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IDENTIFICATION OF A FLUORESCENT GLUCOSIDE ISOLATED FROM THE PROTEIN-FREE EXTRACT OF HUMAN LENS

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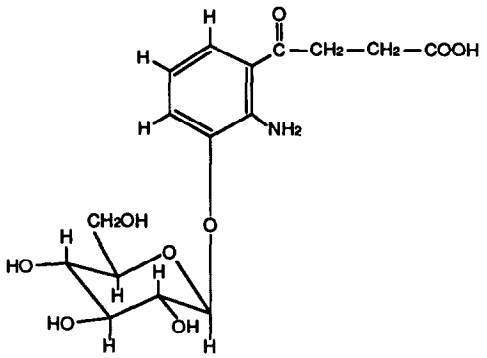
Abstract: One of the fluorescent glucosides associated with yellow pigments was isolated from the protein-free extract of human lens by use of gel filtration and reverse phase HPLC. The isolated compound was identified as 2-amino-3-(β-D-glucopyranosyloxy)-γ-oxobenzenbutanoic acid (**1**) by employing ^{1}H, ^{13}C NMR and mass spectroscopies. The compound was named α-deamino-3-OH-L-Kynurenine-O-β-D-glucoside (DHKN-Glc).

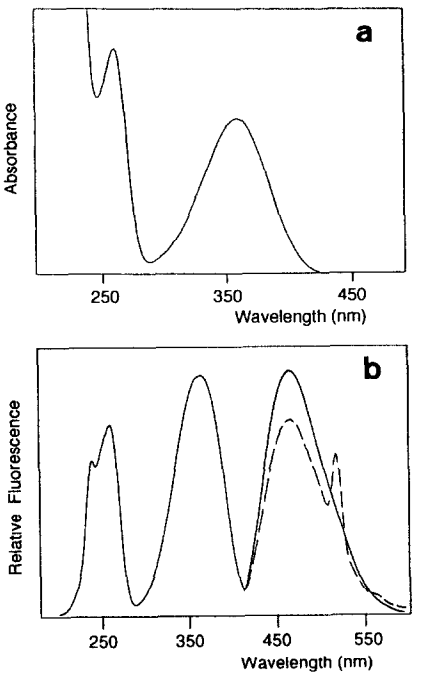
Van Heyningen was the first to identify three fluorescent compounds in the protein-free extract of human lens[1,2,3], the main component as 3-OH-L-kynurenine-O-β-D-glucoside (HKN-Glc) and the minor ones as kynurenine (KN) and 3-OH-L-kynurenine (HKN). However, an additional fluorescent compound present in the extract has been left unidentified since then. This paper describes the structure elucidation of this compound.

The water soluble fraction from surgically enucleated cataractous human lenses (approximately 70 g wet weight) was prepared by the method previously reported[4]. The protein-free low molecular weight fraction was extracted from the water soluble fraction by an extensive dialysis against water using



(1) α-deamino-3-OH-L-Kynurenine-O-β-D-glucoside

dialysis tubing (Spectra/Por 3 membrane MWCO 3500). The protein-free dialysate was concentrated to dryness *in vacuo*. The dried material was taken up with a small portion of 2.8% acetic acid, and was then applied to a Toyoperl HW-40 super fine column (2.2 x 40 cm). Elution was performed with 2.8% acetic acid at a flow rate of 30 ml/hr. The fluorescence rich fractions eluted around 350 ml were pooled. These fractions were further fractionated into a single component by reverse phase HPLC. Separation was made on a Senshu Pak ODS-1151-ss column (4.5 x 150 mm) at a flow rate of 1 ml/min for 24 min using a linear gradient of 0~19.6% aqueous acetonitrile containing either 0.1% trifluoroacetic acid or 0.1% n-heptafluorobutyric acid. The total fluorescent substance recovered was approximately 1 mg.



(2) UV-visible absorption (a) and fluorescent spectra (b) of the fluorescent glucoside

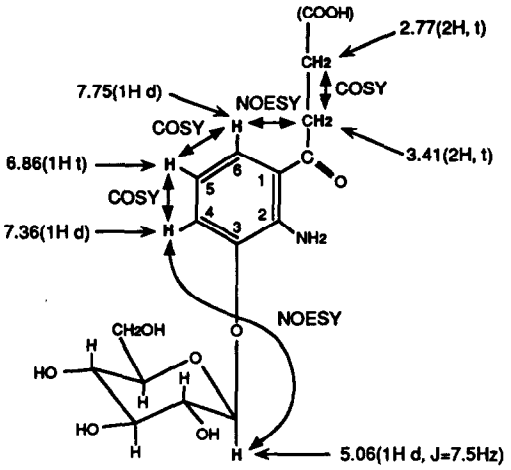
UV-visible spectrum of the purified substance in water revealed absorption maxima at 260 and 360 nm (**2-a**). The molar extinction coefficient of the compound at 260 and 360 nm were found to be 4900 and 3400 $M^{-1}cm^{-1}$, respectively. The fluorescent spectra of the compound in ethanol showed the excitation maxima at 260 and 365 nm and the emission maximum at 465 nm (**2-b**).

In order to confirm the existence of the O-β-D-glucoside linkage in the fluorescent compound, which was suggested by Van Heyningen[1], the compound was treated with almond β-glucosidase (EC 3.2.1.21, Sigma Chemical Company, St. Louis, USA). The TLC on a silica gel plate (Silica gel 60, Merck, Darmstadt, Germany)[solvent; n-butanol: acetic acid : water = 4 : 1 : 1 (v/v)] of the reaction mixture revealed the presence of two components corresponding to D-glucose and the fluorescent aglycone.

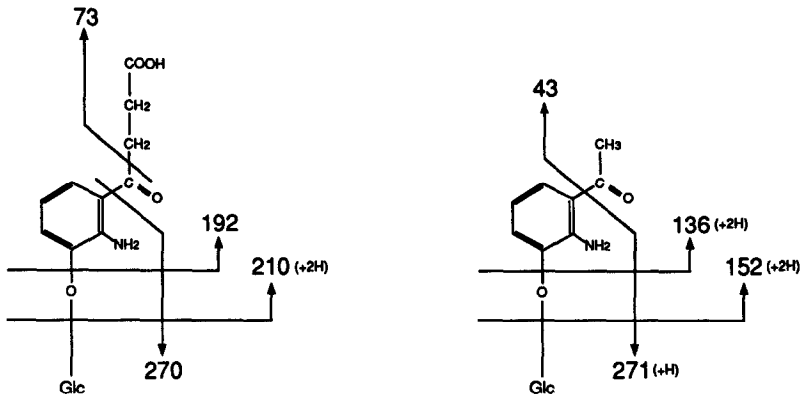
The molecular size of the compound was estimated by the FAB high resolution mass spectrometry. A m/z of 372.0861 was obtained, which is compatible with the molecular formula of $C_{16}H_{21}NO_9$.

Further molecular elucidation of the fluorescent glucoside was carried out by ^1H and ^{13}C NMR spectroscopies. The ^1H NMR spectra (D_2O) (**3**) of the compound showed two doublets, respectively at 7.75 and 7.36 ppm in the aromatic region, and each of these doublets was shown to couple to the triplet at 6.86 ppm, as revealed by a COSY experiment. This observation indicated the presence of three contiguous aromatic protons. Two higher field triplets observed at 2.77 and 3.41 ppm were shown to couple to each other in a COSY experiment. Since the crosspeak was observed between the triplet at 3.41 ppm and the doublet at 7.75 ppm in the NOESY spectrum, $-CH_2-CH_2-$ was assigned to be connected to position 1. The NOESY experiment also showed that an anomeric proton at 5.06 ppm is spacial proximity to the one at 7.36 ppm. The coupling constant of the anomeric proton was shown to be J = 7.5 Hz. These results

indicated the presence of D-glucose in β conformation at position 3. The compound was then subjected to ^{13}C NMR analysis (D_2O). The spectrum showed six signals (147.7, 144.0, 128.8, 123.4, 121.8, 118.8 ppm), which were also found in the signals obtained from 2-amino-3-hydroxyacetophenone-O-β-D-glucoside (AHA-Glc)[5], indicating the carbonyl group be directly bound to the carbon at position 1 and amino group to the one at position 2. In addition, the signal was also observed at 181.4 ppm, indicating the presence of carboxyl group in the side chain. To ascertain the above assignment, fragmentation patterns of this compound was examined by FAB CAD MS/MS, and were compared with those of AHA-Glc (4). As shown in (4), difference in mass thus assessed (~58Da) indicated the presence of $-CH_2-CH_2-COOH$ bound to the carbonyl at position 1.



(3) Configurational assignments based on the ^1H and ^{13}C NMR data



DHKN-Glc (MW: 371) AHA-Glc (MW: 313)

(4) Configurational assignments based on the fragmentation patterns obtained by FAB CAD MS/MS analysis

From these results it was concluded that the isolated fluorescent compound is 2-amino-3-(β-D-glucopyranosyloxy)-γ-oxobenzenbutanoic acid. The compound was designated as α-deamino-3-OH-L-Kynurenine-O-β-D-glucoside (DHKN-Glc)

Including this compound, three structurally related fluorescent glucosides have so far been identified

in human lens, HKN-Glc and DHKN-Glc in the protein-free extract, and AHA-Glc in the protein fraction[6]. HKN-Glc in the protein-free fraction was shown to decrease as the lens ages[7], whereas the fluorescence intensity associated with the lens protein was to increase[8], it is thus suggested that HKN-Glc is first synthesized from tryptophan[2] and is then converted into DHKN-Glc by deamination, which leads to formation of AHA-Glc. The latter may bind to the lens protein via hydrophobic interactions during aging of human lens.

Glucoside formation indeed is unusual in mammalian tissues[9]. It may therefore be interesting to elucidate the *in situ* physiological function of these glucosides in human lens.

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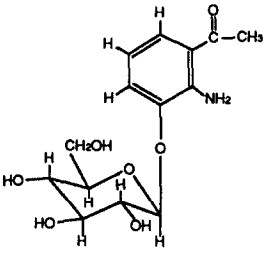
5. ^{13}C NMR (300MHz) spectra of 2-amino-3-hydroxy acetophenone-O-β-D-glucoside(AHA-Glc) (**5**) in D_2O (sodium 3-(trimethylsilyl)-propionic acid as external standard): ring carbons δ147.6, 144.1, 129.9, 123.5, 122.1, 118.5; carbonyl group δ207.2 ; methyl group δ30.4; glucose carbons δ104.4, 79.0, 78.4, 75.7, 72.2, 63.3



(5) AHA-Glc

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