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UPTAKE AND TRANSLOCATION OF 3-AMINO- AND-3-HYDROXY- 1, 2, 4, -TRIAZOLE IN PLANTS

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3-Amino- 1, 2, 4, -triazole (AT) is known to be taken up readily by the leaves of many plants, and to be translocated rapidly (HALL, JOHNSON and LEINWEBER, 1954; ROGERS, 1957; BONDARENKO and WILLARD, 1956). In view of the simplicity of its molecule and the presumed stability of its rings system toward plant enzymes it seemed interesting to compare AT in this respect to a closely related substance, 3-hydroxy- 1, 2, 4, -triazole (OT). Both AT and OT are very soluble in water and alcohol but insoluble in acetone. AT is a weak base with a basic dissociation constant of 10^{-10}, OT a weak acid with a dissociation constant of 1.6. 10^{-9}. Neither of the two compounds diminishes the surface tension of water at a concentration of 0.1 M.

Solutions of AT and OT, labelled with C^{14} in the 5-position, were applied to one leaf of plants of French dwarf bean, tomato, four o'clock plant (*Mirabilis jalapa*) and maize (*Zea mais*), and the uptake and distribution of the tracer measured after 24 hrs. With bean plants, the uptake by leaf injection, through the roots and through the stem was also investigated. The diffusion of the two compounds through the cuticula of the epidermis was studied with isolated cuticles of the upper epidermis of pear leaves, which has no stomata.

MATERIALS AND METHODS

The labelled compounds were synthesized by Dr. Halberstadt (Philips-Roxane, Weesp) with a specific activity of about 2mc/mmole.

All the plants were grown in soil with the exception of some of the bean plants which were grown in a gravel culture. The tomato plants had 4 to 5 developed leaves when they were used. A ring of lanoline with an internal diameter of 12 mm was made on the terminal lobe of the third leaf and 10 μl of an aqueous solution containing AT or OT, 0.05 M and 0.1 % "Nacconol" (an arylalkyl sulfonate wetting agent from the Allied Chem. and Dye Corp.) was spread out within the ring with a micropipet. The four o'clock plants which had three pairs of leaves were treated on one half of one of the second leaves in the same manner. The maize plants were 20 to 30 cm long when they were used. The end of the first leaf was treated in the same manner. The bean plants were used when the first trifoliate leaf was just expanding. The solution was applied to one of the primary leaves on the first main lateral vein.

The leaf injection method of BIDDULPH (1941) was used with bean

and four o'clock plants in order to separate the translocation through the plant from the uptake through the leaf epidermis. The first main lateral vein of a primary leaf was excised so that it was still connected distally with the leaf. The flap thus obtained was bent down into a minuscule beaker containing ca 70 μl of a solution of 1 μc AT or OT.

In one experiment, bean plants grown in a gravel culture were placed with their roots in erlenmeyers containing 20 cc of a 10^{-4} M aqueous solution of AT or OT. In another experiment, bean plants were cut off just above the roots and placed in erlenmeyers containing the same solution.

The treated plants were allowed to stand for 22 to 24 hrs. at room temperature in the light of a row of fluorescent tubes, and then harvested. The treated part of the leaf was punched out and discarded. The treated leaf or the roots were separated from the rest of the plant. The plant parts were ground separately in a mortar with a little sand and formic acid, then extracted for a short time at room temperature, first with 70 % alcohol, then with water. An aliquot of the collected extracts was evaporated on an aluminium planchet and assayed for radioactivity with an end-window Geiger-Müller counter.

The activity of the residue of some plants was checked. The residue was dried at 50° in vacuo and burned in oxygen by SCHÖNINGER's method (1955). The carbon dioxide was precipitated as $BaCO_3$ and assayed for radioactivity. In most cases the residue contained 10 to 20 % of the radioactivity of the extract. In an experiment with maize, however, not more than 20 to 30 % of the radioactivity was extracted after two extractions; in repeating this experiment the plants were not extracted at all but ground as a whole in liquid air, dried, burned and assayed as $BaCO_3$. In one experiment, tomato plants were assayed in the same way.

The cuticulae used for in vitro diffusion experiments were isolated by ORGELL's method (1955). Discs 19 mm in diameter were punched from pear leaves and incubated in a 2 % solution of pectinase (Nutritional Biochemicals Corp., Cleveland, Ohio) in acetate buffer, 0.1 M, pH 4,5, containing 100 ppm ethylmercurithiosalicylate.

The preparation was shaken gently for 2 to 3 weeks at 40° until the punches had disintegrated. The cuticles were washed free from tissue, sorted under the microscope for freedom from stomata and damage, and mounted on a disc of 2 % agar, 23 mm diameter and 2 mm thick. A glass ring of 11,5 mm internal diameter was attached to each cuticle by means of apiezon grease and 20 μl of a solution of radioactive AT or OT, 0.005 M spread out in the ring, or, alternatively, the cuticles were covered with another disc of agar containing the AT or OT. After 6 to 24 hrs. the upper disc of agar of the glass ring with the cuticle was discarded, the undermost disc of agar was dissolved in water, dried on an aluminium planchet and assayed for radioactivity.

The uptake of AT or OT through the epidermis was also studied with detached primary leaves of bean. The leaves were enclosed in Petri dishes with the petiole immersed in 1 cc of water. A disc of agar

containing 15 μg of AT or OT was attached to the leaf. After 40 hrs. in the light at room temperature, the leaves were frozen in liquid air, ground, dried at 50° in vacuo, burned and assayed for radioactivity as $BaCO_3$.

The activity of the water contained in the petri dishes was checked too and found to be negligible.

An autoradiogram was made of one plant of each lot. The plant was pressed between pads of filter paper and kept in an oven for 15 min. at 120°, then for two hrs. at 90°. The dried plant was then placed on a fresh pad of filter paper, and covered with "Mylar Foil" 6 μ thick (DuPont de Nemours). A sheet of X-ray film ("Osray", Gevaert) and another pad of filter paper was placed on top of the mylar foil and the whole exposed for one to three weeks in a cardboard folder.

Results and discussion

Table I shows that the translocation out of the treated leaf is 10 to 60 times faster for AT than for OT. The uptake by intact leaves is also faster for AT, although the difference is not as great as in translocation, at least for bean plants. The diffusion through the isolated cuticula of pear leaves did not differ significantly for the two compounds.

On the autoradiograms of the leaf treated plants (Fig. 1–6) the much stronger translocation of AT is apparent. In addition to that, the distribution of the two compounds is different: AT is accumulated in the stem and in the growing tissues, such as buds, young leaves and roots. OT is translocated mainly to the young leaves and swept into their edges and points (Fig. 2 and 6).

A distribution which is similar to that of AT has been found, besides for AT (Rogers, 1957; Bondarenko and Willard, 1956), also for other radioactive compounds taken up by plants, e.g. phosphate (Colwell 1942; Tukey, Wittwer *et al.*, 1956) and 2, 4- dichlorophenoxy-acetic acid (Ashton, 1957). This distribution would be expected for compounds translocated by the phloem system.

In one experiment with bean plants AT was applied to leaves of different age and the plants pressed and autoradiographed after 24 hrs. of uptake. It was found that the amount of AT translocated out of a leaf was greater the higher the age of the treated leaf. The youngest leaves which were just unfolded didn't export any tracer at all. This effect has also been observed for the translocation of radioactive phosphate by Koontz and Biddulph (1957).

Through the roots or the stem the two compounds were taken up at the same rate; also an assay of the solution which remained in the erlenmeyers after the experiment showed that the concentration of both compounds had not changed during the uptake. The compounds were passively borne along with the transpiration stream and distributed over all the leaves, as is shown in the autoradiograms (Fig. 7 and 8.). AT is accumulated in greater amounts in the roots and in the stem than OT. This is probably due to a preferential adsorption

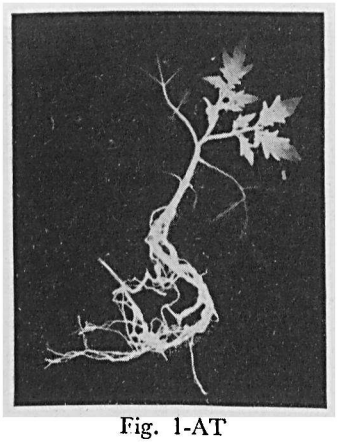

Fig. 1-AT

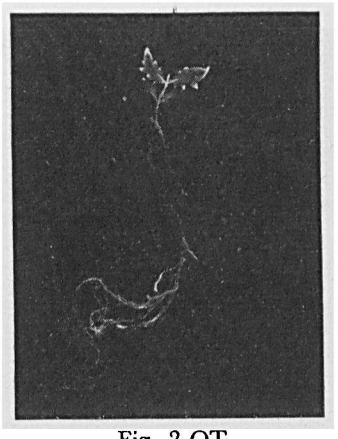

Fig. 2-OT

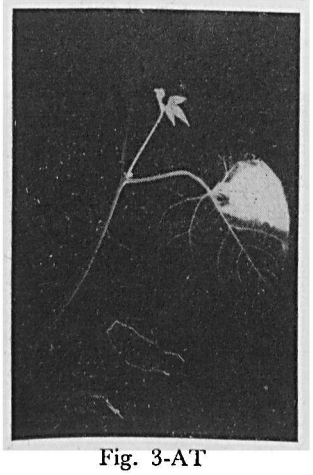

Fig. 3-AT

Fig. 4-OT

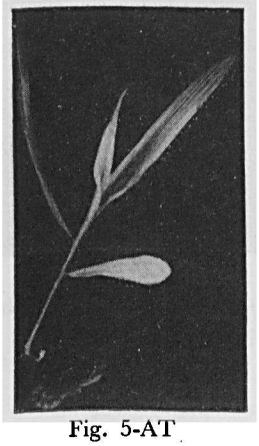

Fig. 5-AT

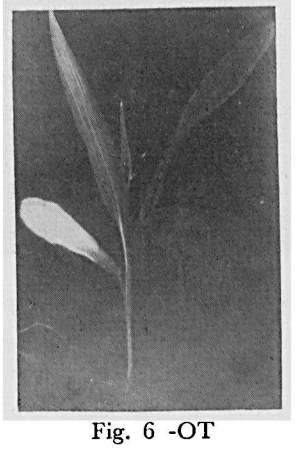

Fig. 6 -OT

Figs. 1–6. Distribution of C^{14} after 22 to 23 hrs. uptake of AT or OT in the light through a leaf; Figs. 1 and 2: tomato (treated leaf discarded); Figs. 3 and 4: bean; Figs. 5 and 6: maize.

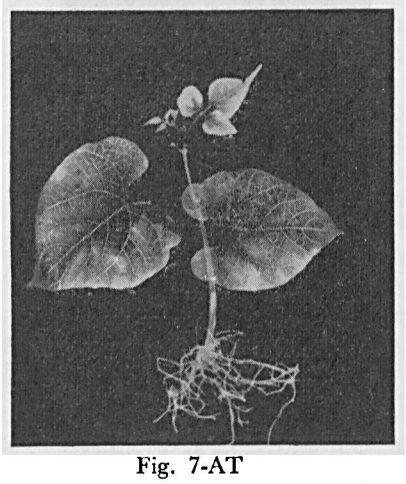

Fig. 7-AT

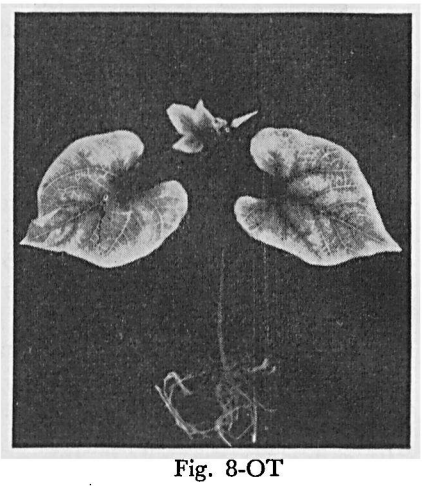

Fig. 8-OT

Figs. 7 and 8. 48 hrs. uptake by bean through roots.

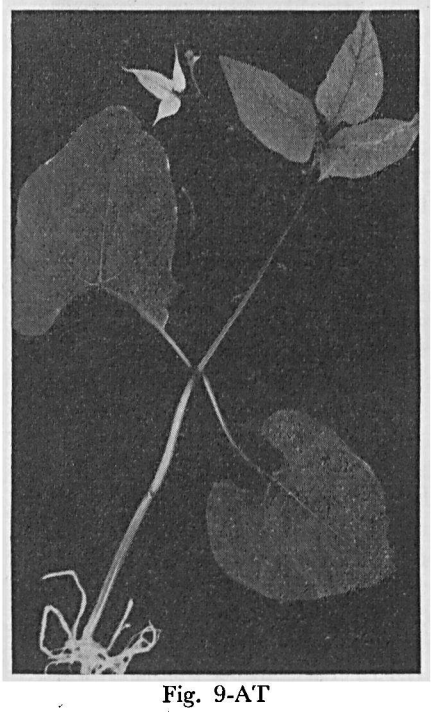

Fig. 9-AT

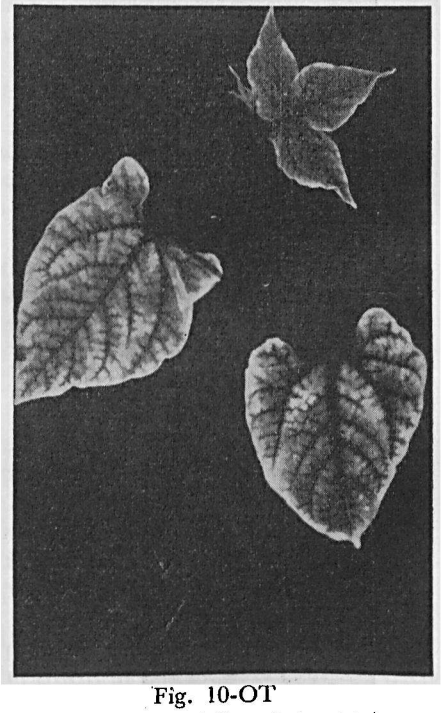

Fig. 10-OT

Figs. 9 and 10. 48 hrs. uptake by bean through roots, followed by 72 hrs. uptake of water.

of basic substances by the xylem tissue, as has also been observed by CHARLES (1953) for dyes and antibiotics.

TABLE I

Radioactivity of extracts of plants treated with AT ot OT

	Nr. of plants per treatment	cpm x 10^{-3} per plant [2] or % of total	
		AT	OT
Bean			
leaf smear, 23 hrs.			
total uptake [1]	2	17.1	10.2
without treated leaf		6.2	0.2
translocated %		36	2
leaf injection, 23 hrs.			
total uptake [1]	2	120	68
without treated leaf		24	0.2
translocated, %		20	0.3
uptake through roots, 48 hrs.	3		
total uptake		53	58
roots alone		18	12
uptake through stem, 24 hrs.	3		
total uptake		124	136
stem alone		39	30
uptake by detached leaves, 40 hrs.	7	3.9 ± 0.4	1.7 ± 0.3 [3]
Tomato			
leaf smear, 23 hrs. [5]	5		
translocated [4]		24 ± 3	1.2 ± 0.2 [3]
Four o'clock plant			
leaf smear, 23 hrs.	2		
total uptake [1]		2.3	0.5
without treated leaf		0.8	0.2
translocated, %		35	40
leaf injection, 23 hrs.	2		
total uptake [1]		23	22
without treated leaf		1.9	0.2
translocated %		8	1
Maize			
leaf smear, 22 hrs.	4		
total uptake [1] [5]		18.5 ± 6	0.9 ± 0.1 [3]
Pear			
diffusion through isolated cuticula			no significant difference

[1] Treated area discarded.
[2] With the specific activity and the counting efficiency used, 1 μg AT corresponds to 6300 cpm, 1 μg OT to 6800 cpm.
[3] Standard errors.
[4] Whole plant without treated lobe.
[5] Plants burned and assayed as $BaCO_3$.

Another difference between Figures 7 and 8 is the beginning of a certain rearrangement of AT within the plant.

In order to make this effect more distinct, two bean plants were placed with their roots into a solution of AT or OT for 48 hrs and then transferred to distilled water for another 72 hrs. The plants were then dried, pressed and exposed to X-ray film. Fig. 9 and 10 show the autoradiograms of those plants. It appears that both compounds are taken up by the xylem system. OT is then swept out of the vessels into the parenchyma and the edges, especially of the oldest, big leaves. On the other hand, AT was taken over by the phloem system as soon as it arrived in the leaf parenchyma, and translocated to the meristematic tissue, so that the distribution finally came to resemble that obtained after uptake through the leaves.

SUMMARY

The uptake and translocation of radioactive 3-amino and-3-hydroxy- 1,2,4, -triazole in various plants has been investigated. The first compound is taken up and translocated much faster than the second one upon administration to a leaf. The rate of uptake through the roots or through the cut off stem is the same for the two compounds. The distribution of aminotriazole in the plants points to a translocation by the phloem system.

REFERENCES

ASHTON, F. M. 1957. Plant Physiol. *32*: suppl. 42.
BIDDULPH, O. 1941. Am. Jour. Bot. *28*: 348.
BONDARENKO, D. D. and C. J. WILLARD. 1956. Proc. 13th Ann. Meet. North Central Weed Control Conf., Chicago.
CHARLES, A. 1953. Nature *171*: 435.
COLWELL, R. N. 1942. Am. Jour. Bot. *29*: 798.
HALL, W. C., S. P. JOHNSON and C. L. LEINWEBER, 1954. Texas Agricult. Exper. Station, Bull. 789.
KOONTZ, H. and O. BIDDULPH. 1957. Plant Physiol. *32*: 463.
ORGELL, W. H. 1955. Plant Physiol. 30–78.
ROGERS, B. J. 1957. Weeds *5*: 5.
SCHÖNINGER, W. 1955. Mikrochim. A. *1955*: 123.
TUKEY, H. B., S. H. WITTWER, F. G. TEUBNER and W. G. LONG. 1956. Proc Intern. Conf. Atom. Energy Geneva 1955, vol. *12*: 138.