

Biotransformation of (-)-Menthol by *Eucalyptus perriniana* Cultured Cells¹

Tsutomu Furuya,* Yutaka Orihara, and Hiromitsu Miyatake

School of Pharmaceutical Sciences, Kitasato University, Shirokane, Minato-ku, Tokyo 108, Japan

Ten biotransformation products have been isolated from *Eucalyptus perriniana* cultured cells administered (-)-menthol. The main product is (-)-menthol 3-*O*-β-D-gentiobioside (**1**) and the minor products are (-)-menthol 3-*O*-β-D-cellobioside (**2**), (+)-neomenthol 3-*O*-β-D-gentiobioside (**3**), (1*R*,3*R*,4*R*)-*p*-menthane-3,8-diol 3-*O*-β-D-glucoside (**4**) and 8-*O*-β-D-glucoside (**5**), (1*R*,3*R*,4*R*)-*p*-menthane-3,8-diol 3-*O*-β-D-gentiobioside (**6**) and 8-*O*-β-D-gentobioside (**7**), (1*S*,3*R*,4*S*)-*p*-menthane-1,3-diol 3-*O*-β-D-glucoside (**8**), (1*R*,2*S*,3*S*,4*S*)-*p*-menthane-2,3-diol 3-*O*-β-D-gentiobioside (**9**), and 2-*O*-β-D-gentiobioside (**10**).

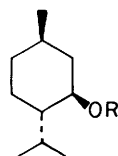
Cultured or immobilized plant cells have been used by many investigators, to study such biotransformations of organic compounds as oxidation, reduction, hydroxylation, glycosylation, esterification, methylation, and isomerization.^{2,3} One of the advantages of biotransformation is that the substrate which is not only in the biosynthetic pathway of plant cells but also irrelevant to it can be metabolized. Glycosylation is characteristic of this in that it occurs readily in plant cells but only with difficulty in micro-organisms.

In the course of our biotransformation experiments, (-)-menthol (**11**) was fed to several *Eucalyptus* cell suspension cultures. Among the cultures tested, only *Eucalyptus perriniana* cultured cells, from which eight triterpenes have already been isolated and their structures determined,⁴ can convert (**11**) into water-soluble products. In this paper, we describe the isolation and structure elucidation of these biotransformation products.

Results and Discussion

The suspension culture of *E. perriniana* was prepared from static cultured cells on a reciprocal shaker. After pre-culture for 3 weeks on BA1 liquid medium (Murashige and Skoog's basal medium⁵ containing sucrose 30 g/l and benzylaminopurine 1 mg/l), (-)-menthol (**11**) (50 mg) dissolved in ethanol was administered to each flask, and the culture allowed to proceed for an additional 7 days. The cells were harvested and twice extracted with methanol and the extract was evaporated. The residue was then extracted with ethyl acetate and butanol. When the butanol fraction was subjected to silica gel plate chromatography (10% H₂SO₄ spray and heating) characteristic red-purple spots that were absent in the control culture were detected. In contrast the t.l.c. characteristics of the ethyl acetate fraction were almost identical with those of the control culture. From 26 culture flasks [total 1 300 mg of (-)-menthol was fed] 6.4 g of butanol fraction was obtained. This fraction was chromatographed on silica gel and the resulting product fractions were further purified by h.p.l.c.: products (**1**), (**2**), (**3**), (**5**), and (**8**) were isolated. Further purification of the mixtures of (**4**) and (**5**), (**6**) and (**7**), (**9**) and (**10**) by h.p.l.c. was performed after acetylation, to give the acetates (**4a**) and (**5a**), (**6a**) and (**7a**), and (**9a**) and (**10a**), respectively. The ¹³C n.m.r. chemical shifts of products (**1**)—(**10**) are presented in Table 1 and of their acetates (**1a**)—(**10a**) in Table 2.

The main product (**1**), yield 26.6% from (-)-menthol (**11**), was isolated as colourless prisms (from ethanol), m.p. 125—126 °C, the field desorption mass spectrum (f.d.-m.s.) of which showed the *M* + 1⁺ ion at *m/z* 481; its molecular weight is, therefore, 480. In the ¹H n.m.r. spectrum of product (**1**) three

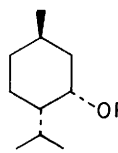


(1) R = Glc $\frac{6-1}{-}$ Glc

(1a) R = Glc $\frac{6-1}{-}$ Glc(Ac)₇

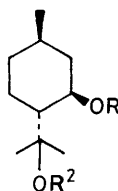
(2) R = Glc $\frac{4-1}{-}$ Glc

(2a) R = Glc $\frac{4-1}{-}$ Glc(Ac)₇



(3) R = Glc $\frac{6-1}{-}$ Glc

(3a) R = Glc $\frac{6-1}{-}$ Glc(Ac)₇



(4) R¹ = Glc, R² = H

(4a) R¹ = Glc(Ac)₄, R² = H

(5) R¹ = H, R² = Glc

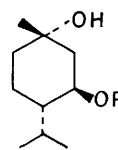
(5a) R¹ = Ac, R² = Glc(Ac)₄

(6) R¹ = Glc $\frac{6-1}{-}$ Glc, R² = H

(6a) R¹ = Glc $\frac{6-1}{-}$ Glc(Ac)₇, R² = H

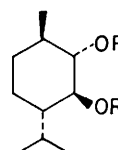
(7) R¹ = H, R² = Glc $\frac{6-1}{-}$ Glc

(7a) R¹ = Ac, R² = Glc $\frac{6-1}{-}$ Glc(Ac)₇



(8) R = Glc

(8a) R = Glc(Ac)₄



(9) R¹ = Glc $\frac{6-1}{-}$ Glc, R² = H

(9a) R¹ = Glc $\frac{6-1}{-}$ Glc(Ac)₇, R² = Ac

(10) R¹ = H, R² = Glc $\frac{6-1}{-}$ Glc

(10a) R¹ = Ac, R² = Glc $\frac{6-1}{-}$ Glc(Ac)₇

Glc = glucose.

methyl signals at δ_{H} 0.68, 0.81, and 0.98 p.p.m. (each d, each *J* 7.0 Hz) and a 3-H signal at δ_{H} 3.82 p.p.m. (ddd, *J* 11.0, 11.0, and 4.0 Hz) were observed; this suggests the presence of the (-)-menthol entity. Other proton signals observed at δ_{H} 3.87—5.07 are considered sugar protons, especially two anomeric proton signals at δ_{H} 4.82 and 5.07 (each d, *J* 8 Hz). Furthermore, in the ¹H n.m.r. of the acetate (**1a**) the coupling constants for 1' to 5' and 1'' to 5'' are all relatively large (8—10 Hz) on the basis of ¹H—¹H shift correlation spectrum; this suggests that the two sugars connected to (**11**) are glucose, each of which has the β-configuration. In the ¹³C n.m.r. spectrum of (**1**) 22 carbon signals are observed and 10 of them are assigned to the (-)-menthol entity, reference being made to the data of

Table 1. ^{13}C Chemical shifts of biotransformation products (1)–(10) in $\text{C}_5\text{D}_5\text{N}$ at 100 MHz

Carbon	(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
1	31.5	31.7	28.6	31.8	31.5	31.5	31.6	70.3	38.4	37.2
2	41.0	41.4	42.3	41.4	45.8	41.5	45.8	45.8	81.4	93.5
3	76.2	76.4	77.9	79.6	72.2	80.0	72.3	75.5	88.5	76.5
4	48.7	48.6	49.4	53.4	53.7	52.6	53.1	49.1	47.4	48.2
5	23.7	23.6	25.3	28.0	26.9	27.5	27.1	20.0	22.9	23.1
6	34.9	34.9	35.7	35.2	35.2	35.0	35.2	39.4	33.0	33.4
7	22.6	22.6	22.9	22.6	22.4	22.3 ^a	22.4 ^a	32.6	19.3	19.7
8	25.8	25.6	26.8	73.5	82.5	73.2	82.3	26.0	25.3	26.3
9	16.9	16.3	21.4	26.1	21.0	27.1 ^a	21.4 ^a	16.7	16.2	16.6
10	21.5	21.4	22.0	30.2	27.7	31.0 ^a	28.1 ^a	21.8	21.8	21.4
1'	100.8	101.6	106.4	101.5	98.2	102.2	98.1	102.0	106.2	106.3
2'	75.1 ^a	74.8 ^a	75.7 ^a	75.5	75.2	75.0 ^d	74.9 ^b	75.5	76.0	76.2
3'	78.4 ^b	77.5 ^b	78.8 ^b	78.8	79.2	78.5 ^b	78.9 ^c	78.9	78.9	78.9 ^a
4'	72.1 ^c	82.3	72.0 ^c	72.3	71.9	71.7 ^c	72.3 ^d	72.3	71.7 ^b	71.9 ^b
5'	77.5	77.1 ^b	77.4	78.9	78.6	77.3	77.1	78.2	77.2	77.6
6'	70.8	62.6 ^d	70.7	63.5	63.0	70.9	70.5	63.4	70.5	70.5
1''	105.6	105.2	105.6			105.4	105.5		105.7	105.6
2''	75.3 ^a	75.0 ^a	75.3 ^a			75.5 ^d	75.3 ^b		75.4	75.6
3''	78.6 ^b	78.7 ^c	78.7 ^c			78.6 ^b	78.7 ^c		78.6	78.5 ^a
4''	71.7 ^c	71.7	71.8 ^c			71.9 ^c	71.8 ^d		71.8 ^b	71.8 ^b
5''	78.6	78.4 ^c	78.6 ^b			78.5 ^b	78.5 ^c		78.6	78.7
6''	62.9	62.9 ^d	62.9			62.8	62.9		63.0	63.0

^{a–d} Assignments may be reversed in each vertical column.**Table 2.** ^{13}C Chemical shifts of acetates of biotransformation products (1a)–(10a) in CDCl_3 at 100 MHz

Carbon	(1a)	(2a)	(3a)	(4a)	(5a)	(6a)	(7a)	(8a)	(9a)	(10a)
1	31.4	31.4	28.3	31.4	31.2	31.2	31.2	71.4	37.3	36.8
2	40.5	40.7	41.5	40.0	41.5	39.9	41.6	44.3	81.9 ^a	84.2 ^a
3	78.1	78.9	78.9	80.0	73.4	79.7	73.3	77.0	79.3 ^a	77.8 ^a
4	47.6	47.4	48.6	52.1	50.2	52.1	50.2	47.5	46.9	47.3
5	23.1	23.0	24.6	27.4	25.5	27.3	25.5	18.7	22.1	22.3
6	34.3	34.2	34.8	34.3	34.1	34.3	34.1	38.4	31.9	32.3
7	22.3	22.2	22.4	22.1	21.7	22.0	21.8	31.7	17.9	18.9
8	25.2	25.1	26.0	72.9	79.9	72.9	79.9	25.0	24.4	25.9
9	16.0	15.4	21.0	23.9	23.6	24.4	23.8	15.5	15.7	15.9
10	20.9	20.8	21.2	29.5	26.8	29.5	26.7	21.0	20.6	20.6
1'	97.9	98.5	102.2	97.3	94.8	96.9	94.6	99.1	100.4	100.4
2'	71.6 ^a	71.8	71.6 ^a	71.2	71.4 ^a	71.4	71.7	71.5	71.0 ^b	71.2
3'	73.0 ^b	72.8	72.9 ^b	72.5	73.2	72.5	73.1	73.1	72.9 ^c	73.2 ^b
4'	69.2 ^c	77.2	69.2	68.5	68.9	69.1	69.3	68.8	69.1 ^d	69.2 ^c
5'	73.4 ^b	72.4	73.2 ^b	71.8	71.7 ^a	74.0	73.2	71.5	73.3 ^c	73.5 ^b
6'	68.2	61.6	68.2	61.9	62.4	68.1	68.1	62.5	68.0	67.9
1''	100.5	100.8	100.6			100.5	100.4		100.4	100.4
2''	71.2 ^a	71.6	71.1 ^a			71.3	71.2		71.2 ^b	71.2
3''	72.9 ^b	73.0	72.9 ^b			72.8	72.9		72.9 ^c	72.8 ^b
4''	68.3 ^c	67.8	68.3			68.3	68.4		68.3 ^d	68.2 ^c
5''	72.0	72.0	72.0			72.0	71.9		72.0	71.9
6''	61.9	62.2	61.9			61.9	61.9		61.8	61.8

^{a–d} Assignments may be reversed in each vertical column.

(–)-menthol 3-*O*- β -D-glucopyranoside.⁶ The other 12 signals observed at δ_{C} 60–105 p.p.m. are in good agreement with the reported values for β -D-glucopyranosides⁶ except for one methylene carbon signal at δ_{C} 70.8 p.p.m., which can be ascribed to C-6' of one glucose moiety, where terminal glucose is linked.

As mentioned above it is supposed that product (1) is (1*R*,3*R*,4*S*)-*p*-menthan-3-yl *O*- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside [(–)-menthol 3-*O*- β -D-gentiobioside]. Furthermore its acetate was synthesized from (11) and D-gentiobiose peracetate, according to Sakata *et al.*,⁷ and the synthesized and biotransformed acetate (1a) are identified with each other by direct comparison of their ^1H n.m.r. and i.r. spectra.

Product (2) was isolated as colourless needles, m.p. 182–

184 °C, and its molecular weight was determined as 480 by fast atom bombardment mass spectrometry (f.a.b.–m.s.). Since 22 carbon signals are observed in the ^{13}C n.m.r. spectrum and signals assigned to the aglycone part are in good agreement with those of product (1), it is considered that the aglycone part of product (2) is also (–)-menthol and that product (2) is the isomer of product (1), the sugar part differing. In the ^1H n.m.r. and ^1H – ^1H shift correlation spectrum of (2a), as for (1a), the coupling constants for 1' to 5' and 1'' to 5'' are all relatively large values (8–10 Hz), so that the two sugars connected to (–)-menthol are also β -glucose. The carbon signal observed at δ_{C} 82.3 p.p.m. is assigned to C-4' of the inner glucose by ^{13}C – ^1H shift correlation spectrum. On the basis of the above results it is considered that product (2) is (1*R*,3*R*,4*S*)-*p*-menthan-3-yl *O*- β -

D-glucopyranosyl-(1→4)-β-D-glucopyranoside [(–)-menthol 3-*O*-β-D-cellobioside].

Product (3) was isolated as colourless prisms, m.p. 104–106 °C, and its molecular weight was determined as 480, the same value for products (1) and (2), on the basis of f.a.b.–m.s. results. In the ¹H n.m.r. spectrum of (3a) 3 methyl signals (δ_H 0.84, 0.84, and 0.88 p.p.m.) were observed as doublets and a 3-H signal was observed at δ_H 3.92 p.p.m. as a broad singlet. Since this is in contrast to product (1) (ddd, *J* 11.0, 11.0, and 4.0 Hz), the oxygen atom in the aglycone part is axial oriented; namely, it is thought oxidation and reduction occurs at C-3 in (11). In the ¹³C n.m.r. spectrum of (3) and (3a) carbon signals assigned to the sugar part agree well with those of products (1) and (1a), respectively, except for C-1', so that it is thought the sugar part is also gentiobiose. On the basis of the above data it is suggested that product (3) is (1*R*,3*S*,4*S*)-*p*-menthan-3-yl *O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside [(+)-neomenthol 3-*O*-β-D-gentiobioside].

Product (5) was isolated as colourless flakes, m.p. 204–206 °C. Compounds (4a) and (5a) were isolated by h.p.l.c. after acetylation of the mixture of products (4) and (5) both as colourless needles, m.p. 175–177 °C and m.p. 116–118 °C, respectively. Product (4), an amorphous solid, was obtained by deacetylation of (4a). In the f.d.–m.s. and f.a.b.–m.s. the molecular weights of (4) and (5) were determined as 334. In the ¹³C n.m.r. spectra of both (4) and (5) 16 carbon signals were observed. Those arising from the sugar part were assignable to glucose, the signals at δ_C 73.5 p.p.m. and 82.5 p.p.m. for the quaternary carbon attached to the oxygen atom in the aglycone part being particularly characteristic. Furthermore, the C-4 signals for products (4) and (5) observed at δ_C 53.4 and 53.7 p.p.m., the C-9 signals observed at δ_C 26.1 and 21.0 p.p.m., and the C-10 signals observed at δ_C 30.2 and 27.7 p.p.m., respectively, are shifted 4–9 p.p.m. to lower field compared with those for (1) and (2). In the ¹H n.m.r. spectra two methyl signals (9-H and 10-H in the aglycone part) are observed as singlets, so that the hydroxylation mentioned above has occurred at C-8 and the aglycone part of products (4) and (5) is *p*-menthane-3,8-diol (15). Since the 3-H proton signal of (5) observed at 3.73 p.p.m. is shifted to lower field (4.77 p.p.m.) by acetylation, it is supposed that the 3-OH group is acetylated and the glucose is connected to 8-*O*. On the other hand, the 3-H signal of (4) and (4a) is observed at δ 4.03 and 3.80, respectively, so that glycosylation has occurred at 3-*O*. Additionally, since only 4 acetyl signals are observed in the ¹H and ¹³C n.m.r. spectra of (4a), but 5 acetyl signals are observed in those of (5a), product (4) possesses the tertiary hydroxy group. It is concluded that products (4) and (5) are (1*R*,3*R*,4*R*)-8-hydroxy-*p*-menthan-3-yl *O*-β-D-glucopyranoside (*p*-menthane-3,8-diol 3-*O*-β-D-glucoside) and (1*R*,3*R*,4*R*)-3-hydroxy-*p*-menthan-8-yl *O*-β-D-glucopyranoside (*p*-menthane-3,8-diol 8-*O*-β-D-glucoside), respectively.

Acetylation of the mixture of products (6) and (7) gave compounds (6a) and (7a) as colourless needles and colourless prisms, m.p. 202–204 °C and m.p. 160–161 °C, respectively. In the ¹H n.m.r. spectra of (6a) and (7a) as well as in those of (4) and (5) two methyl signals were observed as singlets; the 3-H signals appear at δ_H 3.83 and 4.78 (ddd), respectively. In the ¹³C n.m.r. of (6a) and (7a) 22 carbon signals in addition to the acetyl signals were observed and the signals assigned to the aglycone part agreed well with those of (4a) and (5a), respectively (see Table 2) so that it is thought the aglycone parts of both products (6) and (7) are *p*-menthane-3,8-diol (15) with glycosylation occurring at C-3-*O* and C-8-*O*, respectively. Furthermore it is thought that the sugar parts of both products (6) and (7) are β-D-gentiobiose according to the ¹³C n.m.r. chemical shifts and the ¹H n.m.r. coupling constants.

Based on the above results, it is thought that the structures of product (6) and (7) are (1*R*,3*R*,4*R*)-8-hydroxy-*p*-menthan-3-yl

O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (*p*-menthane-3,8-diol 3-*O*-β-D-gentiobioside) and (1*R*,3*R*,4*R*)-3-hydroxy-*p*-menthan-8-yl *O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (*p*-menthane-3,8-diol 8-*O*-β-D-gentiobioside), respectively.

Product (8) was isolated as an amorphous solid by h.p.l.c. and in the ¹³C n.m.r. spectrum 16 carbon signals are observed. In particular one oxygen attached to a quaternary carbon is observed at δ_C 70.3 p.p.m. in the aglycone part, and the sugar part is assigned as glucose. In the ¹H n.m.r. spectrum of product (8) one methyl signal is observed as a singlet so that hydroxylation occurs at C-1. As the 3-H signal observed at δ_H 4.36 is not shifted to lower field upon acetylation, and only 4 acetyl signals are observed (one hydroxy group was not acetylated) in the ¹H n.m.r. spectrum of (8a), it is demonstrated that the glycosylation has occurred at 3-*O* as in the products (4) and (6). The stereochemistry of C-1 was elucidated as follows; in the ¹H n.m.r. spectrum of (8a), 5-axial-H and 3-H are observed at δ_H 1.29–1.42 and 3.71, respectively, shifted to 0.3–0.4 p.p.m. lower field than (1a) and (2a), so that hydroxy group attached to C-1 is oriented to the axial, 1*S* configuration. Additionally, the C-1 and 1-Me signal of product (8) observed at δ_C 70.3 and 32.6 p.p.m. respectively are compared with those of *cis*-1-methyl-3-*t*-butylcyclohexanol observed at δ_C 70.1 and 32.2 p.p.m. and *trans*-1-methyl-3-*t*-butylcyclohexanol observed at δ_C 71.6 and 26.1 p.p.m.,⁸ it is also concluded that methyl group at C-1 is oriented to equatorial and the stereochemistry of C-1 has the *S*-configuration. On the basis of these data it is concluded that product (8) is (1*S*,3*R*,4*S*)-1-hydroxy-*p*-menthan-3-yl *O*-β-D-glucopyranoside (*p*-menthane-1,3-diol 3-*O*-β-D-glucoside).

Both compounds (9a) and (10a) were isolated by h.p.l.c., after acetylation, as colourless needles, m.p. 200–202 and 231–232 °C, respectively, and have the same molecular weight, 832, on the basis of f.d.–m.s. Since in the ¹³C n.m.r. spectra of (9a) and (10a) eight acetyl carbonyl signals, one more than (1a), are observed and 14 carbon signals, one more than (1a), are observed at δ_C 60–105 p.p.m., it is supposed that one carbon of the (–)-menthol moiety is hydroxylated. The sugar part have almost the same chemical shifts as compound (1a), except that for the C-1' signal, so that it is thought the sugar parts of (9) and (10) are also gentiobiose.

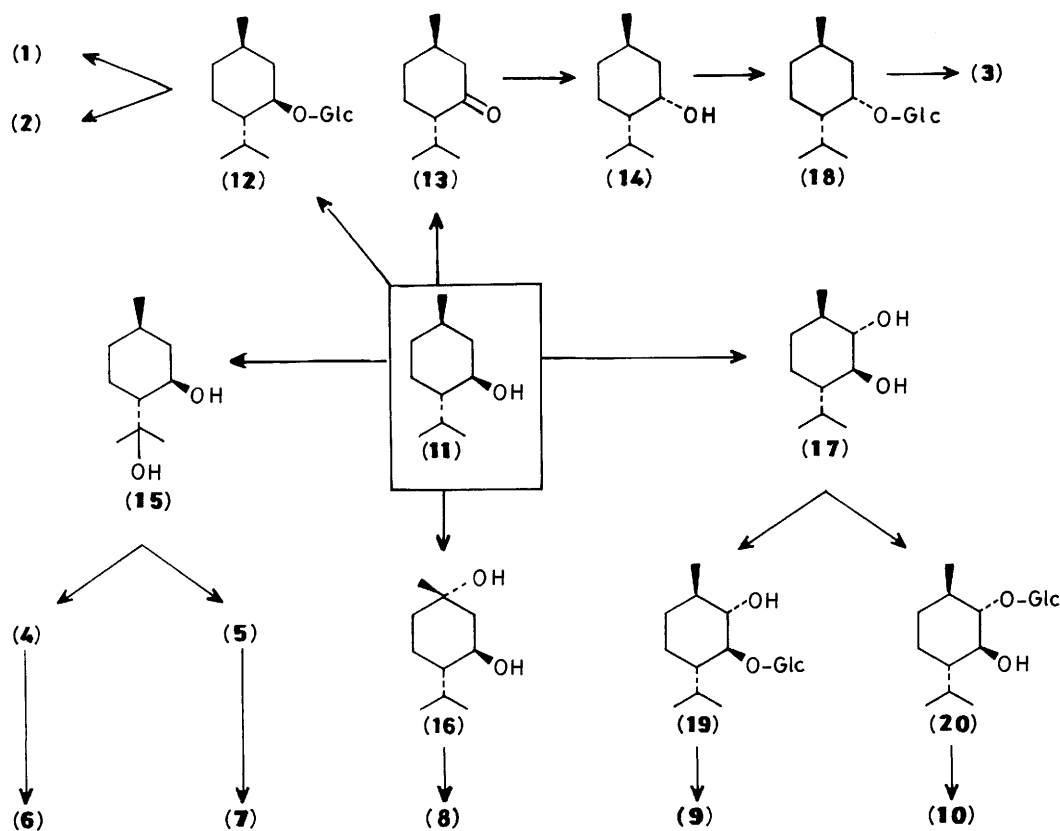
The structure of the aglycone and the position of glycosylation is determined from the ¹H–¹H shift correlation spectrum and ¹H n.m.r. decoupling experiments. In the ¹H n.m.r. of compound (9a) two proton signals attached to oxygen bearing carbon in the aglycone part observed at δ_H 4.65 (1 H, dd, *J* 10.5 and 9.0 Hz, 2-H) and 3.43 (1 H, dd, *J* 10.5 and 9.0 Hz, 3-H) are coupling together, so that the conformation of two hydroxy groups in the aglycone part of compound (9a) should be 2,3-diequatorial, and that the structure of the aglycone part is 2*S*,3*S*-*p*-menthane-2,3-diol (17).

In the ¹H n.m.r. spectrum of (9a) the proton signal attached to the acetoxy carbon observed at δ_H 4.65 is coupled with the 1-H signal observed at δ_H 1.43 and the 1-H signal is coupled with the 7-H methyl signal observed at 0.84, so that the proton signal at δ_H 4.65 is assigned to 2-H. On the other hand the proton signal attached to the glycosyloxy carbon observed at δ_H 3.43 is coupled with the 4-H signal observed at δ_H 1.32, the 4-H signal is coupled with the 8-H methine signal, and the 8-H signal is coupled with the 9-H and 10-H two methyl signals; the proton signal at δ_H 3.43 is, therefore, assigned to 3-H. From these results, it is elucidated that the structure of product (9) is (1*R*,2*S*,3*S*,4*S*)-2-hydroxy-*p*-menthan-3-yl *O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (*p*-menthane-2,3-diol 3-*O*-β-D-gentiobioside).

In the same manner it is demonstrated that the structure of the aglycone part in (10a) is also 2*S*,3*S*-*p*-menthane-2,3-diol (17), and that the proton signals observed at δ_H 3.27 and 4.83

Table 3. Comparison of biotransformation ratio between (–)-menthol and possible intermediates

Substrate	(1)	(2)	(3)	(4) + (5)	(6) + (7)	(8)	(9) + (10)
(–)-Menthol (11)	26%	0.5%	1.2%	4.5%	0.2%	0.6%	0.8%
Menthyl glucoside (12)	31%	1.5%					
(–)-Menthone (13)			20%				
(+)-Neomenthol (14)			47%				
<i>p</i> -Menthane-3,8-diol (15)				15%	2.9%		
<i>p</i> -Menthane-2,3-diol (17)							41%

**Scheme.** Biotransformation pathway of (–)-menthol (11) by *E. perriniana* cultured cells. Compounds (12)–(20) are possible intermediates and could not be isolated. Abbreviation; Glc = glucose

are assigned to 2-H and 3-H, respectively. It becomes clear that the structure of product (10) is (1*R*,2*S*,3*S*,4*S*)-3-hydroxy-*p*-menthan-2-yl *O*-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside (*p*-menthane-2,3-diol 2-*O*-β-D-gentiobioside).

As described above therefore, *E. perriniana* cultured cells can convert the administered (–)-menthol (11) and the oxidized, hydroxylated, and glycosylated products (1)–(10). Their isolated yields from (11) by h.p.l.c. is presented in Table 3. Several (–)-menthol derivatives [(–)-menthol 3-*O*-β-D-glucopyranoside (12), (–)-menthone (13), (+)-neomenthol (14), (–)-*p*-menthane-3,8-diol (15), and (±)-*p*-menthane-2,3-diol (17)] were fed to *E. perriniana* cultured cells to confirm the structures of isolated products and the biotransformation pathway of (11) by these cells. Biotransformation products were isolated by silica gel column chromatography and h.p.l.c. as well as the cells administered (11); their isolation yields are also presented in Table 3. A possible metabolic pathway for (11) in *E. perriniana* cultured cells is shown in the Scheme.

When (12) was administered, only (1) and (2), in higher yields than when (11) was administered, were isolated. If (12) is hydrolysed and the resulting (11) acts as the substrate, other products (3)–(10) should be detected. And if (12) is hydroxylated and then glycosylated, (4), (6), and (9) should be

also detected. In this case (12) should be the intermediate between (11) and (1) or (2), the glycosylation reaction to (11) occurring stepwise.

When (13) and (14) were administered, only product (3) was isolated in higher yield than when (11) was administered; other products could not be detected. It is supposed that (13), which is the oxidation product of (11), is reduced stereoselectively to (14), the latter then undergoing glycosylation.

Compound (15) was synthesized from (+)-citronellal, according to Zimmerman *et al.*⁹ When (15) was administered, products (4), (5), (6), and (7) were isolated, and when (17) was administered, products (9) and (10) were isolated, in higher yield than when (11) was administered. Further, when (11) was administered, the existence of the C-8-*O*-glycosylated products, (5) and (7), and the C-2-*O*-glycosylated product, (10), suggests that hydroxylation occurs at C-2 or C-8 in (11) before glycosylation. The above results also support the metabolic pathway presented in the Scheme.

E. perriniana cultured cells do not contain detectable amounts of glycosides so that it is advantageous to isolate the glycosylation products from the cells. The monoglycosylation reaction of phenolic compounds was observed in *Datura innoxia* suspension culture to produce arbutin from hydro-

quinone¹⁰ and in *Lithospermum erythrorhizon* and *D. innoxia* suspension culture to produce salicin and isosalicin, respectively, from saligenin.¹¹ It was observed that gladiolus corms and tomato plants converted *o*-chlorophenol into its β -D-gentiobioside,¹² and it was suggested that these glycosylations occur for the purpose of detoxification. Recently it was reported that naringenin was biotransformed to naringenin 7-*O*- β -D-gentiobioside by *Swertia japonica*¹³ and *Duboisia myoporoides*¹⁴ cultured cells, and that phenylacetic acid and phenylpropionic acid were converted into their gentiobiosyl esters by *Coffea arabica* cultured cells.¹⁵ This report is the first case of a glycosylation leading to a monoterpene by plant cultured cells.

The stereospecific reduction of menthone was also observed in the cultured cells of *Mentha* species,¹⁶ and the hydroxylation of (1*R*,4*S*)- and (1*R*,4*R*)-*p*-menth-3-ones to (1*R*,4*R*)- and (1*R*,4*S*)-4-hydroxy-*p*-menth-3-ones was observed in *Nicotiana tabacum* cultured cells.¹⁷ Since, however, until now, there has been no report that (11) could be biotransformed to another product by plant cultured cells,¹⁸ the present work constitutes the first report of the biotransformation of (11) by plant cultured cells.

Additionally, it is interesting that *p*-menthane-3,8-diol (15), one of the aglycone biotransformation products of (11), has been isolated from *E. citriodora* leaves and shown to be germination and growth inhibitors against lettuce (*Lactuca sativa*). Since *cis*- and *trans*-isomers existed as a (+)- and (-)-mixture in this plant, it was supposed that the *cis*- and *trans*-diols were formed by cyclization of (\pm)-citronellal in *E. citriodora* enzymatically or non-enzymatically.¹⁹ In contrast, *E. perriniana* cultured cells can hydroxylate (11) regio- and stereospecifically, and the hydroxylation products (15) and (17) are glycosylated. But it is not clear that (8) was formed by hydroxylation of (12) at C-1 or by glycosylation of *p*-menthane-1,3-diol (16) at C-3-O, since only (8) was isolated as the 1-hydroxylated product.

All biotransformation products of (11) isolated here are new compounds. Further the administration of other terpenoids and large-scale conversion experiments are in progress.

Experimental

All m.p.s were determined on a Yanagimoto micromelting point apparatus and are uncorrected. I.r. spectra were recorded as KBr discs with a JASCO IRA-100 spectrometer. ¹H N.m.r. spectra were recorded on a Varian EM390 spectrometer (90 MHz), using tetramethylsilane (TMS) as an internal standard, or on a Varian XL-400 spectrometer (400 MHz), setting the chloroform (CHCl₃) signal at δ_{H} 7.26 p.p.m. and the lowest pyridine (C₅D₄HN) signal at δ_{H} 8.60 p.p.m. ¹³C N.m.r. spectra were also recorded on a Varian XL-400 spectrometer (100 MHz), setting the chloroform (¹³CDCl₃) signal at δ_{C} 77.0 p.p.m. and the lowest pyridine (¹³CC₄D₅N) signal at δ_{C} 150.0 p.p.m. Optical rotations were measured with a JASCO DIP-181 digital polarimeter. Mass spectra were determined with a JEOL JMS DX-300 spectrometer fitted with a fast atom bombardment (f.a.b.) or a field desorption (f.d.) ionization system. Wakogel C-200 silica gel was used for chromatography. Pre-coated silica gel plates F254 (Merck Art. 5715) were used for t.l.c. and visualized by spraying with 10% sulphuric acid and then heating. H.p.l.c. was performed using a Shimadzu LC-3A system, using a differential refractometer Showa Denko RI SE11 and a column Unisil Q C₁₈ (5 μ m, 7.6 \times 300 mm) or Senshu Pak ODS-4301-N (5 μ m, 10 \times 300 mm).

Eucalyptus perriniana Cultured Cells for Biotransformations.—The methods of callus induction and subculture have already been reported.⁴

Culture Conditions and Substrate Feeding Methods.—Static

cultured cells (ca. 15 g) were inoculated into a 250 ml BA1 medium in 1-l Erlenmeyer flask and cultured on a reciprocal shaker (78 strokes/min) at 25 °C in the dark. After 3 weeks substrate (50 mg) dissolved in EtOH (25 mg/ml) was fed to each flask through membrane filter and further cultured for an additional 7 days.

Extraction and Purification of Biotransformation Products.—The cells [26 flasks, 1.30 g of (-)-menthol (11) was fed, fresh weight 1.6 kg] were collected through nylon mesh and extracted twice with MeOH at room temperature. The MeOH extract was concentrated under reduced pressure and partitioned between EtOAc and water. The water fraction was then extracted with BuOH and the extract evaporated. The residue (6.4 g) was chromatographed on silica gel with CH₂Cl₂-MeOH (7:1) as eluant and the resulting fractions were further purified by h.p.l.c.: products (1) (1 047 mg), (2) (19 mg), (3) (49 mg), (5) (28 mg), and (8) (16 mg) were isolated. Mixtures of products (4) and (5) (69 mg), products (6) and (7) (7 mg), and products (9) and (10) (34 mg) also resulted but could not be isolated by h.p.l.c.

(1*R*,3*R*,4*S*)-*p*-Menth-3-yl O- β -D-Glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (1).—Colourless prisms (from EtOH), m.p. 125–126 °C, $[\alpha]_{\text{D}}^{26}$ -78° (c 2.0 in MeOH); δ_{H} (C₅D₅N; 400 MHz) 0.62 [1 H, dddd, *J* 12.0, 12.0, 12.0, and 3.0 Hz, 6-H (axial)], 0.68 (3 H, d, *J* 7.0 Hz, 7-H), 0.81 (3 H, d, *J* 7.0 Hz, 9-H), 0.89 [1H, br ddd, *J* 12.0, 12.0, and 11.0 Hz, 2-H (axial)], 0.89 [1 H, br ddd, *J* 12.0, 12.0, and 11.5 Hz, 5-H (axial)], 0.98 (3 H, d, *J* 7.0 Hz, 10-H), 1.13 (1 H, m, 1-H), 1.22 (1 H, dddd, *J* 11.5, 11.0, 3.0, and 3.0 Hz, 4-H), 1.40 [1 H, br d, *J* 12.0 Hz, 6-H (equatorial)], 1.43 [1 H, br dd, *J* 12.0 and 3.0 Hz, 5-H (equatorial)], 2.18 [1 H, br d, *J* 12.0 Hz, 2-H (equatorial)], 2.53 (1 H, qqd, *J* 7.0, 7.0, and 3.0 Hz, 8-H), 3.82 (1 H, ddd, *J* 11.0, 11.0, and 4.0 Hz, 3-H), 3.87 (2 H, dd, *J* 9.0 and 9.0 Hz), 3.95 (2 H, dd, *J* 9.0 and 9.0 Hz), 4.04 (1 H, dd, *J* 9.0 and 8.0 Hz), 4.09–4.19 (3 H, m), 4.24 [1 H, dd, *J* 11.5 and 7.0 Hz, 6'-H (a)], 4.29 [1 H, dd, *J* 11.5 and 5.0 Hz, 6''-H (a)], 4.45 [1 H, dd, *J* 11.5 and 2.0 Hz, 6''-H (b)], 4.78 [1 H, br d, *J* 11.5 Hz, 6'-H (b)], 4.82 (1 H, d, *J* 8.0 Hz), and 5.07 (1 H, d, *J* 8.0 Hz); *m/z* (f.d.) 481 (*M* + H⁺, 100%).

(1*R*,3*R*,4*S*)-*p*-Menth-3-yl O- β -D-glucopyranosyl-(1 \rightarrow 4)- β -D-glucopyranoside (2). Colourless needles (from EtOH), m.p. 182–184 °C; δ_{H} (C₅D₅N; 400 MHz) 0.60 [1 H, dddd, *J* 12.0, 12.0, 12.0, and 3.0 Hz, 6-H (axial)], 0.68 (3 H, d, *J* 6.5 Hz), 0.72 (3 H, d, *J* 7.0 Hz), 0.76 (3 H, d, *J* 7.0 Hz), 0.81 [1 H, dddd, *J* 12.0, 12.0, 12.0, and 2.5 Hz, 5-H (axial)], 0.92 [1 H, ddd, *J* 12.0, 12.0, and 11.5 Hz, 2-H (axial)], 1.08 (1 H, m, 1-H), 1.18 (1 H, br ddd, *J* 12.0, 10.5, and 2.5 Hz, 4-H), 1.41 [2 H, br d, *J* 12.0 Hz, 5-H (equatorial), 6-H (equatorial)], 2.15 [1 H, br d, *J* 12.0 Hz, 2-H (equatorial)], 2.45 (1 H, qqd, *J* 7.0, 7.0, and 2.5 Hz, 8-H), 3.55 (1 H, ddd, *J* 11.5, 10.5, and 4.0 Hz, 3-H), 3.82 (1 H, ddd, *J* 8.0, 4.0, and 3.0 Hz, 5''-H), 3.88 (1 H, dd, *J* 8.0 and 8.0 Hz, 2''-H), 3.90 (1 H, ddd, *J* 8.0, 5.0, and 2.5 Hz, 5'-H), 3.99 (1 H, dd, *J* 8.0 and 8.0 Hz, 2'-H), 4.07 (1 H, dd, *J* 8.0 and 8.0 Hz, 4'-H), 4.11 (1 H, dd, *J* 8.0 and 8.0 Hz, 3'-H), 4.18 [1 H, dd, *J* 11.0 and 5.0 Hz, 6'-H (a)], 4.19 (1 H, dd, *J* 8.0 and 8.0 Hz, 3''-H), 4.22 (1 H, dd, *J* 8.0 and 8.0 Hz, 4''-H), 4.34 [1 H, br d, *J* 11.0 Hz, 6''-H (a)], 4.43 [2 H, br d, *J* 11.0 Hz, 6'-H (b), 6''-H (b)], 4.76 (1 H, d, *J* 8.0 Hz, 1''-H), and 5.10 (1 H, d, *J* 8.0 Hz, 1'-H); *m/z* (f.a.b.) 503 (*M* + Na⁺, 7%).

(1*R*,3*S*,4*S*)-*p*-Menth-3-yl O- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (3). Colourless prisms (from MeOH), m.p. 104–106 °C; δ_{H} (C₅D₅N; 400 MHz) 0.61–0.78 [2 H, m, 4-H, 6-H (axial)], 0.74 (3 H, d, *J* 7.0 Hz), 0.76 (3 H, d, *J* 6.5 Hz, 7-H), 0.90 [1 H, ddd, *J* 13.0, 13.0, and 1.5 Hz, 2-H (axial)], 0.93 (3 H, d, *J* 6.5 Hz), 1.39 [1 H, dddd, *J* 13.0, 13.0, 13.0, and 3.5 Hz, 5-H (axial)], 1.42–1.50 [1 H, m, 5-H (equatorial)], 1.54 [1 H, br ddd, 13.0, 3.5, and 2.5 Hz, 6-H (equatorial)], 1.97 (1 H, qqd, *J* 7.0, 6.5, and 2.5 Hz, 8-H), 1.91–2.04 (1 H, m, 1-H), 2.49 [1 H, dddd, *J* 13.0, 2.5, 2.5, and 2.5 Hz, 2-H (equatorial)], 3.81–3.90 (1 H, m),

3.86 (1 H, dd, *J* 8.5 and 8.0 Hz, 2'-H), 3.91—4.00 (3 H, m), 4.04 (1 H, dd, *J* 8.5 and 8.5 Hz), 4.08—4.16 (3 H, m), 4.21 [1 H, dd, *J* 11.5 and 5.5 Hz, 6'-H (a)], 4.27 [1 H, dd, *J* 11.5 and 5.0 Hz, 6'-H (a)], 4.43 [1 H, dd, *J* 11.5 and 2.5 Hz, 6'-H (b)], 4.69 (1 H, d, *J* 8.0 Hz, 1'-H), 4.70 [1 H, br d, *J* 11.5 Hz, 6'-H (b)], and 5.05 (1 H, d, *J* 8.0 Hz, 1'-H); *m/z* (f.a.b.) 503 (*M* + Na⁺, 24%).

(1R,3R,4R)-3-Hydroxy-p-menthan-8-yl O-β-D-glucopyranoside (5). Colourless flakes (from MeOH), m.p. 204—206 °C (Found: C, 57.3; H, 9.1. C₁₆H₃₀O₇ requires C, 57.45; H, 9.05%); δ_H(C₅D₅N; 400 MHz) 0.68 [1 H, dddd, *J* 13.0, 13.0, 13.0, and 3.0 Hz, 6-H (axial)], 0.71 (3 H, d, *J* 6.0 Hz, 7-H), 0.76 [1 H, dddd, *J* 13.0, 13.0, 13.0, and 3.0 Hz, 5-H (axial)], 1.13 [1 H, ddd, *J* 11.0, 11.0, and 10.5 Hz, 2-H (axial)], 1.20 (1 H, m, 1-H), 1.37 (3 H, s, 10-H), 1.39 (3 H, s, 9-H), 1.42 [1 H, br d, *J* 13.0 Hz, 6-H (equatorial)], 1.58 [1 H, br ddd, *J* 13.0, 5.0, and 3.0 Hz, 5-H (equatorial)], 1.68 (1 H, br ddd, *J* 13.0, 10.0, and 3.0 Hz, 4-H), 2.07 [1 H, br d, *J* 11.0 Hz, 2-H (equatorial)], 3.73 (1 H, ddd, *J* 10.5, 10.0, and 4.0 Hz, 3-H), 3.80—3.86 (2 H, m, 2'-H, 5'-H), 4.06 (1 H, dd, *J* 9.0 and 9.0 Hz, 4'-H), 4.12 (1 H, dd, *J* 9.0 and 9.0 Hz, 3'-H), 4.22 [1 H, dd, *J* 11.5 and 5.5 Hz, 6'-H (a)], 4.39 [1 H, dd, *J* 11.5 and 2.5 Hz, 6'-H (b)], and 5.05 (1 H, d, *J* 7.5 Hz, 1'-H); *m/z* (f.d.) 335 (*M* + H⁺, 100%).

(1S,3R,4S)-1-Hydroxy-p-menthan-3-yl O-β-D-glucopyranoside (8). Amorphous solid; δ_H(C₅D₅N; 400 MHz) 0.81 (3 H, d, *J* 7.0 Hz, 10-H), 0.89 (3 H, d, *J* 7.0 Hz, 9-H), 1.21 [1 H, ddd, *J* 13.0, 13.0, and 3.5 Hz, 6-H (axial)], 1.25 (3 H, s, 7-H), 1.28—1.40 [2 H, m, 4-H, 5-H (equatorial)], 1.42 [1 H, dd, *J* 12.5 and 11.0 Hz, 2-H (axial)], 1.73 [1 H, dddd, *J* 13.0, 13.0, 13.0, and 3.5 Hz, 5-H (axial)], 1.73 [1 H, br d, *J* 13.0 Hz, 6-H (equatorial)], 2.50—2.58 [1 H, m, 2-H (equatorial)], 2.57 (1 H, qdd, *J* 7.0, 7.0, and 2.5 Hz, 8-H), 3.65 (1 H, ddd, *J* 9.0, 5.0, and 3.0 Hz, 5'-H), 3.89 (1 H, dd, *J* 9.0 and 8.0 Hz, 2'-H), 4.09 (1 H, dd, *J* 9.0 and 9.0 Hz, 3'-H), 4.15 (1 H, dd, *J* 9.0 and 9.0 Hz, 4'-H), 4.23 [1 H, dd, *J* 11.5 and 5.0 Hz, 6'-H (a)], 4.31 [1 H, dd, *J* 11.5 and 3.0 Hz, 6'-H (b)], 4.36 (1 H, ddd, *J* 11.0, 11.0, and 4.5 Hz, 3-H), and 4.79 (1 H, d, *J* 8.0 Hz, 1'-H).

Acetylation of Biotransformation Products.—Products (1), (2), (3), (5), and (8) dissolved in acetic anhydride and pyridine were kept for 16 h after which each reaction mixture was evaporated. Further purification by h.p.l.c. was performed if necessary and the acetates were recrystallized from MeOH or EtOH.

(1R,3R,4S)-p-Menthan-3-yl O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside hepta-acetate (1a). Colourless needles (from MeOH), m.p. 192—193 °C (Found: C, 55.9; H, 7.05. C₃₆H₅₄O₁₈ requires C, 55.8; H, 7.05%); [α]_D²³ -61° (c 0.13, CHCl₃); v_{max}(KBr) 1755 cm⁻¹; δ_H(CDCl₃; 400 MHz) 0.81 (3 H, d, *J* 7.0 Hz), 0.78—0.94 [2 H, m, 2-H (axial), 6-H (axial)], 0.91 (3 H, d, *J* 7.0 Hz), 0.94 (3 H, d, *J* 7.0 Hz, 7-H), 0.99 [1 H, dddd, *J* 13.0, 13.0, 13.0, and 3.0 Hz, 5-H (axial)], 1.23 (1 H, dddd, *J* 13.0, 10.5, 3.0, and 3.0 Hz, 4-H), 1.36 (1 H, m, 1-H), 1.67 [2 H, m, 5-H (equatorial), 6-H (equatorial)], 1.98 [1 H, m, 2-H (equatorial)], 2.01 (3 H, s, OAc), 2.02 (3 H, s, OAc), 2.05 (9 H, s, OAc), 2.07 (3 H, s, OAc), 2.12 (3 H, s, OAc), 2.24 (1 H, qdd, *J* 7.0, 7.0, and 3.0 Hz, 8-H), 3.43 (1 H, ddd, *J* 10.5, 10.5, and 4.0 Hz, 3-H), 3.65 (1 H, ddd, *J* 9.5, 7.0, and 2.0 Hz, 5'-H), 3.67 [1 H, m, 6'-H (a)], 3.68 (1 H, ddd, *J* 9.5, 5.0, and 3.0 Hz, 5'-H), 3.83 [1 H, dd, *J* 12.0 and 2.0 Hz, 6'-H (b)], 4.14 [1 H, dd, *J* 12.0 and 3.0 Hz, 6'-H (a)], 4.27 [1 H, dd, *J* 12.0 and 5.0 Hz, 6'-H (b)], 4.59 (1 H, d, *J* 8.0 Hz, 1'-H), 4.64 (1 H, d, *J* 8.0 Hz, 1'-H), 4.91 (1 H, dd, *J* 9.5 and 8.0 Hz, 2'-H), 4.95 (1 H, dd, *J* 9.5 and 9.5 Hz, 4'-H), 4.99 (1 H, dd, *J* 9.5 and 8.0 Hz, 2'-H), 5.08 (1 H, dd, *J* 9.5 and 9.5 Hz, 4'-H), 5.19 (1 H, dd, *J* 9.5 and 9.5 Hz, 3'-H), 5.20 (1 H, dd, *J* 9.5 and 9.5 Hz, 3'-H); *m/z* (f.d.) 774 (*M*⁺, 100%).

(1R,3R,4S)-p-Menthan-3-yl O-β-D-glucopyranosyl-(1→4)-β-D-glucopyranoside hepta-acetate (2a). Colourless needles (from MeOH), m.p. 182—184 °C (Found: C, 56.1; H, 6.95. C₃₆H₅₄O₁₈

requires C, 55.8; H, 7.05%); v_{max}(KBr) 1750 cm⁻¹; δ_H(CDCl₃; 400 MHz) 0.70 (3 H, d, *J* 7.0 Hz, 9-H), 0.80 [1 H, ddd, *J* 13.0, 13.0, and 10.5 Hz, 2-H (axial)], 0.80 [1 H, dddd, *J* 12.5, 12.5, 12.5, and 3.5 Hz, 6-H (axial)], 0.85 (3 H, d, *J* 7.0 Hz, 10-H), 0.90 (3 H, d, *J* 6.5 Hz, 7-H), 0.93 [1 H, dddd, *J* 12.5, 12.5, 12.5, and 3.5 Hz, 5-H (axial)], 1.17 (1 H, dddd, *J* 12.5, 10.5, 3.5, and 2.5 Hz, 4-H), 1.32 (1 H, m, 1-H), 1.56—1.68 [2 H, m, 5-H (equatorial), 6-H (equatorial)], 1.92 [1 H, m, 2-H (equatorial)], 1.98 (3 H, s, OAc), 2.01 (6 H, s, OAc), 2.02 (6 H, s, OAc), 2.08 (6 H, s, OAc), 2.17 (1 H, qdd, *J* 7.0, 7.0, and 2.5 Hz, 8-H), 3.35 (1 H, ddd, *J* 10.5, 10.5, and 4.5 Hz, 3-H), 3.56 (1 H, ddd, *J* 9.5, 5.5, and 2.0 Hz, 5'-H), 3.65 (1 H, ddd, *J* 9.5, 4.5, and 2.0 Hz, 5'-H), 3.73 (1 H, dd, *J* 9.5 and 9.5 Hz, 4'-H), 4.03 [1 H, dd, *J* 12.0 and 2.0 Hz, 6'-H (a)], 4.06 [1 H, dd, *J* 11.5 and 5.5 Hz, 6'-H (a)], 4.35 [1 H, dd, *J* 12.0 and 4.5 Hz, 6'-H (b)], 4.48 [1 H, dd, *J* 11.5 and 2.0 Hz, 6'-H (b)], 4.50 (1 H, d, *J* 8.0 Hz, 1'-H), 4.51 (1 H, d, *J* 8.0 Hz, 1'-H), 4.85 (1 H, dd, *J* 9.5 and 8.0 Hz, 2'-H), 4.92 (1 H, dd, *J* 9.5 and 8.0 Hz, 2'-H), 5.05 (1 H, dd, *J* 9.5 and 9.5 Hz, 4'-H), 5.14 (1 H, dd, *J* 9.5 and 9.5 Hz, 3'-H), and 5.17 (1 H, dd, *J* 9.5 and 9.5 Hz, 3'-H); *m/z* (f.d.) 775 (*M* + H⁺, 67%).

(1R,3S,4S)-p-Menthan-3-yl O-β-D-glucopyranosyl-(1→6)-β-D-glucopyranoside hepta-acetate (3a). Colourless needles (from MeOH), m.p. 176—181 °C (Found: C, 55.75; H, 7.0. C₃₆H₅₄O₁₈ requires C, 55.8; H, 7.05%); v_{max}(KBr) 1745 cm⁻¹; δ_H(CDCl₃; 400 MHz) 0.76—0.89 [1 H, m, 6-H (axial)], 0.84 (3 H, d, *J* 7.0 Hz), 0.84 (3 H, d, *J* 6.5 Hz), 0.88 (3 H, d, *J* 7.0 Hz), 0.95 [1 H, ddd, *J* 13.0, 13.0, and 2.0 Hz, 2-H (axial)], 1.25 [1 H, dddd, *J* 13.0, 13.0, 13.0, and 3.5 Hz, 5-H (axial)], 1.43 (1 H, m, 8-H), 1.55—1.65 [2 H, m, 5-H (equatorial), 6-H (equatorial)], 1.68 (1 H, ddd, *J* 13.0, 2.5, and 2.5 Hz, 4-H), 1.80 (1 H, m, 1-H), 1.96—2.11 [1 H, m, 2-H (equatorial)], 1.98 (3 H, s, OAc), 1.99 (3 H, s, OAc), 2.00 (3 H, s, OAc), 2.01 (3 H, s, OAc), 2.02 (3 H, s, OAc), 2.04 (3 H, s, OAc), 2.09 (3 H, s, OAc), 3.65 (1 H, ddd, *J* 9.5, 5.5, and 2.0 Hz, 5'-H), 3.65 [1 H, m, 6'-H (a)], 3.67 (1 H, ddd, *J* 9.5, 5.0, and 2.5 Hz, 5'-H), 3.79 [1 H, dd, *J* 14.5 and 5.5 Hz, 6'-H (b)], 3.92 (1 H, br s, 3-H), 4.11 [1 H, dd, *J* 12.0 and 2.5 Hz, 6'-H (a)], 4.25 [1 H, dd, *J* 12.0 and 5.0 Hz, 6'-H (b)], 4.51 (1 H, d, *J* 8.0 Hz, 1'-H), 4.61 (1 H, d, *J* 8.0 Hz, 1'-H), 4.90 (1 H, dd, *J* 9.5 and 9.5 Hz, 4'-H), 4.97 (1 H, dd, *J* 9.5 and 8.0 Hz, 2'-H), 4.98 (1 H, dd, *J* 9.5 and 8.0 Hz, 2'-H), 5.06 (1 H, dd, *J* 9.5 and 9.5 Hz, 4'-H), 5.16 (1 H, dd, *J* 9.5 and 9.5 Hz, 3'-H), and 5.18 (1 H, dd, *J* 9.5 and 9.5 Hz, 3'-H); *m/z* (f.d.) 775 (*M* + H⁺, 37%).

(1R,3R,4R)-3-Hydroxy-p-menthan-8-yl O-β-D-glucopyranoside penta-acetate (5a). Colourless needles (from MeOH), m.p. 116—118 °C; [α]_D²³ -61° (c 0.13, CHCl₃); v_{max}(KBr) 1740 cm⁻¹; δ_H(CDCl₃; 400 MHz) 0.83 [1 H, dddd, *J* 13.0, 13.0, 13.0, and 3.0 Hz, 6-H (axial)], 0.88 (3 H, d, *J* 6.5 Hz, 7-H), 0.95 [1 H, ddd, *J* 12.5, 12.5, and 10.5 Hz, 2-H (axial)], 1.06 [1 H, dddd, *J* 13.0, 13.0, 13.0, and 3.5 Hz, 5-H (axial)], 1.17 (3 H, s), 1.20 (3 H, s), 1.48 (1 H, m, 1-H), 1.62 (1 H, ddd, *J* 13.0, 10.5, and 3.5 Hz, 4-H), 1.57—1.70 [2 H, m, 5-H (equatorial), 6-H (equatorial)], 1.93—2.02 [1 H, m, 2-H (equatorial)], 2.00 (6 H, s, OAc), 2.01 (3 H, s, OAc), 2.02 (3 H, s, OAc), 2.05 (3 H, s, OAc), 3.65 (1 H, ddd, *J* 9.5, 6.0 and 2.5 Hz, 5'-H), 4.10 [1 H, dd, *J* 12.0 and 2.5 Hz, 6'-H (a)], 4.16 [1 H, dd, *J* 12.0 and 6.0 Hz, 6'-H (b)], 4.66 (1 H, d, *J* 8.0 Hz, 1'-H), 4.77 (1 H, ddd, *J* 10.5, 10.5, and 4.5 Hz, 3-H), 4.91 (1 H, dd, *J* 9.5 and 8.0 Hz, 2'-H), 5.01 (1 H, dd, *J* 9.5 and 9.5 Hz, 4'-H), and 5.20 (1 H, dd, *J* 9.5 and 9.5 Hz, 3'-H); *m/z* (f.d.) 545 (*M* + H⁺, 8%).

(1S,3R,4S)-1-Hydroxy-p-menthan-3-yl O-β-D-glucopyranoside tetra-acetate (8a). Amorphous solid; δ_H(CDCl₃; 400 MHz) 0.77 (3 H, d, *J* 7.0 Hz, 9-H), 0.89 (3 H, d, *J* 7.0 Hz, 10-H), 1.17 [1 H, dd, *J* 13.0 and 11.0 Hz, 2-H (axial)], 1.12—1.25 (1 H, m, 4-H), 1.25 (3 H, s, 7-H), 1.35 [1 H, ddd, *J* 9.5, 9.5, and 3.5 Hz, 6-H (axial)], 1.29—1.42 [1 H, m, 5-H (axial)], 1.50 [1 H, ddd, *J* 8.5, 3.5, and 2.5 Hz, 5-H (equatorial)], 1.59 [1 H, br ddd, *J* 9.5, 2.5, and 2.5 Hz, 6-H (equatorial)], 1.97 [1 H, ddd, *J* 13.0, 4.5, and 2.5 Hz, 2-H (equatorial)], 1.99 (3 H, s, OAc), 2.02 (3 H, s, OAc), 2.03

(3 H, s, OAc), 2.05 (3 H, s, OAc), 2.24 (1 H, qdd, J 7.0, 7.0, and 2.5 Hz, 8-H), 3.66 (1 H, ddd, J 9.5, 5.5, and 2.5 Hz, 5'-H), 3.71 (1 H, ddd, J 11.0, 10.5, and 4.5 Hz, 3-H), 4.09 [1 H, dd, J 12.0 and 2.5 Hz, 6'-H (a)], 4.18 [1 H, dd, J 12.0 and 5.5 Hz, 6'-H (b)], 4.53 (1 H, d, J 8.0 Hz, 1'-H), 4.92 (1 H, dd, J 9.5 and 8.0 Hz, 2'-H), 5.03 (1 H, dd, J 9.5 and 9.5 Hz, 4'-H), and 5.18 (1 H, dd, J 9.5 and 9.5 Hz, 3'-H); m/z (f.d.) 503 ($M + H^+$, 100%).

Synthesis of (1a).—Compound (11) (100 mg) and gentiobiose octa-acetate (37 mg) were refluxed for 1 h in dry benzene (3 ml) with anhydrous zinc chloride (100 mg). Benzene (20 ml) was added to the reaction mixture and the latter then washed twice with water, dried (Na_2SO_4), and evaporated to dryness. The residue was purified by h.p.l.c. to give the acetate (1a) (4 mg, yield 9.5%). After recrystallization from MeOH colourless needles were obtained.

Isolation of Compounds (4a) and (5a).—The mixture of products (4) and (5) dissolved in acetic anhydride and pyridine was kept for 16 h at room temperature after which the mixture was evaporated. Compounds (4a) and (5a) were isolated by h.p.l.c. and further purified by recrystallization from MeOH.

(1R,3R,4R)-8-Hydroxy-p-menthan-3-yl O- β -D-glucopyranoside tetra-acetate (4a). Colourless needles (from MeOH), m.p. 175—177 °C (Found: C, 57.15; H, 7.6. $\text{C}_{24}\text{H}_{38}\text{O}_{11}$ requires C, 57.35; H, 7.6%); ν_{max} (KBr) 1 750 cm^{-1} ; δ_{H} (CDCl₃; 400 MHz) 0.88 [1 H, ddd, J 12.0, 12.0, and 11.0 Hz, 2-H (axial)], 0.89 [1 H, dddd, J 12.0, 12.0, 12.0, and 3.0 Hz, 6-H (axial)], 0.94 (3 H, d, J 6.5 Hz, 7-H), 0.94 [1 H, dddd, J 12.0, 12.0, 12.0, and 3.0 Hz, 5-H (axial)], 1.14 (3 H, s, 10-H), 1.19 (3 H, s, 9-H), 1.39 (1 H, m, 1-H), 1.53 (1 H, ddd, J 12.0, 11.0, and 3.5 Hz, 4-H), 1.69 [1 H, br d, J 12.0 Hz, 6-H (equatorial)], 1.80 [1 H, br dd, J 12.0 and 3.0 Hz, 5-H (equatorial)], 1.96—2.05 [1 H, m, 2-H (equatorial)], 2.00 (3 H, s, OAc), 2.03 (6 H, s, OAc), 2.06 (3 H, s, OAc), 3.71 (1 H, ddd, J 10.0, 4.5, and 3.5 Hz, 5'-H), 3.80 (1 H, ddd, J 11.0, 11.0, and 4.0 Hz, 3-H), 4.16 [1 H, dd, J 11.5 and 4.5 Hz, 6'-H (a)], 4.20 [1 H, dd, J 11.5 and 3.5 Hz, 6'-H (b)], 4.68 (1 H, d, J 8.0 Hz, 1'-H), 4.94 (1 H, dd, J 10.0 and 8.0 Hz, 2'-H), 5.06 (1 H, dd, J 10.0 and 10.0 Hz, 4'-H), and 5.21 (1 H, dd, J 10.0 and 10.0 Hz, 3'-H); m/z (f.d.) 503 ($M + H^+$, 32%).

Deacetylation of Compound (4a).—Compound (4a) (19 mg) dissolved in 0.5% NaOMe (4 ml) was stirred for 1 h and then poured into ice-water. The mixture solution was evaporated to half volume under reduced pressure and then extracted with BuOH. The BuOH extract was evaporated to dryness and purified by h.p.l.c. to give product (4) (8 mg, 63%).

(1R,3R,4R)-8-Hydroxy-p-menthan-3-yl O- β -D-glucopyranoside (4). An amorphous solid; δ_{H} ($\text{C}_5\text{D}_5\text{N}$; 400 MHz) 0.63 [1 H, dddd, J 13.0, 13.0, and 3.0 Hz, 6H (axial)], 0.67 (3 H, d, J 6.5 Hz, 7-H), 0.82 [1 H, dddd, J 13.0, 13.0, 13.0, and 3.0 Hz, 5-H (axial)], 0.98 [1 H, ddd, J 11.5, 11.5, and 10.5 Hz, 2-H (axial)], 1.08 (1 H, m, 1-H), 1.21 (3 H, s, 10-H), 1.39 [1 H, m, 6-H (equatorial)], 1.42 (3 H, s, 9-H), 1.60 (1 H, ddd, J 13.0, 10.5, and 3.5 Hz, 4-H), 1.67 [1 H, br dd, J 13.0 and 3.0 Hz, 5-H (equatorial)], 2.24 [1 H, br d, J 11.5 Hz, 2-H (equatorial)], 3.81 (1 H, ddd, J 9.0, 8.0, and 3.5 Hz, 2'-H), 3.92 (1 H, ddd, J 9.0, 5.5, and 2.5 Hz, 5'-H), 3.97—4.07 (1 H, m, 4'-H), 4.03 (1 H, ddd, J 10.5, 10.5, and 4.0 Hz, 3-H), 4.15 (1 H, dd, J 9.0 and 9.0 Hz, 3'-H), 4.23 [1 H, ddd, J 11.5, 5.5, and 5.5 Hz, 6'-H (a)], 4.47 [1 H, br d, J 11.5, 6'-H (b)], 4.93 (1 H, d, J 8.0 Hz, 1'-H), and 5.95 (1 H, dd, J 5.5, and 5.5 Hz, C-6'-OH); m/z (f.a.b.) 357 ($M + \text{Na}^+$, 40%).

Isolation of Compounds (6a) and (7a).—The mixture (7 mg) of products (6) and (7) was acetylated with acetic anhydride and pyridine as described above, and the product purified by h.p.l.c. to give compounds (6a) (4 mg) and (7a) (4 mg). Both were recrystallized from MeOH.

(1R,3R,4R)-8-Hydroxy-p-menthan-3-yl O- β -D-glucopyranoside

syn-(1 \rightarrow 6)- β -D-glucopyranoside hepta-acetate (6a). Colourless needles (from MeOH), m.p. 202—204 °C; ν_{max} (KBr) 1 755 cm^{-1} ; δ_{H} (CDCl₃; 400 MHz) 0.87 [1 H, ddd, J 11.5, 11.5, and 11.0 Hz, 2-H (axial)], 0.90 [1 H, dddd, J 12.5, 12.5, 12.5, and 3.5 Hz, 6-H (axial)], 0.94 (3 H, d, J 7.0 Hz, 7-H), 0.97 [1 H, dddd, J 12.5, 12.5, 12.0, and 3.5 Hz, 5-H (axial)], 1.17 (3 H, s, 10-H), 1.23 (3 H, s, 9-H), 1.40 (1 H, m, 1-H), 1.53 (1 H, ddd, J 12.0, 10.5, and 3.5 Hz, 4-H), 1.68 [1 H, br d, J 12.5 Hz, 6-H (equatorial)], 1.80 [1 H, br dd, J 12.5 and 3.0 Hz, 5-H (equatorial)], 1.93—2.15 [1 H, m, 2-H (equatorial)], 1.98 (3 H, s, OAc), 1.99 (3 H, s, OAc), 2.02 (9 H, s, OAc), 2.04 (3 H, s, OAc), 2.10 (3 H, s, OAc), 3.65—3.74 [3 H, m, 5'-H, 5'-H, 6'-H (a)], 3.79 [1 H, br d, J 9.0 Hz, 6'-H (b)], 3.83 (1 H, ddd, J 11.0, 10.5, and 4.0 Hz, 3-H), 4.12 [1 H, dd, J 12.0 and 2.5 Hz, 6'-H (a)], 4.25 [1 H, dd, J 12.0 and 5.0 Hz, 6'-H (b)], 4.63 (1 H, d, J 8.0 Hz, 1'-H), 4.68 (1 H, d, J 8.0 Hz, 1'-H), 4.89 (1 H, dd, J 9.5 and 9.5 Hz, 4'-H), 4.90 (1 H, dd, J 9.5 and 8.0 Hz, 2'-H), 4.96 (1 H, dd, J 9.5 and 8.0 Hz, 2'-H), 5.05 (1 H, dd, J 9.5 and 9.5 Hz, 4'-H), 5.17 (1 H, dd, J 9.5 and 9.5 Hz, 3'-H), and 5.20 (1 H, dd, J 9.5 and 9.5 Hz, 3'-H); m/z (f.d.) 791 ($M + H^+$, 54%).

(1R,3R,4R)-3-Hydroxy-p-menthan-8-yl O- β -D-glucopyranoside *syn*-(1 \rightarrow 6)- β -D-glucopyranoside octa-acetate (7a). Colourless prisms (from MeOH), m.p. 160—161 °C; ν_{max} (KBr) 1 750 cm^{-1} ; δ_{H} (CDCl₃; 400 MHz) 0.84 [1 H, dddd, J 12.5, 12.5, 12.5, and 3.5 Hz, 6-H (axial)], 0.88 (3 H, d, J 6.5 Hz, 7-H), 0.95 [1 H, ddd, J 12.5, 12.5, and 10.5 Hz, 2-H (axial)], 1.08 [1 H, dddd, J 12.5, 12.5, 12.5, and 3.5 Hz, 5-H (axial)], 1.17 (3 H, s, 9-H), 1.21 (3 H, s, 10-H), 1.48 (1 H, m, 1-H), 1.62 (1 H, ddd, J 12.5, 10.5, and 3.5 Hz, 4-H), 1.66 [1 H, br ddd, J 12.5, 3.0, and 3.0 Hz, 6-H (equatorial)], 1.94—2.07 [2 H, m, 2-H (equatorial), 5-H (equatorial)], 1.98 (3 H, s, OAc), 1.99 (3 H, s, OAc), 2.00 (3 H, s, OAc), 2.01 (3 H, s, OAc), 2.02 (6 H, s, OAc), 2.03 (3 H, s, OAc), 2.09 (3 H, s, OAc), 3.57—3.68 [2 H, m, 5'-H and 6'-H (a)], 3.68 (1 H, ddd, J 9.5, 5.0, and 2.5 Hz, 5'-H), 3.76 [1 H, br d, J 10.0 Hz, 6'-H (b)], 4.12 [1 H, dd, J 12.5 and 2.5 Hz, 6'-H (a)], 4.26 [1 H, dd, J 12.5 and 5.0 Hz, 6'-H (b)], 4.57 (1 H, d, J 8.0 Hz, 1'-H), 4.65 (1 H, d, J 8.0 Hz, 1'-H), 4.78 (1 H, ddd, J 10.5, 10.5, and 4.5 Hz, 3-H), 4.84 (1 H, dd, J 9.5 and 9.5 Hz, 4'-H), 4.87 (1 H, dd, J 9.5 and 8.0 Hz, 2'-H), 4.95 (1 H, dd, J 9.5 and 8.0 Hz, 2'-H), 5.05 (1 H, dd, J 9.5 and 9.5 Hz, 4'-H), 5.16 (1 H, dd, J 9.5 and 9.5 Hz, 3'-H), and 5.18 (1 H, dd, J 9.5 and 9.5 Hz, 3'-H); m/z (f.d.) 833 ($M + H^+$, 11%).

Isolation of Compounds (9a) and (10a).—The mixture (13 mg) of products (9) and (10) was acetylated with acetic anhydride and pyridine as described above, and then purified by h.p.l.c. to give compounds (9a) (5 mg) and (10a) (9 mg). Both were recrystallized from MeOH.

(1R,2S,3S,4S)-2-Hydroxy-p-methan-3-yl O- β -D-glucopyranoside *syn*-(1 \rightarrow 6)- β -D-glucopyranoside octa-acetate (9a). Colourless needles (from MeOH), m.p. 200—202 °C (Found: C, 54.8; H, 6.7. $\text{C}_{38}\text{H}_{56}\text{O}_{20}$ requires C, 54.8; H, 6.8%); ν_{max} (KBr) 1 755 cm^{-1} ; δ_{H} (CDCl₃; 400 MHz) 0.74 (3 H, d, J 7.0 Hz), 0.84 (3 H, d, J 6.5 Hz, 7-H), 0.88 (3 H, d, J 7.0 Hz), 0.97 [1 H, dddd, J 13.0, 13.0, 13.0, and 3.0 Hz, 5-H (axial)], 1.06 [1 H, dddd, J 13.0, 13.0, 13.0, and 3.0 Hz, 6-H (axial)], 1.32 (1 H, m, 4-H), 1.43 (1 H, m, 1-H), 1.60 [1 H, br dd, J 13.0 and 3.0 Hz, 5-H (equatorial)], 1.70 [1 H, br dd, J 13.0 and 3.0 Hz, 6-H (equatorial)], 1.97 (3 H, s, OAc), 2.00 (3 H, s, OAc), 2.02 (6 H, s, OAc), 2.04 (3 H, s, OAc), 2.06 (3 H, s, OAc), 2.09 (3 H, s, OAc), 2.14 (3 H, s, OAc), 2.22 (1 H, qdd, J 7.0, 7.0, and 2.5 Hz, 8-H), 3.43 (1 H, dd, J 10.5 and 9.0 Hz, 3-H), 3.57 [1 H, dd, J 10.5 and 6.0 Hz, 6'-H (a)], 3.63 (1 H, m, 5'-H), 3.66 (1 H, ddd, J 9.5, 5.0, and 2.5 Hz, 5'-H), 3.85 [1 H, dd, J 10.5 and 1.5 Hz, 6'-H (b)], 4.11 [1 H, dd, J 12.5 and 2.5 Hz, 6''-H (a)], 4.26 [1 H, dd, J 12.5 and 5.0 Hz, 6''-H (b)], 4.52 (1 H, d, J 8.0 Hz, 1'-H), 4.65 (1 H, dd, J 10.5 and 9.0 Hz, 2-H), 4.70 (1 H, d, J 8.0 Hz, 1'-H), 4.92 (1 H, dd, J 9.5 and 8.0 Hz, 2'-H), 4.94 (1 H, dd, J 9.5 and 9.5 Hz, 4'-H), 4.99 (1 H, dd, J 9.5 and 8.0 Hz, 2'-H), 5.07 (1 H, dd, J 9.5 and 9.5 Hz, 4'-H), 5.09 (1 H, dd, J 9.5 and 9.5 Hz), and 5.17 (1 H, dd, J 9.5 and 9.5 Hz); m/z (f.d.) 832 (M^+ , 100%).

(1R,2S,3S,4S)-3-Hydroxy-p-menthan-2-yl O- β -D-glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside octa-acetate (**10a**). Colourless needles (from MeOH), m.p. 231–232 °C (Found: C, 54.6; H, 6.55. C₃₈H₅₆O₂₀ requires C, 54.8; H, 6.8%; ν_{\max} (KBr) 1760 cm⁻¹; δ_{H} (CDCl₃; 400 MHz) 0.78 (3 H, d, *J* 7.0 Hz), 0.88 (3 H, d, *J* 7.0 Hz), 1.00 (3 H, d, *J* 6.5 Hz, 7-H), 1.07 [2 H, dddd, *J* 12.5, 12.5, 12.5, and 3.0 Hz, 5-H (axial), 6-H (axial)], 1.27–1.44 [2 H, m, 4-H, 5-H (equatorial)], 1.49 (1 H, m, 1-H), 1.61 (1 H, m, 8-H), 1.73–1.81 [1 H, m, 6-H (equatorial)], 1.97 (3 H, s, OAc), 2.00 (3 H, s, OAc), 2.02 (3 H, s, OAc), 2.03 (3 H, s, OAc), 2.06 (3 H, s, OAc), 2.08 (3 H, s, OAc), 2.09 (3 H, s, OAc), 2.12 (3 H, s, OAc), 3.27 (1 H, dd, *J* 9.5 and 9.0 Hz, 2-H), 3.62 (1 H, ddd, *J* 9.5, 6.5, and 1.5 Hz, 5'-H), 3.64 (1 H, ddd, *J* 9.5, 5.0, and 2.0 Hz, 5''-H), 3.64 [1 H, m, 6'-H (a)], 3.80 [1 H, dd, *J* 10.5 and 1.5 Hz, 6'-H (b)], 4.12 [1 H, dd, *J* 12.0 and 2.0 Hz, 6''-H (a)], 4.25 [1 H, dd, *J* 12.0 and 5.0 Hz, 6''-H (b)], 4.64 (1 H, d, *J* 8.0 Hz, 1'-H), 4.65 (1 H, d, *J* 8.0 Hz, 1''-H), 4.83 (1 H, dd, *J* 11.0 and 9.0 Hz, 3-H), 4.88 (1 H, dd, 9.5 and 9.5 Hz, 4'-H), 4.93 (1 H, dd, *J* 9.5 and 8.0 Hz, 2'-H), 5.00 (1 H, dd, *J* 9.5 and 8.0 Hz, 2''-H), 5.08 (1 H, dd, 9.5 and 9.5 Hz, 4''-H), 5.10 (1 H, dd, *J* 9.5 and 9.5 Hz, 3'-H), and 5.18 (1 H, dd, *J* 9.5 and 9.5 Hz, 3''-H); *m/z* (f.d.) 832 (*M*⁺, 100%).

Biotransformation of Compound (12) by E. perriniana Cultured Cells.—Compound (**12**) was fed (total 100 mg) to *E. perriniana* cells (2 flasks) pre-cultured for 3 weeks and then cultured for a further 7 days. The cells were collected and extracted as well as the cells administered (**11**). The BuOH fraction (495 mg) was analysed by h.p.l.c. and chromatographed on silica gel. The product fraction was further purified by h.p.l.c. and two glycosylated products were acetylated with acetic anhydride and pyridine as described above. The acetates obtained were identified as compounds (**1a**) and (**2a**) by direct comparison of their m.p.s and i.r. and ¹H n.m.r. spectral characteristics.

Biotransformation of Compounds (13) and (14) by E. perriniana Cultured Cells.—Compounds (**13**) and (**14**) were fed (each 100 mg) to *E. perriniana* cultured cells (each 2 flasks) pre-cultured for 3 weeks and then cultured for a further 7 days. The cells were extracted as well as the cells (**12**) administered, and the BuOH fractions, (99 mg) and (196 mg) respectively, were analysed by h.p.l.c. The acetates were obtained by acetylation as described above. They were identified as (**3a**) by direct comparison of m.p.s and i.r. and ¹H n.m.r. spectral characteristics.

Synthesis of Compound (15)⁹.—(+)-Citronellal (5 g) and 5% sulphuric acid (50 ml) were stirred for 2 h after which the reaction mixture was extracted thrice with CH₂Cl₂. The combined extracts were washed with water, dried (Na₂SO₄), and evaporated to dryness. The residue was chromatographed on silica gel using CH₂Cl₂–MeOH (50:1) as eluant. The main fraction was recrystallized from ether–hexane to give the diol (**15**) (794 mg, 14%) as colourless needles.

(1R,3R,4R)-p-Menthane-3,8-diol (**15**). Colourless needles (from Et₂O–hexane), m.p. 71–72 °C; $[\alpha]_{\text{D}}^{26}$ –10.2° (*c* 1.1 in CHCl₃); δ_{H} (CDCl₃; 90 MHz) 0.90 (3 H, d, *J* 6.0 Hz, 7-H), 1.21 (6 H, s, 9-H, 10-H), and 3.72 (1 H, ddd, *J* 10.0, 10.0, and 4.5 Hz, 3-H); *m/z* 157 (*M* – Me⁺, 7%), 154 (*M* – H₂O⁺, 4), 139 (*M* – H₂O – Me⁺, 6), 121 (8), 111 (6), 96 (89), 81 (100), 68 (32), 59 (99), 55 (39), 54 (48), 43 (83), and 41 (49).

Biotransformation of Compound (15) by E. perriniana Cultured Cells.—Compound (**15**) (200 mg) was fed to *E. perriniana* cultured cells (4 flasks) pre-cultured for 3 weeks, and then cultured for further 7 days. The cells were treated as well as the cells (**12**) administered to give the BuOH fraction (229 mg). The BuOH fraction was analysed by h.p.l.c. and then chromatographed

on silica gel. Further purification by h.p.l.c. gave mixtures (a) and (b), with the same retention times as (**4**) or (**5**) and (**6**) or (**7**), respectively. After acetylation of the mixture (a) and (b), four acetates were isolated by h.p.l.c. and identified with compounds (**4a**), (**5a**), (**6a**), and (**7a**) by direct comparison of m.p. [(**4a**) and (**5a**) only], i.r. [(**4a**) and (**5a**) only] and ¹H n.m.r. spectral characteristics.

Biotransformation of Compound (17) by E. perriniana Cultured Cells in a Jar Fermentor.—*E. perriniana* cells, pre-cultured for 3 weeks, were inoculated into a jar fermentor containing 4-l of BA1 medium and cultured for 16 days. Culture conditions were as follows: agitation speed; 50 r.p.m., aeration ratio; 0.25 vol per vol per min (v.v.m.); temperature 25 °C. An ethanolic solution of (±)-*p*-menthane-2,3-diol (**17**) (800 mg) was fed to these cells and the cells were cultured for a further 7 days. The cells (fresh weight 840 g) were collected through nylon mesh and extracted with MeOH. The MeOH extract was treated as well as the MeOH extract obtained from the cells administered (**11**). After the BuOH fraction (6.14 g) had been chromatographed and purified as above mentioned, the main biotransformation product of (**17**) [476 mg, the same retention time as products (**9**) and (**10**)] was obtained. The mixture (99 mg) was acetylated with acetic anhydride and pyridine as mentioned above, and the acetates (c) (45 mg) and (d) (64 mg) were isolated by h.p.l.c. and identified as compounds (**9a**) and (**10a**), respectively, by direct comparison of m.p. and i.r. and ¹H n.m.r. spectral data.

Deacetylation of Compounds (9a) and (10a).—Compound (**9a**) (30 mg) and methanol solution of 0.5% sodium methoxide (65 ml) were stirred for 3 h and then poured into ice–water. The mixture solution was evaporated to half volume and extracted with BuOH ($\times 3$). The combined BuOH fractions were evaporated to dryness and purified by h.p.l.c. to give product (**9**) (18 mg, yield 100%) as colourless flakes (from MeOH).

(1R,2S,3S,4S)-2-Hydroxy-p-menthan-3-yl O- β -D-Glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (**9**).—Colourless flakes (from MeOH), m.p. 200–202 °C; δ_{H} (C₅D₅N; 400 MHz) 0.76 (3 H, d, *J* 7.0 Hz, 10-H), 0.82 [1 H, dddd, *J* 13.5, 13.5, 13.5, and 2.0 Hz, 6-H (axial)], 0.87 (3 H, d, *J* 7.0 Hz, 9-H), 0.89 [1 H, dddd, *J* 13.5, 13.5, 13.5, and 2.0 Hz, 5-H (axial)], 1.07 (3 H, d, *J* 6.5 Hz, 7-H), 1.34–1.42 [2 H, m, 4-H, 5-H (equatorial)], 1.43–1.51 [1 H, m, 1-H), 1.47 (1 H, m, 6-H (equatorial)], 2.75 (1 H, qdd, *J* 7.0, 7.0, and 1.5 Hz, 8-H), 3.33 (1 H, dd, *J* 9.5 and 9.5 Hz, 2-H), 3.47 (1 H, dd, *J* 9.5 and 9.5 Hz, 3-H), 3.81 (1 H, m, 5''-H), 3.85 (1 H, m, 5'-H), 3.90 (1 H, dd, *J* 8.0 and 8.0 Hz, 2''-H), 3.91 (1 H, dd, *J* 8.0 and 8.0 Hz, 2'-H), 4.04 (2 H, dd, *J* 8.0 and 8.0 Hz, 3'-H and 3''-H), 4.08 (1 H, dd, *J* 8.0 and 8.0 Hz, 4'-H), 4.11 (1 H, dd, *J* 8.0 and 8.0 Hz, 4''-H), 4.22 [1 H, dd, *J* 11.0 and 5.5 Hz, 6'-H (a)], 4.25 [1 H, dd, *J* 11.0 and 4.5 Hz, 6''-H (a)], 4.40 [1 H, br d, *J* 11.0 Hz, 6''-H (b)], 4.60 [1 H, dd, *J* 11.0 and 1.5 Hz, 6'-H (b)], 4.92 (1 H, d, *J* 8.0 Hz, 1''-H), and 5.05 (1 H, d, *J* 8.0 Hz, 1'-H); *m/z* (f.a.b.) 497 (*M* + H⁺, 31%).

Compound (**10a**) (37 mg) was treated in a similar fashion to compound (**9a**), to give the product (**10**) (20 mg, yield 91%), as colourless prisms (from EtOH).

(1R,2S,3S,4S)-3-Hydroxy-p-menthan-2-yl O- β -D-Glucopyranosyl-(1 \rightarrow 6)- β -D-glucopyranoside (**10**).—Colourless prisms (from EtOH), m.p. 127–146 °C (decomp.); δ_{H} (C₅D₅N; 400 MHz) 0.73 (3 H, d, *J* 7.0 Hz, 9-H), 0.78 (3 H, d, *J* 7.0 Hz, 10-H), 0.76–0.92 [2 H, m, 5-H (axial), 6-H (axial)], 1.27 (3 H, d, *J* 6.0 Hz, 7-H), 1.24–1.38 [2 H, m, 4-H, 5-H (equatorial)], 1.40–1.55 [2 H, m, 1-H, 6-H (equatorial)], 2.42 (1 H, qdd, *J* 7.0, 7.0, and 2.0 Hz, 8-H), 3.21 (1 H, dd, *J* 9.0 and 9.0 Hz, 2-H), 3.52 (1 H, dd, *J* 9.0 and 9.0 Hz, 3-H), 3.82 (1 H, br, 5''-H), 3.88–4.00 (3 H, m, 5'-H, 2'-H, 2''-H), 4.02 (2 H, dd, *J* 9.0 and 9.0 Hz, 3'-H, 4''-H),

4.08—4.16 (2 H, m, 3''-H, 4'-H), 4.21 [1 H, dd, J 11.0, and 5.5 Hz, 6'-H (a)], 4.21—4.30 [1 H, m, 6''-H (a)], 4.40 [1 H, br d, J 11.0 Hz, 6''-H (b)], 4.69 [1 H, br d, J 11.0, 6'-H (b)], 4.99 (1 H, d, J 8.0 Hz, 1''-H), and 5.02 (1 H, d, J 8.0 Hz, 1'-H); m/z (f.a.b.) 497 ($M + H^+$, 6%).

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