overnight, the mixture was poured into ice-water, a syrupy product was produced, which was solidified on standing for several hours. This was filtered off and washed with water, and air dried; yielding was 14.1 g. in 60% of theory. After several recrystallizations from $CHCl_3$-EtOH, it melted at 201~202°; $[\alpha]_D^{20}$ −35.6°(c=0.92, $CHCl_3$), reported, m.p. 194°,[25] 199~200°,[26] 202~204°[8]; $[\alpha]_D$ −36.4°.[8] *Anal.* Calcd. for $C_{27}H_{22}O_8$: C, 68.35; H, 4.67. Found : C, 68.06; H, 4.66.

1,6-Anhydro-β-D-glucopyranose (II)——A solution of 5.6 g. of 1,6-anhydro-2,3,4-tri-O-acetyl-β-D-glucose in 50 cc. of 0.01*N* MeONa in MeOH was allowed to stand in refrigerator overnight. After a removal of the solvent, the residues were recrystallized from AcOEt to afford 2.8 g. of (II), m.p. 178°; $[\alpha]_D^{20}$ −66°(c=0.9, H_2O), reported,[25] m.p. 178° and $[\alpha]_D$ −66.5° in water. *Anal.* Calcd. for $C_6H_{10}O_5$: C, 44.44; H, 6.22. Found : C, 44.52; H, 6.32.

Phenylosazone of 3,6-anhydro-D-glucose——10 g. of 6-deoxy-6-iodo-1,2,3,4-tetra-O-acetyl-α-D-glucopyranose (IV)[23] was treated with 3.5 g, of MeONa (1.5 g. of Na in 30 cc. of MeOH) in the same manner described in (I). As compared with β-anomer, the reaction mixture was deepen in its color. After being kept standing overnight in refrigerator, the mixture was neutralized with AcOH and the solvent was removed under reduced pressure. The residues dissolved in water were passed through a column of Amberlite IR-120(H^+) and then a column of Amberlite IR-45(OH^-) successively. The effluent was treated with charcoal and concentrated under reduced pressure to slightly colored syrup, which reduced the Fehling's solution, yielding was 3 g. 1.0 g. of syrup, 2.0 g. of freshly distilled phenylhydrazine and 2 cc. of 50% AcOH were dissolved in 30 cc. of water and heated on a steam-bath for 30 min. After cooling, yellow precipitates were filtered off and washed with a small amount of cold water. Recrystallization from EtOH gave yellow needles, m.p. 190°(decomp.); $[\alpha]_D^{20}$ −84.4~−37.5°(after 24 hr.)(c=1.28, pyridine) reported[27] m.p. 187~188° and $[\alpha]_D$ −146° in MeOH. *Anal.* Calcd. for $C_{18}H_{20}O_3N_4$: C, 63.51; H, 16.46. Found : C, 63.66; H, 5.86; N, 16.55.

Thanks are expressed to Mr. K. Narita for performing microchemical analysis.

Summary

1,6-Anhydro-β-D-glucopyranose was synthesized from 6-O-*p*-toluenesulfonyl-1,2,3,4-tetra-O-acetyl-β-D-glucopyranose by a treatment with sodium methoxide. Since 1,6-anhydro-β-D-glucose is not obtainable from 6-deoxy-6-iodo-1,2,3,4-tetra-O-acetyl-α-D-glucopyranose, this procedure is applicable to distinguish α,β-anomers chemically.

Some conformational discussions are made.

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27) H. El. Khadem, E. Schreier, G. Toehr, E. Hardegger : Helv. Chim. Acta., **35**, 232 (1952).

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144. Mitiiti Fujita*1, Tsutomu Furuya*1, and Mitsuyoshi Matsuo*2 : Studies on the Metabolism of Naturally Occurring Anthraquinones. III.[1] The Metabolism of Alizarin Dimethyl Ether.

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The authors were interested in biological demethylation of anthraquinone derivatives, especially on the difference of behavior to demethylation between their 1-methoxyl and 2-methoxyl groups.

From this point of view, urinary metabolites of alizarin dimethyl ether were studied qualitatively and quantitatively in this paper. And as a reference, the metabolism of

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1) Part II : This Bulletin, **9**, 967 (1961).

alizarin and its 1-methyl ether which are present in Rubiaceous plants.[2] have also been reported.

Experimental

Materials——Alizarin dimethyl ether, m.p. 215°, alizarin 1-methyl ether, m.p. 178~179°, and alizarin 2-methyl ether, m.p. 230~231° were synthesized, and alizarin, m.p. 289~290° was purchased.

Animal, Diet and Dosage——Rats (150 g., body wt.) kept on a constant diet as previously described[3] were used, and 25 mg. of the compounds were administered in aqueous emulsion by a stomach tube.

Identification of Metabolites by Paper Chromatography——The 48 hr. urine after oral administration of alizarin dimethyl ether was acidified with 10% H_2SO_4 and extracted continuously with Et_2O, then the solvent was removed and residue was dissolved in 5 cc. of tetrahydrofuran (UF fraction). The residual urine was hydrolysed and treated as mentioned above to obtain the Et_2O extract (UC fraction). The UF and UC fractions were examined by paper chromatography using the developing solvents given in the preceding paper.[3] The result obtained is shown in Table I.

Table I. Identification of Urinary Metabolites by Paper Chromatography

Compound	UF	UC
Alizarin 1-methyl ether	+	+
Alizarin 2-methyl ether	−	−
Alizarin	+	+
Alizarin dimethyl ether (unchanged)	trace	−

+ : present; − : absent.

Determination of Metabolites——Alizarin 1-methyl ether was determined directly on the paper chromatogram by densitometer (Model Kobayashi).

Color Reagent——0.5% Na_2CO_3 solution,

Standard Solution——10 mg. of Alizarin 1-methyl ether was dissolved in 5 cc. of tetrahydrofuran.

Regression Equation between Concentration and Extinction of Alizarin 1-Methyl Ether——Standard solution of alizarin 1-methyl ether was spotted on the starting line of a paper strip (Tōyō Roshi NO. 51, 2.9×40 cm.). The chromatogram developed with the organic phase of BuOH-benzene-$(NH_4)_2CO_3$ buffer (80:5:15) was immersed in 0.5% Na_2CO_3 solution for a moment, air-dried for 1 hr., and then dipped into 30% Et_2O solution of liquid paraffin to make the paper translucent. After a lapse of 1 hr., the color density was read by the densitometer using a 2 mm. slit and a blue filter(460 mμ). Regression equation calculated from $n=19$ by the least square method is as follows :

$$y=0.0489x-0.0460$$

or $$x=20.50y+0.941 \qquad \sigma=0.1099$$

x=Concentration of alizarin 1-methyl ether (γ).
y=Sum of color density.
σ=Standard deviation.

The recovery of alizarin 1-methyl ether from the normal urine is listed in Table II.

Table II. Recovery of Alizarin 1-Methyl Ether

Amount added to normal urine (γ)	Found (γ)	Recovery (%)	Amount added to normal urine (γ)	Found (γ)	Recovery (%)
10.1	10.80	106.9	20.2	21.40	105.9
10.1	10.23	101.9	20.2	20.05	99.3

Results and Discussion

The result of analysis of the metabolites in the 48 hours urine after administration of 25 mg. of alizarin dimethyl ether are shown in Table III.

2) R.H. Thomson : Naturally Occurring Quinones, 162 (1957).
3) M. Fujita, T. Furuya, M. Matsuo : This Bulletin, **9**, 962 (1961).

Table III. The Excretion of Free and Conjugated Alizarin 1-Methyl Ether by Rats receiving Alizarin Dimethyl Ether orally

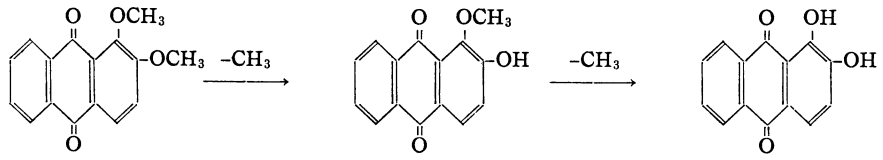
Expt. NO.	Sex	Free		Conjugated		Total
		(mg.)	(% of Dose)	(mg.)	(% of Dose)	(% of Dose)
I	♂	0.181	0.77	0.155	0.65	1.42
II	♂	0.192	0.82	0.161	0.68	1.50
III	♂	0.133	0.56	0.273	1.15	1.61
Average		0.169	0.72	0.196	0.82	1.54

The quantitative analysis revealed that 1.54% of dose was recovered as alizarin 1-methyl ether which was the principal urinary metabolite. The minor metabolites were alizarin and unchanged alizarin dimethyl ether which could not be determined being present in small amounts, but alizarin 2-methyl ether was not detected by paper chromatography.

It has been reported in the previous paper[2,3] that 2-methoxyl group of anthraquinone was easily demethylated than 1-methoxyl group. Therefore, the fact that alizarin 1-methyl ether was solely found as the monomethyl derivative in urinary metabolites, suggests the easiness of demethylation of 2-methoxyl group than 1-methoxyl *in vivo* and it is different from the chemical demethylation.

Furthermore, each 25 mg. of alizarin and its 1-methyl ether were orally administered in rats. Unchanged alizarin was detected by paper chromatography as major urinary metabolites of the former, and other unknown spots were also found. In the case of thelatter, alizarin was solely detected as biological demethylated products.

The authors propose that these metabolic products might be derived from a pathway shown in Chart 1.



Alizarin dimethyl ether — Alizarin 1-methyl ether — Alizarin

Chart 1. Proposed Scheme for the Metabolic Transformation of Alizarin Dimethyl Ether

The authors express their gratitude to Miss K. Ito for her kind cooperation.

Summary

Studies on the metabolic fate of alizarin dimethyl ether in rat has been elucidated. It was mainly demethylated to alizarin 1-methyl ether and alizarin, and a trace of unchanged alizarin dimethyl ether was obtained. The mechanism of biological demethylation at carbon 1 and 2-positions of alizarin dimethyl ether is found to be different from that of chemical reaction. Metabolic products derived from alizarin and its 1-methyl ether were also detected by paper chromatography.

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