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BRYONOSIDE AND BRYOSIDE—NEW TRITERPENE GLYCOSIDES FROM *BRYONIA DIOICA*

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Key Word Index—*Bryonia dioica*; Cucurbitaceae; triterpene glycosides; cucurbitacins; bryonoside; bryoside.

Abstract—The structures of two new triterpene glycosides from *Bryonia dioica* have been shown, on the basis of chemical and FDMS and ^{1}H and ^{13}C NMR spectral evidence, to be 10α-cucurbit-5-en-11-oxo-3β,24ζ,25-triol-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside-25-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside (bryonoside) and 10α-cucurbit-5-in-11-oxo-3β,24ζ,25-triol-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside-25-*O*-β-D-glucopyranoside (bryoside).

INTRODUCTION

The presence of glycosides in the roots of *Bryonia dioica* Jacq. has been known since the beginning of the twentieth century [1]. Only partial structures were elucidated and several apparently different compounds have been referred to by the general name "bryonin(e)" [1, 2]. The only detailed work was carried out in 1959 by Tunmann and Schehrer [2] who began investigation of the structure of the acetates of two glycosides, bryodulcoside (shown to be a trisaccharide) and bryobioside (a disaccharide). Seven years later, Tunmann and Stapel [3] almost completed the structural elucidation by showing them to be derivatives of the simple cucurbitacin-type compound, bryodulcosigenin. It was further shown that bryodulcoside, $C_{49}H_{80}O_{18}$, could be enzymatically hydrolysed to yield bryobioside, $C_{42}H_{70}O_{13}$. We now report the isolation and complete characterization of two tetracyclic triterpene glycosides also from the roots of *Bryonia dioica.* One of the glycosides characterized now is isomeric with the bryodulcoside of Tunmann and Stapel [3].

RESULTS AND DISCUSSION

The new glycosides, named bryonoside (**1**), mp 207–213°, and bryoside (**2**), mp 179–183°, were obtained as colourless powders by the method described in the Experimental.

IR absorption bands of **1** were due to hydroxyl groups (3700–3450 cm^{-1}), a carbonyl group (1685 cm^{-1}) and a trisubstituted double bond (1653 and 811 cm^{-1}). The ^{1}H NMR spectrum of **1** exhibited seven tertiary methyl signals at δ 1.71–0.73 and two secondary methyl signals at 0.93 and 1.71 (both doublets with $J = 6$ Hz). The IR and ^{1}H NMR spectra of **2** showed similar peaks.

Acid hydrolysis of **1** afforded a sapogenin, mp 183°, MS m/z: 474 $[M]^+$ and a related artefact compound, mp 154°, MS m/z: 456 $[M]^+$ together with D-glucose and L-rhamnose. The sugars were identified by paper co-chromatography. Acid hydrolysis of **2** also afforded the same sapogenin, the same related compound and again D-glucose and L-rhamnose. IR, ^{1}H NMR and mass spectral data of the sapogenin and the related compound obtained from **2** were identical with those of the sapogenin and the related compound obtained from **1**. By spectral comparison these sapogenins were readily identified as the known bryodulcosigenin (**3**) [4] and its related dehydration artefact bryogenin (**4**) [3].

Acetates (**5** and **6**) of **1** and **2** respectively were obtained by the usual method (Ac_2O–pyridine, at room temperature for 24 hr). The ^{1}H NMR and IR spectra of **5** and **6** showed no hydroxyl group absorption. The sapogenin bryodulcosigenin has a tertiary hydroxyl at C-25 which resists acetylation by this method. Since IR absorptions due to a hydroxyl group were not present in the spectra of either acetate, **1** and **2** do not possess such a 25-hydroxyl function. Hence, this hydroxyl group cannot be free and must carry a sugar residue in both glycosides.

The permethylated glycosides of **1** and **2** were prepared by the Hakomori method [5] and were subjected to methanolysis to yield, from **1**, three methylated sugars. These were investigated by GLC [6] and identified as the methyl pyranosides of (a) 2,3,4,6-tetra-*O*-methyl-D-glucose, (b) 2,3,4-tri-*O*-methyl-L-rhamnose and (c) 3,4,6-tri-*O*-methyl-D-glucose. The products obtained from the permethylate of **2** gave R_t values identical with those from **1**.

Field desorption mass spectrometry (FDMS) of **1** gave rise to a cationized molecular peak [7] at m/z 1129, which was also the base peak, likely to be $[M + Na]^+$ in which case M would equal $C_{54}H_{90}O_{23}$. Elemental analysis of a hydrate of **1** also indicated this molecular composition. The fragment peaks observed were attributed [8] to the ions formed by proton-induced cleavage: $[(M+Na)—146]^+$ at m/z 983, $[(M+Na)—162]^+$ at m/z 967, $[(M+Na)—308]^+$ at m/z 821 and $[(M+Na)—324]^+$ at m/z 805 which are respectively ascribable to loss of the terminal rhamnose, terminal glucose, rhamnosyl-glucose and glucosyl-glucose residues as shown in Table 1. In the FDMS of **2**, a peak at m/z 983, probably corresponding with $[M+{}^{39}K]^+$ and the base peak at m/z 967, likely to be to the equivalent $[M+Na]^+$ ion, were observed. The projected cationized molecular ion suggested that bryonoside (**1**) consisted of bryodulcosigenin together with three glucose and one rhamnose residues while bryoside (**2**) comprised bryodulcosigenin with two glucose and one rhamnose residues. Moreover, from the fragmentation, two terminal sugar residues (one glucose and one rhamnose) are likely to be present in both glycosides.

Compound **1** was enzymatically hydrolysed with β-glucosidase to obtain a compound the FDMS of which showed peaks at m/z 983 and 967 identical with those observed in the spectrum of **2**. The correct assignment of the cationized molecular ions were confirmed by the appearance of the doubly charged ions, $[M+2Na]^{2+}$ at m/z 495 and $[M+2{}^{39}K]^{2+}$ at m/z 511 [9]. Enzymatic hydrolysis of **2** (or the compound obtained by hydrolysis of **1**) under harsher conditions provided **7**, the FDMS of which corresponded with that of **2** after loss of a further terminal glucose residue. The sugar residues thus appear to be present as two disaccharide moieties—a glucosyl-glucoside and a rhamnosyl-glucoside. The IR spectrum of the acetate (**8**) of **7** showed hydroxyl group absorption at 3510 cm^{-1}, probably due to the tertiary hydroxyl group at C-25. If this is the case, the rhamnosyl-glucoside moiety of **7** cannot be joined to the C-25 hydroxyl. The FDMS of **7** gave rise to a base peak at m/z 805 which could be attributed to a $[M+Na]^+$ ion. The peak at m/z 805 also arose in the FDMS of **1** and **2**, due to the fragmentation product of these compounds which contained one molecule of D-glucose and one of L-rhamnose as shown in Table 1.

In the ^1H NMR spectra of **1**, **2** and **7** (in C_5D_5N), anomeric proton signals were seen in the δ 4.85–6.00 region [10]. In that of **1**, a signal at δ 5.06 (*d*, J = 7 Hz), which was absent from the spectra of **2** and **7**, was identified as an anomeric proton of the terminal β-D-glucose (Gl-3 in Fig. 1). Another doublet signal in the ^1H NMR spectra of **1** and **2** at δ 5.22 and 5.23 (J = 8 Hz) respectively which was absent from that of **7** was also assigned to the anomeric proton of β-D-glucose (Gl-2). As the IR spectra of acetates **5** and **6** showed no hydroxyl absorption, this glucose is likely to be attached to the C-25 hydroxyl group of the aglycone. The ^1H NMR spectra of all three glycosides, **1**, **2** and **7** gave rise to a signal at δ 4.89–4.90 (partially resolved doublet, J = 8 Hz) which was assigned to a further anomeric proton of β-D-glucose (Gl-1). A low-field signal at 5.78–5.79 (*s*) in the spectra of **1**, **2** and **7** must be due to the anomeric proton of L-rhamnose (Rh). The rhamnose residue also gave rise to a methyl doublet (J = 6 Hz) at 1.70. Comparison of the signal for H-6 of bryodulcosigenin (5.70) with that in the spectra of **1**, **2** and **7** (5.98–6.00) shows a difference in chemical shift of about 0.3 ppm (deshielding) indicating that another sugar residue (Rh + Gl-1 in Fig. 1) is probably attached to the C-3 hydroxyl group of the aglycone. ^1H NMR data obtained in CD_3OD confirmed these deductions (see Experimental).

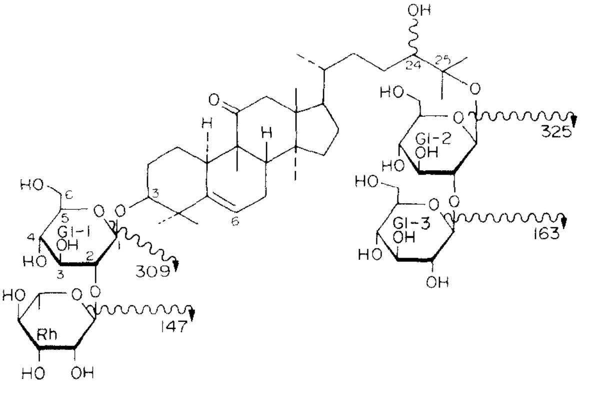


Fig. 1. Bryonoside, showing some FDMS fragmentations and the sugar residue numbering sequence used in Tables 1 and 2. Bryoside has Gl-3 replaced by H.

Compound **1** was partially hydrolysed under mild conditions using a cation exchange resin in the acidic form to obtain a polar glycoside (**9**), mp 229–233°. Compound **9** gave bryodulcosigenin and D-glucose only on acid hydrolysis. Examination of the ^1H NMR spectrum of **9** showed three anomeric proton signals of β-D-glucose (δ 4.89, 5.09 and 5.22, *ds*, J = 8 Hz) and the absence of an anomeric proton signal and of a methyl doublet for a L-rhamnose residue.

Confirmatory evidence was provided by a comparison of the molecular rotation data [11] of **1** ($[M]_D = +289°$) with that of **2** ($[M]_D = +292°$) which indicated that they probably differed by a terminal β-D-glucose residue. Similarly, data for **7** ($[M]_D = +308°$) showed that this material also differed from **2** by the absence of another β-D-glucose moiety. On the other hand, $[M]_D$ for **9** was found to be +366° showing that it was likely that this material had lost an α-L-rhamnose residue compared with **1**. Taken together, all these data indicate that the new glycosides **1** and **2** and their enzymatic and partial hydrolysis products **7** and **9** have the structures indicated.

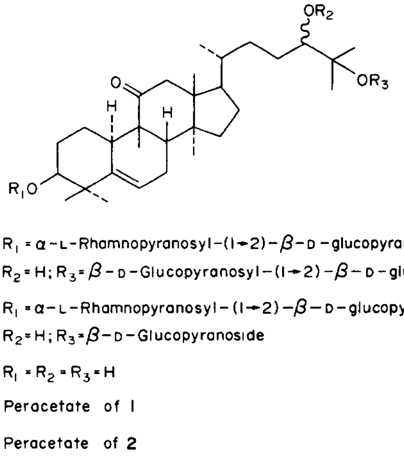
Confirmation of the structures of **1** and **2** was made by ^{13}C NMR spectroscopy. The assignment of ^{13}C NMR signals to the individual carbons in the sapogenin (bryodulcosigenin) and its acetates was aided by chemical shift rules [12], hydroxyl substitution shifts [13], acetylation shifts [14], steric effects [15] and known ^{13}C NMR data of trimethyl *trans*-decanols [16] and perhydrophenanthrene [17]. Comparison of the ^{13}C NMR spectrum of bryodulcosigenin with those of cucurbitacins [18], tetracyclic triterpenes [19] and steroids [20] permitted the complete assignment which was supported by ^{13}C NMR data of the related bryogenin and its acetate. Signals of **1** and **2** were assigned in a similar manner. The spectral data of bryogenin, bryodulcosigenin and the corresponding acetates together with the glycosides **1** and **2** are shown in Table 2. On going from bryodulcosigenin to **1**, a signal (a doublet in the off-resonance spectrum) due to C-3 was deshielded by 10.5 ppm and the C-25 signal (a singlet) deshielded by 8.2 ppm. Compound **2** also gave

Table 1. FDMS of bryonoside (**1**), bryoside (**2**) and the enzymatic hydrolysis products of **1** and **2**. (% relative intensities in parentheses)

m/z	Bryonoside (**1**)	Bryoside (**2**)	Enzymatic hydrolysis product of **1**	Enzymatic hydrolysis product of **2**
1129	[M + Na]$^+$ (100)			
1111	[(M + Na)—H$_2$O]$^+$ (2)			
983	[(M + Na)—146]$^+$ (26)	[M + ^{39}K]$^+$ (10)	[M + ^{39}K]$^+$ (100)	
967	[(M + Na)—162]$^+$ (15)	[M + Na]$^+$ (100)	[M + Na]$^+$ (28)	
949	[(M + Na)—162—H$_2$O]$^+$ (2)	[(M + Na)—162—H$_2$O]$^+$ (2)	[(M + Na)—162—H$_2$O]$^+$ (2)	
837	—	[(M + ^{39}K)—146]$^+$ (3)	[(M + ^{39}K)—146]$^+$ (4)	
821	[(M + Na)—308]$^+$ (12)	[(M + Na)—146]$^+$ (8)	[(M + Na)—146]$^+$ (7)	
805	[(M + Na)—324]$^+$ (8)	[(M + Na)—162]$^+$ (2)	[(M + Na)—162]$^+$ (2)	[M + Na]$^+$ (100)
787	[(M + Na)—324—H$_2$O]$^+$ (4)	—	—	—
659	[(M + Na)—324—146]$^+$ (2)	[(M + Na)—308]$^+$ (2)	[(M + Na)—308]$^+$ (2)	—
576	[M + 2Na]$^{2+}$ (54)	—	—	—
511	—	—	[M + 2^{39}K]$^{2+}$ (2)	—
503	[(M + 2Na)—146]$^{2+}$ (4)	—	[M + ^{39}K + Na]$^{2+}$ (20)	—
495	[(M + 2Na)—162]$^{2+}$ (7)	[M + 2Na]$^+$ (5)	[M + 2Na]$^{2+}$ (36)	—
414	—	—	—	[M + 2Na]$^{2+}$ (54)

rise to C-3 and C-25 signals which were deshielded. This deshielding at the α-carbons (C-3 and C-25) with corresponding shielding at the non-quarternary β-carbons may be considered as characteristic of glycosidation due to steric changes in the environment of the hydroxyl group (Table 2) [21]. The sugar residues are thus shown, in the case of **1** and **2**, to be attached to C-3 and C-25 of the aglycone, respectively. In the spectrum of **1**, a high intensity signal at δ 104.8 was assigned to the two anomeric carbons of β-D-glucose which evidently overlapped. Confirmation was provided by a comparison of this signal with that at δ 104.8 in the spectrum of **2** which was attributed to an anomeric carbon of β-D-glucose, the intensity of the former signal being about twice that of the latter. The shift of this signal (δ 104.8) indicates that the glucose residues concerned are joined to secondary hydroxyl groups and this assignment is confirmed by the data obtained by GLC and ^{1}H NMR studies. A signal at δ 97.4 in the ^{1}H NMR spectrum of **1** was due to an anomeric carbon of β-D-glucose which is bound directly to the sterically more hindered tertiary C-25 hydroxyl and therefore resonates at higher field [22]. In the ^{1}H NMR spectrum of **2**, a signal at δ 97.2 was then immediately recognizable as an anomeric carbon of a tertiary alkyl-β-D-glucoside. Such a glucoside was also suggested by the observation that enzymatic hydrolysis of **2** took far longer to accomplish than did that of **1** [23]. In the ^{13}C NMR spectra of **1** and **2**, signals at δ 100.7 and 100.6 were attributed to the anomeric carbon of the terminal α-L-rhamnoside respectively. Compound **2** is therefore shown to be an α-L-rhamnosyl-β-D-glucoside at C-3 and β-D-glucoside at C-25.

The structure of the new glycosides are thus **1**: 10α-cucurbit-5-en-11-oxo-3β, 24ζ, 25-triol-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside-25-*O*-β-D-glucopyranosyl-(1 → 2)-β-D-glucopyranoside (bryonoside) and **2**: 10α-cucurbit-5-en-11-oxo-3β,24ζ, **25-triol-3-*O*-α-L-rhamnopyranosyl-(1 → 2)-β-D-glucopyranoside-25-*O*-β-D-glucopyranoside (bryoside).**



1 R_1 = α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranoside;
R_2 = H; R_3 = β-D-Glucopyranosyl-(1→2)-β-D-glucopyranoside

2 R_1 = α-L-Rhamnopyranosyl-(1→2)-β-D-glucopyranoside;
R_2 = H; R_3 = β-D-Glucopyranoside

3 R_1 = R_2 = R_3 = H

5 Peracetate of 1

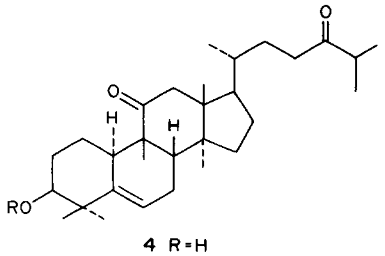
6 Peracetate of 2

7 R_1 = α-L-Rhamnopyranosyl-(1→2)-β-D-Glucopyranoside; R_2 = R_3 = H

8 R_1 = Peracetyl-α-L-rhamnopyranosyl-(1→2)-β-D-glucopyranoside;
R_2 = MeCO; R_3 = H

9 R_1 = β-D-Glucopyranoside; R_2 = H; R_3 = β-D-Glucopyranoside-
(1→2)-β-D-glucopyranoside

10 R_1 = R_2 = MeCO; R_3 = H



4 R = H
11 R = MeCO

Table 2. ^{13}C NMR spectral data of compounds **1**, **2**, **3**, **4**, **10** and **11** at 25.05 MHz in C_5D_5N

Carbon no.	**4**	**11**	**3**	**10**	**1**	**2**		Carbon no.	Sugar residues **1**	Sugar residues **2**
1	34.4	34.4	34.4	34.5	34.1	34.1				
2	29.7	25.0	29.6	25.0	25.3	25.3	Gl-1*	1	104.8	104.8
3	75.4	78.1	75.3	78.0	85.8	85.8		2	80.0	80.0
4	41.8	40.0	41.6	40.0	41.9	41.8		3	78.1	78.0a
5	141.2	139.8	141.0	139.7	139.3	139.2		4	71.7	71.4
6	118.8	119.2	118.6	118.9	119.5	119.5		5	78.1	78.5a
7	24.1	24.0	24.0	23.9	24.2	24.1		6	62.4	62.5
8	43.9	43.8	43.9	43.7	43.9	43.9				
9	48.6	48.6	48.6	48.6	48.8	48.7	Rh†	1	100.7	100.4
10	35.7	35.5a	35.8	35.4	35.8	35.8		2	72.1	72.1
11	213.7	213.3	213.3	212.9	213.4	213.4		3	72.1	72.1
12	48.6	48.6	48.8	48.7	48.8	48.7		4	73.8	73.7
13	49.0	49.0	49.0	48.9	48.9	48.9		5	69.3	69.3
14	49.7	49.4	49.5	49.5	49.4	49.3		6	18.5	18.5
15	27.8	27.8b	27.9a	27.7a	28.1	28.1				
16	27.8	27.5b	27.8a	27.4a	28.1	28.1	Gl-2*	1	97.4	97.2
17	49.7	49.7	49.7	49.5	49.7	49.7		2	80.0	75.1
20	35.7	35.7a	35.8	35.4	35.8	35.8		3	78.7	77.8
22	30.2	30.2	33.7	32.7	34.1	34.1		4	71.3	71.4
23	37.5	37.3	28.5	26.8	28.7	28.6		5	76.7	77.8
24	213.9	213.9	78.7	80.1	76.0	75.9		6	62.4	63.0
25	40.9	40.9	72.5	71.1	80.7	80.1				
26	18.3	18.3	25.8b	25.8	22.3a	22.4a	Gl-3*	1	104.8	
27	18.3	18.3	26.0b	25.8	22.6a	22.8a		2	74.9	
29	26.2	26.3	26.1	26.3	25.8	25.8		3	77.9	
								4	71.7	
								5	77.9	
Other Me groups (18, 19, 21, 28, 30)								6	62.4	
	16.9	16.9	16.9	16.9	16.9	16.9				
	18.3	17.9	18.1	18.0	18.2	18.2				
	18.3	18.3	18.5	18.3	19.2	19.2				
	20.1	20.0	20.0	19.9	20.3	20.3				
	21.1	21.6	21.1	21.6	21.7					
CH_3CO		20.9		20.9						
				21.0						
CH_3CO		170.1		170.7						
				169.8						

*Gl-1, Gl-2 and Gl-3 refer to the numbered sugar residues in Fig. 1 with the carbons numbered in the usual manner.

†Rh refers to the rhamnose residue in Fig. 1 with the carbons numbered in the usual manner.

a–b—signals in columns may be interchanged.

EXPERIMENTAL

Mps were taken with an Electrothermal melting point apparatus and are uncorr. Optical rotations were measured on a Perkin–Elmer 141 automatic polarimeter at 18–22°. IR spectra were obtained from KBr discs (unless otherwise stated). The ^{1}H NMR spectra were recorded at 200 MHz in $CDCl_3$ or in C_5D_5N soln. The ^{13}C NMR spectra were taken in $CDCl_3$ or in C_5D_5N (0.3–0.5 M) soln at 25.05 MHz at room temp. A 5-mm sample tube was used. Tetramethylsilane was used as an int. standard for both ^{1}H NMR and ^{13}C NMR.

The EIMS spectra were measured with direct insertion of the probe into the ion source at an ionizing potential of 70 eV and a sample temperature of 180–240°. The FDMS spectra were measured in a JEOL D-300 mass spectrometer (equipped with FD/FI/EI ion source) at an ion source temperature of 60–70°. The accelerating voltages were +3 kV for the field anode and −5 kV for the slotted cathode plate. The emitter heating currents for examination of the glycosides were between 18 and 22 mA.

Ion exchange resin (Amberlite IR-120, H^+) was used for the partial hydrolysis. Chromatography was on Si gel (G type, Merck) for TLC, PF type (Merck) for prep. TLC, Si gel (Merck) for CC and Whatman No. 1 paper for PC. The following solvents were employed: solvent systems A, $CHCl_3$–MeOH–H_2O (60:33:5); B, CH_2Cl_2–MeOH–H_2O (75:25:2); C, CH_2Cl_2–MeOH–H_2O (95:25:1); D, *n*-hexane–EtOAc (7:1 followed by 3:1); E, *n*-BuOH–AcOH–H_2O (4:1:2), F, $CHCl_3$–MeOH–H_2O (65:30:5); G, *n*-hexane–EtOAc (1:1); H, *n*-hexane–EtOAc (2:3) and I, CH_2Cl_2–MeOH–H_2O (110:25:1). On TLC the glycosides, sapogenins and sugars were detected by heating after spraying with 10% H_2SO_4. Sugars were located on PC (ascending) by ammoniacal $AgNO_3$ soln. GLC of the methylated sugars was carried out on 5 m × 4 mm, 5% butanediol succinated polyester on Chromosorb W; carrier N_2 at 180°.

Isolation of **1** *and* **2**. *Bryonia dioica* roots were collected at the Chelsea Physic Garden, Robal Hospital Road, London SW3, and a voucher specimen of the aerial parts is deposited in the museum at Chelsea College. The dried powdered roots (4 kg) were defatted with petrol in a Soxhlet apparatus and solvent-free powder was likewise extracted with MeOH until the extract became colourless. Removal of solvents from the MeOH extract give a brown syrup. Its soln in H_2O was partitioned with *n*-BuOH–EtOAc (1:2). The aq. phase was evapd to obtain crude **1** (1.91 g). This was purified by successive CC separations first on Si gel (system A followed by system B). It was crystallized as a colourless powder from *n*-PrOH, mp 207–213°; $[\alpha]_D = +26.1°$ ($c = 1.04$, MeOH). (Found: C, 55.83; H, 8.20; $C_{54}H_{90}O_{23}.3H_2O$ requires: C, 55.86; H, 8.27%). IR ν_{max}^{KBr} cm^{-1}: 3650–3300, 1685, 1650, 811; ^{1}H NMR (C_5D_5N): δ 0.73 (3H, *s*), 0.93 (3H, *d*, *J* = 6 Hz), 1.01 (3H, *s*), 1.09 (3H, *s*), 1.41 (3H, *s*), 1.53 (9H, *s*) 1.71 (3H, *d*, *J* = 6 Hz), 2.47 (1H, *d*, *J* = 15 Hz), 2.95 (1H, *d*, *J* = 15 Hz), 3.60 (1H, *s*), 4.89 (1H, *d*, *J* = 8 Hz), 5.06 (1 H, *d*, *J* = 7 Hz), 5.22 (1H, *d*, *J* = 7 Hz), 5.79 (1H, *s*), 5.99 (1H, *br s*). ^{1}H NMR (CD_3OD): δ 4.12 (1H, *d*, *J* = 7 Hz), 4.34 (1H, *d*, *J* = 8 Hz), 4.52 (1H, *d*, *J* = 8 Hz), 5.53 (1H, resolved *d*, *J* = 2 Hz), 5.67 (1H, *br s*). FDMS and ^{13}C NMR data are given in Tables 1 and 2.

The *n*-BuOH–EtOAc phase was worked up in the usual manner and the extract was subjected to Si gel CC (system C).

Pure **2** (1.35 g) was isolated and crystallized as a colourless powder from *iso*-PrOH, mp 179–183°, $[\alpha]_D = 30.9$ (c = 0.86, MeOH). (Found C, 59.35; H, 8.79; $C_{48}H_{80}O_{18}.1\frac{1}{2}H_2O$ requires C, 59.32; H, 8.54%.) IR ν_{max}^{KBr} cm^{-1}, 3600–3300, 1685, 1653, 813; ^{1}H NMR (C_5D_5N): δ 0.74 (3H, *s*), 0.93 (3*H*, *d*, *J* = 6 Hz), 1.00 (3H, *s*), 1.09 (3H, *s*) 1.42 (3H, *s*), 1.54 (9H, *s*), 1.70 (3H, *d*, *J* = 6 Hz), 2.48 (1H, *d*, *J* = 14 Hz), 2.96 (1H, *d*, *J* = 14 Hz), 3.62 (1H, *s*), 4.89 (1H, *d*, *J* = 8 Hz), 5.22 (1H, *d*, *J* = 8 Hz), 5.78 (1H, *s*), 5.99 (1H, *br s*); ^{1}H NMR (CD_3OD): δ 4.36 (1H, *d*, *J* = 7 Hz), 4.51 (H, *d*, *J* = 8 Hz), 5.53 (1H, unresolved *d*, *J* = 2 Hz), 5.68 (1H, *br s*). FDMS and ^{13}C NMR data are given in Tables 1 and 2.

Identification of the sapogenin and sugars. Compound **1** (400 mg) was hydrolysed by refluxing with 1 N HCl for 2.5 hr. The hydrolysate was filtered and the filtrate (neutralized with Ag_2CO_3) subjected to PC (*n*-BuOH–EtOH–H_2O, 4:1:1). The presence of D-glucose and L-rhamnose was revealed. The ppt obtained as above was purified by chromatography on Si gel (system D) to yield bryodulcosigenin as crystalline plates from Et_2O mp 182–184°, IR ν_{max}^{KBr} cm^{-1}: 3550–3430, 1683, 1655, 812; MS *m/z*: 474 $[M]^+$, 59 (base peak); ^{1}H NMR (C_5D_5N): δ 0.74 (3H, *s*), 0.96 (3H, *d*, *J* = 10 Hz), 1.02 (3H, *s*), 1.15 (3H, *s*), 1.25 (3H, *s*), 1.43 (3H, *s*), 1.53 (3H, *s*), 1.56 (3H, *s*), 2.62 (1H, *d*, *J* = 15 Hz), 3.01 (1H, *d*, *J* = 15 Hz), 3.71 (2H, *br s*), 5.68 (1H, unresolved *d*, *J* = 6 Hz). ^{13}C NMR data are given in Table 2. A minor product, bryogenin, was identified by mmp and by direct comparison of R_f value and IR spectrum of an authentic sample. It gave needles from Et_2O, mp 153–156°, ν_{max}^{KBr} cm^{-1}: 2495, 1710, 1685, 1240: MS *m/z*: 456 $[M]^+$, 217 (base peak), ^{13}C NMR data are given in Table 2.

Compound **2** (350 mg) was likewise hydrolysed. The ppt was purified by the above method. The sapogenin and its related artefact compound were identified as bryodulcosigenin and bryogenin respectively by spectral comparison as above. The sugars D-glucose and L-rhamnose were detected on PC (*n*-BuOH–EtOH–H_2O, 4:1:1).

Acetylation of bryodulcosigenin (**3**) *and bryogenin* (**4**). Bryodulcosigenin (**3**) (150 mg) was acetylated with pyridine–Ac_2O (1:1) at room temp. for 24 hr and the reaction mixture was worked up in the usual manner. Bryodulcosigenin diacetate (**10**) isolated was identified by comparison with an authentic specimen.

Bryogenin (**4**) (110 mg) was acetylated by the above method and the bryogenin acetate (**11**) so produced was identical in every respect with an authentic specimen. ^{13}C NMR spectral data of compounds **10** and **11** are given in Table 2.

Acetylation of **1** *and* **2**. Compound **1** (90 mg) was acetylated with pyridine–Ac_2O (1:1) at room temp. for 24 hr and the reaction mixture was worked up in the usual manner. Recrystallization from MeOH gave **5** as needles, mp 167–170°. (Found: C, 58.08; H, 7.05; $C_{82}H_{118}O_{37}$ requires: C, 58.09; H, 6.97%.) IR ν_{max}^{KBr} cm^{-1}: no absorption band; ^{1}H NMR ($CDCl_3$): no signals exchanged with D_2O.

Compound **2** (80 mg) was acetylated as above and the reaction mixture was worked up in the usual manner. Purification gave **6** which recrystallized from MeOH as needles, mp 129–132°. (Found: C, 59.96; H. 7.15; $C_{70}H_{102}O_{29}$ requires C, 56.74; H, 7.25%.) IR ν_{max}^{KBr} cm^{-1}: no OH absorption band; ^{1}H NMR ($CDCl_3$): no signals exchanged with D_2O.

Methylated sugars from **1** *and* **2**. Compound **1** (170 mg) was methylated by the Hakomori method [$(CH_3)_2SO$, NaH, CH_3I] [5]. The methylate was purified by prep. PTLC (system H) to give a syrup; IR $\nu_{max}^{CCl_4}$ cm^{-1}; no OH absorption. The permethylated **1** was methanolysed with dry 1 N HCl in MeOH for 2 hr at about 100° and the reaction mixture was worked up in the usual manner. The methylated sugars were analysed by GLC. Methyl 2, 3, 4, 6-tetra-*O*-methyl-D-glucopyranoside (R_t, 8.1 min), methyl 2,3,4-tri-*O*-methyl-L-rhamnopyranoside (R_t, 3.1 min) and methyl 3,4,6-tri-*O*-methyl-D-glucopyranoside (anomeric carbon α: R_t, 16.2 min and β: R_t, 19.4 min) were identified by comparison with authentic specimens. Compound **2** (140 mg) was likewise treated and the product was purified as above to yield a syrup; IR $\nu_{max}^{CCl_4}$ cm^{-1}: no OH absorption. The permethylated product was methanolysed as above. The methylated sugars were analysed by the same GLC method and three methylated sugars identical with those above were detected.

Enzymatic hydrolysis of **1** *and* **2**. Compound **1** (200 mg) was dissolved in H_2O (10 ml) and emulsin (50 mg, BDH) added. The mixture was incubated at 40° for 2 days. The hydrolysate was purified by prep. TLC (system F). D-Glucose was identified by TLC (system F). The product obtained was crystallized as a colourless powder from *iso*-PrOH, mp 181–184°, $[\alpha]_D = 30.4°$ ($c = 0.84$, MeOH). (Found: C, 60.39: H, 8.35. $C_{48}H_{80}O_{18}$ requires: C, 60.10, H, 8.47%.) IR ν_{max}^{KBr} cm^{-1}: 3650–3300, 1685, 1655, 813; ^{1}H NMR (C_5D_5N): δ 0.73 (3H, *s*), 0.92 (3H, *d*, *J* = 6 Hz), 1.00 (3H, *s*), 1.08 (3H, *s*), 1.42 (3H, *s*), 1.53 (9H, *s*), 1.72 (3H, *d*, *J* = 6 Hz), 2.48 (1H, *d*, *J* = 14 Hz), 2.96 (H, *d*, *J* = 14 Hz), 3.61 (1H, *s*), 4.90 (1H, *d*, *J* = 8 Hz), 5.23 (1H, *d*, *J* = 8 Hz), 5.77 (1H, *s*), 6.00 (1H, *br s*). FDMS data are given in Table 1. This product is thus considered to be identical with **2**, isolated from *Bryonia dioica* roots.

Compound **2** (240 mg) was dissolved in 2 ml dioxan and to this was added emulsin (60 mg, BDH), in 10 ml HOAc–NaOAc buffer solution (pH 5). The mixture was incubated at 40° for 6 days. The hydrolysate was purified by prep. TLC (system F). D-Glucose was identified by TLC (system E). The glycoside crystallized as a colourless powder from aq. MeOH, mp 216–219°, $[\alpha]_D = +39.4°$ ($c = 0.76$, MeOH). (Found: C, 64.29; H, 8.81; $C_{42}H_{70}O_{13}$ requires: C, 64.45; H, 8.95%.) IR ν_{max}^{KBr} cm^{-1}: 3600–3300, 1684, 1655, 812; ^{1}H NMR

(C_5D_5N): δ 0.73 (3H, *s*), 0.93 (3H, *d*, $J = 6$ Hz), 0.98 (3H, *s*), 109 (3H, *s*), 1.40 (3H, *s*), 1.53 (6H, *s*), 1.56 (3H, *s*), 1.73 (3H, *d*, $J = 6$ Hz), 2.48 (1H, *d*, $J = 15$ Hz), 2.96 (1H, *d*, $J = 15$ Hz), 3.62 (1H, *s*), 4.89 (1H, *d*, $J = 8$ Hz), 5.80 (1H, *s*), 5.98 (1H, *br s*). FDMS data are given in Table 1.

Compound **7** (50 mg) was acetylated with pyridine–Ac_2O (1 : 1) at room temp. for 24 hr and the reaction mixture was worked up in the usual manner. The acetate (**8**) was obtained as colourless needles from MeOH, mp 123–125°. (Found: C, 62.08; H, 8.05; $C_{56}H_{84}O_{20}$ requires: C, 62.45; H, 7.81%.) IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3510 (*tert* OH).

Partial hydrolysis of **1**. To a soln of **1** (0.9 g) in H_2O (10 ml) was added 2 g air-dried cation exchange resin (converted to acid form with 10% H_2SO_4) and the mixture was heated at 80° for 1.5 hr [24]. The hydrolysate was purified by Si gel CC (system C, by decreasing the CH_2Cl_2 content by 4–5% in each step). D-Glucose and L-rhamnose were detected by TLC (system E). The glycoside **9** was obtained as a colourless powder from MeOH–Me_2CO, mp 229–233°; $[\alpha]_D = 38.1°$ ($c = 0.81$, MeOH). (Found: C, 59.81; H, 8.52; $C_{48}H_{80}H_{19}$ requires: C, 60.00; H, 8.33%.) IR $\nu_{\max}^{\mathrm{KBr}}$ cm^{-1}: 3650–3300, 1683, 1654, 812; ^{1}H NMR (C_5D_5N): δ 0.73 (3H, *s*), 0.91 (3H, *d*, $J = 7$ Hz), 0.97 (3H, *s*), 1.13 (3H, *s*), 1.18 (3H, *s*), 1.56 (9H, *s*), 2.49 (1H, *d*, $J = 15$ Hz), 2.99 (1H, *d*, $J = 15$ Hz), 3.61 (1H, *s*), 4.89 (1H, *d*, $J = 8$ Hz), 5.09 (1H, *s*, $J = 8$ Hz), 5.20 (1H, *d*, $J = 8$ Hz), 5.54 (1H *br s*). The above glycoside, **9** (40 mg) was then hydrolysed with 1 N HCl for 2.5 hr. The reaction mixture was worked up in the usual manner. The product, bryodulcosigenin, was identified by mmp and by direct comparison of R_f value and IR spectrum of the authentic sample. From the aq. phase only D-glucose was detected on TLC (system E).

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