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ISOLATION AND STRUCTURE OF CUCURBITACIN Q1*

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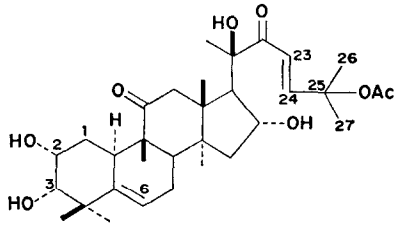
Key Word Index—*Cucumis prophetarum*; Cucurbitaceae; melon; structure elucidation; tetracyclic triterpenoids; anti-tumour agents.

Abstract—Cucurbitacin Q1, a compound isolated from the fruit of *Cucumis prophetarum*, has been shown to be the pure *trans* component of cucurbitacin Q.

THE REPORTED isolation of a number of cucurbitacins with cytotoxic properties[1–3] prompted us to investigate the active principles present in the fruits of *Cucumis prophetarum*, a plant locally known as 'Choti indrayan' or 'Khar indrayan'. It is a perennial, trailing herb with ellipsoidal echinate fruits. The plant grows wild in various regions of Pakistan, Rajputana (India), Arabia and tropical Africa.[4] The fruit is used in indigenous medicine as an emetic and purgative.[5] It is known to contain cucurbitacins B and D and traces of cucurbitacins G and H.[6–9]



Cucurbitacin Q1

* Part I in the projected series "Anti-tumour Cucurbitacins".

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Fresh undried fruits were collected from the suburbs of Karachi and cucurbitacin Q1 isolated as described below. The UV spectrum of the substance exhibited a band at 230 nm (ϵ 10 740) indicative of the presence of an α,β-unsaturated ketone. The IR spectrum also suggested this by the presence of a band at 1695 cm^{-1}. Another band at 1715 cm^{-1} indicated the presence of an ester group. A broad band at 3300–3600 cm^{-1} was indicative of the presence of one or more hydroxyl groups.

Upon electron impact, cucurbitacin Q1 afforded the highest peak at $m/e = 500$ and others at 482, 457, 405, 387, 113, 112, 111 and, interestingly, a strong peak at $m/e = 96$. The prominent peak at $m/e = 96$ suggested that the side chain was of a similar nature as that encountered in cucurbitacins-*A*, -*B*, -*C*, -*D*, -*E* and -*I* which also afford a strong ion at 96 by rupture of the side chain.[10] The highest peak at $m/e = 500$ is attributed not to the molecular ion, which fails to appear, but to $M^+ - 60$ ion formed by the loss of acetic acid from the parent ion. The presence of an acetate grouping was confirmed from the NMR spectrum of cucurbitacin Q1 (discussed below); a similar situation is encountered in the MS of cucurbitacins-*A*, -*B* and -*C*. High resolution mass measurements confirmed the formulae of the ion at $m/e = 500$ as $C_{30}H_{44}O_6$ and of the other major fragments.

Kupchan *et al.* have recently reported the isolation of cucurbitacins O, P and 'Q' from *Brandegea bigelovii*.[11] The MS of cucurbitacin Q1 closely resembled that reported for 'cucurbitacin Q' and the IR spectra were virtually superimposable. There were, however, significant differences in other physical properties. 'Cucurbitacin Q' was obtained as a gum, m.p. 118–135°, soluble in chloroform and with an ϵ value of 9000. Cucurbitacin Q1, however, was a colourless crystalline solid, m.p. 240°, insoluble in chloroform and gave an ϵ value of 10 750. On the basis of the position and multiplicity of the side chain protons and the positions of the C-25 methyl groups in the NMR spectrum of 'cucurbitacin Q', Kupchan suggested that a mixture of *cis*- and *trans* isomers at the side chain double bond were present, but did not isolate the pure substances. Thus the olefinic protons at C_{23}—C_{24} double bond in 'cucurbitacin Q' (in $CDCl_3$) appeared as both a singlet at τ3·72 (*cis* isomer) and an *AB* quartet at τ2·94 and 3·56 (*J* 15·5 Hz) (*trans* isomer). Cucurbitacin Q1 (in CD_3OD) however, showed no corresponding singlet but gave an *AB* quartet for these protons at τ3·06 and τ3·16 (*J* 15·5 Hz). Whereas the C-25 methyl groups of 'cucurbitacin Q' appeared at τ8·37 (*cis*) and τ8·48 (*trans*) those of cucurbitacin Q1 occur at τ8·44 and τ8·46. It may be supposed that the *cis* band is absent from the latter, and that the *trans* band is split into two because of its diastereotropic nature. A 3-proton singlet at τ8·01 in cucurbitacin Q1 is assigned to the methyl of the acetate group and the C-6 vinylic proton resonates as a multiplet at τ4·27.[12]

When the NMR spectrum of cucurbitacin Q1 was recorded in ^{d6} DMSO-, slight differences were apparent. The side chain olefinic protons were no longer resolved into an *AB* quartet but appeared as a singlet at τ3·20. The C-6 vinylic proton was shifted upfield to τ4·40 and the two C-25 methyl groups appeared as a 6-proton singlet at τ8·51.

Catalytic reduction of cucurbitacin Q1 afforded a dihydro compound which showed no UV absorption. The MS dihydrocucurbitacin Q1 no longer showed the strong peak at $m/e = 96$, but showed prominent peaks at $m/e = 502$ ($M^+ - 60$), 405, 387, 369, 351, 219, 177 and 113. The NMR spectrum showed the absence of the side chain olefinic protons, and was otherwise similar to that of cucurbitacin Q1. The identity of physical properties of

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dihydrocucurbitacin Q1 with those of dihydrocucurbitacin Q conclusively established its structure. The *cis* stereochemical disposition of the two hydroxyl groups in ring A of cucurbitacin Q1 was confirmed by acetonide formation. The monoacetonide showed the highest peak in its MS at $m/e = 540$ ($M^+ - 60$) in analogy with the acetonide of cucurbitacin P.

From these results it is evident that cucurbitacin Q1 is the pure *trans* component at the 23–24 double bond of 'cucurbitacin Q'. The comparatively high ϵ value (10 750) of cucurbitacin Q1 is in agreement with typical values for α,β-unsaturated ketones and 'cucurbitacin Q', which affords a lower ϵ value of 9000 (probably due to the presence of impurities) is a mixture of *cis* and *trans* isomers at the C_{23-24} double bond. The cytotoxicity of these cucurbitacins O, P and Q against Eagles KB strain of human carcinoma of the nasopharynx has been demonstrated and it has been shown that the 23–24-double bond and the 25-acetate group are important for such activity.[11] 'Cucurbitacin Q' was shown to have the highest degree of activity of the three cucurbitacins (O, P and Q) reported. The cytotoxic activity of cucurbitacin Q1 would therefore be of interest and is to be determined.

EXPERIMENTAL

M.ps are uncorrected. TLC was carried out on silica gel GF 254 coated plates and silica gel 100–200 mesh (Fisons) was used for column chromatography.

*Cucurbitacin Q*1 (1). The fresh fruits of *Cucumis phrophetarum* (5·4 kg) were cut into small pieces and percolated 3× with EtOH (9 l.) at room temp. for 48 hr. The extracts were combined and concentrated under reduced pressure. The dark green residue was partitioned repeatedly between H_2O and EtOAc until no insoluble material was left. The EtOAc layer was washed, dried and evaporated under reduced pressure. The greenish residue was repeatedly extracted with Et_2O. The ether soluble fractions were combined, concentrated and kept in the cold when crystals were deposited. On recrystallization from MeOH colourless prismatic rods were obtained m.p. 240° $[\alpha]_D^{23}$ +50° (1% MeOH). (0·67 g, yield/kg of dry fruit: 0·8 g.) The substance gave positive Salkowski and Liebermann–Burchard tests for triterpenoids and afforded a single spot on TLC, (EtOAc, R_f 0·7); (Found: C, 68·3; H, 8·4%. $C_{32}H_{48}O_8$ requires: C, 68·6; H, 8·6%); λ_{max} 230 nm (ϵ 10 750); ν_{max} (KBr): 3300–3600 cm^{-1} (–OH), 1712 cm^{-1} (–OCOMe), 1695 cm^{-1} (C=O); NMR (CD_3OD): τ8·01 (3H, *s*, –OCOMe), τ8·44 (3H, *s*, Me [26 or 27]) τ8·46 (3H, *s*, Me [26 or 27]), τ8·6, 8·70, 8·82, 8·93, 9·03, 9·4 (3H each, *s*, 6 quaternary methyls), τ4·27 (1H, *m*, C-6H), τ3·06 and τ3·16 (2H, *AB* quartet [*J* 15·5 Hz], olefinic protons at C_{23} and C_{24}); MS: m/e = 500·3122 ($C_{30}H_{44}O_6$) (M^+—60), 482·3034 ($C_{30}H_{42}O_5$), 457·2946 ($C_{28}H_{41}O_5$), 405·2634 ($C_{24}H_{37}O_5$), 387·2544 ($C_{24}H_{35}O_4$), 113·0605 (C_6H_9O) 112·0882 ($C_7H_{12}O$), 111·0806 ($C_7H_{11}O$), 96·0578 (100% C_6H_8O).

*Dihydrocucurbitacin Q*1. A solution of cucurbitacin Q1 (30 mg) in MeOH (4 ml) was hydrogenated using 10% Pd–C as catalyst until 1 ml H_2 was absorbed. The catalyst was filtered off and the solution evaporated to dryness to afford a homogeneous amorphous solid (31 mg). IR (KBr): 1720 cm^{-1}, 1695 cm^{-1}; UV (MeOH): transparent; NMR (DMSO-d_6): τ4·25 (1H, *m*, C-6 proton), no C_{23}–C_{24} olefinic protons, τ2·0 (3H, *s*, –OCOMe); MS, m/e = 502 (M^+—60), 486, 405, 113.

*Cucurbitacin Q*1 *acetonide*. Cucurbitacin Q1 (20 mg) was dissolved in anhydrous acetone (2 ml) and *p*-toluenesulphonic acid (2 mg) was added. The solution was stirred for 24 hr with Na_2SO_4 (50 mg). The solution was filtered through a short silica gel column and evaporated to a colourless gum. The pure monoacetonide was obtained by preparative TLC (EtOAc) as a colourless homogeneous amorphous solid (12 mg), IR (KBr): 1715 cm^{-1}, 1692 cm^{-1}; UV (MeOH): λ_{max} 230 n.m. (ϵ 10 800); MS: m/e = 540 (M^+ —60), 482, 369, 279, 237, 183, 149, 123, 113, 112, 111, 97, 96 (100%).

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