930. *Bitter Principles of the Cucurbitaceae. Part IX.* Cucurbitacin A.†*

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The structure of the iso-octyl side chain of the tetracyclic triterpenoid acetate, cucurbitacin A, $C_{32}H_{46}O_9$, has been shown to be as in (I; R = COMe). Experiments are described to locate the remaining oxygen atoms and the nuclear, trisubstituted double bond.

Cucurbitaceæ contain a group of highly toxic, closely related bitter principles which have so far not been found in any other plant family. From 64 species (20 genera) investigated, some fourteen crystalline bitter principles have been isolated.[1]

Cucurbitacin A is found in the three related species, *Cucumis hookeri*, *C. leptodermis*, and *C. myriocarpus*, and the formula $C_{28}H_{40}O_8$ has been proposed for it.[2] It was shown to contain an αβ-unsaturated keto-group ($\lambda_{max.}$ 229 mμ), and hydrogenation afforded dihydrocucurbitacin A in which the conjugated double bond had been saturated. Since dihydrocucurbitacin A gave a dioxime which still contained an unreactive keto-group, three keto-groups are present. Of the remaining five oxygen atoms, two are present in an acetoxy-group and at least two in hydroxy-groups.

On the basis of molecular-weight determinations by the *X*-ray method, the formula of cucurbitacin A has been revised [3] to $C_{32}H_{46}O_9$. We now find that it contains four active hydrogen atoms: acetylation gave an amorphous triacetate, $C_{38}H_{52}O_{12}$, which still contained a free hydroxy-group ($\nu_{max.}$ 3448 cm.$^{-1}$).

The dihydrocucurbitacin A described previously has now been found to contain about 10% of a product which is probably deacetoxydihydrocucurbitacin A (compare the reduction products [4] of cucurbitacin B). Both cucurbitacin A and its dihydro-derivative formed mono-2,4-dinitrophenylhydrazones whose ultraviolet absorption spectra indicated that an unconjugated ketone group had reacted. This keto-group is probably part of the α-ketol-system which caused an intense colour with tetrazolium blue.

Dihydrocucurbitacin A gave a pale yellow colour with tetranitromethane and so still

* Part VIII, *J. Sci. Food Agric.*, 1957, **8**, 687.

† For preliminary accounts see *Proc. Chem. Soc.*, 1958, 301, and *Chem. and Ind.*, 1959, 162.

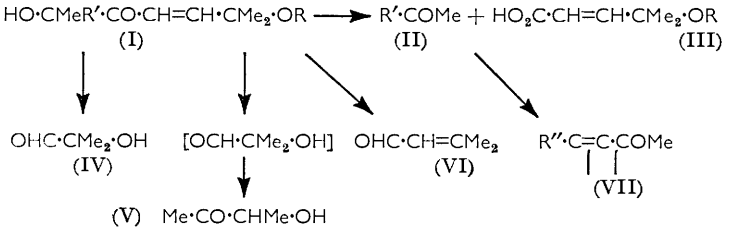
[1] Enslin and Rehm, *Proc. Linnean Soc.*, 1956—1957, 169 Session, p. 230; Lavie and Willner, *J. Amer. Chem. Soc.*, 1958, **80**, 710; Eisenhut and Noller, *J. Org. Chem.*, 1958, **23**, 1984.

[2] Enslin, *J. Sci. Food Agric.*, 1954, **5**, 410.

[3] Rivett and Herbstein, *Chem. and Ind.*, 1957, 393.

[4] Melera, Schlegel, and Noller, *J. Org. Chem.*, 1959, **24**, 291.

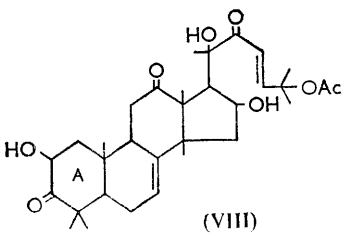
contains a double bond that resists hydrogenation. All these results point to the presence of four alicyclic rings. This is supported by the isolation [5] of 1,2,8-trimethylphenanthrene, a typical dehydrogenation product of tetracyclic triterpenes, on selenium dehydrogenation of reduced cucurbitacin A and now by complete elucidation of the structure of the side chain.



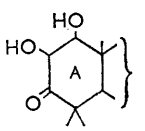
Oxidation of cucurbitacin A or its triacetate by periodic acid gave *trans*-4-acetoxy-4-methylpent-2-enoic acid (III; R = Ac), identified by its alkaline hydrolysis to acetic acid and the known *trans*-4-hydroxy-4-methylpent-2-enoic acid [6] (III; R = H). This oxidation of cucurbitacin A triacetate also afforded the methyl ketone, deoxocucurbitone A (II), $C_{30}H_{40}O_9$, thus establishing partial structure (I; R = COMe) for cucurbitacin A. This structure now offers an explanation for the formation of other side-chain fragments obtained.

Ozonolysis of cucurbitacin A gave α-hydroxy-α-methylpropionaldehyde (IV) (isolated as its 2,4-dinitrophenylhydrazone) by oxidative fission at the double bond and hydrolysis of the acetyl group during hydrazone formation. Alkaline hydrolysis of cucurbitacin A afforded acetoin (V), a rearrangement product of (IV), which is here produced by a retro-aldol condensation.[6,7] Oxidation of cucurbitacin A by chromic acid afforded senecioaldehyde (VI) which requires an allylic rearrangement to precede the oxidation.

The methyl ketone (II) readily lost acetic acid when adsorbed on acid-washed alumina, giving an anhydrodeacetyl substance, $C_{28}H_{36}O_7$ (VII), which showed an ultraviolet maximum at 240 mμ, and infrared bands at 1667 and 1595 cm.$^{-1}$, consistent with the presence of a disubstituted αβ-unsaturated keto-group. If a trimethyl-steroid structure is assumed for cucurbitacin A, then this compound (VII) would be a Δ^{16}-20-ketone formed by elimination of a 16-acetoxy-group as acetic acid.



(VIII)



(IX)

Recently [8] structure (VIII) was proposed for cucurbitacin B, which is the main bitter principle in young, unripe fruits of *Cucumis myriocarpus*. During ripening of the fruits the amount of cucurbitacin A slowly increases at the expense of cucurbitacin B, indicating a probable biogenetic relation.[1] Cucurbitacin A differs from B by having an extra hydroxy-group, which we now tentatively place at position 1 for the following reasons. The α-ketol group in ring A of cucurbitacin B is slowly autoxidised in dilute alkaline solution to a 1,2-diketo-group, as is shown by the development of an enolate band at 312 mμ, which

[5] Enslin and Rivett, *J.*, 1956, 3682.
[6] Lavie, Shvo, and Willner, *J. Amer. Chem. Soc.*, 1959, **81**, 3062.
[7] Rivett and Enslin, *Proc. Chem. Soc.*, 1958, 301.
[8] Lavie and Shvo, *Chem. and Ind.*, 1960, 403.

was displaced to 268 mμ (diosphenol band) on acidification. The solution then exhibited an intense, stable violet colour with ferric chloride. This α-ketol was selectively oxidised to the α-diketone, cucurbitacin E (α-elaterin), by bismuth oxide.[9] Contrary to our earlier report,[10] cucurbitacin A also developed a strong band at 312 mμ in alkaline solution. The enolate chromophore was, however, unstable and the band disappeared on prolonged treatment with alkali. Acidification displaced the band to 268 mμ and the solution then gave, with alcoholic ferric chloride, a violet-green colour which faded within 2 minutes (cf. the oxidation of enediols by ferric chloride[11]). These results are compatible with partial structure (IX) for cucurbitacin A. It is noteworthy, however, that the above acidified solution (from cucurbitacin A) reduced phenol-indo-2,6-dichlorophenol only very slowly, and somewhat more rapidly under alkaline conditions. Oxidation of cucurbitacin A with bismuth oxide in acetic acid resulted in a mixture from which no crystalline product was isolated.

Chromium trioxide oxidises the methyl ketone (II), $C_{30}H_{40}O_9$, or, more conveniently, cucurbitacin A triacetate to cucurbitone A, $C_{30}H_{38}O_{10}$, in good yield. Its molecular formula and the presence of a disubstituted αβ-unsaturated keto-group ($\lambda_{max.}$ 245 mμ, ε 11,900; $\nu_{max.}$ 1667 and 1625 cm.$^{-1}$) indicates that cucurbitone A is formed by oxidation of a methylene group alpha to a trisubstituted double bond. The double bond could then be reduced catalytically or by zinc in acetic acid to afford dihydrocucurbitone A, $C_{30}H_{40}O_{10}$, which showed only saturated keto-bands in the ultraviolet and the infrared spectrum.

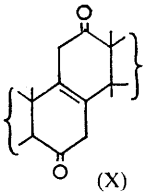
The 16-acetoxy-group in both cucurbitone A and dihydrocucurbitone A was readily eliminated on acid-washed alumina to give, respectively, Δ^{16}-anhydrodeacetylcucurbitone A, $C_{28}H_{34}O_8$ ($\lambda_{max.}$ 240 mμ, ε 19,800; $\nu_{max.}$ 1667, 1631, and 1594 cm.$^{-1}$), and Δ^{16}-anhydrodeacetyldihydrocucurbitone A, $C_{28}H_{36}O_8$ ($\lambda_{max.}$ 239 mμ, ε 8700; $\nu_{max.}$ 1669 and 1594 cm.$^{-1}$). The ultraviolet and infrared spectra are consistent with the presence of a Δ^{16}-20-keto-group in both products. On catalytic reduction of Δ^{16}-anhydrodeacetyldihydrocucurbitone A, 1 mol. of hydrogen was rapidly absorbed to give a saturated tetraketone, $C_{28}H_{38}O_8$ ($\lambda_{max.}$ 292 mμ, ε 154). Δ^{16}-Anhydrodeacetylcucurbitone A contains two αβ-unsaturated keto-groups of which the Δ^{16}-20-keto-group could be reduced readily, yielding a dihydro-derivative, $C_{28}H_{36}O_8$ ($\lambda_{max.}$ 244 mμ, ε 12,500; $\nu_{max.}$ 1667 and 1625 cm.$^{-1}$). The second conjugated double bond was only slowly reduced; this gave the above saturated tetraketone.

On treatment of dihydrocucurbitone A with sodium methoxide in dry methanol, two isomeric substances, $C_{24}H_{32}O_6$, were obtained. The major product, $\lambda_{max.}$ 239 mμ (ε 8900), $\nu_{max.}$ 1669 and 1592 cm.$^{-1}$, on acetylation, gave Δ^{16}-anhydrodeacetyldihydrocucurbitone A, $C_{28}H_{36}O_8$. Accordingly, the alkali caused saponification of two acetyl groups and elimination of the 16-acetoxy-group as acetic acid, to afford a Δ^{16}-20-ketone. The major product was also hydrogenated to a dihydro-derivative which, on acetylation, gave the tetraketone described above.

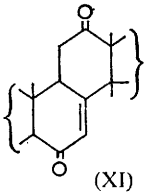
Cucurbitone A developed an unstable red colour ($\lambda_{max.}$ 495 mμ) in dilute alcoholic alkali. This red colour faded rapidly to yellow, probably as the result of autoxidation, since it was more stable in the absence of oxygen. On treatment of cucurbitone A with sodium methoxide in dry methanol under nitrogen, a dark, wine-red solution resulted from which two colourless crystalline isomers, $C_{24}H_{30}O_6$, were isolated. The ultraviolet maximum at 238 mμ and infrared bands at 1667 and 1594 cm.$^{-1}$ of these products showed the presence of a Δ^{16}-20-keto-group and the absence of the αβ-unsaturated keto-group ($\lambda_{max.}$ 245 mμ, $\nu_{max.}$ 1667 and 1625 cm.$^{-1}$) originally present in cucurbitone A. Both substances afforded diacetates, $C_{28}H_{34}O_8$. Catalytic hydrogenation of the diacetate of the major saponification product furnished a dihydro-diacetate, $C_{28}H_{36}O_8$, $\lambda_{max.}$ 289—290 mμ (ε 129), in which only the Δ^{16}-bond has been saturated. The two sodium methoxide products from cucurbitone A, their diacetates, and the above dihydro-diacetate, all gave the same red

[9] Lavie, Shvo, Willner, Enslin, Hugo, and Norton, *Chem. and Ind.*, 1959, 951.
[10] Enslin, Rehm, and Rivett, *J. Sci. Food Agric.*, 1957, **8**, 673.
[11] Arndt, Loewe, and Ayça, *Chem. Ber.*, 1951, **84**, 333.

colour as cucurbitone A with alcoholic alkali. If the inert double bond in cucurbitone A has moved out of conjugation on treatment with sodium methoxide to give a product with two keto-groups and a double bond in rings B and C (all unconjugated), then the product can only have partial structure (X), in which case cucurbitone A must have partial structure (XI) or (XII).*



(X)



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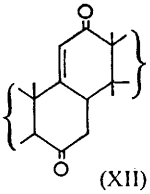

(XII)

However, neither of the sodium methoxide products nor their diacetates gave a colour with tetranitromethane. Experiments are in progress to confirm partial structure (X) for the sodium methoxide products of cucurbitone A.

Experimental

Unless specified to the contrary, $[\alpha]_D$ refers to chloroform, ultraviolet absorption spectra to ethanol, and infrared absorption spectra to chloroform solutions. Infrared frequencies were determined on a Perkin–Elmer Model 21 spectrometer equipped with a calcium fluoride prism. Where compounds were compared over the full range, a sodium chloride prism was used. The descending method of paper chromatography on Whatman No. 1 paper impregnated with a 50% solution of formamide in ethanol was used. The solvent systems employed are indicated in parentheses. Chromatograms were dried for 5 min. at 100°, sprayed with a freshly prepared 0·2% solution of triphenyltetrazolium chloride in N-potassium hydroxide (made up in 50% aqueous ethanol), and held over steam to reveal red spots. Acid-washed alumina was prepared by washing Peter Spence alumina consecutively with dilute hydrochloric acid, hot water, and ethanol and reactivating it at 150° for 12 hr.

Cucurbitacin A.—This substance was isolated as described previously [2] and had m. p. 207—208°, $[\alpha]_D$ +97° (*c* 1·04 in EtOH), $\lambda_{max.}$ 229 and 290 mμ (ε 12,200 and 206 respectively), $\nu_{max.}$ 1730 sh and 1258 (OAc), 1715 (C:O), 1692 and 1631 (CO·C:C) cm.$^{-1}$ [Found: C, 66·7; H, 8·1; active H,[12] 0·75, 0·73%; *M* (*X*-ray method), 587 ± 16. $C_{32}H_{46}O_9$ requires C, 66·9; H, 8·1; 4 active H, 0·7%; *M*, 575]. Cucurbitacin A gave a pale yellow colour with tetranitromethane and an intense colour with tetrazolium blue. The latter test was carried out by mixing equal volumes of a 0·02% alcoholic solution of cucurbitacin A and a freshly prepared 0·1% solution of tetrazolium blue in 0·02N-sodium hydroxide made up in 50% aqueous ethanol. A distinct wine-red colour after 30 min. at 40° was considered a positive result.

Cucurbitacin A 2,4-dinitrophenylhydrazone, prepared as described before [2] and crystallised from ethanol, had m. p. 230°, $\lambda_{max.}$ 233 and 370 mμ (ε 28,300 and 26,200 respectively) (Found: C, 60·4; H, 6·6; N, 7·9. $C_{38}H_{50}O_{12}N_4$ requires C, 60·5; H, 6·7; N, 7·4%).

Hydrogenation of Cucurbitacin A.—Cucurbitacin A (2 g.) was hydrogenated in ethanol (230 ml.) over 2% palladised calcium carbonate (1 g.). The uptake of hydrogen stopped after 5 min. (1·02 mol. absorbed). Crystallisation from ethyl acetate gave a crude product (1·83 g.) which showed two spots of R_F 0·30 and 0·49 on paper chromatograms (1 : 1 ethyl acetate–benzene) and was separated on cellulose powder impregnated with formamide [10] (300 g.). Elution with 3 : 1 benzene–ethyl acetate (1·5 l.) gave fractions containing mainly the minor component (R_F 0·30). Crystallisation from ethyl acetate afforded *deacetoxydihydrocucurbitacin A* (90 mg.), m. p. 135—136°, $[\alpha]_D$ +59° (*c* 0·9), $\lambda_{max.}$ 282 mμ (ε 232), $\nu_{max.}$ (sodium chloride prism) 1712 and 1698 (C:O) cm.$^{-1}$. The substance was hygroscopic and it was difficult to obtain satisfactory analyses (Found: C, 68·7; H, 9·2. $C_{30}H_{46}O_7$ requires C, 69·5; H, 8·9%).

Further elution of the column with the same solvent (600 ml.) gave mixed fractions. The

* Valuable comments by a Referee on this point are gratefully acknowledged.

[12] Perold and Snyman, *Mikrochim. Acta*, 1958, 225.

main substance was then eluted with benzene–ethyl acetate (proportion of ethyl acetate increasing up to 50%; 2·3 l.) and crystallised from ethyl acetate, to give *dihydrocucurbitacin A* (860 mg.), m. p. 138—139°, $[\alpha]_D$ +65° (*c* 1·0), $\lambda_{max.}$ 287 mμ (ε 199), $\nu_{max.}$ (sodium chloride prism) 1714 (OAc and C:O), 1256 (OAc) cm.$^{-1}$ (Found: C, 66·4; H, 8·6; Ac, 7·8. $C_{32}H_{48}O_9$ requires C, 66·6; H, 8·4; Ac, 7·5%). Dihydrocucurbitacin A gave a pale yellow colour with tetranitromethane. The 2,4-*dinitrophenylhydrazone*, prepared in the usual way and crystallised from ethanol, had m. p. 243—244°, $\lambda_{max.}$ 233 and 370 mμ (ε 17,500 and 27,300 respectively) (Found: N, 7·5. $C_{38}H_{52}O_{12}N_4$ requires N, 7·4%).

Oxidation of Cucurbitacin A with Chromium Trioxide.—A solution of chromium trioxide (4 g.) in 80% acetic acid (50 ml.) was added dropwise to cucurbitacin A (2 g.) in acetic acid (10 ml.) through which a current of steam was being passed. The distillate was collected in a solution of 2,4-dinitrophenylhydrazine (3 g.) in 5N-hydrochloric acid (100 ml.), and the resulting precipitate (50 mg.) purified by chromatography over silicic acid [13] (20 g.). Elution with 5 : 1 hexane–ether followed by crystallisation from benzene–hexane and then from ethanol furnished senecioaldehyde 2,4-dinitrophenylhydrazone (10 mg.), m. p. and mixed m. p. 179°, $\lambda_{max.}$ 382 mμ (ε 30,800) (Found: C, 50·1; H, 4·5. Calc. for $C_{11}H_{12}O_4N_4$: C, 50·0; H, 4·6%). No senecioaldehyde was isolated on similar treatment of dihydrocucurbitacin A.

Ozonolysis of Cucurbitacin A.—Ozone (9 mmole) was passed at room temperature through a solution of cucurbitacin A (750 mg., 1·3 mmole) in acetic acid (30 ml.). Decomposition of the ozonide with zinc dust (5 g.) and steam-distillation into a solution of 2,4-dinitrophenylhydrazine (500 mg.) in 3N-hydrochloric acid (200 ml.) afforded a precipitate (205 mg.) which was crystallised twice from benzene to give yellow prisms of α-hydroxy-α-methylpropionaldehyde 2,4-dinitrophenylhydrazone, m. p. and mixed m. p. 181°, $\lambda_{max.}$ 354 mμ (ε 22,000) (Found: C, 44·6; H, 4·6. Calc. for $C_{10}H_{12}O_5N_4$: C, 44·8; H, 4·5%).

Refluxing the above 2,4-dinitrophenylhydrazone with ethanolic hydrochloric acid for 5 min. afforded scarlet needles (from ethanol) of α-methylacraldehyde 2,4-dinitrophenylhydrazone, m. p. and mixed m. p. 201°, $\lambda_{max.}$ 368 mμ (ε 26,500) (Found: C, 48·0; H, 3·7; N, 22·9. Calc. for $C_{10}H_{10}O_4N_4$: C, 48·0; H, 4·0; N, 22·4%).

Periodic Acid Oxidation of Cucurbitacin A.—0·5M-Periodic acid (50 ml.) and water (180 ml.) were added to a solution of cucurbitacin A (4·6 g.) in ethanol (320 ml.), and the mixture was kept at room temperature for 19 hr. (1·9 mol. of periodic acid consumed). Excess of reagent was destroyed with ethylene glycol, and the ethanol evaporated under reduced pressure to give a clear aqueous solution and a gummy precipitate. The aqueous solution was extracted with ether (4 × 50 ml.), and the ether extract washed twice with water and dried (Na_2SO_4). Removal of the ether gave an oil (250 mg.) which afforded crystals (180 mg.) on trituration with ether. Recrystallisation twice from ether and sublimation *in vacuo* at 70° gave *trans*-4-acetoxy-4-methylpent-2-enoic acid, m. p. 84·5—84·9°, $\nu_{max.}$ (in CS_2; sodium chloride prism) 1739 and 1235 (OAc), 1695 and 1656 ($HO_2C{\cdot}C{:}C$) cm.$^{-1}$ (Found: C, 55·6; H, 7·1%; equiv., 171. Calc. for $C_8H_{12}O_4$: C, 55·8; H, 7·0%; *M*, 172).

trans-4-*Hydroxy*-4-*methylpent*-2-*enoic Acid.*—The above ester acid (153 mg.) was hydrolysed for 20 min. with N-sodium hydroxide (20 ml.) on a steam-bath to afford after acidification and isolation with chloroform, *trans*-4-hydroxy-4-methylpent-2-enoic acid, identified by m. p., mixed m. p. (102—103°), and infrared spectrum. A sample of the authentic acid was kindly supplied by Dr. D. Lavie. Acetic acid was isolated from the aqueous layer and identified by paper chromatography.[14]

Cucurbitacin A Triacetate.—Cucurbitacin A with boiling acetic anhydride for 1·5 hr. under nitrogen gave an amorphous *acetate*, which was purified by precipitation with water from alcoholic solution, then having $\lambda_{max.}$ 230 and 293 mμ (ε 11,100 and 192 respectively), $\nu_{max.}$ (sodium chloride prism) 3448 (OH), 1732 (OAc and C:O), 1701 and 1634 (CO·C:C) cm.$^{-1}$ (Found: C, 65·0; H, 7·9; Ac, 22·6. $C_{38}H_{52}O_{12}$ requires C, 65·1; H, 7·5; 4Ac, 24·5%).

Periodic Acid Oxidation of Cucurbitacin A Triacetate.—0·5M-Periodic acid (12 ml.) and water (60 ml.) were added to a solution of cucurbitacin A triacetate (1·32 g.) in ethanol (110 ml.), and the mixture was kept at room temperature for 70 hr. (0·83 mol. of periodic acid consumed). Excess of reagent was destroyed with ethylene glycol, and the ethanol evaporated under reduced pressure. An amorphous solid (800 mg.) separated. From the clear aqueous layer *trans*-4-acetoxy-4-methylpent-2-enoic acid was isolated as described above. Paper chromatography

[13] Carson, *J. Amer. Chem. Soc.*, 1951, **73**, 4652.
[14] Lindquist and Storgårds, *Acta Chem. Scand.*, 1953, **7**, 87.

(1 : 3 ethyl acetate–hexane) showed at least two components, which were separated on acid-washed alumina (100 g.). Elution with benzene (400 ml.), 9 : 1 benzene–chloroform (400 ml.), and 7 : 3 benzene–chloroform (350 ml.) removed only traces of material after which 7 : 3 benzene–chloroform (200 ml.) eluted material (108 mg.) of R_F 0·73. Crystallisation from chloroform–methanol furnished deoxocucurbitone A (91 mg.), m. p. 247—249°, $[\alpha]_D$ +110° (*c* 1·02), $\lambda_{max.}$ 289 mμ (ε 186), $\nu_{max.}$ 1736 (OAc and CO·CH·OAc), 1709 (C:O) cm.$^{-1}$ (Found: C, 66·4; H, 7·5. Calc. for $C_{30}H_{40}O_9$: C, 66·2; H, 7·4%). The substance gave a yellow colour with tetranitromethane and a positive iodoform test. Further elution of the column with 7 : 3 benzene–chloroform (400 ml.) and chloroform (400 ml.) gave mainly material of R_F 0·84 which did not crystallise.

Δ^{16}-Anhydrodeacetyldeoxocucurbitone A.—Deoxocucurbitone A (150 mg.) in chloroform (4 ml.) was adsorbed on acid-washed alumina (5 g.) and kept at room temperature in the dark for 4 weeks. Elution with 10 : 1 chloroform–methanol gave a crude product which was purified by chromatography on acid-washed alumina (8 g.). Elution with benzene (100 ml.) and 9 : 1 benzene–chloroform (60 ml.) gave fractions which contained only one component (paper chromatography; 3 : 1 hexane–ethyl acetate). Crystallisation from ethanol–hexane afforded the *anhydrodeacetyl-compound* (35 mg.), m. p. 209—210°, $[\alpha]_D$ +118° (*c* 0·7), $\lambda_{max.}$ 240 and 291—294 mμ (ε 9750 and 184 respectively), $\nu_{max.}$ 1736 (OAc and CO·CH·OAc), 1704 (hindered C:O), 1667 and 1595 (16-en-20-one) cm.$^{-1}$ (Found: C, 69·3; H, 7·7. $C_{28}H_{36}O_7$ requires C, 69·4; H, 7·5%).

Cucurbitone A.—(a) *From cucurbitacin A*. Cucurbitacin A (10 g.) was acetylated with boiling acetic anhydride (100 ml.) in the usual way. Excess of anhydride was destroyed with water (20 ml.), and the mixture oxidised at 50° by dropwise addition of chromium trioxide (9·4 g.) in 90% acetic acid (30 ml.) during 4 hr. After a further 6 hr. at this temperature and 12 hr. at room temperature, methanol (5 ml.) and water (400 ml.) were added and the mixture was extracted with chloroform. The product crystallised from chloroform–methanol to afford cucurbitone A as needles (6 g.), m. p. 210°, $[\alpha]_D$ +100° (*c* 0·64), $\lambda_{max.}$ 245 mμ (ε 11,900), $\nu_{max.}$ 1742 (OAc and CO·CH·OAc), 1712 (C:O), 1667 and 1625 (CO·C:C) cm.$^{-1}$ (Found: C, 64·4; H, 7·4; Ac, 23·1. $C_{30}H_{38}O_{10}$ requires C, 64·5; H, 6·9; 3Ac, 23·1%). Cucurbitone A gave a negative tetranitromethane, a positive iodoform, and a strongly positive tetrazolium blue test. In 0·01N-alcoholic potassium hydroxide it developed an intense wine-red colour ($\lambda_{max.}$ 495 mμ) which rapidly faded to yellow. The red colour was more stable in the absence of oxygen. Cucurbitone A was also obtained on similar treatment of dihydrocucurbitacin A.

(b) *From deoxocucurbitone A*. Deoxocucurbitone A (41 mg.) in 90% acetic acid (1 ml.) was treated with chromium trioxide (21 mg.) in the same solvent (1 ml.) for 4 hr. at 55°. The product crystallised from methanol to give cucurbitone A (16 mg.), identified by m. p., mixed m. p., paper chromatography (2 : 1 hexane–ethyl acetate), and ultraviolet and infrared spectra.

Cucurbitone A Mono-2,4-dinitrophenylhydrazone, prepared in the usual way and crystallised twice from chloroform–ethanol (yellow needles), had m. p. 263° (Found: C, 58·4; H, 5·8; N, 7·2. $C_{36}H_{42}O_{13}N_4$ requires C, 58·5; H, 5·7; N, 7·6%).

Δ^{16}-Anhydrodeacetylcucurbitone A.—Cucurbitone A (4 g.) in chloroform (110 ml.) was adsorbed on acid-washed alumina (125 g.) and kept at room temperature in the dark for 2 weeks. Paper chromatography (1 : 1 : 2 chloroform–ethyl acetate–hexane) of the product (3·6 g.), eluted with 10 : 1 chloroform–methanol, showed that the reaction was essentially complete. Purification was achieved by chromatography on acid-washed alumina (200 g.). Elution with benzene–chloroform (9 : 1 ⟶ 1 : 1; 1·5 l.) afforded fractions containing only one component (paper chromatography). Crystallisation from chloroform–methanol gave *Δ^{16}-anhydrodeacetylcucurbitone A* (1·3 g.), m. p. 212—213°, $[\alpha]_D$ +155° (*c* 1·04), $\lambda_{max.}$ 240 mμ (ε 19,800), $\nu_{max.}$ 1746 (OAc and CO·CH·OAc), 1704 (hindered C:O), 1667 (conjugated C:O strength equivalent to two groups), 1631 and 1594 (C:C conjugated with C:O) cm.$^{-1}$ (Found: C, 67·7; H, 7·1; Ac, 17·2. $C_{28}H_{34}O_8$ requires C, 67·5; H, 6·9; 2Ac, 17·3%).

Anhydrodeacetyl-16,17-dihydrocucurbitone A.—Δ^{16}-Anhydrodeacetylcucurbitone A (2 g.) was hydrogenated over 2% palladised calcium carbonate (1 g.) in ethanol (200 ml.). The hydrogenation was interrupted after the uptake of 1 mol. of hydrogen (1 hr.). Crystallisation from chloroform–methanol furnished the *dihydro-derivative* (1·2 g.), m. p. 278°, $[\alpha]_D$ +165° (*c* 0·77), $\lambda_{max.}$ 244 mμ (ε 12,500), $\nu_{max.}$ 1745 (OAc and CO·CH·OAc), 1709 (hindered C:O and 20-one), 1667 and 1625 (CO·C:C) cm.$^{-1}$ (Found: C, 66·9; H, 7·6; Ac, 16·9. $C_{28}H_{36}O_8$ requires C, 67·2; H, 7·3; 2Ac, 17·2%).

Dihydrocucurbitone A.—Cucurbitone A (4·45 g.) in acetic acid (200 ml.) was heated for 5 hr. on a steam-bath while zinc dust (20 g.) was added in small portions. Crystallisation of the product from chloroform–methanol afforded *dihydrocucurbitone A* (2·1 g.), m. p. 260—262°, $[\alpha]_D$ +91° (*c* 0·97), $\lambda_{max.}$ 292 mμ (ε 153), $\nu_{max.}$ 1745 and 1736 sh (OAc and CO·CH·OAc), 1704 (C:O) cm.$^{-1}$ (Found: C, 64·3; H, 7·2; Ac, 23·4. $C_{30}H_{40}O_{10}$ requires C, 64·3; H, 7·2; 3Ac, 23·0%). The same product is obtained on prolonged hydrogenation of cucurbitone A over palladised calcium carbonate in ethanol.

Δ^{16}-*Anhydrodeacetyldihydrocucurbitone A*.—Dihydrocucurbitone A (4·04 g.) in chloroform (100 ml.) was adsorbed on acid-washed alumina (120 g.) and kept at room temperature in the dark for 4 weeks. Paper chromatography (3 : 1 benzene–hexane) of the product (3·6 g.) eluted with 10 : 1 chloroform–methanol, showed that it contained no more starting material. Purification was achieved by filtering through acid-washed alumina (200 g.) with benzene–chloroform (2 : 1 ⟶ 1 : 1; 1 l.).

Crystallisation from chloroform–methanol gave Δ^{16}-*anhydrodeacetyldihydrocucurbitone A* (1·6 g.), m. p. 202—203°, $[\alpha]_D$ +119° (*c* 0·97), $\lambda_{max.}$ 239 and 299 mμ (ε 8700 and 142 respectively), $\nu_{max.}$ 1746 and 1733 sh (OAc and CO·CH·OAc), 1701 (C:O), 1669 and 1594 (16-en-20-one) cm.$^{-1}$. Great difficulty was experienced in obtaining consistent analyses of this compound, probably owing to strongly bound solvent of crystallisation (Found: C, 66·7, 66·3, 66·1; H, 7·3, 7·5, 7·2; Ac, 16·9. $C_{28}H_{36}O_8$ requires C, 67·2; H, 7·3; 2Ac, 17·2%).

Anhydrodeacetyltetrahydrocucurbitone A.—Δ^{16}-Anhydrodeacetyldihydrocucurbitone A (610 mg.) was hydrogenated over 2% palladised calcium carbonate (300 mg.) in ethanol (60 ml.) (0·8 mol. of hydrogen absorbed in 30 min.). The crude crystalline product (360 mg.) was a mixture (paper chromatography; 2 : 1 hexane–ethyl acetate) and was purified by chromatography on acid-washed alumina (30 g.). Elution with 9 : 1 benzene–chloroform (250 ml.) gave small amounts of impurities, after which 2 : 1 benzene–chloroform (300 ml.) and chloroform (100 ml.) afforded the major component (R_F 0·67) which, crystallised from chloroform–methanol and sublimed *in vacuo*, afforded a saturated *tetraketone* (230 mg.), m. p. 276—278°, $[\alpha]_D$ +142° (*c* 0·93), $\lambda_{max.}$ 292 mμ (ε 154), $\nu_{max.}$ 1745 and 1733 sh (OAc and CO·CH·OAc), 1704 (C:O) cm.$^{-1}$ (Found: C, 67·2; H, 7·6. $C_{28}H_{38}O_8$ requires C, 66·9; H, 7·6%).

Hydrogenation of anhydrodeacetyl-16,17-dihydrocucurbitone A over palladised calcium carbonate in ethanol for 48 hr. or reduction with zinc and acetic acid also afforded the same tetraketone but the product still contained 10—25% of starting material (ultraviolet spectrum). Attempts to separate the mixture by crystallisation or chromatography were unsatisfactory.

Reaction of Cucurbitone A with Sodium Methoxide in Methanol.—To a suspension of finely ground cucurbitone A (2 g.) in dry methanol (200 ml.) was added at 4°, under nitrogen, 0·1N-sodium methoxide in dry methanol (70 ml.). The mixture was kept, with occasional shaking, at 4° for 4 hr. during which the solid slowly dissolved and the solution became dark wine-red. On addition of 0·1N-hydrochloric acid (75 ml.) and water (200 ml.) the colour changed to light yellow. After 30 min., the product was isolated with chloroform and crystallised from methanol as prisms (570 mg.), m. p. 255—258°. It consisted mainly of two components (paper chromatography; 1 : 1 ethyl acetate–benzene), which were separated by chromatography on acid-washed alumina (60 g.). Elution with 1 : 1 chloroform–benzene (125 ml.) afforded fractions (60 mg.) containing mainly the minor component of R_F 0·73 after which elution with 1 : 1 chloroform–benzene (125 ml.) and chloroform (125 ml.) gave fractions (357 mg.) containing the major component of R_F 0·53.

Crystallisation of the minor *compound* from chloroform–methanol gave prisms, m. p. 259—262°, $[\alpha]_D$ +80° (*c* 0·84), $\lambda_{max.}$ 238 and 298—305 mμ (ε 9650 and 110 respectively), $\nu_{max.}$ 1721 sh, 1712, and 1701 (C:O), 1667 and 1597 (16-en-20-one) cm.$^{-1}$ (Found: C, 69·3; H, 7·3. $C_{24}H_{30}O_6$ requires C, 69·5; H, 7·3%). Boiling in acetic anhydride under nitrogen and crystallisation from chloroform–methanol gave a *diacetate*, m. p. 295—297°, $[\alpha]_D$ +12° (*c* 1·0), $\lambda_{max.}$ 239 and 300—302 mμ (ε 10,900 and 128 respectively) (Found: C, 68·0; H, 7·1. $C_{28}H_{34}O_8$ requires C, 67·5; H, 6·9%).

Crystallisation of the major *compound* from chloroform–benzene and then from chloroform–methanol gave prisms, m. p. 257—259° or 264—265°, $[\alpha]_D$ +48° (*c* 0·84), $\lambda_{max.}$ 238 mμ (ε 10,000), $\nu_{max.}$ 1721 (CO·CH·OH), 1704 (C:O), 1667 and 1594 (16-en-20-one) cm.$^{-1}$ (Found: C, 69·8, 69·6; H, 7·5, 7·4. $C_{24}H_{30}O_6$ requires C, 69·5; H, 7·3%). This substance in boiling acetic anhydride under nitrogen furnished a *diacetate*, m. p. 233—235° (in block at 225°, melts and resolidifies) (from chloroform–methanol), $[\alpha]_D$ +15° (*c* 0·82), $\lambda_{max.}$ 239 and 296—300 mμ (ε 10,100 and 121

respectively), $\nu_{max.}$ 1748 sh and 1739 (OAc and CO·CH·OAc), 1704 (C:O), 1669 and 1595 (16-en-20-one) cm.$^{-1}$ (Found: C, 67·6, 67·4; H, 7·2, 7·1; Ac, 17·9, 17·8. $C_{28}H_{34}O_8$ requires C, 67·5; H, 6·9; 2Ac, 17·3%).

The second of these diacetates (474 mg.) was hydrogenated over 2% palladised calcium carbonate (500 mg.) in ethanol (250 ml.) (complete after 10 min.; 1·03 mol.). Paper chromatography (2 : 1 hexane–ethyl acetate) showed that the product contained a small amount of a second substance. Purification was by filtration through acid-washed alumina (30 g.) with benzene (250 ml.) and benzene–chloroform (9 : 1 ⟶ 1 : 1; 1 l.), and crystallisation from chloroform–methanol to afford a *dihydro-diacetate*, m. p. 222—227°, $[\alpha]_D$ +50° (*c* 1·0), $\lambda_{max.}$ 289—290 mμ (ε 129), $\nu_{max.}$ (sodium chloride prism) 1735 (OAc) and 1710 (C:O) cm.$^{-1}$ (Found: C, 67·1; H, 7·4. $C_{28}H_{36}O_8$ requires C, 67·2; H, 7·3%).

The major and the minor product of the reaction of cucurbitone A with sodium methoxide, their diacetates, and the above dihydro-diacetate, all give a red colour with 0·1N-alcoholic potassium hydroxide and no colour with tetranitromethane or ferric chloride.

Reaction of Dihydrocucurbitone A with Sodium Methoxide in Methanol.—Finely ground dihydrocucurbitone A (600 mg.) was suspended in dry methanol (60 ml.), and 0·1N-sodium methoxide in methanol (25 ml.) added at 4° under nitrogen. The mixture was kept, with occasional shaking, at 4° for 5 hr. The solid slowly dissolved and the solution became light yellow. The solution was acidified, then left for 45 min., and the product isolated with chloroform. Crystallisation from chloroform–methanol gave a crude product (303 mg.), m. p. 270—280°, which showed two spots on paper chromatograms (2 : 1 ethyl acetate–benzene). Chromatography of the mixture (800 mg.) on acid-washed alumina (200 g.) gave a good separation. Elution with chloroform (400 ml.) gave impurities (19 mg.), chloroform + 0·5% of methanol (300 ml.) gave the minor component (150 mg.; R_F 0·36), and chloroform + 0·5% of methanol (500 ml.) and then 3% of methanol (900 ml.) gave the major component (330 mg.; R_F 0·25).

Crystallisation of the minor *compound* from chloroform–methanol afforded prisms, m. p. 280—281°, $[\alpha]_D$ +112° (*c* 0·4), $\lambda_{max.}$ 239 and 294—296 mμ (ε 8400 and 138 respectively), $\nu_{max.}$ 1717 sh and 1695 (C:O), 1667 and 1592 (16-en-20-one) cm.$^{-1}$ (Found: C, 69·5; H, 8·0. $C_{24}H_{32}O_6$ requires C, 69·2; H, 7·7%). Acetylation gave the *acetate*, m. p. 269—270° (from methanol), $[\alpha]_D$ +110° (*c* 0·78), $\lambda_{max.}$ 239 and 291 mμ (ε 7900 and 171 respectively), $\nu_{max.}$ 1751 and 1742 sh (OAc and CO·CH·OAc), 1701 (C:O), 1669 and 1594 (16-en-20-one) cm.$^{-1}$ (Found: C, 66·5; H, 7·4. $C_{28}H_{36}O_8$ requires C, 67·2; H, 7·3%).

The major *compound* crystallised from chloroform–methanol as prisms, m. p. 279—280° (undepressed on admixture with the minor component), $[\alpha]_D$ +175° (*c* 0·17), $\lambda_{max.}$ 239 and 290—298 mμ (ε 8900 and 178 respectively), $\nu_{max.}$ 1715 (CO·CH·OH), 1698 (C:O), 1669 and 1592 (16-en-20-one) cm.$^{-1}$ (Found: C, 69·0; H, 7·9%). Acetylation and crystallisation of the product from chloroform–methanol afforded Δ^{16}-anhydrodeacetyldihydrocucurbitone A (m. p., mixed m. p., and infrared spectrum).

The above major product (1·9 g.) was hydrogenated over 2% palladised calcium carbonate (500 mg.) in ethanol (350 ml.) (0·8 mol. absorbed in 1 hr.); the product crystallised from chloroform–methanol and sublimed *in vacuo*, to afford a *dihydro-derivative* (1·2 g.), m. p. 283—285°, $[\alpha]_D$ +121° (*c* 0·20) (Found: C, 68·9; H, 8·2. $C_{24}H_{34}O_6$ requires C, 68·9; H, 8·2%). Acetylation and crystallisation from chloroform–methanol furnished the saturated tetraketone, m. p. 276—278° (identified by mixed m. p. and infrared spectrum).

Action of Dilute Alkali on Cucurbitacin A and B.—Both cucurbitacin A and B developed a new peak at 312 mμ in dilute alcoholic potassium hydroxide solution (cf. Table).

	$E_{1\,cm.}^{1\%}$ at 312 mμ			$E_{1\,cm.}^{1\%}$ at 312 mμ	
Time (hr.)	Cucurbitacin A	Cucurbitacin B	Time (hr.)	Cucurbitacin A	Cucurbitacin B
	In 0·01N-alkali			In 0·1N-alkali	
0·1	4	6	0·17	56	55
0·33	19	17	0·66	81	90
4	56	67	18	7	103
24	19	98			
47	8	92			

The maximum at 312 mμ, developed after 12 min. in 0·1N-alkali, shifted in both cucurbitacins to 268—272 mμ ($E_{1\,cm.}^{1\%}$ 66) on acidification with 0·1N-hydrochloric acid.

Ferric chloride tests were performed as follows: Solutions of cucurbitacin A and of B (4 mg.)

in 0·1N-ethanolic potassium hydroxide (1 ml.) were kept for 70 min. and then neutralised with 0·1N-hydrochloric acid. On addition of 0·05M-ethanolic ferric chloride (0·4 ml.), a violet colour formed which was stable in the case of cucurbitacin B but faded within 2 min. in the case of cucurbitacin A.

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