931. *Bitter Principles of the Cucurbitaceae. Part X.* Cucurbitacin C.†*

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The tetracyclic triterpenoid acetate, cucurbitacin C, $C_{32}H_{48}O_8$, contains the same iso-octyl side-chain (I) as cucurbitacin A. Experiments are described to determine the positions of the oxygen atoms and the nuclear, trisubstituted double bond.

Cucurbitacin C[1] is a bitter principle found so far only in *Cucumis sativus* var. *Hanzil*[2] but most probably also responsible for the bitterness which often develops in greenhouse cucumbers.[3] On the basis of determinations by the *X*-ray method, its formula has been revised[4] to $C_{32}H_{48}O_8$. Acetylation afforded amorphous cucurbitacin C triacetate which still contained a free hydroxyl group ($\nu_{max.}$ 3448 cm.$^{-1}$). Cucurbitacin C also contains an acetoxy-group, an αβ-unsaturated and an isolated unreactive keto-group ($\lambda_{max.}$ 231 and 298 mμ; ε 11,100 and 131 respectively). Cucurbitacin C failed to form a 2,4-dinitrophenylhydrazone. Hydrogenation over palladised calcium carbonate in ethanol gave dihydrocucurbitacin C in which the conjugated double bond has been saturated.

Oxidation of cucurbitacin C by periodic acid gave *trans*-4-acetoxy-4-methylpent-2-enoic acid and a methyl ketone, hexanorcucurbitacin C, $C_{24}H_{36}O_5$. Cucurbitacin C therefore contains the same side-chain (I) as cucurbitacin A (see preceding paper). This structure is further supported by the formation of senecioaldehyde on oxidation by chromium trioxide and of α-hydroxy-α-methylpropionaldehyde on ozonolysis of cucurbitacin C.

(I) $HO\cdot\underset{|}{C}Me\cdot CO\cdot CH{=}CH\cdot CMe_2\cdot OAc$

The ultraviolet spectrum ($\lambda_{max.}$ 294 mμ; ε 120) and infrared bands at 1704 and 1695 cm.$^{-1}$ of hexanorcucurbitacin C showed the presence of two isolated keto-groups. It was fairly stable towards further attack by periodic acid, and gave a pale yellow colour with tetranitromethane but no colour with tetrazolium blue. The relationship between cucurbitacin C and a third periodic acid oxidation product is not clear.

On treatment of hexanorcucurbitacin C with hydrochloric acid in aqueous methanol, an anhydro-compound, $C_{24}H_{34}O_4$, was obtained. The ultraviolet maximum at 241 mμ

* Part IX, preceding paper.

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

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

[2] Enslin and Rehm, *Proc. Linnean Soc.*, 1956—1957, 169 Session, p. 230.

[3] Rehm and Wessels, *J. Sci. Food Agric.*, 1957, **8**, 687; Andeweg and De Bruyn, *Euphytica*, 1959, **8**, 13.

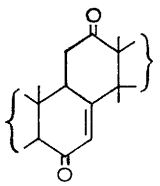
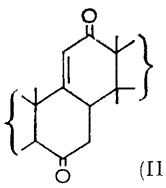
[4] Rivett and Herbstein, *Chem. and Ind.*, 1957, 393; Rivett and Enslin, *Proc. Chem. Soc.*, 1958, 301.

(ε 8500) and infrared bands at 1667 and 1590 cm.$^{-1}$ are consistent with the presence of a Δ^{16}-20-keto-group in an assumed trimethyl-steroid structure. The infrared spectrum still showed the band at 1692 cm.$^{-1}$ of an isolated keto-group.

Dihydrocucurbitacin C slowly developed a band at 270 mμ (ε 4180 after 4 hr.) in dilute alkaline solution. Since this band was unchanged on acidification, it is probably due to a doubly unsaturated keto-group formed by dehydration of hydroxy-groups. This must involve the keto-group in the side-chain and the 16- and 20-hydroxy-groups because hexanorcucurbitacin C developed no band at 270 mμ in alkaline solution, but only a strong band at 242 mμ (ε 7580 after 20 hr.) which is due to the formation of a Δ^{16}-20-keto-group.

Hexanorcucurbitacin C gave a triacetate, deoxocucurbitone C, $C_{30}H_{42}O_8$, which on oxidation with chromium trioxide afforded cucurbitone C, $C_{30}H_{40}O_9$, more conveniently prepared by similar oxidation of cucurbitacin C triacetate. The molecular formula and the presence of an αβ-unsaturated keto-group ($\lambda_{max.}$ 241 mμ, ε 13,200; $\nu_{max.}$ 1664 and 1622 cm.$^{-1}$) showed that, as with cucurbitone A (see preceding paper), this product is also formed by oxidation of a methylene group alpha to a nuclear, trisubstituted double bond. Cucurbitone C lost acetic acid on acid-washed alumina to afford Δ^{16}-anhydrodeacetylcucurbitone C, $C_{28}H_{36}O_7$, which contains two αβ-unsaturated keto-groups ($\lambda_{max.}$ 238 mμ, ε 22,100; $\nu_{max.}$ 1663, 1623, and 1592 cm.$^{-1}$). On catalytic reduction one mol. of hydrogen was rapidly absorbed to give a dihydro-compound, $C_{28}H_{38}O_7$. The ultraviolet spectrum ($\lambda_{max.}$ 241 mμ, ε 12,300) and infrared bands at 1661 and 1623 cm.$^{-1}$ showed that the double bond of a proposed Δ^{16}-20-keto-group had been reduced. On further catalytic reduction a second mol. of hydrogen was slowly absorbed, to afford anhydrodeacetyltetrahydrocucurbitone C, $C_{28}H_{40}O_7$. This saturated triketone ($\lambda_{max.}$ 294 mμ, ε 117) with ethanedithiol gave a monothioketal. Desulphurisation over Raney nickel then afforded a diketone, $C_{28}H_{42}O_6$ ($\lambda_{max.}$ 296 mμ, ε 98). The keto-group originally introduced into the molecule by chromium trioxide oxidation is therefore in a hindered position.

Treatment of cucurbitone C with sodium methoxide in methanol afforded a monoacetate, $C_{26}H_{34}O_6$ ($\lambda_{max.}$ 238 mμ, ε 8200; $\nu_{max.}$ 1727, 1701, 1667, and 1595 cm.$^{-1}$), the ultraviolet and infrared absorption spectra of which are consistent with the presence of a Δ^{16}-20-keto-group and the absence of the originally αβ-unsaturated keto-group.

(II)   (III)

These results can be explained on the basis of partial structure (II) or (III) for cucurbitone C (cf. previous paper).

Experimental

General directions are as given in the preceding paper. Oven-dried chromatograms were sprayed either (*a*) with 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*) with a solution of vanillin (10 g.) in a mixture of ethanol (150 ml.) and 85% phosphoric acid (50 ml.). Spots were 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.

Cucurbitacin C.—The crude bitter principle was isolated from *Cucumis sativus* var. *Hanzii* as described before [1] and crystallised from ethyl acetate as needles, m. p. 207—207·5°, $[\alpha]_D$ +95° (*c* 1·03 in ethanol), $\lambda_{max.}$ 231 and 298 mμ (ε 11,100 and 131 respectively), $\nu_{max.}$ 1731 and 1256 (OAc), 1689 and 1631 (CO·C:C) cm.$^{-1}$ [Found: C, 68·6; H, 8·9%; *M* (*X*-ray method), 577 ± 12. $C_{32}H_{48}O_8$ requires C, 68·5; H, 8·6%; *M*, 561]. No crystalline 2,4-dinitrophenylhydrazone

could be prepared. Cucurbitacin C gave a pale yellow colour with tetranitromethane, and negative ferric chloride, tetrazolium blue (see preceding paper), and iodoform tests.

Cucurbitacin C Triacetate.—Cucurbitacin C was boiled with acetic anhydride under nitrogen for 1 hr. and the product isolated by pouring the whole into water. The crude *acetate* could not be crystallised and was purified by precipitation with water from alcoholic solution, then having $[\alpha]_D$ +36° (*c* 0·99), $\nu_{max.}$ (sodium chloride prism) 3448 (OH), 1730 (OAc and C:O), 1692 and 1631 (CO·C:C) cm.$^{-1}$ (Found: C, 66·5; H, 8·0. $C_{38}H_{54}O_{11}$ requires C, 66·5; H, 7·9%). The acetate gave a yellow colour with tetranitromethane.

Dihydrocucurbitacin C.—Cucurbitacin C (1 g.) was hydrogenated over 2% palladised calcium carbonate (160 mg.) in ethanol (100 ml.) (1·07 mol. of hydrogen absorbed in 30 min.). *Dihydrocucurbitacin C* crystallised from ethanol–hexane as needles (850 mg.), m. p. 226°, $[\alpha]_D$ +66° (*c* 1·03), $\lambda_{max.}$ 289 mμ (ε 116), $\lambda_{max.}$ in 0·1N-alcoholic potassium hydroxide 270 mμ (ε 600, 4180, and 5450 after 20 min., 4 hr., and 20 hr. respectively) (Found: C, 68·6; H, 9·2. $C_{32}H_{50}O_8$ requires C, 68·3; H, 9·0%).

Oxidation of Cucurbitacin C by Periodic Acid.—0·5M-Periodic acid (115 ml.) and water (480 ml.) were added to a solution of cucurbitacin C (10·68 g.) in ethanol (765 ml.), and the mixture kept at room temperature in the dark for 66 hr. (0·98 mol. consumed). Excess of reagent was destroyed with ethylene glycol, and the ethanol evaporated *in vacuo* until the solution became strongly turbid. In the cold a gum (2·81 g.) settled out. This was dissolved in methanol (20 ml.) and poured into water (400 ml.), to afford a gum which crystallised from methanol–water as needles (1·25 g.), m. p. 169°. Recrystallisation from 96% ethanol gave needles, m. p. 112—113°, $[\alpha]_D$ +124° (*c* 1·06), $\lambda_{max.}$ 288—291 mμ ($E^{1\%}_{1\,cm.}$ 1·99), end absorption at 210 mμ ($E^{1\%}_{1\,cm.}$ 348), $\nu_{max.}$ 1721 v.s. (broad), 1695 and 1658 w cm.$^{-1}$ (Found: C, 67·6; H, 8·6%). The substance gave a negative iodoform test.

The aqueous layer from the first precipitate was concentrated further *in vacuo* to afford two successive crops of precipitate which were combined and crystallised from methanol, to give *hexanorcucurbitacin C* (3·8 g.), m. p. 235—236°, $[\alpha]_D$ +185° (*c* 0·96 in ethanol), $\lambda_{max.}$ 294 mμ (ε 120), $\lambda_{max.}$ in 0·1N alcoholic potassium hydroxide 242 mμ (ε 640, 4240, 7580 after 30 min., 4 hr., and 20 hr. respectively), $\nu_{max.}$ 1704 (20-one) and 1695 (hindered C:O) cm.$^{-1}$ (Found: C, 71·4; H, 8·8. $C_{24}H_{36}O_5$ requires C, 71·3; H, 9·0%). It gave a pale yellow colour with tetranitromethane and a positive iodoform and negative tetrazolium blue test. Hexanorcucurbitacin C consumed 0·16 mol. of periodic acid in 3 days. On paper chromatograms (3 : 2 ethyl acetate–benzene, V.P.A. spray reagent) it had R_F 0·13 and the substance of m. p. 112—113° had R_F 0·72.

The final aqueous layer, obtained after precipitation of hexanorcucurbitacin C, was extracted with ether to afford an oil (1·8 g.) which crystallised on trituration with a little ether to give *trans*-4-acetoxy-4-methylpent-2-enoic acid, identified by m. p. and infrared spectrum.

Senecioaldehyde and α-Hydroxy-α-methylpropionaldehyde.—These substances were prepared from cucurbitacin C under the conditions described for cucurbitacin A and identified as before (see preceding paper).

Δ^{16}-Anhydrohexanorcucurbitacin C.—Hexanorcucurbitacin C (200 mg.) was refluxed in methanol (20 ml.) and 2N-hydrochloric acid (20 ml.) for 11 hr. Dilution with water (50 ml.) and isolation with chloroform gave a crude product which contained no more starting material (paper chromatography; 3 : 2 ethyl acetate–benzene, V.P.A. spray reagent). Crystallisation from methanol afforded the *anhydro-substance*, m. p. 178—179° (in block at 170°, melts and resolidifies), $\lambda_{max.}$ 241 and 296—299 mμ (ε 8500 and 108 respectively), $\nu_{max.}$ 1692 (hindered C:O), 1667 and 1590 (16-en-20-one) cm.$^{-1}$ (Found: C, 74·5; H, 8·9. $C_{24}H_{34}O_4$ requires C, 74·6; H, 8·9%).

Deoxocucurbitone C.—Hexanorcucurbitacin C (507 mg.) was boiled for 1·5 hr. in acetic anhydride (5 ml.) under nitrogen, and the product crystallised from chloroform–methanol to afford *deoxocucurbitone C* (463 mg.), m. p. 154—156°, $[\alpha]_D$ +126° (*c* 1·03), $\lambda_{max.}$ 291 mμ (ε 96), $\nu_{max.}$ 1733 (OAc), 1714 (20-one), 1705 sh (hindered C:O) (Found: C, 67·9; H, 8·0. $C_{30}H_{42}O_8$ requires C, 67·9; H, 8·0%), that gave a yellow colour with tetranitromethane.

Cucurbitone C.—(a) *From cucurbitacin C.* Cucurbitacin C (3·2 g.) was boiled with acetic anhydride (35 ml.), the excess of anhydride destroyed with water (8 ml.), and the crude acetate oxidised at 45—50° with chromium trioxide (3 g.) in acetic acid (20 ml.) for 2 days. The final product was isolated in the usual way and crystallised from chloroform–methanol to afford cucurbitone C (1·7 g.), m. p. 246—247° (after drying *in vacuo*), $[\alpha]_D$ +153° (*c* 1·3), $\lambda_{max.}$ 241 and 298 mμ (ε 13,200 and 129 respectively), $\nu_{max.}$ 1739 (OAc), 1712 (20-one and hindered C:O), 1664

and 1622 (CO·C:C) cm.$^{-1}$ (Found: C, 66·1; H, 7·6; Ac, 23·3. $C_{30}H_{40}O_9$ requires C, 66·2; H, 7·4; 3Ac, 23·7%). The same product was obtained on similar treatment of dihydrocucurbitacin C. Cucurbitone C gave no colour with tetranitromethane and a positive iodoform test. Whereas cucurbitacin C and hexanorcucurbitacin C gave no colour with the T.T.C. spray reagent on paper chromatograms, cucurbitone C gave a red spot. It is considered, however, that the colour is not due to an α-ketol system in cucurbitone C since, under the conditions of the controlled tetrazolium blue test (see previous paper), it gave a negative result. In comparison, cucurbitacin A and cucurbitone A gave a strongly positive test.

(b) *From hexanorcucurbitacin C.* Hexanorcucurbitacin C (167 mg.) was refluxed with acetic anhydride (2 ml.) under nitrogen for 1 hr., water (0·4 ml.) added, and the crude acetate oxidised at 50—60° with chromium trioxide (82 mg.) in 90% acetic acid (1 ml.) for 5 hr. The final product crystallised from chloroform–methanol to give cucurbitone C (146 mg.), identified by m. p. mixed m. p., paper chromatography (2 : 1 hexane–ethyl acetate, T.T.C. spray reagent), and ultraviolet and infrared spectra.

Δ^{16}-Anhydrodeacetylcucurbitone C.—Cucurbitone C (2·5 g.) in chloroform (80 ml.) was adsorbed on acid-washed alumina (100 g.) and kept at room temperature in the dark for two weeks. The product was eluted with 10 : 1 chloroform–methanol and crystallised from ethanol, to afford *Δ^{16}-anhydrodeacetylcucurbitone C* (1·3 g.), m. p. 223—224°, $[\alpha]_D$ +218° (*c* 1·14), $\lambda_{max.}$ 238 mμ (ε 22,100), $\nu_{max.}$ 1739 (OAc), 1703 (hindered C:O), 1663 (conjugated C:O; strength equivalent to two groups), 1623 and 1592 (C:C conjugated with C:O) cm.$^{-1}$ (Found: C, 69·5; H, 7·8; Ac, 19·6. $C_{28}H_{36}O_7$ requires C, 69·4; H, 7·5; 2Ac, 17·8%).

Anhydrodeacetyl-16,17-dihydrocucurbitone C.—Δ^{16}-Anhydrodeacetylcucurbitone C (152 mg.) was hydrogenated over 5% palladised calcium carbonate (150 mg.) in ethanol (60 ml.). The hydrogenation was interrupted after the uptake of *ca.* 1 mol. of hydrogen (10 min.). Crystallisation from ethanol gave the *dihydro-derivative* (90 mg.), m. p. 244°, $[\alpha]_D$ +218° (*c* 1·24), $\lambda_{max.}$ 241 and 293 mμ (ε 12,300 and 117 respectively), $\nu_{max.}$ 1739 (OAc), 1706 (C:O), 1661 and 1623 (CO·C:C) (Found: C, 69·2; H, 8·4; Ac, 18·8. $C_{28}H_{38}O_7$ requires C, 69·1; H, 7·9; 2Ac, 17·7%).

Anhydrodeacetyltetrahydrocucurbitone C.—Δ^{16}-Anhydrodeacetylcucurbitone C (1 g.) was hydrogenated over 2% palladised calcium carbonate (1 g.) in ethanol (180 ml.) for 30 hr. (*ca.* 2 mols. absorbed). The product crystallised from ethanol and sublimed *in vacuo*, to afford the *tetrahydro-derivative* (0·6 g.), m. p. 220—221°, $[\alpha]_D$ +133° (*c* 1·64), $\lambda_{max.}$ 294 mμ (ε 117), $\nu_{max.}$ 1739 (OAc), 1704 (C:O) cm.$^{-1}$ (Found: C, 69·0; H, 8·3; Ac, 17·9. $C_{28}H_{40}O_7$ requires C, 68·8; H, 8·3; 2Ac, 17·6%). The *2,4-dinitrophenylhydrazone* crystallised from chloroform–methanol in yellow needles, m. p. 281° (Found: C, 60·9; H, 6·3; N, 8·8. $C_{34}H_{44}O_{10}N_4$ requires C, 61·1; H, 6·6; N, 8·4%).

To a solution of anhydrodeacetyltetrahydrocucurbitone C (406 mg.) in acetic acid (5 ml.) were added ethanedithiol (1 ml.) and boron trifluoride–ether complex (1 ml.), and the mixture was kept at room temperature for 6 days, then concentrated *in vacuo* at 40°. Methanol (8 ml.) was added and the product (230 mg.) recrystallised from ethanol, to furnish the *mono(dithioketal)*, m. p. 270—271° (Found: C, 64·0; H, 7·7; S, 11·4. $C_{30}H_{44}O_6S_2$ requires C, 63·8; H, 7·9; S, 11·3%). From the mother-liquors another product (50 mg.), m. p. 290°, was isolated (Found: C, 65·1; H, 8·5%).

Monodeoxo-substance.—The above thioketal (140 mg.) in absolute ethanol (100 ml.) was refluxed with Raney nickel W_2 (*ca.* 3 g.) for 22 hr. The product crystallised from ethanol to afford fine needles of the *monodeoxo-substance* (60 mg.), m. p. 229°, $[\alpha]_D$ +130° (*c* 1·01), $\lambda_{max.}$ 296 mμ (ε 98), $\nu_{max.}$ 1739 (OAc), 1698 (C:O) cm.$^{-1}$ (Found: C, 70·8; H, 8·9. $C_{28}H_{42}O_6$ requires C, 70·9; H, 8·9%).

Reaction of Cucurbitone C with Sodium Methoxide in Methanol.—To a suspension of finely ground cucurbitone C (1 g.) in dry methanol (100 ml.) was added at 4° under nitrogen 0·1N-sodium methoxide in methanol (35 ml.), and the mixture was kept, with occasional shaking, at 4° for 6 hr. during which the solid slowly dissolved and the solution became light yellow. After acidification with 0·1N-hydrochloric acid and dilution with water (100 ml.), the product was isolated with chloroform and crystallised from chloroform–methanol to afford a *monoacetate* (130 mg.), m. p. 235—237°, $[\alpha]_D$ +24° (*c* 1·0), $\lambda_{max.}$ 238 and 307 mμ (ε 8200 and 92 respectively), $\nu_{max.}$ 1727 and 1252 (OAc), 1701 (C:O), 1667 and 1595 (16-en-20-one) cm.$^{-1}$ (Found: C, 70·2; H, 7·6; Ac, 9·6. $C_{26}H_{34}O_6$ requires C, 70·6; H, 7·7; Ac, 9·7%).

The mother-liquors of the above crystallisations showed two spots on paper chromatograms (2 : 1 benzene–ethyl acetate, T.T.C. spray reagent). Chromatography on acid-washed alumina

and elution with benzene–chloroform (9 : 1 ⟶ 1 : 1) gave further quantities of the above monoacetate and small amounts of a substance, m. p. 257—258° (from methanol), $\lambda_{max.}$ 237 and 308—312 mμ ($E^{1\%}_{1\,cm.}$ 198 and 2·4 respectively), $\nu_{max.}$ 1701, (C:O) 1667 and 1595 (16-en-20-one) cm.$^{-1}$. Neither of these two substances gave a colour with tetranitromethane.

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