Colorado Potato Beetle Antifeedants by Simple Modification of the Birchbark Triterpene Betulin

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Oxidation of the 19-side-chain of betulin 3β,28-diacetate, a pentacyclic triterpene derived from the readily available birchbark triterpene, betulin, yielded compounds active as antifeedants against larvae of the agricultural insect pest Colorado potato beetle, *Leptinotarsa decemlineata*. Of the eight compounds investigated, the most active was 30-hydroxy-20-oxo-29-norlup-3β,28-diacetate (**VI**). Acetylation of **VI** significantly reduced antifeedant activity.

Keywords: *Antifeedant; betulin; Colorado potato beetle; Leptinotarsa decemlineata*

As substances that reduce feeding on plants by insects, antifeedants offer considerable promise as components of emerging integrated pest management (IPM) strategies for agricultural insect control (Alford, 1994; Jermy, 1990; Cutler, 1988; Whitehead and Bowers, 1983). Increasing evidence points to multiple types of activity by some of these substances, including toxicity (Zehnder and Warthen, 1988), oviposition deterrence (Murray et al., 1993; Liu et al., 1989), and growth regulation activity (Liu et al., 1991; Warthen, 1979, 1989). They may also enhance the activity of other insect control agents such as *Bacillus thuringiensis* (Salama and Sharby, 1988; Murray et al, 1993) or synthetic insecticides (Parmar and Dutta, 1986). Unlike conventional pesticides, antifeedants are often highly specific in their action (Bernays, 1983; Dethier, 1980), a significant consideration for IPM applications. Antifeedants that are natural products or closely related analogues also have a high likelihood of ready biodegradation.

In spite of these seemingly favorable considerations, antifeedants are not in common use even though they have been studied for decades. Indeed, to our knowledge neem (*Azadirachta indica*) extract is the only antifeedant currently marketed. There are several reasons for this. The isolation of pure, naturally occurring antifeedants is tedious and expensive, and these compounds are seldom available from natural sources in the quantities necessary for agricultural applications. Furthermore, the total synthesis of these substances on a large scale is generally economically impractical. The apparent activity of most reported antifeedants is not high, especially when compared with that of available pesticides. Most antifeedants have not, however, been studied in enough detail to determine whether synergism with other potential IPM insect control agents, together with other benefits, makes up for the latter common deficiency.

The problem of economically viable production of antifeedants might be solved either by simple synthetic transformation of abundant, easily isolated natural products or by synthesis of natural product models whose structures mimic essential parts of active, more complex natural products. Ley et al. (1987) have reported synthesis of model antifeedants based on azadirachtin, and we have reported active models based on limonin (Bentley et al., 1990). We have also reported preparation of an antifeedant for bollworm larvae (*Helicoverpa zea*, formerly *Heliothis zea*) from betulin (Lugemwa et al., 1990), a pentacyclic triterpene of the lupane group abundant in the bark of the so-called white birches such as paper birch (*Betula papyrifera*) and gray birch (*Betula populifolia*).

We are particularly interested in useful semisynthetic transformations of betulin, since the white birches are widespread in the northern latitudes of the world and there is no current economically significant use for this abundant, easily isolated compound. For paper birch, betulin constitutes about 12% of the dry weight of the bark (O'Connell et al., 1988) and for the Scandinavian species, *Betula verrucosa*, as much as 30% (Ekman, 1983). Here we report the simple preparation from betulin of antifeedants for larvae of the Colorado potato beetle (*Leptinotarsa decemlineata*).

MATERIALS AND METHODS

NMR spectra were obtained on a Varian XL-200 system and FTIR spectra on a Bio-Rad FTS-60 system. Mass spectra were obtained on a HP-5985-B. Uncorrected melting points were determined on a Fisher-Johns apparatus.

A. Preparation of Materials. *1. Isolation of Betulin* (**I**). Betulin was isolated from mixed bark of paper birch (*B. papyrifera*) and gray birch (*B. populifolia*) by extraction with chloroform followed by chromatography of the extract residue on silica gel eluted with 5% EtOAc/hexane as reported earlier from our laboratory (Lugemwa et al., 1990).

2. Betulin 20,29-Epoxy-3β,28-diacetate (**III**). Betulin 3β,-28-diacetate (II) (1 g, 1.9 mmol), prepared from betulin according to the method of Miles et al. (1974), was stirred with *m*-chloroperbenzoic acid (0.38 g, 2.1 mmol) in CH_2Cl_2 for 2 h, and the resulting mixture was sequentially washed with 10% KI, 5% Na_2SO_3, and H_2O. After drying over Na_2SO_4, the solvent was evaporated and the residue recrystallized from MeOH to afford 910 mg (88%) of **III**: mp 191–193 °C [lit. 194–203 °C (Klinot et al., 1970)]; IR (KBr) 2946, 2872, 1743, 1664, 1390, 1374, 1365, 1142, 1030, 978 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.48 (m, 1H, 3α-H), 4.20 (d, J = 11.3 Hz, 1H, 28-H), 3.66 (d, J = 11.3 Hz, 1H, 28-H), 2.56 (d, J = 4.8 Hz, 1H, 29-H), 2.64 (d, J = 4.8 Hz, 1H, 29-H), 2.04 (s, 3H, Ac), 2.02 (s, 3H, Ac), 0.84–1.66 (complex, CH_2, CH), 1.25, 1.03, 0.97, 0.86, 0.85, 0.84 (all

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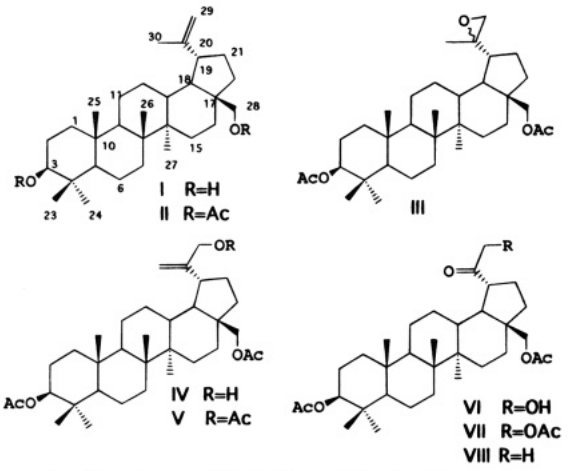


Figure 1. Structures of betulin and derivatives.

s, 18H, 6 × CH_3); MS(EI), *m/e* (rel int.) 542 (M^+, 0.8%), 527 (1%), 424 (11%), 189 (54%), 212 (45%), 107 (60%), 81 (83%), 55 (100%).

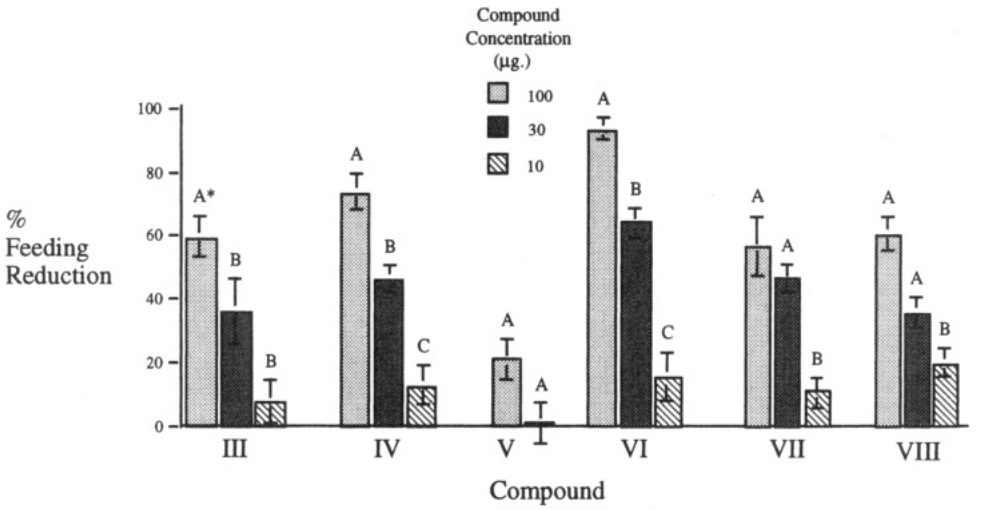
3. 30-Hydroxylup-20(29)-ene 3β,28-Diacetate (**IV**). **III** (500 mg) was dissolved in 40 mL of $CHCl_3$, 5 drops of 35% HCl was added, and the resulting solution was refluxed for 20 min. After washing with 5% aqueous $NaHCO_3$ and water, the organic phase was evaporated under vacuum. The residue was chromatographed over basic aluminum oxide with 10% EtOAc to give **IV** (302 mg, 61%): mp (202−205 °C [lit. 220−221.5 °C (Klinot et al., 1970)]; IR (KBR) 3470, 2946, 2872, 1734, 1645, 1450, 1390, 1374, 1365, 1242, 1030, 1014, 978 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.95 (d, J = 1.07 Hz, 30-H), 4.88 (br s, 1H, 30-H), 4.45 (m, 1H, 3-H), 4.22 (d, J = 11.3 Hz, 1H, 28-H), 4.09 (br s, 2H, 29-H), 3.82 (d, J = 11.3 Hz, 1H, 28-H), 2.3 (m, 1H, 19-H), 2.05 (s, 3H, Ac), 2.02 (s, 3H, Ac), 0.84−1.98 (complex, CH_2, CH), 1.01, 0.85, 0.82, 0.81 (all s, 15H, 5 × CH_3); MS(EI), *m/e* (rel int.) 542 (M^+, 29%), 524 (19%), 482 (22%), 467 (10%), 201 (25%), 189 (100%), 107 (48%).

4. Lup-20(29)-ene 3β,28,30-Triacetate (**V**). A solution containing 200 mg of **IV** in 10 mL of acetic anhydride and 2 drops of pyridine was refluxed for 2 h. The product crystallized upon cooling and was collected by vacuum filtration and washed with methanol to give **V** (191 mg, 91%): mp 90−91 °C [lit. 115−117 °C (Klinot et al., 1970)]; IR (KBr) 2940, 2870, 1740, 1450, 1394, 1245, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.96 (br s, 2H, 29-H), 4.55 (br s, 2H, 30-H), 4.47 (m, 1H, 3α-H), 4.24 (d, J = 10.4 Hz, 1H, 28-H), 3.82 (d, J = 10.4 Hz, 1H, 28-H), 2.38 (m, 1H, 19β-H), 2.07 (s, 3H, Ac), 2.04 (s, 3H, Ac), 2.1 (s, 3H, Ac), 0.86−1.96 (complex, CH_2 CH), 1.03, 0.97, 0.84, 0.83 (s, 15H, 5 × CH_3); MS(EI), *m/e* (rel int.) 584 (M^+, 1.3%), 524 (17.2%), 451 (13.1%), 203 (17.9%), 189 (100%), 159 (26.2%), 135 (39.6%), 121 (38.9%), 107 (44.4%).

5. 30-Hydroxy-20-oxo-29-norlup-3β,28-diacetate (**VI**). **IV** (200 mg) was dissolved in 30 mL of dry CH_2Cl_2, and the solution was cooled to −78 °C. Ozone was bubbled into the solution and the progress of the reaction monitored by TLC. When the reaction was complete, the solution was warmed to room temperature and 2 g of Zn dust and 2 mL of glacial acetic acid were added. After the solution was stirred for 2 h, it was filtered, and the filtrate was washed with aqueous $NaHCO_3$ followed by H_2O. After drying over Na_2SO_4, the solvent was removed under vacuum to yield an oil which was chromatographed over silica gel eluted with 10% ethyl acetate in hexane to give 141 mg (71%) of **VI**: mp 129−130 °C [lit. 131−133 °C (Pouzar and Vystrcil, 1977)]; IR (KBr) 3470, 2948, 2879, 1735, 1718, 1450, 1369, 1245, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.48 (m, 1H, 3α-H), 4.28 (s, 2H, 29-H), 4.20 (d, J = 11.3 Hz, 1H, 28-H), 3.78 (d, J = 11.3 Hz, 1H, 28-H), 2.6 (m, 1H, 19β-H), 2.07 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.26−2.2 (complex, CH_2, CH), 1.01, 1.00, 0.84 (all s, 15H, 5 × CH_3). Compound **VI** was acetylated to give **VII** according to the procedure of Vystrcil and Budesinsky (1970).

6. 20-Oxo-29-norlup-3β,28-diacetate (**VIII**). Betulin 3β,28-diacetate (200 mg) in 15 mg of CH_2Cl_2 was cooled to −78 °C, and O_3 was bubbled into the solution. When the reaction was complete (TLC), the solution was warmed to room temperature, and 1 g of Zn dust and 1.5 mL of HOAc were added. After stirring for 4 h, the suspension was filtered, and the filtrate was washed with aqueous $NaHCO_3$ followed by H_2O. After drying over $MgSO_4$, the solvent was evaporated under vacuum to yield a gum which was chromatographed over silica gel eluted with 10% EtOAc/hexane to give 145 mg (72%) of **VIII**: mp 190−191 °C [lit. 188−189 °C (Vystrcil and Budesinsky, 1970)]; IR (KBr) 2848, 1738, 1710, 1450, 1370, 1030 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.48 (m, 1H, 3 αH), 4.18 (d, J = 11.3 Hz, 1H 28H), 3.72 (d, J = 11.3 Hz, 1H, 28H), 2.65 (m, 1H, 19βH), 2.16 (s, 3H, Ac), 2.07 (s, 3H, Ac), 2.04 (s, 3H, Ac), 1.01−1.92 (complex, CH_2, CH), 1.01, 0.99, 0.84, 0.83 (all s, 15H, 5 × CH_3); MS(EI), *m/e* (rel int.) 528 (M^+, 5.4%), 510 (0.4%), 468 (36%), 453 (14%), 425 (22%), 365 (7%), 203 (25%), 189 (100%), 161 (40%), 135 (57%), 121 (56%), 107 (61%).

B. Bioassays. Colorado potato beetle fourth instar larvae used in the assays were reared at 24 °C, ca. 50% RH, 16:8 (L:D) photoperiod on potato foliage (*Solanum tuberosum* L. cv. Katahdin) from greenhouse-grown plants. No-choice feeding assays to assess antifeedant activity were conducted in feeding arenas made of plastic Petri dishes (15 × 90 mm) lined with moistened filter paper. Disks (1.1 cm diameter, ca. 1 cm^2) cut from potato leaves with a corkborer were coated on the upper surface with 50 μL of a solution of the compound in acetone (treated) or with 50 μL of acetone only. Treated or control disks were placed equidistant from each other on the top of



*Bars within a compound with the same letter are not significantly different, Tukey test, alpha = 0.05.

Figure 2. Antifeedant activity of betulin derivatives.

the filter paper in each dish. At the beginning of each test, one fourth instar that had molted within 24 h prior to the test was placed into each arena for 6–8 h. Assays began 4–5 h after the start of photophase. Arenas were placed in clear plastic ventilated boxes containing moist paper towels and were kept at 24 °C in an environmental chamber. Each compound was tested at three dosages in 10–23 arenas. The amount of leaf material consumed in each arena was determined by weighing to the nearest 0.1 mg the oven-dried (24 h at 100 °C) leaf material remaining uneaten at the end of the test. Initial leaf weight was estimated from the mean dry weight of an additional 30–60 leaf disks. Consumption was calculated as the mean initial leaf disk weight minus the uneaten leaf disk weight. A treatment/control consumption ratio was calculated for each compound at each dosage level. Percentage reduction of feeding (%FR) was calculated as

$$\%FR = (1 - \text{mean treatment consumed/mean control consumed}) \times 100$$

Treatment effects were determined by the method of variance. Mean %FRs were compared using a Tukey test (Tukey, 1953).

RESULTS AND DISCUSSION

Isolation of pure betulin (**I**, Figure 1) was accomplished by extraction of mixed bark of *B. papyrifera* and *B. populifolia* with $CHCl_3$, followed by chromatography on silica gel. In feeding assays against L. decemlineata, betulin was not significantly active even at the highest level of application (100 $\mu g/cm^2$). Modification of betulin by diacetylion (**II**) also did not result in activity at the highest applied dosage. Side-chain oxidation, however, did generate antifeedant activity (Figure 2). Introduction of functionality into the side chain of **II** was accomplished by epoxidation of the 20,29-double bond to form **III**. Epoxide **III** displayed antifeedant activity at application dosages of both 100 and 30 $\mu g/cm^2$. Acid-catalyzed opening of the epoxide yielded **IV**, a betulin diacetate derivative bearing an E-ring allyl alcohol side chain. Compound **IV** exhibited significant activity, and dose response measurements indicated an ED_{50} (dose required for 50% feeding reduction) of 96 $\mu g/cm^2$. Acetylation of **IV** yielded **V**, a compound with greatly reduced activity. Oxidative cleavage of the 20,29-double bond of **IV** are ketone **VI**, a very active betulin derivative displaying an ED_{50} of 15 μg/disk. As was the case with **IV**, acetylation of **VI** reduced its activity considerably. Compound **VIII**, a betulin derivative formed by ozonolysis of betulin diacetate, displayed antifeedant activity, but it was not as high as that observed when the 30-position was also hydroxylated (**VI**).

We have demonstrated that simple oxidative modification of the side chain of betulin diacetate results in products with significant antifeedant activity against the agriculturally important Colorado potato beetle. The level of activity of **VI** is comparable to that of limonin (ED_{50} = 8 $\mu g/cm^2$), a tetranortriterpene occuring in the seeds of citrus species and one of the most active known antifeedants against the Colorado potato beetle (Alford et al., 1987). Although the levels of activity we have observed are low when compared to the effective dosages exhibited by synthetic insecticides, other factors merit consideration in considering the potential for application of antifeedants. For Colorado potato beetle, for example, pesticides are becoming increasingly less effective. In addition, target specificity is often a characteristic of antifeedants not found for synthetic pesticides. The betulin derivatives described here are not active against another agriculturally important insect, *H. zea*; likewise, a betulin-derived diosphenol glucoside antifeedant for *H. zea* which we reported earlier (Lugemwa et al., 1990), is not active against Colorado potato beetle.

As non-naturally occurring semisynthetics, the active compounds we have discovered have played no role in the defensive strategy of the genus *Solanum* in its coevolution with the Colorado potato beetle. The defensive allelochemicals of this genus are the *Solanum* alkaloids (Levinson, 1976), a group of steroidal alkaloid glycosides that are toxic and depress feeding on the plant by the beetle. Biological activity is not a function necessarily of compound classification (i.e. alkaloids or terpenoids) but is the result of interaction of molecules of the correct shape, lipophilicity, and electron density patterns with a receptor protein. It is possible, but by no means certain, that our oxygenated triterpenoids are mimicking the receptor fit provided by the steroidal alkaloids in inducing antifeedant activity.

We have found in field studies with limonin that pretreatment of potato foliage with that antifeedant lowers the effective dosage required for the microbial pesticide, BT, in subsequent applications (Murray et al., 1993). We have also found that oviposition of Colorado potato beetle is adversely affected by both the antifeedants epilimonol and limonin. Our initial studies on betulin derivatives as potentially useful materials in insect control are promising and merit further exploration. Like limonin, there exists a large and potentially low-cost source of raw materials available for utilization. In nature, coevolution has delivered effective plant defense strategies that are multifaceted and depend on a number of substances with different modes of action. Use of a single approach strategy, as has been the case for pesticides, inevitably leads to resistance and decreasing effectiveness. We are further exploring the characteristics of betulin derivatives to examine the possibility of multiple modes of activity for these compounds and to evaluate possible synergism with other substances.

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