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# A *Heliothis zea* Antifeedant from the Abundant Birchbark Triterpene Betulin

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Four triterpenoids bearing α,β-unsaturated A-ring functionality have been prepared from betulin, an abundant and readily isolated triterpene found in the bark of birch (*Betula spp.*). These compounds were evaluated as antifeedants in laboratory leaf-disk choice assays against bollworm larvae, *Heliothis zea*. One triterpenoid, 19β,28-epoxy-2-(β-D-glucopyranosyloxy)-18α-olean-1-en-3-one (VIII), was found to display high antifeedant activity.

The presence of defensive phytochemicals confers upon many species of plants a degree of protection against insect herbivores. These allelochemicals may act as insecticides, repellents, growth regulators, or antifeedants. There is thus considerable interest in application of these compounds, or related synthetic models, as components of integrated pest management (IPM) programs for protection of agricultural crops (Cutler, 1988; Whitehead and Bowers, 1983). Antifeedants, or feeding deterrents, are often highly specific in their action, an advantage in IPM

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applications. In addition, they may be systemic and are likely to be readily biodegradable. A potential disadvantage is the common difficulty of separating pure antifeedants from phytochemical mixtures and the consequent necessity of using complex phytochemical extracts for which quality control may be a problem. Due to the frequently high structural complexity of antifeedants, total synthesis of many of these compounds for use in agriculture is unlikely. We have thus been particularly interested in developing synthetic compounds structurally related to naturally occurring antifeedants, particularly those that can be readily and inexpensively prepared from abundant natural sources or from inexpensive synthetic chemicals.

Betulin (I) is a triterpene that occurs in the bark of birch (*Betula* species). In view of the ease of isolation of I and the widespread occurrence of birch in the northern latitudes of the world, we have directed our attention toward developing applications for this currently little-used resource. In the case of the paper birch, *Betula papyrifera*, betulin constitutes about 12% of the dry weight of the bark (O'Connell et al., 1988) and as much as 30% in *Betula verrucosa*, a Scandinavian species (Ekman, 1983). It can be efficiently extracted from the pulverized bark with toluene, chloroform, or ethanol and isolated in greater than 95% purity by recrystallization. The major impurity is lupeol, but high-purity betulin can be obtained by silica gel column chromatography.

Betulin has been reported to exhibit some antifeedant activity against the aphid (*Myzus persicae*) (Schoonhoven and Derksen-Koppers, 1976), but we have found it to be inactive as an antifeedant against spruce budworm (*Choristoneura fumiferana*), Colorado potato beetle (*Leptinotarsa decemlineata*), bollworm (*Heliothis zea*), and fall armyworm (*Spodoptera frugiperda*). With functionality in the A and E rings, betulin offers the possibility of substantial structural modification. Since $\alpha,\beta$-unsaturated systems have frequently exhibited biological activity, we have focused on preparation and assay of this class of compounds from betulin. Here we report a semisynthetic antifeedant of that class that is active against the bollworm, *Heliothis zea*.

## MATERIALS AND METHODS

NMR spectra were obtained on a Varian XL 200 and FTIR spectra on a Biorad FTS-60 system. Medium-resolution mass spectra were recorded on a HP-5985-B system and high-resolution spectra on a VG-70E at Auburn University by Dr. George Goodloe.

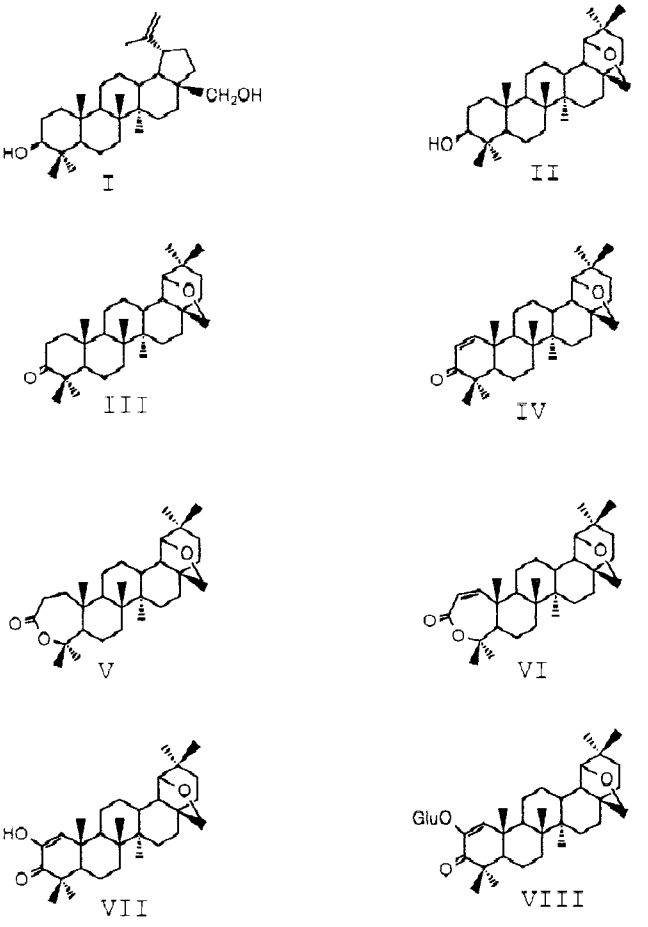
**a. Preparation of Materials.** *1. Isolation of Betulin (I).* Betulin was isolated from mixed bark of paper birch (*B. papyrifera*) and gray birch (*Betula populifolia*). Pulverized, dry bark (1 kg) was extracted overnight with chloroform on a Soxhlet apparatus. The extract was evaporated to dryness under vacuum to give 215 g of crude betulin, which was purified by column chromatography on silica gel eluted with 5% ethyl acetate/hexane. The pure compound [108 g; mp 255–256 °C [lit. mp 254 °C (Schulze and Perot, 1922)]] exhibited the following spectral properties: IR (KBr) 3400, 3080, 1645, 1450, 1380, 1010, 890 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 4.68 (m, 1 H, 29-H), 4.58 (m, 1 H, 29-H), 3.81 (d, $J$ = 10.3 Hz, 1 H, 28-H), 3.35 (d, $J$ = 10.3 Hz, 1 H, 28-H), 3.17 (m, 1 H, 3-H), 2.38 (m, 1 H, 19-H), 1.05–2.05 (complex, $CH_2$, CH), 1.68 (m, 3 H, 30-H), 1.02, 0.98, 0.97, 0.82, 0.76, (all s, 15 H, 5 × $CH_3$); MS (EI) $m/z$ (relative intensity) 442 ($M^+$, 16), 411 (32), 381 (15), 234 (34), 207 (65), 189 (100), 175 (43), 147 (44), 121 (74), 107 (81).

*2. Allobetulin Formate.* After a mixture of I (1 g, 2.26 mmol) was refluxed in 88% formic acid (10 mL) for 50 min, 30 mL of 95% ethanol was added and the mixture cooled to yield, after filtration, 0.86 g of crude product which was recrystallized from 95% ethanol to give pure allobetulin formate: 0.8 g, 75%; mp 310–311 °C [lit. mp 311–312 °C (Schulze and Perot, 1922)]; IR (KBr) 2960, 2940, 2860, 1700, 1185 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 8.10 (s, 1 H, HCOO), 4.60 (m, 1 H, 3-H), 3.76 (d, $J$ = 7.8 Hz, 1 H, 28-H), 3.52 (s, 1 H, 19-H), 3.43 (d, $J$ = 7.8 Hz, 1 H, 28-H), 1.28–1.78 (complex, $CH_2$, CH), 0.97, 0.92, 0.90, 0.86, 0.85, 0.78 (all s, 21 H, 7 × $CH_3$); MS (EI) $m/z$ (% of base peak) 470 ($M^+$, 12), 424 (11), 399 (11), 355 (14), 235 (7), 189 (75), 135 (82), 121 (87), 107 (100).

*3. Allobetulin (II).* To 2.25 g of allobetulin formate was added 20 mL of 1 N KOH in EtOH and the mixture heated to boiling. Toluene was added slowly to the suspension until it became homogeneous, and boiling was continued another 30 min. The solvent was then removed by vacuum evaporation to obtain the crude product (2.02 g), which was recrystallized from 95% EtOH to give II as white crystals: 1.90 g, 90%; mp 265–266 °C [lit. mp 260–261 °C (Schulze and Perot, 1922)]; IR (KBr) 3445, 2950, 1450, 1380, 1040 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 3.76 (d, $J$ = 7.9 Hz, 1 H, 28-H), 3.52 (s, 1 H, 19-H), 3.42 (d, $J$ = 7.9 Hz, 1 H, 28-H), 3.20 (m, 1 H, 3-H), 1.01–1.78 (complex, $CH_2$, CH), 0.96, 0.92, 0.91, 0.83, 0.78, 0.75 (all s, 21 H, 7 × $CH_3$); MS (EI) $m/z$ (% of base peak) 442 ($M^+$, 14.1), 424 (12.7), 411 (6.8), 371 (8.9), 220 (16.2), 207 (58.0), 203 (34.7), 189 (94.7), 135 (79.1), 121 (78.1), 107 (100).

*4. Allobetulone (III).* To compound II (3 g) in acetic acid (100 mL) was added 1 g of chromic acid, and the mixture was stirred at 60 °C for 1 h. The product mixture was poured into water and filtered to obtain 2.3 g of crude product, which was purified by column chromatography on silica gel (Kieselgel 60, 70–130) and eluted with $CHCl_3$ to yield 2.2 g (75%) of III: mp 230–231 °C [Lit. mp 230–231 °C (Schulze and Perot, 1922)]; IR (KBr) 2940, 1700, 1450, 1360, 1040 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) $\delta$ 3.77 (d, $J$ = 7.9 Hz, 1 H, 28-H), 3.52 (s, 1 H, 19-H), 3.44 (d, $J$ = 7.9 Hz, 1 H, 28-H), 2.48 (m, 2 H, 2-H), 1.94 (m, 1 H, 5-H), 0.82–1.56 (complex, $CH_2$, CH), 1.06, 1.02, 1.00, 0.93, 0.92, 0.91, 0.79 (all s, 21 H, 7 × $CH_3$); MS (EI) $m/z$ (% of base peak) 440

**Chart I**



($M^+$, 22.1), 369 (25.4), 220 (14.5), 205 (41.7), 203 (24.4), 191 (34.5), 149 (61.1), 135 (59.6), 121 (75.0), 107 (100).

*5. 19β,28-Epoxyolean-1-en-3-one (IV).* A solution of II (0.5 g) and 0.85 g of benzeneselenenic anhydride in chlorobenzene was refluxed for 25 min, cooled to room temperature, and filtered. The recovered solid was chromatographed on silica gel (Kieselgel 60, 70–130 mesh), eluting with 5% EtOH–hexane to give 0.3 g of crude product, which was purified by recrystallization from toluene–ethyl acetate to yield 0.26 g (50%) of IV: mp 249–250 °C [lit. mp 252 °C (Klinot and Vystrcil, 1960)]; IR (KBr) 3040, 2940, 2870, 1680, 1450, 1385, 1040 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 7.14 (d, $J$ = 10.2 Hz, 1 H, 1-H), 5.81 (d, $J$ = 10.2 Hz, 1 H, 2-H), 3.78 (d, $J$ = 7.8 Hz, 1 H, 28-H), 3.54 (s, 1 H, 19-H), 3.45 (d, $J$ = 7.8 Hz, 1 H, 28-H), 0.96–1.6 (complex, $CH_2$, CH), 1.13, 1.08, 1.05, 0.94, 0.92, 0.80 (all s, 21 H, 7 × $CH_3$); MS (EI) $m/z$ (% of base peak) 438 ($M^+$, 12.0), 367 (12.6), 245 (38.7), 215 (75.5), 150 (92.4), 137 (100), 121 (69.4), 107 (71.7).

*6. 19β,28-Epoxy-A-homo-4-oxaolean-3-one (V).* A mixture of *m*-chloroperbenzoic acid (0.3 g), $NaHCO_3$ (0.3 g), and III in $CH_2Cl_2$ (20 mL) was stirred 4 days at room temperature and then sequentially washed with 10% KI solution, 5% $NaHCO_3$, 1% $Na_2SO_3$, and water. After being dried over $Na_2SO_4$, the solution was evaporated to yield 0.38 g (77%) of V: mp 235–237 °C [lit. mp 246–248 °C (Sejbal et al., 1985)]; IR (KBr) 2950, 2870, 1715, 1450, 1375, 1285, 1115 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 3.77 (d, $J$ = 7.8 Hz, 1 H, 28-H), 3.54 (s, 1 H, 19-H), 3.45 (d, $J$ = 7.8 Hz, 1 H, 28-H), 2.25 (m, 2 H, 2-H), 1.23–1.89 (complex, $CH_2$, CH), 1.48, 1.40, 1.08, 1.02, 0.94, 0.91, 0.80 (all s, 21 H, 7 × $CH_3$); HRMS ($M^+$) for $C_{30}H_{48}O_3$, calcd $m/z$ 456.3603, found $m/z$ 456.3619.

*7. 19β,28-Epoxy-A-homo-4-oxa-18α-olean-1-en-3-one (VI).* A mixture of V (0.5 g) and benzeneselenenic acid (590 mg) in chlorobenzene (10 mL) was refluxed 7 days and then chromatographed on silica gel, eluting with 10% ethyl acetate–hexane. The product was recrystallized from EtOH to yield 0.31 g (61%) of VI as a white solid: mp 235–237 °C; IR (KBr) 3060, 2940, 2870, 1695, 1450, 1380, 1285, 1100 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 6.53 (d, $J$ = 12.2 Hz, 1 H, 1-H), 5.83 (d, $J$ = 12.2 Hz, 1 H, 2-H), 3.77 (d, $J$ = 7.8 Hz, 1 H, 28-H), 3.54 (s, 1 H, 19-H), 3.45 (d, $J$ = 7.8 Hz, 1 H, 28-H), 1.26–1.98 (complex, $CH_3$, CH), 1.43, 1.41, 1.24, 1.05, 0.93, 0.90, 0.88 (all s, 21 H, 7 × $CH_3$); MS (EI) $m/z$ (% of base peak) 454 ($M^+$, 32.5), 436 (19.5), 411 (33.7), 381 (24.2), 245 (100), 215 (52.7), 189 (48.6), 177 (47.0), 149 (49.4), 121 (92.7), 107 (87.3); HRMS ($M^+$) for $C_{30}H_{46}O_3$, calcd $m/e$ 454.34467, found $m/e$ 454.34368.

*8. 19β,28-Epoxy-2-hydroxy-18α-olean-1-en-3-one (VII).* To a solution of III (0.44 g) in dry toluene–*tert*-butyl alcohol (1:1, 20 mL) was added a solution of potassium *tert*-butoxide (0.56 g) in *tert*-butyl alcohol (10 mL), and oxygen was bubbled into the stirred mixture for 30 min. The product mixture was acidified with HOAc and extracted with $CH_2Cl_2$. After being washed with 5% aqueous $NaHCO_3$ and water, the extract was dried over $MgSO_4$ and evaporated to yield 0.42 g of crude solid, which was recrystallized from ether–methanol to give white needles: mp 245–246 °C [lit. mp 245–246 °C (Huneck, 1965)]; IR (KBr) 3420, 2964, 2945, 2867, 1699, 1646, 1457, 1402, 1384, 1234, 1033 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 6.49 (s, 1 H, H-1), 5.91 (s, OH, $D_2O$ exchangeable), 3.77 (d, 1 H, $J$ = 7.8 Hz, 28-H), 3.55 (d, 1 H, $J$ = 7.8 Hz, 28-H), 1.2–1.9 (complex, $CH_2$, CH), 1.21, 1.16, 1.12, 1.04, 0.94, 0.92, 0.81 (all s, 21 H, 7 × $CH_3$); MS (EI) $m/z$ (% of base peak) 454 ($M^+$, 66.5), 229 (25.6), 215 (69.0), 154 (63.6), 151 (65.9), 135 (63.7), 109 (58.5), 107 (63.6), 81 (98.7), 55 (100).

*9. 19β,28-Epoxy-2-(β-D-glucopyranosyloxy)-18α-oleanan-1-en-3-one (VIII).* Compound VII (0.45 g), acetobromo-α-D-glucose (0.4 g), silver oxide (0.46 g), and silver acetate (0.17 g) were refluxed in toluene (100 mL) for 48 h, cooled, and filtered. The filtrate was concentrated under vacuum to furnish a light brown solid that was dissolved in MeOH (30 mL) containing $K_2CO_3$ (1.1 g) and $H_2O$ (1 mL), and the resulting mixture was refluxed for 1 h. The resulting mixture was cooled and filtered to give a light brown solid that was chromatographed on silica gel, eluting with EtOAc–$CHCl_3$ (10–70% gradient) to yield pure VIII: 0.32 g, 52%; mp 157–158 °C; IR (KBr) 3424, 2962, 2945, 2867, 1670, 1646, 1458, 1400, 1384, 1234, 1033 $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 6.77 (s, 1 H, H-1), 4.53 (d, $J$ = 7 Hz, 1 H, 1'-H), 3.42–3.90 (complex, $CH_2OH$, CHOH), 1.16–1.89 (complex, $CH_2$, CH, OH), 1.16, 1.13, 1.10, 1.04, 0.94, 0.92, 0.80 (all s, 21 H, 7 × $CH_3$); HRMS (FAB) (M + 1) for $C_{36}H_{57}O_8$, calcd 617.4053, found 617.4072.

**b. Bioassays.** *H. zea* eggs were obtained from the Southern Grain Insects Research Laboratory, USDA—ARS, Tifton, GA. Insects were reared on pinto bean diet (Burton, 1969) according to the techniques of Shorey and Hale (1965). The colony was kept in environmental chambers at 26 °C and a 18:6 (L:D) photoperiod.

The assays were conducted in arenas constructed from plastic Petri dishes (15 × 90 mm). A circle of moistened filter paper (9-cm diameter) was placed on the floor of each arena. Corn (*Zea mays* L.) foliage disks (11-mm diameter; ca. 1 $cm^2$) were cut with a cork borer. The disks were anchored to the filter paper by small map pins. Treated leaf disks were coated on the upper surface with 50 μL of a solution of the test compound in acetone; control disks received 50 μL of acetone. In each case, solvent was allowed to evaporate prior to using the disks in the assay.

Choice assays were used to assess antifeedant activity. In this assay, five treated disks and five control disks were alternately positioned in each arena (Jermy et al., 1968). Earlier observations indicated a fourth instar would consume 25–50% of the leaf material in a control arena in 19–22 h. Ten arenas were run for each test. Arenas were placed in clear plastic ventilated crisper boxes containing moist paper toweling and placed in an environmental chamber at 26 °C. Consumption was determined by weighing the oven-dried (24 h at 100 °C) remains of disks for each arena. Initial weights were determined by drying an additional 50 disks. Mean daily weights of these blank disks were assumed to be the initial weights of the assay disks. Calculations of amounts of treated or control disks eaten were made by subtracting the weight of the remains from the initial weight for the appropriate test. If this calculation resulted in a negative number, the amount eaten was assumed to be zero. A treatment/control consumption ratio was established for each arena. Percent feeding depressiong (% FD) was determined by the equation (Alford, et al., 1987) % FD = [1 – (treatment consumption/control consumption)] × 100.

## RESULTS AND DISCUSSION

Betulin was readily isolated in high purity from the bark of mixed *Betula* species by extraction and chromatography. In order to simplify the modification of the A ring to various α,β-unsaturated systems, we first converted the E ring to the cyclic ether, allobetulin (II), in two steps. This involved an acid-catalyzed ring closure with ring expansion, followed by saponification of the resulting allobetulin formate. This procedure can also be accomplished successfully without purification of the intermediate formate. α,β-Unsaturated ketone IV was obtained directly from II by reaction with benzeneselenenic anhydride. α,β-Unsaturated lactone VI was obtained from allobetulin by a sequence involving oxidation to ketone III, Baeyer-Villager expansion to lactone V, and finally, oxidation with benzeneselenenic anhydride to VI. Diosphenol (VII) was obtained by oxidation of III with oxygen in the presence of potassium *tert*-butoxide. β-D-Glucoside VIII was formed by the silver-assisted reaction of VII with acetobromo-α-D-glucose, followed by saponification of the acetate esters.

Compounds IV, VI, VII, and VIII were evaluated as antifeedants against larvae of *H. zea* in choice assays. In the initial screening procedures, high doses (100 μg/leaf disk) were utilized. In these assays, only diosphenol glucoside VIII displayed statistically significant activity. Dose–response results for VIII are presented in Table I corresponding to an $ED_{50}$ of 4 μg/disk and an extrapolated threshold of 1.6 μg/disk. For comparison, similar data are also included for limonin ($ED_{50}$ = 70 μg/disk), a tetranortriterpene (limonoid) previously reported to display significant activity against this insect (Klocke and

**Table I. Antifeedant Activity of Compound VIII and Limonin against *H. zea* Larvae in Choice Leaf-Disk Assays**

| | % FD (±SE) | |
|---|---|---|
| dosage, $\mu g/cm^2$ | VIII | limonin |
| 100 | 92(0.04) | 55(0.08) |
| 31.7 | 88(0.04) | 38(0.09) |
| 10 | 83(0.04) | 8(0.01) |
| 3.17 | 40(0.12) | 0(0.13) |

Kubo, 1982). The performance of compound VIII is clearly quite substantial and represents an activity level comparable to that of the better naturally occurring antifeedants reported in the literature against various insects. Naturally occurring antifeedants are often highly selective in their antifeedant activity. We have found that VIII does not display high activity against either the Colorado potato beetle (*L. decemlineata*) or the fall armyworm (*S. frugiperda*).

It is clear that the presence of $\alpha,\beta$-unsaturation does not necessarily result in antifeedant activity against *H. zea*. Obacunone, for example, is a limonoid bearing a seven-membered A-ring $\alpha,\beta$-unsaturated lactone, and it has been reported to have high antifeedant activity against *H. zea*. Our synthetic model compound VI, however, bears the same A ring and is inactive even at high doses. Jacquinic acid, a triterpene having a six-membered A-ring $\alpha,\beta$-unsaturated ketone, is active against the leafcutting ant (*Atta cephalotes*) (Chen et al., 1983) and curcurbitacin E, a triterpene bearing a six-membered A-ring $\alpha,\beta$-unsaturated diosphenol, is active against the flea beetle (*Phylloyeta memorum*) (Nielson et al., 1977). Unfortunately, these triterpenes have not been evaluated against *H. zea*, but we have found the betulin-derived triterpenoids IV and VII to be inactive at high doses against the latter insect. It is interesting that while the diosphenol VII is inactive, the $\alpha$-D-glucoside is a potent antifeedant. Originally, we had decided to examine the glucoside as a means of greatly increasing the polarity and thus altering transport properties. However, at this point, we cannot be certain of the reason for the greatly increased activity of the glucoside. A simple polarity effect may be involved, or there may be some more specific interaction with a glucose receptor involved in food choice discrimination. We are pursuing studies aimed at determining the site of interaction resulting in this observed feeding behavior and are exploring further structure–activity studies in order to more clearly define the structural requirements for this activity.

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