Note

Isolation of Soyasaponin I as an Inhibitor for Glycolate Oxidase

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The photorespiration proceeds on the glycolate pathway to reduce the apparent photosynthesis of C_3 plants and glycolate oxidase (EC 1.1.3.1) is involved in the process. Until now, several chemicals such as α -hydroxypyridinemethanesulfonate (α -HPMS),¹⁾ butyl hydroxybutyrate (BHB),²⁾ and glyoxylate³⁾ have been known to inhibit glycolate oxidase, and it is of interest to study on their effects on the glycolate pathway. Further it has been reported that, under suitable conditions, these inhibitors block the photorespiration and increase the photosynthetic CO_2 uptake.⁴⁾

With a view to find potent inhibitors of the photorespiration, we have continued to search for the inhibitors of glycolate oxidase and have previously reported that some fatty acids inhibit the enzyme.⁵⁾ During the study, an extract of soybean meal showed an inhibitory activity on glycolate oxidase, and in this paper we wish to report isolation of soyasaponin I (1) from the soybean meal as an inhibitor of glycolate oxidase.

The partially purified glycolate oxidase was prepared from spinach leaves, *Spinacia oleracea*, according to the method reported by Zelitch⁶⁾ and the bioassay method used in the present experiments was the same as that in the previous report.⁵⁾

Five hundred grams of soybean meal was dipped in approximately 5 liters of water-methanol mixture (1:1) for a day and, after filtration, the filtrate was concentrated *in vacuo* to 2 liters. The residue was extracted with ethyl acetate at pH 2.0 and the organic layer was extracted with saturated sodium bicarbonate solution. The aqueous layer was acidified to pH 2 and then extracted with ethyl acetate. The extract was concentrated *in vacuo* to afford a solid (5.6 g). The solid was dissolved in 10 ml of methanol and the solution was added to 500 ml of ethyl acetate giving a precipitate (1.3 g). The precipitate was successively chromatographed on a silica gel PF₂₅₄ column using chloroform-methanol-acetic acid-water (12:6:1:1) as a solvent system and then on a Sephadex LH-20 column using ethyl acetate-methanol (8:2) to afford 65 mg of the active principle (1).

The IR spectrum of 1 indicated the presence of a hydroxyl group ($\nu_{\text{max}}^{\text{OH}}$ 3400 cm^{-1}) and a carboxyl (2800–2400 and 1730 cm^{-1}). By treatment with diazomethane, 1 was converted to its methyl ester (2). The ^{13}C -NMR of 2 showed the signals due to 49 carbons including an ester carbonyl carbon ($\delta_{\text{CD}_3\text{OD}}^{\text{TMS}}$ 172.2), two double bond carbons (δ 123.6 and 145.2) and three anomeric carbons (δ 105.7, 102.2 and 102.2).

By refluxing with 1 N hydrochloric acid in dioxan-water (1:2) mixture for 6 hr, 1 was hydrolyzed and the reaction mixture was extracted with ethyl acetate. The aqueous residue was concentrated and treated with TMS-HT reagent to give TMS derivatives of sugars, which were identified as those of rhamnose, galactose and glucuronic acid by GLC. The ethyl acetate extract was chromatographed on a silica gel PF₂₅₄ column (1.5 $\times$ 50 cm) using chloroform-ethyl acetate mixture (8:2) as a solvent system to afford the aglycon (3).

The molecular formula of 3 was determined as $C_{30}H_{50}O_3$ through mass spectrometry: MS m/z , 458 (M^+); High MS m/z , 425.3423 ($M - H_2O - CH_3$, calcd. for $C_{29}H_{45}O_2$ m/z , 425.3427). The ^1H -NMR and ^{13}C -NMR of 3 indicated the presence of 7 methyl groups (^1H -NMR; $\delta_{\text{CDCl}_3}^{\text{TMS}}$ 0.88 (3H), 0.90 (3H), 0.96 (3H), 1.04 (3H) and 1.22 (3H)), a double bond (^1H -NMR; δ 5.25 (1H, t), ^{13}C -NMR; $\delta_{\text{CDCl}_3}^{\text{TMS}}$ 144.0 and 122.4), two secondary hydroxyl groups (^1H -NMR; δ 3.43 (1H, t) and 3.60 (1H, dd), ^{13}C -NMR; δ 81.0 and 76.7) and one hydroxymethyl group (^1H -NMR; δ 3.34 (1H, d) and 4.21 (1H, d), ^{13}C -NMR; δ 64.6). Based on these data, 3 was inferred to be soyasapogenol B,⁷⁾ a triterpenoid with oleanane skeleton.

The FD-MS of 2 showed prominent peaks at m/z 979 ($(M + Na)^+$), 833 ($(M + Na) - C_6H_{10}O_4$)⁺ and 69 ($(M + Na) - C_{12}H_{20}O_9$)⁺ and 691 ($(M + Na) - C_{12}H_{20}O_9$)⁺ indicating the presence of glucuronyl-galactosyl-rhamnose moiety. Thus, the structure of 1 was strongly suggested to be soyasapoinin I⁷⁾ and finally confirmed by direct comparison with authentic soyasaponin I kindly gifted by Dr.

FIG. 1. Double Reciprocal Plots of the Inhibitory Effect of Soyasaponin I on Glycolate Oxidase.

■, soyasaponin I 500 ppm; ●, control; S, substrate (glycolate).

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As shown in Fig. 1, soyasaponin I (**1**) non-competitively inhibited glycolate oxidase ($ID_{50} = 5.0 \times 10^{-4} M$), whereas the sapogenin (**3**) and constituent sugars, glucuronic acid, rhamnose and galactose, were practically inactive. Further, it is of interest in connection with structure-activity relation that methyl ester (**2**) of soyasaponin I does not block the enzyme. Thus the inhibitory activity of **1** might be closely related to the whole structure of molecule. Although the plant physiological role of soyasaponin I has not been clarified, it is worthy to note that **1** inhibits the enzyme involved in the photorespiration and also that **1** has been recently isolated from seedlings of Alaska pea as a Pfr killer of the phytochrome system.⁸⁾

The effects of **1** on the photorespiration is now under investigation.

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