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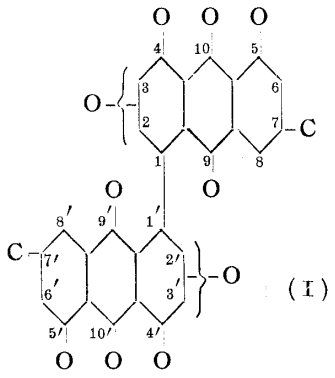
58. Shoji Shibata, Takao Murakami, and Michio Takido : Metabolic Products of Fungi. IX.* Rugulosin. (2). The Structure of Rugulosin and its Relation to the Structure of Flavoskyrin.

(*Pharmaceutical Institute, Medical Faculty, University of Tokyo,*** *and Pharmaceutical Institute, Engineering Faculty, Nihon University****)

Rugulosin

Rugulosin, $C_{30}H_{22}O_{10}$, a bright yellow coloring matter which was isolated by Raistrick and his co-workers[1] from the culture of *Penicillium rugulosum* THOM and *P. wortmanni* KLÖCKER, and was obtained by us[2] from the culture of *Endothia parasitica* (MURR.) ANDERSON ET ANDERSON and *E. fluens* SHEAR ET STEVENS does not exhibit any characteristic properties of anthraquinone, showing no remarkable change of color by the addition of magnesium acetate to the alcoholic solution, while on dehydration it is readily converted into a bianthraquinone derivative named dianhydrorugulosin (=aurantiorugulosin). As reported in our previous paper,[3] dianhydrorugulosin, $C_{30}H_{18}O_8$, was proved to be 4,5,4′,5′-tetrahydroxy-7,7′-dimethylbianthraquinone (1,1′) (IV). Therefore, we suggested that rugulosin possesses the following carbon skeleton and location of substituents, (I).



(I)

The formation of hexaacetate, m.p. 182°, $C_{30}H_{16}O_4(OCOCH_3)_6$, hexamethyl ether, m.p. 280°, $C_{30}H_{16}O_4(OCH_3)_6$, and hexabenzoate, m.p. 226°, $C_{30}H_{16}O_4(OCOC_6H_5)_6$, all of which were reported in the previous paper,[2] led to the conclusion that rugulosin possesses six hydroxyl groups. The presence of an enolic group involved in the six hydroxyls was suggested, since rugulosin is soluble in aq. bicarbonate while its hexaacetate does not dissolve in it.

On boiling in glacial acetic acid or treating with acetyl chloride at room temperature, rugulosin yielded a diacetate, m.p. 194°(decomp.), $C_{30}H_{20}O_8(OCOCH_3)_2$, which on methylation gave diacetyltetramethylrugulosin, m.p. 262°, $C_{30}H_{16}O_4(OCOCH_3)_2(OCH_3)_4$.

The remaining four oxygen atoms in the rugulosin molecule are believed to be present as ketones. Although the position of these ketones should correspond to two pairs of quinone groupings of dianhydrorugulosin, they do not seem to be present initially as quinones in the rugulosin molecule, the evidence for which was provided by the negative magnesium acetate reaction and by the formation of irreversible tetrahydro derivative, m.p. 295°, $C_{30}H_{26}O_{10}$.

A study of the infrared spectrum**** of rugulosin (Fig. 1-A) revealed two distinct bands at 1690 and 1620 cm^{-1}, which would be indicative of a C=O stretching absorption of ketones.

The 1620 cm^{-1} band did not appear in hexaacetylrugulosin (Fig. 1-B) and diacetyltetramethylrugulosin (Fig. 1-D) which retained the 1690 cm^{-1} band. Consequently it

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** Hongo, Tokyo (柴田承二, 村上孝夫).
*** Surugadai, Chiyoda-ku, Tokyo (滝戸道夫).
**** All the infrared spectra cited in this paper were measured in Nujol.

1) J. Breen, J. C. Dacre, H. Raistrick, G. Smith : Biochem. J. (London) **60**, 618(1955).
2) S. Shibata, T. Murakami, O. Tanaka, G. Chihara, M. Sumimoto : This Bulletin, **3**, 274(1955).
3) S. Shibata, T. Murakami, I. Kitagawa, T. Kishi : *Ibid.*, **4**, 111(1956).

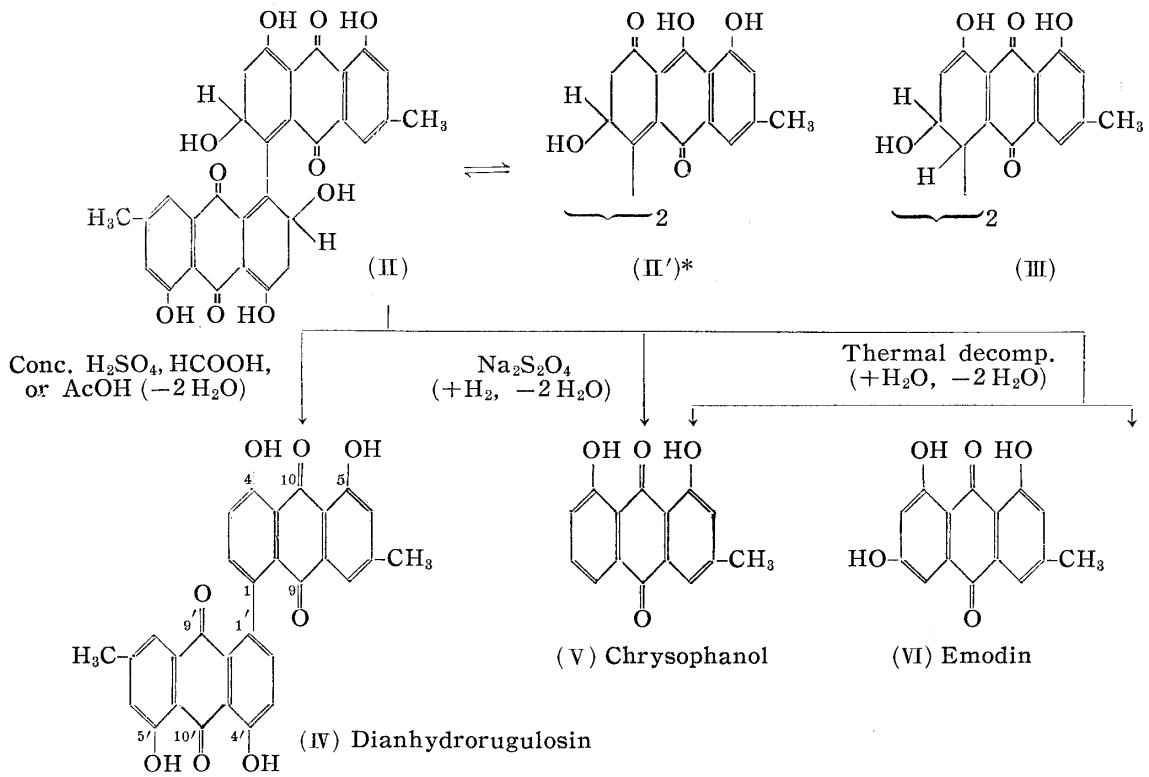
was indicated that the chelated carbonyl grouping which accounts for the 1620 cm^{-1} absorption exists in the rugulosin molecule. The 1690 cm^{-1} band seems to indicate a non-chelated carbonyl, an exact interpretation for which will be given later. The OH stretching vibration at 3360 cm^{-1} which was present in the infrared spectrum of rugulosin disappeared on acetylation with acetic acid or acetyl chloride. Diacetylrugulosin thus obtained exhibited absorption bands at 1754 cm^{-1}(C=O stretching vibration of alcoholic acetate) and 1227 cm^{-1}(C-O stretching absorption of alcoholic acetate)(Fig. 1-C), while hexaacetate of rugulosin showed two split bands at 1773 cm^{-1}(C=O stretching absorption of phenolic and enolic acetate) and 1751 cm^{-1}(C=O band of alcoholic acetate)(Fig. 1-B).

These results from the examination of infrared absorption spectra of rugulosin and its derivatives lead to the conclusion that rugulosin contains 2 alcoholic hydroxyls without hydrogen bonding, 4 chelated phenolic or enolic hydroxyls which would occupy the positions corresponding to that of 4 phenolic hydroxyls in dianhydrorugulosin (i.e. the 4, 5, 4′, and 5′ positions in (I) or (IV)), and 4 ketones, both chelated and non-chelated, whose positions should correspond to the quinone groupings of dianhydrorugulosin (i.e. 9, 10, 9′, and 10′ positions in (I) or (IV)).

The ready dehydration of rugulosin giving dianhydrorugulosin, and the formation of emodin by thermal decomposition suggest that the two alcoholic hydroxyls which account for the formation of diacetate are secondary and present in the 2 and 2′ positions of the formula (I).

Consequently, we propose the structure represented by (II) as the most possible formula of rugulosin. The alternative formula (III) was rejected since rugulosin gave no evidence of possessing a true quinone structure.

On the basis of the structure (II), the degradation reaction of rugulosin shall be considered. Breen, Dacre, Raistrick, and Smith[1] described that (i) chrysophanol was



* The tautomeric form (II′) is possible for rugulosin, but its alcoholic acetate, diacetylrugulosin, at least in its crystalline state, should be present as a diacetate of the form (II), since its infrared spectrum showed no non-chelated hydroxyl band (Fig. 1-C).

isolated in 50% yield from the decomposition products formed by prolonged heating of a solution of rugulosin in 2*N* sodium hydroxide, and (ii) emodin and chrysophanol were obtained in 20% and 24% yield, respectively, by the thermal decomposition of rugulosin in a nitrogen atmosphere at 300~305°. In addition, we found that chrysophanol was formed in 25% yield on heating rugulosin in alkaline sodium dithionite solution, and in 40% yield on heating with zinc dust in glacial acetic acid.

The reductive cleavage of rugulosin can be explained by the formula (II), which is cleaved by the addition of hydrogen and the elimination of alcoholic hydroxyls as 2 moles of water, and forming an aromatic structure (V).

The thermal decomposition results by hydrolytic cleavage with 1 mole of water followed by the elimination of 2 moles of water.

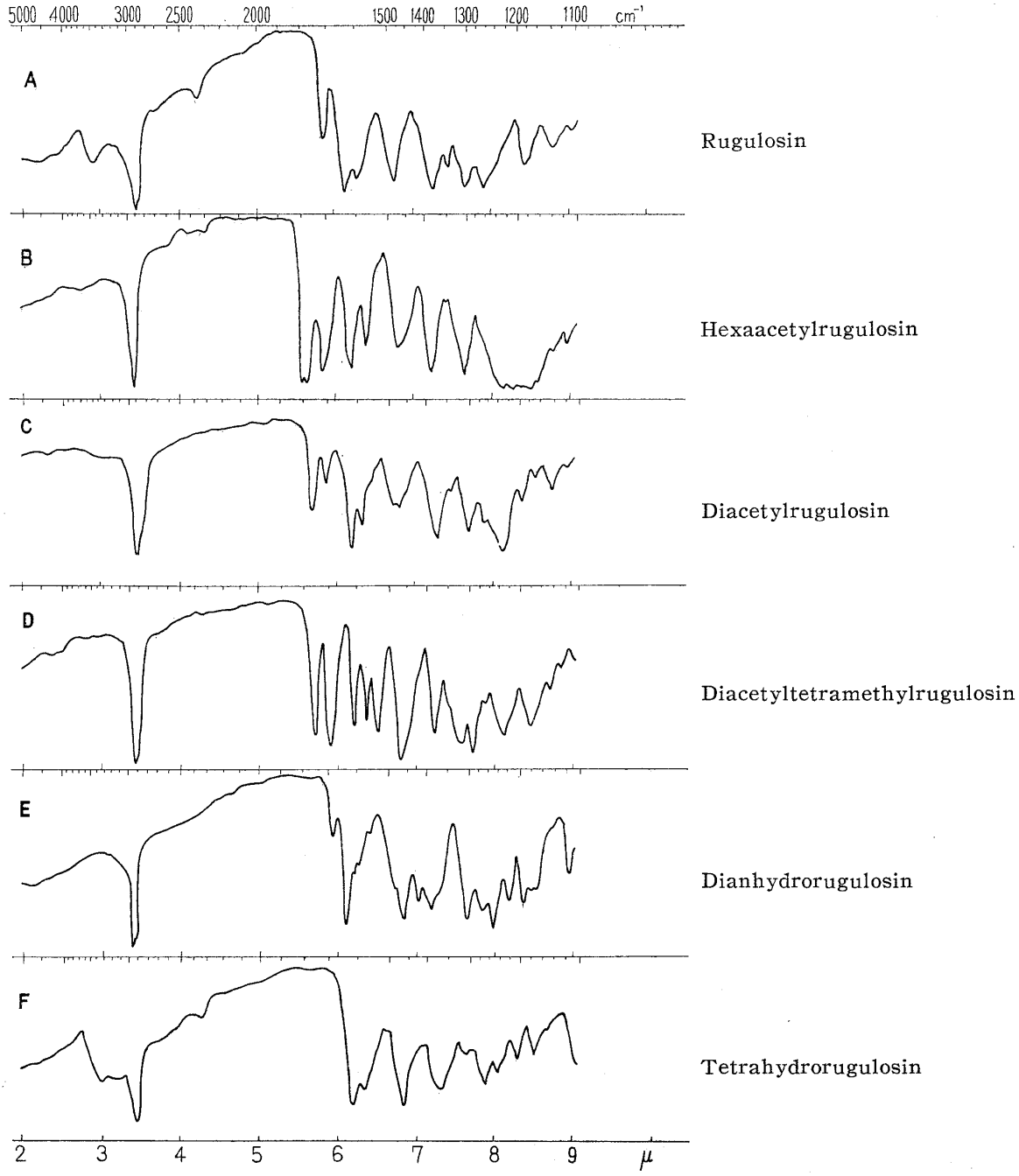
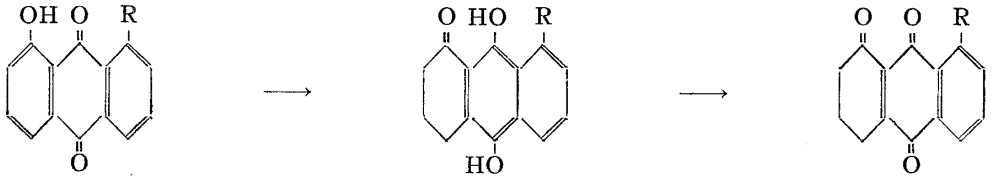
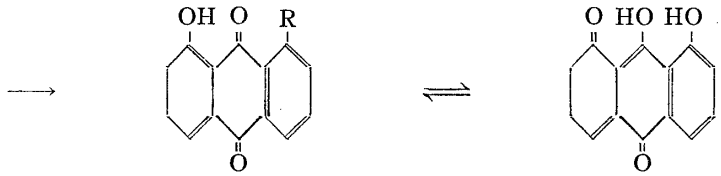


Fig. 1.

The absorption band at 1690 cm^{-1} of rugulosin may be regarded as being shifted toward higher frequencies than expected for the non-chelated carbonyl of aryl-conjugated exocyclic α,β-unsaturated six-membered ring ketone as shown in the 9 and 9′-positions of the formula (II). A precise evidence for the shift of C=O absorption band toward higher frequencies has been provided by a model compound, 1,8-dihydroxy-2,3-dihydroanthraquinone (Xb), prepared by analogy of 1-hydroxy-2,3-dihydroanthraquinone (Xa)[4], which gave C=O stretching absorption bands at 1717 (non-chelated) and 1623 cm^{-1}(chelated).



(VIIa) R=H (VIIb) R=OH (VIIIa) R=H (VIIIb) R=OH (IXa) R=H (IXb) R=OH



(Xa) R=H, (Xb) R=OH (X′)

A tautomeric form (X′) is also possible for the model compound since a hydroxyl absorption band was observed at 3290 cm^{-1}.

Accordingly, the carbonyl absorption at 1690 cm^{-1} observed in rugulosin is not incompatible with the structure represented by (II).

Flavoskyrin

In connection with the structure of rugulosin, we studied the chemical structure of flavoskyrin, m.p. 208°(decomp.),* $[\alpha]_D$ −295°(dioxane), a coloring matter of *Penicillium islandicum* Sopp, N.R.R.L. 1175, which was isolated first by Howard and Raistrick[5] who gave the following equation of dehydration reaction :

$$\underset{\text{Flavoskyrin}}{C_{15}H_{12}O_5} \xrightarrow[\text{heated to the m.p.}]{\text{conc. } H_2SO_4 \text{ or}} \underset{\text{Chrysophanol}}{C_{15}H_{10}O_4} + H_2O$$

The properties and reactions, as well as the infrared spectrum of flavoskyrin suggested its similarity to rugulosin, though the molecular weight of the former is about one-half of the latter. The infrared spectrum of flavoskyrin (Fig. 2) showed the presence of a hydroxyl (3450 cm^{-1}), and chelated (1625 cm^{-1}) and non-chelated (1715 cm^{-1}) carbonyls. A shift of the carbonyl band toward higher frequencies is to the

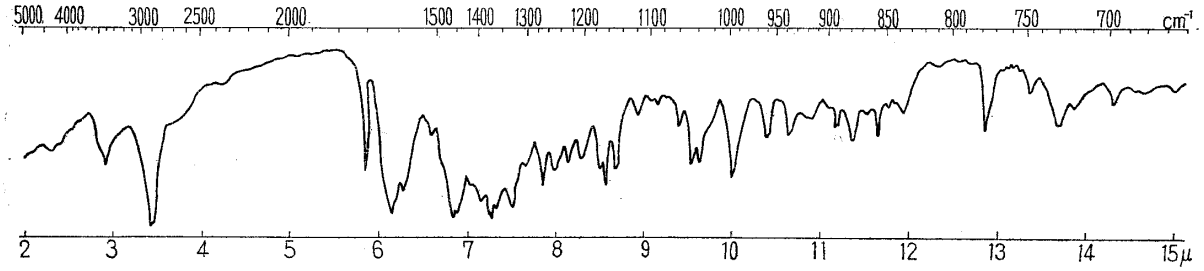


Fig. 2. Flavoskyrin

* Howard and Raistrick[5] recorded m.p. 214~215°(decomp.) for this compound.

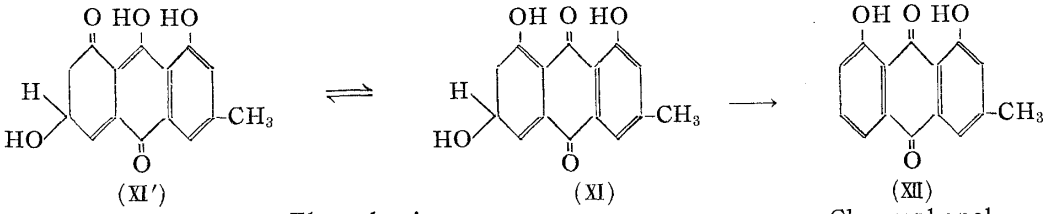
4) K. Zahn, H. Koch : Ber., **71**, 172(1938).

5) B. H. Howard, H. Raistrick : Biochem. J. (London), **56**, 56(1954).

same extent as observed in the model compound (X or X′).

Considering the similarity in the case of rugulosin, its negative magnesium acetate reaction suggested the absence of a true quinone structure in flavoskyrin.

In the present state, it seems not so improbable to advance, by the analogy of rugulosin, the following structural formula (XI or XI′) for flavoskyrin, which corresponds to one-half of the molecule of rugulosin, though its stereochemical relation has not yet been established.



(XI′) Flavoskyrin (XI) (XII) Chrysophanol

We wish to thank Prof. H. Raistrick, Dr. B. H. Howard, and Mr. G. Smith, London School of Hygiene & Tropical Medicine, for their supply of the mold strains and the sample of flavoskyrin, and also for their kind agreement for continuing the present work, the earlier study of which was carried out by them. The infrared spectra were measured by Mr. S. Tanaka, Engineering Faculty, University of Tokyo, and by Mr. H. Shindo, Takamine Research Laboratory, Sankyo Co. Ltd., and the microanalyses were carried out by Mr. D. Ohata, Iatrochemical Laboratory, and by the members of microanalytical laboratories of this Institute, to all of whom our thanks are due.

Experimental

Thermal Decomposition of Rugulosin—On heating rugulosin (100 mg.) in a small Anschutz distillation flask at 250~300° for 2 hrs., orange and yellow needles were obtained in sublimates.

The residue and the sublimates dissolved in $CHCl_3$ were chromatographed on a $CaHPO_4$-column, when chrysophanol (37 mg.) and emodin (23 mg.) were isolated.

Reductive Cleavage of Rugulosin—i) Rugulosin (125 mg.) was dissolved in *N* Na_2CO_3 and mixed with 10% $Na_2S_2O_4$ solution (10 cc.). The mixture was heated on a boiling water bath for 20 mins. when the orange color of the solution changed into red. The precipitate that separated on acidification was dissolved in $CHCl_3$ and chromatographed through a $CaHPO_4$-column to give chrysophanol (32 mg.) (25% of theor. yield).

ii) On heating rugulosin (100 mg.) dissolved in glacial AcOH (15 cc.) and added with Zn dust (1 g.) on a boiling water bath for 10 mins., chrysophanol (40 mg.) was obtained, which was purified by chromatography on a $CaHPO_4$-column eluting with $CHCl_3$.

Diacetylrugulosin—i) Rugulosin (500 mg.) was dissolved in glacial AcOH and boiled for 16 hrs. The precipitate obtained by pouring the mixture into water was recrystallized from EtOH to form faint yellow needles, m.p. 194°(decomp.) (softening from 175°). $[\alpha]_D^{16}$ +196°(0.5% in $CHCl_3$). *Anal.* Calcd. for $C_{30}H_{20}O_8(OCOCH_3)_2$: C, 65.17; H, 4.15; CH_3CO, 13.73. Found: C, 65.50; H, 4.11; CH_3CO, 14.71.

From the mother liquor left after the recrystallization of diacetylrugulosin, chrysophanol (30 mg.) and dianhydrorugulosin (50 mg.) were separated on hydrolysis followed by chromatography on $CaHPO_4$ column.

ii) Rugulosin (500 mg.) dissolved in glacial AcOH (10 cc.) was mixed with AcCl (10 cc.) under ice-cooling and the mixture was allowed to stand over night. The product precipitated on pouring into ice water was recrystallized from a mixture of EtOH-acetone to faint yellow needles, m.p. 194°(decomp., softenning from 175°). $[\alpha]_D^{16}$ +200° (0.5% in $CHCl_3$). *Anal.* Found: C, 65.45; H, 4.25. The identity of the diacetate prepared by the above two different methods was established by the infrared spectra.

Diacetyltetramethylrugulosin—Diacetylrugulosin (1 g.) dissolved in MeI (100 cc.) was added with newly prepared Ag_2O (5 g.) and the mixture was boiled for 50 hrs., shielded from light. During this period, at every 10 hrs., Ag_2O (0.5 g.) and MeI (10 cc.) were added. The reaction mixture was filtered to remove Ag_2O and the filtrate was evaporated.

The residue dissolved in acetone was mixed with $CaHPO_4$ and chromatographed on a $CaHPO_4$-column developing with a mixture of acetone-petr. benzine-H_2O (5 : 5 : 3, upper layer). From the first eluted band yellow needles were obtained by recrystallization from a mixture of EtOAc and

petr. benzine, m.p. 262°(decomp.), $[\alpha]_D^{22}$ +608° (0.25% in acetone). *Anal.* Calcd. for $C_{30}H_{16}O_4(OCH_3)_4(OCOCH_3)_2$: C, 66.86; H, 5.02; CH_3CO, 12.61. Found: C, 66.70; H, 5.42; CH_3CO, 12.84.

Tetrahydrorugulosin—Rugulosin (1 g.) dissolved in EtOH (80 cc.) was catalytically reduced using Pd-black as a catalyst. Two moles of H_2 was absorbed during 4.5 hrs. Colorless needles were obtained by recrystallization from EtOH, m.p. 295°(decomp.; blackening from 210°). $[\alpha]_D^{22}$ +172° (0.55% in acetone). *Anal.* Calcd. for $C_{30}H_{26}O_{10}\cdot H_2O$: C, 63.83; H, 4.96. Found: C, 63.32; H, 5.42.

Octaacetyltetrahydrorugulosin—Prepared by acetylation of tetrahydrorugulosin with Ac_2O and pyridine. Faint yellow, hygroscopic prisms were obtained by recrystallization from EtOH, m.p. 230°(decomp.). *Anal.* Calcd. for $C_{30}H_{18}O_2(OCOCH_3)_8\cdot H_2O$: C, 61.33; H, 4.88; CH_3CO, 38.22. Found: C, 61.09; H, 5.33; CH_3CO, 37.50.

Thermal Decomposition of Tetrahydrorugulosin—Tetrahydrorugulosin (63 mg.) was heated *in vacuo* at 270~310° (bath temp.). The sublimate was dissolved in $CHCl_3$ and passed through a $CaHPO_4$-column. Chrysophanol (31 mg., 49%) and emodin (14 mg., 22%) were obtained.

Tetrahydrochrysazin[6]—Chrysazin (1 g.) was suspended in 4% NaOH (100 cc.) and catalytically reduced using Pd-black as a catalyst, when 1 mole of H_2 was absorbed during the first 1 hr. and another 1 mole during the successive 10 hrs. The reaction mixture was poured into *N* HCl and the orange precipitate obtained was filtered, washed with water, and then recrystallized from benzene to orange red needles, m.p. 180°. Yield, 60%. The product gave a single spot on paper chromatogram developed with acetone-benzine-H_2O (1 : 1 : 3, upper layer) showing no contamination of chrysazin. *Anal.* Calcd. for $C_{14}H_{12}O_4$: C, 68.84; H, 4.95. Found: C, 68.74; H, 5.33.

1-Oxo-8-hydroxy-1, 2, 3, 4-tetrahydroanthraquinone (IXb)—Tetrahydrochrysazin (500 mg.) suspended in glacial AcOH (5 cc.) and added with a solution of $Pb(OAc)_4$ (1 g.) in glacial AcOH (12.5 cc.) was shaken at room temperature for 10 mins. The orange color of the original solution changed instantly and the dark orange-colored precipitate formed by the addition of water was filtered, washed, and dried. m.p. 156°(decomp.). It gave an orange coloration with $Mg(OAc)_2$ in EtOH. It was readily isomerized into (Xb) by the recrystallization process.

2,3-Dihydrochrysazin (1,8-Dihydroxy-2,3-dihydroanthraquinone) (Xb)—i) A mixture of 1-oxo-8-hydroxy-1,2,3,4-tetrahydroanthraquinone (IXb) and pyridine (3 drops) suspended in chlorobenzene (10 cc.) was heated on a boiling water bath for 20 mins., when a clear solution once formed and then yellow prisms separated out. It was recrystallized from dioxane to yellow prisms, m.p. 258° (decomp.) (blackening from 176°).

ii) The compound (IXb) was converted into 2,3-dihydrochrysazin (Xb) by boiling in acetone. Yellow prisms, m.p. 258°(decomp.) (blackening from 176°). It gave no marked change of color by the addition of $Mg(OAc)_2$ in EtOH. *Anal.* Calcd. for $C_{14}H_{10}O_4$: C, 69.42; H, 4.13. Found: C, 69.30; H, 4.28.

Flavoskyrin—The dried mycelium of *Penicillium islandicum* Sopp, N.R.R.L. 1175 strain (80 g.) harvested after incubation on Czapek-Dox solution for 10~15 days, at 28~30°, was extracted with ether for 2 days using a percolator. The reddish brown precipitate that separated (ca. 30 g.) was washed twice with acetone (300 cc. each) and the residue (ca. 20 g.) was extracted 3 times with $CHCl_3$ (500 cc. each) to give yellowish brown crystalline substance (ca. 300 mg.). The yellowish brown crude extract was dissolved in EtOAc and chromatographed on $CaHPO_4$, when it was separated into about 4 bands. The first eluate which contained skyrin along with a small amount of chrysophanol was removed and the middle portion of the chromatogram in which the main part of the coloring matter was present was extracted. The extract was recrystallized from a mixture of acetone and H_2O to give yellow needles, m.p. 208°(decomp.); $[\alpha]_D$ −295°(dioxane). Yield, ca. 100 mg. It was identified with the authentic sample of flavoskyrin given by Prof. Raistrick.

It dissolves in 5% aq. Na_2CO_3 and 5% NaOH, and partly in 5% aq. $NaHCO_3$ to form a yellow solution. It forms an orange solution in conc. H_2SO_4, which changes to red violet. It gives a brown color with $FeCl_3$, and no marked change of color by the addition of $Mg(OAc)_2$ in EtOH.

By the action of conc. H_2SO_4 for 2 hrs. flavoskyrin was converted into chrysophanol, which was identified by paper chromatography.

On thermal decomposition it forms chrysophanol. *Anal.* Calcd. for $C_{15}H_{12}O_5$: C, 66.17; H, 4.41. Found: C, 66.18; H, 4.45.

Summary

i) The structure of rugulosin was established as being formulated by (II).

ii) The infrared spectra of rugulosin and its derivatives were discussed referring to a model compound.

iii) The structure of flavoskyrin (XI) was proposed in relating to the structure of rugulosin.

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