Stereochemistry of Catabolism of the DNA Base Thymine and of the Anti-cancer Drug 5-Fluorouracil [1]

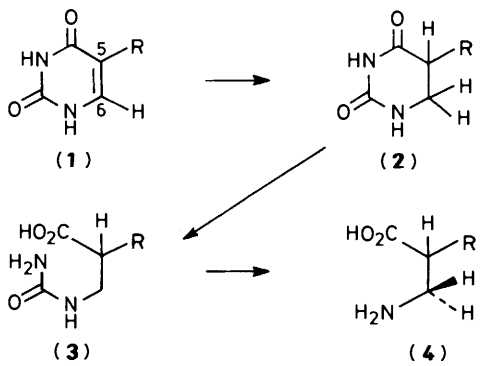
David Gani, Peter B. Hitchcock, and Douglas W. Young *
School of Chemistry and Molecular Sciences, University of Sussex, Falmer, Brighton BN1 9QJ, U.K.

(2*S*)-3-Amino-2-methylpropanoic acid (**6**) and (2*RS*,3*S*)-[3-$^2H_1$]-3-amino-2-methylpropanoic acid (**14**) have been synthesized and used to provide an assay which shows that the catabolism of the DNA base thymine (**1**; R = Me) proceeds by overall *anti* addition of hydrogen to the pyrimidine at the *si* face at C-5 and the *si* face at C-6. *X*-Ray structure analysis of a derivative of the product of reaction of *N,N*-dibenzyl-L-serine methyl ester (**15**; R = Me) with (diethylamino)sulphur trifluoride followed by deprotection has shown it to be (2*R*)-3-amino-2-fluoropropanoic acid (**19**; R = H). This was identical with the product of catabolism of the anti-cancer drug 5-fluorouracil (**1**; R = F). Esters of (2*R*,3*S*)-[3-$^2H_1$]- and (2*R*,3*R*)-[2,3-$^2H_2$]-3-amino-2-fluoropropanoic acids have been synthesized and used to provide an assay which shows that catabolism of the anti-cancer drug 5-fluorouracil (**1**; R = F) proceeds with the same absolute stereochemistry as is found in catabolism of thymine (**1**; R = Me).

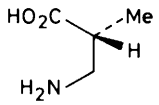
The DNA base thymine (**1**; R = Me) is synthesized as thymidine monophosphate from the RNA base uracil (**1**; R = H) as deoxyuridine monophosphate by a process which is dependent on two enzyme-catalysed reactions. In the first reaction, catalysed by the enzyme thymidylate synthetase, a one-carbon transfer reaction involving the coenzyme tetrahydrofolic acid [2] is coupled to a hydrogen migration leading to thymidine and the oxidized coenzyme. The second reaction, catalysed by the enzyme dihydrofolate reductase, regenerates the coenzyme tetrahydrofolic acid. Both of these enzymes may be inhibited by some compounds which, by virtue of this inhibition, are anti-cancer drugs.

We have recently examined stereochemical aspects of the reaction catalysed by dihydrofolate reductase [3] and important differences have been observed between the binding of this enzyme to its natural substrates and to the anti-cancer drug methotrexate. In the reaction catalysed by thymidylate synthetase, the substrate is deoxyuridinemonophosphate and the corresponding nucleotide of the anti-cancer drug 5-fluorouracil (**1**; R = F) is a suicide inhibitor. It was therefore of interest to us that the same enzyme system which catabolises uracil (**1**; R = H) and thymine (**1**, R = Me) will also catabolise the drug 5-fluorouracil (**1**; R = F).[4] We therefore decided to study the stereochemistry of the catabolism of the 'natural' and 'unnatural' substrates for this enzyme system.

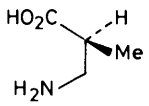
The catabolism of the pyrimidines uracil (**1**; R = H), thymine (**1**; R = Me) and 5-fluorouracil (**1**; R = F) is summarized in Scheme 1. In the first, rate-limiting step the enzyme dihydrothymine dehydrogenase (EC 1.3.1.2) catalyses the reduction of the pyrimidine (**1**) to a dihydropyrimidine (**2**). Subsequent hydrolysis to the substituted β-ureidopropanoic



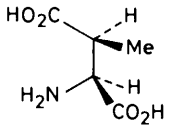
Scheme 1.



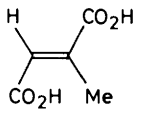
(**5**)



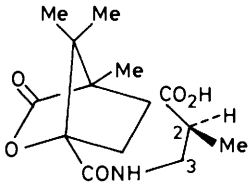
(**6**)



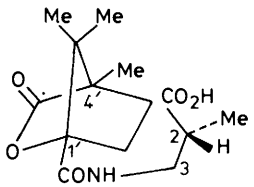
(**7**)



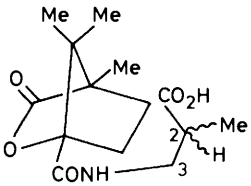
(**8**)



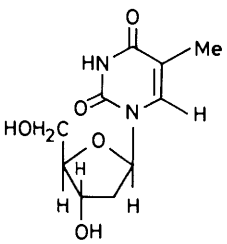
(**9**)



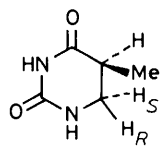
(**10**)



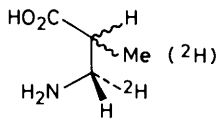
(**11**)



(**12**)



(**13**)



(**14**)

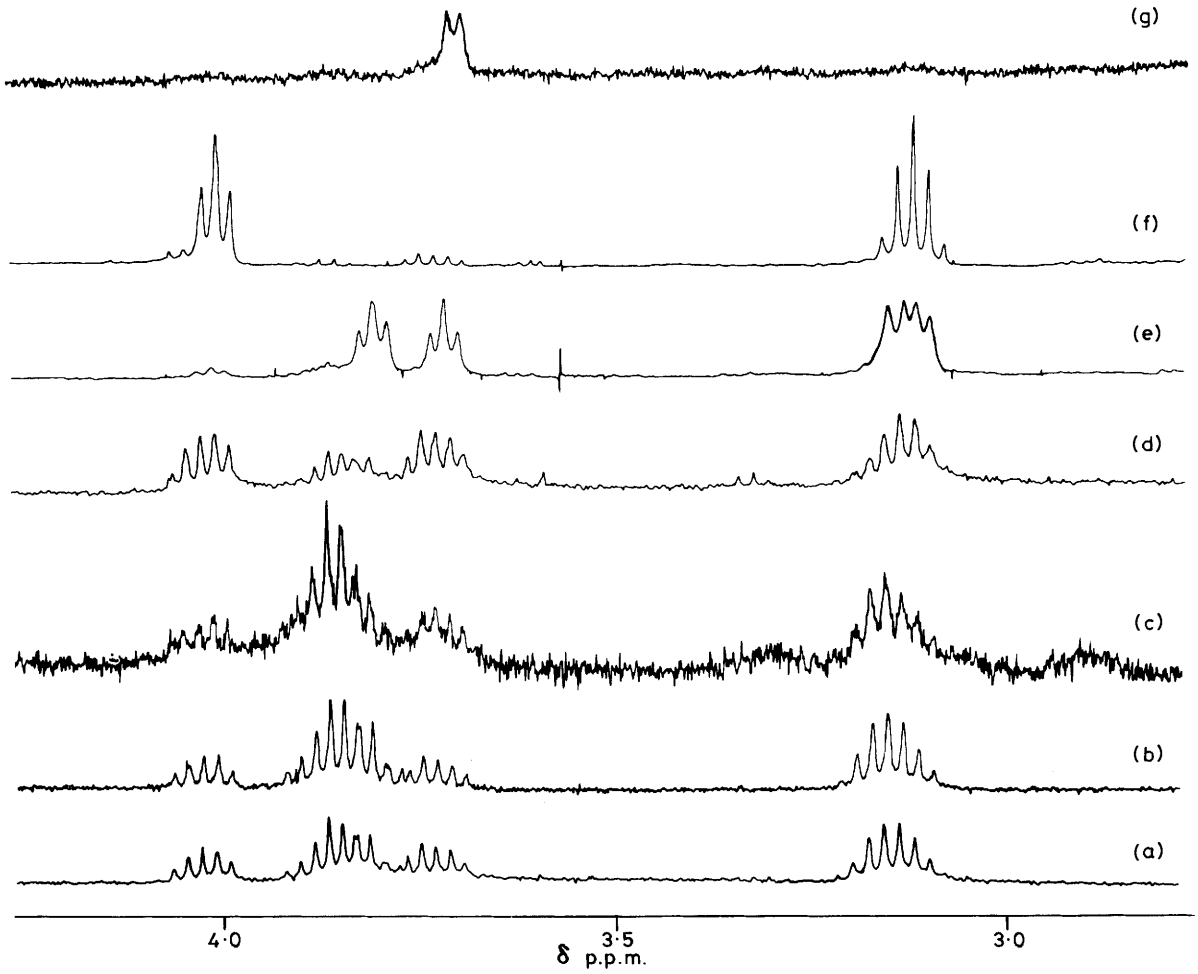


Figure 1. 360 MHz ^1H N.m.r. spectra in $C_5{}^2H_5N$ of samples of 3-camphanoylamino-2-methylpropanoic acid; (a) the (2*RS*)-acid (**11**); (b) predominantly (2*S*)-acid (**9**) by separation of (**11**); (c) predominantly 2*S*)-acid (**9**) by decarboxylation of (2*S*,3*S*)-3-methylaspartic acid; (d) predominantly (2*R*)-acid (**10**) by separation of (**11**); (e) (2*RS*,3*S*)-[3-$^2H_1$]-(**11**) by synthesis; (f) (2*R*,3*R*)-[3-$^2H_1$]-(**10**) from catabolism of [6-^2H]thymine in H_2O; (g) (2*R*,3*S*)-[2,3-$^2H_2$]-(**10**) from catabolism of thymine in ^2H_2O.

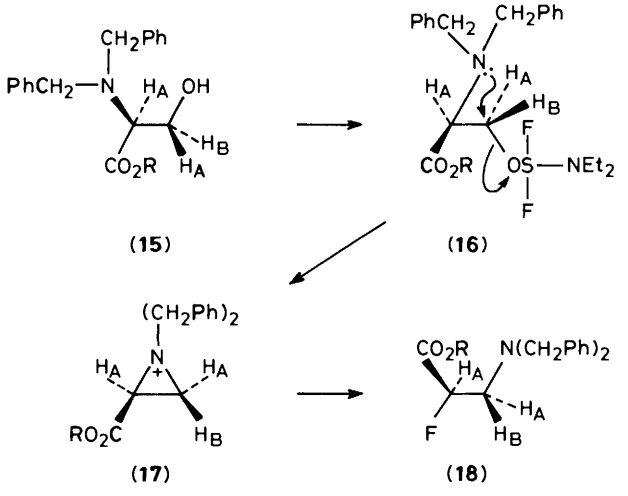
acid (**3**) followed by decarbamoylation yields the 2-substituted 3-aminopropanoic acid (**4**), carbon dioxide, and ammonia. The end product (**4**) may be further degraded. We have obtained a mixed enzyme system from bovine liver[5] which will catabolize pyrimidines (**1**) and have used it to study the stereochemistry of the catabolism of uracil (**1**; R = H).[5] Since we found that this enzyme system would also catabolize thymine and 5-fluorouracil to the corresponding 2-substituted 3-aminopropanoic acids we were also in a position to study the stereochemical outcome of the catabolism of these pyrimidines.

It is known[6] that (2*R*)-3-aminopropanoic acid (**5**) is excreted in human urine so that it might be assumed that the product of catabolism of thymine (**1**; R = Me) had the 2*R* configuration. The enantiomeric amino acid (**6**) has, however, been found in some peptides[7] and it has been reported[8] that the (2*S*)-isomer is more effectively metabolized than the (2*R*)-isomer. It was therefore necessary to confirm the absolute stereochemistry of the product of catabolism of thymine (**1**; R = Me). (2*S*,3*S*)-3-Methylaspartic acid (**7**) of known[9] absolute stereochemistry can be obtained by amination of mesaconic acid (**8**) using the enzyme β-methylaspartase from *Clostridium tetanomorphum*. Since we had isolated this enzyme together with glutamate mutase for studies[10] on vitamin B_{12}-mediated rearrangements, we were able to prepare (2*S*,3*S*)-3-methylaspartic acid (**7**). α-Decarboxylation of this compound by pyrolysis in a melt with *p*-methoxyacetophenone followed by hydrolysis in 3M-aqueous hydrochloric acid gave a sample of predominantly (2*S*)-3-amino-2-methylpropanoic acid (**6**). This was readily converted into the *N*-camphanoyl derivative (**9**). A sample of the camphanamide (**10**) was prepared from crude 3-amino-2-methylpropanoic acid obtained by catabolism of thymine using our bovine liver enzyme system in conjunction with glucose-6-phosphate and glucose-6-phosphate dehydrogenase to regenerate NADPH.[5] A sample of the camphanamide (**11**) of commercial (2*RS*)-3-amino-2-methylpropanoic acid was also prepared and this latter compound could be partially separated into the (2*R*)- and (2*S*)-diastereoisomers (**9**) and (**10**) by trituration with ether to obtain the (2*R*)-diastereoisomer as a solid and the (2*S*)-diastereoisomer from the ethereal solution. The 360 MHz ^1H n.m.r. spectra of these compounds in C^2HCl_3 or in 10% NaO^2H–^2H_2O showed clearly that the C-3 protons had different chemical shifts in the (2*R*)- and (2*S*)-isomers and that, although decarboxylation of (2*S*,3*S*)-3-methylaspartic acid (**7**) had been accompanied by a small amount of racemization at C-2, the product from thymine catabolism was clearly epimeric with this, the authentic (2*S*)-isomer. Later work indicated that clearer separation of the protons C-3 could be obtained in $C_5{}^2H_5N$ and the ^1H n.m.r. spectra of the (2*RS*)-compound (**11**), the authentic (2*S*)-decarboxylation product (**9**) and the partially separated (2*S*)- and (2*R*)-isomers (**9**) and (**10**) in this solvent are shown in Figures 1a, c, b and d respectively.

Having shown that the stereochemistry of the catabolic

product (**4**; R = Me) was clearly that of the 2*R*-isomer (**5**), it was now necessary to have an assay for the stereochemistry of the reduction step in the catabolic process at C-6 of thymine. Since samples of stereospecifically deuteriated (2*R*)-3-amino-2-methylpropanoic acid could be obtained by catabolism of [6-^{2}H]-thymine[11] in H_2O and of thymine in ^2H_2O respectively using our bovine liver/glucose-6-phosphate dehydrogenase system,[5] and since the ^{1}H n.m.r. spectra of the camphanamides of these products (Figures 1f and g respectively) clearly differentiated between the (3*R*)- and (3*S*)-labels, it was necessary to have a sample of camphanamide which was labelled unambiguously at C-3. Witkop[12] has shown that catalytic hydrogenation of thymidine (**12**) is accompanied by asymmetric induction and that hydrolysis of the resultant product gives specifically (5*S*)-dihydrothymine (**13**). Since catalytic hydrogenation would be expected to occur with *syn*-addition of hydrogen, we repeated Witkop's reduction/hydrolysis procedure but used $^2H_2$ and ^2H_2O in the reduction step to obtain thymine which evidently contained deuterium at C-5, C-6, and in the methyl group. Further hydrolysis of this compound was accompanied, as expected,[13] by racemization/exchange at C-2 to yield (2*RS*,3*S*)-[3-$^2H_1$]-3-aminopropanoic acid (**14**) containing additional deuterium in the methyl group. The ^{1}H n.m.r. spectrum of the camphanic acid amide of this compound is shown in Figure 1e and it is evident that the 3-*pro R*-hydrogen in each of the (2*R*)- and (2*S*)-isomers is clearly defined in this spectrum. Thus the spectra of the samples from the catabolic experiments (Figures 1f and g) clearly derive from (2*R*,3*R*)-[3-$^2H_1$]-3-amino-2-methylpropanoic acid and (2*R*,3*S*)-[2,3-$^2H_2$]-3-amino-2-methylpropanoic acid respectively. Addition of hydrogen has therefore occurred at the *si* face at C-6 and at the *si* face at C-5. This *anti* addition is in keeping with our findings on the catabolism of uracil.[5]

On investigating the problem of the catabolism of 5-fluorouracil we noted that Somekh and Shanzer[14] had prepared one enantiomer of the catabolic product (**4**; R = F) without defining its absolute stereochemistry by treating *N*,*N*-dibenzyl-L-serine benzyl ester (**15**; R = CH_2Ph) with (diethylamino)sulphur trifluoride (DAST) and hydrogenolysing the protecting groups. Their proposed mechanism for this reaction (Scheme 2) involving an aziridinium intermediate (**17**) would



Scheme 2.

suggest that the final product was (2*R*)-3-amino-2-fluoropropanoic acid (**19**; R = H). We set about proving this by first repeating their reaction. Since reference to the synthesis of the benzyl ester (**15**; R = CH_2Ph) was obscure, we found it more convenient to prepare the corresponding methyl ester (**15**; R = Me) and treated this with DAST to obtain the ester (**18**). This, on hydrogenolysis and hydrolysis gave 3-amino-2-fluoropropanoic acid (**19**; R = H), α_D +28.4° (H_2O) identical in all respects to a sample which we prepared by incubating 5-fluorouracil with our bovine liver/glucose-6-phosphate dehydrogenase system.

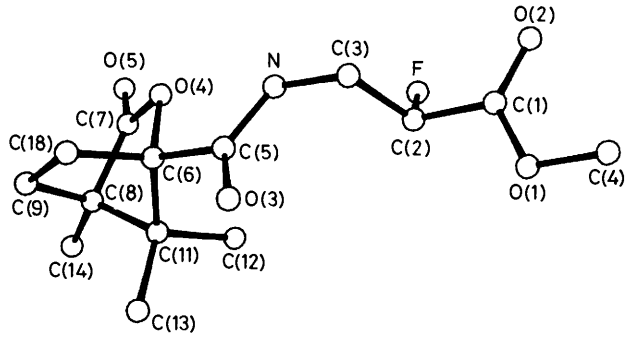
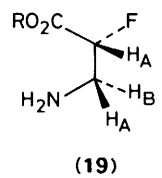
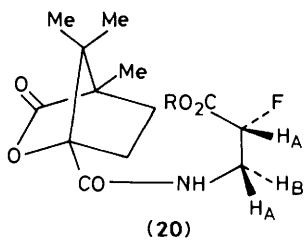


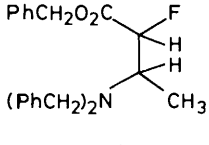
Figure 2. The molecular structure and atom numbering scheme of the amide (**20**; R = Me).

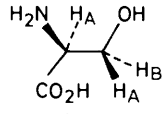
Since the low anomalous dispersion of fluorine made the use of Bijvoet's method difficult, we converted the methyl ester of the amine (**19**; R = H) into the amide (**20**; R = Me) of (−)-camphanic acid. *X*-Ray structural analysis of this compound showed it to have the stereochemistry as shown in Figure 2. Since (−)-camphanic acid is derived[15] from (+)-camphor of known[16] absolute stereochemistry, it has (1*S*,4*R*)-stereochemistry. The stereochemistry shown in Figure 2 therefore represents the absolute stereochemistry of the amide (**20**; R = Me) and so the 5-fluorouracil catabolite must be (2*R*)-3-amino-2-fluoropropanoic acid (**19**; R = H). The enzymic reduction has therefore occurred with the addition of hydrogen from the *si*-face at C-5.

When Somekh and Shanzer[14] replaced the L-serine derivative (**15**; R = CH_2Ph) in their synthesis by the corresponding derivatives of threonine and allothreonine, the products were the *threo*- and *erythro*-isomers respectively of the compound (**21**). This result is in keeping with the mechanism in Scheme 2 and, taken with our *X*-ray study on compound (**20**; R = Me), would imply that the DAST reaction involves inversion of each asymmetric centre. Since we had prepared[17] the stereospecifically deuteriated samples of L-serine (**22**; H_B = ^{2}H) and **22**; H_A = ^{2}H) respectively, we were able to convert these into the *N*,*N*-dibenzyl methyl esters (**15**; R = Me, H_B = ^{2}H) and (**15**; R = Me, H_A = ^{2}H) respectively. Treatment of these with


(19)


(20)


(21)


(22)

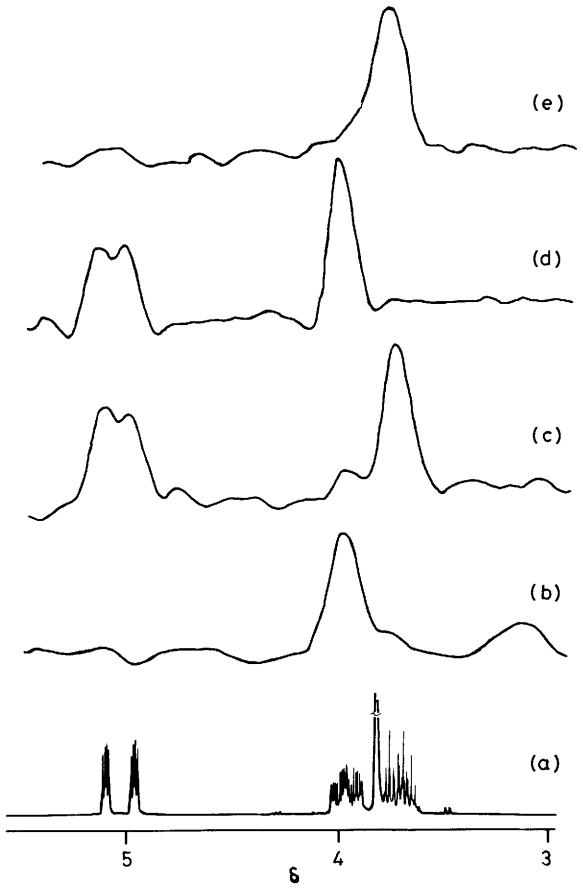


Figure 3. 360 MHz ^{1}H- (a) and 55.3 MHz ^{2}H-(b, c, d, and e) spectra in C^2HCl_3 and $CHCl_3$ respectively of samples of methyl (2*R*)-3-camphanoylamino-2-fluoropropanoate (**20**; R = Me); (a) unlabelled compound; (b) (2*R*,3*S*)-[3-$^2H_1$]- compound by synthesis; (c) (2*R*,3*R*)-[2,3-$^2H_2$]-compound by synthesis; (d) (2*R*,3*S*)-[2,3-$^2H_2$]-compound from catabolism of 5-fluorouracil in ^2H_2O; (e) (2*R*,3*R*)-[3-$^2H_1$]-compound from catabolism of [6-^{2}H]-5-fluorouracil in H_2O.

DAST followed by hydrogenolysis gave methyl (2*R*,3*S*)-[3-$^2H_1$]- and (2*R*,3*R*)-2,3-$^2H_2$]-3-amino-2-fluoropropanoates (**19**; R = Me, H_B = ^{2}H) and (**19**; R = Me, H_A = ^{2}H) respectively. These were converted into the (−)-camphanic acid amides (**20**; R = Me, H_B = ^{2}H) and (**20**; R = Me, H_A = ^{2}H) respectively and the ^{2}H n.m.r. spectra of these (Figures 3b and c respectively) showed unique absorptions for the 3*S*- and 3*R*-deuterium atoms. 5-Fluorouracil was now incubated with our catabolic enzyme system in ^2H_2O and [6^{2}H]-5-fluorouracil was incubated in H_2O to give samples of deuteriated 3-amino-2-fluoropropanoic acid. These were converted into the methyl ester camphanamides (**20**; R = Me) the ^{2}H n.m.r. spectra of which are shown in Figures 3d and e respectively. It is evident from these that enzymic reduction of 5-fluorouracil has occurred from the *si*-face at C-6.

We have therefore shown that catabolism of the DNA base thymine and the anti-cancer drug 5-fluorouracil both occur with *anti*-addition of hydrogen to the pyrimidines at the *si*-face at C-5 and the *si*-face at C-6. Thus, both compounds must bind at the active site of dihydrothymine dehydrogenase in a similar manner when being reduced by the flavin coenzyme. This contrasts with the case of the substrate folic acid and the anti-cancer drug methotrexate which bind differently to dihydrofolate reductase.[3] Methotrexate is not reduced by this enzyme whereas 5-fluorouracil is reduced by dihydrothymine dehydrogenase.

Experimental

M.p.s were determined on a Kofler hot-stage apparatus and optical rotations on a Perkin Elmer PE241 polarimeter using a 1 dm path length cell. I.r. spectra were recorded on Perkin-Elmer 257, 457, and 477 instruments and u.v. spectra on a Pye Unicam SP800 spectrophotometer which, for enzyme assays, was modified to drive an external Kipp and Zonen BD8 chart recorder. ^{1}H N.m.r. spectra were recorded on Perkin-Elmer R12 (60 MHz) and R32 (90 MHz) instruments and on a Bruker WM360 (360 MHz) instrument. ^{2}H (55.3 MHz) N.m.r. spectra were obtained on this latter instrument and ^{19}F (75.4 MHz) n.m.r. spectra were obtained on a Bruker WP80 instrument. Mass spectra were obtained using Kratos MS25 and MS80 instruments.

Isolation of β-*Methylaspartase from* Clostridium tetanomorphum.—*Clostridium tetanomorphum* (ATCC No. 15920) was cultured on the medium recommended by the ATCC[18] according to the method of Barker.[19] The cells were harvested and washed by the method of Barker[19] and were cryogenized in liquid nitrogen as a wet cell paste. The cell paste (25 g) was allowed to thaw (4 h after initial harvesting) and was twice homogenized in a French press, at 0 °C with 0.02M-potassium phosphate (pH 7.4). Acid-washed charcoal (1 g) was added and the suspension was stirred at 0 °C for 10 min. The cell debris was removed by centrifugation at 30 000 g at 0 °C for 20 min.

The supernatant solution was diluted to 140 ml with cold water and 1% protamine sulphate in 0.04M-potassium phosphate (pH 7.0) (32 ml) was added with stirring over 5 min at 0 °C. The stirring was continued for a further 10 min and the precipitated nucleic acids and protein were removed at 12 000 g for 10 min. 1M-Potassium phosphate (pH 7.0, 8 ml) was added to the supernatant clear solution (to bring the total volume to 171 ml) and solid pre-crushed ammonium sulphate (50 g) was added slowly with gentle stirring to 50% saturation, whilst the temperature was kept at 0 °C. The stirring was continued for 10 min and the precipitated protein was removed by centrifugation at 15 000 g at 0 °C for 15 min. The supernatant solution was treated at 0 °C with pre-crushed ammonium sulphate (36 g), as above, to 80% saturation. After an additional 15 min, the protein was collected by centrifugation at 12 000 g for 15 min. The protein pellets were dissolved in 0.01M-potassium phosphate (pH 7.0) to yield 24 ml of a pale red-brown solution. The entire solution was dialysed in Visking tubing against 5 l of 0.005M-potassium phosphate (pH 7.0) at 3 °C for 10 h. The dialysed solution was frozen in 28 × 1 ml vials under nitrogen. The yield of β-methylaspartase was 97 units per ml (total 2 714 units, 45 231 nKat).

Assay for β-*Methylaspartase.*—Assays contained tris buffer (pH 9.0), (0.05M); $MgCl_2 \cdot 6H_2O$ (0.002M); (±)-β-methylaspartate dipotassium salt (0.004M); NaCl (0.001M) and enzyme in a total volume of 3 ml. Assays were conducted in 1 cm path-length cuvettes at 30 °C, Δ(OD) being monitored at 240 nm. 1 Unit is defined as the amount of enzyme forming 1 μmol of mesaconate under the above conditions (ε_{240} for mesaconate = 3 850, *i.e.* a rate of 1.28 OD unit/min = 1 unit under the above conditions).

(2S,3S)-3-*Methylaspartic Acid* (7).—Diammonium mesaconate was prepared from a filtered solution of mesaconic acid (13 g, 0.1 mol) to which aqueous ammonia had been added to pH 6.95. This, sodium chloride (263 mg, 4.5 mmol), magnesium chloride hexahydrate (2.13 g, 10.5 mmol), ammonium chloride (10.7 g, 0.2 mol), and tris buffer (6 g, 0.05 mol) were dissolved in water (150 ml). The pH was adjusted to 9.0 with 0.5M-KOH and 2,2′-bipyridyl (1 mg) was added. The above enzyme mixture containing β-methylaspartase (3 ml, *ca.* 300 units) was added

and the reaction was incubated at 30 °C until no further decrease in absorbance at 240 nm occurred (*ca.* 18 h). The protein was denatured at *ca.* 95 °C for 10 min and then removed by filtration on a Celite pad. The filtrate was evaporated to dryness and was crystallized at pH 3 (by addition of 12M-HCl) from EtOH–H_2O (2:1; 80 ml). The product was recrystallized from EtOH–H_2O (3:1; 100 ml) to yield a solid (9.71 g, 66%), m.p. 276—278 °C, $[\alpha]_D^{22}$ −9.6° (*c* 0.42, H_2O) {lit.,[20] $[\alpha]_D$ −10 ± 2° (*c* 0.42, H_2O)}; δ (10% NaO^2H in ^2H_2O, ^2HOH at 4.6 p.p.m.), 3.69 (1 H, d, *J* 3.1 Hz, 2-CH), 2.62 (1 H, qd, *J* 7.5 and 3.1 Hz, 3-CH), and 0.82 (3 H, d, *J* 7.5 Hz, CH_3).

(2S)-3-*Amino*-2-*methylpropanoic Acid* (**6**).—(2*S*,3*S*)-3-Methyl aspartic acid (**7**) (1 g, 6.8 mmol) was intimately ground with *p*-methoxyacetophenone (2 g, 13 mmol). The mixture was transferred to a small round-bottomed flask and heated to 190 °C for 40 min under nitrogen, when evolution of carbon dioxide ceased (monitored by passing the effluent gas through fresh aqueous barium hydroxide). The reaction was cooled, 3M-hydrochloric acid (10 ml) was added, and the reaction was heated to reflux for 15 min; it was then cooled and extracted with chloroform (3 × 25 ml). The aqueous phase was lyophilized, dissolved in water, and applied to an Amberlite IR45 weakly basic ion-exchange column (10 × 2 cm), with water as eluant. The first fraction (*ca.* 200 ml) gave a pale yellow gum which was recrystallized from Me_2CO–H_2O (9:1) to give small colourless crystals (170 mg, 24%), m.p. 168—170 °C; δ (10% NaO^2H in ^2H_2O, external $SiMe_4$ at 0 p.p.m.) 2.51 (3 H, m, 10 lines, 2-CH and 3-CH_2) and 1.01 (3 H, d, *J* 6 Hz, CH_3). A portion (120 mg) was recrystallized twice to give a sample (43 mg) with m.p. 178—179 °C (lit.,[21] m.p. 192—194 °C), $[\alpha]_D^{20}$ +12.7° (*c* 1.35, H_2O), {lit.,[21] $[\alpha]_D^{23}$ +15.4° (*c* 1.0, H_2O)}.

(2RS)-3-*Camphanoylamino*-2-*methylpropanoic Acid* (**11**).—(2*RS*)-3-Amino-2-methylpropanoic acid (103 mg, 1 mmol) was dissolved in 1.2M-NaOH (2 ml) and the solution was vigorously shaken with (−)-camphanoyl chloride (180 mg, 0.83 mmol) in toluene (1 ml). After 3 h, the organic phase was separated and the aqueous phase was washed with chloroform (2 × 1 ml). The solution was acidified to pH 2 with 6M-hydrochloric acid and extracted with chloroform (3 × 2 ml). The extracts were dried (Na_2SO_4), and the solvent was removed under reduced pressure to yield a colourless syrup (210 mg, 89%); *m/z* (EI) 283 (M^+) and 237 $[(M - HCO_2H)^+]$ (Found: M^+, *m/z* 283.1419. $C_{14}H_{21}NO_5$ requires M^+, *m/z* 283.1420) $\nu_{max.}$(Nujol), 3 400 (NH) and 1 780—1 715 cm^{-1} (lactone, acid, and amide); δ (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m.) 8.08 (1 H, br s, CO_2H), 7.02 (1 H, br d, NH), 3.44 (1 H, m, 3-CH_2 of 2*R*-diastereoisomer), 3.43 (1 H, m, 3-CH_2 of 2*S*-diastereoisomer), 2.70 (1 H, m, 2-CH of 2*R*-and 2*S*-diastereoisomers), 2.46 (1 H, m, CH of camphanoyl), 1.88 (2 H, m, CH_2 of camphanoyl), 1.61 (1 H, m, CH of camphanoyl), 1.16 (3 H, d, *J* 7 Hz, α-CH_3), 1.03 (6 H, s, 2 × CH_3 of camphanoyl), and 0.82 (3 H, 2 × s, CH_3 of camphanoyl); δ (10% NaO^2H in ^2H_2O, ^2HOH at 4.6 p.p.m.; 2- and 3-H only), 3.05 (1 H, 2 × q, J_{AB} 13.3 Hz, $J_{AX} = J_{BX}$ 7.1 Hz, 3-CH_2 of 2*R*-diastereosiomer), 3.02 (1 H, 2 × q, J_{AB} 13.4 Hz, $J_{AX} = J_{BX}$ 6.3 Hz, 3-CH_2 of 2*S*-diastereoisomer), and 2.41 (1 H, m, 2-CH of 2*R*- and 2*S*-diastereoisomers).

Partial Separation of the Diastereoisomers (**9**) *and* (**10**).—After several days crystals began to separate from the oil obtained in the preceding experiment. These were washed with cold diethyl ether and filtered off, m.p. 122—124 °C. The solid had a ^1H n.m.r. spectrum (Figure 1d) which showed a predominance of the 2*R*-diastereoisomer (**10**) containing *ca.* 30% of the 2*S*-diastereoisomer. It was not possible to purify this sample further by crystallization. The mother liquors crystallized slowly with time, m.p. 101—103 °C. The ^1H n.m.r. spectrum (Figure 1b) indicated the compound to contain *ca.* 70% of the pure 2*S*-diastereoisomer (**9**).

(2S)-3-*Camphanolyamino*-2-*methylpropanoic Acid* (**9**). This compound was prepared from (2*S*)-3-amino-2-methylpropanoic acid from decarboxylation of (2*S*,3*S*)-3-methylaspartic acid and (−)-camphanoyl chloride as described above. The ^1H n.m.r. spectrum (Figure 1c) showed that the sample was contaminated with 20—25% of the (2*R*)-isomer (**10**).

[6-^2H]-*Thymine*.—Thymine (378 mg, 3 mmol) was dissolved in 8% NaO^2H in ^2H_2O (*ca.* 95% deuteriated, 10 ml). The solution was heated to 125 °C for 15 h. The reaction was cooled and neutralized with 12M-HCl. White crystals (318 mg, 84%) were deposited with time at 4 °C, m.p. 315—316 °C; *m/z* 129, 128, and 127 (M^+, N^2H present; 6-^2H = 90%); $\lambda_{max.}$(MeOH) 266 nm; δ (8% NaO^2H in ^2H_2O, external $SiMe_4$ at 0 p.p.m.), 7.27 (0.1 H, s, 6-H) and 1.65 (3 H, s, CH_3).

Catabolism of Thymine in H_2O—The incubation mixture contained thymine (56 mg, 0.44 mmol), glucose-6-phosphate (125 mg, 0.44 mmol), and glucose-6-phosphate dehydrogenase (1 unit, 16.7 nKat). NADPH (10 mg, 0.013 mmol) and the bovine liver preparation[5] [6 ml, containing dihydrothymine dehydrogenase (25 units, 7.2 nKat)] in a total volume of 60 ml of a buffer containing potassium phosphate (pH 7.4; 35 mM), mercaptoethanol (5 mM) and $MgCl_2$·$6H_2O$ (2.5 mM). The reaction was incubated at 37 °C for 16 h. The protein was denatured by immersion in boiling water for 2 min and ethanol (10 ml was added. The precipitated protein was removed by centrifugation at 15 000 g for 20 min and the supernatant solution was treated with n-propanol (1 ml) and was reduced in volume to 5 ml. Ethanol (5 ml) was added and the insoluble material was removed by centrifugation. The supernatant solution was applied to two 20 × 20 × 1 mm preparative cellulose plates, with Pr^iOH–NH_3 (*d* 0.880)–H_2O (25:6:5). The product, (2*R*)-3-aminopropanoic acid was an off-white amorphous solid (6 mg), δ (^2H_2O, external $SiMe_4$ at 0 p.p.m.), 2.98 (*ca.* 2 H, m, 3-CH_2, 2.51 (*ca.* 1 H, m, 2-CH) and 1.09 (*ca.* 3 H, d, *J* 7 Hz, CH_3). Other peaks were present in the spectrum.

The camphanate was prepared as described for the (2*RS*)-compound (**11**) above and had δ (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m.; 2- and 3-H only), 3.49 (2 H, m, 3-CH_2), and 2.75 (1 H, m, 2-CH); δ (10% NaO^2H in ^2H_2O, ^2HOH at 4.6 p.p.m., 3-H only) 3.05 (2 H, 2 × q, J_{AB} *ca.* 13 Hz, J_{AX} J_{BX} *ca.* 7 Hz, 3-CH_2); *m/z* (EI) 283 (M^+) and 237 ($[M - HCO_2H]^+$).

Catabolism of Thymine in ^2H_2O.—The incubation contained thymine (56 mg, 0.44 mmol), glucose-6-phosphate (125 mg, 0.44 mmol), glucose-6-phosphate dehydrogenase (3 units, 49.1 nKat), NADPH (10 mg, 0.013 mmol), and our liver enzyme mixture[5] (17 ml, in ^2H_2O containing 64 units (18.4 nKat) of dihydrothymine dehydrogenase) in a total volume of 70 ml of a buffer made up in ^2H_2O containing potassium phosphate (35 mM, pH 7.4), mercaptoethanol (5 mM), and $MgCl_2$·$6H_2O$ (2.5 mM). The reaction was incubated at 37 °C for 71 h and the amino acid was isolated as described above (6.8 mg). This was converted as above into the camphanate, δ(^1H) (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m.) 3.49 (1 H, d, *J* 6 Hz, 3-*H*): δ(^1H) ([$^2H_5$]pyridine, locked on pyridine at 8.71 p.p.m., see Figure 1g) 3.69 (1 H, d, *J* 6 Hz, 3-H_R); δ($^2H_2$) ($CHCl_3$, C^2HCl_3 at 7.24 p.p.m.) 3.98 (1^2H, s, 3-^2H_S) and 3.12 (1^2H, s, 2C^2H); *m/z* (EI) 285 (M^+).

Catabolism of [6-^2H]*Thymine in* H_2O.—The incubation mixture contained [6-^2H]thymine (57 mg, 0.45 mmol), glucose-6-phosphate (125 mg, 0.44 mmol), glucose-6-phosphate

dehydrogenase (5 units, 82 nKat), NADPH (10 mg, 0.013 mmol), and our bovine liver enzyme mixture[5] [12 ml containing *ca.* 48 units (13.8 nKat) of dihydrothymine dehydrogenase], all in a total volume of 60 ml of a buffer containing potassium phosphate (pH 7.4; 35mM), mercaptoethanol (5 mM), and $MgCl_2 \cdot 6H_2O$ (2.5 mM). The reaction was incubated at pH 7.4 for 40 h and the amino acid was purified as described above to yield the crude product (9.1 mg). This was converted as above into the camphanamide; *m/z* (EI) 284 (M^+) and 238 [$(M - HCO_2H)^+$]; δ(^{1}H) ([$^2H_5$]pyridine, locked on pyridine at 8.71 p.p.m., see Figure 1f) 3.98 (1 H, t, *J* 6 Hz, 3-H_S) and 3.11 (1 H, 5 lines, *J* 7 Hz, 2-CH); δ(^{2}H) (pyridine, reference to pyridine at 8.71 p.p.m.) 3.70 (1 ^{2}H, s, 3-^2H_R).

(5S)-*Dihydrothymine* (**13**). This compound was prepared by a modification of the method of Witkop.[12] Thymidine (200 mg, 0.83 mmol) was dissolved in 0.001M-HCl (40 ml) and was hydrogenated at atmospheric pressure in the presence of rhodium (5%) on alumina for 12 h when uptake of hydrogen ceased. The catalyst was filtered off on a pad of Celite and the solvent was removed under reduced pressure. The residue was dissolved in 0.1M-HCl (25 ml) and was heated at 95 °C for 80 min. The solvent was removed under reduced pressure and the residue was dissolved in water (10 ml) and treated with silver carbonate to pH 6.5. The silver chloride was removed by filtration through Celite and the solvent was removed under reduced pressure to yield a sticky off-white solid. Recrystallization from aqueous ethanol yielded pure (5*S*)-dihydrothymine (61 mg, 58%) m.p. 261—263 °C (lit.,[12] 262.5—263 °C), $[\alpha]_D^{22}$ −12.4° (*c* 0.77, pyridine) {lit.,[12] $[\alpha]_D^{20}$ −11.3 ± 1° (*c* 0.49, pyridine)}; *m/z* (EI) 128 (M^+); $\nu_{max.}$(Nujol) 1 690 cm^{-1} (br, amide); δ (^2H_2O, external $SiMe_4$ at 0 p.p.m.) 3.36 (1 H, dd, J_{AB} 13 Hz, $J_{5,6H_S}$ 7 Hz, 6-H_S), 3.02 (1 H, dd, J_{AB} 13 Hz, $J_{5,6H_R}$ 10 Hz, 6-H_R), 2.65 (1 H, m, 5-CH), and 1.07 (3 H, d, *J* 7 Hz, CH_3).

(5S,6S)-[5,6-$^2H_2$]*Dihydrothymine* (**13**; H_S = ^{2}H).—This compound was prepared as described above using thymidine (1 g, 4.13 mmol) in 0.001M-^{2}HCl (99.7%, 150 ml) with 350 mg of catalyst under an atmosphere of deuterium gas to yield a solid (311 mg, 58%), m.p. 260—262 °C; *m/z* (EI), 134 (M^+) showing up to six deuterium atoms present; $\nu_{max.}$(Nujol) 1 680 cm^{-1} (br, amide); δ(^{1}H) (^2H_2O, external $SiMe_4$ at 0 p.p.m.) 3.00 (1 H, br s, 6-H_R) and 1.04 (1 H, br s, C^2H_2H) (there was only a slight trace of non-incorporation at δ 3.33 p.p.m.); δ(^{2}H) (pyridine, ref. to pyridine at 8.71 p.p.m.) 3.3 (1 ^{2}H, s, 6-^2H_S), 2.57 (1 ^{2}H, s, 5-^2H_S), and 1.12 (s, C^2H_3).

(2RS,3S)-[3-$^2H_1$]-3-*Amino*-2-*methylpropanoic Acid* (**14**).—(5*S*,6*S*)-[5,6-$^2H_2$]Dihydrothymine (180 mg, 1.38 mmol) was dissolved in 12M-HCl (10 ml) and the solution was heated to reflux for 5 h. The solution was lyophilized, fresh 12M-HCl was added, and the solution was refluxed for a further 12 h. This process was repeated twice over a further 30 h. After a total of *ca.* 48 h of heating under reflux, the solution was cooled, lyophilized and the residue suspended in ethanol (10 ml). The undissolved material was filtered off and the filtrate was lyophilized. T.l.c. in Pr^iOH–NH_3 (*d* 0.880)–H_2O (25:6:5) on cellulose showed one ninhydrin positive spot co-running with authentic 2-methyl-3-aminopropanoic acid (purple, R_F 0.51), yield 198 mg (containing NH_4Cl). This was used directly in the following step without further purification.

(2RS,3S)-[3-$^2H_1$]-3-*Camphanoylamino*-2-*methylpropanoic Acid*.—Half of the preceding compound, which contained ammonium chloride, was treated with 2M-NaOH (5 ml). The solution was reduced in volume to 3 ml under reduced pressure and the resulting solution was shaken with (−)-camphanoyl chloride (150 mg, 0.69 mmol) in toluene (1 ml) as described earlier to yield a colourless oil (129 mg, 65% based on camphanoyl chloride); *m/z* (EI) 288 (M^+, *ca.* 4.5 atoms ^{2}H); δ(^{1}H) ([$^2H_5$]pyridine, locked on pyridine at 8.71 p.p.m.; see Figure 1e, 2- and 3-positions only), 3.76 (1 H, t, *J* 6 Hz, 3-H_R of 2*S*-diastereoisomer) and 3.68 (1 H, t, *J* 6 Hz, 3-H_R of 2*R* diastereoisomer).

(2S)-N,N-*Dibenzylserine* (**15**; R = H).—This compound was prepared by a modification of the method of Velluz.[22] Benzyl chloride (35 g, 276 mmol) was added to a refluxing solution of L-serine (5.25 g, 50 mmol) and 7M-aqueous KOH (40 ml) in water (50 ml) and ethanol (50 ml) over 30 min. The mixture was heated to reflux for 1 h and then cooled and the ethanol removed under reduced pressure. The solution was adjusted to pH 8.5 with 7M-aqueous KOH and extracted with chloroform (3 × 50 ml). The aqueous phase was adjusted to *ca.* pH 4 with acetic acid and extracted with chloroform (4 × 30 ml). This second organic extract was dried (Na_2SO_4) and the solvent was removed to yield a crystalline residue (6.27 g, 44%). The product was recrystallized from light petroleum (b.p. 40—60 °C)–chloroform (9:1) to yield white crystals (5.21 g, 36%), m.p. 148—149 °C (lit.,[22] m.p. 142—143 °C), $[\alpha]_D^{26}$ −70.1° (*c* 0.896, MeOH) (lit.,[22] $[\alpha]_D$ −79 ± 1°, MeOH) (Found: C, 71.4; H, 7.00; N, 5.05. $C_{17}H_{19}NO_3$ requires C, 71.5; H, 6.7; N, 4.9%); *m/z* (EI) 286 [$(M + H)^+$], 285 (M^+), 254 [$(M - CH_2OH)^+$], and 240 [$(M - CO_2H)^+$]; $\nu_{max.}$(Nujol) 1 612 cm^{-1} (br, CO_2H); δ (C^2HCl_3, external $SiMe_4$ at 0 p.p.m.), 7.3 (10 H, s, ArH), 3.97 (4 H, s, benzyl CH_2), 3.90 (2 H, br m, 3-CH_2 of serine) and 3.65 (1 H, m, 2-CH of serine).

(2S,3R)-[3-$^2H_1$]-N,N-*Dibenzylserine* (**15**; R = H, H_B = ^{2}H).—This compound was prepared as above using (2*S*,3*R*)-[3-$^2H_1$]serine (**22**; H_B = ^{2}H)[17] (80 mg, 0.75 mmol) to yield a solid (74 mg, 34%), which crystallized from light petroleum (b.p. 40—60 °C)–chloroform (9:1), m.p. 146–147 °C; $\nu_{max.}$(Nujol) 1 615 cm^{-1} (br, CO_2H). The ^{1}H n.m.r. spectrum (C^2HCl_3, external $SiMe_4$ at 0 p.p.m.) was identical with that of the unlabelled material but showed one proton less at δ *ca.* 3.90.

(2S,3S)-[2,3-$^2H_2$]-N,N-*Dibenzylserine* (**15**; R = H, H_A = ^{2}H).—This compound was prepared as above using (2*S*,3*S*)-[2,3-$^2H_2$]serine (**22**; H_A = ^{2}H)[17] (80 mg, 0.75 mmol) to yield white crystals (81 mg, 38%) from light petroleum (b.p. 40—60 °C)–chloroform (9:1), m.p. 148—149 °C; $\nu_{max.}$(Nujol) 1 615 cm^{-1} (br, CO_2H). The ^{1}H n.m.r. spectrum (C^2HCl_3, external $SiMe_4$ at 0 p.p.m.) was identical with that of the unlabelled material except that the signal at δ *ca.* 3.90 p.p.m. integrated as one proton less and the signal at δ 3.65 p.p.m. (serine 2-H) was absent.

Methyl (2S)-N,N-*Dibenzylserinate* (**15**; R = Me).—(2S)-*N*,*N*-Dibenzylserine (**15**; R = H) (1.6 g, 5.6 mmol) was dissolved in chloroform–ethanol (3:1; 20 ml) and was treated dropwise at 0 °C with ethereal ethanolic diazomethane, with gentle stirring, until evolution of nitrogen ceased and a yellow colour persisted. After 5 min, acetic acid (2 drops) was added and the solvent was removed under reduced pressure to yield a colourless viscous oil (1.61 g, 96%), which resisted attempts at crystallization; $[\alpha]_D^{26}$ −105° (*c* 1.208, MeOH) (Found: C, 72.0; H, 7.25; N, 4.45. $C_{18}H_{21}NO_3$ requires C, 72.2; H, 7.0; N, 4.70%); *m/z* (EI) 300 [$(M + H)^+$], 268 [$(M - CH_2OH)^+$], and 240 [$(M - CO_2Me)^+$]; $\nu_{max.}$(Nujol) 3 400br (NH) and 1 715 cm^{-1} (CO_2Me); δ (C^2HCl_3, external $SiMe_4$ at 0 p.p.m.) 7.36 (10 H, s, ArH), 3.99—3.59 (10 H, m, benzyl CH_2, serine 2-CH and 3-CH_2 and methyl ester H), and 2.75 (1 H, br s, OH).

Methyl (2S,3R)-[3-$^2H_1$]-N,N-*Dibenzylserinate* (**15**; R = Me, H_B = ^2H).—This compound was prepared as above using (2*S*,3*R*)-[3-$^2H_1$]-*N*,*N*-dibenzylserine (**15**; R = H, H_B = ^2H) (65 mg, 0.23 mmol) to yield the product as an oil (68 mg, 100%). Spectral data were identical with the unlabelled material except that the ^1H n.m.r. spectrum in C^2HCl_3 showed the absence of one proton in the region δ 3.99—3.59; *m/z* (EI) 300 (M^+), 268 [$(M - CH^2HOH)^+$], and 241 [$(M - CO_2Me)^+$].

Methyl (2S,3S)-[3-$^2H_1$]-N,N-*Dibenzylserinate* (**15**; R = Me, Me, H_A = ^2H).—This compound was prepared as above using (2*S*,3*S*)-[3-$^2H_1$]-*N*,*N*-dibenzylserine (**15**; R = H, H_A = ^2H) (65 yield a colourless oil (100%) which showed the expected ^1H n.m.r. spectral data in C^2HCl_3; *m/z* (EI) 302 [$(M + H)^+$], 269 [$(M - CH^2HOH)^+$], and 242 [$(M - CO_2Me)^+$].

Methyl (2R)-3-*Dibenzylamino*-2-*fluoropropanoate* (**18**; R = Me).—A solution of methyl (2*S*)-*N*,*N*-dibenzylserinate (**15**; R = Me) (500 mg, 1.67 mmol) in dry tetrahydrofuran (10 ml) was slowly added dropwise under nitrogen to a solution of diethylaminosulphur trifluoride (0.25 ml, 2 mmol) in dry tetrahydrofuran (10 ml) at 20 °C. After addition, the amber-coloured solution was stirred for 30 min at room temperature. The reaction was quenched by the addition of ice–water (50 ml) followed by ethyl acetate (200 ml). The two-phase mixture was rapidly stirred and solid $NaHCO_3$ was added until effervescence ceased. The layers were separated and the aqueous layer was extracted with ethyl acetate (2 × 50 ml). The organic phases were washed with water (5 ml) and dried (Na_2SO_4). The solvent was removed under reduced pressure to yield an almost colourless oil (437 mg, 86%); $[\alpha]_D^{26}$ −2.5° (*c* 2.198, MeOH) (Found: C, 71.7; H, 7.0; N, 4.4. $C_{18}H_{20}FNO_2$ requires C, 71.7; H, 6.6; N, 4.65%); *m/z* (EI) 301 (M^+) and 210 [$(M - CHFCO_2Me)^+$]; $\nu_{max.}$(Nujol) 1 750 cm^{-1} (CO_2Me); δ (C^2HCl_3, external $SiMe_4$ at 0 p.p.m.) 7.36 (10 H, s, ArH), 5.03 (1 H, dt, J_{AX} = J_{BX} 5 Hz, J_{XF} 50 Hz, 2-CH, *i.e.* H_X), 3.67 (7 H, m, benzyl 2 × CH_2 and OCH_3) and 2.99 (2 H, dd, J_{AX} = J_{BX} 5 Hz, J_{BF} 25 Hz, 3-CH_2, *i.e.* H_A and H_B).

Methyl (2R,3S)-[3-$^2H_1$]-3-*Dibenzylamino*-2-*fluoropropanoate* (**18**; R = Me, H_B = ^2H).—This compound was prepared as above using methyl (2*S*,3*R*)-[3-$^2H_1$]-*N*,*N*-dibenzylserinate (**15**; R = Me, H_B = ^2H) (60 mg, 0.2 mmol) to yield the product as an oil (50 mg, 83%). All spectral data were consistent with the expected structure and labelling pattern; *m/z* (EI) 302 (M^+) and 211 [$(M - CHFCO_2Me)^+$].

Methyl (2R,3R)-[2,3-$^2H_2$]-3-*Dibenzylamino*-2-*fluoropropanoate* (**18**; R = Me, H_A = ^2H).—This compound was prepared as above using methyl (2*S*,3*S*)-[2,3-$^2H_2$]-*N*,*N*-dibenzylserinate (**15**; R = Me, H_A = ^2H) (60 mg, 0.2 mmol) to yield the product as an oil (48 mg, 78%). All spectral data were consistent with the expected structure and labelling pattern; *m/z* (EI) 303 (M^+) and 211 [$(M - C^2HFCO_2Me)^+$].

(2R)-3-*Amino*-2-*fluoropropanoic Acid* (**19**; R = H).—Methyl (2*R*)-3-*N*,*N*-dibenzylamino-2-fluoropropanoate (**18**; R = Me) (1.65 g, 5.48 mmol) was vigorously stirred under hydrogen (at 1 atm) with a suspension of platinum oxide (200 mg) in EtOH–H_2O–HOAc (8:1:1; 80 ml). After 2 days, the uptake of hydrogen had ceased. The solution was diluted with water (40 ml) and filtered through Celite (*ca.* 5 g). The volume of the filtrate was reduced under reduced pressure to yield a colourless glassy syrup (510 mg). The ^1H n.m.r. spectrum (^2H_2O) showed some loss of the methyl ester group and the complete removal of the benzyl groups. The ester/acid mixture (100 mg) was heated to reflux in aqueous 6M-HCl (2 ml) for 90 min. The solvent was removed under reduced pressure and the residue was dissolved in water (20 ml) and applied to a 1 × 10 cm column of Amberlite IR45 (OH) with water as eluant; 150 ml of effluent was collected. The solvent was removed under reduced pressure to yield the crude product (72 mg), δ (^2H_2O, HO^2H at 4.6 p.p.m.), 4.89 (1 H, ddd, J_{XF} 54 Hz, J_{BX} 9 Hz, with a smaller coupling of 2—3 Hz also apparent), 3.23 (1 H, m, H_B of AB) and 3.11 (1 H, m, H_A of AB). This spectrum showed some other signals. The sample was recrystallized from aqueous acetone to yield white needles (51 mg, 58%), m.p. 250—253 °C (decomp.) (lit.,[23] m.p. 261 °C), $[\alpha]_D^{20}$ +28.4° (*c* 0.51, H_2O) {lit.,[14] $[\alpha]_D$ + 29.1° (*c* 1.05, H_2O)}.

(2R)-3-*Camphanoylamino*-2-*fluoropropanoic Acid* (**20**; R = H).—(2*R*)-3-Amino-2-fluoropropanoic acid (**19**; R = H) (21.4 mg, 0.2 mmol) in 2M-NaOH (200 μl) was vigorously shaken with a solution of (−)-camphanoyl chloride (45 mg, 0.21 mmol) in toluene (200 μl) at room temperature. After 2 h, the solution was diluted to 1 ml with water and the pH was adjusted to *ca.* 12 with 2M-NaOH. The solution was extracted with chloroform (2 × 2 ml), acidified to pH 2 with 12M-HCl (3 drops), and again extracted with chloroform (3 × 5 ml). The second extract was dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield a white crystalline solid (43 mg) containing an appreciable amount of camphanic acid. The product was unsuitable for chromatographic purification because of severe streaking on SiO_2 using a wide variety of solvent systems, δ (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m.), 5.08 (1 H, dt, J_{XF} 48 Hz, J_{BX} 4 Hz, $J_{B,NH}$ 4 Hz 2-CH), and 3.95 (1 H, m, 3-CH_B). Peaks due to camphanic acid were also present in this spectrum and obscured 3-CH_A.

Methyl (2R)-3-*Amino*-2-*fluoropropanoate Hydrochloride* (**19**; R = Me).—(2*R*)-3-Amino-2-fluoropropanoic acid (**19**; R = H) (510 mg, 4.77 mmol) was dissolved in dry methanol (8 ml) and thionyl chloride (0.5 ml, 1.2 molar equiv.) was added dropwise with stirring at −10 °C. The solution was warmed to 50 °C for 10 min and then allowed to cool to room temperature. Dry diethyl ether was slowly added with gentle swirling until crystals began to form. The solution was set aside at *ca.* 4 °C for 2 h after which the crystals were filtered off and washed with 10% methanol in diethyl ether to yield, on drying, 591 mg (79%), m.p. 188—189 °C, $[\alpha]_D^{20}$ +10.6° (*c* 0.56, H_2O) (Found: C, 30.55; H, 5.75; N, 8.6. $C_4H_9ClFNO_2$ requires C, 30.5; H, 5.7; N, 8.9%); $\nu_{max.}$(Nujol) 1 763 cm^{-2} (CO_2Me); δ (^2H_2O, corrected for $SiMe_4$ at 0 p.p.m.) 5.63 (1 H, dm, J_{XF} 44 Hz, 2-CH), 3.75 (3 H, s, OCH_3), and 3.49 (2 H, m, 3-CH_2).

Methyl (2R,3S)-[3-$^2H_1$]-3-*Amino*-2-*fluoropropanoate Hydrochloride* (**19**; R = Me, H_B = ^2H).—Methyl (2*R*,3*S*)-[3-$^2H_1$]-3-(*N*,*N*-dibenzylamino)-2-fluoropropanoate (**18**; R = Me, H_B = ^2H) (45 mg, 0.15 mmol) was debenzylated by catalytic hydrogenolysis as described for the unlabelled compound. The product (19 mg) was dissolved in dry methanol (0.5 ml) and treated at −10 °C with thionyl chloride (2 drops), as described above, to yield after crystallization (by the addition of diethyl ether) the hydrochloride (14.7 mg), m.p. 187—189 °C, $\nu_{max.}$(Nujol) 1 762 cm^{-1} (CO_2Me).

Methyl (2R,3R)-[2,3-$^2H_2$]-3-*Amino*-2-*fluoropropanoate Hydrochloride* (**19**; R = Me, H_A = ^2H).—This compound was prepared as described above on the same scale using methyl (2*R*,3*R*)-[2,3-$^2H_2$]-3-(*N*,*N*-dibenzylamino)-2-fluoropropanoate (**18**; R = Me, H_A = ^2H); product yield 13.8 mg, m.p. 188—190 °C, $\nu_{max.}$(Nujol) 1 762 cm^{-1} (CO_2Me).

Methyl (2R)-3-*Camphanoylamino*-2-*fluoropropanoate* (**20**; R = Me).—Methyl (2*R*)-3-amino-2-fluoropropanoate hydrochloride (**19**; R = Me) (35.8 mg, 0.23 mmol) and (−)-

camphanoyl chloride (80 mg, 0.37 mmol) were dissolved in dry pyridine (2 ml) at 0 °C. The solution was allowed to warm to room temperature and after 2 h, a small piece of ice was added. The solution was poured into water (10 ml) and the solvents were removed under reduced pressure. The residue was dissolved in chloroform (10 ml) and the solution washed with 1M-aqueous HCl (2 ml), saturated aqueous Na_2CO_3 (1 ml), and water (1 ml). The organic phase was dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield a colourless glass which crystallized from chloroform–light petroleum (b.p. 40—60 °C) (48 mg, 70%), m.p. 91—3 °C, $[\alpha]_D^{26}$ −27.04° (*c* 0.81, $CHCl_3$) (Found: C, 55.7; H, 7.0; N, 4.0. $C_{14}H_{20}FNO_5$ requires C, 55.8; H, 6.7; N, 4.65%); *m/z* (EI) 301 (M^+) and 255 [$(M - HCO_2H)^+$]; $\nu_{max.}$(Nujol) 3 360 (NH) and 1 780 and 1 744 cm^{-1} (lactone, ester, and amide); δ(^{1}H) (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m., see Figure 3a), 6.76 (1 H, br t, NH), 5.05 (1 H, ddd, J_{XF} 48.3 Hz, J_{AX} 6.3 Hz, J_{BX} 3.7 Hz, 2-H_X), 3.92 (1 H, m, 18 lines, 3-H_B), 3.79 (3 H, s, OCH_3), 3.67 (1 H, m, 3H_A), 2.47, 1.92, and 1.66 (1 H, 2 H, and 1 H respectively, 3 × m, camphanoyl CH_2) and 1.09, 1.08, and 0.89 (total 9 H, 3 × s, camphanoyl methyls); δ(^{19}F) (C^2HCl_3, upfield $CFCl_3$ at 0 p.p.m.) 189.31 (1 F, dt, *J* 48.3 Hz, and 22.5 Hz, 2-CF).

Methyl (2R,3S)-[3-$^2H_1$]-3-*Camphanoylamino-2-fluoropropanoate* (**20**; R = Me, H_B = ^{2}H).—This compound was prepared as above using methyl (2*R*,3*S*)-[3-$^2H_1$]-3-amino-2-fluoropropanoate hydrochloride (**19**; R = Me, H_B = ^{2}H) (8 mg, 0.05 mmol) and recrystallized from light petroleum (b.p. 40—60 °C)–chloroform; yield 10.5 mg (69%). The sample was further recrystallized; yield 7.3 mg (48%), m.p. 89—92 °C. The ^{1}H n.m.r. spectrum (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m.) was similar to that of the unlabelled compound except that the signal at δ 5.05 p.p.m. (3-H_R) had collapsed (dd) and the signal at δ 3.92 p.p.m. was absent; δ(^{2}H) ($CHCl_3$, C^2HCl_3 at 7.24 p.p.m; broad band proton decoupled using Gaussian multiplication line-narrowing, Figure 3b) 3.92 (1^2H, s, 3-^2H_S); *m/z* (EI) 303 [$(M + H)^+$], 302 (M^+), and 256 [$(M - HCO_2H)^+$].

Methyl (2R,3R)-[2,3-$^2H_2$]-3-*Camphanoylamino-2-fluoropropanoate* (**20**; R=Me, H_A = ^{2}H).—This compound was prepared as above on the same scale using methyl (2*R*,3*R*)-[2,3-$^2H_2$]-3-amino-2-fluoropropanoate hydrochloride (**19**; R=Me, H_A = ^{2}H) (8 mg, 0.05 mmol); yield, two recrystallizations, 7.9 mg (52%), m.p. 90—92 °C. The ^{1}H n.m.r. spectrum (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m.) showed the absence of signals at δ 5.05 and 3.67 p.p.m. when compared with the unlabelled compound; δ(^{2}H) ($CHCl_3$, C^2HCl_3 at 7.24 p.p.m., broad band proton decoupled using Gaussian multiplication line-narrowing, Figure 3c) 4.96 (1^2H, d, *J* 7.5 Hz, 2-C^2H) and 3.66 (1^2H, s, 3-^2H_R); *m/z* (EI) 304 ([$M + H$]$^+$), 303 (M^+) and 257 ([$M - HCO_2H$]$^+$).

[6-^{2}H]-5-*Fluorouracil*.—5-Fluorouracil (262 mg, 2.01 mmol) was dissolved in 98% deuteriated 0.5M-NaO^2H in ^2H_2O (10 ml). The solution was heated to 60 °C and monitored by ^{1}H n.m.r. spectroscopy. After 12 h the solution was neutralized with 12M-HCl and the product was allowed to crystallize; yield 231 mg (88%), m.p. 279—282 °C, $\lambda_{max.}$ (H_2O, pH 7) 268 nm (unlabelled material, $\lambda_{max.}$ 268 nm under same conditions); *m/z* (EI) 133 (M^+) [calculated using unlabelled material; *m/z* (EI) 130 (M^+), the compound was found to contain *ca.* 3.8% $^2H_0$, 23.4% $^2H_1$, 45% $^2H_2$ and 27.7% $^2H_3$].

Catabolism of 5-*Fluorouracil in* H_2O.—The incubation mixture contained 5-fluorouracil (58 mg, 0.45 mmol), glucose-6-phosphate (125 mg, 0.44 mmol), glucose-6-phosphate dehydrogenase (1 unit, 16.7 nKat), NADPH (10 mg, 0.013 mmol), and our bovine liver enzyme system[5] (12 ml, containing dihydrothymine dehydrogenase (50 units, 14.3 nKat)) in a total volume of 60 ml of a buffer containing potassium phosphate (pH 7.4; 35 mM), mercaptoethanol (5 mM), and $MgCl_2 \cdot 6H_2O$ (2.5 mM). The reaction was incubated at 37 °C for 70 h. The protein was denatured at 95 °C for 2 min and ethanol (10 ml) was added. The precipitated protein was removed by centrifugation at 15 000 g for 20 min. The supernatant fluid was treated with n-propanol (1 ml) to prevent frothing and the volume of the solution was reduced to *ca.* 5 ml. Ethanol (5 ml) was added and the insoluble material was removed by centrifugation. The supernatant solution was applied to three 20 × 20 cm × 1 mm preparative cellulose plates and developed using Pr^iOH–NH_3 (*d*, 0.880)–H_2O (25:6:5). The product was recrystallized from aqueous acetone; yield 4.8 mg, m.p. 249 °C (decomp.), $[\alpha]_D^{22}$ +27.8° (*c* 0.2742, H_2O). The ^{1}H n.m.r. spectrum (^2H_2O, HO^2H at 4.6 p.p.m.) was identical with that of the synthetic material.

A small sample (1.5 mg) was converted into its *N*-(−)-camphanoyl derivative as described previously and this had spectral data identical with those of the compound derived from synthetic 3-amino-2-fluoropropanoic acid.

Catabolism of [6-^{2}H]-5-*Fluorouracil in* H_2O.—The incubation contained [6-^{2}H]-5-fluorouracil (59 mg, 0.45 mmol), NADPH (10 mg, 0.013 mmol), glucose-6-phosphate (125 mg, 0.44 mmol), glucose-6-phosphate dehydrogenase (5 units, 83.5 nKat), and our freshly isolated bovine liver enzymes[5] [12 ml, containing dihydrothymine dehydrogenase (50 units, 14.3 nKat)] in a total volume of 60 ml of a buffer containing potassium phosphate (35 mM, pH 7.4), mercaptoethanol (5 mM), and $MgCl_2 \cdot 6H_2O$ (2.5 mM). After 40 h at 37 °C the protein was denatured and the amino acid isolated as described above to yield a solid (4.95 mg, 10%); this, after recrystallization from aqueous acetone, had m.p. 250 °C (decomp.). This compound (4 mg) was converted into the methyl ester hydrochloride (4.2 mg) as described above, m.p. 186—188 °C. Spectral data were consistent with the expected structure. The methyl ester hydrochloride (3 mg) was converted into the camphanate (4.68 mg) as described above, δ(^{2}H) ($CHCl_3$, C^2HCl_3 at 7.24 p.p.m., Figure 3e) 3.67 (1^2H, s, 3-^2H_R) using broad band proton decoupling and Gaussian multiplication line-narrowing; *m/z* (EI) 302 (M^+) and 256 ([$M - HCO_2H$]$^+$).

Catabolism of 5-*Fluorouracil in* ^2H_2O.—The incubation contained 5-fluorouracil (58 mg, 0.45 mmol), NADPH (10 mg, 0.013 mmol), glucose-6-phosphate (125 mg, 0.44 mmol), glucose-6-phosphate dehydrogenase (3 units, 50 nKat), and our ^2H_2O–liver enzyme system[5] [18 ml, containing 75 units (21.4 nKat) of dihydrothymine dehydrogenase] in a total volume of 68 ml ^2H_2O–buffer containing potassium phosphate (35 mM, pH 7.4), mercaptoethanol (5 mM), and $MgCl_2 \cdot 6H_2O$ (2.5 mM). After 104 h at 37 °C, the protein was denatured and the amino acid was isolated in the usual way (see above) as white needles from aqueous acetone (5.3 mg), m.p. 252—253 °C (decomp.). This compound (4 mg) was converted into the methyl ester hydrochloride (4.4 mg) by the action of thionyl chloride in dry methanol as described above, m.p. 188—189 °C. Spectral data were consistent with the expected structure. The methyl ester hydrochloride (3 mg) was converted into the camphanamide (4.24 mg) as described above. The ^{1}H n.m.r. spectrum (C^2HCl_3, $CHCl_3$ at 7.24 p.p.m.) showed great diminution of the 2-H signal at δ 5.05 p.p.m. and of 3-H_S at δ 3.92 p.p.m.; δ(^{2}H) ($CHCl_3$, C^2HCl_3 at 7.24 p.p.m., with broad band proton decoupling and Gaussian multiplication line-narrowing, Figure 3d) 4.95 (1 ^{2}H, d, *J* 7.5 Hz, 2-^{2}H) and 3.88 (1 ^{2}H, s, 3-^2H_S); *m/z* (EI) 303 (M^+) and 257 [$(M - HCO_2H)^+$].

X-*Ray Structure Analysis of the Camphanamide* (**20**; R = Me).—Crystal data. $C_{14}H_{20}FNO_5$, *M* = 301.3, orthorhombic,

Table 1. Fractional atomic co-ordinates ($\times 10^4$) with estimated standard deviations in parentheses

	x	*y*	*z*
O(1)	−5 189(9)	−8 313(7)	−4 506(3)
O(2)	−4 021(13)	−6 144(8)	−4 530(3)
O(3)	−5 977(12)	−8 611(6)	−2 274(3)
O(4)	−7 200(10)	−5 289(6)	−1 643(3)
O(5)	−9 076(14)	−3 720(7)	−1 250(4)
N	−5 382(11)	−6 400(7)	−2 581(3)
F	−6 648(11)	−5 490(8)	−3 711(4)
C(1)	−4 902(14)	−7 045(12)	−4 268(4)
C(2)	−5 828(15)	−6 791(13)	−3 664(5)
C(3)	−4 505(13)	−6 772(9)	−3 147(4)
C(4)	−4 368(18)	−8 554(12)	−5 075(4)
C(5)	−6 112(13)	−7 362(8)	−2 209(4)
C(6)	−7 166(13)	−6 866(8)	−1 636(4)
C(7)	−8 704(17)	−4 931(10)	−1 334(5)
C(8)	−9 547(13)	−6 278(9)	−1 080(4)
C(9)	−8 185(16)	−6 827(13)	−622(5)
C(10)	−6 520(15)	−7 248(12)	−1 007(4)
C(11)	−9 217(13)	−7 225(9)	−1 646(4)
C(12)	−10 192(18)	−6 695(15)	−2 241(4)
C(13)	−9 662(18)	−8 754(11)	−1 538(5)
C(14)	−11 518(15)	−6 082(12)	−851(5)

a = 7.375(1), b = 9.522(2), c = 21.793(2) Å, U = 1 530.4 Å^3, Z = 4, D_c = 1.31 g cm^{-3}, $F(000)$ = 640. Monochromated Cu-K_α radiation, λ = 1.5418 Å, μ = 9.2 cm^{-1}. Space group $P2_12_12_1$ from systematic absences of $h00$ for h odd, $0k0$ for k odd, and $00l$ for l odd.

Data were measured on an Enraf-Nonius CAD4 diffractometer using a crystal of size *ca.* 0.5 × 0.2 × 0.1 mm. Preliminary cell dimensions were found using the SEARCH and INDEX routines of the CAD4 and final values were calculated from the setting angles for 25 reflections with $0 \approx 20°$. Intensities for hkl reflections with $2 < \theta < 70°$ were measured by a $\theta/2\theta$ scan with a scan width of $\Delta\theta = (1.0 + 0.35 \tan\theta)°$. The scan rate for each reflection was determined by a rapid prescan at 10° min^{-1} in θ at which point any reflection with $I < \sigma(I)$ was coded as unobserved. The remaining reflections were rescanned at such speed as to give a minimum value of $\sigma(I)/I$ of 0.05 subject to a maximum scan time of 120 s. Two standard reflections monitored every hour showed no significant variation. Data were corrected for Lp effects but not for absorption and after averaging any equivalent reflections 1 132 reflections with $|F^2| > \sigma(F^2)$ were used in the structure refinement. The values of $\sigma(F^2)$ were taken as $[\sigma^2(I) + (0.02I)^2]^{\frac{1}{2}}/Lp$.

The structure was solved by direct methods using the MULTAN[24] program. Refinement of non-hydrogen atoms with anisotropic temperature factors was by full matrix least-squares. The absolute configuration was taken as that giving the known configuration for the camphanoyl group. No attempt was made to include hydrogen atoms in the model. Refinement converged at R = 0.101, R' = 0.129, when the maximum shift-error was 0.02 and the weighting scheme was $w = 1/\sigma^2(F)$. A final difference map was everywhere featureless.

The structure solution and refinement was done on a PDP 11/34 computer using the Enraf-Nonius structure determination package. Scattering factors for neutral atoms were taken from ref. 25. Final atom co-ordinates are listed in Table 1 and bond lengths and angles in Table 2. Temperature factors are available as a Supplementary Publication No. [SUP.56296 (3 pp.)]* and the structure factors may be obtained on request from the editorial office.

Table 2. Intramolecular distances (Å) and Angles (°) with estimated standard deviations in parentheses

(a) Bonds			
O(1)–C(1)	1.331(6)	O(1)–C(4)	1.399(5)
O(2)–C(1)	1.218(6)	O(3)–C(5)	1.202(4)
O(4)–C(6)	1.502(4)	O(4)–C(7)	1.342(6)
O(5)–C(7)	1.200(5)	N–C(3)	1.438(5)
N–C(5)	1.336(5)	F–C(2)	1.382(6)
C(1)–C(2)	1.502(7)	C(2)–C(3)	1.491(7)
C(5)–C(6)	1.545(6)	C(6)–C(10)	1.496(6)
C(6)–C(11)	1.551(6)	C(7)–C(8)	1.529(6)
C(8)–C(9)	1.509(6)	C(8)–C(11)	1.549(6)
C(8)–C(14)	1.547(7)	C(9)–C(10)	1.541(7)
C(11)–C(12)	1.565(6)	C(11)–C(13)	1.510(6)
(b) Angles			
C(1)–O(1)–C(4)	115.1(4)	C(6)–O(4)–C(7)	105.2(3)
C(3)–N–C(5)	122.2(3)	O(1)–C(1)–O(2)	122.8(5)
O(1)–C(1)–C(2)	114.5(5)	O(2)–C(1)–C(2)	122.7(5)
F–C(2)–C(1)	106.1(5)	F–C(2)–C(3)	109.4(4)
C(1)–C(2)–C(3)	111.5(4)	N–C(3)–C(2)	110.9(4)
O(3)–C(5)–N	125.0(4)	O(3)–C(5)–C(6)	116.1(4)
N–C(5)–C(6)	118.9(3)	O(4)–C(6)–C(5)	107.8(3)
O(4)–C(6)–C(10)	105.0(4)	O(4)–C(6)–C(11)	101.8(3)
C(5)–C(6)–C(10)	120.4(4)	C(5)–C(6)–C(11)	114.3(4)
C(10)–C(6)–C(11)	105.7(4)	O(4)–C(7)–O(5)	120.6(5)
O(4)–C(7)–C(8)	107.8(4)	O(5)–C(7)–C(8)	131.2(5)
C(7)–C(8)–C(9)	105.1(4)	C(7)–C(8)–C(11)	97.8(3)
C(7)–C(8)–C(14)	113.4(4)	C(9)–C(8)–C(11)	102.7(4)
C(9)–C(8)–C(14)	117.0(4)	C(11)–C(8)–C(14)	118.3(4)
C(8)–C(9)–C(10)	105.1(3)	C(6)–C(10)–C(9)	100.4(4)
C(6)–C(11)–C(8)	90.7(3)	C(6)–C(11)–C(12)	112.9(4)
C(6)–C(11)–C(13)	115.0(4)	C(8)–C(11)–C(12)	113.6(4)
C(8)–C(11)–C(13)	113.8(3)	C(12)–C(11)–C(13)	109.9(4)

Acknowledgements

We thank Mrs. G. Olney for microanalyses, Mr. A. Greenway for mass spectra, Mr. K. Baker (A.R.C. Unit of Nitrogen Fixation) for growing *C. tetanomorphum*, Mrs. V. Bertram for preparing labelled samples of L-serine and Mr. C. W. Brown, Courtin and Warner Ltd., Lewes, for a gift of thymidine. We thank the S.E.R.C. for financial assistance and for a studentship to one of us (D. G.).

References

1 Preliminary communication, D. Gani, P. B. Hitchcock, and D. W. Young, *J. Chem. Soc., Chem. Commun.*, 1983, 898.
2 D. W. Young in 'Chemistry and Biology of Pteridines,' ed. J. A. Blair, Walter de Gruyter, Berlin, 1983, 321.
3 P. A. Charlton, D. W. Young, B. Birdsall, J. Feeney, and G. C. K. Roberts, *J. Chem. Soc., Chem. Commun.*, 1979, 922; *ibid., J. Chem. Soc., Perkin Trans. 1*, 1985, 1349.
4 T. Shiotani and G. Weber, *J. Biol. Chem.*, 1981, **256**, 219.
5 D. Gani and D. W. Young, *J. Chem. Soc., Chem. Commun.*, 1983, 576; preceding paper.
6 K. Balenovic and N. Bregant, *Tetrahedron*, 1959, **5**, 44.
7 Y. Kakimoto, A. Kanazawa, and I. Sano, *Biochim. Biophys. Acta*, 1965, **97**, 376, ref. 4.
8 M. D. Armstrong, K. Yates, Y. Kakimoto, K. Taniguchi, and T. Kappe, *J. Biol. Chem.*, 1963, **238**, 1447.
9 M. Sprecher and D. B. Sprinson, *J. Biol. Chem.*, 1966, **241**, 868.

* For details of the Supplementary publications scheme, see Introductions for Authors (1985), *J. Chem. Soc., Perkin Trans. 1*, 1985, Issue 1.

10 S. J. Field and D. W. Young, *J. Chem. Soc., Perkin Trans. 1*, 1983, 2387; *ibid.*, unpublished work.
11 Made by an adaptation of the method of J. A. Rabi and J. J. Fox, *J. Am. Chem. Soc.*, 1973, **95**, 1628.
12 Y. Kondo and B. Witkop, *J. Am. Chem. Soc.*, 1968, **90**, 764.
13 V. Rachina and I. Blagoeva, *Synthesis*, 1982, 967 ref. 12.
14 L. Somekh and A. Shanzer, *J. Am. Chem. Soc.*, 1982, **104**, 5836.
15 See H. Gerlach, *Helv. Chim. Acta*, 1968, **51**, 1587, footnotes 3 and 4 and references therein.
16 K. Freudenberg and W. Lwowski, *Liebigs Ann. Chim.*, 1955, **594**, 76.
17 D. Gani and D. W. Young, *J. Chem. Soc., Chem. Commun.*, 1982, 867; *ibid., J. Chem. Soc., Perkin Trans. 1*, 1983, 2393.
18 The American Type Culture Collection Catalogue of Strains I, 14th edn., 1980, 64 (strain 15920).
19 H. A. Barker, R. D. Smyth, R. M. Wilson, and H. Weissbach, *J. Biol. Chem.*, 1959, **234**, 320.
20 H. A. Barker, R. D. Smyth, E. J. Wawszkiewicz, M. N. Lee, and R. M. Wilson, *Arch. Biochem. Biophys.*, 1958, **78**, 468.
21 Y. Kakimoto and M. D. Armstrong, *J. Biol. Chem.*, 1961, **236**, 3283.
22 L. Velluz, G. Amiard, and R. Heymes, *Bull. Soc. Chim. France*, 1955, 201.
23 K. L. Mukherjee and C. Heidelberger, *J. Biol Chem.*, 1960, **235**, 433.
24 G. Germain, P. Main, and M. M. Woolfson, *Acta Crystallogr., Sect. A*, 1971, **27**, 360.
25 International Tables for *X*-Ray Crystallography, vol. 4, Kynock Press, Birmingham, 1974.

Received 1*st October* 1984; *Paper* 4/1679