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BIOSYNTHETIC INCORPORATION OF EMODIN AND EMODINANTHRONE INTO THE ANTHRAQUINONOIDS OF PENICILLIUM BRUNNEUM AND P. ISLANDICUM

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The polyketide origin of fungal anthraquinones has been firmly established by the isotopic tracer studies with acetate and malonate as the precursors.[1] Not only aromatic anthraquinones but also modified bianthraquinones such as rugulosin fall in this category.[2] Gatenbeck examined the sequence of biosynthetic process of the pigments of Penicillium islandicum Sopp to prove that islandicin is not a precursor of its dimer, iridoskyrin.[3] As none of the anthraquinonoids of P. islandicum could be blocked by mutation[4], the various anthraquinonoids in the mould were suggested to be biosynthesized in parallel and not in sequential.[5,6] The structural modifications of anthraquinones seemed to occur at an earlier stage of the biosynthesis. From this viewpoint, incorporation experiment using radioactive emodinanthrone, a possible precursor of anthraquinonoids, was expected to give some reliable evidences for the biosynthesis of modified anthraquinones.

In this communication , we report mainly efficient incorporations of emodinanthrone(^{14}C) as well as emodin(^{14}C) into the anthraquinonoids of P. brunneum Udagawa[7] and P. islandicum Sopp[8], and we discuss a biosynthetical scheme of the anthraquinonoids.

Most of the labelled compounds were prepared biosynthetically by the culture of the above fungi under the presence of diethyl malonate(2-^{14}C), or prepared chemically from the ^{14}C-labelled fungal anthraquinonoids. The results recorded in Table 1 clearly show a high incorporation of emodinanthrone(^{14}C) and emodin(^{14}C) into both skyrin and (+)rugulosin, and only a small amount of the compounds fed were recovered from the culture. When a high quantity of emodin(^{14}C) (51 mg) was fed to the fungus, the specific incorporation ratios into skyrin and (+)rugulosin exceeded 100%.[9] This indicated that more than a half amount of the dimeric anthraquinonoids isolated from the fungus were derived from exogeneous emodin. On the other hand , the feeding of penicilliopsin(^{14}C) , prepared biosynthetically by the culture of Penicilliopsis clavariaeformis [10] showed no incorporation into (+)rugulosin, while a significant radioactivity was observed in skyrin.

Table 1 Feeding Experiments using Penicillium brunneum.

Expt.	Labelled Compounds	Isolated Compounds Incorp. % (Spec. Inc. %) Emodin	Skyrin	(+) Rugulosin
1	Emodinanthrone	1.9 (4.7)	4.4 (12.5)	63.7 (14.0)
2	Emodinanthrone	1.8 (-)	2.5 (-)	7.3 (36.9)
3	Emodin	0.38 (-)	1.6 (12.5)	25.0 (13.6)
4	Emodin (7.1 mg)	0.42 (4.8)	1.6 (19.4)	16.7 (38.7)
5	Emodin (51.0 mg)	21.6 (100.6)	5.6 (159.5)	21.8 (100.1)
6	Penicilliopsin *	0 (0)	3.8 (19.2)	0.7 (0.09)
7	Skyrin	0 (0)	59.3 (61.3)	0.3 (0.12)
8	Skyrin	- (-)	42.1 (45.8)	0.07 (0.12)
9	(+) Rugulosin	- (-)	0.059 (0.012)	3.7 (3.5)
10	(+) Rugulosin	- (-)	0.07 (0.21)	5.2 (2.7)

* = Skyrinanthrone - not tested

It may, however, be caused by the contamination of unchanged penicilliopsin (^{14}C) added to the culture, since it behaves similarly with skyrin on chromatography. When skyrin (^{14}C) was added to the culture of P. brunneum, it was recovered unchanged, and no significant incorporation into (+) rugulosin was observed. On the other hand, the radioactivity of (+) rugulosin recovered from the culture fed with (+) rugulosin (^{14}C), was relatively low. It seems to be caused by an extensive catabolism of exogeneous (+) rugulosin , while endogeneous (+) rugulosin remained unchanged as indicated by the high incorporation of monomeric precursors.

As shown in Table 2,emodinanthrone (^{14}C) was readily incorporated into all the anthraquinonoids of P. islandicum NRRL 1036 so far tested, while emodin (^{14}C) was still significantly incorporated into them, though about 20% of unchanged emodin (^{14}C) was recovered from the culture. A similar observation was obtained also in the experiments with P. islandicum NRRL 1175, where emodinanthrone (^{14}C) highly labelled skyrin, whereas emodin (^{14}C) was incorporated poorly into skyrin and 35% of the added emodin (^{14}C) was recovered from the culture.

As far as these results concern emodinanthrone can be regarded as a normal intermediate, while reluctant incorporation of emodin observed in the feeding experiments in P. islandicum may suggest that it was incorporated indirectly, possibly via anthrone by the reduction or through some intermediates which can be formed from emodin. The metabolites of P. islandicum other than emodin were recovered unchanged to a great extent in the feeding experiments and the incorporations into the metabolites other than themselves were insignificant to reveal them as the end products of

biosynthesis. No incorporation of islandicin (^{14}C) into iridoskyrin was confirmed, and it is in accord with the observation by Gatenbeck.[3]

Table 2 Feeding experiments with P. islandicum NRRL 1036

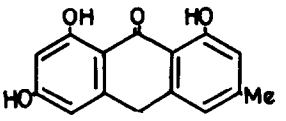
Expt.	Labelled Compds.	Isolated compounds Incorp. % (Spec. Incorp. %) Islandicin	Iridoskyrin	Skyrin	Rubroskyrin	Others
11	Emodinanthrone	20.35 (3.84)	0.35 (10.2)	5.25 (8.12)	2.2 (4.7)	
12	Emodin	0.12 (1.2)	0.053 (1.6)	0.20 (12.0)	- (-)	Emodin 21.8 (47.9)
13	Iridoskyrin	0.11 (0.03)	45.7 (54.3)	0.8 (0.18)	0 (0)	
14	Islandicin	58.6 (7.6)	0.07 (0.25)	0.06 (0.36)	0 (0)	
15	Chrysophanol	0.47 (0.10)	0.02 (0.02)	0.01 (0.36)	- (-)	Chrysophanol 28.6 (65.5)
16	Catenarin	0.14 (0.018)	- (-)	1.3 (0.71)	0.18 (0.57)	Catenarin 13.8 (82.0)
17	Skyrin	0.14 (0.02)	- (-)	22.2 (6.2)	0 (0)	
18	Rubroskyrin	0 (0)	- (-)	0 (0)	8.6 (22.0)	

Table 3 Feeding experiments with P. islandicum NRRL 1175

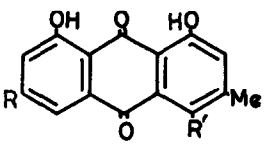
Expt.	Labelled Compds.	Isolated Compds. Emodin	Skyrin
19	Emodinanthrone	- (-)	56.6 (30.0)
20	Emodin	35.5 (32.8)	0.60 (0.89)

Rugulosin, rubroskyrin and luteoskyrin were revealed to be partially hydrogenated bianthraquinones having secondary alcoholic groups.[11] In the biosynthesis of 6-methylsalicylic acid, the loss of oxygen function was shown to be initiated by the dehydration of secondary alcohol group formed by the reduction of carbonyl of polyketomethyene intermediate.[12] By this analogy, the formation of secondary alcoholic function of the modified anthraquinones was regarded to occur before aromatization, at the polyketomethylene stage. The efficient incorporation of aromatic precursors added to the culture now reveals that the secondary alcoholic group of the modified anthraquinones must be formed by the hydrogenation of aromatic ring. This concept is also valid in the case of islandicin where dihydrocatenarin, quinone B, obtained by Bu'Lock and Smith from P. islandicum[13] would be a possible precursor. No incorporation of catenarin (^{14}C) into islandicin

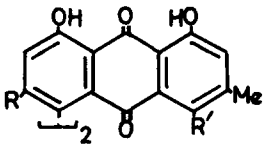
suggests that hydrogenation of aromatic ring must occur in emodinanthrone or its analogues, and not in catenarin, i.e. the hydroxylation must take place after partial hydrogenation of the ring. Further investigation for the exact role of emodinanthrone and emodin as well as the mechanism of the dimerisation to afford modified anthraquinones is now in progress.



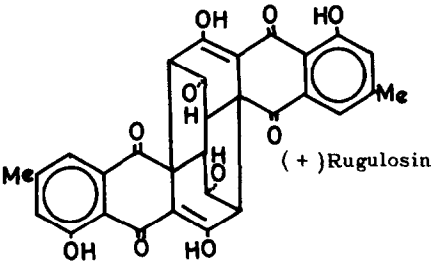
Emodinanthrone



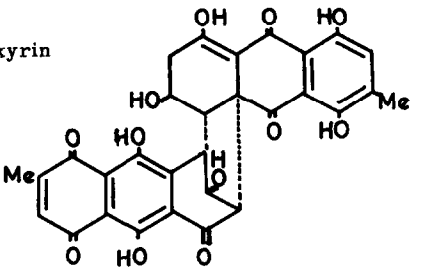
R=R'=H Chrysophanol
R=H, R'=OH Islandicin
R=OH, R'=H Emodin
R=R'=OH Catenarin



R=OH, R'=H Skyrin
R=H, R'=OH Iridoskyrin



(+)Rugulosin



(-) Rubroskyrin

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