°C; $^1$H NMR ($CDCl_3$) δ 8.68 (1 H, d), 9.38 (1 H, d); MS, *m/e* 189 ($M^+$). Anal. ($C_4H_2ClN_3O_2$) C, H, N.

**Methyl 6-chloro-3-nitropyrazinoate (22)** was prepared from **19** as described above for **21**. Crude **22** was purified by flash chromatography on silica gel with 2% methanol/chloroform to afford a yellow oil in 51% yield: $^1$H NMR ($CDCl_3$) δ 4.08 (3 H, s), 8.75 (1 H, s); MS, *m/e* 217 ($M^+$).

**5-[(1,3,4-Trihydroxybutyl)amino]-2-nitropyrazine (23).** To 0.5 g (0.003 mol) of **20** in 10 mL of 2-propanol were added 0.36 g (0.003 mol) of 2-amino-1,3,4-butanetriol and 0.3 g (0.003 mol) of triethylamine in 10 mL of acetonitrile. After the mixture was stirred at room temperature for 18 h, the solvent was removed on the rotary evaporator, and the residue was flash chromatographed on silica gel (230–400 mesh) with 20% methanol/chloroform elution. The product ($R_f$ 0.4) was an oil and was triturated with methanol/ether to give a yellow solid: mp 133–135 °C; $^1$H NMR ($Me_2SO$-$d_6$) δ 3.33 (5 H, br s), 3.51 (1 H, m), 8.02 (1 H, s), 8.28 (1 H, d), 8.95 (1 H, s); MS, *m/e* 244 ($M^+$). Anal. ($C_8H_{12}N_4O_5$) C, H, N.

**2-[(2,3-Dihydroxypropyl)amino]-3-nitropyrazine (24).** To a solution of 1.5 g (0.0094 mol) of **21** and 1.01 g (0.01 mol) of triethylamine in 25 mL of 2-propanol was added 0.91 g (0.01 mol) of 3-amino-1,2-propanediol. After stirring at room temperature, the crude reaction mixture showed a single major yellow spot in TLC ($R_f$ 0.5) with 10% methanol/chloroform. The solvent was removed on the rotary evaporator, and the residue was purified by flash chromatography on silica gel with 8% methanol/chloroform to afford 1.7 g (83%) of **24** as a yellow solid: mp 100–102 °C; $^1$H NMR ($Me_2SO$-$d_6$) δ 3.50 (5 H, m), 4.65 (1 H, t), 5.03 (1 H, d), 7.85 (1 H, d), 8.33 (1 H, t), 8.58 (1 H, d) MS, *m/e* 214 ($M^+$). Anal. ($C_7H_{10}N_4O_4$) C, H, N.

**6-[(2,3-Dihydroxypropyl)amino]-3-nitropyrazinoate (25).** To 2.0 g (0.008 mol) of **22** in 60 mL of 2-propanol at room temperature were added 0.72 g (0.008 mol) of 3-amino-1,2-propanediol and 0.8 g (0.008 mol) of triethylamine. After the mixture was stirred for 16 h, the solvent was removed on the rotary evaporator, and the residue was flash chromatographed on silica gel (230–400 mesh) with 10% methanol/chloroform elution. The product had $R_f$ 0.4 and was isolated as a yellow solid (1.3 g, 60%): mp 107–109 °C; $^1$H NMR ($CD_3OD$) δ 3.57 (5 H, m), 3.92 (3 H, s), 4.70 (2 H, br s), 7.87 (1 H, s); MS, *m/e* 272 ($M^+$). Anal. ($C_9H_{12}N_4O_6$) C, H, N.

**Acknowledgment.** G.D.H. thanks Dr. Edward L. Engelhardt for suggesting this problem and for providing guidance throughout the course of the work. N.R.J., P.W., M.E.W., and M.W. thank the Cancer Research Campaign for financial support.

**Registry No.** **3**, 4853-52-5; **4**, 87155-51-9; **5**, 88793-46-8; **6**, 86845-62-7; **7**, 87155-52-0; **8**, 89083-22-7; **9**, 89690-74-4; **10**, 89083-18-1; **14**, 33332-29-5; **15**, 6863-73-6; **16**, 1458-03-3; **17**, 87885-44-7; **18**, 86536-74-5; **19**, 86536-76-7; **20**, 87885-45-8; **21**, 87885-43-6; **22**, 89690-75-5; **23**, 89690-76-6; **24**, 87885-48-1; **25**, 87885-53-8; **26**, 55153-71-4; $NH_2CH_2CH(OH)CH_2OH$, 616-30-8; $NH_2(CH_2)_2OH$, 141-43-5; *N*-methylpiperazine, 109-01-3; 2-amino-1,3,4-butanetriol, 83168-64-3.

# Pyrido[3′,2′:4,5]thieno[3,2-*d*]-N-triazines: A New Series of Orally Active Antiallergic Agents

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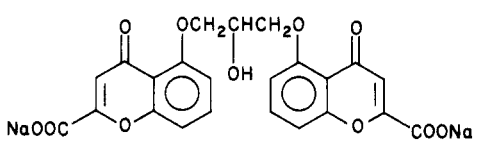
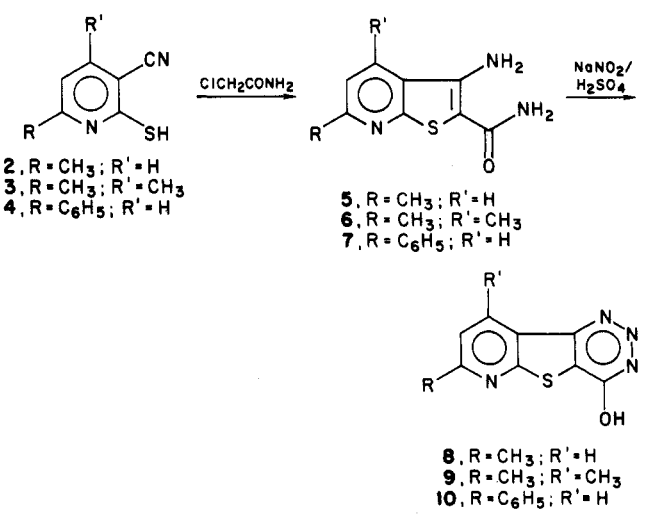
A new series of orally active mediator release inhibitors, pyrido[3′,2′:4,5]thieno[3,2-*d*]-*N*-triazines, was synthesized and evaluated for antiallergic activity. Several products showed high activity as inhibitors or wheal information in the rat passive cutaneous anaphylaxis screen and as inhibitors of histamine release from passively sensitized rat mast cells. Many compounds were orally active in the PCA test. The most potent compound, 7-phenylpyrido-[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazin-4(3*H*)-one (**10**) with an $I_{50}$ value of 0.05 μM, was 60 times more potent than disodium cromoglycate (DSCG) in the RMC assay.

Cromolyn sodium (DSCG, I) is a well-established drug that inhibits release of the mediators of anaphylaxis[1] and thus provides a prophylactic treatment of asthma. The main disadvantage of DSCG is that it is not orally effective and thus must be used as an insufflated powder. A desirable compound should show DSCG-like capacity to inhibit allergy-induced mast cell and be orally effective. Since the discovery of DSCG,[1] there have been intensive efforts in numerous laboratories to find orally active DSCG-like antiallergic agents. Such agents should certainly have an important place in the therapy of asthma and other allergic disorders. Most of the orally active compounds reported are carboxylic acids or derivatives




I

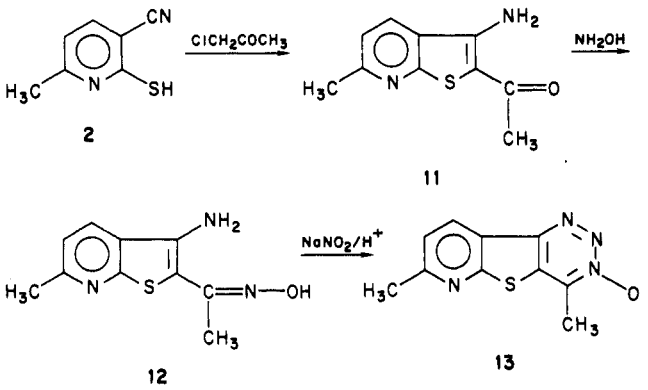
**Scheme I**



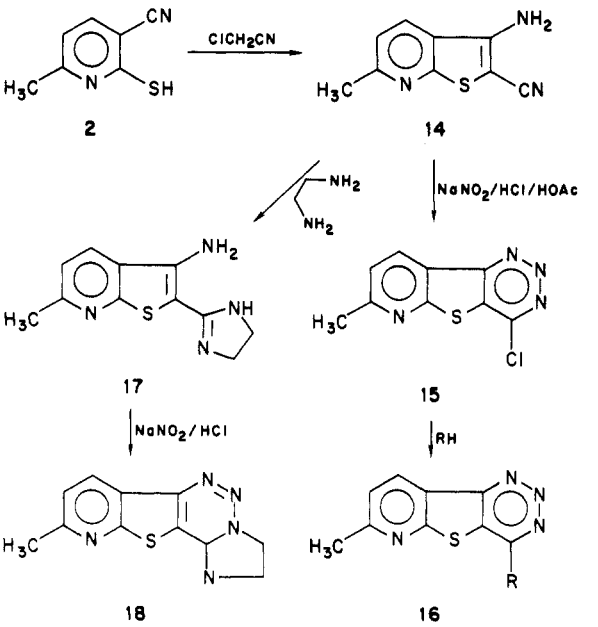

†Department of Medicinal Chemistry.
‡Department of Biochemistry.
§Department of Pharmacology.

0022-2623/84/1827-1639$01.50/0 

**Scheme II**



**Scheme III**



thereof, such as esters or tetrazoles.[2] There are, however, a few exceptions that lack these functionalities.[3]

In this paper we describe the synthesis of a new series of several pyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazines[4] that exhibit oral antiallergy activity. These compounds that lack carboxylic acid derivatives are potent inhibitors of the anaphylactically induced histamine release from rat peritoneal mast cells (RMC) and are orally active as inhibitors of IgE-mediated passive cutaneous anaphylaxis in the rat (PCA).

**Chemistry.** Diazonium ion condensation with an adjacent nucleophilic function to form a five- or six-membered ring has proved valuable for synthesizing various nitrogen heterocycles. Among these are numerous 1,2,3-triazines[5] that are formed via intramolecular attack of an electrophilic nitrogen function. Thus 1,2,3-triazin-4-ones, 4-chloro or 3-oxides, are obtained from the condensation of diazonium ion with an adjacent carboxamide,[6] cyanide,[7] or ketoxime,[8] respectively.

As part of a study designed to investigate tricyclic heterocyclic arrays possessing a pyridothiophene nucleus, it became important to utilize these reactions to prepare a series of new pyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazines. These compounds were synthesized by the routes shown in the Schemes I–III.

The starting 3-cyano-2-mercaptopyridines (**2**–**4**) were readily obtained by a previously described procedure.[9] Condensation of **2** with chloroacetonitrile and chloroacetone led to **14**[10] and **11**,[10] respectively. In this manner, **2**, **3**, and **4** were condensed with chloroacetamide to give **5**, **6**, and **7**, respectively.

Diazotization of **5** with sodium nitrate in concentrated $H_2SO_4$–HOAc gave the triazin-4(3*H*)-one **8** (Scheme I). Treatment of **11** with hydroxylamine (Scheme II) gave the corresponding ketoxime **12**, which, on diazotization, led to the 3-oxide **13**, mp 224–225 °C.

Preparation of **15** utilizing standard reagents such as phosphorous oxychloride, thionyl chloride, and phosphorous trichloride led to decomposition of **8**, apparently owing to the instability of the triazine ring under these conditions. Nevertheless, as shown in Scheme III, **15** was easily obtained from diazotization of an acetic acid solution of **14** with sodium nitrate in concentrated hydrochloric acid. Treatment of the 4-chloro **15** with various nucleophiles such as alkoxide ions, amines, and hydrazines led to normal halide displacement to other 4-substituted derivatives (**16**).

Attempts to synthesize the 4-unsubstituted **16** (R = H) from catalytic hydrogenation of the 4-chloro **15** were unsuccessful. Compound **16** (R = H) was ultimately prepared by conversion of **15** to the hydrazine **21** followed by mercuric oxide oxidation.

Methylation of the thieno[3,2-*d*]-*N*-triazin-4(3*H*)-one with methyl iodide has been reported[11] to produce the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(4) (a) A single compound in this series, pyrido[3′,2′:4,5]thieno-[3,2-*d*]-*N*-triazin-4(3*H*)-one, has been reported by Schneller and Clough: Schneller, S. W.; Clough, F. W. *J. Hetrocycl. Chem.* **1975**, *12*, 513. (b) Youssefyeh, R. D.; Wilson, J. D. *U.S. Patent* 4 239 887; *Chem. Abstr.* **1981**, *94*, 139849t.

(5) Erickson, J. G.; Wiley, P. E.; Wynstrach, "The 1,2,3- and 1,2,4-Triazines, Tetrazines and Pentazines"; Interscience: New York, 1956; pp 1–41.

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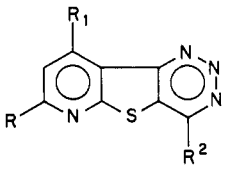
(10) Neiss, R.; Elingsfeld, H. Off. 2 241 717.

(11) Schneller, S. W.; Clough, F. W. *Heterocyles* **1975**, *3*, 135.

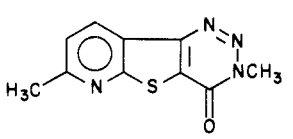
**Table I.** Inhibition of RMC and Rat PCA by Pyridothienotriazines



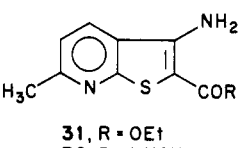
| no. | R | $R_1$ | $R_2$ | RMC[a] $I_{50}$, μM | RMC[a] % I at 100 mg/kg | PCA (po)[a] $ED_{50}$, mg/kg | PCA (po)[a] % I at 25 mg/kg |
|---|---|---|---|---|---|---|---|
| **8** | $CH_3$ | H | OH | 0.25 | | 5.2 | 88[d] |
| **9** | $CH_3$ | $CH_3$ | OH | 80 | | 3.6 | 70[d] |
| **10** | $C_6H_5$ | H | OH | 0.05 | | 3.0 | 68[b,d] |
| **19** | $CH_3$ | H | $NH_2$ | 1.7 | | | 38[e] |
| **20** | $CH_3$ | H | $NHCH_3$ | 11.0 | | | 32[e] |
| **22** | $CH_3$ | H | $NHNHCO_2CH_3$ | | 21 | | 5 |
| **23** | $CH_3$ | H | $OC_2H_5$ | | 18 | 27 | 52[e] |
| **25** | $CH_3$ | H | $O(CH_2)OC_2H_5$ | 6.0 | | | 22[e] |
| **27** | $CH_3$ | H | SH | 0.1 | | | |
| **28** | $CH_3$ | H | $SCH_3$ | | 4 | | |
| **29** | $CH_3$ | H | H | 29 | | 1.5 | 93[c,d] |

[a] All compounds were screened for activity at a single concentration (RMC, 100 μM) or dose level (PCA, 25 mg/kg po). Only those compounds showing 50% inhibition in each screen were retested in a concentration or dose–response assay; for these compounds only $I_{50}$ or $ED_{50}$ values are shown. [b] 30 mg/kg. [c] 18 mg/kg. [d] $p < 0.001$. [e] $p < 0.01$.

3-methylated product. By the same procedure, the triazin-4(3*H*)-one **8** was converted to **30**. To verify the structure of **30**, this compound was synthesized by diazotization[12] of **32**, which was obtained from **31**.



**30**



**31**, R = OEt
**32**, R = $NHCH_3$

The synthetic application of condensation of diazonium ion with an adjacent nitrogen function was extended to the synthesis of the tetracyclic **18**, which was prepared by the diazotization of the intermediate **17**.

## Biological Results and Discussion

Studies of the pyridothienotriazines (Table I) as inhibitors of antigen-induced release of histamine (AIR) in vitro from rat peritoneal mast cells (RMC) showed four pyridothienotriazins (**8**, **10**, **19**, and **27**) were more potent inhibitors of AIR than DSCG ($I_{50}$ = 3 μM) when added simultaneously with antigen. None of the 11 compounds tested had any activity when *pre*incubated for 5 min with RMC prior to the addition of antigen. Compound **10** was the most potent compound of the series and, with an $I_{50}$ value of 0.05 μM, was 60 times more potent than disodium cromoglycate (DSCG) as an inhibitor of AIR.

Eight compounds of this series were tested orally for inhibition of passive cutaneous anaphylaxis (PCA) in the rat, either at a single dose (three compounds, **19**, **22**, and **25**, 25 mg/kg po) or at multiple dose levels (five compounds, **8**–**10**, **23**, and **27**). In this test, **29** was the most potent member of the series ($ED_{50}$ = 1.5 mg/kg po); however, it was virtually inactive in vitro, suggesting in vivo metabolism to an active form. Compound **10** was also an active inhibitor of PCA ($ED_{50}$ = 3 mg/kg). Other compounds with reasonably low oral $ED_{50}$ values were **8** (5.2 mg/kg) and **9** (3.6 mg/kg).

The parent compound **29** had no effect on AIR but was an orally potent inhibitor of the IgE-mediated passive cutaneous anphylaxis in the rat (PCA). Substitution of the hydrogen in the 4-position by an hydroxy (**8**), amino (**19**), ethoxyethyl (**25**), methylamino (**20**), or a sulfhydryl group (**27**) resulted in compounds that inhibited AIR from RMC. However, replacement of the 4-hydrogen by an ethoxy (**23**), chloro (**15**), methyl carbazate (**22**), or methylthio group (**28**) did not result in compounds capable of inhibiting AIR. Replacement of the 7-methyl (**8**) with a phenyl group (**10**) resulted in a compound that was more portent as an inhibitor of AIR from RMC and also oral PCA.

We have described the preparation of a new series of pyrido[3',2':4,5]thieno[3,2-*d*]-1,2,3-triazines. Most of these compounds possess significant antiallergic activity with a mechanism of action similar to that of DSCG. Compounds **8**, **10**, and **29**, which are of high interest as potential antiallergic agents for the prophylaxis of asthma, have been extensively studies.[14]

## Experimental Section

The structures of all compounds were supported by their NMR spectra, which were measured with a Varian Model EM-390 spectrometer using tetramethylsilane as internal standard. Mass spectra were obtained on a Varian Model MAR-112 mass spectrometer. A Cary Model 219 instrument was used for UV spectra measurement. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are not corrected. All compounds were analyzed for C, H, and N and were within 0.4% of calculated theoretical values.

**3-Amino-2-carbamoyl-6-methylthieno[2,3-*b*]pyridine (5).** To a solution of 100 g (1.85 mol) of sodium methoxide in 1200 mL of methanol was first added 135 g (0.9 mol) of 3-cyano-2-mercapto-6-methylpyridine[9] (**2**) and then 90 g (0.967 mol) of chroroacetamide, and the mixture was refluxed for 6 h. It was cooled, filtered, washed with water, and dried to give 122 g (66%) of **5**: mp 240–241 °C; NMR ($Me_2SO$) δ 2.59 (s, 3 H), 7.31 (d, 1 H), 8.40 (d, 1 H); MS, *m/e* 207 ($M^+$, 100). Compounds **7**, **11**, and **14** were prepared by the same procedure (Table II).

**7-Methylpyrido[3',2':4,5]thieno[3,2-*d*]-1,2,3-triazin-4-(3*H*)-one (8).** To a cold solution of 2.7 g (0.04 mol) of sodium nitrite in 65 mL of concentrated sulfuric acid was slowly added a suspension of 8 g (0.039 mol) of 3-amino-2-carbamoyl-6-methylthieno[2,3-*b*]pyridine[10] in 250 mL of acetic acid. Stirring

(12) (a) Henricksen, L.; Autrup, H. *Acta Chim. Scand.* **1972**, *26*, 3342. (b) Beck, J. R.; Yahner, J. A. *J. Org. Chem.* **1973**, *38*, 2450.

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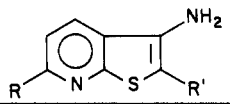
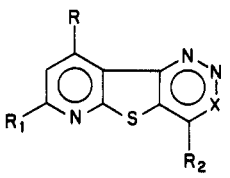
(14) Khanduala, A.; Can Inwegen, R.; Coutts, S.; Dally-Meader, V.; Youssefyeh, R. D. *Int. J. Immunopharmacol.* **1983**, *5*, 491.

**Table II.** Substituted Thieno[2,3-*b*]pyridines



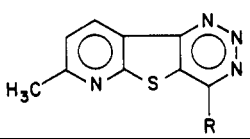
| no. | R | $R^1$ | reagent | reactant | mp, °C | recrystn solvent | % yield |
|---|---|---|---|---|---|---|---|
| 7 | $C_6H_5$ | $CONH_2$ | $ClCH_2CONH_2$ | 4 | 275–278 | $DMF/H_2O$ | 37 |
| 11 | $CH_3$ | $COCH_3$ | $ClCH_2COCH_3$ | 2 | 186–187 | ether | 51 |
| 14 | $CH_3$ | CN | $ClCH_2CN$ | 2 | 143–145 | $DMF/H_2O$ | 74 |

**Table III.** Substituted Pyrido[3′,2′:4,5]thieno[3,2-*b*]-1,2,3-triazines



| no. | R | $R_1$ | $R_2$ | X | reactant | reagent | mp, °C | recrystn solvent |
|---|---|---|---|---|---|---|---|---|
| 9 | $CH_3$ | $CH_3$ | OH | N | 6 | $NO_2$ | 207 | $DMF/H_2O$ |
| 10 | H | $C_6H_5$ | OH | N | 7 | $NO_2$ | 180–183 | $Me_2SO$ |
| 13 | H | $CH_3$ | $CH_3$ | NO | 12 | $NO_2$ | 224–225 | $EtOH/H_2O$ |

**Table IV.** 4-Substituted 7-Methylpyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazines



| no. | R | reagent | mp, °C | recrystn solvent | % yield |
|---|---|---|---|---|---|
| 19 | $NH_2$ | $NH_3$ | 300 | HOAc/ether | 92 |
| 21 | $NHNH_2$ | $NH_2NH_2$ | 218 | dioxane/$H_2O$ | 91 |
| 22 | $NHNHCO_2CH_3$ | $NHNHCO_5CH_3$ | 210–211 | MeOH/ether | 68 |
| 23 | $OC_2H_5$ | $NaOC_2H_5$ | 171–173 | 2-propanol | 40 |
| 24 | $OCH_3$ | $NaOCH_3$ | 190–191 | 2-propanol | 59 |
| 25 | $OCH_2CH_2OC_2H_5$ | $NaOCH_2CH_2OC_2H_5$ | 126–127 | hexane | 23 |
| 26 |  |  | 218–220 | ethanol | 32 |
| 27 | SH | thiourea | 211–214 | NaOH/HOAc | 36 |

was continued for 1 additional hour. It was then filtered and the filtrate was poured on ice. The crude product was filtered, dissolved in 5% sodium hydroxide, treated with charcoal, and acidified with acetic acid, giving 3.1 g (37%) of **8**: mp 215–216 °C; NMR ($CF_3CO_2D$) δ 3.23 (s, 3 H), 8.29 (s, 1 H), 9.62 (d, 1 H). By a similar diazotization procedure, compounds **9**, **10**, and **13** were prepared (Table III).

**4-Chloro-7-methylpyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (15).** To a cold solution of 4.2 g (0.022 mol) of **14** in 30 mL of concentrated hydrochloric acid and 30 mL of acetic acid was added a solution of 1.9 g (0.028 mol) of sodium nitrite in 20 mL of water. After completion of addition, the ice bath was removed and stirring continued for 2 more hours. The crude product was crystallized from DMF–$H_2O$, giving 2.9 g (56%) of **15**: mp 188–189 °C; MS, *m/e* 236 ($M^+$), 201 (100); UV $\lambda_{max}^{EtOH}$ 231 μM (ε 21 352), 249 (14 591), 279 (25 623), 288 (11 566), 322 (4270); NMR ($CF_3CO_2D$) δ 3.27 (s, 3 H), 8.28 (d, 1 H), 9.67 (d, 1 H).

**7-Methyl-4-(methylamino)pyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (20).** To a solution of 5 g (0.02 M) of **15** in 500 mL of ethanol at 70 °C was bubbled methylamine gas for 1 h. After cooling to room temperature, the mixture was evaporated to dryness, diluted with water, and filtered, giving 4.2 g of crude product. Crystallization from acetic acid–ether gave 2.5 g (51%) of **20**: mp 258–260 °C; MS, *m/e* 231 ($M^+$); UV $\lambda_{max}^{EtOH}$ 232 μM (ε 1898) 242 (21 390), 283 (21 685), 320 (5294); NMR ($CF_3CO_2D$) δ 3.23 (s, 3 H), 3.56 (s, 3 H), 8.31 (d, 1 H), 9.54 (d, 1 H). Starting with the intermediate **15**, compounds **19** and **21–27** were prepared by the same method (Table IV).

**4-(Methylthio)-7-methylpyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (28).** To a solution of 5 g (0.02 mol) of **27** in 400 mL of 5% sodium hydroxide was added 5 mL of methyl iodide, and the mixture was stirred for 2 h. The solid precipitate that formed was collected and crystallized from ethanol, giving 2.6 g (49%) of **28**: mp 197–198 °C; UV $\lambda_{max}^{EtOH}$ 238 μM (ε 25 240), 250 (17 572), 295 (25 240); NMR ($CDCl_3$) δ 2.76 (s, 3 H), 2.98 (s, 3 H), 2.45 (d, 1 H), 8.75 (d, 1 H).

**7-Methylpyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazine (29).** A mixture of 10 g of **21** and 11.5 g of mercuric oxide in 700 mL of water was refluxed for 4 h. The reaction mixture was then cooled and filtered. The crude product was dissolved in methylene chloride treated with charcoal, filtered, and evaporated to dryness. After recrystallization from 2-propanol, 1.2 g (14%) of **29** was obtained: mp 264–265 °C; NMR ($CF_3CO_2D$) δ 3.28 (s, 3 H), 8.31 (d, 1 H), 9.75 (d, 1 H), 10.36 (s, 1 H).

**3,7-Dimethylpyrido[3′,2′:4,5]thieno[3,2-*d*]-1,2,3-triazin-4-one (30).** To a mixture of 6 g (0.028 mol) of **8** and 12 g of potassium carbonate in 150 mL of DMF at 75 °C was added 6 mL of methyl iodine and stirring at this temperature was continued for 3 h. The mixture was then diluted with water and the solid precipitate collected by filtration. Crystallization with chloroform gave 2.2 g (34%) of **30**: mp 169–171 °C; MS, *m/e* 232 ($M^+$); UV $\lambda_{max}^{EtOH}$ 236 μM (ε 32 870), 276 (13 426), 310 (8889); NMR ($CDCl_3$) δ 2.76 (s, 3 H), 4.10 (s, 3 H), 7.42 (d, 1 H), 8.60 (d, 1 H).

**2-(2-Imidazolin-2-yl)-3-amino-6-methylthieno[2,3-*h*]-pyridine (17).** To a mixture of 15 g (0.08 mol) of **14** and 9.5 mL of ethylenediamine was slowly added 1.6 mL of carbon disulfide

and this reaction was heated on a steam bath for 6 h. It was then diluted with water and the solid precipitate was filtered. Crystallization from ethanol gave 14.2 g (77%) of **17**: mp 240–242 °C; MS, *m/e* 232 ($M^+$, 100); NMR ($Me_2SO$) δ 2.61 (s, 3 H), 3.54 (4 H), 7.29 (d, 1 H), 8.30 (d, 1 H).

**9-Methyl-2,3-dihydroimidazo[1,2-*c*]pyrido[3′,2′:4,5]thieno[2,3-*c*]-1,2,3-triazine (18).** To an ice-cold suspension of 6.6 g (0.028 mol) of **17** in 100 mL of 5% hydrochloric acid was slowly added a solution of 0.94 g (0.028 mol) of sodium nitrite in 30 mL of water. After completion of addition, the ice bath was removed and stirring continued for 45 min. It was then neutralized with saturated $NaHCO_3$ and cooled in a freezer. The solid that separated was collected, washed with water, and dried. It was dissolved in chloroform, treated with charcoal, and filtered. Upon concentration of the solution, 1.9 g (28%) of **18** was obtained: mp 216–218 °C; MS, *m/e* 243 ($M^+$), 200 (100); NMR ($CDCl_3$) δ 2.72 (s, 3 H), 4.31 (m, 4 H), 7.32 (d, 1 H), 8.43 (d, 1 H).

**Inhibition of Histamine Release from Passively Sensitized Rat Mast Cells (RMC).**[15] RMC were passively sensitized in vitro with rat antiovalbumin serum. Spontaneous histamine release (SR in the absence of antigen) and AIR (in the presence of antigen) from these passively sensitized RMC were measured after 15 min of incubation. Both the histamine released into the incubation medium and the residual histamine extracted from the RMC were measured fluorometrically with a Technicon AutoAnalyzer. Both SR and AIR are expressed as a percent of the total extractable histamine in the RMC. Net AIR was obtained by subtracting the SR from histamine released in the presence of antigen. The effect of the test compound on both SR and AIR was determined.

Test compounds were added simultaneously with antigen. The activity of the test compound is expressed as a percent inhibition of AIR or as the $I_{50}$ value (concentration of the test compound required to inhibit AIR by 50%). Test compounds were dissolved in $Me_2SO$ (final concentration of $Me_2SO$ was 0.17% and did not affect AIR).

**Passive Cutaneous Anaphylaxis in the Rat (PCA).**[16] The effect of compounds on IgE-mediated cutaneous wheal formation in the rat was determined by a modification of the method of Watanabe and Ovary (1977).[16] Antiserum for these studies was prepared according to the following immunization protocol. Male Sprague–Dawley rats (approximately 250 g) were injected intramuscularly on days 0, 2, and 4 with 10 μg of ovalbumin and 20 mg of aluminum hydroxide (Amphojel) in 1 mL of saline. On day 0 each rat was given $10^9$ *Bordatella pertussis* organisms by the intraperitoneal route. Rats were exsanguinated on day 8.

The method of passive cutaneous anaphylaxis was a follows. Naive rats were sensitized at dorsal sites by intradermal injection of the syngeneic IgE antiovalbumin antiserum (1:20 dilution). After a latency period of 48 h to allow cytophilic antibodies to bind to the cutaneous mast cells, groups of four rats were given either vehicle (1% methylcellulose, 3 mL) or graded doses of compound. Rats were challenged intravenously with antigen (4 mg of ovalbumin) in 1% Evans blue dye 10 min after oral administration of the test compound. Thirty minutes after antigen challenge, the rats were sacrificed by cervical dislocation, the dorsal skins reflected, and blued wheal areas measured. Mean values ± SD for wheal areas in control and drug-treated groups were determined and compared statistically by using the Student's *t* test.

**Acknowledgment.** We thank Dr. C. Rehm and his staff in the Department of Analytical Chemistry for mass spectra and analysis data.

**Registry No.** **2**, 3395-04-8; **3**, 54585-47-6; **4**, 92126-72-2; **5**, 59488-61-8; **6**, 67795-42-0; **7**, 76993-15-2; **8**, 76993-12-9; **9**, 76993-13-0; **10**, 76993-14-1; **11**, 52505-52-9; **12**, 92126-73-3; **13**, 88722-19-4; **14**, 52505-53-0; **15**, 76993-16-3; **16** (R = H), 76993-29-8; **17**, 92126-74-4; **18**, 92126-75-5; **19**, 76993-18-5; **20**, 76993-17-4; **21**, 76993-19-6; **22**, 76993-20-9; **23**, 76993-22-1; **24**, 76993-23-2; **25**, 76993-24-3; **26**, 92184-38-8; **27**, 76993-25-4; **28**, 76993-26-5; **29**, 76993-29-8; **30**, 76993-27-6; **31**, 52505-51-8; **32**, 59488-62-9.

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# Effects of Volume and Surface Property in Hydrolysis by Acetylcholinesterase. The Trimethyl Site

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β-Substituted ethyl acetates, $XCH_2CH_2OCOCH_3$, have been prepared, and their hydrolysis by acetylcholinesterase has been studied. Log of enzymic reactivity, normalized for intrinsic reactivity in hydrolysis by hydroxide, log $(k_{cat}/K_m)_n$, rises linearly with increasing refraction volume, MR (or $R_D^{25}$), for substrates with β-X = H, Cl, Br, $CH_3CH_2$, $(CH_3)_2CH$, $(CH_3)_2S^+$, $(CH_3)_3N^+$, and $(CH_3)_3C$. Larger substituents may be accommodated, $(CH_3)_3Si$ and $(CH_3CH_2)_3N^+$, with no further increase in rate. Substrates with β-substituents $CH_3S$, $CH_3S(O)$, $(CH_3)_3N^+(OH)$, and $CH_3S(O_2)$ are less reactive than consistent with the relation with MR by factors of 5–40, indicating that hydrophobic surface and desolvation of the substrate–enzyme interface may be necessary for maximum reactivity correlated with MR. Values of log $(k_{cat}/K_m)_n$ for substrates with β-substituents X = $CH_3S$, Cl, Br, $CH_3CH_2$, $(CH_3)_2CH$, $(CH_3)_3C$, and $(CH_3)_3Si$ rise linearly with increasing hydrophobicity, π, but reactivity of substrates with X = $(CH_3)_3N^+$ and $(CH_3)_2S^+$ are more reactive than consistent with a relation to π by factors of 300 and 40 and with X = $CH_3S(O_2)$, $CH_3S(O)$, and $(CH_3)_2N^+(OH)$, by factors of 7–100. Reactivity appears related to (i) volume of the β-substituent and its fit in its subsite, which is *trimethyl* rather than *anionic*, and (ii) the hydrophobicity of its surface.

Although the part of the active site of acetylcholinesterase at which the trimethylammonium group of acetylcholine, $(CH_3)_3N^+CH_2CH_2OCOCH_3$, binds has generally been considered and depicted as *anionic*,[1-3] our recent studies indicate that it may not contain a specific negative charge and may be better considered *trimethyl*, as complementary to the trimethyl-substituted character of the β-substituent rather than to its positive charge.[4] This was proposed when the enzymic reactivity of a series of β-substituted ethyl acetates, $XCH_2CH_2OCOCH_3$, with cat-

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0022-2623/84/1827-1643$01.50/0 