A NOTE ON THE SPECIFIC ROTATORY POWER OF d-ARGININE.

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The optical activity of d-arginine salts was first measured in 1887 by Schulze and Steiger (1). Using the old Ventzke scale, they found for an aqueous solution of the hydrochloride such a value as, when converted into circular degrees, corresponds to a specific rotatory power of $+11.49^\circ$. 12 years later Gulewitsch (2) reported $[\alpha]_D^{20}$ for the same salt as only $+10.70^\circ$ in water, and as $+21.25^\circ$ in the presence of an excess (6.75 to 13.5 molecules) of hydrochloric acid. Calculated for arginine itself, these values correspond respectively to $+12.93^\circ$ and $+25.70^\circ$. Gulewitsch's estimates have generally been accepted as final; but in 1922 Hunter and Morrell (3) stated that a specimen of the hydrochloride prepared by themselves gave, at $20^\circ$ and with 7 molecules of excess acid, a value of $+21.95^\circ$, on which basis $[\alpha]_D^{20}$ for the base would be $+26.54^\circ$. Their conclusion, that Gulewitsch's results were too low, is supported by the more recent observations of Felix and Dirr (4), who for an aqueous solution of the hydrochloride found $[\alpha]_D^{20} = +12.12^\circ$ to $12.25^\circ$. Hunter and Morrell gave none of the details of their measurements; Felix and Dirr state only that the solution examined contained 2 per cent of the salt, in which case the angle of rotation observed must have been rather small for accurate measurement. Under these circumstances it seems worth while now to publish the actual observations, upon which Hunter and Morrell's value was originally based, as well as to

1 Gulewitsch, misinterpreting Schulze and Steiger's Ventzke degrees as circular, calculated from their data a specific rotatory power of $+33.13^\circ$, and drew the conclusion that there are two arginines, the one of vegetable, the other of animal origin.
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report the wholly confirmatory outcome of a recent repetition of their work.

Preparation of Materials.

Measurements of rotatory power have been made on three preparations obtained and purified in the manner described below.

(a) Free Arginine.—A solution of arginine, prepared from hydrolyzed gelatin by the well known silver-baryta method, and containing about 25 gm. of the base, was neutralized with nitric acid, treated with 25 gm. of silver nitrate, and concentrated on the water bath to a volume of about 150 cc. The crystals of acid arginine-silver nitrate obtained on cooling were twice recrystallized, the yield at each stage being increased by treatment of the mother liquor with a mixture of alcohol and ether. After this process of purification, essentially the same as that recommended and used by Gulewitsch, the arginine was precipitated again by baryta, and from the thoroughly washed precipitate of arginine-silver it was recovered in the usual way as the free base. The final solution, freed from all reagents, was evaporated to a thin syrup and allowed to crystallize in the ice chest. There were obtained thus 10.7 gm. (vacuum-dried) of perfectly white crystalline material, consisting of free arginine without any detectable admixture of carbonate. A second crop of 7.2 gm., obtained by evaporation of the mother liquor, was discarded as possibly less pure.

(b) Arginine Hydrochloride I.—When the preparation just described was found to have a rotatory power higher than the expected, it was at first supposed that it was contaminated by some substance having a higher rotation than arginine. For this reason further purification was sought by conversion into, and recrystallization of, the hydrochloride. The entire first crop of arginine, including that recovered by evaporation of the solution used in the polarimeter, was dissolved in water, and exactly neutralized by hydrochloric acid. The solution was evaporated to dryness, and the completely crystalline residue was twice recrystallized from the smallest possible volume of boiling 70 per cent alcohol, to which was added, as it cooled, just enough ether to produce a permanent opalescence. In this operation a slight difficulty was caused by the fact, noticed already by Gulewitsch, that at certain not com-
pletely defined concentrations of alcohol the salt becomes less soluble as the temperature rises, so that, as the boiling point is approached, it may separate from its solution in the form of oily droplets. The same phenomenon is apt to occur also upon the incautious addition, at a later stage, of too much ether. In either instance the droplets rapidly settle to the bottom in a heavy oily layer. This layer may be left undisturbed, in which case it will usually in time solidify into a mass of crystals; but it was found better to bring it, if it formed, back into solution by the addition, at the first stage, of a drop or two of water, or, at the second, of a

<table>
<thead>
<tr>
<th>Substance analyzed</th>
<th>Total N.</th>
<th>Amino N.</th>
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<tbody>
<tr>
<td></td>
<td>per cent</td>
<td>per cent</td>
</tr>
<tr>
<td>Arginine.</td>
<td>31.90</td>
<td>31.90</td>
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<tr>
<td></td>
<td>31.90</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>8.17*</td>
</tr>
<tr>
<td></td>
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<td></td>
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<tr>
<td>Arginine Hydrochloride I.</td>
<td>26.53</td>
<td>26.47</td>
</tr>
<tr>
<td></td>
<td>26.42</td>
<td></td>
</tr>
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<td>26.45</td>
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* After ½ hour in the deaminizing chamber.
† After 3 hours in the deaminizing chamber.

sufficient quantity of hot alcohol. The complete success of the recrystallization depended then upon a proper adjustment of the concentrations of water, alcohol, and ether—an adjustment easier to attain in practice than to describe.

The product of the double recrystallization was washed with ice-cold 95 per cent alcohol and dried in vacuo over sulfuric acid. It then weighed 7.3 gm.

(c) Arginine Hydrochloride II.—This was prepared in exactly the same manner as the preceding specimen, but 7 years later and from a different lot of gelatin.
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Analysis of Preparations.

In Table I are given the results of determinations of total nitrogen (Kjeldahl) and amino nitrogen (Van Slyke) carried out on the arginine preparation and on Arginine Hydrochloride I. Before analysis each preparation was dried to constant weight, the first at 110°, the other at 140°. Neither lost in drying more than 0.1 per cent of its weight, so that neither contained water of crystallization. Unless otherwise indicated in the table, deamination in the Van Slyke apparatus was allowed to proceed for 5 minutes only. This, it will be seen, yielded the theoretical amount of nitrogen for the one amino group. When the reaction was allowed to go on longer, there was a gradually increasing yield of nitrogen; but the data are far from confirming the statement of Sekine (5) that arginine loses one-half of its total nitrogen in 2 to 3 hours of treatment with nitrous acid.

Arginine Hydrochloride II was not analyzed; but its (corrected) melting point was the same (219°) as that of the earlier preparation. This melting point agrees sufficiently well with those observed by Cox (6) (222°) and by Felix and Dirr (218°), and differs, like them, from that given by Gulewitsch (208-209°). When heated above its melting point the substance solidifies again, and finally decomposes, with copious evolution of gas, at 230° (according to Felix and Dirr at 235°).

Determination of Rotatory Power.

For the determination of rotatory power a weighed quantity of the thoroughly dried substance—about 2.5 gm. in the case of arginine, and about 3 gm. in the case of the salt—was dissolved in a total volume of 25 cc., this volume including as much HCl of constant boiling point as was necessary to provide a total of 8 molecules of acid for each molecule of base. The angles of rotation were measured for the D line, three different polarimeters (by Schmidt and Haensch, Duboscq-Pellin, and Hilger respectively) being made use of from time to time. The results were checked in two ways: (1) by repeating each determination with a second polarimeter, and (2) by conducting, along with the final test, a parallel experiment upon pure glucose. In this control experiment a solution containing 5.196 gm. per 100 cc. gave, as the average of several concordant measurements, a rotation of +6.00°, cor-
responding to a specific rotatory power of $+52.50^\circ$ (theoretical for $d$-glucose, $+52.61^\circ$).

The results with the arginine preparations are given in Table II, in which $c$ is the concentration, in gm. per 100 cc., of the base (in Observations 1 and 2) or the salt (in Observations 3 to 6), and the other symbols have the usual significance.

### TABLE II.

### Determinations of Rotatory Power.

| Observation No. | Description of material | Molecules of HCl for each molecule of arginine | Instrument used | $t$ | $l$ | $c$ | $\alpha$ | $\left[\alpha\right]_D^o$ for: |
|-----------------|-------------------------|---------------------------------------------|----------------|----|----|----|-------|-----------------|-----------------|
| 1 Arginine.     | 8 Schmidt and Haensch.  | 20                                          | 2.2            | 9.973 | +5.80 | +21.85 | +26.43         |
| 2 "             | 8 Duboseq.-Pellin.      | 20                                          | 2.0            | 9.973 | +5.30 | +21.97 | +26.57         |
| 3 Arginine Hydrochloride I. " " | 8 Schmidt and Haensch. | 21                                          | 2.10            | 11.932 | +5.24 | +21.96 | +26.56         |
| 4 "             | 8 Duboseq.-Pellin.      | 21                                          | 2.6            | 11.932 | +5.76 | +21.95 | +26.55         |
| 5 Arginine Hydrochloride II. " " | 8 " "                 | 25                                          | 2.5             | 11.870 | +5.75 | +22.02 | +26.63         |
| 6 "             | 8 Hilger.               | 25                                          | 2.2             | 11.870 | +5.72 | +21.90 | +26.49         |

Average ................................................................. $+21.94^\circ +26.54^\circ$

The observations recorded were made at temperatures varying between 20–25°, but, since Gulewitsch found only slight differences between 20–30°, the average of the six sufficiently concordant results may fairly be taken as the rotatory power at 20°. On this basis $\left[\alpha\right]_D^o$, in the presence of an excess of hydrochloric acid, is found to be $+26.54^\circ$ for $d$-arginine or $+21.94^\circ$ for its hydro-
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chloride. These values, obtained from materials of which the analytical data attest the purity, are 3 per cent higher than Gulewitsch's. It seems fair to conclude that Gulewitsch's material was partly racemized. There is of course no guarantee that the preparations here described were themselves entirely free from the levorotatory isomer, and it is possible that perfectly pure $d$-arginine has a rotatory power even greater than that here given.

SUMMARY.

The specific rotatory power of $d$-arginine, measured for the D line at 20° and in the presence of an excess (8 molecules) of hydrochloric acid, is at least $+26.54°$; the corresponding value for $d$-arginine hydrochloride is $+21.94°$.

Treated with nitrous acid according to the technique of Van Slyke, arginine gives off in 5 minutes at room temperature exactly one-fourth of its total nitrogen. In half an hour the yield is about 5 per cent, in 3 hours about 30 per cent, greater. The statement of Sekine that the nitrogen yield is doubled in 3 hours was not confirmed.

BIBLIOGRAPHY.

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