Bis-Basic-Substituted Polycyclic Aromatic Compounds. A New Class of Antiviral Agents. 1. Bisalkamine Esters of Fluorenone-, Fluorenol-, and Fluorenedicarboxylic Acids

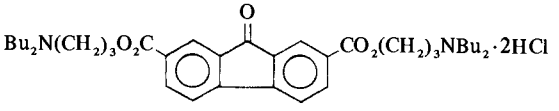
Arthur D. Sill,* William L. Albrecht, Edwin R. Andrews,* Robert W. Fleming, Stephen W. Horgan, Edward McC. Roberts, and F. William Sweet

Organic Chemistry Department, Merrell-National Laboratories, Division of Richardson-Merrell Inc., Cincinnati, Ohio 45215. *Received July 24, 1972*

Bis(3-dibutylaminopropyl) 9-oxofluorene-2,7-dicarboxylate dihydrochloride (**16**) was found to prolong significantly the survival of mice infected with lethal challenges of encephalomyocarditis (EMC) virus and, later, was found to induce interferon in mice. A series of related bisalkamine esters of fluorenone-, fluorenol-, and fluorenedicarboxylic acids was prepared to study structure-activity correlation in the mouse-EMC model. Many of these esters had antiviral potency comparable to **16**, maximal responses being obtained by parenteral administration. The most effective antiviral compounds were bisalkamine esters derived from fluorenone (≥fluorenol > fluorene, where ester groups were identical); those that contained the more strongly basic amine functions; those in which C_3 separated O and N of the ester side chains, although length of this chain (C_2–C_6) was not as critical to activity as other factors. This initial study led ultimately to the development of an extensive new class of antiviral agents and interferon inducers.

The ultimate result of a concerted, long-term research program in our laboratories has been the development of the concept of an extensive new class of antiviral agents and interferon inducers. The scope and significance of this development have been discussed in several previous reports.[1-6]

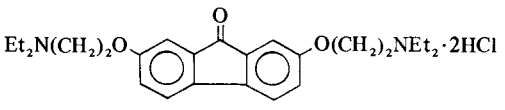
The beginning our our synthetic program dates from the observation that bis(3-dibutylaminopropyl) 9-oxofluorene-2,7-dicarboxylate dihydrochloride[7] (**16**)† was effective in protecting mice against encephalomyocarditis (EMC) virus infections.



16

In this paper, the first of a series, we are reporting the synthesis and antiviral properties of **16** and related bisalkamine esters of fluorenone-, fluorenol-, and fluorenedicarboxylic acids. Many of these esters have the same order of antiviral potency as **16** in the mouse-EMC model with maximal responses occurring upon parenteral administration.

This initial study encouraged us to investigate the antiviral properties of many other related compounds. As our preliminary communications[3,4] have indicated, this general class of antiviral agents now includes many series of bis-basic functional derivatives of polycyclic aromatic compounds in addition to the esters described in this paper. Tilorone hydrochloride, 2,7-bis(2-diethylaminoethoxy)fluoren-9-one dihydrochloride, was the first member of the class to be reported[1,2] and has been the most widely studied and publicized.[6]



tilorone hydrochloride

Reports by Krueger, *et al.*,[1,2,5] on tilorone, **16**, and some analogs representative of other series within this class of antiviral agents show that these compounds stimulate the elaboration of interferon by the host animal. Like other interferon inducers,[8] they exhibit activity against a broad spectrum of viral infections in animal models. Activities have been demonstrated against neurotropic, dermatropic, and respiratory viruses.

In searching for structures having optimum biological properties within this new class of compounds, we have found that even minor molecular and structural modifications produce pronounced effects on antiviral response.[3-5] Such differences in response occur within a given series and, especially, between members of different series. In mice, differences are observed in potency and spectrum of antiviral activity, interferon stimulation, hyporesponsiveness, and acute LD_{50}. Moreover, responses vary with dose of compound, route of treatment, and dosage schedule.[5] These observations will be published in greater detail in this and subsequent papers from our laboratories.

Prior to the reports by Krueger and Mayer on tilorone[1,2] in 1970, the only synthetic substances known to be effective in inducing interferon were polyanionic polymeric substances of high molecular weight.[6,8] As classified by De Clercq and Merigan,[8] these polymers include polycarboxylates (maleic anhydride copolymers, polyacrylic and polymethacrylic acids), polysulfates (polyvinyl sulfate), polyphosphates (polyribonucleotide homopolymer pairs), and polythiophosphates (thiophosphate analogs of polyribonucleotides). Of the four classes, polyribonucleotides and their thio analogs have attracted the greatest interest.[6] Typified by poly(I,C), they are potent inducers of interferon, are active parenterally and by topical application, and are enzymatically degraded to natural mononucleotides which can be incorporated into normal RNA. Polycarboxylates are much less potent inducers than polynucleotides, are active only by parenteral routes, are excreted slowly, and are not known to be biodegraded. None of the polyanions are effective orally.

By their very nature, these polymeric substances are nonhomogeneous and difficult to characterize. Furthermore, as Niblack[6] has noted, the antiviral activity of each class of polymer depends upon a specific critical degree of polymerization. For example, the molecular weight of each homopolymer pair of poly(I,C) must exceed 100,000 for maximum activity. A further requirement for antiviral potency of polynucleotides is a high degree of association between purine and pyrimidine base pairs. These require-

† 16 bears the Code No. RMI 2557DA.

ments have made systematic investigation of structure–activity relationships of the polymeric interferon inducers a difficult synthetic problem.[6]

Unlike the polymeric inducers, compounds in the new class of antiviral agents to be reported in this and subsequent papers are well-defined organic molecules of comparatively low molecular weight. They differ further from the polymers in being dications rather than polyanions. These simple monomeric compounds are prepared by straightforward synthetic methods which have been adapted to systematic modification of both the central polycyclic aromatic system and the amino-substituted side chains. Most importantly, many compounds of this general class are effective orally at doses well below their LD_{50}'s. The therapeutic index of a compound will vary with the nature and challenge of the specific infectious virus used in a test.

In order to evaluate the large number of compounds prepared in this program, we have employed an *in vivo* model that is based upon demonstration of a linear dose–response effect in protecting mice against infection with lethal doses of EMC virus. As explained in the Experimental Section, antiviral activity is expressed in terms of survival time ratio (STR). These primary antiviral data will be the basis for discussion of certain structure–activity relationships in this and each additional series of compounds to be reported. Representative members of each series were further evaluated to determine the spectrum of antiviral activity, induction of interferon, etc., generally by methods described for the evaluation of tilorone.[1,2]

Chemistry. Bisalkamine esters of fluorenonedicarboxylic acids (**16**, *et al.*) were prepared by reaction of the appropriate bisacid chloride with 2 molar equiv of an amino alcohol. The primary aminoalkyl ester **26** was prepared by this method, except that the HCl salt of the amino alcohol was used, and the product was maintained as the dihydrochloride to avoid rearrangement of bisester to bisamide. The bisthioester **4** was prepared similarly by reaction of 2-diethylaminoethanethiol·HCl with the bisacid chloride. The half-ester **45** was obtained from the reaction of 9-oxofluorene-2,7-dicarbonyl chloride with an equimolar amount of 3-dibutylaminopropanol.

Bisalkamine esters of fluorenoldicarboxylic acids (**29–34**) were prepared by reduction of HCl salts of the corresponding fluorenonedicarboxylates with $NaBH_4$. Bisalkamine esters of fluorenedicarboxylic acids were prepared either by catalytic hydrogenation of the corresponding fluorenonedicarboxylates (**35**, **36**, **39–43**) or by esterification of the fluorenedicarbonyl chlorides (**37**, **38**).

Fluorene-2,5-dicarboxylic acid was prepared by Friedel–Crafts acylation of fluorene-4-carboxylic acid with oxalyl chloride. It was oxidized with permanganate to 9-oxofluorene-2,5-dicarboxylic acid. Fluorene-1,7-dicarboxylic acid was prepared by a similar acylation of fluorene-1-carboxylic acid.

Reaction of **16**, lead compound of the series, with Girard's reagent T gave the quaternary derivative **44**. The bismethiodide of **16** was also prepared (**46**).

Biological Activity. As defined in the Experimental Section, the antiviral activity of compounds in Table I is expressed as survival time ratio (STR). By our definition, a ratio of 1.30 or greater indicates high activity.

Inspection of STR values in Table I reveals that a majority of the bisalkamine esters was effective at some dosage level in protecting mice against EMC virus infection, maximal antiviral responses having been obtained by subcutaneous injection.

Compounds **11**, **12**, **14**, **15**, **16**, **19**, **20**, and **21** are considered the most effective antiviral agents of the series. Each had a high degree of activity, gave a good dose–response effect, and showed little or no evidence of lethal effects at the highest dose level. These esters thus provide a basis for defining the structural and functional requirements for optimum antiviral effectiveness in the series.

These eight bisesters are all fluorenones with side chains in the 2,7 positions. Three compounds listed in Table I have side chains substituted in positions other than 2,7. The 2,5-fluorenone **13** is less active than its 2,7 analog **12**. Of three fluorenes, activity of the 1,7-fluorene **38** (high) > the 2,7 analog **36** (medium) > the 2,5 analog **37** (weak).

Fluorenols (**30**, **32**, **34**) were about as active as analogous fluorenones (**12**, **16**, **28**) and both were more effective than corresponding fluorenes (**36**, **39**, **43**). There was one exception; the activity of fluorenol **33** (high) > fluorene **40** (moderate) > fluorenone **18** (weak).

Length of the alkylene chain separating O and N of ester side chains appears to have less effect on antiviral activity than basicity of the amine functions. In the eight highly effective esters cited above, O and N are separated by C_3, the preferred chain length. However, there are compounds with high activity in which the alkylene chain is C_2 (**1**, **3**, **5**, **8**), C_4 (**23**, **24**), C_5 (**25**, **26**, **27**), and C_6 (**28**). Several compounds containing longer alkylene chains (**24–28**), however, did show evidence of lethal effects at 250 mg/kg.

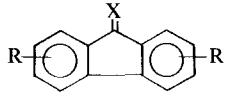
Similarity in basicity of amine functions appears to be common to the most active antiviral compounds. Amine functions in these compounds are all tertiary amines except the primary amine **26**. They include lower molecular weight dialkylamino groups (Me_2N to Bu_2N), of which there are many examples (**1**, **3**, **11**, **12**, **14**, **16**, *et al.*); $(allyl)_2N$ (**15**, **31**); cyclohexyl(Me)N (**8**, **29**, **35**); piperidino (**19**); 1-methyl-4-piperidyl (**20**); and (1-methyl-3-piperidyl)CH_2 (**21**, **41**). The primary amine **26** was comparable in activity to its Me_2N homolog **27** but was toxic at 250 mg/kg. Although examples for comparison are few, esters containing dialkylamino groups larger than Bu_2N were generally less active than their lower homologs (*cf.* **7** with **1**, etc.; **17** and **18** with **11**, etc.; **33** with **32**; **40** with **39**). Esters containing less strongly basic amine functions, such as anilino (**9**), morpholino (**10**), and 2-pyridyl (**22**), were inactive.

The thioester **4** was comparable in activity to the normal ester **2** but was lethal at 250 mg/kg. The quaternary derivatives (**44**, **46**) of **16** were lethal at 250 mg/kg and inactive at lower doses.

Monoalkamine esters were clearly less active than comparable bisesters. The half-ester **45** was only weakly active in contrast to the bisester **16**, one of the most active members of the series. The following esters of fluorenone- and fluorenemonocarboxylic acids were tested and found inactive: 3-dibutylaminopropyl,[9] 3-diethylaminopropyl,[9,10] 2-dimethylaminoethyl,[10] and 2-piperidinoethyl[9] 9-oxofluorene-2-carboxylate (each as the HCl salt), as well as 2-diethylaminoethyl fluorene-2-carboxylate·HCl.[10] Nonbasic esters (**47–50**) were also inactive as were the hydrolysis products of **16**, *i.e.*, 9-oxofluorene-2,7-dicarboxylic acid and 3-dibutylaminopropanol.

Bis(3-dibutylaminopropyl) 9-oxofluorene-2,7-dicarboxylate dihydrochloride (**16**), the representative member of this series, was evaluated in additional test models to determine its spectrum of activity. A single dose of 500 mg/kg sc in mice induced low levels of serum interferon. A 57% increase in mean day of death of mice infected with a fatal challenge of EMC virus was obtained with a dosage

Table I. Chemical and Antiviral Properties of Bisalkamine Esters of Fluorenone-, Fluorenol-, and Fluorenedicarboxylic Acids



No.	X	R	Mp, °C	Yield, %	Recrystn solvent	Formula	Analyses[a]	STR *vs.* EMC virus[b] at various doses (mg/kg sc) 250	100	50	10	2
1	O	2,7-$CO_2(CH_2)_2NMe_2$·HCl	254–256 dec	48	EtOH	$C_{23}H_{26}N_2O_5$·2HCl	C, H, Cl, N	1.66		1.12		
2	O	2,7-$CO_2(CH_2)_2NEt_2$·HCl	260–261 dec	25	EtOH	$C_{27}H_{34}N_2O_5$·2HCl	C, H, Cl	1.21	1.27	1.18	1.00	1.04
3	O	2,7-$CO_2C(Me)_2CH_2NEt_2$·HCl	204–206	5	Me_2CO–EtOH	$C_{31}H_{42}N_2O_5$·2HCl·0.5H_2O	C, H, Cl	1.66		1.28		
4	O	2,7-$COS(CH_2)_2NEt_2$·HCl	265–267 dec	33	MeOH	$C_{27}H_{34}N_2O_3S_2$·2HCl	C, H, Cl, N	Lethal	1.34	1.13		
5	O	2,7-$CO_2(CH_2)_2N(i\text{-}Pr)_2$·HCl	245–247 dec	30	EtOH–H_2O	$C_{31}H_{42}N_2O_5$·2HCl	C, H, Cl, N	1.29	1.35	1.02		
6	O	2,7-$CO_2(CH_2)_2NBu_2$·HCl	194–195 dec	53	*i*-PrOH	$C_{35}H_{50}N_2O_5$·2HCl·0.25H_2O	C, H, Cl	1.22		1.08		
7	O	2,7-$CO_2(CH_2)_2N(hexyl)_2$·HCl	185–186	12	MeCOEt	$C_{43}H_{66}N_2O_5$·2HCl	C, H, Cl, N	1.00		0.98		
								1.15[c]		1.09[c]		
8	O	2,7-$CO_2(CH_2)_2N$(Me)cyclohexyl·HCl	252 dec	59	MeOH–Et_2O	$C_{33}H_{42}N_2O_5$·2HCl	C, H, Cl	1.45		1.27	0.92	0.94
9	O	2,7-$CO_2(CH_2)_2N$(Et)Ph·HCl	212–213 dec	7	MeCOEt–MeOH	$C_{35}H_{34}N_2O_5$·2HCl	C, H, N	1.02		1.09		
10	O	2,7-$CO_2(CH_2)_2$morpholino·HCl	247–248 dec	22	EtOH–MeOH	$C_{27}H_{30}N_2O_7$·2HCl·0.5H_2O	C, H, Cl, N	0.96		0.93		
11	O	2,7-$CO_2(CH_2)_3NMe_2$·HCl	285–286 dec	57	EtOH–H_2O	$C_{25}H_{30}N_2O_5$·2HCl	C, H, Cl	1.67		2.08	1.10	
12	O	2,7-$CO_2(CH_2)_3NEt_2$·HCl	253–254 dec	25	EtOH	$C_{29}H_{38}N_2O_5$·2HCl	C, H, N	1.74		1.63	1.28	1.28
13	O	2,5-$CO_2(CH_2)_3NEt_2$·HCl	203–205	37	MeOH–EtOAc	$C_{29}H_{38}N_2O_5$·2HCl	C, H, Cl	1.49		1.20		
14	O	2,7-$CO_2(CH_2)_3NPr_2$·HCl	229–232 dec	43	EtOH–H_2O	$C_{33}H_{46}N_2O_5$·2HCl	C, H, Cl	2.20		1.30	1.14	
15	O	2,7-$CO_2(CH_2)_3N(allyl)_2$·HCl	236–238 dec	60	EtOH–H_2O	$C_{33}H_{38}N_2O_5$·2HCl	C, H, Cl	1.70		1.19		
16	O	2,7-$CO_2(CH_2)_3NBu_2$·HCl	178–179	38	MeOH–MeCOEt	$C_{37}H_{54}N_2O_5$·2HCl·H_2O	C, H, Cl, N	1.66	1.42	1.50	1.16	1.05
16a	O	2,7-$CO_2(CH_2)_3NBu_2$	Yellow oil			$C_{37}H_{54}N_2O_5$	C, H, N	2.12[d]	1.47[d]			
17	O	2,7-$CO_2(CH_2)_3N(pentyl)_2$·HCl	171–173	10	EtOH–EtOAc	$C_{41}H_{62}N_2O_5$·2HCl	C, H, Cl	1.10[e]		1.17	1.02	
18	O	2,7-$CO_2(CH_2)_3N(isopentyl)_2$·HCl	203–205	47	EtOH	$C_{41}H_{62}N_2O_5$·2HCl	C, H, Cl	1.13		1.07		
								1.17[f]		1.04[f]		
19	O	2,7-$CO_2(CH_2)_3$piperidino·HCl	293–295 dec	34	MeOH–MeCOEt	$C_{31}H_{38}N_2O_5$·2HCl	C, H, Cl	2.03		1.52	1.11	1.07
20	O	2,7-CO_2(1-Me-4-piperidyl)·HCl	303–305 dec	34	MeOH	$C_{27}H_{30}N_2O_5$·2HCl·3.5H_2O	C, H, Cl	2.02		2.08	1.17	
21	O	2,7-CO_2CH_2(1-Me-3-piperidyl)·HCl	257–268 dec[g]	79	H_2O–Me_2CO	$C_{29}H_{34}N_2O_5$·2H_2O	C, H, Cl[h]	1.82[i]		1.50	1.09	
22	O	2,7-$CO_2(CH_2)_2$(2-pyridyl)·HCl	180–182	46	EtOH–MeCOEt	$C_{29}H_{22}N_2O_5$·2HCl·H_2O	C, H, Cl	1.00		0.94	1.06	
23	O	2,7-$CO_2(CH_2)_4NEt_2$·HCl	216–217	38	EtOH	$C_{31}H_{42}N_2O_5$·2HCl	C, H, Cl	1.75		1.40		
24	O	2,7-$CO_2CH(Me)(CH_2)_3NEt_2$·HCl	170–190[g]	9	MeOH–MeCOEt	$C_{33}H_{46}N_2O_5$·2HCl·$^1/_3H_2O$	C, H, Cl	Lethal		1.39	1.14	1.12
25	O	2,7-$CO_2(CH_2)_5NEt_2$·HCl	225–226 dec	67	EtOH	$C_{33}H_{46}N_2O_5$·2HCl	C, H, Cl	0.88	1.71	1.46	1.04	
26	O	2,7-$CO_2CH_2C(Me)_2(CH_2)_3NH_2$·HCl	245 dec	11	H_2O–Me_2CO	$C_{29}H_{38}N_2O_5$·2HCl·$^3/_4H_2O$	C, H, Cl	0.55		1.62	1.20	1.11
27	O	2,7-$CO_2CH_2C(Me)_2(CH_2)_3NMe_2$·HCl	292 dec	40	MeOH	$C_{33}H_{46}N_2O_5$·2HCl	C, H, Cl	1.60[j]		1.29	1.24	
28	O	2,7-$CO_2(CH_2)_6NEt_2$·HCl	216–218 dec	35	EtOH	$C_{35}H_{50}N_2O_5$·2HCl	C, H, Cl	1.07		1.57	1.09	1.04
29	H, OH	2,7-$CO_2(CH_2)_2N$(Me)cyclohexyl·HCl	250–251 dec	50	MeOH-Et_2O	$C_{33}H_{44}N_2O_5$·2HCl	C, H, Cl	1.43		1.17	0.98	0.93
30	H, OH	2,7-$CO_2(CH_2)_3NEt_2$·HCl	213–214	63	EtOH	$C_{29}H_{40}N_2O_5$·2HCl·0.5H_2O	C, H	1.28		1.15		
31	H, OH	2,7-$CO_2(CH_2)_3N(allyl)_2$·HCl	213–215	62	EtOH	$C_{33}H_{40}N_2O_5$·2HCl·0.25H_2O	C, H, Cl	1.57		1.43	1.19	
32	H, OH	2,7-$CO_2(CH_2)_3NBu_2$·HCl	176–178[k]	35	EtOH	$C_{37}H_{56}N_2O_5$·2HCl·0.5H_2O	C, H, Cl	1.80		1.33		
33	H, OH	2,7-$CO_2(CH_2)_3N(isopentyl)_2$·HCl	175–178	65	EtOH–Et_2O	$C_{41}H_{64}N_2O_5$·2HCl	C, H, Cl	1.55		1.29	1.21	
34	H, OH	2,7-$CO_2(CH_2)_6NEt_2$	92–93	53	Et_2O	$C_{35}H_{52}N_2O_5$	C, H, N	1.36[l,m]		1.33[m]	1.09[m]	
35	H_2	2,7-$CO_2(CH_2)_2N$(Me)cyclohexyl·HCl	254 dec	41	MeOH–Et_2O	$C_{33}H_{44}N_2O_4$·2HCl·0.5H_2O	C, H, Cl			1.35	1.10	0.90
36	H_2	2,7-$CO_2(CH_2)_3NEt_2$·HCl	263 dec	40	MeOH–Et_2O	$C_{29}H_{40}N_2O_4$·2HCl	C, H, Cl	1.20		1.09		
37	H_2	2,5-$CO_2(CH_2)_3NEt_2$·HCl	202–203	40	$CHCl_3$–EtOAc	$C_{29}H_{40}N_2O_4$·2HCl	C, H, Cl	1.03	1.14	1.15	1.05	
38	H_2	1,7-$CO_2(CH_2)_3NEt_2$·HCl	246–247	73	MeOH–EtOAc	$C_{29}H_{40}N_2O_4$·2HCl·1.5H_2O	C, H, Cl	1.36	1.55	1.35	1.18	
39	H_2	2,7-$CO_2(CH_2)_3NBu_2$·HCl	183–184	44	EtOH–MeCOEt	$C_{37}H_{56}N_2O_4$·2HCl	C, H, Cl, N	1.30		1.36	1.10	1.12
40	H_2	2,7-$CO_2(CH_2)_3N(isopentyl)_2$·HCl	196–198	57	EtOH–MeCOEt	$C_{41}H_{64}N_2O_4$·2HCl	C, H, Cl	1.24		1.12	1.17	
41	H_2	2,7-CO_2CH_2(1-Me-3-piperidyl)·HCl	255–257 dec	32	EtOH	$C_{29}H_{36}N_2O_4$·2HCl·$^1/_3H_2O$	C, H, Cl	1.71[e]		1.27	1.15	
42	H_2	2,7-$CO_2CH_2C(Me)_2(CH_2)_3NMe_2$·HCl	294–295 dec	24	EtOH	$C_{33}H_{48}N_2O_4$·2HCl	C, H, Cl	Lethal		1.67	1.19	

43	H_2	2,7-$CO_2(CH_2)_6NEt_2 \cdot HCl$	229–230	16	EtOH–MeCOEt	$C_{35}H_{52}N_2O_4 \cdot 2HCl$	C, H, Cl	Lethal	1.25	1.11	
44	n	2,7-$CO_2(CH_2)_3NBu_2 \cdot HCl$	198–199 dec	45	*i*-PrOH–Et_2O	$C_{42}H_{66}ClN_3O_5 \cdot 2HCl \cdot 0.75H_2O$	C, H, N	Lethal	0.90	0.98	0.90
45	O	2-CO_2H, 7-$CO_2(CH_2)_3NBu_2 \cdot HCl \cdot 0.5H_2O$	234–235	9	MeOH–Me_2CO	$C_{26}H_{31}NO_5 \cdot HCl \cdot 0.5H_2O$	C, H, Cl	1.23[m]	1.19[m]	1.19[m]	1.17[m]
46	O	2,7-$CO_2(CH_2)_3N^+(Me)Bu_2I^-$	82–84	57	EtOH	$C_{37}H_{54}N_2O_5 \cdot (CH_3I)_2 \cdot H_2O$	C, H	Lethal	0.84		
47	O	2,7-$CO_2CH_2CONMe_2$	239–241	52	MeCN	$C_{23}H_{22}N_2O_7$	C, H, N	1.10	0.98[f]	1.14[f]	0.98[f]
48	O	2,7-$CO_2(CH_2)_2CN$	229–230	58	MeCN	$C_{21}H_{14}N_2O_5$	C, H, N	1.10[m]	0.98[m]	1.10[m]	
49	O	2,7-$CO_2(CH_2)_2OC_2H_5$	115–117	*o*	EtOH	$C_{23}H_{24}O_7$	C, H	0.98	1.10		
50	O	2,7-$CO_2(CH_2)_2Cl$	184–185	*o*	EtOH	$C_{19}H_{14}Cl_2O_5$	C, H, Cl	0.98	1.02		

[a] All analyses were within ±0.4% of calculated values. Neutralization equivalents (NE) were determined on all hydrated compounds and were within 1% of calculated values except where noted. Method: nonaqueous titration with $HClO_4$ in AcOH with $Hg(OAc)_2$ added and Crystal Violet or *p*-Naphtholbenzein as indicator [USP XVIII, 836 (1970)]. [b] STR, survival time ratio, is defined in description of test method in Experimental Section. In cases where several tests were run, average STR values are given. [c] 5% Tween 80 added to usual injection medium (see Experimental Section). [d] Compound injected in 5% phosphate buffered saline containing 1% Tween 80. [e] Only first five doses of standard regimen given instead of usual six (see Experimental Section). [f] 10% Tween 80 and 5% polyvinylpyrrolidone added to usual injection medium. [g] Probably a mixture of racemates. [h] NE, 305.8; calcd value, 299.8. [i] Only first four doses of standard regimen given. [j] Only first two doses of standard regimen given. [k] Melting point determined in sealed capillary. [l] Only first three doses of standard regimen given. [m] 10% Tween 80 added to usual injection medium. [n] X is $=NNHCOCH_2N^+Me_3Cl^-$ (Girard's T derivative). [o] Yield not calculated; compound prepared by reaction of excess of the alcohol with 9-oxofluorene-2,7-dicarbonyl chloride contained in filtrate from its recrystallization.

of 50 mg/kg sc at 28, 22, and 2 hr before and 2 hr after inoculation with virus (*i.e.*, in this test the STR was 1.57). Severity of vaccinia tail lesions in mice was decreased 63% by administration of 50 mg/kg sc at 24 and 2 hr before and 24 and 48 hr after inoculation with virus. In mice infected with a fatal challenge of Semliki Forest virus, the ED_{50} of **16** was 71 mg/kg administered sc as a single dose 24 hr before inoculation with virus. Acute LD_{50} of **16** in mice was 4000 mg/kg sc.

Experimental Section

Melting points were determined in open capillaries in a Thomas-Hoover apparatus and are uncorrected. Ir and uv spectra of all compounds were obtained and absorptions were as expected. Where analyses are indicated only by symbols of the elements, results obtained were within ±0.4% of theoretical values.

9-Oxofluorene-2,7-dicarboxylic acid, mp >400°, was prepared by the method of Dashevskii and Shamis.[11] The corresponding bisacid chloride was prepared by heating the acid with a large excess of $SOCl_2$ (trace of pyridine) for 5 hr; after removal of $SOCl_2$, the residue was recrystallized from dry C_6H_6 to give yellow needles, mp 212–216°.[7] Amino alcohols were obtained from commercial sources or synthesized by standard procedures reported in the literature. EtOH refers to commercial absolute EtOH.

Antiviral Evaluation Method. The anti-EMC activity of compounds in this study was determined in CF-1 male mice, 15–17 g each, at the several dose levels indicated in Table I. Ten mice were used for each dose level of a compound, and the control group for each compound was 20–30 untreated mice. The test compound was dissolved or suspended in 0.15% hydroxyethylcellulose in H_2O and injected sc in the nape of the neck. For each dose level, the indicated dose was injected at 28, 22, and 4 hr before and 2, 20, and 26 hr after inoculation with virus. The EMC virus was administered sc in the groin at infective doses in the range of 4–62 LD_{50}'s. Simultaneously, untreated control mice were infected with the same viral challenge. The mice were observed for 10 days after inoculation. Deaths were recorded twice daily and the mean day of death of the group was determined. A score of 11 was assigned to each survivor and used in determining the mean. A survival time ratio (STR), which is the mean day of death of the treated group divided by the mean day of death of the controls, was calculated for each dose level.

Activity is interpreted on the basis of parameters derived from standard deviations of the mean of control groups. An STR of less than 0.90 indicates that early deaths were observed; a ratio of 0.90–1.09 indicates that there was no activity; a ratio of 1.10–1.19 indicates low or weak activity (p = 0.2–0.05 by Students's t test); a ratio of 1.20–1.29 indicates medium activity (p = 0.1 to <0.001); and a ratio of 1.30 or greater indicates high activity (p = 0.05 to <0.001).

Bisesters of Fluorenonedicarboxylic Acids. Except for 3, 4, and 26, these esters were prepared essentially by the procedure described below for bis(3-dibutylaminopropyl) 9-oxofluorene-2,7-dicarboxylate·2HCl (**16**).

A suspension of 110 g (0.36 mol) of 9-oxofluorene-2,7-dicarbonyl chloride in 3.5 l. of $CHCl_3$ (EtOH free, stored over molecular sieves) was stirred and treated with 130.5 g (0.72 mol) of redistilled 3-dibutylaminopropanol, which caused a mildly exothermic reaction. The mixture was stirred and refluxed for 2 hr, then reduced in volume to 1.2 l., and washed with saturated $NaHCO_3$, H_2O, and saturated NaCl solution. The $CHCl_3$ solution was dried ($MgSO_4$) and filtered and the $CHCl_3$ evaporated. The residue was dissolved in MeCOEt and treated with ethereal HCl. The precipitate was filtered and recrystallized twice from 15:1 MeCOEt–MeOH to give 95 g (38%) of **16**. This compound on standing in the atmosphere formed a monohydrate: mp 178.5–179.5°; $\lambda_{max}^{H_2O}$ 276 mμ, $E_{1\ cm}^{1\%}$ 1370; % H_2O (Karl Fischer) calcd for $C_{37}H_{54}N_2O_5 \cdot 2HCl \cdot H_2O$, 2.58; found, 2.50, 2.62.

Bis(2-diethylamino-1,1-dimethylethyl) 9-Oxofluorene-2,7-dicarboxylate·2HCl·0.5H_2O (3). A mixture of 39.6 g (0.13 mol) of 9-oxofluorene-2,7-dicarbonyl chloride, 39 g (0.27 mol) of 1-diethylamino-2-methylpropanol-2, and 38 g (0.30 mol) of $PhNMe_2$ in 450 ml of anhydrous C_6H_6 was refluxed for 27.5 hr. The solid which precipitated was suspended in Et_2O and treated with cold 10% NaOH. The Et_2O extract was dried ($MgSO_4$), filtered, and treated with ethereal HCl. The viscous oil was recrystallized from *i*-PrOH, Me_2CO, and Me_2CO–MeOH to give 3.8 g (5%), mp 204–206°.

Bis(2-diethylaminoethyl) 9-Oxofluorene-2,7-dicarbothiolate·

2HCl (4). This compound was prepared from 12.2 g (0.04 mol) of 9-oxofluorene-2,7-dicarbonyl chloride and 14.4 g (0.085 mol) of diethylaminoethanethiol·HCl essentially by procedure described above for preparation of 16 to give 7.5 g (33%): mp 265–267°; $\lambda_{max}^{H_2O}$ 287.5 mμ, $E_{1\ cm}^{1\%}$ 1020.

Bis(5-amino-2,2-dimethylpentyl) 9-Oxofluorene-2,7-dicarboxylate·2HCl Hydrate (26). A solution of 9.4 g (0.072 mol) of 5-amino-2,2-dimethyl-1-pentanol in 360 ml of dry $CHCl_3$ (EtOH free, stored over molecular sieves) was treated with ethereal HCl (0.11 mol) and then with 10.88 g (0.036 mol) of 9-oxofluorene-2,7-dicarbonyl chloride. This suspension was stirred and refluxed for 27 hr. After standing at room temperature for 3 days, the gummy precipitate was filtered and stirred with boiling *i*-PrOH. The mixture was filtered and the filtrate placed under vacuum at room temperature overnight. The resulting moist solid was stirred with Me_2CO, filtered, and recrystallized from MeOH–anhydrous Et_2O and then from H_2O–Me_2CO, and dried to give 2.2 g (11%), mp 245° dec.

Bisesters of Fluorenoldicarboxylic Acids. These esters were prepared by reduction of corresponding fluorenone bisesters with $NaBH_4$ essentially by the procedure described below for bis(3-diallylaminopropyl) 9-hydroxyfluorene-2,7-dicarboxylate·2HCl·0.25H_2O (31). Preparation of 33 was carried out in 1:1 dioxane–H_2O.

A solution of 12.3 g (0.02 mol) of bis(3-diallylaminopropyl) 9-oxofluorene-2,7-dicarboxylate·2HCl (15) in 350 ml of warm H_2O was cooled to room temperature and added to 3 g (0.08 mol) of $NaBH_4$ in 15 ml of H_2O with swirling. The mixture was extracted with Et_2O and the extract washed with H_2O and saturated NaCl solution and then dried ($MgSO_4$). The mixture was filtered, the filtrate treated with ethereal HCl, and most of the Et_2O evaporated. The residue was dissolved in 50 ml of hot EtOH, the solution filtered through filter aid, and the filtrate chilled. The precipitate was filtered and recrystallized twice more from EtOH to give 7.7 g (62%) of 31: mp 213.5–215.5°; $\lambda_{max}^{H_2O}$ 304 mμ, $E_{1\ cm}^{1\%}$ 520.

Bisesters of Fluorenedicarboxylic Acids. Compounds 37 and 38 were prepared from the appropriate bisacid chlorides and amino alcohols by the general procedure described above for 16. The other fluorene bisesters were prepared by catalytic hydrogenation (Pd/C) of the corresponding fluorenone esters essentially by the procedure described below for bis(5-dimethylamino-2,2-dimethylpentyl) fluorene-2,7-dicarboxylate·2HCl (42). EtOH was the solvent used for preparation of 39 and 3:1 dioxane–H_2O for preparation of 40.

A solution of 20.0 g (0.032 mol) of bis(5-dimethylamino-2,2-dimethylpentyl) 9-oxofluorene-2,7-dicarboxylate·2HCl (27) in H_2O (total volume 240 ml) was hydrogenated over 8.0 g of 10% Pd/C for 2 days at 53° in a Paar hydrogenator. The solution was decanted from the catalyst, filtered through filter aid, treated with charcoal, and again filtered. This solution was made alkaline with 20% NaOH and extracted three times wth $CHCl_3$. The extract was washed twice with H_2O, dried (Na_2SO_4), and filtered, and the filtrate was treated with ethereal HCl. The $CHCl_3$ was evaporated under vacuum and the residue recrystallized from MeOH–Et_2O and then from absolute EtOH to give 4.7 g (24%) of 42: mp 294–295° dec; $\lambda_{max}^{0.1\ N\ HCl}$ 317 mμ, $E_{1\ cm}^{1\%}$ 640.

Fluorene-2,5-dicarboxylic Acid and Derivatives. A solution of 45 g (0.35 mol) of oxalyl chloride in 200 ml of CS_2 was added slowly to a stirred suspension of 47.5 g (0.35 mol) of anhydrous $AlCl_3$ and 25 g (0.12 mol) of fluorene-4-carboxylic acid in 700 ml of CS_2 chilled to 0°. After 6 hr, an additional 16 g (0.12 mol) of $AlCl_3$ and 45 g (0.35 mol) of oxalyl chloride was added and the mixture stirred for 64 hr at room temperature. The usual Friedel–Crafts work-up gave 25 g of fluorene-2,5-dicarboxylic acid, mp >360°. Refluxing 13 g of the acid with 150 ml of $SOCl_2$ (trace of pyridine) for 4 hr gave 7.2 g of fluorene-2,5-dicarbonyl chloride [mp 154–156° (from CCl_4). *Anal.* ($C_{15}H_8Cl_2O_2$) C, H, Cl], 800 mg of which was converted to dimethyl fluorene-2,5-dicarboxylate, mp 103–104° (from MeOH). *Anal.* ($C_{17}H_{14}O_4$) C, H. The main portion of the bisacid chloride was used to prepare compound 37.

9-Oxofluorene-2,5-dicarboxylic Acid and Derivatives. A solution of 11.9 g (0.047 mol) of fluorene-2,5-dicarboxylic acid in 150 ml of 40% KOH was added to a solution of 10.2 g (0.065 mol) of $KMnO_4$ in 150 ml of warm water. The mixture was heated on a steam bath 15 min and the precipitated MnO_2 removed by vacuum filtration on a pad of filter aid. The orange filtrate was acidified with 100 ml of 37% HCl and the mixture digested on a steam bath for 2 hr. The yellow precipitate was filtered with suction, washed well with H_2O, and dried in a vacuum oven. The 9-oxofluorene-2,5-dicarboxylic acid (11.7 g), mp 330–335° dec, was heated to reflux with 100 ml of $SOCl_2$ (trace of pyridine) for 15 hr. After removal of $SOCl_2$, the yellow solid was crystallized from $CHCl_3$ to give 6.6 g of 9-oxofluorene-2,5-dicarbonyl chloride, mp 172–174°, 600 mg of which was converted to dimethyl 9-oxofluorene-2,5-dicarboxylate, mp 180–181° (from MeOH). *Anal.* ($C_{17}H_{12}O_5$) C, H. The main portion of the bisacid chloride was used to prepare compound 13.

Fluorene-1,7-dicarboxylic Acid and Derivatives. The procedure described above for preparing fluorene-2,5-dicarboxylic acid was used to prepare 14 g of fluorene-1,7-dicarboxylic acid, mp >330°, from the reaction of 10 g (0.047 mol) of fluorene-1-carboxylic acid, 33.3 g (0.25 mol) of $AlCl_3$, and 38.7 g (0.3 mol) of oxalyl chloride in 550 ml of CS_2. Reaction of 7 g of the acid with refluxing $SOCl_2$ gave 4.5 g of fluorene-1,7-dicarbonyl chloride, mp 196–197° (from CCl_4), most of which was used to prepare compound 38. A 200-mg sample of the bisacid chloride was treated with MeOH to give dimethyl fluorene-1,7-dicarboxylate, mp 165–166°. *Anal.* ($C_{17}H_{14}O_4$) C, H. A solution of 150 mg of this ester and 600 mg of $Na_2Cr_2O_7·2H_2O$ in AcOH was heated to reflux for 30 min, cooled, and diluted with H_2O. The yellow product, mp 165–175°, was recrystallized from C_6H_6 to give dimethyl 9-oxofluorene-2,7-dicarboxylate, mp 184–186° (lit.[12] 184°). *Anal.* ($C_{17}H_{12}O_5$) C, H.

[2,7-Bis(3-dibutylaminopropoxycarbonyl)fluoren-9-yliden]-aminocarbamoylmethyltrimethylammonium Chloride·2HCl (44). A solution of 11.9 g (0.017 mol) of bis(3-dibutylaminopropyl) 9-oxofluorene-2,7-dicarboxylate·2HCl (16), 2.86 g (0.017 mol) of Girard's reagent T, and 125 ml of EtOH was refluxed for 1.5 hr. 1 ml of ethereal HCl added, and the solution refluxed for an additional 1 hr. The solution was diluted with anhydrous Et_2O (350 ml), causing a gum to separate. The supernatant was decanted and the gum triturated with anhydrous Et_2O, filtered, and dried under vacuum at 75°. This material was triturated in boiling Me_2CO, recrystallized from MeCN, triturated in boiling EtOAc, and recrystallized twice from *i*-PrOH–anhydrous Et_2O to give 6.3 g (45%): mp 198–199° dec; $\lambda_{max}^{0.1\ N\ HCl}$ 276 mμ, $E_{1\ cm}^{1\%}$ 1080.

7-(3-Dibutylaminopropoxycarbonyl)-9-oxofluorene-2-carboxylic Acid·HCl·0.5H_2O (45). A solution of 22.5 g (0.12 mol) of 3-dibutylaminopropanol in 150 ml of anhydrous THF (distilled from $LiAlH_4$) was added in 45 min to a stirred, refluxing solution of 36.6 g (0.12 mol) of 9-oxofluorene-2,7-dicarbonyl chloride in 600 ml of anhydrous THF. For 20 min during the addition, the reflux condenser was removed and, as THF evaporated, a thick precipitate separated. This mixture was refluxed for 1 hr, treated with 10 ml of H_2O, and refluxed for an additional 30 min. The precipitate was filtered and recrystallized several times from boiling MeOH to which anhydrous Me_2CO was added until cloudiness occurred: yield 5 g (9%); mp 234–235° (sinters at 185°).

Bis(3-dibutylaminopropyl) 9-Oxofluorene-2,7-dicarboxylate Dimethiodide·H_2O (46). A solution of 21.6 g (0.15 mol) of CH_3I in 50 ml of Me_2CO was added dropwise in 10 min to a stirred solution of 23 g (0.038 mol) of bis(3-dibutylaminopropyl) 9-oxofluorene-2,7-dicarboxylate (16a) in 200 ml of Me_2CO. The mixture was refluxed for 30 min and cooled, and Me_2CO was decanted from the oily precipitate. The oil readily solidified when triturated with Et_2O. Several recrystallizations from EtOH gave 19.7 g (57%) of the monohydrate, mp 82–84°.

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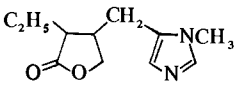
Synthesis and Cholinergic Activity of Some Structural Analogs of Pilocarpine†

Ronald F. Borne,* Hassan Y. Aboul-Enein,‡ I. W. Waters, and Jennifer Hicks§
School of Pharmacy, University of Mississippi, University, Mississippi 38677. *Received July 31, 1972*

In an attempt to clarify the structural requirements of cholinergic activity of pilocarpine, the preparation of 14 analogs was successfully achieved through the Michael addition of the anions derived from 2-picoline, 4-picoline, 4-methylpyrimidine, and 2-methylpyrazine to $\Delta^{\alpha,\beta}$-butenolide, α-methyl- and α-ethyl-$\Delta^{\alpha,\beta}$-butenolide, cyclopentenone, and 2-ethylcyclopent-2-enone. Attempts to generate anions derived from 1,2-dimethylimidazole and 1,2-dimethylpyrrole were unsuccessful. The synthesis and cholinergic activity of these compounds are discussed. Only 3-ethyldihydro-4-(4-pyrimidylmethyl)-2(3*H*)-furanone (**9**) displayed parasympathomimetic activity.

The action of pilocarpine on the parasympathetic nervous system has been widely studied and this alkaloid has achieved limited use in the treatment of glaucoma. Surprisingly, little data are available in the literature with regard to structure–activity relationship requirements for its cholinergic activity. Pilocarpine is assumed to interact with the muscarinic receptor. Molecular configurations of pilocarpine were presented[1] indicating possible modes of receptor binding. The major binding sites have been assumed to be the lactone ring oxygen and carbonyl oxygen atoms and the imidazole nitrogen atoms in the protonated form.[2] Pilocarpine occurs naturally as the cis



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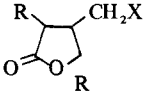
isomer and is more potent than the trans isomer (isopilocarpine).[3] The lactone ring appears to be essential for activity[4,5] while the imidazole ring can be cleaved without completely destroying activity.[6] A number of quaternary salt derivatives of pilocarpine have been recently shown to antagonize the effects of acetylcholine.[7] Although pilocarpine possesses a dual mode of action,[8] we felt it of interest to examine certain structural analogs in an attempt to clarify the binding sites of pilocarpine to the muscarinic receptor. We anticipated that modification of the imidazole ring, the lactone ring oxygen atom, and the alkyl group in the α position of the lactone ring would lead to derivatives which, depending upon their cholinergic activities, could provide information relating to the importance of these functions in cholinergic receptor binding.

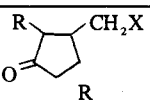
The imidazole ring of pilocarpine can be considered to possess two different types of nitrogen atoms, *viz.*, a pyridine-type nitrogen and a pyrrole-type nitrogen. Delocalization of the positive charge between these nitrogen atoms has been demonstrated upon protonation of 1-methylimidazole[9] and upon quaternization of pilocarpine.[2] To determine whether or not a delocalized positive charge is a requirement for cholinergic activity, we synthesized analogs of pilocarpine in which the imidazole ring is replaced by a pyridine ring while at the same time examining the importance of the ethyl group in the α position of the lactone ring (Table I). The role of the lactone ring oxygen atom could be examined by replacing the oxygen atom with a methylene group to give the corresponding

Table I. γ-Butyrolactone Derivatives



Compd	R	X
2	H	2-Pyridyl
3	CH_3	2-Pyridyl
4	C_2H_5	2-Pyridyl
5	H	4-Pyridyl
6	CH_3	4-Pyridyl
7	C_2H_5	4-Pyridyl
8	H	4-Pyrimidyl
9	C_2H_5	4-Pyrimidyl
10	H	2-Pyrazinyl
11	C_2H_5	2-Pyrazinyl

Table II. Cyclopentanone Derivatives



Compd	R	X
12	H	2-Pyridyl
13	C_2H_5	2-Pyridyl
14	H	4-Pyridyl
15	C_2H_5	4-Pyridyl

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