weighing 20 ± 2 g were divided into random groups, and the compounds were given orally at several dose levels, using at least 10 animals per dose. Thirty minutes after treatment, each animal was individually placed on a metal plate heated to 56 °C by boiling acetone. The paw-licking reaction time was noted, and the ED_2 (dose doubling this time) was determined graphically.

Carrageenin Rat Paw Edema. The procedure employed was a modification of the method of Winter et al.[12] CD1 (Charles River, France) male rats weighing about 150 g were arranged in groups of 8 or 10, and the compounds were given orally at several dose levels. One hour after treatment they received a subplantar injection of 1 mg of carrageenin in 0.1 mL of water in the left hind paw. Paw volume was measured prior and 3 h after the carrageenin injection. The ED_{40} (dose inhibiting by 40% the increase in paw volume) was determined graphically.

In this screening program, compounds were usually given at three dose levels, using a control group and a one-dose reference compound group (glafenine or aminopyrine). If activity of the reference compound proved to be abnormal, the assay was discarded. Standard error of the mean and significance compared to the controls (Dunnett's "t" test)[13] were computed in the carrageenin rat paw edema and in the hot-plate test but not in the writhing test (since writhing counts were not done individually but on six animal groups). These figures do not appear in the tables because of lack of space.

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Synthesis and Antibacterial Activity of 2'-Substituted Chelocardin Analogues

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Chelocardin (1) was condensed with numerous hydrazines, hydrazides, and anilines, yielding 2'-substituted derivatives with antibacterial spectra similar to the parent antibiotic. The hydrazone derivatives **9** and **10** and the two anilino derivatives **42** and **44** had more in vivo antibacterial activity than chelocardin.

Chelocardin, a potent broad-spectrum antibiotic produced by *Nocardia sulphurea* (NRRL-2822), was isolated in 1956. Its structure was established by Mitscher et al. as **1**.[2] The antibacterial spectrum was shown to differ substantially from that of tetracycline, since it is more active against Gram-negative than Gram-positive bacteria.[3]

Despite having the opposite configuration to tetracycline at carbon-4 and being in the anhydrotetracycline series, chelocardin possesses remarkable antibacterial activity. The 4-epi isomer, which has the same configuration as in the tetracycline series, is virtually inactive. Hence, the structure–activity relationship known to be associated with tetracycline antibiotics[4] could not be assumed to apply to chelocardin analogues. In this paper, we report the preparation and antibacterial activity of three classes of 2'-substituted chelocardin analogues.

Chemistry. The 2'-substituted chelocardin derivatives were synthesized as indicated in Scheme I. Chelocardin (**1**) hydrochloride reacted readily with a slight molar excess of substituted hydrazines, **2**, in aqueous tetrahydrofuran to give 2'-substituted hydrazones of chelocardin, **3**. These compounds, described in Table I, were isolated as crystalline hydrochloride salts in yields varying from 12 to 98%.

The 2'-substituted acylhydrazone derivatives of chelocardin, **5** (described in Table II), were prepared by condensation of chelocardin hydrochloride with a variety of hydrazides, **4**, and were also isolated as crystalline hydrochloride salts.

The 2'-substituted anilino compounds, **7** (described in Table III), were prepared by reacting chelocardin hydrochloride with the corresponding aniline **6** in aqueous tetrahydrofuran in the presence of acetic acid.

All the derivatives reported here were characterized and tested as the hydrochloride salts. It is interesting to note that all these compounds show an additional adsorption at approximately 307–312 nm in their UV spectra, indicating the formation of a vinylogous imide system.[5] Hence, the alternative tautomeric structures for **3**, **5**, and **7** represented by structures **3a**, **5a**, and **7a**, respectively, cannot be excluded. The position of substitution, at the 2'-carbonyl rather than at the 1- or 3-carbonyl groups, was

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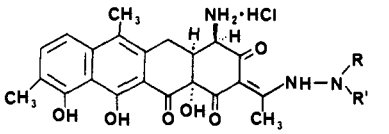
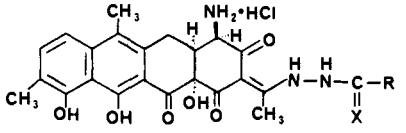
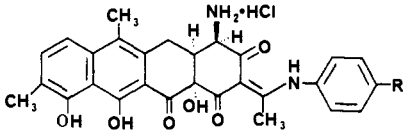


3a



5a



7a

confirmed by ^{13}C NMR analysis.[6] An analogous condensation of the exocyclic carbonyl in the β-tricarbonyl system of usnic acid has recently been reported.[7]

Biology. The chelocardin derivatives presented here show antibacterial activity against a variety of Gram-positive and Gram-negative organisms. The in vitro activities of 2′-substituted hydrazones are shown in Table IV. 4-(2-Hydroxyethyl)piperazinoiminochelocardin dihydrochloride (**10**) has about the same activity as the parent antibiotic; the other compounds are somewhat less active. Taking the increase of molecular weight of the hydrazone derivatives into account, substitution at the 2′-carbonyl by hydrazines does not reduce the antibacterial activity in most cases. On the other hand, substitution by hydrazides gives much less active analogues (with the exception of the acethydrazone **15**) as illustrated by the MIC values shown in Table V.

From the values shown in Table VI, it is seen that substitution by anilines at the 2′-carbonyl of chelocardin gives derivatives which are equipotent (or nearly so) to the parent compound. Of the three series of derivatives prepared in this project, the anilino derivatives were the most potent.

Within each series of analogues, no clear-cut structure–activity correlations could be made with respect to electronic, spatial, or solubility characteristics.

All of the derivatives described here, though they vary in potency, have antibiotic spectra identical with that of chelocardin. In vivo, they are converted to chelocardin at widely varying rates; some of them are excreted unchanged in the urine. To determine whether these derivatives have intrinsic activity, bacterial growth inhibition studies of *Proteus vulgaris* using the *p*-chloroanilino (**35**) and the 4-methylpiperazinoimino (**9**) derivatives were performed. Inhibition curves for chelocardin (**1**) hydrochloride and compounds **35** and **9** shown in Figures 1–3 indicated no time lag in the inhibition effect after addition of antibiotics. Since the derivatives are quite resistant to ordinary chemical hydrolysis and the enzymatic in vivo hydrolysis in rats, monkeys, and mice is relatively slow, the results indicate that the derivatives do have intrinsic activity.

Some of these derivatives offer the advantage of greater water solubility than the parent antibiotic; for example, the hydrochloride salts of 4-methylpiperazinoiminochelocardin (**9**), 4-(hydroxyethyl)piperazinoiminochelocardin (**10**), and chelocardin acethydrazone (**15**) are completely water soluble, whereas chelocardin hydrochloride is only slightly soluble in water.

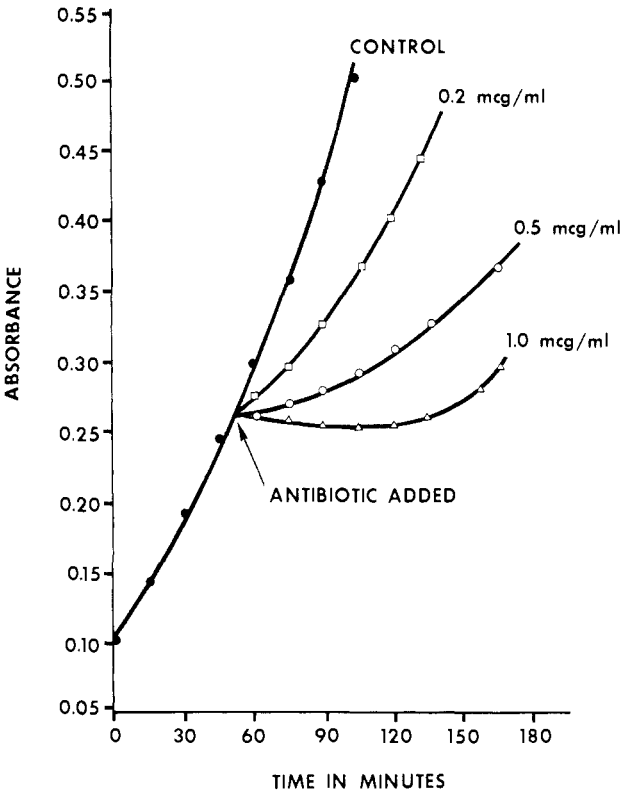


Figure 1. Growth of *P. vulgaris*; inhibition by chelocardin hydrochloride (**1**).

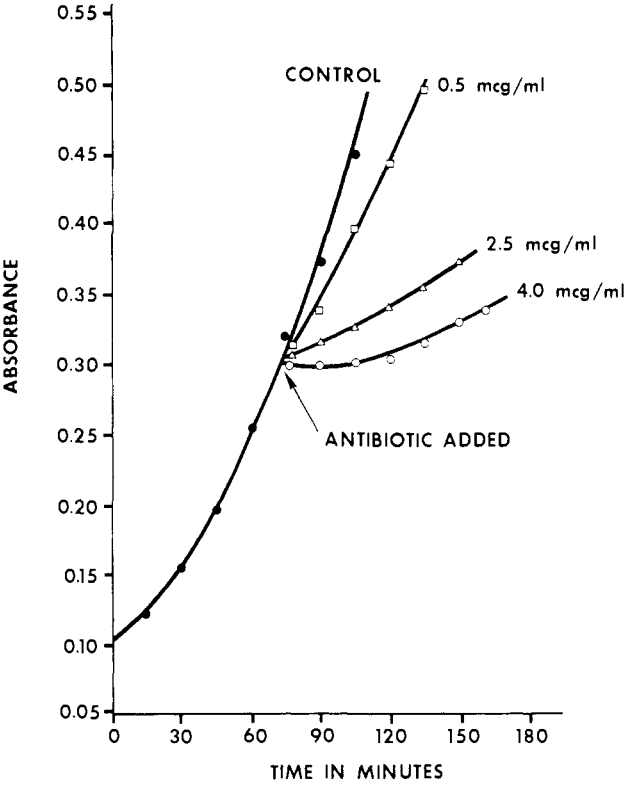


Figure 2. Growth of *P. vulgaris*; inhibition by compound **35**.

The CD_{50} values in the standard mouse protection test using *P. mirabilis* generally parallel the trends of the in vitro activity. However, a few derivatives exceed the in vivo antibacterial activity of the parent antibiotic as shown in Table VII. The most effective compound is the 2-*p*-sulfamylanilino derivative **42**; compound **44** and the hydrazones **9** and **10** are also very active in both in vivo and in vitro studies.

Scheme I

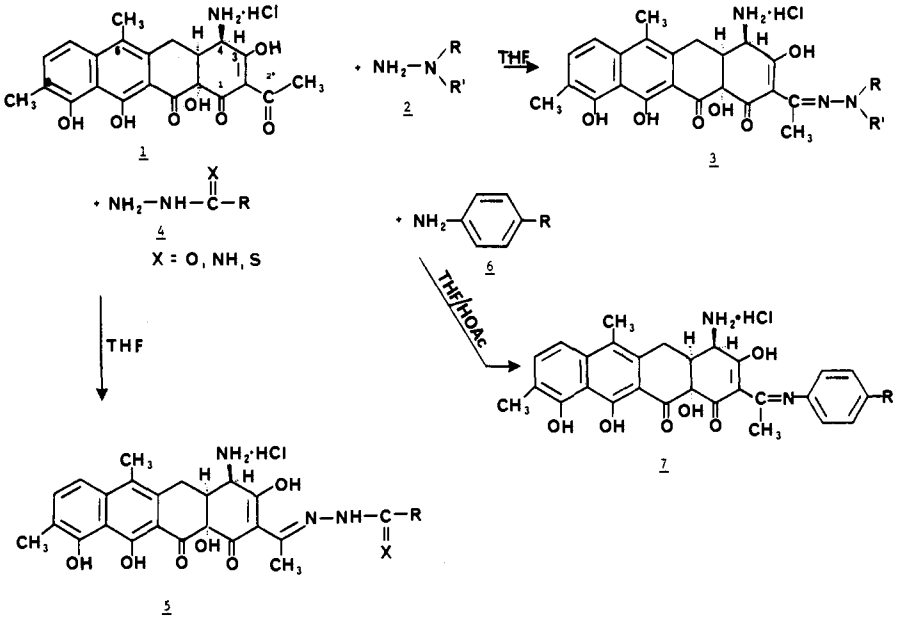
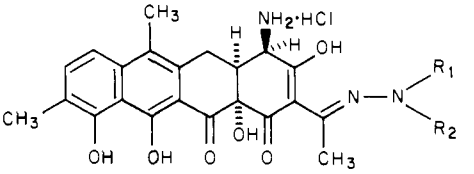
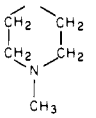
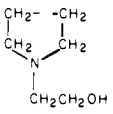
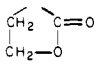


Table I. 2'-Substituted Hydrazones of Chelocardin Hydrochloride



compd	R_1	R_2	yield, %	dp,[a] °C	formula	anal.[b]
8			66		$C_{26}H_{29}N_3O_7 \cdot HCl$	Cl,[c] N
9			98		$C_{27}H_{32}N_4O_6 \cdot 2HCl \cdot 0.5H_2O$	Cl, N
10			50		$C_{28}H_{34}N_4O_7 \cdot 2HCl$	Cl,[d] N
11	H	C_6H_5-	70	237-238	$C_{28}H_{27}N_3O_6 \cdot HCl$	Cl,[e] N
12	H	$-CH_2CH_2N(CH_3)_2$	12		$C_{26}H_{32}N_4O_6 \cdot 2HCl$	Cl,[f] N[g]
13			98	267-275	$C_{25}H_{25}N_3O_8 \cdot HCl$	Cl, N
14	$-CH_3$	$-CH_3$	50.9	240-245	$C_{24}H_{27}N_3O_6 \cdot 2HCl$	Cl,[h] N

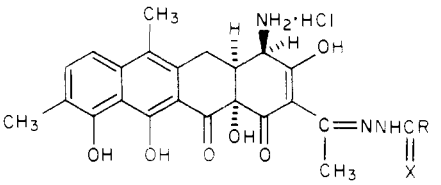
[a] All decomposition points are uncorrected. The decomposition point refers to the temperature at which complete decomposition occurred. [b] Where analyses are indicated by symbols of the elements, except otherwise noted, analytical results were within ±0.4% of the theoretical values. [c] Cl: calcd, 6.66; found, 7.09. [d] Cl: calcd, 11.59; found, 10.97. [e] Cl: calcd, 6.59; found, 6.08. [f] Cl: calcd, 12.45; found, 12.02. [g] N: calcd, 9.84; found, 9.20. [h] Cl: calcd, 13.47; found, 12.36.

Experimental Section

Melting points were determined using a Fisher-Johns melting point apparatus and are uncorrected. The infrared spectra were recorded on Beckman Model IR8 infrared spectrophotometer. The NMR spectra were obtained in Me_2SO-d_6 or Me_2SO-d_6–D_2O on a Varian A-60 and HA-100 spectrometers using tetramethylsilane as an internal standard. The UV spectra were obtained in methanol solution on a Unicam SP-800A ultraviolet spectrophotometer. The high-resolution mass spectra were determined on an Associated Electrical Industries MS-902 double-focusing mass spectrometer and were processed on an IBM 1800 computer. The IR, UV, and NMR data of all compounds were consistent with structure. Elemental analyses of chelocardin as well as its analogues often do not give good agreement with calculated values due to inclusion of nonstoichiometric amounts of solvents.

Preparation of Hydrazones and Acylhydrazones. The general procedure for the preparation of these analogues was to add a slight excess of 1 molar equiv of the corresponding hydrazine or hydrazide to a solution of chelocardin hydrochloride or its free base in 98% aqueous tetrahydrofuran or methanol. The reaction mixture was allowed to stand for a least 1 h at room temperature (a few compounds required a longer reaction time). The product was isolated by precipitation, filtration, and recrystallization. Two

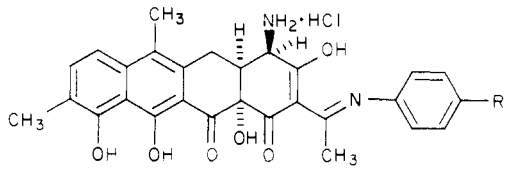
Table II. 2'-Substituted Acylhydrazones and Related Analogues of Chelocardin



compd	R	X	yield, %	dp,[a] °C	formula	anal.[b]
15	CH_3-	O	71	255-265	$C_{24}H_{26}ClN_3O_7$	Cl, N
16	$CH_3CH_2CH_2$-	O	79	235-238	$C_{25}H_{28}ClN_3O_7$	Cl, N[c]
17	$PhCH_2$-	O	83	222-225	$C_{30}H_{30}ClN_3O_7$	Cl,[d] N[e]
18	4-NH_2Ph	O	51		$C_{29}H_{29}ClN_4O_7$	Cl, N
19	4-CH_3OPh-	O	64		$C_{30}H_{30}ClN_3O_8$	Cl, N
20		O	93	239-241	$C_{29}H_{28}ClN_3O_7$	Cl,[f] N
21	4-OHPh-	O	91	239-242	$C_{29}H_{28}ClN_3O_8$	Cl, N
22	2-OHPh-	O	62	237-241	$C_{29}H_{28}ClN_3O_8$	Cl, N
23	NH_2-	O	60		$C_{23}H_{25}ClN_4O_7$	Cl, N
24	NH_2-	S	88		$C_{23}H_{25}ClN_4O_6S$	Cl, N, S
25		O	81	234-236	$C_{28}H_{27}ClN_4O_7$	Cl,[g] N[h]
26		O	86	235-240	$C_{28}H_{27}ClN_4O_7$	Cl,[i] N
27	$CNCH_2$-	O	79	227-231	$C_{25}H_{24}N_4O_7$	N
28		O	93	235-237	$C_{27}H_{26}ClN_3O_8$	Cl, N
29	-NH_2	NH	78		$C_{23}H_{27}Cl_2N_5O_6 \cdot H_2O$	N
30	$CH_3CH_2CH_2NH$-	O	85	230-234	$C_{26}H_{31}ClN_4O_7$	Cl
31	CH_3NH-	O	66.5	238-240	$C_{24}H_{25}ClN_4O_7$	Cl, N
32	$CH_2=CHCH_2NH$-	O	79.4	230-235	$C_{26}H_{29}ClN_4O_7$	Cl,[j] N
33	2-ClPhNH	O	51.4	233-237	$C_{29}H_{28}Cl_2N_4O_7$	Cl, N

[a] All decomposition points are uncorrected. The decomposition point refers to the temperature at which complete decomposition occurred. [b] Where analyses are indicated by symbols of the elements, except otherwise noted, analytical results were within ±0.4% of the theoretical values. [c] N: calcd, 8.11; found, 8.63. [d] Cl: calcd, 6.11; found, 6.60. [e] N: calcd, 7.24; found, 7.70. [f] Cl: calcd, 6.26; found, 5.74. [g] Cl: calcd, 6.25; found, 5.76. [h] N: calcd, 9.88; found, 9.24. [i] Cl: calcd, 6.25; found, 5.68. [j] Cl: calcd, 6.51; found, 5.99.

Table III. Anilino Derivatives of Chelocardin



compd	R	yield, %	formula	anal.[a]
34	-H	96	$C_{28}H_{27}ClN_2O_6$	Cl, N
35	-Cl	91	$C_{28}H_{26}Cl_2N_2O_6$	Cl
36	-Br	86	$C_{28}H_{26}BrClN_2O_6$	Br, Cl, N
37	-OMe	96	$C_{29}H_{29}ClN_2O_7$	N
38	-OH	94	$C_{28}H_{27}ClN_2O_7$	N
39	$-CH_3$	91	$C_{29}H_{29}ClN_2O_6$	N[b], O
40	$-CH_2COOH$	90	$C_{30}H_{29}ClN_2O_8$	N
41	-F	91	$C_{28}H_{26}ClFN_2O_6$	N
42	$-SO_2NH_2$	88	$C_{28}H_{28}ClN_3O_8S$	S
43	-COOH	80	$C_{29}H_{27}ClN_2O_8$	Cl, N
44	$-SO_2NHC(=O)CH_3$	82	$C_{30}H_{30}ClN_3O_9S$	N, S

[a] Where analyses are indicated by symbols of the elements, except otherwise noted, analytical results were within ±0.4% of the theoretical values. [b] N: calcd, 6.62; found, 7.16.

representative examples are given, as follows.

(a) 4-Methylpiperazinoiminochelocardin Dihydrochloride (9). A solution of 1-amino-4-methylpiperazine dihydrochloride (2.62 g, 12.8 mM) in 100 mL of methanol was added to chelocardin free base (5 g, 12.2 mM) suspended in 200 mL of methanol. After stirring the mixture for 2.5 h at room temperature, the chelocardin was completely converted to the hydrazone and the reaction

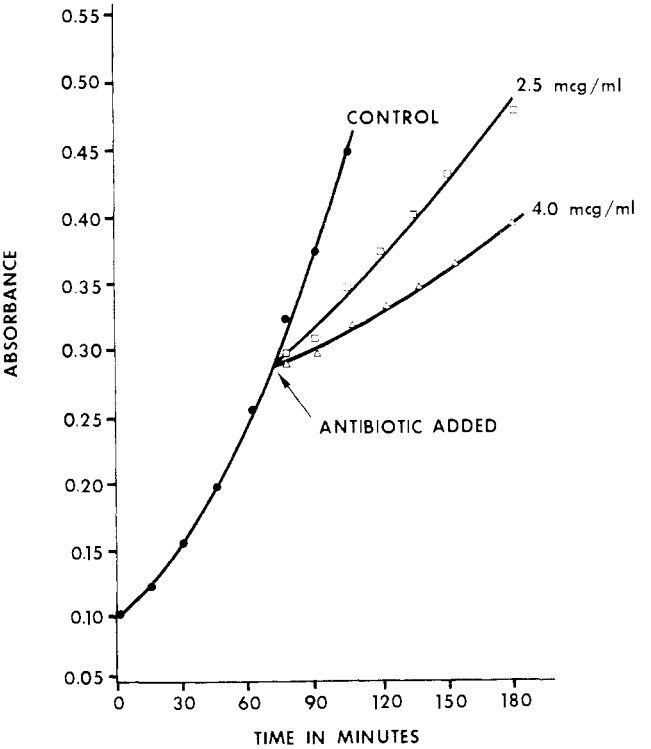
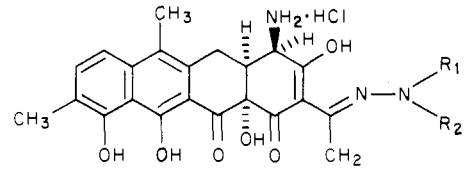


Figure 3. Growth of *P. vulgaris*; inhibition by compound 9.

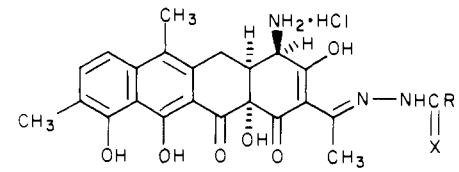
Table IV. In Vitro Activities of 2'-Substituted Hydrazones of Chelocardin



	minimum inhibitory concn, μg/mL[a,b]									
compd	S.a. (45)	S.a.	S.p.	E.	E.c.	K.p.	P.m.	Pr.v.	Pr.m.	S.t.
8	12.5	12.5	12.5	12.5	25	6.2	0.78	6.2	12.5	3.1
9	6.2	6.2	3.1	12.5	6.2	3.1	0.78	6.2	6.2	3.1
10	12.5	6.2	3.1	6.2	6.2	1.56	0.39	3.1	3.1	1.56
11	6.2	6.2	6.2	12.5	50	12.5	0.78	50	50	25
12	50	50	50	100	50	25	3.1	25	25	25
13	25	25	25	25	25	25	1.6	6.2	12.5	25
14	12.5	12.5	6.2	6.2	12.5	6.2	0.78	6.2	6.2	3.1
chelocardin	6.2	3.1	3.1	6.2	6.2	3.1	0.39	1.56	1.56	1.56

[a] The in vitro antibacterial activities are reported as minimum inhibitory concentration (MIC), μg/mL. The MIC's were determined by the twofold agar dilution on brain-heart infusion agar. Organisms selected for inclusion in the table are: S.a. (45), *Straphylococcus aureus* 45; S.a., *Straphylococcus aureus* Smith; S.p., *Streptococcus pyogenes* C-203; E., *Enterococcus* 89; E.c., *Escherichia coli* Juhl; K.p., *Klebsiella pneumoniae* 8045; P.m., *Pasteurella multocida* 10544; Pr.v., *Proteus vulgaris* Abbott JJ; Pr.m., *Proteus mirabilis* Fin no. 9; S.t., *Salmonella typhi*. [b] None of the compounds reported in this table demonstrated significant activity against *Pseudomonas aeruginosa* and these data are not included.

Table V. In Vitro Activities of Chelocardin Acylhydrazones and Related Analogues



	minimum inhibitory concn, μg/mL[a,b]									
compd	S.a. (45)	S.a.	S.p.	E.	E.c.	K.p.	P.m.	Pr.v.	Pr.m.	S.t.
15	12.5	12.5	6.2	12.5	12.5	6.2	0.78	6.2	6.2	6.2
16	50	50	25	50	50	12.5	1.56	12.5	12.5	12.5
17	25	25	12.5	50	50	6.2	6.2	12.5	12.5	12.5
18	50	50	25	50	50	12.5	1.56	12.5	12.5	12.5
19	100	100	50	>100	100	50	3.1	25	25	25
20	100	100	25	100	100	25	3.1	25	25	25
21	50	50	50	100	50	25	3.1	12.5	12.5	25
22	>100	>100	>100	>100	>100	>100	25	>100	>100	>100
23	25	25	25	50	25	12.5	1.56	12.5	25	6.2
24	50	50	25	100	100	25	3.1	50	50	25
25	>100	>100	100	>100	100	100	12.5	>100	>100	100
26	50	50	12.5	50	50	12.5	6.2	12.5	12.5	12.5
27	50	50	50	50	50	12.5	1.56	12.5	12.5	12.5
28	>100	>100	50	>100	>100	50	3.1	50	50	25
29	50	50	25	50	25	12.5	3.1	12.5	12.5	25
30	25	25	12.5	50	25	6.2	0.78	6.2	6.2	6.2
31	50	25	25	50	50	12.5	3.1	25	50	25
32	25	25	12.5	50	25	6.2	0.78	12.5	6.2	12.5
33	100	50	25	100	100	50	6.2	50	50	50
chelocardin	6.2	3.1	3.1	6.2	6.2	3.1	0.39	1.56	1.56	1.56

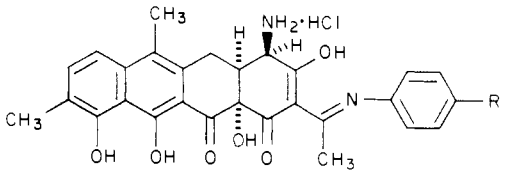
[a,b] See corresponding footnotes to Table IV.

mixture became a clear solution. Activated charcoal (2 g) was added, and the mixture was then stirred for 30 min and filtered. The filtrate was concentrated to a volume of 100 mL under reduced pressure and ether was slowly added until the solution turned slightly tubid. A crystalline product separated out upon standing. A second crop was precipitated from the filtrate by adding additional portions of ether. A total of 6.9 g (98% yield) of 4-methylpiperazinoiminochelocardin dihydrochloride was obtained.

(b) Chelocardin Acethydrazone Hydrochloride (15). A solution of acethydrazide (0.83 g, 11.2 mM) in water (5 mL) was added to chelocardin hydrochloride (2.5 g, 5.6 mM) dissolved in 100 mL of 98% aqueous tetrahydrofuran. The solution was decanted from a small quantity of solid impurity and allowed to stand for 1 h at room temperature. The condensation product crystallized out of the solution (2.2 g, 71% yield); recrystallization from 90% aqueous ethanol produced the pure chelocardin acethydrazone hydrochloride.

Preparation of Anilino Analogues of Chelocardin. The following is a typical example. Acetic acid (0.3 g, 5 mM) in 5 mL of tetrahydrofuran was added to chelocardin hydrochloride (1 g, 2.2 mM) dissolved in 40 mL of 98% aqueous tetrahydrofuran. Aniline (0.3 g, 3.2 mM) dissolved in tetrahydrofuran (5 mL) was added. The mixture was stirred at room temperature for 18 h and was concentrated under reduced pressure to a volume of 5 mL. Ethanol (5 mL) was then added and the solution was added slowly, with vigorous stirring, to ether (250 mL). The suspension was filtered and the residue was washed twice with ether (20 mL),

Table VI. In Vitro Activities of Anilinochelocardin Analogues



compd	minimum inhibitory concn, μg/mL[a,b]									
	S.a. (45)	S.a.	S.p.	E.	E.c.	K.p.	P.m.	Pr.v.	Pr.m.	S.t.
34	12.5	12.5	12.5	25	25	12.5	1.56	6.2	6.2	6.2
35	6.2	6.2	6.2	25	25	3.1	0.39	1.56	3.1	3.1
36	12.5	12.5	12.5	25	25	3.1	0.39	6.2	6.2	3.1
37	12.5	12.5	3.1	6.2	25	3.1	0.78	3.1	6.2	3.1
38	12.5	12.5	12.5	25	50	3.1	0.78	3.1	6.2	6.2
39	12.5	12.5	6.2	25	25	6.2	0.39	6.2	6.2	3.1
40	6.2	6.2	3.1	12.5	6.2	1.56	0.39	1.56	3.1	1.5
41	12.5	6.2	3.1	12.5	6.2	1.56	0.39	1.56	3.1	1.5
42	6.2	3.1	3.1	6.2	6.2	1.56	0.2	1.56	3.1	0.7
43	6.2	3.1	3.1	6.2	6.2	1.56	0.39	1.56	3.1	1.5
44	6.2	3.1	3.1	12.5	6.2	1.56	0.78	3.1	6.2	1.5
chelocardin	6.2	3.1	3.1	6.2	6.2	3.1	0.39	1.56	1.56	1.5

[a,b] See corresponding footnotes to Table IV.

Table VII. In Vivo Activities of Some 2′-Substituted Chelocardin Analogues

compd	mouse protect. CD_{50}[a] vs. *Pr. m.*	
	oral[b]	subcutan[b]
1	200-400	12.5-25
9	25-50	12.5-25
10[c]	100	25
15	50-100	25-50
29[d]	100-200	12.5-25
42[e]	<25	6.25-12.5
44[f]	100-200	6.25-12.5

[a] The CD_{50} values are expressed as the total dose of compound in mg/kg required to protect 50% of the mice challenged intraperitoneally with *P. mirabilis* (Pr.m.). [b] The route of administration of compound. [c] 4-(2-Hydroxyethyl)piperazinoiminochelocardin. [d] Chelocardin guanylhydrazone. [e] *p*-Sulfamylanilinochelocardin. [f] *N*-Acetylsulfamylanilinochelocardin.

yielding 1.1 g of anilinochelocarin hydrochloride (**34**; 96% yield).

Bacterial Growth Inhibition Studies. The antibiotics **1**, **35**, and **9** were dissolved in methanol separately and diluted to 1000 mcg/mL with distilled water. Solutions were stored at 4 °C and used within 2 days of preparation.

One milliliter of an overnight brain–heart infusion (BHI) culture of *Proteus vulgaris* Abbott JJ was inoculated into 250 mL of BHI broth and incubated at 37 °C for 2 h. Aliquots of 9.9 mL were distributed to spectrophotometer tubes which were then incubated in a 37 °C water bath. Changes in absorbance at 645 nm were determined at intervals of 15 min, using a Bausch and Lomb Model 20 spectrophotometer.

After approximately 50–70 min, 0.1 mL of a solution of the antibiotic was added to each tube to give the desired concentration. The growth-inhibition studies were performed in triplicate for each antibiotic level and control. The results are given in Figures 1–3.

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