Chromene Derivatives as Intermediates in the Biosynthesis of Siccanin

By KAZUO T. SUZUKI and SHIGEO NOZOE*

(*Institute of Applied Microbiology, University of Tokyo, Bunkyo-ku, Tokyo, Japan*)

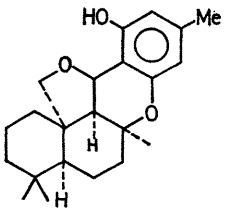
Summary Tritium labelled siccanochromene-A (III) and -B (IV) were incorporated into an antibiotic, siccanin (I), by incubation with the intact cell systems of *Helminthosporium siccans* Drechsler.

RECENTLY we reported the structure of an antibiotic, siccanin (I)[1] and siccanochromene-E (II)[2] both isolated from the cultured broth of the plant pathogenic fungus, *H. siccans*. These compounds occur with triprenyl chromene derivatives, such as siccanochromene-A (III) and -B (IV).[3] All the compounds described were assumed to be derived biogenetically by combination of sesquiterpene and orsellinate entities.

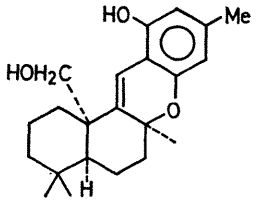
Feeding experiments using isotopically labelled substrates (III) and (IV) now show that chromene derivatives are possible intermediates in the biosynthesis of siccanin (I).

Siccanochromene-A (III) and -B (IV) were labelled by the base catalysed exchange[4] of the aromatic protons marked by asterisks in the figure. Dimethylformamide solutions of (III) and of (IV) were heated in a sealed tube with deuterium oxide in the presence of catalytic amount of triethylamine to yield the deuteriated form of compounds (III) and (IV). N.m.r. spectra showed that *ca.* 80% of the

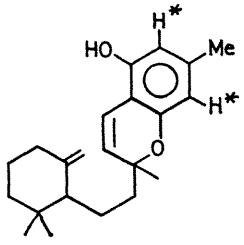
aromatic protons were exchanged. Tritium labelled form of substrates (III) (specific activity: 56 Ci mol^{-1}) and (IV) (specific activity: 64 Ci mol^{-1}) were similarly prepared using tritiated water and were fed to the three-day old culture of *Helminthosporium siccans* at a concentration of 4—5 μCi per 100 ml of culture solution. Incubation was continued aerobically for a further 48 h with shaking. The products of the incubation were extracted and separated by silica gel column chromatography. Siccanin (I) was purified by repeated crystallisation with the carrier substance till constant specific activity was obtained. It was found that 25—40% of the added radioactivity was incorporated into (I) in the case of tritiated (III) and *ca.* 70% in the case of tritiated (IV).



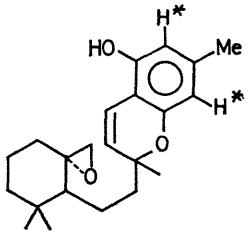
(I)



(II)



(III)



(IV)

The radioactive substrates were also fed to the washed cell suspension (four day old) in 0·1M-phosphate buffer (pH 7·62); 3 and 16% incorporation into (I) were obtained from the tritiated substrates (III) and (IV) respectively. We confirmed that no siccanin was formed by nonenzymatic acid treatment of (IV) or by the incubation with preheated mycelia.

Chromene derivatives, such as (III) and (IV), are thus shown to be possible intermediates in the biosynthesis of siccanin. The unusual *cis*-drimane skeleton might be formed by the epoxyolefin type cyclization of (IV).[5,6]

(*Received, March 15th,* 1971; *Com.* 288.)

[1] K. Hirai, S. Nozoe, K. Tsuda, Y. Iitaka, K. Ishibashi, and M. Shirasaka, *Tetrahedron Letters*, **1967**, 2177.
[2] S. Nozoe and K. T. Suzuki, *Tetrahedron Letters*, 1969, 2457.
[3] S. Nozoe and K. T. Suzuki, *Tetrahadron Letters*, 1968, 3643.
[4] G. W. Kirby and L. Ogunkoya, *J. Chem. Soc.*, 1965, 6941.
[5] D. J. Goldsmith and C. F. Phillips, *J. Amer. Chem. Soc.*, 1969, **91**, 5862.
[6] D. J. Goldsmith and B. C. Clark, *Tetrahedron Letters*, 1967, 1215.