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CUCURBITACIN E: SOME PRELIMINARY OBSERVATIONS

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Abstract—Evidence presented indicates that the functional groupings of cucurbitacin E (α-elaterin) are as follows: an αβ-unsaturated ketone, a 1: 2-diketone (enolised), three hydroxyl groups, one of which is acetylated, and probably a further carbonyl group.

α-ELATERIN is a crystalline compound most readily obtained from the fruit juice of squirting cucumber (*Ecballium elaterium*, Rich.). In addition it may be isolated as a glucoside from *Citrullus colocynthis* Schrad. First isolated in 1831 by Morries[1] and also by Hennel,[2] the compound and details of its structure have claimed the attention of a number of investigators.[3–15] More recently α-elaterin has emerged as one member of a group of related compounds provisionally called the cucurbitacins and peculiar to the family Cucurbitaceae.[16–24] Some eleven members (A to L) of this group have been described to date and α-elaterin appears in this list as cucurbitacin E. In what follows preliminary observations on the functional groupings of this substance are presented.

Recent determination of its molecular weight by means of X-ray unit cell and density measurements has allowed the assignment of a revised and probably definitive molecular formula, which suggests that these substances are triterpenoids[24] and the suggested formula $C_{32}H_{44}O_8$ is used in what follows. Elementary analysis agrees with this formula and in addition cucurbitacin E contains one acetoxy group. (From the evidence on functional groupings presented below, it follows as a corollary that cucurbitacin E is pentacyclic.)

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In the ultra-violet region of the spectrum, cucurbitacin E shews maximum absorption in ethanol at 234 mμ (ϵ 12,500) and 268 mμ (ϵ 8,300). In agreement with previous authors,[19,25] the former band is assigned to an $\alpha\beta$-unsaturated ketone, the latter to an enolised 1:2-diketone. Thus if cucurbitacin E is hydrogenated at a palladium catalyst under conditions under which the double bond of an enone is known to be reduced, the absorption of hydrogen may be stopped at 1 mole. Although the product undergoes secondary changes (viz., loss of acetyl under the action of alkali) and is as yet inadequately characterised, it has lost the former absorption peak at 234 mμ, leaving only that characteristic of the 1:2-diketone. Again, if the spectrum of cucurbitacin E is determined in sodium carbonate solution, the second peak at 268 mμ undergoes a reversible bathochromic shift to 311 mμ (ϵ 5,500) owing to the formation of the enol ion from this 1:2-diketone.[25,26,30] With ferric chloride cucurbitacin E gives a reddish brown coloration, whilst with the benzenediazonium ion a red colour results. The apparent pK_a value for cucurbitacin E, determined spectrophotometrically in 50 per cent ethanol, is 10·8 and the presence of an enol is thus confirmed.

When treated with sodium acetate–acetic anhydride, cucurbitacin E forms an acetyl derivative, which was first described by Hemmelmayr.[8] Two acetyl groups are thus inserted in the molecule. One of these is a normal O-acetyl group, the second forms an enol acetate of the above 1:2-diketone and the acetylcucurbitacin E thus produced shews a high-intensity peak at 233 mμ (ϵ 18,800), the high ϵ value being a summation of the $\alpha\beta$-unsaturated ketone already present and the new $\alpha\beta$-unsaturated carbonyl thus produced by formation of the enol acetate.[30] Unlike cucurbitacin E, the acetate gives no red colour with the benzenediazonium ion. Since, moreover, acetylation produces but a single compound, it would appear that only one of the C atoms adjacent to the 1:2-diketone carries a hydrogen atom. It has been claimed by Lavie[25] that cucurbitacin E forms a quinoxaline: whilst we can confirm that some reaction with *o*-phenylenediamine does occur, the product has so far resisted purification in our hands and no details or physical constants are given by the above author. The amorphous product, however, does shew absorption bands at 238 and 320 mμ characteristic of 2:3-disubstituted quinoxalines[27] and this pattern is not a simple summation of absorption due to cucurbitacin E and *o*-phenylenediamine.

From the infra-red spectrum of cucurbitacin E, bands at 1690 and 1630 cm^{-1} are characteristic, respectively, of the carbonyl group and double bond of the $\alpha\beta$-unsaturated ketone.[28] Bands at 1725 and 1370 cm^{-1} are assigned to the carbonyl and methyl, respectively, of the acetoxy group.[29] A further band in the carbonyl region (1660 cm^{-1}) is not as yet assigned with certainty although probably connected with the 1:2 diketone. It is present in cucurbitacin E and "elateridine" (see below), it is absent both from acetylcucurbitacin E and its glucoside, in which the sugar is likewise attached to the enolic OH.[21] The absence of intense absorption between 1640 and 1530 cm^{-1}, and characteristic of 1:3-diketones,[31] serves to confirm the presence of a 1:2- rather than a 1:3-diketo system. With acetylcucurbitacin E

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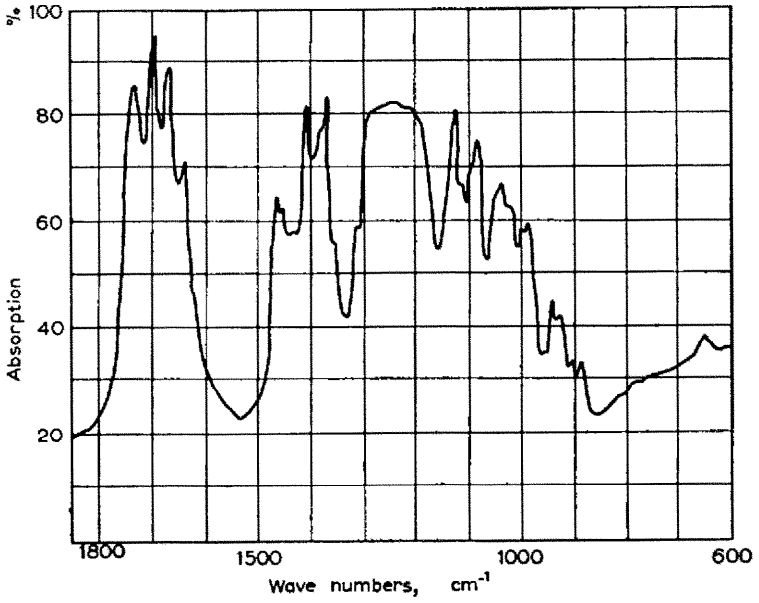


FIG. 1. Infra-red spectrum of cucurbitacin E (2% in chloroform).

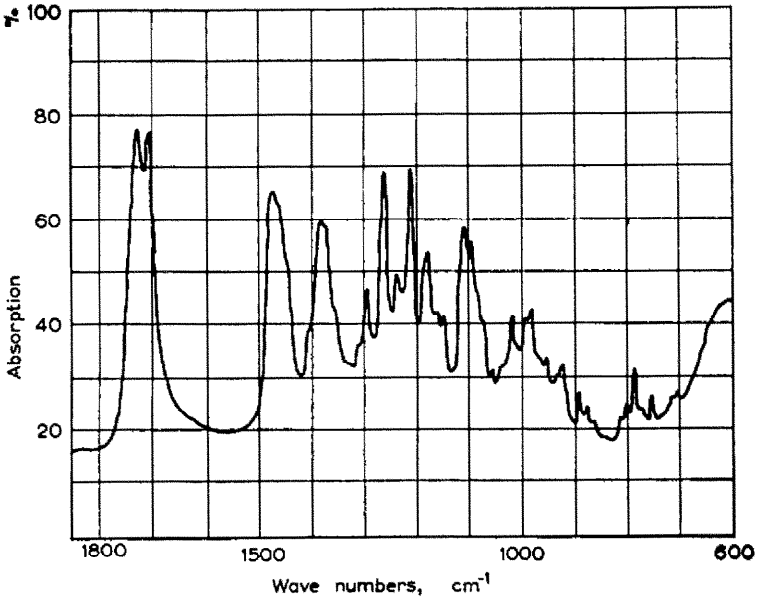


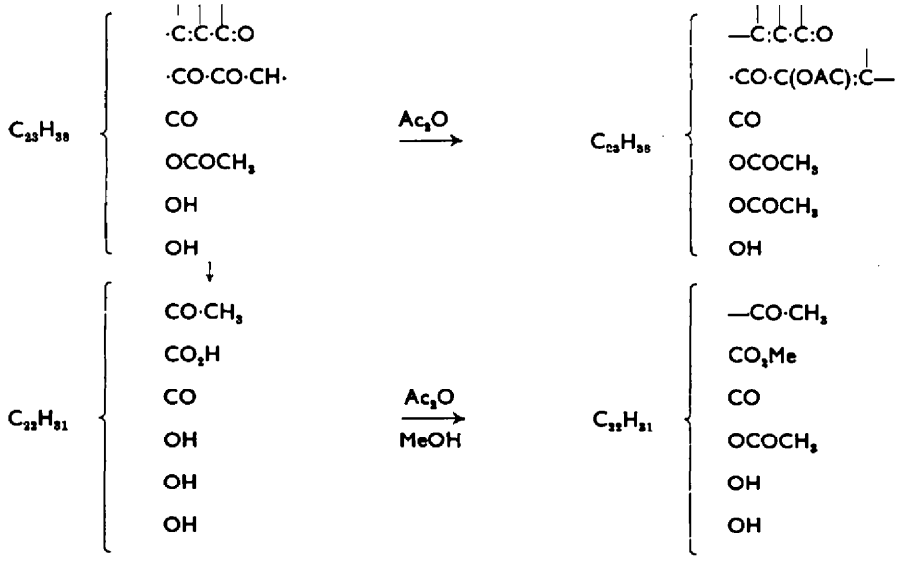
FIG. 2. Infra-red spectrum of methyl ecballate (Nujol mull).

bands at 1770 and 1204 cm^{-1} refer to the enol acetate, whilst those at 1740 and 1240 cm^{-1} are assigned to the two ordinary acetate esters also present. A band at 1370 cm^{-1} due to the C-methyl of these acetate groupings is also observed. An intense band at 1702 cm^{-1} is probably due partly to the carbonyl of the $\alpha\beta$-unsaturated ketones (double bond appears at 1630 cm^{-1}) and partly to a saturated ketone which we believe to be present in addition (see below under ecballic acid).

As stated above, cucurbitacin E contains one hydroxyl group already acetylated. In addition, absorption bands at 3620 and 3546 cm^{-1} and 3468 cm^{-1} correspond, respectively, to free and associated hydroxyl groups. After acetylation, the first two bands disappear and in the acetate there remains but a single peak in this region at 3460 cm^{-1} due to associated hydroxyl.

Active-hydrogen determinations by the method of Zerewitinoff prove difficult in the usual solvents owing to insolubility. Recalculation of previously published determinations[15] carried out in purified quinoline indicates the presence of three active hydrogens in cucurbitacin E and therefore two OH groups in addition to the enolic hydroxyl. We have confirmed this, moreover, by repeating the analysis in N-methyl-1:2:3:4-tetrahydroquinoline as solvent.[32]

Such high concentration of oxygen functions made the action of periodic acid and lead tetra-acetate of obvious interest. When titration of cucurbitacin E with these glycol reagents was carried out, there took place an initial fast consumption of 1 molar equivalent of reagent owing to splitting of the 1:2-diketone. This was followed by a slow continuing uptake. As to acetylcucurbitacin E, only this latter type of slow reaction occurred when periodic acid was used. Lead tetra-acetate had no effect over 3 days. Vicinal hydroxyl functions held in sterically hindered positions are known to shew inactivity towards such reagents,[33,35] and in this connexion it should be noted



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that one of the hydroxyl groups in cucurbitacin E is inert to acetylation under the usual conditions. Cucurbitacin E does not reduce bismuth trioxide and the absence of the simple grouping —CO—CHOH— may be assumed.

Thus far seven of the eight oxygen atoms may be accounted for.

Action of sodium hydroxide on cucurbitacin E: ecballic acid

When cucurbitacin E is treated with sodium hydroxide at 100°, several changes occur in the molecule and the final product is ecballic acid.[15] With the recent finding that acetoin is split from cucurbitacin E under such conditions[24] the overall changes may be represented thus:

$$C_{32}H_{44}O_8 + 3H_2O \rightarrow C_{26}H_{38}O_7 + CH_3COCHOHCH_3 + CH_3COOH$$

Ecballic acid is a monocarboxylic acid. The approximate pK_a is 4·3 and it contains two ketone groups as shewn by the formation of a bis-2:4-dinitrophenylhydrazone. The isolation of iodoform after oxidation with iodine–sodium hydroxide indicates that one of the carbonyl groups is present as a methyl ketone. Ultra-violet absorption bands characteristic of the $\alpha\beta$-unsaturated ketone and the 1:2-diketone have both disappeared and there remains but low-intensity absorption at 285 mμ (ϵ 112), confirming the presence of the above-mentioned saturated carbonyls.

The second carbonyl group is probably also present in the parent compound, cucurbitacin E, and as such would account for the remaining oxygen function in that molecule.

Methyl ecballate shews bands at 1712 and 1210 cm^{-1} characteristic of the methyl ester.

Thus rearrangement of the two main chromophoric systems of cucurbitacin E has occurred, the $\alpha\beta$-unsaturated ketone having undergone a retroaldol rearrangement, giving rise presumably to acetoin and ecballic acid with its methyl ketone residue. The 1:2-diketone has suffered a benzilic acid type of rearrangement and is the source of the carboxylic acid group. Methyl ecballate forms a monoacetate, which possesses bands at 3612, 3564 cm^{-1} and 3465 cm^{-1} characteristic of free and associated hydroxyl, respectively.

Elateridine

When cucurbitacin E is treated with sodium hydroxide at 20°, an amorphous product results—the so called "elateridine" of Berg.[4] This ill defined intermediate has lost the acetoxy group of the original substance and no longer possesses an absorption band at 234 mμ. Under these conditions only retroaldol rearrangement of the enone system can be envisaged, leaving the 1:2-dione intact. Elateridine thus gives a positive ferric chloride reaction and a red colour with the benzenediazonium ion: it also contains the methyl ketone characteristic of ecballic acid. Infra-red bands at 1695 and 1660 cm^{-1} indicate the enolic form of the 1:2-dione ($\alpha\beta$-unsaturated ketone): the acetate band at 1725 cm^{-1} has disappeared.

When elateridine is treated with sodium hydroxide at 100°, ecballic acid may be obtained.

EXPERIMENTAL

Isolation of cucurbitacin E. Commercial elaterium (180 g) was extracted with light petroleum (boiling range 40–60°) until no further colouring matter was removed.

The marc was then percolated to exhaustion with chloroform. Evaporation of the extract to about 50 ml yielded a crystalline crop, which was filtered off and washed with cold acetone to yield a colourless microcrystalline material (19 g). Several crystallisations from chloroform–methanol yielded pure cucurbitacin E, m.p. 234°, $[\alpha]_D^{20}$ — 64·3° (lit.[14] cites m.p. 234°, $[\alpha]_D$ — 52·9°) (*c*, 1·64 in chloroform) (Found: C, 68·9, 68·6, 68·7; H, 8·2, 8·0, 7·9; 0, 23·9; OAc, 8·02; acetic acid equivalent to three *C*-methyl groups (Kuhn–Roth). Calc. for $C_{32}H_{44}O_8$; C, 69·04; H, 8·0; O, 23·0; OAc, 7·7 per cent). Zerewitinoff determination: cucurbitacin E (22 mg) in N-methyl-1:2:3:4-tetrahydroquinoline was treated with methylmagnesium iodide in di*iso*amyl ether to yield 2·54 ml. of methane (S.T.P.); required for three OH groups, 2·65 ml of methane (S.T.P.).

Light absorption (*a*) in ethanol: 234 mμ (ϵ 12,500), 268 mμ (ϵ 8,300); (*b*) in 0·1 M sodium carbonate: 234 mμ (ϵ 12,500), 311 mμ (ϵ 5,500); (*c*) in 0·1 N sodium hydroxide after 30 hr: 270 mμ (ϵ 3,600), 315 mμ (ϵ 5,700). Infra-red peaks (chloroform solution) at 3620, 3546, 3468, 1725, (330),* 1690 (650), 1660 (390), 1630 (190), 1370 cm^{-1}.

Determination of pK_a *value.* By using standard buffers of known pH prepared in 50% ethanol, the optical density was determined at various wavelengths and the pK_a value was calculated in the usual fashion. The results are shown in Table 1.

TABLE 1. DETERMINATION OF pK_a VALUE

Wavelength (mμ)	$E_{1cm}^{1\%}$ at pH				Calculated pK_a	
	2·3	9·7 (*a*)	10·8 (*b*)	11·5	(*a*)	(*b*)
268	142·3	132	87	40·3	10·65	10·73
280	109·5	101·6	74·3	46·5	10·54	10·7
300	18·3	21·7	49·3	79·1	10·93	10·78
310	9·5	14·5	51·5	91·5	10·89	10·78
320	7·5	12·5	48·7	87·4	10·88	10·77
Mean pK_a value, 10·76						

If $E_1 = E_{1cm}^{1\%}$ of the pure enol (pH 2·3) and $E_2 = E_{1cm}^{1\%}$ of the pure enol ion (pH 11·5) at a given wavelength, then, at any given pH, where E is the observed $E_{1cm}^{1\%}$ at that same wavelength:

$$pK_a = pH - \log_{10} \frac{(E_1 - E)}{(E - E_2)}$$

Isosbestic point $E_{1cm}^{1\%}$ at 289 mμ, 59·5.

Acetylcucurbitacin E. Cucurbitacin E (1 g), fused sodium acetate (1 g) and acetic anhydride (10 ml) were heated together for 1 hr at 100°. After the mixture had been poured into cold sodium carbonate solution, extraction with ether gave a crude product (1·07 g), which crystallised from dry ether in colourless needles, m.p. 121°

* Figures in brackets are intensities as peak apparent molar extinction coefficients. They have not been corrected for finite slit width.

(dec.) (lit.[8] cites m.p. 124°), $[\alpha]_D^{22°}$ −68·6° (*c*, 1·94 in chloroform) (Found: C, 67·53, 67·72; H, 7·19, 7·53; OAc, 23·3. Calc. for $C_{36}H_{48}O_{10}$; C, 67·48; H, 7·55; OAc, 20·13 per cent). Zerewitinoff determination: acetylcucurbitacin E (60 mg) in N-methyl-1:2:3:4-tetrahydroquinoline was treated with methylmagnesium iodide in di*iso*amyl ether to yield after 1 hr 1·14 ml of methane (S.T.P.); required for one OH group, 2·10 ml of methane (S.T.P.).

Light absorption in ethanol; 233 mμ (ε 18,800) 320 mμ (ε 210). Infra-red peaks (carbon disulphide) 1770, 1740, 1702, 1240, 1204: (chloroform) 3460, 1726 (970), 1697 (940), 1630 (280). Attempts to prepare cucurbitacin E benzoate failed to produce any crystalline material.

Periodic acid oxidation. This was carried out in aqueous methanol with a 0·5 M solution of periodic acid. At various time intervals samples were withdrawn and analysed by the method of Jackson.[36] Simultaneous control experiments omitting only the substance under investigation, were also run. The results are shown in Table 2.

TABLE 2. PERIODIC ACID OXIDATIONS

Substance	No. of moles of HIO_4 used per mole substance				
	6 hr	20 hr	48 hr	72 hr	100 hr
Cucurbitacin E	1·2	1·7	1·8	—	1·96
Acetylcucurbitacin E	0·2	0·4	0·6	0·85	0·96

Lead tetra-acetate oxidations. This was carried out in glacial acetic acid. Unused reagent was estimated by the method outlined by Hockett and McClenahan.[37] The results are shown in Table 3.

Attempted preparation of an oxime or 2:4-dinitrophenylhydrazone of cucurbitacin E led to no crystalline materials. On warming with Brady's reagent at 100° for 20 min, an orange precipitate was obtained, which could not be purified by chromatography on kieselguhr–bentonite. Partial resinification took place on attempted crystallisation. Previous claims for the formation of such carbonyl derivatives as a phenylhydrazone[8] or an oxime[5] seem doubtful.

Attempted preparation of a quinoxaline from cucurbitacin E.[25] *o*-Phenylenediamine (1·4 g) and cucurbitacin E (1 g) were heated to 130° in a current of nitrogen.[38] Excess of *o*-phenylenediamine was removed by vacuum sublimation. The basic residue could not be crystallised and was several times precipitated with water from a solution in methanol. It contained no primary aromatic amino groups. Light absorption in ethanol: 238 mμ ($E_{1cm}^{1\%}$ 621) and 320 mμ ($E_{1cm}^{1\%}$ 181·5).

Hydrogenation of cucurbitacin E. Cucurbitacin E (275 mg) in glacial acetic acid (10 ml) was hydrogenated at a 10% palladium–charcoal catalyst (20 mg). Hydrogen uptake (11 ml) corresponded to an absorption of 1 mole hydrogen per mole of

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TABLE 3. LEAD TETRA-ACETATE OXIDATION

Substance	No. of moles of $Pb(OAc)_4$ used per mole substance								
	15 min	30 min	1 hr	2 hr	4 hr	8 hr	22 hr	48 hr	114 hr
Cucurbitacin E	1·13	1·2	1·3	1·4	1·6	1·8	2·4	2·9	3·2

cucurbitacin E. The residue could not be satisfactorily crystallised, owing to continuing loss of acetic acid from the material. It gave a brown colour with ferric chloride and a red colour with the benzenediazonium ion. Light absorption in ethanol: 270 mμ ($E^{1\%}_{1cm}$ 122·5). Acetic acid could be removed completely from this material by filtering through a column of activated alumina. Infra-red peaks on this material (KBr disc): 1696, 1660 cm^{-1}.

Ecballic acid. Cucurbitacin E (5 g) and 0·5 N sodium hydroxide (200 ml) were refluxed (2 hr) in a current of nitrogen. The resulting solution was first extracted with ether to remove neutral by-products then added to excess of 10% sulphuric acid and the insoluble residue (*A*) was filtered off. Ether extraction of the filtrate furnished a cream coloured foam (*B*) (1·98 g). The residue (*A*) was reheated in an identical fashion, to yield a further 1·75 g of a foam corresponding to (*B*), whilst a third treatment of residue *A* yielded 0·47 g.

Recrystallisation of bulked fractions *B* from aqueous methanol yielded ecballic acid in colourless prisms containing one molecule of water of crystallisation, m.p. 254–255° (bubbles at 252°) (lit.[15] cites m.p. 257° with bubbling), $[\alpha]_D^{20}$ — 43° (*c*, 2·37 in ethanol) and —61·6° (*c*, 3·46 in acetone). For a sample dried at 100°/10 mm for 16 hr (Found: C, 67·83, 67·0; H, 8·34, 8·2. Acetic acid equivalent to 3·8 C-methyl groups (Kuhn–Roth). Calc. for $C_{28}H_{38}O_7$; C, 67·51; H, 8·28 per cent). Electrometric titration against 0·02 N sodium hydroxide gave a titration equivalent of 472; pH at half neutralisation was 4.28. Light absorption in ethanol: 285 mμ (ϵ, 112).

Methyl ecballate. Ecballic acid (200 mg) in methanol (5 ml) and concentrated sulphuric acid (2 drops) was refluxed for 18 hr. After dilution with water, the sulphuric acid was neutralised with sodium carbonate. Ether extraction yielded 175 mg of solid, which was recrystallised from dry ether, m.p. 207–210° (lit.[15] m.p. 210°), $[\alpha]_D^{20°}$ —47° (*c*, 2·24 in chloroform) (Found: C, 68·05; H, 8·5; O, 23·8. Calc. for $C_{27}H_{40}O_7$: C, 68·04; H, 8·5; O, 23·5 per cent). Light absorption in ethanol: 286 mμ (ϵ 100). Infra-red peaks (Nujol mull): 1712, 1688; (chloroform solution) 1710 (460), 1692 (610). The same product was obtained by the action of diazomethane on the acid.

Methyl ecballate acetate. Methyl ecballate (500 mg) in dry pyridine (10 ml) and acetic anhydride (2 ml) was allowed to stand (2 days) at room temperature in an atmosphere of nitrogen. After dilution with water and making acid with hydrochloric acid, the aqueous portion was extracted with ether. There thus resulted 560 mg of material, which was chromatographed on neutral alumina (Woelhm) grade II (30 g). The material was placed on the column in 20 per cent benzene in light petroleum (boiling range 60–80°) and elution was carried out through the range of solvents light petroleum–benzene–chloroform. The main band (325 mg) was obtained with benzene–

chloroform and crystallised from dry acetone–light petroleum in flat plates, m.p. 181–182°, $[\alpha]_D$ — 85°. (*c*, 1·56 in chloroform) (Found: C, 67·04; H, 8·02. $C_{29}H_{42}O_8$ requires C, 67·16; H, 8·16 per cent). Infra-red peaks (carbon tetrachloride solution): 3612, 3564, 3465; (chloroform solution) 1723 (653) 1700 (550) cm^{-1}.

n-*Butyl ecballate.* Ecballic acid (250 mg) in *n*-butanol (10 ml) and concentrated sulphuric acid (4 drops) were refluxed for 4 hr. On working up as described above for the methyl ester, there resulted 234 mg, which crystallised from dry ether–light petroleum (boiling range 40–60°) in colourless needles, m.p. 166–167° (Found: C, 69·43; H, 9·1. $C_{30}H_{46}O_7$ requires C, 69·47; H, 8·9 per cent).

Ecballic acid 2:4-*dinitrophenylhydrazone.* Ecballic acid)115 mg) in ethanol (10 ml) was heated at 100° with 2:4-dinitrophenylhydrazine (149 mg) and concentrated sulphuric acid (2 drops). After 15 min the orange precipitate was collected and crystallised from methanol, m.p. 165–175° (gradual decomposition) (Found: C, 55·56; H, 5·7; N, 13·67. $C_{38}H_{46}O_{13}N_8$ requires C, 55·47; H, 5·6; N, 13·6 per cent). Main band in chloroform: 367 mμ (ϵ 43,650).

Oxidation of ecballic acid with sodium hydroxide–iodine. Ecballic acid (198 mg) was dissolved in N sodium hydroxide (20 ml) and an aqueous solution of iodine (5% in potassium iodide) was added with shaking until the iodine was no longer decolourised. After 15 min the precipitated iodoform was filtered off, washed, dried and weighed. Yield 78 mg (40 per cent), m.p. and mixed m.p. 121°.

Elateridine. Cucurbitacin E (250 mg) in ethanol (50 ml) was allowed to stand at room temperature (90 hr) with 2% sodium hydroxide (10 ml). Most of the alcohol was removed under reduced pressure and the residue was diluted with water and then extracted with ether. There resulted a colourless foam (249 mg), which was purified by reprecipitation several times with light petroleum from an ether solution. Light absorption in ethanol: 270 mμ ($E^{1\%}_{1cm}$ 121·6). Infra-red bands (chloroform): 1695, 1660 cm^{-1}.

Acknowledgements—We thank Dr. J. E. Page of Glaxo Laboratories Ltd., for determining the infra-red spectra, which were measured on a Perkin-Elmer model 21 spectrophotometer. The hydroxyl region was kindly examined by Mr. W. Kynaston of the Chemical Research Laboratory, Teddington, the instrument used being a modified Hilger D 209 double-beam spectrometer[39] fitted with a quartz prism for measurements in the 3μ region. Compounds were examined in chloroform and carbon tetrachloride at a path length of 0·5 mm. In addition we are indebted to Mr. R. F. Ransom of Messrs. William Ransom and Sons Ltd., who kindly made available to us much of a small stock of α-Elaterin, which enabled this investigation to be initiated. One of us (J.N.T.G.) also thanks the Pharmaceutical Society of Great Britain for a grant during the tenure of which part of this work was carried out.

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