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]

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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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(2) M. Jacobson, "Natural Pest Control Agents," Advances in Chemistry Series, No. 53, American Chemical Society, Washington, D. C., 1966, p 17.

(3) A. M. Stuart, *Nature*, **189**, 419 (1961).

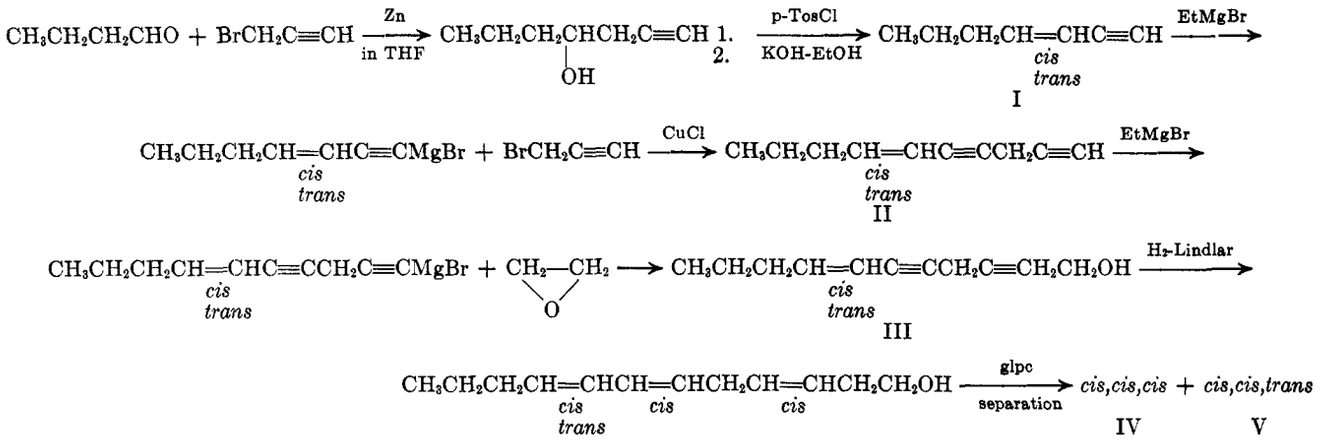
(4) R. V. Smythe and H. C. Coppel, *Ann. Entomol. Soc. Amer.*, **59**, 1008 (1966).

(5) G. R. Esenther, T. C. Allen, J. E Casida, and R. D. Shenefelt, *Science*, **134**, 50 (1961).

(6) R. V. Smythe, H. C. Coppel, S. H. Lipton, and F. M. Strong, *J. Econ. Entomol.*, **60**, 228 (1967).

(7) F. Matsumura, H. C. Coppel, and A. Tai, *Nature*, **219**, 963 (1968).

SCHEME I



8- and *cis*-3,*trans*-6,*cis*-8-dodecatrien-1-ol. The total synthesis of these two compounds and the comparison of their biological potencies appeared to be the only means to positively identify the chemical structure of the termite pheromone. The synthetic route to obtain the above compounds and *cis*-3,*cis*-6,*cis*-8-dodecatrien-1-ol are shown in Schemes I and II.

Altogether 15 mg of *cis,cis,cis* IV, 12 mg of *cis,cis, trans* V, and 35 mg of *cis,trans,cis* XI isomers of 3,6,8-dodecatrien-1-ol were obtained. They were dissolved in ether to make 1 mg/ml of stock solution from which samples of various dilution series were made. A 10-μl portion of the diluted sample solution was used for each bioassay.

The minimum amounts required to stimulate the worker termites to follow artificially laid 10-cm paths along a 120° arc on ground-glass surfaces were 100 pg for *cis,cis,cis*, 10 pg for *cis,trans,cis*, and 0.01 pg for *cis, cis,trans* as compared to the natural termite trail-following pheromone, 0.05 pg. The manner in which the worker termites followed the trails of compound V was identical with that with the natural product.

The result clearly indicated that *cis*-3,*cis*-6,*trans*-8-dodecatrien-1-ol was the termite trail-following pheromone.

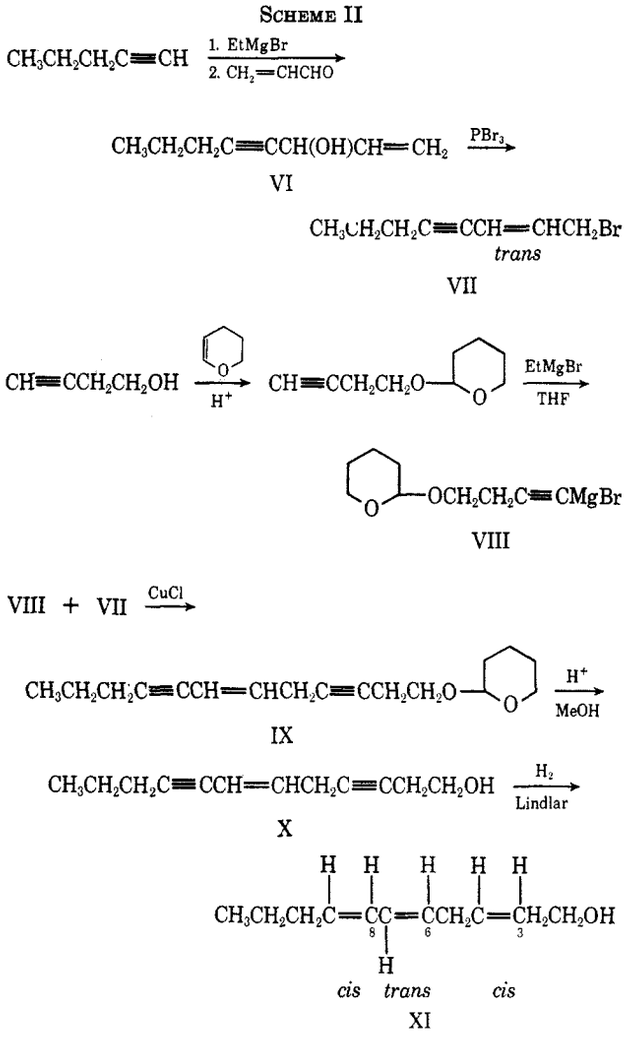
Experimental Section

The instrument used for the chromatographic analyses was a Beckman GC4 with a dual hydrogen-flame detector. A post-column (and predetector) splitter was used to divert nine-tenths of the sample to the *n*-pentane trap at −60°. In all cases, 0.25 in. by 6 ft stainless steel columns were used with the carrier (He) speed at 40 ml/min. One tenth of the sample was used for detecting the sample peaks.

***cis*-3,*cis*-6,*cis*(*trans*)-8-Dodecatrien-1-ol (Scheme I). 3-Hepten-1-yne (I).**—Thirty-five grams of propargyl bromide was treated with 26 g (0.36 mol) of *n*-butyraldehyde[8] (Reformatski reaction); 36 g of crude 1-heptyn-4-ol (*ca.* 0.3 mol) was obtained. It was directly converted to *p*-toluenesulfonyl ester by adding the above product into a solution of 54 g of *p*-toluenesulfonyl chloride in 68 ml of pyridine. The product was transferred into 300 ml of 10% aqueous NaOH solution at 0°. The ester was extracted with ether and the solvent was removed. It was refluxed in 250 ml of 18% ethanolic KOH for 8 hr. The product was extracted with 100 ml of Skelly B, washed with 10% aqueous sulfuric acid, distilled water, 5% of sodium bicarbonate, and distilled water, and dried over sodium sulfate. Removal of solvent and distillation of the residual oil gave 7 g (23.4%) of I, bp 48–55° (135 mm). The catalytic hydrogenation product (with PtO_2), heptane, was checked by a gas chromatographic system; ir (CS_2), 3300 (C≡CH) and 2100 (C≡C), 1600 (C=C), 952 (HC=CH *trans*) and 720 cm^{-1} (HC=CH *cis*). Gas chromatographic analysis indicated the ratio between *trans* and *cis* isomers was approximately 1:1.

(8) L. Crombie and A. G. Jacklin, *J. Chem. Soc.*, 1740 (1955).

SCHEME II



6-Decen-1,4-diyne (II).—A 6-g (0.064 mol) portion of 3-hepten-1-yne (*cis,trans* mixture) was added dropwise to a Grignard reagent, prepared by reacting 8 g of ethyl bromide and 1.8 g of magnesium in 30 ml of ether, and the system was refluxed until evolution of ethane gas ceased. Cuprous chloride (0.5 g) was added to the mixture and the system was stirred for additional 30 min. Propargyl bromide (8.8 g) with ether (26 ml) was added dropwise over a period of 4 hr and the system was refluxed for additional 24 hr.[9] The product in ether was washed

(9) W. J. Gensler, A. P. Mahadevan, Jr., and J. Casella, *J. Amer. Chem. Soc.*, **78**, 163 (1956).

with 15% sulfuric acid, water, 5% sodium bicarbonate, and water, and dried over sodium sulfate. Removal of solvent and distillation of residue gave 3.6 g (42.5%) of decene-1,4-diyne, bp 55–60° (10 mm). Gas chromatographic analyses showed two substances, *cis* and *trans* isomers, one with ir (CS_2) peak at 720 cm^{-1} and the other with 950 cm^{-1}. Both gave *n*-decane upon catalytic hydrogenation with PtO_2; uv, λ_{max} 226 mμ.

8-Dodecene-3,6-diyn-1-ol (III).—A 1.83-g (0.014 mol) portion of 6-decen-1,4-diyne (*cis,trans* mixture) was added to a Grignard reagent, prepared by reacting 1.69 g of ethyl bromide and 0.38 g of magnesium in 25 ml of ether, and the system was refluxed for 1 hr. After cooling the system to 0° ethylene oxide (0.7 g) in 2 ml of ether was added dropwise. Extraction, washing, and distillation steps were repeated as above to obtain 1.42 g (58%) of III: bp 125–135° (1 mm) (*Anal.* Calcd for $C_{12}H_{16}O$: C, 81.82; H, 9.09. Found C, 82.46; H, 10.53.); ir (CS_2) of gas chromatographic peak 1, 3400 (broad) for OH, 3000 for CH=CH, 2200 for C≡C, 1670 for CH=CH, 1040 for C–O, and 720 cm^{-1} for *cis* CH=CH; ir (CS_2) for the peak 2 closely resembled that of the peak 1 except for the absence of absorption at 720 cm^{-1} and the appearance of a new absorption peak at 945 cm^{-1} for *trans* CH=CH. Both compounds gave dodecanol upon catalytic hydrogenation.

3,6,8-Dodecatrien-1-ol (IV and V).—The above product (*i.e.*, the mixture of *cis*-8- and *trans*-dodecen-3,6-diyn-1-ol) was further purified on a Florisil column (1 × 30 m) with ether as a mobile phase. A 0.8-g (4.7 mmol) portion of the column purified product was dissolved in 30 ml of methanol containing 4 drops of quinoline. To hydrogenate selectively the triple bonds only, 0.2 g of Lindlar catalyst[10] was used under 1 atm of H_2 at room temperature. The product was extracted, washed, and dried over sodium sulfate, and the solvent was partially removed as before. The *cis* and *trans* isomers were separated and purified twice on gas chromatographic systems (SE52 and NGA columns). Only one-tenth of the final product was purified in this manner to yield 15 mg of IV and 12 mg of V (IV and V combined yield: 32.0%).

Both compounds were unstable in the concentrated state: they polymerized even under nitrogen at 0°. Elemental analysis was conducted upon their precursor, 8-dodecene-3,6-diyn-1-ol only.

Compound V showed the following spectral properties: ir (CS_2) 3600–3260 (OH), 3000 (=CH), 1055 (C–O), 925 and 980 (CH=CH, *trans–cis* conjugated), 728 cm^{-1} (CH=CH *cis*); uv (*n*-pentane) λ_{max} 234 mμ; nmr (CCl_4) δ 0.92 (t, 3 H, J = 6.5 Hz, CH_3CH_2), 1.25–1.51 (m, 2 H, CH_3CH_2), 1.99–2.32 (m, 5 H, $=CHCH_2CH_2$ and OH), 2.76–2.99 (m, 2 H, $=CHCH_2CH=$), 3.55 (t, 2 H, J = 6.5 Hz, CH_2CH_2OH), 5.0–6.1 (m, 6 H olefinic proton). Compound IV showed the following spectral properties: ir (CS_2) 3600–3260 (OH), 3000 (=CH), 1055 (C–O), 720 cm^{-1} (CH=CH *cis*, prominent); nmr same as V except 1.90–2.35 ($=CHCH_2CH_2$); uv (*n*-pentane) λ_{max} 235 mμ. Both isomers gave dodecan-1-ol by a catalytic hydrogenation with platinum oxide. V behaved in a manner identical with the natural product in all chromatographic tests.

***cis*-3,*trans*-6,*cis*-8-Dodecatrien-1-ol (Scheme II). 1-Octen-4-yn-3-ol (VI).**—To a Grignard reagent (36 g of EtBr and 8 g of Mg in 100 ml of ether) was added dropwise 20 g (0.3 mol) of 1-pentyne, and the mixture was refluxed for 1 hr. After cooling to 10°, 16.8 g of acrolein was added dropwise. The mixture was stirred for an additional 1 hr at 10°, and was then refluxed for 1 hr. After cooling, a 100-ml aliquot of ice–water was added to the reaction vessel and the reaction product was extracted three times with 50 ml each of ether. The solution was dried over sodium sulfate, freed of solvent, and distilled to give 20 g (54.9%) of VI: bp 79–81° (1 mm); ir (CS_2) 3350 (OH), 3010 (CH vinyl), 2225 (C≡C), 1640 (C=C), 920 cm^{-1} ($C=CH_2$).

1-Bromo-*trans*-2-octen-4-yne (VII).—To a cooled solution of 1-octen-4-yn-3-ol (16 g or 0.13 mol in 100 ml ether containing 2.4 g of dry pyridine) was added dropwise a solution of 11.8 g of phosphorus tribromide in 30 ml of dry ether over a 20-min period with continuous stirring. The reaction mixture was then gently heated to reflux for 20 min. Upon cooling, the product was poured over ice. The ether phase was washed successively with 100 ml each of water, 5% of aqueous sodium carbonate, and water and dried over sodium sulfate. Removal of the solvent and distillation of the residue gave 18 g (77.6%) of VII; bp 56–62° (1 mm); ir (CS_2) 2200 (C≡C), 1610 (C=C), 945 cm^{-1} (HC=CH, *trans*).

***trans*-6-Dodecene-3,8-diyn-1-ol (X).**—To a 100 ml of tetrahydrofuran solution with Grignard reagents (6.1 g of EtBr and 1.33 g of Mg) was added dropwise 8.5 g of 1-(tetrahydro-2-pyranoxy)-3-butyne, and the mixture was refluxed for 1 hr with stirring. Cuprous chloride (0.5 g) and then 9 g of VII was added to the reaction mixture at 60°, the latter over a period of 4 hr. The reaction continued overnight, and the product was purified as before to give 1-(tetrahydro-2-pyranoxy)-6-dodecene-3,8-diyne (IX). The product was dissolved in 100 ml of MeOH. Concentrated sulfuric acid (16 ml) was added to the solution, and the system was kept for 24 hr at room temperature. The reaction mixture was poured into 1 l. of 5% sulfuric acid, and the product was extracted three times with 100 ml each of Skellysolve B. Removal of the solvent and distillation of the residue gave 7.5 g (46.0%) of pearl yellow oil, *trans*-6-dodecene-3,8-diyn-1-ol (X), bp 120–125° (0.8 mm). Gas chromatography on NGA (170°) gave a single peak; ir (CS_2) 3300 (OH), 3005 (CH=CH), 2200 (C≡C), 1660 and 1610 (C=C), 1040 (C–O), 950 (CH=CH, *trans*). Catalytic hydrogenation with PtO_2 gave an identical peak as *n*-dodecanol by two glpc systems (SE52 and NGA).

***cis*-3,*trans*-6,*cis*-8-Dodecatrien-1-ol (XI).**—The above product (X) was further purified on a Florisil column (2.5 × 30 cm) with ether. A 1-g (5.9 mmol) portion of the purified material was dissolved in 30 ml of MeOH containing 4 drops of quinoline and was hydrogenated[10] with 0.2 g of Lindlar catalyst at room temperature. Glpc purification of one-twentieth of the reaction product gave 35 mg (66%) of pure *cis*-3,*trans*-6,*cis*-8-dodecatrien-1-ol (XI). Uv, nmr, and ir spectra were identical with those of *cis*-3,*cis*-6,*trans*-8-dodecatrien-1-ol (V). Catalytic hydrogenation gave a compound identical with dodecanol in two glpc systems.

Registry No.—I(*cis*), 764-57-8; I(*trans*), 764-58-9; II(*cis*), 19926-59-1; II(*trans*), 19926-60-4; III(*cis*), 19926-61-5; III(*trans*), 19926-62-6; IV, 19926-63-7; V, 19926-64-8; VI, 19926-65-9; VII, 19926-66-0; X, 19926-67-1.

(10) H. Lindlar, *Helv. Chim. Acta*, **35**, 446 (1952).