The Biotransformation of Foreign Substrates by Tissue Cultures. I. The Hydroxylation of Linalool and Its Related Compounds with the Suspension Cells of *Nicotiana tabacum*

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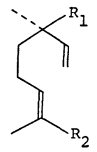
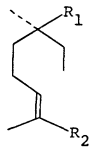
(Received May 22, 1981)

It was found that the tissue cultures of *Nicotiana tabacum* "Bright Yellow" possess the ability to transform selectively the *trans*-methyl group in the 3-methyl-2-butenyl group of such foreign substrates as linalool and the related compounds into the hydroxymethyl group. Also, the cultures were found to have the ability to hydrolize the acetoxyl group of their acetates.

In connection with studies on the biosynthetic ability of plant callus tissues,[1] we are considerably interested in their ability to transform foreign substrates. Recently, it has been documented that some plant cell cultures have the ability to transform administered substrates, and such an ability may be used for the transformation of chemical substances.[2-9] However, there have been few systematic studies on their transformation ability and pattern. We, therefore, began investigating the transformation of acyclic monoterpenoid alcohols by the cultured cells of *Nicotiana tabacum* "Bright Yellow." As the acyclic monoterpenoid alcohols, linalool (**1**), linalyl acetate (**2**), dihydrolinalool (**3**), and dihydrolinalyl acetate (**4**) were used. The results have been partly outlined in the preliminary communication.[10] We will here report details of the results.



1: $R_1=OH$, $R_2=CH_3$
2: $R_1=OAc$, $R_2=CH_3$
5: $R_1=OH$, $R_2=CH_2OH$
7: $R_1=OH$, $R_2=CHO$
8: $R_1=OAc$, $R_2=CH_2OH$
10: $R_1=OAc$, $R_2=CHO$



3: $R_1=OH$, $R_2=CH_3$
4: $R_1=OAc$, $R_2=CH_3$
6: $R_1=OH$, $R_2=CH_2OH$
9: $R_1=OAc$, $R_2=CH_2OH$

Results and Discussion

Callus tissues induced from the stem of *Nicotiana tabacum* "Bright Yellow" were used in this work. The callus tissues were precultured for 3—4 weeks in Murashige and Skoog's medium[11] prior to the administration of the substrates. A sample of the monoterpenoids was administered to the suspension cells. The suspension cultures were incubated at 25 °C for 7 d under shaking in the dark. After incubation, transformation products were detected by comparing the ether-soluble constituents of both the cultured mass and the culture medium with those of the metabolites of *N. tabacum* suspension cells by means of GLC and TLC. The transformation products were separated by chromatographic methods.

In the biotransformation of linalool (**1**), compound **5** was a major product, as shown in Table 1. The compound (**5**) exhibited the ^1H NMR signal at δ 4.00 due to $-C\underline{H}_2OH$, instead of the 8- or 10-methyl signal of **1**. This suggested that the product is a C(8)- or a C(10)-hydroxylated derivative of **1**. The dihydroxy compound (**5**) was selectively hydrogenated with PtO_2 to give a dihydro derivative **6**, which showed a 17% NOE between the hydroxymethyl group (δ 3.94) and the C(6)–H (δ 5.42). The NOE[12] indicated that the hydroxymethyl group is *trans* to the C(1)–C(5) chain portion. This was further supported by the agreement of the observed chemical shift (δ 6.45) of the C(6)–H of the hydroxy aldehyde **7**, with the evaluated shift value (δ 6.40) calculated for the *trans* isomer by fitting Pascual's equation.[13,14] Thus, the product **5** was elucidated to be 8-hydroxylinalool.

When linalyl acetate (**2**) was fed to the tobacco suspension cells, two transformation products, 8-hydroxylinalool (**5**) and 8-hydroxylinalyl acetate (**8**), were found, as shown in Table 1. However, linalool (**1**) resulting from linalyl acetate (**2**) by hydrolysis was not found. Accordingly, the preferential formation of 8-hydroxylinalool (**5**) seems to indicate oc-

TABLE 1. BIOTRANSFORMATION OF LINALOOL (**1**) AND ITS DERIVATIVES (**2**, **3**, AND **4**) BY *Nicotiana tabacum* SUSPENSION CELLS

Substrates	Products	Yield/%[a]
Linalool (**1**)	8-Hydroxylinalool (**5**)	16.5
Linalyl acetate (**2**)	8-Hydroxylinalool (**5**)	14.8
	8-Hydroxylinalyl acetate (**8**)	1.9
Dihydrolinalool (**3**)	8-Hydroxydihydrolinalool (**6**)	14.9
Dihydrolinalyl acetate (**4**)	8-Hydeoxydihydrolinalool (**6**)	15.5
	8-Hydroxydihydrolinalyl acetate (**9**)	2.2

a) The weight percent of the products per the administered substrates.

currence of the hydrolysis of linalyl acetate (**2**) followed by the immediate hydroxylation at C(8) or occurrence of the immediate hydroxylation at C(8) of **2** followed by the hydrolysis of the acetoxyl group. The quite similar results were obtained in the administration of dihydrolinalool (**3**) and dihydrolinalyl acetate (**4**), as shown in Table 1.

We now have established that the suspension cells of *Nicotiana tabacum* "Bright Yellow" have the ability to hydroxylate selectively in the *trans*-methyl group in the 3-methyl-2-butenyl group of linalool (**1**) and its related compounds (**2**—**4**), but not in the *cis*-methyl group. Also, it was found that the cultured cells are capable of hydrolizing the acetoxyl group of the acetates.

Experimental

NOE experiments were carried out on a Hitachi R-22 spectrometer in the frequency-swept and internal-TMS-locked mode. The sample solution was prepared in concentration of 5% (w/v) in CCl_4 and carefully degassed just prior to measurements. GLC analyses were performed on a Shimadzu GC-6A equipped with a FID and a glass column (3 mm×2 m) packed with 2% OV-17 and 10% DEGS on Chromosorb AW-DMCS (80—100 mesh) at 120 °C and 150 °C, respectively, for each case. TLC analyses were carried out with silica gel (Kieselgel GF_{254}; 0.25 mm thick) by using two different solvent systems [(i) EtOAc–hexane (3:7, v/v) and (ii) MeOH–benzene (1:24, v/v)] for each case.

Sample Used. Linalool and its acetate donated from Takasago Perfumery Co. Inc. were purified by column chromatography (silica gel) with a hexane–EtOAc mixture with EtOAc increasing 0 to 15% to give (−)-linalool (**1**) [$[\alpha]_D^{25}$ −19.9° (neat); n_D^{25} 1.4600; d_4^{25} 0.8639; >99.5% pure on GLC] and (−)-linalyl acetate (**2**) [$[\alpha]_D^{25}$ −2.1° (neat); n_D^{25} 1.4550, d_4^{25} 0.8991; >99.5% pure on GLC]. (−)-Dihydrolinalool (**3**) [$[\alpha]_D^{25}$ −2.5° (*c* 1.8, MeOH); n_D^{25} 1.4558; d_4^{25} 0.8601; >99.5% pure on GLC] and (−)-dihydrolinalyl acetate (**4**) [$[\alpha]_D^{25}$ −1.8° (*c* 2.5, MeOH); n_D^{25} 1.4502; d_4^{25} 0.8983; >99.5% pure on GLC] were prepared from **1** and **2**, respectively, by selective hydrogenation with Adams' PtO_2.

Feeding of the Monoterpenoids to the Tobacco Suspension Cells. In this work, we used the callus tissues which were derived from the stem of *Nicotiana tabacum* "Bright Yellow" and then subcultured for about 5 years. Just before use for the transformation, the callus tissues were transplanted to freshly prepared Murashige and Skoog's medium[11] (100 ml per one flask) containing 2 ppm of 2,4-dichlorophenoxyacetic acid and 2% sucrose and then grown with continuous shaking for 3—4 weeks at 25 °C in the dark. To the suspension cells (40—60 g per one flask), the monoterpenoids (20 mg per one flask; total 200 mg) were administered, and then the suspension cultures were incubated at 25 °C for 7 d on a rotary shaker (70 min^{-1}) in the dark.

Isolation of the Products. After incubation as described above, the cells were filtered off and triturated with methanol. The methanol solution, after the removal of the solvent, was extracted with ether. The culture medium filtered from the cells was extracted with ether. These ether soluble fractions were compared by means of GLC and TLC with those of the metabolites of the suspension cells. Transformation products were isolated from the ether soluble fractions by chromatography on a 3% $AgNO_3$–silica-gel plate (1 mm thick) with EtOA–hexane (3:7, v/v) and/or preparative GLC with a glass column (5 mm×2 m) packed with 10% DEGS on Chromosorb AW-DMCS (80—100 mesh) at 150 °C. These products were identified as shown below. The identified products and their quantities are shown in Table 1.

*Identification of the Products. 8-Hydroxylinalool (**5**)*: $[\alpha]_D^{25}$ −12.8° (*c* 1.08, MeOH); IR (Liq.) 3380 (OH) and 1640 cm^{-1} (C=C); ^1H NMR ($CDCl_3$) δ=1.30 (3H, s, C(9)-H_3), 1.68 (3H, bs, C(10)-H_3), 4.00 (2H, s, -C$\underline{H}_2$-OH), and 5.0—6.2 (4H, olefinic H); MS (70 eV), *m/z* (rel intensity) 152 (5, M−H_2O), 137 (7), 119 (9), 71 (51), 67 (37), and 43 (100); direct comparison with a synthetic specimen (co-TLC, co-GLC, IR, ^1H NMR, and MS).

*Derivation of 8-Oxolinalool (**7**) from 8-Hydroxylinalool (**5**)*: A mixture of 8-hydroxylinalool (**5**) (50 mg) and active MnO_2 (40 mg) in CCl_4 (5 cm^3) was stirred at room temp for 12 h. Removal of the solvent from the reaction mixture, after filtration from the inorganic solid, gave an oily product (36 mg). This product was subjected to preparative TLC (silica gel; 1 mm thick) with hexane–EtOAc (4:1, v/v) to give 8-oxolinalool (**7**) (28 mg): IR (Liq.) 2720, 1685 (CHO), and 1640 cm^{-1} (C=C); ^1H NMR (CCl_4) δ=1.33 (3H, s, C(9)-H_3), 1.73 (3H, bs, C(10)-H_3); 6.45 (1H, bt, >C=CH–), and 9.43 (1H, s, CHO); MS (70 eV), *m/z* (rel intensity) 150 (21, M−H_2O), 135 (5), 83 (82), 71 (99), and 43 (100).

*8-Hydroxydihydrolinalool (**6**)*: *m/z* 154 (M−H_2O); IR (Liq.) 3350 (OH) and 1620 cm^{-1} (C=C); ^1H NMR (CCl_4) δ=0.90 (3H, t, *J* 7.5 Hz, C(1)-H_3), 1.19 (3H, s, C(9)-H_3), 1.70 (3H, bs, C(10)-H_3), 3.94 (2H, s, -C$\underline{H}_2$OH), and 5.42 (1H, bt, *J* 7.0 Hz, >C=CH–); direct comparison with a synthetic specimen (co-TLC, co-GLC, MS, IR, and ^1H NMR).

*8-Hydroxylinalyl Acetate (**8**)*: *m/z* 212 (M^+); IR (Liq.) 3500 (OH), 1735 (OAc), 1638 (C=C), and 1250 cm^{-1} (C–O); ^1H NMR ($CDCl_3$) δ=1.55 (3H, s, C(9)-H_3), 1.67 (3H, bs, C(10)-H_3), 2.01 (3H, s, OAc), 4.00 (2H, s, -C$\underline{H}_2$OH), 4.65 (1H, bt, *J* 6.5 Hz, >C=CH–), and 5.0—6.2 (3H, olefinic H). Hydrolysis of **8** with 5% methanolic NaOH gave 8-hydroxylinalool (**5**).

*8-Hydroxydihydrolinalyl Acetate (**9**)*: *m/z* 214 (M^+); IR (Liq.) 3450 (OH) and 1735 cm^{-1} (OAc); ^1H NMR ($CDCl_3$) δ=1.50 (3H, s, C(9)-H_3), 1.69 (3H, bs, C(10)-H_3), 1.95 (3H, s, OAc), 3.99 (2H, s, -C$\underline{H}_2$OH), and 5.0—6.2 (4H, olefinic H). Hydrolysis of **9** with 5% methanolic NaOH gave 8-hydroxydihydrolinalool (**6**).

*Preparation of the Authentic Samples. 8-Hydroxylinalool (**5**)*: Following the reported procedure,[15] linalyl acetate (**2**) (980 mg) was oxidized with SeO_2 (560 mg) in 95% dioxane (5 cm^3) at 70 °C for 30 min. The reaction mixture was subjected to preparative TLC (Si gel; 1 mm thick) with hexane–EtOAc (4:1, v/v) to give 8-oxolinalyl acetate (**10**) (227 mg): IR (Liq.) 2701 and 1686 (CHO), 1734, 1368, and 1246 cm^{-1} (OAc); ^1H NMR (CCl_4) δ=1.54 (3H, s, C(9)-H_3), 1.70 (3H, s, C(10)-H_3), 1.95 (3H, s, OAc), 6.37 (1H, bt, *J* 7.0 Hz, C(6)-H), 9.34 (1H, s, CHO); MS (70 eV), *m/z* (rel intensity) 150 (21, M−AcOH), 135 (14), 121 (18), 107 (17), 71 (45), and 43 (100). Reduction of this oxolinalyl acetate (110 mg) with $LiAlH_4$ (120 mg) in ether under reflux gave dihydroxy compound **5** (56 mg); IR (Liq.) 3400 (OH), 1642, and 922 cm^{-1} (C=C); ^1H NMR ($CDCl_3$) δ=1.29 (3H, s, C(9)-H_3), 1.67 (3H, bs, C(10)-H_3), 4.00 (2H, s, -C$\underline{H}_2$OH), and 5.0—6.2 (4H, olefinic H); MS (70 eV), *m/z* (rel intensity) 152 (3, M−H_2O), 137 (6), 119 (10), 71 (49), 67 (39), and 43 (100).

*8-Hydroxydihydrolinalool (**6**)*: Selective hydrogenation of 8-hydroxylinalool (**5**) (15 mg) on Adams' PtO_2 (5 mg)

in MeOH (3 cm^3) gave **6** (12 mg): IR (Liq) 3350 (OH) and 1620 cm^{-1} (C=C); ^1H NMR (CCl_4) δ=0.90 (3H, t, J 7.5 Hz, C(1)-H_3), 1.19 (3H, s, C(9)-H_3), 1.70 (3H, bs, C(10)-H_3), 3.94 (2H, s, -C$\underline{H}_2$-OH), and 5.42 (1H, bt, J 7.0 Hz, >C=C$\underline{H}$-); MS (70 eV), m/z (rel intensity) 154 (12, M-H_2O), 139 (12), 125 (27), 73 (44), and 43 (100).

The authors wish to express their thanks to Dr. Kunihiko Ojima of Faculty of Agriculture, Tohoku University, for a gift of *Nicotiana tabacum* callus and to the Takasago perfumery Co. Inc., for a gift of sample of linalool and linalyl acetate.

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