Geranyl acetate, a compound with a lemon-like odor has been described previously as a minor constituent of the labial gland secretion of *Bombus* males[6] and it is also found in the mandibular glands of 2 ceratinine bees, *Ceratina*[7] and *Pithitis*[8]. In the latter, as in *S. caementarium,* it is released when the insects are handled roughly. On the other hand, 2-decen-1-ol has never been identified from an arthropod source but its acetate is one of the volatile components of honey bee venom[9].

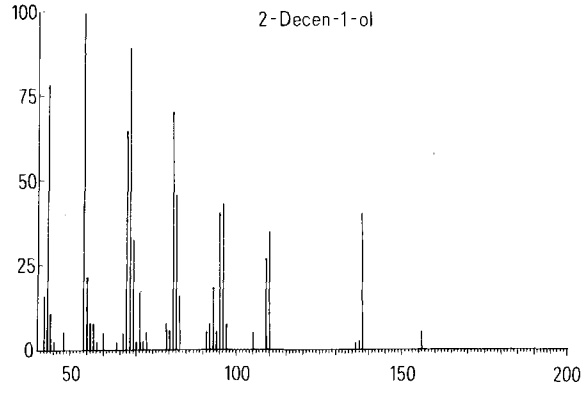

Fig. 2. Mass spectrum of 2-decen-1-ol.

The function of the cephalic secretion of *S. caementarium* has not been determined in field tests. Apparently it is not used in nest site recognition since these wasps locate their nests by visual cues[11]. The odoriferous secretion may aid in the initiation and maintenance of roosting aggregations as is suggested from behavioral data[4], but it may also have a defensive function since it is readily released when the wasps are handled.

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Inhibition of the NADP-linked glutamate dehydrogenase from *Trypanosoma cruzi* by silver nitrate

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Summary. The purified NADP-linked glutamate dehydrogenase from *Trypanosoma cruzi* was strongly inhibited by silver nitrate. The inhibition was reversed by reduced glutathione, and was modified by the presence of the substrates during preincubation of the enzyme with the inhibitor.

The culture, epimastigote form of *Trypanosoma cruzi,* the parasitic protozoa which causes the American trypanosomiasis, Chagas' disease, contains a NADP-linked glutamate dehydrogenase (L-glutamate: NADP oxidoreductase [deaminating] EC 1.4.1.4)[2]. We have recently purified this enzyme to electrophoretic homogeneity, and studied some of its kinetic properties[3]. A comparative study of the biochemistry of the parasite and its host, looking for differences between them suitable for chemotherapeutic attack, is important for the design of a rational approach to the chemotherapy of Chagas' disease[4]. Since this glutamate dehydrogenase differs in many respects from the similar enzyme in the mammalian host, it has recently been considered among the 'targets with possible chemotherapeutic potential in *T. cruzi*'[4]. Silver nitrate, which is able to react with thiol groups with mercaptide formation, although it may also react with other groups on the enzyme protein[5], is known to inhibit glutamate dehydrogenases from several sources, including mammals[6]. We decided to study the effects of this inhibitor on the purified glutamate dehydrogenase from *T. cruzi,* in order to characterize the enzyme further, and as the first part of a project which will include different sulfhydryl reagents and trypanocide drugs.

Methods. Glutamate dehydrogenase was purified to electrophoretic homogeneity from epimastigotes of *T. cruzi,* Tulahuén strain, as previously described[3]. The enzyme activity was assayed spectrophotometrically at 30 °C, in the presence of 20 mM Tris-acetate buffer, at pH 7 (amination of α-oxoglutarate) or 8 (deamination of L-glutamate)[3]; preincubation conditions are described in the legend to table 2.

Results and discussion. The glutamate dehydrogenase from *T. cruzi* was strongly inhibited by silver nitrate. When the enzyme (0.1 µg) was preincubated for 1 min at 30 °C in 20 mM Tris-acetate buffer, pH 7.0, with concentrations of $AgNO_3$ up to 1 µM, before starting the reaction by addition of the substrates α-oxoglutarate, NADPH and NH_4Cl (final concentrations 0.5, 0.01 and 87 mM, respectively), 50% inhibition was attained at about 0.12 µM $AgNO_3$. Since no special precautions were taken to exclude completely chloride ions from the reaction mixtures, the actual Ag^+ concentrations were probably lower. The inhibition could be reversed by incubation of the $AgNO_3$-inhibited enzyme with reduced glutathione; the glutamate dehydrogenase inhibited up to 85% by preincubation with 0.5 µM $AgNO_3$ was completely reactivated by further preincubation for

Table 1. Effect of silver nitrate on the kinetics of the reaction catalyzed by glutamate dehydrogenase from *Trypanosoma cruzi*

Variable substrate	Apparent K_m (M)		Apparent V_{max} (μmoles/min/mg of protein)	
	No $AgNO_3$	0.5 μM $AgNO_3$	No $AgNO_3$	0.5 μM $AgNO_3$
α-oxoglutarate	1.1×10^{-3}	6.6×10^{-3}	214	214
NADPH	3.4×10^{-6}	3.4×10^{-6}	89	62
NH_4Cl	2.3×10^{-2}	2.3×10^{-2}	80	53
	8.8×10^{-2}	8.8×10^{-2}	214	128
L-glutamate	6.2×10^{-3}	1.4×10^{-2}	10.1	10.1
NADP	2.7×10^{-5}	2.7×10^{-5}	7.1	5.3

The enzyme activity was assayed as described under 'methods' and Cazzulo et al.[3], without preincubation with $AgNO_3$. The experiment with NH_4Cl, and those with NADP or L-glutamate, as variable substrates, were performed in the presence of 52 mM or 105 mM KCl, respectively. The 2 apparent K_m- and V_{max}-values for NH_4Cl were calculated from the 2 linear segments of the double reciprocal plots[3].

Table 2. Effect of preincubation with substrates on the inhibition of the glutamate dehydrogenase from *Trypanosoma cruzi* by $AgNO_3$

Concentration of $AgNO_3$ (μM)	Inhibition (%)				
	0	0.1	0.25	0.5	1.0
Experiment A					
Reductive amination of α-oxoglutarate					
Control (no additions)	0	53	66	76	92
Plus 0.5 mM α-oxoglutarate	0	34	50	63	86
Plus 0.1 mM NADPH	0	64	75	82	94
Experiment B					
Oxidative deamination of L-glutamate					
Control (no additions)	0	33	50	62	80
Plus 5 mM L-glutamate	0	50	65	76	87
Plus 0.07 mM NADP	0	17	39	54	75

The enzyme (0.12 μg in experiment A and 0.2 μg in experiment B) was preincubated for 1 min at 30 °C in 20 mM Tris-acetate buffer (pH 7.0, experiment A, or pH 8.0 experiment B), alone or in the presence of 0.1 mM NADPH or 0.5 mM α-oxoglutarate (experiment A), or alone or in the presence of 0.07 mM NADP or 5 mM L-glutamate (experiment B) before preincubating for a further 1-min period with $AgNO_3$, at the concentrations stated. Then the reaction was started by addition of the other substrates at the concentrations stated above, plus 87 mM NH_4Cl (experiment A) or 105 mM KCl (experiment B). The activity of the non-inhibited enzyme, taken as 100% of activity, was 7.2 and 2.3 nmoles/min, in experiments A and B, respectively.

5 min with 2 mM reduced glutathione, before starting the reaction by addition of the substrates.

When the effect of the inhibitor at 0.5 μM, without preincubation with the enzyme, was studied, the inhibition was of competitive type with respect to the main substrate of the reaction, α-oxoglutarate or L-glutamate, and strictly non-competitive towards NADP, NADPH or NH_4Cl (table 1). Inhibitions of competitive type are known for thiol reagents, such as mercurials, despite the high affinity of the reagents towards the enzymes[7], and are evident when the assay is performed before maximal binding of the inhibitor is reached, as in the case of table 1. When the enzyme was preincubated with $AgNO_3$ all the inhibitions were of non-competitive type. Usually this competition reflects a transient protection of the enzyme by the substrates. Table 2 shows that the presence of α-oxoglutarate, and also of NADP, at the assay concentrations during the preincubation of the enzyme with $AgNO_3$ decreased the inhibition. However, when L-glutamate or NADPH were present during preincubation, the inhibition attained was greater than in the controls. Other cases of increase in the inhibition of an enzyme in the presence of a substrate are known[7]. These results differ from what one might expect from those in table 1; however, it must be taken into account that the experimental conditions in the experiment of table 2 were different from those in the experiment of table 1, where all substrates were present when the inhibitor reacted with the enzyme. The effects of preincubation with α-oxoglutarate or L-glutamate, in the absence of the coenzyme, would be difficult to understand if the reaction mechanism was strictly ordered, as suggested initially by Frieden[6]; however, they are compatible with the rapid equilibrium random mechanism suggested by Engel and Chen[8] for the glutamate dehydrogenase from beef liver.

Our present results do not allow us to establish whether the group(s) reacting with $AgNO_3$ is at or in the neighbourhood of the active site, nor, if the latter case is true, whether they are involved in the binding of the substrates or in the catalysis itself. Recent experiments with organic sulfhydryl reagents suggest that there are at least 2, and probably more, sulfhydryl groups involved (Juan, Segura and Cazzulo, unpublished results); this might help to explain the different results obtained by preincubation of the enzyme and the inhibitor in the presence of different substrates, since binding of a substrate might mask one sulfhydryl group, and at the same time increase the exposure of another, placed in the domain which interacts with the other substrate, through the conformational change induced at the active site.

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