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53. Shoji Shibata and Isao Kitagawa : Metabolic Products of Fungi. XVIII.[*1] On the Structure of Lumiluteoskyrin.

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In the previous papers,[1~4] the structure of coloring matters produced by *Penicillium islandicum* Sopp NRRL-1036 was reported and one of them, luteoskyrin (I), a yellow pigment, is noted by its toxicity of causing liver cirrhosis and its photosensibility.

On irradiation by sunlight, yellow color of the solution of luteoskyrin in acetone, ethanol, or acetic acid changes into deep red, separating dark violet crystals. The photo-reaction product has now been designated lumiluteoskyrin on which the present paper is chiefly concerned.

The photo-reaction was markedly suppressed in nitrogen and in carbon dioxide, so that lumiluteoskyrin seems to be the result of photo-oxidation. Lumiluteoskyrin, $C_{30}H_{20}O_{12}$, m.p. above 360°, is sparingly soluble in usual organic solvents except in hot dimethylformamide which gives a deep reddish purple solution. The suspension of lumiluteoskyrin in ethanol gives a sky blue coloration with magnesium acetate. Indigo blue alkaline suspension of the pigment is reduced by sodium dithionite, and the blue color is regenerated on air-oxidation. This indicates that lumiluteoskyrin is a true quinone compound.

Lumiluteoskyrin was not cleaved with alkaline sodium dithionite even under a drastic condition, while luteoskyrin (I) and rubroskyrin (II) were affected to give islandicin[3a] (V). In conc. sulfuric acid, lumiluteoskyrin gave a stable purple color and showed no tendency of forming iridoskyrin which was readily formed from luteoskyrin and rubroskyrin on dehydration reaction.[3b]

The infrared spectrum of lumiluteoskyrin (Table I) showed the presence of non-bonded OH, and non-chelated and chelated C=O groupings. The ultraviolet and visible light absorption spectral curves of lumiluteoskyrin are almost parallel to that given by naphthazarin and shikonin,[5] though the former shows a hyper- and batho-chromic shift in comparison with the latter compounds. (Fig. 1).

Table I. Infrared Absorption Spectra of Lumiluteoskyrin and its Derivatives (cm^{-1})

Compounds	Non-bonded OH	Acetate C=O		Ring C=O	
		Phenolic or enolic	Alcoholic	Non-chelated	Chelated
Lumiluteoskyrin (a)	3453	—	—	1689	1614
Diacetate (a)	—	—	1751	1713	1617
(b)	—	—	1744	1710	1611
Tetraacetate (a)	—	1778	1750	1710, 1662,$^{a)}$ 1642	1620
(c)	—	1775$^{a)}$	1755	1711, 1672, 1646	1620
Hexaacetate (a)	—	1782	1752	1714, 1678, 1650	
(b)	—	1786	1752	1717, 1677, 1650	

State : (a) in Nujol, (b) KBr pellet, (c) $CHCl_3$ solution.
a) Shoulder.

[*1] Part XVII. This Bulletin, 8, 889 (1960).

[*2] Hongo, Tokyo (柴田承二, 北川 勲).

1) S. Shibata, O. Tanaka, I. Kitagawa : This Bulletin, **3**, 278 (1955).

2) S. Shibata, T. Murakami, I. Kitagawa, T. Kishi : *Ibid.*, **4**, 111 (1956).

3) a) S. Shibata, I. Kitagawa : *Ibid.*, **4**, 309 (1956). b) S. Shibata, T. Ikekawa, T. Kishi : *Ibid.*, **8**, 884 (1960).

4) S. Shibata, T. Ikekawa : *Ibid.*, **8**, 889 (1960).

5) R. Majima, C. Kuroda : Acta Phytochim. (Japan), **1**, 43 (1922); cf. H. Brockmann : Ann., **521**, 1 (1936).

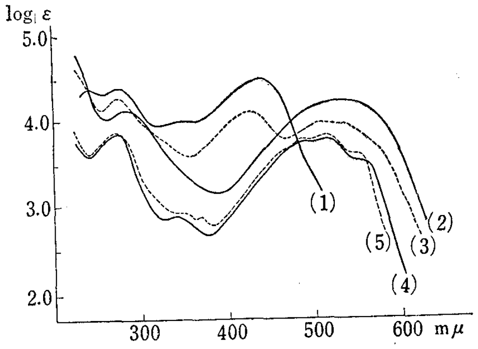
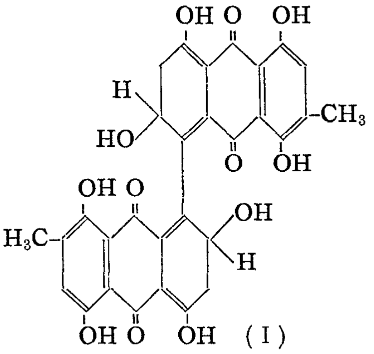
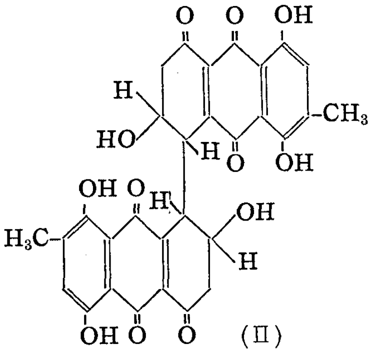


Fig. 1. Ultraviolet Spectra

(1) Luteoskyrin (in EtOH)
(2) Lumiluteoskyrin (in dioxane)
(3) Rubroskyrin (in dioxane)
(4) Naphthazarin (in EtOH)
(5) Shikonin (in EtOH)


(I)


(II)

Thermal decomposition of lumiluteoskyrin under a highly reduced pressure gave islandicin (V) and catenarin (VI) accompanying a brown sublimate which is assumed to be dianhydrolumiluteoskyrin[*3] (IV).

These results suggested that the gross carbon skeleton of luteoskyrin is perfectly retained in the photo-reaction, while the type of junction binding the two tricyclic moieties changes to produce stability in lumiluteoskyrin against reductive cleavage with sodium dithionite.

On acetylation with acetyl chloride in glacial acetic acid, lumiluteoskyrin formed a diacetate and a tetraacetate. The diacetate (VII), $C_{30}H_{18}O_{10}(OCOCH_3)_2$, dark purple rods, m.p. above 360°, exhibits a sky blue coloration with magnesium acetate in ethanol solution as given by lumiluteoskyrin. The infrared absorption spectrum of the diacetate indicated the presence of alcoholic acetate, and non-chelated and chelated C=O groupings (Table I).

The ultraviolet and visible light absorption spectral curves of the diacetate are almost superimposable with that of lumiluteoskyrin (Fig. 2) giving an evidence that the acetylation occurred at alcoholic hydroxyls which are not conjugated with the chromophore system of lumiluteoskyrin molecule.

The diacetate of lumiluteoskyrin was also obtained along with a small amount of tetraacetate by the irradiation of acetylated luteoskyrin. The tetraacetate of lumiluteoskyrin (VIII), $C_{30}H_{16}O_8(OCOCH_3)_4$, small red crystals, m.p. above 360°(darkens from 320°), gave a purple magnesium acetate coloration. The infrared absorption spectrum showed the presence of phenolic and alcoholic acetates, and chelated and non-chelated C=O, but the non-bonded OH band was not observed (Table I). The ultraviolet and visible light absorption showed a hypsochromic effect caused by acetylation of conjugated OH groups. The above results obtained with the acetates showed that the photo-reaction of luteoskyrin proceeded, leaving the secondary alcoholic hydroxyls unaffected.

The hexaacetate of lumiluteoskyrin (IX), $C_{30}H_{14}O_6(OCOCH_3)_6$, orange-red prisms, m.p. 285°(decomp.)(darkens from 262°), was obtained by acetylation of lumiluteoskyrin with

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[*3] Attempted dehydration of lumiluteoskyrin failed to produce dianhydrolumiluteoskyrin. It would be due to the much more strained structure of the expected anhydro compound.

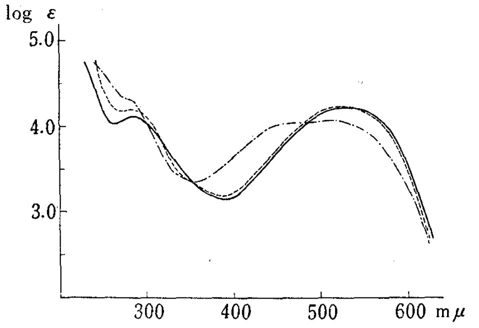
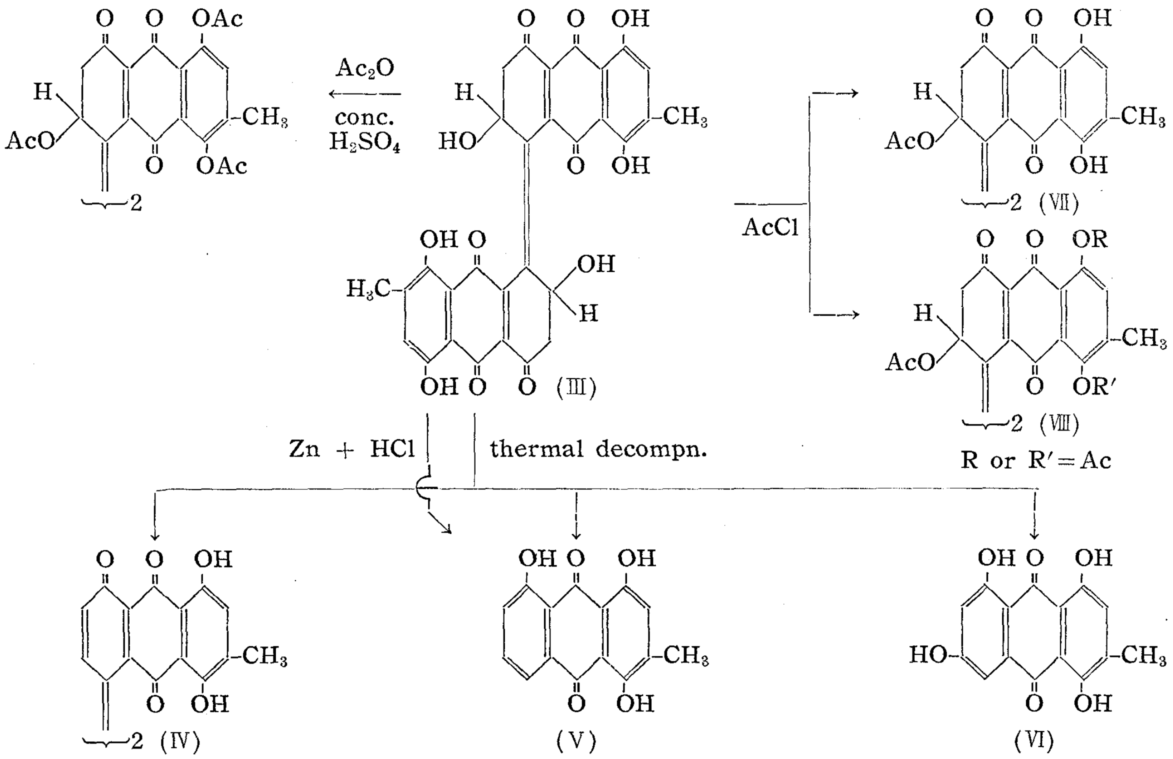


Fig. 2. Ultraviolet Spectra

——— Lumiluteoskyrin (in dioxane)
--------- Lumiluteoskyrin diacetate (in $CHCl_3$)
-·-·-·- Lumiluteoskyrin tetraacetate (in $CHCl_3$)

acetic anhydride and conc. sulfuric acid. The infrared absorption bands are given in Table I showing the presence of phenolic and alcoholic acetates and non-chelated C=O. As indicated by Howard and Raistrick,[6] the naphthazarin system in islandicin, as well as in rubroskyrin and iridoskyrin, is responsible for the instability of these compounds in alkali, fading their initial color on standing for a few days at room temperature. Lumiluteoskyrin looks fairly stable in aqueous alkali due to the less solubility of its alkaline salt, while it undergoes discoloration when its solution in methanoic potassium carbonate is allowed to stand for a few days.

Thus, the naphthazarin system in lumiluteoskyrin which has been suggested by the ultraviolet and visible light absorption spectra is also supported by the above chemical evidence. Consequently, the formula (Ⅲ) is proposed for lumiluteoskyrin. Although the double-bond linkage binding 1,1′-carbon atoms has not directly been proved, reduction of lumiluteoskyrin with zinc and hydrochloric acid successfully yielded islandicin (Ⅴ).



According to the previously mentioned results, the formation of lumiluteoskyrin was proved to result from photo-oxidation of luteoskyrin and this reaction was not affected by the nature of solvent employed. Accordingly, a possible mechanism is proposed for the

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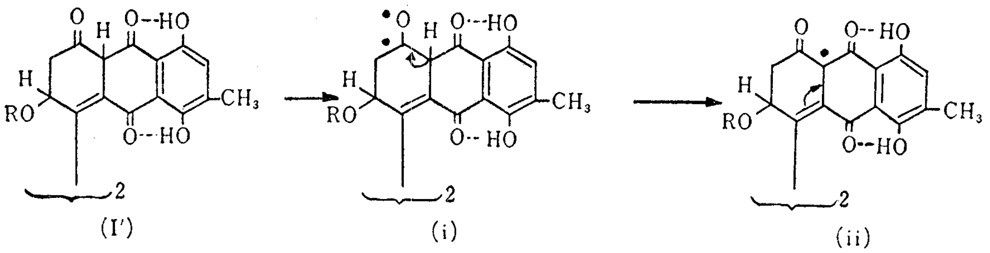
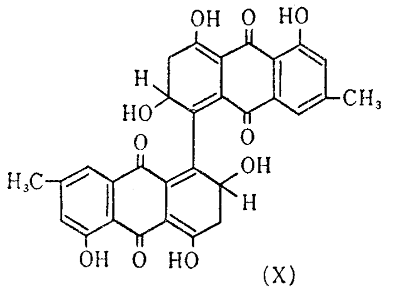
6) B. H. Howard, H. Raistrick : Biochem. J., **57**, 212 (1954).

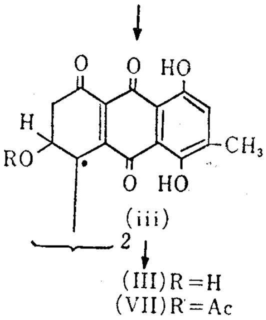
photo-reaction of luteoskyrin to form lumiluteoskyrin, which is shown below.

As the initial step of this reaction, the irradiation activates a non-chelated carbonyl in one of the tautomeric forms[*4] (I′) of luteoskyrin to give a radical (i) which is transformed to produce (ii) by the elimination of hydrogen. The hydroquinone system in the intermediate form (ii), which is strongly bonded with the neighboring ketones, stimulates the completion of naphthazarin structure by the migration of a double bond to the radical form (iii). The intramolecular coupling of the radical (iii : R=H) completes a double bond linkage between the two moieties to yield lumiluteoskyrin (III : R=H). The presence of hydroquinone system which would form strong hydrogen bond with the neighboring carbonyls seems to promote this photo-reaction, because rugulosin (X), a fungal pigment of *p*-hydroxyl-less homolog of luteoskyrin, is not transformed readily by irradiation of sunlight.





(X)



The stability of rubroskyrin (II) against irradiation would probably be explained by the initial stable naphthazarin structure of its molecule, which would prevent radical formation by irradiation.

Experimental

Formation of Lumiluteoskyrin from Luteoskyrin——Luteoskyrin (2 g.) was dissolved in hot Me_2CO (200 cc.) in a 500-cc. flask. After cool, the yellow-colored solution was exposed to sunlight, when the color changed to orange, red, and finally to reddish purple. On exposure for a few days, dark purple rhombic crystals separated out, which were collected and washed with Me_2CO. Yield, 600 mg.

The mother liquor was concentrated to 150 cc. and exposed again to sunlight to obtain 250 mg.

Table II.

Time (min.) / Atmosphere	0	10	15	20	25	30	75	5.5 hr.
Air	Y	Y-Or	Or	R	R	deep R	RP	Purple ppt. (Lumiluteoskyrin)
CO_2	Y	Y	Y-Or	Y-Or	Or	Or	Or	Brown ppt. (not identical with lumiluteoskyrin)
N_2	Y	Y	Y-Or	Y-Or	Or	Or	R	Small amount of purple ppt. (lumiluteoskyrin[a)])

Color observed : Y, yellow; Or, orange; Y-Or, yellowish orange; R, red; P, purple.

a) This might have been formed by O_2 contaminated in N_2 used.

[*4] Enolization of luteoskyrin in the presence of organic bases markedly suppressed this photo-reaction. It, therefore, seems that the keto form (I′) of luteoskyrin participates predominantly in the photo-transformation.

of purple crystals. The crystals separated out were almost pure lumiluteoskyrin, which was recrystallized from a mixture of $HCONMe_2$ and EtOH to crystals of m.p. above 360°. *Anal.* Calcd. for $C_{30}H_{20}O_{12}$: C, 62.93; H, 3.49. Found : C, 62.65, 62.68; H, 3.88, 3.97.

The photo-reaction was proved to be oxidative by the following observation. A solution of luteoskyrin dissolved in Me_2CO (0.02%) was divided into 3 portions and placed in tubes. The tubes were sealed after filling them with N_2, CO_2, or air, and exposed to sunlight. A change in color of the solution was observed by irradiation as recorded in Table II.

Paper Chromatography of Lumiluteoskyrin——Lumiluteoskyrin was developed on filter paper (Toyo Roshi No. 53) using the following solvent systems : Me_2CO-benzene-H_2O (1:2:1) (upper layer) Rf 0.9; Me_2CO-benzine-H_2O[7] (5:5:3.5) (upper layer) Rf 0.0.

Thermal Degradation of Lumiluteoskyrin——Lumiluteoskyrin (200 mg.) was heated in a sublimation tube in high vacuum (0.002 mm. Hg) at 315~320° for about 7 hr. Reddish crystals sublimed on the upper part of the tube and a brown sublimate was obtained from the lower part.

The red sublimate was chromatographed on a $CaHPO_4$ column using $CHCl_3$ as the solvent and separated into two orange-colored bands.

From the lower band, islandicin (40 mg.) was isolated, which was recrystallized from AcOH and identified on mixed fusion with the authentic sample, m.p. and mixed m.p. 216°. From the upper band, catenarin (35 mg.) was obtained, which was recrystallized from EtOH and identified by a mixed fusion with the authentic sample, m.p. and mixed m.p. 247~249°.

The brown sublimate obtained from the lower part of the sublimation tube was purified by repeated sublimation in high vacuum. This product seemed to be dianhydrolumiluteoskyrin, m.p. above 360°. *Anal.* Calcd. for $C_{30}H_{16}O_{10}$: C, 67.16; H, 2.99. Found : C, 66.87; H, 3.32. IR : $\nu_{\max}^{\text{Nujol}}$ 1601 cm^{-1} (chelated C=O; no free OH).

It is almost insoluble in most organic solvents and it forms a sparingly soluble violet Na salt with NaOH. It exhibits violet color with $(AcO)_2Mg$ in EtOH. Its violet suspension in NaOH is reduced with $Na_2S_2O_4$ to an orange red solution on heating in a boiling water bath. The violet color reappears on subsequent 2.5 hr.'s heating.

Acetylation of Lumiluteoskyrin with Acetyl Chloride in Acetic Acid giving Lumiluteoskyrin Diacetate and Tetraacetate——To a suspension of lumiluteoskyrin (100 mg.) in AcOH (20 cc.), AcCl (5 cc.) was added gradually during 15 min. with ice-cooling. After 2 days' standing at room temperature, AcCl (5 cc.) was added and the mixture was allowed to stand for further 2 weeks at room temperature, when the reaction mixture formed a red solution which was poured into ice-water. The precipitate formed was collected by filtration, washed with H_2O, and then chromatographed on a $CaHPO_4$ column, using benzene-Me_2CO (40:1) as the developing solvent. It separated into three main bands, from bottom to top, of yellow (I), red (II), and purple (III), along with several narrow bands.

The yellow band (I) gave a small amount of yellowish orange needles which were recrystallized from Me_2CO-hexane, but failed to give a pure substance.

The red band (II) gave red sandy crystals which were recrystallized from Me_2CO-hexane to crystals of m.p. above 360° (darkens from 320°). *Anal.* Calcd. for $C_{30}H_{16}O_8(OCOCH_3)_4$: C, 61.62; H, 3.78; mol. wt., 740. Found : C, 61.30; H, 3.84; mol. wt. (Barger-Akiya method), 732~882 (tetrahydrofuran as the solvent).

From the purple band (III) dark purple rods, m.p. above 360°, were obtained by recrystallization from $CHCl_3$-EtOH. *Anal.* Calcd. for $C_{30}H_{18}O_{10}(OCOCH_3)_2$: C, 60.67; H, 3.66. Found : C, 60.88, 61.10; H, 3.69, 3.47.

It was noticed that lumiluteoskyrin tetraacetate is partly deacetylated on standing overnight on the chromatographic column to yield lumiluteoskyrin diacetate.

Table III. Rf Values in Paper Chromatography

Compound Solvent system (upper layer)		Lumiluteoskyrin	Lumiluteoskyrin diacetate	Lumiluteoskyrin tetraacetate
Me_2CO-benzene-H_2 (1:1:2)	Rf :	0.90	1.00	1.00
Me_2CO-petr. benzine-H_2O (4:4:3)		0.00	0.00	0.61
Me_2CO-petr. benzine-benzene-H_2O (2:2:0.2:1)		0.00	0.96	0.96

Filter paper : Toyo Roshi No. 53.

Photo-reaction of Acetylated Luteoskyrin giving Lumiluteoskyrin Diacetate and Tetraaetate——Luteoskyrin (300 mg.) was dissolved in warm AcOH (20 cc.), cooled, and AcCl (5 cc.) was added slowly during 5 min. with ice-cooling. After standing overnight in a dark place, the reaction mixture was poured into ice-water (300 cc.) and separated yellow precipitate was collected and washed with H_2O.

Chromatography on $CaHPO_4$ using benzene-Me_2CO (40:1) separated it into several bands. The lowest, main yellow band which consisted of two unseparable bands gave a yellow residue. The

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7) S. Shibata, M. Takido, T. Nakajima : This Bulletin, **3**, 286 (1955).

yellow substance was dissolved in Me_2CO (100 cc.) and the solution was exposed to sunlight for a month, producing dark purple, fine plates (140 mg.) which were recrystallized from a mixture of $CHCl_3$ and EtOH to dark purple, thin rods, m.p. above 360°. *Anal.* Calcd. for $C_{30}H_{18}O_{10}(OCOCH_3)_2$: C, 60.67; H, 3.66. Found : C, 60.79; H, 3.67.

It gave a sky blue coloration with $(AcO)_2Mg$ in EtOH and it was identified with lumiluteoskyrin diacetate by the infrared spectrum and Rf value on paper chromatogram.

The mother liquor of Me_2CO separated from the crystals of lumiluteoskyrin diacetate was evaporated to one-half of its original volume and exposed to sunlight for further 3 days. The irradiated solution was evaporated to dryness and chromatographed on $CaHPO_4$ column, using benzene-Me_2CO (40:1) as the developing solvent, to be separated into 3 bands, from bottom to top, of yellow (I), red (Ⅱ) (main), and purple (Ⅲ). The yellow band (I) and purple band (Ⅲ) were narrow and the band (Ⅲ) was proved to be lumiluteoskyrin diacetate.

The red band (Ⅱ) gave sandy red crystals, m.p. above 360° (darkens from 320°) on recrystallization from Me_2CO-hexane. *Anal.* Calcd. for $C_{30}H_{16}O_8(OCOCH_3)_4$: C, 61.62; H, 3.78. Found : C, 61.49; H, 3.71.

This compound showed a purple coloration with $(AcO)_2Mg$ in EtOH and the same infrared spectrum and Rf value as those of lumiluteoskyrin tetraacetate.

Lumiluteoskyrin Hexaacetate——To a suspension of lumiluteoskyrin (100 mg.) in Ac_2O (3.5 cc.), 2 drops of conc. H_2SO_4 were added at room temperature. The original purple suspension changed immediately to dark yellowish brown and after 1 hr., turned into an orange solution. The mixture was poured into ice-water (300 cc.) and the separated yellowish orange precipitate was chromatographed on $CaHPO_4$, using benzene-Me_2CO (40:1) as the solvent. From the main orange band, an orange substance was isolated which was crystallized from $CHCl_3$-EtOH mixture to orange red prisms, m.p. 285° (decomp., darkens from 262°). *Anal.* Calcd. for $C_{30}H_{14}O_6(OCOCH_3)_6$: C, 61.16; H, 3.91. Found : C, 60.78, 61.21; H, 3.74, 3.88.

Reductive Cleavage of Lumiluteoskyrin with Hydrochloric Acid and Zinc——To a suspension of lumiluteoskyrin (100 mg.) in MeOH (10 cc.) 7% HCl (30 cc.) and Zn powder (1 g.) were added and the mixture was heated in a boiling water bath. After 7 min., color of the suspension changed to brown and then turned orange on further 6 min.'s heating. The mixture was heated for further 2.5 hr. and made alkaline by addition of 20% NaOH. The purple alkaline mixture was heated for 10 min. with occasional shaking, acidified with 25% HCl, and extracted with benzene-AcOEt mixture. The dark brown residue obtained after evaporation of the solvent was chromatographed on $CaHPO_4$, using benzene as the developing solvent. From the main orange band on the chromatogram, an orange substance was eluted which was purified by repeated sublimation *in vacuo* (at 170~180°, 3 mm. Hg) and finally by recrystallization from AcOH to orange red leaflets, m.p. 216° (mixed melting point with authentic sample of islandicin, 216°).

From the top purple band on $CaHPO_4$-column, a small amount of the starting material was recovered, which was identified by paper chromatography.

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Summary

The structure of a photo-reaction product of luteoskyrin, designated lumiluteoskyrin, has been discussed. Lumiluteoskyrin, $C_{30}H_{20}O_{12}$, dark purple crystals, m.p. above 360°, yielded a diacetate, $C_{30}H_{18}O_{10}(OCOCH_3)_2$, a tetraacetate, $C_{30}H_{16}O_8(OCOCH_3)_4$, and a hexa acetate, $C_{30}H_{14}O_6(OCOCH_3)_6$.

On thermal degradation, lumiluteoskyrin gave islandicin and catenarin. On reduction with alkaline sodium dithionite, lumiluteoskyrin was not cleaved, while by the action of hydrochloric acid and zinc, it yielded islandicin. The positive magnesium acetate reaction and the ultraviolet and visible light absorption spectra showed that lumiluteoskyrin is a true quinone compound possessing a naphthazarin system in its molecule. On the basis of the chemical and spectral evidences, a structural formula (Ⅲ) was proposed for lumiluteoskyrin and the photo-reaction mechanism was discussed.

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