

BIOSYNTHETIC RELATIONSHIP BETWEEN TETRAHYDROANTHRACENE AND ANTHRAQUINONE IN *ALOE SAPONARIA*

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(Received 8 November 1977)

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

Abstract—The incorporation of $\text{Me}^{14}\text{COONa}$ 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 ($^{14}\text{CH}_3$) 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, germichryson, 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 $\text{Me}^{14}\text{COONa}$

$\text{Me}^{14}\text{COONa}$ (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 $\text{Me}_2\text{CO}-\text{H}_2\text{O}$, 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 $\text{Me}_2\text{CO}-\text{H}_2\text{O}$, 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 $\text{Me}_2\text{CO}-\text{H}_2\text{O}$, 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.

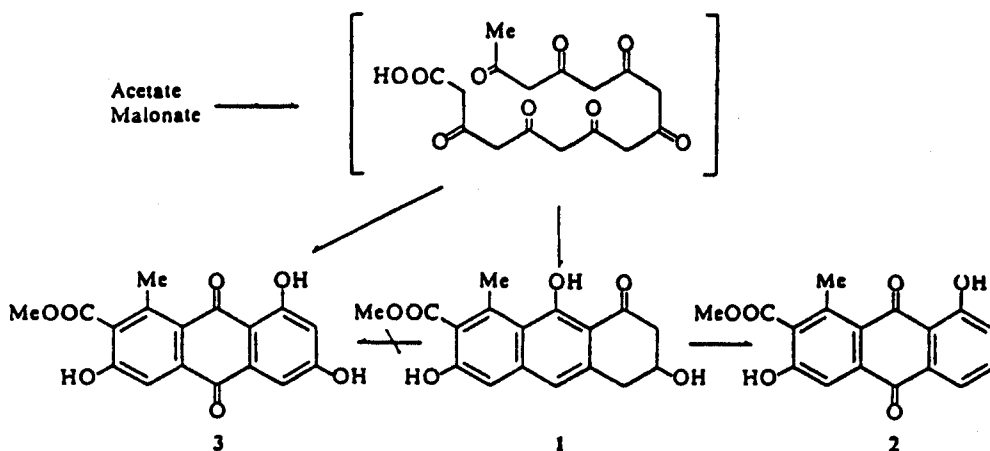
Administration of laccaic acid D methyl ($^{14}\text{CH}_3$) ester (4)

Synthesized laccaic acid D methyl ($^{14}\text{CH}_3$) ester (9.62×10^4 dpm), dissolved in $\text{Me}_2\text{CO}-\text{H}_2\text{O}$, 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 incorporation 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 I. Incorporation results from Me¹⁴COONa administered to *Aloe saponaria*

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

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-CHCl₃ (1:1) for 1, 2, 3 and 4 and CHCl₃-MeOH-H₂O (7:3:1, lower layer) for glycosides. The spots on TLC were monitored by UV or detected with Echtblausalz B(Merk)-KOH. Me¹⁴COONa (1.0 mCi) was purchased from Daiichi Chemical Company (Osaka, Japan) and ¹⁴CH₃I (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 (¹⁴CH₃) 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}^{OH} 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 Me₂CO (12 ml), NaHCO₃ (40 mg) and MeI (6 ml) were added and the reaction mixture was cooled with dry ice. Then ¹⁴CH₃I (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 (¹⁴CH₃) ester was recrystallized from MeOH to give 4, mp 268-270° (1.20 × 10⁴ dpm/mg; 3.94 × 10⁶ dpm/mM).

Feeding of Me¹⁴COONa. An aq. soln (2 ml) of Me¹⁴COONa (1.0 mCi) was administered to the wilted plant. weighine ca

5g. After 48 hr feeding the rhizome and subterranean parts were washed with Me₂CO, chipped and exhaustively extracted with MeOH. The MeOH extract (4.30 × 10⁷ dpm) was purified by PLC and the dilution method to give 1 (mp 249-250°, 2.35 × 10³ dpm/mg; 7.43 × 10³ dpm/mM), 2 (mp 200-203°, 2.30 × 10³ dpm/mg; 7.17 × 10³ dpm/mM), 3 (mp 274-278°, 1.01 × 10⁶ dpm/mg; 3.31 × 10⁶ dpm/mM), 1 (mp 250-253°, 1.35 × 10³ dpm/mg; 4.27 × 10³ dpm/mM) in aloesaponol I 6-O-β-D-glucopyranoside, and aloesin (mp 137-140°, 2.29 × 10⁶ dpm/mg; 9.02 × 10⁶ dpm/mM).

Feeding of 1. 1 (1.17 × 10⁶ dpm; 4.70 × 10³ dpm) dissolved in Me₂CO-H₂O was administered to the wilted plant for 24 or 48 hr and the rhizome and subterranean parts were washed with Me₂CO. The chipped material was exhaustively extracted with MeOH. Each MeOH extract (2.14 × 10² dpm; 2.35 × 10² dpm) was combined and purified by PLC and dilution method to give radioactive 2 (mp 200-202°, 1.20 × 10² dpm/mg; 3.74 × 10⁴ dpm/mM). 1 (4.70 × 10³ dpm; 7.05 × 10³ dpm) dissolved in Me₂CO-H₂O 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

extract (4.50 × 10³ dpm; 5.75 × 10³ dpm) was respectively purified by PLC followed by the dilution method to afford no radioactive 3.

Feeding of 3. 3 (1.45 × 10⁶ dpm; 6.46 × 10⁶ dpm) dissolved in Me₂CO-H₂O 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 × 10⁶ dpm; 5.00 × 10⁶ dpm) was respectively purified by PLC followed by the dilution method to give no radioactive 1.

Feeding of 4. 4 (9.62 × 10⁶ dpm) dissolved in Me₂CO-H₂O 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 × 10⁶ dpm) was purified by PLC followed by the dilution method to give no radioactive 1.

Acknowledgement—The authors express their deep thanks to Prof. Sankawa, Faculty of Pharmaceutical Sciences, University of Tokyo, for his valuable suggestions.

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