mm. to give a virtually quantitative recovery of *N*-carbethoxy-4-(2-hydroxyethyl)-8-methoxy-1,2,3,4-tetrahydrobenz[f]isoquinoline as a glass-like substance, $\nu_{max}^{CHCl_3}$ 3470 (m, OH stretching) and 1665 cm.$^{-1}$ (s, urethan, carbonyl).

Anal. Calcd. for $C_{19}H_{23}NO_4$: C, 69.28; H, 7.04; N, 4.25. Found: C, 69.29; H, 7.32; N, 4.26.

N-Carbomethoxymethyl-4-(2-hydroxyethyl)-8-methoxy-1,2,3,4-tetrahydrobenz[f]isoquinoline (VIf). A solution of 2.62 g. of 4-(2-hydroxyethyl)-8-methoxy-1,2,3,4-tetrahydrobenz-[f]isoquinoline (VId) and 1.54 g. of methyl bromoacetate in 150 ml. of pure tetrahydrofuran was refluxed with stirring for 8 hr. during which time a white precipitate formed. The precipitate was removed by filtration and the filtrate was evaporated under reduced pressure to give a crystalline residue. Recrystallization of this residue from benzene-hexane gave 1.51 g. (45%) of the *N*-alkylated product, m.p. 124–127°. The analytical sample of VIf had m.p. 127.5–128°, $\nu_{max}^{CHCl_3}$ 3450 (m, OH stretching) and 1745 cm.$^{-1}$ (s, ester carbonyl).

Anal. Calcd. for $C_{19}H_{23}NO_4$: C, 69.28; H, 7.04; N, 4.25. Found: C, 69.70; H, 7.26; N, 4.38.

The white precipitate obtained from the reaction mixture proved to be *4-(2-hydroxyethyl)-8-methoxy-1,2,3,4-tetrahydrobenz[f]isoquinoline hydrobromide*. The analytical sample was crystallized from absolute ethanol–ether as a highly hygroscopic material, m.p. 197.5–198°.

Anal. Calcd. for $C_{16}H_{20}BrNO_2$: C, 56.81; H, 5.96; Br, 23.63; N, 4.14. Found: C, 56.76; H, 6.19; Br, 23.23; N, 4.14.

Treatment of the hydrobromide with base gave 1.15 g. of unchanged starting material VId, representing a 44% recovery, m.p. 135–136°.

Acknowledgment. We are indebted to Dr. V. A. Drill and his associates of the Division of Biological Research of G. D. Searle and Company and to the Cancer Chemotherapy National Service Center for bioassays of some of the compounds. Compounds VIb, VIe, and VIf showed essentially no estrogenic or anti-inflammatory activity. Compounds VIb and VIf also lacked any appreciable androgenic activity.

CAMBRIDGE 39, MASS.

[CONTRIBUTION FROM THE DEPARTMENT OF CHEMISTRY, THE UNIVERSITY OF CONNECTICUT AND THE ORGANIC CHEMICAL INSTITUTE OF THE UNIVERSITY OF ZÜRICH]

Catalpa Glycosides. I. The Characterization of Catalposide[1]

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Catalposide has been isolated from several species of *Catalpa* and has been established as a $C_{22}H_{26}O_{12}$ compound; a D-glucoside and an ester of *p*-hydroxybenzoic acid. It contains one additional hydroxyl group and an isolated double bond.

Catalposide was isolated from the fruit of *Catalpa bignonioides* in 1888 by Claassen[3] who named it catalpin. It was reisolated from the same plant fifty-five years later by Colin, Tanret, and Chollet[4,5] who named it catalposide. Still another isolation, this time from several species of *Catalpa*, was made by Plouvier.[6] Catalposide is reported[4,5] to give a positive reducing test when boiled with Fehling's solution, a positive xanthoproteic test, a positive biuret test, to be converted to a black polymer by acid hydrolysis, and to be hydrolyzed by emulsin.[4] In a more recent publication[7] catalposide is postulated to be a complex polysaccharide.

The most striking property of catalposide is its destruction by acidic and enzymatic hydrolysis resulting in the formation of colored solutions and black, amorphous precipitates.[4,5] The sugar portion, however, is stable and remains in the hydrolyzate. From the optical rotation of this solution, it was concluded that the sugar was probably glucose.[4,5] This tendency to decompose is reminiscent of the aucubin type[8] of glucosides, that is, aucubin,[9] monotropein,[10] asperuloside,[11] plumierid,[12] and agnusid.[13] None of the papers on catalposide report any analyses or derivatives or any attempts other than a melting point and a rotation to characterize it in a quantitative manner. This paper reports the

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(2) Abstracted in part from the M.S. Thesis of T. B. Africa, the University of Connecticut, 1959. Present address: Research Laboratories, Chas. Pfizer and Co., Groton, Conn.

(3) E. Claassen, *Am. Chem. J.*, **10**, 328 (1888).

(4) H. Colin, G. Tanret, and M.-M. Chollet, *Compt. rend.*, **216**, 677 (1943).

(5) M.-M. Chollet, *Bull. soc. chim. biol.*, **28**, 668 (1946).

(6) V. Plouvier, *Compt. rend.*, **224**, 670 (1947).

(7) M.-M. Chollet, *Compt. rend.*, **249**, 2611 (1959).

(8) A. R. Trim and R. Hill, *Biochem. J.*, **50**, 310 (1952).

(9) (a) P. Karrer and H. Schmid, *Helv. Chim. Acta*, **29**, 525 (1946); (b) S. Fujise, H. Uda, T. Ishikawa, H. Obara, and A. Fujino, *Chem. & Ind. (London)*, 954 (1959); (c) S. Fujise, H. Obara, and H. Uda, *Chem. & Ind. (London)*, 289 (1960); (d) J. Grimshaw and H. R. Juneja, *Chem. & Ind. (London)*, 656 (1960); (e) M. W. Wendt, W. Haegele, E. Simonitsch, and H. Schmid, *Helv. Chim. Acta*, **43**, 1440 (1960).

(10) M. Bridel, *Compt. rend.*, **176**, 1742 (1923); *Bull. soc. chim. biol.*, **5**, 722 (1923).

(11) L. H. Briggs and B. F. Cain, *J. Chem. Soc.*, 4182 (1954).

(12) O. Halpern and H. Schmid, *Helv. Chim. Acta*, **41**, 1109 (1958).

(13) E. Winde and R. Hänsel, *Archiv. Pharm.*, **293/65**, 556 (1960).

TABLE I

SPECIES OF CATALPA EXAMINED FOR CATALPOSIDE

Species	Source	Yield, %[a]
C. bignonioides Walt.	Tenn.	0.38
	W. Va.	0.23
	Switzerland	0.80
C. speciosa Warder	Conn.	0.06
C. Hybrida Spaeth	Mass.[b]	0.04
C. ovata flavescens	Mass.[b]	0.06
C. bignonioides aurea Lav.	Mass.[b]	0.05

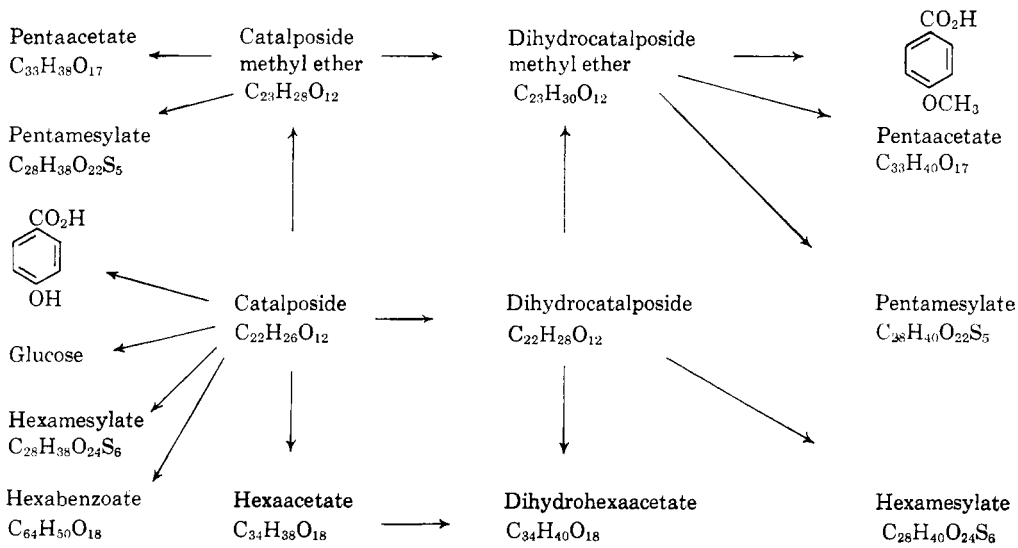
[a] The yield data are questionable since it is certain that the glucoside concentration varies, probably increasing with the age of the fruit, reaching a maximum at the end of the season. The data are based upon weight of green fruit since the glucoside is rapidly destroyed after picking. [b] Arnold Arboretum of Harvard University.

isolation of catalposide from some additional species of *Catalpa* and analyses of the glucoside and twelve crystalline derivatives which establish a molecular formula and some of the functional groups. In addition, three simple degradations are given.

Catalposide has been isolated from *Catalpa bignonioides* Walt.,[3-6] *C. speciosa* Warder,[6] *C. ovata* Don. (*Kaempferi* Sieb.),[6] and *C. bignonioides japonica* Rehd.[6] Using a color test, Plouvier showed[6] that the glucoside was probably present in *C. bignonioides pulverulenta* Hort., *C. bignonioides Koehnei* Dode, *C. bignonioides purpurea* Rehd., and *C. bignonioides semiplena* and that it was absent in *C. Duclouxii* Dode and *C. Fargesi* Bur. The majority of the glucoside used in this investigation was obtained from *C. bignonioides* Walt. and *C. speciosa* Warder, but several other species were investigated. These data are summarized in Table I.

Catalposide exhibited dual melting points (173–178° and 213.7–215.7°) similar to those reported[4,5] (160° and 212°) when crystallized from water but only the higher value when crystallized from methanol–ethyl acetate. Several derivatives showed a similar behavior. The rotation in water, $[\alpha]_D^{21.5}$ −174°, was similar but not identical to the reported[3,4] value, $[\alpha]_D^{15}$ −166°. The ultraviolet spectrum in ethanol showed a single major peak at 260 mμ, log ε 4.27, which shifted in sodium hydroxide to 303 mμ, log ε 4.35. The infrared spectrum showed, among others, peaks at 5.87μ, 6.05μ, and 6.2μ corresponding to a carbonyl, a double bond, and an aromatic system. Catalposide gives a positive ferric chloride test and negative tests for a methylenedioxy group,[14] for a phenol with a vacant *para* position (Gibbs' test),[15] and for an *o*-dihydroxy or 1,3-diketone grouping.[16]

Catalposide acetate gives a positive test with tetranitromethane,[17] confirming the double bond observed in the infrared spectrum. The analysis of catalposide showed the probable absence of methoxyl and acetyl groups and was consistent with the molecular formula $C_{22}H_{26}O_{12}$. The small value for C-methyl (0.36; calcd. for one group, 3.11) may or may not be significant. Molecular weight determinations on catalposide by Rast or ebullioscopic methods gave widely differing values (320 to 1703; calcd., 482) but satisfactory Rast values were obtained on the hexaacetate (723, 800; calcd., 734) and the hexabenzoate (113, 1214; calcd., 1107). Supporting molecular weight data were obtained by quantitative microhydrogenations of catalposide (488, 486) and its hexaacetate (706, 744). Microtitration of catalposide in Methyl Cellosolve-water showed two inseparable acidic functions with a pK_a' of 9.97. The reactions that were carried out are summarized in the diagram.



On hydrogenation with 5% palladium-on-carbon in ethanol, catalposide reacts with one mole of hydrogen to yield dihydrocatalposide which crys-

(14) O. A. Stamm, H. Schmid, and J. Büchi, *Helv. Chim. Acta*, **41**, 2006 (1958).
(15) H. D. Gibbs, *J. Biol. Chem.*, **72**, 649 (1927).
(16) F. Weygand and E. Csendes, *Ber.*, **85**, 45 (1952).
(17) L. Ruzicka, H. W. Huyser, M. Pfeiffer, and C. F. Seidel, *Ann.*, **471**, 25 (1929).

tallized both as a monomethanolate and in an anhydrous form. The infrared spectrum of this compound in potassium bromide no longer shows a peak at 6.05 μ and its acetate gives a negative tetranitromethane test.[17] Perhydrogenation of catalposide with platinum oxide in acetic acid does not yield a crystalline compound, but does show the presence of five unsaturations. Both catalposide and dihydrocatalposide yield monomethyl ethers when treated with diazomethane in methanol-ether. The reduction of catalposide methyl ether to give dihydrocatalposide methyl ether identical with that formed by hydrogenation followed by methylation suggests that both reactions take a normal course. Catalposide methyl ether shows no acidic properties. Thus, a monomethylation *appears* to block both of the acidic functions noted in catalposide. These monomethyl ethers, as well as the crystalline monomethanolate of dihydrocatalposide, gave correct analyses for one methoxyl group, thus further corroborating the molecular formula.

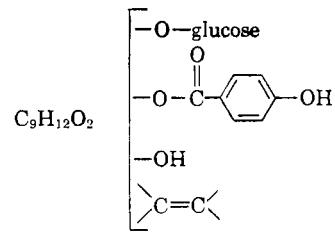
Each of the four compounds, catalposide, dihydrocatalposide, catalposide methyl ether, and dihydrocatalposide methyl ether was converted to an acetate and a mesylate (methanesulfonate). In addition, a benzoate of catalposide was prepared. Catalposide and its dihydro derivative gave hexaacyl or mesyl derivatives while the methyl ethers gave penta- derivatives. The acetates were nicely crystalline compounds with good melting points but did not give conclusive evidence of the number of acylable hydroxyl groups due to the similarity of the carbon-hydrogen ratios of the compounds and their acetates. Thus, it was necessary to prepare the mesylates with their extra sulfur atoms. These mesylates were crystalline and all gave good analyses, but the melting points were catalytic decomposition points and were erratic. The reduction of catalposide hexaacetate to the same dihydrocatalposide hexaacetate obtained by reduction followed by acetylation shows that the reduction and acetylation take a normal course. Thus, it can be concluded that catalposide has six acylable hydroxyl groups. Of these, four are probably in the sugar portion and two are in the aglycone. Of the latter two, one is sufficiently acidic to be reactive to diazomethane.

At present, only three simple degradations have been carried out on catalposide. In the first degradation, catalposide was boiled with 0.05*N* hydrochloric acid. The aglucone was destroyed,[4,5] but D-glucose was isolated from the hydrolyzate and identified, after suitable treatment,[18] as its β-pentaacetate. Thus, catalposide, as previously suggested,[4,5] without adequate proof, is a glucoside. Its lability to emulsin[4,5,7] establishes the glucosidic linkage to be *beta*.

The second and third degradations are similar and explain several of the above experimental facts. Catalposide and dihydrocatalposide methyl ether were saponified with 0.05*N* sodium hydroxide at 35° to give 93 and 96% yields, respectively, of *p*-hydroxybenzoic acid and *p*-methoxybenzoic acid (anisic acid). Thus, catalposide is an ester of *p*-hydroxybenzoic acid or is a 1,3-diketone yielding the acid on base treatment. The negative test for 1,3-diketones mentioned above favors the former conclusion. The free phenol group explains the positive ferric chloride and xanthoproteic tests and clarifies one of the acidic functions. Its *para* relationship explains the negative Gibbs test.[15] The microtitration was carried out slowly and its plateau was at relatively high *p*H values (8.5–11.5 with irregular curve). Since catalposide *mono*methyl ether can not be titrated, the second acidic function of catalposide probably involves saponification and a titration of *p*-hydroxybenzoic acid. The aromatic ester residue accounts for the observed infrared carbonyl and aromatic peaks at 5.87 μ and 6.2 μ. It should be noted that agnusid[13] has been formulated as an ester of *p*-hydroxybenzoic acid and aucubin[9] and that it also has infrared peaks at 5.86 μ and 6.2 μ.

When the ultraviolet spectrum of ethyl *p*-hydroxybenzoate was subtracted from the spectrum of catalposide, a new curve having a maximum at 270 mμ, log ϵ 3.57, was obtained. A similar observation was made when ethyl *p*-methoxybenzoate was compared with catalposide methyl ether and when ethyl *p*-acetoxybenzoate was compared with catalposide hexaacetate. These differences cannot be explained as yet. The ultraviolet spectra of catalposide and dihydrocatalposide are identical. Thus the easily reduced double bond is not conjugated. The shift observed when the ultraviolet spectrum of catalposide is measured in base is readily explained by the phenol group.

The benzene ring and the isolated double bond account for four of the five unsaturations found on perhydrogenation. There is, as yet, no evidence to indicate whether this fifth unsaturation is a double bond or is some grouping sensitive to hydrogenolysis. The hydroxyl groups, the ester linkage, and the glucosidic sugar linkage account for ten of the twelve oxygens. The following diagram illustrates the present state of the problem where $C_9H_{12}O_2$ represents the unknown portion *containing* the double bond carbons. The theoretical aglucone without the *p*-hydroxybenzoyl group is $C_9H_{12}O_5$ in which three oxygens are present as hydroxyl groups.



(18) C. S. Hudson, *J. Ind. Eng. Chem.*, **8**, 380 (1916).

EXPERIMENTAL

The melting points were determined on a Kofler Hot Stage melting point apparatus and are corrected. The microanalyses were performed by Geller Laboratories, Bardonia, N. Y., Drs. Weiler and Straus, Oxford, and H. Frohofer, Chemical Institute, University of Zurich. Before analysis, the compounds were dried under high vacuum over phosphorus pentoxide and potassium hydroxide at 105°, unless noted.

Acetates. The compound to be acetylated, 0.1 g., was dissolved in a mixture of 2 ml. of anhydrous pyridine and 2 ml. of acetic anhydride. After standing at 35° for at least 15 hr., the mixture was poured on ice. The solid was removed by filtration and crystallized from absolute ethanol.

Mesylates. The compound to be mesylated, 0.1 g., was dissolved in 2 ml. of dry pyridine and cooled to −15°. Methansulfonyl chloride, 0.5 ml., was added and the mixture was allowed to warm to 0° over 3 hr. The mixture was cooled again to −12° and water was added, dropwise at first, until a solid precipitated and dissolved and a second precipitate formed. This solid was removed by filtration and dissolved without drying in a minimum of cold 10% pyridine in acetone and crystallized by the addition of methanol. The mesylates were recrystallized from the same solvent system.

Isolation of catalposide. The fruit of *Catalpa bignonioides*, 14.5 kg., was picked, split lengthwise, broken into small pieces, and dropped into 8 l. of boiling ethanol in the shortest possible time. The mixture was boiled for 2 hr. and filtered, and the fruit was boiled for five more hours with a fresh portion of ethanol. The combined, filtered extracts were evaporated under vacuum to about 3 l. and made basic with barium hydroxide. The resulting mixture was treated with concentrated lead acetate solution until no more precipitate formed and filtered through a pad of a filter aid (Hyflo Supercell). Concentrated sulfuric acid was added, dropwise, to the solution until no more precipitate formed and the slurry was again filtered through Hyflo Supercell. The filtrate was extracted three times with ether to remove acetic acid, evaporated, under vacuum, to about 1 l. and allowed to stand overnight at −4°. The resulting precipitate, consisting of small crescent shaped crystals, was removed by filtration and recrystallized three times from 1.5 l. portions of water. After vacuum drying at room temperature over sulfuric acid and potassium hydroxide, 97.6 g. of catalposide, m.p. 213.7–215.7° after partially melting at 173–178°, was obtained (lit.[15] 160° and 212°). An additional 18.6 g. (total 116 g., 0.80%) was obtained from the mother liquors. The homogeneity was demonstrated by paper chromatography using ethyl acetate–acetic acid–water (9:2:2)[19] and butanol-pyridine-water (10:3:3)[19] as eluents and by thin layer (dünnschicht) chromatography[20] on silica-gel with methanol as eluent. The spots on paper were black in ultraviolet light and were also developed by the periodate-permanganate-benzidine spray of Wolfrom and Miller.[21] The spots on the silica-gel layers were developed with concentrated sulfuric acid.

The analytical sample, m.p. 215–216.5°, $[\alpha]_D^{23.2}$ − 184° (*c* 0.87 in methanol), $[\alpha]_D^{21.5}$ − 174° (*c* 0.92 in water) was recrystallized twice from methanol–ethyl acetate.

Anal. Calcd. for $C_{22}H_{26}O_{12}$: C, 54.77; H, 5.43; mol. wt., 482.43. Found: C, 54.79, 54.87; H, 5.52, 5.51; mol. wt. by perhydrogenation, 491, 502 [6.305 mg. (7.174) of catalposide and 53.4 mg. (32.7) of platinum oxide in 5 ml. of acetic acid absorbed 5.03 moles (4.80) of hydrogen in 2 hr. at 20°]; mol. wt. by hydrogenation, 483, 484 [32.043 mg. (30.965) of catalposide and 68.5 mg. (40.0) of 5% palladium on carbon in 5 ml. of ethanol absorbed 0.999 mole (1.004) of hydrogen in 4 hr. at 20°]; mol. wt. by basic microtitration in Methyl Cellosolve-water, 492 (2 moles of base) with pK_a' 9.97; CH_3O, 0.36; CH_3CO, 1.11; CH_3C, 0.36.

The infrared spectrum in potassium bromide showed the following peaks: 2.92 μ (s.), 3.45 (w.), 5.87 (s.), 6.04 (m.), 6.2 (s.), 6.26 (m.), 6.6 (m.), 6.9 to 6.98 (w.), 7.12 (w.), 7.2 (m.), 7.4 (m.), 7.62 (m.), 7.76 (s.), 7.97 (s.), 8.15 (s.), 8.51 (m.), 8.58 (m.), 8.75 (m.), 9.05 (s.), 9.19 (s.), 9.32 (s.), 9.5 (s.), 9.8 to 9.86 (s.), 10.08 (s.), 10.35 (s.), 10.85 (w.), 11.04 (w.), 11.29 (w.), 11.46 (w.), 11.7 (m.), 11.9 (w.), 12.3 (w.), 12.96 (s.), 13.64 (w.), 14.23 (m.), and 14.54 (w.).

The *hexaacetate* was prepared in 71% yield and showed two distinct crystalline forms. When crystallized from acetone-water, the m.p., which persisted after drying, was 100–105°. The analytical sample, m.p. 141.5–142.5°, $[\alpha]_D^{21.7}$ − 106° (*c* 0.75 in chloroform) was recrystallized three times from ethanol.

Anal. Calcd. for $C_{34}H_{38}O_{18}$: C, 55.58; H, 5.22; CH_3CO, 35.15; mol. wt., 734.64. Found: C, 55.45, 55.57; H, 5.40, 5.52; CH_3CO, 35.15, 35.40; mol. wt., Rast, 800, 823; mol. wt. by perhydrogenation, 744, 744 [10.927 mg. (10.413) of catalposide hexaacetate and 35 mg. (53.4) of platinum oxide in 5 ml. of acetic acid absorbed 4.94 moles (4.94) of hydrogen in 1 hr. at 20°]; mol. wt. by hydrogenation, 697, 706 [19.727 mg. (23.235) of catalposide hexaacetate and 68 mg. (18.7) of 5% palladium-on-carbon in 5 ml. of ethanol absorbed 1.05 moles (1.06) of hydrogen in 1 hr. at 20°].

The *hexamesylate* was prepared in 61% yield and showed m.p.'s ranging from 168° to 205°. The analytical sample was recrystallized four times from pyridine–acetone–methanol.

Anal. Calcd. for $C_{28}H_{38}O_{24}S_6$: C, 35.36; H, 4.03; S, 20.23. Found: C, 35.42, 35.53; H, 4.23, 4.13; S, 20.23, 20.46.

The *hexabenzoate* was prepared by allowing catalposide, 2.0 g., to react with 5 ml. of benzoyl chloride in 28 ml. of pyridine. After 14 hr. at 40°, the mixture was poured on ice and filtered. The solid was washed thoroughly with sodium carbonate solution and water and recrystallized four times from ethanol to yield 0.96 g., 21%, of catalposide hexabenzoate, m.p. 174.5–175.5°, $[\alpha]_D^{25}$ − 75.3° (*c* 0.89 in chloroform).

Anal. Calcd. for $C_{64}H_{50}O_{18}$: C, 69.43; H, 4.55; mol. wt., 1107.04. Found: C, 69.39, 69.03; H, 4.80, 4.55; mol. wt., Rast, 1214, 1113.

Dihydrocatalposide. Catalposide, 0.741 g., was hydrogenated at atmospheric pressure and 20° in 20 ml. of ethanol over 0.321 g. of 5% palladium-on-carbon. After the absorption of 1.09 moles of hydrogen, the catalyst was removed by filtration, the solvent was removed under vacuum and the residue was crystallized from methanol to give 0.675 g. (85%) of dihydrocatalposide methanolate. The analytical sample, m.p. 149–151° (loss of solvent), was recrystallized twice from methanol and vacuum dried at 78°.

Anal. Calcd. for $C_{22}H_{28}O_{12}\cdot CH_3OH$: C, 53.48; H, 6.25; CH_3O, 6.01. Found: C, 53.52, 53.42; H, 6.08, 6.04; CH_3O, 6.66.

When dihydrocatalposide was recrystallized from ethanol-benzene, the anhydrous substance was obtained. The analytical sample, m.p. 212–214°, $[\alpha]_D^{22}$ − 139° (*c* 0.85 in methanol) was recrystallized three times.

Anal. Calcd. for $C_{22}H_{28}O_{12}$: C, 54.54; H, 5.83. Found: C, 54.84; H, 5.85.

The *hexaacetate* was prepared in 65% yield and the analytical sample, m.p. 149–150°, $[\alpha]_D^{23.5}$ − 82.3° (*c* 0.92 in chloroform) was recrystallized three times from ethanol.

Anal. Calcd. for $C_{34}H_{40}O_{18}$: C, 55.43; H, 5.47. Found: C, 55.45, 55.53; H, 5.67, 5.52.

Dihydrocatalposide hexaacetate was also obtained in 75% yield by the catalytic hydrogenation of catalposide hexaacetate in ethanol with a 5% palladium-on-carbon catalyst.

The *hexamesylate* was prepared in 55% yield and the analytical sample, m.p. 147–148.5°, was recrystallized four times from pyridine–acetone–methanol.

(19) I. A. Pearl and S. F. Darling, *J. Org. Chem.*, **24**, 731 (1959).

(20) E. Stahl, *Arch. Pharm.*, **292**, 411 (1959).

(21) M. L. Wolfrom and J. B. Miller, *Anal. Chem.*, **28**, 1037 (1956).

Anal. Calcd. for $C_{28}H_{40}O_{24}S_6$: C, 35.29; H, 4.23; S, 20.19. Found: C, 35.11; H, 4.31; S, 20.04.

Catalposide methyl ether. Catalposide, 0.744 g. was dissolved in 75 ml. of methanol, filtered and treated with about 1.5 g. of diazomethane[22] in 120 ml. of ether and allowed to stand at −4° for 15 hr. The excess diazomethane and solvents were distilled and the residue was crystallized from methanol to give 0.708 g. (93%) of catalposide methyl ether, m.p. 210–212°. The analytical sample, m.p. 214–216° dec., was recrystallized once from methanol and twice from water.

Anal. Calcd. for $C_{23}H_{28}O_{12}$: C, 54.64; H, 5.68; CH_3O, 6.25. Found: C, 55.55; H, 5.79; CH_3O, 6.38.

The *pentaacetate* was prepared in 65% yield and the analytical sample, m.p. 148.5–149° was recrystallized three times from ethanol.

Anal. Calcd. for $C_{33}H_{38}O_{17}$: C, 56.09; H, 5.42; CH_3O, 4.39. Found: C, 56.26; H, 5.56; CH_3O, 4.43.

The *pentamesylate* was prepared in 67% yield and the m.p.'s varied between 157° and 175°. The analytical sample was recrystallized five times from pyridine-acetone-methanol.

Anal. Calcd. for $C_{28}H_{38}O_{22}S_5$: C, 37.92; H, 4.32; S, 18.08. Found: C, 38.01; H, 4.55; S, 17.84.

Dihydrocatalposide methyl ether. Dihydrocatalposide, 0.892 g., was methylated by the same procedure described for catalposide. The residue after solvent evaporation was crystallized from methanol–ethyl acetate to give, in two crops, 0.675 g., 74%, of product. The analytical sample, m.p. 194–195°, was recrystallized once from water and twice from methanol–ethyl acetate.

Anal. Calcd. for $C_{23}H_{30}O_{12}$: C, 55.41; H, 6.07; CH_3O, 6.22. Found: C, 55.78; H, 6.38.

When the compound was crystallized from water, another crystalline form was obtained which fused at 120–126°, crystallized and melted again at 175–177°, crystallized again and finally melted at 191°.

Anal. Found: C, 55.26; H, 6.01; CH_3O, 6.63.

Dihydrocatalposide methyl ether was also obtained by the catalytic hydrogenation, in ethanol, of catalposide methyl ether with a 5% palladium-on-carbon catalyst.

The *pentaacetate* was prepared in 88% yield and the analytical sample, m.p. 163–164°, was recrystallized twice from ethanol.

Anal. Calcd. for $C_{33}H_{40}O_{17}$: C, 55.93; H, 5.69; CH_3O, 4.38. Found: C, 55.95; H, 5.45; CH_3O, 4.56.

The *pentamesylate* was prepared in 69% yield and the analytical sample, m.p.'s from 150 to 156°, was recrystallized three times from pyridine-acetone-methanol.

Anal. Calcd. for $C_{28}H_{40}O_{22}S_5$: C, 37.84; H, 4.54; S, 18.04. Found: C, 38.00; H, 4.57; S, 18.15.

Isolation of β-D-glucose pentaacetate. Catalposide, 0.283 g., in 20 ml. of 0.5*N* hydrochloric acid was heated on a water bath until paper chromatography indicated complete hydrolysis (about 17 hr.). The black solid was removed by filtration and the filtrate was treated with decolorizing carbon, filtered, and passed slowly over a column of weakly basic exchange resin (Amberlite IR-45) to remove the hydrochloric acid. The eluate was again treated with carbon, filtered and evaporated, under vacuum, to a colorless sirup. Two portions of benzene were added and evaporated to dry the sample which was subsequently warmed on a water bath for 1.5 hr. with 4 ml. of acetic anhydride and 0.5 g. of freshly fused sodium acetate. The mixture was poured on ice and the resulting precipitate was crystallized from ethanol to give 0.023 g., 10%, m.p. 129–131°, lit.[18] 132°, of β-D-glucose pentaacetate. The infrared spectrum was identical with that of an authentic sample and the mixture melting point was not depressed.

Isolation of p-hydroxybenzoic and p-methoxybenzoic acids. Catalposide, 0.292 g., was treated with 40 ml. of 0.05*N* sodium hydroxide at 35° for 15 hr. Continuous ether extraction yielded nothing. The solution was acidified with concentrated hydrochloric acid and again continuously extracted with ether for 15 hr. The ether extract was dried over sodium sulfate and evaporated to a crystalline residue which was sublimed (150° at 0.05 mm.) to yield 0.078 g., 93%, of *p*-hydroxybenzoic acid, m.p. 212–214°, lit.,[23] m.p. 213°.

In an exactly analogous manner, 0.0724 g., 96%, of *p*-methoxybenzoic acid, m.p. 177.5–180.5°, lit.,[23] m.p. 184° was obtained from 0.248 g. of dihydrocatalposide methyl ether. Recrystallization from ethanol raised the m.p. to 181–183°.

In each case the infrared spectrum of the acid was identical with that of an authentic sample and the mixture melting point was not depressed.

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(22) Prepared from 10 g. of Diactin, *N*-methyl-*N*-nitroso-*p*-toluenesulfonamide, T. J. De Boer and H. J. Backer, *Rec. trav. chim.*, **73**, 229 (1954).

(23) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, *The Systematic Identification of Organic Compounds*, 4th ed., John Wiley and Sons, New York, 1956, p. 279.