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Summary

The effects of iodine on the phenanthrene-bromine reaction have been determined, and from an analysis of the results it is shown that the observations cannot be reconciled with the addition-elimination theory for aromatic substitutions.

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[CONTRIBUTION FROM THE CHEMICAL LABORATORY OF THE UNIVERSITY OF ILLINOIS]

High Vacuum Distillation of N-Acyl Amino Acid and Polypeptide Esters

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An observation that certain N-benzenesulfonyl polypeptide esters suffer no apparent decomposition at temperatures appreciably above their melting points, suggested that such substances might be distilled satisfactorily under suitable conditions. Since Fischer's work upon the vacuum distillation of amino acid esters, very little further extension of this technique to N-acyl amino acid esters has been made; apparently no successful distillation of polypeptide derivatives of this type has been reported.

Acetylglycine ethyl ester,[2] as well as carbethoxyglycine and alanine ethyl esters[3] may be distilled easily. Muhlemann[4] attempted to apply this technique to several benzoylated amino acid esters, but observed considerable decomposition at a pressure of 2 mm. Cherbuliez[5] prepared a number of N-acetyl amino acid esters, and successfully distilled them, although considerable racemization accompanied the distillation of optically active compounds of this type.

It was found that butyl esters of benzenesulfonylated amino acids could be distilled without decomposition or racemization at pressures of 10^{-6}–10^{-7} mm. with the aid of a mercury vapor pump of the type described by Copley, Simpson, Tenney and Phipps.[6] In Table I are described the results obtained with a number of such compounds which were prepared in most cases by the method previously described by Gurin and Clarke.[7] Although butyl esters were first prepared in order to obtain greater stability (in contrast to methyl and ethyl esters),[8] this precaution was later found to be unnecessary since corresponding ethyl esters could be equally well distilled. Under these conditions distillation begins, in most cases, at temperatures ranging from 10 to 35° above the melting point. No racemization was observed to take place when optically active derivatives of this type were distilled. Thus, benzenesulfonyl-*l*-leucine butyl ester as well as dibenzenesulfonyl-*l*-tyrosine butyl ester showed no change in rotation after distillation. In Table I are listed the approximate distillation temperature ranges, as well as mixed melting points which were made with original and distilled material. Di-benzenesulfonyl-*d*-lysine butyl ester[9] distils extremely slowly, and at a temperature consid-

TABLE I

Substance	M. p., °C.[a]	Dist. temp.	Mixed m. p., °C.
$PhSO_2$-Glycine butyl ester[b]	26	50–55	25–26
$PhSO_2$-Alanine butyl ester[b]	114	120–125	113.5–114
$PhSO_2$-*l*-Leucine butyl ester[b,c]	51	68–73	50.5–51
$PhSO_2$-Phenylalanine butyl ester	107	120–125	106–107
N-$PhSO_2$-Serine butyl ester	55	70–75	55
$PhSO_2$-Methionine ethyl ester	45	75–80	45
Di-$PhSO_2$-*l*-tyrosine butyl ester	98	150–155	98
Di-$PhSO_2$-*d*-Lysine butyl ester	62	155–160	62
Dibutyl-$PhSO_2$-*d*-glutamate[b]	58–59	80–85	58
Dibutyl-$PhSO_2$-*i*-β-hydroxyglutamate[b]	74	95–100	73–74

[a] All temperatures are corrected. [b] Prepared according to method previously described.[7] [c] After distillation $[\alpha]^{25}D$ −15.9° (1% in ethyl alcohol).

(1) National Research Fellow in Biochemistry.

(2) T. Curtius, *Ber.*, **17**, 1672 (1884).

(3) E. Fischer and E. Otto, *ibid.*, **36**, 2106 (1903); E. Fischer and W. Axhausen, *Ann.*, **340**, 123 (1905).

(4) G. W. Muhlemann, *C. A.*, **22**, 1756 (1928).

(5) E. Cherbuliez and Pl. Plattner, *Helv. Chim. Acta*, **12**, 317 (1929).

(6) Copley, Simpson, Tenney and Phipps, *Rev. Sci. Instruments*, **6**, 265 (1935).

(7) S. Gurin and H. T. Clarke, *J. Biol. Chem.*, **107**, 395 (1934).

(8) E. Abderhalden and S. Suzuki, *Z. physiol. Chem.*, **176**, 101 (1928).

(9) The author wishes to acknowledge his indebtedness to Dr. Hans T. Clarke for furnishing a supply of this material.

erably higher than its melting point, while dibenzenesulfonyl-*l*-cystine diethyl ester does not distil but slowly decomposes at 185–200° yielding a colorless oily distillate.

In Table II are shown the results obtained with similar peptide derivatives. Again, ready distillation took place, although very little decomposition was observed. Successful distillation of N-benzenesulfonyl peptide ethyl esters indicates that it is unnecessary to prepare butyl esters for this purpose. Furthermore, carbethoxy peptide esters were found to be still more readily and rapidly distilled than their N-benzenesulfonyl analogs. For example, 0.2–0.3 g. of carbethoxyglycylglycine ethyl ester was distilled in approximately fifteen minutes at 95–100°.

TABLE II

Substance	M. p., °C.	Dist. temp., °C.	Mixed m. p., °C.
$PhSO_2$-Glycylalanine butyl ester[a]	101	120–125	100.5–101
$PhSO_2$-Glycylalanine ethyl ester	74	90–95	74
$PhSO_2$-Alanylglycine butyl ester[a]	76.5	100–105	75.5–76
$PhSO_2$-Glycyl-*l*-leucine butyl ester[a]	107	125–130	106.5
$PhSO_2$-Alanylalanine butyl ester	102	115–120	101
Carbethoxyglycylglycine ethyl ester	86	95–100	86
Carbethoxy-diglycylglycine ethyl ester	159	170–175	158–159
$PhSO_2$-Leucylglycylglycine ethyl ester	171	190–195	171

[a] Prepared by method previously described.[7]

Several artificial mixtures were fractionated by this method. Benzenesulfonyl-methionine ethyl ester was almost quantitatively separated from dibenzenesulfonyl-cystine diethyl ester by distillation at 75–95°, while the cystine component was left behind unchanged and undecomposed. Similarly, benzenesulfonyl-glycine butyl ester and benzenesulfonylalanine butyl ester were separated in pure form from a mixture of the two. From a mixture of carbethoxyglycylglycine ethyl ester and carbethoxydiglycylglycine ethyl ester, both components were separated easily in pure form as were the N-benzenesulfonyl butyl ester derivatives of glycylalanine and alanylglycine from a mixture of the two. Finally, benzenesulfonylleucylglycylglycine ethyl ester and benzenesulfonylglycylalanine ethyl ester were similarly separated. Recoveries of 90% or better were obtained in every case.

The application of this method to problems of protein structure is at present in progress.

Experimental

Benzenesulfonyl - *d,l* - alanylalanine Butyl Ester.—*d,l*-Alanylalanine was prepared according to Fischer and Kautzsch[10] (amino-N, 8.60; calcd. amino-N, 8.75). To 0.6 g. of the dipeptide dissolved in 10 cc. of water plus 1 cc. of 5 *N* sodium hydroxide were added with good stirring 1.4 g. of benzenesulfonyl chloride and 2 cc. of 5 *N* sodium hydroxide. The reaction mixture was kept just alkaline to thymolphthalein, and, after stirring for four hours, was acidified to Congo red with concentrated hydrochloric acid. The solution was extracted repeatedly with butyl alcohol and esterification carried out as previously described.[7] The ester crystallized as needles from ether and petroleum ether and weighed 0.569 g. (40%); m. p. 102°.

Anal. Calcd. for $C_{16}H_{24}O_5N_2S$: N, 7.86. Found: N, 8.02.

Benzenesulfonyl - glycyl - *d,l* - alanine Ethyl Ester.—Glycyl-*d,l*-alanine, prepared according to Fischer and Schulze,[11] was benzenesulfonylated as previously described.[7] The precipitate which separated out of the aqueous acid reaction mixture was filtered, dried and esterified by boiling for a few minutes with absolute ethyl alcohol previously saturated with dry hydrogen chloride gas. On evaporation of the alcoholic solution, followed by recrystallization from chloroform and petroleum ether, needles were obtained melting at 74°.

Anal. Calcd. for $C_{13}H_{18}O_5N_2S$: N, 8.90. Found: N, 8.93.

Benzenesulfonyl - *d,l* - leucylglycylglycine Ethyl Ester.—Two and forty-five hundredths grams of *d,l*-leucylglycylglycine prepared according to Fischer[12] (N, 17.30; calcd. 17.12) was dissolved in 10 cc. of *N* sodium hydroxide and treated with 3.5 g. of benzenesulfonyl chloride and 20 cc. of *N* sodium hydroxide; the reaction mixture was kept just alkaline to thymolphthalein. After stirring for three hours, a small amount of insoluble material was removed by filtration, and the filtrate acidified to Congo red with 5 *N* hydrochloric acid. The solution was concentrated *in vacuo* to dryness, and the residue extracted with 3–15 cc. portions of the hot methyl alcohol. After evaporating the methyl alcohol solution to dryness, the residue was suspended in 75 cc. of cold absolute ethyl alcohol previously saturated with dry hydrogen chloride gas, and boiled for a few minutes until a clear solution was obtained. The solution was concentrated *in vacuo* to approximately 10 cc. and, after chilling, the ester was obtained in the form of small bars. A further crop was obtained by addition of petroleum ether to the mother liquor. After recrystallization from hot absolute alcohol, the product weighed 2.51 g. (61% yield); m. p. 171°. *Anal.* Calcd. for $C_{18}H_{27}O_6N_3S$: N, 10.16. Found: N, 10.27.

Carbethoxyglycylglycine Ethyl Ester.—Prepared according to Fischer and Fourneau;[13] m. p. 86°; N, 12.20; calcd. for $C_9H_{16}O_5N_2$: N, 12.07.

(10) E. Fischer and K. Kautzsch, *Ber.*, **38**, 2375 (1905).
(11) E. Fischer and A. Schulze, *ibid.*, **40**, 946 (1907).
(12) E. Fischer, *ibid.*, **38**, 605 (1905).
(13) E. Fischer and E. Fourneau, *ibid.*, **34**, 2868 (1901).

Carbethoxydiglycylglycine Ethyl Ester.—Carbethoxydiglycylglycine was prepared according to the method described by Fischer;[14] m. p. 208–209°; N, 16.02; calcd. for $C_9H_{15}O_6N_3$: N, 16.08. The ester was obtained by treatment with absolute ethyl alcohol and dry hydrogen chloride; the solution obtained after boiling for a few minutes, was concentrated *in vacuo* to a small volume and the ester precipitated by addition of ether. On recrystallization from hot absolute alcohol, needles were obtained melting at 159°: N, 14.44; calcd. for $C_{11}H_{19}O_6N_3$, N, 14.53. This ester was prepared by Fischer[15] using a different method of synthesis.

Dibenzenesulfonylcystine Diethyl Ester.—Dibenzenesulfonylcystine was prepared according to the method described by Fruton and Clarke;[16] m. p. 208°. The diethyl ester was obtained by suspending in absolute ethyl alcohol and saturating with dry hydrogen chloride. After refluxing for ten minutes, the clear solution was concentrated *in vacuo* to dryness, and the residue recrystallized from hot ethyl acetate. The ester (tufts of needles) melted at 121°: N, 4.96; S, 22.40; calcd. for $C_{22}H_{28}O_8N_2S_4$, N, 4.86; S, 22.22.

Benzenesulfonyl-*d,l*-phenylalanine Butyl Ester.—This was prepared from benzenesulfonyl-*d,l*-phenylalanine by vacuum distillation in the presence of excess butyl alcohol by the general method described by Gurin and Clarke.[7] The product is obtained in the form of needles after recrystallization from ether and petroleum ether: m. p. 107°; N, 3.95; calcd. for $C_{19}H_{23}O_4NS$, N, 3.88.

***N*-Benzenesulfonyl-*d,l*-serine Butyl Ester.**—After esterifying in the same manner with butyl alcohol, needles are obtained from ether and petroleum ether, m. p. 55°; N, 4.78; calcd. for $C_{13}H_{19}O_5NS$, N, 4.65.

Dibenzenesulfonyl-*l*-tyrosine Butyl Ester.—Obtained as needles from ethyl acetate and petroleum ether or from amyl ether; m. p. 98°; N, 2.74; calcd. for $C_{25}H_{27}O_7NS_2$, N, 2.72. $[\alpha]^{26}D$ +20.1° (1% in ethyl alcohol). After distillation $[\alpha]^{26}D$ +20.2°.

Benzenesulfonyl-*d,l*-methionine Ethyl Ester.—Prepared from benzene-sulfonyl-*d,l*-methionine by treatment with absolute ethyl alcohol and dry hydrogen chloride gas. The product crystallizes from ether and petroleum ether as tufts of fine needles; m. p. 45°; N, 4.60; calcd. for $C_{13}H_{19}O_4NS_2$, N, 4.41.

Separation of Dibenzenesulfonylcystine Diethyl Ester and Benzenesulfonyl-*d,l*-methionine Ethyl Ester.—From a mixture containing 50 mg. of dibenzenesulfonylcystine diethyl ester and 50 mg. of benzenesulfonyl-*d,l*-methionine ethyl ester, 49 mg. of the methionine derivative was recovered by high vacuum distillation at a temperature range of 75–95°. The benzenesulfonyl-*d,l*-methionine ethyl ester so recovered, melted at 45°; N, 4.50; calcd. for $C_{13}H_{19}O_4NS_2$, N, 4.41. The dibenzenesulfonylcystine diethyl ester remained in the distillation bulb unchanged; m. p. 120–121°.

Benzenesulfonylglycine Butyl Ester and Benzenesulfonyl-*d,l*-alanine Butyl Ester.—A mixture containing 100 mg. of benzenesulfonylglycine butyl ester and 85 mg. of benzenesulfonyl-*d,l*-alanine butyl ester was similarly subjected to high vacuum distillation. On heating to 50–75°, 98 mg. of a colorless oil was recovered, which crystallized on chilling and proved to be benzenesulfonylglycine butyl ester; m. p. 26°; N, 5.01; calcd. for $C_{12}H_{17}O_4NS$, N, 5.16. Upon raising the temperature to 125–135°, 81 mg. of benzenesulfonyl-*d,l*-alanine butyl ester was recovered; m. p. 113.5–114°; N, 4.78; calcd. for $C_{13}H_{19}O_4NS$, N, 4.91.

Carbethoxydiglycylglycine Ethyl Ester and Carbethoxyglycylglycine Ethyl Ester.—A mixture was made up consisting of 35.0 mg. of carbethoxyglycylglycine ethyl ester and 27.0 mg. of carbethoxydiglycylglycine ethyl ester. After distilling at 95–115° for one hour, a first fraction was recovered weighing 34.8 mg. and melting at 86°; N, 12.02; calcd. for $C_9H_{16}O_5N_2$, N, 12.07. The product obviously was pure carbethoxyglycylglycine ethyl ester.

A second fraction weighing 24.1 mg. was obtained at 175–185°, and appeared to be pure carbethoxydiglycylglycine ethyl ester; m. p. 158–159°; N, 14.35; calcd. for $C_{11}H_{19}O_6N_3$, N, 14.53. The remaining few mg. was accounted for by a small amount of decomposed material in the distilling bulb.

Benzenesulfonyl-*d,l*-leucylglycylglycine Ethyl Ester and Benzenesulfonyl-glycyl-*d,l*-alanine Ethyl Ester.—In a similar manner, a mixture consisting of 75.4 mg. of benzenesulfonyl-glycyl-*d,l*-alanine ethyl ester and 54.1 mg. of benzenesulfonyl-*d,l*-leucylglycylglycine ethyl ester was fractionated. At 90–110° a colorless oil was obtained weighing 70.3 mg.; after chilling, crystals were obtained melting at 74°, thereby indicating the product to be benzenesulfonyl-glycylalanine ethyl ester; N, 9.08; calcd. for $C_{13}H_{18}O_5N_2S$, N, 8.90.

The second fraction, obtained at 190–200°, weighed 48.5 mg. and appeared to consist of pure benzenesulfonyl-leucylglycylglycine ethyl ester; m. p. 171°; N, 10.25; calcd. for $C_{18}H_{27}O_6N_3S$; N, 10.16.

Summary

N-Benzenesulfonyl amino acid esters have been distilled at pressures of 10^{-6}–10^{-7} mm. No racemization and very little decomposition were observed under the conditions described.

A number of N-benzenesulfonyl di- and tripeptide esters were prepared and similarly distilled without decomposition. Carbethoxy peptide esters may likewise be distilled.

Several artificial mixtures containing N-acyl amino acid, dipeptide and tripeptide esters were fractionated by this method. In every case reported, the individual components were isolated in pure form, and in yields corresponding to 90% or more.

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(14) E. Fischer, *Ber.*, **36**, 2982 (1903).
(15) E. Fischer, *ibid.*, **36**, 2094 (1903).
(16) J. Fruton and H. T. Clarke, *J. Biol. Chem.*, **106**, 667 (1934).