Co.), and 10 ml of $CHCl_3$ were refluxed for 3 hr. Solvent was removed *in vacuo*. The residue, 0.15 g of pyridine hydrochloride, 5 ml of H_2O, and 10 ml of EtOH were kept overnight at 25–27°, made alkaline with 10% NaOH, and extracted with $CHCl_3$. The residue left from drying and evaporation of the $CHCl_3$ was acidified (in Et_2O) with dry HCl giving 85 mg of **9**·**HCl**, mp 261–262° (needles from MeOH–Me_2CO). *Anal.* ($C_{12}H_{16}ClN$) C, H. The **picrate** (yellow prisms from MeOH) melted at 171–173°. *Anal.* ($C_{18}H_{18}N_4O_7$) C, H.

N-Demethylation of **8** with BrCN[22] gave, after prolonged hydrolysis of the N-cyano intermediate with 6% HCl, 2-cyano-6,7-benzomorphan (λ_{max}^{Nujol} 4.5 μ), 2-carbamido-6,7-benzomorphan (λ_{max}^{Nujol} 5.9 μ), and a 22% yield of **9**. The hydrochloride of **9** and 2-carbamido-6,7-benzomorphan formed a well-characterized "double" compound, mp 165–166°, which was separated into its components by converting to the picrate.

(22) J. von Braun, *Ber.*, **47**, 2312 (1914).

Acknowledgment.—We are indebted to Mr. J. Harrison Ager of the permanent NIH staff and Dr. Robert T. Parfitt, formerly Visiting Associate, now at Glasgow, Scotland, for attempts to synthesize **8**, by some of the synthetic routes mentioned in footnote 7.

Irreversible Enzyme Inhibitors. CLII.[1,2] Proteolytic Enzymes. X.[3] Inhibition of Guinea Pig Complement by Substituted Benzamidines

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The inhibition of guinea pig complement was investigated with 54 amidines, alkylamines, aralkylamines, and guanidines previously synthesized for inhibition of trypsin; based on these results, 15 new candidate inhibitors were synthesized. The best inhibitors were derived from benzamidine, the latter being a fair inhibitor. Inhibition by benzamidine was considerably enhanced by *meta* substituents such as isoamyloxy (**18**), phenoxypropyloxy (**22**), and *p*-acetamidophenylbutyl (**27**). Of 28 *para*-substituted benzamidines, only the benzamidine with an $O(CH_2)_4OC_6H_4$-*p*-$NHCONHC_6H_3$-2-OMe-5-SO_2F substituent (**37**) showed good inhibition. The most potent inhibitor in the literature, maleopimaric acid (**2**), showed about 50% inhibition at 0.5 m*M*; the same concentration of **18**, **22**, and **27** showed 50% inhibition, whereas only 0.062 m*M* of **37** was required. However, maleopimaric acid showed better total inhibition than the three benzamidines when the concentrations of the four compounds were increased.

Among the myriad of serum proteases involved in a number of disease states[4] is the complement system utilized for lysis of bacterial, protozoan, or foreign mammalian cells.[5–7] Complement consists of nine components which arise from eleven distinct proteins, all of which are required for cell lysis.[6] Since cell lysis begins with the combining of an antibody with a foreign cell which then triggers the complement system, two avenues for inhibition of rejection of organ or tissue transplants are available. Either antibody formation[8] or the function of the complement system could be inhibited. Inhibition of antibody formation has the disadvantage that the complement system for control of bacterial infection also cannot operate and infection becomes a serious problem with organ transplantation. Inhibition of the complement system could have two disadvantages: (a) there are a variety of other serum proteases that might be inhibited giving serious side reactions,[4] and (b) the function of the complement system for controlling bacterial infection may also be inhibited. There is little doubt that suppression of antibodies will not be selective with respect to bacterial infection; there is reasonable doubt that the complement systems for lysis of mammalian cells and bacterial cells are identical, since the cell wall composition of bacteria and mammalian cells are so different.

The selective inhibition of the complement system with minimal inhibition of other serum proteases may be possible with active-site-directed irreversible inhibitors that operate by the exo mechanism.[9] These exo-type irreversible inhibitors have an extra dimension of specificity not present with reversible inhibitors, particularly if an area on the enzyme adjacent to the active site[10] is employed for covalent bond formation;[11] thus enzymes closely related mechanistically[12] or even isozymes can be selectively inhibited.[13] At the time we embarked on our studies on proteolytic enzymes, no exo-type irreversible inhibitors of this type were known, although several endo-type irreversible inhibitors had been investigated.[4] Therefore we started studies on two different types of proteolytic enzymes that were readily available, namely trypsin[4] and chymotrypsin,[14] to determine if exo-type irreversible inhibitors could be designed; a variety of irreversible inhibitors of the exo-

(1) This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

(2) For the previous paper of this series see B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **12**, 224 (1969).

(3) For the previous paper on proteolytic enzymes see B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 221 (1969).

(4) For key references see B. R. Baker and E. H. Erickson, *ibid.*, **10**, 1123 (1967).

(5) Ciba Foundation Symposium, Complement, G. E. W. Wolstenholme and J. Knight, Ed., Little, Brown and Co., Boston, Mass., 1965.

(6) For a review see H. J. Müller-Eberhard, *Advan. Immunol.*, **8**, 1 (1968).

(7) P. H. Schur and K. F. Austen, *Ann. Rev. Med.*, **19**, 1 (1968).

(8) G. H. Hitchings and G. B. Elion, *Ann. N. Y. Acad. Sci.*, **129**, 799 (1966).

(9) B. R. Baker, "Design of Active-Site-Directed Irreversible Enzyme Inhibitors. The Organic Chemistry of the Enzymic Active-Site," John Wiley and Sons, Inc., New York, N. Y., 1967.

(10) The active site is defined as those regions of an enzyme necessary for complexing the substrate(s) and catalyzing the reaction; see ref 9, p 188.

(11) See the bridge principle of specificity in ref 9, pp 172–184.

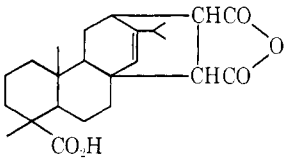
(12) See ref 9, Chapter IX.

(13) (a) B. R. Baker and R. B. Meyer, Jr., *J. Med. Chem.*, **11**, 489 (1968), paper CXIX of this series; (b) B. R. Baker, G. J. Lourens, R. B. Meyer, Jr., and N. M. J. Vermeulen, *ibid.*, **12**, 67 (1969), paper CXXXIII of this series.

(14) B. R. Baker and J. A. Hurlbut, *ibid.*, **10**, 1129 (1967), paper CVII of this series.

type for both trypsin[15] and chymotrypsin[3,16] emerged. These compounds have now been investigated for inhibition of the complement system since the system has both "tryptic" and "chymotryptic" properties;[5,6] the results with the trypsin inhibitors[15] are the subject of this paper and the results with the chymotrypsin inhibitors[3,16] are in the paper that follows.

Inhibition Assay.—The guinea pig complement system, although complex in nature due to the many proteins involved, is relatively simple to assay[17] since all of the components are commercially available. In this study, the lysis of sheep red blood cells by guinea pig complement and guinea pig antibody (hemolysin) was employed; the variety of possible assays[17] were modified slightly as described in the Experimental Section. With the whole complement assay system, it is not possible to discern which of the enzymes in the system are being inhibited; however, the whole complement system serves admirably as a screen for possible active compounds. A variety of inhibitors of the complement system or its components have been described,[5-7] but usually concentrations in the 1–50-m*M* range are required. The most potent are maleopimaric acid (**2**)[18] and a series of irreversible *p*-nitrophenyl phosphonates (**1**);[19] the latter type can phosphorylate the active-site



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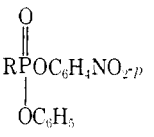

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serine of a number of other enzymes, including trypsin, chymotrypsin, and cholinesterase.

As described in detail in the Experimental Section, 1 ml of solution containing 2.5×10^8 sheep red blood cells (RBC), excess antibody, and sufficient complement to lyse 43–60% of the RBC in 15 min at 37° was quenched with 2.75 ml of citrate–saline.[17] Total lysis gives 0.7 OD unit of hemoglobin; thus the controls should give 0.30–0.42 OD unit. Since many of the compounds were difficultly soluble in water, 5% MeOEtOH was employed as a solvent; no appreciable lysis was caused by this solvent, in contrast to 5% DMSO which gave too much RBC lysis to be useful. RBC lysis catalyzed by complement is not linear with time, but sigmoidal with reasonable linearity at 0.2–0.5 OD unit; the 5% methoxyethanol slowed the reaction somewhat, *i.e.*, the slope of the linear portion of the curve was changed, but was duplicatable.

The data in Tables I–V are recorded as the effect of a given concentration of the compound on complement-catalyzed lysis compared to a control with no compound. In addition to either inhibition of lysis or no effect, another effect was observed; more lysis than the control was sometimes observed which is expressed as a minus amount of inhibition. One cause of more lysis was the lysis of the RBC by the compound in the absence of complement, expressed in the tables as a per cent of the total possible lysis, 0.7 OD unit. When the compound showed no lysis in the absence of complement, but accelerated lysis in the presence of complement, this acceleration was a direct effect on the complement system. Perhaps one of the active components of complement such as the C'4 component was destroyed at a slower rate due to inhibition of the enzyme that normally rapidly destroys the C'4 component of complement.[6,20] Sometimes when lysis was accelerated by the compound, inhibition could be observed at a lower concentration. Although the results on inhibition of the complement system are more difficult to interpret than inhibition of a single enzyme, it is not difficult to see which compounds show inhibition or even which are the most potent inhibitors.

TABLE I

INHIBITION[a,b] OF GUINEA PIG COMPLEMENT BY VARIOUS BASES

No.	Structure	Concn, m*M*	% inhibn[c]	% lysis[d]
3	Benzamidine	3	38	8
		1	15	
4	Phenylacetamidine[e]	3	6	0
5	Hexhydrobenzamidine[f]	3	12	0
6	Phenylguanidine	3	0	0
7	Benzylguanidine	0.25[g]	0	0
8	Phenethylguanidine	3	11	16
9	Phenylbutylguanidine	3	8	0
10	Benzylamine	3[h]	0	0
11	Phenethylamine	3[h]	−8	0
12	Phenylpropylamine	3[h]	−8	0
13	Phenylbutylamine	3[h]	8	0
14	*n*-Butylamine	3[h]	8	9

[a] The technical assistance of Sharon Lafler, Diane Shea, and Susan Black with these assays is acknowledged. [b] See Experimental Section for procedure and ref 4 and 15 for compound synthesis. [c] A minus number indicates more lysis than the complement control without compound. [d] Lysis in the absence of complement corrected for 2–5% lysis in a control in the absence of compound. [e] Prepared in this laboratory by M. Cory according to ref 22. [f] Prepared by hydrogenation of **3**·HCl in EtOH containing 2% HOAc with a rhodium catalyst; see ref 22. [g] Maximum solubility. [h] Assayed as the acetate salt.

Inhibition Results.—Since one or more of the components of complement such as C'1a are "tryptic" in character,[21] some known inhibitors of trypsin were investigated. Trypsin can be inhibited by benzamidine (**3**),[22] phenylguanidine (**6**),[22] or benzylamine (**10**)[23] in decreasing order of effectiveness;[4] the effect of these three compounds on the complement system is shown in Table I. Benzamidine (**3**) at 3 m*M* showed reproducible inhibition of complement with little lysis in the absence of complement. In contrast, the same concentration of **6** and **10** showed no inhibition of complement. The higher homolog (**4**) of benzamidine (**3**) or its hexahydro derivative (**5**) showed much less inhibition. Higher homologs (**7**–**9**) of phenylguanidine (**6**) also showed little inhibition; similarly, higher homologs

(15) (a) B. R. Baker and E. H. Erickson, *J. Med. Chem.*, **11**, 245 (1968), paper CXV of this series; (b) B. R. Baker and E. H. Erickson, *ibid.*, **12**, 112 (1969), paper CXLIV of this series.

(16) (a) B. R. Baker and J. A. Hurlbut, *ibid.*, **11**, 233 (1968), paper CXIII of this series; (b) B. R. Baker and J. A. Hurlbut, *ibid.*, **11**, 241 (1968), paper CXIV of this series; (c) B. R. Baker and J. A. Hurlbut, *ibid.*, **11**, 1054 (1968), paper CXXXII of this series; (d) B. R. Baker and J. A. Hurlbut, *ibid.*, **12**, 118 (1969), paper CXLV of this series.

(17) E. A. Kabat and M. M. Mayer, "Experimental Immunochemistry," 2nd ed, Charles C Thomas, Springfield, Ill., 1967, pp 149–153.

(18) M. M. Glovsky, E. L. Becker, and N. J. Halbrook, *J. Immunol.*, **100**, 979 (1968).

(19) E. L. Becker, *Biochim. Biophys. Acta*, **147**, 289 (1967), and references therein.

(20) H. J. Müller-Eberhard, A. P. Dalmasso, and M. A. Calcott, *J. Exptl. Med.*, **123**, 33 (1966).

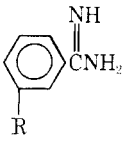
(21) (a) A. L. Haines and I. H. LePow, *J. Immunol.*, **92**, 456 (1964); (b) E. L. Becker and K. F. Austen, *J. Exptl. Med.*, **120**, 491 (1964).

(22) M. Mares-Guia and E. Shaw, *J. Biol. Chem.*, **240**, 1579 (1965).

(23) T. Inagami, *ibid.*, **239**, 787 (1964).

Table II

Inhibition[a,b] of Guinea Pig Complement by



No.	R	Concn, m*M*	% inhibn[c]	% lysis[d]
3	H	3	38	80
		1	15	
15[e]	CH_3	3	25	0
		1	26	
16	OH	3	38	
17[f]	OC_3H_7-*n*	1	36	4
		0.5	20	
18[f]	OC_5H_{11}-*i*	1	55	0
		0.5	46	
19[f]	$OCH_2C_6H_5$	1	53	0
		0.5	21	
20[f]	$O(CH_2)_3C_6H_5$	1	12	0
		0.5	56	
21[f]	$O(CH_2)_2OC_6H_5$	1	56	4
		0.5	42	
22	$O(CH_2)_3OC_6H_5$	1	60	0
		0.5	54	
		0.25	27	
23[f]	$O(CH_2)_4OC_6H_5$	1	3	20
		0.5	36	0
		0.25	26	
24[f]	$O(CH_2)_2OC_{10}H_7$-α	0.5		100
		0.25	−3	23
		0.1	8	0
25	$O(CH_2)_4OC_6H_4NH_2$-*p*	1	38	51
		0.5	44	12
		0.25	12	
26	$O(CH_2)_4OC_6H_4$-*p*-$NHCONHC_6H_4SO_2F$-*p*	0.5		100[g]
		0.1	−3	50
		0.075	33	17
		0.05	26	4
27	$(CH_2)_4C_6H_4NHAc$-*p*	1	59	0
		0.5	59	
		0.25	37	
28[f]	$NHCO(CH_2)_2C_6H_5$	1	23	0
		0.5	0	

[a–d] See corresponding footnotes in Table I. [e] Prepared according to J. B. Ekeley, D. V. Tieszen, and A. Renzio, *J. Am. Chem. Soc.*, **57**, 381 (1935). [f] See Experimental Section for synthesis. [g] *p*-$AcNHC_6H_4SO_2F$ at 0.5 m*M* gave no inhibition and no lysis.

(**11**–**13**) of benzylamine (**10**) showed no appreciable inhibition.

Since benzamidine (**3**) was the most effective base in Table I, derivatives of benzamidine originally prepared as inhibitors of trypsin[4,15] were tested as inhibitors of complement; these could be divided into three classes, *meta* derivatives (Table II), *para* derivatives (Table III), and N derivatives (Table V). *m*-(Phenoxypropyloxy)-benzamidine (**22**), an excellent reversible inhibitor of trypsin,[4] was a considerably more effective inhibitor of complement than the parent **3**; 54% inhibition was seen with 0.5 m*M* of **22** which was not significantly increased at 1 m*M*, but no lysis was observed in the absence of complement. Therefore **17**–**21**, **23**, **24**, and **27** were synthesized for evaluation. That the propyl moiety of **22** was giving a hydrophobic interaction with the enzyme was indicated by the greater effectiveness of the *m*-propoxy derivative (**17**) than the parent **3** and the equivalency of **3** and its *m*-hydroxy derivative (**16**). Activity was further enhanced by increasing the chain to isoamyloxy (**18**); the latter was nearly as effective as **22**, but the benzyloxy derivative (**19**) was less effective at 0.5 m*M*. At 0.5 m*M*, the phenylpropyloxy (**20**) and phenoxyethyloxy (**21**) derivatives were as effective as **22**. However, at 1 m*M*, **20** was a less effective inhibitor than at 0.5 m*M*; that this effect was directly on the complement system was shown by the lack of lysis by **20** in the absence of complement. The phenoxybutyloxy (**23**) and α-naphthoxyethoxy (**24**) derivatives were poorer inhibitors than **22**, both showing lysis in the absence of complement.

When *m*-(phenoxybutoxy)benzamidine (**23**) was substituted with a *p*-NH_2 on the terminal phenyl, the resultant **25** at 1 m*M* also showed lysis of RBC in the absence of complement; however, **25** still showed inhibition of complement. Lysis in the absence of complement was increased when **25** was further bridged to sulfanilyl fluoride (**26**); **26** showed strong lysis even at 0.1 m*M*, but also showed some inhibition of complement at 0.05–0.075 m*M*. That the sulfanilyl fluoride moiety did not cause this enhanced lysis was shown by assay of N-acetylsulfanilyl fluoride (Table II, footnote *g*); the latter at 0.5 m*M* showed no lysis in the absence of complement and no inhibition of complement.

The benzamidine with a *p*-acetamidophenylbutyl substitutent (**27**) on the *meta* position at 0.5 m*M* showed good inhibition of complement with no lysis at 1 m*M* in the absence of complement. The *m*-phenylpropionamido substituent (**28**) gave little change in activity compared to the parent benzamidine (**3**).

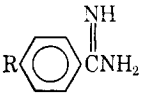
A series of benzamidines with *para* substituents were then investigated (Table III); none of the first eight compounds (**29**–**36**) were effective inhibitors, and many gave extensive lysis in the absence of complement. In three cases, the corresponding *meta* isomers were good inhibitors; compare **22** *vs.* **32**, **20** *vs.* **34**, and **26** *vs.* **36**. Of twenty sulfonyl fluorides related to **36**,[15] only **37** showed good inhibition of complement at their maximum solubility of 0.016–0.12 m*M*; note that the closely related compounds (**38**–**42**) showed no inhibition of complement. Further studies will be necessary to establish the structural requirements for the high potency of **37**.

Even though phenylguanidine (**6**) showed no inhibition, eight derivatives available from another study[4] were assayed (Table IV); only one (**48**) showed some activity. Note that the *m*-phenylpropyloxy and *m*-phenyloxypropyloxy derivatives of benzamidine (**3**) (Table II) enhanced activity (compare **20** *vs.* **48** and **22** *vs.* **47**).

In Table V are a group of miscellaneous compounds. When benzamidine (**3**) was N-substituted by phenyl (**50**), *n*-butyl (**51**) or benzyl (**52**) potency was lost; the cyclic amidine (**53**) showed considerably reduced activity.

Since the *m*-phenoxyethoxy (**21**) and *m*-phenoxypropyloxy substituent (**22**) on benzamidine gave considerably enhanced activity, one of these groups was put on benzoic acid to show that the amidine group was necessary for activity; the resultant **54** showed no inhibition of complement. That the benzamidine moiety could not be replaced by benzylamine was further substantiated with **55** and **56** which did not inhibit complement. Similarly, it was again substanti-

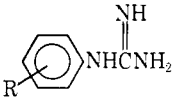
TABLE III
INHIBITION[a,b] OF GUINEA PIG COMPLEMENT BY



No.	R	Concn, m*M*	% inhibn[c]	% lysis[d]
3	H	3	38	8
		1	15	
29	$COCH_3$	1	9	
30	$CON(CH_3)C_6H_5$	1	−51	12
		0.5	0	
31[f]	$NHCO(CH_2)_2C_6H_5$	0.25[e]	12	0
32	$O(CH_2)_3OC_6H_5$	1	−14	59
		0.5	−8	5
		0.25	−5	0
33	$O(CH_2)_3OC_6H_4NO_2$-*p*	0.5	0	
34	$O(CH_2)_3C_6H_5$	1	−13	44
		0.5	9	7
35[f]	$(CH_2)_4C_6H_5$	0.5		100
		0.25	−3	0
		0.125	0	
36	$O(CH_2)_4OC_6H_4$-*p*-$NHCONHC_6H_4SO_2F$-*p*	0.062[e]	9	9
37	$O(CH_2)_4OC_6H_4$-*p*-$NHCONHC_6H_3$-2-OMe-5-SO_2F	0.062[e]	59	6
		0.031	9	0
38	$O(CH_2)_4OC_6H_4$-*p*-$NHCONHC_6H_4SO_2F$-*m*	0.062[e]	−4	0
39	$O(CH_2)_4OC_6H_4$-*p*-$NHCONHC_6H_3$-2-Cl-5-SO_2F	0.016[e]	3	6
40	$O(CH_2)_4OC_6H_4$-*p*-$NHCONHC_6H_3$-4-Me-3-SO_2F	0.05[e]	0	16
41	$O(CH_2)_4OC_6H_4$-*p*-$NHCONHC_6H_3$-3-OMe-4-SO_2F	0.031[e]	3	5
42	$O(CH_2)_4OC_6H_3$-2-Cl-4-$NHCONHC_6H_4SO_2F$-*m*	0.05[e]	6	12

[a–d] See corresponding footnotes in Table I. [e] Maximum solubility. [f] See Experimental Section for synthesis.

TABLE IV
INHIBITION[a,b] OF GUINEA PIG COMPLEMENT BY



No.	R	Concn, m*M*	% inhibn[c]	% lysis[d]
6	H	3	0	0
43	*m*-$COCH_3$	3	10	
44	*p*-$COCH_3$	3	−7	
		0.5	0	
45	*p*-$CH_2CON(CH_3)C_6H_5$	1	−5	
		0.5	0	
46	*p*-$O(CH_2)_3C_6H_5$	1	−24	15
		0.5	−17	6
47	*m*-$O(CH_2)_3OC_6H_5$	0.13[e]	0	0
48	*m*-$O(CH_2)_3C_6H_5$	1	−15	42
		0.5	26	8
		0.25	−7	0
49	*p*-$O(CH_2)_2C_6H_5$	1	3	0
50	N′-C_6H_5	0.5	0	

[a–d] See corresponding footnotes in Table I. [e] Maximum solubility.

ated that an N-benzyl group on the amidine led to greatly reduced activity; compare **21** *vs.* **57**. Since a number of the active bases in Tables I–V were prepared as the tosylate or iodide salt, sodium tosylate (**59**) and sodium iodide were shown to be devoid of effect in the 0.5–1 m*M* range used for many of the bases; similarly, sodium benzoate at 3 m*M* was ineffective.

There are two ways to compare which compounds are best, namely, which give the most inhibition at optimum concentration and which give 50% inhibition at the lowest concentration. Other than maleopimaric acid (**2**) the most inhibition seen at optimum concentrations in Tables I–V was only 55–60% with the *meta*-substituted benzamidines (**18**, **20**, **22**, **27**) and the *para*-substituted benzamidine (**37**); the latter (**37**) was the most potent from the concentration standpoint giving 59% inhibition at 0.062 m*M*.

The most potent compound reported in the literature for inhibition of the whole complement system is maleopimaric acid (**2**) which gave 50% inhibition at 0.7 m*M* and 100% inhibition at 10 m*M*. Since the assay system used by Becker, *et al.*,[19] was different from that used here, **2** was reassayed under our conditions. When **2** was dissolved carefully in MeOEtOH containing 1 equiv of Tris base in order to maintain the anhydride linkage, solubility was achieved at 0.062 m*M*, but precipitation occurred at 0.12 m*M* in the assay medium; at 0.062 m*M*, **2** showed only negligible inhibition. Since Becker, *et al.*,[18] dissolved maleopimaric acid in excess NaOH, then adjusted the pH to about 7.5 for assay, **2** was dissolved in 50% aqueous MeOEtOH containing 3 equiv of NaOH; this solution of maleopimaric acid (**2**) now could be assayed readily at 1 m*M* without precipitation. Thus it is clear that a solution of maleopimaric acid in NaOH is rapidly converted to the corresponding tricarboxylic acid. The tricarboxylic acid from **2** was indeed an excellent inhibitor of the complement system giving 81% inhibition at 1 m*M* and 45% inhibition at 0.5 m*M*.

The Becker assay[18] determines the concentration of compound necessary to return complement-induced lysis with doubled complement concentration to the amount of lysis seen with a not doubled concentration of complement in the absence of compound; that is, one out of two complement units is inhibited. Thus they reported[18] that 0.7 m*M* of maleopimaric acid (**2**) inhibited one out of two complement units. Under similar conditions, 1 m*M* *m*-phenoxypropyloxybenza-

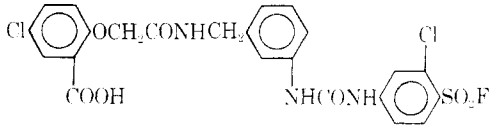
Table V
Inhibition[a,b] of Guinea Pig Complement by Miscellaneous Compounds

No.	Compound	Concn, mM	% inhibn[c]	% lysis[d]
3	$C_6H_5C(NH_2)=NH$	3	38	8
		1	15	
21	m-$C_6H_5O(CH_2)_2OC_6H_4C(NH_2)=NH$	1	56	4
22	m-$C_6H_5O(CH_2)_3OC_6H_4C(NH_2)=NH$	1	60	0
		0.5	54	
50	$C_6H_5C(NHC_6H_5)=NH$	0.5	0	
51	$C_6H_5C(NHC_4H_9$-$n)=NH$	3	6	
52	$C_6H_5C(NHCH_2C_6H_5)=NH$	3	−8	
		1		4
53		3	12	0
54[e]	m-$C_6H_5O(CH_2)_3OC_6H_4COOH$	1[f]	0	18
		0.5	8	
55[e]	m-$C_6H_5(CH_2)_3OC_6H_4CH_2NH_2$	1	−42	
		0.5	0	0
56[e]	m-$C_6H_5O(CH_2)_2OC_6H_4CH_2NH_2$	1	6	8
57[e]	m-$C_6H_5O(CH_2)_2OC_6H_4C(NHCH_2C_6H_5)=NH$	2	−80	
		1	17	6
58	C_6H_5COONa	3	6	
59	p-$CH_3C_6H_4SO_3Na$	0.5	0	
60	NaI	1		0
2	Maleopimaric Acid	1[g]	81	2
		0.5[g]	45	
		0.25[g]	3	
		0.062[f,h]	10	

[a-d] See corresponding footnotes in Table I. [e] See Experimental Section for synthesis. [f] Dissolved in MeOEtOH + 1 equiv of Tris base. [g] Dissolved in 1:1 MeOEtOH–H_2O + 3 equiv of NaOH. [h] Maximum solubility.

midine (**22**) was required, being almost as effective as the tricarboxylic acid from maleopimaric acid. Becker, *et al.*,[18] also established that this maleopimaric acid derivative inhibited the C′1, C′2, and C′5,6,7 components of complement.[5,6]

Although none of the compounds in Tables I–V at optimum concentrations showed 80% inhibition of complement as seen with 1 mM maleopimaric acid (**2**), such high inhibition has been seen in this laboratory with **61**;[24] at 0.25 mM, **61** showed 80% inhibition of the complement system.



61

Our initial studies reported here show that some benzamidines (**18**, **20**, **22**, **27**, **37**) are inhibitors of complement. If potency is defined as the minimum concentration necessary for 50% inhibition of the complement system, then **37** is the most potent compound yet observed. Further studies underway are designed to try to answer the following questions.

(1) Since a m-isoamyloxy group (**18**) enhances the activity over m-hydroxybenzamidine (**16**), the isoamyloxy group contributes to inhibition by a hydrocarbon interaction, probably of the hydrophobic type. What are the nature and dimensions of this hydrophobic bonding region and can more potent compounds be prepared by utilizing optimum hydrophobic bonding as previously done with other enzyme systems?[9]

(2) It has been observed that m-phenylbutylbenzamidine at 1 mM gives 72% lysis in the absence of complement[25] and 28% inhibition at 0.25 mM. Note that a p-acetamido substituent (**27**) on the terminal phenyl (Table II) gives no lysis at 1 mM in the absence of complement, but good inhibition in the presence of complement. Thus appropriate substitution can decrease lysis in the absence of complement without losing inhibition of complement-induced lysis. If appropriate substitution on **26** could remove lysis in the absence of complement, a potent inhibitor for the complement system could emerge.

(3) Which of the components of complement are most inhibited by the better inhibitors in Tables I–V?

(4) Is the potency of **26** or **37** partially due to irreversible inhibition by formation of a covalent bond *via* the SO_2F moiety[26] of one or more of the components of complement?

(5) Of 28 *para*-substituted benzamidines (Table III) why is **37** uniquely active? What are the structural requirements and can more potent related inhibitors be found?

Chemistry.—As noted in Tables I–V, the syntheses of many of the compounds tested had been previously described.[4,15] The new benzamidines in Tables II and III were prepared from the corresponding nitriles *via* the thioamide,[4] except for **17** and **18** where the imino ether precedure[15a] was employed.

The requisite benzonitriles for **17**–**21**, **23**, and **24** were prepared by alkylation of m-cyanophenol[27] with the appropriate bromide.[4] Bromination of m-tolunitrile

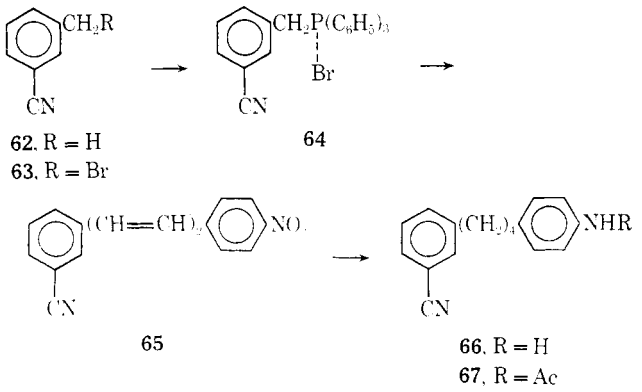
(24) B. R. Baker and J. A. Hurlbut, *J. Med. Chem.*, **12**, 415 (1969), paper CLIII of this series.

(25) B. R. Baker and M. Cory, to be published.

(26) B. R. Baker and G. J. Lourens, *J. Med. Chem.*, **10**, 1113 (1967), paper CV of this series.

(27) T. van Es, *J. Chem. Soc.*, 1564 (1965).

(**62**) with NBS gave **63** which was not purified, but converted directly to the Wittig reagent (**64**) in 81%



over-all yield by reaction with triphenylphosphine in boiling xylene. Condensation of **64** with *p*-nitrocinnamaldehyde[28] in methanolic NaOMe afforded the butadiene (**65**) as a mixture of *cis–trans* isomers in 54% yield. Catalytic reduction of **65** with a Pd–C catalyst gave **66** as oil which was converted to **67** with Ac_2O in good over-all yield. Similarly *p*-phenylbutylbenzonitrile was prepared by Wittig condensation of *p*-cyanobenzaldehyde with cinnamyltriphenylphosphonium chloride[29] followed by catalytic reduction.

p-Phenylpropionamidobenzonitrile was prepared by reaction of hydrocinnamoyl chloride with *p*-aminobenzonitrile in $CHCl_3$–pyridine, then converted to **31** by the H_2S method. The *meta* isomer (**28**) was prepared by reaction of *m*-aminobenzamidine dihydrochloride[30] with hydrocinnamoyl chloride.

The substituted benzylamines (**55**, **56**) were prepared by catalytic reduction of the corresponding nitriles with a PtO_2 catalyst in EtOH containing $EtSO_3H$; **57** was prepared from the corresponding nitrile by reaction of its imino thioether with benzylamine. Alkylation of ethyl *m*-hydroxybenzoate with phenoxypropyl bromide followed by saponification afford **54**.

Experimental Section

Melting points were determined in capillary tubes on a Mel-Temp block and are uncorrected. All analytical samples had ir spectra compatible with their assigned structures and moved as a single spot on tlc on Brinkmann silica gel GF or polyamide MN_{254}; each gave combustion values for C, H, and N within 0.4% of theory.

***m*-Cyanobenzyltriphenylphosphonium Bromide (64).**—A mixture of 25 g (0.14 mole) of NBS, 16.4 g (0.14 mole) of *m*-tolunitrile, 60 ml of CCl_4, and 0.3 g of Bz_2O_2 was refluxed for 90 min; if reaction was not complete, as indicated by succinimide not rising to the surface, additional Bz_2O_2 was added and boiling was continued. The mixture was diluted with 70 ml of $CHCl_3$ and filtered, and the filtrate was washed with 100 ml of H_2O. Dried with $MgSO_4$, the solution was spin-evaporated *in vacuo* to about 25 ml, then chilled. The crude **63**,[31] mp 80–87°, was collected on a filter and washed with petroleum ether (bp 30–60°).

To a solution of the crude **63** in 150 ml of xylene was added 36.7 g (0.14 mole) of triphenylphosphine in 150 ml of xylene. After being refluxed for 60 min, the mixture was cooled and the product was collected on a filter; yield 52.9 g (81%), mp 270–280°, suitable for further transformations. Recrystallization of a sample from *n*-PrOH gave white crystals, mp 311–320°. *Anal.* ($C_{26}H_{21}BrNP$) C, H, N.

1-(*m*-Cyanophenyl)-4-(*p*-nitrophenyl)butadiene (65).—To a solution of 20 g (43 mmoles) of **64** and 7.64 g (43 mmoles) of *p*-nitrocinnamaldehyde[28] in 250 ml of MeOH was added a solution of 2.5 g (46 mmoles) of NaOMe in 100 ml of MeOH. After being stirred 15 hr, the mixture was filtered and the product was washed with MeOH. Recrystallization from MeOEtOH gave 6.6 g (54%) of product as a mixture of *cis–trans* isomers, mp 145–160°, that was suitable for further transformation. Two additional crystallizations gave yellow crystals, mp 148–165°. *Anal.* ($C_{17}H_{12}N_2O_2$) C, H, N.

1-(*p*-Cyanophenyl)-4-phenylbutadiene (80).—Condensation of 1.34 g (10 mmoles) of *p*-cyanobenzaldehyde with 4.15 g (10 mmoles) of cinnamyltriphenylphosphonium chloride,[29] as described for **65** with a 60-min reaction time, gave 0.82 g (34%) of product, mp 164–167°, suitable for further transformation; lit.[32] mp 168–171°.

***m*-(*p*-Acetamidophenylbutyl)thiobenzamide (81).**—A solution of 2.0 g (7.2 mmoles) of **65** in 200 ml of MeOEtOH was shaken with H_2 at 2–3 atm in the presence of 0.30 g of 5% Pd–C; reduction was complete in 45 min. The filtered solution was evaporated *in vacuo* leaving **66** as an oil. The oil was dissolved in 20 ml of $CHCl_3$, cooled in an ice bath, and treated with 1.0 g (10 mmoles) of Ac_2O. After 1 hr at ambient temperature when tlc showed reaction was complete, the solution was washed with H_2O and evaporated *in vacuo* leaving **67** as an oil. The oily **67** was dissolved in 10 ml of pyridine and 0.8 ml of Et_3N, then H_2S was slowly passed through the solution for 2 hr. The solution was allowed to stand about 18 hr, then diluted with 50 ml of H_2O and the oily layer was extracted with two 50-ml portions of $CHCl_3$. The extracts were evaporated *in vacuo* and the residue crystallized from C_6H_6; yield 1.48 g (62% over-all), mp 120–130°, suitable for further transformation. Two recrystallizations from C_6H_6 gave the analytical sample, mp 129–131°. *Anal.* ($C_{19}H_{22}N_2O_2S$) C, H, N.

***p*-Phenylbutylthiobenzamide (82).**—From 0.81 g (3.5 mmoles) of **80**, as described for the preparation of **81** with omission of the Ac_2O step, was obtained 0.61 g (66%) of product, mp 145–160°, that was suitable for further transformation. Two recrystallizations from EtOH gave the analytical sample, mp 163–165°. *Anal.* ($C_{17}H_{19}NS$) C, H, N.

***p*-Phenylpropionamidobenzonitrile (83).**—To an ice-cold solution of hydrocinnamoyl chloride, prepared from 2.25 g (15 mmoles of the acid with $SOCl_2$, in 10 ml of $CHCl_3$ was added 2 ml of pyridine, followed by 1.8 g (15 mmoles) of *p*-aminobenzonitrile in 10 ml of $CHCl_3$. After 90 min at ambient temperature, the mixture was washed successively with 5% HCl (two 20-ml portions), 20 ml of 5% NaOH, and finally H_2O. The organic layer, dried with $MgSO_4$, was evaporated *in vacuo*. Recrystallization from C_6H_6 gave 1.42 g (38%) of product, mp 105–111°, that was used for subsequent transformation. Two recrystallizations of a sample from C_6H_6 gave white crystals, mp 116–118°. *Anal.* ($C_{16}H_{14}N_2O$) C, H, N.

***m*-Phenylpropionamidobenzamidine *p*-Toluenesulfonate (28).**—To a stirred and ice-cooled solution of 1.00 g (5 mmoles) of *m*-aminobenzamidine dihydrochloride[30] in 4 ml of DMF and 2 ml of pyridine was added dropwise a solution of the acid chloride from 0.80 g (5.3 mmoles) of hydrocinnamic acid (prepared with $SOCl_2$) in 5 ml of DMF. After 3 hr at ambient temperature, the mixture was diluted with several volumes of Et_2O. The solution was decanted from the oil; the latter was dissolved in 25 ml of hot H_2O containing 1.1 g of TsOH. The cooled solution deposited 0.77 g of crude product that was twice recrystallized from H_2O; yield 0.27 g (12%) of white crystals, mp 192–194°. *Anal.* ($C_{23}H_{25}N_3O_4S$) C, H, N.

***m*-Phenoxyethoxybenzylamine (56) Ethanesulfonate.**—A solution of 2.39 g (10 mmoles) of **68a** (Table VI) and 1.10 g (10 mmoles) of $EtSO_3H$ in 100 ml of EtOH was shaken with H_2 at 2–3 atm in the presence of 0.1 g of PtO_2 until 30 mmoles were absorbed; during this time the product separated. The mixture was warmed, then sufficient H_2O was added to dissolve the product; the catalyst was removed by filtration. The filtrate deposited 1.8 g of product, mp 155–158°, and an additional 0.3 g (total 60%) could be isolated from the filtrate. Recrystallization from EtOH gave white crystals, mp 164–166°. *Anal.* ($C_{17}H_{23}NO_5S$) C, H, N.

(28) B. R. Baker and J. H. Jordaan, *J. Med. Chem.*, **8**, 35 (1965).

(29) R. N. McDonald and T. W. Campbell, *J. Org. Chem.*, **24**, 1969 (1959).

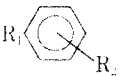
(30) Prepared by catalytic reduction of *m*-nitrobenzamidine hydrochloride with Pd–C catalyst; see A. P. T. Easson and F. L. Pyman, *J. Chem. Soc.*, 2991 (1931).

(31) E. J. Cragoe, Jr., and A. M. Pietruszkiewicz, *J. Org. Chem.*, **22**, 1338 (1957), prepared this compound by an alternate method.

(32) Kodak Society Anon., Belgian Patent 641,415 (1964); *Chem. Abstr.* **63**, P3092H (1965).

Table VI

Physical Constants of



No.	R_1	R_2	Method[a]	% yield	Mp, °C	Formula
17	n-C_3H_7O	m-C(NH$_2$)=NH·TsOH	E[b]	8	145–148[c]	$C_{17}H_{22}N_2O_4S$
18	i-$C_5H_{11}O$	m-C(NH$_2$)=NH·TsOH	E[c]	11	192–194[d]	$C_{19}H_{26}N_2O_4S$
19	$C_6H_5CH_2O$	m-C(NH$_2$)=NH·TsOH	D	23	201–202 dec[e]	$C_{21}H_{22}N_2O_4S$
20	$C_6H_5(CH_2)_3O$	m-C(NH$_2$)=NH·TsOH	D	43	203–205[e]	$C_{23}H_{26}N_2O_4S$
21	$C_6H_5O(CH_2)_2O$	m-C(NH$_2$)=NH·HNO$_3$	D	20	131–133[e]	$C_{15}H_{17}N_3O_5$
23	$C_6H_5O(CH_2)_4O$	m-C(NH$_2$)=NH·HNO$_3$	D	20[f]	150–153[e]	$C_{17}H_{21}N_3O_5$
24	α-$C_{10}H_7O(CH_2)_2O$	m-C(NH$_2$)=NH·HNO$_3$	D	23[f]	153–156[e]	$C_{19}H_{19}N_3O_5$
27	p-AcNHC$_6$H$_4$(CH$_2$)$_4$	m-C(NH$_2$)=NH·TsOH	D	35	187–190[e]	$C_{26}H_{31}N_3O_4S$
31	$C_6H_5(CH_2)_2CONH$	p-C(NH$_2$)=NH·TsOH	D	31	275–276[e]	$C_{23}H_{25}N_3O_4S$
35	$C_6H_5(CH_2)_4$	p-C(NH$_2$)=NH·TsOH	D	19	160–162[f]	$C_{24}H_{28}N_2O_3S$
68a	$C_6H_5O(CH_2)_2O$	m-CN	A	87	80–81[f]	$C_{15}H_{13}NO_2$
68b	$C_6H_5O(CH_2)_4O$	m-CN	A	78	82–84[g]	$C_{17}H_{17}NO_2$
69	α-$C_{10}H_7O(CH_2)_2O$	m-CN	A	60	142–145[h]	$C_{19}H_{15}NO_2$
70	$C_6H_5CH_2O$	m-C(NH$_2$)=S	B	68[i]	97–99[j]	$C_{14}H_{13}NOS$
71	$C_6H_5(CH_2)_3O$	m-C(NH$_2$)=S	B	70[i]	62–64[k]	$C_{16}H_{17}NOS$
72a	$C_6H_5O(CH_2)_2O$	m-C(NH$_2$)=S	B	85	182–185[k]	$C_{15}H_{15}NO_2S$
72b	$C_6H_5O(CH_2)_4O$	m-C(NH$_2$)=S	B	90	107–109[l]	$C_{17}H_{19}NO_2S$
73	α-$C_{10}H_7O(CH_2)_2O$	m-C(NH$_2$)=S	B	100	91–92[j]	$C_{19}H_{17}NO_2S$
74	$C_6H_5(CH_2)_2CONH$	p-C(NH$_2$)=S	B	45	192–199[l]	$C_{16}H_{16}N_2OS$
75a	$C_6H_5CH_2O$	m-C(=NH)SMe·HI	C	60	133–136[m]	$C_{15}H_{16}INOS$
75b	$C_6H_5O(CH_2)_2O$	m-C(=NH)SMe·HI	C	95	138–142[n]	$C_{16}H_{18}INO_2S$
76	$C_6H_5(CH_2)_3O$	m-C(=NH)SMe·HI	C	100	147–150[o]	$C_{17}H_{20}INOS$
77	p-AcNHC$_6$H$_4$(CH$_2$)$_4$	m-C(=NH)SMe·HI	C	60	151–153[p]	$C_{20}H_{25}IN_2OS$
78	$C_6H_5(CH_2)_2CONH$	p-C(=NH)SMe·HI	C	50	175 dec[f]	$C_{17}H_{19}IN_2OS$
79	$C_6H_5(CH_2)_4$	p-C(=NH)SMe·HI	C	45	130–133[o]	$C_{18}H_{22}INS$

[a] For methods A–D, see ref 4: A, alkylation of the cyanophenol; B, addition of H_2S to the nitrile; C, methylation of the thioamide; D, amination of the imino thioether; E,[15a] CN → imino ether → amidine. [b] The intermediate nitrile was an oil that was not purified. [c] Recrystallized from H_2O–EtOH. [d] Recrystallized from H_2O–Me_2CO. [e] Over-all yield from thioamide; the intermediate imino thioether was an oil. [f] Recrystallized from *i*-PrOH. [g] Recrystallized from EtOH. [h] Recrystallized from MeOEtOH. [i] Over-all yield from *m*-cyanophenol; the intermediate nitrile was an oil. [j] Recrystallized from CCl_4. [k] Recrystallized from petroleum ether (bp 60–110°). [l] Recrystallized from MeOH. [m] Recrystallized from Me_2CO. [n] Recrystallized from PhMe. [o] Recrystallized from C_6H_6. [p] Recrystallized from MeEtCO.

***m*-Phenylpropyloxybenzylamine (55) ethanesulfonate** was prepared from oily *m*-phenylpropyloxybenzonitrile as described for **56**; white crystals from acetone, mp 75–78°. *Anal.* ($C_{18}H_{25}NO_4S$) C, H, N.

***m*-(Phenoxypropyloxy)benzoic Acid (54).**—Alkylation of 4.98 g (30 mmoles) of ethyl *m*-hydroxybenzoate with phenoxypropyl bromide by method A[4] gave the crude ether ester. The latter was refluxed with 40 ml of 3.5 *N* NaOH and 40 ml of EtOH for 30 min, then the solution was acidified with 12 *N* HCl. The product (100%, mp 142–146°) was collected and washed with H_2O. Three recrystallizations from EtOH gave 3.6 g (43%) of analytical sample, mp 147–149°. *Anal.* ($C_{16}H_{16}O_4$) C, H.

N-Benzyl-*m*-(phenoxyethoxy)benzamidine (57) Hydrochloride.—A mixture of 0.21 g (0.50 mmole) of **75b** (Table VI), 0.070 g (0.65 mmole) of benzylamine, and 5 ml of EtOH was stirred at ambient temperature for 24 hr when tlc indicated the reaction was complete. The solution was spin-evaporated *in vacuo*. The residue was dissolved in 6 ml of hot 2 *N* HCl by gradual addition of sufficient EtOH. The solution deposited crystals on cooling. Three recrystallizations from H_2O–EtOH containing HCl gave 0.047 g (24%) of white crystals, mp 193–195°. *Anal.* ($C_{22}H_{23}ClN_2O_2$) C, H, N.

Complement Assay.[17] **Materials.**—Sheep red blood cells (RBC) suspended in Alsever's solution, guinea pig hemolysin (antibody), and lyophilized guinea pig complement were purchased from Grand Island Biologicals Co., Oakland, Calif. After sterile withdrawal of 4 ml of RBC, the suspension was centrifuged in a clinical centrifuge, and the cells were washed several times with buffer, then standardized to 10^9 cells/ml of buffer;[17] buffer-diluted cells are stable 2–3 days at 3°. Hemolysin was diluted 1:800 in buffer for assay and was stable indefinitely at 3°; as used in the assay below, this gives five times the amount needed for maximum velocity. The lyophilized complement on receipt was dissolved in its restoring solution; 200-μl aliquots were immediately placed in 15–20-ml vials and kept frozen at −15° until ready for assay. For a day's assays, a vial was diluted with 9.8 ml of buffer (1:50) and stored at 0°; a new vial should be used each day.

Solutions.—Citrate–saline was a 1:4 mixture of 0.075 *M* sodium citrate and 0.15 *M* NaCl; it was kept refrigerated for convenience in assay. Buffer was 5 m*M* Tris containing 0.5 m*M* $MgCl_2$, 0.15 m*M* $CaCl_2$, 0.15 *M* NaCl, and 0.1% gelatin.

Assay.—In seven 12-ml centrifuge tubes were placed 0.25 ml of RBC (10^9/ml). Then 0.25 ml of 1:800 hemolysin was added dropwise with Vortex mixing. The tubes were incubated at 37° for 15 min. To the tubes was added 50 μl of MeOEtOH plus or minus inhibitor. To all but tubes 3, 5, and 7 was added 0.40 ml of 1:50 complement; to the other three tubes was added 0.40 ml of buffer. The tubes were incubated at 37° for 15 min, then lysis was quenched by addition of 2.75 ml of cold citrate–saline; the tubes were kept in an ice bath until ready for assay. Tubes were centrifuged 3 min in a clinical centrifuge, then the optical density of the hemoglobin in the supernatant was read at 541 mμ in a 1-ml glass cuvette.

Tubes 1 and 6 served as standards by omission of inhibitor from the MeOEtOH. Tubes 2 and 4 contained inhibitor at two concentrations or two inhibitors. Tubes 3 and 5 served as controls for inhibitor tubes 2 and 4 where the lysis caused by the compound in the absence of complement was determined. Tube 7 without compound or complement served as a lysis control of the assay; the optical density of this tube should be less than 5% of the total 0.70 optical density possible or the experiment was rejected; it had only to be run once a day. The optical density of the standard tubes 1 and 6 should be 0.30–0.42 unit; if higher or lower, either the second incubation time or the complement dilution was changed appropriately.

Data are recorded in Tables I–V in two ways. Lysis by the compounds in tubes 3 and 5 were corrected for lysis in tube 7 and recorded as a percentage of the 0.70 optical density obtained on 100% lysis. The inhibition of complement by compounds in tubes 2 and 4 are recorded as a fractional percentage of the optical density observed over the average optical density in the standard tubes 1 and 6.