Tetrahedron Letters No.27, pp. 3179-3184, 1968. Pergamon Press. Printed in Great Britain.

THE REVISED STRUCTURES OF LUTEOSKYRIN, RUBROSKYRIN AND RUGULOSIN

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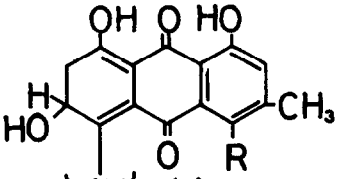
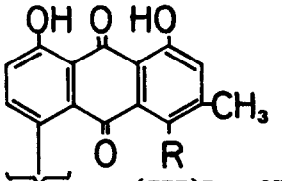
(Received in Japan 23 March 1968; received in UK for publication 11 April 1968)

In our earlier studies (1), we proposed to formulate (-) luteoskyrin, $C_{30}H_{22}O_{12}$, m.p. 281°(decomp.), $[\alpha]_D$ -880°, a hepatotoxic yellow pigment of Penicillium islandicum Sopp (NRRL 1036, UD and E strains) as (I), and (+)rugulosin, $C_{30}H_{22}O_{10}$, m.p. 290°(decomp.), $[\alpha]_D$ +492°, a yellow pigment isolated from P. rugulosum Thom and some other fungi as (II). The formulations were based mainly on the negative quinonic colour reactions, the formation of bianthraquinones, iridoskyrin (III) and dianhydrorugulosin(IV), respectively, by dehydration reaction, and the IR spectra showing chelated carbonyl (1620 cm^{-1}) in luteoskyrin and rugulosin, and non-chelated carbonyl (1690 cm^{-1}) in rugulosin. The presence of phenolic or enolic hydroxyls and alcoholic hydroxyls in the both pigments was proved by the IR spectral absorptions of acetate carbonyl at 1773 and 1751 cm^{-1}, respectively in O-hexaacetylrugulosin and O-octaacetylluteoskyrin.



(I)R = OH
(II)R = H



(III)R = OH
(IV) R = H

The NMR spectral analysis which was not available at the time of the earlier investigation has now been performed to reexamine the structures of the above compounds. The NMR(in d_6-DMSO) signals of aromatic methyls ((R)** 2.42δ;(L)***2.28δ), phenolic hydroxyls ((R)11.37δ;(L)11.28δ,12.38δ), enolic hydroxyls ((R)14.54δ;(L)14.53δ) and aromatic protons ((R)7.16δ(d),7.43δ(d): (L)7.28δ(s)) of the both compounds agreed well with the aromatic part of the

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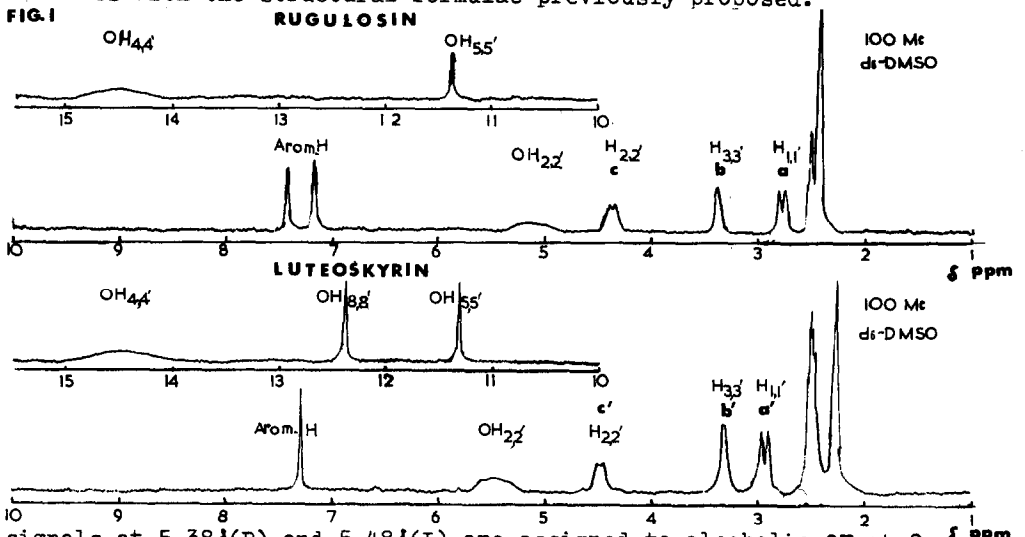
(R): Rugulosin; *(L): Luteoskyrin

structures, whereas the coupling patterns of the non-aromatic part showed some discrepancies with the structural formulae previously proposed.

FIG. 1



The signals at 5.38δ(R) and 5.48δ(L) are assigned to alcoholic O<u>H</u> at 2 and 2' positions, since they disappeared along with phenolic and enolic O<u>H</u> signals in the down field by the addition of D_2O. The proton signal (c or c') was assigned to the proton at the carbon atom (2 or 2') bearing alcoholic hydroxyl, because it showed down-field shift by acetylation of the hydroxyl.

Irradiation at (c)* made (a)**and (b)***sharp singlets,while irradiation at (a) caused collapse of the signal (c) to singlet . According to the formula (I) for luteoskyrin and (II) for rugulosin in which a system of -CH(OH)-CH_2-C(OH)= is involved, a resonance of methylene with a large coupling constant (J: ca 20 cps) would be expected. The experimental results were not the case.

Tetrahydrorugulosin, $C_{30}H_{26}O_{10}$, m.p. 295° (decomp.),$[\alpha]_D$ + 172°(Me_2CO), prepared by the catalytic reduction of rugulosin (1) showed the disappearance of non-chelated carbonyl by the IR spectrum, while the NMR spectrum gave new appearance of the signal of secondary alcohol at 4.70δ(H) and 6.24δ(O<u>H</u>). On acetylation the former signal is shifted to 6.21δ to form a sharp singlet which indicates the absence of proton at the adjacent carbon atom of the newly formed secondary alcohol group. The disappearance of non-chelated carbonyl by the reduction of rugulosin caused a remarkable up-field shift of the proton signal (b) (at 3.38δ) to 2.70δ. A similar effect was shown in the aromatic protons (7.43δ, 7.16δ→ 7.05δ, 6.67δ).

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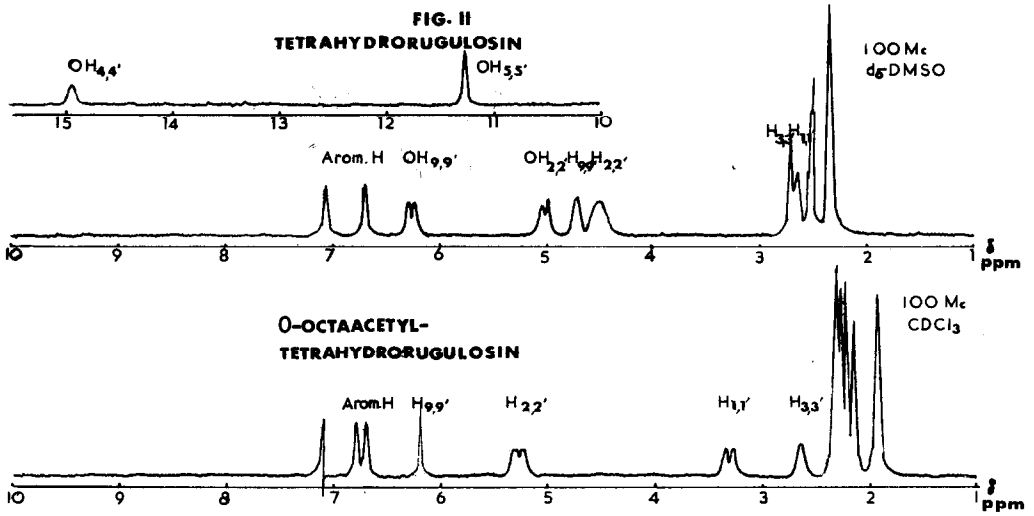
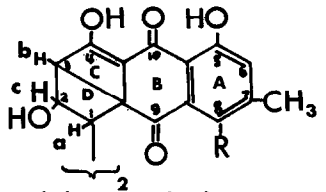
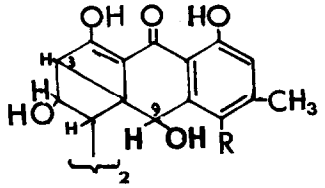
* (c,c'):Doublet,J= 5.5 cps **(a,a'):Doublet,J= 5.5 cps ***(b,b')Broad singlet.



All the results of the NMR spectral analyses are satisfied when the structural formulae of luteoskyrin and rugulosin are partly modified to (V) and (VI), respectively.



(V)R= OH (VI)R=H

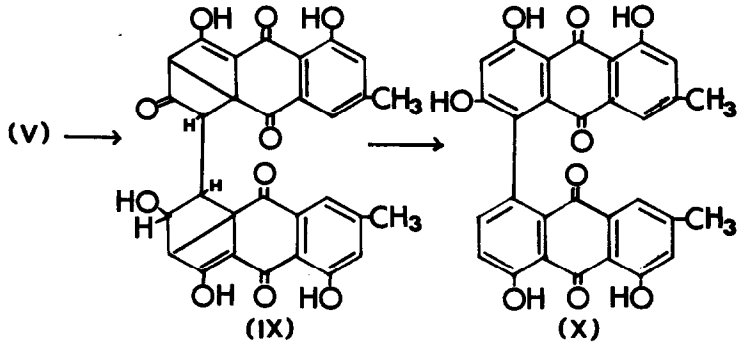


(VII)R= OH (VIII)R=H

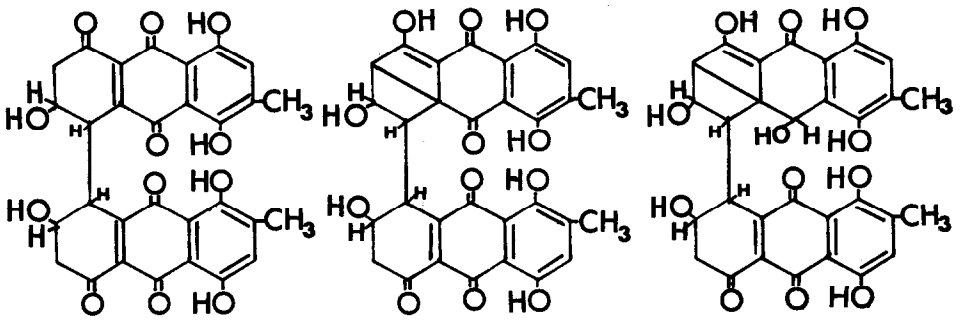
The proton signals (a,a; b,b' and c,c') of the non-aromatic part of luteoskyrin and rugulosin are assigned as shown in Fig.1. The fused four-membered ring system of the C and D rings in the non-aromatic part of luteoskyrin and rugulosin seems to be somewhat peculiar, but it is demonstrated to be less-strained in the molecular model, which also shows a stereochemically close location (s-cis) of the carbonyl at $C_{(9,9')}$ and the proton at $C_{(3,3')}$ to give an agreement with the remarkable up-field shift of the signal of proton at $C_{(3,3')}$ by the reduction of the carbonyl at $C_{(9,9')}$.

In order to give an additional support for the existence of four-membered ring system, the Jones oxidation of secondary alcohol was carried out. The resulted product showed an IR-absorption at 1770 cm^{-1} corresponding to a four-membered ring ketone. The NMR spectrum revealed that only the half of the dimeric molecule was suffered by the oxidation and the other half was remained unchanged. On treatment with boiling formic acid the product (IX)

was aromatized to yield auroskyrin (X) (2), and the reaction would be formulated as follows:



Rubroskyrin, $C_{30}H_{22}O_{12}$, m.p. 281° (decomp.), which is occurring in P. islandicum along with luteoskyrin and other pigments is noted to be readily converted into luteoskyrin by the action of organic bases and into iridoskyrin on dehydration. Rubroskyrin was formulated formerly as (XI) mainly based on its quinonic nature (magnesium acetate reaction: green), the IR spectrum (1706 cm^{-1} non-chelated six-membered ring ketone; 1623 cm^{-1} chelated carbonyl), and the correlation with luteoskyrin and iridoskyrin.

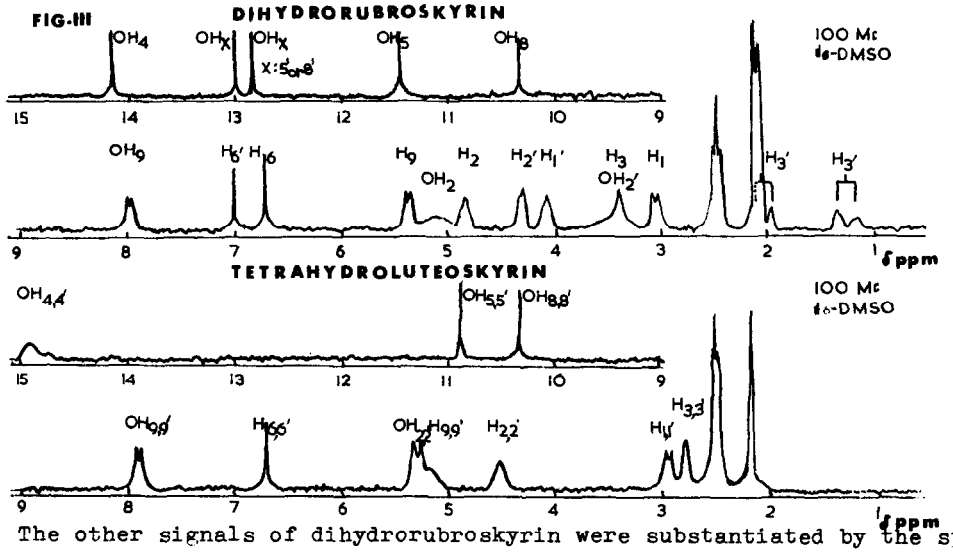


The NMR spectrum of rubroskyrin gave no clear pattern, whereas that of dihydrorubroskyrin obtained by the catalytic reduction of rubroskyrin revealed that the half of the molecule is corresponding to the monomeric moiety of reduced luteoskyrin (VII) and the remaining half retains the original quinonic structure (XIII).

In comparing the NMR spectral patterns of dihydrorubroskyrin (HRb) and tetrahydroluteoskyrin (HL), it has been demonstrated that the following signals are corresponding each other:

HL	2.18 δ ($CH_{3(7,7')}$);	2.79 δ ($H_{(3,3')}$);	2.93 δ ($H_{(1,1')}$);	4.52 δ ($H_{(2,2')}$)
HRb	2.17 δ	3.40 δ	3.07 δ	4.84 δ
HL	5.17 δ ($H_{(9,9')}$);	5.28 δ ($OH_{(2,2')}$);	6.72 δ ($H_{(6,6')}$);	7.91 δ ($OH_{(9,9')}$)
HRb	5.37 δ	5.06 δ	6.72 δ	8.07 δ
HL	10.33 δ ($OH_{(8,8')}$);	10.88 δ ($OH_{(5,5')}$);	14.96 δ ($OH_{(4,4')}$)	
HRb	10.33 δ	11.45 δ	14.14 δ	

FIG-III



The other signals of dihydrorubroskyrin were substantiated by the spin-decoupling experiments: Irradiation at 1.99 δ caused collapse of the doublet (1.17 δ, 1.37 δ); irradiation at 4.31 δ collapsed the same doublet; irradiation at 4.84 δ which corresponds to 4.52 δ (assigned to $H_{(2,2')}$ of HL) collapsed the doublet at 3.07 δ; irradiation at 4.07 δ gave no effect on the signals at 1.17 δ I.37 δ, and 1.99 δ. The addition of D_2O made the signal at 5.37 δ sharp singlet and caused the broad resonances centered on 3.40 δ and 5.06 δ, and the signal at 8.07 δ along with all the signals in the lower field (> 10 δ) to disappear.

All these data have agreed with the structure of quinonic half of rubroskyrin molecule. The extremely high field shift of the signal of methylene (AB type doublets) at 1.17 δ, 1.37 δ (J: 20 cps), 1.99 δ (2.19 δ overlapped with methyl signal at 2,12 δ) (J: 20 cps) would be due to an anisotropic shielding effect of the benzene ring (A) and carbonyl at 10-position of the ring (B) of the other half of the molecule. The signal of $H_{(2')}$ showed up-field shift by the same effect. The signal of $H_{(3)}$ of dihydrorubroskyrin appeared at the lower field (3.40 δ) in comparison with the corresponding signal (2.79 δ) of

tetrahydroluteoskyrin. This would be caused by the deshielding effect of the carbonyl at $C_{(9')}$ of the quinonic half.

Thus rubroskyrin must be represented by the formula (XII). The ready conversion of rubroskyrin into luteoskyrin would be rationalized by this structure.

Iridoskyrin derived from (-)luteoskyrin and rubroskyrin as well as dianhydrorugulosin derived from (+)rugulosin revealed optical activities which would be caused by the restricted rotation of the C-C linkage connecting the monomeric halves.

The para hydroxylation of dianhydrorugulosin prepared from (+)rugulosin resulted an antipode of iridoskyrin of naturally occurring or being derived from (-)luteoskyrin and rubroskyrin. Thus the naturally occurring (-)luteoskyrin and (+)rugulosin are in the opposite stereochemical relation about the C-C linkage suggesting a stereochemical specificity in the process of biosynthetical oxidative coupling.

The structural formula of flavoskyrin of P. islandicum Sopp NRRL 1175, which was deduced from the former structure of rugulosin should also be revised. The experiments on this pigment are now under progress.

Acknowledgements : We wish to thank Dr. H. Shimizu, Kyushu University for discussion on the NMR spectral analyses, and Dr. S. Marumo, Nagoya University for supplying the mould cultures (P. islandicum E.strain).

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