[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF WASHINGTON UNIVERSITY]

The Preparation and Hydrolysis of Some Hydroxyanthraquinone Biosides[1]

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For a number of years a considerable amount of work has been done on the identification of naturally occurring glycosides. The hydroxyanthraquinone glycosides have received particular attention because of their occurence in many of the common laxative drugs. Identification of the aglycone usually offers no difficulty. The sugar component, however, is often hard to characterize. In order to determine if the rate of hydrolysis of these glycosides is a function of the sugar as well as the aglycone it was thought desirable to determine the rates of hydrolysis of several mono and polyhydroxyanthraquinone glycosides.

With these facts in mind Gardner, McDonnell and Wiegand[4] prepared 1-hydroxyanthraquinone-β-*d*-glucoside and -β-*d*-arabinoside, then Foster and Gardner[5] prepared 1,5-dihydroxyanthraquinone, 1,8-dihydroxyanthraquinone and 1,8-dihydroxy - 3 - methylanthraquinone - β - *d* - glucosides and finally Gardner and Demaree[6] prepared 2 - hydroxyanthraquinone - β - *d* - glucoside. For each of these compounds the rates of hydrolysis in hydrochloric acid and potassium hydroxide solutions or suspensions were measured.

From these studies it was found that the rate of hydrolysis was not only a function of the aglycone but of the sugar component as well. With this thought in mind it seemed desirable to prepare glycosides of these anthraquinone derivatives in which the sugar portion was a biose. For this purpose the cellobiosides and maltosides of 1-hydroxyanthraquinone, 1,8-dihydroxyanthraquinone, 1,8-dihydroxy-3-methylanthraquinone and 1,6,8-trihydroxy-3-methylanthraquinone were prepared and their rates of hydrolysis determined in hydrochloric acid and potassium hydroxide solutions. Because of the high degree of solubility of these glycosides in water, a new analytical procedure was established which measured only the aglycone produced in the hydrolysis reaction.

No significant difference is noted in the rates of hydrolysis of the 1-hydroxyanthraquinone, 1,8-dihydroxyanthraquinone and 1,8-dihydroxy-3-methylanthraquinone-α-*d*-cellobioside and -β-*d*-maltoside with those of the corresponding β-*d*-glucosides with the possible exception of the acid hydrolysis of 1,8-dihydroxyanthraquinone-α-*d*-cellobioside which resembled 2-hydroxyanthraquinone-β-*d*-glucoside.[6]

In the hydrolysis of 1,6,8-trihydroxy-3-methylanthraquinone-α-*d*-cellobioside and -β-*d*-maltoside, however, a much slower rate of hydrolysis in both media was observed with reduction to anthranol occurring in the potassium hydroxide hydrolysis of the maltoside.

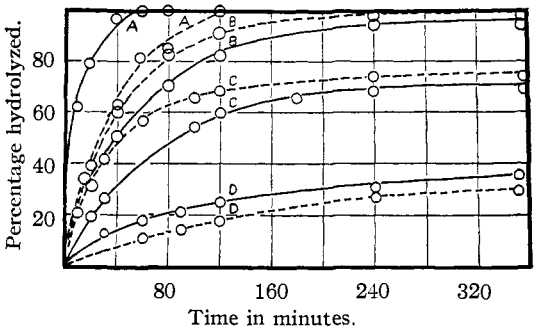

Fig. 1.—Rate of hydrolysis of β-*d*-maltosides of hydroxyanthraquinones, KOH —, HCl — — — — —; A, 1-hydroxyanthraquinone; B, 1,8-dihydroxyanthraquinone; C, 1,8-dihydroxy-3-methylanthraquinone; and D, 1,6,8-trihydroxy-3-methylanthraquinone.

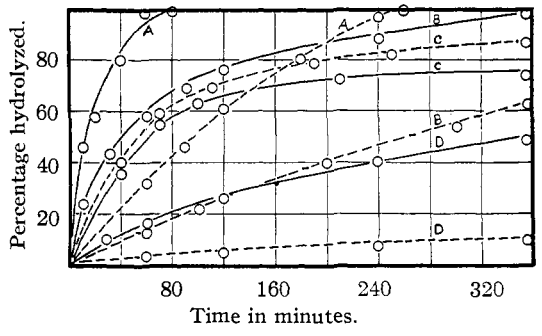

Fig. 2.—Rate of hydrolysis of α-*d*-cellobiosides of hydroxyanthraquinones, KOH ——, HCl — — — — —: A, 1-hydroxyanthraquinone; B, 1,8-dihydroxyanthraquinone; C, 1,8-dihydroxy-3-methylanthraquinone; and D, 1,6,8-trihydroxy-3-methylanthraquinone.

Experimental

Heptaacetylbiosides.—The heptaacetylbiosides were prepared from acetobromo-α-*d*-cellobiose or acetobromo-β-*d*-maltose and the anthraquinone derivatives by solution in quinoline and subsequent reaction with silver oxide.

To illustrate the method used, the preparation of 1-hydroxyanthraquinone-α-*d*-heptaacetyl cellobioside is described.

To 0.9 g. of 1-hydroxyanthraquinone dissolved in 10 cc. of freshly distilled quinoline there was added 3.5 g. of acetobromo-α-*d*-cellobiose and the mixture was shaken until the latter dissolved. To this solution there was then added 2 g. of freshly prepared 100-mesh silver oxide, and the mixture shaken vigorously for twenty minutes and

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(4) Gardner, McDonnell and Wiegand, THIS JOURNAL, **57**, 1074 (1935).

(5) Foster and Gardner, *ibid.*, **58**, 597 (1936).

(6) Gardner and Demaree, *ibid.*, **58**, 757 (1936).

TABLE I

HEPTAACETYL-α-*d*-CELLOBIOSIDES OF HYDROXYANTHRAQUINONES

Anthraquinone	Yield, %	M. p., °C.	Molecular formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Acetyl, % Calcd.	Acetyl, % Found
1-Hydroxy-	66.1	245.5–246[a,7]	$C_{40}H_{42}O_{20}$	56.98	56.81	5.02	4.84	35.7	35.6
1,8-Dihydroxy-	68.5	264–264.5[b]	$C_{40}H_{42}O_{21}$	55.92	56.38	4.93	5.02	35.3	34.9
1,8-Dihydroxy-3-methyl-	81.5	237.8–238.4	$C_{41}H_{44}O_{21}$	56.40	56.81	5.08	5.30	34.6	34.3
1,6,8-Trihydroxy-3-methyl-	81.0	220.8–221.4	$C_{41}H_{44}O_{22}$	55.38	55.09	4.99	5.24	34.0	33.2
HEPTAACETYL-β-*d*-MALTOSIDES OF HYDROXYANTHRAQUINONES									
1-Hydroxy-	73.5	215.4–215.9	$C_{40}H_{42}O_{20}$	56.98	56.63	5.02	5.26	37.5	35.9
1,8-Dihydroxy-	71.5	214.0–214.5	$C_{40}H_{42}O_{21}$	55.92	56.26	4.93	5.10	35.3	35.5
1,8-Dihydroxy-3-methyl-	77.5	218.5–219.0	$C_{41}H_{44}O_{21}$	56.40	56.78	5.08	5.22	34.6	34.2
1,6,8-Trihydroxy-3-methyl-	61.1	159.0–160.0	$C_{41}H_{44}O_{22}$	55.38	55.26	4.99	5.17	34.0	34.4

[a] Mendelényi and Müller[8] give 244–245°. [b] Mendelényi and Müller[8] give 270–271°.

TABLE II

α-*d*-CELLOBIOSIDES OF HYDROXYANTHRAQUINONES

Anthraquinone	Yield, %	M. p., °C.	Molecular formula	Carbon, % Calcd.	Carbon, % Found	Hydrogen, % Calcd.	Hydrogen, % Found	Acetyl, % Calcd.	Acetyl, % Found
1-Hydroxy-	72.5	255.0–255.5	$C_{26}H_{28}O_{13}$	56.91	57.13	5.14	5.08	0.0	1.2
1,8-Dihydroxy-	86.0	246.0–246.8	$C_{26}H_{28}O_{14}$	55.30	55.62	5.00	5.28	.0	1.0
1,8-Dihydroxy-3-methyl-	88.1	231.0–232.0	$C_{27}H_{30}O_{14}$	56.30	56.40	5.23	5.06	.0	0.5
1,6,8-Trihydroxy-3-methyl-	70.7	274.0–274.6	$C_{27}H_{30}O_{15}$	54.52	54.31	5.09	4.88	.0	0.9
β-*d*-MALTOSIDES OF HYDROXYANTHRAQUINONES									
1-Hydroxy-	65.0	241.2–241.8	$C_{26}H_{28}O_{13}$	56.91	56.59	5.14	4.92	0.0	1.1
1,8-Dihydroxy-	40.2	228.0–229.0	$C_{26}H_{28}O_{14}$	55.30	55.51	5.00	5.18	.0	1.4
1,8-Dihydroxy-3-methyl-	81.2	239.0–239.5	$C_{27}H_{30}O_{14}$	56.03	56.48	5.23	5.10	.0	1.3
1,6,8-Trihydroxy-3-methyl-	33.1	226.6–227.2	$C_{27}H_{30}O_{15}$	54.52	54.13	5.09	5.29	.0	0.7

then allowed to stand for two hours. Heat was evolved and the mixture set to a semi-solid mass. To the mass there was then added 100 cc. of chloroform and the mixture again shaken and allowed to stand for one hour. Heat was again evolved. The chloroform solution was filtered to remove silver bromide and any unchanged silver oxide. It was washed with three 200-cc. portions of 5% sulfuric acid solution to remove the quinoline, then with water and finally dried over anhydrous sodium sulfate. The solution was filtered and the major portion of the solvent allowed to evaporate spontaneously overnight. The last portions were taken off in vacuum. The residue remaining was dissolved in hot alcohol and cooled in ice. The precipitate thus formed was the acetylbioside.

In the preparation of the other members of both the cellobioside and maltoside series the same general method was followed using an equivalent amount of the appropriate hydroxyanthraquinone in place of 1-hydroxyanthraquinone. The yields, melting points, and analyses of the acetyl biosides are given in Table I.

Biosides.—The saponification of 1-hydroxyanthraquinone heptaacetyl-α-*d*-cellobioside and heptaacetyl-β-*d*-maltoside was carried out by the method used by Gardner, McDonnell and Wiegand[4] for the saponification of 1-hydroxyanthraquinone tetraacetyl-β-*d*-glucoside with modifications.

A suspension of 2.0 g. of the acetylbioside in 100 cc. of 5% alcoholic potassium hydroxide was stirred rapidly for fifteen minutes. During this time the odor of ethyl acetate became pronounced and the color of the solution became a very faint pink. The bioside was then filtered off and resuspended in 15 cc. of alcohol to which was then added just enough glacial acetic acid to discharge the pink coloration. After a short digestion the solid was filtered off and air dried. The dry material was then washed with chloroform to remove unreacted acetylbioside and free aglycone. A small portion was further recrystallized from alcohol for analysis.

The saponification of the other members of the series was carried out in the same general way but due to the great solubility of the maltosides in alcohol and glacial acetic acid, small amounts of these reagents had to be used in order to obtain a high yield of bioside. The yields, melting points and analyses of the biosides are given in Table II.

Hydrolysis Procedure.—The hydrolysis procedure followed was a modification of that used by Gardner, McDonnell and Wiegand[4] for the hydrolysis in hydrochloric acid and in potassium hydroxide of 1-hydroxyanthraquinone-β-*d*-glucoside. Solutions of exactly 0.050 *N* hydrochloric acid and potassium hydroxide were made up using 90 cc. of 95% alcohol per liter of solution. The bioside samples were ground to pass a standard 100-mesh sieve in order to assure a more uniform particle size.

Acid Hydrolysis.—A suspension of 0.0200 g. of the bioside in the proportion of 1.36 millimoles to 1 cc. of 0.050 *N* hydrochloric acid was heated in a boiling water-bath under reflux for the desired period of time. At the end of the heating time the flask was plunged into an ice-bath and allowed to cool for fifteen minutes. The solution was then transferred to a separatory funnel and the aglycone extracted completely with diethyl ether. The diethyl ether washings were then shaken with 2 *N* potassium hydroxide solution which extracted the aglycone quantitatively. The potassium hydroxide solution was then transferred to a 100-cc. volumetric flask and made up to the mark with 2 *N* potassium hydroxide solution. The concentration of aglycone was then determined using a Model 11 Coleman Universal Spectrophotometer with a PC-4 filter and a wave length setting of 490 for the 1-hydroxyanthraquinone biosides, 500 for the 1,8-dihydroxyanthraquinone biosides, 505 for the 1,8-dihydroxy-3-methylanthraquinone biosides and 520 for the 1,6,8-trihydroxy-3-methylanthraquinone biosides. The wave lengths selected correspond to the absorption maxima for the aglycones in the concentration of 1.5 mg. per 100 cc. of

(7) All melting points in this paper are corrected.

(8) Mendelényi and Müller, *Magyar Biol. Kutationteset Munkái*, **6**, 316 (1933).

2 *N* potassium hydroxide solution. The actual concentration of aglycone was then read from transmittance-concentration curves derived from measurements on solutions of the pure aglycones in 2 *N* potassium hydroxide solution. These curves were found to be linear between 0.4 and 2.0 mg. of aglycone per 100 cc. of 2 *N* potassium hydroxide.

Alkaline Hydrolysis.—The same quantities of the biosides were heated in a boiling water-bath with the same amounts of 0.050 *N* potassium hydroxide as the 0.050 *N* hydrochloric acid referred to in the acid hydrolysis. At the end of the desired period of time the solution was acidified with 0.050 *N* hydrochloric acid and cooled in an ice-bath for fifteen minutes. The solution was then transferred to a separatory funnel and the aglycone extracted and determined as outlined under the acid hydrolysis procedure.

The aglycone obtained by either acid or alkaline hydrolysis showed no depression in melting point with the parent hydroxyanthraquinone indicating simple hydrolysis. Although reduction undoubtedly occurred in the alkaline hydrolysis of 1,6,8-trihydroxy-3-methylanthraquinone-*β*-*d*-maltoside, as indicated by the intense blue fluorescence produced, it was not possible to isolate the small amount of compound formed.

The results obtained in work of this type are relative rather than, in the strictest sense quantitative, since the acid hydrolyses are carried out in heterogeneous systems in which the factor of particle size is very important. As has been mentioned earlier, however, this factor was minimized by grinding the samples to pass a standard 100-mesh sieve.

Although the analytical method used in the present investigation differs in some details from that used by Gardner, McDonnell and Wiegand[4] a preliminary experiment on the hydrolysis of 1-hydroxyanthraquinone-*β*-*d*-glucoside indicated agreement with the results obtained by these workers.

In order to correlate the results obtained in previous hydrolysis experiments with those reported here, the approximate time required for fifty per cent. hydrolysis in hydrochloric acid and potassium hydroxide solutions was estimated from the curves in this and the previous papers. These data are collected in Table III.

It is apparent from the data given in Table III that the rate of hydrolyses of hydroxyanthraquinone glycosides varies both with the nature of the aglycone and the sugar residue. Data of the type presented could be of use in the identification of hydroxyanthraquinone glycosides isolated from plant materials. It is usually easy to identify the aglycone but extremely difficult to identify the sugar by direct means. Consequently if rates of hydrolysis of the glycosides derived from a given hydroxyanthraquinone and a given sugar are known, it should be possible to effect at least a partial identification of a naturally occurring hydroxyanthraquinone glycoside by the determination of the rate of hydrolysis under conditions similar to those used in this investigation. It is obviously necessary to obtain similar data for glycosides derived from additional hydroxyanthraquinones and sugars before this method could be used with certainty.

TABLE III

A COMPARISON OF THE RATES OF HYDROLYSIS OF HYDROXYANTHRAQUINONE GLYCOSIDES IN HYDROCHLORIC ACID AND POTASSIUM HYDROXIDE SOLUTIONS

Aglycone-anthraquinone	Sugar	Time required for 50% hydrolysis, minutes HCl	KOH
1-Hydroxy-	*β*-*d*-Glucose[4]	36	12
	β-*d*-Arabinose[4]	5	4
	α-*d*-Cellobiose	98	13
	β-*d*-Maltose	30	5
2-Hydroxy-	*β*-*d*-Glucose[6]	360	15
1,5-Dihydroxy-	*β*-*d*-Glucose[5]	30	9
1,8-Dihydroxy-	*β*-*d*-Glucose[5]	26	4
	α-*d*-Cellobiose	270	40
	β-*d*-Maltose	32	40
1,8-Dihydroxy-3-methyl-	*β*-*d*-Glucose[5]	40	16
	α-*d*-Cellobiose	50	55
	β-*d*-Maltose	45	85
1,6,8-Trihydroxy-3-methyl-	*α*-*d*-Cellobiose	>360	360
	β-*d*-Maltose	>360	>360

Summary

1. The cellobiosides and maltosides of 1-hydroxy-, 1,8-dihydroxy-, 1,8-dihydroxy-3-methyl- and 1,6,8-trihydroxy-3-methylanthraquinone have been prepared.

2. The rates of hydrolysis of these biosides in hydrochloric acid and potassium hydroxide solutions or suspensions have been measured and compared with those obtained previously for related compounds.

3. A new method has been developed for the measurement of these hydrolysis rates.

4. It has been shown that the hydrolysis of 1,6,8 - trihydroxy - 3 - methylanthraquinone - *β*-*d*-maltoside in potassium hydroxide solution is accompanied by reduction.

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