

# THE FUNCTIONAL GROUPINGS OF CUCURBITACIN E ( $\alpha$ -ELATERIN)

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In addition to the widespread use of cucurbits for their food value there remain many suggestions that members of the family *Cucurbitaceae* are useful for the alleviation of a variety of complaints. Thus claims have been made<sup>1</sup>, and refuted<sup>2</sup>, that several species viz. *Bryonia cretica*, *Cephalandra indica* and *Momordica charantia* exert a hypoglycaemic action in diabetic patients. Extracts of *Bryony*, *Colocynth* and *Elaterium* have been shown to possess a tumour necrosing action<sup>3</sup> whilst extracts of *Cucumis melo*<sup>4</sup> and *C. sativus*<sup>5</sup> have been claimed to inhibit the growth of fungi.

Findings of this sort have not as yet led to important applications and it is only of the purgative action of certain members of this family that any medicinal use is still made. Such use is ancient and mention of *Citrullus colocynthis* is to be found in an Anglo-Saxon herbal<sup>6</sup> (useful "for stirring of the inwards"). The same drug is likewise to be found in the Papyrus Ebers<sup>7</sup> and is believed by some authorities<sup>8</sup> to be the fruit of Elisha's miracle<sup>9</sup>. Interest in biological activity of this sort however is waning for obvious reasons and revival of interest in certain constituents of this family centres on the first-mentioned use of the cucurbit as an article of diet. In

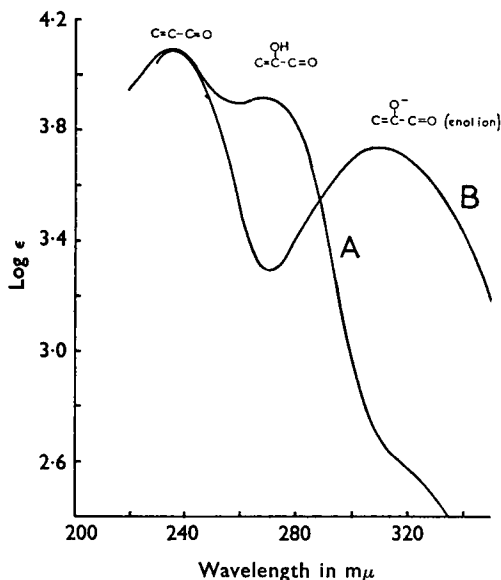


FIG. 1. Ultra-violet absorption spectrum of A, cucurbitacin E in ethanol; B, cucurbitacin E in 0.1M  $\text{Na}_2\text{CO}_3$ .

Europe, Africa and elsewhere, the occurrence of bitterness in cultivated cucurbits presents a problem not only from the economic viewpoint but also as something of a hazard to human health<sup>10</sup>. Systematic search for the bitter principles involved has led to the isolation of eleven toxic substances provisionally called the Cucurbitacins and numbered alphabetically<sup>11</sup>; these represent a new class of triterpenes characterised by their

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unusually high oxygen content. Cucurbitacin E ( $\alpha$ -elaterin) is one member of the above list which constitutes approximately 15 per cent by weight of the crude drug elaterium. It is normally present as a glycoside in many cucurbitaceous fruits but in the case of *Ecballium elaterium* (squirting cucumber) for example, is rapidly hydrolyzed to the genin by the enzyme elaterase<sup>12</sup>. Not all species contain this enzyme—*Citrullus* being one such—and it is of interest to note that this is the probable reason for the failure of later workers<sup>13</sup> to repeat the original isolation by Power and Moore<sup>14</sup> of  $\alpha$ -elaterin from *Citrullus colocynthis*.

Structural studies on Cucurbitacin E ( $\alpha$ -elaterin) have been carried out in these laboratories and full details will be published elsewhere. The following summarises the position with regard to the functional groupings of this substance.

Accurate determination of the molecular weight<sup>15</sup> has allowed the assignment of a revised molecular formula  $C_{32}H_{44}O_8$ . Analysis indicates the presence of one *O*-acetyl group and in the light of what follows, the corollary may be added that Cucurbitacin E is probably pentacyclic. Since this substance forms but few, if any, normal derivatives of carbonyl function and hydroxyl group, evidence for the presence of such is mainly spectroscopic. Figure 1 shows the ultra-violet absorption spectrum of Cucurbitacin E and the assignment of the bands. Thus Cucurbitacin E gives a positive ferric chloride reaction and with the benzene diazonium ion, a red colour. It has an apparent pKa value in 50 per cent ethanol of 10.8. With acetic anhydride-sodium acetate two acetyl groups may be inserted in the molecule; one a normal *O*-acetyl group, the other an enol acetate of the above 1:2-diketone.

The resulting Cucurbitacin E acetate has lost the band characteristic of the 1:2-diketone and retains that due to the enone system with the intensity approximately doubled. (Fig. 2).

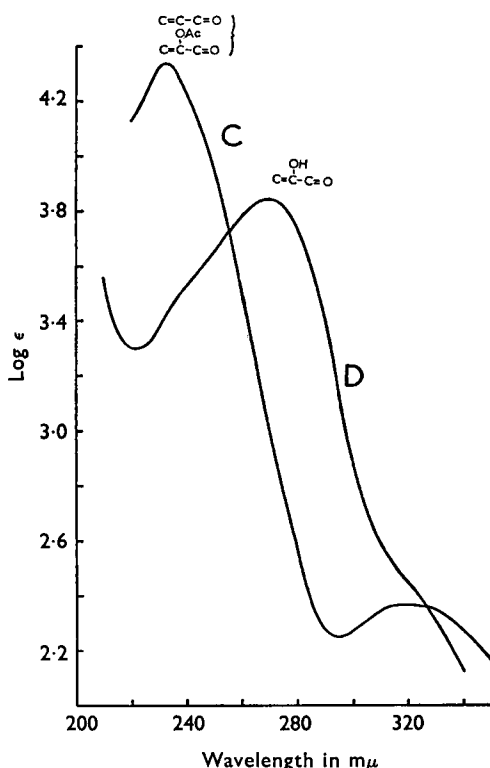


FIG. 2. Ultra-violet absorption spectrum in ethanol of C, cucurbitacin E acetate; D, dihydrocucurbitacin E\*.

\*In calculating values of  $\epsilon$ , a molecular weight of 559 has been assumed.

On the other hand, a "dihydrocucurbitacin E" shows no enone band and only that characteristic of the 1:2-diketone.

Confirmation of these oxygen functions is to be found in the infra-red spectrum. (Fig. 3).

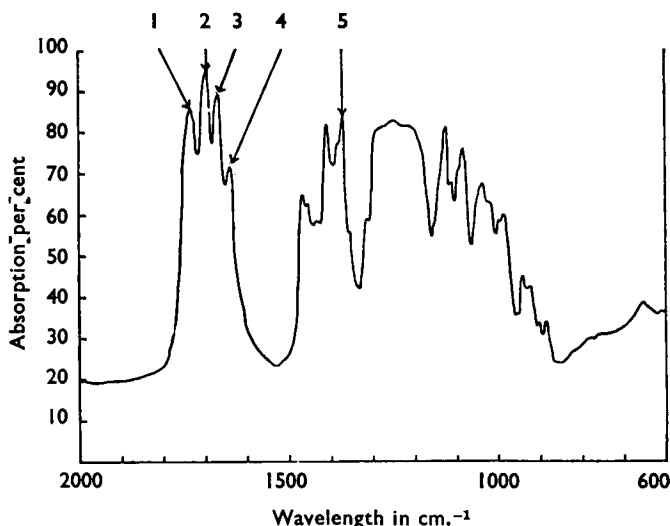
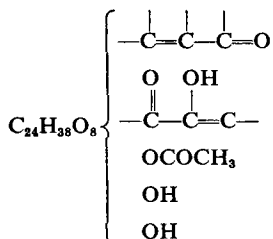


FIG. 3. Infra-red spectrum of cucurbitacin E. Solvent, chloroform. 1, Acetyl carbonyl; 2,  $2 \times$  carbonyl; 3, enolic 1:2-diketone; 4, double bond  $\alpha\beta$  to carbonyl; 5, acetoxy group.

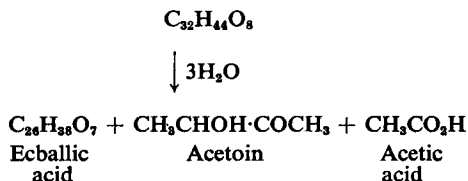
Of the hydroxyl groups present in Cucurbitacin E, one is already acetylated; a second, as noted above, may be so esterified whilst a third fails to react in this wise. The third "inert" hydroxyl is most easily shown by a band in the infra-red region at  $3460\text{ cm.}^{-1}$  and characteristic of associated hydroxyl groups. Zerewitinoff determination of active hydrogen indicates three hydroxyl groups in Cucurbitacin E one of these is the enolic hydroxyl from the 1:2-diketone. Thus far seven of the eight oxygen atoms may be assigned their function.



When Cucurbitacin E is treated with cold sodium hydroxide several changes take place in the molecule. Firstly the acetyl group is split off and secondly the  $\alpha\beta$ -unsaturated ketone undergoes what is probably a reversed aldol shift (Rearrangement A). The intermediate product which results (elateridine) is as yet inadequately characterised but on further

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treatment with caustic alkali at 100°, yields the crystalline ecballic acid in which a benzilic acid change of the 1 : 2-diketone has further taken place (Rearrangement B). Acetoin has recently been shown to be a product of alkaline isomerization and the overall changes resolve themselves thus<sup>15</sup>



Ecballic acid yields iodoform on treatment with iodine and sodium hydroxide and the presence of a methyl ketone is thus indicated. It arises as a result of rearrangement A. The formation of a bis-2 : 4-dinitrophenyl hydrazone indicates a second carbonyl group which we believe is also present in the parent Cucurbitacin E: it thus accounts for the eighth oxygen. Rearrangement B (the benzilic acid change) is the source of the carboxylic acid group of ecballic acid.

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### DISCUSSION

The short communication was presented by DR. D. W. MATHIESON.

THE CHAIRMAN. How had the Authors prepared the Cucurbitacin E? Did any other curcubitacin correspond with  $\beta$ -elaterin? What was the difference in the structure of  $\alpha$  and  $\beta$ -elaterin?

DR. J. B. STENLAKE (Glasgow). An infra-red frequency a little higher than normal had been quoted for a hydroxyl group, might this be a tertiary hydroxyl which would account for its being inert?

DR. SZINAI (Israel). The 1690 line was stated to be due to two carbonyl groups. Which carbonyl group could give rise to this absorption? As one dehydrogenation product of  $\alpha$ -elaterin was 1 : 2 : 8-trimethyl-phenanthrene, was not a tetracyclic triterpene more likely the basic structure?

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MR. S. G. E. STEVENS (London). Had the Authors truly compensated for the solvent? Had they tried carbon disulphide which would give fewer bands in the region investigated?

DR. MATHIESON replied. Crude elaterium was the sediment from the expressed juice of the unripe cucumber. After percolation with light petroleum to remove chlorophyll and then with chloroform,  $\alpha$ -elaterin was obtained by crystallisation from a crude crystalline principle which appeared in the chloroform extract. There were five or six closely related cucurbitacins.  $\alpha$  and  $\beta$ -Elaterin were similar and were probably triterpenoids with seven or eight oxygen atoms. The band at  $3460\text{ cm.}^{-1}$  was probably the tertiary hydroxyl but the presence of eight oxygen atoms might affect the assignments of the bands. When examined using a grating system, the  $1690$  band was seen to consist of two discreet bands. Two carbonyls therefore became more likely. Pentacyclic triterpenes normally gave both phenanthrenes and naphthalenes on dehydrogenation.  $\alpha$ -Elaterin was insoluble in carbon disulphide. As a double beam instrument was used, solvent correction was automatically applied.