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# STUDIES IN THE CHEMICAL CONSTITUENTS OF THE FRUITS OF CUCUMIS PROPHETARUM LINN

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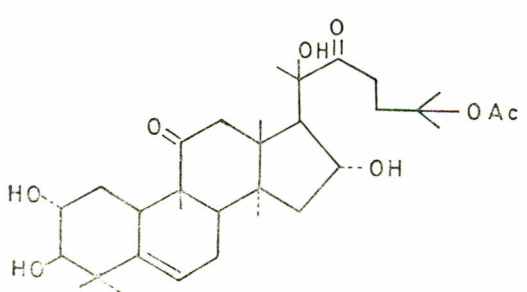
**Abstract.** The isolation and characterisation of a new sterol, m.p. 146°C, and a new cucurbitacin, m.p. 240°C, provisionally named as propheterosterol and cucurbitacin Q1 respectively, have been reported in this communication from fresh and undried fruits of *Cucumis prophetarum*. A quantitative estimation of its amino acids have also been studied.

*Cucumis prophetarum* Linn (vernacular choti indrayan or khar indrayan), belongs to the family Cucurbitaceae. It is perennial mostly trailing 60–20 cm long herb, its fruits are taberculated, ellipsoidal, green with paler vertical stripes, covered with spines, and are medicinally used as emetic and purgative agents.[1] The plant grows wild all over Pakistan, Arabia, Rajputana and Tropical Africa.[2]

Very little work has been reported on this plant; it is known to contain cucurbitacins B and D and traces of cucurbitacins G and H.[3,4] Recently Gopala and Ramchandra[5] reported the isolation of cucurbitacin C from its fruits.

In view of the pharmacological importance which is attached to the constituents of members of the family Cucurbitaceae and cytotoxic properties of cucurbitacins it was considered of interest to carry out a chemical reinvestigation of the fruits of *Cucumis prophetarum* Linn which is abundantly available in the suburbs of Karachi. As a result of studies on the fresh and undried fruits of *Cucumis prophetarum* Linn, a new crystalline cucurbitacin (m.p. 240°C) and a new sterol (m. p. 146°C) provisionally named as cucurbitacin Q1 and propheterosterol respectively were obtained in the pure form. The structure of cucurbitacin Q1 was assigned on the basis of chemical and spectroscopic investigations.[6] It was found to be *trans* component of cucurbitacin Q.




Cucurbitacin Q 1.

The propheterosterol was obtained from the mother-liquor of cucurbitacin Q1 through column chromatography. It recrystallised from methanol in the form of fluffy needles, m.p. 146°C, and analysed for $C_{28}H_{46}O_2$. Its mass spectrum showed the molecular ion peak at *m/e*/414. Its IR spectrum showed the presence of—OH group at 3580 $cm^{-1}$ and UV absorption spectrum indicated maxima at $\lambda_{max}$ 215 nm and shoulders at $\lambda_{max}$ 245 and $\lambda_{max}$ 254 nm. It formed a monoacetyl derivative in the form of white needle-like crystals, m.p. 158–160°C when treated with acetic anhydride and pyridine. The mass spectrum of the acetyl derivative showed $M^+$ peak at *m/e* 456 conforming to the preparation of a monoacetyl derivative. The IR spectrum showed a peak at 1725 $cm^{-1}$ characteristic of acetyl carbonyl group and the UV absorption maximum was shifted to $\lambda_{max}$ 208 nm.

Furthermore, a study of the sugars and amino acids were carried out through paper chromatography. Glucose/galactose and arabinose were found to present. Qualitative and quantitative estimations of amino acids were carried out and the results are shown in Table 1.

## Experimental

The fresh fruits of *Cucumis prophetarum* (5.4 kg) were cut into small pieces and percolated thrice with ethanol (9 litres) at room temperature for 48 hr. The extracts were combined and concentrated in a cyclone evaporator under reduced pressure. The dark green residue was partitioned repeatedly between water and ethyl acetate until no insoluble material was left.

TABLE 1. AMINO ACIDS IN THE FRUITS.

| Amino acids | *Rf* glycine of standard amino acids | *Rf* glycine sample amino acids | Amino acids in mole/g of fruit |
|---|---|---|---|
| Leucine/isoleucine | 3.152 | 3.149 | 0.707 |
| Phenylalanine | 2.650 | 2.528 | 0.238 |
| Valine | 2.464 | 2.360 | 0.587 |
| Tryptophane | 2.148 | 2.144 | 0.753 |
| Tyrosine | 2.048 | 1.940 | 0.524 |
| Proline | 1.601 | 1.584 | — |
| Alanine | 1.346 | 1.328 | 0.138 |
| Threonine | 1.224 | 1.150 | 0.576 |
| Glycine | 1.000 | 1.000 | 0.393 |
| Argenine | 0.880 | 0.880 | 0.641 |
| Aspartic acid | 0.770 | 0.744 | 0.171 |
| Cystine | 0.460 | 0.504 | 0.181 |

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The ethyl acetate layer was washed with water to remove any inorganic material, dried ($Na_2SO_4$) filtered and evaporated under reduced pressure. The greenish residue thus obtained was repeatedly extracted with ether. The ether-soluble fractions were combined, concentrated and kept in the cold when crystals were deposited. On recrystallization from methanol colourless prismatic rods of *Cucurbitacin Q*1 were obtained, m.p. 240°C, $[\alpha]_D^{23} = +50$ (1% methanol). The substance afforded a single spot on TLC *Rf* 0.7. (Found: C, 68.3; H, 8.4%. $C_{32}H_{48}O_8$ requires: C, 68.6; H, 8.2%.)

The mother liquor of cucurbitacin Ql was freed of the organic solvent and combined with ether-insoluble residue. Ten grams of the total residues thus obtained was repeatedly extracted out with benzene, leaving behind 2 g benzene-insoluble residue which was not worked up presently. The benzene-soluble fraction was passed through a 36-in long and 2 in wide column packed with 600 g neutral alumina (grade I) and elution was made with benzene followed by gradual increase in polarity through chloroform. About 100 fractions, 10 ml each, were collected. All these fractions failed to yield any crystallisate. Elution was then carried out with chloroform, the polarity of which was gradually increased with acetone. The fractions collected from acetone and chloroform (20:100) were taken together and freed of the solvent in vacuum. The dark green residue obtained was taken up in methanol and kept in the cold. It deposited crystals which on recrystallization from the same solvent gave white fluffy needle crystals of propheterosterol melting at 146°C and gave a single spot when subjected to TLC and amounted to ca. 0.29 (yield 0.03% calculated on the weight of the dry fruits). It gave positive Liebermann-Buckard test (green colouration) and analysed for $C_{27}H_{42}O_3$. Found: C, 78.77; H, 10.46%. Calcd. for C, 78.26; H, 10.16%), mol. wt. 414 (mass spectrum). The UV absorption spectrum of this compound in absolute alcohol indicated maxima at 215 nm and shoulders at 245 and 254 nm.

*Monoacetyl Propheterosterol.* Propheterosterol (20 mg) was dissolved in warm acetic anhydride (0.5 ml) and a drop of pyridine and allowed to stand overnight at room temperature. Water was added to the reaction mixture and the white crystalline acetyl derivative thus obtained filtered, washed with water and dried over a porous plate (wt 20 mg), m.p.158-60°C. Its IR spectrum showed a peak at 1725cm$^{-1}$ corresponding to acetyl carbonyl group and UV absorption maxima was shifted to 208 nm. The molecular ion peak in the mass spectrum at *m/e* 456 confirmed the preparation of the monoacetyl derivative of propheterosterol.

*Examination of the Water-Soluble Fraction.* The water-soluble fraction was freed of the solvent *in vacuo* and the brownish residue thus obtained was taken up in methanol, concentrated and kept in the cold when it deposited inorganic needle-like crystals which were found to be potassium chloride. It was filtered out and the filtrate was freed of the solvent *in vacuo*. About 0.5 g from this residue was dissolved in 3 ml water and the resulting solution was loaded in a column packed with Amberlite IR-120 ($H^+$) activated resin. The elution of carbohydrate was effected with distilled water and 28 fractions of 10 ml each were collected and tested with phenol-$H_2SO_4$ reagent. Fractions 4-15 were found to contain the maximum amount of carbohyrates. All these fractions were collected together and concentrated *in vacuo* and then subjected to paper chromatography.

Descending paper chromatography was carried out on Whatman filter paper No. 1 with standard sugar in n-butanol-ethanol-water (4:1:6) solvent system for 48 hr. The chromatogram after drying in the air was developed by spraying with aniline phthalate and dried in an oven at 80°C. It showed the presence of two spots corresponding to arabinose and glucose/galactose respectively.

After the separation of the carbohydrates the elution of amino acids was achieved by passing 3 and 5% ammonium hydroxide down the resin column. 150 fractions of 10 ml each were collected and tested for amino acids. It was found that 119 fractions gave positive test for amino acids. These fractions were taken together and concentrated *in vacuo* and studied through paper chromatography.

A number of solvent systems were tried for the separation of amino acids, n-butanol—acetic acid-water (4:1:5) was used in the present work as it gave the best separation.

Whatman filter paper No. 1 spotted with the sample and standard amino acids and was eluted with the solvent by the descending method for about 22 hr. The chromatogram was air-dried, sprayed with cadmium-ninhydrin solution, prepared by dissolving 50 ml acetone and subsequent addition of 6 ml cadmium acetate (5 g cadmium acetate was dissolved in 100 ml distilled water). The chromatogram was developed by keeping it in an oven at 80°C for 10 min when it revealed the presence of 12 amino acids in the sample. The *Rf* values of these were compared with the standards and are shown in Table 2.

TABLE 2. *Rf* OF THE AMINO ACIDS.

| Amino acids | *Rf* glycine of stdanrd amino acids | *Rf* glycine of sample amino acids |
|---|---|---|
| Leucine/isoleucine | 3.152 | 3.149 |
| Phenylalanine | 2.65 | 2.528 |
| Valine | 2.464 | 2.360 |
| Tryptophane | 2.148 | 2.144 |
| Tyrosine | 2.048 | 1.94 |
| Proline | 1.601 | 1.584 |
| Alanine | 1.346 | 1.328 |
| Threonine | 1.224 | 1.15 |
| Glycine | 1.00 | 1.00 |
| Argenine | 0.88 | 0.88 |
| Aspartic acid | 0.77 | 0.744 |
| Cystine | 0.46 | 0.504 |

TABLE 3. OPTICAL DENSITIES OF THE AMINO ACIDS.

| Amino acids | O.D. at 500 nm (sample 5 litre) | O.D. standard (units/mole) | mmole 5 litre | mole/g |
|---|---|---|---|---|
| Leucine/iso-leucine | 0.270 | 5.08 | 31.86 | 0.707 |
| Phenylalanine | 0.075 | 4.19 | 10.74 | 0.238 |
| Valine | 0.252 | 5.84 | 26.40 | 0.587 |
| Tyrptophane | 0.155 | 2.74 | 33.90 | 0.753 |
| Tyrosine | 0.160 | 4.07 | 23.58 | 0.524 |
| Proline* | — | — | — | — |
| Alanine | 0.060 | 5.74 | 6.24 | 0.138 |
| Threonine | 0.240 | 5.60 | 25.58 | 0.576 |
| Glycine | 0.099 | 3.35 | 17.70 | 0.393 |
| Argenine | 0.235 | 4.88 | 28.86 | 0.641 |
| Aspartic acid | 0.058 | 3.78 | 8.70 | 0.171 |
| Cystine | 0.082 | 5.99 | 8.16 | 0.181 |

* Proline could not be estimated as the colour fades away.

*Quantitative Estimation of Amino Acids.* The sample (0.005 ml) was loaded on the paper and was run in the solvent system as described earlier. The chromatogram was left in a tank containing $H_2SO_4$ for 6 hr for complete development of colour, after being sprayed with cadmium–ninhydrin reagent. The observed spots were cut into small pieces and their colour was taken in 5 ml methanol by keeping them for about 1 hr with occasional shaking. The optical densities of these solution were measured at 375 nm. The quantities of different amino acids were calculated from the calibration curve of the optical densities of standard amino acids (Table 3).

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