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BIOSYNTHETIC STUDIES OF ALOENIN. THE INTERMEDIATE AND THE BIOSYNTHETIC SEQUENCE INVOLVED IN THE BIOSYNTHESIS

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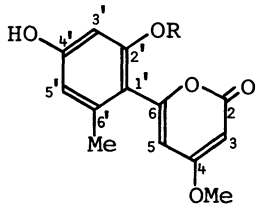
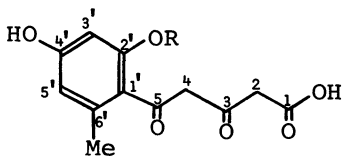
Incorporation of the labels from $^{3}H/^{14}C$-labeled 4-methoxy-6-(2',4'-dihydroxy-6'-methyl)phenyl-2-pyrone (II) and 3,5-dioxo-5-(2'-*O*-glucopyranosyl-4'-hydroxy-6'-methyl)phenylpentanoic acid (III) into aloenin (I) in the Aloe plants demonstrated that the biosynthetic intermediate of aloenin is the pyrone (II) and not the diketo acid (III), and the formation of the aglycone (II) by cyclization of a biological polyketide (IV) and creation of the methoxyl group through methylation is followed by glucosylation which results in the formation of aloenin.

The structure of aloenin, isolated from the leaves of *Aloe arborescens* Mill. var. *natalensis* Berger was first reported as a chromene derivative.[1,2] However, the revised structure was proposed as I,[3] but it remained uncertain due to some ambiguous points in the assignment of the ^{13}C-NMR spectra[3~5] and in the explanation of the anomalous intensity enhancement in the NOE.[3] We recently confirmed the structure of aloenin to be structural formula I by X-ray crystallographic[6] and ^{13}C-NMR spectroscopic[7] studies. In parallel with these studies, feeding experiments of such tracer compounds as ^{14}C-labeled malonate, acetate, phenylalanine, and methionine in the Aloe plants were carried out, and the labeling pattern in aloenin demonstrated that the carbon skeleton of aloenin is generated by the acetate-malonate pathway involving a polyketide intermediate.[8] The biosynthetic pathway of aloenin



I: R=β-D-Glucopyranosyl ($_{1}$"~$_{6}$")

II: R=H



III: R=β-D-Glucopyranosyl ($_{1}$"~$_{6}$")

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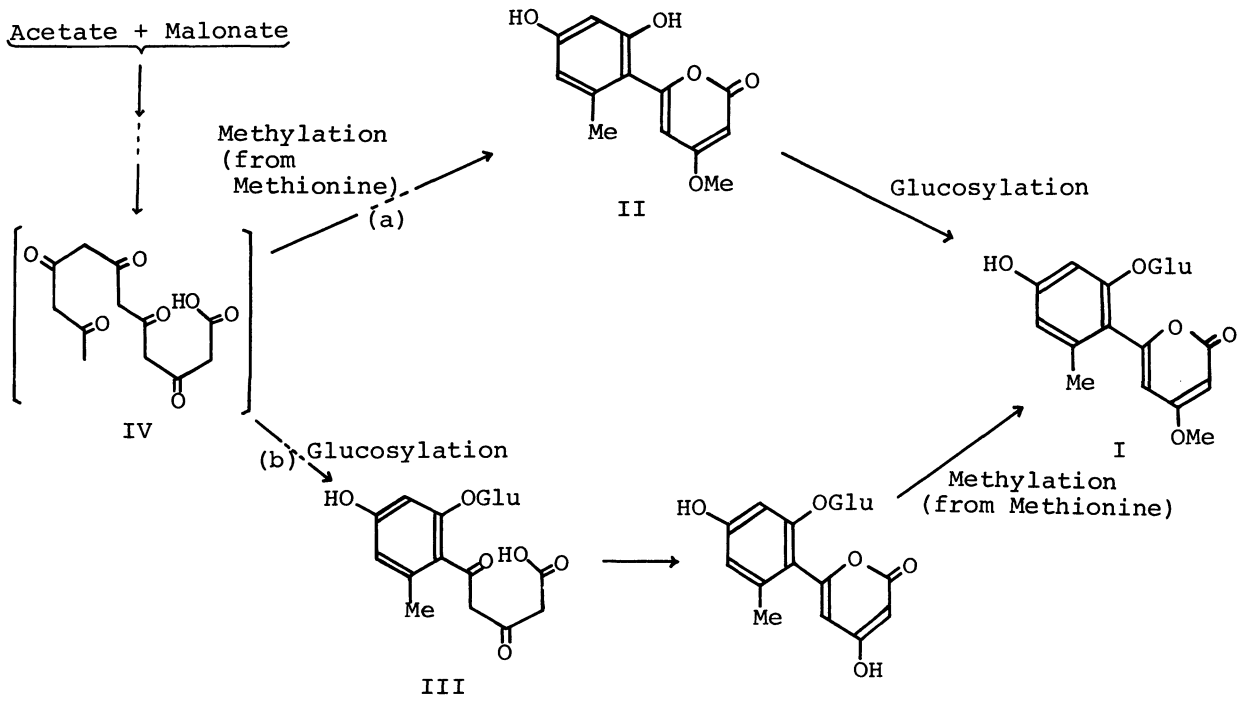
is of considerable interest because of "the masked polyketide structure"[9] and in its biosynthesis the introduction of glucose and the creation of the methoxyl group may occur through pathway (a) or (b) involving a different intermediate, 4-methoxy-6-(2',4'-dihydroxy-6'-methyl)phenyl-2-pyrone (II) or 3,5-dioxo-5-(2'-*o*-glucopyranosyl-4'-hydroxy-6'-methyl)phenylpentanoic acid (III), as shown in Scheme I.[10] We have examined the intermediate and the biosynthetic sequence in stages leading to aloenin (I) from the biological polyketide (IV), and here wish to report the results.



Scheme I. Possible biosynthetic pathways of aloenin (I) in Aloe plants.

In the following way, the $^{3}H/^{14}C$-labeled, two possible intermediates (II) and (III) were prepared from $^{3}H/^{14}C$-labeled aloenin (I) which was synthesized[8] biologically in the Aloe plants by uptake of [1-^{14}C]acetate, L-[methyl-^{3}H]methionine, and D-[3-^{3}H]-glucose, respectively (Table 1). Administration of these labeled compounds to the plants should yield [2,4,6,2',4',6'-^{14}C]-, [methoxy-^{3}H]-, and [3"-^{3}H]-labeled aloenins, respectively, since the carbon skeleton, the methyl of methoxyl group, and the glucose moiety have been established to originate from acetate, methionine, and glucose by previous feeding experiments of the labeled compounds.[8] The [2,4,6,2',4',6'-^{14}C]aloenin (40 mg; 7.61×10^{-3} μCi) was mixed with the [methoxy-^{3}H]aloenin (40 mg; 4.55×10^{-2} μCi), and then the combined material was hydrolyzed[1,3] with 3% HCl/MeOH to yield [methoxy-^{3}H;2,4,6,2',4',6'-^{14}C]pyrone II (32 mg) [ν_{max} (KBr) 3350, 1670, 1628, and 1600 cm^{-1}; δ_{ppm} (acetone-d_6) 2.19 (s, 3H, Ar-Me), 3.92 (s, 3H, OMe), 5.94 (d, J=2.2 Hz, 1H), 6.07 (d, J=2.2 Hz, 1H), and 6.33 (s, 2H, arom. H)]. In addition, a combined sample of [3"-^{3}H]aloenin (70 mg;

TABLE 1. INCORPORATION OF THE RADIOACTIVITY FROM THE RADIOACTIVE PRECURSORS AND THE DOUBLY LABELED POSSIBLE INTERMEDIATES INTO ALOENIN (I) IN THE ALOE PLANTS

Exp. No.	Precursors (μCi) a)	Feeding time (days)	Seasons (month)	Sp. act. of I (dpm/mmole) a)	Incorporation (%)
1	[1-^{14}C]Acetate (1.00×10^{3})	7	April	2.76×10^{6}	0.030
2	[3-^{3}H]Glucose (2.50×10^{3})	7	June	1.12×10^{6}	0.0034
3	[methyl-^{3}H]Methionine (2.50×10^{3})	7	"	5.07×10^{6}	0.036
4	[^{3}H,^{14}C]Pyrone II (1.22×10^{-3})	5	July	8.44×10^{2}	8.1 b)
5	" (1.31×10^{-3})	5	"	6.52×10^{2}	5.5 b)
6	" (4.06×10^{-3})	3	August	8.48×10^{2}	13.4 b)
7	[^{3}H,^{14}C]Diketo Acid III (5.00×10^{-2})	5	July	0	0 b)
8	" (2.55×10^{-2})	3	August	0	0 b)
9	" (2.80×10^{-2})	3	"	0	0 b)

a) Radioactivities of the doubly labeled compounds refer to only ^{14}C.

b) For experiments with the doubly labeled compounds, the incorporation is calculated with respect to only ^{14}C.

TABLE 2. THE ^{3}H/^{14}C LABELING RATIOS IN ALOENIN (I) AFTER UPTAKE OF THE DOUBLY LABELED PYRONE II

Exp. No. a)	Pyrone II			Aloenin (I)		
	Sp. act. (dpm/mmole)		^{3}H/^{14}C Ratio	Sp. act. (dpm/mmole)		^{3}H/^{14}C Ratio
	^{3}H	^{14}C		^{3}H	^{14}C	
4	3.30×10^{5}	5.01×10^{4}	6.59	5.34×10^{3}	8.44×10^{2}	6.33
5	3.30×10^{5}	5.01×10^{4}	6.59	4.07×10^{3}	6.52×10^{2}	6.24
6	2.80×10^{5}	2.46×10^{4}	11.4	8.74×10^{3}	8.48×10^{2}	10.3

a) "Exp. No." corresponds to the number in Table 1.

1.26×10^{-1} μCi) and [2,4,6,2',4',6'-^{14}C]aloenin (36 mg; 6.89×10^{-2} μCi) was treated[3,11] with 2N KOH/MeOH to yield the potassium salt of [3"-^{3}H;1,3,5,2',4',6'-^{14}C]diketo acid III (37 mg) [ν_{max} (Nujol) 3400 (OH) and 1610 (COO^{-}) cm^{-1}; δ_{ppm} (D_2O) 2.30 (s, 3H, Ar-Me) and 6.63 (s, 2H, arom. H)]. These doubly labeled compounds were purified to constant specific activity by preparative TLC and recrystallization.

Administration of the doubly labeled pyrone derivative (II) and the doubly labeled potassium salt of the diketo acid (III) to the Aloe plants and then isolation of aloenin from the plants were carried out in the same manner as described in the previous paper.[8] The labels from the pyrone II was found to be incorporated into aloenin (I) to an extremely large extent in comparison with the incorporation of the labels from such labeled compounds as [1-^{14}C]acetate, [3-^{3}H]glucose, and [methyl-^{3}H]-methionine, as shown in Table 1. In contrast to this, no incorporation of the labels from the diketo acid III into aloenin (I) occurred in neither of the three

trials (Exp. 7~9). These results demonstrate that a direct intermediate for biosynthesizing aloenin (I) is the pyrone (II) and not the diketo acid (III). Further, retention of the $^{3}H/^{14}C$ labeling ratios in aloenin (I) synthesized biologically from the $^{3}H/^{14}C$-labeled pyrone (II) in the Aloe plants, as shown in Table 2, indicates the occurrence of direct glucosylation at the C-2' hydroxyl group of the pyrone (II). Thus, the biosynthesis of aloenin (I) was demonstrated to proceed in pathway (a) shown in Scheme I, that is, formation of the aglycone (II) by cyclization of a polyketide intermediate (IV) and creation of the C-4 methoxyl group through methylation from methionine is followed by glucosylation at the C-2' hydroxyl group.

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