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EFFICIENT ISOLATION OF GLYCOSIDASE INHIBITORY STILBENE GLYCOSIDES FROM *RHEUM PALMATUM*

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ABSTRACT.—A chemical investigation of biologically active compounds from the root of *Rheum palmatum*, an Indonesian Jamu plant known as "kelembak," has led to the isolation and identification of two stilbene glycosides, 4′-*O*-methylpiceid [**1**] and rhapontin [**2**], which exhibited moderate α-glycosidase inhibitory activity. These bioactive glycosides were efficiently isolated by using only dccc.

"Jamu" is the name for traditional medicinal plants in Indonesia. In our continuing search for biologically active natural substances from tropical plants, the root of *Rheum palmatum* L. (Polygonaceae), a Jamu plant known as "kelembak" was studied. Kelembak is widely used to treat malaria and tropical cough (1,2).

Many medicinal plants are used to cure diabetes; however, a rational explanation of how they behave is still in an embryonic stage. α-Glucosidase is one of the most important carbohydrase enzymes (3). Thus, inhibition of α-glucosidase activity may be effective in controlling abnormal levels of blood glucose in metabolic diseases such as diabetes. Although some proteins and sugars have been reported as inhibitors of this enzyme (4,5), phytochemical inhibitors are not yet well investigated. In our preliminary screening, the yellow precipitate from the EtOAc fraction of kelembak inhibited α-glucosidase activity, although assay of its MeOH extract was impossible because of strong interference of the uv absorption.

In general, when the fractionation is guided by bioassays to isolate active principles from crude extracts, the first step is to partition the extracts between H_2O and organic solvents such as *n*-hexane, Et_2O or CH_2Cl_2 or $CHCl_3$), EtOAc, and *n*-BuOH to narrow the spectrum of chemical constituents and concentrate the biological activity. If the biological activity is found in the polar fractions such as the EtOAc and/or *n*-BuOH fractions, countercurrent chromatographic methods can be considered as appropriate isolation techniques (6–8). Therefore, for further purification of the above-mentioned bioactive precipitate, based on tlc analysis, dccc was chosen. In addition, since separation using dccc is time-consuming, reducing the operation time was also attempted. This paper describes an efficient isolation of two α-glucosidase-inhibitory stilbene glycosides from the EtOAc fraction of kelembak by using only dccc.

RESULTS AND DISCUSSION

The roots of *R. palmatum* (397 g), were extracted with MeOH at ambient temperature. The solvent was removed under reduced pressure, and the aqueous layer was freeze-dried to yield the crude extract (45 g). Following suspension of this extract in H_2O, the H_2O-insoluble portion was removed by filtration, and the suspension was successively partitioned into *n*-hexane; $CHCl_3$-, EtOAc-, and H_2O-soluble fractions. A yellow precipitate from the EtOAc extract was obtained after concentration of the solvent. Using an in vitro bioassay, this precipitate was found to inhibit α-

glucosidase activity. Incidentally, the crude MeOH extract of kelembak was also assayed, but analysis of the activity was impossible because of the strong uv absorption of the sample solution.

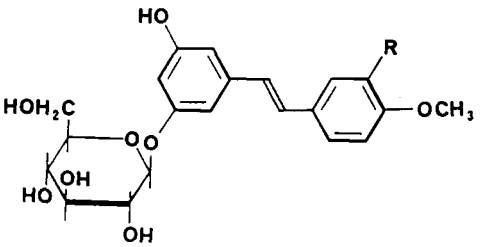
The bioactive yellow material, consisting primarily of three compounds, was subjected to purification by dccc. The two α-glucosidase inhibitors were efficiently isolated without using any solid packing material (see Experimental).

The two biologically active constituents were identified as 4′-*O*-methylpiceid (3,5-dihydroxy-4′-methoxystilbene-3β-D-glucopyranoside) [**1**] and rhapontin (3,5,3′-trihydroxy-4′-methoxystilbene-3β-D-glucopyranoside) [**2**] (2) by comparison of their spectroscopic data (uv, ir, fabms, ^{1}H nmr, and ^{13}C nmr) with those reported in the literature (10).

terial that might cause the irreversible absorption of large amounts of such polar compounds.

An attempt to inject the sample continuously without washing the vertical glass columns of the dccc, so as to reduce the time between injections, failed due to the surface of the glass becoming wettable. Thus, washing the glass columns after each injection, at least in the case of the above separation, seems essential to maintain good resolution. In addition, the sample injection could not exceed 1 g because of solubility limitations.

The 50% α-glucosidase inhibitory concentrations (IC_{50}) of **1** and **2** were 280 μg/ml and 600 μg/ml, respectively, an activity which is much weaker compared to known inhibitors such as acarbose and 1-deoxynojirimycine, which are currently receiving much attention



1 R=H
2 R=OH

Separations using dccc are time-consuming since they require low flow rates and pressures. For example, the isolation of several hundred mg of the above-mentioned stilbene glycosides by dccc required from 6 to 8 days, even though the flow limitation was reduced in part by a modification of the commercial instrument (11). Another limitation of dccc is the selection of solvents that must form droplets. Incidentally, this also limits the maximum elution flow rate. Despite these limitations, dccc is still very useful because of large sample capacity and low solvent consumption. More importantly, this liquid-liquid separation technique required no solid packing material that might cause the irreversible absorption of large amounts of such polar compounds.

(12). However, since the total amounts of **1** and **2** in kelembak are relatively large (about 1% dry wt), they may be considered useful.

Rhapontin [**1**] was previously reported as an antifouling substance against the blue mussel, *Mytilus edulis* (13). However, neither **1** nor **2** exhibited any molluscicidal activity against *Biomphalaria glabratus* at the concentration of 200 ppm (14).

EXPERIMENTAL

GENERAL EXPERIMENTAL PROCEDURE.—Mp's were determined on Sybron Thermolyne Mp-12615 and uncorrected. Uv spectra were obtained on a Hitachi 100-80 in MeOH. Ir spectra were recorded on a Perkin-Elmer 1310 in Nujol.

Fabms spectra were taken by a JEOL DX-303HF using glycerol matrix. Nmr spectra were recorded on a JEOL GSX-500 (500 MHz for ^1H and 125 MHz for ^{13}C). Dccc was performed on a DCC-300-G2 (Tokyo Rikakikai, Tokyo, Japan) with 300 glass columns (2 mm i.d. × 400 mm).

PLANT MATERIAL.—Kelembak, the dried root of *R. palmatum*, was purchased at a marketplace in Bandung, Indonesia, and identified by one of the authors, I. Soediro. A sample specimen was submitted to the Inter University Center.

EXTRACTION AND ISOLATION.—The dried roots (397 g) were pulverized and extracted with MeOH at room temperature. The MeOH was removed under reduced pressure, and the aqueous layer was freeze-dried to yield the crude extract (45 g). Following suspension of a portion of the extract (4 g) in H_2O (200 ml), the H_2O-insoluble portion was removed by filtration, and the suspension was successively partitioned into *n*-hexane- (200 ml × 3), $CHCl_3$- (200 ml × 3), EtOAc- (200 ml × 3), and H_2O-soluble fractions, yielding 102 mg, 147 mg, 1108 mg, and 1310 mg, respectively. A yellow precipitate was obtained from the EtOAc-soluble fraction after concentration of the volume to about 1/20, and a portion of this (1 g) was dissolved in 14 ml of the stationary and mobile phase (1:1) for dccc. The solvent system $CHCl_3$-MeOH-H_2O (7:13:8) was chosen for dccc separation in the descending mode based on tlc analysis. The flow rate of the mobile phase was adjusted to 21 ml/h. Each 21-ml fraction was collected into a test tube and monitored by tlc with vanillin/H_2SO_4 spray reagent. A total of 141 fractions were collected. Fractions 28–35 afforded compound **1**, and fractions 87–141 yielded compound **2**. Following repeated recrystallization from a mixture of CH_2Cl_2 and EtOH, needles of 4′-*O*-methylpiceid [**1**] (98.5 mg) and rhapontin [**2**] (184.5 mg) were obtained. Fractions 5–12 were a mixture of three minor yellow compounds which could be separated only after application of recycling hplc, and the detailed data will be reported separately.

4′-O-Methylpiceid [**1**].—Mp 223–224°; $[\alpha]_D$ −94.4° ($c = 0.5$, MeOH); uv (EtOH) 217, 315 (ε 57000, 77000); ir (Nujol) 3300 (br), 1610, 1575, 1305, 1295, 1240 cm^{-1}; ^1H nmr (C_5D_5N) 3.80 (s, OMe) 6.80 (br s, H-2 or H-6), 6.63 (br s, H-2 or H-6), 6.46 (br s, H-4), 6.90 (d, $J = 8$ Hz, H-2′ and H-6′), 7.45 (d, $J = 8$ Hz, H-3′ and H-5′), 6.89 and 7.05 (d, $J = 16$ Hz, olefinic); ^{13}C nmr (C_5D_5N) 55.1 (OMe), 139.1 (C-1), 104.8 (C-2 or C-6), 158.2 (C-3), 107.1 (C-2 or C-6), 158.7 (C-5), 102.8 (C-4), 129.4 (C-1′), 127.8 (C-3′ and C-5′), 114.1 (C-2′ and C-6′), 100.5 (C-1″), 73.2 (C-2″), 76.5 (C-3″ or C-5″), 69.7 (C-4″), 76.9 (C-3″ or C-5″), 60.6 (C-6″), 128.1 and 126.1 (olefinic).

Rhapontin [**2**].—Mp 240–241°; $[\alpha]_D$ −54.0° ($c = 0.7$, MeOH); uv (EtOH) 220, 328 nm (ε 39,000, 32,000), ir (Nujol) 3395, 1620, 1570, 1440 cm^{-1}; ^1H nmr (C_5D_5N) 3.78 (s, OMe), 6.74 (br s, H-2 or H-6), 6.60 (br s, H-2 or H-6), 6.36 (br s, H-4), 7.04 (d, $J = 2$ Hz, H-2′), 6.91 (d, $J = 8$ Hz, H-5′), 6.97 (dd, $J = 2$, 8 Hz, H-6′, 6.85 and 7.01 (d, $J = 16$ Hz, olefinic), ^{13}C nmr (C_5D_5N) 55.5 (OMe), 139.1 (C-1), 104.9 (C-2), 158.2 (C-3), 107.1 (C-4), 158.7 (C-5), 102.8 (C-6), 129.8 (C-1′), 112.8 (C-2′), 147.7 (C-3′), 146.4 (C-4′), 112.0 (C-5′), 118.6 (C-6′), 100.5 (C-1″), 73.2 (C-2″), 76.5 (C-3″ or C-5″), 69.7 (C-4″), 76.9 (C-3″ or C-5″), 60.6 (C-6″), 128.5 and 126.0 (olefinic).

ENZYME ASSAY.—α-Glucosidase inhibitory activity was measured according to the method of Lai and Axelrod (15). α-Glucosidase from yeast was purchased from Sigma Chemical Co. (St. Louis, MO). The test samples were dissolved in DMSO and added to the test buffer solution. Enzyme solution (0.1 ml), *p*-nitrophenyl-α-D-glucoside (0.1 ml), sample solution (0.15 ml), and 0.1 M phosphate buffer at pH 6.8 (2.55 ml) were mixed and hydrolysis was allowed to proceed for 10 min at 37°. The reactions was quenched by the addition of 1 ml of 16% (w/w) Na_2CO_3. The liberated *p*-nitrophenol was measured at 400 nm with a Beckman DU-64. The IC_{50} values were calculated based on percent inhibition obtained.

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