



PHLOROGLUCINOL GLUCOSIDE AS A NATURAL CONSTITUENT OF *CANNABIS SATIVA*

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Abstract—Phloroglucinol β -D-glucoside was identified from shoot laticifer exudate of *Cannabis sativa* (marihuana) by TLC. Isolation of the aglycone from acid-heat hydrolysis or emulsin treatment yielded the free phenol, phloroglucinol (1,3,5-trihydroxybenzene), as identified by GC-mass and ^1H NMR spectrometry. Phloroglucinol also was identified by TLC as a prominent component in glandular trichomes.

INTRODUCTION

Vegetative shoots of *Cannabis sativa* L. when cut at the level of young internodes produce copious clear exudate which rapidly oxidizes to a red colour indicative of the presence of phenol. Our initial analytical studies showed that this exudate, derived from the specialized non-articulated laticifer [1], contained one abundant and several less abundant phenols. The occurrence of phenols in *Cannabis* is of particular interest in that this plant is characterized by the production of a series of distinctive terpenophenolic compounds, the cannabinoids, which accumulate in glandular trichomes [2-4]. Cannabinoids are believed to originate from the condensation of a phenol with a terpene derivative [5, 6]. This phenol is widely believed to be olivetol [7, 8] based only on structural and synthetic studies, because olivetol is not reported to occur naturally in *Cannabis*. The purpose of this study is to identify the prominent phenolic compound detected in stem exudate and glandular trichomes of *Cannabis*.

RESULTS AND DISCUSSION

Extraction of stem exudate for phenols by acid-heat treatment [9] and separation by TLC yielded a variety of fast blue salt B (fbsb) staining phenols. The most abundant spot was isolated by preparative TLC and identified by GC-mass and ^1H NMR spectrometry to be phloroglucinol. This was further confirmed by TLC comparison with a phloroglucinol standard. Fresh stem exudate in ethanol, not acid-heat treated, yielded by TLC, a single fbsb staining phenol spot at a more polar R_f than phloroglucinol. This phenol, suspected to be a glucoside, liberated phloroglucinol upon treatment with β -D-glucosidase. This polar phenol was identified as phloroglucinol glucoside (phlorin) from TLC by comparison with

R_f and fbsb staining colour of a phlorin standard. Extracts from a large sample of isolated *Cannabis* glandular trichomes that were acid-heat treated yielded by TLC a single phenol spot which was identified by R_f and colour staining with fbsb to be phloroglucinol.

Phloroglucinol or its glucoside form, phlorin, is of rare occurrence as a natural constituent in angiosperms. Phloroglucinol is reported only from scales of onion (*Allium cepa*) [10] and phlorin from leaves of dogwood (*Cornus capitata*) [11] and rind of citrus (*Citrus paradisi*, *C. sinensis*, *C. limon*) [12], although they have not been identified with a particular cell or tissue type. It is also of interest that hops (*Humulus lupulus*), the only other genus in the Cannabaceae may utilize phloroglucinol derivatives in the synthesis of its specialized secondary products, humulone and lupulone [13].

This is the first report of phloroglucinol glucoside to be a prominent component of the nonarticulated laticifer, and of phloroglucinol as the only detectable phenolic component of the glandular trichomes in *Cannabis*. The presence of phloroglucinol as the only phenol in these glandular trichomes suggests that it, rather than olivetol, may play an important role in the *in vivo* enzymatically regulated biogenesis of cannabinoids.

EXPERIMENTAL

General. ^1H NMR spectra were determined in DMSO- d_6 on a Nicolet NT 360 spectrometer operated at 361 MHz. The chemical shifts are given in ppm. GC-MS peaks were recorded on a Carlo Erba gas chromatograph using an OV-1 column programmed from 170 to 180° and interfaced with a Kratos MS-80 mass spectrometer.

Plant material. *Cannabis sativa* was greenhouse grown from a Mexican seed source under vegetative long day or

floral short day conditions. Voucher specimens are on file in the herbarium of Indiana University, Bloomington.

Extraction, fractionation, and isolation. Fresh exudate was collected in H₂O or 80% EtOH from the cut surface of young internodes of robust plants. Exudate was either adjusted to 2 M with HCl, heated for 30–60 min at 105°, and extracted in peroxide-free Et₂O or extracted directly in Et₂O without acid-heat treatment. Extracts were concd to near dryness under N₂ and spotted at 1–3 µl on 5 × 10 cm strips of Eastman Kodak Chromogram Sheet 13179 Sil Gel, and developed in a non-equilibrated capped jar in an EtOAc–MeOH–aq. 2% HOAc (67:7:1) solvent. Visualization of phenolics was by long and short wave UV and by colour detection after spraying with 0.1% aq. MeOH (1:3) fbsb. Isolation of phenolics was by extraction from prep. TLC in Et₂O and concentration to dryness under N₂.

Gland isolation. Glandular heads were isolated in large numbers with high purity from floral regions by blending floral heads in cold NaOAc buffer (pH 5.0), filtration through cheesecloth followed by filtration through 20 mesh Nitex in a Buchner funnel. Glands were rinsed off the Nitex with cold buffer and concentrated by low speed centrifugation. Glands were disrupted with an amalgamator, using 0.5 mm zirconium beads, and the supernatant subjected to phenol extraction described above.

Standards. Phloroglucinol was provided commercially (Sigma P-3502). Phloroglucinol glucoside was prepared in *Vicia* seeds following the procedure of ref. [14] and isolated by prep. paper chromatography.

Glucosidase treatment. Stem exudates were incubated in buffered β-D-glucosidase (Sigma G-8625 Type II from almonds) or buffer alone as a control. The enzyme was prepared as 0.2% in NaOAc buffer, pH 5.0. Two parts of substrate were combined with one part of buffered enzyme and incubated for 1.5 and 3 hr at 37°. Solutions were extracted in Et₂O and concd to near dryness under N₂ prior to TLC screening.

Phloroglucinol. GC-MS peaks: *m/z* 126 (100); 85 (18); 97 (12); 80 (8); 111 (6). ¹H NMR (DSMO-*d*₆): 8.95s; 5.64s; 3.34s; 2.50s. Values were taken from unknown phenol in exudate which are consistent with identification as

phloroglucinol and correspond exactly with comparison to phloroglucinol standard.

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