Structures of New Quassinoid Glycosides, Yadanziosides A, B, C, D, E, G, H, and New Quassinoids, Dehydrobrusatol and Dehydrobruceantinol from *Brucea javanica* (L.) MERR[1)]

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(Received April 16, 1985)

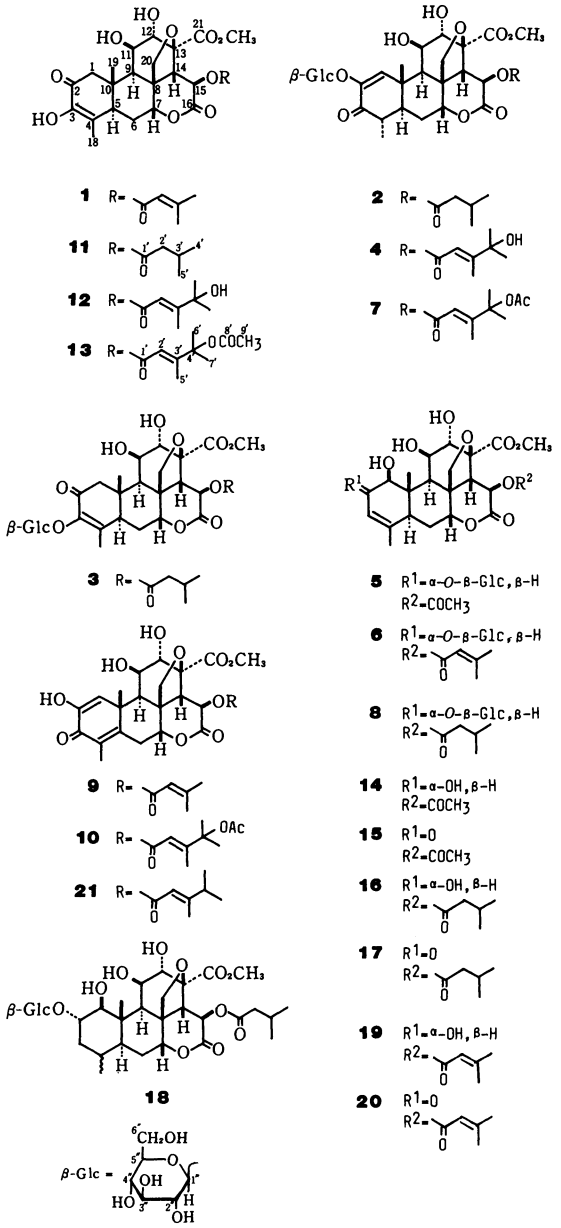
Quassinoid glycosides, yadanziosides A, B, C, D, E, G, and H, and two quassinoids, dehydrobrusatol and dehydrobruceantinol were isolated from seeds of *Brucea javanica*, and their structures were determined. These glycosides showed bitter taste and were demonstrated to have antileukemic activity.

In the preceding paper,[2)] the structure determination of three new quassinoids, yadanziolides A, B, and C, isolated from "Ya-dan-zi", seeds of *Brucea javanica* (L.) MERR was reported. As a continuation of studies on bitter principles of "Ya-dan-zi", quassinoid glycosides were examined, since our preliminary examination revealed the presence of many glycosides in the polar fraction of the methanol extract. Three quassinoid glycosides, bruceoside-A and -B[3)] and bruceantinoside A[4)] have been isolated from the same source as antileukemic constituents.

The methanol extract was subjected to separation to give seven new glycosides, yadanziosides A (**2**; 0.01%), B (**3**; 0.002%), C (**4**; 0.001%), D (**5**; 0.001%), E (**6**; 0.002%), G (**7**; 0.01%), and H (**8**; 0.001%), and two new quassinoids, dehydrobrusatol (**9**; 0.0002%) and dehydrobruceantinol (**10**; 0.0001%) together with brusatol(**1**),[5)] dehydrobrucein-A,[6)] brucein D,[7)] brucein E,[7)] bruceoside-A,[3)] bruceoside-B,[3)] bruceantinoside A,[4)] yadanziolide A,[2)] and yadanzioside F.[2)]

Yadanzioside B (**3**), mp 189—195 °C, showed a peak at *m/z* 707 due to $[M+Na]^+$ in SIMS, from which the molecular formula, $C_{32}H_{44}O_{16}$, was deduced. Its IR and UV spectra showed the presence of hydroxyl, δ-lactone, and conjugated enone moieties. An appearance of a peak at *m/z* 522.2082 ($[M-C_6H_{10}O_5]^+$) in the EI-high-resolution mass spectrum and the presence of a doublet signal (J=7.3 Hz) at δ 5.46 due to an anomeric proton in the ^{1}H NMR spectrum suggested that **3** should be a β-D-glucopyranoside. ^{1}H and ^{13}C NMR spectra (Tables 1 and 2) of **3** revealed that the aglycone must be brucein A (**11**)[6,8)] and glycosylation shifts of signals due to C-3 and C-4 were Δδ +1.9 and +18.1 ppm,[9,10)] respectively, in ^{13}C NMR spectra of **3** and **11**, leading to the structure assignment of yadanzioside B as 3-*O*-(β-D-glucopyranosyl)brucein A (**3**).

This assignment was supported by hydrolysis of **3**; treatment with 1.5 M (1 M=1 mol dm^{-3}) sulfuric acid afforded brucein A (**11**) and hydrolysis with hydrogen chloride in methanol yielded methyl D-glucoside, which was identified as its trimethylsilyl derivative by GLC.



Carbon numbers of picrasane skeleton refer to those described in Chemical Abstracts and numbers of carbon atoms in the side-chain at C-15 are given conventionally.

Yadanzioside A (**2**), mp 200—204 °C, was shown to be a glucoside with the same molecular formula as that of **3** by SIMS. On hydrolysis with β-glucosidase, **2** afforded brucein A (**11**) as the major aglycone. The ^{1}H NMR spectrum of **2** showed the presence of a doublet signal (J=6 Hz) due to $C_{(4)}$-CH_3 at δ 1.13. This fact implies the brucein A moiety isomerizes into a 3-keto-1-ene structure, which forms the β-D-glucoside linkage through an oxygen atom on C-2 of the aglycone. The configuration of the $C_{(4)}$-CH_3 of **2** was assigned as β-equatorial on the basis of the structural similarity to bruceoside-A.[3] Thus the structure of yadanzioside A was determined to be 2-*O*-(β-D-glucosyl)brucein A (**2**).

Yadanzioside C (**4**), mp 204—209 °C, gave a peak at m/z 749 due to $[M+Na]^+$ in the SIMS, which corresponds to the molecular formula $C_{34}H_{46}O_{17}$ and a peak at m/z 564.2230 ($[M-C_6H_{10}O_5]^+$) in the EI-high-resolution mass spectrum. Acid hydrolysis of **3** with 1.5 M sulfuric acid in methanol gave brucein C (**12**)[6,8,11] as the aglycone. On treatment with hydrogen chloride in methanol yielded methyl D-glucoside. The ^{1}H NMR spectrum of **4** showed a doublet signal (J=7.0 Hz) at δ 5.37 due to an anomeric proton and a doublet signal (J=6.7 Hz) due to $C_{(4)}$-CH_3 at δ 1.19. These observations led to the structure, 2-*O*-(β-D-glucopyranosyl)brucein C (**4**) for yadanzioside C.

Yadanzioside G (**7**), mp 180—185 °C, showed the presence of three tertiary methyl groups, one secondary methyl, one olefinic methyl, one acetoxyl, and one methoxycarbonyl groups in the ^{1}H NMR spectrum. The molecular formula, $C_{36}H_{48}O_{18}$, was suggested by a peak at m/z 791 due to $[M+Na]^+$ in the SIMS. On hydrolysis with β-glucosidase, **7** afforded bruceantinol (**13**).[6,11] A doublet signal (J=6 Hz) due to $C_{(4)}$-CH_3 at δ 1.17 indicates the sugar moiety in yadanzioside G (**7**) was linked to C-2 of bruceantinol (**13**). Therefore, the structure of yadanzioside G is formulated as 2-*O*-(β-D-glucopyranosyl)bruceantinol (**7**).

Yadanzioside D (**5**), mp 207—212 °C, exhibited the IR-absorption bands due to hydroxyl and δ-lactone moieties, but no UV-absorption maximum characteristic of a conjugated enone moiety. In the EI-mass spectrum of **5**, a peak at m/z 480 derived from an aglycone was observed and the ^{1}H NMR spectrum suggests the presence of an acetoxyl group. Yadanzioside D (**5**) was hydrolyzed with β-glucosidase to yield the aglycone (**14**), whose molecular formula, $C_{23}H_{30}O_{11}$, was given by the high-resolution mass spectrum. The structure of **14** was determined by oxidation with manganese dioxide in *N*,*N*-dimethylformamide (DMF) leading to isobrucein B (**15**).[12,13] In the ^{1}H NMR spectrum of **14**, the signal due to $C_{(1)}$-H appeared at δ 4.01 as a doublet and its coupling constant between $C_{(1)}$-H and $C_{(2)}$-H was 8 Hz. A glycosylation shift ($\Delta\delta$ +11.0 ppm) due to

Table 1. ^{1}H NMR Spectra[a] of yadanziosides (**2**—**8**), **9**, **10**, and **13**.

	2[b]	**3**[c]	**4**[c]	**5**[b]	**6**[b]	**7**[b]	**8**[b]	**9**[d]	**10**[d]	**13**[e]
1-H	7.22 s	3.25 d (16.2)	7.29 s	f)	f)	7.25 s	f)	6.56 s	6.56 s	f)
2-H	—	—	—	4.45 m	4.42 m	—	4.42 m	—	—	—
3-H	—	—	—	5.72 brs	5.69 brs	—	5.69 brs	—	—	—
15-H	6.84 d (13)	6.9 br	6.9 br	6.78 d (12)	6.76 d (13)	6.81 d (13)	6.82 d (13)	f)	f)	6.23 d (13)
$C_{(4)}$-Me	1.13 d (6)	2.04 d (1.2)	1.19 d (6.7)	1.43 brs	1.43 brs	1.17 d (6)	1.42 brs	2.03 s	2.04 s	1.86 d (2)
$C_{(10)}$-Me	1.61 s	1.71 s	1.63 s	1.43 brs	1.43 brs	1.64 s	1.42 brs	1.66 s	1.66 s	1.41 s
CO_2Me	3.84 s	3.84 s	3.73 s	3.77 s	3.72 s	3.89 s	3.79 s	3.78 s	3.81 s	3.81 s
2′-H	f)	f)	6.77 d (1.2)	—	5.82 brs	6.06 s	f)	5.60 brs	5.74 d (1.2)	5.75 s
3′-H	0.96 d (6)	0.95 d (6.7)	2.40 brs	—	1.67 s	2.26 s	0.94 d (6)	2.16 d (1.2)	2.11 d (1.2)	2.14 d
	0.99 d (6)	0.98 d (6.7)		—	2.14 s		0.97 d (6)	1.91 d (1.2)		
4′-H	—	—	1.41 s	—	—	1.42 s	—	—	1.50 s	1.55 s
			1.42 s			1.46 s			1.52 s	1.55 s
4′-OAc	—	—	—	—	—	1.95 s	—	—	2.01 s	2.03 s
Anomeric-H	f)	5.46 (7.3)	5.37 d (7.0)	4.93 d (8)	4.94 d (6)	f)	f)	—	—	—

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

Table 2. ^{13}C NMR Spectra (22.5 MHz, C_5D_5N) of yadanziosides (YS's) and some their aglycones

No. of carbon	YSA(2)	YSB(3)	YSC(4)	YSD(5)	YSE(6)	YSG(7)	YSH(8)	9[a]	10[b]	11	14	16	19
1	129.6 d	51.1 t	129.3 d	84.9 d	84.8 d	129.7 d	84.9 d	122.3 d	121.9 d	50.1 t	84.5	84.5	84.3
2	148.8 s	193.6 s	149.2 s	84.3 d	84.2 d	148.8 s	84.3 d	145.8 s	145.8 s	192.2 s	73.3	73.3	73.3
3	194.6 s	147.9 s [c]	194.5 s	124.3 d	124.3 d	194.7 s	124.3 d	180.9 s	180.9 s	146.0 s	126.5	126.5	126.5
4	43.8 d	146.2 s [c]	43.8 d	135.8 s	135.9 s	43.9 d	135.8 s	130.8 s	130.9 s	128.1 s	135.8	135.8	135.8
5	40.6 d	43.3 d	40.5 d	43.1 d [c]	43.1 d [c]	40.4 d	43.1 d	156.8 s	156.3 s	42.4 d	43.4[c]	43.4	43.4[c]
6	30.0 t	29.3 t	30.0 t	28.3 t	28.3 t	30.1 t	28.3 t	32.5 t	32.4 t	29.6 t	28.6	28.6	28.7
7	83.5 d	83.5 d	83.5 d	81.2 d	81.2 d	83.5 d	81.2 d	83.9 d	83.7 d	83.7 d	82.3	82.3	82.4
8	46.6 s	46.1 s	46.7 s	46.7 s [d]	46.7 s [d]	46.6 s	46.7 s [c]	45.9 s	45.8 s	46.2 s	46.8[d]	46.9[c]	46.8[d]
9	41.3 d	42.2 d	41.4 d	43.0 d [c]	42.9 d [c]	41.4 d	43.1 d	41.4 d	41.4 d	42.4 d	43.3[c]	43.4	43.3[c]
10	39.5 s	40.8 s	39.7 s	44.4 s [d]	44.4 s [d]	39.6 s	44.4 s [c]	43.3 s	43.2 s	41.4 s	44.6[d]	44.6[c]	44.7[d]
11	73.4 d	73.1 d	73.6 d	75.5 d [e]	75.6[e]	73.4 d	75.5 d [d]	73.5 d [c]	75.9 d [c]	73.1 d	75.8	75.6[d]	75.8[e]
12	75.9 d	76.0 d [d]	76.1 d	75.6 d [e]	75.7[e]	76.0 d	75.7 d [d]	75.9 d [c]	77.2 d [c]	75.8 d	75.8	75.9[d]	75.9[e]
13	82.6 s	82.7 s	82.6 s	82.5 s	82.5 s	82.6 s	82.5 s	81.7 s	81.6 s	82.8 s	82.6	82.6	82.6
14	50.4 d	50.6 d	50.3 d	50.6 d	50.6 d	50.2 d	50.8 d	51.0 d	51	50.7 d	50.4	50.7	50.4
15	68.4 d	68.4 d	68.4 d	68.8 d	68.2 d	68.7 d	68.4 d	66.1 d	66.1 d	68.4 d	68.9	68.6	68.4
16	168.1 s	168.1 s	168.2 s [c]	168.0 s	168.2 s	168.0 s	168.2 s	166.5 s	166.1 s	168.1 s	168.0	168.2	168.3
18	12.5 q	15.3 q	12.6 q	20.7 q	20.8 q	12.5 q	20.7 q	10.9 q	11.9 q	13.4 q	20.9	20.9	21.0
19	17.9 q	15.8 q	18.0 q	12.1 q	12.1 q	18.0 q	12.1 q	23.6 q	23.7 q	15.7 q	12.3	12.3	12.4
20	73.1 t	73.6 t	73.8 t	74.1 t	74.1 t	73.7 t	74.1 t	73.2 t	73.4 t	73.8 t	74.1	74.1	74.1
21	171.1 s [c]	171.6 s [e]	171.2 s	171.5 s	171.4 s	171.7 s	171.6 s	171.5 s	171.5 s	171.6 s	171.6	171.7	171.5
OMe	52.3 q	52.4 q	52.4 q	52.2 q	52.2 q	52.6 q	52.2 q	53.0 q	53.4 q	52.3 q	52.3	52.3	52.2
1′	171.6 s [c]	171.2 s [e]	166.5 s	169.7 s	165.3 s	165.7 s	171.4 s	164.6 s	165.5 s [d]	171.3 s	169.7	171.5	165.4
2′	43.3 t	43.3 t	112.9 d	20.6 q	116.0 d	113.7 d	43.3 t	114.3 d	111.8 d	43.3 t	20.6	43.4	116.1
3′	25.8 d	25.9 d	168.3 s [c]		158.2 s	169.5 s	25.8 d	160.6 s	169.6 s	25.9 d		25.9	158.1
4′	22.4 q	22.4 q	73.2 s		27.0 q	82.3 s	22.5 q	27.5 q	82.2 s	22.4 q		22.5	27.0
5′	22.5 q	22.5 q	15.6 q		20.2 q	14.5 q	22.4 q	20.6 q	14.5 q	22.5 q		22.5	20.2
6′			28.9 q			26.4 q			26.3 q				
7′			28.9 q			25.8 q			26.0 q				
8′						163.3 s			164.7 s [d]				
9′						21.4 q			21.6 q				
1″	102.0 d	104.9 d	102.0 d	107.0 d	106.9 d	102.0 d	107.0 d						
2″	74.5 d	75.7 d [d]	74.7 d	76.1 d	76.0 d	74.6 d	76.1 d						
3″	78.7 d [d]	78.6 d [f]	79.0 d [d]	78.7 d [f]	78.6 d [f]	78.8 d [d]	78.7 d [e]						
4″	71.3 d	71.6 d	71.3 d	71.7 d	71.6 d	71.4 d	71.6 d						
5″	78.3 d [d]	78.4 d [f]	78.6 d [d]	78.6 d [f]	78.5 d [f]	78.4 d [d]	78.6 d [e]						
6″	62.3 t	62.8 t	62.4 t	62.8 t	62.7 t	62.4 t	62.8 t						

a) Measured in $CDCl_3$ at 22.5 MHz. b) Measured in $CDCl_3$ at 100 MHz. c) The assignments of these signals may be reversed in each column. d), e), and f) are the same as c).

C-2 was observed in the ^{13}C NMR spectra of **5** and **14**. These observations suggest that the configuration of $C_{(2)}$-OH of **14** is α-equatorial and the glycoside linkage forms through the C-2 oxygen atom, leading to the structure, 2-β-D-glucoside of the allylic alcohol (**14**) derived from isobrucein B (**15**) for yadanzioside D (**5**).

Yadanzioside H (**8**), mp 180—185 °C, showed a peak at *m/z* 709 due to $[M+Na]^+$ in the SIMS, indicating the molecular formula, $C_{32}H_{46}O_{16}$, and was hydrolyzed with β-glucosidase to give an aglycone (**16**). The aglycone (**16**) showed a similar ^1H NMR spectrum to that of **14**, except for the presence of an isopropyl group (δ 0.94 and 0.97, each d, J=6 Hz) instead of the acetyl group (δ 2.09, s) for **14**. Therefore, the structure (**16**) was deduced for the aglycone and the proposed structure was confirmed by oxidation with manganese dioxide yielding isobrucein A (**17**).[14]

In the ^{13}C NMR spectra of **8** and **16**, a glycosylation shift ($\Delta\delta$ +11.0 ppm) was observed at C-2, indicating the structure of yadanzioside H (**8**) should be assigned as a 2β-D-glucoside of the aglycone (**16**) derived from isobrucein A (**17**).

On hydrogenation, yadanzioside H (**8**) yielded a dihydro derivative (**18**), $C_{32}H_{48}O_{16}$, as the sole product judging from ^{13}C NMR, although the configuration of $C_{(4)}$-CH_3 could not be assigned.

Yadanzioside E (**6**), mp 190—195 °C, was inferred to be a 2′,3′-dehydro derivative of yadanzioside H (**8**) from the following observations. The molecular formula, $C_{32}H_{44}O_{16}$, was determined by the peak at *m/z* 707 due to $[M+Na]^+$ in the SIMS and the presence of $(CH_3)_2C$=CH- grouping (δ 1.67 (3H, s), 2.14 (3H, s), and 5.82 (1H, brs)) was revealed by the ^1H NMR spectrum.

On hydrolysis with β-glycosidase, yadanzioside E (**6**) afforded an aglycone (**19**), the ^1H NMR spectrum being compatible with the proposed structure. The aglycone (**19**) was oxidized with manganese dioxide in DMF to give a keto alcohol (**20**). The molecular formula, $C_{26}H_{32}O_{11}$, of **20** was determined by high-resolution mass spectrum and the presence of the $(CH_3)_2C$=CH- grouping was confirmed by a peak at *m/z* 83 characteristic of $[(CH_3)_2C=CH-C^+=O]$ together with ^1H NMR spectrum. Thus the structure of yadanzioside E (**6**) was proposed to be a 2β-D-glucoside of the aglycone (**19**). The proposed structure was firmly established as follows. Yadanzioside E (**6**) was hydrogenated over 10% palladium on carbon in ethanol to give a tetrahydro derivative, which was completely identical with the dihydro derivative (**18**) obtained from yadanzioside H (**8**).

Prior to yadanziosides, a fraction containing new quassinoids was eluted from the silica-gel column chromatography of the filtrate obtained by the separation of brusatol (**1**) (*loc. cit.*). The fraction was further subjected to separation by silica-gel column chromatography and then by reversed-phase preparative TLC to give new quassinoids, dehydrobrusatol (**9**) and dehydrobruceantinol (**10**).

Dehydrobrusatol (**9**), mp 157—160 °C, gave the molecular formula, $C_{26}H_{30}O_{11}$, in EI-high-resolution mass spectrum, which is two hydrogen atoms less than that of brusatol (**1**). The mass spectrum also showed prominent peaks at *m/z* 436 ($[M-82]^+$), and *m/z* 83, which indicate the presence of $(CH_3)_2C$=CH-CO- grouping. Dehydrobrusatol (**9**) exhibited the UV absorption maximum at 256 nm, which shifted to 340 and 262 nm on addition of alkali. This behavior is characteristic of dehydro-type quassinoids, such as dehydrobruceantin (**21**),[12] which shows the UV maxima at 259 and 225 nm and the former absorption shifts to 340 and 263 nm on addition of alkali. The ^1H NMR spectrum of **9** is similar to that of dehydrobruceantin (**21**) except for the side chain. These observations led to the structure (**9**) for dehydrobrusatol and the structure was supported by chemical transformation; brusatol (**1**) was treated with 2,3-dichloro-5,6-dicyano-*p*-benzoquinone (DDQ) in boiling benzene to give a dehydro product in 64% yield, which was identical with dehydrobrusatol (**9**).

Dehydrobruceantinol (**10**), mp 152—154 °C, showed the peak at *m/z* 627 due to $[M+Na]^+$ in the SIMS, which corresponds to the molecular formula, $C_{30}H_{36}O_{13}$. The EI-high-resolution mass spectrum gave a dehydration peak at *m/z* 586.2064. The ^1H NMR spectrum of **10** measured at 400 MHz was given in Table 1. Comparison with dehydrobrusatol (**9**) as to the skeletal structure and with bruceantinol (**13**) as to the side chain led to the construction of the structure (**10**) for dehydrobruceantinol.

Yadanziosides A, B, C, D, E, and G showed antileukemic activities against the murine P388 lymphocytic leukemia at a 10 mg/kg dose level and their ILS values[15] were 7.1, 4.1, 2.0, 9.2, 7.1, and 4.1%, respectively. These activities were found to be slightly stronger than that (ILS value; 2.0%) of bruceoside A.

Experimental[16]

Extraction and Separation. Pulverized seeds (60 kg) of *B. javanica* were defatted with hexane (100 l) twice and then 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 extracted with dichloromethane five times. The organic layer was concentrated to give a residue, which was dissolved in methanol kept at *ca.* 60 °C. The methanolic solution was allowed to stand at room temperature to afford brusatol (**1**) as crystals, which was filtered off. The filtrate was evaporated to give an oily residue (160 g), a part (36 g) of which was separated by the following procedures.

Column A. The oily residue (36 g) was subjected to separation by silica-gel (575 g) column chromatography.

Elution (each fraction 1 l) with PhH–AcOEt (2:1) gave fractions 1—7, PhH–AcOEt (1:1) fractions 8—16, AcOEt fractions 17—22, and AcOEt–MeOH fractions 23—32. Fraction 20 afforded brucein D (736 mg).

Column **B**. The fraction 9 of Column **A** yielded brusatol (**1**; 4.1 g) as crystals on standing, which was filtered off. The mother liquor, on evaporation, gave a residue (980 mg), which was chromatographed on silica gel (150 g) eluted with 5% MeOH in $CHCl_3$ (each fraction 30 ml). The fraction 20 (56 mg) was further separated by preparative TLC (RP-2, developed with H_2O–MeOH (6:4) twice) to give dehydrobrusatol (**9**; 13.6 mg), dehydrobrucein A (7.5 mg), and dehydrobruceantinol (**10**; 5.5 mg).

Column **C**. The fractions 23 and 24 of Column **A** were combined and evaporated to afford a residue (17.6 g), a part (10.7 g) of which was chromatographed on silica gel (865 g). Low layers of the following solvent-ratios of $CHCl_3$–MeOH–H_2O were used for elution (each fraction 400 ml): fractions 1—6; 25:4:1, fractions 7—10; 20:4:1, and fractions 11—24; 16:4:1.

Column **D**. The fractions 12 and 13 (1.5 g) of Column **C** were subjected to separation by a Lobar column (RP-8) eluted with MeOH–H_2O (4:6) and a total of 142 fractions (each 10 g) were collected. Fractions 30—63 gave bruceoside A (792 mg). Yadanziosides A (**2**; 151 mg) and G (**7**; 300 mg) were obtained from fractions 75—86 and 88—111, respectively. Fractions 113—125 afforded bruceantinoside A (24 mg).

Column **E**. The fraction 14 (932 mg) of Column **C** was purified by partition chromatography on silicic acid (400 g) absorbed with water (270 ml). Elution (each fraction 250 ml) was performed with 0, 3, 6,···24% EtOH in $CHCl_3$ (each 1 l).

Column **F**. The fractions 16 and 17 of Column **E** were combined (524 mg) and chromatographed using a Lobar column (RP-8). Elution was carried out with MeOH–H_2O and 28 fractions (each 10 g) were collected.

Column **G**. The fractions 17—24 (291 mg) of Column **F** were chromatographed on Toyopearl HW-40S eluted with MeOH (each 10 g). Fractions 37—39 (207 mg) were further purified by silica-gel (30 g) column chromatography eluted with a low layer of $CHCl_3$–MeOH–H_2O (16:4:1) to give yadanzioside E (**6**; 123 mg).

Column **H**. The fractions 25—28 (47 mg) of Column **F** were chromatographed using Toyopearl HW-40S. Elution with MeOH afforded yadanzioside H (**8**; 32 mg).

Column **I**. The fractions 16 (958 mg) of Column **C** was subjected to separation by partition chromatography on silicic acid (400 g) absorbed with water (270 ml). Elution (each 250 ml) was performed with 0, 3, 6, 9,···24% EtOH in $CHCl_3$ (each 1 l). Fractions 26—29 and 32—34 afforded yadanzioside D (**5**; 68 mg) and brucein E (35 mg), respectively.

Column **J**. The fractions 17—20 (681 mg) of Column **I** were chromatographed on a Lobar column (RP-8). Elution with MeOH–H_2O (1:1, each fraction 10 g) afforded bruceoside B (147 mg) from fractions 11—13. Fractions 18 and 19 were combined (62 mg) and further purified using Toyopearl HW-40S eluted with MeOH to give yadanzioside B (**3**; 52 mg).

Column **K**. The fractions 22—24 (128 mg) of Column **I** were chromatographed using Toyopearl HW-40S. Elution with MeOH afforded yadanzioside C (**4**; 86 mg).

Yadanzioside B (3). Amorphous solid, mp 189—195 °C (decomp), $[\alpha]_D^{26}$ −8.1° (*c* 0.84, EtOH); IR (KBr) 3425, 1735, 1635, and 1050 cm^{-1}; UV (ethanol) 255 nm (ε 8300); MS (SIMS) *m/z* 707 ($[M+Na]^+$), 523, and 439; High-resolution MS (EI) *m/z* 522.2082. Calcd for $C_{26}H_{34}O_{11}$ ($M-C_6H_{10}O_5$): *m/z* 522.2099.

Acid Hydrolysis of 3. Yadanzioside B (**3**; 36 mg) was treated with 1.5 M sulfuric acid (7 ml) and methanol (7 ml) at reflux temperature for 5 h. The usual work-up gave brucein A (**11**; 6 mg).

A solution of **3** (>1 mg) in a mixture of acetyl chloride (0.05 ml) and dry methanol (0.5 ml) was refluxed for 16 h. The reaction mixture was concentrated *in vacuo* to yield methyl D-glucoside, which was treated with trimethylsilyl chloride (10 μl) in pyridine (50 μl) and hexamethyldisilazane (5 μl). The trimethylsilylated product was identified by GLC retention time (9.4 min) by comparison with an authentic sample.

Yadanzioside A (2). Amorphous solid, mp 200—204 °C (decomp): $[\alpha]_D^{26}$ +3° (*c* 1.8, ethanol); IR (KBr) *ca.* 3400, 1740, 1675, 1625, and 1040 cm^{-1}; UV (ethanol) 256 nm (ε 5900); MS (SIMS) *m/z* 707 ($[M+Na]^+$), 523, 439, 421, and 403; High-resolution MS (EI) *m/z* 522.2127. Calcd for $C_{26}H_{34}O_{11}$ ($M-C_6H_{10}O_5$): *m/z* 522.2099.

Enzymatic Hydrolysis of 2. A mixture of yadanzioside A (**2**; 43 mg) and β-glucosidase (23 mg, Product of Sigma Chemical Company) in water (5 ml) was kept 2 weeks at 37 °C. The mixture was added to ethanol and heated at 80 °C for 10 min. After being cooled, the mixture was filtered and the filtrate was evaporated to dryness *in vacuo*. The residue was subjected to purification by column chromatography (C-200, 10 g) eluted with 10% MeOH in $CHCl_3$ to yield brucein A (**11**; 13 mg) as the aglycone and the starting material (20 mg). The aglycone, brucein A, was completely identical with an authentic sample.

Yadanzioside C (4). Amorphous solid, mp 204—209 °C (decomp); $[\alpha]_D^{26}$ +20° (*c* 0.92, ethanol); IR (KBr) 3445, 1740, 1680, 1640, and 1070 cm^{-1}; UV (ethanol) 221 nm (ε 14800) and 254 nm (ε 7800); MS (SIMS) *m/z* 749 ($[M+Na]^+$), 709, 673, 565, 547, 521, 439, 185, and 127; High-resolution MS (EI) *m/z* 564.2230. Calcd for $C_{28}H_{36}O_{12}$ ($M-C_6H_{10}O_5$): *m/z* 564.2207.

Acid Hydrolysis of 4. A solution of yadanzioside C (**4**; 38 mg) in 1.5 M sulfuric acid (10 ml) and methanol (10 ml) was refluxed for 5 h. The usual work-up gave brucein C (**12**; 13 mg), which was identical with an authentic sample.

A solution of **4** (>1 mg) in a mixture of dry methanol (0.5 ml) and acetyl chloride (0.05 ml) was refluxed for 16 h and the usual work-up afforded methyl D-glucoside.

Yadanzioside G (7). Amorphous solid, mp 180—185 °C (decomp); $[\alpha]_D^{22}$ +19° (*c* 1.2, ethanol); IR (KBr) 3450, 1740, 1685, 1645, 1075, 1050, and 1025 cm^{-1}; UV (ethanol) 222 nm (ε 16000) and 253 nm (ε 7800); MS (SIMS) *m/z* 791 ($[M+Na]^+$), 547, 127, and 109; High-resolution MS (EI) *m/z* 546.2103. Calcd for $C_{28}H_{34}O_{11}$ ($M-C_6H_{10}O_5-C_2H_4O_2$): *m/z* 546.2101.

Enzymatic Hydrolysis of 7. A solution of **7** (66 mg) and β-glucosidase (23 mg) in water (8 ml) was kept at 37 °C for 2 weeks and worked up as before to give bruceantinol

(**13**; 14 mg) and the starting material (31 mg).

Yadanzioside D (5). Amorphous soilid, mp 207—212 °C (decomp); $[\alpha]_D^{20}$ +38° (*c* 0.98, ethanol); IR (KBr) 3430, 1745, 1640, 1065, and 1040 cm^{-1}; MS (EI) *m/z* (%) 480 (0.8), 464 (1.5), 446 (1.4), 438 (1), 169 (27), 73 (100), 60 (72), and 57 (72); High-resolution MS (EI) *m/z* 480.1610. Calcd for $C_{23}H_{28}O_{11}$ ($M-C_6H_{12}O_5$): *m/z* 480.1630.

Enzymatic Hydrolysis of 5. Yadanzioside D (**5**; 29 mg) was hydrolyzed with β-glucosidase (10 mg) for 2 weeks at 37 °C to give an aglycone (**14**; 8 mg) and the starting material (**5**; 7 mg). **14**: mp 218—219 °C (from acetone-hexane); $[\alpha]_D^{26}$ +16° (*c* 0.63, pyridine); IR (KBr) 3450, 1745, 1640, 1060, and 1035 cm^{-1}; ^1H NMR (pyr-d_5, 90 MHz) δ=1.52 (3H, s; 10-Me), 1.55 (3H, brs; 4-Me), 2.09 (3H, s; 15-OAc), 2.60 (2H. br; 5- and 9-H), 3.78 (3H, s; CO_2Me), 3.85 (1H, d, *J*=8 Hz; 20-H), 3.90 (1H, br; 14-H), 4.01 (1H, d, *J*=8 Hz; 1-H), 4.50 (1H, m; 2-H),4.91 (1H, t-like; 7-H), 5.05 (1H, br; 12-H), 5.20 (1H, d, *J*=7 Hz; 20-H), 5.50 (1H, d, *J*=6 Hz; 11-H), 5.71 (1H, br; 3-H), and 6.76 (1H, d, *J*=13 Hz; 15-H); MS (EI) *m/z* (%) 482 (M^+; 2), 464 (6), 446 (8), 422 (5), 404 (7), 60 (100), and 57 (80); High-resolution MS (EI) *m/z* 482.1764. Calcd for $C_{23}H_{30}O_{11}$: *m/z* 482.1786.

Oxidation of 14 with Manganese Dioxide. The aglycone (**14**; 7 mg) was stirred with manganese dioxide (115 mg, preheated at 110 °C) in DMF (3 ml) for 6 h. The reaction mixture was filtered and the residue was washed with methanol. The filtrate and washings were combined and evaporated. The residue was purified by column chromatography on silica gel (C-200, 3 g). Elution with chloroform–methanol–water (16:4:1) gave isobrucein B (**15**; 5 mg), which was identified by comparison with an authentic specimen.

Yadanzioside H (8). Amorphous solid, mp 180—185 °C (decomp); $[\alpha]_D^{24}$ +33° (*c* 1.03, ethanol); IR (KBr) 3450, 1750, 1640, 1065, and 1040 cm^{-1}; MS (SIMS) *m/z* 709 ($[M+Na]^+$), 523, 507, and 439; MS (EI) *m/z* (%) 522 (0.6), 506 (0.6), 488 (2.7), 474 (2.2), 456 (3.8), 438 (4.2), 169 (26), 85 (59), and 57 (100); High-resolution MS (EI) *m/z* 522.2091. Calcd for $C_{26}H_{34}O_{11}$ ($M-C_6H_{12}O_5$): *m/z* 522.2099.

Enzymatic Hydrolysis of 8. Yadanzioside H (**8**; 43 mg) was hydrolyzed with β-glucosidase (15 mg) in water (5 ml) at 37 °C for 2 weeks to give an aglycone (**16**; 15 mg) and the starting material (**8**; 17 mg). **16**: mp 162—165 °C (from acetone-hexane); IR (KBr) 3455, 1750, 1640, 1060, and 1040 cm^{-1}; $[\alpha]_D^{26}$ +10° (*c* 1.0, pyridine); ^1H NMR (pyr-d_5, 90 MHz) δ=0.94 and 0.97 (each 3H, d, *J*=7 Hz; 3′-Me), 1.52 (3H, s; 10-Me), 1.53 (3H, brs; 4-Me), 2.60 (2H, br; 5- and 9-H), 3.80 (3H, s; CO_2Me), 3.85 (1H, d, *J*=8 Hz; 20-H), 3.98 (1H, d, *J*=7 Hz; 1-H), 4.50 (1H, m; 2-H), 4.87 (1H, brs; 7-H), 5.05 (1H, br; 12-H), 5.19 (1H, d, *J*=8 Hz; 20-H), 5.48 (1H, brd, *J*=5 Hz; 11-H), 5.70 (1H, br; 3-H), and 6.82 (1H, d, *J*=13 Hz; 15-H); MS (EI) *m/z* (%) 524 (M^+; 0.1), 506 (1), 85 (60), and 57 (100); Found: *m/z* 524.2279. Calcd for $C_{26}H_{36}O_{11}$: M, 524.2257.

Oxidation of 16 with Manganese Dioxide. The aglycone (**16**; 14 mg) was treated with manganese dioxide (230 mg) in DMF (4 ml) for 6 h. The same work-up as before afforded isobrucein A (**17**; 3 mg), which was identical with an authentic sample.

Hydrogenation of Yadanzioside H (8). A solution of yadanzioside H (**8**; 20 mg) in ethanol (3 ml) was hydrogenated in the presence of 10% palladium on carbon (10 mg) for 10 h. The usual work-up and separation by silica-gel chromatography afforded a dihydro derivative (**18**; 18 mg), mp 201—205 °C (from acetone-hexane); ^1H NMR (pyr-d_5, 90 MHz) δ=0.65 (3H, brs; 4-Me), 0.94 and 0.96 (each 3H, d, *J*=7; 3′-Me), 2.31 (2H, s; 2′-H), 3.80 (3H, s; CO_2Me), and 6.84 (1H, d, *J*=13 Hz; 15-H); ^{13}C NMR (pyr-d_5, 22.5 MHz) δ=12.9, 19.5, 22.5, 22.5, 25.8, 28.7, 29.2, 41.5, 43.4, 44.1, 44.6, 45.9, 46.9, 50.7, 52.3, 62.8, 68.4, 71.6, 73.6, 75.7, 75.9, 76.0, 78.6, 78.7, 82.4, 82.5, 83.5, 84.2, 106.9, 168.3, 171.5, and 171.6; MS (EI) *m/z* (%) 526 (0.2), 508 (0.6), 490 (0.7), 476 (0.7), 442 (0.6), 424 (1.5), 407 (1.7), 406 (2.4), 392 (1.9), 388 (1.6), 376 (1.5), 374 (1.5), 358 (1.6), 348 (2.4), 318 (2.8), 126 (17), 85 (50), 73 (24), 69 (32), 60 (100), and 57 (72).

Yadanzioside E (6). Amorphous solid, mp 190—195 °C (decomp); $[\alpha]_D^{23}$ +59° (*c* 1.6, ethanol); IR (KBr) 3450, 1740, 1645, 1080, 1060, and 1040 cm^{-1}; MS (SIMS) *m/z* 707 ($[M+Na]^+$), 519, 505, and 439; High-resolution MS (EI) *m/z* 520.1928. Calcd for $C_{26}H_{32}O_{11}$ ($M-C_6H_{12}O_5$): *m/z* 520.1943.

Enzymatic Hydrolysis of 6. Yadanzioside E (**6**; 39 mg) in water (5 ml) was enzymatically hydrolyzed with β-glucosidase (17 mg) at 37 °C for 2 weeks. The usual work-up and separation afforded an aglycone (**19**; 18 mg), mp 162—164 °C (from acetone-hexane); IR (KBr) 3450, 1740, 1645, 1055, and 1035 cm^{-1}; ^1H NMR (pyr-d_5, 90 MHz) δ=1.54 (6H, brs; 4- and 10-Me), 1.66 and 2.14 (each 3H, s; 3′-Me), 2.60 (2H, br; 5- and 9-H), 3.74 (3H, s; CO_2Me), 4.02 (1H, d, *J*=8 Hz; 1-H), 4.50 (1H, m; 2-H), 4.94 (1H, br; 7-H), 5.08 (1H, br: 12-H), 5.21 (1H, d, *J*=8 Hz; 20-H), 5.51 (1H, m; 11-H), 5.71 (1H, br; 3-H), 5.80 (1H, brs; 2′-H), and 6.75 (1H, d, *J*=13 Hz; 15-H); MS (EI) *m/z* (%) 522 (M^+; 0.1), 504 (0.4), 486 (3), and 83 (100); Found: *m/z* 522.2108. Calcd for $C_{26}H_{34}O_{11}$: M, 522.2102.

Oxidation of 19 with Manganese Dioxide. Oxidation of the aglycone (**19**; 15 mg) with manganese dioxide (245 mg) in DMF (3 ml) for 6 h gave a keto alcohol (**20**; 3 mg) after purification by silica-gel (5 g) column chromatography eluted with 10% MeOH in $CHCl_3$ **20**: Amorphous, mp 162—164 °C (from acetone-hexane); IR (KBr) 3460, 1750, 1675, 1660, 1085, 1065, and 1040 cm^{-1}; UV (ethanol) 222 nm (ε 17600); ^1H NMR ($CDCl_3$, 90 MHz) δ=1.19 (3H, s; 10-Me), 1.94 (3H, d, *J*=1 Hz; 3′-Me), 1.96 (3H, s; 4-Me), 2.19 (3H, brs; 3′-Me), 3.78 (3H, s; CO_2Me), 4.16 (1H, s; 1-H or 12-H), 4.29 (1H, brs; 12-H or 1-H), 4.77 (1H, br; 7-H), 4.82 (1H, d, *J*=6 Hz; 20-H), 5.64 (1H, brs; 2′-H), 6.12 (1H, brs; 3-H), and 6.23 (1H, d, *J*=14 Hz; 15-H); MS (EI) *m/z* (%) 520 (M^+; 0.7), 502 (1.5), and 83 (100); Found: *m/z* 520.1968. Calcd for $C_{26}H_{32}O_{11}$: M, 520.1943.

Hydrogenation of Yadanzioside E (6). Yadanzioside E (**6**; 46 mg) in ethanol (6 ml) was hydrogenated in the presence of 10% palladium on carbon (30 mg) for 4 h. The reaction product was worked up as usual to give a tetrahydro derivative (46 mg). The physical and spectral data were completely identical with those of **18**, obtained by the hydrogenation of yadanzioside H (**8**).

Dehydrobrusatol (9). Amorphous solid, mp 157—160 °C (from acetone-hexane); $[\alpha]_D^{22}$ +57° (*c* 1.4, ethanol); IR (KBr) 3440, 1740, 1635, 1140, 1080, and 1060 cm^{-1}; UV (ethanol) 256 nm (ε 7500); (ethanol+KOH) 340 nm and 262 nm; MS (EI) *m/z* (%) 518 (M^+; 1), 436 (>0.1), and 83 (100); Found: *m/z* 518.1784. Calcd for $C_{26}H_{30}O_{11}$: M, 518.1787.

Dehydrogenation of Brusatol (1). A solution of brusatol (**1**; 20 mg) in benzene (2 ml) was refluxed with DDQ (12 mg) for 6 h under nitrogen atmosphere. The reaction product was subjected to separation by silica-gel column chromatography. Elution with 3% 2-propanol in dichloromethane gave crude dehydrobrusatol (**9**; 16 mg), which was further chromatographed on silica gel. Elution with 1% methanol in chloroform afforded dehydrobrusatol (**9**; 13 mg), whose physical and spectral data were identical with those of the natural dehydrobrusatol (**9**).

Dehydrobruceantinol (10). Amorphous solid, mp 152—154 °C (from acetone-hexane); $[\alpha]_D^{22}$ +39° (*c* 0.45, ethanol); IR (KBr) 3445, 1740, 1635, 1260, 1165, and 1060 cm^{-1}; UV (ethanol) 255 nm (ε 8400); (ethanol+KOH) 336—340, 262, and 229 nm; MS (SIMS) *m/z* 627 ($[M+Na]^+$) and 567; High-resolution MS (EI) *m/z* 586.2064 and *m/z* 526.1866. Calcd for $C_{30}H_{34}O_{12}$ ($M-H_2O$): *m/z* 586.2051 and $C_{28}H_{30}O_{10}$ ($M-H_2O-AcOH$): *m/z* 526.1839, respectively.

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

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15) $\text{LIS}(\%) = \left(\frac{\text{Mean life span (day) of sample}}{\text{Mean life span (day) of control}} - 1\right) \times 100$

16) General procedures are the same as those described in the preceding paper.[2]