CHEMISTRY LETTERS, pp. 1245-1248, 1976. Published by the Chemical Society of Japan

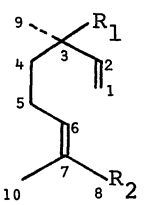
BIOTRANSFORMATION OF MONOTERPENES BY TOBACCO TISSUE CULTURES. SELECTIVE HYDROXYLATION OF *TRANS*-METHYL GROUP IN ISOPROPYRIDENE GROUP

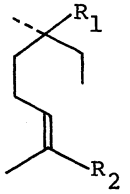
Takayuki SUGA, Toshifumi HIRATA, Yoshio HIRANO, and Takashi ITO
Department of Chemistry, Faculty of Science, Hiroshima University
Higashisenda-machi, Hiroshima 730

The callus cultures of *Nicotiana tabacum* "Bright Yellow" are capable of transforming selectively the *trans*-methyl group in the isopropyridene group of linalool (I) and its derivatives, (V), (VI), and (VII), into the hydroxymethyl group.

The ability of callus tissues to metabolize or biologically transform administered foreign compounds is considerable interest. However, only a few studies[1~3] are seen up to the present. We now tested the ability of tobacco suspension callus tissues to biologically transform administered acyclic monoterpenes, such as linalool (I) and its derivatives, (V), (VI), and (VII).

Tobacco callus tissues[4] used in this work were derived from the stem of *Nicotiana tabacum* "Bright Yellow". The callus tissues were transplanted to the freshly prepared Murashige and Skoog's medium[5] (100 ml per one flask) containing 2 ppm of 2,4-dichlorophenoxyacetic acid as auxin and 3% of sucrose and then were grown with continuous shaking for 3~4 weeks at 25°C in the dark. To the suspension callus





I: R_1=OH, R_2=CH_3
II: R_1=OH, R_2=CH_2OH
IV: R_1=OH, R_2=CHO
VI: R_1=OAc, R_2=CH_3
VIII: R_1=OAc, R_2=CH_2OH

III: R_1=OH, R_2=CH_2OH
V: R_1=OH, R_2=CH_3
VII: R_1=OAc, R_2=CH_3
IX: R_1=OAc, R_2=CH_2OH

tissues (40~60 g per one flask), the acyclic monoterpene (20 mg per one flask; total 200 mg) was administered, and then the suspension callus cultures were incubated at 25°C for 7 days in a shaker. The callus mass was filtered off and triturated with methanol. The methanol solution, after removal of the solvent, was extracted with ether. The culture medium filtered from the callus mass was extracted with ether. These ether soluble fractions were compared, respectively, with the corresponding ether soluble fractions of the callus' own metabolites by means of GLC and TLC. This comparative analysis indicated evidently the formations of one product in the administration of the acyclic monoterpene alcohols, (I) and (V), and two products in the case of the acetates, (VI) and (VII). The ether soluble fractions, after removal of the solvent, were subjected, respectively, to chromatography on a 3% $AgNO_3$-silica gel plate with a mixture of ethyl acetate and *n*-hexane to isolate the products. The homogeneity of the product isolated was, respectively, confirmed by GLC and TLC analyses.

The product obtained from linalool (I) ($[\alpha]_D^{25}$ -19.9°) exhibited the n.m.r. signal at 3.95 ppm (2H, s) due to $-C\underline{H}_2OH$, instead of the 8- or 10-methyl signal of I. This suggests that the product is a C(8)- or a C(10)-hydroxylated derivative of I. This dihydroxy compound (II) ($[\alpha]_D^{25}$ -12.8° (*c* 1.08, MeOH); m/e 152 ($M-H_2O$)) was selectively hydrogenated with PtO_2 to give the dihydro-derivative III, which showed m/e 154 ($M-H_2O$) and a 17% NOE between the hydroxymethyl group (δ 3.94 ppm) and the C(6)-H (δ 5.42 ppm). The NOE[6] indicates that the hydroxymethyl group is *trans* to the C(1)~C(5) chain moiety. This was further supported by the agreement of the observed chemical shift (δ 6.45 ppm) of the C(6)-H of the hydroxy aldehyde IV, which was derived from II on MnO_2-oxidation, with the evaluated shift value (δ 6.40 ppm)[7] calculated for the *trans* isomer by fitting Pascual's equation.[8] Thus, the dihydroxy compound was established to be 8-hydroxylinalool (II).

As shown in the Table, the administration of dihydrolinalool (V) resulted in the formation of only 8-hydroxydihydrolinalool (III) in a similar manner as above. On the other hand, linalyl acetate (VI) was transformed by the tobacco suspension callus tissue to 8-hydroxylinalool (II) and 8-hydroxylinalyl acetate (VIII). However, the formation of linalool, which is the hydrolyzed product of VI, was not observed. The preferential formation of the dihydroxy compound II seems, accordingly, to indicate the occurrence of the hydrolysis of the administered acetate (VI) immediately followed by the hydroxylation at C(8). A quite similar result was also obtained in respect of

dihydrolinalyl acetate (VII) and is shown in the Table.

We now have established that tobacco suspension callus tissues are capable of hydroxylating selectively the *trans*-methyl group in the isopropyridene group, but this is not the case for the *cis*-methyl group.

TABLE. Biotransformation of linalool (I) and its derivatives, (V), (VI) and (VII), by *Nicotiana tabacum* suspension callus cultures

Substrates	C(8)-Hydroxylated products	Yield (%)*
I	II	16.5
V	III	14.9
VI	II	14.8
	VIII	1.9
VII	III	15.5
	IX	2.2

* The weight percent of the products per the administered substrates.

Acknowledgment. We thank to Dr. K. Ojima of Faculty of Agriculture, Tohoku University, for a gift of *Nicotiana tabacum* callus.

References and Notes

1) M. Hirotani and T. Furuya, Phytochemistry, 13, 2135 (1974) and the references cited therein.

2) K. Takeya, M. Akasu, S. Mihashi, and H. Itokawa, The 4th Symposium for Plant Tissue Culture, Tokyo, July (1974).

3) K. Takeya, M. Akasu, and H. Itokawa, The 5th Symposium for Plant Tissue Culture, Sendai, July (1976).

4) The callus tissues have been subcultured continuously for 5 years prior to their use in this study.

5) T. Murashige and F. Skoog, Physiol. Plant, 15, 473 (1962).

6) J. H. Noggle and R. E. Schirmer, "The Nuclear Overhauser Effect. Chemical Applications", Academic Press Inc. (1971).

7) The chemical shift evaluated for the *cis* isomer is δ 6.67 ppm.

8) C. Pascual, J. Meier, and W. Simon, Helv. Chim. Acta, 49, 164 (1966).

(Received August 30, 1976)