SIDE CHAIN MODIFIED STEROLS AS PROBES INTO INSECT MOLTING HORMONE METABOLISM. II: SYNTHESIS OF MONOFLUOROCHOLESTEROLS

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ABSTRACT

The hydroxylations of the cholesterol side chain at C-20, 22, and 25 are key terminal events in ecdysone biogenesis. We have prepared the C-20, C-22, C-24, and C-25 monofluorinated cholesterols as potential inhibitors of these hydroxylation events, and preliminary bioassay results in Manduca sexta are reported. The synthesis of [26^{14}C]-20-fluorocholesterol is also described. Although the 20-,22-, and 25-monofluorocholesterols do not appear to affect larval growth and development, the 24-fluoro isomer shows a moderate retardation of growth and a modest increase in mortality.

INTRODUCTION

Insect steroid metabolism offers an attractive target for arthropod-specific control agents, since the distinctive C-24 dealkylation and ecdysone biogenesis pathways are unique to arthropods [1,2]. One approach we have taken to the design of selective inhibitors of the hydroxylations at C-20, 22, 25, and 26 of the cholesterol side chain has been the substitution of fluorine for hydrogen in several phytosterols [3]. In this paper, we report the synthesis and in vivo bioassay of four monofluorocholesterols, in which hydrogens at C-20 (1), C-22 (2), C-24 (3), and C-25 (4) have been replaced with fluorines (Figure 1).

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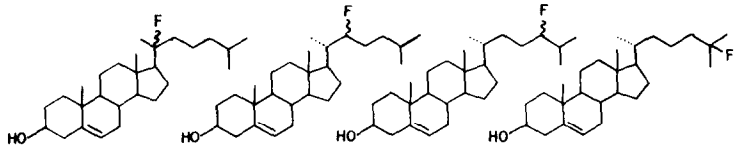
The substitution of fluorine for hydrogen in a substrate molecule may have several effects [4]. Fluorine has a small van der Waals radius (1.35Å) which allows it to masquerade sterically as hydrogen. When remote from labilized hydrogens, it may slightly alter lipid solubility without affecting reactivity. When attached to carbons at which hydrogens are labilized during a reaction, the increased polarity of the C-F bond may alter deprotonation, hydride transfer or radical abstraction reactions. We propose that monofluorocholesterols



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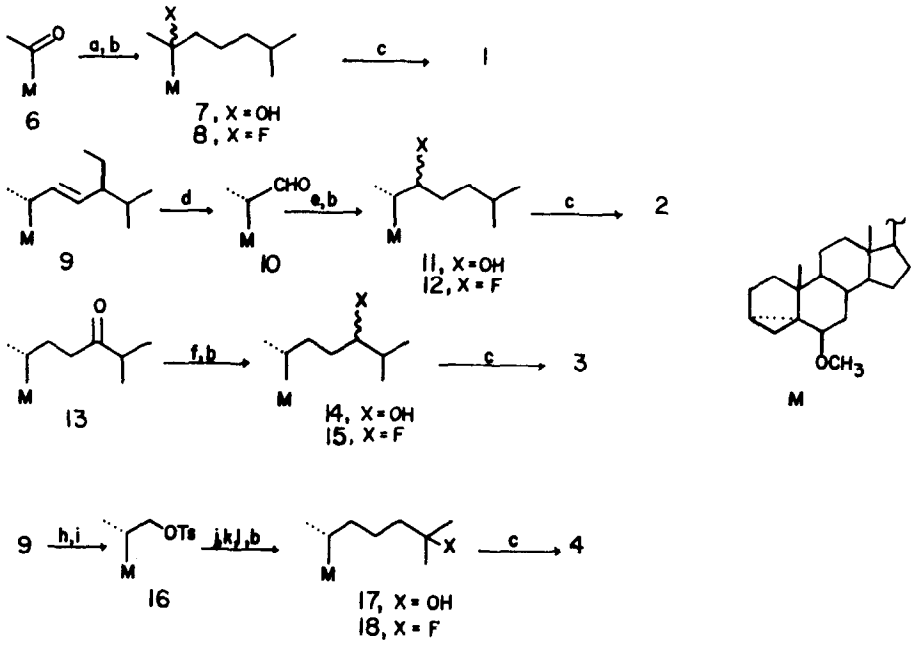
should resemble cholesterol during the initial stages of ecdysone biogenesis (C-2, C-14 hydroxylation and introduction of the 5β-H-6-keto-7-ene moiety). However, we predict that specific hydroxylations at C-20, C-22, and C-25 could not occur on the corresponding monofluoro substrates and that these might serve as inhibitors of these hydroxylations. In particular, 20-fluorocholesterol was designed to block ecdysone 20-hydroxylase, the best-characterized cytochrome P-450 monooxygenase involved in the

crucial conversion of ecdysone to 20-hydroxyecdysone [5,6]. To this end, we also synthesized the radioactively labelled [26-^{14}C]-20-fluorocholesterol to enable metabolic studies on the fate of 20-fluorocholesterol in vivo and in vitro.

RESULTS AND DISCUSSION

Syntheses

The synthesis of the four monofluorocholesterols from the corresponding monohydroxy cholesterols [7] is shown in Scheme I. Pregnenolone (3β-hydroxy-5-pregnen-20-one) was converted to the iso-methyl ether 6 [8] and condensed with isohexylmagnesium bromide to give an epimeric mixture of alcohols 7 in 70% yield after chromatography. The product mixture was crystallized from ethanol, and the precipitate was recrystallized several times, giving 90% pure 20(S)-hydroxy compound containing 10% of the R epimer. The stereochemistry of the C-21 methyl group was assigned by its ^{1}H-NMR resonance [9]. The 90% pure 20(S) epimer 7 was converted to 8 by treating with diethylaminosulfur trifluoride (DAST) [10] in methylene chloride at -78°C in 85% yield after purification by flash chromatography on silica gel. Equal amounts of 20(R) and 20(S)-fluoro compound 8 were detected by GC and by ^{19}F-NMR. The desired 20(ξ)- fluorocholesterol isomers 1 were obtained by deprotection with pTsOH in dioxane in 80% yield. The 20(R) and 20(S)-fluoro compounds were found to be converted into the $\Delta^{20,21}$ compound during esterification of 1 with benzoyl chloride in pyridine. Dehydrofluorination of 1 was caused by use of pyridine as solvent in the esterification. The dehydrofluorination also happened during GC-MS analysis of 1.



Scheme I. Synthesis of Monofluorocholesterols.
Reagents: (a) isohexylmagnesium bromide, THF ; then H_3^+O, (b) diethylaminosulfurtrifluoride (DAST), CH_2Cl_2, -78°C; (c) pTsOH, 2:1,dioxane-H_2O, 80°; (d) O_3, CH_3OH-CH_2Cl_2(1:3), -78°; then $(CH_3O)_3P$; (e) isopentylmagnesium bromide, THF, ; then H_3^+O; (f) $NaBH_4$, C_2H_5OH; (g) O_3, 1% Pyridine-CH_3OH, -78°C; (h) $NaAlH_2(OCH_2CH_2OCH_3)_2$; then H_3^+O; (i) TsCl, pyridine, 0°; (j) $LiC{\equiv}CCH_2C(CH_3)_2OTHP$, THF, reflux; (k) H_2, 10% Pd/C, dioxane; (l) pTsOH, CH_3OH, 0°C.

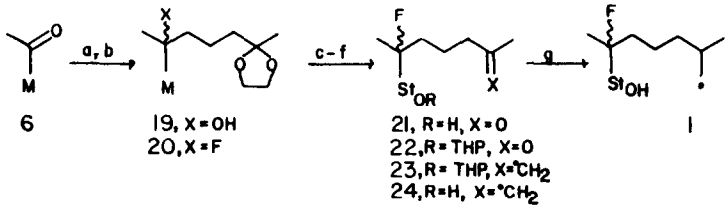
The 22 (ξ)-fluorocholesterols 2 were prepared from the aldehyde 10. The crude stigmasteryl-iso-methyl ether 9 was treated with ozone in a mixture of methylene chloride and methanol (ratio 3:1) at -78°C and reduced by trimethylphosphite to obtain the get crude aldehyde 10 [11]. The crude aldehyde 10 was then converted to the mixture of alcohols by a Grignard reaction with excess isopentylmagnesium bromide in tetrahydrofuran [12]. The mixture of 22(S) and 22(R)-hydroxy-isomers

11 (74% yield after chromatography) was converted to 22(S)-and 22(R)-fluoro-isomers 12 in 91% yield by using DAST as described above. Finally, the 22(ξ)-fluorocholesterol isomers (2) were obtained by deprotection as described above in 84% yield.

The 24(ξ)-fluorocholesterol 3 was prepared from 24-oxocholesterol 13 [7]. Reduction with sodium borohydride gave a mixture of C-24 epimeric alcohols 14 which were converted to epimeric fluoro compounds 15 with DAST. Deprotection as described above gave the epimeric 24(ξ)-fluorocholesterols 3.

The 25-fluorocholesterol 4 was prepared starting with alkylation of the 22-tosylate 16 with the lithium acetylide of 3-methyl-1-butyn-3-ol tetrahydropyranyl (THP) ether [13]. The acetylenic bond was catalytically reduced and the THP ether selectively cleaved [14] to afford the protected 25-hydroxy compound 17 in 72% yield from 16. Fluorination with DAST and deprotection as described above gave the 25-fluorocholesterol 4 in 78% yield. This compound was reported independently [15] during the course of our work in this series.

The synthesis of [26-^{14}C]-20-fluorocholesterol required modification of the synthetic scheme, so that the sequence shown in Scheme II was initially carried through to authentic 1 epimers without the isotopic label. 5-Bromopentan-2-one was prepared according to the literature (95% yield) [16] and converted to the corresponding ethylene ketal in 95% yield. The Grignard reagent of the ketal was prepared in THF, added to the 20-keto compound 6, to give the 20(S)-hydroxy compound 19 which was the dominant product (80% chromatograped yield). The 20ξ-fluoro isomers 20 were obtained equally in



Scheme II. Synthesis of ^{14}C-labelled 20-fluorocholesterol. Reagents: (a) $BrMgCH_2CH_2C(OCH_2CH_2O)CH_3$, THF ; then H_3^+O; (b) DAST, CH_2Cl_2, -78°; (c) pTsOH, $(CH_3)_2CO_2$-H_2O; (d) DHP, pTsOH; (e) $Ph_3P=^{14}CH_2$, C_6H_6; (f) CH_3OH, pTsOH; (g) $(Ph_3P)_3RhCl$, H_2, C_6H_6.

the reaction of alcohol 19 with DAST in 90% yield. Deprotection of 25-keto and of the isomethyl ether of 20 was done in acetone-water solution with a trace of p-toluenesulfonic acid monohydrate, because ethylene ketal at C-25 could not be deprotected selectively. The desired product 21 (84% yield) was converted to the tetrahydropyranyl ether 22 in 85% yield. Condensation with methylenetriphenyl-phosphorylid, which was made according to Pettler et al [17], gave the olefinic compounds 23, contaminated with triphenylphosphine even after chromatography. The THP group was removed with pTsOH/methanol to give homogeneous 24 in 64% yield from 22. Finally, catalytic hydrogenation of 24 in dry benzene with a trace of Wilkinson's catalyst [18] gave 20(ξ)-fluorocholesterol 1 in 75% yield which was identical to that made above (Scheme I). Use of ^{14}C-methyl iodide (500μCi, 58mCi/mmol) in the latter steps then resulted in the production of 4 μmmol of [26-^{14}C]-1 (26.5mCi/mmol) in an overall 80% chemical yield from 22 and 21% radiochemical yield from ^{14}C-methyl iodide.

Bioassays

Monofluorocholesterols were incorporated into cholesterol-free artificial diets, and 2nd instar larvae were fed in individual containers through to pupation. The dietary concentrations chosen for the fluorosterols were at least ten-fold higher than the effective doses for several known azasterol inhibitors [1]. The results are presented in Table 1, which shows little effect on the growth of Manduca in the presence of 20-F (1), 22-F (2), and 25-F (4). The 24-fluorocholesterol caused unexpectedly high mortality and growth disruption at 50 ppm. The 25-F compound had been shown to serve as a sterol supplement at 0.2% in the diet of Bombyx mori [15].

Table 1. Effects of in vivo feeding of Fluorinated Sterols to Manduca sexta (2nd instars). Values are means for 20 larvae (except 10 when asterisked).

Compound	Concentration in Diet (w/w%)	% Survival		Max Wt. Gain Time Elapsed; % of Control for Survivors	
		5th instar	Pupa		
Stigmasterol (control)	50 ppm	100%	85%	17days	100%
20-fluorocholesterol	50	80	80	16	86
	150	100	95	16	103
22-fluorocholesterol	50	85	85	15	116
24-fluorocholesterol	45	65	65	18	107
24-fluorositosterol	50	95	90	14	103
25-fluorocholesterol	30	90	85	16	98
25-fluorositosterol*	50	100	90	17	78
26-fluorositosterol (24R)*	50	100	100	17	84
(24S)*	50	100	100	17	92

The fluorocholesterols also have little effect on the sterol composition of the larvae. Analysis of the sterol composition of experimental and control prepupae revealed essentially unaltered ratios of sitosterol, campesterol, desmosterol, and cholesterol (Table 2). Slightly elevated desmosterol levels in 25-F fed larvae suggests metabolism by dehydrofluorination as previously hypothesized [15].

Table 2. Sterol distribution in experimental hornworms. Values are relative area % (n = 4 hornworms, 3 determinations each) ± 1 s.d.

Compound	Concentration in Diet (ppm)	Relative Percent Sterol			
		Cholesterol	Desmosterol	Campesterol	Sitosterol
Stigmasterol (control)[a]	50 ppm	66.66(0.55)	2.60(0.15)	12.19(0.16)	9.52(0.21)
20-fluorocholesterol (1)	50 ppm	81.86(0.34)	3.66(0.55)	9.29(0.18)	5.19(0.22)
	150 ppm	82.01(1.81)	3.08(0.69)	9.24(0.50)	5.67(0.75)
22-fluorocholesterol (2)	50 ppm	80.97(0.77)	3.04(0.51)	10.08(0.70)	5.91(0.25)
24-fluorocholesterol (3)	45 ppm	81.13(0.33)	4.26(0.75)	9.37(0.24)	5.34(0.80)
25-fluorocholesterol (4)	30 ppm	70.8%(1.53)	13.88(1.44)	9.59(0.50)	5.75(0.13)

[a]Stigmasterol also present, 9.03% (0.14).

The lack of efficacy of the majority of these substrates as *in vivo* disruptors of growth and development can be attributed to several possible causes, including (1) removal of the fluorine by elimination of HF or by hydrolysis or (2) normal participation in the biogenetic sequence and then non-interaction of the resulting fluoroecdysone analogs with the regulatory proteins. Due to the lack of significant *in vivo* activity, we have not explored this further.

EXPERIMENTAL

General procedures. Solvent purifications: Anhydrous tetrahydrofuran (THF) was distilled under N_2 from Na metal and benzophenone prior to use, and 1,4-dioxane was distilled under nitrogen from $LiAlH_4$. All other solvents were Fisher HPLC grade and used without further purification.

IR spectra were taken on a Perkin-Elmer 727 spectrophotometer either as a thin film between NaCl salt plates or as a 10% solution in CCl_4. Melting points were determined with a Thomas Hoover capillary melting point apparatus and are uncorrected. NMR spectra were obtained in $CDCl_3$ or C_6D_6 solutions on Varian Associated CFT-20 instruments operating at 20 MHz for ^{13}C and 80 MHz for ^1H. High resolution ^1H-NMR spectra were obtained on a Bruker 360 spectrometer and on a Nicolet 300 NB instrument.

Low-resolution electron-impact mass spectra were obtained using a Hewlett Packard Model 5980A mass spectrometer interfaced to a HP5710A G.C. High resolution mass spectra were obtained on an MS-30 instrument interfaced to an HP7210A G.C. and a DS-50 data system.

Gas chromatography separations were performed on a Varian 3700 gas chromatograph interfaced to a Varian Vista CDS-401. The glass columns (2 mm x 2m) used were 3% OV-17 or with 1% SP-2100. Thin layer chromatography (TLC) was carried out on Polygram SilG, UV254 (Macherey-Nagel and Co.).The spots were visualized with a vanillin spray reagent (9.0 g vanillin, 300 mL ethyl alcohol and 1.5 mL concentrated sulfuric acid). Flash chromatography using 230-400 mesh silica gel was conducted according to Still et al [19].

Radioactivity determinations were performed by liquid scintillation counting in Fisher Scintiverse on a Packard TriCarb with quench correction by automatic external standardization.

Epimeric mixture of 20(R) and 20(S)-6-methoxy-20-isopentyl-3α, 5-cyclo-5α-pregnan-20-ol, (7). To a solution of isohexylmagnesium bromide [from 0.265 g (10 mmole) of Mg and 1.2 g (7 mmole) of isohexyl bromide under N_2 at room temperature] was added dropwise 20 mL of THF containing 2.31 g (7 mmole) of 3α, 5-cyclopregnan-6β-ol-20-one methyl ether 6, m.p. 123-126°C [8]. The mixture was stirred for 3 hr at 20° and refluxed for 1 hour. The excess Grignard reagent was destroyed with aq. Na_2SO_4. Filtration and evaporation of the solvent in vacuo gave 2.21 g (70%) of 7 as a mixture of epimers. IR ($CHCl_3$): 3600 cm^{-1}; ^1H-NMR: δ3.30 (s, 2H); 2.76 (m, 1H); 1.24 (s, 3H), 1.00 (s, 3H), 0.88 (s, 6H); 0.81 (s, 3H); LRMS: m/e (rel. intensity) 416 (M^+, 1); 398 (5), 385 (10); 345 (18); 275 (70); 159 (90); 145 (94); 129 (100); 111 (82). This material was homogeneous by TLC (R_f 0.33, 20% ethyl acetate-hexane). GLC (1% SP-2100 column, 200°-275°C) analysis of this material indicated that it was 90% of 20(S)-hydroxy epimer (rt 15.60 min) and 20(R)-hydroxy epimer (rt 15.02 min). The assignment was based on the known chemical shift (^1H-NMR) of C-21 methyl group in analogous C-20 epimeric hydroxy compounds.

Mixtures of 20(R)-and 20(S)-fluoro-6β-methoxy-3α, 5-cyclo-5α-cholestane,(8). A solution of 360 mg (0.85 mmole) of 7 in 15 mL HPLC grade CH_2Cl_2 was added dropwise to a cooled (-78°C) mixture of 153 mg of diethylaminosulfur trifluoride (DAST) (0.93 mmole) in 10 mL of CH_2Cl_2. The mixture was stirred at -78 to 20° for 20 min. Saturated aqueous $NaHCO_3$ (10 mL) and 20 mL CH_2Cl_2 were then added. The organic phase was separated, washed with water, dried over anhydrous $MgSO_4$, and the solvent was removed. The residue was chromatographed on silica gel flash column to yield 289 mg (0.69 mmole, 85%) of the epimeric fluoro compounds 8. ^{1}H-NMR: δ3.30 (s, 3H); 2.75 (m, 1H); 1.00 (s, 3H); 0.87 (s, 6H); 0.80 (s, 3H); LRMS: m/e (rel. intensity) 398 (5); 383 (5); 366 (30); 281 (30); 213 (63); 145 (100); 105 (92); 91 (80). This material was homogeneous by TLC (R_f 0.65, 30% ethyl acetate-hexane). GLC analysis (SP-2100 column, 200°-275°C) of this material indicated that it contained a 1:1 mixture of 20R and 20S fluoro epimers.

Mixture of 20(R)-and 20(S)-fluorocholesterols (1). A solution of 508 mg (1.2 mmole) of mixture 8 in 15 mL dioxane, 5 mL H_2O, and 0.025 g of pTsOH was stirred at 80°C for 4 hours and cooled. The thick white precipitate was collected by filtration, taken up in CH_2Cl_2, and washed with aq. $NaHCO_3$. The solution was dried over anhydrous $MgSO_4$ to yield 392 mg (0.97 mmole, 80%) of 1. ^{1}H-NMR: δ5.36 (m, 1H), 3.48 (m, 1H), 0.98 (s, 3H), 0.88 (s, 6H), 0.81 (s, 3H); m/e (rel. intensity) 404 (M^+, 5); 384 (20), 271 (100); 145 (38); 105 (48); 91 (48); high-resolution mass spectrum, calcd. for $C_{27}H_{45}OF$, 404.3434; found, 404.3444. This material was homogeneous by TLC (R_f 0.30, 20% ethyl acetate-hexane). GLC analysis (SP-2100 column, 200°-275°C) of this material indicated that it was of equal amount of the two C-20 epimers.

Mixture of 22(S)- and 22(R)-hydroxy-6β-methoxy-3α, 5-cyclo-5α-cholestane (11). To a solution of isopentylmagnesium bromide, which was made from 0.7 g (25.9 mmole) of Mg and 2.8 g (23.24 mmole) of isopentyl bromide under a nitrogen condition at room temperature, was added dropwise 20 mL anhydrous THF containing 2.0 g (5.81 mmole) of aldehyde 10 [11]. The mixture was stirred at room temperature for 3 hours and refluxed for 3 hours. The excess Grignard reagent was destroyed with aq. Na_2SO_4. Filtration, evaporation of the solvent *in vacuo*, and chromatography on silica gel flash column gave 1.8 g (4.32 mmole, 74%) mixture of epimeric alcohols 11. ^{1}H-NMR δ3.62 (m, 1H); 3.31 (s, 3H); 2.75 (m, 1H); 1.01 (s, 3H); 0.91 (s, 3H); 0.84 (s, 3H); 0.71 (s, 3H). IR ($CHCl_3$) 3400, 1096, 1080 and 755 cm^{-1}. This material was homogeneous by TLC (R_f 0.39, 25% ethyl acetate-hexane).

Mixture of 22(R), 22(S)-fluoro-6β-methoxy-3α, 5-cyclo-5α-cholestane (12). A solution of 557 mg (1.33 mmole) of mixture of alcohols 11 in 20 mL (HPLC grade) CH_2Cl_2 was added dropwise to a cooled (-78°C) mixture of 646 mg (4.0 mmole) DAST in 10 mL of CH_2Cl_2. The mixture was stirred for 5 minutes at -78°C; the cooling bath was removed, and the contents were warmed to ambient temperature for 15 minutes. Aqueous $NaHCO_3$ (10 mL) and 20 mL CH_2Cl_2 were then added.

The organic phase was separated, and the solvent was removed. The residue was chromatographed on silica gel flash column to yield 508 mg (1.2 mmole, 91%) of 12: ^{1}H-NMR: δ3.31 (s, 3H); 2.75 (m, 1H); 1.01 (s, 3H); 0.91 (s, 3H); 0.84 (s, 3H); LRMS: m/e (rel. intensity) 398 (10); 383 (7); 366 (44); 145 (100); 95 (89); 91 (84). This material was homogeneous by TLC (R_f 0.64, 25% ethyl acetate-hexane).

Mixture of 22(R) - and 22 (S)-fluorocholesterols (2). A solution of 508 mg (1.21 mmole) of 12, 15 mL dioxane, 5 mL H_2O and 0.025 g of pTsOH was stirred at 80°C for 4 hours and cooled. The thick white precipitate was collected by filtration, taken up in CH_2Cl_2, and washed with aq. $NaHCO_3$. The solution was dried over anhydrous $MgSO_4$. Solvent was evaporated to yield 415 mg (1.02 mmole, 84%) of 2: IR ($CHCl_3$) 1080, 1020 cm^{-1}; ^{1}H-NMR: δ5.36 (m, 1H); 3.45 (m, 1H); 1.11 (s, 3H); 0.90 (s, 3H); 0.83 (s, 3H); LRMS: m/e (rel. intensity) 404 (M^+, 30); 386 (20); 384 (40); 351 (30); 145 (100); 105 (98); 95 (87); high resolution mass spectrum calcd. for $C_{27}H_{45}OF$, 404.3454; found, 404.3490. This material was homogeneous by TLC (R_f 0.29, 25% ethyl acetate-hexane).

Mixture of 24(R), 24(S)-fluoro-6β-methoxy-3α,5-cyclo-5α-cholestane (15). A solution of 150 mg (0.35 mmol) of the epimeric alcohols 14 (obtained by $NaBH_4$ reduction of the known [7] ketone 13) in 2.0 mL of CH_2Cl_2 was added to a -78°C solution of 4.2 μl (0.35 mmol) of DAST in 2.0 mL of CH_2Cl_2. The mixture was stirred at -78° to 20° for 1 hr, at which time TLC indicated incomplete reaction. A second portion (10 μl) of DAST was added and the reaction was quenched by successive addition of 0.5g of Na_2CO_3 and 5 mL of H_2O. The product 15 was extracted with ether (5 x 5 mL), dried ($MgSO_4$), concentrated in vacuo. Flash chromatography (SiO_2, 3% ethyl acetate-hexane) gave a less polar minor product shown to be a mixture of Δ^{23} and Δ^{24} cholesterols, and the desired 24-fluoro compound 15 as the major product (37 mg, 24% of analytically pure material). ^{1}H-NMR: δ3.32 (s, 3H), 2.75 (m, 1H); 1.47 (s, 3H), 1.19 (s, 3H), 1.02 (s, 3H), 0.97 (d, 6 Hz, 3H), 0.71 (s, 3H).

Mixture of 24(R) and 24(S)-fluorocholesterols (3). The homogeneous protected 24-fluoro compound 15 (37 mg, 0.086 mmol) was deprotected with catalytic pTsOH in aqueous dioxane as described above. Workup as usual followed by chromatography afforded 28 mg (0.067 mmol, 75%) of TLC-homogeneous 24-fluorocholesterol 3. ^{1}H-NMR: δ5.36 (m, 1H), 3.45 (m, 1H); 1.02 (s, 3H), 0.97 (d, 6 Hz, 3H), 0.71 (s, 3H). No aliphatic R_2CHF signal is seen due to the presence of two diastereoisomers, each of which shows extensive 2- and 3-bond couplings. LRMS: m/e (rel. intensity), 404 (M^+, 20); 389 (M^+-15, 7). 386 (M^+-18, 16), 384 (M^+-20, 17), 371 (M^+-33, 15), 351 (M^+-53, 9), 319 (20), 293 (25), 273 (19), 255 (22), 213 (50), 161 (52), 107 (100), 105 (98).

25-Fluoro-6β-methoxy-3α, 5-cyclo-5α-cholestane (18). A solution of 500 mg (1.20 mmole) of 25-hydroxycholesterol isomethyl ether 17, mp 153-154° [11] in 15 mL CH_2Cl_2 was added dropwise to a -78° solution of 212 mg (1.32 mole) of DAST in 10 mL of CH_2Cl_2. The mixture was stirred for 5 minutes; the cooling bath was removed, and the contents were warmed to ambient temperature for 15 minutes. Aq. $NaHCO_3$ (10 mL)

and 20 mL CH_2Cl_2 were then added, the organic phase was separated, washed with water, dried over $MgSO_4$ and the solvent was removed. The residue was chromatographed on silica gel flash column to afford 432 mg (85%) of oily 18. IR ($CHCl_3$) 1080, 1020 cm^{-1}. ^1H-NMR ($CDCl_3$) δ3.25 (s, 3H), 2.70 (bs, 1H); 1.25 (d, J = 22Hz, 6H); 0.90 (s, 3H); 0.65 (s, 3H); LRMS: m/e (rel. intensity) 398 (6), 383 (18), 366 (32), 343 (38), 159 (60), 91 (75), 105 (88), 145 (100). This material was homogeneous by TLC (R_f 0.77, 25% ethyl acetate-hexane).

25-Fluorocholesterol (4). A solution of 306 mg (0.73 mmole) of 18, 9 mL of dioxane, 3 mL of water, and 0.02 g of pTsOH was stirred at 80°C for 4 hours and cooled. The thick white precipitate was collected by filtration, taken up in CH_2Cl_2, and washed with aq. $NaHCO_3$ solution. The solution was dried over anhydrous $MgSO_4$ to yield 230 mg (78%) of 4, m.p. 140-142°C, IR ($CHCl_3$) 3630, 1080, 1020 cm^{-1}; ^1H-NMR δ5.36 (m, 1H); 3.48 (m, 1H); 1.25 (d, J = 22Hz, 6H); 0.90 (s, 3H); 0.85 (d, J = 7Hz, 3H); 0.65 (s, 3H); m/e (rel. intensity) 404 (M+, 12), 386 (10), 384 (18), 145 (91), 107 (78), 105 (100), 91 (83); high-resolution mass spectrum, calcd for $C_{27}H_{45}OF$, 404.3436; found, 404.3445. This material was homogeneous by TLC (R_f 0.36, 20% ethyl acetate-hexane).

2-Methyl-2-(3-bromopropyl)-1,3-dioxolane. A mixture of 16.5g (0.1 mole) of 5-bromo-2-pentanone, 6.8 g (0.11 mole) of ethylene glycol and a trace of pTsOH in 40 mL benzene was refluxed for 3 hours during separation of water with a Dean-Stark trap. The desired ketal (16.7 g, 80%) was obtained by distillation (bp, 96-97°C/17 torr.) ^1H-NMR δ1.29 (s, 3H); 1.79-2.3 (m, 4H); 3.41 (t, 2H, J = 6Hz); 3.91 (s, 4H). GLC analysis (OV-17 column, 100°C) of this material indicated that it was 99% pure.

27-Nor-20(ξ)-hydroxy-6β-methoxy-3α, 5-cyclo-5α-cholestan -25-one ethylene ketal (19). The Grignard reagent of the bromoketal above was prepared by adding 2 g (9.5 mmole) of freshly distilled ketal in 50 mL dry THF to 0.348 g (143 mmole) of Mg under N_2 condition. The reaction was initiated by adding few drops of 1,2-dibromoethane and refluxed under stirring. After refluxing for 1 hour, 262 mg (7.9 mmole) of ketosterol 6 in 5 mL THF were added dropwise. The mixture was stirred for 4 hours at room temperature. The excess Grignard reagent was decomposed with sat. NH_4Cl aqueous solution. The desired product 19 was extracted with ether and washed with water and $NaHCO_3$ aq., dried over $MgSO_4$, filtered and concentrated in vacuo. The oily residue was purified by flash column to obtain (2.72 g) in 75% yield. ^1H-NMR δ3.92 (s, 4H); 3.31 (s, 3H); 2.70 (bs, 1H); 1.30 (s, 3H, C-26); 1.26 (s, 3H, C-21); 1.00 (s, 3H, C-19); 0.88 (s, 3H, C-18). LRMS: m/e (rel. int.) 445 (M^+-CH_3,1); 253 (20); 187 (5.4); 173 (100); 155 (94); 87 (76). This material was homogeneous by TLC (R_f, 30% ethyl acetate-hexane).

27-Nor-20(ξ)-fluoro-6β-methoxy-3α, 5-cyclo-5α-cholesten-25-one ethylene ketal (20). A solution of 928 mg (2.01 mmole) of 19 in 10 mL CH_2Cl_2 was added dropwise to a cooled (-78°C) mixture of 1.62 g of DAST (10.0 mmole) in 10 mL of CH_2Cl_2. The mixture was stirred at -78° to 20° for 30 min and worked up as described above to give crude

mixture 20 which was further purified by flash column to give 835 mg of 20 in 90% yield. ^{1}H-NMR δ3.92 (s, 6H); 3.31 (s, 3H); 2.65 (bs, 1H); 1.30 (s, 3H, C-26); 0.95 (s, 3H, C-19); 0.80 (s, 3H, C-18). LRMS, m/e (rel. int.) 462 (M^+, 0.9), 447 (M^+-CH_3, 1), 430 (1), 427 (0.6), 253 (100), 87 (63). This material was homogeneous by TLC (R_f 0.55, 30% ethyl acetate-hexane).

27-Nor-25-oxo-20(ξ)-fluoro-cholesterol (21). To a solution of 600 mg (1.73) mmole) of 20 in 50 mL acetone-H_2O (10:1, v:v) was added trace of p-toluenesulfonic acid monohydrate. The mixture was refluxed for 5 hours, then was poured into ethyl acetate-water and the organic layer was separated and dried over $MgSO_4$. The ethyl acetate was removed to give 1.40 mg of 21 om 84% yield. ^{1}H-NMR: δ5.37 (bs, 1H); 3.48 (bs, 1H); 2.15 (s, 3H, C-26); 1.02 (s, 3H, C-18), 0.85 (s, 3H, C-18). This material was homogeneous by TLC (R_f 0.12, 30% ethyl acetate-hexane).

3-O-Tetrahydropyranyl derivative of 27-nor-25-oxo-20 (ξ)-fluoro-cholesterol (22). To a stirred solution of mixture of 21 (300 mg, 0.742 mmole) and a trace of p-toluenesulfonic acid monohydrate in anhydrous dioxane (20 mL) at room temperature was added dihydro-4H-pyran (0.2 mL, 2.2 mmole). After stirring for 1 hour the desired product was extracted from aqueous solution using ethyl acetate. The organic solvent was removed and purified by flash column to give 307 mg of oily 22 in 85% yield. IR (CCl_4) 1720, 1180, 1142, 1061 cm^{-1}. ^{1}H-NMR δ5.31 (m, 1H); 4.67 (m, 1H); 3.88 (m, 1H); 3.47 (m, 2H); 2.09 (s, CH_3, C-26); 0.96 (s, CH_3, C-19); 0.79 (s, CH_3, C-18); LRMS, m/e (rel. intensity); 386 (30.5); 371 (22); 351 (10.2); 289 (22.0); 145 (86.4); 105 (100); 91 (84). This material was homogeneous by TLC (R_f 0.32, 25% ethyl acetate-hexane).

20(ξ)-fluoro-25-cholesta-5, 25-dien-3β-ol (24). In order to make ^{14}C labelled 20-fluorocholesterol for metabolic study the reaction sequences were initially carried out in non-radioactive form. To a solution of 8.72 mg (0.06 mmole) of methyl iodide in 2 mL dry benzene was added 32 mg (0.12 mmole) of triphenylphosphine in 1 mL dry benzene. The mixture was stirred at room temperature and kept for 48 hours, during which time crystals of methyltriphenylphosphonium iodide were obtained. The benzene was removed under a stream of dry N_2 and three drops of dry benzene were added, followed by addition of 4 drops of n-butyllithium in hexane. The solution turned orange immediately. Then 11 mg (0.022 mmole) of 25-oxo- 22 in a minimum volume of benzene was added. The resulting gel was kept for 24 hour and the crude product was extracted with ethyl acetate and purified through a pipet flash column to remove the unreacted starting material. The crude product obtained from the flash column was contaminated with triphenylphosphine. In order to remove the triphenylphosphine, the 3-tetrahydropyranyl group was removed in methanol and trace of p-toluensulfonic acid monohydrate at 0°C for 2 hours. Organic solvents were evaporated, and the residue was purified through flash column to give 6 mg (0.012 mmole) of 24 in 64% yield from 22. IR (CCl_4) 3600, 1640, 1138, 1075 cm^{-1}. ^{1}H-NMR δ5.38 (m, 1H); 4.70 (s, 2H); 3.53 (m, 1H); 1.53(s, 3H, C-27); 1.01 (s, 3H, C-19); 0.85 (s, 3H, C-18); LRMS, m/e (rel. intensity); 382 (M^+-HF, 1.0); 349 (1.2); 271

(100); 109 (37.3); 105 (29.0); 91 (29). This material was homogeneous by TLC (R_f 0.34, 25% ethyl acetate-hexane).

20(ξ)-fluorocholesterol (1). A solution of 6 mg (0.012 mmole) of 24 in 2 mL benzene was hydrogenated in the presence of a trace of Wilkinson's catalyst [tris(triphenylphosphine) rhodium chloride] for 16 hours, then organic solvents were evaporated, and the residue was purified through a flash column of silica gel to give pure 4 mg (0.009 mmole) of 1 in 75% yield. This was identical to the mixture of compounds 1 which was prepared as in Scheme I.

3-O-Tetrahydropyranyloxy-20(ξ)-fluoro-[26-^{14}C]-cholesta-5, 25-diene (23) was prepared by a modification of the procedure of Pettler et al [17]; [^{14}C] methyl iodide (Amersham, 500 μCi, 58 mCi/mmole) (1.22 mg, 0.0086 mmole) was cooled to -78°C by solid CO_2/acetone and 10.5 mg (0.040 mmole) of Ph_3P in 2 mL of dry benzene was added to [^{14}C] methyl iodide. The mixture was allowed to warm to room temperature and kept for 48 hours during which time crystals of [^{14}C] methyl triphenylphosphonium iodide were obtained. The benzene was removed under a stream of dry N_2 and 1 mL of dry benzene was added, followed by addition of 23.7 μL (0.025 mmole) of n-butyllithium in hexane. The solution turned orange immediately and was left for 30 minutes, after which 20 mg (0.04 mmole) of [26-^{14}C]-22 dissolved in the minimum volume of dry benzene was added. The reaction mixture was kept for 24 hours and finally the benzene was exaporated. The residue in the test tube was transferred to a small pipet flash column and eluted with hexane-ethyl acetate (5:1, v:v) to give 2.4 mg (0.0047 mmole) of [26-^{14}C]-23 in 56% chemical and 24% radioactive yield. The TLC showed the same R_f value of both labelled and unlabelled compound 23, and contamination of Ph_3P was evident.

20 (ξ)-fluoro-[26-^{14}C]-cholesta-5, 25-dien-3β-ol (24) was prepared by removing of 3-tetrahydropyranyl group in acidic methanol. To a solution of 2.4 mg (0.0048 mmole) of radiolabeled 23 in 2 mL methanol at 0°C was added a trace of pTsOH. The solution was stirred at 0°C for 2 hours, then the methanol was evaporated, and the residue was passed through a pipet flash column to give 1.7 mg (0.0042 mmole) of labelled 24 free from Ph_3P contamination in 92% chemical yield from 23 and 30% radioactive yield from [^{14}C] CH_3I (500 μCi, 58 mCi/mmole). This labelled compound showed the same R_f value as the unlabelled compound did upon TLC plate. The specific activity of [26-^{14}C]-24 was 32.4 mCi/mmole.

20(ξ)-fluoro-[26-^{14}C]-cholesterol (1) was prepared by hydrogenation in the presence of Wilkinson's catalyst in benzene for 16 hr. The benzene was evaporated and the residue was chromatographed on a pipet flash column to give 1.5 mg (0.0037 mmole) of radiolabelled 1 (26.5 mCi/mmole) in 90% chemical and 70% radioactive yield. The TLC plate showed identical R_f value in both labelled and unlabelled 1 compounds.

Bioassays. For each compound, twenty first instar larvae were removed from stock culture at the point of apolysis to the second instar. The experimental diet [1] was modified to use cholesterol-free casein (4 extractions with 1:1 $CH_3OH:CHCl_3$), and the test sterols were coated as CH_2Cl_2 solutions on the wheat germ-methyl paraben-Wesson salt premix. No supplemental phytosterols were added to the test diets. Hornworms were reared individually and their growth and development was monitored daily. Four prepupae (after gut voiding) were removed from each experiment for sterol analysis as follows: a) homogenization (1:1 chloroform-methanol); b) centrifugation and evaporation of lower (lipid) layer; c)saponification (10% KOH-CH_3OH, 1 hr, 80°); d) extraction of non-saponifiable lipids with diethyl ether; e) pipet flash column to isolate sterols (elution with hexane, 10% ethyl acetate in hexane, and then diethyl ether elutes sterols); capillary GC analysis (260°C isothermal, DB-5 0.25 mm I.D., 0.25 μm coating, 30m column).

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REFERENCES

1. Svoboda, J.A., Thompson, M.J., Robbins, W.E., Kaplanis, J.N., LIPID 13, 742 (1978).
2. Rees, H.H., Davies, T.G., Dinan, L.N., Lockley, W.J.S., and Goodwin, T.W., In J.A. Hoffman, ed., Progress in Ecdysone Research (Elsevier/North-Holland, 1980), pp. 125-138.
3. Kline, T.B. and Prestwich, G.D., TETRAHEDRON LETT., 23, 3043 (1982).
4. Schlosser, M., TETRAHEDRON 34, 3 (1978).
5. Bollenbacher, W.E., Smith, S.L., Wielgus, J.J., and Gilbert, L.I., NATURE 268, 660 (1977).
6. (a) Smith, S.L., Bollenbacher, W.E., and Gilbert, L.I., In, J.A. Hoffman, ed., Progress in Ecdysone Research (Elsevier/North-Holland, 1980), pp. 139-162. (b) Kaplanis, J.N., Weirich, G.F., Svoboda, J.A., Thompson, M.J., and Robbins, W.E., ibid, pp. 163-186.
7. Piatak, D.M. and Wicha, J. CHEM REV. 78, 199 (1978).
8. Morisaki, M., Sato, S., and Ikekawa, N., CHEM. PHARM. BULL. 25, 2576 (1977).
9. William, R.N. and Thankamma, E.V., J. ORG. CHEM. 41, 1652 (1976).
10. Middleton, W.J., J. ORG. CHEM. 40, 574 (1975).
11. Salmond, W.G. and Sobala, M.C., TETRAHEDRON LETT. 1695 (1977).
12. Poyer, J.P. and Ourisson, G.J., J. CHEM. SOC. PERKIN I, 2061 (1974).
13. Partridge, J.J., Stephanie, F., and Uskokovic, M.R., HELV. CHIM. ACTA 57, 764 (1974).
14. Narwid, T.A., Cooney, K.E. and Uskokovic, M.R., HELV. CHIM. ACTA 57, 771 (1974).

15. Isaka, Y., Morisaki, M., and Ikekawa, N., STEROIDS 38, 2841 (1981).
16. Schaltegger, H. and Joss, U., HELV. CHIM. ACTA 52, 2465 (1969).
17. Pettler, P.J., Lockley, W.S., Rees, H.H., and Goodwin, T.W., BIOCHEM. J. 174, 397 (1978).
18. Birch, A.J. and Walken, K.A.M., J. CHEM. SOC. C, 1894 (1966).
19. Still, W.C., Kahn, M., and Mitra, A., J. ORG. CHEM 43, 2923 (1978).