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

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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 The isosteric 1,3-dioxanes in which the boron atom has been replaced by a methine group are inactive under the conditions of test.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 activity against *Staph. aureas* [12] and patents granted for enhanced chemotherapeutic properties of phenyl boronate esters of



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medicinal diols [13,14], including the phenyl, 4-tolyl, 4-anisyl, and 1-naphthyl boronate esters of chloramphenicol[15].

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 antimicrobial 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 Chloramphnicol

### 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-dioxa-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) $\delta$ 4.42 (2H, dd, J= 4 Hz), 4.65 (1H, d, J= 4Hz), 4.85 (1H, d, J= 4Hz), 5.66 (1H,s), 6.55 (1H,s*), 7.40-8.40(9H, m). Uv. (EtOH) $\lambda$ max 274 nm Shoulder at 218 nm, $\varepsilon$ = 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-dioxa-2-boracyclohexane (3 b) Pale yellow crystalline solid, m.p. 175-6°. Found. C, 42.24; H, 3.02; N, 5.71; Cl, 14.49, $C_{17}H_{14}B\,Br\,Cl_2$

$N_2O_5$ 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) $\delta$ 4.40 (2H, dd, J= 4Hz), 4.66 (1H, d, J= 4Hz), 4.84 (1H, d, J= 4Hz), 5.65 (1H, s), 6.62 (1H, s*), 7.40-8.40 (8H,m). Uv. (buffer at pH 7.8) $\lambda$ 274 nm, $\varepsilon$ 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-dioxa-2-boroacyclohexane (3c) Pale yellow crystalline solid, m.p. 204-5°. Found. C, 44.71; H, 3.16; N, 9.04; Cl, 15.68, $C_{17}H_{14}BCl_2N_3O_7$ 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. ($d_6$DMSO from TMS) $\delta$ 4.10 (2H, dd, J=12Hz), 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. ($H_2O$) $\lambda$ max 274 nm, $\varepsilon$ 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 wahed with water several times and dried under vacuum.

2-(3',5'-Ditrifluoromethylphenyl)-4-(4''-nitrophenyl)-5-(dichloro) acetamido)-1,3-dioxa-2-boracyclohexane (3d) White microcyrstals 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) $\delta$ 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. ($H_2O$) $\lambda$ max 274 nm. $\varepsilon$ 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., 0.00619 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. $\varepsilon$ 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

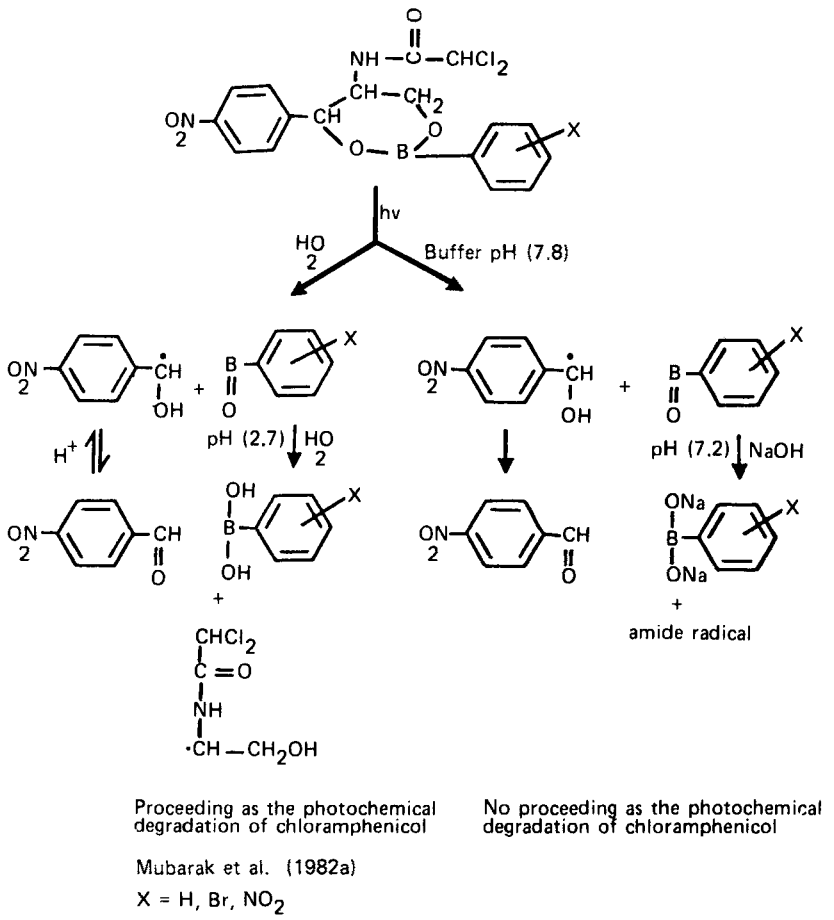
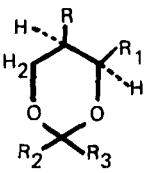



Figure - Proposed photochemical degredation of substituted phenyl boronate esters of chloramphenicol in deionized water and in Clark Lubs borate buffer pH (7.8)

(1H,s), 7.08-8.48 (8H,m). Uv. (MeOH) $\lambda$ max 266 nm. $\varepsilon$ 7500. MS 70 e/v 305, 303, 220, 153, 150, 116, 105, 104, 70, 40, . Yield 1.1 g.

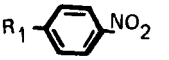
Photochemical Degradation of Substituted Phenyl Boronate Esters of Chloramphenicol

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:-

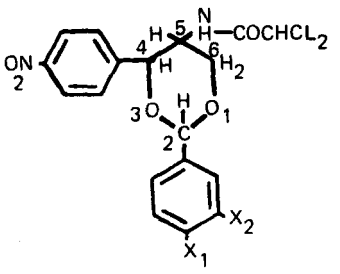


(1)

$R_2$ = H; $R_3$ = Me
$R_2$ = $R_3$ = Me
$R_3$ = $CH_2N(CH_3)_2$

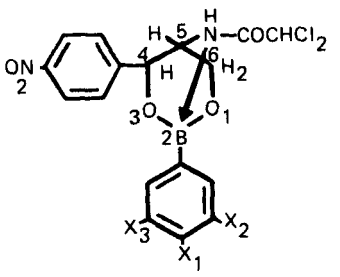


R = $NHCOCHCl_2$



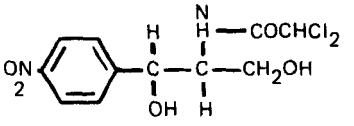
(2)

a: $X_1$ = $X_2$ = H
b: $X_1$ = H; $X_2$ = $NO_2$

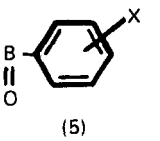


(3)

a: $x_1$ = $x_2$ = $x_3$ = H
b: $x_1$ = Br; $x_2$ = $x_3$ = H
c: $x_1$ = $x_2$ $x_2$ = H; $x_3$ $NO_2$
d: $x_1$ = H; $x_2$ = $x_3$ = $CF_3$



(4)



(5)

D (-) Threo-1-(4-nitrophenyl) -2-dichloracetamide, 1,3 - propandiol

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 method[18], h.p.l.c method [19]. 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. method[20]. Subsequent assessment of compounds which showed significant activity was carried out by the B.P. method [21] at three concentrations (25,50, and 100 ug/$cm^3$) 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' method [22] and screening was carried out against:- Escherichia coli (NCTC5933) Proteus vulgaris (NCTC 4636), Bacillus substilis (NCTC 8236), Candida albicans (NCYC 597) and Staphylococcus aureas (NCTC 7447). MIC determinations were carried out against E.coli and Staph. aureas in deionised water. Compounds (3 a), (3 b) and (3 c) all showed 5-10 ug / $cm^3$ and (3 d) showed 20-40 ug / $cm^3$ in the MIC measure--ments. Chloramphenicol showed MIC values of 5-10 ug/ $cm^3$ against Staph. aureas and 2.5-5 ug/$cm^3$ against E. coli.

Attempted Hydrolysis of Compounds of Types 2 and 3

The hydrolysis was carried by the method of Guven and Topaloghu[23], with the modifiaction of the pepsin being ommited from the present work. T.l.c examination [18] of the reaction mixture showed a single spot, Rf 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 test[20] 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 mg / $cm^3$). A second cont--rol 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 chloramphen--icol have between 57.4 to 72.8 of the potency of the parent drug, when assessed against Staph. aureas at 100 ug/ $cm^3$. 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)

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 Pharmacopoeia [21]

| Concentration ($\mu g/cm^3$) | 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 |
| Chloramphenicol ($\mu g.cm^3$) | | | | |
| 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. sublitis (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 | 16.46 | 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 Pharmacopoeia [11]

| Concentration ($\mu g/cm^3$) | 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 | 9,8,10,12,12 |
| Chloramphenicol ($\mu g/cm^3$) | | | | | |
| 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. 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 | 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 ($\mu g/cm^3$) | Diameter of zone of Inhibition (mm) | | | |
|---|---|---|---|---|
| | E.coli | S. abony | Staph. aureas | B. subtilis (spores) |
| 100 | 19,21,20,19,21,<br>20,21,21,20,20,<br>20,20,20,21,21 | 17,18,18,19,18,<br>17,20,18,18,17,<br>19,19,18,19,18 | 22,21,23,22,21,<br>23,24,22,23,22,<br>22,22,22,22,21 | 28,26,27,30,27,<br>30,29,29,26,28,<br>29,27,28,26,27 |
| 50 | 16,16,15,15,16,<br>17,14,16,17,16,<br>16,16,15,15,17 | 14,13,13,14,14,<br>14,16,14,14,16,<br>14,14,12,14,14 | 18,18,18,18,18,<br>18,19,19,20,20,<br>19,18,18,18,19 | 24,23,23,26,24,<br>25,23,22,22,23,<br>25,23,24,26,24 |
| 25 | 9, 9, 9, 9,12,<br>9,11,11, 9, 9,<br>9, 9,11, 9,12 | 9, 9, 9, 9, 9,<br>9, 9,11,11, 9,<br>9, 9, 9, 9, 9 | 15,14,14,15,15,<br>13,14,15,14,15,<br>15,14,15,15,16 | 22,20,20,21,21,<br>18,19,17,16,17,<br>22,20,20,21,21 |
| Chloramphenicol ($\mu g/cm^3$) | | | | |
| 100 | 21,21,22,22,22 | 19,22,22,20,20 | 24,24,23,23,24 | 29,29,28,25,25 |
| 50 | 18,17,17,16,17 | 16,17,17,16,17 | 19,19,18,18,18 | 20,21,23,19,20 |
| 25 | 13,14,13,14,14 | 9,13,13, 9, 9 | 17,16,15,15,15 | 17,19,17,16,17 |

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. sublitis (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 [21]

| Concentration ($\mu g/cm^3$) | 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 |
| Chloramphenicol ($\mu g/cm^3$) | | | | | |
| 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.subtilis (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 | 3.13 | 17.33 | 30.33 | 52.7 | 36.93 | 34.3 | 17.5 | 14.33 | 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 | |

have a rigid structure due to bonding between the amide nitrogen atom and the boron atom[15]. 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 work[25] 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 irradiation [17-19]. In both cases the subst--ituted 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 4-nitrosobenzoic acid to give the brown precipitate of 4,4'-azoxy benzoic acid. The precipitate is the usual visible indication of photolysis of chloramphenicol[18]. Cognate experiments in de-

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 | |
| 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 | $k_a$ |
| 9.5 | 72.5 | 0.00561 | -2.250 | |
| 23.15 | 49.8 | 0.00385 | -2.414 | |
| 29.75 | 44.4 | 0.00343 | -2.463 | |
| 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 | $k_b$ |
| 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 | -1.397 | -2.17 | -2.133 | -2.239 | | | |
| Corr.Coeff. (r) | -0.303 | -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 | $k_a$ |
| 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 | $k_b$ |
| 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 | Log C/ log T | 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 | | | |

-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.

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 | |
| 1.0 | 92.3 | 0.00180 | -2.743 | |
| 4.0 | 74.9 | 0.00146 | -2.834 | $k_a$ |
| 6.0 | 54.0 | 0.00106 | -2.976 | |
| 7.5 | 50.1 | 0.000979 | -3.008 | |
| 21.45 | 23.6 | 0.00462 | -3.335 | |
| 25.0 | 20.3 | 0.000397 | -3.401 | $k_b$ |
| 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 | Log C/T | | | $k_a + k_b$ | $k_a$ | $k_b$ |
| | | | a + b | a | b | | | |
| slope (m) [d] | -1971.0 | -568.0 | -23.27 | -29.0 | -17.34 | 53.59 | 66.78 | 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 | $k_a$ |
| 3.0 | 35.3 | 0.000863 | -3.063 | |
| 5.0 | 20.7 | 0.000506 | -3.295 | $k_b$ |
| 6.4 | 16.0 | 0.000391 | -3.406 | |

| 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] | -12517.9 | -861.79 | -124.5 | -148.92 | -101.91 | 286.72 | 342.96 | 234.69 |
| 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 | | | |

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 Rose[26] 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

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 | 0.00139 | -2.869 | $k_a$ |
| 1.0 | 49.2 | 0.00067 | -3.173 | $k_a$ |
| 3.15 | 17.8 | 0.00024 | -3.619 | $k_b$ |
| 5.15 | 10.4 | 0.00014 | -3.853 | $k_b$ |

| 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 | -16029.38 | -1177.87 | -189.3 | -233.22 | -117.0 | 435.95 | 537.10 | 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 | | | |

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.

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 | |
| 1.0 | 89.6 | 0.000735 | -3.133 | $k_a$ |
| 4.0 | 76.3 | 0.000626 | -3.202 | |
| 7.3 | 47.4 | 0.000389 | -3.409 | |
| 21.45 | 34.1 | 0.000280 | -3.552 | $k_b$ |
| 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 | 31.64 |
| Intercept(c) | 88.168 | -3.079 | -3.13 | -3.076 | -3.30 | | | |
| 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 | | | |

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