Biological Fate Studies of 2-Nitro-1,1-bis-p-chlorophenylpropane

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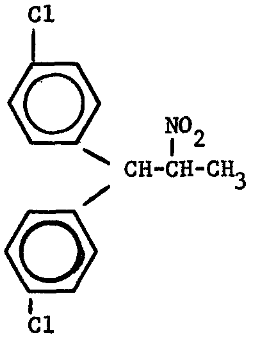
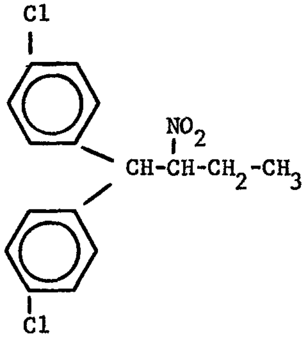
As a result of the work conducted by Muller, et al., (1) which led to the discovery of the insecticidal properties of DDT, Hass, et al. (2) became interested in some of the DDT analogues, especially the nitroalkane derivatives. From the latter work, two compounds emerged as potentially useful insecticides. These compounds were 2-nitro-1,1-bis-p-chlorophenylpropane (I) and 2-nitro-1,1-bis-p-chlorophenylbutane (II):



I



II

These compounds have been shown to be about five times as effective as DDT when measured against thrips on beans and aphids on okra. A concentrate of these two substances in the ratio of 1 of I to 2 of II, known as Dilan 80 LC[b], has been studied for its insecticidal (3,4) and biological effects (5-9).

The suggested use of Dilan 80 LC as an insecticide on edible foods prompted this study of the absorption and translocation of I in a plant which produces food. Thus, as a prelude to this study, the radio-labelled synthesis of I with ^{14}C (referred to in this paper as I-^{14}C) uniformly distributed in the phenyl rings was developed (10) and the labelled compound studied after application to the kidney bean, Phaseolus vulgaris.

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Experimental

The labelled insecticide I-^{14}C was applied to a single leaf of two different groups of the plant which were grown under greenhouse conditions. Group A, 49 day old plants, consisted of 30 plants which had already reached the blooming stage and were in the process of fruiting. Group B, 30 day old plants, consisted of 44 plants which were in the pre-blooming stage.

A solution of I-^{14}C in methanol was prepared so that 300 micro-liters would contain 20 mg. of the insecticide and have an activity between 1 and 1.5 million counts per minute. Methanol was used as the solvent because of its relative high volatility and solubility characteristic of compound I-^{14}C. In addition, the rapid evaporation of methanol deposited the insecticide on the leaf in a manner analogous to a dust spray with a minimum of plant damage.

Three hundred micro-liters of the methanolic solution of I-^{14}C was applied uniformly on the entire upper surface of one of the leaves comprising the first trifoliate. The two primary leaves were removed from all plants prior to the application of the labelled insecticide because some of these leaves had already fallen from the plant and others were in the process of drying.

At intervals of 0, 3, 6, 9, 12, 15, 20, 25, 30, 35, and 40 days after application of I-^{14}C to a single leaf of each group, 3 plants from Group A and 4 plants from Group B were selected at random for analysis. The total plant was then separated as follows: (a) treated leaves (leaves on which the labelled insecticide had been applied originally), (b) untreated leaves (all leaves excluding the labelled leaf), (c) stems, (d) roots, and (e) beans. Like parts from all 3 plants of Group A and 4 plants of Group B for a particular time interval were pooled. The root samples were washed free of soil under a gently-running stream of water. The different parts of the plant upon removal were placed in a plastic bag and stored at 0°C prior to analysis. Before beginning the analysis, the contents of each bag were weighed and recorded without drying. The different parts were homogenized in a blender and a 3-6 gm. aliquot was removed and used for the oxidation. In the case of the treated leaves, the total sample was oxidized because of the small size of the samples.

A modification of the techniques described by Van Slyke and Folch (11) and Jaffey and Alvarez (12) was employed to analyze the various parts for compound I-^{14}C and/or its metabolites. The apparatus consisted of a glass joint 500 ml. suction flask connected to a 250 ml. separatory funnel provided with a similar joint. The suction flask was connected through the side-arm to two gas washing towers connected in series to a water aspirator to provide a negative pressure to aid in the removal and subsequent trapping of the CO_2 gas. The tower directly connected to the oxidizing (suction) flask was provided with 200 ml. of a 1:2 mixture of 2-amino-ethanol and 2-ethoxyethanol. The second

TABLE I

The ^{14}C Content Representing 2-Nitro-1, 1-bis-p-chlorophenylpropane-^{14}C and/or Its Metabolites of Leaves and Stems of *Phaseolus vulgaris* at Various Time Intervals After Foliar Application

Time[a] Interval	^{14}C Content[b]					
	Treated Leaves[c] (%)		Untreated Leaves[d] (%)		Stems (%)	
(Days)	Group A[e]	Group B[f]	Group A[e]	Group B[f]	Group A[e]	Group B[f]
0	100	100	0	0	0	0
3	96.6	80.1	0	3.3	2.5	0.5
6	94.6[g]	71.9	0.2	0	2.6	0.2
9	93.0	84.2	0.6	0.2	1.2	1.0
12	97.1	76.2	0.1	0	0.2	0.4
15	----	81.4	---	0	---	0.4
20	87.4	78.8	0.2	0	1.1	0.4
25	88.2	74.5	0.3	0.1	0.7	0
30	86.1	73.8	0.2	0.7	0.4	0.4
35	86.8	75.8	0.3	0.2	0	0.1
40	----	77.5	---	0.4	---	0.3

[a] Time interval after application to the leaf.

[b] ^{14}C content of the plant part expressed as percent of the total activity found at time 0. Activity per plant part is expressed in disintegrations per minutes and includes a correction for counter efficiency and quenching by internal standardization. The corrected total activity at time zero was 2,167,472 dis./min. for Group A and 2,057,212 dis./min. for Group B.

[c] Treated leaves has reference to leaves on which the labelled insecticide had been applied.

[d] Untreated leaves refers to all remaining leaves of the same plant to which the labelled insecticide had not been applied.

[e] Group A consisted of 49-day old plants in the blooming stage.

[f] Group B consisted of 30-day old plants in the pre-blooming stage.

[g] The sum of the values for each group at each time interval is the percent recovery in the total plant of the applied radioactivity (e.g. at time interval 6, the total recovery for Group A is 94.6 + 0.2 + 2.6 = 97.4%).

washing tower contained 100 ml. of the CO_2 trapping solution. The separatory funnel carried a drying tube containing a mixture of Drierite and Ascarite to provide dry, carbon dioxide free, atmospheric air for flushing the system during the CO_2 trapping process.

The plant sample, 3.0 gms. of potassium iodate and 10.0 gms. of chromic tioxide was placed into the oxidizing flask. One hundred milli-liters of a mixture of 1 part 85% phosphoric acid and 2 parts of 20% fuming sulfuric acid were dropped into the oxidizing chamber from the separatory funnel in small portions to control the reaction. Following the addition of the acid, the stop-cock of the funnel was closed and the reaction mixture was heated to reflux. Boiling was continued for ten minutes; the stopcock was opened, and dry, carbon dioxide free air was flushed throughout the system for an additional fifteen minutes. The 200 ml. of absorbing solution was transferred to an amber glass bottle and stored prior to preparation of the scintillation samples for counting. A 3 ml. sample was taken after each run from the second tower and analyzed for ^{14}C content to determine if the first tower had quantitatively absorbed the total CO_2. No activity was found in the solution from the second tower.

Following oxidation of the sample, 3 ml. aliquots of the CO_2 trapping solution were removed and added to a scintillator vial containing 15 ml. of a scintillation cocktail consisting of 0.4% PPO (2,5-diphenyloxazole) and 0.01% of dimethyl POPOP /1,4-bis (4-methyl-5-phenyl-2-oxazolyl)benzene/ contained in a mixture of equal volumes of toluene and 2-ethoxyethanol. The vials were cooled and counted in a Packard TRI-CARB liquid scintillation spectrometer at a window setting of 50-1000 and a gain of 19%. The samples were corrected for quenching. Counter efficiency was calculated by using the internal standardization technique employing the use of toluene-^{14}C standard with an activity of 50,100 disintegrations per minute per 100 micro-liters.

A limit was established for the minimum activity which was to be considered significant. The value chosen for this purpose was 0.1% of the activity at time 0 found on the leaf to which the compound had been applied. Samples were counted for appropriate periods of time to have no more than 10% error at the 95% confidence level (13). Table I gives the ^{14}C content representing Compound I-^{14}C and/or its metabolites found in the various plant parts. Root and bean results are not included in Table I because in all but two cases the ^{14}C content was zero percent. The two exceptions were (a) in which the ^{14}C content of the root was 0.7% and (b) the bean 0.2%. These values are minor and could be attributed to experimental error.

Conclusion

The most significant conclusion from the data collected is that the 2-nitro-1,1-bis-p-chlorophenylpropane and/or its metabolites are very poorly absorbed from the surface of the leaf and accordingly are not translocated throughout the plant to any significant degree. No measurable amount of the insecticide was found in the roots or in the bean at any time intervals over the 40 day span of study following foliar application. Only minor amounts of the compound representing about 3 percent of that originally applied to the leaf was observed in the stem and untreated leaves, signifying a small amount of absorption and translocation within the first 9 days after application.

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