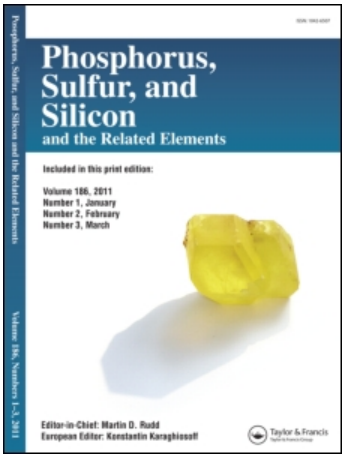

Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Chemically Modified Phosphocitrate and Entrapment in Microparticles for Sustained Inhibition of Biomineralization

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To cite this Article Sallis, John , Meehan, John , Kamperman, Harold and Anderson, Maree(1993) 'Chemically Modified Phosphocitrate and Entrapment in Microparticles for Sustained Inhibition of Biomineralization', Phosphorus, Sulfur, and Silicon and the Related Elements, 76: 1, 281 — 284
To link to this Article: DOI: 10.1080/10426509308032413
URL: http://dx.doi.org/10.1080/10426509308032413

Phosphorus, Sulfur, and Silicon, 1993, Vol. 76, pp. 281–284

CHEMICALLY MODIFIED PHOSPHOCITRATE AND ENTRAPMENT IN MICROPARTICLES FOR SUSTAINED INHIBITION OF BIOMINERALIZATION

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ABSTRACT Synthesis of glycolamide esters of phosphocitrate is described. Incorporation of salts or modified forms of phosphocitrate into albumin/polylactide based microspheres markedly improves efficiency in delivery. Strong inhibition of hydroxyapatite deposition into rat skin plaques was observed.

INTRODUCTION

Phosphorylated carboxylic acids are powerful inhibitors of hydroxyapatite crystallization with phosphocitric acid (PC: see Figure 1) the most potent[1]. Beside restricting calcium phosphate, formation of other calcium salts and also magnesium phosphates can be influenced[2]. Potential exists then for the application of PC to diverse biological and industrial fields.

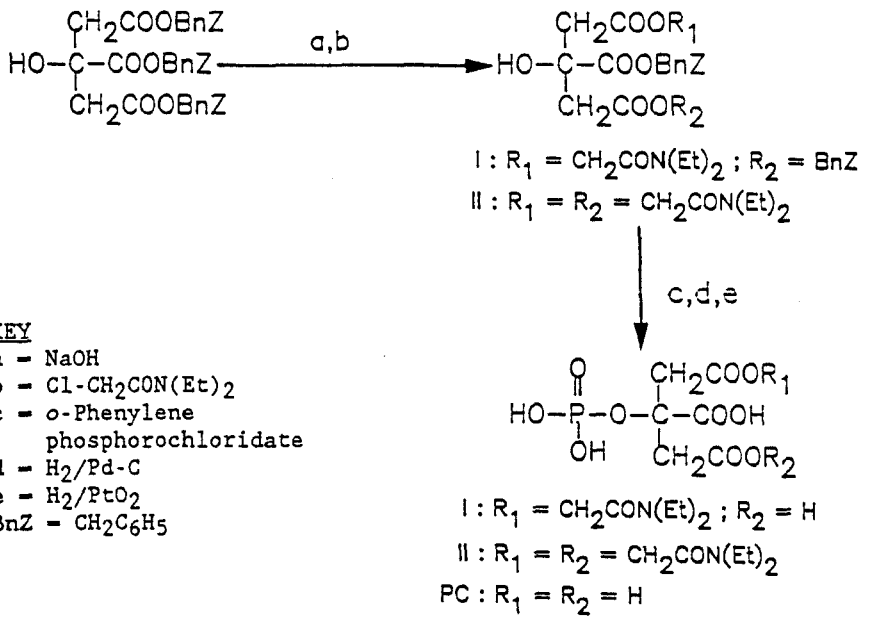
In previous studies we have demonstrated that PC can control important aspects associated with urolithiasis[2], atherosclerosis and dystrophic soft tissue calcification[3]. Of importance, PC is found in soft tissues probably arising from the cytosolic phosphorylation of citric acid[4], which may be why the compound appears non-toxic and environmentally friendly. Many of the present and projected future applications would greatly benefit if targeted and or slow constant release forms of PC were to become available. The long term control of a pathologically calcifying site in a vertebrate might be more efficiently managed by an "in tissue" drug depot or alternately, by relatively infrequent injections of a slow release form of the drug. With this in mind, our recent studies have focussed on the synthesis of prodrug forms of PC and the potential of sustained release microparticles as a delivery system for selected salts or prodrugs of PC.

For many compounds, ester prodrugs are beneficial because of their inherent lipophilicity allowing improved transport across

membranes. Methyl or ethyl esters of PC are not suitable because there is difficulty in their later total removal *in vivo*. We considered an alternate strategy might be to synthesize glycolamide esters because such compounds are known to exhibit aqueous stability but are rapidly hydrolysed by blood cholinesterase[5].

Synthesis of PC glycolamide esters

The following synthetic strategy was adopted (Figure 1)



KEY
a = NaOH
b = Cl-$CH_2CON(Et)_2$
c = o-Phenylene phosphorochloridate
d = H_2/Pd-C
e = H_2/PtO_2
BnZ = $CH_2C_6H_5$

FIGURE 1 Scheme for the synthesis of mono (I), and di- (II) glycolamide esters of PC

Proton NMR was used to confirm the structures i.e. for asym-mono-ester (I) δ 1.1-1.3 (6H; 2x tr), 3.0-3.2 (4H; d; J= 16.75ppm), 3.4 (4H; m), 4.0 (2H;d). The bioreversibility of the prodrugs was tested by incubation of the prodrugs *in vitro* with pseudo-cholinesterase. Rapid conversion of prodrug to drug was observed with over 90% occurring within 10 minutes.

PC MICROSPHERES

Microspheres containing salt or modified forms of PC (prodrugs) were prepared (Figure 2). The sodium salt of PC was first incorporated into glutaraldehyde cross-linked albumin microspheres through modifying the method of Tomlinson and Burger[6]. Submicron uniform spheres with prodrug loading of 30% by weight (90% trapping efficiency) were obtained. The *in vitro* release characteristics however were unsatisfactory with >80% released within 5 mins. Marked improvement was achieved however by coating the albumin microspheres with polylactide incorporating CaPC and/or PC prodrug. Release was sustained over several hours.

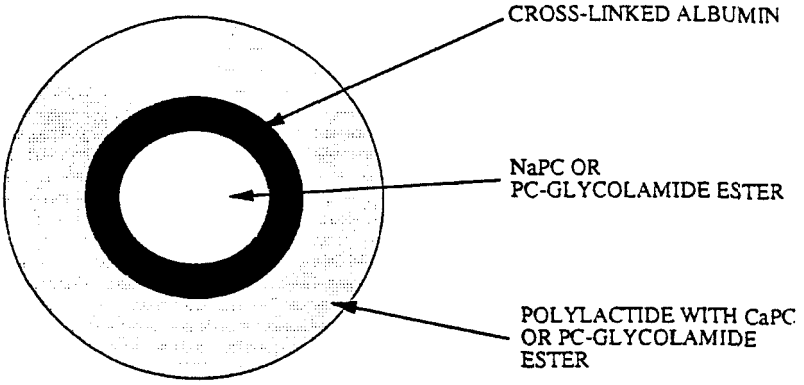


FIGURE 2 Schematic representation of drug laden microparticles.

CALCERGY STUDIES

The effectiveness of the preparations to prevent hydroxyapatite formation was assessed in an animal calcergy model[7]. Rats were challenged on day 1 with two dorso-ventral subcutaneous injections of a chemical calcergic agent which subsequently produced two calcified skin plaques within 10 days when untreated (Table 1). In contrast to rats given a single I.M. dose of either free Na or CaPC, the drug trapped within the microparticles given as a single injection effectively blocked calcified plaque formation. A comparable result could only be achieved by multiple intraperitoneal injections of the free Na or Ca salts. Reduced dosage and less frequent administration were significant features of the new system.

TABLE I Calcergic plaque inhibition as a function of PC formulation and dose frequency. Suspensions of solid PC or microspheres in sunflower oil (0.2 ml) were administered i.m. or i.p.[#] either on day 1 alone[a] or days 1 and 5[b], before plaque recovery on day 10. Plaques (n = 10 / group) differ from control, one-tailed t-test, ** $p < 0.01$; *** $p < 0.001$.

Treatment Group	Dose (mg/kg body wt.)	Frequency (per 10 days)	Plaque wt. (mg ± s.e.m.)	inhibition %
Expt. I				
None	---		186.3 ± 12.9	----
NaPC	100	2x[b]	146.1 ± 10.8	21.6**
CaPC	70	2x	48.8 ± 9.2	73.8***
Expt. II				
None	---		157.7 ± 9.3	----
NaPC	150	1x[a]	157.3 ± 10.4	0.0
CaPC	105	1x	60.8 ± 7.4	61.4***
Expt. III				
None	---		178.1 ± 8.9	----
NaPC/CaPC microspheres	105	1x	22.4 ± 5.1	87.4***
Expt. IV[#]				
None	---		146.8 + 10.3	----
CaPC	50	daily	0.0 ± 0.0	100.0***

CONCLUSIONS

PC glycolamide ester prodrugs have been synthesized and a microparticulate delivery system developed. The CaPC salt proved more inhibitory than the sodium form. Versatility has been achieved through modification of the free acid and microsphere entrapment thus providing sustained delivery for controlling pathological biomineralization.

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