δ 6.31 (s, 1 H, C-6 H), 6.82 (d, 1 H, C-1′ H, $J_{1'2'}$ = 3.5 Hz), 2.53 (s, 3 H, SCH_3).

Anal. Calcd for $C_{14}H_{17}N_5O_6S$: C, 43.99; H, 4.45; N, 18.30. Found: C, 43.68; H, 4.63; N, 18.41.

4-Amino-5-carboxamido-2-methylthio-7-oxo-8-(2′,3′-*O*-isopropylidene-β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (17). To DMF (4 ml) was added 1 drop of concentrated HCl and 0.6 ml of dimethoxypropane. After stirring for 1 h, **16** (60 mg) was added and the suspension stirred for 1 h. Ammonium hydroxide solution was added until pH 8 was obtained. The solution was evaporated in vacuo to give a white solid. The solid was dissolved in MeOH (3 ml)–H_2O (3 ml). Removal of MeOH in vacuo and filtration gave 44 mg (80%) of **17**: MS *m/e* 423 (M^+); ^1H NMR δ 6.35 (s, 1 H, C-6 H), 7.02 (s, 1 H, C-1′ H, $J_{1'2'}$ < 1.0 Hz), 1.32, 1.52 [2 s, 6 H, $C(CH_3)_2$], 2.50 (s, 3 H, SCH_3).

4-Amino-5-carboxamido-7-oxo-8-(β-D-ribofuranosyl)pyrido-[2,3-*d*]pyrimidine (3). Compound **15** (1.0 g, 1.44 mmol) was refluxed in EtOH (100 ml) containing Raney nickel (3 g) for 12 h. Additional Raney nickel (3 g) was added and reflux continued for an additional 24 h. The reaction mixture was filtered while hot through Celite and the nickel washed with an additional 100 ml of hot EtOH. Evaporation of the filtrate to dryness gave 797 mg of oily solid which was dissolved in MeOH (100 ml). MEOH (10 ml), in which Na (100 mg) was previously dissolved, was added and the solution was stirred at room temperature overnight. H_2O (30 ml) was added, the pH was adjusted to 7 with Dowex 50-X8 (H^+), and the solution was filtered. Evaporation, followed by coevaporation with EtOH–H_2O three times, gave a white solid. Recrystallization from H_2O gave 209 mg (43%) of **3**: mp 240 °C dec; MS *m/e* 697 (M^+) for penta-Me_3Si derivative; UV (pH 1) 297 nm (ε 9500), 313 (sh, 7800); (pH 7) 250 (11 800), 322 (9000); (pH 11) 250 (11 100), 322 (8300); ^1H NMR δ 6.45 (s, 1 H, C-6 H), 8.33 (s, 1 H, C-24), 6.87 (d, 1 H, C-1′ H, $J_{1'2'}$ = 3.4 Hz), 7.50 (br s, 2 H, 4-NH_2), 8.30 8.60 (2 br s, 2 H, $CONH_2$).

Anal. Calcd for $C_{13}H_{15}N_5O_6$: C, 46.29; H, 4.48; N, 20.76. Found: C, 46.58; H, 4.78; N, 20.48.

5-Carboxamido-2,4-diamino-7-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine (18). Compound **15** (795 mg, 1.0 mmol) was dissolved in $CHCl_3$ (50 ml) containing m-chloroperbenzoic acid (400 mg, 2 mmol). After stirring for 3 h, the solvent was removed in vacuo. The solid was triturated with Et_2O and filtered. The white powder was treated with liquid NH_3 (30 ml) in a glass bomb for 18 h. Evaporation of the ammonia gave an oily solid which was dissolved in MeOH (50 ml) in which Na (23 mg) was previously dissolved. After stirring for 3 h at room temperature, the pH was adjusted to 7 with Dowex 50-X8 (H^+). The resin was removed by filtration and the filtrate evaporated in vacuo, then coevaporated three times with EtOH–H_2O. The residue was triturated with $CHCl_3$ (50 ml) and filtered. The solid was dissolved in H_2O–EtOH by heating. Cooling gave a precipitate, which was filtered and washed with EtOH and Et_2O to give 195 mg (55%) of **18**: mp 192 °C dec; UV (pH 1) 300 nm (ε 13 500), 327 (13 500); (pH 7) 342 (14 600); (pH 11) 342 (14 600); ^1H NMR δ 5.93 (s, 1 H, C-6 H), 6.77 (d, 1 H, C-1′ H, $J_{1'2'}$ = 3.0 Hz).

Anal. Calcd for $C_{13}H_{16}N_6O_6 \cdot 2H_2O$: C, 40.21; H, 5.19; N, 21.64. Found: C, 39.99; H, 5.31; N, 21.57.

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Registry No.—**3**, 61140-07-6; **4**, 36707-44-5; **5**, 61140-08-7; **6**, 61140-09-8; **8**, 61140-10-1; **8** sulfoxide, 61140-11-2; **8** sulfone, 61140-12-3; **9**, 61140-13-4; **10**, 61140-14-5; **11**, 61140-15-6; **12**, 61140-16-7; **13**, 61140-17-8; **14**, 61129-19-9; **15**, 61140-18-9; **16**, 61140-19-0; **17**, 61140-20-3; **18**, 61140-21-4; methyl α-chloromethyl ether, 107-30-2; 4-amino-6-carboxamido-2-methylthio-5-oxo-8-(β-D-ribofuranosyl)pyrido[2,3-*d*]pyrimidine, 36707-04-7; 2,3,5-tri-*O*-benzoylribofuranosyl bromide, 16205-60-0.

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Synthesis and Stereochemistry of 3-Hydroxy-5-methylproline, a New Naturally Occurring Imino Acid

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3-Hydroxy-5-methylproline was synthesized via Dieckmann cyclization of methyl *N*-methoxycarbonyl-3-methoxycarbonylmethylaminobutyrate (**6**) to *N*,2-dimethoxycarbonyl-5-methylpyrrolid-3-one (**8**). Reduction of the latter to *N*,2-dimethoxycarbonyl-5-methylpyrrolidin-3-ol (**9**) and subsequent hydrolysis afforded a mixture (**10**) of the four diastereoisomers (**1–4**) of 3-hydroxy-5-methylproline, which were separated by ion-exchange chromatography. From ^1H NMR data and epimerization studies, the relative stereochemistry of these stereoisomers was established. The NMR study also revealed conformational differences between the various isomers. Isomer **1**, which was reported earlier to correspond with a component of the peptide antibiotic actinomycin Z_1, has 2,3-trans-2,5-cis stereochemistry and a C_3-exo, C_4-endo ("twist") conformation.

A preliminary communication[1] reported the identification of 3-hydroxy-5-methylproline as a component of the peptide antibiotic, actinomycin Z_1, and the same imino acid has also been identified in some members of an actinomycin complex from *Micromonospora floridensis* NRRL8020.[2] A synthesis of the four racemic diastereoisomers (racemates of **1–4**, Figure 1) and an investigation of their stereochemistry are described here.

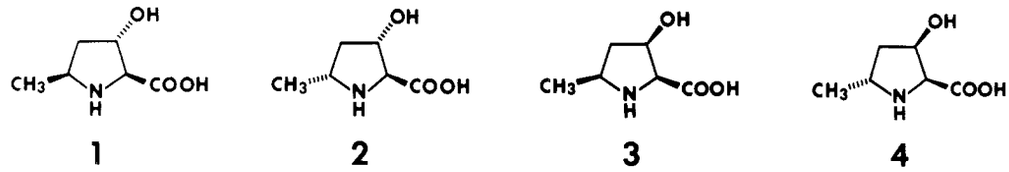


Figure 1. The four diastereoisomeric 3-hydroxy-5-methylprolines. The same numerals are used to denote the corresponding racemates.

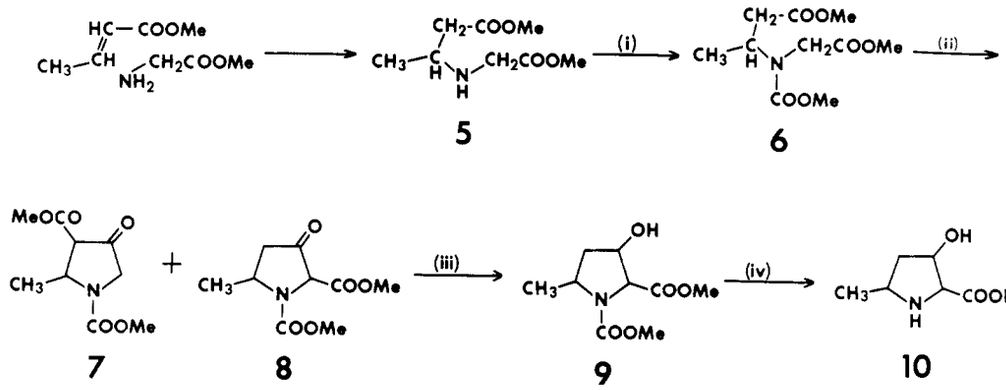


Figure 2. Synthesis of 3-hydroxy-5-methylproline. Reagents: (i) ClCOOMe; (ii) *t*-BuOK/PhMe; (iii) $NaBH_4$; (iv) $Ba(OH)_2/H_2O$.

Table I. Relative Intensities in the Mass Spectra of the Diastereoisomers of *N,O*-Ditrifluoroacetyl-3-hydroxy-5-methylproline Methyl Ester

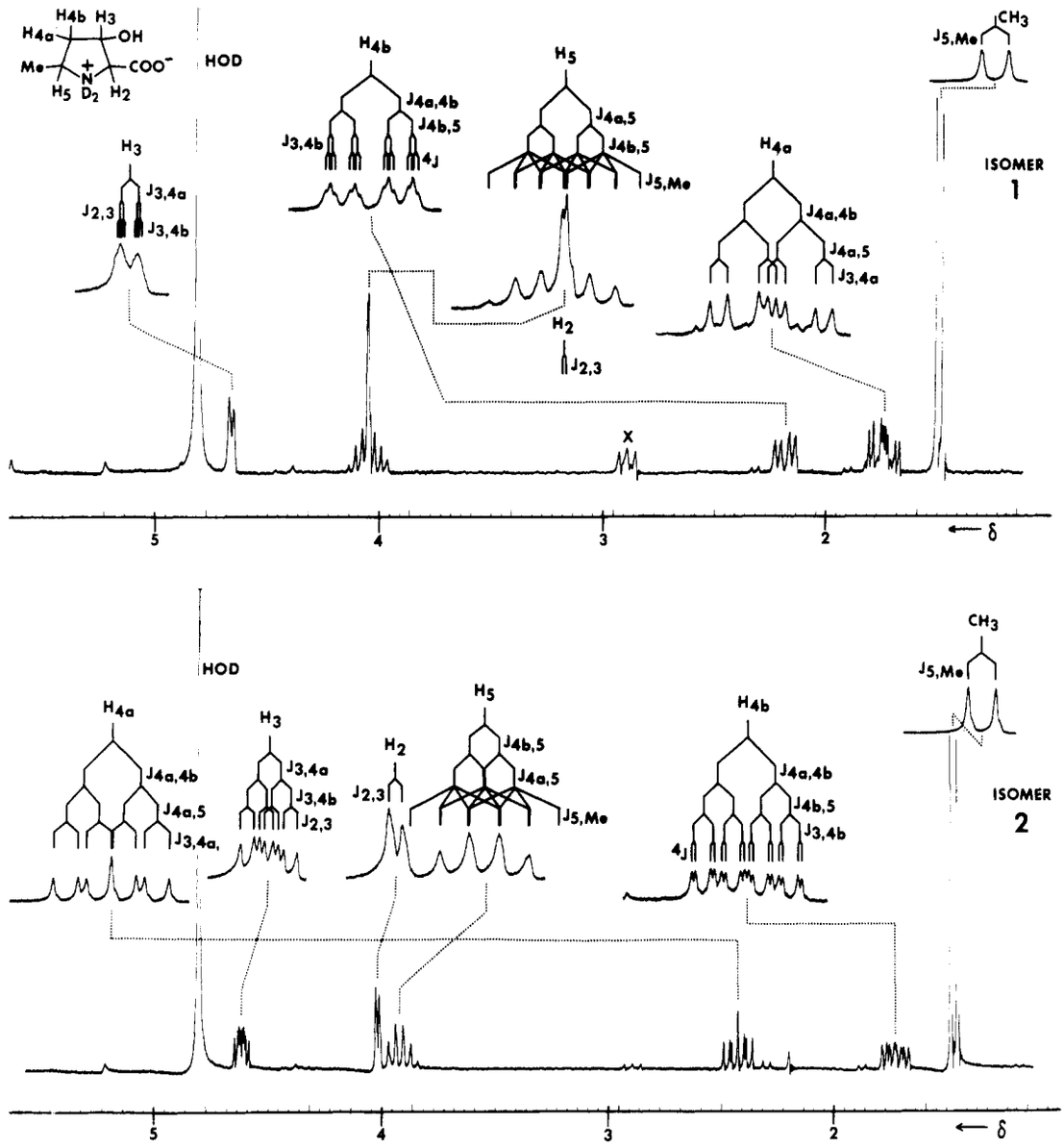
m/e	Natural (61218-64-2)[a]	Isomer 1 (61247-99-2)	Isomer 2 (61248-00-8)	Isomer 3 (61248-01-9)	Isomer 4 (61248-02-0)	Ion$^+$
351	0.002	0.002	0.033	0.023	0.028	M
292	1.00	1.00	1.00	1.00	1.00	M − COOMe
237	0.16	0.18	0.13	0.051	0.087	M − CF_3COOH
178	0.28	0.30	0.35	0.24	0.32	M − COOMe − CF_3COOH
153	0.04	0.05	0.14	0.043	0.22	
83	0.27	0.31	0.34	0.24	0.37	M − COOMe − CF_3COOH − CF_3CO
81	0.19	0.21	0.25	0.17	0.25	
69	0.76	0.65	0.75	0.54	0.78	CF_3
65	0.10	0.11	0.12	0.090	0.13	
59	0.32	0.35	0.43	0.31	0.36	$COOCH_3$
55	0.19	0.22	0.25	0.17	0.26	

[a] Registry no.

The synthetic route (Figure 2) parallels that reported for a synthesis of 3-hydroxyproline,[3] except that in the initial step the imino diester **5** was prepared by addition of methyl glycinate to methyl crotonate. Dieckmann cyclization of **6** gave a mixture of isomeric β-keto esters (**7** and **8**) and reduction of the latter provided a derivative (**9**) of 3-hydroxy-5-methylproline. Hydrolysis of **9** afforded 3-hydroxy-5-methylproline as an isomeric mixture (**10**) which was separated by ion-exchange chromatography into four crystalline diastereoisomeric racemates designated **1**–**4** according to their emergence from the column. The four diastereoisomers were distinguishable by paper electrophoresis, ion-exchange chromatography, and gas chromatography (GC) of their *N,O*-ditrifluoroacetyl methyl esters,[1] and by these criteria the natural imino acid was found to correspond in relative stereochemistry with **1**. In addition, these derivatives were subjected to combined GC–mass spectrometry and electron impact mass spectra were obtained (Table I). The derivative of **1** differed from those of **2**–**4** in producing a spectrum with a less intense molecular ion.

Stereochemical relationships between the various synthetic isomers were investigated by C_2-epimerization experiments. In aqueous alkali at 140 °C, **1** and **4** were each converted to an equilibrium mixture of these two isomers in the ratio of 2:1. Likewise, **2** and **3** each formed a 2:1 mixture of these isomers, and it follows that **1** and **4**, and also **2** and **3**, are pairs of C_2 epimers. Furthermore, since 3-hydroxyproline forms a 2:1 equilibrium mixture of trans and cis isomers in hot alkali,[3] it appears likely that **1** and **2** are the isomers which possess 2,3-trans stereochemistry. These observations permit an understanding of the relative yields of **1**–**4** during the synthesis. When **9** is hydrolyzed with alkali, the stereoisomeric composition of the resulting 3-hydroxy-5-methylproline is **1**, 16%; **2**, 50%; **3**, 27%; **4**, 7%, whereas acid hydrolysis gives **1**, 2%; **2**, 11%; **3**, 55%; **4**, 32%. The latter composition reflects the stereochemistry of **9**, whereas the former results from C_2-epimer equilibration. Since **9** is expected to be predominantly 2,3-cis, resulting from borohydride reduction of **8** (by analogy with studies on 3-hydroxyproline[4]), the results are consistent with 2,3-cis stereochemistry for **3** and **4**. ^1H NMR studies (discussed below) established 2,3-trans-2,5-cis stereochemistry for **1**, and it follows from the foregoing discussion that the relative stereochemistry is as shown for isomers **1**–**4**. The stereochemical composition resulting from the synthesis is explained by concluding that **8** is predominantly cis and **9** mainly cis,cis.

^1H NMR spectra of **1**–**4** were obtained in D_2O at 220 MHz. At this resolution all four spectra were first order (Figures



Figures 3 and 4. ^{1}H NMR spectra of 1 and 2. H_{4a} and H_{4b} are cis and trans, respectively, to the carboxyl group. "X" denotes signals caused by the internal standard (DSS).

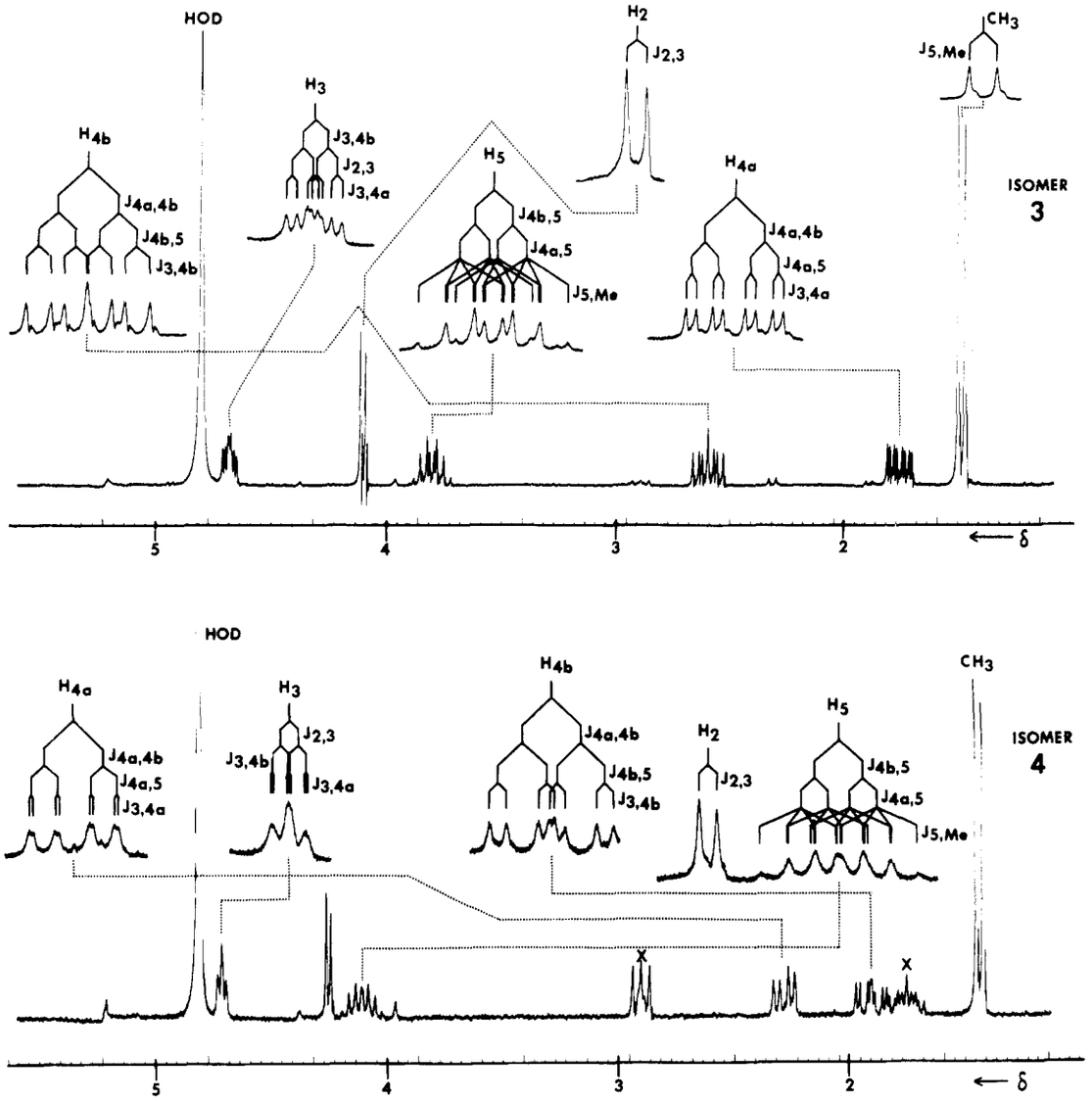
Table II. Coupling Constants[a] (Hz)

Isomer	$J_{2,3}$	$J_{3,4a}$	$J_{3,4b}$	$J_{4a,4b}$	$J_{4a,5}$	$J_{4b,5}$	$J_{5,Me}$	$^4J_{2,4}$
1	~0	4.4	1.0	14.3	11.8	6.1	6.7	~1
2	3.2	5.8	4.6	14.0	8.0	7.2	6.8	1.0
3	4.9	2.5	5.9	14.3	6.5	9.1	6.8	
4	4.2	1.0	4.0	14.1	6.0	11.4	6.9	

[a] H_{4a} and H_{4b} are cis and trans, respectively, to the carboxyl group.

3–6), permitting direct measurement of all the coupling constants (Table II). The striking chemical shift differences (Table III) between the geminal 4 protons cannot be attributed to the effects of the hydroxyl or carboxyl substituents, to judge by analogy with the 4 protons in proline[5,6] or the 3 protons in hydroxyproline and *allo*-hydroxyproline.[7,8] They can be explained by the known shielding effect of a methyl group upon the adjacent cis proton in five- or six-membered rings.[9] This effect produces a shift difference of δ 0.66 in the geminal 2 protons of methylcyclopentane[10] and δ 0.53 in the 5 protons of 2,3-*trans*-3,4-*cis*-3-hydroxy-4-methylproline[11] and can be used to assign the 4-proton signals in the isomers of 3-hydroxy-5-methylproline (Figures 3–6).

The stereochemistry of **1** and **4** can be deduced from their vicinal coupling constants as follows. The minimal value of $J_{2,3}$ in **1**, which implies a dihedral angle close to 90°, requires 2,3-trans stereochemistry and parallels the case of *trans*-3-hydroxyproline.[3,4] Likewise, the minimal $J_{3,4b}$ reveals that H_{4b} is trans to H_3 and hence to the carboxyl group. The value of 11.8 Hz for $J_{4a,5}$ must result from a trans dihedral angle close to 180°. It follows that H_5 is trans to the carboxyl group and hence the methyl is cis. These dihedral angles comprise a 3-



Figures 5 and 6. ^{1}H NMR spectra of **3** and **4**. H_{4a} and H_{4b} are cis and trans, respectively, to the carboxyl group. "X" denotes signals caused by the internal standard (DSS).

Table III. Chemical Shifts (δ)

Isomer	Me	H_{4a}	H_{4b}	H_5	H_2	H_3
1	1.49	1.76	2.20	4.05	4.05	4.67
2	1.47	2.43	1.75	3.92	4.01	4.61
3	1.49	1.76	2.59	3.80	4.10	4.69
4	1.43	2.28	1.91	4.09	4.23	4.70

exo-4-endo ("twist") conformation, which explains the long-range coupling (^4J) between H_2 and H_{4b}, since these protons are joined by a "W" bond conformation. In comparing the coupling constants of **4** with those of **1** (in Table II, note that for this purpose protons 4a and 4b are exchanged), these are seen to be almost identical except for $J_{2,3}$. It follows that their relative stereochemistry at C_3 and C_5 is the same, in accord with the observation that they are C_2 epimers. Furthermore, the ring conformation (3-endo,4-exo) of **4** is the same, relative to the hydroxyl and methyl substituents, as that of **1**. This conformation renders the hydroxyl group quasi-axial and the methyl quasi-equatorial, and the same conformation meets the same requirements in the case of 2,3-*trans*-3,4-*cis*-3-hydroxy-4-methylproline.[11,12] The latter imino acid has three coupling constants (0, 4, and 11 Hz for $J_{2,3}$, $J_{3,4}$, and $J_{4,5}$, respectively) very similar to corresponding vicinal couplings in **1**. It is also noteworthy that the conformations established for hydroxyproline[7,13] and *allo*-hydroxyproline[7] compel the hydroxyl group to adopt a quasi-axial configuration.

Interpretation of the coupling constant data for **2** and **3** in terms of stereochemistry and conformation presents a more difficult task than for **1** and **4**. Attempts to establish mutually compatible dihedral angles using various forms of the Karplus relationship are still under study. There is a possibility that rapid interconversion of different conformational populations prevails, as in the case of proline.[5] The relative stereochemistry at C_2 and C_3 cannot be deduced with certainty from the $J_{2,3}$ values for **2** and **3**, but these parameters, in the case of their *N,O*-di-*p*-toluenesulfonyl methyl esters ($J_{2,3}$ = 0 and 6.9 Hz, respectively), revealed that **2** undoubtedly possesses 2,3-trans stereochemistry and that its derivative has a ring conformation more similar to that of **1** than does the free imino acid. Conformational alteration in the conversion of substituted prolines to their *N*-*p*-toluenesulfonyl derivatives has been reported previously.[14] Isomer **2** also shares with **1** the presence of a long-range coupling between H_2 and H_{4b}, which was confirmed when irradiation at the frequency of the latter produced sharpening of the H_2 doublet. The ^4J value (1.0 Hz)

requires a cis arrangement of these protons in order to approximate a "W" four-bond conformation, and this serves to confirm the assignment of H_{4b} based upon the shielding effect of the methyl group. Although four-bond couplings have been observed with other bond conformations, the resulting ^4J values are generally smaller (0.4–0.8 Hz) than that observed here.[15]

The possibility that relative lanthanide-induced shifts could be utilized to confirm the stereochemistry and the assignments of H_{4a} and H_{4b} in **2** and **3** was investigated using europium(III) nitrate. In the case of hydroxyproline it was reported that the 3 and 5 protons cis to the carboxyl group were shifted more than those which were trans, in accord with the expected geometry of the 1:1 complex in which the europium is bound to the carboxylate anion.[16] The observed upfield shifts of the various protons in **2** and **3** were expressed relative to the shift of H_2, and as such varied little during eight incremental additions of reagent up to a reagent/substrate molar ratio of 0.8. The mean relative shifts (Hz) were as follows: for **2**, H_2, 1.00; H_3, 1.87; H_{4a}, 0.59; H_{4b}, 0.55; H_5, 0.77; Me, 0.32. For **3**, H_2, 1.00; H_3, 1.31; H_{4a}, 0.65; H_{4b}, 0.45; H_5, 0.52; Me, 0.16. The markedly greater shift of H_3 in **2** than in **3** confirms the 2,3 stereochemistry proposed for these isomers, and the greater shift of H_5 in **2** than **3** is in accord with their postulated 2,5 stereochemistry. Comparison of the induced shifts in H_{4a} and H_{4b}, while in accord with the assignments in the case of **3**, is invalid in the case of **2** because the difference is too small to be significant. Ambiguity occurs because an induced shift depends not only upon the distance of the proton from the lanthanide ion, but also upon the angle this vector makes with the magnetic axis, and is therefore strongly dependent upon conformation.[16] It is concluded that the comparisons discussed here, while valid for H_3, become increasingly unreliable as the proton–lanthanide distance increases. In considering the comparatively small methyl proton shifts, which appear to contradict the result for H_5, the latter must be considered a more dependable stereochemical criterion.

Evaluation of all the evidence confirms that structures **1–4** correctly depict the stereochemistry of the four diastereoisomers. That the naturally occurring imino acid corresponds with **1** is not unexpected. The ^{1}H NMR spectrum of actinomycin Z_1 was reported[17] to include a "singlet" in the region ($\sim\delta$ 6.0) occupied only by the α protons of proline and its congeners in the actinomycin series. The data presented here explain that observation, which is compatible only with the presence of a trans 3-substituted proline residue. *cis*-5-Methylproline occurs in actinomycin Z_5, another component of the same complex[18,19] and examination of space-filling (CPK) molecular models reveals that *trans*-5-methylproline, in contrast to the cis isomer, could not be accommodated without alteration of the peptide backbone conformation which is common to all the actinomycins which have been investigated.[20,21]

Experimental Section

For gas chromatography (GC) a Shimadzu Model 4BM, equipped with flame ionization detectors, was employed with argon (60 ml/min) as carrier gas. Glass columns (2.5 m × 3 mm) contained 3% OV225 (column A) or 3% OV17 (column B) on Gas-Chrom Q (100–120 mesh). The derivatization procedure for the *N,O*-ditrifluoroacetyl methyl esters of **1–4**, and their retention times, were reported previously.[1]

For combined GC–mass spectrometry, an LKB9000 instrument was used, with a 6-ft column of 1% OV17 on Gas-Chrom Q at 108 °C. Electron impact mass spectra (Table I) were obtained for the *N,O*-ditrifluoroacetyl methyl esters of each synthetic diastereoisomer and of the natural compound in an actinomycin Z_1 hydrolysate. The conditions for paper chromatography and high-voltage paper electrophoresis were described earlier.[1]

Infrared (IR) spectra were obtained on a Perkin-Elmer Model 337. ^{1}H NMR spectra were obtained on a Varian HR-220 in the cw mode. Solutions of **1–4** were in D_2O with DSS as internal standard. The temperature was 18 °C, pH 6.4, and the concentration was 0.2 M for **1–3** and 0.1 M for **4**. Eight additions of a 0.8 M solution of $Eu(NO_3)_3$ in D_2O were made to the sample solutions (0.8 ml) of **2** and **3** in increments of 25 μl, each representing a 1:10 molar ratio of reagent to imino acid. Chemical shifts were determined and the upfield induced shifts (Hz) expressed relative to H_2 = 1.00 at each reagent concentration.

Methyl 3-Methoxycarbonylmethylaminobutyrate (5). Methyl crotonate (15.21 g, 0.15 mol) and glycine methyl ester hydrochloride (40.8 g, 0.33 mol) were stirred in methanol (200 ml) during addition of triethylamine (35 g, 0.35 mol). After 2 days at 26 °C, the precipitate was filtered off and the filtrate evaporated. The residue, in ethyl acetate (500 ml), was washed with aqueous $NaHCO_3$ and water, dried (Na_2SO_4), and evaporated. The residual oil was distilled at 0.28 Torr and the fraction bp 76–78 °C collected: yield 13.46 g (47%); IR ($CHCl_3$) 1730 cm^{-1} (ester C=O).

Anal. Calcd for $C_8H_{15}NO_4$: C, 50.78; H, 7.99; N, 7.40. Found: C, 50.52; H, 8.03; N, 7.82.

Methyl *N*-Methoxycarbonyl-3-methoxycarbonylmethylaminobutyrate (6). A solution of **5** (3.35 g, 71 mmol) in ethyl acetate (100 ml) was stirred vigorously with water (150 ml) and $NaHCO_3$ (10.0 g) during addition of methyl chloroformate (8.0 g, 85 mmol). After 2 h at room temperature, ethyl acetate (150 ml) was added and the layers separated. The organic phase was washed with water, dried (Na_2SO_4), and evaporated. The residual oil was distilled at 0.4 Torr and the fraction bp 107–110 °C collected: yield 16.39 g (94%); IR ($CHCl_3$) 1690 (urethane C=O) and 1740 cm^{-1} (ester C=O).

Anal. Calcd for $C_{10}H_{17}NO_6$: C, 48.57; H, 6.93; N, 5.67. Found: C, 48.65; H, 7.14; N, 5.60.

***N*,2-Dimethoxycarbonyl-5-methylpyrrolid-3-one (8).** To a solution of potassium *tert*-butoxide (88 mmol) in toluene (prepared using 3.45 g of potassium by the published method[22]) at 0 °C was added a solution of **6** (14.27 g, 58 mmol) in dry toluene (30 ml) with stirring during 15 min in a stream of nitrogen. After 90 min, acetic acid (6 ml) and chloroform (300 ml) were added and the solution was washed with 10% aqueous NaH_2PO_4 (300 ml). The chloroform extracts were washed with pH7 phosphate buffer, dried (Na_2SO_4), and evaporated. The resulting mixture of **7** and **8** was separated via partition between toluene (350 ml) and pH 9.5 carbonate buffer (3 × 200 ml) at 0 °C. The combined aqueous layers were reextracted with chloroform until complete separation of the two components was apparent from GC. The chloroform extracts were dried (Na_2SO_4) and evaporated. The residual **8** was distilled at 0.025 Torr and the fraction bp 97.5–99 °C collected: yield 3.57 g (29%); IR ($CHCl_3$) 1700 (urethane C=O), 1750 (ester C=O), and 1770 cm^{-1} (ketone C=O).

Anal. Calcd for $C_9H_{13}NO_5$: C, 50.23; H, 6.09; N, 6.51. Found: C, 50.10; H, 5.99; N, 6.68.

***N*,2-Dimethoxycarbonyl-5-methylpyrrolidin-3-ol (9).** Phosphate buffer, pH 7.2 (250 ml), was cooled to 0 °C and $NaBH_4$ (8.0 g) was gradually added with stirring, followed by **8** (3.50 g, 16 mmol) in methanol (165 ml). The pH was maintained at 8–9 by addition of $NaH_2PO_4 \cdot H_2O$, and after 1 min further $NaBH_4$ (4.0 g) was added. After 4 min the mixture was adjusted to pH 3 with aqueous H_2SO_4, then neutralized with NaOH and extracted with chloroform (3 × 500 ml). The chloroform extracts were dried (Na_2SO_4) and evaporated, and the residue chromatographed on a column (40 × 4.7 cm) of silica gel 60 (70–230 mesh) using 25% ethyl acetate in chloroform. Appropriate fractions were pooled and evaporated and the residual oil distilled (short path) at 150 °C (0.20 Torr): yield 2.18 g (62%); IR ($CHCl_3$) 1680 (urethane C=O) and 1760 cm^{-1} (ester C=O).

Anal. Calcd for $C_9H_{15}NO_5$: C, 49.76; H, 6.96; N, 6.45. Found: C, 49.56; H, 7.07; N, 6.26.

3-Hydroxy-5-methylproline (10). Aqueous 0.3 N $Ba(OH)_2$ (130 ml) was added to **9** (1.97 g, 9.1 mmol) and the mixture was heated under reflux in a stream of nitrogen for 60 h. The cooled solution was neutralized with H_2SO_4, filtered, washed with ethyl acetate (250 ml), and evaporated. The residue was dissolved in water and applied to a column (2.2 × 27 cm) of cation exchange resin AG50W-X2. After washing with water (600 ml) the product was eluted with 2 N NH_4OH, and evaporation gave a white solid (1.02 g, 77%) which gave four spots on paper electrophoresis (yellow with ninhydrin) and four peaks on the amino acid analyzer.[1]

Separation of the 3-Hydroxy-5-methylproline Diastereoisomers. The isomeric mixture **10** (1.02 g) was dissolved in 0.2 M ammonium acetate buffer (pH 3.8) containing 40% methanol (20 ml) and divided into four equal aliquots. Each aliquot was chromatographed on a column (61 × 3.6 cm) of cation exchange resin (Baker CGC-241, 8% cross-linked, 200–400 mesh) using the same solvent. Fractions (10 ml) were collected and aliquots examined by paper chromatography and high-voltage paper electrophoresis;[1] **1** was located in fractions

46–53, **2** in fractions 56–67, **3** in fractions 81–90, and **4** in fractions 94–100. Similar fractions from the four separations were pooled and evaporated, and each isomer was desalted on a column (26 × 2.0 cm) of Dowex 50W-X8 as described above (for **10**). After evaporation, each isomer was crystallized from water/acetone. **1** formed needles, mp 267–628 °C dec, yield 71 mg. Anal. Calcd for $C_6H_{11}NO_3$: C, 49.64; H, 7.64; N, 9.55. Found: C, 49.72; H, 7.89; N, 9.80. **2** formed plates, mp 242–243 °C dec, yield 369 mg. Found: C, 49.58; H, 7.79; N, 9.72. **3** formed plates, mp 204–205 °C dec, yield 253 mg. Found: C, 49.24; H, 7.84; N, 9.52. **4** formed plates, mp 259–260 °C dec, yield 15 mg.

Epimerization Studies. Each diastereoisomeric racemate **1–4** (0.1 mg) in 1 N NaOH (0.1 ml) was kept at 140 °C (sealed tube) for 22 h. HCl (1.5 N, 0.1 ml) was added and the solution was evaporated in vacuo. Each residue was derivatized as reported previously[1] (*N,O*-ditrifluoroacetyl methyl esters) and analyzed by GC[1] on column A at 130 °C. The following compositions were observed: **1** → 68% **1** + 32% **4**; **2** → 68% **2** + 32% **3**; **3** → 70% **2** + 30% **3**; **4** → 62% **1** + 28% **4** + 10% **3**.

Hydrolysis of 9 with Acid and Alkali. A. 9 (6 mg) in acetic acid (0.5 ml) was heated with concentrated HCl (0.5 ml) at 110 °C (sealed tube) for 16 h, then evaporated in vacuo. After derivatization as before,[1] GC on column B at 115 °C indicated the following isomeric composition: **1**, 2%; **2**, 11%; **3**, 55%; **4**, 32%.

B. Reverse Reaction. Each isomer **1–4** (0.1 mg) was converted to the *N*-methoxycarbonyl methyl ester by treatment with 5 N methanolic HCl (0.5 ml, 80 °C, 1 h) followed by evaporation and treatment with methyl chloroformate (10 mg) in ethyl acetate (0.2 ml) and $NaHCO_3$ (20 mg) in water (0.2 ml). GC on column B at 165 °C gave the following retention times (min): **1**, 8.3; **2**, 8.1; **3**, 7.5; **4**, 7.1. GC analysis of **9** indicated an isomeric composition of almost entirely **3** and **4** in approximately 2:1 ratio.

C. 9 (2 mg) in 0.3 N $Ba(OH)_2$ (1 ml) was heated at 110 °C (sealed tube) for 16 h, then neutralized with 1 N H_2SO_4, filtered, and evaporated. After derivatization and GC as above (A) the isomeric composition was **1**, 16%; **2**, 50%; **3**, 27%; **4**, 7%.

***N,O*-Di-*p*-toluenesulfonyl Methyl Ester of 3.** A solution of **3** (62 mg) in 5 N methanolic HCl (2 ml) was kept at 80 °C (sealed tube) for 1 h. After evaporation, the residue was treated with *p*-toluenesulfonyl chloride (260 mg) in pyridine (27 ml) containing triethylamine (0.06 ml) at 5 °C for 2.5 days. After evaporation in vacuo, the residue was partitioned between 0.1 N HCl (15 ml) and ethyl acetate (30 ml) and the ethyl acetate extract was washed with aqueous $NaHCO_3$ and water and dried (Na_2SO_4). After evaporation, the residue was chromatographed on a column (16 × 1.5 cm) of silica gel 60 (70–230 mesh) with chloroform and the product located by TLC on fluorescent silica gel. The product crystallized from ethyl acetate/petroleum ether as needles: mp 116–118 °C; yield 121 mg (61%); NMR ($CDCl_3$, internal Me_4Si) δ 1.35 (d, J = 7.5 Hz, 5-CH_3, 3), 2.01 (m, 4-H, 1), 2.05 (m, 4-H, 1), 2.44 (s, Tos CH_3, 3), 2.46 (s, Tos CH_3, 3), 3.63 (s, OCH_3, 3), 3.87 (m, 5-H, 1), 4.54 (d, J = 6.9 Hz, 2-H, 1), 4.85 (m, 3-H, 1), 7.31 (d, $J \sim 8$ Hz, ArH, 2), 7.35 (d, $J \sim 8$ Hz, ArH, 2), 7.70 (d, $J \sim 7$ Hz, ArH, 2), and 7.74 (d, $J \sim 7$ Hz, ArH, 2).

Anal. Calcd for $C_{21}H_{25}NO_7S_2$: C, 53.94; H, 5.39; N, 3.00; S, 13.72. Found: C, 54.03; H, 5.43; N, 2.92; S, 13.58.

***N,O*-Di-*p*-toluenesulfonyl methyl ester of 2** was prepared as described above using **2** (48 mg) with methanolic HCl (1.4 ml), then pyridine (1.6 ml), triethylamine (0.04 ml) and *p*-toluenesulfonyl chloride (140 mg), yield 44 mg (28%). The product could not be crystallized: NMR ($CDCl_3$, internal Me_4Si) δ 1.22 (d, J = 6.7 Hz, 5-CH_3, 3), 2.42 (s, Tos CH_3, 3), 2.47 (s, Tos CH_3, 3), 2.48 (m, 4-H, 2), 3.69 (s, OCH_3, 3), 4.19 (m, 5-H, 1), 4.50 (~s, 2-H, 1), 4.95 (d, J = 4.8 Hz, 3-H, 1), 7.26 (d, $J \sim 8$ Hz, ArH, 2), 7.36 (d, $J \sim 8$ Hz, ArH, 2), 7.67 (d, $J \sim 8$ Hz, ArH, 2), and 7.75 (d, $J \sim 8$ Hz, ArH, 2).

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