

Formation of Deoxyshikonin in Callus Cultures of *Lithospermum erythrorhizon*

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(Received February, 28, 1974)

Callus tissues of *Lithospermum erythrorhizon* Sieb. et Zucc. are capable of synthesizing shikonin derivatives including acetyl-, β -hydroxyisovaleryl-, and isovaleryl-shikonin when cultured in the dark.²⁾ The present paper describes that callus tissues grown in the dark also form deoxyshikonin, which is a shikonin derivative isolated recently from the roots of *L. erythrorhizon* and *Macrotomia euchroma* by Kyōgoku *et al.*³⁾

A red pigment, which corresponded to deoxyshikonin in its chromatographic behavior on TLC, was isolated from the CHCl_3 extract of callus tissues. This compound was identified as deoxyshikonin by direct comparison with authentic sample by mixed mp, TLC, UV and IR spectra.

The content of deoxyshikonin in callus tissues was approximately 0.02 % per dry weight of tissue, which is comparable to 0.016 % in the intact roots.⁴⁾ By contrast, no deoxyshikonin was detectable in callus tissues irradiated with white or blue light during the culture period.

Experimental

Tissue culture

The stock culture of callus M-0231a (22-month-old) originally derived from the germinating seed of *Lithospermum erythrorhizon* SIEB. et ZUCC. was grown on Linsmaier and Skoog's basal agar medium⁵⁾ containing 10^{-6} M IAA and 10^{-5} M kinetin at 25° in the dark for 6 weeks.

Extraction and identification of deoxyshikonin

Fresh callus tissues (800 g) harvested from cultured flasks were homogenized in a mixer with CHCl_3 . The filtrate of the homogenate was washed with water, and the CHCl_3 layer containing red pigments was dried, filtered, and evaporated under reduced pressure. The pigments were separated on a silica gel column, and the first pigment fraction obtained by elution of the column with *n*-hexane—benzene (7 : 3) was purified by preparative TLC on silica gel G with CHCl_3 . The isolated fraction on recrystallization with *n*-hexane gave a red crystalline substance (8.0 mg), mp 90—90.5°, undepressed on admixture with an authentic sample of deoxyshikonin. The UV and IR spectra of this compound were identical with those of the authentic sample of deoxyshikonin.

Acknowledgment We are thankful to Drs. Manki Komatsu and Kazuaki Kyōgoku, Research Laboratory, Taishō Pharmaceutical Co. Ltd. for the precious gift of the authentic specimen of deoxyshikonin.

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