(26%) of **63** sulfate; mp 78 °C; NMR (Me_2SO-d_6) 1.97 (d, 3H, J = 1.5 Hz), 3.68 (s, 2 H), 6.58 (br, 1 H), 7.2–7.4 (m, 5 H).

Pharmacology. Multicolored guinea pigs of both sexes (supplied by K. Meckel, Lauback, FRG), about 8 weeks old and weighing between 300 and 400 g, were used for the pharmacological investigations.

About 20 h before the start of the study, feed was withdrawn from the guinea pigs, the animals being fasted for the entire study period but having free access to drinking water. A typical experiment included six animals per dose group. The test substance was given by intraperitoneal injection of a neutral solution. A control group was treated in parallel with physiological NaCl solution ip. To determine the glucose concentrations, 10-μL blood samples were withdrawn by puncture of a peripheral ear vein immediately before the start of the study and thereafter once an hour up to the sixth hour after administration of the substance. The blood glucose determinations were performed by the hexokinase method on the hemolysate.[13] The therapeutic effect of each test substance was calculated as the percentage difference between the lowest blood glucose concentrations measured in each case, referred to the initial value.

Differences in the blood glucose concentrations were considered to be relevant at a significance level of $p \leq 0.05$ (U-test of Welcoxon for paired samples).

Registry No. 1, 70082-23-4; 2, 70081-97-9; 3, 70082-03-0; 4, 70082-18-7; 5, 70082-06-3; 6, 97294-10-5; 7, 97294-11-6; 8, 97294-12-7; 9, 97294-13-8; 10, 97294-14-9; 11, 70082-10-9; 12, 70081-99-1; 13, 70082-16-5; 14, 81866-87-7; 15, 81866-96-8; 16, 97294-15-0; 17, 70081-94-6; 18, 70082-20-1; 19, 97294-16-1; 20, 66700-68-3; 21, 69717-72-2; 22, 69717-74-4; 23, 69717-69-7; 24, 97294-17-2; 25, 69717-67-5; 26, 69717-70-0; 27, 69717-66-4; 28, 69717-65-3; 29, 69717-71-1; 30, 93689-83-9; 31, 97294-18-3; 32, 69717-75-5; 32 (free acid), 97314-99-3; 33, 97294-19-4; 34, 81866-93-5; 35, 97294-20-7; 36, 97294-21-8; 37, 69717-76-6; 38, 95791-38-1; 39, 97294-22-9; 40, 97294-23-0; 41, 97294-24-1; 42, 97294-25-2; 43, 97294-26-3; 44, 88333-74-8; 45, 97294-27-4; 46, 97294-28-5; 47, 97294-29-6; 47·HCl, 97294-46-7; 48, 97294-30-9; 48·HCl, 97294-48-9; 49, 97294-31-0; 50, 81866-74-2; 50·HCl, 97294-50-3; 51, 81866-76-4; 52, 97294-32-1; 53, 97294-33-2; 53·HCl, 97294-51-4; 54, 97294-34-3; 55, 69717-88-0; 56, 97294-35-4; 57, 97294-37-6; 57·HCl, 97294-53-6; 58, 97294-38-7; 59, 97294-39-8; 60, 97294-40-1; 61, 93049-07-1; 62, 97294-42-3; 62 (free base), 97294-41-2; 62·HCl, 97294-54-7; 63, 97294-44-5; 63a, 97294-57-0; 63a·H_2SO_4, 97294-58-1; 63·HCl, 97294-55-8; 64, 97294-45-6; (*X*,*Z*)-$CH_3CH(CH_3)CH{=}NNHCH_2C(CH_3){=}CHPh$, 97294-59-2; CH_3NHNH_2·HCl, 7339-53-9; $CH_3CH_2CH_2NHNH_2$·HCl, 56795-66-5; $CH_3(CH_2)_3NHNH_2$·HCl, 56795-65-4; $CH_3(CH_2)_4NHNH_2$·HCl, 1119-68-2; $CH_3(CH_2)_5NHNH_2$·HCl, 79201-41-5; $CH_3(CH_2)_4CH(CH_3)NHNH_2$·HCl, 97294-47-8; $CH_3(CH_2)_6NHNH_2$·HCl, 79201-42-6; $CH_3(CH_2)_7NHNH_2$·HCl, 97294-49-0; $PhCH_2NHNH_2$·HCl, 1073-62-7; $Ph(CH_2)_2NHNH_2$·HCl, 5470-36-0; *o*-$Me_6H_4(CH_2)_2NHNH_2$·HCl, 69717-86-8; *m*-$MeC_6H_4(CH_2)_2NHNH_2$·HCl, 97294-52-5; *p*-$MeC_6H_4(CH_2)_2NHNH_2$·HCl, 69717-83-5; *p*-$FC_6H_4(CH_2)_2NHNH_2$·HCl, 69717-87-9; *p*-$ClC_6H_4(CH_2)_2NHNH_2$·HCl, 69717-82-4; *p*-$MeOC_6H_4(CH_2)_2NHNH_2$·HCl, 69717-81-3; $PhCH_2CH(CH_3)NHNH_2$·HCl, 66-05-7; $PhCH(CH_3)CH_2NHNH_2$·HCl, 1743-28-8; $Ph(CH_2)_3NHNH_2$·HCl, 24214-86-6; $PhO(CH_2)_2NHNH_2$·HCl, 4230-21-1; $Ph(CH_2)_4NHNH_2$·HCl, 24215-06-3; $PhCH{=}CHCH_2NHNH_2$·HCl, 69717-89-1; pyruvic acid, 127-17-3; ethyl pyruvate, 617-35-6; (*E*)-(3-chloro-2-methyl-1-propenyl)benzene, 97294-56-9; hydrazine, 302-01-2; (*Z*)-(2-methyl-3-phenyl)acrylonitrile, 26157-51-7; hydroxylamine-*O*-sulfonic acid, 2950-43-8; isobutyraldehyde, 78-84-2.

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Synthesis and Activity of 5-(Aminomethylene)-1,3-cyclohexanediones: Enolic Analogues of γ-Aminobutyric Acid

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Eight 1,3-cyclohexanediones with an aminoalkyl side chain in the 5-position were synthesized as rigid enolic analogues of GABA (γ-aminobutyric acid). Biochemical investigations about their abilities to displace [^{3}H]GABA and [^{3}H]baclofen [β-(*p*-chlorophenyl)-γ-aminobutyric acid] in binding studies or to inhibit the high-affinity sodium-dependent GABA uptake showed that these compounds were generally devoid of affinity for the two GABA receptors and for the GABA carrier. Only compound 1 exhibited a weak affinity in the GABA-A binding experiments ($IC_{50} = 6.5 \times 10^{-5}$ M). Graphic computer modeling was applied in an attempt to explain this activity in comparison to some reference GABA agonists. Electrophysiological studies on dorsal root ganglia (DRG) also excluded agonistic or antagonistic properties on GABA-A or GABA-B receptor models but pointed out an atypical prolongation of Ca^{2+}-dependent action potential for compound 1.

Structure–activity relationships show that for designing GABA agonists both the amino and the carboxyl groups of GABA can be replaced by other basic or acidic isosteric subunits without significant loss of affinity or intrinsic activity at GABA receptors. Particularly, the carboxylic acid function of GABA can be replaced by various acidic groups covering a broad range of pK_a values. Examples include the sulfonic acid group in homotaurine (3-aminopropanesulfonic acid)[1] and 4-piperidinesulfonic acid,[2] a tetrazole group in "tetrazolyl-GABA" [5-(3-aminopropyl)tetrazole],[3] an iminol group in muscimol [5-(aminomethyl)-3-hydroxyisoxazole] or THIP (4,5,6,7-tetrahydroisoxazolo[5,4-*c*]pyridin-3-ol,[4] or an enol group in kojic

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[§] Institut de Physiologie.

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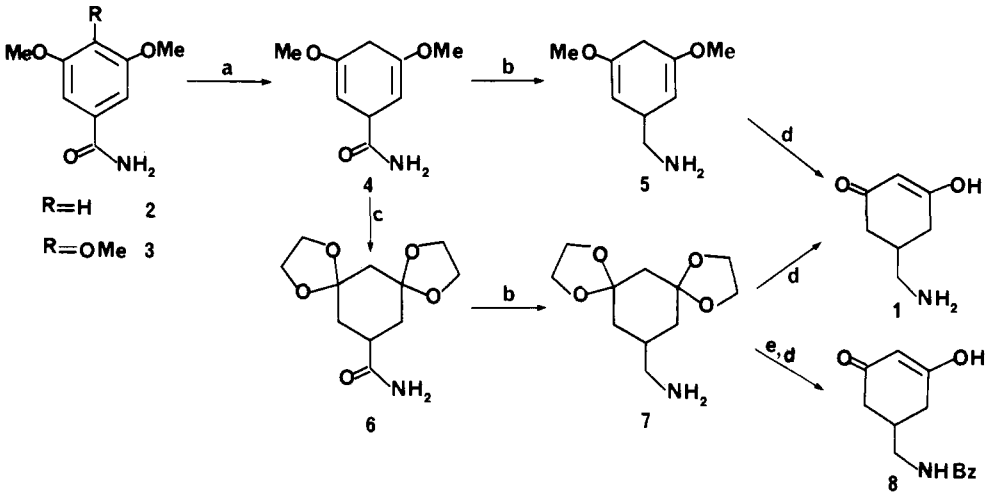
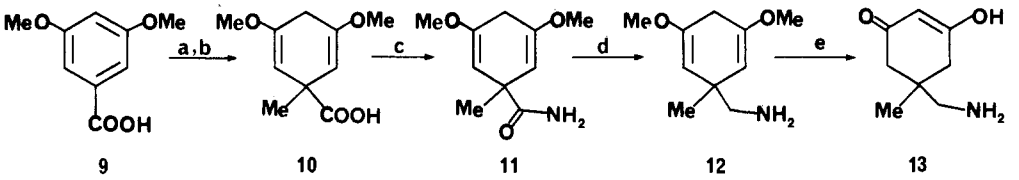
Scheme I[a]



[a] Reagents: (a) Na, EtOH, NH_3; (b) LAH; (c) ethylene glycol, H^+; (d) H^+, THF; (e) benzaldehyde, H_2Pt/C.

Scheme II[a]



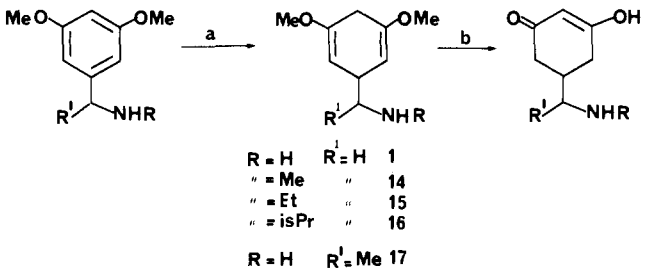
[a] Reagents: (a) Li, NH_3; (b) CH_3I; (c) ClCOOEt, NH_3; (d) LAH; (e) H^+, THF.

amine [2-(aminomethyl)-5-hydroxy-4*H*-pyran-4-one[5] (Figure 1). On the other hand, the partial or total incorporation of the GABA sequence in a rigid, planar framework seems to be a favorable factor, as illustrated by the high affinity of muscimol,[4] *trans*-amino-4-crotonic (TACA),[6] isoguvacine,[4] THIP,[4] and 4-aminocyclopent-1-en-1-carboxylic acid[7] to GABA binding sites. Accordingly, it seemed of interest to prepare 5-(aminomethyl)-1,3-cyclohexanedione (1) and some substituted analogues and to investigate their biological properties. The pK_a value of 1,3-cyclohexanedione is 5.3;[8] this value is close to that of an aliphatic carboxylic acid such as GABA whose pK_a is 3.6.[9] Moreover, compound 1 in aqueous solution would predominantly exist in the enolic form, which is structurally similar to GABA and which retains the folded conformation in which GABA is presumed to interact with its postsynaptic recognition sites[10,11] (Figure 1).

Chemistry

The most convenient route to the 5-substituted 1,3-cyclohexanediones (Table I) consists of the Birch reduction of the corresponding 1,3-diphenolic compounds, starting either from carboxamides (method A), carboxylic acids (method B), or aminomethylenes (method C).

Scheme III[a]



[a] Reagents: (a) Li, NH_3, *t*-BuOH; (b) H^+, THF.

Method A. As outlined in Scheme I either 3,5-dimethoxybenzamide (2) or 3,4,5-trimethoxybenzamide (3) gave rise to the same compound: 3-carbamoyl-1,5-dimethoxy-1,4-cyclohexadiene (4) when submitted to a Birch reduction under classical conditions.[12] A further reduction of the carboxamide 4 with lithium aluminum hydride (LAH) in tetrahydrofuran led to an approximate 1:1 mixture of the expected 3-(aminomethyl)-1,5-dimethoxy-1,4-cyclohexanediene (5) and of undesired 3,5-dimethoxybenzylamine, which was clearly characterized by GC. However on a preparative scale, the separation required a rather tedious fractional distillation. The hydrolysis of pure 5 was achieved in a mixture of concentrated hydrochloric acid and tetrahydrofuran and directly yielded 5-(aminomethyl)-1,3-cyclohexanedione (1) as the crystalline hydrochloride. Alternatively, the bis(enolic ether) 4 was first converted into the corresponding bis(dioxolane) 6; this protection prevents the return to aromaticity and allows an LAH reduction exclusively into the aminomethyl bis(dioxolane) 7 that, after hydrolysis under the same conditions as described above, yielded the desired cyclo-

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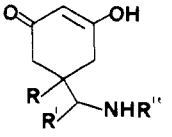
Table I. Analytical Data for the (Aminomethylene)-1,3-cyclohexanediones and Related Compounds



compd	R	R'	R''	method	formula[a]	yield,[b] %	mp,[c] °C
1	H	H	H	A, C	$C_7H_{11}NO_2 \cdot HCl$[d]	14, 63	181
8	H	H	Bzl	C[f]	$C_{14}H_{17}NO_2 \cdot HCl$	23	166
13	CH_3	H	H	B	$C_8H_{13}NO_2 \cdot HCl$	51	234
14	H	H	CH_3	C	$C_8H_{13}NO_2 \cdot HCl$	57	195
15	H	H	C_2H_5	C	$C_9H_{15}NO_2 \cdot HCl$	45	179
16	H	H	*i*-Pr	C	$C_{10}H_{17}NO_2 \cdot HCl$	46	187
17	H	CH_3	H	C	$C_8H_{13}NO_2 \cdot HCl$	47	*e*
18		5-$CH_2CH_2NH_2$		C	$C_8H_{13}NO_2 \cdot HCl$[d]	43	138

[a] The compounds were pure directly (TLC) without recrystallization. [b] Overall yield for the two last steps. [c] All the compounds decompose. [d] Crystallizes with 1 mol of water. [e] Highly hygroscopic compound. [f] See Experimental Section.

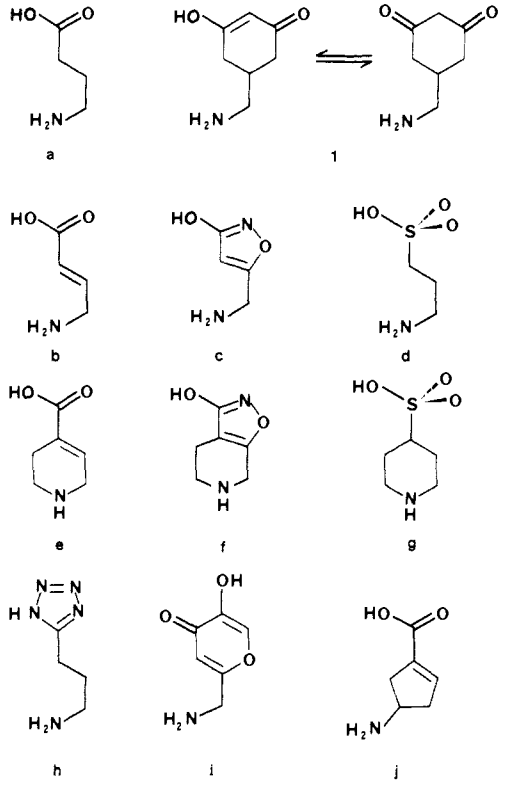


Figure 1. (a) GABA in the "active" conformation; (b) TACA; (c) muscimol; (d) homotaurine; (e) isoguvacine; (f) THIP; (g) 4-piperidinesulfonic acid; (h) tetrazolyl-GABA; (i) kojic amine; (j) 4-aminocyclopent-1-ene-carboxylic acid.

hexanedione 1. Compound **7** was also used in the synthesis of the *N*-benzyl derivative **8** by means of reductive amination and subsequent hydrolysis.

Method B. 5-Methyl-5-(aminomethyl)-1,3-cyclohexanedione (**13**) was prepared as follows: the carbanion of 3,5-dimethoxybenzoic acid (**9**) (generated from a lithium–ammonia mixture, see ref 13) was reacted with an excess of methyl iodide. The obtained α-methyl carboxylic acid **10** was converted in its primary amide **11** by the mixed-anhydride method. The amide was then reduced with LAH to the corresponding bis(enolic ether) **12** that was hydrolyzed into **13** (Scheme II).

Method C. This method utilized a direct reduction of the appropriate 3,5-dimethoxybenzylamines by a modified Birch reduction recently described by Olson et al.[14] that uses *tert*-butyl alcohol as proton source. This procedure allows the formation of radical anions even from deactivated molecules.[15] Starting from 3,5-dimethoxybenzylamine, the dihydro derivative **5** was obtained in 95% purity (GC) and was easily hydrolyzed to **1** under acidic conditions. The same method was used for the preparation of the N-alkylated derivatives **14–16**, the α-methyl derivative **17**, and the aminoethyl homologue **18** (Scheme III).

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Table II. Binding Results for Compound 1

compd[a]	IC_{50},[b] μM [^{3}H]GABA binding	[^{3}H]baclofen binding	uptake inhibn
1	65	>100	>100
GABA	0.03	0.06	1.8
ipecotic acid	>100	>100	5.6

[a] For the other compounds no significant affinity was observed with concentrations up to 10^{-4} μM. [b] Results are means of two experiments done in triplicate.

Biological Results

(a) Biochemical Studies. All the compounds were tested for their ability to displace [^{3}H]GABA from rat brain membranes prepared according to the method described by Enna and Snyder.[1] A possible interaction with the GABA-B receptor was also examined on rat brain membranes with [^{3}H]baclofen as described by Hill and Bowery.[20] Finally a possible inhibition of the high-affinity sodium-dependent GABA uptake was studied.[32] As illustrated in Table II, only compound **1** shows some affinity in GABA-binding assays, but its potency is 2200 times less than that of GABA.

(b) Electrophysiological Studies. Primary afferent sensory neurons were used to test the 5-(aminoalkyl)-cyclohexane-1,3-diones with regard to the different electrical phenomena that follow activation of either GABA-A or GABA-B receptors. Cells in groups Aβ and Aδ were used as they show the highest sensitivity to the GABA agonists that act at the GABA-A site and produce depolarization due to changes in Cl^- conductance.[16,17] Group C neurons and those Aα neurones that display action po-

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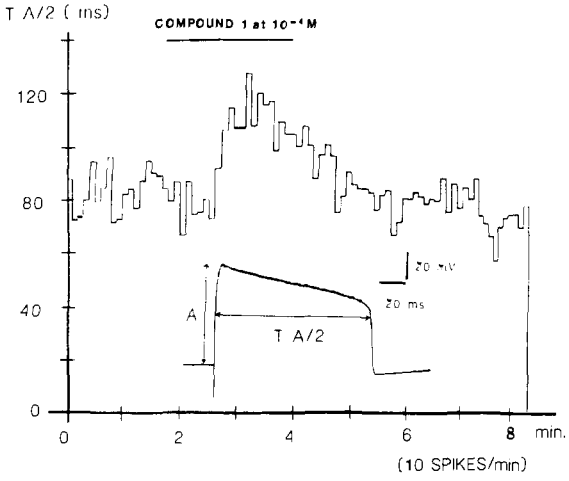

Figure 2. Prolongation of the Ca^{2+} component of action potentials by compound 1 recorded in a slow Aδ spinal ganglion neuron (Ca^{2+} content of the superfusing medium 5 mmol). Inset examplifies spike shape resulting from a 10-min stimulation rate. Graphs shows reversible effect of compound 1 on the time to half-decay of each spike: *TA/2*, delay of spike repolarization to half it maximum amplitude; *A*, membrane potential remained stable at −55 mV between spiking sequences and while superfusing 100 μM of compound 1. See details of the relationships between membrane potential and spike duration in ref 19.

tentials with a plateau phase in the presence of K^+ channel blockers (Figure 2 inset; details in ref 18 and 19) were used to test drug selectivity toward both GABA-A and GABA-B receptors. The latter receptors were activated by (−)-baclofen.[20] GABA-B receptor activation reduced spike duration as a function of Ca^{2+} entry in cells and slow conducting Aδ cells (<15 m/s) while in other Aδ cells (>15 m/s) it caused a blockade of accommodation when the cells were repeatedly depolarized.[21] Neither the desensitization of GABA-A receptors with isoguvacine (*c* 10^{-5} M) nor their blockade by bicuculline given with GABA in equimolar amounts (*c* 10^{-6} M) had any apparent effect on the typical GABA and baclofen responses seen on Ca^{2+} spikes. according to these tests, which permit the electrophysiological identification of both types of GABA receptors, we found that all compounds (Table I) were devoid of either agonist or antagonist properties. However compound 1 showed some atypical properties: (a) High doses of compound 1 caused a 10% reduction of GABA-A-induced responses (9 out of 17 neurons). (b) Under voltage clamp, where bicuculline has no intrinsic effect, compound 1 provoked a slight outward current (possibly K^+ or closing of Cl^- channels). (c) An extensive analysis with C and Aδ neurons (GABA-B receptors) showed that spike duration was commonly increased rather than diminished (Figure 2 graph). This observation is similar to data reported for bicuculline.[19,22]

Graphic Computer Modeling and Discussion

The common structural requirements encountered in potent GABA-A-related compounds such as TACA, THIP muscimol, isoguvacine, and kojic amine led to the description of a transoid, partially folded conformation that brings the charged ends (i.e., NH^+_3, COO^-) at a distance of about 5 Å.[10,11] According to this active conformation, compound 1 was a likely candidate since it possesses these structural requirements (see Figure 3).

The structures of THIP, kojic amine, and dihydromuscimol[23] have been selected for comparison purposes since they present either the essential structural requirements or some similarities with compound 1. All compounds have been built and compared with the TRIGRAPH system and its SYBYL software package.[24] When available, crystal data were extracted from the Cambridge Data Files.[25] Standard fragments available on the system were further added to complete the molecules. In a next step, the geometry was optimized through a simple minimization procedure,[24] and when necessary, a systematic conformational search was achieved to locate the sterically allowed conformers. THIP, which by its conformationally restricted bicyclic structure constitutes the best guess about the biologically significant conformation, was then used to select the closest sterically allowed conformer of kojic amine, dihydromuscimol, and the title compound 1. A least-squares fit was achieved for each compound in order to line up the charged ends; this is illustrated in Figure 3.

Depending on the negative charge delocalization extension, there are two possible comparisons between compound 1 and the selected structures (Figure 4a,b). In both cases, compound 1 verifies the 5-Å distance but it occupies various exclusive van der Waals volumes with respect to THIP, kojic amine, and dihydromuscimol envelopes as shown in Figure 4, parts a and b. This particular steric occupation can be thought as hindering the interaction at the receptor.

In an attempt to explain the inactivity of isomuscimol,[26] it had been stated that a three-center delocalization process of the negative charge was a strict requirement for GABA activity. According to this, compound 1 which can extend the delocalization on the whole diketone moiety should in fact be inactive. To better evaluate the delocalization process, we determined the point charges (Gasteiger method, available in TRIGRAPH) for the molecules of interest (see Figure 3). All active molecules (THIP, kojic amine, TACA, and dihydromuscimol) share a negative region including the carboxyl or equivalent function; this negative area is eventually extended. The inactive compounds (isomuscimol and compound 1) present a positive gap splitting this area. A β-diketone derivative of THIP[27] was recently reported to be a very weak GABA agonist. This compound presents the same charge distribution as encountered for isomuscimol and compound 1. It must be noted however that its cis configuration brings it completely away from the model GABA-A conformation as illustrated in Figure 5. This is probably the essential cause of its inactivity. In conclusion, from the available structure–activity data, it remains unclear whether or not the inactivity of the title compound should rather be attributed to steric hindrance or electronic properties. Further work is now being done to decide whether or not it is possible to distinguish between these two possible causes of inactivity.

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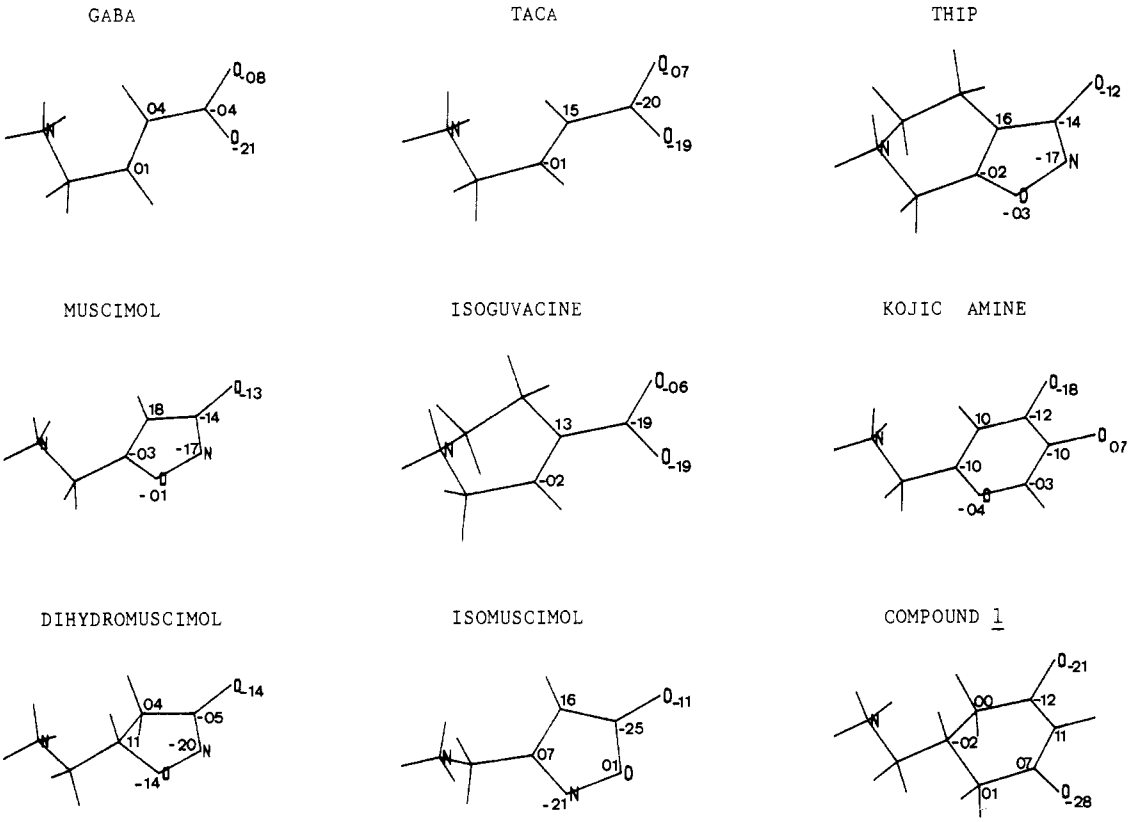

Figure 3. Molecular structures of the various compounds mentioned through this work. The point charges in election units ($\times 10^2$) determined by the Gasteiger method available in TRIGRAPH are presented for the atoms of interest.

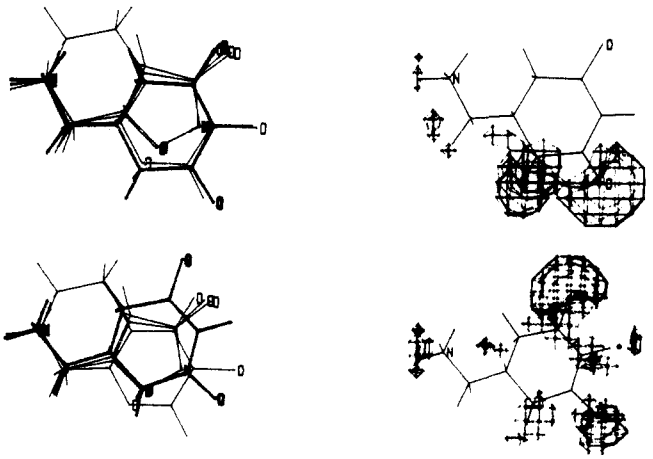

Figure 4. Comparison of compound 1 (thick lines): (a) considering a three-center delocalization and the supplementary van der Waals volumes particular to compound 1; (b) taking into account a complete delocalization on the diketone moiety and the extra volumes occupied by compound 1 with respect to this charge disposition.

The results in the GABA reuptake system are less surprising since the spatial disposition of the two electronic centers in the (aminomethyl)cyclohexane-1,3-dione compounds cannot adopt the same organization as the one observed in the reference compound of this field, namely nipecotic acid.[28] The inactivity in the [^{3}H]baclofen binding cannot be interpreted with precision: very little is known about the stereoelectronic requirements of the GABA-B receptor.

Concerning the electrophysiological data, the negative results on DRG cells are in accordance with the biochemical results insofar as the electrophysiological tests were designed to reveal agonism or antagonism at either GABA-A or GABA-B sites. However, the study was complicated by the fact that compound 1 showed consistently intrinsic activity in the tests used. The reduction of the GABA-A responses (reduction of the GABA-induced depolarization as well as the GABA-B response (increase of the Ca^{2+} spike duration) does not simply result from a bicuculline-like activity. First, the antagonist activity observed with compound 1 on GABA-A responses was slight, even at the highest dose used, and, second, the inward current (fast and carried by Cl^-) is apparently diminished by slight outward currents caused by compound 1 itself. Such currents combine to reduce the depolarizing response (GABA-A), and the flow of outward currents (possibly K^+ or delayed Cl^- movements) might be of interest in analyzing the process by which GABA-B receptors regulate Ca^{2+} spike duration. On the electrophysiological tests for GABA receptors, compound 1 has effects opposite to that of baclofen but the biochemical data are not consistent (no binding) with a classical antagonism so allosteric interactions between receptor and ionophore are raised.

The present data might fit with other data showing less typical GABA actions. For instance, there is evidence that GABA or muscimol can reversibly decrease, rather than increase, membrane conductance[29,30] and that the modulatory action of GABA on benzodiazepine binding yields slightly different structure–activity relationships than that observed directly on GABA receptors.[2,31] Also, recently,

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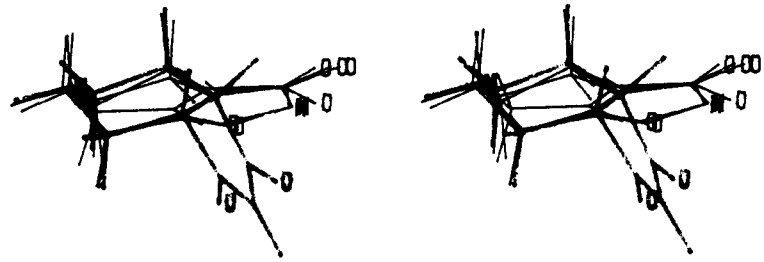

Figure 5. Stereoscopic views of the *cis*-β-diketone derivative recently reported[27] compared to the GABA structures used in the pharmacophore model description.

three distinct elementary ionic currents have been demonstrated at the crustacean neuromuscular junction.[29] Furthermore, in vertebrate sensory cells at the earliest stage of development the bicuculline-sensitive GABA-A receptor is coupled to a channel for cations (Na^+/K^+) and not to the familiar Cl^- ionophore.

Experimental Section

Melting points were obtained on a calibrated Kofler hot-stage apparatus and are uncorrected. IR spectra were measured with a Beckman Acculab-4 spectrophotometer and NMR recorded on a Perkin-Elmer R12 A spectrometer using Me_4Si as an internal reference. GC was effected with a Girdel E 300 gas chromatograph. All the compounds were analyzed for C, H, and N and gave results within ±0.4% of the theoretical values. Starting amides and benzylamines were prepared according to published procedures; only the 3,5-dimethoxy-α-methylbenzylamine was new; for its preparation, see below.

Method A. 3-Carbamoyl-1,5-dimethoxy-1,4-cyclohexanediene (4). This compound was prepared according to the procedure of Kuehne,[12] with some modifications. In a 1-L flask equipped with a mechanical stirrer, a dry ice condenser, a thermometer, and a gas inlet tube was condensed 600 mL of anhydrous ammonia. A solution of **2** (17 g, 80 mmol) in absolute alcohol (80 mL) was added and followed by the portionwise addition of sodium metal (9.2 g, 0.4 mol) under vigorous stirring. After disappearance of the blue color, ammonium chloride (35 g) was cautiously added; the ammonia was evaporated overnight. The remaining mixture was diluted with water (200 mL) and extracted with CH_2Cl_2; the organic layer was washed with brine and evaporated under reduced pressure, to leave a solid residue. Crystallization from C_6H_6–$CHCl_3$ (4:1) gave 10 g (68%) of **3**: mp 156 °C (lit.[12] mp 156 °C); NMR ($CDCl_3$) δ 2.90 (2 H, m), 3.65 (6 H, s), 3.80 (1 H, m), 4.82 (2 H, m), 5.90 (2 H, s br). The same procedure applied to **3** gave also **4** with a better yield (76%).

3-(Aminomethyl)-1,5-dimethoxy-1,4-cyclohexadiene (5). A slurry of LAH (5 g, 120 mmol) in dry THF (250 mL) was stirred in a 500-mL three-necked flask; **4** (10 g, 54 mmol) was added portionwise to maintain a gentle reflux that was continued for 3 h. The mixture was chilled, and the excess of LAH was destroyed cautiously with 25 mL of water. The inorganic material was filtered off and carefully washed with ether. After evaporation of the combined organic phases, the oily residue was distilled on short-path column, to yield 5 g of a colorless oil, bp 100–120 °C (bath temperature) (0.1 mmHg), containing **5** and 3,5-dimethoxybenzylamine (1:1). The mixture of amines was submitted to a fractional distillation (Fisher column): 1.7 g of pure compound **5** distilled at 130 °C (19 mmHg) (purity 95% by GC, 10% Carbowax, 2% KOH on Chromosorb); NMR ($CDCl_3$) δ 1.11 (2 H, s) 2.62–3.30 (3 H, m) 3.55 (6 H, s), 4.55 (2 H, m).

5-(Aminomethyl)-1,3-cyclohexanedione Hydrochloride (1). A solution of **5** (1.5 g, 9 mmol) in THF (100 mL) was stirred, and concentrated HCl (2.3 mL, 27 mmol) was added slowly. After one night, the white crystalline material was collected, washed with ether, and dried (0.1 mm over P_2O_5) for 2 days, to give 900 mg of **1** pure by TLC (MeOH–1% NH_4OH); mp 180–182 °C. For analytical data, see Table I.

The Bis(dioxolane) of 5-Carbamoyl-1,3-cyclohexanedione (6). A mixture of **4** (2.7 g, 15 mmol), ethylene glycol (2.4 g, 40 mmol), a few crystals of *p*-toluenesulfonic acid, and benzene (100 mL) were heated and stirred in a distillation flask so that about 50 mL of benzene distilled in 3 h. After cooling, the crystals were collected and recrystallized in $CHCl_3$, to give 2.4 g (66%) of **6**: mp 233–235 °C; IR (KBr) 3460, 3360, 1680 cm^{-1}; NMR ($CDCl_3$) δ 1.92 (6 H, m), 2.70 (1 H, m), 3.96 (8 H, m), 5.85 (2 H, br). Anal. ($C_{11}H_{17}NO_5$) C, H, N.

The Bis(dioxolane) of 5-(Aminomethyl)-1,3-cyclohexanedione (7). Compound **6** (1.2 g, 5 mmol) was added to a slurry of LAH (0.5 g, 12 mmol) in THF, and the mixture was refluxed for 3 h. After the usual hydrolysis (H_2O, 0.6 mL), filtration, and evaporation, high-vacuum distillation provided 750 mg of **7** (65%): bp 210 °C (bath) (0.1 mmHg); NMR ($CDCl_3$) δ 1.65 (2 H, s), 3.90 (8 H, m). Hydrolysis of 270 mg (12 mmol) of **7** with 0.4 mL of concentrated aqueous HCl in 50 mL of THF gave 105 mg of a white product identical with **1** obtained from **5** (IR, NMR, mp, TLC).

5-[(Benzylamino)methyl]-1,3-cyclohexanedione Hydrochloride (8). Compound **7** (1.1 g, 5 mmol) and benzaldehyde (500 mg, 5 mmol) in MeOH (100 mL) were shaken in a Parr bottle with 100 mg of 10% Pt/C at 50 psi for 12 h. After concentration, the residue was purified through a short chromatography column (Al_2O_3, CH_2Cl_2) and taken up in THF for acidic hydrolysis (1.5 mL, HCl concentrated, as above for **7**) to obtain **8** (for analytical data, see Table I).

Method B. 3-Carboxy-3-methyl-1,5-dimethoxy-1,4-cyclohexadiene (10). Ammonia (300 mL) was collected in a three-necked 1-L flask equipped with a dry ice condenser, an all-glass mechanical stirrer, and an addition funnel. Compound **9** (5.4 g, 30 mmol) was added, followed by lithium (0.6 g, 85 mmol), portionwise to maintain the blue color. The flask was cooled at −70 °C under argon, and 10 g of CH_3I was slowly injected over 15 min. Ammonia was evaporated overnight. The residue was dissolved in 200 mL of water, and the residual ammonia was eliminated by evaporation under reduced pressure. The solution was cooled to −5 °C, acidified at pH 5 with 4 N HCl, and repeatly extracted with Et_2O. The organic phase was washed with brine and concentrated; the residue gave after trituration with hexane 4.6 g of the unstable acid **10** (utilized as such in the next step): NMR ($CDCl_3$) δ 1.40 (3 H), 2.68 (2 H, m), 3.55 (6 H, s), 4.75 (2 H, m), 11.3 (1 H, m).

3-Carbamoyl-3-methyl-1,5-dimethoxy-1,4-cyclohexanediene (11). Compound **10** was dissolved in THF (150 mL); triethylamine (28 g, 28 mmol) was added followed by a portionwise addition of isobutyl chloroformate (3.4 g, 25 mmol) at −20 °C. After 1 h, $CHCl_3$ (25 mL) saturated with ammonia (4 g) was slowly added at 0 °C to the mixture. The next day the solvent was evaporated, and the residue taken up in CH_2Cl_2, washed with brine, dried on Na_2SO_4, and concentrated under reduced pressure (in vacuo) to give after recrystallization in benzene and addition of hexane 2.9 g of **11** (50%): mp 130–131 °C; NMR ($CDCl_3$) δ 1.35 (3 H, s), 6.0 (2 H, m). Anal. ($C_{10}H_{15}NO_3$) C, H, N.

3-(Aminomethyl)-3-methyl-1,5-dimethoxy-1,4-cyclohexanediene (12). To a slurry of LAH (0.8 g, 20 mmol) in Et_2O (80 mL) was added compound **11** (2 g, 10 mmol) portionwise; the mixture was stirred at room temperature for 6 h. The hydrolysis and the workup process were the same as described above for **10**. Kugelrohr distillation gave 1.35 g of **12** (73%): bp 150 °C (0.1 mm); purity 95% by GC; NMR ($CDCl_3$) δ 1.08 (5 H, m), 2.45 (2 H, s), 2.78 (2 H, m), 3.57 (6 H, s), 4.30 (2 H, m). The acid hydrolysis conditions for **12** yielding **13** were the same as for the preparation of **1** (for analytical data, see Table I).

Method C. 3-(Aminomethyl)-1,5-dimethoxy-1,4-cyclohexanediene (5). Ammonia (60 mL) was condensed in a 250-mL

three-necked flask equipped with a mechanical stirrer and a dry ice condenser. *tert*-Butyl alcohol (5 mL) and 3,5-dimethoxybenzylamine (1.67 g, 10 mmol) were added, followed by lithium metal (0.42 g, 60 mmol), which was introduced portionwise to the well-stirred solution. After 2 h, NH_4Cl (6 g) was added to quench the reaction. The ammonia was evaporated, and the residue was extracted with CH_2Cl_2–H_2O; the organic phase was washed with brine, dried, and concentrated to yield an oil that was distilled in the Kugelrohr apparatus (bp 120 °C, 0.1 mm) to give 1.2 g of **5** (78%), completely identical with the compound obtained by method A (NMR, GC).

3,5-Dimethoxy-α-methylbenzylamine Hydrochloride. A mixture of 3,5-dimethoxyacetophenone (5 g, 28 mmol) and hydroxylamine hydrochloride (5 g, 70 mmol) in pyridine (50 mL) was refluxed for 6 h and then poured in water. The precipitated crude oxime was filtered off, dried, and hydrogenated (Pd/C) at 55 psi in a mixture of MeOH (180 mL) and concentrated HCl (20 mL). After filtration and concentration, the residue was taken up in water, the solution was washed with ether and alkalinized with NaOH (20%), and the free base was repeatedly extracted with ether. The organic phase was dried and evaporated under reduced pressure. The oily residue was distilled in a Kugelrohr apparatus to give 2.62 g of the title compound: bp 130 °C (0.1 mm); yield 67%; NMR ($CDCl_3$) δ 1.35 (3 H, d, J = 5 Hz), 1.45 (2 H, s), 3.75 (6 H, s), 4.05 (1 H, q, J = 5 Hz), 6.45 (3 H, m); hydrochloride mp 192 °C. Anal. ($C_{10}H_{15}NO_2Cl$) C, H, N.

Biochemical Assays. [^{3}H]GABA Binding Assay. Compounds were investigated for their ability to displace [^{3}H]GABA from its postsynaptic receptor site (GABA-A). Experiments were performed using classical Triton-treated membranes prepared according to Enna and Snyder.[1] Binding assays were carried out at 4 °C for 5 min. The reaction mixture in a final volume of 2 mL contained 0.2 mL of synaptic membrane suspension (about 1 mg of protein), 0.2 mL of [^{3}H]GABA [aminobutyric acid, γ-[2,3-^{3}H(N)], 25–40 Ci/mmol, New England Nuclear, Boston, MA] in a final concentration of 2.9 nM, 0.2 mL of unlabeled drug, and 1.4 mL of 50 mmol Tris/citrate buffer, pH 7.1. At the end of the incubation the mixture was quickly filtered through Whatman GF/C filters. The filters were washed with 10 mL of ice-cold 50 mM Tris/citrate buffer, pH 7.1, and bound radioactivity was evaluated by scintillation counting.

[^{3}H]Baclofen Binding Assay. Affinity of compounds for the GABA-B site was estimated by the [^{3}H]baclofen binding assay [baclofen, DL-[butyl-4-^{3}H(N)], 30–50 Ci/mmol, New England Nuclear, Boston, MA]. Experiments were performed according to Hill and Bowery[20] with minor modifications (final concentration of [^{3}H]baclofen was 26.7 nM instead of 20 nM and incubation time was 30 min instead of 10 min).

[^{3}H]GABA Synaptosomal Uptake. Experiments were slightly modified from Ramsay et al.[32] Rats were killed by decapitation, and corpus striatum were rapidly dissected. Tissues were pooled and homogenized in 20 volumes of 0.32 M sucrose on a Potter Elvehjem tissue grinder. Homogenates was centrifuged at 1000*g* at 4 °C for 15 min. Supernatant was centrifuged at 20000*g* for 15 min at 4 °C, and the resulting pellet was resuspended in cold 0.32 M sucrose.

Incubation was carried out at 37 °C for 2 min in glass tubes containing 50 μL of synaptosomes (1 mg of protein), 750 μL of pH 7.4 Krebs Ringer phosphate buffer supplemented with NaCl (0.15 M), 100 μL of [^{3}H]GABA [aminobutyric acid, γ-[2,3-^{3}H(N)], 25–40 Ci/mmol, New England Nuclear, Boston, MA] in a final concentration of 1.1 μmol, and 100 μL of compounds to be tested. Blanks were treated identically except that NaCl was not added in the incubation medium. Uptake was terminated by dilution with 5 mL of incubation medium without NaCl. Samples were centrifuged at 20000*g* at 4 °C for 15 min, and radioactivity was evaluated in pellets after dissolution in 1 mL of Protosol (New England Nuclear).

GABA-mimetic Activity and Antagonism in Spinal Ganglia Neurons. Compounds were administered by transient superfusion of rat dorsal root ganglia in vitro (200-μL chamber) as described previously.[16,17] Single-barrel intracellular electrodes were used to record the membrane protential and to pass constant-current transmembrane pulses, by means of which the membrane conductance could be estimated. Ionic currents (Cl^- and Ca^{2+}) were measured by a single-electrode switch clamp technique.[17,21] To calculate drug concentration in the superfusing ringer (pH 7.4, 37 °C, 5–20 μL s^{-1}), membrane depolarization caused by calibrated injections of K^+ was measured;[16] in some instances a direct measurement of transient increases of extracellular K^+ activity was sensed by ion-selective micropipettes. Under these experimental conditions, threshold responses (1 mV) were obtained with concentrations of 5×10^{-6} to 5×10^{-5} M of GABA. The maximum response (up to 25 mV) was generally obtained at concentrations up to 10^{-4} M (with either GABA, isoguvacine, muscimol, ...). For the study of GABA-B receptors, spikes were generated by stimulating either cell bodies (50 μs, 1–5 nA) and/or the slow conduction fibers (groupes Aδ/C). The Ca^{2+} component of the action potentials was then revealed by blocking most of the repolarizing currents carried by K^+ as shown previously (intracellular injection of cesium and 7 mM tetraethylammonium outside).[19]

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Registry No. 1, 97294-64-9; 1 (base), 97294-86-5; **2**, 17213-58-0; **3**, 3086-62-2; **4**, 54118-32-0; **5**, 97294-65-0; **5** (*N*-methyl derivative), 97294-82-1; **5** (*N*-ethyl derivative), 97294-83-2; **5** (*N*-isopropyl derivative), 97294-84-3; **5** (α-methyl derivative), 97294-85-4; **6**, 97294-66-1; **7**, 97294-67-2; **8**, 97294-68-3; **9**, 1132-21-4; **10**, 64286-79-9; **11**, 97294-69-4; **12**, 97294-70-7; **13**, 97294-71-8; **14**, 97294-72-9; **15**, 97294-73-0; **16**, 97294-74-1; **17**, 97294-75-2; **18**, 97294-76-3; GABA, 56-12-2; 3,5-$(MeO)_2C_6H_3CH_2NH_2$, 34967-24-3; C_6H_5CHO, 100-52-7; 3,5-$(MeO)_2C_6H_3COCH_3$, 39151-19-4; 3,5-$(MeO)_2C_6H_3C(=NOH)CH_3$, 97294-77-4; 3,5-$(MeO)_2C_6H_3CH(NH_2)CH_3$, 97294-78-5; 3,5-$(MeO)_2C_6H_3CH(NH_2)CH_3$·HCl, 97294-79-6; 3,5-$(MeO)_2C_6H_3CH_2NHMe$, 77775-71-4; 3,5-$(MeO)_2C_6H_3CH_2NHEt$, 97294-80-9; 3,5-$(MeO)_2C_6H_3CH_2NHPr$-*i*, 97294-81-0; 3,5-$(MeO)_2C_6H_3(CH_2)_2NH_2$, 3213-28-3; 3-(2-aminoethyl)-1,5-dimethoxy-1,4-cyclohexadiene, 61273-81-2.

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