Isotope dilution methodology was used to determine if the tissue residues contained a 1-methyl-5-nitroimidazole nucleus. Previous work in our laboratory had shown that the imidazole nucleus is resistant to oxidation by nitric acid (Baer et al., 1977). Studies with ronidazole showed that a high yield of 1-methyl-5-nitroimidazole resulted from the oxidation of ronidazole with nitric acid in the presence of ammonium vanadate catalyst. Hence, if the tissue residues contained the nitroimidazole functionality of ronidazole with a substituent on the 2-position, the residue should yield 1-methyl-5-nitroimidazole on similar treatment.

Studies with muscle samples showed that about 1% of the tissue residues are converted to 1-methyl-5-nitroimidazole on oxidation with nitric acid. By this analysis only a small fraction of the residue retains the biologically important structural features of the drug.

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Outdoor Dissipation of the Experimental Acaricide 2-Naphthylmethyl Cyclopropanecarboxylate on Apple Trees: Formation of Lipophilic Metabolites

Andrew Pryde* and Roland P. Hänni

Fruit and foliage of an apple tree (Golden Delicious) were treated with the experimental acaricide 2-naphthylmethyl cyclopropanecarboxylate (Ro 12-0470; ^{14}C labeled) and samples analyzed over a period of 7 days. In apples the recovery of radioactivity was high throughout (88–96%) but on leaves fell from 89.5% to 28.5% during the trial period. The nature of the radioactivity washed from the surface of both commodities and extracted from the surface-washed commodities was investigated. The results indicate a half-life for Ro 12-0470 of ca. 12 h on apples and less than 6 h on foliage. Similar results were found in a trial with nonradioactive material under field conditions. Four lipophilic metabolites isolated from apples in the radioactive trial were esters of 2-naphthalenemethanol with naturally occurring C_{16}, C_{18}, C_{20}, and C_{22} saturated fatty acids. This therefore represents a further example of the rather unusual phenomenon of the formation of highly lipophilic metabolites between a xenobiotic alcohol and naturally occurring fatty acids.

The conversion of xenobiotic compounds into lipophilic metabolites in plants and mammals is a rather unusual phenomenon. In general, xenobiotics are modified by oxidation and/or conjugation, giving rise to more polar or water-soluble metabolites which are more readily excreted from the organism. Highly lipophilic metabolites have been formed between xenobiotic alcohols and naturally occurring fatty acids in mammals (Leighty et al., 1976, 1980). In addition, there are also reports of xenobiotic acids being converted to lipophilic metabolites (triglycerides) after esterification with endogenous alcohols (Quistad et al., 1976; Fears et al., 1978; Schooley et al., 1978; Crayford and Hutson, 1980). In their studies of the metabolism of the acaricide hexadecyl cyclopropanecarboxylate in mammals and plants (Schooley et al., 1978), the compound with a ^{14}C radiolabel in the carboxyl group was used. The fate of the cyclopropanecarboxylic acid moiety could thus be investigated in detail. As part of our studies of the environmental behavior of the experimental acaricide 2-naphthylmethyl cyclopropanecarboxylate (Ro 12-0470), the fate of the compound in apple fruits and foliage under outdoor conditions was investigated. The compound was available with a ^{14}C radiolabel in the naphthylmethyl group, and thus the fate of the 2-naphthylmethanol moiety could be elucidated.

DR. R. MAAG, Ltd., Department of Registration and Environmental Studies, CH-8157 Dielsdorf, Switzerland.

EXPERIMENTAL SECTION

Liquid Scintillation Counting (LSC). Triplicate aliquots of the sample solution were counted in 10 mL of Insta-Gel scintillation cocktail (Packard, Ltd., Zurich). The samples were counted twice in a Packard Model 3375 liquid scintillation spectrometer and the counts averaged; the automatic external standard ratio method was used to determine counting efficiency.

Thin-Layer Chromatography. Precoated Alox E (alumina) and Kieselgel 60 F 254 (silica) thin-layer chromatography (TLC) plates were obtained from E. Merck (Darmstadt, GFR). A Model LB 2723 radio-TLC scanner

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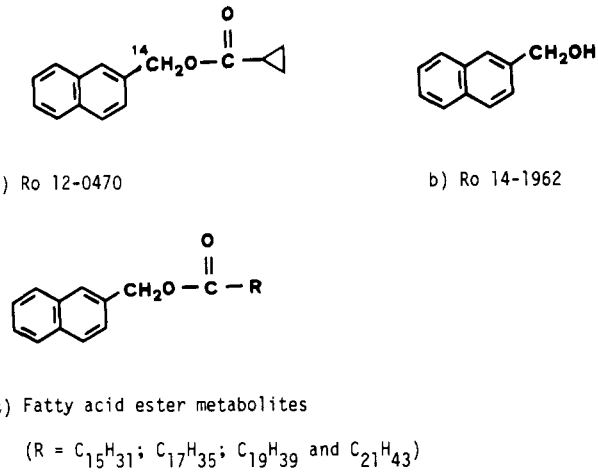

Figure 1. Structures of (a) 2-naphthylmethyl cyclopropanecarboxylate (Ro 12-0470) showing the position of ^{14}C label, (b) its hydrolysis product (Ro 14-1962), and (c) the fatty acid ester metabolites.

was used (Berthold, Wildbad, GFR). Nonradioactive standards were visualized under a UV lamp.

High-Performance Liquid Chromatography (HPLC). The high-performance liquid chromatograph was laboratory assembled and comprised a Model 28646-4 pneumatic pump (Haskel, Burbank, CA), a 5025 BF radioactivity detector with a 50-μL flow cell packed with cerium-activated lithium glass beads (Berthold, Wildbad, GFR), a Rheodyne injection valve with a 100-μL loop, and a 100 × 5 mm i.d. stainless steel column slurry packed with Hypersil 5-μm spherical silica particles (Shandon Southern Products, Ltd., Runcorn, U.K.).

Gas Chromatography (GC). For residue analysis a Carlo Erba Fractovap GI-450 gas chromatograph equipped with a flame ionization detector was used. The column was 3 m × 3 mm packed with 3% OV-17 on Gas-Chrom Q (100–120 mesh) and operated at 195 °C with 30 mL/min nitrogen gas. The injector was maintained at 245 °C.

Gas Chromatography/Mass Spectrometry (GC/MS) System. GC/MS results were obtained by using a Varian 1740 gas chromatograph coupled to a Varian MAT CH7 mass spectrometer operated in the electron impact (EI) mode (70 eV; source temperature, 250 °C).

Radiolabeled a.i. The ^{14}C-labeled form of the acaricide Ro 12-0470 (Figure 1) was available for the present study (from F. Hoffmann-La Roche, Basel). The compound was characterized as follows: empirical formula, $C_{15}H_{14}O_2$; molecular weight, 226; specific activity, 15.9 μCi/mg (3.6 mCi/mmol); radiochemical purity (radio-TLC, radio-GC), >99%.

Dosage. An emulsion was prepared containing Ro 12-0470 (12 mg), plant seed oil poly(ethylene glycol ether) adduct (2 μL), alkylarylsulfonate calcium salt (0.6 μL), and alkylated benzene (9.8 μL) in distilled water (12 mL) previously sterilized to prevent breakdown of a.i. by microorganisms. Twenty-one apples growing randomly on a Golden Delicious apple tree were each treated with 250 μL of the radioactive emulsion (≡8.836 × 10^6 dpm/apple; 250 μg of a.i./apple = ca. 4 ppm) by using a precalibrated Hamilton syringe. The upper surfaces of 18 leaves on the same tree were each treated with 100 μL of the radioactive formulation (≡3.534 × 10^6 dpm/leaf = ca. 2 μg/cm^2). These levels correspond to those found in previous residue analysis trials with nonradioactive material. The tree was covered with netting to prevent entry of birds. Samples (three apples per three leaves) were taken at times t = 0 (i.e., on drying of the formulation; 1–2 h after application), 6 h, 24 h, and 3 days. At t = 7 days, the six remaining leaves and nine remaining apples were collected for workup.

Location. The Golden Delicious apple tree was situated in plot J 134, Waisenhof location 5, DR. R. MAAG, Ltd., Dielsdorf, Switzerland.

Application date was July 19, 1979.

Weather. During application the weather was overcast but dry. Data from the Dielsdorf weather station for the 7-day trial period are as follows: T_{max}, +28 °C; T_{min}, +9 °C; cumulative rainfall, 0.0 mm; cumulative sunshine, 29 h.

Workup. *Apples.* The surfaces of the three apples taken at each sampling time were washed with around 300 mL of methanol immediately after sampling. The radioactivity content of the solution obtained was determined by liquid scintillation counting (LSC), and the nature of the radioactivity present was determined by TLC analysis (Table I). The Ro 12-0470 contents in the surface-washed samples were confirmed by GC. The surface-washed apples were chopped into small pieces and macerated on an Omni-mixer (J. Sorvall Inc., Connecticut) in a 1-pt Mason jar with 300 mL of methanol at 0 °C for 5 min. The homogenate was filtered through a glass sinter containing a thin layer of Hyflo Super-Cell and the filter cake washed thoroughly with methanol. The radioactivity in the filtrate (total volume ca. 550 mL) was determined by LSC; the nature of the radioactivity present could not be determined by TLC since the viscous residues obtained on evaporation of aliquots of the solution were unsuitable. Instead, an aliquot (containing ca. 500 000 dpm) of the methanol filtrate was evaporated to dryness and the residue transferred with hexane (150 mL) and water (50 mL) into a separating funnel and partitioned between the two phases. The radioactivity in both phases was determined by LSC: the nature of the radioactivity in the hexane phase was determined by TLC analysis (Table II). (For the residue analyses with nonradioactive material, unwashed apples were homogenized directly.)

The residue from the apple extraction remaining on the glass sinter was air-dried in a crystallizing dish (3 days), ground to obtain a homogeneous sample, and weighed. Aliquot samples (100 mg) were combusted in duplicate [by the method of Kalberer and Rutschmann (1961)] to determine unextracted radioactivity. (The combustion blank value was 39 cpm/100 mg.)

At t = 7 days where nine apples were worked up, the scale was increased accordingly. Results are shown in Table III.

Foliage. The surfaces of the three leaves taken at each sampling time were washed with ca. 200 mL of methanol immediately after sampling. The radioactivity content of the solution obtained was determined by LSC; the nature of the radioactivity present was determined by TLC. The surface-washed leaves were extracted in a Virtis 45 homogenizer (Virtis Co., New York) with 75 mL of methanol at 0 °C for 5 min. The homogenate was filtered through a glass sinter containing a thin layer of Hyflo Super-Cell and the filter cake washed thoroughly with methanol. The radioactivity in the filtrate (total volume ca. 150 mL) was determined by LSC and the nature of the radioactivity by TLC. The residue from the leaf extraction remaining on the glass sinter was combusted as described above for the apple residues. (A combustion blank value of 42 cpm/100 mg was found for the foliage samples.)

RESULTS AND DISCUSSION

Fate of the Radioactivity in Apples. The radioactivity recovered by washing the apple surfaces decreased steadily from a value of 71.1% (of the radioactivity ap-

Table I. Nature and Percentage Distribution of Residual Radioactivity in Apple Surface Washes

time	radioact in surface wash[a]	TLC analysis[a,b] Ro 12-0470	FAEMs	Ro 14-1962	polar[c]
0	71.1	69	0	1	1
6 h	51.8	41	5	5	1
24 h	29.9	17	11	1	1
3 days	11.9	3	8	0	0.5
7 days	12.3	0.5	11	<0.5	<0.5

[a] As percentage of initially applied radioactivity or a.i. [b] Calculated from the results obtained with the alumina and silica TLC systems (see Experimental Section). [c] Remained at the origin of the alumina plate.

Table II. Nature and Percentage Distribution of Residual Radioactivity in Apple Extracts

time	radioact in apple extract[a]	hexane/water partitioning[a,b] hexane	water	TLC analysis of hexane phase[a,c] Ro 12-0470	Ro 14-1962	FAEMs
0	23.2	20.6	1.8	19	1	0
6 h	36.8	28.6	7.7	19	5	5
24 h	46.8	24.8	19.8	10	4	11
3 days	47.8	11.9	35.8	3	0.5	8
7 days	39.3	3.5	37.1	0.5	0.5	3

[a] As percentage of initially applied radioactivity or a.i. [b] Aliquots of methanolic apple extract evaporated and partitioned between hexane/water (see the text). [c] Calculated from the results obtained with the alumina and silica TLC systems (see Experimental Section). Polar radioactivity (located at the origin of the alumina plate) accounted for ≤0.5% of the dose at all time points.

Table III. Summary of Nature and Percentage Distribution of Residual Radioactivity in Apples

time	total Ro 12-0470[a,b]	total FAEMs[a,b]	total Ro 14-1962[a,b]	radioact in extracted residues by combustion[a]	total radioact recovery[a,c]
0	88	0	2	1.6	95.9
6 h	60	10	10	4.7	93.3
24 h	27	22	5	12.2	88.9
3 days	6	16	<1	30.5	90.2
7 days	1	14	<1	36.6	88.2

[a] Values are percentages of initially applied a.i. or radioactivity. [b] Sum of values from surface wash plus extract (data from Tables I and II, respectively). [c] Sum of radioactivity from surface wash plus extract plus combustion.

plied) at t = 0 to ca. 12% at t = 3 and 7 days (Table I), whereas the radioactivity extracted (23.2% at t = 0) was at a maximum at t = 3 days (47.8%) (Table II) and the radioactivity in the extracted residues increased steadily from 1.6% at t = 0 to 36.6% at t = 7 days (Table III). In spite of the variations in the nature of the radioactivity over the 7-day period, the total recovery of radioactivity remained high (95.9% at t = 0 and 88.2% at t = 7 days) as shown in Table III.

The decrease in the amount of unchanged Ro 12-0470 in the apples (obtained by summing amounts in the surface wash plus extract) indicated rapid dissipation of a.i., the half-life determined graphically being around 12 h. This result from the radioactive trial was in good agreement with the value from the parallel experiment carried out under practical conditions with nonlabeled material in

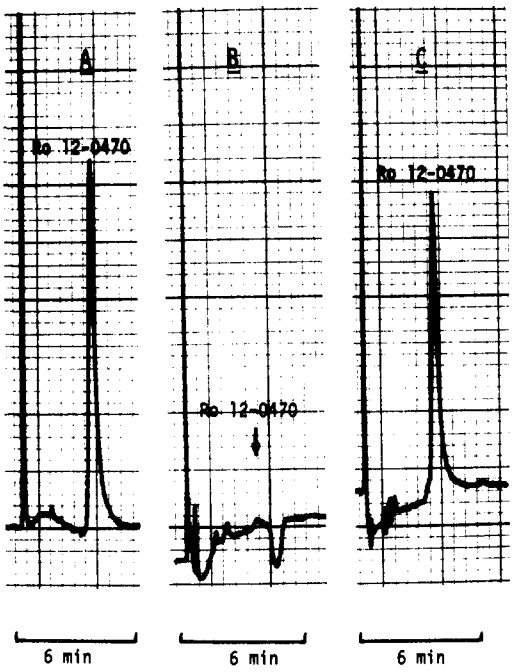

Figure 2. Gas chromatograms of Ro 12-0470 samples: (A) 1.0 ng of Ro 12-0470 standard sample; (B) 1 μL of control sample corresponding to 10 mg of apple fruit; (C) 1 μL of sample corresponding to 1 mg of apple fruit spiked at 0.8 ppm with Ro 12-0470 (recovery: 103%). Detector input, 10^{10} ohm; output, ×4; recorder full scale deflection, 2.5 mV. Other conditions are described in the text.

Table IV. Dissipation of Nonlabeled Ro 12-0470 on Apple Fruits

time, days	Ro 12-0470 concn,[a] ppm[b]
0	2.86
1	0.83
3	0.10
7	0.01
14	<0.01

[a] By GC (see Figure 2). [b] Micrograms of a.i. per gram of fruit, fresh weight.

Table V. TLC R_f Values of the Compounds Investigated

TLC system	R_f values Ro 12-0470	FAEMs	Ro 14-1962
alumina/chloroform	0.7	0.7	0.2
silica/toluene	0.3	0.5	0.04

which a graphically determined half-life of ca. 12 h was also found by GC (Table IV and Figure 2).

Isolation and Characterization of the Lipophilic Metabolites. On TLC analysis of the surface washes and extracts of the apple samples, it was evident that a lipophilic metabolite or metabolite mixture had been formed. As described below, the unknown radio-TLC peak was shown to correspond to a mixture of fatty acid ester metabolities (FAEMs) formed by the esterification of Ro 14-1962 with the naturally occurring saturated C_{16}, C_{18}, C_{20}, and C_{22} fatty acids (Figure 1). On alumina TLC plates with chloroform as the developing solvent, FAEMs and Ro 12-0470 were not well separated from each other but were well resolved as a group from Ro 14-1962 and any radioactivity remaining at the origin. On silica plates with toluene as the developing solvent, Ro 12-0470 was well separated from FAEMs while Ro 14-1962 remained at the origin. R_f values are shown in Table V. Unfortunately,

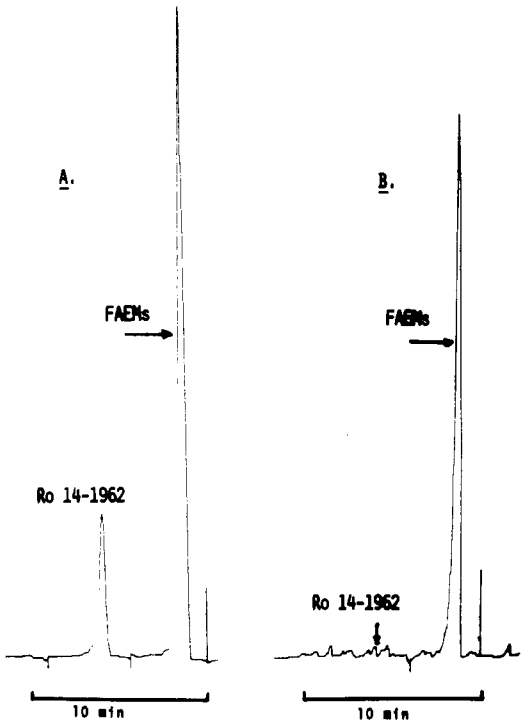

Figure 3. High-performance liquid chromatograms of (A) Ro 14-1962 plus FAEMs mixture and (B) sample of purified FAEMs (30 000 dpm) after preparative HPLC. Mobile phase, hexane/chloroform (80/20); flow rate, 2.2 mL/min; pressure, 400 psi; radiodetector sensitivity, 10K full scale. Other conditions are described in the text.

preparative TLC of a (3 day plus 7 day) combined apple surface-wash sample on silica/toluene to remove Ro 12-0470 also resulted in partial hydrolysis of the FAEMs to Ro 14-1962. A similar effect was observed when an aliquot of the Ro 12-0470 free sample was subsequently chromatographed preparatively on the alumina/$CHCl_3$ system in an attempt to remove the Ro 14-1962. However, HPLC using a short silica column and a $CHCl_3$/hexane (20/80) mobile phase proved satisfactory for the final preparative separation of FAEMs from Ro 14-1962. The recovery of radioactivity in the separated HPLC peaks was 96% (by off-line LSC). A sample containing ca. 500 000 dpm was thus fractionated preparatively by using this system. Chromatograms of the mixture and the purified FAEMs are shown in Figure 3. The sample of FAEMs (394 000 dpm = 11 μg) purified in this manner was shown by GC with a radioactivity detector to contain four radioactive peaks. These were shown by GC/MS techniques to correspond to four novel lipophilic metabolites formed by esterification of Ro 14-1962 with naturally occurring saturated C_{16}, C_{18}, C_{20}, and C_{22} fatty acids. (Parent peaks were found in the mass spectra at m/e 396, 424, 452, and 480, respectively.) The palmitate ester was synthesized for confirmatory purposes by mixing palmitoyl chloride and Ro 14-1962 at room temperature. The mass spectrum, GC retention time, and TLC R_f values of the synthesized material were in agreement with those of the metabolite.

The mixture of FAEMs was not separated into individual components but as a group was present in substantial amounts (21% at t = 24 h) as shown in Table III. In apple surface washes, Ro 12-0470 plus FAEMs accounted for most of the radioactivity present (Table I). However, in the extracts of surface-washed apples the increasing amounts of water-soluble radioactivity (Table II) suggested that polar metabolities were also present.

The isolation of lipophilic metabolites formed between a xenobiotic alcohol (Ro 14-1962) and naturally occurring fatty acids in apples was unexpected and rather unusual. One day after treatment, 22% of the applied dose was present in the form of FAEMs (Table III). As a rule xenobiotic compounds are converted to more polar metabolites, for example, by oxidation, conjugation of which gives rise to water-soluble products. Several examples of the formation of lipophilic metabolities have, however, been reported. Leighty et al. (1976) identified lipophilic metabolites formed in the rat between Δ^8- and Δ^9-tetrahydrocannabinol and naturally occurring fatty acids. A hydroxylated DDT metabolite was also shown by Leighty et al. (1980) to form esters with naturally occurring fatty acids in the rat.

Xenobiotic acids have been shown to be esterified with naturally occurring alcohols (glycerols). Quistad et al. (1976) showed that in chickens the insect growth regulator methoprene gave acid metabolites which formed triglyceride conjugates (as well as cholesterol esters).

Fears et al. (1978) found that 4-benzyloxybenzoic and related acids participated in glycerolipid turnover in the rat, the resulting triglycerides (containing the exogenous acid) being stored in adipose tissue. Similar results were found by Crayford and Hutson (1980) using 3-phenoxybenzoic acid. Schooley et al. (1978) studied the metabolism of the acaricide hexadecyl cyclopropane[*carboxyl*-^{14}C]-carboxylate in mammals and plants. They demonstrated that cyclopropanecarboxylic acid participated in fatty acid biosynthesis, giving a homologous series of ω-cyclopropyl fatty acids by successive additions of up to eight acetate groups. These novel fatty acids could be retained in rats as triglycerides.

Fate of the Radioactivity in Foliage. In contrast to the situation in the fruits where the radioactivity recovery remained high during the trial period, the recovery from the leaves fell from 89.5% at t = 0 to 28.5% at t = 7 days. The loss may have been due to translocation of radioactivity away from the treated leaves or to evaporation. The half-life of a.i. on leaves was very short (<6 h). Radioactivity in leaf surface washes was predominantly unchanged a.i. at early sampling times but became more polar (remained at origin of TLC plates) with time; in leaf extracts, the radioactivity remained at the origin of TLC plates at all sampling times. Little evidence was found for the production of FAEMs or Ro 14-1962 in or on leaves (<5% and <1%, respectively, of the radioactivity in the sample investigated).

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Registry No. Ro 12-0470, 72395-08-5; FAEM (R = $C_{15}H_{31}$), 84849-00-3; FAEM (R = $C_{17}H_{35}$), 84849-01-4; FAEM (R = $C_{19}H_{39}$), 84849-02-5; FAEM (R = $C_{21}H_{43}$), 84849-03-6.

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