Infrared Absorption Spectra—The infrared spectra of 5-dehydro-α-santenic acid and santenic acid are given in Fig. 1, A and B (Perkin-Elmer Model 21 double-beam). From Fig. 1 A, it seems most likely that the bands at 6.07 and 11.00~11.24 μ indicate the presence of $>C=C<{}^{H}_{H}$ and a band corresponding to hydroxyl group (2.77 or 3.03~3.15 μ) is not observed.

Summary

d-5-Amino-α-santenic acid was prepared from *d*-isoketopinic acid as the starting material. The diazotization of this produced a dehydrosantenic acid, the dehydration product of the expected 5-hydroxy-α-santenic acid. The structure of the unsaturated acid was assumed as 5-dehydrosantenic acid.

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24. Shoji Shibata, Takao Murakami, Isao Kitagawa, and Teruo Kishi :

Metabolic Products of Fungi. Ⅷ.* Rugulosin. (1). The Structure of Dianhydrorugulosin and Its Relation to the Structure of Iridoskyrin.

(*Pharmaceutical Institute, Medical Faculty, University of Tokyo***)

In the previous paper,[1] we reported that radicalisin,[2] $C_{30}H_{22}O_{10}$, a yellow pigment isolated from the laboratory cultures of *Endothia parasitica*(Murr.) Anderson et Anderson and *E. fluens* Shear et Stevens (syn. *E. radicalis* Fr.), is identical with rugulosin which was isolated by Raistrick and his co-workers[3] from the cultures of *Penicillium rugulosum* Thom and *P. wortamanni* Klöcker grown on a Czapek-Dox solution.

The general properties of rugulosin and its derivatives were described in the papers by Raistrick[3] and by the present writers,[1,2] though the conclusive evidences for the chemical structure have not yet been elucidated. One of the noticeable reactions of rugulosin is the dehydration reaction that involves conversion of rugulosin, which is not an anthraquinone itself, into a compound having anthraquinone properties.

Breen, Dacre, Raistrick, and Smith[3] showed that on heating in conc. H_2SO_4, rugulosin, $C_{30}H_{22}O_{10}$, was converted into a compound m.p. 325°, with a molecular formula $C_{30}H_{18}O_8$, which was named aurantio-rugulosin and was suggested to be a homolog of iridoskyrin,[4] with two hydroxyls less. Iridoskyrin was derived from rubroskyrin[4] by an analogous reaction.

We have also obtained a dehydrated product of rugulosin, m.p. 321°, orange red crystals having a molecular formula $C_{30}H_{18}O_8$, in 60% yield by boiling in 95% formic acid or in 10% yield by boiling either in 55% H_2SO_4 or in glacial acetic acid. It was named dianhydrorugulosin and would be identical with aurantio-rugulosin though there are some discrepancies in the melting points of its derivatives.

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* Part Ⅶ : This Bulletin, **3**, 286(1955).

** Hongo, Tokyo (柴田承二, 村上孝夫, 北川 勳, 貴志光雄).

[1] S. Shibata, T. Murakami, O. Tanaka, G. Chihara, M. Sumimoto : This Bulletin, **3**, 274(1955).

[2] S. Shibata, O. Tanaka, G. Chihara, H. Mitsuhashi : *Ibid.*, **1**, 302(1953).

[3] J. Breen, J.C. Dacre, H. Raistrick, G. Smith : Biochem. J. (London), **60**, 618(1955).

[4] B.H. Howard, H. Raistrick : *Ibid.*, **57**, 212(1954).

Present paper is chiefly concerned with the work of establishing the structure of dianhydrorugulosin with a special reference to the structure of iridoskyrin which has also been confirmed.

Dianhydrorugulosin shows the properties of polyhydroxyanthraquinone and gives tetraacetate, m.p. 248°(decomp.), and tetramethyl ether, m.p. 347~348°(decomp.). On reductive cleavage with alkaline $Na_2S_2O_4$, dianhydrorugulosin yields chrysophanol in 43% yield. The ultraviolet absorption maxima of dianhydrorugulosin and its acetate are very similar, though slightly bathochromic, to that of chrysophanol and its acetate, respectively (Figs. 1 and 2).

It was, therefore, strongly suggested that dianhydrorugulosin might be a bianthraquinone consisting of two molecules of chrysophanol joined together, as emodins constituting skyrin[5] (di-emodin (8,8′) (Me in the 2-position)).

On oxidation with CrO_3 followed by alkaline hydrolysis, tetraacetate of dianhydrorugulosin afforded a carboxylic acid, m.p. 370°(decomp.), named dianhydrorugulosic acid. The decarboxylation of dianhydrorugulosic acid gave orange needles, m.p. 342°, with a molecular formula, $C_{28}H_{14}O_8$, which was named bisnordianhydrorugulosin whose ultraviolet absorption maxima closely resemble that of chrysazin (1,8-dihydroxy-anthraquinone) though slightly bathochromic (Fig. 3).

The structure of bisnordianhydrorugulosin was finally established as 4,5,4′,5′-tetrahydroxybianthraquinone-(1,1′)[6] by a mixed fusion and comparison of the infrared spectra with a sample synthetically prepared by Brockmann's procedure of condensing 5-iodochrysazin dimethyl ether and followed by demethylation. Thus the bianthraquinone-(1,1′) structure of dianhydrorugulosin and the 4,5,4′,5′-position of the four phenolic hydroxyls in the rings have been proved.

Consequently, two alternative possibilities have been advanced for the positions of the methyl groups of dianhydrorugulosin, i.e. the 2,2′- or 7,7′-positions (C-C linkage at 1,1′). The conclusive evidence for the positions of the methyls in the structure of anhydrorugulosin was finally provided by establishing the relation with iridoskyrin, by which the structure of iridoskyrin itself was conversely established.

Iridoskyrin, deep red colored crystals exhibiting a green iridescence, was isolated by Howard and Raistrick[4] from cultures of *P. islandicum* Sopp and was also found as being derived by the action of conc. H_2SO_4 from rubroskyrin, a red pigment,[4] and pigment A, a yellow pigment[7], both of which were produced by the same strain of mold. Howard and Raistrick[4] considered that iridoskyrin might be a bianthraquinone consisting of two molecules of islandicin,[8] though they have not provided conclusive evidence while they noticed similarities between iridoskyrin and aurantiorugulosin.[3]

Although Howard and Raistrick reported that iridoskyrin has been recovered unchanged by the action of alkaline $Na_2S_2O_4$, we have found that by the reductive cleavage under a more drastic condition, iridoskyrin yields islandicin in 16% yield. This and the ultraviolet data (Fig. 4) provided a decisive evidence that islandicin is a component of iridoskyrin.

It should be noted, however, that we previously described[5] that 4,4′-dihydroxybianthraquinone-(1,1′) gave no evidence of cleavage by alkaline $Na_2S_2O_4$, whereas the bianthraquinones-(1,1′) having hydroxyls or methoxyls in the *ortho*-positons of the

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[5] S. Shibata, O. Tanaka, I. Kitagawa: This Bulletin, **3**, 278(1955); O. Tanaka, C. Kaneko: *Ibid.*, **3**, 284(1955); B. H. Howard, H. Raistrick: Biochem. J. (London), **56**, 56(1954).

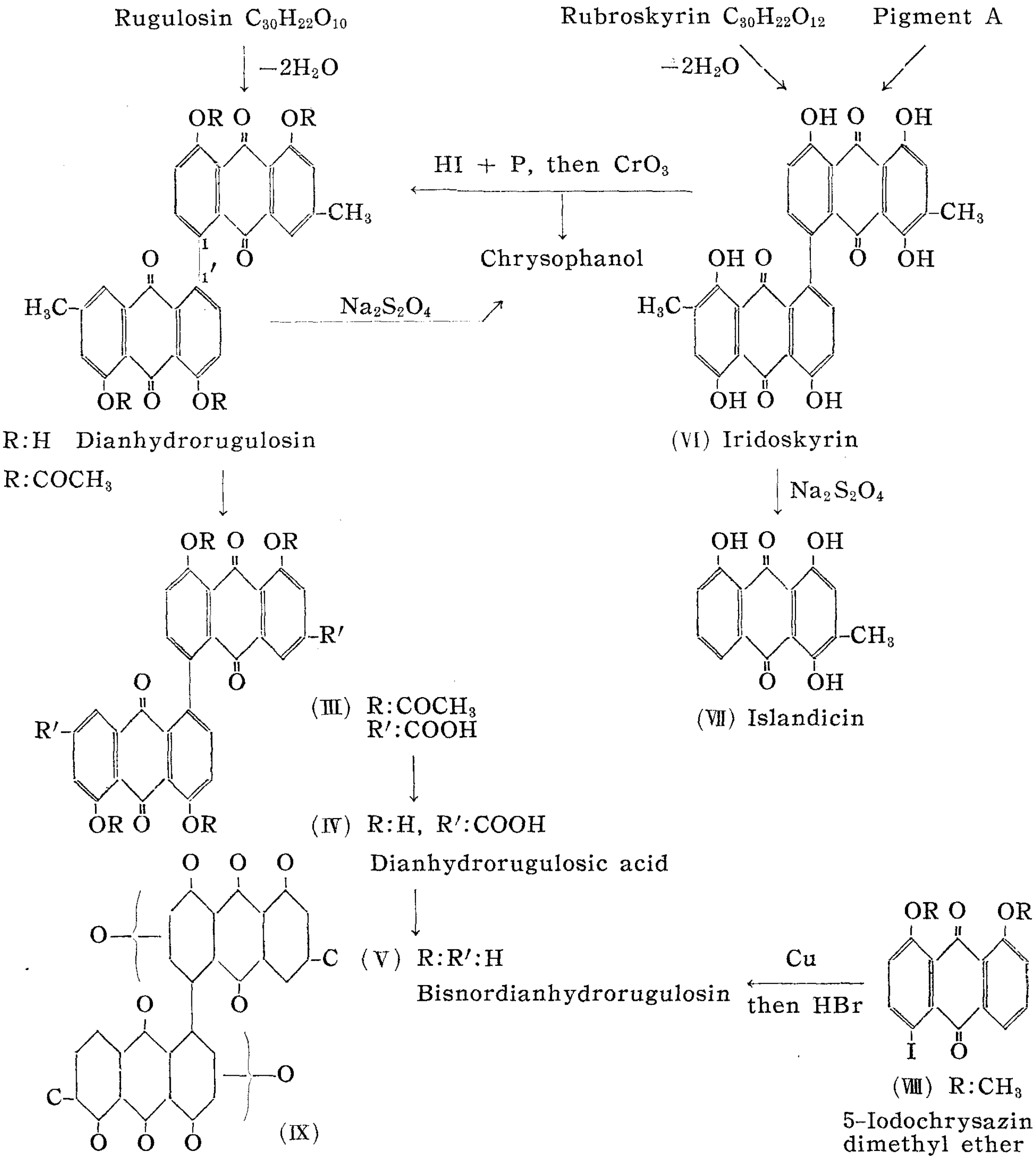
[6] H. Brockmann: Chem. Ber., **83**, 467(1950).

[7] S. Shibata, M. Takido, T. Nakajima: This Bulletin, **3**, 286(1955); cf. T. Tatsuno, M. Tsukioka, Y. Sakai, Y. Suzuki, Y. Asami: *Ibid.*, **3**, 476(1955); Y. Yamamoto, T. Yamamoto, K. Kanatomo, K. Tanimichi: J. Pharm. Soc. Japan, **76**, 192(1956).

[8] B. H. Howard, H. Raistrick: Biochem. J. (London), **44**, 227(1949).

carbon–carbon linkage were cleaved. We have now revised the former conclusion after a repeated examination and have confirmed that even 4,4′-dihydroxybianthraquinone-(1,1′) undergoes reductive cleavage when the reaction mixture is heated for about 20 mins. in a boiling water bath.

On the other hand, by the action of HI follwed by oxidation with CrO_3 iridoskyrin was converted into dianhydrorugulosin with a loss of two hydroxyls. It has been shown that dianhydrorugulosin is undoubtedly a dihydroxy-less homolog of iridoskyrin possessing their methyl groups in the same position. As the location of hydroxyl groups in dianhydrorugulosin has been confirmed by the synthesis of bisnordianhydrorugulosin, the structure of dianhydrorugulosin as well as that of iridoskyrin have unequivocally been established as shown by the formulae (I) and (VI), respectively.



The establishment of the structure of dianhydrorugulosin enables us to advance a fundamental carbon skeleton of rugulosin and the position of C–O bonds on it as shown by the formula (IX).

We are greatly indebted to Prof. H. Raistrick, Dr B. H. Howard, and Mr. G. Smith, London School of Hygiene & Tropical Medicine, for supplying us the strains of *Penicillium rugulosum* Thom and *P. islandicum* Sopp N.R.R.L. 1036 which were used for this part of work. We thank the Sankyo Co. Ltd. for the measurement of infrared spectra. The microanalyses were carried out by the members of the Microanalytical Laboratories of this Institute and by Mr. D. Ohata, Iatrochemical Institute, to all of whom the authors' thanks are due.

Experimental

Dianhydrorugulosin—Rugulosin (1 g.) was dissolved in 95% HCOOH (150 cc.) and the solution was boiled gently for 48 hrs. The orange red crystalline substance which separated out during the reaction was collected every 8 hrs. The combined crystals were dissolved in benzene and chromatographed on a $CaHPO_4$ column to be separated into several bands. From the first eluted fraction, chrysophanol (40 mg.) was isolated and from the second band, orange red prisms, m.p. 321°, were obtained by recrystallization from $CHCl_3$. Yield: 600 mg. (60%). It dissolves in aq. NaOH solution and gives orange red color with $(AcO)_2Mg$ in ethanolic solution. *Anal.* Calcd. for $C_{30}H_{18}O_8$: C, 71.15; H, 3.56. Found: C, 70.80; H, 3.66. $[\alpha]_D^{17}$: nil (0.5% in $CHCl_3$).

Rugulosin (100 mg.) was suspended in 55% H_2SO_4 and the mixture was boiled gently for 1 hr. The dark-colored reaction mixture was poured into water. The precipitate obtained was dissolved in benzene and was purified by chromatography as above. Dianhydrorugulosin (10 mg.) was obtained along with a few mg. of chrysophanol.

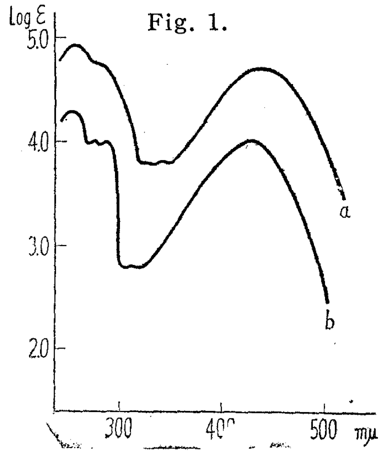


Fig. 1.

(a) Dianhydrorugulosin
(b) Chrysophanol (in $CHCl_3$)

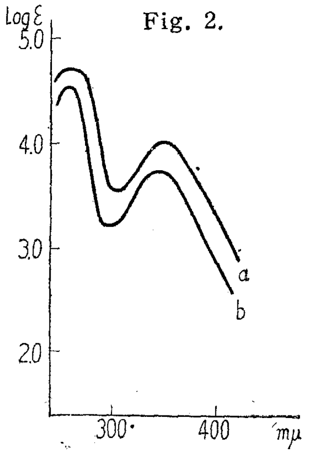


Fig. 2.

(a) Tetraacetyldianhydrorugulosin
(b) Diacetylchrysophanol (in $CHCl_3$)

Tetramethyldianhydrorugulosin—A mixture of dianhydrorugulosin (250 mg.), anhyd. K_2CO_3 (1.5 g.), and Me_2SO_4 (2.3 cc.) suspended in dehyd. acetone (40 cc.) was refluxed for 30 hrs. The yellow precipitate formed during the reaction was collected and washed with $CHCl_3$. The washings were passed through Al_2O_3 column. From the eluate of the lowest band, yellow needles, m.p. 347~348°, were obtained after recrystallization from $CHCl_3$. *Anal.* Calcd. for $C_{30}H_{14}O_4(OCH_3)_4$: C, 72.60; H, 4.62. Found: C, 72.05; H, 5.00.

Reductive Cleavage of Dianhydrorugulosin with Alkaline $Na_2S_2O_4$—Dianhydrorugulosin (100 mg.), was dissolved in 2*N* NaOH (50 cc.) and added with $Na_2S_2O_4$ (1 g.). The mixture was heated in a boiling water bath for 30 mins. The precipitate obtained from the reaction mixture by acidification with dil. HCl was dissolved in dehyd. benzene and the solution was chromatographed on $CaHPO_4$ column. From the lowest band chrysophanol (43 mg.) was obtained by elution.

Tetraacetyldianhydrorugulosic Acid—Tetraacetyldianhydrorugulosin (0.8 g.) (yellowish needles, m.p. 244~248°, recrystallized from $CHCl_3$-acetone mixture) was dissolved in a mixture of glacial AcOH (25 cc.) and Ac_2O (25 cc.). To this solution, a solution of CrO_3 (1.7 g.) in water (2 cc.) and glacial AcOH (16 cc.) was dropped under stirring for 40 mins. keeping the temperature of the reaction mixture at 55~60°. The stirring was continued further for 3 hrs. at temperature 60~65°. The green-colored reaction mixture was poured into cold water and the precipitate was collected, which was reacetylated with Ac_2O and pyridine. The product was recrystallized from glacial AcOH to yellowish needles, m.p. 375° (decomp.). *Anal.* Calcd. for $C_{30}H_{10}O_8(OCOCH_3)_4 \cdot H_2O$: C, 60.63; H, 3.19. Found: C, 60.33, 61.01, 61.05; H, 3.55, 3.61, 3.41.

Dianhydrorugulosic Acid—The acetate obtained as above was hydrolyzed with *N* NaOH and the product was recrystallized from dioxane to orange red needles, m.p. 370° (decomp.). It gave a single spot (Rf 0.8) on the paper chromatogram developed with the lower layer of acetone-benzene-water (1:1:1). *Anal.* Calcd. for $C_{30}H_{14}O_{12} \cdot C_4H_8O_2$ (with dioxane as the solvent of crystallization) : C, 62.39; H, 3.36. Found : C, 62.30, 62.82; H, 3.47, 3.70.

Dimethyl ester : Prepared by the action of CH_2N_2 in ether. Orange red needles, m.p. 305~308°.

Bisnordianhydrorugulosin—Dianhydrorugulosic acid mixed with Cu powder was heated at 300° under a reduced pressure. The crystalline sublimate was dissolved in benzene and the solution was chromatographed on $CaHPO_4$ column. The crystals obtained from the lowest band were recrystallized from a mixture of $CHCl_3$ and MeOH to orange red needles, m.p. 342°. It gave no melting point depression when admixed with 4,5,4′,5′-tetrahydroxybianthraquinone-(1,1′) synthesized by Brockmann's method.[6] *Anal.* Calcd. for $C_{28}H_{14}O_8$: C, 70.29; H, 2.92. Found : C, 70.31; H, 2.89.

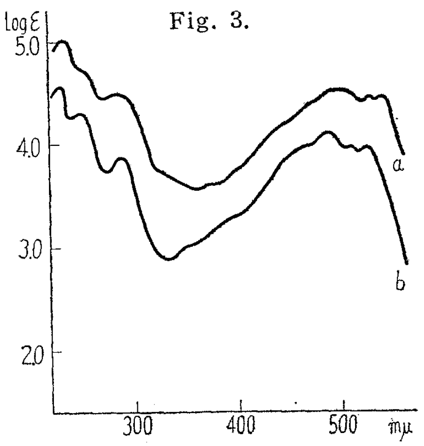


Fig. 3.

(a) Bisnordianhydrorugulosin
(b) Chrysazin (in $CHCl_3$)

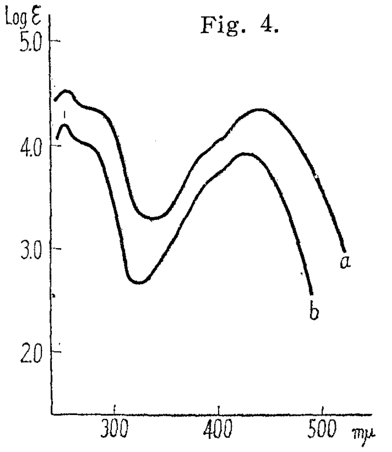


Fig. 4.

(a) Iridoskyrin*
(b) Islandicin (in EtOH)
* Measured in a saturated solution

Iridoskyrin—*Penicillium islandicum* Sopp N.R.R.L. 1036 was inoculated in a Czapek-Dox solution and incubated at 25° for 3 weeks. The harvested mycelium was dried and extracted with ether and acetone, successively, to obtain the crude pigments. The mixture of the pigments was chromatographed on $CaHPO_4$ column developing with $CHCl_3$, when the pigments split into several bands which were, from bottom to top, orange (islandicin), purple (iridoskyrin), yellow (erythroskyrin (?)), red (catenarin), yellow (Pigment A), brownish red (rubroskyrin), and yellowish orange (skyrin). The lowest two bands were eluted and the solvent was removed to give a dark red crystalline residue which was shown by paper chromatopraphy developing with petr. ether[7] to be a mixture of islandicin and iridoskyrin. On heating under a reduced pressure (180°, 2 mm. Hg), islandicin sublimed first and iridoskyrin was obtained as a brownish red crystalline sublimate when the temperature was raised to 320°. It gave a characteristic greenish iridescence and exhibited a single spot on paper chromatogram. *Anal.* Calcd. for $C_{30}H_{18}O_{10}$: C, 66.91; H, 3.35. Found : C, 67.03; H, 3.28.

Hexamethyliridoskyrin—Prepared by methylation of iridoskyrin with Me_2SO_4 and anhyd. K_2CO_3 in acetone. Orange crystals, m.p. 343~345°. *Anal.* Calcd. for $C_{36}H_{30}O_{10}$: C, 69.45; H, 4.82. Found : C, 70.21; H, 4.80.

The Formation of Dianhydrorugulosin from Iridoskyrin—A mixture of iridoskyrin (300 mg.), 52% HI (3 cc.), and red P (700 mg.) in glacial AcOH (30 cc.) was boiled at 140° (bath temp.) for 4.5 hrs. HI (3 cc.) was added during the reaction. The reaction mixture was filtered while hot to remove red P and to recover iridoskyrin (53 mg). The filtrate was poured into water and extracted with ether. The ethereal extract was washed successively with water, aq. $NaHCO_3$, and $Na_2S_2O_3$ solutions. The ether was distilled off to obtain a dark brown residue which was dissolved in glacial AcOH (10 cc.). A 5(w/v)% solution of CrO_3 in AcOH (3 cc.) was dropped into the above solution while heating at 50° for 35 mins. The reaction mixture was then poured into water and the precipitate was extracted with ether. The extracts were examined by paper chromatography developed with the upper layer of acetone-benzine-water (1:1:3), giving 2 spots (Rf 0.97 and 0.91).

The mixture was dissolved in $CHCl_3$, mixed with $CaHPO_4$, and filled on the top of a $CaHPO_4$ column. The chromatography was carried out using a mixture of benzine and AcOEt (8:1) as a developing solvent. The two lowest bands, (i) yellow and (ii) orange, were mechanically separated and extracted with $CHCl_3$.

The chloroform extracts were chromatographed again on $CaHPO_4$ column developing with benzene. From the yellow band of the extract (i) yellowish orange crystalline substance was obtained, which was recrystallized from EtOH to leaflets, m.p. 191° (yield : a few mg.) It was identified as chrysophanol by a mixed fusion.

From the orange band of the extract (ii), orange red crystals were separated, which were recrystallized from $CHCl_3$-MeOH mixture to orange plates, m.p. 321°. It was identified as dianhydrorugulosin by a mixed fusion (mixed m.p. 321°).

Reductive Cleavage of Iridoskyrin with Alkaline $Na_2S_2O_4$—To a solution of iridoskyrin (50 mg.) dissolved in 2*N* NaOH (15 cc.), $Na_2S_2O_4$(300 mg.) was added while heating in a boiling bath. The solution exhibited at first a deep violet blue color which instantly became dark yellowish brown, and finally turned deep bluish violet during 15 mins.' heating. After further 5 mins.' heating the reaction mixture was neutralized with dil. HCl and the precipitate formed was extracted with $CHCl_3$. $CHCl_3$ solution showed a greenish fluorescence. The residue obtained by evaporation of the solvent was heated *in vacuo* (170~180°, 2 mm. Hg) when brick red sublimate (8 mg.) was obtained, which was purified by chromatography on $CaHPO_4$ column using $CHCl_3$ as a developing solvent. From an orange band, red crystals separated, which were identified as islandicin by a mixed fusion (mixed m.p. 213°). The residue (21 mg.) from the sublimate was the recovered iridoskyrin, which was proved by paper partition chromatography.

Summary

i) Dianhydrorugulosin, $C_{30}H_{18}O_8$, which was derived from rugulosin, $C_{30}H_{22}O_{10}$, a pigment of *P. rugulosum* Thom, by a dehydration reaction, was converted into bisnordianhydrorugulosin which was synthetically proved to be identical with 4,5,4′,5′-tetrahydroxybianthraquinone-(1,1′).

ii) Iridoskyrin, $C_{30}H_{18}O_{10}$, a pigment of *P. islandicum* Sopp, yielded islandicin (1,4,5-trihydroxy-2-methylanthraquinone) by reductive cleavage with alkaline $Na_2S_2O_4$. Dianhydrorugulosin gave chrysophanol by the same reaction.

iii) Dianhydrorugulosin was derived from iridoskyrin by the action of HI and red phosphorus, then with CrO_3, losing two hydroxyls.

iv) By the above reactions, the structure of dianhydrorugulosin was established as being 4,5,4′,5′-tetrahydroxy-7,7′-dimethylbianthraquinone-(1,1′), while iridoskyrin was proved conversely to be 4,5,8,4′,5′,8′-hexahydroxy-7,7′-dimethylbianthraquinone-(1,1′).

v) A fundamental skeleton of rugulosin has been forwarded.

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