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TRITERPENOIDAL SAPONINS FROM *BAPTISIA AUSTRALIS**

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Key Word Index—*Baptisia australis*; Leguminosae; saponin; triterpene; sophoradiol; baptisiasaponin I.

Abstract—Extraction of the roots of *Baptisia australis* afforded a new triterpenoid saponin, baptisiasaponin I together with kaikasaponin III. The structure of baptisiasaponin I was elucidated as 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl sophoradiol on the basis of its spectral data and chemical degradation. © 1998 Elsevier Science Ltd. All rights reserved

INTRODUCTION

Baptisia australis (L.) R. Br. is called Blue Wild Indigo in English. Some flavonoids and alkaloids were identified from this plant [1]. However, there are no reports on the saponins of *Baptisia* spp.[1]. As a part of our study on leguminous plants, we investigated the saponins of *B. australis*. We now report the isolation and structural determination of a new saponin along with kaikasaponin III.

RESULTS AND DISCUSSION

A methanolic extract of the dried roots of *B. australis* afforded compounds **1** (0.026%) and **2** (0.024%) after various chromatographic purification. Compound **2** was identified as kaikasaponin III [2, 3] by comparison with various data.

Compound **1**, baptisiasaponin I showed a peak due to $[M-H]^-$ at *m/z* 925 in the negative FAB-mass spectrum. Acid hydrolysis of **1** gave sophoradiol (**1a**), glucuronic acid, xylose and rhamnose. The absolute configurations of sugars were determined to be D-form (glucuronic acid, xylose) and L-form (rhamnose), according to the procedure developed by Hara *et al.*[4]. In the ^{13}C NMR spectrum of **1**, signals due to a sugar moiety were identical with those of astragaloside VIII [5, 6]. Since the signals ascribable to the aglycone were superimposable on those of **2**, the structure of **1** was deduced to be 3-*O*-α-L-rhamnopyranosyl-(1→2)-β-D-xylopyranosyl-(1→2)-β-D-glucuronopyranosyl sophoradiol.

Although some saponins of soyasapogenol B [5, 6], complogenin [7], wistariasapogenols A and B [6, 8] contained the same oligosaccharide chain as **1**, baptisiasaponin I is the first example of sophoradiol glycoside having the oligosaccharide.

EXPERIMENTAL

General

^1H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded in C_5D_5N. TMS was used as internal standard. FAB-MS (negative ion mode) was measured with glycerol matrix. CC were performed with Sephadex LH-20 (Pharmacia) and Bondapak C_{18} (Waters). HPLC was carried out on a system of a pump: CCPM (Tosoh), UV detector: UV-970 (JASCO) and a column heater: U-620 (Sugai). The preparative HPLC conditions were as follows: column, Nova-Pak HR C_{18} (6 μmm, 25 × 100 mm) with Radial-Pak 40 × 10 module (Waters/Millipore), solvent, CH_3CN-H_2O-TFA(30→50:70→50:0.05) (v/v).

Plant material

The roots of *B. australis* were collected in the Medicinal Garden of our faculty. A voucher specimen has been deposited in the Medicinal Garden, Faculty of Pharmaceutical Sciences, Kumamoto University.

Extraction and isolation

The dried aerial parts of *B. australis* (42 g) was percolated with MeOH. The MeOH extract (17 g) was

* Part 60 in a series of studies on the constituents of the leguminous plants.

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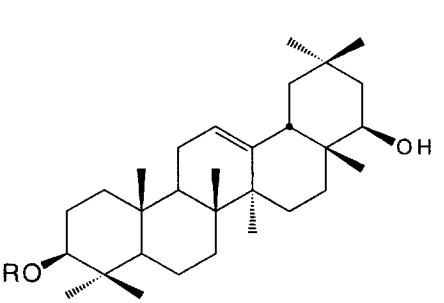

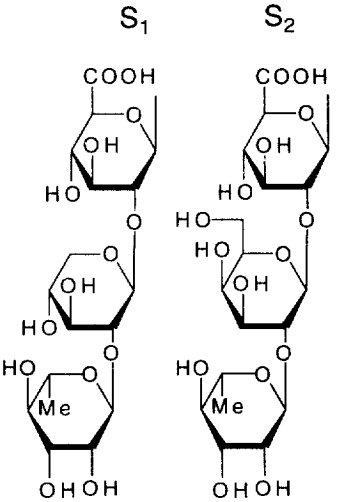

baptisiasaponin I (**1**) : R = S_1

sophoradiol (**1a**) : R = H

kaikasaponin III (**2**) : R = S_2

chromatographed on a Sephadex LH-20 column using MeOH to yield a crude saponin fraction. The combination of Bondapak C_{18} chromatography [H_2O-MeOH = 1:1 → H_2O-MeOH = 0:1] and preparative HPLC for the crude saponin fraction afforded compounds **1** (11 mg) and **2** (10 mg).

Baptisiasaponin I (**1**)

A white amorphous powder. $[\alpha]_D^{28} - 6.5°$ (pyridine, *c* 0.52). FAB-MS *m/z*: 895 $[M-H]^-$, 749 $[M-H-rha]^-$, 617[M-H-rha-xyl]$^-$. ^1H NMR: δ 0.88, 1.00, 1.01, 1.10, 1.24, 1.28, 1.29, 1.34 (each 3H, *s*, Me-23 ~ 30), 1.80 (3H, *d*, *J* = 6.1 Hz, rha H_3-6), 5.33 (1H, *s*, H-12), 5.67 (1H, *d*, *J* = 7.3 Hz, xyl H-1). ^{13}C NMR: δ 38.9, 26.4, 89.4, 39.8, 55.9, 18.6, 33.3, 40.7, 47.8, 36.9, 23.9, 122.6, 144.9, 42.5, 26.6, 28.8, 38.1, 45.5, 46.8, 30.9, 42.1, 75.6, 28.7, 15.8, 16.7, 17.2, 25.7, 21.1, 33.1, 28.3 (C-1 ~ 30), 105.4, 78.5, 76.9, 73.7, 78.4, 174.1 (glc A C-1 ~ 6), 102.8, 79.1, 78.4, 71.5, 66.7 (xyl C-1 ~ 5), 102.1, 72.3, 72.4, 74.2, 69.5, 19.0 (rha C-1 ~ 6).

Acid hydrolysis of **1**

A small amount of **1** was dissolved in 2 N HCl/H_2O (2 ml) and heated at 90°C for 2 hr. After addition of $CHCl_3$, the organic layer was to be sophoradiol, by TLC. R_fs: 0.43 [$CHCl_3$-MeOH (19:1)], 0.53 [*n*-hexane-acetone (2:1)]. The aq. layer was neutralized with 2 N KOH/H_2O. The sugar mixture was subjected to TLC analysis [TLC, Kieselgel 60 F_{254} (Merck Art 5554), $CHCl_3$-MeOH $-H_2O$ = 6:4:1, R_fs: 0.11 (glucuronic acid), 0.40 (xylose), 0.48 (rhamnose).

D, L Determination of sugars of **1**

A small amount of **1** was methylated with ethereal CH_2N_2. To a soln of the methylated sample of **1** was added $NaBH_4$, and the mixture was kept at room temp. for 30 min. The reaction mixture was worked up with MCI gel CHP 20P. The MeOH eluate was evaporated and heated in 2 N HCl/H_2O at 90°C for 3 hr. The hydrolysate was subjected to MCI gel CHP 20P and Amberlite IRA-400 to give a sugar fraction. This fraction was dissolved in pyridine (0.1 ml), then the soln was added to a pyridine soln(0.2 ml) of L-cysteine methyl ester hydrochloride (0.1 mol/l) and warmed at 60°C for 2 hr. The solvent was evaporated under N_2 stream and dried *in vacuo*. The remaining syrup was trimethylsilylated with trimethylsilylimidazole (0.1 ml) at 60°C for 1 hr. After addition of *n*-hexane and H_2O, the *n*-hexane layer was taken out and checked by GC. The retention times (R_t) of the peaks were 14.2 min (D-glucose), 7.3 min (D-xylose) and 8.8 min (L-rhamnose).

Kaikasaponin III (**2**)

A white amorphous powder, $[\alpha]_D^{25} - 8.3°$ (pyridine, *c* 0.48). FAB-MS *m/z*: 925 $[M-H]^-$, 779 $[M-H-rha]^-$, 617 [M-H-rha-xyl]$^-$. HPLC, conditions see ref.[9], (R_t: 32.4 min) and TLC, Kieselgel 60 F_{254} (Merck Art 5554), $CHCl_3$-MeOH-H_2O (6:4:1), R_f: 0.47; *n*-BuOH-AcOH-H_2O (4:1:5, upper), R_f: 0.37].

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