Walden Inversion of Amino Acids. VII. The Formation of N-*p*-Toluenesulfonylproline from N^{δ}-*p*-Toluenesulfonylornithine[1]

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(Received June 16, 1952)

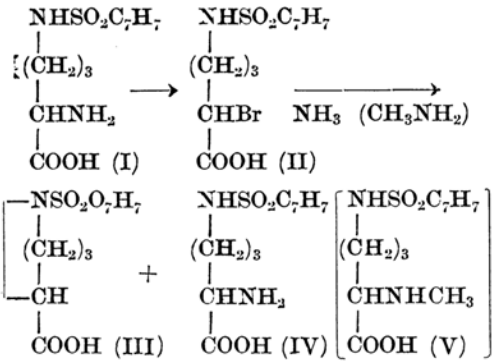
Formation of proline by the reaction of ammonia on δ-nitroguanidylamino-α-bromovaleric acid prepared by the action of nitrosyl bromide on nitroarginine was distinctly shown on a paper chromatogram.[2] The author has assumed for explanation of the phenomenon that the formation of N-nitroguanidylproline had occurred after the splitting-off of hydrogen bromide from α-bromine atom and δ-imino hydrogen atom, and the subsequent hydrolysis of the N-nitroguanidyl group took place. By the amination of δ-benzoylamino-α-bromovaleric acid, only N^{δ}-benzoylornithine was obtained, and there was no formation of N-benzoylproline.[3] Therefore, it may be concluded that the degree of formation of a pyrolidinyl ring depends upon the degree to which the functional group combined with the δ-amino group activates the δ-imino hydrogen atom.

It is well known that the toluenesulfonyl group activates the imino hydrogen atom belonging to the same group on a compound which has the toluenesulfonylamino group. In the present paper, the formation of N-*p*-toluenesulfonylproline was practically occurred in the case of the reaction of ammonia and methylamine on [L]-δ-*p*-toluenesulfonylamino-α-bromovaleric acid, and the configurations of resulting N-*p*-toluenesulfonylprolines were studied comparing with the specific rotation of N-*p*-toluenesulfonyl-L-proline. The reactions were carried out as follows:

(1) The present paper was read at the 5th Annual Meeting of the Chemical Society of Japan, April 4, 1952, Tokyo.

(2) N. Izumiya, unpublished.

(3) N. Izumiya, *J. Chem. Soc. Japan, Pure Chem. Sect.*, **72**, 149 (1951).



By the amination at 0° of [L]-δ-*p*-toluenesulfonylamino-α-bromovaleric acid (II), N-*p*-toluenesulfonylproline (m. p. 129—132°, D:DL=11:89) was obtained in a good yield and N^{δ}-*p*-toluenesulfonylornithine (IV) was detected by paper chromatography. The amination of (II) at 100°, gave the compound (III) (m. p. 130—133°, pure DL-form) in a good yield, and the compound (IV) in a small quantity. The methylamination at 0° as well as 100° gave the compound (III) (oily state, D:DL=27—31:73—69), and N^{δ}-*p*-toluenesulfonyl-N^{α}-methylornithine (V) which was detectable only by paper chromatography. In order to confirm the configurational ratios of the compounds (III), these were hydrolysed with hydrobromic acid, prolines isolated through rhodanilate, and their specific rotations measured.

Kapfhammer and Eck[4] reported the synthesis of N-*p*-toluenesulfonyl-L-proline (VI) melting at 130—133°, and that it was easily crystallized, while McChesney,[5] however, reported that the substance (VI) could not be crystallized. The specific rotation of (VI) was not given by either of them. In order to find the specific rotation of it, the present author synthesized (VI) in the usual way, and it was not easily crystallizable. After various trials, however, (VI) melting at 41—43° was crystallized, while N-*p*-toluenesulfonyl-DL-proline was easily obtained crystalline and melted at 134°. Hence, Kapfhammer and Eck must have synthesized N-*p*-toluenesulfonyl-DL-proline instead of N-*p*-toluenesulfonyl-L-proline.

The author had supposed that there was some difference in the mechanism of reaction between amination or methylamination on δ-*p*-toluenesulfonylamino-α-bromovaleric acid (II) and that on δ-benzoylamino-α-bromovaleric acid (VII). So that, the splitting-off velocity of bromine ion was measured under various conditions, but no difference in the velocity was observed between (II) and (VII).

(4) J. Kapfhammer and E. Eck, *Z. physiol. Chem.*, **170**, 294 (1927).
(5) E. W. McChesney, *J. Am. Chem. Soc.*, **59**, 1116 (1937).

Experimental

N-*p*-Toluenesulfonyl-DL-proline.—DL-Proline[6] (0.01 mol.), *p*-toluenesulfonyl chloride (0.014 mol.) and 2N-sodium hydroxide (0.03 mol.) were made to react in the usual way.[7] The solution was acidified with hydrochloric acid, and the oily substance formed was crystallized immediately (m. p. 130—133°). This was recrystallized twice from 40% alcohol. m. p. 134°, rhombic plates. Found: N, 5.10%; neut. equiv., 273. Calcd. for $C_{12}H_{15}O_4NS$: N, 5.19%; neut. equiv., 270.3.

N-*p*-Toluenesulfonyl-L-proline.—L-Proline (0.01 mol), having $[\alpha]_D^{19}=-84.6°$ (c=2.55, water), *p*-toluenesulfonyl chloride (0.01 mol., recrystallized twice from acetone-water) and 2N-sodium hydroxide (0.02 mol.) were made to react. The oil produced by the addition of hydrochloric acid was extracted with chloroform. The chloroform solution was washed with water, and reextracted with aqueous sodium bicarbonate. It was acidified with hydrochloric acid, and the resulting oil was allowed to stand in an ice box for 1 to 2 days, and crystallized in needles (m. p. 39—42°). The extraction with chloroform and sodium bicarbonate and the addition of hydrochloric acid were repeated. m. p. 41—43° (reported m. p. 130—133°,[4] oily state[5]), $[\alpha]_D^{19}=-158.5°$ (c=2.21, 2 equivs. of NaOH) and $[\alpha]_D^{19}=-92.5°$ (c=1.78, alcohol). Found: N, 5.14%; neut. equiv., 276. Calcd: N, 5.19%; neut. equiv., 270.3.

N^{δ}-*p*-Toluenesulfonyl-L-ornithine(I).—This was prepared by applying Kurtz's procedure[8] from L-ornithine monohydrochloride. m. p. 236—238° (decomp.) (reported m. p. 210—215°(9)), $[\alpha]_D^{20}=+14.9°$ (c=2.23, 2 equivs. of HCl) and $[\alpha]_D^{20}=+23.4°$ (c=1.85, 3N-HCl). The regenerate copper complex melted at 240—242° with decomposition. Found: N, 8.71%. Calcd. for $(C_{12}H_{17}N_2O_4S)_2Cu$: N, 8.83%.

[L]-δ-*p*-Toluenesulfonylamino-α-bromovaleric acid (II).—(I) (0.01 mol) and potassium bromide (0.035 mol) were dissolved in 2.5N-sulfuric acid (0.052 mol). To the mixture was added sodium nitrite (0.016 mol) in portions during 1 hour with stirring at ice cooling, and the stirring was continued thereafter for 2 hours at room temperature. The resulting pale yellow oil was washed with water by decantation until bromine ion became undetectable. It was then dried in a vacuum desiccator. The yield was 95%. $[\alpha]_D^{15}=-35.2°$ (c=3.05, alcohol). Found: N, 4.14%. Calcd. for $C_{12}H_{16}NO_4SBr$: N, 4.00%. Though the dried substance and the alcoholic solution were allowed to stand at room temperature (10—20°) for 2 weeks, there was no change in their specific rotation.

Amination of (II) at 100°.—(II) (3.50 g.) was

(6) D. Hamer and J. P. Greenstein, *J. Biol. Chem.*, **193**, 81 (1951).
(7) E. Fischer and W. Lipschitz, *Ber.*, **48**, 360 (1915).
(8) A. C. Kurtz, *J. Biol. Chem.*, **180**, 1253 (1949).
(9) H. N. Christensen, *J. Biol. Chem.*, **160**, 75 (1945).

dissolved in 30 cc. of 28% ammonia and heated in a sealed tube at 100° for 20 minutes. The solution was evaporated to dryness under reduced pressure, and the residue was collected by the aid of water, washing it several times with water (the filtrate and washings were to be used in the next step). It crystallized out in small rectangular plates from hydrochloric acid and ammonia, and there was obtained 0.17 g. (yield 6%). This was N^{δ}-*p*-toluenesulfonyl-DL-ornithine, and had m. p. 229-232° (decomp.) and no rotation. Found: N, 9.85%. Calcd. for $C_{12}H_{18}N_2O_4S$: N, 9.79%. Its copper complex had m. p. 244—246° (decomp.). Found: N, 8.68%. Calcd.: N, 8.83%. The filtrate and washings combined were acidified with hydrochloric acid, and the oil formed solidified immediately. This was recrystallized once from 40% alcohol, and obtained 1.95 g. (yield 72%). This was N-*p*-toluenesulfonyl-DL-proline (III), and had m. p. 131—133° and no rotation. Found: neut. equiv., 277. Calcd.: neut. equiv., 270.3. (III) (0.01 mol) was refluxed with 48% hydrobromic acid (30 cc.) for 2 hours. The solution was evaporated under reduced pressure, and the concentration was repeated with the addition of water. The residue was dissolved in water (30 cc.). To the solution was added ammonium rhodanilate (0.008 mol) dissolved in methylalcohol (55 cc.). DL-Proline rhodanilate was obtained 72% yield, and had m. p. 130—133°. DL-Proline was isolated from rhodanilate by Bergmann's method,[10] and had m. p. 202—204° (decomp.) (reported m. p. 203—204°[11]).

Amination of (II) at 0°.—(II) was dissolved in ammonia in the manner described above and kept at 0° for 10 days. N-*p*-Toluenesulfonylproline (III) was obtained in 69% yield, having m. p. 129—132° and $[\alpha]_D^{10}=+17.4°$ (c=2.12, 2 equivs. of NaOH). Found: neut. equiv., 279. Calcd.: neut. equiv., 270.3. It was shown that (III) are composed of approximately 11% D-form and 89% DL-form. In another experiment, (III) had $[\alpha]_D^{10}=+15.5°$ (c=1.98, 2 equivs. of NaOH). From the filtrate of the compound (III), N^{δ}-*p*-toluenesulfonylornithine was detected by a paper chromatography, though its isolation was unsaccessful. Proline was isolated from (III) ($[\alpha]_D^{19}=+17.4°$) in the manner described above, and had $[\alpha]_D^{10}=+8.4°$ (c=1.88, water).

Methylamination of (II) at 100°.—(II) (0.01 mol) was dissolved in 35 cc. of aqueous methylamine (33%) and heated at 100° for 10 minutes. The solution was concentrated under reduced pressure, and the residue was dissolved in water, and acidified with hydrochloric acid. The oily N-*p*-toluenesulfonylproline (III) formed could not be solidified even when it had been allowed to stand in an ice-box for several weeks. This was washed with water by decantation, and dried in a vacuum desiccator. The yield was 77%. It had $[\alpha]_D^{10}=+42.6°$ (c=2.02, 2 equivs. of NaOH), showing it to be a mixture of 27% D-form and 73% DL-form. Found: neut. equiv., 283. Calcd.: neut. equiv., 270.8. Proline isolated had $[\alpha]_D^{19}=+21.6°$ (c=1.85, water). N^{δ}-*p*-Toluenesulfonyl-N^{α}-methylornithine (V) has never been synthesized, so that its *Rf* value on paper chromatogram is unknown. The mother liquid obtained by decantation was tested by paper chromatography, and it showed only one spot of ninhydrine reaction. The *Rf* values of this spot and N^{δ}-*p*-toluenesulfonylornithine in various solvents were compared with *Rf* values of N^{δ}-benzoylornithine and N^{δ}-benzoyl-N^{α}-methylornithine. From the observed regularity, the substance showing a spot of ninhydrine reaction was considered to be the compound (V) which could not be isolated. *Rf* values are shown in Table 1. The composition of each solvent was the same as in Part VI of this Series.[12]

Table 1

Rf values

Amino acid	Solvent: Phenol	Collidine + Lutidine	Acetic Acid + Butanol
N^{δ}-*p*-T. S.-L-ornithine	0.91	0.72	0.71
N^{δ}-*p*-T. S.-N^{α}-methylornithine	0.97	0.77	0.74
N^{δ}-Benz.-L-ornithine	0.94	0.63	0.69
N^{δ}-Benz.-DL-N^{α}-methylornithine	0.98	0.67	0.72

Methylamination of (II) at 0°.—(II) was kept at 0° for 4 days. The oily N-*p*-toluenesulfonylproline had $[\alpha]_D^{19}=+48.3°$ (c=2.08, 2 equivs. of NaOH), showing it to be a mixture of 31% D-form and 69% DL-form. The yield was 81%. Found: neut. equiv., 285. Calcd.: neut. equiv., 270.3. Proline isolated had $[\alpha]_D^{19}=+24.7°$ (c=1.91, water).

Splitting-off Velocity of Bromine Ion from

Table 1

Splitting-off % of Br Ion at 0°

Hours	Compound (II) NH_3	Compound (II) CH_3NH_2	Compound (VII) NH_3	Compound (VII) CH_3NH_2
1		37.0		36.8
2	2.2	50.5	2.3	51.1
4		70.1		71.2
10	11.1	91.5	11.7	90.9
20	23.1	98.3	23.3	98.6
40	45.0	98.6	46.1	98.7
100	77.9		78.8	
200	95.6		95.5	
300	98.5		99.1	

(10) M. Bergmann, *J. Biol. Chem.*, **110**, 471 (1935).
(11) N. F. Albertson and J. L. Fillman, *J. Am. Chem. Soc.*, **71**, 2818 (1949).
(12) N. Izumiya and A. Nagamatsu, This Bulletin, **25**, 265 (1952).

Table 2

Minutes	Splitting-off % of Br Ion at 100° Compound (II)		Compound (VII)	
	NH_3	CH_3NH_2	NH_3	CH_3NH_2
0.25		78.0		81.4
0.5	60.5	93.3	64.0	91.8
1	81.6	99.1	80.9	100.0
2	92.8	100.1	93.2	99.8
4	98.6	99.9	98.9	100.2
6	99.5		99.7	
10	100.2		99.9	

α-Bromo Acids.—[L]-δ-*p*-Toluenesulfonylamino-α-bromovaleric acid (II) and [L]-δ-benzoylamino-α-bromovaleric acid (VII)[3] were used as α-bromo acids. 0.001 mol. of (II) or (VII) was dissolved in 2.72 cc. of 28% ammonia (0.04 mol.) or in 3.77 cc. of 33% methylamine (0.04 mol.). At adequate intervals at 0° or 100°, to 0.2 cc. of the reaction mixture was added 2 cc. of 0.05 N-silver nitrate (its solvent was 7 N-nitric acid), and 1 cc. of the filtrate was titrated with 0.05 N-thiocyanate solution. The results are shown in Tables 1 and 2.

Summary

(i) Kapfhammer and Eck's report touching the synthesis of N-*p*-toluenesulfonyl-L-proline was corrected.

(ii) N-*p*-Toluenesulfonylproline was resulted by the amination and methylamination of [L]-δ-*p*-toluenesulfonylamino-α-bromovaleric acid. In this case, racemization took place in consequence of amination at 100°, and partial Walden inversions were caused by amination at 0° and methylamination at 0° and 100°.

This work was carried out partly with a grant from the Scientific Research Funds of the Ministry of Education. The author wishes to thank Prof. Dr. R. Hirohata for his guidance in carrying out this work, and also Mr. S. Saeki for his microanalysis of the substances.

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