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Synthesis of isoacteoside, a dihydroxyphenylethyl glycoside

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Abstract The total chemical synthesis of isoacteoside (**1**), 2-(3′,4′-dihydroxyphenyl)ethyl 6-*O*-caffeoyl-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside, is described. An acteoside acetate with benzyl groups at the catechols (**3**: 2-(3′,4′-dibenzyloxyphenyl)ethyl 2,6-di-*O*-acetyl-4-*O*-[3′,4′-bis(*O*-benzyl)caffeoyl]-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside) was treated with a solution of methylamine in methanol ($MeNH_2$ in MeOH) to perform both deacetylation and caffeoyl migration, affording an isoacteoside derivative with benzyl groups at the catechols – **4b**: 2-(3′,4′-dibenzyloxyphenyl)ethyl 6-*O*-[3′,4′-bis(*O*-benzyl)caffeoyl]-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside – in 34% yield. Debenzylation of **4b** was successfully accomplished by catalytic transfer hydrogenation using 1,4-cyclohexadiene to give the target compound isoacteoside (**1**) in 54% yield. ^1H and ^{13}C nuclear magnetic resonance spectral data of the synthesized isoacteoside (**1**) were identical with those of the natural isoacteoside isolated from *Paulownia tomentosa* (Thumb.) Steud.

Key words Wood extractives · Carbohydrates · Caffeic acid sugar ester · Dihydroxyphenylethyl glycoside · Phenylpropanoid glycoside

Introduction

Dihydroxyphenylethyl glycosides comprise an interesting family of plant extractives distributed in several species.[1,2] More than 80 compounds have been isolated,[3] many of which are found in medicinal plants and have various bioactivities.[4] They have a common structure, consisting of a dihydroxyphenethyl β-D-glucopyranoside, a phenylpropenoic acid (cinnamic, *p*-coumaric, caffeic, and ferulic acids) as an ester, and a monosaccharide residue (rhamnose, glucose, xylose).

To understand the relation between bioactivities and chemical structures, there must be systematic study of bioactivities using a series of phenylpropanoid glycosides. Hence, the systematic synthesis method for these substances is important. We have already reported the basic method for systematic synthesis of a series of dihydroxyphenylethyl glycosides having a 4-*O* caffeoyl group.[5,6] In this report we describe the chemical synthesis of isoacteoside (**1**) [2-(3′,4′-dihydroxyphenyl)ethyl 6-*O*-caffeoyl-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside] (Fig. 1) as an example of synthesizing phenylpropanoid glycosides with a 6-*O* caffeoyl group using caffeoyl migration.

Isoacteoside (**1**), an isomer of acteoside (verbascoside), is one of the dihydroxyphenylethyl glycosides family with several bioactivities (e.g., hepatoprotective activity[4] and cytotoxic activity,[7] among others). It was first isolated in 1963 by Scarpati and Monache[8] as an "isoverbascoside," but its chemical structure was not described. In 1982, two independent research groups reported the chemical structure of isoacteoside from ^{13}C-nuclear magnetic resonance (NMR) spectral data. Miyase et al.[9] isolated isoacteoside from *Leucosceptrum japonicum* (MIQ.) Kitamura et Murata as an "acteoside isomer," and Schilling, et al.[10] isolated it from *Paulownia tomentosa* (Thumb) Steud. as an "isoverbascoside." In 1989, Sasaki et al.[11] first used the name "isoacteoside" for the compound. The detailed data of ^1H- and ^{13}C-NMR for the "isoacteoside," isolated from *Paulownia tomentosa* (Thumb) Steud., were first described by Ota et al.[12] in 1993. As noted above, three names have been used for this compound; in this report we prefer to use "isoacteoside."

Recently, two reports on total synthesis of acteoside[5,11] and three reports on partial synthesis of some other member of the dihydroxyphenylethyl glycosides family[6,14,15] have

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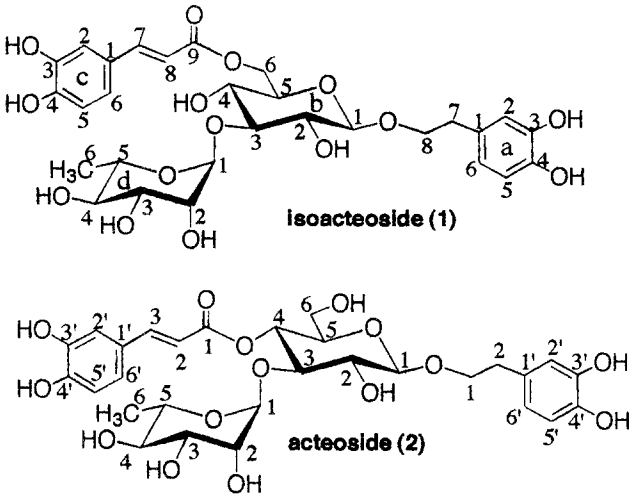

Fig. 1. Isoacteoside (**1**) and acteoside (**2**). The carbon-numbering system of (**1**) is used to describe nuclear magnetic resonance (NMR) data. The carbon numbering of (**2**) is according to IUPAC rules. It is used to describe compound names

appeared. However, there have been no reports dealing with the total chemical synthesis of isoacteoside, although Schilling et al.[10] reported the transformation of natural acteoside into isoacteoside by isomerization of the caffeoyl group using sodium hydroxide solution. They also reported the ^{13}C-NMR data [20MHz; (dimethyl sulfoxide)-d_6] of isoacteoside but without full assignment.

Experiment

Analytical and preparative thin-layer chromatography (TLC) was done on silica gel plates (Kieselgel 60 F_{254}, Merck). ^{1}H- and ^{13}C-NMR spectra were recorded with a JNM-500 FT-NMR (JEOL) with tetramethylsilane as an internal standard. Coupling constants (J) are given in hertz. The signals were assigned using ^{1}H-^{1}H correlated spectroscopy, a ^{13}C-^{1}H heteronuclear multiple-quantum correlation technique, or both.

2-(3′,4′-Dibenzyloxyphenyl)ethyl 6-*O*-[3′,4′-bis(*O*-benzyl)caffeoyl]-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (**4b**)

To a solution of 2-(3′,4′-dibenzyloxyphenyl)ethyl 2,6-di-*O*-acetyl-4-*O*-[3′,4′-bis(*O*-benzyl)caffeoyl]-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside[5] (**3**) (900mg, 0.75mmol) in dichloromethane (CH_2Cl_2; 12ml) was added 40% methylamine solution in methanol (MeOH) (18ml) at −20°C. The reaction mixture was stirred at −20°C for 19h and for 3h at 0°C; it was then concentrated in vacuo. The residue was purified by TLC using a mixture of MeOH/CH_2Cl_2 (1:9, v/v) to give a colorless syrup. Crystallization of the syrup from ethanol (EtOH) gave **4c** (251mg, 34%) as colorless crystals: R_f 0.50 (MeOH/CH_2Cl_2 1:9, v/v); mp 132°–133°C (uncorrected); $[\alpha]_D$ −30.0 (c 1.00, $CHCl_3$); ^{1}H NMR δ (ppm, $CDCl_3/CD_3OD$ 3:1, v/v) 1.30 (d, 3H, $J_{5d,6d}$ = 6.1, H_3-6d), 2.85–2.89 (m, 2H, *H*-7a, *H*-7′a), 3.34–3.50 (m, 1H, *H*-2b), 3.41 (t, 1H, $J_{3b,4b} = J_{4b,5b}$ = 8.8, *H*-4b), 3.43 (t, 1H, $J_{3d,4d} = J_{4d,5d}$ = 9.5, *H*-4d), 3.51 (t, 1H, $J_{2b,3b} = J_{3b,4b}$ = 8.8, *H*-3b), 3.52–3.58 (m, 1H, *H*-5b), 3.68–3.76 (m, 1H, *H*-8a), 3.72 (dd, 1H, $J_{2d,3d}$ = 3.4, $J_{3d,4d}$ = 9.5, *H*-3d), 3.88–3.96 (m, 1H, *H*-5d), 3.98–4.07 (m, 1H, *H*-8′a), 3.99 (dd, 1H, $J_{1d,2d}$ = 1.7, $J_{2d,3d}$ = 3.4, *H*-2d), 4.32 (d, 1H, $J_{1b,2b}$ = 7.8, *H*-1b), 4.41 (dd, 1H, $J_{5b,6b}$ = 6.0, $J_{6b,6'b}$ = 12.0, *H*-6b), 4.54 (dd, 1H, $J_{5b,6'b}$ = 2.0, $J_{6b,6'b}$ = 12.0, *H*-6′b), 5.05, 5.09 (2s, 2 × 2H, CH_2Ph), 5.11 (br.s, 1H, *H*-1d), 5.14 (2s, 2 × 2H, CH_2Ph), 6.30 (d, 1H, $J_{7c,8c}$ = 15.9, *H*-8c), 6.74 (d, 1H, $J_{5a,6a}$ = 8.0, *H*-6a), 6.82 (d, 1H, $J_{5a,6a}$ = 8.0, *H*-5a), 6.87 (br.s, 1H, *H*-2a), 6.92 (d, 1H, $J_{5c,6c}$ = 8.3, *H*-5c), 7.02 (d, 1H, $J_{5c,6c}$ = 8.3, *H*-6c), 7.14 (br.s, 1H, *H*-2c), 7.25–7.47 (m, 20H, *H*-aromatic), 7.59 (d, 1H, $J_{7c,8c}$ = 15.9, *H*-7c); ^{13}C NMR δ (ppm, $CDCl_3/CD_3OD$ 3:1, v/v) 17.5 (*C*-6d), 35.9 (*C*-7a), 64.0 (*C*-6b), 69.3 (*C*-5d), 69.6 (*C*-4b), 71.0 (*C*-2d), 71.3 (*C*-8a, *C*-3d), 71.8, 71.9 (4 × CH_2Ph), 73.0 (*C*-4d), 73.9 (*C*-2b), 74.3 (*C*-5b), 84.6 (*C*-3b), 101.8 (*C*-1d), 103.4 (*C*-1b), 114.2 (*C*-2c), 114.7 (*C*-5c), 115.7 (*C*-8c), 115.8 (*C*-5a), 116.5 (*C*-2a), 122.2 (*C*-6a), 123.6 (*C*-6c), 127.4–128.8 (*C*-1c, *Ph*), 132.3 (*C*-1a), 137.1, 137.5, 137.6 (3 × *Ph*), 145.6 (*C*-7c), 147.8, 149.2, 151.6 (*C*-3a, *C*-3c, *C*-4a, *C*-4c), 167.9 (*C*-9c). Anal. Calcd. for $C_{57}H_{60}O_{15} \cdot 2.0\ H_2O$: C, 67.05; H, 6.12. Found: C, 67.16, H, 5.87.

2-(3′,4′-Dihydroxyphenyl)ethyl 6-*O*-caffeoyl-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (Isoacteoside, **1**)

A mixture of **4b** (160mg, 0.162mmol), 5% Pd-C (160mg), and 1,4-cyclohexadiene (305ml, 3.26mmol) in *N,N*-dimethylformamide (DMF)/EtOH (1:1, v/v; 1.0ml) was stirred at 40°C for 9h. The catalyst was filtered off, and the filtrate was concentrated in vacuo to give a yellow oily residue. The residue was purified by preparative TLC using a solvent mixture of $CHCl_3$/MeOH/H_2O (30:8:1, v/v/v) to give isoacteoside (**1**) (55mg, 54%) as a pale-yellow powder: R_f 0.51 ($CHCl_3$/MeOH/H_2O = 30:10:1, v/v/v); $[\alpha]_D$ −23.5 (c 0.31, MeOH); ^{1}H NMR (Table 1); ^{13}C NMR (Table 2). Anal. Calcd. for $C_{29}H_{36}O_{15} \cdot 2.7\ H_2O$: C, 51.74; H, 5.79. Found: C, 51.46, H, 5.71.

Results and discussion

Our previous paper[5] reported total synthesis of acteoside via deacetylation of the intermediate, 2-(3′,4′-dibenzyloxyphenyl)ethyl 2,6-di-*O*-acetyl-4-*O*-[3′,4′-bis(*O*-benzyl)caffeoyl]-3-*O*-(α-L-rhamnopyranosyl)-β-D-glucopyranoside (**3**), into **4a** using a solution of methylamine in methanol ($MeNH_2$ in MeOH) (Fig. 2). The reaction produced acteoside derivative **4a** in 49% yield and trace amounts of many unknown by-products. We identified one of these by-products as **4b**, with a 6-*O* caffeoyl group migrated from the 4-*O* position. Although only a trace amount of **4b** was afforded by the reported reaction conditions, the reaction is useful for the synthesis of isoacteoside (**1**) if the yield of

Table 1. ^{1}H-NMR spectral data of isoacteoside

Proton	Synthetic	Natural[a]
3,4-Dihydroxyphenylalcohol		
2a	6.66 (d, 2.2)	6.66 (d, 2.1)
5a	6.63 (d, 8.1)	6.62 (d, 8.2)
6a	6.52 (dd, 2.2, 8.1)	6.53 (dd, 2.1, 8.2)
7a	2.74–2.79 (m)	2.76 (m)
8a	3.66–3.74 (m)	3.70 (m)
8′a	3.90–4.04 (m)	3.97 (m)
D-Glucose		
1b	4.32 (d, 8.1)	4.32 (d, 8.1)
2b	3.28–3.33 (m)	ca. 3.30
3b	3.53 (t, 9.3)	3.52 (t, 9.1)
4b	3.39 (t, 9.3)	3.39 (t, 9.5)
5b	3.50–3.57 (m)	3.53 (m)
6b	4.34 (dd, 5.8, 12.0)	4.34 (dd, 5.9, 12.1)
6′b	4.49 (dd, 2.0, 12.0)	4.48 (dd, 2.0, 12.1)
Caffeic acid		
2c	7.03 (d, 2.0)	7.02 (d, 2.2)
5c	6.76 (d, 8.3)	6.76 (d, 8.0)
6c	6.88 (dd, 2.0, 8.3)	6.88 (dd, 2.2, 8.0)
7c	7.55 (d, 15.9)	7.55 (d, 15.9)
8c	6.28 (d, 15.9)	6.28 (d, 15.9)
L-Rhamnose		
1d	5.17 (d, 1.2)	5.17 (d, 1.7)
2d	3.90–4.04 (m)	3.93 (dd, 1.7, 3.3)
3d	3.66–3.74 (m)	3.69 (dd, 3.3, 9.7)
4d	3.37–3.43 (m)	3.38 (t, 9.7)
5d	3.90–4.04 (m)	3.98 (m)
6d	1.24 (d, 6.1)	1.23 (d, 6.2)

Splitting patterns and coupling constants (Hz) are given in parentheses
[a] Data are from Ota et al.[12]

Table 2. ^{13}C-NMR spectral data of isoacteoside

Carbon	Synthetic	Natural[a]
3,4-Dihydroxyphenylalcohol		
1a	131.4	131.3
2a	116.4	116.3
3a	144.6	144.6
4a	146.1	146.1
5a	117.1	117.0
6a	121.3	121.3
7a	36.7	36.7
8a	72.4	72.4
D-Glucose		
1b	104.4	104.3
2b	75.4	75.4
3b	83.9	83.8
4b	70.0	70.0
5b	75.7	75.7
6b	64.6	64.6
Caffeic acid		
1c	127.7	127.6
2c	114.8	114.3
3c	149.6	149.6
4c	146.8	146.8
5c	116.5	116.5
6c	123.2	123.2
7c	147.3	147.3
8c	115.1	115.0
9c	169.1	169.1
L-Rhamnose		
1d	102.7	102.7
2d	72.3	72.3
3d	72.2	72.2
4d	74.0	74.0
5d	70.4	70.3
6d	17.9	17.8

[a] Data are from Ota et al.[12]

compound **4b** is improved. Reaction conditions of the deacetylation and the caffeoyl migration have been examined.

Compound **3** was dissolved in CH_2Cl_2 and treated with $MeNH_2$ in MeOH at −20°C (Fig. 2). The reaction was monitored by TLC (MeOH/CH_2Cl_2, 1:9, v/v). At 9h the spot of the starting compound **3** at R_f 0.85 became small, and the spot of deacetylated acteoside derivative **4a** at R_f 0.54 became a major one. With another reaction time of 10h at the same temperature, the spot of **4a** was smaller, and two spots at R_f 0.50 and 0.16 were larger (the ratio of the spot size at R_f 0.50 and R_f 0.16 was about 2:1). After 10h the spot at R_f 0.16 had enlarge, and the spots of **3** and **4a** at R_f 0.50 had diminished.

The purified compound at R_f 0.50 was analyzed by ^{1}H-NMR. There was no signal for the acetyl group, indicating that the compound was a deacetylated product. Two doublets at δ 6.30 and 7.59 have the same coupling constant of 15.9Hz, assignable as *trans* olefinic protons of a caffeoyl moiety (*H*-7 and *H*-8), which suggested that the caffeoyl group remained. Signals of *H*-4 of the glucose moiety at δ 3.41 showed a higher-field shift (by 1.55ppm) compared with the corresponding signal of **4a**.[5] Signals of *H*-6a and *H*-6b of the glucose moiety appeared at δ 4.41 and 4.54, whereas the corresponding protons of **4a** appeared as a multiplet at δ 3.51–3.68, exhibiting a lower-field shift by 0.73–1.03ppm. These higher- and lower-field shifts suggested that the caffeoyl group of compound **4a** migrated from the 4-*O* position to 6-*O* position. All other signals were successfully assigned, and so the compound at R_f 0.50 was determined to be **4b**. Because the compound at R_f 0.16 gave no signals derived from the acetyl and caffeoyl groups, it was determined to be **4c**.

Based on these results, deacetylation of compound **3** seems to be almost completed in 9h to afford **4a**. After deacetylation, the 4-*O* caffeoyl group of **4a** started to migrate to the 6-*O* position, but the caffeoyl group was cleaved simultaneously. When the reaction was stopped at 19h, **4b** was isolated in about 20% yield, and a certain amount of **4a** remained. The longer reaction decreased the yield of **4b**.

Next, the reaction temperature was increased to 0°C after the 19h of stirring at −20°C. The spot of **4b** enlarged to reach a maximum (34% yield) within 3h. After this time, the spot of **4b** was not increased, whereas the spot of **4c** became larger.

The final step, debenzylation of **4b**, was performed via catalytic transfer hydrogenation using 1,4-cyclohexadiene as a proton source.[5] It was reported that the debenzylation rate under these reaction conditions was affected by the reaction solvent: increasing in the order of EtOH > MeOH > DMF.[16] In this case, because of the insolubility of **4b** in EtOH and MeOH, a 1:1 mixture of DMF/EtOH was used. The reaction temperature was also important because no reaction occurred at 0°, 10°, 20°, or 30°C; and the reaction began at 40°C.

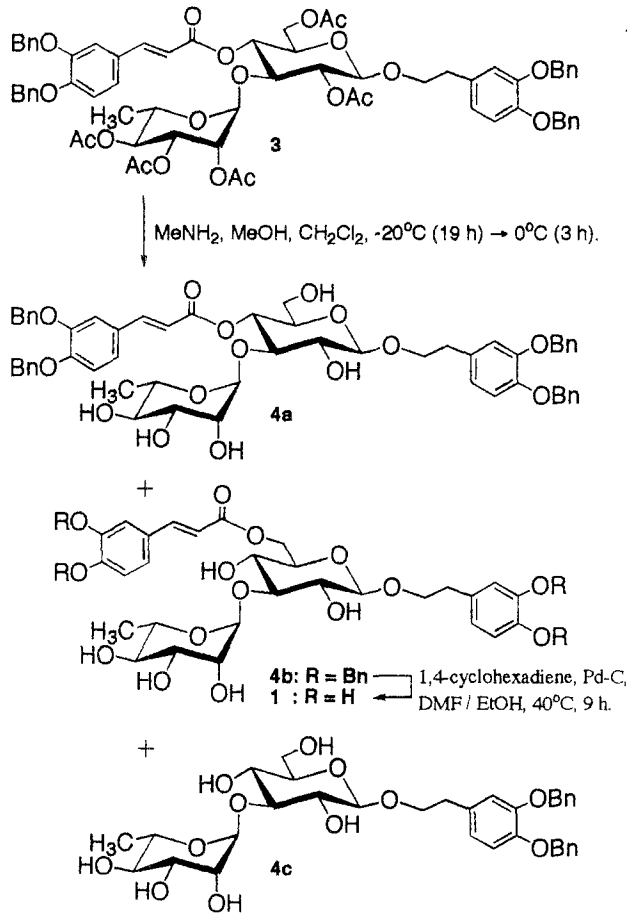

Fig. 2. Synthesis of isoacteoside (**1**)

Hence, **4b** was dissolved in a mixture of DMF and EtOH (1:1, v/v) and was treated with 1,4-cyclohexadiene in the presence of 5% Pd-C at 40°C for 9h. The reaction was monitored by TLC (solvent A: $CHCl_3$/MeOH/H_2O, 30:10:1 v/v/v; solvent B: CH_2Cl_2/MeOH, 9:1 v/v). At 9h a spot corresponding to the starting compound **4b** at R_f 0.55 (solvent A) disappeared, and two spots at R_f 0.50 and 0.55 (solvent B) appeared. The reaction was stopped at 9h because a longer reaction time decreased the spot size at R_f 0.50 (solvent B) and increased it at R_f 0.55 (solvent B).

The major product at R_f 0.50 was purifed and its chemical structure was determined by ^{1}H-NMR. Two doublets of *H*-7c and *H*-8c at δ 6.28 and δ 7.55, respectively, had a coupling constant typical of *trans* olefinic protons (15.9Hz). All other signals supported the identification of isoacteoside (**1**). In addition, the compound at R_f 0.55 is assumed to be an unexpected compound in which a double bond of caffeoyl moiety was saturated.

The ^{1}H-NMR spectral data of synthesized isoacteoside (**1**) were identical with those of the natural isoacteoside isolated by Ota et al.[12] (Table 1). ^{13}C-NMR data, shown in Table 2, were also identical to those of the natural compound.[12]

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References

1. Jiménez C, Riguera R (1994) Phenylethanoid glycosides in plants: structure and biological activity. Nat Prod Rep 11:591–606
2. Cometa F, Tomassini L, Nicoletti M (1993) Phenylpropanoid glycosides: distribution and pharmacological activity. Fitoterapia 64:195–217
3. Mølgaard P, Ravn H (1988) Evolutionary aspects of caffeoyl ester distribution in dicotyledons. Phytochemistry 27:2411–2421
4. Xiong Q, Hase K, Tezuka Y, Tani T, Namba T, Kodata S (1997) Hepatoprotective activity of phenylethanoids from *Cistanche deserticola*. Planta Med 64:120–125
5. Kawada T, Asano R, Hayashida S, Sakuno T (1999) Total synthesis of the phenylpropanoid glycoside, acteoside. J Org Chem 64:9268–9271
6. Kawada T, Asano R, Makino K, Sakuno T (2000) Synthesis of conandroside: a dihydroxyphenylethyl glycoside from *Conandron ramaidioides*. Eur J Org Chem 2000:2723–2727
7. Pettit GR, Numata A, Takemura T, Ode RH, Narula AS, Schmidt JM, Cragg GM, Pase CP (1990) Antineoplastic agents, 107: isolation of acteoside and isoacteoside from *Castilleja linariaefolia*. J Nat Prod 53:456–458
8. Scarpati ML, Monache FD (1963) Isolamento dal verbascum sinuatum di due nuovi glucosidi: il verbascoside e l'isoverbascoside. Ann Chem 53:356–367
9. Miyase T, Koizumi A, Ueno A, Noro T, Kuroyanagi M, Fukushima S, Akiyama Y, Takemoto T (1982) Studies on the acyl glycosides form *Leucosceptrum japonicum* (MIQ.) Kitamura et Murata. Chem Pharm Bull (Tokyo) 30:2732–2737
10. Schilling G, Hügel M, Mayer W (1982) Verbascoside und Isoverbascoside aus *Paulownia tomentosa* Steud. Z Naturforsch 37b:1633–1635
11. Sasaki H, Nishimura H, Morota T, Chin M (Chen Z), Mitsuhashi H, Komatsu Y, Maruyama H, Tu G, He W, Xiong Y (1989) Immunosuppressive principles of *Rehmannia glutinosa* var. *hueichingensis*. Planta Med 55:458–562
12. Ota M, Azuma T, Onodera S, Taneda K (1993) The chemistry of color changes in kiri wood (*Paulownia tomentosa* Steud.) III. A new caffeic acid sugar from kiri wood. Mokuzai Gakkaishi 39:479–485
13. Duynstee HI, de Koning MC, Ovaa H, van der Marel GA, van Boom JH (1999) Synthesis of verbascoside: a dehydroxyphenylethyl glycoside with diverse bioactivity. Eur J Org Chem 1999:2623–2632
14. Zhang SQ, Li ZJ, Wand AB, Cai MS, Feng R (1998) Synthesis of a phenylpropanoid glycoside, osmanthuside B6. Carbohydr Res 308:281–285
15. Ota M, Takahashi K, Kofujita H (1998) Role of caffeic glucoside esters in defense-repair processing of trees. II. Synthesis of 2-(3,4-dihydroxyphenyl)-ethyl 3-*O*-α-L-rhammopyranosyl-β-D-glucopyranoside. J Wood Sci 44:320–326
16. Felix AM, Heimer EP, Lambros TJ, Taougraki C, Meinenhofer J (1978) Rapid removal of protecting groups from peptides by catalytic transfer hydrogenation with 1,4-cyclohexadiene. J Org Chem 21:4194–4196