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

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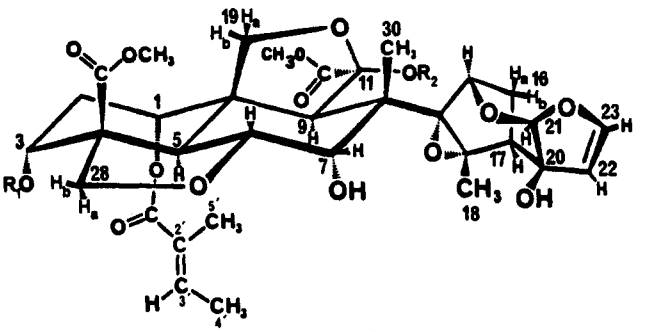
Eight derivatives of azadirachtin were prepared and bioassayed for their growth-inhibitory and lethal activities against the major agricultural insect pest *Heliothis virescens*. Neither deacetylation nor hydrogenation of the two carbon–carbon double bonds of azadirachtin had any significant effect on 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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pharate condition (i.e., during attempted ecdysis).

High-Performance Liquid Chromatography. Preparative HPLC was carried out with a Micromeritics Model 750 solvent delivery system equipped with a Negretti and Zambra injector, a Micromeritics Model 787 variable-wavelength UV/visible detector, a Hewlett-Packard 3388A integrator/recorder, and a Gilson Model 201 fraction collector. Chromatography was accomplished with either a normal-phase Alltech Associates silica gel (10-μm particle size) stainless-steel column (25 × 1.0 cm (i.d.)), protected with an Alltech Associates stainless-steel guard column (5.0 × 0.46 cm (i.d.)) packed with Alltech Associates pellicular silica gel, or a reversed-phase Regis Chemical Co. octadecylsilyl–silica gel (ODS) (10-μm particle size) stainless-steel column (25 × 1.0 cm (i.d.)), protected with an Alltech Associates stainless-steel guard column (5.0 × 0.46 cm (i.d.)) packed with Alltech Associates pellicular ODS. The solvent (vide infra) was eluted at a flow rate of 5.00 mL/min.

Analytical HPLC was performed as described elsewhere (Yamasaki et al., 1986). All prepared derivatives of azadirachtin were purified to >98% purity.

Spectroscopy. Infrared (IR) spectra of samples prepared as 2% (w/w) KBr pellets were recorded on a Perkin-Elmer Model 710B infrared spectrophotometer. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on a 200-MHz IBM AC200 spectrometer equipped with a Cryomagnetic Systems Inc. magnet. All chemical shifts are expressed as parts per million (δ) downfield from tetramethylsilane internal standard. Only the chemical shifts that differ from those of the starting compound are listed. Fast atom bombardment mass spectra (FAB-MS) were run in the positive-ion mode in glycerol and recorded on a Varian MAT 112S mass spectrometer using an SS100C computer.

Azadirachtin (1a). **1a** was isolated and purified from neem (*A. indica*) seeds by the method of Yamasaki et al. (1986). All ^{1}H NMR assignments of **1a** have been taken from the literature (Kraus et al., 1985; Klenk et al., 1986): FAB-MS, m/z 721 (MH^+); IR ν_{max} (cm^{-1}) 3450 (br), 1735 (s), 1710 (sh), 1655 (w), 1620 (w), 740 (m); ^{1}H NMR ($CDCl_3$) δ 1.31 (br d, J 12.9 Hz, 1 H, H-16$_b$), 1.71 (br d, J 12.9 Hz, 1 H, H-16$_a$), 1.75 (s, 3 H, 30-Me), 1.78 (br d, J 7.1 Hz, 3 H, 4′-Me), 1.85 (br s, 3 H, 5′-Me), 1.95 (s, 3 H, 3-Ac), 2.01 (s, 3 H, 18-Me), 2.14 (ddd, J 16.9, 3.6, 2.6 Hz, 1 H, H-2$_b$), 2.33 (ddd, J 16.9, 2.6, 2.6 Hz, 1 H, H-2$_a$), 2.38 (br d, J 5.0 Hz, 1 H, H-17), 2.92 (br s, D_2O-exchangeable, 1 H, 7-OH), 2.99 (br s, D_2O-exchangeable, 1 H, 20-OH), 3.33 (s, 1 H, H-9), 3.37 (d, J 12.5 Hz, 1 H, H-5), 3.63 (d, J 10.2 Hz, 1 H, H-19$_b$), 3.69 (s, 3 H, MeOOC), 3.77 (d, J 9.0 Hz, 1 H, H-28$_a$), 3.80 (s, 3 H, MeOOC), 4.07 (d, J 9.0 Hz, 1 H, H-28$_b$), 4.15 (d, J 10.2 Hz, 1 H, H-19$_a$), 4.60 (dd, J 12.5, 2.7 Hz, 1 H, H-6), 4.68 (br d, J 3.4 Hz, 1 H, H-15), 4.73 (unresolved d, 1 H, H-7), 4.75 (unresolved dd, 1 H, H-1), 5.03 (s, D_2O-exchangeable, 1 H, 11-OH), 5.05 (d, J 2.9 Hz, 1 H, H-22), 5.51 (unresolved dd, 1 H, H-3), 5.65 (s, 1 H, H-21), 6.46 (d, J 2.9 Hz, 1 H, H-23), 6.93 (br q, J 7.1 Hz, 1 H, H-3′).

3-Deacetylazadirachtin (1b). **1a** (30 mg, 0.042 mmol) was stirred with sodium methoxide (2.9 mg, 0.055 mmol) in 1.0 mL of dry methanol at 25 °C for 150 min. The reaction mixture was then poured into 10 mL of saturated aqueous sodium bicarbonate and extracted three times with 10-mL portions of dichloromethane. The dichloromethane phases were combined, washed once with 10 mL of saturated aqueous sodium chloride, and rotary evaporated in vacuo. Purification of the crude product by silica gel preparative HPLC (solvent 2-propanol–*n*-hexane, 12:88, v/v), followed by ODS preparative HPLC (solvent methanol–water, 1:1, v/v) gave **1b**: 15 mg; FAB-MS, m/z 679 (MH^+); IR ν_{max} (cm^{-1}) 3460 (br), 1730 (s), 1710 (sh), 1655 (w), 1620 (w), 740 (m); ^{1}H NMR ($CDCl_3$) δ 2.20 (m, 2 H, H-2$_{ab}$), 2.2 (obscured, D_2O-exchangeable, 1 H, 3-OH), 3.24 (d, J 11.7 Hz, 1 H, H-5), 4.07 (d, J 8.8 Hz, 1 H, H-28$_a$), 4.26 (d, J 8.8 Hz, 1 H, H-28$_b$), 4.36 (d of unresolved dd, J 9.0 Hz, changes to unresolved dd in D_2O-exchange experiment, 1 H, H-3), 4.85 (unresolved dd, 1 H, H-1).

11-*O*-Acetylazadirachtin (1c). **1c** was prepared by a modification of the method of Butterworth et al. (1972). **1a** (30 mg, 0.042 mmol) was dissolved in 1.0 mL of acetic anhydride and the resultant mixture heated at reflux under nitrogen for 15 min. The mixture was dried in vacuo, and the residue, taken up in 15 mL of dichloromethane, was washed once with 10 mL of saturated aqueous sodium bicarbonate. The aqueous phase was back-extracted twice with 10-mL portions of dichloromethane. The dichloromethane layers were combined, washed once with 10 mL of saturated aqueous sodium chloride, and rotary evaporated in vacuo. The crude product was purified by silica gel preparative HPLC (solvent 2-propanol–*n*-hexane, 17:83, v/v) to afford **1c**: 15 mg; FAB-MS, m/z 763 (MH^+); IR ν_{max} (cm^{-1}) 3460 (br), 1745 (s), 1710 (sh), 1655 (w), 1620 (w), 740 (m); ^{1}H NMR ($CDCl_3$) δ 1.89 (s, 3 H, 18-Me), 2.06 (s, 3 H, 11-Ac), 3.19 (s, 1 H, H-9), 3.80 (d, J 10.2 Hz, 1 H, H-19$_b$), 4.28 (d, J 10.2 Hz, 1 H, H-19$_a$), 5.19 (unresolved dd, 1 H, H-1).

11-*O*-Methylazadirachtin (1d). **1a** (30 mg, 0.042 mmol) in 0.3 mL of iodomethane was stirred vigorously with 50 mg (0.21 mmol) of silver(I) oxide under nitrogen in the dark at 37 °C for 24 h. The reaction mixture was then filtered and rotary evaporated in vacuo. Analytical HPLC showed that the reaction had gone to only 60% completion. Therefore, the residue was redissolved in 0.3 mL of iodomethane and the resultant mixture stirred with 50 mg of fresh silver(I) oxide under the same conditions for 21 h. The crude product (after filtration and evaporation) was purified by silica gel preparative HPLC (solvent 2-propanol–*n*-hexane, 1:9, v/v), followed by ODS preparative HPLC (solvent methanol–water, 1:1, v/v) to yield **1d**: 15 mg; FAB-MS, m/z 735 (MH^+); IR ν_{max} (cm^{-1}) 3480 (br), 1740 (s), 1710 (sh), 1650 (w), 1620 (w), 740 (m); ^{1}H NMR ($CDCl_3$) δ 1.59 (s, 3 H, 30-Me), 1.89 (s, 3 H, 18-Me), 2.28 (br d, J 4.9 Hz, 1 H, H-17), 3.33 (s, 3 H, 11-MeO), 3.47 (s, 1 H, H-9), 4.57 (br s, 1 H, H-7).

22,23-Dihydroazadirachtin (2a). **2a** was prepared by a modification of the method of Butterworth et al. (1972). In a typical preparation, **1a** (40 mg, 0.056 mmol) in 0.5 mL of ethyl acetate was stirred with 13 mg of platinum(IV) oxide at 20 °C under hydrogen (5 atm) for 3 h. The reaction mixture was then filtered through glass wool and rotary evaporated in vacuo. The residue was chromatographed by ODS preparative HPLC (solvent methanol–water, 1:1, v/v) to afford **2a**: 33 mg; FAB-MS, m/z 723 (MH^+); IR ν_{max} (cm^{-1}) 3460 (br), 1740 (s), 1710 (sh), 1650 (w), 740 (m); ^{1}H NMR ($CDCl_3$) δ 1.53 (br d, J 12.9 Hz, 1 H, H-16$_b$), 2.1 (m, 2 H, H-22$_{ab}$), 2.47 (br d, J 4.9 Hz, 1 H, H-17), 3.95 (m, 2 H, H-23$_{ab}$), 5.27 (s, 1 H, H-21).

2′,3′,22,23-Tetrahydroazadirachtin (2b). **1a** (30 mg, 0.042 mmol) in 0.5 mL of ethanol was stirred with 20 mg of 5% palladium on alumina at 20 °C under hydrogen (10 atm) for 3 h. The reaction mixture was then filtered and rotary evaporated in vacuo. The crude product was purified by silica gel preparative HPLC (solvent 2-propanol–*n*-hexane, 17:83, v/v), followed by ODS preparative HPLC (solvent methanol–water, 1:1, v/v) to give **2b**: 18 mg; FAB-MS, m/z 725 (MH^+); IR ν_{max} (cm^{-1}) 3450 (br),

1735 (s); ^{1}H NMR ($CDCl_3$) δ 0.93 (t, J 7.4 Hz, 2.25 H, 4′-Me), 0.95 (t, J 7.4 Hz, 0.75 H, 4′-Me), 1.16 (d, J 6.8 Hz, 2.25 H, 5′-Me), 1.17 (d, J 7.1 Hz, 0.75 H, 5′-Me), 1.45 (m, 2 H, H-3′$_{ab}$), 1.58 (br d, J 12.0 Hz, 1 H, H-16$_b$), 1.78 (m, 1 H, H-2′), 2.06 (s, 3 H, 3-Ac), 2.10 (m, 2 H, H-22$_{ab}$), 2.22 (s, 3 H, 18-Me), 2.53 (br d, J 5.0 Hz, 1 H, H-17), 3.07 (d, J 12.5 Hz, 1 H, H-5), 3.61 (d, J 8.9 Hz, 1 H, H-28$_a$), 3.8–4.1 (m, 2 H, H-23$_{ab}$), 5.24 (s, 1 H, H-21).

1-Detigloyl-22,23-dihydroazadirachtin (2c). **2a** (25 mg, 0.035 mmol) and osmium tetroxide (8.8 mg, 0.035 mmol) in 1.0 mL of 50% aqueous acetonitrile were stirred together at 20 °C for 30 min. Sodium periodate (52 mg, 0.25 mmol) was added, and, after being stirred for an additional 3 h, the reaction mixture was diluted with 10 mL of water and extracted three times with 10-mL portions of dichloromethane. The dichloromethane extract was dried in vacuo, and the residue, taken up in 2.0 mL of methanol, was dissolved in 10 mL of saturated aqueous sodium bicarbonate. After 150 min at 20 °C, the mixture was extracted three times with 15-mL portions of dichloromethane. The dichloromethane layers were combined, washed once with 10 mL of saturated aqueous sodium chloride, and dried in vacuo. The residue was chromatographed twice by ODS preparative HPLC (solvent methanol–water, 1:1, v/v) to give **2c**: 4.8 mg; FAB-MS, m/z 641 (MH$^+$); IR ν_{max} (cm^{-1}) 3455 (br), 1730 (s); ^{1}H NMR ($CDCl_3$) δ 1.62 (m, 1 H, H-16$_b$), 2.1 (m, 2 H, H-22$_{ab}$), 2.11 (s, 3 H, 3-Ac), 2.12 (s, 3 H, 18-Me), 2.48 (br d, J 4.8 Hz, 1 H, H-17), 2.57 (s, D_2O-exchangeable, 1 H, 1-OH), 3.14 (d, J 12.4 Hz, 1 H, H-5), 3.45 (d, J 9.4 Hz, 1 H, H-19$_a$), 3.7–4.1 (m, 2 H, H-23$_{ab}$), 3.87 (d, J 8.9 Hz, 1 H, H-28$_a$), 3.45 (obscured dd, 1 H, H-1), 5.20 (s, 1 H, H-21).

2′,3′-Dihydroxy-2′,3′,22,23-tetrahydroazadirachtin (2d). **2a** (30 mg, 0.042 mmol) and osmium tetroxide (10.5 mg, 0.042 mmol) in 1.5 mL of 50% aqueous acetonitrile were stirred together at 25 °C for 30 min. Sodium periodate (10.8 mg, 0.050 mmol) was added to the darkened solution in portions over a 10-min period. After being stirred for another 230 min, the reaction mixture was poured into 10 mL of saturated aqueous sodium bicarbonate, stirred for 30 min, and extracted three times with 15-mL portions of dichloromethane. The dichloromethane phases were combined, washed once with 15 mL of saturated aqueous sodium chloride, and rotary evaporated in vacuo. The residue was chromatographed twice by ODS preparative HPLC (solvent methanol–water, 1:1, v/v) to give **2d**: 16 mg; FAB-MS, m/z 757 (MH$^+$); IR ν_{max} (cm^{-1}) 3460 (br), 1735 (s); ^{1}H NMR ($CDCl_3$) δ 1.19 (d, J 6.3 Hz, 3 H, 4′-Me), 1.35 (s, 3 H, 5′-Me), 1.49 (br d, J 13.0 Hz, 1 H, H-16$_b$), 2.10 (s, 3 H, 3-Ac), 2.1 (m, 2 H, H-22$_{ab}$), 2.15 (s, 3 H, 18-Me), 2.52 (br d, J 5.0 Hz, 1 H, H-17), 2.55 (br s, D_2O-exchangeable, 1 H, 3′-OH), 3.11 (d, J 12.4 Hz, 1 H, H-5), 3.17 (br s, D_2O-exchangeable, 1 H, 2′-OH), 3.60 (d, J 9.0 Hz, 1 H, H-28$_a$), 3.9–4.1 (m, 3 H, H-3′ and H-23$_{ab}$), 5.29 (s, 1 H, H-21).

11,20-*O*,*O*-Dicarbomethoxy-22,23-dihydroazadirachtin (2e). **2a** (20 mg, 0.028 mmol) was dissolved in 0.20 mL of dimethyl pyrocarbonate and the resultant mixture heated at 75 °C under nitrogen for 30 min. The reaction mixture was chromatographed by silica gel preparative HPLC (solvent 2-propanol–n-hexane, 15:85, v/v) to yield **2e**: 18 mg; FAB-MS, m/z 839 (MH$^+$); IR ν_{max} (cm^{-1}) 3460 (br), 1770 (sh), 1745 (s), 1710 (sh), 1655 (w), 800 (m), 740 (m); ^{1}H NMR ($CDCl_3$) δ 1.69 (s, 3 H, 18-Me), 2.34 (s, D_2O-exchangeable, 1 H, 7-OH), 2.4 (m, 2 H, H-22$_{ab}$), 3.24 (s, 1 H, H-9), 3.34 (br d, J 5.9 Hz, 1 H, H-17), 3.74 (s, 3 H, MeOCOO), 3.78 (s, 3 H, MeOCOO), 4.1 (m, 2 H, H-23$_{ab}$), 4.57 (unresolved d, 1 H, H-7), 5.18 (unresolved dd, 1 H, H-1), 5.80 (s, 1 H, H-21).

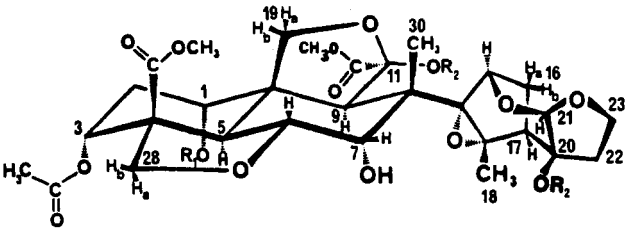
RESULTS AND DISCUSSION

Eight derivatives of azadirachtin (**1a**) were prepared, and their structures were confirmed by FAB-MS, IR, and ^{1}H NMR. Transesterification of **1a** with sodium methoxide gave 3-deacetylazadirachtin (**1b**); the acetate ester was much more reactive than the tiglate ester. ^{1}H NMR showed a loss of the acetyl protons (δ 1.95) and the appearance of a new D_2O-exchangeable signal at δ 2.2.

Heating a solution of **1a** in acetic anhydride at reflux gave only a monoacetylated product as reported previously (Butterworth et al., 1972; Zanno et al., 1975; Nakanishi, 1975). ^{1}H NMR showed a new singlet at δ 2.06, concomitant with the disappearance of the 11-hydroxyl proton signal at δ 5.03 and a shift of the 18-methyl singlet from δ 2.01 to 1.89. The structure of the monoacetylated derivative most consistent with the data is 11-*O*-acetylazadirachtin (**1c**).

Treatment of **1a** with iodomethane and silver(I) oxide, as described above, gave only a monomethylated product. The absorption at 3480 cm^{-1} in the IR spectrum was reduced by approximately 30%. ^{1}H NMR showed a loss of the 11-hydroxyl proton signal at δ 5.03 and the appearance of a new singlet at δ 3.33 (consistent with a methoxyl group). The monomethylated derivative appears to be 11-*O*-methylazadirachtin (**1d**) since the protons at positions 7, 9, 18, and 30 displayed significant changes in their chemical shifts (δ 4.73, 3.33 , 2.01, and 1.75 to δ 4.57, 3.47, 1.89, and 1.59, respectively), whereas the protons at positions 5 and 21 showed little or no change.

22,23-Dihydroazadirachtin (**2a**) was made easily by hydrogenation of **1a** over platinum(IV) oxide at 5 atm pressure. The IR absorption of **1a** at 1620 cm^{-1}, due to



	R_1	R_2
2a	HC=C(CH$_3$)CO	H
2b	CH$_3$CH$_2$CH(CH$_3$)CO	H
2c	H	H
2d	HO OH HC—C(CH$_3$)CO	H
2e	HC=C(CH$_3$)CO	CH$_3$OCO

the carbon–carbon double bond of the dihydrofuran ring moiety (Butterworth et al., 1972), disappeared in **2a**, and the ^{1}H NMR signals of the vinylic protons at positions 22 (δ 5.05) and 23 (δ 6.46) were replaced by unresolved multiplets at δ 2.1 and 3.95, respectively. As reported by

Butterworth et al. (1972), **1a** resisted hydrogenation at atmospheric pressure. In our experience, hydrogenation of **1a** with platinum(IV) oxide did not give 2′,3′,22,23-tetrahydroazadirachtin (**2b**).

2b was made by hydrogenation of **1a** over palladium catalyst, but only at elevated pressures was this reaction successful. The IR absorptions at 1710, 1655, and 740 cm^{-1}, due to the tigloyl group (Butterworth et al., 1972), disappeared along with the absorption at 1620 cm^{-1} (dihydrofuran ring moiety). In addition to the changes in the ^1H NMR signals seen with **2a**, the 3′-vinyl (δ 6.93), 5′-methyl (δ 1.85), and 4′-methyl (δ 1.78) signals were replaced by a multiplet (δ 1.45), two overlapping doublets (δ 1.16 and 1.17, 3:1 ratio), and two overlapping triplets (δ 0.93 and 0.95, 3:1 ratio), respectively. A new multiplet at δ 1.78 was also seen for the proton at the 2′-position. The two sets of doublet and triplet signals observed for the 5′- and 4′-protons (respectively) most likely reflect the fact that a new chiral center at position 2′ had been created (Silverstein et al., 1981).

The tigloyl group was removed by oxidizing **2a** with osmium tetroxide and excess sodium periodate and then hydrolyzing the resulting pyruvate ester with mild aqueous alkali. 1-Detigloyl-22,23-dihydroazadirachtin (**2c**) lacked the IR bands of the tigloyl group and the dihydrofuran ring moiety at 1710, 1650, 1620, and 740 cm^{-1}. All of the ^1H NMR signals of the tigloyl group (i.e., δ 1.78, 1.85, and 6.93) had vanished, and a new D_2O-exchangeable singlet (δ 2.57) appeared. When this reaction sequence was repeated using an amount of sodium periodate approximately equimolar with **2a**, 2′,3′-dihydroxy-2′,3′,22,23-tetrahydroazadirachtin (**2d**) was obtained in about 50% yield. The IR absorption at 3460 cm^{-1} was noticeably greater, and the bands at 1710, 1650, 1620, and 740 cm^{-1} were gone. The ^1H NMR spectrum of **2d** showed two new D_2O-exchangeable signals (δ 2.55 and 3.17) and no vinylic signal at δ 6.93.

Heating **2a** with dimethyl pyrocarbonate cleanly gave a dicarbomethoxylated product, unlike the acetylation reaction in which only one hydroxyl group was esterified. A shoulder was seen at 1770 cm^{-1} in the IR spectrum, consistent with the presence of dialkyl carbonates (Nakanishi and Solomon, 1977), and the ^1H NMR spectrum showed only one D_2O-exchangeable proton (δ 2.34) remaining. Two new singlets (δ 3.74 and 3.78) were also observed in the region expected for carbomethoxy protons. The protons at positions 9, 17, 18, 21, and 22 in **2a** displayed significant changes in their chemical shifts (δ 3.31, 2.47, 2.02, 5.27, and 2.1 to δ 3.24, 3.34, 1.69, 5.80, and 2.4, respectively) in **2e**, whereas the proton at position 5 remained relatively unchanged. 11,20-*O*,*O*-Dicarbomethoxy-22,23-dihydroazadirachtin (**2e**) is the structure most consistent with the data.

The growth-inhibitory and lethal activities of azadirachtin against first-instar *H. virescens* are compared with those activities of eight derivatives (Table I). Similar to the results of Morgan (1982) with *S. gregaria*, neither deacetylation at position 3 nor hydrogenation of the carbon–carbon double bond of the dihydrofuran ring moiety of **1a** had any significant effect on the activity against *H. virescens* (EC_{50} values of 0.07–0.09 ppm; LC_{50} values of 0.37–0.80 ppm). The latter result was particularly significant since the double bond in the dihydrofuran ring can interfere with other reactions (e.g., those involving the tiglate moiety).

Also similar to the structure–activity results of Morgan (1982) was the finding that acetylation of the hydroxyl group at position 11 resulted in a slight decrease in the activity of the natural product (EC_{50} = 0.18 ppm compared to 0.07 ppm; LC_{50} = 0.95 ppm compared to 0.80 ppm). More effective in the reduction of activity at this position was O-methylation (EC_{50} = 5.10 ppm; LC_{50} = 30.0 ppm). Other derivatives made at the hydroxyl groups at positions 11 and 20 also resulted in diminished activity. For example, trimethylsilylation (Morgan, 1982) or carbomethoxylation (EC_{50} = 29.0 ppm; LC_{50} = 90.0 ppm) of these hydroxyl groups resulted in decreased activity. Abolishment of activity was not found in any of these derivatives. However, we have not ruled out the possibility that the insect enzymatically (with *O*-demethylase and carboxylesterase) hydrolyzed these derivatives back to the natural product. Efforts are under way to make and bioassay derivatives that have been dehydrated at one or both of the C-11 and C-20 positions.

Table I. Mortality and Growth Inhibition in First-Instar *H. virescens* Larvae Fed Azadirachtin and Eight Derivatives in an Artificial Diet[a]

test compound	LC_{50},[b] ppm	EC_{50},[c] ppm
azadirachtin (**1a**)	0.80 (0.46–1.39)[d]	0.07 (0.05–0.10)
3-deacetylazadirachtin (**1b**)	0.37 (0.17–0.80)	0.09 (0.05–0.17)
22,23-dihydroazadirachtin (**2a**)	0.47 (0.32–0.67)	0.08 (0.04–0.15)
11-*O*-acetylazadirachtin (**1c**)	0.95 (0.59–1.54)	0.18 (0.08–0.40)
11-*O*-methylazadirachtin (**1d**)	30.0 (21.7–41.5)	5.10 (2.80–9.40)
11,20-*O*,*O*-dicarbomethoxy-22,23-dihydroazadirachtin (**2e**)	90.0 (37.8–214.5)	29.0 (20.3–41.5)
1-detigloyl-22,23-dihydroazadirachtin (**2c**)	2.40 (1.15–5.02)	0.59 (0.30–1.14)
2′,3′,22,23-tetrahydroazadirachtin (**2b**)	0.30 (0.11–0.80)	0.08 (0.05–0.13)
2′,3′-dihydroxy-2′,3′,22,23-tetrahydroazadirachtin (**2d**)	42.0 (32.3–54.6)	3.80 (1.95–7.41)

[a] Similar preliminary results were obtained against the corn earworm (*Heliothis zea*) and the fall armyworm (*Spodoptera frugiperda*). [b] LC_{50} values are the concentrations (ppm) causing 50% mortality. [c] EC_{50} values are the concentrations (ppm) causing 50% growth inhibition. [d] Numbers in parentheses are the 95% confidence limits determined using the method of Litchfield and Wilcoxon (1949).

Removal of the tigloyl group from **2a** resulted in a moderately reduced (EC_{50} = 0.59 ppm; LC_{50} = 2.40 ppm) activity (Table I). The possibility that the tiglate moiety contributes to the activity of azadirachtin by acting as an alkylating center via Michael-type addition was ruled out with the discovery that the tetrahydro derivative (EC_{50} = 0.08 ppm; LC_{50} = 0.30 ppm) was at least as active as the natural product. The activity of the tetrahydro derivative was decreased dramatically by forming the polar 2′,3′-dihydroxy derivative (EC_{50} = 3.8 ppm; LC_{50} = 42.0 ppm). We suspect that the tiglate group, or a more lipophilic substituent, is necessary at the C-1 position for transport of the molecule upon ingestion by the insect. We, therefore, plan to bioassay derivatives that have been esterified with alkyl chains of varying lengths at position 1 of **2c**.

Our data seem to indicate that, for azadirachtin, requirements for maximum activity include a lipophilic side of the molecule (possibly for transport phenomena) and a hydrophilic side containing hydroxyl groups [possibly for binding to hypothetical binding proteins (Rembold et al., 1984)]. Not necessary for activity are the two carbon–carbon double bonds and the acetyl group. The former result is particularly significant since the tetrahydro derivative of azadirachtin is fully active and more stable (results to be published elsewhere) than the natural product. While none of our derivatives enhanced the potent insecticidal activity of azadirachtin, the data provide useful information for both formulation and synthetic purposes.

We are continuing our investigations of the structure–bioactivity relationships of azadirachtin as a potential insect control agent. Ultimately, these investigations should

prove useful in the development of azadirachtin as a model compound for the chemical synthesis of a new class of insecticides.

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Feasibility Study of Constant Energy Synchronous Luminescence Spectrometry for Pesticide Determination: Application to Carbaryl, Naphthol, and Carbofuran

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Constant energy synchronous luminescence spectrometry (CESLS) at low temperature (77 K) is applied to pesticide determination. This method is inexpensive, selective, and sensitive. Limits of detection, linear dynamic ranges, and results obtained using a variety of scan parameters are given for carbaryl, naphthol, and carbofuran.

Carbamate pesticides are widely used to protect plants from insects (Haskell, 1985). Due to the toxicity of these compounds and the possibility of residual presence in the environment and crops, there is an obvious need for a sensitive and reliable method for determining them. A method for distinguishing pesticides from their hydrolysis products and/or metabolites could also have a wide variety of applications for studies involving optimizing application concentrations and times, as well as metabolism of pesticides by insects, animals, and humans.

Pesticide determination has been done by a wide variety of methods ranging from liquid chromatography followed

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