

THE BIOSYNTHESIS OF PEDERIN

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Biosynthetically, pederin (1),^{1,2} the toxic principle from Paederus fuscipes Curt., seems unique among the natural products isolated from insects.

We report here on feeding experiments designed to establish the origin of the carbon framework of pederin (1). Although the results obtained so far are neither complete nor conclusive we should like to present them now as they give a fairly definite idea of the origin of the carbon skeleton of this substance.

The insects, collected in the Pavia area (by M.P. and M.D.V.) between December and April, were kept at 20° for 1-2 weeks and then fed with an aqueous solution of the precursors containing 1% sucrose. Typically, 4000 insects (16 g, wet, containing ca. 5 mg of pederin) take up 25 ml of solution during 4 days, and, surprisingly, in preliminary parallel runs with [2-¹⁴C] acetate, with precursors with 0.12 and 60 mCi/mmole of specific activity (0.1 mCi fed), 0.1% and 0.04% incorporations were observed respectively. However, later in the season during larger scale feeding the incorporations were much lower.

Incorporation into pederin (1) was observed in feeding experiments with [1-¹⁴C] acetate, [2-¹⁴C] acetate, [1,2-¹⁴C] glycine, and [2-¹⁴C] propionate but only with the first two did we obtain enough material for specific degradation.

The crude extract of the insects was chromatographed on alumina to give radioactive but impure pederin (1). This was further purified by partition between water and hexane, followed by

ether extraction from the aqueous layer. Occasionally in this procedure a mixture of pederin (1) and pseudopederin (2) was obtained. Therefore, pederin (1) was completely transformed into (2) by shaking the benzene solution with 0.5N H_2SO_4 at room temperature for 30 min, and the radiochemical purity of (2) was carefully determined.

The specific incorporation of the precursors was determined as follows. Pseudopederin (2) upon lead tetraacetate oxidation gives isopederolactone (3) and meropederoic acid (8).

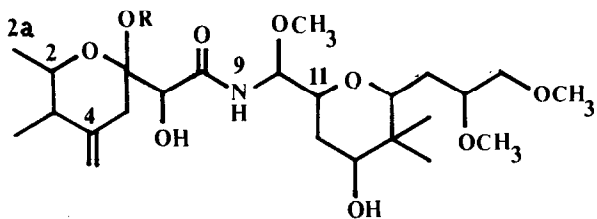
Isopederolactone (3) is ozonised in $CHCl_3$ at -15° for 10 min. The solvent is evaporated and the residue is treated with 50% aqueous acetic acid and Zn powder. The filtered solution is partially distilled into dimedone solution, and the derivative of formaldehyde (4) is collected. The residue is made alkaline with 50% NaOH and slowly distilled into dimedone solution to give the derivative of acetaldehyde (5). Pederolactone (6), which is obtained from isopederolactone (3) on alumina chromatography, is ozonised in the above mentioned conditions, and the residue is oxidatively decomposed with 50% performic acid. The excess of oxidant is destroyed (Pd/C), the solution is diluted, and lead oxalate is precipitated upon addition of lead acetate. The collected material is treated with HCl, the solvent is evaporated, and the residue is sublimed to give oxalic acid (7). In this way the radioactivity from the methylene at C-4, from the two carbons unit represented from C-2 and from the methyl attached to it, and from the two carbon atoms at positions 5 and 6 is determined.

TABLE

Relative ^{14}C molar activities and (incorporations%) ^a										
expt.	precursor	pseudo- pederin (2)	pedero- lactone (6)	meropedero- ic acid (8)	formalde- hyde ^b (4)	acetal- dehyde ^b (5)	oxalic acids (7)	(9)	pederenale (117)	(10)
1 ^c	[1- ^{14}C]acetate	100 (10^{-3})	35		<2	28	10	12	20	65
2	[2- ^{14}C]acetate	100 (10^{-2})	38	62	6	20	8	12	7	54

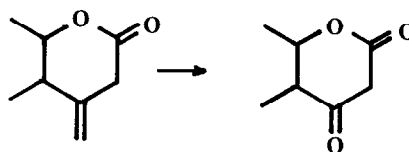
^a Average values of several experiments are reported. ^b Isolated as dimedone-derivatives.

^c Radioactivity assays hold at least $\pm 10\%$, owing to the low specific activity. The degradation in expt. 1 was performed once



(1) R= Me

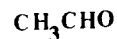
(2) R= H



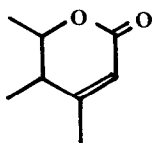
(3)



(4)



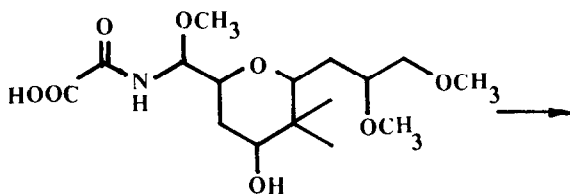
(5)



(6)



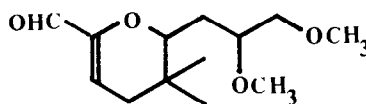
(7)



(8)



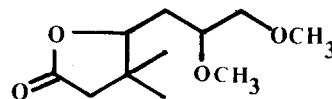
(9)



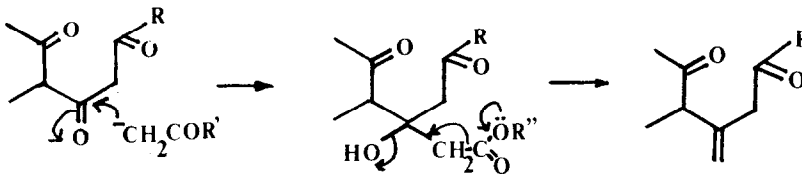
(10)



(11)



(12)



Meropederic acid (8), purified as methyl ester, is hydrolysed in a two phase system with 1N HCl-hexane on the water bath. From the aqueous layer oxalic acid (9) from the two carbon atoms at positions 7 and 8 of (1) is obtained as before. The organic solvent contains pederone (10) which is purified by distillation. The latter compound (10) gives on ozonolysis oxalic acid (11) from the two carbon atoms at positions 10 and 11, and pedelactone (12). The activity of (12) was not determined owing to the very small amount of material available.

The relative ^{14}C molar activities of the abovementioned products obtained in the two degradation series (Table) indicate that $[1-^{14}\text{C}]$ acetate and $[2-^{14}\text{C}]$ acetate provide the carbon atoms at positions 2 and 2a with far better efficiency than the carbon atoms at positions 5 and 6, 7 and 8, 10 and 11, and that a considerable part of the total activity is present in the undegraded pedelactone (12). Further, expt. 2 with $[2-^{14}\text{C}]$ acetate shows specific incorporation of the carbon atom at position 2 in the fed acetate into the methylene group at C-4. The results obtained so far with $[1-^{14}\text{C}]$ acetate indicate an insignificant incorporation of ^{14}C activity in the same position.

The evidence therefore suggests that much of the framework of pederin is derived via polyketide biosynthesis. This view is also supported from the incorporation of $[2-^{14}\text{C}]$ propionate into pederin (1). Lack of radioactive material did not allow specific degradation, however ca. 40% of the ^{14}C activity was in pederolactone (6).

Further, the incorporation of activity from the methyl group of acetate (expt. 2) into the methylene at C-4, might be explained by means of condensation of acetate onto the polyketide chain, followed by fragmentation, as shown in the Scheme.

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