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STEREOCHEMISTRY OF THE 4-HYDROXYISOLEUCINE FROM *TRIGONELLA FOENUM-GRAECUM*

NATHANIEL W. ALCOCK, DAVID H. G. CROUT,* MARIA V. M. GREGORIO, EDWARD LEE, GRAHAM PIKE and CHRISTOPHER J. SAMUEL

Department of Chemistry, University of Warwick, Coventry, CV4 7AL, U.K.

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Key Word Index—*Trigonella foenum-graecum*; Leguminosae; fenugreek; (2*S*,3*R*,4*S*)-2-amino-4-hydroxy-3-methylpentanoic acid; 4-hydroxyisoleucine.

Abstract—The stereochemistry of the 4-hydroxyisoleucine from fenugreek (*Trigonella foenum-graecum*) has been reinvestigated. The absolute configuration was shown to be (2*S*,3*R*,4*S*) by a combination of chemical, spectroscopic and X-ray crystallographic techniques.

INTRODUCTION

In 1973, Fowden *et al.* [1] reported the occurrence of (2*S*,3*R*,4*R*)-4-hydroxy-3-methylpentanoic acid (4-hydroxyisoleucine) (**1**) in *Trigonella foenum-graecum* L. (fenugreek) (Leguminosae). The stereochemistry of this amino acid was assigned by comparison with the 4-hydroxyisoleucine isolated by Wieland *et al.* [2] as a hydrolysis product of γ-amanitin, with which it was stated to be identical. As part of our studies of branched-chain amino acid metabolism, we wished to investigate the biosynthesis of 4-hydroxyisoleucine. For this, we have reinvestigated the preparation of the lactone of 4-hydroxyisoleucine by the photochemical chlorination of L-isoleucine following the procedure of Wieland *et al.* [2, 3]. We have compared the compounds obtained in this way with the lactone of the 4-hydroxyisoleucine isolated from fenugreek.

RESULTS AND DISCUSSION

Photochemical chlorination of L-isoleucine followed by cyclization leads to two diastereoisomeric lactones (**2**) and (**3**) [2]. In the ^{1}H NMR spectra of the hydrochlorides of these compounds, the signals due to the C-4 methyl groups were reported to appear at δ1.39 and 1.46, respectively. The compounds giving the signal at δ1.46 on electrophoresis appeared to be identical with the lactone of the 4-hydroxyisoleucine isolated from γ-amanitin. Because the C-4 methyl group *cis* to the ammonium group in the hydrochlorides of the lactones **2** and **3** was expected to give a signal downfield of the corresponding signal for a *trans*-methyl group, the isomer giving the signal at δ1.46 was assigned the (2*S*,3*R*,4*R*)-configuration (**3**). Accordingly, this configuration was assigned also to the 4-hydroxyisoleucine from fenugreek.

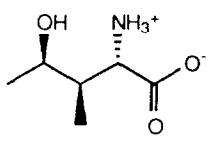
We have repeated the synthesis of the 4-hydroxyisoleucine lactones and have obtained as described one lactone by the recrystallization of the mixture of hydrochlorides from ethanol. This lactone was converted into the corresponding *N*-tosylamide for which full analytical data were obtained and which we show below to have the structure **4**. We also reisolated the 4-hydroxyisoleucine from fenugreek as described by Fowden *et al.* [1] and converted this amino acid into the *N*-tosylamide of the corresponding lactone by treatment with *p*-toluenesulphonyl chloride in pyridine. The product obtained differed from the synthetic compound produced by the photochemical chlorination procedure. Although lactonization of hydroxy-acids in *p*-toluenesulphonyl chloride–pyridine is reported to proceed without inversion at the carbinol carbon atom [4], this possibility was eliminated by conversion of the 4-hydroxyisoleucine into the lactone hydrochloride, as described by Fowden *et al.* [1], followed by *N*-tosylation. The product obtained was identical with that obtained directly from the 4-hydroxyisoleucine.

The situation was further complicated by the revision of the structure of the 4-hydroxyisoleucine from γ-amanitin by Wieland *et al.* [5]. An X-ray crystal structure of a lactone hydrobromide obtained from L-isoleucine by photochemical chlorination followed by lactonisation showed that this compound had the (2*S*,3*R*,4*S*)-configuration (**2**). This stereochemistry was assigned to the 4-hydroxyisoleucine from γ-amanitin and by implication to the 4-hydroxyisoleucine from fenugreek.

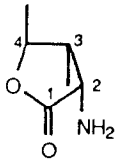
Further difficulties arise in interpreting the published data when the data for the hydrochlorides of lactones **2** and **3** are compared. If the lactones are identified by the relative chemical shifts due to the C-4 methyl group in the corresponding hydrochlorides, the isomer giving the signal at lower field can be designated lactone A and the C-4 epimer, lactone B. Lactone A, according to Wieland *et al.* [2] corresponds to the lactone of the 4-hydroxyisoleucine from γ-amanitin. In their 1968 paper [2], the hydrochloride of lactone A was reported to have a mp of 212°, whereas in the repetition of this work in 1974 [5], a mp of 230° was reported. In 1968 [2], a mp of 200° for the hydrochloride of lactone B was reported, but in 1974, a mp of 255°. In 1968 [2] it was reported that on heating in 6 M hydrochloric acid at 110°, lactone A was converted into an apparent equilibrium mixture of lactones A and B, containing 33–38% of lactone B. This is a rather surprising result given that the mechanism of epimerization is likely to involve elimination to the corresponding 3,4-unsaturated amino acid, relactonization of which [6]

would be expected to give a mixture that might contain besides lactones A and B (*cf.* **2** and **3**), the diastereoisomeric lactones **5** and **6** (Scheme 1). Photochemical chlorination of L-isoleucine [7] (**7**, Scheme 2) leads to a mixture of C-4 and C-5 chlorinated products. Treatment of the product with sodium hydroxide leads to a mixture of 3-methylproline (**8**) and isomeric 4-hydroxyisoleucines (**9**) (Scheme 2).

In view of the reported epimerization of lactone A on heating with hydrochloric acid, the relative yields of lactones A and B are likely to depend on the method of work-up used in the photochemical chlorination exper-



(1)



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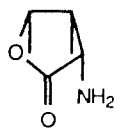

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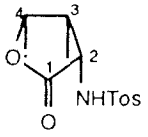

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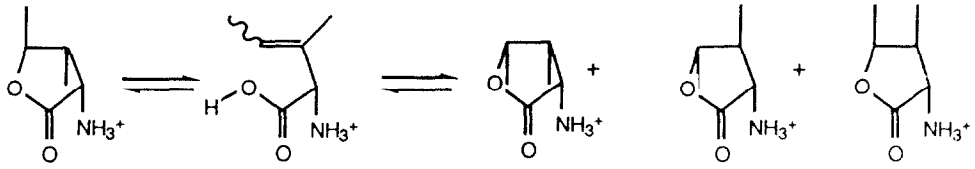
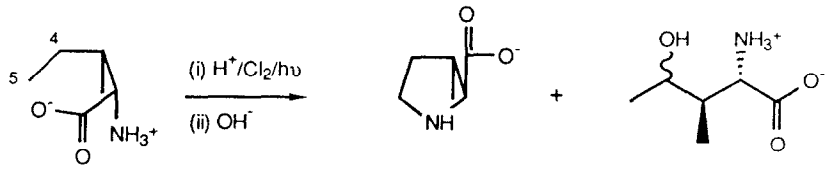


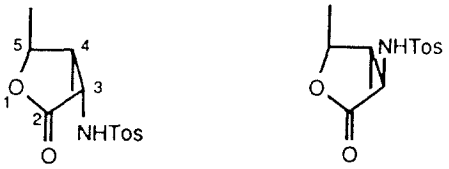
(2) (?) (3) (?) (5) (6)

Scheme 1



(7) (8) (9)

Scheme 2



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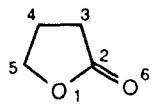

(12)

iments. Two methods are reported by Wieland *et al.* [2]. In their 1968 paper [2], the photochemical chlorination products were brought to pH 8–9 with phosphate buffer to bring about lactonization. However, in 1973, in a further description of the procedure [3], lactonization was brought about, following photochemical chlorination, by boiling the solution in hydrochloric acid for four hours. The duration of the photochemical chlorination in the two procedures also differed (5 and 8 hr, respectively). The paper describing the reinvestigation of the stereochemistry of the 4-hydroxyisoleucine from γ-amanitin referred back to both previous procedures without specifying which procedure for lactonization was followed. The relative amounts of lactones A and B produced by the photochemical chlorination of L-isoleucine are likely to depend both on the conditions used for the photochemical chlorinations, on their durations and on the procedure used for lactonization. In the original procedure, on which the procedure of Wieland *et al.* was based, considerable amounts of L-isoleucine were recovered [7]. In our experiments, the chlorination was continued until it could be seen from the NMR spectra of withdrawn aliquots, that all of the L-isoleucine had been consumed. At this point, the product contained considerable amounts of 4-chlorinated species as indicated by the appearance of a doublet at δ1.60 in the ^{1}H NMR spectrum. (Separate signals attributable to the C-4 epimers could not be distinguished.) Although it was not possible to be precise, integration of the NMR spectrum indicated the formation of approximately 70% of the 4-chlorinated species. This value is in accord with the reported isolation of 33% (based on converted L-isoleucine) of 3-methylproline (derived from the 5-chlorinated species) following photochemical chlorination of L-isoleucine [7]. These factors provide an explanation of the observation that in our experiments the relative amounts of the lactone products differed from those in the experiments of Wieland *et al.* and that the hydrochloride of lactone B crystallized preferentially from the mixture. That this was undoubtedly the case could be deduced from the observation that from the mother liquor, further quantities of the hydrochloride of lactone B could be recovered which, from the ^{1}H NMR spectrum, appeared to contain minor amounts (*ca* 25%) of lactone A. The identity of the 4-hydroxyisoleucine from fenugreek with lactone A was confirmed by comparison of ^{1}H NMR data for solutions in DMSO-d_6, the solvent used by Wieland *et al.* [5]. These data are given in Table 1, from which it can be seen that the data for the lactone hydrochloride from fenugreek correspond closely to those reported for lactone A but differ significantly from those for lactone B. (From Table 1 it appears that the chemical shift data for H-2 and H-4 in lactone hydrochloride A (as **2**) inadvertently may have been transposed in ref. [5]).

The poor reproducibility with respect to the isolation of a particular lactone from these photochemical chlorination experiments points to a difficulty in the interpretation of the X-ray crystallographic data for the hydrobromide of the lactone studied by Wieland *et al.* This hydrobromide was prepared by treatment with HBr of the lactone mixture in ether. From 15 g of mixed hydrobromides 1 g was crystallized from isopropanol for the X-ray analysis. In their publication, Wieland *et al.* [5] do not state how they confirmed that the compound obtained was the hydrobromide of lactone A. It is not clear therefore, that the isomer subjected to X-ray analysis

Table 1. ^{1}H NMR chemical shifts (δ) and coupling constants in DMSO-d_6 of the hydrochlorides of the lactone **2** from the 4-hydroxyisoleucine from fenugreek and lactones A and B

Compound (as the hydrochloride)	H-2	$J_{H\text{-}2,H\text{-}3}$	H-3	$J_{H\text{-}2,H\text{-}3}$	$J_{H\text{-}3,H\text{-}4}$	$J_{H\text{-}3,Me\text{-}3}$	H-4	$J_{H\text{-}3,H\text{-}4}$	$J_{H\text{-}4,Me\text{-}4}$	Me-3	$J_{H\text{-}3,Me\text{-}3}$	Me-4	$J_{H\text{-}4,Me\text{-}4}$
Lactone **2** from the 4-hydroxyisoleucine from fenugreek*	4.50	8.4	2.51	—	3.03	7.2	4.48	3.04	6.52	1.07	7.2	1.36	6.52
Lactone A†‡	4.48	—	2.55	8.5	3.3	—	4.51	—	—	1.10	7.4	1.37	6.5
Lactone B†‡	4.64	—	2.85	7.5	4.6	—	4.83	—	—	0.90	7.0	1.28	6.5

*400 MHz.
†100 MHz.
‡Ref. [5].

corresponded to the 4-hydroxyisoleucine from γ-amanitin, still less to the 4-hydroxyisoleucine from fenugreek. Accordingly we carried out an X-ray crystal structure determination of the hydrochloride of the lactone derived from the 4-hydroxyisoleucine from fenugreek which showed that this compound had the (2*S*,3*R*,4*S*)-or (2*R*,3*S*,4*R*)-configuration (Fig. 1).

In order to determine the absolute configuration, the circular dichroism of the corresponding tosylamide was studied. The curve obtained showed a positive maximum at 227 nm (Table 2). A very similar maximum was observed in the CD analysis of the tosylamide of lactone B.

As the relevant absorption is that of the tosylamide group, which would dominate the CD, it was concluded that the tosylamide lactone of the 4-hydroxyisoleucine from fenugreek has the same (*S*)-configuration at C-2 as L-isoleucine. It can therefore be assigned structure **10**. In order to confirm this assignment, the tosylamide lactone was epimerized at C-2 by treatment with sodium methoxide in methanol. Epimerization appeared to go to completion to give a crystalline tosylamide **11**, the CD of which showed a negative peak at 223 nm (Table 2). In order to confirm that the experimental conditions led only to epimerization at C-2, the procedure was repeated with MeOD as solvent. The product was shown by both ^1H and ^2H NMR to have incorporated deuterium only at C-2. Accordingly the absolute (2*S*,3*R*,4*S*)-configuration (**2**) can be assigned to the 4-hydroxyisoleucine from fenugreek. These data also confirm the structure of lactones A and B as **2** and **3**, respectively. The (2*S*,3*S*,4*R*)-isomer of 4-hydroxyisoleucine occurs in lactone form as a component of the alkaloid funebrine from *Quararibea funebris* [8].

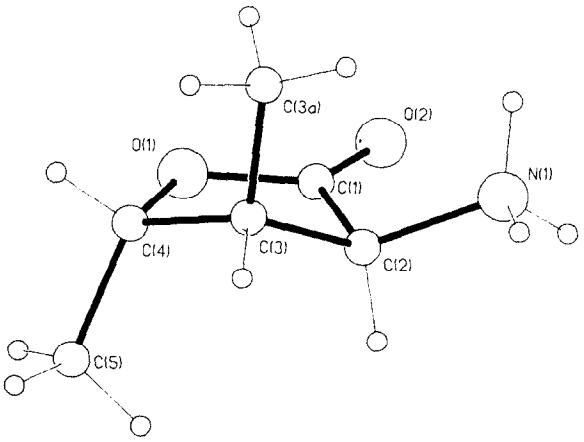

Fig. 1. X-ray Structure of the cation of the lactone **2** hydrochloride showing the atomic numbering, the configuration and the conformation of the lactone ring.

Table 2. CD Maxima (nm) in methanol of tosylamide lactones **10**, **11** and **4**

Compound	λ_{max}nm
10	227 (+4.63)*
11	223 (−2.64)
4	223 (+3.53)

*Δε values in parentheses.

Although NMR spectroscopy is extremely useful for structural analysis in this series, it could not be applied to give unambiguous conclusions as to stereochemistry. Nuclear Overhauser effects were determined for the three tosylamide lactones **4**, **10** and **11**. The results are given in Table 3. In compound **4**, the NOE's between H-3 and the *cis*-related H-2 is very similar to that between H-3 and the *trans*-related H-2 in lactone **10**. The NOE's between H-3, and the *trans*-related protons H-2 and H-4 in **11** are smaller than the corresponding NOE's between the *cis*-related protons in lactone **4**. However, the NOE's are not sufficiently different to allow one with confidence to assign relative configurations to ring substituents in this series. The difficulty in drawing structural conclusions from NMR data is attributable to the flexibility of the γ-lactone ring. The Cambridge Crystallographic Data Base contains structural information on more than one hundred γ-lactones with a hydrogen atom at C-3, C-4 and C-5 (*cf* **12**). For only eight of these are NMR data on coupling constants between H-3, H-4 and H-5 available. These data, together with the corresponding crystallographic dihedral angles, are given in Table 4. Corresponding values for the coupling constants calculated from the crystallographic data using the Karplus equation are also given. The values observed (for solution spectra) and calculated (from crystallographic data) are in reasonable agreement. Also given in Table 4 are the deviations of C-3 and C-4 from the plane defined by atoms 1, 2, 3 and 6 (*cf* **12**) in the crystal structures. These data show that these compounds exist in the crystal in the familiar envelope conformation in which C-5 deviates only slightly, but C-4 may deviate significantly from this plane.

The data indicate the difficulties in assigning relative configurations in five-membered ring systems using ^1H NMR. For example although the relative configurations at C-3 and C-4 in tosylamide lactones **10** and **11** are the same, the corresponding coupling constants between H-3 and H-4 are 0.8 and 10 Hz, respectively (Table 7).

The dihedral angles between the C-H bonds at C-2, C-3 and C-4 in the three tosylamide lactones **4**, **10** and **11** obtained during this study were calculated from the observed ^1H NMR coupling constants using the Karplus equation (Table 5). These calculated angles vary considerably. Taken together with the crystallographic data of Table 4, they indicate the inherent difficulties in assigning relative configurations in γ-lactones from ^1H NMR data alone. Further, the rate of change of coupling constant with dihedral angle, as predicted from the Karplus equation ($^3J = 4.22 - 0.5\cos\theta + 4.5\cos 2\theta$) by taking the second differential of the coupling constant with dihedral angle, reaches maxima at 134° and 45°. Consequently

Table 3. NOE data* for tosylamide lactones **4**, **10** and **11**

Compound	Proton irradiated	Proton signal enhanced (% enhancement)
4	H-3	H-2 (3.5), H-4 (2.9)
10	H-3	H-2 (5.6), H-4 (2.7)
11	H-3	H-2 (1.4), H-4 (0.9)

*400 MHz ($CDCl_3$).

Table 4. X-ray crystal structure data, observed and calculated coupling constants for some γ-lactones

Compound	∠2,3*	$J_{2,3}$(Hz)		∠3,4	$J_{3,4}$(Hz)		Deviations (Å) from plane defined by atoms 1,2,3,6 (cf 12)		Reference
		Observed	Calculated†		Observed	Calculated	C-4	C-3	
Cis, cis-2-methyl-3-hydroxy-4-iodomethyl-γ-butyrolactone	44.9	3.06	3.9	−36.2	4.88	5.2	−0.023	−0.521	[9]
Laurycolactone A	82.5	~0	−0.19	−66.7	~1	0.9	0.145	−0.579	[10]
Zeylena	45	5.0	6.3	−53.5	4.5	2.5	−0.040	−0.734	[11]
Hymenosignin	−26.89	8	6.4	26.64	11	6.5	−0.42	−0.523	[12]
Eregoyazidin	−145.87	10	6.3	145.69	—	—	−0.86	−0.562	[13]
Erivanin	−174.33	9.7	9.1	161.49	—	—	−0.139	0.505	[14]
Picrotoxinin	−50	5.0	3.1	51.5	4.5	3.0	0.038	0.744	[15]
Heliangolidin	−135.2	2.5	4.6	117.3	3.6	1.8	−0.002	−0.013	[16]

* ∠2,3 = dihedral angle between C-H bonds at C-2 and C-3 (numbering as in **4**).
†Using the standard Karplus equation ($J = 4.22 - 0.5 \cos\theta + 4.5 \cos 2\theta$).

dihedral angles close to these values are expected to be particularly sensitive to small conformational changes in the γ-lactone ring.

EXPERIMENTAL

Plant material. Seeds of fenugreek (*Trigonella foenum-graecum*) were obtained from Thompson and Morgan Ltd, Ipswich.

Extraction and isolation of (2*S*,3*R*,4*S*)-2-*amino*-4-*hydroxy*-3-*methylpentanoic acid* (4-*hydroxyisoleucine*) (**2**). Fenugreek seed (500 g) was macerated with $CHCl_3$ (800 ml) in a Waring Blender. The residue after separation from $CHCl_3$, was macerated ×4 with EtOH–H_2O (7:3, 250 ml). The combined aq. ethanolic extracts were evapd to dryness under red. pres. The residue was dissolved in H_2O and passed through a column of Dowex 50W-X8 ion exchange resin (H^+ form, 250 g). The column was washed thoroughly with H_2O and the amino acids were eluted with 2M NH_4OH until the eluate gave a negative reaction with ninhydrin. The ammoniacal eluate was evapd to dryness under red. pres., the residue was triturated with EtOH–Et_2O and crystallized from aq. EtOH to give 4-hydroxyisoleucine (**1**). (1.5 g), mp 224–225°. ^{1}H NMR (100 MHz; D_2O): δ0.95 (3H, *d*, *J* = 7.1 Hz, MeCH), 1.24 [3H, *d*, *J* = 6.3 Hz, Me C(OH)], 1.91 (1H, *m*, H-3), 3.84 (1H, *m*, H-4) 3.89 (1H, *d*, *J* = 4.4 Hz, H-2).

Lactonization of 4-*hydroxyisoleucine.* The amino acid (500 mg) was boiled under reflux in HCl (1 M, 10 ml) for 15 min. The soln was evapd to dryness under red. pres. and the residue was twice recrystallized (EtOH) to give the hydrochloride of lactone **2**, 200 mg, mp 206°. ^{13}C NMR (MHz, DMSO-d_6, proton noise decoupled): δ12.58 (3-Me), 19.22 (4-Me), 37.51 (C-3), 50.55 (C-2), 82.3 (C-4), 172.34 (C-1). The ^{1}H NMR data are given in Table 6. Recrystallization from $CHCl_3$–petrol gave well-formed block crystals suitable for X-ray structural analysis (see below).

Tosylamide lactone (**10**) *from the* 4-*hydroxyisoleucine from fenugreek.* To a soln of the amino acid **1** (98 mg) in freshly distilled pyridine (5 ml) at 0° was added *p*-toluenesulphonyl chloride (255 mg) in several portions. The mixture was kept at 0° for 1 hr, and it was then left at room temp. for 48 hr. Et_2O (50 ml) was added. The soln was washed with H_2SO_4 (2 M) until the extracts were acidic, and then with H_2O. The remaining ethereal soln was dried ($MgSO_4$) and evapd to dryness to give the tosylamide lactone **10** (95 mg), pure by TLC ($CHCl_3$–MeOH, 7:3). Recrystallization from EtOAc–petrol (bp 40–60°) gave 39 mg, mp 153–154°. (Found: C, 55.00; H, 6.05; N, 4.55. $C_{13}H_{17}NSO_4$ requires C, 55.11; H, 6.05; N, 4.94%). EIMS, 70 eV, *m/z* (rel. int.): 283 $[M]^+$ (13), 239 (23), 224 (34), 155 (46), 91 (84), 84 (100). CIMS (NH_3) *m/z* (rel. int.): 301 $[M+18]^+$ (54), 284 $[M+1]^+$ (9), 239 (6), 224 (3), 155 (2), 128 (5), 108 (3), 84 (23.7). IR ν_{max}^{nujol} cm^{-1}: 3300 (NH), 3000–2800 (CH), 1790 (CO), 1600. UV λ_{max}^{MeOH} nm (ε): 228.5 (11100), 256.3 (458), 263.6 (483), 268.8 (354), 274.5 (267). The ^{1}H NMR data are given in Table 7.

C-2 Epimer **11** *of tosylamide lactone* **10**. A soln of the tosylamide lactone **10** in MeOH (50 ml) containing NaOMe (from the addition of 25 mg Na) was boiled under reflux and the disappearance of the lactone was followed by TLC ($CHCl_3$–MeOH, 7:3), (I_2 visualization). After 2.5 hr, the starting material was no longer detectable. The soln was evapd to dryness. Trituration with H_2O of the residue gave the tosylamide lactone **11**, 69 mg, pure by ^{1}H NMR, mp ($CHCl_3$–Et_2O) 160–161°. HRMS *m/z*: Calc. for $C_{13}H_{17}NSO_4$ 283.0878. Found 283.0883. EIMS, 70 eV, *m/z* (rel. int.): 283 $[M]^+$ (17), 239 (33), 224 (50), 155 (83), 91 (95), 84 (100). IR ν_{max}^{nujol} cm^{-1}: 3305 (NH) 2960, 2930, 2855 (CH), 1782 (CO), 1600, 1338, 1162. The ^{1}H NMR data are given in Table 7.

Epimerization of tosylamide lactone **10** *in* MeOD. The tosylamide lactone **10** (70 mg) was boiled under reflux for 3 hr in MeOD (1.2 ml) containing NaOMe (prepared by addition of 13 mg Na

Table 5. Calculated dihedral angles in tosylamide lactones **4**, **10** and **11**

Compound	$J_{H-2,H-3}$*	∠2,3†(calc.)	$J_{H-3,H-4}$	∠3,4 (calc.)
4	6.9	23	43	42
10	7.8	13	0.8	109
11	12	180	10	180

*400 MHz NMR data from Table 7.
† ∠2,3 = dihedral angle between C-H bonds at C-2 and C-3.

Table 6. ^{1}H NMR data for lactone hydrochlorides **2** and **3** (in D_2O)

H/Compound	2*	3†
2§	4.56 *d* (8.2)‡	4.67 *d* (7.3)
3	2.69 *ddq* (2.0, 7.3, 8.2)	3.00 *m*
4	4.55 *dq* (2.0, 6.6)	4.9 *m*
Me-3	1.08 *d* (7.3)	1.00 *d* (7.8)
Me-4	1.36 *d* (6.7)	1.41 *d* (7.3)

*δ, Measured at 400 MHz.
†δ, Measured at 220 MHz.
‡Coupling constants (Hz) in parentheses.
§Numbering as in **2**.

to 21 g MeOD). The epimer **11** was isolated as before. Accurate integration of the ^{1}H NMR spectrum (400 MHz) indicated 43% D at H-2. The ^{2}H NMR spectrum (61.40 MHz) showed a single peak at δ3.70 corresponding to H-2 (*cf* Table 7). MS *m/z* (rel. int.): 284 (2.56), 283 (2.55), corresponding to 43% d_1 species.

Benzyloxycarbonyl derivative of γ-hydroxyisoleucine. The γ-hydroxyisoleucine (147 mg) and benzyl chloroformate (187 mg) were added alternately and dropwise to a vigorously stirred soln of $NaHCO_3$ (210 mg) in H_2O (1.25 ml). Stirring was continued for 1 hr. The aq. soln was extracted with EtOAc. The extracts were dried ($MgSO_4$) and evapd to give the *Z*-derivative, 134 mg, mp 106–107° [after recrystallization ($CHCl_3$–Et_2O)]. (Found C, 63.9; H, 6.6; N, 5.65. $C_{14}H_{17}NO_4$ requires C, 63.87; H, 6.51; N, 5.32%). EIMS, *m/z* (rel. int.): 263 $[M]^+$ (4), 160 (2), 132 (3), 128 (2), 114 (4), 108 (40), 107 (10), 91 (100). IR ν_{max}^{nujol} cm^{-1}: 3440 (NH), 1785 (CO). ^{1}H NMR (100 MHz, $CDCl_3$): δ0.99 (3H, *d*, *J* = 7.0 Hz, Me-3), 1.44 (3H, *d*, *J* = 6.5 Hz, Me-4), 1.61 (1H, *br s*, NH), 2.61 (1H, *m*, H-3), 4.40 (1H, *m*, H-2), 4.65 (1H, *m*, H-4), 5.15 (2H, *s*, CH_2) 7.37 (5H, *s*, Ar).

Photochemical chlorination of L-*isoleucine.* L-Isoleucine (10.9 g) was dissolved in conc. HCl (150 ml). The soln was cooled to 0°. Cl_2 gas was bubbled through the reaction mixture which was irradiated using a low-pressure Hg vapour lamp (254 nm). Progress of the reaction was monitored by NMR (60 MHz) on aliquots withdrawn from the reaction mixture. When reaction was complete (*ca* 5 hr), the Cl_2 supply was removed, and N_2 bubbled through the soln for 30 min to remove remaining Cl_2. H_2O (150 ml) was added to the soln, which was left to stand for 12 hr. The soln was concd at 30° under red. pres. The residual, highly concd soln was treated with a mixture of Na_3PO_4 and Na_2HPO_4 (1 : 1). The pH of the resulting paste was adjusted to 8–8.5 by careful addition of NaOH soln (2 M). The paste was extracted with Et_2O (5 × 100 ml). The Et_2O extracts were dried ($MgSO_4$) and cooled to 0°. Dry HCl was passed into the soln, the Et_2O was removed under red. pres. and the residue crystallized (EtOH). The product (0.59 g) was recrystallized (EtOH) to give the hydrochloride of the lactone **3**, 110 mg, mp 227°. The ^{1}H NMR data are given in Table 6. From the original mother liquor by addition of Et_2O, an additional fraction was obtained which on recrystallization gave a mixture of hydrochlorides of lactones **3** and **2** (28 mg).

Tosylamide of the lactone (**3**). The lactone **3** 190 mg, was treated with *p*-toluenesulphonyl chloride as described above to give the tosylamide lactone **4**, 53 mg, mp 148°. (Found: C, 54.9; H, 5.9; N, 4.7. $C_{13}H_{17}NSO_4$ requires C 55.11; H, 6.05; N, 4.94%). EIMS, 70 eV, *m/z* (rel. int.): 283 $[M]^+$ (7), 239 (26) 224 (24), 155 (40), 139 (6), 91 (78), 84 (100). CIMS (NH_3): 301 $[M+18]^+$ (33), 284 $[M+1]^+$ (5), 239 (2), 224 (1), 155 (1), 139 (2), 128 (2), 108 (1), 91 (1), 84 (6), 18 (100). IR ν_{max}^{nujol} cm^{-1}: 3303 (OH), 2990, 2980, 2961, 2921, (CH), 1768 (CO), 1601, 1340, 1166. The ^{1}H NMR data are given in Table 7.

X-Ray structure analysis. 3-Amino-4,5-dimethyl-2-oxotetrahydrofuran hydrochloride, $C_8H_{12}NO_2Cl$. Note: this compound (the hydrochloride of lactone **2**) is numbered throughout the text and in Fig. 1 as a derivative of the parent amino acid.

Table 7. ^{1}H NMR data* of tosylamide lactones **4**, **10** and **11** (in $CDCl_3$)

H/Compound	4	10	11
2‡	4.02 *dd* (4.0, 6.9)†	4.09 *dd* (4.5, 7.8)	3.63 *dd* (12, 6.8)
3	2.75 *m*	2.53 *m*	1.95 *m*
4	4.54 *dq* (4.3, 6.5)	4.36 *dq* (0.80, 6.8)	3.99 *dq* (10, 6.15)
Me-3	0.91 *d* (7.2)	1.07 *d* (6.8)	1.16 *d* (6.5)
Me-4	1.35 *d* (6.5)	1.36 *d* (6.8)	1.34 *d* (6.2)
Ar	7.77 *d* (8.3)	7.78 *d* (8.4)	7.74 *d* (8.4)
	7.33 *d* (8.0)	7.33 *d* (7.9)	7.25 *d* (7.9)
MeAr	2.43 *s*	2.43 *s*	2.36 *s*
NH	4.99 (3.7)	4.90 *d* (4.3)	5.05 *d* (6.8)

*δ, 400 MHz.
†Coupling constants in parentheses.
‡Numbering as in **4**.

$M = 165.5$, orthorhombic, space group $P2_12_12_1$, $a = 5.247(3)$, $b = 12.371(2)$, $c = 13.199(3)$ Å, $U = 857.19$ Å^3, $Z = 4$, $D_c = 1.67$ g/cm^3, Mo$K\alpha$ radiation, $\lambda = 0.71069$ A, μ(Mo$K\alpha$) $= 3.90$ cm^{-1}, $T = 298$ K, $F(000) = 352$. Data were collected with a Syntex $P2_1$ four circle diffractometer. Maximum 2θ was 50, with scan range $\pm 1.1°$ (2θ) around the $K_{\alpha1}$–$K_{\alpha2}$ angles, scan speed 3–29° min^{-1}, depending on the intensity of a 2 sec prescan; backgrounds were measured at each end of the scan for 0.25 of the scan time. *hkl* ranges were: 0, 0, 0 to 7, 14, 15. Three standard reflections were monitored every 200 reflections, and showed some irregular changes during data collection apparently due to crystal movement. Unit cell dimensions and standard deviations were obtained by least-squares fit to 15 reflections ($2\theta < 2\theta < 22$). Reflections were processed using profile analysis to give 879 unique reflections. 651 were considered observed ($I/\sigma(I) \geqslant 1.5$) and used in refinement; they were corrected for Lorentz, polarisation and absorption effects, the last by the Gaussian method; maximum and minimum transmission factors were 0.94 and 0.92. Crystal dimensions were $0.16 \times 0.18 \times 0.32$ mm. Systematic absences: $h00$, $h \neq 2n$, $0k0$, $k \neq 2n$, $00l$, $l \neq 2n$, indicate space group $P2_12_12_1$ uniquely.

The structure was solved by direct methods using SHELXTL. Anisotropic temperature factors were used for all non-H atoms. Hydrogen atoms were given fixed isotropic temperature factors, $U = 0.07$ Å^2. Those defined by the molecular geometry were inserted at calculated positions and not refined; the methyl group and the NH_3^+ were treated as rigid CH_3 units, with their initial orientation taken from the strongest H-atom peaks on a difference Fourier synthesis. The absolute structure of the individual crystal chosen was identified from the known conformation at C(3). Final refinement was on F by cascaded least squares methods refining 100 parameters. Largest positive and negative peaks on a final difference Fourier synthesis were of height ± 0.5 Å^{-3}.

A weighting scheme of the form $W = 1/[\sigma^2(F) + gF^2]$ with $g = 0.0160$ was used and shown to be satisfactory by a weight analysis. Final $R = 0.080$ $R_w = 0.083$. The relatively high R-value probably results from the low level of intensity cut-off chosen to maximise the number of contributing reflections coupled with apparent slight movement of the crystal during data collection. Maximum shift/error in the final cycle was 0.3. Computing with SHELXTL [17] on a Data General DG30. Scattering factors in the analytical form and anomalous dispersion factors taken from *International Tables for X-ray Crystallography*. Coordinates, anisotropic thermal parameters and bond lengths and angles have been deposited with the Cambridge Crystallographic Data Centre.

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