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Structure–Bioactivity Relationships of Salannin as an Antifeedant against the Colorado Potato Beetle (*Leptinotarsa decemlineata*)

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Fourteen derivatives of salannin were prepared and bioassayed for antifeedant activity against larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*). Several of the derivatives, including 3-*O*-methyl-3-deacetyl-2′,3′,20,21,22,23-hexahydrosalannin and 3-deacetoxy-2′,3′,20,21,22,23-hexahydrosalannin, were over 40-fold more active than salannin as Colorado potato beetle antifeedants. Changes in the antifeedant activity were observed with chemical modification at four points of the salannin molecule: (1) hydrogenation of the furan ring, (2) replacement of the acetoxyl group, (3) modification of the tigloyl group, and (4) saponification of the methyl ester.

Salannin ($C_{34}H_{44}O_9$) is a limonoid of the tetranortriterpenoid type found to occur in at least four species of plants in the Meliaceae, including *Azadirachta indica* A. Juss. (neem) (Henderson et al., 1964, 1968), *Melia azedarach* L. (chinaberry) (Srivastava, 1986), *Melia dubia* Cav. (de Silva et al., 1969), and *Melia volkensii* Gurke (Rajab et al., 1988). The biological effects of salannin include insect antifeedant or feeding deterrency activity against *Musca domestica* L. (house fly) (Warthen et al., 1978), *Acalymma vittatum* F. (striped cucumber beetle), Diabrotica *undecimpunctata howardi* Barber (spotted cucumber beetle) (Reed et al., 1982), *Spodoptera littoralis* Boisd. (Egyptian cotton leafworm), *Earias insulana* Boisd. (spiny bollworm) (Meisner et al., 1981), *Aonidiella aurantii* Maskell (California red scale), and *Locusta* sp. (locust) (Warthen, 1979).

Although the insect antifeedant activity of salannin is well documented, little is known about its structure–bioactivity relationships. In this paper, we report on the preparation of 14 derivatives of salannin and their antifeedant activity against the agricultural pest insect *Leptinotarsa decemlineata* (Say) (Colorado potato beetle).

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.

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Bioassay. Compounds were examined for antifeedant activity by leaf disk choice bioassays. Third-instar (colony reared) larvae of *L. decemlineata* were used as the test organism.

Leaves of uniform size and thickness were taken from potato plants (Russet Burbank variety) grown in a greenhouse. Disks (1 cm^2) were punched out from the leaves, randomized, and arranged (6 disks/cup) in a circle sandwiched between moistened filter paper (Gelman) inside plastic cups (52 × 36 mm). Only the upper surfaces of the disks were exposed. The upper surfaces of alternating disks were treated with either 25 μL of acetone (control) or between 1 and 400 μg of a test substance dissolved in 25 μL of acetone. Newly molted third-instar *L. decemlineata*, reared from hatching on Russet Burbank leaves and weighing between 35 and 40 mg, were placed 1 larva/cup at ca. 27 °C in continuous light. The disks were examined visually every 2 h until >95% of the control disks were eaten. The PC_{95} value, the minimal protective concentration (micrograms/disk) at which >95% of the control disks, while <5% of the treated disks, were eaten in the choice bioassay, was determined for each test substance from 15–20 replicates/concentration. The PC_{50} value, the minimal protective concentration (micrograms/disk) at which >50% of the control disks, while <5% of the treated disks, were eaten in the choice bioassay, was determined for certain test substances from 15–20 replicates/concentration.

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

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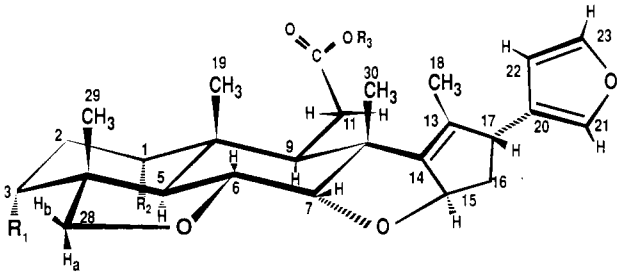
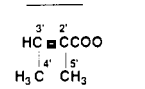
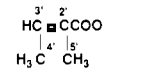
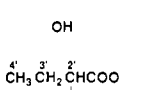
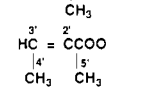
a reversed-phase Regis Chemical octadecylsilyl silica gel (ODS) (10-μm particle size) stainless steel column (25 × 1.0 cm (i.d.)), protected with an Alltech Associates stainless steel guard column (5.0 × 0.46 cm (i.d.)) packed with Alltech Associates pellicular ODS. The solvent (vide infra) was eluted at a flow rate of 5.0 mL/min.

Analytical HPLC was performed as described elsewhere (Yamasaki et al., 1988). All prepared derivatives of salannin were purified to >99% 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 magnet. All chemical shifts are expressed as parts per million (δ) downfield from a tetramethylsilane internal standard. Only the chemical shifts that differ by more than ±0.05 ppm from those of the starting compound are listed. All J coupling constants are expressed in hertz. Electron impact mass spectra (EI-MS) were recorded at 70 eV on a Varian MAT 112S mass spectrometer using an SS100C computer.

Salannin (1a). **1a** was isolated and purified from neem (*A. indica*) seeds by the method of Yamasaki et al. (1988). The spectral assignments of **1a** have been reported elsewhere (Henderson et al., 1968; Kubo et al., 1986).



	R_1	R_2	R_3
1a	CH_3COO		CH_3
1b	OH		CH_3
1c	OH	OH	H
1d	OH	OH	CH_3
1e	CH_3COO		CH_3
1f	CH_3O		CH_3
1g	CH_3O	OH	CH_3
1h	CH_3O	CH_3O	CH_3
1i	CH_3COO	CH_3COO	CH_3

3-Deacetylsalannin (1b). **1b** was isolated and purified from *A. indica* seeds as described by Yamasaki et al. (1988). The spectral assignments of **1b** have been reported elsewhere (Henderson et al., 1964, 1968; Kraus and Cramer, 1981; Kubo et al., 1986).

Salannic Acid (1c). **1c** was prepared by a modification of the method of de Silva et al. (1969). **1a** (30 mg, 0.050 mmol) was dissolved in 2.0 mL of 0.5 M sodium hydroxide in 50% aqueous methanol and the resultant mixture incubated at 70 °C for 3 h. The reaction mixture was poured into 10 mL of 0.5 N hydrochloric acid and extracted three times with 10-mL portions of dichloromethane. The organic layers were combined, washed once with 10 mL of water, followed by 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–acetic acid, 10:90:1, v/v/v) yielded **1c** (20 mg): IR ν_{max} (cm^{-1}) 3400 (v br, includes COOH), 1700 (s), 1500 (w), 870 (m); ^{1}H NMR ($CDCl_3$) δ 1.12 (br s, $J_{29,28a} < 1.0$, 3 H, 29-Me), 1.75 (d, $J_{18,15} = 1.5$, 3 H, 18-Me), ca. 2.0–2.3 (br s, D_2O exchangeable, 1 H, OH), ca. 2.5–2.6 (m, 1 H, H-9), 2.67 (d, $J_{5,6} = 12.5$, 1 H, H-5), ca. 3.4–4.2 (br, D_2O exchangeable, 2 H, OH, COOH), 3.67 (dd, $J_{1,2\alpha} = J_{1,2\beta} = 3.0$, 1 H, H-1), 3.91 (dd, $J_{3,2\alpha} = J_{3,2\beta} = 3.0$, 1 H, H-3), 4.06 (br d, $J_{28a,28b} = 6.8$, $J_{28a,29} < 1.0$, 1 H, H-28$_a$), 4.26 (d, $J_{7,6} = 3.4$, 1 H, H-7), 6.18–6.21 (m, 1 H, H-22), 7.15–7.19 (m, 1 H, H-23); EI-MS, m/z 458 (M$^{\cdot+}$, 100%), 443 (M – CH_3, 7), 440 (M – H_2O, 8), 385 (21), 283 (51), 230 (48), 173 (40), 147 (49), 91 (48), 69 (44), 55 (43).

1-Detigloyl-3-deacetylsalannin (1d). **1d** was prepared by methylating the carboxylic acid group of **1c** via a modification of the method of Mehta (1972). To a solution of **1c** (75 mg, 0.16 mmol) in 1.5 mL of dimethyl sulfoxide were added 0.5 mL of 1.0 M aqueous sodium bicarbonate and 0.3 mL of iodomethane. The reaction mixture was stirred at 25 °C for 15 h under nitrogen, poured into 20 mL of saturated aqueous sodium thiosulfate, and extracted three times with 20-mL portions of dichloromethane. The organic layers were combined, washed once with 20 mL of water, followed by 20 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, 2:23, v/v) yielded **1d** (61 mg) as white rosette crystals. The spectral assignments of **1d** have been reported elsewhere (Henderson et al., 1964, 1968).

2′,3′-Dihydrosalannin (1e). **1a** (40 mg, 0.066 mmol) in 0.75 mL of ethanol was stirred with 20 mg of 5% palladium on alumina at 25 °C under hydrogen (5 atm) for 10 min. The reaction mixture was then filtered and evaporated in vacuo. The residue was chromatographed by silica gel preparative HPLC (solvent 2-propanol–n-hexane, 2:23, v/v) to afford **1e** (27 mg): IR ν_{max} (cm^{-1}) 1735 (s), 1500 (w), 870 (m); ^{1}H NMR ($CDCl_3$) δ 0.95 (t, $J_{4',3'} = 7.4$, 1 H, 4′-Me), 0.96 (t, $J_{4',3'} = 7.4$, 2 H, 4′-Me), 1.23 (d, $J_{5',2'} = 6.5$, 2 H, 5′-Me), 1.30 (d, $J_{5',2'} = 6.9$, 1 H, 5′-Me), ca. 1.4–2.0 (m, 2 H, H-3′$_{a,b}$), 2.07 (s, 3 H, 3-Ac), ca. 2.3–2.6 (m, 1 H, H-2′), 2.68 (dd, $J_{9,11b} = 8.3$, $J_{9,11a} = 2.7$, 1 H, H-9), 2.73 (d, $J_{5,6} = 12.5$, 1 H, H-5), 3.60 (apparent br s, 2 H, H-28$_{a,b}$), 4.87 (dd, $J_{3,2\alpha} = J_{3,2\beta} = 2.9$, 1 H, H-3); EI-MS, m/z 598 (M$^{\cdot+}$, 100%), 583 (M – CH_3, 7), 567 (M – OCH_3, 2), 513 (M – $COCH(CH_3)CH_2CH_3$, 4), 504 (10), 496 (M – $HOCOCH(CH_3)CH_2CH_3$, 5), 421 (17), 283 (30), 259 (23), 235 (17), 173 (14), 147 (13), 85 ($CH_3CH_2CH(CH_3)CO^+$, 14), 57 ($CH_3CH_2CH(CH_3)^+$, 46).

3-*O*-Methyl-3-deacetylsalannin (1f). **1f** was prepared by methylating the hydroxyl group of **1b** via a modification of the method of Johnstone and Rose (1979). To a solution of **1b** (100 mg, 0.18 mmol) and iodomethane (0.2 mL) in 0.5 mL of dimethyl sulfoxide was added, with vigorous stirring, 0.1 g of finely powdered potassium hydroxide. The suspension was stirred vigorously at 25 °C for 40 min, poured into 10 mL of saturated aqueous sodium bicarbonate, and extracted three times with 10-mL portions of dichloromethane. The organic layers were combined, washed successively with 10-mL portions of saturated aqueous sodium thiosulfate, water, and saturated aqueous sodium chloride, and rotary evaporated in vacuo. Chromatography of the residue by silica gel preparative HPLC (solvent 2-propanol–n-hexane, 1:49, v/v), followed by ODS preparative HPLC (solvent methanol–water, 7:3, v/v) afforded **1f** (39 mg): IR ν_{max} (cm^{-1}) 1735 (s), 1705 (s), 1650 (w), 1500 (w), 870 (m), 735 (m); ^{1}H NMR ($CDCl_3$) δ 1.90 (ddd, $J_{2\alpha,2\beta} = 16.4$, $J_{2\alpha,1} = J_{2\alpha,3} = 3.8$, 1 H, H-2$_\alpha$), 2.36 (ddd, $J_{2\beta,2\alpha} = 16.5$, $J_{2\beta,1} = J_{2\beta,3} = 2.6$, 1 H, H-2$_\beta$), 2.70 (dd, $J_{9,11b} = 8.5$, $J_{9,11a} = 4.3$, 1 H, H-9), 2.89 (d, $J_{5,6} = 12.7$, 1 H, H-5), 3.13 (s, 3 H, 3-MeO), 3.32 (dd, $J_{3,2\alpha} = J_{3,2\beta} = 2.5$, 1 H, H-3), 3.56 (d, $J_{28b,28a} = 7.1$, 1 H, H-28$_b$), 4.76 (dd, $J_{1,2\alpha} = J_{1,2\beta} = 2.7$, 1 H, H-1); EI-MS, m/z 568 (M$^{\cdot+}$, 100%), 553 (M – CH_3, 7), 485 (M – $COC(CH_3)$=$CHCH_3$, 11), 468 (M – $HOCOC(CH_3)$=$CHCH_3$, 11), 421 (11), 397 (15), 283 (55), 267 (15), 259 (19), 230 (13), 173 (12), 147 (11), 83 (CH_3CH=$C(CH_3)CO^+$, 62), 55 (CH_3CH=$C(CH_3)^+$, 49).

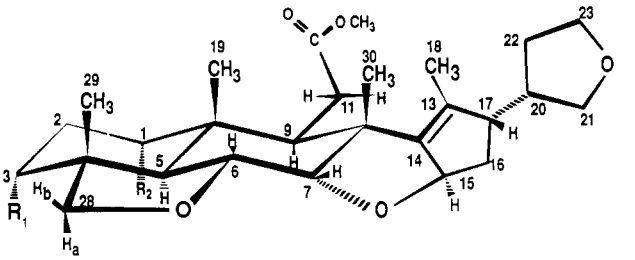
1-Detigloyl-3-*O*-methyl-3-deacetylsalannin (1g). **1f** (50 mg, 0.086 mmol) was dissolved in 2.0 mL of 0.5 M sodium hydroxide in 50% aqueous methanol and stirred at 70 °C for 42 h. The reaction mixture was poured into 10 mL of 0.5 N hydrochloric acid and extracted three times with 10-mL portions of dichloromethane. The organic layers were combined, washed once with 10 mL of water, followed by 10 mL of saturated aqueous sodium chloride, and rotary evaporated in vacuo. The residue was shown by IR to be the desired 3-*O*-methylsalannic acid. The

residue, dissolved in 1.0 mL of dimethyl sulfoxide, was added to a mixture of 0.25 mL of 1.0 M aqueous sodium bicarbonate and 0.2 mL of iodomethane and stirred under nitrogen at 25 °C for 20 h. The reaction mixture was poured into 10 mL of saturated aqueous sodium thiosulfate and extracted three times with 10-mL portions of dichloromethane. The organic layers were combined, washed once with 10 mL of water, followed by 10 mL of saturated aqueous sodium chloride, and rotary evaporated in vacuo. Chromatography of the residue by silica gel preparative HPLC (solvent 2-propanol–*n*-hexane, 3:47, v/v) yielded **1g** (38 mg) as white rosette crystals: IR ν_{max} (cm^{-1}) 3490 (sharp), 1740 (s), 1500 (w), 870 (m); ^1H NMR ($CDCl_3$) δ 1.99 (ddd, $J_{2\alpha,2\beta}$ = 15.7, $J_{2\alpha,1}$ = $J_{2\alpha,3}$ = 2.8, 1 H, H-2$_\alpha$), 2.18 (ddd, $J_{2\beta,2\alpha}$ = 14.8, $J_{2\beta,1}$ = $J_{2\beta,3}$ = 3.0, 1 H, H-2$_\beta$), 2.26 (dd, $J_{11a,11b}$ = 14.2, $J_{11a,9}$ = 5.9, 1 H, H-11$_a$), 2.38 (dd, $J_{11b,11a}$ = 14.2, $J_{11b,9}$ = 6.3, 1 H, H-11$_b$), 2.58 (d, $J_{5,6}$ = 12.6, 1 H, H-5), 2.79 (dd, $J_{9,11a}$ = $J_{9,11b}$ = 6.1, 1 H, H-9), 3.37 (s, 3 H, 3-MeO), 3.41 (dd, $J_{3,2\alpha}$ = $J_{3,2\beta}$ = 2.7, 1 H, H-3), 3.48 (s, 3 H, MeOOC), 3.51 (ddd, $J_{1,OH}$ = 9.6, $J_{1,2\alpha}$ = $J_{1,2\beta}$ = 2.9, changes to dd in D_2O-exchange experiment, $J_{1,2\alpha}$ = $J_{1,2\beta}$ = 2.9, 1 H, H-1), 3.66 (d, D_2O exchangeable, $J_{OH,1}$ = 9.6, 1 H, 1-OH), 3.96 (br d, $J_{28a,28b}$ = 7.0, $J_{28a,29}$ < 1.0, 1 H, H-28$_a$), 5.52 (ddq, $J_{15,16a}$ = $J_{15,16b}$ = 7.0, $J_{15,18}$ = 1.8, 1 H, H-15), 6.34–6.38 (m, 1 H, H-22); EI-MS, *m/z* 486 ($M^{\cdot+}$, 100%), 471 (M – CH_3, 4), 283 (11), 259 (6), 230 (5), 202 (9), 173 (7), 147 (7), 59 (11), 55 (9).

1,3-*O*,*O*-Dimethyl-1-detigloyl-3-deacetylsalannin (1h). To a solution of **1d** (60 mg, 0.13 mmol) and iodomethane (0.2 mL) in 0.5 mL of dimethyl sulfoxide was added, with vigorous stirring, 0.1 g of finely powdered potassium hydroxide. The suspension was stirred vigorously at 25 °C for 60 min, poured into 10 mL of saturated aqueous sodium bicarbonate, and extracted three times with 10-mL portions of dichloromethane. The organic layers were combined, washed successively with 10-mL portions of saturated aqueous sodium thiosulfate, water, and saturated aqueous sodium chloride, and rotary evaporated in vacuo. Purification of the crude product by silica gel preparative HPLC (solvent 2-propanol–*n*-hexane, 1:24, v/v) yielded **1h** (40 mg): IR ν_{max} (cm^{-1}) 1735 (s), 1500 (w), 870 (m); ^1H NMR ($CDCl_3$) δ 1.73 (ddd, $J_{2\alpha,2\beta}$ = 16.4, $J_{2\alpha,1}$ = $J_{2\alpha,3}$ = 3.1, 1 H, H-2$_\alpha$), 2.36 (ddd, $J_{2\beta,2\alpha}$ = 16.2, $J_{2\beta,1}$ = $J_{2\beta,3}$ = 2.6, 1 H, H-2$_\beta$), 2.64 (d, $J_{5,6}$ = 12.7, 1 H, H-5), 2.88 (dd, $J_{9,11a}$ = $J_{9,11b}$ = 6.0, 1 H, H-9), 3.02 (dd, $J_{1,2\alpha}$ = $J_{1,2\beta}$ = 2.6, 1 H, H-1), 3.27 (dd, $J_{3,2\alpha}$ = $J_{3,2\beta}$ = 2.6, 1 H, H-3), 3.29 (s, 3 H, 1-MeO), 3.32 (s, 3 H, 3-MeO), 3.43 (s, 3 H, MeOOC), 4.01 (br d, $J_{28a,28b}$ = 7.5, $J_{28a,29}$ < 1.0, 1 H, H-28$_a$), 4.16 (d, $J_{7,6}$ = 3.3, 1 H, H-7), 5.56 (ddq, $J_{15,16a}$ = $J_{15,16b}$ = 7.0, $J_{15,18}$ = 1.5, 1 H, H-15), 6.31–6.34 (m, 1 H, H-22), 7.24–7.28 (m, 1 H, H-23); EI-MS, *m/z* 500 ($M^{\cdot+}$, 100%), 485 (M – CH_3, 7), 421 (7), 406 (13), 397 (16), 283 (63), 259 (20), 101 (21), 85 (26), 55 (20).

1-*O*-Acetyl-1-detigloylsalannin (1i). The diacetate, **1i**, was prepared by a modification of the method of Henderson et al. (1968). **1d** (96 mg, 0.21 mmol) and acetic anhydride (195 μL, 2.1 mmol) were dissolved together in 0.8 mL of dry pyridine, and the resultant mixture was heated at 70 °C under nitrogen for 20 h. The reaction mixture was poured into 20 mL of 0.5 N hydrochloric acid and extracted three times with 20-mL portions of dichloromethane. The organic layers were combined, washed once with 20 mL of saturated aqueous sodium bicarbonate, followed by 20 mL of saturated aqueous sodium chloride, and rotary evaporated in vacuo. Chromatography of the residue by silica gel preparative HPLC (solvent 2-propanol–*n*-hexane, 1:24, v/v), followed by ODS preparative HPLC (solvent methanol–water, 7:3, v/v), gave **1i** (70 mg) as white needles: IR ν_{max} (cm^{-1}) 1735 (s), 1500 (w), 870 (m); ^1H NMR ($CDCl_3$) δ 1.20 (br s, $J_{29,28a}$ < 1.0, 3 H, 29-Me), 1.65 (d, $J_{18,15}$ = 1.4, 3 H, 18-Me), 2.07 (s, 3 H, Ac), 2.14 (s, 3 H, Ac), 2.73 (dd, $J_{9,11b}$ = 8.8, $J_{9,11a}$ = 3.3, 1 H, H-9), 3.31 (s, 3 H, MeOOC), 3.71 (br d, $J_{28a,28b}$ = 7.6, $J_{28a,29}$ < 1.0, 1 H, H-28$_a$), 4.17 (d, $J_{7,6}$ = 3.3, 1 H, H-7), 4.79 (dd, $J_{3,2\alpha}$ = $J_{3,2\beta}$ = 2.9, 1 H, H-3), 4.94 (dd, $J_{1,2\alpha}$ = $J_{1,2\beta}$ = 2.9, 1 H, H-1), 6.32–6.35 (m, 1 H, H-22), 7.26–7.30 (m, 1 H, H-23); EI-MS, *m/z* 556 ($M^{\cdot+}$, 100%), 541 (M – CH_3, 5), 421 (13), 283 (4), 259 (6), 235 (5), 100 (7).

2′,3′,20,21,22,23-Hexahydrosalannin (2a). **1a** (40 mg, 0.066 mmol) in 0.5 mL of ethanol was stirred with 30 mg of 5% palladium on alumina at 24 °C under hydrogen (10 atm) for 6 h. The reaction mixture was then filtered and rotary evaporated in vacuo. The residue was chromatographed by silica gel preparative HPLC (solvent 2-propanol–*n*-hexane, 1:9, v/v) to give **2a** (30 mg): IR ν_{max} (cm^{-1}) 1735 (s); ^1H NMR ($CDCl_3$) δ 0.94 (t, $J_{4',3'}$ = 7.1, 1 H, 4′-Me), 0.95 (t, $J_{4',3'}$ = 7.1, 2 H, 4′-Me), 1.19 (d, $J_{5',2'}$ = 6.9, 2 H, 5′-Me), 1.27 (d, $J_{5',2'}$ = 7.8, 1 H, 5′-Me), ca. 1.4–2.0 (m, 3 H, H-3′$_{a,b}$, H-20), 1.67 (d, $J_{18,15}$ = 1.2, 2 H, 18-Me), 1.74 (d, $J_{18,15}$ = 1.5, 1 H, 18-Me), 1.80–2.35 (m, 8 H, H-2$_{\alpha,\beta}$, H-11$_{a,b}$, H-16$_{a,b}$, H-22$_{a,b}$), 2.07 (s, 3 H, 3-Ac), 2.30–2.65 (m, 2 H, H-17, H-2′), 2.60 (dd, $J_{9,11b}$ = 9.5, $J_{9,11a}$ = 3.3, 1 H, H-9), 2.68 (d, $J_{5,6}$ = 12.6, 1 H, H-5), 3.32 (dd, $J_{21a,20}$ = $J_{21a,21b}$ = 8.5, 0.33 H, H-21$_a$), 3.38 (dd, $J_{21a,20}$ = $J_{21a,21b}$ = 8.2, 0.67 H, H-21$_a$), 3.52 (s, 1 H, MeOOC), 3.53 (s, 2 H, MeOOC), 3.59 (apparent s, 2 H, H-28$_{a,b}$), 3.60–3.95 (m, 3 H, H-21$_b$, H-23$_{a,b}$), 4.09 (d, $J_{7,6}$ = 3.2, 0.33 H, H-7), 4.10 (d, $J_{7,6}$ = 3.2, 0.67 H, H-7), 4.85 (dd, $J_{3,2\alpha}$ = $J_{3,2\beta}$ = 3.0, 1 H, H-3), 5.26 (ddq, $J_{15,16a}$ = $J_{15,16b}$ = 7.0, $J_{15,18}$ = 1.5, 0.33 H, H-15), 5.29 (ddq, $J_{15,16a}$ = $J_{15,16b}$ = 7.0, $J_{15,18}$ = 1.2, 0.67 H, H-15); EI-MS, *m/z* 602 ($M^{\cdot+}$, 64%), 587 (M – CH_3, 6), 571 (M – OCH_3, 3), 490 (6), 397 (8), 287 (44), 235 (36), 193 (46), 133 (32), 119 (26), 85 ($CH_3CH_2CH(CH_3)CO^+$, 33), 57 ($(CH_3CH_2CH(CH_3)^+$, 100).



	R_1	R_2
2a	CH_3COO	$CH_3CH_2CH(CH_3)COO$
2b	OH	OH
2c	CH_3O	$CH_3CH_2CH(CH_3)COO$
2d	CH_3O	OH
2e	CH_3O	CH_3O
2f	H	$CH_3CH_2CH(CH_3)COO$

1-Detigloyl-3-deacetyl-20,21,22,23-tetrahydrosalannin (2b). **2a** (35 mg, 0.057 mmol) was stirred with sodium methoxide (40 mg, 0.74 mmol) in 1.0 mL of dry methanol at 25 °C for 24 h. 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 portions were combined, washed once with 10 mL of saturated aqueous sodium chloride, and rotary evaporated in vacuo. Chromatography of the residue by silica gel preparative HPLC (solvent 2-propanol–*n*-hexane, 1:9, v/v) afforded **2b** (18 mg) as white rosette crystals: IR ν_{max} (cm^{-1}) 3420 (br), 1735 (s); ^1H NMR ($CDCl_3$) δ 1.10 (br s, $J_{29,28a}$ < 1.0, 3 H, 29-Me), 1.75 (d, $J_{18,15}$ = 1.6, 2 H, 18-Me), 1.82 (d, $J_{18,15}$ = 1.5, 1 H, 18-Me), ca. 2.3–2.5 (m, 1 H, H-9), 3.49 (ddd, $J_{1,OH}$ = 4.7, $J_{1,2\alpha}$ = $J_{1,2\beta}$ = 3.4, changes to dd in D_2O-exchange experiment, $J_{1,2\alpha}$ = $J_{1,2\beta}$ = 3.4, 0.33 H, H-1), 3.50 (ddd, $J_{1,OH}$ = 3.8, $J_{1,2\alpha}$ = $J_{1,2\beta}$ = 3.4, changes to dd in D_2O-exchange experiment, $J_{1,2\alpha}$ = $J_{1,2\beta}$ = 3.4, 0.67 H, H-1), 3.58 (d, $J_{28b,28a}$ = 7.2, 1 H, H-28$_b$), 3.70 (s, 3 H, MeOOC), 3.82 (ddd, $J_{3,OH}$ = 8.7, $J_{3,2\alpha}$ = $J_{3,2\beta}$ = 3.4, changes to dd in D_2O-exchange experiment, $J_{3,2\alpha}$ = $J_{3,2\beta}$ = 3.4, 1 H, H-3), 4.04 (d, D_2O exchangeable, $J_{OH,3}$ = 8.7, 1 H, 3-OH), 4.10 (br d, $J_{28a,28b}$ = 7.0, $J_{28a,29}$ < 1.0, 1 H, H-28$_a$), 4.19 (d, $J_{7,6}$ = 3.4, 0.33 H, H-7), 4.20 (d, $J_{7,6}$ = 3.4, 0.67 H, H-7), 4.59 (d, D_2O exchangeable, $J_{OH,1}$ = 4.7, 0.33 H, 1-OH), 4.61 (d, D_2O exchangeable, $J_{OH,1}$ = 3.8, 0.67 H, 1-OH); EI-MS, *m/z* 476 ($M^{\cdot+}$, 100%), 461 (M – CH_3, 15), 445 (M – OCH_3, 6), 403 (M – CH_2COOCH_3, 4), 287 (42), 271 (20), 235 (17), 219 (10), 206 (10), 193 (29), 162 (20), 147 (16), 135 (28), 133 (28), 119 (31), 107 (28), 105 (28), 91 (26).

3-*O*-Methyl-3-deacetyl-2′,3′,20,21,22,23-hexahydrosalannin (2c). **1f** (40 mg, 0.069 mmol) in 0.5 mL of ethanol was stirred with 30 mg of 5% palladium on alumina at 24 °C under hydrogen (10 atm) for 2 h. The reaction mixture was then filtered and rotary evaporated in vacuo. The residue was chromatographed by silica gel preparative HPLC (solvent 2-propanol–*n*-hexane, 1:9, v/v), followed by ODS preparative HPLC (solvent methanol–water, 7:3, v/v), to obtain **2c** (23 mg): IR ν_{max} (cm^{-1}) 1735 (s), 1725 (s); ^1H NMR ($CDCl_3$) δ 0.94 (t, $J_{4',3'}$ = 7.6, 1 H, 4′-Me), 0.95 (t, $J_{4',3'}$ = 7.4, 2 H, 4′-Me), 1.16 (d, $J_{5',2'}$ = 7.0, 2 H, 5′-Me), 1.24 (d, $J_{5',2'}$ = 7.1, 1 H, 5′-Me), ca. 1.4–1.7 (m, 2 H, H-3′$_a$, H-20), 1.66 (d, $J_{18,15}$ = 1.3, 2 H, 18-Me), ca. 1.7–2.0 (m, 1 H, H-3′$_b$), 1.73 (d, $J_{18,15}$ = 1.4, 1 H, 18-Me), ca. 1.8–2.3 (m, 2 H, H-22$_{a,b}$), ca. 2.3–2.6 (m, 1 H, H-2′), ca. 2.4–2.6 (m, 1 H, H-17), 2.62 (dd, $J_{9,11b}$ = 9.5, $J_{9,11a}$ = 3.4, 1 H, H-9), 2.77 (d, $J_{5,6}$ = 12.6, 1 H, H-5), 3.20 (s, 3 H, 3-MeO), 3.26 (dd, $J_{3,2\alpha} = J_{3,2\beta}$ = 3.0, 1 H, H-3), 3.31 (dd, $J_{21a,20} = J_{21a,21b}$ = 8.6, 0.33 H, H-21$_a$), 3.37 (dd, $J_{21a,20} = J_{21a,21b}$ = 8.5, 0.67 H, H-21$_a$), 3.51 (s, 1 H, MeOOC), 3.52 (s, 2 H, MeOOC), 3.60–3.95 (m, 3 H, H-21$_b$, H-23$_{a,b}$), 4.06 (br d, $J_{28a,28b}$ = 7.6, $J_{28a,29}$ < 1.0, 1 H, H-28$_a$), 4.09 (d, $J_{7,6}$ = 3.3, 1 H, H-7), 4.70 (dd, $J_{1,2\alpha} = J_{1,2\beta}$ = 3.0, 1 H, H-1), 5.26 (ddq, $J_{15,16a} = J_{15,16b}$ = 7.0, $J_{15,18}$ = 1.4, 0.33 H, H-15), 5.29 (ddq, $J_{15,16a} = J_{15,16b}$ = 7.0, $J_{15,18}$ = 1.3, 0.67 H, H-15); EI-MS, m/z 574 ($M^{\bullet+}$, 100%), 559 (M – CH_3, 13), 287 (37), 267 (16), 234 (12), 193 (27), 133 (11), 119 (11), 85 ($CH_3CH_2CH(CH_3)CO^+$, 20), 57 ($CH_3CH_2CH(CH_3)^+$, 48).

1-Detigloyl-3-*O*-methyl-3-deacetyl-20,21,22,23-tetrahydrosalannin (2d). **2c** (50 mg, 0.085 mmol) was dissolved in 2.0 mL of 0.5 M sodium hydroxide in 50% aqueous methanol and the resultant mixture stirred at 70 °C for 42 h. The reaction mixture was poured into 10 mL of 0.5 N hydrochloric acid and extracted three times with 10-mL portions of dichloromethane. The organic layers were combined, washed once with 10 mL of water, followed by 10 mL of saturated aqueous sodium chloride, and rotatory evaporated in vacuo. The residue was shown to be, by IR, the desired 3-*O*-methyl-20,21,22,23-tetrahydrosalannic acid. The residue, dissolved in 1.0 mL of dimethyl sulfoxide, was added to a mixture of 0.25 mL of 1.0 M aqueous sodium bicarbonate and 0.2 mL of iodomethane and stirred under nitrogen at 25 °C for 20 h. The reaction mixture was poured into 10 mL of saturated aqueous sodium thiosulfate and extracted three times with 10-mL portions of dichloromethane. The organic layers were combined, washed once with 10 mL of water, followed by 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, 3:17, v/v) to give **2d** (32 mg): IR ν_{max} (cm^{-1}) 3510 (br), 1735 (s); ^1H NMR ($CDCl_3$) δ 1.97 (ddd, $J_{2\alpha,2\beta}$ = 15.9, $J_{2\alpha,1} = J_{2\alpha,3}$ = 3.7, 1 H, H-2$_\alpha$), 2.18 (ddd, $J_{2\beta,2\alpha}$ = 15.5, $J_{2\beta,1} = J_{2\beta,3}$ = 3.0, 1 H, H-2$_\beta$), 2.55 (d, $J_{5,6}$ = 12.2, 1 H, H-5), 2.72 (dd, $J_{9,11a} = J_{9,11b}$ = 6.0, 1 H, H-9), 3.37 (s, 3 H, 3-MeO), 3.41 (dd, $J_{3,2\alpha} = J_{3,2\beta}$ = 2.8, 1 H, H-3), 3.48 (ddd, $J_{1,OH}$ = 9.7, $J_{1,2\alpha} = J_{1,2\beta}$ = 3.0, changes to dd in D_2O-exchange experiment, $J_{1,2\alpha} = J_{1,2\beta}$ = 3.0, 1 H, H-1), 3.60 (s, 3 H, MeOOC), 3.63 (d, D_2O exchangeable, $J_{OH,1}$ = 9.7, 1 H, 1-OH), 3.94 (br d, $J_{28a,28b}$ = 7.1, $J_{28a,29}$ < 1.0, 1 H, H-28$_a$), 5.36 (ddq, $J_{15,16a} = J_{15,16b}$ = 7.0, $J_{15,18}$ = 1.6, 0.33 H, H-15), 5.40 (ddq, $J_{15,16a} = J_{15,16b}$ = 7.0, $J_{15,18}$ = 1.6, 0.67 H, H-15); EI-MS, m/z 490 ($M^{\bullet+}$, 100%), 475 (M– CH_3, 4), 403 (11), 287 (16), 193 (16), 107 (11), 91 (14), 85 (11), 81 (11), 59 (13), 55 (19).

1,3-*O*,*O*-Dimethyl-1-detigloyl-3-deacetyl-20,21,22,23-tetrahydrosalannin (2e). **1h** (30 mg, 0.060 mmol) in 0.5 mL of ethanol was stirred with 25 mg of 5% palladium on alumina at 25 °C under hydrogen (10 atm) for 2 h. The reaction mixture was then filtered and rotary evaporated in vacuo. The residue was chromatographed by silica gel preparative HPLC (solvent 2-propanol–*n*-hexane, 1:9, v/v) to afford **2e** (26 mg): IR ν_{max} (cm^{-1}) 1735 (s); ^1H NMR ($CDCl_3$) δ ca. 1.4–1.8 (m, 1 H, H-20), 1.70 (d, $J_{18,15}$ = 1.5, 2 H, 18-Me), 1.77 (d, $J_{18,15}$ = 1.8, 1 H, 18-Me), ca. 1.8–2.4 (m, 8 H, H-2$_{\alpha,\beta}$, H-11$_{a,b}$, H-16$_{a,b}$, H-22$_{a,b}$), ca. 2.5–2.6 (m, 1 H, H-17), 2.79 (dd, $J_{9,11a} = J_{9,11b}$ = 6.2, 0.33 H, H-9), 2.81 (dd, $J_{9,11a} = J_{9,11b}$ = 6.2, 0.67 H, H-9), 2.96 (dd, $J_{1,2\alpha} = J_{1,2\beta}$ = 3.0, 0.33 H, H-1), 3.00 (dd, $J_{1,2\alpha} = J_{1,2\beta}$ = 3.0, 0.67 H, H-1), 3.26 (s, 1 H, 1-MeO), 3.27 (s, 2 H, 1-MeO), 3.34 (dd, $J_{21a,20} = J_{21a,21b}$ = 8.5, 0.33 H, H-21$_a$), 3.39 (dd, $J_{21a,20} = J_{21a,21b}$ = 8.5, 0.67 H, H-21$_a$), 3.45–3.95 (m, 3 H, H-21$_b$, H-23$_{a,b}$), 3.58 (s, 2 H, MeOOC), 3.59 (s, 1 H, MeOOC), 5.35–5.48 (m, 1 H, H-15); EI-MS, m/z 504 ($M^{\bullet+}$, 100%), 489 (M – CH_3, 11), 403 (48), 287 (61), 235 (13), 193 (18), 119 (11), 101 (28), 85 (24), 59 (11), 55 (15).

3-Deacetoxy-2′,3′,20,21,22,23-hexahydrosalannin (2f). **1b** (100 mg, 0.18 mmol) was stirred with methanesulfonyl chloride (70 μL, 0.90 mmol) in 0.4 mL of pyridine at 25 °C for 3 h. The reaction mixture was poured into 10 mL of 0.5 N hydrochloric acid and extracted three times with 10-mL portions of dichloromethane. The organic layers were combined, washed once with 10 mL of 0.5 N hydrochloric acid, followed by 10 mL of saturated aqueous sodium chloride, and rotary evaporated in vacuo. The residue, shown by IR to contain no remaining hydroxyl, was dissolved in 1.0 mL of dimethyl sulfoxide, and then 0.31 g (0.54 mmol) of tetra-*n*-butylammonium oxalate disalt (Corey and Terashima, 1972) was added. The reaction mixture was heated at 70 °C for 16 h before pouring into 20 mL of saturated aqueous sodium bicarbonate and extracting three times with 20-mL portions of dichloromethane. The organic layers were combined, washed once with 20 mL of water, followed by 20 mL of saturated aqueous sodium chloride, and rotary evaporated in vacuo. The residue, following chromatography by silica gel preparative HPLC (solvent 2-propanol–*n*-hexane, 3:97, v/v), was stirred with 20 mg of 5% palladium on alumina in 0.5 mL of ethanol at 25 °C under 10 atm of hydrogen for 3 h. The reaction mixture was filtered and rotary evaporated in vacuo. The residue was chromatographed by silica gel preparative HPLC (solvent 2-propanol–*n*-hexane, 1:19, v/v) to yield **2f** (24 mg): IR ν_{max} (cm^{-1}) 1730 (s); ^1H NMR ($CDCl_3$) δ 0.94 (t, $J_{4',3'}$ = 7.4, 1 H, 4′-Me), 0.95 (t, $J_{4',3'}$ = 7.4, 2 H, 4′-Me), 1.16 (d, $J_{5',2'}$ = 6.9, 2 H, 5′-Me), 1.25 (d, $J_{5',2'}$ = 7.3, 1 H, 5′-Me), ca. 1.4–2.6 (m, 16 H, H-2$_{\alpha,\beta}$, H-2′, H-3$_{\alpha,\beta}$, H-3′$_{a,b}$, H-9, H-11$_{a,b}$, H-16$_{a,b}$, H-17, H-20, H-22$_{a,b}$), 1.66 (apparent br s, $J_{18,15}$ < 1.0, 2 H, 18-Me), 1.73 (d, $J_{18,15}$ = 1.6, 1 H, 18-Me), 2.27 (d, $J_{5,6}$ = 12.3, 1 H, H-5), 3.31 (dd, $J_{21a,20} = J_{21a,21b}$ = 8.6, 0.33 H, H-21$_a$), 3.37 (dd, $J_{21a,20} = J_{21a,21b}$ = 8.3, 0.67 H, H-21$_a$), 3.51 (s, 1 H, MeOOC), 3.53 (s, 2 H, MeOOC), 3.54 (br d, $J_{28a,28b}$ = 7.3, $J_{28a,29}$ < 1.0, 1 H, H-28$_a$), 3.60–3.95 (m, 3 H, H-21$_b$, H-23$_{a,b}$), 3.69 (d, $J_{28b,28a}$ = 7.2, 1 H, H-28$_b$), 3.91 (dd, $J_{6,5}$ = 12.6, $J_{6,7}$ = 3.3, 1 H, H-6), 4.08 (d, $J_{7,6}$ = 3.3, 0.33 H, H-7), 4.09 (d, $J_{7,6}$ = 3.3, 0.67 H, H-7), 4.76 (unresolved dd, 1 H, H-1), 5.23 (ddq, $J_{15,16a} = J_{15,16b}$ = 7.0, $J_{15,18}$ = 1.5, 0.33 H, H-15), 5.26 (apparent br dd, $J_{15,16a} = J_{15,16b}$ = 7.0, $J_{15,18}$ < 1.0, 0.66 H, H-15); EI-MS, m/z 544 ($M^{\bullet+}$, 73%), 529 (M – CH_3, 5), 412 (9), 339 (11), 287 (22), 263 (19), 237 (28), 235 (24), 234 (24), 193 (86), 133 (39), 85 ($CH_3CH_2CH(CH_3)CO^+$, 45), 57 ($CH_3CH_2CH(CH_3)^+$, 100).

RESULTS AND DISCUSSION

Fourteen derivatives of salannin (**1a**) were prepared, and their structures were confirmed by IR, ^1H NMR, and EI-MS. 3-Deacetylsalannin (**1b**) was purified from neem seeds as described by Yamasaki et al. (1988). Alternatively **1b** could be obtained, albeit at somewhat low (25%) yields, through transesterification of **1a** with sodium methoxide, a reaction that yielded equal amounts of 1-detigloyl-3-deacetylsalannin (**1d**) (unpublished data). The spectral data obtained from **1b** and **1d** were in agreement with those reported elsewhere (Henderson et al., 1964, 1968; Kraus and Cramer, 1981; Kubo et al., 1986).

Saponification of **1a** with aqueous methanolic sodium hydroxide yielded salannic acid (**1c**). The IR spectrum of **1c** showed the loss of the bands at 1710, 1655, and 735 cm^{-1}, assigned to the tigloyl group (Henderson et al., 1968; Butterworth et al., 1972; Yamasaki and Klocke, 1987), and the appearance of a very broad, intense absorption at 3400 cm^{-1} characteristic of a carboxylic acid (Silverstein et al., 1981a). All of the ^1H NMR signals of the tigloyl (δ 1.82, 1.92–1.96, 6.96), acetyl (δ 1.94), and carbomethoxyl (δ 3.24) groups had vanished. EI-MS showed a parent ion at m/z 458, as reported by de Silva et al. (1969).

Although **1d** could be prepared by transesterification of **1a** with sodium methoxide, we could obtain better yields (81% compared to 25%) of **1d** by methylating the sodium salt of the carboxylic acid group of **1c** with iodomethane in dimethyl sulfoxide. The very broad, intense absorption at 3400 cm^{-1} (carboxylic acid and two hydroxyl groups) in the IR spectrum of **1c** became a less broad and less intense band in **1d**. In addition, the carbonyl stretching band at

1700 cm^{-1} of **1c** changed to 1735 cm^{-1} in the IR spectrum of **1d**, indicating the conversion of the carboxylic acid to the methyl ester. ^1H NMR showed the presence of a carbomethoxyl group (singlet at δ 3.59) in **1d** as well as two D_2O-exchangeable hydroxyl groups at positions 1 and 3.

2′,3′-Dihydrosalannin (**1e**) was made by hydrogenation of **1a** over palladium on alumina. The IR bands of the tigloyl group at 1710, 1655, and 735 cm^{-1} had disappeared. In the ^1H NMR spectrum, the 4′-methyl (δ 1.82), 5′-methyl (δ 1.92–1.96), and 3′-vinyl (δ 6.96) signals of **1a** were replaced in **1e** by two overlapping triplets (δ 0.95 and 0.96, 1:2 ratio), two overlapping doublets (δ 1.23 and 1.30, 2:1 ratio), and an unresolved multiplet (δ 1.4–2.0), respectively. Another multiplet (δ 2.3–2.6) was 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., 1981b; Yamasaki and Klocke, 1987). The two diastereomers appear to have been produced in a 2:1 ratio, although the favored configuration at position 2′ is unknown.

Methylation of the hydroxyl group of **1b** afforded 3-*O*-methyl-3-deacetylsalannin (**1f**). Absence of a free hydroxyl group in **1f** was indicated by the disappearance of the hydroxyl band at 3420 cm^{-1} in the IR spectrum and the hydroxyl signal at δ 2.42 in the ^1H NMR spectrum of **1b**. In the ^1H NMR spectrum of **1f**, a new singlet appeared at δ 3.13, consistent with a methoxyl group.

1-Detigloyl-3-*O*-methyl-3-deacetylsalannin (**1g**) was made by first saponifying the tiglate and methyl esters of **1f** and then remethylating the carboxylic acid group according to a modification of the method of Mehta (1972). The IR spectrum of **1g** contained a hydroxyl band at 3490 cm^{-1} but lacked the bands of the tigloyl group at 1705, 1650, and 735 cm^{-1}. The ^1H NMR spectrum of **1g** contained a D_2O-exchangeable proton signal at δ 3.66 and lacked the signals of the tigloyl group.

Methylation of the two hydroxyl groups of **1d** in a manner similar to the preparation of **1f** yielded 1,3-*O*,*O*-dimethyl-1-detigloyl-3-deacetylsalannin (**1h**). The IR band at 3420 cm^{-1} and the two D_2O-exchangeable ^1H NMR signals (δ 4.02 and 4.56) seen with **1d** were absent in the corresponding spectra of **1h**. Singlets were observed (δ 3.29 and 3.32) in the ^1H NMR spectrum of **1h** for the methoxyl groups at positions 1 and 3, respectively.

The hydroxyl groups of **1d** were acetylated according to a modification of the method of Henderson et al. (1968) to afford the known 1-*O*-acetyl-1-detigloylsalannin (**1i**). The ^1H NMR spectrum of **1i** was in agreement with those reported by Henderson et al. (1964, 1968) and de Silva et al. (1969).

When **1a** was hydrogenated over a palladium catalyst at higher pressure (10 versus 5 atm) and for a longer reaction time (360 versus 10 min) than that used to make **1e**, the furan ring was reduced as well as the tigloyl group. The IR spectrum of 2′,3′,20,21,22,23-hexahydrosalannin (**2a**) showed the loss of the furan moiety bands at 1500 and 870 cm^{-1} (de Silva et al., 1969) and the loss of the tigloyl bands at 1710, 1655, and 735 cm^{-1}. In addition to the changes in the ^1H NMR spectrum observed with **1e**, the signals of the furan ring protons at positions 21, 22, and 23 (δ 7.31–7.35, 6.28–6.31, and 7.24–7.28, respectively) in **1a** were replaced by two doublet of doublets at δ 3.32 and 3.38 (1:2 ratio, respectively, proton 21a) and unresolved multiplets at δ 3.60–3.95 (protons 21b and 23a,b) and 1.80–2.35 (protons 22a,b) in **2a**. A new multiplet at δ 1.4–2.0 (proton at position 20) was also observed. The doublet ($J_{18,15}$ = 1.3) at δ 1.67 of the 18-methyl group and the singlet at δ 3.24 of the carbomethoxyl group of **1a** were replaced in **2a** by two doublets at δ 1.67 and 1.74 (2:1 ratio, $J_{18,15}$ = 1.2 and 1.5, respectively) of the 18-methyl group and two singlets at δ 3.52 and 3.53 (1:2 ratio) of the carbomethoxyl group. The signal for the proton at position 17 had also changed from a broadened doublet (δ 3.63) to a multiplet within the envelope at δ 2.30–2.65. The two sets of signals observed for each of the 18-methyl and carbomethoxyl protons (as well as for protons 7, 15, and 21a) is probably a consequence of the hydrogenation at either face of the prochiral carbon at position 20. Similar to our result with the hydrogenation of the tigloyl group, one face was preferentially reduced by a 2:1 ratio.

Transesterification of **2a** with sodium methoxide afforded 1-detigloyl-3-deacetyl-20,21,22,23-tetrahydrosalannin (**2b**). The IR spectrum of **2b** showed the loss of the tigloyl and furan ring bands and the presence of new hydroxyl bands at 3420 cm^{-1}. ^1H NMR showed two D_2O-exchangeable protons, the loss of the acetyl and tigloyl signals, and the same changes in the furan ring signals as those seen with **2a**. In this case, partitioning of the carbomethoxyl signal (δ 3.70) into two sets was not observed.

Hydrogenation of **1f** and **1h** over a palladium catalyst gave 3-*O*-methyl-3-deacetyl-2′,3′,20,21,22,23-hexahydrosalannin (**2c**) and 1,3-*O*,*O*-dimethyl-1-detigloyl-3-deacetyl-20,21,22,23-tetrahydrosalannin (**2e**), respectively. The appropriate spectral changes observed in the hydrogenation of **1a** to give **2a** were likewise observed in the hydrogenation of **1f** and **1h** to give **2c** and **2e**, respectively.

1-Detigloyl-3-*O*-methyl-3-deacetyl-20,21,22,23-tetrahydrosalannin (**2d**) was prepared from **2c** in a manner analogous to the preparation of **1g** from **1f**. The IR spectrum of **2d** showed a hydroxyl band at 3510 cm^{-1} and lacked the carbonyl stretching band at 1725 cm^{-1} (α-methylbutyrate ester). ^1H NMR showed the presence of a D_2O-exchangeable proton (δ 3.63) and the absence of all of the α-methylbutyryl group protons.

3-Deacetoxy-2′,3′,20,21,22,23-hexahydrosalannin (**2f**) was smoothly prepared from **1b** in three steps. The hydroxyl group of **1b** was first mesylated and then eliminated in an E2 process using a modification of the method of Corey and Terashima (1972). The resulting olefin, as well as the furan ring and tiglate carbon–carbon double bonds, was subsequently hydrogenated over palladium.

In the ^1H NMR spectra of **1a** and nearly all of the derivatives reported in this study (two exceptions will be discussed below), broadening ($J_{28a,29} = J_{29,28a} < 1.0$) of the signal was observed for protons 28a (doublet) and 29-methyl (singlet). This example of the *w* conformation long-range coupling (Silverstein et al., 1981b) was reported previously for **1i** (Henderson et al., 1964, 1968) and helped distinguish proton 28a from 28b. In two of the derivatives in the present study (**1e** and **2a**), this broadening effect was not observed since the ^1H NMR signals of protons 28a and 28b, normally doublets due to geminal coupling, coalesced into an apparent singlet. These two derivatives represent examples of structures containing chemically and magnetically nonequivalent (diastereotopic) coupled protons that have fortuitously identical chemical shift values.

In every case, the molecular ion was observed in the EI-MS. A major peak was also seen at *m/z* 283 in **1a** and all of its derivatives whose furan ring was not reduced. The identity of this fragment is unknown. However, it probably includes the furan ring moiety since the six derivatives, whose furan ring was completely hydrogenated (series **2a–f**), showed a major peak at *m/z* 287 instead of 283.

The antifeedant activities of salannin and 14 of its derivatives against third-instar *L. decemlineata* are shown

Table I. Antifeedant Activity of Salannin and 14 Derivatives, Azadirachtin, and Kelthane against Colorado Potato Beetle (*L. decemlineata* Say) Larvae by a Leaf Disk Choice Bioassay

compd	PC_{95},[a] μg	PC_{50},[b] μg
1a	>400	150
1b	>400	25
1d	>400	25
1g	>400	400
1i	>400	>400
1e	200	
1f	100	
2e	100	
1c	50	
1h	50	
2b	50	
2d	50	
2a	25	10
2c	10	2
2f	10	
azadirachtin	75	19
kelthane	6	2

[a] PC_{95} is the minimal protective concentration at which >95% of the control disks were eaten, while <5% of the treated disks were eaten in choice bioassays. [b] PC_{50} is the minimal protective concentration at which >50% of the control disks were eaten, while <5% of the treated disks were eaten in choice bioassays.

in Table I. Modifications of four chemical points of the parent salannin molecule resulted in changes in the antifeedant activity.

First, hydrogenation of the furan ring to the tetrahydrofuran ring increased the antifeedant activity. For example, the activity of **1d** (PC_{95} > 400 μg), a diol of **1a**, was increased more than 8-fold via hydrogenation to the diol of tetrahydrosalannin (**2b**; PC_{95} = 50 μg). In addition, the activity of **1e** (PC_{95} = 200 μg), hydrogenated only at the tigloyl moiety, was increased 8-fold via hydrogenation of its furan ring to yield **2a** (PC_{95} = 25 μg).

Second, replacement of the acetoxyl group at position 3 by a methoxyl group increased the antifeedant activity. For example, the activity of **1a** (PC_{95} > 400 μg) increased via derivatization to **1f** (PC_{95} = 100 μg), as did that of **2a** (PC_{95} = 25 μg) via derivatization to **2c** (PC_{95} = 10 μg). A similar increase in antifeedant activity was observed when the acetoxyl group at position 3 was replaced by hydrogen (**2f**; PC_{95} = 10 μg).

Third, modifications of the tigloyl group resulted in some changes in antifeedant activity. Hydrogenation of the tigloyl moiety of **1a** (PC_{95} > 400 μg) to give the α-methylbutyryl group of **1e** (PC_{95} = 200 μg) resulted in at least a 2-fold increase in the activity. In general, deesterification of the tigloyl or the α-methylbutyryl groups resulted in a reduction of activity. Examples include **2c** (PC_{95} = 10 μg), which was 5-fold more active than **2d** (PC_{95} = 50 μg), and **1f** (PC_{95} = 100 μg), which was more than 4-fold more active than **1g** (PC_{95} > 400 μg). O-Methylation at position 1 of the detigloyl derivatives had opposite effects on the activity depending on whether the derivatives contained a furan or a tetrahydrofuran ring. For example, **1h** (PC_{95} = 50 μg), with a methoxyl group at position 1 and a furan ring at position 17, was found to be more than 8-fold more active than **1g** (PC_{95} > 400 μg), which contained a hydroxyl group at position 1 instead of the methoxyl group. On the other hand, **2d** (PC_{95} = 50 μg), with a hydroxyl group at position 1 and a tetrahydrofuran ring at position 17, was twice as potent as **2e** (PC_{95} = 100 μg), which contained a methoxyl group at position 1 instead of the hydroxyl group.

Fourth, saponification of the methyl ester at position 11 increased the antifeedant activity. For example, salannic acid (**1c**, PC_{95} = 50 μg) was at least 8-fold more active than the 1,3-diol, **1d** (PC_{95} > 400 μg). More derivatives, in which the methyl ester is chemically modified, should be prepared in order to ascertain the structure–activity relationship of the carbomethoxyl group.

Salannin and four of its nonhydrogenated derivatives (**1b, 1d, 1g, 1i**) did not exhibit 95% protection (i.e., PC_{95} values) at 400 μg/disk, the highest concentration tested. However, some antifeedant activity was observed at 400 μg/disk such that PC_{50} values, the protective concentration at which >50% of the control disks were eaten, while <5% of the test disks were eaten, were determined for these compounds (Table I). For comparative purposes, the PC_{50} values were also determined for two of the most active of the salannin derivatives (**2c**, PC_{50} = 2 μg; **2a**, PC_{50} = 10 μg), azadirachtin (PC_{50} = 19 μg), and kelthane (PC_{50} = 2 μg) (Table I). The PC_{50} values for these compounds were 2- to 5-fold lower than their respective PC_{95} values.

Replacement of the tigloyl moiety of salannin (**1a**; PC_{50} = 150 μg) to form the 1,3-diacetate (**1i**; PC_{50} > 400 μg) resulted in a loss of the antifeedant activity. More active was the 1,3-diol (**1d**; PC_{50} = 25 μg). Its activity was unaffected by the presence of a tigloyl group esterified at position 1 (**1b**; PC_{50} = 25 μg). However, methylation of its C-3 hydroxyl group to form **1g** (PC_{50} = 400 μg) resulted in a 16-fold reduction in the antifeedant activity.

Our data indicate that the potency of salannin as an antifeedant against third-instar *L. decemlineata* larvae is increased by over 40-fold by a combination of hydrogenation of the furan ring and the tigloyl group and replacement of the acetoxyl group by a methoxyl group or a hydrogen atom. The two derivatives prepared in this manner (**2c** and **2f**) were found to be 7.5-fold more active as antifeedants against *L. decemlineata* than the natural plant compound, azadirachtin (PC_{95} = 75 μg), and they were about as active as the commercial miticide, kelthane (PC_{95} = 6 μg) (Table I). Azadirachtin is currently being investigated as a source of and model compound for a new commercial insect antifeedant (Klocke, 1989). Kelthane has been reported as an antifeedant against the Colorado potato beetle in laboratory and field studies (Walgenbach and Wyman, 1987).

Although hydrogenation of the furan ring and the tigloyl group of salannin increased its antifeedant potency against the Colorado potato beetle, no difference in the antifeedant activity of salannin and 2′,3′,20,21,22,23-hexahydrosalannin was observed against the fall armyworm, *Spodoptera frugiperda*. By using cotton leaf disk choice bioassays (Klocke and Kubo, 1982), we found the PC_{95} value for both **1a** and **2a** against *S. frugiperda* to be 75 μg. The effectiveness of salannin and its derivatives as antifeedants for various species of insects is difficult to predict and should be determined empirically.

We will attempt to further increase the insect antifeedant activity of salannin through derivatization. We will test these derivatives against the Colorado potato beetle and other economically important insect species. Our aim is to develop salannin as a source of and a model compound for new commerical insect control agents.

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Registry No. **1a**, 992-20-1; **1b**, 1110-56-1; **1c**, 29803-85-8; **1d**, 1062-24-4; **1e** (diastereomer 1), 121212-30-4; **1e** (diastereomer 2), 121251-66-9; **1f**, 121212-31-5; **1g**, 121212-32-6; **1h**, 121212-33-7; **1i**, 18184-23-1; **2a**, 121232-47-1; **2b**, 121212-34-8; **2c**, 121232-48-2;

2d, 121212-35-9; **2e**, 121212-36-0; **2f**, 121232-49-3.

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Molecular Orbital Approaches to the Photolysis of Organophosphorus Insecticide Fenitrothion

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Photodegradation pathways of the organophosphorus insecticide fenitrothion (I) [*O,O*-dimethyl *O*-(3-methyl-4-nitrophenyl) phosphorothioate] in water are discussed from a theoretical point of view, using semiempirical molecular orbital (MO) calculations. Spectroscopic analyses and MO calculations showed that the Norrish type II intramolecular abstraction of the aryl methyl protons by the nitro group in the excited state is involved in the formation of *O,O*-dimethyl *O*-(3-carboxy-4-nitrophenyl) phosphorothioate (IV). The photooxidation of P=S to P=O was considered to proceed via the reaction of I with a hydroxyl radical and not with singlet molecular oxygen.

Photochemical processes play an important role in the degradation of the organophosphorus insecticide fenitrothion (I) in the environment (Mikami et al., 1985a). It undergoes various reactions such as oxidation of the P=S and the aryl methyl moieties and cleavage in the P—O aryl and the PO-CH_3 moieties with subsequent degradation to carbon dioxide. Although the environmental fate of I has been studied in detail, the degradation mechanism, especially photolysis, is still obscure at the molecular level.

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In the case of photochemical transformation of a pesticide in pure water, direct photolysis, i.e. where the pesticide absorbs the incident irradiation directly, can occur (Zepp and Cline, 1977). However, active oxygen species generated by direct photolysis can react to give rise to further reactions often referred to as "indirect" photolysis. From this viewpoint, the reactions of pesticides with hydroxyl radicals, singlet molecular oxygen (Draper and Crosby, 1981), hydrogen peroxide (Draper and Crosby, 1984), and superoxide anion radicals (Draper and Crosby, 1983a) have been examined. Although both direct and indirect transformations of pesticides have been studied extensively, the influence of molecular properties such as electronic configurations in both the ground and excited

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