Articles

Synthesis and Hypoglycemic Activity of Phenylalkyloxiranecarboxylic Acid Derivatives

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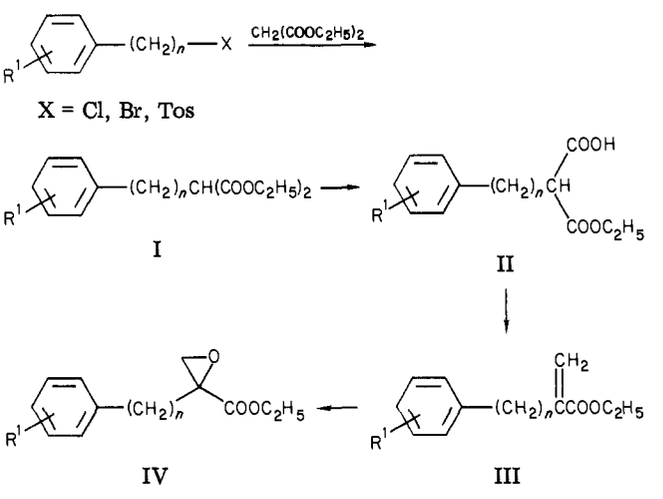
A series of new 2-(phenylalkyl)oxirane-2-carboxylic acids has been synthesized and studied for its effects on the concentration of blood glucose. Most of the compounds exhibit remarkable blood glucose lowering activities in fasted rats. Structure–activity studies reveal that substituents like Cl or CF_3 on the phenyl ring and a chain length of three to five carbon atoms lead to the most effective substances. Among these compounds, ethyl 2-[5-(4-chlorophenyl)pentyl]oxirane-2-carboxylate (**36**) exhibits the most favorable activity.

During the last 10 years, evidence has accumulated showing that several substituted or modified alkanoic acids are able to inhibit gluconeogenesis by inhibiting various sites of the fatty acid oxidation sequence in the liver mitochondria[1-7] and that this inhibition, in connection with Randle's[8] "glucose fatty acid cycle", can cause a decrease in blood glucose concentration in vivo.

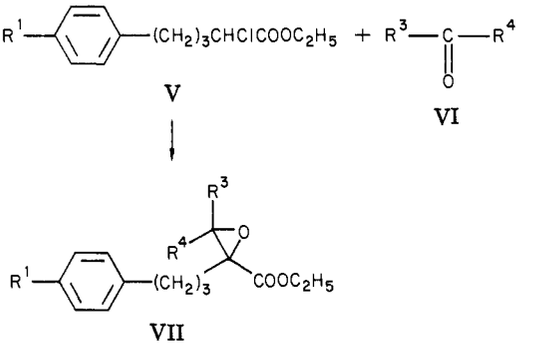
Chase and Tubbs[7] were the first to report 2-bromopalmitoyl-CoA appearing to be an active-site-directed inhibitor of carnitine acyltransferase (CAT), a rate-limiting enzyme of fatty acid utilization reviewed by McGarry and Foster.[9] Tutwiler et al.[2,3] reported on the pharmacology and biochemistry of a more effective compound, methyl tetradecylglycidate (McN-3716), a long-chain alkyl oxiranecarboxylic acid with a similar site of action.

Chemistry. The substituted ethyl oxiranecarboxylates IV (**21–40**) shown in Table II were prepared by the general reaction sequence outlined in Scheme I. The alkylated diethyl malonates (I) were synthesized by reaction of phenylalkyl halides or phenylalkyl tosylates with diethyl malonate in the presence of NaOEt. Treatment of esters I with alcoholic KOH afforded the monoesters II, which on treatment with paraformaldehyde, pyridine, and piperidine according to the method of Stetter and Kuhlmann[16] yielded the ethyl 2-methylenecarboxylates III (Table I, **1–20**). Oxidation with 3-chloroperbenzoic acid or permaleic acid[11] furnished the desired ethyl oxiranecarboxylates (IV, **21–40**). The sodium salts (**41–44**) were obtained by alkaline hydrolysis of the corresponding ethyl carboxylates. Ethyl oxiranecarboxylates VII (**45–48**, Table II) were synthesized by Darzen–Claisen condensation of ethyl 2-chloro-5-phenylpentanoates[12] (V) with the appropriate ketones VI (Scheme II) and **49** by using ethyl chloroacetate and hydrocinnamaldehyde. Compound **50** was prepared by oxidation of methyl 6-phenyl-2-hexenoate.[13] Amides **55–57** were obtained by ester cleavage of **3** and subsequent treatment with $SOCl_2$ and the appropriate amine to give 2-methylenecarboxamides **52–54**, followed by oxidation with 3-chloroperbenzoic acid.

Scheme I



Scheme II



Pharmacological Results and Discussion

The compounds listed in Table II were evaluated for

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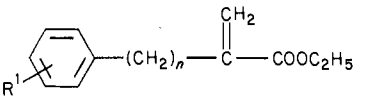
Table I. Ethyl 2-Methylene-ω-phenylalkylcarboxylates



no.	R^1	n	bp (mm), °C	yield, %	formula	anal.
1[a]	H	1	64–65 (0.01)	82	$C_{12}H_{14}O_2$	
2[b]	H	2			$C_{13}H_{16}O_2$	
3[b]	H	3	78–79 (0.02)	66	$C_{14}H_{18}O_2$	
4	2-Cl	3	95–98 (0.005)	84	$C_{14}H_{17}ClO_2$	C, H, Cl
5	3-Cl	3	105–110 (0.005)	76	$C_{14}H_{17}ClO_2$	C, H, Cl
6	4-Cl	3	120–123 (0.05)	72	$C_{14}H_{17}ClO_2$	C, H, Cl
7	4-F	3	84–86 (0.01)	67	$C_{14}H_{17}FO_2$	C, H
8	3,4-Cl_2	3	128–130 (0.02)	68	$C_{14}H_{16}Cl_2O_2$	C, H, Cl
9	4-CH_3	3	85 (0.06)	55	$C_{15}H_{20}O_2$	C, H
10[c]	4-$C(CH_3)_3$	3	oil[d]	82	$C_{17}H_{24}O_2$	C, H
11	5-Cl, 2-OCH_3	3	136–138 (0.01)	82	$C_{15}H_{19}ClO_3$	C, H, Cl
12	4-OCH_3	3	136–139 (0.01)	71	$C_{15}H_{20}O_3$	C, H
13	3-CF_3	3	110 (0.07)	59	$C_{15}H_{17}F_3O_2$	C, H
14	H	4	120–125 (0.005)	78	$C_{15}H_{20}O_2$	C, H
15	H	5	103–106 (0.07)	53	$C_{16}H_{22}O_2$	C, H
16	4-Cl	5	125–130 (0.08)	66	$C_{16}H_{21}ClO_2$	C, H, Cl
17	H	6	oil[d]	65	$C_{17}H_{24}O_2$	C, H
18	H	7	136 (0.05)	71	$C_{18}H_{26}O_2$	C, H
19	4-Cl	7	134–136 (0.005)	53	$C_{18}H_{25}ClO_2$[e]	C, H, Cl
20	H	8	oil[f]	77	$C_{19}H_{28}O_2$	C, H

[a] Reference 23. [b] Reference 12. [c] Methyl ester. [d] Chromatographed over silica gel (CH_2Cl_2). [e] C: calcd, 70.00; found, 70.75. Cl: calcd, 11.48; found, 10.01. [f] Chromatographed over silica gel ($CHCl_3$).

their ability to lower blood glucose levels in fasted rats. They were characterized by the ED_{50} values and relative changes of glucose after a single dose; a structure–response relationship is better described by the comparison of ED_{50} values.

The potency of these compounds differs according to the chain length between the phenyl and oxirane residue. Within the series of compounds with an unsubstituted phenyl moiety, optimum strength was found with a chain length of four carbon atoms between the phenyl and oxirane ring (**34**). Compounds with chain lengths of three (**23**) or five to seven (**35**, **37**, and **38**) carbon atoms were less potent. When the length was changed to one, two, or eight carbon atoms (**21**, **22**, and **40**), the activity disappeared.

Substitution of the phenyl moiety by Cl, F, CF_3, or CH_3 in the 3- or 4-position resulted in increased activity (**25–29**, **33**, **36**, and **39**), whereas compounds substituted by OCH_3 or $C(CH_3)_3$ revealed no activity (**30–32**).

Salts (**41–44**) of the active esters exhibited comparable activity, while other substitution variations resulted in total loss of activity: Amides (**55–57**), 3-substituted oxiranes (**45–48**), and oxiranes **49** and **50** show the limit of substitution variations.

The blood glucose lowering effects of the active compounds were noticed only in fasted rats. In parallel to the hypoglycemic effect, a pronounced decrease of ketone bodies in the blood was observed.[14a] This permits us to conclude that the substances may act as inhibitors of fatty acid oxidation, which would result in inhibition of ketogenesis and gluconeogenesis in the liver. This effect, in addition to the consequence of Randle's glucose fatty acid cycle,[8] causes a decrease of blood glucose in vivo. In perfusion experiments with isolated rat liver,[14b] a simultaneous inhibition of gluconeogenesis, ketogenesis, and oxidation of long-chain fatty acids was observed in the presence of effective oxiranes. However, oxidation of octanoic acid or palmitoylcarnitine, a substrate and a metabolite which permeate into the mitochondrial matrix without implicating the CAT system, was not affected.

The results of these experiments suggest that the mechanism of hypoglycemic activity involves the structure-related specific inhibition of the transport of long-chain fatty acids across the mitochondrial membrane.

For therapeutic use of the oxiranecarboxylic acids it is necessary to establish whether these compounds have any mutagenic activity, since several oxirane derivatives are known to be mutagens and carcinogens.[15] Therefore, selected compounds of the series listed in Table II were investigated for their mutagenic ability. Compounds **26**, **33**, **35**, **43**, and **44** were subjected to the host-mediated assay[16,17] and the Ames test,[18] and compounds **35**, **36**, **43**, and **44** were subjected to the micronucleus test.[19] The results did not indicate any mutagenic activity. Compounds **21–23**, **26**, **28**, **29**, **33–36**, and **42–44** were tested for their effect on hepatic epoxide hydrolase (EC 4.2.1.63), which plays an important part in the detoxication of epoxide metabolites.[20] None of the compounds exhibited significant inhibition up to 10^{-3} mol/L.

In conclusion, our study shows that the new oxiranecarboxylic acids are potent blood glucose lowering compounds. In comparison to other orally effective compounds, i.e., tolbutamide or buformin, ethyl 2-[5-(4-chlorophenyl)pentyl]oxirane-2-carboxylate (**36**) is about 5 and 30 times more potent, respectively, in fasted rats.[14a]

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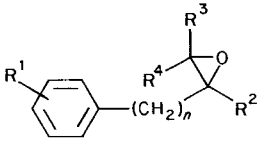
Table II. (ω-Phenylalkyl)oxiranecarboxylates



no.	R^1	R^2	R^3	R^4	n	yield, %	mp or bp (mm), °C	formula	anal.	max blood glucose decrease, %,[a] after 0.6 mmol/kg dose	ED_{50} (25%) ± SD,[b] mmol/kg	LD_{50} (mice), mmol/kg po
21	H	$COOC_2H_5$	H	H	1	41	79–80 (0.003)	$C_{12}H_{14}O_3$	C, H	no effect		
22	H	$COOC_2H_5$	H	H	2	75	95–100 (0.01)	$C_{13}H_{16}O_3$	C, H	no effect		
23	H	$COOC_2H_5$	H	H	3	56	115 (0.03)	$C_{14}H_{18}O_3$	C, H	19***	1.17 ± 0.17	
24	2-Cl	$COOC_2H_5$	H	H	3	34	110–113 (0.005)	$C_{14}H_{17}ClO_3$	C, H, Cl	11**		
25	3-Cl	$COOC_2H_5$	H	H	3	73	135–138 (0.005)	$C_{14}H_{17}ClO_3$	C, H, Cl	26***	0.68 ± 0.14	
26	4-Cl	$COOC_2H_5$	H	H	3	41	110 (0.005)	$C_{14}H_{17}ClO_3$	C, H, Cl	30***	0.20 ± 0.04	2.72
27	4-F	$COOC_2H_5$	H	H	3	86	115–117 (0.02)	$C_{14}H_{17}FO_3$	C, H	37***		3.96
28	3,4-Cl_2	$COOC_2H_5$	H	H	3	77	oil[c]	$C_{14}H_{16}Cl_2O_3$	C, H, Cl	29***	0.33 ± 0.01	2.87
29	4-CH_3	$COOC_2H_5$	H	H	3	49	116 (0.2)	$C_{15}H_{20}O_3$	C, H	27***		
30	4-$C(CH_3)_3$	$COOCH_3$	H	H	3	77	oil[c]	$C_{17}H_{24}O_3$	C, H	no effect		
31	5-Cl, 2-OCH_3	$COOC_2H_5$	H	H	3	42	150 (0.005)	$C_{15}H_{19}ClO_4$	C, H, Cl	no effect		
32	4-OCH_3	$COOC_2H_5$	H	H	3	4	134–138 (0.07)	$C_{15}H_{20}O_4$	C, H	no effect		
33	3-CF_3	$COOC_2H_5$	H	H	3	41	110 (0.07)	$C_{15}H_{17}F_3O_3$	C, H	47***	0.17 ± 0.02	0.86
34	H	$COOC_2H_5$	H	H	4	59	105 (0.005)	$C_{15}H_{20}O_3$	C, H	44***	0.60	
35	H	$COOC_2H_5$	H	H	5	47	131 (0.07)	$C_{16}H_{22}O_3$	C, H	18***	0.08[d]	0.88
36	4-Cl	$COOC_2H_5$	H	H	5	47	148–150 (0.3)	$C_{16}H_{21}ClO_3$	C, H, Cl	80[e]	0.02 ± 0.02	0.93
37	H	$COOC_2H_5$	H	H	6	50	oil[f]	$C_{17}H_{24}O_3$	C, H	24*	not feasible	
38	H	$COOC_2H_5$	H	H	7	33	126–132 (0.15)	$C_{18}H_{26}O_3$	C, H	18	not feasible	
39	4-Cl	$COOC_2H_5$	H	H	7	57	145–148 (0.005)	$C_{18}H_{25}ClO_3$	C, H, Cl	49***	0.27 ± 0.10	0.95
40	H	$COOC_2H_5$	H	H	8	87	oil[c]	$C_{19}H_{28}O_3$	C, H	no effect		
41	4-Cl	COO^-Na^+	H	H	3	84	160–167	$C_{12}H_{12}ClNaO_3$	C, H, Cl	68***	0.18 ± 0.04	
42	3-CF_3	COO^-Na^+	H	H	3	46	160–164	$C_{13}H_{12}F_3NaO_3$	C, H	77***	0.08 ± 0.02	
43	H	COO^-Na^+	H	H	5	70	82–86	$C_{14}H_{17}NaO_3 \cdot 2H_2O$	C, H	29***[g]	not feasible[h]	1.06
44	4-Cl	COO^-Na^+	H	H	5	75	136–142	$C_{14}H_{16}ClNaO_3$	C, H, Cl	80[e]	0.03 ± 0.01	1.64
45	H	$COOC_2H_5$	CH_3	CH_3	3	31	110–115 (0.1)	$C_{16}H_{22}O_3$	C, H	no effect		
46	4-Cl	$COOC_2H_5$	CH_3	CH_3	3	50	oil[f]	$C_{16}H_{21}ClO_3$	C, H, Cl	10		
47	H	$COOC_2H_5$	$-(CH_2)_5-$		3	23	110–120 (0.001)	$C_{19}H_{26}O_3$	C, H	no effect		
48	4-Cl	$COOC_2H_5$	$-(CH_2)_5-$		3	19	oil[f]	$C_{19}H_{25}ClO_3$	C, H, Cl	no effect		
49	H	H	$COOC_2H_5$	H	2	13	123 (0.06)	$C_{13}H_{16}O_3$	C, H	no effect		
50	H	H	$COOCH_3$	H	3	54	oil[f]	$C_{13}H_{16}O_3$[i]	C, H	no effect		
55	H	$CONH_2$	H	H	3	48	88.5–89.5[k]	$C_{12}H_{15}NO_2$	C, H, N	no effect		
56	H	$CONHC_2H_5$	H	H	3	40	55–56[k]	$C_{14}H_{19}NO_2$	C, H, N	no effect		
57	H	$CON(C_2H_5)_2$	H	H	3	45	120–123 (0.001)	$C_{16}H_{23}NO_2$	C, H, N	no effect		

[a] Two-sided Student's t test: * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$. [b] ED_{50} (25%) was calculated from the dose–response relationship and is defined as 50% of the rats showing at least a 25% decrease of blood glucose levels compared to the controls. [c] Chromatographed over silica gel (CH_2Cl_2). [d] ED_{50} (23%), reverse effect at higher dose. [e] Animals died in hypoglycemic coma. [f] Chromatographed over silica gel (petroleum ether/ethyl acetate 9:1). [g] Dose = 0.11 mmol/kg. [h] No dose–response relationship. [i] C: calcd, 67.75; found, 67.15. [k] Cyclohexane.

The potency of **36** also appears to be slightly greater than that described for McN-3716.[3] Studies are in progress to evaluate therapeutic employment of the new compounds in diabetics.

Experimental Section

Chemistry. Melting points were determined in open glass capillary tubes using a Büchi melting point apparatus and are uncorrected. Elemental analyses were performed by the Institute of Organic Chemistry, Stuttgart University, and were within ±0.4% of the calculated values, unless otherwise noted. Compounds were checked by NMR on a JEOL C-60 HL spectrometer, the spectral data being consistent with the assigned structure in all cases. Silica gel 60 (Machery Nagel, Düren) was used for column chromatography. TLC was performed on silica gel plates (F 1500 LS 254, Schleicher & Schüll, Dassel). Solutions were dried over Na_2SO_4 and concentrated under reduced pressure (20 mm) using a rotatory evaporator.

General Procedure for the Preparation of Ethyl 2-Methylenecarboxylates 1–20. Ethyl 5-(3-Chlorophenyl)-2-methylenepentanoate (5). Diethyl malonate (91.3 g, 0.57 mol) was added within 15 min to a freshly prepared solution of 13.1 g (0.57 mol) of sodium in ethanol (650 mL) and stirred for 1 h at 50 °C. 2-(3-Chlorophenyl)propyl tosylate (185 g, 0.57 mol) was added within 1 h to this solution, and the mixture was maintained, under stirring, at 50 °C for an additional 4 h. Water (2 L) was added and the mixture was extracted with ether (2 × 700 mL). The combined organic extracts were concentrated to an oily residue, which was distilled to yield 74.2 g (42%) of diethyl 3-(3-chlorophenyl)propylmalonate: bp 145–152 °C (0.005 mm). A solution of 13.5 g (0.237 mol) of KOH in 200 mL of ethanol was added under stirring within 1 h to a solution of 74 g (0.237 mol) of diethyl 3-(3-chlorophenyl)propylmalonate in ethanol (200 mL). After being stirred for 12 h, the reaction mixture was concentrated, and the residue was treated with 500 mL of water and extracted with ether (2 × 100 mL). The aqueous phase was acidified with HCl, extracted with ether (2 × 200 mL), and evaporated to give 57.6 g of ethyl 3-(3-chlorophenyl)propylmalonate as oil. Ethyl 3-(3-chlorophenyl)propylmalonate (57 g, 0.47 mol), pyridine (37.7 mL), piperidine (2.5 mL), and paraformaldehyde (8.36 g, 0.28 mol) were heated under stirring to 50–55 °C until CO_2 evolution ceased (~4 h). The mixture was treated with 100 mL of ice–water, acidified with HCl, and extracted with ether (3 × 100 mL). The combined extracts were concentrated to an oily residue, which was distilled to yield 35.7 g (75%) of ethyl 5-(3-chlorophenyl)-2-methylenepentanoate (**5**), bp 105–110 °C (0.005 mm).

General Procedure for the Preparation of Oxiranes 21–40, 50, and 55–57. Ethyl 2-[3-(3-Chlorophenyl)propyl]oxirane-2-carboxylate (25). Ethyl 5-(3-chlorophenyl)-2-methylenepentanoate (**5**; 14 g, 0.055 mol) and 3-chloroperoxybenzoic acid (22.5 g, 0.11 mol) in CH_2Cl_2 (170 mL) were refluxed for 21 h. The precipitated 3-chlorobenzoic acid was filtered off and washed with CH_2Cl_2 (50 mL). The combined filtrates were evaporated, and the residue was treated under stirring with acetone (100 mL) and saturated aqueous $NaHCO_3$ solution (100 mL) for 30 min. Water was added (100 mL) and extracted with ether (3 × 100 mL). The combined organic solutions were dried, stirred with solid sodium hydrogen sulfite if the iodine–starch test was positive, filtered, and evaporated, and the oily residue was distilled to yield 10.9 g (73%) of ethyl 2-[3-(3-chlorophenyl)propyl]oxirane-2-carboxylate (**25**), bp 135–138 °C (0.005 mm).

General Procedure for the Preparation of the Salts 41–44. Sodium 2-[3-[3-(Trifluoromethyl)phenyl]propyl]oxirane-2-carboxylate (42). A solution of 10 g (0.033 mol) of ethyl 2-[3-[3-(trifluoromethyl)phenyl]propyl]oxirane-2-carboxylate in THF (30 mL) was added under stirring to a mixture of 33 mL of 1 N NaOH and THF (50 mL). After 1 h, the solution was evaporated to dryness, and the solid residue was crystallized with light petroleum ether/acetone to yield 4.5 g (46%) of sodium 2-[3-[3-(trifluoromethyl)phenyl]propyl]oxirane-2-carboxylate (**42**), mp 160–164 °C.

General Procedure for the Preparation of Oxiranes 45–48. Ethyl 3,3-Dimethyl-2-(3-phenylpropyl)oxirane-2-carboxylate (45). A freshly prepared solution of 1.65 g (0.042 mol) of potassium in 50 mL of *tert*-butyl alcohol was added within 25 min to a stirred mixture of 15 g (0.052 mol) of ethyl 2-bromo-5-phenylpentanoate and acetone (3.1 g) in *tert*-butyl alcohol (20 mL). Stirring was continued for an additional 4 h, and the reaction mixture was treated with 200 mL of water and extracted with ether (2 × 100 mL). The combined organic phases were washed with 2 N HCl and water, dried, and evaporated. The crude oily residue (13.1 g) was chromatographed on silica gel. The major fraction eluted with ethyl acetate/light petroleum ether (1:9) was distilled to give 4.25 g (31%) of pale yellow **45**, bp 110–115 °C (0.1 mm).

Ethyl *trans*-3-(2-Phenylethyl)oxirane-2-carboxylate (49). A mixture of 53.9 g (0.4 mol) of 3-phenylpropanal and 73.5 g (0.6 mol) of ethyl chloroacetate was added under stirring within 0.5 h at −10 °C to a freshly prepared solution of 13.8 g (0.6 mol) of sodium in ethanol (400 mL). The mixture was allowed to stand at room temperature for 12 h and then treated with 30 g (0.5 mol) of acetic acid and ice–water (500 mL). The mixture was extracted with ether (2 × 300 mL), and the combined organic phases were dried and evaporated. The oily residue was distilled in vacuo, and the fraction with bp 105–110 °C (0.01 mm) was purified via column chromatography (silica gel; elution with ethyl acetate/light petroleum ether, 1:9). Evaporation of the volatiles, with distillation of the residue, gave 11.2 g (12.7%) of **49**, bp 123 °C (0.06 mm).

2-Methylene-5-phenylpentanoic Acid (51). Ethyl 2-methylene-5-phenylpentanoate (**3**; 100 g, 0.458 mol), 2 N NaOH (460 mL), and EtOH (275 mL) were stirred for 3 h at 50 °C. The cold reaction mixture was acidified with HCl and then extracted with ether (4 × 200 mL), and the combined organic solutions were dried and evaporated to yield 84.3 g (96%) of 2-methylene-5-phenylpentanoic acid (**51**) as an oil.

2-Methylene-5-phenylpentanamide (52). 2-Methylene-5-phenylpentanoic acid (25 g, 0.13 mol) and $SOCl_2$ (20 mL) were stirred at 50 °C for 7 h, excess $SOCl_2$ was distilled off in vacuo, and the residue, dissolved in 25 mL of $CHCl_3$, was dropped with ice-cooling into a stirred solution of 4.2 g (0.25 mol) of NH_3 in $CHCl_3$ (70 mL). After stirring at room temperature for 7 h, the reaction mixture was treated with water (100 mL), and the organic phase was separated, dried, and evaporated. The oily residue was crystallized with ethanol/cyclohexane to yield 13.9 g (56%) of 2-methylene-5-phenylpentanamide (**52**), mp 70–71 °C. Anal. ($C_{12}H_{15}NO$) C, H, N.

***N*-Ethyl-2-methylene-5-phenylpentanamide (53)** was prepared in a similar manner from ethylamine and crystallized from light petroleum ether: yield 77%; mp 39–41 °C. Anal. ($C_{14}H_{19}NO$) H, N; C: calcd, 77.38, found, 76.17.

***N*,*N*-Diethyl-2-methylene-5-phenylpentanamide (54)** was prepared similarly from diethylamine: yield 69%; bp 112–115 °C (0.001 mm). Anal. ($C_{16}H_{23}NO$) C, H, N.

Pharmacology. Groups of 6–12 male rats (Sprague–Dawley SIV-50 or Mus Rattus, body weight 160–200 g) were fasted overnight. Using an oral tube, the substances were administered as neutral, aqueous solutions or emulsions (ratio 1:1) with Cremophor EL (BASF, Germany) and water. Administration volume in all cases was 10 mL/kg of body weight at a dose range of 2.5 to 600 μmol/kg of body weight. Blood samples were collected by puncturing the retro-orbital plexus prior to and at 2, 4, and 6 h after dosing. Blood glucose was measured enzymatically according to the method of Richterich[21] (HK/G6PDH method). The results were expressed as changes relative to the control group and, in some cases, as the effective dose [ED_{50} (25%)], calculated from the dose–response relationship by linear or log-linear regression with data obtained from at least four different doses. ED_{50} (25%) means that 50% of the animals showed at least a 25% decrease in the blood glucose level compared to the controls.

Acute Toxicity. Groups of five female mice (NMR strain, 22–26 g) received water ab libitum, and food was reduced to 50 g/kg of body weight 18 h prior to dosing. The test compounds were administered orally. The LD_{50} was calculated according to the method of Lichtfield and Wilcoxon.[22]

Acknowledgment. The authors are indebted to Pro-

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Aminomethyl-1,2,4-benzothiadiazines as Potential Analogues of γ-Aminobutyric Acid. Unexpected Discovery of a Taurine Antagonist

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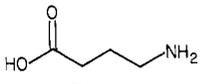
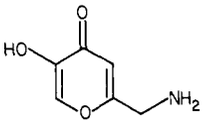
A series of 6- and 8-(aminomethyl)-4*H*-1,2,4-benzothiadiazine 1,1-dioxides has been synthesized and tested for interaction with various GABA systems. None of the compounds showed significant GABA-mimetic properties, but unexpectedly, compound **7** [6-(aminomethyl)-3-methyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide] possessed the properties of a selective antagonist of taurine, as measured by the antagonism of taurine-induced inhibition of rat cerebellar Purkinje firing.

Ever since the discovery of its presence in the nervous system,[2] γ-aminobutyric acid (GABA) has been the object



GABA



kojic amine

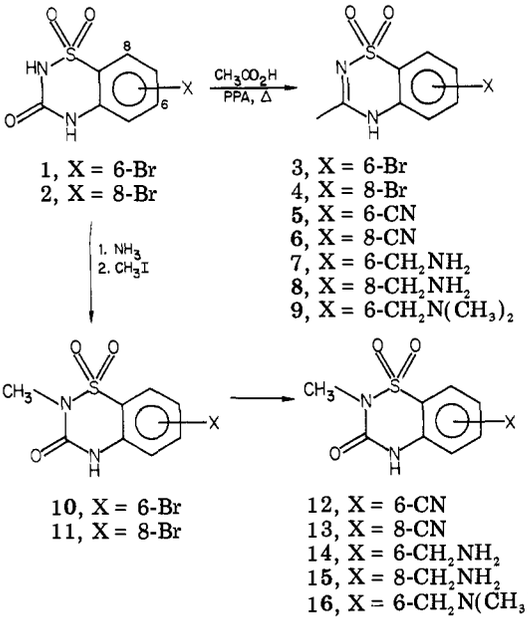
of intense and continuous research in efforts to unravel its various functions, its role in the pharmacological action of a number of drugs acting through the central nervous system (CNS), and as a basis for the development of new and useful drugs.[3] One of the most fruitful approaches to the study of the action of GABA has been the synthesis of relatively rigid molecules of known, restricted conformations as discussed recently by O'Donnell, Johnson, and Azzaro.[4]

We have previously reported on the synthesis and muscle-relaxant activity of the planar GABA analogue kojic amine,[5] and others have reported on further aspects of its pharmacology and mechanism of action.[6,7] In the present article we report the synthesis of a series of aminomethyl-1,2,4-benzothiadiazines (**7–9**, **14–16**; Scheme I), which like kojic amine, incorporate the elements of an acidic and a basic center in a rigid, planar framework.

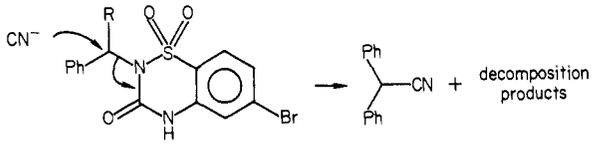
Of further encouragement in considering these structures, in analogy with kojic amine, was the fact that the 1,2,4-benzothiadiazine structure has a weakly acidic pK_a value of about 9,[8] similar to the value of 8 found for kojic amine.[5] In addition, several lines of evidence point to the tautomer with the proton located on N(4) as being the predominant form in solution,[9,10] which places the acidic and basic centers in the target compounds the same distance apart as found in GABA.

Chemistry. We have recently reported[11] a new one-step synthesis of 1,2,4-benzothiadiazines, which provided the basis for the present syntheses. At that time we made the incidental observation that the 2*H*-1,2,4-benzothiadiazine-3(4*H*)-one 1,1-dioxide system could be converted directly to the 3-methyl-4*H*-1,2,4-benzothiadiazine 1,1-dioxide structure by simply heating in a mixture of acetic acid and hydrochloric acid. This "side reaction" was turned to advantage when it was found that the bromo

Scheme I



Scheme II



17, R = H
18, R = Ph

compounds **1** and **2** (as a mixture) could be converted to a mixture of **3** and **4** (Scheme I) in 65% yield by heating

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