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# Enzymatic Resolution of O-Methyl-N-acetyl-DL-serine. Amino Acids. XXXII*

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Received December 23, 1955

Enzymatic resolution of *O*-methyl-*N*-acetyl-DL-serine was carried out following Greenstein's procedure. The thus obtained *O*-methyl-L-serine was converted under partial racemisation into *O*-methyl-*N*-phthaloyl-L-serine, by reaction with phthalic anhydride.

Greenstein and coworkers have used hog kidney enzymes for the resolution of forty-six different amino acids[1]. It has been shown that the majority of amino acids obtained through enzymatic resolution were 99.9% optically pure[1,2]. In the first reports of the use of hog kidney enzymes a filtered crude homogenate was used as the resolving agent[3]. The activity of this material was satisfactory for most resolutions but was ineffective in some cases. Acetone and salt fractionation of the crude homogenate yielded a fraction which was thirty times more active than the original solution[4]. This fraction is known as acylase I. Acyl-DL-aspartic acid was resolvable with the crude enzyme but not with acylase I. From the crude enzyme a fraction was isolated four times more active toward acyl-DL-aspartic acid than was the crude homogenate. This fraction is known as acylase II.

Resolution was found to be possible with all aliphatic amino acids from $C_2$ to $C_{11}$ using acylase I. In all these resolutions the D-isomer was unchanged and the L-isomer was isolated as the free amino acid.** Isolation of some amino acids has been facilitated by ion exchange chromatography[5].

The only *O*-methyl-substituted amino-hydroxy-acid resolution has been that carried out on *O*-methyl-threonine, following Bergmann and Fruton[6], with activated papain[7].

In our laboratory a synthesis of *threo*-DL-1-*p*-nitrophenyl-2-dichloroacetoamido-1,3-propanediol from DL-serine ethyl ether was performed[8-10]. For this synthesis optically active serine ethyl ether of known configuration was needed. Optically active *N*-phthaloyl-*O*-alkyl-serine of unknown configuration could be obtained by fractional crystallization of their brucine salts[11].

Therefore, we subjected *O*-methyl-*N*-acetyl-serine[12] to enzymatic resolution and converted the thus obtained *O*-methyl-L-serine to *O*-methyl-*N*-phthaloyl-L-serine with $[\alpha]_D$-17.7°, and compared this compound with optically active *O*-methyl-*N*-phthaloyl-serine obtained by fractional crystallization of the brucine salt[11]. It seems that during the reaction of *O*-methyl-L-serine with phthalic anhydride considerable racemization occurs***, but for determination

* Communication No. 50 from the Chemical Institute. Paper No. VI of Studies in the Chloramphenicol Series; for No. V see ref. 11.

** For an excellent review on the resolution of racemic α-amino acids see ref. 18.

*** For similar behaviour of *S*-benzyl-L-cystein in reaction with phthalic anhydride see ref. 13.

of the configuration of *O*-methyl-*N*-phthaloyl-serine, in this case only the sign of rotation is important. It was thus established that laevorotatory *O*-methyl-*N*-phthaloyl-serine belongs to the L-series of amino acids.

We also prepared *O*-ethyl-*N*-acetyl-serine, and subjected it to enzymatic resolution.

*O*-Methyl-*N*-acetyl-D-serine and *O*-ethyl-*N*-acetyl-D-serine had $[\alpha]_D$—10° and $[\alpha]_D$—17° respectively.

## EXPERIMENTAL

All melting points are uncorrected.

### *Starting materials*

O-*Methyl*-DL-*serine* was prepared according to Schiltz and Carter[14].

O-*Ethyl*-DL-*serine* was prepared according to Wood and du Vigneaud[15].

O-*Methyl*-N-*acetyl*-DL-*serine* was prepared according to Synge[12]. O-*Ethyl*-N-*acetyl*-DL-*serine* was also prepared in this manner, for the first time.

*Acylase* I was prepared according to Greenstein, Birnbaum and Levintow[16].

O-*Ethyl*-N-*acetyl*-DL-*serine* was prepared from α-bromo-β-ethoxypropionic acid (25 g.) after reaction with ammonia. The thus obtained crude mixture of α-amino-β-ethoxypropionic acid and ammonium bromide was treated with excess 5% sodium hydroxide and evaporated to dryness on a water bath. The obtained ammonia free mixture of α-amino-β-ethoxypropionic acid and sodium bromide was dissolved in 5% sodium hydroxide (100 ml.), magnesium carbonate (6 g.) was added, and this mixture treated dropwise with acetic anhydride (18 g.) during an hour, under stirring, at 5°. The reaction mixture was kept at pH 9 with 4 *N* sodium hydroxide during the addition of acetic anhydride. O-*Ethyl*-N-*acetyl*-DL-*serine* was isolated using ethyl acetate, in the usual manner, yield 17.8 g. (80%, based on the α-bromo-β-ethoxypropionic acid used), m. p. 128—133°. Recrystallization from ethyl acetate-petroleum ether gave clusters of colourless needles, m. p. 133—135°.

*Anal.* 9.57 mg. subst.: 16.89 mg. $CO_2$, 6.42 mg. $H_2O$
$C_7H_{13}NO_4$ (175.18) calc'd.: C 47.99; H 7.49%
found: C 48.14; H 7.50%

### *Enzymatic resolution of* O-*methyl*-N-*acetyl*-DL-*serine*

*O*-Methyl-*N*-acetyl-DL-serine (20 g.) was dissolved in water (220 ml.) and the solution brought to pH 7.8—7.9 with 2 *N* ammonia. An acylase I solution (36 ml.) corresponding to 1 g. of acylase I powder was added, and the mixture thoroughly stirred and allowed to stand at 37° for 24 hours. The enzymic hydrolysis was followed by the Van Slyke manometric procedure for α-carboxyl nitrogen analysis[17]. After 24 hours the reaction mixture was removed from the thermostat, cooled to 20° and treated dropwise with glacial acetic acid to pH 4.5. Charcoal was added, and the mixture filtered. The protein-free filtrate was evaporated *in vacuo* at 40°. A part of this residue (5 g.) was dissolved in water (10 ml.) and poured on the top of a column (3.5 × 38.5 cm.) composed of 20—50 mesh Dowex 50 resin in the acid phase (the resin was regenerated by two cycles of washing with 5 *N* hydrochloric acid, water, 1 *N* sodium hydroxyde, and water, followed by a final 5 *N* hydrochloric acid and water wash). Dilution with water was carried out at the flow rate of 20 ml. per hour. After elution with 800 ml. of effluent, all of the *O*-methyl-*N*-acetyl-D-serine was eluted, yield 1.9 g, (76%). After recrystallization from ethyl acetate-petroleum ether, m. p. 70—74°, $[\alpha]_D^{17}$ —9.7° ± 0.9° (c, 1.04 in 1 *N* NaOH).

*Anal.* 9.02 mg. subst.: 14.85 mg. $CO_2$, 5.66 mg. $H_2O$
$C_6H_{11}NO_4$ (161.16) calc'd.: C 44.71; H 6.88%
found: C 44.95; H 7.03%

After further washing of the column with one liter of water, elution was begun with 2.5 *N* hydrochloric acid. The entire *O*-methyl-L-serine was eluted after 400 ml. of solution had passed through the column. The combined fractions containing

O-methyl-L-serine were neutralized with a sodium hydroxide solution (10%) to pH 7, evaporated to dryness *in vacuo* and converted in the usual manner to O-methyl-L-serine methyl ester by successive treatment with absolute methanol, dry hydrogen chloride, and a chloroform solution of dry ammonia. O-Methyl-serine methyl ester was hydrolysed by refluxing with a tenfold quantity of water for 10 hours, and evaporated to dryness. The O-methyl serine thus obtained was optically inactive; after recrystallization from water-ethanol white leaflets were obtained, with m. p. 228—230° (decomp.) (Schiltz and Carter[14] reported 200—210°; Nobuo Izumiya[19] reported 233—234°).

*Anal.* 7.81 mg. subst.: 11.56 mg. $CO_2$, 5.26 mg. $H_2O$
$C_4H_9NO_3$ (119.09) calc'd.: C 40.35; H 7.59%
found: C 40.42; H 7.54%

In subsequent isolation experiments of O-methyl-DL-serine from Dowex 50 columns, 4% ammonia was used instead of 2.5 *N* hydrochloric acid. After evaporation of the effluent, crude O-methyl-L-serine was obtained, showing $[\alpha]_D^{18}$ + 16° (c, 1 in 1 *N* HCl), which was converted into O-methyl-*N*-phthaloyl-L-serine without further purification.

O-*Methyl*-N-*phthaloyl*-L-*serine* was prepared from equimolar quantities of O-methyl-L-serine ($[\alpha]_D$ + 16°) and phthalic anhydride, by heating the mixture for half an hour at 130°. The oily O-*methyl*-N-*phthaloyl*-L-*serine* was purified by precipitation from dichloromethane-petroleum ether, $[\alpha]_D^{18}$ —18° (c, 5 in methanol).

*Anal.* 9.15 mg. subst.: 19.32 mg. $CO_2$, 3.84 mg. $H_2O$
$C_{12}H_{14}NO_5$ (249.22) calc'd.: C 57.83; H 4.45%
found: C 57.61; H 4.69%

The resolution of O-*ethyl*-N-*acetyl*-DL-*serine* was carried out in the same manner, and O-*ethyl*-N-*acetyl*-D-*serine* was obtained as white needles, m. p. 150—154°, with $[\alpha]_D^{18}$ —17° (c, 0.9 in 1 *N* NaOH).

*Anal.* 6.01 mg. subst.: 10.62 mg. $CO_2$, 4.04 mg. $H_2O$
$C_7H_{13}NO_4$ (175.18) calc'd.: C 47.99; H 7.49%
found: C 48.20; H 7.52%

*Acknowledgments.* One of us (M. J.) is indebted to Dr. R. Seiwerth, Head of the Research Institute of »Pliva«, Pharmaceutical and Chemical Works, for facilities in carrying out this work.

The authors are further indebted to Mrs. Z. Štefanac for the microanalyses, and to Professor K. Balenović for his interest in this work.

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## IZVOD

### Enzimatsko cijepanje O-metil-N-acetil-DL-serina. Aminokiseline. XXXII

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Izvedeno je enzimatsko cijepanje O-metil-N-acetil-DL-serina prema Greensteinu. Tako dobiveni O-metil-L-serin daje sa ftalanhidridom, uz djelomičnu racemizaciju, O-metil-N-ftaloil-L-serin.

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Primljeno 23. decembra 1955.