The Synthesis of N(τ)-(2-Hydroxypropyl)histidine, N(τ)-(2-Hydroxyethyl)histidine, and Their Deuteriated Analogues

John B. Campbell †
M.R.C.Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF

The synthesis of N(τ)-(2-hydroxypropyl)histidine (1), N(τ)-(2-hydroxy[$^2H_5$]propyl)histidine (2), N(τ)-(2-hydroxyethyl)histidine (3), and N(τ)-(2-hydroxy[$^2H_4$]ethyl)histidine (4) by reaction of protected histidine derivatives with bromoacetone, bromo[$^2H_5$]acetone, 2-benzyloxyethyl toluene-*p*-sulphonate (17), and 2-benzyloxy[1,1,2,2-$^2H_4$]ethyl toluene-*p*-sulphonate (18), respectively, is described.

It has been shown [1-4] that when humans or animals are exposed to alkylating agents haemoglobin is partially alkylated and that the amount of alkylation is directly related to the degree of exposure. Quantitative measurement of the extent of haemoglobin alkylation provides a means of determining total exposure to an alkylating agent, and early work has shown a linear relationship between *S*-methylcysteine levels in haemoglobin and dosage of methylating agents to rats.[1,2,5] Further work [6,7] on the alkylation of haemoglobin histidine by the important industrial chemicals ethylene oxide and propylene oxide required the synthesis of the alkylated histidines and their deuteriated analogues for use as internal standards for gas chromatography–mass spectrometry (GC-MS) quantification.

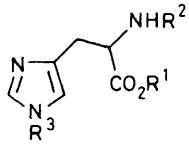
It was decided initially to concentrate attention on the 1,4- or N(τ)-isomers (1) and (3) as, for steric reasons, these are most likely to be the major isomers produced by the alkylation reaction.[8-10] Similarly, of the two positional isomers possible by nucleophilic attack at propylene oxide at either the terminal carbon or the secondary carbon, the former is likely to predominate,[11] and the latter may be disregarded.

The N(τ)- and N(π)-isomers of 2-hydroxyethylhistidine have been synthesised by Calleman *et al.*[12] by a lengthy and tedious procedure in modest yields. The method was unsuitable for the synthesis of a deuteriated hydroxyethylhistidine as ethylene oxide is used in a 25 molar excess.

Jones and co-workers [10] have used the reaction of N(α)-benzyloxycarbonyl-L-histidine methyl ester silver salt (5) with phenacyl bromide in dimethyl sulphoxide solution to obtain almost exclusively the N(τ)-phenacyl derivative. The reaction with bromoacetone was tried and, as expected, the N(τ)-(2-oxopropyl)histidine derivative (6) was obtained, in 44% yield after chromatography. The amino acid (1) was readily produced from this by sodium borohydride reduction followed by deprotection. The acid was a mixture of the expected diastereoisomers.

When the reaction was repeated using bromo[$^2H_5$]acetone, the N(τ)-(2-oxopropyl)histidine derivative was shown by n.m.r. to be a mixture of $^2H_5$—$^2H_1$ substituted compounds. This isotopic exchange had occurred despite the rigorous exclusion of moisture from the reaction mixture during the reaction. It was hoped that the deuterium atoms lost so easily might have been replaced by acid- or base-catalysed exchange in methan[^2H]ol solution, but no exchange was observed. The use of dimethyl sulphoxide, damp only with deuterium oxide, as the reaction solvent failed to prevent the deuterium loss.

Jones and Hysert [9] have prepared N-substituted histidine derivatives by the reaction of N(α)-benzoyl-L-histidine methyl ester in methanol solution over sodium hydrogen carbonate with reactive electrophiles such as phenacyl bromide and allyl bromide. Mixtures were obtained of both mono-substituted derivatives and the disubstituted derivative. The reaction was repeated with bromoacetone as electrophile; as expected, three products were obtained and the major product, of higher R_F, was tentatively assigned as the N(τ)-oxopropyl derivative (8). When bromo[$^2H_5$]acetone was used with methan[^2H]ol as solvent, it was apparent from the n.m.r. spectrum that no loss of deuterium atoms in the products had occurred. Mass spectrometry indicated the presence of the $^2H_5$, $^2H_4$, $^2H_3$, $^2H_2$, and $^2H_1$ species, presumably due to exchange at the source, as sodium borohydride reduction of the reaction mixture gave after work-up chiefly two products, and mass spectrometry of the main product showed *ca.* 90% N(α)-benzoyl-N(τ)-(2-hydroxy[$^2H_5$]propyl)histidine methyl ester (11) with only 10% of the [$^2H_4$]-species. Separation of (11) from the mixture by chromatography followed by acid hydrolysis gave the deuteriated amino acid (2) in 20% overall yield.

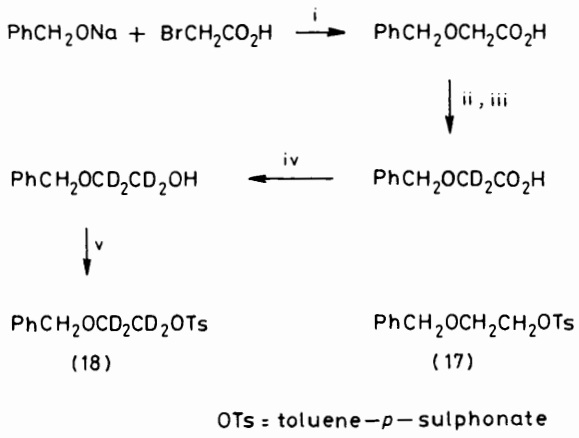


	R^1	R^2	R^3
(1)	H	H	$CH_2CH(OH)Me$
(2)	H	H	$CD_2CH(OH)CD_3$
(3)	H	H	CH_2CH_2OH
(4)	H	H	CD_2CD_2CH
(5)	Me	$PhCH_2OCO$	Ag
(6)	Me	$PhCH_2OCO$	CH_2COMe
(7)	Me	$PhCH_2OCO$	$CH_2CH(OH)Me$
(8)	Me	PhCO	CH_2COMe
(9)	Me	PhCO	$CH_2CH(OH)Me$
(10)	Me	PhCO	CD_2COCD_3
(11)	Me	PhCO	$CD_2CH(OH)CD_3$
(12)	Me	PhCO	CH_2CH_2OCOMe
(13)	Me	PhCO	$CH_2CH_2OCH_2Ph$
(14)	Me	PhCO	$CD_2CD_2OCH_2Ph$
(15)	Me	PhCO	$CH_2CH{=}CH_2$
(16)	Me	PhCO	CH_2CHO

Neither of these reaction schemes was suitable for the preparation of N(τ)-(2-hydroxyethyl)histidine as bromoacetaldehyde proved too unstable to the reaction conditions and iodoethyl acetate too unreactive. The aldehyde (16), obtained from the allyl derivative (15), was also too unstable to be isolated.

Johnstone and Rose have shown [13] that peptides may be

† *Present address:* Amersham International plc, Forest Farm, Whitchurch, Cardiff.



Scheme. *Reagents:* i, heat; ii, $NaOD-D_2O$; iii, H^+; iv, $LiAlD_4$; v, TsCl, C_5H_5N

rapidly permethylated with methyl iodide in dimethyl sulphoxide solution over solid sodium hydroxide and it is apparent that the imidazole ring in histidine is methylated under these conditions.[13]

Benzoylhistidine methyl ester proved to be unstable in dimethyl sulphoxide solution in the presence of sodium hydroxide, but when one equivalent of solid sodium methoxide (solubility in dimethyl sulphoxide *ca.* 1.5 mg ml^{-1}) was used in place of sodium hydroxide, reaction with iodoethyl acetate occurred in 1 h at ambient temperature to give almost exclusively the *N*(τ)-acetoxyethylhistidine derivative (12). This was converted into the amino acid (3) by acid hydrolysis. The acid (3) obtained was identical with that of Calleman *et al.*[12] The overall yield was *ca.* 40%, and in a similar reaction with 2-benzyloxyethyltoluene-*p*-sulphonate, some unchanged tosylate was found after work-up.

Synthesis of the deuteriated analogue (4) was achieved by reaction of benzoylhistidine methyl ester with compound (18) under similar conditions for 1.5 h. The synthesis of (18) is outlined in the Scheme. The main product, the *N*(τ)-benzyloxyethylhistidine derivative (14), was isolated chromatographically, and was readily deprotected to give the amino acid (4), which was shown by n.m.r. and mass spectrometry to have 98% [$^2H_4$]-species, with no detectable non-deuteriated compound. Application of the method to synthesise the acetone (8) was also successful.

Experimental

M.p.s were determined on a Mettler FP5 hot-stage apparatus. N.m.r. spectra were recorded on a Perkin-Elmer R12B 60 MHz spectrometer with tetramethylsilane as internal standard, in deuteriochloroform solution except where indicated. I.r. spectra were recorded on a Perkin-Elmer 457 spectrometer in chloroform solution. Microanalyses were determined by Elemental Microanalysis Ltd., Beaworthy, Devon. Mass spectra were determined using a VG Micromass 70/70 high resolution mass spectrometer. All chromatography was performed using Merck silica gel. Solvents were dried over sodium sulphate, and were removed under reduced pressure.

Bromoacetone.—This was synthesised by the published procedure[14] in 20% yield and was stored at −15 °C in the dark.

Bromo[$^2H_5$]*acetone.*—This was synthesised by a published procedure[15] for bromoacetone except that [$^2H_6$]acetone (99.5%; Aldrich Chemical Co.) was used instead of acetone; b.p. 41—46 °C at 14 Torr. Redistillation through a helices column gave the fraction b.p. 40—42 °C at 14 Torr (4.2 g, 18%). No ^1H n.m.r. spectrum, *m/z* 143, 141 ($M^{+\cdot}$, 11%), 95, 97 ($M^+ - COCD_3^{\cdot}$, 11), and 46 ($COCD_3^+$, 100).

N(α)-*Benzyloxycarbonyl*-L-*histidine Methyl Ester.*—This was prepared by the method of Holle and Sondheimer[16] in 60% yield, but was not obtained in a crystalline form.[10] The silver salt (5) was prepared from this by the method of Seltzman[17] as a white solid in 90% yield.

N(α)-*Benzoyloxycarbonyl*-N(τ)-(2-*oxopropyl*)-L-*histidine Methyl Ester* (6).—The silver salt (1.52 g, 3.7 mmol) was dissolved in dry dimethyl sulphoxide (20 ml) and bromoacetone (600 mg, 4.25 mmol) in chloroform (4 ml) was added in the dark to the stirred solution during 40 min. 20 Min later the mixture was diluted with chloroform (40 ml) and the suspension removed by centrifugation. The supernatant was washed with water (5 × 20 ml) and was dried. The dry solution was clarified by further centrifugation and was evaporated under reduced pressure to an oil (1.2 g). Column chromatography (Merck 7734; 50 g; eluant 3% methanol in dichloromethane) gave the pure ester, assigned as the N(α)-*ester* (6) (590 mg, 44%), as an oil. δ 7.38 (6 H, s, Ph + 2-H of imidazole), 6.68 (1 H, s, 4-H of imidazole), 6.40 (1 H, br d, CONHCH), 5.13 (2 H, s, $PhCH_2O$), 4.67 (2 H, s, CH_2CO), 4.6—4.3 (1 H, m, CH_2CH), 3.68 (3 H, s, CO_2CH), 3.06 (2 H, d, CH_2CH), and 2.10 (3 H, s, $COCH_3$); $\nu_{max.}$ 3 430, 1 720br, and 1 500 cm^{-1}; *m/z* 359 ($M^{+\cdot}$, 4.5%), 300 [$(M - CO_2CH_3)^+$], and 137 [$(CH_3COCH_2$–imidazole=$CH_2)^+$] (Found: M^+, 359.1492. $C_{18}H_{21}N_3O_3$ requires *M*, 359.1481). R_F on t.l.c. 0.5 (15% methanol in dichloromethane). Negative reaction to the Pauly test.

N(α)-*Benzyloxycarbonyl*-N(τ)-(2-*hydroxypropyl*)-L-*histidine Methyl Ester* (7).—The oxopropyl derivative (6) (0.3 g, 1.06 mmol) was dissolved in methanol (20 ml) and an excess of sodium borohydride (200 mg) was added with stirring. After 15 min the methanol was evaporated and the residue was partitioned between dichloromethane (50 ml) and water (50 ml). The organic phase was separated and the aqueous phase was extracted with more dichloromethane (2 × 30 ml). The organic extracts were combined, and the solution was dried and evaporated to give the *hydroxypropyl derivative* (7) as an oil (250 ml, 85%), virtually pure by t.l.c. and n.m.r. analysis. Preparative layer chromatography of this gave the pure compound: δ 7.42 (6 H, s, Ph + 2-H of imidazole), 6.78 (1 H, s, 4-H of imidazole), 6.33 (1 H, br d, NH), 5.18 (2 H, s, $PhCH_2O$), 4.68 (1 H, m, CH_2CH), 3.85 (2 H, d, CH_2CHOH), 3.78 (3 H, s, CO_2CH_3), 4.1—3.6 (1 H, m, CH_2CHOH), 3.10 (2 H, d, CH_2CH), and 1.26 (3 H, d, $CHOHCH_3$); $\nu_{max.}$ 3 610, 3 430, and 1 720 cm^{-1}; *m/z* 361 ($M^{+\cdot}$, 5%), 302 [$(M - CO_2CH_2)^+$, 15], 210 [$(M - C_6H_5CO_2NH_2)^+$, 38], and 139 [$(CH_3CHOHCH_2$–imidazole=$CH_2)^+$, 100] (Found: M^+ 361.1642. $C_{18}H_{23}N_3O_5$ requires *M*, 361.1641).

N(τ)-(2-*Hydroxypropyl*)-L-*histidine* (1).—The hydroxypropyl derivative (7) (600 mg, 1.67 mmol) was dissolved in acetic acid (10 ml), and cyclohexa-1,4-diene (2 ml) and 10% palladium-on-charcoal (250 mg) were added. The mixture was stirred under nitrogen for 1 h and, after filtration, the solution was evaporated. The residue was dissolved in 6M-hydrochloric acid (5 ml) and the solution was boiled under reflux for 4 h, after which time t.l.c. analysis (acetic acid–water–butanol 35 : 35 : 30) showed only one spot, R_F 0.2, which gave a brown colour with ninhydrin. Removal of the hydrochloric acid gave an oil, the amino acid dihydrochloride, which was applied to a column of Dowex 50 × 4 cation

exchange resin (10 × 40 mm). The column was washed with water (100 ml) and the amino acid was eluted from the column with 2M-ammonium hydroxide solution (50 ml). Evaporation of the solution gave the crude amino acid (1) as a straw-coloured oil. This was crystallised from hot isopropyl alcohol (10 ml) and ethanol (0.2 ml) to give buff hygroscopic needles, m.p. 185—187 °C (260 mg, 73%). Further crystallisation after treatment with charcoal gave prisms, m.p. 199—201 °C. δ (D_2O) (Me_4Si in $CDCl_3$ as external standard) 7.60 (1 H, s, 2-H of imidazole), 7.02 (1 H, s, 4-H of imidazole), 4.2—3.7 [4 H, m, CH_2CHOH and $CH_2CH(NH_2)CO_2H$], 3.08 and 2.97 (2 H, s + d, CH_2CH), and 1.10 (3 H, d, $CHOHCH_3$). A sample was derivatised for GC-MS analysis. The acid (10 μg) was esterified in 3M-HCl in methanol (200 μl) for 1 h at 80 °C. Methanol was removed under a stream of nitrogen and the residue was heated with heptafluorobutyric anhydride (30 μl) in anhydrous ethyl acetate (100 μl) at 120 °C for 10 min in a sealed tube. The solution was evaporated under a stream of nitrogen, and the residue dissolved in ethyl acetate (200 μl). The solution (1 μl) was injected onto a fused silica column coated with SE52 stationary phase with a 10 : 1 split injector run isothermally at 220 °C with total ion-current monitoring. Two partially resolved peaks of equal height were obtained. Mass spectral analysis of each peak gave identical *m/z* 619 ($M^{+\cdot}$, 2.5%) and 560 [$(M - CO_2CH_3)^+$, 100%]. In later preparations, formic acid instead of cyclohexa-1,4-diene in acetic acid was used in the catalytic transfer hydrogenation step.[18]

N(α)-*Benzoyl*-L-*histidine Methyl Ester*.—To a stirred mixture of histidine methyl ester dihydrochloride (24.2 g, 0.1 mol) and triethylamine (freshly distilled, 27 ml, 0.2 mol) in anhydrous chloroform (250 ml) cooled in an ice-bath was added, in four portions, benzoyl chloride (23.2 ml, 0.2 mol), alternating with the addition, in four portions, of triethylamine (27 ml, 0.2 mol) during 30 min. 30 Min later the mixture was diluted with chloroform (300 ml) and the solution was extracted with water (2 × 100 ml). The dried solution was evaporated to an oily residue (40 g), the dibenzoyl derivative, which was treated with cold methanol and sodium methoxide (5.4 g, 0.1 mol). Acetic acid (4 ml) was added after 10 min and the solution was partitioned between chloroform (300 ml) and water (300 ml). The chloroform layer was washed with water (2 × 100 ml) and the aqueous extracts were back-extracted with chloroform (100 ml). The combined dried chloroform extracts gave, on evaporation, an oil (39 g), which was triturated with hexane (100 ml) twice to remove methyl benzoate, and dissolved in hot methyl acetate. *N*(α)-Benzoylhistidine methyl ester was obtained as prisms (14.4 g), m.p. 148—149 °C (lit.,[9] 159—160 °C). Further product was obtained from the mother-liquors (4.24 g, total yield 68%). A recrystallised sample had m.p. 158—159 °C. The crude product was pure enough by n.m.r. analysis for further reaction.

N(α)-*Benzoyl*-N(τ)-(2-*oxopropyl*)*histidine Methyl Ester* (8).—A solution of *N*(α)-benzoylhistidine methyl ester (0.48 g, 1.59 mmol) and bromoacetone (0.26 g, 1.88 mmol) in methanol (5 ml) was stirred for 17 h over anhydrous sodium carbonate (120 mg, 1.25 mmol). T.l.c. indicated three new compounds. The mixture was partitioned between dichloromethane and water, and the dried dichloromethane layer was evaporated to a foam (450 mg). A portion was separated by preparative layer chromatography (10% methanol in dichloromethane). The mixture (100 mg) yielded the N(τ)-*oxopropyl derivative* as the major product (R_F 0.7) (33 mg, gum, 25%). This crystallised on standing, and was recrystallised from ethyl acetate, m.p. 146—148 °C (Found: C, 62.0; H, 5.8; N, 12.6%; M^+, 329.1377. $C_{17}H_{19}N_3O_4$ requires C, 62.0; H, 5.8; N, 12.8%; *M*, 329.1375), δ 8.20 (1 H, br d, NH), 8.0—7.7 (2 H, dd, *o*-H of Ph), 7.6—7.2 (4 H, m, *m*- and *p*-H of Ph, +2-H of imidazole), 6.75 (1 H, s, 4-H of imidazole), 4.95—4.65 (1 H, m, $CHCH_2$), 4.65 (2 H, s, CH_2CO), 3.62 (3 H, s, CO_2CH_3), 3.02 (2 H, d, CH_2CH), and 2.08 (3 H, s, $COCH_3$); $\nu_{max.}$, 3 425, 3 340, 1 740, and 1 660 cm^{-1}; *m/z* 329 ($M^{+\cdot}$, 4%), 270 [$(M - CO_2CH_3)^+$, 7], 208 [$(M - C_6H_5CONH_2)^+$, 10], and 105 ($C_6H_5CO^+$, 100).

The N(π)-*oxopropyl derivative* was obtained as a minor product (15 mg, gum, R_F 0.4) (Found: M^+ 329.1377. $C_{17}H_{19}N_3O_4$ requires *M*, 329.1375). The other two bands (R_F 0.5 and 0.1) were eluted to give starting material (18 mg, gum) and presumably the dialkyl species (5 mg, gum), respectively.

N(α)-*Benzoyl*-N(τ)-(2-*hydroxypropyl*)*histidine Methyl Ester* (9).—The foregoing reaction was repeated on a 3.5-mmol scale. To the stirred reaction mixture was added sodium borohydride (100 mg, excess) and 10 min later a further addition (40 mg) was made. After 1 h the mixture was partitioned between dichloromethane (50 ml) and water (30 ml). The aqueous layer was extracted further with dichloromethane (30 ml) and the combined organic extracts were dried. Evaporation to an oil (1.02 g) allowed preparative layer chromatography of the two main products and unchanged starting material (15% methanol in dichloromethane). The N(τ)-*hydroxypropyl derivative* (9) was obtained thus as an oil (26 mg from 100 mg total). δ 8.2 (1 H, d, NH), 8.0—7.7 (2 H, dd), 7.6—7.3 (4 H, m), 6.75 (1 H, s), 4.9 (1 H, m), 3.9 (1 H, m, CH_2CHOH), 3.9—3.6 (2 H, d, CH_2CHOH), 3.72 (3 H, s), 3.05 (2 H, d), and 1.05 (3 H, d, $CHOHCH_3$); $\nu_{max.}$ 3 620, 3 435, 3 320, 1 740, and 1 660 cm^{-1}; *m/z* 331 ($M^{+\cdot}$, 12%), 272 [$(M - CO_2CH_3\cdot)^+$, 16], 209 [$(M - C_6H_5CONH_3\cdot)^+$, 14], and 105 ($C_6H_5CO^+$, 100) (Found: M^+, 331.1532. $C_{17}H_{21}N_3O_4$ requires *M*, 333.115 33).

N(α)-*Benzoyl*-N(τ)-(2-*hydroxy*[$^2H_5$]*propyl*)*histidine Methyl Ester* (11).—*N*(α)-Benzoylhistidine methyl ester (0.96 g, 3.5 mmol) was dissolved in methan[^{2}H]ol (Aldrich Chemical Co.; 99.5+% deuterium) (10 ml) and to the stirred solution over sodium carbonate (anhydrous; 250 mg, 2.6 mmol) was added bromo[$^2H_5$]acetone (550 mg, 3.85 mmol). After 18 h sodium borohydride (150 mg) was added and, after work-up, an oil was obtained (0.77 g). After column and preparative layer chromatography the hydroxypropyl derivative (11) was isolated as an oil (200 mg). The ^{1}H n.m.r. spectrum was identical with that of the non-deuteriated compound except that no signals were evident at δ 3.9—3.6 nor at 1.05; *m/z* 337 (4%), 336 ($M^{+\cdot}$, 10.7), 335 {$(M - 1)^{+\cdot}$ [$^2H_4$]}, no ions at 334, 333, 332 or 331.

In one experiment the *N*(π)-isomer was also isolated, *m/z* 336 (M^+). In another experiment the intermediate oxo-[$^2H_5$]propyl derivative (10) was isolated. Some exchange was noticeable in the mass spectrum: *m/z* 335 {([$^2H_5$]-M + 1)$^{+\cdot}$, 0.8%}, 334 ([$^2H_5$]-$M^{+\cdot}$, 1.9), 333 ([$^2H_4$]-$M^{+\cdot}$, 4.1), 332 ([$^2H_3$]-$M^{+\cdot}$, 4.5), 331 ([$^2H_1$]-$M^{+\cdot}$, 2.5), and 330 ([$^2H_1$]-$M^{+\cdot}$, 1.1). This exchange probably occurs at the source, as the ^{1}H n.m.r. spectrum shows no detectable protonation of the $-COCD_3$ group, and up to 50% protonation of the $-CD_2CO-$ group, *i.e.* a predominance of the [$^2H_4$]-compound.

N(τ)-(2-*Hydroxy*[$^2H_5$]*propyl*)*histidine* (2).—The hydroxypropyl derivative (11) (200 mg) was dissolved in 6M-HCl (3 ml) and hydrolysed overnight under reflux. The straw-coloured solution was diluted with water (5 ml) and extracted with methyl acetate (3 × 5 ml). The dried extracts gave on evaporation a solid (*ca.* 50 mg), m.p. 116—120 °C, presumably benzoic acid. The aqueous solution was evaporated to a brown oil (170 mg), which was pure by t.l.c. and which gave a positive ninhydrin reaction. The crude dihydrochloride was converted

into the free amino acid by ion-exchange chromatography as previously described for (1), which was obtained as a foam (100 mg, 20% yield from benzoylhistidine methyl ester). This crystallised with difficulty, from ethanol–isopropyl alcohol to give prisms (m.p. 182—184 °C). The *N*(α),*O*-diheptafluorobutyryl methyl ester derivative was prepared, and showed very similar GC properties to the non-deuteriated analogue, *m/z* 625 ($M^+ + 1$, 6.5%), 624 (M^+, 22), 623 [($^2H_4$)-M^+, 3), no ions at 623, 622, 621, or 620.

Benzyloxyacetic Acid.—This was prepared from bromoacetic acid and sodium benzylate in benzyl alcohol in 60% yield, b.p. 163—165 °C at 1 Torr (lit.,[19] b.p. 136 °C at 0.2 Torr), δ 11.4 (1 H, s), 7.35 (5 H, s), 4.60 (2 H, s, PhCH_2O), and 4.10 (2 H, s, OCH_2CO$_2$H).

2-*Benzyloxyethanol.*—Benzyloxyacetic acid (3.4 g, 20 mmol) was reduced with lithium aluminium hydride (760 mg, 20 mmol) in dry tetrahydrofuran (10 ml) for 1 h. Ether extraction of the mixture after addition of saturated sodium chloride solution gave 2-benzyloxyethanol as an oil (2.9 g, 94%), virtually pure by ^{1}H n.m.r. analysis, δ 7.35 (5 H, s), 4.53 (2 H, s), 3.8—3.4 (4 H, m, CH_2CH_2), and 3.10 (1 H, br s, OH).

2-*Benzyloxyethyl Toluene*-p-*sulphonate* (17).—Toluene-*p*-sulphonyl chloride (1.05 g, 5 mmol) and 2-benzyloxyethanol (0.76 g, 5 mmol) in dry pyridine (5 ml) were stirred at 0 °C for 1 h and at 5 °C for 3 h. The solution was poured into cold 1M-H_2SO_4. The oil formed was separated and the aqueous solution was extracted with dichloromethane (2 × 30 ml). The oil and combined extracts were washed once with sodium hydrogen carbonate solution, dried and evaporated to an oil (1.2 g, 78%), which did not crystallise (lit.,[20] m.p. 45 °C), δ 7.86 (2 H, d, *o*-tosyl H, *J* 9 Hz), 7.33 (2 H, d, *m*-tosyl H, *J* 9 Hz), 7.36 (5 H, s, Ph), 4.50 (2 H, s, PhCH_2O), 4.4—4.1 and 3.8—3.6 (4 H, m, CH_2CH_2), and 2.44 (3 H, s, tosyl-CH_3).

N(α)-*Benzoyl*-N(τ)-(2-*benzyloxyethyl*)*histidine Methyl Ester* (13).—Sodium methoxide (1 mmol, 54 mg) was added to dry dimethyl sulphoxide (8 ml) with stirring, followed after 5 min by 2-benzyloxyethyl toluene-*p*-sulphonate (1 mmol, 306 mg) and 1 min later by benzoylhistidine methyl ester (1 mmol, 273 mg). The mixture was stirred for 1.5 h, acetic acid (1 ml) was added, and the solution was separated between chloroform and water. The chloroform layer was separated, the aqueous layer was extracted with more chloroform, and the combined chloroform layers were washed with water (5 × 50 ml). The dried chloroform solutions were evaporated to an oil (320 mg), a mixture of product and unchanged (17). Column chromatography (2% methanol in dichloromethane) gave the unchanged toluene sulphonate (17) (50 mg) and the pure N(τ)-*benzyloxyethylhistidine derivative* (13), an oil (150 mg, 37%). δ 8.40 (1 H, d, NH), 7.95 (2 H, q, *o*-H), 7.6—7.3 (4 H, m, *m*- and *p*-H + 2-H of imidazole), 7.28 (5 H, s, Ph), 6.80 (1 H, s, 4-H of imidazole), 4.98 (1 H, m, CH), 4.45 (2 H, s, PhCH_2O), 4.2—3.9 and 3.8—3.5 (4 H, m, CH_2CH_2), 3.62 (3 H, s, CO_2CH_3), and 3.15 (2 H, d, CH_2CH); $\nu_{max.}$ 3 440, 3 320, 1 745, 1 660, and 1 520 cm^{-1}; *m/z* 407 ($M^{+\cdot}$, 35%), 348 [(M − ·CO_2CH_3)$^+$, 24], 105 ($C_6H_5CO^+$, 93), and 91 ($C_7H_7^+$, 100) (Found: M^+, 407.1845. $C_{23}H_{25}N_3O_4$ requires M, 407.1845).

N(α)-*Benzoyl*-N(τ)-(2-*acetoxyethyl*)*histidine Methyl Ester* (12).—The foregoing procedure was used on a 6-mmol scale using 2-iodoethyl acetate instead of 2-benzyloxyethyl toluenesulphonate. The crude reaction product was purified by column chromatography (1—5% methanol in dichloromethane) from unchanged iodoethyl acetate, giving an oil (420 mg) (Found: $M^{+\cdot}$, 359.1485. $C_{18}H_{21}N_3O_5$ requires M, 359.1481); δ 8.30 (1 H, d, NH), 8.1—7.85 (2 H, dd), 7.67—7.35 (4 H, m), 6.82 (1 H, s), 5.02 (1 H, sext., CH), 4.5—4.0 (4 H, m, CH_2CH_2), 3.72 (3 H, s), 3.19 (2 H, d), and 2.03 (3 H, s, CH_3CO_2); $\nu_{max.}$ 3 430, 3 330, 1 745, and 1 660 cm^{-1}; *m/z* 359 ($M^{+\cdot}$, 23%), 300 [(M − ·CO_2CH_3)$^+$, 19], 105 ($C_6H_5CO^+$, 93), and 87 [($C_4H_7O_2$)$^+$, 100].

N(τ)-(2-*Hydroxyethyl*)*histidine* (3).—The foregoing 2-acetoxyethylhistidine derivative was hydrolysed in 6M-HCl and the free amino acid was obtained by the method described for hydroxy[$^2H_5$]propylhistidine as a foam (230 mg, 19% yield from benzoylhistidine methyl ester). This was crystallised from ethanol–isopropyl alcohol to give compound (3), buff prisms (80 mg), m.p. 195—197 °C (lit.,[12] m.p. 209—211 °C). An authentic sample of *N*(τ)-(2-hydroxyethyl)histidine (kindly donated by Dr. C. Calleman) had m.p. 195.5—198 °C. A mixed m.p. determination showed no significant depression. The *N*(α),*O*-diheptafluorobutyryl methyl ester derivative of (3) showed only one peak on a capillary gas chromatogram, *m/z* 605 ($M^{+\cdot}$, 18%), 546 [(M − CO_2CH_3)$^+$, 90], and 321 [($C_3F_7CO_2CH_2CH_2$–imidazole=CH_2)$^+$, 100].

Benzyloxy[$^2H_2$]*acetic Acid.*—Sodium hydride (200 mg, 57% dispersion in oil, 45 mmol) was added cautiously to deuterium oxide (10 ml, 99.5% ^{2}H-atom, Ryvan Ltd.) and to the mixture was added benzyloxyacetic acid (1.7 g, 10 mmol). The solution was flushed with nitrogen and heated in an evacuated hydrolysis tube at 115 °C for 46 h. The cooled contents were acidified, the silica gel suspension was removed by centrifugation and the deuteriated acid was extracted with ether (3 × 30 ml). The dried extracts gave an oil (1.3 g), whose n.m.r. spectrum showed *ca.* 75% deuterium incorporation. The exchange reaction was repeated twice more at 115 °C for 24 h, except that the sodium salt (formed from the acid by addition of sodium hydride to a solution in dry tetrahydrofuran) was used in place of the acid. The benzyloxy[$^2H_2$]acetic acid was obtained as an oil (1.1 g, 98.5% ^{2}H-atom % by n.m.r. analysis), *m/z* 168 ($M^{+\cdot}$, 5.4%), 167 [(M − H·)$^+$, 13], 107 ($C_7H_7O^+$, 66), and 91 ($C_7H_7^+$, 100).

2-*Benzyloxy*[1,1,2,2-$^2H_4$]*ethanol.*—Reduction of the acid (1.1 g) obtained above with lithium aluminium deuteride (Aldrich Chemical Co., 98+ % ^{2}H atom) (300 mg) gave the [$^2H_4$]ethanol (0.9 g), 98% [$^2H_4$]-material by n.m.r. analysis, *m/z* 156 ($M^{+\cdot}$, 8.1%), 155 [(M − H·)$^+$, 1.2], 107 ($C_7H_7O^+$, 28), and 91 ($C_7H_7^+$, 100).

2-*Benzyloxy*[1,1,2,2-$^2H_4$]*ethyl Toluene*-p-*sulphonate* (18).—The toluene-*p*-sulphonate of benzyloxy[$^2H_4$]ethanol (3 mmol, 460 mg) was prepared, but found to be impure. Flash column chromatography gave the pure toluenesulphonate (18) as an oil (220 mg), δ 7.90 and 7.75 (2 H, d), 7.35 and 7.15 (2 H, d, tosylate), 7.2 (5 H, s, Ph), 4.35 (2 H, s), and 2.3 (3 H, s).

N(α)-*Benzoyl*-N(τ)-(2-*benzyloxy*[1,1,2,2-$^2H_4$]*ethyl*)*histidine Methyl Ester* (14).—The preparation of the non-deuteriated material (13) was repeated with the deuteriated benzyloxyethyl toluenesulphonate (18) (220 mg, 0.7 mmol). The crude product (260 mg) was purified by column chromatography to yield the deuteriated histidine derivative (14) (110 mg), δ 8.35 (1 H, d), 7.95 (2 H, q), 7.6—7.4 (4 H, m), 7.30 (5 H, s), 6.80 (1 H, s), 5.00 (1 H, m), 4.49 (2 H, s), 3.70 (3 H, s), and 3.17 (2 H, d); *m/z* 411 (411 ($M^{+\cdot}$, 35.), 352 [(M − ·CO_2CH_3)$^+$, 24], 306 [(M − C_6H_5CO·)$^+$, 18], 105 [$C_6H_5CO^+$, 90), and 91 (C_7H_7, 100%).

N(τ)-(2-*Hydroxy*[1,1,2,2-$^2H_4$]*ethyl*)*histidine* (4).—The foregoing compound (14) (100 mg, 0.27 mmol) in formic acid (5 ml) was stirred with 10% palladium-on-charcoal (50 mg) for 2 h. The filtrate was evaporated and hydrolysed in 6M-HCl as previously described, and converted into the amino acid, an oil (70 mg). Crystallisation from methanol–isopropyl alcohol gave a buff microcrystalline powder (35 mg), m.p. 196—198 °C. The *N*(α),*O*-diheptafluorobutyryl methyl ester derivative gave the following mass spectrum: *m/z* 610 [$(M + 1)^{+\cdot}$, 7%], 609 ($M^{+\cdot}$, 23), 608 (1), 550 [$(M - CO_2CH_3)^+$, 100], and 325 [$(C_3H_7COCD_2CD_2$–imidazole=$CH_2]^+$, 95].

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