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THE ROLES OF ALANINE, ASPARTATE AND GLUTAMATE IN LAWSONE BIOSYNTHESIS IN IMPATIENS BALSAMINA

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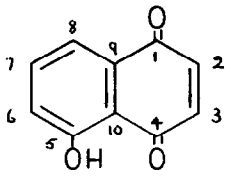
It is now well established that shikimic acid (I) provides seven of the ten carbon atoms of the naphthoquinone ring system of materials such as juglone (II), lawsone (III), and vitamin K_2 (IV) (see reference 1 and references therein). Although it has been noted that in juglone biosynthesis in Juglans regia (2), and in vitamin K_2 biosynthesis in Mycobacterium phlei (3), the methyl group of acetate contributes activity specifically to C-2 and/or C-3, the immediate precursor of the remaining three ring carbons has not yet been identified.



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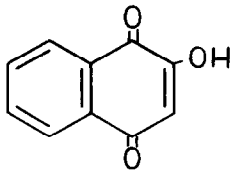

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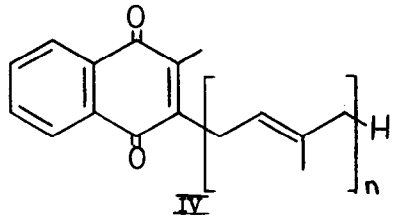


IV

In pursuing the source of this unit we have assessed the effectiveness of ^{14}C labelled alanine (V) and aspartate (VI) as precursors of the plant product lawsone (III). The substrates were administered in aqueous solution to excised shoots of 6-8 weeks old Impatiens balsamina plants. After twelve hours metabolism in ordinary daylight the plants were homogenised in chilled ethyl acetate: acetone: water, 1:1:1. The homogenate was filtered free of cell debris, and the two phases were separated. The lower phase, containing glycosidically bound lawsone (4), was reduced to 50% its volume, was acidified with concentrated hydrochloric acid and was warmed on the steam bath for 10 minutes. Extraction of this cooled solution with chloroform provided crude lawsone which was further purified by buffer extraction, thin

Table 1

Tracer	U-^{14}C alanine (L)	1-^{14}C alanine (DL)	U-^{14}C aspartate (L)	2-^{14}C glutamate (DL)
Amount of tracer fed (μc)	50	96	100	30
Incorporation (%)	0.33	0.03 *	0.11	1.36 *
Degradation:-				
C_1 and C_4	13	27	46	99
C_2 and C_3	73	12	35	6
C_5 - C_{10}	14	61	10	0

* Incorporation based on exclusive L-isomer utilisation

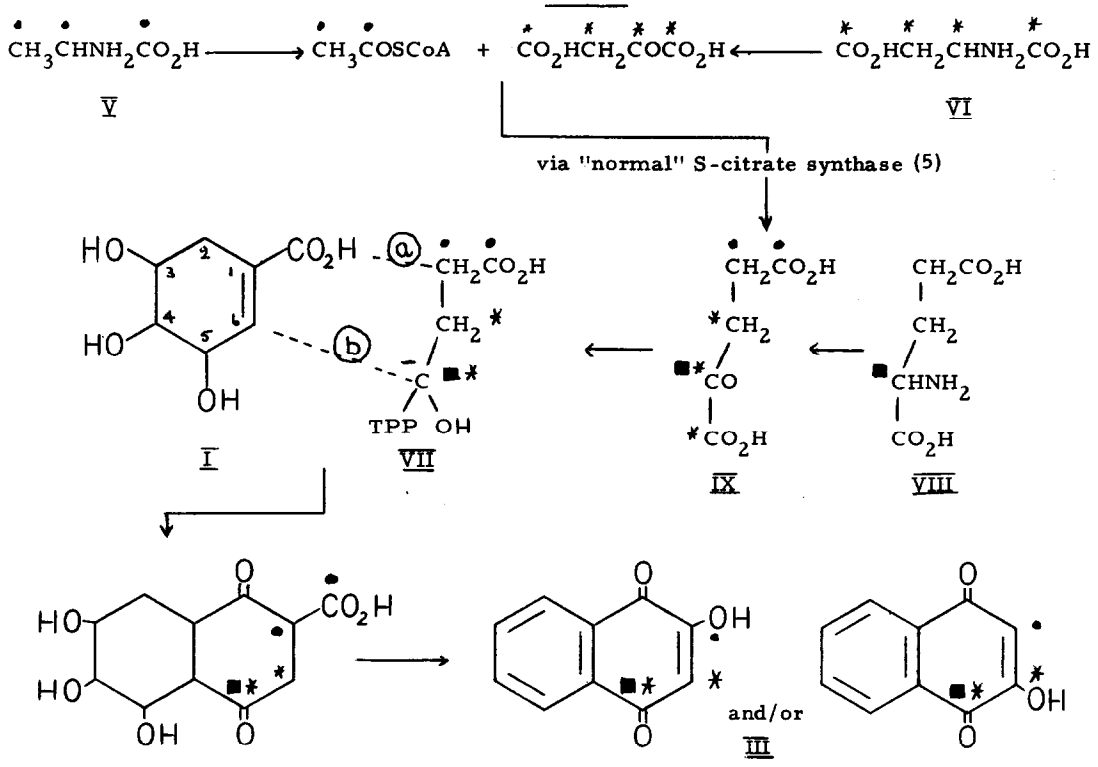
layer chromatography, sublimation and crystallisation to constant specific activity.

After dilution with cold carrier the lawsone was degraded to phthalic acid with alkaline hydrogen peroxide, the liberated carbon dioxide being collected as barium carbonate. Schmidt degradation of the phthalic acid afforded access to lawsone C-1 and C-4. All radioactive measurements were made with a scintillation counter.

The results of feeding U-^{14}C-alanine, 1-^{14}C-alanine and U-^{14}C-aspartate are shown in Table 1. It is clear that both alanine and aspartate have a role in lawsone biosynthesis. Since however, neither of the uniformly labelled substrates yielded in lawsone a C_2 and C_3: C_1 and C_4 ratio of 2:1, neither substance is a direct source of the quested C_3 unit. Donation of two carbon atoms by aspartate and of one or two by alanine appears more in line with the experimental data. The 1-^{14}C-alanine distribution is considered due to random incorporation from CO_2.

An hypothesis which would effectively rationalise these results, the acetate observations mentioned earlier, and additional juglone biosynthesis data previously obtained by Leistner and Zenk (2), is one that considers a thiamine pyrophosphate - succinate semialdehyde complex (VII), the immediate source of the C_3 unit. Its synthesis from acetate and oxaloacetate via the Krebs cycle is well documented and is summarised in Chart 1. Also in Chart 1 is presented a possible mechanism for coupling TPP-succinyl semialdehyde with shikimic acid (TPP =

Chart 1



a = Claisen condensation; b = Michael addition; • = label from U-^{14}C-alanine; ✱ = label from U-^{14}C-aspartate; ■ = label from 2 ^{14}C-glutamate.

thiamine pyrophosphate). The mechanism requires a Michael addition and a Claisen-type condensation and takes into account the finding that in juglone biosynthesis C-1 and C-6 of shikimic acid become C-9 and C-10 of juglone (2).

To test this hypothesis, 2-^{14}C-glutamate (VIII) was fed to I. balsamina plants to serve as a source of 2-^{14}C-2-ketoglutarate (IX) and the postulated complex, (VII). The high level of incorporation of this substrate and its specific donation of activity to lawsone C-1 and/or C-4, are results fully in keeping with the proposed mechanism and confer on it an additional measure of credibility. (Table 1)

Currently we are seeking further corroboration of this naphthoquinone biosynthetic pathway in I. balsamina and other organisms.

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