C-Methyl Determinations.—A weighed quantity of pure "adduct" contained in the oxidation flask was dissolved in dry methylene chloride and ozonized at room temperature for 1.5 hr. After removal of the solvent *in vacuo*, the oxidation mixture was introduced, and the determination performed according to Pregl.[35] The "adduct" yielded 0.16 mole of acetic acid. Identical treatment of caryophyllene gave 1.11 moles of acetic acid. Two independent[36] C-methyl determinations, performed on the "adduct" without the precautionary ozonolysis, afforded 0.29 (and 0.29) mole of acetic acid, and consumed 7.87 (and 8.23) moles of the dichromate reagent.

In the normal C-methyl determination, caryophyllene is known to give 0.77 mole of acetic acid (5.94 moles of dichromate consumed), and caryophyllene oxide (X) gives 0.63 mole of acid (5.52 moles of dichromate reagent consumed).[37]

(35) F. Pregl, "Quantitative Organic Microanalysis," 3rd ed., J. & A. Churchill Ltd., London, 1937, p. 201.

(36) We are indebted to E. J. Eisenbraun at the University of Wisconsin, Madison, for these determinations.

(37) E. J. Eisenbraun, S. M. McElvain and B. F. Aycock, THIS JOURNAL, **76**, 607 (1954).

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, WELLESLEY COLLEGE]

Flower Pigments. I. Further Studies on the Structure of Stillopsin

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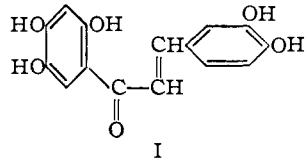
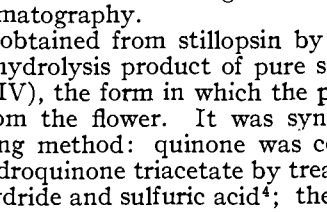
By synthesis of 3′,4′,6,7-tetraacetoxyflavanone and its isolation as a degradation product of stillopsin octaacetate and by paper chromatography of the sugar obtained on hydrolysis of the glycosidic pigment, the structure of the yellow pigment stillopsin, from *Coreopsis stillmanii*, has been verified as a glucoside of 3,4,2′,4′,5′-pentahydroxychalcone. An anion exchange resin has been used to deacidify hydrolysis filtrates preceding analysis by paper chromatography.

Previous work[3] had shown that stillopsin, the yellow pigment of *Coreopsis stillmanii*, was a glycoside of 3,4,2,′,4′,5′-pentahydroxychalcone (I); the proof of the position of the hydroxyl groups was based on the identity of the sample of tetra-



I

methoxyflavanone derived from the pigment with a synthetic sample of 3′,4′,6,7-tetramethoxyflavanone (II). This structure has now been substanti-



II, R = CH_3; III, R = CH_3CO; VI, R = H

ated by means of a similar comparison of 3′,4′,6,7-tetraacetoxyflavanone (III) derived from synthetic and natural sources. In addition the glycosidic group has been shown to be glucose by means of paper chromatography.

III was obtained from stillopsin by acetylating the crude hydrolysis product of pure stillopsin octaacetate (IV), the form in which the pigment was isolated from the flower. It was synthesized by the following method: quinone was converted to hydroxyhydroquinone triacetate by treatment with acetic anhydride and sulfuric acid[4]; the ring acetyl group was introduced by means of acetic acid and zinc chloride,[5] the phenolic acetyl groups being removed in the process; the resultant 2,4,5-trihydroxyacetophenone (V) was condensed in cold basic solution under nitrogen with protocatechualdehyde to give chalcone I; this was isomerized by acid to 3′,4′,6,7-tetrahydroxyflavanone (VI) which was acetylated to the tetraacetyl derivative III. The two samples of III were shown to be identical by analysis, mixed melting point, the distinctive ink-blue color test with ethanol, magnesium and hydrochloric acid[3] and by absorption spectra (Table I).

Since this work was completed, King, King and Neill[6] have reported the isolation of both the free chalcone I, called neoplathymenin, and the corresponding flavanone VI, called plathymenin, from the heartwood of *Plathymenia reticulata*, and they have also prepared the acetyl derivatives. They proved the structure of their compounds by preparing the tetramethyl ether of plathymenin, identical with the known compound II and by oxidative degradation of this ether to 2-hydroxy-4,5-dimethoxybenzoic acid and veratric acid. The present complete synthesis of 3,4,2′,4′,5′-pentaacetoxychalcone (VII) and 3′,4′,6,7-tetraacetoxyflavanone (III) substantiates their identification.[7]

The sugar solution obtained after hydrolyzing IV with hydrochloric acid and separating VI was deacidified by the anion exchange resin Amberlite IR-4-BA.G.[8] After evaporation, it was chromatographed on paper with butanol–acetic acid–water[9] as the developing solvent. Spraying with *m*-phenyl-

(1) Submitted, June, 1952, in partial fulfillment of the requirements for the B.A. degree with honors.

(2) Chromatographic analysis from Miss Thompson's M.A. thesis, June, 1953.

(3) M. K. Seikel and T. A. Geissman, THIS JOURNAL, **72**, 5720 (1950).

(4) E. B. Vliet, "Organic Syntheses," Coll. Vol. I, John Wiley and Sons, Inc., New York, N. Y., 1941, p. 317.

(5) M. Healey and R. Robinson, *J. Chem. Soc.*, 1628 (1934); T. C. Chadha and K. Venkataraman, *ibid.*, 1074 (1933).

(6) F. E. King, T. J. King and K. G. Neill, *ibid.*, 1055 (1953).

(7) A comparison of the acetyl derivatives reported by King, *et al.*, with those synthesized in the present work offers more convincing proof of the identity of the two series than a comparison of the free hydroxy compounds since the latter show a considerable divergency in melting points. This probably resulted because the hydroxy compounds melt with decomposition.

(8) Rohm and Haas, Philadelphia, Penna.

(9) S. M. Partridge, *Biochem. J.*, **42**, 238 (1948).

TABLE I

DATA ON ABSORPTION SPECTRA[a,b]

Compound	Band I λmax, mμ	log ε	λmin, mμ	log ε	Band II λmax, mμ	log ε	λmin, mμ	log ε
2,4,5-Trihydroxyacetophenone (V)	349	3.85	309	3.37	*a*, 280	4.03	258	3.52
					b, 241	4.08	225	3.80
					c, 214	4.12		
3',4',6,7-Tetrahydroxyflavanone (VI)[c]	345.5	3.81	307.5	3.38	*a*, 281.5	4.14	257.5	3.67
					b, 240	4.26	225	4.11
3',4',6,7-Tetraacetoxyflavanone (III), synthetic	320.5	3.65	281.5	2.98	*a*, 253.5	4.05	241.5	3.95
					b, 219	4.55		
III from stillopsin	320.5	3.64	281.5	2.96	*a*, 253	4.05	242	3.94
					b, 219	4.55		
3,4,2',4',5'-Pentahydroxychalcone (I)	393[d]	4.37	293	3.81	268[e]	4.08	238	3.93
3,4,2',4',5'-Pentaacetoxychalcone (VII)	307	4.17	273.5	3.99				

[a] Determined with a Beckman quartz spectrophotometer, model DU. [b] In absolute alcohol solution. [c] Sample may have been slightly impure. [d] With a shoulder at 320 mμ, log ε 4.03. [e] With two minor bands (such as have been noticed in the spectra of other chalcones) at 252 and 245 mμ, log ε 3.96.

enediamine dihydrochloride[10] yielded yellow spots with R_f values identical with those given by an authentic glucose solution.

The absorption spectra of the various compounds resembled in general those of similar compounds. Band I, the color band, of the chalcone I was very broad and as far in the visible as bands of any tetra- or pentahydroxychalcones,[11] the result of the contribution of five hydroxyl groups to the resonance of the molecule. The spectrum of its acetyl derivative, 3,4,2',4',5'-pentaacetoxychalcone (VII), resembled that of the corresponding glycoside IV[3] and of butein tetraacetate[3] although the absorption was considerably less intense than that of the former. The absorption bands of the acetoxyflavanone III were shifted as expected toward the ultraviolet in comparison with the bands of the corresponding hydroxy and methoxy[3] compounds, and the spectrum resembled that of naringenin triacetate (4',5,7-triacetoxyflavanone).[12] The maxima of these bands were at wave lengths almost identical (within ±1 mμ) with those of unsubstituted flavanone itself.[13] The free tetrahydroxyflavanone VI showed three maxima similar to those of its tetramethyl ether II[3] and so characteristically different from the bands of other polymethoxyflavanones.[13] The 6-hydroxyl or -methoxyl group in these compounds apparently caused considerable shift to the visible of the long wave length band (band I) of the unsubstituted flavanone (λ_{max}, 320 mμ[13]); thus it was not concealed by the shift and exaltation of the 252 mμ band of flavanone as it was in flavanones without a methoxyl group in the 6-position.[13] The great similarity of the spectrum of this flavanone VI to that of the acetophenone V from which it is derived seemed to show that the absorption of the flavanone is caused mostly by the acetophenone part of the molecule where the hydroxyl groups can interact with the carbonyl group in resonance. The position of the long wave length band of V (at 349 mμ) is distinctive, likewise, since other hydroxyacetophenones do not show maxima above 327 mμ.[11b,14] The effect of the hydroxyl groups in these positions is being studied further.

Acknowledgment.—The authors wish to express their appreciation to the Research Corporation, New York, for a Frederick Gardner Cottrell grant which made possible the purchase of a Beckman spectrophotometer.

Experimental[15,16]

Synthesis of 3',4',6,7-Tetraacetoxyflavanone (III). 2,4,-5-Trihydroxyacetophenone (V).—Attempts to prepare V from resacetophenone by the Elbs persulfate oxidation by a variety of modifications reported in the literature[17] failed to give yields better than the 9% yield of pure material obtained orginally in this research,[8,18] a result undoubtedly due to the instability of the trihydric phenol in the alkaline oxidizing medium. Nuclear acetylation of hydroxyhydroquinone was studied and found to promise better results although all the details were not satisfactorily worked out. The following method was adapted from those of Healey and Robinson and of Chadha and Venkataraman.[5]

Fused zinc chloride (139 g.) was dissolved with stirring in 133 ml. of almost boiling acetic acid, a process requiring 30 minutes. To these reagents kept at 120°, 93 g. of hydroxyhydroquinone triacetate (prepared within 24 hours[4]) was added with stirring. The resulting mixture was heated for 2 hours at 140–150°.[19] The color changed from deep brown to wine-red. After being cooled, the product was hydrolyzed by refluxing it for 2 hours with 83 ml. of concentrated hydrochloric acid and 455 ml. of saturated sodium bisulfite solution. A 32% yield of crude V, 19.8 g., m.p. 196–198°, was isolated in two portions. The first portion was obtained by extracting with 100 ml. of water the purple gum which precipitated during the hydrolysis and re-extracting the water with ether; after the ether extract was washed with small portions of 10% sodium acetate solution until most of the color was removed, it was evaporated and orange crystals were obtained. The second portion was obtained from the aqueous mother liquor, from which more purple gum was forced out on attempted extraction with ether; this gum was dried and extracted with ether in a Soxhlet

(10) E. Chargaff, C. Levine and C. Green, *J. Biol. Chem.*, **175**, 70 (1948).

(11) For examples see: (a) footnote 3; (b) A. Russell, J. Todd and C. L. Wilson, *J. Chem. Soc.*, 1942 (1934).

(12) M. K. Seikel and T. A. Geissman, THIS JOURNAL, **72**, 5725 (1950).

(13) B. Skarzynski, *Biochem. Z.*, **301**, 150 (1939).

(14) R. A. Morton and A. L. Stubbs, *J. Chem. Soc.*, 1347 (1940).

(15) All melting points are uncorrected.

(16) Microanalyses performed by Drs. Weiler and Strauss, Oxford, England.

(17) (a) By the method of W. Baker, N. C. Brown and J. A. Scott, *J. Chem. Soc.*, 1924 (1939), a 16.7% yield of crude tan material, m.p. 195–198°, was obtained after many extractions of the aqueous hydrolysis mixture with ether and washing of the dark ether solutions with small portions of sodium acetate solution; (b) by the modification of W. Baker and N. C. Brown, *ibid.*, 2304 (1948), for water-soluble phenols a 6.9% yield of tan material, m.p. 170–195°, was isolated; (c) by the method of K. V. Rao and T. R. Seshadri, *Proc. Indian Acad. Sci.*, **25A**, 422 (1947), only a small amount of gum was obtained.

(18) Note also S. M. Sethna, *Chem. Revs.*, **49**, 91 (1951).

(19) Lower or higher temperatures yielded none of the desired product.

and when the ether solution was treated as before, a yellow product was isolated.[20] The crude product was decolorized most efficiently by recrystallizing it from a minimum amount of water containing an equal weight of Norite decolorizing carbon. Glistening straw-colored needles, m.p. 200–202° (recorded 200–202°,[21] 206–207°[5]) separated and one or two extractions of the Norite with alcohol produced further material of the same color and melting point. The recovery was 70–80% when this method was applied to once-recrystallized material. The over-all yield of this run was actually 5.93 g. or 9.5%.[22]

3,4,2',4',5'-Pentahydroxychalcone (I), Stillopsidin.—V, 3.61 g. was condensed with 2.97 g. of protocatechualdehyde[23] by a cold condensation method modified by the use of an atmosphere of nitrogen.[24] After solution of the reactants in 7.2 ml. of ethanol by heating and displacement of the air by nitrogen, the flask was cooled to 0° and 51 ml. of 60% potassium hydroxide was added slowly. After the yellowish mixture had stood 1–2 hours in the ice-bath, it was allowed to stand, with occasional shaking, for two weeks at room temperature. The resultant deep red solution containing a dark red oil was poured onto ice and acidified slowly to *p*H 3 with 6 *N* hydrochloric acid. The crude orange chalcone which separated was dried in air and weighed 3.46 g. From the filtrates 2.20 g. of very impure V, m.p. 170–174°, was recovered by extraction with ether. Compound I was purified first by dissolving it in ether and removing an insoluble brown residue which was mainly inorganic, then by two recrystallizations of the residue, after evaporation of the ether, from water, and finally by recrystallization from a methanol–benzene mixture. After the first recrystallization from water 1.02 g. (16.5% yield) of golden yellow needles was obtained; from methanol and benzene they separated as a brilliant orange powder. The melting point was extremely difficult to determine accurately because of decomposition and could not be used as a criterion of purity. Values observed included 237°, 225–226° and 210–211°, of which *ca.* 225°, obtained by plunging the melting point tube into a preheated bath, seems best. King, *et al.*,[6] report 232° dec. for the chalcone isolated from *Plathymenia reticulata*. Compound I gave the following color tests: intense blue-red with 10% sodium hydroxide; bright orange with concentrated sulfuric acid; blue with ethanol, magnesium and hydrochloric acid after 15 minutes of preliminary heating in acidified alcohol to isomerize the chalcone to flavanone.

Anal. Calcd. for $C_{15}H_{12}O_6$: C, 62.50; H, 4.20. Found: C, 62.50; H, 4.29.

3,4,2',4',5'-Pentaacetoxychalcone (VII).—Compound I, 0.2 g., was acetylated with acetic anhydride and sodium acetate.[3] The delicate cream-yellow fibrous crystals obtained after three recrystallizations from methanol and one from ethanol melted at 154.5° (155–156° was reported by King, *et al.*,[6] for the derivative of the "natural" chalcone). With 10% sodium hydroxide VII develops a blue-red color in 5–10 seconds. With concentrated sulfuric acid it produces a red-orange color at once. When heated for 1 minute with ethanol–hydrochloric acid (1:1) to deacetylate it and then treated with magnesium and ethanol only a yellow color or sometimes a pale blue color is obtained in contrast to the intense blue given by the flavanone III under the same conditions.

Anal. Calcd. for $C_{25}H_{22}O_{11}$: C, 60.24; H, 4.45. Found: C, 60.35, 60.21; H, 4.79, 4.52.

3',4',6,7-Tetrahydroxyflavanone (VI).—Compound I was isomerized to the corresponding flavanone by the acid isomerization method[24] modified because of the instability of VI. The chalcone (0.1 g.) was dissolved in 4 ml. of hot methanol and 20 ml. of 0.6 *N* hydrochloric acid. The mixture was allowed to stand overnight, during which time compound I reprecipitated, and was then refluxed for 3 hours.[25] The color then changed from red-orange to yellow-orange. The resulting solution which contained a mixture of I and VI was extracted with eleven 1-ml. portions of peroxide-free isopropyl ether[26] to remove the more soluble chalcone, the progress of the extraction being followed by color tests with base and with ethanol, magnesium and hydrochloric acid. Ten to forty per cent. of I was recovered after evaporation of the isopropyl ether. VI was isolated by saturating the residual aqueous solution with ammonium sulfate and extracting it with peroxide-free ether.[26,27] The ether was washed with saturated ammonium sulfate solution until it was pale yellow in color, dried over magnesium sulfate and evaporated. The residual red oil crystallized to a tan powder weighing 0.065 g. (65% yield) and melting at 216°; it was not purified further before acetylation.

VI also was obtained in purer form from the acid hydrolysis of the pentaacetoxychalcone VII. For this preparation 0.45 g. of VII was refluxed for 17.5 hours with 18 ml. of methanol and 90 ml. of 0.6 *N* hydrochloric acid until a brown residue began to collect. The extractions with peroxide-free isopropyl ether and ethyl ether were done as before. In this way a yellow glass was obtained which crystallized to 0.14 g. of a light yellow powder (54% yield), m.p. 213° dec. The best method for decolorizing this tetrahydroxyflavanone was to dissolve it in acetone, add chloroform until the solution just clouded, clear it by adding acetone, boil the solution with Norite, filter and boil the nearly colorless filtrate for 1–2 minutes until it clouded. After standing at 0° for several hours the solution deposited 0.01 g. of pure white material, m.p. 216° dec. on the third recrystallization. All attempts to recover more product by concentration of filtrates led to darker material. The pure white crystals tended to darken to a dirty white even on standing at room temperature in a vacuum desiccator and they could not be analyzed satisfactorily. VI gives the following color tests: dirty yellow slowly changing to blue-red with 10% sodium hydroxide; red-orange with concentrated sulfuric acid, and deep ink-blue in the ethanol–magnesium–hydrochloric acid test. Similar color tests were reported by King, *et al.*,[6] for the sample isolated from *Plathymenia reticulata*, but they gave 229° dec. for the melting point.

3',4',6,7-Tetraacetoxyflavanone (III).—VI (0.065 g.) was acetylated by the same method used for I. After one recrystallization of the crude tan precipitate from methanol 0.05 g. of cream-colored material, a 48.5% yield melting at 147°, was obtained. It was completely decolorized (the contaminating pentaacetoxychalcone being removed) only by using large amounts of Norite and then by extracting the Norite with more methanol and evaporating the filtrates. After three recrystallizations the product (18.7 mg.) melted at 149.5–151° (King, *et al.*,[6] report 149–151° for the derivative of their "natural" flavanone). When III and VII were mixed, the melting point was lowered to 132°. With 10% sodium hydroxide III slowly gave a yellow-red color which changed to blue-red after several minutes; with concentrated sulfuric acid a bright orange color developed; with ethanol, magnesium and hydrochloric acid, after hydrolysis for 1 minute with ethanol–hydrochloric acid (1:1), it gave a deep ink-blue, stable to excess acid.

Anal. Calcd. for $C_{23}H_{20}O_{10}$: C, 60.52; H, 4.42. Found: C, 60.20, 60.53; H, 4.76, 4.70.

3',4',6,7-Tetraacetoxyflavanone (III) from Degradation of Stillopsin.—Dried petals (ray-florets) of *Coreopsis stillmanii*[28] (32 g.) were extracted with ether as described pre-

(20) In addition to V, two by-products were isolated by further ether extractions of the original purple gum. Five grams of material melting at 155–163° was probably 2,4,5-trihydroxyacetophenone diacetate, m.p. 165–166° ("Beilstein," Vol. 8, 1st Supplement, 1931, p. 687), since a purified sample of similar material melted at 163–165°. A similar amount of material melting around 180° may have been the compound, m.p. 187°, isolated by Bargellini from the action of aluminum trichloride on 2,4,5-triacetoxyacetophenone ("Beilstein," *loc. cit.*). These could not be hydrolyzed to the desired product, since unchanged material, m.p. 184°, was obtained after they had been refluxed with hydrochloric acid and saturated bisulfite solutions, and since refluxing this material with 60% sulfuric acid yielded only tars.

(21) G. Bargellini, *Gazz. chim. ital.*, **43**, I, 164 (1913) [*C. A.*, **7**, 1725 (1913)].

(22) This yield could undoubtedly be raised by (a) using larger volumes of hydrochloric acid and sodium bisulfite solution in the hydrolysis and (b) applying the more efficient method of decolorization to all of the crude material.

(23) J. S. Buck and F. J. Zimmerman, "Organic Syntheses," Coll. Vol. II, John Wiley and Sons, Inc., New York, N. Y., 1943, p. 549.

(24) T. A. Geissman and R. O. Clinton, THIS JOURNAL, **68**, 697 (1946). Hot condensation, even in the presence of nitrogen, produced only tars plus unchanged ketone.

(25) More refluxing yielded large amounts of black by-products.

(26) C. E. Redemann and C. Niemann, *Org. Syntheses*, **22**, 3 (1942).

(27) When ordinary ether was used, dark products were obtained after evaporation of the ether.

(28) Grown in Wellesley, Mass., the preceding summer.

viously, but no extraction with methanol was attempted.[3] After extraction for 6 weeks, 3.23 g. of a vivid orange, ether-insoluble gum was obtained. This was acetylated as before,[3] and after two recrystallizations from methanol 0.445 g. of stillopsin octaacetate (IV), m.p. 191° (recorded 191.5–192.5°[3]) was isolated representing 1.4% of the original weight of the dried petals and being over four times the recovery reported before.[3] IV (0.3 g.) was hydrolyzed as described previously.[3] Partially granular, red-orange VI (0.10 g., a 53% yield, m.p. *ca.* 213–215° dec.) was obtained by extraction with ether, and the aqueous solution was reserved for identification of the sugar. The crude flavanone VI gave the typical ink-blue color with ethanol, magnesium and hydrochloric acid. It was acetylated in the usual manner,[3] and 0.060 g. of cream-white III (36% yield), m.p. 147.8–148°,[29] was obtained after three recrystallizations from methanol. It gave the following color tests: 10% sodium hydroxide, red-orange developing slowly; concentrated sulfuric acid, brilliant orange; ethanol, magnesium and hydrochloric acid, after hydrolysis for 1 minute with ethanol–hydrochloric acid (1:1), deep blue, stable to excess acid.

Anal. Calcd. for $C_{23}H_{20}O_{10}$: C, 60.52; H, 4.42. Found: C, 60.31, 60.26; H, 4.76, 4.60.

The sample of III obtained by degradation of IV was shown to be identical with that prepared by synthesis as follows: the two formed oblong crystals, their color tests were identical when conducted simultaneously, a mixture melted at 147° (natural 147.5–148°, synthetic 147.5–148.5°), and the absorption spectra were superimposable (see Table I).

Identification of Glucose. Removal of Hydrochloric Acid.—Preliminary experiments to develop a method for the use of an ion exchange resin for the removal of hydrochloric acid from the hydrolysis filtrates of glycosidic pigments were done using the resins listed in Table II and three synthetic solutions: namely, (a) 1 *N* hydrochloric acid, (b) methanol–1 *N* hydrochloric acid (1:17 v./v.), (c) methanol–1 *N* hydrochloric acid–1% glucose (1:17:3 v./v.). Solution c is approximately the mixture present in hydrolysis filtrates. The columns, 2 × 11 cm., were prepared for use by regenerating the resins with 50 ml. of 1 *N* sodium hydroxide and washing them with about a liter of water, until the washings showed a *p*H of 4–5. Table II shows the results of passing 25 ml. of solution a through the columns.

TABLE II

EFFECTIVENESS OF ANION EXCHANGE RESINS IN DEACIDIFICATION

Resin	Ml. 1 *N* NaOH Method I[e]	Method II[f]
Nalcite SAR[a]	0.00[g]	0.00[g]
Nalcite WBR[a]	6.2	...
Amberlite IRA-400[b]	10.95	...
Amberlite IR-4-BA.G.[b]	0.60	0.10
Deacidite[c]	[h]	...
Ionac A-300[d]	4.45[h]	...

[a] National Aluminate Corporation. [b] Footnote 6. [c] Permutit Company. [d] Dow Chemical Company. [e] Method I: after the acid was passed through once, the column was washed free of acid and the eluate and washings recirculated. The column was rewashed and the eluate and washings (total volume 300 ml.) were titrated with base. [f] Method II: the 25 ml. of eluate was recirculated five times without the intermediate washing, then the column was washed with 50 ml. of water. The total volume, 80 ml., was titrated as before. [g] The eluates were slightly basic and required 0.5–0.05 ml. of acid for back-titration. [h] The resins imparted a yellow color to the solution. Deacidite was less effective than Amberlite IR-4-BA.G.

Nalcite SAR and Amberlite IR-4-BA.G. operated just as effectively with solution b. Solution c was passed through these two resins and the eluate collected in 10-ml. portions. Benedict test showed that the sugar was being discharged in the fifth, sixth and seventh 10-ml. portions with the Amberlite column but that the Nalcite column had adsorbed the sugar. The sugar-rich portions were recirculated through the former resin; the fifth and sixth portions again contained the sugar but in considerably lessened amounts. Amberlite IR-4-BA.G. was chosen as the resin best suited for the removal of hydrochloric acid from filtrates from the hydrolysis of glucosides.

The acidic aqueous solution[30] from the hydrolysis of stillopsin octaacetate was clarified by filtration through a Celite[31] mat and then half (35 ml.) of the yellow filtrate was deacidified with Amberlite IR-4-BA.G. by the method described for solution c, the seventh and eighth 10-ml. portions being recirculated. The resultant sixth portion alone gave evidence of sugar, so it was evaporated under reduced pressure to 0.5 ml.[32]

Paper Chromatography.—Preliminary experiments on the paper chromatography of sixteen hexoses, pentoses and uronic acids showed that butanol–acetic acid–water (4:1:5 v./v.)[9,33] was the most satisfactory developing solution from the point of view of speed, separation of sugars and absence of streaking. Ethyl acetate–acetic acid–water (3:1:3 v./v.)[34] was fairly satisfactory; it was faster but did not separate as many sugars as well. In butanol–ethanol–water (4:1:5 v./v.)[9,35] the sugars gave more diffuse spots and the uronic acids streaked. Ethyl acetate–pyridine–water (2:1:2 v./v.)[34,36] moved rapidly but was unsatisfactory because the solvent in the trays could not be reused, the R_f values were difficult to check and galacturonic acid streaked. The most satisfactory spraying reagents were aniline phthalate (0.47 g. of aniline and 0.83 g. of phthalic acid in 50 ml. of water-saturated butanol[37]) and *m*-phenylenediamine dihydrochloride (0.2 *M* in 76% ethanol[10]), since they gave the best color distinctions between the different types of sugars and since the colors in the case of the latter are particularly vivid (Table III). The clear distinction of the colors was not permanent in either case since the spots all grew brown with time. The entire sheets sprayed with *m*-phenylenediamine began to turn brown within a day, but they could be preserved to some extent for a few weeks by dipping in melted paraffin. With benzidine spray[38] dis-

TABLE III

COLORS OF SUGAR SPOTS WITH AMINE SPRAYS

Sugar	Aniline phthalate	*m*-Phenylenediamine[a]
Glucose	Tan	Yellow-brown
Galactose	Brown	Yellow-brown
Mannose	Deep tan	Yellow-brown
Rhamnose	Tan	Yellow-brown
Fructose	Pale yellow	Yellow-brown
Xylose	Deep mauve	Pink-brown
Arabinose	Deep mauve	Pink-brown
Ribose	Deep mauve	Pink-brown
Maltose	None	Yellow
Lactose	Light tan	Yellow
Sucrose	None	Yellow
Fucose	...	Yellow
Apiose	...	Yellow
Glucuronic acid	Pale yell.-or.	Pink[b]
Galacturonic acid	Orange	Pink

[a] The fluorescence under ultraviolet light mentioned by Chargaff, *et al.*, also was noticed. [b] With a discernible orange-brown cast in comparison with galacturonic acid.

(29) Compare with the melting point of 146.5–148.5° obtained in the earlier work, footnote 3.

(30) A year old at the time.

(31) Johns-Manville.

(32) The maximum concentration of glucose was 1% as it was derived from one-half of the hydrolysis filtrate from 0.3 g. of IV.

(33) J. R. Hawthorne, *Nature*, **160**, 715 (1948). It has been used frequently since then.

(34) M. A. Jermyn and F. A. Isherwood, *Biochem. J.*, **44**, 402 (1949). It also has been used frequently.

(35) S. M. Partridge, *Nature*, **158**, 270 (1946).

(36) P. S. Rao, R. M. Beri and P. R. Rao, *Proc. Indian Acad. Sci.*, **34A**, 263 (1951).

(37) S. M. Partridge, *Nature*, **164**, 443 (1949). It has been reported frequently since.

(38) R. H. Horrocks, *Nature*, **164**, 444 (1949).

tinction was poor since the spots ranged from yellow to brown in color and soon became brown. Anisidine phosphate[39] likewise gave tan colors with variations toward yellow and pink, but both the sheets and the spots became dark gray on standing. In contrast to the wide applicability, considerable color distinction and ease of use of the aryl amine sprays, phenolic sprays were found to be less satisfactory.[40]

The unknown sugar solution was chromatographed by the descending method on Whatman No. 1 filter paper with butanol–acetic acid–water as the developing agent and *m*-phenylenediamine as the spray. Two, four, six and ten applications of the solution were made to separate areas 5 mm. in diameter,[41] and the chromatogram was run for 14 hours after the chromatography box had been saturated overnight. The spots, after development of the color, were clear and well-defined, showing that the anion exchange resin had been effective in removing the acid. The results listed in Table IV were obtained, proving that the sugar was glucose.

TABLE IV

CHROMATOGRAPHY OF UNKNOWN SUGAR

Sugar[a]	R_f[b]	Color
Unknown	0.205[c]	Yellow-tan
Glucose	.206	Yellow-tan
Galactose	.189	Yellow-tan
Mannose	.241	Yellow-tan
Fructose	.243	Pale yellow

[a] These sugars were used for comparison since the unknown was known from analysis to be a hexose and since it appeared to yield glucosazone.[3] [b] Average of 4 values. [c] Average of 12 values.

(39) S. Mukherjee and H. C. Srivastava, *ibid.*, **169**, 330 (1952).

(40) They were, however, more selective for certain types of sugars. Those tried included orcinol and trichloroacetic acid, A. Benvenue and K. T. Williams, *Arch. Biochem. Biophys.*, **34**, 225 (1951); resorcinol, V. V. Rachinskii and E. I. Knyazyatova, *Doklady. Akad. Nauk S.S.S.R.*, **85**, 1119 (1952) [*C. A.*, **47**, 448 (1953)] and W. G. Forsyth, *Nature*, **161**, 240 (1948); and naphthoresorcinol, W. G. Forsyth, above, and footnote 7.

(41) Each 5 mm. spot was approximately equivalent to 3–5 μl. of a 1% sugar solution or 30–50 μg. of sugar. Extra applications were made since the strength of the unknown solution was undoubtedly less than 1%.

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[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY OF WAYNE UNIVERSITY]

Terpenoids. XI.[1] Investigation of Nine Cactus Species. Isolation of Two New Triterpenes, Stellatogenin and Machaeric Acid[2]

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The chemical examination of nine cacti from Mexico, Costa Rica and Peru is described. In addition to the known triterpenes oleanolic acid (I) and betulinic acid (III), two new triterpenes were encountered. From *L. stellatus* and *M. eruca* there was isolated a new dihydroxy lactone, stellatogenin, which was shown to possess one secondary and one tertiary hydroxyl function by direct correlation with the cactus triterpene thurberogenin. The isolation of a second new triterpene, machaeric acid (21-keto-oleanolic acid) (II), from *M. gummosus* is recorded.

Our initial observation[1,3] of the occurrence of triterpene glycosides in certain giant cacti has prompted us to undertake a more extensive study of this plant family. We should now like to report on nine cactus species which range, as far as their natural habitats are concerned, from Northern Mexico to Peru.

The genus *Lemaireocereus* has so far been the one most extensively studied in our laboratory[1,3] since it appears to be particularly rich in triterpene glycosides and it has now been possible to obtain four additional species.

L. pruinosus[4] occurs rather widely in Central and Southern Mexico[5] where it is known as "Pitayo" and the presently employed specimens were obtained from the gardens of Mr. Howard E. Gates of Corona, California.[6] The plant contained glycosidic material and upon hydrolysis yielded a single triterpene identified as oleanolic acid (I).

L. stellatus[6,7] is quite common in Southern Mexico, principally in the States of Oaxaca and Puebla and is generally referred to as "Xoconochtle" or "Pitayo." This cactus has so far proved to be the richest source of triterpenes, approximately 3.5% of a triterpene mixture being obtained after acid hydrolysis. The acidic fraction was separated only with difficulty after chromatography of the methyl esters, whereupon oleanolic (I) and betulinic (III) acids[8] could be identified. This represents the second isolation of a member of the lupeol group of triterpenes from cacti[1]; as will become apparent from subsequent publications several new triterpenes encountered in our cactus studies also belong to this group.

The neutral fraction was processed by chromatography of the acetates which resulted in the clean separation of two components. The earlier eluted material (*ca.* 15%) was identical with the acetate of thurberogenin, a new triterpene lactone ($C_{30}H_{46}O_3$) isolated recently[3a] from the cactus *Lemaireo-*

(1) Paper X, C. Djerassi and A. E. Lippman, THIS JOURNAL, **76**, 5780 (1954).

(2) We are greatly indebted to the Division of Research Grants (Grant No. G-3863) of the U. S. Public Health Service and to the Rockefeller Foundation for financial support.

(3) (a) C. Djerassi, L. E. Geller and A. J. Lemin, THIS JOURNAL, **75**, 2254 (1953); (b) C. Djerassi, R. N. McDonald and A. J. Lemin, *ibid.*, **75**, 5940 (1953); (c) C. Djerassi, E. Farkas, A. J. Lemin, J. C. Collins and F. Walls, *ibid.*, **76**, 2969 (1954).

(4) N. L. Britton and J. N. Rose, "The Cactaceae," Carnegie Institution of Washington, Washington, D. C., 1920, Vol. II, p. 88.

(5) H. Bravo, "Las Cactaceas de Mexico," Mexico, D. F., 1937, p. 256.

(6) We are greatly indebted to Mr. Howard E. Gates for his cooperation in supplying specimens for our work.

(7) Reference 4, p. 92; ref. 5, p. 261.

(8) In contrast to oleanolic acid (see ref. 3a where all plant sources described up to 1952 are summarized), the occurrence of betulinic acid has been reported in only relatively few plants (*cf.* list of plant sources by A. Stabursvik, *Acta Chem. Scand.*, **7**, 446 (1953)). It is quite probable that the presence of this acid has been overlooked in other plants because of the difficult separation from the isomeric oleanolic acid.