Scientific Research

Microtropins J-P: 6'-*O*-(2"*S*,3"*R*)-2"-Ethyl-2",3"-Dihydroxybutyrates of Phenolic Alcohol *β*-D-Glucopyranosides from the Branches of *Microtropis japonica*

Yuka Uemura[1], Sachiko Sugimoto[1], Katsuyoshi Matsunami[1], Hideaki Otsuka[1,2*], Yoshio Takeda[2]

[1]Department of Pharmacognosy, Graduate School of Biomedical and Health Sciences, Hiroshima University, Hiroshima, Japan;
[2]Department of Natural Products Chemistry, Faculty of Pharmacy, Yasuda Women's University, Hiroshima, Japan.
Email: *hotsuka@hiroshima-u.ac.jp, *otsuka-h@yasuda-u.ac.jp

ABSTRACT

From the branches of *Microtropis japonica* (Celastraceae), seven phenolic alcohol glucosides, named microtropins J-P (**1-7**), were isolated. The 6-position of glucose was esterified with 2-ethyl-2,3-dihydroxybutyric acid. Microtropin K (**2**) was hydrolyzed under a mild basic condition to give methyl (2*S*,3*R*)-2-ethyl-2,3-dihydroxybutyrate, whose absolute structure was determined by the comparison of NMR data and the optical rotation value with that reported.

Keywords: *Microtropis japonica*; Celastraceae; Microtropin; (2*S*,3*R*)-2-Ethyl-2,3-Dihydroxybutyrate

1. Introduction

Celastraceous plants appeared in the spotlight after a potent antileukemic ansa-type macrolide, maytansine, was isolated from an Ethiopian shrub, *Maytenus serrata* (formerly *M. ovatus*) [1,2]. In continuous research on subtropical resource plants, a Celestraceous plant, *Microtropis japonica*, collected in Okinawa attracted our attention and its constituents were investigated. In previous papers, the isolation of *ent*-labdane diterpene glucosides, microtroipiosides A-F [3,4], from the leaves of *M. japonica*, and the 2-ethyl-2,3-dihydroxybutyrate of nine aliphatic glucosides, microtropins A-I [4], from its branches has already been reported. Further extensive isolation work on a MeOH extract of the branches of *M. japonica* resulted in the isolation of seven new aromatic glucoside (2*S*,3*R*)-2-ethyl-2,3-dihydroxybutyrates (**1-7**), named microtropins J-P, along with two known compounds, vanillic (**8**) [5] and salicylic (**9**) acids (**Figure 1**). Isolation of 2-ethyl-2,3-dihydroxybutyrate may be interesting in a chemotaxonomic point of view.

2. Results and Discussion

Seven new 2-ethyl-2,3-dihydroxybutyrates of various phenolic glucosides, named microtropins J-P (**1-7**), were isolated from the 1-BuOH-soluble fraction of a MeOH extract of the branches of *M. japonica* by a combination of various separation procedures. Their structures were elucidated from spectroscopic evidence. Microtropin J (**1**), $[\alpha]_D^{24}$ −59.8, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{26}O_{11}$ by high-resolution (HR)-electrospray ionization (ESI) mass spectrometry (MS). The IR spectrum exhibited absorption bands assignable to hydroxy (3380 cm^{-1}), ester carbonyl (1730 cm^{-1}), carboxylic acid (1705 cm^{-1}), aromatic ring (1606, and 1511 cm^{-1}), phenolic alcohol (1239 cm^{-1}), and aliphatic alcohols (1073 cm^{-1}). The UV absorption band at 245 nm also supported the presence of an aromatic ring. In the ^{13}C-NMR spectra, six signals assignable to 2-ethyl-2,3-dihydroxybutyrate were observed together with ones assignable to glucose, which had a substituent at the 6-position. The 1H-NMR spectrum of the aglycone moiety comprised seven signals, which included one for a *para*-substituted aromatic ring with a carboxyl functional group. Acid hydrolysis liberated D-glucose, which was identified by HPLC analysis with a chiral detector. In the heteronuclear multiple bond correlation spectrum (HMBC), the anomeric proton (δ_H 5.03) showed a correlation peak with C-4 (δ_C 162.7), and H-6'

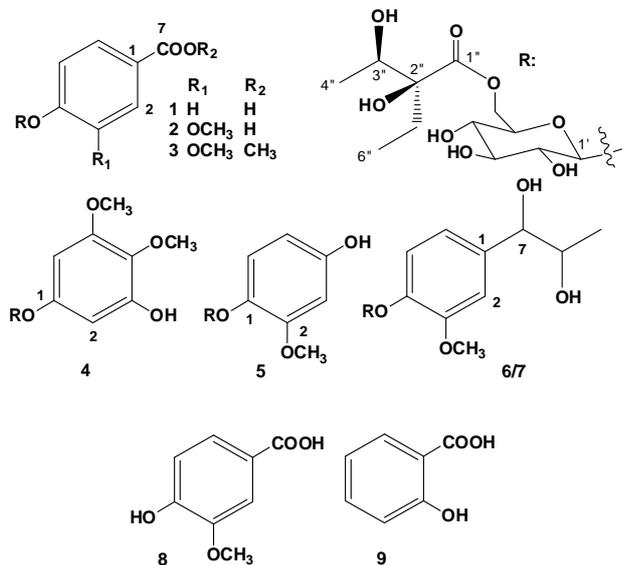**Figure 1. Structures of compounds isolated.**

(δ_H 4.21 and 4.65) with the carbonyl signal at δ_C 176.2. Therefore, the structure of microtropin J (**1**) was elucidated to be *p*-hydroxybenzoic acid *O*-*β*-D-glucopyranoside 6'-*O*-2"-ethyl-2",3"-dihydroxybutyrate, as shown in **Figure 1**. The absolute configuration of the acyl moiety was expected to be the same (2"*S*,3"*R*) as that of microtropin A [4].

Microtropin K (**2**), $[\alpha]_D^{24}$ –50.6, was isolated as an amorphous powder and its elemental composition was determined to be $C_{20}H_{28}O_{12}$ by HR-ESI-MS. The IR and UV spectra showed similar absorption bands to those of **1**, and in the NMR spectra, a methoxy signal [δ_H 3.90 (3H, s) on δ_C 56.8] was observed. The AA'BB' type coupled four protons observed in **1** were replaced by three aromatic protons coupled in an ABX system. In the HMBC spectrum, one of the aromatic protons [H-2, δ_H 7.625 (s)] showed correlation peaks with C-1 (δ_C 126.4), C-4 (δ_C 151.8), and C-7 (δ_C 169.5), and the anomeric proton (δ_H 5.03) with C-4. From the above evidence, the structure of aglycone was determined to be vanillic acid and the overall structure is shown as **2** in **Figure 1**. Since microtropin K (**2**) was isolated in a good quantity, it was hydrolyzed under a mild alkaline condition to give vanllic acid *β*-D-glucopyranoside (**2a**) [5] and methyl 2-ethyl-(2*S*,3*R*)-dihydroxybutyrate (**2b**) [4].

Microtropin L (**3**), $[\alpha]_D^{23}$ –31.6, was isolated an amorphous powder and its elemental composition was determined to be $C_{21}H_{30}O_{12}$ by HR-ESI-MS. Spectroscopic data were almost superimposable on those of **2**, except for the presence of an ester methoxy group (δ_H 3.89 on δ_C 52.6), and the molecular weight was 14 mass units larger than that of **2**, which accounted for a labile hydrogen atom being replaced by a methyl group. Therefore, the structure of **3** was elucidated to be ester of **2**, and it may

be an artifact produced during the extraction and isolation procedures.

Microtropin M (**4**), $[\alpha]_D^{25}$ –96.7, was isolated as an amorphous powder and its elemental composition was determined to be $C_{20}H_{30}O_{12}$ by HR-ESI-MS. The IR spectrum exhibited a strong absorption band for a carbonyl functional group and two methoxy signals were observed in the NMR spectra (δ_H 3.73 on δ_C 61.1 and 3.80 on δ_C 56.5). Judging from the NMR data, the aromatic ring had two *meta*-coupled protons [δ_H 6.27 (d, *J* = 2.7 Hz) and 6.32 (d, *J* = 2.7 Hz)] and thus was suggested to have unsymmetrically substituted benzene, that is, four electro-negative functional groups are substituted at the 1, 3, 4 and 5 positions. The relatively deshielded methoxy signal (δ_C 61.1) was implied that it was located between the substituents. The HMBC correlations from H-1' (δ_H 4.77) to δ_C 155.6 (C-1), those from H-2 and H-6 to δ_C 133.6, to which the methoxy signal at δ_H 3.73 on δ_C 61.1 also correlated, and those from H-2 and H-6 to C-6 and C-2 respectively were observed (**Figure 2**). Therefore, the structure of aglycone was expected to be 4,5-dimethoxybenzene-1,3-diol 1-*O*-*β*-D-glucopyranoside, which was also supported by the NOESY spectrum in which the anomeric proton showed significant correlation cross peaks with H-2 and H-6. The structure was finally elucidated to be **4** shown in **Figure 1**.

Figure 2.Microtropin N (**5**), $[\alpha]_D^{25}$ –11.5, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{28}O_{11}$. In the NMR spectra, one methoxy carbon and three protons coupled in an ABX system were observed in the aglycone region. Other NMR spectral data for the aglycone were essentially the same as those of isotachioside isolated from *Isotachis japonica* [6]. Therefore, the structure of **5** was elucidated to be isotachioside 6'-*O*-(2"*S*,3"*R*)-2"-ethyl-2",3"-dihydroxybutyrate, as shown in **Figure 1**.

Microtropin O (**6**) and microtropin P (**7**), $[\alpha]_D^{25}$ –32.1 and $[\alpha]_D^{26}$ –49.0, respectively, were isolated as amorphous powders and the elemental compositions of both compounds were $C_{22}H_{34}O_{12}$. In the ^{1}H-NMR spectrum,
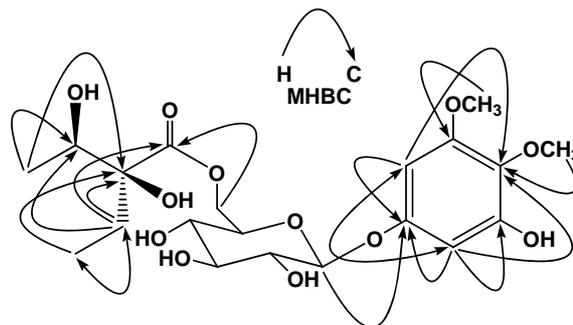

Figure 2. HMBC correlations of 4. Dual arrow curve denote HMBC correlations were observed in both directions.

one methoxy signal and three protons couple in an ABX system, one doublet methyl and two oxymethines were observed. The methyl protons were coupled with one of the oxymethine protons and the two oxymethine protons were coupled with each other. Thus, the structure of the aglycone moiety was expected to be 4-hydroxy-2-methoxy or 4-hydroxy-3-methoxyphenylpropane-7,8-diol. In the HMBC spectrum, H-2 and 6 ($δ_H$ 7.03 and 6.87, respectively) showed correlation peaks with C-7 ($δ_C$ 79.9) as well as C-4, to which also the anomeric proton ($δ_H$ 4.87) was correlated. The key correlation from H-5 ($δ_H$ 7.10) to C-3 ($δ_C$ 151.0) established the structure of the aglycone to be 4-hydroxy-3-methoxy-phenylpropane-7,8-diol. Therefore, the structure of **6** was elucidated to be as shown in **Figure 1**. From spectroscopic data, microtropin P (**7**) was expected to be a similar compound to **6**. Although the ^{13}C-NMR data of both compounds were almost superimposable (**Table 1**), they were separated in the same HPLC runs and exhibited significantly different retention times. The stereochemistry of both side chains has not fully been clarified yet, but, judging from the

fairly large coupling constants of H-7 and H-8 (6.7 Hz for **6** and 6.9 Hz for **7**) in the ^{1}H-NMR, their aglycones are in a *threo*-form, accordingly the stereochemistry of the diol-part must be enantiomers, each other [7].

3. Material and Method

3.1. Plant Material

Branches of *M. japonica* Hallier f. (Celastraceae) were collected in Kunigami Village, Kunigami County, Okinawa, Japan, in July 1997, and a voucher specimen was deposited in the Herbarium of Pharmaceutical Sciences, Graduate School of Biomedical Sciences, Hiroshima University (97-MJ-Okinawa-0716).

www.ramble-among-flora-of-miyazaki.com/sub73-46.html

3.2. General Experimental Procedures

Optical rotations were measured on a JASCO P-1030 digital polarimeter. IR and UV spectra were measured on Horiba FT-710 and JASCO V-520 UV/Vis spectrophotometers, respectively. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL JNM *α*-400 at 400 MHz and 100 MHz, respectively, with tetramethylsilane as an internal standard. Positive-ion HR-ESI-MS was performed with an Applied Biosystems QSTAR XL NanoSpray™ System.

A highly-porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel column chromatography (CC) was performed on silica gel 60 (E. Merck, Darmstadt, Germany), and ODS open CC on Cosmosil 75C$_{18}$-OPN (Nacalai Tesque, Kyoto) [($Φ$ = 5 cm, L = 25 cm, H$_2$O-MeOH (9:1) (2 L) → H$_2$O-MeOH (1:9) (2 L), linear gradient, 10-g fractions being collected]. The droplet counter-current chromatograph (DCCC) (Tokyo Rikakikai, Tokyo, Japan) was equipped with 500 glass columns ($Φ$ = 2 mm, L = 40 cm), the lower and upper layers of a solvent mixture of CHCl$_3$-MeOH-H$_2$O-*n*-PrOH (9:12:8:2) being used as the

Table 1. ^{13}C NMR spectroscopic data for micotropins J-P (1–7) (CD$_3$OD, 100 MHz).

C	1	2	3	4	5	6	7
1	125.9	126.4	125.8	155.6	140.7	138.7	138.7
2	132.7	114.7	114.5	99.1	152.5	112.7	112.8
3	117.4	150.7	150.8	151.9	102.0	151.0	150.9
4	162.7	151.8	152.0	133.6	155.2	147.3	147.4
5	117.4	117.0	117.1	154.8	107.6	118.3	118.2
6	132.7	124.7	124.4	95.8	121.4	120.9	120.9
7	169.6	169.5	168.3			79.9	79.9
8						72.9	72.9
9						19.4	19.3
2-OMe					56.6		
3-OMe		56.8	56.9			56.8	56.8
4-OMe				61.1			
5-OMe				56.5			
-COOMe			52.6				
1'	101.6	104.9	101.9	103.0	104.3	104.0	103.0
2'	74.8	75.2	74.8	74.9	75.1	75.0	75.0
3'	77.8	77.8	77.7	77.8	77.7	77.7	77.7
4'	71.5	71.3	71.5	71.6	71.6	71.5	71.5
5'	75.6	75.2	75.6	75.5	75.5	75.6	75.6
6'	65.4	65.1	65.3	65.4	65.3	65.4	65.4
1"	176.2	176.1	176.2	176.2	176.1	176.2	176.2
2"	82.9	82.9	82.9	83.0	82.9	82.9	82.9
3"	72.9	72.8	72.8	72.8	72.8	72.8	72.8
4"	16.9	16.8	16.8	16.8	16.8	16.9	16.9
5"	29.3	29.2	29.3	29.3	29.2	29.3	29.3
6"	8.4	8.4	8.4	8.3	8.3	8.4	8.4

stationary and mobile phases, respectively. Five-gram fractions were collected and numbered according to their order of elution with the mobile phase. HPLC was performed on an ODS column (Inertsil; ODS-3, GL Science, Tokyo, Japan; $\Phi = 6$ mm, $L = 250$ mm, 1.6 mL/min), and the eluate was monitored with a UV detector at 254 nm, and a refractive index monitor.

3.3. Extraction and Isolation

Air-dried branches of *M. japonica* (13.0 kg) were extracted three times with MeOH (30 L × 3) at room temperature for one week and then concentrated to 3 L *in vacuo*. The concentrated extract was washed with *n*-hexane (3 L, 53.8 g), and then the MeOH layer was concentrated to a gummy mass. The latter was suspended in water (3 L) and then extracted with EtOAc (3 L) to give 103 g of an EtOAc-soluble fraction. The aqueous layer was extracted with 1-BuOH (3 L) to give a 1-BuOH-soluble fraction (40.9 g), and the remaining water-layer was concentrated to furnish 107 g of a water-soluble fraction. The 1-BuOH-soluble fraction (39.9 g) was subjected to Diaion HP-20 CC ($\Phi = 50$ mm, $L = 40$ cm), using H$_2$O-MeOH (4:1, 2 L), (3:2, 2 L), (2:3, 2 L), and (1:4, 2 L), and MeOH (2 L), 500 mL-fractions being collected. The residue (7.53 g out of 8.73 g) in fractions 7 - 10 was subjected to silica gel (250 g) CC with increasing amounts of MeOH in CHCl$_3$ [CHCl$_3$ (3 L), and CHCl$_3$-MeOH (49:1, 1.5 L), (24:1, 1.5 L), (23:2, 1.5 L), (9:1, 1.5 L), (17:3, 1.5 L), (4:1, 1.5 L), (3:1, 1.5 L), and (7:3, 1.5 L)], CHCl$_3$-MeOH-H$_2$O (35:15:2, 1.5 L), and MeOH (1.5 L), 300 mL-fractions being collected. The residue (0.962 g) in fractions 23 - 29 was separated by ODS open CC and the residue (108 mg) in fractions 81 - 87 was applied to DCCC. The residue (4.03 mg) in fractions 51 - 61 was purified by HPLC (H$_2$O-MeOH, 3:1) to give 2.4 mg of **5** from the peak at 21 min. The residue (64.0 mg) in fractions 99 - 105 obtained on ODS open CC was subjected to DCCC and the residue (6.7 mg) in fractions 84 - 93 was purified by HPLC (H$_2$O-MeOH, 13:7) to give 2.8 mg of **4** from the peak at 14 min. The residue (2.91 g out of 3.10 g) in fractions 30 - 38 obtained on silica gel CC was purified by ODS open CC to give three fractions. The residue (306 mg) in the second lot of fractions, 81 - 93, was separated by DCCC and the residue (45.5 mg) in fractions 33 - 35 was then purified by HPLC (H$_2$O-MeOH, 33:7) to give 3.96 mg of **7** and 10.1 mg of **6** from the peaks at 46 min and 50 min, respectively. The residue (1.67 g) in fractions 94 - 124 obtained on ODS open CC was subjected to DCCC to give 1.21 g of **2** in fractions 63 - 86, 21.6 mg of 8 in fractions 142 - 164, and 10.1 mg of **3** in fractions 165 - 183.

The residue (1.17 g out of 1.27 g) in fractions 39 - 47 obtained on silica gel CC was separated by ODS open

CC and the residue (351 mg) in fractions 121 - 144 was applied to DCCC. The residue (86.6 mg out of 279 mg) in fractions 30 - 46 was purified by HPLC (H$_2$O-MeOH, 4:1) to yield 14.2 mg of **1** and 21.0 mg of **2** from the peaks at 50 min and 65 min, respectively.

The residue (5.68 g out of 6.22 g) in fractions 11 - 14 obtained on Diaion HP-20 CC was subjected to silica gel CC (180 g) with increasing amounts of MeOH in CHCl$_3$ [CHCl$_3$ (2 L), and CHCl$_3$-MeOH (49:1, 1 L), (24:1, 1 L), (23:2, 1 L), (9:1, 1 L), (17:3, 1 L), (4:1, 1 L), (3:1, 1 L), and (7:3, 1 L)], CHCl$_3$-MeOH-H$_2$O (35:15:2, 1 L), and MeOH (1 L), 200 mL-fractions being collected. The residue (1.30 g out of 1.41 g) in fractions 27 - 34 was separated by ODS open CC to give 13.6 mg of **9** in fractions 10 - 22.

3.4. Microtropin J (1)

Amorphous powder, $[\alpha]_D^{24}$ −59.8 (*c* 0.92, MeOH); IR ν_{max} (film): 3380, 2934, 1730, 1705, 1606, 1511, 1454, 1239, 1073, 1016 cm^{-1}; UV λ_{max} (MeOH): 245 (4.10), 209 (3.90) nm (log ε); ^{1}H-NMR (CD$_3$OD, 400 MHz) δ: 7.98 (2H, d, $J = 8.7$ Hz, H-2 and 6), 7.14 (2H, d, $J = 8.7$ Hz, H-3 and 5), 5.03 (1H, d, $J = 7.7$ Hz, H-1'), 4.65 (1H, dd, $J = 11.9, 2.1$ Hz, H-6'a), 4.21 (1H, dd, $J = 11.9, 6.3$ Hz, H-6'b), 3.89 (1H, q, $J = 6.4$ Hz, H-3"), 3.72 (1H, ddd, $J = 9.5, 6.3, 2.1$ Hz, H-5'), 3.51 - 3.40 (3H, m, H-2', 3' and 4'), 1.73 (1H, dq, $J = 14.8, 7.4$ Hz, H-5"a), 1.55 (1H, dq, $J = 14.8, 7.4$ Hz, H-5"b), 1.15 (3H, d, $J = 6.4$ Hz, H$_3$-4"), 0.84 (3H, dd, $J = 7.4, 7.4$ Hz, H$_3$-6"); ^{13}C-NMR (CD$_3$OD, 100 MHz): **Table 1**; HR-ESI-MS (positive-ion mode) *m/z*: 453.1367 [M+Na]$^+$ (calcd for C$_{19}$H$_{26}$O$_{11}$Na, 453.1367).

3.5. Microtropin K (2)

Amorphous powder, $[\alpha]_D^{24}$ −50.6 (*c* 1.20, MeOH); IR ν_{max} (KBr): 3410, 2978, 1735, 1706, 1602, 1513, 1462, 1270, 1073, 1022; UV λ_{max} (MeOH): 285 (3.58), 250 (4.00), 214 (4.15) nm (log ε); ^{1}H-NMR (CD$_3$OD, 400 MHz) δ: 7.626 (1H, d, $J = 8.8$ Hz, H-5), 7.625 (1H, s, H-2), 7.19 (1H, d, $J = 8.8$ Hz, H-6), 5.03 (1H, d, $J = 7.3$ Hz, H-1'), 4.64 (1H, dd, $J = 11.9, 2.1$ H-6'a), 4.19 (1H, dd, $J = 11.9, 6.4$ Hz, H-6'b), 3.90 (3H, s, -OCH$_3$), 3.87 (1H, q, $J = 6.4$ Hz, H-3"), 3.69 (1H, ddd, $J = 9.7, 6.4, 2.1$ Hz, H-5'), 3.57 - 3.42 (3H, m, H-2', 3' and 4'), 1.70 (1H, dq, $J = 14.6, 7.4$ Hz, H-5"a), 1.53 (1H, dq, $J = 14.6, 7.4$ Hz, H-5"b), 1.14 (3H, d, $J = 6.4$ Hz, H$_3$-4"), 0.82 (3H, dd, $J = 7.4, 7.4$ Hz, H$_3$-6"); ^{13}C-NMR (CD$_3$OD, 100 MHz): **Table 1**; HR-ESI-MS (positive-ion mode) *m/z*: 483.1474 [M+Na]$^+$ (calcd for C$_{20}$H$_{28}$O$_{12}$Na, 483.1472).

3.6. Microtropin L (3)

Amorphous powder, $[\alpha]_D^{23}$ −31.6 (*c* 0.67, MeOH); IR

ν_{max} (film): 3392, 2938, 1733, 1716, 1512, 1458, 1274, 1115, 1073, 1028 cm^{-1}; UV λ_{max} (MeOH): 335 (2.75), 287 (3.60), 248 (3.80), 220 (3.84) nm (log ε); ^{1}H-NMR (CD$_3$OD, 400 MHz) δ: 7.62 (1H, d, *J* = 8.6 Hz, H-6), 7.61 (1H, s, H-2), 7.20 (1H, d, *J* = 8.6 Hz, H-5), 5.03 (1H, d, *J* = 7.3 Hz, H-1'), 4.62 (1H, dd, *J* = 11.9, 2.2 Hz, H-6'a), 4.19 (1H, dd, *J* = 11.9, 5.2 Hz, H-6'b), 3.90 (3H, s, -OCH$_3$), 3.89 (3H, s, -COOCH$_3$), 3.87 (1H, m, H-3"), 3.69 (1H, ddd, *J* = 9.5, 5.2, 2.2 Hz, H-5'), 3.56 - 3.22 (3H, m, H-2', 3' and 4'), 1.71 (1H, dq, *J* = 13.7, 7.4 Hz, H-5"a), 1.53 (1H, dq, *J* = 13.7, 7.4 Hz, H-5"b), 1.13 (3H, d, *J* = 6.6 Hz, H$_3$-4"), 0.82 (3H, dd, *J* = 7.4, 7.4 Hz, H$_3$-6"); ^{13}C-NMR (CD$_3$OD, 100 MHz): **Table 1**; HR-ESI-MS (positive-ion mode) *m/z*: 497.1630 [M+Na]$^+$ (calcd for C$_{21}$H$_{30}$O$_{12}$Na, 497.1630).

3.7. Microtropin M (4)

Amorphous powder; $[\alpha]_D^{25}$ –96.7 (*c* 0.18, MeOH); IR ν_{max} (film): 3393, 2939, 1736, 1508, 1457, 1231, 1170, 1102, 1075, 1017 cm^{-1}; UV λ_{max} (MeOH): 275 (3.18), 216 (3.79) nm (log ε); ^{1}H-NMR (CD$_3$OD, 400 MHz) δ: 6.32 (1H, d, *J* = 2.7 Hz, H-2), 6.27 (1H, d, *J* = 2.7 Hz, H-6), 4.77 (1H, d, *J* = 7.5 Hz, H-1'), 4.64 (1H, dd, *J* = 11.9, 2.2 Hz, H-6'a), 4.21 (1H, dd, *J* = 11.9, 6.2 Hz, H-6'b), 3.95 (1H, q, *J* = 6.4 Hz, H-3"), 3.80 (3H, s, 5-OCH$_3$), 3.73 (3H, s, 4-OCH$_3$), 3.62 (1H, ddd, *J* = 9.5, 6.2, 2.2 Hz, H-5'), 3.48 - 3.36 (3H, m, H-2', 3' and 4'), 1.75 (1H, dq, *J* = 13.9, 7.4 Hz, H-5"a), 1.52 (1H, dq, *J* = 13.9, 7.4 Hz, H-5"b), 1.16 (3H, d, *J* = 6.4 Hz, H$_3$-4"), 0.84 (3H, dd, *J* = 7.4, 7.4 Hz, H$_3$-6"); ^{13}C-NMR (CD$_3$OD, 100 MHz): **Table 1**; HR-ESI-MS (positive-ion mode) *m/z*: 485.1633 [M+Na]$^+$ (calcd for C$_{20}$H$_{30}$O$_{12}$Na, 485.1629).

3.8. Microtropin N (5)

Amorphous powder; $[\alpha]_D^{25}$ –11.5 (*c* 0.16, MeOH); IR ν_{max} (film): 3362, 2938, 1736, 1512, 1457, 1233, 1213, 1167, 1072, 1025 cm^{-1}; UV λ_{max} (MeOH): 279 (3.30), 213 (3.71) nm (log ε); ^{1}H-NMR (CD$_3$OD, 400 MHz) δ: 6.96 (1H, d, *J* = 8.6 Hz, H-6), 6.46 (1H, d, *J* = 2.7 Hz, H-3), 6.28 (1H, dd, *J* = 8.6, 2.7 Hz, H-5), 4.67 (1H, d, *J* = 7.7 Hz, H-1'), 4.63 (1H, dd, *J* = 11.8, 2.2 Hz, H-6'a), 4.15 (1H, dd, *J* = 11.8, 6.2 Hz, H-6'b), 3.88 (1H, q, *J* = 6.5 Hz, H-3"), 3.80 (3H, s, -OCH$_3$), 3.50 (1H, ddd, *J* = 9.5, 6.2, 2.2 Hz, H-5'), 3.44 - 3.31 (3H, m, H-2', 3' and 4'), 1.66 (1H, dq, *J* = 13.9, 7.4 Hz, H-5"a), 1.50 (1H, dq, *J* = 13.9, 7.4 Hz, H-5"b), 1.15 (3H, d, *J* = 6.5 Hz, H$_3$-4"), 0.80 (3H, dd, *J* = 7.4, 7.4 Hz, H$_3$-6"); ^{13}C-NMR (CD$_3$OD, 100 MHz): **Table 1**; HR-ESI-MS (positive-ion mode) *m/z*: 455.1524 [M+Na]$^+$ (calcd for C$_{19}$H$_{28}$O$_{11}$Na, 455.1523).

3.9. Microtropin O (6)

Amorphous powder; $[\alpha]_D^{25}$ –32.1 (*c* 0.34, MeOH); IR ν_{max} (film): 3366, 2932, 1736, 1512, 1457, 1265, 1241, 1167, 1073, 1038 cm^{-1}; UV λ_{max} (MeOH): 271 (3.35), 224 (3.82) nm (log ε); ^{1}H-NMR (CD$_3$OD, 400 MHz) δ: 7.10 (1H, dd, *J* = 8.3, 0.4 Hz, H-5), 7.03 (1H, d, *J* = 1.9 Hz, H-2), 6.87 (1H, ddd, *J* = 8.3, 1.9, 0.4 Hz, H-6), 4.87 (1H, d, *J* = 7.5 Hz, H-1'), 4.63 (1H, dd, *J* = 11.9, 2.1 Hz, H-6'a), 4.31 (1H, d, *J* = 6.7 Hz, H-7), 4.18 (1H, dd, *J* = 11.9, 5.6 Hz, H-6'b), 3.89 (1H, q, *J* = 6.4 Hz, H-3"), 3.87 (3H, s, -OCH$_3$), 3.79 (1H, dq, *J* = 6.7, 6.4 Hz, H-8), 3.61 (1H, ddd, *J* = 9.5, 5.6, 2.1 Hz, H-5'), 3.50 - 3.38 (3H, m, H-2', 3' and 4'), 1.71 (1H, dq, *J* = 14.9, 7.4 Hz, H-5"a), 1.53 (1H, dq, *J* = 14.9, 7.4 Hz, H-5"b), 1.15 (3H, d, *J* = 6.4 Hz, H$_3$-4"), 0.98 (3H, d, *J* = 6.4 Hz, H$_3$-9), 0.83 (3H, dd, *J* = 7.4, 7.4 Hz, H-6"); ^{13}C-NMR (CD$_3$OD, 100 MHz): **Table 1**; HR-ESI-MS (positive-ion mode) *m/z*: 513.1947 [M+Na]$^+$ (calcd for C$_{22}$H$_{34}$O$_{12}$Na, 513.1942).

3.10. Microtropin P (7)

Amorphous powder; $[\alpha]_D^{26}$ –49.0 (*c* 0.13, MeOH); IR ν_{max} (film): 3362, 2933, 1736, 1512, 1457, 1266, 1231, 1165, 1073, 1025 cm^{-1}; UV λ_{max} (MeOH): 273 (3.44), 223 (3.94) nm (log ε); ^{1}H-NMR (CD$_3$OD, 400 MHz) δ: 7.10 (1H, d, *J* = 8.4 Hz, H-5), 7.03 (1H, d, *J* = 1.9 Hz, H-2), 6.87 (1H, dd, *J* = 8.4, 1.9 Hz, H-6), 4.87 (1H, d, *J* = 7.5 Hz, H-1'), 4.62 (1H, dd, *J* = 11.8, 2.2 Hz, H-6'a), 4.31 (1H, d, *J* = 6.9 Hz, H-7), 4.18 (1H, dd, *J* = 11.8, 6.1 Hz, H-6'b), 3.90 (1H, q, *J* = 6.4 Hz, H-3"), 3.87 (3H, s, -OCH$_3$), 3.79 (1H, dq, *J* = 6.9, 6.4 Hz, H-8), 3.61 (1H, ddd, *J* = 9.5, 6.1, 2.2 Hz, H-5'), 3.52 - 3.30 (3H, m, H-2', 3' and 4'), 1.70 (1H, dq, *J* = 14.9, 7.5 Hz, H-5"a), 1.54 (1H, dq, *J* = 14.9, 7.5 Hz, H-5"b), 1.15 (3H, d, *J* = 6.4 Hz, H$_3$-4"), 0.98 (3H, d, *J* = 6.4 Hz, H$_3$-9), 0.83 (3H, dd, *J* = 7.5, 7.5 Hz, H-6"); ^{13}C-NMR (CD$_3$OD, 100 MHz): **Table 1**; HR-ESI-MS (positive-ion mode) *m/z*: 513.1944 [M+Na]$^+$ (calcd for C$_{22}$H$_{34}$O$_{12}$Na, 513.1942).

3.11. Sugar Analysis

About 500 μg of each of microtropins J-P (**1-7**) was hydrolyzed with 1M HCl (0.2 mL) at 90°C for 2 h. The reaction mixtures were partitioned with an equal amount of EtOAc (0.2 mL), and then the water layers were analyzed with a chiral detector (JASCO OR-2090 *plus*) on an amino column [Asahipak NH$_2$P-50 4E, CH$_3$CN-H$_2$O (3:1), 1 mL/min]. The hydrolyzates gave a peak for D-glucose at 8.8 min with each positive optical rotation sign. The peaks were identified by co-chromatography with authentic samples.

3.12. Mild Alkaline Hydrolysis of 2

Microtropin K (**2**) (19.9 mg) in 450 μL of MeOH was

hydrolyzed with 50 µL of 1M CH_3ONa at 25°C for 34 hrs. The reaction mixture was neutralized with Amberlite IR-120B (H^+) and filtered. The filtrate was evaporated and the residue was purified by HPLC (H_2O-MeOH, 17:3) to give 14.1 mg of **2a** from the peak at 3 min and 2.0 mg of **2b** from the peak at 21 min. Vanillic acid β-D-glucopyranoside (**2a**): amorphous powder; $[\alpha]_D^{22}$ −61.8 (*c* 0.85, MeOH); IR ν_{max} (film): 3334, 2937, 1603, 1559, 1413, 1383, 1261, 1216, 1073, 1026 cm^{-1}; UV λ_{max} (MeOH): 328 (3.02), 287 (3.51), 246 (3.85) nm (log ε); ^{1}H-NMR (CD_3OD, 400 MHz) δ: 7.65 (1H, d, *J* = 1.9 Hz, H-2), 7.57 (1H, dd, *J* = 8.4, 1.9 Hz, H-6), 7.13 (1H, d, *J* = 8.4 Hz, H-5), 4.96 (1H, d, *J* = 7.5 Hz, H-1'), 3.89 (3H, s, -OCH_3), 3.87 (1H, dd, *J* = 11.2, 2.0 Hz, H-6'a), 3.70 (1H, dd, *J* = 11.2, 4.9 Hz, H-6'b), 3.34 - 3.54 (4H, m, H-2', 3', 4' and 5'), ^{13}C-NMR (CD_3OD, 100 MHz): 173.0 (C-7), 150.7 (C-4), 150.1 (C-3), 130.9 (C-1), 124.2 (C-6), 116.5 (C-5), 114.7 (C-2), 102.4 (C-1'), 78.3 (C-5'), 77.9 (C-3'), 74.9 (C-2'), 71.4 (C-4'), 62.5 (C-6'), 56.7 (-OCH_3); HR-ESI-MS (positive-ion mode) *m/z*: 353.0848 [M+Na]$^+$ (calcd for $C_{14}H_{18}O_9Na$, 353.0843). Methyl 2-ethyl-(2*S*,3*R*)-2,3-dihydroxybutyrate (**2b**): colorless liquid, $[\alpha]_D^{22}$ −3.2 (*c* 0.09, MeOH); IR ν_{max} (film): 3411, 2978, 2940, 1735, 1558, 1511, 1457, 1381, 1172, 1078 cm^{-1}; ^{1}H-NMR (CD_3OD, 400 MHz) δ: 3.88 (1H, q, *J* = 6.5 Hz, H-3), 3.75 (3H, s, -OCH_3), 1.70 (1H, dq, *J* = 13.8, 7.4 Hz, H-5a), 1.53 (1H, dq, *J* = 13.8, 7.4 Hz, H-5b), 1.16 ((3H, d, *J* = 6.5 Hz, H$_3$-4), 0.84 (3H, dd, *J* = 7.4, 7.4 Hz, H$_3$-6); ^{13}C-NMR (CD_3OD, 100 MHz): 176.9 (C-1), 82.9 (C-2), 72.8 (C-3), 52.7 (-OCH_3), 29.3 (C-5), 16.9 (C-4), 8.3 (C-6); HR-ESI-MS (positive-ion mode) *m/z*: 185.0786 [M+Na]$^+$ (calcd for $C_7H_{14}O_4Na$, 185.0784).

4. Acknowledgements

REFERENCES

[1] S. M. Kapchan, Y. Komoda, W. A. Court, G. J. Thomas, R. M. Smith, A. Karim, C. J. Gilmore, R. C. Haltiwagner and B. F. Bryan, "Maytansine, a Novel Antileukemic Ansa Macrolide from *Maytenus ovate*," *Journal of American Chemical Society*, Vol. 94, No. 4, 1972, pp. 1354-1956. http://dx.doi.org/10.1021/ja00759a054

[2] J. M. Cassady, K. K. Chan, H. G. Floss and E. Leistner, "Recent Developments in the Maytansinoid Antitumor Agents," *Chemical and Pharmaceutical Bulletin*, Vol. 52, No. 1, 2004, pp. 1-26. http://dx.doi.org/10.1248/cpb.52.1

[3] Y. Koyama, S. Sugimoto, K. Matsunami and H. Otsuka, "Microtropiosides A-F: *ent*-Labdane Diterpenoid Glucosides from the Leaves of *Microtropis japonica* (Celastraceae)," *Phytochemistry*, Vol. 71, No. 5-6, 2010, pp. 67-681. http://dx.doi.org/10.1016/j.phytochem.2010.01.004

[4] Y. Uemura, S. Sugimoto, K. Matsunami, H. Otsuka, Y. Takeda, M. Kawahata and K. Yamaguchi, "Microtropins A-I: 6'-*O*-(2"*S*,3"*R*)-2"-ethyl-2",3"-dihydroxy-butyrates of Aliphatic β-D-Glucopyranosides from the Branches of *Microtropis japonica*," *Phytochemistry*, Vol. 87, No. 1, 2013, pp. 140-147. http://dx.doi.org/10.1016/j.phytochem.2012.11.007

[5] A. Sakushima, M. Coşkun and T. Maoka, "Hydroxy-Benzoic Acids from *Boreava orientalis*," *Phytochemistry*, Vol. 40, No. 1, 1995, pp. 257-261. http://dx.doi.org/10.1016/0031-9422(95)00059-G

[6] Y. Asakawa, M. Toyota and L. Harrison, "Isotachin A and Isotachin B, Two Sulphur-Containing Acrylates from the Liverwort *Isotachis japonica*," *Phytochemistry*, Vol. 24, No. 7, 1985, pp. 1505-1508. http://dx.doi.org/10.1016/S0031-9422(00)81055-9

[7] B. A. A. A. Balboul, A. A. Ahmed, H. Otsuka and A. de Adams, "4-Hydroxyphenylpropan-7,8-diols and Derivatives from *Narvalina domingensis*," *Phytochemisty*, Vol. 42, No. 4, 1996, pp. 1191-1193. http://dx.doi.org/10.1016/0031-9422(96)00116-1