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SYNTHESIS OF 29-FLUOROPHYTOSTEROLS: A NOVEL CLASS OF INSECT-ACTIVATED SELECTIVE POISONS

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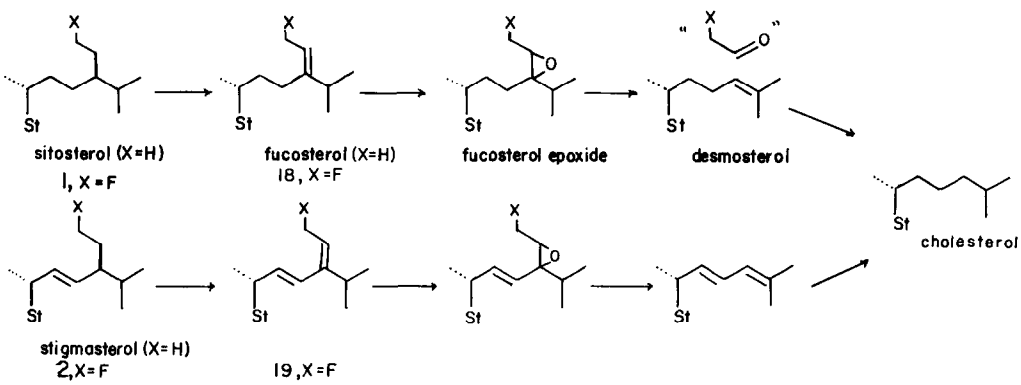
Summary. Four 29-monofluorophytosterols are prepared from stigmasterol. When fed to insects, evidence is obtained for fluoroacetate poisoning via dealkylation at C-24.

Insect steroid metabolism is an attractive target for the design of selective insecticides, since insects are unable to biosynthesize sterols de novo and rely on dietary sources for their sterol requirement.[1] Many plant-eating insects satisfy their need for cholesterol (C_{27}) by the dealkylation of C_{29} phytosterols such as sitosterol and stigmasterol (Scheme 1).[1,2] We predicted that the substitution of F for H at C-29 would not significantly perturb the dehydrogenation, epoxidation, and fragmentation steps.[3] However, the released C_2 fragment for X=F should give rise to the latent poison fluoroacetate.[4] To test this prediction, four monofluorinated phytosterols 1-4 were prepared stereospecifically (Scheme 2) by modification of methods developed for the preparation of the oogoniol side chain.[5]



Scheme 1. Phytosterol dealkylation in insects.

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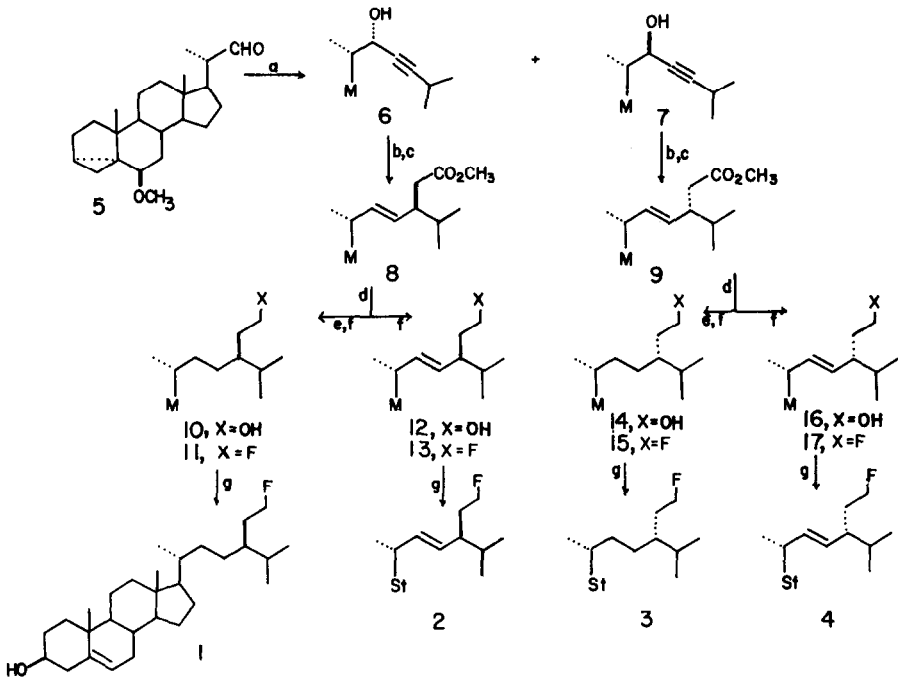

Scheme 2. Synthesis of 29-fluorophytosterols. Reagents (isolated yields): (a) $LiC{\equiv}CCH(CH_3)_2$, THF (78%); (b) H_2, $Pd/BaSO_4$, quinoline, C_2H_5OH (96%); (c) 10 eq. $CH_3C(OCH_3)_3$, 2,4,6-trimethylbenzoic acid (10 mole %), xylene, 140° (86%); (d) $LiAlH_4$, THF; then H_3O+ (90%); (e) H_2, 5% Pt/C, C_2H_5OH (90%); (f) DAST, CH_2Cl_2, -78° to 25°; $CaCO_3$ then aq. $NaHCO_3$ (63%); (g) pTsOH, dioxane-water, reflux (75%).

Aldehyde 5[6] was converted to a mixture of epimeric propargylic alcohols 6 and 7 by alkylation with the lithium acetylide of 3-methyl-1-butyne, generated *in situ* by a modification of the Negishi procedure.[7] The propargylic alcohols were separated[5] by flash chromatography, reduced to the Z-allylic alcohols, and subjected to the orthoester Claisen reaction with trimethylorthoacetate using the hindered 2,4,6-trimethylbenzoic acid as catalyst.[8] The resulting C-29 esters 8 and 9 were transformed along two parallel pathways. To obtain the 22,23-saturated materials, hydride reduction of the C-29 esters to the C-29 alcohols was necessary to achieve efficient hydrogenation to 10 and 14. Fluorination of the 29-alcohols with diethylaminosulfur trifluoride (DAST)[9] gave the A/B protected 29-fluoro compounds 11, 13, 15 and 17.[10] Finally, deprotection of the sterol A/B rings and crystallization of the products from methanol gave 29-fluorositosterol 1, mp 119-122°, 29-fluorostigmasterol 2, mp 147-151°C;

Table 1. Effects of 29-fluorophytosterols (50 ppm in diet) on growth of Manduca sexta.[12]

Sterol	% Survival at 21 days	% Maximum Weight Gain	Days to Maximum Weight	% Pupal Weight (No. of survivors)
1	85	53.5	25	67.9 (17)
2	20	<2.5	26	- (0)
3	90	48.8	26	63.6 (18)
4	25	52.1	33	66.3 (2)
Control	100	100	16	100 (20)

29-fluoroclionosterol 3, mp 113-116°C, and 29-fluoroporiferasterol 4, mp 141-144°C.[11]

The 29-fluorophytosterols all show significant impairment of growth and development of larval tobacco hornworms (Table 1).[12] It is clear that the $\Delta^{22,23}$ sterols 2 and 4 are more toxic and cause more severe stunting than the 22,23-dihydro analogs 1 and 3. Moreover, C-24 sterochemistry does not appear to be as important in determining relative toxicity as the absence or presence of the $\Delta^{22,23}$ olefinic bond. The toxicity and abnormal development seen for the 29-fluorophytosterols is similar to that seen in larvae fed sodium fluoroacetate or ω-fluorofatty acids.[12] Moreover, no toxicity or abnormal growth is found for 25- or 26-monofluorophytosterols[13] or for four monofluorocholesterols[14] incorporated into diets fed to M. sexta.

The slow step in sterol dealkylation thus appears to be 24,28-desaturation to give 29-fluorofucosterol 18 or the corresponding 29-fluorostigmastatrienol 19. The substitution of the weakly electron withdrawing fluoromethyl for methyl causes desaturation to proceed sluggishly on this substrate. With the $\Delta^{22,23}$ bond present, electron release into an incipient conjugated diene can compensate for the electron withdrawing effect of the fluoromethyl group.

We have therefore harnessed the insect phytosterol dealkylation pathway to release the latent toxin fluoroacetate in vivo.[14,15] The identity of the proposed intermediates and the metabolic fate of C-28 and C-29 are being explored to confirm the production of fluorocitrate[15] as the ultimate biochemical lesion leading to mortality.

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10. Protected 29-fluorosterols (all oils): 11, ^{1}H-NMR ($CDCl_3$, 80 MHz) δ0.30-0.60 (m, H-4), 0.71 (s, CH_3-18), 0.84 (d, CH_3-26, CH_3-27, J = 6.5 Hz, coincidental overlap), 0.97 (d, CH_3-21, J = 5.8 Hz), 1.01 (s, CH_3-19), 2.75 (m, H-6), 3.32 (s, OCH_3), 4.42 (d of m, H-29, J_{HF} = 46.8 Hz); 13, ^{1}H-NMR as in 11 except δ4.39 (d of m, H-29, J_{HF}= 46.8 Hz, J_{HH} unresolved), 5.12 (m, H-22,23); ^{13}C-NMR(C_6D_6), δ33.5 (d, $^2J_{CF}$ = 19.7 Hz, C-28), 45.2 (d, $^3J_{CF}$ = 4.5 Hz, C-24) 82.3 (d, $^1J_{CF}$ = 165.2 Hz, C-29); LRMS: m/z (rel. int.) 444 (10, M^+), 429 (38, M-CH_3), 412 (32, M-MeOH), 397 (12, M-CH_2CH_2F), 389 (65, ring A fission), 255 (M-side chain -CH_3OH), 253 (40), 213 (35), 81 (100) HRMS: calcd. for $C_{30}H_{49}OF$, 444.3768; found 444.3835; 15, ^{1}H-NMR, as in 11 except δ4.46 (d of m, H-29, J_{HF} = 47.3 Hz); 17, ^{1}H-NMR as in 13 except δ4.38 (d of m, H-29, J = 46.8 Hz); ^{13}C-NMR (C_6D_6), δ33.5 (d, 19.4 Hz, C-28), 45.1 (d, 4.6 Hz, C-24); 82.4 (d, 165.0 Hz, C-29); HRMS, found 444.3765.
11. Spectral data for 29-fluorosterols: 1, ^{1}H-NMR, (300 MHz), δ0.67 (s, CH_3-18), 0.83 (d, CH_3-26, J = 6.8 Hz), 0.84 (d, CH_3-27, J = 6.8 Hz), 0.91 (d, CH_3-21, J = 6.5 Hz), 0.99 (s, CH_3-19), 3.51 (m, H-3), 4.45 (d of m, H-29, J_{HF} = 47.3 Hz), 5.34 (m, H-6); ^{13}C-NMR ($CDCl_3$, 75 MHz), δ31.40 (d, 18.2 Hz, C-28), 40.29 (d, 4.4 Hz, C-24), 83.43 (d, 163.9 Hz, C-29); LRMS: m/z (rel. int.) 432 (18, M^+), 414 (15, M-H_2O), 399 (17, M-CH_3-H_2O), 347 (22), 273 (15), 255 (26), 213 (48), 107 (100); HRMS, calcd for $C_{29}H_{49}OF$, 432.3768; found, 432.3793; 2, ^{1}H-NMR as in 1 except δ4.39 (d of m, $^2J_{HF}$ = 46.8 Hz, H-29), 5.12 (m, H-22,23); ^{13}C-NMR, δ33.2 (d, 19.8 Hz, C-28), 45.2 (d,4.5 Hz, C-24), 82.3 (d, 165.1 Hz, C-29); LRMS, m/z (rel. int.), 430 (M^+, 34), 412 (10, M-H_2O), 397 (5, M-CH_3-H_2O), 345 (5), 300 (21), 273 (10) 271 (20), 255 (43), 213 (32), 133 (100); HRMS, calcd for $C_{29}H_{47}OF$, 430.3610; found, 430.3571; 3, ^{1}H-NMR (300 MHz), as in 1 except δ4.47 (d of m, H-29, J = 46.5 Hz), ^{13}C-NMR (20 MHz), δ31.5 (d, 19.2 Hz, C-28) 40.4 (d, 5.2 Hz, C-24), 82.8 (d, 164.0 Hz, C-29); LRMS like 1; 4, ^{1}H-NMR as in 2 except δ4.39 (d, 46.8 Hz, H-29); ^{13}C-NMR, δ33.5 (d, 19.6 Hz, C-28); 45.1 (d, 4.4 Hz, C-24), 82.5 (d, 165.0 Hz, C-29); HRMS, found 430.3599.
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