Dihydrorhizobitoxine, a New Ether Amino-acid from *Rhizobium japonicum*

By LOWELL D. OWENS*†

(*U.S. Soils Laboratory, Soil and Water Conservation Research Division, U.S. Department of Agriculture, Agriculture Research Service, Plant Industry Station, Beltsville, Maryland*)

JOHN F. THOMPSON

(*U.S. Plant, Soil and Nutrition Laboratory, Soil and Water Conservation Research Division, U.S. Department of Agriculture, Agriculture Research Service, Ithaca, New York* 14850)

and PAUL V. FENNESSEY

(*Department of Chemistry, Massachusetts Institute of Technology, Cambridge, Massachusetts*)

Summary The structure of dihydrorhizobitoxine isolated from *Rhizobium japonicum* has been determined as *O*-(2-amino-3-hydroxypropyl)homoserine.

A NEW amino-acid, formerly called unknown Y, was originally isolated from nodules produced by *Rhizobium japonicum* on roots of soybean[1] *Glycine max* (L.) Merr., and was subsequently isolated from solution cultures of *Rhizobium japonicum* with mannitol as a carbon source.[2] It is here shown to be *O*-(2-amino-3-hydroxypropyl)homoserine ($CH_2OH{\cdot}CHNH_2{\cdot}CH_2{\cdot}O{\cdot}CH_2{\cdot}CH_2{\cdot}CHNH_2{\cdot}CO_2H$) and given the trivial name dihydrorhizobitoxine to indicate that it is a structural analogue of the antimetabolite rhizobitoxine[3] produced by the same bacterium.

Dihydrorhizobitoxine was isolated[2] as a hygroscopic non-crystalline solid [*m/e* 193·1184 ($M^+ + H$) ν_{max} (KBr) 1620 and 1395 (carboxylate C=O) and 1085 cm^{-1} (C–O–C); pKa_2 7·2, pKa_3 8·6, isoionic point 7·9]. Free amino-acids commonly show protonated molecular ions in conventional mass spectrometry. Fragments at *m/e* 75·0352, 161·0941, and 133·0731 show the presence of an α-amino-acid,[4] the 3′-OH group and the 2′-NH_2 group respectively. Dihydrorhizobitoxine was stable to acid and alkali and formed a purple product with ninhydrin. The presence of an ether function on C-4 was suggested by oxidation with periodate which yielded HCHO (1·8 mol. equiv.) (g.l.c.), NH_3 (0·5 mol. equiv.), and homoserine (2-amino-4-hydroxybutanoic acid) (1·0 mol. equiv.). The latter was the sole amino-product reactive to ninhydrin and was identified by co-chromatography with authentic homoserine on two-directional paper chromatograms, by co-electrophoresis on paper at pH 2·2 and 5·6, and by oxidation with $Cr_2O_7^{2-}$ to aspartic acid (identified by paper chromatography and by paper electrophoresis at pH 5·9). The amount of homoserine produced by complete oxidation with periodate was determined by reaction with ninhydrin after evolution of ammonia.[5] The presence of an ether function on C-4 of dihydrorhizobitoxine was confirmed by reductive cleavage with HI which yielded homoserine (1·0 mol. equiv.), 2-aminopropane-1,3-diol (0·7 mol. equiv.) (both determined by co-chromatography with authentic materials on ion-exchange resin of amino-acid analyser and by co-chromatography on two-directional paper chromatograms), and a small amount of an unidentified amino-compound (probably an iodo-derivative of the latter product).

The 100 MHz n.m.r. spectrum of dihydrorhizobitoxine [D_2O; Me_4Si external reference; multiplets at δ 2·60 (2H, distorted q, $CH_2{\cdot}CH_2{\cdot}CH$) and 4·13 (8H) p.p.m.] strongly supported the proposed structure. Decoupling the multiplet at δ 4·13 p.p.m. collapsed the multiplet at 2·60 p.p.m. to a singlet, so both the C-4 methylene and C-2 methine protons were at low-field. The presence of only one high-field CH_2 group and no *N*-CH_2 groups (the latter would be observed at a higher field than δ 4·13) suggests the partial structure: $O{\cdot}CH_2{\cdot}CH_2{\cdot}CHNH_2{\cdot}CO_2H$. One resonance of the multiplet at δ 4·13 was almost separate (δ 3·82 p.p.m.). Decoupling the high-field multiplet from the low-field protons indicated that this proton gives a quintet and confirms the presence of a $O{\cdot}CH_2{\cdot}CH{\cdot}CH_2{\cdot}O$ group.

Reaction of dihydrorhizobitoxine with phenyl isothiocyanate[6] formed the 2′-phenylthiourea phenyl thiohydantoin derivative, λ_{max} (EtOH) 268 nm (ε 24,800); *m/e* 426·1218 $M^+ - H_2O$. An ether function joining the CH_2 units C-4 and C-1′ would lead to the fragments at *m/e* 250·0752, 236·0611, 219·0588, and 205.0440. Further, a CH_2 unit at C-3 would lead to the additional fragment at *m/e* 192·0360. The latter fragment, phenyl thiohydantoin, also confirms the α-amino-acid group of dihydrorhizobitoxine. Thus the data support the proposed structure and together with the mass spectral data on C-2′ and C-3′ of dihydrorhizobitoxine completely establish the structure of dihydrorhizobitoxine.

Alkyl-ether derivatives of homoserine have previosly been identified in bacterial cultures containing an alcohol as the sole carbon source,[7] but this is the first instance of an alkyl-ether derivative of homoserine being found in the absence of an added alcohol.

We thank Mr. R. G. Pitcher, Hoffmann-La Roche Inc. for the n.m.r. analysis.

(*Received, 5th April* 1972; *Com.* 570.)

† Present address: P.O. Box 1706, South Carolina State College, Orangeburg, South Carolina 29115.

[1] L. D. Owens and D. A. Wright, *Plant Physiol.*, 1965, **40**, 927.
[2] L. D. Owens and D. A. Wright, *Plant Physiol.*, 1965, **40**, 931; J. Giovanelli, L. D. Owens, and S. H. Mudd, *Biochim Biophys. Acta*, 1971, **227**, 671.
[3] L. D. Owens, J. F. Thompson, R. G. Pitcher, and T. Williams, preceding communication.
[4] K. Bieman and J. A. McCloskey, *J. Amer. Chem. Soc.*, 1962, **84**, 3192.
[5] G. E. Connell, G. H. Dixon, and C. S. Hanes, *Canad. J. Biochem. Physiol.*, 1955, **33**, 416.
[6] I. Smith, 'Chromatographic and Electrophoretic Techniques. Vol. I, Chromatography,' Interscience, New York, 1960, p. 159.
[7] T. Harada, Y. Murooka, and Y. Izumi, *Biochem. Biophys. Res. Comm.*, 1967, **28**, 485; Y. Murooka and T. Harada, *Biochim. Biophys. Acta*, 1970, **215**, 333.