Biosynthesis of Rutacridone in Cell Cultures of *Ruta graveolens*: Incorporation Studies with [^{13}C]Acetate

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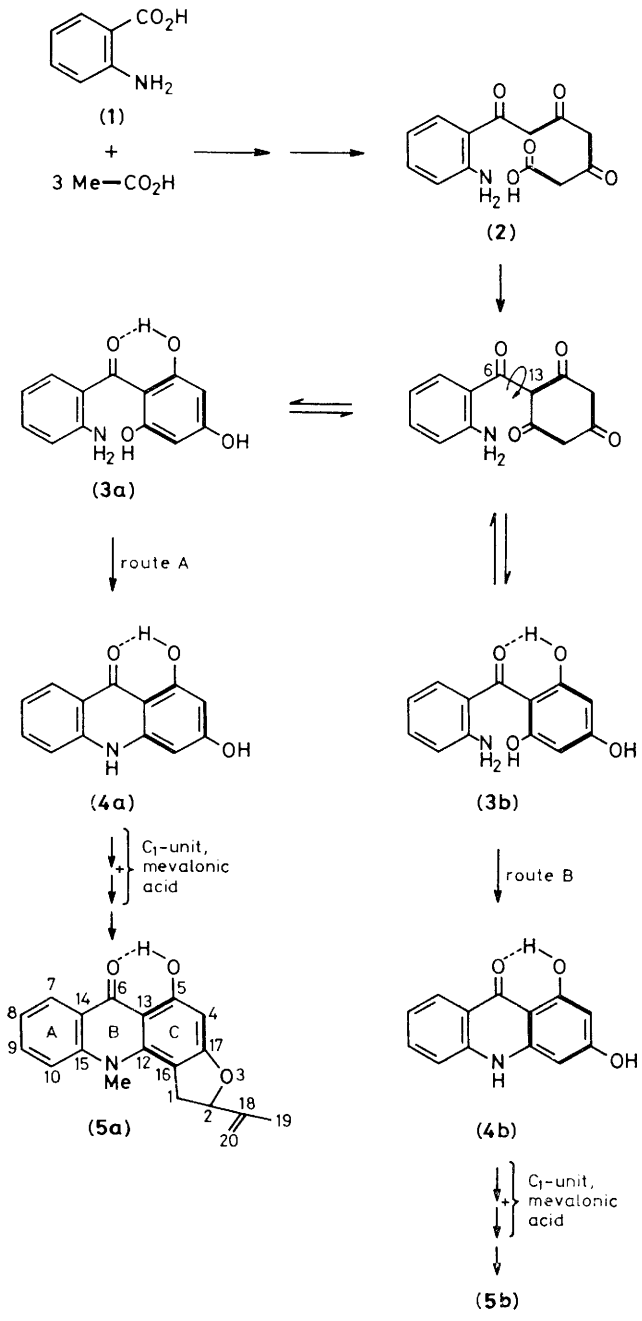
Carbon-13 labelling experiments have shown that ring C of the acridone alkaloid rutacridone is acetate-derived and that an aminobenzophenone derivative is a probable intermediate in the biosynthetic pathway.

The acridone alkaloids comprise about forty members found solely in the *Rutaceae* family of higher plants. A number of them have been isolated from the intact plant and cell cultures of *Ruta graveolens* L.[1]



Robinson[2] has postulated that acridones are biosynthetically derived from anthranilic acid (**1**) and acetate *via* a polyketo-acid. As potential intermediates 2-aminobenzophenones have been discussed.[3] Leete[4] has suggested the stepwise addition of acetate to anthranilic acid. In this case a quinolone may be envisaged as an intermediate.

The specific incorporation of anthranilic acid into various acridones present in different species of *Rutaceae* is well documented.[5] Recent experiments[6] with cell cultures of *Ruta graveolens* confirm the precursor role of (**1**) previously demonstrated with intact plants. This particular cell line R-19 of *R. graveolens*[7] accumulates mainly the dihydrofuroacridone derivative rutacridone (**5**). We could not show unambiguously that the isopropylidenedihydrofuran part of (**5**) is derived from mevalonic acid. After administration of [2-^{14}C]acetate, labelled rutacridone was isolated and degraded to 1-methyl-4-quinolone-3-carboxylic acid. Based on the labelling pattern some evidence was provided that acetate might be involved in acridone biosynthesis, but this assumption was not proved unequivocally.

Table 1. ^{13}C-Chemical shifts (δ), enrichments from [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetate and coupling constants (Hz) from [1,2-$^{13}C_2$] acetate observed in rutacridone (**5**).

Carbon	δ/ p.p.m.	Enrichment factors[a] after feeding of: [1-^{13}C]-acetate	[2-^{13}C]-acetate	[1,2-$^{13}C_2$]-acetate	$^1J(^{13}C–^{13}C)$: route A	route B
1	38.1	0.88	0.99	0.76		
2	86.2	0.88	0.99	0.92		
4	92.6	0.88	3.5	3.36	70.5	70.9
5	166.3	4.7	1.1	3.36	62.8	70.9
6	180.9	1.18		1.43		
7	120.4	0.88	0.99	0.92		
8	121.6	0.88	0.99	1.01		
9	133.9	0.88	1.1	1.09		
10	114.6	1.18	0.9	0.92		
12[b]	143.1				74.7	62.8
13	106.3	1.18	4.7	5.55	62.8	62.8
14	121.3	0.59		1.18		
15	142.7					
16	99.7	1.76	5.3	3.7	74.7	62.3
17	167.4	3.5		3.03	70.5	62.3
18	143.5	0.88				
19	17.1	0.88	0.9	1.01		
20	112.9	0.88	0.99	0.84		
N-Me	37.1	1.18	1.1	0.92		

[a] Ratios between peak heights of the observed resonances of ^{13}C-enriched and natural abundance rutacridone recorded under identical conditions. [b] The signals for C(12) are superimposed by the signals of C(15) and C(18) and have not been integrated.

We therefore performed feeding experiments with [1-^{13}C]-, [2-^{13}C]-, and [1,2-$^{13}C_2$]-acetate using cell suspension cultures of *R. graveolens*, cell line R-19, grown in the dark. On the sixth day after inoculation each round-bottomed flask containing 120 ml of medium[6] was supplemented with a daily dose of 1 mg of ^{13}C-enriched sodium acetate (90% ^{13}C-enriched) for 16 days. The cells were then harvested and fractionated giving 9—10 mg of (**5**) per flask. The ^{13}C n.m.r. data[8] of rutacridone biosynthesized from [^{13}C]acetate are given in Table 1. The proton noise decoupled ^{13}C n.m.r. spectra of [1-^{13}C]- and [2-^{13}C]-acetate-derived rutacridone showed enhancements of signals of carbon atoms C(5), C(17) and also C(4), C(13), and C(16).

The ^{13}C n.m.r. spectrum of rutacridone enriched with [1,2-$^{13}C_2$]acetate showed intense satellite resonances due to $^{13}C–^{13}C$ spin–spin couplings. Six bonded $^{13}C–^{13}C$ pairs were identified by matching coupling constants in the positions (route A): C(5)–C(13), C(12)–C(16), and C(17)–C(4) and (route B): C(5)–C(4), C(17)–C(16), and C(12)–C(13). The intensities of the doublets of both types are roughly equal. Thus the six carbons were enriched from the incorporation of three intact acetate units, the enrichment distribution arising from rotation about the C(6)–C(13) bond of the acetate-derived ring in the intermediate (**3**). This rotation, which interchanges carbon atoms on opposite sides of the axis, can occur only if, at the same stage, (**3**) is not bound rigidly to an enzyme surface. A similar biosynthetic pattern, *e.g.* two different arrangements of acetate units, has been found in the formation of some xanthones[9] and the antibiotic griseofulvin.[10] In these cases the rotation occurs at the stage of benzophenone intermediates.

Our results provide conclusive evidence that, besides shikimate-derived anthranilic acid, a polyketide is involved in acridone alkaloid biosynthesis. Furthermore, they are in accord with the assumption that aminobenzophenones are probable intermediates on this route.

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