N-Acetylchondrosine (2-acetoamido-2-deoxy-3-O-β-D-glucopyranuronosyl-D-galactopyranose)—Chondrosine (142 mg.) was treated as described above giving fine prisms of the barium salt (178 mg.), m.p. 155~157°(decomp.), $[\alpha]_D^{20}$ −10°(c=1, H_2O). *Anal.* Calcd. for $C_{14}H_{22}O_{12}NBa_{1/2}$: N, 3.01. Found : N, 3.02.

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Ching-Tan Chen, Koji Nakanishi,*1 and Shinsaku Natori*2 : Biosynthesis of Elsinochrome A, the Perylenequinone from *Elsinoë* spp. I.

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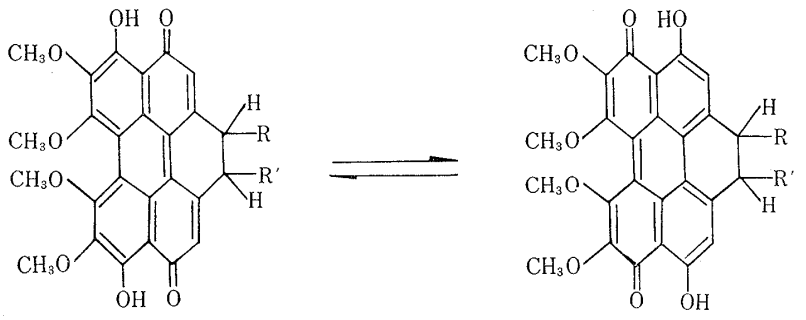
The formation of red pigments by the fungus *Elsinoë* (*Myrianginales, Ascomycetes*) and its conidial stage *Sphaceloma* had been noticed for a long time. Weiss, *et al.*[1,2] isolated the pigments, designated elsinochromes, and proposed the rapidly interconverting tautomeric structures (Ia) for the major pigment, elsinochrome A, chiefly from spectral evidences.



Ia : R, R′=CH_3CO
Ib : R=CH_3CO, R′=$CH_3CH(OH)$
Ic : R, R′=$CH_3CH(OH)$

The same pigment was also studied by Hackeng, *et al.*,[3] and by collaboration with Weiss' group, they established the structures of the minor pigments, elsinochrome B and C as Ib and Ic.[4]

Formation of dimeric phenolics through the oxidative coupling of two identical units[5] is now a widely accepted concept. As regards the binaphthyls, binaphthoquinonyls, and

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perylenequinone derivatives isolated from mould cultures, the structures of xylindein,[6] xanthomegnin,[7] mycochryson,[8] ustilaginoidins,[9] and cephalochromin[10] have been elucidated. On the other hand, Allport and Bu'Lock[11] have studied the biogenetic relationships of naphthalene, binaphthyl and perylenequinone derivatives produced by *Daldinia concentrica*. Although the oxidative coupling of phenolics as induced by growing moulds and enzymic preparations from the moulds has been reported,[12] the biosyntheses of these phenolic dimers from the corresponding monomeric unit has not been confirmed by tracer experiments. The biosynthesis of elsinochrome A from [1-^{14}C]acetate and [^{14}C]formate has been studied and the results are reported in the following.

Experimental

Cultures——Four strains of *Elsinoë*,*3 *E. ampelina* IFO 5263 and IFO 6359, *E. araliae* IFO 6166, *E. faucetti* IFO 6442, were incubated on a potato-dextrose medium for 5 weeks at 25°. Extraction of the dried mycelia and examination of the extract with thin-layer chromatography, using silica gel G treated with 2% oxalic acid, revealed that the four strains showed nearly the same formation of the pigments. However, because of the relatively high yield of elsinochrome A, *E. araliae* IFO 6166 was selected for further work.

Cultivation——The mycelial suspension of *E. araliae* was inoculated into 500 ml.-flasks each containing 50 ml. of potato-dextrose medium.*4 After incubation at 25° for 2 weeks, the mycelia had covered the surface of the fluid and the pigment formation had started. At this stage, $CH_3{}^{14}COONa$ (0.2 mc.) and $H^{14}COONa$ (0.1 mc.), dissolved in a small amount of water were distributed evenly in 10 and 5 flasks, respectively. During the course of further incubation for 3 weeks, the mycelia became intensively red. The mycelia were then filtered through a cotton guage and dried under an infrared light (cf. Table I).

Isolation of [^{14}C]Elsinochrome A——The dried and powdered mycelia were extracted with acetone and the residue, after evaporation of the solvent, was chromatographed on a column of silica gel (Mallinckrodt) treated with 2% oxalic acid in benzene solution. Five colored bands appeared and were eluted successively with benzene, mixtures of benzene-EtOAc, and MeOH. Each fraction was examined by thin-layer chromatography and the less polar and major band, eluted by benzene-EtOAc (100:3), was collected. Recrystallization from benzene-hexane afforded red crystals, m.p. 255~256°, of elsinochrome A.

A minor component not identical with the known elsinochromes (A, B and C) has also been isolated from the column as crystals in minute quantities. The structure of this compound, which is closely related to the known elsinochromes, is under investigation.

Determination of Radioactivity——All determination of ratioactivities were carried out with a Nuclear Chicago Model 725 liquid scintillation counter using the channel ratio method. The colored samples or $BaCO_3$ were converted into CO_2 by Van Slyke-Folch oxidation and the liberated CO_2 was absorbed in hyamine hydroxide and counted.

Degradation of Labelled Elsinochrome A (Ia)——The radioactive elsinochrome A was diluted with carrier*5 and recrystallized from benzene-hexane until constant specific activity was attained.

a) Kuhn-Roth Oxidation of Elsinochrome A (Ia): Ia (200 mg.) was treated with the oxidizing mixture and acetic acid was recovered by steam-distillation, the fraction being neutralized with LiOH (0.05*N*) and evaporated to dryness. The residue was dissolved in EtOH (8 ml.) and filtered. A portion of the filtrate (3 ml.) was used for the preparation of the *p*-bromophenacyl ester of acetic acid (II), m.p. 83~85° (prepared by conventional methods).

b) The Schmidt Reaction of Acetate (II): The other part of [^{14}C]LiOAc in EtOH was evaporated and the residue was degraded and the CO_2 formed was collected as $BaCO_3$.

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*3 We are indebted to Dr. K. Tsubaki, Institute for Fermentation, Osaka, for his kind donation of the strains.

*4 Since the mycelia are apt to become waterlogged, a small amount of the medium was placed in each flask.

*5 The authors thank Professor H. Nishikawa for his generous gift of the specimen.

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Table I. Incorporation of [1-^{14}C]Acetate and [^{14}C]Formate

Substrate	Total activity of substrate (mc.)	Cultural medium (ml.)	Wt. of dried mycelia (g.)	Wt. of acetone extract (g.)	Total yield of Ia[a] (mg.)	Sp. activity of Ia[a] (d.p.m./mM)	Total activity of Ia[a] (μc.)	Incorporation (%)
[1-^{14}C]acetate	0.2	50×10	10.8	0.93	73.8	6.0×10^{7}	3.67	1.8
[^{14}C]formate	0.1	50×5	4.5	0.40	30.2	1.3×10^{8}	3.25	3.3

a) Ia: elsinochrome A

The reaction mixture was diluted with water, made alkaline and distilled. Methylamine was absorbed in 1N HCl (20 ml.) and converted to N-methyl-2,4-dinitroaniline, m.p. 174~176°, by conventional methods.

c) Nitric Acid Oxidation of Ia: Ia (300 mg.) was heated with conc. HNO_3 (12 ml.) on a water-bath for 2 hr. and then evaporated to dryness. The residue was treated in the same way once again and the excess HNO_3 was completely removed by the addition of water and evaporation. The yellow solid thus obtained was dissolved in MeOH and treated with diazomethane. The reaction product was purified through a column of alumina (neutral) and recrystallized from MeOH-H_2O to afford hexamethyl mellitate (III), colorless needles, m.p. 187~189°.

d) Demethylation of Ia: A mixture of Ia (100 mg.), phenol (200 mg.), Ac_2O (7 ml.) and HI (3 ml.), was boiled under N_2 stream for 2 hr. and the generated methyl iodide was absorbed in 5% ethanolic triethylamine. After evaporation of the solvent, the residue was recrystallized from EtOH-ether to afford colorless needles, m.p. >360°. The triethylmethylammonium iodide was converted into the picrate, m.p. 288~289° (from MeOH).

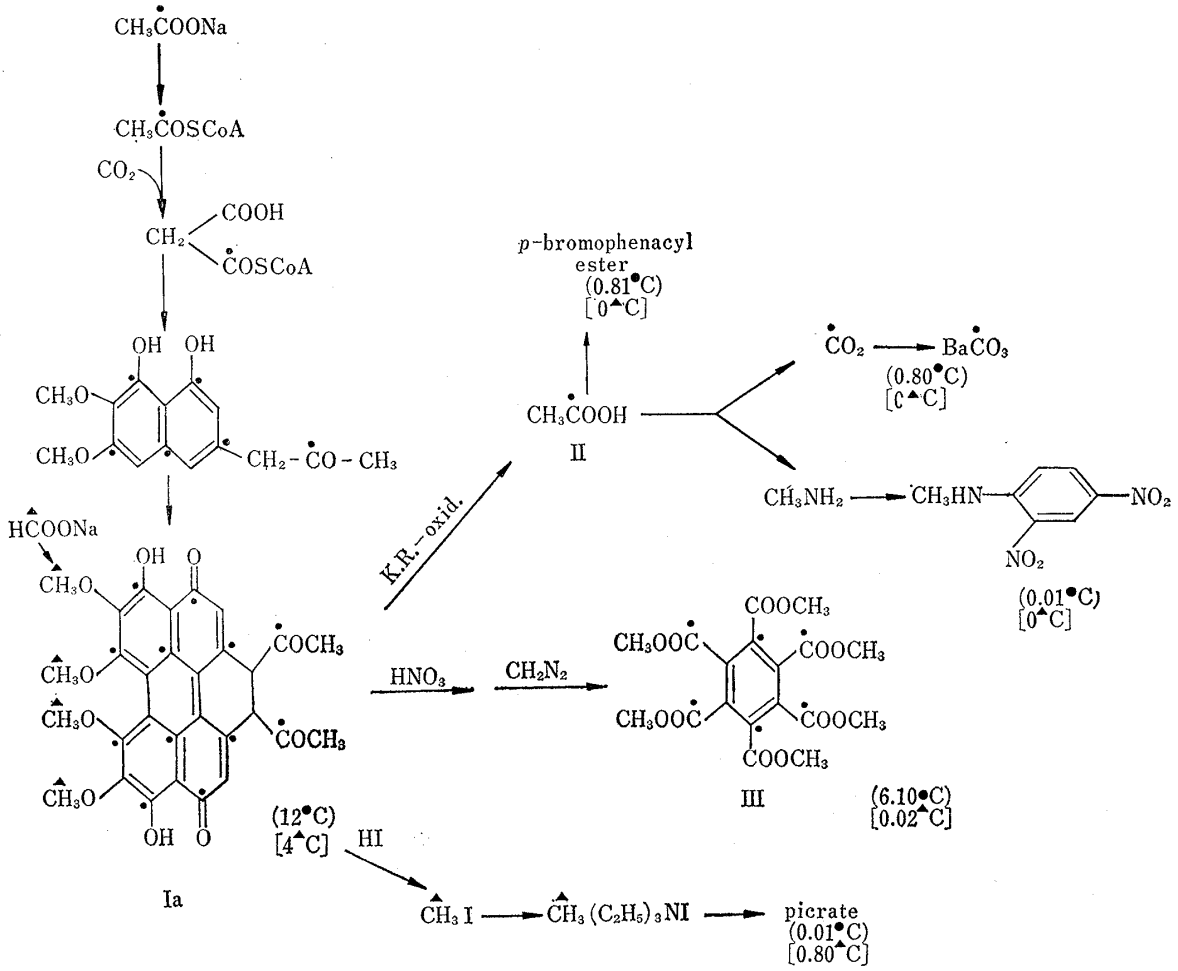

Chart 1. Biosynthesis and Degradation of Radioactive Ia and Relative Activities of Degradation Products

Results and Discussion

Although the yield of the pigment was inferior than reported,[4] incorporation of both radioactive precursors into elsinochrome A (Ia) was satisfactory (Table I).

A diluted specimen of [^{14}C]elsinochrome A was then degraded as described in Experimental and Chart 1. The specific activity of the sample of Ia actually used for degradations, and of degradation products, are shown in Table II.

Table II. Specific Activities of Diluted Elsinochrome A and of the Degradation Products (d.p.m./mM)

	from [1-^{14}C]acetate	from [^{14}C]formate
Elsinochrome A (Ia)	5.9×10^5	1.2×10^6
p-Bromophenacyl ester of acetic acid (II) from the Kuhn-Roth oxidation of Ia	4.0×10^4	4.4×10^2
$BaCO_3$ from the Schmidt reaction of II	3.9×10^4	2.8×10^2
N-Methyl-2,4-dinitroaniline from methylamine formed by the Schmidt reaction of II	4.2×10^2	3.6×10^2
Hexamethyl mellitate (III) after HNO_3 oxidation of Ia	3.0×10^5	4.9×10^3
Methyltriethylammonium picrate from the Zeisel reaction of Ia	4.7×10^2	2.4×10^5

The structure (Ia) of elsinochrome A postulates that Ia has been formed from one acetate plus six malonate units, followed by decarboxylation, hydroxylation, *O*-methylation, and dimerization. Thus Ia from [1-^{14}C]acetate must contain twelve labelled carbon atoms and that from [^{14}C]formate, four. The observed activities of the degradation products calculated from this assumption are shown in Chart 1. The calculations were made by taking the number of carbons incorporated into Ia from active acetate and formate as 12 and 4, respectively. The activities are indicated in number of active carbons thus calculated, and are enclosed in round and square brackets, respectively, for degradation products resulting from acetate and formate incorporation.

Acetic acid (II) formed by the Kuhn-Roth oxidation of Ia from [1-^{14}C]acetate retained its activity in the carboxyl-C. The activities of II and mellitate (III) are in agreement with the calculated values. Finally, the activity of Ia formed from [^{14}C]formate is located exclusively in the methoxyl-C.

The results exclude all conceivable biogenetic routes in which the carbon skeleton is built by cyclization of one long "polyketomethylene" chain, and although the biosynthetic sequence should be established by further work the results are in agreement with the scheme shown in Chart 1. Recently the acetate-polymalonate origin of two mould naphthoquinones, javanicin[13] and mollisin,[14] has been reported.

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