Degradation of p^{32}-Bromophos by Microorganisms and Seedlings

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Biological degradation of bromophos has been studied by Rowlands (1) and Stiansi et al. (2). They conclude that among other products desmethylbromophos (mbPS) is formed in wheat grains and rats. No bis-desmethyl bromophos (bPS) was found in their studies.

This paper describes the degradation of p^{32}-bromophos by microorganisms and some seedlings, using qualitative thin layer chromatography to detect the products.

METHODS

Materials

p^{32}-labeled bromophos was commersially obtained from Amersham radiochemical centre, U.K. The compound was purified by elution through a column of activated silica gel by tetrachlorcarbon. Unlabeled bromophos and methyl parathion were obtained as gifts from producers of these insecticides. Methyl phenyl phosphorothionate (mØPS) and sodium hydrogen phenyl phosphorothionate (ØPS) were synthetised in our

laboratory. The potassum salt of m_2PS was obtained as a gift from Dr. Sutherland, Cyanamide Company, Princeton, U.S.A. The ammonium salt of m_2PS, obtained as a gift from Bayer Chemie AG, Leverkusen, Germany, was spontaniously converted to mPS. The sodium salt of mbPS was a gift from C.H. Boehringer und Sohn Chemische Fabrik, Ingelheim, Germany.

No reference substance of bPS was obtained. The identity of bPS could therefore not be directly determined. The spots, supposed to be bPS, gave positive reaction with phosphate- and phenyl detecting sprays. The colour was exactly the same as that obtained with ØPS when DQC (2.6-dibromobenzoequinone-4-chloroimide) was used as the spray reagent. The R_F value was higher than the value for mbPS, as ØPS has a higher value than mØPS. Although mbPS is rather stable against alkali, hydrolysis of this compound gave products with R_F values and colour reactions with DQC identical with mPS and bPS.

A solution of m_2PS, mbPS and bromophos in ethanol was found after several weeks, to contain two more substances which was chromatographically identified as mPS and bPS. This solution was used as reference solution throughout the study.

Analysis

Analysis of the incubation mixture were done by thin layer chromatography. Activated silica gel G layers with the thickness 0.5 mm or 0.25 mm were used. The chromatograms were developed in acetonitril : water (85 : 15) or (80 : 20) v/v (3). p^{32}-labeled compounds were located on the chromatogram by laying an X-ray film in close contact with the plate for 24 hours. The reference substances were localised by spraying with the DQC reagent (3) (1% 2.6-dibromobenzoequinone-4-chlorimide in acetic acid). This reagent gives red colours with aryl phosphorothionates (aPS), and m_2PS, and yellow colours with maPS and mPS.

Microbiological studies

Preliminary studies with the fungi Alternaria tenius and Trichoderma lignorum had shown that all the water soluble metabolites were in the medium. The mycel contained most of the unmetabolised bromophos which was absorbed or adsorbed, but no water soluble metabolite was detected. After the myceles were macerated in a mortar with quartz sand and partioned between petrolether and water, all the radioactivity was found in the petrolether phase.

Therefore only the medium was analysed in these investigations.

50 strains of microorganisms were isolated from garden soil. They were isolated on soil extract agar (4) (which contained 15 g agar, 1.0 g glucose 0.5 g K_2HPO_4, 100 ml soil extract and 900 ml water) and were grown in 1 ml of the same medium with no agar added. 48 hours after the inoculation, P^{32}-bromophos, dissolved in ethanol was added. 48 hours after the bromophos addition, the standard solution was added in sufficient amounts to give a concentration of 5 µg/25 µl of its compounds in the medium. 25 µl of the medium was then applied on chromatograms of 0.5 mm thickness and developed as described.

Laboratory strains of Alternaria tenius, Trichoderma lignorum, Aspergillus niger, Fusarium solani and Phizopus nigricans were also tested using the same medium.

Studies on seedlings

Seeds of onion, carrot and wheat were treated with 1 µl P^{32}-bromophos solution pr. seed and placed on a moistened filter paper. A forthnight after

the treatment, the roots, and the leaves were separated from the seeds, and the parts were separately macerated with quartz in a mortar, and then extracted in ethanol, with some of the standard solution added. The extracts were then analysed as already described. In some of the experiments, the unmetabolised bromophos was removed by extraction with petrolether.

RESULTS

Microbial degradation

All the microorganisms degraded bromophos to some extent, but the activity varied considerably. Some produced m_2PS and mPS, and others produced mainly bPS. It was not possible to detect mbPS. Fig. 1 is a typical chromatogram of microorganisms. Other products, possibly the oxygen analoges of m_2PS and mPS were sometimes detected. A compound with slightly less R_F value than mbPS may be its oxygen analoge (mbPO). T. lignorum and A. tenius seem to be particularly active in the bromophos metabolism.

Metabolism by the seedlings

The seedlings also produced metabolites. Wheat was most active, and the carrot least active. The

main metabolites are inorganic phosphates or other compounds with $R_F O$. The onion seedlings produced most bPS, which was located mainly in the leaves. Onion seeds which did not germinate, produced no metabolites. In the chromatogram shown (fig. 2) bPS appeared as double spots, there was, however, excellent coincidence between the carrier and the radioactivety.

DISCUSSION

The results indicate that aPS can be formed directly by biological degradation of dimethyl aryl phosphorothionates. However, mbPS is rather stable. If formed as an intermediate one should expect it to accumulate to detectable amounts. By methyl transfer reactions, mbPS and other maPS compounds are easily formed from the analogous dimethyl compounds. Hilgetag (5) has shown that nucleophilic reagents as dimethyl sulfide, thiourea and tertiary amines react with m_2aPS to give salts of maPS. Also in biological systems such methyl transfer reactions occurs. Fukami and Shishido (6) found a soluble enzyme system which transferred a methyl group from methyl parathion to glutathione.

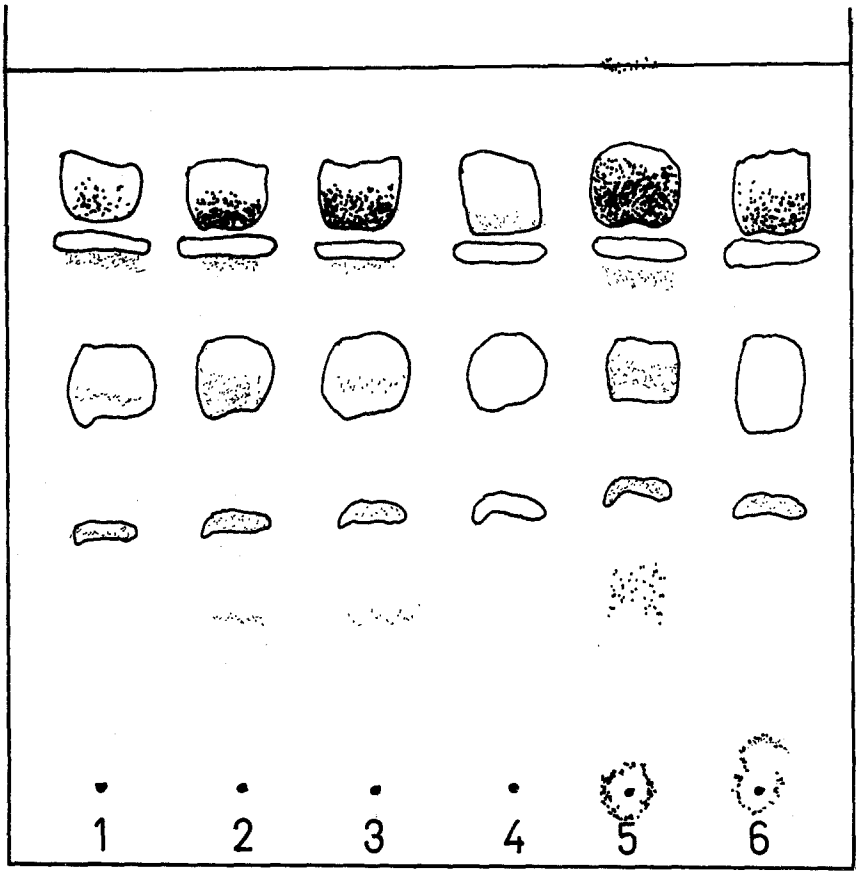

Fig. 1. TLC of P^{32}-bromophos metabolites formed by some fungi. The radioactive metabolites are dotted and the non-radioactive reference substances are encirceled (from top downward: bPS, mbPS, m_2PS and mPS) 1, 2, 4, 6: Unidentified soil fungi, 3: Trichoderma lignorum, 5: Alternaria tenius. Incubation time was 9 days. Unmetabolised P^{32}-bromophos was removed by petrolether extraction before chromatography.

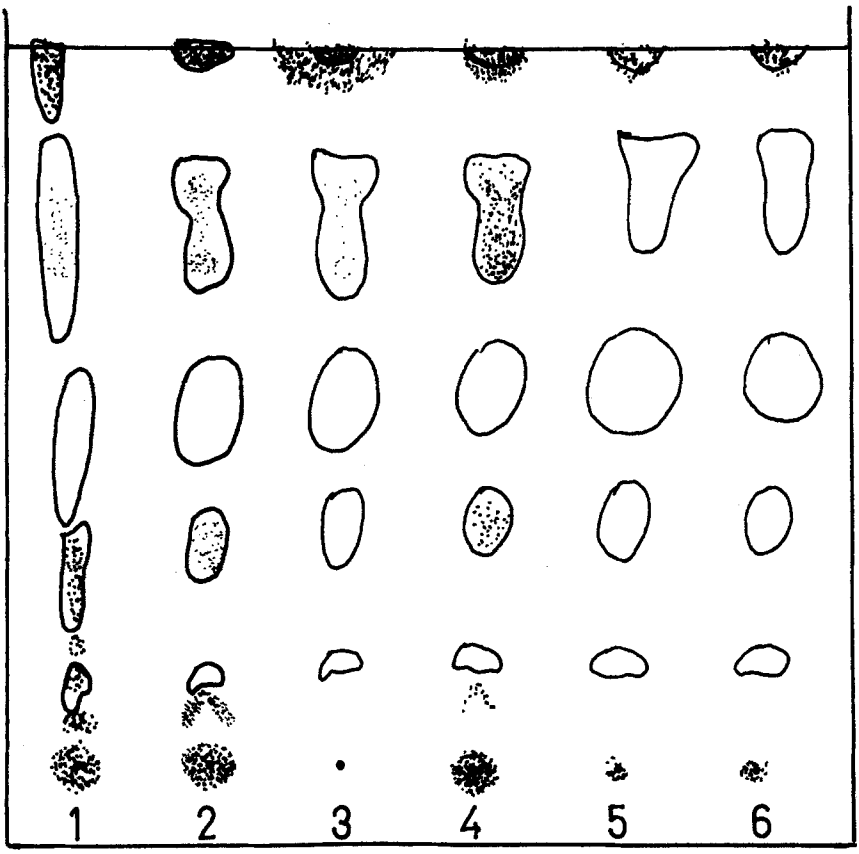

Fig. 2. TLC of P^{32}-bromophos metabolites formed by seedlings. The radioactive metabolites are dotted and the non-radioactive reference substances (from the top downwards: bromophos, bPS, mbPS, m_2PS and mPS) are encirceled. 1: The leaves of germinated wheat. 2: The seed of germinated wheat. 3: Purified P^{32}-bromophos. 4: The leaves of germinated onion, and 6: ungerminated onion seeds. Growing time was 14 days.

Hydrolytic degradation by biological systems seems, however, to give the bisdesmethyl compound directly. This may also be the case for other dimethyl aryl phosphorothionates.

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