Anal. Calcd for $C_{40}H_{56}O_3$: C, 82.14; H, 9.65; O, 8.21. Found: C, 81.83; H, 9.97; O, 8.36.

2,3-Dialkoxy-1-propanols IV–XIV.—The synthesis of VIII is described in detail. All other compounds were prepared under the same conditions, working under nitrogen in the case of compounds XI–XIV. The yields and characteristic data of compounds IV–XIV are quoted in Table I.

2-Hexadecyloxy-3-octadecyloxy-1-propanol (VIII).—In a 250-ml, three-necked flask, fitted with water-separation head, reflux condenser, dropping funnel, calcium chloride tubes, magnetic stirrer, and heating mantle, were placed 3.0 g of powdered potassium hydroxide, 80 ml of xylene, and 3.5 g (6 mmoles) of tritylated octadecyl glyceryl-(1) ether II. The mixture was refluxed for 1 hr to remove water by azeotropic distillation. Hexadecyl methanesulfonate[2] (2.1 g, 6.6 mmoles) dissolved in 20 ml of xylene was added dropwise, and refluxing was continued for 6–8 hr. After removing about 50 ml of xylene by distillation, cooling, and addition of 100 ml of water and 150 ml of ether, the water phase was extracted twice with 100 ml of ether. Drying the organic phase over anhydrous potassium carbonate and evaporation yielded 2-hexadecyloxy-3-octadecyloxy-1-trityloxypropane.

This product was dissolved in 100 ml of 95% methanol, a stream of hydrogen chloride was led through the vigorously stirred reaction mixture and refluxing was continued for 5–6 hr. The solvent was removed by distillation, 100 ml of water and 200 ml of ether were added, and the phases were separated in a 1-l funnel. After a second extraction with 100 ml of ether, the combined organic layers were washed consecutively with 50 ml of water, 1% potassium carbonate solution (until basic), and 50 ml of water, and were dried over anhydrous sodium sulfate. The solvent was evaporated. The residue was taken up in 80 ml of Skellysolve F, and the precipitate of triphenylcarbinol formed on storing the solution in the refrigerator was filtered off and was washed with a small amount of ice-cold Skellysolve F. Crystallization from the filtrate at freezer temperature (−30°), separation on a Büchner funnel, and recrystallization first from ethanol, then from Skellysolve F, yielded 3.0 g (88%) of VIII, mp 58.5–59°.

Compound XI was collected at −30° on a chilled Büchner funnel. The unsaturated compounds XIII and XIV were preferably purified by preparative tlc.

1,2,3-Trialkoxypropanes XV–XIX.—Compounds XV–XVIII were prepared from alkyl glyceryl-(1) ethers by alkylation with 2 equiv of alkyl methanesulfonates; XIX was obtained from VIII by alkylation with 1 equiv of methanesulfonate. The procedure used is described for the synthesis of XVIII.

1-Dodecyloxy-2,3-dioctadecyloxypropane (XVIII).—Powdered potassium hydroxide (3.0 g), 80 ml of xylene, and 0.8 g (3.1 mmoles) of dodecyl glyceryl-(1) ether[2] were refluxed in a 250-ml, three-necked flask equipped as described for the preparation of VIII. After 1 hr a xylene solution of 2.4 g (6.8 mmoles) of octadecyl methanesulfonate[2] was added dropwise, and refluxing was continued for 5–6 hr. The extraction was carried out in a similar manner to that with VIII.

The residues of XV–XIX obtained after evaporation of the solvents were purified from contaminating dialkyl ether, dialkyl glyceryl ethers, and other by-products by adsorption chromatography in columns according to Hirsch and Ahrens.[13] Samples of 500 mg were fractionated on 36 g of silicic acid.[16] After checking 20-ml subfractions of fraction 3 by tlc, evaporation of the solvents and recrystallization of the residues from Skellysolve F at −30°, 150–250 mg of pure trialkyl glyceryl-(1,2,3) ethers were obtained. The yields and physical properties of XV–XIX are summarized in Table II.

The by-products formed during the synthesis of the trialkyl glyceryl ethers were isolated and identified. Fractions 1 and 2 contained dioctadecyl ether (mp 61.5°). 2,3-Dioctadecyloxy-1-propanol (IX) was isolated from fraction 5 (mp 64–65°). The characterization data (infrared spectrum, R_f value, melting point) of these compounds were identical with those of reference materials.

1,3-Dioctadecyloxy-2-propanol was obtained from fraction 4 after recrystallization from Skellysolve F: mp 63–63.5°.

Anal. Calcd for $C_{39}H_{80}O_3$: C, 78.46; H, 13.51; O, 8.04. Found: C, 78.34; H, 13.27; O, 8.66.

Alkylation of the products isolated from fractions 4 and 5 with octadecyl methanesulfonate, as described for XVIII, and purification by preparative tlc led to identical compounds (infrared spectrum, R_f 0.7), which were shown to be 1,2,3-trioctadecyloxypropane (XVI).

Acknowledgment.—The authors are indebted to Dr. J. R. Chipault and Mr. W. Deutsch for recording infrared spectra, and to Mr. L. L. Jones for skilled technical assistance.

(16) Bio-Rad Laboratories, Richmond, Calif.

Catalpa Glycosides. III.[1,2] The Structure of Catalposide

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Catalposide and its derivatives have been degraded by acidic and basic hydrolysis. Through a study of the chemical properties and the n.m.r. spectra of catalposide, its derivatives, and its degradation products, structure **1** is proposed for the compound.

Catalposide is the major glucoside of the *Catalpa* genus.[4] It has been shown to be a β-D-glucoside and an ester of *p*-hydroxybenzoic acid.[4] Furthermore, it contains one additional hydroxyl group and one easily reduced double bond.[4] Additional structures **1a**[5] and **1b**[6] have been proposed for catalposide (see Chart I). Structure **1** is now proposed for the compound.

Structure **1a** was based primarily on the alleged presence of seven hydroxyl groups in catalposide and its reduction to a derivative,[7] **2**, of aucubin (**11**)[8,9] with lithium and liquid ammonia. After an n.m.r. study, **1b** was suggested.[6] The major differences between **1** and **1a–1b** lie in the number of double bonds and the

(1) Portions of this work have been published in a preliminary communication which is paper II of the series: J. M. Bobbitt, D. W. Spiggle, S. Mahboob, H. Schmid, and W. von Philipsborn, *Tetrahedron Letters*, 321 (1962). Furthermore, the work was discussed at the 1962 I.U.P.A.C. Natural Products Symposium in Brussels, Belgium.

(2) This work was sponsored, in part, by the Cancer Institute of the National Institutes of Health, Public Health Service, Grants CY-4015 and CY-4512, and by the National Science Foundation through a regular postdoctoral fellowship given to J. M. Bobbitt in 1959–1960. The work of H. Schmid and W. von Philipsborn was sponsored by the Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forchung.

(3) Deceased, 1964.

(4) For a historical summary, see J. M. Bobbitt, H. Schmid, and T. B. Africa, *J. Org. Chem.*, **26**, 3090 (1961).

(5) W. H. Lunn, D. W. Edward, and J. T. Edward, *Chem. Ind.* (London), 1488 (1961).

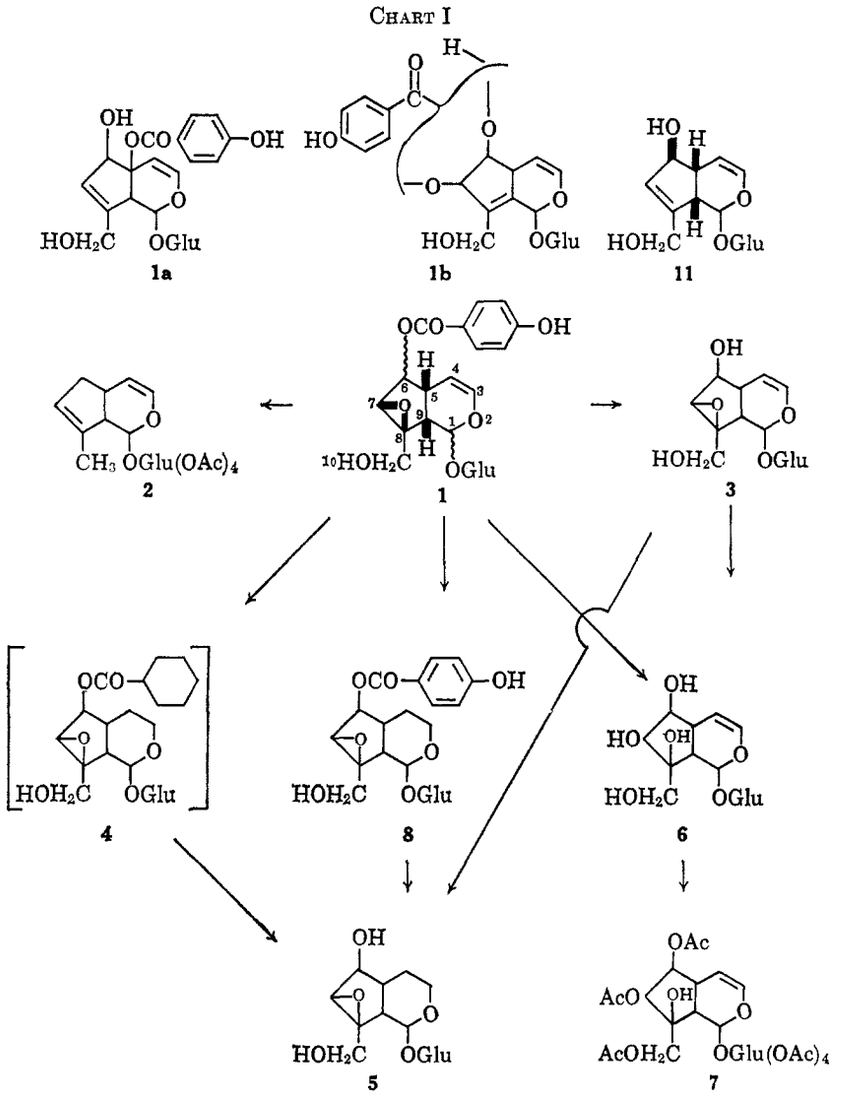
(6) W. H. Lunn, D. W. Edward, and J. T. Edward, *Can. J. Chem.*, **40**, 104 (1962).

(7) J. Grimshaw and H. R. Juneja, *Chem. Ind.* (London), 656 (1960); A. J. Birch, J. Grimshaw, and H. R. Juneja, *J. Chem. Soc.*, 5194 (1961).

(8) J. Fujise, H. Obara, and H. Uda, *Chem. Ind.* (London), 289 (1960).

(9) M. W. Wendt, W. Haegele, E. Simonitsch, and H. Schmid, *Helv. Chim. Acta*, **43**, 1440 (1960).

CHART I



number of hydroxyl groups. Structure **1** contains one double bond, six hydroxyl groups, and an epoxide, while **1a** and **1b** have two double bonds and seven hydroxyl groups.

The presence of one reducible double bond has been established by the following experiments. Catalposide (**1**) and its acetate[4] react with 1 mole of hydrogen under mild conditions (ethanol–palladium on carbon) and with 5 moles of hydrogen under vigorous conditions (acetic acid–platinum). Since aucubin (**11**) always reacts with at least 2 moles of hydrogen,[10] it is highly unlikely that either of the two double bonds in **1a** and **1b** will resist hydrogenation. Under identical, vigorous conditions, catalposide takes up 5 moles of hydrogen and ethyl *p*-hydroxybenzoate takes up about 4 moles (3.78, three for the aromatic ring and one for hydrogenolysis of the hydroxyl group[11]). The difference corresponds to the one double bond in the aglucone. This was further confirmed by the saponification of dihydrocatalposide[4] (**8**) and the crude decahydrocatalposide (**4**) to the same compound, dihydrodeshydroxybenzoylcatalposide (**5**).

The second major difference between **1** and **1a–1b**, the number of hydroxyl groups, was resolved by the following experiments. As detailed previously,[4] catalposide and its derivatives produce mesylates (methanesulfonates) on which the analytical data (mainly the sulfur analyses) agree only with a hexaacyl form. Since the mesylates show no hydroxyl absorptions in their infrared spectra, it must be concluded that there are only six hydroxyl groups in catalposide. This was substantiated by a study of the n.m.r. spectra of the acetates. Careful integration of the spectrum of catalposide acetate[4] shows a ratio of aromatic acetyl protons to aliphatic acetyl protons of 1:5 ± 0.2 (Figure 1) and a ratio of vinyl protons (on C-3) to the total acetyl protons of 1:18 ± 1. A heptaacetate would show ratios of 1:6 and 1:21, respectively. Furthermore, when the spectrum is measured in pyridine (Figure 1), the protons on the six acetyl groups are clearly resolved into six separate peaks. A similar study of deshydroxybenzoylcatalposide hexaacetate (acetate of **3**) gave the same results.

In the first paper from this laboratory[4] and in the second paper of Lunn, Edward, and Edward,[6] a slight discrepancy was noted when the ultraviolet spectra of catalposide, its acetate, and its methyl ether were compared with the model compounds, ethyl *p*-hydroxybenzoate, its acetate, and its methyl ether. In order to clarify this ester linkage, the following experiment was performed. Catalposide methyl ether (**9**)[4] gave,

(10) P. Karrer and H. Schmid, *Helv. Chim. Acta*, **29**, 525 (1946).
(11) R. H. Levin and J. H. Pendergrass, *J. Am. Chem. Soc.*, **69**, 2436 (1947).

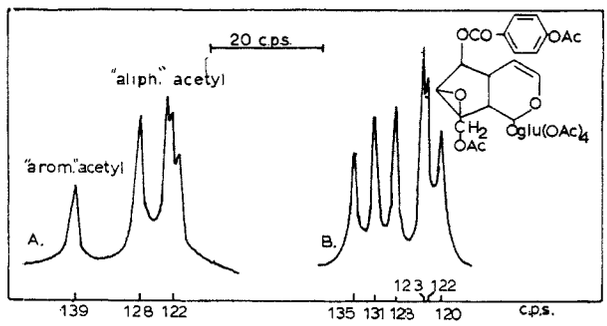


Figure 1.—The 60-Mc./sec. n.m.r. spectra of catalposide hexaacetate in deuteriochloroform (A) and in pyridine (B).

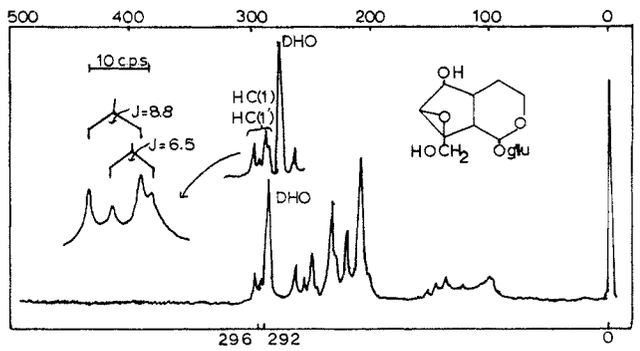
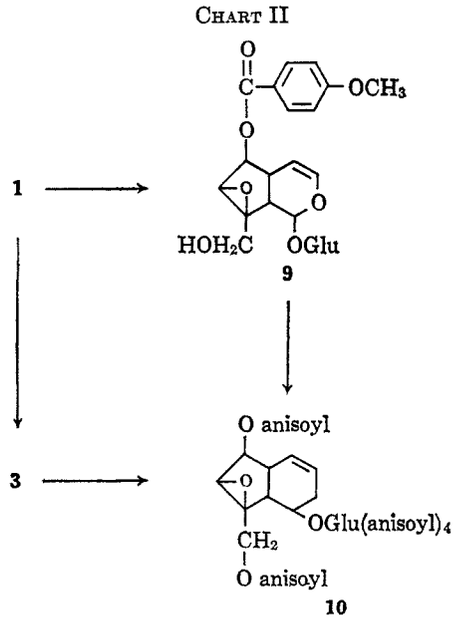


Figure 2.—The 60-Mc./sec. n.m.r. spectrum of deshydroxybenxoyldihydrocatalposide (5) in deuterium oxide.

on treatment with anisoyl chloride in pyridine, the same hexaanisate (**10**) which was obtained from deshydroxybenzoylcatalposide (**3**) by a similar reaction. This is equivalent to the re-formation of catalposide from **3** and *p*-hydroxybenzoyl chloride and shows the ester to be quite normal. (See Chart II.)

CHART II



The known portions of the catalposide molecule account for 10 of the 12 oxygens. Since the infrared spectrum of deshydroxybenzoylcatalposide (**3**) contains no carbonyl absorption, the last two oxygens must be ethers.

The reduction of catalposide with lithium–ammonia[5,6] proved that the carbon skeleton and certain aspects of the stereochemistry of catalposide are the same as in aucubin (**11**). This is not surprising, since catalposide has long been considered[12] to be an "aucubin-type" glycoside and several of these such as plumereid,[13] verbenalin,[14] asperuloside,[15] agnusid,[16] and aucubin[7–9] have the same basic carbon skeleton.

Treatment of catalposide (**1**) and its methyl ether (**9**) with aqueous sodium hydroxide gave near quantitative yields of *p*-hydroxybenzoic acid and anisic acid, respectively.[4] Under these conditions, the solutions discolored and it was impossible to obtain a good yield of deshydroxybenzoylcatalposide (**3**).[17] However, when **1** was treated with the strong base resin, Amberlite IRA-400-OH, no color resulted and a smooth reaction was accomplished. Both **1** and its methyl ether (**9**) were saponified to yield **3**. In a similar manner dihydrocatalposide (**8**) and its methyl ether (**12**) were saponified to yield deshydroxybenzoyldihydrocatalposide (**5**). Neither **3** nor **5** appeared to contain a carbonyl (infrared spectrum). Thus, of the original 12 oxygen atoms of catalposide, 11 can be accounted for (five in glucose, two in acetal at C-1, two in ester, one phenol, and one additional hydroxyl group) and the 12th is present as an ether, since it is neither carbonyl nor hydroxyl. Reduction of **3** to **5** with hydrogen over palladium on carbon indicated the absence of unpredicted reactions.

The 60-Mc./sec. n.m.r. spectrum of **5** in deuterium oxide is given in Figure 2.

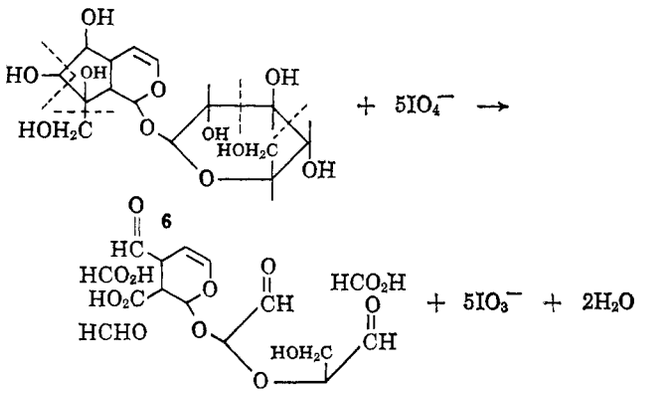
Both **3** and **5** gave crystalline acetates, but no mesylates. Therefore, there was some concern about whether the acetates were hexa- or heptaacetates (acetyl values were half-way between). If the compounds were heptaacetates, some anomalous reaction would be indicated. The dilemma was resolved in two ways. First, compounds **3** and **5** were regenerated from their acetates by transesterification with sodium methoxide in methanol according to Leacock.[18] Second, a careful integration of the n.m.r. spectrum of **3** acetate yielded a ratio of 1:18 ± 1 for the single vinyl proton on C-3 to acetyl protons. Thus, the acetates are hexaacetates and the reaction is normal.

The treatment of catalposide with Amberlite IRA-400-OH led not only to the formation of **3**, but to a second compound, **6**, which appeared to result from the addition of one molecule of water to **3**. The sequential reaction of **1** to **3** to **6** was demonstrated by a qualitative kinetic study using thin layer chromatography.[19] Compound **6** was isolated and analyzed as a monohydrate. Attempts to dry it completely led to poor analytical values. The actual presence of a reacting molecule of water was learned from a study of the heptaacetate **7**. The seven acetyl groups were confirmed by n.m.r. as above. The acetate **7** also had a free hydroxyl group according to the infrared spectrum in Nujol. Since no portion of the established oxygenation could be expected to add water under these conditions to produce two hydroxyl groups, it was con-

(12) A. R. Trim and R. Hill, *Biochem. J.*, **50**, 310 (1952).
(13) O. Halpern and H. Schmid, *Helv. Chim. Acta*, **41**, 1109 (1958).
(14) G. Büchi and R. E. Manning, *Tetrahedron Letters*, 5 (1960).
(15) J. Grimshaw, *Chem. Ind.* (London), 403 (1961).
(16) E. Winde and R. Hänsel, *Arch. Pharm.*, **293/65**, 556 (1960).
(17) This compound has been given the trivial name of "catalpol."[5,8]
(18) D. H. Leacock, *J. Chem. Soc.*, 3166 (1960).
(19) J. M. Bobbitt, "Thin-Layer Chromatography," Reinhold Publishing Co., New York, N. Y., 1963, p. 8.

cluded that the 12th oxygen, the unknown ether, must be involved. The ether must then be present as an epoxide or a trimethylene oxide. Structure **6** was completely established by the knowledge of the carbon skeleton and its reaction with periodate ion.[20]

Three compounds were oxidized with sodium periodate. The reduced periodate was determined by the arsenite method,[20-22] liberated formaldehyde was estimated as the dimedon derivative,[23] and the acids were determined by titration, before and after distillation. Formic acid was confirmed through its sodium salt.[20] The results are shown in Table I. When the data on α-methyl-D-glucoside were subtracted from those of **3** and **6**, it became evident that the aglucone portion of **3** does not reduce periodate, whereas the aglucone portion of **6** reduces 3 molar



equiv. of periodate, yielding 1 molar equiv. of formaldehyde and 2 equiv. of acid, one of which is formic as shown above. Assuming the carbon skeleton, only structure **6** satisfies all of these requirements.

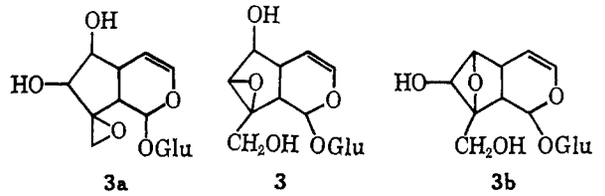
TABLE I

PERIODATE OXIDATION OF CATALPOSIDE DERIVATIVES[a]

Compd.	IO_4^- reduced	Formaldehyde	Acid
α-Methyl-D-glucoside	2	0	1 (formic)
3	2	0	1 (formic)
6	5	1	3 (2 formic)

[a] All values are in molar equivalents.

Both an epoxide and a trimethylene oxide could give **6** on hydrolysis. Therefore, the action of sodium thiosulfate on several catalposide derivatives was studied. Sodium thiosulfate reacts specifically[24] with epoxide rings to yield a sulfur-containing ester and hydroxide ion, which colors phenolphthalein and can be titrated. Only those derivatives in which the phenol is blocked would be expected to show the presence of the hydroxide ion and give a positive test. These



3a **3** **3b**

(20) J. M. Bobbitt, *Advan. Carbohydrate Chem.*, **11**, 1 (1956), and references cited therein.

(21) P. Fleury and J. Lange, *J. Pharm. Chim.*, **17**, 107, 196 (1933).

(22) E. L. Jackson, *Org. Reactions*, **2**, 341 (1944).

(23) R. E. Reeves, *J. Am. Chem. Soc.*, **63**, 1476 (1941).

(24) W. C. J. Ross, *J. Chem. Soc.*, 2257 (1950); see also J. M. Ross, D. S. Tarbell, W. E. Lovett, and A. D. Cross, *J. Am. Chem. Soc.*, **78**, 4675 (1956).

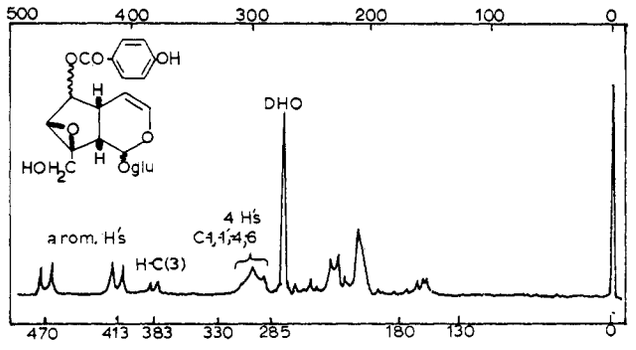


Figure 3.—The 60-Mc./sec. n.m.r. spectrum of catalposide (**1**) in deuterium oxide.

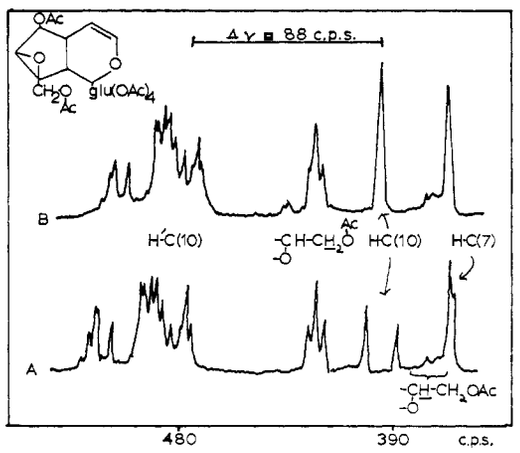


Figure 4.—The 100-Mc./sec. n.m.r. spectra of deshydroxybenxoylcatalposide (**3**) hexaacetate in deuteriochloroform: (A) the normal spectrum; and (B) the spectrum irradiated at 478 c.p.s.

data are summarized in Table II. It should be noted that only the glycol **6** and trimethylene oxide gave negative tests. It would seem that three structures are now possible for deshydroxybenzoylcatalposide.

TABLE II

REACTION OF CATALPOSIDE DERIVATIVES WITH SODIUM THIOSULFATE

Compd.	Qual. test	Quant. estd., %
Glycol **6**	−	
Trimethylene oxide	−	
Catalposide methyl ether[4] (**9**)	+	
Dihydrocatalposide methyl ether (**12**)	+	
Deshydroxybenzoylcatalposide (**3**)	+	
Deshydroxybenzoyldihydrocatalposide (**5**)	+	75[a]
Dihydrocatalpogenin methyl ether (**13**)	+	39[a]

[a] At this point, decomposition was sufficient to mask the titration end point.

Of these structures, **3** is the most logical, but it is conceivable that the trimethylene oxide in **3b** is sufficiently strained to react with thiosulfate or that the two hydroxyl groups in **3a** are so securely locked in a *trans* situation that they do not react with periodate.[20] Structure **3a** can be excluded from a study of the n.m.r. spectra of catalposide (Figure 3), its acetate (Figure 1), and of deshydroxybenzoylcatalposide and its acetate (Figure 4); structure **3b** can be excluded by a study of the acid hydrolysis of dihydrocatalposide methyl ether (**12**).

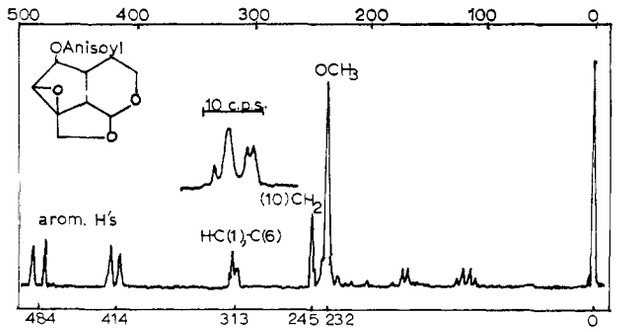
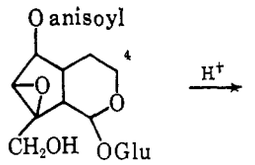


Figure 5.—The 60-Mc./sec. n.m.r. spectrum of anhydrodihydrocatalpogenin methyl ether (**14**) in deuteriochloroform.

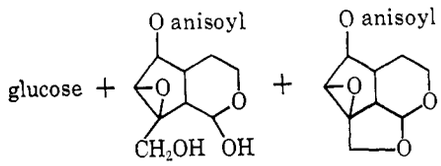
In the region from 180 c.p.s. toward higher fields, the n.m.r. spectrum of catalposide (deuterium oxide) (Figure 3) shows the signals of only two protons, probably those on C-9 and C-5.[25] Structure **3a** appears unlikely, since the total number of protons in the 270–325-c.p.s. region of the 60-Mc./sec. spectrum of deshydroxybenzoylcatalposide hexaacetate has now[26] been found to contain eight protons which must be assigned in the following way. They correspond to the two acetal protons at C-1 and C-1′ to the vinyl proton at C-4, to *one* of the protons on carbon atom 10 bearing the primary, acetylated hydroxyl group [as found by a spin-decoupling experiment at 100-Mc./sec. (Figure 4)], and, consequently, to only *four* protons from carbon atoms bearing secondary acetylated hydroxyl groups. Since there are three such groupings in the sugar moiety, only one secondary hydroxyl group can be present in the aglycone of **3**. In the 100-Mc./sec. spectrum of the free alcohol in deuterium oxide, the AB quartet of the C-10 methylene protons appears at *ca.* 440 and *ca.* 385 c.p.s. (centers of the doublets) and shifts to low field on acetylation (Figure 4). Furthermore, in the same spectrum of **3** in deuterium oxide a quartet is found at 275 c.p.s., which must be assigned to the proton at C-9, showing two large coupling constants (7 and 9 c.p.s.) with the protons on C-1 and C-5. The signal from the proton on C-5 is a clearly resolved multiplet at *ca.* 240 c.p.s., showing several small couplings, as expected, with protons on C-4, C-3, and possibly C-6.

A study of the acid degradation of catalposide and its derivatives established the presence of glucose,[4] established the position of the aromatic ester, and, along with the data of Lunn, Edward, and Edward,[5,6] established the stereochemistry as shown in **1**.

As previously noted,[4] catalposide decomposes on acid treatment. However, dihydrocatalposide methyl ether (**12**)[4] can be degraded with dilute sulfuric acid to glucose, an aglucone, **13**, and a transformation product, **14**, of the aglucone. A qualitative kinetic study using thin layer chromatography[19] showed that **14** was formed from **13**. The structure of **13** is confirmed by a positive Tollens test[27] (when **12** and **14**



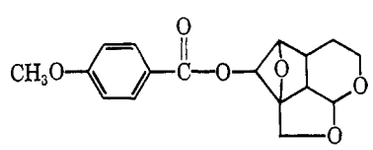
12[4]



13 **14**

are negative) and the formation of a diacetate. Compound **14** contains no hydroxyl groups (infrared spectrum) and can be recovered from an acetylation reaction. The type of ring closure, **13** to **14**, has been noted in the aucubin series.[8] Thus, the hydroxyl on C-10 must be free in catalposide and the *p*-hydroxybenzoyl group must be on C-6.

A study of the 60-Mc./sec. n.m.r. spectrum of **14** (Figure 5) allows the exclusion of structure **3b** for deshydroxybenzoylcatalposide. If the structure of **3b** were correct, the structure of **14** would have to be modified to **14b**. The spectrum shows the expected



14b

aromatic and methoxyl signals, a broad singlet at 245 c.p.s. (two protons on C-10), and a well-defined multiplet at 313 c.p.s., corresponding to the proton on the ester-bearing carbon and the proton on C-1. On the basis of its integration, this multiplet must consist of one doublet and some other signal which is *more* than a doublet. Since the doublet corresponds to the proton on C-1, the proton on the ester-bearing carbon must be coupled with more than one other proton. This would be expected in **14** (two neighboring protons) but not in **14b**. Therefore, deshydroxybenzoylcatalposide must be **3**, and catalposide must be **1**.

The degradation[5,6] of catalposide to an aucubin derivative establishes the ring structure and stereochemistry to be *cis* as in aucubin.[28] The steric requirements of structure **14** make it necessary for the epoxide ring to be *cis* with respect to the ring hydrogens. The stereochemistry at C-1 and at C-5 is not yet known with certainty although work is in progress on the problem. Recently, the absolute configuration **11** for aucubin has been proposed.[29,30] Accordingly, the absolute configuration for catalposide may be formulated as in **1**.

The course of reaction during the reduction of **1** to **2** (isolated as its acetate) as carried out by Lunn, Edward, and Edward[5,6] and repeated in this laboratory

(25) In the n.m.r. spectrum of aucubin (**11**) the signal on C-5 is at 160 c.p.s.

(26) The integral for this region reported previously[1] had to be rechecked according to a suggestion from Dr. A. R. Forrester of the University of Aberdeen. An analysis of this spectral region analogous to the one given above has been carried out by us and is reported in Dr. Forrester's paper on the naturally occurring deshydroxybenzoylcatalposide 5-methyl ether (catalpol methyl ether): R. B. Duff, J. S. D. Bacon, C. M. Mundie, V. C. Farmer, J. D. Russell, and A. R. Forrester, *Biochem. J.*, **96**, 1 (1965).

(27) R. L. Shriner, R. C. Fuson, and D. Y. Curtin, "The Systematic Identification of Organic Compounds," John Wiley and Sons, Inc., New York, N. Y., 1956, p. 162.

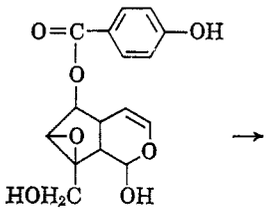
(28) W. Haegele, F. Kaplan, and H. Schmid, *Tetrahedron Letters*, 110 (1961).

(29) H. Inouye and K. Fuji, *Chem. Pharm. Bull.* (Tokyo), **12**, 901 (1964).

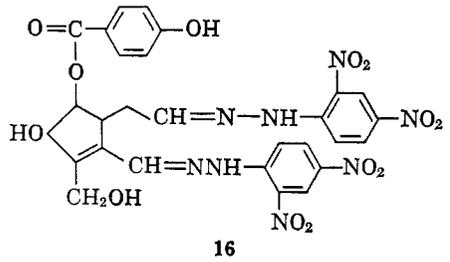
(30) W. Haegele, F. Kaplan, and H. Schmid, unpublished results.

is not clear. There appear to be two possible routes. The first involves the reduction of the epoxide to a tertiary alcohol on C-8 followed by dehydration and loss of the two subsequently activated oxygen functions on C-6 and C-10. The second route assumes that an epoxide will behave like a double bond to activate the oxygen functions to reduction and is then subsequently reduced to the tertiary alcohol on C-8. The alcohol is dehydrated during the acetylation process to yield **2**. The latter route is more probable because a dehydration is unlikely with lithium in ammonia.

The bis-2,4-dinitrophenylhydrazone (**16**), produced[5,6] from catalposide aglucone (**15**) (from an emulsin hydrolysis), can easily be considered to arise from an epoxide–allyl alcohol rearrangement. Structure **16** was suggested by Lunn, Edward and Edward[5,6] and appears to be reasonable.



15



16

Experimental Section[31]

Deshydroxybenzoyldihydrocatalposide (5).—Dihydrocatalposide methyl ether (**12**,[4] 0.094 g.) was dissolved in 10 ml. of water and stirred with 2 ml. of Amberlite IRA-400-OH at 85° for 2 hr. The resin was removed by filtration and the filtrate was washed with ether and evaporated to dryness under vacuum. The resulting glass was crystallized from methanol–ethyl acetate to give 0.047 g. (69%) of **5**, m.p. 214–216°. The analytical sample, m.p. 216–217°, was recrystallized once from the same solvents.

Anal. Calcd. for $C_{15}H_{24}O_{10}$: C, 49.45; H, 6.64. Found: C, 49.39; H, 6.90.

The **hexaacetate** was prepared as previously described,[4] crystallized from benzene–hexane, and recrystallized twice to yield an analytical sample, m.p. 62–66°, which crystallized on further heating and finally melted at 150.5–151.5°.

Anal. Calcd. for $C_{27}H_{36}O_{16}$: C, 52.59; H, 5.89; CH_3CO, 41.89. Found: C, 52.60; H, 5.86; CH_3CO, 44.47.

Complete Reduction and Saponification of Catalposide (1 → 4 → 5).—Catalposide (0.557 g.) was hydrogenated in 100 ml. of acetic acid over 1.8 g. of prehydrogenated platinum oxide at 25° and atmospheric pressure. After 5 moles of hydrogen had been absorbed, the catalyst was removed by filtration and the filtrate was concentrated under vacuum to a brown gum (0.550 g.). The gum was saponified with Amberlite IRA-400-OH as described later in the preparation of **3** from **1**. The product of the saponification (0.286 g.) was absorbed on 2 g. of silica gel and chromatographed over a column consisting of 20 g. of silica gel G using benzene–methanol–ether (60:30:10) as an eluent.[32] The major component was isolated to yield 0.080 g. of deshydroxybenzoyldihydrocatalposide (**5**), identical in every respect (infrared spectra, melting points, and mixture melting point) with **5** isolated by saponification of **12**.

Catalposide Methyl Ether Pentaanisate (10).—Anisoyl chloride (0.5 ml.) was added, with gentle shaking, to a solution of 0.23 g. of **3** in 3 ml. of dry pyridine. The solution was heated to 100° for 2 hr. and kept at 40° overnight. The reaction mixture was poured on ice and filtered after 15 min. The precipitate was dissolved in benzene, washed with 5% sodium bicarbonate and water, and dried over sodium sulfate. Thin layer chromatography (benzene) showed that the major product contained a colored impurity that remained at the origin of the chromatogram. Therefore, the product was purified by passing it in benzene solution over a short column of silica gel G under 5 p.s.i. of nitrogen. Evaporation of the benzene eluate yielded 0.7 g. (88%) of **10**, m.p. 97–100°. The analytical sample, m.p. 98–99.5°, was recrystallized three times from benzene–acetone–methanol and dried under vacuum at 25°. It clung to 1 mole of benzene.

Anal. Calcd. for $C_{63}H_{58}O_{22}\cdot C_6H_6$: C, 66.51; H, 5.12. Found: C, 66.82; H, 4.96.

The same compound was obtained in 75% yield from the reaction of anisoyl chloride and **9**. Identity was shown by infrared spectra, melting points, and mixture melting point.

Saponification of Catalposide with Amberlite IRA-400-OH (1 → 3 + 6).—Catalposide (3.3 g.) was dissolved in 190 ml. of water and stirred at 85° with 185 ml. of Amberlite IRA-400-OH for 2 hr. The mixture was filtered and the filtrate was washed with ether and evaporated to dryness to give 1.16 g. of a glass. Thin layer chromatography (methanol–ether, 1:1) showed it to contain a very small amount of a contaminant, **6**, which could be removed by recrystallization. The glass was crystallized from methanol–ethyl acetate to yield 1.04 g. (42%) of **3**. The analytical sample, m.p. 201–203°, was recrystallized three times from methanol–ethyl acetate and was identical with the "catalpol" of Lunn, Edward, and Edward,[33] m.p. 207–209°.

Anal. Calcd. for $C_{15}H_{22}O_{10}$: C, 49.72; H, 6.12. Found: C, 49.69; H, 6.08.

The **hexaacetate** was prepared[4] in 69% yield and recrystallized twice from ethanol to give the analytical sample, m.p. 142–143°.

Anal. Calcd. for $C_{27}H_{34}O_{16}$: C, 52.67; H, 5.58; CH_3CO, 42.03. Found: C, 52.96; H, 5.66; CH_3CO, 46.32.

The resin which was removed by filtration from the original reaction mixture was boiled with 300 ml. of water for 3 hr. The cooled, filtered aqueous solution was washed with ether and evaporated under vacuum to yield 0.814 g. of a glass. Thin layer chromatography (methanol–ether, 1:1) of the glass showed that it consisted mainly of the contaminant **6**, mentioned above, along with some **3**. The mixture was separated by column chromatography over silica gel (Davison) using gradient elution. Successive amounts of methanol, up to 25%, were added to benzene–ether, 3:1. The fractions were analyzed by thin layer chromatography. The first compound which came off was 0.260 g. of **3** (total 52% yield). The next fractions contained 0.064 g. of a mixture of **3** and product. The last fractions yielded 0.490 g. (18%) of the glycol, **6**. The glycol was crystallized and recrystallized once from wet methanol to give the hygroscopic analytical sample, m.p. 120–122°. The sample was dried under vacuum at 25°.

(31) All melting points were determined on a Kofler melting point apparatus and are corrected. Analyses were carried out by Geller Laboratories, Charleston, W. Va., and H. Fröhofer, Zürich, Switzerland. Before analysis, all compounds were dried over phosphorus pentoxide and potassium hydroxide at 100°, unless noted. N.m.r. spectra were measured on a Varian A-60 instrument. Chemical shifts for those spectra taken in deuteriochloroform are relative to tetramethylsilane as an internal standard. For the spectra in deuterium oxide, a dilute solution of tetramethylsilane was used as an external standard: HDO signal at 286 c.p.s. For integration, the water signals were shifted thermally. The samples measured in deuterium oxide were dissolved in the solvent and evaporated to dryness several times before the spectra were measured. The spectra in Figure 4 were taken with a Varian HR-100 Mc./sec. spectrometer equipped with an integrator–decoupler, V-3521 a. Thin layer chromatography[19] was carried out on silica gel G layers 0.25 mm. thick. The developed layers were visualized by spraying them with sulfuric acid and heating them to 100°.

(32) The solvent was predicted from thin layer chromatography by the process described in ref. 19, p. 11. The column silica gel G was prepared by wetting the commercial product with water (2 ml./g.), drying it at 110° for 1.5 hr., grinding it in a ball mill, and finally drying it again at 110° for 1.5 hr.

(33) We appreciate a sample of "catalpol" furnished by Professor J. T. Edward of McGill University.

Anal. Calcd. for $C_{15}H_{24}O_{11}\cdot H_2O$: C, 45.23; H, 6.59. Found: C, 45.32; H, 6.35.

The **heptaacetate** was prepared[4] in 32% yield. The analytical sample, m.p. 131–133°, was recrystallized twice from ethanol. It had an infrared peak at 2.9 μ.

Anal. Calcd. for $C_{29}H_{38}O_{18}$: C, 51.62; H, 5.69; CH_3CO, 40.83. Found: C, 51.69; H, 5.77; CH_3CO, 43.13.

Regeneration of 3 and 5 from Their Acetates.—Compound **3** hexaacetate (0.255 g.) was slurried with 10 ml. of anhydrous methanol and treated with 1 drop of 2 *N* sodium methoxide in anhydrous methanol.[18] More methanol was added, dropwise and with stirring, until total solution was attained. The mixture was allowed to stand at 27° for 1 hr. and at 5° overnight. Qualitative thin layer chromatography (methanol–ether, 1:1) showed a complete reaction after 3 hr. The product was precipitated with ethyl acetate to yield 0.062 g. (47%) of **3** which was identical in every respect (infrared, melting points, and mixture melting point) with a known sample.

Similar results were obtained when compound **5** hexaacetate was treated in a similar fashion. However, the yield was 72%.

Reduction of 3 to 5.—Compound **3** (0.414 g.) was dissolved in 20 ml. of ethanol and hydrogenated at room temperature and 1 atm. over 0.13 g. of 10% palladium on carbon. The reaction mixture was filtered and the filtrate was evaporated to dryness. The residue was crystallized from methanol–ethyl acetate to yield 0.277 g. (68%) of **5**, m.p. 214–216°, identical in all respects with an authentic sample.

Acid Degradation of 12.—A mixture of **12**[4] (1.0 g.), 30 ml. of water, and 50 ml. of benzene was stirred and heated to reflux (about 65°). Sulfuric acid (30 ml., 1 *N*) was added and the reflux was continued for *exactly 15 min.* The mixture was just neutralized with solid sodium bicarbonate and the product was salted out by the addition of 60 ml. of saturated sodium chloride. The benzene was removed under vacuum and the mixture was kept at −4° for 2 hr. The crystalline product was removed by filtration and dried to give 0.527 g. of a mixture. The combined products of six such reactions totaled 3.473 g. Thin layer chromatography (ether–methanol, 9:1) showed the presence of two major components and one minor component. The total product was chromatographed over silica gel (Davison, 100–200 mesh) which had been dried at 120° for 6 hr. The first eluent was benzene–ether–methanol (300:160:25), which was changed after the elution of the first zone to benzene–ether–methanol (300:160:46). The first compound eluted yielded 0.188 g. (5% from **12**) of **14** after crystallization from benzene–hexane. The next fraction, 0.215 g., consisted of a mixture and was discarded. The third and last fraction consisted of 2.471 g. of **13** (61% from **12**) after crystallization from acetone–water.

Compound **13** gave a positive Tollens test. Two analytical samples were prepared: one, m.p. 95–96.5°, recrystallized from benzene and a second, m.p. 87–105°, recrystallized from water. The melting points were erratic, but both were homogeneous and identical chromatographically and both gave the same analysis.

Anal. Calcd. for $C_{17}H_{20}O_7$: C, 60.71; H, 5.99. Found: C, 60.85; H, 6.14.

Acetylation by the described method[4] gave a **diacetate**, m.p. 114–117°, in 57% yield. The analytical sample, m.p. 114.5–116°, was recrystallized twice from ethanol.

Anal. Calcd. for $C_{21}H_{24}O_9$: C, 59.99; H, 5.75; CH_3CO, 20.48; OCH_3, 7.38. Found: C, 59.96; H, 5.99; CH_3CO, 22.69; OCH_3, 7.57.

Compound **14** gave a negative Tollens test and yielded only starting material when acetylated. The analytical sample, m.p. 119–120.5°, was recrystallized twice from hexane–benzene.

Anal. Calcd. for $C_{17}H_{18}O_6$: C, 64.14; H, 5.70. Found: C, 64.20; H, 5.66.

Periodate Oxidations.—Sodium metaperiodate solution (5 ml., 0.25 *M*, 1.25 mmoles) was added to 0.13-mmole portions of the substances to be oxidized (0.0472 g. of **3**, 0.0517 g. of **6** hydrate, and 0.0252 g. of α-methyl-D-glucoside), and the solutions were made up to 25 ml. Aliquots of 2 ml. were removed at timed intervals and analyzed by the arsenite method.[22] Interpretation of the curves yielded the data shown in Table I.

Total acidity was measured[20] by treating one of the above aliquots with ethylene glycol to remove the excess periodate and titrating with 0.01 *N* sodium hydroxide to a phenolphthalein end point. Formic acid was estimated on a second series of reaction mixtures which contained only the theoretical amount of oxidant as determined previously. Ethylene glycol was added and the formic acid was distilled.[20] The distillate was titrated with 0.01 *N* sodium hydroxide to a phenolphthalein end point and the results are shown in Table I. After the distillate was titrated, it was extracted with ether to remove phenolphthalein and evaporated to dryness to yield sodium formate. Although the infrared spectra of the samples of sodium formate obtained in this fashion were not exactly identical with that of a commercial sample, they were identical with one another and similar to that of the known compound.

Formaldehyde was estimated[23] with dimedon (5,5-dimethyl-1,3-cyclohexanedione). The theoretical amount of oxidant was used for the oxidation of each of the three compounds, **3**, **6**, and α-methyl-D-glucoside. Only **6** yielded any of the methone of formaldehyde. The methone, obtained in 92% yield (14.7 mg. of **6** yielded 10 mg. of methone) had m.p. 188–190° (lit.[23] m.p. 189–190°) and was identical (infrared and mixture melting point) with a known sample.

Qualitative Tests with Sodium Thiosulfate.[24]—The sample to be tested (5–10 mg.) was dissolved in about 2 ml. of acetone. Sodium thiosulfate (2 ml., 0.1 *N*) was added with 1 drop of phenolphthalein indicator and the solution was heated to reflux. A pink color showed a positive test. The experiment with freshly distilled trimethylene oxide was carried out in a sealed tube at 100° and was negative.

Quantitative Estimation of Epoxide with Sodium Thiosulfate.[24]—A mixture of **5** (0.270 g.), 10 ml. of 0.4 *N* sodium thiosulfate (previously neutralized with acetic acid), 10 ml. of acetone, and 3 drops of phenolphthalein indicator were heated to a gentle reflux in an apparatus so arranged that the tip of a 5-ml. buret, containing 0.2 *N* acetic acid, was directly over the surface. As the pink color developed from the reaction, acid was added. A very faint pink color was maintained. After 4.5 hr., the added acid was equivalent to 75% of the theoretical amount. At this point decomposition became evident.

In a similar reaction with **13**, decomposition obscured the end point after 1.5 hr. during which time 39% of the theoretical reaction was obtained.

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