New Cytotoxic Principles from *Datisca glomerata*

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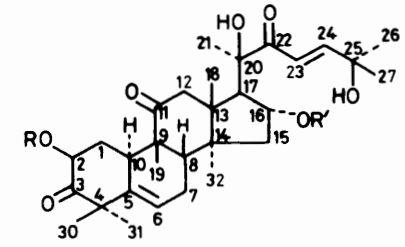
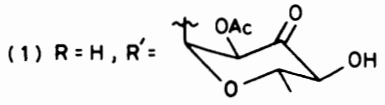
Datisca glomerata has been systematically fractionated by following cellular toxicity in an effort to identify previously uncharacterized cytotoxic principles. Several new cucurbitacin glycosides, including datiscosides B(8), C(3), D(9), E(4), F(5), G(6), and H(10) and the known compound datiscoside (1), as well as cucurbitacins B(11), D(2), and F(7) have been isolated and characterized. Structures were assigned to the compounds on the basis of their high field ^{1}H n.m.r., ^{13}C n.m.r., high-resolution mass spectra (CI, EI, and FD) and chemical interconversions. The structure of datiscoside C(3) was independently established by single-crystal *X*-ray analysis at 193 and 293 K.

The cucurbitacins are a class of tetracyclic triterpenes that have undergone extensive chemical and biological investigation over the last 20 years.[1] They occur widely in the plants of the family *Cucurbitaceae* and are thought to be involved in plant protection against herbivores. Along these lines, the cucurbitacins have been shown to function as kairomones for the co-evolved diabroticite beetles.[2]

The acute mammalian toxicity of the cucurbitacins has been known for some time and several of these compounds have been explored as possible anticancer agents.[1] In spite of extensive biological investigations, the molecular basis of this toxicity is unclear.[3] The report by Kupchan *et al.*[4] of an unusual cucurbitacin glycoside from *Datisca glomerata* has prompted us to reinvestigate *D. glomerata* for the presence of additional cucurbitacins. We felt that their possible regulation of glucocorticoid action [3] might make them viable agents for the treatment of hormone sensitive tumours.

Dried twigs of *Datisca glomerata* (Datiscaceae) were extracted successively with n-hexane, ether, methanol, and water at room temperature and the extracts were tested against KB cells. The greenish yellow ether extract showed significant *in vitro* cytotoxicity and on partition between water and chloroform the activity was concentrated in the precipitate. The insoluble residue was freed from inorganic salts and chlorophyll by passage through Sephadex LH-20 in methanol. The biologically active fractions were successfully separated by repeated silica-gel column chromatography, flash chromatography, preparative thin-layer chromatography and, finally, by preparative h.p.l.c. (see Fractionation scheme). This Scheme shows the active principles isolated from *Datisca glomerata*. The new datiscosides B—H were named in accordance with previous work.[4]





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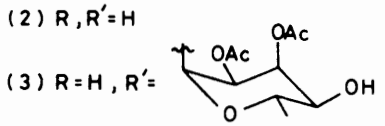
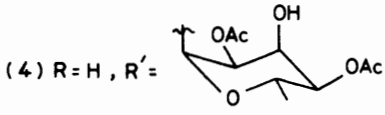


(2) R, R′ = H

(3) R = H, R′ =



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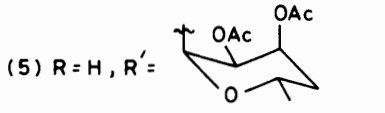

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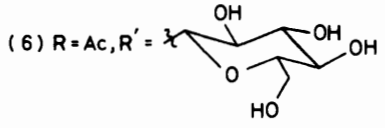


(6) R = Ac, R′ =

Structures of Datiscosides C(3), *E*(4), *and F*(5).—Datiscosides C(3), E(4), and F(5) bear a close structural resemblance to datiscoside (1), first isolated from *Datisca glomerata*.[5] Their highfield ^{1}H n.m.r. spectra revealed that each had cucurbitacin D(2) as an aglycone. Datiscosides (3) and (4) were found to be isomeric with molecular formula $C_{40}H_{58}O_{13}$, whereas (5) had one less oxygen atom. All the functional groups of (1), including the α,β-unsaturated ketone system in the side chain (23-H, δ 6.48, d, *J* 15.0 Hz; 24-H, δ 7.04, d, *J* 15 Hz), a hindered carbonyl group at C-11 (12α-H, 3.23br d, *J* 14.5 Hz; 12β-H, δ 2.73, d, *J* 14.5 Hz), a secondary hydroxy-group at C-2 (2-H, δ 4.42), a trisubstituted double bond between C-5 and C-6 (6-H, δ 5.77br, d, *J* 5.04), and 16-H carbinyl proton at δ 4.42, could be readily identified in the n.m.r. spectra of (3), (4), and (5) (see Tables 1—3), suggesting that they differed only in their sugar moieties. Datiscosides C and E have resonances at δ 5.43br (d, *J* 4 Hz), and 4.15 (dm, *J* 4 Hz), respectively, suggesting that the C-3′ ketone of (1) had been reduced in these datiscosides.

The anomeric proton of datiscoside C(3) appeared as a doublet at δ 4.67 (*J* 4 Hz) which is at slightly higher field than in datiscoside (1).

Table 1. 360 MHz N.m.r. chemical shifts [a] of relevant protons of compounds isolated from *Datisca glomerata*

Proton on carbon no.	Cucurbitacin-D glycosides							Cucurbitacin-F glycosides			
	(2)	(1)	(3)	(4)	(5)	(6) †	(20)	(7)	(8)	(9)	(10)
1α	2.33ddd (12.5, 6.5, 3.5)	2.33dd (12.5, 6.0, 3.5)	2.33dd (12.5, 5.5)	2.33dm (12.5)	2.31dm (12.5)	2.27dm (12.5, 5.5)	2.05	1.91m	2.36br d (12)	1.91ddd (11, 3.5, 2)	2.30dm (12.5)
1β	1.23q (12.5)	1.22q (12.5)	1.22 *	1.26 *	1.25 *	1.10 *	1.19 *	1.10q (12.0)	1.18 *	1.10ddd (12.5, 11, 11)	1.28 *
2	4.44ddd (12.5, 6.5, 3)	4.42 *	4.41dd (12.5, 5.5)	4.44 *	4.41 *	4.53dd (12.5, 5.5)	4.51dd (13.5, 5)	3.59dt (10, 3.5)	3.60t (9)	3.57ddd (11, 9.0, 3.5)	3.60ddd (11.0, 3.5, 9)
3	—	—	—	—	—	—	—	2.98d (10)	2.99d (9)	2.98d (9.0)	2.99d (9)
6	5.79dt (5.5, 3)	5.77br d (5.05)	5.73br m	5.78br d (5.5)	5.75br d (6.0)	5.77d (5.0)	5.71br d (5.0)	5.75br d (5.5)	5.73br d (5.5)	5.68br d (5.5)	5.73br d (5.5)
7α	2.41ddd (19, 7.5, 3)	2.45ddd (18.5, 8.0, 3)	2.44ddd (18, 7.5, 2)	2.46dm (18.0)	2.45dm (18.0)	2.40dm (18)	2.44dm (19.5)	2.39dm (19.5)	2.44ddd (15, 8.0, 1)	2.43ddd (19.5, 7.0, 3.0)	2.45ddd (18, 7.0, 7)
7β	1.96dd (19, 5.5)	1.95dd (18.5, 5)	1.91dd (18, 5.5)	1.96dd (18.0, 5.5)	1.92dd (18.0, 6.0)	1.9dd (18, 5.5)	1.95 *	1.87dm (19.5)	1.93dd (15, 5.5)	1.87dd (19.5, 5.5)	1.90dd (18, 5.5)
8	1.98d (7.5)	2.01d (8.0)	1.99d (7.5)	2.00d (7.5)	1.97d (7.5)	2.05d (8.0)	2.00 *	1.94d (7.0)	1.96dd (8.0)	1.95d (7.0)	1.95d (7.5)
10	2.76dd (12.5, 3)	2.72br d (12.5)	2.72br d (12.5)	2.76d (12.5)	2.73br d (12.5)	2.77br d (12.5)	2.71br d (12.5)	2.35br d (12.5)	1.94m	2.36dd (12.5, 2)	2.35 *
12α	3.29d (14.5)	3.23br d (14.5)	3.26d (14.5)	3.28br d (14.5)	3.24br d (14.5)	3.27d (14.5)	3.29br d (14.0)	3.19br d (14.0)	3.18br d (14.5)	3.21br d (14.0)	3.18br d (14.5)
12β	2.71dd (12.5, 3)	2.73d (14.5)	2.73d (14.5)	2.77d (14.5)	2.72d (14.5)	2.70d (14.5)	2.72d (14)	2.62d (14.0)	2.66d (14.5)	2.66d (14)	2.68d (14.5)
15α	1.86dd (14.5, 7.5)	1.62dd (14.5, 7.5)	1.63dd (14.5, 7.5)	1.68dd (14.5, 7.5)	1.68dd (14.5, 7.5)	1.72dd (14.5, 7.0)	1.55dd (14.5, 7.5)	—	1.16dd (14, 6.8)	1.62dd (14, 7.0)	1.67dd (14.5, 7.2)
15β	1.40d (14.5)	1.54d (14.5)	1.52d (14.5)	1.41d (14.5)	1.56d (14.5)	1.40d (14.5)	1.50d (14.5)	—	1.52d (14)	1.52d (14)	1.55d (14.5)
16	4.36t (7.5)	4.42 *	4.33t (7.5)	4.43 *	4.39t (7.5)	4.30t (7.5)	5.13t (7.5)	4.36dis t	4.40t (7)	4.32br t (7.0)	4.38dis t
17	2.56d (7.5)	2.70d (7.5)	2.65d (7.5)	2.68d (7.5)	2.68d (7.5)	2.50d (7.5)	2.67d (7.5)	2.54d (7.0)	2.68d (7)	2.64d (7.0)	2.74d (7.0)
23	6.65d (15.0)	6.48d (15.0)	6.73d (14.5)	6.60d (14.5)	6.78d (15)	6.48d (15)	6.37d (15)	6.66d (15)	6.47d (14.5)	6.72d (14.5)	6.49d (14.5)
24	7.33d (15.0)	7.04d (15.0)	7.06d (14.5)	7.29d (14.5)	7.05d (15)	7.04d (15)	7.11d (15)	7.10d (15)	7.04d (14.5)	7.06d (14.5)	7.00d (14.5)

[a] Spectra were taken in $CDCl_3$; d = doublet, s = singlet, br d = broad doublet, dd = double doublet, ddd = triple doublet, dt = doublet of triplet, dis t = distorted triplet, t = triplet, q = quartet, dm = doublet of multiplet. Chemical shifts are in δ units relative to $SiMe_4$. Coupling constants (J) in parentheses are given in Hz.

* Protons assigned by selective decoupling experiments.

† Additional signal for C-2-acetoxy group at δ 2.0 in datiscoside G(6).

Table 2. Chemical shifts of tertiary methyls of compounds isolated from *Datisca glomerata* [a]

(2)	(1)	(3)	(4)	(5)	(6)	(20)	(7)	(8)	(9)	(10)
0.99	1.01	1.01	1.00	1.00	0.91	0.99	0.92	0.95	0.93	0.94
1.18	1.09	1.09	1.09	1.09	1.07	1.05	1.10	1.00	1.00	1.00
1.29	1.29	1.26	1.26	1.26	1.27	1.10	1.11	1.11	1.11	1.11
1.35	1.33	1.31	1.32	1.32	1.31	1.11	1.19	1.19	1.16	1.19
1.35	1.33	1.40	1.34	1.34	1.35	1.11	1.26	1.26	1.33	1.25
1.37	1.34	1.42	1.40	1.40	1.44	1.40	1.37	1.34	1.38	1.25
1.37	1.42	1.42	1.42	1.42	1.54	1.53	1.37	1.42	1.39	1.30
1.41	1.45	1.42	1.44	1.44	1.58	1.54	1.40	1.42	1.40	1.31

[a] Spectra were taken in $CDCl_3$, δ values, 360 MHz NMR. All signals were singlets.

Table 3. 360 MHz N.m.r. chemical shifts [a] of relevant protons in the sugar moiety of glycosides from *Datisca glomerata*

Proton on Carbon no.	Cucurbitacin D-glycosides						Cucurbitacin F-glycosides		
	(2)	(3)	(4)	(5)	(6)	(20)	(8)	(9)	(10)
1′	5.25 *	4.67d (4.0)	4.97d (4)	4.72d (4)	4.28d (9)	4.69d (8.0)	5.25 *	4.66d (4.0)	5.16d (5.5)
2′	5.25 *	4.77t (4)	4.69t (4)	4.81t (4)	3.42t (9)	4.98dd (9.0, 7.5)	5.25 *	4.77t (4)	5.29t (5.5)
3′	—	5.44br d	4.15dm	5.33m	3.52t	5.20t	—	5.45br d	3.8dd (14.5, 7.0)
		(4)	(4)		(9)	(9.0)		(4)	2.35dd (14.5, 4)
4′	3.86d (9.0)	3.39br d (9.5)	4.45dd (9.5, 4)	1.66dm	3.27 *	5.08t (9.0)	3.86d (9.0)	3.40br d (9.5)	—
5′	3.48dq (9.0, 6.0)	3.76dq (9.5, 7.0)	3.76dq (9.5, 7.0)	4.03m	3.35dm (3.5, 9, 6.0)	3.59dm (9.0)	3.46dq (9.0, 8.0)	3.76dq (9.5, 7.0)	3.35q (7.0)
6′	1.45d	1.30d	1.24d	1.23d	3.82dd (6.0, 11.0)	4.19dd (13, 4)	1.45d	1.24d	1.40d
	(6.0)	(7.0)	(7.0)	(7.0)	3.96dd (11.0, 3.5)	4.12dd (13, 2)	(8.0)	(6)	(7.0)
2′-OCOCH_3	2.11s	2.01s	2.11s	2.10s	—	1.99s	2.11s	2.01s	2.08s
3′-OCOCH_3	—	2.13s	—	2.02s	—	2.00s	—	2.13s	—
4′-OCOCH_3	—	—	2.08s	—	—	2.09s	—	—	—
6′-OCOCH_3	—	—	—	—	—	2.10s	—	—	—

[a] Spectra were taken in $CDCl_3$. Chemical shifts are in δ units relative to $SiMe_4$. Coupling constants (J) in parentheses are given in Hz.
* Protons assigned by selective decoupling experiments.

2′-H At δ 4.76 (J 4 Hz, t), was coupled to a broad doublet at δ 5.43 (J 4 Hz) indicating that both of these protons must be on carbons bearing acetoxy groups. Since the dihedral angle between the two C–H bonds at C-3′ and C-4′ was *ca.* 90°, the 3′-H was weakly coupled with 4′-H. The latter proton appeared at δ 3.39 as a broad doublet (J 9.6 Hz) and was coupled to 5′-H (δ 3.76, dq, J 9.6, 6.8 Hz). The large coupling constant (9.6 Hz) between 4′-H and 5′-H suggested a *trans* diaxial relationship. The latter proton was further coupled to the methyl doublet at δ 1.30 (J 6.8 Hz). The above results indicated that datiscoside C(3) contained a reduced 3′-carbonyl in which the hydroxy group was acetylated and *cis* to 2′-OAc. These data indicated the structure of datiscoside C as the 16-*O*-(2,3-di-*O*-acetyl-6-deoxy-α-L-allopyranoside) of cucurbitacin D.*

To confirm this conclusion, and to establish the overall stereochemistry of the molecule, single-crystal *X*-ray analyses of datiscoside C, in the form of its dibenzene solvate, were carried out at room temperature (293 K) and at 193 K. A stereoscopic view of the datiscoside C unit at 193 K is shown in Figure 1. The absolute configuration has been assumed to be the same as that established for datiscoside by the *X*-ray analysis of its bis(*p*-iodobenzoate).[4] The crystallographic numbering scheme adopted is shown in Figure 2. This scheme is the same as that for the bis(*p*-iodobenzoate), and differs from the chemical numbering scheme only in that the C(4) methyl groups are labelled as C(28) and C(29) rather than C(30) and C(31), and the C(14) methyl group is labelled as C(30) rather than C(32).

Atomic parameters defining the crystal structure at 193 K are given in Table 4. Bond lengths and angles in the datiscoside C moiety are given in Table 5. Torsion angles are given in Table 6, which also includes, for purposes of comparison, those found for datiscoside in the analysis of the bis(*p*-iodobenzoate). As had been deduced from the n.m.r. analysis, datiscoside C differs chemically from datiscoside only in the nature of the sugar moiety, with an acetoxy substituent being present on C-3′ [C(33)] rather than the oxo group present in datiscoside. The chemical identification given above is thus fully confirmed.

This *X*-ray analysis of a non-heavy atom derivative provides a much more accurate definition of the molecular geometry and conformation in the cucurbitacin D ring system than was possible with the bis(*p*-iodobenzoate), with standard deviations in most bond lengths of 0.005 Å and in bond angles of 0.3. There is good agreement between the bond lengths and angles in the molecule at 193 and 293 K, the apparently significant differences which do occur being attributable to neglect of any corrections for the effects of thermal motion. Typically, these cause C=O bond lengths to be shorter and

* In fact, acid hydrolysis of datiscoside C afforded cucurbitacin D, albeit in low yield.

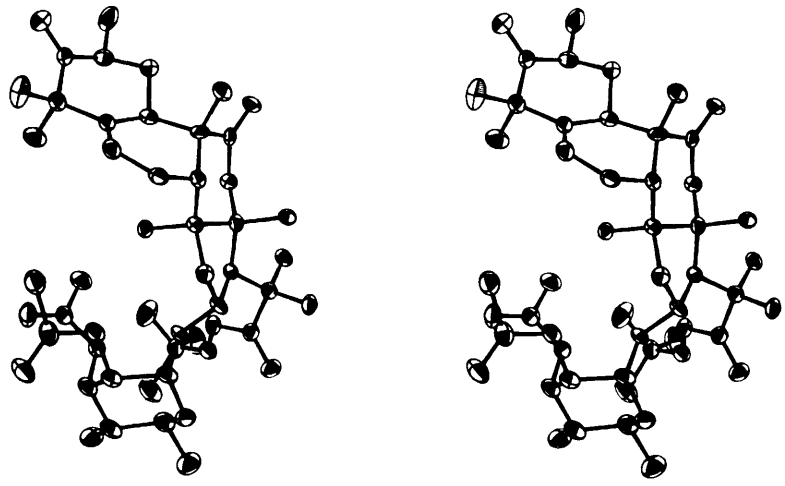


Figure 1. Stereoscopic view of the molecule at 193 K. Thermal ellipsoids are drawn to enclose the 50% probability envelope

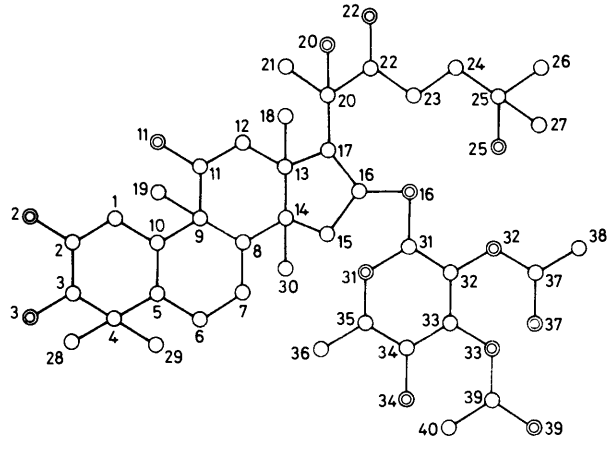


Figure 2. Numbering scheme adopted in the crystallographic work. Single circles represent carbon atoms, double circles oxygen

C–CH_3 bond lengths to be longer at 293 than at 193 K. The first effect is attributable to enhanced pendulum motion of the bonds at the higher temperature, the second to smearing of the hydrogen electron density producing a displacement in the centroid of the methyl carbon atom.

Closely similar conformations are found in datiscoside C(3) and datiscoside bis(*p*-iodobenzoate) for the cucurbitacin ring systems, the sugar rings, and the attachment of the sugar to ring D. Rings A and C have flattened chair conformations, the cyclohexene ring B and ring D both have half-chair conformations, and the sugar ring is a chair. A more detailed analysis of the conformational features of the cucurbitacin ring system was given in the report of the bis(*p*-iodobenzoate) structure [4] and need not be repeated here.

The molecular conformation of datiscoside C is little affected by change in temperature from 293 to 193 K, but two systematic effects are noted: a flattening of ring A and a puckering of the sugar ring at the lower temperature. The mean change in torsion angle in each ring is *ca.* 2°.

There is an increase in molar volume of 38.5 Å^3 at the higher temperature and markedly enhanced rotation of methyl groups is observable for C(26), C(27), C(28), C(36), and C(40). There is also a marked increase in the thermal vibrations of the benzene molecules at the higher temperature.

The datiscoside units are linked in the crystal by O–H · · · O hydrogen bonds which, as may be seen from Table 7, are slightly shorter at the lower temperature. Other intermolecular contacts are of normal van der Waals type; the shortest C · · · C contact at 193 K [between C(38) and C(3b2) of a benzene ring] being 3.44 Å at 193 K and 3.54 Å at 293 K.

Datiscoside E(4) had the same mass ion (*m/z* 746; negative chemical ionization) as datiscoside C and contained the cucurbitacin D aglycone. Therefore, a comparison of the highfield ^{1}H n.m.r. corresponding to the sugar portion of these two compounds was made to permit the structure of (4) to be assigned.

The anomeric proton in (4) appeared as a doublet at δ 4.97 (*J* 4 Hz) and was coupled to a 1H triplet at δ 4.69 (*J* 4 Hz). 2′-H Was further coupled to a doublet of multiplets at δ 4.15 (*J* 4 Hz), a signal that was at 1.3 p.p.m. higher field than 3′-H in datiscoside C(3). Likewise, the 4′-H doublet at δ 4.45 (*J* 9.5, 4 Hz) was 1.1 p.p.m. further downfield than the analogous proton in datiscoside C and established the presence of the acetate at C-4′ rather than C-3′. 4′-H Was again coupled to 5′-H (δ 3.76, *J* 9.5, 6.8 Hz) which appeared as a doublet of quartets as in datiscoside C(3). 5′-H Was further coupled to a methyl doublet at δ 1.24 (*J* 6.8 Hz).

From the foregoing evidence the sugar moiety of datiscoside E(4) was established as 2,4-di-*O*-acetyl-6-deoxy-α-L-allopyranoside and the aglycone as cucurbitacin D. The mass spectra of both datiscosides (3) and (4) underwent similar fragmentation to datiscoside (1) [6] with the loss of the sugar ($M^+ - 231$) being the most prominent ion in both the spectra.

Datiscoside F, (5), is another compound containing the curcurbitacin-D aglycone. It has a diacetate like datiscosides C and E, however its negative chemical ionization and field desorption mass spectra indicated a molecular weight of 730, 16 units less than for (3) and (4). The mass spectrum also showed a prominent peak at *m/z* 215, corresponding to a sugar fragment, 16 units less than the value of *m/z* 231 measured for the sugar moieties of datiscosides C and E.

The sugar anomeric proton of (5) appeared as a doublet at δ 4.72 (*J* 4 Hz) coupled to a triplet at δ 4.81 (*J* 4 Hz). 2-H Was further coupled to a downfield shifted multiplet at δ 5.33 suggestive of a 3′-acetoxy group. Irradiation of 3′-H collapsed a two-proton multiplet centered at δ 1.68. Irradiation of 5′-H at δ 4.03 also affected the signal at δ 1.68, as well as the methyl doublet at δ 1.23, thus confirming the structure and configuration of the sugar moiety of datiscoside F as 2,3-di-*O*-acetyl-4,6-dideoxy-α-L-allopyranoside.

Table 4. Fractional co-ordinates (× 10^4) and equivalent isotropic thermal parameters (Å^2) for the non-hydrogen atoms at 193 K,* with standard deviations in parentheses

Atom	*x*	*y*	*z*	B_{eq}
C(1)	8 333(4)	10 245(2)	2 329(1)	2.5(2)
C(2)	8 898(4)	10 998(2)	2 060(1)	2.8(2)
O(2)	9 911(3)	11 457(3)	2 239(1)	5.0(1)
C(3)	7 823(4)	11 646(3)	1 937(1)	2.5(2)
O(3)	7 894(3)	12 509(2)	2 009(1)	3.8(2)
C(4)	6 676(3)	11 172(2)	1 738(1)	2.2(2)
C(5)	6 298(3)	10 231(2)	1 946(1)	1.8(2)
C(6)	5 095(4)	9 920(2)	1 948(1)	2.2(2)
C(7)	4 647(3)	8 973(2)	2 104(1)	1.9(2)
C(8)	5 696(3)	8 244(2)	2 204(1)	1.9(2)
C(9)	6 871(3)	8 763(2)	2 382(1)	1.8(2)
C(10)	7 353(3)	9 624(2)	2 120(1)	1.9(2)
C(11)	8 042(3)	8 093(2)	2 421(1)	1.9(2)
O(11)	8 782(2)	8 192(2)	2 689(1)	2.5(1)
C(12)	8 288(3)	7 335(2)	2 108(1)	2.0(2)
C(13)	7 051(3)	6 788(2)	2 022(1)	1.7(2)
C(14)	6 028(3)	7 519(2)	1 874(1)	1.7(2)
C(15)	4 951(3)	6 824(2)	1 755(1)	1.8(2)
C(16)	5 651(3)	5 975(2)	1 556(1)	2.1(2)
O(16)	5 625(2)	6 057(1)	1 136(1)	2.0(2)
C(17)	7 073(3)	6 052(2)	1 678(1)	1.8(2)
C(18)	6 677(3)	6 278(2)	2 404(1)	2.1(2)
C(19)	6 501(3)	9 139(2)	2 785(1)	2.2(2)
C(20)	7 698(3)	5 048(2)	1 723(1)	1.9(2)
O(20)	6 938(2)	4 464(1)	1 969(1)	2.3(1)
C(21)	9 077(3)	5 091(2)	1 885(1)	2.4(2)
C(22)	7 828(3)	4 547(2)	1 321(1)	2.1(2)
O(22)	7 321(3)	3 765(2)	1 270(1)	3.0(1)
C(23)	8 628(3)	5 007(2)	1 022(1)	2.2(2)
C(24)	9 153(3)	4 491(3)	742(1)	2.4(2)
C(25)	10 005(4)	4 833(3)	424(1)	2.6(2)
O(25)	10 105(3)	5 868(2)	464(1)	4.7(2)
C(26)	11 322(4)	4 383(3)	457(1)	4.7(2)
C(27)	9 407(4)	4 576(4)	30(1)	4.5(2)
C(28)	5 617(4)	11 901(3)	1 700(1)	3.7(2)
C(29)	7 140(4)	10 908(3)	1 319(1)	3.1(2)
C(30)	6 421(3)	8 044(2)	1 497(1)	1.8(2)
C(31)	4 487(3)	5 810(2)	956(1)	2.0(2)
O(31)	4 515(2)	4 890(2)	783(1)	2.4(1)
C(32)	4 157(4)	6 556(2)	643(1)	2.5(2)
O(32)	4 163(2)	7 502(2)	828(1)	2.3(1)
C(33)	5 053(4)	6 523(3)	299(1)	2.3(2)
O(33)	6 338(2)	6 771(2)	418(1)	2.2(1)
C(34)	5 118(4)	5 486(3)	148(1)	2.6(2)
O(34)	5 952(3)	5 405(2)	−173(1)	3.3(1)
C(35)	5 465(4)	4 794(3)	478(1)	2.7(2)
C(36)	5 454(4)	3 741(3)	354(1)	4.0(2)
C(37)	3 494(4)	8 218(3)	658(1)	2.4(2)
O(37)	2 841(2)	8 098(2)	372(1)	3.2(1)
C(38)	3 659(4)	9 143(3)	873(1)	3.2(2)
C(39)	6 731(4)	7 695(3)	376(1)	2.6(2)
O(39)	6 085(3)	8 333(2)	240(1)	3.4(1)
C(40)	8 025(4)	7 819(3)	539(1)	3.0(2)
C(1b1)	2 695(5)	2 702(3)	1 175(1)	5.2(2)
C(2b1)	2 812(5)	2 039(3)	876(1)	5.0(2)
C(3b1)	1 753(6)	1 545(3)	747(1)	5.1(2)
C(4b1)	582(5)	1 706(3)	915(1)	5.5(3)
C(5b1)	485(5)	2 352(4)	1 215(2)	5.4(3)
C(6b1)	1 526(7)	2 825(3)	1 351(1)	5.6(3)
C(1b2)	8 855(5)	3 951(4)	3 188(2)	6.1(3)
C(2b2)	9 192(5)	4 122(4)	3 548(2)	7.1(4)
C(3b2)	9 290(5)	3 391(8)	3 810(2)	8.2(4)
C(4b2)	8 985(6)	2 455(6)	3 698(2)	7.8(4)
C(5b2)	8 589(5)	2 295(4)	3 324(3)	7.3(4)
C(6b2)	8 562(4)	3 053(5)	3 075(1)	6.0(3)

* Fractional co-ordinates and equivalent isotropic thermal parameters recorded at 293 K form part of the Supplementary publication.

Datiscoside G(6).—Datiscoside G was found to be a 2-*O*-acetylcucurbitacin D glycoside, which exhibited less cytotoxicity as compared to the other datiscosides and cucurbitacins. Its molecular ion (negative ion chemical ionization-CH_4, N_2O) appeared at *m/z* 719 which required $C_{38}H_{56}O_{13}$ as a molecular formula (confirmed by its high-resolution mass spectrum). The ^{1}H n.m.r. spectrum proved very similar to that of cucurbitacin D except for the 2-H resonance and the protons of the sugar moiety. The 2-H proton appeared at δ 4.53 (dd, *J* 12.6, 5.4 Hz), indicating the presence of a 2-acetoxy group. The anomeric proton of datiscoside G(6) appeared as a doublet at δ 4.28 (*J* 9 Hz), very unlike the other datiscosides. The large coupling to 2′-H (δ 3.42, t, *J* 9.0 Hz), and the appearance of C-1′ at δ 103.7 in the ^{13}C n.m.r. spectrum indicated that 1′-H was axially oriented and that the sugar linkage to the aglycone was β-equatorial. 5′-H Had a different multiplicity from the other datiscosides (δ 3.35, dm, *J* 9.0, 6.5, 3.5 Hz) and was coupled to an AB pattern (δ 3.82, dd, *J* 11.0, 6.5 Hz; and 3.94 dd, *J* 11.0, 3.5 Hz) indicative of the hydroxy group at C-6′.

Datiscoside G(6) formed a penta-acetate when treated with acetic anhydride–pyridine and the mass spectrum of this derivative showed a fragment ion, *m/z* 331, arising from the loss of a peracetylated hexose. The ^{1}H n.m.r. spectrum of the acetate supported the assignment of β-glucose as the sugar unit and hydrolysis of (6) (methanolic HCl, 100 °C, 8 h) permitted the identification of glucose by paper chromatography and the assignment of datiscoside G as the 16-*O*-glucoside of 2-*O*-acetylcucurbitacin D.

An inactive glycoside was also isolated along with datiscoside G(6). This species was identified as β-sitosterol glucoside [7] on the basis of its melting point, spectral data, and finally by hydrolysis with methanolic HCl, which gave β-sitosterol and β-glucose, both of which were identified by direct comparison with authentic samples.

Structures of Datiscosides B(8), *D*(9), *and H*(10).—From their highfield ^{1}H n.m.r. spectra, these datiscosides seemed to possess a 2,3-vicinal glycol in ring A. The structural relationship between datiscoside (1) and datiscoside B (8) was established by conversion of the aglycone of datiscoside (2; Cucurbitacin D) to a synthetic transformation product (tetrahydrocucurbitacin F) having the same geometry in ring A as cucurbitacin derivatives (7)—(10) as judged by n.m.r. spectroscopy. The synthetic transformations have been outlined previously; [6] the procedures are given in the Experimental section. Thus the 2-hydroxy groups of both types of aglycone were shown to have the same (β-)configuration and the aglycone of datiscosides B, D, and H was established as cucurbitacin F.

Datiscoside B(8) *and Datiscoside D*(9).—Datiscosides B and D were eluted in successive fractions along with cucurbitacin F(7) during silica gel column chromatography and were further purified by preparative t.l.c. and h.p.l.c. The ^{1}H n.m.r. spectra of (8) and (9) showed them to be closely related to cucurbitacin F(7) [6] (see Table 1); compound (8) was found to contain the same carbohydrate present in datiscoside itself.

The field desorption mass spectrum of datiscoside B gave a molecular ion, M^+, at *m/z* 704 which was two mass units higher than datiscoside (*m/z* 702). Exact mass measurements of the aglycone fragment, *m/z* 518.3241 ($C_{30}H_{46}O_7$), suggested that the additional saturation was present in the aglycone and the formation of a tetra-acetate (pyridine, acetic anhydride, room temp.) suggested that one of the carbonyl groups of datiscoside was reduced in (8). The absence of the carbonyl signal at 213 p.p.m. in the ^{13}C n.m.r. spectrum of (8) and the appearance of a doublet at 80 p.p.m. were also consistent with

Table 5.

(a) Bond lengths (Å)

Bond	193 K	293 K	Bond	193 K	293 K
C(1)–C(2)	1.511(5)	1.502(6)	C(1)–C(10) *	1.518(5)	1.543(5)
C(2)–O(2)	1.382(5)	1.406(6)	C(2)–C(3)	1.499(5)	1.503(6)
C(3)–O(3) *	1.217(4)	1.193(4)	C(3)–C(4)	1.531(5)	1.543(6)
C(4)–C(5)	1.533(4)	1.510(4)	C(4)–C(28) *	1.504(5)	1.540(6)
C(4)–C(29)	1.561(5)	1.558(6)	C(5)–C(6)	1.334(5)	1.341(5)
C(5)–C(10)	1.512(5)	1.528(5)	C(6)–C(7)	1.488(5)	1.474(5)
C(7)–C(8)	1.529(5)	1.541(5)	C(8)–C(9) *	1.552(5)	1.578(5)
C(8)–C(14) *	1.552(4)	1.522(5)	C(9)–C(10)	1.572(4)	1.551(5)
C(9)–C(11)	1.544(5)	1.555(5)	C(9)–C(19)	1.530(4)	1.551(5)
C(11)–O(11)	1.214(4)	1.223(4)	C(11)–C(12) *	1.521(4)	1.499(5)
C(12)–C(13)	1.530(5)	1.537(5)	C(13)–C(14) *	1.558(4)	1.587(5)
C(13)–C(17)	1.560(4)	1.551(4)	C(13)–C(18)	1.537(4)	1.550(5)
C(14)–C(15)	1.537(4)	1.554(5)	C(14)–C(30)	1.538(4)	1.542(4)
C(15)–C(16)	1.541(4)	1.541(5)	C(16)–O(16)	1.449(3)	1.442(4)
C(16)–C(17)	1.555(5)	1.567(5)	O(16)–C(31) *	1.388(4)	1.412(4)
C(17)–C(20)	1.538(4)	1.540(5)	C(20)–O(20)	1.416(4)	1.430(4)
C(20)–C(21)	1.552(5)	1.545(6)	C(20)–C(22)	1.549(4)	1.545(5)
C(22)–O(22)	1.214(4)	1.213(4)	C(22)–C(23)	1.471(5)	1.487(6)
C(23)–C(24)	1.317(5)	1.314(6)	C(24)–C(25)	1.488(5)	1.493(6)
C(25)–O(25)	1.436(4)	1.403(6)	C(25)–C(26)	1.520(6)	1.519(8)
C(25)–C(27)	1.532(5)	1.544(7)	C(31)–O(31)	1.401(4)	1.401(5)
C(31)–C(32)	1.526(5)	1.510(5)	O(31)–C(35)	1.454(4)	1.463(5)
C(32)–O(32) *	1.451(4)	1.417(5)	C(32)–C(33) *	1.511(5)	1.540(6)
O(32)–C(37)	1.344(4)	1.359(5)	C(33)–O(33)	1.451(4)	1.465(5)
C(33)–C(34)	1.522(5)	1.490(7)	O(33)–C(39) *	1.346(4)	1.320(5)
C(34)–O(34)	1.413(4)	1.423(5)	C(34)–C(35)	1.524(5)	1.511(6)
C(37)–O(37)	1.210(4)	1.211(5)	C(35)–C(36)	1.512(5)	1.515(7)
C(37)–C(38)	1.482(5)	1.478(7)	C(39)–O(39)	1.206(5)	1.209(5)
C(39)–C(40)	1.480(5)	1.516(7)			

(b) Bond angles (°)

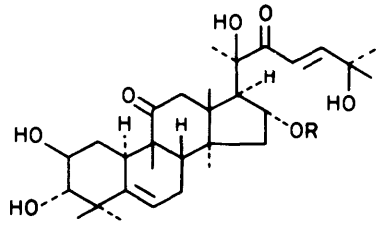
Angle	193 K	293 K	Angle	193 K	293 K
C(10)–C(1)–C(2)	111.4(3)	110.0(4)	O(2)–C(2)–C(1) *	110.2(3)	108.6(4)
C(3)–C(2)–C(1)	106.6(3)	106.4(4)	C(3)–C(2)–O(2) *	115.7(3)	111.9(3)
O(3)–C(3)–C(2)	118.5(3)	120.2(5)	C(4)–C(3)–C(2) *	117.6(3)	115.6(3)
C(4)–C(3)–O(3)	123.8(3)	124.3(5)	C(5)–C(4)–C(3)	110.9(3)	111.5(3)
C(28)–C(4)–C(3) *	109.6(3)	108.0(4)	C(28)–C(4)–C(5)	114.4(3)	113.8(4)
C(29)–C(4)–C(3)	105.4(3)	106.2(4)	C(29)–C(4)–C(5)	108.3(3)	108.2(3)
C(29)–C(4)–C(28)	107.9(3)	108.8(4)	C(6)–C(5)–C(4)	121.3(3)	122.0(3)
C(10)–C(5)–C(4)	117.6(3)	117.7(3)	C(10)–C(5)–C(6)	120.9(3)	120.2(3)
C(7)–C(6)–C(5)	125.7(3)	126.0(4)	C(8)–C(7)–C(6)	115.4(3)	116.2(3)
C(9)–C(8)–C(7) *	111.0(2)	109.6(3)	C(14)–C(8)–C(7)	114.9(2)	114.8(3)
C(14)–C(8)–C(9)	114.0(3)	114.1(3)	C(10)–C(9)–C(8)	112.2(2)	112.3(3)
C(11)–C(9)–C(8) *	113.1(3)	111.2(3)	C(11)–C(9)–C(10)	104.2(3)	104.4(3)
C(19)–C(9)–C(8)	108.1(3)	107.2(3)	C(19)–C(9)–C(10) *	110.1(2)	112.0(3)
C(19)–C(9)–C(11)	109.1(3)	109.9(3)	C(5)–C(10)–C(1)	111.8(3)	110.0(3)
C(9)–C(10)–C(1)	112.0(3)	111.6(3)	C(9)–C(10)–C(5)	114.1(3)	114.3(3)
O(11)–C(11)–C(9) *	120.6(3)	118.9(3)	C(12)–C(11)–C(9)	119.0(3)	119.8(3)
C(12)–C(11)–O(11)	120.4(3)	121.2(4)	C(13)–C(12)–C(11)	109.2(3)	109.8(3)
C(14)–C(13)–C(12) *	109.3(2)	107.4(3)	C(17)–C(13)–C(12)	117.0(3)	117.6(3)
C(17)–C(13)–C(14)	100.4(2)	101.2(3)	C(18)–C(13)–C(12)	106.2(2)	106.8(3)
C(18)–C(13)–C(14)	113.5(3)	112.5(3)	C(18)–C(13)–C(17)	110.7(2)	111.3(3)
C(13)–C(14)–C(8)	109.4(2)	109.8(3)	C(15)–C(14)–C(8)	115.4(3)	115.4(3)
C(15)–C(14)–C(13) *	101.1(2)	99.5(3)	C(30)–C(14)–C(8) *	111.8(2)	113.2(3)
C(30)–C(14)–C(13)	113.3(3)	112.7(3)	C(30)–C(14)–C(15)	105.5(2)	105.3(3)
C(16)–C(15)–C(14)	103.9(3)	104.7(3)	O(16)–C(16)–C(15)	111.9(2)	112.3(3)
C(17)–C(16)–C(15)	106.7(2)	106.5(3)	C(17)–C(16)–O(16)	106.3(2)	106.2(3)
C(31)–O(16)–C(16)	116.2(2)	116.0(3)	C(16)–C(17)–C(13)	103.5(2)	103.6(3)
C(20)–C(17)–C(13)	121.0(2)	120.6(3)	C(20)–C(17)–C(16)	112.0(3)	112.3(3)
O(20)–C(20)–C(17)	109.3(3)	109.4(3)	C(21)–C(20)–C(17)	113.6(3)	113.6(3)
C(21)–C(20)–O(20)	109.4(2)	109.7(3)	C(22)–C(20)–C(17)	110.4(2)	109.9(3)
C(22)–C(20)–O(20)	109.2(2)	108.5(3)	C(22)–C(20)–C(21)	104.7(3)	105.6(3)
O(22)–C(22)–C(20)	119.0(3)	119.0(4)	C(23)–C(22)–C(20)	118.7(3)	118.8(3)
C(23)–C(22)–O(22)	122.2(3)	121.9(4)	C(24)–C(23)–C(22)	121.1(3)	120.5(4)
C(25)–C(24)–C(23) *	128.1(3)	126.2(4)	O(25)–C(25)–C(24) *	106.7(3)	108.4(4)
C(26)–C(25)–C(24)	111.3(3)	110.7(5)	C(26)–C(25)–O(25) *	109.3(3)	111.4(5)
C(27)–C(25)–C(24)	109.1(3)	109.4(5)	C(27)–C(25)–O(25)	110.2(3)	110.3(5)
C(27)–C(25)–C(26) *	110.2(3)	106.6(5)	O(31)–C(31)–O(16) *	113.1(3)	111.5(3)
C(32)–C(31)–O(16)	110.1(3)	110.3(4)	C(32)–C(31)–O(31)	108.4(2)	109.9(3)

Table 5 (*continued*)

(*b*) Bond angles (°)

Angle	193 K	293 K	Angle	193 K	293 K
C(35)–O(31)–C(31)	113.7(2)	114.2(3)	O(32)–C(32)–C(31)	107.2(2)	108.5(3)
C(33)–C(32)–C(31)	112.9(3)	112.3(4)	C(33)–C(32)–O(32)	111.5(3)	112.3(4)
C(37)–O(32)–C(32)	117.9(2)	118.7(3)	O(33)–C(33)–C(32) *	110.6(3)	109.1(3)
C(34)–C(33)–C(32)	108.8(3)	110.0(4)	C(34)–C(33)–O(33)	106.0(3)	106.5(4)
C(39)–O(33)–C(33)	118.5(3)	118.9(4)	O(34)–C(34)–C(33)	111.6(3)	111.6(4)
C(35)–C(34)–C(33) *	110.2(3)	111.8(4)	C(35)–C(34)–O(34)	112.5(3)	112.1(4)
C(34)–C(35)–O(31)	108.4(3)	108.4(4)	C(36)–C(35)–O(31)	106.5(3)	106.2(4)
C(36)–C(35)–C(34)	113.0(3)	114.3(4)	O(37)–C(37)–O(32)	123.2(3)	121.5(5)
C(38)–C(37)–O(32)	110.7(3)	111.7(4)	C(38)–C(37)–O(37)	126.0(3)	126.9(5)
O(39)–C(39)–O(33)	124.0(4)	125.1(5)	C(40)–C(39)–O(33)	110.5(3)	111.8(4)
C(40)–C(39)–O(39) *	125.4(3)	123.0(4)			

* $\Delta \geq 3\sqrt{(\sigma_1^2 + \sigma)_2^2}$.



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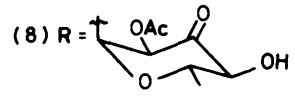

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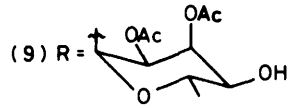

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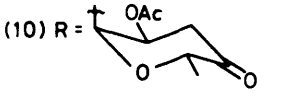

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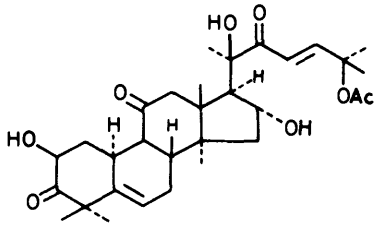


(11)

the suggestion that datiscoside B(8) contained a reduced carbonyl group. The ^{1}H n.m.r. spectrum of (8) showed two coupled protons at δ 3.60 (t, *J* 9.0 Hz) and 2.99 (d, *J* 9.0 Hz) which are readily assigned to the same 1,2-*trans* glycol functionality on ring A that is found in cucurbitacin F. Thus structure (8) was assigned to datiscoside B.

Datiscoside D (9) had the same 2,3-*trans* glycol system as (8) (2-H, δ 3.57, ddd, *J* 11, 9.0, 3.5 Hz; 3-H, δ 2.98, d, *J* 9.0 Hz) and it was shown to contain the same sugar present in (3) by double resonance ^{1}H n.m.r. experiments. This permitted the assignment of structure (9) to datiscoside D.

Datiscoside H(10).—Datiscoside H co-eluted with datiscoside C on silica gel flash chromatography and was further purified by h.p.l.c. (5% MeOH–$CHCl_3$). The mass spectrum of datiscoside H(10) showed a molecular ion at *m/z* 688, $C_{38}H_{56}O_{11}$. That part of the highfield n.m.r. spectrum due to the aglycone was very similar to that of cucurbitacin F(7), showing a 2,3-*trans* glycol system (2-H, δ 3.6 ddd, *J* 11.0, 9, 3.5 Hz; 3-H, δ 2.99, d, *J* 9 Hz).

That portion of the n.m.r. spectrum corresponding to the carbohydrate moiety appeared quite different from the spectra of the other datiscosides. The anomeric proton appeared as a doublet at δ 5.16 (*J* 5.5 Hz) coupled to a triplet at δ 5.29. 5′-H Appeared as a neat quartet at δ 3.38 (*J* 7.0 Hz), suggesting that C-4′ contained no protons and was at the oxidation state of a carbonyl group. Two additional protons δ 3.8 (dd, *J* 14.4, 7.2 Hz) [6a] and δ 2.35 (dd, *J* 14.4 and 4 Hz) were coupled to 2′-H and were geminally coupled to each other. The 5.5 Hz coupling between 1′-H and 2′-H along with the 7.2 Hz *trans* diaxial coupling between 2′-H and one of the C-3′ protons supported the equitorial orientation of the 2′-acetoxy group and the α orientation of the sugar. This established the sugar of (10) as a 2-*O*-acetyl-3′-deoxy-4′-ketohexose.

Chemical ionization mass spectroscopy (CH_4) gave a base peak at *m/z* 171 ($C_8H_{11}O_4$) corresponding to the sugar unit, as well as the aglycone fragment, *m/z* 518 (M^+ − sugar + H^+). The mass spectrum further showed fragments at *m/z* 575, 457, and 439 corresponding to *m/z* 405, 387, and 369, fragments of cucurbitacin F.

^{13}C N.m.r. of *Cucurbitacin Glycosides*.—The study of ^{13}C n.m.r. data and their applicability in structural and conformational analyses in the area of naturally occurring substances such as steriods [8] and related terpenoids [9] has grown enormously in the past decade. However, the application of ^{13}C n.m.r. spectroscopy to the structure elucidation of this family of heavily oxygenated tetracyclic triterpenes known as cucurbitacins has not been reported.

Here we report the ^{13}C n.m.r. spectral assignments of various cucurbitacins and their glycosides and discuss the way in which this study can be used for structure elucidation of these highly oxygenated tetracyclic triterpenes and their glycosides. The chemical shifts were assigned with the aid of ^{13}C n.m.r. (coupled and proton noise decoupled), and by the application of known chemical shift rules, steric effects, and comparisons of the results between compounds possessing identical structural features. For convenience, the assignments of the carbons in the aglycone moiety will be discussed separately from those of the sugar.

The ^{13}C n.m.r. results of datiscoside (1) showed four downfield singlets at δ 212.52, 211.4, 201.5, and 201.0 p.p.m. which could be readily assigned to the carbonyl carbons at C-11, C-3, C-22 of the α,β-unsaturated ketone and C-3′ of the sugar moiety, respectively. The chemical shift at 212.5 p.p.m. corresponded to the isolated ketone at C-11 and was in excellent agreement with the C-11 chemical shift of corticosteriods and androstane derivatives.[10] The signal at 211.4

Table 6. Torsion angles (°) *

	193 K	293 K	DI †
Ring A			
C(10)–C(1)–C(2)–C(3)	62.5	66.2	65
C(1)–C(2)–C(3)–C(4)	−57.1	−59.6	−63
C(2)–C(3)–C(4)–C(5)	43.0	44.4	46
C(3)–C(4)–C(5)–C(10)	−34.6	−36.3	−40
C(4)–C(5)–C(10)–C(1)	42.7	44.2	46
C(5)–C(10)–C(1)–C(2)	−57.0	−59.2	−54
Ring B			
C(10)–C(5)–C(6)–C(7)	−1.6	−2.2	4
C(5)–C(6)–C(7)–C(8)	−11.1	−10.3	−18
C(6)–C(7)–C(8)–C(9)	37.7	37.1	39
C(7)–C(8)–C(9)–C(10)	−52.4	−53.0	−54
C(8)–C(9)–C(10)–C(5)	40.9	42.6	44
C(9)–C(10)–C(5)–C(6)	−13.8	−14.7	−18
Ring C			
C(14)–C(8)–C(9)–C(11)	−38.2	−39.3	−41
C(8)–C(9)–C(11)–C(12)	36.1	36.9	43
C(9)–C(11)–C(12)–C(13)	−47.5	−49.1	−52
C(11)–C(12)–C(13)–C(14)	60.6	60.5	60
C(12)–C(13)–C(14)–C(8)	−65.0	−65.7	−70
C(13)–C(14)–C(8)–C(9)	53.4	55.3	59
Ring D			
C(17)–C(13)–C(14)–C(15)	49.2	49.0	50
C(13)–C(14)–C(15)–C(16)	−40.9	−40.7	−40
C(14)–C(15)–C(16)–C(17)	16.8	17.4	17
C(15)–C(16)–C(17)–C(13)	13.8	13.6	13
C(16)–C(17)–C(13)–C(14)	−38.5	−38.6	−39
Sugar ring			
O(31)–C(31)–C(32)–C(33)	−55.6	−53.1	−44
C(31)–C(32)–C(33)–C(34)	52.8	50.0	50
C(32)–C(33)–C(34)–C(35)	−53.8	−52.2	−60
C(33)–C(34)–C(35)–O(31)	58.1	56.7	63
C(34)–C(35)–O(31)–C(31)	−63.8	−61.8	−68
C(35)–O(31)–C(31)–C(32)	61.2	60.0	57
Ring junctions			
C(6)–C(5)–C(10)–C(4)	−175.1	−174.6	−172
C(1)–C(5)–C(10)–C(9)	128.4	126.4	124
C(14)–C(8)–C(9)–C(10)	79.2	77.3	75
C(7)–C(8)–C(9)–C(19)	69.2	70.4	74
H(8)–C(8)–C(9)–C(19)	−44.2	−53.6	−42
H(8)–C(8)–C(9)–C(11)	76.7	66.5	74
C(8)–C(14)–C(13)–C(18)	53.2	51.6	47
C(15)–C(14)–C(13)–C(18)	−69.0	−69.9	−68
C(30)–C(14)–C(13)–C(17)	−63.2	−62.1	−66
C(30)–C(14)–C(13)–C(12)	60.4	61.7	59
C(17)–C(16)–O(16)–C(31)	167.2	167.6	−180
C(15)–C(16)–O(16)–C(31)	−76.7	−76.4	−63
C(16)–O(16)–C(31)–C(32)	136.1	135.9	152
C(16)–O(16)–C(31)–O(31)	−102.4	−101.8	−91

* E.s.d.s for the present analyses *ca.* 1° [except for angles involving H(8)] and for datiscoside bis(*p*-iodobenzoate) *ca.* 4°.
† DI indicates datiscoside bis(*p*-iodobenzoate).

p.p.m. was assigned to the C-3 ketone. A similar shift was observed for 3-ketones in triterpenes having an almost identical ring A substitution [11] and this carbonyl resonance was absent in cucurbitacin F(7). The same assignments could be made for the carbonyl carbons in all the reported structures containing the cucurbitacin D aglycone (Table 8).

Table 7. Hydrogen bond lengths (Å) *

Bond	193 K	293 K
O(2) · · · O(11^{I})	2.768	2.814
O(20) · · · O(3^{II})	2.878	2.913
O(25) · · · O(39^{III})	2.850	2.861
O(34) · · · O(37^{III})	2.943	2.980

* Bonds are from the donor in the base molecule to the acceptor in the molecule denoted by the Roman superscripts
(I) $2 - x, 0.5 + y, 0.5 - z$
(II) $x, y - 1, z$
(III) $0.5 + x, 1.5 - y, -z$

The two doublets at δ 156.1 and 119.9 were assigned to the β and α carbons, respectively, of the α,β-unsaturated ketone in the side chain of datiscoside. The remaining sp^2 carbons at δ 140.8 (s) and 118.8 (d) could be accommodated only by the double bond between C-5 and C-6, analogously to observations made for Δ^5-steroids [12] and physalins.[13] The chemical shifts for C-5 and C-6 in compounds (2)—(11) were observed in the same region with 0.5 p.p.m. variation. The above results confirm the assignment of the α,β-unsaturated ketone in the side chain and a trisubstituted double bond in ring B which are characteristic features of this class of compounds.

The resonances at 77.8, 77.6, 71.5, and 70.5 in the oxycarbon region are assigned, respectively, to C-20, C-16, C-25, and C-2 in the aglycone moiety of datiscoside (1).

20-OH.—The side chain of the cucurbitacins and their glycosides can be analyzed readily by comparison with other tetracyclic triterpenes. Introduction of a C-20-hydroxy group into the side chain of cucurbitacin shifts C-16 to higher field by 3 p.p.m. through a γ-gauche effect, whereas C-13 (40.1 p.p.m.) is virtually unchanged, as would be expected for a quaternary carbon. The γ-effect of C-16 due to C-20 hydroxy group is not unusual in 17β-oriented side chain compounds.[12] The considerable deshielding of C-21 caused by the C-20 hydroxy group is similar to related steroids.[8,12] The chemical shift of C-20 was observed around 78 p.p.m. in all cucurbitacins and their glycosides with 1 p.p.m. variation, which is in excellent agreement with related steroids [12] and triterpenes.[14]

25-OH.—The hydroxy group at this position resulted in deshielding of C-27 and C-25 by 6–7 p.p.m. when compared to non-hydroxylated side chain compounds and 2,6-dimethyloctane.[8] Acetylation of this tertiary hydroxy group in cucurbitacin B(11) does not affect the side chain carbons.[15] This is similar to the observed effect in acetylated sugars and other aliphatic compounds.[8]

16-OH.—The C-16 and C-20 hydroxy groups deshielded the C-17 carbon. The chemical shift of C-16 indicated that it is subject to the γ-effect by two axial substituents at C-13 and C-14, as in the withanolides. The C-16 chemical shift in cucurbitacins B(11), D(2), and F(7) was observed at δ 71.34, 71.5, and 70.2 respectively. In the case of the glycosides, there was a downfield shift of 8—9 p.p.m. in all cases. Such a shift, due to the glycoside linkage, is also observed in naturally occurring terpenoid glycosides such as memordicosides.[15]

C-2 and C-3-OH.—The chemical shift of C-2 in all cucurbitacins and their glycosides appeared around 70 p.p.m. Hydroxylation of C-2 caused a β-effect on C-1 and a γ-effect on C-10 of the order of 2 p.p.m. The appearance of the C-2 resonance at *ca.* 70 p.p.m. indicated that the OH group was

Table 8. ^{13}C Chemical shifts for cucurbitacin (1)—(11)

Carbon No.	(1)	(2)	(3)	(4)	(5)	(6)	(7) †	(8)	(9)	(10)	(11)
1	33.7	33.7	33.7	33.7	34	34	33.5	33.1	33.2	34	33.4
2	70.5	71.4	70.6	69.8	71	70	70	70.5	70.3	70.2	70.8
3	211.4	211	211.7	211.2	212	211	80.2	80.6	80.7	80.7	212
4	48.5	48.5	48.6	48.6	48.5	49	48.7	48.6	48.8	48.4	48.4
5	140.8	140.5	140.8	140.8	141	139.5	140.5	141	141.4	141.2	140
6	118.8	118.8	119.3	117.8	119	120.3	118.6	118.7	118.5	118.7	120
7	29.4	29.5	29.3	29.5	29.5	29.6	29.3	29.2	29.5	29.5	29.4
8	38.7	42.3	40.9	42	40.8	42	42.4	41.8	41.8	42	42
9	50.3	50.7	50.7	50	50	51	50.3	50.4	50.8	50.3	50.3
10	42	45.3	42.1	47	42.4	45.2	45	42.3	42.5	42.5	45
11	212.5	212	212.4	212	213	212.8	213	212.3	213	212	212.7
12	50.1	50.2	50	50.7	50.7	50.5	50.1	50.4	50.8	48.8	50
13	48.1 [a]	48.5 [a]	48.1 [a]	48.2 [a]	48.2 [a]	48	48.2 [a]	48.1 [a]	48.1	48.1 [a]	48 [a]
14	47.9 [a]	48.2 [a]	48 [a]	47.8 [a]	48 [a]	48	48.1 [a]	47.8 [a]	48	47.9 [a]	47.8 [a]
15	35.8	35.9	35.8	33.7	35.8	36.2	32.8	33.8	34.1	34	35.7
16	77.6	71.5	77.9	77.5	78.3	80.8	70.2	77.6	78.1	77.8	71.3
17	55.5	57.2	56	55.5	56.3	58	56.9	55.4	56.3	55.9	57.8
18	19.8	19.2	19.8	19.8	19.9	20	19.4	19.7	20	19.7	19.6
19	18.3	19.1	17.4	18.1	19.7	18	18.4	19.4	17.6	18.4	18.6
20	77.8	78	78.7	78.1	78.3	79	77.9	77.9	78.7	78.1	78
21	23.8	23.8	24	23.8	24	22.3	23.6	23.3	24	24.4	23.7
22	201	201	201	200.6	202	202	202	200.5	201	202	202
23	119.9	120.1	119.7	119.7	119.9	120.3	118.8	118.9	119.7	119.3	120
24	156	155.7	154.7	158	154.5	151.8	155.1	156	154.6	156	151.3
25	71.5	71.1	71.5	71.4	71.6	71.1	70.3	70.9	70.9	70.9	71
26	29.4 [b]	29.5	29.2 [b]	28.7	30 [b]	28.9 [b]	28.4 [b]	29	29.4 [b]	29.1 [b]	29
27	28.7	28.9	28	28	29	25.8	28	28.3	25	25	25.8
30	20.2	21.1	20.5	20.6	21	21.9	21.1	24	20.1	20.9	20
31	29.2 [b]	28.9	29.1 [b]	29.4	29.3 [b]	26.4 [b]	28 [b]	29	29.2 [b]	28.3 [b]	
32	20	20	19.9	19.9	20.5	21.2	19.7	20.2	20.4	20	19.7
1′	96.8	—	94.9	95	95.6	103.7	—	96.8	94.9	96.6	—
2′	76.5	—	69.5	69	66.3	74.7	—	76.6	69.6	67	—
3′	201.4	—	64.7	61.9	61	80.3	—	201	64.7	41.7	—
4′	74	—	68.3	68.4	30	73.8	—	74.4	68.4	200	—
5′	72.6	—	70	72.3	68.6	81	—	72.6	70.6	70.8	—
6′	18.1	—	16.5	16.4	17.6	62.4	—	18.4	16.6	16.5	—
2′-OCOCH$_3$	168.9	—	169	169.2	169.4	—	—	169	169	169	—
3′-OCOCH$_3$	—	—	171	—	170	170	—	—	171	—	—
4′-OCOCH$_3$	—	—	—	169.6	—	—	—	—	—	—	—
2′-OCOCH$_3$	21.2	—	21.2	21.3	21.3	—	—	21.7	21.1	21	—
3′-OCOCH$_3$	—	—	20.5	—	21	21.9	—	—	—	—	—
4′-OCOCH$_3$	—	—	—	21.2	—	—	—	—	—	—	—

[a,b] The assignments of signals with the same superscript within any vertical column are uncertain and may be interchanged.

† Methanol added to improve solubility.

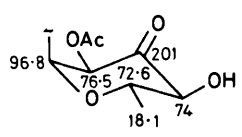
• Spectra were recorded in $CDCl_3$ on a JEOL, JNM-PFT-100 FT spectrometer operating at 25.15 MHz.

β-equatorial; this value was comparable to the chemical shift of carbon to which the β-equatorial OH group is attached in triterpenes.[9]

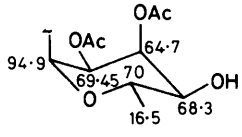
The C-3 hydroxylated carbons in cucurbitacin F(7) and datiscosides B(8), D(9), and H(10) were observed, respectively, at δ 80.2, 80.6, 80.7, and 80.7. This OH group resulted in a γ-effect on the equatorial 4-methyl carbon (C-30) by 6—7 p.p.m., as in normal pentacyclic and tetracyclic triterpenes.[16] The chemical shift of C-2 in both cucurbitacin D and F type compounds was observed in the same region. The oxidation state at C-3 did not affect the chemical shifts of the ring A carbons. This was also true of the equatorial methyl carbons at C-4, which had essentially the same chemical shifts for all compounds studied.

The doublet at δ 55.5 due to C-17 indicated the β-orientation of the side chain in cucurbitacins, as in the 17β-withanolides.[12] C-17 Is shifted downfield by the C-16 substituent (2—3 p.p.m.), as may be judged by comparison with the spectra of appropriate model compounds.[12] A similar effect was observed for cucurbitacins B(11), D(2), F(7), and the other datiscosides. The chemical shifts at δ 50.1 and 50.3 were assigned to C-12 and C-9, respectively, which were deshielded by the carbonyl group at C-11 as has been found in cortisones and other 11-oxo triterpenes.[10] In corticosteroids, C-9 and C-12 were observed at 60 and 50 p.p.m., respectively, but in this series of compounds C-9 was observed at 50.3 p.p.m. (s) and C-8 at *ca.* 40 p.p.m. The shielding of these carbons is believed to be due to the homoallylic effect of the C(5)–C(6) double bond in ring B. For C-8, a compensatory β-effect due to the two adjacent methyl substituents (at C-19 and C-22) was also noted.

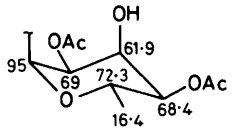
The two singlets at δ 48.1 and 47.1 are assigned to two non-protonated carbons (C-13 and C-14) which are very similar to normal tetracyclic triterpenes.[9] C-13 and C-14, being quaternary, were not subject to a γ-effect due to C-16 oxygen substitution. Since there is an axial methyl at C-14, a γ-effect on C-17 and C-16 might have been expected. In fact, only C-16 was shielded (by 3 p.p.m.); C-17 was unaffected. As a rule, it seems that no more than one carbon in a rigid 5-membered ring experiences a γ-effect.[12] The methylene carbons C-1, C-7, and C-15 in all of the compounds were observed almost in the same region and were in agreement



Datiscoside (1)



Datiscoside C(3)



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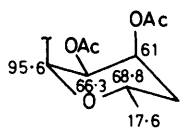

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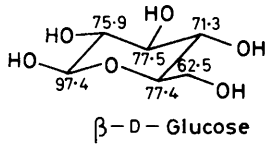
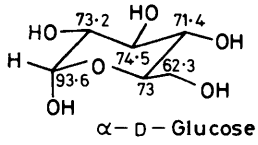
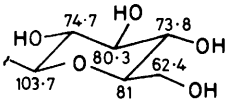


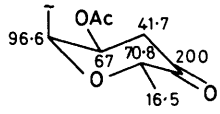
β-D-Glucose



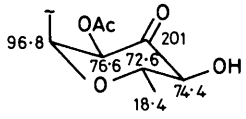
α-D-Glucose



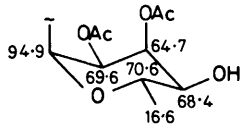
Datiscoside G(6)



Datiscoside H(10)



Datiscoside B(8)

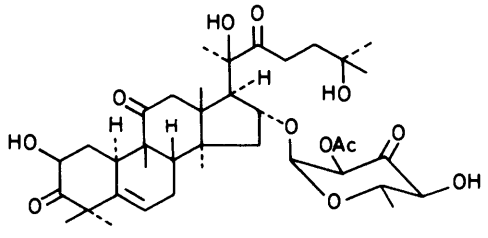


Datiscoside D(9)

with chemical shifts observed in related compounds. C-1 and C-15 Were slightly deshielded relative to lanosterol [17] due, presumably, to β-oxygen substitution.

The methyl carbons were rather difficult to distinguish; however, the oxygen substitution helped with the assignment of chemical shifts to respective carbons. C-26 and C-27 Were deshielded by 6 p.p.m. when compared with the normal steroids and triterpenes,[9] due to C-25 hydroxylation in compounds (1)—(11). One of the gem-dimethyl carbons in ring A, *i.e.* the equatorial methyl, was deshielded in both the 3-oxocucurbitacins and 3-hydroxy compounds because of its greater proximity to the oxygen. Similar effects have been observed in many triterpenes and 4,4-dimethyl steroids.[9] The 19-, 18-, and 32-methyl carbons were not affected by any of the structural changes in the isolated compounds; their chemical shifts were in good agreement with those of other terpenoids.

Carbohydrate Moiety.—Datiscoside, (1), contains the novel sugar, 2′-*O*-acetyl-6′-deoxy-α-L-*gluco*-hexos-3′-ulopyranoside, whose ^{13}C n.m.r. spectrum has been assigned by comparisons with α- and β-D-glucose and the sugar portion of datiscoside G which contains a β-D-glucoside. The C-1′ of datiscoside appears at δ 96.8 as a doublet, characteristic of an α-glycoside (Table 8). The ketone moiety at C-3′ resulted in deshielding of carbons 2′ and 4′ by roughly 4 p.p.m., to δ 76.5 and 74.0, respectively. The actual factors involved in the effects on chemical shift are complicated by the presence of an acetoxy group at C-2′ and the absence of a hydroxy group at C-6′, although relatively minor contributions would be anticipated from these substituents. The 6′-methyl group in datiscoside resonated at δ 18.1 and the oxidation state of the pyranose ring had little effect on its chemical shift, as may be judged by comparison with the other cucurbitacin derivatives reported here. Reduction of the carbonyl moiety at C-3′ to an axial group in datiscosides C, D, and E resulted in the shift of C-2′ and C-4′ to higher field (Table 8). The presence of a 3′-acetoxy group resulted in a downfield shift (*ca.* 3 p.p.m.) of the C-3′ carbon relative to the alcohol. The C-4′ deoxy sugar in datiscoside F reflected an increase in shielding around C-5′ and C-3′ that resulted in a highfield shift of 2 and 3 p.p.m., respectively. Likewise, the 3′-deoxy sugar of datiscoside H showed a C-2′ resonance shifted 2 p.p.m. to higher field.



(12)

Chemical Modification of Datiscoside.—To permit an assessment of the contribution of the C(23)–C(24) double bond to the cytotoxicity of datiscoside, reduction of this bond was affected by catalytic hydrogenation over Pd–$CaCO_3$, affording cucurbitacin glycoside (12) in quantitative yield.

Biological Assays.—The datiscosides and cucurbitacins were isolated by following the cytoxicity of fractionated materials against Eagle's KB strain of human carcinoma of the nasopharynx.[18] When tested at 10 μg/ml, essentially no viable cells remained in the case of compounds (1), (2), (4), (5), (7), (8), and (12), relative to a control sample that lacked added compounds. Even at lower dose (1μg/ml), compounds (1) and (2) killed all the cells in the culture. Treatment with compounds (3), (9), (10), and (11) also produced cytoxicity (3—5 viable cells; 60—90 non-viable cells) relative to control assays (210—220 viable cells; 5—10 non-viable cells). Interestingly, compound (6) was much less toxic when tested at 10μg/ml (80 viable cells; 30 non-viable cells) *versus* a control assay that yielded 100 viable cells and 5 non-viable cells. ED_{50} values were measured for compounds (1), (2), and (12), and are presented in Table 9.

The datiscosides showed similar activity to their cucurbitacin aglycones, but reduction of the side chain double bond significantly reduced the activity (Table 9). Although the basis for the correlation is not entirely clear, this was consistent with reports that the cucurbitacins displaced labelled glycocorticoids more effectively than the dihydro analogues.[3] It has recently been shown that the datiscosides also induce terminal differentiation in cultured lymphocytes and this activity is also

Table 9. Cytotoxicity of compounds isolated from *Datisca glomerata* against Eagle's KB strain of human carcinoma of the nasopharynx

Compound	E.D.$_{50}$ mg/ml
(1)	0.05 *
(2)	0.05
(12)	0.73

* Estimated.

reduced upon saturation of the side chain double bond.[19] The relevance of these findings is under investigation.

Experimental

M.p.s were measured on a Thomas Hoover Capillary Melting Point Apparatus and are uncorrected. Preliminary fractionation was carried out with Sephadex LH-20, particle size 25—100 μm. Column chromatography was carried out with Silica gel 60, particle size 0.063—0.200 mm, Merck. T.l.c. monitoring of all reactions was performed with Merck silica gel 60 F254 pre-coated sheets (0.2 mm) and preparative t.l.c. separations were carried out on 20 × 20 cm glass plates coated with Merck G.F.-254 silica gel and developed with 15% methanol–$CHCl_3$, or as indicated. Paper chromatography was performed on Whatman No. 1 paper, development with Bu^nOH–EtOH–H_2O (5 : 1 : 4). Chromatograms were visualized with aniline hydrogen phthalate spray, heated at 100 °C for 15 min. Flash column chromatography was carried out using Merck silica gel, particle size 0.40—0.062 mm, 230—400 mesh. T.l.c. spots were visualized with $Ce(SO_4)_2$ (5%) solution and also using u.v. light. High pressure liquid chromatography (h.p.l.c.) was performed on a Waters Associates instrument with a u.v. detector (254 nm). A Whatman Partisil M9 10/50 column was employed for separations; the solvent was 5% methanol in $CHCl_3$. U.v. spectra were measured with a Cary 15 spectrophotometer; i.r. spectra with a Perkin-Elmer 257 grating spectrophotometer. ^1H N.m.r. spectra were obtained on JOEL FX-100 or Nicolet NT 360 spectrometers. All values are reported as p.p.m. downfield from $SiMe_4$. ^{13}C N.m.r. were obtained on a JEOL, JNM-PFT-100 spectrometer operating at 25.15 MHz in the Fourier transform mode. Spectral widths of 5 000 Hz and the use of 8 K data points resulted in acquisition times and pulse delays of 0.8 s. The pulse widths used correspond to flip angles of 42—45° with a repetition rate of 1—2 s. Spectra were taken at probe temperature 23 ± 2 °C. All the reported ^{13}C chemical shifts are in p.p.m. downfield from internal $SiMe_4$, for solutions in $CDCl_3$. In one case, as mentioned in the Tables, a few drops of methanol were necessary to improve the solubility. Electron-impact mass spectral data were obtained with a Hitachi RMU-6E, chemical ionization high-resolution mass spectral data with an AEI-MS 902 modified for chemical ionization.

Extraction and Fractionation of the Twigs of Datisca glomerata.—Powdered air-dried twigs (3.3 kg) of *Datisca glomerata* were extracted successively with n-hexane (11.0 l, 29 days) and ethyl ether (33.0 l, 42 days) at room temperature. The pale yellow hexane extract on evaporation yielded a residue (8.0 g) which was inactive against KB cells and which would not crystallize from any of several organic solvents; it was not examined further.

The ether extract was concentrated under reduced pressure to give a brown residue (154 g) which was partitioned between $CHCl_3$ and water. The insoluble residue was filtered and examined separately. The $CHCl_3$ layer of the soluble portion was evaporated and partitioned successively between n-hexane and 10% aqueous methanol. The aqueous methanol was concentrated and the residue was partitioned between CCl_4 and 20% aqueous methanol. The CCl_4 layer was concentrated and tested for activity against KB cells; no significant activity was observed. The insoluble portion yielded an active fraction after filtration through Sephadex LH-20 in methanol, which was studied in detail in this paper. The fractionation and isolation of the active components are outlined in the Scheme.

Table 10. Separation of datiscosides from the twigs of *Datisca glomerata*

Compound	R_F * value	M.p. (°C)	Yield (g) †
Cucurbitacin B (11)	82.3	178—179	0.021
Datiscoside E (4)	80	152—153	0.025
Datiscoside (1)	69.8	174—176	0.177
Datiscoside F (5)	71.3	150—151	0.031
Cucurbitacin D (2)	63.2	152—154	0.044
Datiscoside C (3)	61.0	173—175	0.032
Datiscoside H (10)	59.5	149—150	0.017
Datiscoside B (8)	52.9	144—145	0.195
Cucurbitacin F (7)	49.2	187—189	0.279
Datiscoside D (9)	47	165—167	0.102
Datiscoside G (6)	42.2	154—156	0.065

* Silica gel t.l.c., $CHCl_3$–MeOH (17 : 3), R_F × 100.
† Yields obtained from 3.3 kg of the dried plant material.

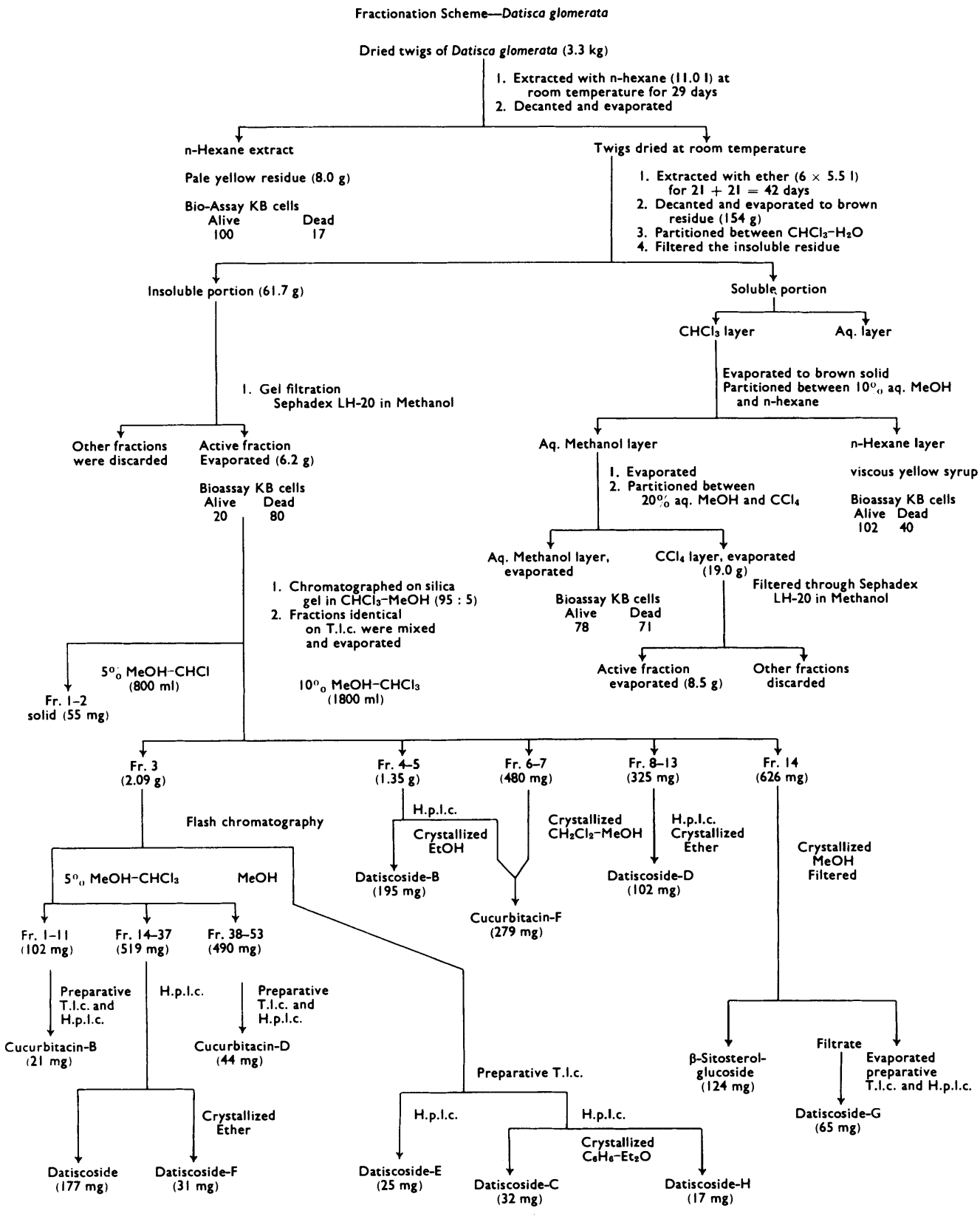
Isolation of Active Components.—The insoluble portion (61.7 g) of the $CHCl_3$ aqueous partition was filtered through Sephadex LH-20 (25-100μ, 1.5 kg); elution was with methanol. Several fractions were collected and the fractions active against KB cells were combined. A residue (6.1 g) was obtained on evaporation of the active fractions.

Identification of Cucurbitacin B(11).—This compound was eluted with 10% MeOH–$CHCl_3$ during column chromatography of the active fraction (see isolation scheme) which was purified by flash chromatography, preparative t.l.c., and finally by preparative h.p.l.c. It yielded colourless crystals from benzene and ether, $[\alpha]_D^{25}$ +87° (*c* 1.0, $CHCl_3$) (Table 10). It was identified as cucurbitacin B by ^1H and ^{13}C n.m.r. (see Tables 1, 2, 4), by chemical ionization mass spectrometry, and by direct comparison with an authentic sample [20] (t.l.c., mixed m.p., and co-injection in h.p.l.c.); mass spectrum: chemical ionization-negative ion (CH_4/N_2O) *m/z* 557 (M − H)$^-$, 498 (M − AcOH), 479 (M − H_2O − AcOH), 385, 367, 127, and 113.

Cucurbitacin D(2).—This compound eluted along with cucurbitacin B during silica gel column chromatography and was further purified by additional silica gel flash chromatography, preparative thin layer chromatography, and finally by preparative h.p.l.c. It provided colourless crystals from ether, m.p. 152—154 °C (lit.,[21] 151—152 °C), $[\alpha]_D^{23}$ +47° (*c* 1.0, $CHCl_3$; lit.,[21] +46°); mass spectrum, *m/z* 517 (M + H)$^+$, 515 (M − H)$^-$ (negative chemical ionization), 498 (M − H_2O), 480 (M − 2 H_2O), 465, 403, 385, and 113.

The structure assignment was verified by direct comparison with an authentic sample of cucurbitacin D (co-t.l.c., mixed m.p., and h.p.l.c. co-injection).

Dihydrocucurbitacin D.—Cucurbitacin D(2) (10 mg) was dissolved in reagent grade methanol (5 ml) and hydrogenated at room temperature over 2.2 mg of 5% $Pd/CaCO_3$. After 8 h,


Fractionation Scheme—*Datisca glomerata*
Dried twigs of *Datisca glomerata* (3.3 kg)
1. Extracted with n-hexane (11.0 l) at room temperature for 29 days
2. Decanted and evaporated
n-Hexane extract
Pale yellow residue (8.0 g)
Bio-Assay KB cells
Alive 100
Dead 17
Twigs dried at room temperature
1. Extracted with ether (6 × 5.5 l) for 21 + 21 = 42 days
2. Decanted and evaporated to brown residue (154 g)
3. Partitioned between $CHCl_3$–H_2O
4. Filtered the insoluble residue
Insoluble portion (61.7 g)
Soluble portion
$CHCl_3$ layer
Aq. layer
Evaporated to brown solid
Partitioned between 10% aq. MeOH and n-hexane
1. Gel filtration
Sephadex LH-20 in Methanol
Other fractions were discarded
Active fraction
Evaporated (6.2 g)
Bioassay KB cells
Alive 20
Dead 80
Aq. Methanol layer
n-Hexane layer
viscous yellow syrup
Bioassay KB cells
Alive 102
Dead 40
1. Evaporated
2. Partitioned between 20% aq. MeOH and CCl_4
Aq. Methanol layer, evaporated
CCl_4 layer, evaporated (19.0 g)
Filtered through Sephadex LH-20 in Methanol
Bioassay KB cells
Alive 78
Dead 71
1. Chromatographed on silica gel in $CHCl_3$–MeOH (95 : 5)
2. Fractions identical on T.l.c. were mixed and evaporated
5% MeOH–CHCl (800 ml)
Fr. 1–2 solid (55 mg)
10% MeOH–$CHCl_3$ (1800 ml)
Active fraction evaporated (8.5 g)
Other fractions discarded
Fr. 3 (2.09 g)
Fr. 4–5 (1.35 g)
Fr. 6–7 (480 mg)
Fr. 8–13 (325 mg)
Fr. 14 (626 mg)
Flash chromatography
H.p.l.c.
Crystallized EtOH
Datiscoside-B (195 mg)
Crystallized CH_2Cl_2–MeOH
Cucurbitacin-F (279 mg)
H.p.l.c.
Crystallized Ether
Datiscoside-D (102 mg)
Crystallized MeOH Filtered
5% MeOH–$CHCl_3$
MeOH
Fr. 1–11 (102 mg)
Fr. 14–37 (519 mg)
Fr. 38–53 (490 mg)
Preparative T.l.c. and H.p.l.c.
Cucurbitacin-B (21 mg)
H.p.l.c.
Preparative T.l.c. and H.p.l.c.
Cucurbitacin-D (44 mg)
Crystallized Ether
Datiscoside (177 mg)
Datiscoside-F (31 mg)
Preparative T.l.c.
H.p.l.c.
H.p.l.c.
Crystallized C_6H_6–Et_2O
Datiscoside-E (25 mg)
Datiscoside-C (32 mg)
Datiscoside-H (17 mg)
β-Sitosterol-glucoside (124 mg)
Filtrate
Evaporated preparative T.l.c. and H.p.l.c.
Datiscoside-G (65 mg)

Scheme.

the reaction was stopped and the product was obtained by filtration of the catalyst and concentration of the solution. The crude reaction product was purified by chromatography on a column of silica gel in $CHCl_3$, which yielded an amorphous powder (10 mg); the n.m.r. ($CDCl_3$) indicated the absence of the 23,24-double bond.

Conversion of Dihydrocucurbitacin D into Tetrahydrocucurbitacin F.[6]—Dihydrocucurbitacin D (5 mg, 0.01 mmol) was dissolved in methanol (5 ml) and treated with 3 mg (0.07 mmol) of $NaBH_4$ at room temperature for 3 days, after which a single new spot was observed on t.l.c. The reaction mixture was concentrated to small volume and treated dropwise with dilute aqueous HCl and the product was extracted into $CHCl_3$ (3 × 7 ml). The combined $CHCl_3$ extract was dried over Na_2SO_4 and concentrated. The product was obtained as a gummy solid that would not crystallize. T.l.c. analysis indicated that it was more polar than the starting material, and that it was identical with the product obtained from cucurbitacin F(7) on similar hydrogenation, and reduction with $NaBH_4$.

The product was converted into its tetra-acetate with pyridine–acetic anhydride (25 °C, 12 h); ^{1}H n.m.r. δ 1.99—2.2 (4 s, 1212 H) and four multiplets at δ 4.6—5.2; mass spectrum: chemical ionization-negative ion (CH_4/N_2O) *m/z* 689 (M − H)$^-$, 673, 655, 569, 553, 531, 471, 451, 411, 399, 369, 337, 309, 221, 163, and 115. From the spectral data this product was identified as 2β,3α,16α,22-tetra-*O*-acetyltetrahydrocucurbitacin F, which could also be obtained from cucurbitacin F(7).

Datiscoside (1).—This compound was obtained from fraction no. 12 of the column chromatography of the crude active fraction (see Fractionation scheme) by h.p.l.c. It crystallized from methanol as colourless needles; $[\alpha]_D^{23}$ +25.7° (*c* 1.0, $CHCl_3$); $\nu_{max.}$ (KBr) 3 500—3 350br, 3 280, 1 740br, 1 710, 1 685, 1 620, 1 450, 1 370, 1 310, 1 230br, 1 090, 1 070, 970, 910, 840, and 770 cm^{-1}. From the highfield ^{1}H and ^{13}C n.m.r. and chemical ionization and field desorption mass spectra, this compound was identified as datiscoside, isolated previously by Kupchan *et. al.*[5]; $C_{30}H_{44}O_7$ (M − sugar + H calculated 516.3087; found 516.306); mass spectrum: *m/z* 703 [(M + H)$^+$, FD] 684 (M − H_2O), 642 (M − AcOH), 606 (M − AcOH − 2 H_2O), 589, 571, 561, 553, 516.306, 498.297, and 480.289.

Datiscoside (10 mg, 0.014 mmol) was converted into the peracetate by treatment with pyridine (0.2 ml) and acetic anhydride (0.1 ml) at 25 °C for 12 h. The reaction mixture was poured into ice–water (15 ml) and extracted with $CHCl_3$ (3 × 10 ml). The combined $CHCl_3$ extract was washed successively with 1M-HCl (2 × 15 ml) and water (2 × 15 ml) and dried over Na_2SO_4. The $CHCl_3$ extract after evaporation yielded the crude acetate (19 mg) which was purified further by h.p.l.c. and finally precipitated from methanol as an amorphous powder, m.p. 151—152 °C; δ ($CDCl_3$) 1.9—2.2 (3 s, 9 H), 4.42 (d, 1 H, *J* 4 Hz), 4.92 (d, *J*, 1 H 10 Hz), 5.25br (s, 1 H), 5.45 (dd, 1 H, *J* 13.5, 5 Hz), 5.75 (d, 1 H, *J* 7 Hz), 6.5 (d, 1 H, *J* 14.5 Hz), and 7.15 (d, 1 H, *J* 14.5 Hz).

Dihydrodatiscoside (12).—Datiscoside (10 mg) was dissolved in methanol (5 ml) and hydrogenated at 25 °C over $Pd/CaCO_3$ (2 mg). After 15 min, the reaction was stopped, the catalyst filtered off and the filtrate concentrated, to give dihydrodatiscoside (10 mg), which gave a single spot, R_F 0.50, on silica gel t.l.c. (development with 15% MeOH in $CHCl_3$) and lacked u.v. absoprtion; δ ($CDCl_3$) 1.0 (s, 3 H), 1.09 (s, 3 H), 1.22 (s, 3 H), 1.24 (s, 3 H), 1.35 (s, 3 H), 1.41 (s, 3 H), 1.46 (d, 3 H, *J* 6 Hz), 2.0 (d, 1 H, *J* 7.5 Hz), 2.13 (s, 3 H), 2.70 (d, 1 H, *J* 7 Hz), 2.72 (d, 1 H, *J* 13 Hz), 2.75 (d, 1 H, *J* 14.5 Hz), 3.22 (d, 1 H, *J* 14.5 Hz), 3.48 (dq, 1 H, *J* 9, 5.5 Hz), 3.88 (d, 1 H, *J* 9 Hz), 4.35 (t, 1 H, *J* 7 Hz), 4.45 (ddd, 1 H, *J* 13.5, 7, 4 Hz), 5.27 (m, 2 H), and 5.77br (d, 1 H, *J* 6 Hz).

Datiscoside F (5).—This compound was obtained from the column fractions eluted with 5% MeOH in $CHCl_3$ during the flash chromatography (see Fractionation scheme), and was purified further by preparative h.p.l.c. It crystallized as colourless plates from diethyl ether; $[\alpha]_D^{23}$ +16.6° (*c* 1.0, $CHCl_3$); $\lambda_{max.}$ (MeOH) 230 nm (ε 8 800); $C_{40}H_{58}O_{12}$ (Found: 730.393; M^+ calc. 730.3928); *m/z* 731 (M + H$^+$, FD), 712, 694, 670, 617, 614, 540, 480, 382, 215, and 96.

Datiscoside E(4).—This compound was co-eluted with datiscoside C(3) during silica gel column chromatography and was purified by preparative t.l.c. and h.p.l.c. Crystallization from ether afforded colourless plates; $\nu_{max.}$ (KBr) 3 500—3 400br, 3 270, 1 740, 1 710, 1 685, 1 630, 1 450, 1 365, 1 315, 1 240, 1 220, 1 090, 1 040, 980, and 900 cm^{-1}; *m/z* 745 (M − H)$^-$ (negative chemical ionization) 728 (M − H_2O), 713, 686, 515, 497, 479, 255, 165, 149, 138, 127, and 113.

Datiscoside C(3).—After final purification by preparative h.p.l.c., compound (3) crystallized from benzene–ether as colourless prisms; $[\alpha]_D^{23}$ +6.6° (*c* 0.5, $CHCl_3$), $\nu_{max.}$ (KBr) 3 520—3 400br, 3 290, 1 740br, 1 710, 1 685, 1 640, 1 450, 1 365, 1 310, 1 240br, 1 040, 970, 900, 850, and 770 cm^{-1}; $C_{40}H_{56}O_{12}$ (M − H_2O calculated 728.3771. Found: 728.378); *m/z* 745 [(M − H)$^-$, negative chemical ionization], 728, 713, 686, 630, 515, 497, 480, 383, 340, 255, 231, 228, 165, 157, 127, and 113.

Datiscoside C(3) (10 mg, 0.013 mmol) was converted into the respective peracetate by treatment with pyridine (0.2 ml) and acetic anhydride (0.1 ml) at 25 °C for 12 h. The reaction mixture was poured into ice–water and extracted with portions of $CHCl_3$. The combined $CHCl_3$ extract was washed successively with 1M-HCl and water and then dried over Na_2SO_4. Concentration of the extract provided the crude acetate, which was purified further by h.p.l.c. and then precipitated from methanol as an amorphous powder, m.p. 150—151 °C; δ ($CDCl_3$) 1.00 (s, 3 H), 1.09 (s, 3 H), 1.22 (d, 3 H, *J* 7 Hz), 1.35 (s, 3 H), 1.40 (s, 3 H), 1.41 (s, 3 H), 2.0—2.2 (4 s, 12 H), 2.50br (d, 1 H, *J* 12 Hz), 2.64 (d, 1 H, *J* 14.5 Hz), 2.67 (d, 1 H, *J* 7 Hz), 3.17 (d, 1 H, *J* 14.5 Hz), 3.70 (dq, 1 H, *J* 9.5, 7 Hz), 4.92 (d, 1 H, *J* 7 Hz), 5.0 (ddd, 1 H, *J* 12, 7, 3.5 Hz), 5.27 (t, 1 H, *J* 4 Hz), 5.27 (d, 1 H, *J* 6 Hz), 5.75 (d, 1 H, *J* 5.5 Hz), 6.42 (d, 1 H, *J* 14.5 Hz), and 7.05 (d, 1 H, *J* 14.5 Hz).

Datiscoside H(10).—This compound co-eluted with compounds (3) and (4) during silica gel flash chromatography and was purified further by preparative h.p.l.c. Crystallization from benzene and ether afforded (10) as colourless plates; mass spectrum: *m/z* 670 (M − H_2O), 652, 575, 556, 528, 518, 500, 482, 476, 457, 452, 439, 433, 428, 405, 387, 369, 368, 359, 354, 351, 339, 219, 201, 171 (sugar), and 113.

Datiscoside B(8).—This compound co-eluted with cucurbitacin F(7) during silica gel column chromatography in methanol–$CHCl_3$. It was purified further by preparative h.p.l.c.; crystallization from ethanol afforded colourless plates; $[\alpha]_D^{23}$ +20.7° (*c* 1.0, $CHCl_3$); $\lambda_{max.}$ (MeOH) 231 nm (ε 9 000); $\nu_{max.}$ (KBr) 3 500—3 380br, 1 740, 1 695, 1 680, 1 620, 1 450, 1 380, 1 315, 1 235s, 1 065, 1 000, 915, 840, and 770 cm^{-1}; $C_{30}H_{46}O_7$ (M − sugar, calculated 518.3243; found 518.324); *m/z* 705 [(M + H)$^+$, FD], 685 (M − H_2O), 591, 547, 518, 500, 482, 369, 187, and 113.

Datiscoside B (10 mg, 0.014 mmol) was converted into its peracetate by analogy with the acetylation of datiscoside and

datiscoside C. The crude product (11 mg) was obtained as a white solid; after purification by preparative h.p.l.c., elution with 1% CH_3OH in $CHCl_3$, the acetate crystallized from methanol, m.p. 138—140 °C; δ ($CDCl_3$) 3.70 (dq, 1 H, *J* 10, 7 Hz), 4.61 (d, 1 H, *J* 4 Hz), 4.66 (d, 1 H, *J* 10 Hz), 4.87 (t, 1 H, *J* 7 Hz), 4.97 (ddd, 1 H, *J* 12, 10, 5 Hz), 5.2br (s, 1 H), 5.75 (d, 1 H, *J* 7.5 Hz), 6.41 (d, 1 H, *J* 14.5 Hz), and 7.04 (d, 1 H, *J* 14.5 Hz).

Cucurbitacin F(7).—This compound crystallized from $CHCl_3$–MeOH as colourless needles, and was identified by highfield n.m.r. and mass spectrometry and by direct comparison with an authentic sample;[6] *m/z* 519 [$(M + H)^+$, FD], 517 [$(M - H)^-$, negative chemical ionization], 500 ($M - H_2O$), 405, 387, 369, 361, and 113.

Dihydrocucurbitacin F.—Cucurbitacin F (10 mg, 0.02 mmol) was dissolved in methanol (5 ml) and hydrogenated over 5% $Pd/CaCO_3$ (3 mg) for 1 h at 25 °C. Filtration of the reaction mixture and concentration of the solution afforded a residue that was deposited as an amorphous solid from methanol. The ^1H n.m.r. spectrum ($CDCl_3$) indicated the absence of the 23,24-double bond.

Tetrahydrocucurbitacin F.—Dihydrocucurbitacin F (5 mg, 0.01 mmol) was dissolved in methanol (5 ml) and treated with $NaBH_4$ (3 mg, 0.07 mmol) at room temperature for 1 h, after which a single new spot was observed on t.l.c. The reaction mixture was concentrated to small volume and treated with dilute aqueous HCl and the product was extracted into $CHCl_3$ (3 × 5 ml). The $CHCl_3$ extract was dried over Na_2SO_4 and concentrated to afford a gummy solid (4 mg) that would not crystallize. T.l.c. analysis indicated that the product was more polar than the starting material and identical with the same species obtained from dihydrocucurbitacin B.

The new product was converted into the respective acetate by treatment with pyridine (5 drops) and acetic anhydride (3 drops) at 25 °C for 12 h. The crude product was purified by silica gel column chromatography and identified as 2β,3α,16α,-22-tetra-*O*-acetyltetrahydrocucurbitacin F by highfield ^1H n.m.r. and chemical ionization mass spectrometry; δ ($CDCl_3$) 0.9 (s), 1.05 (s), 1.10 (s), 1.19 (s), 1.2 (s), 1.3 (d), 1.35 (s), 2.0—2.10 (4 s), 2.42 (d, *J* 18 Hz), 2.47 (d, *J* 7 Hz), 2.65 (d, *J* 14.5 Hz), 3.10 (d, *J* 14.5 Hz), 4.68 (d, *J* 10 Hz), 5.0 (dd, *J* 10 Hz), 5.05 (t, *J* 7 Hz), 5.45 (dd, *J* 14.5, 10 Hz), and 5.75 (d, *J* 7 Hz); *m/z* 691 $(M + H)^+$, 673, 655, 613, 569, 553, 531, 471, 451, 399, 307, 278, 248, 219, 191, 163, 143, 139, 112, and 109. The same product, having identical ^1H n.m.r. and mass spectra, was also obtained from dihydrocucurbitacin D.

Datiscoside D(9).—This compound crystallized directly from one of the silica gel column fractions and was recrystallized from anhydrous ether as colourless plates; $[\alpha]_D^{23}$ +19.3 (*c* 1.0, $CHCl_3$); $\nu_{max.}$ (KBr) 3 520—3 380br, 1 740br, 1 685, 1 440, 1 360, 1 310, 1 240, 1 040, 1 005, 850, 800, and 770 cm^{-1}; $C_{40}H_{58}O_{12}$ ($M - H_2O$ calculated 730.3929. Found: 730.393); *m/z* 749 [$(M + H)^+$, FD], 668, 672, 517, 493, 491, 482, 231, 157, and 113.

β-*Sitosterol Glucoside.*—This compound was co-eluted with datiscoside G(6) during silica gel column chromatography and was purified by fractional crystallization from methanol, m.p. 287—288 °C (decomp.); *m/z* 396 ($M - H_2O$), 382, 367, 329, 255, 175, 145, 135, and 105.

β-Sitosterol glucoside (10 mg, 0.017 mmol) was converted into the respective acetate by treatment with pyridine (0.2 ml) and acetic anhydride (0.2 ml) at 25 °C for 12 h. The reaction mixture was poured onto crushed ice and the product was extracted into $CHCl_3$ (3 × 5 ml). The $CHCl_3$ extract was washed successively with 1M-HCl and saturated $NaHCO_3$ solution, and then dried over Na_2SO_4. After concentration, the product was crystallized from methanol, m.p. 219—220 °C; δ ($CDCl_3$) 0.7 (s, 3 H), 0.79 (d, 3 H, *J* 7 Hz), 0.81 (s, 3 H), 0.85 (d, 3 H, *J* 7 Hz), 0.99 (s, 3 H), 2.0—2.10 (4 s, 12 H), 3.5 (m, 1 H), 3.64 (m, 1 H), 4.10 (dd, 1 H, *J* 11.5, 2.5 Hz), 4.25 (dd, 1 H, *J* 11.5, 5.5 Hz), 4.6 (d, 1 H, *J* 8 Hz), 4.95 (t, 1 H, *J* 11 Hz), 5.07 (t, 1 H, *J* 11 Hz), 5.20 (t, 1 H, *J* 11 Hz), 5.35 (d, 1 H, *J* 7 Hz); *m/z* 743 [$(M - H)^-$, negative chemical ionization], 684, 654, 626, 598, 564, 522, 496, 460, 424, 396, 382, 331, 288, 255, 213, 169, 109, and 105.

Hydrolysis of β-*Sitosterol Glucoside.*—β-Sitosterol glucoside (10 mg) was heated at reflux with 2M-methanolic HCl (10 ml) for 5 h. The reaction mixture was cooled and the HCl was removed under a stream of nitrogen. The resulting residue was partitioned between water and $CHCl_3$, and the $CHCl_3$ layer after evaporation yielded a white solid (3 mg) that was identified as β-sitosterol by direct comparison with an authentic sample isolated from *Myrcia fallax*.[22] The aqueous layer was heated at 80 °C for 1 h, concentrated, and then tested for carbohydrates; only D-glucose [R_F value 0.23; development with 5 : 1 : 4 Bu^nOH–HOAc–H_2O] was identified by paper chromatography, by comparison with an authentic sample.

Datiscoside G(6).—This compound was isolated from the mother liquors that deposited β-sitosterol glucoside. Purification was effected by h.p.l.c. and crystallization from anhydrous ether; $\nu_{max.}$ (KBr) 3 500—3 400br, 1 740br, 1 690, 1 680, 1 625, 1 460, 1 375, 1 300, 1 260br, 1 130, 1 070, 1 010, 980, 870, and 770 cm^{-1}; *m/z* 719 [$(M - H)^-$, negative chemical ionization], 659 (M – AcOH), 641, 617, 563, 497, 480, and 113.

The penta-acetate of (6) was obtained by treatment of datiscoside G (10 mg, 0.013 mmol) with pyridine (0.2 ml) and acetic anhydride (0.2 ml) at 25 °C for 12 h; the product was purified by extractive work-up and h.p.l.c.; *m/z* 480, 423, 411, 331, 285, 271, 157, 137, 125, and 111.

Hydrolysis of Datiscoside G(6).—A sample of datiscoside G (2 mg) was treated with 1M-hydrochloric acid (2 ml) in 50% aqueous methanol. The reaction mixture was maintained at room temperature for 2 h and then heated at reflux for 8 h. The reaction mixture was cooled and the HCl was removed under a stream of N_2. The resulting residue was partitioned between $CHCl_3$ and water. The $CHCl_3$ layer after evaporation yielded a gummy residue which showed three spots on t.l.c., one of which corresponded to cucurbitacin D. The aqueous layer was heated at 90 °C for 30 min, then cooled and concentrated under diminished pressure. The residue was tested for the presence of carbohydrates; only glucose [R_F value 0.23; development with 5 : 1 : 4 Bu^nOH–HOAc–H_2O] was identified by paper chromatography, by comparison with an authentic sample.

Biological Assays.—Cytotoxicity was measured against Eagle's KB strain of human carcinoma of the nasopharynx.[18] Monolayers of the cultured cells were split 4 to 1 with trypsin and the cells were recultured for 48 h after the addition of the extract, which was added routinely in 5 μl of ethanol. Cell counts and viability using Trypan Blue were performed at 48 h (Table 9).

Crystal Data.—$C_{40}H_{58}O_{13}\cdot 2C_6H_6$, M = 903.08. Orthorhombic, (293 K) a = 10.649(4), b = 13.858(5), c = 34.733(12) Å, U = 5125.6 Å^3, Z = 4, D_c = 1.170; (193 K) a = 10.502(4), b = 13.780(5), c = 34.355(12) Å, U = 4971.8 Å^3, Z = 4,

D_c 1.206; $F(000) = 1\,944$. Space group $P2_12_12_1$ from systematic absence of axial reflections of odd order. Cu-K_α radiation, $\lambda = 1.5418$ Å; μ(Cu-K_α) = 6.2 cm^{-1}.

Unit cell parameters were derived from a least-squares fit to the optimum diffractometer setting angles for 15 strong general reflections.

Intensity Measurements.—These were made from single crystals by means of the $2\theta/\omega$ scan technique, using Nicolet P3m diffractometers. Precession photographs of the small crystals initially available, taken with Mo-K_α radiation, had shown a very rapid fall-off in intensity with increasing 2θ, so a set of intensity measurements was made at 193 K with Cu-K_α radiation and an upper limit of 100° in 2θ. Though more reflections could have been obtained at low temperature from the crystals used, better crystals became available which gave reasonable scattering at room temperature. To achieve adequate resolution, but to limit the time spent measuring very weak reflections, an upper limit of 116° was chosen for 2θ for the room temperature set. At 293 K, 3 661 of 3 968 unique reflections were significant at the 2σ level and were treated as observed. Measurements at 193 K were made using a Nicolet LT-1 cooling system with N_2 gas, and 2 904 of 2 911 unique reflections were significant at the 3σ level and were treated as observed.

Structure Determination and Refinement.—Independent attempts were made to solve the structure from both room temperature and low temperature data sets by a variety of approaches using direct methods, but all were uniformly unsuccessful. The structure was finally solved from the 193 K data by a real space Patterson search using a program written by Hornstra.[23] The search fragment consisted of 25 atoms and was taken from the known structure of datiscoside bis(*p*-iodobenzoate),[4] excluding the large substituents at C(16) and C(17). The best solution after the vector search was readily expanded to the complete structure by an *E*-map recycling procedure; a second difference electron-density map was only necessary to locate the benzene molecules.

Refinement of the 193 K data proceeded smoothly with $R = 0.115$ for isotropic refinement of C and O atoms, and $R = 0.092$ for anisotropic refinement. All hydrogen atoms were located from successive difference electron-density maps, and included in the refinement with fixed isotropic thermal parameters. Convergence was reached with $R = 0.038$, $R_w = 0.042$. The weighting scheme used had $w = [\sigma^2(F) + 0.0001(F)^2]^{-1}$.

The heavy atom positions for the 193 K data were used as the starting point for the refinement with the 293 K data. Isotropic refinement gave $R = 0.134$, and anisotropic refinement gave $R = 0.083$. Hydrogen atoms were placed in calculated positions (C–H 0.9 Å) and their positional and isotropic thermal parameters included in the refinement. At convergence, $R = 0.047$, $R_w = 0.044$. The *B*-values of the hydrogens of the methyl groups attached to C(26), C(27), C(28), C(36), and C(40) were all >11 Å^2, and those of the hydrogens of the benzene rings >15 Å^2, so that these atoms are not considered to have been located by the analysis.

Calculations involving the 193 K data (Göttingen) were carried out using the SHELX-76 program system; those involving the 293 K data (Virginia) were carried out using local programs written for the SDS Sigma 2 computer. The stereoscopic view in Figure 1 was prepared using ORTEP.[24] Observed and calculated structure amplitudes, atomic co-ordinates for the 293 K data set, parameters for hydrogen atoms, and anisotropic thermal parameters are listed in Supplementary Publication No. SUP 23581 (49 pp.).*

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* See note about Supplementary Publications in the Instructions for Authors (1983), *J. Chem. Soc., Perkin Trans. 1*, 1983, Issue 1.

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