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Mitiiti Fujita,*2 Tsutomu Furuya,*2 and Mitsuyoshi Matsuo*3 : Studies on the Metabolism of Naturally Occurring Anthraquinones. II.*1
The Metabolism of 1-Methoxyanthraquinone and 2-Methoxyanthraquinone.

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Following the preceding report on the metabolism of 1-hydroxy- and 2-hydroxyanthraquinones, studies were extended to their methyl ethers, 1-methoxy- and 2-methoxyanthraquinones. Each of their urinary and fecal metabolites was examined by paper chromatography and mechanism of their demethylation was discussed.

Experimental

Material——1-Methoxyanthraquinone, m.p. 169°, was prepared from 1-chloroanthraquinone, and 2-methoxyanthraquinone, m.p. 195~196°, was synthesized from 2-hydroxyanthraquinone.

Animal, Diet and Dosage——Rats were kept on the same constant diet as described in the previous paper,*1 and 25 mg. each of compounds was administered as an aqueous emulsion by stomach tube.

Qualitative Examination of Urine——The free and conjugated fractions were examined by the same technique as that for 1-hydroxyanthraquinone, described in the previous paper, and 2-hydroxyanthraquinone, therefrom 1-hydroxy- and 2-hydroxyanthraquinones as the demethylated substances, alizarin as the hydroxylate, and the unchanged forms were detected in the urinary and fecal excretes of the original compounds as shown in Tables I and II.

Table I. Identification of Metabolites of 1-Methoxyanthraquinone by Paper Chromatography

Compound	Urine		Feces	
	Free	Conjugated	Free	Conjugated
1-Methoxyanthraquinone (unchanged)	−	−	+	−
1-Hydroxyanthraquinone	+	+	(+)	(+)
Alizarin	+	+	(+)	(+)

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

Table II. Identification of Metabolites of 2-Methoxyanthraquinone by Paper Chromatography

Compound	Urine		Feces	
	Free	Conjugated	Free	Conjugated
2-Methoxyanthraquinone (unchanged)	−	−	+	−
2-Hydroxyanthraquinone	+	+	+	−
Alizarin	+	+	(+)	(+)

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

Determination of Metabolites——The method was essentially same as described in the previous paper.*1 After adjusting the urine to pH 2 with 10% H_2SO_4, the free fraction was obtained by continuous extraction with Et_2O.

Extraction of the residual urine after hydrolysis with 10% H_2SO_4 gave the conjugated fraction. Each of the free and conjugated fractions of 1-hydroxy-, 2-hydroxyanthraquinones and alizarin was determined by paper chromatography. The following method was employed for the determination of 1-methoxy- and 2-methoxyanthraquinones.

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The chromatogram was developed with the MeOH-saturd. petr. benzine and then the fluorescent spots corresponding to anthraquinone derivatives were cut out, and the paper strips were eluted with EtOH for 5 min. on a boiling water bath. After cool, it was adjusted to 10 cc., and then read at 253 mμ (1-methoxyanthraquinone) or 268 mμ (2-methoxyanthraquinone) by using Cary Model-11 Recording Spectrophotometer.

Calibration curves of standard 1-methoxy- and 2-methoxyanthraquinones are shown in Fig. 1.

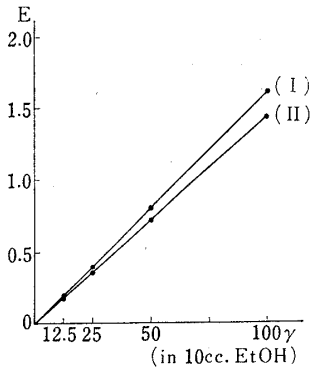

Fig. 1. Calibration Curves of 1-Methoxy- and 2-Methoxyanthraquinone

I : 1-Methoxyanthraquinone (λ_{max} 253 mμ)

II : 2-Methoxyanthraquinone (λ_{max} 268 mμ)

The recovery rate of all the compounds is approximately 99.00~101.21% from three experiments.

Results and Discussion

The 48 hours urinary and fecal metabolites of 25 mg. each of two original methoxyanthraquinones after oral administration were determined, and the results obtained are summarized in Tables III, IV, and V.

Table III. The Urinary and Fecal Metabolites of 1-Methoxyanthraquinone

Expt. No.	Urine: 1-Hydroxyanthraquinone Free (mg.)	Free (% of dose)	Conjugated (mg.)	Conjugated (% of dose)	Total (% of dose)	Urine: Alizarin Free (mg.)	Free (% of dose)	Conjugated (mg.)	Conjugated (% of dose)	Total (% of dose)	Total (% of dose)	Feces: 1-Methoxyanthraquinone Free (mg.)	Free (% of dose)
I (♀)	0.320	1.36	0.692	2.94	4.30	0.330	1.31	1.515	6.01	7.32	11.62	undetermined	
II (♂)	0.125	0.53	0.585	2.49	3.02	0.355	1.41	1.445	5.79	7.20	10.22	4.027	16.11
III (♂)	0.130	0.55	0.390	1.66	2.21	0.420	1.67	1.552	6.16	7.45	10.04	2.700	10.80
Average	0.192	0.81	0.556	2.36	3.17	0.368	1.46	1.504	5.99	7.45	10.62	3.364	13.46

Table IV. The Urinary Metabolites of 2-Methoxyanthraquinone

Expt. No.	2-Hydroxyanthraquinone Free (mg.)	Free (% of dose)	Conjugated (mg.)	Conjugated (% of dose)	Total (% of dose)	Alizarin Free (mg.)	Free (% of dose)	Conjugated (mg.)	Conjugated (% of dose)	Total (% of dose)	Total (% of dose)
I (♂)	0.833	3.54	1.338	5.69	9.23	0.298	1.18	0.440	1.55	2.73	11.96
II (♂)	0.523	2.22	0.588	2.50	4.72	0.220	0.87	0.230	0.91	1.78	6.50
III (♂)	0.925	3.93	1.538	6.54	10.47	0.565	2.24	0.698	2.77	5.01	15.48
Average	0.760	3.23	1.155	4.91	8.14	0.341	1.43	0.456	1.75	3.18	11.32

It has been found that 2-methoxyanthraquinone is demethylated in rat in accordance with the general rule for the metabolism of aromatic ethers, giving 4.91% of 2-hydroxyanthraquinone, and 1.75% of alizarin as conjugated forms in the urine, which appears probably to be excreted as the glucuronide and also ethereal sulfate (Table IV).

Table V. The Fecal Metabolites of 2-Methoxyanthraquinone

Expt. No.	2-Methoxyanthraquinone Free		2-Hydroxyanthraquinone Free		Total (% of dose)
	(mg.)	(% of dose)	(mg.)	(% of dose)	
I (♂)	6.302	25.21	1.563	6.64	31.85
II (♂)	8.297	33.19	2.075	8.82	42.01
III (♂)	5.807	23.23	1.193	5.07	28.30
Average	6.802	27.21	1.610	5.85	33.06

While, it is notable in the case of 1-methoxyanthraquinone that it splits to 1-hydroxyanthraquinone (3.17% of dose) with the loss of a methyl group at the ether link and undergoes finally hydroxylation to alizarin (7.45%) (Table III). Biotransformation of these compounds is shown in Chart 1.

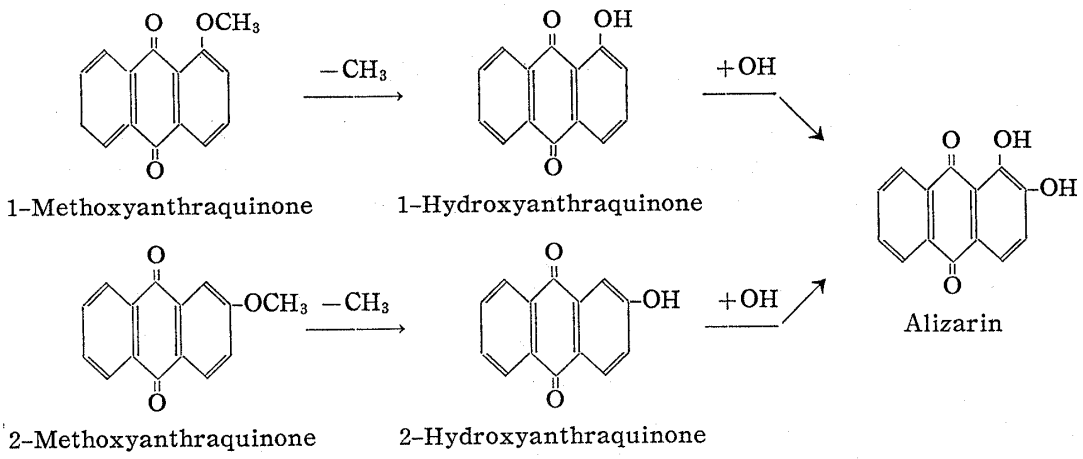

Chart 1. Metabolic Process of 1-Methoxy- and 2-Methoxyanthraquinones

It is known that hydroxylation of the aromatic ring usually takes place at *para* position, when it is free, to the ether linkage. The authors, however, could not detect 1-methoxy-4-hydroxyanthraquinone as a metabolite of 1-methoxyanthraquinone. Therefore, it seems that the latter was not hydroxylated at *para* position *in vivo*, and would be hydroxylated at *ortho* position after demethylation.

From the experimental results it might be possible to assume that the direct oxidation or methanol splits after hydration of aromatic compounds should be occurred on the way of process of their biological dealkylation.

In accordance with the same procedure as described in the preceding paper, the biological demethylation and hydroxylation indices of 1-methoxy- and 2-methoxyanthraquinones are calculated as follows:

$$\text{Demethylation index of 1-methoxyanthraquinone} = \frac{A}{100-\text{1-methoxyanthraquinone excreted in feces (\% of dose)}}$$

$$= \frac{3.17+7.45}{100-13.46} = 0.12$$

$$\text{Demetylation index of 2-methoxyanthraquinone} = \frac{B}{100-\text{2-methoxyanthraquinone excreted in feces (\% of dose)}}$$

$$= \frac{13.99+3.18}{100-27.21} = 0.24$$

Hydroxylation index of 1-methoxyanthraquinone

$$= \frac{C}{100-\text{1-methoxyanthraquinone excreted in feces (\% of dose)}}$$

$$= \frac{7.45}{100-13.46} = 0.086$$

Hydroxylation index of 2-methoxyanthraquinone

$$= \frac{C}{100-\text{2-methoxyanthraquinone excreted in feces (\% of dose)}}$$

$$= \frac{3.18}{100-27.21} = 0.044$$

A: Alizarin and 1-hydroxyanthraquinone excreted in urine (free+conjugated, % of dose).
B: Alizarin and 2-hydroxyanthraquinone excreted in urine and feces (free+conjugated, % of dose).
C: Alizarin excreted in urine (free+conjugated, % of dose).

It is conjectured from those indices that 2-methoxyanthraquinone is easily demethylated at the double rate of 1-methoxyanthraquinone in opposition to usual chemical reaction, and the hydroxylation of the latter compound would be occurred at double speed as easily as that of the former.

The authors wish to express their thanks to Miss H. Ueda for her cooperation in a part of this work.

Summary

Each of 1-methoxy- and 2-methoxyanthraquinones was demethylated in rats to be metabolized to the 1-hydroxy compound and they were finally hydroxylated to alizarin. Every form in conjugation of their metabolites was determined using a densitometer and an ultraviolet spectrophotometer.

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150. Noboru Shigematsu: Studies on the Synthetic Analgesics. XVI.[1] Synthesis of 1-(2-*tert*-Aminoalkyl)-3,4-dihydrocarbostyrils.

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Acetanilide (I: R′=H) and acetophenetidine (I: R′=EtO) have been regarded as a nonnarcotic antipyretic-analgesic for a long time. Boréus[2] recently reported that 4′-hydroxyacetanilide (I: R′=OH) showed the same antipyretic-analgesic action as acetophenetidine with less toxicity and diminished degree of methohemoglobin formation.

It is also noted that Wright[3] reported strong analgesic effect of a new series of propionanilide derivatives (II), the structure of which had likely been hinted by those of Methadone and Isomethadone.

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