Microbial Degradation of the Acaricide *N*-(4-Chloro-*o*-tolyl)-*N*,*N*-Dimethylformamidine[1]

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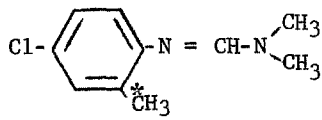
The acaricidal efficacy of N'-(4-chloro-o-tolyl)-N,N-dimethylformamidine (Galecron®, CIBA Agrochemical Co.; Fundal®, NOR-AM Agricultural Products, Inc.) is well documented (1,2). Also, studies have been conducted on the photodecomposition of Galecron (3) and on the metabolic fate of Galecron in apple seedlings (4). In this paper we report on the degradation of Galecron by bacteria, actinomycetes, and fungi.

MATERIALS AND METHODS

Galecron-C^{14} was provided by the CIBA Agrochemical Co., Vero Beach, Fla.; structure of the acaricide and position of the radiocarbon are shown below:



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The Galecron-C^{14} was diluted to a specific activity of 1.96 mc/mmole with the pure nonradioactive compound.

Separation of Galecron and radiocarbon-containing metabolites after extraction from the microbial preparations was accomplished on glass plates coated with silica gel GF_{254} (500 μ). The chromatograms were developed with benzene-diethylamine (95:5) in an unsaturated chamber (3); average R_f values were 0.85 for Galecron, 0.41 for demethyl-Galecron, 0.18 for N-formyl-4-chloro-o-toluidine, 0.51 for 4-chloro-o-toluidine, 0.19 for 4-chloro-2-methylacetanilide, 0.95 for 2,2'-dimethyl-4,4'-dichloroazobenzene, and 0.00 for 5-chloroanthranilic acid and its N-formyl derivative. The C^{14}-containing metabolites were tentatively identified by cochromatography with these authentic standards.

Cultures of Aerobacter aerogenes, Serratia marcesens, Streptomyces griseus, Fusarium moniliforme, and Rhizopus nigricans were obtained from the Fish-Pesticide Research Laboratory Collection. The actinomycete and bacteria cultures were regenerated from lyophiles in brain-heart broth in a 50-ml Delong flask. A 2-3 mm^2 disc from the fungi stock slant was added to a Delong flask containing the brain-heart broth, and the mixture was incubated for 48 hr. After growth periods of 24 hr in the case of the actinomycete and bacteria and 48 hr in the case of the fungi, 100 μl of an ethanolic solution of Galecron-C^{14} (approximately 10^5 dpm) were added to each flask. One flask containing the brain-heart broth and Galecron-C^{14} but no microorganisms served as the control. All flasks were placed in a rotatory water bath shaker and incubated at 28°C for 48 hr.

After incubation with the acaricide, the contents of each flask were extracted twice with 50-ml aliquots of chloroform. The actinomycete and fungi samples were sonicated for 20 seconds prior to chloroform extraction. The chloroform was dried over anhydrous sodium sulfate and concentrated to approximately 0.3 ml under reduced pressure. The chloroform concentrate was then spotted on a

thin-layer plate, and the chromatogram was developed. A radioautograph was prepared, and the silica gel corresponding to the darkened images on the film was scraped into test tubes and extracted two times with 10 ml of acetone. The acetone was added to a scintillation vial and allowed to evaporate. The scintillation "cocktail" was added, and the sample was radioassayed (3).

All analyses were conducted in duplicate and the results were averaged.

RESULTS AND DISCUSSION

Radioautographs showing the radiocarbon-containing compounds formed when Galecron-C^{14} was incubated for 48 hr with bacteria (A. aerogenes and S. marcesens), fungi (F. moniliforme and R. nigricans), and an actinomycete (S. griseus) are shown in Fig. 1; quantitative results of these studies are given in Table 1. Compared with the control (Table 1), Galecron was metabolized extensively by all five microbial species. N-Formyl-4-chloro-o-toluidine was apparently the major degradation product in the case of the two bacterial and two fungal species. However, it is noteworthy that the chromatographic system used in this study did not provide complete separation of N-formyl-4-chloro-o-toluidine from 4-chloro-2-methylacetanilide. Thus, the possibility of the presence of some 4-chloro-2-methylacetanilide cannot be excluded. With S. griseus, the single actinomycete used, 4-chloro-o-toluidine was by far the predominant metabolite. Appreciable amounts of 4-chloro-o-toluidine were detected in all cases. Demethyl-Galecron was not extracted from any of the microorganisms in large amounts by 48 hr; therefore, if N-demethylation is an important pathway the compound must have been hydrolyzed rapidly to N-formyl-4-chloro-o-toluidine. Unknown I cochromatographed with the azo derivative; however, it was not present in microbial extracts in amounts significantly greater than that in the control. Moreover, it was shown in a previous study that small amounts of Unknown I formed when Galecron was stored as a solid or in solution, and the

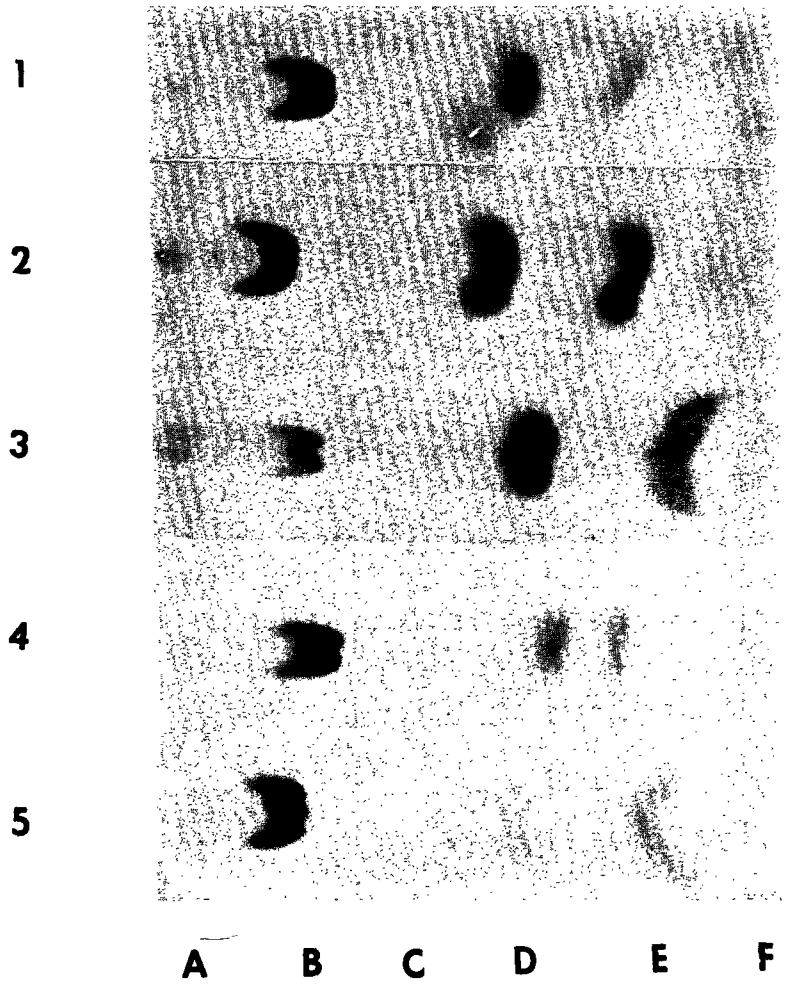

Fig. 1. Radioautographs of C^{14}-containing compounds formed when Galecron-C^{14} was incubated for 48 hr with cultures of Aerobacter aerogenes (1), Serratia marcesens (2), Streptomyces griseus (3), Fusarium moniliforme (4), and Rhizopus nigricans (5). A = origin, B = N-formyl-4-chloro-o-toluidine, C = demethyl-Galecron, D = 4-chloro-o-toluidine, E = Galecron, and F = Unknown I.

TABLE 1

Degradation of Galecron-C^{14} by Aerobacter aerogenes (AA), Serratia marcesens (SM), Streptomyces griseus (SG), Fusarium moniliforme (FM), and Rhizopus nigricans (RN).

Nature of radioactive compounds	% Recovered radioactivity 48 hr after inoculation with Galecron-C^{14}					
	AA	SM	SG	FM	RN	Control*
Galecron	5.7	19.9	20.1	6.5	7.7	68.5
Demethyl-Galecron	1.2	1.4	1.4	1.0	0.8	0.5
N-Formyl-4-chloro-o-toluidine	72.9	43.2	10.0	81.9	88.0	27.7
4-Chloro-o-toluidine	18.6	33.8	65.2	9.2	2.3	1.9
Unknown I	0.2	0.3	0.7	0.2	0.1	0.4
Origin	1.4	1.4	2.6	1.2	1.1	1.0

*Galecron-C^{14} (10^5 dpm) was added to a 50-ml Erlenmeyer flask containing 10 ml of brain-heart broth; the mixture was incubated at 28°C with shaking for 48 hr prior to extraction.

compound was not the symmetrical azo derivative of 4-chloro-o-toluidine (3). An additional unknown can be seen between the origin and the N-formyl-4-chloro-o-toluidine in some of the radioautographs (Fig. 1); no attempts were made to identify this compound.

The extensive degradation of Galecron in the control flask (Table 1) was not surprising; Knowles and Sen Gupta (3) reported that Galecron was unstable in aqueous medium and that N-formyl-4-chloro-o-toluidine was the major decomposition product.

The percentage of the applied radioactivity recovered was somewhat low in most cases. Recovery figures averaged 70% for A. aerogenes, 55% for S. marcesens, 50% for S. griseus, 70% for F. moniliforme, 90% for R. nigricans, and 80% for the control. Binding of Galecron to components of the brain-heart broth or conversion of Galecron to chloroform insoluble metabolites could

account for a portion of the loss. Other factors possibly involved which would result in a lower recovery could include volatilization of Galecron during the incubation and degradation of Galecron with either incorporation of the radiocarbon fragment of the molecule into natural microbial components or release as volatile $C^{14}O_2$.

Because of the narrow spectrum of microorganisms used in these studies it is difficult to make any generalizations relative to degradation of Galecron by the three groups (i.e., bacteria, actinomycetes, and fungi). However, those organisms selected degraded the acaricide extensively, and it would appear that the Galecron molecule could be an attractive target for microorganisms.

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