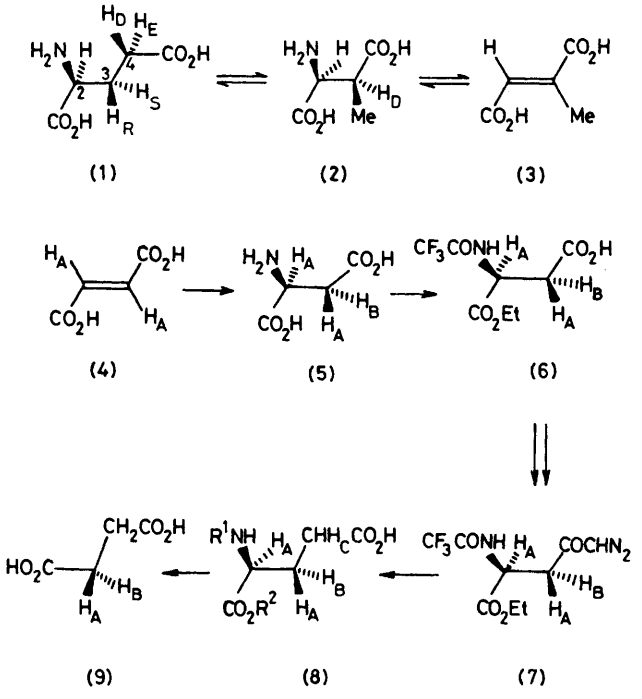
Synthesis of (2*S*,3*S*)-[3-$^2H_1$]-, (2*S*,3*R*)-[2,3-$^2H_2$]-, (2*S*,3*S*,4*RS*)-[3-$^2H_1$,4-$^3H_1$]-, and (2*S*,3*R*,4*RS*)-[2,3-$^2H_2$,4-$^3H_1$]-Glutamic Acids

By STEVEN J. FIELD and DOUGLAS W. YOUNG*
(*School of Molecular Sciences, University of Sussex, Falmer, Brighton* BN1 9QJ)

Summary Synthesis of (2*S*,3*S*)-[3-$^2H_1$]-, (2*S*,3*R*)-[2,3-$^2H_2$]-, (2*S*,3*S*,4*RS*)-[3-$^2H_1$,4-$^3H_1$]-, and (2*S*,3*R*,4*RS*)-[2,3-$^2H_2$,4-$^3H_1$]- glutamic acids in useful amounts has been achieved; the route involves a step where Wolff rearrangement occurs with retention of stereochemistry at a secondary chiral centre.

GLUTAMIC ACID (**1**) and its derivatives glutamine and glutathione play a central role in the metabolism of amino-acids and of ammonia.[1] In one pathway, the bacterial fermentation of glutamate by *Clostridium tetanomorphum* is initiated by the rearrangement of glutamate (**1**) to β-methylaspartate (**2**) which is catalysed by the coenzyme vitamin B_{12} and the enzyme glutamate mutase. Subsequent elimination of ammonia by β-methylaspartase yields mesaconate (**3**). The rearrangement involves migration of the carbon C-2 of glutamate to C-4, and of the 4- *pro-S* hydrogen to C-3[2] thus creating the methyl group. The stereochemistry of the rearrangement with respect to C-3 of glutamate (**1**) which becomes the methyl group in β-methylaspartate (**2**) and in mesaconate (**3**) is as yet unknown. This point could be examined if samples of glutamic acid were available stereospecifically labelled at C-3 with two of the isotopes of hydrogen and labelled in the 4- *pro-S* position with the third isotope of hydrogen.



We now report synthesis of a variety of stereospecifically labelled glutamic acids which should be useful in the study of glutamic acid metabolism. Two of these fulfil the criteria outlined above for the study of the glutamate-mutase system.

Fumaric acid (**4**) and [2,3-$^2H_2$]fumaric acid (**4**, H_A = ^2H)[3] were incubated with the commercially available† enzyme L-aspartase and buffered ammonium chloride in (respectively) ^2H_2O and ^1H_2O. L-Aspartase is known[4] to add ammonia to the olefin with *trans* stereospecificity so that 25% yields of (2*S*,3*R*)-[3-$^2H_1$]- and (2*S*,3*S*)-[2,3-$^2H_2$]-aspartic acid [(**5**; H_B = ^2H) and (**5**; H_A = ^2H), respectively] were available from these reactions. Selective protection of the α-carboxylic acid was achieved by reaction with trifluoroacetic anhydride (85% yield) and treatment of the resultant anhydride with ethanol[5] when the esters (**6**; H_B = ^2H)‡ and (**6**; H_A = ^2H)‡ were obtained in 88% yield. Formation of the acid chlorides followed by reaction with diazomethane gave the diazoketones (**7**; H_B = ^2H)‡ and (**7**; H_A = ^2H)‡ in 79% yield. Wolff rearrangement was achieved by photolysis in dioxan containing water to hydrolyse the intermediate ketens, and the products (**8**; R^1 = CF_3CO,R^2 = Et), obtained in *ca.* 64% yield, were hydrolysed using aqueous HCl to yield (2*S*,3*S*)-[3-$^2H_1$]- and (2*S*,3*R*)-[2,3-$^2H_2$]-glutamic acid [(**8**; R^1 = R^2 = H,H_B = ^2H) and (**8**; R^1 = R^2 = H,H_A = ^2H)], respectively, in *ca.* 92% yield.

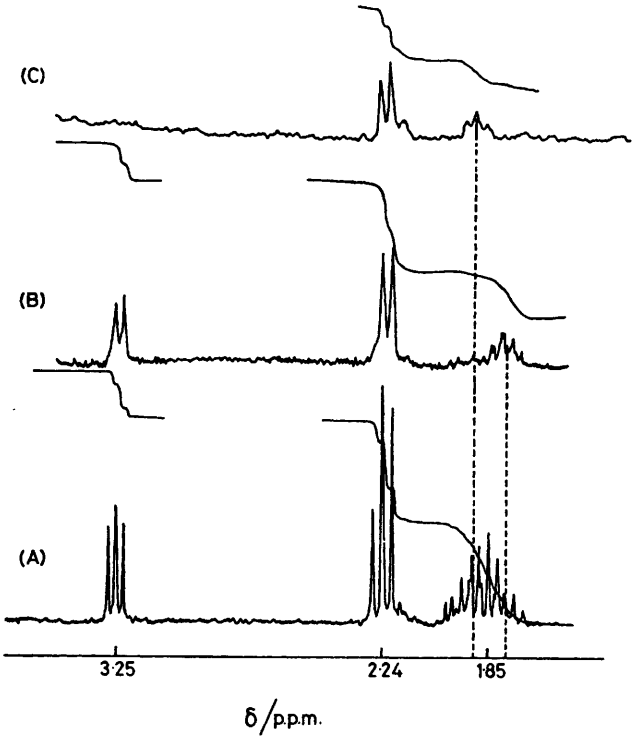


FIGURE. ^1H-n.m.r. spectra in 10% NaO^2H–^2H_2O of (A) (2*S*)-glutamic acid (**1**); (B) (2*S*,3*S*)-[3-$^2H_1$]glutamic acid (**8**; R^1 = R^2 = H,H_B = ^2H; and (C) (2*S*,3*R*)-[2,3-$^2H_2$]glutamic acid (**8**; R^1 = R^2 = H,H_A = ^2H).

Although the Wolff rearrangement involved migration of the prochiral centre, C-3, it is known that this rearrangement is stereospecific and involves retention of stereochemistry for tertiary and quaternary chiral migrating groups.[6] The stereochemical integrity at C-3 in the [3-^2H]-glutamates could be ascertained from the ^1H-n.m.r. spectra (Figure) where the complex multipet centred at δ 1·85 (Figure A) showed specific absences in the spectra of the deuteriated analogues (Figures B and C). The absolute stereochemistry at C-3 was verified by degradation of the glutamates to succinic acid (**9**) using chloramine-T. O.r.d.

† From Sigma Ltd. In a typical experiment we have used 10 units of the enzyme to prepare 3—4 g of labelled aspartate.

‡ These compounds had the expected spectral properties and stereochemical integrity was confirmed by the observation of selective omissions in the AB part of the ABX system for H-2 and H-3.

and c.d. measurements[7] showed that (2*S*,3*S*)-[3-$^2H_1$]-glutamic acid (**8**; $R^1 = R^2 = H, H_B = {}^2H$) gave (*S*)-[2-$^2H_1$]succinic acid (**9**; $H_B = {}^2H$) and that (2*S*,3*R*)-[2,3-$^2H_2$]- glutamic acid (**8**; $R^1 = R^2 = H, H_A = {}^2H$) gave (*R*)-[2-$^2H_1$]succinic acid (**9**; $H_A = {}^2H$). The Wolff rearrangement had therefore taken place with retention of configuration at the secondary chiral centre.

To achieve non-chiral labelling at C-4, the Wolff rearrangement was first conducted using undeuteriated diazoketone (**7**) in the presence of ^2H_2O to yield [4-$^2H_1$]-glutamic acid in which the resonance at δ 2·24 in the ^{1}H-n.m.r. spectrum (Figure) integrated as one proton. The reaction was therefore repeated using the deuteriated diazoketones (**7**; $H_B = {}^2H$) and (**7**; $H_A = {}^2H$) in the presence of ^3H_2O to yield (2*S*,3*S*,4*RS*)-[3-$^2H_1$, 4-$^3H_1$]- and (2*S*,3*R*,4*RS*)-[2,3-$^2H_2$, 4-$^3H_1$]-glutamic acids [(**8**; $R^1 = R^2 = H, H_B = {}^2H, H_C = {}^3H$) and (**8**; $R^1 = R^2 = H, H_A = {}^2H, H_C = {}^3H$)], respectively.

One of us (S.J.F.) thanks the S.R.C. for a studentship.

(*Received, 12th September* 1979; *Com.* 976.)

[1] 'Glutamic Acid—Advances in Biochemistry and Physiology,' ed. L. J. Filer, Raven Press, New York, 1979.
[2] M. Sprecher, R. L. Switzer, and D. B. Sprinson, *J. Biol. Chem.*, 1966, **241**, 864.
[3] E. M. Richards, J. C. Tebby, R. S. Ward, and D. H. Williams, *J. Chem. Soc.* (*C*), 1969, 1542.
[4] D. W. Young in 'Isotopes in Organic Chemistry,' eds. E. Buncel and C. C. Lee, Elsevier, Amsterdam, 1978, vol. 4, p. 231.
[5] F. Weygand, P. Klinke, and I. Eigen, *Chem. Ber.*, 1957, **90**, 1896.
[6] P. A. S. Smith in 'Molecular Rearrangements,' ed. P. DeMayo, Wiley–Interscience, New York, 1967, part 1, p. 528.
[7] Ref. 4, p. 185—186; We thank Dr. G. Ryback, Shell Biosciences Laboratory, Sittingbourne, for o.r.d. and c.d. spectra.