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BIOSYNTHETIC RELATIONSHIP BETWEEN TETRAHYDROANTHRACENE AND ANTHRAQUINONE IN *ALOE SAPONARIA*

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Key Word Index—*Aloe saponaria*; Liliaceae; aloesaponol I; laccaic acid D methyl ester; aloesaponarin I; biosynthesis.

Abstract—The incorporation of $Me^{14}COONa$ into aloesaponol I, laccaic acid D methyl ester and aloesaponarin I was demonstrated. The biosynthetic relation between aloesaponol I and aloesaponarin I was established, but incorporation of aloesaponol I into laccaic acid D methyl ester, or *vice versa* was not demonstrated and this result was confirmed by an investigation using labelled laccaic acid D methyl ($^{14}CH_3$) ester. It was possible to show that aloesaponol I and laccaic acid D methyl ester were biosynthesized in parallel in *Aloe saponaria*.

INTRODUCTION

In previous papers we described the identification of 1,2,3,4-tetrahydroanthracenes (aloesaponol I, -II, -III, -IV, the glucoside) anthraquinones (aloesaponarin I, -II, laccaic acid D methyl ester, deoxyerythrolaccin, chrysophanol, helminthosporin, isoxanthorin) and phenolics (aloesin, aloenin and isoeleutherol) from the rhizoma of *Aloe saponaria* [1]. In a biosynthetic study on anthraquinones the acetate–malonate or shikimate pathway has been demonstrated [2]. Sankawa [3] reported that modified bianthraquinoids must be produced by hydrogenation of the aromatic ring of emodin or emodin anthrone in *Penicillium* spp. but Takahashi [4] indicated that the tetrahydroanthracene, germichrysone, is not derived from the anthraquinone originally contained in the seed of *Cassia torosa*, but is a product of *de novo* biosynthesis in the seedling.

In the formation of the hydroxyl group Dimroth [5] pointed out that the partial reduction of a carbonyl of the polyketomethylene intermediate followed by dehydration of the secondary alcohol group occurred at the triacetic acid level before the final condensation with malonyl CoA to give 6-methylsalicylic acid in *Penicillium patulum*. Stipanovic [6] reported that (−)-vermelone was derived from (+)-scytalone via 1,3,8-trihydroxynaphthalene in *Verticillium dahliae*. The biosynthetic relationship between anthraquinone and tetrahydroanthracene still remains obscure. This paper deals with the biosynthetic relation between aloesaponol I, aloesaponarin I and laccaic acid D methyl ester in *Aloe saponaria*.

RESULTS AND DISCUSSION

Administration of $Me^{14}COONa$

$Me^{14}COONa$ (1.0 mCi) was administered to the plant and, after feeding, the MeOH extract was purified by PLC and the dilution method to give radioactive aloesaponol I, aloesaponarin I, laccaic acid D methyl ester, aloesaponol I 6-*O*-β-D-glucopyranoside and aloesin.

Conversion of aloesaponol I to aloesaponarin I

Aloesaponol I (1.17×10^4 dpm; 4.70×10^3 dpm) (**1**), dissolved in Me_2CO–H_2O, was administered to the plant. After the feeding the plant was extracted with MeOH. The MeOH extract was purified by PLC and the dilution method to give radioactive aloesaponarin I (incorporation, 1.47%; sp. incorpn., 5.03%).

Administration of aloesaponol I (**1**)

Aloesaponol I (4.70×10^3 dpm; 7.05×10^3 dpm) (**1**), dissolved in Me_2CO–H_2O, was administered to the plant. After the feeding the same procedure as that employed with aloesaponarin I was carried out. Laccaic acid D methyl ester (**3**) purified by PLC and the dilution method showed no radioactivity.

Administration of laccaic acid D methyl ester (**3**)

Laccaic acid D methyl ester (1.45×10^4 dpm; 6.46×10^4 dpm) (**3**), dissolved in Me_2CO–H_2O, was fed to the plant which was then extracted as for **1**. Aloesaponol I (**1**) purified by PLC and the dilution method showed no radioactivity.

Administration of laccaic acid D methyl ($^{14}CH_3$) ester (**4**)

Synthesized laccaic acid D methyl ($^{14}CH_3$) ester (9.62×10^4 dpm), dissolved in Me_2CO–H_2O, was administered to the plant and the same procedure as that for **1** was carried out. Aloesaponol I purified by PLC and the dilution method showed no radioactivity. The incoporation of aloesaponol I (**1**) to aloesaponarin I (**2**) indicated that a chrysophanol type anthraquinone was derived from 1-oxo-3(equatorial),8,9-trihydroxy-6-methyl-1,2,3,4-tetrahydroanthracene by dehydration followed by oxidation. This evidence is coincident with the dehydration process in (+)-scytalone to give 1,3,8-trihydroxynaphthalene [6]. Comparison of the incorporation ratio in **1**, **2** and **3** from acetate indicates that an emodin type anthraquinone might be biosynthesized at an earlier stage. The results that no incorporation of aloesaponol I (**1**) into laccaic acid D methyl ester (**3**) or

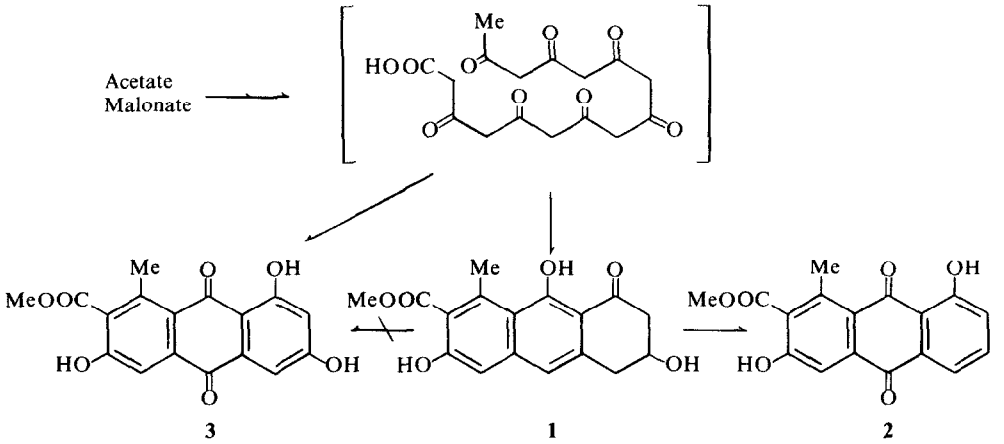
Table 1. Incorporation results from $Me^{14}COONa$ administered to *Aloe saponaria*

Metabolites	$Me^{14}COONa$ (dpm/mg)	(dpm/mM)
Aloesaponol I (**1**)	2.35×10^3	7.43×10^5
Aloesaponarin I (**2**)	2.30×10^3	7.17×10^5
Laccaic acid D methyl ester (**3**)	1.01×10^4	3.31×10^6
Aloesaponol I in aloesaponol I 6-*O*-*β*-D-glucopyranoside	1.35×10^3	4.27×10^5
Aloesin	2.29×10^4	9.02×10^6

vice versa occurred, suggested that aloesaponol I (**1**) and laccaic acid D methyl ester (**3**) are biosynthesized in parallel and not in sequence. The partial hydrogenation at either the polyketomethylene level or before the aromatization was indicated. The biosynthetic pathway is shown in Scheme 1.

5 g. After 48 hr feeding the rhizome and subterranean parts were washed with Me_2CO, chipped and exhaustively extracted with MeOH. The MeOH extract (4.30×10^7 dpm) was purified by PLC and the dilution method to give **1** (mp 249–250°, 2.35×10^3 dpm/mg; 7.43×10^5 dpm/mM), **2** (mp 200–203°, 2.30×10^3 dpm; 7.17×10^5 dpm/mM), **3** (mp 274–278°, 1.01×10^4 dpm/mg; 3.31×10^6 dpm/mM), **1** (mp 250–253°, 1.35×10^3 dpm/mg; 4.27×10^5 dpm/mM) in aloesaponol I 6-*O*-*β*-D-glucopyranoside, and aloesin (mp 137–140°, 2.29×10^4 dpm/mg; 9.02×10^6 dpm/mM).

Feeding of **1**. **1** (1.17×10^4 dpm; 4.70×10^3 dpm) dissolved in Me_2CO–H_2O was administered to the wilted plant for 24 or 48 hr and the rhizome and subterranean parts were washed with Me_2CO. The chipped material was exhaustively extracted with MeOH. Each MeOH extract (2.14×10^2 dpm; 2.35×10^2 dpm) was combined and purified by PLC and dilution method to give radioactive **2** (mp 200–202°, 1.20×10^2 dpm/mg, 3.74×10^4 dpm/mM). **1** (4.70×10^3 dpm; 7.05×10^3 dpm) dissolved in Me_2CO–H_2O was administered to the wilted plant for 24 or 48 hr. After the feeding the rhizome and subterranean parts were extracted with MeOH and the MeOH



Scheme 1. The biosynthetic relation between aloesaponol I (**1**), laccaic acid D methyl ester (**3**) and aloesaponarin I (**2**) in *Aloe saponaria*.

EXPERIMENTAL

General procedure. Mps are uncorr. TLC was performed on Si gel developing with EtOAc–$CHCl_3$ (1:1) for **1**, **2**, **3** and **4** and $CHCl_3$–MeOH–H_2O (7:3:1, lower layer) for glycosides. The spots on TLC were monitored by UV or detected with Echtblausalz B(Merck)-KOH. $Me^{14}COONa$ (1.0 mCi) was purchased from Daiichi Chemical Company (Osaka, Japan) and $^{14}CH_3I$ (1.0 mCi) from New England Nuclear (Mass., U.S.A.). Radioactivity was measured with a scintillator soln made up of PPO (0.7 g), POPOP (0.005 g) and naphthalene (10 g) in dioxane (100 ml). All radioactive products were recrystallized to constant sp. act.

Preparation of laccaic acid D methyl ($^{14}CH_3$) ester (**4**). Laccaic acid D was synthesized by saponification of laccaic acid D methyl ester (30 mg) with NaOMe (800 mg) in DMF (10 ml) by refluxing for 1 hr. Laccaic acid D, orange needles (recrystallized from EtOAc), mp > 300°, UV λ_{max}^{MeOH} nm (log ε): 236 (3.92), 286 (3.88), 436 (3.75); IR ν_{max}^{KBr} cm^{-1}: 3415, 1690, 1662, 1630 [7]. To a soln of laccaic acid D (12 mg) dissolved in Me_2CO (12 ml), $NaHCO_3$ (40 mg) and MeI (6 ml) were added and the reaction mixture was cooled with dry ice. Then $^{14}CH_3I$ (1.0 mCi) was passed to the reaction mixture via manifolds and the mixture was allowed to stand for 5 hr at room temp. After purification by PLC laccaic acid D methyl ($^{14}CH_3$) ester was recrystallized from MeOH to give **4**, mp 268–270° (1.20×10^4 dpm/mg; 3.94×10^6 dpm/mM).

Feeding of $Me^{14}COONa$. An aq. soln (2 ml) of $Me^{14}COONa$ (1.0 mCi) was administered to the wilted plant, weighing *ca* extract (4.50×10^3 dpm; 5.75×10^3 dpm) was respectively purified by PLC followed by the dilution method to afford no radioactive **3**.

Feeding of **3**. **3** (1.45×10^4 dpm; 6.46×10^4 dpm) dissolved in Me_2CO–H_2O was administered to the wilted plant for 24 or 48 hr. After the feeding the rhizome and subterranean parts were extracted with MeOH and MeOH extract (1.17×10^4 dpm; 5.00×10^4 dpm) was respectively purified by PLC followed by the dilution method to give no radioactive **1**.

Feeding of **4**. **4** (9.62×10^4 dpm) dissolved in Me_2CO–H_2O was administered to the wilted plant. After feeding for 48 hr the rhizome and subterranean parts were extracted with MeOH and the MeOH extract (7.34×10^4 dpm) was purified by PLC followed by the dilution method to give no radioactive **1**.

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