Synthesis and Anthelmintic Activity of 4-Alkoxy-1-naphthamidines

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The preparation, isolation, and melting points of 45 new 4-alkoxy-*N*,*N*-dialkyl-1-naphthamidine hydrochlorides with the alkoxy alkyl = Me through $C_{13}H_{27}$ and the *N*-alkyl = Et through C_8H_{17} are outlined. A brief discussion of the limitations of several synthetic routes to sterically hindered amidines is given. Approximate oral LD_{50} values and therapeutic indices (TI) against the cestodes (tapeworms) *Hymenolepis nana* and *Oochoristica symmetrica* of these and related α-naphthamidines in the mouse are given. A broad band of effective compounds is found at the sum of the number of carbons on O (C_{OR}) plus twice the number of carbons on each (of two identical) alkyl groups on one N ($2C_{NR}$) = 12–*ca.* 23. A narrow band of maximum TI exists at $C_{OR} + C_{NR}$ = 10–13. Branching at the N (comparison of *N*,*N*-di-*i*-Pr with *N*,*N*-di-Et) does not effect the TI. Presence of hydrophilic groups or benzene rings on an amidine N destroyed activity, in the few cases studied. *H. nana* was much more readily eliminated than *O. symmetrica* from the mouse. However results with neither of these adequately matched the structure–activity relationships found in the cestodes of dog and cats: *Taenia pisiformis*, *Dipylidium caninum*, *Hydatigera taeniaformis*, or *Spirometra mansonoides*. Individual amidines were effective against each of these, but the best compounds against all 4 species in the dog and cat are grouped at $C_{OR} \geq 4$ and $C_{NR} \leq 4$. Bunamidine (4-hexyloxy-*N*,*N*-dibutyl-1-naphthamidine) has been found to be a "broad spectrum" cestocide effective against a variety of cestodes of veterinary significance in farm animals, and to be effective against adult *Echinococcus* spp. in dogs. These cestodes, in the larval form, are significant pathogens of man as well as farm animals.

An earlier study of structure–activity relationships for anthelmintics active against the nematode *Syphacia obvelata* (a "mouse pinworm") in the mouse, disclosed that four *N*,*N*-dialkyl-4-methoxynaphthamidines had substantial activity against that parasite.[2] The criteria used to select compounds for testing were at first a high numerical ratio of oral LD_{50} to ip LD_{50}, and later the existence (at physiological pH values) of a formal positive charge adjacent to a ring. The first criterion was taken to indicate that such compounds had a high inherent toxicity (shown by the low ip LD_{50}) and so might well be toxic to the parasite "immersed" in the substance, but were poorly absorbed by the host (large value of the oral LD_{50}). The second criterion was presented as a rewarding but completely empirical relationship.

The amidines reported at that time were not quite good enough to be acceptable candidates for human pinworm treatment. A variety of them, however, were submitted for other anthelmintic screening. Although usually there is no overlap between effective teniacides and compounds useful against nematodes, preliminary screening revealed that many of these naphthamidines had marked activity against tapeworms in the mouse and in the dog. A preliminary communication[3] from our group and collaborating groups has reported the activity of one of these, *N*,*N*-dibutyl-4-hexyloxynaphthamidine (bunamidine),[4] which is outstandingly effective against a variety of cestodes. It is the purpose of this communication to give the synthesis, properties, and aspects of the chemistry of these amidines, and to discuss the evidence, primarily based on tapeworm removal in the mouse, on which the decision to take testing to larger host animals was based. The results of experiments using larger animals are summarized briefly, and the usefulness of these mouse screening tests as a guide to results in larger animals is evaluated on the basis of experience with this series.

Results and Discussion

Chemistry.—These amidine salts were made, in general, by only minor modifications of a method reported earlier.[5] This involves the reaction of the appropriate 4-alkoxynaphthonitrile, itself made from 1-naphthol,[6] with the dialkylaminomagnesium halide made from the secondary amine and a Grignard reagent, usually Et-MgBr. However, the isolation method reported for the lower molecular weight amidines had to be modified because the amidine bases decomposed to give nitrile at the temperatures required to distil off the excess of some of the higher-boiling amines. In the case of reactions involving amines of intermediate size and 4-alkoxynitriles with large alkyl groups, it was generally possible to keep the starting amine and the Mg salts in solution with HCl while precipitating the amidine·HCl, by decomposition of the reactions with aq HCl. An example of this is given in the Experimental Section as procedure A. Occasionally a mixture of amidinium bromide with the chloride was obtained, which could be converted into pure chloride, *e.g.*, by stirring in MeOH with AgCl.

When the starting amine·HCl precipitated with the amidine salt on such HCl treatment, a tedious separation could be avoided by utilizing the very rapid reac-

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(1) (a) Department of Organic Chemistry. (b) Department of Experimental Therapeutics.

(2) M. Harfenist, *J. Med. Chem.*, **6**, 361 (1963).

(3) R. Baltzly, R. B. Burrows, M. Harfenist, K. A. Fuller, J. E. D. Keeling, O. D. Standen, C. J. Hatton, V. J. Nunns, D. A. Rawes, B. D. Blood, V. Moya, and J. L. Lelijveld, *Nature (London)*, **206**, 408 (1965).

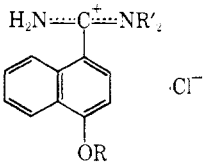
(4) Scoloban.

(5) E. Lorz and R. Baltzly, *J. Amer. Chem. Soc.*, **73**, 93 (1951), and earlier papers.

(6) 1-Naphthol → 1-naphthyl alkyl ether → 1-alkoxy-4-bromonaphthalene → 4-alkoxynaphthonitrile.

TABLE I

PROPERTIES OF *N,N*-DIALKYL-4-ALKOXY-1-NAPHTHAMIDINE HYDROCHLORIDES



Number	R of OR	R' of NR'_2	Yield, %[a]	Mp,[b] °C	Recryst[c] solvent	Formula[b] (HCl omitted)	Anal.
1	CH_3	C_7H_{15}	78	183	EA	$C_{26}H_{40}N_2O$	C, H
2	CH_3	C_8H_{17}	70	176	A–EA–E	$C_{28}H_{44}N_2O$	C, H
3	C_2H_5	C_7H_{15}	44	231–231.5	A–EA–E	$C_{27}H_{42}N_2O$	C, H
4	C_2H_5	C_8H_{17}	42	222–223	Ac–E	$C_{29}H_{46}N_2O$	C, H
5	C_3H_7	C_7H_{15}	54	221–223	Ac	$C_{28}H_{44}N_2O$	C, H
6	C_3H_7	C_8H_{17}	45	212–214	A–EA–E	$C_{30}H_{48}N_2O$	C, H
7	C_4H_9	C_5H_{11}[d]	45	222	A–EA–E	$C_{25}H_{38}N_2O$	C, H
8	C_4H_9	C_6H_{13}	65	218–219	A–E	$C_{27}H_{42}N_2O$	C, H
9	C_4H_9	C_7H_{15}	68	214	A–E	$C_{29}H_{46}N_2O$	C, H
10	C_4H_9	C_8H_{17}	39	205–206	A–E	$C_{31}H_{50}N_2O$	C, H
11	C_5H_{11}	C_3H_7	25	219.5	W–HCl	$C_{22}H_{32}N_2O$	C, H
12	C_5H_{11}	$CH(CH_3)_2$	49	207	W–HCl	$C_{22}H_{32}N_2O$	C, H
13	C_5H_{11}	C_4H_9	40	209–210	A–E	$C_{24}H_{36}N_2O$[e]	C, H
14	C_5H_{11}	C_5H_{11}	48	220–221	A–E–H	$C_{26}H_{40}N_2O$	C, H
15	C_5H_{11}	C_6H_{13}	55	213	A–EA–E	$C_{28}H_{44}N_2O$	C, H
16	C_5H_{11}	C_7H_{15}	60	207	A–E	$C_{30}H_{48}N_2O$	C, H
17	C_6H_{13}	C_3H_7	66	205	W–HCl	$C_{23}H_{34}N_2O$	C, H
18	C_6H_{13}	C_4H_9[f]	76	214–215	A–E; W–HCl	$C_{25}H_{38}N_2O$	C, H
19	C_6H_{13}	C_5H_{11}[d]	60	217–218	A–EA–E	$C_{27}H_{42}N_2O$	C, H
20	C_6H_{13}	C_6H_{13}	69	200.5–201	N	$C_{29}H_{46}N_2O$	C, H
21	C_6H_{13}	C_7H_{15}	81	205.5–206.5	A–EA–E	$C_{31}H_{50}N_2O$	C, H
22	C_6H_{13}	C_8H_{17}	71	190.5–191.5	N	$C_{33}H_{54}N_2O$	C, H
23	C_7H_{15}	C_3H_7	93	203–203.5	W–HCl	$C_{24}H_{36}N_2O$	C, H
24	C_7H_{15}	$CH(CH_3)_2$	46	209.5	W–HCl	$C_{24}H_{36}N_2O$	C, H
25	C_7H_{15}	C_4H_9	42	205	W–HCl	$C_{26}H_{40}N_2O$	C, H
26	C_7H_{15}	C_6H_{13}	73	197–198	N	$C_{30}H_{48}N_2O$	C, H
27	C_7H_{15}	C_7H_{15}	52	196.5–198.5	A–EA–E	$C_{32}H_{52}N_2O$	C, H
28	C_7H_{15}	C_8H_{17}	79	186–188	N	$C_{34}H_{56}N_2O$	C, H
29	C_8H_{17}	C_3H_7	60	205	W–HCl; Ac	$C_{25}H_{38}N_2O$[g]	C, H
30	C_8H_{17}	$CH(CH_3)_2$	41	210	W–HCl; Ac	$C_{25}H_{38}N_2O$	C, H
31	C_8H_{17}	C_4H_9	75	197–198	W–HCl	$C_{27}H_{42}N_2O$	C, H
32	C_9H_{19}	C_3H_7	13	205–207	Ac–E	$C_{26}H_{40}N_2O$	C, H
33	C_9H_{19}	C_4H_9	37	198–199	W–HCl	$C_{28}H_{44}N_2O$	C, H
34	$C_{10}H_{21}$	C_3H_7	47	195–196	W	$C_{27}H_{42}N_2O$	C, H
35	$C_{10}H_{21}$	$CH(CH_3)_2$	41	184–186	W–HCl; Ac–E	$C_{27}H_{42}N_2O$	C, H
36	$C_{10}H_{21}$	C_4H_9	43	195–196	W	$C_{29}H_{46}N_2O$	C, H
37	$C_{11}H_{23}$	C_2H_5	27	144	A–E	$C_{26}H_{40}N_2O$	C, H
38	$C_{11}H_{23}$	C_3H_7	64	186.5–187	W–HCl	$C_{28}H_{44}N_2O$	C, H
39	$C_{11}H_{23}$	$CH(CH_3)_2$	19	181.5	W–HCl	$C_{28}H_{44}N_2O$	C, H
40	$C_{12}H_{25}$	C_2H_5	13	151.6–152	EA–E	$C_{27}H_{42}N_2O$	C, H
41	$C_{12}H_{25}$	C_3H_7	4	181–181.5	W–HCl	$C_{29}H_{46}N_2O$	C, H
42	$C_{12}H_{25}$	$CH(CH_3)_2$	15	179–180	W–HCl	$C_{29}H_{46}N_2O$	C, H
43	$C_{13}H_{27}$	C_3H_7	21	175–176	W–HCl	$C_{30}H_{48}N_2O$	C, H
44	$CH_2C_6H_5$	C_4H_9	90	239–241	A–W	$C_{26}H_{32}N_2O$	C, H
45	$CH_2C_6H_5$	C_6H_{13}	81	243.5–245	A–W	$C_{30}H_{40}N_2O$	C, H

[a] All yields are anal. pure material isolated. [b] All data on mp, anal., etc. refer to monohydrochloride salts. [c] Solvents: A = anhyd EtOH; Ac = Me_2CO; E = anhyd Et_2O; EA = EtOAc; HCl = 6 *N* aq HCl; N = $MeNO_2$; W = H_2O. [d] Mixed diamylamines used. [e] Monohydrate analyzed. [f] Bunamidine. [g] A hemihydrate sometimes cryst (C, H, N).

tion of methyl formate with amines in MeOH.[7] This was done by converting the reaction product to the base (See Caution in Experimental Section) and treating in MeOH solution with methyl formate in excess (procedure B). If the amidine formylates under these conditions, the resulting compound reverts to amidine(·HCl) rapidly in ethanolic HCl at room temp, while the formylated amine apparently remains unhydrolyzed. Addition of Et_2O or hexane to an ethanolic HCl solution of the formylated base(s) then precipitates the pure amidinium salt while the formamide (and any starting naphthonitrile) remains in solution.

Other methods of synthesis of amidines were also investigated. The classical Pinner method[8] is ineffective for our naphthamidines apparently because of the com-

(7) A. A. Rothstein, Thesis, Polytechnic Institute of Brooklyn, Brooklyn, N. Y. (1949). Rothstein showed that methyl formate reacts extremely rapidly with secondary amines, esp in MeOH. Probably a few min would be adequate for quant reaction between our amines and excess methyl formate. Compare B. W. Horrom, M. Freifelder, and G. R. Stone, *J. Amer. Chem. Soc.*, **77**, 753 (1955).

(8) A. Pinner, *Ber.*, **23**, 161 (1890).

bination of deactivation of the (protonated) CN group by the electron-supplying para-alkoxy group (and presumably the adjacent benzene ring), and the steric hindrance of the naphthalene 8-H to conversion of the cylindrical CN group to the more bulky iminoether function. Although Pinner had said that his method failed with the sterically hindered *o*-tolunitrile and α-naphthonitrile, it was felt that this point should be reinvestigated, since Pinner's criterion for reaction was the precipitation of the iminoether·HCl from the reaction mixture. Solomon and Turner[9] were able to convert α-naphthonitrile under Pinner reaction conditions, but without isolation of the intermediate iminoether·HCl, to a 15–20% yield of isolated α-naphthamidine, after reaction with NH_3. Reactions of α-naphthonitrile under Pinner conditions with secondary amines gave poorer yields, and in all reactions of 4-alkoxynaphthonitriles under Pinner conditions, conversions were smaller still (5% or less).

Some attention was also given to other alternative syntheses. Heating, *e.g.*, 4-butoxynaphthonitrile with salts made from various sec amines and *p*-TosOH[10] resulted in 90% recovery of the alkoxynaphthonitrile, and formation of a trace of 4-hydroxynaphthonitrile. However, the formally similar process of warming the nitrile with 2 moles of secondary amine and 1 mole of $AlCl_3$,[11] preferably by mixing the last two components before adding nitrile to diminish dealkylation at the ether bond, gave acceptable yields of amidines. An example of this, procedure C, is given in the Experimental Section.

A single attempt was made to treat 1-hexyloxynaphthalene with BrCN and $AlCl_3$ in C_6H_6, rather than in CS_2 as reported by others,[12] with the hope that the resulting nitrile could then be converted directly into amidine using the same $AlCl_3$, by our procedure C. This led to an excellent yield of what appeared to be hexylbenzene, and so was not studied further.

Activity against Mouse Tapeworms.—The results of tests of selected naphthamidines against *H. nana* and *O. symmetrica* in the mouse are summarized in Table II. Other naphthamidines tested, but which would be expected to be inactive based on the correlations reported below, are omitted. Results have been converted to a therapeutic index (TI) for each compound and each parasite where possible, to allow intercomparisons. The TI in each case has as its numerator the acute oral LD_{50} in the mouse, and the denominator is the single oral dose needed to remove 100% of *H. nana* (CD_{100}) or 67% of *O. symmetrica* (CD_{67}) (two-thirds removal of *O. symmetrica* was selected because of the difficulty of removing 100% of this cestode). Thus, for *H. nana* the TI = LD_{50}/CD_{100}, while for *O. symmetrica* the TI = LD_{50}/CD_{67}. In many cases, the LD_{50} or the appropriate CD was not reached, so the maximum dose is shown preceded by a "greater than" or "less than" sign.

(9) W. Solomon and A. G. Turner, Wellcome Chemical Research Laboratories, Beckenham, Kent, England, private communication. We are grateful for permission to summarize this work.

(10) W. F. Short, M. W. Partridge, and P. Oxley, British Patent 604,032 (1948). P. Oxley and W. F. Short, *J. Chem. Soc.*, 147 (1946), used benzene sulfonates of amines with aromatic nitriles including α-napthonitrile to get acceptable yields of amidines.

(11) W. F. Short and M. W. Partridge, British Patent 598,453 (1948); P. Oxley, M. W. Partridge, and W. F. Short, *J. Chem. Soc.*, 1110 (1947).

(12) G. W. Grey and B. Jones, *ibid.*, 678 (1954); P. Karrer, A. Rebmann, and E. Zeller, *Helv. Chim. Acta*, **3**, 261 (1920).

Inspection of the results shows that *H. nana* was markedly the more sensitive species to these amidines in the mouse. Table II shows that 29 of the 42 compounds for which results are given, expelled all of the *H. nana* at or below a dose level of 200 mg/kg (calcd as base). However, only 10 of these compounds expelled all of the *O. symmetrica* at 400 mg/kg or less, *i.e.*, at twice the *H. nana* dosage. Nevertheless the same compounds had the most favorable TI values against both species of parasites in the mouse, where comparisons were possible.

Activities (without consideration of toxicity) can be matched as a first approximation to the sum of the carbons of the alkoxyl group (defined = C_{OR}) and those on the amidine N (defined = $2C_{NR}$ for the symmetrical compounds shown). Significant activity against these cestodes is found when $12 < (C_{OR} + 2C_{NR}) \lesssim$ *ca.* 23, and among a few scattered lower amidines which apparently are less specific in their actions. The broad activity range might be considered as a plateau of the 3-dimensional surface obtained by plotting the 2 independent variables C_{OR} and $2C_{NR}$ against activity.[13] This surface, when cut by planes representing constant values of either C_{OR} or $2C_{NR}$, would give the usual sort of curves of activity *vs.* number of C, rising to a maximum and then decreasing. This type of curve is commonly attributed to the existence of an optimum lipid–water partition coefficient. It should be pointed out that in the case of compounds cationic at physiological pH values, such as these amidines, lowering of the activity with increased chain length can also be due to decreased solubility of salts formed by combination with any of a variety of acids present in the gut of the host.

The TI values must reflect not only the activity against the appropriate cestode, but also the toxicity to the host. Inspection of Table II suggests that the oral LD_{50} values[14] are not a neat function of the individual numbers of carbons, but are most easily summarized in terms of $C_{OR} + C_{NR}$ for the amidines made from sym amines. Since our LD_{50} data are scanty, the best statement for present purposes is that compounds with relatively lower LD_{50} values are found in the region of low values of $C_{OR} + C_{NR}$ while greater values of this sum lead to variable, but numerically higher values of the mouse LD_{50}. Because of this relationship, combined with the activity relationship outlined above, Table II shows no compounds in the lower range of $C_{OR} + C_{NR}$ whose TI exceeds 1, while most compounds whose value of $C_{OR} + C_{NR}$ is greater than 9–10 have TI values, at least against *H. nana*, greater than 1. We could not decide whether the TI again fell off at higher values of OR and NR_2, because it was not convenient to give the large dose required to reach the CD, and generally impossible to give an LD_{50} dosage. As a practical matter, this question was felt not worth pursuing because of the large amount of compound which would have to be administered for any therapeutic effect.

Surprisingly, inspection of Table II reveals that for those groups of amidines for which reasonably precise TI values have been determined, the compounds of

(13) There obviously is no such continuous surface, since C_{OR} and $2C_{NR}$ can only have integral values. As in all structure–activity curves, continuity is assumed for convenience.

(14) Oral LD_{50} values determined by R. V. Fanelli by the procedure given in M. Harfenist, R. V. Fanelli, R. Baltzly, H. W. Brown, K. L. Hussey, and K. F. Chan, *J. Pharmacol. Exp. Ther.*, **121**, 347 (1957).

TABLE II

ACTIVITY AND TOXICITY OF 4-ALKYLOXY-1-NAPHTHAMIDINES[a] AGAINST *Hymenolepis nana* AND *Oochoristica symmetrica* IN MICE

Compd from Table I	R of OR	R′ of NR′$_2$	Oral LD_{50}, mg/kg (base)	*H. nana* Oral CD_{100}, mg/kg (base)	*H. nana* TI[b] = LD_{50}/CD_{100}	*O. symmetrica* Oral CD_{67}, mg/kg (base)	*O. symmetrica* TI[b] = LD_{50}/CD_{67}
e	CH_3	C_2H_5	200	>200	<1	>50	*d*
c	CH_3	C_3H_7	100	>100	<1	>200	<1
c	CH_3	C_4H_9	400	>400	<1	>200	*d*
c	CH_3	C_5H_{11}	160	>200	<1	>200	<1
c	CH_3	C_6H_{13}	244	400	<1[e]	>500	<1
1	CH_3	C_7H_{15}	2000 ?	400	5[e] ?	>500	<4 ?
2	CH_3	C_8H_{17}	3000 ?	200	15[e] ?	400	8 ?
c	C_2H_5	C_3H_7	>200	200	>1	>50	*d*
c	C_2H_5	C_4H_9	200	200	1	>200	<1
c	C_2H_5	C_5H_{11}	*ca.* 200	>400	<1	>200	<1
3	C_2H_5	C_7H_{15}	*ca.* 1000	200	*ca.* 5	>400	<2
4	C_2H_5	C_8H_{17}	>1500	100	>15	<400	>4
5	C_3H_7	C_7H_{15}	1400	200	7	400	4
6	C_3H_7	C_8H_{17}	>1500	100	>15	400	4
c	C_4H_9	C_4H_9	200	200	1	>300	<1
7	C_4H_9	C_5H_{11}[f]	200	200	1	>300	<1
8	C_4H_9	C_6H_{13}	*ca.* 1400	200	*ca.* 7	200	*ca.* 7
9	C_4H_9	C_7H_{15}	*ca.* 1400	⩽400	*ca.* 4	*ca.* 300	*ca.* 5
10	C_4H_9	C_8H_{17}	1800	200	9	>500	*d*
11	C_5H_{11}	C_3H_7	200			>200	*d*
13	C_5H_{11}	C_4H_9	*ca.* 200	100	*ca.* 2	>300	<1
14	C_5H_{11}	C_5H_{11}	375	200	2	>300	*d*
15	C_5H_{11}	C_6H_{13}	>1000	200	>5	250	>4
16	C_5H_{11}	C_7H_{15}	>5000	100	>50	450	>11
17	C_6H_{13}	C_3H_7	>200	>100	?		
18	C_6H_{13}	C_4H_9[g]	540	200	3	>300	<2[d]
19	C_6H_{13}	C_5H_{11}[f]	360	200	2	>300	*d*
20	C_6H_{13}	C_6H_{13}	*ca.* 1700	200	9	200	9
21	C_6H_{13}	C_7H_{15}	>2000	<400	>5	*ca.* 400	>5
22	C_6H_{13}	C_8H_{17}	>5000	400[h]	>12	>500	*d*
23	C_7H_{15}	C_3H_7	440	150	3	>400	<1
25	C_7H_{15}	C_4H_9	650	200	3	>400	*d*
26	C_7H_{15}	C_6H_{13}	650	200	3	450	1
27	C_7H_{15}	C_7H_{15}	7000	400	18	>300	*d*
28	C_7H_{15}	C_8H_{17}	10500	>400	<26	>500	*d*
29	C_8H_{17}	C_3H_7	500	100	5	300	2
31	C_8H_{17}	C_4H_9	620	200	3	>200	?
32	C_9H_{19}	C_3H_7	750	100	8	>200	*d*
33	C_9H_{19}	C_4H_9	750	200	4	400	2
34	$C_{10}H_{21}$	C_3H_7	>1000	100	>10	100	>10
36	$C_{10}H_{21}$	C_4H_9	700	200	4	150	5
38	$C_{11}H_{23}$	C_3H_7	>1000	100	>10	300	>3
41	$C_{12}H_{25}$	C_3H_7	>400	100	>4	300	>1
43	$C_{13}H_{27}$	C_3H_7	>200	>200	?	>400	*d*

[a] Tests on the hydrochlorides. [b] TI = therapeutic index. See text for definition. [c] Prepn reported by Lorz and Baltzly.[5] [d] Essentially inactive at the highest dose tested. [e] Activity against *Syphacia obvelata* reported by Harfenist.[2] [f] Made from inhomogeneous Am_2NH. [g] Bunamidine. [h] Two mice of 5 cleared.

maximum TI against the 2 screening cestodes in the mouse are found to be narrowly grouped about $C_{OR} + C_{NR} = 10–13$.

Table III shows the results obtained when selected amidines containing branched rather than linear alkyl groups on the amidine N were tested against the same screening systems. Intercomparisons (*e.g.*, **34** with **35**, **38** with **39**, and **41** with **42**) show no clearcut consistent superiority of either linear or the branched series over the other. Apart from its therapeutic significance, the equivalence of the two series clearly shows that the amidine linkage does not need to bind at a highly sterically hindered site for either activity or toxicity.

Further studies directed along lines suggested by the maximum activity data in the mouse were aborted when work with larger animals and their cestode parasites showed that therapeutic indices for these did not parallel TI values found in the mouse (see below).

Table IV gives a cross-section of 4-alkoxy-1-naphthamidines which do not fit into the categories of the other tables. Some of these have two different groups on one amidine N, one of which is Ph, substituted Ph, or benzyl. The others have a piperazine or morpholine ring whose N is part of the amidine moiety. None of these showed activity against our test tapeworms in the mouse at the dosage tabulated.

Effectiveness against the Cestodes of Larger Animals.—Detailed studies have been published (see

TABLE III

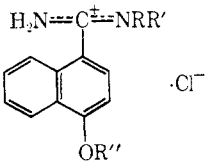
ACTIVITY OF N-BRANCHED 4-ALKYLOXY-1-NAPHTHAMIDINES[a] AGAINST *H. nana* AND *O. symmetrica* IN MICE

Table I No.	R of OR	R′ of NR′$_2$	LD$_{50}$, mg/kg (base)	*Hymenolepis nana* CD$_{100}$, mg/kg (base)	*Hymenolepis nana* TI[b] = LD$_{50}$/CD$_{100}$	*Oochoristica symmetrica* CD$_{67}$ mg/kg	*Oochoristica symmetrica* TI[b] = LD$_{50}$/CD$_{67}$
c	CH_3	$CH(CH_3)_2$	<400	400	<1	>200	*d*
c	CH_3	$CH_2CH(CH_3)_2$	*ca.* 200	>200	<1	>200	*d*
c	CH_3	$CH(CH_3)C_2H_5$	*ca.* 200	>200	<1	>200	*d*
c	CH_3	$(CH_2)_2CH(CH_3)_2$	*ca.* 170	>200	<1	>200	*d*
c	C_2H_5	$CH_2CH(CH_3)_2$	<200	400	<1	>200	*d*
c	C_2H_5	$CH(CH_3)C_2H_5$	<200	>200	<1	>25	*d*
c	C_2H_5	$(CH_2)_2CH(CH_3)_2$	*ca.* 170	>200	<1	>200	*d*
12	C_5H_{11}	$CH(CH_3)_2$	>200	>200	?		
24	C_7H_{15}	$CH(CH_3)_2$	800	100	8	350	2
30	C_8H_{17}	$CH(CH_3)_2$	400	200	2	400	1
35	$C_{10}H_{21}$	$CH(CH_3)_2$	>1000	40	>25	200	>5
39	$C_{11}H_{23}$	$CH(CH_3)_2$	>1000	50	>20	*ca.* 200[d]	>5
42	$C_{12}H_{25}$	$CH(CH_3)_2$	>1000	100	10	400	3

[a] Tested as hydrochloride salts. [b] TI = therapeutic index. [c] Preparation reported by Lorz and Baltzly.[5] [d] Variable activity leading to CD$_{67}$ = 400 and 300 mg, and CD$_{100}$ = 200 mg.

TABLE IV

MISCELLANEOUS NAPHTHAMIDINES SHOWING NO SIGNIFICANT ACTIVITY AGAINST *H. nana* AND *O. symmetrica* IN MICE



R″ of OR″	R′	R	LD$_{50}$, mg/kg	Maximum dose tested, mg/kg × 1 day: *H. nana*	Maximum dose tested, mg/kg × 1 day: *O. symmetrica*
CH_3	C_2H_5	$C_6H_4CH_3$-*o*	190	200	150
CH_3	C_2H_5	$CH_2C_6H_5$	~200	50	200
CH_3	C_4H_9	$C_6H_4OCH_3$-*p*	~210	200	300
C_2H_5	C_2H_5	$C_6H_4CH_3$-*m*	>200	200	25
C_2H_5	C_2H_5	$C_6H_4CH_3$-*p*	>200	200	200
C_2H_5	C_2H_5	$CH_2C_6H_5$	>200	200	200
C_2H_5	NRR′ = morpholine		<400	200	400
C_2H_5	NRR′ = 4-methylpiperazine		500	500	500
C_3H_7	NRR′ = 4-methylpiperazine		>400	400	200

especially ref 15–23) on this subject. However, it is necessary to summarize briefly certain of the findings here, to allow evaluation of the usefulness of the mouse-cestode tests in terms of the discovery of the 4-alkoxy-naphthamidines that are the subject of this paper.

Secondary screening of those amidines thought most likely to be of further interest was carried out in the dog, mostly using *Taenia pisiformis* but also with *Dipylidium caninum*, and in the cat infected with *Hydatigera* (*Taenia*) *taeniaeformis*, *Spirometra mansonoides*, or *D. caninum*. Quantitative data were difficult to arrive at because killed cestodes were often at least partially digested in the host. The patterns of activity of these 4 parasites in the dog and cat differed markedly from the structure–activity pattern in mice, and, indeed, to an extent from each other. Thus some amidines in all portions of the spectrum of variations in size of the alkyl groups both on O and on N had substantial activity against at least one dog or cat cestode. However a group of amidines with high activity against *all four* species in the dog and cat screens were those with ethereal substituent (OR) Bu or higher, combined with the *N*-alkyl groups (NR) Bu or lower, *e.g.*, **13**, **17**, **18**, **23**, **25**, **29-31**.

Comparison of these results with the mouse-cestode data showed that all compounds of high activity against all 4 dog and cat parasites were also at least somewhat active against *H. nana* in the mouse. However, a number of the amidines active against *H. mana* were not appreciably active in the larger animals. Thus screening with *H. nana* did not result in the better compounds being missed, but did give a large number of positive results inapplicable to, and a distorted structure–activity profile for, the target species. The *O. symmetrica* screen gave fewer inapplicable positives, but would have dismissed as essentially inactive some of the amidines found best in the larger animal screens.

(15) C. J. Hatton, *Vet. Rec.*, **77**, 408, (1965).
(16) R. B. Burrows and W. G. Lillis, *Amer. J. Vet. Res.*, **27**, 1381 (1966).
(17) D. A. Czipri, V. J. Nunns, and G. C. Shearer, *Vet. Rec.*, **82**, 505 (1968).
(18) B. McCullough and S. Kasimbala, *ibid.*, **81**, 219 (1967).
(19) D. A. Czipri, private communication.
(20) L. Hromatka, E. Kutzer, and W. Stettner, *Wien. Tieraerztl. Monatsschr.*, **53**, 616–617 (1966).
(21) C. J. Hatton, *Vet. Rec.*, **81**, 104 (1967).
(22) B. D. Blood, V. Moya, and J. J. Lelijveld, *Bull. W. H. O.*, **39**, 67 (1968); M. A. Gemmell and G. C. Shearer, *Vet. Rec.*, **82**, 252 (1968); G. C. Shearer and M. C. Gemmell, *Res. Vet. Sci.*, **10**, 296 (1969).
(23) R. B. Burrows, C. J. Hatton, W. G. Lillis, and G. R. Hunt, *J. Med. Chem.*, **14**, 87 (1971).

A balancing of activity and toxicity led to the selection of *N,N*-dibutyl-4-hexyloxynaphthamidine·HCl (bunamidine·HCl) as the amidine of choice for the treatment of cestode infections in large animals. It should be noted that while this compound (**18**) is within the limits set for the region of superior TI in the mouse ($C_{OR} + C_{NR} = 10$), the results with it in Table II would hardly recommend it, if only the mouse tests were considered. Salts of bunamidine have subsequently been shown to have a broad spectrum of useful activity against cestodes of veterinary importance including *H. (Taenia) taeniaeformis*,[15,16] *D. caninum*,[16] and *S. mansonoides*[16] in the cat, *Moniezia expansa* in sheep,[17] *Raillietina cesticillus, R. echinobothrida,* and *R. tetragona* in poultry,[18] *Anoplocephala* spp. in horses,[19] and *Taenia pisiformis*,[15,16] *D. caninum*,[15] *Mesocestoides lineatus*,[20] *Multiceps multiceps*,[21] and *Echinococcus granulosus*[22] in dogs.

E. granulosus is of special interest in that this cestode, though normally going through a carnivore–herbivore cycle leading to hydatid disease of farm livestock, has public health significance. Its larval (cystic) form causes a serious and sometimes fatal parasitic disease of humans,[24] especially those humans closely associated with sheep and dogs. Bunamidine·HCl has been recognized as the drug of choice for individual treatment of potentially infected dogs[25] to prevent infection of humans, and is being evaluated for mass treatment of all dogs in areas of *Echinococcus* spp. prevalence, to break the chain of transmission to humans.

Experimental Section

Caution.—We wish to emphasize the fact that the free amidine bases discussed here are frequently irritating to the skin, and some are apparently sensitizing. Repeated contact with these bases may cause symptoms resembling those due to poison ivy exposure. No such symptoms attributable to exposure to the amidine salts used in anthelmintic treatment have been observed.

Chemistry.—The new amidines reported in this work are tabulated (Table I) together with pertinent details of the preparative method and the physical properties of the salts (generally hydrochlorides) isolated. These amidines were synthesized initially by the method of Lorz and Baltzly,[5,26] but repreparations were made by the $AlCl_3$ method (procedure C) on occasion. Examples of two work-up procedures (procedures A and B) are given below.

Most of the 4-alkoxynaphthonitriles required for this work have been reported elsewhere,[12] made by a different method, while the lower members of the series were reported from our laboratories earlier.[5] The procedure that we used is essentially that of Newman[27] modified as mentioned. Three new 4-alkoxynaphthonitriles were prepared for this work. Their properties of interest are: 4-undecyloxynaphthonitrile, mp 60.0–60.8° (hexane), 56% yield, *anal.* ($C_{22}H_{29}NO$) C, H, N; 4-tridecyloxynaphthonitrile, mp 66.5–67° (hexane, 80% yield, *anal.* ($C_{24}H_{33}NO$) C, H, N; 4-tetradecyloxynaphthonitrile, mp 71–72° (hexane), 81% yield, *anal.* ($C_{25}H_{35}NO$) C, H, N.

***N,N*-Dipropyl-4-tridecyloxynaphthamidine·HCl (43). Procedure A.**—A Grignard reagent from 1.81 g (0.074 g-atom) of Mg turnings and 7.35 g (0.067 mole) of EtBr in 100 ml of dried Et_2O was heated for 0.5 hr with 8.16 g (0.081 mole) of Pr_2NH. A soln of 4-tridecyloxy-1-naphthonitrile (20 g, 0.056 g-atom) in 250 ml of dried C_6H_6 was added, and the mixture was heated under reflux for 2 days. Cautious addition of first 50 ml of H_2O, then 50 ml of 6 *N* HCl, and cooling at −10° overnight led to formation of 13 g of mp 155°. This was recrystd 3 times from hot H_2O by addition of 6 *N* HCl to incipient turbidity, mp 175–176°. *Anal.* ($C_{30}H_{40}N_2O \cdot HCl \cdot H_2O$) C, H, N.

***N,N*-Dihexyl-4-hexyloxynaphthamidine (20). Procedure B (Use of Methyl Formate).**—A Grignard reagent from 19.7 g (0.18 mole) of EtBr and 4.85 g (0.2 g-atom) of Mg turnings in 250 ml of anhyd Et_2O was treated with 40 g (0.216 mole) of Hex_2NH in 220 ml of anhyd Et_2O, and the mixture was heated under reflux for 30 min. A soln of 38 g (0.15 mole) of 4-hexyloxynaphthonitrile in 100 ml of hot, dry C_6H_6 was added as rapidly as possible, followed by 100 ml of hot C_6H_6 washes. The reaction mixture was stirred and heated under reflux for 44 hr and decompd (foaming!) by slow addition of 160 ml of 7 *N* HCl. The solid was 88 g of *impure* material. It was dissolved in MeOH and partitioned between 500 ml of 12 *M* NaOH and C_6H_6–Et_2O contg approx 10% MeOH (see caution about amidine bases), reextracting the aq gel with C_6H_6–Et_2O twice more. The resulting org layers were combined and vacuum concd on the steam bath at aspirator pressure. The 85 g of oil so produced was dissolved in 600 ml of MeOH, treated with 15 ml of HCO_2Me, and stored at 4° overnight. It was then vacuum concd to remove solvents and treated with an excess of dry ethanolic HCl followed by much hexane. Crystals formed (64.2 g), mp 196–198°, after hexane washings. Two recrystns from $MeNO_2$ gave 50 g, mp 201.8–202.8° (vacuum dried, but 201–201.5° air equilibrated).

***N,N*-Dibutyl-4-hexyloxynaphthamidine·HCl (18). Procedure C ($AlCl_3$ Method).**—A mixture of 13 g (0.1 mole) of Bu_2NH and 7 g (0.053 mole) of powdered anhyd $AlCl_3$ (heat evolved on mixing) in a 100-ml flask was treated with 12.7 g (0.05 mole) of 4-*n*-hexyloxy-1-naphthonitrile, and heated on a steam bath for 4 hr. The mixture was cooled and cautiously treated with 20 ml of 6 *N* HCl with stirring. An additional 50 ml of cold H_2O was added, and the solid was filtered off after 2 hr. The filtrate was discarded. The solid was washed with aq HCl and with Et_2O and recrystd from H_2O containing a little HCl, or from Me_2CO. Yields varied from 35 to 75%.

Screening Procedures. *Hymenolepis nana.*—The general maintenance technique was described earlier by Standen.[27] Gravid worms were crushed gently in a mortar to free the eggs. These were suspended in tap water and large pieces of worm tissue were removed. After counting the eggs in three 0.1-ml aliquots, the total vol of the suspension was adjusted to 500 eggs/ml. Each mouse received by stomach tube 0.2 ml of suspension contg approx 100 eggs. Tapeworm eggs appeared in the feces 14–15 days after infection.

The test compound was prepared in distd H_2O as a soln or as an emulsion obtained by roller milling with gum tragacanth for 16 hr. The dose vol was finally adjusted to give 0.1 ml per 10 g of body wt. Groups of at least 5 mice were employed. On the 17th or 18th day after infection the mice received the test compound as a single dose by stomach tube. In these tests it is considered impracticable to exceed a dose level of 400 mg/kg (calcd as base).

At necropsy the contents of the entire small intestine were expressed under H_2O in a large petri dish and examined against a black background. Special care was taken to detect and count all scolices.

The cecum and rectum were opened and examined for both entire worms and strobilae recently removed from the small intestine by peristalsis. Estimates of activity were based on a comparison of the numbers of worm-free mice in the test group and an untreated control group. This is necessary rather than merely desirable, because tapeworms eliminated after treatment with these naphthamidines were found to be partially digested.

Oochoristica symmetrica.—The maintenance technique used was that previously described.[28] Cysticercoids of this tapeworm

(24) *E.g.*, see A. Neglime and R. Silvo, *Bol. Chil. Parasitol.*, **23**, 59 (1968).

(25) *WHO Chronicle*, **22**, 535 (1968).

(26) Our brominations were carried out in CCl_4 with a slight excess of Br_2 and gave nearly quant yields. The conversion of the alkoxybromonaphthalenes to the nitriles with CuCN in pyridine was extremely sensitive to the proportion of pyridine used, but the yield of isolated nitrile was normally over 90% if this was properly selected. Apparently too low a ratio of pyridine to CuCN led to too high an internal temp (under reflux), with consequent destruction of reactants, while no perceptible reaction occurred at too low a temp. In small-scale runs the standard proportion of pyridine had to be increased slightly, apparently to allow for the amount resident in the reflux condenser, for optimum yields to be obtained. The best guide to proper proportions was the internal temp, which for the 4-bromo-1-alkoxynaphthalenes was best adjusted to about 182°, while we have found in other work that an internal temp about 10° higher is best for displacements of Cl by CN in 4-chloro-1-alkoxynaphthalenes. Excessive tar formation indicates too high a temp was used.

(27) M. S. Newman, *J. Amer. Chem. Soc.*, **59**, 2472 (1937).

(28) O. D. Standen, "Experimental Chemotherapy", Vol. 1, R. J. Schnitzer and F. Hawking, Ed., Academic Press, New York, N. Y., 1963, p 701.

were dissected from larvae of the museum beetle, *Trogoderma versicolor*, and coned in a Syracuse watch glass. Each mouse received 5–10 cysticercoids by stomach tube. Sixteen to twenty days after infection, mice were placed in individual cages and those passing proglottids into the pans beneath the cages were used in the experiments.

During the test each mouse was kept in an individual cage, which contained a bottle of H_2O and a special feeding rack. Feces and worms were collected in a pan of H_2O beneath the cage. The test compound was prepared as a suspension made by grinding it in a mortar with Tween 80 and H_2O or, if sol, as a soln. It was then given in a single dose by stomach tube. The max dosage employed was 500 mg/kg, and at least 2 mice were used at each dose level.

Twenty-four hours after treatment the pan of H_2O was replaced by a clean one. The first pan was searched for worms or fragments and the findings were recorded. After another period of 24 hr the mouse was necropsied and any worms remaining were counted and recorded. Worms recovered from both pans and fragments of worms recovered from the cecum and large intestine were considered to have been dislodged by the treatment. The number of worms removed was expressed as a percentage of the total worm burden, and the results from all mice used in the experiment were averaged.

Mitomycin Derivatives. 1. Preparation of Mitosane and Mitosene Compounds and Their Biological Activities

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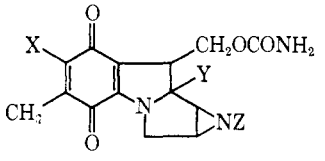
Several derivatives of mitomycin were prepared from natural mitomycins and subjected to antibacterial tests. The structure–activity relationship was investigated for enzymatic activation, inhibition of DNA synthesis, and prophage induction. Substituents X, Y, and Z in mitomycins (1) are closely related to the rate of activation, which has a great effect on the biological activity. The aziridine group is responsible for inhibition of DNA synthesis and also for alkylating actions of mitomycins.

Mitomycins (**1**) prossess strong activities against Gram-positive and Gram-negative bacteria, as well as against several kinds of tumors. These formulas[1,2] are



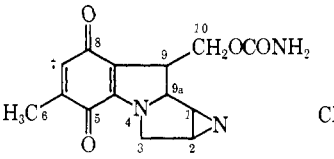
1

	X	Y	Z
1a, mitomycin A	CH_3O	OCH_3	H
b, mitomycin B	CH_3O	OH	CH_3
c, mitomycin C	NH_2	OCH_3	H
d, porifiromycin	NH_2	OCH_3	CH_3

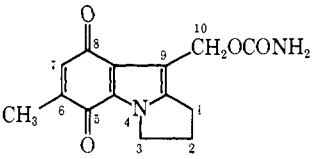
unique not only in natural products chemistry, but also among antitumor substances in that they have three carcinostatic groups—quinone, aziridine, and carbamoyloxymethyl—in their structures. It appeared important to elucidate the role of these 3 groups, and that of the X, Y, and Z substituents, on the biological activities of mitomycins.

Many derivatives were synthesized from the natural mitomycins in a search for less toxic and more effective substances. The present paper deals with their synthetic methods, biological activities, and structure–activity relationship.

A. Synthesis of Derivatives.—The derivatives of mitomycins in this report are classified in two groups, mitosane compounds **2** and mitosene compounds **3.**



2



3

I. Mitosane Compounds.—Several kinds of mitosane compounds were prepared from natural mitomycins as shown in Charts I and II.

Mitomycin A (**1a**) and C (**1c**) were acylated (**4**) with acyl chlorides in the presence of Et_3N. Using the same procedure 1a-sulfonyl derivatives **5** were also prepared. Alkylation of the 1a position was performed with alkyl iodide in the presence of K_2CO_3. Mitomycin A (**1a**) and B (**1b**) were treated with NH_3, and primary or secondary amines to give 7-aminomitosane compounds **7**.

Reduction with LAH of **1b** followed by oxidation with potassium nitrosodisulfonate (Fremy's salt) gave a new quinoid compound **8** which had the same chromophor as mitomycin B. In the ir and nmr spectra of **8**, no C=O ($\nu_{C=O}$1700 cm^{-1}) of CH_2OCONH_2 and MeO (3.7 ppm in $CDCl_3$) at C-9 was found, while those absorptions exist in mitomycin A and C. Compound **8** was converted into dehydroxymitomycin B (**10**) and demethoxyporfiromycin (**11**) through the phenoxycarbonyl derivative **9**. Compounds **10** and **11** were also prepared by $NaBH_4$ reduction of mitomycins. Compounds **8–11** were not converted into mitosene or decarbamoylmitosene by acid hydrolysis, and unchanged original products were recovered, while mitomycins gave mitosene by

(1) F. S. Webb, D. B. Cosulich, J. H. Mowat, R. W. Broschard, W. E. Meyer, R. P. William, C. F. Wolf, W. Fulmor, C. Pidacks, and J. E. Lancaster, *J. Amer. Chem. Soc.*, **84**, 3185 (1962).

(2) J. S. Webb, D. B. Cosulich, J. H. Mowat, R. W. Broschard, W. E. Meyer, R. P. William, C. F. Wolf, W. Fulmor, C. Pidacks, and J. E. Lancaster, *ibid.*, **84**, 3187 (1962).