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148. Mitiiti Fujita,[*1] Tsutomu Furuya,[*1] and Mitsuyoshi Matsuo[*2]: Studies on the Metabolism of Naturally Occurring Anthraquinones. I. The Metabolism of 1-Hydroxyanthraquinone and 2-Hydroxyanthraquinone.

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The homologues of hydroxyanthraquinones containing two or more hydroxyl groups are of interest on account of their distribution in the plant kingdom, particularly in the anthraglucoside-bearing drugs, such as senna, rhubarb, frangula, and others which are popularly used as purgatives. It has been observed in the earlier work[1] that about 40% of the total activity of senna pod and leaf may be due to sennoside content. English workers showed that besides sennosides A and B, the pod and leaf contain a third anthracene glycoside, which may bear the similar pharmacological activity as the formers.

Although many studies have been hitherto made on various anthraquinones present in those drugs, but none gave any sufficient explanation about the relationship of chemical conversion of the compounds and their mode of action as purgative *in vivo*.

This work was undertaken to study the metabolic fate of anthraquinones in rat and the authors have attempted to make it the basis for the elucidation of the chemical form of their active principles and the physiological action in living tissues.

In this field, however, nothing has been reported so far on any other anthraquinone derivative except the metabolism of anthraquinone itself[2,3] and its 1,8-dihydroxy derivative[4] (chrysazin).

This paper describes some experiments on the metabolism of 1-hydroxy- and 2-hydroxyanthraquinones. Even though the former substance has not been found in nature, the latter was obtained from chay root (*Oldenlandia umbellata* L.).[5] All the hydroxyanthraquinones were administered orally to the rats, and then investigated by paper chromatography described in the experimental part.

Experimental

Material——1-Hydroxyanthraquinone, m.p. 194~195°, was prepared from 1-methoxyanthraquinone by the boiling with HBr and 2-hydroxyanthraquinone, m.p. 306°, was synthesized from 2-aminoanthraquinone.

Animal, Diet and Dosage——Rats (150 g., body wt.) were reared in the metabolism cages and kept on a constant diet consisted of solid food and water. Each of 1-hydroxy- and 2-hydroxyanthraquinones was administered by stomach tube as a suspension with gum arabic and water using the continuous procedure. The dose level of 50 mg. per rat of the compounds did not show any sign of diarrhoea in any animal.

Paper Chromatography——In addition to the paper chromatography described by Takido,[6] some new procedure was adopted in the present work. 20% dimethylformamide solution containing $NaBH_4$[7] was first used as a detecting reagent of anthraquinones and anthrones. This reagent was sprayed on the paper chromatograms. It gave a strong yellow, green or blue fluorescence under ultraviolet

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ray, such as a bright greenish yellow fluorescence to 2-hydroxyanthraquinone and a bright yellow to 2-hydroxyanthrone. The Rf values and color reaction of other related compounds are also shown in Table I.

Table I. Rf Values and Color Reactions of Anthraquinone and Anthrone Derivatives

Compound	Solvent System				UV-Fluorescence			10% Na_2CO_3
	a	b	c	d	untreated	$(CH_3)_2NCHO$	10% Na_2CO_3	
Anthraquinone	0.85	0.84	0.86	0.98	—	V	—	—
1-Hydroxy-	0.88	0.89	0.91	0.98	O	Y	O	Y O
2-Hydroxy-	0.57	0.89	0.88	0.72	Y	B G	O R	O
1,2-Dihydroxy-	0.26	0.68	0.86	0.96	V	Y G	V	V
1,3-Dihydroxy-	0.55	0.84	0.88	0.90	O Y	Y	O	O
1,4-Dihydroxy-	0.83	0.84	0.87	0.98	Y O	B	O R	V
1,5-Dihydroxy-	0.77	0.00	0.00	0.98	p O Y	f O	O	f O Y
1,8-Dihydroxy-	0.81	0.87	0.90	0.98	O	Y	O	P R
2,3-Dihydroxy-	0.38*	0.78	0.82	0.13	V	Y G	V	V
2,6-Dihydroxy-	0.13	0.86	0.85	0.12	O Y	B G	O	Y
2,7-Dihydroxy-	0.13	0.89	0.88	0.16	f O Y	B	P R	P
1,2,3-Trihydroxy-	0.37*	0.72	0.80	0.25	—	Y G	—	Gray
1,2,4-Trihydroxy-	0.27	0.54	0.83	0.94	O	O	O	P
1,2,5,8-Tetrahydroxy-	0.19	0.34*	0.84	0.96	—	O	—	—
1,2,3,5,6,7-Hexahydroxy-	0.00	0.07	0.00	0.00	—	B	—	Br
1-Methoxy-	0.82	0.85	0.88	0.98	G Y	B	p B	Y
2-Methoxy-	0.80	0.87	0.87	0.98	G Y	Y G	Y G	—
1-Methoxy-2-hydroxy-	0.50	0.86	0.89	0.95	O Y	Y	R	R
1-Methoxy-3-hydroxy-	0.42	0.85	0.89	0.72*	O Y	B G	O	O
1-Methoxy-5-hydroxy-	0.82	0.88	0.91	0.98	O	B G	O	O Y
1-Methoxy-8-hydroxy-	0.82	0.85	0.90	0.97	O	f B	O	O Y
1-Hydroxy-2-methoxy-	0.69	0.75	0.81	0.98	O Y	—	O	P
1,2-Dimethoxy-	0.82	0.85*	0.87	0.97	f G Y	B	f O	—
Anthrone		0.86	0.89	0.97	p B	Y→B	Y	Y
1-Hydroxy-		0.89	0.90	0.98	Y	Y→Y G	Y→O Y	Y
2-Hydroxy-		0.84	0.90	0.98	B	B G	B	O
1,2-Hydroxy-		0.82	0.86	0.36	p B→G Y	Y G	G Y	V
1,8-Hydroxy-		0.92*	0.90	0.98	Y→O Y	Y G	G Y	O Y→O
10:10-Dianthrone		0.90	0.90*	0.97	—	f O Y	f O Y	—
10:10-Dehydrodianthrone		0.32*	0.78*	0.98	Y→G Y	V	O	Y

Solvent System : (a) BuOH-benzene-$(NH_4)_2CO_3$ buffer (80:5:15) (b) Water-saturated BuOH
(c) BuOH-AcOH-H_2O (4:1:5) (d) Benzene-AcOH-H_2O (2:2:1)
* Tailing spot

B : blue Y : yellow R : red G : green O : orange Br : brown
V : violet P : pink f : faint p : pale

Qualitative Examination of Urine——The urine excreted during 48 hr. after oral administration of each of 1-hydroxy- or 2-hydroxyanthraquinones was adjusted to pH 2.0 with 10% H_2SO_4, and then continuously extracted with peroxide-free Et_2O for 3 hr. After drying over anhyd. Na_2SO_4, the brownish extract was evaporated to dryness on a water bath. To the residual urine 50% H_2SO_4 was added to dilute it up to 10% solution which was subjected to hydrolysis on a boiling water bath for 2 hr. The hydrolysate was extracted with Et_2O for 3 hr., and then the solvent was evaporated.

Table II. Identification of Metabolites of 1-Hydroxyanthraquinone by Paper Chromatography

Compound	Urine		Feces	
	Free	Conjugated	Free	Conjugated
1-Hydroxyanthraquinone (Unchanged)	+	+	+	+
Alizarin	+	+	+	+
1,3-Dihydroxyanthraquinone	—	—	—	—
1,2,4-Trihydroxyanthraquinone	—	—	—	—

+ : present — : absent

On the other hand, feces excreted during 48 hr. by the same procedure as described above, were extracted with Et_2O for 3 hr., using Soxhlet's extractor. The residual feces were submitted to hydrolysis on a boiling water bath with 50 cc. of 10% H_2SO_4 for 2 hr., and then extracted with Et_2O for 3 hr. Each of the Et_2O extracts was evaporated to dryness.

All the free and conjugated fractions (I) extracted with Et_2O before and after hydrolysis were developed by paper chromatography. The results are shown in Tables II and III.

Table III. Identification of Metabolites of 2-Hydroxyanthraquinone by Paper Chromatography

Compound	Urine		Feces	
	Free	Conjugated	Free	Conjugated
2-Hydroxyanthraquinone (Unchanged)	+	+	+	+
Alizarin	+	+	(+)	(+)
2,3-Dihydroxyanthraquinone	−	−	−	−
1,2,4-Trihydroxyanthraquinone	−	−	−	−

+ : present (+) : present in trace − : absent

Determination of Metabolites——All the fractions (I) were evaporated to dryness and each of the residues was dissolved in 10 cc. of tetrahydrofuran, 0.05 cc. or 0.1 cc. of which was applied to a sheet of filter paper (Toyo Roshi No. 51, 2.9×40 cm.). The chromatogram developed with a solvent system of MeOH-saturd. peter. benzine was colored by 0.5% Na_2CO_3 solution. Just after 1 hr., the paper was dipped into 30% liquid paraffin Et_2O solution to render it translucent and then subjected to the following procedure. The color density was read by a densitometer (Model Kobayashi) using a slit of 2 mm. and a blue filter (for 1-hydroxy- and 2-hydroxyanthraquinones) or an orange one (for alizarin).

Standard regression equations between concentration and extinction calculated from $n=40$ by the least squares method are as follows:

1-Hydroxyanthraquinone: $y=0.037x+0.0156$ or $x=64.10y-2.372$ $\sigma=0.053$
2-Hydroxyanthraquinone: $y=0.027x-0.028$ or $x=37.04y+1.074$ $\sigma=0.081$
Alizarin: $y=0.232+0.0524x$ or $x=19.08y-4.427$ $\sigma=0.150$

x=Concentration of anthraquinones (γ); y=Extinction; σ=Standard deviation

The recovery of 1-hydroxy-, 2-hydroxyanthraquinone, and alizarin resulting from urine and feces are listed in Table IV.

Table IV. Recovery of 1-Hydroxy-, 2-Hydroxyanthraquinone and Alizarin

Compound	Urine		Feces	
	Free[a] (%)	Conjugated[b] (%)	Free[a] (%)	Conjugated[b] (%)
1-Hydroxyanthraquinone	98.72～106.57	92.95～105.90	94.23～ 99.68	92.36～108.97
2-Hydroxyanthraquinone	98.53～105.32	95.30～104.30	95.48～100.35	93.67～106.27
Alizarin	98.76～104.20	89.13～100.15	94.32～108.97	96.47～110.88

The results are quoted as the range for three experiments.

a) Added to normal urine.

b) Added to hydrolysed urine after Et_2O extraction.

Results and Discussion

The 48 hours urinary and fecal metabolites after oral administration of 50 mg. each of 1-hydroxy- and 2-hydroxyanthraquinones to rats were determined by the above mentioned method. All the results are shown in Tables V, VI, VII, and VIII.

Table V. The Urinary Metabolites of 1-Hydroxyanthraquinone

Expt. No.	1-Hydroxyanthraquinone					Alizarin					Total (% of dose)
	Free		Conjugated		Total (% of dose)	Free		Conjugated		Total (% of dose)	
	(mg.)	(% of dose)	(mg.)	(% of dose)		(mg.)	(% of dose)	(mg.)	(% of dose)		
I (♀)	1.15	2.31	4.00	8.00	10.31	0.473	0.88	1.50	2.78	3.66	13.97
II (♂)	0.560	1.12	1.09	2.17	3.29	0.473	0.88	1.08	2.02	2.90	6.19
III (♂)	0.235	0.47	0.735	1.47	1.94	0.208	0.38	0.293	0.55	0.93	2.87
Average		1.30		3.88	5.18		0.71		1.78	2.49	7.67

Table VI. The Fecal Metabolites of 1-Hydroxyanthraquinone

Expt. No.	1-Hydroxyanthraquinone					Alizarin					Total (% of dose)
	Free		Conjugated		Total (% of dose)	Free		Conjugated		Total (% of dose)	
	(mg.)	(% of dose)	(mg.)	(% of dose)		(mg.)	(% of dose)	(mg.)	(% of dose)		
I (♀)	7.68	15.37	—	—	15.37	—	—	0.340	0.63	0.63	16.00
II (♂)	5.21	10.41	2.18	4.36	14.77	—	—	0.744	1.39	1.39	16.16
III (♂)	4.50	9.00	—	—	9.00	0.101	0.19	0.110	0.20	0.39	9.39
Average		11.59		1.45	13.04		0.06		0.74	0.80	13.84

Table VII. The Urinary Metabolites of 2-Hydroxyanthraquinone

Expt. No.	2-Hydroxyanthraquinone					Alizarin					Total (% of dose)
	Free		Conjugated		Total (% of dose)	Free		Conjugated		Total (% of dose)	
	(mg.)	(% of dose)	(mg.)	(% of dose)		(mg.)	(% of dose)	(mg.)	(% of dose)		
I (♂)	1.13	2.25	1.57	3.14	5.39	—	—	0.010	0.02	0.02	5.41
II (♂)	1.90	3.80	1.81	3.60	7.40	0.289	0.54	—	—	0.54	7.94
III (♂)	1.05	2.11	1.40	2.79	4.90	0.325	0.59	0.493	0.92	1.51	6.41
Average		2.72		3.17	5.89		0.38		0.31	0.69	6.58

Table VIII. The Fecal Metabolites of 2-Hydroxyanthraquinone

Expt. No.	2-Hydroxyanthraquinone					Alizarin					Total (% of dose)
	Free		Conjugated		Total (% of dose)	Free		Conjugated		Total (% of dose)	
	(mg.)	(% of dose)	(mg.)	(% of dose)		(mg.)	(% of dose)	(mg.)	(% of dose)		
I (♂)	18.64	37.28	5.47	10.93	48.21	—	—	0.325	0.59	0.59	48.80
II (♂)	17.65	35.32	0.975	1.95	37.27	—	—	—	—	—	37.27
III (♂)	20.96	41.92	2.23	4.46	46.38	—	—	—	—	—	46.38
Average		38.17		5.78	43.95				0.20	0.20	44.15

From the results shown in the above Tables it is quite clear that 1-hydroxy- and 2-hydroxyanthraquinones were converted *in vivo* into alizarin which would be mainly excreted as an end-product in conjugation with glucuronic and sulfuric acids.

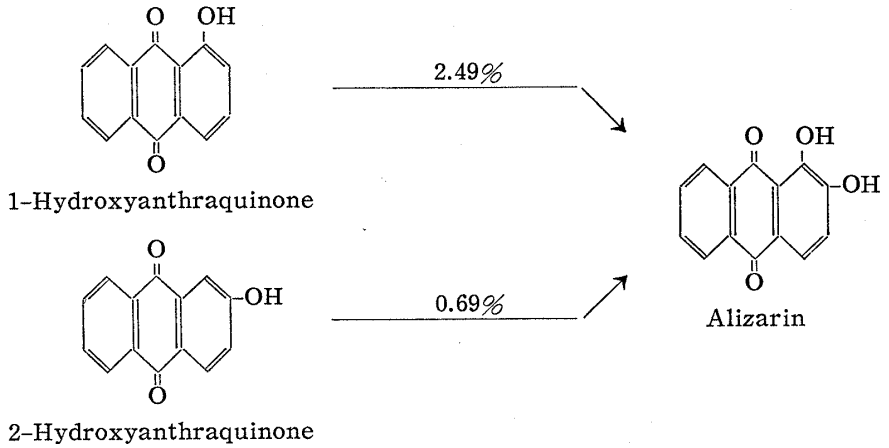

Chart 1. Metabolic Process of 1-Hydroxy- and 2-Hydroxyanthraquinones

1-Hydroxyanthraquinone was excreted in urine by the ratio of unchanged form 1.30%, conjugated form 3.88%, free alizarin 0.71% and conjugated alizarin 1.78% (Table V). In the case of urinary metabolites of 2-hydroxyanthraquinone it was determined as follows:

Unchanged form 2.72%, conjugated form 3.17%, free alizarin 0.38%, and conjugated alizarin 0.31% (Table Ⅶ). While, 13.04% of the 1-hydroxyanthraquinone fed and 43.95% of the 2-hydroxyanthraquinone fed can be accounted for as each of unchanged forms excreted in feces (Tables Ⅵ and Ⅷ).

The authors would like to propose now that the biological hydroxylation rate of both the hydroxyanthraquinones to alizarin should be designated by the following comparative indices :

$$\text{Hydroxylation index of 1-hydroxyanthraquinone} = \frac{A}{100-\text{1-hydroxyanthraquinone excreted in feces (free, \% of dose)}} = \frac{2.49+0.80}{100-13.04} = 0.038$$

$$\text{Hydroxylation index of 2-hydroxyanthraquinone} = \frac{A}{100-\text{2-hydroxyanthraquinone excreted in feces (free, \% of dose)}} = \frac{0.69+0.20}{100-43.95} = 0.016$$

(A : Alizarin excreted in urine and feces (free+conjugated, % of dose))

From the numbers of those hydroxylation indices it is assumed that 1-hydroxyanthraquinone would be hydroxylated to alizarin *in vivo* at about twice the rate as that of 2-hydroxyanthraquinone.

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Summary

The metabolic fate of 1-hydroxy- and 2-hydroxyanthraquinone in rat has been elucidated. Both the compounds were mainly hydroxylated to alizarin. Appearance of two or more hydroxylated anthraquinones was not detected. The urinary and fecal metabolites were determined directly by a densitometer.

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