713. *The Constitutions of the Cucurbitacins.*

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The functional groups in rings A and B of cucurbitacins A and B have been connected in several different ways. The presence of a hydroxymethyl group attached to a quaternary carbon atom and placed α to a ketone group has been demonstrated for both cucurbitacins A and C. The functional groups of cucurbitacin C have been clarified and the presence of a 3-hydroxyl group has been established. By means of an unusual intramolecular rearrangement of a formyl group (from C-9 to C-2) the hydroxymethyl group in cucurbitacin C has been proved to be at position 9. Cucurbitacins B, D, E, and I have five methyl groups attached to quaternary atoms in their tetracyclic nucleus; cucurbitacins A and C have only four such groups. Cucurbitacins A, B, and C have been inter-related and thus have the same carbon skeleton.

By having regard to the extensive prior work on the cucurbitacins and taking cognisance of the results now reported, complete constitutional and (tentative) stereochemical formulæ have been advanced for cucurbitacins A, B, C, D, E, and I.

The main conclusions and most of the evidence have already been presented in preliminary form.[1]

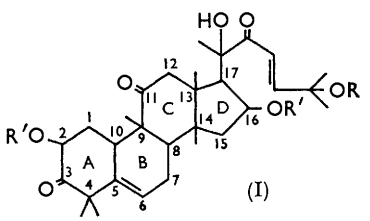
THE cucurbitacins, a group of triterpenoid bitter principles found in the *Cucurbitaceae*,[2-5] have medicinal,[6] cytostatic,[7] and toxic [8] properties that compound the interest attached to structural work on such complex compounds. Numerous papers have already been

[1] de Kock, Enslin, Norton, Barton, Sklarz, and Bothner-By, *Tetrahedron Letters*, 1962, 309.
[2] Enslin and Rehm, *Proc. Linn. Soc.* (*London*), 1956—1957, **3**, 230; Enslin, Rehm, and Rivett, *J. Sci. Food Agric.*, 1957, **8**, 673.
[3] Bhakuni, Sharma, and Kaul, *J. Sci. Ind. Res., India*, 1961, **20**, *B*, 232.
[4] Lavie, Shvo, Gottlieb, Desai, and Khorana, *J.*, 1962, 3259.
[5] Eisenhut and Noller, *J. Org. Chem.*, 1958, **23**, 1984.
[6] Lavie and Szinai, *J. Amer. Chem. Soc.*, 1958, **80**, 707.
[7] Gitter, Gallily, Shohat, and Lavie, *Cancer Res.*, 1961, **21**, 516.
[8] Steyn, *S. African Med. J.*, 1950, **24**, 713.

published on their chemistry and many different formulæ suggested. When we initiated our joint investigations (I.U.P.A.C. Meeting, Australia, August 1960) a normal tetracyclic triterpenoid carbon skeleton was generally accepted. It seemed to us, however, that the sensitivity of cucurbitone A (see below) to alkali with generation of a chromophore at 495 mμ could hardly be explained by a normal skeleton. Instead, we presumed that the α-ketolic system of ring A (see below) could in some way be conjugated with the enone chromophore now placed in ring B (see below). Such an hypothesis would preclude the presence of the usual blocking groups at C-4 and/or C-10. Our initial experiments were, therefore, devoted to attempts to connect the functional groups of rings A, B, and C in a rational manner. Success was, in fact, quickly attained.

We shall present in this paper constitutions for cucurbitacins A, B, C, D, E, and I. Cucurbitacins B, D, E, and I have been inter-related [9] in a simple way. For convenience in exposition we shall at once give the correct formulæ and summarise later the relevant evidence. Cucurbitacin B has structure (I; R = Ac, R′ = H), D has (I; R = R′ = H), E has (II; R = Ac, R′ = H), and I has (II; R = R′ = H).

Cucurbitacin B is found in most species of *Cucurbitaceae*, almost invariably in association with cucurbitacin D. It has been isolated and studied in some detail by Noller and his collaborators.[5,10–14] Since it is already well established that all cucurbitacins which are discussed in this paper have the same fundamental side-chain (sometimes in acetylated form; see above) and are hydroxylated at position 16 we shall not comment on these features further. Cucurbitacin B contains a secondary α-ketol system (in ring A), a strongly hindered isolated ketone group, and a trisubstituted ethylenic linkage. Oxidation with bismuth oxide affords cucurbitacin E (see above).[9] Periodate oxidation of cucurbitacin B affords the non-crystalline aldehydo-acid (III).[15] On treatment with dilute acid this cyclised to the β-dicarbonyl compound (IV) (enolic form) which with sodium carbonate gave a mixture of nor-compounds [(V; R = OH, X = H_2) and (VI)] obtained earlier [15] from cucurbitacin D. By using alkali for the deformylation, mainly the di-unsaturated triketone (VI) was obtained.



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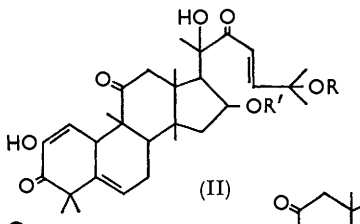

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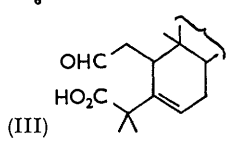

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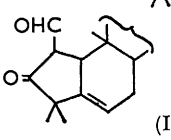

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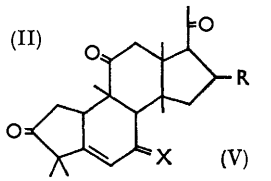
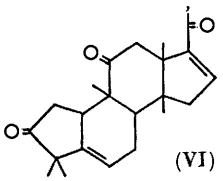


(V)

Hydrogenation of this product (VI) gave the derivative (V; R = H, X = H_2), whilst acetylation of the nor-compound (V; R = OH, X = H_2) afforded its acetate (V; R = OAc, X = H_2). Oxidation of the latter and of compound (V; R = H, X = H_2) with chromic acid gave the corresponding αβ-unsaturated ketones (V; R = OAc, X = O) and (V; R = H, X = O), respectively. Both these ketones rapidly absorbed 1 mol. of oxygen in dilute alkali solution at room temperature, to furnish the fully conjugated

[9] Lavie, Shvo, Willner, Enslin, Hugo, and Norton, *Chem. and Ind.*, 1959, 951.
[10] Melera, Gut, and Noller, *Tetrahedron Letters*, 1960, No. 14, 13.
[11] Noller, Melera, Gut, Shoolery, and Johnson, *Tetrahedron Letters*, 1960, No. 15, 15.
[12] Schlegel, Melera, and Noller, *J. Org. Chem.*, 1961, **26**, 1206.
[13] Schlegel and Noller, *J. Org. Chem.*, 1961, **26**, 1211.
[14] Melera and Noller, *J. Org. Chem.*, 1961, **26**, 1213.
[15] Lavie and Shvo, *J. Amer. Chem. Soc.*, 1960, **82**, 966.

dienediones (VII) and (VIII), that both showed strong ultraviolet absorption at 284 mμ and the appropriate carbonyl stretching bands in the infrared region. The dienedione (VIII) has also been prepared by Lavie and his collaborators.[16]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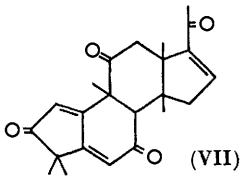
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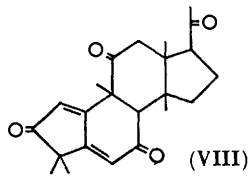
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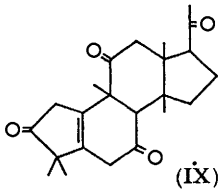
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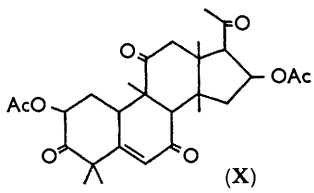
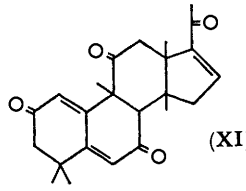
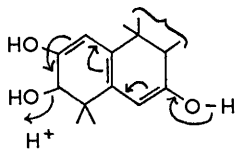

(X)


(XI)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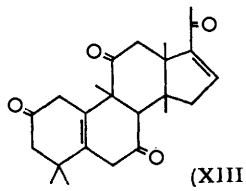
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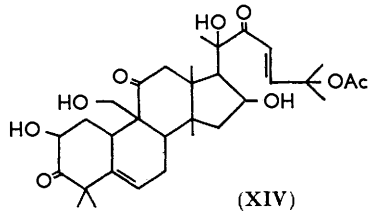

(XIV)

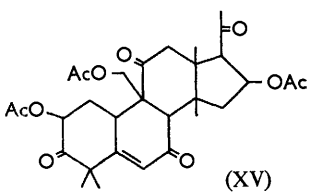
The dienedione character of compound (VIII) was shown by the easy reduction of the main chromophore with zinc dust and acetic acid at room temperature. This reduction gave the expected βγ-unsaturated ketone (IX) and re-oxidation with oxygen in dilute alkaline solution gave back compound (VIII).

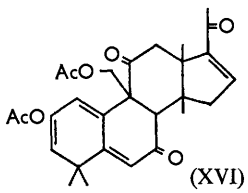
These experiments define the relationship between the α-ketol system and the tri-substituted ethylenic linkage in cucurbitacin B. Further evidence on this subject was secured from the following experiments. Oxidation of cucurbitacin B diacetate (I; R = R′ = Ac) with chromic acid gave the so-called " cucurbitone B " (X).[12] The nuclear magnetic resonance spectrum of this compound showed two acetate-methyl groups, a methyl ketone, and five *quaternary* carbon atoms carrying methyl groups. With methanolic hydrochloric acid cucurbitone B underwent an interesting rearrangement. A further conjugated dienedione was produced which we formulate as (XI). This had $\lambda_{max.}$ 238 and 288 mμ (ε 12,800 and 19,800, respectively) and showed the appropriate infrared carbonyl bands.* The nuclear magnetic resonance spectrum showed five quaternary *C*-methyl groups, and a methyl ketone and three vinyl protons [τ = 3·30 (multiplet), C-16; 3·73 and 4·16 (doublets), C-1 and C-6]. The splitting of the signal for the 1- and 6-protons arises from long-range coupling through the conjugated system. The rearrangement of oxygen functions in the acetoxy-tetraketone (X), giving the tetraketone (XI), is not difficult to understand if the enol (XII) is involved (see arrows) and is not without precedent.[17] Mild reduction of the dienedione (XI) with zinc dust gave the βγ-unsaturated ketone (XIII) which was readily reoxidised with oxygen in dilute alkali to the parent compound (XI).

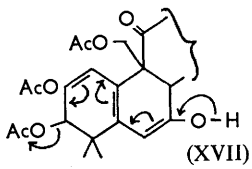
* This dienedione had been obtained in prior and independent work by Professor C. R. Noller and his collaborators (Stanford University). We thank Professor Noller for an authentic specimen of his compound and for personal communications to one of us (P. R. E.).

[16] Lavie, Shvo, Gottlieb, and Glotter, *Tetrahedron Letters*, 1961, 615.
[17] Clarke, *J. Amer. Chem. Soc.*, 1960, **82**, 4629.

Before discussing further the constitution of cucurbitacin B we shall consider cucurbitacins A and C. Cucurbitacin A has the constitution (XIV) and thus differs from cucurbitacin B by one primary hydroxyl group. Previous investigation had established [18] the usual side-chain, C-16 hydroxylation, ring A α-ketol, and trisubstituted ethylenic linkage. The " extra " hydroxyl was not characterised except by acetylation. Chromic acid oxidises cucurbitacin A triacetate to " cucurbitone A," which we now formulate as (XV). The nuclear magnetic resonance spectrum of the latter compound showed, besides three acetate-methyl groups and one methyl ketone, four quaternary *C*-methyl groups. Since a biogenetic relationship between cucurbitacins A and B was almost certain [2] the implication was that the " extra " hydroxyl group was primary. The nuclear magnetic resonance spectrum of cucurbitone A confirmed this since it also showed an AB system centred at τ 5·59 and 6·02 (*J* 11·0 c./sec.), indicative of the grouping $-CH_2-OAc$ attached to an asymmetric quaternary carbon atom. We considered, therefore, that cucurbitacin A had the same constitution as B, but with one of the methyl groups at quaternary C-4, C-9, C-13, or C-14 hydroxylated.


(XV)


(XVI)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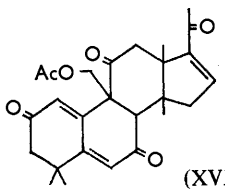
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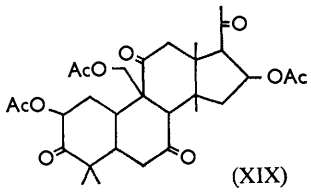
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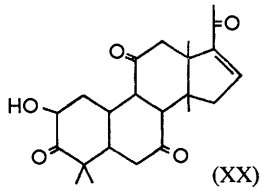

(XX)

We first attempted to inter-relate the α-ketol system in cucurbitone A (XV) with the αβ-unsaturated ketone grouping in ring B. Enol-acetylation of cucurbitone A with sodium acetate–acetic anhydride under reflux [19] gave a yellow crystalline compound having $\lambda_{max.}$ 238 and 357 mμ (ε 14,400 and 9200, respectively). The long-wavelength band of relatively low ε (*cisoid* chromophore) is in agreement with the formulation (XVI) for this compound. It showed the appropriate infrared bands The nuclear magnetic resonance spectrum indicated, besides two acetate groups and one methyl ketone, four olefinic protons [τ 3·2 (multiplet), 3·75 (doublet), 4·10 (triplet), and 4·35 (doublet)]. These bands are ascribed to protons at positions 16, 6, 1, and 3, respectively. In addition, an AB system with doublets at τ 5·49 and 6·04 (*J* 10·8 c./sec.) proved that the $-CH_2{\cdot}OAc$ group of cucurbitone A (XV) was still present. The mechanism for the conversion of (XV) into (XVI) may be understood as outlined in (XVII) (see arrows).

Mild acid hydrolysis (0·1% aqueous perchloric acid in acetic acid) converted compound (XVI) into the dienedione (XVIII). This had ultraviolet and infrared spectra closely comparable with those found for the cucurbitone B derivative (XI) (see above). In addition, both compounds had closely similar strongly positive ($[\alpha]_D$ ~500°) optical rotations. The nuclear magnetic resonance spectrum of the dienedione (XVIII) was also essentially identical with that of the " B " dienedione (XI), but with the additional bands characteristic of the quaternary-attached $-CH_2{\cdot}OAc$ grouping and with four instead of five quaternary-attached methyl groups.

The position of the hydroxymethyl group in cucurbitacin A was revealed by the

[18] Enslin, Hugo, Norton, and Rivett, *J.*, 1960, 4779.
[19] Jones and Verrill, *J.*, 1940, 1512.

following experiments. On treatment with aqueous-ethanolic alkali, dihydrocucurbitone A [18] (XIX) gave formaldehyde and the nor-compound (XX). Nuclear magnetic resonance measurements confirmed the absence of the primary hydroxyl group. However, four quaternary *C*-methyl groups were clearly demonstrated. Since the loss of formaldehyde must represent a reversed aldol reaction as found, for example, in the chemistry of isterogenin,[20] the quaternary-attached hydroxymethyl group must be α to a ketone group. Since a grouping of the type (CHMe) is *not* produced in the reaction (see above) the hydroxymethyl group cannot be attached to C-4. Nor can it be attached to C-13, for then a 13,17-ene-12,20-dione would have resulted. Position 14 is excluded because there is no 15-carbonyl group (cyclopentanone). Positions 5 and 10 are excluded because of the connections established between rings A and B. Position 8 was excluded relative to 9 for reasons which emerged from our studies on cucurbitacin C (see below).

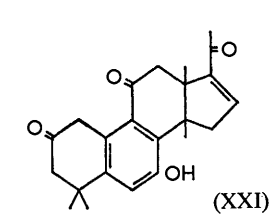
On treatment with dilute alkali at room temperature both the enol acetate (XVI) and the dienedione (XVIII) gave formaldehyde and the phenolic ketone (XXI). The latter showed a complex ultraviolet spectrum consistent with a 3-hydroxyacetophenone chromophore, and the appropriate infrared spectrum. Its nuclear magnetic resonance spectrum showed four quaternary *C*-methyl groups, one methyl ketone group, one aromatic hydrogen (τ 2·9), and one olefinic hydrogen (τ 3·1).

Cucurbitacin C [21] has the same side-chain and 16-hydroxyl group as cucurbitacin A (XIV). In addition, there are a hindered ketone group, a trisubstituted ethylenic linkage, and two easily acylated hydroxyl groups. We show that cucurbitacin C has the constitution (XXII) on the basis of the following experiments. Chromic acid oxidises cucurbitacin C triacetate to " cucurbitone C " [21] (XXIII). The nuclear magnetic resonance spectrum of the latter showed two secondary alcohol acetates and a primary alcohol acetate, the methyl ketone group, and four quaternary *C*-methyl groups. The primary alcohol acetate group, as in cucurbitone A (XV), could be detected by signals at τ 5·52 and 5·98 (doublets, *J* 11·4 c./sec.).

Hexanorcucurbitacin C (XXIV; R = OH) (obtained [21] by periodate oxidation of cucurbitacin C), its anhydro-derivative (XXV), and the dihydro-derivative (XXIV; R = H) of the latter all gave weak Zimmermann tests showing the absence of a normal triterpenoid 3-ketone.[20] In their nuclear magnetic resonance spectra all three compounds showed bands for four quaternary-attached methyl groups and one quaternary-attached hydroxymethyl group. With refluxing aqueous-ethanolic alkali anhydrohexanorcucurbitacin C (XXV) gave formaldehyde and a heptanorcucurbitacin C derivative (XXVI; R = H). Similarly, the compound (XXIV; R = H) furnished formaldehyde and an analogous heptanor-derivative (XXVII) (see further below). Both heptanor-compounds gave weak Zimmermann tests and had four quaternary *C*-methyl groups (nuclear magnetic resonance spectra). Oxidation of the alcohols (XXVI; R = H) and (XXVII) with chromium trioxide in acetone afforded the corresponding 3-ketones, (XXVIII; R = H) and (XXIX; R = H), respectively, both of which gave positive Zimmermann tests. A secondary hydroxyl group at position 3 was, therefore, indicated for cucurbitacin C (XXII). Partial oxidation of anhydrohexanorcucurbitacin C (XXV) with chromium trioxide gave a mixture of products from which the keto-aldehyde (XXVIII; R = CHO) was isolated as well as the hydroxy-aldehyde (XXVI; R = CHO) and the hydroxy-trione (XXVIII; R = CH_2·OH). The constitutions assigned to these compounds are confirmed by their nuclear magnetic resonance spectra. Chromic acid oxidation of the alcohol (XXVIII; R = CH_2·OH) gave the keto-aldehyde (XXVIII; R = CHO). Similarly the derivative (XXIV; R = H) gave an analogous aldehyde (XXIX; R = CHO). The keto-alcohol (XXIX; R = CH_2·OH) was also isolated as a by-product from the latter oxidation. Its constitution was proved by treatment with alkali, which gave formaldehyde and the

[20] Barton and de Mayo, *J.*, 1954, 887.
[21] Enslin, Hugo, Norton, and Rivett, *J.*, 1960, 4787.

triketone (XXIX; R = H). Both aldehydes (XXVIII; R = CHO) and (XXIX; R = CHO) resisted acid-catalysed enolisation and enol-acetylation under normal conditions. Nevertheless, in ethanolic alkali at room temperature they rapidly developed strong absorption in the ultraviolet spectrum. From the reaction of aldehyde (XXVIII; R = CHO) with alkali two isomeric enolic compounds were obtained. When the presence of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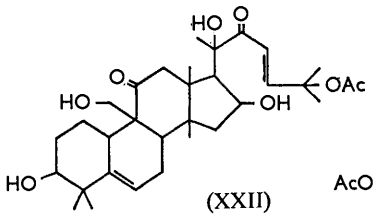
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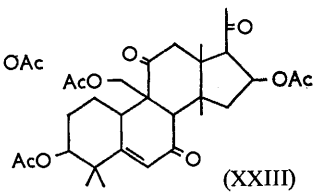
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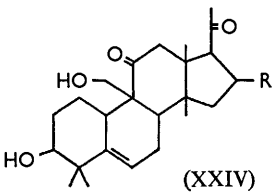
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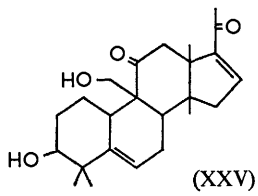
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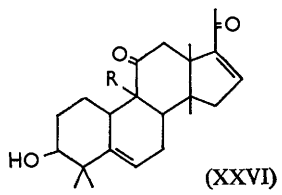
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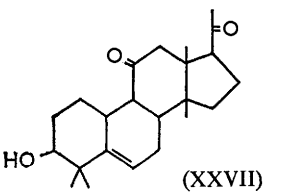
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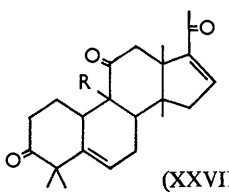
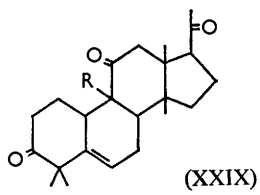

(XXVIII)

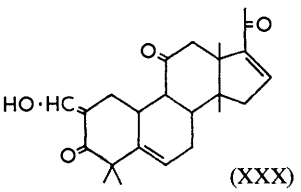

(XXIX)

acid was avoided enol-I, m. p. 214—219°, $[\alpha]_D$ −247°, $\lambda_{max.}$ 241 and 286 mμ (ε 10,600 and 8700, respectively), was obtained. In ethanolic alkali this compound developed a new band at 316 mμ (ε 18,100). On acidification and extraction in the presence of excess acid an isomeric enol-II, m. p. 269—275°, $[\alpha]_D$ −245°, $\lambda_{max.}$ 242 and 277 mμ (ε 10,900 and 11,200, respectively) was formed. Enol-II could be reconverted into enol-I by dissolution in alkali, neutralisation, and working up in the absence of acid. These two enols are represented by formulæ (XXX) and (XXXI). In so far as the nuclear magnetic resonance spectrum of enol-II, after equilibration with deuterium oxide, showed a band at τ +1·55 which can be ascribed to the proton in the grouping (>C=CH–O–), enol-II can be tentatively formulated as (XXX), and enol-I as (XXXI). Analogous pairs of enols from 2-hydroxymethylene-3-ketones of the steroid and triterpenoid series are well known,[22] and where appropriate show comparable spectral behaviour.

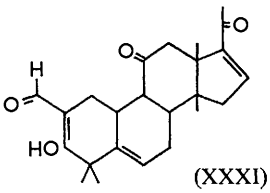
Clearly the conversion of aldehyde (XXVIII; R = CHO) into enols-I and -II is not a simple enolisation of a β-formyl-ketone. It is most convincingly formulated as an intramolecular transfer of a formyl group. For this to happen two ketone groups are needed. That at position 3 must be readily enolisable so that the anionic 2-carbon can attack the 9-formyl group. The resulting aldol (XXXII) can be cleaved in two ways, either to give back the starting ketone or to give (with formyl transfer) the 2-hydroxymethylene-3-ketone. Formation of the latter is, of course, irreversible. Proof that the rearrangement was indeed intramolecular was provided by the fact that the aldehyde

[22] Clinton, Manson, Stonner, Neumann, Christiansen, Clarke, Ackerman, Page, Dean, Dickinson, and Carabateas, *J. Amer. Chem. Soc.*, 1961, **83**, 1478; Tsuda and Nozoe, *Chem. and Pharm. Bull.* (*Japan*), 1959, **7**, 232; Holker, Powell, Robertson, Simes, and Wright, *J.*, 1953, 2414.

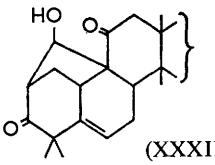
(XXVI; R = CHO) was stable under the conditions needed for formation of the hydroxymethylene-ketone. An intermolecular reaction *via* ethyl formate is thus excluded. However, with refluxing 0·1N-potassium hydroxide, the aldehyde (XXVI; R = CHO) gave anhydroheptanorcucurbitacin C (XXVI; R = H) and formate ion.



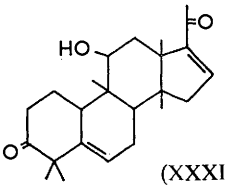
(XXX)



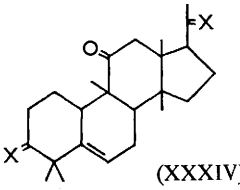
(XXXI)



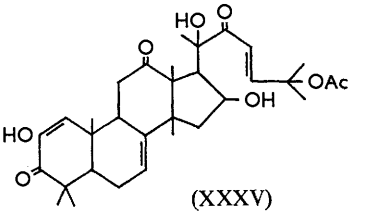
(XXXII)



(XXXIII)



(XXXIV)



(XXXV)

From an inspection of molecular models the intramolecular transformylation appears to place considerable restriction upon the formulæ possible for the cucurbitacins. The elimination of formaldehyde by base from compounds (XXIV; R = H) and (XXV) shows that the hydroxymethyl group must be α to the hindered ketone. The ready loss of formaldehyde from cucurbitacins A and C, but not from B, on treatment with alkali is also in agreement with this view. If we accept that there is a ketone group at position 3 in compound (XXVIII; R = CHO) then transfer of a formyl group can take place only if the B/C ring fusion is *cis* and the formyl group is at position 9 (not 8) and thus the hindered ketone group is at position 11. Further, the configuration of the 10-hydrogen has to be opposite to the configuration of the 9-formyl group. We take up these stereochemical points again in the sequel.

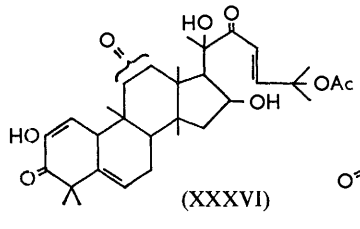
The constitutions that we suggest for the cucurbitacins are considerably strengthened by chemical inter-relations of cucurbitacins A, B, and C. Reduction of cucurbitacin A triacetate with calcium in liquid ammonia,[23] oxidation of the product with periodic acid, and treatment with alkali under mild conditions afforded a mixture; this was resolved by chromatography over alumina into the triketone (XXVIII; R = H), the hydroxy-triketone (XXIX; R = CH_2·OH), and the hydroxy-triketone (XXVIII; R = CH_2·OH), all three being derivatives of cucurbitacin C (see above). The position of the hydroxymethyl group in cucurbitacin A is thus the same as that (9) in cucurbitacin C (see above).

Reduction of cucurbitacin B diacetate with calcium in liquid ammonia, periodate fission of the product, and mild treatment with alkali afforded the hydroxy-diketone (XXXIII). This was prepared earlier by Melera, Gut, and Noller [10] by essentially the same procedure. Hydrogenation followed by chromic acid oxidation gave the triketone (XXXIV; X = O). Reaction with ethanedithiol to furnush the bisdithioketal followed by desulphurisation with Raney nickel afforded the monoketone (XXXIV; X = H_2). The same monoketone was obtained when the triketo-aldehyde (XXIX; R = CHO) (see above) from cucurbitacin C was treated successively with ethanedithiol and Raney nickel in the same way.

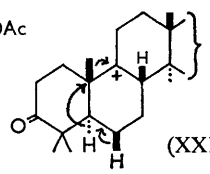
Amongst the many formulæ that have been suggested earlier for the cucurbitacins we shall mention two. Lavie, Shvo, and Gottlieb [24] at first favoured formula (XXXV) for cucurbitacin E. Later, as a result of the cogent work of Noller and his collaborators,[5,10-14]

[23] Chapman, Elks, Phillips, and Wyman, *J.*, 1956, 4344.
[24] Lavie, Shvo, and Gottlieb, *Tetrahedron Letters*, 1960, No. 22, 23.

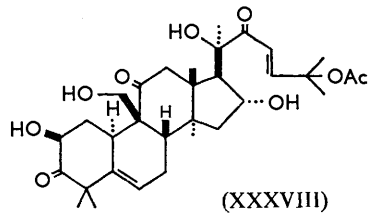
the revised formula (XXXVI) was suggested.[16] The latter is in agreement with our own work in so far as comparison can be made.



(XXXVI)



(XXXVII)



(XXXVIII)

The stereochemistry of cucurbitacins B, D, E, and I was tentatively discussed by Lavie, Shvo, and Gottlieb [24,25] in terms of the " wrong " constitutional formula. However, the conclusions reached about configurations of the C/D ring junction, the 16-hydroxyl group, and the side-chain are still relevant and we accept them. The ring B/C junction must be *cis*, and the 9,10-stereochemistry *anti*, from the arguments given above. If we accept a view that the biogenesis of the cucurbitacins involves rearrangement of carbonium ion (XXXVII) (see arrows) and that, as in all the natural tetra- and penta-cyclic triterpenoids so far known, the 10-methyl group is β, then full stereochemical formulæ, including absolute configuration [24] can be given for all the cucurbitacins. For brevity we give the key compound cucurbitacin A (XXXVIII); other stereoformulæ can, of course, be at once derived from the work discussed earlier in the paper.

Selected nuclear magnetic resonance results have been cited in the text above. More detailed results are presented in the Table. It will be seen that as a whole they provide good support for the structural suggestions in the present paper.

Experimental

Unless specified to the contrary, $[\alpha]_D$ refer to chloroform, ultraviolet absorption spectra to ethanol, and infrared absorption spectra to chloroform solutions. Infrared spectra were determined on a Perkin-Elmer model 21 or 221 spectrometer, and ultraviolet absorption spectra on a Unicam model S.P. 500 or 700 spectrometer. The descending method of paper chromatography on Whatman No. 1 paper impregnated with a 50% solution of formamide in ethanol was used. Oven-dried chromatograms were sprayed with either (*a*) a freshly prepared 0·2% solution of triphenyltetrazolium chloride in N-sodium hydroxide (made up in 50% aqueous ethanol) and heated over steam to reveal red spots (T.T.C. spray reagent), or (*b*) a solution of vanillin (10 g.) in a mixture of ethanol (150 ml.) and 85% phosphoric acid (50 ml.), spots being revealed by heating at 90° for *ca.* 2 min. (V.P.A. spray reagent). The solvent systems and spray reagents employed are indicated in parentheses. Acid-washed alumina was prepared by washing Peter Spence's alumina consecutively with dilute hydrochloric acid, hot water, and ethanol and reactivating it at 150° for 12 hr. Silica for chromatography refers to F. Smith's supply (50—200 mesh).

For recording nuclear magnetic resonance spectra, samples (15—30 mg.) were introduced into thin-walled precision-ground Pyrex tubes, which were then fixed to a vacuum-line. Solvent (0·2 ml.) (usually deuteriochloroform, obtained from Merck Inc. of Canada), which had been degassed by freezing and thawing under a vacuum, was distilled in from a graduated vessel. Tetramethylsilane, similarly treated, was added in the amount of 0·5—1·0%. The tubes were then sealed off.

Spectra were for the most part obtained on a Varian A-60 spectrometer. Resolution of the order of 0·2—0·3 c./sec. was maintained as routine. Calibration was checked daily. A conservative estimate of probable error of line positions is ±0·01 p.p.m.

Oxidation of Cucurbitacin B by Periodic Acid.—0·5M-Periodic acid (60 ml.) and water (290 ml.) were added to a solution of cucurbitacin B (5 g.) in ethanol (450 ml.), and the mixture kept at room temperature in the dark for 18 hr. (2·2 mol. consumed). The excess of reagent

[25] Lavie and Gottlieb, *Chem. and Ind.*, 1960, 929.

TABLE.

Nuclear magnetic resonance spectral data for cucurbitacins and their derivatives.

Compound	Chemical shift, τ scale *	Assignment	Comments † (*J* in c/sec.)
Cucurbitacin A (XIV)	2·98	αβ-Unsat. ketone (C-24)	*J* 14·0
	3·46	αβ-Unsat. ketone (C-23)	*J* 14·0
	4·20	Olefin (C-6)	Broad
	7·95	Acetate	
	8·43	2 Quat. Me	
	8·56	2 Quat. Me	
	8·68	2 Quat. Me	
	8·94	Quat. Me (C-13)	
Cucurbitone A (XV)	3·75	Olefin (C-6)	
	4·37	CH–O·COR (C-3, C-16)	
	4·50	CH–O·COR (C-3, C-16)	
	5·59	CH_2·O·COR	*J* 11·0
	6·02	CH_2·O·COR	*J* 11·0
	7·83	3 Acetates + 1 Me ketone	
	7·87	3 Acetates + 1 Me ketone	
	8·02	3 Acetates + 1 Me ketone	
	8·10	3 Acetates + 1 Me ketone	
	8·60	3 Quat. Me	Partially resolved
	9·15	Quaternary Me (C-13)	Rather broad
Enol acetate A (XVI)	3·20	Olefin (C-16)	
	3·75	Olefin (C-6)	d, *J* 2·0
	4·10	Olefin (C-1)	t, *J* 2·0
	4·35	Olefin (C-3)	d, *J* 2·0
	5·49	CH_2·O·COR	neq, *J* 10·8
	6·04	CH_2·O·COR	neq, *J* 10·8
	7·70	2 Acetates + Me ketone	
	7·83	2 Acetates + Me ketone	
	8·02	2 Acetates + Me ketone	
	8·66	Quat. Me	
	8·71	Quat. Me	
	8·89	2 Quat. Me	
Dienedione A (XVIII)	3·20	Olefin (C-16)	
	3·62	Olefins (C-1, C-6)	*J* 2·0
	3·97	Olefins (C-1, C-6)	*J* 2·0
	5·54	CH_2·O·COR	neq, *J* 11·2
	5·88	CH_2·O·COR	neq, *J* 11·2
Phenol A (XXI)	2·90	Aromatic ring (C-6)	Sharp
	3·10	Olefin (C-16)	Doublet of doublets *J* 2, 3·5
	6·90	2 CH_2 (C-3, C-12)	Sharp
	7·42	2 CH_2 (C-3, C-12)	Sharp
	7·60	Me ketone	
	8·64	3 Quat. Me	Not well resolved
	8·67	3 Quat. Me	Not well resolved
	8·70	3 Quat. Me	Not well resolved
	8·99	Quat. Me	
Cucurbitacin B (I; R = Ac, R′ = H)	2·95	αβ-Unsat. ketone (C-24)	neq, *J* 16·2
	3·48	αβ-Unsat. ketone (C-23)	neq, *J* 16·2
	4·27	Olefin (C-6)	m, broad
	8·01	Acetate	
	8·45	2 Quat. Me	
	8·58	2 Quat. Me	
	8·64	6 Quat. Me	Not completely resolved
	8·68	6 Quat. Me	Not completely resolved
	8·72	6 Quat. Me	Not completely resolved
	8·95	6 Quat. Me	Not completely resolved
	9·04	6 Quat. Me	Not completely resolved
Cucurbitacin C (XXII)	3·19	αβ-Unsat. ketone (C-24)	neq, *J* 15·1
	3·26	αβ-Unsat. ketone (C-23)	neq, *J* 15·1
	3·77	Olefin (C-6)	m, broad
	8·04	Acetate	
	7·50	CH_2 (C-3)	Sharp
	7·68	Acetate + Me ketone	
	7·99	Acetate + Me ketone	
	8·62	Quat. Me	
	8·74	Quat. Me	
	8·89	2 Quat. Me	Broad, not resolved

TABLE. (*Continued.*)

Compound	Chemical shift, τ scale *	Assignment	Comments † (*J* in c./sec.)
Dienedone A (XVIII)	8·45	7 Quat. Me	Not completely resolved
	8·44		
	8·63		
	8·65		
	8·86		
	9·01		
	9·05		
Cucurbitone C (XXIII)	3·73	Olefin (C-6)	d, *J* 2·0
	4·32	CH·O·COR (C-16)	t, *J* 7·2
	5·35	CH·O·COR (C-3)	m
	5·52	CH_2·O·COR	neq, *J* 11·4
	5·98		
	7·82	3 Acetates + Me ketone	
	7·92		
	8·00		
	8·08		
	8·72	Quat. Me	
	8·83	2 Quat. Me	
	9·13	Quat. Me	
Anhydroheptanor-cucurbitacin C (XXVI; R = H)	3·20	Olefin (C-16)	t, *J* 2·5
	4·45	Olefin (C-6)	m, broad
	7·68	Me ketone	
	8·89	3 Quat. Me	
	8·93		
	8·97		
	9·08	Quat. Me (C-13)	Rather broad
Anhydrodihydro-heptanorcucurbi-acin C (XXVII)	4·45	Olefin (C-6)	m, broad
	7·92	Me ketone	
	8·87	3 Quat. Me	
	8·92		
	8·97		
	9·32	Quat. Me (C-13)	Rather broad
Anhydrotriketone (XXVIII; R = H)	3·20	Olefin (C-16)	t, *J* 2·4
	4·43	Olefin (C-6)	m, broad
	7·72	Me ketone	
	8·72	3 Quat. Me	
	8·81		
	8·88		
	9·08	Quat. Me (C-13)	Rather broad
Anhydrodihydro-triketone (XXIX; R = H)	4·42	Olefin (C-6)	m, broad
	7·42	Me ketone	
	8·72	3 Quat. Me	
	8·82		
	8·88		
	9·35	Quat. Me (C-13)	Rather broad
Anhydrotriketo-aldehyde (XXVIII; R = CHO)	0·10	Aldehyde	d, |*J*| 1·0 ‡
	3·25	Olefin (C-16)	t, |*J*| 2·5
	4·20	Olefin (C-6)	m, broad
	7·69	Me ketone	
	8·75	3 Quat. Me	
	8·79		
	8·89		
	9·02	Quat. Me (C-13)	Rather broad
Anhydrodihydro-triketo-aldehyde (XXIX; R = CHO)	0·19	Aldehyde	Unresolved fine structure ‡
	4·20	Olefin (C-6)	m, broad
	7·87	Me ketone	
	8·72	3 Quat. Me	
	8·76		
	8·86		
	9·22	Quat. Me (C-13)	Rather broad
Cucurbitacin E diacetate (deuterio-acetone solution) (II; R = R′ = R″ = Ac)	2·85	αβ-Unsat. ketone	neq, *J* 15·0
	3·18	αβ-Unsat. ketone	
	3·71	Olefin (C-1)	d, *J* 3·0
	4·15	Olefin (C-6)	m, broad
	7·85	3 Acetates	
	8·01		
	8·13		
	8·43	2 Quat. Me (C-25)	Sharp
	8·56	2 Quat. Me	
	8·72	2 Quat. Me	
	8·95	2 Quat. Me	Not resolved
	8·97		

* Tiers, *J. Phys. Chem.*, 1958, **62**, 1151. † Abbreviations: d = doublet, t = triplet, m = multiplet, neq = non-equivalence quartet. ‡ Small long-range splitting; cf. Davis, Lutz, and Roberts, *J. Amer. Chem. Soc.*, 1961, **83**, 246.

was destroyed with ethylene glycol, and water (800 ml.) was added. Extraction with chloroform and separation with aqueous sodium hydrogen carbonate gave a neutral fraction (2 g.) and an acid fraction (3 g.). The acid fraction was chromatographed on silica (100 g.). Elution with 49 : 1 chloroform–methanol (600 ml.) gave crude *trans*-4-acetoxy-4-methylpent-2-enoic acid (1·3 g.). Further elution with 19 : 1 chloroform–methanol (500 ml.) gave fractions of an aldehyde acid of R_F 0·15 (4 : 1 benzene–ethyl acetate, V.P.A. spray reagent). The combined fractions (1·3 g.) were dissolved in ether, and a small amount of enolic material was removed by extraction of the acid into sodium hydrogen carbonate solution. The purified aldehyde acid (III) showed only a single spot on paper chromatograms, but failed to crystallise; it had $\nu_{max.}$ 2715, 1718, and 1695 cm.$^{-1}$.

Enol (cf. IV) *from the Aldehydo-acid* (III).—The aldehydo-acid (III) (650 mg.) in 0·02N-hydrochloric acid (100 ml.) was heated on a steam-bath for 1 hr. After dilution with water (100 ml.), the product was isolated in chloroform and separated by sodium hydrogen carbonate solution from an acid fraction (46 mg.). Crystallisation of the product (390 mg.) from chloroform–methanol gave a crude enol (247 mg.) which was purified by chromatography on silica (12 g.). Elution with 99 : 1 chloroform–methanol (200 ml.) and crystallisation from chloroform–methanol gave the *keto-aldehyde* (IV) (deep violet colour with ferric chloride), m. p. 239—241°, $[\alpha]_D$ +114° (*c* 1·0), $\lambda_{max.}$ 282 mμ (ε 2740 in EtOH) and 309 mμ (ε 8450 in 0·1N-potassium hydroxide in 50% aqueous ethanol), $\nu_{max.}$ 1742, 1701, 1697, 1663, and 1592 cm.$^{-1}$ (Found: C, 72·3; H, 8·0. $C_{24}H_{32}O_5$ requires C, 72·0; H, 8·1%).

A-*Nor-hexanorcucurbitacin B* (V; R = OH, X = H_2) *and Anhydro-*A*-nor-hexanorcucurbitacin B* (VI) *from the Keto-aldehyde* (IV).—The finely ground enol (IV) (967 mg.) was heated and stirred on a steam-bath for 2 hr. with a saturated sodium carbonate solution (160 ml.). After isolation with chloroform, the product was chromatographed on acid-washed alumina (40 g.). Elution with 1 : 1 chloroform–benzene (100 ml.) gave fractions (293 mg.) of m. p. 195—203°. Crystallisation from chloroform–methanol gave the anhydro-A-nor-compound (VI), m. p. 200—205°, $[\alpha]_D$ +35° (*c* 1·0), $\lambda_{max.}$ 239 and 298 mμ (ε 8300 and 160, respectively), $\nu_{max.}$ 1742, 1695, 1667, and 1592 cm.$^{-1}$ (Found: C, 77·6; H, 8·6. Calc. for $C_{23}H_{30}O_3$: C, 77·9; H, 8·5%).

Further elution of the column with the same solvent (200 ml.) gave fractions (409 mg.), m. p. 227—231°. Crystallisation from chloroform–methanol and chloroform–benzene gave the A-nor-compound (V; R = OH, X = H_2), m. p. 226—229°, $[\alpha]_D$ +61° (*c* 1·0), $\lambda_{max.}$ 294 mμ (ε 156), $\nu_{max.}$ 1741 and 1702 cm.$^{-1}$ (Found: C, 73·9; H, 8·6. Calc. for $C_{23}H_{32}O_4$: C, 74·2; H, 8·7%).

*Direct Preparation of Anhydro-*A*-nor-hexanorcucurbitacin B* (VI) *from Cucurbitacin B* (I; R = Ac, R′ = H).—0·5M-Periodic acid (180 ml.) and water (870 ml.) were added to a solution of cucurbitacin B (15 g.) in ethanol (1350 ml.), and the mixture kept at room temperature in the dark for 18 hr. (2·06 mol. consumed). The excess of reagent was destroyed with ethylene glycol, and the mixture concentrated *in vacuo* to half its volume. Extraction with chloroform gave a crude product which was dissolved in ethanol (810 ml.). N-Sodium hydroxide (90 ml.) was added, and the mixture refluxed for 2 hr. under nitrogen. After dilution with water (2 l.), the mixture was extracted with chloroform, and the extract washed with water, dilute hydrochloric acid, again with water, and evaporated, to give a gum which was crystallised from methanol, affording crude anhydro-A-nor-product (VI) (3·15 g.), m. p. 194—198°. The gum from the mother-liquors was filtered in 4 : 1 benzene–chloroform through acid-washed alumina (250 g.) to give more (VI) (1 g.) and the 16-hydroxy-compound (V; R = OH, X = H_2), (343 mg.).

16-*Deoxy-*A*-nor-hexanorcucurbitacin B* (V; R = H, X = H_2).—The anhydro-A-nor-compound (VI) (1 g.) was hydrogenated over 5% palladised calcium carbonate (200 mg.) in ethanol (150 ml.) (0·95 mol. of hydrogen absorbed in 30 min.). The crude crystalline product (852 mg.), m. p. 148—152°, crystallised from chloroform–benzene–ether to give the 16-deoxy-A-nor-hexanor-compound (V; R = H, X = H_2), m. p. 177—180°, $[\alpha]_D$ +77° (*c* 1·0), $\lambda_{max.}$ 293 mμ (ε 161) (Found: C, 76·9; H, 9·1. Calc. for $C_{23}H_{32}O_3$: C, 77·5; H, 9·1%).

Chromium Trioxide Oxidation of 16-*Deoxy-*A*-nor-hexanorcucurbitacin B* (V; R = H, X = H_2).—The 16-deoxy-A-nor-compound (V; R = H, X = H_2) (770 mg.) in 90% acetic acid (10 ml.) was oxidised at 50—60° by dropwise addition of chromium trioxide (566 mg.) in 90% acetic acid (7 ml.) during 3 hr. After a further 2 hr. at this temperature, methanol was added, the mixture diluted with water, and the product isolated with chloroform. Crystallisation from chloroform–benzene and chloroform–ether gave the *tetraketone* (V; R = H, X = O)

(426 mg.), m. p. 253—258°, $[\alpha]_D$ +98° (*c* 1·0), $\lambda_{max.}$ 243 mμ (ε 11,400), $\nu_{max.}$ 1751, 1706, and 1664 cm.$^{-1}$ (Found: C, 74·5; H, 8·0. $C_{23}H_{30}O_4$ requires C, 74·6; H, 8·2%).

Conjugated Dienedione (VIII) *from Tetraketone* (V; R = H, X = O).—The tetraketone (V; R = H, X = O) (566 mg.) in 0·1N-85% alcoholic potassium hydroxide (300 ml.) was shaken for 30 min. under oxygen (0·9 mol. absorbed). The ultraviolet absorption spectrum ($\lambda_{max.}$ 283 mμ; ε 16,900) of the solution remained practically unchanged for another 2 hr. in the dark. After dilution with water (600 ml.) the mixture was extracted with chloroform. Most of the yellow colour remained in the aqueous layer. The chloroform extract was washed consecutively with water, dilute hydrochloric acid, and water, and evaporated to give a semi-crystalline product (386 mg.). The crude substance was filtered through acid-washed alumina (40 g.) with benzene–chloroform (19 : 1, 100 ml.; 9 : 1, 400 ml.). The first crystalline fractions (74 mg.), m. p. 196—216°, still showed a band at 1745 cm.$^{-1}$ of an unconjugated five-membered keto-group. These were followed by a middle fraction (258 mg.), 150—187°, and by an end fraction (12 mg.), m. p. 193—205°. The middle fraction was again filtered through acid-washed alumina (30 g.) with 19 : 1 benzene–chloroform, and fractions (total 62 mg.) of m. p. 165—179° were combined and crystallised from benzene–hexane, to give the light yellow conjugated *dienedione* (VIII), m. p. 176—179°, $[\alpha]_D$ +377° (*c* 0·5), $\lambda_{max.}$ 284 mμ (ε 22,800), $\nu_{max.}$ 1712, 1661, and 1565 cm.$^{-1}$ (Found: C, 75·3; H, 7·7. $C_{23}H_{28}O_4$ requires C, 75·0; H, 7·7%).

Reduction of Conjugated Dienedione (VIII) *with Zinc in Acetic Acid.*—The dienedione (VIII) (29 mg.) was stirred with acetic acid (5 ml.) and zinc dust (150 mg.) for 2·5 hr. at room temperature. The product crystallised from chloroform–methanol and chloroform–ether, to give the colourless reduction *product* (IX) (10 mg.), m. p. 186—191°, $[\alpha]_D$ +190° (*c* 0·6), $\lambda_{max.}$ 284 mμ (ε 1140), $\nu_{max.}$ 1751 and 1706 cm.$^{-1}$ (Found: C, 74·0; H, 8·2. $C_{23}H_{30}O_4$ requires C, 74·55; H, 8·2%). This product (40 mg.) in 0·1N-alcoholic sodium hydroxide (20 ml.) was shaken for 1·5 hr. in air. Extraction of the yellow solution with chloroform and crystallisation from benzene–hexane gave the dienedione (VIII) (m. p., mixed m. p., ultraviolet and infrared spectra).

Elimination of Formaldehyde from Cucurbitacin A and C.—Samples (40 mg.) of each of cucurbitacins A, B, and C were dissolved in 1 : 1 aqueous-ethanolic N-potassium hydroxide (5 ml.) and kept at room temperature for 17 hr. After extraction with chloroform, the aqueous layer was tested for the presence of formaldehyde (chromotropic colour test [26]). Formaldehyde was detected in the case of cucurbitacin A and C, whereas cucurbitacin B gave a negative result.

Acetylation and Chromium Trioxide Oxidation of the A-Nor-compound (V; R = OH, X = H_2).—This A-nor-compound (500 mg.) was boiled for 1 hr. in acetic anhydride (10 ml.) under nitrogen, the excess of anhydride destroyed with water (2 ml.), and a solution of chromium trioxide (364 mg.) in 90% acetic acid (5 ml.) added in 3 hr. at 50—60°. After a further 2 hr. at the same temperature, methanol and water were added and the product was isolated with chloroform. The product was purified by chromatography on silica. Elution with benzene–chloroform (1 : 1, 250 ml.; 1 : 2, 200 ml.) gave crystalline fractions which were crystallised from chloroform–benzene, to give the *tetraketone* (V; R = OAc, X = O) (380 mg.), m. p. 268—269°, $[\alpha]_D$ +12° (*c* 1·2), $\lambda_{max.}$ 242 mμ (ε 11,100), $\nu_{max.}$ 1745, 1706, and 1661 cm.$^{-1}$ (Found: C, 69·9; H, 7·5. $C_{25}H_{32}O_6$ requires C, 70·1; H, 7·5%).

Conjugated Dienedione (VII) *from the Tetraketone* (V; R = OAc, X = O).—The tetraketone (584 mg.) in 9 : 1 aqueous-alcoholic 0·1N-potassium hydroxide (300 ml.) was shaken for 1 hr. under oxygen (0·91 mol. absorbed). The light yellow mixture was kept in the dark for another 2 hr., diluted with water (400 ml.), and extracted with chloroform, giving yellow crystals (477 mg.). For further purification these (288 mg.) were chromatographed on acid-washed alumina (20 g.). Elution with benzene (400 ml.) gave semi-crystalline material (57 mg.); further elution with 9 : 1 benzene–chloroform (100 ml.) afforded yellow crystals (167 mg.), m. p. 197—206°. Further elution with the same solvent gave a small amount of colourless crystals. The yellow crystals were filtered through acid-washed alumina (20 g.) in 19 : 1 benzene–chloroform. Fractions of yellow crystalline material of m. p. 207—211° were combined (66 g.) and crystallised from benzene–hexane to give the *dienedione* (VII), m. p. 210·5—211°, $[\alpha]_D$ +438° (*c* 0·56), $\lambda_{max.}$ 239 and 284 mμ (ε 13,400 and 21,300, respectively), $\nu_{max.}$ 1706, 1659, 1597, and 1564 cm.$^{-1}$ (Found: C, 75·2; H, 7·2. $C_{23}H_{26}O_4$ requires C, 75·4; H, 7·2%).

Cucurbitone B (X).—Cucurbitacin B (10 g.) was treated with boiling acetic anhydride (90 ml.) for 1·5 hr. The excess of anhydride was destroyed with water (15 ml.), and the mixture

[26] Bricker and Johnson, *Ind. Eng. Chem., Analyt.*, 1945, **17**, 400.

oxidised at 50—55° by dropwise addition of chromium trioxide (9 g.) in 90% acetic acid (85 ml.) during 3·5 hr. After a further 4 hr. at this temperature and 14 hr. at room temperature, methanol (10 ml.) and water (600 ml.) were added and the mixture was extracted with chloroform. The chloroform extract was washed with water, formamide, and again with water. The product crystallised from benzene–hexane, to afford cucurbitone B (4·4 g.), m. p. 232—234°. The gum from the mother-liquors was filtered in 49 : 1 chloroform–methanol through silica, to yield a further 1·2 g. of cucurbitone B. Crystallisation of the crude product from acetone–hexane gave cucurbitone B, m. p. 233—234° (Pyrex capillary) or 217—220° (soda-glass capillary). In both cases the sample melted and resolidified when placed in a preheated block at 210°, and had $[\alpha]_D$ +104° (*c* 0·98), $\lambda_{max.}$ 244 and 335 mμ (ε 13,000 and 105, respectively), and $\nu_{max.}$ 1736, 1712 sh, 1704, 1664, and 1621 cm.$^{-1}$ (Found: C, 67·4; H, 7·4. Calc. for $C_{28}H_{36}O_8$: C, 67·2; H, 7·3%). Cucurbitone B is identical (m. p., mixed m. p., and infrared spectrum) with " ketone A " [12] kindly supplied by Professor C. R. Noller.

Conjugated Dienedione (XI) *from Cucurbitone B* (X).—Cucurbitone B (X) (505 mg.) was heated in boiling methanol (30 ml.) and concentrated hydrochloric acid (20 ml.) for 2 hr. under nitrogen. Water (50 ml.) was added and the product isolated with chloroform and purified by chromatography on silica (30 g.). Elution with 1 : 1 benzene–chloroform (300 ml.) and crystallisation from chloroform–methanol gave the yellow dienedione (XI) (206 mg.), m. p. 236—237°, $[\alpha]_D$ +506° (*c* 1·2), $\lambda_{max.}$ 238 and 288 mμ (ε 12,800 and 19,800, respectively), $\nu_{max.}$ 1704, 1662, 1594, and 1572 cm.$^{-1}$ (Found: C, 75·6; H, 7·5. Calc. for $C_{24}H_{28}O_4$: C, 75·8; H, 7·4%). The substance is identical (m. p., mixed m. p., and infrared spectrum) with a sample (kindly supplied by Professor C. R. Noller) of a substance first prepared by him in a similar way from cucurbitone B.

Reduction of the Dienedione (XI) *with Zinc in Acetic Acid.*—The dienedione (XI) (216 mg.) was stirred with acetic acid (15 ml.) and zinc dust (1 g.) for 4 hr. at room temperature. The product, crystallised from chloroform–methanol, gave the colourless reduction *product* (XIII) (147 mg.), m. p. 222—223°, $[\alpha]_D$ +145° (*c* 1·1), $\lambda_{max.}$ 238 and 287 mμ (ε 9400 and 560, respectively), $\nu_{max.}$ 1718 sh, 1701, 1665, and 1594 cm.$^{-1}$ (Found: C, 75·3; H, 7·9. $C_{24}H_{30}O_4$ requires C, 75·4; H, 7·9%). This product (49 mg.) in 0·1N-alcoholic sodium hydroxide (10 ml.) was shaken for 5 min. under oxygen (0·87 mol. absorbed). Extraction of the red solution with chloroform and crystallisation from chloroform–methanol gave the dienedione (XI) (m. p., mixed m. p., ultraviolet and infrared spectra).

Trienone (XVI) *from Cucurbitone A* (XV).—Cucurbitone A (XV) (0·86 g.) was treated with sodium acetate (1 g.) in boiling acetic anhydride (40 ml.) for 5½ hr. The mixture was poured into benzene (100 ml.), extracted with water, and dried. Evaporation under reduced pressure at moderate temperature and then under a high vacuum left a brown oil. This was taken up in di-isopropyl ether (*ca.* 80 ml.). On cooling, the *trienone* (XVI) crystallised as yellow needles (560 mg.). Recrystallised from the same solvent, this had m. p. 88—90°, $[\alpha]_D$ +593° (*c* 1·0), $\lambda_{max.}$ 238 and 357 mμ (ε 14,400 and 9200, respectively), $\nu_{max.}$ (in Nujol mull) 1764, 1739, 1701, 1660, 1645, 1592, and 1570 cm.$^{-1}$ (Found: C, 69·75; H, 6·9; Ac, 18·6. $C_{28}H_{32}O_7$ requires C, 70·0; H, 6·7; 2Ac, 17·9%).

Dienedione (XVIII) *from the Trienone* (XVI).—The trienone (XVI) (670 mg.) in glacial acetic acid (40 ml.) containing 60% perchloric acid (0·06 ml.) was warmed overnight at 50—55°. The solution was poured into benzene (100 ml.), and the mixture extracted with water. The aqueous extracts were washed with benzene, and the combined benzene solutions were washed with water, dried, and evaporated. The residual oil crystallised when scratched. After two recrystallisations from ethanol the *dienedione* (XVIII) (340 mg.) was obtained; it had m. p. 223—224° (transition to long needles at 215°), $[\alpha]_D$ +487° (*c* 1·0), $\lambda_{max.}$ 238 and 287 (ε 13,600 and 19,500, respectively in EtOH), or 250 (shoulder) and 417 mμ (ε 20,000 and 9400, respectively, in EtOH with one drop of 4N-NaOH), or 227 and 347 mμ (in EtOH on reacidification), $\nu_{max.}$ 1747, 1711, 1668, and 1596 cm.$^{-1}$ (Found: C, 71·5; H, 6·85; Ac, 10·3. $C_{26}H_{30}O_6$ requires C, 71·2; H, 6·9; 1Ac, 9·8%).

Phenolic Compound (XXI) *from the Dienedione* (XVIII).—The dienedione (XVIII) (108 mg.) in methanol (3 ml.) and benzene (1·2 ml.) (both previously boiled) was treated under nitrogen with boiled N-sodium hydroxide (1 ml.). The deep red solution was acidified after 30 min. and the mixture extracted with chloroform. The washed and dried solution gave a foam which crystallised when triturated with freshly distilled methylene dichloride (65 mg.). Recrystallisation from methylene dichloride gave the *phenol* (XXI), m. p. 248—252°, $[\alpha]_D^{25}$ −11°

(*c* 1·0), $\lambda_{max.}$ 215, 227 (shoulder), 265 (shoulder), and 334 mμ (ε 23,000, 21,000, 7700, and 4100, respectively, in EtOH) or 244 and 415 mμ (ε 16,300 and 5260, respectively, in EtOH with one drop of 4N-NaOH), $\nu_{max.}$ 3268, 1706 (shoulder), 1669, and 1595 cm.$^{-1}$ (Found: C, 63·65; H, 5·95; Cl, 17·65. $C_{23}H_{26}O_4,CH_2Cl_2$ requires C, 63·85; H, 6·25; Cl, 15·7%). A sample was dried to constant weight (Found: loss in wt., 18·15. $C_{23}H_{26}O_4,CH_2Cl_2$ requires CH_2Cl_2, 18·85%).

Phenolic Compound (XXI) *from the Trienone* (XVI).—The trienone (XVI) (153 mg.) in previously boiled methanol (10 ml.) was treated under nitrogen with 2N-sodium hydroxide (15 ml.). After 30 min. the red solution was acidified and extracted with chloroform. Working up as above gave the phenol (XXI) (35 mg.) (m. p. and mixed m. p.). The combined aqueous layer was distilled and the distillate (250 ml.) treated with dimedone (500 mg.) in ethanol, to furnish the derivative of formaldehyde (m. p., mixed m. p., and crystal form).

Nor-product (XX) *from Dihydrocucurbitone A* (XIX).—The crude sodium methoxide product, m. p. 278—279° (802 mg.), prepared from dihydrocucurbitone A,[18] was dissolved in ethanol (216 ml.), N-sodium hydroxide (24 ml.) added, and the mixture kept under nitrogen at room temperature for 19 hr. The red solution was acidified with dilute hydrochloric acid and, after 20 min., extracted with chloroform. The aqueous layer was steam-distilled.

To the distillate (chromotropic colour test [26] strongly positive), dimedone (900 mg.) was added and the solution kept for 2 days. On concentration to 400 ml. and cooling, the formaldehyde derivative (227 mg.) crystallised (m. p. and mixed m. p.).

The chloroform extract yielded on evaporation a foam (846 mg.) which was filtered in chloroform solution through acid-washed alumina (50 g.). The first 100 ml. of eluate gave material (390 mg.) showing mainly a spot of R_F 0·86 on paper chromatograms (2 : 1 ethyl acetate–benzene, T.T.C. spray reagent), and crystallised from chloroform–methanol, to give the *nor-product* (XX) (200 mg.), m. p. 242—247°, $[\alpha]_D$ +187° (*c* 1·07), $\lambda_{max.}$ 240 mμ (ε 9400), $\nu_{max.}$ 1709, 1669, and 1600 cm.$^{-1}$ (Found: C, 71·2; H, 8·0. $C_{23}H_{30}O_5$ requires C, 71·5; H, 7·8%).

Anhydrodihydrohexanorcucurbitacin C (XXIV; R = H).—Crude anhydrohexanorcucurbitacin C (XXV) (2·2 g.) was hydrogenated over 2% palladised calcium carbonate (1 g.) in ethanol (300 ml.) for 25 hr. (0·99 mol. absorbed). The product was dissolved in chloroform (50 ml.), filtered from insoluble material (262 mg.), and poured on a column of acid-washed alumina (200 g.). Elution with chloroform containing 0·5% of methanol afforded fractions containing one component (paper chromatography, 1 : 1 ethyl acetate–benzene, V.P.A. spray reagent). Crystallisation from chloroform–methanol gave the *dihydro-compound* (XXIV; R = H), m. p. 234—237°, $\lambda_{max.}$ 290 mμ (ε 96) (Found: C, 73·7; H, 9·1. $C_{24}H_{36}O_4$ requires C, 74·2; H, 9·0%).

Anhydrodihydroheptanorcucurbitacin C (XXVII).—Anhydrodihydrohexanorcucurbitacin C (XXIV; R = H) (382 mg.) in ethanol (180 ml.) and N-sodium hydroxide (20 ml.) was refluxed under nitrogen for 2 hr. Water (100 ml.) was added, the solution acidified with dilute hydrochloric acid and concentrated to half its volume. The distillate gave a strongly positive chromotropic colour test for formaldehyde.[26] The concentrate was diluted with water (100 ml.) and extracted with chloroform. The chloroform extract was washed consecutively with 0·5N-aqueous sodium hydroxide, dilute hydrochloric acid, and water and evaporated to a foam (380 mg.), which crystallised from chloroform–ether and sublimed *in vacuo* at 150—170° to furnish the *hydroxy-diketone* (XXVII) (100 mg.), m. p. 197—201°, $[\alpha]_D$ −75° (*c* 1·0) (Found: C, 76·9; H, 9·4. $C_{23}H_{34}O_3$ requires C, 77·1; H, 9·7%).

Triketone (XXIX; R = H) *from the Hydroxy-diketone* (XXVII).—Anhydrodihydroheptanorcucurbitacin C (XXVII) (400 mg.) in acetone (100 ml.) was treated with an aliquot part (0·45 ml.) of chromium trioxide (2·67 g.) in 11·5% sulphuric acid (10 ml.) at 5—10° during 15 min. After a further 10 min., methanol (4 ml.) and water (100 ml.) were added, and the product was isolated with chloroform and separated with sodium hydrogen carbonate solution to give a neutral fraction (380 mg.). Crystallisation from chloroform–methanol and chloroform–ether gave the *triketone* (XXIX; R = H) (250 mg.), m. p. 238—243°, $[\alpha]_D$ −116° (*c* 1·0), $\lambda_{max.}$ 295 mμ (ε 188) in ethanol and in 0·1N-potassium hydroxide in 50% ethanol, $\nu_{max.}$ 1706 cm.$^{-1}$ (Found: C, 77·2; H, 9·0. $C_{23}H_{32}O_3$ requires C, 77·5; H, 9·1%).

Anhydroheptanorcucurbitacin C (XXVI; R = H).—Anhydrohexanorcucurbitacin C (XXV) (400 mg.) in ethanol (180 ml.) and N-aqueous sodium hydroxide (20 ml.) was refluxed under nitrogen for 2 hr. Water (150 ml.) was added, and the solution acidified (pH 4·0) with dilute sulphuric acid and concentrated until 150 ml. of distillate had been collected. Extraction of the concentrate with chloroform gave a crude foam (360 mg.). A further (100 ml. of distillate from the aqueous solution was combined with the first and dimedone (450 mg.) added. The

solution was left overnight, then concentrated further until turbid; on cooling, the formaldehyde derivative (26%) crystallised (m. p. and mixed m. p.). The formation of formaldehyde during the reaction was confirmed by the positive chromotropic reaction [26] given by the distillate.

The above-mentioned foam was purified by chromatography on acid-washed alumina (25 g.). Elution with 1 : 1 chloroform–benzene (200 ml.) gave fractions which showed only one spot on paper chromatograms (2 : 1 benzene–ethyl acetate; V.P.A. spray reagent). Crystallisation from chloroform–benzene gave *anhydroheptanorcucurbitacin C* (XXVI; R = H) (165 mg.), m. p. 252—258°, $[\alpha]_D$ −191° (*c* 1·0), $\lambda_{max.}$ 239 and 312 mμ (ε 9200 and 61, respectively), $\nu_{max.}$ 1695, 1667, and 1592 cm.$^{-1}$ (Found: C, 77·4; H, 9·1. $C_{23}H_{32}O_3$ requires C, 77·5; H, 9·1%).

Acetylation in boiling acetic anhydride under nitrogen gave the *monoacetate*, m. p. 216—219°, $\nu_{max.}$ 1724, 1701, 1668, and 1595 cm.$^{-1}$ (Found: C, 75·1; H, 8·6. $C_{25}H_{34}O_4$ requires C, 75·3; H, 8·6%).

Triketone (XXVIII; R = H) *from Compound* (XXVI; R = H).—Anhydroheptanorcucurbitacin C (XXVI; R = H) (400 mg.) was oxidised in acetone (100 ml.) with 8N-chromic acid (0·45 ml.) and worked up as above, yielding a neutral fraction. This was purified by chromatography on acid-washed alumina (20 g.). Elution with benzene (300 ml.) and benzene–chloroform (300 ml.) gave fractions which crystallised from chloroform–methanol and chloroform–ether to give the *triketone* (XXVIII; R = H) (300 mg.), m. p. 247—250°, $[\alpha]_D$ −243° (*c* 1·0), $\lambda_{max.}$ 239 and 297 mμ (ε 9390 and 186, respectively) in ethanol and in 0·1N-potassium hydroxide in 50% ethanol, $\nu_{max.}$ 1704, 1669, and 1595 cm.$^{-1}$ (Found: C, 77·5; H, 8·5. $C_{23}H_{30}O_3$ requires C, 77·9; H, 8·5%).

Oxidation of Anhydrohexanorcucurbitacin C (XXV).—Anhydrohexanorcucurbitacin C (XXV) (2 g.) was partially oxidised in acetone (300 ml.) with 8N-chromic acid (1 ml.), giving a neutral fraction. This was shown to be a mixture on paper chromatography (5 : 1 benzene–ethyl acetate, V.P.A. spray reagent), showing spots at R_F 0·73 (grey, weak), 0·68 (purple, weak), 0·51 (green), and 0·22 (purple, strong). Chromatography on acid-washed alumina (100 g.) and elution with benzene–chloroform (4 : 1 ⟶ 1 : 1) and chloroform separated the mixture into four fractions (a, b, c, and d) enriched in the substances of R_F 0·73, 0·68, 0·51, and 0·22, respectively. Fraction d (606 mg.) contained unchanged starting material.

Fraction a (76 mg.) contained the fully oxidised triketo-aldehyde (XXVIII; R = CHO). This compound was obtained in better yield on oxidation of the anhydrohexanor-compound (XXV) (1 g.) in acetone (250 ml.) with an excess (1·4 ml.) of 8N-chromic acid; the neutral fraction was purified by chromatography on acid-washed alumina (40 g.). Elution with 9 : 1 benzene–chloroform (600 ml.) and crystallisation from methanol–ether and chloroform–ether gave the *triketo-aldehyde* (XXVIII; R = CHO) (250 mg.), m. p. 205—207°, $[\alpha]_D$ −2° (*c* 1·0), $\lambda_{max.}$ 239 and 298 mμ (ε 9280 and 186, respectively, in EtOH) or 242 and 316 mμ (ε 10,100 and 12,700, respectively, in 0·1N-potassium hydroxide in 50% EtOH), $\nu_{max.}$ 1718, 1695. 1671, and 1595 cm.$^{-1}$ (Found: C, 75·1; H, 7·7. $C_{24}H_{30}O_4$ requires C, 75·4; H, 7·9%).

Fraction b (96 mg.) was rechromatographed on acid-washed alumina (10 g.). Elution with 2 : 1 benzene–chloroform (190 ml.) and crystallisation from chloroform–ether gave the *hydroxy-diketo-aldehyde* (XXVI; R = CHO) (16 mg.), m. p. 200—202°, $[\alpha]_D$ *ca.* 0° (*c* 0·77), $\lambda_{max.}$ 239 mμ (ε 10,600) (unchanged in 0·1N-potassium hydroxide in 50% ethanol for 50 hr. at room temperature or on boiling under nitrogen for 1 hr.), $\nu_{max.}$ 3540, 1724, 1692, 1669, and 1590 cm.$^{-1}$ (Found: C, 74·5; H, 8·3. $C_{24}H_{32}O_4$ requires C, 75·0; H, 8·4%). The nuclear magnetic resonance spectrum showed a band for an aldehyde group at τ −0·02. This aldehyde (4 mg.) was boiled in 1 : 1 aqueous-alcoholic 0·1N-potassium hydroxide (5 ml.) under nitrogen for 1 hr. After dilution with water (5 ml.), the product was isolated with chloroform and crystallised from chloroform–benzene, to give anhydroheptanorcucurbitacin C (XXVI; R = H) (1·1 mg.), m. p. 243—247°, identified by paper chromatography and infrared spectrum. The aqueous layer was concentrated to half its volume, acidified with hydrochloric acid, and distilled. Formic acid was detected in the distillate by a positive chromotropic reaction after reduction.[27]

Fraction c (550 mg.) was rechromatographed on acid-washed alumina (50 g.). Elution with benzene–chloroform (2 : 1, 600 ml.; 3 : 2, 500 ml.) and crystallisation from benzene–ether and chloroform–ether gave the hydroxy-triketone (XXVIII; R = CH_2·OH) (260 mg.), m. p. 191—193°, $[\alpha]_D$ +167° (*c* 1·1), $\lambda_{max.}$ 241 mμ (ε 8500) in ethanol and in 0·1N-potassium hydroxide in 50% ethanol, $\nu_{max.}$ 3528, 1712, 1692, 1669, and 1590 cm.$^{-1}$ (Found: C, 74·55;

[27] F. Feigl, " Qualitative Analysis by Spot Tests," Elsevier Publ. Co., Amsterdam, 1939, p. 329.

H, 8·4. $C_{24}H_{32}O_4$ requires C, 75·0; H, 8·4%). The nuclear magnetic resonance spectrum showed signals at τ 5·64 and 6·69 (doublets, J 10·9 c./sec.) of a hydroxymethyl group, and no signal for an aldehyde group. Oxidation of this hydroxy-triketone (35 mg.) in acetone with 8N-chromic acid as above gave the triketo-aldehyde (XXVIII; R = CHO) (8 mg.) (m. p., mixed m. p., and infrared spectrum).

Isomerisation of Triketo-monoaldehyde (XXVIII; R = CHO) *in Alkali.*—The triketo-monoaldehyde (200 mg.) in ethanol (70 ml.) and N-sodium hydroxide (5 ml.) was kept at room temperature for 30 min. After acidification, the product was extracted with chloroform. The extract, washed free from all trace of acid, gave, after crystallisation from chloroform–methanol and chloroform ether, *enol-I*, m. p. 214—219°, $[\alpha]_D$ −247° (*c* 1·0), $\lambda_{max.}$ 241 and 286 mμ (ε 10,600 and 8700, respectively), $\lambda_{max.}$ in 0·1N-alcoholic potassium hydroxide 242 and 316 mμ (ε 10,900 and 18,100, respectively, after 2 min.), $\nu_{max.}$ 1700, 1668, 1637 (shoulder), and 1595 cm.$^{-1}$ (Found: C, 75·8; H, 8·1. $C_{24}H_{30}O_4$ requires C, 75·4; H, 7·9%).

After incomplete washing of the chloroform extract, or on boiling enol-I in 0·01N-alcoholic hydrochloric acid, *enol-II* was obtained. Recrystallised from chloroform–ethanol and chloroform ether this had m. p. 269—275°, $[\alpha]_D$ −245° (*c* 1·0), $\lambda_{max.}$ 242 and 277 mμ (ε 10,900 and 11,200, respectively) (Found: C, 75·2; H, 8·1%). In 0·1N-alcoholic potassium hydroxide a band at 316 mμ slowly developed (ε 9250, 15,000, and 17,500 after 20 min., 1 hr., and 18 hr., respectively); $\nu_{max.}$ were at 1701, 1694, and 1594 cm.$^{-1}$. Enol-II was converted into enol-I by enolisation in alkaline solution, acidification, and removal of all trace of acid from the chloroform extract.

Oxidation of Anhydrodihydrohexanorcucurbitacin C (XXIV; R = H) *with Chromium Trioxide.*—The compound (XXIV; R = H) (300 mg.) in acetone (70 ml.; stabilised over potassium permanganate) was treated with an aliquot part (0·59 ml.) of chromium trioxide (2·67 g.) in 11·5% sulphuric acid (10 ml.) at 5—10°. After dilution with water, the product was isolated with chloroform and separated into acid (15 mg.) and neutral (260 mg.) fractions. The latter was partially crystalline and was separated on acid-washed alumina (10 g.). Elution with benzene (160 ml.) and 9 : 1 benzene–chloroform; (200 ml.) and crystallisation from chloroform–methanol and chloroform–ether afforded the *triketo-aldehyde* (XXIX; R = CHO) as prisms, m. p. 202—206°, $[\alpha]_D$ +100° (*c* 1·0), $\lambda_{max.}$ 295 mμ (ε 190), $\nu_{max.}$ 1709 cm.$^{-1}$ (Found: C, 74·9; H, 8·3. $C_{24}H_{32}O_4$ requires C, 75·0; H, 8·4%).

Further elution of the column with 1 : 1 chloroform–benzene (100 ml.) and chloroform (100 ml.) gave fractions (51 mg.) of m. p. 242—251°. Crystallisation from chloroform–methanol and chloroform–ether afforded the *hydroxy-triketone* (XXIX; R = CH_2OH) as needles, m. p. 251—253°, $\lambda_{max.}$ 290 mμ (ε 139) in neutral solution and in alcoholic 0·1N-potassium hydroxide, $\nu_{max.}$ 3509 and 1706 cm.$^{-1}$ (Found: C, 74·0; H, 8·9. $C_{24}H_{34}O_4$ requires C, 74·6; H, 8·9%). On treatment of the above hydroxy-triketone (XXIX; R = CH_2OH) with boiling ethanolic 0·1N-potassium hydroxide the triketone (XXIX; R = H) was obtained, identified by m. p., mixed m. p., and infrared spectrum.

Correlation of Cucurbitacins A and C.—Cucurbitacin A triacetate (4·2 g., 6 mmoles) in toluene (70 ml.) was concentrated at normal pressure to 40 ml. and added during 5 min. with stirring to calcium turnings (1·82 g.) in liquid ammonia (120 ml.) and stirred for a further 30 min. The excess of calcium was destroyed by dropwise addition of bromobenzene (3 ml.). The mixture was protonated with solid ammonium chloride (4 g.), and the ammonia allowed to evaporate. The resulting slurry was added to water (500 ml.) and made weakly acid with hydrochloric acid, and the crude product (2·5 g.) was isolated with chloroform.

0·5M-Periodic acid (25 ml.) and water (100 ml.) were added to the above crude product (2·5 g.) in ethanol (160 ml.) and the whole was kept at room temperature for 40 hr. The excess of reagent was destroyed with ethylene glycol, the solution diluted with water (300 ml.), and the product isolated with chloroform. The crude product yielded a neutral fraction (2·03 g.) which was dissolved in 90% ethanolic 0·1N-sodium hydroxide and kept at 20° under nitrogen for 17 hr. Dilution with water (300 ml.) and extraction with chloroform gave a neutral fraction (1·42 g.) which was shown to be a mixture on paper chromatography (4 : 1 benzene–ethyl acetate, V.P.A. spray reagent). Chromatography on acid-washed alumina (75 g.) and elution with benzene–chloroform (9 : 1 ⟶ 1 : 1) separated the crude product into two main fractions which were enriched in substances of R_F 0·87 and 0·68, respectively. Rechromatography of the above two fractions and crystallisation from methanol–ether gave the following substances which, on further crystallisation, were found to be identical (m. p., mixed m. p., and infrared

spectra) with the corresponding compounds prepared from cucurbitacin C: (i) triketone (XXVIII; R = H) (113 mg.); (ii) hydroxy-triketone (XXVIII; R = CH_2·OH) (78 mg.); and (iii) hydroxy-triketone (XXIX; R = CH_2OH) (14 mg.).

Oxidation of material (ii) gave a triketo-aldehyde identical (m. p., mixed m. p., and infrared spectrum) with compound (XXVIII; R = CHO) prepared from cucurbitacin C (see above).

Correlation of Cucurbitacins B and C.—Cucurbitacin B diacetate (7·7 g.) in toluene (120 ml.) was concentrated at normal pressure to 70 ml., and added during 10 min. with vigorous stirring to calcium turnings (3·64 g.) in liquid ammonia (240 ml.). After a further 30 minutes' stirring, the excess of calcium was destroyed by dropwise addition of bromobenzene (6 ml.), and the mixture protonated with solid ammonium chloride (8 g.). The ammonia was allowed to evaporate, the resulting slurry was poured into water (1 l.) and acidified with hydrochloric acid, and the product (5·06 g.) was isolated with chloroform. The crude reduction product (5 g.) in ethanol (450 ml.) and water (300 ml.) was treated with 0·5M-periodic acid (60 ml.) at 20° for 90 hr. (1·2 mol. consumed) and the product (3·8 g.) was isolated with chloroform. The crude product in 90% ethanolic 0·1N-sodium hydroxide (400 ml.) was refluxed under nitrogen for 1 hr. Dilution with water (600 ml.) and extraction with chloroform gave a neutral fraction (3 g.). Chromatography on acid-washed alumina (200 g.) and elution with 1 : 1 benzene–chloroform, chloroform, and 98 : 2 chloroform–methanol separated the crude product into three main fractions: (i) an oil (770 mg.), (ii) a crystalline fraction (668 mg.), and (iii) an amorphous fraction (790 mg.).

The crystalline fraction, crystallised from methanol–ether and chloroform–ether, gave the diketo-alcohol (XXXIII), m. p. 218—220°, $[\alpha]_D$ +52° (*c* 1·0), $\lambda_{max.}$ 244 and 295 mμ (ε 9100 and 123, respectively), $\nu_{max.}$ 3509, 1709, 1667, and 1589 cm.$^{-1}$ (Found: C, 77·8; H, 9·4. Calc. for $C_{24}H_{34}O_3$: C, 77·8; H, 9·3%). This compound should be identical with a substance obtained by the same procedure by Melera, Gut, and Noller.[10]

Reduction and Oxidation of the Diketo-alcohol (XXXIII).—The diketo-alcohol (XXXIII) (425 mg.) was hydrogenated over 5% palladised calcium carbonate (140 mg.) in ethanol (150 ml.) (0·91 mol. absorbed in 10 min.). The crude product in acetone (50 ml.) was treated with an aliquot part (0·75 ml.) of chromium trioxide (2·67 g.) in 11·5% sulphuric acid (10 ml.) during 1 hr. After addition of methanol (2 ml.) and water (150 ml.), the product was extracted with chloroform, and the extract washed with 0·1N-sodium hydroxide and water to give a crude crystalline product. This was purified by chromatography on acid-washed alumina (20 g.). Elution with 4 : 1 benzene–chloroform and crystallisation from chloroform–ether gave the *triketone* (XXXIV; X = O) (280 mg.), m. p. 201—202°, $[\alpha]_D$ +261° (*c* 1·0), $\lambda_{max.}$ 292 mμ (ε 142), $\nu_{max.}$ 1708 cm.$^{-1}$ (Found: C, 77·8; H, 9·3. $C_{24}H_{34}O_3$ requires C, 77·8; H, 9·3%).

Further quantities of the above triketone were obtained from the oily (i) and amorphous (iii) fractions (see above). Oxidation of the oily fraction (770 mg.) with chromic acid in acetone and filtration of the product through acid-washed alumina (30 g.) with 9 : 1 benzene–chloroform gave the above triketone (118 mg.).

The amorphous fraction (iii) (790 mg.) was oxidised with chromic acid in acetone and hydrogenated in ethanol over palladised calcium carbonate; it gave a crude product which was filtered through acid-washed alumina (30 g.) with 19 : 1 benzene–chloroform, affording a further amount (122 mg.) of the triketone.

Monoketone (XXXIV; X = H_2).—The triketone (XXXIV; X = O) (423 mg.) was treated with ethane-1,2-dithiol (0·5 ml.) and boron trifluoride–ether complex (0·5 ml.) for 2·5 hr. The crystals formed were washed with methanol, to give a bisthioketal (536 mg.). Desulphurisation with Raney nickel W_2 (*ca.* 6 g.) in boiling dioxan (100 ml.) for 16 hr. gave a crude crystalline product (332 mg.) which was purified by chromatography on acid-washed alumina (30 g.). Elution with 9 : 1 hexane–benzene and repeated crystallisations from chloroform–methanol gave the *monoketone* (XXXIV; X = H_2), m. p. 150—151°, $[\alpha]_D$ +244° (*c* 0·74), $\lambda_{max.}$ 295 mμ (ε 61), $\nu_{max.}$ (in CS_2) 1697 cm.$^{-1}$ (Found: C, 84·4; H, 11·1. $C_{24}H_{38}O$ requires C, 84·2; H, 11·2%).

Monoketone (XXXIV; X = H_2) *from the Triketo-aldehyde* (XXIX; R = CHO).—The aldehyde (XXXI) (344 mg.) was treated with ethane-1,2-dithiol (0·75 ml.) and boron trifluoride–ether complex (0·75 ml.) for 2·5 hr. The crude crystalline product (532 mg.) was treated in boiling dioxan (150 ml.) with Raney nickel W_2 (*ca.* 6 g.) for 16 hr. Chromatography of the crude crystalline product (230 mg.) on acid-washed alumina (30 g.), elution with 9 : 1 hexane–benzene, and repeated crystallisation from chloroform–methanol gave the monoketone (XXXIV; X = H_2), m. p. 154—156°, $[\alpha]_D$ +251° (*c* 0·26) (Found: C, 84·5; H, 11·3. Calc. for $C_{24}H_{38}O$:

C, 84·2; H, 11·2%). The compound was identical (mixed m. p. and infrared spectrum) with the substance prepared from cucurbitacin B (see above).

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