adduct (retention time 1.8 min) were collected, combined, and extracted with methylene chloride (2 × 40 mL). The combined extracts were dried over Na_2SO_4, and the solvent was removed at 5 °C under vacuum to yield crude **6**: 240-MHz ^1H NMR ($CDCl_3$) δ 2.50 (s, 3 H, NCH_3), 4.0 (m, 1 H, C2), 6.1 (m, 1 H, C5), 7.3–7.5 (m, 5 H, Ar H). The remaining assignments could not be made due to the presence of interfering signals from sample impurities.

2-Cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (6) from Reaction of NaCN with 2,3-MPDP$^+$ClO$_4^-$. The perchlorate salt of 2,3-MPDP$^+$ (200 mg, 0.74 mmol) and NaCN (180 mg, 3.7 mmol) were dissolved in 450 mL of 0.2 M phosphate buffer (pH 7.4). This solution was swirled in a water bath at 37 °C for 12 h and then was extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were dried over Na_2SO_4, filtered, and concentrated to give a yellow solid. Recrystallization from hot heptane yielded 112 mg (0.56 mmol, 76%) of pure, crystalline product: mp 88 °C; 240-MHz ^1H NMR ($CDCl_3$) δ 2.50 (s, 3 H, NCH_3), 2.74 (br d, 1 H, $C3_{eq}$), 3.0–3.1 (m, 2 H, $C3_{ax}$ and $C6_{eq}$), 3.45 (d of m, 1 H, $C6_{ax}$), 4.0 (m, 1 H, C2), 6.1 (m, 1 H, C5), 7.3–7.5 (m, 5 H, Ar H); CIMS *m/e* 199 $(MH)^+$. Anal. ($C_{13}H_{14}N_2$) C, H, N. Treatment of this product (50 mg, 0.25 mmol) in ethanol (2 mL) with 70% perchloric acid (20 μL) for 5 days at 5 °C led to the formation of crystalline 2,3-MPDP$^+$ClO$_4^-$ (47 mg, 0.17 mmol, 69%): mp 120–122 °C (lit.[13] mp 122.5 °C).

Rearrangement of 6-Cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (5) to 2-Cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (6). A suspension of the 6-cyano isomer **5** (20.7 mg, 0.11 mmoles) in 20 mL of 0.1 M KH_2PO_4 buffer (pH 7.4) was sonicated to facilitate dissolution and then incubated at 37 °C with constant swirling for 18 h. The reaction mixture was extracted with CH_2Cl_2 (3 × 10 mL), and the combined extracts were dried over Na_2SO_4 and evaporated to dryness to yield 17 mg (82% recovery) of the crude 2-cyano isomer which was identified by its ^1H NMR spectrum.

Timed Studies on the Metabolism of MPTP by Brain Mitochondrial Preparations. Timed aliquots (15 mL) were removed from an incubation mixture (45 mL) prepared from brain mitochondrial fraction (5 mg of protein/mL), 0.5 mM NaCN, and 0.2 mM MPTP in pH 7.4 potassium phosphate buffer at 10, 20, and 30 min. The percent metabolism was estimated by analytical HPLC on a 100-μL sample following the procedure described previously.[13] The remaining portion of each aliquot was cooled on ice and then was centrifuged for 5 min at 20000*g* to sediment the protein. Each of the resulting supernatant fractions was extracted with CH_2Cl_2 (2 × 15 mL), and the resulting extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure. The ^1H NMR data are summarized in Table I.

Disproportionation of 2,3-MPDP$^+$ClO$_4^-$ in D_2O Buffer. A solution of 2,3-MPDP$^+$ClO$_4^-$ (20 mg, 0.074 mmol) in 10 mL of 0.1 M phosphate buffer prepared in D_2O (pD 7.4) was incubated at 37 °C with gentle swirling. After 20 h, the clear yellow solution was basified to pH 8 with a 5% K_2CO_3 and then was extracted with ether (3 × 10 mL) and then with CH_2Cl_2 (3 × 10 mL). The ether extracts were combined, dried over Na_2SO_4, and concentrated to yield MPTP. Proton NMR and CI mass spectral analysis established that no deuterium had been incorporated into the product. The CH_2Cl_2 extracts were combined, dried over Na_2SO_4, and concentrated under reduced pressure to yield the 1-methyl-4-phenylpyridinium species in which one of the β-pyridinium ring protons was substituted with a deuterium atom: 240-MHz ^1H NMR (CD_3CN) δ 8.61 (br s, 2 H, pyridinium α-H), 8.21 (d, 1 H, pyridinium β-H); CIMS *m/e* 157 $(MH - CH_3)^+$.

Deuterium Incorporation Studies. Solutions (80 mL) prepared in D_2O and containing 0.7 mM 2,3-MPDP$^+$ClO$_4^-$, 4.3 mM or 1.8 mM NaCN, and 0.2 M phosphate buffer (pD 7.4) were swirled in a 37 °C water bath, and aliquots (15 mL) were removed after 35 min and 1, 2, and 5 h. The aliquots were extracted (15 mL CH_2Cl_2) and the extracts dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Each residue was analyzed by 240-MHz ^1H NMR for deuterium content (see Table I). In a separate experiment, the double-bond rearrangement of 6-cyano-1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine to the corresponding 2-cyano isomer (as described above) was carried out in D_2O. The following 240-MHz ^1H NMR spectrum ($CDCl_3$) was obtained for the resulting 2-cyano product: δ 2.5 (s, 3 H, NCH_3), 2.9 (m, 0.2 H, $C3_{eq}$), 3.1 (d, 0.2 H, $C3_{ax}$) 3.15 (d, J = 17.7 Hz, 1 H, $C6_{eq}$), 3.48 (dd, J_{gem} = 17.7 Hz, J_{vic} = 1.9 Hz, 1 H, $C6_{ax}$), 4.01 (s, 0.37 H, C2), 6.12 (m, 1 H, C5), 7.3–7.5 (m, 5 H, Ar H).

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Registry No. 1, 28289-54-5; 2^+, 94613-45-3; $2^+ \cdot ClO_4^-$, 97467-07-7; 3^+, 48134-75-4; **5**, 76113-47-8; **5**-d_1, 97467-08-8; **6**, 97187-62-7; **6**-d_2, 97467-09-9; **6**-d_3, 97467-10-2; MAO, 9001-66-5.

Synthesis and Hypoglycemic Activity of N-Alkylated Hydrazonopropionic Acids

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A series of N-alkylated 2-hydrazonopropionic acids have been synthesized and evaluated for their hypoglycemic activity. Most of the compounds exhibit a remarkable blood glucose lowering activity in fasted guinea pigs. Some of the structural variables studied were the effects of branching, unsaturation, or substitution on the alkyl side chain and the effect of nuclear substitution on the aralkyl analogues. From these compounds, 2-[[(*E*)-2-methyl-3-phenyl-2-propenyl]hydrazono]propionic acid (BM 42.304; **42**) was selected for further investigation.

Haeckel and Oellerich recently established in the course of investigations into the effects of hydrazines in perfused guinea pig liver that the addition of these substances to the perfused solution inhibits gluconeogenesis. The hypoglycemic effect of monoamine oxidase inhibitors with a hydrazine structure has already been known for some time, but the observation that the gluconeogenesis-inhibiting effect is due not to the hydrazine itself but to its condensation product with pyruvate, formed in vivo, was new.[1]

Further work on synthetically derived condensation products of this kind in the whole animal showed that the lowering of the blood sugar level was much more pronounced after these products than after the hydrazines. In addition, the inhibitory action on monoamine oxidase was substantially attenuated in the case of the condensation products.[2]

In parallel with the lowering of the blood glucose level, the lactate level underwent an increase. However, the oxidative metabolism of the cells was not inhibited in the

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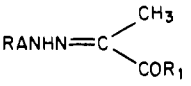
Table I. N-Substituted 2-Hydrazonopropionic Acids



no.	R	A	R_1	method	yield, %	mp, °C	formula	blood glucose dec,[a,i] %
ref[j]							$C_{10}H_{15}N_5$	70*
1	H	CH_2	OH	B	57	89–91	$C_4H_8N_2O_2$	9[b] *
2	H	$(CH_2)_3$	OH	B	46	56–58	$C_6H_{12}N_2O_2$	24+
3	H	$(CH_2)_4$	OH	B	63	56–57	$C_7H_{14}N_2O_2$	41*
4	H	$(CH_2)_5$	ONa	B	59	225–228	$C_8H_{15}NaN_2O_2$	45*
5	H	$(CH_2)_6$	OH	B	59	44	$C_9H_{18}N_2O_2$	72*
6	H	$CH{=}CH(CH_2)_4$	ONa	B	67	180–183	$C_9H_{15}NaN_2O_2$	60*
7	H	$(CH_2)_2CH{=}CH(CH_2)_2$	OH	B	73	oil	$C_9H_{16}N_2O_2$	82*
8	H	$(CH_2)_3CH{=}CHCH_2$	OH	B	71	oil	$C_9H_{16}N_2O_2$	43*
9	H	$(CH_2)_5CH(CH_3)$	OH	B	45	oil	$C_{10}H_{20}N_2O_2$	45*
10	H	$(CH_2)_2CH(CH_3)(CH_2)_3$	OH	A	91	oil	$C_{10}H_{20}N_2O_2$	41*
11	H	$(CH_2)_7$	OH	B	48	48–50	$C_{10}H_{20}N_2O_2$	70*
12	H	$(CH_2)_8$	OH	B	45	37–38	$C_{11}H_{22}N_2O_2$	69*
13	H	$(CH_2)_9$	OH	B	91	46–47	$C_{12}H_{24}N_2O_2$	25[b] +
14	H	$CH_2O(CH_2)_6$	OH	B	71	oil	$C_{10}H_{20}N_2O_3$	22+
15	H	$(CH_2)_3O(CH_2)_4$	OH	A	50	oil	$C_{10}H_{20}N_2O_3$	22+
16	c-C_5H_9	$(CH_2)_3$	OH	B	61	oil	$C_{11}H_{20}N_2O_2$	68*
17	c-C_6H_{11}	$(CH_2)_2$	OH	B	91	47	$C_{11}H_{20}N_2O_2$	48*
18	c-C_6H_{11}	$(CH_2)_3$	ONa	B	44	224–226[c]	$C_{12}H_{21}NaN_2O_2$	13+
19	C_6H_5	CH_2	OH	B	60	105–106[d]	$C_{10}H_{12}N_2O_2$	7
20	C_6H_5	$(CH_2)_2$	OH	B	91	74–76	$C_{11}H_{14}N_2O_2$	40*
21	C_6H_5	$(CH_2)_2$	OC_2H_5	C	64	oil	$C_{13}H_{18}N_2O_2$	14+
22	C_6H_5	$(CH_2)_2$	NH_2	B	50	75–78[c]	$C_{11}H_{15}N_3O$	15[b] +
23	2-$CH_3C_6H_4$	$(CH_2)_2$	OH	B	78	92	$C_{12}H_{16}N_2O_2$	14[b]
24	3-$CH_3C_6H_4$	$(CH_2)_2$	ONa	B	48	165[c]	$C_{12}H_{15}NaN_2O_2$[e]	24+
25	4-$CH_3C_6H_4$	$(CH_2)_2$	OH	B	40	69–70	$C_{12}H_{16}N_2O_2$	8
26	4-FC_6H_4	$(CH_2)_2$	OH	B	49	103–104	$C_{11}H_{13}FN_2O_2$	21+
27	4-ClC_6H_4	$(CH_2)_2$	OH	B	42	87–89	$C_{11}H_{13}ClN_2O_2$[e]	11
28	4-$CH_3OC_6H_4$	$(CH_2)_2$	OH	B	86	101–102	$C_{12}H_{16}N_2O_3$	7
29	3-$CF_5C_6H_4$	$(CH_2)_2$	OH	B	61	64–66	$C_{12}H_{13}F_3N_2O_2$	13[b]
30	C_6H_5	$CH_2CH(CH_3)$	ONa	B	66	199–204[f]	$C_{12}H_{15}NaN_2O_2$	14[b]
31	C_6H_5	$CH(CH_3)CH_2$	ONa	B	56	188–190	$C_{12}H_{15}NaN_2O_2$	15[b] +
32	C_6H_5	$(CH_2)_3$	ONa	B	62	201–203	$C_{12}H_{15}NaN_2O_2$	23[b] +
33	C_6H_5	$CH_2CH(CH_3)CH_2$	OH	B	98	oil	$C_{13}H_{18}N_2O_2$	22[b] +
34	C_6H_5	$O(CH_2)_2$	OH	B	76	90–92	$C_{11}H_{14}N_2O_3$[g]	22+
35	C_6H_5	$(CH_2)_4$	ONa	B	59	180–182	$C_{13}H_{17}NaN_2O_2$	34*
36	C_6H_5	$CH{=}CH(CH_2)_2$	OH	B	47	72–74	$C_{13}H_{16}N_2O_2$	15+
37	C_6H_5	$CH{=}CHCH_2$	ONa	B	50	210	$C_{12}H_{13}NaN_2O_2$	15
38	3-$CH_3C_6H_4$	$CH{=}CHCH_2$	OH	A	80	118–119	$C_{13}H_{16}N_2O_2$	22+
39	3-ClC_6H_4	$CH{=}CHCH_2$	OH	A	42	86–88	$C_{12}H_{13}ClN_2O_2$	6[b]
40	3-ClC_6H_4	$CH{=}CHCH_2$	OH	A	37	101–103	$C_{12}H_{13}ClN_2O_2$	14+
41	4-ClC_6H_4	$CH{=}CHCH_2$	OH	A	48	100–102	$C_{12}H_{13}ClN_2O_2$	9
42	C_6H_5	(*E*)-$CH{=}C(CH_3)CH_2$	OH	B	95	42–43[h]	$C_{13}H_{16}N_2O_2$	73*
43	C_6H_5	(*Z*)-$CH{=}C(CH_3)CH_2$	OH	B	76	78–81	$C_{13}H_{16}N_2O_2$	7
44	C_6H_5	$C(CH_3){=}CHCH_2$	OH	B	86	117–118	$C_{13}H_{16}N_2O_2$	20+

[a] Differences between the mean changes in the control and the treated groups after a dose of 25 mg/kp ip. [b] Results for a dose of 50 mg/kg ip. [c] With decomposition. [d] Reported mp 106 °C.[14] [e] Contains 0.5 mol of H_2O. [f] Reported mp 199–200 °C. [g] Contains 1 mol of H_2O. [h] Polymorph, mp 92–94 °C (unstable). [i] Key: +, $p < 0.05$; *, $p < 0.01$. [j] Phenformin.

concentration range studied.

With this class of compounds, therefore, there is no risk of lactate acidosis such as occasionally occurs under the influence of the biguanides.[3]

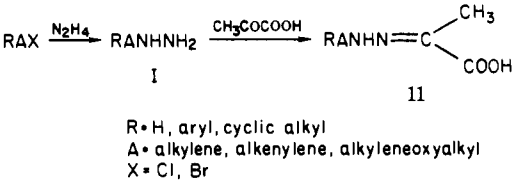
The novel pharmacological properties led us to investigate structure–activity relationships more closely. The present paper reports on the synthesis and the hypoglycemic properties of N-substituted 2-hydrazonopropionic acids.

Chemistry

The N-substituted 2-hydrazonopropionic acids of formula II are obtained from monoalkylated hydrazines I and pyruvic acid (Scheme I).

For condensation with pyruvic acid, either the free base I is reacted in the partition system, water–methylene chloride (method A), or a salt thereof is reacted in acetate-buffered aqueous medium (method B). Derivatives of other ketocarboxylic acids are obtained in an analogous manner.

Scheme I



The ester **21** is synthesized by reacting I with ethyl pyruvate in a mixture of acetic acid and water (method C). The results are summarized in Table I.

The hydrazines of type I, most of which are known, are obtained, in accordance with the method of Drain et al.[4]

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Table II. Monosubstituted Hydrazines

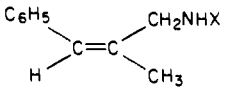
$RANHNH_2$

no.	R	A	yield, %	mp, °C/bp, mmHg	formula
45	H	$CH{=}CH(CH_2)_4$	55	130–135	$C_6H_{14}N_2{\cdot}HCl$
46	H	$(CH_2)_2CH{=}CH(CH_2)_2$	45	166–167	$C_6H_{14}N_2{\cdot}HCl$
47	H	$(CH_2)_3CH{=}CHCH_2$	44	166–167	$C_6H_{14}N_2{\cdot}C_2H_2O_4$
48	H	$(CH_2)_2CH(CH_3)(CH_2)_3$	72	86–88/14	$C_7H_{18}N_2$
49	H	$(CH_2)_9$	69	115[a]	$C_9H_{22}N_2{\cdot}HCl$
50	H	$CH_2O(CH_2)_6$	76	83–87	$C_7H_{18}N_2O$
51	H	$(CH_2)_3O(CH_2)_4$	36	97–104/15	$C_7H_{18}N_2O$
52	c-C_5H_9	$(CH_2)_3$	48	197–202	$C_8H_{18}N_2{\cdot}HCl$
53	c-C_6H_{11}	$(CH_2)_2$	52	185–187[a]	$C_8H_{18}N_2{\cdot}H_2SO_4$
54	c-C_6H_{11}	$(CH_2)_3$	72	220–225	$C_9H_{20}N_2{\cdot}HCl$
55	3-$CF_3C_6H_4$	$(CH_2)_2$	48	148–149	$C_6H_{14}F_3N_2{\cdot}HCl$
56	C_6H_5	$CH_2CH(CH_3)CH_2$	41	108–110	$C_{10}H_{16}N_2{\cdot}HCl$
51	C_6H_5	$CH{=}CH(CH_2)_2$	50	148–150[a]	$C_{10}H_{14}N_2{\cdot}C_2H_2O_4$
58	3-$CH_3C_6H_4$	$CH{=}CHCH_2$	55	110–113/0.1	$C_{10}H_{14}N_2$
59	2-ClC_6H_4	$CH{=}CHCH_2$	73	108–112/0.1	$C_9H_{11}ClN_2$
60	3-ClC_6H_4	$CH{=}CHCH_2$	87	124–128/0.1	$C_9H_{11}ClN_2$
61	4-ClC_6H_4	$CH{=}CHCH_2$	61	113–118/0.1	$C_9H_{11}ClN_2$
62	C_6H_5	(*E*)-$CH{=}C(CH_3)CH_2$	80	156–158	$C_{10}H_{14}N_2{\cdot}H_2SO_4$
63	C_6H_5	(*Z*)-$CH{=}C(CH_3)CH_2$	26	78	$C_{10}H_{14}N_2{\cdot}H_2SO_4$
64	C_6H_5	$C(CH_3){=}CHCH_2$	54	120	$C_{10}H_{14}N_2{\cdot}HCl$

[a] With decomposition.

from the corresponding alkyl halides, as per Scheme I, by reaction with hydrazine hydrate in boiling ethanol. New physical data are given in Table II.

[(*Z*)-2-methyl-3-phenyl-1-propenyl]hydrazine (**63**) is synthesized by a different scheme, from the known (*Z*)-2-methyl-3-phenylacrylonitrile,[5] by reduction with lithium aluminum hydride/aluminum trichloride to [(*Z*)-2-methyl-3-phenyl-2-propenyl]amine (**63a**) and subsequent reaction of **63a** with hydroxylamine-*O*-sulfonic acid.



63, X = NH_2
63a, X = H

The configurations of **62** (*E* isomer) and **63** (*Z* isomer) were confirmed by comparing their NMR data.

The stereochemistry of the other alkenyl analogues has not been evaluated.

Results and Discussion

The hypoglycemic activities of the 2-hydrazonopropionic acids are listed in Table I. The values are given as percentage differences between the mean blood glucose concentrations in treated groups of animals and in untreated controls.

The activity of the substances changes with the length of the alkyl residue R–A on the nitrogen. If R–A is an unsubstituted alkyl group (Table I; **1–13**), the maximum activity is found at a chain length of six to eight carbons (**5, 11, 12**). Modification of the degree of saturation slightly increases (**6**) or weakens (**7, 8**) the activity, depending on the position of the double bond. The introduction of methyl branches (**9, 10**), on the other hand, seems generally to weaken the activity. Incorporation of heteroatoms in the alkyl chain (**14, 15**) seems to result in a distinct loss of activity, whereas substitution with alicycles has no effect on the activity if the whole R–A residue is of an optimal length (**16, 18**).

The aralkyl-substituted analogues (R = aryl; Table I; **19–36**) have a pronounced activity maximum when the aryl residue and the imino group are separated by two carbon atoms (**20** as compared to **19, 32** and **35**). In this type of compound, too, the activity tends to be weakened by methyl branches (**30, 31, 33**) or by the introduction of heteroatoms into the alkylene chain A (**34**). Substitution on the phenyl ring likewise causes a loss of activity (**23–29**). In comparison with the purely aliphatic derivatives, the aralkyl-substituted compounds II are on the whole less active.

A similar situation is discernible in the case of the aralkenyl derivatives with a conjugated double bond cinnamyl structure; Table I; **37–44**). The activity is comparable with that of the saturated series (**37** as compared to **32**). By contrast, a different behavior is observed in the compounds with methyl branching. The introduction of a methyl group in position β leads in this case to a clear increase in activity (**42**), whereas the introduction of a branch in the saturated series has practically no effect on activity (**32, 33**). In addition to the substitution pattern of the cinnamyl derivatives, steric factors evidently have a bearing on the activity level. Thus, the *Z* isomer of the β-methyl branch (**43**) has an activity only about one-tenth as strong as that of the *E* isomer (**42**).

Other structural variations (e.g., the introduction of ester and amide groups, dialkylation, and acylation of the hydrazono nitrogen) resulted in all cases in virtually inactive compounds.

For the pharmacological investigations performed with the compounds listed in Table I, we used guinea pigs, as this species approximates most closely to the situation in man in its mitochondrial cytosol distribution of the key enzymes of gluconeogenesis.[6,7] In other animal species such as the rat, rabbit, and Chinese hamster the substances likewise exhibited a hypoglycemic activity, but to a clearly smaller extent compared to the guinea pig.

The substances described showed in in vitro investigations a marked inhibition of the intestinal absorption of glucose in rats and guinea pigs, which was comparable with that of phenformin. The cellular ratio of ATP/ADP was, however, unaffected.[8]

As has been demonstrated by investigations on the model of perfused guinea pig liver and on isolated liver cells, the blood glucose lowering action of the compounds

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is based on inhibition of gluconeogenesis.

In addition to the influence on the fasting glycemia, after administration of the substances, a pronounced reduction of the blood concentration of ketone bodies was observed. Further investigations revealed that the inhibition of gluconeogenesis from various substrates in the perfused guinea pig liver could be eliminated by simultaneous perfusion of octanoate, a medium-length-chain fatty acid that passes through the mitochondrial membrane without the help of Carnitine-acyl transferase (CAT). On the other hand, when a long-chain fatty acid is used, such as oleate, which is transported with the aid of the CAT system, the pharmacological activity of the substance remains unchanged.[9]

From the above findings it can be concluded that the new hydrazonopropionic acids should be regarded as inhibitors of CAT, an effect that leads ultimately to reduced gluconeogenesis.

It has been well documented that the transport of long-chain free fatty acids through the mitochondrial membrane proceeds with the aid of two transferases, CAT I and CAT II (EC 2.3.1.21).[10,11] Whereas previously described inhibitors of this metabolic pathway exert their effects on CAT I, the substances described here were found to exert their activity predominantly on CAT II.

Two of the compounds described (**17**, **42**) were tested for mutagenic action and underwent comprehensive investigations to establish their general pharmacological properties. The results of this work are reported elsewhere. The findings obtained and the results of the toxicological studies are not suggestive of any effects that would prohibit the compounds' use in man.

The LD_{50} on oral administration to rats was found to be 520 mg/kg for **17** and 680 mg/kg for **42**.

The therapeutic efficacy of **42** is similar to that of phenformin; in view of the different modes of action of these two classes of substances, the side effects seen with the biguanides (e.g., lactic acidosis) are not to be expected.

Experimental Section

Chemistry. The melting points were determined on a Büchi melting point apparatus and are uncorrected. Microanalyses were performed by the Microanalytical Laboratory of the Chemical Research Department of Boehringer Mannheim and were within ±0.4% of the theoretical values. The compounds were checked for consistency of their spectroscopic data with the assigned structure by ^1H NMR on a Bruker HFX 90 spectrometer. Mass spectra were recorded with Varian spectrometer, Model CH 7 A. Solutions were dried over sodium sulfate and evaporated under reduced pressure (15 mm) on a rotary evaporator.

2-[(3-Chlorocinnamyl)hydrazono]propionic Acid (39). Method A. A solution of 8.7 g (48 mmol) of (3-chlorocinnamyl)hydrazine in 100 mL of methylene chloride was mixed with a solution of 4.2 g (48 mmol) of pyruvic acid in 100 mL of water and stirred for 30 min at room temperature. The organic phase was then separated off, washed with water, dried, and concentrated. The residue was recrystallized from a mixture of ethyl acetate and 50–70 °C petroleum ether: yield of **39** 5.9 g (49%); mp 101–103 °C.

Sodium 2-[(3-Phenylpropyl)hydrazono]propionate (32). Method B. A solution of 3.0 g (16 mmol) of (3-phenylpropyl)-hydrazine hydrochloride in 10 mL of water was mixed with a solution of 1.4 g (16 mmol) of pyruvic acid and 2.1 g (25 mmol) of sodium acetate in 5 mL of water and stirred for 2 h. Extraction was then performed with methylene chloride, and the organic phase was dried and evaporated, leaving behind an oily residue. The oil (3.5 g, 16 mmol) was dissolved in a solution of sodium methoxide, prepared from 0.35 g (14.6 mmol) of sodium and 20 mL of methanol, and the solution was evaporated. The residue was stirred with ether, and the crystals were collected: yield of **32** 2.4 g (62%); mp 201–203 °C.

2-(Phenylethylhydrazono)propionic Acid Ethyl Ester (21). Method C. A mixture of 1.7 g (9.9 mmol) of phenethylhydrazine hydrochloride, 1.2 g (10.3 mmol) of ethyl pyruvate, 7 mL of water, and 15 mL of glacial acetic acid was stirred for 4 h at room temperature and then diluted with 30 mL of water. The organic layer was taken up in ether, and the solution was washed with sodium bicarbonate solution, dried, and evaporated. An oily residue of 1.5 g (65%) of **21** was obtained.

[(*E*)-2-Methyl-3-phenyl-2-propenyl]hydrazine (62). [(*E*)-3-chloro-2-methyl-1-propenyl]benzene[12] (33.3 g, 0.2 mol) was quickly run into a solution of 78 mL (1.6 mol) of hydrazine hydrate in 500 mL of ethanol at 60 °C while stirring, and after the reaction and subsided, the mixture was refluxed for 18 h. The solution was then evaporated, and the residue was mixed with 30 g of anhydrous potassium carbonate and extracted with chloroform. The chloroform extracts were dried and evaporated, and the residue was distilled under vacuum: yield of **62** 28.6 g (88%); bp 101–105 °C (0.05 mm).

The distillate (28.6 g) was dissolved in 275 mL of ethanol and treated dropwise with 9.8 mL of concentrated sulfuric acid while cooling in ice. The resulting crystal suspension was allowed to stand in the cold bath for 1 h further and was finally filtered with suction. The precipitate was washed first with 45 mL of ethanol and then with 45 mL of ether and was dried under vacuum at 40 °C: yield of **62** (sulfate) 41.7 g (80% total yield); mp 156–158 °C dec; NMR (Me_2SO-d_6) 1.89 (d, 3 H, J = 1.5 Hz), 3.59 (s, 2 H), 6.56 (br, 1 H), 7.1–7.6 (m, 5 H).

The remaining hydrazines I were obtained analogously from the corresponding alkyl bromides or chlorides (see also Table II).

[(*Z*)-2-Methyl-3-phenyl-2-propenyl]hydrazine (63). [(*Z*)-2-Methyl-3-phenyl]acrylonitrile was prepared as described in the literature[5] from the potassium salt of 2-(diethylphosphono)propionitrile and benzaldehyde at −78 °C. The crude product contained about 10% of the *E* nitrile, which was separated off by preparative HPLC.

[(*Z*)-2-Methyl-3-phenyl-2-propenyl]amine (63a). A suspension of 40.0 g (0.3 mol) of aluminum chloride in 320 mL of absolute ether was mixed with 11.4 g (0.3 mol) of lithium aluminum hydride, with stirring and cooling in ice. The cold bath was then removed, and a solution of 47.4 g (0.33 mol) of (*Z*)-2-methyl-3-phenylacrylonitrile in 50 mL of absolute ether was added dropwise at room temperature. The reaction mixture was left to stand at room temperature for 18 h and subsequently decomposed with water and 20% sodium hydroxide. The ether phase was separated off, and the aqueous phase was extracted several times with portions of ether. The combined organic extracts were dried and evaporated. The residue was dissolved in dry ether, and the sulfate was precipitated with sulfuric acid. Recrystallization from methanol yielded 41 g (63%) of **63a** sulfate: mp 228–229 °C; NMR (D_2O) 2.03 (d, 3 H, J = 1.5 Hz), 3.83 (s, 2 H), 6.79 (br, 1 H), 7.2–7.7 (m, 5 H).

[(*Z*)-2-Methyl-3-phenyl-2-propenyl]hydrazine (63). To a mixture of 13.0 g (88 mmol) [(*Z*)-2-methyl-3-phenyl-2-propenyl]amine (**63a**) and 50 mL of water was slowly added at room temperature a solution of 2.8 g (22.5 mmol) of about 90% hydroxylamine-*O*-sulfonic acid in 8 mL of water. The reaction mixture was stirred for a further 20 min, and the pH was adjusted to 1 with dilute sulfuric acid. The aqueous solution was washed with ether and mixed with 8 mL of isobutyraldehyde, and the pH was adjusted to 3 by the addition of sodium hydroxide. After 30 min the hydrazone thus formed was extracted with ether. The combined extracts were evaporated, and the residue (2.3 g) was treated with 11 mL of 2 N sulfuric acid. The isobutyraldehyde thus formed was distilled off with steam, and the aqueous residue was cooled, washed with ether, and evaporated. The evaporation residue was recrystallized from ethyl acetate. This gave 1.5 g

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(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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