A Glucuronidase Inhibitor from the Seeds of *Baphia racemosa:* Application of Fast Atom Bombardment coupled with Collision Activated Dissociation in Natural Product Structure Assignment

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The novel imino acid, 2(*S*)-carboxy-3(*R*),4(*R*),5(*S*)-trihydroxypiperidine has been isolated from the seeds of *Baphia racemosa,* characterized primarily by fast atom bombardment mass spectrometry, and found to be a specific inhibitor of human liver β-D-glucuronidase.

Nojirimycin and deoxynojirimycin[1,2] represented the first naturally occurring examples of imino sugars containing a nitrogen atom in place of the ring oxygen of the sugar. These same materials[3,4] as well as related compounds[5] have been found in higher plants. The discovery that these imino sugars are able specifically to inhibit certain glycosidase enzymes have made them useful in understanding the processing of complex oligosaccharides[6] and cell wall polysaccharides.[7] In our search for other such naturally occurring factors, an imino acid from the legume *Baphia racemosa* was discovered which is an analogue of glucuronic acid and possesses specific enzyme inhibition activity.

The seeds of *Baphia racemosa* (Hoechst.) Bak. on preliminary screening revealed a neutral compound which gave a yellow colour with ninhydrin but was distinguished from proline by giving no reaction with isatin or acetaldehyde–nitroprusside reagent.[8] The finely ground seed (100 g) was defatted with acetone and extracted with 50% aqueous methanol (1 l). The filtered extract was applied to Amberlite IR-120 resin, (H^+ form, 14—52 mesh, 15 × 2 cm) prepared in

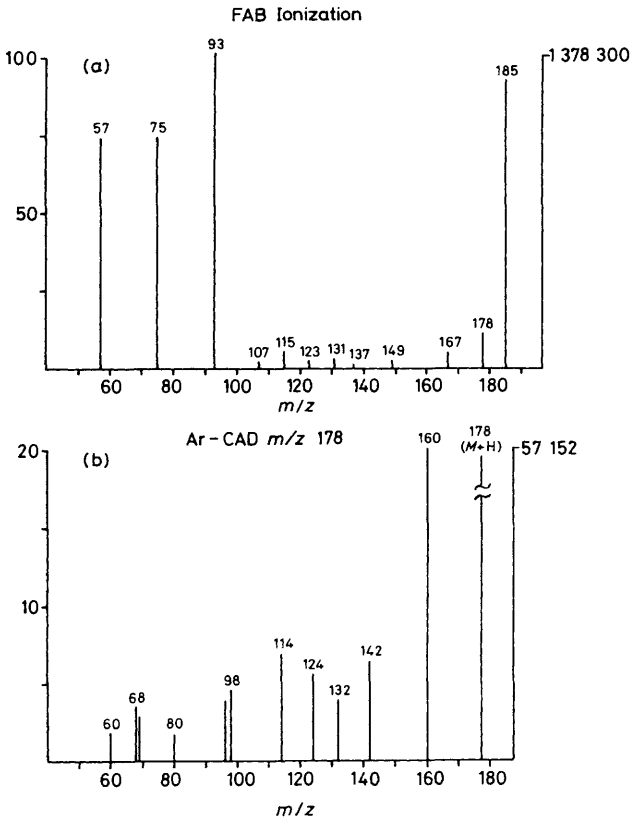
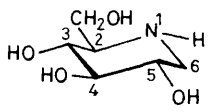
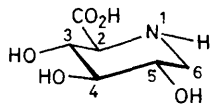


Figure 1. (a) The ionization of compound (**2**) from a glycerol matrix by 6 keV xenon FAB. (b) The $M + H$ ion at m/z 178 was selected and subjected to CAD with argon as the reagent gas; the resulting fragmentation pattern is shown. The data were obtained using a Finnigan 4500 triple stage quadrapole mass spectrometer equipped with an Ion Tech FAB gun.

50% aqueous methanol. After washing with excess of 50% aqueous methanol, the amino acids and amines were eluted with 1 M NH_3. This fraction was dried, dissolved in water, and applied to Amberlite CG-400 (acetate form, 100—200 mesh, 15 × 2 cm). Unbound material and water washings were pooled, evaporated to *ca.* 50 ml, and applied to Amberlite CG-120 (NH_4^+ form, 100—200 mesh, 20 × 2 cm). The imino acid passed through the column immediately and was thereby separated from another contaminating neutral amino acid which was held back. Fractions containing the desired compound were evaporated to dryness, dissolved in a minimum of water, and applied to Amberlite CG-400 (OH^- form, 100—200 mesh, 5 × 1 cm). After washing with water the imino acid was eluted with 0.25 M acetic acid. The acetic acid fraction was evaporated to dryness, the residue dissolved in water and applied to Amberlite CG-120, (H^+ form, 100—200 mesh, 5 × 2 cm). The imino acid was eluted with 0.2 M HCl and the acid was removed under vacuum. The colourless crystals which appeared spontaneously were recrystallized from water–ethanol–acetone to yield 25 mg (a satisfactory analysis for $C_6H_{11}NO_5 \cdot 2H_2O$ was obtained).

The imino acid had a melting point of 228—230 °C, an end absorption in the u.v. spectrum, and a small positive rotation ($[\alpha]_D^{25} = +18.3°$, 1% w/w in H_2O). The compound volatilized easily from a glycerol matrix with xenon fast atom bombardment (FAB),[9] producing the spectrum shown in Figure 1(a). Although the major ions are characteristic of different glycerol adducts, the ion at m/z 178 was distinct from the





background. Selection of this ion with a triple quadrapole mass spectrometer[10] and collision activated dissociation (CAD) with argon yielded the spectrum shown in Figure 1(b).

The $M + H$ ion at m/z 178 loses H_2O to give the fragment ion at m/z 160. The fragmentation of this ion may be rationalized *via* two distinct pathways. Two additional losses of H_2O give ions at m/z 142 and 124; the fragment at m/z 124 loses 44 (CO_2) to give the protonated pyridinium cation at m/z 80. Alternatively, the m/z 160 ion loses CO to produce the resonance stabilized piperidinium cation, m/z 132. Two successive dehydrations of the piperidinium cation give ions at m/z 114 and 96. This latter fragmentation pattern is similar to that reported for deoxynojirimycin, nojirimycin, and 2-piperidinemethanol.[1] These data established the imino acid as a trihydroxypipecolic acid.

The position and relative stereochemistry of each hydroxy group were assigned with ^1H n.m.r. spectroscopy. Capitalizing on the weak complexation between the carboxylic acid and pyridine, a 27% [$^2H_5$]pyridine–D_2O solution resolved each of the resonances: (360 MHz), δ 3.52 (5-Hax, m, J 12, 9.0, 6.0 Hz), 3.43 (3-Hax, t, J 10 Hz), 3.30 (4-Hax, t, J 9.0 Hz), 3.14 (6-Heq, dd, J 12, 6.0 Hz), 3.08 (2-Hax, d, J 10 Hz). 2.52 (6-Hax, t, J 12 Hz). The observed coupling constants and multiplicities are consistent with the placement of an equitorial carboxy group at position 2 and equatorial hydroxy groups at positions, 3, 4, and 5 as shown in (**2**).

The c.d. spectra of carboxy groups of amino acids have been analysed as a superposition of two carbonyl chromophores resulting in a 16 sector system for the analysis of substituent effects.[11] The c.d. spectrum of (**2**) shows a small positive Cotton effect at 201 nm (H_2O, $\Delta\varepsilon$ + 1.14). This positive effect is consistent with the absolute configuration shown for (**2**) and is supported by the small positive Cotton effect measured for *trans*-4-hydroxypipecolic acid (206 nm, $\Delta\varepsilon$ + 0.49, H_2O)[12] and for L-proline (212 nm, + 0.29, H_2O).[13].

The characterization of (**2**) was greatly facilitated by the use of FAB volatilization followed by ion selection and CAD in a triple quadrapole mass spectrometer. This combination gave intense molecular ions for the very polar compound (**2**) without derivatization and allowed the fragmentation to be analysed without interference from the intense background ions of the glycerol matrix.

Our previous suggestion that (**1**) is biosynthetically derived from amino sugar precursors[5] was based on an analogy to the biosynthetic studies of the bacterial metabolite nojirimycin.[1] However, the demonstration that (**2**) is a pipecolic acid derivative suggests that the biosynthesis of (**1**) could occur through the reduction of an hydroxylated pipecolic acid.† Hydroxylated derivatives of these acids have now been found in several higher plants.[15]

It is now known[16] that (**2**) is a specific inhibitor of human liver β-D-glucuronidase and iduronidase and that it has no effect on α- and β-glucosidase or α- and β-mannosidase. Therefore this compound should prove to be useful in the specific inhibition of certain enzymes involved in polysaccharide processing. The specific enzyme inhibitory activity

† Further support comes from the observation that both *cis*-5-hydroxypipecolic acid (ref. 14) and deoxynorjirimycin (ref. 4) have been found in *Morus spp.*

and the relatively large amounts of this compound in the seeds of *B. racemosa* suggest that it may be involved in herbivore protection,[17,18] and this is being investigated.

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