle between the aromatic rings of approximately 90°. In this conformation the distance between the 'onium group and the carbonyl oxygen is 5.6 Å, which indicates the presence in bicuculline of the structural features and dimensions corresponding to the extended conformation of GABA. Andrews and Johnston's data [66] do not conflict with this hypothesis and show that rotation about the $C_{(4)}-C_{(5)}$ bond is possible (Fig. 4*a*) in the range from −70° to −110°† and that preferred rotational conformers should therefore exist. It was then noted that the free alkaloid in solution can have a conformation different to that of bicuculline salts. Within the limits of the angular rotation indicated, there is a possibility of the attainment of an isosteric correspondence between a fragment of the alkaloid, on the one hand, and the structure of GABA and a number of its agonists (in which there is some variation of the distances between N^+ and O^-), on the other. The N-containing ring of the protonated alkaloid has a preferred "half-chair" conformation with a pseudoaxial disposition of the phthalide ring.

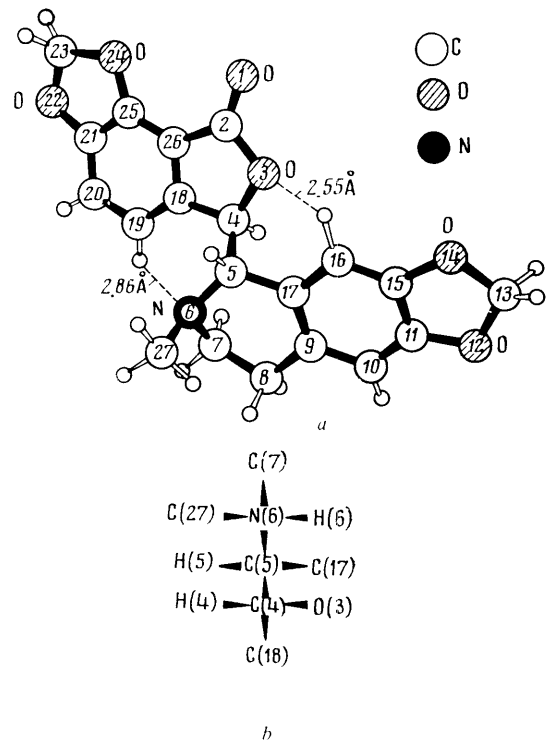


Figure 4. The configuration of bicuculline in the crystal: *a*) absolute configuration; *b*) relative configuration of the asymmetric atoms of the protonated molecule (the numbering of the atoms is in accordance with Gilardi's paper [61]).

The distribution of the charges on the atoms in three-dimensions has a definite importance for the determination of the preferred conformations of bicuculline, for distinguishing GABA agonists and antagonists, and for the elucidation of the mechanism of the interaction of GABA with its receptor. Fig. 5 illustrates the molecular skeletons of GABA (extended conformation) and protonated

† The torsional angle is positive if $N_{(6)}-C_{(5)}$ rotates clockwise about $C_{(4)}-C_{(5)}$.

bicuculiine with calculated atomic charges [67]. Calculations have shown [67] that the $O_{(10)}-C_{(11)}=O_{(12)}$ group (Fig. 5*a*) exhibits a greater similarity to the carboxylate group of the GABA zwitterion than does the $C_{(2')}-C_{(11)}=O_{(12)}$ group, the negatively charged atoms of the lactone group of bicuculline (Fig. 5*a*) having smaller charges than the atoms of the GABA carboxylate group (in its partly coiled conformer the charges change insignificantly).

The part of the bicuculline molecule isosteric with GABA contains two asymmetric centres, which corresponds to the possibility of the existence of four optical isomers. Since earlier studies [31,34] were performed on (+)-bicuculline only (Fig. 3), Collins and Hill [68] synthesised by the method of Teitel et al. [69] the methochloride of its (−)-isomer (Fig. 6*a*), which is rarely encountered in nature [70], and tested its biological activity. The results of the tests showed that only the (+)-form of bicuculline methochloride‡ exhibits an antagonistic effect on both the depressant properties of GABA and on its presynaptic inhibition. Furthermore, the (+)-isomer is a stronger convulsant than the (−)-isomer (approximately by a factor of 400). Evidently the conformation of the (+)-isomer is more isosteric with the preferred biologically active conformation of GABA than that of the (−)-isomer.

(+)-Bicuculline has the 1*S*,9*R*-configuration [its (−)-form has the 1*R*,9*S*-configuration [64,73]] and one can probably expect that only analogues with a similar configuration at the asymmetric centres will exhibit a physiological GABA inhibiting activity. The study of the structural and functional analogues of bicuculline will therefore help in the elucidation of the problem of the optical selectivity of this alkaloid and the mechanism of its antagonism. One of such compounds, which has the same configuration as (+)-bicuculline (1*S*, 9*R*), [68] is corlumine [74] (Fig. 6*b*), which, like the methyl ester of bicucine (Fig. 6*c*) is an effective GABA antagonist [60]. However, their close analogues bicucine [75] and laudanosine (Figs. 6*d* and 6*e*) do not exhibit a GABA-inhibiting activity [31,60]. It is therefore of interest to investigate the biological activity of bicuculline epimers and related compounds in order to determine whether the 1*S*,9*R*-configuration can serve as a criterion of antagonism in relation to GABA in this series of alkaloids [60,68].

It has recently been suggested [76] and then confirmed experimentally [77,78] that 2,6-dithia-1,3,5,7-tetrazaadamantane 2,2,6,6-tetraoxide or tetramethylenedisulphotetramine [79] (Fig. 6*f*) can serve as a GABA antagonist. This substance is a powerful convulsant and, like

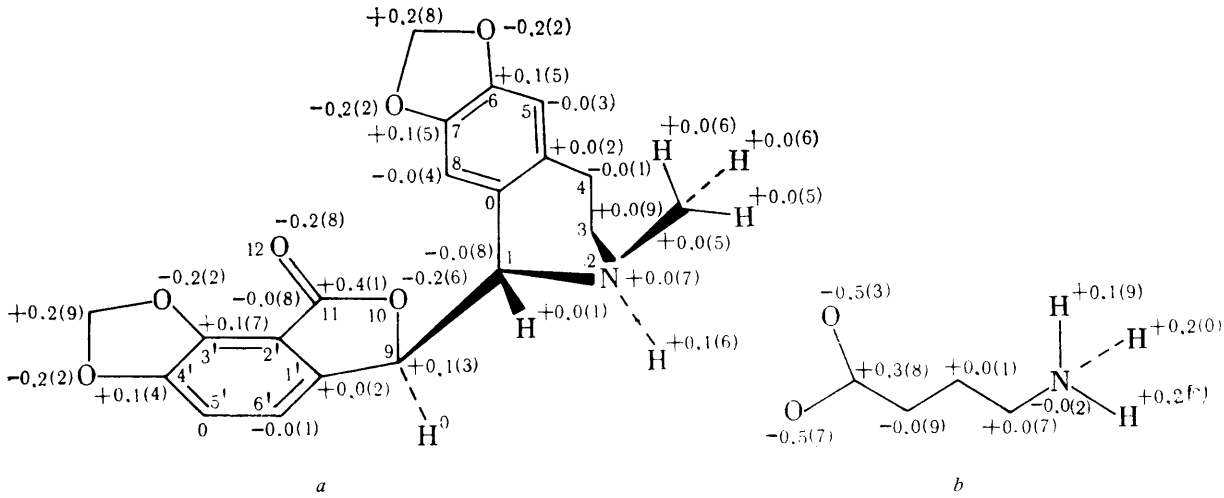


Figure 5. The distribution of charges in bicuculline and GABA: *a*) protonated bicuculline (in the conformation preferred for a solution of the alkaloid); *b*) GABA zwitterion (the numbering of the atoms is in accordance with the paper of Steward et al. [67]).

‡ One should note that the solubility of bicuculline hydrochloride at the "biological" pH is very low and this has led to certain contradictions in the determination of the efficiency of the action of the (+)-form of the alkaloid [17,72]; furthermore, it has been found recently [60] that the (+)-form of bicuculline methochloride is an effective GABA antagonist and is 100 times more soluble in water than the hydrochloride of the alkaloid.

bicuculline, inhibits the depolarising effect of GABA in the sympathetic ganglion of the rat. However, in contrast to bicuculline, the action of tetramethylenedisulphotetramine is non-competitive and probably non-specific. It may be that the nature of its antagonism is different to that of bicuculline[80]. It is noteworthy that pentamethylenemonosulphotetramine (Fig. 6*g*) a very close analogue of the new antagonist, does not exhibit a GABA-inhibiting activity[80]. The study of the nature of the antagonism of tetramethylenedisulphotetramine, which at first sight bears no structural analogy to GABA, is of special interest in connection with the possibility of a new approach to the explanation of the physiological activity of GABA and its analogues.

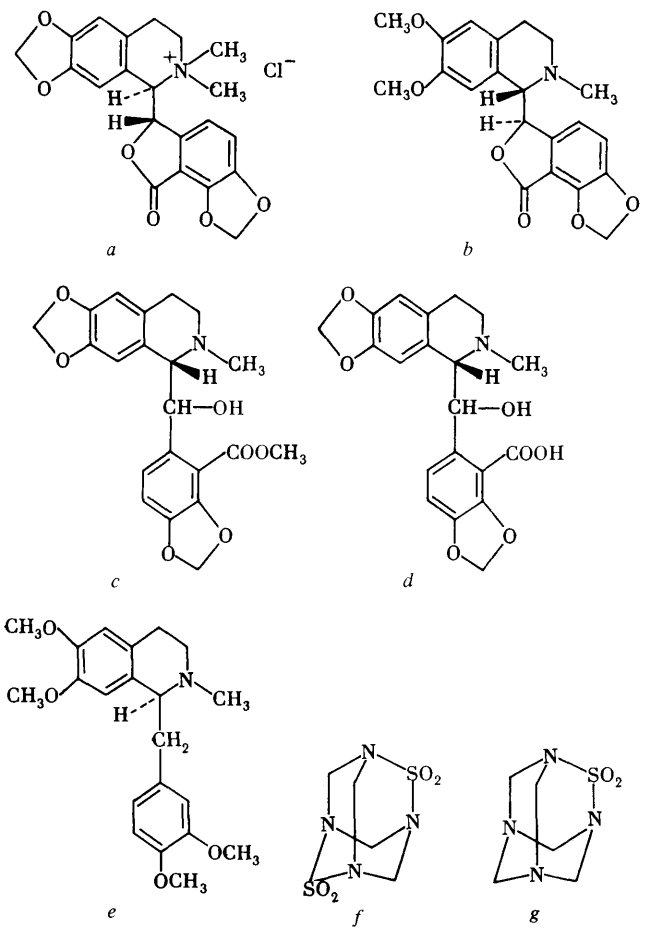


Figure 6. Structural formulae of certain GABA antagonists and their analogues: *a*) (−)-bicucullinemethochloride; *b*) corlumine; *c*) methyl ester of bicucine; *d*) bicucine; *e*) laudanosine; *f*) tetramethylenedisulphotetramine; *g*) pentamethylenemonosulphotetramine.

III. MODELLING OF THE INTERACTION OF γ-AMINOBUTYRIC ACID WITH ITS RECEPTOR

The depressant effect of GABA on nerve structures is achieved by the interaction with its receptor. Information about the chemical nature of GABA receptors is extremely fragmentary and to a large extent speculative. The study of the steric structures of many GABA agonists, which are structurally similar and have a qualitatively similar physiological activity, helps to elucidate in greater detail the structure of the receptors. According to modern ideas, these receptors consist of a protein material[81] in a lipid (hydrophobic) phase, which is located in presynaptic or postsynaptic membranes[82,83]. Receptors capable of capturing GABA can apparently differ from receptors associated with its physiological activity.

One of the probable mechanisms of the interaction of the receptor with the GABA molecule—an agent transmitting the nerve pulse—is based on the idea[49,84] that GABA can exist in the form of two conformers. The receptor initially "recognises" one of the GABA conformations in terms of molecular size, the presence of N^+ and O^- charges, their magnitude, and the distance between them, and attracts this conformation. This is followed by the stage of the interaction of the initial conformer with the receptor, which apparently proceeds with the fixation of a definite GABA conformation on the receptor. The ionised state of the GABA molecule, which varies as a function of the pH of the medium, is responsible for the specific sorption of its molecule on the outer side of the membrane, where sodium is the dominant cation. The sites where GABA is bound to sodium apparently constitute the receptor sections on the membrane where the adsorbed GABA exhibits its physiological effects. This bound form of GABA freely exchanges molecules with the GABA molecules present in solution.

The second conformer can participate in the formation of a GABA – receptor complex in the course of the change of membrane conductivity. The interaction of GABA in a definite "rigid" conformation with the receptor leads to a change in the orientation of the double layer of lipid and phospholipid molecules, an increase of the strength of the bond between GABA and the receptor, and the formation of pores in the membrane. The pore dimensions are determined by the distance between the two charged groups in the GABA molecule, whose position on the membrane is fixed by electrostatic forces and hydrogen bonds. Univalent potassium or chloride ions[85,86], which ensure the inhibition process in neurons, or hydrated sodium ions, responsible for the transmission of excitation[85], enter the cell or emerge from it via the membrane pores. The ability of GABA to exhibit both an inhibiting and an exciting influence on neurons has been observed earlier by Kerkut and Walker[87,88]. The selective change in the permeability of the membrane to various ions is apparently associated not only with conformational changes in the GABA molecule but also with changes in the geometry of the active sections of the receptor, which bears a definite analogy to stereospecific reactions.

The formation of hydrogen bonds ensures the rigidity of the GABA conformer structure within the membrane, which is particularly important in solution, where the GABA molecules can exist in several conformations. The distance between the two charged groups of the extended GABA conformer is between 5.4 and 6.3 Å and, when the conformer reacts with the receptor, it can open pores within the membrane whose size is about 5.4 Å (Fig. 7*a*),[84] which ensures the transport of hydrated sodium ions (whose size is 4.8–5.1 Å) and hence the excitation of the neuron and the transmission of the nerve pulse[89]. In the partly folded GABA conformation the pore size in the membrane diminishes to 4.4 Å (Fig. 7*b*),[84] which is sufficient for the passage of hydrated chloride ions only (their size is 3.32–3.86 Å),[90] the latter being responsible for the

inhibition of nerve signals. This model of the interaction of GABA with the receptor is consistent with all the theoretical and experimental facts obtained previously. Thus the difference between the distances separating the N^+ and O^- charges in a series of GABA agonists showed that the active section in the GABA receptors is somewhat elastic, which permits the variation of the distance between the charged reaction centres of the receptor within a certain range[53]. This idea is consistent with the flexible nature of membrane proteins.

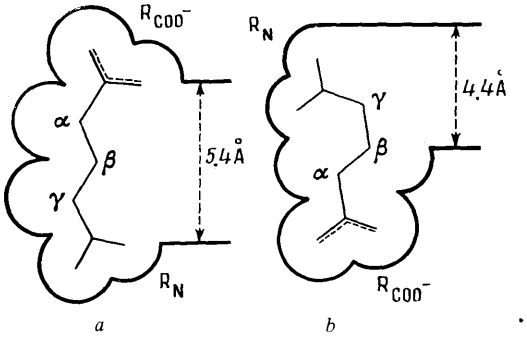


Figure 7. Schematic illustration of the interaction of the conformers of GABA with its receptor (R_{COO^-} and R_N are the active sections of the receptor interacting with a carboxylate and amino-groups of GABA and its analogues)[84]: *a*) extended conformer; *b*) folded conformer.

The inhibiting and exciting effect of GABA on two types of cells in the neurons of the snail *Helix aspersa*[87-89] can be accounted for by the flexibility and the ready change of the structural specificity of this molecule (the interconversion of the conformers) under the influence of external conditions and also by the possibility of structural changes of the receptors themselves in different types of cells[84]. A GABA molecule in the extended conformation interacts with one of the receptors (under these conditions, β-alanine in the two possible conformers of which the N^+-O^- distances are 3.4 and 4.7 Å,[53] does not exhibit a physiological activity, while muscimol is 10–100 times more active than GABA[84]), while the partly folded GABA conformer interacts with the other receptor (in this case β-alanine exhibits a GABA-like activity, while the effectiveness of muscimol does not differ from that of GABA[84]).

The antagonism of bicuculline and related compounds can be associated with the fact that, after the "recognition" by the receptor of the GABA-like element in the alkaloid structure, the latter cannot assume the second conformation[49,53]. This is apparently associated with the charge distribution in the remainder of the cumbersome three-dimensional bicuculline molecule[67]. Its competitive antagonism may depend on the smaller negative charges on the atoms of the lactone ring in bicuculline compared with the COO group in GABA, which leads to a weaker (hindered) binding of the bulky molecule of the alkaloid to the receptor; this weak binding cannot be induced by conformational changes corresponding to the terminal stages in the action of GABA. It has also been suggested[49] that the charges on the residue of the bicuculline molecule can interact with the environment of the active section of the receptor and can thus prevent a rapid liberation of the latter. Furthermore, there is a possibility of a large energy barrier in bicuculline to conversion into another conformer[49]. Bicuculline thus inhibits the activity of GABA, imitating its "initial" conformer and occupying the reaction centres of the receptor without manifesting at the same time a GABA-like physiological activity.

The absence of elements of structural similarity in another GABA antagonist (tetramethylenedisulphotetramine) suggests the possibility of the complete blocking of the receptor and the prevention of access to the latter by GABA molecules as a result of the electrostatic interaction of the charged groups of the receptor and the antagonist. This hypothesis is supported by the non-specificity of the action of the antagonist and the non-competitive antagonism.

Van Gelder[90] suggested a different mechanism for the interaction of GABA with the receptor. According to his model, the inhibition process is associated solely with the configuration at the γ-carbon atom in GABA, namely with the steric influence of the γ-hydrogen atom. When GABA interacts with the receptor, the γ-hydrogen atoms determine the pore dimensions in the membrane, which are 3.7–4.5 Å according to calculations[90], corresponding to the diameter of hydrated chloride ions. However, this theory cannot explain the activity of certain GABA antagonists and also the exciting activity of GABA.[88,89]

In conclusion, the following structural features of compounds with GABA-like activity should be noted.

(1) Free amino- and carboxy-(or sulphoxy-)groups are necessary for the manifestation of GABA activity, because derivatives with ester or amide groups have almost no activity[7]. The comparative ineffectiveness of branched or substituted GABA derivatives can apparently be accounted for by the fact that the substituents interfere with the orientation of GABA relative to the receptor groups.

(2) The optimum length of the atomic chain between the nitrogen of the basic group and the oxygen of the acid group corresponds to four atoms. Molecules with 2–3 or 5–6 atoms in this chain exhibit a low activity or are completely inactive.

(3) GABA receptors react with a molecule having at least two highly charged regions whose charges are of opposite sign to those of the centres of positive and negative charges on the amino- and carboxylate groups respectively.

(4) A GABA-like compound must exhibit a structural stereospecificity, i.e. must correspond to one of the GABA conformations. For activity to be shown, the charges on the cationic (N^+) and anionic (O^-) groups of the zwitterionic form must be at a distance of 5.0–6.0 Å (exciting activity) or 3.7–4.5 Å (inhibiting activity)[84,90].

(5) Not only the properties indicated above are important for the interaction with the receptor; in certain cases the "recognition" of the molecule by the receptor requires a specific optical configuration. In order to account for the chiral specificity of the receptor, further investigation of the physiological activity of alkaloids of the phthalide–isoquinoline series is required.

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The interaction of a biologically active molecule with a macromolecular receptor is possible by virtue of the influence of intermolecular forces and presupposes a definite intermolecular orientation, which apparently does

not rule out a change in the conformation of the molecule when it approaches the receptor, The final solution of the problem of the preferred conformation of medicinal drugs depends on direct experimental observations on the complexes of the active molecules with the receptor. The most promising methods for the determination of the "topography" of the active centres of the receptors and for the elucidation of the mechanism of the action of GABA and other medicinal preparations may be X-ray diffraction and nuclear magnetic resonance of GABA–receptor complexes in the biophase itself. Computer quantum-chemical analysis is of great assistance to chemists, biochemists, and pharmacologists in the solution of these problems. In addition, when account is taken of the influence of the surrounding medium and the receptor on the energetically favourable GABA conformation and of the conformational and electronic changes in the structure of the macromolecular drug–receptor complex for various GABA-like medicinal preparations, such analysis may be used in future to predict and design new effective drugs with a desirable range of action on the central nervous system of man.

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