Tobacco Caterpillar Antifeedent from the Gotti Stem Wood Triterpene Betulinic Acid

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Betulinic acid (**I**), a pentacyclic triterpene, on derivatization gives six compounds: 3*β*-hydroxylup-20(29)-en-28 oic acid methyl ester (**II**), 3*β*-acetoxylup-20(29)-en-28-oic acid (**III**), 3*β*-allyloxylup-20(29)-en-28-oic acid (**IV**), 3*β*-*p*-methylcinnamatoxylup-20(29)-en-28-oic acid (**V**), 3*β*-*p*-methoxycinnamatoxylup-20(29)-en-28-oic acid (**VI**), and 3*β*-tri-*O*-methylgallotoxylup-20(29)-en-28-oic acid (**VII**). Their antifeedent activity against the agricultural pest tobacco caterpillar larvae (*Spodoptera litura* F) in a no-choice laboratory study showed the active compounds are **V**, **VI**, and **VII**.

Keywords: *Antifeedent; betulinic acid derivatives; tobacco caterpillar; Spodoptera litura (F)*

There is a revival of interest in the isolation of insecticidally active compounds from indigenous plants (Benerji et al., 1982). Indeed, to our knowledge neem (*Azadirachta indica*) seed extract is the only antifeedent currently marketed. Plant products have distinct advantage over commercial organic synthetic insecticides. Some of these compounds have multiple types of activity including toxicity (Zehnder et al., 1988), growth regulation activity (Liu et al., 1991), and oviposition deterrence (Murray et al., 1993; Liu et al., 1989). They also enhance the activity of other insect control agents such as *Bacillus thuringiensis* (Salama and Sharby, 1988). Antifeedents of natural origin or their derivatives are specific to target (Benerji et al., 1982; Liu et al., 1989, 1991) and generally do not cause toxicity or residue problems.

Isolation of pure naturally occurring antifeedents is tedious and expensive, and these substances are seldom available from natural sources in the quantities necessary for agricultural applications. Furthermore, the total synthesis of these compounds on a large scale is generally econmically impractical. The problem of economically viable production of antifeedents might be solved either by simple synthetic transformation, derivatization of easily isolated natural products (Bently et al., 1995), or by synthesis of natural product model compounds having structures that mimic the essential part of the more active and complex natural products. Ley et al. (1987) have reported the synthesis of model antifeedents based on azdirachtin and have also reported models based on limonin (Bentley et al., 1990).

We are particularly interested in the study of the antifeedent activity of plant products (Srimannarayana et al., 1989) and semisynthetic derivatives of betulinic acid. These pentacyclic triterpenes of the lupane group are abundant in the stem wood of *Zizyphus xylopyrus* (Gotti) and *Diospyros perigrina*. Betulinic acid is also reported (Macias et al., 1994) to have allelopathic activity on monocotyledon species *Hordeum vulgara* and *Triticum aestivum* and dicotyledon species *Lactuca sativa* and *Lepidium sativum*. Here we report the simple preparation from betulinic acid of antifeedents for the tobacco caterpillar (*Spodoptera litura* F).

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MATERIALS AND METHODS

NMR spectra were obtained on a Varian Gemini 200 MHz system and FT-IR spectra on a Perkin-Elmer 1605 system. Mass spectra were obtained on a Perkin-Elmer Hitachi RMU-6L and MS-30. Uncorrected melting points were determined in a sulfuric acid bath. Plant voucher (No. GSN/23/94) was kept in the Department of Botany, Osmania University, Hyderabad.

Preparation of Materials. *Isolation of Betulinic Acid (**I**).* Betulinic acid was isolated from *Zizyphus xylopyrus* collected from the Mannnnoor forest, Mahaboobnager District, AP. Pulverized, dry stem wood (6 kg) was extracted overnight with methanol on a Soxhlet apparatus. The extract was evaporated to dryness under vacuum to give 110 g of crude, which was purified by column chromatography on silica gel (100−200 mesh) eluted with 5% EtOAc/benzene. One hundred twenty fractions, each 500 mL, were collected. Fractions 42−65 yielded betulinic acid, which was recrystallized from methanol: pure compound [2 g, 0.033%, mp 280 °C (lit. mp 285 °C (Ikuta et al., 1988))].

*3β-Hydroxylup-20(29)-en-28-oic Acid Methyl Ester (**II**).* **I** (100 mg) was dissolved in 50 mL of freshly prepared ethereal diazomethane kept at 0−5 °C for 24 h and the ether evaporated. The product was recrystallized from methanol to afford 93 mg (90%) of **II**: mp 220−222 °C [lit. 223−225 °C (Ritti Kawaguiti et al., 1940)]; IR (KBr) 3538, 3065, 2941, 2849, 1707, 1639, 1451, 1373, 1167, 1067, and 978 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.76 and 4.48 (each bs, 2H, 29-H), 3.68 (s, 3H, OCH_3), 3.18 (m, 1H, 3α-H), 2.97 (m, 1H, 19-H), 1.70 (s, 3H, 30-H), and 0.97−0.72 (s, 15H, 5 × CH_3); MS (EI), *m/e* (relative intensity) 470 M^+ (10), 452 (5), 411 (8), 262 (23), 234 (6), 207 (28), 205 (23), 189 (56), 149 (100), and 135 (30).

*3β-Acetoxylup-20(29)-en-28-oic Acid (**III**).* **I** (100 mg) was dissolved in 10 mL of acetic anhydride, two drops of pyridine was added, and the resulting solution was stirred for 20 h at room temperature. The solution was poured onto crushed ice

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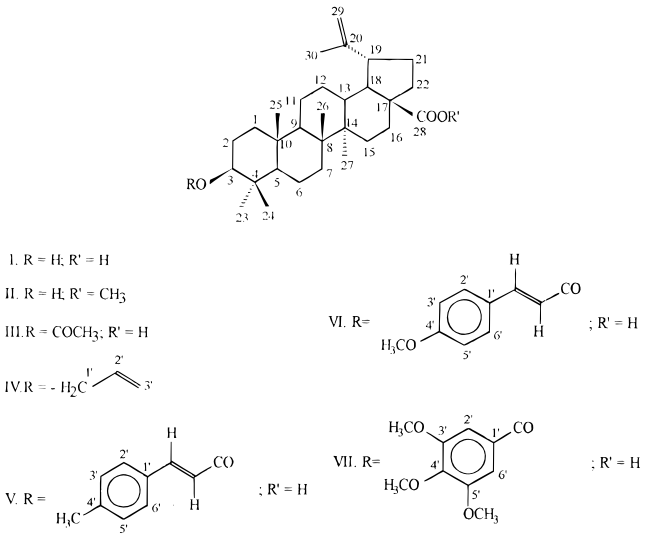

Figure 1. Structures of betulinic acid derivatives.

(100 g), filtered, and washed with water, and the product was recrystallized from methanol to give **III** (80 mg, 73%): mp 288−290 °C [lit. 291−293 °C (Ritti Kawaguiti et al., 1940)]; IR (KBr) 2943, 2870, 1735, 1693, 1640, 1367, 1318, 1195, 1026, and 979 cm^{-1}; ^1H NMR ($CDCl_3$) δ 4.67 and 4.46 (each bs, 2H, 29-H), 2.98 (m, 1H, 3α-H), 2.01 (s, 3H, Ac), 1.67 (s, 3H, 30-H), and 0.99−0.75 (s, 15H, 5 × CH_3); MS (EI), *m/e* (relative intensity) 498 M^+ (3), 454 (4), 438 (12), 248 (6), 189 (25), and 149 (56).

*3β-Allyloxylup-20(29)-en-28-oic Acid (**IV**).* A THF (35 mL) solution of **I** (100 mg) and NaH (150 mg) was stirred at room temperature for 1 h, then allyl bromide (1 mL) was added after 24 h, and the excess NaH was decomposed with methanol (10 mL). The methanol was evaporated under vacuum, and the resulting residue was macerated with ether and washed with water, 5% AcOH, and water (3 × 50 mL) to afford a residue that was chromatographed over silica gel eluting with $CHCl_3$ to give **IV**: 40 mg (37%); IR (KBr) 3071, 2941, 2860, 1689, 1454, 1377, 1190, 1135, 1036, and 943 cm^{-1}; ^1H NMR ($CDCl_3$) δ 5.92 (m, 1H, 2′-H), 5.25 (m, 2H, 3′-H), 4.72 and 4.56 (each bs, 2H, 29-H), 3.95 (m, 2H, 1′-H), 3.21 (m, 1H, 3α-H), 1.71 (s, 3H, 30-H), and 0.98−0.72 (s, 15H, 5 × CH_3); MS (EI), *m/e* (relative intensity) 496 M^+ (1), 438 (4), 411 (30), 393 (4), 220 (25), 207 (50), 189 (89), 175 (30), 135 (56), and 41 (100).

General Procedure for Preparation of Compounds V−VII. A solution of acid (*p*-methylcinnamic acid, *p*-methoxycinnamic acid, or tri-*O*-methylgallic acid, respectively) (1 equiv), DCC (1 equiv), **I** (1 equiv), and DMAP (0.1 equiv) in CH_2Cl_2 (30 mL) was stirred for 24 h at room temperature; DCC urea was filtered and the filtrate washed with water, 5% aqueous AcOH, and water (3 × 25 mL) and then dried ($MgSO_4$) and evaporated under vacuum. The residue was chromatographed by eluting with petroleum ether/EtOAc (6:4 v/v) to give **V**, **VI**, and **VII**, respectively.

*3β-p-Methylcinnamatoxylup-20(29)-en-28-oic Acid (**V**):* yield, 70%; mp 225 °C; IR (KBr) 3269, 2932, 2854, 1708, 1644, 1624, 1596, 1451, 1375, 1152, and 890 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.61 (d, 1H, α-H, J = 16 Hz), 7.38 (d, 2H, 2′,6′-H, J = 8 Hz), 7.16 (d, 2H, 3′,5′-H, J = 8 Hz), 6.66 (d, 1H, β-H, J = 16 Hz), 4.71 and 4.56 (each bs, 2H, 29-H), 2.37 (s, 3H, 4′-CH_3), 1.72 (s, 3H, 30-H), and 0.97−0.72 (s, 15H, 5 × CH_3) ppm; MS (EI), *m/e* (relative intensity) 600 M^+ (2), 509 (5), 368 (18), 288 (6), 243 (17), 205 (6), 160 (38), 145 (100), 117 (25), 115 (25).

*3β-p-Methoxycinnamatoxylup-20(29)-en-28-oic Acid (**VI**):* yield, 76%; mp 245 °C; IR (KBr) 3354, 2933, 2855, 1721, 1638, 1604, 1511, 1451, 1304, 1170, 1037, and 977 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.58 (d, 1H, β-H, J = 16 Hz), 7.45 (d, 2H, 2′,6′-H, J = 8 Hz), 6.88 (d, 2H,3′,5′-H, J = 8 Hz), 6.28 (d, 1H,α-H, J = 16 Hz), 4.72 and 4.56 (each bs, 2H, 29-H), 3.81 (s, 3H, 4′-OCH_3), 1.65 (s, 3H, 30-H), and 0.92−0.71 (s, 15H, 5 × CH_3); MS(FAB), *m/e* (relative intensity) 616 M^+ (3), 438 (8), 393 (12), 367 (25), 248 (10), 221 (48), 207 (56).

*3β-Tri-O-methylgallotoxylup-20(29)-en-28-oic Acid (**VII**):* yield, 86%; mp 256 °C; IR (KBr) 3449, 2999, 2854, 1701, 1637, 1508, 1458, 1338, and 938 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.22 (s, 2H, 2′,6′-H), 4.68 and 4.52 (each bs, 2H, 29-H), 3.88 (s, 6H, 3′,5′-OCH_3), 3.83 (s, 3H, 4′-OCH_3), 1.68 (s, 3H, 30-H), and 0.92−0.67 (s, 15H, 5 × CH_3); MS (EI), *m/e* (relative intensity) 650 M^+ (2), 510 (45), 279 (12), 220 (35), 205 (10), 189 (12), 168 (40), 149 (100), 121 (15).

Bioassays. The antifeedent activity of the compounds was assessed on tobacco caterpillar (*S. litura* F). The tobacco caterpillars were reared on fresh castor leaves (*Ricinus communis*) grown on the Osmania University campus at 27 ± 1 °C, relative humidity 65 ± 5%, and 14:10 light/dark photo-

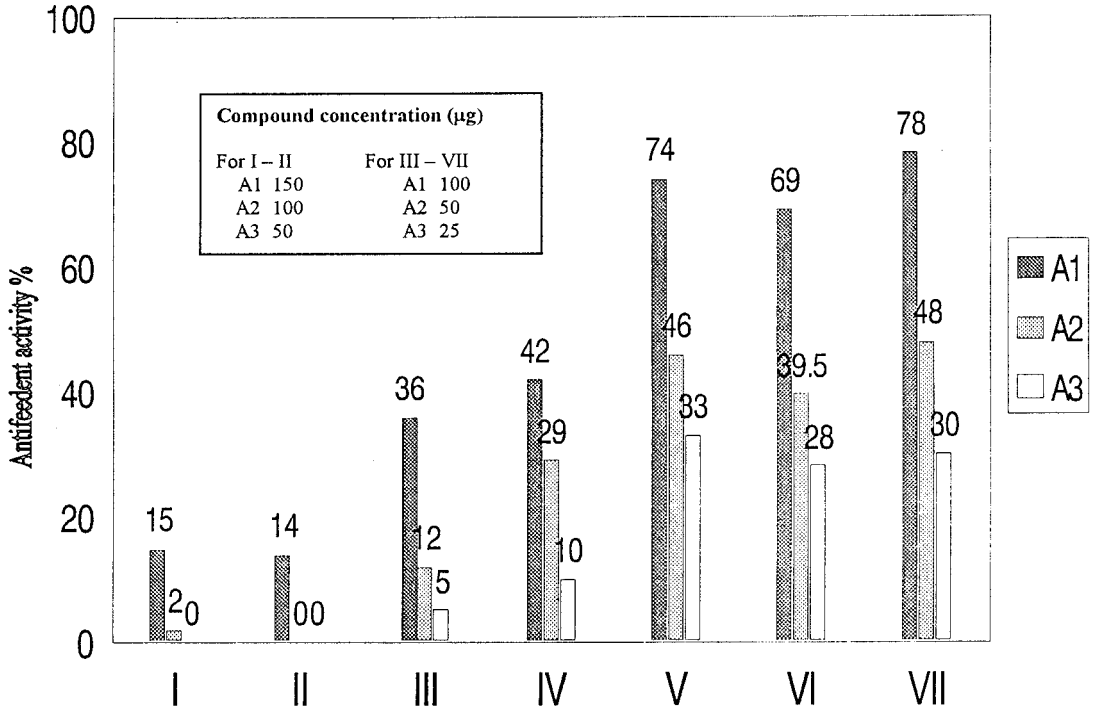

Figure 2. Antifeedant activity of betulinic acid derivatives.

period. Freshly molted fourth-instar larvae were used in the assays. The assays were conducted as described by Ascher and Rones (1964) 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. Castor leaf disks (2 cm diameter) were cut with a cork borer from leaves with well-developed primary leaflets. Treated leaf disks were coated on the upper surface with 100 μL of solution having 5% Triton X-100 of the test compound in acetone; control leaf disks coated with 100 μL of acetone having 5% Triton X-100 only. Acetone was allowed to evaporate before assays were initiated. For these no-choice assays (Jermy et al., 1968), 10 treated and 10 untreated control disks were run for each test and each test was replicated three times. In each Petri dish one prestarved fourth-instar larva was placed. Assays began 4−5 h after the start of the photophase. Arenas were placed in clear plastic ventilated Crisper boxes containing moist paper toweling and placed in an environmental chambers at 27 ± 1 °C. The time period of the experiment was 48 h. Leaf consumption (damaged areas) was measured with the help of a Planimeter, and the percentage of protection was calculated using the following formula by adopting the method of Singh and Panth (1979):

$$\% \text{ of antifeedent activity} = \left[\frac{(\% \text{ protection in treated} - \% \text{ protection in control})}{(100 - \% \text{ protection in control})}\right] \times 100$$

A treatment/control consumption was calculated for each compound at each dosage level.

Variation within the controls of between 0.5 and 1.3% was observed; variation in the treated experiments was between 1 and 3%.

RESULTS AND DISCUSSION

Betulinic acid (**I**) (Figure 1) was extracted from stem wood of *Z. xylopyrus* with MeOH, followed by chromatography on silica gel and recrystallization from methanol. In feeding assays against *S. litura* F, betulinic acid (**I**) was active only at high dose of application (150 μg/cm^2). Derivatization of betulinic acid by methylation with diazomethane gave 3β-hydroxylup-20(29)-en-28-oic acid methyl ester (**II**), which was also active only at high dose of application (150 μg/cm^2). Derivatization of the 3β-hydroxyl group by acetylation with acetic anhydride/pyridine gave 3β-acetoxylup-20(29)-en-28-oic acid (**III**), and allylation with allyl bromide gave 3β-allyloxylup-20(29)-en-28-oic acid (**IV**). Compounds **III** and **IV** have antifeedent activity at both 100 and 50 μg/cm^2. Introduction of an aryl group at the 3β-hydroxyl group by esterification with *p*-methylcinnamic acid, *p*-methoxycinnamic acid, and tri-*O*-methylgallic acid gave 3β-*p*-methylcinnamatoxylup-20(29)-en-28-oic acid (**V**), 3β-*p*-methoxycinnamatoxylup-20(29)-en-28-oic acid (**VI**), and 3β-tri-*O*-methylgallotoxylup-20(29)-en-28-oic acid (**VII**) structures, respectively. These compounds (**IV**−**VII**) showed antifeedent activity at dosages of 100 and 50 μg/cm^2. Compounds **V**−**VII** displayed antifeedent activity at even lower dosages of 25 μg/cm^2. The EC_{50} values for the compounds are 125 μg/cm^2 for **IV**, V is 50 μg/cm^2 for **V**, 70 μg/cm^2 for **VI**, and 50 μg/cm^2 for **VII**. Figure 2 showes the antifeedent activity of betulinic acid and its derivatives at different concentrations. Our study shows the derivatization of the betulinic acid structure by the addition of a methylcinnamic, methoxycinnamic, or tri-*O*-methylgallic acid moiety in the C-3 position of compound **I** increases the antifeedent property of the compounds against the agriculturally important pest tobacco caterpillar (*S. litura* F) in the no-choice laboratory assay.

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LITERATURE CITED

Ascher, K. R. S.; Rones, G. Fungicide has residual effect on larval feeding. *Int. Pest Control* **1964**, *6* (3), 6−8.

Banerji, R.; Misra, G.; Nigam, S. K.; Prasad, N.; Pandey, R. S.; Mathur, Y. K. Indigenous plant as antifeedents. *Indian J. Entomol.* **1982**, *44* (1), 71−76.

Bentley, M. D.; Rajab, M. S.; Mendel, M. J.; Alford, A. R. Limonoid model insect antifeedents. *J. Agric. Food Chem.* **1990**, *38* (6) 1400−1403.

Bentley, M. D.; Huang, F.-Y.; Chung, B. Y.; Alford, A. R. Colorado potato beetle antifeedents by simple modification of the Birchbark Triterpene Betulin. *J. Agric. Food Chem.* **1995**, *43*, 2513−2516.

Ikuta, A.; Itokawa, H. *Phytochemistry* **1988**, *27*, 2813.

Jermy, T.; Hanson, F. E.; Dethier, V. G. Induction of Specific Food Preference in *Lepidoptopterous* Larvae. *Entomol. Exp. Appl.* **1968**, *11*, 211−230.

Ley, S. V.; Santufianos, D.; Blaney, W. M.; Simmonds, M. S. J.; Synthesis of hydrofuran acetal related to azadirichtin; a potent insect antifeedents. *Tetrahedron Lett.* **1987**, *28* (2), 221−224.

Liu, Y.-B.; Alford, A. R.; Bentley, M. D. Effects of limonoids and starvation on feeding and oviposition by *Leptiotarsa decemlineata*. *Entomol. Exp. Appl.* **1989**, *60*, 13−18.

Liu, Y.-B.; Bentley, M. D.; Rajab, M. S.; Alford, A. R. Effects and modes of action of citrus limonoids against *Leptiotarsa decemlineata*. *Physiol. Entomol.* **1991**, *15*, 37−45.

Macias, F. A.; Simonet, A. M.; Esteban, M. D. Potential allelopathic lupane from bioactive fractions of *Melilotus messanesis*. *Phytochemistry* **1994**, *36* (6), 1369−1374.

Murray, K. D.; Alford, A. R.; Drummong, F.; Geoden, E.; Storch, R. H.; Bentley, M. D. Interactive effects of an antifeedeent used with *Bacillus thuringiensis* subsp *Sandiego delta* endotoxin on Colorado potato beetle. *J. Econ. Entomol.* **1993**, *86*, 1793−1801.

Riiti Kawaguiti; Kim, K. W. Constituents of seeds of *Zizyphus vulgaris* Lamark ver, Spinosus Bungi. *J. Pharm. Soc. Jpn.* **1940**, *60*, 343; *Chem. Abstr.* **1941**, *35*, 1396[9].

Salama, H. S.; Sharby, A. Feeding induced by nsome plants in *Spodoptera littorlis* and their pottentianting effects of *Bacillus thuringiensis* Berliner. *Entomol. Exp. Appl.* **1988**, *46*, 93−100.

Singh, R. P.; Panth, N. C. Lycoine—a resistance factor in the plants of subfamily Amaryllidoideae (Amaryllidaceae) against desert lacust *Schitocerca gregri* F. *Experientia* **1980**, *36*, 552−553.

Srimannarayana, G.; Jyostna, D.; Purohit, P. Antifeedent activity of indigenous plant extracts against larvae of castor semi-looper. *Pesticides* **1989**, 23−26.

Zehnder, G.; Wartyhen, J. D. Feeding inhibition and mortality effects of neem seed extracts on the Colorado potato beetle (*Coleoptera*, *Chrysomelidae*). *J. Econ. Entomol.* **1988**, *81*, 1040−1044.

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