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Incorporation of Labelled Acetate in Emodin in *Penicillium islandicum*

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The distribution of the isotopic content in emodin obtained from *Penicillium islandicum* when grown on $CH_3-^{14}COOH$ has been studied. The results clearly indicate that the biosynthesis of emodin involves a head to tail condensation of acetate.

The field of aromatic biosynthesis has been extended in recent years with the demonstration by Birch of a head to tail condensation of acetate as the source of the aromatic rings in various phenolic products [1,2]. Birch and others have employed the acetate theory as a working hypothesis for the determination of the then unknown structures of complex ring systems [3]. Since there are many natural polyhydroxyanthraquinones which fit such a theory of origin, it was decided to attempt to confirm the direct incorporation of acetate into emodin.

If the biosynthesis of emodin involves head to tail condensations of acetic acid units it is reasonable to suppose that the methyl group of emodin is an unchanged methyl group from acetic acid. Starting with that methyl group, the acetic acid residues must be orientated as indicated in Fig. 1. The methyl groups from acetic acid will then be found in the positions m, 1, 3, 6, 8, 9, 11 and 14 as indicated in Fig. 2.

Emodin is produced in very small amounts in organisms suitable for growth experiments with labelled compounds. Skyrin [4], a dimer of emodin,

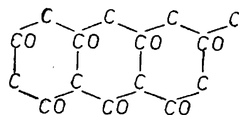

Fig. 1.

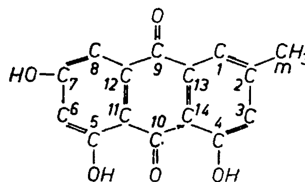

Fig. 2.

occurs in large amounts in the mycelium of *Penicillium islandicum* when supplied with glucose as a carbon source. Skyrin is easily transformed into emodin by reductive cleavage with sodium dithionite. As it is highly probable that skyrin is formed by a condensation of emodin, or a common precursor, skyrin has been used as a source of emodin. Free emodin is produced by *P. islandicum* in amounts that are scarcely detectable by paper chromatographic techniques.

P. islandicum Sopp N.R.R.L. 1175 was supplied with $CH_3{}^{14}COONa$ and the emodin was isolated from the skyrin fraction of the mycelium. The radioactive emodin was diluted with inactive emodin obtained from chrysarobin [5]. The emodin was totally oxidized to carbon dioxide collected as barium carbonate, and the radioactivity was measured. Acetic acid was obtained from the carbons m and 2 after a Kuhn-Roth oxidation. The degradation of the acetic acid, according to Phares [6], gave the radioactivity of each of these carbon atoms. Nitration of emodin to 1,3,6,8-tetranitroemodin and hypobromite cleavage of the nitrocompound gave the carbons 1, 3, 6 and 8 as bromopicrin which was converted to carbon dioxide and the radioactivity measured as above.

EXPERIMENTAL

Culture conditions. Penicillium islandicum Sopp, N.R.R.L. 1175 was grown under the conditions described by Howard and Raistrick. 4 flasks, each containing 500 ml of medium, were grown for 12 days, at this time a solution containing about 0.3 mC of carboxyl labelled sodium acetate was added to each flask. Growth was continued for another 10 days.

Isolation of emodin. The mycelium, from each flask, was removed by filtration, washed with distilled water, and air dried. The dried mycelium was extracted with petroleum ether (b.p. 40—60°) for 1.5 h in a Soxhlet apparatus. The extraction was continued with acetone until nearly all of the pigment had been extracted. The acetone solution was then evaporated to dryness on a steam bath. The dry weight of the acetone extract was 3.32 g. It was dissolved in 750 ml of 1 N Na_2CO_3. The solution was heated on a steam bath and 700 ml of water containing 100 g of $Na_2S_2O_4$ was added during a 10—15 min period with stirring. The solution was stirred continuously for another 20 min and then cooled to room temperature under running water, and neutralized with concentrated HCl. The neutralized solution was then extracted several times with ether. The ether phase was next washed with 1 N Na_2CO_3. The sodium carbonate phase was separated, acidified with concentrated HCl, and again extracted with ether. The ether solution was dried over anhydrous Na_2SO_4 and then evaporated to dryness. The residue was run through a silica column with a mixture of chloroform-acetone (4—1) as solvent. The fraction with a yellow-green fluorescence in UV-light was collected, evaporated to dryness and washed with petroleum ether. The weight of the dried residue was 72.4 mg with m.p. 254—255°. This substance gave all the color reactions of emodin and the melting point was not depressed on admixture of authentic emodin.

The radioactive emodin was diluted about 5-fold with inactive emodin and recrystallized from chloroform yielding 282 mg, m.p. 256—257°.

Radioactive assay. Radioactivity measurements were made with a Tracerlab Autoscaler Type SC-51 in conjunction with a Tracerlab TGC-2 Geiger tube. All the determinations of radioactivity were made after converting the material to barium carbonate. Only self-absorption corrections were made, since all other variables were kept constant.

Combustion. Emodin and bromopicrin were oxidized by the van Slyke-Folch method [7]. The evolved carbon dioxide was trapped in a carbonate free barium hydroxide solution.

Kuhn-Roth oxidation of emodin: The Kuhn-Roth oxidation of emodin (75 mg) gave acetic acid that was isolated by steam distillation. The carbon dioxide formed during the reaction was trapped in barium hydroxide solution. The Kuhn-Roth reaction was quantitative, both for the acetic acid and the carbon dioxide. The acetic acid was degra-

Table 1. Determined and calculated isotopic content of emodin.

Material	Carbon atoms isolated	Numbers of theoretical COOH and CH_3 groups	Specific activity *			
			Found			Calculated
			Experiment 1	Experiment 11	Average	
(1)	(2)	(3)	(4)	(5)	(6)	(7)
Total combustion	all	COOH 7; CH_3 8	32.7	—	32.7	32.1
K—R ox:carbon dioxide	1, 3, 4, 5, 6, 7, 8, 9, 10, 11,12,13,14	COOH 6; CH_3 7	32.4	32.4	32.4	31.8
K—R ox:carboxyl group of acetic acid	2	COOH 1	61.0	64.0	62.5	62.5
K—R ox:methyl group of acetic acid	m	CH_3 1	5.2	5.3	5.3	5.3
Bromopicrin	1, 3, 6, 8	CH_3 4	9.9	6.6	8.3	5.3
Hypobromite degradation: carbon dioxide	4,5,7,9,10, 11,12,13,14	COOH 6; CH_3 3	44.5	39.8	42.2	43.4

* Specific activity in counts/min/mg $BaCO_3$ corrected for selfabsorption.

ded by a Schmidt reaction and the carboxyl group isolated as barium carbonate. The methylamine produced by the Schmidt reaction was oxidized with alkaline permanganate to carbon dioxide, which, after acidification, was trapped as barium carbonate.

1,3,6,8-Tetranitroemodin. Emodin (90.3 mg) was dissolved in sulfuric acid (2.0 ml, *d* 1.84) and nitric acid (0.33 ml, *d* 1.52) was added dropwise. The mixture was heated on a steam bath for 45 min and then cooled with ice and diluted with ice-water. The yellow crystalline product was filtered and washed with distilled water. The air dried substance was recrystallized from glacial acetic acid, yielding tetranitroemodin (61.4 mg) m. p. 301—302° (decomp.). Tetranitroemodin was prepared in the same way about ten times from non-labelled material. The nitrogen content in these experiments varied between 11.4 and 12.5 %, the theoretical value being 12.4 %.

Hypobromite degradation of 1,3,6,8-tetranitroemodin. Tetranitroemodin (60.0 mg) was suspended in 5 ml aqueous, carbonate-free, saturated barium hydroxide. 40 ml of cold (0°), carbonate-free, aqueous barium hypobromite was added to the nitroemodin suspension. The barium hypobromite solution was prepared from barium hydroxide (2.5 g) and bromine (0.3 ml) in 40 ml of water. The reaction mixture was kept at 0° for 24 h with occasionally shaking. The bromopicrin formed by this process, was isolated by steam distillation. The distillate was trapped in a conical centrifuge tube cooled with ice-water. The bromopicrin was washed with distilled water by centrifugation, and oxidized to carbon dioxide according to van Slyke-Folch. The barium carbonate that was formed during the hypobromite reaction was filtered off and washed with distilled water. The carbon dioxide was regenerated by acidification with perchloric acid and again trapped in aqueous barium hydroxide. Tests with unlabelled nitroemodin showed that the yield of the bromopicrin and carbon dioxide was about 80 %, indicating that all the nitrogen atoms in tetranitroemodin were represented in the bromopicrin and that the central ring in the anthraquinone molecule was oxidized to carbon dioxide.

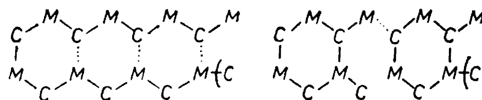

Fig. 3.

RESULTS AND DISCUSSION

The data of the various radioactivity measurements are given in Table 1 in counts/min/mg $BaCO_3$. All radioactive measurements were counted for a sufficient time to give a probable error of less than 1 %. The data in columns 4 and 5 are obtained from two different experiments. In column 3 are found the numbers of carboxyl and methyl groups from acetic acid which the emodin molecule will contain if the acetate theory is valid. Since the radioactivity of the carboxyl group and the methyl group of the acetate obtained in the Kuhn-Roth oxidation are measured directly, these values have been taken as a basis of calculating the theoretical activities of the samples measured which are listed in column 7. These theoretical values show a very good agreement to the experimentally determined. The results support entirely the acetate theory of the biosynthesis of emodin, but do not distinguish between various possible intermediary compounds between acetic acid and emodin. The work by Birch on 2-hydroxy-6-methylbenzoic acid indicates that the formation of emodin by intermolecular condensation of a polyketoacid or a condensation of two phenol carboxylic acids will give the same labelling of emodin from acetic acid, Fig. 3.

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REFERENCES

1. Birch, A. J., Massy-Westropp, R. A. and Moye, C. J. *Austral. J. Chem.* **8** (1955) 539.
2. Birch, A. J., Massy-Westropp, R. A., Rickards, R. W. and Smith, H. *J. Chem. Soc.* **1958** 360.
3. Birch, A. J. and Massy-Westropp, R. A. *J. Chem. Soc.* **1957** 2215.
4. Howard, B. H. and Raistrick, H. *Biochem. J.* **56** (1954) 56.
5. Gardner, J. H. *J. Am. Pharm. Assoc.* **28** (1939) 143.
6. Phares, E. F. *Arch. Biochem. Biophys.* **33** (1951) 173.
7. *Cf.* Calvin, Heidelberger, Reid, Tolbert and Yankwich *Isotopic carbon* Wiley, New York 1949, p. 92.

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