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

Helianthates of Amino Acid and Polypeptide Esters

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Sulfonic acid salts of amino acid and polypeptide esters have been infrequently described, or prepared for the characterization of such substances. Colombano[2] reported the preparation of the *d*-camphor-10-sulfonates and *d*-bromocamphorsulfonates of glycine and alanine ethyl esters. In addition to their mineral acid salts, the tartrates and picrates of amino acid esters have been described.[3] The preparation of helianthates of a number of bases by Dehn,[4] as well as the use of helianthin by Williams[5] for the removal of bases from aqueous solution, suggested the possible application of this method to the characterization of amino acid and polypeptide esters.

It was found that such salts could be prepared by the addition of methyl orange to amino acid ester hydrochlorides in aqueous solution (Table I). Orange colored crystalline precipitates of low solubility in water were obtained in yields ranging from 65–95%. These substances were generally recrystallized from hot water, alcohol or aqueous acetone; they are sparingly soluble in ether, chloroform and other organic solvents.

$$RCH(NH_2)COOR\cdot HCl + (CH_3)_2NC_6H_4N_2C_6H_4SO_3Na \longrightarrow RCH(NH_2)COOR\cdot(CH_3)_2NC_6H_4N_2C_6H_4SO_3H + NaCl$$

Similar salts likewise may be prepared by the addition of helianthin to the free esters in appropriate solvents. This method has been employed only with butyl esters since they show considerably less tendency to form piperazines than do their corresponding methyl and ethyl esters.[6] For this purpose, several amino acids and peptides were esterified successfully at temperatures below 55° by prolonged vacuum distillation in the presence of a continuous excess of butyl alcohol containing just enough mineral acid for neutralization of the amino groups. The free esters were liberated with sodium hydroxide and sodium carbonate, followed by extraction with ether; after drying, and evaporating *in vacuo*, the residual sirup was usually dissolved in aqueous acetone and converted to the helianthate by the addition of an equivalent amount of helianthin.

Similar well crystallized salts are obtained with peptide esters (Table II). In these cases, hot aqueous solutions of methyl orange were added in equivalent proportion to cold and well-stirred aqueous solutions of peptide ester hydrochlorides.

In general, all of the helianthates are orange in color, and decompose over a narrow temperature range upon heating; in most cases darkening and gas formation were observed before the salts melted.

Colorimetric helianthin estimations were carried out in 50% aqueous acetic acid against helianthin standards dissolved in the same solvent. Concentrations of approximately 10^{-5} molar were used, since readings can be taken quite easily at this strength. The analytical figures given in the tables were obtained with a "Step" photometer equipped with an appropriate light filter, or with a K. and E. color analyzer using monochromatic light at 5100 Å.

In Table III are listed helianthates prepared from esters having one or more functional basic groups. It will be observed that lysine, histidine, arginine and cystine esters, as well as α-glycyllysine methyl ester,[7] form di-helianthates whereas ε-carbobenzoxylysine ester[7] yields a mono-helianthate. Within limits, therefore, colorimetric estimation of helianthin in such compounds affords another method for the titration of combining basic groups.

Guanidine salts are rapidly and almost completely precipitated from aqueous solution by methyl orange. It is interesting to note that free amino acids and polypeptides as well as their N-acyl derivatives do not yield similar products; cystine hydantoic acid and 5-methylhydantoin likewise failed to react.

Although helianthates of volatile bases have been decomposed by heating at temperatures sufficiently high to produce appreciable dissociation,[8] this method of recovering the base appar-

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(2) (a) A. Colombano and G. Lama, *Atti accad. Lincei*, **22**, 234 (1914); A. Colombano, G. Sanna and I. Delitala, *Gazz. chim. ital.*, **44**, 97 (1914).

(3) E. Fischer, *Ber.*, **34**, 433 (1901).

(4) Dehn, THIS JOURNAL, **39**, 1348, 1377 (1917); **40**, 1573 (1918).

(5) R. R. Williams, R. E. Waterman and J. C. Keresztesy, *ibid.*, **56**, 1187 (1934).

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

(7) The authors wish to acknowledge their indebtedness to Dr Max Bergmann and Dr. W. F. Ross for kindly supplying samples of these compounds.

(8) Hantzsch, *Ber.*, **41**, 1187 (1908).

TABLE I

Helianthates	Formula	M. p., dec., °C.	Crystal shape	Helianthin, % Calcd.	Helianthin, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Glycine ethyl ester	$C_4H_9O_2N\cdot(C_{14}H_{15}O_3N_3S)$	203.0	Blocks	74.7	78.0	13.72	13.55
Alanine ethyl ester	$C_5H_{11}O_2N\cdot(C_{14}H_{15}O_3N_3S)$	211.5	Needles	72.3	73.5	13.27	13.38
l-Leucine ethyl ester	$C_8H_{17}O_2N\cdot(C_{14}H_{15}O_3N_3S)$	210.5	Needles	65.7	65.5	12.07	11.86
Phenylalanine ethyl ester	$C_{11}H_{15}O_2N\cdot(C_{14}H_{15}O_3N_3S)$	210.0	Bars	61.5	62.7	11.24	11.44
l-Tyrosine ethyl ester	$C_{11}H_{15}O_3N\cdot(C_{14}H_{15}O_3N_3S)$	209.0	Blocks	59.3	62.5	10.89	10.72
Methionine ethyl ester	$C_7H_{15}O_2NS\cdot(C_{14}H_{15}O_3N_3S)$	210.5	Needles	63.3	68.1	11.62	11.53
Alanine butyl ester	$C_7H_{15}O_2N\cdot(C_{14}H_{15}O_3N_3S)$	201.5	Needles	67.8	68.1	12.44	12.64
d-Glutamic dibutyl ester	$C_{13}H_{25}O_4N\cdot(C_{14}H_{15}O_3N_3S)$	199.0	Bars	54.1	56.1	9.93	9.95

TABLE II

Helianthates	Formula	M. p., dec., °C.	Crystal shape	Helianthin, % Calcd.	Helianthin, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
Glycylglycine ethyl ester	$C_6H_{12}O_3N_2\cdot(C_{14}H_{15}O_3N_3S)$	210	Plates	65.6	66.0	15.05	15.09
Glycyl-*l*-leucine ethyl ester	$C_{10}H_{20}O_3N_2\cdot(C_{14}H_{15}O_3N_3S)$	216	Prisms	58.5	59.7	13.43	13.60
Alanylalanine butyl ester	$C_{10}H_{20}O_3N_2\cdot(C_{14}H_{15}O_3N_3S)$	210	Needles	58.5	59.1	13.43	13.32
Glycylalanine butyl ester	$C_9H_{18}O_3N_2\cdot(C_{14}H_{15}O_3N_3S)$	...	Blocks	60.2	59.6	13.80	13.71
Alanylglycine butyl ester	$C_9H_{18}O_3N_2\cdot(C_{14}H_{15}O_3N_3S)$	210	Plates	60.2	63.0	13.80	13.93
Diglycylglycine ethyl ester	$C_8H_{15}O_4N_3\cdot(C_{14}H_{15}O_3N_3S)$	215	Plates	58.4	58.1	16.09	15.85
Leucylglycylglycine ethyl ester	$C_{12}H_{23}O_4N_3\cdot(C_{14}H_{15}O_3N_3S)$	187	Blocks	52.8	54.6	14.53	14.35

TABLE III

Substance	Formula	M. p., °C.	Crystal shape	Helianthin, % Calcd.	Helianthin, % Found	Nitrogen, % Calcd.	Nitrogen, % Found
d-Lysine methyl ester di-helianthate	$C_7H_{16}O_2N_2\cdot2(C_{14}H_{15}O_3N_3S)$	242.5	Needles	79.2	78.3	14.54	14.43
ϵ-Carbobenzoxy-*d*-lysine methyl ester helianthate	$C_{15}H_{22}O_4N_2\cdot(C_{14}H_{15}O_3N_3S)$	183.0	Plates	50.9	50.6	11.68	11.70
α-Glycyl-*d*-lysine methyl ester di-helianthate	$C_9H_{19}O_3N_3\cdot2(C_{14}H_{15}O_3N_3S)$	232.5	Plates	73.8	75.5	15.23	15.16
l-Cystine diethyl ester di-helianthate	$C_{10}H_{20}O_4N_2S_2\cdot2(C_{14}H_{15}O_3N_3S)$	212.0	Bars	67.3	70.1	12.36	12.20
l-Histidine methyl ester di-helianthate	$C_7H_{11}O_2N_3\cdot2(C_{14}H_{15}O_3N_3S)$	221.0	Bars	78.3	76.3	16.17	16.05
d-Arginine methyl ester di-helianthate	$C_7H_{16}O_2N_4\cdot2(C_{14}H_{15}O_3N_3S)$	229.5	Blocks	76.4	77.0	17.54	17.65
Guanidine helianthate	$CH_5N_3\cdot(C_{14}H_{15}O_3N_3S)$	270.0	Needles	83.8	84.9	23.07	23.08

ently cannot be applied to the more heat stable amino acid ester helianthates; alanine butyl ester could not be recovered by dry distillation of the helianthate. A satisfactory method of recovery involves acidification of an alcoholic solution of the salt with dry hydrogen chloride. A very small amount of acid is generally sufficient to precipitate the greater part of the helianthin, leaving the ester hydrochloride in solution. Glycine ethyl ester hydrochloride was recovered in this manner from its helianthate in a yield of 79%. Attempts to decompose helianthates with alkali, followed by extraction with ether, have in several cases resulted in poor recovery of the amino acid esters.

Preliminary experiments indicate that salts of similar sparing solubility in water are apparently formed by Congo red, although this reagent appears to offer no advantage over methyl orange. Furthermore, ethane sulfonic, *n*-butane-sulfonic, benzyl-sulfonic, *m*-nitrobenzenesulfonic and *d*-camphor-10-sulfonic acids were found to yield hygroscopic and extremely water soluble sulfonates of amino acid and polypeptide esters. These substances were not investigated further.

Experimental

Glycine Ethyl Ester Helianthate.—To 10 cc. of a chilled aqueous solution of 1.39 g. of glycine ethyl ester hydrochloride was added with good stirring and cooling, 75 cc. of a hot water solution containing 3.27 g. of methyl orange. After stirring for twenty minutes, a heavy orange precipitate was filtered and washed several times with cold water. The product after recrystallization from hot methyl alcohol weighed 3.2 g. (78%). It may also be recrystallized from hot water.

Dibutyl-*d*-glutamate Helianthate.—To a solution of 5 g. of *d*-glutamic acid in 20 cc. of water containing 2 cc. of

concentrated sulfuric acid, 100 cc. of butyl alcohol was added, and the material subjected to prolonged vacuum distillation at a temperature not higher than 55°. Butyl alcohol was added from time to time as required, and distillation continued for eight to ten hours, although esterification is practically complete in four to five hours. After removal of excess butyl alcohol by vacuum distillation, the residual yellow oil was dissolved in 20 cc. of cold water, made alkaline, and the butyl ester extracted into ether by the usual Fischer method. From the ether solution, 7.445 g. (84%) of the ester was obtained as a yellow oil. It was then dissolved in 50 cc. of 50% aqueous acetone, and a solution of 8.7 g. of helianthin in 100 cc. of the same solvent added. On evaporation to approximately 75 cc., a heavy precipitate of orange needles was filtered, and washed with 50 cc. of cold water. After recrystallization from hot methyl alcohol (1 g. requires 40 cc. boiling alcohol), 11.83 g. of helianthate was obtained (75% from ester). The product is sparingly soluble in cold water, ether or petroleum ether, but somewhat more so in acetone, aqueous acetone, and hot methyl alcohol, dioxane or water.

d,l-**Alanine Butyl Ester Helianthate.**—Alanine (5 g. in 50 cc. of water containing 3.5 cc. concentrated sulfuric acid) was converted to its butyl ester sulfate by vacuum distillation in the presence of excess butyl alcohol as described above. From this material, 4.37 g. of free ester was obtained and converted to the helianthate by the addition of 9.2 g. of helianthin in aqueous acetone solution. After recrystallization from hot alcohol, the salt weighed 10.45 g. (77% from ester).

Methionine Ethyl Ester Helianthate.—Methionine ethyl ester hydrochloride was prepared from synthetic methionine with absolute ethyl alcohol and dry hydrogen chloride gas; it was recrystallized from alcohol and ether; m. p. 212° dec., N, 6.61; calcd. for $C_7H_{16}O_2NSCl$, N, 6.55. To a solution of 1 g. of methionine ethyl ester hydrochloride in 10 cc. of cold water, was added 1.5 g. of methyl orange in 25 cc. of hot water. After stirring and cooling for thirty minutes, the precipitate was filtered, washed with cold water and recrystallized from boiling methyl alcohol (1 g. requires 150 cc. of methyl alcohol). The product weighed 1.8 g. (81%).

Glycylglycine Ethyl Ester Helianthate.—Ninety-eight hundredths gram of glycylglycine ethyl ester hydrochloride prepared according to Fischer and Fourneau[9] (m. p. 181–182°), was dissolved in 5 cc. of cold water, and a solution of 1.63 g. of methyl orange in 25 cc. of hot water added with good stirring and chilling. The precipitate was filtered, washed with cold water and, after recrystallization from boiling alcohol, weighed 1.97 g. (85%).

Glycyl-*l*-leucine Ethyl Ester Helianthate.—Glycyl-*l*-leucine ethyl ester hydrochloride was prepared in the usual way from glycyl-*l*-leucine.[10] The product is appreciably soluble in alcohol from which it may be precipitated by 2–3 volumes of ether in the form of glistening plates; m. p. 163–164°; N, 11.20; calcd. for $C_{10}H_{21}O_3N_2Cl$, N, 11.09. The helianthate was obtained as described above in 92% yield after recrystallization from hot ethyl alcohol.

Guanidine Helianthate.—To a solution of 2 g. of guanidine sulfate in 50 cc. of water was added with stirring a hot solution of 6.1 g. of methyl orange in 150 cc. of water. After filtering and washing with water, the precipitate was recrystallized from 800 cc. of boiling water. The product weighed 6.35 g. (95%).

Glycine Ethyl Ester Hydrochloride from Glycine Ethyl Ester Helianthate.—Two-tenths gram of glycine ethyl ester helianthate was dissolved in 100 cc. of hot absolute ethyl alcohol and acidified with a small amount of dry hydrogen chloride gas producing an almost immediate precipitate of helianthin. After cooling, the helianthin was removed by filtration, and the alcoholic solution concentrated *in vacuo* to a volume of 10 cc. On chilling, glycine ethyl ester hydrochloride was obtained, and, after recrystallization from 10–15 cc. of hot absolute alcohol, weighed 0.0539 g. (78.9%); m. p. 142°. A mixed m. p. with synthetic glycine ethyl ester hydrochloride showed no depression.

(9) Fischer and Fourneau, *Ber.*, **34**, 2868 (1901).

(10) Fischer and Steingroever, *Ann.*, **365**, 167 (1909).

Summary

By the addition of methyl orange to amino acid and polypeptide ester hydrochlorides, crystalline helianthates have been prepared. These salts are non-hygroscopic and sufficiently insoluble in water to serve as a convenient means for isolating or characterizing amino acid and polypeptide esters. Similar salts may likewise be prepared by the addition of helianthin to the free esters.

Butyl esters of amino acids and polypeptides may be prepared conveniently by vacuum distillation with excess butyl alcohol containing small amounts of mineral acid.

Amino acids and polypeptides do not yield similar products; guanidine forms a characteristic helianthate having a low solubility in water.

Colorimetric estimations of helianthin in these salts have been carried out in 50% aqueous acetic acid, thereby furnishing a simple method for the titration of combining basic groups as well as analysis of the substances. Lysine, histidine, arginine and cystine esters have been found to form di-helianthates.

A method is described for the recovery of ester hydrochlorides from their helianthates.

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