°C (20 mm)) gave a residue which was dissolved in 50 mL of CH_2Cl_2 and passed through 3 × 20 g of neutral alumina in three equal portions. A large yellow band was eluted first, the three fractions from these bands being combined and evaporated (50 °C (20 mm)) to give 0.460 g (56%) of **17**, mp 265–268 °C, suitable for condensation with **6**. A second chromatography provided an analytical sample as pale yellow prisms, mp 272–273 °C: IR ($CHBr_3$) 3010–2840 (m), 1660 (vs), 1610 (w), 1595 (s), 1555 (s), 1255 (s), 985, 890, 805, 735 cm^{-1}; NMR ($CDCl_3$, 60 MHz) δ 2.5–3.0 (m, 12 H), 7.04 (s, 2 H), 8.18 (s, 2 H), 10.30 (s, 2 H); ^{13}C NMR ($CDCl_3/CF_3CO_2H$, 25.05 MHz, ppm from $SiMe_4$) 21.5 (t), 26.6 (t), 28.7 (t), 122.4 (d), 128.4 (d), 130.8 (s), 131.4 (s), 133.1 (s), 139.9 (s), 143.1 (s), 152.4 (s), 195.0 (d).

Anal. Calcd for $C_{24}H_{18}Cl_2O_2$: C, 70.43; H, 4.43; Cl, 17.32. Found: C, 70.36; H, 4.45; Cl, 17.31.

1,2,4,5,7,8,10,11,13,14,24,25-Dodecahydro-18,21-dioxonia-15,23:16,22-dimethenobenzo[1,2-*a*:5,4-*a*]dipentaphene Bis(trifluoromethanesulfonate) (18). To a mixture of 46.0 mg (14.5 mmol) of **6** and 59.3 mg (14.5 mmol) of **17** was added 0.170 g (1.13 mmol) of CF_3SO_3H; the mixture was well stirred and heated at 100 °C for 2 h. The residue was triturated with 10 × 5 mL of Et_2O and filtered to give 124 mg (86%) of **18**, brownish-red microprisms, mp > 300 °C: IR ($CHBr_3$) 3500 (w, bd), 2950–2840, 1620 (m), 1560 (bd), 1475 (s), 1430, 1395, 1330 (w), 1260 (vs, v, bd), 1220, 1025 (s), 890 (bd, w), 870 (vw), 760 (vw), 740 (vw), 730 (vw) cm^{-1}; NMR ($CDCl_3/CF_3CO_2H$, 300 MHz) δ 2.5–3.1 (m, 24 H), 7.19 (s, 4 H, external), 8.11 (s, 4 H, internal), 8.66 (s, 2 H, γ in pyrylium ring); fast atom bombardment MS, M^+ 618 (100), 619 (65), 620 (22), 621 (11). $C_{46}H_{42}O_2$ requires M 618. No deductions of background (ca. 10%) have been made.

Anal. Calcd for the tetrahydrate $C_{48}H_{42}F_6S_2O_{12}$: C, 58.30; H, 4.28; S, 6.48. Found: C, 57.95; H, 3.78; S, 6.63.

5,6,8,9-Tetrahydrodibenzo[*c*,*h*]xanthylium Trifluoromethanesulfonate (21). To a mixture of 0.963 g (5.00 mmol) of **23** and 0.730 g (5.00 mmol) of 1-tetralone (Aldrich Co.) was added 0.750 g (5.00 mmol) of CF_3SO_3H; the mixture was well stirred and heated at 100 °C for 2 h. The residue was triturated with 3 × 50 mL of Et_2O and filtered to give 1.473 g (68%) of **21**. Recrystallization from HOAc gave orange needles, mp 204–206 °C: IR($CHBr_3$) 1610, 1560, 1475 (s), 1430 (s), 1420, 1350 (w), 1335 (w), 1265 (vs), 1225, 1190 (w), 1025 (s), 960 (w), 925 (bd, w), 890 (w), 735 (vs) cm^{-1}; NMR, see Table II.

Anal. Calcd for $C_{22}H_{17}F_3SO_4$: C, 60.83; H, 3.94; S, 7.38. Found: C, 60.81; H, 3.97; S, 7.41.

5,6,8,9-Tetrahydrodibenz[*c*,*h*]acridine (25). A mixture of 0.146 g of 1-tetralone (1.00 mmol), 0.193 g of 1-chloro-3,4-dihydro-2-naphthalenecarboxaldehyde and 0.3 mL of 4% ethanolic KOH were stirred at 20 °C for 4 h. Extraction with 20 mL of CH_2Cl_2 gave a solution which was washed with 3 × 20 mL of Et_2O, dried over $MgSO_4$, and evaporated (50 °C (20 mm)) to give 0.340 g of an oil; this was not identified but was refluxed with 0.500 g (6.49 mmol) of NH_4OAc and 0.500 g (8.47 mmol) of $MeCONH_2$ for 3 days. Addition of water, extraction with 3 × 20 mL of CH_2Cl_2, followed by washing with 3 × 20 mL of water, drying the organic layer over Na_2SO_4, and evaporating (50 °C (20 mm)) gave an oil which was chromatographed (alumina; 1:9 (v/v) EtOAc–light petroleum) to give 96.0 mg (33%) of **25**, crystallizing as needles from ethanol: mp 158–159 °C (lit.[21] 162 °C).

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Synthesis and Photoreactivity of Cholesteryl Diazoacetate: A Novel Photolabeling Reagent[1]

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Abstract: Cholesteryl diazoacetate (**1**), a new photolabeling reagent, has been synthesized for use in biological systems. Photolysis of the reagent proceeds by first-order kinetics at 254 and 350 nm. High-field ^1H and ^{13}C NMR spectroscopy allowed identification of separated products. The reagent undergoes intermolecular insertion reactions; C–H and O–H insertion are responsible for the major products when **1** is photolyzed in cyclohexane and methanol, respectively. Cholesterol and cholesteryl formate were unexpected side products of the cyclohexane reaction. Cholesterol was also a product of the photolysis in methanol, where O–H insertion was found to be favored by 16:1 over C–H insertion. In both solvents, photolysis produced little Wolff rearrangement.

Photoaffinity labeling, introduced with the photolysis of diazoacetylchymotrypsin,[2] has developed into a major technique for the investigation of biological systems.[3,4] An ideal photolabel should (a) be capable of introduction at a site of interest while remaining chemically "inert", (b) generate a highly reactive species upon photolysis which will react rapidly with the immediate environment to form a covalent bond, (c) react not only with nucleophiles, but also with hydrophobic regions (C–H bonds), and (d) generate a reactive species which is not readily deactivated by nonproductive side reactions such as H abstraction, intramolecular reaction, or rearrangement.[5]

Diazo compounds have demonstrated their utility as photolabels. Upon photolysis a molecule of N_2 is eliminated, followed by insertion of the resulting carbene into C–H or O–H bonds. α-Diazocarbonyl compounds are known, however, to undergo Wolff rearrangement to varying degrees, thereby wasting a percentage of the reactive carbene intermediate.[6] In the Wolff rearrangement an intermediate is formed[7] that may be relatively long lived and fail to react at the site where it was produced.

Cholesterol is the most abundant sterol found in mammalian cells. The molar ratio of cholesterol to phospholipid varies, de-

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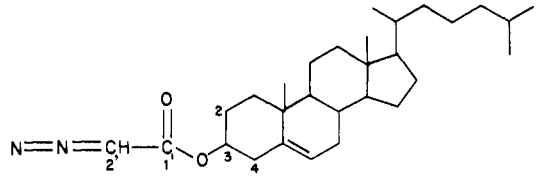
pending upon the location and function of the cell within the body. Its upper limit is generally about 1.00.[8] Cholesterol is carried in human serum by high-density lipoproteins[9] and has been shown to associate with certain proteins such as band-3 protein from human erythrocyte[10] or Ca^{2+} ATPase of sarcoplasmic reticulum.[11]

A cholesterol derivative containing a diazo group appeared to be a good candidate for use as a membrane photolabeling reagent. Therefore, we now report the synthesis and properties of cholesteryl diazoacetate (**1**). Compound **1** is thermally stable and undergoes



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photolysis with high yields of insertion into cyclohexane and methanol, accompanied by little Wolff rearrangement. Interestingly, cholesterol was isolated as a minor photoproduct in both cyclohexane and methanol. Furthermore, cholesteryl formate was unexpectedly generated (10% yield) during photolysis of **1** in cyclohexane. It seems highly unlikely that this formate arises from a carbene intermediate. Therefore, it may result from cleavage of the excited diazo compound.

Experimental Section

Materials. Thionyl chloride ($SOCl_2$) was distilled from triphenyl phosphite[12a] immediately before use. Triethylamine (Et_3N) was distilled, bp 89 °C (lit.[12b] bp 89.5 °C), and stored over potassium hydroxide until use. Methanol and benzene were dried over 3-Å molecular sieves for at least 48 h and filtered (Millipore type BD, 0.6 μm) or decanted just before use. Cyclohexane was ACS grade, spectroanalyzed. All other reagents were ACS grade or better and were used as purchased. For column chromatography Merck Silica Gel 40 (200–500-μm particle size) or Merck Silica Gel 60 (63–200-μm particle size) was used. Merck Silica Gel 60 (40–63-μm particle size) was used for flash chromatography columns.

Physical Methods. Infrared spectra were obtained on a Perkin-Elmer 735 spectrophotometer. Ultraviolet studies were conducted on a Perkin-Elmer 202 UV–visible spectrophotometer. Proton NMR spectra were obtained on a Bruker WM 250 spectrometer. Carbon NMR spectra were accumulated on either a JEOL PFT-100 (25 MHz) or Bruker WM 250 (62.9 MHz) spectrometer. All NMR chemical shifts are reported in parts per million (ppm) downfield from internal Me_4Si. ^1H NMR spectra used for integration were run at a 30° pulse width, with 8 total scans and a 5-s repetition time. Although full data are given in the supplementary material, agreement of corresponding NMR absorptions for the steroid nucleus was very close for the compounds studied, except where noted below. The statement that the absorptions for a particular compound conformed to the steroid nucleus means agreement generally within ±0.02 ppm for ^1H and 0.5 ppm (±1.5 ppm for olefin peaks only) for ^{13}C NMR spectra. TLC plates (Merck Silica Gel 60) were visualized by using phosphomolybdic acid solution, 35 g in 1 pt of ethanol. Elemental analyses on samples dried under vacuum for 12 h were performed by either Galbraith Analytical Laboratories, Knoxville, TN or Midwest Microlab, Ltd., Indianapolis, IN.

Photolysis Reactions. Prior to photolysis all reaction mixtures were warmed to 33 °C and purged of oxygen by bubbling oxygen-free N_2[12c] through the solution for a minimum of 5 min. The reaction vessel was a 46-cm long quartz tube (2.5-cm i.d.) the contents of which were agitated by magnetic stirrer. Temperature was maintained during the reaction by a flow of forced air. Photolysis took place at 33 °C under N_2 for 15 min in a Rayonet RPR-100 photoreactor using 254-nm lamps (16). During the order of reaction determination, 350-nm lamps (8) were also employed. Although all product isolations were carried out on solutions photolyzed at 254 nm, it was shown by TLC that essentially identical product mixtures were formed at 350 nm.

Synthesis. The synthesis of 3-cholesteryl diazoacetate (**1**) was patterned after the preparation of estrogen photolabels developed by Katzenellenbogen et al.[13] Glyoxylic acid chloride *p*-toluenesulfonylhydrazone (4 g, 15.3 mmol) in 50 mL of methylene chloride was treated dropwise at 0 °C with a solution of cholesterol (5.72 g, 14.8 mmol) and redistilled triethylamine (1.5 g, 14.8 mmol) in 40 mL of methylene chloride–THF (1:1). After 0.5 h, an additional 2.25 g (22.2 mmol) of triethylamine was added. The resulting solution was allowed to warm to room temperature and to stir for an additional hour. The reaction mixture was filtered to remove triethylamine hydrochloride and the solvent was removed at 25 °C under reduced pressure. Purification was achieved by column chromatography (silica gel 40, packing: 5.3-cm diameter × 20-cm high) followed by column chromatography (silica gel 60–80 g, 2.5-cm i.d.) using toluene as the eluent in both cases. From the latter column 60 fractions of 25 mL each were collected. Recrystallization from ethyl acetate gave **1** as pale yellow prisms in 40% yield, mp 141–142 °C dec. IR, ^1H NMR, and ^{13}C NMR spectra conformed to those found for the steroid nucleus with the addition of the following: UV λ_{max} 250 nm (log ε 4.05); IR (CCl_4) 2100 (vs), 1700 (s), 1385 (s), 1240 (m), 1135 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.678 (overlapping s, 1 $H_{2'}$; m, 1 H_3; see **1** for numbering); ^{13}C NMR (CD_2Cl_2, 25 MHz) δ 28.33 (C_2), 38.64 (C_4), 74.65 (C_3), 46.11 ($C_{2'}$), 166.60 ($C_{1'}$).

Anal. Calcd for $C_{29}H_{46}N_2O_2$: C, 76.60; H, 10.20; N 6.16. Found: C, 76.61; H, 10.43; N, 6.30.

Products. Cyclohexane Photolysis. A toluene–hexane (1:4) solution of the total product mixture (from 2 mmol of **1** in 2 L of cyclohexane) was applied to a flash column[14] (silica gel 60, packing: 2.5-cm diameter × 13.75–15-cm high). The column was eluted with 1000 mL of toluene–hexane (1:4) followed by toluene–$CHCl_3$ (1:1), 500 mL; $CHCl_3$, 500 mL; and MeOH–$CHCl_3$ (1:19), 1000 mL. Cholesteryl cyclohexylacetate (**2**) eluted from the column in toluene–hexane (1:4) with partial overlap of cholesteryl formate (**3**). Any residue of **3** eluted from the column in toluene. Complete separation of **2** and **3** was achieved by repeat flash chromatography of the unresolved fractions. Cholesterol (**4**) eluted from the column in an impure state in toluene–$CHCl_3$ (1:1) and $CHCl_3$. Final purification was achieved by flash chromatography (silica gel 60, packing: 2.5-cm diameter × 13.75–15-cm high), using EtOAc–hexane (1:3), 1000 mL. Product **5**, cholesteryloxyacetic acid, eluted from the column in MeOH–$CHCl_3$ (1:19).

Cholesteryl Cyclohexylacetate (2). Yield: 70% of **1** photolyzed; mp 137.5–138 °C dec. Spectra conformed to the steroid nucleus with the following additions: IR (CCl_4) 1735 (s), 1240 (m), 1120 (m) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.612 (m, 1 H_3), 2.145 (d, 2 $H_{2'}$, J = 6.8 Hz), 1.702 (m, 1 $H_{3'}$), $H_{4'}$, $H_{5'}$, $H_{6'}$ detected as extra absorption overlapped by steroid protons; ^{13}C NMR (CD_2Cl_2, 25 MHz) δ 73.82 (C_3), 172.18 ($C_{1'}$), 42.73 ($C_{2'}$), 35.33 ($C_{3'}$), 33.38 ($C_{4'}$), 26.46 ($C_{5'}$), unresolved shoulder downfield side of 26.46 peak ($C_{6'}$).

Anal. Calcd for $C_{35}H_{58}O_2$: C, 82.29; H, 11.44. Found: C, 82.41; H, 11.06.

Cholesteryl Formate (3). Yield: 10% of **1** photolyzed; mp 90–92 °C. Spectral assignments conformed to the steroid nucleus plus: IR (CCl_4) 1725 (vs), 1235 (w), 1135(w) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.716 (m, 1 H_3), 8.021 (s, 1 $H_{1'}$); ^{13}C NMR (CD_2Cl_2, 25 MHz) δ 160.57 ($C_{1'}$; gated decoupled spectrum: d, J = 223.8 Hz), 74.22 (C_3); CI mass spectrum (NH_4^+), m/e (relative intensity) 432 (M^+ + 18, 58), 387 (42), 386 (74), 369 (100), 368 (90), 353 (62), 255 (50), 247 (46), 148 (66), 147 (56).

Anal. Calcd for $C_{28}H_{46}O_2$: C, 81.10; H, 11.18. Found: C, 81.21; H, 11.08.

Cholesterol (4). Yield: 10% of **1** photolyzed. IR and ^1H NMR were identical with those of authentic cholesterol within experimental uncertainty.

Cholesteryloxyacetic Acid (5). Yield: 6% of **1** photolyzed. Spectral data conformed to the steroid nucleus plus: IR ($CHCl_3$) 1730 (s), 1590 (s), 1110 (s) cm^{-1}, carboxyl OH obscured by aliphatic CH bands; ^1H NMR ($CDCl_3$) δ 3.318 (m, 1 H_3), 4.123 (s, 2 $H_{2'}$), 6.3 (br s, 2.1 H, COOH and H_2O H's exchanging); ^{13}C NMR (CD_2Cl_2, 62.9 MHz) δ 171.70 ($C_{1'}$), 65.76 ($C_{2'}$), 81.10 (C_3).

Further IR data used for structure determination (toluene) 1720 (br s; acid C=O), 1580 (w, acid salt C=O) cm^{-1}; (toluene + Et_3N) 1720 (w), 1590 (s) cm^{-1}.

Methanol Photolysis. The product mixture (from 2 mmol of **1** in 4 L of MeOH) was separatated on a flash column (silica gel 60 packing: 2.5-cm diameter × 13.75–15-cm high) by elution with toluene, 500 mL, followed by EtOAc–$CHCl_3$ (1:4), 1000 mL, and EtOAc, 1000 mL. Cholesteryl methoxyacetate (**6**), cholesteryl 3-hydroxypropionate (**7**), and cholesterol (**4**) eluted from the column in EtOAc–$CHCl_3$ (1:4). Cho-

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lesteryl methoxyacetate (**6**) was recrystallized from methanol. Products **7** and **4** were separated by repeat flash chromatography, using methylene chloride as the elution solvent.

Cholesteryl Methoxyacetate (6). Yield: 62% of **1** photolyzed. Spectral data conformed to the steroid nucleus plus: IR (CCl_4) 1760 (s), 1290 (w), 1135 (s) cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.718 (m, 1 H_3), 3.43 (s, 3 $H_{3'}$), 3.99 (s, 2 $H_{2'}$); ^{13}C NMR (CD_2Cl_2, 25 MHz) δ 169.81 ($C_{1'}$), 70.38 ($C_{2'}$), 59.43 ($C_{3'}$), 74.8 (C_3).

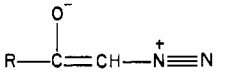
Anal. Calcd for $C_{30}H_{50}O_3$: C, 78.54; H, 10.98. Found: C, 78.30; H, 10.83.

Cholesteryl 3-Hydroxypropionate (7). Yield: 12% of **1** photolyzed. ^1H NMR ($CDCl_3$) was consistent with the steroid nucleus plus: δ 4.663 (m, 1 H_3), 2.540 (t, 2 $H_{2'}$), 3.847 (t, 2 $H_{3'}$), 2.7 (br s, OH).

Cholesterol (4). Yield: 16% of **1** photolyzed. ^1H NMR spectrum and TLC were identical with those of authentic cholesterol within experimental uncertainty.

Results and Discussion

Synthesis. Cholesteryl diazoacetate (**1**) has been synthesized and purified according to our recently developed three-step synthesis patterned after the preparation of estrogen photolabels developed by Katzenellenbogen et al.[13] Purification of **1** in quantities necessary for photolysis in model membranes was complicated by the presence of a reaction side product, tentatively identified as cholesteryl *p*-toluenesulfinate, which has nearly the same R_f as **1**. Final purification was achieved by multiple use of silica gel column chromatography, using toluene as the eluent. The average yield of **1** was 40%. Spectral data were uniquely consistent with the proposed structure. The IR exhibited bands attributable to the diazoacetate moiety.[15] In diazo compounds the carbonyl and the diazo frequencies are shifted (lower and higher, respectively) due to a resonance contribution from structures such as that seen in eq 1.

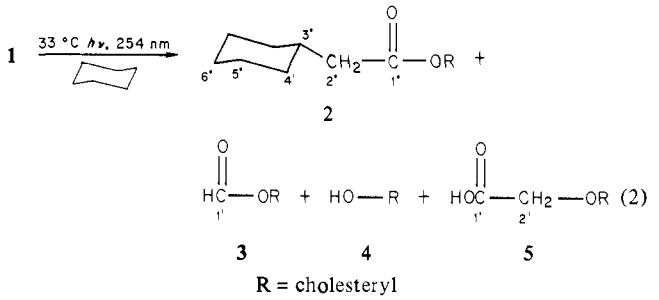


(1)

Secondly, the ^1H NMR spectrum displayed the methine proton at 4.708 ppm, which is consistent with previously reported values for α-diazo esters.[16] A 1-ppm downfield shift was noted for H_3, which is typical when a hydroxyl group is replaced by an ester function. The magnitude of the shift may be the result of a stronger electron-withdrawing inductive effect by the ester group or the 3-position proton being oriented in the deshielding cone of the carbonyl on conformational averaging. Finally, the carbon NMR spectrum exhibited the carbonyl resonance at 166.60 ppm and the 2′-carbon at 46.11 ppm, close to the values reported by Lichter et al.[17]

Photolysis Studies. The photolabel **1** was photolyzed in two solvent systems to determine its ability to insert into C–H or O–H bonds and its potential to undergo Wolff rearrangement. Cyclohexane[18] was chosen as a model for the hydrophobic portions of a phospholipid molecule, while methanol was used as a model for water insertion. During the photolysis of **1** in both solvents the diazo compound disappeared (followed by UV absorption) according to first-order kinetics. The half-time of the disappearance (under conditions described in the Experimental Section) was estimated as $t_{1/2}$ = 61 s for cyclohexane and $t_{1/2}$ = 21.5 s for methanol. Photolysis was also run at 350 nm, to determine if the reaction would proceed under gentler conditions which will be required for more complex membrane systems. The disappearance of **1** again followed first-order kinetics, for cyclohexane $t_{1/2}$ = 3 h and for methanol $t_{1/2}$ = 2.6 h. Reactions run at both wavelengths in a particular solvent system produced the same products in approximately the same yields according to TLC.

Reaction of **1** in cyclohexane produced four noteworthy products (eq 2). The predominant product was the result of C–H insertion into cyclohexane, **2**. Its yield was 70% of the diazo compound



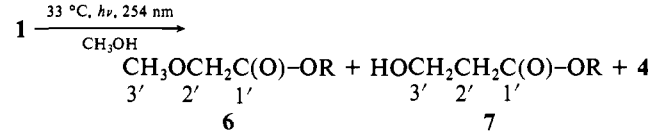
R = cholesteryl

used. The spectra were uniquely consistent with the proposed structure. In the proton NMR only the 3′ proton of the cyclohexyl group is well resolved (1.720 ppm). Protons at positions 4′, 5′, and 6′ are partially obscured by the protons of the steroid ring system.

The Wolff rearrangement product (**5**) accounted for only 6% of **1** used in the reaction. It was formed by reaction of the initially formed ketene with traces of water in the cyclohexane. Spectral data for the cholesteryl group are closely similar to those for **1** and **2** with the exception of the ^1H NMR resonance for H_3. In **5** this resonance is shifted upfield to 3.318 ppm (H_3 of cholesterol at 3.529 ppm) compared to 4.678 and 4.612 ppm observed for **1** and **2** respectively. Proton NMR indicates structure **5**, but is not sufficient to completely differentiate **5** from the product which would be formed by direct insertion of the carbene into water ($HOCH_2C(O)$–O–cholesteryl). The spectral grade cyclohexane used contained 0.2% water which at 1 mM concentration of **1** could produce a significant amount of water insertion, as well as **5**. Actual confirmation of the Wolff rearrangement product structure was achieved by IR. When the IR of **5** was run in toluene, a carbonyl resonance was observed at 1720 cm^{-1} (br) with a small peak at 1580 cm^{-1}. Upon addition of Et_3N, the 1720-cm^{-1} peak diminished considerably and a prominent peak appeared at 1590 cm^{-1}. The 1720-cm^{-1} band corresponds to the carbonyl of a free acid, while the 1580-cm^{-1} band corresponds to the carbonyl of an acid salt.

Surprisingly, cholesteryl formate (**3**) and cholesterol (**4**) were the other two isolated photoproducts. Both **3** and **4** were found in 10% yield (there does not seem to be any significance to their presence in equal amounts). Cholesterol was identified by IR, ^1H NMR, and TLC comparisons with authentic cholesterol. Cholesteryl formate characterization was more interesting. The proton NMR spectrum was distinguished by a one-proton singlet at 8.021 ppm, while the ^{13}C spectrum exhibited a slight upfield shift of the carbonyl carbon and the loss of a carbon resonance. Chemical ionization mass spectrometry confirmed a molecular weight of 414. Thus, it became evident that a formate ester was one of the photolysis products. A gated decoupled carbon spectrum was run to complete the characterization. The parameters were adjusted to allow maximum NOE to accumulate while retaining the maximum C–H coupling constants. $C_{1'}$, the carbonyl carbon, appeared as a doublet with J_{CH} = 223.8 Hz. This J_{CH} value agrees well with values reported for similar formyl carbons.[19]

Reaction of cholesteryl diazoacetate in methanol produced three major products: cholesteryl methoxyacetate (**6**), cholesterol (**4**), and cholesteryl 3-hydroxypropionate (**7**) (eq 3). Cholesteryl



R = cholesteryl (3)

methoxyacetate (**6**) is the predominant product, generated by carbene insertion into the O–H bond. Its yield was 62% of the diazo compound photolyzed. Spectral data were uniquely consistent with the proposed structure. Product **6** is not the result

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of Wolff rearrangement ($ROCH_2C(O)$–OCH_3). Proton NMR spectroscopy indicates that the H_3 proton multiplet is at 4.718 ppm in excellent agreement with the H_3 chemical shift for the cholesteryl acetate derivatives **1**, **2**, and **3** (4.678, 4.612, and 4.716 ppm, respectively). It is also significantly downfield from the H_3 chemical shift noted for the cyclohexane Wolff rearrangement product, **5** (3.726 ppm). The three-proton singlet of the methyl ether group was located at 3.430 ppm in agreement with literature values, i.e., CH_3–O–CH_2–C(O)–CH_3 at 3.45 ppm,[20a] and significantly upfield from the chemical shift expected for a methyl ester moiety, ca. 3.7 ppm.[20b]

The second expected product was **7**, tentatively identified by ^1H NMR, which is the result of carbene insertion into C–H bonds.[21] On the basis of the integration of the proton NMR spectrum of a mixture of **4** and **7**, cholesteryl 3-hydroxypropionate was found to be approximately 12% of **1** used in the photolysis.

Cholesterol (**4**) was found in a yield of 16% of **1** photolyzed. In the methanol photolysis, the Wolff rearrangement product was not isolated. According to TLC, production of **5** by reaction of the rearranged ketene intermediate with water present in methanol, was negligible. However, the methyl ester of **5**, from reaction of the rearranged intermediate with methanol, could be one of several minor products (less than 5%) noted on TLC.

The total recovery for **1** reacted was 96% for cyclohexane photolysis and 90% for methanol photolysis. Control experiments under identical conditions but without irradiation gave negligible amounts of decomposition of **1** in both solvents. Cholesteryl diazoacetate exhibits a strong tendency to insert into C–H or O–H bonds with little formation of Wolff rearrangement product, which is somewhat surprising compared to results reported for other diazoester compounds.[21] This particular diazo compound is also fairly nonselective since the yield of O–H insertion is favored over the yield of C–H insertion into methanol by only 5:1, a selectivity ratio of 16:1.

Cholesterol is a direct photoproduct of the reaction in both cases. Control experiments under identical conditions, but without irradiation, also produced negligible amount of **4**. Cholesterol was found to be in the reaction mixture immediately following photolysis and is not the result of further workup of the product mixture. Production of **4** could be the result of an ester exchange process noted in a paper by DoMinh et al.[22] During the photolysis of diazoacetate compounds in alcohols, eq 4, **8** is formed in addition

$$N_2CHCO_2R \xrightarrow[i\text{-PrOH}]{h\nu} \underset{\mathbf{8}}{(CH_3)_2CHOCH_2CO_2CH(CH_3)_2} + \underset{\mathbf{9}}{HOR} \quad (4)$$

to the expected reaction products. The analogous exchange products would be $HOCH_2CO_2H$ (from H_2O), $CH_3OCH_2CO_2CH_3$ (from CH_3OH), or $CH_3OCH_2CO_2H/HOCH_2CO_2CH_3$ (from H_2O and CH_3OH). Cholesterol would be the corresponding alchohol (**9**) side product.

Another possible mechanism for **4** formation would be homolytic α-cleavage of the (O=C)—O bond followed in this case by hydrogen abstraction. This process has been detected in special cases[23] such as *o*- and *p*-acylphenols or γ-butyrolactones and has been suggested by ESR studies of the photolysis of aliphatic carboxylic acids and esters.[24] Our data are insufficient to differentiate between these two processes; however, published data strongly indicate that C–O bond cleavage in aliphatic esters is very minor.[23]

With simple alkyl esters, carbon–carbon α-cleavage is the major photolysis process.[23,24] This results in the formation of O=ĊOR which may further abstract a hydrogen atom from solvent to give formate esters. Product **3** may result from this process. Cholesteryl formate (**3**) could also result from (O=C)C α-cleavage similar to that noted for β,γ-unsaturated ketones[25,26] followed by hydrogen abstraction. It is highly unlikely that **3** is a result of reaction of the carbene; therefore, this unusual product may be formed from an excited diazo ester intermediate.

Conclusion

Cholesteryl diazoacetate shows great promise as a photolabel of biological membranes. Assuming the label orients in a manner similar to cholesterol, the carbene should insert readily into C–H or O–H bonds of the hydrophilic portion of a bilayer, phospholipid, water, or protein. Only 30% of the label should be wasted by nonproductive side reactions. It may also prove to be an excellent probe of high-density lipoproteins which carry cholesterol in human serum[9,27] or the association of cholesterol with certain proteins.[10,11] The production of cholesterol and cholesteryl formate in significant yields also deserves further study.

In fact, we have shown that photolysis of **1** in membranes does lead to insertion into phospholipid (to be published).

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Supplementary Material Available: Tables of ^1H and ^{13}C NMR chemical shifts of cholesteryl diazoacetate and its photolysis products (2 pages). Ordering information is given on any current masthead page.

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