# 7-O-Methyl-luteone Metabolism in *Botrytis cinerea:* Identification of the Epoxy-Intermediate and Absolute Configuration of the Pyrano-isoflavone Metabolite

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Fungal Metabolism, *Botrytis cinerea,* 7-O-methyl-luteone, Prenylisoflavone, Epoxy-Intermediate, Stereochemistry

7-O-Methyl-luteone was metabolized in a buffer solution by resting cells of *Botrytis cinerea* which is known as a fungus detoxifying prenylated isoflavones into further oxygenated metabolites, and we could isolate an unstable epoxy-intermediate. The epoxy-intermediate was easily cyclized under acidic conditions to yield the corresponding isoflavone with a hydroxychroman part structure (= dihydropyrano-isoflavone). The optically active (dextrorotatory) dihydropyrano-isoflavone metabolite was derivatized into the [*R*]-α-methoxy-α-trifluoromethyl-phenylacetic acid ester and its absolute configuration [*S*] was unambiguously elucidated by the modified Mosher's method.

## Introduction

As shown in our previous paper, prenylated isoflavones were metabolized by *Botrytis cinerea* into the corresponding dihydrofurano- and dihydropyrano-isoflavone derivatives, and glycol derivatives [1]. Those metabolites have been thought to be derivated through epoxy-intermediates at the prenyl double bond [1, 2]. As a matter of fact, 7-O-methyl-2,3-dehydrokievitone (**1**) which has no hydroxyl group *ortho* to the prenyl substituent was transformed into the corresponding epoxy-intermediate (**3**), which was gradually hydrolyzed into the glycol derivative (**4**) in the reaction medium of *B. cinerea* [3], whilst 2,3-dehydrokievitone (**2**) was metabolized into three metabolites (**5, 6,** and **7**) [4]. The stereochemistry at C-2″ in both epoxide (**3**) and glycol (**4**) was established to be *S* [3]. The absolute configuration *R* at the dihydrofurano side structure both in luteone (**9**) and licoisoflavone A metabolites are well compatible with the *S* configuration of the epoxy-intermediate [3], because the dihydrofurano ring formation by attacking of an *ortho*-phenolic oxygen at C-2″ is most plausibly due to a stereo-inverting $S_{N2}$ manner. Even though we could isolate an optically active (dextrorotatory) dihydropyrano-metabolite (**10**) in addition to metabolites **11** and **12** in the *Botrytis* medium administered 7-O-methyl-luteone (**8**), the stereochemistry of the side attachment remained uncertain [5].

In the former experiment of 7-O-methyl-luteone (**8**) metabolism in the culture of *B. cinerea,* we could not detect the corresponding epoxy-intermediate [5]. However, the present study using resting cells of *B. cinerea* and a buffer solution of the substrate revealed that the epoxy-intermediate of 7-O-methyl-2,3-dehydrokievitone produced in the buffer solution (pH 7.0) was found to be stable. The fact prompted us to conduct an experiment to isolate the epoxy-intermediate from 7-O-methyl-luteone (**8**) and analyze the stereochemistry of the dihydropyrano-metabolite (**10**).

## Results and Discussion

A preliminary experiment using 7-O-methyl-2,3-dehydrokievitone (**1**) and resting cells of *Botrytis cinerea* (10 mg of substrate and 25 g of wet mycelia in 200 ml of a buffer solution) was performed and revealed that almost all of the substrate (HPLC *tR* 16.74 min) disappeared from the reaction medium right after being added and the major metabolite eluted at 6.90 min by HPLC agreed to that of authentic 7-O-methyl-2,3-dehydrokievitone epoxide (**3**), increased gradually and went up to the maxi-

*Abbreviations:* *m*CPBA, *meta*-chloroperbenzoic acid; MTPA, α-methoxy-α-trifluoromethyl-phenylacetic acid.

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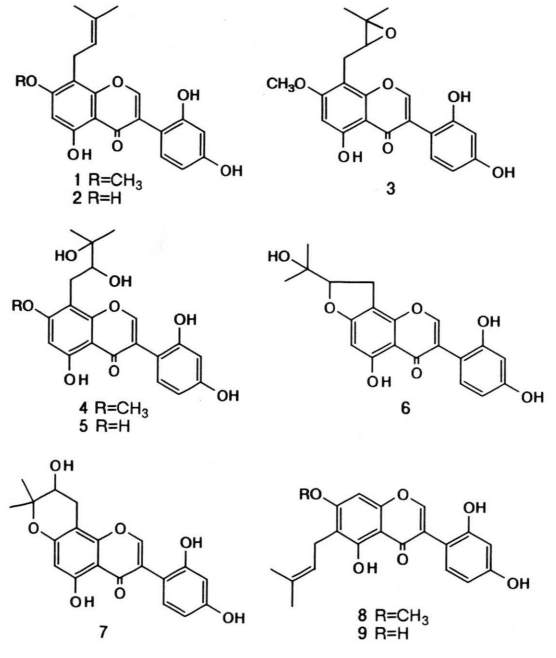
mum after 15–24 h at which point however a part of the substrate extractable with acetone still remained in the mycelia. As shown in our previous paper [3], the epoxide was also produced in the growing medium administered **1.** However, the epoxide was rapidly hydrolyzed into the corresponding glycol (**4**) probably due to the acidity of the medium (*ca.* pH 4.1) [3]. In the buffer solution at pH 7.0, no glycol derivative was detected, whereas standing in a buffer solution at pH 4.0 for 14 h at room temperature, *ca.* 2/3 of the epoxide was hydrolyzed to the glycol (**4,** *tR* 5.21).

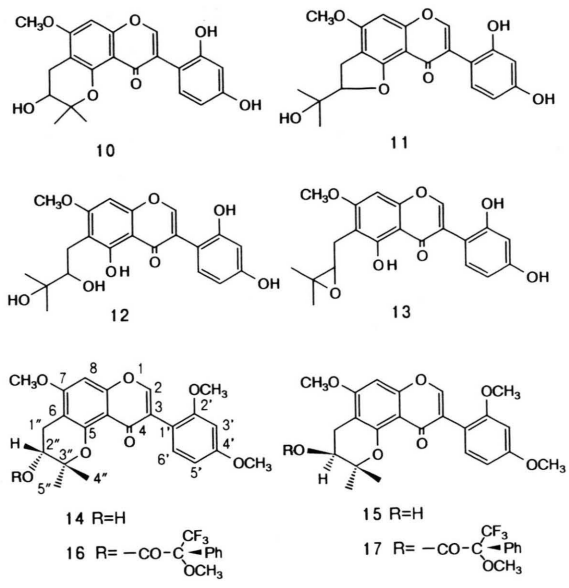
When 3% glucose was added to the reaction medium (M/20 phosphate buffer pH 7.0), the epoxidation was suppressed to one-seventh of that in the glucose free medium.

*Identification of 7-O-methyl-luteone epoxide* (**13**) *as a key intermediate for the metabolism of prenylated isoflavones*

When 7-O-methyl-luteone (**8**) was metabolized for 7 h in 100 ml of the buffer solution pH 7.0 containing 5.8 g of wet mycelia and 3.0 mg of the substrate, the HPLC chromatogram of the reaction mixture showed three major peaks with peak areas 6:3:1 respectively corresponding to dihydrofurano-type metabolite (**11,** *tR* 5.79 min), the epoxide metabolite (**13,** *tR* 7.15), and the unchanged substrate (**8,** *tR* 17.04). The epoxide metabolite (**13**) derived from 6 mg of the substrate was adsorbed to a Sep-Pak C-18 column (Environmental, Waters) followed by elution with acetonitrile. The eluate was subjected to preparative HPLC to give *ca.* 0.5 mg of pure epoxide, showing the FD-MS $M^+$ at *m/z* 384 (100%), and UV $\lambda_{max}$ (MeOH): 264 nm, 282 (sh) nm. $^1H$ NMR analysis revealed that signals due to the major constituent agreed well with those of synthetic 7-O-methyl-luteone epoxide (**13**). However the spectrum associated with minor signals corresponding to those of dihydrofurano-type derivative (**11**) which may be spontaneously derived from the epoxide after the isolation by preparative HPLC.

We can thus confirm the presence of an epoxy-intermediate in the course of oxidative metabolism of prenylated isoflavones into dihydrofurano-, dihydropyrano- and 2,3-dihydrodihydroxyprenyl-isoflavones by using 7-O-methyl-luteone (**8**) which contains a prenyl side chain and a phenolic OH *ortho* to the side chain. In a buffer solution at pH 7.0, the epoxy-intermediate (**13**) produced by *B. cinerea* was transformed predominantly into dihydrofurano-isoflavone (**11**), whereas in the growing medium (pH *ca.* 4.1) **13** was metabolized mainly into the corresponding dihydropyrano-de-



rivative and glycol (**10** and **12,** 1:1, 32% yield each) in association with minute amounts of dihydrofurano-derivative (**11,** 2%) [5]. The difference in the quantity of the metabolites was presumably attributable to the hydrogen ion concentration of the reaction medium. Since it is quite reasonable to consider that only the epoxidation of the substrate depends on biological process and the following steps for cyclic-ether formation to yield dihydrofurano- and dihydropyrano-derivatives, and hydrolysis to yield the glycol derivative are chemical events.

Epoxy-derivatives have long been postulated as biosynthetic intermediates from prenylated phenolic precursors to dihydrofurano- or dihydropyrano-ring fused part structures in many secondary metabolites [6, 7]. However, no supporting evidence has been obtained so far, for the presence of an epoxy-intermediate in prenylated phenol metabolism for example, in biosyntheses of coumarins [8], rotenoids [9], pterocarpans [10], and so on.

### *Absolute configuration of dextrorotatory dihydropyrano-isoflavone* (**10**)

As shown in our earlier paper [5], stereochemistry of the laevorotatory epoxy-intermediate (**3**) and the glycol derivative (**4**) from 7-O-methyl-2,3-dehydrokievitone (**1**), together with that of several dihydrofurano-isoflavones has been established [11]. However, no stereochemical study on dihydropyrano-isoflavone has been done so far. Even though 7-O-methyl-luteone (**8**) was metabolized into the dihydropyrano-derivative (**10**) only in minute amounts in the resting cell system buffered at pH 7.0, the epoxy-intermediate (**13**) was quantitatively transformed in 99% HCOOH solution into **10** which has been isolated as a major metabolite from the growing medium of *B. cinerea* administered **8** [5]. Therefore, stereochemical analysis of dihydropyrano-metabolite was conducted by using the modified Mosher's method [12, 13] and dextrorotatory **10.** This method has already been applied successfully to the stereochemical study on isoflavone atropisomers bearing a dimethyldihydrohydroxypyrano part structure [14].

At first, [*RS*]-dihydropyrano-isoflavone ([*RS*]-**10**) prepared from 7-O-methyl-luteone (**8**) and *m*CPBA was derivatized into dimethyl ether (**14** and **15**), and further esterified with [*R*]-MTPA. The resulting diastereomers ([*R,R*]- and [*R,S*]-isomers (**16** and **17**); the former *R* indicates the configuration in MTPA and the latter, that in the pyrano side structure) were isolated by PTLC in benzene-ethyl acetate = 5:1, as lower (**16**) and upper (**17**) bands respectively, both exhibiting the $M^+$ ion at *m/z* 628. $^1H$ NMR spectra of these isolates in $CDCl_3$ are shown in Table I. Since the shielding effect caused by the phenyl group in MTPA esters

Table I. $^1H$ NMR data for diastereomeric MTPA esters (**16** and **17**) and trimethyl ether (**15**).

| Compound<br>Proton | δ ppm (*J* [Hz])<br>**16** | <br>**17** | <br>**15** |
|---|---|---|---|
| H-2 | 7.70 s | 7.71 s | 7.72 s |
| $CH_3O$-7 | 3.88 s | 3.90 s | 3.90 s |
| H-8 | 6.39 s | 6.42 s | 6.43 s |
| $CH_3$-2′ | 3.75 s | 3.74 s | 3.75 s |
| H-3′ | 6.50–6.56 m (2H) | 6.50–6.55 m (2H) | 6.51 d, *J* = 2.4 |
| H-5′ | | | 6.53 dd, *J* = 8.3, 2.4 |
| H-6′ | 7.26 d, *J* = 8.2 | 7.26 d, *J* = 8.2 | 7.27 d, *J* = 8.3 |
| Ha-1″ | 3.15 dd, *J* = 17.2, 5.8 | 3.15 dd, *J* = 17.5, 5.7 | 2.89 dd, *J* = 17.4, 5.2 |
| Hb-1″ | 2.60 br. dd, *J* = 17.2, 7.4 | 2.74 br. dd, *J* = 17.5, 7.2 | 2.70 dd, *J* = 17.4, 5.3 |
| H-2″ | 5.17 dd, *J* = 7.4, 5.8 | 5.19 dd, *J* = 7.2, 5.7 | 3.85 t-like, *J* = 5.3 |
| $H_3$-4″ | 1.45 s | 1.37 br. s | 1.43 s |
| $H_3$-5″ | 1.29 s | 1.25 s | 1.37 s |
| $CH_3O$-MTPA | 3.51 s | 3.50 s | |
| aromatic proton in MTPA | 7.50 m (2H)<br>7.39 m (3H) | 7.50 m (2H)<br>7.38 m (3H) | |

NMR spectra were determined at 270 or 500 MHz in $CDCl_3$ (TMS reference).
$CH_3O$-4′ in each compound (**15**–**17**) was detected at δ 3.82 as a singlet.

are expected only on the protons neighbouring to the secondary carbinol carbon and oriented to the same direction as that of the phenyl group (see Fig. 1). When compared the chemical shift values (Table I), it is apparent that the protons located to the opposite direction from the carbinol carbon (C-2″, an asymmetric centre), $H_2$-1″ in **16** and two $CH_3$ on C-3″ in **17** are relatively more shielded than the corresponding protons respectively in **17** and **16**. In the light of the modified Mosher's method [12, 13], the absolute configuration [*R,R*] and [*R,S*] were thus attributed to **16** and **17,** respectively.

The dextrorotatory dihydropyrano-type metabolite was methylated in the same manner as described to methylation of a mixture of synthetic [*RS*]-isomers, followed by esterification with [*R*]-MTPA. The MTPA ester was purified by PTLC in benzene-ethyl acetate and revealed to be identical to the former [*R,S*]-MTPA ester (**17**) in $^1$H NMR spectroscopy. Thus the absolute configuration *S* was attributed to the asymmetric centre in the dihydropyrano side structure of the dextrorotatory 7-O-methyl-luteone metabolite (**15**). Together with our earlier results [3, 11], the present study made the stereochemistry for the fungal metabolism of prenylated isoflavones completely clear as shown in Fig. 2.

Optically active dihydropyrano-isoflavone mundulone was isolated from *Mundulea sericea* [15], however the absolute configuration remained to be solved. To our knowledge, the metabolite (+)−**10** may be the first dihydropyrano-isoflavone for which its stereochemistry has been established,

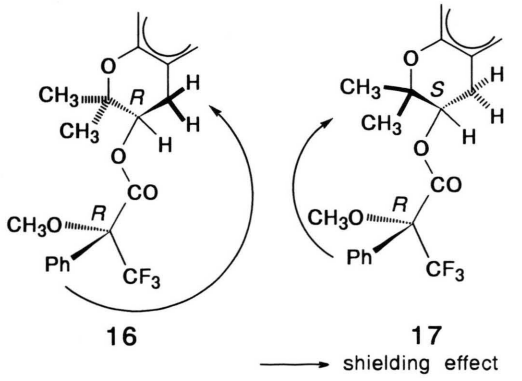



Fig. 1. Configuration of MTPA esters and expectable shielding effects.

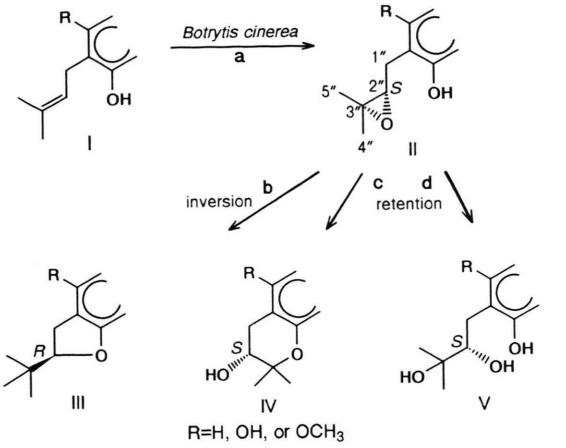



Fig. 2. Stereochemistry for fungal transformation of prenylated isoflavones. I: substrate, II: epoxy-intermediate, III: dihydrofurano-metabolite, IV: dihydropyrano-metabolite, and V: glycol metabolite. **a:** stereospecific oxygenation by *Botrytis cinerea;* **b:** back-side attack by the *o*-phenolic oxygen at C-2″ of the epoxy-intermediate resulting in stereo-inversion; **c:** attack by the *o*-phenolic oxygen directly at C-3″ of the epoxy-intermediate or the protonated epoxide; **d:** hydrolysis of the epoxy-intermediate under acidic conditions. Steps **b**−**d** presumably take place spontaneously.

whereas the stereochemistry of dihydropyrano-ring fused coumarins has been reported [16].

## Experimental

### *General procedures*

Spectroscopic data were obtained by the following instruments, FD-MS: JEOL JMS-O1SG-2, EI-MS: JEOL JMS-DX300, UV: Hitachi U-3210, and $^1$H NMR: Bruker AM 500 or JEOL JNM GX 270. TLC was performed by using Merck precoated glass plates Silica Gel 60 $F_{254}$ (0.25 and 0.50 mm thickness). Hitachi L-6000 HPLC equipped with an L-4000 UV detector, a D-2500 chromato-integrator and an Inertsil Prep-ODS 6.0 × 250 mm column was used for analytical purpose of isoflavonoids and their derivatives. A mixture of MeOH−$H_2O$ = 4:1 as an eluting solvent was flowed at 1 ml/min and eluates were monitored at UV 260 nm.

### *Fungus and metabolic experiments*

*Botrytis cinerea* AHU 9424 grown in the PYG medium (peptone 1%, yeast extract 0.1% and glu-

cose 5%) at 25 °C for 4–5 days was harvested by filtration and washed with M/20 pH 7.0 phosphate buffer solution. The sucked-dry mycelia (*ca.* 78% moisture) were resuspended in the phosphate buffer, and a substrate was added to the suspension as ethanol solution and shaken gently at 25 °C for a set time. An aliquot (*ca.* 3 ml containing less than 0.15 mg of isoflavonoid) of this metabolic reaction mixture (100–200 ml) was pretreated with a Sep-Pak cartridge (C-18, Light, Waters) which had been previously washed with 0.6 ml of acetonitrile and equilibrated with 3 ml of the phosphate buffer. The adsorbed isoflavonoids were eluted with 0.6 ml of acetonitrile and 10 µl of the eluate was injected into the chromatograph.

*Chemicals*

7-O-Methyl derivatives of 2,3-dehydrokievitone and luteone (**1** and **8**) were prepared as previously described [3, 17]. Additional data for dihydropyrano-isoflavone derivative (+)–**10**; $[\alpha]_D$ + 64° ($c = 0.13$, MeOH), in ref. [2] we incorrectly reported the specific rotation of **10** as + 6.4°. [*R*]-MTPA (α-methoxy-α-trifluoromethyl-phenylacetic acid) purchased from Aldrich was derivatized into its chloride according to literature [18].

[*RS*]-7-O-Methyl-luteone epoxide ([*RS*]-**13**): [*RS*]-**13** was prepared according to the method of Crombie *et al.* [19]. To **8** (10 mg) in 0.3 ml of $CHCl_3$ was added 5.4 mg of anhydrous disodium hydrogen phosphate and mixed with *m*CPBA (6.5 mg in 0.2 ml of $CHCl_3$) by stirring for 30 min at which point the mixture was diluted with 0.5 ml of EtOAc. The resulting organic layer was washed successively with aq. 5% $NaHCO_3$, and saturated aq. NaCl. The organic layer dried over $Na_2SO_4$ concentrated to yield 4.3 mg of the epoxide of 7-O-methyl-luteone showing more than 94% purity (analyzed by HPLC). [*RS*]-**13**: Gibbs test, (+) rapid, purple. FD-MS *m/z* (rel. int.): 385 ($[M+1]^+$, 42), 384 ($[M]^+$, 100). $^1$H NMR (acetone-$d_6$) $\delta_{TMS}$ ($J$ = Hz): 13.12 (s, 5-OH), 8.48 (br. s, phenolic OH), 8.31 (br. s, phenolic OH), 8.24 (s, H-2), 7.14 (d, $J$ = 8.2, H-6′), 6.73 (s, H-8), 6.50 (d, $J$ = 2.4, H-3′), 6.45 (dd, $J$ = 8.2, 2.4, H-5′), 4.02 (3H, s, $CH_3O$-7), 3.01 (dd, $J$ = 13.5, 4.7, Ha-1″), 2.89 (dd, $J$ = 7.5, 4.7, H-2″), 2.75 (dd, $J$ = 13.5, 7.5, Hb-1″), 1.38 (3H, s, $H_3$-4″), 1.20 (3H, s, $H_3$-5″). UV $\lambda_{max}$ in MeOH (nm): 264, 282sh; + $AlCl_3$ 273, 302sh.

[*R*]-MTPA esters: [*R*]-MTPA ester of [*RS*]-dihydropyrano-isoflavone derivative (**16** and **17**) was prepared as follows. [*RS*]-7-O-Methyl-luteone epoxide ([*RS*]-**13,** 20 mg) was solved in HCOOH and stood for 90 min to yield dihydropyrano-isoflavone ([*RS*]-**10,** 9.5 mg). Physicochemical properties of thus prepared [*RS*]-**10** agreed well with those of the dihydropyrano-type 7-O-methyl-luteone metabolite [5] except its optical property. [*RS*]-**10** was then methylated as usual in dry acetone containing potassium carbonate [20] to yield the corresponding trimethyl ether (**14** and **15**). **14** and **15:** Gibbs test (–). EI-MS *m/z* (rel. int.): 413 ($[M+1]^+$, 28), 412 ($[M]^+$, 84), 382 (22), 381 (81), 353 (12), 342 (20), 341 (78), 340 (48), 339 (24), 325 (22), 311 (13), 309 (35), 179 (27), 163 (12), 162 (28), 161 (100), 148 (14), 119 (14), 57 (10). $^1$H NMR (acetone-$d_6$) $\delta_{TMS}$ ($J$ = Hz): 7.79 (s, H-2), 7.15 (d, $J$ = 8.3, H-6′), 6.60 (d, $J$ = 2.4, H-3′), 6.55 (s, H-8), 6.54 (dd, $J$ = 8.3, 2.4, H-5′), 4.25 (d, $J$ = 5.4, OH-2″), 3.95 (3H, s, $CH_3O$-7), 3.81 (3H, s, $CH_3O$-4′), 3.80 (m, H-2″), 3.75 (3H, s, $CH_3O$-2′), 2.91 (dd, $J$ = 17.2, 5.7, Ha-1″), 2.55 (dd, $J$ = 17.2, 7.4, Hb-1″), 1.37 (3H, s, $H_3$-4″), 1.27 (3H, s, $H_3$-5″).

Thus derivatized 7,2′,4′-tri-O-methyl ether ([*RS*]-**14,** 2.8 mg) was esterified in a mixture of 50 µl of anhydrous $CH_2Cl_2$, 50 µl of pyridine and 10 µl of MTPA chloride. After standing for 20 h, the excess chloride was decomposed by the addition of 3-dimethylamino-1-propylamine, and the reaction mixture diluted with ether was washed successively with 2 N aq. HCl, 5% $NaHCO_3$ and aq. NaCl solution. The organic layer was dried over $MgSO_4$ and the diastereomeric constituents were separated from each other by preparative TLC (PTLC) in benzene-EtOAc = 5:1 after triplicated development; the upper band corresponding to **17** moved to $R_f$ 0.57 and the lower one corresponding to **16,** $R_f$ 0.50 both in *ca.* 1.5 mg. **16:** EI-MS *m/z* (rel. int.): 628 ($[M]^+$, 17), 597 (19), 413 (43), 395 (19), 380 (26), 379 (100), 363 (21), 340 (20), 233 (19), 217 (22), 190 (18), 189 (88), 162 (23), 161 (68), 148 (18), 139 (22), 127 (20), 119 (60), 105 (95), 92 (21), 91 (44), 77 (71). $^1$H NMR data, see Table I. **17:** EI-MS *m/z* (rel. int.): 628 ($[M]^+$, 15), 597 (16), 413 (37), 395 (19), 380 (25), 379 (100), 363 (20), 341 (17), 340 (19), 233 (18), 217 (22), 190 (17), 189 (92), 162 (24), 161 (68), 148 (17), 141 (18), 139 (22), 127 (20), 119 (63), 105 (94), 92 (22), 91 (44), 77 (65). $^1$H NMR data, see Table I.

[*R*]-MTPA ester of the dextrorotatory dihydropyrano metabolite (**17**): The dextrorotatory dihydropyrano-type metabolite ([*S*]-**10**) was methylated and esterified with [*R*]-MTPA in the same manner as described to the preparation of diastereoisomeric [*R*]-MTPA esters (**16** and **17**). $^1$H NMR data of the trimethyl ether (**15**) in $CDCl_3$ are shown in Table I. The chromatographic (silica gel TLC) and physicochemical properties (EI-MS and $^1$H NMR) were indistinguishable from those of not **16** ([*R,R*]-isomer), but **17** ([*R,S*]-isomer).

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[1] S. Tahara, J. L. Ingham, and J. Mizutani, Z. Naturforsch. **46c,** 341 (1991).
[2] S. Tahara, S. Nakahara, J. Mizutani, and J. L. Ingham, Agric. Biol. Chem. **48,** 1471 (1984).
[3] S. Tahara, J. L. Ingham, and J. Mizutani, Phytochemistry **28,** 2079 (1989).
[4] S. Tahara, E. Misumi, J. Mizutani, and J. L. Ingham, Z. Naturforsch. **42c,** 1055 (1987).
[5] S. Tahara, J. L. Ingham, and J. Mizutani, Nippon Nogeikagaku Kaishi **63,** 999 (1989).
[6] R. D. H. Murray, Fortschr. Chem. Org. Naturst. **35,** 199 (1978).
[7] L. Crombie, Nat. Prod. Rep. **1,** 3 (1984).
[8] D. Hamerski and U. Matern, Eur. J. Biochem. **171,** 369 (1988).
[9] L. Crombie, J. T. Rossiter, N. Van Bruggen, and D. A. Whiting, Phytochemistry **31,** 451 (1992).
[10] R. Welle and H. Grisebach, Arch. Biochem. Biophys. **263,** 191 (1988).
[11] S. Tahara, J. L. Ingham, and J. Mizutani, Agric. Biol. Chem. **51,** 211 (1987).
[12] J. L. Dale and H. S. Mosher, J. Am. Chem. Soc. **95,** 512 (1973).
[13] I. Ohtani, T. Kusumi, Y. Koshman, and H. Kakisawa, J. Am. Chem. Soc. **113,** 4092 (1991).
[14] M. Moriyama, Master Thesis, Graduate School of Agriculture, Hokkaido University, 1992.
[15] B. F. Burrows, N. Finch, W. D. Ollis, and I. O. Sutherland, Proc. Chem. Soc. **1959,** 150.
[16] J. Lemmich and B. E. Nielsen, Tetrahedron Lett. **1969,** 3.
[17] S. Tahara, K. Watanabe, and J. Mizutani, J. Fac. Agr. Hokkaido Univ. **63,** 155 (1987).
[18] J. A. Dale, D. L. Dull, and H. S. Mosher, J. Org. Chem. **34,** 2543 (1969).
[19] L. Crombie, G. W. Kilbee, F. Moffatt, G. Proudfoot, and D. A. Whiting, J. Chem. Soc. Perkin Trans. I, **1991,** 3143.
[20] S. Tahara, J. L. Ingham, S. Nakahara, J. Mizutani, and J. B. Harborne, Phytochemistry **23,** 1889 (1984).