

Chemical Studies on the Oriental Plant Drugs. XXI.<sup>1)</sup>  
The Constituents of *Cassia tora* L. (2).  
A Glycoside of Rubrofusarin

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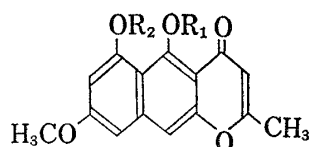
A new rubrofusarin glycoside was isolated from the seeds of *Cassia tora* L. (Leguminosae) and its structure was established as rubrofusarin-6- $\beta$ -gentiobioside (III).

Rubrofusarin (I) was isolated from the seeds of *Cassia tora* L. by Rangaswami<sup>3)</sup> and Kimura, *et al.*<sup>4)</sup> We also obtained rubrofusarin from the benzene extracts of the seeds while we found that rubrofusarin was also isolated from the acid hydrolysate of methanolic extracts of the seeds which were extracted once with benzene. This suggests that rubrofusarin is present in the seeds as a form of glycoside, along with a free aglycone. The present paper concerns with the isolation and the structural determination of the glycoside of rubrofusarin.

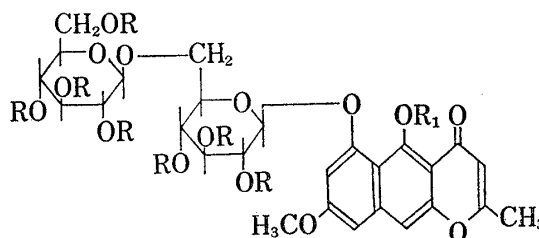
The crude methanolic extracts of *Cassia tora* L. was acetylated with acetic anhydride and pyridine at room temperature, and the mixture of acetates was chromatographed on silica gel to separate yellow needles, C<sub>41</sub>H<sub>46</sub>O<sub>22</sub> (II), mp 245—246°,  $[\alpha]_D^{25}$  -78.8° (CHCl<sub>3</sub>). The UV spectrum of II resembled that of I and its derivatives, in particular, 6-O-methyl-rubrofusarin (IV) to suggest that II is an acetate of glycoside of I (Table I). On deacetylation

TABLE I. UV Spectra of Rubrofusarin Derivatives and Rubrofusarin  
Glycoside  $\lambda_{\max}^{\text{EtOH}}$  m $\mu$  (log  $\epsilon$ )

II	223 (4.46), 255 (inflex.) (4.46), 276 (4.71), 322 (3.49), 395 (3.79)
IV	225 (4.44), 254 (inflex.) (4.43), 275 (4.69), 322 (3.51), 395 (3.81)
VI	224 (4.41), 252 (inflex.) (4.46), 270 (4.66), 325 (3.65), 341 (3.72), 368 (3.77)
VIII	226 (4.47), 252 (inflex.) (4.46), 271 (4.67), 326 (3.53), 342 (3.62), 373 (3.78)



I : R<sub>1</sub>=R<sub>2</sub>=H  
IV : R<sub>1</sub>=H, R<sub>2</sub>=CH<sub>3</sub>  
V : R<sub>1</sub>=CH<sub>3</sub>, R<sub>2</sub>=H  
VII : R<sub>1</sub>=R<sub>2</sub>=CH<sub>3</sub>



II : R<sub>1</sub>=H, R=Ac  
III : R<sub>1</sub>=R=H  
VI : R<sub>1</sub>=CH<sub>3</sub>, R=Ac  
VIII : R<sub>1</sub>=R=CH<sub>3</sub>

Chart 1

- 1) Part XX: S. Shibata, E. Morishita, M. Kaneda, Y. Kimura, M. Takido and S. Takahashi, *Chem. Pharm. Bull.* (Tokyo), 17, 454 (1969).
- 2) Location: Hongo, Bunkyo-ku, Tokyo; a) Present Address: Research Laboratory of Takasago Kōryō Co. Ltd.
- 3) S. Rangaswami, *Proc. Indian Acad. Sci.*, A 57, 88 (1963).
- 4) Y. Kimura, M. Takido and S. Takahashi, *Shoyakugaku Zasshi*, 18, 28 (1964).

of II with sodium methoxide at room temperature, pale yellow needles,  $C_{27}H_{32}O_{15} \cdot 2H_2O$  (III), mp 186—188°,  $[\alpha]_D^{25} -62.5^\circ$ , was yielded.

The acid hydrolysis of III afforded, along with D-glucose, a red crystalline compound which was identified to be rubrofusarin. D-Glucose was proved by paper and thin-layer chromatography. The NMR spectrum of II (Fig. 1) was compared with those of IV and V to find that a singlet (1H) at  $\tau -4.58$  is assigned to OH at C(5) which is chelated with carbonyl of the  $\gamma$ -pyrone system. Consequently, the sugar moiety is linked with the hydroxyl at C(6) (Table II).

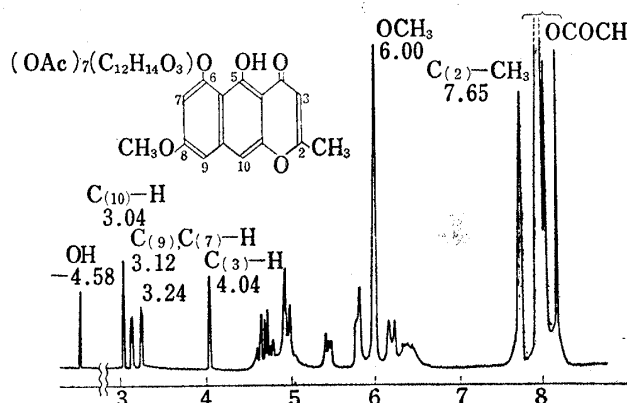


Fig. 1. NMR Spectrum of II ( $\tau$  in  $CDCl_3$ )

TABLE II. The NMR Spectra of 6-O-Methylrubrofusarin (IV) and 5-O-Methylrubrofusarin (V) ( $\tau$  in  $CDCl_3$ )

	$C_{(10)}-H$	$C_{(9)}-H$ , $C_{(7)}-H$	$C_{(3)}-H$	$OCH_3$	$C_{(2)}-CH_3$	OH
IV	3.10	3.46 (d), 3.64 (d)	4.04	6.03, 6.11	7.66	-4.95
V	2.64	3.40 (d), 3.48 (d)	4.18	5.87, 6.11	7.66	0.05

Methylation of II with dimethyl sulphate and potassium carbonate in dry acetone afforded  $C_{42}H_{48}O_{22}$  (VI), mp 136—137°,  $[\alpha]_D^{25} -45.5^\circ$ , which was hydrolyzed with acid to yield V. The position linking sugar moiety was thus proved chemically to be C(6)-OH.

The sugar moiety of the glycoside was proved to be a disaccharide as seven acetyl signals were observed in the NMR spectrum of II and VI. The molecular weight determination of VI by osmometry also agreed with the formula of the glycoside having a glucosyl glucoside moiety. The permethyl ether (VII) prepared by the methylation of III with methyl iodide and silver oxide in dimethylformamide (Kuhn's method)<sup>5</sup> afforded on methanolysis with 5% methanolic hydrogen chloride methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 2,3,4-tri-O-methyl-D-glucopyranoside in almost equal amount.

The enzymatic hydrolysis with emulsin gave I and D-glucose to prove the  $\beta$ -linkage between two glucose moieties and also that of the sugar moiety with the aglycone. The structure of the rubrofusarin glycoside of *Cassia tora* has now been represented by 6-O-(6-O- $\beta$ -D-glucopyranosyl- $\beta$ -D-glucopyranosyl) rubrofusarin (=rubrofusarin-6- $\beta$ -gentiobioside).

### Experimental

**Extraction**—The pulverized seeds of *Cassia tora* L. (2 kg) were first treated with *n*-hexane to remove fat and oil, then extracted with boiling benzene, and finally four times with hot MeOH to give a dark brown syrup (ca. 170 g).

**Rubrofusarin Gentiobioside Acetate (II)**—MeOH extract (22 g) was dissolved in dry pyridine (80 ml), and  $Ac_2O$  (70 ml) was added under ice cooling. The mixture, after standing overnight at room temperature, was poured into ice water, and then extracted with  $CHCl_3$ . The  $CHCl_3$  solution was washed with water, dried, and evaporated to dryness. The residual dark brown syrup (27 g) was chromatographed on silica-gel using a mixture of benzene and acetone (4:1) as the solvent. The fourth band from the bottom which gave a dark green fluorescence under UV illumination was eluted and evaporated. The residue obtained was purified by fractional crystallization to give yellow needles (II), ca. 1 g, mp 245—246°,  $[\alpha]_D^{25} -78.8^\circ$  ( $c=0.60$ ,  $CHCl_3$ ). *Anal.* Calcd. for  $C_{41}H_{46}O_{22}$ : C, 55.33; H, 5.21.

6) R. Kuhn and H. Trischmann, *Angew. Chem.*, **67**, 32 (1955).

Found: C, 55.16; H, 5.42. UV  $\lambda_{\text{max}}^{\text{EtOH}}$  m $\mu$  (log  $\epsilon$ ): 223 (4.46), 255 (infl.) (4.46), 276 (4.71), 322 (3.49), 395 (3.79). IR  $\nu_{\text{max}}^{\text{CHCl}_3}$  cm $^{-1}$ : no OH, 1760 (OAc), 1652 ( $\gamma$ -pyrone C=O), 1627, 1587 (aromatic).

**Rubrofusarin Gentiobioside (III) (Deacetylation of II)**—The compound (II) (170 mg) was suspended in dry MeOH (30 ml) to which was added 2 ml of a freshly prepared solution of NaOMe (0.5 g of Na dissolved in 100 ml of MeOH). The suspension was stirred at room temperature for 3 hr. When Na ion was removed by stirring with a slight excess of ion exchange resin (Amberlite IR-120 (H $^{+}$ )), the crude glycoside (III) began to separate as pale yellow solid. Recrystallization from MeOH gave pale yellow needles (97 mg), mp 186–188°,  $[\alpha]_D^{25}$   $-62.5^\circ$  ( $c=0.16$ , pyridine). Anal. Calcd. for  $\text{C}_{27}\text{H}_{32}\text{O}_{15} \cdot 2\text{H}_2\text{O}$ : C, 51.31; H, 5.74. Found: (on a sample dried *in vacuo* at 110° for 10 hr): C, 51.04; H, 5.70. UV  $\lambda_{\text{max}}^{\text{EtOH}}$  m $\mu$  (log  $\epsilon$ ): 223 (4.34), 255 (infl.) (4.32), 277 (4.61), 323 (3.31), 399 (3.73). IR  $\lambda_{\text{max}}^{\text{KBr}}$  cm $^{-1}$ : 3400 (broad, OH), 1660 ( $\gamma$ -pyrone C=O), 1623, 1587 (arom.).

**Acid Hydrolysis of III**—The solution of III (100 mg) in 7%  $\text{H}_2\text{SO}_4$  (20 ml) was heated on a boiling water bath for 5 hr. The red precipitate separated was collected with  $\text{CHCl}_3$ , and the  $\text{CHCl}_3$  solution was washed with water, dried, and evaporated to dryness. The residue was recrystallized from EtOH to give orange red needles of rubrofusarin (I) (42 mg), mp 210°. It was identified with the authentic sample of rubrofusarin by a mixed fusion (mixed mp 210°) and comparison of IR spectra (KBr) and thin-layer chromatograms.

The water layer was neutralized by stirring with ion exchange resin (Amberlite IR-4B (OH $^{-}$ )) and evaporated to dryness. The paper chromatography of this residue (solvent: BuOH–pyridine– $\text{H}_2\text{O}$  (6:4:3). Colour reagent: Aniline hydrogen phthalate) showed the presence of D-glucose only. It was also confirmed by thin-layer chromatography (microcrystalline cellulose powder, solvent: pyridine–AcOEt–AcOH– $\text{H}_2\text{O}$  (5:5:1:3). Colour reagent: aniline hydrogen phthalate).

**Methyl Ether of Rubrofusarin Gentiobioside Acetate (VI)**—A mixture of II (200 mg), anhydrous  $\text{K}_2\text{CO}_3$  (500 mg),  $\text{Me}_2\text{SO}_4$  (0.5 ml) and dry acetone (25 ml) was refluxed for 3 hr under stirring. The product obtained by the usual treatment was purified by chromatography on silicagel column using a mixture of benzene and acetone (4:1) as the solvent. The band giving yellowish green fluorescence under UV-illumination was eluted and recrystallized from 50% aqueous MeOH to form colourless plates (142 mg). mp 136–137°,  $[\alpha]_D^{25}$   $-45.5^\circ$  ( $c=1.0$ ,  $\text{CHCl}_3$ ). UV  $\lambda_{\text{max}}^{\text{EtOH}}$  m $\mu$  (log  $\epsilon$ ): 224 (4.41), 252 (4.46), 270 (4.66), 325 (3.65), 341 (3.72), 368 (3.77). IR  $\nu_{\text{max}}^{\text{CHCl}_3}$  cm $^{-1}$ : 1758 (OAc), 1654 ( $\gamma$ -pyrone C=O), 1625, 1612 (sh.), 1561 (arom.). Anal. Calcd. for  $\text{C}_{42}\text{H}_{48}\text{O}_{22}$ : C, 55.80; H, 5.35; mol. wt. 904. Found: C, 55.72; H, 5.55; mol. wt. 911 (vapour pressure method, in benzene).

**Hydrolysis of VI**—The compound (VI) (55 mg) was refluxed with 7%  $\text{H}_2\text{SO}_4$  (10 ml) and EtOH (5 ml) for 3 hr. The reaction mixture was diluted with water and extracted with  $\text{CHCl}_3$ . The  $\text{CHCl}_3$  solution was washed with water, dried, evaporated, and chromatographed on silica gel using a mixture of benzene–acetone (4:1) as the solvent. The yellow fluorescent band was eluted and recrystallized from EtOH to give pale yellow needles (10 mg), mp 207–208°. This product was identified with the authentic sample of 5-O-methylrubrofusarin (V) by a mixed fusion (the mixed mp 207–208°) and comparison of IR spectra and thin-layer chromatograms.

**Permethyl Ether of Rubrofusarin Gentiobioside (VII)**—To a solution of III (100 mg) and  $\text{CH}_3\text{I}$  (2 ml) in dimethylformamide (8 ml) was added dry, freshly prepared  $\text{Ag}_2\text{O}$  (500 mg) in an equal portions at some intervals, and the mixture was stirred at room temperature.

After 24 hr the mixture was filtered and a KCN solution (5%) was added to the filtrate, which was extracted with  $\text{CHCl}_3$ . The  $\text{CHCl}_3$  solution was washed with water, dried, and evaporated to dryness. The residue was chromatographed on silica gel using benzene–acetone mixture (4:1) as the solvent, and the second, greenish yellow fluorescent band from the bottom was eluted, and the evaporation of the solvent gave an almost colourless syrup, which gave one spot on a thin-layer chromatogram. IR  $\nu_{\text{max}}^{\text{CCl}_4}$  cm $^{-1}$ : no OH, 1660 (C=O), 1623, 1562 (arom.).

The above methyl ether (VII) (30 mg) was heated with 5% methanolic HCl in a sealed tube in a boiling water bath for 7 hr. The reaction mixture was diluted with water, and extracted with  $\text{CHCl}_3$ . The  $\text{CHCl}_3$  solution was washed with water, dried, and concentrated. The presence of equimolecular methyl 2,3,4,6-tetra-O-methyl-D-glucopyranoside and methyl 2,3,4-tri-O-methyl-D-glucopyranoside in this residue was demonstrated by gas-liquid chromatography: condition: column; 5% neopentyl glycol succinate on Gaschrom C.L. H. (2 m  $\times$  3 mm); column temperature, 180°; carrier gas:  $\text{N}_2$  (1 kg/cm $^2$ ). On Hitachi–Perkin Elmer F-6 type with flame ionization detector (Fig. 2).

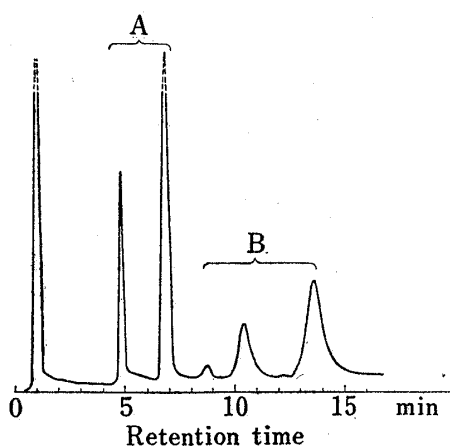


Fig. 2. Gas Chromatogram of the Methanolysis Product of VII

- A: methyl 2,3,4,6-tetra-O-methyl-D-glucopyranosides  
B: methyl 2,3,4-tri-O-methyl-D-glucopyranosides

**Enzymatic Hydrolysis of III**—To a solution of III (10 mg) in purified water (10 ml) was added almond emulsin (1 mg) (Sigma Chemical Co., 1 mg of this enzyme liberates approx.  $3.4 \mu$  moles glucose per min. from salicin at pH 5.25 at 37°) and the mixture was incubated at 37° for 27 days. The red precipitate separated was extracted with  $\text{CHCl}_3$ , and the chloroform layer was dried and evaporated. The residue was recrystallized from EtOH to form orange red needles. This compound was identified with rubrofusarin. The water layer was evaporated to dryness. In this residue the paper chromatography showed the presence of D-glucose only.

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