

SOME ASPECTS OF THE ANTIMICROBIAL AND CHEMICAL PROPERTIES OF PHENYL
BORONATE ESTERS OF CHLORAMPHENICOL

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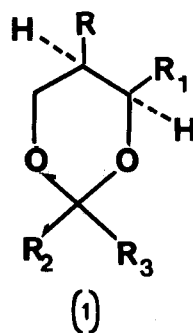
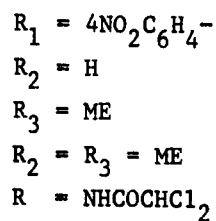
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ABSTRACT

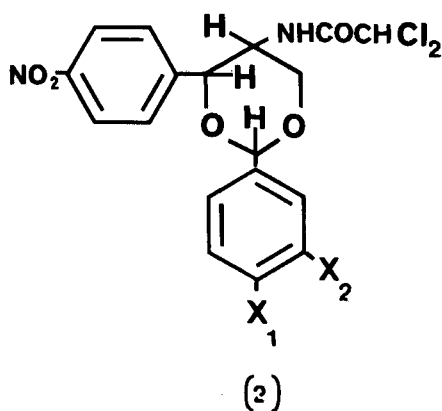
Substituted phenyl boronate esters of chloramphenicol have significant antimicrobial potency against common pathogens. The isosteric 1,3-dioxanes in which the boron atom has been replaced by a methine group are inactive under the conditions of test. The boronate esters are regarded as having the same conformation as the parent antibiotic. Photochemical degradation of these esters by simulated sunlight proceeds much faster than it does with chloramphenicol.

INTRODUCTION

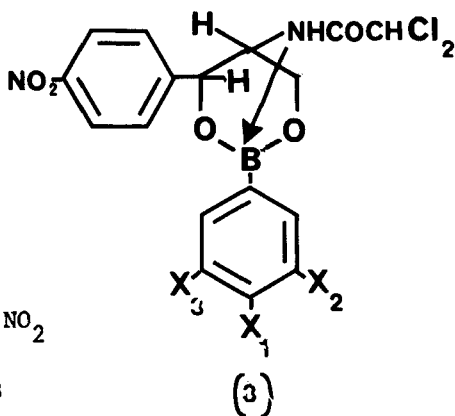
There are many reports in the literature which indicate that modification of the chloramphenicol molecule results in reduced antimicrobial potency ¹⁻⁹, the exceptions being the replacement of the side chain CHCl_2 by CF_3 ¹⁰ and changing the aromatic nitro group for a perchloryl group ¹¹. Substituted phenyl boronic acids have mild antimicrobial

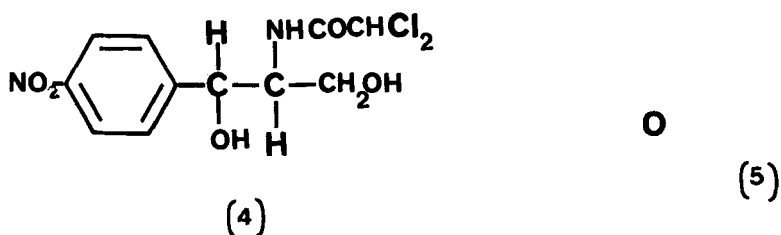


- (a) $X_1 = X_2 = \text{H}$
 (b) $X_1 = \text{H}, X_2 = \text{NO}_2$



- (a) $X_1 = X_2 = X_3 = \text{H}$
 (b) $X_1 = \text{Br}, X_2 = X_3 = \text{H}$
 (c) $X_1 = \text{H}, X_2 = \text{H}, X_3 = \text{NO}_2$
 (d) $X_1 = \text{H}, X_2 = X_3 = \text{CF}_3$





Chloramphenicol

activity against Staph. aureus¹² and patents granted for enhanced chemotherapeutic properties of phenyl boronate esters of medicinal diols^{13,14}, including the phenyl, 4-tolyl, 4-anisyl, and 1-naphthyl boronate esters of chloramphenicol¹⁵.

The objects of the present work are to show that phenyl boronate esters of chloramphenicol in which the phenyl boronate group carries an electron withdrawing substituent retain the anti-microbial potency of the parent drug and that the boron atom is essential to this activity. It is also intended to demonstrate that these esters are less stable to light than is the parent drug.

MATERIALS AND METHODS

Synthesis of Substituted Phenyl Boronate Esters of Chloramphenicol

Method A

Equimolar amounts of chloramphenicol (2.0 g., 0.00619 mol.) and each of the following:- phenyl, 4-bromophenyl, 3-nitrophenylboronic acids were reacted by the method of Irwin et. al.¹⁶.

2-(Phenyl)-4-(4'-nitrophenyl)-5-(dichloroacetamido)-1,3-dioxo-2-boracyclohexane (3a) Pale yellow crystalline solid, m.p.

172-3°. Found. C, 49.48; H, 3.73; N, 6.87; Cl, 17.44. $C_{17}H_{15}BN_2Cl_2O_5$

requires, C, 49.92; H, 3.69; N, 6.84; Cl, 17.33 %. Ir. (KBr disc)

major peaks at 3220, 1660, 1600, 860, 845, 825, 800, 745 cm^{-1} .

P.m.r. (CDCl_3 from TMS) δ 4.42 (2H, dd, $J = 4$ Hz), 4.65 (1H, d, $J = 4$ Hz), 4.85 (1H, d, $J = 4$ Hz), 5.66 (1H, s), 6.55 (1H, s^{*}), 7.40–8.40 (9H, m). Uv. (EtOH) λ_{max} 274 nm Shoulder at 218 nm, $\epsilon = 12000$. MS 70/ev, M^+ 409, 281, 258, 257, 222, 153, 146, 118, 105, 70, 43. Yield 0.45 g

2-(4'-Bromophenyl)-4-(4''-nitrophenyl)-5-(dichloroacetamido)-1,3-

dioxo-2-boracyclohexane (3b) Pale yellow crystalline solid, m.p.

175–6°. Found. C, 42.24; H, 3.02; N, 5.71; Cl, 14.49, $\text{C}_{17}\text{H}_{14}\text{BrCl}_2\text{N}_2\text{O}_5$

requires, C, 41.85; H, 2.87; N, 5.74; Cl, 14.53 %. Ir. (KBr disc)

) major peaks at 3240, 1665, 1600, 845, 820, 800, 750 cm^{-1} . P.m.r.

(CDCl_3 from TMS) δ 4.40 (2H, dd, $J = 4$ Hz), 4.66 (1H, d, $J = 4$ Hz), 4.84

(1H, d, $J = 4$ Hz), 5.65 (1H, s), 6.62 (1H, s^{*}), 7.40–8.40 (8H, m). Uv.

(buffer at pH 7.8) λ_{max} 274 nm, ϵ 13950. MS 70/ev, M^+ 487.6, 360, 334,

301, 225, 183, 153, 118, 70, 44. Yield 0.54 g.

2-(3'-Nitrophenyl)-4-(4''-nitrophenyl)-5-(dichloroacetamido)-1,3-

dioxo-2-boracyclohexane (3c) Pale yellow crystalline solid, m.p.

204–5°. Found. C, 44.71; H, 3.16; N, 9.04; Cl, 15.68, $\text{C}_{17}\text{H}_{14}\text{Cl}_2\text{N}_3\text{O}_7$

requires, C, 44.97; H, 3.06; N, 9.25; Cl, 15.62 %. Ir. (KBr disc)

major peaks at 3220, 1660, 1605, 860, 845, 830, 820, 800, 765 cm^{-1} .

P.m.r.: (d_6DMSO from TMS) δ 4.10 (2H, dd, $J = 12$ Hz), 5.18 (1H, d, $J = 12$ Hz),

9.12 (1H, s^{*}), 6.22 (1H, s), 6.52 (1H, s), 7.52–9.12 (8H, m). Uv. (H_2O)

λ_{max} 274 nm, ϵ 16900. MS 70/ev M^+ 453, 326, 268, 191, 153, 121, 70, 44.

Yield 0.41 g.

Method B

Equimolar amounts of chloramphenicol and 3,5-ditrifluoromethyl phenyl boronic acid in ethyl acetate (100 cm^3) were treated with

phosphorus pentoxide (1 g.) and the mixture shaken for 0.5 hr before being heated at 50-60° for 2 hrs. After cooling the reaction mixture was filtered and the filtrate evaporated under vacuum to dryness. The residue was washed with water several times and dried under vacuum.

2-(3',5'-Ditrifluoromethylphenyl)-4-(4'-nitrophenyl)-5-(dichloroacetamido)-1,3-dioxane-2-boracyclohexane (3d) White microcrystals

m.p. 130-3° (dec.). Found. C, 41.82; H, 2.59; N, 4.95; Cl, 12.72
 $C_{19}H_{13}BCl_2F_6N_2O_5$ requires C, 41.87; H, 2.40; N, 5.13; Cl, 13.00 %.
 Ir. (KBr disc) major peaks at 3400, 3320, 1710, 1685, 1620, 840, 800, 740 cm^{-1} . P.m.r. ($CDCl_3$ from TMS) δ 4.50 (2H, dd, J= 4Hz), 4.90 (1H, d, J= 4 Hz), 4.75 (1H, d, J= 4Hz), 5.73 (1H, s), 6.72 (1H, s*), 7.52-8.40 (7H, m). Uv. (H_2O) λ_{max} 274 nm. ϵ 9100. MS 70 e/v 417, 394, 359, 282, 153, 70, 61, 43. Yield 0.83 g.

Synthesis of 4-(4'-nitrophenyl)-5-(dichloroacetamido)-2-(aryl)-1,3-dioxanes

Chloramphenicol (2.0g., 0.00619⁴ mol.), substituted benzaldehyde (0.00619 mol.) and 4-toluene sulphonic acid (0.3 g.) were dissolved in ethyl acetate (100 cm^3) and the mixture heated under reflux in a Dean & Stake apparatus until approximately 10 cm^3 of liquid remained in the flask. The last traces of solvent were removed under vacuum and the residue treated with water (100 cm^3) and recrystallised from methanol.

4-(4'-Nitrophenyl)-5-(dichloroacetamido)-2-(phenyl)-1,3-dioxane (2a)

White microcrystals, m.p. 82-3°. Found. C, 52.15; H, 4.11; N, 6.69; Cl, 16.73. $C_{18}H_{16}Cl_2N_2O_5$ requires, C, 52.57; H, 3.92; N, 6.81; Cl, 17.24 %. Ir. (KBr disc) major peaks at 3560, 3270, 1685, 1665, 1600, 840, 820, 800, 785 cm^{-1} . P.m.r ($CDCl_3$ from TMS) 3.50 (1H, s*), 4.4

(2H,s), 4.56 (1H,d, J= 4Hz), 5.40 (1H,s), 5.74 (1H,s), 5.90 (1H,s), 7.26-8.34 (9H,m). Uv. (MeOH) $\lambda_{\max}$ 268 nm. ϵ 10,240. MS 70 e/v M^+ 411, M^{+1} 412, M^{-1} 410, 259, 241, 221, 177, 153, 105, 91, 70, 51.

Yield. 1.1 g.

4-(4'-Nitrophenyl)-5-(dichloroacetamido)-2-(3'-nitrophenyl)-1,3-dioxane (2b).

Pale yellow microcrystals m.p. 135-6°. Found. C, 47.30; H, 3.39; N, 9.25; Cl, 15.53. $C_{18}H_{15}Cl_2N_3O_7$ requires C, 47.42; H, 3.31; N, 9.21; Cl, 15.55 %. Ir (KBr disc) major peaks at 3390, 1710, 1600, 875, 850, 820, 810, 760 cm^{-1} P.m.r. ($CDCl_3$ from TMS) 3.80 (1H,s*), 4.43 (2H,s), 4.60 (1H,d, J= 4Hz), 5.45 (1H,s), 5.73 (1H,s), 5.98 (1H,s), 7.08-8.48 (8H,m). Uv. (MeOH) $\lambda_{\max}$ 266 nm. ϵ 7500. MS 70 e/v 305, 303, 220, 153, 150, 116, 105, 104, 70, 40, Yield 1.1 g.

Photochemical Degradation of Substituted Phenyl Boronate Esters of Chloramphenicol

The following substituted phenyl boronate esters of chloramphenicol were dissolved in and made up to 200 cm^3 in both Clark Lubs borate buffer (pH 7.8) and in deionized water to give concentrations:- phenyl, 0.1 %, 0.08 %, 4-bromophenyl, 0.066 % and 0.04 % respectively. These solutions were irradiated by the method of Evans et. al.¹⁷, samples were taken at time intervals and evaluated:- T.l.c method¹⁸, h.p.l.c method¹⁹. The H.p.l.c method had the following modification Compound (3 a): Flow rate, 1.6 cm^3 /minute, absorbance 0.5, Mobile phase, acetonitrile/ water (1:4). Compound (3 b) Flow rate, 2.0 cm^3 / minute, absorbance 0.2, Mobile phase, acetonitrile/water (1:1). The following retention times were recorded:- (3 a) 5.5 minutes, (3 b) 2.5 minutes.

Microbiological Evaluation of the Test Compounds

This was carried out for initial screening by the B.P. method²⁰.

Subsequent assessment of compounds which showed significant activity was carried out by the B.P. method²¹ at three concentrations (25, 50, and 100 $\mu\text{g}/\text{cm}^3$) using the cup plate assay, randomisation of experiments was achieved by means of a Latin Square test. Minimum inhibitory concentrations (MIC) were measured by Jones' method²² and screening was carried out against:- Escherichia coli (NCTC 5933), Proteus vulgaris (NCTC 4636), Bacillus subtilis (NCTC 8236), Candida albicans (NCYC 597) and Staphylococcus aureus (NCTC 7447).

MIC determinations were carried out against E. coli and Staph. aureus in deionised water. Compounds (3 a), (3 b) and (3 c) all showed 5-10 $\mu\text{g} / \text{cm}^3$ and (3 d) showed 20-40 $\mu\text{g} / \text{cm}^3$ in the MIC measurements. Chloramphenicol showed MIC values of 5-10 $\mu\text{g} / \text{cm}^3$ against Staph. aureus and 2.5-5 $\mu\text{g}/\text{cm}^3$ against E. coli.

Attempted Hydrolysis of Compounds of Types 2 and 3

The hydrolysis was carried by the method of Guven and Topaloglu²³, with the modification of the pepsin being omitted from the present work. T.l.c examination¹⁸ of the reaction mixture showed a single spot, R_f 0.36, corresponding to the starting material. Supportive evidence was obtained by testing a sample of the reaction mixture by the B.P. microbiological screening test²⁰ compounds of type 2 were inactive whilst those of type 3 were active.

Acute Toxicity Studies on Compounds of Type 3

Male Swiss mice (body weight 20-25 g.) were injected i.p. with 0.1 cm^3 of solutions containing 60, 30, 15 and 60 mg / cm^3 of compounds (3 a-

d) respectively in ethanol. Control experiments were carried out using chloramphenicol at 0.1 cm^3 dose ($60 \text{ mg} / \text{cm}^3$). A second control group of mice were injected with the same dose of ethanol. the animals were kept for several days and all survived. The control group treated with chloramphenicol died after two days.

DISCUSSION

Examination of Tables 1-4 shows that the substituted phenyl boronate esters of chloramphenicol have significant in vitro antimicrobial activity against the organisms tested. The results were evaluated by the 'ex log' programme which incorporated the results of the control experiments on chloramphenicol. Direct comparisons of potency are unrealistic since they do not account for differences in molecular weight. Molecular activity calculations indicate that the substituted phenyl boronate esters of chloramphenicol have between 57.4 to 72.8 of the potency of the parent drug, when assessed against Staph. aureas at $100 \text{ ug} / \text{cm}^3$. These boronate esters are inactive against both Pseudomonas aeruginosa and Candida albicans

Previous work ²⁴ has shown that compounds of type (1) are inactive as are those of type (2) in contrast to those of (3) which are active. The structural difference between (2) and (3) lie in the boron atom of (3) being replaced by a methine group in (2).

P.m.r spectral data of these two groups of compounds suggests that those of type (3) have a similar conformation to the D-threo form of chloramphenicol. Some supportive information is obtained from examination of molecular models. These observations agree with

those of previous workers who maintain that compounds of type (3) have a rigid structure due to bonding between the amide nitrogen atom and the boron atom¹⁵. Additional experimental work in this laboratory has shown that substituted phenyl boronic acids will not condense with 2-amino-3-(4'-nitrophenyl)-propane-1,3-diol. Recent work²⁵ has shown that under certain conditions aryl boronate derivatives act as electron donors. In the reaction with chloramphenicol, the amide nitrogen atom would act as an electron acceptor with respect to the boron atom. However, the amino group of 2-amino-3-(4'-nitrophenyl)-propane-1,3-diol could not act in this manner. It was of interest to note that the nature and positions of the substituents on the phenyl ring had no effect on the antibacterial potency of (3).

Compounds of type (3) were found to be resistant to acid hydrolysis and may be expected to be absorbed intact and so the acute toxicity studies are the more interesting and worthy of further study.

Photochemical studies on (3 a & b) showed a decomposition pattern similar to that reported for chloramphenicol under the same conditions of irradiation¹⁷⁻¹⁹. In both cases the substituted phenyl boronic acid from which the compound was made was liberated. The acids released then reacted with the sodium hydroxide in the Clark Lubs borate buffer, forming salts which act as an additional buffer system. The pH of the reaction mixture fell to 7.2 which was too alkaline to permit the condensation of the photolysis products, 4-nitrobenzaldehyde and

Table 1: - Antimicrobial activity of phenyl boronate ester of chloramphenicol in Clark Lubs borate buffer (pH 7.8) as assessed by the method of the British Pharmacopoeia²¹

Concen- tration (ug/cm ³)	Diameter of zone of Inhibition (mm)			
	E.coli	S. abony	Staph.aureas	B. subtilis (spores)
100	19, 19, 19, 20, 23	19, 20, 20, 19, 21	23, 23, 23, 23, 23	22, 22, 22, 22, 22
50	14, 15, 15, 15, 15	16, 18, 17, 16, 16	20, 20, 21, 20, 19	16, 16, 17, 17, 16
25	11, 11, 10, 11, 13	12, 14, 15, 12, 12	17, 17, 17, 17, 17	10, 11, 10, 9, 9
Chloram- phenicol (ug. cm ³)				
100	19, 20, 20, 22, 22	21, 21, 22, 22, 22	25, 24, 25, 25, 25	20, 20, 21, 21, 20
50	17, 18, 17, 17, 17	16, 17, 17, 16, 17	20, 20, 20, 20, 20	17, 18, 18, 18, 18
25	10, 11, 12, 13, 15	12, 14, 13, 13, 13	18, 18, 19, 19, 19	15, 15, 16, 16, 14

Clark Lubs borate buffer ph 7.8 assessed under the same conditions gave no zone of inhibition.
The hole diameter in the plate was 9 mm.

Analysis of results by 'Exlog' programme
a) Phenyl boronate ester of chloramphenicol, b) Chloramphenicol

Statistical parameters	E. coli		S. abony.		Staph aureas		B. subtilis (spores)	
	a	b	a	b	a	b	a	b
Mean of log x,	1.69	1.69	1.69	1.69	1.69	1.69	1.69	1.69
Mean of y	15.33	16.66	16.45	17.00	20.00	21.1	16.00	17.80
Slope m	14.61	13.95	11.29	14.28	9.96	10.2	20.2	8.63
Correlation coefficient r	0.952	0.936	0.940	0.980	0.980	0.940	0.993	0.966
Sum of square regression	193.5	176.3	115.5	184.9	90	96	372	67.5
Sum of square residual	19.73	24.9	14.1	6.03	1.99	11.63	4.83	4.83
t-value comparing the slopes	0.544		-3.61		-0.49		20.81	
Variance ratio	1.263		2.34		5.81		20.81	

Table 2: - Antimicrobial activity of 4-bromophenyl boronate ester of chloramphenicol in Clark Lubs borate buffer (ph 7.8) as assessed by the method of the British Pharmacopoeia²¹

Concentration (ug/cm ³)	Diameter of zone of Inhibition (mm)			
	E. coli	S. abony.	Staph aureus	B. subtilis (spores) P. vulgaris
100	20,19,18,21, 19	19,5,19,18,5, 19,18.5,	21,20,21,23 23	20,5,19,18 18.5,20 21,20,5,22.5, 23,21
50	17.5,17.5,17 15,16	15,14,14,15 15	19,18.5,18.5 19,20	14.5,15,15 16,15 18.5,17.5, 20.5,18,18.5
25	8,8,8,8,8	8,8,8,8,8	12.5,14,12, 14,14	8,8,8,8,8 9,9,10,12,12
Chloram- phenicol (ug/cm ³)				
100	21,22,21,22 22.5	23,23,23,22, 23.5	25,24,23,26, 26	21.5,20.5, 21,21,22 24.5,24.5,26 25,26
50	18,19.5,19 19.5,20	16,17,16,15, 16	21,20.5,20.5, 22.5,22	18,18.5,18, 18.5, 18 21,21,22,21, 21
25	13.5,15,10 11,14	12.5,12,12, 10.5,11.5	16,16,17.5, 17.5,17	12,10,8,8, 14 14,14,16,18 16

Clark Lubs borate buffer assessed under the same conditions gave no zone of inhibition.
The hole diameter in the plate was 8 mm.

Analysis of results by 'Exlog' programme

a) 4-Bromophenyl boronate ester of chloramphenicol, b) Chloramphenicol

Statistical parameters	E. coli		S. abony		Staph aureas		B.sublitis		P. vulgaria	
	a	b	a	b	a	b	a	b	a	b
Mean of log x	1.69	1.69	1.69	1.69	1.69	1.69	1.69	1.69	1.69	1.69
Mean of y	14.6	17.86	13.8	16.86	17.96	20.96	14.10	16.60	16.80	20.66
Slope m	18.9	14.94	18.10	18.60	13.78	13.28	18.60	17.93	18.93	15.94
Correlation coefficient r	0.94	0.92	0.989	0.98	0.94	0.96	0.98	0.93	0.93	0.96
Sum of square regression	324	202	297	313.6	172.2	160	313	291.6	324.9	230.4
Sum of square residual	37.9	35.2	6.3	11.13	20.5	13.23	13.0	48.0	47.0	16.43
t-value comparing slopes	2.55		-0.653		0.470		0.466		2.056	
variance ratio	1.07		1.764		1.54		3.69		2.860	

Table 3: - Antimicrobial activity of 3-nitrophenyl boronate ester of chloramphenicol in Clark Lubs borate buffer (pH 7.8) as assessed by the method of the British Pharmacopoeia 21

Concentration (ug/cm ³)	Diameter of zone of Inhibition (mm)		
	E. coli	S. abony	Staph. aureus
100	19,21,20,19,21, 20,21,21,20,20, 20,20,20,21,21	17,18,18,19,18, 17,20,18,18,17, 19,19,18,19,18	22,21,23,22,21, 23,24,22,23,22, 22,22,22,22,21
	16,16,15,15,16, 17,14,16,17,16, 16,16,15,15,17	14,13,13,14,14, 14,16,14,14,16, 14,14,12,14,14	28,26,27,30,27, 30,29,29,26,28, 29,26,28,26,27
	9, 9, 9, 9, 12, 9,11,11, 9, 9, 9, 9,11, 9,12	9, 9, 9, 9, 9, 9, 9, 11,11, 9, 9, 9, 9, 9, 9	24,23,23,26,24, 25,23,22,22,23, 25,23,24,26,24
50	21,21,22,22,22, 18,17,17,16,17, 13,14,13,14,14	19,22,22,20,20, 16,17,17,16,17, 9,13,13, 9, 9	22,20,20,21,21, 18,19,17,16,17, 22,20,20,21,21
25			
Chloram- phenicol (ug/cm ³)			
100			
50			
25			
The hole diameter in the plate was 9 mm.			

Analysis of Results by "Exlog" programme
a) 3-nitrophenyl boronate ester of chloramphenicol, b) chloramphenicol

Statistical parameters	E. coli		S. abony.		Staph. aureas		B. subtilis (spores)	
	a	b	a	b	a	b	a	b
mean of log x	1.69	1.69	1.69	1.69	1.69	1.69	1.69	1.69
mean of y	15.28	17.40	13.82	15.93	18.42	19.20	23.82	21.66
slope m	17.38	12.95	14.83	16.60	12.51	13.20	13.75	16.60
Correlation coefficient r	0.974	0.982	0.974	0.943	0.971	0.969	0.910	0.929
Sum of square regression	821.6	152.0	598.5	250.0	425.6	159.9	512.5	249.9
Sum of square residual	43.61	5.23	32.0	30.93	25.3	10.4	105.77	39.33
t value comparing slopes	4.900		-1.729		-1.00		-1.851	
Variance ratio	8.33		1.035		2.436		2.689	

Table 4:-- Antimicrobial activity of 3,5-ditrifluoromethylphenyl boronate ester of chloramphenicol in deionized water as assessed by the method of the British Pharmacopoeia 71

Concen- tration (ug/cm ³)	E. coli	S. abony	Staph aureas	B. subtilis (spores)	P. vulgaris
100	18,18,17,18, 18	20,19,17,15 16	24,27,22,25, 24	23,24,23,23, 23	19,20,19,18, 19
50	15,15,15,15, 14	16,12,14,14, 15	21,21,18,21, 19	17,19,20,18, 19	19,13,14,14, 14
25	9,9,9,9,9	10,9,11,9,11	10,14,10,12, 11	16,16,17,18, 18	12,11,11,10, 11
Chloram- phenicol (ug/cm ³)					
100	20,20,20,22, 20	22,20,22,19, 23	27,30,25,28, 29	24,25,25,24, 25	22,23,22,22, 24
50	18,18,17,19, 17	21,14,17,16 18	24,25,21,25, 23	20,20,21,20, 18	19,18,19,18, 20
25	12,15,12,15, 14	16,13,15,11, 14	16,18,16,18, 17	14,17,17,17, 17	13,12,14,13, 14

Hole diameter in the plate was 9 mm.

Analysis of results by "Exlog" programme
a) 3,5-ditrifluoromethylphenyl boronate ester of chloramphenicol
b) Chloramphenicol

Statistical parameter	E. coli.		S. abony		Staph aureas		B.bustilis (spores)		P.vulgaris	
	a	b	a	b	a	b	a	b	a	b
Mean of log x	1.69	1.69	1.69	1.69	1.69	1.69	1.69	1.69	1.69	1.69
Mean of y	13.86	17.26	13.86	17.4	18.9	22.8	19.6	20.26	14.6	18.2
Slope m	14.61	11.29	12.29	12.29	23.2	17.93	10.29	13.61	13.28	15.61
Correlation coefficient r	0.98	0.932	0.903	0.849	0.95	0.948	0.919	0.95	0.97	0.974
Sum of square regression	193.6	115.6	136.9	136.8	489.9	291.5	96.0	168.0	159.9	220.9
Sum of square residual	8.13	17.33	30.83	52.7	36.93	34.8	17.5	14.83	9.3	11.50
t value comparing slopes	3.605		1.585		3.43		-3.199		-2.79	
Variance ratio	2.131		1.709		1.06		1.17		1.23	

Table 5:- Assay of residual chloramphenicol after irradiation in various solvents. Values obtained by h.p.l.c. analysis
Mubarak et al., (1982b)

In deionized water

Irradiation Time (hours)	Residual Drug (% of original)	Residual Drug (Gramme moles/litre)	log ₁₀ of Residual Drug (Gramme moles/litre)	
0.0	100.0	0.00773	-2.11	k_a
1.0	94.0	0.00727	-2.138	
2.0	88.3	0.00683	-2.165	
4.0	80.7	0.00624	-2.204	
7.0	76.0	0.00588	-2.230	
9.5	72.5	0.00561	-2.250	
23.15	49.8	0.00385	-2.414	
29.75	44.4	0.00343	-2.463	k_b
33.5	42.2	0.00326	-2.485	
46.5	35.6	0.00275	-2.559	
57.0	30.9	0.00239	-2.621	
71.15	25.2	0.00195	-2.709	
79.15	24.6	0.00190	-2.720	
94.75	18.2	0.00140	-2.851	
144.5	7.25	0.00056	-3.250	

Linear line regression analysis						first order reaction rate K		
Regression analysis	C/T	Log C/ log T	Log C/T			$k_a + k_b$	k_a	k_b
			a+b	a	b			
slope(m) ^d	-642.8	-391.39	-7.45	-12.41	-6.729	17.15	28.58	15.47
Intercept(c)	78.48	-1.997	-2.17	-2.133	-2.239			
Corr.coeff.								
(r)	-0.903	-0.893	-0.991	-0.989	-0.993			
standard deviation	12.90	0.142	0.0412	0.0143	0.0291			

C	=	Residual chloramphenicol percent of original
T	=	Irradiation time in hours
log C	=	Log percent of residual chloramphenicol (grammoles/litre)
d	=	All values must be multiplied by 10 ⁻³ units:hr ⁻¹
k	=	2.303 x -slope (hr ⁻¹)
t ₅₀	=	Time for 50% degradation of chloramphenicol
k _a	=	The primary reaction rate
k _b	=	The secondary reaction rate
k _a +k _b	=	The overall reaction rate

Table 5 (continued)

In Clark Lubs borate buffer (pH 7.8)

Irradiation Time (hours)	Residual Drug (% of original)	Residual Drug (Gramme moles/ litre)	log ₁₀ of Residual Drug Gramme moles/litre)
0.0	100	0.00773	-2.111
2.0	88.4	0.00684	-2.165
3.5	79.5	0.00615	-2.211
12.5	60.5	0.00468	-2.329
18.0	58.4	0.00452	-2.345
22.0	41.3	0.00319	-2.495
37.0	38.0	0.00294	-2.531
45.0	32.6	0.00252	-2.598
54.0	27.8	0.00215	-2.667
69.0	24.7	0.00191	-2.718
93.0	16.8	0.00130	-2.886

Linear line regression analysis						first order reaction rate k		
Regression analysis	C/T	LogC/ logT	Log C/T			k _a +k _b	k _a	k _b
			a+b	a	b			
slope(m) ^d	-824.76	-361.0	-7.97	-14.94	-5.63	18.35	34.40	12.96
Intercept (c)	78.32	-2.014	-2.20	-2.131	-2.348			
Corr.coeff. (r)	-0.897	-0.929	-0.971	-0.971	-0.990			
standard deviation	12.287	0.0941	0.0584	0.033	0.0197			

Table 6:- Assay of the residual phenylboronate ester of chloramphenicol
(0.08% w/v) irradiated in deionized water

Irradiation Time (hours)	Residual Drug (% of original)	Residual Drug (Gramme moles/ litre)	log ₁₀ of Residual Drug (Gramme moles/litre)	
0.0	100	0.00195	-2.708	k _a
1.0	92.3	0.00180	-2.743	
4.0	74.9	0.00146	-2.834	
6.0	54.0	0.00106	-2.976	
7.5	50.1	0.000979	-3.008	
21.45	23.6	0.000462	-3.335	k _b
25.0	20.3	0.000397	-3.401	
29.0	16.9	0.000330	-3.480	
45.45	9.0	0.000176	-3.754	

Linear line regression analysis				first order reaction rate k		
Regression analysis	C/T	log C/ log T	$\frac{\log C/T}{a+b}$	$\frac{\log C/T}{a}$	$\frac{k + k_b}{a}$	k_b
slope (m) ^d	-1971.0	-568.0	-23.27	-29.0	-17.34	39.93
Intercept (c)	79.54	-2.626	-2.77	-2.74	-2.968	
Corr.Coeff. (r)	-0.902	-0.953	-0.986	-0.982	-0.999	
standard deviation	14.69	0.1106	0.0597	0.0431	0.0061	

Table 7:— Assay of the residual phenylboronate ester of chloramphenicol
(0.1% w/v) irradiated in Clark Lubs borate buffer (pH 7.8)

Irradiation Time (hours)	Residual Drug (% of original)	Residual Drug (Gramme moles/ litre)	$\log_{10}$ of Residual Drug (Gramme moles/litre)
0.0	100	0.00244	-2.611
1.0	66.9	0.00163	-2.786
3.0	35.3	0.000863	-3.063
5.0	20.7	0.000506	-3.295
6.4	16.0	0.000391	-3.406

 k_a k_b

Linear line regression analysis				first order reaction rate K			
Regression analysis	C/T	Log C/	Log C/T		$k_a + k_b$	k_a	k_b
			a+b	a b			
slope (m) ^d	-12517.9	-861.79	-124.5	-148.92	-101.91	286.72	342.96
Intercept (c)	86.33	-2.69	-2.648	-2.62	-2.765		
Corr.Coeff. (r)	-0.946	-0.980	-0.994	-0.998	-0.995		
standard deviation	11.41	0.0590	0.0366	0.0137	0.0173		

Table 8:- Assay of the residual 4-bromophenyl boronate ester of chloramphenicol
(0.066% w/v) irradiated in Clark Lubs borate buffer (pH 7.8)

Irradiation Time (hours)	Residual Drug (% of original)	Residual Drug (Gramme moles/ litre)	$\log_{10}$ of Residual Drug (Gramme moles/litre)	
0.0	100	0.00135	-2.869	k_a
1.0	49.2	0.00067	-3.173	
3.15	17.8	0.00024	-3.619	k_b
5.15	10.5	0.00014	-3.853	

Linear line regression analysis				first order reaction rate k			
Regression analysis	C/T	Log C/	Log C/T		$k_a + k_b$	k_a	k_b
			a+b	$\frac{a}{b}$			
slope (m) ^d	-16029.38	-1177.87	-189.3	-233.22	-117.0	435.95	269.45
Intercept (c)	81.61	-3.022	-2.938	-2.897	-3.25		
Corr.Coeff. (r)	-0.903	-0.959	-0.984	-0.995	-1.0		
standard deviation	17.44	0.1243	-0.0766	0.0372	0		

Table 9:— Assay of the residual 4-bromophenyl boronate ester of chloramphenicol
(0.04% w/v) irradiated in deionized water

Irradiation Time (hours)	Residual Drug (% of original)	Residual Drug (Gramme moles/litre)	log ₁₀ of Residual Drug (Gramme moles/litre)	
0.0	100	0.000821	-3.085	k _a
1.0	89.6	0.000735	-3.133	
4.0	76.3	0.000626	-3.202	
7.3	47.4	0.000389	-3.409	k _b
21.45	34.1	0.000280	-3.552	
25.0	25.5	0.000209	-3.678	

Linear line regression analysis				first order reaction rate k			
Regression analysis	C/T	Log C/ log T	Log C/T		$k_a + k_b$	k_a	k_b
			a + b	a b			
slope (m) ^d	-2657.23	-377.63	-21.64	-42.4	-13.74	49.83	97.64
Intercept (c)	88.168	-3.079	-3.13	-3.076	-3.30		31.64
Corr.Coeff. (r)	-0.930	-0.965	-0.966	-0.977	-0.956		
standard deviation	11.29	0.0629	0.0617	0.0302	-0.039		

4-nitrosobenzoic acid to give the brown precipitate of 4,4'-azoxy benzoic acid. The precipitate is the usual visible indication of photolysis of chloramphenicol¹⁸. Cognate experiments in de-ionized water resulted in the formation of the brown precipitate and the pH had fallen to 2.7. It was also found that the liberated substituted phenyl boronic acids as shown by t.l.c. (Rf 0.60) act as catalysts for the photochemical degradation of (3 a & b). There was no evidence to indicate that chloramphenicol was liberated in these photochemical reactions.

Examination of tables 5- 9 shows that the rate of photolysis of (3 a & b) was much faster than that of chloramphenicol. Mass spectral fragmentation studies suggest that (5) was produced, this supports the findings of Longstaff and Rose²⁶ who examined substituted phenyl boronate esters of 1,2 and 1,3-diols. It is probable that (5) would act as a sensitizer for the photochemical degradation of (3). The observation that the rate of photolysis in Clark Lubs borate buffer was much faster than in deionized water may be explained by the proposal that the alkaline buffer could facilitate the formation of hydroxyl radicals, which may be regarded as the primary photochemical reagent. The difference in photolysis rates between 3 a and 3 b could be attributed to the poor solubility of the latter in both Clark Lubs borate buffer and in deionized water.

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