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IDENTIFICATION OF A NEW FLUORESCENT COMPOUND ISOLATED FROM HUMAN LENS INSOLUBLE PROTEIN FRACTION

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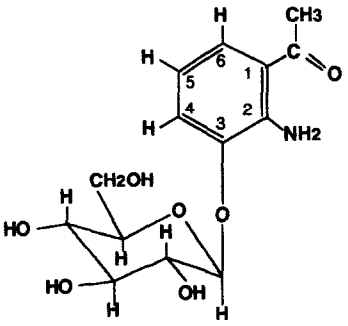
Abstract: A new fluorescent compound was isolated from human lens insoluble protein fraction, and was identified as 2-amino-3-hydroxyacetophenone-O-β-D-glucoside(1).

The fluorescence substance in human lens has been thought to serve as a color filter to remove the near-ultraviolet light to protect retina from the photooxidative damages[1]. It is also well known that there is an increase in the intensity of the fluorescence during aging of human lens and the fluorescence is associated mostly with the insoluble protein fraction[2]. Many investigators have long been struggled for identification of the fluorescent compounds[3, 4, 5, 6], it is therefore urgently needed to document the exact nature of the fluorescence in the aging lens. We report here the structure elucidation of a newly isolated fluorescent compound associated with the human lens insoluble protein fraction.

The water insoluble fraction from surgically enucleated cataractous human lens was prepared by the method previously reported[7]. The water insoluble fraction thus obtained (2g) was then treated with 400ml 80% ethanol in 5% KOH for 48 hr at room temperature in dark. The extract was neutralized with 1N HCl and concentrated by evaporation in vacuo, and was then fractionated on a Toyopearl HW-40 superfine column (2.2 x 40 cm) equilibrated with 2.8% acetic acid. The fluorescence rich fraction thus obtained was successively purified by reverse phase HPLC on a Senshu Pak ODS-1151-ss column (4.6 x 150 mm) at a flow rate of 1 ml/min for 24 min using a linear gradient from 0 to 19.6% aqueous acetonitrile containing either 0.1% trifluoroacetic acid or 0.1% n-heptafluorobutyric acid as counter ion. Total amount of the final preparation thus obtained was 460μg.



(1) 2-amino-3-hydroxy-acetophenone-O-β-D-glucoside(AHA-Glc)

Absorption maxima of the purified fluorescent compound in water were observed at 260 (ε=5000) and 360nm (ε=3570). The compound

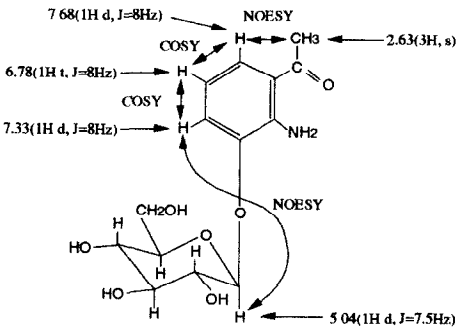
in water was also shown to fluoresce at 480 nm as excited at both 260 and 360 nm.

The molecular formula $C_{14}H_{19}NO_7$ was attributed to the isolated fluorescent compound on the basis of the molecular ion peak at 314.1140 in FAB-high resolution mass spectrum.

When the fluorescent compound was treated with almond β-glucosidase (EC 3.2.1.21), D-glucose was found to be released from the fluorophore. The result coincides with the fact that D-glucose was bound to the fluorophore by O-β-glucosidic linkage through hydroxy group on the conjugate[8].

IR spectrum of the fluorophore was measured in KBr. The presence of amino group was suggested by IR absorption at 3481 and 3354 cm^{-1} and carbonyl group by absorption at 1684 cm^{-1}.

The ^1H NMR spectrum (D_2O)(2) of the glucosidic fluorescent compound showed two doublets, respectively at 7.68 and 7.33 ppm in the aromatic region, and each of the doublet was coupled to the triplet at 6.78 ppm, as revealed by a COSY experiment. This configuration indicated the presence of three adjacent aromatic protons. Since the crosspeak was observed between the singlet at 2.63 ppm and the doublet at 7.68 ppm in the NOESY spectrum, the presence of acethyl group at position 1 was assigned. The NOESY experiment also showed that an anomeric proton at 5.04 ppm was close to the proton at 7.33 ppm, and the coupling constant of the anomeric proton was shown to be J=7.5Hz. These results indicate the presence of glucose in β conformation at position 3. Consequently, amino group was assigned at position 2, where is the only one space left for an existing functional group. From the data provided, it is concluded that the newly isolated fluorescent compound is 2-amino-3-hydroxyacetophenone-O-β-D-glucoside (**1**). The origin and route of formation of the compound in aging human lens remain to be investigated to elucidate the role that may play in physiology of the lens.



(2) Configurational assignments based on the ^1H NMR data from AHA-Glc

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