Tetrahedron. Vol. 31, pp. 2625 to 2627. Pergamon Press 1975. Printed in Great Britain

A SIMPLE METHOD FOR DETERMINING THE ABSOLUTE CONFIGURATION OF α-AMINO ACIDS

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(*Received in U.S.A.* 17 *January* 1975; *Received in UK for publication* 1 *April* 1975)

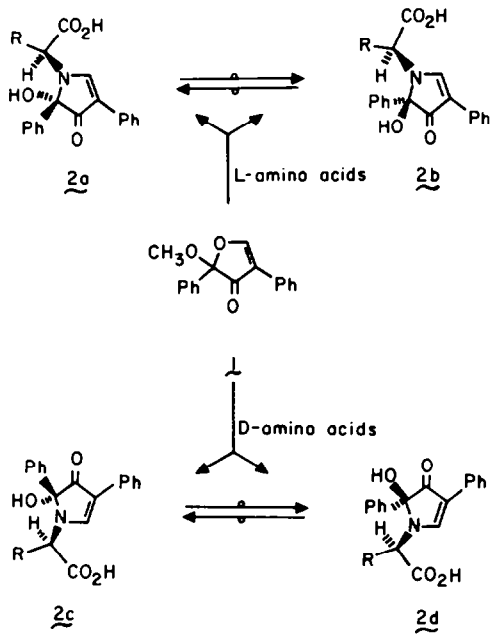
Abstract—Chiral α-amino acids react with 2-methoxy - 2,4 - diphenyl - 3 (2*H*) - furanone **1** to afford *N*-substituted 3,5 - diphenyl - 5 - hydroxy - 2 - pyrrolin - 4 - ones **2**. The characteristic cotton effects given by these chromophoric derivatives provide a means for the determination of the absolute configuration of the parent amino acids. The longest wavelength(first) extremum in the chiroptical spectra (ORD and CD) of the L-amino acid derivatives is always positive, while it is negative for the D-amino acid derivatives.

In addition to the twenty common building elements of proteins there are now several hundred amino acids known to occur in nature.[1] Most of these possess the L-configuration at the asymmetric α-C atom. However, D-amino acids are also frequently encountered, notably in microbial products. As new amino acids continue to be uncovered, the establishment of their absolute stereostructure poses an important recurring task.

Chiroptical methods have been utilized in various ways for the configurational analysis of amino acids.[2] The optically active $n \rightarrow \pi^*$ transition of the carboxyl group gives rise to a Cotton effect, the sign of which has been correlated with the absolute configuration of simple aliphatic amino acids.[3] In the presence of additional chromophoric substituents (as in aromatic amino acids) more complex chirospectral patterns have to be considered. In such cases it has been the practice to derivatize the amino acid under investigation with a reagent capable of producing a new chromophore, whose absorption is shifted to a region where the substituents do not absorb. Several methods have been suggested for this purpose[2,4] and some of their adherent disadvantages have also been noted.[4,5] For example, derivatization is often accompanied by partial racemization, and in other instances, the sign of the Cotton effect of the derivative is not a function of the configuration alone, but remains dependent upon the nature of the substituents at the α-carbon as well.

In recent communications from this laboratory,[6] it was reported that 2 - methoxy - 2,4 - diphenyl - 3(2*H*) - furanone (MDPF) **1** reacts with substances containing primary amino groups to form the corresponding N-substituted 3,5-diphenyl - 5 - hydroxy - 2 - pyrrolin - 4 - ones. Thus, L-amino acids afford the two diastereomeric products **2a** and **2b**, and D-amino acids yield the enantiomers of these compounds, **2c** and **2d** (Scheme 1). It has now been found that the characteristic multiple Cotton effects given by such chromophoric derivatives provide a means for the determination of the absolute configuration of chiral α-amino acids.

For the preparation of derivatives of type **2**, the amino acid under study (1 Eq) is allowed to react with MDPF **1** (1·1 Eq) in aqueous methanolic solution in the presence of triethylamine (1 Eq). Characteristically, the product is obtained upon workup and purification in 50 to 80% yield as an amorphous solid, consisting of two disatereomeric components.



Scheme 1.

The presence of two isomers is clearly revealed in the NMR spectra, which generally consist of two sets of signals. Most notably, two singlets between 8·1 and 9·2 ppm (in DMSO-d_6) with relative magnitudes of 1 : 1 to 1 : 2 are observed for the vinylic protons of the conjugated enamine systems. Attempts to separate the diastereomeric components have been unsuccessful due to rapid epimerization of the carbinolamine function.

The UV absorption spectra of compounds **2** (Fig. 1) contain an inflection at 215 to 220 nm ($\epsilon \sim 11{,}000$) and maxima at 280 to 285 nm ($\epsilon \sim 16{,}000$–$18{,}000$) and 380 to 390 nm (ϵ 6,000–6,500). As expected, these electronic transitions recognize the chirality of the α-C atom, and consequently the derivatives **2a,b** and **2c,d**, respectively, afford ORD and CD spectra with characteristic multiple Cotton effects.

Twenty-four amino acids, representing a wide variety of structural types, were derivatized with MDPF (**1**), and the chiroptical spectra of the resulting products were

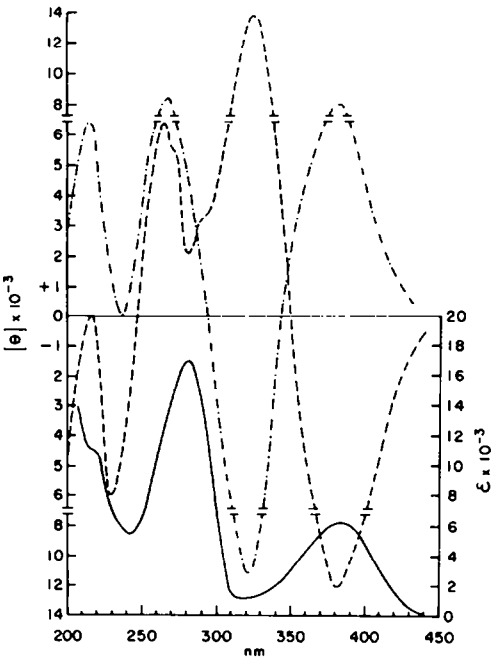


Fig. 1. UV-absorption (–) and CD-spectrum (–·–) of the L-threonine derivative **2a,b** [R = –CH(CO₂H)CH(OH)CH₃]; CD-spectrum (––) of the D-phenylalanine derivative **2c,d** [R = –CH(CO₂H)CH₂Ph] in ethanol.

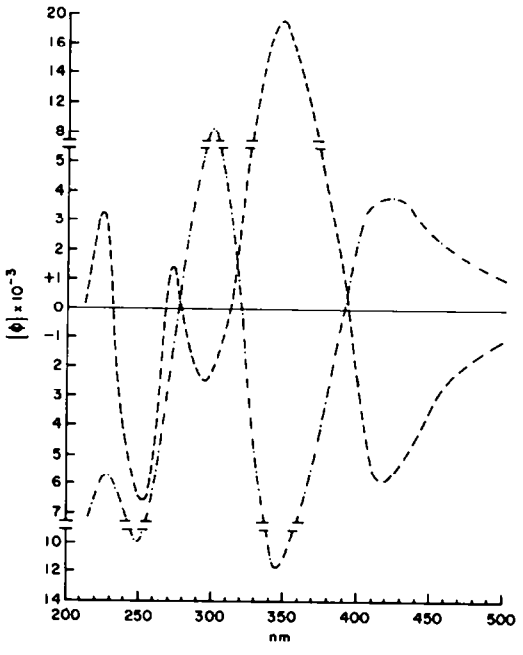


Fig. 2. ORD-spectra of the L-threonine derivative **2a,b** [R = –CH(CO₂H)CH(OH)CH₃] (–·–) and of the D-phenylalanine derivative **2c,d** [R = –CH(CO₂H)CH₂Ph] (––) in ethanol.

measured in ethanol† (Table 1). As examples, the CD- and ORD-spectra of the D-phenylalanine and L-threonine derivatives are depicted in Figs. 1 and 2.

At lower wavelengths (i.e. below 300 nm) the chiroptical spectra of compounds **2** show characteristic differences in position and magnitude of the extrema, depending on the substituents present in the starting amino acid. However, the sign of the longest wavelength ("first") extremum appears to be solely a function of the absolute configuration at the α-C atom of the parent amino acid, regardless of the nature of the substituents (Table 1). Without exception, the first extremum in the chiroptical spectra of the L-amino acid derivatives **2a,b** is positive, whereas it is negative in the spectra of the D-analogs **2c,d**. Accordingly, the ORD spectra of **2a,b** have a peak, and those of **2c,d** have a trough at 415–430 nm. The CD spectra of **2a,b** possess a maximum, and those of **2c,d** have a minimum at 376–386 nm.

Thus, the ease with which amino acids react with MDPF (**1**) combined with the consistent chirospectral properties of the resulting derivatives furnish a most convenient method for the determination of the absolute configuration of α-amino acids. It is believed that this method can be adapted to other classes of chiral amines as well.‡

EXPERIMENTAL

The reported chiroptical spectra were measured in EtOH (0·002–0·01 *M*) on a Durrum–Jasco Spectropolarimeter, Model ORD/CD/UV-5. No significant differences were observed when dioxane was used as the solvent. The requisite amino acid derivatives were prepared by a standard procedure, which was found to be very adaptable in scale.‡ The following example (with leucine) illustrates the general method.

†Dioxane is an equally suitable solvent.

‡For an example see the subsequent paper.[7]

Table 1. First and second extrema in the ORD and CD spectra of pyrrolinones **2**

	ORD*				CD*				
	1st		2nd			1st		2nd	
Derivative of:	λnm	[Φ]x10⁻³	λnm	[Φ]x10⁻³	a**	λnm	[θ]x10⁻³	λnm	[θ]x10⁻³
L-Alanine	428	+ 1.13°	347	- 2.87°	40	384	+ 1.58	327	- 8.41
D-Alanine	428	- 1.25°	348	+ 2.25	35	385	- 1.61	325	+ 7.38
L-Leucine	420	+ 6.25°	345	-13.75°	200	385	+10.12	324	-15.20
D-Leucine	422	- 6.75°	348	+10.62°	173	385	- 9.89	323	+14.05
L-Isoleucine	422	+ 4.25°	347	- 8.00°	123	385	+ 8.00	323	- 7.60
D-Isoleucine	423	- 5.75°	348	+ 5.75°	115	385	- 7.65	323	+ 6.52
L-Alloisoleucine	419	+ 4.75°	347	- 5.73°	105	385	+ 5.12	322	- 6.96
D-Alloisoleucine	422	- 5.00°	346	+ 5.50°	105	385	- 7.30	322	+ 7.90
L-Lysine	423	+ 4.00°	346	- 9.25°	111	391	+ 8.73	323	-11.00
L-Threonine	417	+ 5.75°	345	-11.75°	175	381	+ 8.15	322	-11.22
D-Threonine	417	- 6.24°	344	+11.99°	182	380	-11.16	323	+13.82
L-Leucine Amide	419	+ 9.49°	343	- 4.43°	139	380	+ 6.69	322	-11.03
L-Methionine	419	+ 5.75°	349	-13.25°	190	385	+11.20	325	-12.26
D-Methionine	422	- 6.25°	348	+11.75°	180	387	-10.62	325	+10.15
L-Glutamic Acid	415	+ 1.98°	344	- 2.88°	49	376	+ 2.59	321	- 5.90
D-Glutamic Acid	415	- 1.86°	348	+ 2.50°	44	376	- 2.78	320	- 5.08
L-Glutamine	415	+ 4.25°	347	- 7.50°	118	383	+ 5.82	325	- 6.60
D-Glutamine	420	- 4.00°	350	+ 6.25°	103	383	- 5.45	324	+ 4.36
L-Cystine	419	+ 4.50°	345	-26.09°	306	386	+15.22	323	-25.75
L-Phenylalanine	417	+ 4.75°	347	-18.75°	235	382	+11.48	323	-13.83
D-Phenylalanine	417	- 6.00°	347	+18.75°	247	382	-12.00	325	+13.82
D-Phenylglycine	420	-10.59°	346	+13.87°	248	385	-16.14	322	+16.14
L-Tryptophan	415	+ 4.21	343	-25.25°	295	384	+12.49	317	-19.40
D-Tryptophan	415	- 5.13°	342	+24.50°	296	383	-13.90	318	+19.02

* Recorded in ethanol (0.002 - 0.01M) on a DURRUM-JASCO Spectropolarimeter, Model ORD/CD/UV-5.

** Amplitude $a = \frac{[\Phi]_1 - [\Phi]_2}{100}$

(α - *S*) - 2,3 - *Dihydro* - 2,4 - *diphenyl* - 2 - *hydroxy* - 3 - *oxo* - α - (*isobutyl*)*pyrrole* - 1 - *acetic acid* [**2a,b**, R = $-CH_2CH(CH_3)_2$].

To a solution of L-leucine (52·5 mg; 0·4 mmol) and triethylamine (40 mg; 0·4 mmol) in 5 ml water/MeOH (1:1, v/v) at 50° was added, with stirring, a soln of MDPF, **1** (118 mg; 0·44 mmol) in 2·5 ml hot MeOH. The mixture was kept for 10 min at 50° and then allowed to cool to room temp. MeOH was removed under reduced pressure and the aqueous remainder was distributed between 0·01 *N* HCl and EtOAc. The organic phase was washed with water, dried over Na_2SO_4, and evaporated *in vacuo*. The residue was purified on a silica gel column (16 g) with a gradient mixture of $CHCl_3$/MeOH as the eluent. The chromatography was monitored by TLC, and fractions containing the desired material were combined and evaporated. The residue was triturated with ether to afford the product (pure by TLC) as a yellow powder (97 mg; 68%).

UV max (EtOH) 283 (ε 16,500), 387 nm (6,200); IR (KBr) 1635, 1600, 1555 cm^{-1}; NMR (DMSO-d_6) δ 8·91, 9·13 ppm [2s(1:1) > N–C*H* =].

ORD (0·01 *M*, EtOH) $[\phi]_{700}$ + 210; $[\phi]_{589}$ + 410; $[\phi]_{418}$ + 3,250 (peak); $[\phi]_{394}$ 0; $[\phi]_{345}$ − 13,750 (trough); $[\phi]_{332}$ 0; $[\phi]_{299}$ + 13,500 (pk); $[\phi]_{280}$ 0; $[\phi]_{260}$ − 8,750 (tr.); $[\phi]_{242}$ − 7,500 (sh.); $[\phi]_{228}$ − 4,250 (pk); CD (0·01 *M*, EtOH) $[\theta]_{460}$ 0; $[\theta]_{385}$ + 10,000 (max); $[\theta]_{350}$ 0; $[\theta]_{324}$ − 15,200 (min); $[\theta]_{300}$ 0; $[\theta]_{281}$ + 14,800 (max); $[\theta]_{255}$ + 3,400 (sh); $[\theta]_{238}$ + 400 (min); $[\theta]_{217}$ + 8,600 (max).

Acknowledgements—We thank Mrs. Bogda Wegrzynski for spectral measurements and Dr. Milan Uskokovic for helpful suggestions.

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