Structure of Rhizobitoxine, an Antimetabolic Enol-ether Amino-acid from *Rhizobium japonicum*

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Summary The antimetabolite rhizobitoxine has been identified as 2-amino-4-(2-amino-3-hydroxypropoxy)-*trans*-but-3-enoic acid.

RHIZOBITOXINE is the trivial name given to an amino-acid first isolated from root nodules produced by *Rhizobium japonicum* in soybean *Glycine max* (L.) Merr., wherein it was shown to cause the symptoms of the disease rhizobial-induced chlorosis.[1] It irreversibly inactivates β-cystathionase in bacteria[2] and plants[3] and inhibits the conversion of methionine into ethylene in plants.[4] Rhizobitoxine is here identified as 2-amino-4-(2-amino-3-hydroxypropoxy)-*trans*-but-3-enoic acid ($CH_2OH{\cdot}CHNH_2{\cdot}CH_2{\cdot}O{\cdot}CH{:}CH{\cdot}CHNH_2{\cdot}CO_2H$). It is an unsaturated analogue of dihydrorhizobitoxine, a new ether amino-acid also produced by *Rhizobium japonicum* and reported in the following communication.[5]

Rhizobitoxine was isolated from whole culture extracts of *Rhizobium japonicum*[2] as a hygroscopic, colourless, non-crystalline solid, ν_{max} (KBr) 1610 and 1390 (carboxylate C=O), 1660, and 1195 cm^{-1} (O–C=C).[6] Attempts to obtain a mass spectrum were unsuccessful. Hydrogenation with Raney-Ni catalyst under weakly alkaline conditions yielded 79% by weight of *O*-(2-amino-3-hydroxypropyl)homoserine (dihydrorhizobitoxine).[5] The identity of the product was established by co-chromatography with naturally produced dihydrorhizobitoxine on ion-exchange resin (amino-acid analyser), and on paper (3 solvent systems), by co-electrophoresis on paper, and by various chemical tests. Reduction of rhizobitoxine in water (H_2O–PtO_2; ambient temperature; 1 h) unexpectedly produced 2-aminobutanoic acid as the major ninhydrin-reactive product and much smaller amounts of homoserine, as determined by two-directional paper chromatography. These conditions reduced homoserine to 2-aminobutanoic acid (*ca.* 25%). The presence of an enol-ether group was further indicated by the acid lability[7] and contrasting alkaline stability of rhizobitoxine, and the yellow product of the reaction of rhizobitoxine with ninhydrin is consistent with a double bond character at a carbon atom one or two removed from that bearing the amino group.[8]

A detailed analysis of the 220 MHz n.m.r. spectrum revealed the complete structure of rhizobitoxine. The spectrum (D_2O; Me_4Si external reference) showed two separate spin systems. Thus, the protons at δ 3·70 (1H, quintet, *J* 6 Hz, 2′-C*H*), 4·06 and 4·12 (2H, d of d, *J* 6 and 11 Hz, 3′-CH_2O), and 4·27 and 4·35 p.p.m. (2H, d of d, *J* 6 and 11 Hz, 1′-CH_2O) form a characteristic five-spin system, similar to that observed with dihydrorhizobitoxine[5] for the seryl group. The resonances at δ 4·56 (1H, d, *J* 10 Hz, 2-C*H*), 5·45 (1H, d of d, *J* 10 and 13 Hz, 3-C*H*), and 7·18 p.p.m. (1H, d, *J* 13 Hz, 4-C*H*) are similar to the corresponding ones in the analogous L-2-amino-4-methoxy-*trans*-but-3-enoic acid[9]. The coupling constant of 13 Hz indicates a *trans*-configuration.[10]

The ^{13}C n.m.r. spectrum supported the proposed structure [δ 56·0 (C-2), 53·0 (C-2′), 63·8 (C-3′), and 72·2 (C-1′), 101·5 (C-3) and 154·0 (C-4) (olefinic), and 171·3 (vw; C-1).

In many of the chemical and physical properties reported above rhizobitoxine closely resembled the antimetabolite analogue L-2-amino-4-methoxy-*trans*-but-3-enoic acid[9], the first enol-ether amino-acid to be reported.

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