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STUDIES ON FUNGAL METABOLITES—XXXI[1]

ANTHRAQUINONOID COLOURING MATTERS OF *PENICILLIUM ISLANDICUM* SOPP AND SOME OTHER FUNGI (−)LUTEOSKYRIN, (−)RUBROSKYRIN, (+)RUGULOSIN AND THEIR RELATED COMPOUNDS

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Abstract—The structures of (+)rugulosin, (−)luteoskyrin and (−)rubroskyrin have been reexamined by NMR and new structures **18**, **19** and **20** proposed respectively. Their absolute structures were established on the basis of the X-ray analysis of (+)dibromodehydrotetrahydrorugulosin (**27**). The minor analogous metabolites, (−)4a-oxyluteoskyrin (**31**) of *P. islandicum* and (+)4a-oxyrugulosin (**32**) of *P. brunneum*, have been formulated. On oxidation of (−)luteoskyrin and (+)rugulosin with pertrifluoroacetic acid (−)4a,4a′-dioxyluteoskyrin (**33**) and (+)4a,4a′-dioxyrugulosin (**34**) were formed, while with MnO_2 (+)4a,4a′-dehydrorugulosin (**35**) was obtained. The structures of lumiluteoskyrin (**37**), and deoxylumiluteoskyrin (**38**), photooxidation products of luteoskyrin and deoxyluteoskyrin, respectively, have been elucidated.

INTRODUCTION

The colouring matter of *Penicillium islandicum* Sopp was first studied by Raistrick *et al.*,[2] and later investigated by our research group.[3a–x] From the cultures of the mould more than twenty compounds have been isolated, almost all of which, except erythroskyrine, are grouped as (a) monomeric anthraquinones, (b) bianthraquinones and (c) modified bianthraquinones (Table 1). All the combinations of the monomeric anthraquinones isolated from the mould occur naturally as the bianthraquinones. The bianthraquinones possess no asymmetric C atom but nevertheless show optical activity due to the atropisomerism about the C–C linkage connecting two monomeric moieties, whose chiralities must be same, since their ORD curves give similar features with (+) Cotton effects in their longest wavelength regions. One of the modified bianthraquinones, (−)rugulosin recently found in the culture of *P. islandicum* Sopp NRRL 1175 was also isolated from *Myrothecium verrcaria* (Alb. et Schw.) Ditmer ex Fr.[4] The antipode, (+)rugulosin, was originally isolated from *Penicillium rugulosum* Thom,[5] and has been found along with (+)skyrin in various moulds,[6] even in some lichens.[7] (Table 2).

The hepatotoxicity of rice polluted with *Penicillium islandicum* has been extensively studied, and (−)luteoskyrin and a chlorine containing peptide, cyclochlorotine, were found to be responsible for the toxicity of the mould. Although the acute toxicity of luteoskyrin (LD_{50} 147 mg/kg (s.c.); 221 mg/kg (p.o.)) (mice) is not so high, the selective toxicity on liver is very remarkable in experimental animals causing morphological and functional damage and sometimes liver carcinoma.[8–10]

Both (+) and (−)enantiomers of rugulosin were also recognized as mycotoxins of the same series, but are less toxic than (−)luteoskyrin.[11–14]

Biochemical investigations on (−)luteoskyrin and its analogues have revealed that (−)luteoskyrin forms a complex with DNA in the presence of Mg^{2+} inactivating irreversibly DNA-dependent RNA polymerase.[15–17]

In an earlier investigation,[3d–f] (+)rugulosin $C_{30}H_{22}O_{10}$, m.p. 290° (dec), $[\alpha]_D$ +492° (dioxan), (−)luteoskyrin, $C_{30}H_{22}O_{12}$, m.p. 281° (dec.) and (−)-rubroskyrin, $C_{30}H_{22}O_{12}$, m.p. 281° (dec), were formulated as **15**, **16** and **17**, respectively.

Quinonic (−)rubroskyrin was transformed into non-quinonic (−)luteoskyrin, and (−)luteoskyrin was converted into a deep coloured quinonic compound, lumiluteoskyrin,[31] under illumination of light.

Dehydrating reagents convert luteoskyrin and rubroskyrin into iridoskyrin, and rugulosin into dianhydrorugulosin, whose structure was established by correlation with synthetic dichrysazin (4,4′).

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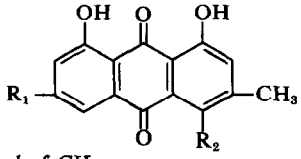
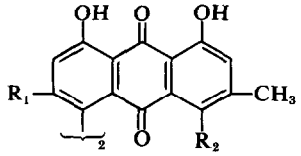
Table 1. The colouring matters produced by *Penicillium islandicum* Sopp

I. *Monomeric Anthraquinones*



Chrysophanol*	(1) $R_1 = R_2 = H$
Islandicin	(2) $R_1 = H$, $R_2 = OH$
Emodin	(3) $R_1 = OH$, $R_2 = H$
ω-Hydroxyemodin	(3′) $R_1 = OH$, $R_2 = H$, CH_2OH instead of CH_3
Endocrocin† = Emodin 2-carboxylic acid	
Catenarin	(4) $R_1 = R_2 = OH$

II. *Dimeric Anthraquinones*



(+)Dianhydrorugulosin	(5) $R_1 = R_2 = H$
(+)Iridoskyrin	(6) $R_1 = H$, $R_2 = OH$
(+)Skyrin	(7) $R_1 = OH$, $R_2 = H$
(+)Oxyskyrin	(7′) One of the methyls of skyrin is replaced by CH_2OH.
(+)Skyrinol	(7″) Both the methyls of skyrin are replaced by CH_2OH.
(+)Dicatenerin	(8) $R_1 = R_2 = OH$
(+)Roseoskyrin	(9) 1,1′-Dimer consisting of chrysophanol and islandicin.
(+)Auroskyrin	(10) chrysophanol and emodin.
(+)Rhodoislandin A	(11) chrysophanol and catenarin.
(+)Rhodoislandin B	(12) emodin and islandicin.
(+)Punicoskyrin	(13) islandicin and catenarin.
(+)Aurantioskyrin	(14) emodin and catenarin.

(On reductive cleavage with alkaline $Na_2S_2O_4$, these bianthraquinones are readily decomposed to give the monomeric anthraquinone components.)

III. *Modified bianthraquinones*

(−)Luteoskyrin
(−)Rubroskyrin
(−)Flavoskyrin*
(−)Rugulosin*
(−)Deoxyluteoskyrin
(−)Deoxyrubroskyrin
(−)4a-Oxyluteoskyrin

Erythroskyrine, a red pigment, $C_{26}H_{33}O_6N$,[3m.n] occurring in all the strains of *P. islandicum* is not an anthraquinonoid.

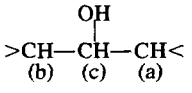
*Isolated from *P. islandicum* NRRL 1175.
†Isolated from a mutant of *P. islandicum* by Gatenbeck (*Acta Chem. Scand.* **13**, 386 (1959)).

The presence of aromatic methyls, phenolic and enolic hydroxyls and secondary alcohols and their relative dispositions were shown by the IR analysis and some chemical reactions.

*Renewed investigations on the structures of (−)-luteoskyrin, (−)rubroskyrin and (+)rugulosin**

The NMR data obtained recently are incompatible with the earlier proposed structures for (−)luteoskyrin, and (+)rugulosin, since there was no evidence to support the presence of a methylene at the position adjacent to the enol and secondary hydroxyls, which would be expected in the hydroaromatic ring of the former structures with an ABX type quartet with a large coupling constant in the higher field.

The signals (1 H each) marked a, b and c were characteristic in the NMR spectra of rugulosin and luteoskyrin. Decoupling experiments revealed the spin couplings between H(a)↔H(c), and H(b)↔H(c) to indicate the following partial structural system.



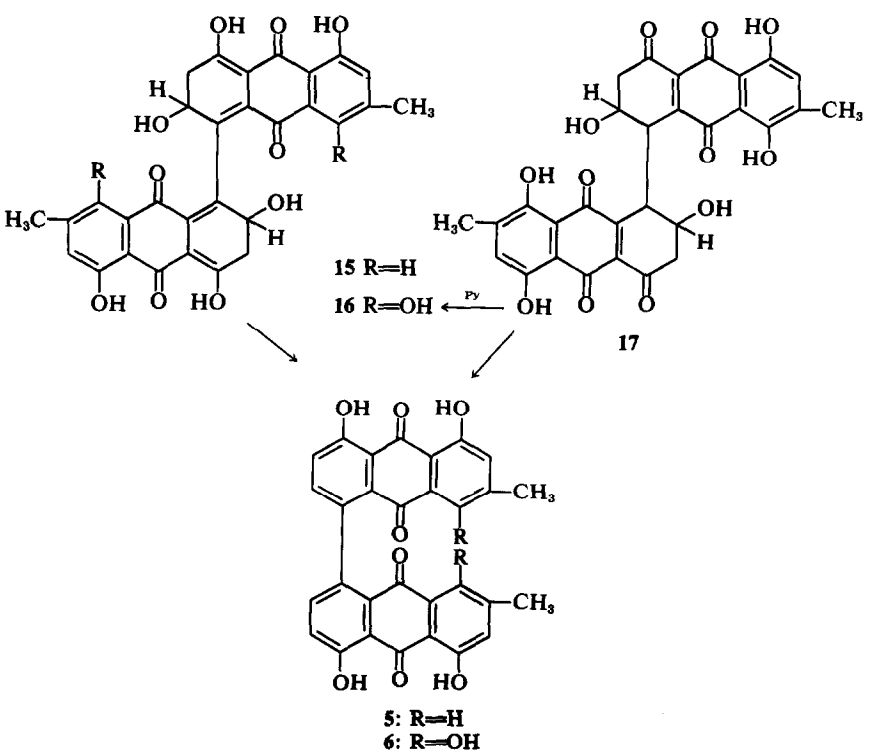
On catalytic reduction rugulosin afforded tetrahydrorugulosin whose IR spectrum showed the absence of non-chelated C=O at C_9. This has also been demonstrated by the NMR of tetrahydrorugulosin in giving a secondary alcoholic (>CH(OH)) group (δ 4·70 (H) and δ 6·24 (OH)).

*This part was preliminarily reported in Ref (3r and 3s).

Table 2. Occurrence of (+)rugulosin and (+)skyrin in fungi and lichens

Fungi:	(+)Skyrin		(+)Rugulosin		Ref.
Penicillium islandicum	+		−	((−)Rugulosin) in NRRL 1175)	
P. rugulosum	+		+		*a*
P. wortmanni	+		+		*a*
P. tardum	+		+		*b*
P. brunneum	+		+	((+)4a-Oxy-	*c*
P. variabile	−		+	rugulosin)	*d*
Sepedonium ampullosporum	+		+		*e*
Penicilliopsis clavariaeformis	+		−	(Penicilliopsin)	*e*
Endothia parasitica	+		+		*f*
E. fluens	+		+		*f*
E. gyrosa	+		+		*g*
E. longirostris	+		−		*g*
E. tropicalis	+		−		*g*
			−		*h*
Lichens					
Acroscyphus sphaerophoroides	+		+		*h*
Trypethliopsis boninensis	+		−	(Oxyskyrin, skyrinol)	*i*
Physcia obscura var. *endococcina*	+	(=Rhodo-physcin (Zopf))	−		*j*
Pyxine endochrysina	+		−		*k*

References: [a]J. Breen, J. C. Dacre, H. Raistrick and G. Smith, *Biochem. J.* **60**, 618 (1955); [b]Y. Yamamoto, A. Hamaguchi, I. Yamamoto and S. Imai, *Yakugaku Zasshi* **76**, 1428 (1956); [c]S. Shibata and S. Udagawa, *Chem. Pharm. Bull. Tokyo* **11**, 402 (1961); [d]M. Yamazaki, private comm; [e]S. Shibata, J. Shoji, A. Ohta, and M. Watanabe, *Pharm. Bull. Tokyo* **5**, 380 (1957); [f]S. Shibata, O. Tanaka, T. Murakami, G. Chihara and M. Sumimoto, *Ibid.* **3**, 274 (1955); [g]L. H. Briggs and P. W. Le Quesne, *J. Chem. Soc.* 2290 (1965); [h]S. Shibata, O. Tanaka, U. Sankawa, Y. Ogihara, R. Takahashi, S. Seo, D. N. Yang, Y. Iida, *J. Jap. Bot. Tokyo* **43**, 335 (1968); [i]J. Santesson, *Acta Chem. Scand.* **23**, 3331 (1970); [j]W. Steglich, Private comm. (Sept. 1971); [k]I. Yosioka, K. Morimoto, K. Murata, H. Yamaguchi and I. Kitawawa, *Chem. Pharm. Bull. Tokyo* **19**, 2420 (1971).



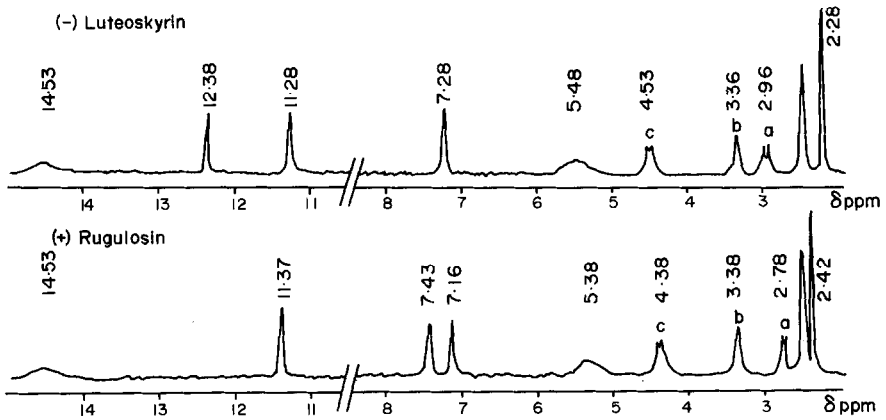


Fig 1. The NMR spectra of (−)Luteoskyrin and (+)Rugulosin (in d_6-DMSO).

The broad singlet signal of the proton attached to carbon (C_9) bearing an OH was shifted on acetylation to the down-field (δ 6·21) very sharp singlet.

This indicated that the C atoms at 9a and 9'a adjacent to the reduced carbonyls at 9 and 9'-positions are quaternary. A remarkably higher shift of the signal (b) of rugulosin was observed on reduction of the carbonyls at 9 and 9' positions into secondary alcohols. This can be explained by assigning the signal (b) to the protons at C_1 and $C_{1'}$, which must be shifted higher by removal of the deshielding effect of the carbonyls at 9 and 9' positions. The same result was obtained in the catalytic reduction of the carbonyls at 9 and 9'-positions of luteoskyrin. Thus the following partial structures are supported by the spectral data.

dimer. But the NMR data of the triacetate indicated that it must be a non-equivalent dimer having a quinonic structure and a methylene bearing ring system in one monomeric moiety, while the other monomeric half is the same as that of luteoskyrin. The quinonic nature of rubroskyrin is demonstrated by an intensive green colouration with magnesium acetate in alcohol.

Partial acetylation of rubroskyrin yielded a triacetate, $IR_{max}^{CHCl_3}$ ν cm^{-1}: 1770 (sh) (enolic acetate C=O), 1745 (alcoholic acetate C=O), 1705 (6-membered ring C=O), 1655 (sh), 1640 (chelated C=O), 1620 (benzene ring). Since rubroskyrin was recovered in a good yield on hydrolysis of the acetate, no rearrangement of the skeletal structure occurred during acetylation.

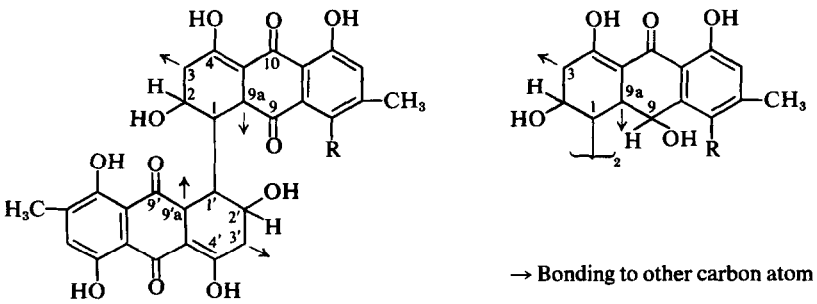
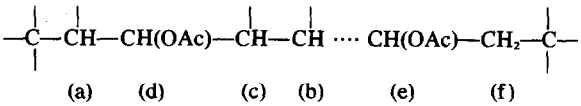


CHART 1

There are two possibilities to satisfy the above partial structure by the formation of C–C linkages, e.g. (i) C_3–$C_{9'a}$ and $C_{3'}$–C_{9a} (inter-monomeric moieties), or (ii) C_3–C_{9a} and $C_{3'}$–$C_{9'a}$ (intra-monomeric moieties). The latter possibility was discarded by the NMR analysis of rubroskyrin triacetate as mentioned later.

Rubroskyrin, which is isomerized readily into nonquinonic luteoskyrin by the action of basic reagents, was regarded earlier as being a symmetric

The NMR spectrum of rubroskyrin triacetate showed the signals (e and d) of protons on the carbon bearing acetoxyl at δ 5·53 (br.s.) and δ 5·30 (br.tr.), respectively. An ABX type pair of doublets (f) at δ 1·56, 1·21 (J = 17·5 Hz) was observed to reveal the presence of —CH_2—, which is coupled with a proton (e). Apart from this, the spin decoupling experiments showed the following coupling correlation: a↔d↔c and c↔b. This can be assigned to the following system:



Since the coupling constants of the signals of protons at C_1, C_2 and C_3 of rubroskyrin triacetate are different from those given by the corresponding protons of rugulosin and luteoskyrin, the structures involving the inter-monomeric linkages are more plausible for these modified bianthraquinones than the intramonomeric linking formulae, otherwise the coupling pattern of those protons of rubroskyrin triacetate must be same as those of acetates of rugulosin and luteoskyrin.

Thus rugulosin, luteoskyrin and rubroskyrin are formulated as **18**, **19** and **20**, respectively, which could be formed biogenetically by the base-catalysed Michael-type intramolecular condenzation of a hypothetical intermediate, a partially hydrogenated biantraquinone (**21**).

On the other hand, Bu'Lock[18] isolated from a strain of *Penicillium islandicum* monomeric modified anthraquinones, tetrahydrocatenarin (quinone A) and dihydrocatenarin (quinone B), the latter corresponding to the quinonic monomeric half of rubroskyrin, though he formulated it with the quinonic system in the middle ring. By analogy with the findings by Scheuer *et al.*[19,20] as the signal of proton on the quinone ring of alkyl naphthazarin derivatives appears in higher field than the benzenoid protons, the proton signals at δ 6·80 and δ 7·15 given by rubroskyrin triacetate should be assigned to the

Table 3. NMR spectra of rugulosin and luteoskyrin (in d_6-DMSO)

Positions of proton	Chemical shift (δ) Rugulosin (**18**)	Luteoskyrin (**19**)
(a) H (3,3′)	2·78 (d. J = 5·5 Hz)	2·96 (d. J = 5·5 Hz)
(b) H (1,1′)	3·38 (br.s.)	3·36 (br.s.)
(c) H (2,2′)	4·38 (br.d. J = 5·5 Hz)	4·53 (br.d. J = 5·5 Hz)
CH_3 (7,7′)	2·42 (s.)	2·28 (s.)
Arom. H	7·16 (d. J = 1 Hz)	7·28 (s.)
	7·43 (d. J = 1 Hz)	—
Phenolic OH (5,5′)	11·37 (s.)	11·28 (s)
	—	12·38 (s.)
Enolic OH (4,4′)	14·54 (s.)	14·53 (s.)
Alcoholic OH (2,2′)	5·38	5·48

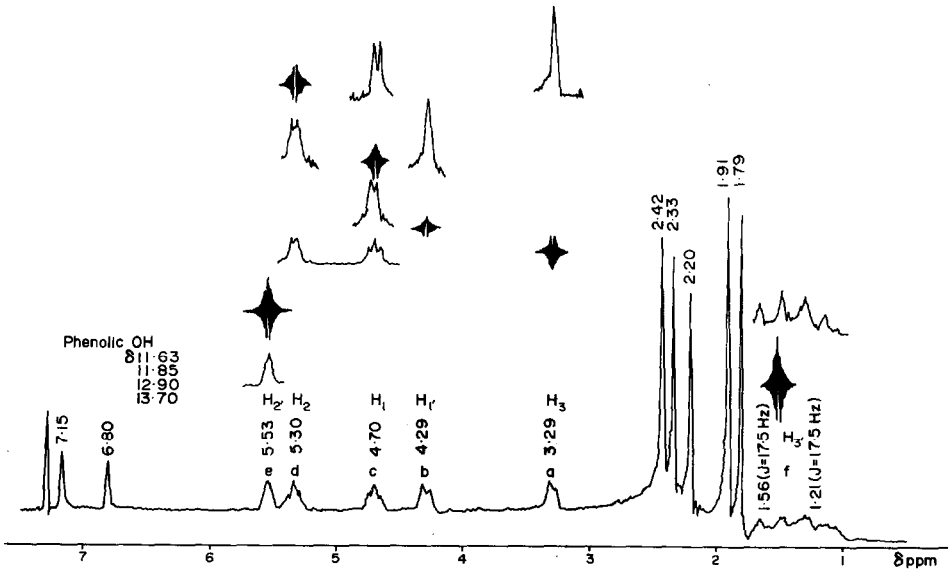
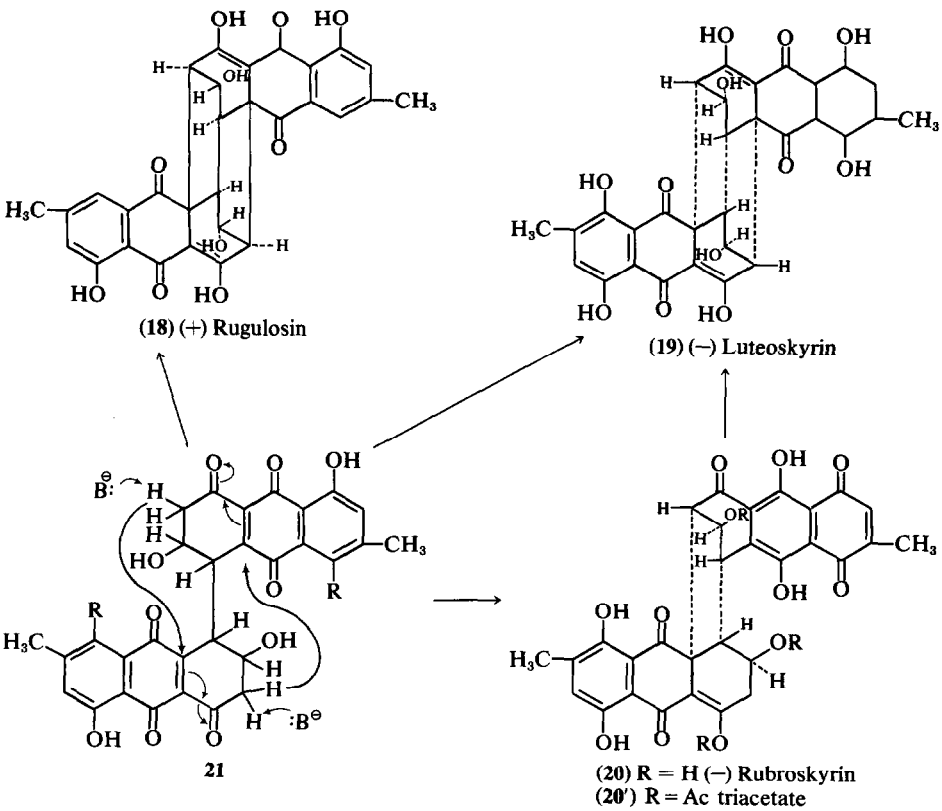


Fig 2. The NMR spectrum of (−)Rubroskyrin Triacetate (in $CDCl_3$).

Table 4. NMR spectrum of rubroskyrin triacetate (in $CDCl_3$)

Positions of proton	Chemical shift (δ)
(f) 2H (3′)	1·21, 1·56 (a pair of AB doublets $J = 17·5$ Hz)
(e) H (2′)	5·53 (br.s.)
(b) H (1′)	4·29 (br.d. $J = 5$ Hz)
(c) H (1)	4·70 (br.tr. $J = 5$ Hz)
(d) H (2)	5·34 (br.tr. $J = 5$ Hz)
(a) H (3)	3·29 (br.d. $J = 5$ Hz)
CH_3 (7)	2·20 (s.)
CH_3 (7′)	2·33 (s.)
Arom. H(6)	6·80 (s.)
Arom. H (6′)	7·15 (s.)
Acetyl CH_3 (4′)	2·42 (s.)
(2) or (2′)	1·91 (s.), 1·79 (s.)
Phenolic OH (5,5′ 8,8′)	11·63, 11·85, 12·90, 13·70

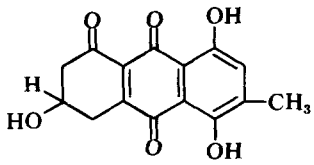


quinonoid and benzenoid proton, respectively, and the former signal is coupled weakly with the Me signal at δ 2·20. Thus the *p*-quinone system of rubroskyrin must be on the ring bearing the Me group. Scheuer's quinonoid directing effect also favours the existence of a *p*-quinone system on the end ring of a monomeric half of ruboskyrin.

The stereochemistry of these compounds indicated by the prefix (+) or (−) is based on the following stereochemical correlations.

On dehydration with thionyl chloride and pyridine at 0°, (+)rugulosin afforded (−)dianhydrorugulosin, (**5**), while (−)luteoskyrin and (−)rubroskyrin yielded (+)iridoskyrin (**6**). (−)Dianhydrorugulosin was treated with pertrifluoroacetic acid in dichloromethane at 0° to give (−)iridoskyrin and (−)roseoskyrin (**9**). The ORD curve of (−)roseoskyrin, m.p. 280° (dec.), is the reverse of the naturally occurring (+)roseoskyrin.[3p]

Thus the chiralities about the C–C linkage con-



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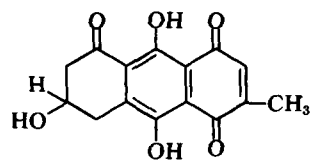

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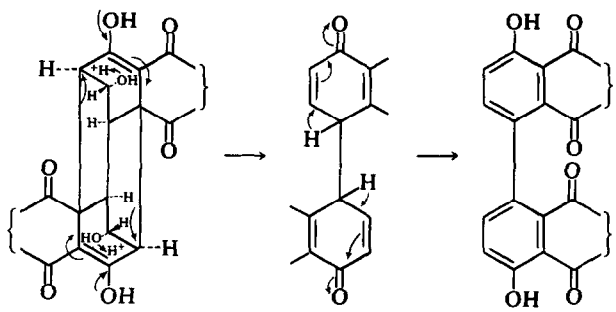
necting two monomeric halves of (−)luteoskyrin and (+)rugulosin were proved to be opposite each other.

(−)Deoxyluteoskyrin and (−)Deoxyrubroskyrin

As the minor colouring matter of *Penicillium islandicum* NRRL 1036, (−)deoxyluteoskyrin, giving IR absorptions at 1625 cm^{-1} (chelated C=O) and 1690 cm^{-1} (non-chelated C=O), and (−)deoxyrubroskyrin, $C_{30}H_{22}O_{11}$, red crystals, m.p. 255°, giving a blue colouration with magnesium acetate in alcohol and IR absorptions at 1690 and 1670 cm^{-1} (non-chelated C=O) and 1618 cm^{-1} (chelated C=O), were isolated.

(−)Deoxyrubroskyrin is readily converted into (−)deoxyluteoskyrin by the action of bases similar to the conversion of rubroskyrin into luteoskyrin.

The NMR pattern of (−)deoxyluteoskyrin is such



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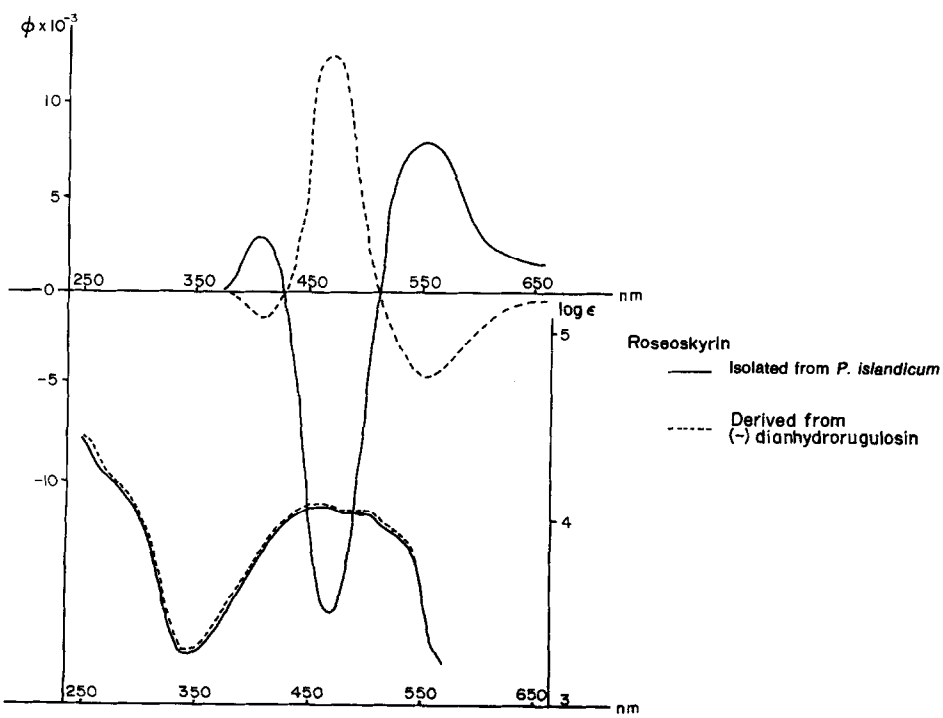
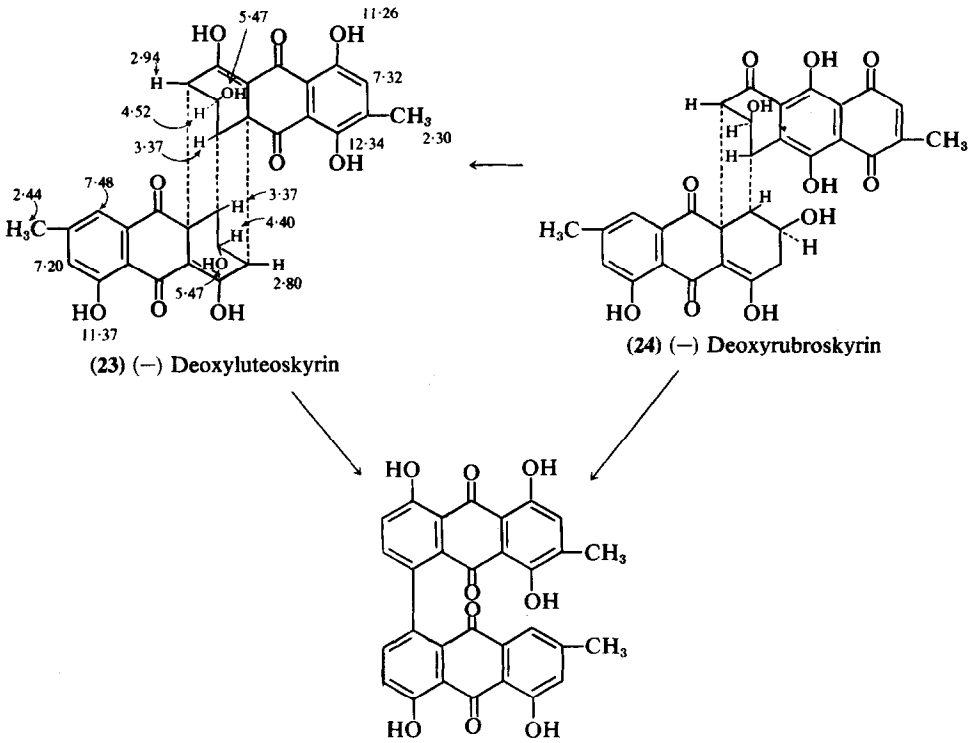


Fig 3. The ORD and UV absorption spectra of (+) and (−)roseoskyrin (in Dioxan).



(Figures indicated are NMR signals (δ ppm) measured in d_6-DMSO)

that it must be a non-equivalent dimer consisting of monomeric halves of (−)luteoskyrin and (−)-rugulosin. The UV spectrum of (−)deoxyrubroskyrin suggests a combination of those of rugulosin and rubroskyrin and therefore is a non-equivalent dimer consisting of the monomeric half of (−)rubroskyrin and (−)rugulosin.

On dehydration with thionyl chloride in pyridine (−)deoxyluteoskyrin and (−)deoxyrubroskyrin afforded (+)roseoskyrin which yielded islandicin and chrysophanol on reductive cleavage with alkaline sodium dithionite.

As previously reported, on thermal decomposition (−)luteoskyrin and (−)rubroskyrin yielded catenarin and islandicin, while (+)rugulosin afforded emodin and chrysophanol. Under the same conditions, (−)deoxyluteoskyrin and (−)deoxyrubroskyrin gave chrysophanol, emodin, islandicin and catenarin in the same molar ratio.

Electron-impact also induced similar fragmentations with these bimolecular modified anthraquinones: Luteoskyrin (M^+ 574) gave catenarin (*m/e* 286) and islandicin (*m/e* 270); rugulosin (M^+ 542) afforded emodin (*m/e* 270) and chrysophanol (*m/e* 256); deoxyluteoskyrin (M^+ 558 (5%)) showed an overlapped pattern of fragmentation signals given by luteoskyrin and rugulosin (*m/e* 286 (40%), 270 (100%), 256 (50%) (1:2:1)), and the same result was given by deoxyrubroskyrin.

*The absolute stereochemical structures of (+)-rugulosin, (−)luteoskyrin and (−)rubroskyrin on the basis of X-ray analysis of (+)dibromodehydrotetrahydrorugulosin**

In order to undertake an X-ray crystallographic analysis a bromination product of (+)tetrahydrorugulosin was prepared.

(+)Tetrahydrorugulosin was prepared by catalytic hydrogenation of (+)rugulosin. Bromination of this compound, (+)dibromodehydrotetrahydrorugulosin, as yellow plates, $C_{30}H_{22}O_{10}Br\cdot H_2O\cdot 2CH_3OH$, on recrystallization from acetone–methanol–water.

The crystal was placed in sealed thin walled capillaries filled with the vapour of the solvent to avoid the deterioration of crystal form. The crystals, mol. wt. 784, $[\alpha]_D + 317°$ (in dioxan), are monoclinic with space group P_{2_1} and the unit-cell dimensions are $a = 9{\cdot}78$, $b = 17{\cdot}04$, $c = 9{\cdot}45$ Å, and $\beta = 98{\cdot}0°$. Two formular units are contained in the cell. Dm = 1 1·64 g.cm^{-3} in dibromomethane–methanol solution. Dx = 1·671 g.cm^{-3}.

The crystal structure was solved by the heavy

*The detail of the X-ray crystallographic experiment was reported in *Acta Cryst* **B26**, 188 (1970).

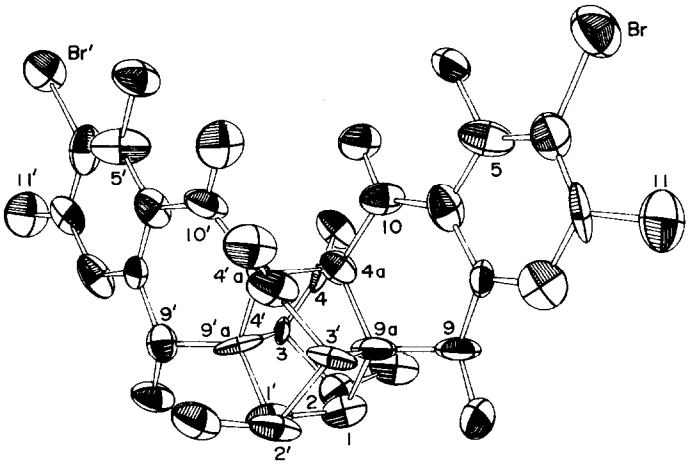


Fig 4. A perspective drawing of the absolute structure of (+)dibromodehydrotetrahydroruglosin drawn by the plotter program ORTEP.

atom method and refined by the block-matrix-squares method including anisotropic thermal parameters.

The final R-value for 1482 non-zero observed structure factors was 0·109.

The absolute configuration was determined by the use of the anomalous dispersion of bromine atoms for CuKα radiation.

It is obvious that during the bromination process introducing bromine to the aromatic rings at the 6 and 6'-positions of (+)tetrahydrorugulosin a dehydrogenation takes place simultaneously to form a new linkage between 4a and 4'a of the molecule.

This has been demonstrated in the IR and NMR spectra by the disappearance of enolic OH of tetrahydrorugulosin and appearance of 5-membered ring C=O on bromination. The positions of bromination at C_6 and $C_{6'}$ are also indicated by the disappearance of proton signal at δ 6·67 of tetrahydrorugulosin on bromination.

Since the hydrogenation takes place from the less hindered side of C=O at C_9 and C_9 of (+)rugulosin, the secondary alcoholic hydroxyls at $C_{(9)}$ and $C_{(9')}$ of (+)tetrahydrorugulosin must be equatorial (δ 4·70 ($C_{9,9'}$–H); δ 6·24 ($C_{9,9'}$–OH)).

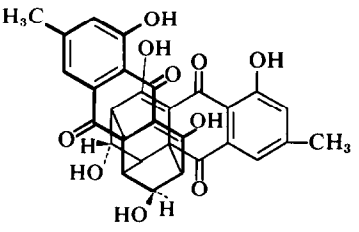
From the established absolute stereochemical structure of (+)dibromodehydrotetrahydrorugulosin (**27**), the absolute structures of (+)tetrahydrorugulosin (**26**) as well as that of (+)rugulosin (**25** = **18**) have been deduced unequivocally as illustrated above. Referring the stereochemical evidence previously mentioned, the absolute stereochemical structures of (−)rugulosin, (−)luteoskyrin and (−)-rubroskyrin are now formulated as **28**, **29** and **30**, respectively.

(−)4a-*Oxyluteoskyrin and* (+)4a-*oxyrugulosin*

As a minor component of the pigments of *Penicillium islandicum* Sopp NRRL 1036, (−)4a-oxyluteoskyrin, $C_{30}H_{22}O_{13}$, yellow needles, m.p.>

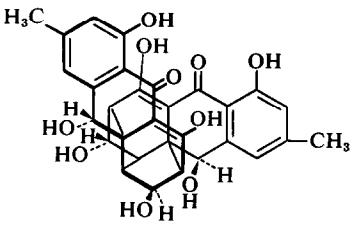
Table 5. Spectral evidences for the derivation of (+)dibromodehydrotetrahydrorugulosin from (+)rugulosin

	(+)Rugulosin	(+)Tetrahydro-rugulosin	(+)Dibromodehydro-tetrahydrorugulosin
IR ν_{max}^{KBr} cm^{-1}	1603 C_{10}=O	1605 C_{10}=O	1608 C_{10}=O
	1688 C_9=O	—	—
	—	—	1755 C_4=O (5-membered ring C=O)
NMR d_6 DMSO (δ ppm)	—	4·70 C_9—H	4·93 C_9—H
	—	6·24 C_9—OH	6·48 C_9—OH
	7·16 C_6—H	6·67 C_6—H	—
	14·54 C_4—OH	14·90 C_4—OH	—



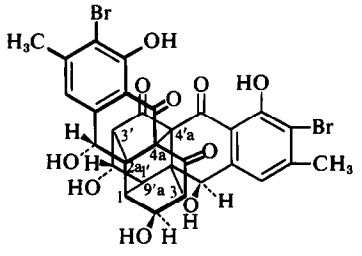
25 = 18

H_2/PtO_2



26

↓ Br_2



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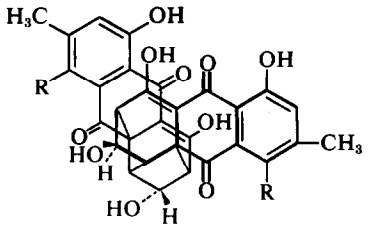
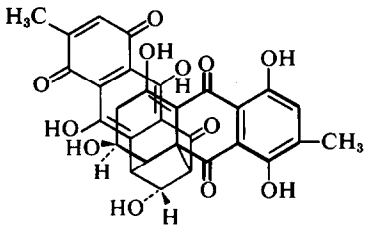


(28) R = H (−) Rugulosin
(29) = (19) R = OH(−) Luteoskyrin



(30) = (20) (—) Rubroskyrin

250° (dec.) (**31**), was isolated, while (+)4a-oxyrugulosin, $C_{30}H_{22}O_{11}$, yellow rods, m.p. >270° (dec.) (**32**), was obtained from *P. brunneum* Udagawa.

These compounds could also chemically be derived from (−)luteoskyrin and (+)rugulosin, respectively, by the oxidation with pertrifluoroacetic acid. In this case dihydroxylated analogues of both compounds were also formed. The IR and NMR data indicated that hydroxyls are introduced at the angular positions, 4a and 4'a, (**33** and **34**).

The IR absorptions at 1735 or 1740 cm^{-1} showed the presence of a strained 6-membered ring C=O at C_4 or $C_{4\text{and}4'}$ of these compounds.

On oxidation of (+)rugulosin with MnO_2 yielded (+)4a,4'a-dehydrorugulosin, $C_{30}H_{18}O_{10}$, yellow needles, m.p. 278° (dec.).

The NMR spectrum of this compound in comparison with that of (+)rugulosin indicated structure **35**. The IR absorption at 1760 cm^{-1} showed the presence of a fused 5-membered ring C=O.

*Lumiluteoskyrin**

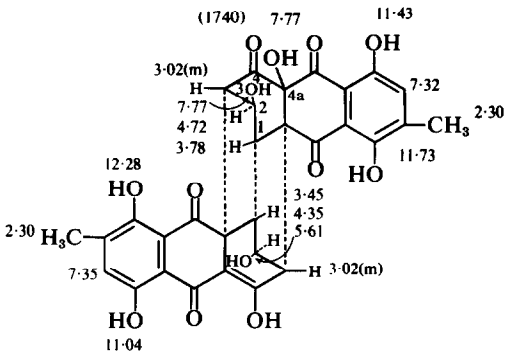
(−)Luteoskyrin is highly photosensitive in solution being converted into a deep coloured quinonic compound, lumiluteoskyrin, $C_{30}H_{20}O_{12}$, m.p. >370°, for which we earlier proposed the structural formula **36**.

As the earlier structural formula (**16**) of luteoskyrin has been revised, the structure of lumiluteoskyrin should also be corrected.

The UV spectral curve of lumiluteoskyrin resembles closely those given by dihydrocatenarin (Bu'Lock's quinone B) (**22'**) and rubroskyrin (**20**). The blue colouration of lumiluteoskyrin with magnesium acetate is very similar to that given by quinone B indicating the presence of a similar quinonic chromophore.

The IR absorption of C=O at 1693 cm^{-1} ($\alpha\beta$-unsaturated 6-membered ring C=O) given by lumiluteoskyrin corresponds to that given by rubroskyrin at 1697 cm^{-1}. The NMR spectrum and its decoupling experiments of lumiluteoskyrin tetraacetate, $C_{30}H_{16}O_8(OCOCH_3)_4$, which is reversible to lumiluteoskyrin on hydrolysis, revealed that it is a symmetric dimer possessing the following func-

*This part was preliminarily reported in Ref (3u).



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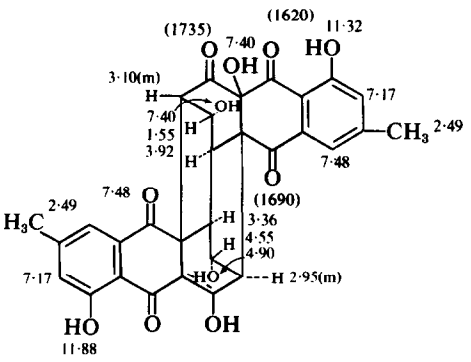

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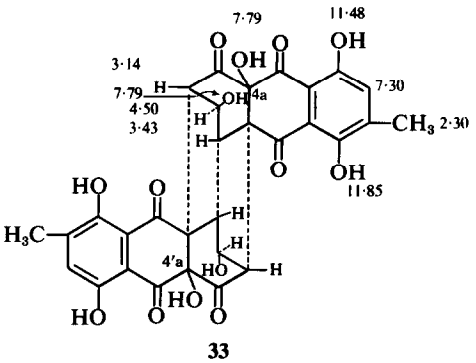

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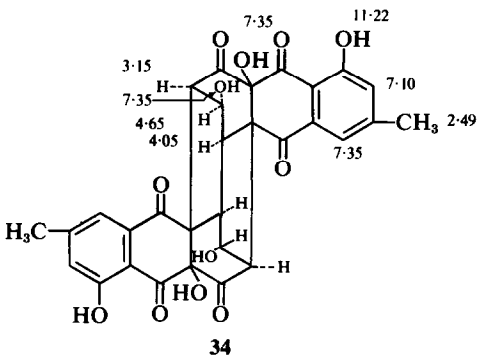

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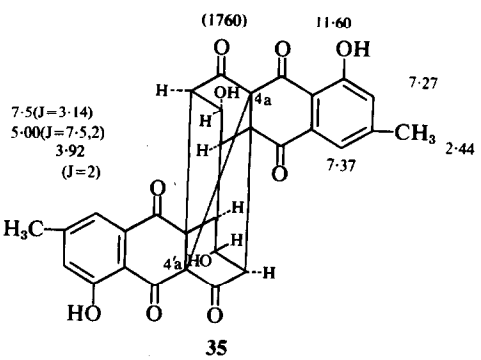
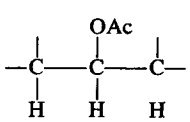


35

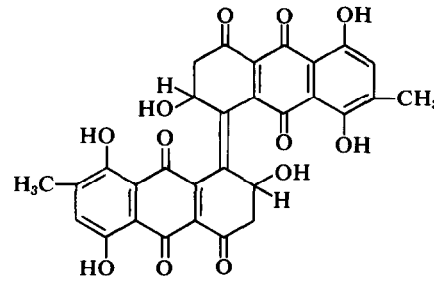
Figures indicated are NMR signals measured in d_6-DMSO, and those in parentheses are IR absorptions in KBr (cm^{-1})

tional groups or a partial structure:



On the basis of the established structure of (−)-luteoskyrin (**19**), the above NMR data can be assigned to the following structural formula of lumiluteoskyrin (**37**), and the photoreaction which has also been observed with (−)deoxyluteoskyrin (**23**) is elucidated by the following mechanism involving the split of C_3–C_9 linkage and the 1–4 migration of C_3–C_{9a} bond to form a new C_3–$C_{3'}$ linkage.

As (+)rugulosin is stable under illumination of light, the presence of a monomeric half of luteoskyrin is essential for the photo-sensibility. In this case, the first triplet state might be stabilized by the resonance with the neighbouring 1,4-diphenolic system.



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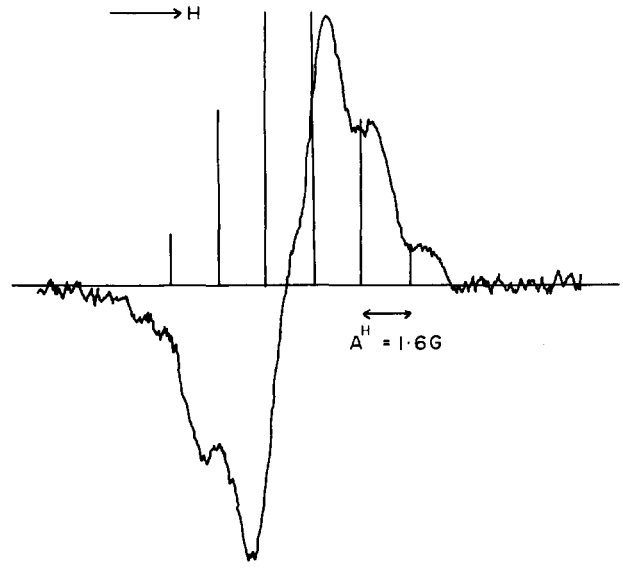


Fig 5. The ESR spectrum of (−)Luteoskyrin under the illumination of light (> 350 nm) in DMSO.

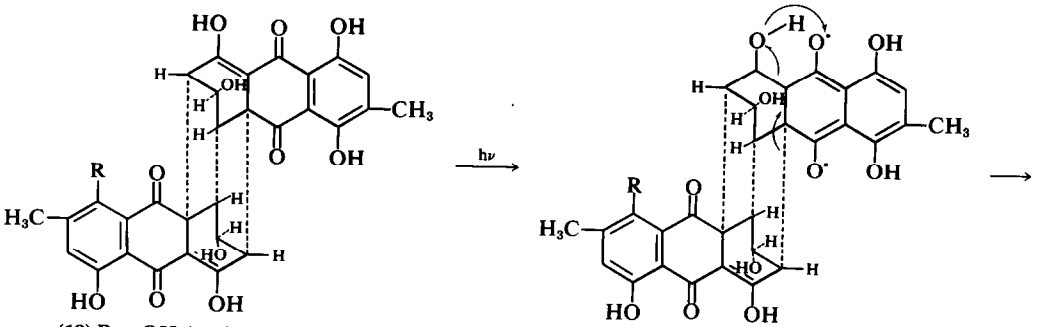
The ESR spectrum of luteoskyrin under illumination of light (> 350 nm) exhibited 5 fine structure to reveal the formation of a radical state with 5 protons in the neighbouring positions. Referring Scheuer's *quinonoid directing effects of substituents* and his observation on the NMR signals of quinonoid and benzenoid protons, the quinone structure of lumiluteoskyrin (**37**) and deoxylumiluteoskyrin (**38**) must be located in the end of the ring system (δ 6·79, q, $J = 1$ Hz, quinonic ring proton coupled with CH_3). As previously reported, luteoskyrin tetraacetate is converted into lumiluteoskyrin tetraacetate. This could be explained in an analogy of the acetyl migration of naphthazarin diacetate system.

*The full details and experiments of the X-ray crystallographic studies of the (+)dibromodehydrotetrahydrorugulosin is described in a paper published in *Acta Cryst.* **B26**, 188 (1970).[3b]

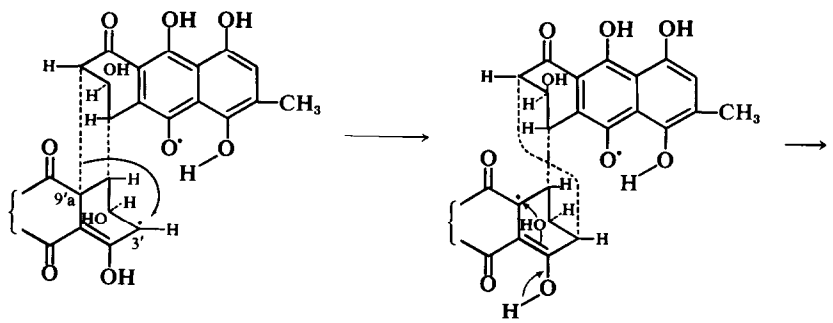
EXPERIMENTAL

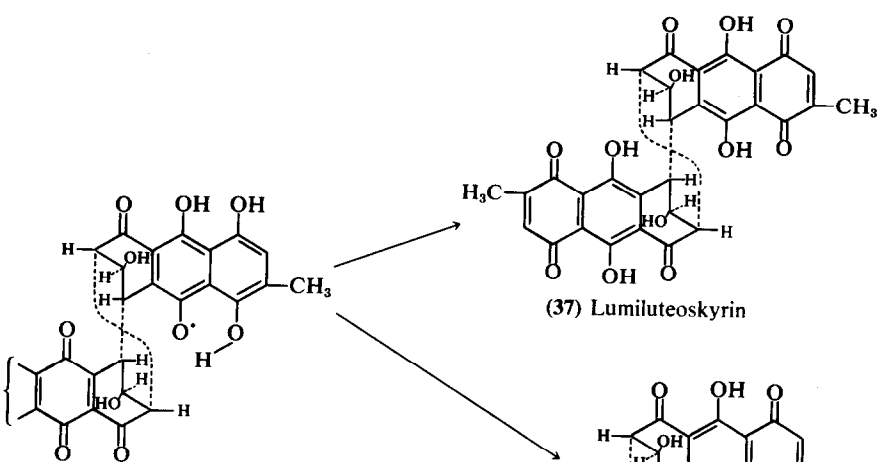
M.ps were determined on a Yanagimoto m.p. apparatus and are uncorrected. NMR spectra were measured on a Japan Electron Optics Lab. JMH-4H-100 (100 MHz) instrument with TMS as the internal standard. Optical rotations were measured with a Yanagimoto Photomagnetic Polarimeter Model OR-50. ORD curves were recorded with JASCO ORD Recorder Model ORD/UV-5. UV absorption spectra were measured in EtOH on a Cary Spectrometer Model 11 or a Hitachi ESP-3T. IR spectra were taken on a JASCODS-402G spectrophotometer. ESR spectra were measured with JEOL JES-P-10 type, X-band with 100 Kc modulation.

Isolation of the pigments of Penicillium islandicum Sopp *NRRL 1036, Ud and E-stains.* The moulds were cultivated stationarily on Czapek-Dox medium for 2 weeks at 27°. The dried mycelia were extracted with acetone in the dark. The acetone extracts were refluxed with benzene to dissolve islandicin, chrysophanol, iridoskyrin, roseoskyrin and dianhydrorugulosin. The residual portion was dissolved in acetone and chromatographed over an activated charcoal column to adsorb anthraquinones and bianthraquinones, which were recovered by elution with 1 N

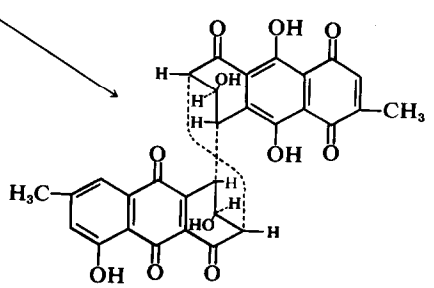


(19) R = OH (−) Luteoskyrin
(23) R = H (−) Deoxyluteoskyrin





(37) Lumiluteoskyrin



(38) Deoxylumiluteoskyrin

CHART 3

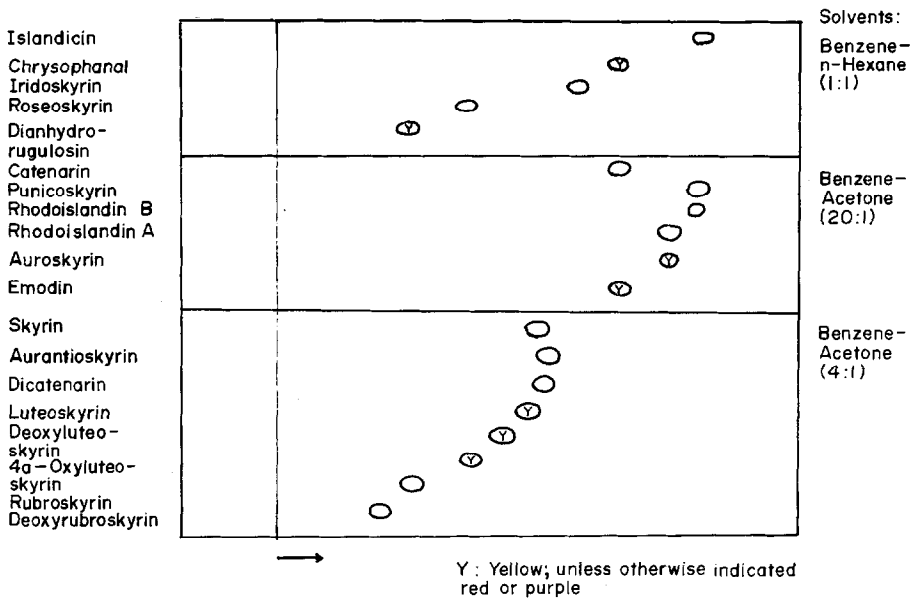


Fig 6. The TLC of the pigments of *Penicillium islandicum* Sopp developed on silica gel G impregnated with 0·5 N oxalic acid.

NaOH followed by acidification. The recovered pigments were chromatographed again on silicic acid to isolate catenarin, emodin, skyrin, auroskyrin, and aurantioskyrin, rhodoislandin A and rhodoislandin B. Some anthraquinones were separated after acetylation.

The modified bianthraquinones were eluted from the carbon column with acetone, and rechromatographed on silicic acid or silica gel using benzene–acetone (9:1) as the solvent. (−)Luteoskyrin, (−)rubroskyrin, (−)deoxyluteoskyrin and (−)deoxyrubroskyrin and (−)4a-oxyluteoskyrin were separated from this fraction.

TLC was undertaken using a plate of silica gel G impregnated with 0·5 N oxalic acid and the solvent systems, benzene–hexane (1:1), benzene–acetone (9:1) and benzene–acetone (4:1).

The properties of the pigments of Penicillium islandicum Sopp

Anthraquinones. Chrysophanol, islandicin, emodin and catenarin are all known.

Table 6. NMR spectrum of lumiluteoskyrin tetraacetate (in $CDCl_3$)

	Chemical shift (δ)	
Acetyl CH_3	1·79	
	2·46	
Quinone ring—CH_3	2·20	(d. $J = 1$ Hz)
(a) H (3,3′)	3·25	(d. $J_{ac} = 2{\cdot}5$ Hz)
(b) H (1,1′)	4·45	(q. $J_{bc} = 3$ Hz, $J_{ab} = 1$ Hz)
(c) H (2,2′)	5·23	(br.d. $J_{ac} = 2{\cdot}5$ Hz, $J_{bc} = 3$ Hz)
Quinone ring—H	6·79	(q. like $J = 1$ Hz)
Phenolic OH	12·95	

Bianthraquinones. The properties of skyrin, iridoskyrin, dianhydrorugulosin and dicatenarin were described in Raistrick's and our earlier papers.

The bianthraquinones newly isolated are characterized by their R_f-values on TLC, their original colours, their magnesium acetate colourations and the products of reductive cleavage with alkaline $Na_2S_2O_4$. (See Table 1.)[3a]

The 1,1′-dimeric structures were proposed for these pigments by the analogy of skyrin, iridoskyrin and dianhydrorugulosin whose structures were established earlier. Neither rhodoislandins A and B nor their acetates were separated chromatographically. The yields of the products of reductive cleavage of rhodoislandins A and B mixture were determined spectrometrically. In this case, chrysophanol and catenarin were obtained 1:1 in a higher grade of yield, while emodin and islandicin were afforded 1:1 in a lower grade of yield. Thus the dimer of the former combination was named rhodoislandin A and the latter rhodoislandin B. The NMR data of the acetates of anthraquinones and bianthraquinones of *P. islandicum* are tabulated as follows in Table 7.

The optical activities were observed with these bianthraquinones as illustrated in the ORD curves (Fig 3). A prefix (+) is attached to all the bianthraquinones which show (+) Cotton effect at the highest wavelength region of ORD curves.

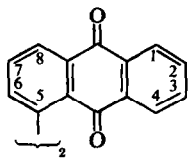
Modified bianthraquinones. The physical and chemical properties of (−)luteoskyrin and (−)rubroskyrin were described in our earlier papers.

(−)*Deoxyluteoskyrin.* Yellow crystals, m.p. 293° (from acetone), $[\alpha]_D - 610°$ ($c = 0{\cdot}0059$ in dioxan), M: 558. IR ν_{max}^{KBr} cm^{-1}: 1690, 1618. (Found: C, 63·50; H, 4·29. $C_{30}H_{22}O_{11}$ $\frac{1}{2}H_2O$ requires C, 63·49; H, 4·05). By the action of $SOCl_2$ in pyridine, it was converted into (+)roseoskyrin which yielded islandicin and chrysophanol on reductive cleavage with alkaline $Na_2S_2O_4$.

(−)*Deoxyrubroskyrin.* Red crystals, m.p. 255° (from

Table 7. The NMR spectra of peracetates of anthraquinones and bianthraquinones of *Penicillium islandicum* (δ (ppm)) in $CDCl_3$

Positions of protons		Peracetate of Chrysophanol	Islandicin	Emodin	Catenarin	Dianhydro-rugulosin	Iridoskyrin	Skyrin	Oxyskyrin	Dicatenarin	Roseoskyrin	Punicoskyrin	Auroskyrin	Aurantioskyrin
—OCOCH$_3$	6			2·38	2·37			1·93	1·91	1·96				1·93
	6′							1·93	1·91	1·96		1·99	1·85	1·94
	8	2·45	2·42	2·46	2·45	2·52	2·47	2·50	2·49	2·46	2·49	2·46	2·50	2·48
	8′					2·52	2·47	2·50	2·49	2·46	2·49	2·46	2·50	2·48
	1	2·45	2·42	2·46	2·45	2·50	2·45	2·47	2·49	2·46	2·49	2·46	2·50	2·48
	1′					2·50	2·45	2·47	2·49	2·46	2·49	2·46	2·50	2·48
	4		2·49		2·51		1·86			1·96		1·88		
	4′						1·86			1·96	1·89	1·92		1·90
—CH$_3$	3	2·50	2·30	2·53	2·36	2·41	2·22	2·42	2·41	2·22	2·42	2·23	2·42	2·39
	3′					2·41	2·22	2·42	(CH_2OAc) 5·12 2·12	2·22	2·21	2·23	2·42	2·19
Arom. —H	5	8·20	8·05	8·01	7·87									
	6	7·73	7·68			7·42	7·40				7·42	7·32	7·40	
	6′					7·42	7·40				7·42			
	7	7·37	7·32	7·20	7·17	7·42	7·40	7·38	7·40	7·38	7·42	7·37	7·40	7·41
	7′					7·42	7·40	7·38	7·40	7·38	7·42	7·31	7·30	7·46
	2	7·20	7·24	7·23	7·23	7·16	7·20	7·15	7·17	7·20	7·18	7·23	7·20	7·16
	2′					7·16	7·20	7·15	7·38	7·20	7·18	7·23	7·20	7·16
	4	7·98		7·94		7·66		7·69	7·69		7·70		7·70	7·72
	4′					7·66		7·69	7·69				7·70	



Numbering of bianthraquinones referred in this table

acetone), IR ν_{max}^{KBr} cm^{-1} 1710, 1690, 1670. UV λ_{max}^{dioxan} nm (log ε): 280 (4·89), 369 (4·00), 380 (4·00), 510 (4·93), 531 (3·92), 570 (3·63). On treatment with pyridine, it was transformed into (−)deoxyluteoskyrin.

(−)*4a-Oxyluteoskyrin.* Yellow needles m.p. >250° (dec.) (from acetone). IR ν_{max}^{KBr} cm^{-1}: 1740. (Found: C, 60·26; H, 4·07. $C_{30}H_{22}O_{13}\cdot\frac{1}{2}H_2O$ requires C, 60·10; H, 3·84.)

Isolation of the pigments of Penicillium brunneum Udagawa. The mycelia (166 g) of *Penicillium brunneum* Udagawa cultivated stationarily on Czapek-Dox media at 27° for 3 weeks were extracted first with *n*-hexane and then ether and acetone subsequently. The orange red solid obtained on evaporation of the extracts was dissolved in acetone and chromatographed over an active carbon column to adsorb (+)skyrin. (+)Rugulosin was obtained in a yellow crystalline form on concentration of the acetone eluate. The mother liquor was chromatographed on a column of silica gel impregnated with 0·5 N oxalic acid using benzene–acetone (10:1) as the solvent, when (+)rugulosin and (+)4a-oxyrugulosin (87 mg) were separated.

The properties of the pigments of Penicillium brunneum

The physical and chemical properties of skyrin and (+)-rugulosin were described in Raistrick's and our earlier papers.

(+)*4a-Oxyrugulosin.* Yellow prisms, m.p. > 270° (from acetone–benzene). It shows an orangered fluorescence under UV-illumination. IR ν_{max}^{KBr} cm^{-1}: 1735, 1690. (Found: C, 64·21; H, 3·89. $C_{30}H_{22}O_{11}$ requires C, 64·51; H, 3·94.)

(−)*Dihydrorubroskyrin.* (−)Rubroskyrin (400 mg) was reduced catalytically for 6 hrs in EtOH using PtO_2 as a catalyst. The reaction mixture which was separated from the catalyst was evaporated, and the residue was

chromatographed over a silicic acid column using benzene–acetone (4:1) as the eluting solvent. After removing unchanged (−)rubroskyrin first, a dark red coloured band was separated as the main part. Dark red crystals, m.p. 270° (from EtOH). (Found: C, 61·48; H, 4·54. $C_{30}H_{24}O_{12}\cdot\frac{1}{2}H_2O$ requires: C, 61·33; H, 4·29%); IR ν_{max}^{KBr} cm^{-1}: 1670, 1615, 1585; UV λ_{max} nm (log ϵ): 280 (5·08), 400 (3·89), 510 (3·80), 534 (3·78), 574 (3·46).

(−)*Rubroskyrin triacetate.* Yellow needles, m.p. 262–263°, $[\alpha]_D^{31}$ −333° (dioxan) was prepared by acetylation of (−)rubroskyrin (50 mg) with Ac_2O (2 ml) and *p*-toluenesulphonic acid (10 mg) at room temp. The ppts obtained by pouring the mixture into ice water were chromatographed on silica gel impregnated with acid using benzene–acetone (20:1) to separate an orange band as the main product (yield: 25 mg). Orange crystals, m.p. 262–263° (from acetone–EtOH). (Found: C, 61·85; H, 4·17. $C_{36}H_{28}O_{15}$ requires: C, 61·85; H, 4·03%).

(−)*Dianhydrorugulosin from* (+)*rugulosin.* To the soln of (+)rugulosin (100 mg) dissolved in pyridine (5 ml) was added dropwise $SOCl_2$ under stirring and ice-cooling until a yellow brown colour of the soln turned into faint yellow. Stirring was continued further for a few min, and then water was added to the mixture. Orange ppt was recrystallized from $CHCl_3$ as yellow needles, m.p. > 300°, which showed an optical activity, $[\alpha]_D^{23}$ −1600°. Under some other conditions, only orange crystals which showed no optical activity were obtained. This product was proved to be identical with (±)dianhydrorugulosin prepared earlier by the action of HCOOH on (+)rugulosin. The IR spectra taken in KBr tablets showed a slight difference between (−) and racemic dianhydrorugulosins.

(+)*Iridoskyrin from* (−)*luteoskyrin.* (−)Luteoskyrin was treated as above described for (+)rugulosin. The addition of $SOCl_2$ was stopped when the original red violet colour of the solution changed to greenish yellow. The brown red ppt from the mixture on addition of water was taken up in ether, and the residue obtained on evaporation of the solvent was chromatographed over silicic acid with benzene. (+)Iridoskyrin was obtained as red crystals, m.p. > 300°. The same compound was also formed from (−)rubroskyrin under the same conditions.

Hydroxylation of (−)*dianhydrorugulosin (formation of* (−)*roseoskyrin and* (−)*iridoskyrin).* To a soln of (−)-dianhydrorugulosin (60 mg) dissolved in CH_2Cl_2 (20 ml) was added dropwise pertrifluoroacetic acid reagent under stirring and ice-cooling.

The pertrifluoroacetic acid reagent was prepared by mixing trifluoroacetic acid (0·2 ml) in CH_2Cl_2 (5 ml) with 80% H_2O_2 (0·2 ml) in CH_2Cl_2 (2 ml) under ice-cooling. After the addition of the peracid reagent was complete, the mixture was kept overnight at room temp. under stirring. The excess of peracid was decomposed carefully by washing the mixture with 10% $NaHCO_3$.

The solvent was removed from the organic layer after washing with water and drying over Na_2SO_4. The products were separated on TLC using silica gel impregnated with 0·5 N oxalic acid as the plate and benzene-n-hexane (2:1) as the solvent. (−)Roseoskyrin, red crystals, m.p. 275–280° (dec.) was isolated in a yield of 4·6 mg, whose TLC and IR spectrum were proved to be identical with (+)roseoskyrin isolated from *Penicillium islandicum* NRRL 1036. (−)Iridoskyrin, deep red crystals, m.p. > 300°, was obtained in a yield of 2·5 mg, whose TLC and IR spectral patterns were identical with those of (+)iridoskyrin naturally occurring in *P. islandicum* NRRL 1036.

(±)*Roseoskyrin pentaacetate.* (±)Roseoskyrin was acetylated with Ac_2O and pyridine at room temp. on standing overnight. The product was separated chromatographically using a silica gel column impregnated with 0·5 N oxalic acid and benzene–acetone). (Found: C, 65·45; H, 3·77. $C_{30}H_{13}O_9(COCH_3)_5$ requires C, 65·57; H, 3·85%).

Reductive cleavage of (±)*roseoskyrin.* (±)Roseoskyrin was dissolved in 1 N NaOH. $Na_2S_2O_4$ was added to the soln and heated on a boiling water-bath, when the colour of the soln changed from red violet to colourless, and again red violet. The mixture, neutralized with 10% HCl gave an orange ppt which was taken up in ether and treated as usual. The products were separated on a silicic acid column using benzene-n-hexane (1:1) as the solvent to isolate chrysophanol and islandicin.

(+)*Tetrahydrorugulosin.* (+)Tetrahydrorugulosin was prepared by the catalytic hydrogenation of (+)rugulosin (470 mg) using PtO_2 (200 mg) in EtOH (200 ml), pale yellow needles, m.p. 285° (dec.), $[\alpha]_D^{28\cdot5}$ +210° (in dioxan), IR ν_{max}^{KBr} cm^{-1}: 1605 (chelated C=O).

Octaacetate. (+)Tetrahydrorugulosin was acetylated with Ac_2O and pyridine at room temp. m.p. 230° (dec.), $[\alpha]_D^{28}$ = +60° (in dioxan).

(+)*Dibromodehydrotetrahydrorugulosin.* To a soln of (+)tetrahydrorugulosin (155 mg) in THF newly prepared dioxane dibromide* was added dropwise. After 1·5 hr stirring, the mixture was allowed to stand at room temp further 3 hr. The excess of Br_2 was consumed by the addition of $NaHSO_3$. The product was chromatographed over silica gel impregnated with 0·5 N oxalic acid using benzene–acetone as the developing solvent. On recrystallization from acetone–MeOH–water, it formed yellow prisms, m.p. 298° (dec.), $[\alpha]_D^{25\cdot5}$ +345° (in dioxan), yield: 100 mg. (Found: C, 50·65; H, 3·28. $C_{30}H_{22}O_{10}Br_2\cdot\frac{1}{2}H_2O$ requires: C, 50·63; H, 3·37%); UV: λ_{max}^{dioxan}, nm (log ϵ): 225 (4·40), 279 (inflex. 4·03), 289 (4·22), 3·46 (3·86); IR: ν_{max}^{KBr} cm^{-1}: 1608 (chelated C=O), 1755 (5-membered ring C=O); ORD (in dioxan) nm ($\phi \times 10^4$): 368 (+2·48), 342 (−1·82), 316 (+1·16), 294 (−5·78), 247 (+4·13).

Acetylation of lumiluteoskyrin (formation of lumiluteoskyrin tetraacetate). Lumiluteoskyrin (100 mg) was suspended in Ac_2O (4 ml) and *p*-toluenesulphonic acid (20 mg) was added. The mixture was stirred for 4 hr at room temp to form a yellowish soln. On a silicic acid column developed with benzene–acetone (7:1) an orange yellow band was separated as the main product, which was proved by TLC and IR spectrum (KBr) to be identical with lumiluteoskyrin tetraacetate, yield: 60%).

Lumiluteoskyrin leucoacetate. A mixture of crude peracetate (110 mg) of lumiluteoskyrin, Ac_2O (3 ml) and AcONa (500 mg) was added with Zn dust (300 mg) under stirring. The colour of mixture turned into pale greenish yellow on heating the mixture under stirring. After 1 hr AcOH (10 ml) was added, and the mixture was heated to dissolve NaOAc. Zn- dust was filtered off and the filtrate was poured into ice water. The ppt formed was separated, washed and dried. On recrystallization from acetone–EtOH it formed pale yellow crystals, m.p. > 280°; IR $\nu_{max}^{CHCl_3}$ cm^{-1}: 1770, 1753, 1633, 1190; NMR (in $CDCl_3$) δ 1·80 ($C_{2,2'}$-OAc), 2·35 ($C_{7,7'}$-CH_3), 2·45 (8×OAc), 3·45 ($C_{3,3'}$-H $W_{\frac{1}{2}}$ 6 Hz), 3·73 ($C_{1,1'}$-H $W_{\frac{1}{2}}$ 5 Hz), 5·20 ($C_{2,2'}$-H $W_{\frac{1}{2}}$ 8 Hz), 7·16 ($C_{6,6'}$-H sharp singlet). (Found: C, 58·41; H, 4·99. $C_{50}H_{44}O_{12}\cdot 2H_2O$ requires: C, 58·14; H, 4·68%).

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