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Assessment of the Anti-HIV Activity of a Pine Cone Isolate

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Abstract

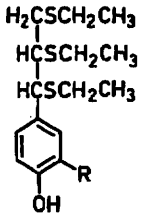
Characterization of an alkali-soluble isolate from *Pinus nigra* Arnold (Pinaceae) seed cones by thioacidolysis revealed a significant phenylpropanoid (i.e. lignin) component which may provide the observed anti-HIV-1 activity for this substance.

Numerous reports claim that an aqueous-alkali treatment of previously extracted pine cones affords, after fractionation, a "lignin-related" isolate having antiviral and antitumor activities (reviewed in reference 1). Interest in pine cones as a source of medicinals appears to stem from a frequently mentioned folklore which suggests that the ingestion of a hot-water extract from *Pinus parviflora* seed cones is an effective treatment for gastric cancers in humans (1–4). Thus far, research has suggested that the most active agent(s) from pine cones is not among the aqueous-soluble extractives, but in the above-mentioned alkali-soluble substance (1–5).

Intrigued by suggestions that such an alkali-soluble isolate from *P. parviflora* seed cones provides a viable treatment for AIDS (2), we applied the reported isolation procedure to mature seed cones of *Pinus nigra*. The isolate of interest, known in the literature as "Fraction VI", was thus obtained and subjected to chemical analysis and anti-HIV-1 screening.

Analysis of our isolate (Fraction VI) by IR and ^{13}C-NMR spectroscopy gave signals coincident with those demonstrated for isolated lignins and polysaccharides (data not shown; spectra are available from the author of correspondence). Klason lignin determinations, commonly used to quantify, but not prove the existence of lignin, gave a value (Table 1) for our isolate which was similar to that reported by Kikuchi et al. (5). Using thioacidolysis, a method which effectively cleaves the β-*O*-4 linkages in lignin to give the lignin monomer units as trithioethyl phenylpropane adducts (6) (Fig. 1), we now provide the first definitive evidence for a phenylpropanoid component in this isolate. A predominance of guaiacyl moieties (typical for conifer woods) was demonstrated for our isolate and a sample of the seed cone tissue. The lower

yields of thioacidolysis products for the seed cone isolate and tissue, relative to that for conifer woods, could suggest either a more condensed lignin structure (i.e. carbon-carbon linked monomers) and/or the inclusion of non-lignin substances (e.g. suberin, protein) in the Klason lignins as often noted for non-wood plant tissues.



p-Hydroxyphenyl: R = H
Guaiacyl: R = OCH_3

Fig. 1 Lignin derived thioacidolysis products.

Sugar analysis of our isolate gave a total amount of neutral sugars (10.2%) similar to that reported by Sakagami et al. (3). For *P. parviflora*, the isolate gave almost no arabinose and no reported value for xylose; fucose was detected in significant amounts (3). In contrast, data from our *P. nigra* seed cone tissue and isolate showed significant amounts of arabinose and xylose but not fucose (Table 1). Data for our standard (*Pinus taeda* wood) matched that well established in the literature (7). Analyses of 5 additional species of pine and spruce cones gave data (submitted for publication) reflecting that shown here and therefore the data for *P. parviflora* appears atypical.

Anti-HIV-1 screening of our isolate also gave an apparent reversal of HIV-1 cytopathic effects (EC_{50} = 9.95 μg/ml) for infected T4 lymphocytes (CEM cell line); cytotoxicity was observed at a ca. 5 fold higher concentration of the isolate (IC_{50} = 56.3 μg/ml). The reported anti-HIV-1 activity for *P. parviflora* therefore does not appear to be species specific. Moreover, since analysis of our isolate (Fraction VI) demonstrated a significant phenylpropanoid component, the anti-HIV-1 activity observed may parallel that shown for other polyphenols (e.g. tannins) (8) by mechanisms suggested to involve protein and/or polysaccharide complexation (9).

Table 1 Lignin and sugar analysis of *P. nigra* seed cones and cell wall derived isolate.

Analysis	Seed Cones	Isolate (Fraction VI)
Klason Lignin (%)	26.9[a]	63.5
Thioacidolysis Products: (μmoles/g Klason Lignin)		
p-Hydroxyphenyl	7.5	4.3
Guaiacyl	454.8	223.4
Sugars: (%)		
Arabinose	2.6[a]	0.7
Galactose	3.0	2.7
Glucose	38.5	3.9
Xylose	6.7	1.3
Mannose	14.9	1.6

[a] Values for Klason lignin and sugars are expressed as weight percents of the dry extractive-free tissue or the dry isolate.

Materials and Methods

Plant material

Mature seed cones of *Pinus nigra* Arnold (Pinaceae) were collected at a local site, rinsed with distilled water, and dried in an oven at 35 °C. After the removal of any attached seeds, the samples were ground in a Wiley mill (4 mm diameter mesh openings). A voucher specimen (Thomas L. Eberhardt *s.n.*) was also collected and deposited in the Botany Department Herbarium at the University of Wisconsin, Madison, Wisconsin 53706, U.S.A.

Extraction and alkali treatment

Seed cone tissue (75 g) was extracted in sequence with methanol, ethanol:water (85:15), and boiling water as previously described (3). Extracts were evaporated in vacuo and/or lyophilized. Total weights of the methanol, ethanol:water, and boiling water extracts were 1.8, 0.5, and 2.6 g, respectively. After extraction, the seed cone tissue was treated twice with 4% NaOH (500 ml) for 4 hours at room temperature (3, 4) to give dark brown suspensions which were centrifuged (20 min, 10,000 × *g*). Adjustment of the pH of the combined supernatants to 5 with glacial acetic acid gave a precipitate which was collected by centrifugation, dialyzed (Spectra/Por 3 membrane) against distilled water, and then lyophilized (Fraction VI, 253 mg). Additional precipitates were collected in the same manner after the addition of 1, 2, and 5 volumes of ethanol to the supernatants (Fractions: VII, 128 mg; VIII, 119 mg; IX, 78 mg).

Chemical analyses and anti-HIV-1 screening

Klason lignin and sugar analyses were carried out at the USDA Forest Products Laboratory using an established method (10). Thioacidolyses were performed using the reported procedure (6). Screenings for anti-HIV-1 activity by the soluble-formazan assay (11) were conducted through the Developmental Therapeutics Program at the National Cancer Institute. All analyses were carried out in duplicate.

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