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A Note on the Racemization of Serine

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According to Daft & Coghill (1931), the successful preparation of DL-serine from sericin by the method of Cramer (1865) depended on the intermediate mixture of serine and other amino-acids being kept for 1 hr. at room temperature in the presence of baryta at pH 9 before the final crystallization stages were carried out. These authors believed that the baryta treatment caused racemization of the serine, but no experimental evidence was produced in support of this idea. In view of the resistance to racemization by alkali observed in the case of certain

amino-acids, it seemed remarkable that serine should be so labile. L-Tyrosine, for example, is completely racemized only after 30 hr. boiling with 5N-sodium hydroxide (Gunness, Dwyer & Stokes, 1946). L-Serine was required for certain investigations in this laboratory, and therefore it was a matter of practical importance as well as of theoretical interest to study the effect of alkali in more detail.

The results obtained under various conditions are shown in Table 1. It will be seen that even in the

Table 1. *Optical rotation and racemization studies of D-serine and D-alanine at various pH values*

Amino-acid	$[\alpha]_D^{20}$	Solvent	<i>c</i>	Exp. no.	Initial $[\alpha]_D$ in NaOH† solution*	pH	Duration of racemization exp. (hr.)	Temp. (°)	Final $[\alpha]_D$ of solution*
Serine	$+6.65 \pm 0.05^\circ$	Water	9.7	1	$+6.4^\circ \pm 0.3^\circ$ (18°; <i>c</i> , 1.0)	8.9	192	18	$+6.4^\circ \pm 0.3^\circ$ (18°; <i>c</i> , 1.0)
				2	$+4.6^\circ \pm 0.3^\circ$ (18°; <i>c</i> , 0.9)	9.9	18	18	$+4.6^\circ \pm 0.3^\circ$ (18°; <i>c</i> , 0.9)
							2.25	100	$+4.6^\circ \pm 0.3^\circ$ (18°; <i>c</i> , 0.9)
				3	$+3.0^\circ \pm 0.1^\circ$ (19°; <i>c</i> , 10.5)	11.4	2.5	18	$+3.0^\circ \pm 0.1^\circ$ (18°; <i>c</i> , 10.5)
							4.25	105	$+2.7^\circ \pm 0.1^\circ$ (18°; <i>c</i> , 10.5)
							17	105	$+1.9^\circ \pm 0.1^\circ$ (18°; <i>c</i> , 10.5)
Alanine	$-10.0^\circ \pm 0.2^\circ$	N-HCl	5.8	4	$+3.2^\circ \pm 0.1^\circ$ (19°; <i>c</i> , 10.0)	> 13	22	18	$+3.0^\circ \pm 0.1^\circ$ (18°; <i>c</i> , 10.0)
				5†	$+3.4^\circ \pm 0.1^\circ$ (20°; <i>c</i> , 4.3)	10	18	18	$+3.4^\circ \pm 0.1^\circ$ (20°; <i>c</i> , 4.3)
				6	$-3.52^\circ \pm 0.1^\circ$ (20°; <i>c</i> , 8.0)	12.4	16	105	$-3.52^\circ \pm 0.1^\circ$ (20°; <i>c</i> , 8.0)
	$-10.7^\circ \pm 0.2^\circ$	N-HCl	5.8	7	$-3.95^\circ \pm 0.1^\circ$ (20°; <i>c</i> , 7.3)	13.4	16	105	$-3.95^\circ \pm 0.1^\circ$ (20°; <i>c</i> , 7.3)

* *t* and *c* values in parentheses.

† Exp. no. 5, Ba(OH)₂ and not NaOH was used.

presence of excess of *N*-sodium hydroxide (Exp. 4) the optical rotation of *D*-serine was unchanged after 22 hr. at room temperature. The assumption of Daft & Coghill (1931) regarding the racemization of serine was therefore incorrect. On the other hand, comparison of Exp. 3 with Exps. 6 and 7 showed that *D*-serine was relatively easily racemized at 105° and pH 11.4, whereas *D*-alanine remained unchanged even at pH 13.4. It was necessary to consider the possibility that part at least of the observed fall in rotation was due to decomposition of the serine and not to racemization. Daft & Coghill (1931) have already shown that serine is slowly decomposed by hot concentrated alkali into a complex mixture of products including ammonia, pyruvic acid, alanine and glycine. In view of the vigorous conditions necessary to decompose serine it seemed probable that racemization alone had occurred in our case. This was confirmed by the observation that at pH 11.4 and 105° serine evolved no ammonia and no amino-acid except serine remained in solution.

The observation made by Daft & Coghill (1931) in their preparation of *DL*-serine is difficult to explain. It is possible that some racemization of the serine residues occurred during the acid hydrolysis of the protein, but in the absence of a method for the quantitative isolation of serine in an amount sufficient for accurate polarimetric measurement this must remain an open question. Serine having full optical activity can be isolated in fairly good yield from silk as the *p*-hydroxyazobenzene-*p'*-sulphonate by the method of Stein, Moore, Stamm, Chou & Bergmann (1942), but this does not shed any light on the problem because of the lower solubility of the optically active salt compared with that of the *DL*-isomer (see Experimental section).

EXPERIMENTAL

Resolution of *DL*-serine. *N*-*p*-Nitrobenzoylserine was prepared in 75% yield by the method used recently by Zambito, Peretz & Howe (1949) for the *p*-nitrobenzoylation of *DL*-threonine. This method is superior to that of Fischer & Jacobs (1906). Resolution of the nitrobenzoyl derivative was carried out according to the latter authors.

Racemization studies on *D*-serine and *D*-alanine. Optical rotations of the solutions immediately after preparation are

recorded in Table 1. pH values were determined by the glass electrode. Experiments with serine at 100° were carried out in sealed tubes to avoid darkening. The serine from Exp. 3 was isolated by acidifying the solution with conc. HCl, evaporating to dryness under reduced pressure, extracting the residue with hot ethanol, filtering and adding conc. ammonia solution to the filtrate. After cooling, the serine was collected and crystallized from aqueous ethanol. The yield was 0.21 g. of serine, $[\alpha]_D^{20} + 3.9^\circ \pm 0.4^\circ$ in water (*c*, 1.5); the experiment was commenced with 0.6 g. serine. This sample was therefore 42% racemized, comparing well with the value of 35% expected from the rotation of the solution.

The stability of *DL*-serine under the conditions of Exp. 3 was demonstrated as follows. A pure sample of *DL*-serine (0.6 g.) and *N*-NaOH (5.7 ml.) were placed in one limb of a double-limbed tube. The second limb contained 2 ml. of *N*-H₂SO₄ which was approximately isopiestic with the serine solution. After 17 hr. at 105°, back-titration of the H₂SO₄ revealed that not more than 1% of the serine nitrogen had been evolved as volatile material. The alkaline serine solution was neutralized with HCl and submitted to paper chromatography using aqueous phenol as solvent; as a control, a solution of serine containing the same amount of NaCl as the unknown was used. No other amino-acid was found in the alkali-treated serine, and the two serine spots were in identical positions on the chromatograms.

Determination of the solubilities of *p*-hydroxyazobenzene-*p'*-sulphonic acid salts of *DL*- and *D*-serine at 20°. The pure salts were dissolved in hot water and the solutions allowed to crystallize for 16 hr. Measured volumes of the supernatant were evaporated to dryness in a vacuum desiccator and the residue weighed. Experiments were carried out in duplicate. Solubility was also determined by shaking the powdered solids for 16 hr. with water, then filtering and proceeding as before. The solubility of the *DL*-salt was found to be 3.5 g. $\pm$ 0.1 g./100 ml. of solution; that of the *D*-isomer was 1.9 g. $\pm$ 0.1 g./100 ml. of solution.

SUMMARY

Serine was not racemized at pH 9 at room temperature as Daft & Coghill (1931) supposed, neither was it racemized by excess of *N*-NaOH at room temperature. On the other hand, it was found to be racemized at 105° and pH 11.4, whereas alanine was unchanged at pH 13.4 and 105°.

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