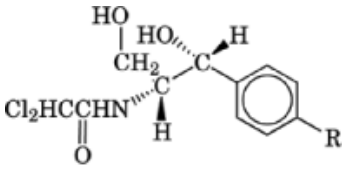
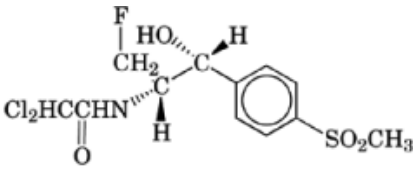
Kirk-Othmer Encyclopedia of Chemical Technology.

CHLORAMPHENICOL AND ANALOGUES

Chloramphenicol [56-76-7] (**1**, R = NO_2), $C_{11}H_{12}Cl_2N_2O_5$, is a commercially significant antibacterial agent and its status in clinical practice has been reviewed (1–6). Although widespread use of this antibiotic declined in the United States in the 1960s because of reports of serious toxic effects, this situation changed a decade later when ampicillin-resistant Hemophilus influenzae emerged on the clinical scene (3, 6). The appearance of *Bacteroides* species and of *Streptococcus pneumoniae* (6) resistant to β-lactam antibiotics contributed further to the resurgence. In the 1970s, chloramphenicol also became important in the treatment of serious *Salmonella* invasive gastroenteritis in infants less than three months of age (5). Because chloramphenicol crosses the blood–brain barrier, it is indicated in infections of the central nervous system caused by susceptible organisms (5, 7). The antibacterial activities of chloramphenicol and thiamphenicol [15318-45-3] (**1**, R = SO_2CH_3), $C_{12}H_{15}Cl_2NO_5S$, a close analogue, against a number of chloramphenicol-sensitive gram-positive and gram-negative organisms are given in Table 1.



(**1**)



(**2**)

The emergence of quinolones (see Antibacterial agents, synthetic) and other antibiotics is expected to curtail the use of chloramphenicol in the future, but this drug is relatively inexpensive, orally active, and the toxicity, except for the rare idiosyncratic aplastic anemia (1–6), can be managed through monitoring of blood levels by sensitive modern analytical procedures (3). However, clinical use is being further curtailed by the emergence of chloramphenicol-resistant organisms. In Table 2, the median *in vitro* susceptibilities of chloramphenicol, thiamphenicol, and a fluoroanalogue, florfenicol [76639-94-6] (**2**), $C_{12}H_{14}Cl_2FNO_4S$, against a host of chloramphenicol-sensitive and -resistant organisms are given. Bacteria resistant to chloramphenicol are also resistant to thiamphenicol.

Table 1. Minimum Inhibitory Concentration (MIC) of Chloramphenicol and Thiamphenicol Against Sensitive Organisms

	MIC[a], μg/mL	
Organism	Chloramphenicol	Thiamphenicol
Enterobacter ridant	2	32
E. coli ATCC 10536	1	32
Klebsiella 0217604	4	64
Proteus mirabilis 12453	2	4
Proteus valgaris Napolitano	2	32
Proteus rettgeri Hewitt 104	4	8
Serratia 0213605	16	>128
B. subtilis 66333	2	4
Staphylococcus aureus 209P	4	8
Streptococcus pyogenes Bolden	4	4
Streptococcus 2040	4	4
Salmonella Gr. B. typhimurium	4	64
Shigella 1313	0.5	0.5

[a] Agar dilution, 48 h, Mueller-Hinton agar.

Table 2. *In Vitro* Susceptibilities of Amphenicols

			MIC, μg/mL[a]		
Organism strain	No. of strains tested	Susceptibility[b]	Chloramphenicol	Thiomphenicol	Florfenicol[c]
Enterobacter	4	S	4	64	4
	14	R	512	1024	8
Citrobacter	3	S	4	32	8
	3	R	512	1024	128
E. coli	9	S	4	64	8
	20	R	256	1024	8
Klebsiella	9	S	4	64	8
	20	R	512	1024	4
Providencia	4	S	16	128	8
	12	R	128	1024	8
Pseudomonas	13	R	128	128	256
Serratia	6	S	16	512	64
	18	R	512	1024	64
Salmonella	15	S	4	32	8
	7	R	256	1024	8
Shigella	9	S	1	2	2
Proteus	23	R	256	512	8
Acinetobacter	4	R	64	512	128
Staphylococcus	9	S	4	8	8
aureus	7	R	64	512	8
Streptococcus pneumonae	3	R	8	64	4

[a] Agar dilution, 24 h, Mueller-Hinton agar.
[b] S = susceptible; R = resistant.
[c] Florfenicol data are from Ref. 8.

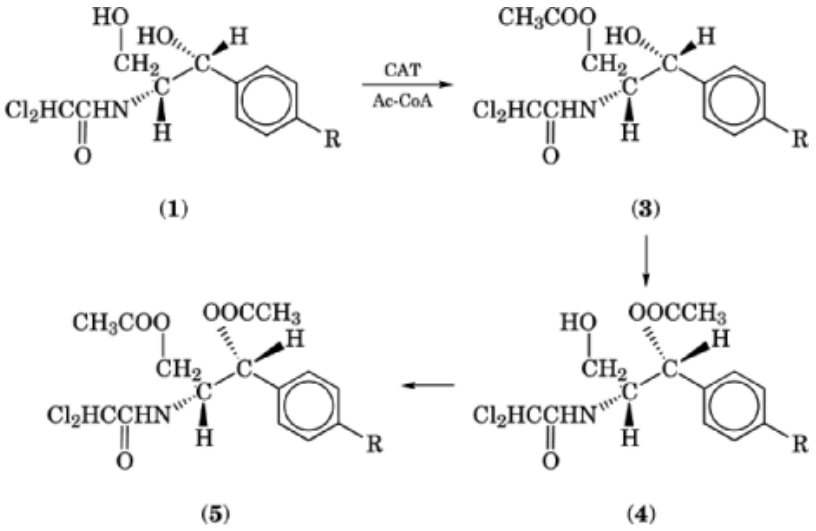


Fig. 1. CAT (catalyzed acetylation) of chloramphenicol (**1**, R= NO_2), and thiamphenicol (**1**, R= CH_3SO_2) where Ac-CoA is acetyl coenzyme A [72-89-9].

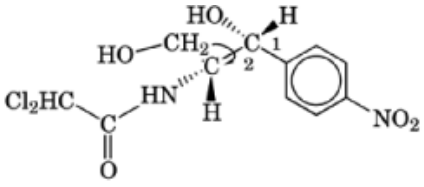
Both chloramphenicol and thiamphenicol cause reversible bone marrow suppression (9) The irreversible, often fatal, aplastic anemia, however, is only seen for chloramphenicol (9). This rare (1 in 10,000–45,000) chloramphenicol toxicity has been linked to the nitroaromatic function (1, 9). Thiamphenicol, which is less toxic than chloramphenicol in regard to aplastic anemia, lacks potency as can be seen in Table 1, and thiamphenicol has never found much usage in the United States. An analogue of thiamphenicol having antimicrobial potencies equivalent to chloramphenicol was sought. Florfenicol (**2**) was selected for further development from a number of closely related structures.

1. Bacterial Resistance of Amphenicol

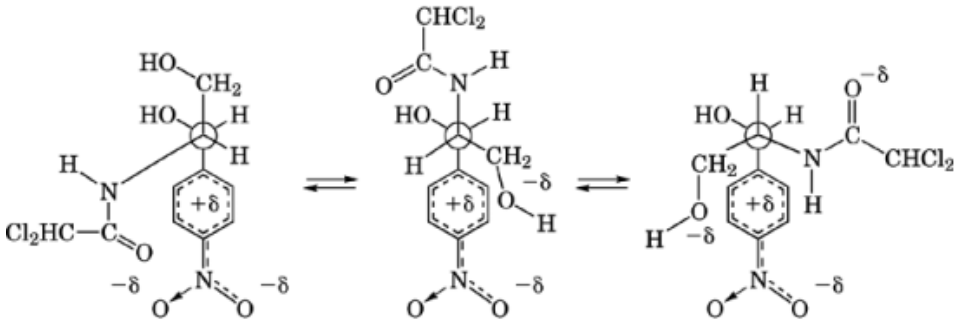
Of the many mechanisms of bacterial resistance to chloramphenicol and thiamphenicol, the plasmid-mediated transmissible resistance conferred by the presence in resistant bacteria of chloramphenicol-acetyltransferases (CAT) is the most important. This enzyme catalyzes the acetyl-CoA dependent acetylation of chloramphenicol and thiamphenicol (1, 10–12). CAT is a cytoplasmic enzyme of which there are three main types: I, II, and III, type III being the most catalytically active (13). The most commonly observed variant of CAT appears to be type I, and type I and type III proteins are known to associate with one another to form hybrids possessing properties of both (13, 14). The type III variant of CAT has been studied in detail by steady-state kinetics (13). The data indicate the formation of a ternary complex in the rate determining step involving the enzyme, acetyl-CoA, and chloramphenicol, but the tightness of substrate binding is not a contributing factor. Four types of CAT, type A, B, C, and D, have been characterized in *Staphylococcus aureus* (15) and type C seems to be the most common variant in this species.

Plasmid-mediated bacterial inactivation of chloramphenicol and thiamphenicol can potentially lead to three products, the 3-*O*-acetyl (**3**), 1-*O*-acetyl (**4**), and 1,3-di-*O*-acetyl (**5**) derivatives as shown in Figure 1.

It was postulated (16–18) and later shown (10) that acetylation of the 3-hydroxyl group was the only enzymatic step in the inactivation process leading to products (**3**) and (**5**), and that formation of the 1-*O*-acetyl derivatives (**4**) resulted from a nonenzymatic intramolecular migration of the acetyl group from



(**1**)



(**a**) (**b**) (**c**)

Fig. 2. Conformers (**a**), (**b**), and (**c**) of chloramphenicol (**1**, R=NO_2).

the 3- to the 1-position. Thus both the parent amphenicols (**1**) and the 1-*O*-acetyl derivatives (**4**) would seem to be substrates for the CAT enzyme (15, 16, 19) and prevention of enzymatic *O*-acetylation at the 3-position by replacement of that hydroxyl group using a suitable nonacylable function was proposed to block both modes of antibiotic inactivation (16). Extensive work on the modification of chloramphenicol at the 3-position had however, produced no therapeutically useful derivatives. Additionally, structure-activity relationship studies using chloramphenicol led to the conclusion that the 1,3-propanediol moiety was absolutely essential for amphenicol-type activity (20, 21).

2. Structure-Activity Relationship of Chloramphenicol

Structure-activity and mechanism of action studies indicate that the requirements for chloramphenicol activity are: the D-threo-configuration, the 1,3-propanediol moiety, and a strong electron withdrawing group on the aromatic ring. The L-threo, the mirror image of (**1**), and the D-erythro and L-erythro isomers are not biologically active. Thus the speculation arose that certain specific intramolecular dipolar attractive interactions must exist in chloramphenicol leading to greater stabilization of one particular conformer over the others (16) where biological activity results from the most stable conformer.

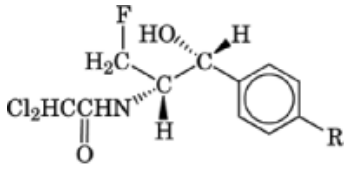
The three basic conformational isomers of chloramphenicol are shown in Figure 2. In solution, the rotamers (**a**), (**b**), and (**c**) are expected to be in equilibrium and the concentration of any one of the three species at any given time is dependent on the nature of the solvent and temperature. Chloramphenicol has a specific rotation, $[\alpha]_D$, of +18.5° in ethanol and −30° in dimethyl formamide (22).

In solution, as in the crystalline state (23), the amide carbonyl bond is expected to be in a near syn-periplanar orientation with the C-2 hydrogen bond for all three rotameric forms (24–26). In the crystalline

state, chloramphenicol exists in the rotameric form (**a**) (23) where the carbonyl group and the aromatic ring are in close proximity and the carbonyl atom is directed toward the π-electron system of the nitroaromatic ring suggesting strong dipolar attractive forces between the two moieties. In the rotameric form (**b**) such a dipolar interaction is not possible. Although, in conformer (**c**) a dipolar attractive interaction similar to the one seen in (**a**) is possible, it would require that the amide carbonyl bond depart from its thermodynamically preferred near syn-periplanar orientation to become near anti-periplanar. Therefore, rotamer (**a**) is the only conformation accommodating the preferred orientation of the amide carbonyl group with respect to both the C-2 methine hydrogen and the nitroaromatic group. Theoretical calculations (27) and molecular modeling (28) have also suggested that (**a**) is the most preferred conformation. Although the possibility of an intramolecular hydrogen bond between the 1,3 hydroxyl groups is implied in the x-ray crystal structure of chloramphenicol, the existence of such a bond in solution has been ruled out (27, 29).

3. Fluoroanalogues

Because the lack of biological activity of 3-substituted chloramphenicols reported previously might result from the inability to exist in the "active" (**a**) type conformation, it was speculated that the size and nature of the C-3 substituent, maintenance of a low barrier to rotation about the C-2–C-3 bond, and the length of the carbon-substitutent atom bond at C-3 were highly critical for achieving a conformational preference of the (**a**) type. Thus, on the basis of the van der Waals radii of fluorine and oxygen being the same (0.14 nm) and the average $C-O$ and $C-F$ bond lengths being close (0.131 nm, 0.138 nm, respectively), the C-3-hydroxyl group of chloramphenicol was replaced by a fluorine atom. Optical rotation measurements of 3-fluoro-chloramphenicol [73212-55-2], (**6**, $R = NO_2$), $C_{11}H_{11}Cl_2FN_2O_4$, in ethanol, gave $[\alpha]_D = +24.4^\circ$ and in dimethylformamide gave -23.4°. Thus, as in the case of chloramphenicol, the optical rotation changed from a positive to a negative value on going from a protic to a dipolar aprotic solvent. The solid-state conformation was determined by single crystal x-ray structure analysis (30) and the crystals contained two rotameric structures in the asymmetric unit. The first conformer (Fig. 3**a**), corresponds to rotamer (**a**) of chloramphenicol where all the chloramphenicol conformational features are maintained. The other conformer, shown in Figure 3**b**, corresponds to the extended form (**b**) seen in Figure 2. The fluorine atom in this case is located close to the plane of the aromatic ring system supporting the explanation given for the stability of conformer (**b**) of chloramphenicol.



(**6**)

In solution, it is to be expected that rotamers (**a**) and (**b**) of structure (**6**) would exist in equilibrium with a third rotamer comparable to (**c**) of Figure 2, and that the thermodynamically preferred conformer **(6a)** would be responsible for biological activity. Removal of the nitro group from the aromatic ring was expected to have a destabilizing effect on the carbonyl-aromatic ring attraction in **(6a)** and in the solid state, the desnitro analogue (**6**, $R = H$) exists only in an extended form, comparable to rotamer (**b**), where the fluorine atom is no longer close to the plane of the aromatic ring. This desnitro compound is biologically inactive and it is therefore concluded that for activity a conformation of type (**a**) is required. The absence of the aromatic nitro group is thought to induce repulsive forces between the flourine atom and the electron system resulting in the deflection of the flourine away from the plane of the aromatic ring.

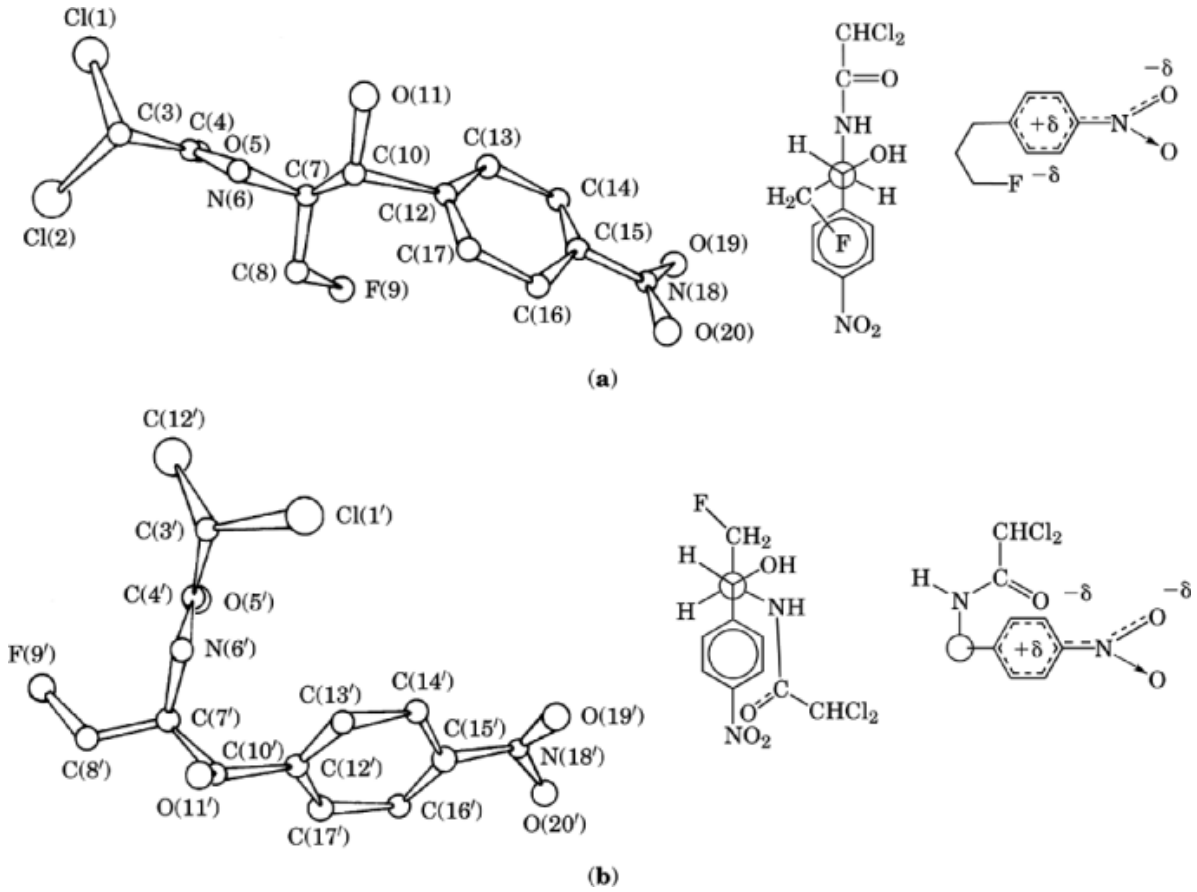


Fig. 3. The solid-state conformations of 3-fluoro-chloramphenicol (**6**, $_{R=}$ NO_2) showing the corresponding rotamers and schematics of the stabilizing attractive forces.

3.1. Biological Activity

The biological activity of 3-fluoro-chloramphenicol (**6**, R = NO_2) against chloramphenicol-sensitive and -resistant organisms was determined and is given in Table 3. Potencies against sensitive strains are similar to those of chloramphenicol. Additionally, this fluoroderivative is highly active against chloramphenicol-resistant organisms having MICs ranging from 1 to 16 μg/L. This result prompted the synthesis and biological evaluation of a number of amphenicols containing a fluorine atom at the 3-position. The activity of the most promising, florfenicol (**2**), 3-fluorothiamphenicol, is also given in Table 3. Florfenicol is not only active against the chloramphenicol–thiamphenicol-resistant strains, but the potency of florfenicol against sensitive organisms is also superior to any of the other amphenicols.

The antimicrobial properties of florfenicol have been evaluated by several laboratories (8, 31). As seen in Table 2, the median MICs of florfenicol against chloramphenicol-susceptible strains of *Enterobacter*, *Citrobacter*, *Providenia*, *Serratia*, *Salmonella*, and *Shigella* are similar to chloramphenicol whereas against *Klebsiella* the median MIC is half. However, against sensitive *Staphylococci* florfenicol is twice as potent as chloramphenicol. Thiamphenicol is considerably less potent than florfenicol against all of the above strains. Against 103 resistant strains, the MICs of chloramphenicol and thiamphenicol ranged from 64 to 1,026 μg/mL. Mean

Table 3. Activity of Chloramphenicol and Analogues Against Chloramphenicol-Sensitive and -Resistant Organisms

	MIC, μg/mL[a]			
Organism	Chloramphenicol	Thiamphenicol	3-Fluoro-chloramphenicol	Florfenicol
Ent. aerogenes Ridant	2	8	4	0.5
Ent. aerogenes Jackson	>128	>128	8	4
E. coli ATCC 10536	1	32	0.5	0.5
E. coli 0128604	>128	>128	16	16
Kleb. pneumoniae 0217604	4	64	8	8
Kleb. pneumoniae 1117501	>128	>128	2	2
Prov. stuartll Rahal	16	64	2	1
Prov. stuartll 4GR	>128	>128	16	2
Serr. marcescens 0213605	8	> 128	8	8
Serr. marcescens Brooke 4	>128	>128	4	2
Staph. aureus 209P	4	4	4	0.5
Staph. aureus Ziegler	8	4	4	0.5
Staph. aureus 59N	4	4	1	0.5
Staph. aureus 1613	2	4	2	0.25
Strep. pyogenes Bolden	0.5	4	2	0
Strep. pyogenes Cruz	2		4	4
Salm. Gr. B. typhimurium	2	32	4	4
Salm. Gr. C2 Newport	2	8	4	2
Shig. dysenteriae ATCC 13213	0.5	0.25	0.25	0.25
Shig. dysenteriae SS1NO1	0.25	0.25	0.25	0.25
Prot. mirabilis ATCC 12453	4	1	4	1
Prot. mirabilis ATCC 12453	4	1	4	1
Prot. morgani Daly	8	32	8	1
Prot. mirabilis Charlot. Va.	32	32	8	0.5
Prot. morgani Garro	128	32	4	1
Prot. rettgeri 120	128	>128	16	2

[a] Mueller-Hinton broth, 24 h.

values were 256 and 512 μg/mL, respectively. For florfenicol, the MICs ranged from 1 to 64 μg/mL against the same resistant organism; the median value was 8 μg/mL.

Florfenicol inhibited 91% of the 399 bacterial isolates at a concentration of 12.5 μg/mL (31). At the same concentration, chloramphenicol and thiamphenicol inhibited only 70% and 24% of the isolates, respectively. Other work has also confirmed the superior activity of florfenicol against chloramphenicol-resistant strains (32–35). More recently it has been shown that florfenicol is active against *E. coli* strains that produce type I, II, or III CAT enzymes (36).

3.2. Veterinary Potential or Florfenicol

The absolute ban on the use of chloramphenicol in food producing animals in the United States and Canada has accentuated the need for an effective broad spectrum antibiotic in animal food medicine. Florfenicol and other antibiotics commonly used in veterinary medicine have been evaluated *in vitro* against a variety of important veterinary and aquaculture pathogens. Some of these data are shown in Tables 4 and 5, respectively. Florfenicol was broadly active having MICs lower than those of chloramphenicol in each of the genera tested (Table 4).

Table 4. Activity of Florfenicol (2) and Chloramphenicol (1, R = NO_2) Against Veterinary Bacterial Pathogens

		MIC_{90}[a] (μg/mL)	
Organism	No. of strains tested	Florfenicol	Chloramphenicol
Streptococcus spp.	81	4.0	8.0
Staphylococcus spp.	23	5.0	64.0
Staphylococcus spp.	57	8.0	16.0
Corynebacterium spp.	14	2.0	16.0
Clostridium spp.	21	8.0	4.0
Pasteurella multocida	32	1.0	16.0
E. coli	272	16.0	>128.0
Salmonella spp.	68	16.0	>128.0
Klebsiella spp.	54	32.0	>128.0
Proteus spp.	26	16.0	>128.0
Pseudomonas spp.	35	>128.0	>128.0

[a] MIC_{90} is the antibiotic concentration at which 90% of the bacteria tested are inhibited.

Table 5. Minimum Inhibitory Concentrations (MIC_{90}[a]) of Florfenicol (2) and Other Antibiotics Against Bacterial Pathogens Isolated from Fish in Japan, μg/mL

	Organism (No. of strains tested)		
Antibiotic	*Pasteurella piscicida* (50)	*Edwardsiella tarda* (50)	*Vibrio anguillarum* (35)
florfenicol	0.4	0.8	0.8
chloramphenicol	12.5	50.0	25.0
thiamphenicol	>100.0	>100.0	>100.0
oxytetracycline	6.3	50.3	
ampicillin	100.0		
oxolinic acid		1.6	

[a] MIC_{90} is the antibiotic concentration at which 90% of the bacteria tested are inhibited.

Florfenicol was also superior to chloramphenicol, thiamphenicol, oxytetracycline [79-57-2], ampicillin [69-53-4], and oxolinic acid [14698-29-4] against the most commonly isolated bacterial pathogen of fish in Japan (Table 5) (37).

Florfenicol (**2**) has been approved in Japan for the treatment of pseudo-tuberculosis caused by *Pasteurella piscicida* and *streptococcosis* in yellowtail fish. The recommended dose is 10 mg/kg for up to one week and the drug withdrawal time is five days after cessation of treatment. Florfenicol is active in bovine respiratory disease caused by *Pasteurella* species and mastitis caused by *Staphylococci* and *Streptococci*. It is also effective in neonatal colibacillosis caused by *E. coli*. The drug is being developed worldwide by Schering-Plough Animal Health for the treatment of aquatic and bovine diseases.

3.3. Structure–Activity Relationships of 3-Fluoro-amphenicols

A number of analogues of 3-fluorochloramphenicol (**6**, R = NO_2) and florfenicol (**2**) have been synthesized and the biological activities examined. Replacement of the dichloroacetyl group by a difluoroacetyl function in both series led basically to retention of potency and the spectrum of activity of the parent structures. However, changing the difluoroacetyl to a trifluoro-or a chlorodifluoroacetyl group abolished the antimicrobial activity almost completely. Reduced level of potency was also seen when the dichloroacetyl group was changed to a chlorofluoroacetyl group. Other amide functions such as methoxyacetyl or methylsulfonylacetyl did not give

Table 6. Comparative Toxicities in Mice for Amphenicols

	Route of Administration[a]			
Compound	Oral	Ip	Sc	Iv[b]
chloramphenicol	2000	700	2450	90
thiamphenicol	>3000	>2500	>3000	370
florfenicol	>3000	1500	3000	100

[a] The vehicle employed, unless otherwise noted, was 50% propylene glycol plus 50% of a biological vehicle where complete solubility of the drugs was not attained at the concentrations employed.
[b] The vehicle employed was 25% propylene glycol plus 75% water and complete solubility was attained at the indicated concentrations.

any appreciable activity. In the florfenicol structure, changing the methylsulfonyl group to methylsulfoxide greatly reduced the potency and the methylthio analogue was practically inactive.

3.4. Mechanism of Action of Florfenicol

The inhibitory activities of chloramphenicol (**1**, R = NO_2), thiamphenicol (**1**, R = SO_2CH_3), and florfenicol (**2**) against a sensitive *E. coli* strain have been studied (36). In two different liquid media, both chloramphenicol and florfenicol allowed only 20–30% residual growth at a drug concentration of 2 mg/L, whereas a thiamphenicol concentration of 25 mg/L was required to produce a similar effect. Florfenicol was also found to be a selective inhibitor of prokaryotic cells. At concentrations of 1 mg/L chloramphenicol and florfenicol, and at a concentration of 25 mg/L, thiamphenicol, inhibited protein synthesis. Florfenicol inhibited peptidyl transferase selectively on 70S ribosomes and florfenicol, like chloramphenicol and thiamphenicol, was inactive against *E. coli* strain A19-CM which is resistant at the ribosome level. The binding site for the three amphenicols on the prokaryotic 70S ribosome is different from that on the eukaryotic 80S ribosomes which accounts for the observed selective action. Both chloramphenicol and thiamphenicol have a higher affinity than florfenicol for a common ribosomal-receptor site that represents the peptidyl transferase domain. Although florfenicol is the most potent of these three drugs as an inhibitor in a cell-free transcription system, it is least effective in inhibiting the puromycin [53-79-2] reaction which is a measure of inhibition of puromycin-induced release of ribosome-associated nascent peptides.

3.5. In Vivo Effects of Florfenicol

Comparative acute toxicities of florfenicol, chloramphenicol, and thiamphenicol in mice are given in Table 6. As can be seen, florfenicol is similar to thiamphenicol in acute toxicity by oral and subcutaneous (sc) administration, but is comparable to chloramphenicol by intraperitoneal (ip) and intravenous (iv) routes. Serum levels in mice following either a single or subcutaneous dose of 200 mg/kg of amphenicol have been determined and the results are given in Figure 4. The serum drug levels attained following oral administration were similar to those obtained by parenteral administration for all compounds. Serum levels of chloramphenicol and thiamphenicol were generally similar whereas florfenicol levels were much higher.

In rats, the observed serum drug levels were generally much lower than those seen at the same dose in mice which would seem to indicate a greater metabolic degradation of these compounds in rats. Once again, levels after oral administration were very similar to those given parenterally for all of the compounds. Serum levels attained using florfenicol were similar to those seen using thiamphenicol, however, these levels were lower after parenteral administration than after oral administration. Binding of florfenicol to serum

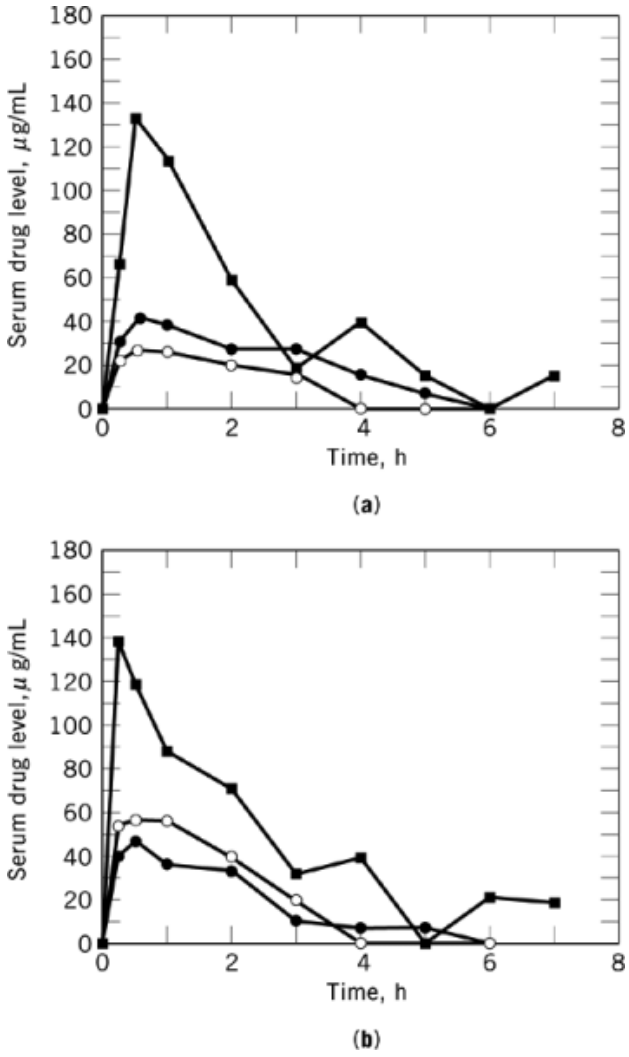


Fig. 4. Serum levels in mice of chloramphenicol (**1**, R= NO_2) •, thiamphenicol (**1**, R= CH_3SO_2) ○, and florfenicol (**2**) ■, following a single dose of 200 mg/kg given (**a**) subcutaneously, and (**b**) orally.

protein is substantially higher than thiamphenicol binding at 60% and 16%, respectively, but is similar to chloramphenicol binding which is 47%.

The efficacy of florfenicol *in vivo* was determined by measuring the dose required to obtain values for protection from infection in 50% of the animals (PD_{50}) against 10 chloramphenicol-resistant strains and two chloramphenicol-sensitive isolates. Florfenicol, chloramphenicol, and thiamphenicol were evaluated concurrently against each strain. Against sensitive *Enterobacter*, PD_{50} by the subcutaneous and oral routes were similar for florfenicol and chloramphenicol (25 mg/kg sc, 5 mg/kg oral), but higher for thiamphenicol (30 mg/kg sc, 20 mg/kg oral). A dramatic effect was seen for florfenicol against *Shigella* (3 mg/kg sc, 2 mg/kg oral) as compared to chloramphenicol and thiamphenicol (100 mg/kg by both routes). Against resistant strains of

Enterobacter, *Klebsiella*, *Providencia*, *Serratia*, *Salmonella*, and *Staphylococcus*, the PD_{50} values for florfenicol ranged from 5 to 60 mg/kg whereas chloramphenicol and thiamphenicol were practically ineffective.

4. Pharmacokinetics in Nonrodents

The pharmacokinetic disposition of florfenicol (**2**) was studied in preruminant veal calves after administration of a single 22 mg/kg dose intravenously, orally after a 12-h fast, and orally 5 min postfeeding. The disposition of florfenicol in veal calves following a single iv dose was adequately described by a two-compartment open model where there was no significant effect of the animal's age on the pharmacokinetic parameters. Calves given the oral doses had a complex absorption pattern and delayed absorption. Administering florfenicol with milk delayed the onset of absorption and therefore the time to peak concentration. The disposition of the serum concentration of florfenicol in veal calves given by either oral method could be adequately described by a one-compartment pharmacokinetic model with first-order drug absorption and first-order drug elimination (38). The bioavailability of florfenicol was significantly less when given with milk replacer than when given on an empty stomach: after a 12-h fast median bioavailability was 88% of the dose; and given 5 min postfeeding, median bioavailability of the drug was 65%.

The elimination half-life of florfenicol after a single iv dose of 22 mg/kg (138–204 min) compares well with the elimination half-life of chloramphenicol (**1**, R = NO_2) reported in cattle, except in very young calves. Half-lives of 350 min (39) and 302 min (40) in 1-week-old calves, 207 min (40) in 4-week-old calves, 210 min in 6-week-old calves (40), and 264 min (39) and 210 min (41) in adult cows have been reported. The apparent volume of distribution (V_z) for florfenicol following iv administration ranged from 0.68 to 0.84 L/kg as compared to V_z values following iv administration of chloramphenicol in calves of from 0.905 to 1.23 L/kg (39, 40). The total body clearance (CL) for florfenicol, 2.77–4.00 mL/(kg·min), is also similar to the CL of chloramphenicol, 1.9–4.03 mL/(kg·min), in calves (39, 40).

Florfenicol concentrations in tissues and body fluids of male veal calves were studied after 11 mg/kg intramuscular doses administered at 12-h intervals (42). Concentrations of florfenicol in the lungs, heart, skeletal muscle, synovia, spleen, pancreas, large intestine, and small intestine were similar to the corresponding serum concentrations indicating excellent penetration of florfenicol into these tissues. Because the florfenicol concentration in these tissues decreased over time as did the corresponding serum concentrations, it was deemed that florfenicol equilibrated rapidly between these tissues and the blood. Thus serum concentrations of florfenicol can be used as an indicator of drug concentrations in these tissues.

High florfenicol concentrations were found in the kidney, urine, bile, and small intestine of three calves and in the large intestine of one calf. High florfenicol concentrations in the kidneys and urine indicate that florfenicol may be an excellent drug for treating urinary tract infection caused by susceptible organisms. On the basis of the high concentrations of florfenicol in the bile and the good absorption of the drug after oral administration, florfenicol may undergo some degree of enterohepatic recirculation.

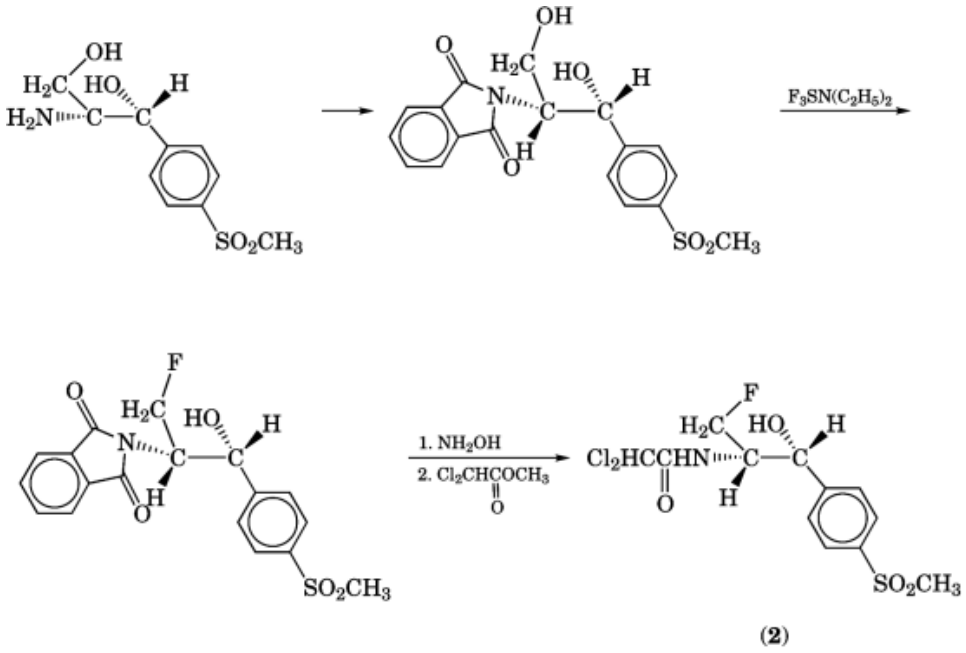
Florfenicol concentrations in the brain, cerebrospinal fluid (CSF), and aqueous humor were one-fourth to one-half the corresponding serum concentrations. Concentrations in these tissues and fluids did not decrease as rapidly, maintaining a low, but fairly constant value. Because the brain, CSF, and aqueous humour are separated from the blood by specialized barriers, florfenicol can seemingly only cross these barriers to a limited extent.

Florfenicol has a wide tissue distribution, similar to that reported for chloramphenicol in calves and thiamphenicol in humans (43, 44). Chloramphenicol attains concentrations higher than the corresponding plasma concentrations in bile and urine, as does florfenicol (43). Unlike florfenicol, chloramphenicol concentrations in the liver, kidney, spleen, and lungs are less than corresponding plasma concentrations. However, chloramphenicol penetrates the brain and CSF much better than does florfenicol, reaching values equal to plasma concentrations in the brain. The distribution of thiamphenicol into the kidney, urine, and muscles of humans

compared with corresponding plasma concentration is similar to what was observed for florfenicol in calves (44). The penetration of thiamphenicol into the CSF is much smaller than that of florfenicol in calves.

5. Synthesis

The first syntheses of florfenicol (**2**), 3-fluorochloramphenicol, (**6**, R = NO_2) and other fluoroanalogues were accomplished beginning with thiamphenicol (**1**, R = SO_2CH_3) according to the reaction sequence



(**2**)

(16, 22). Because the starting materials were optically active, the products were all pure enantiomers. Later, the synthetic scheme shown in Figure 5 was developed (22, 45). Resolution of the racemic mixture was accomplished at the penultimate stage and the optically active D-threo-amine (**7**) was converted to florfenicol (**2**). This synthetic process also resulted in the synthesis of thiamphenicol shown in Figure 6 using 1,1,2,3,3,3-hexafluoropropyl diethylamine (FPA) (46). More recently an improved method of synthesis of florfenicol has been developed (17).

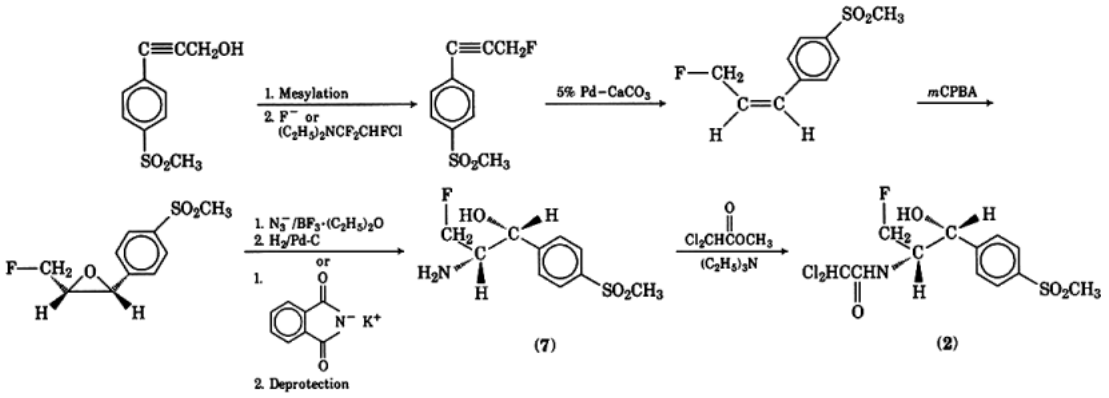
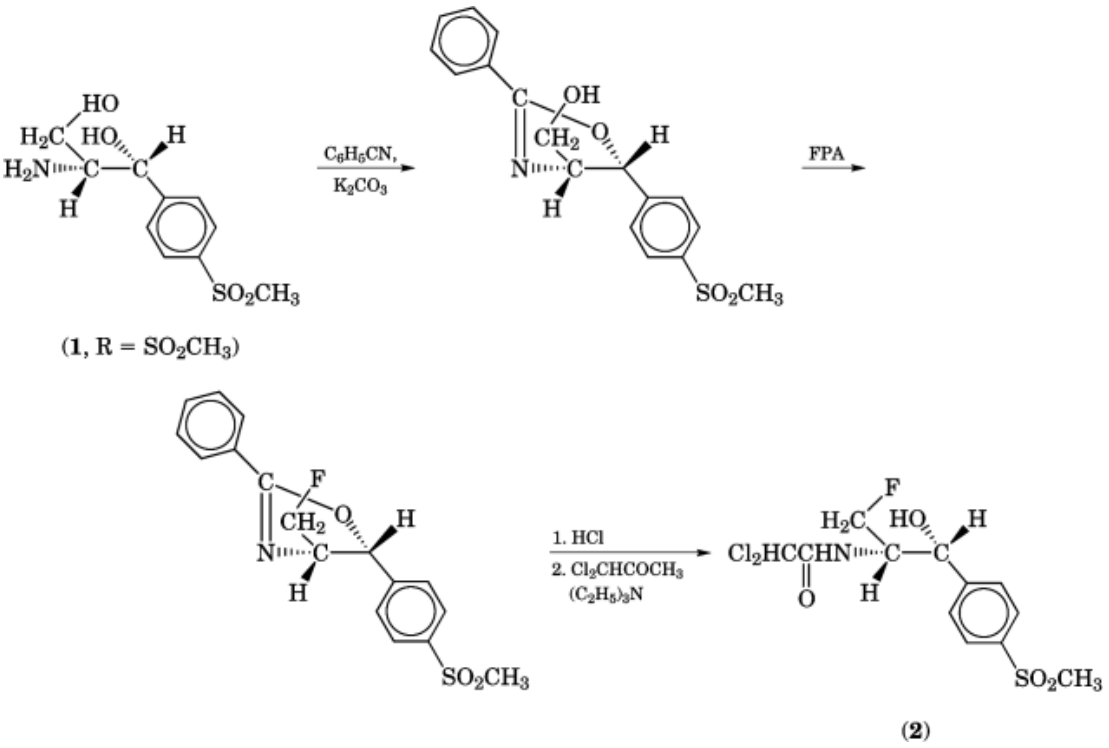


Fig. 5. Alternative synthesis of florfenicol (**2**) where *m*CPBA is *m*-chloroperbenzoic acid.



The C – H bond of the dichloracetyl group in chloramphenicol is readily oxidized by liver enzymes and the resulting oxalylchloride derivative is presumed to be implicated in chloramphenicol's toxic manifestation (48). Because such an oxidative mechanism might also be operative in bacterial systems, leading to inactivation of the antibacterial activity, the C – H bond was replaced by deuterium exchange under mildly basic conditions, in both the amphenicol and the 3-fluoroamphenicol series, to see if the rate of oxidation might be retarded. The deuterio analogues have been observed to be consistently twice as potent as the hydrogen analogues (49).

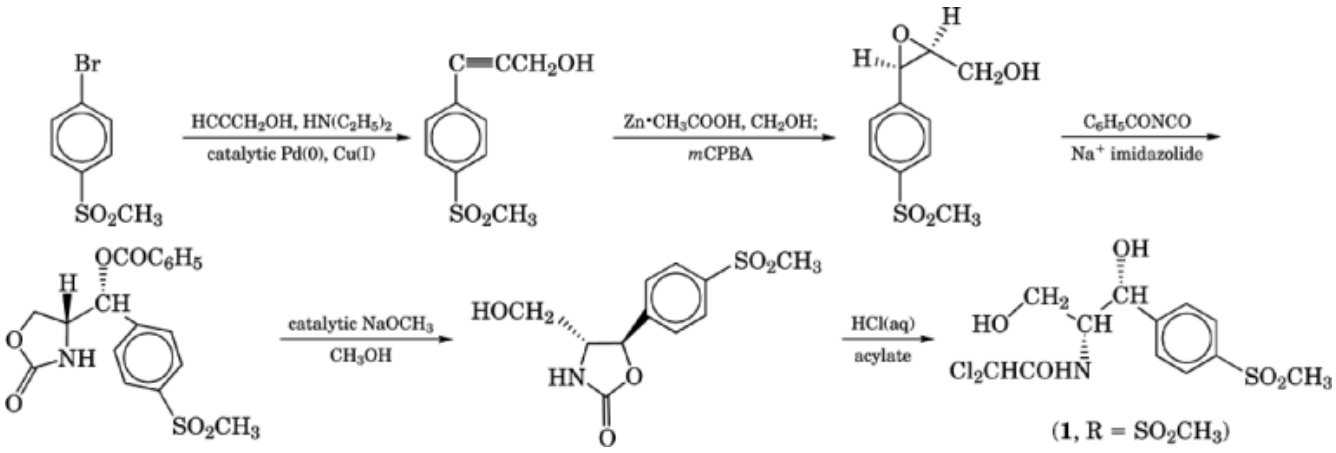


Fig. 6. Synthesis of thiamphenicol (**1**, R= SO_2CH_3) (47) where *m*CPBA is *m*-chloroperbenzoic acid.

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