INTOXICATION OF APHELENCHUS AVENAE BY ETHYLENE DIBROMIDE

BY

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The exposure of Aphelenchus avenae to low concentrations of ethylene dibromide results in a very small conversion of the halide by the nematodes just preceding death. Using ethylene dibromide 1,2-C14, two primary products are characterized. These are ethylene (~5%) and O-acetylserine (~95%). These products account for all of the ethylene dibromide converted. The radiolabel in O-acetylserine resides in the carbons of the acetyl moiety. These transformations are taken to indicate two primary modes of intoxication of the nematodes. These are formulated as: (1) a direct reaction of the halide with an iron center in the respiratory sequence and (2) the substitution of a serine at the active site of an esterase or protease.

Alkyl halides are well established as effective soil fumigants. Yet the capacity of these simple molecules to control nematodes, fungi and other plant pathogenic organisms is wholly uncharted at the molecular level. While the metabolism of some of these substances, principally by Flavobacteria and Pseudomonas spp has been noted (Castro & Bartnicki, 1965, 1968, 1969; Castro & Belser, 1968, 1971) their molecular interaction with nematodes remains undefined.

We report here our studies of the very low level metabolism of the fumigant ethylene dibromide by the nematode Aphelenchus avenae. The work was undertaken with the aim of pinpointing the first chemical processes that occur preceding death. We take these processes to reflect interaction with the most sensitive sites in the animal and the means of intoxication by the chemical.

MATERIALS AND METHODS

Materials — Cultures of Aphelenchus avenae were grown on Rhizoctonia solani on sterilized wheat in quart mason jars at room temperature (Evans, 1969). Animals were harvested by washing in 250 μm sieves and collected via the Baermann Funnel technique in a mist chamber. Aqueous suspensions of the nemas were filtered on a sterile medium porosity sintered glass funnel, washed five times with 100 ml portions of sterile distilled water, scraped from the funnel and weighed.

Ethylene dibromide 1,2-C14 from New England Nuclear was diluted with pure non-radioactive substance to give a specific activity of 1.02 × 10^8 disintegrations/minute/millimole. O-Acetylserine, m.p. 156-158° (decomp); mass spec: 147 (parent), 102 (P-CO2H); infrared carbonyls (H2O): 1725 (ester), ~1625 (amino acid carboxyl); was prepared by the acetylation of L-serine (Mann Biochemicals) in acidic milieu (Sakami & Toennies, 1942). N-Acetylserine was
obtained from 1-serine via the sequence: serine → O-benzoylserine → O-benzoyl-
N-acetylserine → N-acetylserine (Synge 1932). An aqueous solution of the sub-
stance showed a mass spectrum nearly identical to the o-acetyl isomer: 147 (parent),
102 (P-CO₂H). Major carbonyl absorption in the infrared was a broad composite:
1618 (sh) (Carboxylate and 1650 cm⁻¹ (amide I).

Methods. — Bromide ion was determined by direct potentiometry (Castro &
Bartnicki, 1965) using an Orion specific ion electrode. Ethylene was characterized
by gas chromatography of itself and its dibromide derivative (Belser & Castro,
1968), and by its gas infrared spectrum. The substance was quantitated from
reactions by flame ionization gas chromatography on an Aerograph A-600C
machine equipped with a 15 cm Porapak P column that was coated with 3%
diethylene glycol succinate. Propylene was employed as an internal standard.
O-Acetyl serine, ninhydrin positive, was characterized by final co-chromatography
on thin layer silica Gel-G plates with an authentic sample and by its mass and
infrared spectrum. These latter were identical with authentic o-acetyl serine. Ninhy-
drin negative N-acetyl serine was characterized in similar fashion. The rather com-
plicated chromatographic procedure employed is detailed below.

Reaction conditions. — A typical run is depicted below.

Nematodes (23 g wet) were placed in a sterile, round bottom flask fitted with
a serum-stoppered stopcock. Fifty ml of ethylene dibromide 1,2-C¹⁴ 5.8 × 10⁻⁸ M
in sterile water was added to the flask (2.89 × 10⁻⁴ moles EDB) and shaken at
room temperature on a reciprocal shaker for 24 hours. At this time 10 µl of pro-
pylene were added to the gas phase in the flask. Three 0.6 ml samples of the
gas phase were withdrawn and analyzed for ethylene by gas chromatography. The
yield of ethylene was 1.53 × 10⁻⁷ moles. The flask was opened and the material
transferred to a beaker. Bromide ion was determined to be 2.75 × 10⁻⁴ M
(1.92 × 10⁻⁵ moles). The beaker was placed in an ice bath and the material
sonicated with a Bronwill probe sonicator for a total of approximately 12 min in
short bursts of about 2 min each. No whole nematodes were observed under a
dissecting scope after this procedure. The sonicate was transferred to a separatory
funnel and extracted with three 100 ml volumes of ether and centrifuged after
each shaking to break the emulsion. The ether fractions were pooled and tested
for radioactivity. All counts were accounted for by unreacted EDB as determined
by gas chromatography of this solution. The aqueous sonicate was centrifuged for
30 min at 15,000 RPM. Three layers were formed: a thin top crust, a liquid layer,
and a pellet. The sixty ml aqueous layer was removed. It contained 1.32 × 10⁶
DPM. The top layer and the pellet contain 9 × 10⁶ DPM and were discarded.
The aqueous supernatant was placed on a G25 coarse Sephadex column (1.5 ×
30 cm) and eluted with water. The radioactive fractions were collected, pooled,
and reduced in volume to ~5 ml.

The clear somewhat thick greenish material was placed on a column of DEAE
cellulose (1.5 × 9 cm) and eluted with water. The turbid, green radioactive frac-