the acetonitrile solution (after filtration) gave an oil which was chromatographed over tlc plates (silica gel G; benzene–ethyl acetate, 4:1). A band (R_f 0.65) afforded **13** as an oil which did not crystallize after long standing: yield 30 mg; ir bands ($CHCl_3$) 3450 (hydroxyl), 1750 (carbonyl), and 1250 (acetate) cm^{-1}. The nmr spectrum (Table I) indicated that the substance was pure and clearly indicated a vinylic methyl group as shown in the formula of **13**.

Registry No.—**1**, 25383-30-6; **2**, 25383-32-8; **6**, 25383-33-9; **7**, 25383-34-0; **8**, 25383-35-1; **9**, 22555-70-0; **10**, 25383-37-3; **11**, 25383-31-7; **13**, 25383-38-4.

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Tumor Inhibitors. LVI.[1a] Cucurbitacins O, P, and Q, the Cytotoxic Principles of *Brandegea bigelovii*[1b]

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An ethanolic extract of *Brandegea bigelovii* Cogn. was found to have significant activity against human carcinoma of the nasopharynx (KB). Systematic fractionation of the extract led to the characterization of the major active principles as the new tetracyclic triterpenes cucurbitacin O (**1a**), cucurbitacin P (**3a**), and cucurbitacin Q (**2**). The structures were deduced from their formulas and spectra, and the relationships were confirmed by conversion of both **1a** and **2** to **3a**. Interrelation of **3a** with the known cucurbitacin B confirmed the structure and stereochemistry of **3a** at all positions but C-3 and C-20. Conversion of **3a** to a 2,3-acetonide showed it to be the 3α-hydroxy isomer of dihydrocucurbitacin F. The mass spectra of these compounds have been studied and are discussed.

In the course of a continuing search for tumor inhibitors of plant origin, an ethanolic extract of *Brandegea bigelovii* Cogn. (*Cucurbitaceae*)[3] was found to show significant activity against human carcinoma of the nasopharynx carried in cell culture (KB).[4] We report herein the systematic fractionation of the crude extract and the characterization of the three major cytotoxic principles as the new tetracyclic triterpenes cucurbitacin O (**1a**), cucurbitacin P (**3a**), and cucurbitacin Q (**2**).

The dried stems, leaves, flowers, and fruit of *B. bigelovii* Cogn. were continuously extracted with ethanol and the crude extract (A) was partitioned into a water-soluble fraction (B) and a chloroform-soluble fraction (C). The activity was concentrated into the latter, which was partitioned between petroleum ether (D) and aqueous methanol (1:9, E). The active fraction E was chromatographed on silica gel and successive elution with chloroform and 3 and 4% methanol in chloroform gave two active fractions (F and G, respectively, Table I).

Further chromatography of fraction G on silica gel gave a fraction which was crystallized from acetone to yield colorless crystals (H), mp 226–227°. A study of the mass spectrum and the elemental analysis suggested that H was a mixture of two similar compounds, $C_{30}H_{46}O_7$ [*m/e* 518 (M^+) and 500 ($M^+ - 18$)] and $C_{30}H_{48}O_7$ [*m/e* 520 (M^+) and 502 ($M^+ - 18$)]. On tlc two overlapping spots were present, the less polar of which absorbed ultraviolet light and thus probably contained a conjugated system. The infrared spectrum, 5.90, 5.94, and 6.14 μ, and ultraviolet spectrum, λ_{max} 230 mμ (ε 5500), indicated the presence of an α,β-unsaturated ketone, but the intensity of the ultraviolet absorption was unusually low.

TABLE I
CYTOTOXICITY OF FRACTIONS AND COMPOUNDS FROM *B. Bigelovii* AGAINST EAGLE'S KB STRAIN OF HUMAN CARCINOMA OF THE NASOPHARYNX

Fraction	ED_{50}, μg/ml	Compd	ED_{50}, μg/ml
A	2.70	**1b**	20
B	>100	**2**	0.032
C	0.61	**3a**	0.54
D	>100	**3b**	45
E	0.21	**4**	2.9
F	0.021		
G	0.20		
H	0.24, **1a**–**3a** (1:1)		
I	0.19, **1a**–**3a** (3:1)		

Although no work has previously been reported on *Brandegea* species, other members of the family *Cucurbitaceae* have yielded cucurbitacins, a series of highly oxygenated tetracyclic triterpenes which often contain α,β-unsaturated ketones in their side chains. A number of the cucurbitacins have been shown to have cytotoxic properties.[5–7]

(1) (a) Presented at the Southeastern Regional Meeting, American Chemical Society, Richmond, Va., Nov 5–8, 1969. Part LV: S. M. Kupchan in "Plants and the Future of Medicine," T. Swain, Ed., in preparation. (b) This work was supported by grants from the National Institutes of Health (CA-04500 and CA-11718) and the American Cancer Society (T-275), and a contract with Chemotherapy, National Cancer Institute, National Institutes of Health (PH 43-64-551).

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(3) Plant collected in California in May 1967. The authors acknowledge receipt of the dried plant material from Dr. Robert E. Perdue, Jr., U. S. Department of Agriculture, under a program developed by the Cancer Chemotherapy National Service Center (CCNSC) with the USDA.

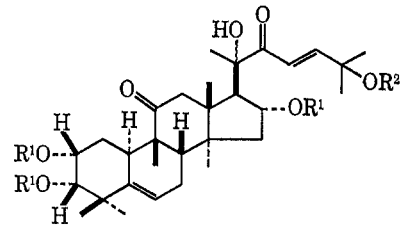
(4) *In vitro* testing was carried out under the auspices of the CCNSC using the techniques described in *Cancer Chemother. Rep.*, **25**, 1 (1962), and also by differential agar diffusion, by Dr. D. Perlman of the School of Pharmacy, University of Wisconsin, Madison, as described in *J. Pharm. Sci.*, **58**, 633 (1969).

(5) S. M. Kupchan, A. H. Grey, and M. D. Grove, *J. Med. Chem.*, **10**, 337 (1967).

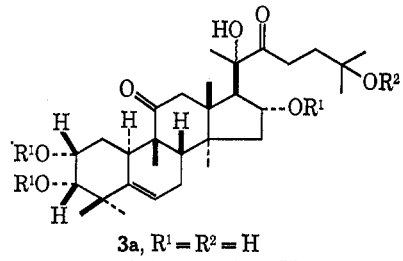
(6) S. Gitter, R. Gallily, B. Shohat, and D. Lavie, *Cancer Res.*, **21**, 516 (1961).

(7) R. Gallily, B. Shohat, J. Kalish, S. Gitter, and D. Lavie, *ibid.*, **22**, 1038 (1962).

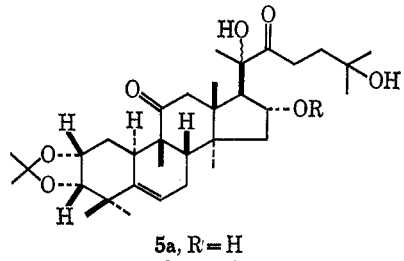
The nmr spectrum of fraction H contained a peak at τ 4.50 typical for a cucurbitacin C-6 vinylic proton.[8] The spectrum also showed a singlet at τ 3.17, which was resolved into an AB quartet (J = 16 Hz) when examined at 100 MHz. However, although this peak could be assigned to the C-23,24 vinylic proton signals of a typical α,β-unsaturated ketone, its integration corresponded to only a single proton. Thus, it appeared that fraction H was a 1:1 mixture of a compound containing a conjugated double bond and its dihydro derivative.



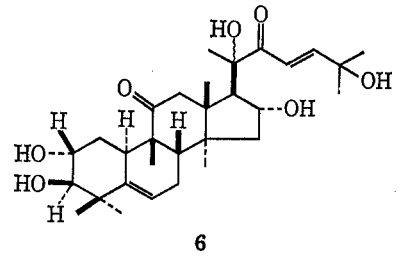
1a, $R^1 = R^2 = H$
b, R^1 = Ac; $R^2 = H$
2, $R^1 = H$; R^2 = Ac



3a, $R^1 = R^2 = H$
b, R^1 = Ac; $R^2 = H$
4, $R^1 = H$; R^2 = Ac



5a, R = H
b, R = Ac



6

Attempts to separate the two compounds by column chromatography on silica gel or on Florisil and by tlc were unsuccessful. Repeated recrystallization from a number of solvents led only to a mixture (I), mp 246–247°, whose nmr spectrum indicated a 1:3 ratio of saturated to α,β-unsaturated ketone. The ultraviolet spectral absorption intensity (ϵ 8250) corresponded to a mixture containing 75% typical α,β-unsaturated carbonyl chromophore [*i.e.*, cucurbitacin B (ϵ 11,000)].[9]

(8) P. R. Enslin, C. W. Holzapfel, K. B. Norton, and S. Rehm, *J. Chem. Soc. C*, 964 (1967).
(9) W. O. Eisenhut and C. R. Noller, *J. Org. Chem.*, **23**, 1984 (1958).

Acetylation of mixture H yielded two amorphous triacetates, which were inseparable on silica gel chromatography but could be separated by chromatography on Florisil. The less polar compound **1b**, λ_{max} 230 mμ (ϵ 12,200), contained, in its nmr spectrum, an AB quartet at τ 2.82 and 3.32 (J = 15 Hz, 2 H) as well as the C-6 proton signal at τ 4.32. The infrared spectrum contained bands at 5.89 and 6.12 μ corresponding also to an α,β-unsaturated ketone. The spectrum of the more polar compound, **3b**, contained only the C-6 vinylic proton (τ 4.32) and the compound showed no ultraviolet absorption bands. The nmr spectra of both compounds contained three singlets at τ 7.9–8.2 (9 H, $COCH_3$) and a multiplet at τ 4.7–5.1 (3 H, -CHOAc). Their mass spectra contained characteristic ions (see later) corresponding to an unsaturated and saturated cucurbitacin side chain, respectively, and to a common tetracyclic nucleus.

Hydrogenation of mixture H over palladium yielded a homogeneous compound, cucurbitacin P (**3a**), $C_{30}H_{48}O_7$ [m/e 520 (M^+)], identical on tlc with the lower portion of mixture H. The compound showed no ultraviolet absorption, and the melting point (157–159°, resolidifying, remelting 211–212°) and infrared spectrum (5.90 μ) suggested that it could be dihydrocucurbitacin F, mp 155–156°. This compound had previously been obtained by Enslin and his coworkers[10] by hydrogenation of cucurbitacin F (**6**), mp 244–245°. However, direct comparison of mixture H and its hydrogenation product by nmr and infrared spectroscopy and tlc with cucurbitacin F[11] and its dihydro derivative showed them to be different. In particular, instead of containing a peak at τ 4.48 for the C-6 proton as in the spectrum of **3a**, the nmr spectrum of cucurbitacin F and its dihydro derivative contained peaks at τ 4.34 and 4.32, respectively. These results suggested that the compounds in hand differed in ring-A stereochemistry from **6** (2α, 3β).

During the work on cucurbitacin F, Enslin found that, if dihydrocucurbitacin D (2α-hydroxy-3-ketone) was reduced with sodium borohydride, dihydrocucurbitacin F was formed. However, if dihydrocucurbitacin D was reduced catalytically, an uncharacterized isomer of dihydrocucurbitacin F was obtained. Cucurbitacin B[5], which is the C-25 acetyl derivative of cucurbitacin D, was therefore catalytically hydrogenated to yield a tetrahydro derivative. Hydrolysis of the acetoxyl group then led directly to a product identical with **3a**. Thus the compounds in hand were considered to differ from cucurbitacin F only at C-3 and thus had a 2α,3α configuration. Confirmation of the *cis* configuration was obtained by treatment of **3a** with acetone and *p*-toluenesulfonic acid, which yielded a mixture of a 2,3-monoacetonide (**5a**) and a diacetonide (probably 2,3:16,25). The structure of **5a** was confirmed by its conversion to only the monoacetate **5b** (τ 8.08, 3 H) on acetylation. The mass and nmr spectra of both acetonides were in agreement with the proposed structures.

Thus mixture H contained a 1:1 mixture of the two new cucurbitacins O (**1a**) and P (**3a**). Cucurbitacin P

(10) K. J. van der Merwe, P. R. Enslin, and K. Pachler, *J. Chem. Soc.*, 4275 (1963).
(11) We cordially thank Professor Enslin, of National Chemical Research Laboratory, South African Council for Scientific and Industrial Research, Pretoria, South Africa, for an authentic sample of cucurbitacin F.

appears to be the same as hexahydrocucurbitacin I,[12] mp 156–157°, and tetrahydrocucurbitacin D,[13] mp 152–156°, obtained previously by hydrogenation and shown to be similar to but different from dihydrocucurbitacin F.[10]

The recent work of Barton and coworkers[14] has directly related cucurbitacin A with lanosterol and thus the absolute stereochemistry has been defined for most positions in the cucurbitacins with the exception of C-20. However, there is contradictory evidence in the literature for the relative stereochemistry at C-2. The configurations used in this paper are based on the proposals of Snatzke, *et al.*,[15] and of Barton, *et al.*[14]

The second active fraction (F) from the original separation was repeatedly chromatographed on silica gel but the nmr spectrum showed it still to be a mixture. Finally, after chromatography on Florisil and tlc, cucurbitacin Q (2), λ_{max} 229 mμ (ϵ 9500), was obtained as a single-spot product. The nmr spectrum contained an AB quartet τ 2.94 and 3.56 (J = 15.5 Hz), a vinylic proton signal at τ 4.23 (m), and an acetyl group singlet at τ 8.00. Its mass spectrum contained peaks at m/e 500 ($M^+ - 60$), 482, 405, 387, 113, 112, 111 (strong), and 96 (strong), suggesting that it was the C-25 acetyl derivative of cucurbitacin O (see later). Mild hydrogenation gave a dihydro derivative (**4**, m/e 502 ($M^+ - 60$), τ 4.27, 1 H), which on alkaline hydrolysis yielded cucurbitacin P (**3a**). The dihydro derivative (**4**) was similar to the crude hydrogenation product of cucurbitacin B on examination by tlc and nmr spectroscopy. The nmr spectrum of cucurbitacin Q showed that the side chain was undergoing partial transformation to a 23,24 *cis* form, as the C-25 methyl groups (total 6 H) appeared as two peaks, τ 8.48 (*trans*) and τ 8.37 (*cis*), and the olefinic protons appeared partially as the AB quartet and also as a singlet at τ 3.72 in the same ratio (total 2 H), whereas the remainder of the spectrum was unchanged. On hydrogenation only one compound was formed. The acetyl group was assigned to C-25, the typical substitution position for the cucurbitacins, and the assignment was in agreement with the low-field position of the C-25 methyl group signals in the nmr spectrum (*cf.*, *e.g.*, cucurbitacin B,[5] τ 8.45).

From a study of the mass spectra[16] of the three new compounds and cucurbitacins B and F, a number of correlations were determined. The main fragmentations were those involving the side chain. Loss of the side chain by fission of the 20,22 bond gave a series of peaks unaffected by substitution at C-25 or by the saturation of the 23,24 bond. These peaks appeared at m/e 405, 387, and 369 in the spectra of the 2,3-diols and were shown in the case of **3a** to have the formulas $C_{24}H_{37}O_5$, $C_{24}H_{35}O_4$, and $C_{24}H_{33}O_3$, respectively. In compounds containing a C-3 carbonyl group, peaks appeared instead at m/e 403 and 385. In the spectra of the triacetates **1b** and **3b** there were corresponding peaks at m/e 531, 471, and 411, in the 2,3-acetonide (**5a**) at m/e 445 and 427, and in its acetate (**5b**) at m/e 487 and 427.

The second fragment formed from the 20,22 fission varied markedly. In the case of the Δ-23,24 compounds a very intense and characteristic peak, often the base peak, was found at m/e 96 (C_6H_8O), corresponding to a loss of the C-25 substituent from the side chain to give a vinylic radical. However, when the side chain was reduced, relatively smaller peaks were found. In the case of **3a** these appeared at m/e 115 ($C_6H_{11}O_2$) and m/e 97 (C_6H_9O). Instead a second characteristic fragmentation, by fission of the 17,20 bond, gave a strong peak at m/e 142 ($C_8H_{14}O_2$) corresponding to the entire side chain, minus the C-25 substituent; no corresponding m/e 140 peak was found from the unsaturated compounds.

A second intense peak also occurred in the mass spectra of these compounds, at m/e 111 ($C_7H_{11}O$) if the 23,24 bond was unsaturated or at m/e 113 ($C_7H_{13}O$) if the bond was saturated. Its origin is unclear although it is possibly formed by migration of the C-20 methyl group to C-22 with fission of the 20,22 bond and loss of the C-25 substituent.

As expected the spectrum of mixture H contained peaks at m/e 142, 113, 111, and 96, characteristic of both the saturated and unsaturated side chains. The presence of peaks at m/e 405 and 387 in the spectrum of **2** were strong confirmation of the position of the acetyl group at C-25 rather than C-20. The 20-acetate would be expected to give, instead, peaks at m/e 447 and 429.

Previously,[17] the peaks at m/e 113 and 95 had been assigned to a ring A fragmentation in the C-3 carbonyl compounds tetrahydrocucurbitacin I and dihydrocucurbitacin B, but the present work shows these assumptions to have been incorrect. However, the reported spectra agree with the present arguments as they contain strong m/e 113 and 142 peaks as well as m/e 403 and 385 peaks.

The cytotoxicity of the purified samples (Table I) has been determined and confirmed previous observations that the 23,24 double bond and 25-acetyl groups are important for cytotoxicity. In general, however, the new compounds were much less active than the corresponding cucurbitacin B derivatives,[5] although the only difference was the oxidation level at C-3.

Experimental Section

Melting points were determined on a Thomas–Hoover melting point apparatus and are corrected. Infrared spectra were determined on a Beckman IR-9 spectrometer. Ultraviolet spectra were measured on a Beckman DK-2A spectrometer. Nmr spectra were determined on Varian A-60A and HR-100 spectrometers, using deuteriochloroform as solvent, except for fraction H, **3a**, and **6**, which were measured in DMSO-d_6. Analytical and preparative tlc were carried out on silica gel plates, using ethyl acetate as solvent unless otherwise stated. Petroleum ether refers to the fraction, bp 60–68°. Evaporations were carried out at reduced pressure below 40°. Analyses were carried out by Spang Microanalytical Laboratories, Ann Arbor, Mich.

Extraction and Fractionation.—The dried ground stems, leaves, flowers, and fruits of *Brandegea bigelovii* Cogn. (1.6 kg) were continuously extracted with 95% ethanol for 15 hr. Evaporation gave the crude extract (A, 250 g). A portion of fraction A (200 g) was partitioned between water (500 ml) and chloroform (1.5 l.). The chloroform was washed with water (200 ml) and evaporated to give a brown foam (C, 138 g). The combined

(12) D. Lavie and D. Willner, *J. Amer. Chem. Soc.*, **80**, 710 (1958).
(13) D. Lavie and Y. Shvo, *ibid.*, **81**, 3058 (1959).
(14) D. H. R. Barton, C. F. Garbers, D. Giacopello, R. G. Harvey, J. Lessard, and D. R. Taylor, *J. Chem. Soc. C*, 1050 (1969).
(15) G. Snatzke, P. R. Enslin, C. W. Holzapfel, and K. B. Norton, *ibid.*, 972 (1967).
(16) We cordially thank Dr. D. Rosenthal, Research Triangle Institute, N. C., and Dr. W. E. Baitinger and Dr. W. L. Budde, Department of Chemistry, Purdue University, for the high- and low-resolution mass spectra.
(17) G. R. Duncan, D. D. Levi, and R. Pyttel, *Planta Med.*, **16**, 224 (1968).

aqueous solutions were evaporated to yield fraction B (62 g). Fraction C (138 g) was partitioned between aqueous methanol (1:9, 1 l.) and petroleum ether (four 400-ml portions). Evaporation of the aqueous methanol yielded a brown foam (E, 36 g) and the combined petroleum ether extracts yielded a residue (D, 100 g).

Isolation of Active Principles.—Repeating the separation yielded a larger aqueous methanol-soluble fraction E (250 g), which was fractionated by chromatography on silicAR (3 kg). Elution with chloroform followed by 3% methanol in chloroform yielded the first active fraction (F, 15.2 g); further elution with 4% methanol in chloroform yielded a second active fraction (G, 27 g).

Fraction F was repeatedly rechromatographed on silicic acid and silica gel to give a fraction apparently homogeneous by tlc but which was found to be a mixture on examination by nmr spectroscopy. Further chromatography using Florisil, on elution with ether–ethyl acetate (2:1), gave a fraction, which on preparative tlc yielded a single-spot gum, cucurbitacin Q (**2**, 48 mg): R_f 0.6; mp 118–135°; uv λ_{max}^{MeOH} 229 mμ (ε 9000); ir $\lambda_{max}^{CHCl_3}$ 2.9, 5.82, 5.95, and 6.17 μ; mass spectrum, *m/e* 500 ($M^+ - 60$), 405, 387, 369, 111.0800 ($C_7H_{11}O$), and 96.0575 (C_6H_8O).

Chromatography of fraction G on silica gel, on elution with 5% methanol in chloroform and crystallization from acetone, yielded the major component as a crystalline solid (H), which was a mixture of **1a** and **3a** (1:1): R_f 0.46–0.40 (upper part absorbed uv light); mp 226–227°; uv λ_{max}^{MeOH} 230 (ε 5500) and 292 mμ (ε 25); ir λ_{max}^{KBr} 2.9, 5.90, 5.94, and 6.14 μ; mass spectrum, *m/e* 520 (M^+), 518 (M^+), 500 ($M^+ - 18$), 502 ($M^+ - 18$).

Anal. Calcd for $C_{30}H_{46}O_7$: C, 69.47; H, 9.94; mol wt, 518. Calcd for $C_{30}H_{48}O_7$: C, 69.30; H, 9.29; mol wt, 520. Found: C, 69.61; H, 9.17.

Rechromatography of fraction H on silica gel or Florisil failed to achieve any resolution into its two components. Recrystallization of fraction H (98 mg) from chloroform gave crystals (45 mg): mp 238–240°; λ_{max}^{MeOH} 231 mμ (ε 8400). On recrystallization from aqueous methanol and then from ethyl acetate, crystals (I, 18 mg) were obtained [mp 247–248°; uv λ_{max}^{MeOH} 230 mμ (ε 8250); ir $\lambda_{max}^{CHCl_3}$ 2.9, 5.89, 5.93 (sh), and 6.12 μ; nmr τ 3.19 and 4.44 (3:2)], which thus consisted of a mixture of cucurbitacin P (**3a**)–cucurbitacin O (**1a**) (1:3). Fraction I was unchanged on further recrystallization.

Cucurbitacin P. a. From Fraction H.—A solution of fraction H (95 mg) in ethanol was hydrogenated using 10% Pd–C catalyst to give a white solid. Recrystallization twice from ethyl acetate–petroleum ether yielded crystals, mp 190–191°, and further recrystallization from aqueous methanol and then ethyl acetate–petroleum ether gave cucurbitacin P (**3a**, 22 mg): R_f 0.40; mp 157–159°, then resolidifying, mp 211–212° dec; ir λ_{max}^{KBr} 2.9, 5.90, and 6.13 μ; mass spectrum, *m/e* 502.3305 ($M^+ - 18$, $C_{30}H_{46}O_6$), 405.2633 ($C_{24}H_{37}O_5$), 387.2526 ($C_{24}H_{35}O_4$), 369.2419 ($C_{24}H_{33}O_3$), 142.0985 ($C_8H_{14}O_2$), and 113.0961 ($C_7H_{13}O$).

Anal. Calcd for $C_{30}H_{48}O_7$: C, 69.20; H, 9.29. Found: C, 68.81; H, 9.23.

b. From Dihydrocucurbitacin Q.—A solution of dihydrocucurbitacin Q (**4**, 20 mg) in methanol (3 ml) and 2 *N* aqueous sodium hydroxide (1 ml) was stirred at 25° for 6 hr. The solution was partially evaporated, then diluted with water (4 ml), and extracted with ethyl acetate (three 10-ml portions). The extract on drying ($MgSO_4$) and evaporation gave a white solid, which was separated by tlc to give the major component (10 mg). Recrystallization from ethyl acetate–ether–petroleum ether gave cucurbitacin P (**3a**, 5 mg) [mp 220–221°, mmp 215–217° dec] the infrared spectrum (KBr) was identical with that of **3a** obtained by method a.

c. From Cucurbitacin B.—A solution of cucurbitacin B (30 mg) in acetic acid was hydrogenated using platinum as catalyst. The product was twice separated by tlc to yield crude dihydrocucurbitacin Q (16 mg). This material was hydrolyzed as in method b. Separation by tlc and recrystallization twice from ethyl acetate–petroleum ether gave cucurbitacin P (1 mg) identical by infrared spectroscopy (KBr) and tlc with **3a** from method a.

Dihydrocucurbitacin Q (4).—A solution of cucurbitacin Q (**2**, 37 mg) in methanol (5 ml) was hydrogenated using 10% Pd–C as catalyst. The product was centrifuged and the supernatant liquid was evaporated to give a homogeneous (tlc) oil (**4**, 37 mg): ir $\lambda_{max}^{CHCl_3}$ 2.75, 2.9, 5.81, and 5.90 μ; mass spectrum, *m/e* 502 ($M^+ - 60$), 405, 387, 369, 142, 113, and 97.

Triacetates 1b and 3b.—A solution of fraction H (110 mg) in pyridine (5 ml) and acetic anhydride (5 ml) was left overnight at room temperature. Working up in the normal way gave a solid, which was separated by chromatography on silica gel to give a mixture of two triacetates (99 mg) and an unidentified less polar product. Repeating the acetylation gave a mixture of two triacetates (174 mg), which was separated by chromatography on Florisil (100 g). Elution with ether and then 3% ethyl acetate in ether yielded first the acetate **1b** as a homogeneous (tlc) amorphous solid (40 mg): R_f 0.6, absorbed uv light [ether–ethyl acetate (4:1)]; uv λ_{max}^{MeOH} 230 mμ (ε 12,200); ir $\lambda_{max}^{CHCl_3}$ 2.9, 5.74, 5.89, and 6.12 μ; mass spectrum, *m/e* 644 (M^+), 531, 471, 411, 111, and 96.

Further elution yielded a second amorphous homogeneous (tlc) triacetate **3b** (146 mg): R_f 0.5 [ether–ethyl acetate (4:1)]; uv end absorption; ir $\lambda_{max}^{CHCl_3}$ 2.8, 5.74, 5.90, and 6.10 μ; mass spectrum, *m/e* 531, 471, 411, 142, and 113.

Acetonide of Cucurbitacin P (5a).—A solution of cucurbitacin P (**3a**, 1.1 g) and *p*-toluenesulfonic acid (91 mg) in acetone (40 ml) was stirred overnight with anhydrous Na_2SO_4 (1.5 g). The solution was filtered through silica gel and evaporated to give a brown solid (1.3 g), which was fractionated by chromatography on silica gel (120 g). Elution successively with chloroform, 2% methanol in chloroform, and 7% methanol in chloroform yielded first a crude diacetonide (152 mg), R_f 0.8, and then a crude monoacetonide (544 mg), R_f 0.7. The diacetonide was chromatographed on Florisil and on elution with ether gave a homogeneous (tlc) oil: $\lambda_{max}^{CHCl_3}$ 2.8, 5.91 μ; mass spectrum, *m/e* 600 (M^+), 567, 142, and 113. The crude monoacetonide was twice recrystallized from ether–petroleum ether to give crystals of the monoacetonide (**5a**, 80 mg): mp 181–182°; ir $\lambda_{max}^{CHCl_3}$ 2.67, 2.90, 5.90, and 5.91 μ; mass spectrum, *m/e* 542 ($M^+ - 18$), 524.3516 ($C_{33}H_{48}O_5$), 445, 142, and 113.

Anal. Calcd for $C_{33}H_{52}O_7$: C, 70.68; H, 9.35; mol wt, 560. Found: C, 71.14; H, 9.45.

Acetate of Acetonide 5a.—Acetylation of the acetonide (**5a**, 30 mg) with acetic anhydride in pyridine yielded a mixture. Separation by tlc gave a homogeneous (tlc) amorphous compound (**5b**, 11 mg): R_f 0.7; ir $\lambda_{max}^{CHCl_3}$ 2.78, 2.90, 5.72, and 5.90 μ; nmr τ 8.08 (acetyl CH_3CO); mass spectrum, *m/e* 602 (M^+), 487, 427, 115, and 113.

Registry No.—**1a**, 25383-23-7; **1b**, 25383-24-8; **2**, 25383-25-9; **3a**, 25383-26-0; **3b**, 25383-27-1; **4**, 25383-28-2; **5a**, 25383-29-3; **5b**, 25383-31-7.