Enzymic Formation of Siccanochromen-A, a Key Intermediate in the Biosynthesis of Siccanin

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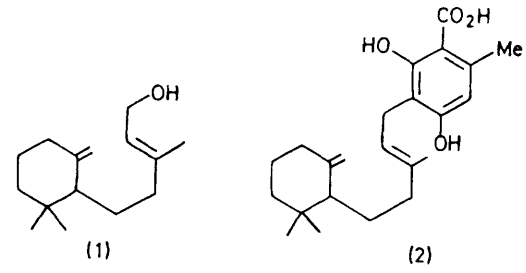
Summary Enzymic formations of siccanochromene-A (**4**) by incubation of cell-free systems of *Helminthosporium siccans* Drechsler with mevalonic acid lactone and orsellinic acid, *trans,trans*-farnesyl pyrophosphate and orsellinic acid, presiccanochromenic acid (**2**), and siccanochromenic acid (**3**) are described.

RECENTLY we demonstrated that *trans*-γ-monocyclofarnesol (**1**),[1] a likely precursor of siccanin (**6**),[2] is derived in cell-free systems from mevalonic acid lactone. The importance of the intermediary chromen derivatives, siccanochromen-A (**4**)[3] and -B (**5**),[3] in siccanin biosynthesis has also been established by growing cell systems.[4] We now report the formation of siccanochromen-A (**4**) in cell-free systems of *H. siccans*.

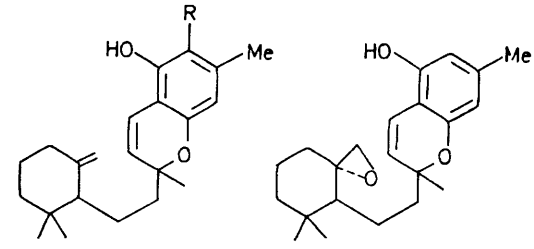
[2-^{14}C]Mevalonic acid lactone was incubated for 3 h at 36° in the presence of ATP and orsellinic acid† with the cell-free supernatant prepared by disrupting the cells from *H. siccans* with washed sea sand and centrifuging at 11,000 *g* in phosphate buffer (0·1 M) containing Mg^{2+} (0·1 mM). The autoradiograph of the nonsaponifiable fraction of the extract contained a spot identical with (**4**) (conversion ratio: ~30%). The incubation of *trans,trans*-[4,8,12-^{14}C]-farnesyl pyrophosphate‡ with the same cell-free supernatant and orsellinic acid, but without ATP, also afforded (**4**) in quantitative yield. The isolated siccanochromen-A (**4**) and its acetate were identical with authentic samples by co-chromatographs on silica gel, silica gel–silver nitrate, and reversed phase-silica gel plates, as well as by radio gas chromatography.

Other aromatic compounds such as 2-n-propyl-4,6-dihydroxybenzoic acid,§ α- and β-resorcylic acid, orcinol, and resorcinol were found not to be effective as co-substrates for the enzyme systems.

When unlabelled presiccanochromenic acid[5] (**2**) (1 mg/ml) was incubated aerobically with the same supernatant, (**4**) was obtained in 40% yield with recovered (**2**) (50%) and (**3**)

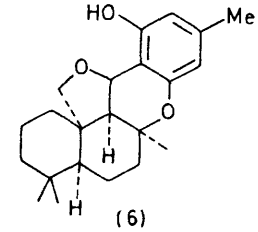


(1) (2)



(3) R = CO_2H
(4) R = H

(5)



(6)

† Hydrolysis product of gyrophoric acid from *Gyrophora esculenta*.

‡ Prepared by the incubation of [2-^{14}C]mevalonic acid lactone with pig liver homogenate and purified by DEAE cellulose column. G. Popjak, J. Clifford, V. Williams, and J. Edmond, *J. Biol. Chem.*, 1969, **244**, 1897.

§ Hydrolysis product of divaricatic acid from *Ramalia subbreviuscula*.

(4%). Anaerobic incubation however, afforded no transformation products, indicating that oxygen is required for the formation of the chromen ring.

Siccanochromenic acid[5] obtained from *H. siccans* was found to be a diastereomeric mixture of (**3**) and episiccanochromenic acid which differ in the configuration of the methyl group on the chromen ring.[6] Incubation of this mixture (1·1 mg/ml) with cell-free systems of *H. siccans* gave a mixture of siccanochromen-A (**4**) and episiccanochromen-A in quantitative yield. The ratio of products was identical to that of the precursor (**3**), suggesting that the decarboxylase is nonspecific for the chromen ring of the substrate. Since (**2**) yields isomerically pure (**3**) and (**4**), the mixture of (**3**) and its diastereoisomer isolated from *H. siccans* may be formed non-enzymically during the isolation process.

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[3] S. Nozoe, K. T. Suzuki, and S. Okuda, *Tetrahedron Letters*, 1968, 3643.
[4] K. T. Suzuki and S. Nozoe, *Chem. Comm.*, 1971, 527.
[5] S. Nozoe and K. T. Suzuki, *Tetrahedron Letters*, 1969, 2457.
[6] S. Nozoe and K. T. Suzuki, *Tetrahedron*, 1971, **27**, 6063.