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THE BIOSYNTHESIS OF ALOENIN IN ALOE ARBORESCENS MILL. VAR. NATALENSIS BERGER*

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The ^{14}C-labeling pattern in aloenin (I) biosynthesized from [2-^{14}C]malonate, [1-^{14}C]acetate, [1-^{14}C]- and [3-^{14}C]phenylalanine, and [methyl-^{14}C]methionine in Aloe arborescens Mill. var. natalensis Berger demonstrated that the carbon skeleton of aloenin is generated by the acetate-malonate pathway and that the methyl of the methoxyl group is originated from methionine.

Aloe arborescens Mill. var. natalensis Berger (Japanese name: Kidachirokai or Kidachiaroe) has been widely used in the domestic medicines.[1-3] Recently we isolated from the plant a new bitter glucoside, named as aloenin, exhibiting anti-gastric ulcer activity, and reported its revised structure as I.[4] The biosynthetic pathway of this compound is of considerable interest because of its "masked polyketide structure."[5] In order to establish the biosynthetic pathway, we have now investigated the labeling pattern of aloenin (I) biosynthesized from ^{14}C-labeled tracers, such as sodium malonate, sodium acetate, phenylalanine, and methionine, in a terrestrial part of Aloe arborescens.

Feeding experiments were carried out on the potted plants (ca. 30 cm long) in the months of April, July, and October. A phosphate buffered solution (pH 7.38) of each of the ^{14}C-labeled compounds, such as sodium [2-^{14}C]malonate, sodium [1-^{14}C]acetate, DL-[1-^{14}C]- and DL-[3-^{14}C]phenylalanine, and L-[methyl-^{14}C]methionine, was fed to the whole plant through the stem perforated with a cotton thread for sucking up the solution.[6] The leaves were then minched mechanically and extracted with methanol using a Soxhlet extractor. Removal of the solvent gave a brown viscous oil, which was column-chromatographed over silica gel to yield crude aloenin. This was subsequently purified to constant specific activity by repeated recrystallization.

It was observed that sodium [2-^{14}C]malonate and sodium [1-^{14}C]acetate were incorporated to a considerable extent in contrast to the other ^{14}C-labeled compounds, as shown in Table 1. This suggests that aloenin (I) is biosynthesized by the acetate-malonate pathway, which, probably, involves a biological polyketide intermediate (II). Further, the seasonal variation observed in the incorporation of the ^{14}C-labeled malonate shows that aloenin (I) is biosynthesized more favorably in April rather than in July or October.

In order to establish the labeling pattern of I after the uptake of the precursors,

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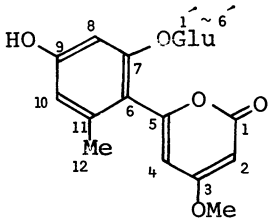
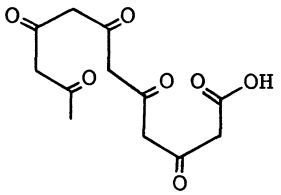
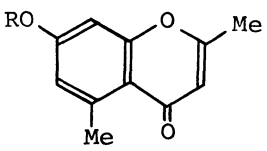
it was degraded[4] using 5% hydrochloric acid to 2,5-dimethyl-7-hydroxychromone (III), glucose, and carbon dioxide, which, in turn, are originated from the C-2~C-12, C-1'~C-6', and C-1 moieties,[7] respectively, of I. The carbon dioxide produced was converted to barium carbonate by absorption in a barium hydroxide solution. The methyl ether (IV) of III was further degraded using 50% sodium hydroxide solution to 2-hydroxy-4-methoxy-6-methylacetophenone (V)[8] corresponding to the C-4~C-12 moiety. Methylation of V by Hakomori's method[9] gave 2,4-dimethoxy-6-methylacetophenone (VI), which was transformed to orcinol dimethyl ether (VII) in the presence of 3N hydrochloric acid. Nitration of the dimethyl ether (VII) afforded a trinitro-compound (VIII), which afforded bromopicrin (IX) involving C-6, C-8, and C-10, respectively, by the bromopicrin cleavage. The radioactivities of these compounds were directly determined in Bray's scintillation solvent[10] using a Packard Tri-Carb liquid scintillation spectrometer (Tables 2~6).



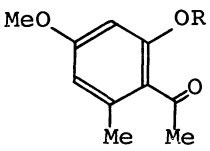
(I)



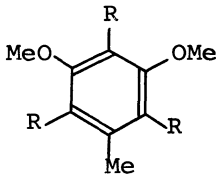
(II)



(III) R=H
(IV) R=Me



(V) R=H
(VI) R=Me



(VII) R=H
(VIII) R=NO_2

TABLE 1. INCORPORATION OF RADIOACTIVE TRACERS INTO ALOENIN (I) IN THE ALOE SPECIES

Exp. No.	Precursors[a] (mCi)	Feeding time (day)	Seasons	Specific radioactivity of I (dpm/mmole)	Incorporation (%)
1	2-^{14}C-SM; 0.005	3	April	2.17×10^4	0.020
2	3-^{14}C-PA; 0.005	3	"	2.28×10^3	0.007
3	2-^{14}C-SM; 0.005	7	"	1.21×10^5	0.180
4	3-^{14}C-PA; 0.005	7	"	4.00×10^3	0.004
5	1-^{14}C-SA; 0.1	7	"	1.87×10^5	0.062
6	2-^{14}C-SM; 0.1	7	July	2.73×10^4	0.049
7	3-^{14}C-PA; 0.02	7	"	1.18×10^3	0.002
8	3-^{14}C-PA; 0.05	7	"	4.44×10^3	0.004
9	m-^{14}C-ME; 0.03	7	"	4.36×10^3	0.012
10	2-^{14}C-SM; 0.1	7	October	3.79×10^3	0.004
11	1-^{14}C-SA; 0.1	7	"	1.85×10^4	0.008
12	1-^{14}C-PA; 0.05	7	"	5.03×10^2	0.001

a) 2-^{14}C-SM, 3-^{14}C-PA, 1-^{14}C-SA, m-^{14}C-ME, and 1-^{14}C-PA denote sodium [2-^{14}C]-malonate, DL-[3-^{14}C]phenylalanine, sodium [1-^{14}C]acetate, L-[methyl-^{14}C]-methionine, and DL-[1-^{14}C]phenylalanine respectively.

TABLE 2. DISTRIBUTION OF RADIOACTIVITY IN ALOENIN (I) AFTER THE UPTAKE OF SODIUM [1-^{14}C]ACETATE

Compounds	Carbons originated from I	Specific radioactivity (dpm/mmole)	Distribution (%) Found	Calcd.[a]
Aloenin (I)	C-1~C-12, MeO, and C-1'~C-6'	2.27×10^4	100	100
Glucose	C-1'~C-6'	8.30×10^3	36.6	——
Chromone (IV)	C-2~C-12	1.29×10^4	56.8	52.8
Acetophenone (V)	C-4~C-12	1.05×10^4	46.3	42.2
Orcinol dimethyl ether (VII)	C-6~C-12	7.56×10^3	33.3	31.6
Bromopicrin (IX)	C-6, C-8, and C-10	0	0	0

a) Theoretical values calculated on the basis of the acetate-malonate pathway, after subtracting the radioactivity of glucose moiety from that of I.

TABLE 3. DISTRIBUTION OF RADIOACTIVITY IN ALOENIN (I) AFTER THE UPTAKE OF SODIUM [2-^{14}C]MALONATE

Compounds	Carbons originated from I	Specific radioactivity (dpm/mmole)	Distribution (%) Found	Calcd.[a]
Aloenin (I)	C-1~C-12, MeO, and C-1'~C-6'	3.82×10^4	100	100
Glucose	C-1'~C-6'	1.20×10^4	31.4	——
Chromone (IV)	C-2~C-12	2.60×10^4	68.1	68.6
Acetophenone (V)	C-4~C-12	2.25×10^4	58.9	57.2
Orcinol dimethyl ether (VII)	C-6~C-12	1.76×10^3	46.2	45.8
Bromopicrin (IX)	C-6, C-8, and C-10	1.14×10^4	29.8	34.2

a) Refer a) in Table 2.

TABLE 4. DISTRIBUTION OF RADIOACTIVITY IN ALOENIN (I) AFTER THE UPTAKE OF [1-^{14}C]PHENYLALANINE

Compounds	Carbons originated from I	Specific radioactivity (dpm/mmole)	Distribution (%) Found	Calcd.[a]
Aloenin (I)	C-1~C-12, MeO, and C-1'~C-6'	5.03×10^2	100	100
Glucose	C-1'~C-6'	8.30×10	16.5	——
Chromone (IV)	C-2~C-12	2.92×10^2	58.1	83.5
Acetophenone (V)	C-4~C-12	2.26×10^2	44.9	0

a) Theoretical values calculated on the basis of the shikimate pathway, after subtracting the radioactivity of glucose moiety from that of I.

As shown in Tables 2 and 3, it was observed that the ^{14}C-labeling pattern of I biosynthesized from [2-^{14}C]malonate and [1-^{14}C]acetate respectively was in complete agreement with that of the acetate-malonate pathway. Further, low incorporation and random distribution of radioactivity were observed in the feeding experiments of [1-^{14}C]- and [3-^{14}C]phenylalanine (Tables 1, 4, and 5). These facts unambiguously establish that the aloenin skeleton is biosynthesized by the acetate-malonate pathway

TABLE 5. DISTRIBUTION OF RADIOACTIVITY IN ALOENIN (I) AFTER THE UPTAKE OF [3-^{14}C]PHENYLALANINE

Compounds	Carbons originated from I	Specific radioactivity (dpm/mmole)	Distribution (%) Found	Calcd.[a]
Aloenin (I)	C-1~C-12, MeO, and C-1'~C-6'	4.44×10^3	100	100
Glucose	C-1'~C-6'	7.50×10^2	16.8	——
Chromone (IV)	C-2~C-12	3.20×10^3	72.0	83.2
Acetophenone (V)	C-4~C-12	2.85×10^3	64.1	83.2
Orcinol dimethyl ether (VII)	C-6~C-12	2.38×10^3	53.5	0

a) Refer a) in Table 4.

TABLE 6. DISTRIBUTION OF RADIOACTIVITY IN ALOENIN (I) AFTER THE UPTAKE OF L-[METHYL-^{14}C]METHIONINE

Compounds	Carbons originated from I	Specific radioactivity (dpm/mmole)	Distribution (%) Found	Calcd.[b]
Aloenin (I)	C-1~C-12, MeO, and C-1'~C-6'	4.36×10^3	100	100
Chromone (IV)	C-2~C-12	9.79×10	2.3	0
Glucose	C-1'~C-6'	0	0	0
CO_2	C-1	5.50×10	1.3	0
——	MeO	——	(96.4)[a]	100

a) Balance amount.
b) Theoretical values calculated on the basis that the methyl of the methoxyl group at C-3 is originated from L-methionine.

and not by the shikimate pathway. The results obtained in the uptake of phenylalanine are explained by the participation of this precursor in the formation of the polyketide (II) via acetoacetyl-CoA which originates from phenylalanine through the intricate metabolic pathway[11] involving even the aromatic ring cleavage. After the uptake of L-[methyl-^{14}C]methionine, a substantial amount of the total radioactivity was located mainly in the methyl group of the methoxyl moiety of I. The distribution of radioactivity on the C-2~C-12, C-1'~C-6', and C-1 moieties was negligibly small and was almost within the experimental error. This demonstrates that the methyl of the methoxyl group at C-3 in aloenin is originated from L-methionine.

References and Footnote

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