(18) (a) T. Sheradsky, *Tetrahedron Lett.*, 5225 (1966); (b) A. Mooradian, *Tetrahedron Lett.*, 407 (1967); (c) D. Kaminsky, J. Shavel, Jr., and R. I. Meltzer, *Tetrahedron Lett.*, 859 (1967).
(19) For example, *O*-(2,4-dinitrophenyl)hydroxylamine (**12**) is prepared from 2,4-dinitrofluorobenzene by a displacement reaction with N-(protected) hydroxylamine followed by removal of the protecting group.[22b,23]
(20) C. L. Bumgardner and R. L. Lilly, *Chem. Ind. (London)*, 559 (1962).
(21) J. S. Nicholson and D. A. Peak, *Chem. Ind. (London)*, 1244 (1962).
(22) (a) T. Sheradsky, *J. Heterocycl. Chem.*, **4**, 413 (1967); (b) T. Sheradsky, G. Salemnick, and Z. Nir, *Tetrahedron*, **28**, 3833 (1972).
(23) Y. Tamura, J. Minamikawa, K. Sumoto, S. Fujii, and M. Ikeda, *J. Org. Chem.*, **38**, 1239 (1973).
(24) For a recent review, see R. K. Brown in "Indoles", Part 1, W. J. Houlihan, Ed., Wiley-Interscience, New York, 1972, pp 232–316.
(25) A. Mooradian and P. E. Dupont, *J. Heterocycl. Chem.*, **4**, 441 (1967).
(26) The formation of **9** probably arises from a Beckmann rearrangement. For example, treatment of oxime acetates with aluminum chloride promoted a Beckmann rearrangement instead of formation of the desired α-acetoxyimines [H. O. House and F. A. Richey, Jr., *J. Org. Chem.*, **34**, 1430 (1969)].
(27) P. E. Eaton, G. R. Carlson, and J. T. Lee, *J. Org. Chem.*, **38**, 4071 (1973).
(28) G. Caporale and A. M. Bareggi, *Gazz. Chim. Ital.*, **98**, 444 (1968).
(29) (a) A. Mooradian and P. E. Dupont, *Tetrahedron Lett.*, 2867 (1967); (b) T. Sheradsky and A. Elgavi, *Isr. J. Chem.*, **6**, 895 (1968); (c) ref 26; (d) T. Sheradsky and G. Salemnick, *J. Org. Chem.*, **36**, 1061 (1971); (e) T. Sheradsky, E. Nov, S. Segal, and A. Frank, *J. Chem. Soc., Perkin Trans. 1*, 1827 (1977).
(30) F. A. Davis and E. B. Skibo, *J. Org. Chem.*, **39**, 807 (1974).
(31) Solvent evaporations were carried out in vacuo using a Berkeley Rotary Evaporator after drying over $MgSO_4$. All melting points are uncorrected. Infrared (IR) spectra were recorded on a Perkin-Elmer 137 spectrometer, ultraviolet (UV) spectra were recorded on a Cary Model 14 spectrometer, and nuclear magnetic resonance (NMR) spectra were recorded using $CDCl_3$ solutions (unless otherwise indicated) on a Varian T-60 spectrometer with Me_4Si as internal standard. Mass spectra were obtained on an AEI MS 12 instrument. All reactions were carried out under a nitrogen atmosphere unless otherwise indicated. T_i is internal temperature, and T_b is bath temperature. Column chromatography was performed on Merck silica gel (70–230 mesh) or on Camag Kieselgel (>250 mesh). Elemental analyses were performed by the Analytical Laboratory, Department of Chemistry, University of California, Berkeley.

Antitumor Agents. 33. Isolation and Structural Elucidation of Bruceoside-A and -B, Novel Antileukemic Quassinoid Glycosides, and Brucein-D and -E from *Brucea javanica*[1]

Kuo-Hsiung Lee,* Yasuhiro Imakura, Yoshio Sumida, Rong-Yang Wu, and Iris H. Hall

Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina, Chapel Hill, North Carolina 27514

Huan-Chang Huang

School of Pharmacy, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China

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The active extract of the seeds of *Brucea javanica* has yielded bruceoside-A (**1**) and -B (**2**), two novel potent antileukemic quassinoid glycosides of bruceosin (**15**) and brusatol (**8**), respectively, as well as brucein-D (**3**) and -E (**4**). The structure and stereochemistry of these compounds have been established from chemical transformation, correlations, and spectral analyses.

The seeds of *Brucea javanica* (Linn.) Merr (Simaroubaceae) are known as "Ya-Tan-Tzu" in Chinese folklore and as herbal remedies for human amebiasis, as well as for cancer.[2-4] As a result of the continuing search among plants for new and novel naturally occurring potential antitumor agents, the methanolic extract of the seeds of *B. javanica* was found to show significant inhibitory activity in vivo against the Walker 256 carcinosarcoma in rats and the P-388 lymphocytic leukemia in the mouse.[5] A preliminary communication[6] described the structural determination of the potent novel antileukemic principle, bruceoside-A (**1**). The purpose of this paper is to present the full account of the isolation and structural elucidation of bruceoside-A (**1**) and bruceoside-B (**2**), the new antileukemic substance, as well as the companion quassinoids brucein-D (**3**) and brucein-E (**4**). Bruceoside-A and -B appear to be the first two quassinoid glucosides which have been demonstrated to have antileukemic activity.[6] Brucein-D demonstrated significant antisarcoma activity in the Walker 256 screen.[6] The seeds of *B. javanica* were first defatted by *n*-hexane, and the marc was then extracted with methanol. The active methanol extract was concentrated and partitioned between chloroform and water (1:1). Guided by the in vivo P-388 assay, the active principles were concentrated in both the chlorofrom extract as well as in the water extract. The active water extract was further extracted with saturated butanol–water. Chromatography of the active butanol extract over silica gel led to the isolation of the active principles, bruceoside-A and -B and brucein-D and -E. Isolation of the active principles from the chlorofrom extract is in progress.

Bruceoside-A (**1**), $C_{32}H_{42}O_{16}$, showed the presence of hydroxyl, conjugated enone, and lactone moieties in its IR spectrum. Its NMR spectrum revealed the presence of a senecioyl group at δ 1.93, 2.16 (CH_3-23), and 5.36 (H-22), a carbomethoxyl group (δ 3.76), an angular methyl group (δ 1.60), a secondary methyl group [δ 1.15 (d, J = 6.0 Hz)], and low-field protons at δ 6.02 (H-15) and 6.84 (H-1) as a doublet and a singlet, respectively (Table I).

Acid hydrolysis of **1** with 3 N sulfuric acid–methanol (1:1) yielded D-glucose, identified by GLC as its trimethylsilyl derivative, and the major aglycon, which was identified as brusatol (**8**).[8] Structural characterization of **8** was based upon spectral evidence described before[6] as well as decoupling experiments (100 MHz, $CDCl_3$). For example (Table I), irradiation of H-14 (dd, J = 1.0 and 13.0 Hz) at δ 3.12 collapsed the H-15 doublet (J = 13.0 Hz) to a singlet. Conversely, irradiation of H-15 converted H-14 to a singlet. Irradiation of H-22 (m) at δ 5.64 changed the Me_2-23 doublets (J = 1.5 Hz each) at either δ 1.93 or 2.20 to a singlet. Conversely, irradiation of Me_2-23 at either δ 1.93 or 2.20 converted the H-22 multiplet to a doublet (J = 1.5 Hz). Final identification of **8** was established by a direct comparison with an authentic sample of brusatol, isolated from *Brucea sumatrana* by Geissman and co-workers.[8] As is apparent from the ^1H NMR data given in Table I, compound **8** lacks the characteristic signals of H-1 and Me-4 as found in **1** and shows the presence of a C-4 vinyl methyl group as a doublet (J = 2.0 Hz) at δ 1.84. Consequently, it was concluded that compound **8** was a secondary product formed during the acid hydrolysis of **1**. En-

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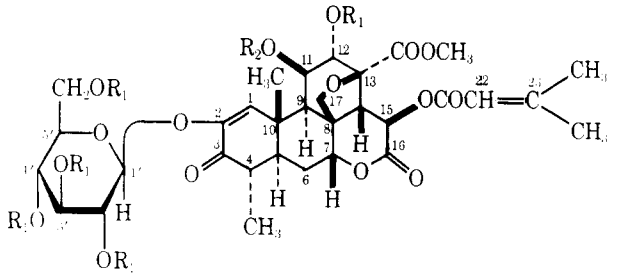

1, $R_1 = R_2 = H$
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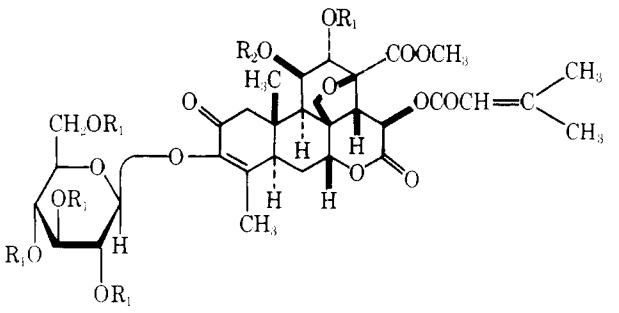
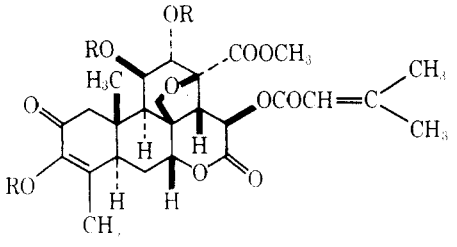
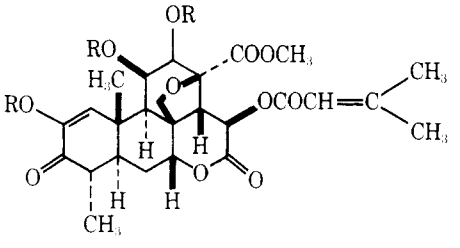
6, $R_1 = COCH_3$; $R_2 = H$
7, $R_1 = R_2 = CH_3$



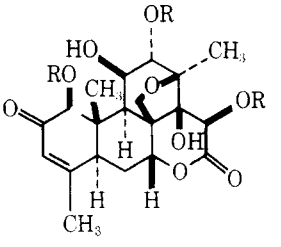
2, $R_1 = R_2 = H$
10, $R_1 = R_2 = COCH_3$
11, $R_1 = COCH_3$; $R_2 = H$
16, $R_1 = R_2 = CH_3$



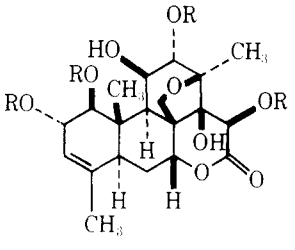
8, R = H
12, R = $COCH_3$



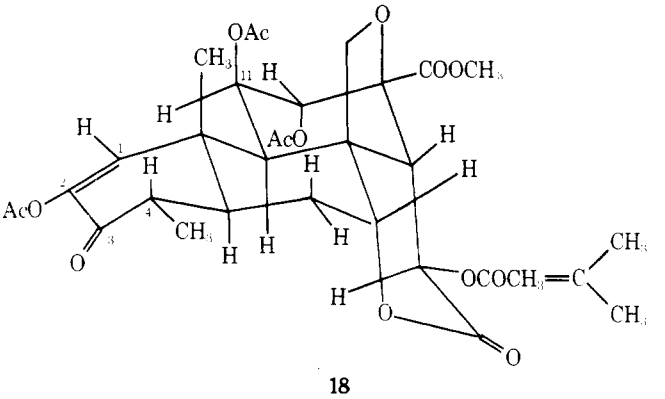
9, R = $COCH_3$
15, R = H



3, R = H
13, R = $COCH_3$
17, =O replaces 11-OH; R = $COCH_3$



4, R = H
14, R = $COCH_3$



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zymatic hydrolysis of 1 with β-glucosidase yielded brusatol (8) as the major aglycon and a D-glucose, demonstrating the presence of a β-D-glucosidic linkage of the glucoside.

Acetylation of 1 with pyridine–acetic anhydride at room temperature for 17 h afforded a hexaacetate (5) and a pentaacetate (6), which could be converted into 5 by treatment with acetic anhydride in *p*-toluenesulfonic acid. The assignments of protons between C-14, C-15, C-17, C-22, C-23, C-5′, and CH_2OCOCH_3 of 5 were achieved by extensive decoupling experiments (100 MHz, 1:1 $CDCl_3$–C_6D_6). For example, irradiation at the frequency of H-5′ at δ 3.80 (dd, J = 3.0 and 4.0 Hz) converted the doublet of doublets at δ 4.30 (CH_2O-$COCH_3$, J = 3.0 and 12.0 Hz) to an AB quartet (J = 12 Hz). Irradiation of H-17 at δ 3.49 collapsed the doublet at δ 4.47 (J = 8.0 Hz) to a singlet. Irradiation of H-22 at δ 5.63 collapsed the two methyl doublets (Me_2-23, J = 1.5 Hz each) at δ 2.09 and 1.71 to two singlets. Conversely, irradiation of the Me_2-23 either at δ 2.09 or at δ 1.71 collapsed H-22 (m) to a doublet (J = 1.5 Hz). Irradiation at the frequency of H-14 at δ 3.14 collapsed the H-15 doublet (J = 14.0 Hz) at δ 6.05 to a singlet. The singlets at δ 1.32 and 1.52 corresponding to the Me-10 of 5 and 6, respectively, were clearly indicative of a 1,3-diaxial relationship between Me-10 and OAc(OH)-11 in 5 and 6. Thus, the steric structure of the genuine aglycon of 1 agrees with that of brusatol exclusive of ring A.

In order to clarify the structure of the genuine aglycon of 1, acetolysis of 1 with acetic anhydride–zinc chloride[13] was undertaken. This reaction gave rise to a known pentaacetyl glucopyranoside and a triacetate whose NMR data (Table I) revealed the presence of the characteristic Me-4 and H-1 signals at δ 1.18 (d, J = 6.0 Hz) and 6.62 (s), respectively, as found in 1 and were in accord with the assigned structure 9 (i.e., bruceosin triacetate).[6] Consequently, the structure of the genuine aglycon (i.e., bruceosin) of 1 was established as 15. The downfield shift of H-1 in 9 at δ 6.62, which was due to the introduction of an acetoxyl group at C-2, compared to H-1 in 5 at δ 6.17 indicated that the sugar moiety in bruceoside-A (1) was linked to C-2 of bruceosin (15).

Methylation of 1 with methyl iodide–silver oxide in *N*,*N*-dimethylformamide according to Kuhn's method[7] led to the formation of a hepta-*O*-methyl derivative (7) which lacks the hydroxyl absorption bands in its IR spectrum. Its NMR spectrum exhibited signals due to six *O*-methyls, one carbomethoxy, and one senecioyl group (Table I), as well as one anomeric proton [δ 4.59 (d, J = 8 Hz)], which indicated the presence of a β-glucopyranoside linkage and confirmed the aforementioned result of the enzymatic hydrolysis of 1 with β-glucosidase. Thus, the complete structure of bruceoside-A is now expressed as bruceosin 2-β-D-glucopyranoside (1). Methanolysis of 7 with anhydrous 7% hydrogen chloride–methanol yielded methyl 2,3,4,6-tetra-*O*-methylglucopyranoside (identified by TLC and GLC).

Table I. ^{1}H NMR SPectra of Bruceoside-A (1) and -B (2) Brucein-D (3) and -E (4), and Derivatives[a]

compd	H-1	H-3	H-7	H-11	H-12	H-14	H-15	H-17	H-22	Me-4	Me-10	Me-23	COOMe	misc
1[b]	6.84						6.02 d (13)		5.36 m	1.15 d (6)	1.60	1.93 d (1.5) 2.16 d (1.5)	3.76	
1[c]	7.30						6.99 d (14)			1.11 d (6)	1.59	1.68, 2.13 (1)	3.77	
2[b]							6.09 br d (13)		5.70 m	2.01	1.42	1.95 d (1.5) 2.18 d (1.5)	3.74	
3	5.26	6.09 m	5.18 t (2)	4.58 dd (1.5)	3.81 d (1)		4.20	3.87 dd (2, 8) 4.55 d[d] (8)		1.98 m	1.44			2.33 dd (2, 5) (H-9), 2.90 d (13) (H-5), 1.19 (13-CH_3), 2.44 dd (2, 14) H-6α), 1.78 dd (13, 14) (H-6β)
4[c]										2.08	1.58[d]			1.58[d] (13-CH_3)
5[f]	6.17			5.61 dd[d] (1.5, 5)		3.18 d (14)	6.05 d (14)	3.49 d (8) 4.47 d (8)	5.63 m	1.00 d (6)	1.02	1.71 d (1.5) 2.09 d (1.5)	3.60	1.86, 1.87, 1.94, 1.98 (9 H) (6OCOCH$_3$), 4.30 dd, (3, 12) (CH_2OCOCH$_3$), 3.80 dd (3, 4) (H-5′), 4.18, 4.95, 5.30–5.40 m (sugar H)
5	see Experimental Section													
6	6.74						6.02 d (14)			1.10 d (6)	1.55	1.92 d (1.5) 2.18 d (1.5)	3.78	2.01, 2.04, 2.07, 2.14, 2.16 (5OCOCH$_3$)
7	6.37						5.79 d (14)		5.69 m	1.14 d (6)	1.50	1.91 d (1.5) 2.18 d (1.5)	3.77	3.37, 3.41, 3.48, 3.56, 3.66, 3.69 (6OCH$_3$), 4.59 (8) (H-1′)
8	2.40 d[d] (16) 2.90 d[d] (16)		4.80 m	4.25 d-like (5)	4.22 d (1)	3.12 dd (1, 13)	6.26 (13)	3.80 d[d] (8) 4.76 d[d] (8)	5.64 m	1.84 d (2)	1.39	1.93 d (1.5) 2.20 d (1.5)	3.80	2.40, 2.90 m (H-6), 3.46, 4.22, 6.15 (3OH)[e]
9	6.62		4.78 m	5.52 dd (2, 5)	5.32 d (d)	3.31 d (13)	6.00 d (13)	3.87 dd (2, 8) 4.73 d (8)	5.63 m	1.18 d (6)	1.39	1.92 d (2) 2.18 d[d] (2)	3.74	2.04, 2.18 (2OCOCH$_3$), 2.24 (OCOCH$_3$-2)
10							6.10 d (13)		5.62 m	1.82	1.19	1.92 d (1.5) 2.18 d (1.5)	3.72	ca. 4.80 m, 5.20, m (sugar H), 4.10 dd, (3, 12) (CH_2OCOCH$_3$), 2.01, 2.03, 2.04, 2.12 (6OCOCH$_3$)
11							6.06 d (13)		5.65 m	1.85 d (1.5)	1.40	1.92 d (1.5) 2.19 d (1.5)	3.76	2.04, 2.06, 2.12 (5OCOCH$_3$)
12			4.85 m	5.21 dd (2.5)	5.35 t-like (2)		6.08 d (13)	3.83 dd (2, 8) 4.77 d (8)	5.62 m	1.80 d (1.5)	1.31	1.93 d (1.5) 2.19 d (1.5)	3.75	2.03, 2.12, 2.26 (3OCOCH$_3$)
13	5.41	6.08 m	5.21 t (2)		4.87 d (1)		6.13	3.96 dd (2, 8) 4.64 d (8)		1.95 m	1.40			1.31 (13-CH_3), 2.20 ($COCH_3$), 2.25 (2$COCH_3$)
14	5.22 d (7)		5.12 t-like	3.83 dd (1, 5)	4.76 d (1)		6.13	3.95 dd (1, 8) 4.67 d (8)		1.67 d (1)	1.42			5.39 br s (H-2, H-3), 1.29 (13-CH_3), 2.01, 2.02, 2.07, 2.24 (4$COCH_3$)
17					4.85 d (1)		6.67	3.96						

[a] Run in $CDCl_3$ at 100 MHz, and values are in parts per million. Multiplicities are indicated by the usual symbols: d, doublet; t, triplet; m, multiplet whose center is given; br, slightly broadened singlet. Unmarked signals are singlets. Figures in parentheses are coupling constants in hertz. [b] Run in CD_3OD at 100 MHz. [c] Run in pyridine-d_5. [d] Overlapped signals. [e] Signals disappeared upon D_2O exchange. [f] Run in $CDCl_3$–C_6D_6 (1:1).

The configuration of the C-4 methyl group of **1** was tentatively assigned as α-equatorial on the basis of the following reasons (1). A half-chair conformation of ring A is generally more stable than the boat conformation. (2) The chemical shift of the C-10 methyl group is at δ 1.60, which would be shielded to higher field due to the positive anisotropic effect of the C-3 carbonyl if it possesses a boat conformation of ring A. (3) No nuclear Overhauser effect (NOE) was observed when the C-10 methyl group of bruceosin triacetate (**9**) was irradiated; i.e., there was no increase in the intensity of the C-4 methyl group, suggesting that the C-4 methyl group is in an α-equatorial disposition as shown (**18**).

Bruceoside-B (**2**), mp 223–224 °C dec, was isolated as colorless prisms and is quite as bitter as bruceoside-A (**1**). Its IR bands showed the presence of hydroxyl, lactone, and conjugated enone carbonyl moieties. The NMR spectrum of bruceoside-B (Table I) disclosed the presence of many hydroxyl groups, a senecioyl group at δ 2.18 (Me-23), 1.95 (Me-23), and 5.70 (H-22), a carbomethoxyl group at δ 3.74, and two methyl groups at δ 1.42 (Me-10) and 2.01 (Me-4), as well as a one-proton doublet at δ 6.09 (J = 13 Hz) for H-15. The absence of further additional low-field olefinic protons in comparison with those of bruceoside-A (**1**) was indicative of the presence of an α,β-substituted enone system for ring A as shown in **2**.

Bruceoside-B is a glucoside of brusatol (**8**) since upon acid hydrolysis with 3 N sulfuric acid–methanol it yielded a D-glucose and an aglycon which was shown to be identical with brusatol by mixture melting point determination and IR and NMR spectral comparison. The isolation of the same brusatol from an enzymatic hydrolysis of bruceoside-B by β-glucosidase confirmed the presence of a β-D-glucosidic linkage of the glucoside.

Acetolysis of bruceoside-B with acetic anhydride–zinc chloride gave a triacetate (mp 247–250 °C), identified as brusatol triacetate (**12**), and a pentaacetyl glucopyranoside.

Acetylation of bruceoside-B with acetic anhydride–pyridine afforded a hexaacetate and a pentaacetate whose elemental analyses and NMR and mass spectral data were in accord with the assigned structures **10** and **11**, respectively. Methylation of bruceoside-B with methyl iodide–silver oxide in *N,N*-dimethylformamide gave a hepta-*O*-methyl derivative (**16**). The appearance of the characteristic anomeric proton in the NMR spectrum of **16** at δ 4.60 (1 H, d, J = 8 Hz), similar to that of **7**, indicated the presence of a β-glucopyranoside linkage. Methanolysis of **16** with anhydrous hydrogen chloride–methanol furnished methyl 2,3,4,6-tetra-*O*-methylglucopyranoside, which was identified by TLC and GLC with a sample prepared from methylation of D-glucose.

The foregoing evidence led to the structural assignment of bruceoside-B as brusatol 3-β-D-glucopyranoside (**2**).

Compound **3**, mp 294–296 °C, $C_{20}H_{26}O_9$, was isolated as colorless needles. The NMR spectrum of **3** revealed the presence of three methyl groups at δ 1.98, 1.44, and 1.19 which were assigned as vinyl methyl and methyl at C-10 and C-13, respectively. The assignments of protons at C-3, C-5, C-6, C-7, C-17, C-9, C-11, and C-12 of structure **3** were achieved based upon extensive NMR decoupling experiments (100 MHz, CD_3OD). For example, irradiation at the frequency of the C-4 vinyl methyl group at δ 1.98 (m) collapsed the H-3 multiplet at δ 6.09 to a doublet-like signal. The H-3 and Me-4 multiplets also collapsed to a broad singlet and a doublet (J = 1.0 Hz), respectively, while the H-5 multiplet at δ 2.90 was irradiated. Irradiation of H-9 (dd, J = 2.0 and 5.0 Hz) at δ 2.33 collapsed the doublet of doublets at δ 4.58 (H-11, J = 1.0 and 5.0 Hz) and 3.87 (H-17, overlapped, J = 2.0 and 8.0 Hz) to a singlet-like signal and a doublet (J = 8.0 Hz), respectively. Conversely, irradiation at δ 4.58 collapsed the doublet of doublets at δ 2.33 (H-9) to a doublet (J = 2.0 Hz) as well as the doublet at δ 3.81 (H-12, J = 1.0 Hz) to a singlet. Irradiation at δ 3.87 (H-17) changed the doublet of doublets at δ 2.33 (H-9) to a doublet (J = 5.0 Hz). Irradiation at δ 5.18 (H-7, t, J = 2.0 Hz) collapsed the doublet of triplets at δ 2.40 (H-6α) and 1.78 (H-6β) to a doublet of doublets with J = 2.0 and 4.0 Hz and J = 13.0 and 14.0 Hz, respectively. The remaining signals at δ 5.26 and 4.20 were thus assigned to H-1 and H-15, respectively. The ^{13}C NMR spectrum of **3** displayed two carbonyl carbon signals at δ 146.83 and 166.46 which were due to the lactonic and enonic carbonyls, respectively. The signals at δ 16.67, 22.56, and 25.58 were assigned to methyl groups at C-10, C-13, and C-4, respectively, on the basis of the selective decoupling technique. Further assignments of carbons corresponding to structure **3** by off-resonance proton decoupling are listed in the Experimental Section. These assignments were also comparable with those of other similar quassinoids.[9]

Acetylation and oxidation of **3** with acetic anhydride in pyridine with Jones' reagent gave a triacetate (**13**) and a ketone (**17**), respectively. The NMR spectra of **13** and **17** (Table I) were identical with those of brucein-D triacetate and its corresponding 11-keto derivative, respectively, reported by Polonsky and co-workers.[10] To confirm the structure of **3**, the identity of **3** with an authentic sample of brucein-D was established by TLC, mixture melting point, and IR and NMR spectral comparison.

Compound **4**, mp 261–265 °C, $C_{20}H_{28}O_9$, was isolated as colorless needles. Compound **4** gave, upon acetylation with acetic anhydride in pyridine, a tetraacetate (**14**) which showed four acetyl groups at δ 2.01, 2.02, 2.07, and 2.24 in its NMR spectrum. The NMR spectrum of **14** was also comparable with that of brucein-D triacetate (**13**) except for the slight difference of signals due to H-1 and H-2. The fact that H-1 was a doublet at δ 5.22 in **14** instead of a further downfield singlet at δ 5.41 as seen in **13** was a strong indication of the presence of an acetyl group in **14**, and consequently a hydroxyl group in **4**, at the C-2 position. This means that **4** possesses an α-glycol moiety for its ring A instead of an enone system as found in brucein-D (**3**). The identity of **4** and brucein-E[10] was established by comparative TLC and IR and NMR spectral analyses.

Studies on the structure–activity relationships and mechanism of action among the bruceoside related quassinoids are currently in progress.

Experimental Section

Melting points were determined on a Thomas-Hoover melting point apparatus and were uncorrected. Specific rotations were obtained on a Perkin-Elmer Model 141 polarimeter (l = 1 dm). Infrared spectra were recorded with a Perkin-Elmer 257 grating IR spectrometer. ^1H nuclear magnetic resonance (NMR) spectra were determined with a Varian XL-100 NMR spectrometer (Me_4Si as an internal standard). ^{13}C NMR spectra were recorded on a Varian XL100 spectrometer functioning at 25.20 MHz. All NMR spectra were obtained with the use of the Fourier transform technique. Mass spectra were determined on an A.E.I. MS-902 instrument at 70 eV using a direct inlet system. Gas–liquid chromatography was performed on a Packard gas chromatograph. Silica gel (Merck silica gel 60, 70–325 mesh) was used for column chromatography, and precoated silica gel (Merck silica gel 60 F-254) was used for thin-layer chromatography (TLC). Detection of components was made either by spraying with 1% cerium sulfate–10% sulfuric acid solution followed by heating or by use of a UV lamp. Elemental analyses were performed by either Integral Microanalytical Laboratories, Inc., Raleigh, N.C., or M-H-W Laboratories, Phoenix, Ariz.

Isolation of Bruceoside-A (1) and -B (2) and Brucein-D (3) and -E (4) from *Brucea javanica*. The seeds of *B. javanica* were procured and identified by H. C. Huang in Kaohsiung, Taiwan. Guided by the in vivo assay (P-388 lymphocytic leukemia in mice),[5] the ground air-dried seeds (800 g) were exhaustively extracted with *n*-hexane. The residue, after the removal of hexane, was further extracted with methanol. The concentrated active methanolic extract (10 g) was partitioned between 1:1 chlorofrom and water to yield an

active chloroform extract (1.41 g) as well as an active water layer. The water layer (7.35 g) was extracted with saturated 1-butanol–water. The active 1-butanol layer was evaporated to give a dark brown syrup (5.61 g). The 1-butanol extract (10 g) was chromatographed on silica gel (900 g) and eluted with the lower layer of a solvent mixture of chloroform–methanol–water (50:12:3). Fractions of 150–200 mL each were collected and combined on the basis of TLC similarity. Fractions 20–25 gave, upon evaporation of solvent, 250 mg of crystalline residues which were recrystallized from methylene chloride–methanol–ether to afford brucein-D (**3**, 74 mg). Fractions 29–32 yielded 553 mg of crude bruceoside-A (**1**), which was purified by preparative TLC (Merck silica gel; 50:12:3 chloroform–methanol–water, lower layer). Fractions 47–51 gave bruceoside-B (**2**, 183 mg), which was fairly homogeneous and was purified by preparative TLC over silica gel using 65:35:10 chloroform–methanol–water (lower layer) and detected by UV. Fractions 54–68 yielded brucein-E (**4**, 105 mg) upon further purification by preparative TLC (silica gel; 65:35:10 chloroform–methanol–water, lower layer).

Bruceoside-A (1). Bruceoside-A was obtained as an amorphous powder: mp 175–180 °C; $[\alpha]^{25}_D$ +9.2° (c 0.50, MeOH); IR (Nujol) 3433 (OH), 1732 (δ-lactone and ester CO), 1674 (α,β-unsaturated C=O), 1640 (C=C) cm^{-1}.

Anal. Calcd for $C_{32}H_{42}O_{16}$: C, 56.30; H, 6.20. Found: C, 56.79; H, 6.51.

Acid Hydrolysis of 1. A solution of 1 (100 mg) in 3 N sulfuric acid–methanol (1:1, 20 mL) was refluxed for 5 h and then poured into ice water. The reaction mixture was further concentrated in vacuo and extracted with chloroform. The combined chloroform layers were dried over anhydrous sodium sulfate and evaporated under reduced pressure to give a product (54 mg) which was subjected to preparative TLC (10:1 chloroform–methanol detected by UV) to yield a pure compound (34 mg). This compound was recrystallized from methanol–acetone to afford **8** as colorless needles: mp 274–277 °C; MS *m/e* 83 (base peak, $Me_2C{=}CHCO^+$); IR 3450 (OH), 1734 (δ-lactone and ester CO), 1680 (sh) and 1676 (conjugated CO), 1630 (C=C) cm^{-1}. The physical data of **8** are in agreement with literature data[8] for brusatol (**8**), and identity was confirmed by a direct comparison (mixture melting point, TLC, and IR, NMR, and mass spectra) with an authentic sample.

Anal. Calcd for $C_{26}H_{32}O_{11}$: *m/e* 520.1951. Found: *m/e* 520.1945.

The aqueous layer was passed through a column of anion exchange resin (Bio-Rad AG-3, hydroxide form, 10 g) and evaporated under reduced pressure to give a residue which was identified as D-glucose by partition paper chromatography (detected by aniline hydrogen phthalate, R_f 0.52) and by gas–liquid chromatography (as the trimethylsilyl derivative).

Enzymatic Hydrolysis of 1. A mixture of **1** (40 mg) and β-glucosidase (50 mg, 2.5–6.0 units/mg)[12] in water was stirred for 6 days at 38–42 °C. The mixture was added to ethanol (20 mL) and heated at 80 °C for 10 min. After being cooled, the mixture was filtered and the residue was washed with ethanol–water (1:1, 5 mL). The combined filtrates and washings were evaporated to dryness in vacuo. The residue was purified by preparative TLC (10:1 chloroform–methanol, detected by UV) to yield a crystalline residue of the aglycon and a sugar component. One recrystallization of the aglycon from acetone–methanol provided colorless crystals (11 mg) which showed no depression in melting point on admixture with the foregoing brusatol (**8**) isolated from the acid hydrolysis of **1**, and IR spectra were identical. The sugar component was identified as D-glucose by partition paper chromatography (7:1:2 isopropanol–1-butanol–water, developed for 24 h, and detected by aniline hydrogen phthalate).

Bruceoside-A Hexaacetate (5) and Pentaacetate (6). Bruceoside-A (77 mg) was acetylated with pyridine and acetic anhydride for 17 h at 25–30 °C followed by the usual workup to give a mixture of hexaacetate (**5**) and pentaacetate (**6**). This mixture was purified and separated by preparative TLC (20:2 chloroform–acetone, detection with UV, double development) to yield, after recrystallization from ether–dichloromethane, 31.4 mg of **5** and 32.5 mg of **6**. Compound **5**: mp 162–165 °C; IR ($CHCl_3$) no OH, 1756, 1751 (ester and lactone C=O), 1701 (α,β-unsaturated C=O), 1644 (C=C) cm^{-1}; NMR ($CDCl_3$) δ 6.21 (1 H, s, H-1), 6.02 (1 H, d, J = 14 Hz, H-15), 5.66 (1 H, m, H-22), 3.31 (1 H, d-like, J = 14 Hz, H-14), 3.76 (3 H, s, $COOCH_3$), 1.92 (3 H, d, J = 1.0 Hz), 2.18 (3 H, d, J = 1.0 Hz) (23-CH_3), 1.32 (3 H, s, 10-CH_3), 1.11 (3 H, d, J = 7.0 Hz, 4-CH_3), 2.01, 2.06, 2.09, 2.18 (3 H, s each, 4$OCOCH_3$), 2.03 (6 H, s, 2$OCOCH_3$).

Anal. Calcd for $C_{44}H_{54}O_{22}$: C, 56.52; H, 5.82. Found: C, 56.60; H, 5.83.

Compound **6**: mp 124–125 °C; IR ($CHCl_3$) 3500 (OH), 1765, 1758, 1751 (ester and lactone C=O), 1696 (a,b-unsaturated C=O), 1651 (C=C) cm^{-1}.

Anal. Calcd for $C_{42}H_{52}O_{21}$: C, 56.47; H, 5.87. Found: C, 56.70; H, 5.58.

Compound **6** could also be converted to compound **5** quantitatively by treatment of **6** (1 mg) with *p*-toluenesulfonic acid (2 mg) in acetic anhydride (0.2 mL) followed by stirring the mixture at room temperature for 2 h.

Acetolysis of 1. To a solution of 1 (15 mg) in acetic anhydride (1.5 mL) was added zinc chloride (15 mg). After being heated at 90–110 °C for 2 h, the mixture was poured into ice water, neutralized with sodium bicarbonate, and extracted with chloroform (150 mL). The chloroform extract was worked up in the usual manner to give a product (15 mg) which was purified by preparative TLC (10:1 chloroform–acetone, detected by iodine vapor) to afford an amorphous triacetate (**9**).

Anal. Calcd for $C_{32}H_{38}O_{14}$: *m/e* 646.2250. Found: *m/e* 646.2267

The above preparative TLC also yielded a pentaacetyl glucopyranoside (an α and β mixture) which was identified with a synthetic sample prepared from D-glucose by direct comparison (TLC and mass spectra).

Methylation of Bruceoside-A(1). A solution of bruceoside-A (140 mg) in *N,N*-dimethylformamide (7 mL) was added to methyl iodide (6 mL) and silver oxide (1.5 g). After being stirred at 27–30 °C for 24 h in the dark, the reaction mixture was filtered. The filtrate was diluted with water, extracted with chloroform, and dried ($MgSO_4$). The dried chloroform extract was evaporated under reduced pressure to furnish a product (**7**). Subsequent purification of **7** by column chromatography on silica gel (25 g) (7:1 benzene–acetone) and recrystallization of the eluates from tetrachloromethane gave the hepta-*O*-methyl derivative (**7**, 80 mg) as colorless needles: mp 118–121 °C (from carbon tetrachloride); IR ($CHCl_3$) no OH, 1750 (lactone and ester C=O), 1700 (α,β-unsaturated C=O), 1650 and 1630 (C=C) cm^{-1}.

Anal. Calcd for $C_{38}H_{54}O_{16}$: C, 59.52; H, 7.09. Found: C, 59.49; H, 7.26.

Methanolysis of 7. A solution of **7** (27 mg) in anhydrous 7% HCl–MeOH (6 mL) was refluxed for 2 h. The reaction mixture was neutralized with Ag_2CO_3 and filtered. The filtrate was concentrated under reduced pressure, and the product was purified by preparative TLC (3:1 benzene–acetone, detected by iodine vapor) to yield methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside, which was identified by TLC and GLC retention time [3.75 min (2 mm × 2 m 3% OV-17 on Chromosorb W (80–100 mesh), column temperature 170 °C, 10 mL/min N_2 gas flow)] by comparison with an authentic sample prepared by methylation of D-glucose.

Brucoeside-B (2). Bruceoside-B was isolated as colorless prisms: mp 223–224 °C dec; $[\alpha]^{25}_D$ +3.73° (*c* 1.02, MeOH); IR (KBr) 3410 (OH), 1730 (δ-lactone and ester C=O), 1670 (conjugated C=O), 1655 (C=C) cm^{-1}.

Anal. Calcd for $C_{32}H_{42}O_{16}$: C, 56.30; H, 6.20. Found: C, 56.75; H, 6.45.

Acid and Enzymatic Hydrolyses of 2. Hydrolysis of **2** by either 3 N sulfuric acid–methanol (1:1) or β-glucosidase according to similar procedures to those described above for the hydrolysis of **1** led to the isolation and identification of brusatol (**8**) as the only aglycon.

Acetolysis of 2. Treatment of **2** (50 mg) with zinc chloride (20 mg) in acetic anhydride (2 mL) in a similar manner as described above for the acetolysis of **1** afforded **12** as colorless needles, mp 247–250 °C. The identity of **12** was established by direct comparison with a sample of brusatol triacetate obtained by acetylation of brusatol with pyridine–acetic anhydride (mixture melting point, TLC, and superimposable IR spectra).

The sugar portion isolated from this reaction was identified as pentaacetyl glucopyranoside.

Bruceoside-B Hexaacetate (10) and Pentaacetate (11). Bruceoside-B (100 mg) was acetylated with acetic anhydride and pyridine for 24 h at room temperature and purified by preparative TLC (4:1 chloroform–acetone) to yield the pure hexaacetate (**10**, 28 mg) and pentaacetate (**11**, 23 mg) after one recrystallization from ethyl acetate–*n*-hexane each, respectively.

Compound **10**: mp 266.5–268.5 °C dec; mass spectrum, *m/e* 934 (M^+).

Anal. Calcd for $C_{44}H_{54}O_{22}$: C, 56.53; H, 5.82. Found: C, 56.50; H, 5.86.

Compound **11**: mp 275.5–278 °C dec; mass spectrum, *m/e* 832 [M^+ − 60 (CH_3COOH)].

Anal. Calcd for $C_{42}H_{52}O_{21}\cdot H_2O$: C, 55.99; H, 6.04. Found: C, 55.75; H, 5.99.

Methylation of Bruceoside-B (2). Bruceoside-B (**2**) was methylated, and the product was isolated in an analogous manner as described for **7** to yield the hepta-*O*-methyl derivative (**16**) as colorless crystals, mp 118–119 °C.

Anal. Calcd for $C_{38}H_{54}O_{16}$: *m/e* 766.3409. Found: *m/e* 766.3400.

Methanolysis of 16. This was carried out by a procedure exactly identical with that for the methanolysis of **7**. The methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside isolated was identified by TLC and GLC with the compound prepared from methylation of D-glucose.

Brucein-D (3). Brucein-D showed the following: mp 294–296 °C (dichloromethane–ether–methanol) (lit.[10] mp 285–290 °C); IR (Nujol) 3380 (br), 1710, 1655, 1620 cm^{-1}; ^{13}C NMR (25.20 MHz, Me_2SO-d_6) 73.0 (C-1), 166.5 (C-2), 107.1 (C-3), 138.6 (C-4), 41.8 (C-5), 29.6 (C-6), 63.1 (C-7), 46.9 (C-8), 42.9 (C-9), 46.1 (C-10), 70.8 (C-11), 71.7 (C-12), 72.6 (C-13), 74.4 (C-14), 66.9 (C-15), 146.9 (C-16), 62.7 (C-17), 25.6 (Me-4), 16.7 (Me-10), 22.6 (Me-13).

Anal. Calcd for $C_{20}H_{26}O_9$: C, 58.53, H, 6.39. Found: C, 58.61; H, 6.39.

Brucein-D Triacetate (13). Acetylation of **3** (10 mg) with acetic anhydride (1 mL) and pyridine (1 mL) at room temperature for 46 h followed by the usual workup yielded a product which was purified by preparative TLC (5:1 chloroform–acetone) to give a triacetate [**13**, 8 mg, mp 243–246 °C (ether–chloroform)] whose NMR spectrum (Table I) was in accord with the assigned structure.

Anal. Calcd for $C_{26}H_{32}O_{12}$: C, 58.20; H, 6.01. Found: C, 58.10; H, 6.00.

Oxidation of 13 with Jones' Reagent. To a solution of **13** (2.8 mg) in acetone (2 mL) was added one drop of Jones' reagent. After the mixture was kept at room temperature for 3 h, it was diluted with water and the product was extracted with ether. The ether extract was washed, dried, and evaporated to give a ketone (**17**, 2 mg), mp 254–256 °C (acetone–carbon tetrachloride) (lit.[9] mp 259–262 °C). The NMR spectrum of **17** is identical with that of 11-ketobrucein triacetate reported by Polonsky and co-workers.[10]

Brucein-E (4). Brucein-E was obtained as colorless crystals (chloroform–methanol–water): mp 261–265 °C (lit.[10] mp 260–264 °C); IR (Nujol) 3500, 3250, 1710, 1660 cm^{-1}.

Anal. Calcd for $C_{20}H_{28}O_9$: C, 58.24; H, 6.84. Found: C, 58.56; H, 6.72.

Brucein-E Tetraacetate (14). Acetylation of **4** (20 mg) by treatment with acetic anhydride–pyridine yielded a product (13 mg) which gave an NMR spectrum which was consistent with the structure of brucein-E tetraacetate (**14**). The product (**14**) formed colorless needles from ether–chloroform and had mp 250–253 °C (lit.[10] mp 250–254 °C).

Anal. Calcd for $C_{28}H_{36}O_{13}$: C, 57.92; H, 5.79. Found: C, 57.58; H, 5.60.

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Registry No.—**1**, 63306-30-9; **2**, 69687-69-0; **3**, 21499-66-1; **4**, 21586-90-3; **5**, 63344-09-2; **6**, 69687-70-3; **7**, 63344-10-5; **8**, 14907-98-3; **8**, 14907-98-3; **9**, 63344-08-1; **10**, 69687-71-4; **11**, 69687-72-5; **12**, 14907-99-4; **13**, 21586-89-0; **14**, 21499-68-3; **16**, 69745-20-6; **17**, 21499-67-2; methyl 2,3,4,6-tetra-*O*-methyl-β-D-glucopyranoside, 3149-65-3.

References and Notes

(1) (a) Dedicated to the memory of the late Dr. T. A. Geissman, Professor Emeritus of the Department of Chemistry, University of California at Los Angeles. (b) For part 32, see K.-H. Lee, E. C. Mar, M. Okamoto, and I. H. Hall, *J. Med. Chem.*, **21**, 819 (1978). Presented in part before the Academy of the 125th American Pharmaceutical Association Annual Meeting in Montreal, Canada, May 16, 1978.
(2) "Ku Chin Chung Yao Chi Ch'eng", Ta Chung Shu Chu, 1957, p 448.
(3) W. S. Kan, "Pharmaceutical Botany", National Research Institute of Chinese Medicine, Taiwan, Republic of China, 1969, p 356.
(4) M. Sugi and Y. Nagashio in "Cancer Therapy in Modern China", K. Kondo, Ed., Shizen Sha, Japan, 1977, p 109.
(5) In vivo activity was assayed by the standard National Cancer Institute procedures described by R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep., Part 3*, **3**, 1 (1972). Bruceoside-A and -B showed significant (T/C ≥ 120%) antileukemic activity in P-388 leukemia (T/C = 156 and 132%) at the 6 and 1.5 mg/kg/day levels, respectively. Brucein-D demosntrated significant inhibitory activity against the Walker 256 carcinosarcoma in rats at T/C 144% at the 0.5 mg/kg/day level.
(6) K.-H. Lee, Y. Imakura and H.-C. Huang, *J. Chem. Soc., Chem. Commun.*, 69 (1977).
(7) R. Kuhn, H. Trischman, and I. Löw, *Angew. Chem.*, **67**, 32 (1955).
(8) K. Y. Sim, J. J. Sims, and T. A. Geissman, *J. Org. Chem.*, **33**, 429 (1968).
(9) J. Polonsky, Z. Baskévitch, H. E. Gottlieb, E. W. Hagaman, and E. Wenkert, *J. Org. Chem.*, **40**, 2499 (1975).
(10) J. Polonsky, Z. Baskévitch, M. B. Das, and J. Müller, *C. R. Hebd. Seances Acad. Sci., Ser. C*, **267**, 1346 (1968).
(11) A voucher specimen is available for inspection at the Herbarium of the Department of Botany, Kaohsiung Medical College, Kaohsiung, Taiwan, Republic of China.
(12) Product of Aldrich Chemical Co., Inc., Milwaukee, Wis.
(13) R. D. Guthrie and J. F. McCarthy, *Adv. Carbohydr. Chem.*, **22**, 11 (1967).

Novel Conversion of Aromatic Ester Groups to Methyl. Catalytic Effect of Iodine in Reactions Involving Iodotrimethylsilane

Robert A. Benkeser,* Edward C. Mozdzen, and Charles L. Muth

Department of Chemistry, Purdue University, West Lafayette, Indiana 47907

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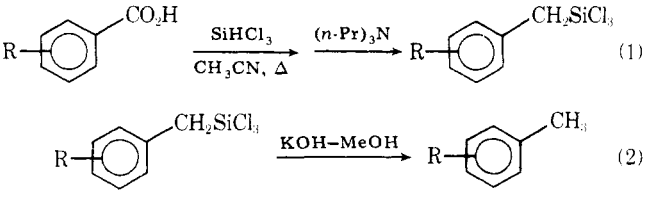
A three-step sequential procedure has been developed whereby a "one-pot" reduction of aromatic ester groups directly to methyl is now possible. One of these steps involves conversion of the aromatic ester to a trimethylsilyloxy ester by means of iodotrimethylsilane or one of its variants. A strong catalytic effect by iodine in reactions involving iodotrimethylsilane has been discovered. The intermediacy of trimethylsilyl triiodide is proposed to explain certain spectral effects noted in the NMR. Iodine catalysis also permits an alternative suggestion as to the reason for the superiority of trimethylphenylsilane–iodine combinations as a substitute for iodotrimethylsilane itself.

Some years ago, we reported[1] that trichlorosilane, in the presence of tertiary aliphatic amines, was a powerful reducing agent. For example, the reagent was capable of reducing aromatic carboxylic acids to benzylic trichlorosilanes[2] (eq 1). The Si–C bond of such benzylic silanes was very susceptible to base cleavage[3] (eq 2). A procedure was devised such that steps 1 and 2 could be carried out sequentially in the same reaction flask,



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