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Synthesis of Potential Inhibitors of the Enzyme Pantothenate Synthetase

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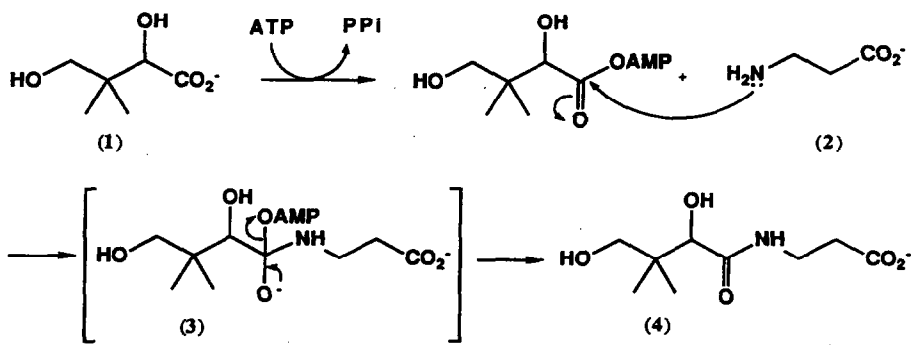
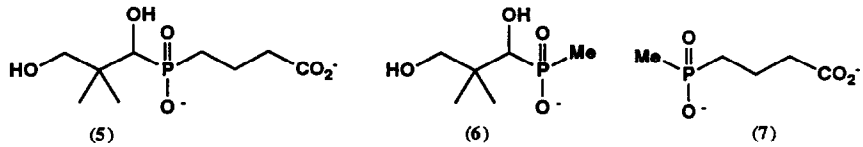
Abstract: Three phosphinate salts (5), (6) and (7) were prepared as potential inhibitors of the enzyme pantothenate synthetase. The synthesis of compound (5) utilises a (diethoxymethyl)-protected phosphinate (8) as a new reagent for the formation of unsymmetrical phosphinic acids and esters. Initial P-alkylation of (8) followed by a selective deprotection sequence yields intermediate P-H phosphinates (16) and (21) which can be P-alkylated a second time. Enzyme assays have been performed with the target compound and the results are discussed.

For many years, we have been involved in the rational design of novel agrochemicals using biochemical reasoning.[1] In some of these studies we have utilised tetrahedral phosphorus species, for instance to mimic the shape and properties of the reaction intermediate formed during nucleophilic addition-elimination reactions at a carbonyl group.[2,3] Such compounds have been well documented and offer the potential for very tight binding.[4,5] We were interested in applying the same principle to the design of inhibitors of coenzyme A biosynthesis, and in particular of the enzyme pantothenate synthetase (E.C. 6.3.2.1), as potential herbicides. This enzyme catalyses the condensation of pantoate (**1**) and β-alanine (**2**) to form pantothenate (**4**).



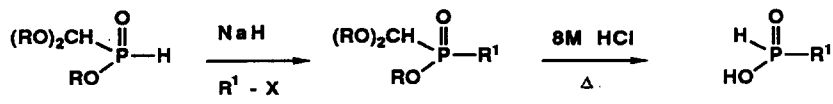
Investigations using the enzyme from *E.coli*[6] support a mechanism in which (**1**) is first adenylated by ATP. Subsequent nucleophilic attack by (**2**) gives rise to a tetrahedral high energy intermediate (**3**) which breaks down with loss of AMP to give (**4**). At present only simple, competitive inhibitors of this enzyme have been reported.[7] We wished to prepare the phosphinic acid (**5**) as a potential multisubstrate analogue.[8] In order to improve the stability, the NH group has been replaced by an isosteric methylene group. We also hoped that (**5**) might be adenylated by the enzyme to give a very close mimic of the reaction intermediate (**3**).

This compound and two simpler but related analogues (**6**) and (**7**) were synthesised; this communication describes their preparation and biological activity.



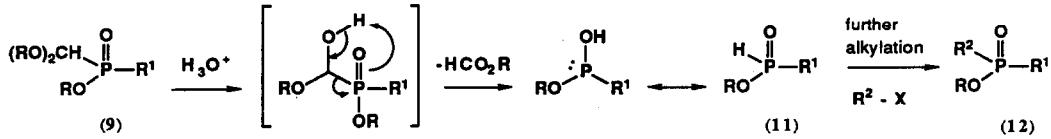
(5) (6) (7)

To synthesise compound (**5**) we envisaged the use of an alkyl (dialkoxymethyl)phosphinate (**8**) as a key starting material. Such compounds are readily synthesised by the reaction of anhydrous hypophosphorus acid with a trialkylorthoformate.[9] Dingwall *et al.*[10] have shown that reaction of (**8**) (as the free phosphinate, metal salt or silyl ether) with a variety of electrophiles leads to phosphinates (**9**) which upon acid hydrolysis afford phosphinic acids (**10**).



(8) (9) (10)

However, to the best of our knowledge, the selective acidic hydrolysis of (**9**) to prepare phosphinate esters (**11**) has not been investigated.



(9) (11) (12)

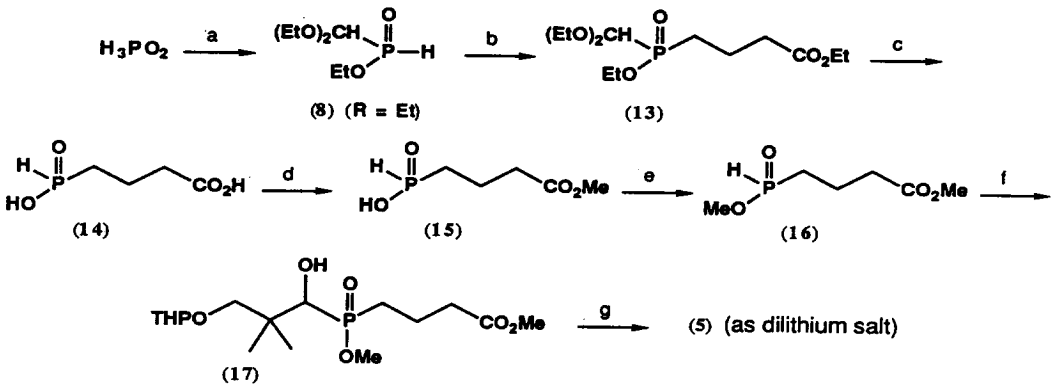
Phosphinate esters (**11**) have an acidic hydrogen available for further alkylation and thus compounds (**8**) can be regarded as useful precursors for the preparation of unsymmetrical phosphinic esters and acids (**12** R = H, alkyl).

Target compound (**5**) was synthesised as illustrated in Scheme I. The sodium salt of (**8**) was alkylated with ethyl-4-bromobutyrate to give (**13**) which upon prolonged reflux with 8M HCl led to complete hydrolysis of the acetal, carboxylic and phosphinic ester groups to give (**14**). Refluxing (**14**) in methanol led to esterification of the carboxylic acid to give (**15**), etherial diazomethane being required to esterify the phosphinic acid group giving (**16**). 2,2-Dimethyl-3-(tetrahydropyran-2-yl) propanal[11] and (**16**) were stirred with anhydrous caesium fluoride according to the method of Texier-Boullet *et al.*[12] affording (**17**) which was hydrolysed with 8M HCl to give the desired product (**5**) as a viscous gum which was most easily isolated as its crystalline dilithium salt.

In order to avoid the tedious two step re-esterification of (**14**), we attempted a selective hydrolysis of the acetal group in (**18**), prepared analogously to (**13**). A variety of acidic conditions were investigated but we were unable to selectively hydrolyse the acetal without accompanying hydrolysis of the carboxylate ester. However, we were able to selectively hydrolyse the carboxylic ester in (**18**) (Scheme II) using 2M HCl in THF to give (**19**). By increasing the amount of acid and reflux time, we were able to selectively hydrolyse both the acetal and carboxylic ester moieties affording (**20**) which could be esterified with etherial diazomethane to give (**21**) (Scheme III). Like phosphinate (**16**), compound (**21**) could also be converted into target (**5**), further illustrating the usefulness of phosphinate (**8**) as a reagent for the synthesis of unsymmetrical phosphinic acids or esters.

Target compound (6) was prepared by reaction of freshly distilled monomeric hydroxypivalaldehyde (22) and ethyl methyl phosphinate[13] (23) to give phospholane (24) which upon acidic hydrolysis gave phosphinate (6), most easily isolated as its lithium salt (Scheme IV). Target compound (7) was prepared by Arbuzov reaction between diethyl methylphosphonite[14] and ethyl 4-bromobutyrate followed by acidic hydrolysis.

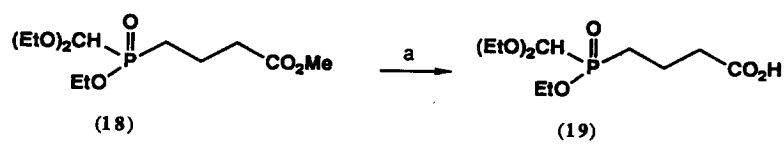
Scheme I



THP = Tetrahydropyran-2-yl

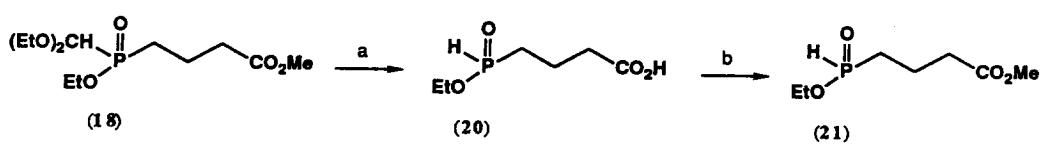
a) $2HC(OEt)_3$, cat pTSA, r.t., 2 days; 48%. b) NaH, THF, 12 hr, r.t. then $Br(CH_2)_3CO_2Et$, reflux, 6 hr; 42%. c) 8M HCl, reflux, 8 hr, N_2; 94%. d) MeOH, reflux, 4 hr; 99%. e) xs CH_2N_2, Et_2O, 5°C, 30 mins; 96%. f) $THPOCH_2C(Me)_2CHO$, CsF, r.t., 20 hr; 47%. g) 8M HCl, reflux, 8hr, then remove organics by CH_2Cl_2 extraction, evap. to dryness, then LiOH to pH7; (86%).

Scheme II



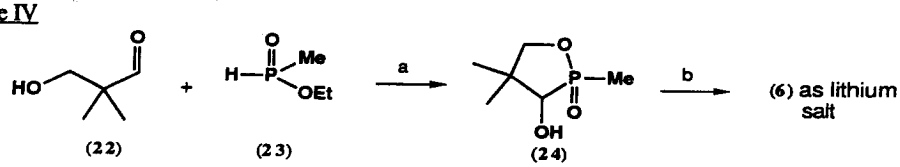
a) 2M HCl : THF (1 : 2), reflux, 40 mins; 98%.

Scheme III



a) 2M HCl : THF (1 : 1), reflux, 18 hr; 97%. b) CH_2N_2, Et_2O, 5°C, 30 mins; 98%.

Scheme IV



a) Neat, 140°C, 2 hr; 48%. b) 8M HCl reflux 4 hr, then evap. to dryness, then LiOH to pH7; 94%.

Compounds (**5**), (**6**) and (**7**) were tested for activity against pantothenate synthetase from *E. coli*, extracted and assayed by the method of Miyatake *et al*[7]. Disappointingly, the compounds did not show any significant inhibition of the enzyme under a variety of assay conditions.[16] The compounds were essentially inactive in our biological screens.

Although we did not try to establish why our compounds were not inhibitors, there are, of course, several possible explanations for these disappointing results. Despite the close analogy with the proposed enzyme intermediate, our inhibitors may simply not fit well enough at the enzyme active site, perhaps because the electron density around the phosphorus atom is wrong or because the NH moiety is very important for binding. Alternatively, adenylation of such inhibitors may well be required to allow effective binding to the enzyme and this may not occur. Finally, the enzyme may involve a "closed transition state" so that inhibitors based on the reaction intermediate may be unable to bind to the enzyme.[17]

Acknowledgements: This communication is dedicated to the memory of the late Mr B.J. Wright. The authors wish to thank Dr C.G. Earnshaw for helpful discussions during this work and Mr H.W. Eyton-Jones for performing the enzyme assays.

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15. All new compounds exhibited physical and spectroscopic properties consistent with their structure. All yields quoted are unoptimised.
16. No compound gave evidence of competitive inhibition (at 0.3mM; [ATP] was 10mM, [pantoate] was 5 mM and [β-alanine] was 5mM) nor was there any time-dependent inhibition observed following various conditions of preincubation (enzyme + compound (5mM) + (i) ATP (ii) ATP + β-alanine (iii) ATP + pantoate (iv) pantoate + β-alanine); ATP, pantoate and β-alanine were at the concentrations given above. A parallel set of experiments was carried out in which AMP replaced ATP.
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