Identification and Sequencing of Sugars in Saponins using 2D ^{1}H N.M.R. Spectroscopy

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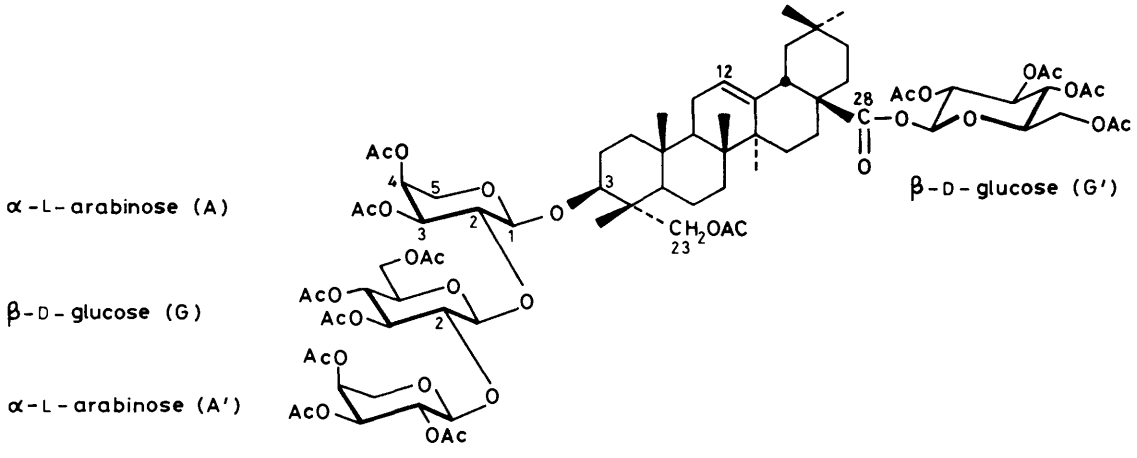
The structure of a saponin, including sequencing of its sugars has been determined using ^{1}H n.m.r. spectroscopy only.

Saponins are a group of natural substances which possess a broad spectrum of biological properties.[1] The structure of these molecules composed of one or several sugar chains linked to an aglycone is usually determined by derivatization followed by degradation[2] or by ^{13}C n.m.r. spectroscopy.[3] Faced with the problem of determining the structures of antifeedant alfalfa saponins, we have identified and sequenced their sugars using ^{1}H n.m.r. spectroscopy alone.[4]

Whereas the ^{1}H n.m.r. spectra of underivatized saponins show considerable line broadening at temperatures below



The structure of saponin (**1**).

80 °C, the spectra of the corresponding peracetates are well resolved and display resonances for the sugar protons spread over 2.5 p.p.m. If the field strength is sufficient (>300 MHz) the sugar proton resonances split into two zones: one between δ 4.75 and 5.4 assigned to C*H*OAc, and the other between δ 3.0 and 4.3, assigned as CH_2OAc, C*H*OR, and CH_2OR. Anomeric protons are located between these two zones in the case of ether linkages or at a higher frequency than δ 5.5 for ester linkages. An example of such a distribution of resonances is shown in Figure 1(top), which is part of the 500 MHz ^{1}H n.m.r. spectrum of a four-sugar alfalfa saponin.

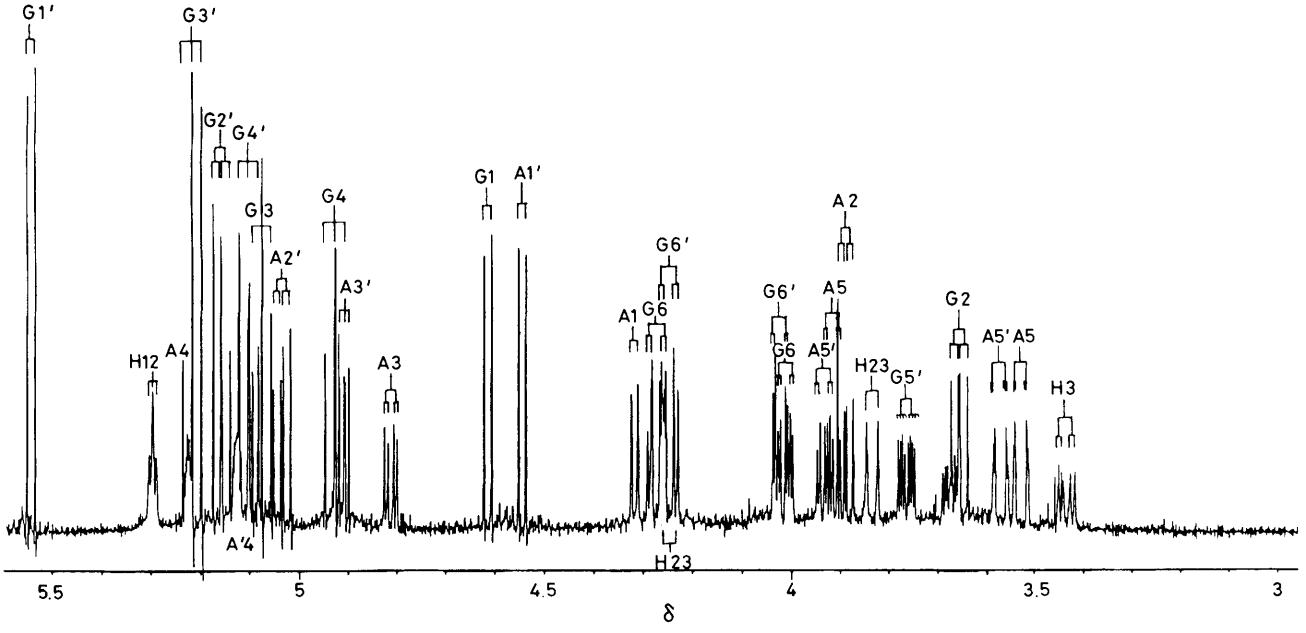
A single COSY experiment[4] reveals the interproton connectivities and therefore identifies the location of the branching points of each sugar. The nature of individual sugars is determined by counting their protons and determining their coupling constants. In this case, they are terminal glucose and arabinose, plus glucose and arabinose substituted on their arabinose C(2) carbons. The chemical shift of the anomeric proton of the terminal glucose corresponds to an ester linkage and this places this sugar on the carboxylic C(28) carbon of the genine (hederagenine as determined by ^{13}C n.m.r. spectroscopy and by microhydrolysis). The three remaining sugars form a chain bound to the C(3) oxygen since the C(23) oxygen has been acylated.

The sequence of these three sugars is the only other uncertainty; there are two possible formulae; (**1**) or (**2**).

Ara-(1→2)-Glu-(1→2)-Ara-(1→3)-Hederagenine-(28→1)-Glu
(**1**)

Ara-(1→2)-Ara-(1→2)-Glu-(1→3)-Hederagenine-(28→1)-Glu
(**2**)

A long range COSY experiment[5] offers a solution to the problem since it allows the observation of a ^4J intersugar



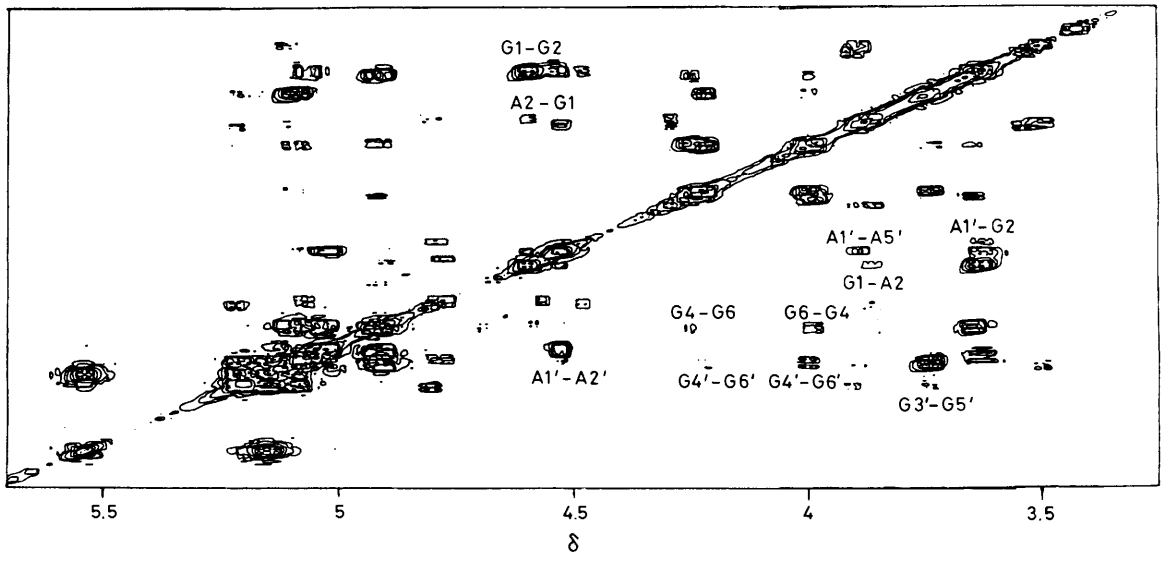


Figure 1. (Top) Part of the 500 MHz ^{1}H n.m.r. spectrum of peracylated saponin (**1**), 5 mg sample dissolved in 0.5 ml $CDCl_3$ at 27 °C. (Bottom) Part of the long range COSY contour plot of (**1**) with assignment of some important correlations. The sugar protons are numbered after their position on the sugar and after the name of the sugar, *e.g.* A2 refers to the C(2) proton of the inner arabinose.

coupling between the anomeric proton of the inner glucose and H(2) of the inner arabinose; this favours structure (**1**). In practice, three n.m.r. experiments: a 1D-normal spectrum, 2D COSY, and 2D long range COSY [Figure 1(bottom)] are sufficient to identify and sequence the sugars of a reasonably complex saponin. The observation of typical splitting patterns for characteristic protons [H(5) of glucose, rhamnose, arabinose, xylose] provides a shortcut in the identification of sugars but the existence of intra-ring long range couplings often multiplies the possible interpretations if a correct and complete proton assignment has not been carried out.

In many cases however such a complete assignment is not mandatory and the full structure of a saponin may be obtained in *ca.* 6 h of instrument time using a 5—10 mg sample.

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