Optical Rotatory Dispersion and Circular Dichroism of Amino Acid Hydantoins

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Optical rotatory dispersion (ORD) and circular dichroism (CD) of 17 amino acid hydantoins were measured between 190 and 600 nm. Most of hydantoins exhibited the negative Cotton effect which showed the trough between 238 and 245 nm. The negative trough of CD was also observed between 212 and 236 nm. The Cotton effect of hydantoins was attributable to $n \rightarrow \pi^*$ transition of carbonyl group at C-4 of hydantoin ring.

Amino acid hydantoins are prepared with urea or potassium cyanate followed by the acidification with hydrochloric acid. The potassium cyanate method maintains the optical activity of hydantoin, whereas the urea method proceeds with loss of activity. Neuberger¹⁾ mentioned that the hydantoins derived from L-amino acids showed the negative molecular rotations at sodium D-line (589 nm), and that the derivatives were available to the stereochemistry of amino acids.

Stark²⁾ reported the application of hydantoin for the amino termini determination of peptide.

In this paper, ORD and CD of hydantoins are described.

EXPERIMENT

Preparation of the sample

Representative procedures were as follows.

1. *Preparation of 5-methylhydantoin (hydantoin derived from alanine).* L-Alanine 4.5 g (0.05 mole) and 4.9 g of potassium cyanate were dissolved in 30 ml water and were allowed to react for 30 min at 70°C with stirring. The reaction mixture was refluxed with 30 ml of 6 N hydrochloric acid. After filtration, the solution was allowed to stand overnight in a refrigerator. Recrystallization from hot water gave 4.5 g of the hydantoin in 79.0% yield.

2. *Preparation of 5-isobutylhydantoin (hydantoin derived from leucine).* L-Leucine 6.6 g (0.05 mole)

and 4.9 g of potassium cyanate were dissolved and suspended in 30 ml of water. Pyridine (*ca.* 25 ml) was added to dissolve the amino acid. The solution was allowed to react for 1 hr at 60°C with stirring. Pyridine was extracted with 300 ml of ethylacetate. The aqueous layer was refluxed for 30 min by the addition of 30 ml of 6 N hydrochloric acid and 30 ml of glacial acetic acid. The solution was allowed to stand overnight in a refrigerator. Recrystallization from hot ethanol-water or hot ethanol gave 5.8 g of the hydantoin in 74.3% yield.

According to the procedure-1, 12 hydantoins were prepared, including the derivatives of alanine, phenylalanine, aspartic acid, glutamic acid, lysine, arginine, histidine, methionine, cysteine, proline, serine and threonine. Histidine hydantoin was obtained by the adjustment of pH to 5 with 2 N sodium hydroxide after cyclization. Arginine hydantoin was prepared according to the Boon and Robson.³⁾ Lysine, which has ϵ -amino group, reacted with double the molar quantity of potassium cyanate, as a similar manner of phenylisothiocyanate. Reaction of other functional groups was in a similar manner of phenylthiohydantoin with the exception of serine and threonine.

Five hydantoins were prepared according to the procedure-2, including the derivatives of valine, leucine, isoleucine, tyrosine and tryptophan. Pyridine and acetic acid were added to dissolve the amino acid.

The hydantoins from glycine, asparagine and glutamine were not prepared, since glycine has no asymmetric carbon, and asparagine and glutamine were hydrolyzed to aspartic acid and glutamic acid respectively.

The all 17 hydantoins were checked by thin-layer chromatography (developer; *n*-butanol:acetic acid: water=4:1:2 or 3:1:1). They were confirmed to be one spot on each chromatogram, except threonine hydantoin (which is discussed later), both by the

detection with ninhydrin and tert-butylhypochlorite.

Equipments and operation of spectroscopy

a. *Infra-red spectroscopy.* Japan Spectroscopic Co., JASCO model IR-S. Samples were measured on KBr disk. Spectra were calibrated with polystyrene film.

b. *Nuclear magnetic resonance spectroscopy (NMR).* Varian T-60. Samples (ca. 30 mg) were dissolved in about 0.3 ml of d_6 -DMSO. Tetramethylsilane was used as the internal standard.

c. *ORD and CD.* JASCO model ORD/UV-5. Samples (ca. 100 mg) were weighed exactly and dissolved. ORD and CD were measured with 1 mm cell after the proper dilution by ethanol or water. Molecular rotations at 589 nm were measured with 10 mm cell. Region near 230 nm was also measured with 0.1 mm cell.

TABLE II. INFRA-RED ABSORPTION (cm^{-1}) OF CARBONYL GROUPS OF HYDANTOINS

Ala	1720	1740
Val	1720	1760
Leu	1730	1770
Ile	1700~1740 (broad)	
Asp	1680	1770
Glu	1630~1750 (broad)	
Phe	1730	1770
Tyr	1690~1740 (broad)	
Try	1710~1725 (broad)	1770
Met	1730	1770
CySO ₃ H	1690~1720 (broad)	1760
Ser	1690~1760 (broad)	
Thr	1690~1760 (broad)	
Lys	1700~1725 (broad)	1750
Arg	1720	1760
His	1725	1760
Pro	1695~1760 (broad)	

RESULTS AND DISCUSSION

1. Preparation of hydantoin—Especially serine and threonine hydantoin—

Melting points and elementary analysis were showed in Table I. Infra-red spectrum of leucine hydantoin showed in Fig. 1 as the representative. Peaks near 1770 cm^{-1} and 1730 cm^{-1} were attributable to the carbonyl groups at C-2 (ureido form) and at C-4 (amide-form) respectively (Table II).

In the case of thiohydantoins from serine and threonine (for example, phenylthiohydantoin), hydroxyl group is dehydrated and polymerized during cyclization, resulting colored compounds. Serine and threonine hydantoins were, however, scarcely dehydrated which were supported by elementary analysis and NMR. Especially, serine hydantoin seemed to be more stable than threonine hydantoin.

TABLE I. ELEMENTARY ANALYSIS AND Mp OF HYDANTOINS

Compd.	mp ($^{\circ}\text{C}$)	Calculated (%)			mp ($^{\circ}\text{C}$)	Found (%)		
	(lit.)	C	H	N	(exp.)	C	H	N
Ala-	174~7	42.10	5.30	24.55	177	41.72	5.31	24.47
Val	146~8	50.69	7.07	19.71	145	50.45	7.02	19.43
Leu	212~4	53.83	7.74	17.94	214	53.63	7.77	18.24
Ile	150~1	53.83	7.74	17.94	148	53.83	7.67	18.04
Asp	210~3	37.98	3.83	17.72	218	37.67	3.64	18.01
Glu	175~6	41.86	4.68	16.28	174	41.70	4.58	16.17
Phe	181~3	63.15	5.30	14.73	182	63.35	5.13	15.00
Tyr	261~4	58.25	4.89	13.58	262	58.10	4.82	13.50
Try	248~51	62.87	4.84	18.33	244	62.64	4.69	18.41
Met	103~5	41.36	5.79	16.08	104	41.23	5.65	16.40
CySO ₃ H	—	20.83	3.06	12.15	273d	20.54	2.11	12.24
Ser	187~8	36.93	4.65	21.53	188	36.61	4.49	21.54
Thr	193~6	41.66	5.59	19.44	200	41.87	5.34	19.33
Lys	195~7	44.85	6.59	26.16	193	44.85	6.64	26.15
Arg	100	34.36	6.18	28.62	100	34.30	6.04	28.45
His	235	28.81	4.19	25.86	280<	28.99	4.14	25.78
Pro	163~5	51.42	5.75	19.99	165	49.22	5.61	19.81

FIG. 1. Infra-red Spectrum of Leucine Hydantoin. 5-isobutylhydantoin

In NMR of serine hydantoin, protons of imino groups at N-1 and N-3 showed $\delta=7.87$ and 11.70 respectively. These peaks disappeared by the dilution with deuterium oxide. Proton of hydroxyl group, though not clear, appeared at 3.5~4.3. The triplet at 4.03 and doublet at 3.58 were accounted for a proton attached at asymmetric carbon-5 and two protons at β -carbon respectively ($J=3.0$ cps). Temperature was elevated to 60°C in order to shift the peak which was attributable to water.

In NMR of threonine hydantoin, protons attached to asymmetric carbon-5 and β -carbon appeared at 3.8~4.2 and two imino protons attached to N-1 and N-3 appeared at 7.86 and 10.53 respectively. Protons of hydroxyl and methyl group appeared at 4.87 and 1.15 respectively. In the case of threonine hydantoin, small amount of dehydration proceeds simultaneously. Methine and methyl group of dehydrated threonine hydantoin showed at 5.60 (quartet) and 1.76 (doublet) ($J=7.5$ cps) respectively.

2. ORD and CD.

Molecular rotations of 17 hydantoins had usually larger magnitude than those of phenylthiohydantoins⁴⁾ and methylthiohydantoins.⁵⁾

The found magnitude of molecular rotation, however, was not constant, because of tautomerization which has been studied with ultraviolet spectra⁶⁾ and with several kinds of peptide derivatives.⁷⁾ Found molecular rotations at 589 nm of prepared hydantoins were smaller than those in the literature¹⁾ (Table III).

Thirteen hydantoins, including the derivatives of alanine, valine, leucine, isoleucine, phenylalanine, serine, threonine, aspartic acid,

TABLE III. " $[\alpha]_D$ " OF HYDANTOINS

	Found Temp.	Solv.	Literature ¹⁾ Solv.
Ala	- 48°	28°C E	- 58°C W
Val	-134	28 E	
Leu	- 94	28 E	-148 50%-E
Ile	- 99	28 E	
Asp	-150	24 W	-198 1 N NaOH
Glu	-119	26 W	-136 1 N NaOH
Phe	-182	24 E	-183 50%-E
Tyr	-165	23 E	-292 1 N NaOH
Try	-161	28 E	
Met	- 62	28 E	
CySO ₃ H	0	24 W	
Ser	-281	28 E	
Thr	-168	26 E	
Lys	-118	28 W	
Agr	-101	28 W	
His	- 10	24 W	
Pro	-169	28 E	-334 W

solvent: E; ethanol, W; water.

FIG. 2. ORD Curves of Hydantoins.

Alanine, valine, leucine, isoleucine, aspartic acid, glutamic acid, methionine, phenylalanine, lysine, arginine, histidine, serine and threonine.

glutamic acid, lysine, arginine, histidine and methionine, showed the negative Cotton effect between 238 and 245 nm (Fig. 2). The Cotton effect revealed between 240 and 244 nm in ethanol. The Cotton effect shifted by solvent effect in water. Dispersion curves crossed over the base line which agreed theoretically with the minimum wave length of CD. The peak of ORD were not-measurable, even by 0.1 mm cell, because of the disturbance of the strong ultraviolet absorption below 220 nm, mainly ring absorption.

Three hydantoins, including tyrosine, tryptophan and proline, showed the characteristic curves (Fig. 3).

Tyrosine hydantoin showed the negative Cotton effect at 248 nm. The trough of CD was observed at 234 nm and the peak was at 207 nm. Measurement between 270 and 300 nm, however, was not clear. It likely showed an additional Cotton effect in the range of absorption band of the aromatic group.

FIG. 3. ORD Curves of Hydantoins.

Tyrosine, proline and tryptophan.

Proline hydantoin

Tryptophan hydantoin showed the negative Cotton effect which had the trough at 256 nm. Measurement was not clear between 260 and 290 nm. The CD peak and trough were observed at 274 nm and 240 nm. Crossing over of CD showed at 258 nm.

Proline hydantoin showed an unique ORD curve. Molecular rotation enlarged gradually from 290 nm and the peak was at 253 nm. Curve also showed the trough at 223 nm and crossed over about 245 nm, and the peak showed about 214 nm. It predicted that the asymmetric structure of proline showed the positive Cotton effect according to C-4 carbonyl group.

Molecular rotations of hydantoins were

not related with the side chains which were derived from amino acids. Molecular rotations of amino acid hydantoins, except serine hydantoins, were varied between 870 and 8,000. Serine hydantoin indicated large molecular rotation (10,000~12,000). It was interesting that serine hydantoin showed large

molecular rotation in contrast to thiohydantoin which lost the optical activity by dehydration and formation of double bond or polymerization.

3. Absolute configuration of hydantoin—Application of "octant rule"—

Djerassi *et al.*⁴⁾ reported that the positive Cotton effect of L-amino acid phenylthiohydantoin was attributable to $n \rightarrow \pi^*$ transition of thiocarbonyl group at C-2. The positive Cotton effect could be expected for the carbonyl group at C-2 of L-amino acid hydantoin. L-Amino acid hydantoin, however, gave the negative Cotton effect. The negative Cotton effect, therefore, was contributable to the carbonyl group at C-4. In D-alanine hydantoin, ORD and CD curve showed symmetrically for L-alanine hydantoin.

TABLE IV. $[\theta]$ OF HYDANTOINS

	$\lambda_{\min}$	$\lambda_{\max}$ (nm)
Ala ⁻	231(nm)	-2610
Val	232	-8110
Leu	231	-5130
Ile	234	-5350
Asp [*]	215	-16900
Glu	221	-7570
Phe	233	-8990
Tyr [*]	234	-3810
Try [*]	241	-4290
Met	236	-3860
Ser	232	-20160
Thr [*]	232	-15050
Lys [*]	212	-9000
Arg [*]	224	-7910
His	Not measured	
Pro	214	238 + 8300

* 0.1 mm cell.

TABLE V. $[M]$ OF HYDANTOINS

	350	300	280	260 nm	$[M]_{\min}$ nm	Cross over nm
Ala	-240	-450	-730	-1600	244	-3050
Val	-600	-1100	-1640	-3350	246	-5480
Leu	-400	-690	-990	-1890	244	-3480
Ile	-460	-860	-1290	-2520	246	-4000
Asp [*]	-630	-1200	-1640	-2840	238	-5990
Glu	-590	-1030	-1520	-2790	241	-6030
Phe	-840	-1590	-2410	-4600	244	-7920
Met	-280	-520	-770	-1640	245	-2910
Ser	-1230	-2140	-3320	-6950	245	-12350
Thr [*]	-730	-1380	-1940	-3230	245	-5820
Lys [*]	-510	-930	-1450	-2380	240	-5170
Arg [*]	-460	-850	-1270	-2340	240	-5045
His	-70	-150	-180	-400	244	-870

Tyr^{*} ; 350 nm (-460), 301 (min -760), 290 (-720), 272 (cross over), 270 (-210), 260 (-330), 250 (-1340), 248 (min -1510), 240 (-1240), 234 (cross over), 230 (+1340), 220 (+3820), 218 (max +4230), 210 (+2060).

Try^{*} ; 350 (-570), 344 (min -590), 340 (-510), 330 (-450), 320 (-390), 310 (-280), 304 (cross over), 275 (cross over), 256 (-1060), 250 (-1000), 240 (-830).

Pro ; 350 (-710), 300 (-990), 290 (-1030), 280 (-940), 270 (-420), 267 (cross over), 260 (+1360), 253 (max +3080), 245 (cross over), 240 (-3790) 223 (min —).

* ; 0.1 mm cell. Tyr, Try, Pro; wave length nm ($[M]$).

In this way, "octant rule" may apply to these derivatives in a similar manner. This agreed with the absolute configuration of amino acids which are determined with X-ray diffraction.⁸⁾

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