5.5 cc. of acetic anhydride, the excess acetic acid and acetic anhydride were then removed *in vacuo*, and the residual nepetic acid anhydride was evaporatively distilled at 0.05 mm. The crystalline distillate was immediately heated under reflux for 2 hr. with 6 cc. of anhydrous methanol, a solution of 0.5 g. of sodium metal in 10 cc. of anhydrous methanol was added and heating was continued for 30 min. Water (5 cc.) was now added and the reaction mixture was heated under reflux for a further 1 hr. in order to saponify the half-ester XVIIIb. The methanol was distilled off under reduced pressure and the solution was evaporated repeatedly with water. The residue was dissolved in water, acidified, and the crude acid obtained after ether extraction was converted into its barium salt. This proved to be water-soluble, thus indicating the absence of any *cis, cis* isomer XIIIa (insoluble barium salt) and after acidification and repeated ether extraction, there was obtained (after several recrystallizations from ether–petroleum ether) 0.546 g. of (−)-*trans, cis*-nepetic acid (XVIIIa), m.p. 95–100° (Kofler block), 98–100° (capillary), $[\alpha]_D$ −66.2° (*c*, 1.08 in chloroform). The melting point remained unchanged after countercurrent distribution (ether *vs.* phosphate-citrate buffer of *p*H 6.05) or silica gel chromatography.

Anal. Calcd. for $C_8H_{12}O_4$: C, 55.80; H, 7.03; O, 37.17. Found: C, 55.71; H, 7.16; O, 37.01.

Lithium aluminum hydride reduction of 1,10-anhydro-7,8-dihydrogenipin (XXVII). A solution of 5.203 g. of 1,10-anhydro-7,8-dihydrogenipin (XXVII) in 70 cc. of anhydrous ether was reduced with 9.6 g. of lithium aluminum hydride exactly as described above for the analogous reduction of IIa. The crude product (4.623 g.) was chromatographed in benzene solution on 150 g. of Merck acid-washed alumina to afford in the ether-methanol (9:1) eluates 2.09 g. of the *unsaturated aldehyde* XXVIII. The analytical samples was distilled at 120°/0.05 mm. and exhibited $[\alpha]_D$ +40.3° (*c*, 1.34 in chloroform), $\lambda_{max}^{C_2H_5OH}$ 216 mμ, log ε 3.73, $0_{max}^{CHCl_3}$ 2.77, 2.92, 3.69 (w), 5.92 (s), and 6.03 (w) μ.

Anal. Calcd. for $C_{10}H_{14}O_3$: C, 65.91; H, 7.74. Found: C, 65.36; H, 7.35.

Treatment of a sample of the aldehyde XXVIII with 2,4-dinitrophenylhydrazine in methanolic hydrochloric acid for 30 min. at room temperature led to the *2,4-dinitrophenylhydrazone* XXX, which was recrystallized from methanol-chloroform; m.p. 182–184°, $\lambda_{max}^{CHCl_3}$ 366 mμ, log ε 4.42.

Anal. Calcd. for $C_{17}H_{22}N_4O_6$: C, 53.96; H, 5.86; N, 14.81; O, 25.37; OCH_3, 8.20. Found: C, 54.16; H, 5.59; N, 14.72; O, 25.59; OCH_3, 8.04.

The ether-methanol (7:3) eluates of the original chromatogram furnished 0.303 g. of the *triol* XXIXa as a sticky oil, which was distilled at 150°/0.03 mm, $\lambda_{max}^{CHCl_3}$ 2.90, 6.13 (w) μ. For purposes of characterization, the triol was acetylated by heating under reflux for 6 hr. with acetic anhydride–pyridine, evaporating to dryness and extraction with ether. The crude *triacetate* XXIXb was purified by chromatography on 12 g. of Merck acid-washed alumina, elution with benzene-ether (9:1 and 6:1) and finally distillation at 120°/0.05 mm.; $[\alpha]_D$ +25° (*c*, 1.00 in chloroform), $\lambda_{max}^{CHCl_3}$ 5.79 (s), 6.06 (w), 8–8.4 μ and 11.06 μ.

Anal. Calcd. for $C_{16}H_{24}O_6$: C, 61.52; H, 7.75; O, 30.73. Found: C, 61.89; H, 7.84; O, 30.32.

Reduction of 0.547 g. of the unsaturated aldehyde XXVIII with 1.4 g. of lithium aluminum hydride followed by acetylation of the resulting triol produced 0.44 g. of the triacetate XXIXb.

STANFORD, CALIF.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY AND CHEMICAL ENGINEERING, STANFORD UNIVERSITY]

Reduction and Oxidation Products of Cucurbitacin B[1]

W. SCHLEGEL, A. MELERA, AND C. R. NOLLER

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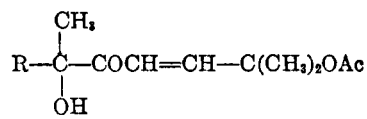
Catalytic hydrogenation of cucurbitacin B yields two products, a dihydro derivative and a dihydrodeacetoxy derivative. Reaction with zinc dust gives a deacetoxy derivative with migration of the double bond out of conjugation with a carbonyl group. Oxidation of acetylated cucurbitacin B with chromic acid gives β,β-dimethylacrylic acid and two methyl ketones. These results can be accommodated by a side chain having the structure



Cucurbitacin B, present in numerous members of the *Cucurbitaceae*,[2] is one of the bitter principles isolated from *Echinocystis fabacea*.[3] A second product, called fabacein, also was isolated, separation being accomplished by fractional crystallization. Since then it has been found that the separations can be made readily by the use of a chromatographic column in which the immobile phase is formamide on Celite (4:5 by weight) and the elutant is benzene or benzene–ethyl actate, thus providing a means of obtaining these substances in relatively large amounts with little difficulty. The present paper reports the details of a number of experiments on cucurbitacin B.

Cucurbitacin B (I) contains an acetoxy group, an α,β-unsaturated carbonyl group, two additional unconjugated carbonyl groups and three hydroxyl groups.[3,4] A determination of the molecular weight

(1) The results of some of these investigations were published in a preliminary report, *J. Org. Chem.*, 24, 291 (1959).

(2) P. R. Enslin, *J. Sci. Food Agri.*, 5, 411 (1954); S. Rehm, P. R. Enslin, A. D. J. Meeuse, and J. H. Wessels, *J. Sci. Food. Agr.*, 8, 679 (1957).

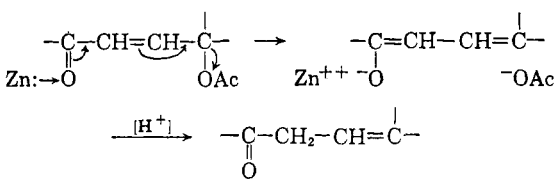
(3) W. O. Eisenhut and C. R. Noller, *J. Org. Chem.*, 23, 1984 (1958).

(4) P. R. Enslin, S. Rehm, and D. E. A. Rivett, *J. Sci. Food Agri.*, 8, 674 (1957).

from the density of crystals and the size of the unit cell,[5] indicated the molecular formula $C_{32}H_{48}O_8$. Numerous analyses of our product checked better with the formula $C_{30}H_{44}O_8$.[3] However, it was later noted[1] that our analyses checked equally well with the formula $C_{32}H_{46-48}O_8 \cdot 0.5\ H_2O$ and that the analyses of derived products agree better with a C_{32} formula. Subsequent work indicates that the correct formula of the anhydrous product must be $C_{32}H_{46}O_8$.

It was reported[3] that catalytic hydrogenation of cucurbatacin B in ethyl acetate, using palladium on carbon as catalyst, involves partial hydrogenolysis of the acetoxy group, along with hydrogenation of the double bond. From this mixture, two pure products have been isolated by chromatography on a column of Celite impregnated with formamide. One product is dihydrocucurbitacin B (II), $C_{32}H_{48}O_8$, and the other is dihydrodeacetoxycucurbitacin B (III), $C_{30}H_{46}O_6$. For both products, ultraviolet and infrared spectra show that the α,β-unsaturated carbonyl system has disappeared, and hence the double bond conjugated with the carbonyl group has been reduced. Both reduction products give a yellow color with tetranitromethane, indicating the presence of an additional unconjugated and unreactive bond. The NMR spectra to be reported later indicate that this double bond is trisubstituted, a conclusion that is supported by absorption in the infrared at 12.1 μ (825 cm.$^{-1}$).

Removal of the acetoxy group by hydrogenolysis suggests that it occupies an allylic position with respect to a double bond. If this is true, one would expect the possibility of removal of the acetoxy group by zinc dust and acetic acid by a mechanism analogous to that proposed for the easy removal of an acetoxy group that is *alpha* to a carbonyl group.[6] In fact, treatment of cucurbitacin B with zinc dust in acetic acid at room temperature gave a deacetoxycucurbitacin B. This product gave a deep yellow color with tetranitromethane, and the ultraviolet and infrared spectra show that it lacks α,β-unsaturation. Evidently the double bond moved out of conjugation with the carbonyl group. When



hydrogenated over palladium on charcoal, one mole of hydrogen was absorbed, and the resulting product was identical with dihydrodeacetoxycucurbitacin B (III).

It has been reported[3] that attempts to convert cucurbitacin B into a crystalline acetate were unsuccessful. The same difficulty has been experienced with the reduction products. However, paper chromatograms indicate that the acetates are homogeneous. Because they have been used in oxidative investigations, their properties are reported. It should be noted that the results of carbon-hydrogen analyses of our acetylated products from cucurbitacin B and its reduction products may be interpreted as indicating the presence of three acetylateable hydroxyl groups, or that two hydroxyl groups have been acetylated and the products contain one-half mole of water (despite the fact that they were dried at 120° under 0.1 mm. of mercury pressure). We have not been able to distinguish between these formulations by acetyl determinations because the results have been extremely erratic. Analyses by three different commercial laboratories using several methods of hydrolysis gave wholly inconsistent and frequently impossible results. The infrared spectra of all of the acetylated products still showed the presence of a hydroxyl group, and subsequent work agrees best with the assumption of the presence of two acetylateable secondary hydroxyl groups and one nonacetylateable tertiary hydroxyl group.

Another difficulty in handling these acetates is that an acetyl group may be removed easily by hydrolysis. Thus when the acetylated dihydrodeacetoxycucurbitacin B, $C_{34}H_{50}O_8 \cdot 0.5H_2O$, was chromatographed on acidic alumina, partial hydrolysis took place and a monoacetate, $C_{32}H_{48}O_7$, was isolated.

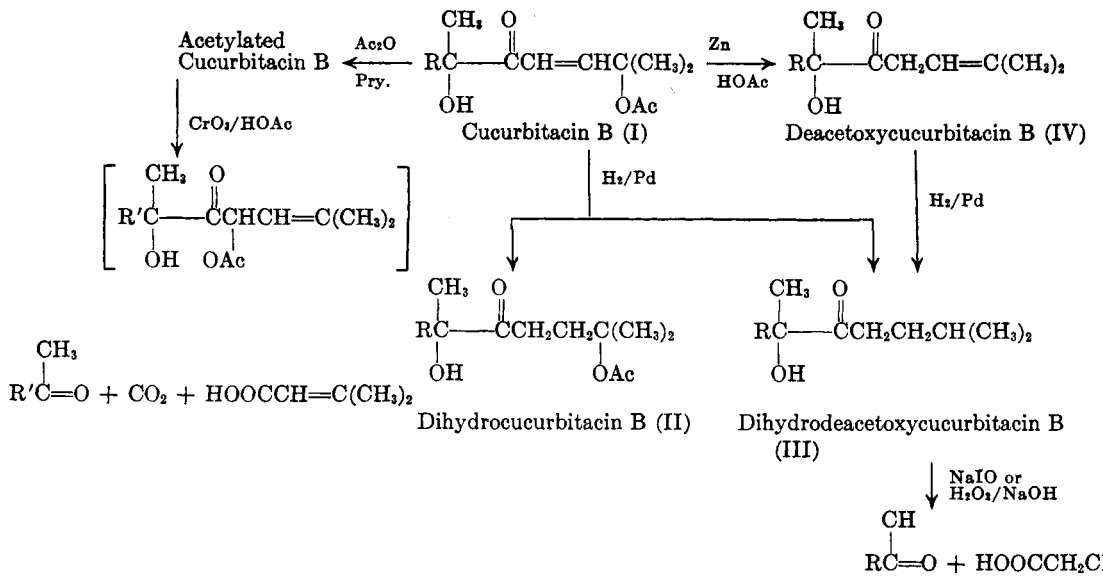
One other point should be noted. The product obtained by hydrogenating acetylated cucurbitacin B is different from but isomeric with that obtained by acetylating dihydrocurcurbitacin B. Inasmuch as the difference in molecular rotation between dihydrocucurbitacin B and its acetylation product, $\Delta[M]_D$ −412, is the same as the difference between dihydrodeacetoxycucurbitacin B and its acetylation product, $\Delta[M]_D$ −424, the same change in structure or configuration is involved. On the other hand, the molecular rotation difference between cucurbitacin B and its acetylation product, $\Delta[M]_D$ −288, is considerably different. Hence, a different change in structure or configuration is involved during the acetylation of cucurbitacin B from that involved during the acetylation of its reduction products.

When dihydrodeacetoxycucurbitacin B was oxidized in aqueous dioxane either with sodium periodate or with alkaline hydrogen peroxide, the chief volatile product was isocaproic acid. Thus the carbon skeleton of isocaproic acid is present in the side chain and an α-diketo or α-hydroxy keto group at the sixth and seventh carbon atoms of the side chain is indicated.

Oxidation of acetylated dihydrodeacetoxycucurbitacin B with chromium trioxide likewise gave iso-

(5) D. E. A. Rivett and F. H. Herbstein, *Chem. and Ind., (London)*, 393 (1957).

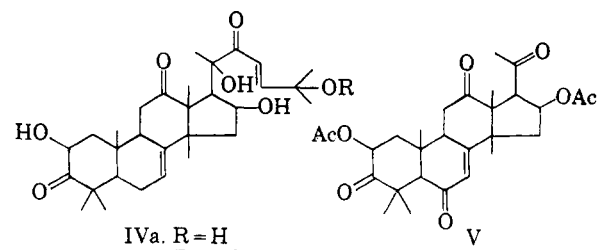
(6) R. B. Woodward, F. Sondheimer, D. Taub, K. Heusler, and W. M. McLamore, *J. Am. Chem. Soc.*, **74**, 4225 (1952); R. B. Woodward, F. E. Bader, H. Bickel, A. J. Frey, and R. W. Kierstead, *Tetrahedron*, **2**, 10 (1958).



caproic acid as the chief volatile acid. Acetylated cucurbitacin B, however, gave chiefly β,β-dimethylacrylic acid. Both materials gave the same neutral products, which appear to be methyl ketones (positive iodiform reactions). These facts, together with the observations concerning the reduction products, can be explained by partial formula I for cucurbitacin B. The formation of β,β-dimethylacrylic acid would involve an allylic shift of the acetoxyl group. After this work was completed, the presence of this side chain in cucurbitacin B was proved by Enslin and Norton[7] by the isolation of *trans*-4-acetoxy-4-methyl-2-pentenoic acid from the periodic acid oxidation products of acetylated cucurbitacin B. Previously the same side chain had been shown by Lavie and co-workers[8] to be present in elatericin A and B (cucurbitacins D and I) and in α-elaterin (cucurbitacin E).

The neutral fractions from the chromic acid oxidation of acetylated cucurbitacin B, dihydrocucurbitacin B, dihydrodeacetoxycucurbitacin B, or fabacein[3] appear from the paper chromatogram to be identical and to consist of a mixture of at least six substances. When passed through a column of Celite impregnated with formamide, two main fractions were obtained. Both appear to be methyl ketones (positive iodoform reaction). The larger of the two fractions was designated as ketone A and has the formula $C_{28}H_{36}O_8$. It contains an α,β-unsaturated carbonyl group and is formed by the scission of the side chain between the carbonyl group and the adjacent carbon bearing the tertiary hydroxyl group and by introduction of a new carbonyl group into the remainder of the molecule by oxidation of an allylic methylene group. A joint communication by Lavie and Enslin and their co-workers[9] reported the isolation of a product of higher melting point and rotation which was considered to be identical or isomeric with ketone A. Dr. Enslin has kindly compared a sample of ketone A with his product and in a private communication has informed us that the two products are indeed identical. The smaller fraction designated ketone B appears to have the molecular formula $C_{28}H_{38}O_8$. No α,β-unsaturated carbonyl group is present. The additional oxygen probably is a new carbonyl group resulting from rearrangement of an oxide formed by addition of an oxygen atom to the nuclear double bond.

Structure IVa recently has been proposed[10] for elatericin A from which it follows that cucurbitacin B should have structure IVb and ketone A structure V.



IVa. R = H
b. R = $COCH_3$

V

A serious objection to these formulas is the fact that under the conditions under which ketone A is produced, one would expect further oxidation to a conjugated dienedione.

EXPERIMENTAL

Separation of cucurbitacin B and fabacein. The portion of Celite No. 545 which passed a 100-mesh screen and did not

(7) P. R. Enslin and K. B. Norton, *Chem. and Ind. (London)*, 162 (1959).

(8) D. Lavie and Y. Shvo, *Proc. Chem. Soc.*, 220 (1958); D. Lavie, Y. Shvo, and D. Willner, *Chem. and Ind. (London)*, 1361 (1958); *J. Am. Chem. Soc.*, 81, 3062 (1959).

(9) D. Lavie, Y. Shvo, D. Willner, P. R. Enslin, J. M. Hugo, and K. B. Norton, *Chem. and Ind. (London)*, 951 (1959).

(10) D. Lavie and Y. Shvo, *Chem. and Ind. (London)*, 403 (1960).

pass a 200-mesh screen was washed with 6 *N* hydrochloric acid, then with water until the washings were neutral, and dried at 100° for 50 hr. Five parts by weight of the treated Celite was thoroughly mixed with four parts of formamide and a slurry in benzene saturated with formamide[11] was poured into a column 100 cm. high and 50 cm. in diameter. Packing was accomplished by forcing the liquid phase through the column several times by air pressure. At the end of the procedure, the adsorbent occupied about two-thirds of the height of the column.

In a typical run, a benzene solution of 12.8 g. of the crystalline mixture of cucurbitacin B and fabacein, which was obtained from the syrupy concentrate of extracts of *Echinocystis fabacea*,[3] was placed on the column and eluted with benzene. Each 100-cc. portion of eluate was evaporated to dryness and the residue weighed. Fractions 1–7, amounting to 5.45 g., were crystallized from ethanol and gave 4.61 g. of fabacein, m.p. 203–208°.[12] After recrystallization it melted at 207–210°, and a paper chromatogram indicated that it was homogeneous.[13] Fractions 8–10 (0.18 g.) were mixtures of cucurbitacin B and fabacein together with some third substance. Fractions 11–25 (5.71 g.) were eluted with benzene and with benzene–ethyl acetate (9v.:1v.). Crystallization from acetone-hexane gave 4.16 g. of cucurbitacin B, m.p. 170–175°, which after recrystallization melted at 177–179°. A paper chromatogram showed that it was homogeneous. Fractions 27–30 (0.55 g.), eluted with benzene–ethyl acetate (4v.:1v. and 3v.:2v.), did not crystallize.

Reduction products of cucurbitacin B. To a solution of 3.15 g. of cucurbitacin B in 25 cc. of ethanol and 20 cc. of ethyl acetate was added 0.5 g. of hydrogen-saturated catalyst (Baker and Co., 10% palladium on charcoal), and the suspension was shaken in a hydrogen atmosphere until no more hydrogen was absorbed (155 cc. = 1.28 moles per mole of compound). After filtration and evaporation of the solvent, the residue was separated on a Celite-formamide column. Fractions 1–8 (0.23 g.), eluted with benzene-hexane (1v.:1v. and 3v.:1v.), could not be obtained crystalline. Fractions 9–26 (1.04 g.) were crystallized from acetone-hexane and gave 0.85 g. m.p. 200–205°. After recrystallization from ether it melted at 208–210°; $[\alpha]_D^{25}$ +57° (*c* 0.93);[14] ultraviolet λ_{max} 279 mμ, log ε 2.46[14]; infrared 2.92 (OH) 5.85 sh (C=O), 5.90 (C=O).[14] It gave a negative test with ferric chloride and a positive test with Tollens reagent. Analysis showed that this product was a *dihydrodeactoxycucurbitacin B.*

Anal.[15] Calcd. for $C_{30}H_{46}O_6$: C, 71.68; H, 9.22. Found: C, 71.59; H, 9.37.

Fraction 27 (0.02 g.) eluted with benzene was a mixture. Fractions 28–37 (1.26 g.) were eluted with benzene and when crystallized from acetone-hexane gave 0.95 g., m.p. 158–164°. After recrystallization, it melted at 160–163°; $[\alpha]_D^{25}$ +57 (*c* 0.91); ultraviolet λ_{max} 282 mμ, log ε 2.32; infrared 2.92 (OH), 5.79 (AcO); 5.85 sh (C=O), 5.89 (C=O), 8.10 (AcO). It gave a negative test with ferric chloride and a positive test with Tollens reagent. Analysis of a sample dried in air at room temperature indicated that the product was *dihydrocucurbitacin B.*

Anal. Calcd. for $C_{32}H_{48}O_8$: C, 68.54; H, 8.63. Found: C, 68.38; H, 8.80.

When dried at elevated temperature and reduced pressure, the analytical values for carbon were high.

To a solution of 1.0 g. of cucurbitacin B in 50 cc. of glacial acetic acid was added 2 g. of zinc dust, and the mixture was stirred at room temperature for 1 hr. At this time a paper chromatogram (formamide-benzene) showed the complete absence of cucurbitacin B (R_f 0.51) and the presence of a new compound (R_f 0.81). The mixture was filtered, the filtrate evaporated to dryness at reduced pressure, and the residue dissolved in ether. Evaporation of the ether gave 0.85 g. which could not be crystallized from ethanol. The mixture was chromatographed on a 2.5 × 50-cm. Celite-formamide column and eluted with benzene-hexane (1:1) taking 50-cc. fractions. Fractions 4–11 amounted to 0.08 g. A paper chromatogram showed five spots having R_f values of 0.68–0.89. A second maximum in the chromatogram appeared in fractions 15–23 which amounted to 0.45 g. Crystallization from ethanol followed by recrystallization from acetone-hexane gave 0.31 g., m.p. 178–179°; $[\alpha]_D^{25}$ +78° (*c* 0.76 in ethanol); ultraviolet λ_{max} 292 mμ, log ε 2.66; infrared 2.91 (OH), 5.87 (C=O), 5.90 (C=O). Tetranitromethane gave an orange color, in contrast to the pale yellow color obtained with cucurbitacin B. The product is *deacetoxycurbitacin B.*

Anal. Calcd. for $C_{30}H_{44}O_6$: C, 71.97, H, 8.86. Found: C, 71.72; H, 9.05.

When deacetoxycucurbitacin B in ethyl acetate was hydrogenated in the presence of palladium on carbon, the product was indistinguishable from dihydrodeacetoxycucurbitacin B by melting point and mixture melting point, by rotation, and by infrared absorption spectra.

Anal. Calcd. for $C_{30}H_{46}O_6$: C, 71.68; H, 9.22. Found: C, 71.39; H, 9.14.

This two-step procedure was preferable to direct hydrogenation of cucurbitacin B for the preparation of dihydrodeacetoxycucurbitacin B in quantity, because no dihydrocucurbitacin B is obtained. The crude deacetoxycucurbitacin B was dissolved in ethanol and hydrogenated over 10% palladium on carbon and the product chromatographed on a Celite-formamide column using benzene-hexane (2:3) for elution. Crystallization of the main fraction gave 75–80% of the calculated amount based on cucurbitacin B. From the early fractions, a faster running component was isolated in small amounts (0–5%). This product is identical with *isodihydrodeacetoxycucurbitacin B* obtained by the action of 0.05*N* aqueous methanolic sodium hydroxide on dihydrodeacetoxycucurbitacin B.[16]

Acetylations. A solution of 3.0 g. of cucurbitacin B in a mixture of 10 cc. of pyridine and 7.5 cc. of acetic anhydride was allowed to stand for 20 hr. at 25°. The solution was diluted with water and extracted with three portions of ether. The combined extract was washed with water, 2*N* sulfuric acid, water, saturated sodium bicarbonate solution, and water, and dried over sodium sulfate. Because it was not possible to crystallize the product, it was chromatographed on a Celite-formamide column. The main fraction was concentrated and evaporated to dryness at reduced pressure. The resulting foam gave a homogeneous paper chromatogram; $[\alpha]_D^{25}$ +4.4 (*c* 0.95); ultraviolet λ_{max} 228 mμ and 288 mμ, log ε 4.02[17] and 2.41; infrared 2.90 (OH), 5.80 (AcO), 5.92 (C=O), 6.13 (C=C), 8.10 (AcO). It gave a negative test with ferric chloride and a positive test with Tollens reagent.

Anal. Calcd. for $C_{38}H_{52}O_{11}$: C, 66.65; H, 7.65; O, 25.70; for $C_{36}H_{50}O_{10}\cdot{}^1/_2\ H_2O$: C, 66.34; H, 7.89; O, 25.78. Found: C, 66.25, 66.65, 66.37; H, 7.78, 7.88, 7.84; O, 25.38.

Dihydrocucurbitacin B when acetylated and worked up in the same way likewise gave an amorphous tetraacetate,

(11) In all column or paper chromatography, the mobile phase was equilibrated with the immobile phase before use.

(12) All melting points were determined on a Monoscope IV hot-stage.

(13) Unless otherwise noted all paper chromatograms were made on Whatman No. 1 paper impregnated with formamide, using benzene as the elutant and aqueous permanganate as the developer. *Cf.* P. R. Enslin, T. G. Joubert, and S. Rehm, *J. S. African Chem. Inst.*, **7**, 131 (1954).

(14) Unless otherwise noted, all rotations and infrared spectra are in chloroform and all ultraviolet spectra in ethanol.

(15) Unless otherwise noted all analytical samples were dried at 60–100° and 0.2 mm. for 24 hr. Microanalyses were made by commercial laboratories.

(16) A. Melera and C. R. Noller, *J. Org. Chem.*, **26**, 1213 (1961).

(17) The value 4.33 reported in Ref. 1 is a misprint.

or a triacetate with 0.5 mole of water; $[\alpha]_D^{25}$ −16.3° (*c* 0.92); ultraviolet λ_{max} 286 mμ, log ε 2.28; infrared 2.90 (OH), 5.80 (AcO), 5.90 sh (C=O), 8.10 (AcO).

Anal. Calcd. for $C_{38}H_{54}O_{11}$: C, 66.45; H, 7.92; for $C_{36}H_{52}O_{10}$. $^1/_2$ H_2O: C, 66.13; H, 8.17. Found: C, 66.36, 66.14; H, 8.28, 8.24.

Catalytic hydrogenation of acetylated cucurbitacin B gave a product which was different from but isomeric with that from the acetylation of dihydrocucurbitacin B; $[\alpha]_D^{25}$ +3.5° (*c* 1.49); ultraviolet λ_{max} 286 mμ, log ε 2.43; infrared 2.90 (OH), 5.80 (AcO), 5.90 sh (C=O), 8.10 (AcO).

Anal. Calcd. for $C_{38}H_{54}O_{11}$: C, 66.45; H, 7.92; for $C_{36}H_{52}O_{10}$. $^1/_2$ H_2O: C, 66.13; H, 8.17. Found: C, 66.55, 66.65, 66.71, 66.56, 66.77; H, 8.19, 7.98, 8.21, 8.13, 8.08.

Analysis of the amorphous acetylated dihydrodeacetoxycucurbitacin B indicated that it was either a triacetate, or a diacetate having one-half molecule of water of hydration; $[\alpha]_D^{25}$ −20.4 (*c* 1.37, $CHCl_3$), +5.7° (*c* 0.88, ethanol); ultraviolet 282 mμ, log ε 2.44; infrared 2.90 (OH), 5.80 (AcO), 5.90 (C=O), 8.10 (AcO).

Anal. Calcd. for $C_{36}H_{52}O_9$: C, 68.76; H, 8.34; for $C_{34}H_{50}O_8$. $^1/_2H_2O$: 68.54, 8.63. Found: C, 68.55; H, 8.46.

When 1.15 g. of the amorphous diacetate was placed on a column of 25 g. of Woelm alumina, activity II, and eluted with hexane-benzene (1v.:1v.) 400 mg. of the diacetate was recovered in ten 50-cc. fractions. Elution of the residue with benzene gave a second amorphous product, analysis of which indicated a partial hydrolysis to give a monoacetate; $[\alpha]_D^{25}$ −4° (*c* 0.99, ethanol); infrared 2.90 (OH), 5.80 (AcO), 8.10 (AcO).

Anal. Calcd. for $C_{32}H_{48}O_7$: C, 70.56; H, 8.88; O, 20.56. Found: C, 70.38; H, 8.76; O, 20.58.

Oxidations. To a solution of 1.08 g. of dihydrodeacetoxycucurbitacin B in 40 cc. of dioxane was added a solution of 1 g. of sodium periodate in 40 cc. of water. After standing at 25° for 35 hr. the solution was diluted with water, extracted with ether, and the ether solution washed with water, saturated sodium bicarbonate solution, and water, and dried over sodium sulfate. Evaporation of the ether gave 0.47 g. of neutral products. The bicarbonate extract was immediately acidified and extracted with ether to give 0.60 g. of acidic products which were converted to the methyl esters with an ether solution of diazomethane. The methyl esters were steam distilled, the distillate extracted with ether, the combined extracts dried, and the ether evaporated through a column. The residue was subjected to gas chromatography on a 1.5-m. Carbowax column. Three peaks were observed after 15, 22, and 28 min., which were identified by comparison with known mixtures as methyl isovalerate, dioxane, and methyl isocaproate. The ratio of methyl isocaproate to methyl isovalerate varied from 4:1 to 9:1 in different experiments. The same results were obtained when 1.02 g. of dihydrodeacetoxycucurbitacin B in 30 cc. of dioxane was treated with 15 cc. of 30% hydrogen peroxide in 15 cc. of 2*N* sodium hydroxide for 2 hr. at 25° and 0.5 hr. at 90°.

In a three-necked flask fitted with gas inlet and outlet tubes and a dropping funnel was placed a solution of 2.3 g. of acetylated cucurbitacin B in 25 cc. of acetic acid. Dry nitrogen was passed through the solution and the exit gases were passed through a solution of 2,4-dinitrophenylhydrazine, a drying tower, and a tared U-tube containing Ascarite. A total of 110 cc. of a 2% solution of chromium trioxide in acetic acid was added dropwise and the mixture allowed to stand for 50 hr. During this time a yellow precipitate formed in the dinitrophenylhydrazine solution, which was identified as acetone dinitrophenylhydrazone. The Ascarite tube increased in weight by 151 mg. (calcd. for 1 equiv. of carbon dioxide: 148 mg.). The excess of chromium trioxide was destroyed by adding methanol, and the bulk of the acetic acid was removed at reduced pressure. The residue was diluted with water, extracted with ether, and the ether solution washed with water, bicarbonate solution, and water and dried over sodium sulfate. Acidification of the bicarbonate extract gave only small amounts of acidic products. It was possible, however, to show by paper chromatography the absence of isocaproic acid and the presence of β,β-dimethylacrylic acid by comparison with synthetic samples.

The neutral fraction weighed 1.9 g. and the paper chromatogram showed the presence of at least six substances, although two compounds predominate. The combined neutral fractions from three oxidations, weighing 9.7 g., were chromatographed on a Celite-formamide column using benzene-hexane (1:1) as elutant. Fractions 26–34 gave 1.07 g. and fractions 42–57 gave 2.86 g. of two relatively pure substances. The larger, slower running fraction has been designated as *ketone A*. After several recrystallizations from acetone-hexane, it melted at 219–221°; $[\alpha]_D^{25}$ 86.6 (*c* 0.96); ultraviolet λ_{max} 243 mμ, log ε 4.1; 335 mμ, log ε 2.0; infrared 5.77 (AcO), 5.85 (C=O), 6.00, 6.15 (C=C—C=O), 8.10 (AcO); positive iodoform reaction.

Anal. Calcd. for $C_{28}H_{36}O_8$: C, 67.18; H, 7.25. Found: C, 67.13, 67.07; H, 7.44, 7.51. The next largest, faster-running fraction has been designated *ketone B*. After repeated crystallization from acetone-hexane, it melted at 154–157°; $[\alpha]_D^{25}$ −84.9° (*c* 1.14); ultraviolet λ_{max} 295 mμ, log ε 2.37; infrared 5.78 (AcO), 5.91 (C=O), 8.10 (AcO); positive iodoform reaction.

Anal. Calcd. for $C_{28}H_{38}O_8$: C, 66.91; H, 7.62. Found: C, 67.04, 67.13; H, 7.09, 7.21.

The same ketones were obtained by similar oxidations of acetylated fabacein, of acetylated dihydrocucurbitacin B and dihydrodeacetoxycucurbitacin B, and of the hydrogenation product of acetylated cucurbitacin B. However, acetylated dihydrodeacetoxycucurbitacin B gave isocaproic acid and a small amount of isovaleric acid rather than β,β-dimethylacrylic acid. Peaks in the chromatogram of the neutral products of oxidation were obtained also from fractions 4–5 (0.26 g.), 6–7 (0.25 g.), 11–15 (0.45 g.), and 16–18 (0.23 g.) but these materials have not yet been investigated.

When sodium dichromate in acetic acid was used as an oxidizing agent, ketone A was the chief product and could be obtained in a purer form by direct crystallization of the neutral fraction without requiring a chromatographic separation. A solution of 10.5 g. of acetylated cucurbitacin B in 50 cc. of acetic acid was heated on the steam bath to 95° and a solution of 22 g. of sodium dichromate in 100 cc. of acetic acid was added dropwise with continued heating for 3 hr. Then a solution of 6 g. of sodium bisulfite in 30 cc. of water was added and the solvent evaporated at reduced pressure. The residue was dissolved in 500 cc. of water and the mixture extracted with ether-chloroform (3v.:1v.). The ether-chloroform layer was washed twice with water, once with sodium bicarbonate solution, and then with water until the washings were neutral. After drying over sodium sulfate and evaporation of the solvents, the residue weighed 7.5 g. On solution in 10 cc. of acetone and addition of 100 cc. of ether, crystallization took place. After standing overnight the crystals were removed and repeatedly crystallized from acetone-hexane to give a product, m.p. 230–231°, $[\alpha]_D^{25}$ +92.8 (*c* 0.98). It was indistinguishable by paper chromatography and infrared spectrum from the previously obtained ketone A.

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