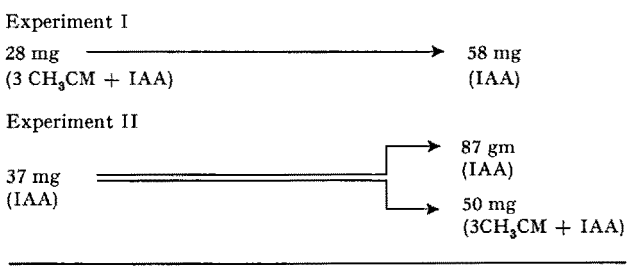
Table III. Control of 3-methylcoumarin inhibition on *Helianthus tuberosus* dormant tuber explants in vitro



Each datum is dry weight average (mg) of 20 explants grown first for 21 days and after another 26 days. Concentrations are: 3CH_3CM $10^{-3}M$ and IAA $2\times10^{-6}M$.

Table IV. 3-hydroxycoumarin effect on root neoformation in *Cichorium intybus* explants in vitro

3 OH-CM (M)	No. neoformed roots	No. roots (%)
0	7.1 ± 0.7	100
10^{-4}	5.1 ± 0.4[a]	72
5×10^{-4}	5.4 ± 0.6[a]	76
10^{-3}	0.5 ± 0.1[a]	7

Average values ± SE were made on 28 explants, 25 days old, growing in continuous light. [a] The difference of each average with its control is significant at least at 5% Student's t-test.

Root neoformation in chicory is inhibited (more than 90%) particularly by mM 3 OH-CM (Table IV). Also 3 CH_3-CM causes a similar effect. The rhyzogenetic activity also of *H. tuberosus* explants was verified according to TRIPATHI[10] and TRIPATHI and GAUTHERET[14]. Unfortunately our explants were not able to form roots on TRIPATHI medium probably because this phenomenon is related to variety.

Two parallel experiments were made in alternating light or in the dark, with 3 CH_3-CM and 4 OH-CM, at concentrations between 0.1 and 1 mM, on *C. intybus* and *H. tuberosus*. The data show, referred to the controls, no significant differences of growth except for *C. intybus* cultivated on 3 CH_3-CM in the dark, which is more inhibited. 3 CH_3-CM shows a greater effect on bud formation in the dark than in the light, while 4 OH-CM exerts the same effect in both cases. However, the number of buds formed by explants grown on the same coumarin concentrations, was constantly lower in those grown in the dark (about 33%).

The results were also examined to discover a possible relationship between the type and position of substituting radicals in the molecule and its action. The coumarin (CM) generally inhibits the callogenesis to the same degree as 3 CH_3-CM and the organogenesis more than the other coumarins essayed. The methyl substitution in position 3 has a greater inhibitory effect, at mM concentrations, than hydroxylation and carboxylation in the same position on bud formation in *C. intybus*. The hydroxy-groups in position 3 and 4 cause a similar effect on chicory, while the 4 OH-CM exerts a greater inhibition on *H. tuberosus*. The substitution in position 6 with amino or 6, 7 with hydroxy-groups has very little effect on callogenesis of *H. tuberosus* and *C. intybus*, while 6-chloro-group substituted coumarin is more inhibiting.

Probably the plants do not metabolize coumarins simply by detaching the radicals: in fact the effects of various derivatives are often very different from those of parent compound. Generally *H. tuberosus* appears to be more sensitive than *C. intybus* to coumarin action, especially expressed as fresh weight. Besides chicory does not show significant variations of dry weight percentage. It is known that the IAA activation of dormant tissues as *H. tuberosus* raises its hydration. In such explants, treated with increasing coumarin concentrations, the dry weight percentage generally grows parallel. This fact could indicate that, in addition to the inhibitory effect on callogenesis, these compounds, at suitable concentrations, probably exercise some inhibition also on extention growth as confirmed by numerous authors[3,4-6], perhaps modifying the cellular permeability[15].

In conclusion, the inhibitory effect on growth and organogenesis of coumarins essayed are obviously different according to the tissues utilized, but overall they are dependent on position and type of substituting radicals[16].

Riassunto. La cumarina ed alcuni suoi derivati (3 metil-, 3 idrossi-, 3 carbossi-, 4 idrossi-, 6 amino-, 6 cloro- e 6,7 diidrossi-cumarina), specialmente alla concentrazione mM, hanno effetto inibitorio sia sulla callogenesi di tuberi di *Helianthis tuberosus* che sulla organogenesi (neoformazione di gemme e radici) e callogenesi di radici di *Cichorium intybus* coltivati in vitro. Il grado di inibizione dipende dal tipo di radicale sostituente della cumarina e dalla pianta utilizzata come test.

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Antimicrobial Activity of Cyclacillin against *Escherichia coli* in vivo and in vitro

Cyclacillin [6-(1-aminocyclohexanecarboxamido)penicillanic acid] is a semisynthetic penicillin with a wide antibacterial spectrum; it resembles ampicillin in being effective against a wide range of gram-positive and gram-negative pathogens, but, unlike ampicillin, is also effective against the penicillinase-producing staphylococci[1-3]. However, in laboratory experiments in which cyclacillin was tested against a number of gram-positive and gram-negative pathogens, an inconsistency was encountered between the in vivo and in vitro susceptibility[4]. In vitro, cyclacillin was less active than ampicillin against both gram-negative and gram-positive bacteria, while in vivo,

cyclacillin and ampicillin were about equally active against *Streptococcus pyogenes*, *Diplococcus pneumoniae* and penicillin-sensitive *Staphylococcus aureus*. In addition, cyclacillin was clearly and consistently superior to ampicillin in mice infected with multiples of the standard LD_{95} dose of *S. aureus* and *Strep. pyogenes*.

Quantitative tests have shown that many organisms which appear resistant to cyclacillin in vitro are quite susceptible in vivo[4]. An example is the previously stated susceptibility of the penicillin-resistant staphylococci; when tested against infection with graded numbers of these bacteria, cyclacillin was consistently superior to ampicillin. Thus, the high level of therapeutic activity exhibited by cyclacillin could not have been predicted by the in vitro susceptibility data.

Although there is no satisfactory explanation for this discrepancy, it is widely recognized that many factors influence the apparent susceptibility of bacteria in vivo. Many of these factors may be absent under in vitro conditions. The experiments presented here were undertaken to clarify the apparent differences between the in vivo and in vitro antibacterial results with cyclacillin. The effectiveness of this antibiotic was compared with that of ampicillin in mice infected with *Escherichia coli*, and the in vivo data on both antibiotics were compared with the results of in vitro susceptibility assays.

Adult NIH random-bred Albino A mice were used as the experimental animals. They were obtained from a commercial dealer in the Philadelphia area and weighed approximately 20–25 g at the time of testing. *E. coli* isolated from a blood specimen of an infected patient was used as the test organism. The bacteria were cultured on brain heart infusion agar for 18 h, and washed cell suspensions were prepared with sterile saline. The number of organisms per ml was standardized by routine bacteriologic pour plate assay, and approximately 5×10^7 organisms, in 0.1 ml saline, were injected i.v. by the tail vein into groups of 20–30 mice. Some of the mice were treated by i.p. injection of 100 µg of either cyclacillin or ampicillin. The antibiotics were obtained from commercial lots prepared at Wyeth Laboratories. Stock solutions were prepared in sterile saline at a concentration of 1000 µg of each antibiotic per ml saline. The solutions were sterilized by filtration and stored in 5 ml aliquots at –20 °C until used.

To test the effect of antibiotic on clearance of bacteria from the circulation, 0.1 ml volumes of blood were obtained, by either retroorbital venous plexus puncture or from the tail vein, at closely spaced intervals during the first 6–10 h after injection of the bacteria. At given intervals 4 to 5 mice were killed and their spleens and livers removed aseptically. The organs were homogenized with 10.0 ml sterile saline, using aseptic procedures. The number of viable bacteria in each organ was determined by a standard pour plate procedure, using nutrient agar plates.

For in vitro susceptibility tests, a conventional tube dilution technique was used. Two-fold serial dilutions of each antibiotic were made in 0.5 ml volumes of sterile brain heart broth in small glass tubes. To each dilution was added 0.1 ml of an 18 h broth culture diluted so as to contain approximately 5×10^6 viable organisms per ml. The tubes were incubated at 37 °C for 18 h and the MIC was recorded as that concentration of antibiotic which prevented growth of the bacteria.

As is evident in Table I, there was a rapid clearance of *E. coli* from the blood of untreated as well as treated mice. Few if any bacteria were detected in the blood after 6–8 h, even without antibiotics. On the other hand, there was a rapid increase in the number of *E. coli* in the spleen and liver of all mice, the largest number occurring in the liver. Much greater numbers of bacteria were recovered from the untreated mice; for example, there were over 2000 *E. coli* per ml of spleen or liver homogenate 8 h after injection of bacteria in control mice, whereas less than 200 were present in equivalent tissue samples from the antibiotic-treated groups.

As shown in Table II, ampicillin was about 6–8 times more effective than cyclacillin against *E. coli*. This difference is similar to that reported in other studies[4] and con-

Table I. Blood clearance and tissue localization of *E. coli* in untreated and treated mice

Test sample[a]	Time after injection (h)	Treatment[b] None	Cyclacillin	Ampicillin
Blood	0	>2000	>2000	>2000
	2	50 ± 19	70 ± 31	80 ± 22
	4	105 ± 26	120 ± 68	98 ± 37
	8	<5	<5	<5
	12	<5	<5	<5
Spleen	0	<100	<100	<100
	2	850 ± 360	240 ± 43	210 ± 39
	4	1150 ± 630	300 ± 52	600 ± 130
	8	>2000	<100	238 ± 52
Liver	0	<100	<100	<100
	2	>2000	515 ± 65	480 ± 70
	4	>2000	800 ± 93	1000 ± 21
	8	>2000	1020 ± 130	1150 ± 360

[a] Groups of 30 mice each injected i.p. with *E. coli* (approximately 1.0×10^8 organisms) after treatment with indicated antibiotic; 4–6 mice were tested per group at indicated time interval (0 time = within 10 min of injection of bacteria) for number of *E. coli* per 0.1 ml test sample. [b] 100 µg of indicated antibiotic injected into each test mouse 1 h before challenge injection.

Table II. Minimal inhibitory concentrations for in vitro inhibition of growth of *E. coli* in presence or absence of homogenate of normal mouse liver or spleen

Antibiotic	Mouse tissue added[a]	MIC with test bacteria[b] (µg/ml)
Cyclacillin	None	6.7
	Spleen	6.7
	Liver	6.7
Ampicillin	None	0.8
	Spleen	0.8
	Liver	0.8

[a] Equal volume of 10% homogenate (v/v) of indicated normal mouse tissue added to antibiotic dilution before testing for MIC. [b] MIC determined after 18–24 h incubation at 37 °C.

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trasts markedly with the in vivo results. Thus, in an additional series of experiments, the addition of 10% (v/v) saline homogenates of liver and spleen cells from normal mice had no significant effect on the MIC of either antibiotic (Table II).

The results of these experiments confirm the marked difference between the in vitro and in vivo susceptibility of *E. coli* to cyclacillin. Whereas there was little difference between the effects of cyclacillin and ampicillin in vivo, there was a marked difference in the MIC tests. The reason for these differences is not clear.

Further studies, now in progress, on the in vitro effect of tissue homogenates from normal and antibiotic-treated mice should provide relevant information concerning the comparative efficacy of cyclacillin and other semisynthetic penicillins. The in vivo vs in vitro paradox is also being investigated by determining the effects of cyclacillin on RE cell funtion, antibody formation and nonspecific resistance.

Résumé. Chez la souris, l'effet de la cyclacilline diffère peu de celui de l'ampicilline, en ce qui concerne la concentration ou localisation de *E. coli* dans le sang, la rate et la foie. Une différence importante est cependant notée dans les concentrations minima inhibitrices (MIC) de ces deux antibiotiques pour les bactéries gram-négatives.

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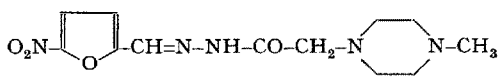
Nifurpipone, a New Nitrofuran with a Large Antimicrobial Spectrum

Since the low water solubility of nitrofurans is a limiting factor for pharmaceutical formulation and for some therapeutic uses, we synthesized a series of 5-nitro-2-furaldehyde aminoacethydrazones to obtain new nitrofurans with a better water solubility. Among these the 5-nitro-2-furaldehyde N'-methyl-N-piperazinoacethydrazone (Rec 15-0122 nifurpipone) showed a very good antimicrobial activity in vitro and in vivo, and also a high urinary excretion, so that this compound appears as a potential drug for urinary tract infections[1-4].



nifurpipone

Nifurpipone is a microcrystalline yellow powder with m.p. 167–168° (dec.). Its UV-spectrum in water shows 2 absorption maxima at 360 and 252 nm. ($E^{1\%}_{1cm}$ = 582 at 360 nm). The substance is very soluble in methanol, and chloroform, soluble in ethanol, acetone, and benzene, slightly soluble in water (0.2%). Its salts are very soluble in water. The monoacetate and the dihydrochloride are microcrystalline powders with m.p. at 126–129° and at 250 respectively.

The in vitro antimicrobial activity was tested by conventional methods and the results are reported in Table I. Nitrofurantoin was investigated for comparison.

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Table I. Antimicrobial activity in vitro

Compound	Minimal inhibitory concentrations (μg/ml)												
	Escherichia coli 100	*Salmonella breslau* 1090	*Salmonella typhymurium* 1086	*Klebsiella*	*Pseudomonas aeruginosa* H2	*Proteus Vulgaris* OX	*Staphylococcus aureus* SG 511	*Streptococcus pyogenes humanus* A88	*Bacillus subtilis* ATCC 9466	*Chlostridium novyi*	*Mycobacterium tuberculosis* H37 Ra	*Tricophyton mentagrophytes* 1236	*Candida albicans* 28
Nifurpipone	20 10[a]	40	20 10[a]	160 160[a]	160	160 80[a]	10 10[a]	5 2.5[a]	20	160	>160	>160	>160
Nitrofurantoin	10 10[a]	40	20 20[a]	80 >160[a]	160	80 80[a]	10 10[a]	2.5 2.5[a]	10	160	160	>160	>160

[a] With 10% bovine serum.