PROTONATION AND SULFONATION OF GRAMINE IN STRONG SULFURIC ACID

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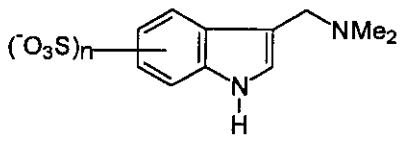
Abstract - Gramine (3-dimethylaminomethylindole) when treated with concentrated sulfuric acid at room temperature, after protonation at the dimethylamino group and at position 3, was sulfonated at the aromatic positions. Initial sulfonation in either the 5- or 6-position was succeeded by introduction of a second sulfonic acid group giving a mixture of gramine-4,6-disulfonic acid, gramine-5,7-disulfonic acid and gramine-2,6- or gramine-2,5-disulfonic acid.

Indole derivatives bearing a nucleophilic group in the β-position of a 3-ethyl derivative may undergo electrophilic attack at the 3-position followed by nucleophilic ring closure to the 2-position.[1] When the electrophile is a proton the reaction is usually reversible. In order to study the initial protonation reaction we chose gramine because a subsequent ring closure reaction was excluded. During experiments in conc. sulfuric acid it became evident that competitive fast reactions occurred and caused disturbance of the monitoring of the protonation reaction. We now wish to report on the character of these reactions and the implications for the results reported in the literature for the protonation of indoles.

When gramine is dissolved in conc. sulfuric acid immediate protonation of the dimethylamino group is followed (^{1}H, ^{13}C nmr) by a fast protonation of the 3-position of the indole nucleus in analogy to the findings for skatole.[2] Also the parent heterocyclic system, indole, underwent 3-protonation under these conditions. For

reference purposes the nmr data are given in the experimental section. When gramine was dissolved in conc. hydrochloric acid, 85% phosphoric acid or trifluoroacetic acid 3-protonation was not observed by nmr techniques.

In conc. sulfuric acid protonation is succeeded by sulfonation of the aromatic system. Initially two monosulfonated gramines emerge soon to be replaced by disulfonated products which eventually dominate the reaction mixture. The introduction of an additional sulfonic acid group is a slower process, and no further sulfonation reaction is observed in conc. sulfuric acid at room temperature. The reactions are fairly fast in conc. sulfuric acid, slow in 14 M sulfuric acid and in 12 M sulfuric acid neither protonation nor sulfonation is observed at room temperature. However, oleum (30% SO_3 in H_2SO_4) introduce a third sulfonic acid group in gramine forming a mixture of gramine 2,4,6- and 2,5,7-trisulfonic acids (**5**, **6**). All compounds isolated were studied as the sodium salts.



n=1	**1a** gramine 5- or 6-sulfonic acid
	1b gramine 5- or 6-sulfonic acid
n=2	**2** gramine 2,5- or 2,6-disulfonic acid
	3 gramine 4,6- or 5,7-disulfonic acid
	4 gramine 4,6- or 5,7-disulfonic acid
n=3	**5** gramine 2,4,6- or 2,5,7-trisulfonic acid
	6 gramine 2,4,6- or 2,5,7-trisulfonic acid

The structures of the monosulfonated gramines were determined as gramine 5- and 6-sulfonic acids (**1a**, **1b**). Analogously, the disulfonated products must be gramine 2,5- or 2,6-disulfonic acid (**2**) and gramine 4,6- and 5,7-disulfonic acid (**3**, **4**). Attempts to characterize the disulfonic derivatives by performing the sulfonation on 5- and 6-deuteriogramine, prepared by Br/D exchange of 5-and 6-bromogramine,[3] failed because of a fast proton exchange reaction of the aromatic protons. In order to unequivocally assign the proton signal originating from H2 2-deuteriogramine[4] was synthesized, characterized and subjected to nmr analysis in conc. sulfuric acid. In other indoles the general trend for the velocity of exchange of the protons in acid are H1 > H3 > H2.[4] In skatole (in 18 M D_2SO_4) the exchange of H1 is very fast and H2 in the order of 30 min.[5] In protonated gramine (D_2SO_4), on the contrary, H3 is exchanged so fast that the corresponding nmr signal disappear when the signal from the solvent is decoupled. The protons H5 and H6 exchange faster than H4 and H7. The protons

most stable towards exchange is H2 and the extremely stable H1. This sequence of tendency for exchange is in contrast to findings for solutions in deuterio trifluoroacetic acid where the order of reactivity is H1 and H5 > the remaining protons. The latter result is also in contrast to the observations for tryptophan in the same solvent where the reactivity was H2 ≈ H6 > the remaining protons.[6] It should be borne in mind however that tryptophan is 3-protonated under these conditions[7] while that is not the case for gramine. The sulfonated derivatives exhibit a strongly reduced disposition for proton exchange (D_2SO_4) and reacted too slowly to allow detection within 5 h. In addition no sign of 3-protonation was observed in any of the sulfonated derivatives.

The reactions can be monitored by uv spectroscopy as well as nmr spectroscopy. The results of studies of gramine in sulfuric acid at concentrations higher than 12 M must be viewed with suspicion unless the products have been subjected to analysis by independent techniques. When gramine was investigated by uv spectroscopy in sulfuric acid of concentrations between 0.05 and 17 M[7] the progress of what was believed to be the 3-protonation reaction coincide exactly with our findings for the uv monitoring of the sulfonation reactions. Furthermore, the reaction was reported to be reversible, a result we have not been able to confirm. We conclude that the latter study actually describes the sulfonation of gramine instead of the protonation reaction.

EXPERIMENTAL

Methods:-Ir spectra were recorded with a Perkin Elmer 1760X FT-IR spectrophotometer in potassium bromide pellets. ^1H and ^{13}C nmr spectra were recorded on a Bruker AM-250 FT-NMR instrument at 25°C using TMS as internal standard. The nmr spectra are presented in the order δ value in ppm, peak multiplicity, coupling constant in Hz, integrated number of protons and assignment. Silica gel 60 F_{254} (Merck) and RP-18 $F_{245}s$ (Merck) plates were used for thin layer chromatography (tlc). Column chromatography was carried out on Lobar Si-60 (Merck) and Sephadex G10 (Pharmacia).

Materials:-Gramine (Fluka) and 5-bromogramine (Sigma) were used without purification. Solutions of n-BuLi (2.5 M in hexane) and *tert.*-BuLi (1.7 M in pentane) were from Aldrich Chemical Company. Gramine-6-D was prepared by a literature procedure.[3] Tetrahydrofuran and ether were dried by distillation from sodium.

Nmr spectra of gramine in concentrated H_2SO_4. ^1H Nmr spectra were recorded using the H_2SO_4 signal at 11.3 ppm as internal standard. ^{13}C Nmr spectra were recorded using an internal standard sealed capillary insert containing TMS. *Ca.* 100 mg of gramine was dissolved in 0.6 ml of concentrated sulfuric acid on ice bath, and the solution was transferred to a nmr tube. Progress of the sulfonation reaction was monitored by recording ^1H nmr spectra immediately and with 10-20 min interval until the reaction was completed. The first spectrum was obtained after 5 min and exhibited signals from both the protonated form of gramine and the sulfonated derivatives. The signals from the protonated form of gramine appeared at 3.4 (s, 6H, CH_3), 4.0 and 4.1 (two br s, 2H, CH_2), 5.0 (br s, 1H, H3), 7.8-8.2 (m, 4H, H4-H7), 9.6 (s, 1H, H2) and 13.2 ppm (br s, 1H, H1). These signals faded over a period of 2 h. The transient signals of the monosulfonated derivatives arose at 3.2 (s, CH_3), 4.7 (s, CH_2), 7.2 (br s, H1) and 7.8-8.6 ppm (m, H4-H7). After 4 h the spectrum showed only the signals of the disulfonated derivatives (**2**, **3** and **4**).

^{13}C Nmr spectrum obtained immediately after dissolving gramine showed the signals from the protonated form at 42.0 (CH_3), 48.9 (C3), 53.9 (CH_2), 117.8, 124.1, 129.8, 130.8, 131.6, 139.2, 174.6 (C2).

Protonation of skatole in conc. H_2SO_4: Skatole (120 mg) dissolved in conc. H_2SO_4 (1 ml) was subjected to nmr spectroscopy. ^1H Nmr: 1.8 (d, J=7.8, 3H, CH_3), 4.5 (q, J=7.8, 1H, H3) 7.5-8.0 (m, 4H, H4-H7), 9.2 (d, J=5.6, 1H, H2), 12.7 (br s, 1H, H1). To assure the assignments of the signals the resonance of the methyl protons at 1.8 ppm was saturated resulting in the quartet at 4.5 ppm to be replaced with a singlet. Saturation of the H2 resonance at 9.2 ppm does not affect the other signals.

^{13}C Nmr: 10.3 (CH_3), 48,1 (C3), 116.4, 123.7, 128.7, 130.1, 136.8, 139.1, 180.9 (C2).

Protonation of indole in conc. H_2SO_4: *Indole (ca. 100 mg) dissolved in conc. H_2SO_4 (1 ml) was subjected* to nmr spectroscopy. ^1H Nmr: 4.8 (s, 2H, H3), 8.0 (m, 4H, H4-H7), 9.5 (d, J=7.7, H2), 13.0 (br s, 1H, H1).

^1H Nmr spectra of gramine in strong acids. Saturated solutions of gramine in acid were subjected to nmr spectroscopy. In no cases was protonation detected. Trifluoroacetic acid (the signal at 11.3 ppm was used as internal standard): 2.8 (d, J=6.6, 6H, CH_3), 4.4 (d, J=6.6, 2H, CH_2), 7.0 (br s, 1H, H1), 7.1 (m, 2H, H5

and H6), 7.3 (s, 1H, H2), 7.3 (d, J=8.2, 1H, H7), 7.4 (d, J=7.3, 1H, H4). Conc. hydrochloric acid (TMS was used as internal standard): 1.3 (d, J=4.8, 6H, CH_3), 2.8 (d, J=5.4, 2H, CH_2), 5.9 (m, 2H, H5 and H6), 6.1 (s, 1H, H2), 6.2 (d, J=6.0, 2H, H4 and H7). Phosphoric acid (TMS was used as external standard): 1.5 (d, J=3.0, 6H, CH_3), 3.0 (d, J=3.0, 2H, CH_2), 5.9 (br s, 1H, H1), 6.1 (m, 2H, H5 and H6), 6.2 (s, 1H, H2), 6.3 (m, 2H, H4 and H7).

Sulfonation of gramine: Gramine (2.245 g, 12.9 mmol) was dissolved in conc. H_2SO_4 (20 ml) at 0^0C. After 20 h at room temperature, the mixture was poured on ice and made alkaline with conc. sodium hydroxide. The solution was washed with chloroform (5 x 100 ml), and the aqueous phase was neutralised with conc. H_2SO_4. After freeze drying the products were extracted from the Na_2SO_4 with hot ethanol (9 x 150 ml). Evaporation of the solvent at reduced pressure yielded 3.46 g of product. Tlc (silica gel with methanol/water 4:1 as eluent) revealed a mixture of four components and nmr spectroscopy (D_2O) showed a mixture of **1a**, **2**, **3** and **4** in the ratio 5:25:40:30. The crude material was fractionated by column chromatography on a Sephadex column eluted with H_2O. The fractions were further purified on Si-60 (MeOH/H_2O, 4:1) to give **1a**, **2**, **3** and **4**. Ir ν_{max}: **1a** 3412, 1621, 1476, 1176, 1026, 648, 620 cm^{-1}. **2** 3420, 1633, 1191, 1040, 653, 620, 600 cm^{-1}. **3** 3431, 1634, 1190, 1042, 658, 600 cm^{-1}. **4** 3421, 1641, 1476, 1196, 1039, 653, 600 cm^{-1}. Anal. Calcd for $C_{11}H_{12}N_2O_6Na_2S_2$, $2H_2O$: C: 31.89 H: 3.89 N: 6.76 S: 15.47. Found: **3** C: 32.11 H: 3.59 N: 6.72 S: 15.50. ^{1}H Nmr: **1a** (CD_3OD) 3.0 (s, 6H), 4.6 (s, 2H), 7.7 (d, J=8.7, 1H), 7.8 (s, 1H), 7.9 (dd, J=8.6 J=1.7, 1H) 8.4 (d, J=1.8, 1H). **2** (CD_3OD) 3.1 (s, 6H), 4.9 (s, 2H), 7.7 (d, J=8.6, 1H), 7.9 (dd, J=8.6 J=1.6, 1H), 8.4 (d, J=1.6, 1H). **2** (H_2SO_4) 3.3 (s, 6H), 5.0 (s, 2H), 7.0 (br s, 1H), 8.1 (d, J=8.6, 1H), 8.3 (d, J=8.6, 1H), 8.8 (s, 1H). **3** (CD_3OD) 3.0 (s, 6H), 4.9 (s, 2H), 7.9 (s, 1H), 8.3 (d, J=1.6, 1H), 8.5 (d, J=1.6, 1H). **3** (H_2SO_4) 3.0 (s, 6H), 4.6 (s, 2H), 6.8 (br s, 1H), 8.0 (s, 1H), 8.4 (d, J=1.6, 1H), 8.5 (d, J=1.6, 1H). **4** (CD_3OD) 2.8 (s, 6H), 4.4 (s, 2H), 7.8 (s, 1H), 8.4 (d, J=1.5, 1H), 8.4 (d, J=1.5, 1H). **4** (H_2SO_4) 3.0 (s, 6H), 4.6 (s, 2H), 6.6 (br s, 1H), 8.9 (s, 1H), 8.4 (s, 1H), 8.7 (s, 1H).

Quenching the reaction after 5 min made it possible to isolate two monosulfonated gramines. ^{1}H Nmr: **1a** (D_2O) 2.7 (s, 6H, CH_3), 4.3 (s, 2H, CH_2), 7.6 (d, 8.8, 1H), 7.6 (s, 1H, H2), 7.9 (dd, J=8.6, J=1.6, 1H), 8.4 (d, J=1.2, 1H). **1b** (D_2O) 2.7 (s, 6H, CH_3), 4.3 (s, 2H, CH_2), 7.7 (s, 1H, H2), 7.7 (dd, J=8.4, J=1.5, 1H), 7.8 (d, J=8.6, 1H), 8.2 (d, J=0.9, 1H). Sulfonation of gramine (1.0 g, 5.7 mmol) in oleum (10 ml, 30%

SO_3 in H_2SO_4) following the directions given above yielded 1.8 g crude material mainly consisting of two trisulfonated derivatives **5** and **6** in the ratio 9:5 revealed by ^1H nmr (D_2O). ^1H Nmr **5** (CD_3OD): 3.1 (s, 6H, CH_3), 5.3 (s, 2H, CH_2), 8.3 (d, J=1.6, 1H), 8.5 (d, J=1.6, 1H). **6** (CD_3OD): 3.1 (s, 6H, CH_3), 4.9 (s, 2H, CH_2), 8.4 (d, J=1.5, 1H), 8.5 (d, 1.5, 1H).

3-Dimethylaminomethylindole-5-D was prepared from 5-bromogramine following the litterature procedure given for 3-dimethylaminomethylindole-6-D.[3] 5-Bromogramine (0.9571 g, 3.8 mmol) was dissolved in Et_2O (60 ml) and THF (20 ml) and cooled to -78°C under a nitrogen atmosphere. n-BuLi (4.7 mmol) was added and the mixture allowed to warm to 0°C. *tert.*-BuLi (9.9 mmol) was added to the yellow solution and the reaction mixture was stirred for 1 h at 30°C, before quenching with D_2O (0.3 ml) followed by H_2O (10 ml). The reaction mixture was extracted with CH_2Cl_2 (5x10 ml) and the organic phase was dried ($MgSO_4$). Evaporation of the solvent left 3-dimethylaminomethylindole-5-D (650 mg, 97%) as colorless crystals. Ir (KBr, cm^{-1}) 3044, 2818, 2265, 1451, 1169, 1114, 810, 751, 667. ^1H Nmr ($CDCl_3$) 2.3 (s, CH_3), 3.7 (s, CH_2), 7.1 (d, J=2.2, H2), 7.2 (d, J=8.3, H6), 7.4 (d, J=8.2, H7), 7.7 (s, H4), 8.4 (br s, H1). ^{13}C Nmr ($CDCl_3$): 45 (CH_3), 54 (CH_2), 111 (C7), 112 (C3), 119 (C4), 122 (C6), 124 (C2).

3-Dimethylaminomethylindole-2-D was prepared by a litterature procedure.[4] Gramine (3.38 g, 19 mmol) was converted to its sodium salt with NaH (1.44 g of a 80 % dispersion in mineral oil, 48 mmol). Acylation with *p*-toluenesulphonyl chloride (4.0 g, 21 mmol) yielded 4.24 g of yellow oil. Purification of 2.4 g of this oil on a Lobar Si-60 column using EtOH/conc. NH_4OH 99:1 as eluent yielded 0.87 g (36%) of 1-(*p*-toluenesulphonyl)-3-dimethylaminomethylindole. Nmr ($CDCl_3$) 2.1 (s, 3H, CH_3Ar), 2.3 (s, 6H, $N(CH_3)_2$), 3.5 (s, 2H, CH_2N), 7.0 (d, J=8.5, 2H, Ar), 7.1-7.3 (m, 2H, H5, H6), 7.5 (s, 1H, H2), 7.6 (dd, J=2.1, 7.7, 1H, H7), 7.7 (d, J=8.3, 2H, Ar), 8.0 (dd, J=1.8, 6.7, 1H, H4). Lithiation of 1-(*p*-toluenesulphonyl)-3-dimethylaminomethylindole (0.87 g, 2.7 mmol) with n-BuLi (2 ml of a 2.5 M solution in hexane, 5 mmol) and subsequent quenching with D_2O gave 0.77 g (85%) of 1-(*p*-toluenesulphonyl)-3-dimethylaminomethylindole-2-D. Nmr ($CDCl_3$) 2.19 (s, 3H, CH_3Ar), 2.20 (s, 6H, $N(CH_3)_2$), 3.48 (s, 2H, CH_2), 7.08 (d, J=8.0, 2H, Ar), 7.14-7.30 (m, 2H, H5, H6), 7.60 (dd, J=1.4, 7.6, 1H, H7), 7.72 (d, J=8.3, 2H, Ar), 7.98 (dd, J=0.8, 8.1, 1H, H4). The *p*-toluenesulphonyl group of 1-(*p*-toluenesulphonyl)-3-dimethylaminomethylindole-2-D (0.77 g,

2.3 mmol) was removed by treatment with sodium in liquid ammonia (30 ml). Yield: 0.2 g (48%) of 3-dimethylaminomethylindole-2-D as colorless crystals. Nmr ($CDCl_3$) 2.3 (s, 6H, $N(CH_3)_2$), 3.7 (s, 2H, CH_2), 7.1-7.2 (m, 2H, H5, H6), 7.3 (dd, J=7.6, 1.3, H7), 7.7 (dd, J=7.6, 1.4, H4), 8.6 (br s, 1H, H1).

Uv spectra of gramine in H_2SO_4. A standard solution (2.6 mM) of gramine in methanol was prepared. Samples of this solution (0.5 ml) was evaporated to dryness and dissolved in sulfuric acid (25 ml) giving a final concentration of gramine of 0.05 mM. Sulfuric acid at various concentrations between 5 and 18 M was used. The spectrum was recorded immediately and the changes with time was observed. Between 5 and 12 M sulfuric acid absorbtion maxima appeared at 212 and 276 nm and no change was observed for a period of 1 h. Above 12 M sulfuric acid the position of the absorbtion maxima changes to 200, 230 and 290 nm. The absorbance at 200 and 230 nm increases for at least 1 h. The position of the absorbtion maxima remained unchanged after dilution with water.

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