Isolation of 2-Fluorocitrate Produced by in Vivo Dealkylation of 29-Fluorostigmasterol in an Insect*

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A novel pro-insecticide, 29-fluorostigmasterol, is proposed to cause mortality due to release of fluoroacetate during side chain dealkylation. The 29-3H-labeled substrate was fed to third instar tobacco hornworms (Manduca sexta) and erythro-2-fluoro-[2-3H]citrate was isolated in 0.012% yield by ion-exchange, silica gel, and reverse-phase chromatography of the tricarboxylic acid, trimethyl ester, and trimethyl ester benzoate, respectively. The less toxic 29-fluoro-[29-3H]sitosterol did not provide sufficient labeled fluoroacetate to allow isolation, while a more toxic 16-3H-labeled 16-fluorofatty acid gave nearly 1% conversion to labeled fluorocitrate. This is the first direct chemical evidence for the fate of the two carbons removed during phytosterol dealkylation in an insect. It is also the first use of labeled fluoroacetate precursors to identify labeled 2-fluorocitrate as an in vivo metabolite of these precursors.

The 29-fluorophytosterols (1, 2) are novel pro-insecticides which we have proposed to be metabolically activated by dealkylation (3-5) at C-24 of the steroid side chain to release fluoroacetate or its equivalent (Fig. 1). Fluoroacetate then undergoes a "lethal synthesis" (6, 7) to (−)-erythro-2-fluoro-citrate (LD50 10-50 µg/kg for susceptible animals), an irreversible inhibitor of a citrate transport protein (8, 9), although other interpretations of fluoroacetate toxicity are possible (10). We reported a markedly higher toxicity of 29-fluorostigmastrol relative to the 22,23-saturated analog, 29-fluorositosterol to Manduca sexta larvae. Moreover, we noted a lower toxicity of the 29-fluorophytosterol relative to equivalent molar amounts of sodium fluorocitrate or 16-fluorohexadecenoic acid fed to hornworms (1).

To obtain further support for the proposed metabolic activation for the 29-fluorosterols, the 29-3H-labeled analogs were required. We also required an efficient method for the isolation of trace quantities of labeled 2-fluorocitrate from a crude insect homogenate. We report herein the synthesis of 29-fluoro-[29-3H]sitosterol (2), 29-fluoro-[29-3H]stigmasterol (4), and (E)-16-fluoro-[16-3H]-9-hexadecenoic acid as substrates for in vivo conversion to 2-fluoro-[2-3H]citrate. The isolation of fluoro-[2-3H]citrate from insects fed these substrates is described and a comparison of the phytosterol dealkylation and fatty acid β-oxidation pathways for fluorocitrate production in Manduca larvae is presented.

MATERIALS AND METHODS

General—Unless otherwise noted, materials were obtained from commercial suppliers and used without further purification. CH2Cl2 was distilled from CaCl2 and stored over 4 Å molecular sieves. Hexane, ethyl acetate, and methanol were Fisher HPLC grade and used without further purification. Sodium [3H]borohydride was purchased from New England Nuclear. All reactions were performed under N2. IR spectra were determined with a Perkin-Elmer Model 721 instrument and are reported in wave numbers (cm⁻¹). 80 MHz 1H-NMR and 20 MHz 13C-NMR spectra were obtained on an AFT 20 spectrometer. 300 MHz 1H-NMR were obtained in CDC6 or CDCl3 using a Nicolet NT-300 instrument. Proton-noise decoupled 1H-NMR spectra were obtained by J. Balschi (Stony Brook) at 10.6 MHz on a modified Varian XL-100 spectrometer. Chemical shifts are expressed in parts per million downfield from internal tetramethylsilane.

TLC was performed using MN Polygram Sil G/UV 254 (4 x 8 cm) TLC plates. Flash chromatography (11) on nondiadebabeled material was performed under N2 pressure on Merck Silica Gel G (400-230 mesh) using hexane/CH2Cl2 mixtures by applying pressure with a pipette bulb. The chromatograms of nonradiolabeled materials were visualized with an ethanol/vanillin/H2SO4 reagent. Visualization of radiolabeled material was accomplished at 254 nm followed by staining with I2. Radioactive samples were counted in a Packard Tri-Carb liquid scintillation counter using an Omnifluor/toluene mixture for organic scintillants and Biofluor (New England Nuclear) for aqueous samples. Counting was 52-57% efficient as determined by quench curves, and all counts were corrected using automatic external standardization.

HPLC1 was performed using a Waters M6000 pump and a Whatman C8 XDS 10/25 column with detection at 204 nm. Gas chromatography was performed using a Varian 3700 capillary instrument equipped with a flame ionization detector and one of the following columns: Durabond DB-1 (0.25 mm x 30 m) or Durabond DB-5 (0.25 mm x 30 m) (J&W Scientific) at the temperatures indicated for citrate analyses and at 280 °C for sterol analyses.

Synthesis of Labeled Substrates (Fig. 2) — 29-Fluoro-[29-3H]sitosterol (2) was synthesized in five steps from 29-hydroxystigmasterol isomethyl ether, an intermediate in the synthesis of the unlabeled 29-fluorostigmasterol (2). First, a CH2Cl2 solution of the 29-alcohol (162 mg, 0.354 mmol) was added to a suspension of 1.1 mg (0.531 mmol) of pyridinium chlorochromate in 5 ml of CH2Cl2. The mixture was stirred 1 h at 20 °C, diluted with ether, and filtered through a small silica gel column to remove chromate salts. The crude aldehyde was purified by flash chromatography on 230-400 mesh silica gel under N2 pressure by elution with 5% ethyl acetate/hexane to yield 140 mg (88%) of aldehyde. 1H-NMR showed a characteristic RCHO resonance at 9.75 (t, 2 Hz) and a carbon 13C resonance at 192.3 ppm.

Next, a solution of 116 mg (0.262 mmol) of the 29-aldehyde in 2.0 ml of dry isopropyl alcohol was added to an ampoule containing 300

1The abbreviations used are: HPLC, high-pressure liquid chromatography; PCC, pyridinium chlorochromate; DAST, diethylaminosulfur trifluoride; GLC, gas-liquid chromatography; TMPO, trimethyl fluorocitrate; TMC, trimethyl citrate; TMIC, trimethyl isocitrate.

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mCi of sodium borotritide (0.90 mg, 11.4 Ci/mmol). The reaction mixture was stirred for 4 h under N₂, and then excess NaBH₄ (150 mg) was added to complete the reduction. After 1 h, the salts were hydrolyzed with 3 ml of 50% acetic acid, the mixture was extracted with 20-ml portions of ether (to ensure complete recovery of homogenous A/B-protected alcohol 3 was used as starting material (2). Thus, PCC was added to 100 mCi of NaBT₃ (0.18 mmol, 5.5 Ci/mmol) in 1 ml of isopropyl alcohol, and the reaction was stirred under N₂ for 2 h at 20 °C. The reduction was completed by adding 50 mg (1.3 mmol) of unlabeled NaBH₄ and then quenched with acetic acid and worked up as described above to give 25 mg (0.56 mmol, 99 Ci/mmol) of [29-3H]₄ after flash chromatography.

The flotation was performed using diethylaminodimethylsilane to a solution of 7.7 mg (0.048 mmol) of diethylaminotrimethylsilane to a solution of 7.7 mg (0.048 mmol) of diethylaminotrimethylsilane to a solution of 7.7 mg (0.048 mmol) of diethylaminotrimethylsilane to a solution of 7.7 mg (0.048 mmol) of diethylaminotrimethylsilane in CH₂Cl₂ at -78 °C. The mixture was warmed to 20 °C. The reduction was completed by adding 50 mg (1.3 mmol) of A/B-protected 29-fluoro-[29-3H]sterol (2.0 Ci/mmol) after flash chromatography. Deprotection as described above gave 10.4 mg (88%) of the [29-3H]₄ (120 mCi/mmol) after flash chromatography.

(E)-16-Fluoro-[16-3H]hexadec-9-enoic acid (6) was synthesized in five steps from methyl-(E)-16-hydroxy-9-hexadecenoate following procedures described previously (12). First, a solution of 225 mg (0.833 mmol) of hydroxyester 5 was oxidized with 350 mg (1.6 mmol) of PCC in 25 ml of CH₂Cl₂. After workup as described above and evaporation distillation (120 °C/0.1 torr), TLC and GLC homogeneous aldehyde ester was obtained in 90% yield: ¹H-NMR (CDCl₃) δ 2.2 (t, 3 H), 3.63 (s, 3 H); ¹³C-NMR (CDCl₃), 129.6, 129.9 (E-RCH=CHR).

The aldehyde (48 mg, 0.180 mmol) was dissolved in absolute ethanol and added to a freeze-degassed solution of 100 mCi of sodium borotritide (0.7 mg, 5.4 Ci/mmol) in 2 ml of ethanol in a vacuum-sealed break-seal apparatus. After 2 h at 20 °C, a solution of 0.6 mCi of unlabeled NaBH₄ in 1 ml of ethanol was added and the reaction was stirred for 30 min at 20 °C. Acetone was added to react with excess hydride and the volatiles were removed in vacuo. The residue was taken up in 2 ml of H₂SO₄ and 2 ml of hexane, extracted three additional times, and the combined hexane layers were washed (saturated NaCl), dried (MgSO₄), and concentrated in vacuo. The crude product was flash chromatographed (5 g of SiO₂, 22% EtOAc/hexane) to give 86 mg (74%) of [29-3H]₄ (specific activity, 540 Ci/mmol); ¹H-NMR (CDCl₃) showed a pair of singlet resonances in a 1:1 ratio, 63.84 and 3.80, corresponding to the diastereotopic carbonyl tiritons at C-29.

A solution of 16 mg (0.0385 mmol) of [29-3H]₄ in 2 ml of CH₂Cl₂ was cooled to -5 °C, and a solution of 0.070 mCi of diethylaminosulfur trifluoride as a 1 mCi solution in CH₂Cl₂ was added slowly. The reaction was stirred overnight at -20 °C and quenched by addition of K₂CO₃ (100 mg) followed by 1 ml of H₂O and 5 ml of ether. The aqueous layer was extracted 2 ml of ether, and the extracts were washed (H₂O, saturated NaHCO₃, saturated NaCl), dried (MgSO₄), and concentrated in vacuo. The crude product was flash chromatographed (5% EtOAc/hexane) to give 9 mg (56%) of the TLC homogeneous A/B-protected 29-fluoro-[29-3H]sterol. Proton-decoupled ¹H-NMR showed a doublet at 44.22 with J₆₇ = 51.4 Hz. Finally, 9 mg (0.020 mmol) of the A/B-protected 29-fluoro-29-tritiossterol was dissolved in 2 ml of dioxane containing 2 mg of p-toluenesulfonic acid, water was added until cloudiness developed, and the mixture was refluxed 1 h. After cooling, the mixture was washed (H₂O, saturated NaHCO₃, saturated NaCl), dried (MgSO₄), and chromatographed (32% EtOAc/hexane) to give 6 mg (68%) of 29-fluoro-[29-3H]sterol ([29-3H]₂) with a specific activity of 550 mCi/mmol.

29-Fluoro-[29-3H]sterol (4) was prepared by the same procedures as for [29-3H]₂, except that the 22,23-unsaturated A/B-protected alcohol 3 was used as starting material (2). Thus, PCC oxidation of 190 mg (44 mmol) of alcohol 3 provided 160 mg (77%) of the corresponding aldehyde after flash chromatography. A solution of 65 mg (0.15 mmol) of this aldehyde in 1 ml of isopropyl alcohol was added to 100 mCi of NaBT₃ (0.18 mmol, 5.3 Ci/mmol) in 1 ml of isopropyl alcohol, and the reaction was stirred under N₂ for 2 h at 20 °C. The reduction was completed by adding 50 mg (1.3 mmol) of unlabeled NaBH₄ and then quenched with acetic acid and worked up as described above to give 25 mg (0.56 mmol, 99 Ci/mmol) of [29-3H]₃ after flash chromatography.

The fluorination was performed using diethylaminodimethylsulfur trifluoride generated in situ by adding 7 mg (0.048 mmol) of diethylaminodimethylsulfur trifluoride to a solution of 7.7 mg (0.048 mmol) of diethylaminodimethylsulfur trifluoride to a solution of 7.7 mg (0.048 mmol) of diethylaminodimethylsulfur trifluoride to a solution of 7.7 mg (0.048 mmol) of diethylaminodimethylsulfur trifluoride to a solution of 7.7 mg (0.048 mmol) of diethylaminodimethylsulfur trifluoride to a solution of 7.7 mg (0.048 mmol) of diethylaminodimethylsulfur trifluoride in CH₂Cl₂ at -20 °C. The mixture was warmed to 20 °C, cooled to -78 °C, and then 8 mg (0.013 mmol) of the alcohol [29-3H]₃ and 1.2 mg of unlabeled 3 in 3 ml of CH₂Cl₂ were added dropwise. After 3 h, the reaction was worked up as described above to give 12.6 mg (0.028 mmol) of the A/B-protected 29-fluoro-29-tritiossterol (120 mCi/mmol) after flash chromatography. Deprotection as described above gave 10.4 mg (88%) of the [29-3H]₄ (120 mCi/mmol) after flash chromatography.

(E)-16-Fluoro-[16-3H]hexadec-9-enoic acid (6) was synthesized in five steps from methyl-(E)-16-hydroxy-9-hexadecenoate following procedures described previously (12). First, a solution of 225 mg (0.833 mmol) of hydroxyester 5 was oxidized with 350 mg (1.6 mmol) of PCC in 25 ml of CH₂Cl₂. After workup as described above and evaporation distillation (120 °C/0.1 torr), TLC and GLC homogeneous aldehyde ester was obtained in 90% yield: ¹H-NMR (CDCl₃) δ 2.2 (t, 3 H), 3.63 (s, 3 H); ¹³C-NMR (CDCl₃), 129.6, 129.9 (E-RCH=CHR).

The aldehyde (48 mg, 0.180 mmol) was dissolved in absolute ethanol and added to a freeze-degassed solution of 100 mCi of sodium borotritide (0.7 mg, 5.4 Ci/mmol) in 2 ml of ethanol in a vacuum-sealed break-seal apparatus. After 2 h at 20 °C, a solution of 0.6 mCi of unlabeled NaBH₄ in 1 ml of ethanol was added and the reaction was stirred for 30 min at 20 °C. Acetone was added to react with excess hydride and the volatiles were removed in vacuo. The residue was taken up in 2 ml of 0.5 N H₂SO₄ and 2 ml of hexane, extracted three additional times, and the combined hexane layers were washed (saturated NaCl), dried (MgSO₄), and concentrated on 3 g of 230–400 mesh silica gel. Elution with 10% ethyl acetate/hexane gave 29.8 mg of TLC homogeneous alcohol ester with a specific activity of 826 mCi/mmol (total of 91 mCi).

To a solution of 29.8 mg of the tritiated ω-hydroxy ester in 10 ml of dry CH₂Cl₂ at 0 °C was added 0.5 ml of freshly distilled N-(1,1,2-
The reaction was stirred at 0–20 °C for 2 h, the solvents were removed in vacuo, and then 1 ml of 2-propanol in 2 ml of 5% ethyl acetate/hexane was added and stirred 10 min. This mixture was washed (saturated NaCl), dried (MgSO₄), and filtered through silica gel to give 6.5 mg (45 mCi) of the desired tritium-labeled, fluorinated ethyl ester (due to ethanol transesterification catalyzed by basic impurities). Spectral data were analogous with those of the previously reported unlabeled methyl ester (12). In addition, the proton-decoupled 1H-NMR showed a doublet, 64.05, J = 51.2 Hz for the CHF₃ tritiofluoromethyl group.

To an aliquot of 4.5 ml of this ester in methanol were added 21 mg of the unlabeled fluoro ester, 30 mg of K₂CO₃, and 100 μl of H₂O. The hydrolysis mixture was stirred 16 h at 20 °C, concentrated in vacuo, and then 1 ml of ethanol was added as a carrier. The residue was resuspended in three additional 10-ml portions of 1:1 ethyl acetate/water, and the combined aqueous fractions (about 30 ml) were passed through a 2-ml column (AG 1-X8 formate form) to bind to organic acids. After washing with water (5 ml) and 5 N formic acid (5 ml), the tricarboxylic acids were eluted with 2 N ammonium formate (10 ml). The eluate was acidified (pH < 1), lyophilized, methylated (CH₃N₂), and the trimethyl 2-fluorocitrates (13) were not resolved on the less polar C₈ reverse-phase columns. Table 1 summarizes the radioactivity recovered at each purification step for 29-fluoro-[29-3H]stigmasterol (2), 29-fluoro-[29-3H]sitosterol (4), and 16-fluoro-[16-3H]hexadecenoic acid (6).

**RESULTS AND DISCUSSION**

Tritium-labeled 29-fluorostigmasterol 2 and 29-fluorostigmasterol 4 were prepared from the corresponding A/B ring-protected compounds 1 and 3 (Fig. 2). Thus, oxidation (PCC, CH₂Cl₂) to the 29-aldehyde was followed by sodium borotri fluoride (300 mCi, 5 Ci/mmol) reduction, fluorination ((CH₃)₂NSF₂), and deprotection as described previously (2) to give 29-fluoro-[29-3H]sitosterol 2 (246 mCi/mmol) and 29-fluoro-[29-3H]stigmasterol 4 (120 mCi/mmol). The labeled ω-fluorofatty acid 6 was prepared from methyl (E)-16-hydroxyhexadec-9-enoate (12) by an analogous sequence to give the 16-fluoro-[16-3H]-labeled fatty acid 6 (73 mCi/mmol). Proton-decoupled tritium NMR was employed to unambiguously identify ³H resonance due to the triitiofluromethyl groups of 2 and 6. Flash chromatography was performed after each reaction with labeled substrates to ensure removal of radioactive side products.

The in vivo data for Manduca larvae fed unlabeled 2, 4, and (−)-erythro-2-fluorocitrate (1) showed a 500- to 1000-fold higher toxicity for fluorocitrate relative to 29-fluorostigmasterol. Thus, we estimated that less than 0.1% of the administered labeled 29-fluorostigmasterol 4 (e.g., less than 10 ng) would be converted to the lethal fluorocitrate stereoisomer. Unlabeled fluorocitrate was thus required as a carrier to facilitate chromatographic detection and improve recovery of labeled material. In preliminary experiments following this protocol, mass recovery of trimethylfluorocitrate benzoate exceeded 70%.

Initial experiments with labeled 2, 4, and 6 employed older 5th instar M. sexta larvae, and the labeled compounds were administered by impregnation into small blocks of artificial diet (15). These experiments gave suboptimal incorporations into labeled fluorocitrate, due to the enormous bulk (10 g) of the insect and relatively low dealkylation rates for this stage. We recently determined that sterol dealkylation rates for [29-3H]sitosterol vary during the insect life cycle, from a maximum of 7.5 nmol of sterol/g fresh weight insect/h in 3rd instar larvae to 0.57 nmol/g/h in 5th instars and 0.18 nmol/g/h in pupae (15). These assays employed a peroral injection technique followed by a simple partition assay to measure aqueous tritium-labeled metabolites of a [29-3H]phytosterol. Thus, the experiments reported here employed were optimized in terms of 1) peroral injection to standardize equantity of labeled precursor administered, 2) use of 3rd instars to maximize phytosterol turnover, and 3) use of 10 insects/replicate to minimize the effects of individual variation on the incorporation results.

A key requirement for our isolation of labeled fluorocitrate was that the ultimate HPLC step would resolve the erythro-

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**Fluorocitrate from 29-Fluorosterols in Insects**

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![Diagram](image-url)
**Fluorocitrate from 29-Fluorosterols in Insects**

**Purification of erythro-2-fluoro-[2-3H]citrate from precursors injected perorally into third instar hornworm larvae**

For two replicates of 10 third instar *M. sexta* each, 1 day after apolysis. Each insect received 1 μl of dimethyl formamide containing one-tenth of the indicated amount. After 8-h incubation at 26 °C, insects were frozen and stored at −17 °C until homogenization.

| Stage of purification | Metabolites present | Radioactivity remaining* |
|-----------------------|---------------------|-------------------------|
|                       | [3H]Precursor        | 9.6 × 10^6              |
|                       | Aqueous metabolites  | 1.6 × 10^6              |
|                       | Tricarboxylic acids  | 1.9 × 10^5              |
|                       | Trimethyl esters     | <10^5                   |
|                       | Trimethyl ester benzoates | 4.1 × 10^5        |
|                       | erythro-2-Fluorocitrate | 1.7 × 10^6              |
| Conversion to [2-3H]erythro-2-fluorocitrate (%) |                      | 0.012%                  |

* Averages of duplicate assay; counts per min were corrected for background and converted to disintegrations per min using quench corrections from automatic external standardization.

**Fig. 4. Reverse-phase HPLC purification of trimethyl erythro-2-fluoro-[2-3H]citrate benzoate.**

A, standard mixture of trimethyl isocitrate, trimethyl citrate, and *erythro* and *threo-*trimethyl fluorocitrate benzoates; B, labeled *erythro*-TMFC benzoate from [29-3H]4, C, labeled *erythro*-TMFC benzoate from [16-3H]6. The nontoxic (2S,3S)-enantiomer, which has an identical retention time to the toxic (2R,3R)-enantiomer, was used for the HPLC standard for the *erythro*-fluorocitrate.

and *threo*-2-fluorocitrate diastereoisomers from each other as well as from nonfluorinated citrates. For this reason, the initial stages of purification were designed not to discriminate among these compounds and increasing discrimination was achieved by increasing the degree of replacement of polar groups with less polar derivatives. At the final stage, HPLC conditions for this separation were critical; use of a C18 (octadecyl)-capped silica rather than C8 (octyl), or deviation by more than 6% from the 34% CH3CN/H2O mixture resulted in loss of base-line resolution of the trimethyl (fluoro)citrate benzoates. The final separations showing UV absorbance of 3H]4; C, labeled erythro-TMFC benzoate from [29-3H]6. The nontoxic (2S,3S)-enantiomer, which has an identical retention time to the toxic (2R,3S)-enantio-1.4mer, was used for the HPLC standard for the *erythro*-fluorocitrate.

The data in Table I demonstrate that the efficiency of conversion of labeled precursor to labeled fluorocitrate decreases in the order 16-fluoro-[16-3H]fatty acid 6 > 29-fluoro-[29-3H]stigmasterol 4 > 29-fluoro-[29-3H]sitosterol 2. The toxicity of the unlabeled materials in vivo parallels the relative quanties of fluorocitrate produced (1, 15). In fact, of the two 29-fluorosterols only the 100-fold more toxic 29-fluorostigmasterol 4 led to an isolable quantity of labeled fluorocitrate.

A theoretical pathway can be proposed which integrates the known aspects of sterol dealkylation (or fatty acid β-oxidation) and fluorocitrate synthesis with the 29-fluorostigmasterol toxicity and labeled citrate isolation results (Fig. 5). In a formal sense, β-oxidation produces fluoroacetyl-CoA directly. This circumvents one inefficient step in the lethal synthesis of fluorocitrate itself into fluorocitrate (Vmax for fluorocitrate < 1% that of acetate) (8, 10). The next step, condensation with oxaloacetate to give (2R,3R)-fluorocitrate, also has a Vmax for fluoroacetyl-CoA of 0.3% that of acetyl-CoA (8). Moreover, fluoroacetyl-CoA can enter many of the pathways, e.g. thiolase, malate synthase, and acetyl-coA carboxylase, for which acetyl-CoA is the starting material (10). Thus, the 1% conversion from a 16-fluorocid acid 6 to *erythro*-fluorocitrate is in accord with our best expectations.

For the 29-fluorostigmasterols, the two-carbon fragment released during dealkylation is formally fluoroacetaldehyde. This requires conversion into fluoroacetyl-CoA prior to the condensation to give fluorocitrate. This conversion should involve an aldehyde dehydrogenase and could proceed directly to the CoA derivative or via oxidation, hydrolysis, and reactivation at a later time. Although the toxicities of fluoroo-
ethanol and higher even-carbon homologs (and their corresponding aldehydes) are known (17), the enzymatic conversions have not been examined in detail. In any event, the dealkylative pathway leading to fluorocitrate is considerably more tortuous than that for β-oxidation, and has at least five separate enzymic steps in which the substitution of fluorine for hydrogen could exert an adverse electronic effect on $K_m$ or $V_{max}$ of the reaction. In particular, the desaturation-oxidation-fragmentation has been shown to be sensitive to 29-fluorine substitution. Lower fluoroacetate-produced toxicity is observed for the 22,23-saturated sterols than for the 22,23-unsaturated analogs (1, 2, 15). In addition, one expects fluoroacetaldehyde to be less efficiently oxidized than acetate. The observed conversion of labeled sterol 4 to fluorocitrate in 0.012% yield, nearly 100-fold lower than for the fluorofatty acid 6, is reasonable given the numerous opportunities for enzymic bottlenecks. That any fluorocitrate at all can be isolated testifies to the efficiency of sterol utilization in this phytophagous insect, which increases in weight 1000-fold during its 2-week development from egg to 5th instar.

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