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## Solution to Spectroscopy Challenge 10

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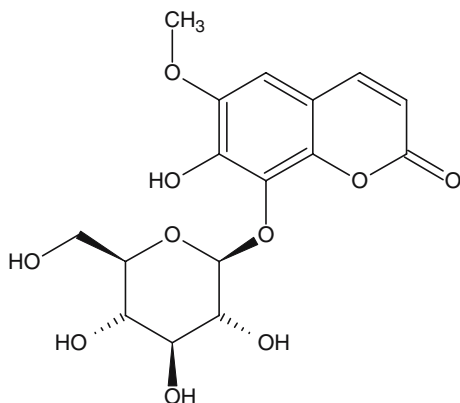
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### Solution

Fraxin [7,8-dihydroxy-6-methoxycoumarin-8- $\beta$ -D-glucopyranoside or 8-( $\beta$ -D-glucopyranosyloxy)-7-hydroxy-6-methoxycoumarin; C<sub>16</sub>H<sub>18</sub>O<sub>10</sub>; MW=370.31; CAS number: 524-30-1]



Fraxin is a coumarin glycoside found in the bark of the Horse Chestnut (*Aesculus hippocastanum*, Linn.) and the Ash (*Fraxinus excelsior*, Linn.). The name *Fraxinus* is derived from the Greek word *Phraxis* for separation owing to the fact that the wood of the ash tree is easy to chop. There

are about fifty species of the genus *Fraxinus*, and cultivation has produced and perpetuated a large number of distinct varieties. The Common Ash, a tall, handsome tree, is readily distinguished by its light grey bark and by its large compound leaves, divided into four to eight pairs of lance-shaped leaflets. Ash can be used for more purposes than the wood of other trees: it is one of the toughest and most elastic of timbers and hence used to be used for spears and bows. Ash logs are excellent for burning, giving out no smoke, and the ashes of the wood afford potash. Both bark and the leaves have medicinal uses and fetch prices which should repay the labour for collecting them, especially the bark. The bark is collected from the trunk and the root, the latter being preferred. Besides the glucoside fraxin, the bark contains the bitter substances fraxetin, tannin, quercetin, mannite, a little volatile oil, gum and malic acid, free and combined with calcium.

For structure determination we should first consider the mass spectrum. In the ESI mass spectrum the molecular ions  $[M+Na]^+$  are seen clearly at  $m/z$  393. The small signal at  $m/z$  409 results from  $[M+K]^+$  ions. The even molecular mass number of 370 is a first indication of a nitrogen-free molecule. The singular fragment ion at  $m/z$  231 of the 7,8-dihydroxy-6-methoxycoumarin (208+23) resulted from fragmentation of the glucopyranose.

We observe 16 carbon signals and 13 proton signals in the <sup>13</sup>C and <sup>1</sup>H NMR spectra, respectively. It can be concluded that the intensity of the <sup>1</sup>H NMR signal at 3.3 ppm arises from just one proton only and represents a CH signal (see HSQC and DEPT spectra). The molecule obviously contains a few exchangeable (XH) protons (<sup>1</sup>H NMR signal at 3.9 ppm without C–H correlation in the HSQC spectrum and the strong IR signal at about 3,400 cm<sup>-1</sup>). The 16 carbon and 13 hydrogen atoms afford a mass difference of 165 (i.e. 370–192–13). Assuming this mass difference originates from five oxygen atoms and five hydroxyl groups, an empirical formula of C<sub>16</sub>H<sub>18</sub>O<sub>10</sub> can be concluded. We can now calculate the number of double bond equivalents (DBEs):  $DBE = (2 \times 16 + 2 - 18) / 2 = 8$ . One DBE is for the carbonyl group (<sup>13</sup>C at 163.9 ppm) and one

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for a C=C double bond ( $^1\text{H}/^{13}\text{C}$  signals at 7.8/146.9 and, 6.2/113.5 ppm, respectively). The five quaternary carbon signals and the singlet proton signal at about 7 ppm give a hint to an aromatic ring system accounting for four DBEs. The only solution is to assign the two remaining DBEs to two further rings. The  $^1\text{H}$  NMR multiplet signals in the 3.2- to 4-ppm region points to a carbohydrate moiety with an anomeric proton (doublet at 5 ppm) and carbon (CH signal at 106 ppm).

The results of the COSY, HSQC and HMBC spectra clarify the presence of a coumarin base structure substituted with both a methoxy and hydroxyl group and a carbohydrate (glucopyranose) ring. Finally, the positions of the substituents in the 6, 7 and 8 positions need to be determined. In the COSY spectrum a small coupling is observed between the aromatic proton signal in position 5 ( $^3J_{\text{CH}}$  coupling in HMBC to the carbon 4 in the low-field shifted  $\beta$ -position to the carbonyl group C2) and the methoxy singlet (position 6).

The assignment of the position of the glucose moiety requires verification, and  $^{13}\text{C}$  chemical shift values are most suitable for a consistency check. Calculation of the chemical shifts (ppm) for the two possible regioisomers (A and B) with different substituted aromatic carbons 7 and 8 with Pretsch and co-workers' UpSol NMR prediction program yields:

A	C7 (-OH)	132.5	C8 (-Glu)	145.8
B	C7 (-Glu)	137.2	C8 (-OH)	141.1
Observed		133.6		146.1

This allows an unambiguous assignment to regioisomer A in which the glucose ring is located at position 8.