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

CHARACTERISTICS OF THE TWO CRYSTALLINE FORMS OF GLYCINE

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Emil Fischer[1] observed that the needle-shaped form of glycine, precipitated from water solution by absolute alcohol, reacts smoothly with phosphorus pentachloride in presence of acetyl chloride to form the hydrochloride of glycyl chloride (amino-acetyl chloride). The plate form, prepared by evaporating the water solution and drying at 100°, did not react in the same manner. Fischer believed that this behavior had to do with isomerism. There are only two structures for glycine theoretically possible, the straight chain (NH_2CH_2COOH) and the inner salt $\underline{NH_3CH_2COO}$. There is much controversy concerning the correct form and much additional experimentation is needed before a satisfactory conclusion can be reached.

Falk and Sugiura[2] studied the two forms of glycine and observed that the needle form began to decompose at a slightly higher temperature than the plate; that the time interval after solution of the plate form caused a slight difference in amino nitrogen content by the Van Slyke method, whereas the needle form underwent no change; and that the plate form took up about one atomic proportion of bromine while the needle form took up about two, the bromine, however, leaving the glycine readily. Their conclusion was that a difference existed between the two forms.

H. Biltz and H. Paetzold[3] observed that the two forms of glycine did not react with diazomethane in the absence of moisture. When moisture was present, both forms acted exactly alike, yielding betaine hydrochloride. When the two forms were pulverized and dried at 130° both plates and needles decomposed at the same temperature and in the same manner. They believed that the difference noted by Falk and Sugiura in decomposition temperature for the two forms, a fact confirmed by them when the crystals were dried at 100–103°, was due to the plates occluding mother liquor which depressed the decomposition temperature. Biltz and Paetzold then repeated the bromination experiments of Falk and Sugiura and observed that bromine taken up by both forms was lost on standing in a vacuum desiccator over moist potassium hydroxide; that each form took up about the same quantity of bromine which was adsorbed or loosely attached and variable in quantity in different experiments and was not affected whether the glycine was strongly dried or not. In repeating E.

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[1] Fischer, *Ber.*, **38**, 2914 (1905).

[2] Falk and Sugiura, *J. Biol. Chem.*, **34**, 29 (1918).

[3] Biltz and Paetzold, *Ber.*, **55**, 1066 (1922).

Fischer's acetylation experiments,[1] they obtained similar results with both forms when dried at 130°, whereas when the substances were dried at 100° the results agreed with those of Fischer, the needles reacting to form the glycyl chloride hydrochloride in about 50% yield, whereas the plates reacted only to a slight extent. It thus becomes possible to obtain this acid chloride from glycine in plate form, prepared by evaporation of the water solution, by heating to 130° for 9 hours or possibly less. The questions then arise, since the supposition can be made that the needle form has the open structure, (1) does the action of heat at 130° effect a transformation of the plate form into the needle form; (2) does solution or drying at 130° effect an equilibrium between the two forms; (3) does glycine in crystalline forms and in the presence of water have a different structure? We hope to furnish a little evidence from the following experiments to aid in answering these questions.

Experimental Part

The two forms of glycine were prepared as described by Fischer[1] from imported glycine and glycine prepared according to the method of Klages,[4] T. Curtius and E. Welde[5] and K. Kraut.[6] The material was pulverized, sifted, and dried at 100–103°. Photomicrographs of the plate and needle forms before pulverization indicated that there was water of occlusion in the plates at times. The processes of pulverizing and drying at 100–103° and sifting to 0.3 mm. mesh apparently remove this water.

Decomposition Temperature of the Two Forms

From the following and similar results, using a long thermometer, it appears that there is no difference in decomposition temperature between the two forms.

	Decomposition temperatures			
Appearance	Needle form ° C.	Plate form ° C.	Needle and plates equal parts ° C.	Needle form and plate form, 10–90 ratio ° C.
Light brown	228	228	227	224
Dark brown	229	229	228	228
Black spots	230	230	230	229
Decomp., gas formed	233	233	233	233

Content of Moisture or Alcohol

Although Fischer[1] in his experiments on the action of phosphorus pentachloride and acetyl chloride, carefully dried each form similarly at 103° and although Falk and Sugiura[2] also first dried the 2 forms of glycine, it appeared desirable to determine the amounts of moisture present in the air-dry material since, as will be shown, the presence of moisture has an effect upon the degree and intensity of the reaction.

[4] Klages, *Ber.*, **36**, 1507 (1903).

[5] Curtius and Welde, *ibid.*, **43**, 868 (1910).

[6] Kraut, *Ann.*, **266**, 295 (1891).

The two forms were prepared as usual, ground uniformly, in dried air and the moisture was determined by heating at 100°, and cooling in a desiccator.

Needles			Plates		
Sample G.	Loss in weight G.	%	Sample G.	Loss in weight G.	%
1.0308	0.0036	0.34	1.9878	0.0076	0.38
1.7547	.0034	.19	1.9243	.0060	.31
1.0291	.0005	.05	1.0314	.0015	.13
0.9164	.0010	.10	0.9435	.0013	.16
.1756	.0010	.57	.2093	.0008	.38
.1774	.0011	.62	.2525	.0009	.35
	Av.	.31		Av.	.28

In one experiment, after pulverized plates and needles had been dried over phosphorus pentoxide for 24 hours, the plates lost 0.05% in weight at 100° in 24 hours, and the needles lost 0.20% under the same conditions. From these results it appears that the small quantity of moisture or alcohol held by the air-dry glycine is the same for both forms.

Action of Hydrogen Chloride

Dry hydrogen chloride was passed over each of the two powdered forms of glycine after it had been sieved to 0.3 mm. mesh, and dried at 100°. Bell jars were used with a tray of fused calcium chloride at the bottom. Before removing the samples for weighing, the bell jar was first swept out with dry air, or the treated glycine was placed in a vacuum desiccator.

Sample G.	Weight		Glycine hydrochloride	
	After 3 days	After 6 days	Calc. G.	Found %
	Glycine needles			
0.1336	...	0.1386	0.1985	69.7
.1210	...	.1264	.1790	70.6
.1112	0.1160	.1190	.1648	72.2
.5000	.7408	...	.7425	99.7
	Glycine plates			
.1270	.1368	.1462	.1880	77.8
.1265	.1356	.1456	.1874	77.7
.1052	.1085	.1115	.1556	71.7
.5000[a]	.7040	...	.7425	84.0

[a] In this experiment the dried gas was conducted over the powdered crystals in fractions, allowed to remain in contact during the intervals and then the excess removed in a vacuum desiccator. The results of this series show that dry hydrogen chloride acts slowly on dry glycine in either form.

In the presence of moisture, for instance, when glycine was treated with hydrogen chloride in a bell jar with a dish of water present the hydrochloride was more easily and completely formed. The samples were dried at 100° after the reaction, before weighing.

The results expressed in the last tabulation show that moisture permits hydrogen chloride to react quantitatively with glycine in 6 days. It is

Sample G.	Weight after 6 days	Glycine hydrochloride Calc. G.	Glycine hydrochloride Found %
	Needles		
0.1045	0.1528	0.1547	98.77
.1071	.1550	.1585	97.79
.1139	.1675	.1687	99.30
.1077	.1546	.1595	96.90
	Plates		
.1122	.1611	.1611	100.00
.1150	.1693	.1704	99.35
.1093	.1556	.1618	96.20
.1305	.1907	.1932	98.60

also apparent that no difference exists between the two forms chemically, in the presence of moisture.

Action of Sulfur Dioxide

Sulfur dioxide was allowed to react with the two forms. They were first exposed in a jar under ordinary moisture, temperature and pressure conditions. Here the reaction proceeds according to the equation: $2CH_2(NH_2)COOH + H_2SO_3 \longrightarrow (CH_2.NH_2.COOH)_2H_2SO_3$ and goes practically to completion with both forms.

Needles				Plates			
		Diglycine hydrosulfite				Diglycine hydrosulfite	
Sample G.	Weight after 6 days	Calc. G.	Found %	Sample G.	Weight after 6 days	Calc. G.	Found %
0.1017	0.1538	0.1566	98.22	0.1123	0.1667	0.1729	96.42
.1127	.1709	.1736	98.45	.0894	.1197	.1377	86.93

When dry gas and powdered crystals were employed, practically no sulfur dioxide was taken up, the action at best being only very slight, less than one part per hundred in the case of the needle form, and none at all with the plates.

Action of Bromine

Even after 6 days, not enough bromine (an average of 67%) added to the dry powdered crystals to form a monobromo addition product. The needle form, however, was observed to react somewhat more readily than the plates.

Under atmospheric moisture conditions bromine acted upon the two forms as follows.

Sample G.	Weight After 3 days	Weight After 6 days	Calc. wt. for glycine monobromide	Calc. wt. for glycine dibromide
		Needles		
0.1166	0.4420	...	0.2390	0.3653
.1206(b)	.2000	0.2480	.2473	.3780
.1167	.2044	.2244	.2394	.3656
		Plates		
.1260	.3800	...	.2587	.3950
.1066	.3305	.4055	.2187	.3340
.1007	.2947	.3417	.2064	.3160

In this series of experiments, the results are irregular. Sometimes the needle form or the plate form takes up more bromine than the theoretical quantity for a dibromo addition product. In (b) the quantity taken up approximated the monobromo product. According to Falk and Sugiura[2] the needles form the dibromo and the plates the monobromo product. These investigators state, however, that at times when conditions were apparently suitable for the one form the other was obtained.

When moist bromine was allowed to come in contact with glycine, enough bromine was taken up by both forms in 45 minutes to form about 67% of the monobromo product. After 5 hours, a dark red, liquid mass was obtained. When this was dried, colorless glycine was recovered.

In view of the facts that relatively little bromine adds to glycine in dry form, that varying quantities of moisture change the speed and quantity of bromine addition or absorption, or adsorption, and that the bromine is immediately given off when the compound is allowed to stand in the air, it seems probable that bromine forms no definite chemical compound with either variety of glycine. If all moisture were to be excluded from the apparatus, the glycine and the bromine, it is probable that no bromine would adsorb or add to the glycine at all. Various inorganic salts containing water of crystallization or hydration also add bromine temporarily. Crystallized borax, for instance, adds bromine when exposed under ordinary conditions to bromine vapors in a bell jar. In one experiment 30.2305 g. of borax (weight with container) added 0.0138 g. of bromine in 3 days and 0.0820 g. in 6 days.

Formation of Amino-acetyl Chloride

Attempts were next made to investigate the nature of the compounds formed in Fischer's original experiment with phosphorus pentachloride and acetyl chloride. When 2 g. of glycine was treated with phosphorus pentachloride and acetyl chloride according to Fischer's procedure, the needle form yielded 0.353 g. of the hydrochloride of glycyl chloride, while the plate form yielded 0.345 g., amounting to about 10%. These results indicate that one form might have reverted to the other upon standing after pulverization. The filtrates from the solid residues were distilled under 170 mm. pressure and at from 20–25°. The distillates in both cases contained no nitrogen and consisted chiefly of acetyl chloride. A red oily substance remained in the flasks. When alcohol was added to the oil, that from the needle form warmed but deposited no crystalline substance, while that from the plate form warmed to a greater degree and yielded some crystals of glycine ethyl ester hydrochloride. H. T. Clark[7] states that acetyl chloride reacts with glycine to form acetyl-glycine.

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[7] Clark, "An Introduction to the Study of Organic Chemistry," Longmans, Green and Co., **1914**, p. 290.

The second set of experiments was carried out with 10 g. of the freshly prepared forms without intermission. In these experiments Fischer's results were duplicated, that is, 8.5 g. of the acid chloride was separated from the reaction mixture from needle glycine, corresponding to 49.2% of that calculated for glycyl chloride hydrochloride. The plate form gave a solid residue which when dried weighed 3.0 g., corresponding to 17.3% of the hydrochloride of glycyl chloride. Aniline, water and alcohol all reacted violently and exothermically with the hydrochloride of glycyl chloride from needle glycine.

The 3 g. of substance separated from the reaction mixture from the plate form did not react with aniline or alcohol and dissolved in water without rise in temperature. Its melting temperature was 202°, it contained chlorine and was probably glycine hydrochloride. Analogous to ammonium chloride, its heat of solution was negative. When dissolved in water, treated with silver oxide and hydrogen sulfide, filtered, the filtrate evaporated and the residue crystallized, glycine was obtained. It thus appears that the plate form did not react to form an acid chloride and that the substance that was formed dissolved in the acetyl chloride.

The filtrates were examined as before. They were distilled under reduced pressure at 30°, the residual oil was washed in petroleum ether and was then found to contain phosphorus and chlorine. It was soluble in acetone and ethyl acetate but could not be purified.

In order to study the influence of one constituent at a time, the action of phosphorus trichloride alone on glycine was examined. Even prolonged action or treatment of a suspension of the pulverized needle or plate glycine in phosphorus trichloride yielded no new product, the glycine being recovered unchanged.

Phosphorus pentachloride was next dissolved in carbon disulfide and treated with glycine. No action was observed. Ten g. of each form was mixed with 200 cc. of carbon disulfide and 31.5 g. of phosphorus pentachloride and the mixture stirred for 4 hours. The plate form yielded 11 g. of a white crystalline salt which fumed strongly in the air (due to the presence of hydrogen chloride) even after being washed with carbon disulfide and petroleum ether. After the mass had stood in a vacuum desiccator over phosphorus pentoxide for 12 hours it darkened and became oily and resinous. This action was probably the effect of some phosphorus pentachloride that had not been washed out, as a positive test for phosphorus was obtained. When phosphorus pentachloride and glycine were mixed dry and allowed to stand in a desiccator, an oily mass was formed, similar to that obtained in the experiment with acetyl chloride when phosphorus pentachloride was present.

In the next trial, 10 g. of the plate form of glycine was treated with a larger quantity of carbon disulfide (400 cc.) and much effort was made to wash out all phosphorus pentachloride with carbon disulfide and petroleum ether. In this manner 7 g. of a white crystalline solid was obtained. This did not fume in moist air, contained only a slight trace of chlorine, melted at 210° and was soluble in carbon disulfide. It was, therefore, not an acid chloride. Upon purification, it proved to be glycine. The needle form under exactly the same conditions yielded 10.4 g. of a white crystalline solid that was soluble in carbon disulfide, insoluble in alcohol and melted at 210°.

From these experiments, we conclude that phosphorus trichloride and phosphorus pentachloride in carbon disulfide solution have no action on either form of glycine. This would indicate that the substance causing the reaction in the Fischer experiment, yielding a chlorine derivative was the acetyl chloride, although the phosphorus pentachloride may have had some influence.

An attempt was made to determine whether any difference existed between the 2 forms of glycine in their action toward esterification reagents; 5 g. of each was boiled under a reflux condenser for 2 days with ethyl iodide and absolute alcohol. There was no evidence of reaction in either case, the glycine separating unchanged after removal of heat. After filtration, the filtrates were extracted with ether and when the ether extract was evaporated only a small quantity of resinous material was obtained. Evaporated with strong hydrochloric acid solution, no crystals were obtained. According to Beilstein, the plate form yields the ethyl ester when treated with absolute alcohol and ethyl iodide in a closed tube.

Summary

1. The action of hydrogen chloride, bromine vapor, and sulfur dioxide, with and without moisture, shows that moisture rapidly brings about an equilibrium between the plate and needle forms of glycine, and that any difference to be definitely established must be determined in the absence of water or even atmospheric moisture. The dry crystals do not differ chemically when dried at 103° and pulverized to pass a 0.3 mm.-mesh sieve; neither is there a difference in their moisture content when air-dried and pulverized, or in their decomposition temperatures.

2. Bromine did not form a chemical compound with glycine. By varying the quantity of moisture and length of time of contact, varying quantities of bromine can be taken up. The addition, absorption, or adsorption, however, is only temporary.

3. It is probable in the Fischer experiment that phosphorus pentachloride does not act directly on glycine to form any acid chloride. From the results obtained with phosphorus trichloride and phosphorus pentachloride, both dissolved in carbon disulfide, it appears that acetyl chloride itself reacted with glycine in the presence of the phosphorus halide to form glycyl

chloride indirectly, together with a number of other substances, some containing phosphorus.

This study of the characteristics of glycine is being continued.

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[CONTRIBUTION FROM THE PHARMACOGNOSY LABORATORY, BUREAU OF CHEMISTRY, UNITED STATES DEPARTMENT OF AGRICULTURE]

NEW SOURCES OF SANTONIN[1]

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The discovery of a new source of santonin should attract attention, especially because it is now almost impossible to obtain the plant material, wormseed, as well as santonin itself, owing to the unsettled conditions in Russia. Both are now marketed at prices unusually high. Levant wormseed is quoted at $3.25 to $3.50 per pound, and santonin at $172 to $175 per pound.[2]

Supplies other than the normal source of santonin, *Artemisia cina* Bg. (*Artemisia maritima* var. *stechmanniana* Bees.), which grows exclusively in the region of Turkestan and Siberia, have been utilized by manufacturers of santonin. It is reported that the plant species *Artemisia gallica* Willdenow, which is indigenous to France, and *Artemisia brevifolia* Wallich contain santonin. No definite quantities of santonin are reported in the investigations of Heckel and Schlagdenhauffen[3] and, as far as is known, this plant (*Artemisia gallica*) was never utilized in the manufacture of santonin. *Artemisia brevifolia*, a form very closely related to *Artemisia maritima* Linné, growing abundantly in Thibet, has more recently been mentioned as a possible commercial source. In 1921 Greenish and Pearson[4] called attention to this source in which they found 0.85% of santonin. Another sample of the same plant, collected at Gurez, yielded 1.09% in the leaves dried at 100°, or 0.76%, calculated upon the air-dry leaves and stems as received.[5] Whether this material can be utilized as a commercial source is still undecided.

In 1920[6] a German firm patented a process to isolate santonin from the

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[1] A preliminary note was presented at the meeting ot the American Chemical Society, Birmingham, Ala., April 3–7, 1922. An abstract was published in *Am. J. Pharm.*, **94**, 446 (1922).

[2] Oil, Paint and Drug Reporter, Jan. 10, 1923.

[3] Heckel, Ed., and Schlagdenhauffen, Fr., "De l'Artemisia gallica Willd., comme plante à santonin, et de sa composition chimique." *Compt. rend.*, **100**, 804 (1885).

[4] Greenish, H. C., and Pearson, C. E., "A new source of santonin." *Pharm. J.*, **52**, (4th ser.), 243 (1921).

[5] Greenish and Pearson, "Note on the Occurrence of Santonin," *Pharm. J.*, **109**, 85 (1922).

[6] Patentschrift Nr. 346,947, Klasse 12_0, Gruppe 25, Jan. 11, 1922.