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BETULIN AND LUPEOL IN BARK FROM FOUR WHITE-BARKED BIRCHES

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Key Word Index—*Betula X caerulea; Betula cordifolia; Betula papyrifera; Betula populifolia*; Betulaceae; birch; chemotaxonomy; triterpenoids; betulin; lupeol.

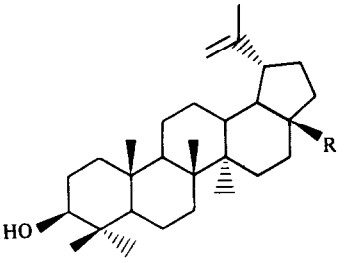
Abstract—Levels of betulin in the outer bark of four species of white-barked birch range from 5.0 to 22.0%. This variation in the level of betulin is taxonomically useful. The percentage of betulin in chloroform extracts is significantly different between two taxa, *Betula cordifolia* and *B. papyrifera*, that are often considered conspecific. In *B. X caerulea*, a putative hybrid of *B. cordifolia* and *B. populifolia*, betulin levels are intermediate between and significantly different from those of the parents. Levels of the codominant triterpenoid, lupeol, are less than 1.3% and are significantly different only between *B. populifolia* and *B. X caerulea*.

INTRODUCTION

Betulin (**1**, lup-20(29)-ene-3, 28-diol) has been extracted from the bark of white-barked birches (*Betula* series *Albae*) in amounts up to 30% dry weight [1, 2]. The dominance of betulin in extractives is reported to give white birch bark its white colour [3–5]. Lupeol (**2**) the precursor of betulin, occurs in smaller amounts [3, 4].

Levels of betulin have been reported to increase with age [3, 5], and vary within the bark and with location in the tree. Generally the base of the tree has fewer extractives than the apex [6]. The outer bark of *Betula platyphylla* var. *japonica* contains four times as many extractives as the inner bark with betulin being the dominant extractive in the outer bark [2]. A similar ratio of outer to inner bark extractives has been reported in *B. verruccosa* (*B. pendula*) [1, 7].

Betulin is the major extractive component of bark of *Betula papyrifera* Marsh. [8], but precise levels of this, or any other component of bark from North American white-barked birch trees, have not been determined. We used gas chromatography to quantify the levels of the dominant components of the chloroform extracts. betulin and lupeol, from the bark of four white-barked birches native to Maine: *Betula papyrifera* (paper or canoe birch), *B. populifolia* Marsh. (gray birch), *B. cordifolia* Regel. (mountain paper birch), and *B. X caerulea* Blanch. (blue birch).



1 R = CH_2OH
2 R = Me

Our specific objectives were (i) to ascertain whether betulin and/or lupeol levels support separate species status for *Betula papyrifera* and *B. cordifolia*, the latter often having been considered a variety of the former [9–11], and (ii) to determine whether betulin and/or lupeol levels in the putative interspecific hybrid, *B. X caerulea*, fall between those of its parents, *B. cordifolia* and *B. populifolia* [12–16].

RESULTS AND DISCUSSION

The significant differences in the amounts of betulin present in *Betula cordifolia* and *B. papyrifera* (Table 1) support species status for *B. cordifolia*, as do many cytological, morphological and phenological differences between the two taxa [12, 15–17]. Lupeol amounts were not significantly different between *B. papyrifera* and *B. cordifolia*. Chemotaxonomically, triterpenoids limited in distribution have been more useful characters at the genus and species level than those of widespread occurrence [18, 19]. Betulin is very restricted in its distribution in plants relative to lupeol [20], and, in our study, lupeol levels were significantly different only between *B. populifolia* and *B. X caerulea* (Table 1).

Betula caerulea was described by Blanchard in 1904 and later designated an interspecific hybrid [12]. That *B. cordifolia* and *B. populifolia* are the parents is supported by multivariate morphometric studies [13, 15, 16], numerical taxonomy [14], and levels of the dominant triterpenoid, betulin, in the outer bark (Table 1). *Betula X caerulea* contains $11.4 \pm 1.6\%$ betulin and *B. cordifolia* and *B. populifolia* $6.8 \pm 0.4\%$ and $17.6 \pm 0.6\%$, respectively. Betulin levels in *B. X caerulea* have the largest vari-

Table 1. Mean percent (±s.e.) betulin and lupeol in bark extracts of species of *Betula*

	n	Betulin	s.e.	Lupeol	s.e.
B. X caerulea	7	11.4[a]	±1.6	0.6[a]	±0.12
B. cordifolia	10	6.8[b]	±0.4	0.4[a,b]	±0.08
B. papyrifera	10	11.9[a]	±1.2	0.6[a,b]	±0.19
B. populifolia	10	17.6[c]	±0.6	1.0[b]	±0.10

[a–c] Values sharing at least one superscript are not significantly different at $P < 0.05$. (Student Newman Keuls Test). Copies of the raw data, on which these mean values are based, are available on request from the authors.

ability (Table 1). Extreme values might be either F_2-segregants, backcrosses, or misclassified individuals.

The seven *Betula X caerulea* individuals in this study were part of DeHond's [16] multivariate morphometric demonstration that this taxon is morphologically intermediate and nearly spans the range of morphological variation between *B. cordifolia* and *B. populifolia*. Percent betulin of these trees corresponds to their location on DeHond's principal components and canonical variates plots. Individuals of *B. X caerulea* with relatively low percent betulin have values on the first principle component and first canonical variate near those of *B. cordifolia*. Similarly trees with relatively high betulin levels are nearer to *B. populifolia*. These results also suggest that inheritance of betulin is polygenic, with numerous loci contributing to the rate and amount of betulin production.

Our measures of betulin in *Betula papyrifera* ($\bar{X} = 11.9\%$) are much larger than the 1.5% previously reported [8]. Our quantitative extractions of North American birches yielded lower percentages than those reported for *B. verrucosa* (24.6%) [1] and *B. platyphylla* var. *japonica* (26%) [2].

It has been reported that betulin amounts increase with age in *Betula verrucosa* [3, 5]. In our study, no statistically significant correlation between betulin and age ($r = 0.436$) or lupeol and age ($r = 0.207$) was seen. Correlation between age and percent betulin would seem unlikely for bark samples from mature trees. After betulin is deposited in dead cork cells, it probably does not undergo degradation and seems to be highly resistant to fungal attack as suggested by exceptional preservation of materials made with birch bark. Had we sampled during the first 15 years of growth, when the trunks turn from brown to white, we might have detected an increase in betulin.

EXPERIMENTAL

Extraction. Outer bark was collected from trees at 1 m above the base of the tree. Trees were cored with an increment borer to determine age.

Bark was ground into 1 mm pieces and dried to constant weight at 100° (*ca* 2 hr). 5–10 50 mg samples of bark were weighed into 10 ml Erlenmeyer flasks and boiled in 5 ml of $CHCl_3$ for 10 min, replenishing the $CHCl_3$ to keep it at about 5 ml. After 10 min, samples were filtered into pear-shaped flasks and the $CHCl_3$ evapd. This procedure was repeated until a constant weight of extract was attained (usually ×3); about 45 ml of $CHCl_3$ or 15 ml per extraction per sample were used.

GC/MS. 1 ml of an int. standard soln (25 mg allobetulone in 50 ml $CHCl_3$) was used to dissolve 0.5–1.0 mg of crude extract. Four μl of each sample were injected into a gas chromatograph equipped with a 30 m × 0.25 mm SE-30 column and FID (using a 100:1 split ratio). The temp. of the injection port and detector were 350°, and the oven temp. was isothermal at 300°. Flow rates for the carrier gas (He) and make-up gas (N_2) were 1.0 ml/min and 40 ml/min, respectively. Identities of betulin and lupeol were confirmed by comparison of mass spectra with those of standard samples using an HP 5970 series mass selective detector.

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