Biosynthesis of Aloenin in *Aloe arborescens* var. *natalensis**

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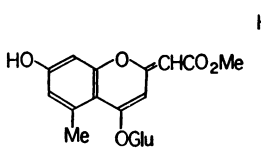
(Received September 12, 1977)

The ^{14}C- and ^{3}H-labeling patterns in aloenin biosynthesized by uptake of acetate-1-^{14}C, malonate-2-^{14}C, phenylalanine-1-^{14}C and -3-^{14}C, methionine-*methyl*-^{14}C, and glucose-3-^{3}H to *Aloe arborescens* var. *natalensis* demonstrated that the carbon skeleton of aloenin is arisen *via* a biological polyketide by the acetate-malonate pathway and the *O*-methyl group originates from methionine. Further, feeding experiments of $^{3}H/^{14}C$-doubly labeled 4-methoxy-6-(2,4-dihydroxy-6-methylphenyl)-2-pyrone and 5-(2-β-D-glucopyranosyloxy-4-hydroxy-6-methylphenyl)-3,5-dioxopentanoic acid in the Aloe plant revealed that an immediate intermediate is the pyrone derivative and not the dioxopentanoic acid derivative. Aloenin was demonstrated to be biosynthesized in such a way that the first formation of the pyrone by cyclization of the biological polyketide and then *O*-methylation of the cyclized intermediate is followed by introduction of a glucose molecule into the pyrone derivative.

Aloe arborescens Mill. var. *natalensis* Berger has been widely used for folk remedies.[3-5] A new bitter glucoside, named aloenin, with an inhibitory activity for the gastric juice secretion of rats was isolated from the plant, and its structure was reported to be a chromene derivative (**1**).[6-7] However, reexaminations of the structure by means of spectroscopic and chemical methods had proposed revised structure **2**,[8] which was recently confirmed by X-ray crystallographic and ^{13}C NMR spectroscopic studies.[9-11] We also examined other bioactive components and chemical constituents of the plant.[12]

The biosynthetic pathway of aloenin (**2**) is of considerable interest because of "the masked polyketide structure".[13-15] In parallel with the structural studies of aloenin, we carried out feeding experiments of the tracer compounds, such as ^{14}C-labeled acetate, malonate, phenylalanine, and methionine, ^{3}H-labeled glucose, and $^{3}H/^{14}C$-doubly labeled 4-methoxy-6-(2,4-dihydroxy-6-methylphenyl)2-pyrone (**3**) and 5-(2-β-D-glucopyranosyloxy-4-hydroxy-6-methylphenyl)-3,5-dioxopentanoic acid (**4**), in the Aloe plants to elucidate the biosynthetic pathway for generating the carbon skeleton of aloenin, the immediate intermediate for the biosynthesis of aloenin, and the biosynthetic sequence in the stage leading to aloenin from the biological polyketide. The results have been partly outlined in the preliminary communications,[1,2] but we wish to describe *en bloc* details of the results.



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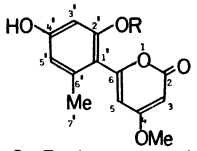
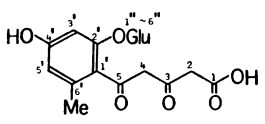


2: R=glucopyranosyl (1''~6'')

3: R=H



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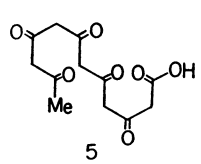

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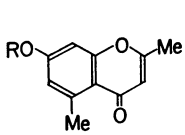
* Preliminary accounts of this work have been communicated in references 1 and 2.

Results and Discussion

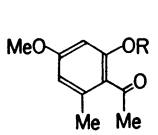
Feeding experiments of the ^{14}C- and ^{3}H-labeled tracer compounds, such as sodium acetate-1-^{14}C, sodium malonate-2-^{14}C, DL-phenylalanine-1-^{14}C, DL-phenylalanine-3-^{14}C, L-methionine-*methyl*-^{14}C, and D-glucose-3-^{3}H, were carried out in the potted plants by the cotton thread method.[16] Sodium acetate-1-^{14}C and sodium malonate-2-^{14}C were incorporated to a considerable extent in contrast to the other ^{14}C- and ^{3}H-labeled tracers, as shown in Table 1. This suggests that aloenin (**2**) may be biosynthesized by the acetate-malonate pathway, which probably involves a biological polyketide intermediate **5**. Further, the seasonal changes in the incorporations of ^{14}C-labeled acetate and malonate into aloenin indicated that the biosynthesis of aloenin (**2**) was favored in April rather than in July or October.

In order to establish the biosynthetic pathway suggested by the foregoing feeding experiments, aloenin biosynthesized by incorporation of the above-described ^{14}C- and ^{3}H-labeled precursors, respectively, was subjected to the degradation to determine the labeling pattern. Aloenin was first degraded using 5% hydrochloric acid to 2,5-dimethyl-7-hydroxychromone (**6**), glucose, and carbon dioxide,[8,11] which, in turn, originate from the C-3—C-6 and C-1′—C-7′, the C-1″—C-6″, and the C-2 moieties of **2**,** respectively. The alkaline degradation of the methylated compound (**7**) derived from **6** gave 2-hydroxy-4-methoxy-6-methylacetophenone (**8**)[17] corresponding to the C-5—C-6 and C-1′—C-7′ moiety. Methylation of **8** by Hakomori's method gave 2,4-dimethoxy-6-methylacetophenone (**9**), which was then transformed to 3,5-dimethoxytoluene (**10**) on treatment with 10% hydrochloric acid. Nitra-



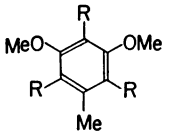
6: R=H

7: R=Me



8: R=H

9: R=Me



10: R=H

11: R=NO_2

** In this paper, for convenience, the carbon atoms of aloenin (**2**), the aglycone (**3**), and the diketo acid (**4**) are numbered as shown in the formulae.

Table 1. Incorporation of the radioactive tracers into aloenin (**2**) by the aloe plants

Exp. No.	Precursor[a] (μCi)	Feeding time (day)	Season	Sp. act. of **2** (dpm/mmol)	Incorporation (%)
1	AcNa-1-^{14}C (1.00×10^{2})	7	April	1.87×10^{5}	0.062
2	AcNa-1-^{14}C (1.00×10^{3})	7	April	2.76×10^{6}	0.030
3	AcNa-1-^{14}C (1.00×10^{2})	7	October	1.85×10^{4}	0.008
4	MaNa-2-^{14}C (5.00)	3	April	2.17×10^{4}	0.020
5	MaNa-2-^{14}C (5.00)	7	April	1.21×10^{5}	0.180
6	MaNa-2-^{14}C (1.00×10^{2})	7	July	2.73×10^{4}	0.049
7	MaNa-2-^{14}C (1.00×10^{2})	7	October	3.79×10^{3}	0.004
8	Phe-1-^{14}C (5.00×10)	7	October	5.03×10^{2}	0.001
9	Phe-3-^{14}C (5.00)	3	April	2.28×10^{3}	0.007
10	Phe-3-^{14}C (5.00)	7	April	4.00×10^{3}	0.004
11	Phe-3-^{14}C (2.00×10)	7	July	1.18×10^{3}	0.002
12	Phe-3-^{14}C (5.00×10)	7	July	4.44×10^{3}	0.004
13	Met-*m*-^{14}C (3.00×10)	7	July	4.36×10^{3}	0.012
14	Met-*m*-^{3}H (2.50×10^{3})	7	June	5.07×10^{6}	0.036
15	Glc-3-^{3}H (2.50×10^{3})	7	June	1.12×10^{6}	0.003

a) AcNa-1-^{14}C, MaNa-2-^{14}C, Phe-1-^{14}C, Phe-3-^{14}C, Met-*m*-^{14}C, Met-*m*-^{3}H, and Glc-3-^{3}H denote sodium acetate-1-^{14}C, sodium malonate-2-^{14}C, D,L-phenylalanine-1-^{14}C, DL-phenylalanine-3-^{14}C, L-methionine-*methoxyl*-^{14}C, L-methionine-*methoxyl*-^{3}H, and D-glucose-3-^{3}H, respectively.

Table 2. Distribution of radioactivity in aloenin (**2**) after uptake of sodium acetate-1-^{14}C

Compound	Carbons originated from **2**	Sp. act. (dpm/mmol)	Distribution (%)	
			Found	Expected[a]
2	C-2—C-6, C-1′—C-7′, MeO, and C-1″—C-6″	2.27×10^{4}	100	100
Glucose	C-1″—C-6″	8.30×10^{3}	36.6	—
7	C-3—C-6 and C-1′—C-7′	1.29×10^{4}	56.8	52.8
8	C-5, C-6 and C-1′—C-7′	1.05×10^{4}	46.3	42.2
10	C-1′—C-7′	7.56×10^{3}	33.3	31.7
Bromopicrin	C-1′, C-3′, and C-5′	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 **2**.

Table 3. Distribution of radioactivity in aloenin (**2**) after uptake of sodium malonate-2-^{14}C

Compound	Carbons originated from **2**	Sp. act. (dpm/mmol)	Distribution (%)	
			Found	Expected[a]
2	C-2—C-6, C-1′—C-7′, MeO, and C-1″—C-6″	3.82×10^{4}	100	100
Glucose	C-1″—C-6″	1.20×10^{4}	31.4	—
7	C-3—C-6 and C-1′—C-7′	2.60×10^{4}	68.1	68.6
8	C-5, C-6 and C-1′—C-7′	2.25×10^{4}	58.9	57.2
10	C-1′—C-7′	1.76×10^{4}	46.1	45.8
Bromopicrin	C-1′, C-3′, and C-5′	1.14×10^{4}	29.8	34.2

a) Refer a) in Table 2.

Table 4. Distribution of radioactivity in aloenin (**2**) after uptake of DL-phenylalanine-1-^{14}C

Compound	Carbons originated from **2**	Sp. act. (dpm/mmol)	Distribution (%)	
			Found	Expected[a]
2	C-2—C-6, C-1′—C-7′, MeO, and C-1″—C-6″	5.03×10^{2}	100	100
Glucose	C-1″—C-6″	8.30×10	16.5	—
7	C-3—C-6 and C-1′—C-7′	2.92×10^{2}	58.1	83.5
8	C-5, C-6, and C-1′—C-7′	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 **2**.

Table 5. Distribution of radioactivity in aloenin (**2**) after uptake of DL-phenylalanine-3-^{14}C

Compound	Carbons originated from **2**	Sp. act. (dpm/mmol)	Distribution (%)	
			Found	Expected[a]
2	C-2—C-6, C-1′—C-7′, MeO, and C-1″—C-6″	4.44×10^3	100	100
Glucose	C-1″—C-6″	7.50×10^2	16.9	—
7	C-3—C-6 and C-1′—C-7′	3.20×10^3	72.0	83.1
8	C-5, C-6, and C-1′—C-7′	2.85×10^3	64.1	83.1
10	C-1′—C-7′	2.38×10^3	53.5	0

a) Refer a) in Table 4.

Table 6. Distribution of radioactivity in aloenin (**2**) after uptake of L-methionine-*methyl*-^{14}C

Compound	Carbons originated from **2**	Sp. act. (dpm/mmol)	Distribution (%)	
			Found	Expected[b]
2	C-2—C-6, C-1′—C-7′, MeO, and C-1″—C-6″	4.36×10^3	100	100
7	C-3—C-6 and C-1′—C-7′	9.79×10	2.3	0
Glucose	C-1″—C-6″	0	0	0
CO_2	C-2	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-4 is originated from L-methionine.

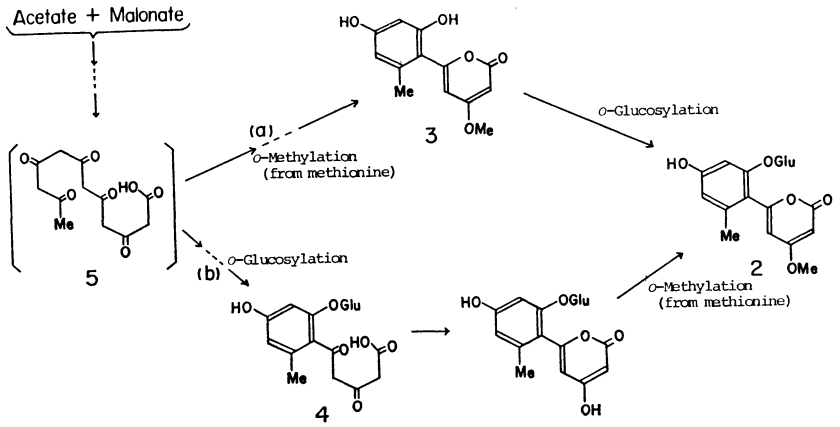
Table 7. Distribution of radioactivity in aloenin (**2**) after uptake of D-glucose-3-^3H

Compound	Carbons originated from **2**	Sp. act. (dpm/mmol)	Distribution (%)	
			Found	Expected[a]
2	C-2—C-6, C-1′—C-7′, MeO, and C-1″—C-6″	4.80×10^3	100	100
Glucose	C-1″—C-6″	4.52×10^3	94.2	100
7	C-3—C-6 and C-1′—C-7′	2.00×10^3	4.1	0

a) Theoretical values calculated on the basis that the glucose moiety of **2** is originated from D-glucose.

tion of **10** afforded the trinitro derivative (**11**), which on bromopicrin cleavage[18,19] afforded bromopicrin originated, respectively, from C-1′, C-3′, and C-5′ of **2**. The radioactivities of these compounds are listed in Tables 2—7. It was observed that the ^{14}C-labeling patterns of **2** biosynthesized from acetate-1-^{14}C and malonate-2-^{14}C, respectively, were in complete agreement with the pattern anticipated from the acetate-malonate pathway (Tables 2 and 3). In feeding experiments of phenylalanine-1-^{14}C and -3-^{14}C (Tables 1, 4, and 5), the low incorporation of the tracers and the random distribution of radioactivities were observed in contrast with the incorporations of the acetate and the malonate. These facts unambiguously establish that the carbon skeleton of aloenin is generated by the acetate-malonate pathway and not by the shikimate pathway. The above results obtained in the incorporations of phenylalanine may be explained by participation of these precursors in the formation of polyketide **5** *via* acetoacetyl-CoA which is probably produced from phenylalanine through such an intricate metabolic pathway[20] as involving the cleavage of even the aromatic ring.

After uptake of L-methionine-*methyl*-^{14}C, almost all the amount of the total radioactivities were located on the *O*-methyl group of aloenin, as shown in Table 6. The distribution of radioactivity on the C-2—C-6, C-1′—C-7′, and C-1″—C-6″ carbon atoms was negligibly small and was within the experimental error. This demonstrates that the *O*-methyl group at C-4 of aloenin (**2**) originates from the *S*-methyl group of L-methionine. On the other hand, the ^3H-labeling pattern in aloenin biosynthesized by incorporation of D-glucose-3-^3H are shown in Table 7. The pattern indicates that the glucose moiety (C-1″—C-6″) is produced by introduction of a molecule of glucose. In the stage leading to aloenin from the biological polyketide (**5**), the introduction of the glucose molecule and the creation of the methoxyl group may occur through pathway (a) or (b) involving a different compound, 4-methoxy-6-(2,4-dihydroxy-6-methylphenyl)-2-pyrone (**3**) or 5-(2-β-D-glucopyranosyloxy-4-hydroxy-6-methylphenyl)-3,5-dioxopentanoic acid (**4**), which are possible as an immediate intermediate for the biosynthesis of aloenin, as shown in Scheme 1. In order to prove the immediate intermediate and the biosynthetic sequence in the stage, the $^3H/^{14}C$-doubly labeled compounds, (**3**) and (**4**), were prepared from the labeled aloenin and subjected to feeding experiments as follows. The ^{14}C- and ^3H-labeled specimens of aloenin were synthesized biologically by feeding acetate-1-^{14}C, L-methionine-*methyl*-^3H, and D-glucose-3-^3H to the Aloe plant (Table 1). The incorporation of these labeled compounds into aloenin should yield 2, 4, 6, 2′, 4′, 6′-^{14}C-, *methoxyl*-^3H-, and



Scheme 1. Possible biosynthetic pathways of aloenin (**2**) in aloe plants.

Table 8. Incorporation of the doubly labeled tracers, **3** and **4**, into aloenin (**2**) by the aloe plants

Exp. No.	Precursor[a,b] (μCi)	Feeding time (day)	Season	Sp. act. of **2** (dpm/mmol)[b]	Incorporation (%)[c]
16	Pyrone-^3H; ^{14}C (1.22×10^{-3})	5	July	8.44×10^2	8.1
17	Pyrone-^3H; ^{14}C (1.31×10^{-3})	5	July	6.52×10^2	5.5
18	Pyrone-^3H; ^{14}C (4.06×10^{-3})	3	August	8.48×10^2	13.4
19	Acid-^3H; ^{14}C (5.00×10^{-2})	5	July	0	0
20	Acid-^3H; ^{14}C (2.55×10^{-2})	3	August	0	0
21	Acid-^3H; ^{14}C (2.80×10^{-2})	3	August	0	0

a) Pyrone-^3H; ^{14}C and acid-^3H; ^{14}C denote 4-methoxy-6-(2,4-dihydroxy-6-methylphenyl)-2-pyrone-*methoxyl*-^3H; 2,4,6,2′,4′,6′-^{14}C (**3**) and potassium salt of 5-(2-β-D-glucopyranosyloxy-4-hydroxy-6-methylphenyl)-3,5-dioxopentanoic acid-3″-^3H; 1,3,5,2′,4′,6′-^{14}C (**4**), respectively. b) Radioactivities of the doubly labeled compounds refer to only ^{14}C. c) The incorporations were calculated with respect to only ^{14}C.

Table 9. The $^3H/^{14}C$ labeling ratios in aloenin (**2**) after uptake of doubly labeled pyrone **3**

Exp. No.[a]	Pyrone **3**			Aloenin (**2**)		
	Sp. act. (dpm/mmol)		$^3H/^{14}C$ Ratio	Sp. act. (dpm/mmol)		$^3H/^{14}C$
	^3H	^{14}C		^3H	^{14}C	
16	3.30×10^5	5.01×10^1	6.59	5.34×10^3	8.44×10^2	6.33
17	3.30×10^5	5.01×10^4	6.59	4.07×10^3	6.52×10^2	6.24
18	2.80×10^5	2.46×10^1	11.4	8.74×10^3	8.48×10^2	10.3

a) "Exp. No." corresponds to the number in Table 8.

3″-^3H-labeled aloenin, respectively, since the carbon skeleton, the methoxyl group, and the glucose moiety have been established to be arisen from acetate, methionine, and glucose as described above. Aloenin-2,4,6,2′,4′,6′-^{14}C was mixed with aloenin-*methoxyl*-^3H and then the combined material was hydrolyzed[8,11] with 3% HCl–MeOH to yield 4-methoxy-6-(2,4-dihydroxy-6-methylphenyl)-2-pyrone-*methoxyl*-^3H; 2,4,6,2′,4′,6′-^{14}C (**3**). On the other hand, following the method established for the synthesis of the corresponding diketo acid by alkaline degradation of the 6-substituted 4-methoxy-2-pyrone derivatives,[8,11] a combined sample of aloenin-3″-^3H and aloenin-2,4,6,2′,4′,6′-^{14}C was treated with 5% KOH–MeOH to yield potassium salt of 5-(2-β-D-glucopyranosyloxy-4-hydroxy-6-methylphenyl)-3,5-dioxopentanoic acid-3″-^3H; 1,3,5,2′,4′,6′-^{14}C (**4**).

Feeding experiments of the doubly labeled pyrone (**3**) and the potassium salt of diketo acid **4** to the Aloe plant were carried out in the same manner as above. The labeled pyrone **3** was incorporated into aloenin to an extremely large extent (Exp. 16—18) in comparison with the incorporation of such labeled compounds as acetate-1-^{14}C, malonate-2-^{14}C, glucose-3-^3H, and methionine-*methyl*-^3H, as shown in Tables 1, 8, and 9. In contrast to this, no incorporation of the diketo acid (**4**) into aloenin occurred in all of the three trials (Exp. 19—21). These results demonstrate that the pyrone **3**, but not the diketo acid **4**, is an immediate intermediate for the biosynthesis of aloenin. Further, the $^3H/^{14}C$ ratio of the doubly labeled pyrone (**3**) was retained in aloenin biosynthesized by uptake of the labeled pyrone, as shown in Table 9. This indicates the occurrence of *O*-glucosylation at the C-2′ hydroxyl group of the pyrone **3**. Thus, the biosynthesis of aloenin (**2**) was demonstrated to proceed in pathway (a) as shown in Scheme 1, that is, the first formation of aglycone **3** by cyclization of biological polyketide 5

produced by the acetate-malonate pathway and then by creation of the C-4 methoxyl group by *O*-methylation with methionine is followed by introduction of a glucose molecule into the C-2′ hydroxyl group of the aglycone (**3**).

Experimental

The radioactive products were purified to constant specific radioactivity by a combination of preparative TLC (silica gel; 0.75 mm in thickness) and repeated recrystallization. All the products had PMR, IR, and MS spectra consistent with the structure, and the melting points and spectral data agreed with the corresponding authentic samples.[8,11] Radioactivities were measured on a liquid scintillation spectrometer using Bray's scintillation solvent.[21] The counting error was within ±2%.

Incorporation of the Labeled Compounds into Aloenin by the Aloe Plants. A phosphate buffered solution (pH 7.38) of each of the ^{14}C- and ^{3}H-labeled compounds was fed to the potted whole plants (*ca.* 30 cm in length) through the stem perforated with a cotton thread for sucking up the solution.[16] After 3—7 days, the leaves (*ca.* 200 g) were minced mechanically and extracted with MeOH by means of a Soxhlet apparatus. Removal of the solvent gave a brown viscous mass, which was chromatographed on a silica gel column with $CHCl_3$–MeOH (3:1) to give aloenin (**2**) (*ca.* 220 mg): Mp 145—147 °C (lit,[8,11] mp 145—147 °C); IR (Nujol) 3350 (OH), 1710 (C=O), and 1640 cm^{-1} (C=C); PMR (acetone-d_6) δ 2.19 (s, 3H, Ar-Me) and 3.86 (s, 3H, OMe). The radioactivities are shown in Table 1.

*Degradation of **2** with Hydrochloric Acid.* Aloenin (**2**) (100 mg), obtained by incorporating the tracer compounds, was refluxed[8,11] with 5% HCl (10 ml) for 2 h using the $Ba(OH)_2$ trap attached to the reflux apparatus. Carbon dioxide liberated was converted to $BaCO_3$ by absorbing it in the $Ba(OH)_2$ solution. The reaction mixture was then extracted with ether to give 2,5-dimethyl-7-hydroxychromone (**6**) (45 mg): Mp 245 °C (dec) (lit,[17] mp 245 °C (dec)); IR (Nujol) 1665 (C=O) and 1618 cm^{-1} (C=C). The aqueous layer was passed through an Amberlite resin IR-45 (OH) column until all chloride ions had been removed to give D-glucose (18 mg): Mp 144—145 °C. Methylation of **6** (30 mg) with CH_2N_2 gave 2,5-dimethyl-7-methoxychromone (**7**) (29 mg): Mp 116—117 °C (lit,[17] mp 116—117 °C); IR (Nujol) 3050, 1650, 1630, and 1570 cm^{-1}; PMR ($CDCl_3$) δ 2.25 (s, 3H, C(2)-Me), 2.77 (s, 3H, Ar-Me), and 3.81 (s, 3H, OMe).

*Degradation of **7** with Sodium Hydroxide.* To a solution of **7** (45 mg) in MeOH (4 ml), 30% KOH solution (10 ml) was added, and the mixture was refluxed under an atmosphere of N_2.[17] The reaction mixture, after acidification, was extracted with ether to give 2-hydroxy-4-methoxy-6-methylacetophenone (**8**) (15 mg): Mp 78.0—78.5 °C (lit,[17,22] mp 78.0—78.5 °C); IR (KBr) 1612, 1590, 1500, 859, and 821 cm^{-1}; PMR ($CDCl_3$) δ 2.53 (s, 3H, Ar-Me), 2.58 (s, 3H, COMe), 3.77 (s, 3H, OMe), 6.25 (s, 2H, arom. H), and 13.48 (s, 1H, OH). Methylation of **8** (10 mg) by Hakomori's method[23] gave 2,4-dimethoxy-6-methylacetophenone (**9**) (7 mg): Mp 41—42 °C (lit,[22] mp 41—42 °C); IR ($CDCl_3$) 1682 and 1600 cm^{-1}; PMR ($CDCl_3$) δ 2.23 (s, 3H, Ar-Me), 2.44 (s, 3H, COMe), 3.76 (s, 6H, 2×OMe), and 6.28 (s, 2H, arom. H).

*Degradation of **9** with Hydrochloric Acid.* A solution of **9** (140 mg) in 5% HCl (25 ml) was refluxed for 10 h, and the reaction mixture was extracted with ether to give an oily product (103 mg), which was separated on preparative TLC with hexane–EtOAc (3:1) to afford 3,5-dimethoxytoluene (**10**) (95 mg): Bp 130—132 °C (7.0 mmHg); n_D^{25} 1.5239; IR (liq.) 1600, 1298, and 1207 cm^{-1}; PMR ($CDCl_3$) δ 2.32 (s, 3H, Ar-Me), 3.73 (s, 6H, 2×OMe), and 6.30 (s, 3H, arom. H).

*Nitration of **10**.* To a solution of **10** (250 mg) in concd H_2SO_4 (3 ml), a mixture of concd HNO_3 (3 ml) and concd H_2SO_4 (3 ml) was added under ice-cooling. After heating for 3 min at 60 °C, the reaction mixture was poured into H_2O (60 ml) and then allowed to stand overnight to give yellow powders (119 mg). Recrystallization of the powders afforded pale yellow crystals of the trinitro derivative (**11**): Mp 70—71 °C; *m/e* 287 (M^+); IR (KBr) 1625, 1570, 1500, 1380, and 882 cm^{-1}; PMR ($CDCl_3$) δ 2.38 (s, 3H, Ar-Me) and 4.12 (s, 6H, 2×OMe).

*Bromopicrin Cleavage of **11**.* According to the procedure of bromopicrin cleavage,[18,19] a paste of $Ca(OH)_2$ (7.0 g in 30 ml of H_2O) was added at 0 °C to a solution of **11** (20 mg) in a saturated solution of $Ca(OH)_2$ (1.0 ml), and Br_2 (2.5 ml) was then dropped into the mixture under stirring. After the mixture had been kept at 10 °C for 2 h until disappearance of the yellow color, it was steam-distilled to give bromopicrin (6 mg): IR (liq.) 1560 and 1351 cm^{-1} (NO_2).

Preparation of 4-Methoxy-6-(2,4-dihydroxy-6-methylphenyl)-2-pyrone-methoxy-^{3}H;*2,4,6,2′,4′,6′-*^{14}C (**3**).* The singly labeled tracers, aloenin-2,4,6,2′,4′,6′-^{14}C and *-methoxyl-*^{3}H, were synthesized biologically by administration of acetate-1-^{14}C and L-methionine-*methyl-*^{3}H, respectively, into the Aloe plant in the same manner as described above (Table 1). A mixture of the ^{14}C-labeled aloenin (40 mg) and the ^{3}H-labeled aloenin (40 mg) was refluxed[8,11] with 3% HCl–MeOH (10 ml) for 2 h. The reaction mixture was diluted with H_2O (30 ml) and extracted with ether using a continuous extractor to give a crude product (32 mg), which was purified to constant specific radioactivity by preparative TLC with MeOH–$CHCl_3$ (1:4) followed by recrystallization to afford pure doubly-labeled pyrone **3**: Mp 213—214 °C; IR (KBr) 3350, 1670, 1628, and 1600 cm^{-1}; PMR (acetone-d_6) δ 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. 2H). The radioactivities are shown in Table 8.

Preparation of Potassium Salt of 5-(2-β-D-Glucopyranosyloxy-4-hydroxy-6-methylphenyl)-3,5-dioxopentanoic acid-3″-^{3}H;*1,3,5,2′,4′,6′-*C-^{14} (**4**).* The singly labeled tracers, aloenin-2,4,6,2′,4′,6′-^{14}C and -3″-^{3}H, were prepared biologically from acetate-1-^{14}C and D-glucose-3-^{3}H, respectively (Table 1). According to the reported procedure,[8,11] a mixture of the ^{14}C-labeled aloenin (36 mg) and the ^{3}H-labeled aloenin (70 mg) dissolved in 5% KOH–MeOH (10 ml) was stirred at room temperature under an atmosphere of N_2. After 3 days, the reaction mixture was subjected to preparative TLC with 1-BuOH–MeOH–H_2O (7:3:3) to give potassium salt (37 mg) of doubly labeled **4**: IR (Nujol) 3400 (OH) and 1610 cm^{-1} (COO^-); PMR (D_2O) δ 2.30 (s, 3H, Ar-Me) and 6.63 (s, 2H, arom. 2H). The radioactivities are shown in Table 8.

This investigation was partially supported by a Grant-in-Aid (074176 and 047089) for Scientific Research in 1975 from the Ministry of Education.

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