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SHORT COMMUNICATIONS

Metabolic activation of alkylphenyl phosphates

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SOME triaryl phosphates are neurotoxic and cause ataxia in various animals. Except tri-*p*-ethylphenyl phosphate (TPEP), they have at least one *o*-tolyl or *o*-alkylphenyl ester group.[1–3] Eto and his co-workers[4–6] demonstrated that *o*-tolyl phosphates are metabolized to saligenin cyclic aryl phosphates, which are responsible for the neurotoxic action,[7–10] through hydroxylation of *o*-methyl group and subsequent cyclization. This scheme is, however, not applicable to TPEP.

In the feces of rats administered TPEP and of mice administered *O,O*-diethyl *O*-*p*-ethylphenyl phosphorothioate *p*-hydroxyacetophenone was found. This suggests a possibility that *p*-ethylphenyl phosphates may be oxidized *in vivo* to *p*-acetylphenyl phosphates. Though the active metabolites of tri-*o*-tolyl phosphate were easily detected by their antiesterase activity,[4] any distinct inhibitors of plasma cholinesterase were not detected in the feces of hens administered TPEP. Then radiophosphorus-labeled TPEP was used for the further study of metabolism in hens, mice and houseflies.

Adult oriental houseflies were each treated topically with 14 μg of TPEP-^{32}P dissolved in acetone. After 48 hr the flies were homogenized and extracted with acetone. Ether soluble neutral fraction was obtained in the normal way and was subjected to silica gel thin layer chromatography. Seven metabolites were detected on autoradiogram (Table 1). Four of them were identified as mono-, di- and tri-α-hydroxy and mono-α-oxo derivatives of TPEP respectively by co-chromatography with each authentic compound. The other two metabolites were assumed to be mono-α-hydroxy-mono-α-oxo and di-α-hydroxy-mono-α-oxo derivatives on the basis of their R_f values. Male mice were treated intraperitoneally with TPEP-^{32}P in a dose of 500 mg/kg. After 24 hr livers and intestines were removed for the extraction of metabolites. In a similar way the extract was analyzed and essentially the

TABLE 1. *In vivo* AND *in vitro* METABOLITES OF TPEP-^{32}P

Metabolite		*In vivo* conversion (%)	*In vitro* conversion (mμmole)‡		
Derivative of TPEP	R_f*	Houseflies†	Microsome§ (I)	Soluble fraction‖ (II)	I+II
mono-α-hydroxy	0·50	1·0	2·2	0·4	10·2
di-α-hydroxy	0·10	0·7	0·6	—	6·7
tri-α-hydroxy	0·01	0·5	0·4	—	3·8
mono-α-oxo	0·71	0·2	trace	—	3·8
mono-α-oxo, mono-α-hydroxy	0·25	0·2	trace	—	4·3
mono-α-oxo, di-α-hydroxy	0·04	trace	—	—	3·1
unknown	0·57	trace	trace	trace	trace

* Silica gel thin layer chromatography developed with ethyl acetate–hexane (5:6). Hydroxylated esters were visualized by spraying 2,6-dibromoquionone-chloroimide after hydrolysis with alkali. Carbonyl compounds were with 2,4-dinitrophenylhydrazine. Metabolites were detected by autoradiography. Another solvent system, chloroform–ether (10:1), was also used for identification.

† Two days after treatment.

‡ TPEP-^{32}P (1·46 μmole) was incubated in 0·025 M tris buffer (pH 7·5) including 12·5 μmole nicotinamide and indicated additives for 4·5 hr at 37°.

§ Microsome corresponding to 0·8 g liver fortified with 2·5 μmole TPNH.

‖ Soluble fraction corresponding to 0·4 g liver fortified with 2·5 μmole DPN.

same metabolites as from houseflies were detected. Hens were given a dose of 500 mg/kg through a stomach tube. Hydroxy and mono-α-oxo derivatives were detected in the feces. The mono-α-hydroxy derivative was the main metabolite in all cases. It was isolated by repeating column and thin layer chromatographies from the feces of hens after the oral administration of a total of 8·5 g of TPEP. Its chemical structure was confirmed by mass spectrometry; m/e = 426·1595 ($C_{24}H_{27}O_5P$ = M), 411 (M—CH_3), 408 (M—H_2O).

Similar results were also obtained in *in vitro* experiments. Rat liver microsome fortified with NADPH afforded three hydroxy derivatives from TPEP-^{32}P. By the addition of liver soluble fraction and NAD to the microsome system, the dehydrogenation of the hydroxy compounds took place and, moreover, the hydroxylation itself was promoted (Table 1). The dehydrogenation was demonstrated by the conversion of isolated mono-hydroxy metabolite-^{32}P to the corresponding oxo derivative by the incubation with the soluble fraction with NAD. In another experiment, synthesized tri-α-hydroxy derivative was converted to three oxo derivatives by the action of the soluble fraction fortified with NAD. One of the products was identical with an authentic sample of tri-oxo derivative on thin layer chromatography. The metabolic pathway of TPEP is, thus, summarized as shown in Fig. 1; *p*-ethyl group is hydroxylated by the action of microsomal "mixed function oxidase" to give α-hydroxyethyl group, which is then transformed to acetyl group by the action of a soluble dehydrogenase.

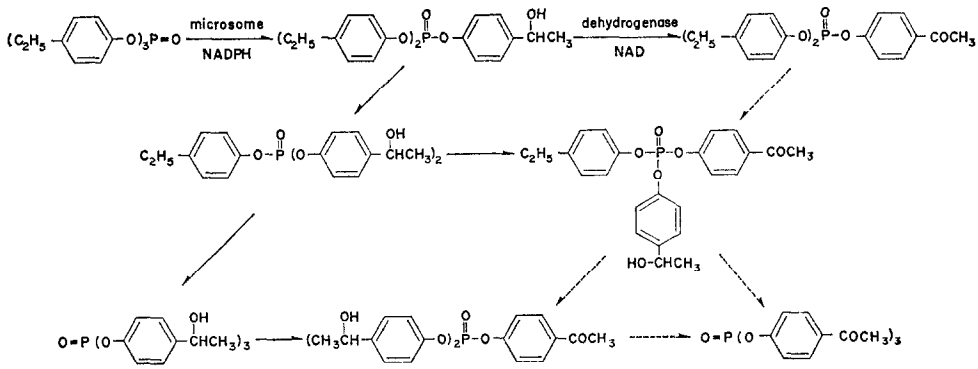

FIG. 1. Metabolic pathway of tri-*p*-ethylphenyl phosphate.

The strong electron-withdrawing property of *p*-acetyl group, whose Hammett's sigma constant is +0·874, suggests that the α-oxo metabolites may be biologically active. Therefore, some *p*-acetylphenyl phosphate esters were synthesized and examined for some biological activities. Mono-α-oxo derivative of TPEP (di-*p*-ethylphenyl *p*-acetylphenyl phosphate; b.p. 225–230° (0·075 mmHg), nD^{24} 1·5577) caused ataxia in hen by oral administration of 100 mg/kg. Tri-oxo derivative (tri-*p*-acetylphenyl phosphate; m.p. 123°) also caused ataxia by an oral dose of 50 mg/kg, if the same amount of TPEP was mixed to the dose. It is reasonable to consider that these oxo metabolites may contribute, at least in part, to the neurotoxicity of TPEP.

All the known neurotoxic organophosphorus compounds are active inhibitors of certain esterases either *in vitro* or *in vivo*.[8,11] TPEP had a poor antiesterase activity *in vitro*. It inhibited 46 per cent of chicken liver esterase activity against phenyl acetate at 2×10^{-4} M and 30 per cent of human plasma cholinesterase activity at 4×10^{-3} M. The mono-oxo derivative showed a moderate activity; I_{50} values for these esterases were 2×10^{-4} M and 9×10^{-5} M respectively. The tri-oxo derivative was a potent inhibitor of these esterases; I_{50} values for them were $1{\cdot}1 \times 10^{-7}$ M and $1{\cdot}6 \times 10^{-7}$ M respectively. Thus, the conversion of TPEP to tri-oxo compounds is an activation of as much as 2000 to 20,000 times in respect of antiesterase activity.

These tri-aryl phosphates having *p*-acetyl group were not insecticidal. However, dialkyl homologs, for example diethyl *p*-acetylphenyl phosphate, showed high insecticidal activity (LD_{50} = 0·48 μg/housefly) and potent anticholinesterase activity (I_{50} for housefly cholinesterase = $8{\cdot}6 \times 10^{-9}$ M). The corresponding *p*-ethyl analogs had almost the same insecticidal activity but no antiesterase activity *in vitro*. This suggests that they should be metabolized to the active oxo analogs *in vivo*.

Tri-*m*-tolyl phosphate is not neurotoxic.[1-3] It was administered orally to rabbit and *m*-(α-hydroxy)-cresol was found in urine. Hook *et al.*[1,2] found that a low toxic insecticide Sumithion, *O,O*-dimethyl *O*-3-methyl-4-nitrophenyl phosphorothioate, was metabolized to 2-nitro-5-hydroxybenzoic acid in several species of mammals. It appears that the hydroxylation of meta alkyl group may not relate with the activation of the esters, but with degradation.

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Determination of human urinary kinin levels by radioimmunoassay using a tyrosine analogue of bradykinin*

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BRADYKININ (BK), kallidin, and methionyl-lysyl-bradykinin, are a group of closely related vasodilator peptides. It has been shown that the flushing attacks experienced by patients with the carcinoid syndrome[1,2] are frequently associated with elevated blood kinin levels and it is widely believed that they are important mediators of inflammation. The existence of these peptides in the blood,[3] urine,[4] and other body fluids of normal individuals, suggests that they may also have a physiological function but difficulties in quantitating the kinin levels in various situations have limited progress in defining any such role.