Stereoselectivity of a radioimmunoassay for the insecticide S-bioallethrin[1]

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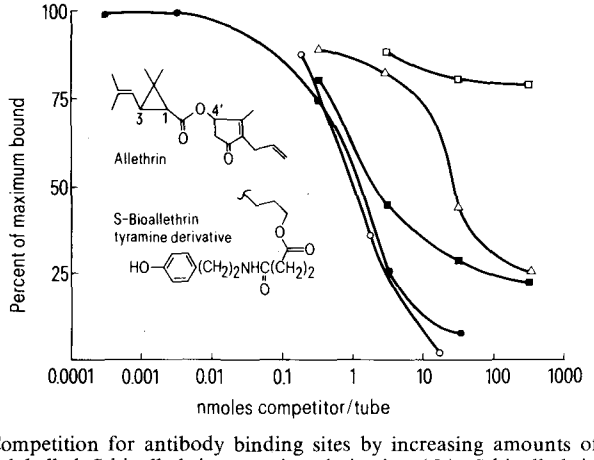
Summary. The stereoselectivity of a radioimmunoassay (RIA) system using an S-bioallethrin specific antiserum was studied by observing the abilities of the 8 allethrin isomers and other selected compounds to compete with a radiolabelled S-bioallethrin tyramine derivative for antibody binding sites. The results demonstrate the feasibility of RIA as a rapid, sensitive and stereoselective residue technique for compounds difficult to analyze by classical methods.

Although immunochemical techniques and especially radioimmunoassay (RIA) have demonstrated excellent sensitivity, reproducibility and cost effectiveness in other fields[2,3], they have received little attention for the monitoring of environmental contaminants[4-7], an operation necessary for the maintenance of environmental quality and worker safety. Recently, pyrethroids (synthetic mimics of the pyrethrins from *Chrysanthemum cinerariaefolium*) have emerged as a promising class of insecticides due to their favorable toxicological and environmental properties[8]. Present residue techniques for these compounds are insensitive, laborious, and/or expensive[9-11]; in addition, they are unable to distinguish between stereoisomers which may differ markedly in toxicity[8,12]. This study demonstrates the optical selectivity of an RIA for the marketed insecticidal isomer of allethrin, S-bioallethrin (1R, 3R, 4'S; figure).

Materials and methods. Generation of an S-bioallethrin specific antibody titer is described previously[7]. Reaction of an S-bioallethrin hemisuccinate derivative with ^{3}H-tyramine (5–10 Ci/mmole, New England Nuclear Corp.) provided a radioligand (S-bioallethrin-tyramine derivative, figure). The 8 geometrical and optical isomers of allethrin were synthesized by slight modification of classical methods[7,13,14] from highly purified corresponding chrysanthemic acids and allethrolones (> 96%, Sumitomo Chemical Co.). Structures were verified by ^{1}H-magnetic resonance, IR and UV spectroscopy, co-chromatography and optical rotation.

All competition assays were performed under equilibrium conditions in 6×50 mm glass tubes containing 200 µl of serum diluted with sodium phosphate buffer (I=0.2 M, pH 7.4, 0.01% NaN_3) to bind 42% of 65.5 nCi of the tritiated S-bioallethrin tyramine derivative. Radioligand and competitors were each added to the serum in 1 µl of ethanol, incubated at 37 °C for 10 min and at 4 °C for 12 h. Unbound radioligand was precipitated by the addition of 100 µl of a charcoal suspension in phosphate buffer (10% acid washed Mallinckrodt charcoal and 0.01% dextran T70) followed by centrifugation (0 °C, 15 min, 5000× g). Radioactivity in the supernatant was determined by liquid scintillation counting of a 100 µl aliquot.



Competition for antibody binding sites by increasing amounts of unlabelled S-bioallethrin tyramine derivative (○), S-bioallethrin (● **1**), and other selected allethrin isomers (■ **2**, △ **3**, □ **8**) with tritiated S-bioallethrin tyramine derivative. The absolute configuration of the allethrin isomers are presented in the table.

Results and discussion. S-Bioallethrin reproducibly competed with the radioligand for antibody binding sites in a dose-dependent manner. At 1.2 nmoles (figure, table, **1**), 50% of the radioactivity was displaced and ultimately > 93% radioligand displacement could be obtained. The unlabelled S-bioallethrin tyramine derivative was a better competitor (50% radioactivity displacement at 1 nmole, maximum displacement > 99%) than S-bioallethrin as was its anti-Markovnikov allyl side chain alcohol derivative (50% radioactivity displacement at 0.95 nmoles). This result could be anticipated since these 2 compounds more closely resemble the injected hapten than the parent allethrin. Based on the estimated specific activity of the radioligand, the unlabeled S-bioallethrin tyramine derivative was expected to displace the radioligand at much lower concentrations than those indicated (figure). No cause could be elucidated for this anomoly. R-Bioallethrin (figure, table, **2**) was only half as active a competitor as S-bioallethrin, and the competition curve displayed a different shape.

Competition displayed by other isomers (**3–8**) may be explained in part by trace contaminants of more competitive isomers. Absolute configuration at the 4' position of the allethrolone moiety is generally less critical than the 1 and 3 positions of the chrysanthemate moiety in determining competitive ability, illustrating the accepted doctrine that the portion of the hapten farthest from the protein is usually the most important in leading to antibody population specificity[3,15]. In addition, esters of the trans-chrysanthemates having the 1R, 3R configuration (**1, 2**) are better competitors than the 1S, 3S isomers (**3, 6**) and, with the exception of compound (**6**), the trans-isomers were better competitors than the cis-isomers (**4, 5, 7, 8**), with the 1S, 3R, 4'R isomer (**8**) demonstrating the weakest competition as expected.

Specificity of anti-S-bioallethrin serum for the 8 isomers of allethrin

Compound Number	Absolute configuration 1	3	4'	nmoles needed to reduce antibody bound radioactivity by 50%
1	R	R	S	1.2
2	R	R	R	2.1
3	S	S	S	24
4	R	S	R	41
5	S	R	S	60
6	S	S	R	160
7	R	S	S	> 1000
8	S	R	R	≫ 1000

Absolute configuration denotes stereochemistry at specified carbon of chrysanthemic acid (1, 3) or allethrolone (4') moiety (see figure).

The free chrysanthemic acids and allethrolones, and the pyrethroids permethrin and S-5602[16] were negligible competitors at their solubility limits. Even when 1R, 3R chrysanthemic acid was esterified to 5-benzyl-3-furyl methanol or 3-phenoxybenzyl alcohol[16] (bioresmethrin and (+)-trans phenothrin, respectively), little competition was observed. However, an extract of *Chrysanthemum* flowers containing ~43% pyrethrins[17] (all of which closely resemble S-bioallethrin) gave 50% radioactivity displacement at 1.5 nmoles/assay. These experiments indicate that this antibody population selectively recognized the entire S-bioallethrin molecule.

Antibody stereoselectivity for the haptenic optical or geometric configuration thus represents a powerful potential tool to the environmental chemist. Development of RIA's for pesticides may not only simplify and improve detection of parent compound residues, but may also be applied to metabolism studies, monitoring of compound shelf life, and forensic toxicology.

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Effect of hydroxydopamine on the morphine-induced reduction in brain acetylcholine turnover

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Summary. Morphine reduced brain acetylcholine turnover in normal and 6-hydroxydopamine-pretreated rats and mice. Morphine probably has a direct effect on cholinergic neurons rather than modifying acetylcholine indirectly through catecholamine neurons. Acetylcholine is not directly involved in morphine's antinociceptive action in the mouse but it could be implicated in the rat.

Morphine interacts with brain acetylcholine (Ach). It reduces Ach release in vivo[1] and in vitro[2], increases brain Ach content[3] and reduces its turnover[4,5]. It is not clear whether cholinergic neurons are affected directly by morphine or if Ach has a major role in morphine's antinociceptive action. Interactions occur in brain between cholinergic and catecholaminergic neurons[6] so it is possible that morphine could have an indirect effect on Ach. There is an established connection between morphine, Ach and the catecholamines because dopamine drugs alter Ach turnover[7] and morphine increases the turnover of brain dopamine[8]. Drugs that affect brain catecholamines alter morphine's antinociceptive action[9]. 6-Hydroxydopamine (6-OHDA), which depletes brain noradrenaline and dopamine[10–12], antagonises morphine's antinociceptive action in mice[13]. The possibility that Ach could have a direct, major role in morphine's antinociceptive action has been investigated by measuring morphine-induced reductions in Ach turnover in rats and mice pretreated with 6-OHDA.

Materials and methods. Female Sprague-Dawley rats (150–155 g) and male albino mice (25–35 g) were used. Rats, anaesthetized with ether, received 2 intraventricular injections[14] of either saline solution or 6-OHDA (250 µg) 48 h apart. Mice, anaesthetized with ether, received 2 doses of 6-OHDA (75 µg) intraventricularly[15]. 10 days were allowed after the 6-OHDA for the depletion of catecholamines. The turnover of Ach was estimated by measuring Ach levels following inhibition of its synthesis with hemicholinium-3 (HC-3)[4]. Groups of saline- and 6-OHDA-pretreated rats and mice were given 16 mg kg^{-1} of morphine i.p. 10 min before HC-3 intraventricularly[14,15]. Each rat received 20 µg of HC-3 and the mice received 1 µg for each 15 g of b.wt. The animals were killed 30 min later using the brain rapid ejection and freezing apparatus[16]. The discs of frozen brain were extracted in 5 ml g^{-1} of acidified ethanol solution[17] and the Ach content was measured by bioassay. Rats and mice pretreated with saline or 6-OHDA intraventricularly were killed by decapitation for the fluorimetric measurement[18] of brain noradrenaline and dopamine.

Brain noradrenaline and dopamine in rats and mice following intraventricular administration of saline and 6-OHDA

Species	Treatment	Noradrenaline (µg g^{-1} ± SEM)	Dopamine (µg g^{-1} ± SEM)
Rat	Saline	0.32 ± 0.02 (7)	1.09 ± 0.03 (6)
Rat	6-OHDA	0.07 ± 0.01[b] (8)	0.75 ± 0.03[a] (6)
Mouse	Saline	0.44 ± 0.02 (6)	1.31 ± 0.04 (6)
Mouse	6-OHDA	0.16 ± 0.02[b] (6)	0.70 ± 0.03[a] (6)

Significance of difference between saline- and 6-OHDA-treated. (Student's t-test) [a] $p < 0.01$; [b] $p < 0.001$. Number of animals used shown in brackets.