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Note

Separation of glyceollin isomers I–III by thin-layer chromatography

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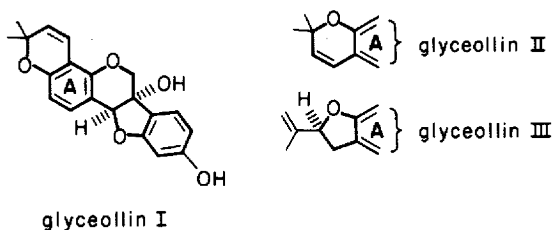
Environmental stress of soybeans by chemical treatment (herbicide[1], heavy metal[2], ozone[3], etc.) or microbial infection[2,4–15] leads to accumulation of the antifungal metabolites glyceollin I, II and III (GI–III), the levels and relative ratios of which strongly depend on the plant tissue studied and the nature of the elicitor[1,6,11,12]. These isomeric phytoalexins can be analyzed by gas–liquid chromatography (GLC)[4,8] or high-performance liquid chromatography (HPLX)[1,6,11,12,15] but no separation is achieved with the usual thin-layer chromatographic (TLC) systems for phenolic compounds[1–5,7–15]. Accordingly, GI–III are usually quantitated after several TLC purification steps as "glyceollin", a mixture of the three compounds[1–5,7–15].



The present study evaluated a large number of cellulose, polyamide or silica gel TLC systems suggested for analysis of phenols[16,17] but without success for separation of GI–III (data now shown). However, these compounds are easily separated by using multiple developments of formamide-impregnated silica gel layers, a sorbent described for TLC of *Umbelliferae* drugs[18]. Quantitation of GI–III in plant extracts is then achieved by using this system in place of the last purification–quantitation steps[1–15], followed by normal UV spectrometry of the eluted spots. This method, when combined with radioautography, may also find application in radiotracer studies of the biosynthesis of GI–III.

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EXPERIMENTAL

Cut soybean seedlings (5 g) were treated with the diphenyl ether herbicide acifluorfen (sodium 5-[2-chloro-4-(trifluoromethyl)phenoxy]-2-nitrobenzoate; 5 ppm, stem uptake for 72 h). The GI–III mixture was extracted with 40% ethanol and purified by TLC according to Ingham *et al.*[11]. The purified GI–III mixture and standards of GI, II and III (0.2, 1 and 5 μg; both separately and together) were spotted onto formamide-impregnated silica gel TLC plates [prepared by developing silica gel 60 F254, 0.25 mm thick TLC plates (Merck) twice with 5% formamide in acetone and drying at room temperature] and developed four times with diethyl ether–hexane (3:1). UV-absorbing spots of GI, II and III were detected at R_F values of 0.50, 0.42 and 0.35, respectively, and the compounds were quantitated by UV spectrophotometry (using the following absorption maxima and molar absorption coefficients: GI, 285 nm, 8300 $l\ mol^{-1}\ cm^{-1}$; GII, 285 nm, 8700 $l\ mol^{-1}\ cm^{-1}$; GIII, 292 nm, 9600 $l\ mol^{-1}\ cm^{-1}$)[4,6] after scraping off the spots and eluting with 2 × 1 ml ethanol. The levels of GI, II and III found in acifluorfen-treated soy bean seedlings were 7, 19 and 38 μg/g fresh weight, respectively (compared to 4, 28 and 44 μg/g fresh weight determined by HPLC in a separate experiment[1]). The GI–III content in the untreated leaves was < 1 μg/g fresh weight.

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