521. *The Ionisation of Guanidine and of Amino-acids in Sulphuric Acid.*

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Cryoscopic *i*-factors in 99·9% sulphuric acid have been measured for three guanidine salts and for the corresponding sodium or ammonium salts. The results indicate that not more than 28% of the singly charged guanidinium cations accept a further proton in this medium. The guanidinium cation is therefore a very weak base, having $pK_a \sim -11$; and ΔpK for the two stages of ionisation of guanidine is unusually large, being of the order 25.

Measurements of *i*-factors for amino-acids in sulphuric acid closely confirm the results of O'Brien and Niemann (*J. Amer. Chem. Soc.*, 1951, **73**, 4264). The maximum proton uptake by the carboxyl group in glycine and α-alanine is 20%. It increases for amino-acids in which the amino- and carboxyl groups are more widely separated by methylene groups.

GUANIDINE is a very strong monoacid base, with $pK_a = 13{\cdot}65$ at 25° (Hall and Sprinkle, *J. Amer. Chem. Soc.*, 1932, **54**, 3469; Angyal and Warburton, *J.*, 1951, 2492); and many guanidine salts have been prepared, with both strong and weak acids (*e.g.*, by Marckwald and Struwe, *Ber.*, 1922, **55**, 457). The singly charged guanidinium cation still contains free amino-groups, but in none of the salts which have been isolated does guanidine behave as a diacid base. On the other hand, Hantzsch (*Ber.*, 1930, **63**, 1782) took the depression of freezing point of sulphuric acid by guanidine hydrogen carbonate (after subtraction of the depression caused by water formed in chemical reaction) to mean that the guanidine cation is triply charged in nearly anhydrous sulphuric acid, so that a proton is accepted (presumably) at each of the three nitrogen atoms in very acid conditions. However, Hantzsch's result is not free from ambiguity; for the dissolution of guanidine hydrogen carbonate is a complex process, and the cryoscopic constant which he used for sulphuric acid was not correct. Whether, or not, guanidine forms a multiply-charged cation in concentrated sulphuric acid is significant for its reactions in very acid media: for example, for its reversible reaction with nitric acid in sulphuric acid, to form nitroguanidine (Simkins and Williams, *J.*, 1952, 3086). Multiplication of charge on the cation would be expected to retard nitration and would help to account for the unfavourable equilibrium constants observed in the most concentrated acids. New cryoscopic measurements with guanidine salts in sulphuric acid (Table 1) fail to support Hantzsch's conclusion for guanidine, and indicate, rather, that the singly-charged guanidinium cation is a very weak base.

The measurements are not claimed to be of the very highest precision (cf. Gillespie, Hughes, and Ingold, *J.*, 1950, 2473); but, where comparison is possible, the results agree with those obtained in the most accurate experiments (Gillespie, *ibid.*, pp. 2537, 2542). In order to eliminate idiosyncracy of apparatus or method, each of three guanidine salts has been paired with a sodium or ammonium salt having the same anion. The comparison salts are of known behaviour in sulphuric acid (Gillespie, *loc. cit.*): sodium benzoate should give an *i*-factor of 4 owing to formation of benzoic acid and then of its conjugate acid; and the benzenesulphonate and perchlorate, which form acids, too strong to take up a further proton, should give *i*-factors of 3. The reasons for minor deviations from these figures have been discussed by Gillespie (*loc. cit.*).

Each guanidine salt (Table 1) gives an *i*-factor only slightly higher than that of the uni-univalent comparison salt, instead of giving the *i*-factors of 5 for the benzoate and 4 for the two other salts which would be observed if the guanidine molecule took up two protons quantitatively in sulphuric acid. *i*-Factors of 6 and 5, corresponding to a triply charged guanidine cation, are out of the question.

TABLE 1. i-*Factors in sulphuric acid.*

		Comparison salt		Guanidine salt	
Anion	Cation	*i*-Factor	*i* (mean)	*i*-Factor	*i* (mean)
Benzoate	Na	3·98, 3·98, 4·08, 4·06, 4·07, 4·03	4·03	(4·16), 4·27, 4·28, 4·32, 4·23, 4·28	4·28
Benzenesulphonate	Na	3·16, 3·15, 3·21, 3·11, 3·14	3·15 (3·13) *	3·39, 3·43, 3·48, 3·50, 3·41, 3·46, 3·44	3·44
Perchlorate	NH_4	2·92, 2·91, (2·85), 2·92, 2·94	2·92 (2·93) *	3·28, 3·25, 3·21, 3·19, 3·24, 3·20	3·23

* *v*-Factors, Gillespie (*locc. cit.*).

The Basic Ionisation Constant of the Guanidinium Cation.—If the mean difference of 0·28 between the *i*-factors of the guanidine salts and the comparison salts can be taken to mean that the singly charged guanidinium cation is about 28% ionised to the doubly charged cation in 99·9% sulphuric acid, then the guanidinium cation is a weaker base than 2 : 4 : 6-trinitroaniline, which is about 61% ionised in the same medium and has $pK_a = -9{\cdot}29$ (Hammett and Deyrup, *J. Amer. Chem. Soc.*, 1932, **54**, 2721; Hammett and Paul, *ibid.*, 1934, **56**, 827). In principle, the ionisation constant,* pK_a^I, might be derived (Hammett *et al.*, *locc. cit.*) from the equations

$$pK_a^I = -\log\,(a_{H^+}\,a_{GH^+}/a_{GH_2^{2+}}) = H_+ + \log\,\{[GH_2^{2+}]/[GH^+]\} \quad . \quad . \quad (1)$$

in which G stands for the neutral guanidine molecule. If $H_0 = -10{\cdot}5$ for 99·9% sulphuric acid is substituted in (1) for the unknown value of H_+, then with $[GH_2^{2+}]/[GH^+] = 0{\cdot}39$, $pK_a^I \sim -10{\cdot}9$. With certain assumptions, Brand, Horning, and Thornley (*J.*, 1952, 1374) have estimated that $H_+ \gtrsim H_0 - 0{\cdot}28$ for 100% sulphuric acid. With $H_+ \approx -10{\cdot}8$, $pK_a^I \gtrsim -11{\cdot}2$. These results for pK_a^I for guanidine can be no more than estimates of order of magnitude, for both the ionisation ratio and the value of the acidity function (very sensitive to change of medium composition in this region) are approximate; but even the rough estimate implies a prodigious stretch between the first and second ionisation constants for guanidine (Table 3).

The Behaviour of Amino-acids.—A similar influence of an existing positive charge upon basic ionisation in sulphuric acid occurs with amino-acids. By analogy with acetic acid (Hantzsch, *Z. physikal. Chem.*, 1907, **61**, 257), these might be expected to take up a proton at the carboxyl group, when dissolved in sulphuric acid. We had made preliminary

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* Since the constant pK_a refers, strictly, to the ionisation of the conjugate acid of a base, it seems logical to use the symbols pK_a^{II} and pK_a^I, respectively, for *acceptance* of the first and second protons by a base.

In the text, the symbol ΔpK_I^{II} stands for $pK_a^{II} - pK_a^I$.

measurements of *i*-factors for amino-acids when the results of similar experiments were published by O'Brien and Niemann (*J. Amer. Chem. Soc.*, 1951, **73**, 4264). Our results are given, without further detail, in Table 2, since they agree closely with those of O'Brien and Niemann, shown in parentheses in the Table.

TABLE 2. *i-Factors for amino-acids,* $H_3\overset{+}{N}\cdot[CHR]_n\cdot CO_2H$, *in sulphuric acid.*

R	H	Me	H	H	H
n	1	1	2	3	5
i	2·2 (2·2)	2·2	2·7 (2·7)	(2·9)	3·0 (3·0)

The existing positive charge almost inhibits proton uptake at the carboxyl group in glycine and α-alanine. If 20% proton uptake occurs with glycine (as supposed by O'Brien and Niemann), pK_a^I from a similar equation to (1) is −11·1.

First and Second Ionisation Constants of Bases.—For ethylenediamine, $\Delta pK_I^{II} = 3{\cdot}0$ (Schwarzenbach, *Helv. Chim. Acta*, 1933, **16**, 522). For adjacent amino-groups, it has been estimated (*idem, ibid.*, 1936, **19**, 178) that in hydrazine $\Delta pK_I^{II} \approx 9$. It seems surprising, therefore, that the residual amino-groups in the guanidinium cation are so weakly basic. On the other hand, it has already been noted (Flexser, Hammett, and Dingwall, *J. Amer. Chem. Soc.*, 1935, **57**, 2103) that when a base accepts a second proton only in very strong acid ΔpK_I^{II} may be large. Figures are given in Table 3. Comparison of pK values determined in water with those based on measurements in sulphuric acid is significant, since the latter are calculated by means of the acidity function, H_0, and are thus referred to a standard state in water.

TABLE 3. *First and second ionisation constants of bases.*

Base	$Ph\cdot CO_2^-$	$Ph\cdot CO_2H$	$CH_2(NH_3^+)\cdot CO_2^-$	$CH_2(NH_3^+)\cdot CO_2H$	$(NH_2)_2C{:}NH$	$(NH_2)_2C{:}NH_2^+$
pK_a	4·16 *	−7·26 *	2·34 †	~ −11·1	13·65	~ −10·9
$pK_a^{II} - pK_a^I$	11·4		~13·4		~24·6	

* Flexser, Hammett, and Dingwall (*loc. cit.*).

† Cohn and Edsall, "Proteins, Amino Acids and Peptides," Reinhold, New York, 1943, p. 84.

Structural influences upon ionisation in guanidine are large. Thus, although guanidine itself is such a strong base, nitroguanidine is so weak that its salts are almost completely hydrolysed in water (Thiele, *Annalen*, 1892, **70**, 1). Wood (*J.*, 1903, **83**, 568) estimated the extent of hydrolysis by a catalytic method and obtained a (rough) figure corresponding to $pK_a \sim -0{\cdot}2$ for nitroguanidine. Instead of $\Delta pK_a \sim 2$ for the pair aniline and *m*-nitroaniline, introduction of the nitro-group into guanidine causes $\Delta pK_a \sim 14$. Lack of the special resonance stabilisation present in the guanidinium cation (estimated by Pauling, "Nature of the Chemical Bond," Cornell Univ. Press, Ithaca, 1939, p. 198, as about 7 kcal./mole, corresponding to $\Delta pK \sim 5$) no doubt contributes to the relative weakness of nitroguanidine as a base, in comparison with guanidine. The same factor must oppose acceptance of a further proton by the guanidinium cation.

Electrostatic effects may also be considerable in the planar, symmetrical guanidinium cation, in which the C–N distance is 1·18 Å (Theilacker, *Z. Krist.*, 1931, **76**, 303; 1935, **90**, 51, 256). Bjerrum's original treatment of the first and second ionisations of dibasic acids (*Z. physikal. Chem.*, 1923, **106**, 219; cf. Greenspan, *Chem. Reviews*, 1933, **12**, 339) is not adequate when a charge is very near the centre of ionisation, but if Kirkwood and Westheimer's more elaborate theory (*J. Chem. Phys.*, 1938, **6**, 506; 1939, **7**, 437) is applied to the process $GH_2^{2+} + G \rightleftharpoons 2GH^+$, their expression for a spherical cavity (having low dielectric constant, but situated in a medium with the dielectric constant of water) of radius 2·65 Å (calculated from a Traube partial molar volume of 67 c.c. for guanidine; cf. Partington, "Physical Chemistry," Vol. II, Longmans, London, 1951, p. 24), containing unit positive charge at 1·18 Å from the centre, leads to $\Delta pK_I^{II} \sim 19{\cdot}5$. This amounts to omission of the N–H distance in locating the charge within the cavity [cf. Brand, Horning, and Thornley's treatment (*loc. cit.*) of the basic ionisation of the nitro-group]. If the distance from the centre of the cavity to the ionisable hydrogen atoms of the NH_3^+ group is to be taken into account (Westheimer and Shookhof, *J. Amer. Chem. Soc.*, 1939, **61**, 555), then a large value ($\Delta pK_I^{II} \sim 13$) is obtainable only if the guanidinium cation is supposed to occupy a spherical cavity of radius about 4 Å. These calculations show only that a large electrostatic contribution to the value of ΔpK_I^{II} is not impossible if a charge is near enough to the centre of ionisation. The fact that the ion GH_2^{2+} is not a symmetrical dibasic acid has been neglected, except for the omission of any statistical factor.

EXPERIMENTAL

Materials.—Guanidine benzenesulphonate, m. p. 209—210° (Found: C, 39·2; H, 5·4; N, 19·3; S, 14·3. Calc. for $C_7H_{11}O_3N_3S$: C, 38·7; H, 5·1; N, 19·3; S, 14·8%), was made from guanidine carbonate and benzenesulphonyl chloride (Clarke and Gillespie, *J. Amer. Chem. Soc.*, 1932, **54**, 1966). Guanidine benzoate, m. p. 226—227° (Found: C, 53·3; H, 6·1; N, 23·2. Calc. for $C_8H_{11}O_2N_3$: C, 53·0; H, 6·1; N, 23·2%), was made from the carbonate and sodium benzoate (Walker, *J.*, 1949, 1996). Guanidine perchlorate, m. p. 247·5—248·5° (Found: C, 7·9; H, 3·9; N, 26·8; Cl, 22·2. Calc. for $CH_6O_4N_3Cl$: C, 7·5; H, 3·8; N, 26·3; Cl, 22·2%), was made by neutralising an aqueous solution of the carbonate with perchloric acid (Marckwald and Struwe, *loc. cit.*; Mazzucchelli and Rossi, *Gazzetta*, 1927, **57**, 383; cf. Glasner and Makovky, *J.*, 1953, 182).

The sodium or ammonium salts were purified and dried before use. The amino-acids (cf. Table 2) were commercial specimens.

The sulphuric acid was prepared by diluting redistilled oleum with 98% sulphuric acid to an acid of f. p. approx. 9·9°.

Measurements.—The cryoscopic cell was made entirely of glass except for a rubber collar holding a Beckmann thermometer in a close-fitting neck, and was essentially similar to that of Newman, Kuivila, and Garrett (*J. Amer. Chem. Soc.*, 1945, **67**, 704). It had a hand- or motor-operated stirrer with mercury seal. The cell was surrounded by an air-jacket with upward sloping side-arm and the whole was supported in a Dewar vessel.

Before a measurement, the cell was cooled in ice–salt and was then transferred to a similar Dewar vessel containing water at a temperature 2° below the freezing point to be measured. A supercooling of about 1·5° was permitted and was corrected for by the formula of Gillespie, Hughes, and Ingold (*loc. cit.*). F. p.s, read on the Beckmann mercury thermometer, were reproducible to within 0·005°, and did not change appreciably with time. For example, a sample of sulphuric acid solvent had f. p. (Beckmann reading) 1·405°, 1·403°, and 1·406° at times zero, 1 hr., and 7 hr. respectively; and a solution of sodium benzoate in sulphuric acid had f. p. 3·053°, 3·054° at zero time and 3·050° after 20 hr. in the apparatus.

i-Factors were calculated from the expression $ik = \Delta\theta/\Delta m$ where $\Delta\theta$ is the depression in the f. p. caused by a change Δm in the molality of the solute; and k, the cryoscopic constant of sulphuric acid, is 5·98 deg. mole⁻¹ kg. (*idem, ibid.*). Only experiments in which $0{\cdot}5° < \Delta\theta < 1{\cdot}55°$ were included in the mean *i*-factors in Table 1. The accuracy of the results did not warrant the application of the corrections used for the calculation of υ-factors (*idem, ibid.*).

The results for one pair of salts are given in full.

Ammonium perchlorate			Guanidinium perchlorate		
Δm	$\Delta\theta$	i	Δm	$\Delta\theta$	i
0·04388	0·765°	2·92	0·04449	0·873°	3·28
0·07980	1·388	2·91	0·06256	1·217	3·25
0·1086	1·881	2·90	0·08019	1·541	3·21
0·03632	0·620°	2·85	0·0411	0·785°	3·19
0·06067	1·061	2·92	0·05507	1·067	3·24
0·08082	1·425	2·94	0·07901	1·511	3·20

We thank the Department of Scientific and Industrial Research for a maintenance allowance and Imperial Chemical Industries Limited for financial help.

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[*Received, May 4th*, 1953.]