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INHIBITION OF MONOAMINE OXIDASE BY ISOGENTISIN AND ITS 3-O-GLUCOSIDE

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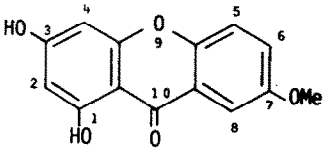
Isogentisin (1,3-dihydroxy-7-methoxyxanthone) is a characteristic constituent found in plants such as Guttiferae and Gentianaceae [1]. These plants have been used in popular medicine both in Europe and Asia. In particular, in the Indian system of medicine, extracts of Canscora decussata and several Swertia species are used for the treatment of melancholia. Recently, some xanthones were shown to produce central stimulating action [2,3]. By observing the behavioral effects of xanthones on rats, mice and dogs, Ghosal et al. [4] suggested that these central stimulating effects of xanthone may be mediated via monoamine oxidase (MAO) inhibition. Accordingly, the present study was undertaken to determine if isogentisin and its 3-O-glucoside inhibit MAO in vitro.

Isogentisin has the following structure:



Male albino rats of the Sprague-Dawley breed were used. The brains were homogenized with 9 vol. of 0.25 M sucrose in a Potter-Elvehjem homogenizer fitted with a Teflon pestle being cooled in an ice bath and centrifuged at 1500 g for 5 min to remove cellular debris. The resulting supernatant fraction was centrifuged at 18000 g for 20 min and the crude mitochondrial pellet was suspended in the sucrose solution. The suspension was recentrifuged

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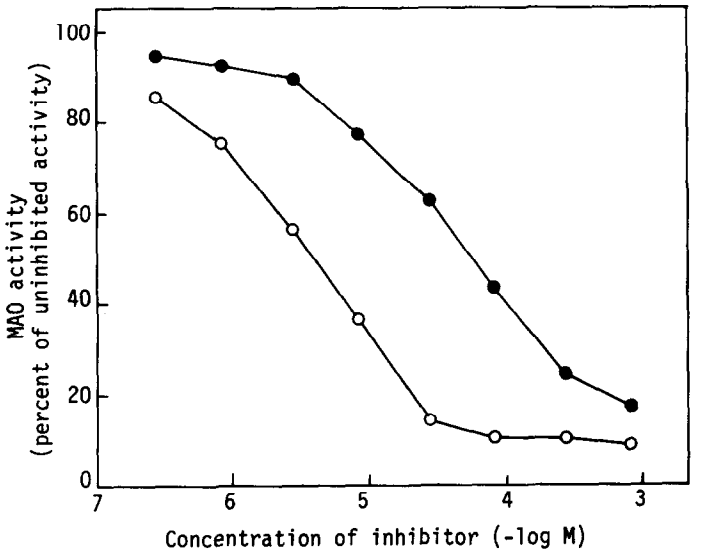

Fig. 1. Effect of various concentrations of isogentisin and its 3-O-glucoside on deamination of kynuramine by rat brain mitochondrial MAO. Open circles: isogentisin; closed circles: isogentisin-3-O-glucoside. The concentration of the substrate was 82 μM. Each point represents the mean obtained from duplicate determinations.

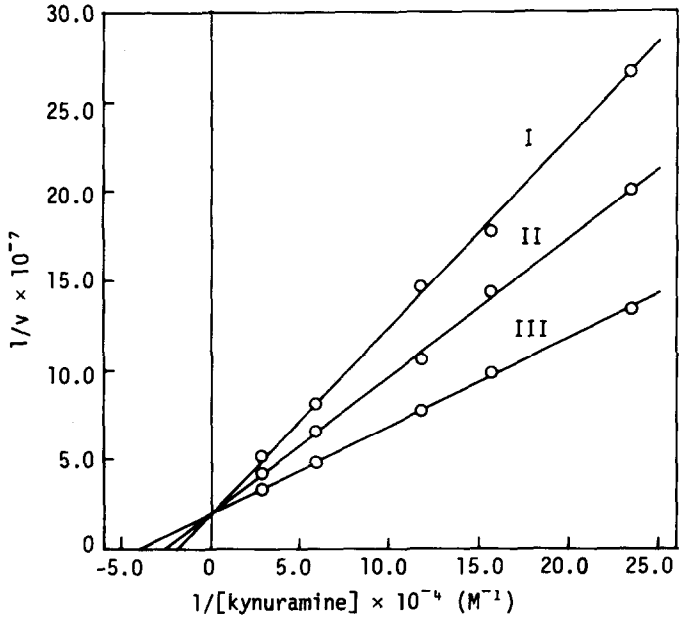

Fig. 2. Lineweaver-Burk plots showing the inhibition of rat brain mitochondrial MAO by isogentisin and its 3-O-glucoside. I: 1.0×10^{-6} M isogentisin; II: 1.0×10^{-5} M isogentisin-3-O-glucoside; III: no inhibitor; v: moles 4-hydroxyquinoline formed/mg of protein/30 min. Each point represents the mean obtained from duplicate determinations.

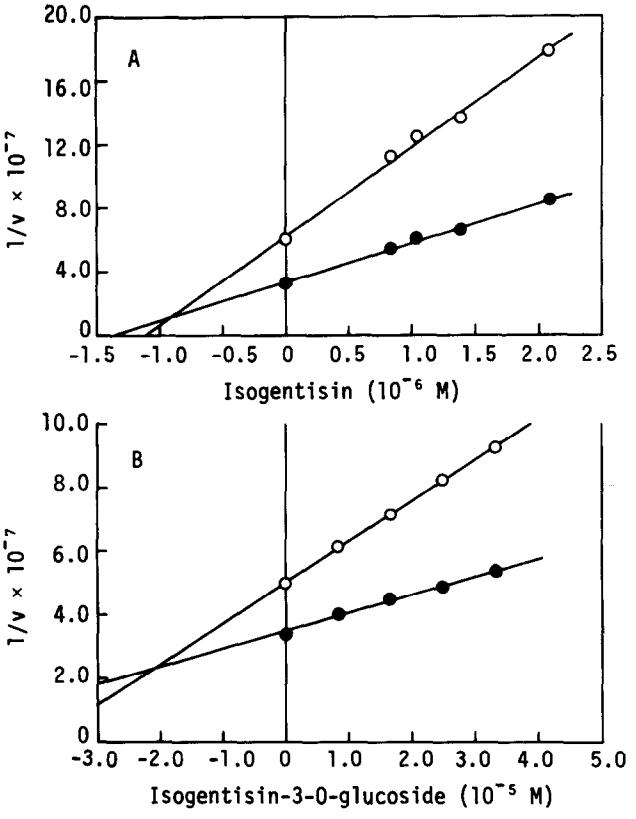

Fig. 3. Dixon plots of deamination of kynuramine by rat brain mitochondrial MAO. A: isogentisin; B: isogentisin-3-O-glucoside. Open circles: 15.3 μM kynuramine; closed circles: 40.9 μM kynuramine; v: moles 4-hydroxyquinoline formed/mg of protein/30 min. Each point represents the mean obtained from duplicate determinations.

at 18000 g for 30 min and the pellet was resuspended in 0.1 M sodium phosphate buffer (pH 7.4). This suspension was used as an enzyme source.

Isogentisin was prepared according to the method of Grover et al. [5]. Isogentisin-3-O-glucoside was synthesized as a model compound following the procedure used in the flavonoid field [6]. It was confirmed by thin-layer chromatography, melting points, nuclear magnetic resonance and mass spectrometry that the compounds were absolutely pure.

MAO activity with kynuramine as substrate was determined fluorometrically by a minor modification [7] of the method of Kraml [8]. The assay mixture was preincubated with the compounds at 37° for 10 min. Since the xanthone derivatives are not easily water-soluble, we added ethanol to all assay mixtures to give a final concentration of 1% (v/v) to ensure the complete dissolution of the compounds. At this concentration of ethanol, MAO activity was almost unaffected. Neither isogentisin nor its 3-O-glucoside quenched the fluorescence

of 4-hydroxyquinoline, the product of enzyme reaction.

The initial experiment was designed to examine the effects of various concentrations of isogentisin and its 3-O-glucoside on MAO activity. As can be seen in Fig. 1, both compounds revealed potent MAO inhibition. Isogentisin was much more effective than its 3-O-glucoside. Fifty per cent inhibition by isogentisin and its 3-O-glucoside with a substrate concentration of 82 μM was achieved at 4.0×10^{-6} M and 5.6×10^{-5} M respectively.

The Lineweaver-Burk plots for both compounds are presented in Fig. 2. As shown in the figure, inhibition of rat brain MAO by these compounds was fully competitive.

The binding constants for both compounds were determined from the Dixon plots [9] as shown in Fig. 3. The K_i values of isogentisin and its 3-O-glucoside were 9.2×10^{-7} M and 2.1×10^{-5} M respectively.

In the present communication, we clearly demonstrated that isogentisin and its 3-O-glucoside, with kynuramine as substrate, inhibit rat brain mitochondrial MAO *in vitro*. The potency of inhibition by isogentisin is comparable to that by MAO inhibitors such as pargyline and harmaline [10]. Since we are synthesizing various xanthone derivatives other than the above compounds, extensive studies on their MAO inhibition are in progress in our laboratories.

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