Tetrahedron Letters No. 19, pp 1969 - 1972, 1972. Pergamon Press. Printed in Great Britain.

BIOSYNTHESIS OF TRICHOTHECIN AND RELATED COMPOUNDS

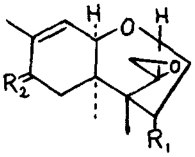
Yoshimasa Machida and Shigeo Nozoe
Institute of Applied Microbiology, University of Tokyo
Bunkyo-ku, Tokyo, Japan

(Received in Japan 25 March 1972; received in UK for publication 5 April 1972)

As part of a study on the biosynthesis of trichothecin (I) and related compounds, we recently isolated a hydrocarbon from a trichothecin producing fungus, *Trichothecium roseum* Link, and the name trichodiene was proposed.[1] The hydrocarbon had been suggested to be a hypothetical precursor of trichothecin.[2] This paper deals with the role of the hydrocarbon as a precursor in the biosynthesis of trichothecin.

Trichodiene (II) with tritium labeling at either the olefinic methyl group or the olefinic hydrogen in the cyclohexene moiety was prepared by the following method which would be generally applicable to the terpenic compounds having similar groups. Trichodiene (II) was treated with *m*-chloroperbenzoic acid to afford the monoepoxide III,[1] which on reaction with lithium diethylamide in refluxing benzene[3] gave the allylic alcohol IV, $C_{15}H_{24}O$: NMR (δ): 0.95 (3H), 0.97 (3H), 3.95 (1H), 4.58 (1H), 4.68 (1H), 4.75 (1H), 4.87 (1H). This compound was converted to the chloride V, $C_{15}H_{23}Cl$: NMR (δ): 0.86 (3H), 1.04 (3H), 3.90 (2H), 4.70 (1H), 4.92 (1H), 5.65 (1H), by the action of $SOCl_2$ in ether and successively reduced with lithium aluminum tritiide in ether. The product was diluted with a pure sample of trichodiene and was carefully purified by repeated preparative thin layer chromatographies on $AgNO_3$-impregnated silica gel plate to yield the labeled trichodiene (specific activity, 6.2×10^5 dpm/μmol). An attempt to prepare trichodiene having ^{14}C-labeled exocyclic methylene group was unsuccessful because the Wittig reaction of the ketone VI[1] and methylenetriphenylphosphorane did not proceed.

The tritium labeled trichodiene (1×10^6 dpm) in acetone (15 μl) was fed to a 2-day-old culture (potato medium, 5 ml) of *T. roseum* and fermentation was continued for further 9 days. The culture was harvested and the extract was saponified with alcoholic KOH.



(I) $R_1 = OCOCH\underset{t}{=}CHCH_3$

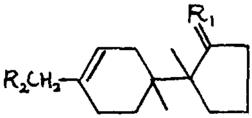
$R_2 = O$

(VII) $R_1 = OH$

$R_2 = O$

(VIII) $R_1 = H$

$R_2 = H_2$



(II) $R_1 = CH_2$

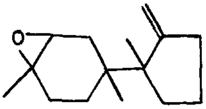
$R_2 = H$

(V) $R_1 = CH_2$

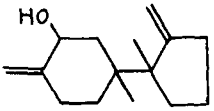
$R_2 = Cl$

(VI) $R_1 = O$

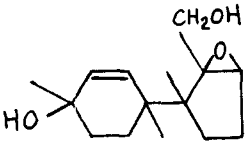
$R_2 = H$



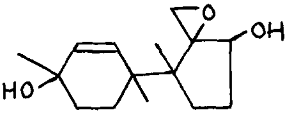
(III)



(IV)



(IX)



(X)

The nonsaponifiable fraction was found to contain *ca.* 20 % of the original activity. To this fraction was added cold trichothecolone (VII, 60 mg), which was then recrystallized four times. Both of the samples obtained after the third and the fourth crystalization showed the same specific activity, 5.4×10^2 dpm/mg, which corresponds to 3.2 % incorporation into trichothecolone, whereas the first and the second crystals showed 4.9×10^2 and 5.1×10^2 dpm/mg, respectively. Preparative TLC of the mother liquor followed by measurement of radioactivity showed incorporation of the hydrocarbon into 12,13-epoxytrichothec-9-ene (VIII)[4] and trichodiol-A (IX)[5] (*ca.* 0.6 % and 2 %, respectively) as well.

Recently we isolated a new sesquiterpene, trichodiol (X), from the cultured broth of the same fungus and trichodiol-A was found to be an artefact produced from trichodiol during alkaline saponification.[5]

Thus, trichodiene was shown to be a precursor of trichothecin, 12,13-epoxytrichothec-9-ene, and trichodiol

Feeding experiments to follow the metabolic fate of the hydrocarbon in *Fusarium nivale*[6] and other trichothecane type mycotoxin producing fungi are underway.

We thank Dr. Nakayama, Nippon Kayaku Co. Ltd., for supplying the fermentation materials of *T. roseum*.

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2. (a) J. D. Bu'Lock, "The Biosynthesis of Natural Products," McGraw-Hill, London, 1965: (b) R. Bentley and I. M. Campbell, in "Comprehensive Biochemistry," ed. M. Florkin and E. H. Stotz, Elsvier, Amsterdam, 1968, Vol. XX, p. 452.
3. J. K. Crandall and Luan-Ho Chang, *J. Org. Chem.*, **32**, 435 (1967).
4. Y. Machida and S. Nozoe, *Tetrahedron*, in press.
5. We had proposed the name trichodiol to compound IX, but as it was found to be an artefact, and we assigned the name trichodiol-A to this compound and trichodiol to compound X; see S. Nozoe and Y. Machida, *Tetrahedron Letters*, 1177 (1970); *Tetrahedron*, in press.
6. T. Tatsuno, Y. Fujimoto, and Y. Morita, *Tetrahedron Letters*, 2823 (1969).