  Bull. Chem. Soc. Jpn., 59, 3541—3546 (1986)

Structures of Yadanziosides K, M, N, and O, New Quassinoid Glycosides from *Brucea javanica* (L.) MERR[1)]

Toshiro SAKAKI, Shin YOSHIMURA, Takahiko TSUYUKI,* Takeyoshi TAKAHASHI,*
Tadashi HONDA,† and Toshihiro NAKANISHI†
Department of Chemistry, Faculty of Science, The University of Tokyo,
Hongo, Bunkyo-ku, Tokyo 113
†Suntory Institute for Biomedical Research, Wakayamadai,
Shimamotocho, Mishimagun, Osaka 618
(Received May 15, 1986)

Four new bitter quassinoid glycosides, yadanziosides K, M, N, and O were isolated from *Brucea javanica* (L.) MERR and their structures were determined by spectral measurement and chemical means. The aglycone of yadanzioside O was found to exhibit a significant antitumor activity against the murine P388 lymphocytic leukemia and this aglycone could be prepared from brusatol.

"Ya-dan-zi," seed of *Brucea javanica* (L.) MERR has been used as a Chinese medicine for the treatment of cancer, and the main components with antileukemic activity have been investigated.[2)] We examined the minor components of "Ya-dan-zi," and reported structure elucidation of sixteen new bitter principles.[3)] Further investigation led to the isolation of four new quassinoid glycosides, yadanziosides K, M, N, and O (**1**—**4**). This paper describes structure determination of these bitter principles (**1**—**4**) and a preparation and antileukemic activity of the aglycone of yadanzioside O (**4**).

The methanol extract of defatted seeds of *B. javanica* was partitioned between dichloromethane and water. The organic layer was subjected to separation by a silica-gel column chromatography, followed by a Lobar column Lichroprep RP-8, Toyopearl HW-40S, or silicic acid column chromatography to afford yadanzioside K (**1**; ca. 0.001% yield), yadanzioside M (**2**; ca. 0.0005% yield), yadanzioside N (**3**; ca. 0.0001% yield), and yadanzioside O (**4**; ca. 0.0002% yield).

Yadanzioside K (**1**) crystallized from methanol, mp 214.5—216.5 °C and showed the presence of three tertiary methyl, two vinyl methyl, an acetoxyl, and a methoxycarbonyl groups and an olefinic proton in the ^{1}H NMR spectrum (Table 1), and the presence of an α,β-unsaturated carbonyl group was shown by the UV spectrum. The molecular formula, $C_{36}H_{48}O_{18}$, was inferred from ^{13}C NMR spectrum (Table 2) together with a peak at m/z 769 ($[M+H]^+$) in the secondary ion mass spectrometry (SI-MS) and hydrolysis with β-glucosidase to give bruceantinol (**5**)[4)] as the major aglycone. The glycosylation shifts of signals due to C-3 and C-4 were $\Delta\delta$+2.1 and +18.3 ppm, respectively (Table 2), and the signal due to the $C_{(4)}$–CH_3 was observed at δ 2.04 as a singlet in the ^{1}H NMR spectrum of **1**. Thus the structure of yadanzioside K (**1**) was determined to be 3-*O*-(β-D-glucosyl)bruceantinol.

Yadanzioside M (**2**) was obtained as amorphous powder from acetone–hexane, mp 208—213 °C (decomp). The EI-MS showed a peak at m/z 542 ($[M-C_6H_{10}O_5]^+$) and the SI-MS afforded a peak at m/z 727 ($[M+Na]^+$). Enzymatic hydrolysis of **2** with β-glucosidase afforded bruceantarin (**6**)[5)] as the major aglycone. These observations suggest the molecular formula, $C_{34}H_{40}O_{16}$, for **2**. The ^{1}H NMR spectrum of **2** showed the presence of a doublet signal (J=7 Hz) due to $C_{(4)}$–CH_3 at δ 1.18 and a singlet signal at δ 7.26 assignable to $C_{(1)}$–H. This fact suggests the bruceantarin moiety enolizes into a 2-hydroxy-3-keto-1-ene structure, which forms the β-glucoside linkage through the oxygen atom on C-2 of the aglycone.[3b)] The configuration of the $C_{(4)}$–CH_3 of **2** was inferred to be α-equatorial from the structural similarity to bruceoside A.[2c)] The structure (**2**) is, therefore, proposed for yadanzioside M.

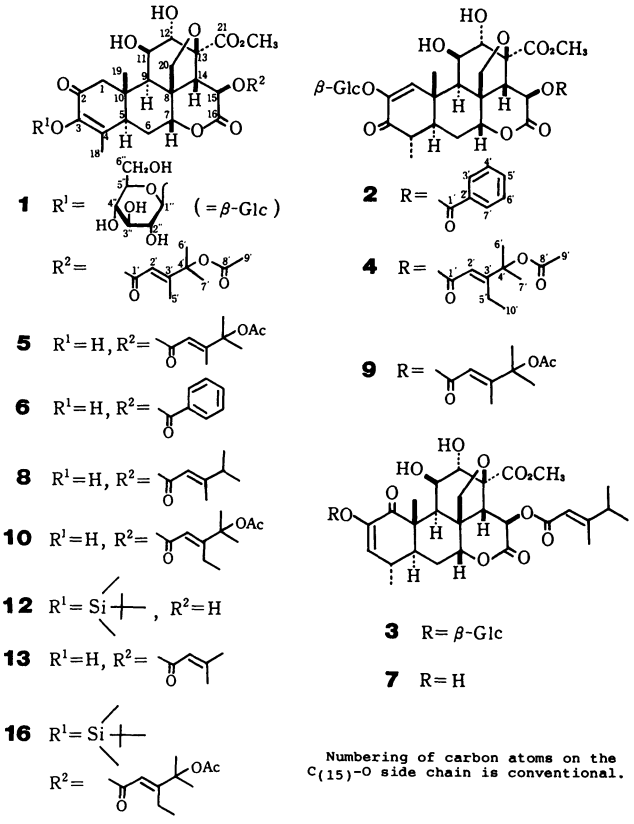

Table 1. ^{1}H NMR Spectra[a] of Yadanziosides K (**1**), M (**2**), N (**3**), and O (**4**) and Some Aglycones

	K (**1**)[b]	M (**2**)[b]	N (**3**)[c]	O (**4**)[d]	**7**[e]	**10**[e]
1-H	f)	7.26 s	—	7.27 s	—	f)
3-H	—	—	6.10 d (2.2)	—	5.74 d (2.4)	—
4-H	—	f)	2.24 brt	2.42 m	f)	—
5-H	f)	f)	0.98 dd (9.5, 6.6)	f)	f)	f)
6α-H	f)	f)	2.15 brd	f)	2.25 brtd (2.9, 14.8)	f)
6β-H	f)	f)	1.56 brt (13)	f)	1.94 brt (11.1)	f)
7-H	f)	f)	5.13 brs	4.96 brs	4.66 brs	4.82 brs
9-H	f)	f)	2.95 d (4.0)	2.50 d (5.4)	f)	f)
11-H	f)	f)	6.20 d (4.4)	5.13 d (5.4)	5.44 br	4.26 brd (4.3)
12-H	f)	f)	4.90 brs	5.08 brs	4.29 brs	4.22 brs
15-H	6.85 d (13)	f)	f)	f)	f)	f)
20-H	f)	f)	5.12 d (7.7)	5.05 d (8.1)	4.70 d (7.9)	4.73 d (7.6)
4-Me	2.04 brs	1.18 d (7)	0.87 d (8.8)	1.16 d (8.1)	1.11 d (7.0)	1.85 d (2.2)
10-Me	1.68 s	1.64 s	1.87 s	1.63 s	1.62 s	1.40 s
CO_2Me	3.89 s	3.56 s	3.77 s	3.86 s	3.78 s	3.81 s
2′-H	6.06 s		5.89 s	6.10 s	5.66 brs	5.78 s
3′-Me	2.26 s		2.18 s		2.14 d (1.1)	—
4′-Me	1.42 s		0.85 d (7.0)	1.49 s	1.06 d (6.8)	1.57 s
	1.47 s			1.51 s		
4′-OAc	1.98 s		—	1.94 s	—	2.03 s
5′-H	—		—	2.67 m	—	2.59 q (7.2)
				2.78 m		
5′-Me	—		—	1.24 t (8.1)	—	1.13 t (7.3)
1″-H	f)	f)	5.47 d (7.0)	5.28 d (8.1)		
6″-H	f)	f)	4.57 d (11)	4.42 dd (10.8, 2.7)		

a) Coupling constants in Hz in parentheses. b) 90 MHz, C_5D_5N. c) 400 MHz, C_5D_5N. d) 270 MHz, C_5D_5N. e) 270 MHz, $CDCl_3$. f) Not measured.

Yadanzioside N (**3**), mp 175—180 °C, showed a doublet signal due to an anomeric proton at δ 5.47 (J=7.0 Hz) in the ^{1}H NMR spectrum, a peak at m/z 733 ($[M+Na]^+$) in SI-MS, and a peak at m/z 548 ($[M-C_6H_{10}O_5]^+$) in EI-MS. These spectral data together with ^{13}C NMR spectrum (Table 2) indicate that **3** is a hexoside with the molecular formula, $C_{34}H_{46}O_{16}$. Yadanzioside N (**3**) was hydrolyzed with 1.5 M (1 M=1 mol dm^{-3}) sulfuric acid in boiling methanol to afford an aglycone (**7**) and D-glucose, the latter of which was identified by GLC after trimethylsilylation. The aglycone (**7**), mp 180—183 °C, was shown to have the molecular formula, $C_{28}H_{36}O_{11}$, by high-resolution mass spectrum (HR-MS), and a peak at m/z 111 in EI-MS suggested the presence of a heptenoyl group ($C_6H_{11}CO$) in the side chain. Since the ^{1}H NMR spectrum revealed the presense of an isopropyl group and a vinyl methyl group (δ 1.06 d (J=6.8 Hz) and 2.14 d (J=1.1 Hz), respectively) in the side chain and the shift values of signals due to these groups are similar to those of bruceantin (**8**)[6] in ^{1}H and ^{13}C NMR spectra, the side chain might be 3,4-dimethyl-2-pentenoyl group. The UV absorption maxima appeared at 220 and 267 nm in ethanol solution, and the latter was shifted to 314 nm on addition of alkali, indicating the presence of a diosphenol moiety.[7]

In the ^{1}H NMR spectrum of **3**, a doublet signal due to $C_{(11)}$-H appeared at δ 6.20, which is lower than that of bruceantin (**8**).[6] On irradiation of a broad triplet signal due to $C_{(4)}$-H at δ 2.24, signals at δ 0.87 due to $C_{(4)}$-CH_3 and at δ 6.10 due to $C_{(3)}$-H became sharp. On the other hand, the aglycone (**7**) showed a doublet signal due to $C_{(4)}$-CH_3 at δ 1.11 and a doublet signal due to an olefinic proton at C-3 at δ 5.74 with a coupling constant, J=2.4 Hz. These spectral features suggest that the configuration of $C_{(4)}$-CH_3 is α-equatorial and that A-ring of the aglycone (**7**) is the same as that of norquassin.[7] From these observations and ^{1}H and ^{13}C NMR spectral comparison with bruceantin (**8**), the aglycone (**7**) was shown to be an isomer with 1-keto-2-ene structure of bruceantin (**8**). Since no bathochromic shift of the UV absorption maxima of **3** was observed on addition of alkali, the glycoside linkage was shown to be formed through an oxygen atom on C-2 of the aglycone (**7**). The structure of yadanzioside N (**3**) was, therefore, determined to be methyl 2-(β-D-glucopyranosyloxy)-13β,20-epoxy-15β-

Table 2. ^{13}C NMR Spectra of Yadanziosides K (**1**), M (**2**), N (**3**), and O (**4**), Bruceantin (**8**), and Some Aglycones

No. of carbon	K (**1**) [a]	M (**2**) [a]	N (**3**) [a]	O (**4**) [a]	(**5**) [a]	(**7**) [b]	(**8**) [c]	(**10**) [b]
1	51.1 t	129.4 d	199.6 s	129.6 d	50.0 t	201.0 s	48.7 t	48.6
2	193.6 s	148.8 s	146.4 s	148.8 s	192.9 s	143.7 s	192.2 s	192.1
3	148.0 s [d]	194.5 s	125.5 d	194.6 s	145.9 s	121.3 d	144.2 s	144.1
4	146.6 s [d]	43.9 d	31.6 d	43.9 d	128.3 s	30.7 d	127.9 s	127.8
5	43.4 d	40.8 d	36.9 d	40.4 d	42.5 d	36.5 d	41.2 d	44.1
6	29.3 t	30.1 t	28.8 t	30.0 t	29.6 t	28.5 t	29.2 t	29.1
7	83.4 d	83.7 d	83.0 d	83.4 d	83.6 d	82.4 d	82.4 d	82.6
8	46.0 s	46.9 s	46.7 s	46.6 s	46.2 s	46.1 s	45.5 s	45.4
9	41.9 d	41.4 d	44.2 d	41.4 d	42.1 d	43.9 d	41.9 d	41.9
10	40.8 s	39.7 s	48.9 s	39.6 s	41.4 s	47.3 s	41.2 s	41.1
11	73.0 d	73.5 d	75.1 d	73.5 d	73.1 d	72.9 d	71.1 d	71.0
12	75.9 d	76.1 d	76.3 d	76.0 d	75.9 d	76.1 d	75.9 d	75.7
13	82.6 s	82.7 s	83.0 s	82.6 s [l]	82.7 s	81.2 s	81.4 s	81.3
14	50.2 d	50.7 d	50.9 d	50.3 d	50.4 d	51.6 d	51.7 d	51.5
15	68.4 d	69.3 d	68.8 d	68.6 d	68.6 d	66.7 d	66.0 d	66.2
16	168.0 s	168.0 s	168.2 s	168.0 s [m]	168.0 s	168.2 s	167.0 s	166.9
18	15.2 q	12.6 q	14.5 q	12.5 q	13.3 q	14.8 q	13.3 q	13.3
19	15.8 q	18.0 q	18.9 q	18.0 q	15.7 q	19.0 q	15.5 q	15.5
20	73.5 t	73.8 t	73.7 t	73.6 t	73.7 t	73.6 t	74.1 t	74.1
21	171.2 s	171.2 s	171.1 s	171.1 s	171.2 s	171.6 s	171.8 s	171.8
OMe	52.5 q	52.3 q	52.3 q	52.6 q	52.5 q	52.8 q	52.9 q	53.4
1′	165.7 s	165.3 s	166.0 s	165.2 s	165.7 s	165.2 s	165.0 s	164.2
2′	113.5 d	130.4 s [f]	113.6 d	113.8 d	113.6 d	111.8 d	111.8 d	112.0
3′	169.5 s	130.2 d [g]	167.2 s	169.5 s	169.5 s	169.6 s	169.6 s	169.6
4′	82.3 s	128.7 d [h]	38.1 d	82.7 s [l]	82.4 s	38.3 d	38.4 d	82.4
5′	14.3 q	133.6 d [i]	16.7 q	22.0 t	14.5 q	17.1 q	17.0 q	22.1
6′	25.8 q	128.7 d [h]	20.7 q	26.2 q	26.4 q	20.8 q	20.8 q	26.5
7′	26.3 q	130.2 d [g]	20.7 q	26.5 q	25.9 q	20.8 q	20.8 q	26.5
8′	163.4 s			168.6 s [m]	163.3 s			170.9
9′	21.4 q			21.7 q	21.4 q			21.9
10′				14.6 q				14.2
1″	104.8 d	102.0 d	100.9 d	102.0 d				
2″	75.6 d	74.7 d	74.7 d	74.6 d				
3″	78.5 d [e]	78.8 d [j]	78.9 d [k]	78.8 d [n]				
4″	71.5 d	71.4 d	71.5 d	71.3 d				
5″	78.3 d [e]	78.5 d [j]	78.6 d [k]	78.4 d [n]				
6″	62.8 t	62.4 t	62.5 t	62.4 t				

a) 22.5 MHz, C_5D_5N. b) 67.5 MHz, $CDCl_3$. c) 22.5 MHz, $CDCl_3$. d) The assignment of signals may be reversed. e) and j)—n) are the same as d). f) Aromatic carbon atom adjacent to the carbonyl carbon. g) Carbon atom at *o*-position. h) Carbon atom at *m*-position. i) Carbon atom at *p*-position.

[(2*E*)-3,4-dimethyl-2-pentenoyloxy]-11β,12α-dihydroxy-1,16-dioxo-2-picrasen-21-oate, which is a new type bruceoside with a quassin-type A-ring.

Yadanzioside O (**4**), mp 183—188 °C, was found to be a hexose with the same skeleton as yadanzioside G (**9**)[3b] except for an additional triplet signal (δ 22.0) due to a methylene group from the comparison of ^{13}C NMR spectra (see Table 2). The ^1H NMR spectrum of **4** also revealed the presence of an acetoxyl, an ethyl, and two methyl groups assignable to the side chain together with an olefinic proton. Therefore the structure of the side chain could be formulated as 15-*O*-(4-acetoxy-3-ethyl-4-methyl-2-pentenoyl), and geometry of the double bond was assigned to be 2*E* by the following difference NOE measurement of **4** at 400 MHz. Irradiation of the signal due to $C_{(2')}$–H at δ 6.10 resulted in an enhancement of the signals at δ 1.49 and 1.51 due to the geminal methyl group at C-4′.

Yadanzioside O (**4**) was hydrolyzed with β-glucosidase to yield an aglycone (**10**), mp 138—143 °C, which gave no molecular ion but a fragment ion at *m/z* 560 due to a loss of acetic acid in the EI-MS. The ^1H and ^{13}C NMR spectra of **10** were very similar to those of bruceantinol (**5**)[4] except for signals due to the

additional methylene group in the side chain. This fact implies that A-ring of **10** possesses 3-hydroxy-3-en-2-one structure; the signal due to $C_{(4)}$-CH_3 appeared at δ 1.85 as a doublet signal with a small coupling constant (J=2.2 Hz). An anomeric proton was observed at δ 5.28 as a doublet signal (J=8.1 Hz) and a signal due to $C_{(4)}$-CH_3 appeared at δ 1.16 as a doublet signal (J=8.1 Hz) in the ^{1}H NMR spectrum of **4**. Since these spectral features are almost the same as those for yadanzioside G (**9**) and also yadanziosides A,[3b] C,[3b] F,[3a] and J,[3a] the structure of yadanzioside O (**4**) was determined to be methyl 2-(β-D-glucopyranosyloxy)-13β,20-epoxy-15β-[(2*E*)-4-acetoxy-3-ethyl-4-methyl-2-pentenoyloxy]-11β,12α-dihydroxy-3,16-dioxo-1-picrasen-21-oate. Yadanzioside O (**4**) is a bruceoside with the largest carbon number in the 15-*O* side chain so far in nature.

The structure (**10**) proposed for the aglycone of yadanzioside O was confirmly established by the partial synthesis, which was achieved by the reaction of (2*E*)-4-acetoxy-3-ethyl-4-methyl-2-pentenoic acid (**11**) with 3-*O*-(*t*-butyldimethylsilyl)bruceolide (**12**) derived from brusatol (**13**).

Methyl (2*E*)-3-ethyl-4-hydroxy-4-methyl-2-pentenoate (**14**) was prepared by the Wittig-Honer reaction[8] of 2-hydroxy-2-methyl-3-pentanone (**15**)[9] with a Wittig reagent derived from dimethyl (methoxycarbonylmethyl)phosphonate using sodium hydride as a base in dimethoxyethane (DME). The reaction did not occur at room temperature, but took place at boiling temperature to afford the reaction product (**14**) together with a considerable amount of undesirable by-products. Separation of **14** from the reaction mixture was attained by preparative liquid chromatography to afford **14** in 27% yield. Geometry of the double bond of **14** was determined after hydrolysis and acetylation. Although a double bond isomer, the starting material (**15**), and unidentified by-products were obtained from the reaction mixture, neither further purification nor identification for these products was carried out. Hydrolysis of **14** with alkali followed by acetylation yielded 4-acetoxy-3-ethyl-4-methyl-2-pentenoic acid (**11**) in 95% yield from **14**. The synthetic unsaturated acid (**11**) showed a singlet signal due to the olefinic proton at δ 5.85, which was almost the same as the shift value (δ 5.78) observed for yadanzioside O aglycone (**10**). Thus the double bond geometry of **11** was concluded to be 2*E*.

The derivation of the skeletal part and introduction of the side chain into $C_{(15)}$-OH are as follows; brusatol (**13**) was treated with *t*-butyldimethylsilyl chloride in the presence of imidazole in DME to afford 3-*O*-(*t*-butyldimethylsilyl)brusatol in 94% yield, which, on hydrolysis with potassium methoxide in dry methanol, gave 3-*O*-(*t*-butyldimethylsilyl)bruceolide (**12**) in 65% yield.[10] The esterification of the acid (**11**) with **12** was effected in the presence of dicyclohexylcarbodiimide (DCC) in dichloromethane,[11] and the reaction product (**16**) was subjected to deprotection by a treatment with 5% hydrofluoric acid-acetonitrile or with tetrabutylammonium fluoride in tetrahydrofuran (THF) at 0 °C. The synthetic specimen was found to be completely identical with yadanzioside O aglycone (**10**).

Yadanzioside O aglycone (**10**) exhibited a significant antitumor activity against the murine P388 lymphocytic leukemia[12] and the ILS values for the natural specimen were 8.2, 37.1, and 47.2% at 1, 2, and 4 mg/kg/d dose levels, respectively. The ILS values for the synthetic one were 42.3 and 46.4% at 4 and 6 mg/kg/d dose levels, respectively, but a toxicity was observed at 8 mg/kg/d dose level.

Experimental[13]

Extraction and Separation. Pulverized seeds (49.5 kg) of *B. javanica*, "Ya-dan-zi" were defatted with hexane (100 l) twice and then with extracted with methanol (100 l) twice. The methanol extract was concentrated in vacuo to give a syrup, to which the equal volume of water was added. The aqueous solution was completely defatted with hexane (7 l) three times and then extracted with dichloromethane five times. The organic layer was concentrated to give an oily residue (158 g), which was subjected to the following separation.

Column A. The residue (158 g) was dissolved in chloroform and absorbed on silica gel (335 g). After the solvent was removed in vacuo, the silica gel was placed on the top of a column of silica gel (2.5 kg) and elution was performed with the following solvents; benzene (frs 1—3, each 1.5 l), benzene-ethyl acetate (2:1, frs 4—10; 1:1, frs 11—19; 1:2, frs 20—23, each 1.5 l), ethyl acetate (frs 24—30, each 1.5 l), 10% methanol in ethyl acetate (frs 31—35, each 2 l), and 20% methanol in ethyl acetate (frs 36—40, each 2 l).

Column B. The fractions 35—37 of Column A were combined to give a residue (73 g), a part (10.7 g) of which was chromatographed on silica gel (850 g) eluted with lower layer of chloroform-methanol-water in the following ratio; frs 1—6 (25:4:1), frs 7—11 (20:4:1), and frs 11—24 (16:4:1) in 400 ml fraction each.

Column C. A part (55.3 g) of the fractions 35—37 of Column A was separated by column chromatography on silica gel (2.5 kg) eluted with lower layer of chloroform-methanol-water (25:4:1). Thirty fractions (each 500 ml) were collected.

Column D. The fraction 21 of Column C gave a residue (3.8 g), a part (500 mg) of which was separated by reversed phase chromatography using Lobar column Lichroprep RP-8 size B. Elution (each fraction, 10 g) was performed with methanol-water (4:6—5:5). Fractions 55—63 gave yadanzioside O (**4**; 16 mg).

Column E. A part (826 mg) of the fraction 24 (2.2 g) of Column C was subjected to separation using Lobar column eluted with methanol-water (4:6). Eighty-two fraction (each 10 g) were collected. Fractions 62—65 gave yadanzioside M (**2**; 25 mg).

Column. F. Tne fraction 27 (1.2 g) of Column C was separated using Lobar column eluted with methanol-water

(4:6—5:5). Seventy-six fractions (each 10 g) were collected. Fractions 70—76 gave yadanzioside N (**3**; 25 mg).

Column G. The fractions 12 and 13 of Column B were combined and evaporated to afford a residue (5.2 g), a part (1.5 g) of which was separated using Lobar column. Elution (each 10 g) was performed with methanol–water (4:6) and 142 fractions were collected.

Column H. The fractions 64—74 (89 mg) of Column G were combined and separated by gel chromatography using Toyopearl HW-40S. Elution with methanol (each 10 g) gave yadanzioside M (**2**; 22 mg) in fractions 56—62.

Column I. The fractions 127—142 of Column G gave a residue (17 mg), which was separated using Toyopearl HW-40S. Elution (each 10 g) with methanol gave yadanzioside O (**4**; 5 mg).

Column J. The fraction 14 (932 mg) of Column B was purified by partition chromatography on silicic acid (400 g) pretreated with water (270 ml). Elution (each 250 ml) was performed with 0, 3, 6, ···, 24% ethanol in chloroform (each 1 l).

Column K. The fractions 16 and 17 of Column J were combined (524 mg) and chromatographed using a Lobar column. Elution (each 10 g) was performed with methanol–water.

Column L. The fractions 17—24 of Column K were combined and evaporated to afford a residue (291 mg), which was separated using Toyopearl HW-40S. Elution (each 10 g) with methanol gave yadanzioside K (**1**; 24 mg) from fractions 41 and 42.

Yadanzioside K (1). $[\alpha]_D^{23}$ +15° (*c* 1.0, EtOH); $[\alpha]_D^{18}$ −31° (*c* 0.9, C_5H_5N); IR (KBr) 3450, 1740, 1645, and 1065 cm^{-1}; UV (EtOH) 222 nm (ε 17000) and 252 nm (ε 9000); ^1H NMR (Table 1); ^{13}C NMR (Table 2); EI-MS *m/z* (%) 546 ([M−162−60]$^+$: 1.4), 438 (3), 420 (1.4), 402 (1.9), 392 (1.8), 149 (18), 109 (90), 83 (70), 60 (100), and 57 (82); SI-MS *m/z* 769 ([M+H]$^+$); HR-MS (EI) *m/z* 546.2100. Calcd for $C_{28}H_{34}O_{11}$: *m/z* 546.2100.

Enzymatic Hydrolysis of 1. Yadanzioside K (**1**; 67 mg) in water (5 ml) was treated with β-glucosidase (20 mg) at 37 °C for 2 weeks and the usual work-up afforded bruceantinol (**5**; 17 mg) together with the starting material (**1**; 35 mg).

Yadanzioside M (2). $[\alpha]_D^{26}$ +39° (*c* 1.3, EtOH); IR (KBr) 3450, 1740, 1680, 1640, 1075, 1050, and 720 cm^{-1}; UV (EtOH) 231 nm (ε 16000) and 255 nm (ε 9000); ^1H NMR (Table 1); ^{13}C NMR (Table 2); EI-MS *m/z* (%) 542 ([M−162]$^+$; 3.8), 438 (6.6), 420 (3.5), 402 (3.4), 392 (2.4), 374 (2), 369 (2), and 105 (100); SI-MS *m/z* 727 ([M+Na]$^+$); HR-MS (EI) *m/z* 542.1804. Calcd for $C_{28}H_{30}O_{11}$: *m/z* 542.1789.

Enzymatic Hydrolysis of 2. Yadanzioside M (**2**; 60 mg) in water (6 ml) was hydrolyzed with β-glucosidase (28 mg) under the same conditions as before to yield bruceantarin (**6**; 11 mg) and the unchanged material (**2**; 24 mg).

Yadanzioside N (3). $[\alpha]_D^{24}$ +7.6° (*c* 1.8, EtOH); IR (KBr) 3420, 1740, 1680, 1640, 1080, 1045, and 1020 cm^{-1}; UV (EtOH) 222 nm (ε 13400) and 255 nm (ε 5100); ^1H NMR (Table 1); ^{13}C NMR (Table 2); EI-MS *m/z* (%) 548 ([M−$C_6H_{10}O_5$]$^+$; 0.1), 530 (0.8), 438 (0.6), 420 (0.9), 402 (5), 372 (4), 345 (3), 315 (4), and 111 (100); SI-MS *m/z* 733 ([M+Na]$^+$), 549, 531, 439, 421, 185, and 111; HR-MS (EI) *m/z* 548.2264. Calcd for $C_{28}H_{36}O_{11}$: *m/z* 548.2258.

Acid Hydrolysis of 3. A solution of yadanzioside N (**3**; 35 mg) in 1.5 M sulfuric acid–methanol (14 ml) was heated under reflux for 4 h. The reaction product, after removal of the solvent, was extracted with dichloromethane and divided into an organic layer and an aqueous layer. The usual work-up of the organic layer gave a residue, which was purified by column chromatography on silica gel (1 g). Elution with 5% methanol in chloroform yielded an aglycone (**7**; 11 mg), mp 180—183 °C; $[\alpha]_D^{24}$ +30° (*c* 0.7, EtOH); IR (KBr) 3460, 1750, 1685, 1645, 1050, and 1010 cm^{-1}; UV (EtOH) 220 nm (ε 16100) and 267 nm (ε 5500); on addition of potassium hydroxide, the latter maximum shifted to 314 nm (ε 4000); ^1H NMR (Table 1); ^{13}C NMR (Table 2); EI-MS *m/z* (%) 548 (M$^+$; 0.3), 530 (3), 512 (0.2), 420 (1), 402 (10), 372 (6), 345 (4), 315 (4), and 111 (100); HR-MS *m/z* 548.2282. Calcd for $C_{28}H_{36}O_{11}$: M, 548.2257. The aqueous layer, obtained from the acid hydrolysis of **3**, was passed through a short column of anion-exchange resin (Amberlite IRA-400, hydroxide form) and the eluate was evaporated in vacuo to give a residue. The residue was identified as D-glucose by GLC after trimethylsilylation.

Yadanzioside O (4). $[\alpha]_D^{21}$ +20° (*c* 2.0, EtOH); IR (KBr) 3430, 1740, 1680, 1640, 1065, 1050, and 1020 cm^{-1}. UV (EtOH) 224 nm (ε 14900) and 254 nm (ε 8000); ^1H NMR (Table 1); ^{13}C NMR (Table 2); EI-MS *m/z* (%) 560 ([M−$C_6H_{10}O_5$−AcOH]$^+$; 0.7), 438 (3), 420 (0.8), 402 (1), 392 (0.9), 141 (8), 123 (29), and 60 (100); SI-MS *m/z* 805 ([M+Na]$^+$), 561, 439, and 421.

Enzymatic Hydrolysis of 4. Yadanzioside O (**4**; 72 mg) was hydrolyzed with β-glucosidase (20 mg) to afford an aglycone (**10**; 13 mg) together with the starting material (**4**; 33 mg). **10**: Mp 138—143 °C; $[\alpha]_D^{27}$ +23° (*c* 0.6, EtOH); IR (KBr) 3450, 1740, 1640, and 1060 cm^{-1}; UV (EtOH) 222 nm (ε 14800) and 278 nm (ε 7000); ^1H NMR (Table 1); ^{13}C NMR (Table 2); EI-MS *m/z* 560 ([M−AcOH]$^+$; 1.5), 438 (4), 420 (3), 402 (4), 392 (6), 354 (18), 278 (36), 140 (56), 123 (68), 111 (82), and 60 (100); HR-MS *m/z* 560.2258. Calcd for $C_{29}H_{36}O_{11}$: *m/z* 560.2258.

Methyl (2*E*)-3-Ethyl-2-hydroxy-4-methyl-2-pentanoate (14). A Wittig reagent was prepared from dimethyl (methoxycarbonylmethyl)phosphonate (3.4 ml; 4 equiv) and sodium hydride (750 mg; 4 equiv) in DME (60 ml) at room temperature for 1 h under nitrogen atmosphere in a three-necked flask and then the solution was heated under reflux. To the solution 2-hydroxy-2-methyl-3-pentanone (**15**; 500 mg, 4.3 mmol) in DME (10 ml) was added and the heating was continued for 4 h. The usual work-up gave a residue, which was subjected to separation by preparative LC (Liquid Chromatograph LC-08, Japan Analytical Industry, column: JAIGEL-1H and 2H; solvent: chloroform) to afford methyl (2*E*)-3-ethyl-4-hydroxy-4-methyl-2-pentenoate (**14**; 203 mg) as an oil, IR (neat) 3460, 1710, 1640, and 750 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ=1.13 (3H, t, *J*=8 Hz), 1.39 (6H, s), 2.57 (2H, q, *J*=8 Hz), 3.68 (3H, s), and 6.03 (1H, s); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ=14.8q, 22.4t, 29.0q, 29.0q, 50.9q, 77.4s, 112.7d, 167.3s, and 170.6s; EI-MS *m/z* (%) 157 ([M−15]$^+$; 33), 154 (94), 139 (28), 129 (100), 122 (57), 111 (20), 97 (91), and 85 (65).

(2*E*)-4-Acetoxy-3-ethyl-4-methyl-2-pentenoic Acid (11). Methyl (2*E*)-3-ethyl-4-hydroxy-4-methyl-2-pentenoate (**14**; 468 mg) was treated with 5% potassium hydroxide solution (15 ml) at 80 °C for 10 h. Acidification by addition of 0.25 M sulfuric acid and the usual work-up afforded (2*E*)-3-ethyl-4-hydroxy-4-methyl-2-pentenoic acid as an oil quantitatively,

IR (film) 3450, 3300—2500, 1700, and 1640 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ=1.13 (3H, t, J=8 Hz), 1.43 (6H, s), 2.56 (2H, q, J=8 Hz), and 6.07 (1H, s); EI-MS m/z (%) 143 ([M−15]$^+$; 22), 140 (39), 125 (28), 115 (100), and 97 (38).

The carboxylic acid (430 mg) above obtained was acetylated with acetic anhydride (1 ml) in the presence of triethylamine (5 ml) and 4-dimethylaminopyridine (DMAP, 330 mg) at room temperature for 3 h with stirring. To the cooled solution was added 2 M hydrochloric acid (50 ml) and the reaction product was extracted with ether (30 ml×3). The extracts were combined and washed with 2 M hydrochloric acid (30 ml) and then with water. The usual work-up gave a residue, which was purified by column chromatography on silica gel (25 g). Elution with 0—2% methanol in chloroform yielded (2*E*)-4-acetoxy-3-ethyl-4-methyl-2-pentenoic acid (**11**; 95% yield) as an oil, IR (neat) 3500—2500, 1740, 1700, and 1640 cm^{-1}. ^1H NMR (90 MHz, $CDCl_3$) δ=1.13 (3H, t, J=8 Hz), 1.59 (6H, s), 2.62 (2H, q, J=8 Hz), 2.02 (3H, s), and 5.85 (1H, s); ^{13}C NMR (22.5 MHz, $CDCl_3$) δ=14.4q, 21.9q, 21.9q, 26.6q, 26.6q, 82.9s, 113.9d, 169.1s, 169.6s, and 171.0s; EI-MS m/z (%) 158 ([M−42]$^+$; 5), 140 (100), 125 (81), 122 (33), 112 (38), 97 (85), 60 (23), and 43 (56).

Reaction of 3-*O*-(*t*-Butyldimethylsilyl)bruceolide (12) with 11. 3-*O*-(*t*-Butyldimethylsilyl)bruceolide (**12**; 970 mg),[10] DMAP (21 mg), and DCC (362 mg) were dissolved in dry dichloromethane (15 ml) under nitrogen atmosphere and the acid (**11**; 351 mg) in dichloromethane (15 ml) was added. The mixture was stirred at 25 °C overnight and filtered through a column of Celite. The filtrate was washed with water (50 ml), 5% acetic acid (50 ml), and then water (50 ml). The usual work-up gave a residue, which was purified by column chromatography on silica gel (50 g). Elution with 1% methanol in chloroform afforded a 3-*O*-(*t*-butyldimethylsilyl) derivative (**16**; 469 mg) of the aglycone (**10**) together with the starting material (**12**; 579 mg). **16**: Mp 281.5—283 °C, colorless needles from chloroform–acetone; IR (KBr) 3560, 3380, 1745, 1680, 1650, 1060, and 1020 cm^{-1}; ^1H NMR (90 MHz, $CDCl_3$) δ=0.13 (3H, s), 0.17 (3H, s), 0.97 (9H, s), 1.13 (3H, t, J=7 Hz), 1.39 (3H, s), 1.56 (6H, s), 1.85 (3H, brs), 2.01 (3H, s), 3.78 (3H, s), 4.22 (2H, br), 4.62 (1H, d, J=8 Hz), 4.80 (1H, brs), 5.78 (1H, s), and 6.20 (1H, d, J=13 Hz); EI-MS m/z (%) 677 ([M−$(CH_3)_3C$]$^+$; 6), 659 (4), 635 (14), 617 (92), 598 (16), 537 (47), 495 (8), 478 (27), 424 (16), 326 (13), 123 (100), and 60 (58); HR-MS (EI) m/z 677.2656. Calcd for $C_{33}H_{45}O_{13}Si$: m/z 677.2629.

Deprotection of 3-*O*-(*t*-Butyldimethylsilyl) Group of 16. i) A solution of 3-*O*-(*t*-butyldimethylsilyl) derivative (**16**; 50 mg) in acetonitrile–47% hydrofluoric acid (10:1, 3 ml) was stirred at room temperature for 4 h. After addition of dichloromethane and water, the organic layer was separated. The aqueous layer was extracted with dichloromethane twice and the organic layers were combined. The usual work-up gave a residue, which was chromatographed on silica gel (5 g). Elution with 1% methanol in chloroform yielded yadanzioside O aglycone (**10**; 21 mg). ii) To a solution of 3-*O*-(*t*-butyldimethylsilyl) derivative (**16**; 244 mg) in THF (5 ml) was added tetrabutylammonium fluoride (230 mg) in THF (5 ml) at 0 °C under nitrogen atmosphere. The solution was stirred for 30 min at the same temperature and chloroform (100 ml) was added. The organic solution was washed with a saturated sodium hydrogencarbonate solution. The washings were extracted with chloroform (50 ml×2) and the organic solution and the extracts were combined and worked up as usual. Column chromatographic separation on silica gel (15 g, eluted with 3% methanol in chloroform) afforded yadanzioside O aglycone (**10**; 206 mg).

The authors wish to thank Mr. Hideo Naoki of Suntory Institute for Bioorganic Research for measurement of SI-MS.

References

1) A part of this work was reported in a preliminary form: T. Sakaki, S. Yoshimura, T. Tsuyuki, T. Takahashi, T. Honda, and T. Nakanishi, *Tetrahedron Lett.*, **27**, 593 (1986).

2) a) J. Polonsky, Z. Baskévitch, A. Gaudemer, and B. C. Das, *Experientia*, **23**, 424 (1967); b) K. Y. Sim, J. J. Sims, and T. A. Geissman, *J. Org. Chem.*, **33**, 429 (1968); c) K. H. Lee, Y. Imakura, Y. Sumida, R.-Y. Wu, and I. H. Hall, *ibid.*, **44**, 2180 (1979).

3) a) S. Yoshimura, T. Sakaki, M. Ishibashi, T. Tsuyuki, T. Takahashi, and T. Honda, *Bull. Chem. Soc. Jpn.*, **58**, 2673 (1985); b) T. Sakaki, Y. Yoshimura, M. Ishibashi, T. Tsuyuki, T. Takahashi, T. Honda, and T. Nakanishi, *ibid.*, **58**, 2680 (1985).

4) a) J. D. Phillipson and F. A. Darwish, *Planta Med.*, **41**, 209 (1981); b) J. Polonsky, J. Varenne, T Prangé, and C. Pascard, *Tetrahedron Lett.*, **21**, 1853 (1980).

5) S. M. Kupchan, R. W. Britton, J. A. Lacadie, M. F. Ziegler, and C. W. Sigel, *J. Org. Chem.*, **40**, 648 (1975).

6) S. M. Kupchan, R. W. Britton, M. F. Ziegler, and C. W. Siegel, *J. Org. Chem.*, **38**, 178 (1973).

7) T. Murae, T. Tsuyuki, T. Ikeda, T. Nishihama, S. Masuda, and T. Takahashi, *Tetrahedron*, **27**, 1545 (1971).

8) The hydroxy ketone (**15**) was prepared by the Grignard reaction of acetone cyanohydrin 2-ethoxyethyl derivative with ethylmagnesium bromide.

9) a) W. S. Wadsworth, Jr. and W. D. Emmons, *J. Am. Chem. Soc.*, **83**, 1733 (1961); b) J. Boutagy and R. Thomas, *Chem. Rev.*, **74**, 87 (1974); c) K. H. Dahm, B. M. Trost, and H. Röller, *J. Am. Chem. Soc.*, **89**, 5292 (1967).

10) Suntory Ltd., Jpn. Kokai Tokkyo Koho JP 59 67,287 (84 67,287) (cl. C07D493/08) 16Apr 1984, Appl. 82/178,210 09 Oct 1982; 16pp.

11) A. Hassner and V. Alexanian, *Tetrahedron Lett.*, **1978**, 4475.

12) In vivo activity was assayed according to the standard National Cancer Institute procedure.

$$\text{ILS}(\%) = \left(\frac{\text{Mean survival time(day) of the test group}}{\text{Mean survival time(day) of the control group}} - 1\right) \times 100$$

The ILS value of bruceantin (**8**) was 70.1% at 2 mg/kg/d dose level and a toxicity was observed at 4 mg/kg/d dose level.

13) General procedures are the same as those described in lit, 3a.