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WHY THE YEW TREE (*TAXUS BACCATA*) IS NOT ATTACKED BY INSECTS

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Key Word Index—*Taxus baccata*; 10-deacetylbaccatin III; 10-deacetylbaccatin V; antifeedant activity; storage pests; *Tribolium confusum*; *Trogoderma granarium*; *Sitophilus granarius.*

Abstract—In recent years, the yew tree has attracted considerable attention due to the isolation from it of the important antitumour drug Taxol®. The fact that the wood is not attacked by woodworm and its needles are attacked by very few insects has so far neither been examined nor exploited. Extracts of *Taxus baccata* have strong antifeedant activity against storage pests *Tribolium confusum*, *Trogoderma granarium* and *Sitophilus granarius.* Nine pure compounds were isolated from the extract and very strong antifeedant activity was exhibited by 10-deacetylbaccatin III and 10-deacetylbaccatin V, which most probably are responsible for the activity and the resistance to insects. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

In recent years, the yew tree has attracted considerable attention, due to the isolation from *Taxus brevifolia* Nutt. of the antitumour drug Taxol®. The content of taxol in the bark of Pacific yew is very small (0.01%) and insufficient for its development as a drug. Alternative sources were thus required, and investigations of other species from the genus of *Taxus* were undertaken. The needles of *T. baccata* L. were found to contain a compound (10-deacetyl-baccatin III) useful for the semisynthesis of Taxol®.

The yew tree (*T. baccata*) exhibits a very strong resistance to insect pests; its needles are attacked by very few insects e.g. yew scale (*Lecaniinae*) and some mites (*Eriophyoidea*) [1], and the wood is not affected by woodworm. In ancient times the tree was used to make arms and poisons, and its wood is practically imperishable. There are numerous finds of prehistoric yew spears, one of which is thought to date back to 50,000 years ago [2]. The yew is poisonous because of a mixture of alkaloids, known collectively as taxine [2]. In a continuation of our studies on the resistance to insect pests of various plants and higher fungi [3, 4], we decided to identify the compounds responsible for this phenomenon in the methylene chloride extract of needles of *T. baccata*.

RESULTS AND DISCUSSION

The methylene chloride extract of needles of *T. baccata* showed strong antifeedant activity against the storage pests *Tribolium confusum*, *Trogoderma granarium* and *Sitophilus granarius*. Subsequently the extract was separated into various fractions by column chromatography on silica gel with a hexane–EtOAc gradient solvent system. The antifeedant activity of the fractions was evaluated and fractions exhibiting good activity were separated further and pure compounds isolated. The structures of the compounds were substantiated by exhaustive spectroscopic investigations especially ^{1}H and ^{13}C NMR. Altogether nine pure compounds were isolated and identified by comparison of their spectroscopic data with those found in the literature; these are shown in Scheme 1.

The antifeedant activities of the compounds are shown in Table 1. It was found that the most active compounds were 10-deacetyl baccatins III (**1**) and V (**2**), and no doubt they are responsible for the activity of the extract. Both derivatives of taxicine (**3** and **4**) exhibited moderate activity. The phenolic derivatives betuligenol (**8**) and 3,5-dimethoxy phenol (**9**) are weak antifeedants against the storage pests.

EXPERIMENTAL

Frontal analysis

This is an isolation technique which allows the separation of mixtures of compounds possessing different

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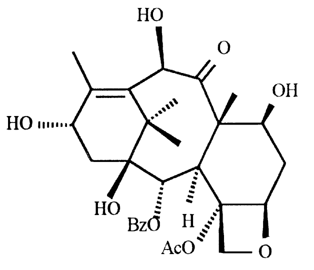
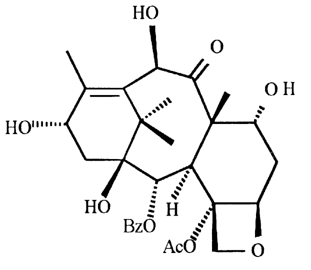
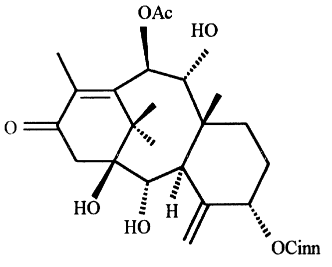


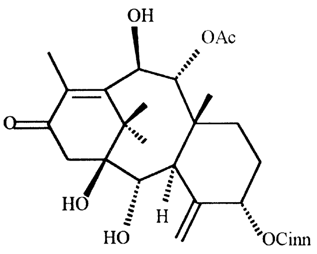
10-deacetyl-baccatin III (1)



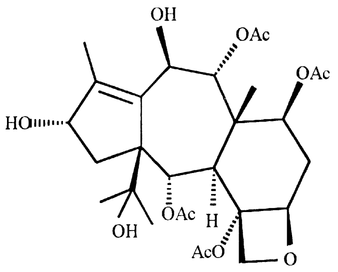
10-deacetyl-baccatin V (2)



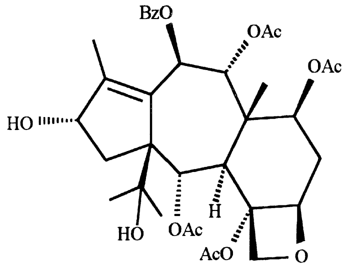
5-cynamoyl-10-acetyl-taxicin I (3)



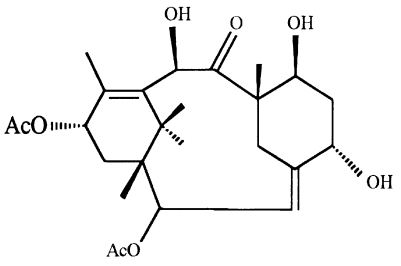
5-cynamoyl-9-acetyl-taxicin I (4)



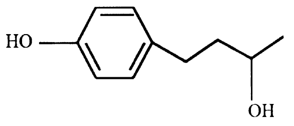
taxacustin (5)



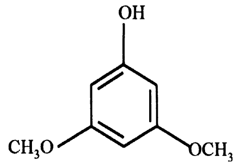
11(15->)*abeo*-taxane-1,4,7,9-tetraacetoxy-10-benzoyloxy (6)



deaminoacyltaxine A (7)



betuligenol (8)



3,5-dimethoxyphenol (9)

Scheme 1.

Table 1. Antifeedant activity of various compounds isolated from *T. baccata*

Compound no	*Tribolium confusum* Adults	*Tribolium confusum* Larvae	*Trogoderma granarium* Larvae	*Sitophylus granarius* Adults	Average activity
1	180.2	149.4	115.5	185.0	157.5
2	145.1	146.4	191.9	172.1	163.8
3	75.5	128.8	9.0	7.3	55.1
4	103.4	129.0	79.0	115.5	106.7
5	126.3	76.3	65.5	47.1	78.8
6	37.5	48.5	112.4	74.1	68.1
7	112.3	129.1	73.6	110.3	106.3
8	−42.1	55.7	44.9	63.5	30.5
9	95.0	22.6	−34.5	136.3	54.8

polarities or solubility by means of a relatively small amount of stationary phase (solid support). The mixture of compounds (extract) dissolved in a suitable solvent is deposited on the solid support (silica gel, × 3 the wt of the extract) by evaporation in a rotary evaporator. The freely running powder, thus obtained, is packed into a chromatographic column (Jobling), which is eluted with a slow gradient solvent system. The separation process starts when the mixture is deposited on the solid support during the evaporation, as the most polar compounds, or the least soluble ones, are deposited first and deeper into the pores of the solid support (silica gel). Subsequently, the constituents of the mixture are eluted in reverse order from the front of the solid phase, hence the term frontal analysis. The elution and collection of fractions is monitored by TLC.

Isolation of compounds **5**, **6** and **8**

Needles and stems of *T. baccata* were supplied by the Plant Survey Division of Regional Research Laboratory (CSIR), Canal Road, Jammu Tawi, India. Dried plant material (1 kg) was extracted with MeOH (5 l) overnight at room temp. The procedure was repeated × 2 and the combined extract (125 g) after evaporation of the solvent, was partitioned between hexane and H_2O and subsequently H_2O and $CHCl_3$. The solvents were removed in a rotary evaporator to give a hexane extract (18 g) and a $CHCl_3$ extract (27 g). The $CHCl_3$ extract (27 g) was deposited on silica gel (100 g) and packed over the top of a glass column filled with silica gel (500 g). The column was eluted using a hexane–EtOAc gradient solvent system. The chromatography was monitored by TLC. Fractions of similar R_f values were pooled together, evaporated, rechromatographed, crystallized to give several compounds from which **5**, **6** and **8** were evaluated for their antifeedant activity.

Taxacustin (**5**). 0.412 g; TLC R_f 0.34 (C_6H_6–Me_2CO 1 : 1); mp 225–228°C; $[\alpha]_D^{20}$ $-39.8°$ (MeOH; *c* 0.89); UV: λ_{max}^{EtOH} nm: 204 (ε 9664); IR ν_{max}^{nujol} cm^{-1}: 3598, 3474, 3417, 1748, 1717; ^1H NMR (500 MHz, MeOH-d_4): δ 5.97 (1H, *d*, $J_{2,3} = 7.5$ Hz, H-2), 3.05 (1H, *d*, $J_{3,2} = 7.5$ Hz, H-3), 4.99 (1H, *d*, $J_{5,6\alpha} = 8.6$ Hz, H-5), 1.66, 1.98 (2H, *m*, $J_{5,6\alpha} = 8.6$, $J_{6\alpha,7} = J_{6\beta,7} = 7.3$ Hz, H-6β, H-6α), 4.50 (1H, *t*, H-7), 5.82 (1H, *d*, $J_{9,10} = 10.2$, H-9), 4.63 (1H, *d*, $J_{10,9} = 10.2$, H-10), 5.42 (1H, *t*, $J_{13,14\alpha} = J_{13,14\beta} = 8.5$ Hz, H-13), 1.80, 2.41 (2H, *m*, H-14α, H-14β), 1.02 (3H, *s*, H-16), 1.15 (3H, *s*, H-17), 1.84 (3H, *s*, H-18), 1.59 (3H, *s*, H-19), 4.37, 4.43 (2H, *d*, $J_{20\alpha 20\beta} = 7.2$ Hz, H-20); ^{13}C NMR (125.7 MHz, MeOH-d_4) δ 68.3 (*s*, C-1), 69.4 (*d*, C-2), 46.2 (*d*, C-3), 80.3 (*s*, C-4), 86.2 (*d*, C-6), 77.6 (*d*, C-7), 44.5 (*s*, C-8), 80.7 (*d*, C-9), 67.5 (*d*, C-10), 138.2 (*s*, C-11), 147.9 (*s*, C-12), 72.4 (*d*, C-13), 35.8 (*t*, C-14), 76.6 (*s*, C-15), 25.3 (*q*, C-16), 28.1 (*q*, C-17), 11.2 (*q*, C-18), 13.0 (*q*, C-19), 75.5 (*q*, C-20); EI MS 70 eV, *m/z* (rel. int.): 550 $[M–H_2O]^+$ (0.6), 532 (0.5), 490 (5), 447 (15), 390 (8), 372 (11), 330 (8), 312 (15), 297 (12), 270 (14), 252 (25), 237 (10), 224 (10), 209 (7), 195 (5), 60 (60), 45 (58), 43 (100); HR-MS: calcd for $C_{28}H_{40}O_{12}$ 568.25298, found *m/z* 568.2518. Structure reported [11].

11(15→1) *abeo*-Taxane-1,4,7,9-tertraacetoxy-10-benzoyloxy (**6**). 1.13 g; TLC R_f 0.46 (C_6H_6–Me_2CO 1 : 1); mp 230°; $[\alpha]_D^{20}$ $-51.8°$ (MeOH; *c* 1.88); All spectroscopic data the same as in references [12, 13].

Betuligenol (**8**). 2.88 g; TLC R_f 0.40 (C_6H_6–Me_2CO 1 : 1); amporphous solid mp 67–68°; HR-MS: calcd for $C_{10}H_{14}O_2$ 166.0994 found *m/z* 166.09955; Spectral data exactly the same as those described in [9].

Isolation of compounds **1**, **2**, **3**, **4** and **7**

Dry needles and stems (4.0 kg) of *T. baccata* grown in our Institute were extracted the same way as described above; however, CH_2Cl_2 instead of $CHCl_3$ was used as the partition extraction solvent. The CH_2Cl_2 extract (117 g) was subjected to "frontal analysis" by depositing it on silica gel (3 × 117 g) which was then packed in a chromatographic column. The column was eluted with a gradient system beginning with pure hexane through hexane–EtOAc (1 : 1) plus an increasing amount MeOH. The analysis was monitored by TLC (hexane–EtOAc–MeOH 47 : 47 : 5). The fraction eluted with hexane–EtOAc–MeOH (49 : 49 : 2) was collected, evaporated and rechromatographed by prep. HPLC on silica gel with CH_2Cl_2–MeOH (99 : 1) solvent system and gave pure 10-deacetylbaccatin III (**3**) (85 mg) identical in all respects with that described in [6, 7].

Similarly from 40 g of the CH_2Cl_2 extract, using frontal analysis, a fraction (3 g) eluted with hexane–EtOAc (2 : 3) was obtained. This was rechromatographed with C_6H_6–Me_2CO (4 : 1) as solvent system (TLC, R_f 0.3 C_6H_6–Me_2CO 7 : 3) and gave a fraction (0.5 g) from which, by prep. HPLC (series of three columns (30 cm × 20 mm) eluted with CH_2Cl_2–MeOH 99 : 1), pure 10 deacetylbaccatin V (**2**) ($K' = 1.26$) (84 mg) identical in all respects with that described in [6] was obtained.

The CH_2Cl_2 extract (200 g, obtained directly from needles of *Taxus baccata*) was subjected to frontal analysis using a CH_2Cl_2–MeOH gradient system. The fraction eluted with 2.5% MeOH in CH_2Cl_2 (40 g) was rechromatographed with a C_6H_6–Me_2CO gradient system. Fractions having R_f 0.35 (TLC C_6H_6–Me_2CO 4 : 1) were collected and subjected to rechromatography with hexane–Me_2CO (7 : 3) as solvent system. The fraction having R_f 0.3 was separated by HPLC using a series of prep. columns (5 col. 30 cm × 8 mm eluted with CH_2Cl_2–MeOH 99 : 1). Two major peaks were isolated and gave 5-cinnamoyl-10-acetyltaxicin I (**3** $K' = 0.83$) (31 mg) and 5-cinnamoyl-9-acetyl-taxicin I (**4** $K' = 0.1.33$) (29 mg), both **3** and **4** having the same physico-chemical as well as spectroscopic data as those described in [8].

The fraction obtained by elution of the above column with CH_2Cl_2–MeOH (19 : 1) was purified by passing through a reverse phase column in MeOH–H_2O (7 : 3). Subsequently, it was purified further by passing through Al_2O_3 (neutral) with C_6H_6–EtOH (7 : 3) as solvent system. CC of the prepurified material in a hexane–EtOAc gradient solvent system allowed the isolation of deaminoacyltaxine A (**7**) identical with that described in [10].

The hexane layer obtained on partition of the CH_2Cl_2 extract was evaporated and subjected to frontal analysis in a hexane–CH_2Cl_2 gradient system. The fraction eluted with hexane–CH_2Cl_2 (1 : 1) was rechromatographed in hexane–EtOAc (93 : 7) and gave 3,5-dimethoxy-phenol (**9**) (190) with the same physicochemical and spectroscopic data as those described [14].

Antifeedant activity test

The test is described in detail in Ref. [5]. Insects (adults and larvae) used for the test were reared under laboratory condition at a temp. of 26° and 75% humidity. All compounds investigated were dissolved in EtOH at a concentration of 10 mg ml^{-1}. Air dry wheat wafer discs were used as the test food. The discs (1 cm in diameter) were saturated with EtOH sols of pure compounds to produce 0.5% (by wt) contamination of the wafer in every test. Feeding of insects was recorded under three conditions: (1) on pure food (control); (2) on food with the possibility of choice (choice test); (3) on food with the compounds tested (no choice test). The wafer discs were weighed after saturation and drying in air for 30 min. before the experiments and again after 7 days of feeding by beetles or larvae. On the basis of eaten food, the index of activity of the compounds tested was calculated in the following way: three values of the food eaten were obtained in the control KK, in the no-choice test EE, and in the choice test KE.
Thus:
The absolute coefficient of antifeedancy

$$A = \frac{\mathrm{KK} - \mathrm{EE}}{\mathrm{KK} + \mathrm{EE}} \times 100,$$

The relative coefficient of antifeedancy

$$R = \frac{\mathrm{K} - \mathrm{E}}{\mathrm{K} + \mathrm{E}} \times 100$$

The total coefficient of antifeedancy is equal to $T = A + R$, and the maximum value of the coefficient can reach 200 for a perfect antifeedant.

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