New Cucurbitacin Derivatives from *Bryonia dioica* Jacq.

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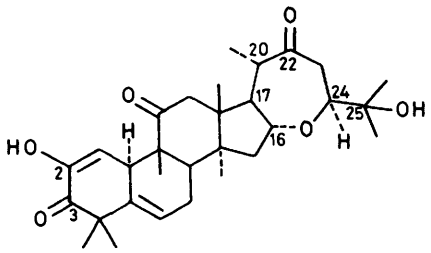
The isolation and structural elucidation of some new methoxy-containing artefacts from methanol extracts of *Bryonia dioica* roots is reported. Their isolation supports the suggestion that the isocucurbitacins (3-hydroxy-2-ketones) are not natural compounds but are really artefacts formed from the more usual 2-hydroxy-3-keto compounds.

The presence of groups such as hydroxy ketones in tetracyclic triterpenes may lead to possibilities for the formation of artefacts. The cucurbitacins are such a class due to the presence of diosphenols (*e.g.* cucurbitacin E) or α-ketols (*e.g.* cucurbitacin B) in ring A. Thus, cucurbitacin B has a 2-hydroxy-3-ketone function but the isomeric 3-hydroxy-2-ketones are also known.[1] These latter have been called the isocucurbitacins.

The present work reports the isolation of some further cucurbitacin and isocucurbitacin derivatives but in this case further complications were caused by additional reactions in the side-chain. The new compounds described here are related to cucurbitacin S, which until recently was thought to be derived from a 23-ketone compound but has now been shown to be a derivative (1) in which the 16-hydroxy group has cyclised to the side-chain in a more usual 22-carbonyl compounds.[2,3]



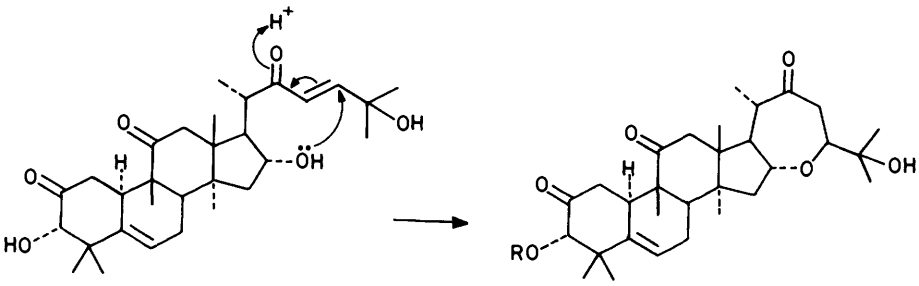
(1)

Results and Discussion

One of the fractions isolated after column chromatography of an acid-hydrolysed methanolic extract of *Bryonia dioica* root showed a series of compounds with very similar R_F values. Attempted isolation of these materials by preparative thin-layer chromatography (p.t.l.c.) did not produce single compounds and there was no doubt that the materials were being interconverted during the chromatography. I.r. examination of the mixture showed a strong band at 3 500 cm^{-1}, indicative of the presence of a hydroxy group. The mass spectrum showed a weak molecular ion peak at *m/z* 500 (*cf.* $M^{+\cdot}$ for cucurbitacin S is at *m/z* 498)[2] suggesting that the major component was a dihydro-derivative of cucurbitacin S. After acetylation of the mixture p.t.l.c. did allow isolation of a major compound, (2), m.p. 186—188 °C, which was both relatively stable and identical with a substance isolated from another column chromatographic separation carried out on a previously acetylated extract of *Bryonia dioica*.

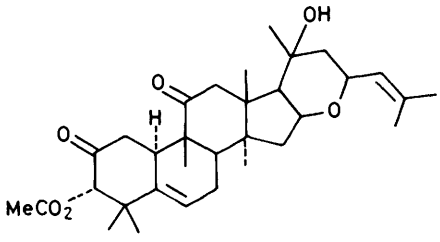
Mass spectrometry of (2) showed a molecular ion peak at *m/z* 542 (corresponding with a monoacetate derivative) but this peak was again of very low intensity compared with the peak at *m/z* 524, probably due to ready dehydration of the compound. Evidently, the material still possessed an hydroxy group which, since it was not acetylated under the normal conditions, indicated that it was probably tertiary. The ^1H n.m.r. spectrum of this compound was very similar to that of cucurbitacin S[2] with respect to the signals of the side-chain but differed in the signals due to the hydrogens in ring A. The diosphenolic 1-H signal was absent but a 1 H signal at δ 4.94 was present. This value is very close to the chemical shift (δ 4.95) of 3-H in the spectrum of the acetate of anhydro-22-deoxo-3-*epi*-isocucurbitacin D (3)[1,4,5] which clearly points to an α-acetoxy-ketone structure for ring A in compound (2). The i.r. spectrum showed several carbonyl absorptions, one of which (at 1 715 cm^{-1}) may be considered as being due to such an α-acetoxy carbonyl.[6] Such a structure could also explain the observed instability of the naturally occurring unacetylated alcohol, since cucurbitacin-type α-ketol compounds are known to isomerise readily. In the ^1H n.m.r. spectrum of (2) the signal at δ 4.94 (already assigned to a methine hydrogen of an acetyl function) was a very sharp singlet. Thus, no hydrogens could be present on adjacent carbon atoms indicating that the material was a 3-acetate, *i.e.* of the isocucurbitacin series. Moreover, such a value of this chemical shift (δ 4.94) can be considered as evidence for an equatorial hydrogen [*i.e.* an axial (α) acetyl group],[7] since the equivalent hydrogen in the spectra of the 3β-compounds 22-deoxo-isocucurbitacin D and tetrahydroisocucurbitacin I resonates at the significantly lower field of δ 5.10.[1,4,5] The other spectral characteristics were very similar to those of cucurbitacin S[3] and readily led to the proposal of the structure of the new acetate as (2).

A further group of methoxy-containing acetals, which were clearly also related to the new α-ketol series, was also isolated. Thus, further column chromatography of the acetylated extract allowed isolation of three minor compounds (4), (5), and (6). Compounds (5) and (6) contained one methoxy function but (4) gave signals in the ^1H n.m.r. spectrum for two oxygen-bearing methyl groups at δ 3.24 and 3.42. Overall, the whole ^1H n.m.r. spectrum of (4) was very similar to that of the cucurbitacin S acetal (which was previously isolated along with cucurbitacin S)[2] except that diosphenol hydrogen resonances were absent and had been replaced by a signal at δ 4.02 (dd, *J* 6 and 13 Hz). Since it was considered likely that the second methoxy group in (4) was present as part of an acetal function (probably produced during the acidic methanol hydrolysis of the plant extract before chromatography) treatment of (4) with acid in aqueous acetone should destroy this acetal function in a manner analogous to the interconversion of cucurbitacin S with its methoxy derivative.[2] This reaction was therefore carried out and two products were obtained—identical with (5) and (6) from the extract—which, as has been stated previously, both still contained one methoxy-group. Conversely, treatment of (5) with methanolic HCl provided a mixture of unchanged starting material with *ca.* 40% of (6). It may thus be stated that (6) is indeed likely to be an artefact, produced during the initial hydrolysis from a compound similar to either (4) or (5).

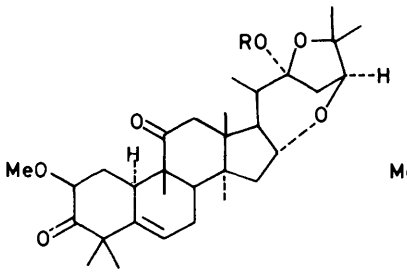


(8)

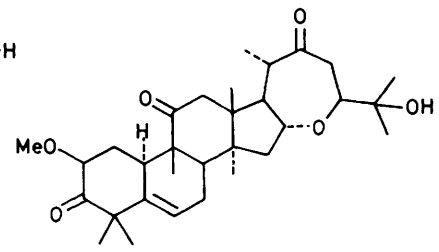
(2) R = Ac
(7) R = H



(3)



(4) R = Me
(6) R = H



(5)

Accurate mass measurements on the spectrum of (6) pointed to a molecular formula of $C_{31}H_{46}O_6$. In addition to signals for seven tertiary methyl groups and one secondary methyl (which must be C-21), the ^1H n.m.r. spectrum showed signals for four low-field hydrogens of which the broad singlet at δ 5.76 may be assigned to 6-H and the characteristic multiplet at δ 3.78 to 16-H.[8] Its cucurbitacin-like nature was confirmed by the presence of the lower half of an AB system due to the (equatorial) 12β-H at δ 3.07. Two low-field signals remain to be accounted for. As has already been mentioned, the spectrum of (4) showed a doublet of doublets at δ 4.02. A similar signal was also present at δ 4.04 in the spectra of both (5) and (6). From the shift value, it is likely that this resonance is due to a methine hydrogen on a carbon carrying an oxygen function. Moreover, since the signal remains unchanged after treatment with aqueous acid, it cannot be part of an acetal system. A methoxy signal is also present in the spectra of (5) and (6) and it is thus likely that this δ 4.04 signal is due to the system CH_3O–CH. Methoxy-containing triterpenes are unlikely to be naturally occurring especially in this case since the products were isolated from an extract which had been previously treated with methanolic acid. It is likely then that this methoxy group is also an artefact but produced in such a way that it is stable in the product.

The new acetate isolated earlier was probably derived from a 3-hydroxy-2-keto compound and it is reasonable to suggest that reaction of this latter material with methanol, catalysed by the presence of acid, to give the 2,3-dihydroxy-2-methoxy compound followed by dehydration would produce the 2-methoxy-3-keto compound, *via* the enol. Furthermore, from the coupling constants of this signal (6 and 13 Hz) in the spectrum of (6) the methoxy group must be β.[8,9]

Still with regard to (6) it may now be stated, in summary, that its structure is that of a cucurbitacin derivative with a 2β-methoxy-3-keto function, an oxygen function at C-16 which is probably attached to the side-chain as an ether (*cf.* cucurbitacin S[2]), the usual C-11 keto-function, and a 5-double bond and no substituent at C-20 (since C-21 is secondary). C-25 Must carry an oxygen atom since there is only one secondary methyl group (C-21). The only structure compatible with these data is thus (6).

The mass spectrum of the ketonic product (5) shows a molecular ion formula of $C_{31}H_{46}O_6$, *i.e.* isomeric with (6). In the ^1H n.m.r. spectrum the hydrogens of the side-chain methylene group experience pronounced downfield shifts of *ca.* 1.19 p.p.m. and 0.1 p.p.m. implying that they must be adjacent to the newly formed carbonyl. In fact one of these methylene hydrogens resonates at a field as low as δ 3.04 (*cf.* 12β-H which gives a signal at *ca.* δ 3.00 in the cucurbitacins)[2] and must lie in the plane of the carbonyl. The structure for the ketone-containing compound is thus (5). Mass spectrometry of the methoxy group containing acetal indicates a formula of $C_{32}H_{48}O_6$ and is thus likely to be (4). The non-ketonic hydrolysis product is thus probably the corresponding hemiacetal (6). As confirmation, absorption for a hydroxy group is not present in the i.r. spectrum of (4).

A likely structure for the major compound of the original alcohol mixture is thus the corresponding 3-hydroxy compound (7). However this latter material could not be isolated in a single form probably due to the ready isomerisation to the corresponding 2-hydroxy-3-keto compound.

From a detailed consideration of the spectra of known cucurbitacins,[10,11] it has been possible to investigate the ^{13}C n.m.r. spectra of the new compounds. The data are shown in the Table. Unequivocal assignments were not possible for every carbon in every compound but the Table shows likely assignments. All the observed signals are compatible with the proposed structures, and multiplicities in the off-resonance spectra were also appropriate to the assignments. The point of interest lies in the data for the 3α-acetoxy compound (2). A singlet was observed at δ 105.0, as expected for a quaternary carbon carrying two oxygen atoms, readily assignable to C-22 in the proposed open form. This is similar to the signals in the spectra of (4) (δ 108.6) and (6) (δ 105.5). However, in addition

Table ^{13}C N.m.r. data for compounds (2), (4), (5), and (6) (δ, $CDCl_3$)

Carbon no.	(2)	(4)	(5)	(6)
1	35.9	34.3	34.4	34.2
2	205.0	80.6	80.5	82.0
3	81.4	209.7	209.7	209.7
4	47.5	46.5	46.0	47.8
5	138.0	140.6	140.6	140.0
6	121.7	119.8	119.9	119.8
7	24.0	24.1	24.0	24.1
8	35.8	33.8	34.2	33.8
9	43.1	43.2	48.5	43.1
10	40.0	40.3	43.3	40.8
11	212.6	212.7	212.5	212.6
12	43.2 *	49.1	48.6	43.1
13	48.6	47.8	49.0	49.1
14	48.8	48.1	50.6	49.5
15	49.7	46.0	49.7	41.8
16	82.0	76.1	80.2	80.6
17	49.9	50.6	51.2	51.3
20	32.1	33.2	33.1	33.2
22	203.0, 105.5	108.6	212.6	105.5
23	43.1 *	48.6	47.8	43.1
24	85.5	81.3	81.1	85.6
25	71.1	71.2	71.1	73.7
18, 19, 21,	29.6	29.9	29.6	29.9
26, 27, 28,	28.9	29.5	29.3	29.7
29, 30	24.5	28.9	28.9	28.9
	20.5	21.5	24.7	21.5
	20.1	20.8	21.4	20.7
	19.9	20.2	20.8	20.2
	18.1	18.2	19.9	18.0
	11.0	15.3	18.6	11.0
OCH_3	—	57.8, 57.8	57.8	57.9
CO_2CH_3	170.0	—	—	—
CO_2CH_3	20.5	—	—	—

* This assignment may be reversed.

to this, an *extra* signal at δ 203.0 was observed. This is fairly close to the observed signal (δ 212.6) for the C-22 carbonyl carbon in the spectrum of (5), the 25-hydroxy-22-keto compound in the 2β-methoxy series. This can only be explained by the proposal that an equilibrium existed between the open hydroxy ketone and the cyclised hemiacetal form of (2) in the $CDCl_3$ solution used to obtain the ^{13}C n.m.r. spectrum. This also goes some way to account for the difficulty experienced in the isolation of these series of compounds.

As a result of these detailed ^1H n.m.r. studies it is possible to make some stereochemical deductions. In the doubly cyclised side-chain, two asymmetric centres are present so four isomers are theoretically possible. Examination of Dreiding models however indicates that if the 24 centre is *R*, *i.e.* 24-H is β, only the 22*R* compound may form. Conversely, if the 24 centre is assumed to be *S*, only the 22*S* isomer is possible. This assumes the normal cucurbitacin stereochemistry at 16, 17, and 20. From the n.m.r. data it may be stated that the more likely structure for the compound with the acetal side-chain is the 22*S*, 24*S*-isomer. By similar considerations the structures of all the new compounds isolated in this work may be summarised as follows: (2), (24*S*)-3α-acetoxy-16, 24-anhydro-16,-24,25-trihydroxy-2,11,22-trioxocucurbit-5-ene; (4), (22*S*,24*S*)-16,24;22,25-dianhydro-16,22,24,25-tetrahydroxy-2β,22-dimethoxy-3,11-dioxocucurbit-5-ene; (5); (24*S*)-16,24-anhydro-16,24,25-trihydroxy-2β-methoxy-3,11,22-trioxocucurbit-5-ene; and (6), (22*S*,24*S*)-16, 24;22,25-dianhydro-16,22,22,24,25-pentahydroxy-2β-methoxy-3,11-dioxocucurbit-5-ene. The structure of the alcohol which could not be isolated is thus likely to be (24*S*)-16,24-anhydro-3α,16,24,25-tetrahydroxy-2,11,22-trioxocucurbit-5-ene.

As has already been mentioned, most cucurbitacins which have two oxygen-containing functions in ring A exist either as diosphenols (*e.g.* cucurbitacin E), diols (*e.g.* cucurbitacin F), or α-ketols (*e.g.* cucurbitacin B). The latter has, more specifically, a 2-hydroxy-3-keto function. The isomeric 3-hydroxy-2-keto compounds (the isocucurbitacins) such as tetrahydroisocucurbitacin I have been isolated from *Bryonia dioica* but only from extracts which have been stored for several months.[1] In view of this, and the co-occurrence of tetrahydrocucurbitacin I in the same extracts,[1] it is likely that the iso-material is an artefact, formed by slow enolisation of the α-ketol system in ring A. This had been previously suggested [2] and extra evidence is thus provided for this conclusion by the present work, which has demonstrated the extreme lability of the α-ketol systems in that they readily form 2-methoxy derivatives in acidic methanol. The increased stability of the 2-acetate confirms this, but it is unfortunate that, in the series of materials examined in the present work, further problems were caused by additional isomerisations in the side-chain. The whole question of the status of the isocucurbitacins is thus open but it is likely that isocucurbitacins are *not* natural substances but formed as artefacts as indicated above.

The present series of compounds in which an extra ring is formed by cyclisation across a 16-hydroxy group and C-24 are unusual. Apart from cucurbitacin S and its derivatives,[2] the only other similar compound is an anhydro-derivative of a cucurbitacin D-type substance. The latter was isolated from a hybrid of *Lagenaria siceraria* [4] and it was considered that acid treatment of 22-deoxocucurbitacin D led to elimination of water to yield the cyclised anhydro derivative. Similarly, since the compounds in the present study were all isolated after acid-catalysed hydrolysis of a methanolic extract, it is considered likely that a similar reaction occurred in this series. Thus, the most probable structure for the natural compound is (8) which could cyclise to give the 7-membered ring ether (2) in the manner shown. Such a proposal is particularly attractive since a variety of products could be produced, all bearing oxygen at C-22 either as a ketone, acetal or hemiacetal. A search for this compound in the extract has, however, not yet been successful.

The isolation of the present series of compounds has implications for the biosynthesis of the cucurbitacin side-chain, since, because the C-20 hydroxy substituent is absent, it appears to indicate that, in this series at least, C-20 hydroxylation occurs as the *last step* in the reaction sequence to yield the (typical) cucurbitacin side-chain, as in (3).

Experimental

^1H N.m.r. spectra were recorded at either 400 MHz (Bruker WH 400 n.m.r. spectrometer), 200 MHz (Nicolet NT 200), or 90 MHz (Perkin-Elmer R 32). ^{13}C N.m.r. spectra were recorded using the Bruker instrument. Mass spectra were recorded on an AEI MS 902 high resolution mass spectrometer having a direct inlet system and operating at 18 and 70 eV. Elemental analytical data were not good for the new compounds presumably due to the isomerisation described in the text and to a variable degree of hydration. The compounds gave single spots on t.l.c. and homogeneity was ensured by repeated p.t.l.c. (systems indicated individually below).

Roots of *Bryonia dioica* were collected from the Royal Botanic Gardens, Kew, London in June 1976. A voucher specimen of the dried aerial parts of the plants has been

deposited in the museum, Chelsea College. Air-dried powdered root (11.3 kg) was exhaustively extracted successively with light petroleum b.p. 40—60 °C and then with methanol.

The methanolic extract (1.33 kg) was hydrolysed by refluxing with 2M-HCl in methanol (95%) for 2 h. The solution was concentrated by evaporation under reduced pressure, diluted with a large volume of water, neutralised with Na_2CO_3 solution, and extracted many times with chloroform. Evaporation under reduced pressure yielded a black oily residue (278 g), part of which (244 g) was coarsely fractionated by column chromatography (750 g Silica gel G) by eluting successively with toluene, ethyl acetate, and methanol. The ethyl acetate-eluted fractions were bulked together and evaporated under reduced pressure to yield an oily black residue (135 g), part of which was acetylated in the usual way. Part of the acetylated extract (67 g) was adsorbed on the top of a column of silica gel G (2.75 kg).

In addition to known substances,[1,10,12] elution of the column with 13% ethyl acetate in toluene gave a colourless solid, repeated crystallisation of which from ethyl acetate gave white fan-shaped crystals (65 mg) of (22S,24S)-16,24;22,25-*dianhydro*-16,22,24,25-*tetrahydroxy*-2β,22-*dimethoxy*-3,11-*dioxocucurbit*-5-*ene* (4), m.p. 237—239 °C; $\lambda_{max.}$ (EtOH) (log ε) 224 (3.25) and 295 nm (2.38); $\nu_{max.}$ (Nujol) 1 715 (3-ketone), 1 690 cm^{-1} (11-ketone); δ(400 MHz, $CDCl_3$), 0.78 (3 H, s), 1.00 (3 H, d, *J* 7 Hz, 21-H), 1.08 (3 H, s), 1.25 (3 H, s), 1.29 (3 H, s), 1.30 (3 H, s), 1.36 (3 H, s), 1.41 (3 H, s), 1.48 (1 H, dd, *J* 4.5, 11 Hz, 15α- or 15β-H), 1.83 (1 H, dd, *J* 4.5, 11 Hz, 15β or 15α-H), *ca.* 1.94 (2 H, *m*, 2 × 23-H), 2.60 (2 H, m, 2 × 1-H), 2.38 (1 H, d, *J* 12 Hz, 12α-H), 2.40 (1 H m), 2.67 (1 H, m, 10-H), 3.60 (1 H, d, *J* 12 Hz, 12β-H), 3.24 (3 H, s, CH_3O), 3.43 (3 H, s, CH_3O), 3.70 (1 H, d, *J* 2.5 Hz, 24-H), 4.00 (1 H, sext, *J* 9.5, 9.5, 4.5 Hz, 16-H), 4.02 (1 H, dd, *J* 6, 13 Hz, 2-H), and 5.62 (1 H, m, 6-H); *m/z* (rel. int.) 528 (12), 513 (17), 496 (46), 481 (33), 464 (6), 453 (12), 446 (10), 444 (8), 429 (4), 403 (3), 382 (1), 364 (3), 349 (18), 333 (7), 239 (46), 189 (24), 180 (29), 128 (100), and 73 (98). Accurate mass measurements: Found: 528.3454, $C_{32}H_{48}O_6$ requires 528.3451; Found: 496.3189, $C_{31}H_{44}O_5$ requires 496.3189; Found: 349.2177, $C_{24}H_{29}O_2$ requires 349.2167. Found: 239.1442, $C_{17}H_{19}O$ requires 239.1436. ^{13}C n.m.r. data are given in the Table.

Elution with 19% ethyl acetate in toluene gave a white solid, crystallisation of which from ethyl acetate gave white needles (10 mg), m.p. 185—187 °C, identical in every respect with (24S)-16,24-*anhydro*-16,24,25-*trihydroxy*-2β-*methoxy*-3,11,22-*trioxocucurbit*-5-*ene* (5), m.p. 187—189 °C; $\lambda_{max.}$ (EtOH) (log ε) 234 (3.30) and 297 nm (2.16); $\nu_{max.}$ (Nujol) 3 450 (OH stretching), 1 725 (3-ketone), 1 710 (22-carbonyl) and 1 685 cm^{-1} (11-carbonyl); δ(400 MHz, $CDCl_3$), 0.80 (3 H, s), 0.98 (3 H, d, *J* 7 Hz, 21-H), 1.22 (6 H, s), 1.28 (6 H, s), 1.30 (6 H, s), 1.42 (1 H, dd, *J* 4.5, 11 Hz, 15α- or 15β-H), 1.82 (1 H, dd, *J* 4.5, 11 Hz, 15β- or 15α-H), 2.20 (2 H, m, 1-H_2), 2.24 (1 H, dd, *J* 9.5, 12 Hz, 17-H), 2.38 (1 H, dd, *J* 5, 12 Hz, 23-H), 2.46 (1 H, d, *J* 12 Hz, 12α-H), 2.72 (1 H, br m, 10-H), 3.04 (1 H, d, *J* 12 Hz, 23-H), 3.13 (1 H, d, *J* 12 Hz, 12β-H), 3.41 (3 H, s, 2β-OMe), 4.04 (1 H, dd, *J* 5.5, 13 Hz, 2α-H), 4.23 (7 H, sext, *J* 9.5, 9.5, 4.5 Hz, 16-H), 4.34 (1 H, d, *J* 5 Hz, 24-H), and 5.80 (1 H, br m, 6-H); *m/z* (rel. int.) 514 (5), 496 (100), 494 (12), 483 (12), 482 (24), 481 (48), 467 (13), 464 (14), 438 (5), 233 (8), 215 (9), 203 (8), 188 (22), 187 (20), 180 (15), 178 (11), 175 (14), 161 (14), 152 (10), 121 (15), 108 (21), and 83 (12); ^{13}C n.m.r. data are given in the Table.

Elution with 22% ethyl acetate in toluene gave a material, crystallisation of which from ethyl acetate gave white needles (15 mg), m.p. 186—188 °C, of (24S)-3α-*acetoxy*-16,24-*anhydro*-16,24,25-*trihydroxy*-2,11,22-*trioxocucurbit*-5-*ene* (2); $\nu_{max.}$ (Nujol) 3 500 (OH str.), 1 735 (2-acetate), 1 715 and 1 700 (2- and 22-carbonyl), and 1 685 cm^{-1} (11-carbonyl); δ(400 MHz, $CDCl_3$), 0.78 (3 H, s), 0.96 (3 H, s), 1.00 (3 H, d, *J* 7 Hz, 21-H), 1.18 (3 H, s), 1.20 (3 H, s), 1.26 (3 H, s), 1.37 (6 H, s), 1.43 (1 H, dd, *J* 4.5, 11 Hz, 15α- or 15β-H), 1.84 (1 H, dd, *J* 4.5, 11 Hz, 15β- or 15α-H), 1.85 (1 H, d, *J* 12 Hz, 23-H), 2.12 (1 H, dd, *J* 9.5, 12 Hz, 17-H), 2.19 (3 H, s, CH_3CO_2), 2.26 (1 H, dd, *J* (5,12 Hz, 23-H), 2.34 (1 H, d, *J* 12 Hz, 12α-H), 2.51 (2 H, br m, 1-H_2), 2.70 (1 H, m, 10-H), 2.94 (1 H, d, *J* 12 Hz, 12β-H), 3.78 (1 H, sext, *J* 9.5, 9.5, 4.5 Hz, 16-H), 3.97 (1 H, d, *J* 5 Hz, 24-H), 4.94 (1 H, s, 3β-H), 5.96 (1 H, br m, 6-H); *m/z* (rel. int.) 542 (2), 524 (100), 512 (2), 496 (17), 482 (13), 467 (98), 449 (14), 352 (4), 336 (10), 309 (8), 189 (11), 179 (10), 164 (10), 148 (13), and 135 (11). Accurate mass measurement: Found: 542.3242, $C_{32}H_{46}O_7$ requires 542.3421; ^{13}C n.m.r. data are given in the Table.

Elution with 23% ethyl acetate in toluene gave a colourless amorphous material. Repeated p.t.l.c. (30% ethyl acetate in cyclohexane, run 6×) and crystallisation from ethyl acetate afforded white crystals (35 mg), m.p. 227—229 °C, identical in every respect with (22*S*,24*S*)-16,24;22,25-dianhydro-16,22,-22,24,25-pentahydroxy-2β-methoxy-3,11-dioxocucurbit-5-ene (6) which could also be obtained by synthesis from (22*S*,24*S*)-16,24; 22,25-dianhydro-16,22,23,25-tetrahydroxy-2β,22-dimethoxy-3,11-dioxocucurbit-5-ene (4) (*q.v.*).

Reaction of (22S,24S)-16,24;22,25-*Dianhydro*-16,22,24,25-*tetrahydroxy*-2β,22-*dimethoxy*-3,11-*dioxocucurbit*-5-*ene* (4) *with* HCl.—The title compound (44 mg) was dissolved in acetone (3 ml) in a small round bottomed flask, 10% aqueous HCl (7 ml) was added and the mixture refluxed for 6 h. The solution was concentrated and diluted with water (*ca.* 100 ml). Neutralisation with aqueous Na_2CO_3 and extraction with chloroform gave, after drying and evaporation of the solvent, a residue (44.5 mg), which showed 3 components by t.l.c. (cyclohexane–ethyl acetate 3 : 2, run 4×). These were separated by p.t.l.c. (cyclohexane–ethyl acetate 7 : 3, run 8×). The major (most polar) compound crystallised from ethyl acetate to afford white needles (26 mg) of (22S, 24S)-16,24;22,25-*dianhydro*-16,22,22,24,25-*pentahydroxy*-2β-*methoxy*-3,11-*dioxocucurbit*-5-*ene* (6), m.p. 227—229 °C; $\lambda_{max.}$ (EtOH) (log ε) 229 (2.75) and 295 nm (2.05); $\nu_{max.}$ (Nujol) 3 500 (OH str), 1 715 (3-ketone), and 1 684 cm^{-1} (11-ketone); δ(400 MHz, $CDCl_3$), 0.78 (3 H, s), 1.01 (3 H, d, *J* 7 Hz, 12-H), 1.07 (3 H, s), 1.24 (6 H, s), 1.28 (3 H, s), 1.29 (3 H, s), 1.36 (3 H, s), 1.42 (1 H, dd, *J* 4.5, 11 Hz, 15α- or 15β-H), 1.82 (1 H, dd, *J* 4.5, 11 Hz, I5β- or 15α-H), 1.85 (1 H, d, *J* 12 Hz, 23-H), 2.16 (1 H, dd, *J* 9.5, 12 Hz, 17-H), 2.26 (2 H, m, 1-H_2), 2.28 (1 H, dd, *J*, 5,12-Hz, 23-H), 2.40 (1 H, d, *J* 12 Hz, 12α-H), 2.68 (1 H, m, 10-H), 3.07 (1 H, d, *J* 12 Hz, 12β-H), 3.42 (3 H, s, OCH_3), 3.78 (1 H, sext., *J* 9.5, 9.5, 4.5 Hz, 16-H), 4.00 (1 H, d, *J*, 5 Hz, 24-H), 4.04 (1 H, dd, *J* 6, 13 Hz, 2a-H), and 5.76 (1 H, br m, 6-H); *m/z* (rel. int.) 514 (10), 496 (100), 481 (29), 470 (12), 467 (19), 439 (8), 421 (4), 397 (5), 369 (4), 293 (5), 335 (7), 239 (20), 189 (33), 187 (29), 121 (43), 119 (48), and 43 (95). Accurate mass measurements: Found: 514.3298, $C_{31}H_{46}O_6$ requires 514.3295; Found: 496.3188, $C_{31}H_{44}O_5$ requires 496.3189; Found: 482.3041, $C_{30}H_{42}O_5$ requires 482.3032; Found: 481.2963, $C_{30}H_{41}O_5$ requires 481.2954; Found: 239.1436, $C_{17}H_{19}O$ requires 239.1436; ^{13}C n.m.r. data are given in the Table.

Purification of the second component of this product by p.t.l.c. allowed isolation of a colourless solid which crystallised from ethyl acetate as needles (12 mg) of (24*S*)-16,24-anhydro-16,24,25-trihydroxy-2β-methoxy-3,11,22-trioxocucurbit-5-ene (5), *q.v.*

Reaction of (24S)-16,24-*Anhydro*-16,24,25-*trihydroxy*-2β-

methoxy-3,11,22-*trioxocucurbit*-5-*ene* (5) *with Hydrochloric Acid in Methanol*.—The title compound (4 mg) was dissolved in pyridine (1 ml), and 3M-HCl in methanol (4 ml) added. The solution was refluxed for 10 h and then diluted with distilled water (30 ml), neutralised with aqueous Na_2CO_3 and extracted with $CHCl_3$. After evaporation of the solvent the residue so obtained showed two main components by t.l.c. the R_F values of which were identical with those of the starting material and a product which was identical with (22*S*,24*S*)-16,24;-22,25-dianhydro-16,22,24,25-tetrahydroxy-2β,22-dimethoxy-3,11-dioxocucurbit-5-ene (4). This latter compound appeared to represent *ca.* 40% of the total mixture.

Similar chromatography of part of the unacetylated plant extract allowed isolation of compounds (4), (5), and (6) and a substance assumed to be (7) which could only be purified after acetylation; this gave a substance identical in all respects with (2).

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