Antibacterial and Antifungal Properties of β-Naphthol Derivatives VI

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The bacteriostatic and fungistatic activities of a number of β-naphthol, 2-hydroxy-6-naphthoic acid, and 2-hydroxy-3-naphthoic acid derivatives are reported against *Staphylococcus aureus, Salmonella typhosa,* and *Aspergillus niger.* Selected compounds from the initial screening have been further tested for their activity against *Staphylococcus albus, Salmonella paratyphi* A, *S. paratyphi* B, *Escherichia coli, Shigella dysenteriae, Trichophyton rubrum, Trichophyton gypseum, Candida albicans,* and *Candida tropicalis.* Few compounds exhibited activity against the selected Gram-negative organisms at concentrations below 50 mcg./ml. For *S. aureus* and *S. albus,* the bacteriostatic concentrations of 6-alkyl-, 3-alkyl-, and 1,6-dialkyl-β-naphthols, esters of 2-hydroxy-6-naphthoic acid, and substituted anilides of 1,6-dibromo-, 1-nitro-, and 1-nitro-6-bromo-2-hydroxy-3-naphthoic acid are between 1 and 10 mcg./ml. Marked inhibition of the few fungus pathogens tested is shown by 3-propyl, 3-butyl, 1-methyl-6-ethyl-, 1-methyl-6-propyl- and 1,6-diethyl-β-naphthol, and alkyl esters of 2-hydroxy-6-naphthoic acid.

ANTI-INFECTIVE ACTIVITY of a number of β-naphthol derivatives has been reported previously (1–5) from this laboratory. As a further extension of this work another 200 derivatives have been prepared and tested. The syntheses of these compounds have already been published (6–11).

In the preliminary screening all the compounds were tested against *S. aureus*, *S. typhosa*, and *A. niger*. Various derivatives of 3-keto- (9), 6-keto- β-naphthols (7), 6-naphthyl-alkyl acids (8, 11), dinaphthols (7), *etc.*, are not included in this publication as they showed little activity in the screening.

EXPERIMENTAL

Bacteriostatic Activity

The serial broth dilution method of McKee, Rake, and Menzel (12) was followed, using the organisms *S. aureus* (FDA 209) and *S. typhosa* (Lister). Different concentrations of the test compounds were prepared by adding the requisite quantities of alcoholic (95%) stock solutions to double strength broth to make up the volume to 5 ml. Stock solutions were so prepared that in no case did the quantity of the alcoholic solution added to broth exceed 0.1 ml. A similar quantity of alcohol alone added to the broth did not show bacteriostasis. The results were observed after incubation at 37° for 24 hr. and further confirmed after 48 hr. The minimum inhibition concentrations, in mcg./ml., are recorded in Table I.

Fungistatic Activity

Screening for fungistatic activity against *A. niger* was done following the agar cup-plate (13) as well as the agar-streak (14) methods. The results of the former method gave an idea of the power of diffusion of the substances; that of the latter gave the minimum inhibition concentrations.

Agar Cup-Plate Method (13)—One per cent alcoholic solutions of the compounds were added to prepared cups and the plates were incubated for 48 hr. at 25–27°. The average diameters of the zones of complete inhibition have been recorded in Table I. Alcohol alone gave no zone of inhibition.

Agar Streak Method (14)—Spore suspensions of test organisms were streaked on Sabouraud's agar plates containing the test substances in concentrations of 200, 100, 50, 10, and 5 mcg./ml. Control plates containing alcohol exhibited no inhibition. Results were noted after incubation for 5 days at 25–27° and these have been recorded in Table I.

Antimicrobial Spectra—Compounds which were fairly active in the preliminary screening were next tested against organisms such as *S. aureus, S. albus, S. typhosa, S. paratyphi* A, *S. paratyphi* B, *S. dysenteriae*, and *E. coli*. The results of these tests are presented in Table II.

Similarly, the antifungal activity of these compounds was determined against *T. rubrum* and *T. gypseum* by the agar-streak method, and against *C. tropicalis* and three strains of *C. albicans* by both the serial broth dilution and the agar-streak methods. The methods used were essentially the same as described above except that Sabouraud's glucose broth and glucose agar were used as the culture media.

DISCUSSION

Alkylation increases the bacteriostatic activity of β-naphthol. Compared to 6-alkyl derivatives reported in a previous publication (4), the corresponding 1,6-dialkyl derivatives now tested are more active. The lower members of 1,6-dialkyl derivatives, such as 1-methyl-6-ethyl-, 1,6-diethyl-, and

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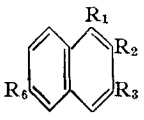
TABLE I—BACTERIOSTATIC AND FUNGISTATIC ACTIVITIES OF β-NAPHTHOL DERIVATIVES



Compd.				Activity Against			
				S. aureus[a]	*S. typhosa*[a]	*A. niger*[b]	*A. niger*[a]
	Alkyl-β-naphthols (R_2 = OH)						
	R_1	R_3	R_6				
1	H	H	H	200	100	43	50
2	H	H	(*iso*)C_5H_{11}	10	100	12	[c]
3	H	H	(*iso*)C_6H_{13}	5	100	13.5	[c]
4	H	H	C_7H_{15}	5	100	12	[c]
5	H	CH_3	H	50	100	28	100
6	H	C_2H_5	H	20	20	40	10
7	H	C_3H_7	H	10	20	30	10
8	H	C_4H_9	H	2	20	28	10
9	CH_3	H	H	100	100	42	50
10	C_2H_5	H	H	100	100	45	50
11	C_3H_7	H	H	100	100	40	50
12	CH_3	H	CH_3	20	100	24	50
13	CH_3	H	C_2H_5	10	20	35	20
14	CH_3	H	C_3H_7	5	200	25	20
15	CH_3	H	C_4H_9	2	200	23	200
16	CH_3	H	C_5H_{11}	2	200	18	200
17	CH_3	H	C_6H_{13}	1	200	15	[c]
18	C_2H_5	H	C_2H_5	10	10	35	20
19	C_2H_5	H	C_3H_7	2	10	24	50
20	C_2H_5	H	C_4H_9	2	[c]	19	200
21	C_2H_5	H	C_5H_{11}	1	[c]	15	[c]
22	C_2H_5	H	C_6H_{13}	1	[c]	12	[c]
	Bromo-alkyl-β-naphthols (R_2 = OH)						
	R_1	R_3	R_6				
23	Br	Br	C_2H_5	10	100	28	100
24	Br	Br	C_3H_7	5	100	28	100
25	Br	Br	C_4H_9	2	100	28	[c]
26	Br	Br	C_5H_{11}	10	100	16	[c]
27	Br	Br	C_6H_{13}	10	100	16	[c]
	Ethers (R_6 = H)						
	R_1	R_2	R_3				
28	H	OCH_3	H	200	200	18	[c]
29	CH_3	OCH_3	H	50	100	32	50
30	C_2H_5	OCH_3	H	10	[c]	18	[c]
31	C_2H_5	OC_2H_5	H	10	[c]	20	[c]
32	H	OCH_3	COC_2H_5	5	5	[d]	[c]
	Aldehydes and Derivatives (R_6 = H)						
	R_1	R_2	R_3				
33	H	OH	CHO	50	20	23	100
34	H	OH	CH=N—$C_6H_4Cl(4')$	200	200	[d]	200
35	H	OH	CH=N—$C_6H_4NO_2(4')$	200	200	[d]	200
36	NO_2	OH	CHO	50	50	25	100
37	NO_2	OH	CH=N—C_6H_4Cl	200	200	[d]	[c]
38	H	OH	CH=CH—$COCH_3$	50	200	[d]	[c]
	Esters of 2-Hydroxy-6-naphthoic Acid (R_3 = H)						
	R_1	R_2	R_6				
39	H	OH	$COOCH_3$	50	50	24	100
40	H	OH	$COOC_2H_5$	25	25	22	100
41	H	OH	$COOC_3H_7$	25	25	20	100
42	H	OH	$COOC_4H_9$	25	100	22	...[e]
43	H	OH	$COOC_3H_7$(*iso*)	25	100	18	...
44	H	OH	$COOC_4H_9$(*iso*)	25	100	20	...
45	H	OH	$COOC_6H_5$	50	100	18	...
46	H	OH	$COOC_6H_4OH$ (4′)	100	100	18	...
47	H	OH	$COOC_6H_3OH(3')Cl(4')$	100	100	18	...
48	CH_3	OH	$COOCH_3$	[c]	[c]	14	...
49	CH_3	OH	$COOC_2H_5$	[c]	[c]	22	...
50	CH_3	OH	$COOC_3H_7$	[c]	[c]	14	...
51	CH_3	OH	$COOC_3H_7$(*iso*)	10	[c]	18	...
52	CH_3	OH	$COOC_4H_9$(*iso*)	5	[c]	17	...
53	H	OH	COOH	200	[c]	[d]	...
54	H	OCH_3	COOH	[c]	[c]	[d]	...
55	H	$OCOCH_3$	COOH	200	[c]	[d]	...
56	CH_3	OH	COOH	[c]	[c]	17	...

(*Continued on next page.*)

TABLE I—(*Continued.*)

Compd.	Anilides of 2-Hydroxy-3-naphthoic Acid (R_2 = OH)			Activity Against			
				S. aureus[a]	*S. typhosa*[a]	*A. niger*[a]	*A. niger*[b]
	R_1	R_3	R_6				
57	Br	COOH	Br	[c]	[c]	...	[c]
58	Br	$CONH_2$	Br	100	100	12	[c]
59	Br	CONH—C_6H_5	Br	50	200	...	[c]
60	Br	$CONHC_6H_4OCOCH_3(4')$	Br	25	100	...	[c]
61	Br	$CONHC_6H_4NO_2(4')$	Br	1	100	...	[c]
62	Br	$CONHC_6H_4Cl(4')$	Br	0.5	100	...	[c]
63	Br	$CONHC_6H_3OH(3')$-$COCH_3(4')$	Br	25	100	...	[c]
64	Br	$CONHC_6H_3NO_2(3')CH_3(4')$	Br	2	100	...	[c]
65	Br	$CONHC_6H_4COOC_2H_5(4')$	Br	1	100	14	...
66	Br	$CONHC_4H_3S$	Br	25	100	16	...
67	Br	$CONHC_5H_4N$	Br	25	100	12	....
68	NO_2	COOH	H	[c]	200	...	...
69	NO_2	$CONH_2$	H	200	100	14	...
70	NO_2	$CONHC_6H_5$	H	25	100	20	...
71	NO_2	$CONHC_6H_4NO_2(4')$	H	0.5	100	24	100
72	NO_2	$CONHC_6H_4Cl(4')$	H	25	100	...	...
73	NO_2	$CONHC_6H_4COCH_3(4')$	H	50	100	16	...
74	NO_2	$CONHC_6H_3NO_2(3')CH_3(4')$	H	1	100	...	250
75	NO_2	$CONHC_4H_3S$	H	50	100	20	250
76	NO_2	$CONHC_5H_4N$	H	100	100	14	250
77	NO_2	$CONHC_6H_4COOC_2H_5(4')$	H	50	100	16	...
78	NO_2	COOH	Br	[c]	[c]	[d]	[c]
79	NO_2	$CONHC_6H_5$	Br	10	200	[d]	[c]
80	NO_2	$CONHC_6H_4Cl(4')$	Br	10	200	[d]	[c]
81	NO_2	$CONHC_6H_4Cl(3')$	Br	100	200	[d]	[c]
82	NO_2	$CONHC_6H_4Cl(2')$	Br	200	200	[d]	[c]
83	NO_2	$CONHC_6H_4NO_2(4')$	Br	10	200	[d]	[c]
84	NO_2	$CONHC_6H_4NO_2(3')$	Br	100	200	[d]	[c]
85	NO_2	$CONHC_6H_5OCH_3(4')$	Br	10	200	[d]	[c]

[a] Minimum inhibition concentration (MIC) in mcg./ml. [b] Average diameter of zone of inhibition in mm. with 1% w/v solution. [c] MIC more than 200 mcg./ml. [d] No zone of inhibition. [e] Not tested.

1-ethyl-6-propyl-β-naphthol (compounds *13*, *18*, *19*) are active against all the microorganisms tested, while the higher homologs, such as 1-methyl-6-hexyl-, 1-ethyl-6-pentyl-, and 1-ethyl-6-hexyl-β-naphthol (compounds *17*, *21*, *22*) show specific activity against *S. aureus* and *S. albus* only. The position of the alkyl group has a marked influence. The 3-alkyl derivatives possess activity against a wider microbial spectra than the 1-alkyl-, 6-alkyl-, or 1,6-dialkyl derivatives. Of all the alkyl derivatives screened, 3-butyl-β-naphthol (compound *8*), is the most active compound.

Introduction of bromine in 6-alkyl-β-naphthols shows variable effects. The activity increases as the chain-length is increased to butyl (compound *25*) and then drops down. In general, bromination does not appear to enhance considerably the antimicrobial activity of the parent alkyl compounds.

Conversion of β-naphthol to alkyl ethers has been reported (4) to depress the activity. However, the ethers of 1-alkyl-β-naphthols now tested show enhanced activity especially against *S. aureus* and *S. albus* (compounds *29*, *30*, *31*).

All the ketonaphthols, their methyl ethers, and bromo derivatives tested show poor activity; the only exception is 3-propionyl-2-methoxy-naphthalene (compound *32*) and this compound is bacteriostatic at a concentration of 5 mcg./ml. against *S. aureus* and *S. albus* and some Gram-negative organisms.

2-Hydroxy-3-naphthaldehyde (compound *33*) is much more active than 2-naphthol, itself, against the few Gram-positive and Gram-negative organisms tested. However, introduction of an additional nitro group, conversion of the aldehydes to anils or chalkones depresses the activity considerably.

Introduction of a carboxyl group in β-naphthol has been reported (3) to reduce its bacteriostatic activity. The activity of 1-methyl-2-naphthol (compound *9*) is similarly reduced by the introduction of a carboxyl group (compound *56*). However, esterification of the carboxyl group appears to enhance the antimicrobial activity. Among the straight-chain alkyl esters of 2-hydroxy-6-naphthoic acid studied, the bacteriostatic activity increases until the propyl (compound *41*) derivative is reached, and then it drops. The branched-chain esters are as active as the straight-chain esters against *S. aureus* and *S. albus* (Table II).

The anilides of 2-hydroxy-3-naphthoic acid have been reported (4) to be bacteriostatic at low concentrations against Gram-positive organisms. Various substituted anilides of 1,6-dibromo-, 1-nitro-, and 1-nitro-6-bromo-2-hydroxy-3-naphthoic acids, now studied, exhibit marked activity against *S. aureus* and *S. albus*. The activity varies considerably with the position and nature of the substituent on the aniline ring; in general, the *para*-substituted

TABLE II—ANTIBACTERIAL ACTIVITY OF SELECTED COMPOUNDS[a]

Compd.[b]	*S. aureus*	*S. albus*	*S. typhosa*	*S. paratyphi* A	*S. paratyphi* B	*E. coli*	*S. dysenteriae*
2	10	10	[d]	[d]	[d]	[d]	[d]
3	5	5	[d]	[d]	[d]	[d]	[d]
4	5	5	[d]	[d]	[d]	[d]	[d]
6	20	20	20	20	20	50	50
7	10	10	20	20	20	50	50
8	2	5	20	20	20	50	50
13	10	10	20	50	50	[d]	[d]
14	5	10	[d]	[d]	[d]	[d]	[d]
15	2	5	[d]	...[e]	...	...	...
16	2	2	[d]	...	...	...	...
17	1	1	[d]	...	...	...	...
18	10	10	10	50	50	[d]	[d]
19	2	5	10	...	...	...	...
20	2	2	[c]	...	...	...	...
21	1	1	[c]	...	...	...	...
22	1	1	[c]	...	...	...	...
24	5	5	[d]	[d]	[d]	[d]	[d]
25	2	5	[d]	[d]	[d]	[d]	[d]
32	5	5	5	10	20	20	20
33	50	50	20	20	20	50	20
39	50	50	50	50	50	[d]	50
40	25	25	25	50	50	[d]	50
41	25	25	25	50	50	[d]	50
42	25	25	[d]	...	...	...	...
43	25	25	[d]	...	...	...	...
44	25	25	[d]	...	...	...	...
51	10	10	[c]	...	...	...	...
52	5	5	[c]	...	...	...	...
60	25	25	[c]	...	...	...	...
61	1	1	[c]	...	...	...	...
62	0.5	0.5	[d]	...	...	...	...
65	1	1	[d]	...	...	...	...
71	0.5	0.5	[d]	[d]	[d]	[d]	[d]
74	1	1	[d]	[d]	[d]	[d]	[d]

[a] Minimum inhibition concentration (MIC) in mcg./ml. [b] Numbers refer to compounds listed in Table I. [c] MIC more than 200 mcg./ml. [d] MIC more than 50 mcg./ml. [e] Not tested.

TABLE III—FUNGISTATIC ACTIVITY OF SELECTED COMPOUNDS[a]

Compd.[b]	*A. niger*	*T. rubrum*	*T. gypseum*	*C. albicans* Strain I	*C. albicans* Strain II	*C. albicans* Strain III	*C. Tropicalis*
1	50	50	50				
6	10	10	10				
7	10	5	5	50	50	20	[c]
8	10	5	5	20	20	10	[c]
13	20	50	50	50	50	10	[c]
14	20	20	20	10	10	2	[c]
18	20	50	50	50	50	5	[c]
19	50	50	50	20	50	5	[c]

[a] Minimum inhibition concentration (MIC) in mcg./ml. [b] Numbers refer to compounds listed in Table I. [c] MIC more than 50 mcg./ml.

derivatives show the highest and the *ortho*-substituted ones the lowest activity. Of the *para*-substituted compounds, the chloro and nitro derivatives (compounds *61*, *62*, *71*) are bacteriostatic at 1 mcg./ml.

Table II, which records the activity of the selected compounds against some other microorganisms, shows that while very few compounds are bacteriostatic against the four Gram-negative microorganisms used for the study below the concentration of 20 mcg./ml., several of them are active against *S. aureus* and *S. albus* at fairly low concentrations. The specificity of activity shown by the alkyl derivatives is noteworthy and further data on these compounds will be reported separately.

The fungistatic activity reported in Table I indicates that as the length of the alkyl chain in alkyl naphthols increases, the zone of inhibition against *A. niger* first increases and then decreases, the ethyl derivatives being the most active.

Of the dialkyl derivatives, 1-methyl-6-ethyl-, 1-methyl-6-propyl-, and 1,6-diethyl-β-naphthol (compounds *13*, *14*, *18*) exhibit good activity against *A. niger* and a few other fungi. Of the esters of 2-hydroxy-6-naphthoic acid, the methyl ester (compound *39*) shows the highest activity and the

ethyl and propyl esters (compounds *40*, *41*) come next. However, in general they have lower fungistatic activity than the alkyl naphthols.

Comparing the activities against all the fungi included in the tests (Table III), it is observed that while compounds *8* and *14*, 3-butyl- and 1-methyl-6-propyl-β-naphthol, are fungistatic at 20 mcg./ml., all others are fungistatic only at 50 mcg./ml.

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Effects of Adrenochrome Semicarbazone on Blood Loss in the Mouse

By JACK H. KEITH, WILLIAM W. KEITH, and MARY H. NELSON

A method for determining the blood loss and bleeding time of a standardized wound is described, and it is applied in examining the activities of a clinically-used hemostatic agent—adrenochrome semicarbazone. It is shown that adrenochrome semicarbazone has an effect in decreasing blood loss at doses as low as 0.1 mg./Kg., and is effective in the presence of anticoagulants. A mechanism of action is suggested.

THE DIFFICULTY in showing a decrease in blood loss, clinically or experimentally, is apparent when one attempts to produce a standard wound. Variations of up to 1000% may occur on the same animal with the same size wound.

Roskam (1) measured blood loss from rabbit ears, achieving reproducible results, but the authors found that his method gave insufficient information. The major deficiency in his procedure was the measurement of bleeding times rather than blood loss. Blood loss was assumed to parallel bleeding time, and the authors have found that this is not always true.

After many attempts at determining blood loss, with and without adrenochrome semicarbazone, it was found that blood loss could be most accurately and reproducibly measured in mice. The clipping of 1.5 cm. from the tips of the tails of animals of the same size and sex gives an easily measurable method of determining blood loss.

The mouse tail was used because the vessels are large enough to produce a bleeding condition that could be classified as traumatic hemorrhage. The major vessels in the mouse tail vary from 0.05 mm. to 0.25 mm. in diameter. During the peak period of hemorrhage, the first 30 sec., the blood loss may be as great as 0.2 ml., which would represent 10% of total blood volume.

This paper concerns the effect of adrenochrome semicarbazone upon initial blood loss from the clipped tail of the mouse, and an indication of its mechanism of action. We emphasize the term "initial blood loss," since anticoagulant-treated animals stop bleeding only for a short period, and then begin again.

METHOD

Determination of Blood Loss and Bleeding Time—Male mice[1] weighing 18–22 Gm. were used. The tail was severed 1.5 cm. from the tip with a frequently changed razor blade to ensure keenness. The animals were placed in a holder at least 5 min. before cutting. This holder consisted of a plastic vial 1 in. × 2.5 in. (Fig. 1) with holes for ventilation. In some of the experiments the tails were bled into a beaker containing 100 ml. of deionized water at 37°; 3–5 mice were bled into the same 100 ml. of water containing disodium ethylendiaminetetraacetate, 2 mg./100 ml., as an anticoagulant. The tail was observed until bleeding stopped and was then removed immediately from the water. The

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[1] ICR strain random breed, obtained from Dublin Animal Farm, Dublin, Va.