Pentachlorophenylcellulose Preparation and Investigation on its Applicability as a Controlled Release Fungicide

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Synopsis

Pentachlorophenylethers (PCP-ethers) of cellulose are prepared using cellulosetosylates as intermediates. The products release no measurable quantities of PCP into buffered solutions (pH 4, 7, and 9, resp.). However, the modified cellulose shows no fungicidal effect in agar plate tests with the usually cellulose-metabolizing fungus, *Trichoderma viride*. The lack of biological activity is attributed to the stability of the cellulose pentachlorophenyl ether bond.

INTRODUCTION

Low molecular weight bioactive substances dissipate quickly and lose their efficacy when applied by standard methods. By fixation of the active agent onto or in polymeric matrices this problem can be possibly avoided (*Controlled Release Technologies*).[1] This fixation can be achieved not only by adsorption onto and solution[2-4] or dispersion[5] in polymers, but also by covalently attaching the active agent to the macromolecular backbone.[6,7]

Cellulose, a renewable raw material of widespread use, presents itself as matrix of a controlled release formulation. Different types of bonding and different spacer molecules to attach pesticides to the cellulose backbone have been described. It was found that biological activity depends strongly on the combination of spacer molecule and active agent used. Allan and Halabisky[8] reported that pentachlorophenol (PCP) bound to cellulose by triazole bonds maintains its fungicidal activity. On the other hand, Hüttinger[9] could not find any biological effects of chlorophenylderivatives linked to cellulose via aliphatic groups and nonhydrolyzable bonds. Up to now, no work has been published in which PCP had been fixed hydrolytically stable and without spacer to the cellulose molecule. But it has been shown that pentachlorophenylvinyl copolymerisates have fungicidal efficiency without releasing considerable amounts of PCP.[10]

The work reported here has been carried out to investigate the possibilities of binding a biologically active substance without a spacer molecule and at the same time hydrolytically stable to cellulose. PCP was attached to cellulose by an ether bond. The biological effectiveness was tested in simple bioassays.

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EXPERIMENTAL

IR spectra were recorded on a Perkin-Elmer Spectrophotometer 1400. Elemental analyses were done by microanalytical laboratory Pascher, Bonn (FRG). PCP concentrations were determined by HPLC. A reversed phase column (ODS-Hypersil, 4 × 100 mm) and a mobil phase mixture (90 : 10 v/v) of methanol and phosphate buffer (5 mM, pH = 2.5) were used. PCP was detected by a Knauer variable wavelength monitor set to 230 nm. Quantification was done by an external standard method with a HP 3390 A integrator. For 10 succesive injections of 10 ng PCP, a relative standard error of 1% was obtained. The detection limit was 1 ng PCP. All reagents and solvents used in synthesis were of analytical grade or purified by appropriate methods.

Cellulosetosylate (I)

Cellulosetosylates (I) were prepared according to the literature.[12] The reaction was quenched by pouring into methanol. The product was filtered by suction and extracted with dried acetone for 5 h in a soxhlet apparatus.

A reaction time of 24 h yielded a cellulose derivative (Ia) containing 8.8% (by weight) S (DS* = 0.75), while after 3 days a product (Ib) with 12.8% S (DS = 1.64) was obtained.

IR (KBr; cm^{-1}): 3500 (s, CellOH), 1600 (s, arylsubstituent), 1350 (s, OSO_2Ar).

Pentachlorophenylcellulose (II)

One gram (I) was suspended in 50 mL dry DMSO and warmed to 50°C. The higher substituted cellulose derivative (Ib) went thereby into solution. A solution of 2 eq (based on tosyl groups) of sodium pentachlorophenolate in 10 mL DMSO were poured into this mixture with stirring. The reaction mixture was stirred at 100°C for 48 h. The resulting dark-brown liquid was poured into 500 mL acetone. The precipitate was filtered off and washed at least for 5 h by soxhlet extraction with acetone. The material contained 5.4% Cl (IIb) [DS = 0.1, higher substituted starting material (Ib)] or 2.7% Cl (IIa) [DS = 0.03, less substituted material (Ia)]. 9.6 and 4.0% S, resp., remained in the products. IR spectra showed no change compared to the starting polymers.

Biological Assay

The fungus *Trichoderma viride* was cultured on malt agar slabs (Maltextract agar, Merck Art. 5398) until good sporulation was visible. The sporules were suspended in sterile 0.9% NaCl solution. Petridishes with 25 mL maltextract agar were inoculated with 1 mL (approx. 10^6 sporules) of this suspension, after a test piece (round filter paper ∅ 2 cm, Schleicher and Schüll Nr. 595) had been placed onto the agar surface. Test pieces were either untreated (control), or they had been powdered with different amounts of pentachlorophenylcellulose (IIa or IIb) (corresponding to 500 and 2500 ppm PCP relative to filter paper weight). For positive control filter papers were impregnated with 500

*DS: degree of substitution (mol tosyl substituents/mol anhydroglucose units).

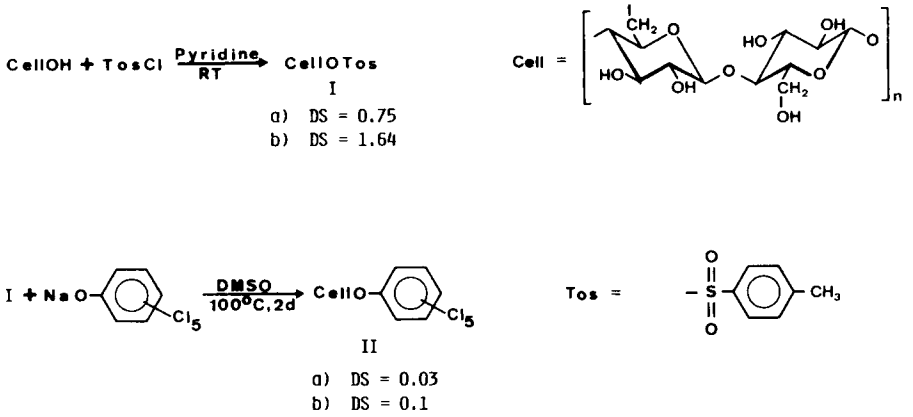

Fig. 1. Reaction sequence to pentachlorophenylcellulose.

ppm free PCP. All agar plates were prepared in triplicate. The growth of microorganisms was observed for 5 days.

RESULTS AND DISCUSSION

Two procedures can be contemplated for the preparation of pentachlorophenylcelluloseethers (II). On the one hand, the nucleophilic substitution of efficient leaving groups bound to the cellulose by phenolate; on the other hand, the nucleophile aromatic substitution on hexachlorobenzene by cellulosate ions. The latter method, which would correspond to one of the technical syntheses of PCP, proved to be not feasible, because — even with the use of phase transfer catalysts[11] — only negligible amounts of PCP were bound to cellulose. Introduction of tosylgroups into the cellulose backbone is a well-known[12] and reliable method to get a reactive cellulose derivative (Fig. 1).

Although the preparative potential of cellulosetosylates was recognized very early, only a few publications are known, which describe the reactions of this intermediate with mostly relatively small nucleophiles.[13] In the present work, two differently substituted cellulosetosylates [DS = 0.75 (Ia) and DS = 1.64 (Ib), resp.] were reacted in DMSO with sodium pentachlorophenolate. While the highly substituted cellulose (Ib) is soluble under the reaction conditions, the weakly substituted cellulose (Ia) is only slightly swollen. It is known that because of steric requirements of the Walden inversion tosyl groups on primary carbon atoms only are substituted in cellulosetosylates.

Furthermore, primary OH groups in cellulose are preferentially tosylated. It is, therefore, desirable to conduct substitution reactions with cellulosetosylates having a degree of substitution < 1 to get products which contain the least possible number of excess tosyl groups.

The weakly substituted cellulosetosylate (Ia) (DS = 0.75) yielded a product (IIa) which contains 0.03 PCP residues per anhydroglucose unit (4% PCP by weight). Additionally, 0.25 tosylgroups per anhydroglucose unit remained on the cellulose backbone. For the cellulosetosylate (Ib) with DS = 1.64 these values (product IIb) are 0.1 (7.6% by weight) and 1.05, resp. Presently, we are studying the reactions with different chlorophenols to find out, whether these low substitution yields can be attributed to steric or electronic factors. At

room temperature no measurable quantities (less than 0.5% of bound amount) of PCP are released during 150 days from the pentachlorophenyl celluloses into stirred buffer solutions of pH = 4, 7, and 9, resp.

The described polymeric pentachlorophenolderivatives (IIa and IIb) have been tested for fungicidal activity in agar plate tests; the cellulose metabolizing fungus, *Trichoderma viride*, was used as test organism.

While the control filter papers with free PCP inhibited fungal growth considerably, the polymeric samples were overgrown by fungus as quickly as untreated controls. This observation agrees with the results from other investigations[9] and can be explained by means of the results from the release experiments and the mode of fungicidal action of PCP. The biocidal effect of PCP results from its interference with oxidative phosphorylation, i.e., with processes inside the cellular organism.[14] Since PCP bound to cellulose comes only into contact with the cell membrane and no measurable amounts of PCP are hydrolyzed from the polymer, the absence of any biological effect is by all means understandable. However, results[10] obtained with pentachlorophenylvinylether copolymerisates contrast with our observations. Those polymers are not attacked by *Trichoderma viride*. Those products release indeed small amounts of PCP into alkaline solution. But the data in hand up to now do not allow a final conclusion whether the different behavior of the two polymeric PCP derivatives can exclusively be attributed to the stability to hydrolysis.

CONCLUSIONS

The reported results show that it is possible to prepare pentachlorophenylcelluloseethers with a low degree of substitution (DS = 0.03–0.1). However, the stable bond between PCP and macromolecule leads to a complete loss of the biocidal activity of the fungicide. It cannot be determined by agar plate tests with the available powdery material whether the modified cellulose is attacked and (partially) metabolized or only overgrown by the fungus. More sensitive biological tests (Warburg assay) will be applied to reveal any undiscovered influences of the pentachlorophenylcelluloseether on microorganisms. It can be imagined that suitable molecules can be found, which enable the PCP to show fungicidal activity even in a nonhydrolyzable formulation. For example, vinyl graft polymers could be one possible way in the right direction, and will be explored.

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