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CUCURBITACIN S, A NEW CUCURBITACIN FROM *BRYONIA DIOICA*

PETER J. HYLANDS and AHMED M. SALAMA

Pharmacognosy Research Laboratories, Department of Pharmacy, Chelsea College, University of London, Manresa Road, London, SW3 6LX, England

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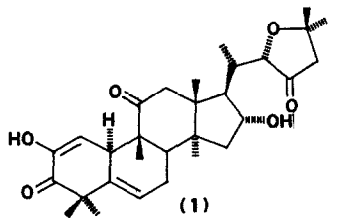
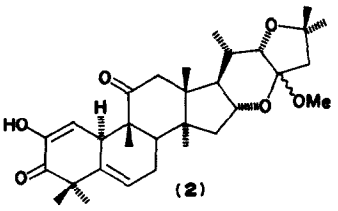
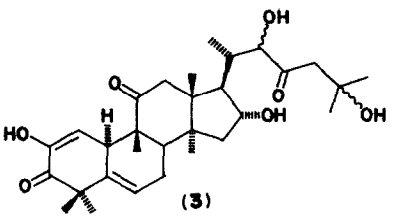
Cucurbitacins possess a wide range of biological activities [1] and a recent report cites their possible rôle as antigibberellins [2]. *Bryonia dioica* Jacq. has previously been shown to yield, in addition to other triterpenes, cucurbitacins B, D, E, I, tetrahydro-I, J, K and L [1, 3] together with the less highly substituted bryodulcosigenin, bryosigenin and bryogenin [4]. They are characterized by the presence of a unique carbon skeleton and a more or less highly oxygenated side chain. Although compounds have been isolated which bear oxygen functions on carbons 20, 22, 24 and/or 25, until now no cucurbitacin has been shown to possess an oxygen substituent on C-23.

A methanolic extract of *Bryonia diocia*, treated with 2N HCl [3], gives a black oil which on fractionation by chromatography on silica gel provides cucurbitacin S, assigned structure **1** on the basis of chemical and spectral evidence, in 0·004% yield. Mass spectrometry shows the M^+ peak to be at *m/e* 498 but this was of very low intensity compared with the peak at *m/e* 480 (shown by accurate mass measurement to correspond with $C_{30}H_{40}O_5$) due to ready dehydration of the compound. The base peak appears at *m/e* 164; this fragment, characteristic of cucurbitacins with a diosphenol group in ring A, arises by retro-Diels–Alder fission in ring B [5]. Confirmation of the presence of this function is provided by the UV maximum at 268 nm shifted to 313 nm in the presence of base and the NMR spectrum (the occurrence of a sharp 1H doublet (*J*, 2 Hz) at δ 5.96 assigned to the vinyl H-1). Both results are very strong indications of a cucurbitacin skeleton [6, 7]. The NMR spectrum has a signal for one H, exchangeable with D_2O, at δ 2.27 and an IR band at 1750 cm^{-1} may be assigned to a 5-membered ring ketone.

A second highly crystalline compound **2** obtained from the less polar fractions of the column (0.002% yield) may also be generated by treatment of cucurbitacin S with methanolic HCl suggesting that **2** is probably an artifact of the extraction procedure. High resolution mass spectrometry shows the molecular formula of **2** to be $C_{31}H_{44}O_6$ (i.e. with one more carbon than **1**). The UV is still consistent with the presence of a diosphenol chromophore but the IR does not show any carbonyl absorption above 1685 cm^{-1}. The MS has peaks at *m/e* 512 (M^+) and *m/e* 480 ($M^+ - 32$, indicative of the possible presence of a methoxyl group). This is substantiated by the 3H singlet in the NMR spectrum at δ 3.23. Thus **2** appears to be an acetal derivative of cucurbitacin S. The NMR spectrum of **2** also shows signals due to the







presence of seven tertiary methyl groups (δ 1.34–0.78) and one secondary methyl group (δ 0.95, *d J*, 7 Hz). Of the tertiary methyl groups five are accounted for by a cucurbitacin nucleus and if a normal C_8 side chain is assumed the secondary methyl must be C-21.

Excluding that of the methoxyl function, there are signals for four hydrogens between δ 3.00 and δ 5.00, one of which (δ 3.47, broad *m*) is assigned to the allylic H-10 (irradiation at δ 3.47 collapses the H-1 doublet at δ 5.96 (*J*, 2.5 Hz) to a singlet). The pair of signals centred at δ 3.03 is assigned to the H-12β [8]. The presence of two further signals at δ 3.69 (1H, *d*, *J*, 3 Hz) and δ 4.04 (1H, broad *m*) indicates that the molecule possesses two hydrogens which are attached to carbons that also bear oxygen atoms. An INDOR experiment, irradiating the C-21 methyl signal, shows that the adjacent C-20 methine hydrogen absorbs at *ca* δ 2.00. Irradiation at this frequency collapses the δ 3.69 doublet to a singlet, showing that C-20 is adjacent to another carbon bearing an oxygen function and only one hydrogen. In addition, because the signal of H-22 appears as a doublet, C-23

carries no hydrogens. The other low field signal at δ 4.04 (1H, *sextuplet*, *J*, 9.5, 9.5, 4.0 Hz) is reminiscent of the resonance due to H-16 in those cucurbitacins bearing an oxygen substituent at C-16 [8]. Since no signals due to D_2O exchangeable hydrogens are seen in the NMR spectrum it must be concluded that the C-16 substituent in **2** is an ether. Because the methoxyl group must be present as part of an acetal function it is clear that C-16 is joined via an ether oxygen to the side chain at either C-23, C-24 or C-25. Also, since only one secondary methyl group is present, the C-26 and C-27 methyls must be tertiary; i.e. C-25 cannot bear a hydrogen atom. **2** is the only structure consistent with all these data.

It thus follows that cucurbitacin S must be represented by **1**, with a 2,2-dimethyl-4-oxo-tetrahydrofuran side chain, rather like the 3,4-dihydroxy-2,2-dimethyl-tetrahydrofuran side chain of mexicanol [9]. **2** presumably arises by formation of the 23-acetal of cucurbitacin S followed by intramolecular dehydration (cf. the formation of anhydro-22-deoxo-cucurbitacin D by treatment of 22-deoxo-cucurbitacin D with acid [10]), indicating that **2** is 23-methoxy-16,23-anhydro-23-deoxo-cucurbitacin S.

By analogy with cucurbitacin D (the stereochemistry of which has been determined by X-ray crystallography [11]) the 16-OH group in cucurbitacin S is assigned the α configuration; the possibility that **2** is a 16-β ether is discounted because cucurbitacin S is regenerated by treatment of **2** with aqueous acid. Also by analogy with cucurbitacin D, if the C-17 C-20 bond and the C-21 methyl group are assumed to be β and α respectively and since $J_{20,22}$ is 3 Hz, H-20 and H-22 must be *syn* (i.e. H-22 is β). The stereochemistry of cucurbitacin S is thus best represented by **1**.

Several examples of tetracyclic triterpenes with cyclic ether side chains have been reported [9, 12, 13] and in the cucurbitacin series gratiogenin (from *Gratiola officinalis* L., Scrophulariaceae) possesses a substituted isopropyl tetrahydrofuran side chain [14]. These compounds presumably arise by spontaneous cyclisation/dehydration in the acid hydrolysis of diols or unsaturated alcohols (e.g. protopanaxadiol from ginseng yields panaxadiol [14]). In the case of cucurbitacin S a likely natural precursor is the 22,25-dihydroxy compound **3**, but a search for this compound in an elaterase hydrolysed extract [15] has so far been unsuccessful.

EXPERIMENTAL

NMR spectra were recorded at 90 MHz in $CDCl_3$. MS were determined with an AEI MS 902 high resolution instrument having a direct inlet system and operating at 70 eV. Roots of *Bryonia dioica* were collected at The Royal Botanic Gardens, Kew in July 1974.

The dried powdered roots (1.69 kg) were exhaustively extracted successively with light petroleum bp 40–60° then with MeOH. The MeOH extract was hydrolysed by heating in 2N HCl (methanolic) for 2 hr, diluted with a large volume of water and extracted into $CHCl_3$. The resultant black oil (83.4 g) was adsorbed onto a column of Si gel G (750 g). Elution with increasing concentrations of EtOAc in C_6H_6 allowed isolation [after preparative TLC on Si gel G plates; EtOAc–C_6H_6 (3:7)] of **2** followed by **1**. *Cucurbitacin S* (**1**) Colourless amorphous solid from EtOAc, mp 75°; UV: λ_{max}^{EtOH} 268 nm (log ε 3.95) shifted to 315 nm in the presence of alkali; IR: $\nu_{max}^{CHCl_3}$ 3420 (OH), 1750 (5 m-ring ketone), 1685 (diosphenol), 1658 (C=C), 1222 (diosphenol) cm^{-1}; NMR: (90 MHz, $CDCl_3$) δ 5.96 (1H, *d*, *J*, 2 Hz, C-1), 5.78 (1H, broad *m*, C-6), 4.24 (1H, broad *m*, C-16), 3.97 (1H, *d*, *J*, 3 Hz, C-22), 3.47 (1H, broad *m*, C-10), 3.03 (1H, *d*, *J*, 15 Hz, C-12), 2.27 (1H, *s*, exchangeable with D_2O, C-16 OH), 1.35 (6H, *s*), 1.33 (3H, *s*), 1.24 (3H, *s*), 1.21 (3H, *s*), 1.02 (3H, *s*), 0.93 (3H, *d*, *J*, 6.5 Hz, C-21), 0.90 (3H, *s*,); MS: *m/e* (rel. int.): 498 (0.3), 481 (8), 480 (27), 465 (7), 462 (3), 441 (3), 395 (37), 379 (9), 317 (10), 257 (7), 256 (7), 255 (10), 233 (7), 229 (9), 204 (13), 203 (14), 189 (17), 175 (13), 165 (18), 164 (100). Accurate mass measurement: Found 480.2902. $C_{30}H_{40}O_5$ requires 480.2877.

23-Methoxy-16,23-anhydro-23-deoxo-cucurbitacin S (**2**) Colourless needles from EtOAc mp 265–267° (decomp.); UV: λ_{max}^{EtOH} 269 nm (log ε 4.09) shifted to 315 nm in the presence of alkali; IR: $\nu_{max}^{CHCl_3}$ 1685 (diosphenol), 1664 (C=C) cm^{-1}; NMR: (90 MHz, $CDCl_3$) δ 5.96 (1H, *d*, *J*, 2.5 Hz, C-1), 5.77 (1H, broad *m*, C-6), 4.04 (1H, *sextuplet*, *J*, 9.5, 9.5, 4.0 Hz, C-16), 3.69 (1H, *d*, *J*, 3 Hz, C-22), 3.47 (1H, broad *m*, C-10), 3.23 (3H, *s*, OMe), 3.03 (1H, *d*, *J*, 15 Hz, C-12β), 1.34 (6H, *s*), 1.30 (3H, *s*), 1.27 (3H, *s*), 1.23 (3H, *s*), 1.03 (3H, *s*), 0·95 (3H, *d*, *J*, 7 Hz, C-21), 0.78 (3H, *s*); MS: *m/e* (rel. int.): 512 (20), 497 (8), 494 (5), 481 (6), 480 (8), 350 (8), 349 (30), 317 (29), 257 (11), 256 (33), 255 (30), 247 (11), 230 (9), 229 (20), 165 (28), 164 (100). Accurate mass measurement: Found 512.3175. $C_{31}H_{44}O_6$ requires 512.3139.

Interconversion of cucurbitacin S and **2**. Cucurbitacin S was dissolved in 2N methanolic HCl and the solution refluxed for 2 hr. Usual work up gave (**2**). Similarly, treatment of **2** with 2N HCl in Me_2CO–H_2O (1:1) yielded cucurbitacin S.

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