Chemical Constituents of *Prangos tschimganica*; Structure Elucidation and Absolute Configuration of Coumarin and Furanocoumarin Derivatives with Anti-HIV Activity

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The methanol extract of the dried aerial parts of *Prangos tschimganica* gave three new coumarin derivatives and 30 known coumarin derivatives. Their structures were established on the basis of chemical and spectroscopic evidence. Absolute configuration of the isolated compounds were determined by using a modified Mosher's method. Some of the isolated compounds showed anti-HIV activity.

Key words *Prangos tschimganica*; Umbelliferae; coumarin derivative; anti-human immunodeficiency virus (HIV) activity; optical purity

Prangos tschimganica belongs to the Umbelliferae and is distributed throughout Central Asia. This family is well known for producing a large number of coumarins with isoprenoid units disposed in multifarious ways.[1—3] This family also has been found to be relatively rich in secondary metabolic products, such as furocoumarin,[4,5] which have attracted considerable interest due to their biological activity, and their chemical and physical properties have been investigated extensively.[6] The aerial parts of *P. tschimganica* are used in Uzbekistan as a folk medicine for skin conditions such as leukoplakic disease. In the course of our search for bioactive metabolites from herbal plants in Uzbekistan, we have been studying the chemical components of this plant. We described here in the structural determination of three new coumarin derivatives (**1**—**3**) and 30 known compounds (**4**—**33**) isolated from *P. tschimganica.*

Repeated column chromatography of the *n*-BuOH and EtOAc soluble fractions from the methanol extracts of the dried aerial parts of *P. tschimganica* yielded compounds **1**—**33**.

Compound **1** was obtained as a colorless oil, and its molecular formula was determined to be $C_{22}H_{28}O_{10}$ on the basis of high resolution (HR)-FAB-MS. Its IR spectrum showed the presence of an α,β-unsaturated lactone (1730 cm^{-1}), ester (1700 cm^{-1}), a hydroxy group (3422 cm^{-1}) and an aromatic ring (1625 cm^{-1}). The UV spectrum exhibited absorption bands characteristic of coumarin at 321, 264 and 250 nm. The ^{1}H-NMR spectrum of **1** showed the presence of two α-pyron protons [δ_H 7.78, 6.23 (each 1H, d, J=9.5 Hz)], one AB-type aromatic proton [δ_H 7.47, 7.01 (each 1H, d, J=8.7 Hz)], two methyl groups [δ_H 1.31, 1.29 (each 3H, s)] and one methoxyl group [δ_H 3.99 (3H, s)]. The ^{13}C-NMR spectrum exhibited 22 carbon resonances including two methyls, one methoxy, three methylenes, eight methines, two carbonyl carbons and six quaternary carbons (Table 1). The ^{13}C-NMR spectral data of **1** were very similar to those of meranzin hydrate (see Experimental),[7] except for the presence of seven carbon signals in compound **1**. In the ^{1}H–^{1}H correlation spectroscopy (COSY) spectrum of **1**, the proton signal at δ_H 1.63 (H2-6″) was correlated with δ_H 3.86 (H-5″); δ_H 3.26 (H-4″) with δ_H 3.86 and 3.90 (H-3″); δ_H 1.41 (H_2-2″) with δ_H 1.89 (H_2-6″) and 3.90, suggesting that the remaining signals reflected a quinic acid moiety. In the heteronuclear multiple bond correlation (HMBC) spectrum of **1**, the proton signals at δ_H 3.45 (H_2-1′) and 3.08 (H_2-1′) were correlated with the carbon signals at δ_C 162.5 (C-7), 154.6 (C-8a) and 114.6 (C-8); δ_H 7.78 (H-4) with δ_C 154.6; δ_H 5.23 (H-2′) with δ_C 175.6 (C-7″); and δ_H 3.99 (OCH_3) with δ_C 162.5. These findings clearly indicated that the isoprene unit was connected to C-8 of the coumarin ring (Fig. 1), and carboxylic acid (C-7″) was connected to C-2′ of the isoprene unit, while a methoxy function was connected to C-7. The depicted relative stereochemistry of the quinic ester moiety was established on the basis of multiplicities and the nuclear Overhauser enhancement and exchange spectroscopy (NOESY) spectrum. Significant correlations were observed between the proton signal at δ_H 3.26 (H_{ax}-4″, dd, J=2.9, 9.3 Hz) and the proton signals at δ_H 1.71 and 1.63 (H_{ax}-2″, dd, J=2.6, 14.5 Hz, H_{ax}-6″, t, J=12.9 Hz); between δ_H 3.86 (H_{ax}-5″, ddd, J=3.4, 9.3, 12.9 Hz) and δ_H 1.89 (H_{eq}-6″, dd, J=3.4, 12.9 Hz); between δ_H 3.90 (H_{eq}-3″, br s) and δ_H 1.71 (H_{ax}-2″) and 1.41 (H_{eq}-2″, dd, J=2.9, 14.5 Hz). These spectral results led us to propose the structure for tschimganic ester A (**1**) except absolute configurations (Fig. 1).

Compound **2**, obtained as a colorless oil, showed an $[M-H]^-$ peak at m/z 477 in its negative FAB-MS. Its UV absorption spectrum indicated the presence of a linear-type furanocoumarin (309, 267, 249, 221 nm), and its IR spectrum showed absorption bands due to an α,β-unsaturated lactone (1727 cm^{-1}), a hydroxy group (3400 cm^{-1}), an aromatic ring (1630 cm^{-1}) and a furan ring (883 cm^{-1}). The ^{1}H-NMR spectrum showed two pairs of doublets in the downfield region, one at δ_H 8.12 and 6.23 (J=9.8 Hz) was attributed to the C-3 and C-4 protons of the coumarin nucleus while a second pair of signals at δ_H 7.78 and 7.19 (J=2.0 Hz) confirmed the presence of the benzofuran moiety. The single aromatic pro-

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ton signal at δ_H 7.12 was assigned to the C-8 proton. The ^{13}C-NMR spectrum showed 23 atoms, among which were 11 carbon atoms of the furanocoumarin nucleus, 5 carbons (δ_C 80.4, 73.2, 72.1, 27.5, 26.0) of the isoprene unit and 7 carbons consisting of two methylenes (δ_C 42.9, 38.7), three methines (δ_C 77.0, 71.8, 68.4) and two quaternary carbons (δ_C 77.5, 175.4) (Table 1). These results were consistent with $C_{23}H_{26}O_{11}$ as molecular formula of **2**, which was supported by HR mass spectral data. The ^{1}H- and ^{13}C-NMR data of furanocoumarin and isoprene units in **2** were very similar to those of oxypeucedanin hydrate (**12**),[8] except for the presence of a signal assignable to the quinic acid moiety and the chemical shifts at C-2′ (**2**: δ 80.4, **12**: δ_C 76.5). In the HMBC spectrum, the proton signals at δ_H 8.12 (H-4) and 4.89 (H_2-1′) were correlated with the carbon signal at δ_C 150.4 (C-5), suggesting that the isoprene unit was connected to C-5. The proton signals at δ_H 5.34 (H-2′) and 1.89 (H-2″) were correlated with the carbon signal at δ_C 175.4 (C-7″), indicating that the quinic acid group was located at C-2′. The relative stereochemistry was identified in the same manner as described for **1**. To determine the absolute stereochemistry of positions 1″, 3″, 4″ and 5″ of the quinic acid moiety, compound **2c** and **2c′** were prepared by the reactions shown in Chart 1. As shown, commercially available (−)-quinic acid was treated with acetic anhydride in pyridine, to give tetraacetylquinic acid (**2a**), which was treated with dicyclohexylcarbodiimide (DCC), 4-pyrrolidinopyridine and **12** in dichloromethane led to the compound **2b**.[9] The compound **2b** (40 mg) was acetylated by using the same method with acetic anhydride as that of esterification of oxypeucedanin hydrate, to give the compound **2c** (2 mg). Tetraacetylation of **2** with DCC and acetic anhydride gave **2c′** which was identical to compound **2c** by comparison of TLC, ^{1}H-NMR and FAB-MS findings. In this synthesis, we used racemic compound **12** (see later) so, compound **2** was concluded to be diastereomer on C-2′. Based of these results, tschimganic ester

Table 1. ^{13}C-NMR Data for **1**, **2** and **3**

	1	**2**	**3**
C-2	163.0	163.5	162.8
C-3	113.5	113.6	115.3
C-4	146.8	141.7	147.0
C-4[a)]	115.1	108.1	118.2
C-5	129.4	150.4	115.5
C-6	109.4	114.8	128.1
C-7	162.5	160.2	149.2
C-8	114.6	94.8	133.4
C-8[a)]	154.6	154.3	144.5
C-9		147.3	147.0
C-10		106.1	108.2
C-1′	24.0	73.2	73.4
C-2′	80.5	80.4	80.3
C-3′	73.4	72.1	72.2
C-4′	28.9	27.5	27.1
C-5′	28.6	26.0	26.2
C-1″	77.4	77.5	77.6
C-2″	38.5	38.7	38.6
C-3″	72.6	71.8	72.2
C-4″	77.6	77.0	77.6
C-5″	68.0	68.4	68.2
C-6″	42.9	42.9	42.6
C-7″	175.6	175.4	175.1
OCH_3	57.2		

a) CD_3OD.

Table 2. Anti-HIV Activity of Compounds **4**, **5**, **9**, **11** and **13**

Compound	IC_{50} (μg/ml)	EC_{50} (μg/ml)	TI
4	19.1	0.1	191
5	19.8	7.29	2.71
9	16.7	6.38	2.61
11	26.3	2.25	11.7
13	24.8	0.354	69.9
AZT	500	<0.001	>500.000

AZT: azidothymidine

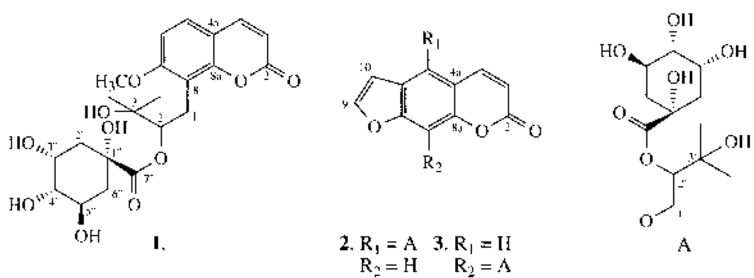
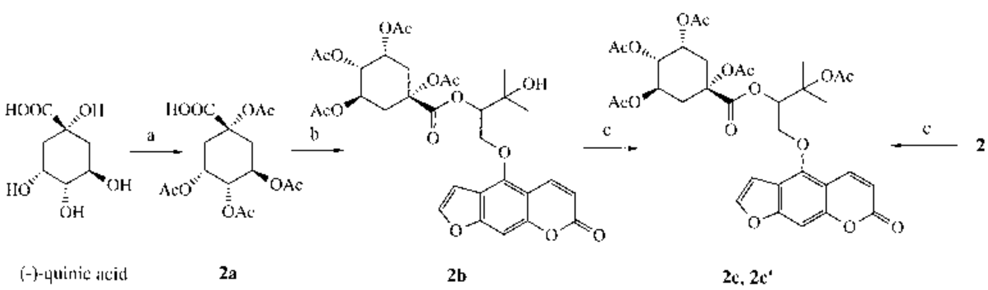


Fig. 1



Reagents : (a) Ac_2O, pyr; (b) **12**, DCC, 4-pyrrolidinopyridine; (c) Ac_2O, DCC, 4-pyrrolidinopyridine

Chart 1

B could be represented by the formula **2** (Fig. 1).

Compound **3** was obtained as a colorless oil, and exhibited a quasi-molecular ion peak at 477 in negative FAB-MS to give a molecular formula of $C_{23}H_{26}O_{11}$ in combination with ^{1}H- and ^{13}C-NMR data. The ^{13}C-NMR spectra were very close to those of **2**, except for the signals due to C-5 and C-8 [**3**: δ_C 115.5 (d), 133.4 (s), **2**: δ_C 150.4 (s), 94.8 (d)] (Table 1). This suggested the difference between these compounds should be the linkage position of the isoprene unit. In the HMBC spectrum of **3**, the correlation of δ_H 4.62 (H_2-1′) with 133.4 (C-8) indicated the isoprene group was connected to C-8. Other ^{1}H- and ^{13}C-NMR signals were assigned in the same manner as described above. The structure of tschimganic ester C was confirmed to be formula **3** (Fig. 1). While many coumarin compounds have been isolated from natural sources, this is the first isolation of two quinic acid ester of coumarins (**2**, **3**).

Based on a detailed study of spectral data, the known compounds were identified to be psoralen (**4**), heraclenin (**5**), imperatorin (**6**), xanthotoxin (**7**), (±)-heraclenol (**8**), (±)-pabulenol (**9**), isoimperatorin (**10**), (±)-saxalin (**11**), (±)-oxypeucedanin hydrate (**12**), bergapten (**13**), (±)-8-(3-chloro-2-hydroxyl-3-methylbutoxy)-psoralen (**14**) and xanthotoxol (**15**),[9] pabularinone (**16**), osthol (**17**),[10] columbianetin (**18**), columbianetin-*O*-β-D-glucopyranoside (**19**), (±)-auraptenol (**20**), isomeranzin (**21**),[11] osthenol (**22**), scopoletin (**23**), umbelliferone (**24**),[12] *tert-O*-methylheraclenol (**25**) marmesin (**26**), (+)-ulopterol (**27**),[13] (±)-oxypeucedanin methanolate (**28**),[14] desmethyl-7 suberosine (**29**),[15] isogospherol (**30**),[16] (+)-peucedanol (**31**),[17] yuehgesin-B (**32**),[18] and marmesinine (**33**).[19] Thus, *P. tschimganica* is rich source of coumarin derivatives.

To determine the absolute configurations and optical purity of the isolated compounds, the (*R*) and (*S*) methoxytrifluoromethyl phenylacetic acid (MTPA) esters of coumarin derivatives (**20**, **27**, **31**) and furanocoumarin derivatives (**8**, **9**, **11**, **12**, **14**, **28**) were prepared by using a modified Mosher's method.[20] The ^{1}H-NMR spectral of coumarin-MTPA esters (**27**, **31**) showed a optically pure, and these were determined to be *R* by comparison of its optical rotation with that reported in the literature. However, the ^{1}H-NMR spectral of coumarin-(*R* or *S*) MTPA esters (**20**) showed separated pairs on signals. This finding suggested that compound **20** should be an enantiomeric mixture, and the ratio of two enantiomeric isomers is about 1 : 3 [*R* : *S*] according to the integral value of the separated signals. On the other hand, the ^{1}H-NMR spectral of furanocoumarin-MTPA esters also showed separated pairs of signals. These results indicated that these compounds should be an enantiomeric mixture, and the ratio of two isomers is about 1 : 1 (**8**, **11**, **12**, **28**), 6 : 4 (**9**) and 2 : 1 (**14**) according to the integral value of the separated signals. Furthermore, the optical rotation values of furanocoumarin derivatives (**8**, **9**, **11**, **12**, **28**) are near zero.

In our previous paper,[21] we reported the isolation of coumarin derivatives with anti-HIV activity. In this paper, we report the anti-HIV activity[22,23] of the isolated compounds (Table 2). Psoralen (**4**) inhibited HIV-1 (IIIB strain) replication in H9 lymphocytes with an EC_{50} value of 0.1 μg/ml, and it inhibited uninfected H9 cell growth with an IC_{50} value of 19.1 μg/ml; the therapeutic index (TI) was calculated to be 191. In general, TI>5.0 is considered to denote significant activity; compounds **11** and **13** also showed potent anti-HIV activities with TI values greater than 5.0.

Experimental

General Experimental Procedures NMR (400 MHz for ^{1}H-NMR, 100 MHz for ^{13}C-NMR, both use tetramethylsilane (TMS) as int. stand.) were measured on a Bruker AM 400 spectrometer and MS spectra on a JEOL JMS D-300 instrument; CC: Silica gel 60 (Merck), Sephadex LH-20 (Pharmacia) and Toyo pearl HW-40 (TOSOH); HPLC: GPC (shodex H-2001, 2002, $CHCl_3$; shodex GS-310 2G, MeOH), silica gel (Si 60, Hibar RT 250-25) and ODS (YMC-R-ODS-5; yamamura). IR spectra on a JASCO FT-IR spectrometer (FT/IR-420) and 1720 FT-IR spectrometer (Perkin-Elmer), UV spectra on a UV2100 UV-Vis recording spectrometer (Shimadzu). Optical rotations were measured with a JASCO DIP-370 digital polarimeter.

Plant Material The dried aerial parts of *Prangos tschimganica* were collected in June 1998 from Uzbekistan. Herbarium specimens were deposited in the herbarium of the Institute of Botany, Academy of Sciences, Uzbekistan. This plant was identified by Dr.Olimjon K. Kodzhimatov.

Extraction and Fractionation The dried aerial parts of *P. tschimganica* were extracted three times with MeOH (10 l and 8 h each time) at 60 °C. The combined extracts were concentrated under reduced pressure, the residue (357 g) was diluted with water, and then extracted with AcOEt and *n*-BuOH, respectively. The *n*-BuOH layer (44 g) was chromatographed over a silica gel column (11×100 cm, Merck Silica gel 60, 1 kg) and eluted with $CHCl_3$–MeOH (8 : 2 to 1 : 1). Thirteen fractions were obtained. Fraction 3 (3.8 g) was chromatographed on Toyo pearl (5×70 cm) with $CHCl_3$–MeOH (2 : 1) to give 4 fractions (3.1—3.4). Fraction 3.2 (2.7 g) was chromatographed over silica gel (5×80 cm, 300 g) and eluted with $CHCl_3$–MeOH (8 : 2) to give 3 fractions (3.2.1—3.2.3). Fraction 3.2.2 (852 mg) was separated by general permeation chromatography (GPC) (MeOH), and gave further 8 fractions (3.2.2.1—3.2.2.8). Fraction 3.2.2.8 (48 mg) was isolated by HPLC (ODS, MeOH–H_2O, 3 : 1) to give compound **3** (4 mg). Fraction 3.2.3 (1 g) was isolated by GPC (MeOH) to give 10 fractions (3.2.3.1—3.2.3.10). Compound **1** (2 mg) was obtained after the purification of fraction 3.2.3.5 (26 mg) using HPLC (ODS, MeOH–H_2O, 3 : 1). Fraction 3.2.3.8 (53 mg) was purified by HPLC (ODS, MeOH–H_2O, 3 : 1) to give compound **2** (24 mg). Another compounds were obtained following yield **4** (28 mg), **5** (100 mg), **6** (40 mg), **7** (31 mg), **8** (10 mg), **9** (19 mg), **10** (126 mg), **11** (278 mg), **12** (183 mg), **13** (8 mg), **14** (24 mg), **15** (5 mg), **16** (28 mg), **17** (5.5 g), **18** (16 mg), **19** (2 mg), **20** (39 mg), **21** (15 mg), **22** (16 mg), **23** (80 mg), **24** (7 mg), **25** (325 mg), **26** (4 mg), **27** (21 mg), **28** (20 mg), **29** (2 mg), **30** (32 mg), **31** (15 mg), **32** (57 mg) and **33** (14 mg).

Tschimganic Ester A (**1**): $[\alpha]_D^{25}$ −5.0° (*c*=0.2, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3422, 3109, 2975, 1730, 1700, 1625, 1573, 1451, 1249, 1100; UV λ_{max}^{MeOH} nm (log ε): 321 (3.89), 264 (4.02), 250 (4.10); HR-FAB-MS *m/z* 475.1594 [M+Na]$^+$, (Calcd for $C_{22}H_{28}O_{10}Na$, 475.1580); ^{1}H-NMR (CD_3OD) δ_H: 7.78 (H-4, d, *J*=9.5 Hz), 7.47 (H-5, d, *J*=8.7 Hz), 7.01 (H-6, d, *J*=8.7 Hz), 6.23 (H-3, d, *J*=9.5 Hz), 5.23 (H-2′, dd, *J*=1.9, 9.3 Hz), 3.99 (3H, s, OCH_3), 3.90 (H-3″, br s), 3.86 (H-5″, ddd, *J*=3.4, 9.3, 12.9 Hz), 3.45 (H-1′, t, *J*=12.5 Hz), 3.26 (H-4″, dd, *J*=2.9, 9.3 Hz), 3.08 (H-1′, dd, *J*=1.9, 12.5 Hz), 1.89 (H-6″, dd, *J*=3.4, 12.9 Hz), 1.71 (H-2″, dd, *J*=2.6, 14.5 Hz), 1.63 (H-6″, t, *J*=12.9 Hz), 1.41 (H-2″, dd, *J*=2.9, 14.5 Hz), 1.31 (H-4′, 3H, s), 1.29 (H-5′, 3H, s); ^{13}C-NMR data see Table 1.

Tschimganic Ester B (**2**): $[\alpha]_D^{25}$ −8.0° (*c*=0.2, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3400, 3071, 1727, 1707, 1630, 1597, 1458, 1402, 1331, 1216, 883; UV λ_{max}^{MeOH} nm (log ε): 309 (4.02), 267 (4.21), 249 (4.30), 221 (4.42); HR-FAB-MS *m/z* 501.1386 [M+Na]$^+$, (Calcd for $C_{23}H_{26}O_{11}Na$, 501.1373); ^{1}H-NMR (CD_3OD) δ_H: 8.12 (H-4, d, *J*=9.8 Hz), 7.78 (H-9, d, *J*=2.0 Hz), 7.19 (H-10, d, *J*=2.0 Hz), 7.12 (H-8, s), 6.23 (H-3, d, *J*=9.8 Hz), 5.34 (H-2′, d, *J*=9.8 Hz), 4.89 (H-1′, d, *J*=9.8 Hz), 4.67 (H-1′, t, *J*=9.8 Hz), 4.09 (H-3″, m), 4.02 (H-5″, ddd, *J*=3.2, 9.2, 12.5 Hz), 3.40 (H-4″, m), 2.18 (H-6″, m), 2.12 (H-2″, 2H, m), 1.89 (H-6″, t, *J*=12.5 Hz), 1.32 (H-5′, 3H, s), 1.30 (H-4′, 3H, s); ^{13}C-NMR data see Table 1.

Tschimganic Ester C (**3**): $[\alpha]_D^{25}$ −8.4° (*c*=0.2, MeOH); IR ν_{max}^{KBr} cm^{-1}: 3420, 3075, 1729, 1707, 1623, 1610, 1589, 1460, 1334, 1212, 1101, 874; UV λ_{max}^{MeOH} nm (log ε): 309 (3.97), 267 (4.15), 248 (4.25), 218 (4.29); HR-FAB-MS *m/z* 501.1342 [M+Na]$^+$, (Calcd for $C_{23}H_{26}O_{11}Na$, 501.1373); ^{1}H-NMR (CD_3OD) δ_H: 7.98 (H-4, d, *J*=9.8 Hz), 7.87 (H-9, d, *J*=2.0 Hz), 7.51 (H-5, s), 6.87 (H-10, d, *J*=2.0 Hz), 6.31 (H-3, d, *J*=9.8 Hz), 5.28 (H-2′, d, *J*=9.8 Hz), 4.92 (H-1′, d, *J*=9.8 Hz), 4.62 (H-1′, t, *J*=9.8 Hz), 4.09 (H-3″, m), 3.99 (H-5″, ddd, *J*=3.2, 9.5, 12.5 Hz), 3.40 (H-4″, m), 2.20 (H-6″, m), 2.12 (H-2″, 2H, m), 1.92 (H-6″, t, *J*=12.5 Hz), 1.28 (H-5′, 3H, s), 1.26 (H-4′, 3H, s); ^{13}C-NMR data see Table 1.

Meranzin Hydrate: ^{13}C-NMR (CD_3OD) δ_C: 163.8 (C-1), 162.5 (C-7), 154.6 (C-8a), 146.4 (C-4), 128.4 (C-5), 117.2 (C-4a), 114.3 (C-8), 113.0 (C-3), 109.1 (C-6), 78.8 (C-2′), 74.1 (C-3′), 26.3 (C-1′), 25.6 (C-4′, 5′), 56.8 (OCH_3).

Peracetylation of (−)-Quinic Acid (−)-Quinic acid (400 mg) was acetylated with acetic anhydride (10 ml) and pyridine (10 ml) at room temperature overnight. The products were purified by using GPC eluted with $CHCl_3$, obtained the pure peracetate of quinic acid (**2a**).

2a: ^{1}H-NMR ($CDCl_3$) δ_H: 5.55 (1H, d, J=1.5 Hz), 5.42 (1H, m), 4.95 (1H, dd, J=2.6, 9.1 Hz), 2.70 (1H, br d, J=12.1 Hz), 2.61 (1H, br d, J=12.6 Hz), 2.36 (1H, dd, J=1.5, 12.1 Hz), 2.15 (3H, s), 2.10 (3H, s), 2.06 (3H, s), 2.04 (3H, s), 1.98 (1H, t, J=12.6 Hz). EI-MS: m/z 360 $[M]^+$.

Esterification of Oxypeucedanin Hydrate (12) A solution of oxypeucedanin hydrate (**12**) (100 mg), quinic acid peracetate (**2a**) (100 mg), DCC (60 mg) and 4-pyrrolidinopyridine (40 mg) in CH_2Cl_2 (20 ml) was allowed to stand at room temperature overnight. The *N*,*N*-dicyclohexyl urea was filtered and the filtrate washed with water, 5% acetic acid solution and again with water, dried over $MgSO_4$ and the solvent evaporated *in vacuo* to give the ester. The residue was purified by GPC, quinic ester of oxypeucedanin hydrate (**2b**; 45 mg) was obtained.

2b: ^{1}H-NMR ($CDCl_3$) δ_H: 8.08, 8.06 (H-4), 7.62 (2H, H-9), 7.15 (2H, H-8), 6.97, 6.95 (H-10), 6.29, 6.27 (H-3), 2.14 (3H, OAc), 2.10 (3H, OAc), 2.08 (3H, OAc), 2.07 (3H, OAc), 2.01 (9H, OAc×3), 2.00 (3H, OAc), 1.38 (3H, CH_3), 1.36 (3H, CH_3), 1.31 (3H, CH_3), 1.29 (3H, CH_3). FAB-MS: m/z 647 $[M+H]^+$.

Acethylation of 2b The compound **2b** (40 mg) was acetylated by using the same method with acetic anhydride as that of esterification of oxypeucedanin hydrate, to give the compound **2c** (2 mg).

2c: ^{1}H-NMR ($CDCl_3$) δ_H: 8.08 (2H, H-4), 7.64 (2H, H-9), 7.18 (2H, H-8), 6.97, 6.95 (H-10), 6.31, 6.27 (2H, H-3), 2.14 (3H, OAc), 2.13 (3H, OAc), 2.09 (3H, OAc), 2.08 (3H, OAc), 2.07 (3H, OAc), 2.03 (6H, OAc×2), 2.02 (3H, OAc), 2.00 (3H, OAc), 1.98 (6H, OAc×2), 1.59 (6H, CH_3×2), 1.57 (6H, CH_3×2). FAB-MS: m/z 711 $[M+Na]^+$, 689 $[M+H]^+$.

Acetylation of Tschimganic Ester B Tschimganic ester B (10 mg) was acetylated by using the same method as that of esterification of oxypeucedanin hydrate, obtained the tetraacetate of tschimganic ester B (**2c′**; 1 mg). This was identified by ^{1}H, TLC and FAB-MS comparison to the compound **2c**.

Esterification with Chiral Anisotropic Reagents [(*R*,*S*)-MTPA] Three equivalent of 2,4,6-trinitrochlorobenzene, MTPA and an alcohol [**8** (8 mg), **9** (17 mg), **11** (10 mg), **12** (16 mg), **14** (3 mg), **20** (5 mg), **27** (3 mg), **28** (3 mg), **31** (5 mg)] were dissolved in pyridine dehydrated (5 ml). After the mixture was stirred for 8 h, $CHCl_3$ was added, and the organic layer was washed with 8% aqueous sodium hydrogen carbonate and brine, dried over Na_2SO_4 and concentrated to yield a crude ester. A crude ester was purified by GPC using $CHCl_3$.

(2′*R*)-**20** [(*R*)-MTPA] Ester: ^{1}H-NMR ($CDCl_3$) δ_H: 7.53 (0.25H, H-4), 7.32 (0.25H, H-5), 6.77 (0.25H, H-6), 6.15 (0.25H, H-3), 5.81 (0.25H, H-2′), 5.05 (0.25H, H-4′), 4.95 (0.25H, H-4′), 3.08 (0.25H, H-1′), 1.90 (0.75H, H-5′).

(2′*S*)-**20** [(*R*)-MTPA] Ester: ^{1}H-NMR ($CDCl_3$) δ_H: 7.60 (0.75H, H-4), 7.40 (0.75H, H-5), 6.84 (0.75H, H-6), 6.22 (0.75H, H-3), 5.72 (0.75H, H-2′), 4.96 (0.75H, H-4′), 4.90 (0.75H, H-4′), 3.15 (0.75H, H-1′), 1.80 (2.25H, H-5′).

(2′*R*)-**20** [(*S*)-MTPA] Ester: ^{1}H-NMR ($CDCl_3$) δ_H: 7.61 (0.25H, H-4), 7.34 (0.25H, H-5), 6.84 (0.25H, H-6), 6.23 (0.25H, H-3), 5.73 (0.25H, H-2′), 4.96 (0.25H, H-4′), 4.90 (0.25H, H-4′), 3.16 (0.25H, H-1′), 1.81 (0.75H, H-5′).

(2′*S*)-**20** [(*S*)-MTPA] Ester: ^{1}H-NMR ($CDCl_3$) δ_H: 7.53 (0.75H, H-4), 7.32 (0.75H, H-5), 6.78 (0.75H, H-6), 6.16 (0.75H, H-3), 5.82 (0.75H, H-2′), 5.05 (0.75H, H-4′), 4.96 (0.75H, H-4′), 3.10 (0.75H, H-1′), 1.91 (2.25H, H-5′).

27 [(*S*)-MTPA] Ester: ^{1}H-NMR ($CDCl_3$) δ_H: 7.48 (H-4), 6.81 (H-5), 6.24 (H-3), 5.47 (H-2′), 3.93 (OCH_3), 3.24 (H-1′), 2.82 (H-1′), 1.33, 1.31 (CH_3×2).

31 [MTPA] Ester: ^{1}H-NMR ($CDCl_3$) δ_H: 6.38 (H-3), 5.34 (H-2′), 2.92 (H-1′), 2.79 (H-1′), 1.11, 1.09 (CH_3×2).

8 [(*R*)-MTPA] Ester: ^{1}H-NMR ($CDCl_3$) δ_H: 7.75, 7.73 (each 0.5H, H-4), 6.81, 6.79 (each 0.5H, H-10), 6.34, 6.33 (each 0.5H, H-3), 5.59 (1H, H-2′), 4.92, 4.83 (each 0.5H, H-1′), 4.69 (1H, H-1′), 1.40, 1.39, 1.28, 1.24 (each 1.5H, CH_3×4).

9 [(*S*)-MTPA] Ester: ^{1}H-NMR ($CDCl_3$) δ_H: 8.01 (0.6H, H-4), 7.92 (0.4H, H-4), 6.89 (0.6H, H-10), 6.83 (0.4H, H-10), 6.23 (0.6H, H-3), 6.20 (0.4H, H-3), 5.90 (1H, H-2′), 5.26, 5.19 (each 0.6H, H-4′), 5.14, 5.12 (each 0.4H, H-4′), 1.89 (1.2H, CH_3), 1.81 (1.8H, CH_3).

11 [(*R*)-MTPA] Ester: ^{1}H-NMR ($CDCl_3$) δ_H: 7.94, 7.91 (each 0.5H, H-4), 7.62, 7.60 (each 0.5H, H-9), 6.92, 6.87 (each 0.5H, H-3), 5.59 (1H, H-2′), 4.92, 4.83 (each 0.5H, H-1′), 4.69 (1H, H-1′), 1.40, 1.39, 1.28, 1.24 (each 1.5H, CH_3×4).

12 [(*R*)-MTPA] Ester: ^{1}H-NMR ($CDCl_3$) δ_H: 7.97, 7.95 (each 0.5H, H-4), 6.96, 6.89 (each 0.5H, H-10), 6.21, 6.18 (each 0.5H, H-3), 5.57, 5.51 (each 0.5H, H-2′), 1.36, 1.35, 1.33, 1.28 (each 1.5H, CH_3×4).

14 [(*S*)-MTPA] Ester: ^{1}H-NMR ($CDCl_3$) δ_H: 7.76 (0.5H, H-4), 7.75 (1H, H-4), 6.84 (0.5H, H-10), 6.81 (1H, H-10), 6.38 (0.5H, H-3), 6.36 (1H, H-3), 5.78 (0.5H, H-2′), 5.77 (1H, H-2′), 5.04 (0.5H, H-1′), 4.91 (1H, H-1′), 4.77 (0.5H, H-1′), 4.72 (1H, H-1′), 1.79, 1.76 (each 1.5H, CH_3), 1.64, 1.57 (each 3H, CH_3).

28 [MTPA] Ester: ^{1}H-NMR ($CDCl_3$) δ_H: 7.95, 7.93 (each 0.5H, H-4), 6.96, 6.89 (each 0.5H, H-10), 6.21, 6.15 (each 0.5H, H-3), 5.65 (1H, H-2′), 4.84 (each 0.5H, H-1′), 4.67 (1H, H-1′), 4.56 (each 0.5H, H-1′), 1.28, 1.27, 1.23, 1.21 (each 1.5H, CH_3×4).

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