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A NEW AMINE, STIZOLAMINE, FROM *STIZOLOBIUM HASSJOO*

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Key Word Index—*Stizolobium hassjoo*; Leguminosae; amine; stizolamine; permanganate oxidation; pyrazine; 1,3,5-triazine.

Abstract—A new pyrazine derivative, stizolamine (1-methyl-3-guanidino-6-hydroxymethylpyrazin-2-one), has been isolated from seeds of *Stizolobium hassjoo*. This amine, which has a blue fluorescence, gives guanidine, *N*-methylalanine, oxalic acid, alanine and glycine on treatment with 6 N HCl. The permanganate oxidation product of stizolamine is 4-amino-6-methylcarbamoyl-1,3,5-triazine-2-carboxylic acid.

INTRODUCTION

A number of interesting nitrogenous compounds, alkaloids, amines and non-protein amino acids, have been isolated from various parts of plants belonging to the family Leguminosae. Particularly, in the subfamily Lotoideae, N-rich compounds, e.g. canavanine, vicine and lathyrine, accumulate, and there seem to be nitrogen storage substances. From the etiolated seedlings of *Stizolobium hassjoo* non-protein amino acids, DOPA [1], stizolobic and stizolobinic acids [2], have been isolated. During a study of the metabolism of these non-protein amino acids, an amine having blue fluorescence under UV light was detected in seeds and etiolated whole plants. Its chemical structure was studied and the new amine has been named as stizolamine. This paper deals with the structure of this amine.

RESULTS AND DISCUSSION

Stizolamine (1 in Fig. 1) was shown to be a N-rich compound, $C_7H_{11}N_5O_2$, $M^+ = 197$, which was Sakaguchi—alkaline nitroprusside–ferricyanide—and Dragendorff—positive—indicating that it is a mono-substituted guanidyl compound. The pK value of 11.9 also suggests the existence of a guanidino group in its molecule. The PMR spectrum (DMSO-d_6, δ, ppm) exhibits >NMe (3.24, *s*, 3 H), $-CH_2-$ group adjacent to an O atom (4.28, *s*, 2 H), methine group in a –C=C– bond (6.62, *s*, 1 H) and 4 protons derived from amino and/or imino groups (7.10, *m*). The IR spectrum and UV absorption bands indicate the similarity to those of pyrazine derivatives [3–5]. Hydrolysis with 6 N HCl gave guanidine, *N*-methylalanine, oxalic acid, Ala and glycine in which the former 3 compounds were obtained crystalline and the latter 2 compounds were detected by PC.

The $KMnO_4$ oxidation product (4 in Fig. 1) had an empirical formula of $C_6H_7N_5O_3$ ($M^+ - CO_2 = 153$). Disappearance of the colour reactions with the Sakaguchi and alkaline nitroprusside–ferricyanide reagents indicated the loss of the mono-substituted guanidyl group. Hydrolysis of the oxidation product with 6 N HCl gives guanidine, methylamine and 2 mol oxalic acid. The oxidation product was esterified with MeOH and dry HCl and the PMR spectrum of the Me ester exhibits >NMe (2.78, *d*, 3 H; *J* 8 Hz), the ester Me (3.88, *s*, 3 H), 2 protons derived from an amino group (8.26, *m*) and 1 proton from an imino group (8.52, *m*). It has been proved by means of spin decoupling that the 3 protons at 2.78 are coupling to the proton of –NH– at 8.52. The IR spectrum suggests that the Me ester contains a primary amine (3370, 3290, 1632, 1257 cm^{-1}), a secondary amide (3210, 1677, 1550) and an esterified carboxyl group (1720), but there are neither –N=N– nor –C≡N groups.

From these results, the functional groups, –COOH, $-NH_2$ and –CONHMe, were present in the molecule of

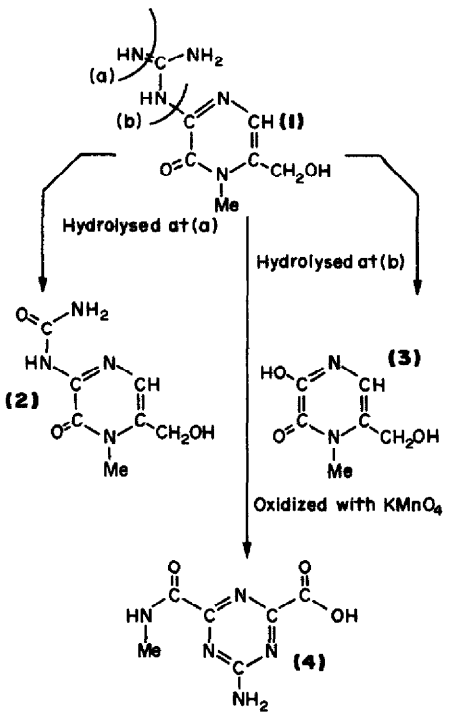

Fig. 1. Degradation patterns of stizolamine.

the oxidation product. Therefore, the residual nucleus should have the formula of C_3N_3. The IR spectrum of the Me ester shows the >C=N– group (1370, 1190 cm^{-1}) so that the oxidation product should be 4-amino-6-methylcarbamoyl-1,3,5-triazine-2-carboxylic acid (**4** in Fig. 1). The structure is supported by MS and UV absorption spectra. The UV absorption spectrum of the oxidation product resembles that of the 1,3,5-triazine derivatives, melamine and azacytidine [6].

When stizolamine was decomposed in H_2O at 95° for 48 hr, two pyrazine-like compounds were obtained. One of them (**2** in Fig. 1) has the empirical formula $C_7H_{10}N_4O_3$. The PMR spectrum shows >NMe (3.48, *s*, 3 H), $-CH_2-$ group adjacent to an O atom (4.48, *s*, 2 H), methine group in –C=C– group (6.72, *s*, 1 H) and an $-NH_2$ group (9.20, *m*, 2 H). Its reactivity with the Sakaguchi and alkaline nitroprusside–ferricyanide reagents is lost but the colour reaction with Ehrlich's reagent is positive. So this compound is a ureido compound.

The other compound (**3** in Fig. 1) has the empirical formula $C_6H_8N_2O_3$ ($M^+ = 156$), the IR spectrum of which shows the presence of –OH (3440 cm^{-1}), $>NCH_3$ (2824), >NC=O (1672, 1640) and –N=C (1630, 1379, 1195). The PMR spectrum exhibits >NMe (3.37, *s*, 3 H), $-CH_2-$ group adjacent to an O atom (4.25, *s*, 2 H) and a methine group of a –C=C– bond (6.35, *s*, 1 H). The colour reactiin with $FeCl_3$ (in EtOH) also indicates the existence of a phenolic OH group next to a carbonyl group. The UV spectrum of this compound resembles that of stizolamine and 2-hydroxy-3-amidopyrazine (synthesized compound).

From the above results the structure of stizolamine was 1-methyl-3-guanidino-6-hydroxymethylpyrazin-2-one (**1** in Fig. 1). This structure has also been proved by X-ray diffraction [7]. The structure of the compounds, **2** and **3**, produced by mild alkaline hydrolysis should be 1-methyl-3-ureido-6-hydroxymethylpyrazin-2-one and 1-methyl-3-hydroxy-6-hydroxymethylpyrazin-2-one, respectively. The processes of hydrolysis with 6 N HCl of stizolamine are summarized in Fig. 2.

EXPERIMENTAL

Isolation of stizolamine. Seeds (2 kg) of *S. hassjoo* were ground, extracted with 5 l. of 1% HOAc (containing 50 ml sulfurous acid) for 18 hr and then squeezed through cotton cloth. Residue was extracted again with 3 l. of the same solvent. Combined extracts were treated with 20% Pb acetate, allowed to stand for 18 hr and centrifuged at 9000 *g* for 15 min to remove proteins. Supernatant was adjusted to pH 7.0–7.5 with 2 N NH_4OH and centrifuged. The supernatant was treated with H_2S after acidification with HOAc to pH 4–5 and then filtered. Filtrate was concentrated *in vacuo* and applied to a cellulose column. Stizolamine was eluted from the column

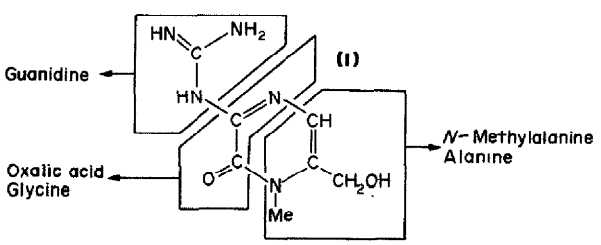

Fig. 2. Hydrolysis of stizolamine with 6 N HCl.

with *iso*-BuOH saturated with H_2O: the eluate having a blue fluorescence was concentrated to dryness. It was dissolved in 2 l. of H_2O and passed through an Amberlite IRA 410 (OH^- form) column. Effluent was concentrated to dryness, dissolved in min vol. of hot N HCl and kept in refrigerator for 18 hr. A brownish crystalline mass of stizolamine HCl was obtained (1500 mg). Crude crystals dissolved in H_2O (500 mg/l.) were passed through an IRA 410 (OH^- form) column and concentrated. In this process a white crystalline powder of stizolamine was obtained. Purification of both stizolamine and its HCl was achieved by recrystallization from H_2O.

Stizolamine. Fine colourless needles from H_2O, mp 208° (decomp.). (Found: C, 41.24; H, 5.78; N, 33.89. $C_7H_{11}N_5O_2\frac{1}{2}H_2O$ requires: C, 40.76; H, 5.87; N, 33.97%.) UV λ_{max}^{nm}: 242, 326 (in H_2O); 242, 326 (in N HCl); 262, 338 (in N NaOH). Fluorescence (in 0.1 M HOAc buffer, pH 5.0); 336 nm (Exc.), 391 (Ana.). IR ν_{max}^{KBr} (cm^{-1}); 3350, 3230, 2930, 2860, 1655, 1627, 1600, 1500, 1445, 1428, 1397, 1363, 1341, 1320, 1224, 1158, 1017, 855, 745. PMR (100 MHz, DMSO-d_6, δ, ppm); 3.24 (*s*, 3 H), 4.28 (*s*, 2 H), 6.62 (*s*, 1 H), 7.10 (*m*, 4 H). MS *m/e* (%); 197 (27; M^+), 180 (10), 169 (8), 155 (100), 138 (33), 126 (23), 111 (26), 110 (48), 95 (10), 83 (11), 69 (13), 55 (16). Titration with NaOH gave p*K* values of 9.7 and 11.9. Reactions with ninhydrin, $FeCl_3$ (in EtOH), Ehrlich's and 2,4-dinitrophenylhydrazine reagents were all negative.

Stizolamine HCl. Colourless needles from H_2O, mp 294–296° (decomp.). (Found: C, 35.97; H, 5.30; N, 29.93; Cl, 15.21. $C_7H_{11}N_5O_2HCl$ requires: C, 35.97; H, 5.18; N, 29.97; Cl, 15.17%.) IR ν_{max}^{KBr} (cm^{-1}); 3270, 3140, 3110, 1680, 1635, 1605, 1565, 1530, 1437, 1410, 1236, 1215, 1150, 1100, 1028, 988, 968, 916, 847, 810, 736.

Mild hydrolysis of stizolamine. Stizolamine (250 mg) was suspended in 120 ml of H_2O and warmed at 90–95° for 48 hr in a sealed tube. After cooling, the reaction mixture was concentrated and separated by PC developed with *n*-BuOH–HOAc–H_2O (6:1:2). On the chromatogram 8 components were detected under UV light. Two main components, **2** and **3**, were eluted from large scale chromatograms with 50% MeOH. **2** had a blue fluorescence (R_f 0.3) and **3** had a dull blue fluorescence (R_f 0.4). **2** was purified by Sephadex LH20 column chromatography using MeOH as solvent and crystallized from MeOH, yield 11 mg. **3** was purified by TLC and development with EtOAc–Me_2CO–MeOH–HOAc (8:4:7:1) and crystallized from MeOH, yield, 3.4 mg.

1-Methyl-3-ureido-6-hydroxymethylpyrazin-2-one (**2**). Yellow needles from MeOH, mp 200° (decomp.). (Found: C, 42.66; H, 5.19; N, 27.30. $C_7H_{10}N_4O_3$ requires: C, 42.42; H, 5.09; N, 28.27%.) MS *m/e* (%); 155 ($M^+ - 43$; 100), 138 (48), 126 (16), 111 (20), 110 (40). UV λ_{max}^{nm}: 247, 321 (in 50% MeOH), 241, 320, 333 (in 0.2 N HCl–50% MeOH), 248, 321 (in 0.2 N NaOH–50% MeOH). PMR (100 MHz, DMSO-d_6, δ, ppm); 3.48 (*s*, 3 H), 4.48 (*s*, 2 H), 6.78 (*s*, 1 H), 9.20 (*m*, 2 H). This compound did not react with the Sakaguchi reagent and alkaline nitroprusside–ferricyanide but reacted with Ehrlich's reagent. Reactions with ninhydrin, $FeCl_3$ (in EtOH) and bromocresol green (BCG) were all negative.

1-Methyl-3-hydroxy-6-hydroxymethylpyrazin-2-one (**3**). White powder from MeOH, mp 175–178°. (Found: C, 43.36; H, 4.93; N, 15.79. $C_6H_8N_2O_3\frac{1}{2}H_2O$ requires: C, 43.64; H, 5.49; N, 16.96%.) MS *m/e* (%); 156 (M^+; 100), 141 (7), 138 (8), 127 (11), 111 (42), 110 (22), 96 (8), 81 (18), 55 (28), 53 (29). UV λ_{max}^{nm}: 239, 312, 324 (in 50% MeOH), 239, 324 (in 0.2 N HCl–50% MeOH), 250, 317 (in 0.2 N NaOH–50% MeOH). PMR (60 MHz, DMSO-d_6, δ, ppm); 3.35 (*s*, 3 H), 4.20 (*s*, 2 H), 6.34 (*s*, 1 H). IR ν_{max}^{KBr} (cm^{-1}); 3440, 3370, 3100, 2880, 2824, 2740, 1672, 1640, 1630, 1598, 1565, 1530, 1420, 1379, 1367, 1336, 1218, 1195, 1143, 1125, 1020, 940, 918, 842, 782. This compound was Sakaguchi, alkaline nitroprusside–ferricyanide, Ehrlich's and ninhydrin negative. Spraying with (BCG) gave a yellow colour, and $FeCl_3$ (in EtOH) gave a brown colour.

Acid hydrolysis of stizolamine. Stizolamine (500 mg) was refluxed with 100 ml of 6 N HCl for 10 hr. After cooling, the reaction mixture was shaken with Et_2O (3 × 100 ml) Et_2O

was evaporated and the residue was crystallized from ligroin–MeOH. This substance was determined as oxalic acid by elementary analysis, PC and colour reaction. The remaining aq. layer was concentrated and applied to a IRA410 (OH^- form) column and the effluent concentrated. One substance was separated from soln by PC, developed with *n*-BuOH–HOAc–H_2O (6:1:2), and crystallized from Me_2CO–H_2O. This compound was dissolved in N HCl, dried in a desiccator and crystallized from EtOH–Et_2O. These 2 compounds were determined as guanidine acetate and guanidine hydrochloride by elementary analyses, MS and colour reactions. The adsorbate on the IRA410 column was eluted with N HCl, concentrated and applied to a IR120B (H^+ form) column. Adsorbate was eluted with N NH_4OH, concentrated and separated by PC developed with 80% pHOH. Three amino acids were detected with ninhydrin reagent. Two of them could not be crystallized, because their yields were small, but they were purified and identified as Ala and glycine. Another amino acid was prepared by large scale PC, purified by Sephadex G-10 column chromatography (1.5 × 49 cm) and crystallized from ligroin–MeOH. Its chemical and physical properties indicated *N*-methylalanine.

Oxalic acid. Colourless plates from ligroin–MeOH. Yield 28.4 mg, mp 60° (loss of H_2O), 72–75° (sublim. and decomp.). (Found: C, 19.86; H, 5.09. Calc. for $C_2H_2O_4 2H_2O$: C, 19.05; H, 4.80%.) IR ν_{max}^{KBr} (cm^{-1}); 3020, 2880, 2680, 2515, 2300, 1720, 1480, 1401, 1298, 1224, 914, 865, 717. Acidic nature of this compound was indicated by BCG. The reaction with diphenylamine gave the same colour as that of authentic material.

Guanidine monoacetate. White powder from Me_2CO–H_2O. Yield 46.5 mg, mp 141–145° (decomp.). (Found: C, 32.21; H, 7.74; N, 34.09. Calc. for $CH_5N_3C_2H_4O_2$: C, 30.25; H, 7.62; N, 35.29%.) MS *m/e* (%); 119 (0.8), 60 (51), 59 (60), 45 (46), 43 (100). This compound did not react with the Sakaguchi reagent but reacted with alkaline nitroprusside–ferricyanide. Basic nature of this compound was indicated by BCG.

Guanidine hydrochloride. Colourless prisms from EtOH–Et_2O, mp 179–180°. (Found: C, 13.30; H, 6.32; N, 43.34; Cl, 38.18. Calc. for CH_5N_3HCl: C, 12.57; H, 6.33; N, 43.99; Cl, 37.11%.)

N-*Methylalanine.* Colourless prisms from ligroin–MeOH. Yield 43.7 mg. (Found: C, 45.91; H, 8.94; N, 14.08. Calc. for $C_4H_9NO_2$: C, 46.60; H, 8.80; N, 13.59%.) IR ν_{max}^{KBr} (cm^{-1}); 3445, 3060, 2980, 2835, 2615, 2420, 1570, 1479, 1393, 1360, 1327, 1286, 1198, 1147, 1098, 1072, 1050, 1017, 926, 897, 772. This compound gave a brown colour with ninhydrin and reacted with *p*-nitrobenzylchloride reagent for detection of *N*-methyl amino acids.

Permanganate oxidation of stizolamine. Stizolamine HCl (300 mg) was dissolved in 800 ml of H_2O and 200 ml of 0.2% $KMnO_4$ aq. soln was added dropwise for 20 min. After reaction, the mixture was decolorized with a min vol. of 0.1% H_2O_2, warmed to aggregate the sludge of MnO_2, and then filtered. Filtrate was acidified with 2 N HCl to pH 3, concentrated *in vacuo* to 30 ml, and it was stood in the refrigerator for 18 hr. The amorphous ppt was purified from boiling H_2O.

4-*Amino-6-methylcarbamoyl-1,3,5-triazine-2-carboxylic acid.* White powder from boiling H_2O, mp 230° (decomp.). Yield 100 mg. (Found: C, 34.61; H, 4.01; N, 33.83. $C_6H_7N_5O_3\frac{1}{2}H_2O$ requires: C, 34.95; H, 3.91; N, 33.97%.) MS *m/e* (%); 153 ($M^+ - 44$; 50), 125 (34), 96 (94), 69 (58), 44 (100). UV λ_{max}^{nm} 233 (in H_2O), 240 (in N HCl), below 210 (in N NaOH). This compound did not react with the Sakaguchi reagent, alkaline nitroprusside–ferricyanide, Ehrlich's and $FeCl_3$ (in EtOH) reagents. Its acidic nature was indicated by BCG.

2-*Methoxycarbonyl-4-amino-6-methylcarbamoyl-1,3,5-triazine.* The oxidation product (4) (100 mg) was suspended in 20 ml of MeOH and saturated with dry HCl gas. After standing for 5 hr, the reaction mixture was concentrated in a desiccator and crystallized from MeOH–EtOH, yield 74.2 mg. White crystalline powder, mp 210° (decomp.). (Found: C, 38.67; H, 4.46; N, 30.06. $C_7H_9N_5O_3\frac{1}{2}H_2O$ requires: C, 38.17; H, 4.58; N, 31.81%.) UV λ_{max}^{nm} (in EtOH); 238. IR ν_{max}^{KBr} (cm^{-1}); 3370, 3290, 3210, 2930, 1720, 1677, 1632, 1550, 1503, 1440, 1408, 1364, 1257, 1204, 1177, 968, 943, 836, 775, 744. MS *m/e* (%); 211 (M^+; 17), 182 (40), 179 (32), 154 (100), 139 (60), 95 (31), 69 (32), 68 (34), 58 (30). PMR (100 MHz; DMSO-d_6, δ, ppm); 2.78 (*d*, 3 H; *J* 8 Hz), 3.88 (*s*, 3 H), 8.26 (*m*, 2 H), 8.52 (*m*, 1 H).

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