of the blank. Calculations were made with and without this correction, however; in the actual titration the amount of thiosulfate accounted for in this way is probably much smaller since the excess of bromine is much less. We know of no way of calculating the actual excess of thiosulfate used in the titration due to the impurity of the β-naphthol. In the previous work a correction of 0.5 cc. was made in the results.

DATA

Temp., °C.	$Na_2S_2O_3$, cc.	Sample, g.	% Enol Titrated	% Enol Corrected
21.5	19.8	0.7353	31.15	30.68
22.0	16.0	.6213	29.76	29.18
22.0	17.9	.6911	29.93	29.44
22.0	13.9	.5256	30.59	29.93
		Average	30.36	29.81

Conclusions.—Our results agree within the limits of experimental error with those of our former paper. In view of the discrepancy still existing between these values and those of von Auwers, we are forced to conclude that the only further light which can be thrown on the question should come from an isolation of enol and keto in the pure state, followed by a determination of the refractive index and the bromine titration value of each.

Summary

The enol content of ethyl α-phenylacetoacetate has been redetermined both by bromine titration and by the determination of the refractive index. The values so obtained check within the limits of experimental error with those previously published by the authors.

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BENZENESULFONYLGUANIDINES[1]

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In the course of an investigation, now in progress, of the benzenesulfonyl derivatives of proteins and peptides, it became necessary to ascertain the effect of benzenesulfonyl chloride upon guanidine and its derivatives under various conditions. Ackermann[2] treated guanidine carbonate with benzenesulfonyl chloride in the presence of excess sodium hydroxide, thereby obtaining the sparingly soluble benzenesulfonylguanidine. He also reported failure to produce a sparingly soluble derivative of arginine under analogous conditions. The corresponding β-naphthalenesulfonyl

[1] Work supported by a research grant from The Chemical Foundation.

[2] Ackermann, *Z. physiol. Chem.*, **47**, 366 (1906).

derivatives of certain guanidines have recently[3] been employed for their characterization in urine.

We have been able to confirm Ackermann's observation with guanidine, but find that the yield of benzenesulfonylguanidine depends upon the amount and the concentration of the sodium hydroxide, as well as upon the amount of benzenesulfonyl chloride employed. The reason for this is not far to seek. Guanidine is a strong base which furnishes a titration curve[4] almost indistinguishable from that obtained with sodium hydroxide, and replacement of a hydrogen atom apparently takes place with guanidine only in its undissociated form.

This view is borne out by the behavior of guanidine carbonate toward benzenesulfonyl chloride in the presence of potassium carbonate, under which conditions the sole product is a well crystalline benzenesulfonate of guanidine.

Analogous results are obtained with methylguanidine, asymmetrical dimethylguanidine and piperidoguanidine, which are bases as strong as guanidine.[4] The resulting benzenesulfonyl derivatives display weakly basic properties, forming crystalline hydrochlorides which appear to be readily hydrolyzed in aqueous solution. Benzenesulfonylguanidine also forms an acetyl derivative and an unstable picrate. It shows no tendency to form salts with alkalies, thereby differing from the majority of primary sulfonamides. This may be interpreted as being due either to the preponderance of the benzenesulfonimino form or, more plausibly, to the inability of the single benzenesulfonyl group entirely to suppress the basic properties of the guanidine molecule. Guanidine nevertheless shows no tendency to form derivatives containing more than one benzenesulfonyl group, nor do the benzenesulfonyl guanidines yield nitrogen by the action of sodium nitrite in presence of acetic acid, therein resembling guanidine.[5]

The guanidine group in arginine appears, from the dissociation curve of arginine[6] and from the fact that benzylidenearginine is incapable of forming a sodium salt,[7] to be as strongly dissociated as guanidine itself. It should therefore be possible, by treating arginine with benzenesulfonyl chloride in the presence of sodium carbonate, to attach a benzenesulfonyl group to the α-nitrogen atom, while leaving the terminal guanidine group untouched. This appears to be the case; monobenzenesulfonylarginine, isolated in the form of its picrate, yields very little nitrogen under the conditions adopted by Van Slyke[5] and must, in consequence, possess the anticipated structure. This is confirmed by the regeneration of the amino group on acid hydrolysis.

[3] Stockholm and Cerecedo, *Proc. Soc. Exptl. Biol. Med.*, **29**, 78 (1931).

[4] Davis and Elderfield, THIS JOURNAL, **54**, 1499 (1932).

[5] Van Slyke, *J. Biol. Chem.*, **9**, 185 (1911).

[6] Foster and Schmidt, *ibid.*, **56**, 551 (1923).

[7] Bergmann and Zervas, *Z. physiol. Chem.*, **152**, 282 (1926); **172**, 277 (1927).

On the other hand, when the benzenesulfonylation is carried out in presence of a large excess of concentrated sodium hydroxide, benzenesulfonyl groups attach themselves to the guanidine group as well. While it was not found possible to isolate the resulting product in crystalline condition, analysis showed it unquestionably to consist of dibenzenesulfonylarginine.

Attempts to prepare benzenesulfonyl derivatives of acetamidine were unsuccessful, benzenesulfonamide being the only product isolated; analogous experiments with creatine also failed.

Experimental

Guanidine Benzenesulfonate.—To a solution of 18 g. of guanidine carbonate (0.2 m. of guanidine) in 125 cc. of water were added 26 cc. (0.2 m.) of benzenesulfonyl chloride and 14 g. (0.2 m.) of potassium carbonate. The mixture was stirred vigorously; the temperature rose to 43°. The crystals which formed on cooling were collected (22 g.) and recrystallized from 50 cc. of water from which they separated in diamond-shaped plates melting at 209–210° (corr.). The product was soluble in nine parts of water at 26°; it was readily soluble in alcohol and insoluble in ether. It could be recrystallized unchanged from dilute sodium hydroxide solution.

Anal. Calcd. for $C_7H_{11}O_3N_3S$: C, 38.7; H, 5.07; N, 19.4; S, 14.8. Found: C, 38.7; H, 5.01; N, 19.0; S, 15.1.

On adding a hot concentrated solution of picric acid to a solution of the salt, guanidine picrate melting at 330° (uncorr.) separated; this picrate contained no sulfur.

Benzenesulfonylguanidine.—Solutions of 3 g. of guanidine carbonate in 35 cc. of water were treated with varying amounts of sodium hydroxide and 6-cc. quantities of benzenesulfonyl chloride. After stirring mechanically until the odor of benzenesulfonyl chloride had disappeared (thirty to sixty minutes), the resulting precipitate was collected, washed with cold water and weighed. The crude product so obtained was recrystallized from the minimum quantity of 95% alcohol, when it separated in leaflets melting at 212° (corr.). The maximum yield (42%) of recrystallized product was obtained with 6.4 g. of sodium hydroxide; with 3.2 g. of alkali the yield was 17%; intermediate yields were obtained with intermediate quantities of alkali. With 8.6 g. the yield was only 30%. Decreasing the amount of benzenesulfonyl chloride to 4 cc. had only slight effect on the yield.

The pure product is no more readily soluble in cold aqueous alkali than in cold water; on boiling 0.250 g. with 200 cc. of *N* sodium hydroxide for twenty-four hours, 94.6% of the total amount of nitrogen was recovered as ammonia.

Benzenesulfonylguanidine hydrochloride was prepared by dissolving 0.5 g. of benzenesulfonylguanidine in 10 cc. of concentrated hydrochloric acid and evaporating the excess acid at room temperature in a current of air under reduced pressure. It melted at 160–163° (corr.).

Anal. Calcd. for $C_7H_{10}O_2N_3SCl$: Cl, 15.1. Found: Cl, 14.9.

The picrate separated in yellow needles, melting at 190–191° (corr.), on adding picric acid to a solution of benzenesulfonylguanidine in hot alcohol. On attempting to recrystallize from alcohol or ethyl acetate, dissociation took place with loss of picric acid.

The acetyl derivative was prepared by boiling benzenesulfonylguanidine for fifteen to thirty minutes with eight parts of acetic anhydride; m. p. 197–197.5° (corr.) from ethyl acetate.

Anal. Calcd. for $C_9H_{11}O_3N_3S$: S, 13.3. Found: S, 13.4.

The benzenesulfonyl derivatives of alkyl guanidines were prepared by the procedure which had been found to give the best yields with guanidine.

Benzenesulfonylmethylguanidine.—M. p. 180.5–181° (corr.).

Anal. Calcd. for $C_8H_{11}O_2N_3S$: C, 45.1; H, 5.20; N, 19.7; S, 15.0. Found: C, 45.2; H, 5.26; N, 19.2; S, 15.1.

Hydrochloride: M. p. 123–126° (corr.).

Anal. Calcd. for $C_8H_{12}O_2N_3SCl$: Cl, 14.2. Found: Cl, 14.0.

Benzenesulfonyl-*as*-dimethylguanidine.—M. p. 164.5–165.5° (corr.).

Anal. Calcd. for $C_9H_{13}O_2N_3S$: C, 47.6; H, 5.75; N, 18.4; S, 14.1. Found: C, 47.8; H, 5.65; N, 17.2; S, 14.1.

Benzenesulfonylpiperidoguanidine.—Three and one-half grams of a pure product, m. p. 168.5–169° (corr.), was obtained from 4 g. of piperidoguanidine sulfate.[4]

Anal. Calcd. for $C_{12}H_{17}O_2N_3S$: C, 53.9; H, 6.36; N, 15.7; S, 12.0. Found: C, 53.7; H, 6.28; N, 15.1; S, 11.7.

Monobenzenesulfonylarginine.—To a solution of 6 g. of arginine nitrate (0.025 m.) in 30 cc. of water were added 11 g. (0.08 m.) of potassium carbonate and 5 cc. of benzenesulfonyl chloride (0.039 m.). After stirring mechanically for a half hour at room temperature the clear solution was weakly acidified with 7 cc. of concentrated hydrochloric acid and evaporated to dryness under reduced pressure. The residue was extracted with ethyl alcohol; the alcoholic solution was again evaporated to dryness and taken up in the minimum quantity of alcohol. The glassy sirup left on evaporation was dissolved in 100 cc. of water and treated with 6 g. of picric acid in hot concentrated aqueous solution. The oily precipitate became crystalline on standing. On recrystallization from alcohol, 9.5 g. of yellow needles, melting at 161–162° (corr.), was obtained.

Anal. Calcd. for $C_{18}H_{21}O_{11}N_7S$: C, 39.8; H, 3.87; N, 18.0; S, 5.89; picric acid, 42.2. Found: C, 38.9; H, 4.02; N, 16.9; S, 5.60; picric acid, 41.9.

A 3% solution of the picrate in $N/10$ sodium hydroxide when treated by Van Slyke's method gave 4.2% of the amount of amino nitrogen theoretically obtainable from a corresponding quantity of arginine. This solution was mixed with an equal volume of concentrated hydrochloric acid, freed of precipitated picric acid by filtration, and boiled under reflux; 2-cc. portions removed at intervals were analyzed for amino nitrogen, the hydrochloric acid being neutralized by first adding 1.5 cc. of 25% sodium hydroxide and 0.5 cc. of acetic acid to the reaction mixture.

Hours boiling.....	0	1	3	8	16	24	44
Amino nitrogen, % of theoretical.....	4.2	17.0	24.1	43.8	68.6	79.7	106.0

Dibenzenesulfonylarginine.—To a solution of 4 g. of arginine monohydrochloride in 8 cc. of water was added 20 cc. of 25% sodium hydroxide solution; 6 cc. of benzenesulfochloride was added in two equal portions, the mixture being shaken vigorously after each addition. The temperature was not allowed to rise above 25°. After standing for half an hour, the mixture was freed of crystals (about 6 g., largely sodium benzenesulfonate), and the filtrate shaken with a further 3-cc. portion of benzenesulfochloride. After standing overnight, the mixture was rendered strongly acid with concentrated hydrochloric acid; the gummy precipitate was taken up by shaking with a mixture of butyl alcohol (75%) and ethyl acetate (25%). Undissolved salts were removed and the solution concentrated to a sirup. This was taken up in dioxane; the filtered solution was evaporated to a sirup and treated with excess of ethyl ether. The

sticky insoluble product was washed with ether, treated with 60 cc. of boiling water until free of steam-volatile and water-soluble impurities, and finally dried at 100°. On cooling, it formed a colorless resin, soluble in acetic acid, acetone, chloroform, ethyl acetate and ethyl alcohol, but insoluble in benzene, carbon tetrachloride, ethyl ether and water. A sample gradually dissolved in boiling sodium hydroxide solution, giving off the odor of butyl alcohol; it thus appeared to consist principally of the butyl ester of dibenzenesulfonylarginine.

Anal. Calcd. for $C_{22}H_{36}O_6N_4S_2$: C, 51.34; H, 6.79; N, 10.87; S, 12.42. Found: C, 50.4; H, 5.97; N, 10.43; S, 11.45.

Hydrolysis was effected by warming on the steam-bath a solution in ethyl alcohol to which small quantities of concentrated ammonia were added from time to time. After twelve hours no precipitation occurred on diluting with water; the solution was evaporated to dryness, and the residue taken up in boiling water, filtered hot and allowed to cool. The clear resin which separated was rinsed with water and dried at 100°; it appeared to consist of the ammonium salt.

Anal. Calcd. for $C_{18}H_{25}O_6N_5S_2$: N, 14.85; S, 13.59; acid equiv., 471. Found: N, 15.25; S, 11.9; acid equiv., 465.

The free acid was thrown out as an oil on adding a slight excess of mineral acid to a saturated solution of the ammonium salt in cold water. After rinsing with cold water and drying at 100° it solidified to a colorless resin, soluble in acetone, acetic acid, ethyl alcohol and hot water, but insoluble or sparingly soluble in benzene, carbon tetrachloride, chloroform, ethyl acetate and ethyl ether.

Anal. Calcd. for $C_{18}H_{22}O_6N_4S_2$: N, 12.34; S, 13.84. Found: N, 12.39; S, 13.07.

The authors wish to express their indebtedness to Mr. William Saschek for carrying out the microanalyses recorded in this paper.

Summary

The introduction of the benzenesulfonyl group into guanidine and its derivatives takes place only in the presence of strong alkalies, and not with alkali carbonates. Similarly, with arginine, the benzenesulfonyl group attaches itself only to the α-nitrogen atom in the presence of carbonate, but when alkali hydroxide is present in excess, the guanidine group is acylated as well.

Benzenesulfonyl guanidine is a weak base, and exhibits no tendency to form a sodium derivative analogous to that formed by benzenesulfonamide. It yields an acetyl derivative on treatment with acetic anhydride.

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