mp 99–100°; R_f 0.61 (3% acetone in benzene; R_f of methyl 3-keto-5β-cholanoate, 0.37, and methyl lithocholate, 0.16).

Acetic Acid-t[48,49].—Acetic anhydride (3.5 g), water (0.5 g), and tritiated water (0.1 g) with an original activity of 10 mC were mixed together and refluxed for 1 hr. The product was cooled, left overnight at room temperature, and distilled. After rejecting the first fraction, the second fraction was collected as tritiated acetic acid.

Methyl 3-Keto-4α-^{3}H-5β-cholanoate (13).—Methyl 3-keto-4β-bromocholanoate (300 mg) was dissolved in 10 ml of dry ether which was added directly into the reaction flask by distillation from lithium aluminum hydride. Tritiated acetic acid (0.3 ml) and zinc dust (0.6 g), previously dried *in vacuo*, were added to the ether solution which was maintained at 15° and magnetically stirred for 1 hr in an atmosphere of nitrogen. Ether was added and the mixture was filtered. The ethereal filtrate was washed with sodium bicarbonate solution and then with water, and finally dried over anhydrous sodium sulfate. On evaporation of ether a residue of 249.4 mg was obtained which on tlc had the same mobility as methyl 3-keto-5β-cholanoate,[7] R_f 0.62 (5% acetone in benzene). On crystallization of the residue from acetone–hexane short needles of methyl 3-keto-4α-^{3}H-5β-cholanoate,[17] mp 121–122°, were obtained: specific activity, 2.36×10^4, 2.38×10^4 dpm/mg.

Action of Raney Nickel on Methyl 3-Keto-4α-^{3}H-5β-cholanoate.—A mixture of compound **13** (152 mg), Raney nickel catalyst (*ca.* 400 mg), and *p*-cymene (10 ml) was refluxed in the usual way. After separation of the catalyst and the solvent, the product (120 mg) was purified by plc in 3% acetone in benzene. The major compound (96 mg) on crystallization from aqueous methanol yielded shining plates of methyl 3-keto-5α-cholanoate: mp 115–116°; specific activity 4.3×10^2, 4.6×10^2 dpm/mg.

***p*-Toluic Acid-^{3}H.**—*p*-Cymene-^{3}H (specific activity 1.24×10^2 dpm/mg, 5 ml), obtained by steam distillation of the product from the previous reaction, was oxidized by dilute nitric acid by the procedure of Tuley and Marvel.[18] The crude *p*-toluic acid-^{3}H (1.5 g, mp 173–174°) was purified by extraction with toluene in a Soxhlet and chilling the product. *p*-Toluic acid-^{3}H was separated, dissolved in sodium hydroxide solution, precipitated by hot dilute hydrochloric acid, and crystallized from toluene: mp 177–178°; specific activity 0.57×10,[2] 0.59×10^2 dpm/mg after two crystallizations.

Action of Raney Nickel on Cholestanol.—A mixture of cholestanol (510 mg), 20 ml of *p*-cymene, and Raney nickel catalyst (*ca.* 1.2 g) was heated for 10 hr under reflux in the usual way. After separation of the catalyst and the solvent, the product (440 mg) was separated by plc in 5% acetone in benzene into the following compounds: (a) 5α-cholestan-3-one [270 mg, mp 128°, R_f 0.80 (R_f of cholestanol 0.30, methyl 3-keto-5α-cholanoate 0.60), R_t 0.41 (R_t of cholestanol 0.35)]; (b) 5β-cholestan-3-one [32 mg, mp 62°, R_f 0.84, R_t 0.35]; (c) Δ^4-cholestenone [36 mg, mp 82°, R_f 0.56 (R_f of methyl 3-keto-Δ^4-cholenoate 0.41), R_t 0.53]. No cholestanol was detected in the above reaction product.

Registry No.—**2**, 1173-32-6; methyl 3-keto-12α-hydroxy-Δ^4-cholenoate, 19684-72-1; *p*-toluic acid-^{3}H, 19689-62-4; 5β-cholestan-3-one, 601-53-6; Δ^4-cholestenone, 601-57-0.

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Neighboring Group Participation in Reactions of Alcohols with Lead Tetraacetate

PETER MORAND AND M. KAUFMAN

Department of Chemistry, University of Ottawa, Ottawa, Canada

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The participation of the neighboring group in two related steroidal α-methoxy alcohols has been studied. 3β-Acetoxy-5α-methoxycholestan-6β-ol, **2a**, and 3β-acetoxy-6β-methoxycholestan-5α-ol, **5a**, were prepared by cleavage of the corresponding 5β,6β- and 5α,6α-epoxides, **1a** and **1b**, in methanol in the presence of acetic acid or boron trifluoride. The course of the lead tetraacetate oxidation of these alcohols was strongly influenced by the adjacent methoxyl group. The structures of the products isolated have been established and mechanisms for their formation are discussed.

Since the discovery, in 1959, that CH_3, CH_2, and CH groups δ, and sometimes ε, to a secondary alcohol group could be oxidized by lead tetraacetate[1] to give rise to cyclic ethers, reactions of this type have been extensively studied.[2] Much of this work has been done with steroids[3] which are convenient models on which to study the geometrical factors involved in this interesting reaction. However, alcohols in diterpene,[4] bridged bicyclic,[5] and aliphatic[6–8] systems have also been shown to form cyclic ethers as well as other products. Conditions for this reaction vary; in the work described here the alcohol was treated with lead tetraacetate and irradiated in refluxing cyclohexane or benzene in the presence of iodine.[9]

Certain correlations have been made[2b] regarding the favorable internuclear distance between the oxyradical and the carbon atom carrying hydrogen atoms which can be abstracted intramolecularly. When a molecule has more than one alkyl group appropriately situated for hydrogen atom abstraction, other factors may influence the preferential abstraction of one hydrogen

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(4) U. Scheidegger, K. Schaffner, and O. Jeger, *Helv. Chim. Acta.*, **45**, 400 (1962).
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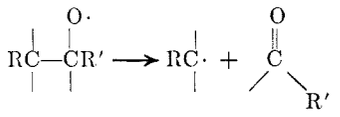
atom over another. For example, hydrogen atoms attached to an oxygen-bearing carbon atom are more reactive[10] than the hydrogen atoms of a methyl group and, as expected, the reactivity of hydrogen atoms decreases[11] in the order tertiary > secondary > primary.

In addition to intramolecular abstraction of suitably situated hydrogen atoms, oxy radicals produced by oxidation with lead tetraacetate are also known to undergo fragmentation, as shown. The amount of



cleavage which occurs increases with the stability[12,13] of the product, RĊ<. The stability of the ketone formed, the decrease in strain by the loss of bulky groups, entropy factors in the resonance stabilization of the radical formed by cleavage, and, in cyclic compounds (particularly small rings), the decrease in strain as a result of ring opening are also factors which determine the outcome of the competition between intramolecular hydrogen abstraction and fragmentation.

Results

Lead Tetraacetate Oxidation of 3β-Acetoxy-5α-methoxycholestan-6β-ol (2a).—Epimerization at the carbon atom bearing the hydroxyl group and at a carbon atom further removed has been shown to occur in the lead tetraacetate oxidation of 4β-hydroxy steroids[14] and of 6α-methyl-6β-hydroxy steroids.[15] A reversible fragmentation reaction was postulated by the authors in both cases to explain the formation of the products isolated. Intermediates in which bond cleavage had occurred at C-4, C-5 and at C-5, C-6, respectively, were invoked.

In steroids having a secondary hydroxyl group at C-6, apart from small quantities of C-6 ketone, only the 6β,19-oxide[16] has been isolated.[17] It was therefore decided to introduce a 5α-methoxy group in a suitable 6β-hydroxy steroid to investigate what influence it would have on the course of the lead tetraacetate oxidation of the alcohol.

3β-Acetoxy-5α-methoxycholestan-6β-ol, **2a**, was prepared[18] by heating 3β-acetoxy-5β,6β-oxidocholestane, **1a**, in methanol in the presence of acetic acid (Scheme I). The relative positions of the hydroxyl and methoxyl groups were confirmed by oxidation of **2a** to a ketone identified as 3β-acetoxy-5α-methoxycholestan-6-one, **6**. The ORD curve of this compound had a negative Cotton effect curve with a trough at 327 mμ, consistent with a C-6 ketone[19] in a *trans* A/B ring system.

Treatment of 3β-acetoxy-5α-methoxycholestan-6β-ol, **2a**, with excess lead tetraacetate and irradiation in refluxing cyclohexane in the presence of iodine gave a crude product from which three pure substances were isolated by column chromatography. The first compound to be eluted from the column was the methylenedioxy compound **3b** in a yield of 36%. On further elution, 3β-acetoxy-5α-methoxy-6β,19-oxidocholestane, **4** (27%), and 3β-acetoxy-5α-methoxycholestan-6-one, **6** (4%), were isolated.

The structure of **4** was deduced from its analysis and by examination of its ir and nmr spectra. The infrared spectrum showed a low intensity band at 1497 cm^{-1} which has been assigned[3a] to the C–H scissoring of the protons of the C-19 methylene group of the 6β,19-oxide. The nmr spectrum confirmed the presence of an acetate group, a methoxyl group, and a C-19 methylene group.[3a] A broad signal at 4.75 ppm for the hydrogen atom at C-3 indicated an equatorial configuration[20] for the C-3 acetate group which would be expected if the A/B ring was *trans*.

Examination of the nmr spectrum of compound **3b** showed that the acetate group at C-3 and the C-19 methyl group were intact. The presence of a five-membered methylenedioxy group was suggested[21] by the appearance of a singlet at 5.1 ppm which integrated for two protons. When the spectrum was determined in benzene instead of CD_3Cl an unresolved quartet was observed.

Examination of Drieding models shows that three isomeric methylenedioxy compounds are possible (Figure 1). Since the proton attached to C-6 would not be

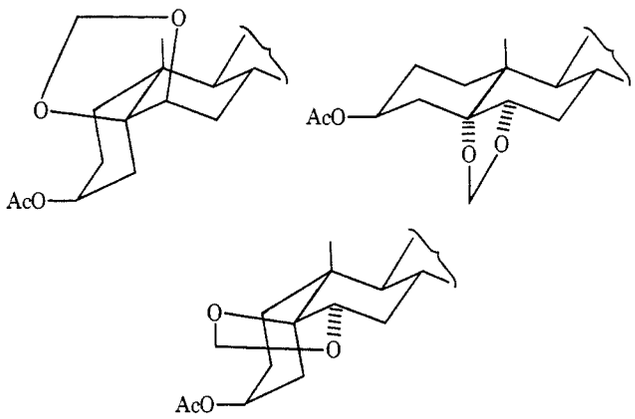


Figure 1.—Possible isomers of the methylenedioxy compound.

expected to be in too different an environment in any of these compounds the triplet centered at 3.86 ppm in the nmr spectrum and attributed to the C-6 proton did not enable one to establish configurations with any degree of certainty. Furthermore, since the band attributed to the 3α-H coincided with the signal of the methylenedioxy group, examination of the half-band width of the former was not possible.

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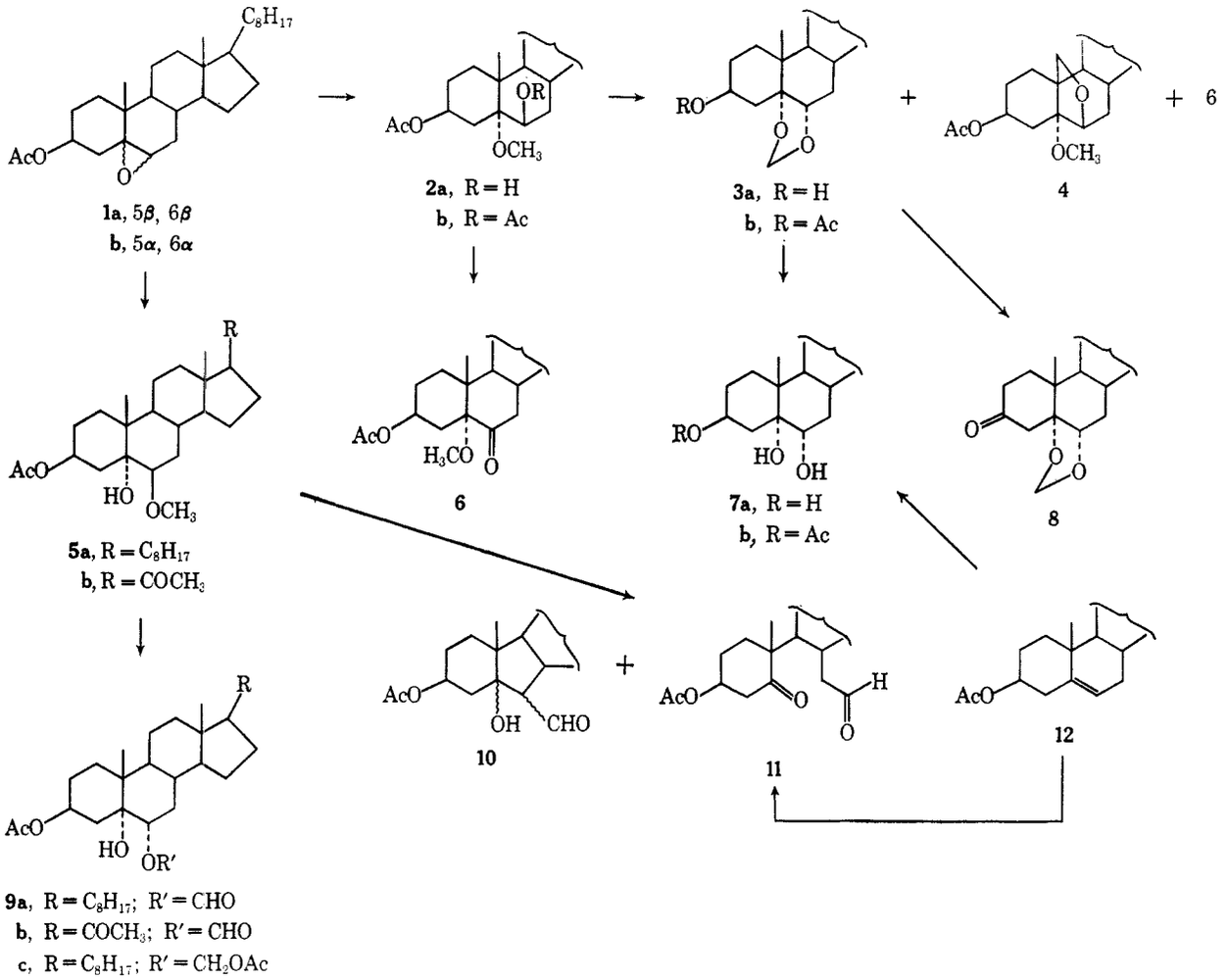
SCHEME I



Hydrolysis of compound **3b** under basic conditions gave the 3β-hydroxy compound **3a** which was subsequently oxidized with chromic acid in pyridine to ketone **8**. The ORD curve of this compound showed a positive Cotton effect as would be expected with a C-3 ketone in a *trans* A/B ring system.[22]

Treatment of **3b** with hydrogen chloride gas in ether–methanol cleaved the methylenedioxy group and hydrolyzed the C-3 acetate group giving a triol. The structure of this triol was identical with 3β,5α,6α-trihydroxycholestane, **7a**, obtained by hydroxylation of cholesterol acetate, **12**, with osmium tetroxide[23] and subsequent hydrolysis. The configuration at C-5 and C-6 of the methylenedioxy compound must therefore be as indicated in **3b**.

Lead Tetraacetate Oxidation of 3β-Acetoxy-6β-methoxycholestan-5α-ol (5a).—The lead tetraacetate oxidation of 5α- and 5β-hydroxy steroids has been shown[24] to yield products in which the C-5, C-10 bond is cleaved. The introduction of a methoxyl group at C-6 in steroids having a 5α-hydroxy group has a strong influence on the course of the lead tetraacetate oxidation of such compounds and a discussion of these results follows.

The mixture of the epimeric epoxides, **1a** and **1b**, obtained by treatment of cholesterol acetate, **12**, with perphthalic acid was treated with boron trifluoride etherate[25] in methanol and the product isolated was subsequently acetylated. By chromatography it was possible to isolate 5α-methoxycholestane-3β,6β-diol diacetate, **2b**, and 3β-acetoxy-6β-methoxycholestan-5α-ol, **5a**, in a ratio of 1:2.

When 3β-acetoxy-6β-methoxycholestan-5α-ol, **5a**, was oxidized with lead tetraacetate under the same conditions used for the reaction with **2a**, two products were isolated from the reaction mixture by chromatography on silica gel. The major product was the keto aldehyde **11** in which cleavage of the C-5, C-6 bond had occurred. The structure of this substance was confirmed by direct comparison with the same substance obtained by ozonolysis[26] of cholesterol acetate. The other product isolated was assigned the structure **10** on the basis of its elemental analysis and spectral properties. It was also shown that when the pure keto aldehyde **11** was eluted over silica gel it was partially converted into the hydroxy aldehyde **10**.

Under slightly different conditions and using less of an excess of lead tetraacetate, Lunn[27] has reported the isolation of the formate ester **9b** from 3β-acetoxy-5α-

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hydroxy-6β-methoxypregnan-20-one, **5b**. We have confirmed these results by isolation of the analogous formate ester **9a** (30%) from 3β-acetoxy-6β-methoxycholestan-5α-ol, **5a**, under the conditions described by Lunn. The structure of **9a** was assigned on the basis of its elemental analysis, its nmr and ir spectra, and the fact that triol **7a** was obtained on hydrolysis of this substance in base.

A small quantity (8%) of a second product, identified as acetyl 3β-acetoxy-5α-hydroxy-6α-cholestanyl formal, **9c**, was also isolated in this experiment. The empirical formula ($C_{32}H_{54}O_6$) of this compound was confirmed by its mass spectrum and by elemental analysis. Examination of the nmr spectrum of this substance indicated the presence of two acetate groups and an AB quartet centered at 5.22 ppm was attributed to a methylene group flanked by two oxygen atoms. The group at C-6 was assigned the α-configuration on the basis of the half-band width (20 Hz) of the signal attributed to the 6β-H.

Discussion

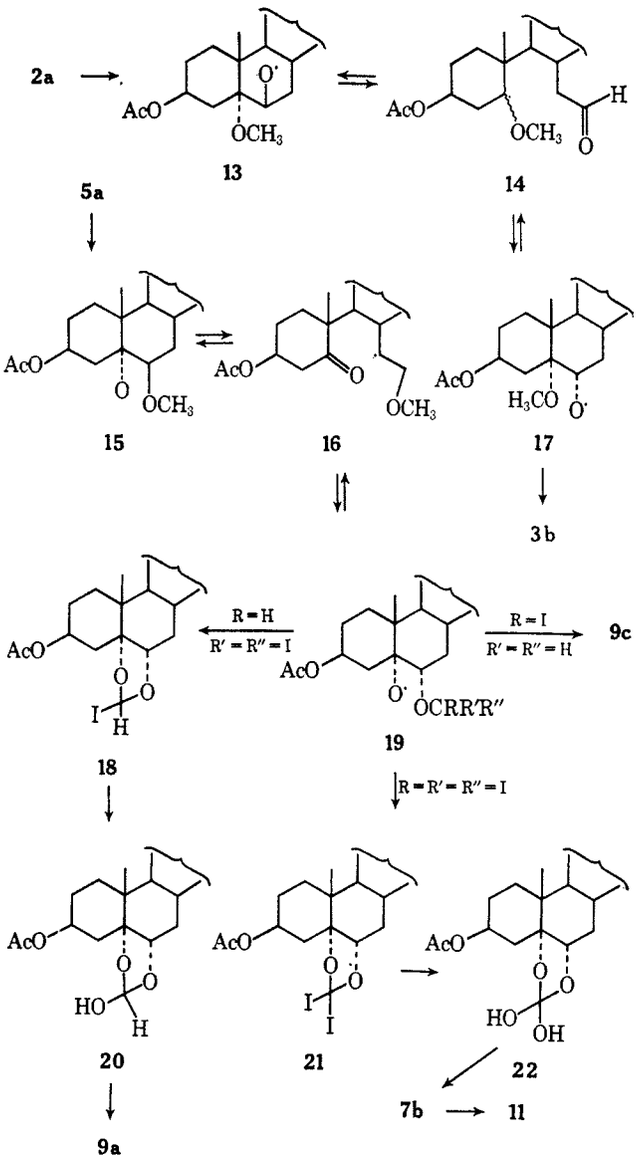
As already mentioned, epimerizations have been observed in the lead tetraacetate reaction of certain steroidal alcohols and these results have been explained[14,15] by assuming a reversible fragmentation reaction. Bearing this in mind an attempt will now be made to correlate the results obtained from the oxidation of both methoxy alcohols. For the sake of simplicity, it is assumed in the mechanisms discussed below that decomposition of the lead(IV) alkoxide intermediates formed in these reactions occurs homolytically.

Looking at Scheme II, it is seen that homolysis of the lead(IV) alkoxide of **2a** leads to the formation of an oxy radical (**13**) which can give rise to the intermediate **14** in which cleavage of the C-5, C-6 bond has occurred. This intermediate can then undergo cyclization with epimerization of the substituent at C-6 to give the oxy radical **17** in which the substituents at C-5 and C-6 are geometrically disposed to allow for abstraction of a hydrogen atom from the methoxyl group, resulting in the formation of 3β-acetoxy-5α,6α-methylenedioxycholestane, **3b**.

Lead tetraacetate oxidation of 3β-acetoxy-6β-methoxycholestan-5α-ol, **5a**, using a 2 molar excess of reagent, can proceed in an analogous manner. Cleavage of the C-5, C-6 bond (**16**), followed by cyclization with epimerization at C-6, leads to the formation of the intermediate **19** (R = R′ = R″ = H). From this intermediate it is possible to explain the formation of acetyl 3β-acetoxy-5α-hydroxy-6α-cholestanyl formal, **9c**, by the abstraction of a proton from the methoxyl group and concomitant addition of an acetoxyl radical. Alternatively, abstraction of two hydrogen atoms from the methoxyl group followed by the addition of two atoms of iodine would give the intermediate **19** (R = H, R′ = R″ = I). Upon displacement of one atom of iodine by the oxy radical the monoiodomethylenedioxy intermediate **18** could be formed which, under the reaction conditions, could decompose to give 3β-acetoxycholestane-5α,6α-diol 6-formate, **9a**.

With an excess of lead tetraacetate present the hydrogen atoms of the methoxyl group in **19** (R = R′ = R″ = H) can be substituted by three atoms of iodine.

SCHEME II



Displacement of one of these atoms by the oxy radical may lead to intermediate **21** which could then decompose to *cis* glycol **7b**. Further reaction with lead tetraacetate would cleave this glycol in the usual manner giving ketoaldehyde **11** as the final product.

The difference in behavior of the two methoxy alcohols toward lead tetraacetate can be rationalized on the basis of steric hindrance. By using Dreiding models it is revealed that the methoxyl group in **17** is restricted in its rotation about the C–O bond at C-5. The least hindered position for this group appears to be just under the oxy radical. Abstraction of more than one hydrogen atom with concomitant addition of iodine is therefore not favored and even if excess lead tetraacetate is used the only other products that can be isolated are the 6,19-oxide **4** (formed *via* intermediate **13**) and methoxy ketone **6**.

In the case of the intermediate **19** (R = R′ = R″ = H) rotation about the C–O bond at C-6 does not involve any serious steric interactions. Abstraction of more than one hydrogen atom with concomitant addition of iodine is therefore possible, leading to the formation of formate ester **9a** when 2 molar excess reagent was used

and to ketoaldehyde **11** when a larger excess of the reagent was used.

Experimental Section[28]

3β-Acetoxy-5α-methoxycholestan-6β-ol (2a).—3β-Acetoxy-5β,6β-oxidocholestane[29] (**1a**, 0.25 g) was dissolved in 9:1 methanol–acetic acid (30 ml) and the solution was stirred at 60° until no starting material remained (tlc). Usual work-up gave a crude product (0.24 g) which, upon crystallization from MeOH, gave a substance identified as 3β-acetoxy-5α-methoxycholestan-6β-ol, **2a**: mp 152.5–154°; $[\phi]_D$ −119° (*c* 0.6, $CHCl_3$); nmr ($CDCl_3$) δ 4.8 (m, 1, *w*/2 = 20 Hz, CHOAc), 3.9 (m, 1, *w*/2 = 9 Hz, CHOH), 3.2 (s, 3, OCH_3), 2.0 (s, 3, $OCOCH_3$).

Anal. Calcd for $C_{30}H_{52}O_4$: C, 75.58; H, 11.00. Found: C, 75.83; H, 10.84.

Oxidation of 3β-acetoxy-5α-methoxycholestan-6β-ol (**2a**, 0.15 g) with Jones reagent[30] led to the isolation of a crude product (0.12 g) after working up the reaction mixture in the usual manner. Crystallization of this material from methanol gave an analytical sample of a substance identified as **3β-acetoxy-5α-methoxycholestan-6-one, 6**: mp 149–150°; $[\phi]_D$ −206° (*c* 0.6, $CHCl_3$); ORD (dioxane) $[\phi]_{600}$ −280°, $[\phi]_{327}$ −5680°, $[\phi]_{315}$ −1860°, $[\phi]_{285}$ +2620°; nmr ($CDCl_3$) δ 4.8 (m, 1, *w*/2 = 25 Hz, CHOAc), 3.14 (s, 3, OCH_3), 2.0 (s, 3, $OCOCH_3$); ir ($CHCl_3$) 1725 (acetate C=O), 1700 cm^{-1} (C=O).

Anal. Calcd for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62. Found: C, 75.68; H, 10.55.

Lead Tetraacetate Oxidation of 3β-Acetoxy-5α-methoxycholestan-6β-ol (2a).—Lead tetraacetate (6.5 g), previously dried over P_2O_5, and anhydrous calcium carbonate (3.5 g) were added to benzene (150 ml) and the system was refluxed for 40 min by means of a 500-W lamp.[9] Freshly sublimed I_2 (2.0 g) and 3β-acetoxy-5α-methoxycholestan-6β-ol (**2a**, 0.50 g) were then added and the reaction mixture was refluxed for 1 additional hr. The insoluble white residue was removed by filtration over Celite and the filtrate was washed with an aqueous 30% $Na_2S_2O_3$ solution (200 ml). After working up in the usual manner, a crude product (0.48 g) consisting of three major components (tlc) was isolated.

Chromatography of this material over silica gel (500 g) and elution with benzene separated the first component (19 mg) which, after crystallization from methanol, was found to be identical in all respects with an authentic sample of **3β-acetoxy-5α-methoxycholestan-6-one, 6**.

The second substance (182 mg) to be eluted from the column was identified as **3β-acetoxy-5α,6α-methylenedioxycholestane, 3b**, on the basis of its spectral properties and of its subsequent chemical reactions. An analytical sample of this substance was obtained by crystallization from methanol: mp 150–152°; $[\phi]_D$ −137° (*c* 0.7, $CHCl_3$); nmr ($CDCl_3$) δ 5.1 (m, 1, CHOAc), 5.1 (s, 2, OCH_2O), 3.86 (t, 1, CHOC), 2.0 (s, 3, $OCOCH_3$), 1.0 (s, 3, CCH_3); nmr (benzene) δ 4.29, 4.19 (m, 2, OCH_2O).

Anal. Calcd for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62. Found: C, 75.78; H, 10.42.

Further elution with benzene led to the isolation of a third substance (136 mg) which could be purified by crystallization from methanol–acetone–water. It was identified as **3β-acetoxy-5α-methoxy-6β,19-oxidocholestane, 4**: mp 110.5–112°; $[\phi]_D$ +32° (*c* 0.9, $CHCl_3$); ir ($CHCl_3$) 1720 (acetate C=O), 1497 cm^{-1} (CH_2OC); nmr ($CDCl_3$) δ 4.8 (s, 1, *w*/2 = 26 Hz, CHOAc), 3.8 (m, 3, CH_2OC and CHOC), 3.27 (s, 3, OCH_3), 2.0 (s, 3, $OCOCH_3$).

Anal. Calcd for $C_{30}H_{50}O_4$: C, 75.90; H, 10.62. Found: C, 75.60; H, 10.62.

Hydrolysis and Oxidation of 3β-Acetoxy-5α,6α-methylenedioxycholestane (3b).—Treatment of **3b** (0.13 g) with a solution of potassium hydroxide (0.10 g) in 9:1 methanol–water (10.0 ml) for 12 hr at room temperature gave, after usual work-up, a crude product (0.10 g) which could be purified by crystallization from acetone. The pure substance was identified as **3β-hydroxy-5α,6α-methylenedioxycholestane, 3a**: mp 153–155°, $[\phi]_D$ −80.8°.

Sarett[31] oxidation of **3a** (25 mg) led to the isolation of a crude product which, on crystallization from methanol, afforded a pure sample of **5α,6α-methylenedioxycholestan-3-one, 8**: mp 167–169°; ir ($CHCl_3$) 1710 cm^{-1} (C=O); ORD (dioxane) $[\phi]_{600}$ −117°, $[\phi]_{350}$ +292°, $[\phi]_{308}$ +2250°, $[\phi]_{284}$ −3600°.

Acid Hydrolysis of 3β-Acetoxy-5α,6α-methylenedioxycholestane (3b).—Treatment of **3b** (180 mg) in a dry methanolic solution of HCl gave, on usual work-up, a crude product which afforded an analytical sample of **3β,5α,6α-trihydroxycholestane, 7a**, on crystallization from acetone: mp 236–237° (lit.[23] mp 236–238°).

Osmium tetroxide oxidation of 3β-acetoxycholest-5-ene,[23] **12**, and subsequent hydrolysis of the product gave an authentic sample of **3β,5α,6α-trihydroxycholestane, 7a**, which was identical in all respects with the substance obtained above by treatment of **3b** in a dry methanolic solution of HCl.

3β-Acetoxy-6β-methoxycholestan-5α-ol (5a).—Epoxidation of 3β-acetoxycholest-5-ene (**12**, 10.0 g) was effected by treatment of the latter in an ethereal solution containing an excess of monoperphthalic acid for 12 hr at room temperature. The reaction solution was then washed with an aqueous 20% solution of Na_2SO_3. Phthalic acid formed in the reaction was removed by filtering the dried ethereal solution through a column of alumina (500 g). The crude product (10.6 g) which was isolated was found (by tlc) to consist of two major components.

Part of the product (5.0 g) obtained by epoxidation of **12** was treated with distilled boron trifluoride etherate (5.0 ml) in dry MeOH (40 ml) for 4 hr at room temperature.[25] Usual work-up gave a crude product (4.56 g), part (2.5 g) of which was acetylated with acetic anhydride and pyridine for 18 hr at room temperature. Ice–water was added to the reaction solution and the solid material (2.4 g) which precipitated was collected by filtration. The crude product was found to consist of two major components (tlc) which were subsequently separated by chromatography on silica gel (600 g).

The first fraction obtained by elution with benzene consisted of an oil (0.60 g) which consisted mostly of **3β,6β-diacetoxy-5α-methoxycholestane, 2b**: nmr ($CDCl_3$) δ 5.0 (m, 1, *w*/2 = 6 Hz, 6α-H), 4.82 (m, 1, *w*/2 = 25 Hz, 3α-H), 3.38 (s, 3, OCH_3), 2.0 (s, 3, $OCOCH_3$), 1.95 (s, 3, $OCOCH_3$).

Further elution with benzene gave a solid (1.26 g) which was purified by crystallization from methanol and subsequently identified as 3β-acetoxy-6β-methoxycholestan-5α-ol, **5a**: mp 139–141° (lit. mp 139.5–140.5°,[32] 138–139°,[25] 124–125°[33]); nmr ($CDCl_3$) δ 5.1 (m, 1, 3α-H), 3.3. (s, 3, OCH_3), 2.99 (s, 1, *w*/2 = 5 Hz, 6α-H), 2.05 (s, 3, $OCOCH_3$).

Oxidation of 3β-Acetoxy-6β-methoxycholestan-5α-ol (5a) with Excess Lead Tetraacetate.—3β-Acetoxy-6β-methoxycholestan-5α-ol (**5a**, 0.50 g) was treated with lead tetraacetate (7.0 g) under conditions identical with those previously described for the oxidation of **2a**. The crude product isolated appeared to consist of at least two major components (tlc) and these were separated by chromatography over silica gel (300 g).

On elution with benzene an oil (148 mg) was isolated which was identified as **3β-acetoxy-5,6-secocholestan-5-on-6-al, 11**: ir ($CHCl_3$) 2715 (CHO), 1735 (acetate C=O), 1725 (aldehyde C=O), 1700 cm^{-1} (C=O), ORD (dioxane) $[\phi]_{600}$ +318°, $[\phi]_{316}$ +7600°, $[\phi]_{309}$ +5400°, $[\phi]_{305}$ +6000°, $[\phi]_{295}$ +1600°, $[\phi]_{265}$ +5500°; nmr ($CDCl_3$) δ 9.35 (m, 1, CHO), 5.35 (m, 1, 3α-H), 2 (s, 3, $OCOCH_3$).

A second substance was obtained as a solid (36 mg) on further elution with benzene. Crystallization from ethyl acetate gave

(28) Melting points were determined on a Hoover Uni-Melt apparatus and are uncorrected. Infrared and nmr spectra were recorded on a Beckman IR-8 infrared spectrophotometer and on a Varian V-4302 60 Mc spectrometer, respectively. A Durrum-Jasco automatic spectropolarimeter, Model ORD-5, and a Perkin-Elmer 141 recording polarimeter were used to determine optical rotatory dispersion curves and optical rotations, respectively. Microanalyses were performed in the Microanalytical Laboratory of Dr. A. Bernhardt, Max Planck Institute, West Germany. SilicaR (200–300 mesh) and neutral alumina (Woelm, activity I) were used as adsorbants for column chromatography. Silica gel G (according to Stahl) was used as adsorbant for thin layer chromatography and sulfuric acid was used as spraying agent. In working up the products of reactions the organic extracts were washed with dilute HCl solution and/or $NaHCO_3$ solution, dried over anhydrous $MgSO_4$, and evaporated to dryness under reduced pressure.

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an analytical sample of a substance identified as **3β-acetoxy-B-nor-6ζ-formylcholestan-5ζ-ol, 10**: mp 91–92°; ir ($CHCl_3$) 3600 (OH), 2720 (CHO), 1725 (acetate C=O), 1710 cm^{-1} (aldehyde C=O); ORD (dioxane) $[\phi]_{600}$ −74°, $[\phi]_{316}$ −277°, $[\phi]_{280}$ +166°; nmr ($CDCl_3$) δ 9.35 (m, 1, CHO), 5.1 (m, 1, 3α-H), 2.05 s, 3, $OCOCH_3$).

Anal. Calcd for $C_{29}H_{48}O_4$: C, 75.60; H, 10.50. Found: C, 75.34; H, 10.53.

Preparation of 11 by Ozonolysis of 3β-Acetoxycholest-5-ene (12).—Ozonized oxygen was passed through a saturated solution of 3β-acetoxycholest-5-ene (**12**, 5.0 g) in hexane (100 ml) for 24 hr.[25] The solvent was removed under vacuum and the product was washed with petroleum ether and dried under vacuum at room temperature.

The ozonide was reduced by shaking with zinc powder (7.5 g) in acetic acid (50 ml) for 60 hr at room temperature. The zinc was removed by filtration over Celite and ether (50 ml) was added to the filtrate which was washed repeatedly with an aqueous 60% solution of $NaHCO_3$. After usual work-up part (2.7 g) of the crude product was chromatographed over silica gel (300 g). Elution with benzene gave pure **3β-acetoxy-5,6-secocholestan-5-on-6-al (11**, 1.0 g, oil) which was identical in all respects with the substance produced in the lead tetraacetate oxidation of 5a.

Oxidation of 3β-Acetoxy-6β-methoxycholestan-5α-ol (5a) with 2 Mol of Lead Tetraacetate.—A solution of 3β-acetoxy-6β-methoxycholestan-5α-ol (**5a**, 0.50 g), lead tetraacetate (0.89 g, previously dried over P_2O_5), and iodine (1.0 g) in dry benzene (50 ml) was refluxed for 2.75 hr. The reaction mixture was cooled and water (0.5 ml) was added with rapid stirring, followed 15 min later by the addition of an aqueous 10% solution of sodium bisulfite (30 ml). Usual work-up gave a crude product (474 mg) which was chromatographed over silica gel (300 g).

Elution with benzene afforded a fraction (144 mg) of a solid substance which was crystallized from methanol and identified as **3β-acetoxycholestane-5α,6α-diol 6-formate, 9a**: mp 141.5–143°; nmr ($CDCl_3$) δ 8.05 (m, 1, OCHO), 5.05 (m, 2, 3α-H, 6β-H), 2.0 (s, 3, $OCOCH_3$), 1.03 (s, 3, CH_3 at C-10).

Anal. Calcd for $C_{30}H_{50}O_5$: C, 73.43; H, 10.27. Found: C, 73.73; H, 10.47.

A second substance (40 mg) was isolated on further elution with benzene. Crystallization from methanol gave an analytical sample identified as **acetyl 3β-acetoxy-5α-hydroxy-6α-cholestanyl formal, 9c**: mp 156.5–157.5°; nmr ($CDCl_3$) δ 5.31, 5.25, 5.20, 5.15 (m, 2, J_{AB} = 6 Hz, OCH_2O), 5.2 (m, 1, 3α-H), 3.5 (m, 1, $w/2$ = 20 Hz, 6β-H), 2.06 (s, 3, $OCOCH_3$), 2.0 (s, 3, $OCOCH_3$), 0.94 (s, 3, CH_3 at C-10); mass spectrum (70 eV) *m/e* (relative intensity) 456 (48), 444 (49), 426 (40), 414 (60), 396 (61), 384 (99), 368 (100), 360 (86).

Anal. Calcd for $C_{32}H_{54}O_6$: C, 71.87; H, 10.18. Found: C, 71.72; H, 10.13.

Hydrolysis of 9a to 3β,6α,6α-Trihydroxycholestane (7a).—3β-Acetoxycholestane-5a,6a-diol 6-formate (**9a**, 20 mg) was treated with a 0.5 *N* methanolic potassium hydroxide solution (5 ml) for 12 hr at room temperature. The product which was obtained by working up in the usual manner was identical in all respects with the 3β,5α,6α-trihydroxycholestane, **7a**, prepared by the osmium tetroxide oxidation of 3β-acetoxycholest-5-ene, **12**, and subsequent hydrolysis.

Registry No.—**2a**, 19317-73-8; **2b**, 2515-24-4; **3a**, 19289-39-5; **3b**, 19289-40-8; **4**, 19289-41-9; **5a**, 2515-20-0; **6**, 19289-48-6; **8**, 19289-49-7; **9a**, 19289-50-0; **9c**, 19289-51-1; **10**, 19289-52-2; **11**, 19289-53-3; lead tetraacetate, 546-67-8.

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Chemical Identification of the Trail-Following Pheromone for a Southern Subterranean Termite[1]

AKIRA TAI, F. MATSUMURA, AND H. C. COPPEL

Department of Entomology, University of Wisconsin, Madison, Wisconsin

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The synthetic procedures for the three isomers of a trail-following pheromone of a southern subterranean termite were described. Two of the isomers had identical spectroscopic properties as the natural pheromone. Only one of the isomers, *cis*-3,*cis*-6,*trans*-8-dodecatrien-1-ol, showed, however, an outstanding biological activity comparable to the natural product: less than 1 pg of the synthesized pheromone (like the natural pheromone) stimulated worker termites to follow artificially laid trails on ground-glass surfaces.

The presence of insect pheromones that chemically control the behavior of highly specialized social insect species has been well documented.[2] One such pheromone, "termite trail-following substance," is secreted by the sternal gland of various species of termite workers to mark the source of suitable wood to other workers of the same species.[3,4] The substance, when streaked across the surface of a solid object, creates a trail-following response in termite workers allowing them to follow the exact streak. Esenther, *et al.*,[5] discovered that woods decayed by the fungus *Lenzites trabea* Pers. ex Fr. also produced a substance attractive to the eastern subterranean termite, *Reticulitermes flavipes*. This substance was later found to work also as a "trail-following substance" against *R. flavipes* and a southern subterranean termite, *Reticulitermes virginicus*.[6] The active principle was purified and analyzed spectroscopically.[7] We now report the synthetic aspects of the pheromone leading to its identification.

Synthesis of Candidate Compounds and Bioassay.—As a result of various spectroscopic analyses of the purified termite pheromone,[7] two candidate compounds were considered to have spectroscopic properties identical with the natural product: *i.e.*, *cis*-3,*cis*-6,*trans*-

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