

**INVESTIGATION OF THE ANTIBACTERIAL ACTIVITY OF 3-O-OCTANOYL(-)-
EPICATECHIN**

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Abbreviated running title

Activity of 3-O-oct(-)-epicatechin

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ABSTRACT

Aims: To measure antibacterial activity of the semi-synthetic flavonoid 3-*O*-octanoyl(-)-epicatechin and investigate the mechanism of action.

Methods and results: MICs determined by the broth microdilution method were 50 µg ml⁻¹ for β-lactam sensitive and resistant *Staphylococcus aureus*, and 100 µg ml⁻¹ for vancomycin sensitive and resistant enterococci. In time-kill studies, 100 µg ml⁻¹ 3-*O*-octanoyl(-)-epicatechin reduced colony forming unit numbers of antibiotic sensitive and methicillin resistant *Staph. aureus* below detectable levels within 120 min. Bacterial aggregation was not observed when 3-*O*-octanoyl(-)-epicatechin treated cells were examined by light microscopy. It was also shown that 50 µg ml⁻¹ 3-*O*-octanoyl(-)-epicatechin is capable of reducing colony forming unit numbers of high cell density *Staph. aureus* populations by 80-fold within 60 min incubation, and inducing leakage of 50 % of their internal potassium within just 10 min.

Conclusions: 3-*O*-octanoyl(-)-epicatechin is active against Gram positive bacteria, has bactericidal activity against both antibiotic sensitive and resistant strains, and is likely to exert its primary antibacterial effect by damaging the cytoplasmic membrane.

Significance and impact of study: 3-*O*-octanoyl(-)-epicatechin has significant antibacterial activity and additional structural modification and / or formulation studies may allow this to be potentiated.

Keywords

flavonoid; flavan-3-ol; catechin; octanoyl; antibacterial; bactericidal; cytoplasmic membrane

INTRODUCTION

The emergence and spread of antibiotic resistant bacteria such as vancomycin resistant enterococci (VRE) and methicillin resistant *Staphylococcus aureus* (MRSA) is a cause for serious concern (Croft et al. 2007). MRSA, in particular, is being seen with increasing prevalence in many parts of the world (Gould 2005), and the recent discovery of vancomycin resistance among such strains (Schwaber et al. 2003) is a stark reminder of the almost limitless capacity that bacteria have for adaptation and survival. In the past, medicine and science were able to stay one step ahead of the resistance problem through the discovery of potent new classes of antimicrobial agent, but this process has slowed to a virtual standstill since the 1970s and the pipeline of new drugs is running dry (IDSA 2004; Croft et al. 2007). Given that it takes between 12 and 15 years for the development of new agents (Watkins 2002), considerable efforts in this area of research are now urgently required.

In a recent report by the World Health Organization (WHO), it was estimated that 25 % of modern medicines have been derived from plants that were first used in traditional medicine (WHO 2003). Flavonoids are a group of phytochemicals with a rich history of use in the treatment of human diseases, and are increasingly becoming the subject of medical research (Havsteen 2002; Cushnie & Lamb 2005a). Compounds of the flavan-3-ol (or catechin) class are found at high concentrations in woody plants such as *Camellia sinensis* (Japanese green tea; Daniel 2005; Taylor et al. 2005) and *Elaeagnus glabra* Thunb. (an Okinawan shrub used in medicine; Nishino et al. 1987), and have been reported to possess antibacterial activity on numerous occasions (Taguri et al. 2004; Taylor et al. 2005; Friedman 2007). Several research groups have postulated that the mechanism of action underlying this activity involves an interaction between the catechins and the cytoplasmic membrane (Ikigai et al. 1993; Tsuchiya 1999; Caturla et al. 2003).

The incorporation of fatty acid hydrocarbon chains into certain compounds is known to increase their propensity to interact with lipid bilayers, and in the past this strategy has successfully been used to increase the antibacterial activity of the human peptide, cathepsin G (Mak et al. 2003). Working on the hypothesis that catechins target at the bacterial membrane,

and with the intention of improving the ability of catechins to interact with this cell structure, a number of 3-*O*-acyl derivatives were synthesised. Subsequent analysis indicated that 3-*O*-octanoyl-(-)-epicatechin (Fig. 1) has superior anti-MRSA activity than the naturally occurring flavonoid (-)-epicatechin gallate, and it was suggested that this acyl derivative and some of its structural relatives may be useful as topical therapeutic agents (Stapleton et al. 2004). The purpose of the present investigation was to ascertain the range of bacteria against which 3-*O*-octanoyl-(-)-epicatechin is effective. In addition, time-kill assays and aggregation studies were performed to determine whether or not the agent has bactericidal properties, and potassium loss assays were conducted to establish cytoplasmic membrane damage as the primary antibacterial mechanism of action.

MATERIALS AND METHODS

Chemicals

Cephalosporin C (potassium salt; ~90 %), dimethylsulphoxide (DMSO), (-)-epicatechin, novobiocin (sodium salt; minimum 90 %), phosphate buffered saline (PBS; pH 7.4) tablets, penicillin G and sodium octanoate were obtained from Sigma (Sigma-Aldrich Co. Ltd., Poole, UK). In addition, D-glucose monohydrate and sodium chloride (general purpose grade) were from Fisher Scientific UK Ltd. (Loughborough, UK), ethanol was from Hayman Ltd. (Witham, UK), Iso-sensitest nutrient broth and agar were from Oxoid Ltd. (Basingstoke, UK), and vancomycin hydrochloride hydrate and galangin were from Aldrich (Sigma-Aldrich Co. Ltd., Gillingham, UK).

Bacteria

Strains of bacteria (listed in Table 1 along with corresponding phenotype) were obtained from the National Collection of Type Cultures (NCTC; London, UK), the American Type Culture Collection (ATCC; Manassas, Virginia, USA) and the National Collections of Industrial, Food and Marine Bacteria (NCIMB; Aberdeen, UK). At least one antibiotic sensitive and two antibiotic resistant strains of each species were selected for testing, and these were sub-

cultured and maintained on agar as described previously (Cushnie et al. 2003). All of the bacteria used in experiments in the present investigation were prepared in broth, harvested after 18 hours incubation at 37 °C, and washed and resuspended in aqueous 0.9 % (w/v) sodium chloride (Cushnie et al. 2003).

MIC determinations using the broth microdilution assay

Assays were performed in 96-well microtitre trays (gamma-irradiated; Bibby Sterilin Ltd., Stone, UK) with an inoculum density of $\sim 5 \times 10^5$ CFU ml⁻¹ in 100 µL of broth. In the experiments carried out to assess activity of the catechins and sodium octanoate, positive control wells containing broth supplemented with solvent only [1% (v/v) ethanol] were included. All commercial antibacterial agents were dissolved in sterile distilled water without the aid of additional solvents. Negative control wells containing an uninoculated dilution series of test agent and broth were included in all experiments. MICs were obtained after trays had been incubated at 37°C for 18 hours (Mark II proportional temperature controller; LEEC Ltd., Nottingham, UK). Experiments were performed in duplicate to confirm the reproducibility of results.

Time-kill studies

Two control flasks were prepared by pipetting either 20 ml broth or 20 ml broth supplemented with 1 % (v/v) ethanol into sterile glass flasks. A third sterile glass flask, containing 20 ml broth supplemented with the desired concentration of 3-*O*-octanoyl(-)-epicatechin and 1 % (v/v) ethanol, was prepared after bacterial harvest, washing and enumeration. Flasks were then inoculated so as to contain $\sim 5 \times 10^5$ CFU ml⁻¹ of test bacterium and incubated at 37°C in an orbital incubator set at 100 rpm (IOX400.XX2.C; Sanyo Gallenkamp PLC, Loughborough, UK). Colony forming unit counts were performed for each suspension after various time intervals by removing 0.1 ml samples, preparing a dilution series in PBS (10⁻¹ to 10⁻⁹) and plating out this dilution series (ten 20 µl drops per dilution) on agar. Experiments were performed in duplicate to confirm the reproducibility of results.

Examination of 3-*O*-octanoyl(-)-epicatechin treated bacteria for aggregation

In this section of work, 3-*O*-octanoyl(-)-epicatechin treated *Staph. aureus* cells were examined by light microscopy to establish if results from time-kill studies were attributable to bacterial killing, bacterial aggregation or a combination of both. Preparatory experiments involved performing time-kill assays with $\sim 5 \times 10^7$ CFU ml⁻¹ *Staph. aureus* NCTC 6571 and 3-*O*-octanoyl(-)-epicatechin to identify a suitable concentration(s) of agent and an appropriate treatment time. Untreated and 3-*O*-octanoyl(-)-epicatechin treated samples were then examined by standard brightfield light microscopy (1000x magnification) as described previously (Cushnie et al. 2007), observations were made and representative photographs were taken (Leitz DMR microscope and DMRD camera; Leica UK Ltd., Milton Keynes, UK). Samples treated with MIC levels of the flavonoid galangin were also examined for comparative purposes.

Detection of cytoplasmic membrane damage in 3-*O*-octanoyl(-)-epicatechin treated bacteria

Potassium leakage was identified as an appropriate means of examining 3-*O*-octanoyl(-)-epicatechin treated bacteria for cytoplasmic membrane damage, as this is one of the first signs of an increase in bacterial membrane permeability, and because potassium leakage can be readily measured over time. In order to identify a suitable concentration of 3-*O*-octanoyl(-)-epicatechin with which to treat and examine *Staph. aureus* cells for potassium leakage, time-kill studies were performed with the semi-synthetic flavonoid and $\sim 1 \times 10^9$ CFU ml⁻¹ *Staph. aureus* NCTC 6571. This involved preparing two sterile glass flasks with 18 ml unsupplemented iso-osmotic [1.96 % (v/v)] DMSO and 18 ml iso-osmotic DMSO supplemented with 3-*O*-octanoyl(-)-epicatechin, inoculating each of these with 2 ml of $\sim 1 \times 10^{10}$ CFU ml⁻¹ *Staph. aureus* NCTC 6571, incubating at 37°C and 100 rpm, and performing colony forming unit counts. Experiments were repeated to verify the reproducibility of results.

The effect of 3-*O*-octanoyl(-)-epicatechin upon potassium loss from populations of $\sim 1 \times 10^9$ CFU ml⁻¹ *Staph. aureus* NCTC 6571 was then investigated. The effects of MIC levels of novobiocin (62.5 ng ml⁻¹) and penicillin G (15.6 ng ml⁻¹), determined using the broth microdilution method, were also examined for comparative purposes. Assays were performed by preparing two sterile polymethylpentene flasks containing 27 ml iso-osmotic DMSO, inoculating these with 2 ml of $\sim 1.6 \times 10^{10}$ CFU ml⁻¹ *Staph. aureus*, shaking the flasks to evenly distribute bacteria and removing two 1 ml samples from each for processing as described previously (Cushnie and Lamb 2005b). Three ml of test agent (dissolved in iso-osmotic DMSO and at ten times the desired final concentration) was then added to the first flask, and 3 ml of unsupplemented iso-osmotic DMSO was added to the second flask, before mixing and removal of another two 1 ml samples for processing. At this point, both flasks were placed into a shaking water bath (Clifton Model NE5-28D; Nickel-Electro Ltd., Weston-super-Mare, UK) set to a temperature of 37°C and a speed of 400 (arbitrary units). Two 1 ml samples were removed from each flask every 10 min for 60 min. In order to determine the total potassium pool, flasks were then boiled for 30 min and four 1 ml samples were removed for processing. After all samples had been collected, extracellular potassium concentrations were measured by flame atomic emission spectrophotometry (Model AA3100; Perkin-Elmer Ltd., Beaconsfield, UK) and graphs of percentage potassium loss versus time were plotted. Each assay was performed in duplicate to verify the reproducibility of results.

RESULTS

MIC determinations using the broth microdilution assay

(-)-Epicatechin showed no activity against any of the eighteen test strains when used at concentrations up to and including 200 µg ml⁻¹ (Table 1). The acylated derivative of this compound 3-*O*-octanoyl(-)-epicatechin exhibited inhibitory activity against antibiotic sensitive and resistant strains of *Staph. aureus* and enterococci, but not against Gram negative bacteria. On its own, the octanoate chain exhibited no antibacterial activity at concentrations up to and including 200 µg ml⁻¹.

Time-kill studies

In time-kill assays with 50 $\mu\text{g ml}^{-1}$ 3-*O*-octanoyl(-)-epicatechin (and an inoculum density of $\sim 5 \times 10^5$ CFU ml^{-1}), a 600-fold reduction was seen in numbers of *Staph. aureus* NCTC 6571 colony forming units, and a 1300-fold reduction was seen in *Staph. aureus* NCTC 11940 colony forming unit numbers within 2 hours incubation (data not shown). However, bacterial re-growth was seen with both strains with recovery to a level of $\sim 5 \times 10^5$ CFU ml^{-1} reached after 24 to 28 hours. Experiments were therefore repeated with the semi-synthetic flavonoid at twice the MIC. At a concentration of 100 $\mu\text{g ml}^{-1}$, 3-*O*-octanoyl(-)-epicatechin reduced colony forming unit numbers of $\sim 5 \times 10^5$ CFU ml^{-1} *Staph. aureus* NCTC 6571 and *Staph. aureus* NCTC 11940 by more than 1000-fold in just 60 min (Figs. 1a and 1b). Within 120 min, colony forming unit numbers of both strains had been reduced below the minimum detectable level (~ 50 CFU ml^{-1}). No bacterial re-growth was observed in the 34 hours that followed, and colony forming unit numbers in flavonoid treated populations were found to have remained below the minimum detectable level when additional samples were taken after 80 hours and 168 hours (7 days) incubation.

Examination of 3-*O*-octanoyl(-)-epicatechin treated bacteria for aggregation

At concentrations of 50 $\mu\text{g ml}^{-1}$ and 100 $\mu\text{g ml}^{-1}$, and within 60 min after incubation, 3-*O*-octanoyl(-)-epicatechin reduced colony counts of $\sim 5 \times 10^7$ CFU ml^{-1} *Staph. aureus* NCTC 6571 by approximately 800-fold and 165,000-fold respectively (data not shown). These results suggested that 50 $\mu\text{g ml}^{-1}$ and 100 $\mu\text{g ml}^{-1}$ would be suitable concentrations for the detection of flavonoid-induced aggregation of *Staph. aureus* cells, as they both produced readily detectable decreases in colony counts of bacterial populations. Sixty min was selected as the treatment time in order to minimise *Staph. aureus* cell growth and the incidence of naturally occurring clusters in treated and untreated flasks.

In subsequent light microscopy experiments, samples of untreated *Staph. aureus* were found to contain bacteria that were evenly distributed and typically occurring as single cells, cell pairs and small clusters (Figs. 2a). Samples of *Staph. aureus* that had been incubated

with $50 \mu\text{g ml}^{-1}$ 3-*O*-octanoyl(-)-epicatechin for 60 min had a similar appearance (Fig. 3b). When $100 \mu\text{g ml}^{-1}$ 3-*O*-octanoyl(-)-epicatechin treated samples were examined, the majority of bacteria were again seen as single cells, cell pairs and small clusters (Fig. 3c). It should be noted that a large amount of detritus (possibly cell debris) was detected in the $100 \mu\text{g ml}^{-1}$ 3-*O*-octanoyl(-)-epicatechin treated samples though, and it is possible that this prevented observation of all of the treated cells. High levels of aggregation were observed in the positive control samples (Fig. 3d).

Detection of cytoplasmic membrane damage in 3-*O*-octanoyl(-)-epicatechin treated bacteria

Experiments in this section of the study were performed using aqueous iso-osmotic DMSO, as *Staph. aureus* cells remained viable for longer in this solution than in aqueous iso-osmotic ethanol. From Fig. 4, it can be seen that populations of $\sim 1 \times 10^9$ CFU ml^{-1} *Staph. aureus* NCTC 6571 underwent an 80-fold reduction in their colony forming unit numbers when incubated in the presence of $50 \mu\text{g ml}^{-1}$ 3-*O*-octanoyl(-)-epicatechin for 60 min. After 6 hours incubation with the semi-synthetic flavonoid, colony forming unit numbers had decreased by 1900-fold. These results indicated that MIC levels of 3-*O*-octanoyl(-)-epicatechin were capable of producing a readily detectable effect against high cell densities of *Staph. aureus*, and suggested that it would be possible to perform subsequent potassium loss assays using $50 \mu\text{g ml}^{-1}$ semi-synthetic flavonoid and 1×10^9 CFU ml^{-1} *Staph. aureus* NCTC 6571 suspended in an aqueous DMSO medium.

In the experiments that followed, 3-*O*-octanoyl(-)-epicatechin was found to induce rapid potassium loss from cells of *Staph. aureus* (Fig. 5a). Flavonoid treatment of *Staph. aureus* populations resulted in leakage of 50 % of their total potassium pool within just 10 min. MIC levels of novobiocin and penicillin G, by sharp contrast, induced no increase in potassium loss from populations of $\sim 1 \times 10^9$ CFU ml^{-1} *Staph. aureus* NCTC 6571 during 60 min incubation (Figs. 4b and 4c). It should be noted that a low level of potassium loss was detected from untreated control populations of *Staph. aureus* throughout this work (Figs. 4a to

4c). As in the case of the previous study with galangin (Cushnie and Lamb 2005b), this is likely to have occurred as a consequence of starvation-induced autolysis.

DISCUSSION

Results from the present investigation show that (-)-epicatechin does not inhibit the growth of *Staph. aureus*, *Ent. faecalis*, *Ent. faecium*, *E. coli* or *Ps. aeruginosa* when tested at concentrations up to and including 200 $\mu\text{g ml}^{-1}$ (Table 1). These results indicate that (-)-epicatechin has negligible antibacterial activity. Importantly however, the acylated derivative of this compound 3-*O*-octanoyl(-)-epicatechin was found to have antibacterial activity, inhibiting growth of staphylococcal strains at 50 $\mu\text{g ml}^{-1}$, and enterococcal strains at 100 $\mu\text{g ml}^{-1}$. These results are in broad agreement with the previous study, which found that 3-*O*-octanoyl(-)-epicatechin has more potent antibacterial activity than (-)-epicatechin gallate (Stapleton et al. 2004). Results presented here also correlate well with those of Mellou et al., who report that acylation of the flavonoid chrysoeriol-7-[6'''-*O*-acetyl- β -D-allosyl-(1 \rightarrow 2)- β -D-glucopyranoside] leads to increased antibacterial activity against *Staph. aureus* and *B. cereus* (Mellou et al. 2005). On its own, the octanoate structure is inactive at concentrations up to and including 200 $\mu\text{g ml}^{-1}$ (Table 1), indicating that 3-*O*-octanoyl(-)-epicatechin activity is not solely attributable to the presence of this hydrocarbon chain. In addition to identifying some of the bacterial species against which 3-*O*-octanoyl(-)-epicatechin is effective, results presented in Table 1 show that there is no detectable cross-resistance between 3-*O*-octanoyl(-)-epicatechin and either the β -lactams or vancomycin. This finding is important because the existence of cross-resistance between 3-*O*-octanoyl(-)-epicatechin and an antibiotic in current use would almost certainly make redundant any plans to try and develop 3-*O*-octanoyl(-)-epicatechin as an antibacterial agent.

When populations of antibiotic sensitive and methicillin resistant *Staph. aureus* were exposed to 100 $\mu\text{g ml}^{-1}$ 3-*O*-octanoyl(-)-epicatechin in time-kill assays, the colony forming unit numbers decreased more than 1000-fold within 60 min of incubation (Fig. 2). Unlike time-kill experiments with galangin (Cushnie et al. 2003), concentrations of treated bacteria

remained below detectable levels for the duration of the assays (36 h). Furthermore, no viable colony forming units were recovered from flasks containing 3-*O*-octanoyl(-)-epicatechin after 80 h or even 7 days incubation. These results strongly suggest that 3-*O*-octanoyl(-)-epicatechin has bactericidal activity. However, given the evidence that natural flavonoids (-)-epigallocatechin gallate (Ikigai et al. 1993) and galangin (Cushnie et al. 2007) induce substantial clumping of bacterial cells, it was considered prudent to check that decreases in colony forming unit numbers caused by 3-*O*-octanoyl(-)-epicatechin were not the result of a similar aggregatory effect.

In subsequent light microscopy studies, samples of *Staph. aureus* NCTC 6571 that had been treated with 50 $\mu\text{g ml}^{-1}$ (Fig. 3b) and 100 $\mu\text{g ml}^{-1}$ (Fig. 3c) 3-*O*-octanoyl(-)-epicatechin were found to have a similar appearance to those of untreated samples (Fig. 3a). The predominance of single cells, cell pairs and small clusters in 50 $\mu\text{g ml}^{-1}$ treated samples, and frequent observation of single cells, cell pairs and small clusters in 100 $\mu\text{g ml}^{-1}$ treated samples indicates that 3-*O*-octanoyl(-)-epicatechin does not induce a significant aggregatory effect. These results support the suggestion that 3-*O*-octanoyl(-)-epicatechin has bactericidal activity.

Data from Figs. 4 and 5a show that 50 $\mu\text{g ml}^{-1}$ 3-*O*-octanoyl(-)-epicatechin is capable of causing an 80-fold reduction in colony counts of $\sim 1 \times 10^9$ CFU ml^{-1} *Staph. aureus* NCTC 6571 during 60 min of incubation and inducing substantial potassium loss (approximately 50 % of the total pool) within just 10 min. MIC levels of the bacteriostatic antibiotic novobiocin and the bactericidal antibiotic penicillin G (Block and Beale 2004), by comparison, induced no increase in potassium loss from *Staph. aureus* populations during 60 min treatments (Figs. 5b and 5c). These experiments indicate that 3-*O*-octanoyl(-)-epicatechin causes damage to the cytoplasmic membrane of bacteria and strongly suggest that this is the primary antibacterial mechanism of action underlying the semi-synthetic flavonoid's activity. The absence of novobiocin-induced potassium loss implies that data shown in Fig. 5a is not the result of a general emergency response by cells to the presence of an antibacterial agent. Also, the failure of penicillin G to induce potassium loss, and the speed with which 3-*O*-

octanoyl(-)-epicatechin induces potassium loss, indicates that results in Fig. 5a were not caused by bactericidal agent induced autolysis. Data presented here correlates well with previous research, which showed that structurally related 3-*O*-octanoyl(+)-catechin promotes labelling of *Staph. aureus* cells with membrane impermeable propidium iodide (Stapleton et al. 2004). The means by which sub-MIC levels of 3-*O*-octanoyl(-)-epicatechin induced the formation of pseudomulticellular bacteria [cells which have divided but failed to separate (Giesbrecht et al. 1998)] in the previous study (Stapleton et al. 2004) is not clear at the current time. Any damage caused to a component of the bacterium as important as the cytoplasmic membrane is likely to trigger a cascade of secondary events though, one of which may be inhibition of cell wall autolysins. Alternatively, it could be that sub-MIC levels of 3-*O*-octanoyl(-)-epicatechin induce the formation of pseudomulticellular bacteria by inhibiting cell wall autolysins directly, and that this represents an as-yet-uncharacterised activity of this compound which is separate and distinct from its cytoplasmic membrane activity.

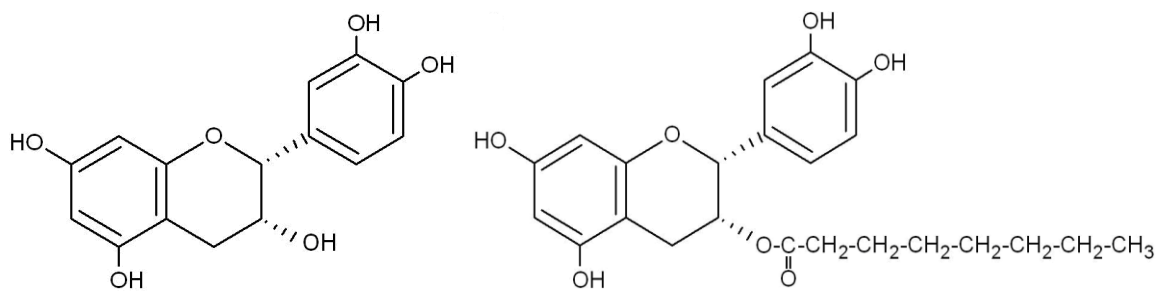
In summary, results from the present investigation indicate that 3-*O*-octanoyl(-)-epicatechin has inhibitory activity against antibiotic sensitive, β -lactam resistant and vancomycin resistant Gram positive bacteria. Aggregatory studies with the semi-synthetic flavonoid failed to generate definitively negative results, but it is clear that this agent does not induce clumping of bacterial cells on the same scale as naturally occurring flavonoids such as galangin (Cushnie et al. 2007). This finding, together with data from time-kill and potassium loss assays, very strongly suggests that 3-*O*-octanoyl(-)-epicatechin has bactericidal activity. Results from potassium loss assays indicate that the primary target of this agent is the cytoplasmic membrane. Further investigations are warranted to establish whether acyl catechins such as 3-*O*-octanoyl(-)-epicatechin would be useful as topical therapeutic agents. Though the antibacterial activity of these compounds is only of moderate potency, future optimisation through structural alteration and formulation may allow the development of a pharmacologically acceptable class of antibacterial agents. Research by Kajiya and colleagues (Kajiya et al. 2004) and Stapleton and Taylor (unpublished results), for example, indicates that catechin activity is potentiated by the presence of sodium chloride, and synergy

studies with 3-*O*-octanoyl-(-)-epicatechin and sodium chloride might yield a formulation with improved antibacterial activity.

Table 1 The MICs of 3-*O*-octanoyl(-)-epicatechin and its component structures [(-)-epicatechin and octanoate] against a selection of antibiotic sensitive and antibiotic resistant Gram positive and Gram negative bacteria.

Bacterial strain	Phenotype	MIC ($\mu\text{g ml}^{-1}$)					
		Penicillin G	Vanco- mycin	Cephalo- sporin C	3- <i>O</i> -oct- (-)-EC	(-)-EC	Sodium octanoate
<i>Staph. aureus</i> NCTC 6571	Antibiotic sensitive	0.0156	ND	ND	50	-	-
<i>Staph. aureus</i> NCTC 10788	Antibiotic sensitive	0.007813	ND	ND	50	-	-
<i>Staph. aureus</i> NCTC 11940	Methicillin resistant	32	ND	ND	50	-	-
<i>Staph. aureus</i> ATCC 33591	Methicillin resistant	32	ND	ND	50	-	-
<i>Staph. aureus</i> NCTC 11561	β -lactamase producing	128	ND	ND	50	-	-
<i>Staph. aureus</i> NCIMB 9968	Resistant to penicillin, streptomycin & tetracycline	64	ND	ND	50	-	-
<i>Ent. faecalis</i> NCIMB 775	Antibiotic sensitive	ND	0.5	ND	100	-	-
<i>Ent. faecalis</i> NCTC 12201	Vancomycin resistant	ND	256	ND	100	-	-
<i>Ent. faecalis</i> NCTC 12203	Vancomycin resistant	ND	256	ND	100	-	-
<i>Ent. faecium</i> NCTC 7171	Antibiotic sensitive	ND	1.0	ND	100	-	-
<i>Ent. faecium</i> NCTC 12202	Vancomycin resistant	ND	256	ND	100	-	-
<i>Ent. faecium</i> NCTC 12204	Vancomycin resistant	ND	256	ND	100	-	-
<i>E. coli</i> NCTC 12241	Antibiotic sensitive	50	ND	ND	-	-	-
<i>E. coli</i> NCTC 11954	β -lactamase producing	80	ND	ND	-	-	-
<i>E. coli</i> NCTC 11560	β -lactamase producing	1600	ND	ND	-	-	-
<i>Ps. aeruginosa</i> NCTC 10662	Antibiotic sensitive	ND	ND	1600	-	-	-
<i>Ps. aeruginosa</i> NCTC 8203	Cephalosporinase producing	ND	ND	1600	-	-	-
<i>Ps. aeruginosa</i> NCTC 8506	Cephalosporinase & streptomycin antagonist producing	ND	ND	3200	-	-	-

3-*O*-oct(-)-EC, 3-*O*-octanoyl(-)-epicatechin; (-)-EC, (-)-epicatechin; ND, not determined; -, no antibacterial activity detected at concentrations up to and including 200 $\mu\text{g ml}^{-1}$

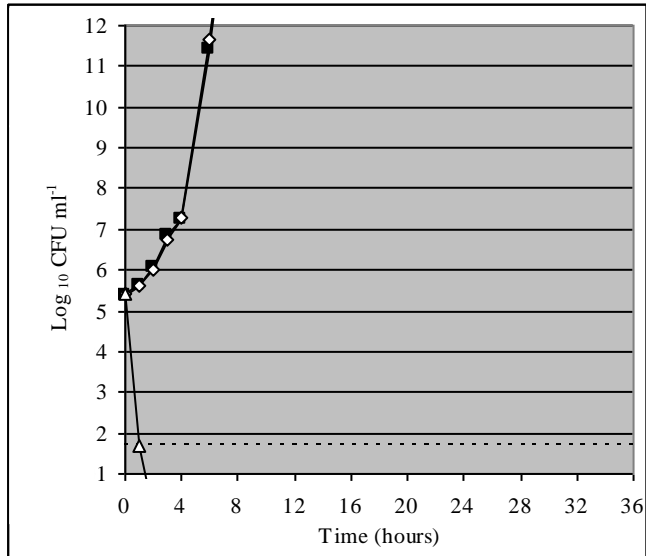


(-)-Epicatechin
(*cis*-3,5,7,3',4'-pentahydroxyflavane)

3-*O*-octanoyl(-)-epicatechin
(*cis*-3,5,7,3',4'-pentahydroxyflavane 3-octanoate)

Figure 1 The structure of (-)-epicatechin and its acylated derivative 3-*O*-octanoyl(-)-epicatechin.

(a)



(b)

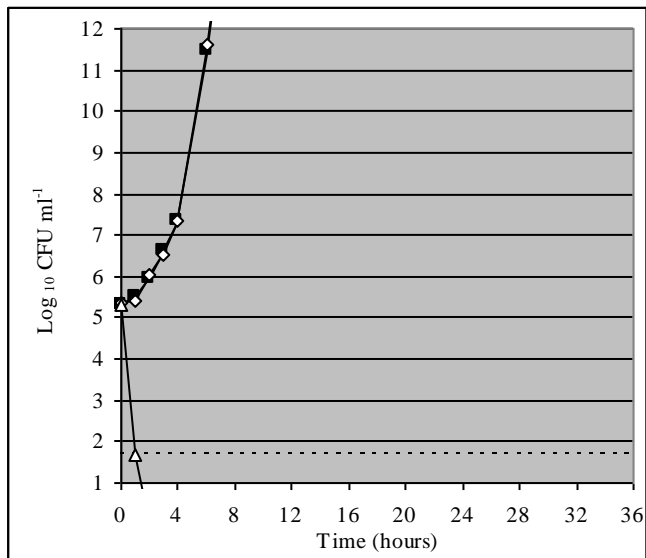


Figure 2 The effect of 3-*O*-octanoyl(-)-epicatechin on populations of $\sim 5 \times 10^5$ CFU ml⁻¹ (a) *Staph. aureus* NCTC 6571 & (b) *Staph. aureus* NCTC 11940. ◇: Broth only (positive control); ■: Broth with 1 % (v/v) ethanol (positive control); △: Broth with 100 µg ml⁻¹ 3-*O*-octanoyl(-)-epicatechin; ----: minimum detectable number of CFU ml⁻¹

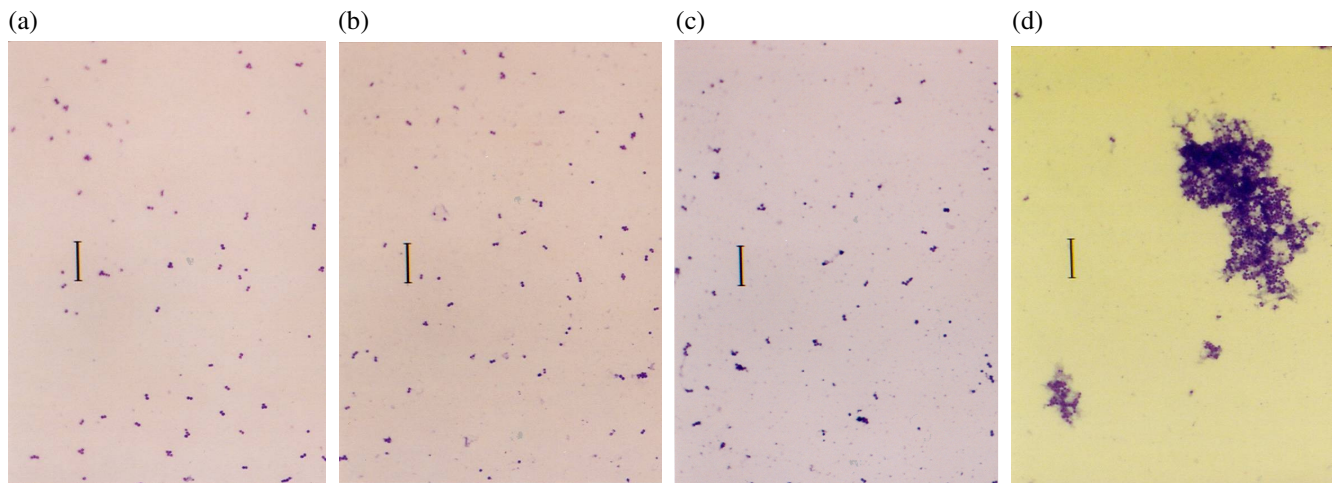


Figure 3 Light microscopy images of (a) *Staph. aureus* NCTC 6571 incubated in broth for 60 min (negative control), (b) *Staph. aureus* NCTC 6571 incubated in broth supplemented with $50 \mu\text{g ml}^{-1}$ 3-*O*-octanoyl(-)-epicatechin for 60 min, (c) *Staph. aureus* NCTC 6571 incubated in broth supplemented with $100 \mu\text{g ml}^{-1}$ 3-*O*-octanoyl(-)-epicatechin for 60 min and (d) *Staph. aureus* NCTC 6571 incubated in broth supplemented with $50 \mu\text{g ml}^{-1}$ galangin for 60 min (positive control). Bar = $10 \mu\text{m}$

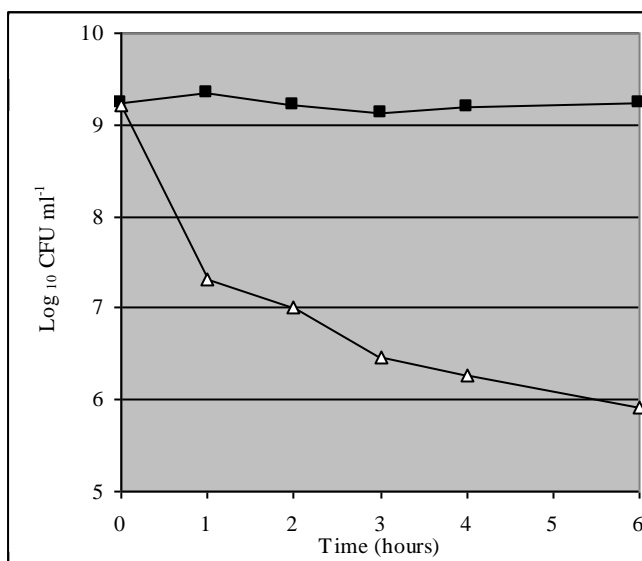


Figure 4 The effect of 3-*O*-octanoyl(-)-epicatechin on a population of $\sim 1 \times 10^9$ CFU ml⁻¹ *Staph. aureus* NCTC 6571. ■: aqueous 1.77 % (v/v) DMSO & 0.09 % (w/v) sodium chloride; △: 50 µg ml⁻¹ 3-*O*-octanoyl(-)-epicatechin, 1.77 % (w/v) DMSO & 0.09 % (w/v) sodium chloride

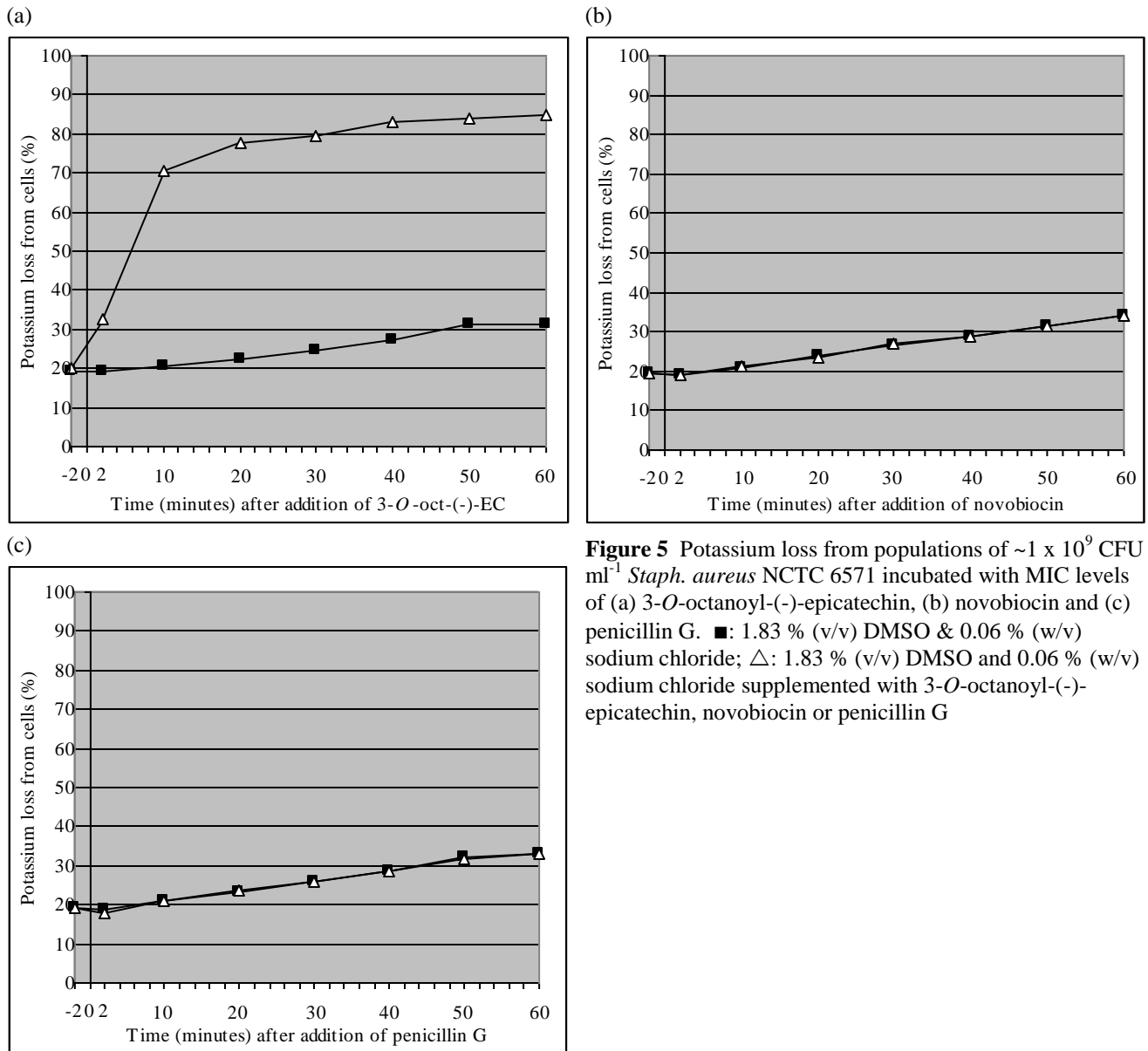


Figure 5 Potassium loss from populations of $\sim 1 \times 10^9$ CFU ml^{-1} *Staph. aureus* NCTC 6571 incubated with MIC levels of (a) 3-O-octanoyl(-)-epicatechin, (b) novobiocin and (c) penicillin G. ■: 1.83 % (v/v) DMSO & 0.06 % (w/v) sodium chloride; △: 1.83 % (v/v) DMSO and 0.06 % (w/v) sodium chloride supplemented with 3-O-octanoyl(-)-epicatechin, novobiocin or penicillin G

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