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Constituents of Seeds of *Brucea javanica*. Structures of New Bitter Principles, Yadanziolides A, B, C, Yadanziosides F, I, J, and L.[1,2]

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Three new quassinoids and four new quassinoid glycosides were isolated from water-soluble fraction of methanol extract of seeds of *Brucea javanica* (L.) MERR, known as "Ya-dan-zi" in Chinese folklore and their structures were determined by spectral and chemical means. Yadanziosides F, I, J, and L were demonstrated to have antileukemic activity.

Quassinoids, bitter principles of Simaroubaceous plants have been extensively investigated from the interest in antileukemic activity and structure determination,[3] and some of them have been shown to exhibit useful biological activities.[4] In connection with our studies on bitter principles of *Picrasma ailanthoides* PLANCHON and *Ailanthus altissima* SWINGLE,[5] constituents of seeds of *Brucea javanica* (L.) MERR were examined and several new bitter principles were reported.[1,2] Seeds of *B. javanica* are known as "Ya-dan-zi" in Chinese folklore and have been used as a Chinese medicine for cancer. The main active compounds of the plant have been extensively investigated by Polonsky,[6] Geissman,[7] and Lee.[8] We examined minor components in the polar fraction. This paper deals with isolation and structure elucidation of new bitter quassinoids, yadanziolides A, B, and C (**1**, **2**, and **3**) and bitter quassinoid glycosides, yadanziosides F, I, J, and L (**4**, **5**, **6**, and **7**).

The methanol extract of defatted seeds of *B. javanica* was partitioned between dichloromethane and water. The aqueous layer was separated by silica-gel column chromatography eluted with a lower layer of chloroform–methanol–water. Each fraction was further purified by gel chromatography on Toyopearl HW-40S eluted with methanol and then a reversed phase chromatography on Lobar column Lichroprep RP-8 eluted with methanol–water. These isolation procedures afforded several new bitter principles together with known compounds, bruceosides A (**8**), B (**9**),[8] bruceins D (**10**), E (**11**),[8,9] and F (**12**).[10]

Yadanziolide A (**1**; *ca.* 0.01% yield) crystallized from methanol as colorless needles, mp 283—285 °C (decomp) and $[\alpha]_D^{28}$ −10.5° (pyridine). Elemental analysis indicates the formula $C_{20}H_{26}O_{10}$. The IR and UV spectra showed the presence of hydroxyl(s), a δ-lactone and an α,β-unsaturated carbonyl groups. Comparison of ^1H and ^{13}C NMR spectra of **1** with those of brucein F (**12**)[10] could lead to the structure (**1**) for yadanziolide A, which is a conjugated ketone corresponding to an oxidation product of brucein F (**12**). Brucein F (**12**) was treated with manganese dioxide in *N,N*-dimethylformamide to give the oxidation product, which was identical with **1** in respect to mp, IR, ^1H NMR, and TLC. Thus the structure of yadanziolide A (**1**) was determined to be 13β,20-epoxy-1β,11β,12α,14,15β,21-hexahydroxypicras-3-ene-2,16-dione.

When treated with acetic anhydride in pyridine at room temperature for 48 h, **1** give a tetraacetyl derivative (**13**), mp 202—203 °C. The ^1H NMR spectrum of **13** indicates that **13** is 1,12,15,21-tetra-*O*-acetylyadanziolide A.

Yadanziolide B (**2**; *ca.* 0.002% yield), mp 279—282 °C (decomp), was shown by elemental analysis to have the molecular formula, $C_{20}H_{26}O_{11}$, which possesses one more oxygen atom than **1**. The IR and UV spectra showed the presence of hydroxyl(s), a δ-lactone, and an α,β-unsaturated carbonyl groups. In the 400 MHz ^1H NMR and COSY spectra, all protons were assigned unambiguously (Table 1). A doublet signal at δ 5.70 (J=1.5 Hz) due to H-7 and a double-doublet signal at δ 4.24 (J=11.5 Hz) due to H-6 are shifted to a lower field than those of yadanziolide A (**1**). These observations suggest that the structure of yadanziolide B (**2**) is 6-hydroxy-substituted yadanziolide A (**1**). The coupling constants, $J_{5,6}$=11.5 Hz and $J_{6,7}$=1.5 Hz, suggest a trans-relationship between H-6 and an α(axial)-proton on C-5, and therefore the hydroxyl group on C-6 was determined to be in 6α(equatorial)-configuration. This assignment is firmly established by the fact that the NOE was observed between H-6 and $C_{(10)}$-CH_3 in the NOESY spectrum.

Yadanziolide B (**2**) was acetylated with acetic anhydride in pyridine to afford a pentaacetate (**14**), mp 278—283 °C (decomp). The ^1H NMR spectrum showed two methyl signals at δ 1.52 (s, 10-CH_3) and 2.03 (br s, 4-CH_3) together with signals due to acetoxyl groups at δ 1.96 (3H, s), 2.09 (6H, s), and 2.22 (6H, s). Thus the structure of yadanziolide B (**2**) was concluded to be 13β,20-epoxy-1β,6α,11β,12α,14,

15β,21-heptahydroxypicras-3-ene-2,16-dione.

Yadanziolide C (**3**; *ca.* 0.001% yield) crystallized from methanol–diethyl ether as colorless prisms, mp 292—297 °C (decomp). The elemental analysis indicates the molecular formula, $C_{20}H_{26}O_9$, which is the same as that of brucein D (**10**).[8,9] The IR and UV spectra showed the presence of hydroxyl(s), a δ-lactone, and an α,β-unsaturated carbonyl groups. The ^{1}H NMR spectrum revealed that the signals of H-1, H-5, and H-9 of yadanziolide C (**3**) shifted to a lower field than those of brucein D (**10**; see Table 2). Comparison of ^{13}C NMR spectra showed that the signals of C-5 and C-9 of **3** (δ 37.7 and 38.3, respectively) shifted to a higher field than those of **10** (δ 43.6 and 45.8, respectively).

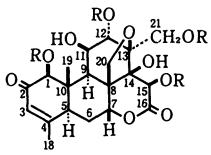
When treated with acetic anhydride in pyridine at room temperature for 1 week, **3** gave 1,15-di-*O*-acetyl derivative (**15**; 35% yield) and 1,12,15-tri-*O*-acetyl derivative (**16**; 52% yield). Under the same conditions, brucein D (**10**) gave 1,12,15-tri-*O*-acetyl derivative in 96% yield.

These evidences suggest that the configuration of C-1, C-5, or C-9 might be defferent from that of brucein D (**10**). The extensive double resonance experiments at 400 MHz led to an unambiguous assignment of all protons for **3** (Table 2). Two broad doublet signals due to H-1 and H-12 were assigned as follows. Two signals at δ 4.46 and δ 4.66 were changed into broad singlets on addition of D_2O. Irradiation at δ 1.63 due to $C_{(10)}$–CH_3 resulted in a slight sharpening of the broad doublet at δ 4.66, while the signal at δ 4.46 remained unchanged. Therefore, the broad doublet signals at δ 4.66 and δ 4.46 are assigned to H-1 and H-12, respectively.

The coupling constants, $J_{5,6\alpha}$=3 Hz and $J_{5,6\beta}$=13.5 Hz, being the same as those of other picrasane derivatives, the proton at C-5 was determined to be in α(axial)-configuration.

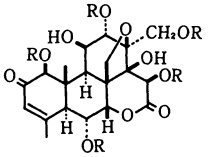
The configurations at $C_{(1)}$–H and $C_{(9)}$–H were determined by differential NOE measurement. On saturation of the signal due to $C_{(10)}$–CH_3, increases in area of signals due to $C_{(1)}$–H and one of $C_{(20)}$–H_2 were observed, while an increase in area of the signal due to $C_{(9)}$–H was not detected, indicating $C_{(1\beta)}$–H and $C_{(9\alpha)}$–H orientations.

Thus the structure of yadanziolide C (**3**) was established to be 13β,20-epoxy-1α,11β,12α,14,15β-pentahydroxypicras-3-ene-2,16-dione, which corresponds to 1-epibrucein D.



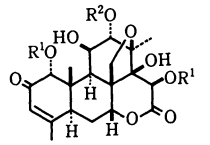
1 R=H

13 R=Ac



2 R=H

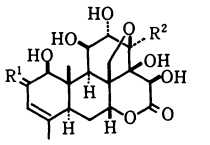
14 R=Ac



3 R^1-R^2-H

15 R^1-Ac, R^2-H

16 R^1-R^2-Ac



10 R^1=O, R^2=CH_3

11 R^1=α-OH, β-H, R^2=CH_3

12 R^1=α-OH, β-H; R^2=CH_2OH

Table 1. ^{1}H NMR Spectra of Yadanziolides A (**1**) and B (**2**) in C_5D_5N

		1[a]		**2**[b]	
		δ	*J*/Hz	δ	*J*/Hz
1-H		4.32 s [d]		4.35 s [d]	
3-H		6.10 br s		6.22 br s	
5-H		3.06 br d	12	3.39 br d	11.5
6-H	(6α-H)	2.31 dd	14, 3	4.24 dd	11.5, 1.5
	(6β-H)	c)			
7-H		5.44 br s		5.70 br s	
9-H		2.89 d	5	3.00 br s	
11-H		5.33 d	5	5.48 br s	
12-H		4.71 s [d]		4.79 s [d]	
15-H		6.06 s		6.21 s	
20-H		4.29 d	8	4.42 d	7
20-H′		5.00 d	8	5.06 d	7
21-H		4.63 d	12	4.71 d	11.5
21-H′		5.03 d	12	5.08 d	11.5
4-CH_3		1.75 br s		2.51 br s	
10-CH_3		1.43 s		1.62 s	

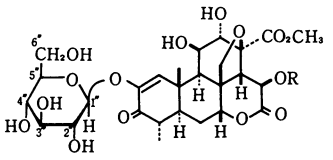
a) Measured at 90 MHz. b) Measured at 400 MHz. c) Not assignable. d) The assignments of these signals described in Ref. 1 were found to be revised.

Yadanzioside F (**4**; *ca.* 0.03% yield), a bitter glycoside, mp 202—207 °C, showed the presence of a secondary methyl, a tertiary methyl, an acetoxyl, and a methoxycarbonyl groups in 90 MHz ^{1}H NMR spectrum. The ^{13}C NMR spectrum and a peak observed at *m/z* 643 in the secondary ion mass spectrometry (SIMS) suggested the molecular formula, $C_{29}H_{38}O_{16}$, for **4**, which was supported by the following observations. In the EI mass spectrum, an abundant fragment ion at *m/z* 480 (M^+—162) indicated the presence of a hexose moiety. Yadanzioside F (**4**) was hydrolyzed with β-glucosidase to give brucein B (**17**).[10,11] Since a doublet signal due to $C_{(4)}$–CH_3 of the aglycone part of **4** was observed at δ 1.15 (*J*=5 Hz), it is indicated that the brucein B moiety isomerizes into a 3-keto-1-ene structure and the glycoside linkage is formed through an oxygen atom on C-2. The structure of yadanzioside F (**4**) was therefore formulated as 2-*O*-(β-D-glucosyl)brucein B.

Table 2. ^{1}H NMR Spectra of Yadanziolide C (**3**) and Brucein D (**10**) in C_5D_5N

	3[a]		**10**[b]	
	δ	J/Hz	δ	J/Hz
1-H	4.66 d	3	4.30 s [d]	
3-H	6.09 br s		6.10 br s	
5-H	3.60 br d	13.5	3.05 br d	13
6α-H	2.38 ddd	13.5, 3, 3	2.30 dt	14, 2
6β-H	1.72 ddd	13.5, 13.5, 3	c)	
7-H	5.52 t	3	5.47 t	2
9-H	3.62 d	5.5	2.84 d	5
11-H	4.97 t	5.5	5.37 d	5
12-H	4.46 d	3	4.52 s [d]	
15-H	6.03 s		6.04 s	
20-H	4.39 d	7.5	4.20 d	8
20-H′	5.01 d	7.5	4.91 d	8
4-CH_3	1.71 br s		1.74 br s	
10-CH_3	1.63 s		1.46 s	
13-CH_3	2.05 s		2.07 s	
-OH	5.50 d	5.5		
	7.05 s			
	7.53 d	3		
	7.66 br s			
	8.23 d	3		

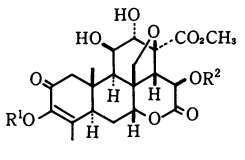
a) Measured at 400 MHz. b) Measured at 90 MHz. c) Not assignable. d) The assignments of these signals described in Ref. 1 were found to be revised.



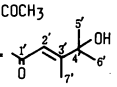
4 R=COCH$_3$

6 R= 

8 R= 



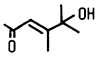
5 R^1=β-Glc, R^2=COCH$_3$

7 R^1=β-Glc, R^2= 

9 R^1=β-Glc, R^2= 

17 R^1=H, R^2=COCH$_3$

18 R^1=H, R^2= 

19 R^1=H, R^2= 

Yadanzioside I (**5**; *ca.* 0.02% yield) crystallized from ethanol as colorless needles, mp 287—290 °C (decomp). The molecular formula, $C_{29}H_{38}O_{16}$, was inferred by a peak at m/z 643 in the SIMS together with the ^{13}C NMR spectrum and hydrolysis with β-glucosidase giving brucein B (**17**). In the ^{1}H NMR spectrum, the $C_{(4)}$-CH_3 was observed at δ 2.04 as a singlet. In ^{13}C NMR spectrum, glycosylation shifts (see Table 3) of the signals due to C-3 and C-4 were observed, respectively, indicating that D-glucose is attached at C-3 of the aglycone. Thus the structure of yadanzioside I (**5**) was determined to be 3-*O*-(β-D-glucosyl)brucein B.

Yadanzioside J (**6**; *ca.* 0.002% yield), mp 198—202 °C, was suggested to have a molecular formula, $C_{32}H_{44}O_{17}$ by SIMS and ^{13}C NMR spectrum. The IR and UV spectra showed the presence of hydroxyl(s), a δ-lactone, and an α,β-unsaturated carbonyl groups. In the ^{1}H NMR spectrum, a doublet signal due to $C_{(4)}$-CH_3 of the aglycone part was observed at δ 1.16 (J=6.6 Hz), and a doublet signal due to the anomeric proton of the sugar part was observed at δ 5.35 (J=7.3 Hz). In the ^{13}C NMR spectrum, all of carbons were assigned (see Table 3). These indicate that the structure of **6** must be a 2-β-D-glucoside like bruceoside A or yadanzioside F and the aglycone must be 15-*O*-(3-hydroxy-3-methylbutanoyl)bruceolide.

On hydrolysis with 1.5 M sulfuric acid (1 M=1 mol dm^{-3})-methanol (1:2), **6** gave the aglycone (**18**) and D-glucose. The latter was identified as its trimethylsilyl derivative by GLC. The aglycone (**18**) was shown to have the molecular formula, $C_{26}H_{34}O_{12}$ by high-resolution mass spectrum. By comparison of the ^{1}H and ^{13}C NMR spectra with those of known bruceolides, the signals δ 1,34 (6H, s) and 2.43 (2H, s) in the ^{1}H NMR, and δ 170.7s, 48.6t, 69.2s, 29.8s, and 29.9s in the ^{13}C NMR (see Table 3) were well assigned to 3-hydroxy-3-methylbutanoate moiety. Thus the structure of **18** was determined to be 15-*O*-(3-hydroxy-3-methylbutanoyl)bruceolide, which is corresponding to 3′-hydroxybrucein A. From these evidences, the structure (**6**) is proposed for yadanzioside J.

Yadanzioside L (**7**; *ca.* 0.01% yield), mp 199—204 °C, was suggested to have a molecular formula, $C_{34}H_{46}O_{17}$, by a peak observed at m/z 727 in the SIMS and the ^{13}C NMR spectrum. On hydrolysis with 1.5 M sulfuric acid-methanol, **7** afford brucein C (**19**)[6,11,12] and D-glucose. The former was identified in respect to mp, IR, and ^{1}H NMR, and the latter was identified as its trimethylsilyl derivative by GLC examination. Glycosylation shifts of the signals due to C-3 and C-4 were observed in ^{13}C NMR, respectively, (see Table 3) which implies that D-glucose is attached at C-3 of the aglycone. An anomeric proton was observed at δ 5.40 as a doublet (J=7 Hz) in the ^{1}H NMR spectrum. Thus the structure of yadanzioside L (**7**) was determined to be 3-*O*-(β-D-glucosyl)-brucein C.

Yadanziosides F (**4**), I (**5**), J (**6**), and L (**7**) were demonstrated to have *in vivo* antileukemic activity against the murine P-388 lymphocytic leukemia at same strength as bruceoside A (**8**).

Experimental

General Procedures. All melting points were measured

Table 3. ^{13}C NMR Spectra of Yadanziosides F (**4**), I (**5**), J (**6**), L (**7**), bruceins B (**17**),[15] C (**19**),[15] and 15-*O*-(3-hydroxy-3-methylbutanoyl)-bruceolide (**18**)

No. of Carbon	4[a]	5[a]	6[a]	7[a]	17[b]	19[c]	18[a]
1	129.5 d	51.0 t	129.5 d	51.1 t	48.7	47.8	50.1 t
2	148.8 s	193.6 s	148.9 s	193.6 s	192.9	193.0	193.0 s
3	194.5 s	146.8 s [d]	194.5 s	146.6 s [d]	144.1	144.3	146.0 s
4	43.9 d	148.0 s [d]	43.8 d	147.9 s [d]	128.3	129.4	128.2 s
5	40.6 d	43.4 d	40.7 d	43.4 d	39.9	41.1	41.4 d
6	30.0 t	29.3 t	30.0 t	29.4 t	28.7	29.1	29.6 t
7	83.6 d	83.5 d	83.6 d	83.4 d	82.8	83.2	83.8 d
8	46.6 s	46.0 s	46.8 s	46.0 s	44.7	45.5	46.3 s
9	41.4 d	42.1 d	41.4 d	42.1 d	40.4	41.6	42.4 d
10	39.6 s	40.8 s	39.6 s	40.8 s	40.9	41.1	42.5 d
11	73.4 d	72.9 d	73.5 d	73.0 d	71.4	71.2	73.1 d
12	76.1 d	76.0 d	76.0 d	76.0 d	74.7	75.4	75.8 d
13	82.7 s	82.7 s	82.7 s	82.7 s	81.4	81.6	82.8 s
14	50.5 d	50.3 d	50.5 d	50.5 d	48.7	49.7	50.4 d
15	68.8 d	68.7 d	68.7 d	68.3 d	67.3	66.7	68.6 d
16	168.0 s	168.0 s	168.1 s	168.2 s	167.0	168.0	168.1 s
18	12.6 q	15.3 q	12.5 q	15.3 q	13.3	13.1	13.4 q
19	18.0 q	15.8 q	17.9 q	15.9 q	15.0	15.2	15.7 q
20	73.7 t	73.5 t	73.7 t	73.6 t	72.3	73.6	73.7 t
21	171.3 s	171.3 s	171.2 s	171.2 s	169.9	168.0	171.3 s
OMe	52.3 q	52.4 q	52.4 q	52.4 q	52.3	52.7	52.4 q
1′	169.7 s	169.8 s	170.7 s	166.4 s	168.7	165.9	170.7 s
2′	20.6 q	20.6 q	48.6 t	112.8 d	20.4	111.5	48.6 t
3′			69.2 s	168.2 s		171.3	69.2 s
4′			29.7 q	73.2 s		73.6	29.8 q
5′			29.8 q	28.9 q		27.9	29.9 q
6′				28.9 q		27.9	
7′				15.5 q		15.2	
1″	102.0 d	104.8 d	102.1 d	104.9 d			
2″	74.7 d	75.9 d	74.7 d	75.9 d			
3″	78.8 d [e]	78.5 d [e]	78.8 d [e]	78.6 d [e]			
4″	71.3 d	71.5 d	71.4 d	71.6 d			
5″	78.5 d [e]	78.3 d [e]	78.5 d [e]	78.4 d [e]			
6″	62.4 t	62.8 t	62.4 t	62.9 t			

a) Measured in C_5D_5N at 22.5 MHz. b) Measured in $(CD_3)_2SO$. c) Measured in $CDCl_3$. d, e) Signals within any vertical column may be reversed.

on a Mel-temp capillary melting point apparatus (Laboratory Devices) and uncorrected. Optical rotations were determined on a JASCO polarimeter DIP-181. Infrared (IR) spectra were measured on a Hitachi 260-30 spectrometer. Ultraviolet absorption (UV) spectra were measured on a Hitachi 340 spectrometer. Low and high resolution mass spectra (MS and HRMS, respectively) were run on a JEOL JMS-D300 mass spectrometer operating at 70 eV and secondary ion mass spectra (SIMS) on a Hitachi M-80 mass spectrometer. Proton nuclear magnetic resonance (^1H NMR) spectra (90 MHz) were taken using a Varian EM390 and carbon-13 nuclear magnetic resonance (^{13}C NMR) spectra (22.5 MHz) JEOL FX 90Q. Measurement of ^1H NMR spectra at 400 MHz was carried out on a JNM GX 400 (JEOL) spectrometer. Chemical shifts were expressed in δ-value (ppm downfield from tetramethylsilane as an internal standard) and coupling constants in Hz. Thin-layer chromatography (TLC) was carried out on Kieselgel 60 GF_{254} or Kieselgel 60 HF_{254} silasiert coated in 0.25 mm or on TLC plates Kieselgel 60/Kieselgur F_{254} (E. Merck). Wakogel C-200 (Wako), TSK-gel Toyopearl HW-40S (Toyo Soda), or Lobar column Lichroprep RP-8 size B (E. Merck) was used for column chromatography. Commercialized β-glucosidase available from Sigma Chemical Company was used.

Plant Material. Seeds of *Brucea javanica* (L.) Merr as Chinese medicine, "Ya-dan-zi", were ground to afford 60 kg of materials.

Extraction and Separation. Seeds (60 kg) were defatted with haxane (*ca.* 100 l) twice, and then extracted with methanol (*ca.* 100 l) twice. The extract was concentrated to give a syrup, to which the same volume of water was added, and defatted with hexane (*ca.* 7 l) four times. The aqueous layer was extracted five times with dichloromethane. The aqueous layer, on evaporation, gave a bitter residue (*ca.* 1 kg). A part of the residue (53 g) was absorbed on silica gel

(60 g), placed on a top of a silica-gel (1.2 kg) column, and eluted with a lower layer of chloroform–methanol–water (50:12:3; *ca.* 9 l, 65:35:10; *ca.* 9 l, 50:50:18; *ca.* 12 l), and 40 fractions (each 700 ml) were collected (Column A).

The each fraction was further separated by the following procedures.

Procedure A: Gel chromatography using Toyopearl HW-40S (2.4×100 cm). Elution was performed with methanol, and fractions (each 10 g) were collected by an automatic fraction collector. Flow rate was about 0.2 ml/min.

Procedure B: Reversed phase chromatography using Lobar column Lichroprep RP-8 size B. Elution was performed with methanol–water, and fractions were collected by an automatic fraction collector. Flow rate was about 1 ml/min. The ratio in volume of methanol–water is given in each chromatographic procedure.

Fractions 8 and 9 (1.02 g) of Column A were combined and separated by Procedure A. Fractions 44—52 (730 mg; Fraction A) and fractions 59—65 (Fraction B; 149 mg) were combined respectively on the basis of TLC examination. Recrystallization of Fraction B from methanol gave brucein D (**10**).

Fractions 10 and 11 (1.24 g) of Column A were combined together and separated by Procedure A. On the basis of TLC, fractions 32—36 (467 mg; Fraction C), fractions 37—41 (193 mg; Fraction D), fractions 42—49 (193 mg; Fraction E), and fractions 50—55 (241 mg; Fraction F) were combined, respectively.

Fraction A and Fraction C were combined and separated by Procedure B (methanol–water, 1:1). Fractions 13—25 (790 mg) were combined and identified to be bruceoside A (**8**).[7] Fractions 26—35 (138 mg) were combined and evaporated to give yadanzioside A (**20**).[2]

Fraction D was separated by Procedure B (methanol–water, 3:7). Fractions 18—23 and fractions 24—45 were identified to be yadanziolide C (**3**; 20 mg) and yadanzioside F (**4**; 58 mg), respectively.

Fraction E was recrystallized from methanol to give brucein D (**10**; 150 mg).

Fraction 12 and 13 (1.63 g) of Column A were separated by Procedure A. Fractions 38—47 (1.18 g; Fraction G) and fractions 52—57 (243 mg; Fraction H) were collected.

Fraction G was separated by Procedure B (methanol–water, 1:1). Fractions 17—23 were combined to give yadanziolide C (**3**; 59 mg). Fractions 24—36 (960 mg) were combined and further separated by Procedure B (methanol–water, 3:7). Fractions 24—51 were combined and evaporated to give yadanzioside F (**4**; 534 mg), and fractions 52—80 were combined and identified to be bruceoside B (**9**; 85 mg).[8]

Fraction F and Fraction H were combined and recrystallized from methanol to afford yadanziolide A (**1**; 410 mg).

Fractions 14 and 15 (1.98 g) of Column A were separated by Procedure A. Fractions 38—47 (1.57 g) were further sepatated by Procedure B (methanol–water, 3:7). Fractions 24—45 (419 mg) were ascertained to be a mixture of yadanzioside F (**4**), brucein E (**11**), and yadanzioside D (**21**)[2] by TLC examination (Kieselgel 60 HF_{254} silasiert, methanol–water, 4:6). Fractions 46—90 (761 mg) were also identified to be a mixture of yadanzioside D (**21**) and bruceoside B (**9**).

Fraction 16 of Column A was crystallized from methanol to give crystalline brucein E (**11**; 1.2 g). The mother liquor (1.16 g) was separated by Procedure A. Fractions 33—38 (372 mg; Fraction I) and fractions 39—43 (353 mg; Fraction J) were combined, respectively. Fraction I was further separated by Procedure B (methanol–water, 3:7). Fractions 44—46 were combined to give yadanzioside J (**6**; 50 mg) and fractions 47—53 were identified to be yadanzioside C (**22**; 200 mg). Fraction J was also separated by Procedure B (methanol–water, 3:7). Fractions 15—23 were combined to give yadanzioside I (**5** 178 mg) and fractions 24—50 were combined and evaporated to give brucein E (**11**; 150 mg).

Fractions 17 and 18 of Column A were crystallized from methanol to give crystalline brucein E (**11**; 450 mg). The mother liquor (2.7 g) was separated by Procedure A. Fractions 35—40 (935 mg; Fraction K), fractions 41—48 (526 mg; Fraction L) and fractions 52—56 (71 mg; Fraction M) were combined, respectively, on the basis of TLC examination. Fraction K was separated by Procedure B (methanol–water, 3:7). Yadanzioside I (**5**; 79 mg) was given from fractions 16—21, brucein E (**11**; 273 mg) from fractions 22—42, and yadanzioside L (**7**; 192 mg) from fractions 43—56. Fraction L was separated by Procedure B (methanol–water, 3:7). Fractions 15—23 were combined to give yadanzioside I (**5**; 178 mg) and fractions 24—50 gave brucein E (**11**; 220 mg).

Fractions 19 and 20 (2.36 g) of Column A were separated by Procedure A. Fractions 43—50 (880 mg; Fraction N) and fractions 51—70 (220 mg; Fraction O) were combined, respectively. Fraction N was crystallized from methanol to give brucein F (**12**).[10] Fraction M and Fraction O were combined and separated by procedure B (methanol–water, 1:1) to give yadanziolide B (**2**; 70 mg) from fractions 12—15.

Yadanziolide A (1). Colorless needles crystallized from methanol, mp 283—285 °C (decomn); $[\alpha]_D^{28}$ −10.5° (*c* 1.7, pyridine); IR (KBr) 3430, 1700, 1645, 1620, and 1025 cm^{-1}; UV (methanol) 240 nm (ε 9300); ^1H NMR (Table 1); ^{13}C NMR (C_5D_5N, 22.5 MHz) δ=11.5q, 22.2q, 28.0t, 43.6d, 45.7d, 48.6s, 50.7s, 64.7t, 70.6t, 70.7d, 75.4d, 78.3d, 79.1d, 83.0d, 83.8s, 84.6s, 124.9d, 163.5s, 174.9s, and 198.4s; MS (EI) *m/z* (%) 426 (M^+; 0.4), 408 (2), 390 (34), 372 (16), 151 (100), and 135 (88); Found: C, 56.04; H, 6.16%. Calcd for $C_{20}H_{26}O_{10}$: C, 56.33; H, 6.15%.

Oxidation of Brucein F (12) with Manganese Dioxide. A solution of brucein F (**12**; 52.6 mg) in *N,N*-dimethylformamide (10 ml) was treated with manganese dioxide (1 g, prepared by Attenburrow's procedure[13]) for 24 h at room temperature. After the usual work-up, the reaction mixture was separated by silica-gel column chromatography (C-200, 12 g) eluted with a lower layer of chloroform–methanol–water (50:12:3). The oxidation product, yadanziolide A (**1**; 9.2 mg) was identified by mp, IR, ^1H NMR, and TLC, and the starting material, brucein F (**12**; 11 mg) was recovered.

1,12,15,21-Tetra-O-acetylyadanziolide A (13). Yadanziolide A (**1**; 25 mg) was acetylated with acetic anhydride (1.5 ml) in pyridine (1.5 ml) at room temperature for 64 h. Addition of methanol and the usual work-up afforded a reaction mixture, which was purified by silica-gel column chromatography (C-200, 10 g) eluted with dichloromethane–ethyl acetate (2:1) to give 1,12,15,21-tetra-*O*-acetylyadanzi-

olide A (**13**; 34 mg), mp 202—203 °C (colorless prisms crystallized from methanol–diethyl ether); IR (KBr) 3430, 1750, 1685, 1630, 1380, 1245, and 1055 cm^{-1}; UV (ethanol) 239 nm (ε 9000); ^1H NMR ($CDCl_3$ 90 MHz) δ=1.38 (3H, s, 10-CH_3), 1.97 (6H, s, 4-CH_3 and OAc), 2.09 (3H, s, OAc), 2.18 (3H, s, OAc), 2.34 (1H, d, J=5 Hz, 9-H), 3.04 (1H, br d, J=11 Hz, 5-H), 4.00 (1H, d, J=5 Hz, 11-H), 4.02, 4.68 (each 1H, d, J=8 Hz, 20-H), 4.30, 4.49 (each 1H, d, J=13 Hz, 21-H), 5.04 (1H, s, 12-H), 5.16 (1H, br s, 7-H), 5.40 (1H, s, 1-H), 6.02 (1H, br s, 3-H), and 6.09 (1H, s, 15-H); MS (EI) m/z (%) 594 (M^+, 1), 576 (0.6), 552 (2), 534 (10), 492 (10), 474 (22), 432 (100), 267 (25), 151 f(25), 135 (26), and 60 (28); Found: m/z 594.1953. Calcd for $C_{28}H_{34}O_{14}$: M, 594.1948.

Yadanziolide A Monohydrate. Colorless prisms crystallized from aqueous methanol, mp 310—314 °C (decomp), IR (KBr) 3540, 3440, 1750, 1665, 1625, 1160, and 1075 cm^{-1}; Found: C, 53.78; H, 6.38%. Calcd for $C_{20}H_{26}O_{10}\cdot H_2O$: C, 54.05; H, 6.34%.

Yadanziolide B (2). Colorless needles crystallized from ethanol–diethyl ether, mp 279—282 °C (decomp); $[\alpha]_D^{23}$ +83° (c 4.3, methanol); IR (KBr) 3500, 3360, 1710, 1645, 1630 (sh), 1255, 1125, and 1070 cm^{-1}; UV (methanol) 244 nm (ε 10000); ^1H NMR (Table 1); ^{13}C NMR (C_5D_5N, 22.5 MHz) δ=12.5q, 27.1q, 44.6d, 49.2d, 51.1s, 51.2s, 64.6t, 68.2d, 70.0t, 70.9d, 75.5d, 78.6d, 83.3d, 83.8s, 83.8d, 84.3s, 126.9d, 166.4s, 174.7s, and 198.2s; MS (EI) m/z (%) 442 (M^+, 0.8), 424 (12), 406 (28), 388 (10), 378 (36), 360 (12), and 111 (100); Found: C, 54.11; H, 5.77%. Calcd for $C_{20}H_{26}O_{11}$: C, 54.30; H, 5.92%.

1,6,12,15,21-Penta-O-acetylyadanziolide B (14). Yadanziolide B (**2**; 27 mg) was acetylated with acetic anhydride (1.5 ml) in pyridine (1.5 ml) at room temperature for 5 d. The usual work-up and silica-gel column chromatography (dichloromethane–ethyl acetate, 2:1) yielded, after crystallization from methanol–diethyl ether, a pentaacetate (**14**; 28 mg), as colorless needless, mp 278—283 °C (decomp); IR (KBr) 3460, 1740, 1680, 1640, 1375, 1240, and 1030 cm^{-1}; UV (ethanol) 239 nm (ε 10000); ^1H NMR ($CDCl_3$, 90 MHz) δ=1.52 (3H, s, 10-CH_3), 1.96 (3H, s, OAc), 2.03 (3H, br s, 4-CH_3, 2.09 (6H, s, OAc), 2.22 (6H, s, OAc), 2.37 (1H, d, J=6 Hz, 9-H), 3.10 (1H, br d, J=11 Hz, 5-H),4.07 (1H, d, J=6 Hz, 11-H), 4.15, 4.70 (each 1H, d, J=8 Hz, 20-H), 4.27, 4.50 (each 1H, d, J=12 Hz, 21-H), 5.03 (1H, s, 12-H), 5.08 (1H, dd, J=11, 2 Hz, 6-H), 5.10 (1H, d, J=2 Hz, 7-H), 5.45 (1H, s, 1-H), 6.07 (1H, br s, 3-H), and 6.12 (1H, s, 15-H); MS (EI) m/z (%) 652 (M^+, 0.8), 634 (0.8), 610 (1.2), 592 (6), 567 (8), 550 (14), 532 (13), 490 (22), 472 (10), 430 (18), and 60 (100); Found: m/z 652.2031. Calcd for $C_{30}H_{36}O_{16}$: M, 652.2004.

Yadanziolide C (3). Colorless prisms crystallized from methanol–diethyl ether, mp 292—297 °C (decomp); $[\alpha]_D^{23}$ +29° (c 1.2, MeOH); IR (KBr) 3575, 3550, 3500, 3420, 1705, 1675, 1620, 1160, and 1060 cm^{-1}; UV (methanol) 244 nm (ε 11000); ^1H NMR (Table 2); ^{13}C NMR (C_5D_5N, 22.5 MHz) δ=15.0q, 19.5q, 22.0q, 28.0t, 37.7d, 38.3d, 44.6s, 49.7s, 70.5d, 70.5t, 73.2d, 76.2d, 79.7d, 82.0s, 82.6s, 85.5s, 125.0d, 161.6s, 174.9s, and 198.4s; MS (EI) m/z (%) 410 (M^+, 4), 392 (64), 374 (32), 151 (60), and 135 (100); Found: C, 58.27; H, 6.36%. Calcd for $C_{20}H_{26}O_9$: C, 58.53; H, 6.39%.

Acetylation of Yadanziolide C (3). Yadanziolide C (**3**; 21 mg) was treated with acetic anhydride (1 ml) and pyridine (1 ml) at room temperature for 1 week. After the usual work-up, the reaction mixture was separated by silica-gel column chromatography (C-200, 10 g) eluted with dichloromethane–ethyl acetate (2:1) to give the diacetate (**15**; 8.8 mg) and the triacetate (**16**; 14.2 mg). 1.15-Di-*O*-acetylyadanziolide C (**15**): mp 299—303 °C (colorles prisms from methanol–diethyl ether); IR (KBr) 3600, 3500, 1740, 1725, 1660, 1620, 1235, and 1075 cm^{-1}; UV (methanol) 246 nm (ε 10000); ^1H NMR ($CDCl_3$, 90 MHz) δ=1.37 (3H, s, 10-CH_3), 1.47 (3H, s, 13-CH_3), 1.97 (3H, br s, 4-CH_3), 2.14 (3H, s, OAc), 2.21 (3H, s, OAc), 2.42 (1H, br d, J=12 Hz, 6α-H), 2.50 (1H, 9-H), 3.11 (1H, br d, J=12 Hz, 5-H), 3.9—4.1 (3H, 11-H, 12-H, and 20-H), 4.51 (1H, d, J=8 Hz, 20-H'), 5.10 (1H, br s, 7-H), 5.15 (1H, s, 1-H), 5.92 (1H, br s, 3-H), and 6.40 (1H, s, 15-H); MS (EI) m/z (%) 494 (M^+, 0.4), 476 (0.8), 434 (3), 416 (3), 392 (2), 374 (15), 356 (5), 135 (100), and 60 (100); Found: m/z 494.1776. Calcd for $C_{24}H_{30}O_{11}$: M, 494.1787. 1,12,15-Tri-*O*-acetylyadanziolide C (**16**): mp 183—188 °C (powder); IR (KBr) 3450, 1750, 1680, 1640, 1380, 1235, 1070, and 1040 cm^{-1}; UV (methanol) 246 nm (ε 10000); ^1H NMR ($CDCl_3$, 90 MHz) δ=1.30 (3H, s, 10-CH_3), 1.41 (3H, s, 13-CH_3), 1.97 (3H, br s, 4-CH_3), 2.00 (3H, s, OAc), 2.11 (3H, s, OAc), 2.23 (3H, s, OAc), 2.45 (1H, br d, J=12 Hz, 6α-H), 3.05 (1H, br d, J=12 Hz, 5-H), 3.97, 4.55 (each 1H, d, J=8 Hz, 20-H), 4.10 (1H, d, J=5 Hz, 11-H), 4.89 (1H, d, J=1.5 Hz, 12-H), 5.18 (1H, br s, 7-H), 5.27 (1H, s, 1-H), 5.95 (1H, br s, 3-H), and 6.13 (1H, s, 15-H); MS (EI) m/z (%), 536 (M^+, 2), 494 (3), 476 (5), 458 (1), and 135 (100); Found: m/z 536.1917. Calcd for $C_{26}H_{32}O_{12}$: M, 536.1894.

Yadanzioside F (4). Amorphous solid, mp 202—207 °C; $[\alpha]_D^{23}$ +10° (c 4.1, ethanol); IR (KBr) 3450, 1740, 1680, 1630, and 1065 cm^{-1}; UV (ethanol) 256 nm (ε 6500); ^1H NMR (C_5D_5N, 90 MHz) δ=1.15 (3H, s, J=5 Hz, 4-CH_3), 1.63 (3H, s, 10-CH_3), 2.10 (3H, s, 15-OAc), 3.81 (3H, s, $COOCH_3$), 6.76 (1H, d, J=13 Hz, 15-H), and 7.22 (1H, s, 1-H); ^{13}C NMR (Table 3); MS (SIMS) 643 (MH^+), 481, and 439; Found m/z 480.1614. Calcd for $C_{23}H_{28}O_{11}$ (M−$C_6H_{10}O_5$): m/z 480.1629.

Enzymatic Hydrolysis of Yadanzioside F (4). A mixture of yadanzioside F (**4**; 143 mg) and β-glucosidase (41 mg) in water (10 ml) was allowed to stand for 2 weeks at 37 °C. Methanol (5 ml) was added and the mixture was heated at boiling-water temperature for 10 min. After being cooled, the mixture was filtered and the filtrate was evaporated *in vacuo*. The residue was separated by silica-gel column chromatography (chloroform–methanol, 9:1) to yield a pure compound (**17**; 37 mg). The physical and spectral data of **17** (IR, ^1H NMR, MS) are in agreement with literature[11] for brucein B (**17**). Found: m/z 480.1618. (M−$C_6H_{10}O_5$): m/z 480.1629.

Yadanzioside I (5). Colorless needles crystallized from ethanol, mp 287—290 °C (decomp); $[\alpha]_D^{28}$ −21° (c 1.0, methanol); IR (KBr) 3450, 1755, 1650, 1620 (sh), and 1065 cm^{-1}; UV (ethanol) 254 nm (ε 9700); ^1H NMR (C_5D_5N, 90 MHz) δ=1.69 (3H, s, 10-CH_3), 2.04 (3H, s, 4-CH_3), 2.10 (3H, s, 15-OAc), 3.81 (3H, s, $COOCH_3$), and 6.80 (1H, d, J=13 Hz, 15-H); ^{13}C NMR (Table 3); MS (SIMS) m/z 643 (MH^+) and 481; Found: m/z 480.1646. Calcd for $C_{23}H_{28}O_{11}$ (M−$C_6H_{10}O_5$): m/z 480.1629.

Enzymatic Hydrolysis of Yadanzioside I (5). A mixture of yadanzioside I (47 mg) and β-glucosidase (23 mg) in water (5 ml) was treated by the same procedure

as before. Brucein B (**17**; 11 mg) was isolated and identified as the sole aglycone.

Yadanzioside J (6). Amorphous solid, mp 198—202 °C; $[\alpha]_D^{22}$ −6.4° (*c* 2.8, methanol); IR (KBr) 3430, 1740, 1680, 1640, and 1075 cm^{-1}; UV (methanol) 254 nm (ε 8000); ^1H NMR (C_5D_5N, 400 MHz) δ=1.16 (3H, d, *J*=6.6 Hz, 4-CH_3), 1.61 (6H, s, 3′-CH_3×2), 1,64 (3H, s, 10-CH_3), 2.01 (1H, br d, *J*=15.0 Hz, 5-H), 2.52 (1H, d, *J*=5.1 Hz, 9-H), 2.88, 2.94 (each 1H, d, *J*=13.7 Hz, 2′-H), 3.80 (3H, s, $COOCH_3$), 3.90, 5.07 (each 1H, d, *J*=7.7 Hz, 20-H), 4.48 (1H, br d, *J*=10.3 Hz, 6″-H), 4.94 (1H, br s, 11-H), 5.13 (1H, br s, 12-H), 5.18 (1H, br s, 7-H), 5.35 (1H, d, *J*=7.3 Hz, 1″-H), and 7.27 (1H, s, 1-H); ^{13}C NMR (Table 3); MS (SIMS) *m/z* 701 (MH^+), 683, 539, 521, and 439; Found: *m/z* 538.2068. Calcd for $C_{26}H_{34}O_{12}$ (M−$C_6H_{10}O_5$): *m/z* 538.2051.

Acid Hydrolysis of Yadanzioside J (5). A solution of **5** (42 mg) in 1.5 M sulfuric acid–methanol (1:2, 6 ml) was refluxed for 5 h. The reaction mixture was concentrated *in vacuo* and extracted with dichloromethane four times. The combined dichloromethane layers were dried over anhydrous magnesium sulfate and evaporated under reduced pressure to give a product (21 mg) which was separated by silica-gel column chromatography (C-200, 10 g) eluted with methanol–chloroform (1:9) to give a pure compound (20 mg). This compound crystallized from methanol–diethyl ether to afford 15-*O*-(3-hydroxy-3-methylbutanoyl)-bruceolide (**18**) as colorless prisms: mp 251—254 °C (decomp); $[\alpha]_D^{19}$ +13° (*c* 1.4, methanol); IR (KBr) 3575, 3460, 1745, 1730, 1675, 1650, 1195, 1150, and 1070 cm^{-1}; UV (methanol) 278 nm (ε 8000); ^1H NMR ($CDCl_3$, 90 MHz) δ=1.34 (6H, s, 3′-CH_3×2), 1.39 (3H, s, 10-CH_3), 1.84 (3H, d, *J*=2 Hz, 4-CH_3), 2.40, 2.95 (each 1H, d, *J*=18 Hz, 1-H), 2.43 (2H, s, 2′-H_2), 3.13 (1H, d, *J*=13 Hz, 14-H), 3.75, 4.73 (each 1H, d, *J*=7 Hz, 20-H), 3.82 (3H, s, $COOCH_3$), 4.23 (2H, br s, 11-H and 12-H), 5.77 (1H, br s, 7-H), and 6.38 (1H, d, *J*=13 Hz, 15-H); ^{13}C NMR (Table 3); MS (EI) *m/z* (%) 538 (M^+, 1.5), 520 (12), 480 (26), 438 (50), 420 (20), 402 (20), 392 (28), 297 (46), 151 (74), and 83 (100); Found: *m/z* 538.2048. Calcd for $C_{23}H_{34}O_{12}$: M, 538.2051.

The aqueous layer, obtained from the acid hydrolysis of **5**, was passed through a short column of anion-exchange resin (Amberlite IRA-400, hydroxide form) and the eluate was evaporated under reduced pressure to give a residue which was identified as D-glucose (as trimethylsilyl derivative) by gas-liquid chromatography.

Yadanzioside L (7). Colorless needles crystallized from methanol–diethyl ether, mp 199—204 °C; $[\alpha]_D^{26}$ −0.7° (*c* 6.2, methanol); IR (KBr) 3420, 1735, 1640, and 1060 cm^{-1}; UV (methanol) 220 nm (ε 14000) and 254 nm (ε 9000); ^1H NMR (C_5D_5N, 90 MHz) δ=1.43 (6H, s, 4′-CH_3×2), 1.69 (3H, s, 3′-CH_3) 2.03 (3H, s, 4-CH_3), 2.38 (3H, s, 10-CH_3), 3.0—3.3 (2H, 1-H, and 14-H), 3.71 (3H, s, $COOCH_3$), 5.40 (1H, d, *J*=7 Hz, anomeric-H), 6.70 (1H, s, 2′-H), and 6.85 (1H,d, *J*=13 Hz, 15-H); ^{13}C NMR (Table 3); MS (SIMS) *m/z* 727 (MH^+) and 565; Found *m/z* 564.2182. Calcd for $C_{28}H_{36}O_{12}$ (M−$C_6H_{10}O_5$): *m/z* 564.2205.

Acid Hydrolysis of Yadanzioside L (7). The same treatment of a solution of **7** (94 mg) in 1.5 M sulfuric acid–methanol (1:2, 10 ml) as that described above led to the isolation of an aglycone (**19**; 47 mg). The physical and spectral data of **19** are in agreement with those[11] for brucein C (**19**). Found: *m/z* 564.2230. Calcd for $C_{28}H_{36}O_{12}$: M, 564.2205.

D-Glucose was also identified from aqueous layer by the same procedure described above.

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References

1) S. Yoshimura, T. Sakaki, M. Ishibashi, T. Tsuyuki, T. Takahashi, K. Matsushita, and T. Honda, *Chem. Pharm. Bull.*, **32**, 4698 (1984).

2) T. Sakaki, S. Yoshimura, M. Ishibashi, T. Tsuyuki, T. Takahashi, T. Honda, and T. Nakanishi, *Chem. Pharm. Bull.*, **32**, 4702 (1984).

3) J. Polonsky, *Fortschr. Chem. Org. Naturst.*, **30**, 101 (1973).

4) I. H. Hall, Y. F. Liou, K. H. Lee, S. G. Chaney, and W. Willingham, Jr., *J. Pharm. Sci.*, **72**, 626 (1983); C. Moretti, J. Polonsky, M. Vuilhorgne, and T. Prangé, *Tetrahedron Lett.*, **23**, 647 (1982) and references cited therein.

5) M. Ishibashi, S. Yoshimura, T. Tsuyuki T. Takahashi, A. Itai, and Y. Iitaka, *Bull. Chem. Soc. Jpn.*, **57**, 2885 (1984); S. Yoshimura, M. Ishibashi, T. Tsuyuki, T. Takahashi, and K. Matsushita, *Bull. Chem. Soc. Jpn.*, **57**, 2496 (1984) and references cited therein.

6) J. Polonsky, Z. Baskévitch, A. Gaudemer, and B. C. Das, *Experientia*, **23**, 424 (1967).

7) K. Y. Sim, J. J. Sims, and T. A. Geissman, *J. Org. Chem.*, **33**, 429 (1968).

8) K. H. Lee, Y. Imakura, Y. Sumida, R. -Y. Wu, and I. H. Hall, *J. Org. Chem.*, **44**, 2180 (1979).

9) J. Polonsky, Z. Baskévitch, M. B. Das, and J. Müller, *C. R. Acad. Sci., Ser. C*, **267**, 1346 (1968).

10) J. Polonsky, Z. Baskévitch, and J. Müller, *C. R. Acad. Sci., Ser. C*, **268**, 1392 (1969).

11) J. D. Phillipson and F. A. Darwish, *Planta Med.*, **41**, 209 (1981).

12) J. Polonsky, J. Varenne, T. Prangé and C. Pascard, *Tetrahedron Lett.*, **21**, 1853 (1980).

13) J. Attenburrow, A. F. B. Cameron, J. H. Chapman, R. M. Evans, B. A. Hems, A. B. A. Jensen, and T. Walker, *J. Chem. Soc.*, **1952**, 1094.

14) J. Polonsky and Z. Baskévitch, *J. Org. Chem.*, **40**, 2499 (1975).