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PSORALEN, A POWERFUL GERMINATION INHIBITOR

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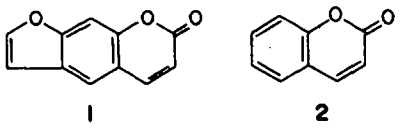
Key Word Index—Psoralen; coumarin; germination inhibitor; photodeactivation.

The furocoumarin psoralen **1** has figured prominently in recent investigations on photodynamic action and photochemical cross linking by several groups of workers [1–4]. In all this work the activation of the furocoumarin has been brought about by irradiation with UV or visible light. We reported on the antibiotic properties [5, 6] of psoralen and on the oral use of psoralen as the best chemotherapeutic agent against vitiligo [7, 8]. The compound in these cases was effective even without activation by UV or visible light. It appeared, therefore, that in these biological effects, photo activation of the furocoumarin is not essential. The seed germination and root growth inhibitory properties of psoralen were reported earlier [10, 11]. The germination inhibitory property of the coumarin **2** has been found to be reversed by light [12, 13]. During a study of the activation of some biological properties of **1** and related compounds, an investigation on the effect of light on the seed germination and root growth inhibitory properties was undertaken. We now report the deactivation of the germination and root growth inhibitory properties of psoralen under the influence of light.



1 **2**

RESULTS AND DISCUSSION

Almost 50% inhibition of germination and root growth were noticed at 10 ppm concentration. The inhibitory activity persisted even at a concentration of 0.1 ppm (Fig. 1). The inhibitory effect on seed germination is lost after illumination (Fig. 2). The higher the intensity of exposed light the greater is the loss of inhibitory power of the compound.

The inhibitory effect was enhanced when a suspension of 200 or 500 ppm of psoralen was used as test solution.

Audus and Quastel [14] reported that **2** inhibits seed germination and root growth at a concentration of 100 ppm while **1** was recorded [10] to be active at 2×10^{-5} M. We have found **1** to be active against *Lactuca sativa* even at a concentration of 0.1 ppm showing it to be a very powerful inhibitor. The higher activity of psoralen is due probably to its furanocoumarin structure which also augments the antibiotic action of the coumarin nucleus [5, 6]. The light sensitive inhibitory action observed by us is in agreement with previous findings for **2**. Since psoralen acts as an inhibitor of seed germination and of some microbes, it is possible that

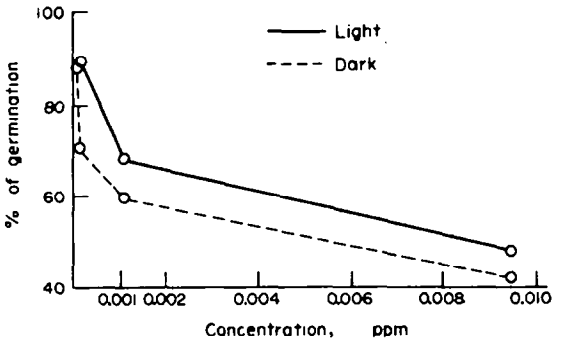


Fig. 1. Effect of psoralen on germination of lettuce in light and dark.

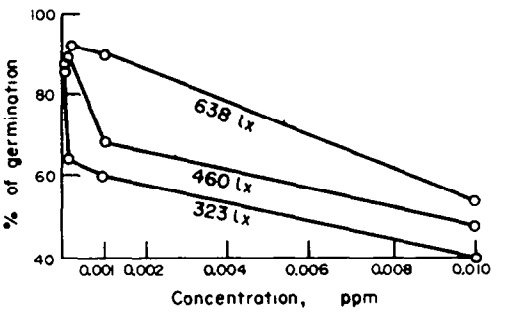


Fig. 2. Effect of psoralen on germination of lettuce at different intensities of light.

the compound may act as an inhibitor for the growth of weeds and microbes detrimental to the growth of the plant. The behaviour of light on the seed germination activity show that seed germination inhibitory property of psoralen is qualitatively different from the photodynamic action of psoralen. This is similar to those biological properties for which UV or visible light is not essential for its activation (antibiotic action or antivitiligo action).

EXPERIMENTAL

Psoralen, mp 162° was isolated from the ripe fruits of *Aegle marmelos* Corr. [9] in our laboratory. Psoralen (1 mg) dissolved in 100 ml (10 ppm) of double dist H_2O was taken as a stock soln. Different dilutions of this soln were applied to test plates. Lettuce (*Lactuca sativa* cv White) was used as the test plant for the measurement of germination inhibitory activity. Achenes were placed in sterile petri dishes (100 per dish) containing discs of filter paper and 4 ml dist H_2O or test soln. They were incubated at 25° for 72 hr and at intervals of 24 hr, the number of seeds germinated was counted. Each

assay was carried out ×5. While some of the dishes were exposed to diffuse light at 25°, the rest were covered with black paper and kept in darkness at the same temp. Subsequent expts were carried out with solns of concn 1, and 0.1 ppm. Some expts were carried out with a suspension of 2.5 mg psoralen in 100 ml. To observe the effect of light, the test dishes were exposed to 323, 460 and 638 lx respectively at 25°.

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STILBENE GLUCOSIDES IN THE BARK OF *PICEA SITCHENSIS*

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Key Word Index—*Picea sitchensis*; Pinaceae; spruce; glucosides; prenylation reactions.

Abstract—The fresh bark of *Picea sitchensis* contains astringin as the major stilbene; isorhapontin and piceid are present in minor amounts. The aglycones astringenin, isorhaponhgenin and resveratrol are absent in samples of fresh bark. Prenylation reactions on 5,3′,4′-tri-*O*-methylastringenin are described.

INTRODUCTION

The bark from *Picea sitchensis*, a waste product of the pulp and paper industry, was examined for a quantitative assessment of the compounds of economic importance. Our initial interest was in the occurrence of stilbenes, a group of phenolic compounds considered [1] as the possible inhibitors of some forest pathogens (e.g. *Fomes annosus*), the control of which plays an important role in the economic cultivation of spruce forests.

RESULTS AND DISCUSSION

The dried fresh spruce bark yielded 6% astringin; other than UV spectra [2, 3] and GLC behaviour [4] no physical data for the unstable and easily oxidized 3,5,3′,4′-tetrahydroxystilbene 3-β-D-glucopyranoside is recorded. The structure of the isolate from *P. sitchensis* was confirmed by acidic hydrolysis of the methylated derivative *trans*-5,3′,4′-tri-*O*-methylastringin. The aglycone from the hydrolysate was ethylated and subsequently oxidized to give an equimolar mixture of 5-ethoxy-3-methoxybenzoic and 3,4-dimethoxybenzoic acids. The name astringin was originally proposed by Hillis to describe an amorphous mixture (mp 75–125°) isolated from *Eucalyptus sideroxylon* [5].

The minor phenolic components of the bark were isolated using $Ph(OAc)_2$ treatment. Isorhapontin was found to be present in 0.4% yield and was characterized as its hexaacetate [6]. Also obtained was the stilbene glucoside, piceid (0.08% yield) which had not previously been found in *P. sitchensis*. The structure of piceid was confirmed by isolation of resveratrol-3,4′-dimethyl ether, on hydrolysis of the methylated glucoside.

(+)-Catechin (0.08%) and sitosterol (0.01%) were also identified in the bark extract.

Chromatograms of Sitka spruce bark extracts were reported [2, 7] as showing 15 fluorescent spots, which, though the compounds were not isolated, were inferred from R_f values to be *trans*- and *cis*-stilbene glucosides and their corresponding aglycones. In the fresh bark extracts of *P. sitchensis* analysed by us, the aglycones were absent.

The presence of stilbenes and their glucosides has also been observed in the barks of *P. abies* L (Karst) [8–10]; *P. glauca* (Moench) Voss [2, 10]; *P. marianna* (Mill) B.S.P., *P. rubens*, *P. engelmannii* (Parry) [2, 11]; *P. obovata* (Ledebour) [12].

The instability of astringin in air prohibited its use in the standard antifungal and antibacterial tests, and the preparation of stable derivatives of the glucoside and its aglycone was undertaken.