Synthesis of new C-19-functionalized cholesterols

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A series of new derivatives of cholesterol bearing polar functional groups on carbon-19 were synthesized, including 19-oximino-, 19-amino-, 19-(methylimino)-, 19-(methylamino)-, 19-mercapto-, and 19-(methylthio)cholesterol, and 3β-hydroxycholest-5-en-19-oic acid, as well as an unusual cyclosterol, 5,19-cyclocholest-6-en-3β-ol. (*Steroids* **59**:244–247, 1994)

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Introduction

Ergosterol is the principal sterol of the parasitic trypanosomatid protozoa,[1–5] and thus inhibitors of any stage of ergosterol biosynthesis are likely to be inhibitors of protozoan growth. As part of a program to develop new antiparasitic agents, we continue to synthesize a variety of inhibitors of sterol and fatty acid biosynthesis and to examine the effects of these compounds on growth and lipid biosynthesis in the representative trypanosomatid flagellate *Crithidia fasciculata*.[6–8] A source of inspiration for the present work was the observation by Miller et al that 14α-hydroxymethylcholest-6-en-3β,15α-diol is a potent and specific inhibitor of the mammalian sterol $\Delta^8 \rightarrow \Delta^7$ isomerase.[9] The mechanism of inhibition is not known, but one may guess that the pendant hydroxyl groups of this compound form strong hydrogen bonds with catalytic amino acid residues in the isomerase active site. More generally, the placement of polar functional groups with some degree of conformational flexibility, above or below the plane of a steroid nucleus, will yield compounds which may form hydrogen bonds with amino acid residues in the active sites of isomerases, desaturases, or reductases required for normal ergosterol biosynthesis, thus inhibiting these enzymes.

The abundant chemistry of steroids functionalized at C-19 and the well-known inhibition of aromatase by steroids bearing olefinic, acetylenic, and haloalkyl groups at this position[10–12] identified C-19 as a reasonable site for attachment of polar functional groups to give sterols with significant biological activity. However, we were surprised to find that although numerous 19-*oxygenated* derivatives of cholesterol have been prepared,[13–15] simple nitrogen- or sulfur-containing functional groups at this position were apparently unknown in the cholestane or ergostane series. A variety of azasterols[16,17] and more recently thiasterols[8,18] have been shown to be potent inhibitors of reactions involved in the elaboration of the ergosterol side chain, so the introduction of these heteroatoms at C-19 was of particular interest. We report herein the syntheses of 19-substituted cholesterols bearing amino, methylamino, oximino, hydroxy, mercapto, methylthio, and carboxyl groups at this position.

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Experimental

Melting points, UV absorption spectra, ^{1}H NMR spectra, and low and high resolution mass spectra were obtained as previously described.[7] 19-Hydroxycholesteryl acetate [**1**, mp 115–116 C (lit.[13] 115–116 C)] was synthesized by the literature procedure.

3β-Acetoxycholest-5-en-19-one (2)

Pyridinium chlorochromate (72 mg, 0.33 mmol) was suspended in CH_2Cl_2 (75 mL), and a mixture of 19-hydroxycholesteryl acetate (**1**, 100 mg, 0.22 mmol) and celite (72 mg) in CH_2CL_2 were added in one portion to the magnetically stirred solution. After 2 h, ether (100 mL) was added, and the supernatant was decanted from the dark brown solid. The insoluble residue was washed thoroughly with ether (3 × 15 mL) and the combined ether extracts were passed through a short pad of florisil. The solvent was evaporated to yield compound **2** (75 mg, 75%); mp 106–107 C (lit.[14] 106–107 C). ^{1}H NMR (270 MHz, $CDCl_3$) δ 9.65 (s, 1H, 19-H), 5.88 (d, J = 5 Hz, 1H, 6-H), 4.59 (m, 1H, 3-H), 2.00 (s, 3H, $COCH_3$), 0.88 (d, J = 6 Hz, 3H, 21-Me), 0.85 (d's, J = 7 Hz, 6H, 26-Me and 27-Me), 0.62 (s, 3H, 18-Me); MS m/z 442 (M^+, 12), 382 (M-AcOH, 20), 353 (M-AcOH-CHO, 100).

19-Oximinocholesterol (3)

A solution of hydroxylamine hydrochloride (36 mg, 0.5 mmol) in water (2 mL) was added to 3β-acetoxycholest-5-en-19-one (**2**,

100 mg, 0.22 mmol) in ethanol (20 mL). The mixture was made alkaline with Na_2CO_3 and refluxed until all starting material had been consumed. The reaction mixture was acidified with acetic acid and the ethanol was distilled off under reduced pressure. Ether and water were added, and the organic phase was separated, dried over Na_2SO_4, and concentrated to give compound **3** (65 mg, 70%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.32 (s, 1H, 19-H), 5.64 (d, J = 5 Hz, 1H, 6-H), 3.57 (m, 1H, 3-H), 0.89 (d, J = 6 Hz, 3H, 21-Me), 0.86 (d's, J = 7 Hz, 6H, 26-Me and 27-Me), 0.62 (s, 3H, 18-Me); MS, m/z 415 (M^+, 10), 398 (M-OH, 62), 397 (M-H_2O, 68), 380 (M-OH-H_2O, 100), 353 (M-H_2O-HONCH, 68). Exact mass 415.3447, calculated for $C_{27}H_{45}O_2N$ 415.3450.

19-Aminocholesterol (4)

19-Oximinocholesterol (**3**, 1.0 g, 2.3 mmol) was dissolved in ether (50 mL). Lithium aluminum hydride (300 mg, 8 mmol) was added and the mixture was refluxed overnight under argon. The reaction mixture was cooled, poured into ice water containing a few drops of KOH, and extracted with ether (5 × 10 mL). The organic layer was dried over Na_2SO_4 and concentrated to dryness to give compound **4** (850 mg, 87%); mp 128–129 C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.67 (d, J = 5 Hz, 1H, 6-H), 3.55 (m, 1H, 3-H), 3.01 (d, J = 14 Hz, 1H, 19-H), 2.68 (d, J = 14 Hz, 1H, 19-H), 0.91 (d, J = 6 Hz, 3H, 21-Me), 0.86 (d's, J = 7 Hz, 6H, 26-Me and 27-Me), 0.72 (s, 3H, 18-Me); MS m/z 402 (M^+ + H, 29), 384 (M-OH, 51), 370 (M-CH_3NH_2, 54), 354 (M-OH-CH_2NH_2, 100). Exact mass 401.3637, calculated for $C_{27}H_{47}ON$ 401.3657.

19-(Methylimino)cholesterol (5)

A large excess of methylamine (40% in water) was added to 3β-acetoxycholest-5-en-19-one (**2**, 100 mg, 0.22 mmol) in ethanol (5 mL). The reaction mixture was stirred overnight, diluted with water, and extracted with ether. The organic layer was washed with water a few times and then dried over Na_2SO_4.

Concentration of the extract left imine **5** (90 mg, 96%). ^{1}H NMR (270 MHz, $CDCl_3$) δ 7.56 (s, 1H, 19-H), 5.66 (d, J = 5 Hz, 1H, 6-H), 3.56 (m, 1H, 3-H), 3.36 (s, 3H, N-CH_3), 0.89 (d, J = 6 Hz, 3H, 21-Me), 0.86 (d's, J = 6 Hz, 6H, 26-Me and 27-Me), 0.57 (s, 3H, 18-Me); MS, m/z 413 (M^+, 17), 395 (M-H_2O, 50), 354 (M-H_2O- CH_3CN, 12). Exact mass 413.3634, calculated for $C_{28}H_{47}ON$ 413.3657.

19-(Methylamino)cholesterol (6)

Lithium aluminum hydride (150 mg, 0.4 mmol) was added to 19-(methylimino)cholesterol (**5**, 90 mg, 0.21 mmol) in ether (10 mL), and the mixture was refluxed overnight under Ar. The solution was cooled, poured into ice water containing a few drops of concentrated KOH, and extracted with ether. The organic phase was dried over Na_2SO_4, and concentrated to dryness to give compound **6** (60 mg, 66%); mp 79–80 C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.61 (d, J = 5 Hz, 1H, 6-H), 3.55 (m, 1H, 3-H) 2.79 (d, J = 12 Hz, 1H, 19-H), 2.48 (d, J = 12 Hz, 1H, 19-H), 2.38 (s, 3H, N-CH_3), 0.90 (d, J = 6 Hz, 3H, 21-Me), 0.85 (d's, J = 7 Hz, 6H, 26-Me and 27-Me), 0.72 (s, 3H, 18-Me); MS, m/z 415 (M^+, 3), 397 (M-H_2O, 4), 384 (M-CH_3NH_2, 22), 354 (M-CH_2NHCH_3-OH, 100), 353, (M-CH_2NHCH_3-H_2O, 37). Exact mass 415.3788, calculated for $C_{28}H_{49}ON$ 415.3814.

3β-Acetoxycholest-5-en-19-oic acid (7)

19-Hydroxycholesteryl acetate (**1**, 650 mg, 1.5 mmol) was dissolved in acetone (100 mL). Jones reagent (2.2 mL) was added dropwise with stirring while maintaining the temperature below 5 C. The stirring was continued overnight. Methanol (20 mL) was added to destroy the excess reagent and the mixture was poured into chilled water saturated with NaCl. The aqueous solution was extracted with ether (5 × 50 mL), and the combined organic extracts were dried and concentrated to yield compound **7** (250 mg, 40%); mp 201–202 C (lit.[15] 202–203 C). ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.74 (d, J = 5 Hz, 1H, 6-H), 4.64 (m, 1H, 3-H), 2.02 (s, 3H, $COCH_3$), 0.89 (d, J = 6 Hz, 3H, 21- Me), 0.85 (d's, J = 7 Hz, 6H, 26-Me and 27-Me), 0.65 (s, 3H, 18-Me); MS, m/z 458 (M^+, 0.6), 398 (M-AcOH, 58), 382 (M-AcO-OH, 36), 368 (M-2CO_2H, 89), 353 (M-CO_2H-AcOH, 74). Exact mass 458.3389, calculated for $C_{29}H_{46}O_4$ 458.3396.

3β-Hydroxycholest-5-en-19-oic acid (8)

3β-Acetoxycholest-5-en-19-oic acid (**7**, 50 mg, 0.11 mmol) was dissolved in ethanol (3 mL). Aqueous NaOH (10%) (0.5 mL) was added, and the reaction mixture was refluxed for 2 h and acidified with dilute HCl. Water and $CHCl_3$ were added and the organic phase was separated. The extraction was repeated 3–4 times, and the combined organic layers were dried over Na_2SO_4 and then concentrated to give the compound **8** (20 mg, 44%); mp 231–233 C. ^{1}H NMR (270 MHz, $CDCl_3$-DMSO-d_6) δ 5.63 (m, 1H, 6-H), 3.57 (m, 1H, 3-H), 0.88 (d, J = 6 Hz, 3H, 21-Me), 0.85 (d's, J = 7 Hz, 6H, 26-Me and 27-Me), 0.64 (s, 3H, 18-Me); MS, m/z 416 (M^+, 1), 398 (M-H_2O, 40), 370 (M-CO_2H-H, 32), 353 (M-CO_2H-H_2O, 84). Exact mass 416.3289, calculated for $C_{27}H_{44}O_3$ 416.3290.

19-Bromocholesteryl acetate (9)

18-Me); MS, m/z 425 (M-CH_3SO_3, 6), 365 (M-CH_3SO_3-AcOH, 100). The pure 19-(mesyloxy)-cholesteryl acetate (750 mg, (3.6 mL, 5 mmol) was added, and the mixture was stirred for 48 h. After addition of water (10 mL), the mixture was extracted with $CHCl_3$. The organic phase was washed with water and dilute HCl, and then dried over Na_2SO_4. Evaporation of the solvent gave the mesylate, which was recrystallized from methanol (750 mg, 64%); mp 108–110 C. ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.69 (d, J = 5 Hz, 1H, 6-H), 4.65 (m, 1H, 3-H), 4.42 (d, 1H, J = 10 Hz, 19-H), 4.20 (d, 1H, J = 10 Hz, 19-H), 3.01 (s, 3H, OSO_2CH_3), 2.03 (s, 3H, $COCH_3$), 0.91 (d, J = 6 Hz, 3H, 21-Me), 0.86 (d's, J = 7 Hz, 6H, 26-Me and 27-Me), 0.71 (s, 3H, 18-Me); MS, m/z 425 (M-CH_3SO_3, 6), 365 (M-CH_3SO_3-AcOH, 100). The pure 19-(mesyloxy)-cholesteryl acetate (750 mg, 2.2 mmol) was dissolved in anhydrous acetone (20 mL). Lithium bromide (390 mg, 4.5 mmol) was added, and the mixture was refluxed for 20 h under Ar. After cooling, the solution was filtered and concentrated to dryness. The residue was dissolved in ether (25 mL) and washed with water. The ether was evaporated, and the residue was chromatographed on silica gel (solvent, 9:1 hexane-ethyl acetate) to yield compound **9** (300 mg, 41%); mp 93–95 C (lit.[19] 93.5–94 C). ^{1}H NMR (270 MHz, $CDCl_3$) δ 5.67 (d, J = 5 Hz, 1H, 6-H), 4.62 (m, 1H, 3-H), 3.74 (d, 1H, J = 11 Hz, 19-H), 3.50 (d, 1H, J = 11 Hz, 19-H), 2.03 (s, 1H, $COCH_3$), 0.91 (d, J = 6 Hz, 3H, 21-Me), 0.86 (d's, J = 7 Hz, 6H, 26-Me and 27-Me), 0.75 (s, 3H, 18-Me); MS, m/z 447 (M-AcOH, 18), 367 (M-AcOH-Br, 100).

19-(Acetylthio)cholesteryl acetate (10)

19-Bromocholesteryl acetate (**9**, 400 mg, 0.8 mmol) was dissolved in DMF (10 mL), and argon was bubbled through the solution for 15 min. $KSCOCH_3$ (88 mg, 0.8 mmol) was added, and the mixture was refluxed overnight. The reaction mixture was extracted with $CHCl_3$, and the extract was

washed 5–6 times with dilute HCl. The organic phase was dried over Na_2SO_4 and concentrated to dryness to give compound **10** (150 mg, 38%); mp 72 C (cloudy liquid), 91 C (clears). ^1H NMR (270 MHz, $CDCl_3$) δ 5.58 (d, J = 5 Hz, 1H, 6-H), 4.60 (m, 1H, 3-H), 3.40 (d, 1H, J = 14 Hz, 19-H), 3.01 (d, 1H, J = 14 Hz, 19-H), 2.33 (s, 3H, $SCOCH_3$), 2.02 (s, 3H, $OCOCH_3$), 0.90 (d, J = 6 Hz, 3H, 21-Me), 0.86 (d's, J = 7 Hz, 6H, 26-Me and 27-Me), 0.70 (s, 3H, 18-Me); MS, m/z 459 (M-$COCH_3$, 5), 416 (M-2$COCH_3$, 26), 413 (M-CH_2SCOCH_3, 33), 399 (M-AcOH-$COCH_3$, 46), 367 (M-AcOH-$SCOCH_3$, 26), 353 (M-AcOH-CH_2SCOCH_3, 100).

19-Mercaptocholesterol (11)

19-(Acetylthio)cholesteryl acetate (**10**, 150 mg, 0.3 mmol) was dissolved in ethanol (10 mL), 10% NaOH (4 mL) was added, and the solution was refluxed for 2 h. The reaction mixture was neutralized with glacial acetic acid, water was added, and the mixture was extracted (5 × 10 mL) with $CHCl_3$. Concentration of the combined organic phases yielded the product **11** (80 mg, 64%); mp 200–204 C. ^1H NMR (270 MHz, $CDCl_3$) δ 5.62 (d, J = 4 Hz, 1H, 6-H), 3.56 (m, 1H, 3-H), 3.14 (d, 1H, J = 12 Hz, 19-H), 3.02 (d, 1H, J = 12 Hz, 19-H), 0.90 (d, J = 6 Hz, 3H, 21-Me), 0.86 (d's, J = 7 Hz, 6H, 26-Me and 27-Me), 0.74 (s, 3H, 18-Me); MS m/z 418 (M^+, 10), 400 (M-H_2O, 28), 385 (M-SH, 47), 371 (M-CH_2SH, 59), 367 (M-SH-H_2O, 56) 353, (M-CH_2SH-H_2O, 100). Exact mass 418.3234, calculated for $C_{27}H_{46}OS$ 418.3269.

3β-Acetoxy-5,19-cyclocholest-6-ene (12)

19-Bromocholesteryl acetate (**10**, 50 mg, 0.1 mmol) was dissolved in DMF (5 mL), and argon was bubbled through the solution for 15 min. Na_2SO_3 (25 mg, 0.2 mmol) was added, and the mixture was refluxed overnight. The reaction mixture was acidified with HCl, extracted with $CHCl_3$, and washed with dilute HCl. Concentration of the organic phase gave the product **12** (30 mg, 60%); mp 132–133 C (lit.[20] 133–133.5 C). ^1H NMR (500 MHz, $CDCl_3$) δ 5.69 (dd, 1H, J = 10, 3 Hz, 7-H), 5.26 (dd, 1H, J = 10, 1 Hz, 6-H), 4.63 (m, 1H, 3-H), 2.00 (s, 3H, $COCH_3$), 0.91 (d, J = 6 Hz, 3H, 21-Me), 0.86 (d's, J = 7 Hz, 6H, 26-Me and 27-Me), 0.66 (s, 3H, 18-Me), 0.52 (d, J = 5 Hz, 1H, 19-H); MS, m/z 426 (M^+, 17), 366 (M-AcOH, 100), 351 (M-AcOH-CH_3, 17).

5,19-Cyclocholest-6-en-3β-ol (13)

Compound **12** (30 mg, 0.6 mmol) was dissolved in ethanol (5 mL), 10% NaOH (0.5 mL) was added, and the solution was refluxed for 2 h. The reaction mixture was acidified with HCl, water and $CHCl_3$ were added, the organic layer was separated, and this extract was washed with water. The solution was dried over Na_2SO_4 and concentrated to give the product **13** (18 mg, 66%); mp 138–139 C. ^1H NMR (270 MHz, $CDCl_3$) δ 5.72 (dd, 1H, J = 10, 3 Hz, 7-H), 5.23 (d, 1H, J = 10 Hz, 6-H), 3.55 (m, 1H, 3-H), 0.91 (d, J = 6 Hz, 3H, 21-Me), 0.86 (d's, J = 7 Hz, 6H, 26-Me and 27-Me), 0.66 (s, 3H, 18-Me), 0.50 (d, J = 5 Hz, 1H, 19-H); MS m/z 384 (M^+, 80), 368 (M-H_2O, 48). Exact mass 384.3383, calculated for $C_{27}H_{44}O$ 384.3392.

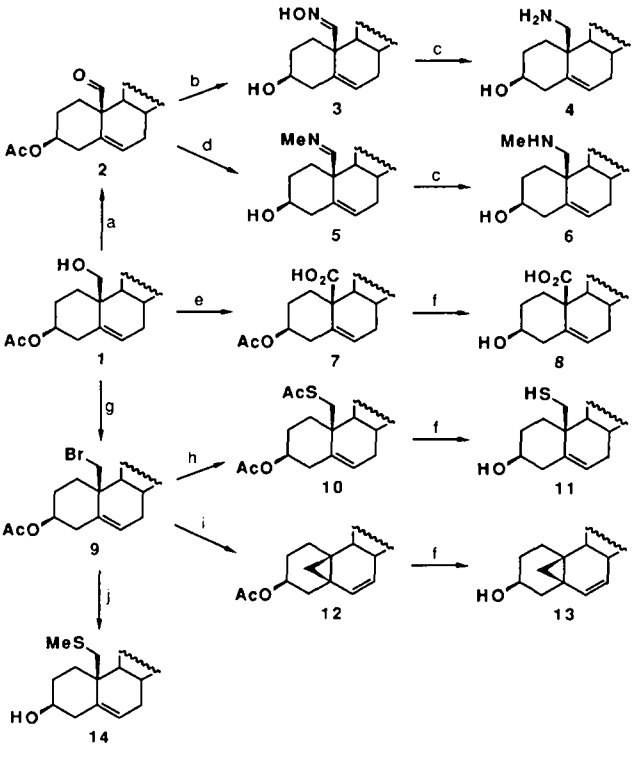
19-(Methylthio)cholesterol (14)

Compound **9** (140 mg, 0.28 mmol) was dissolved in DMF (10 mL), sodium methanethiolate (100 mg, 1.5 mmol) was added, and the mixture was refluxed overnight under Ar. The reaction mixture was extracted with $CHCl_3$, and the extract was washed 5 times with dilute HCl. The organic phase was dried over Na_2SO_4 and evaporated, and the residue was chromatographed on silica gel (solvent, 9:1 hexane-ethylacetate) to yield compound **14** (75 mg, 63%); mp 84–85 C. ^1H NMR (270 MHz, $CDCl_3$) δ 5.59 (d, J = 5 Hz, 1H, 6-H), 3.58 (m, 1H, 3-H), 2.91 (d, J = 13 Hz, 1H, 19-H), 2.57 (d, J = 13 Hz, 1H, 19-H), 2.09 (s, 3H, SMe), 0.91 (d, J = 7 Hz, 3H, 21-Me), 0.85 (d's, J = 7 Hz, 6H, 26-Me and 27-Me), 0.77 (s, 3H, 18-Me); MS, m/z 432 (M^+, 1.5), 417 (M-CH_3, 46), 384 (M-$HSCH_3$, 13), 371 (M-CH_2SCH_3, 85), 353 (M-CH_2SCH_3-H_2O, 100). Exact mass 432.3376, calculated for $C_{28}H_{48}OS$ 432.3426.

Results and discussion

The syntheses of the 19-functionalized sterols are illustrated in Scheme 1. All of the new compounds were derived from 19-hydroxycholesteryl acetate (**1**), which was easy to prepare in relatively large quantities by means of the literature procedure.[13] The C-19 carboxylic acid **8** was synthesized by Jones oxidation of compound **1** to the known acid **7**,[15] followed by simple base hydrolysis.

In order to prepare the new 19-amino sterols, the known aldehyde **2**,[14] which we obtained by pyridinium chlorochromate oxidation of compound **1**, was subjected to a two-step reductive amination procedure. The imines **3** and **5** formed readily upon treatment of **2** with the appropriate amine in excess, which also removed the acetate group. It is notable that the Schiff's base **5** is stable to aqueous solutions. Ordinarily such imines undergo rapid hydrolysis unless the nitrogen is part of a conjugated system, but the steric encumbrance of this



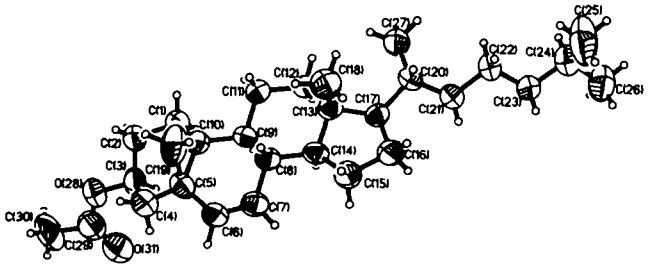
Scheme 1 Conditions: (a) PCC/CH_2Cl_2; (b) $HONH_2$·HCl/EtOH/H_2O/reflux; (c) $LiAlH_4/Et_2O$/reflux; (d) $MeNH_2$/EtOH/H_2O; (e) Jones reagent/acetone; (f) NaOH/EtOH/reflux; (g) MsCl/pyridine; LiBr/acetone/reflux; (h) $KSCOCH_3$/DMF/reflux; (i) Na_2SO_3/DMF/reflux; (j) NaSMe/DMF/reflux.

imine lowers its reactivity. Presumably for the same reason, compounds **3** and **5** proved to be somewhat resistant to reduction by borohydride reagents, but overnight treatment with $LiAlH_4$ in refluxing ether gave the amines **4** and **6** in good yield.

The synthesis of 19-mercaptocholesterol (**11**) was more difficult. In preliminary experiments compound **1** was converted to the tosylate and mesylate, but these hindered sulfonates did not react readily with a variety of sulfur nucleophiles. However, the mesylate was converted to the bromosterol **9** by treatment with lithium bromide in acetone,[19] and compound **9** in turn reacted with potassium thioacetate in refluxing DMF to give 19-(acetylthio)cholesteryl acetate (**10**) in modest yield. Base hydrolysis than gave the desired 19-mercaptocholesterol (**11**). In a similar manner, 19-(methylthio)cholesterol was prepared by displacement of the bromide with sodium methanethiolate, which simultaneously removed the acetate.

However, an attempt to prepare cholesterol 19-sulfonic acid took an unexpected turn. After treating the bromide **9** with sodium sulfite in refluxing DMF, mass spectrometric analysis of the product suggested that only a very small amount of the sulfonic acid had been formed. The major product contained no sulfur, and its NMR spectrum showed new olefinic resonances (δ 5.69 and 5.26) as well as a signal at δ 0.52 which suggested the presence of a cyclopropane. X-ray analysis unambiguously established the product to be the 5,19-cyclosterol **12**,* which, interestingly, had recently been reported as a minor product from the solvolysis of 19-tosyloxycholesteryl acetate.[20] It appears that sulfite is too bulky a nucleophile to attack the very hindered bromide an appreciable rate, and so the solvolysis of **9** becomes the dominant reaction. Compound **12** was easily hydrolyzed to the free sterol **13**.

* A single crystal of **12** measuring 0.725 mm × 0.125 mm × 0.125 mm was used for X-ray analysis. Crystal data: $C_{29}H_{46}O_2$; orthorhombic, space group $P2_12_12_1$; $a = 9.247$ (4) Å, $b = 12.487$ (5) Å, $c = 22.825$ (8) Å, $V = 2635$ (2) Å^3, $Z = 4$, $D_{calcd} = 1.075$ g/cm^3. Intensity measurements were made with $3^\circ \leq 2\theta \leq 114^\circ$ by using graphite-monochromated Cu Kα radiation ($\lambda = 1.54178$ Å) at room temperature on a Nicolet R3m diffractometer. A total of 2039 unique reflections were measured, and after Lorentz, polarization, and background corrections were applied, 1476 were considered to be observed [$|F_o| > 3\sigma(F_o)$]. The structure was solved by direct methods and refined by using the SHELXTL PLUS software. Refinement converged at $R = 0.060$, $R_w = 0.066$. The structure of compound **12** is illustrated below. We thank Ms. Natalie Smyth for the X-ray structure determination. Full details will be published elsewhere.



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