Regular Article

Highlighted Paper selected by Editor-in-Chief

Microtropins Q–W, *ent*-Labdane Glucosides: Microtropiosides G–I, Ursane-Type Triterpene Diglucoside and Flavonol Glycoside from the Leaves of *Microtropis japonica*

Saori Terazawa,[a] Yuka Uemura,[a] Yuka Koyama,[a] Susumu Kawakami,[b] Sachiko Sugimoto,[a] Katsuyoshi Matsunami,[a] Hideaki Otsuka,*[,a,b] Takakazu Shinzato,[c] Masatoshi Kawahata,[d] and Kentaro Yamaguchi[d]

[a] *Graduate School of Biomedical and Health Sciences, Hiroshima University; 1–2–3 Kasumi, Minami-ku, Hiroshima 734–8553, Japan:* [b] *Faculty of Pharmacy, Yasuda Women's University; 6–13–1 Yasuhigashi, Asaminami-ku, Hiroshima 731–0153, Japan:* [c] *Subtropical Field Science Center, Faculty of Agriculture, University of the Ryukyus; 1 Sembaru, Nishihara-cho, Nakagami-gun, Okinawa 903–0213, Japan: and* [d] *Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Kagawa Campus; 1314 Shido, Sanuki, Kagawa 769–2193, Japan.*

Received June 6, 2017; accepted July 31, 2017

Microtropins Q–W, (2*S*,3*R*)-2-ethyl-2,3-dihydroxybutyrate of various glucosides and glucose, as well as three *ent*-labdane diterpenoid glucosides, named microtropiosides G, H and I, an ursane-type triterpene diglucoside and a flavonoid glycoside were isolated from the MeOH extract of the leaves of *Microtropis japonica*. The structure of microtropioside A, also isolated from the branches of *M. japonica*, was elucidated spectroscopically in a previous experiment and was found to possess a rare seven-membered oxyrane ring. Its structure was confirmed by X-ray crystallographic analysis of its pentaacetate.

Key words *Microtropis japonica*; Celastraceae; microtropin; microtropioside; *ent*-labdane glucoside; flavonol glycoside

Celastraceous plants moved into the limelight, after a potent antileukemic ansa-type macrolide, maytansine, was isolated from an Ethiopian shrub, *Maytenus serrata* (formerly *M. ovatus*).[1,2] In ongoing research on subtropical resource plants, a Celestraceous plant, *Microtropis japonica*, collected in Okinawa, attracted our attention and its constituents were investigated. *Microtropis japonica* Hallier f. (Celastraceae) is an evergreen tree of about 5 m in height, and has a distinct distribution in restricted southern parts of Kanto, Kyushu, Okinawa islands and Taiwan.[3] In previous papers, we have reported the isolation of six new *ent*-labdane diterpenoid glucosides, named microtropiosides A–F, from the 1-BuOH-soluble fraction of a MeOH extract of the leaves of *Microtropis japonica*[4] and microtropins A–P from the branches of the title plant.[5,6] Further extensive isolation work on the leaf extract afforded seven esters of (2″*S*,3″*R*)-2″-ethyl-2″,3″-dihydroxybutyric acid, named microtropins Q–W (**1**–**7**) and the acid itself (**8**) (Fig. 1), three new *ent*-labdane glucosides, named microtropiosides G–I (**9**–**11**), a triterpene glucoside (**12**), and a flavonol glycoside (**13**) (Fig. 2) along with microtropin D (**14**),[5] microtropiosides A and F (**15**, **16**),[4] a flavonol glycoside, kaempferol 3-*O*-*β*-D-(2″-*O*-*β*-D-glucopyranosyl)galactopyranoside (lilyn) (**17**)[7,8] and 2-ethyl-3-methylmaleimide *N*-*β*-D-glucopyranoside (**18**).[9] This paper deals with the structural elucidation of these new compounds.

Results and Discussion

New compounds were isolated by a combination of various types of chromatography. and the structures were elucidated mainly by spectroscopic evidence. The structures of known compounds, microtropin D (**14**),[5] microtropiosides A and F (**15**, **16**),[4] lilyn (**17**)[7,8] and 2-ethyl-3-methylmaleimide *N*-*β*-D-glucopyranoside (**18**)[9] were identified by comparison of their spectroscopic data with those reported in the literature.

Microtropin Q (**1**), $[\alpha]_D^{21}$ −20.4, was isolated as an amorphous powder and its elemental composition was determined to be $C_{17}H_{27}O_{10}N$ by observation of a quasi-molecular ion peak ($[M+Na]^+$) in high-resolution (HR)-electrospray ionization (ESI)-MS. In the IR spectrum, three typical absorption bands at 3384, 2225, and 1735 cm^{-1}, were assignable to hydroxy groups, triple bond and ester functional groups, respectively. The ^{13}C-NMR spectrum exhibited five signals for an aglycone together with six signals for a 2-ethyl-2,3-dihydroxybutyrate moiety and six for 6-substituted glucopyranoside, which were seen in microtropins A–P.[5,6] The absolute structure of glucose was determined to be D by HPLC analysis of its hydrolyzate using a chiral detector, and thus the mode of sugar linkage was β from the coupling constant of the anomeric proton. The absolute configuration of 2-ethyl-2,3-dihydroxybutyrate was expected to be the same as that of microtropin A, whose structure was confirmed by X-ray crystallographic analysis.[5] Signals for the aglycone comprised one *sp* signal (δ_C 118.7), two primary alcohols and a trisubstituted double bond; these functionalities were the same as those of microtropin D (**14**),[5] but these were obviously different compound judged from the ^{13}C-NMR chemical shifts (Table 1). Since, in the phase-sensitive (PS) nuclear Overhauser effect (NOE) spectroscopy, significant correlations were observed between H-4 (δ_H 4.41 and 4.51) and H_2-5 (δ_H 4.20), the structure of microtropin Q (**1**) was elucidated to be a *Z* isomer of microtropin D (**14**), as shown in Fig. 1.

Microtropin R (**2**), $[\alpha]_D^{24}$ −90.2, was isolated as an amorphous powder and its elemental composition was determined to be $C_{18}H_{32}O_9$ by HR-ESI-MS. The NMR spectra indicated that **2** was analogous compound to **1** with a different aglycone. The aglycone moiety was revealed to comprise one trip-

* To whom correspondence should be addressed. e-mail: hotsuka@hiroshima-u.ac.jp; otsuka-h@yasuda-u.ac.jp

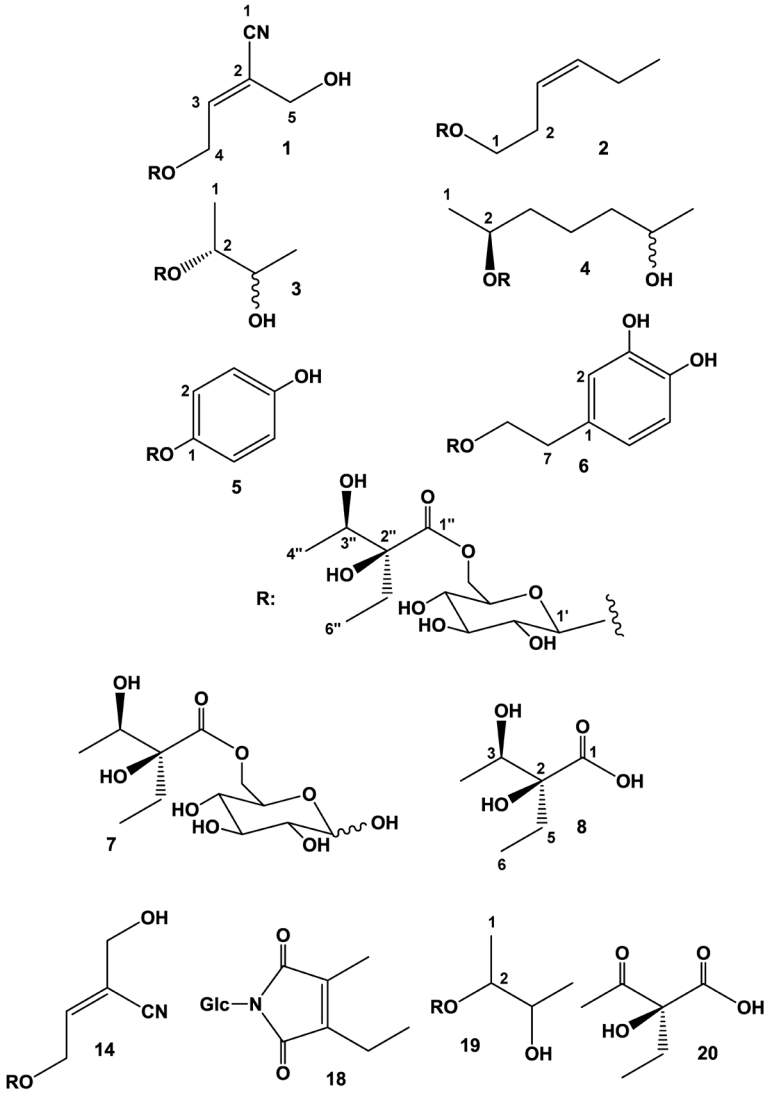

Fig. 1. The Structures of New Compounds (**1**–**8**) and Compounds Appeared in the Text (**14**, **18**–**20**)

let methyl, two methylenes, one primary alcohol and a disubstituted double bond. ^{1}H–^{1}H correlation spectroscopy (COSY) exhibited sequential proton signals from H_3-6 to H_2-1 and the geometry of the double bond was determined to be in a *Z*-form from the rather small coupling constant (J=10.9 Hz) of olefinic protons in the ^{1}H-NMR spectrum. Therefore, the structure of **2** was elucidated to be *Z*-hex-3-en-1-ol *β*-D-glucopyranoside 6′-*O*-(2″*S*,3″*R*)-2″-ethyl-2,″3″-dihydroxybutyric acid ester, as shown in Fig. 1.

Microtropin S (**3**), $[\alpha]_D^{24}$ −12.5, was isolated as an amorphous powder and its elemental composition was determined to be $C_{16}H_{30}O_{10}$ by HR-ESI-MS. From the spectroscopic data, microtropin S (**3**) was found to be an analogous compound to the aforementioned compounds with a different aglycone. The aglycone constituted only four carbons, two methyls and two secondary alcohols, namely, 2,3-butanediol. The ^{13}C-NMR chemical shifts of *meso*- and *rac*-2,3-butanediol were almost identical (C-1 and C-4: δ_C 18.6 and 18.7 for *meso*- and *rac*-2,3-butanediols, respectively, and C-2 and C-3: δ_C 72.6 and 72.7, respectively. On *β*-D-glucopyranosylation to the aglycone of the 2-position, chemical shift of C-1 methyl shifted up by 2.5 or 2.6 ppm, whereas that of C-3 was by 0.9 or 1.0 ppm.[10] Thus, the absolute configuration of the 2-position was determined to be *R*, while since the H-2 and H-3 appeared at the same chemical shift (δ_H 3.67), there was not a clue to determine the *threo*- or *erythro*-relationship. Therefore, the structure of **3** was tentatively elucidated to be 2*R*,3ξ-2,3-butandiol 2-*O*-*β*-D-glucopyranose 6′-*O*-(2″*S*,3″*R*)-2″-ethyl-2,″3″-dihydroxybutyric acid ester, as shown in Fig. 1. Microtropin G (**19**), isolated from the same plant, also possessed 2,3-butanediol as the aglycone.[5] Its ^{13}C-NMR chemical shifts of the aglycone moiety were obviously different from those of **3** (Table 1), indicating that their aglycones were stereoisomers each other, as shown in Fig. 1.

Microtropin T (**4**), $[\alpha]_D^{25}$ −12.9, was isolated as an amorphous powder and its elemental composition was determined to be $C_{19}H_{36}O_{10}$ by HR-ESI-MS. The NMR spectroscopic data indicated that **4** was also analogous compound with seven carbons as the aglycone moiety. Signals for two doublet methyls and two oxygenated methines were observed in the NMR spectra and both of the methyl signals were correlated with the oxygenated methine protons in the COSY spectrum. Thus, the aglycone was assigned to be 2,6-heptanediol 2-*O*-*β*-D-glucopyranoside and the absolute configuration of the 2-postion was deduced by an application of the *β*-D-glucopyranosylation-induced shift trend rule between 2-hexa-

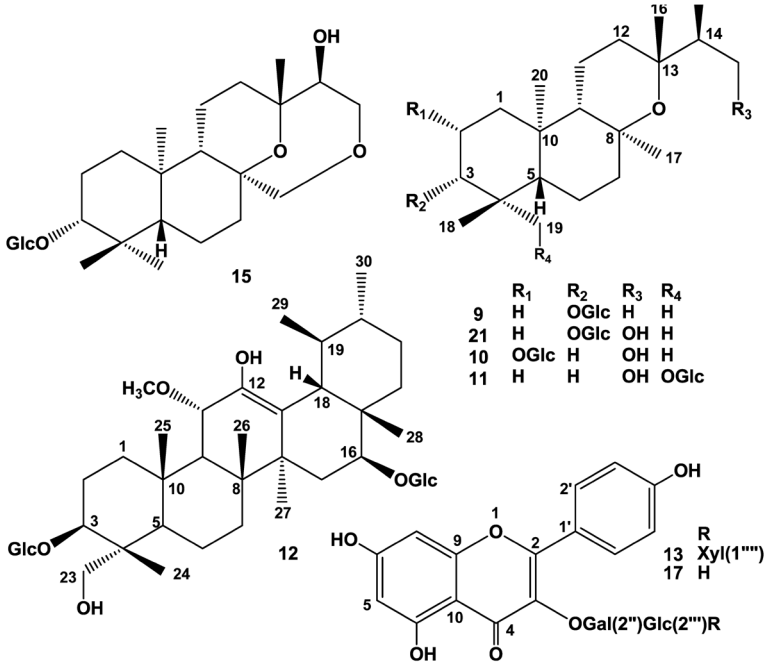

Fig. 2. Structures of Diterpenoids, Triterpenoid and Flavonol Glycosides

Table 1. ^{13}C-NMR Spectroscopic Data for Microtropins Q–W (**1**–**7**), (2″*S*,3″*R*)-2″-Ethyl-2,″3″-dihydroxybutyric Acid (**8**) and Reference Compounds, Microtropins D (**14**)[a] and G (**19**)[a] (100 MHz, CD_3OD)

C	**1**	**14**	**2**	**3**	**19**	**4**	**5**	**6**	**7**		C	**8**
1	118.7	116.7	70.6	16.2	18.5	22.2	154.1	132.1				
2	119.4	118.2	28.9	80.9	83.5	78.0	119.9	120.4				
3	146.3	144.6	134.6	71.5	72.2	37.9	116.6	146.4				
4	65.8	68.2	126.4	18.7	18.7	22.7	152.2	147.3				
5	58.6	63.2	21.6			40.2	116.6	117.2				
6			14.7			68.3	119.9	125.7				
7						23.5		39.6				
8								64.5				
									α	*β*		
1′	104.0	104.2	104.4	104.3	105.7	104.3	103.8	104.8	94.1	98.4		
2′	74.9	74.9	75.1	75.1	75.2	75.1	75.0	74.9	73.9	76.3		
3′	77.7	77.7	77.9	77.9	77.7	77.9	77.8	77.4	74.8	78.0		
4′	71.4	71.4	71.7	71.6	71.5	71.6	71.6	71.4	72.8	71.9		
5′	75.5	75.5	75.3	75.4	75.5	75.4	75.4	75.7	70.8	75.4		
6′	65.0	65.0	65.3	65.3	65.2	65.3	65.4	65.2	65.4	65.6		
1″	176.2	176.2	176.2	176.1	176.1	176.1	176.2	176.2	176.30	176.30	1	178.1
2″	82.9	82.9	82.9	82.9	82.9	82.9	82.9	83.0	82.94	82.99	2	82.2
3″	72.9	72.8	72.9	72.8	72.8	72.8	72.8	72.9	72.86	72.86	3	72.6
4″	16.9	16.9	16.9	16.8	16.8	16.8	16.8	16.9	16.86	16.88	4	17.1
5″	29.3	29.3	29.3	29.2	29.2	29.2	29.2	29.4	29.31	29.30	5	29.1
6″	8.4	8.4	8.2	8.4	8.4	8.4	8.3	8.4	8.38	8.29	6	8.3

a) Data from ref. 5.

nol and the aglycone. On *β*-D-glucopyranosylation, the methyl signal shifted up by 1.2 ppm, whereas, the methylene carbon at the 3-position shifted by 2.0 ppm, indicating that the 2-position had the *S* configuration.[10] Therefore, the structure of **4** was elucidated to be 2*S*,6*ξ*-heptanediol 2-*O*-*β*-D-glucopyranoside 6′-*O*-(2″*S*,3″*R*)-2″-ethyl-2,″3″-dihydroxybutyric acid ester, as shown in Fig. 1.

Microtropin U (**5**), $[\alpha]_D^{24}$ −26.9, was isolated as an amorphous powder and its elemental composition was determined to be $C_{18}H_{26}O_{10}$. The NMR spectra indicated that the aglycone moiety was a *para*-substituted benzene [δ_H 6.70 (2H, d, *J*=8.1 Hz, H-3 and 5) and 6.95 (2H, d, *J*=8.1 Hz, H-2 and 6)], namely *p*-hydroquinone. Therefore, the structure of **5** was elucidated to be arbutin, 6′-*O*-(2″*S*,3″*R*)-2″-ethyl-2,″3″-dihydroxybutyric acid ester, as shown in Fig. 1.

Microtropin V (**6**), $[\alpha]_D^{21}$ −68.9, was isolated as an amorphous powder and its elemental composition was $C_{20}H_{30}O_{11}$. NMR spectroscopic data indicated the presence of three aromatic protons coupled in an ABX system [6.77 (1H, d, *J*=8.2 Hz, H-5), 6.81 (1H, dd, *J*=8.2, 2.0 Hz, H-6) and 7.01

(1H, d, J=2.0 Hz, H-2)], methylene and a primary alcohol. In the heteronuclear multiple bond connectivity (HMBC), correlation of the anomeric proton (δ_H 4.74) with the primary alcohol carbon (δ_C 64.5) established the linkage position of the sugar moiety. Other HMBC correlations of H-2 and H-6 with C-7 revealed the 1,3,4-subsitution of the benzene ring and, therefore, the structure of **6** was elucidated to be 3,4-dihydroxyphenylethanol 8-*O*-*β*-D-glucopyranoside 6′-*O*-(2″*S*,3″*R*)-2″-ethyl-2,″3″-dihydroxybutyric acid ester, as shown in Fig. 1.

Microtropin W (**7**), $[\alpha]_D^{24}$ −90.2, was isolated as an amorphous powder and its elemental composition was $C_{12}H_{22}O_9$. The ^{13}C-NMR spectrum implied the presence of a 2″-ethyl-2,″3″-dihydroxybutyric acid segment; however, all the signals appeared as close dual resonances. D-Glucose was detected by the sugar analysis and two typical anomeric signals observed at δ_H 5.08 (d, J=3.8 Hz, H-1′*α*) on δ_C 94.1 and δ_H 4.48 (d, J=7.8 Hz, H-1′*β*) on δ_C 98.4 were those of *α*- and *β*-anomers, respectively. The ester linkage was expected on C-6′ from its ^{13}C-NMR signals at 65.4 (*α*) and 65.6 (*β*) and therefore its structure was elucidated to be D-glucose 6′-*O*-(2″*S*,3″*R*)-2″-ethyl-2,″3″-dihydroxybutyric acid ester. From the integrals of the anomeric signals in the ^{1}H-NMR spectrum, the ratio of *α*- and *β*-isomers was calculated to be nearly equal. The single prime mark system was used according to previous compounds for the reader's convenience.

(2*S*,3*R*)-2-Ethyl-2,3-dihydroxybutyric acid (**8**), $[\alpha]_D^{24}$ −3.5, was isolated as a colorless syrup and its elemental composition was determined to be $C_6H_{12}O_4$ by HR-ESI-MS. NMR spectra indicated that compound **8** comprised of the same carbon framework as the acid moiety of microtropins and the free acid form was isolated for the first time. Instead of a rearrangement of the ethyl group (C-5 and C-6) of (*S*)-2-hydroxy-2-ethyl-3-oxobutanoic acid (**20**) to C-3 to proceed the biosynthesis of isoleucine, the ketone functional group at C-3 was directly reduced to the secondary alcohol to form **8** and needed to be derailed from the leucine biosynthetic route.

Microtropioside G (**9**), $[\alpha]_D^{24}$ −12.6, was isolated as an amor-

Table 2. ^{13}C- and ^{1}H-NMR Spectroscopic Data for Microtropiosides G–I (**9**–**11**) and ^{13}C-NMR Spectroscopic Data for Microtropioside B (**21**) (^{13}C-NMR at 100 MHz and ^{1}H-NMR at 400 MHz with Multiplicities and Coupling Constants in Hz, CD_3OD)

	9		**10**		**11**		**21**[a)]
	C	H	C	H	C	H	
1	38.5	0.97 m 1.67 m	43.3	1.41 m 1.88 dd 13.3 4.6	40.5	1.60 ddd 13.7, 6.3, 1.6 1.65 m	38.4
2	24.2	1.31 m 1.62 m	75.4	4.17qui-like *ca.* 5	19.3	0.97 m 1.47 m	24.1
3	85.9	3.28 dd 12.0, 2.4	45.9	1.54 dd 13.6, 4.6 1.86 dd 13.6, 6.6	37.9	0.96 m 1.83 m	85.8
4	39.3	—	33.6	—	39.0	—	39.3
5	57.4	0.96 m	55.3	1.03 br d 11.8	58.8	1.07 dd 9.8, 2.1	57.5
6	20.7	1.67 2H m	21.4	1.42 m 1.68 br d 13.1	21.7	1.27 m 1.65 m	20.9
7	44.8	1.32 m 1.74 m	44.6	1.41 br d 11.9 1.76 ddd 11.9, 2.8, 2.7	45.5	1.30 m 1.74 m	45.0
8	76.7	—	76.7	—	76.8	—	76.6
9	58.5	1.30 dd 8.9, 2.8	57.9	1.40 dd 11.0, 5.7	57.2	1.38 m	56.7
10	37.9	—	38.7	—	38.3	—	38.0
11	16.2	1.48 m 2.02 dddd 12.0, 6.4, 6.4, 6.4	15.9	1.63 m 1.67 m	15.7	1.50 m 1.60 m	15.7
12	32.8	1.56 m 1.80 ddd 12.4, 6.4, 2.5	32.7	1.42 m 2.25 ddd 14.0, 5.7, 5.5	33.0	1.39 m 2.18 ddd 13.8, 6.5, 5.9	32.8
13	76.8	—	76.8	—	76.7	—	76.8
14	77.8	3.37 m	77.3	3.76 dd 8.3, 2.5	77.6	3.69 m	77.8
15	16.1	1.29 3H d 6.4	64.3	3.47 dd 11.2, 8.3 3.95 dd 11.2, 2.5	64.3	3.31 m 3.85 m	64.3
16	24.8	1.12 3H s	24.8	1.07 3H s	24.6	1.05 3H s	24.6
17	25.1	1.31 3H s	25.5	1.30 3H s	25.8	1.26 3H s	25.7
18	28.7	1.02 3H s	24.7	1.01 3H s	28.4	1.01 3H s	28.8
19	16.9	0.81 3H s	33.4	0.96 3H s	74.2	3.34 d 9.7 3.97 d 9.7	16.9
20	16.4	0.85 3H s	18.6	1.10 3H s	16.3	0.85 3H s	16.0
1′	102.0	4.32 d 7.8	102.9	4.35 d 7.8	105.0	4.14 d 7.8	102.0
2′	75.2	3.16 dd 9.2, 7.8	75.4	3.16 dd 9.0, 7.8	75.3	3.16 dd 8.8, 7.8	75.2
3′	78.3	3.37 m	78.4	3.37 dd 9.0, 9.0	78.4	3.34 m	78.3
4′	72.0	3.35 m	71.8	3.30 dd 9.5, 9.0	71.8	3.28 m	72.0
5′	77.8	3.23 ddd 8.6, 4.8, 2.6	77.9	3.28 ddd 9.5, 5.9, 2.2	77.8	3.23 m	77.8
6′	63.1	3.67 dd 11.8, 4.8 3.85 dd 11.8, 2.6	62.9	3.69 dd 11.9, 5.9 3.88 dd 11.9, 2.2	62.8	3.44 dd 11.2, 5.5 3.90 dd 11.2, 2.3	63.1

a) Data from ref. 4.

phous powder and its elemental composition was determined to be $C_{26}H_{46}O_8$ by HR-ESI-MS. The strong IR absorption band at 3360 cm^{-1} indicated that **9** was a glycosidic compound and D-glucose was detected by sugar analysis. In the ^{13}C-NMR spectrum, six signals assignable to those of β-glucopyranoside were observed and, of the remaining 20 resonances, those assignable to rings A, B and C showed indistinguishable similarity to those of microtropioside B (**21**) (Table 2), except that the primary alcohol signal (C-15) observed in **21** was replaced by a methyl group (δ_C 16.1).[4] In the ^{1}H-NMR spectrum, the primary alcohol protons also disappeared and instead of those, a methyl proton signal [δ_H 1.29 (3H)] was newly observed. The position and mode of sugar linkage were found to be the same as those of **21**. Therefore, the structure of **9** was elucidated as shown in Fig. 2.

Microtropioside H (**10**), $[\alpha]_D^{23}$ −26.7, was isolated as an amorphous powder and its elemental composition was determined to be $C_{26}H_{46}O_9$ by HR-ESI-MS. In the ^{13}C-NMR spectrum, 26 resonances were observed, of which six were assigned to those of glucopyranoside and, of the remaining 20 resonances, those assignable to ring C and the side chain (C-14 and C-15) were indistinguishable from those in **9**. The positions of the hydroxy group were assigned by HMBC and ^{1}H–^{1}H COSY experiments and a D-glucose moiety was found to be attached to the hydroxy group at the 2-position in β mode (Fig. 3). Judged from coupling constants of H-1a, H-3a and H-3b protons together with that of H-2 itself, the glucopyranoxy group must be in an axial orientation, which was confirmed by the difference NOE experiment. Upon irradiation of the anomeric proton, significant signal enhancements were observed in the H_3-19 and H_3-20 proton signals. Therefore, the structure of microtropioside H (**10**) was elucidated, as shown in Fig. 2.

Microtropioside I (**11**), $[\alpha]_D^{25}$ −30.6, was isolated as an amorphous powder and its elemental composition was determined to be $C_{26}H_{46}O_9$ by HR-ESI-MS. The ^{13}C-NMR spectrum displayed 26 resonances, of which six were also assigned to those of glucoyranoside and a slightly shielded chemical shift of the anomeric carbon (δ_C 105.0) was indicative that the glucosidic linkage was on the primary alcohol. Of the remaining signals, those assignable to rings B and C, and a side chain and a methyl group at the 13-position were essentially the same as the corresponding signals of **9**, **10** and **21** (Table 2). In the ^{1}H–^{1}H COSY spectrum, correlations of protons on three consecutive methylene carbons (H_2-1, H_2-2 and H_2-3) were observed, and the methyl protons at C-20 (δ_H 0.85) showed correlation cross peaks with one end (δ_C 40.5) of the methylene carbon and the methyl protons at C-18 (δ_H 1.01) and the primary alcohol methylene protons (δ_H 3.34 and 3.97) correlated with the methylene carbon (δ_C 37.9) at the other end in the HMBC spectrum, establishing the scaffold of ring A (Fig. 4). In a general trend, oxidation of the equatorial methyl group of the geminal ones at the 4-position, followed by transglucosyaltion, proceeded to form a glucopyranosyl group at the C-18 position (normal type). A related compound, sagittarioside a,[11] which has a glucopyranoxyl group at the C-19 position, showed a close resemblance with the ^{13}C-NMR chemical shifts of the C-5, C-18 and C-19 positions with those of **11**. Figure 5 shows the ^{13}C-NMR chemical shifts of C-5, C-18 and C-19 of labdanes (normal type) which have a primary alcohol at the axial (**a**) and equatorial (**b**) positions.[12,13] Differences in the

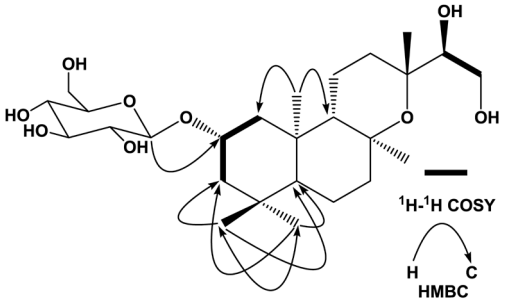

Fig. 3. Diagnostic Two Dimensional NMR Correlations of Microtropioside I (**10**)

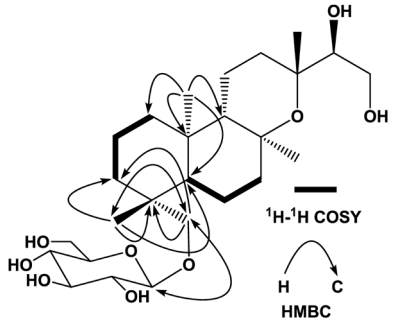

Fig. 4. Diagnostic Two Dimensional NMR Correlations of Microtropioside J (**11**)

Dual arrow curves denote HMBC correlations were observed in the both directions.

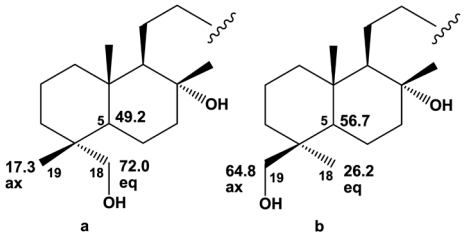

Fig. 5. NMR Spectroscopic Data of C-18 and C-19 Region

chemical shifts between **a** and **b** are able to discriminate two structures in a straightfoward manner. Therefore, the structure of **11** is shown in Fig. 2.

The stereochemistry of C-14 of compounds **9**, **10** and **11** was determined to be *S* by indistinguishable similarity of ^{13}C-NMR chemical shifts to that of **21** (Table 2). However, similar compounds which have the opposite stereochemistry to **21** were reported to have close chemical shifts to that of **21**.[14] Therefore, the stereochemistry of C-14 of compounds **9**, **10** and **11** was tentatively determined and further investigations were required to draw the unambiguous conclusion.

Compound (**12**), $[\alpha]_D^{22}$ −2.8, was isolated as amorphous powder and its elemental composition was determined to be $C_{43}H_{72}O_{15}$. The IR spectrum exhibited absorptions assignable to hydroxy groups (3362 cm^{-1}), aliphatic C–H stretching (2927 and 2874 cm^{-1}) and a double bond (1651 cm^{-1}). Of the 43 ^{13}C-NMR resonances observed, 12 were assignable to those of two terminal glucopyranosides. The remaining 31 resonances included one methoxy, seven methyls, seven methylenes, five methines and three oxygenated methines as well as six quaternary carbons along with a tetrasubstituted double bond and

a primary alcohol. These functional groups were assigned by the aid of distortionless enhancement by polarization transfer (DEPT) and two-dimensional NMR experiments. Two doublet methyls (δ_H 0.94 and 0.95), observed in the ^{1}H-NMR spectrum (Table 3), suggested that compound **12** had an ursane-type skeleton. The positions of the oxygenated carbons were mainly examined by the HMBC experiment (Fig. 6). The methylene protons of the primary alcohol showed correlation peaks with C-3, C-5 and C-24, and the position of the primary alcohol was confirmed by the PS-nuclear Overhauser effect spectroscopy (NOESY) experiment in which a correlation peak between two axial methyls, H_3-24 and H_3-25, was observed. From the HMBC correlations between H-11 and C-12, C-13 and the methoxy carbon, the methoxy group must be placed adjacent to the double bond (Fig. 6). Further HMBC correlation from H-11 to C-10, indicated that the methoxy group was at the 11-position and the double bond at C-12 and C-13. C-12, one of carbons of the tetrasubstituted double bond, must have an oxygen substituent from its highly deshielded chemical shift value (δ_C 145.8); thus, one hydroxy group was placed on C-12. This enol hydroxy hydrogen is probably stabilized by a chelate formation with the methoxy oxygen atom. HMBC correlations between H_3-28 and C-16, and H-27 and C-15 together with the ^{1}H–^{1}H COSY correlation between H-15 and H-16 placed the remaining hydroxy group at the 16-position. From fairy large ^{1}H-NMR coupling constants of H-3 (J=8.1 Hz with H-2ax), H-11 (J=9.7 Hz with H-9ax) and H-16 (J=11.2 Hz with H-15ax), the hydrogens at those positions were determined to be in an axial orientation, namely the substituents were in an equatorial orientation. The absolute configuration of the sugar was analyzed by HPLC analysis of its hydrolyzate using a chiral detector and the mode of linkage was determined to be β from the coupling constant of the anomeric protons. The position of the sugar linkage were established by the HMBC correlations shown in Fig. 6. Therefore, the structure of **12** was elucidated to be 3β,12,16β,23-tetrahydroxy-11α-methoxyurs-12-ene 3,16-di-*O*-β-D-glucopyranoside, as shown in Fig. 2. A similar compound, 3β,12,15α-trihydroxy-11α-methoxyurs-12-ene, was isolated from the stems of *Siphondon*

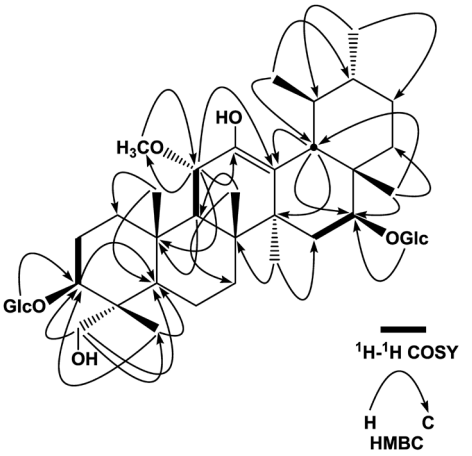

Fig. 6. Diagnostic Two Dimensional NMR Correlations of Triterpenoid Diglucoside (**12**)

Table 3. ^{13}C- and ^{1}H-NMR Spectroscopic Data for Compound **12** (150 MHz and 600 MHz, Respectively, CD_3OD)

	C	H		C	H
1	40.2	1.23 ddd 13.6, 9.6, 3.2	21	32.5	1.33 m
		2.14 ddd 13.6, 3.0, 3.0			1.44 m
2	26.6	1.77 dddd 13.6, 3.2, 3.0, 2.4	22	36.7	1.59 m
		1.94 dddd 13.6, 9.6, 8.1, 3.0			1.72 m
3	83.1	3.85 dd 8.1, 2.4	23	67.4	3.68 m
4	44.26	—			3.83 m
5	48.4	1.28 m	24	13.6	0.74 3H s
6	18.8	1.36 m	25	17.62	1.16 3H s
		1.51 br dd 12.6, 7.9	26	18.9	1.11 3H s
7	34.6	1.35 m	27	25.2	1.29 3Hs
		1.65 ddd 12.6, 10.6, 3.6	28	23.7	0.82 3H s
8	44.2	—	29	17.59	0.94 3H d 6.3
9	49.6	1.90 d 9.7	30	21.7	0.95 3H d 6.3
10	39.1	—	–OCH_3	53.5	3.22 3H s
11	78.36	4.10 d 9.7	1′	105.8	4.40 d 7.8
12	145.8	—	2′	75.7	3.16 m
13	116.9	—	3′	78.40	3.34 m
14	44.29	—	4′	71.8	3.31 m
15	36.4	1.03 dd 13.7, 3.6	5′	77.6	3.26 m
		2.36 dd 13.7, 11.2	6′	62.8	3.68 m
16	78.30	4.19 dd 11.2, 3.6			3.84 dd 11.6, 2.1
17	39.7	—	1″	106.1	4.32 d 7.8
18	50.9	2.48 d 11.5	2″	75.68	3.15 m
19	42.1	1.41 m	3″	78.29	3.33 m
20	41.0	—	4″	71.6	3.29 m
			5″	77.8	3.28 m
			6″	62.9	3.68 m
					3.82 dd 11.6, 2.2

Letters after ^{1}H-NMR data are multiplicities and coupling constants (J) in Hz.

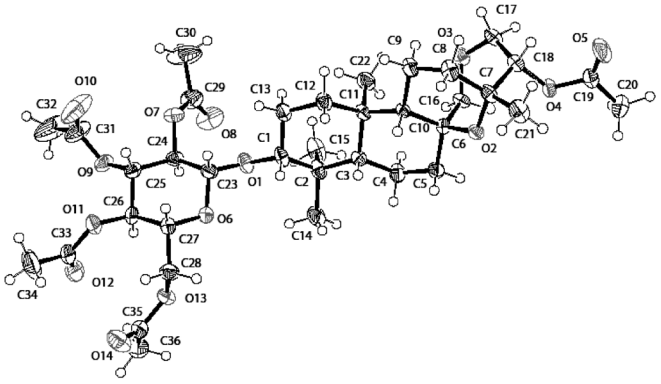

Fig. 7. An ORTEP Drawing of Microtropioside A Pentaacetate (**15a**)
The structure has crystallographic numbering.

celastrineus, which belongs to the same family (Celastraceae) as the title plant used in this experiment. The enol hydrogen was reported to appear as a singlet at δ_H 4.67 in the ^{1}H-NMR spectrum ($CDCl_3$).[15)]

Kaempferol 3-*O*-*β*-D-(2‴-*O*-*β*-D-xylopyranosyl)(2″-*O*-*β*-D-glucopyranosyl)galactopyranoside (**13**) $[\alpha]_D^{23}$ −45.7, was isolated as a pale yellow amorphous powder. Its elemental composition was determined to be $C_{32}H_{38}O_{20}$ by HR-ESI-MS. The IR spectrum showed absorption bands at 3406, 1651 and 1606 cm^{-1}, assignable to hydroxy groups, ketone functional groups and aromatic ring(s), respectively. The UV absorption bands at 265, 332 and 349 nm also indicated the presence of aromatic ring(s). In the ^{1}H-NMR spectrum, two aromatic proton signals coupled in an AA'BB' system, two *meta*-coupled aromatic protons, and three anomeric protons were observed (Table 4). The sugar analysis revealed the presence of D-xylose, D-glucose and D-galactose and the ^{13}C-NMR spectral resonances of the aromatic region and the inner most sugar showed a close resemblance to those of kaempferol 3-*O*-*β*-D-(2″-*O*-*β*-D-glucopyranosyl)galactopyranoside (lilyn) (**17**), isolated from this plant and originally from *Lilium candilum*[7)] and *Trigonella foenum-graecum*.[8)] Thus, the terminal sugar was found to be *β*-D-xylopyranose and the position of the linkage was deduced by cross peaks between H-1‴ (δ_H 4.58) and C-2‴ (δ_C 84.9) in the HMBC and H-2‴ (δ_H 3.46) on C-2‴ was correlated with the anomeric proton of *β*-D-glucopyranoside (H-1‴, δ_H 4.72) in the COSY spectrum. Therefore, the structure of **13** was elucidated to be lilyn 2‴-*O*-*β*-D-xylopyranoside, as shown in Fig. 2.

Microtropioside A (**15**) was first isolated from the branches of the title plant and also from the leaves as well in this experiment. Its structure was spectroscopically elucidated in the previous paper and its pentaacetate (**15a**) was prepared to back up the structure.[4)] After we published the above results, the pentaacetate formed a good crystal suitable for X-ray analysis; Fig. 7 shows the ORTEP drawing of the crystal structure. The absolute structure was finally confirmed using D-glucose as a chiral clue. The structure deduced by the spectroscopic method was found to be correct and the diterpene was confirmed to belong to an *enatio* series.

Experimental

General Procedure Optical rotations were measured on a JASCO P-1030 polarimeter and IR spectra on a Horiba FT-710 spectrophotometer. ^{1}H- and ^{13}C-NMR spectra were taken on a JEOL *α*-400 or Brucker Avance III 600 spectrometer at 400 or 600 MHz, and 100 or 150 MHz, respectively, with tetramethylsilane as an internal standard. Positive- and negative-ion HR-MS were taken with an Applied Biosystem QSTAR XL system ESI (Nano Spray)-MS.

A highly-porous synthetic resin (Diaion HP-20) was purchased from Mitsubishi Kagaku (Tokyo, Japan). Silica gel CC and reversed-phase [octadecylsilanized silica gel (ODS)] open CC were performed on silica gel 60 (Merck, Darmstadt, Germany) and Cosmosil 75C_{18}-OPN (Nacalai Tesque, Kyoto, Japan), [Φ=40 mm, *L*=25 cm, linear gradient: MeOH–H_2O (1 : 9, 1 L) → (9 : 1, 1 L), 5 g-fractions being collected], respectively. 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_2O–*n*-PrOH (9 : 12 : 8 : 2) being used as the 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 (5 *μ*m), 6 mm ×250 mm (GL Science, Tokyo, Japan), flow rate: 1.6 mL/min], and the eluate was monitored with a UV detector at 254 nm and a refractive index monitor. *meso*- and *rac*-Butane-2,3-diols, 2-hexanol were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan).

Plant Material Leaves of *M. japonica* Hallier f. (Celastraceae) were collected in Kunigami Village, Kumigami 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). The plant species was identified by one (T.S.) of the authors.

Extraction and Isolation Leaves of *M. japonica* (3.25 kg) were extracted three times with MeOH (4.5 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, 17.9 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 171 g of an EtOAc-soluble fraction. The aqueous layer was extracted with 1-BuOH (3 L) to give a 1-BuOH-soluble fraction (32.1 g), and the remaining water-layer was concentrated to furnish 136 g of a water-soluble fraction.

The 1-BuOH-soluble fraction (32.0 g) was subjected to Diaion HP-20 CC (Φ=60 mm, *L*=46 cm), using H_2O–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 (5.16 g) in fraction 3 was subjected to silica gel (75 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (2 L), and $CHCl_3$–MeOH (49 : 1, 2 L), (24 : 1, 2 L), (23 : 2, 2 L), (9 : 1, 1 L), (17 : 3, 1 L), (4 : 1, 1 L), (3 : 1, 1 L) and (7 : 3, 1 L)], 500-mL fractions being collected. The residue (1.09 mg) was subjected to ODS open CC to give a residue (409 mg) in fractions 19–35, which was purified by DCCC to give 77.7 mg of **8** in fractions 14–19.

The reside (3.18 g) in fractions 4–6 obtained on Diaion HP-20 CC was subjected to silica gel (75 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (1.2 L), $CHCl_3$–MeOH (49 : 1, 600 mL), (24 : 1, 600 mL), (23 : 2, 600 mL), (9 : 1, 600 mL), (17 : 3, 600 mL), (4 : 1, 600 mL), (3 : 1, 600 mL) and (7 : 3, 600 mL), and MeOH (1 L)], 100-mL fractions being collected. The residue (334 mg) in fractions 41–46 was subjected

to ODS open CC to give the residue (47.3 mg) in fractions 23–47 which was then applied to DCCC. The residue (8.57 mg) in fractions 50–54 was finally purified by HPLC [H_2O–MeOH (3 : 1)] to furnish 1.56 mg of **3** from the peak at 13 min. The residue (25.1 mg) in fractions 55–57 was purified by HPLC [H_2O–MeOH (3 : 1)] to yield 5.04 mg of **17** and 5.98 mg of **18** from the peaks at 15 min and 18 min, respectively. The residue (334 mg) in fractions 41–46 obtained on silica gel CC was subjected to ODS open CC [Φ=40 mm, *L*=25 cm, linear gradient: MeOH–H_2O (1 : 9, 2 L) → (9 : 1, 2 L), 5 g-fractions being collected] to give the residue (90.2 mg) in fractions 51–89) was then applied to DCCC. The residue (41.1 mg) in fractions 24–30 was purified by HPLC [H_2O–MeOH (17 : 3)] to give 11.4 mg of **1** and 7.50 mg of **16** from the peaks at 22 min and 28 min, respectively.

The residue (1.92 g) in fraction 7 obtained on Diaion-HP-20 CC was subjected to silica gel (75 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (1 L), $CHCl_3$–MeOH (49 : 1, 500 mL), (24 : 1, 500 mL), (23 : 2, 500 mL), (9 : 1, 500 mL), (17 : 3, 500 mL), (4 : 1, 500 mL), (3 : 1, 500 mL) and (7 : 3, 500 mL), $CHCl_3$–MeOH–H_2O (35 : 15 : 2, 500 mL) and MeOH (500 mL)], 100-mL fractions being collected. The residue (452 mg) in fractions 24–34 was subjected ODS open CC. The residue (45.8 mg) in fractions 1–16 was purified by HPLC [Inertsil, 6 mm ×250 mm, H_2O–MeOH (17 : 3), 1.6 mL/min] to give two peaks at 6 min and 7 min (4.04 mg and 2.30 mg, respectively) which were interconvertible and the compound was assigned as **8**. The residue (95.1 mg) in fractions 36–72 was applied to DCCC to give two resides (16.7 mg and 34.0 mg) in fractions 35–46 and 65–82, respectively. The former was purified by HPLC [Inertsil, 6 mm ×250 mm, H_2O–MeOH (3 : 1), 1.6 mL/min] to give 5.75 mg of **5** from the peak at 6 min. The latter was also purified by HPLC to afford [H_2O–MeOH (4 : 1)] to give **9**.80 mg of **18** from the peak at 23 min.

The residue (3.20 g) in fractions 8–10 obtained on Diaion HP-20 CC was subjected to silica gel (75 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (1 L), $CHCl_3$–MeOH (49 : 1, 500 mL), (24 : 1, 500 mL), (23 : 2, 500 mL), (9 : 1, 500 mL), (17 : 3, 500 mL), (4 : 1, 500 mL), (3 : 1, 500 mL) and (7 : 3, 500 mL), $CHCl_3$–MeOH–H_2O (35 : 15 : 2, 500 mL) and MeOH (500 mL)], 100-mL fractions being collected. The residue (640 mg) in fractions 32–37 was subjected to ODS open CC. The residue (127 mg) in fractions 58–100 was applied to DCCC and the residue (12.1 mg) in fractions 21–23 was purified by HPLC [H_2O–MeOH (4 : 1)] to give 1.60 mg of **6** from the peak at 57 min. The residue (49.4 mg) in fractions 144–146 was applied to DCCC and the residue (10.9 mg) in fractions in 90–101 was purified by HPLC [H_2O–MeOH (3 : 2)] to give 2.51 mg of **4** from the peak at 22 min. The residue (578 m) in fractions (41–47) was subjected ODS open CC. The residue (95.7 mg) in fractions 144–161 was purified by DCCC to give 11.0 mg of **15** in fractions 20–29.

The residue (6.16 g) in fractions 11–14 obtained on Diaion HP-20 CC was subjected silica gel (170 g) CC with increasing amounts of MeOH in $CHCl_3$ [$CHCl_3$ (2 L), $CHCl_3$–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_2O (35 : 15 : 2, 1 L) and MeOH (1 L)], 200-mL fractions being collected. The residue (439 mg) in fractions 25–28 was subjected to ODS open CC. The residue (42.5 mg) in fractions 211–215 was applied to DCCC to give two residues (4.85 and 9.17 mg) in fractions 167–180 and 181–216, respectively. The former was purified HPLC [H_2O–MeOH (7 : 3)] to give 1.12 mg of **2** from the peak at 6 min and the latter 3.09 mg of **9** from the peak at 4 min. The residue (891 mg) in fractions 29–33 was subjected ODS open CC. The residue (261 mg) in fractions 194–275 was applied to DCCC and the residue (20.6 m) in fractions 64–72 was purified by HPLC [H_2O–MeOH (7 : 3)] to give 1.80 mg of **10** and 0.93 mg of **11** from the peaks at 23 and 35 min, respectively. The residue (594 mg) in fractions 39–41 was subjected to ODS open CC. The residue (13.5 mg) in fractions 206–210 was purified by HPLC [H_2O–MeOH (13 : 7)] to give 2.30 mg of 12 from the peak at 8 min. The residue (522 mg) in fractions 42–44 was subjected ODS open CC to give 7.17 mg of 14 in fractions 280–282. The residue (419 mg) in fractions 45–48 was subjected to ODS open CC. The residue (179 mg) in fractions 10–13 was applied to DCCC. The residue (68.1 mg) in fractions 12–17 was purified by HPLC [H_2O–MeOH (3 : 2)] to give 30.6 mg of **13** from the peak at 7 min.

Microtropin Q (**1**)

An amorphous powder; $[\alpha]_D^{21}$ −20.4 (*c*=0.76, MeOH); IR ν_{max} cm^{-1}: 3384, 2977, 2225, 1735, 1647, 1454, 1243, 1171, 1080, 1019; ^{1}H-NMR (600 MHz; CD_3OD) δ: 6.66 (1H, dd, *J*=6.3, 5.7, 0.7 Hz, H-3), 4.60 (1H, dd, *J*=11.8, 5.2 Hz, H-6′a), 4.51 (1H, dd, *J*=14.9, 5.7 Hz, H-4a), 4.41 (1H, dd, *J*=14.9, 6.3 Hz, H-4b), 4.31 (1H, d, *J*=7.8 Hz, H-1′), 4.27 (1H, dd, *J*=11.8, 2.0 Hz, H-6′b), 4.20 (2H, s, H_2-5), 3.93 (1H, q, *J*=6.4 Hz, H-3″), 3.49 (1H, ddd, *J*=9.0, 5.2, 2.0 Hz, H-5′), 3.37 (1H, dd, *J*=9.4, 9.0 Hz, H-3′), 3.34 (1H, dd, *J*=9.4, 9.0 Hz, H-4′), 3.18 (1H, dd, *J*=9.9, 7.8 Hz, H-2′), 1.74 (1H, dq, *J*=14.3, 7.5 Hz, H-5″a), 1.55 (1H, dq, *J*=14.3, 7.5 Hz, H-5″b), 1.18 (3H, d, *J*=6.4 Hz, H_3-4″), 0.88 (3H, t, *J*=7.5 Hz, H_3-6″); ^{13}C-NMR (150 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) *m/z*: 428.1522 $[M+Na]^+$ (Calcd for $C_{17}H_{27}O_{10}NNa$: 428.1527).

Microtropin R (**2**)

An amorphous powder; $[\alpha]_D^{24}$ −90.2 (*c*=0.08, MeOH); IR ν_{max} cm^{-1}: 3351, 2932, 1735, 1651, 1457, 1370, 1241, 1168, 1081, 712; ^{1}H-NMR (600 MHz; CD_3OD) δ: 5.45 (1H, dtt, *J*=10.9, 7.3, 1.2 Hz, H-4), 5.37 (1H, dtt, *J*=10.9, 7.3, 1.2 Hz, H-3), 4.63 (1H, dd, *J*=11.7, 2.3 Hz, H-6′a), 4.60 (1H, dd, *J*=11.7, 2.3 Hz, H-6′a), 4.27 (1H, d, *J*=7.6 Hz, H-1′), 4.23 (1H, dd, *J*=11.7, 5.5 Hz, H-6′b), 3.92 (1H, q, *J*=6.4 Hz, H-3″), 3.80 (1H, dt, *J*=9.5, 7.3 Hz, H-1a), 3.53 (1H, dt, *J*=9.5, 7.1 Hz, H-1b), 3.48 (1H, ddd, *J*=9.7, 5.5, 2.3 Hz, H-5′), 3.30–3.38 (2H, overlapped, H-3′ and 4′), 3.17 (1H, dd, *J*=9.1, 7.8 Hz, H-2′), 2.37 (2H, dddd, *J*=7.3, 7.3, 7.1, 1.2 Hz, H_2-2), 2.07 (2H, qdd, *J*=7.6, 7.3, 1.2 Hz, H_2-5), 1.73 (1H, dq, *J*=13.7, 7.5 Hz, H-5″a), 1.17 (3H, d, *J*=6.4 Hz, H_3-4″), 0.96 (2H, d, *J*=7.6 Hz, H_3-6), 0.88 (3H, t, *J*=7.5 Hz, H_3-6″); ^{13}C-NMR (100 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) *m/z*: 415.1946 $[M+Na]^+$ (Calcd for $C_{18}H_{32}O_9Na$: 415.1944).

Microtropin S (**3**)

An amorphous powder; $[\alpha]_D^{24}$ −12.5 (*c*=0.10, MeOH); IR ν_{max} cm^{-1}: 3340, 2934, 1736, 1457, 1371, 1238, 1170, 1078; ^{1}H-NMR (400 MHz; CD_3OD) δ: 4.60 (1H, dd, *J*=11.8, 2.0 Hz, H-6′a), 4.34 (1H, d, *J*=7.8 Hz, H-1′), 4.20 (1H, dd, *J*=11.8, 5.8 Hz, H-6′b), 3.94 (1H, q, *J*=6.4 Hz, H-3″), 3.67 (2H, m, H-2 and 3), 3.50 (1H, ddd, *J*=9.5, 5.8, 2.0 Hz, H-5′), 3.35 (1H, m, H-3′), 3.34 (1H, m, H-4′), 3.19 (1H, dd, *J*=9.0, 7.8 Hz, H-2′), 1.74 (1H, dq, *J*=13.8, 7.5 Hz, H-5″a), 1.54 (1H, dq, *J*=13.8 Hz, H-5″b), 1.16 (3H, d, *J*=6.1 Hz, H_3-1), 1.17 (3H, d, *J*=6.4 Hz, H_3-4″), 1.12 (3H, d, *J*=6.2 Hz, H_3-4), 0.87 (3H, t, *J*=7.5 Hz,

H_3-6″); ^{13}C-NMR (100 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) *m/z*: 405.1730 $[M+Na]^+$ (Calcd for $C_{16}H_{30}O_{10}Na$: 405.1731).

Microtropin T (**4**)

An amorphous powder; $[\alpha]_D^{25}$ −12.9 (*c*=0.55, MeOH); IR ν_{max} cm^{-1}: 3340, 2934, 2880, 1736, 1651, 1457, 1371, 1237, 1169, 1077, 1040; ^{1}H-NMR (400 MHz; CD_3OD) *δ*: 4.61 (1H, dd, *J*=11.8, 2.0 Hz, H-6′a), 4.34 (1H, d, *J*=7.8 Hz, H-1′), 4.20 (1H, dd, *J*=11.8, 5.8 Hz, H-6′b), 3.92 (1H, q, *J*=6.4 Hz, H-3″), 3.76 (1H, m, H-6), 3.72 (1H, m, H-2), 3.46 (1H, ddd, *J*=9.1, 5.8, 2.0 Hz, H-5′), 3.34 (1H, m, H-3′), 3.32 (1H, m, H-4′), 3.15 (1H, dd, *J*=8.9, 7.8 Hz, H-2′), 1.73 (1H, dq, *J*=14.0, 7.3 Hz, H-5″a), 1.65 (1H, m, H-3a), 1.54 (1H, dq, *J*=14.0, 7.3 Hz, H-5″b), 1.48 (2H, m, H_2-3), 1.46 (1H, m, H-5a), 1.42 (1H, m, H-3b), 1.37 (1H, m, H-5b), 1.21(3H, d, *J*=6.5 Hz, H_3-1), 1.17 (3H, d, *J*=6.4 Hz. H_3-4″), 1.15 (3H, d, *J*=6.4 Hz, H_3-7), 0.88 (3H, t, *J*=7.3 Hz, H_3-6″); ^{13}C-NMR (100 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) *m/z*: 447.2197 $[M+Na]^+$ (Calcd for $C_{19}H_{36}O_{10}Na$: 447.2200).

Microtropin U (**5**)

An amorphous powder; $[\alpha]_D^{24}$ −26.9 (*c*=0.38, MeOH); IR ν_{max} cm^{-1}: 3389, 2978, 2936, 1736, 1511, 1217, 1075, 1013, 894, 834, 777; UV λ_{max} (MeOH) nm (log*ε*): 333 (3.01), 285 (3.35), 222 (3.74); ^{1}H-NMR (400 MHz; CD_3OD) *δ*: 6.70 (2H, d, *J*=8.1 Hz, H-3 and 5), 6.95 (2H, d, *J*=8.1 Hz, H-2 and 6), 4.71 (1H, d, *J*=7.2 Hz, H-1′), 4.65 (1H, dd, *J*=12.0, 1.8 Hz, H-6′a), 4.18 (1H, dd, *J*=12.0, 6.1 Hz, H-6′b), 3.92 (1H, q, *J*=6.5 Hz, H-3″), 3.57 (1H, ddd, *J*=9.4, 6.1, 1.8 Hz, H-5′), 3.45 (1H, dd, *J*=9.0. 8.6 Hz, H-3′), 3.42 (1H, m, H-2′), 3.40 (1H, m, H-4′), 1.71 (1H, dq, *J*=14.0, 7.0 Hz, H-5″a), 1.54 (1H, dq, *J*=14.0, 7.0 Hz, H-5″b), 1.17 (3H, d, *J*=6.5 Hz, H_3-4″), 0.84 (3H, t, *J*=7.0 Hz, H_3-6″); ^{13}C-NMR (100 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) *m/z*: 425.1417 $[M+Na]^+$ (Calcd for $C_{18}H_{26}O_{10}Na$: 425.1418).

Microtropin V (**6**)

An amorphous powder; $[\alpha]_D^{21}$ −68.9 (*c*=0.11, MeOH); IR ν_{max} cm^{-1}: 3362, 2934, 1736, 1650, 1457, 1279, 1170, 1071, 1015; UV λ_{max} (MeOH) nm (log*ε*): 275 (3.88), 225 (3.32); ^{1}H-NMR (600 MHz; CD_3OD) *δ*: 7.01 (1H, d, *J*=2.0 Hz, H-2), 6.81 (1H, dd, *J*=8.2, 2.0 Hz, H-6), 6.77 (1H, d, *J*=8.2 Hz, H-5), 4.74 (1H, d, *J*=7.6 Hz, H-1′), 4.65 (1H, dd, *J*=12.0, 2.2 Hz, H-6′a), 4.25 (1H, dd, *J*=12.0, 5.7 Hz, H-6′b), 3.94 (1H, q, *J*=6.5 Hz, H-3″), 3.70 (2H, t, *J*=7.1 Hz, H_2-8), 3.62 (1H, m, H-5′), 3.48 (1H, m, H-1′), 3.47 (1H, m, H-3′), 3.42 (1H, m, H-4′), 2.72 (2H, t, *J*=7.1 Hz, H_2-7), 1.72 (1H, dq, *J*=14.0, 7.5 Hz, H-5″a), 1.53 (1H, dq, *J*=14.0, 7.5 Hz, H-5″b), 1.17 (3H, d, *J*=6.5 Hz, H_3-4″), 0.85 (3H, t, *J*=7.5 Hz, H_3-6″); ^{13}C-NMR (150 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) *m/z*: 469.1680 $[M+Na]^+$ (Calcd for $C_{20}H_{30}O_{11}Na$: 469.1680).

Microtropin W (**7**)

An amorphous powder; $[\alpha]_D^{24}$ −90.2 (*c*=0.08, MeOH); IR ν_{max} cm^{-1}: 3399, 2980, 2932, 2855, 1733, 1641, 1391, 1240, 1106, 1078, 1043, 670; ^{1}H-NMR (400 MHz; CD_3OD) *δ*: 5.08 (d, *J*=3.8 Hz, H-1′*α*) 4.48 (d, *J*=7.8 Hz, H-1′*β*), 4.45 (1H, m, H-6′a*α* and 6′a*β*), 4.26 (1H, m, H-6′b*α* and 6-'b*β*), 3.99 (m, H-5′*α*), 3.96 (1H, m, H-3″), 3.68 (m, H-3′*β*), 3.67 (m, H-3′*α*), 3.48 (m, H-5′*β*), 3.37 (m, H-4′*β*), 3.33 (m, H-2′*α*), 3.32 (m, H-4′*α*), 3.13 (dd, *J*=9.2, 7.8 Hz, H-2′*β*), 1.72 and 1.73 (each dq, *J*=14.1, 7.3 Hz, H-2″a), 1.53 and 1.54 (each dq, *J*=14.1, 7.3 Hz, H-5″b), 1.172 and 1.174 (each d, *J*=6.5 Hz, H-4″), 087 (3H, t, *J*=7.3 Hz, H-6″); ^{13}C-NMR (100 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) *m/z*: 333.1160 $[M+Na]^+$ (Calcd for $C_{12}H_{22}O_9Na$: 333.1156).

(2*S*,3*R*)-2-Ethyl-2,3-dihydroxybutyric Acid (**8**)

Colorless syrup; $[\alpha]_D^{24}$ −3.5 (*c*=5.15, MeOH); IR ν_{max} cm^{-1}: 3384, 2979, 2927, 2885, 1732, 1458, 1242, 1171, 1087, 991, 771; ^{1}H-NMR (400 MHz; CD_3OD) *δ*: 3.90 (1H, q, *J*=6.5 Hz, H-3), 1.71 (1H, dq, *J*=13.9, 7.5 Hz, H-5a), 1.53 (1H, dq, *J*=13.9, 7.5 Hz, H-5b), 1.17 (3H, d, *J*=6.5 Hz, H_3-4), 0.89 (3H, d, *J*=7.5 Hz, H_3-6); ^{13}C-NMR (100 MHz, CD_3OD): Table 1; HR-ESI-MS (positive-ion mode) *m/z*: 171.0627 $[M+Na]^+$ (Calcd for $C_6H_{12}O_4Na$: 171.0628).

Microtropioside G (**9**)

An amorphous powder; $[\alpha]_D^{24}$ −12.6 (*c*=0.21, MeOH); IR ν_{max} cm^{-1}: 3360, 2964, 2934, 2874, 1735, 1457, 1161, 1070, 1040; ^{1}H-NMR (400 MHz, CD_3OD): Table 2; ^{13}C-NMR (100 MHz, CD_3OD): Table 2; HR-ESI-MS (positive-ion mode) *m/z*: 509.3098 $[M+Na]^+$ (Calcd for $C_{26}H_{46}O_8Na$: 509.3084).

Microtropioside H (**10**)

An amorphous powder; $[\alpha]_D^{23}$ −26.7 (*c*=0.12, MeOH); IR ν_{max} cm^{-1}: 3362, 2931, 2359, 1650, 1370, 1161, 1075, 1020; ^{1}H-NMR (600 MHz, CD_3OD): Table 2; ^{13}C-NMR (150 MHz, CD_3OD): Table 2; HR-ESI-MS (positive-ion mode) *m/z*: 525.3029 $[M+Na]^+$ (Calcd for $C_{26}H_{46}O_9Na$: 525.3034).

Microtropioside I (**11**)

An amorphous powder; $[\alpha]_D^{25}$ −30.6 (*c*=0.06, MeOH); IR ν_{max} cm^{-1}: 3360, 2929, 2871, 1370, 1077, 1027; ^{1}H-NMR (600 MHz, CD_3OD): Table 2; ^{13}C-NMR (100 MHz, CD_3OD): Table 2; HR-ESI-MS (positive-ion mode) *m/z*: 525.3031 $[M+Na]^+$ (Calcd for $C_{26}H_{46}O_9Na$: 525.3034).

3*β*,12,16*β*,23-Tetrahydroxy-11*α*-methoxyurs-12-ene 3,16-Di-*O*-*β*-D-glucopyranoside (**12**)

An amorphous powder; $[\alpha]_D^{22}$ −2.8 (*c*=0.15, MeOH); IR ν_{max} cm^{-1}: 3362, 2927, 2874, 1651, 1512, 1457, 1077, 1020; ^{1}H-NMR (600 MHz, CD_3OD): Table 3; ^{13}C-NMR (150 MHz, CD_3OD): Table 3; HR-ESI-MS (positive-ion mode) *m/z*: 851.4757 $[M+Na]^+$ (Calcd for $C_{43}H_{72}O_{15}Na$: 851.4763

Kaempferol 3-*O*-*β*-D-(2‴-*O*-*β*-D-Xylopyranosyl)(2″-*O*-*β*-D-glucopyranosyl)galactopyranoside (**13**)

Pale yellow amorphous powder; $[\alpha]_D^{23}$ −45.7 (*c* 0.91, MeOH); IR ν_{max} (film) cm^{-1}: 3406, 2921, 2901, 1651, 1606, 1362, 1176, 1070, 1040, 894: UV λ_{max} (MeOH) nm (log*ε*): 349 (4.11), 332 (4.12), 265 (4.21), 209 (4.28); ^{1}H-NMR (400 MHz, CD_3OD): Table 4; ^{13}C-NMR (100 MHz, CD_3OD): Table 4; HR-ESI-MS (positive-ion mode) *m/z*: 765.1840 $[M+Na]^+$ (Calcd for $C_{32}H_{38}O_{20}Na$, 765.1840).

Sugar Analysis About 500 *μ*g each of microtropins Q–W (**1–7**), microtropiosides G–I (**9–11**) and compound **12** was hydrolyzed with 1 M HCl (0.1 mL) at 90°C for 2 h. The reaction mixtures were partitioned with an equal amount of EtOAc (0.1 mL) and the water layers were analyzed with HPLC on an amino column [Shodex Asahipak NH_2P-5 4E (Showa Denko K.K., Tokyo, Japan); CH_3CN–H_2O (3 : 1), Φ=4.6 mm, *L*=250 mm; flow rate: 1 mL/min]. using a chiral detector (JASCO OR-2090*plus*) to give a sole peak with a positive sign, which had an identical retention time with that of authentic D-glucose at 9.5 min.

Similar HPLC analysis [InertSustain NH_2 (GL Science); Φ=6 mm, *L*=250 mm; CH_3CN–H_2O (4 : 1); flow rate: 1.6 mL/min] of the hydrolyzate of **13** gave three peaks, which had identical retention times with those of authentic D-xylose, D-glucose and D-galactose with positive signs at 7.7, 10.7 and

Table 4. ^{13}C- and ^{1}H-NMR Spectroscopic Data for Kaempferol Glycosides (**13**) and **17** (150 MHz and 600 MHz, Respectively, CD_3OD)

	13		**17**
	C	H	C
2	158.6	—	158.9
3	134.7	—	134.9
4	179.7	—	179.8
5	163.1	—	163.1
6	100.3	6.17 1H d 2	99.9
7	167.2	—	165.9
8	95.0	6.38 1H d 2	94.7
9	158.3	—	158.5
10	105.5	—	105.8
1′	122.9	—	122.8
2′,6′	132.3	8.08 2H d 9	132.9
3′,5′	116.3	6.90 2H d 9	116.3
4′	161.5	—	161.5
1″	101.1	5.49 1H d 8	101.5
2″	82.7	3.95 1H m	80.3
3″	74.5	3.73 1H dd 10, 3	74.8
4″	69.8	3.89 1H dd 3, 2	70.1
5″	77.1	3.45 1H m	76.9
6″	62.1	3.54 1H m	62.0
		3.63 1H m	
1‴	104.0	4.72 1H d 8	104.8
2‴	84.9	3.46 1H dd 9, 8	75.5
3‴	78.0	3.57 1H m	78.2
4‴	70.8	3.51 1H dd 10, 9	71.4
5‴	77.7	3.18 1H ddd 10, 5, 2	77.9
6‴	62.7	3.59 1H m	62.7
		3.64 1H m	
1⁗	106.7	4.58 1H d 8	
2⁗	75.8	3.28 1H dd 9, 8	
3⁗	77.5	3.38 1H dd 9, 9	
4⁗	70.8	3.51 1H ddd 10, 9, 2	
5⁗	67.2	3.25 1H dd 11, 10	
		3.93 1H dd 11, 2	

m: multiplet or overlapped signal.

11.5 min, respectively.

X-Ray Analysis of Microtropioside A Pentaacetate (15a) A suitable crystal (0.25×0.10×0.06 mm) was used for analysis. The data were measured using a Rigakur AFC-5 four circle diffractometer, using Mo*Kα* graphite-monochromated radiation (λ=0.71073 Å). The structure was solved by a direct method using the program SHELXTL-97 (Sheldrick, 2008). The refinement and all further calculations were carried out using SHELXTL-97.[16] The absorption correction was carried out utilizing the SADABS routine.[17] The H atoms were included at calculated positions and treated as riding atoms using the SHELXTL default parameters. The non-H atoms were refined anisotropically, using weighted full-matrix least-squares on F^2. Figure 5 was drawn with ORTEP32 (Farrugia, 1997). Crystal data: $C_{37}H_{56}O_{13}$, M=708.82l, monoclinic, $P2_1$, a=18.715(4) Å, b=6.582(2) Å, c=15.495(4) Å, β=92.798(18)°, V=1906.4(9) Å^3, T=173 K, Z=2, D_c=1.235 Mg/m^3, μ(Mo*Kα*)=0.770 mm^{-1}, F(000)=764, 3913 reflections were measured in the range of $2\theta \leq 136.1°$, 3771 being unique (R_{int}=0.0363) and used in all calculations. The final goodness-of-fit on F^2 was 1.078, and the final R indices were R_1=0.0427 and wR_2=0.0993 based on $I>2\sigma(I)$, and R_1=0.0809 and wR_2=0.1330 with all data. The largest differences of the peak and the hole were 0.262 and −0.257 eÅ^{-3}, respectively. The CCDC deposit contains supplementary crystallographic data (No. 1553754). These data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223 336 033; or e-mail: deposit@ccdc.cam.ac.uk.

Acknowledgments The authors are grateful for access to the superconducting NMR instrument and the HR-ESI-MS at the Analytical Center of Molecular Medicine and the Analysis Center of Life Science, respectively, of the Graduate School of Biomedical Sciences, Hiroshima University. This work was supported in part by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology of Japan, and the Japan Society for the Promotion of Science (Nos. 22590006, 23590130 and 15H04651). Thanks are also due to the Takeda Science Foundation for the financial support.

Conflict of Interest The authors declare no conflict of interest.

References

1) Kupchan S. M., Komoda Y., Court W. A., Thomas G. J., Smith R. M., Karim A., Gilmore C. J., Haltiwagner R. C., Bryan R. F., *J. Am. Chem. Soc.*, **94**, 1354–1356 (1972).
2) Cassady J. M., Chan K. K., Floss H. G., Leistner E., *Chem. Pharm. Bull.*, **52**, 1–26 (2004).
3) Hatusima S., "Flora of The Ryukyus (Including Amami Islands, Okinawa Islands and Sakishima Archipelago) (Added and Corrected)," Biological Society of Okinawa, Naha, Japan, 1975, p. 382.
4) Koyama Y., Matsunami K., Otsuka H., Shinzato T., Takeda Y., *Phytochemistry*, **71**, 675–681 (2010).
5) Uemura Y., Sugimoto S., Matsunami K., Otsuka H., Takeda Y., Kawahata M., Yamaguchi K., *Phytochemistry*, **87**, 140–147 (2013).
6) Uemura Y., Sugimoto S., Matsunami K., Otsuka H., Takeda Y., *Am. J. Plant Sci.*, **4**, 1954–1959 (2013).
7) Nagy E., Sere I., Verzár-Petri G., Neszmélyi A., *Z. Naturforsch. C*, **39b**, 1813–1815 (1984).
8) Han Y., Nishibe S., Noguchi Y., Jin Z., *Phytochemistry*, **58**, 577–580 (2001).
9) Krajewski D., Tóth G., Schreier P., *Phytochemistry*, **43**, 141–143 (1996).
10) Kasai R., Suzuo M., Asakawa J., Tanaka O., *Tetrahedron Lett.*, **18**, 175–178 (1977).
11) Yoshikawa M., Yoshizumi S., Murakami T., Matsuda H., Yamahara J., Murakami N., *Chem. Pharm. Bull.*, **44**, 492–499 (1996).
12) Abraham W.-R., *Phytochemistry*, **36**, 1421–1424 (1994).
13) González A. G., Arteaga J. M., Bretón J. L., Fraga B. M., *Phytochemistry*, **16**, 107–110 (1977).
14) Garcia-Alvarez M. C., Rodriguez B., *J. Org. Chem.*, **46**, 1915–1918 (1981).
15) Kaweetripob W., Mahidol C., Prawat H., Ruchirawat S., *Phytochemistry*, **96**, 404–417 (2013).
16) Sheldrick G. M., *Acta Crystallogr. A*, **64**, 112–122 (2008).
17) Sheldrick G. M., "SADABS," University of Göttingen, Germany, 1996.