Nuclear Relaxation and Molecular Properties. Part VIII.[1] Molecular Dynamics of Glycine and Glycylglycine

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A preparation of [$^2H_1$]glycine and [$^2H_1$]glycyl[$^2H_1$]glycine is described. Deuterium nuclear quadrupole relaxation times have been obtained from proton lineshape analysis of the CHD resonances and are used to calculate the correlation times for molecular motions in these compounds for various conditions. The results show that the local dynamic properties of the molecules depend on ionic state and on the presence of internal motions. Different flexibilities are found for the two ends of the diglycine molecule.

NUCLEAR relaxation is being used more and more for studying the microdynamic behaviour of molecules.[2] Applications to biological systems or models especially are becoming more frequent and important, for instance in the study of the binding of substrates or inhibitors to enzymes.[3] These biophysical applications generally make use of dipole–dipole relaxation of protons or fluorine or, more recently, carbon-13 nuclei.

In the case of nuclei with spin $\geqslant 1$ the dominant relaxation mechanism is due to the modulation of the nuclear quadrupole–molecular field gradient interaction by the molecular motions. Such quadrupolar relaxation presents several advantages over dipole–dipole relaxation, especially in the case of the deuterium nucleus (spin $I = 1$). (See detailed discussion in refs. 1 and 4). However, the n.m.r. sensitivity of deuterium being 100 times lower than that of hydrogen, it is often difficult to measure relaxation times from the deuterium resonance.

When a proton is spin–spin coupled to the deuterium to be studied, the lineshape of the proton resonance depends on the deuterium quadrupolar relaxation time T_q. It is then possible to determine the variations of T_q with proton sensitivity by performing a lineshape analysis of the n.m.r. signal of the coupled proton(s). We first developed this method for determining nitrogen-14 relaxation times[5] and we have extended it to the study of molecular motions using deuterium relaxation times.[1,4,6] Application to various systems has been described. It is thus possible to establish a microdynamical map of local molecular motions and to separate these local motions into overall and internal reorientations.[1,6] We describe here a study of molecular motions in [$^2H_1$]glycine (I) in [$^2H_1$]glycine methyl ester (II), in *N*-acetyl[$^2H_1$]glycine methyl ester (III), and in [$^2H_1$]glycyl[$^2H_1$]glycine (IV) using deuterium quadrupolar relaxation times determined by lineshape analysis of the α-proton.

RESULTS

Preparation of the Substrates.—Compound (I) was prepared by reduction of hydroxyiminoacetic acid with sodium amalgam in heavy water (99·8% D_2O). This method

[1] Part VII, Ch. Brévard, J. P. Kintzinger, J. M. Lehn, *Tetrahedron* 1972, **28**, 2447.
[2] H. G. Hertz, *Progr. in NMR Spectroscopy*, 1967, **3**, 159.
[3] 'Magnetic Resonance in Biological Systems,' eds. A. Ehrenberg, B. G. Malmström, and T. Vänngård, Pergamon, Oxford, 1967; 'Molecular Associations in Biology,' ed. B. Pullman, Academic Press, New York, 1968.
[4] Ch. Brévard, J. P. Kintzinger, and J. M. Lehn, *Tetrahedron*, 1972, **28**, 2429.
[5] J. P. Kintzinger, J. M. Lehn, and R. L. Williams, *Mol. Phys.*, 1969, **17**, 135.
[6] Ch. Brévard, J. P. Kintzinger, and J. M. Lehn, *Chem. Comm.*, 1969, 1193.

was found to be the best among several which were tried and afforded a compound containing 87% ^2H, 7% $^2H_2$, and 6% $^2H_0$ according to the mass spectrum. The methyl ester (II) and *N*-acetyl compound (III) were prepared by standard procedures (see Experimental section).

[$^2H_1$]Glycyl[$^2H_1$]glycine (IV) was obtained by rearrangement of the anhydride of benzyloxycarbonyl[$^2H_1$]glycine (Z-glycine) in the presence of triethylamine.[7]

broadening of the resonance lines, which may be included in the non-quadrupolar ' natural ' line-width Δ (see Experimental section) during the process of lineshape analysis.

The attribution of the low and high field resonances to the carboxy- and amino-terminal groups respectively, at pD 11·4, rests on previous literature data.[8] Because of overlap of the signals, only one CHD resonance of compound (IV) could be studied at pD 5·7.

$$CHO\cdot CO_2H + H_2N\cdot OH,HCl \longrightarrow [HON{=}CH\cdot CO_2H] \xrightarrow[D_2O]{Na,Hg} H_2N\cdot CHD\cdot CO_2H \quad (I)$$

$$ZNH\cdot CHD\cdot CO_2H \xrightarrow{i} (ZNH\cdot CHD\cdot CO)_2O$$

$$(ZNH\cdot CHD\cdot CO)_2O \xrightarrow{ii} ZNH\cdot CHD\cdot CO\cdot NZ\cdot CHD\cdot CO_2H \xrightarrow{iii} H_2N\cdot CHD\cdot CO\cdot NH\cdot CHD\cdot CO_2H \quad (IV)$$

Reagents: i, Dicyclohexylcarbodi-imide; ii, NEt_3–dioxan; iii, H_2, Pd–C

Variable Temperature N.m.r. Spectra.—The n.m.r. spectra of solutions of [^2H]glycine in D_2O have been measured at pD 6·1 ($H_3\overset{+}{N}\cdot CO_2^-$) and 11·7 ($H_2N\cdot CO_2^-$) and at four different temperatures, 0, +10, +20, and +33 °C. The

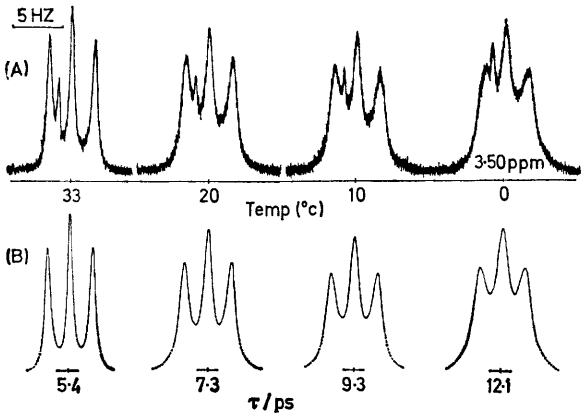


FIGURE 1 A, Temperature dependence of the α-proton n.m.r. signal of [$^2H_1$]glycine (containing 6% undeuteriated material). B, Lineshape analysis showing calculated curves and the corresponding deuterium quadrupolar correlation times τ_q calculated from the relaxation times T_q using equation (1)

resonance of the α-proton at +33 °C is a sharp triplet due to H–D spin–spin coupling, which gradually coalesces as the temperature is lowered (Figure 1). Because of extensive signal broadening no accurate data could be obtained for $H_3N^+\cdot CHD\cdot CO_2H$ in acid. [$^2H_1$]Glycine methyl ester hydrochloride was studied instead at pD 3·8.

In the case of diglycine (IV) it is clearly apparent that the changes in proton lineshape with temperature are more pronounced for the carboxy-terminal than for the amino-terminal group (Figure 2). Compound (IV) is a mixture of two diastereoisomers which in principle have different n.m.r. spectra. However, the effect of deuterium substitution is so weak that it contributes at most to a slight

[7] H. Kotake and T. Saito, *Bull. Chem. Soc. Japan*, 1966, **39**, 853; H. Kotake and T. Saito, *Nippon Kagaku Zasshi*, 1966, **87**, 999.

Deuterium Quadrupolar Relaxation Times.—The modification of the α-proton signals at low temperatures is due to the decrease of the deuterium quadrupolar relaxation time. Lineshape analysis, performed according to the method developed previously,[4-6] leads to the quadrupolar relaxation times at each temperature. An example is shown in Figure 1. The T_q values obtained for compounds (I)—(IV) at +33 and at 0 °C are listed in the Table.

Molecular Correlation Times. Activation Parameters.—Assuming nearly isotropic reorientation, the quadrupolar relaxation time T_q is related to the correlation time τ_q for local molecular motions by equation (1) where χ_D is the

$$T_q^{-1} = \frac{3\pi^2}{2}\chi_D\tau_q \qquad (1)$$

quadrupolar coupling constant of the deuterium nucleus in the C–D bond. χ_D May be taken equal to 170 kHz

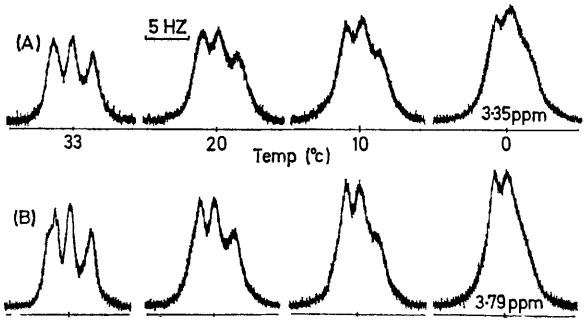


FIGURE 2 Temperature dependence of the α-proton n.m.r. signal of the amino-terminal group (A) and the carboxy-terminal group (B) of [$^2H_1$]glycyl[$^2H_1$]glycine (containing *ca.* 6% undeuteriated material)

as in $CD_2(CO_2H)_2$;[9] this value should be valid for compounds (I)—(IV) within 10% since χ_D is not very sensitive to changes in molecular structure not affecting the C–D

[8] A. Nakamura and O. Jardetzky, *Biochemistry*, 1968, **7**, 1226.

[9] W. Derbyshire, T. C. Gorvin, and D. Warner, *Mol. Phys.*, 1969, **17**, 401.

carbon atom itself.* Equation (1) thus simplifies to equation (2). Typical values of τ_q (in 10^{-12} s) are listed in the Table.

$$T_q^{-1} = 0{\cdot}43\ 10^{12}\tau_q \qquad (2)$$

Using the Eyring rate equation, the temperature dependence of T_q and of τ_q leads to the activation parameters

Deuterium quadrupolar relaxation times T_q, molecular correlation times τ_q, and activation parameters $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$ for molecular reorientation

Compound (0·1M, D_2O)	pD ±0·1	Temp. (°C ± 2)	Δ/Hz (±0·1)	T_q/ms (±10%)	τ_q/ps (±15%)	$\Delta G^{\ddagger}$/ kcal mol^{-1} (±0·1)	$\Delta H^{\ddagger}$/ kcal mol^{-1} (±0·5)	$\Delta S^{\ddagger}$/ cal mol^{-1} K^{-1} (±5)
(I), $H_3\overset{+}{N}{\cdot}CHD{\cdot}CO_2^-$	6·1	33	0·4	430	5·5	2·1	3·4	4
		0	0·7	190	12·3	2·3		
(I), $H_2N{\cdot}CHD{\cdot}CO_2^-$	11·7	33	0·6	595	3·9	1·9	2·9	3
		0	1·4	290	8·0	2·1		
(II), $H_3\overset{+}{N}{\cdot}CHD{\cdot}CO_2Me$	3·8	33	1·3	450	5·2	2·1	3·4	4
		0	1·4	200	11·6	2·2		
(III), $AcNH{\cdot}CHD{\cdot}CO_2Me$	7·4	33	1·4	270	8·6	2·4	5·1	8
		0	1·9	85	27·2	2·7		
(IV), $H_2N{\cdot}CHD{\cdot}CONH{\cdot}CHD{\cdot}CO_2^-$	11·4	33	1·0	320	7·3	2·3	4·9	8
		0	1·4	105	22·1	2·6		
(IV), $H_2N{\cdot}CHD{\cdot}CONH{\cdot}CHD{\cdot}CO_2^-$	11·4	33	0·7	305	7·7	2·4	5·8	11
		0	1·0	85	27·5	2·8		
(IV), $H_3\overset{+}{N}{\cdot}CHD{\cdot}CONH{\cdot}CHD{\cdot}CO_2^-$	5·7	33	1·3	180	13·0	2·7		

The H–D coupling constant is J_{HD} 2·40 ± 0·1 Hz. Δ Is the ' natural ' linewidth of the proton resonance in the absence of quadrupolar broadening (see Experimental section). The temperature range used for determining $\Delta H^{\ddagger}$ and $\Delta S^{\ddagger}$ (Figure 3) extends from 0 to +33 °C.

for molecular reorientation, $\Delta G^{\ddagger}$, $\Delta H^{\ddagger}$, and $\Delta S^{\ddagger}$, the free energy, enthalpy, and entropy of activation respectively. $\Delta G^{\ddagger}$ Is expressed by equation (3) [4,6] which by rearranging

$$\Delta G^{\ddagger} = 4{\cdot}57T[10{\cdot}32 + \log(\tau_q T)] \qquad (3)$$

and using equation (2) becomes equation (4). The slope

$$\log(T_q/T) = (\Delta S^{\ddagger}/4{\cdot}57) - 1{\cdot}31 - (\Delta H^{\ddagger}/4{\cdot}57T) \qquad (4)$$

and intercept of $\log(T_q/T) = f(1/T)$ plots (Figure 3) according to equation (4) respectively lead to $\Delta H^{\ddagger}$ and to $\Delta S^{\ddagger}$. The values of these two parameters for the compounds studied here are listed in the Table. Because of the narrow temperature range which could be used, the absolute values are probably quite inaccurate, but relative values should be usable for comparison purposes. The following discussion is based on the much more accurate $\Delta G^{\ddagger}$ values.

DISCUSSION

The results permit the analysis of two main features of the molecular dynamic behaviour of glycine and diglycine in water, the effect of ionic state on molecular motions and the nature of the local motions.

In addition, the effects on molecular size are also notable. One expects an increase in the reorientational correlation time τ_q when the molecular size increases. It is seen that the molecules listed in the Table fall clearly into two classes with respect to the values of τ_q and of the activation parameters, the glycine-type molecules and the diglycine-type molecules which include also *N*-acetylglycine methyl ester. The smaller molecules of the first type reorient appreciably faster and have lower activation parameters than those of the second type. However reorientation is anisotropic in the present systems (see also below) and a more detailed correlation with molecular size is in general not feasible with complex systems.

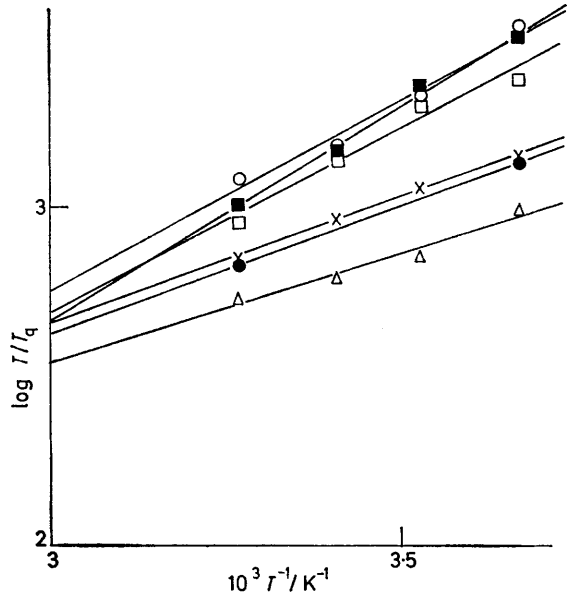


FIGURE 3 Activation plots [equation (4); see text] for molecular reorientation of [$^2H_1$]glycine at pD 11·7 (△) and at pD 6·1 (×); of [$^2H_1$]glycine methyl ester (●); of *N*-acetyl [$^2H_1$]glycine methyl ester (○); of the amino-terminal (□) and carboxy-terminal groups (■) of [$^2H_1$]glycyl[$^2H_1$]glycine (at pD 11·4)

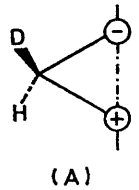
Effect of Ionic State.—Changing the ionic state of the present systems does not alter appreciably their molecular size but modifies markedly their electric properties. Comparing the carboxylate states in basic medium of glycine and diglycine to their respective zwitterionic

* The probable accuracy of ±5% in χ_D has been estimated by considering all the presently reported values of χ_D for C–D bonds on saturated carbon atoms. With ±5% and ±10% accuracies on χ_D and T_q respectively, the accuracy on τ_q is ±15%. This leads to an accuracy on $\Delta G^{\ddagger}$ of better than ±0·1 kcal mol^{-1}.

states, it is seen that the latter species reorient much more slowly and have much larger activation parameters than the former ones. In addition, although glycine methyl ester hydrochloride is a somewhat larger molecule than glycine because of the presence of the methyl group, it seems that much of the effect in the zwitterion is due to the presence of the positive charge on the nitrogen atom. These changes in rates of molecular reorientation may be rationalized by considering that positive ions are more strongly solvated than negative ones[10] and that NH_3^+ may form three $\overset{+}{N}-H\cdots O$ hydrogen bonds to water molecules and CO_2^- only two $O^-\cdots H-O$ bonds. Thus, one would expect an increase in correlation time from the carboxylate, to the ammonium and to the zwitterionic form of glycine (and of amino-acids in general) in agreement with the available data.

Local Molecular Motions.—Aqueous solutions of the glycine zwitterion have also been studied by dielectric relaxation.[11-13] The relaxation times obtained in this way describe the reorientation of the zwitterionic dipole moment and should be three times larger than the n.m.r. correlation times.[2]

A molar solution in water leads to a dielectric relaxation time of 71·6 ps at +20 °C for glycine. At the same temperature the n.m.r. measurements lead to $3\tau_q = 21{\cdot}7$ ps. Thus, the n.m.r. correlation time is still too small by a factor of *ca.* 3. This may indicate that there is another reorientation process present which leads to faster reorientation of the C–D bond than of the dipole-moment axis. This internal process may be rotation of the molecule around the electric dipole axis; such a motion contributes to n.m.r. relaxation but not to dielectric relaxation. Internal motions of rate τ_i decrease the local correlation times τ_q with respect to the overall molecular correlation time τ_M (here τ_M is the correlation time for the reorientation of the dipole axis). Assuming that the C–D bond is



(A)

approximately perpendicular (as in A) to the dipole axis one has equation (5).[1,2,14] Use of $\tau_q = 7{\cdot}2$ and

$$\tau_q = 0{\cdot}25\ \tau_M + 0{\cdot}75\left(\frac{1}{\tau_M} + \frac{1}{\tau_i}\right)^{-1} \qquad (5)$$

$\tau_M = \tau_{diel}/3 = 23{\cdot}8$ ps at +20 °C gives a time constant τ_i of 1·8 ps for the reorientation of the glycine zwitterion around the dipole-moment axis. Use of n.m.r. and dielectric data at different temperatures give also $\Delta H^{\ddagger} = 5{\cdot}2$ kcal mol^{-1} and $\Delta S^{\ddagger} = +13$ cal mol^{-1} K^{-1} for this motion. These values are however expected to be quite inaccurate. The large $\Delta S^{\ddagger}$ value *might* indicate that reorientation about this axis leads to a marked disordering of the surroundings and this is perhaps not unexpected.

Another interesting result about local motions is found in dilabelled diglycine at pD 11·4. From the data in the Table it is clear that the molecular motions are different at the two ends of the dipeptide. They are slower and change more rapidly with temperature (higher activation energy) at the negatively charged carboxylate end than at the amino-end. The central C–CON–C grouping may be considered as the molecular framework. The difference in local motions arises from a different local flexibility at the two chain terminals due both to different intrinsic CHD–CO and N–CHD rotation barriers and to different intermolecular associations. It would have been interesting to study also both ends of the corresponding zwitterion, but this was unfortunately not feasible because of signal overlap.

Because of the high sensitivity of the method and of the special features of deuterium quadrupolar relaxation,[4] it should be possible to extend the present studies to other biophysical systems in order to gain insight into their dynamic molecular properties. For instance, an extension to higher members of the oligoglycines would give information about local flexibilities along the chain. In addition the comparison of quadrupolar deuterium and dipolar carbon-13 relaxation data (which are now becoming available) would also be of much interest, especially in biophysical systems. Such studies are under way in this laboratory.

EXPERIMENTAL

N.m.r. Spectra and Lineshape Analysis.—The proton n.m.r. spectra have been measured on a Varian XL-100-15 spectrometer with internal lock on the proton HDO signal, using a 12 mm O.D. sample tube. Field homogeneity was adjusted on 0·1% internal Bu^{t}OH. Lineshape analysis has been performed as described earlier.[1,4,5] A small amount of undeuteriated compound is present in all samples (*ca.* 6%) and leads to a deformation of the downfield half of the CHD resonances. The CH_2 resonance is isotopically downfield (*ca.* 2 Hz) from the centre of the CHD resonance. Thus the upfield half of the CHD signal is unperturbed and has been used for lineshape analysis. The part of the curve at the foot of the signal has not been included in the analysis since it may be distorted by field inhomogeneity (dotted section of calculated lineshapes; Figure 1). Temperature changes were produced using the Varian variable temperature accessory and the temperatures were measured using the Varian MeOH calibration sample (accuracy ±2°). Some linewidth data have been obtained by deuterium decoupling experiments. The ' natural ' linewidth has been adjusted for best agreement between experimental and calculated curves. A

[10] J. F. Hinton and E. S. Amis, *Chem. Rev.*, 1967, **67**, 367.
[11] O. Sandus and B. B. Lubitz, *J. Phys. Chem.*, 1961, **65**, 881.
[12] M. W. Aaron and E. H. Grant, *Trans. Faraday Soc.*, 1963, **59**, 85.
[13] H. Hartman, E. Lertes, and R. Jaenicke, *Z. Naturforsch.*, 1967, **22a**, 2118.
[14] M. D. Zeidler, *Ber. Bunsengesellschaft Phys. Chem.*, 1965, **69**, 659.

long range spin–spin coupling of *ca.* 0·4 Hz between the two CH_2 groups in diglycine has been found and confirmed by double irradiation experiments. This small coupling leads to a slight broadening of the CHD resonances in [$^2H_1$]glycyl[$^2H_1$]glycine and has been taken into account in the linewidth parameter.

Samples.—All samples were 0·1M solutions in 99·8% D_2O. pD Was adjusted using a 2M-NaOD solution in D_2O.

Preparation of Compounds (I)—(IV).—[$^2H_1$]*Glycine* (I). In order to ensure a high enough isotopic purity of the final compound the exchangeable hydrogens in hydroxylamine hydrochloride and in glyoxylic acid was first replaced by deuterium by applying the sequence, dissolution in D_2O (0·2M in 15 ml), 30 min agitation at room temperature, and lyophilization three times. The final isotopic purity was >95%. The two reagents were then condensed and the resulting oxime was directly reduced without isolation in order to avoid decomposition to HCN, H_2O, and CO_2. ND_2OD, DCl (0·2 mol), and $CHO{\cdot}CO_2D$ (0·2 mol) were dissolved in 99·8% D_2O (40 ml) at room temperature. 15% Sodium amalgam (123 g) was then added in small portions over *ca.* 3 h while keeping the temperature below 40 °C using an ice-bath. After completion of the reduction the mixture was acidified (pH 3) with 6N-HCl and evaporated to dryness. The solid residue was dried over P_2O_5 for 12 h under vacuum (water-pump). A mixture (60 g) of [$^2H_1$]glycine hydrochloride and NaCl was obtained. This residue was suspended in absolute methanol (150 ml) at −25 °C and thionyl chloride (20 ml) was added dropwise. After stirring for 60 h at room temperature, the solid material was filtered, washed thoroughly with methanol, and the filtrate was evaporated to dryness under vacuum. [$^2H_1$]Glycine methyl ester hydrochloride (22 g, 85%) was thus obtained. This compound (16 g) was then hydrolysed by refluxing for 24 h in 2N-hydrochloric acid. The solution was then passed over charcoal (500 mg) and filtered. A solution of triethylamine (22 ml) in acetone (180 ml) was then added. After vigorous stirring for 30 min, the precipitated [$^2H_1$]glycine was filtered, thoroughly washed with chloroform and ether (9·2 g, 97%). The product was recrystallized from water–ethanol, m.p. 240 °C (decomp.) (lit.,[15] 240—245°).

[$^2H_1$]*Glycine methyl ester hydrochloride* (II). This was obtained in the course of the purification process of [$^2H_1$]glycine.

N-*Acetyl*[$^2H_1$]*glycine methyl ester* (III). This was obtained by a literature procedure.[16]

[$^2H_1$]*Glycyl*[$^2H_1$]*glycine* (IV).[7]—*Anhydride of* Z-[$^2H_1$]-*glycine.* Benzyloxycarbonyl[$^2H_1$]glycine (2·2 g) prepared from [$^2H_1$]glycine according to the literature procedure [17] was dissolved in anhydrous dioxan (80 ml) and dicyclohexylcarbodi-imide (2 g) in dioxan (20 ml) was added dropwise at room temperature. After stirring for 5 h the precipitate was filtered and washed with dioxan. The filtrate was evaporated under vacuum at 40 °C and the residue (70%) was recrystallized from anhydrous benzene, m.p. 123 °C (lit.,[7] 122—123°).

Rearrangement of the anhydride. The anhydride (2 g) was dissolved in anhydrous dioxan (20 ml) and dry triethylamine (0·35 ml) was added. The mixture was stirred at room temperature for 1 h. The solvent was evaporated and the residue crystallized directly or could be crystallized from water–ethanol. The bisbenzyloxycarbonyldiglycine obtained was used directly for the next step.

Hydrogenolysis. Bisbenzyloxycarbonylglycylglycine (1 g) in absolute ethanol (20 ml) containing Pd–C (5%; 200 mg) was hydrogenated at atmospheric pressure. The hydrogen uptake was completed after 1 h. The precipitate was filtered, dried, and treated with boiling water (2 ml). The mixture was filtered and the product was precipitated with ethanol (25 ml). The [$^2H_1$]glycyl[$^2H_1$]glycine (65%) thus obtained was recrystallized from water–ethanol, m.p. 230° (decomp). [lit.,[7] m.p. 220° (decomp.)].

[2/269 *Received, 7th February,* 1972]

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[16] *Org. Synth.*, 1943, Coll. Vol. II, p. 11.
[17] S. Guttmann and R. A. Boissonnas, *Helv. Chim. Acta*, 1958, **41**, 1852.