Bruceoside-A, a Novel Antileukaemic Quassinoid Glycoside from *Brucea javanica*

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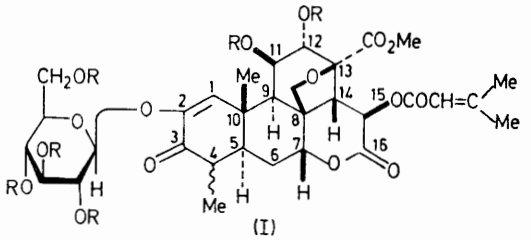
Summary The structure and stereochemistry (exclusive of the configuration of the methyl group at C-4) of bruceoside-A, a novel antileukaemic quassinoid glycoside isolated from *Brucea javanica* (Linn) Merr., have been established from chemical transformations, correlations, and spectral analyses.

As a result of the continuing search among Formosan plants for new and novel naturally occurring potential antitumour agents,[1] the methanolic extract of the seeds of *Brucea javanica* (Linn.) Merr. (Simaroubaceae) (procured and identified by H.C.H.; also known as 'Ya-Tan-Tzu' in folklore) was found to show significant inhibitory activity *in vivo* against the Ehrlich ascites carcinoma and the Walker 256 carcinosarcoma, as well as the P-388 lymphocytic leukaemia.† We report herein the structural elucidation of a novel and potent antileukaemic principle, bruceoside-A (Ia),† which was isolated from this active extract. Bruceoside-A appears to be the first quassinoid glycoside which has been demonstrated to have such activity.

Bruceoside-A (Ia), m.p. 175—180 °C, $[\alpha]_D^{25}$ + 9·2 (*c*, 0·50, MeOH), has the composition $C_{32}H_{42}O_{16}$, and is very bitter. It shows i.r. bands (Nujol) at 3433, 1732, 1674, and 1640 cm^{-1}. Its n.m.r. spectrum (CD_3OD) disclosed the presence of many OH groups, a senecioyl‡ group [δ 1·93 and 2·16 (each 3H d, *J* 1·5 Hz) (senecioyl Me), and 5·36 (1H, m, senecioyl α-H)], a CO_2Me group [δ 3·76 (3·78 in C_5D_5N) (3H, s)] at C-13 and 2 Me groups at C-4 [δ 1·15 (3H, d, *J* 6·0 Hz)] and C-10 [δ 1·60 (3H, s)]. 15-H and 1-H signals were seen at δ 6·02 (1H, d, *J* 13·0 Hz) and 6·84 (1H, s), respectively.

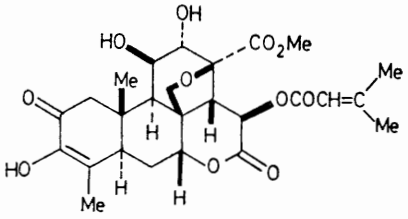
Acid hydrolysis of (Ia) with MeOH–3N H_2SO_4 (1 : 1) yielded D-glucose [identified by paper partition chromatography and g.l.c. (as its Me_3Si derivative)] and the major aglycone, compound (II) which gave a dark green colour with 2% aqueous ferric chloride. The n.m.r. spectrum ($CDCl_3$) of (II) [m.p. 274—277 °C; $C_{26}H_{32}O_{11}$; *m/e* 520·1951 (M^+), 83 (base peak, $Me_2C{=}CHCO^+$] revealed the absence of the characteristic 4-Me and 1-H signals at δ 1·15 and 6·84, respectively, in the spectra of (Ia), and also (Ib), (Ic), and (IIIb) and the presence of peaks [δ 1·39 (3H, s, 10-Me), 1·84 (3H, d, *J* 2·0 Hz, 4-Me), 1·93 and 2·20 (each 3H, d, *J* 1·5 Hz, senecioyl‡ Me), 3·12 (1H, d-like, *J* 13·0 Hz, 14-H), 3·80 (3H, s, 13-CO_2Me), 4·80 (1H, m, 7-H), 5·64 (1H, m, senecioyl‡ α-H), and 6·26 (1H, d, *J* 13·0 Hz, 15-H)] identical to those of brusatol.[2] A direct comparison (t.l.c., and n.m.r. and mass spectra) established the identity of (II) with brusatol.

Acetolysis of (Ia) with Ac_2O–AcONa gave a triacetate [(IIIb); amorphous; $C_{32}H_{38}O_{14}$; *m/e* 646·2267 (M^+), 83 (base peak, $Me_2C{=}CHCO^+$)] and a penta-acetyl glucopyranoside (an α and β mixture).§ Compound (IIIb) showed n.m.r. ($CDCl_3$) signals assignable to 1 CO_2Me [δ 3·74 (3H, s)], 3 Ac

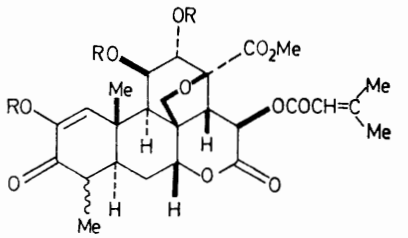


(I)

a, R = H (bruceoside-A)
b, R = COMe
c, R = Me



(II) (brusatol)



(III)

a, R = H (bruceosin)
b, R = COMe

[δ 2·04, 2·18, and 2·24 (each 3H, s)], 1 senecioyl‡ [δ 1·92 and 2·18 (each 3H, d, *J* 1·5 Hz), and 5·63 (1H, m)], and 2 Me groups [δ 1·18 (3H, d, *J* 6·0 Hz, 4-Me) and 1·39 (3H, s, 10-Me)]. Extensive double resonance experiments identified other proton signals at δ 3·31 (1H, d-like, *J* 13·0 Hz,

† *In vivo* activity was assayed by Dr. I. H. Hall, Department of Medicinal Chemistry, School of Pharmacy, University of North Carolina at Chapel Hill, by a literature method [R. I. Geran, N. H. Greenberg, M. M. MacDonald, A. M. Schumacher, and B. J. Abbott, *Cancer Chemother. Rep.* (*Part* 3), 1972, 3, 1]. Bruceoside-A showed significant ($T/C \geq 125\%$) antileukaemic activity in P-388 leukaemia (T/C = 156%) at the 6 mg kg^{-1} day^{-1} level.

‡ Senecioyl = $Me_2C{=}CHCO$–.

§ The identity of this compound with a synthetic sample prepared from D-glucose was established by direct comparison (t.l.c. and mass spectra).

14-H), 3·87 (1H, dd, *J* 8·0 and 2·0 Hz, 8-CH_2O), 4·73 (1H, d, *J* 8·0 Hz, 8-CH_2O), 4·78 (1H, m, 7-H), 6·00 (1H, d, *J* 13·0 Hz, 15-H), and 6·62 (1H, s, 1-H).

This evidence led to the conclusion that (II) was a secondary product of the acid hydrolysis of (Ia). Consequently, the structure of the aglycone (*i.e.* bruceosin) of bruceoside-A was established as (IIIa).

Acetylation of (Ia) with acetic anhydride in pyridine afforded a hexa-acetate [(Ib); $C_{44}H_{54}O_{22}$; m.p. 162—165 °C; δ ($CDCl_3$–C_6D_6, 1:1) 1·00 (3H, d, *J* 6·0 Hz, 4-Me), 1·02 (3H, s, 10-Me), 1·71 and 2·09 (each 3H, d, *J* 1·5 Hz, senecioyl‡ Me), 1·86, 1·87, and 1·94 (each 3H, s, Ac), 1·98 (9H, s, 3 × Ac), 3·49 and 4·47 (each 1H, d, *J* 8·0 Hz, 8-CH_2O), 3·58 (3H, s, 13-CO_2Me), 3·14 (1H, d-like, *J* 14·0 Hz, 14-H), 6·05 (1H, d, *J* 14·0 Hz, 15-H), and 6·17 (1H, s, 1-H)]. Methylation of (Ia) with MeI–Ag_2O–dimethylformamide[3] led to the formation of a hepta-*O*-methyl derivative [(Ic); $C_{38}H_{54}O_{16}$; m.p. 118—121 °C] which lacks the OH absorption bands in the i.r. spectrum ($CHCl_3$). Its n.m.r. ($CDCl_3$) spectrum exhibited signals due to 6 OMe [δ3·37, 3·41, 3·48, 3·56, 3·66, and 3·69 (each 3H, s)] 1 CO_2Me [δ 3·77 (3H, s)], and 1 senecioyl groups [δ 1·91 and 2·18 (each 3H, d, *J* 1·5 Hz) and 5·69 (1H, m)], and one anomeric proton [δ 4·59 (1H, d, *J* 8·0 Hz)] which indicated the presence of a β-glucopyranoside linkage. Methanolysis of (Ic) with 10% HCl–MeOH yielded methyl 2,3,4,6-tetra-*O*-methyl glucopyranoside (identified by t.l.c. and g.l.c.). The above evidence led to the structural assignment of bruceoside-A as bruceosin 2-β-D-glucopyranoside (Ia).

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