observed. The fact that the dihydro derivatives bind 50 times less effectively than the substrate or product does not support any transient formation of a noncovalently bound dihydro intermediate in thymidylate synthetase catalysis. Furthermore, these results suggest that the dihydro forms of the substrate or product do not occur as free intermediates in the reaction.

Experimental Section

IR spectra were measured with a Beckmann IR-33, UV spectra with a Cary 219 recording spectrophotometer, and NMR spectra with a Varian Model EM-360 or T-60. Microanalyses (±0.4% of theory) were obtained from a Hewlett-Packard 185B at the Department of Medicinal Chemistry, University of Kansas. The starting nucleotides were purchased from Sigma Chemical Co., St. Louis, Mo.; DEAE-cellulose was the product of Whatman Biochemicals, Ltd.

Thymidylate synthetase purified from methotrexate-resistant *Lactobacillus casei* was purchased from the New England Enzyme Center, Tufts University, at a specific activity of 0.91 μmol of dTMP formed min^{-1} (mg of protein)$^{-1}$. The substrate 2′-deoxy[5-^{3}H]uridine 5′-monophosphate at a specific activity above 15 Ci/mmol was purchased from Moravek Biochemicals, Industry, Calif., and diluted with cold substrate purchased from Sigma Chemical Co. The cofactor *dl*-tetrahydrofolic acid was also purchased from Sigma Chemical Co.

5,6-Dihydro-2′-deoxyuridine 5′-[Bis(triethylammonium) Phosphate] (2). 2′-Deoxyuridine 5′-(disodium phosphate)·2.5H_2O (200 mg, 0.39 mmol) was dissolved in 20 mL of water, and the pH of the solution was adjusted to 2 with 1 N HCl. Rhodium catalyst (5% rhodium on alumina, 50 mg) was added and the flask flushed with nitrogen for 10 min. The hydrogenation was done at atmospheric pressure for 5 h; within 1 h, almost all of the calculated hydrogen was consumed. After removal of catalyst by centrifugation, the solution was lyophilized to a glassy solid residue. The solid material was dissolved in 3 mL of water and applied on a 2.5 × 50 cm column of DEAE-cellulose. Gradient elution was performed using 0.01 and 0.3 M of triethylammonium bicarbonate (pH 7.5). The corresponding fractions (retention volume 530 mL) were collected and lyophilized to give a white solid, which was dried at 60 °C for 24 h to give 150 mg (55%) of the product **2** as a hygroscopic powder: UV λ_{max} (H_2O) 208 nm; NMR (D_2O) δ 6.2 (t, 1 C_1-H), 4.6–3.4 (m, 6), 3.2 (q, 12, NCH_2), 2.9–1.9 (m, 4), 1.25 (t, 18, CH_2CH_3). Anal. Calcd for $C_{21}H_{45}N_4O_8P$·1.5H_2O: C, 46.74; H, 8.97; N, 10.38. Found: C, 46.65; H, 8.95; N, 10.20.

5,6-Dihydro-2′-deoxythymidine 5′-[Bis(triethylammonium) Phosphate] (3). Using the same procedure as described in the synthesis of **2**, 2′-deoxythymidine 5′-(disodium phosphate)·2H_2O (150 mg) was hydrogenated with 80 mg of 5% rhodium on alumina at pH 2.0 for 15 h. The same treatment as described for **2** gave 70 mg (56%) of the product **3** as a hygroscopic powder: UV λ_{max}(H_2O) 208 nm; NMR (Me_2SO-d_6) δ 6.15 (t, 1, C_1-H), 3.8–3.3 (m, 6), 3.0 (q, 12, NCH_2), 2.4–1.6 (m, 3), 1.2 (apparent t, 21, NCH_2CH_3 and $CHCH_3$). Anal. Calcd for $C_{22}H_{47}N_4O_8P$·2.5H_2O: C, 46.22; H, 9.17; N, 9.80. Found: C, 46.05; H, 9.18; N, 9.62.

Enzyme Assay. The enzyme was assayed by a modification of the radioisotope assays described by Roberts[17] and Lomax and Greenberg.[18] The solution, 0.1 mL, contained 25 mM mercaptoethanol; 0.22 mM *dl*-tetrahydrofolic acid; 6.75 mM formaldehyde; 5 mM sodium bicarbonate; 3 mM magnesium chloride; 0.12 mM EDTA; 6 mM Tris–acetate buffer (pH 6.8); 5 μL of the diluted enzyme solution (0.1 μg); substrate; and, when indicated, inhibitor. Control reactions lacked the cofactor, tetrahydrofolic acid. The substrate 2′-deoxy[5-^{3}H]uridine 5′-monophosphate was used at a specific activity of 500 μCi/μmol. The assays were started by the addition of the enzyme to the complete mixture and then incubated at 30 °C. Incubation was stopped at 30 s by the addition of 50 μL of 20% trichloroacetic acid. A 20% aqueous suspension of charcoal (0.25 mL) was added, and the solution was vortexed and allowed to stand for 15 min. The suspension was filtered through a glass-wool plugged Pasteur pipette, and 0.1 mL of the filtrate was counted in a scintillation fluid containing 0.5% 2,5-diphenyloxazole and 10% Beckman BBS-3 solubilizer in toluene. Counting efficiency was 33%; control samples lacking the cofactor were found to have less than 5% of the respective sample counts.

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References and Notes

(1) M. Friedken, *Adv. Enzymol. Relat. Areas Mol. Biol.*, **38**, 235 (1973).
(2) D. V. Santi and C. S. McHenry, *Proc. Natl. Acad. Sci. U.S.A.*, **69**, 1855 (1972).
(3) R. I. Langenbach, P. V. Danenberg, and C. Heidelberger, *Biochem. Biophys. Res. Commun.*, **48**, 1565 (1972).
(4) See P. V. Danenberg, *Biochim. Biophys. Acta*, **473**, 73 (1977), for a review of this topic.
(5) M. P. Mertes, C. T.-C. Chang, E. DeClercq, G.-F. Huang, and P. F. Torrence, *Biochem. Biophys. Res. Commun.*, **84**, 1054 (1978).
(6) W. E. Cohen and D. G. Doherty, *J. Am. Chem. Soc.*, **78**, 2863 (1956).
(7) C. Janion and D. Shugar, *Acta Biochim. Pol.*, **7**, 309 (1960).
(8) R. B. Dunlap, N. G. L. Harding, and F. M. Huennekens, *Biochemistry*, **10**, 88 (1971).
(9) T. C. Crusberg, R. Leary, and R. L. Kisliuk, *J. Biol. Chem.*, **245**, 5292 (1970).
(10) H. H. Daron and J. L. Aull, *J. Biol. Chem.*, **253**, 940 (1977).
(11) R. S. Wilson and M. P. Mertes, *Biochemistry*, **12**, 2879 (1973).
(12) R. E. Cline, R. M. Fink, and K. Fink, *J. Am. Chem. Soc.*, **81**, 2521 (1959).
(13) A. M. Michelson, *Synth. Proced. Nucleic Acid Chem.* 1, 491–492 (1968).
(14) G.-F. Huang and P. F. Torrence, *J. Org. Chem.*, **42**, 3821 (1977).
(15) A. H. Alegria, *Biochim. Biophys. Acta*, **149**, 317 (1967).
(16) R. Wolfenden, *Annu. Rev. Biochem.*, **5**, 271 (1976).

Preparation and Antidiabetic Activity of Some Sulfonylurea Derivatives of 3,5-Disubstituted Pyrazoles

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Four series of *p*-[3,5-dimethyl- (and 5-methyl-3-carboxy-) pyrazole-1]benzenesulfonylureas, thioureas, 2-thiohydantions, and 5,6-dihydro-4(3*H*)-oxo-2(1*H*)-pyrimidinethiones were prepared for evaluation as hypoglycemic agents. Biological testing of these compounds showed that some possessed antidiabetic activity.

Dulin, Gerritsen, and co-workers showed that 3,5-dimethylpyrazole and its active metabolite 5-methylpyrazole-3-carboxylic acid had a potent hypoglycemic activity.[1-5] Later, they found that several 3,5-dimethylpyrazoles possess hypoglycemic activities as great as 100 times that of tolbutamide in glucose-primed intact rats.

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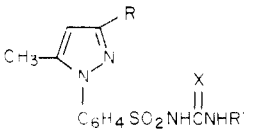
Table I. 3,5-Disubstituted Pyrazolesulfonylureas and Pyrazolethioureas



compd	R	R′	X	proced[a]	yield, %	mp, °C	formula	hypoglycemic act. % decrease vs. control, at 2 and 4 h[b]
12	CH_3	C_2H_5	O	A, B	70	157-158	$C_{14}H_{18}N_4O_3S$	<1
13	CH_3	$CH_2CH_2CH_3$	O	A, B	72	160-161	$C_{15}H_{20}N_4O_3S$	<1
14	CH_3	$(CH_2)_3CH_3$	O	A, B	75	147-148	$C_{16}H_{22}N_4O_3S$	2
15	CH_3	C_6H_{11}	O	A, B	80	185-186	$C_{18}H_{24}N_4O_3S$	10
16	CH_3	$CH_2C_6H_5$	O	B	66	188-189	$C_{19}H_{20}N_4O_3S$	<1
17	CH_3	$NHC(NH)NH_2$	O	B	82	200-201	$C_{13}H_{17}N_5O_3S$	3
18	COOH	C_2H_5	O	A, C	65	239-240	$C_{14}H_{16}N_4O_5S$	0
19	COOH	$CH_2CH_2CH_3$	O	A, C	68	235-236	$C_{15}H_{18}N_4O_5S$	1
20	COOH	$(CH_2)_3CH_3$	O	A, C	72	198-199	$C_{16}H_{20}N_4O_5S$	2
21	COOH	C_6H_{11}	O	A, C	78	224-225	$C_{18}H_{22}N_4O_5S$	1
22	COOH	$CH_2C_6H_5$	O	C	60	160-161	$C_{19}H_{18}N_4O_5S$	0
23	CH_3	C_2H_5	S		55	147-148	$C_{14}H_{18}N_4O_2S_2$	0
24	CH_3	$CH_2CH=CH_2$	S		52	171-172	$C_{15}H_{18}N_4O_2S_2$	-1
25	CH_3	$(CH_2)_2CH_3$	S		62	143-144	$C_{15}H_{20}N_4O_2S_2$	<1
26	CH_3	$(CH_2)_3CH_3$	S		68	135-136	$C_{16}H_{22}N_4O_2S_2$	1
27	CH_3	C_6H_{11}	S		79	174-175	$C_{18}H_{24}N_4O_2S_2$	<2
28	CH_3	$CH_2C_6H_5$	S		80	134-135	$C_{19}H_{20}N_4O_2S_2$	0
29	CH_3	C_6H_5	S		76	225-226	$C_{18}H_{18}N_4O_2S_2$	-3
30	COOH	C_2H_5	S		60	145-146	$C_{14}H_{16}N_4O_4S_2$	-2
31	COOH	$CH_2CH=CH_2$	S		65	136-137	$C_{15}H_{16}N_4O_4S_2$	-6
32	COOH	$(CH_2)_2CH_3$	S		69	140-141	$C_{15}H_{18}N_4O_4S_2$	<1
33	COOH	$(CH_2)_3CH_3$	S		73	134-135	$C_{16}H_{20}N_4O_4S_2$	<1
34	COOH	C_6H_{11}	S		77	170-171	$C_{18}H_{22}N_4O_4S_2$	1
35	COOH	$CH_2C_6H_5$	S		73	154-155	$C_{19}H_{18}N_4O_4S_2$	<2
36	COOH	C_6H_5	S		68	225-226	$C_{18}H_{16}N_4O_4S_2$	0

[a] See Experimental Section. [b] See biological testing method in text. Alloxan-diabetic mice were used. Dose; phenformin, 1.0 mmol/kg body weight of mice; test compounds, 0.4 mmol/kg body weight of mice. A negative value indicates a rise in blood glucose; phenformin = 21.

The present study was undertaken in an attempt to prepare compounds analogous to, but varying in structure from, the arylsulfonylurea derivatives. The proposed compounds might provide valuable information concerning the structural requirements for hypoglycemic activity. Therefore, the synthesis of some sulfonylurea derivatives of 3,5-dimethylpyrazoles and their expected metabolites, 5-methylpyrazole-3-carboxylic acids, was undertaken. Also, the evaluation of the antidiabetic activity of these compounds was carried out.

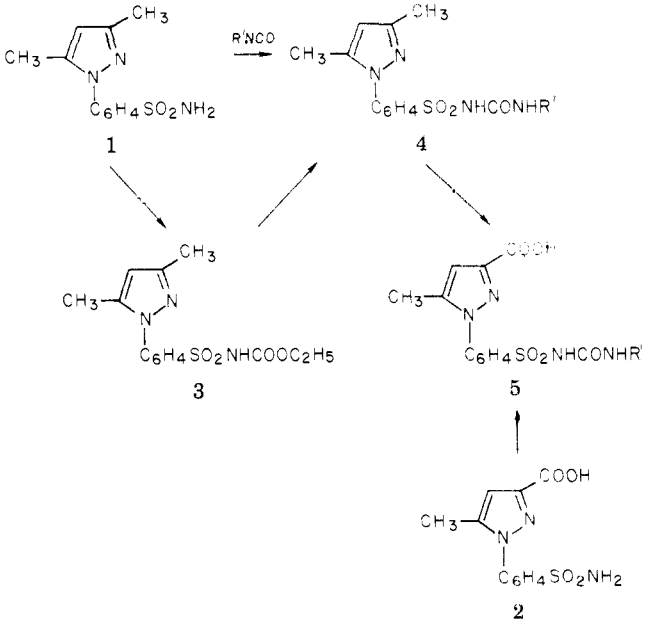
Three main series of 3,5-disubstituted pyrazole derivatives were synthesized. The first series was designed to simulate the clinically useful group of the open-chain benzenesulfonylurea derivatives (Table I). In the second and third series (Tables II and III), the cyclic thio analogues were synthesized in order to study the effect of fixing the open chain of thiourea derivatives in less mobile structures.

Chemistry. The new pyrazoles that were prepared are listed in Tables I-III, together with their antidiabetic activity.

1-(*p*-Sulfamylphenyl)-3,5-dimethylpyrazole (1) was prepared by treating *p*-sulfamylphenylhydrazine with an equivalent amount of 2,4-pentanedione. Alkaline potassium permanganate oxidation of 1 afforded 1-(*p*-sulfamylphenyl)-5-methylpyrazole-3-carboxylic acid (**2**).

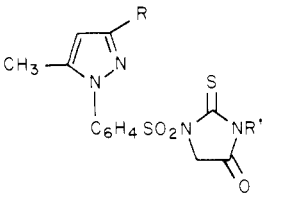
Substituted *p*-(3,5-dimethylpyrazole-1)benzenesulfonylurea derivatives (**4**, Scheme I) were prepared either by (method A; see Experimental Section) reaction between 1 and an alkyl isocyanate[6] or (method B; see Experimental Section) reaction of 1 with ethyl chloroformate,[7] followed by treatment of the resulting sulfonylcarbamate **3** with the appropriate amine.[8]

Scheme I



Substituted *p*-(5-methyl-3-carboxypyrazole-1)benzenesulfonylurea derivatives (**5**, Scheme I) were prepared either by (method A) reaction between **2** and an alkyl isocyanate or (method C; see Experimental Section) treatment of **4** with powdered potassium permanganate in dry acetone.

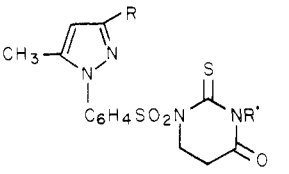
Table II. 3,5-Disubstituted Pyrazole-1-benzenesulfonyl-2-thiohydantoins



compd	R	R′	yield, %	mp, °C	formula	hypoglycemic act. % decrease vs. control, at 2 and 4 h[a]
37	CH_3	C_2H_5	65	128-129	$C_{16}H_{18}N_4O_3S_2$	<1
38	CH_3	$CH_2CH=CH_2$	60	110-111	$C_{17}H_{18}N_4O_3S_2$	4
39	CH_3	$(CH_2)_2CH_3$	68	114-115	$C_{17}H_{20}N_4O_3S_2$	<1
40	CH_3	$(CH_2)_3CH_3$	70	108-109	$C_{18}H_{22}N_4O_3S_2$	1
41	CH_3	C_6H_{11}	80	170-171	$C_{20}H_{24}N_4O_3S_2$	2
42	CH_3	$CH_2C_6H_5$	76	139-140	$C_{21}H_{20}N_4O_3S_2$	1
43	CH_3	C_6H_5	82	188-189	$C_{20}H_{18}N_4O_3S_2$	8
44	COOH	C_2H_5	63	108-109	$C_{16}H_{16}N_4O_5S_2$	<1
45	COOH	$CH_2CH=CH_2$	67	112-113	$C_{17}H_{16}N_4O_5S_2$	<1
46	COOH	$(CH_2)_2CH_3$	70	125-126	$C_{17}H_{18}N_4O_5S_2$	1
47	COOH	$(CH_2)_3CH_3$	75	111-112	$C_{18}H_{20}N_4O_5S_2$	1
48	COOH	C_6H_{11}	78	171-172	$C_{20}H_{22}N_4O_5S_2$	1
49	COOH	$CH_2C_6H_5$	66	105-106	$C_{21}H_{18}N_4O_5S_2$	<1
50	COOH	C_6H_5	80	229-230	$C_{20}H_{16}N_4O_5S_2$	

[a] See biological testing method in text. Alloxan-diabetic mice were used. Dose; phenformin and test compounds, 1.0 mmol/kg body weight of mice; phenformin = 21.

Table III. 3,5-Disubstituted Pyrazole-1-benzenesulfonyl-5,6-dihydro-4(3*H*)-oxo-2(1*H*)-pyrimidinethiones



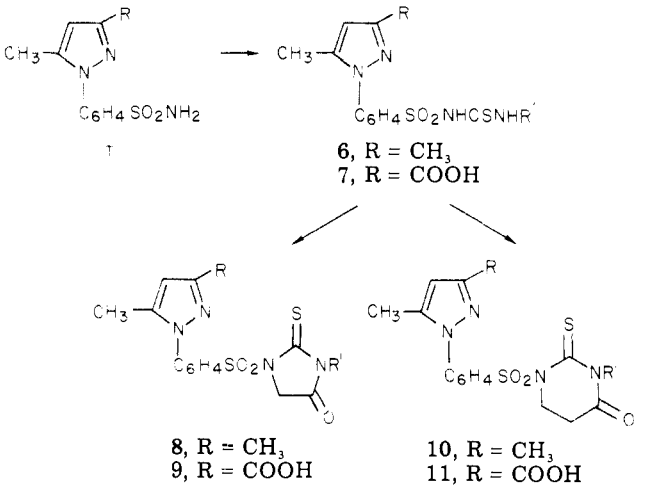
compd	R	R′	yield, %	mp, °C	formula	hypoglycemic act. % decrease vs. control, at 1 and 3 h[a]
51	CH_3	C_2H_5	70	180-181	$C_{17}H_{20}N_4O_3S_2$	4
52	CH_3	$CH_2CH=CH_2$	65	154-155	$C_{18}H_{20}N_4O_3S_2$	
53	CH_3	$(CH_2)_2CH_3$	68	146-147	$C_{18}H_{22}N_4O_3S_2$	1
54	CH_3	$(CH_2)_3CH_3$	76	132-133	$C_{19}H_{24}N_4O_3S_2$	1
55	CH_3	C_6H_{11}	80	168-169	$C_{21}H_{26}N_4O_3S_2$	4
56	CH_3	$CH_2C_6H_5$	72	152-153	$C_{22}H_{22}N_4O_3S_2$	18
57	CH_3	C_6H_5	83	231-232	$C_{21}H_{20}N_4O_3S_2$	<1
58	COOH	C_2H_5	66	224-225	$C_{17}H_{18}N_4O_5S_2$	1
59	COOH	$CH_2CH=CH_2$	64	219-220	$C_{18}H_{18}N_4O_5S_2$	0
60	COOH	$(CH_2)_2CH_3$	70	140-141	$C_{18}H_{20}N_4O_5S_2$	<1
61	COOH	$(CH_2)_3CH_3$	75	136-137	$C_{19}H_{22}N_4O_5S_2$	2
62	COOH	C_6H_{11}	78	169-170	$C_{21}H_{24}N_4O_5S_2$	2
63	COOH	$CH_2C_6H_5$	72	152-153	$C_{22}H_{20}N_4O_5S_2$	16
64	COOH	C_6H_5	77	233-234	$C_{21}H_{18}N_4O_5S_2$	1

[a] See biological testing method in text. Alloxan-diabetic mice were used. Dose; phenformin and test compounds, 1.0 mmol/kg body weight of mice; phenformin = 21.

Substituted *p*-[3,5-dimethyl- (and 5-methyl-3-carboxy-) pyrazole-1]benzenesulfonylthiourea derivatives (**6** and **7**, Scheme II) were prepared by treatment of **1** and **2** with the appropriate isothiocyanate.

3-Substituted 1-*p*-[3,5-dimethyl- (and 5-methyl-3-carboxy-) pyrazole-1]benzenesulfonyl-2-thiohydantions (**8** and **9**, Scheme II) and 3-substituted 1-*p*-[3,5-dimethyl- (and 5-methyl-3-carboxy-) pyrazole-1]benzenesulfonyl-5,6-dihydro-4(3*H*)-oxo-2(1*H*)-pyrimidinethiones (**10** and **11**, Scheme II) were prepared by refluxing an alcoholic solution of the appropriate thiourea derivative with ethyl bromoacetate and ethyl 3-bromopropionate, respectively. The intermediate pseudothio derivatives underwent rapid rearrangement with the formation of 2-thiones. The structures of the latter were confirmed by the characteristic violet color developed with cold alkaline sodium nitroprusside solution[9] and showed the characteristic absorption bands in the infrared spectra at 1120–1140 cm^{-1} indicative of C=S and at 1300 cm^{-1} indicative of C–N (amide III bands). A further confirmation for the cyclic structures (**8**, **9**, **10** and **11**) can be achieved by refluxing these compounds with freshly prepared mercuric oxide in ethanol to give the corresponding 2-oxo derivatives.[10,11] Also, it is known that the pseudothiohydantions that are unsubstituted at the 5 position possess a highly active methylene group and readily react with aromatic aldehydes,[12] nitrous acid, or alkyl nitrites.[13] No such reactions were obtained with the series prepared.

Scheme II



Biological Testing Method. The compounds listed in Tables I–III were tested for hypoglycemic activity using alloxanized female albino mice weighing 20 g. Alloxan, 100 mg/kg of body weight, was injected into the tail vein in 10 mg/mL saline solution. Six days later the mice were given test compounds orally in suspension in 1% carboxymethylcellulose solution at the rate of 1.0 or 0.4 mmol/kg of body weight.

Each day, two groups of six mice were used as controls, and one group of six mice was given the standard drug, phenformin. Up to six groups of six mice received test compounds. Blood samples were taken at either 0, 2, and 4 h or 0, 1, and 3 h.

Blood was collected into 0.04% NaF solution. Glucose was determined by the automated glucose oxidase method of Tinder.

Results are expressed as a percentage compared to the control value. Statistical significance for compounds **15**, **56**, and **63**: $p < 0.01$.

Statistical Treatment of Results. All results are expressed as the mean ± SEM. The statistical significance of the difference between the mean of two differently treated groups of mice was determined using Student's t test. Statistically significant differences were assumed to be present if the calculated value for "t" was greater than the tabulated value for "t" at the 0.05 level of p.

Results and Discussion

The combination of the 3,5-dimethylpyrazole or 5-methylpyrazole-3-carboxylic acid moiety, respectively, with functional groups of sulfonylureas provided compounds with hypoglycemic activity in alloxan-diabetic mice. Compounds **15**, **56**, and **63** (Tables I–III) showed significant hypoglycemic activity. This finding is of interest, since 3,5-dimethylpyrazole is effective in alloxan-diabetic animals while sulfonylureas (e.g., tolbutamide) are not. The potency of the new compounds, however, is less than that of phenformin. As in regards to structure–activity relationships, it is known that variation in the substituents attached to the terminal urea nitrogen can exert a profound influence on the hypoglycemic activity.[14] In the first series, the cyclohexyl group (compound **15**) showed the highest activity as compared to the other substituents. This is in accordance with the reported structure–activity relationships, where the group attached to the terminal urea nitrogen should be of certain size and lipophilicity.

In the second and third series, it appears that there is a remarkable difference in activity between the five- and six-membered rings. The highest activity was demonstrated in compound **56** as compared to compound **42**. It seems that the six-membered ring had provided better spatial configuration for the substituent to exert its biological activity.

Experimental Section

Melting points were taken in open capillary tubes in a Gallenkamp melting point apparatus and are uncorrected. Infrared spectra were measured as Nujol mulls with a Beckman IR-4210. ^{1}H NMR spectra were determined using a Perkin-Elmer R 12 spectrometer and the mass spectra using an AE1 MS9 spectrometer. Microanalyses were performed by the Microanalytical Unit, Faculty of Science, Cairo, Egypt. Analyses for the elements C, H, N, and S were within ±0.4% of the calculated figures for the molecular formula provided, except where noted.

***p*-Sulfamylphenylhydrazine Hydrochloride.** A cold, stirred mixture of sulfanilamide (34.2 g, 0.2 mol), hydrochloric acid (100 mL) and crushed ice (200 g) was diazotized by the dropwise addition of sodium nitrite (14 g, 0.2 mol) in water (25 mL) over 30 min. The cold diazonium salt solution thus formed was rapidly added to a well-cooled solution of stannous chloride (100 g) in hydrochloric acid (150 mL) with vigorous stirring, and the resulting mixture was left in the ice box overnight. The precipitated *p*-sulfamylphenylhydrazide hydrochloride was collected at the pump and dried: white crystals; mp 225 °C (lit.[15] mp 225 °C); yield 39 g (88%).

1-(*p*-Sulfamylphenyl)-3,5-dimethylpyrazole (1). A mixture of *p*-sulfamylphenylhydrazine (18.7 g, 0.1 mol) and 2,4-pentanedione (10 g, 0.1 mol) was mixed throughly while cooling. After 15 min, the solid mass which formed was crystallized from acetic acid as colorless prisms: mp 241 °C (lit. 239.5–241.5 °C); yield 20 g (80%).

1-(*p*-Sulfamylphenyl)-5-methylpyrazole-3-carboxylic acid (2). A stirred mixture of 1 (25.1 g, 0.1 mol) and sodium hydroxide (6 g) in water (250 mL) was treated with powdered potassium permanganate (30 g) over 4 h with stirring and cooling (temperature should not exceed 20 °C), stirring was continued for a further 2 h, and the mixture was left standing overnight. The mixture was then filtered, decolorized with charcoal, and neutralized with 2 N hydrochloric acid, and the product thus obtained was separated and crystallized from ethanol as colorless needles: mp 234–235 °C; yield 21 g (75%). Anal. ($C_{11}H_{11}N_3O_4S$) C, H, N, S.

Ethyl *p*-(3,5-Dimethylpyrazole-1)benzenesulfonylcarbamate (3). A mixture of 1 (25.1 g, 0.1 mol), ethyl chloroformate (15 g, 0.13 mol), and anhydrous potassium carbonate (20 g) in dry acetone (150 mL) was refluxed with stirring for 18 h. Acetone was removed under reduced pressure, the residue was dissolved in water (150 mL) and neutralized with 2 N hydrochloric acid, and the product which formed was separated and crystallized from ethanol as colorless needles: mp 195–196 °C; yield 22.5 g (70%). Anal. ($C_{14}H_{17}N_3O_4S$) C, H, N, S.

***p*-(3,5-Dimethylpyrazole-1)benzenesulfonylureas (4). Method A.** A mixture of 1 (0.05 mol) and anhydrous potassium carbonate (0.1 mol) in dry acetone (100 mL) was stirred and refluxed for 1.5 h. At this temperature there was then added, at a dropwise rate, a solution of the appropriate alkyl isocyanate (0.075 mol) in dry acetone (20 mL). After stirring and refluxing the mixture overnight, acetone was removed under reduced pressure and the solid residue was dissolved in water. The crude product was isolated by acidification with 2 N hydrochloric acid and purified by recrystallization from ethanol.

Method B. To a warm solution of **3** (0.02 mol) in toluene (75 mL) was added, dropwise, with stirring a solution of the desired amine (0.022 mol) in toluene (25 mL). The mixture was refluxed for 3 h and cooled. If the product crystallized out, it was separated; otherwise, the toluene was removed under reduced pressure. The product was then crystallized from dilute ethanol, with acidification with 1 N hydrochloric acid just prior to cooling. Further crystallization was carried out from ethanol.

***p*-(5-Methyl-3-carboxypyrazole-1)benzenesulfonylureas (5). Method C.** A stirred mixture of **4** (0.01 mol) in dry acetone (100 mL) was treated with powdered potassium permanganate (3 g) over 1 h, stirring was continued for further 2 h, and the mixture was left standing overnight. The mixture was then filtered, the residue was washed with hot ethanol, and the

combined filtrate and washing was concentrated and allowed to crystallize.

p-[3,5-Dimethyl- (and 5-methyl-3-carboxy-) pyrazole-1]benzenesulfonylthioureas (6 and 7). A mixture of 1 or 2 (0.05 mol) and anhydrous potassium carbonate (0.1 mol) in dry acetone (100 mL) was stirred and treated with the appropriate isothiocyanate (0.06 mol). After stirring and refluxing the mixture for 10 h, acetone was removed under reduced pressure, and the solid mass thus obtained was dissolved in water and acidified with 2 N hydrochloric acid. The crude product isolated was purified by recrystallization from dilute ethanol.

1-p-[3,5-Dimethyl- (and 5-methyl-3-carboxy-) pyrazole-1]benzenesulfonyl-2-thiohydantions (8 and 9). A mixture of 6 or 7 (0.01 mol) and ethyl bromoacetate (0.011 mol) in absolute ethanol (50 mL) was refluxed with stirring for 2 h, concentrated, and allowed to crystallize. The products obtained were recrystallized from ethanol.

1-p-[3,5-Dimethyl- (and 5-methyl-3-carboxy-) pyrazole-1]benzenesulfonyl-5,6-dihydro-4(3H)-oxo-2(1H)-pyrimidinethiones (10 and 11). A mixture of 6 or 7 (0.01 mol) and ethyl 3-bromopropionate (0.011 mol) in absolute ethanol (60 mL) was refluxed with stirring for 2 h, concentrated, and allowed to crystallize. The products obtained were recrystallized from ethanol.

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References and Notes

(1) J. B. Wright, W. E. Dulin, and J. H. Markillie, *J. Med. Chem.* **7**, 102 (1964).
(2) G. C. Gerritsen and W. E. Dulin, *Diabetes*, **14**, 507 (1965).
(3) D. L. Smith, A. A. Forist, and W. E. Dulin, *J. Med. Chem.*, **8**, 350 (1965).
(4) G. C. Gerritsen and W. E. Dulin, *J. Pharmacol. Exp. Ther.*, **150**, 491 (1965).
(5) D. L. Smith, A. A. Forist, and G. C. Gerritsen, *J. Pharmacol. Exp. Ther.*, **150**, 316 (1965).
(6) F. J. Marshall, M. V. Sigal, and M. A. Root, *J. Med. Chem.*, **6**, 60 (1963).
(7) F. J. Marshall and M. V. Sigal, *J. Org. Chem.*, **23**, 927 (1958).
(8) J. Mills and F. J. Marshall, U.S. patent, 3 155 721; *Chem. Abstr.*, **62**, 3978 (1965).
(9) I. W. Grote, *J. Biol. Chem.*, **93**, 25 (1931).
(10) E. Schmidt, *Arch. Pharm. (Weinheim, Ger.)*, **254**, 626 (1916); **255**, 338 (1917) [*Chem. Abstr.*, **12**, 1552 (1917)].
(11) F. Mehler, *Chem. Abstr.*, **19**, 57 (1921).
(12) H. L. Wheeler and G. S. Jamieson, *J. Am. Chem. Soc.*, **25**, 366 (1903).
(13) Walther and Roch, *J. Prakt. Chem.*, **2**, 27 (1913).
(14) A. Bander, "Oral Hypoglycemic Agents", New York, Academic Press, 1969, p 23.
(15) K. Itano, *Yakugaku Zasshi*, **75**, 441 (1955).

Differential Solubilities in Subregions of the Membrane: A Nonsteric Mechanism of Drug Specificity

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We studied the effect of four volatile anesthetics and convulsants on the GABA- and glutamate-induced conductance change in crab muscle. The intensity of drug action correlated with the *solubility parameter* (δ) values of the four drugs. Thus, the higher that value was for a given drug, the stronger was its effect on the glutamate response but the weaker was it on the response to γ-aminobutyric acid (GABA). We suggest that different gating molecules are housed in specific subregions of the membrane, each characterized by a particular value of the solubility parameter. The differential distribution of drugs in these subregions may be a nonsteric mechanism for drug specificity.

It has been proposed that the volatile anesthetics and convulsants and perhaps other simple compounds act on the nervous system by dissolving in the hydrophobic core of the membrane, thus causing volume expansion and disordering of membrane lipids and possibly proteins.[1-5] This view dwells on the assumption that such drugs do not act on specific binding sites because they lack structural specificity and fail to show evidence for saturable binding to membranes.[6] Yet, the remarkable specificity of these agents in causing either anesthesia or convulsions has remained a challenge to the current theory. We have recently shown[7] that the potent convulsant fluothyl, $CF_3CH_2OCH_2CF_3$, blocks preferentially the response of the γ-aminobutyric acid (GABA) receptor in crab muscle fibers, whereas the anesthetic methoxyflurane, $CHCl_2CF_2OCH_3$, blocks preferentially that of the glutamate receptor in the same fiber. We now report on a correlation between the relative potency of four fluorinated ethers at each of these sites and their solubility parameters (δ).[8,9] On the strength of this and earlier experimental[10,11] and theoretical[12] data, we propose a model for a nonsteric mechanism of drug specificity.

Experimental Section

Experiments were performed on the adductor muscle fiber cells from the walking legs of the crab, *Ocypoda cursor*, in vitro. The procedure has been presented in greater detail in an earlier publication.[7] The preparation was continuously perfused with medium alone or medium containing the desired drug, at a temperature of 22 ± 2 °C. Muscle cells were impaled with two microelectrodes, one for voltage recording and the other for current injection. Hyperpolarizing current pulses of 100–500 nA for 100–200 ms were used to measure the effective input impedance of the cells. The voltage, V, and current, I, traces were suitably amplified and recorded. Membrane conductance, G, was computed as $G = I/V$ and was normally of the order of 100 kmho. The addition of glutamate (5×10^{-5} M) to the bath resulted in depolarization and a conductance increase 1.5 to 2.5 times that of the control. The addition of GABA (5×10^{-5} M) was not followed by a consistent change in membrane potential but induced a conductance change of the same order as that induced by glutamate. Typical intracellular recordings have been published earlier.[7] In addition to fluothyl and methoxyflurane, two more drugs of intermediate δ values have now been submitted to the same evaluation: fluroxene, $CF_3CH_2OCH{=}CH_2$ ($\delta = 7.77$), and enflurane, $CHFClCF_2OCHF_2$ ($\delta = 8.26$).

Results and Discussion

There exists a correlation between the solubility parameter of a drug and its relative effect on the conductance change mediated by each of glutamate and GABA (Figure 1). The higher the value of δ, the more powerful is the effect on the glutamate response but the weaker it is on the GABA response. The opposite is true for low δ values. This finding is consistent with earlier results implicating

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