Keto Esters from the Tobacco Hornworm, *Manduca sexta*. Corroboration of Structure by Synthesis of 12-Oxooctacosanol and Its Acetate, Acetoacetate, and 3-Hydroxybutyrate

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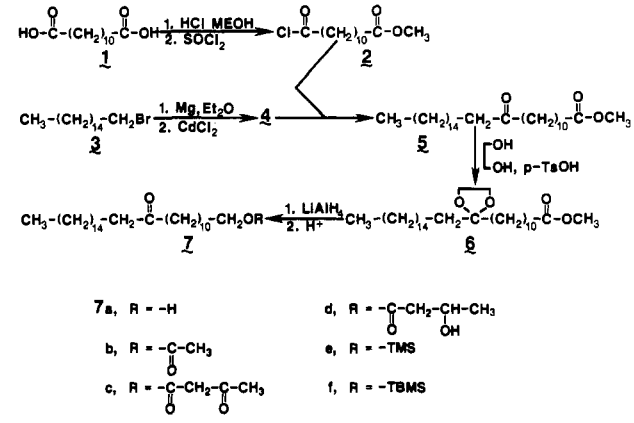
The structures of some keto esters that had been isolated from the surface waxes of the pupa of the tobacco hornworm, *Manduca sexta*, were confirmed by synthesis. The syntheses of the acetate, acetoacetate, and 3-hydroxybutyrate esters of 12-oxo-1-octacosanol are reported.

Insects synthesize and secrete complex mixtures of lipids some of which are composed of wax esters formed from long-chained alcohols and long-chained acids (Tulloch 1970, 1971). From the cochineal scale insect *Dactylopius confusus* and the Wooly Alder aphid *Prociphilus tesselatus* were isolated wax esters that yielded on hydrolysis some keto alcohols and keto esters whose structures were confirmed by synthesis and by spectroscopy (Meinwald et al., 1975). Buckner et al. (1984a,b) isolated and characterized short-chain acid esters of long-chain keto alcohols from the cuticular waxes of the tobacco hornworm, *Manduca sexta*. The major components in the ester fraction were tentatively identified as the acetate (**7b**), acetoacetate (**7c**), and 3-hydroxybutyrate (**7d**) of 12-oxo-1-octacosanol (**7a**). The specific functions of these compounds in insects are not known. It is speculated that they may protect the pupa that overwinters in the soil from desiccation and from attack by soil microorganisms (Buckner et al., 1984a,b). The natural products were characterized by their mass and infrared spectra as well as their trimethylsilyl (**7e**) and *tert*-butyldimethylsilyl (**7f**) derivatives of the hydrolysis product **7a**. Characterization of **7b** and **7c** from the insect by Buckner et al. (1984a,b) was based solely on the comparison of major mass spectral fragments and infrared spectra of the natural products to those of the model compound 12-oxo-1-octadecanol. We are reporting here the synthesis of some of these natural products and their derivatives to confirm these structural assignments.

An attempt at a one-step synthesis of **7a** using hexadecyl tosylate, 11-bromo-1-undecanol, and disodium tetracarbonylferrate (Colmann et al., 1972) did not yield the product. We speculate that the failure was due to poor solubility of the longer chain length reactants. Synthesis of the natural products by an alternative route is shown in Scheme I. Keto esters have been synthesized by the well-known condensation of the half-esters of dicarboxylic acid chlorides with dialkylcadmiums (Cason and Prout, 1955; Shirley, 1954; Cason, 1946). Compound **1** was converted to the half-ester acid chloride **2** (Cason, 1955; Zantour et al., 1972). Reaction of **2** with preformed dihexadecylcadmium (**4**) made from hexadecylmagnesium chloride and cadmium chloride yielded the keto ester **5**. The keto group was masked as the cycloethylene ketal **6**, and the ester group was subsequently reduced with lithium aluminum hydride. Hydrolysis of the blocking group yielded the keto alcohol **7a** in 40% overall yield from **1**.

Three natural derivatives of **7a**, the acetate (**7b**), acetoacetate (**7c**), and 3-hydroxybutyrate (**7d**), as well as two derivatives, **7e** and **7f**, were synthesized. The infrared and mass spectra of the synthetic compounds were identical with those of the natural products. We are currently attempting to resolve **7d** into the component enantiomers.

Scheme I



EXPERIMENTAL PART

Melting points are uncorrected and were determined with a Thomas-Hoover apparatus. Gas chromatography (GC) was performed on a Varian Model 3700 instrument fitted with a flame ionization detector (FID) and a 12-m methyl silicone coated fused silica capillary column with helium as carrier gas at 0.7 mm/min flow rate and an injector split ratio of 46:1. Infrared spectra were determined with a Perkin-Elmer Model 337. Gas chromatography–mass spectrometry (GC–MS) was performed on a modified Varian 3700 GC with the same capillary column and coupled to a Varian-MAT 112S MS in the electron impact mode and fitted with a jet separator. Data acquisition and storage were made with a SS200 data system connected to the MS.

Methyl 12-Oxooctacosanoate (5). The general method for synthesis of keto esters using dialkylcadmium compounds has been described (Cason and Prout, 1955; Shirley, 1954; Cason, 1946). Grignard reagent was formed from hexadecyl bromide (**3**; 13.51 g, 44.29 mmol in 50 mL of anhydrous Et_2O) and iodine-activated Mg (1.08 g, 44.25 mmol). The dialkylcadmium **4** was then formed by reaction of 4.6 g (24.34 mmol) of $CdCl_2$ with the Grignard mixture. Compound **4** was then reacted with the half-ester acid chloride **2** (9.8 g, 35.4 mmol). The reaction mixture was poured into 10 times its volume of ice and enough 20% H_2SO_4 to dissolve the metal salts, and when the ice had melted the mixture was extracted with benzene. The solvents were removed, and the solid product was chromatographed on alumina with the fractions eluted by hexane–EtOAc (4:1), yielding 11.3 g of **5**. Recrystallization of **5** from 95% EtOH yielded 8.9 g (44%) from **3**: mp 65–66 °C; IR (KBr pellet) 2940, 2910, 2840, 1775, 1745, 1450, 1405, 1205, 1185, 730, 720 cm^{-1}; MS, m/z (rel intens) M^+ for $C_{29}H_{56}O_3$ 452 (1), 421 (M – 31, 10), 379 (5), 268 (51), 253 (31), 242 (90), 227 (37), 210 (28), 185 (55), 184 (44), 153 (80), 152 (71), 135 (42), 125 (30), 112 (96), 98 (100), 85 (91).

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12-Oxo-1-octacosanol (7a). A solution of 5.0 g (10.73 mmol) of compound **5** and 2.5 mL of 1,2-ethanediol (44 mmol) in 25 mL of dry toluene was heated under reflux overnight in a Dean–Stark apparatus. The reaction mixture was extracted with benzene and the separated organic layer washed with H_2O, 5% $NaHCO_3$, and brine. After drying (Na_2SO_4), the solvents were removed in vacuo to yield 5.03 g (92%) of the cycloethylene ketal **6**.

Compound **6** (4.89 g, 9.6 mmol) in 20 mL of dry THF was added dropwise to a well-stirred solution of 1 g (24 mmol) of $LiAlH_4$ in 20 mL of dry THF so as to maintain gentle reflux. When addition was complete, the mixture was heated at reflux overnight. The reaction mixture was cooled and 5 mL of EtOAc added to consume the excess hydride after which water and dilute HCl was added to dissolve the Al salts and to hydrolyze the cycloethylene protective group. Extraction with Et_2O and recrystallization from MeOH yielded 4.0 g (97%) of **7a**: mp 91–92 °C; IR (KBr pellet) 3320, 2945, 2925, 2900, 2850, 1700, 1455, 1425, 1380, 1230, 1070, 730, 720 cm^{-1}; MS, m/z (rel intens) M^+ 424 (2), 406 (1), 281 (5), 268 (22), 253 (19), 214 (28), 208 (7), 181 (9), 163 (6), 138 (15), 125, 123 (8, 8), 111, 109 (14, 16), 97, 95 (37, 29), 96 (36), 83 (47), 71 (72), 69 (56), 57 (74), 55 (87), 43 (100); M_r, calcd for $C_{28}H_{56}O_2$ m/e 424.4280, found 424.4291.

12-Oxo-1-octacosanol Acetate (7b). A solution of 100 mg of **7a** in 5 mL each of C_5H_5N and Ac_2O was stirred at room temperature overnight. After the reaction mixture was poured on ice and then extracted with ether, the dried (Na_2SO_4) organic layer was evaporated to yield **7b**. The solid was recrystallized from acetone: mp 73–74 °C; IR (KBr pellet) 2950, 2925, 2900, 2850, 1745, 1710, 1455, 1445, 1360, 1263, 1243, 1060, 890, 715, 725 cm^{-1}; MS, m/z (rel intens) M^+ 466 (0.8), 424 (M – 42, 0.8), 423 (0.6), 407 (M – 59, 0.5), 281 (4), 269 (24), 268 (35), 256 (57), 253 (15), 241 (5), 214 (6), 213 (3), 209 (4), 208 (7), 206 (5), 199 (16), 181 (9), 138 (54), 111 (15), 109 (16), 97 (60), 96 (46), 95 (30), 83 (100), 71 (77), 69 (66); M_r, calcd for $C_{30}H_{58}O_3$ m/z 466.4385, found 466.4422.

12-Oxo-1-octacosanol Acetoacetate (7c). *Method A (Lawesson et al., 1973).* Into a 5-mL conical screw-cap reaction vial was placed 220 mg (0.58 mmol) of **7a**, the resultant mixture was heated to 120 °C in an oil bath to form a melt, and then anhydrous NaOAc (90 mg, 1.36 mmol) was added. The mixture was cooled to 80–90 °C, 0.2 mL (0.75 mmol) of diketene was added all at once, and the vial was stoppered and magnetically stirred at 92 °C for 4 h and finally at ambient temperature overnight. A brown precipitate formed. The reaction mixture was dissolved in 2-propanol, filtered, and allowed to crystallize. The compound was recrystallized from 2-propanol; 185 mg (70%); mp 71–72 °C dec.

Method B (Clemens and Hyatt, 1985). **7a** (100 mg, 0.236 mmol) and 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one (33.5 mg, 40 μL, 0.236 mmol) dissolved in 4 mL of xylenes were heated with vigorous stirring in an oil bath at 150 °C for 30 min and cooled to 20 °C. The solvents were removed in vacuo, and the solid was recrystallized from methanol: mp 71–72 °C; IR 2960, 3020, 2850, 1740, 1700, 1680, 1470, 1410, 1350, 1320, 1160, 715 cm^{-1}; MS, m/z (rel intens) M^+ 508 (3), 425 (3), 407 (1), 298 (33), 283 (8), 268 (27), 253 (20), 241 (25), 139, 138 (26, 30), 111 (22), 103 (100), 97 (70); chemical ionization MS (isobutane), m/z ($M^+ + 1$) 509 (80), 426 (100), 408 (40); M_r, calcd for $C_{32}H_{60}O_4$ m/z 508.4492, found 508.4484.

12-Oxooctacosanol 3-Hydroxybutyrate (7d). A mixture of 169 mL of benzene and 27 mL of ethylene glycol was refluxed for 5 h until no more water was removed. To this mixture was added a solution of 500 mg of **7a** (2.3 mmol) and 83 mg of *p*-TsOH in 50 mL of benzene, and reflux was continued until no more water could be removed (4 h). The solution was cooled, transferred to a separatory funnel, and washed with 3% bicarbonate, brine, and finally distilled water. The dried ($MgSO_4$) organic extract was evaporated to dryness to yield 400 mg of the cycloethylene ketal of **7a** (78%); mp 42–43 °C; IR (KBr pellet), no carbonyl, 3400, 2940, 2900, 2840, 1460, 1360, 1210, 1055, 950, 720 cm^{-1}.

The cycloethylene ketal of **7a** (300 mg, 0.6 mmol) and 120 μL of 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one were dissolved in 8 mL of xylenes, the resulting solution was heated in an oil bath at 150 °C with good stirring for 15 min, and then an additional 120 μL of the dioxinone was added and heating continued for 15 min. The solvents were removed in vacuo to yield a waxy solid, mp 41–43 °C.

The solid obtained from above was dissolved in 2-propanol, the mixture cooled in an ice bath, 400 mg of $NaBH_4$ added, and the solution allowed to slowly warm to room temperature overnight. The reaction mixture was poured onto 100 mL of ice water containing 5 mL of 6% H_2SO_4 and the mixture extracted with methylene chloride. The solvent was dried and removed in vacuo. The solid residue was chromatographed on silica gel. Compound **7d** was eluted from the column with $CHCl_3$. Compound **7d** was recrystallized from ethyl acetate: mp 86–88 °C; IR, identical with natural product (Buckner et al., 1984b) 3410, 2940, 2910, 2840, 1740, 1700, 1460, 1375, 1190, 1070 720 cm^{-1}; MS, calcd for $C_{32}H_{62}O_4$ m/z 510.4648, found 510.4602.

1-[(Trimethylsilyl)oxy]-12-oxooctacosane (7e). Into a 1-mL conical screw-cap vial were placed 5–10 mg of **7a**, 100 μL of DMF, and 100 μL of *N,O*-bis(trimethylsilyl)-acetamide. The vial was sealed and heated with stirring at 68 °C for 15 min and the derivative immediately analyzed by GC–MS: MS, m/z (rel intens) M^+ 496 (2), 481 (M – 15, 68), 352 (5), 340 (15), 286 (23), 271 (10), 253 (4), 229 (20), 143 (30), 130 (62), 103 (28), 97 (44), 83 (75), 75 (100).

1-[(*tert*-Butyldimethylsilyl)oxy]-12-oxooctacosane (7f). Into a 1-mL conical screw-cap vial were placed 5–10 mg of **7a**, 100 μL of DMF, and 100 μL of *N*-methyl-*N*-(*tert*-butyldimethylsilyl)trifluoroacetamide. The vial was sealed and heated with stirring at 68 °C for 15 min and the derivative analyzed by GC–MS: MS, m/z (rel intens) M^+ 538 (1), 523 (M – 15, 2), 481 (M – 57, 100), 313 (1), 282 (2), 271 (17), 253 (2), 213 (5) 75 (89).

Registry No. **2**, 4082-57-9; **3**, 112-82-3; **4**, 108344-61-2; **5**, 108344-62-3; **6**, 108344-63-4; **7a**, 91660-17-2; **7a** cycloethylene ketal, 108344-64-5; **7b**, 91660-00-3; **7c**, 91660-05-8; **7d**, 108344-65-6; **7e**, 108344-66-7; **7f**, 108344-67-8; diketene, 674-82-8; 2,2,6-trimethyl-4*H*-1,3-dioxin-4-one, 5394-63-8; 2-hexadecyl-2-[11-(1,3-dioxobutoxy)undecyl]dioxolane, 108344-68-9.

LITERATURE CITED

Buckner, J. S.; Nelson, D. R.; Pomonis, J. G.; Hakk, H. *J. Biol. Chem.* **1984a**, *259*, 8452–8460.

Buckner, J. S.; Nelson, D. R.; Fatland, C. L.; Hakk, H.; Pomonis, J. G. *J. Biol. Chem.* **1984b**, *259*, 8461–8470.

Cason, J. *Chem. Rev.* **1946**, *40*, 15–33.

Cason, J. In *Organic Syntheses*; Horning, E. C., Ed.; Wiley: New York, 1955; Collect. Vol. III, pp 169–171.

Cason, J.; Prout, F. S. In *Organic Syntheses*; Horning, E. C., Ed.; Wiley: New York, 1955; Collect. Vol. III, pp 601–605.

Clemens, R. J.; Hyatt, J. A. *J. Org. Chem.* **1985**, *50*, 2431–2435.

Colmann, J. P.; Winter, S. R.; Clark, D. R. *J. Am. Chem. Soc.* **1972**, *94*, 1788–1789.

Lawesson, S.-O.; Gronwall, S.; Sandberg, R. In *Organic Syntheses*; Baumgarten, H. E., Ed.; Wiley: New York, 1973; Collect. Vol. V, pp 155–157.

Meinwald, J.; Smolanoff, J.; Chibnall, A. C.; Eisner, T. *J. Chem. Ecol.* **1975**, *1*, 269–274.
Shirley, D. A. In *Organic Reactions*; Adams, R. A., Ed.; Wiley: New York, 1954; Vol. VIII, pp 28–58.
Tulloch, A. P. *Lipids* **1970**, *5*, 247–258.
Tulloch, A. P. *Chem. Phys. Lipids* **1971**, *6*, 235–265.
Zantour, H.; Pousse, A.; Brini, M. *Bull. Soc. Chem. Fr.* **1972**, *12*, 4715–4722.

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Structure–Bioactivity Relationships of Azadirachtin, a Potential Insect Control Agent

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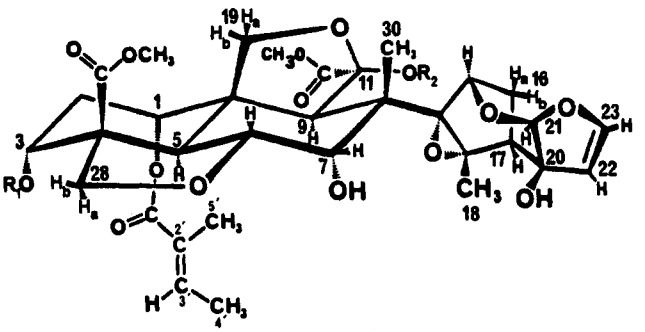
Eight derivatives of azadirachtin were prepared and bioassayed for their growth-inhibitory and lethal activities against the major agricultural insect pest *Heliothis virescens*. Neither deacetylation nor hydrogenation of the two carbon–carbon double bonds of azadirachtin had any significant effect on the bioactivities. Removal of the tigloyl group resulted in a moderately less active derivative. However, converting the relatively hydrophobic tigloyl group to the hydrophilic α,β-dihydroxy-α-methylbutyryl moiety caused a dramatic reduction in activity. The greatest losses of activity were observed when the hydroxyl groups were modified, either by carbomethoxylation or by O-methylation. The results suggest that the hydroxyl groups on azadirachtin are essential for activity and that, for maximum activity, the molecule must also have a lipophilic region (possibly for transport phenomena).

Azadirachtin ($C_{35}H_{44}O_{16}$) is a limonoid of the tetranortriterpenoid type found to occur thus far only in the neem (*Azadirachta indica*) and chinaberry (*Melia azedarach*) trees (Schmutterer et al., 1982; Schmutterer and Ascher, 1984). This compound has generated wide academic (Schmutterer et al., 1982; Schmutterer and Ascher, 1984; Kubo and Klocke, 1986) and industrial (Jacobson et al., 1984; Balandrin et al., 1985) interests because it is one of the most potent naturally occurring insect feeding deterrents known (Kubo and Klocke, 1982). Furthermore, azadirachtin causes metamorphic disorders in a wide variety of insects (Rembold and Sieber, 1981; Kubo and Klocke, 1982; Rembold, 1984; Kubo and Klocke, 1986) yet is nonmutagenic (Jacobson, 1982) and has no apparent mammalian toxicity (Nakanishi, 1975; Morgan, 1982). A molecular structure of azadirachtin (**1a**) has been proposed recently (Kraus et al., 1985; Klenk et al., 1986), which appears also to be supported by X-ray crystallographic data obtained from a chemically modified derivative of azadirachtin (Broughton et al., 1986).

Although the potential for azadirachtin as an insect control agent is well documented, little is known about its structure–bioactivity relationships with respect to the disruption of metamorphosis. Hydrogenation of the olefinic bond of the dihydrofuran ring moiety of azadirachtin has been reported (Rembold, 1984; Rembold et al., 1984) to have little or no effect on its metamorphosis-inhibiting activity against *Epilachna varivestis* and *Locusta migratoria*. The same derivative, as well as the deacetylated one, was fully active as an antifeedant against the desert locust (*Schistocerca gregaria*) (Morgan, 1982). Acetylation and/or trimethylsilylation of the hydroxyl groups of azadirachtin gave less active antifeedants against *S. gregaria* (Morgan, 1982). In this paper, we report on the preparation of eight derivatives of azadirachtin and their growth-inhibitory and lethal activities against the major agricultural insect pest *Heliothis virescens*.



	R_1	R_2
1a	CH_3CO	H
1b	H	H
1c	CH_3CO	CH_3CO
1d	CH_3CO	CH_3

MATERIALS AND METHODS

Materials. Solvents used for high-performance liquid chromatography (HPLC) were of HPLC grade. Other chemicals were of reagent grade or better and were used without further purification unless noted otherwise.

Bioassay. Compounds were examined for growth-inhibitory and lethal activities by an artificial diet "no choice" feeding bioassay (Kubo and Klocke, 1982, 1983). First-instar (colony reared) larvae of *H. virescens* (tobacco budworm) were used as the test organism. Growth inhibition was determined as the percentage difference in larval wet weight between treated and control insects. Mortality occurred either in the first-instar or in the

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