Short Communications

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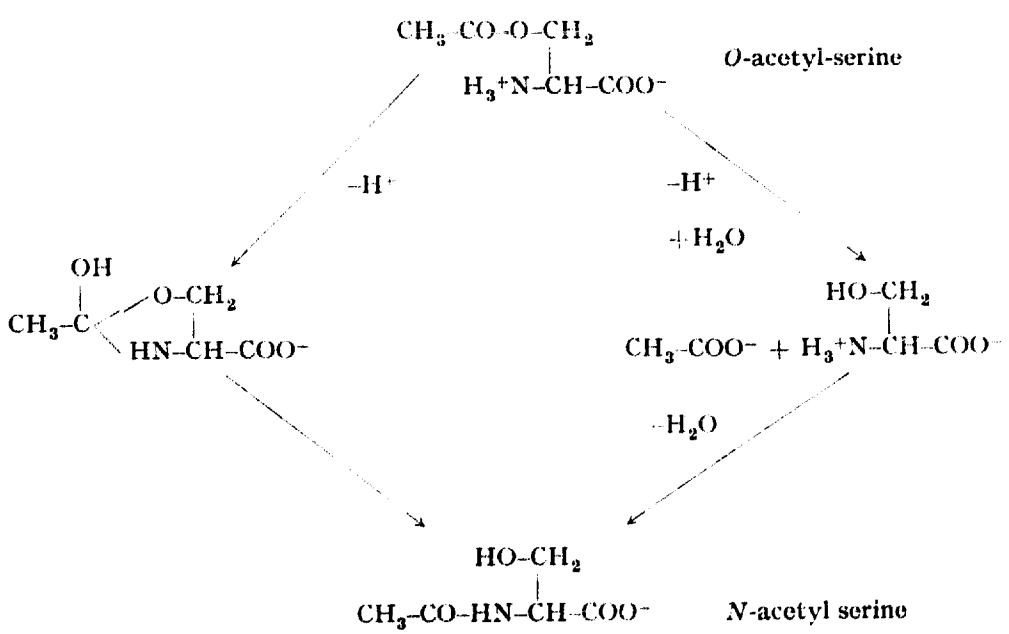
Mechanism of O,N-acyl shift in O-acetyl-DL-serine

The conversion of *O*-acetyl-DL-serine to *N*-acetyl-DL-serine has previously been shown to occur when titrated in a pH-stat at pH 7.5 in aqueous solution[1]. By performing the rearrangement with a high concentration of *O*-acetyl-DL-serine (prepared according to SAKAMI AND TOENNIES[2], m.p. 144°, calculated for $C_5H_9O_4N$: N, 9.5; amino N, 9.5. Found: N, 9.5; amino N, 9.47), it has now been found possible to crystallize the *N*-acetyl-DL-serine formed, directly from the solution. After completion of the alkali consumption at pH 7.5 the solution was concentrated to a few ml by distillation *in vacuo*. Ethanol was then added and after further addition of ethyl ether *N*-acetyl-DL-serine crystallized with cooling. The yield varied between 80 and 90 % in different experiments. The melting point was 121–122°, and nitrogen analysis gave: N, 9.5; amino N, 0.05; these values were the same as found when prepared according to the procedure described previously[3]. Thus it was evident that the alkali consumption must be directly related to the acyl shift, but it was not yet possible to define the mechanism of the rearrangement.

In earlier studies on the *O*,*N*-acyl shift[1,4–6] the formation of a hydroxyoxazolidine derivative as an intermediate had been considered as the most probable explanation. However, the existence of such a compound has never been clearly demonstrated.

The other possibility for the rearrangement would be a migration of the acetyl group from the hydroxyl group to the amino group of the serine molecule via a free acetate ion, since an oxazoline derivative would be stable in the alkaline solution and thereby would accumulate under the conditions used for the migration.



Earlier attempts to distinguish between the two possibilities have failed owing to the fact that the hydroxyoxazolidine derivative, if it exists, must be very labile and therefore present only in very small concentration during the shift. However, by means of *O*-acetyl-DL-serine labeled in the C-1 of the acetyl group with ^{14}C, the two possibilities of transformation can be tested if the reaction is carried out in the presence of an acetate pool. If the *O,N*-acyl shift occurs via free acetate ion one would then expect the specific activity of the *N*-acetyl-DL-serine formed to be considerable decreased. Conversely if the labeled *O*-acetyl-DL-serine did not equilibrate with the acetate pool during the shift to the *N*-acetyl-DL-serine derivative, the specific activity of the two compounds would be approximately equal. The latter finding would lend additional support to the postulate of the formation of a hydroxyoxazolidine intermediate.

TABLE I

O,N-ACYL SHIFT IN *O*-[1-^{14}C]-ACETYL-DL-SERINE

		Counts/min/mg
Initial	0.5 mequiv *O*-acetyl-DL-serine	1650
	1.0 mequiv acetic acid	0
Recovered	0.405 mequiv *N*-acetyl-DL-serine	1610
	0.950 mequiv sodium acetate	30

The transformation of *O*-[1-^{14}C]-acetyl-DL-serine (prepared as above by using [1-^{14}C]acetic acid) was carried out in the usual manner at pH 7.5, but in the presence of a high concentration of acetate ions. After the completion of the reaction the pH was adjusted to pH 2 by hydrochloric acid. After lyophilization, washing with 1 *N* HCl and repeated lyophilization, the *N*-acetyl-serine was analyzed and assayed by using copper planchets with a thin end-window Geiger–Müller tube on samples of infinitesimal thickness. The acetic acid fraction collected in a freezing trap was converted to sodium acetate before assaying. The results presented in Table I strongly support the existence of a hydroxyoxazolidine derivative as an intermediate in the rearrangement, in accordance with the expectations.

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