acetates of the two inosamines have been compared and the results tend to confirm the configurational assignments made. Acyl migration data for tetra-O-acetylstreptamine are also reported.

3. The nomenclature of these compounds is discussed. The use of the names *meso*-inosamine-2 for the epimer having the configuration of *meso*-inositol and *scyllo*-inosamine for the one having the configuration of scyllitol is suggested.

4. *meso*-Inosamine-2 is identical with the "inosamine-SA" and *scyllo*-inosamine with the "inosamine-SB" of Carter, Clark, Lytle and McCasland.

5. A series of new derivatives of these two inosamines and of streptamine has been prepared. These derivatives should be useful for separating and identifying inosamine mixtures.

MADISON, WISCONSIN RECEIVED NOVEMBER 15, 1949

[CONTRIBUTION FROM THE DEPARTMENT OF BIOCHEMISTRY, COLLEGE OF AGRICULTURE, UNIVERSITY OF WISCONSIN]

The Preparation and Alkali Sensitivity of Some New Enol Glucosides and Glucosides of β-Hydroxy Carbonyl Compounds[1]

BY CLINTON E. BALLOU AND KARL PAUL LINK

Alkali lability of glycosides has been attributed to a unique activation of the glycosidic linkage by the aglycon.[2,3,4] Electronic interpretation of this phenomenon usually involves the formation of a cationoid center on the aglycon or the anomeric carbon atom of the sugar. The induced shift of the free electron pair on the glycosidic oxygen will govern the approach of the cleaving anion, and this effect is related to certain properties of the aglycon. Hibbert[5] ascribed this property to aglycon acidity. Though aglycon acidity may be useful in correlating relative rates of alkaline cleavage of some phenolic glycosides, all of the types of alkaline cleavage of glycosides cannot thus be rationalized.[3,4]

In previous papers we have reported the alkaline methanolysis of β-D-glycosides of 3-phenyl-4-hydroxycoumarin[3,6] and the glucoside of theobromine.[4] The former undergo methanolysis to the aglycon and a methyl α-D-glycoside, indicating cleavage between the glycosidic oxygen and the sugar residue. However, when theobromine D-glucoside tetraacetate is cleaved in the same manner, glucose and a methoxy theobromine are obtained.

The alkali sensitivity of these glycosides may be associated with the activating conjugated carbonyl structure of the aglycon. The lability of the glucoside of salicylic acid[7] was thus rationalized.[2] It has been suggested[8] that the biosidic structure, Gl—O—C=C—, is sufficient to labilize the glycosidic linkage to alkali. This paper concerns studies on the basic activating system, Gl—O—C=C—C=O.

A series of glucosides containing variations of the minimum activating system as represented above was synthesized. The glucoside of the enolic form of ethyl acetoacetate is representative.[9] The Koenigs–Knorr conditions produced uncrystallizable sirups. However, a modification of the Robertson synthesis[10] introduced in this Laboratory by Dr. C. F. Huebner[6] gave success. It was first used in the synthesis of the acetylated diglucoside of 3,3′-methylenebis-(4-hydroxycoumarin) (Dicumarol^{R}), and consists of treating tetraacetyl-D-glucosyl bromide (I) with the enolic aglucon, in the presence of silver oxide and a *trace* of quinoline. In the synthesis of glucosides of phenol Robertson[10] employed a relatively large amount of quinoline. The success of the syntheses reported below depends largely on the amount of quinoline used, an observation previously made.[6]

Inspection of the structural formula of the glucoside of ethyl acetoacetate reveals that *cis–trans* isomerism is possible, and two forms of the glucoside were isolated. The method of separation involved fractional crystallization. The yields were low, and it was difficult to obtain the fractions in a pure form. Chromatographic separation on Silene EF, following the procedure of Binkley and Wolfrom,[11] was used with success, and offered a more reliable method of separation.

Since it is not known which of the compounds is *cis* and which is *trans*, the higher melting product was arbitrarily designated *trans*(?), and the

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(2) Isbell, *Ann. Rev. Biochem.*, XII, 215 (1943).

(3) Spero, Ballou and Link, THIS JOURNAL, **71**, 3740 (1949).

(4) Ballou and Link, *ibid.*, **71**, 3743 (1949).

(5) Fisher, Hawkins and Hibbert, *ibid.*, **63**, 3031 (1941).

(6) Huebner, Karjala, Sullivan and Link, *ibid.*, **66**, 906 (1944).

(7) Helferich and Lutzmann, *Ann.*, **537**, 11-21 (1938).

(8) Evans and Benoy, THIS JOURNAL, **52**, 294 (1930); Gehman, Kreider and Evans, *ibid.*, **58**, 2388 (1936).

(9) The preparation of a glucoside of ethyl acetoacetate, m. p. 170–171°, was reported by Gonzales and Aparicio, *Anales fis y quim*, **41**, 846–859 (1945). Neither rotation nor elemental analysis are given in the paper. Thus the structure of their product is questionable.

(10) Robertson and Waters, *J. Chem. Soc.*, 2730 (1930).

(11) Binkley and Wolfrom, Sugar Res. Found., Inc., Scientific Report Series No, 10, August, 1948.

lower melting one *cis*(?).[12] The higher melting compound proved to be the least soluble. The rotations were both negative, and of comparable magnitude, indicating that both were β-D-glucosides.

To show that the two products were *cis–trans* forms of a β-D-glucoside, the double bond in each was reduced with palladium and hydrogen. This reduction produced the identical glucoside of ethyl β-hydroxybutyrate from each compound. Since a new asymmetric carbon atom is formed in the reduction one would expect two and the same products from each glucoside. Only one has been isolated in a crystalline form, although chromatographic analysis indicates that another product is present.

As final evidence for the structure of the enol glucoside of ethyl acetoacetate, the glucoside of ethyl β-hydroxybutyrate was synthesized directly from the aglucon and tetraacetyl-D-glucosyl bromide (I) by the Koenigs–Knorr method. The product proved to be identical with those obtained by the reduction of *trans*(?) and *cis*(?)-O-(tetraacetyl-β-D-glucosyl)-ethyl acetoacetate (II and III).

By the same general method, enol glucosides of acetoacetanilide, ethyl benzoylacetate, benzoylacetone, dibenzoylmethane and dimethylcyclohexanedione were prepared. Only one form was isolated in some of the reactions, and in these cases no attempt is made to designate it as either the *cis* or *trans* form. The products of reduction are described. In general the reduction went smoothly and resulted in saturation of the carbon–carbon double bond. However, when the aglucon contained an aryl ketone anomalous results were obtained, two mole equivalents of hydrogen were absorbed, and crystalline products were isolated which could not be separated into fractions with definitive melting points. It is likely that both the double bond and the aryl ketone underwent reduction, or reductive benzyl cleavage may have occurred.

Some of the glucosides were deacetylated by the catalytic barium methoxide method of Isbell.[13] The attempted deacetylation of the glucosides of ethyl acetoacetate by this method probably resulted in partial ester interchange, as only sirups were obtained.[14] Deacetylation of O-(tetraacetyl-β-D-glucosyl) ethyl benzoylacetate (X) gave a small yield of O-(β-D-glucosyl) *methyl* benzoylacetate. Apparently side reactions occur during the ester interchange of the aglucon which result in the low yields or the formation of sirups.

The glucoside of acetoacetaldehyde (XVII) was synthesized by the method of Michael[15] which has in the past been generally applied to phenolic glucosides. This method was used because the free aldehyde is too unstable for the quinoline method. Glucoside formation could have taken place through the enolized aldehyde or the enolized ketone group. Only one of the *cis–trans* isomers was isolated. Reduction of the double bond produced 2-oxo-*n*-butyl β-D-glucoside tetraacetate (XVIII). The latter was also synthesized from 3-oxo-*n*-butanol and I by the Koenigs–Knorr reaction. Thus the structure of XVII is established as $Gl(OAc)_4—O—\underset{H}{C}=\underset{H}{C}—\overset{O}{\overset{\|}{C}}—CH_3$.

Attempts to prepare the glucosides of ethyl acetoacetate and some of the other enols by the method of Michael failed.

The glucosides were tested for stability to alkali by their reaction with hot Benedict solution (Table I). The *cis* and *trans*-O-(tetraacetyl-β-D-glucosyl) ethyl acetoacetate were weakly reducing. The glucosides were readily hydrolyzed by dilute acid, indicating that they possess a *typical* glucosidic linkage.

TABLE I

DETERMINATION OF RELATIVE ALKALI-SENSITIVITY

The alkali-sensitivity of the glucosides was based on the rate with which 20 mg. of the compound caused appreciable reduction of 1 ml. of Benedict solution at 95°. If the glucoside was insoluble (acetylated) several drops of alcohol were added to effect solution.

Compound	Time to reduce Benedict solution
O-(Tetraacetyl-β-D-glucosyl) ethyl acetoacetate	15 minutes
O-(β-D-Glucosyl) acetoacetanilide	8 minutes
O-(Tetraacetyl-β-D-glucosyl) acetoacetaldehyde	15 seconds[a]
O-(β-D-Glucosyl) ethyl benzoylacetate	Non-reducing for 30 minutes
O-(β-D-Glucosyl) benzoylacetone	2–4 minutes
O-(β-D-Glucosyl) dibenzoylmethane	5–7 minutes
3-oxo-*n*-Butyl β-D-glucoside tetraacetate	30 seconds[a]
Ethyl β-hydroxybutyrate β-D-glucoside tetraacetate	1 minute[a]
β-Hydroxybutyranilide β-D-glucoside tetraacetate	2 minutes
Methyl α-D-glucoside	Non-reducing for 30 minutes
Pentaacetyl β-D-glucose	15–30 seconds[a]

[a] These compounds are cleaved in dry methanol with catalytic amounts of barium methoxide.

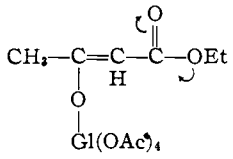
Thus it is seen that the simple system as represented by II is not sufficiently activated to make the glucosidic linkage strikingly labile to alkali. In this respect, it is of interest to compare II with XVII, since the carbonyl effect in XVII is not deactivated by the —OEt group as in II. XVII, in contrast with II, reduces Benedict solution readily. The glucosides of benzoylacetone (XIII) and dibenzoylmethane (XV) were also labile to

(12) The use of the terms *cis*(?) and *trans*(?) seems desirable until absolute configurations can be assigned.

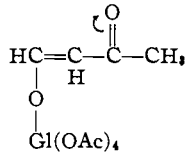
(13) Isbell, *Bur. Standards J. Res.*, **5**, 1185 (1930).

(14) The enol glucosides of the *methyl* ester of acetoacetic acid were made, and the deacetylated products were easily obtained. The results of this work, as well as the application of the synthesis to other sugars, will be described at a later date.

(15) Michael, *Am. Chem. J.*, **1**, 307 (1879).

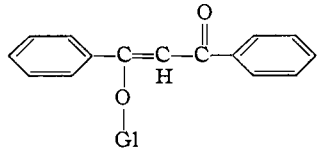


II



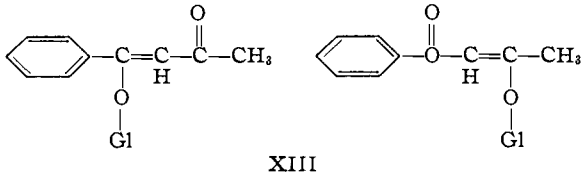
XVII

alkali, as demonstrated by their ready reduction of hot Benedict solution. XV is assigned the structure



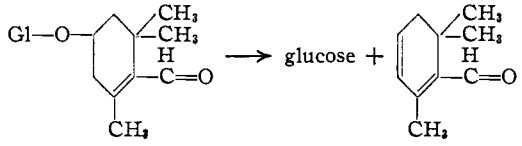
XV

while XIII has two possible structures, depending upon which carbonyl group is enolized. The iodoform reaction was not used in the characterization since the glucoside is labile to alkali.



XIII

Of equal interest are the results obtained with ethyl β-hydroxybutyrate β-D-glucoside tetraacetate (IV). It does not possess the activating conjugated system described above, yet it is readily cleaved by alkali. This sensitivity appears to be related to that of the glucosides of 2-hydroxyethanesulfonic acid reported by Helferich and Schnorr,[16] and is also similar to that described by Kuhn and Löw[17] with picrocrocin [see Isbell, reference (2)].[18] Picrocrocin is quite labile to alkali. It has the structure

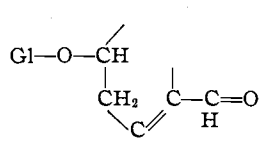


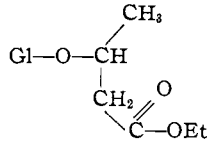
and in alkaline cleavage there are formed free glucose and the aglucon containing a new point of unsaturation. The similarity in activating structure between this glucoside and that of ethyl β-hydroxybutyrate is obvious. The carbonyl effect of the one is a vinylog of the other, and it is to be expected that the activating influence of the carbonyl group would be essentially the same in each case, the only difference being due to the comparable effect of an aldehyde and ester group.

(16) Helferich and Schnorr, *Ann.*, **547**, 201 (1941).

(17) Kuhn and Löw, *Ber.*, **74**, 219 (1941).

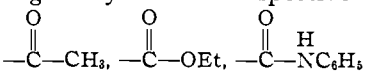
(18) To this group may be added the glucoside of β-nitroethanol prepared by Helferich and Hase, *Ann.*, **554**, 261 (1943). It is reported by these authors to be extremely labile to alkali.





If the cleavage of the glucoside of ethyl β-hydroxybutyrate proceeds with the formation of crotonic acid or ethyl crotonate, then these two reactions could be assumed to follow the same type of mechanism. The products of this cleavage have not been determined. However, the glucoside of 3-oxo-*n*-butanol cleaves in alkali (barium methoxide in dry methanol) to glucose and a lachrymatory substance, methyl vinyl ketone.

The glucoside of 3-oxo-*n*-butanol (XVIII) should exhibit still greater sensitivity to alkali than that of ethyl β-hydroxybutyrate, and this was found to be so. The order of decreasing sensitivity to alkali of the glucosides of the β-hydroxy carbonyl compounds was as follows: the glucoside tetraacetate of 3-oxo-*n*-butanol, ethyl β-hydroxybutyrate and β-hydroxybutyranilide. This order follows that of decreasing negativity of the respective carbonyl groups,



The above results are further evidence that the alkaline cleavage of glycosides of the type represented by 3-phenyl-4-hydroxycoumarin D-glucoside tetraacetate and theobromine D-glucoside tetraacetate proceeds by a mechanism that is different from that of the type represented by picrocrocin, 2-hydroxyethanesulfonic acid glycosides, ethyl β-hydroxybutyrate and 3-oxo-*n*-butyl β-D-glucoside tetraacetate. In addition, the activating system as represented in O-(tetraacetyl-β-D-glucosyl) ethyl acetoacetate does not induce the sensitivity to alkali found in the class of 3-substituted-4-hydroxycoumarin glycosides. While the glucoside of acetoacetaldehyde is labile to alkali, the cleavage proceeds with the formation of glucose not methyl α-D-glucoside.

Experimental

Condensation of the Enolic Aglucon and Tetraacetyl-D-glucosyl Bromide.—Mole equivalents of the aglucon and tetraacetyl-D-glucosyl bromide (I) were dissolved in dry ether (100 ml. per 10 g. of the bromosugar), and an amount of drierite equal in weight to I was added. This mixture was shaken in a dark bottle for fifteen minutes.

Silver oxide (1.5 mole equivalents) and quinoline (8 drops per 100 ml. of ether) were then added and the shaking was resumed. The mixture was shaken until the test for bromide ion was negative, usually six to twenty-four hours.

The mixture was filtered through a thin bed of carbon on a Buchner funnel. (It is more convenient to remove the solids by centrifuging.) The solid was washed twice with chloroform by resuspending it in a beaker and then filtering again. The combined filtrate, light orange to dark red in color, was washed with enough ice-cold 1% sodium hydroxide to remove the unreacted aglucon, then with ice-cold 1% hydrochloric acid to remove the quinoline. It was washed three times with cold water, dried over calcium chloride, filtered and concentrated to a thick sirup

in vacuo at 50°. The sirups obtained were worked up as indicated below.

When practical, an excess of the aglucon may be used, as with ethyl acetoacetate, and the excess may be removed by distillation at reduced pressure.

Preparation of *cis* and *trans*-O-(Tetraacetyl-β-D-Glucosyl) Ethyl Acetoacetate (II and III).—A mixture of 47.5 g. (0.365 mole) of ethyl acetoacetate (Eastman Kodak Co., practical grade, redistilled), 15.0 g. (0.036 mole) of I, 15 g. of drierite, and 100 ml. of dry ether was shaken on a mechanical shaker for fifteen minutes. To the mixture were added 6.0 g. of silver oxide and 8 drops of quinoline. Shaking was resumed, and continued until the reaction mixture gave a negative bromide test (about twelve hours).

The suspension was filtered through a layer of carbon on a Buchner funnel, and the solids were washed with ether. The ether was removed *in vacuo*, and the excess aglucon by distillation at 1 mm. and 55–60°. The light yellow sirup was dissolved in one volume of warm dry ether, stirred with a glass rod, and left in the icebox until crystallization had started. It was kept there for a day, with occasional stirring and addition of more ether. The total volume of ether added amounted to about three times the volume of the original sirup.

The gummy, crystalline mass was collected on a Buchner funnel, and washed with ether. The yield was 4 g. This solid was dissolved in boiling ether (400 ml.), and the solution was filtered and concentrated to a volume of about 100 ml., or until crystallization started. After standing in the icebox overnight, long, hair-like needles were deposited; yield 2.9 g., m. p. 129–131°. One more recrystallization gave m. p. 130–131.5°, $[\alpha]^{23}D$ −28.25° (*c*, 1.2, chloroform).

Anal. Calcd. for $C_{20}H_{28}O_{12}$: C, 52.2; H, 6.08. Found: C, 52.2; H, 6.16.

The filtrate from which the first yield of crystals was obtained was concentrated to a thick sirup, and then taken up in an equal volume of absolute methanol. The flask was scratched with a glass rod, and placed in the icebox. Crystals formed over a period to two or three days. These were collected on a funnel and washed with a little 1:1 ether–Skelly "B" mixture. The yield was 3 g. The material was recrystallized twice by dissolving it in a little warm ether and adding Skelly "B" to turbidity. It formed short, heavy needles, m. p. 116.5–117.5°; $[\alpha]^{23}D$ −24.80° (*c*, 1.2, chloroform).

Anal. Calcd. for $C_{20}H_{28}O_{12}$: C, 52.2; H, 6.08. Found: C, 52.3; H, 6.26.

Formation of Ethyl β-Hydroxybutyrate β-D-Glucoside Tetraacetate (IV) by Reduction of II and III.—The reduction was carried out the same in both cases. One-fourth gram of catalyst (5% palladium chloride on activated carbon)[19] was suspended in 50 ml. of absolute ethanol and shaken with hydrogen in a boat until the catalyst was completely reduced. The reduced catalyst was then washed three times with absolute ethanol (to remove the acid) and resuspended in 50 ml. of absolute ethanol. After shaking with hydrogen until a constant volume was maintained in the chamber, 0.5 g. of II was added, and the hydrogen uptake was followed volumetrically. The reduction was complete in two hours with absorption of 30 ml. of hydrogen (747 mm., 23°), the theoretical uptake for one mole equivalent is 26.6 ml. under the same conditions.

The catalyst was removed by filtration, and the filtrate concentrated to dryness *in vacuo* to yield a white crystalline solid. The solid was recrystallized from 5 ml. of ether. The oblong flat plates were collected and dried; yield 0.25 g., m. p. 91–94°. One more recrystallization gave m. p. 93.5–95°.

Anal. Calcd. for $C_{20}H_{30}O_{12}$: C, 51.9; H, 6.55. Found: C, 51.9; H, 6.63.

The product from the reduction of III showed m. p. 93.5–95°, and gave no depression of the melting point when mixed with the reduction product of II.

Anal. Calcd. for $C_{20}H_{30}O_{12}$: C, 51.9; H, 6.55. Found: C, 51.9; H, 6.69.

Both products showed $[\alpha]^{22}D$ −1.5° (*c*, 20, chloroform).

Since the reduction of the double bond produces an asymmetric carbon atom in the aglucon, two different products should have been formed. Chromatographic studies show two bands, but only one could be crystallized. One would not expect asymmetric reduction of both forms of the glucoside (II and III) to give the same product. It is significant that only one glucoside could be isolated from the coupling of I with DL-ethyl β-hydroxybutyrate, which product (IV) was identical with each of those formed in the above reductions.

Preparation of IV by the Koenigs–Knorr Reaction.—A mixture of 60.0 g. of I, 20.0 g. of DL-ethyl β-hydroxybutyrate,[20] 60 g. of drierite and 380 ml. of dry ether was shaken for fifteen minutes. To the mixture was added 30 g. of silver oxide, and the shaking was continued until the bromide test was negative. The solids were removed and the filtrate was concentrated to a thick colorless sirup which was dissolved in an equal volume of dry ether, and left in the icebox overnight. To the crystalline mass was added 70 ml. of ether and the crystallization allowed to continue for another day.

The crystals were collected on a funnel, washed with a little 1:1 ether–Skelly "B" mixture, and dried in the air; yield 6.5 g., 20% of theory for the glucoside of either the D or L form of the aglucon. The product was recrystallized by dissolving in warm ether, and adding Skelly solvent to turbidity. After two recrystallizations, the melting point was 93–95°; $[\alpha]^{22}D$ −1.5° (*c*, 20, chloroform).

Anal. Calcd. for $C_{20}H_{30}O_{12}$: C, 51.9; H, 6.55. Found: C, 52.1; H, 6.61.

This glucoside (IV) reduces Fehling solution readily, as do the two products from the reduction of II and III. It gave no depression of the melting point when mixed with each of those reduction products. The melting point, rotation, analysis, and pronounced reducing property indicate that the two glucosides produced by the reduction of II and III are identical, and that they are likewise identical with the (D or L) ethyl β-hydroxybutyrate β-D-glucoside tetraacetate formed by the direct condensation of I with the aglucon.

Preparation of O-(Tetraacetyl β-D-glucosyl) Acetoacetanilide (V).—A mixture of 50 g. of I, 21 g. of acetoacetanilide, 20 drops of quinoline, 15 g. of silver oxide, 25 g. of drierite and 300 ml. of ether gave a sirup which crystallized upon adding ether and stirring. The product was filtered off immediately and washed with ether; otherwise it became contaminated with a lower melting fraction. The yield was 22.5 g., m. p. 160–170°. After recrystallization from 60 ml. of 95% ethanol the melting point was 174–176°; $[\alpha]^{24}D$ −27.3° (*c*, 3, chloroform).

Anal. Calcd. for $C_{24}H_{29}O_{11}N$: C, 56.8; H, 5.72. Found: C, 56.9; H, 5.84.

A second fraction of 8.0 g. was obtained from the ether filtrate which melted from 90–120°. This could be separated by fractional crystallization from absolute ethanol into well defined needles having m. p. 90–98°, and granular, light yellow crystals melting from 120–130°. The granular crystals could be converted into the needles by dissolving them in absolute ethanol and then cooling the solution rapidly. Neither form could be obtained with a sharp melting point. Both fractions had the same rotation and gave the same analysis, the analysis corresponding with that of the *trans*(?) form (V) above; $[\alpha]^{22}D$ −62.5° (*c*, 5, chloroform).

Anal. Calcd. for $C_{24}H_{29}O_{11}N$: C, 56.8; H, 5.72. Found: C, 56.8; H, 5.83.

(19) The palladium catalyst was prepared according to Method C, "Organic Syntheses," Vol. 26, p. 77 (1946).

(20) We are indebted to the late Professor Homer Adkins of the Chemistry Department of the University of Wisconsin for a generous supply of DL-ethyl β-hydroxyhutyrate.

The structure of this tentatively named *cis*(?)-O-(tetraacetyl-β-D-glucosyl) acetoacetanilide (VI) must remain in question. It is not reduced by palladium and hydrogen (under the conditions described above) as is V. Theoretically, N-glucosyl formation is possible. However, the expected product would have enolic properties, while VI is insoluble in 5% sodium hydroxide.

Reduction of V resulted in the uptake of one mole equivalent of hydrogen and yielded a product (VII) melting at 149–151°, which was crystallized from 50% ethanol; $[\alpha]^{25}D$ + 10.3° (*c*, 1, chloroform).

Anal. Calcd. for $C_{24}H_{31}O_{11}N$: C, 56.6; H, 6.09. Found: C, 56.5; H, 6.24.

Deacetylation of VII gave β-hydroxybutyranilide β-D-glucoside (VIII), crystallized from isopropyl alcohol; m. p. 152–153°; $[\alpha]^{22}D$ −22.8° (*c*, 2, methanol).

Anal. Calcd. for $C_{16}H_{23}O_7N$: C, 56.3; H, 6.74. Found: C, 56.2; H, 6.50.

Deacetylation of V.—The same general method was used in all deacetylations reported here. Ten grams of the glucoside (V) was dissolved in 250 ml. of dry methanol. The rotation was −1.10° (1 dcm.). After the addition of 5.0 ml. of 0.400 *N* barium methoxide the rotation changed rapidly and stopped thirty minutes later at a value of +0.20° (1 dcm.). An amount of 0.1 *N* sulfuric acid equivalent to the barium ions present was added and the salt that precipitated was removed by filtration through an asbestos mat.

The filtrate was concentrated to a thick sirup, redissolved in methanol, and ether was added to turbidity. After several hours, fine needles aggregated into balls appeared. The yield was 4.3 g., m. p. 163–166°. A second crop of 1.3 g. made the yield 85%. Upon recrystallization from methanol and ether, the melting point was 164–166° (dec.); $[\alpha]^{25}D$ + 5.2° (*c*, 5, methanol).

Anal. Calcd. for $C_{16}H_{21}O_7N$: C, 56.6; H, 6.19. Found: C, 56.6; H, 6.28.

This product, *trans*(?)-O-(β-D-glucosyl) acetoacetanilide (IX) was soluble in water and methanol. It was relatively stable to alkali, but easily hydrolyzed with dilute acid.

Separation by Chromatography.—The enol glucosides could usually be obtained crystalline from ether solution as a mixture of the *cis* and *trans* forms. Separation by fractional recrystallization was not always reproducible. Chromatographic separation was more reliable. Table II lists the adsorption characteristics of some of the *cis–trans* pairs. On a preparative scale, 5 g. of the mixed crystals was dissolved in benzene, poured on an 8 × 33 cm. column packed with 500 g. of the adsorbent, and developed with 1 V. of developer. The column was extruded, streaked with alkaline permanganate, and the zones were cut out. Upon elution with acetone, the compounds were obtained in a reasonably pure form, and were recrystallized from a suitable solvent.

Condensation of Ethyl Benzoylacetate with I.—From 50 g. of I, 25 g. of ethyl benzoylacetate, 20 g. of silver oxide, 10 drops of quinoline, 50 g. of drierite and 250 ml. of dry ether was obtained a sirup which was taken up in an equal volume of methanol, and left in the ice-box until crystals formed (several hours). These were collected and washed with a little cold methanol, yield 20 g., air-dried. Recrystallization from methanol or ethanol gave a product that melted from 48–70°, and was probably a mixture of the *cis* and *trans* forms. By recrystallization from ether three times, a product melting 71.5–73.5° was obtained (X); $[\alpha]^{21}D$ −32.3° (*c*, 3, chloroform).

Anal. Calcd. for $C_{25}H_{30}O_{12}$: C, 57.5; H, 5.74. Found: C, 57.7; H, 5.84.

Deacetylation of the above product (X) with barium methoxide in methanol gave a sirup which was crystallized from absolute ethanol. It melted from 159–161°; $[\alpha]^{23}D$ 0.0 (*c*, 2, water). The substance analyzed correctly for O-(β-D-glucosyl) *methyl* benzoylacetate (XI), indicating that *trans* esterification had taken place during the deacetylation.

Anal. Calcd. for $C_{16}H_{20}O_8$: C, 56.5; H, 5.88. Found: C, 56.5; H, 5.82.

TABLE II

CHROMATOGRAPHIC SEPARATION OF *cis–trans* ENOL GLUCOSIDES

Adsorbent, Silene EF–Celite 545 (5:1); developer, benzene–ethanol (200:1); column, 0.9 × 80 mm. in 0.9 × 130 mm. tube; 1.2 g. of adsorbent; volume of developer, 1V.

Compound	Zone position, mm. from top of column
O-(Tetraacetyl-β-D-glucosyl) ethyl acetoacetate	25–35 *trans*(?)
	40–45 *cis*(?)
O-(Tetraacetyl-β-D-glucosyl) acetoacetanilide	5–10 *trans*(?)
	26–34 *cis*(?)
O-(Tetraacetyl-β-D-glucosyl) benzoylacetone	31–38 *trans*(?)

Preparation of O-(Tetraacetyl-β-D-glucosyl) Benzoylacetone (XII).—The thick, red sirup obtained from 20 g. of benzoylacetone, 50 g. of I, 32 drops of quinoline, 18 g. of silver oxide, 50 g. of drierite and 400 ml. of ether, was dissolved in 150 ml. of ether and stirred with a glass rod. Crystals formed immediately, which were collected and washed with ether. The yield was 16 g. After recrystallization from absolute ethanol, the product amounted to 15 g., with a melting point of 143–145°; $[\alpha]^{24}D$ +46.9° (*c*, 3, chloroform).

Anal. Calcd. for $C_{24}H_{28}O_{11}$: C, 58.6; H, 5.69. Found: C, 58.7; H, 5.80.

The ether filtrate from above deposited an additional 10.5 g. of an impure product melting from 105–125°. This probably contains the second form, but it could not be fractionated.

Deacetylation of XII gave O-(β-D-glucosyl) benzoylacetone (XIII) in a yield of 30%. It was recrystallized from dioxane, and had a melting point of 152–154°, $[\alpha]^{25}D$ −9.2° (*c*, 2.5, methanol).

Anal. Calcd. for $C_{16}H_{20}O_7$: C, 59.3; H, 6.17. Found: C, 59.1; H, 6.41.

Preparation of O-(Tetraacetyl-β-D-glucosyl) dibenzoylmethane (XIV).—From 10 g. of dibenzoylmethane, 18.3 g. of I, 18 g. of drierite, 6.7 g. of silver oxide, 24 drops of quinoline and 250 ml. of dry ether was obtained a sirup which was not washed with acid and alkali. It was taken up in two volumes of ether and left in the ice-box. Crystals formed over a period of a month. The yield was 7 g., and upon recrystallization from absolute ethanol light yellow needles were obtained. The melting point was 139–141°; $[\alpha]^{30}D$ +26.7° (*c*, 2, chloroform).

Anal. Calcd. for $C_{29}H_{30}O_{11}$: C, 62.8; H, 5.42. Found: C, 62.8; H, 5.54.

Deacetylation of XIV gave a small yield of O-(β-D-glucosyl) dibenzoylmethane (XV) which crystallized as very fine needles from absolute ethanol. The glucoside sinters at 190° and melts at 203–204°. It is insoluble in hot and cold water, and in cold ethanol. It is slightly soluble in hot ethanol. XV does reduce Fehling solution. It is unusually stable to hydrolysis with 5% hydrochloric acid, possibly due to its low solubility in water; $[\alpha]^{30}D$ +3.9° (*c*, 1, pyridine).

Anal. Calcd. for $C_{21}H_{22}O_7$: C, 65.2; H, 5.70. Found: C, 65.1; H, 5.91.

Glucoside of 5,5-Dimethylcyclohexanedione-1,3 (XVI).—From 10.5 g. of 5,5-dimethylcyclohexanedione-1,3, 30.8 g. of I, 30 g. of drierite, 100 ml. of ether, 8 drops of quinoline and 12 g. of silver oxide was obtained a sirup, which was treated as in the preparation of XIV. A yield of 2.7 g. of yellow crystals resulted. Most of the color was removed by washing the solid with water on the funnel. After recrystallization from 95% ethanol the substance melted at 142–144°. It was light yellow in color; $[\alpha]^{30}D$ −26.3° (*c*, 2, chloroform).

Anal. Calcd. for $C_{22}H_{30}O_{11}$: C, 56.2; H, 6.38. Found: C, 55.9; H, 6.52.

The reduction of XVI proceded with the uptake of one mole equivalent of hydrogen. A nicely crystalline compound was isolated which melted over a range of ten degrees, and probably was a mixture of the glucosides of the D and L aglucon, 5,5-dimethyl-3-oxycyclohexanol.

Attempted deacetylation of XVI gave a gummy crystalline product which could not be purified for analysis.

Condensation of I with the Sodium Salt of Acetoacetaldehyde.—To 17.7 g. of I dissolved in 60 ml. of acetone was added a solution of 4.5 g. of the sodium salt of acetoacetaldehyde[21] dissolved in 60 ml. of water and 60 ml. of acetone. The reaction mixture was left at room temperature for four hours.

The acetone was removed by concentration *in vacuo*, and the sirup which separated from the aqueous phase was extracted with 100 ml. of chloroform. The chloroform layer was washed with 100 ml. of cold 2% potassium carbonate, then twice with cold water. It was dried over calcium chloride, and the chloroform was removed *in vacuo*. The resulting sirup was diluted with 75 ml. of dry ether, and crystallization took place immediately. The flask was set in the ice-box for several hours. The crystals were collected and washed with ether; yield 2.8 g., m. p. 135–160°. This product is probably a mixture of the *cis* and *trans* forms that are possible. After two recrystallizations from 95% ethanol, the melting point was 158–160°; $[\alpha]^{22}D$ −19.5° (*c*, 1, chloroform).

Anal. Calcd. for $C_{18}H_{24}O_{11}$: C, 52.0; H, 5.77. Found: C, 51.9; H, 6.00.

Another product corresponding to a *cis* and *trans* pair could not be isolated. The above compound O-(tetraacetyl-β-D-glucosyl) acetoacetaldehyde (XVII) is very labile to alkali. It reduces hot Fehling solution immediately. When 0.1 g. in 10 ml. of dry methanol was treated with a catalytic amount of barium methoxide, the rotation of the solution changed from −0.20° (1 dcm.) to +0.25° (1 dcm.). Conversion of the glucoside to free glucose would give a rotation of +0.26° (1 dcm.).

On reducing 0.4 g. of XVII in ether, one mole equivalent of hydrogen was absorbed in one hour, and 0.24 g. of the reduced product, 3-oxo-*n*-butyl β-D-glucoside tetraacetate (XVIII) was obtained. After two recrystallizations from 95% ethanol, the substance showed m. p. 93–94.5°; $[\alpha]^{22}D$ −12.31° (*c*, 1.5, chloroform).

Anal. Calcd. for $C_{18}H_{26}O_{11}$: C, 51.7; H, 6.22. Found: C, 51.5; H, 6.35.

When 1.0 g. of XVIII was dissolved in 25 ml. of dry methanol and 0.2 ml. of 0.400 *N* barium methoxide added, the rotation changed from the original −0.65° (1 dcm.) to +1.12° (1 dcm.) in one hour. Complete conversion to glucose would give a rotation of +1.10° (1 dcm.). The residue was strongly lachrymatory. Glucose was isolated from the reaction mixture.

Preparation of XVIII by the Koenigs–Knorr Reaction.—A sirup was obtained in the condensation of I with 3-oxo-*n*-butanol[22] according to the directions for synthesis of IV. It was crystallized from 95% ethanol, the yield being 35%. It melted at 92–94° and gave no depression of the melting point when mixed with XVIII formed in the reduction of XVII. The rotation showed $[\alpha]^{21}D$ −11.95° (*c*, 2.5, chloroform).

Anal. Calcd. for $C_{18}H_{26}O_{11}$: C, 51.7; H, 6.22. Found: C, 51.5; H, 6.50.

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Summary

The preparation of the β-D-glucoside of enolic ethyl acetoacetate is described. It was isolated in both a *cis* and *trans* form. Glucosides of other activated ketones such as acetoacetanilide, ethyl benzoylacetate, benzoylacetone, dibenzoylmethane, and dimethylcyclohexanedione-1,3 were prepared by the same general method.

The glucoside of acetoacetaldehyde was made by the method of Michael.

The double bond of the enolic glycosides was readily reduced with hydrogen and palladium to produce glucosides of β-hydroxy carbonyl compounds. From *cis* and *trans*-O-(tetraacetyl-β-D-glucosyl) ethyl acetoacetate was produced the same glucoside tetraacetate of ethyl β-hydroxybutyrate. The latter was also synthesized from tetraacetyl-D-glucosyl bromide and ethyl β-hydroxybutyrate.

Some of the glucosides were deacetylated by the catalytic barium methoxide method, although this treatment was sufficient to effect alkaline cleavage of a number of the starting compounds.

The lability to alkali of the various glucosides was determined by noting the ease with which they reduced Benedict solution. The enol glucosides of ethyl acetoacetate were weakly reducing. However, the glucosides of acetoacetaldehyde, benzoylacetone and dibenzoylmethane were readily reducing. The glucosides of the activated β-hydroxy carbonyl compounds showed the same lability to alkali observed in glycosides of β-nitroethanol, 2-hydroxyethanesulfonic acid and picrocrocin. This lability was correlated with the negativity of the respective carbonyl groups.

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(21) Claisen and Stylos, *Ber.*, **21**, 1144 (1888).

(22) Dreyfus and Drewitt, British Patent 559,209, Feb. 9, 1944.