REVERSIBLE N,O-HALOACYL SHIFT IN SERINE DERIVATIVES WITH ANTITUMOR ACTIVITY[1]

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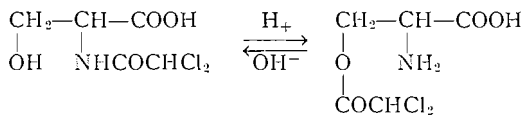
ABSTRACT

Large doses of N-dichloroacetyl-DL-serine sodium salt were required to cause regression of Sarcoma-37 in mice. About 56% of the unchanged compound was recovered from the urine. These observations suggest that the antitumor activity may reside in a metabolic product of the N-dichloracetyl serine. It is postulated that the active compound is the corresponding O-dichloroacetyl-DL-serine formed from the N-acyl compound by an in vivo enzymatically controlled shift which takes place via the hydroxyoxazolidine and (or) the oxazoline rings. O-Dichloroacetyl-DL-serine hydrochloride was prepared by treating a suspension of N-dichloroacetyl-DL-serine in anhydrous ether with gaseous hydrogen chloride. The free base, O-dichloroacetyl-DL-serine, is an extremely labile compound and reverts to the N-compound in neutral aqueous solution at room temperature. The hydrochloride salt, however, is stable, in which form it was isolated and characterized. The same compound was prepared from serine and dichloroacetic anhydride in dichloroacetic acid. O-Dichloroacetyl-DL-serine hydrochloride displays an antitumor effect against Sarcoma-37 and Sarcoma-180 in mice. The work has been extended to the monochloro- and trichloro-acetyl derivatives of serine.

INTRODUCTION

It was reported recently (1) that the sodium salt of N-dichloroacetyl-DL-serine (2) depresses the growth of Sarcoma-37 in mice, causing in some animals complete regression of the tumors. Most of the presently known agents which are effective against animal tumors are usually administered in relatively small doses such as milligrams or even fractions of a milligram per kilogram of body weight. The above compound, however, had to be administered in doses of 1 g per kilogram of body weight in order to be effective against the tumor. This considerable non-toxic dose is largely excreted by the kidney as unchanged drug, since approximately 56% of the pure crystalline product was isolated from the urine of cats as well as of humans (3). No metabolic or hydrolytic products were identified.

We would like to suggest, therefore, that the active antitumor agent was not the administered N-dichloroacetyl derivative itself, but rather a metabolic product of this compound. One of the specific characteristics of acyl derivatives of hydroxy amino acids having the hydroxyl and the amino groups in vicinal position is the ability of the acyl residue to migrate reversibly between the nitrogen and the oxygen. It is therefore possible that the compound could readily change into the heretofore unreported O-dichloroacetyl-DL-serine isomer and that, in vivo, both forms of the compound exist in equilibrium



I II

FIG. 1.

(Fig. 1). Such a concept would explain why large doses of the N-derivative are required to elicit an antitumor effect, since the equilibrium favors the stabilized, less active

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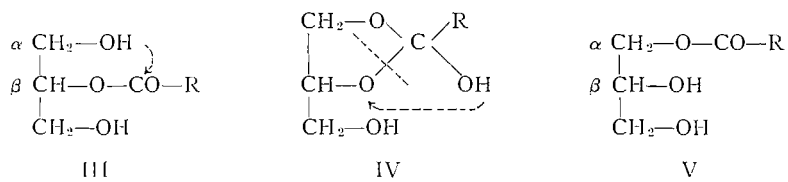
Contribution from Charles E. Frosst & Co., Montreal, Que.

N-dichloroacetyl-DL-serine. We therefore synthesized O-dichloroacetyl-DL-serine hydrochloride as well as its methyl ester, and preliminary experiments with the former compound do indeed indicate that it is active against Sarcoma-37 in mice at approximately 1/40th the dose of the N-compound and against Sarcoma-180 at approximately 1/20th the dose.

Similar reversible acyl migrations have been studied extensively in vitro over a number of years, in a large variety of compounds, and this shift is now well documented in the chemical literature for the N ⇄ O, N ⇄ S, and O ⇄ O systems. For example, acyl migrations for the N ⇄ O system have been reported for orthoaminophenols (4–12), alkylaminoethanols (13–23), adrenaline (24), noradrenaline (25, 26), ephedrine (26–30), phenylserine (31), aminophenylpropanediols including chloramphenicol (32–35), 2-aminocyclohexanol (36, 37), 2-aminocyclopentanol (38), and aminosugars (39, 40). Similar acyl shifts from oxygen to oxygen have been observed in sugars (41, 42) and glycerides (43–47), and more recently in the N ⇄ S system of cysteine (48) and glutathione (49, 50).

Emil Fischer (51) first proposed a mechanism to explain such migrations from oxygen to oxygen in the case of glycerides of fatty acids. He postulated an unstable five-membered hydroxy-orthoester as an intermediate when an acyl group migrated from one hydroxyl group to another in a 1,2-diol, as shown in Fig. 2. This hypothesis received its first confirmatory support from Hibbert and co-workers (52, 53), who isolated 2-hydroxy-2′-trichloromethyl-1,3-dioxolane, which is analogous to the cyclic intermediate IV postulated by Fischer.



III IV V

FIG. 2. Acyl migration in glycerides.

Today this mechanism involving transient cyclic intermediates is widely accepted because it satisfactorily accounts for a variety of reversible acyl shifts. Various workers have postulated ortho esters in the case of glycerides (47) and sugars (42), oxazoline or hydroxyoxazolidine rings in the N ⇄ O shifts (20, 28, 38, 39), and the corresponding thiazoline and hydroxythiazolidine rings in the case of N ⇄ S shifts (48). In some cases these cyclic intermediates were actually detected or isolated (20, 48).

More recently Doerschuk (44) carried out the rearrangement of 2-monopalmitin glyceride to the 1-monopalmitin product in the presence of glycerol-1-C^{14} and showed conclusively that the rearrangement mechanism is entirely intramolecular, thus eliminating the possibility of hydrolysis and re-esterification. van Lohuizen and Verkade (45) found that α- or β-monoglycerides in ethanolic hydrogen chloride mutually rearranged to an equilibrium mixture, while Raiford and Lankelma (8) showed a similar equilibrium in the case of acylated orthoaminophenols. It is also established for nearly all the above classes of compounds that the equilibrium distribution of the two different forms in which the acylated molecules can exist in solution depends on pH. Migration takes place from N to O when N-acyl compounds are treated with anhydrous hydrogen chloride with formation of the O-acyl-N-hydrochloride derivatives which in alkaline medium revert to the original N-acyl forms (8, 18, 20, 28, 31, 32, 35, 36, 38, 50, 54). In every case so far studied the N-acyl derivatives were much more stable than either the corresponding

O or S derivatives and indeed the latter always easily reverted to the more stable N-derivatives.

Since most chemical reactions which are catalyzed by enzymes are also susceptible to catalysis by simpler organic and inorganic agents, we postulate as a working hypothesis that the reversible acyl shifts in amino acid derivatives such as serine and cysteine, which, in vitro, are dependent on changes in pH, can be similarly mediated in vivo via the hydroxyoxazolidine (or less likely, the oxazoline) ring by acyl migratases resulting in an equilibrium mixture of the two tautomeric forms (Fig. 3).

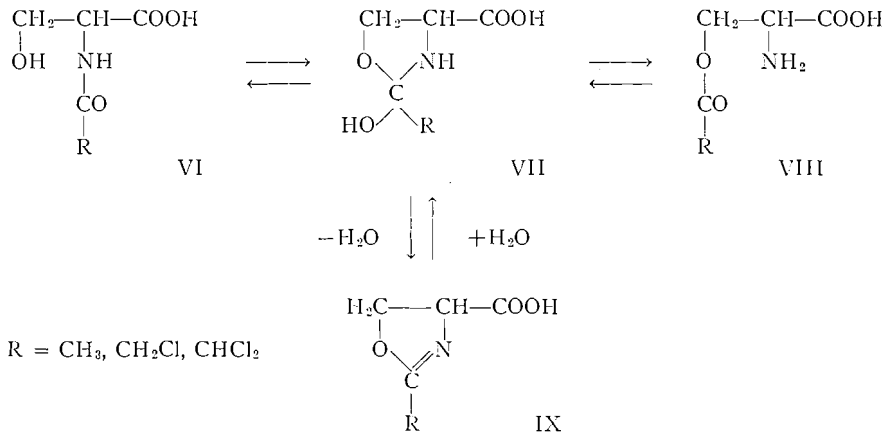


FIG. 3. Reversible N ⇄ O shifts in amino acids.

A corresponding biological shift from O to O, catalyzed by an enzyme, was reported recently by Uziel and Hanahan (55). They described a migratase, which catalyzes the β to α migration of the fatty acyl group on a β-lysolecithin. This enzyme, found in the extracts of *Penicillium notatum* and in commercial pancreatin, is nonhydrolytic in nature. The same migration was effected in the absence of the enzyme by treating the β-lysolecithin with dilute hydrochloric acid. Here again the intermediate formation of an ortho acid was favored to explain this enzymatically controlled intramolecular transesterification. A similar enzyme capable of catalyzing the intramolecular acyl migration in the N ⇄ O or N ⇄ S systems has not yet been described.

The relationship between this type of shift and biological activity has already been observed in the amino acid derivative azaserine (O-diazoacetyl-DL-serine). Here it was shown by Fusari and co-workers (56) that azaserine, in aqueous solution treated with barium hydroxide solution, lost its antibiotic activity as measured against *Kloechera brevis*, and by correlation, its antitumor activity (57), while the diazoacyl group underwent a simultaneous shift from O to N. The resulting barium salt of N-diazoacetyl serine which was isolated possessed little or no microbiological activity in vitro.

DISCUSSION OF RESULTS

It is known that strong acids act as catalysts in the esterification of hydroxy groups with acetic anhydride (58). Acylation of primary amines by acetic anhydride, however, is greatly inhibited when they are present as salts of strong acids (59–61). These facts were utilized in the preparation of O-dichloroacetyl-DL-serine by treating DL-serine with a mixture of dichloroacetic acid and dichloroacetic anhydride. The compound is extremely

labile, tending to change to the more stable N-dichloroacetyl-DL-serine. The hydrogen chloride salt of the O-derivative, however, is sufficiently stable to allow its isolation and storage at room temperature. All attempts to obtain the O-dichloroacetyl base from the crystalline hydrochloride, even under the mildest conditions, failed. For example, neutralization of the hydrogen chloride moiety with an equimolar amount of sodium bicarbonate, at room temperature, resulted in a shift of the dichloroacetyl group from O to N. Similarly, a methanolic solution of O-dichloroacetyl-DL-serine methyl ester hydrochloride (which was prepared by the above procedure from DL-serine methyl ester), upon treatment with one equivalent of triethylamine at room temperature, also underwent this shift to N-dichloroacetyl-DL-serine methyl ester.

The reverse shift of the dichloroacetyl group from N to O was accomplished in the case of N-dichloroacetyl-DL-serine by treating a suspension of the compound in anhydrous ether with a stream of hydrogen chloride gas over a long period of time. Anhydrous conditions are essential, otherwise complete hydrolysis takes place, yielding DL-serine hydrochloride and dichloroacetic acid. When this shift was attempted with the methyl ester of N-dichloroacetyl-DL-serine in anhydrous ether, the unchanged ester was recovered quantitatively, indicating that this compound does not undergo the N → O acyl shift as readily as does the non-esterified compound. Using anhydrous methanol instead of anhydrous ether an intermolecular shift from the N of the amino acid to the O of the alcohol took place rather than the expected intramolecular shift, with the result that serine methyl ester hydrochloride and methyl dichloroacetate were formed. These experiments are summarized in Fig. 4. Although O-dichloroacetyl-DL-serine is a labile compound, it is nevertheless sufficiently stable under the right conditions to be capable of more than transitory existence.

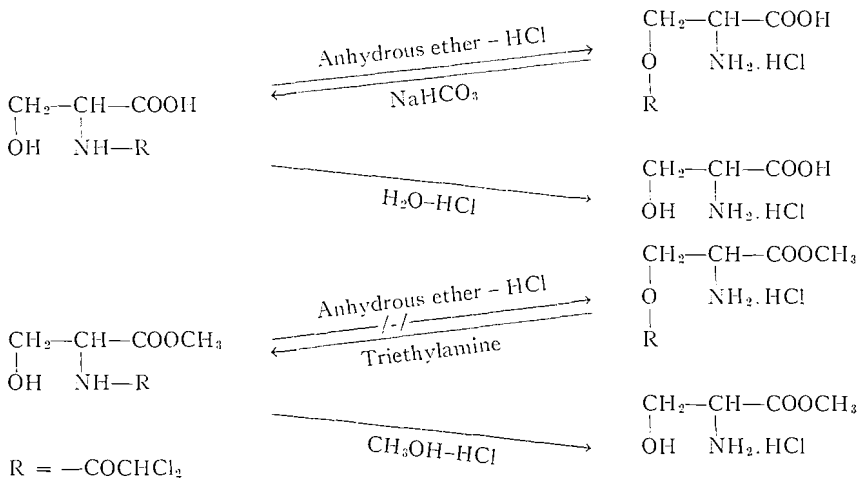


FIG. 4. N ⇄ O dichloroacyl shifts in serine.

Since we were able to show that the postulated reversible N ⇄ O shift of a dihaloacyl group could be effected in vitro, we extended this investigation to the monochloro- and trichloro-acyl compounds of serine. O-Monochloroacetyl-DL-serine was synthesized according to the procedure developed by Sakami and Toennies for the N-acyl derivative (61), and N-monochloroacetyl-DL-serine according to Fischer and Roesner (62). The complete reverse shift of the monochloroacyl group from N to O and O to N was carried

out as for the dichloroacyl compounds. However, in this case O-monochloroacetyl-DL-serine was sufficiently stable to be isolated as the free base as well as the hydrochloride. After this work was completed Benoiton (63) reported the same reversible shift for N-monochloroacetylserine.

All attempts to date to prepare the O-trichloroacetyl derivative have failed. The first synthetic procedure employed was essentially that used for the preparation of O-dichloroacetyl-DL-serine hydrochloride, using benzene as a solvent due to the crystalline state of trichloroacetic anhydride. This yielded instead of the expected O-trichloroacetyl-DL-serine hydrochloride the hitherto unreported O,N-bis(trichloroacetyl)-DL-serine compound. Titration of this compound with alkali indicated an equivalent weight of 199. This agrees with the calculated equivalent weight of 198 based on the finding that the O,N-bis compound is hydrolyzed during the neutralization by the alkali to N-trichloroacetyl-DL-serine (which was isolated and identified) and trichloroacetic acid. The compound gave a negative ninhydrin test in aqueous solution. Its infrared absorption curve indicated the presence of three different carbonyl groups and elemental analyses agreed with the theoretical values for O,N-bis(trichloroacetyl)-DL-serine.

N-Trichloroacetyl-DL-serine was prepared according to the usual Schotten-Baumann procedure (2). An attempt to shift the trichloroacetyl group from N to O under the same anhydrous conditions which converted the N-monochloro- and N-dichloro-acetyl derivatives to the corresponding O-compounds also failed, the unchanged starting material being recovered quantitatively. It is interesting to note that the stability of the amide bond was such in this case that it was not necessary to observe the stringent anhydrous conditions required for the N $\rightarrow$ O dichloroacetyl shift in order to prevent hydrolysis to serine hydrochloride. Ether saturated with water gave the same result, i.e., there was no hydrolysis of the N-trichloroacetyl group and no N $\rightarrow$ O shift took place. This unexpected stability to acid of the N-trichloroacetyl group was also observed in the failure of aqueous hydrochloric acid to hydrolyze the latter compound.

This stability was again evident when the O,N-bis(trichloroacetyl) compound was treated with acid in an attempt to prepare O-trichloroacetyl-DL-serine by hydrolysis of the N-acyl group. Contrary to expectations, the O-trichloroacetyl group was the more labile one under these conditions and again N-trichloroacetyl-DL-serine was obtained. This instability of the O-trichloroacetyl group in an acidic medium accounts for the failure, to date, to isolate O-trichloroacetyl-DL-serine.

It therefore appears that in the O-haloacyl series, the stability of the ester linkage decreases as the number of halogen atoms increases. In the N-haloacyl series, however, the opposite situation seems to exist, namely, the stability of the amide bond increases as the number of halogen atoms increases. These effects are no doubt the result of the inductive displacement of the electrons in the direction of the strong electron-attracting chlorine atoms; the greater the number of the latter the more stable the amide bond and the weaker the ester linkage.

These various haloacyl derivatives of serine are presently being evaluated for their antitumor activity in the mouse and rat to determine whether a relationship exists between such activity and the ease of interconversion of the three pairs of isomers.

EXPERIMENTAL

Infrared absorption spectra were determined with a Beckman IR-5 spectrophotometer equipped with a sodium chloride prism, using potassium bromide pellets. Melting points obtained with a Fisher–Johns apparatus, boiling points, and temperature readings are uncorrected and are in degrees Centigrade.

O-Dichloroacetyl-DL-*serine Hydrochloride*

DL-Serine (105 g, 1.0 mole) was dissolved in redistilled dichloroacetic acid (500 ml) in a 3-liter, three-necked, round-bottomed flask fitted with a dropping funnel, thermometer, and calcium chloride drying tube. The clear solution was cooled in an ice-water bath and stirred continuously with a magnetic stirrer. A slow stream of dichloroacetic anhydride (312 g, 1.3 mole) was added during 2½ hours at such a rate that the temperature of the reaction mixture did not exceed 32–33°. The flask was allowed to stand overnight at room temperature, and the product was then converted to the hydrochloride salt by passing a slow stream of hydrogen chloride gas into the solution for 1 hour. The excess hydrogen chloride was removed under reduced pressure, anhydrous benzene (1250 ml) was added, and the flask was refrigerated. The product crystallized as a fine white granular material which was removed by filtration, washed with anhydrous benzene, followed by petroleum–ether (30–60°), and dried at 45° in a vacuum oven. Weight 85.0 g, m.p. 115–120°. A second crop of product (5.4 g) was recovered from the filtrate, total yield 35.8%. The product was recrystallized from anhydrous acetone–ether, yielding 29.4% pure crystalline O-dichloroacetyl-DL-serine hydrochloride, m.p. 125–126°, which was soluble in water, ethanol, acetone, tetrahydrofuran, and dioxane, and insoluble in ether, chloroform, and ethylene dichloride. It gave a positive ninhydrin test. Anal. Calc. for $C_5H_8Cl_3NO_4$: C, 23.78; H, 3.19; N, 5.55. Found: C, 23.88; H, 3.29; N, 5.61.

This product was also prepared by saturating a suspension of 3.0 g (0.0286 mole) of DL-serine in 100 ml of dichloroacetic acid with hydrogen chloride gas at 0°. The resulting clear solution was kept at room temperature for 48 hours. The addition of anhydrous ether (100 ml) caused a rapid crystallization of crude product as white crystals. Weight 5.4 g (74.9% yield). The infrared absorption curve of this product was identical with that of the O-dichloroacetyl-DL-serine hydrochloride prepared above.

O-Dichloroacetyl-DL-*serine Hydrochloride from N-Dichloroacetyl*-DL-*serine: N → O Dichloroacyl Shift*

Finely pulverized N-dichloroacetyl-DL-serine (2.0 g) (2) was suspended in 100 ml anhydrous ether in a 200-ml round-bottomed flask. A moderately rapid stream of hydrogen chloride was passed into the suspension for 6½ hours. The flow was discontinued overnight and then resumed for 1½ hours. Anhydrous ether was added occasionally to maintain the volume of the reaction mixture. There was no sensible heat effect during this reaction. The ether suspension was evaporated to dryness, anhydrous ethyl acetate (75 ml) added, and the contents warmed for approximately ½ hour. The insoluble residue of O-dichloroacetyl-DL-serine hydrochloride (1.65 g, 70% yield) was removed by filtration, washed with ethyl acetate and ether, and dried, m.p. 125–126°. The infrared absorption spectrum of this material was identical with that of the product synthesized by the route described above and a mixed melting point of the two was not depressed. The ninhydrin test was positive.

N-Dichloroacetyl-DL-*serine from O-Dichloroacetyl*-DL-*serine Hydrochloride: O → N Dichloroacyl Shift*

A solution of O-dichloroacetyl-DL-serine hydrochloride (5.04 g, 0.02 mole) in 50 ml distilled water was treated at room temperature with a solution of 3.36 g (0.04 mole) sodium bicarbonate in 50 ml of water. The pH of the solution changed from an initial value of 1.10 to 5.60. The neutralized clear solution was concentrated to dryness from a water bath at 30–35° and under reduced pressure. The residue crystallized from water. An infrared absorption curve of the crystalline product was identical with that of pure

N-dichloroacetyl-DL-serine sodium salt (2). It melted at 172–173° and did not depress the melting point of authentic N-dichloroacetyl-DL-serine sodium salt (2). It no longer gave a positive ninhydrin test, indicating a practically theoretical shift from O to N at pH less than 7.

*O-Dichloroacetyl-*DL*-serine Methyl Ester Hydrochloride*

DL-Serine methyl ester hydrochloride (15.5 g, 0.1 mole) (2) was dissolved, with gentle heating, in dichloroacetic acid (100 ml) in a 500-ml, two-necked, round-bottomed flask fitted with a dropping funnel, calcium chloride drying tube, and magnetic stirrer. Dichloroacetic anhydride (30.0 g, 0.125 mole) was added dropwise to the clear, stirred solution. No attempt was made to control the very small heat rise during this addition, which required 45 minutes. The solution was allowed to stir for 24 hours at room temperature (23–25°) to complete the esterification. Diethyl ether (100 ml) was then added, followed by sufficient petroleum ether (30–60°) to produce a faint permanent cloudiness, and the flask was refrigerated for 5 hours. The white crystalline precipitate of O-dichloroacetyl-DL-serine methyl ester hydrochloride was removed by filtration, washed with ether (50 ml) then petroleum ether (50 ml), and dried at 30–40° in a vacuum oven. The product (18.8 g, 70.6% yield) was recrystallized from methanol–ether, m.p. 106° (with prior softening at 101°), white needles. It was soluble in water, ethanol, and dioxane, and insoluble in ether, petroleum ether, chloroform, and ethyl acetate. Anal. Calc. for $C_6H_{10}Cl_3NO_4$: C, 27.00; H, 3.78; N, 5.25. Found: C, 27.30; H, 4.19; N, 5.26.

*Attempted Conversion of N-Dichloroacetyl- to O-Dichloroacetyl-*DL*-serine Methyl Ester: Attempted N → O Dichloroacyl Shift*

(i) Hydrogen chloride gas was passed through a suspension of N-dichloroacetyl-DL-serine methyl ester (2) (2.0 g) in 100 ml anhydrous ether for 4 hours whereby complete solution took place. Upon evaporation of the ether a quantitative yield of unreacted starting ester was recovered.

(ii) Dry hydrogen chloride gas was passed through a solution of N-dichloroacetyl-DL-serine methyl ester (5 g) in anhydrous methanol (25 ml), for 1 hour. During this period the flask was cooled in an ice–salt bath. The solution was allowed to stand at room temperature for 24 hours and then concentrated to dryness under reduced pressure and at room temperature. The residual pale yellow syrup which solidified completely on refrigeration for several days was crystallized from methanol–ether, yielding 2.65 g of white crystals, m.p. 132–134°, which were identified by mixed melting points and infrared absorption spectra as DL-serine methyl ester hydrochloride.

*Conversion of O-Dichloroacetyl- to N-Dichloroacetyl-*DL*-serine Methyl Ester: O → N Dichloroacyl Shift*

Triethylamine (0.696 ml, 0.005 mole) was added at room temperature to a solution of O-dichloroacetyl-DL-serine methyl ester hydrochloride (1.33 g, 0.005 mole) in anhydrous methanol (15 ml). The solution was shaken mechanically for 10 minutes and then concentrated to dryness under reduced pressure and on a water bath maintained at 25°. The residue, which was a mixture of crystalline triethylamine hydrochloride and oil, was extracted with anhydrous ether (75 ml) whereby the latter dissolved leaving 0.7 g of triethylamine hydrochloride. The ethereal solution on evaporation to dryness under reduced pressure at 25° yielded a pale yellow residual oil which slowly solidified on refrigeration. This was crystallized from ether – petroleum ether (30–60°), yielding 0.95 g (82.6%) of white, fluffy crystals of N-dichloroacetyl-DL-serine methyl ester, m.p.

82–83°, which, by mixed melting point and infrared absorption spectra, was shown to be identical with N-dichloroacetyl-DL-serine methyl ester (2).

*O-Monochloroacetyl-*DL*-serine*

Monochloroacetic anhydride (121.0 g, 0.708 mole) was dissolved in ethyl monochloroacetate (100 ml) in a 500-ml round-bottomed flask with gentle warming. To the cooled solution there was added, dropwise, a solution of 10.6 g (0.10 mole) DL-serine in 20 g (0.12 mole) of 60% $HClO_4$. The first few increments of this solution did not cause any significant evolution of heat, but, after a period of induction, external cooling was required. On completion of the addition, the solution was heated for 3 hours on a water bath at 55°, then allowed to stand overnight. Slight application of heat was required to redissolve all solids the next day. The dark brown homogeneous solution was diluted with 4 ml of distilled water, which reacted with residual anhydride. Triethylamine (45 g, 0.45 mole) was then added intermittently, with occasional cooling, to neutralize the perchloric acid moiety of the salt. The reaction mixture was poured into 2 liters of ether, whereupon the product separated as a finely divided crystalline powder. It was recovered by filtration, washed and triturated with ether and ethanol, and dried in a vacuum oven. Wt. 16.3 g (89.8% yield), m.p. 122–123° (decomp.). Literature m.p. 122–123° (decomp.) (64). A mixed m.p. with N-monochloroacetyl-DL-serine was depressed. The product was soluble in water, hot ethanol, hot methanol, hot acetone, and insoluble in most other organic solvents. It reacted positive to ninhydrin and positive for chlorine in the flame test with copper wire.

*O-Monochloroacetyl-*DL*-serine Hydrochloride*

Finely powdered O-monochloroacetyl-DL-serine (15.0 g) was suspended in ether (100 ml), and a slow stream of hydrogen chloride gas was passed in for 1 hour. During this process the hydrochloride precipitated as it formed. The particle size of the product was much larger than that of the original O-monochloroacetyl-DL-serine. The excess hydrogen chloride and some of the ether were removed under vacuum. The product (13.2 g, 73.5% yield) was recovered by filtration, washed with ether, and dried in a vacuum oven, m.p. 140–142°. Crystallization from acetone–ether containing a little alcohol yielded 11.0 g of a white crystalline material melting at 141–142°, which was very soluble in the lower alcohols and water, and insoluble in ethyl acetate, chloroform, and ether. Anal. Calc. for $C_5H_9Cl_2NO_4$: C, 27.54; H, 4.16; N, 6.42. Found: C, 27.58; H, 4.16; N, 6.24.

*Conversion of N-Monochloroacetyl- to O-Monochloroacetyl-*DL*-serine: N → O Monochloroacyl Shift*

Finely pulverized N-monochloroacetyl-DL-serine (2.0 g) prepared according to the method of Fischer and Roesner (62) was treated in essentially the same manner as described above for the N → O shift in N-dichloroacetyl-DL-serine. The resulting O-monochloroacetyl-DL-serine hydrochloride 1.27 g (53% yield) after crystallization from ether–alcohol melted at 141–142° and did not depress the melting point of O-monochloroacetyl-DL-serine hydrochloride prepared as above. The infrared absorption spectra of the two compounds were identical.

*Conversion of O-Monochloroacetyl- to N-Monochloroacetyl-*DL*-serine: O → N Monochloroacyl Shift*

O-Monochloroacetyl-DL-serine (10.0 g, 0.055 mole) was dissolved in 27.5 ml of 2 *N* sodium hydroxide solution (0.055 mole NaOH), allowed to stand at room temperature for $3\frac{1}{2}$ hours, and then neutralized with concentrated hydrochloric acid. The solution was

evaporated to dryness, and the residue extracted with warm ethyl acetate (2×40 ml), which was dried over magnesium sulphate. The ethyl acetate was removed by distillation and the residual oil which solidified at room temperature was crystallized from ethyl acetate – petroleum ether (30–60°), yielding 3.35 g (33.5% yield) of product which melted at 120° and was identical by mixed melting point and infrared absorption with N-monochloroacetyl-DL-serine (62).

O,N-Bis-(Trichloroacetyl)-DL-*Serine*

A solution of trichloroacetic anhydride (250.0 g) and trichloroacetic acid (132.0 g) in anhydrous benzene (600 ml) was placed in a water bath at 25–30° and maintained at this temperature throughout the reaction. DL-Serine (69.4 g) was added in small portions to the rapidly stirred solution. When addition was complete, solids began to separate out and in a short while the mixture set to a gel. It was allowed to stand at room temperature overnight, then the product was removed by filtration, washed with benzene, and air-dried. The product (90.9 g), which was insoluble in water and gave a negative ninhydrin test, was crystallized from ether – petroleum ether (30–60°), m.p. 176–180° (a highly purified sample melted at 185–186°). Anal. Calc. for $C_7H_5Cl_6NO_5$: C, 21.21; H, 1.27; N, 3.54. Found: C, 21.42; H, 1.28; N, 3.51.

A sample of this O,N-bis-(trichloroacetyl)-DL-serine (1.0 g) was suspended in distilled water and neutralized at room temperature to the phenol red endpoint with 4.9 ml of 1.027 *N* sodium hydroxide solution. The material dissolved completely as the neutralization proceeded. The aqueous solution was concentrated to dryness on a water bath at 25° under reduced pressure. The residual oil, upon the addition of ether, solidified immediately to a white, friable powder which was removed by filtration, washed with ether, and dried. The product melted at 193–195° and weighed 0.5 g, representing a yield of 72.6% based on hydrolysis to N-trichloroacetyl-DL-serine, sodium salt, with which it was identified by mixed melting point and infrared absorption.

N-Trichloroacetyl-DL-*Serine*

DL-Serine (10.5 g, 0.10 mole) was dissolved in 2 *N* sodium hydroxide solution (50 ml, 0.1 mole) in a 500-ml, four-necked, round-bottomed flask fitted with a thermometer, motor driven stirrer, and two dropping funnels. The clear solution of the sodium salt of DL-serine was cooled in an ice–salt mixture to approximately 5°. Trichloroacetyl chloride (24.2 g, 0.133 mole) and 2 *N* sodium hydroxide solution (100 ml, 0.2 mole) were then added dropwise and simultaneously from the two dropping funnels to the stirred, cooled solution at such a rate that the reaction mixture remained on the basic side and below 10° throughout the additions. These additions required about 1 hour. The clear, colorless reaction solution was then stirred for 1 hour during which time it was allowed to warm up to room temperature. The solution was then acidified with concentrated hydrochloric acid (10 ml) and evaporated to dryness using a water bath at 55° and reduced pressure. The residual mixture of sodium chloride and clear yellow oil was extracted with hot ethyl acetate (3×100 ml). The combined ethyl acetate extracts, dried over anhydrous magnesium sulphate, were filtered and again evaporated, and the resulting oil was well shaken with petroleum ether (30–60°) (2×50 ml) to remove residual trichloroacetic acid. The product was crystallized from ethyl acetate – petroleum ether, yielding 8.9 g (35.6%) of white crystalline N-trichloroacetyl-DL-serine, m.p. 106–108°. Anal. Calc. for $C_5H_6Cl_3NO_4$: C, 23.97; H, 2.41; N, 5.59. Found: C, 23.62; H, 2.28; N, 5.56.

The sodium salt was prepared by neutralizing a solution of 87 g of N-trichloroacetyl-DL-serine in 100 ml of water with a 2 *N* sodium hydroxide solution, equivalent to 13.9 g NaOH. The clear solution was evaporated under vacuum at 60° to a dry solid, which

was recrystallized from ethanol, yielding 71 g of N-trichloroacetyl-DL-serine, sodium salt, m.p. 192–193°. Anal. Calc. for $C_5H_5Cl_3NO_4Na$: C, 22.04; H, 1.85; N, 5.14. Found: C, 21.96; H, 1.85; N, 5.32.

*Attempted Conversion of N-Trichloroacetyl- to O-Trichloroacetyl-*DL*-serine: Attempted $N \rightarrow O$ Trichloroacyl Shift*

N-Trichloroacetyl-DL-serine (2.0 g) was suspended in anhydrous ether (125 ml) and treated with a stream of hydrogen chloride gas for 4 hours. After approximately ½ hour, solution was complete and, upon evaporation to dryness, yielded an oil which rapidly crystallized. The product, 2.0 g, m.p. 106–109°, was identical by mixed melting point and infrared absorption with the starting material.

DL-*Serine Hydrochloride*

An analytical sample of DL-serine hydrochloride was prepared by evaporating to dryness an aqueous solution of serine (5 g in 50 ml water) containing 4 ml concentrated hydrochloric acid and crystallizing the residue from hot anhydrous ethanol. The product was recrystallized from ethanol–ether. Weight 5.4 g, m.p. 136°. Anal. Calc. for $C_3H_8ClNO_3$: C, 25.45; H, 5.70; N, 9.90. Found: C, 25.51; H, 5.48; N, 10.05.

Dichloroacetic Anhydride

This compound was prepared according to the procedure employed by Tedder (65). A mixture of phosphoric anhydride (141 g, 1.0 mole) and dichloroacetic acid (129 g, 1.0 mole) in a 500-ml round-bottomed flask was distilled under reduced pressure. The total distillable liquid came over at 116–120° at 16.5 mm, and was collected without regard to boiling point. Infrared absorption analysis indicated that it contained only a trace of unreacted acid. The product was redistilled in the same manner from approximately 3 g of fresh phosphoric anhydride and the distillate was collected at essentially the same boiling point as above. Weight of dichloroacetic anhydride 87.1 g (72.7% yield). In other preparations yields up to 84% were obtained.

Trichloroacetic Anhydride

This product was prepared in the same manner from trichloroacetic acid with phosphoric anhydride and boiled at 145–150° at 110 mm, yield 77.8%.

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