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STUDIES IN HARMINE SERIES OF ALKALOIDS

Part II. Further New Derivatives of Tetrahydroharmine

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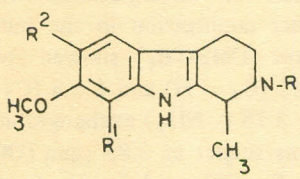
In connection with the studies of structure and activity relationship in the harmine series of indole alkaloids a number of new derivatives of tetrahydroharmine have been prepared. Furthermore, it was found during the course of these reactions that nitration of tetrahydroharmine derivatives (X) and (XI) through HNO_3/CH_3COOH under mild reaction conditions yields tryptamine derivatives (XIII) and (XIV) respectively through substitution of the nitro group at C-2 of the indole nucleus with ring opening.

INTRODUCTION

In continuation of studies in the correlation of the structure and activity in harmine series of alkaloids a number of synthetic analogues of tetrahydroharmine have been reported in earlier communications [1,2]. As an extension of researches in this direction further derivatives of tetrahydroharmine have been prepared and characterized through spectral and chemical studies:-



I. 8-Nitrotetrahydroharmine,
$R = R^2 = H, R^1 = -NO_2$

II. PyN-methyl-8-nitrotetrahydroharmine,
$R = CH_3, R^2 = H, R^1 = -NO_2$

III. PyN-benzenesulfonyl-8-nitrotetrahydroharmine,
$R = -SO_2C_6H_5, R^2 = H, R^1 = -NO_2$

IV. PyN-acetyl-8-nitrotetrahydroharmine,
$R = -COCH_3, R^2 = H, R^1 = -NO_2$

V. pyN-benzoyl-8-nitrotetrahydroharmine
$R = -COC_6H_5, R^2 = H, R^1 = -NO_2$

VI. pyN-methyl-8-amino-tetrahydroharmine
$R = CH_3, R^2 = H, R^1 = -NH_2$

VII. pyN-methyl-8-acetamidotetrahydroharmine,
$R = CH_3, R^2 = H, R^1 = -NHCOCH_3$

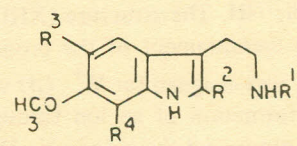
VIII. pyN-methyl-8-benzenesulfonamidotetrahydroharmine,
$R = CH_3, R^2 = H, R^1 = -NHSO_2C_6H_5$

IX. Tetrahydroharmine, $R = R^1 = R^2 = H$

X. py-N-acetyltetrahydroharmine
$R = -COCH_3, R^1 = R^2 = H$

XI. Tetrahydroharmine-6-sulfonic acid,
$R = R^1 = H, R^2 = -SO_3H$

XII. pyN-acetyltetrahydroharmine-6-sulfonic acid,
$R = -COCH_3, R^2 = SO_3H, R^1 = H$



XIII. 3-(2-Acetamidoethyl)-2, 7-dinitro-6-methoxyindole,
$R^1 = -COCH_3, R^2 = R^4 = -NO_2, R^3 = H$

XIV. 3 (2-Aminoethyl)-2-nitro-5-sulpho-6-methoxyindole,
$R^1 = R^4 = H, R^2 = -NO_2, R^3 = -SO_3H$

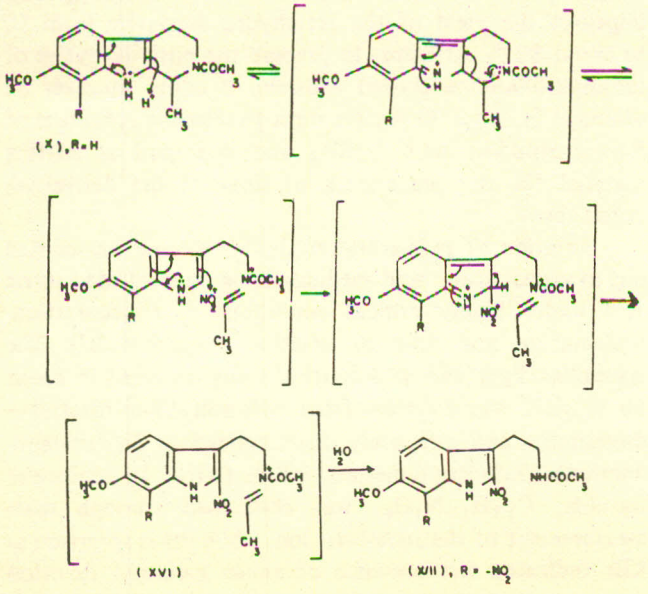
XV. 3-(2-Acetamidoethyl)-2-nitro-5-sulpho-6-methoxyindole,
$R^1 = -COCH_3, R^2 = -NO_2, R^3 = -SO_3H, R^4 = H$

Except (IX). [3] and (X) [6] all these products are hitherto unreported.

As reported earlier, harmidine/harmaline on reduction with Zn/HCl yields tetrahydroharmine [3]. In the present work however it has been found that the addition of NH_4Cl prior to the basification with ammonia greatly improves the yield of the tetrahydro derivative from 50 to about 80 %. Attempts to prepare the nitro derivative of tetrahydroharmine proved fruitless. It could however be obtained in about 90 % yield through selective reduction of 8-nitroharmidine with $NaBH_4$ and was used as starting material for the preparation of some of the derivatives noted above.

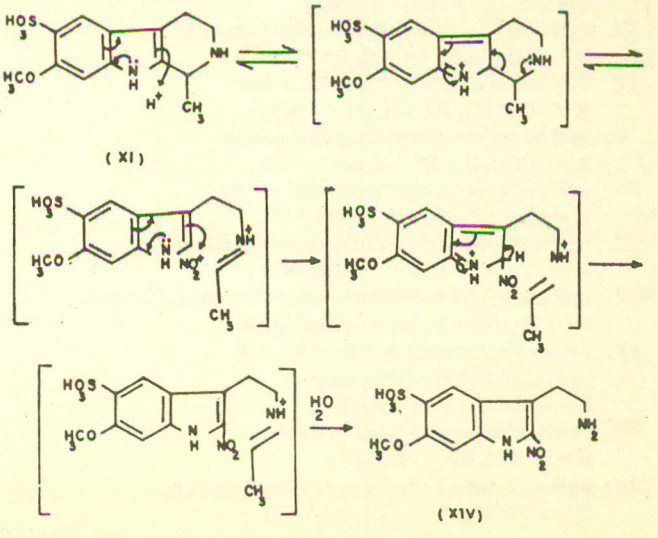
Nitration of pyN-acetyltetrahydroharmine was carried out in glacial acetic acid medium using concentrated nitric acid under highly critical conditions of concentration, temperature and time of reaction (experimental). The crystalline light yellow product thereby obtained in about 50 % yield was different from pyN-acetyl-8-nitrotetrahydroharmine and ultimately characterized as 3-(2-acetamidoethyl)-2,7-dinitro-6-methoxyindole (XIII). Its molecular formula, $C_{13}H_{14}N_4O_6$, was confirmed through mass measurement of the molecular ion, while the ir spectrum in KBr indicated the presence of amide carbonyl function

(1650 cm^{-1}), nitro group (1320 and 1540 cm^{-1}), indolic NH and amido NH (3350 – 3250 cm^{-1}). The ^1H –NMR spectrum of the nitro product in DMSO-d_6 did not show the signals for C_1–CH_3 and C_1 – H of tetrahydroharmine nucleus and contained a three – protons singlet at δ 1.67 ($NCOCH_3$), a three-protons singlet at δ 4.02 (aromatic OCH_3) and a one – proton broad signal at 7.93 ppm (indolic NH). In the aromatic region a one–proton doublet appeared at δ 8.05 (J=8.8 cps) due to C_4–H and a one – proton doublet at δ 7.33 (J=8.8 cps) due to C_5–H, which located one of the nitro groups at C-7 of the indole moiety. The other nitro group was located at C-2 of the indole nucleus due to the absence of any further aromatic proton in the nmr spectrum. Its UV spectrum in methanol indicated further conjugation in the 6-methoxyindole which exhibited bathochromic shift in the basic medium. This shift may be due to the presence of the electron withdrawing nitro group at C - 2 and C - 7 of the indole which increases the acidity of the indolic NH. The structure (XIII) was further confirmed by mass measurement of the prominent peaks. A peak at *m/e* 305 was assigned for M^+–OH which may be explained by the formation of an ion through the interaction of the nitro group and the indolic N - H of the nitro-product. Four further prominent peaks at *m/e* 288, 276,263 and 246 may be attributed to the $(M–OH–OH)^+$, $(M–NO_2)^+$, $(M–OH–COCH_2)^+$ and $(M–OH–NH_2–COCH_3)^+$ ions. Formation of these daughter ions has been proved by link scanning. Substitution of the nitro group at C - 7 of the product is a simple electrophilic substitution which occurs either before or after ring opening process, while the substitution of the nitro group at C - 2 may be rationalized in terms of acid catalyzed opening of tetrahydropyrido ring [5] followed by substitution of the nitro group at C - 2 of the indole nucleus and subsequent hydrolysis of the intermediate iminium (XVI) during the work up. (Scheme I).



Reaction of tetrahydroharmine with concentrated sulfuric acid at 80° for 10 to 15 min yielded tetrahydroharmine-6-sulfonic acid in about 85 % yield. It analyzed for $C_{13}H_{16}N_2O_4S$ which was further confirmed through the mass spectrum of its TMS derivative. The position of sulfonic group was ascertained through ^1H –NMR data of its sodium salt which was recorded in D_2O, the sulfonic acid itself being insoluble in any of the organic solvents as well as in water. The ^1H –NMR suggested its position at C - 6 [δ 7.83, J = 0 (C_5 – H) and δ 6.95, J = 0 (C_8 – H)]. It showed a three - protons doublet at δ 1.27 for C_1 – CH_3 and a one - proton quartet at δ 4.1 for C_1 – H. The pyNH and indolic NH could not be observed being exchanged in D_2O. The ^1H –NMR spectrum of the acetyl derivative showed a singlet for pyN-acetyl (δ 2.2) and a quartet for C_1 – H (δ 5.35) alongwith other signals of sulfonic acid derivative.

Nitration of tetrahydroharmine-6-sulfonic acid, with a view to obtain nitrotetrahydroharmine through substitution of sulfonic group by nitro group, led instead to a product which analyzed for $C_{11}H_{13}N_3O_6S$ and could be characterized as 3-(2-aminoethyl)-2-nitro-5-sulpho-6-methoxyindole (XIV). The mass spectrum was recorded through its TMS derivative while other spectral studies were based on its sodium salt. IR spectrum in KBr showed absorption for $–NO_2$ group at 1360 and 1560 cm^{-1}. Its UV spectrum showed absorptions towards higher wave length as compared with that of tetrahydroharmine-6-sulfonic acid indicating further conjugation in the indole nucleus. ^1H –NMR spectrum (DMSO-d_6) showed two one - proton singlets at δ 8.12 (C_4 – H) and δ 6.8 (C_7 – H), two - protons signal at δ 3.75 ($–NH_2$) exchangeable with D_2O, and a three - protons singlet at 3.85 ppm (OCH_3). Characteristic signals for C_1–CH_3 and C_1–H of tetrahydroharmine nucleus were not observed. Further, there was no signal for C_2–H of indole, indicating the nitro group at this position. A plausible mechanism for the reaction is presented in Scheme II.



(XIV) was further characterized through spectral studies and mass measurement as well as link scanning of prominent peaks of its mono-acetate (XV) as described in detail in the experimental.

EXPERIMENTAL

Melting points were recorded in glass capillary tubes and are uncorrected. IR and UV spectra were measured on IRA-1 Diffraction Grating Infrared Spectrophotometer and Shimadzu UV-VIS Recording Spectrophotometer UV-240 respectively. Proton NMR spectra were determined on Bruker WP 100 SY instrument with TMS as internal reference or otherwise stated. Mass spectra were recorded on MAT-112 and Varian MAT-312 Mass Spectrometer. The purity of the samples was checked on tlc (silica gel).

Borohydride Reduction of 8-nitroharmidine to 8-nitrotetrahydroharmine (I). 2 g of 8-nitroharmidine [4] were dissolved in 75 ml of ethanol and to it was added an aqueous solution of 0.5 g sodium borohydride with stirring at room temperature. Stirring was continued for about an hour and excess of borohydride was decomposed with dilute acetic acid. The reaction mixture was concentrated under reduced pressure, basified with dilute ammonia and shaken out with ethyl acetate. The darkish residue obtained on working up the ethyl acetate layer in the usual manner was taken up in little ethyl acetate and purified through solvation in ether and precipitation with the addition of petroleum ether to remove the darkish impurities. The purified product yielded 8-nitrotetrahydroharmine as light orange rods on recrystallization from methanol m.p. 140–142°. It is soluble in methanol, ethanol, acetone, ethyl acetate and chloroform, sparingly soluble in ether, insoluble in petroleum ether and water (yield 90 %).

IR (KBr) ν_{max}: 3400 (indolic NH), 3260 (Sec. amino NH), 1630 (aromatic C = C), 1350 and 1510 cm^{-1} (N = 0 stretchings of NO_2 group).

UV λ_{max} (MeOH): 217, 250 and 360 nm. λ_{min}: 237 and 300 nm.

Mass: M^+ = 261.1109 (Calcd. for $C_{13}H_{15}N_3O_3$ = 261.11132). Other diagnostic peaks are at *m/e* 246 (B.P.), 244, 231 and 215.

^{1}H–NMR ($CDCl_3$) δ : 7.65 (1H, d, C_5 – H = 8.8 cps), 6.82 (1H, d, C_6 – H, J = 8.8 cps), 4.17 (1H, q, C_1 – H), 4.0 (3H, s, OCH_3), and 1.47 (3H, s, C_1 –CH_3).

–Hydrochloride: elongated rods from methanol m.p. 265–7° (decomp.), soluble in methanol, ethanol and water, sparingly soluble in acetone.

–Hydroiodide; orange plates from methanol; melted at 197–8° (decomp.) readily soluble in acetone, methanol, ethanol and water, sparingly soluble in chloroform and ethyl acetate.

–Picrate: yellow needles from methanol m.p. 240–42° (decomp.); readily soluble in acetone, methanol, ethanol and insoluble in water.

pyN-Methyl-8-nitrotetrahydroharmine (II). 2 g of 8-nitrotetrahydroharmine were refluxed with 6 ml of 40 % aqueous formic acid and 3 ml of 40 % formaldehyde for about an hour, and the reaction mixture basified with dilute ammonia. The liberated base was extracted out with ethyl acetate and worked up in the usual manner. On concentrating the ethyl acetate solution under reduced pressure to a small volume pyN-methyl-8-nitrotetrahydroharmine was obtained as orange coloured crystallizate which on recrystallization from methanol formed aggregates of orange coloured rods, m.p. 106–7° (yield 90 %). It is soluble in methanol, acetone, ethyl acetate and chloroform, sparingly soluble in ether and insoluble in petroleum ether.

IR (KBr) ν_{max}: 3450 (indolic N-H), 1630 (aromatic C = C), 1340 and 1580 cm^{-1} (–NO_2).

UV (MeOH) : λ_{max} 217, 250 and 360 nm. λ_{min} 236 and 302 nm.

Mass: M^+ = 275.12681 (calcd. for $C_{14}H_{17}N_3O_3$ = 275.12696). Other important peaks at *m/e* 260 (B.P.), 258, 245, 230, 214 and 46.

–Hydrochloride: yellow fine needles from methanol, m.p. 234–35° (decomp.), readily soluble in methanol and water.

–Picrate: yellow plates from moist acetone, m.p. 243–44° (decomp.); readily soluble in acetone, sparingly soluble in methanol and insoluble in water.

pyN-Benzenesulfonyl-8-nitrotetrahydroharmine (III). A solution of 1 g of 8-nitrotetrahydroharmine in 2 ml of pyridine and 1 ml of benzenesulfonyl chloride was left at room temperature for about an hour. The reaction mixture was diluted with water, basified with ammonia and extracted out with ethyl acetate. After usual working the ethyl acetate solution was concentrated under reduced pressure, when pyN-benzenesulfonyl-8-nitrotetrahydroharmine came out as yellow coloured crystallizate, which on recrystallization from methanol formed yellowish flowers of needles which melted at 172–73°. It is soluble in methanol, ethyl acetate, acetone, sparingly soluble in ether and insoluble in petroleum ether (yield theoretical).

IR (KBr) ν_{max} : 3400 (indolic N - H), 1630 (aromatic C = C), 1160 (O = S = O), 1340, 1360 (–NO_2 and O = S = O) and 1520 cm^{-1} (–NO_2).

UV (MeOH) λ_{max} : 217, 248 and 358 nm. λ_{min} 238 and 300 nm.

Mass: M^+ = 401.10517 (Calcd. for $C_{13}H_{19}N_3O_5S$ = 401.104505). Other important peaks at *m/e* 386, 260, 259, 245, 141 and 77 (B.P.).

pyN-Acetyl-8-nitrotetrahydroharmine (IV). 1 ml of acetic anhydride was added to a solution of 30 mg 8-nitrotetrahydroharmine in 0.5 ml dry pyridine. On working up the reaction mixture in the usual manner, after keeping it at room temperature for about an hour, pyN-acetyl-8-nitrotetrahydroharmine was obtained as yellow crystallizate,

which on recrystallization from ethyl acetate-ether (2:1) formed aggregates of slender rods, m.p. 218–20°. It is soluble in methanol, acetone and chloroform, insoluble in ether and petroleum ether. (Yield 95 %).

IR (KBr) ν_{max} : 3300 (indolic N – H), 1640 (C = O), 1620 (aromatic C = C), 1360 and 1510 cm^{-1} (– NO_2).

UV (MeOH) λ_{max} : 215, 247 and 357 nm. λ_{min} : 235 and 300 nm.

Mass: 303.12227 (Calcd. for $C_{15}H_{17}N_3O_4$ = 303.12187). Other important peaks at *m/e* 288, 260, 246, 242 and 43 (B.P.).

pyN-Benzoyl-8-nitrotetrahydroharmine (V). pyN-Benzoyl derivative, prepared with benzoyl chloride and pyridine following the reaction conditions noted for pyN-acetyl-8-nitrotetrahydroharmine, formed fine needles on recrystallization from ethyl acetate-methanol (3:1), m.p. 252–54° (yield theoretical). It is soluble in methanol, acetone, and chloroform, sparingly soluble in ethyl acetate and insoluble in ether and petroleum ether.

IR (KBr) ν_{max} : 3360 (indolic N – H), 1630 (C = O/ arom. C = C), 1330 and 1510 cm^{-1} (–NO_2).

UV (MeOH) λ_{max} : 215, 245, 310 and 355 nm. λ_{min} 238, 290 and 320 nm.

Mass: M^+ = 365.1377 (Calcd. for $C_{20}H_{19}N_3O_4$ = 365.13749). Other prominent peaks at *m/e* 350, 260, 245, 105 (B.P.) and 77.

pyN-Methyl-8-aminotetrahydroharmine (VI). 0.5 g pyN-Methyl-8-nitrotetrahydroharmine was taken in 10 % aqueous hydrochloric acid and heated on water bath with zinc dust till the orange solution was decolorized. Unreacted zinc was filtered off and the filtrate ammoniated after the addition of ammonium chloride. The liberated base was extracted out with ethyl acetate and worked up in the usual manner. The darkish residue left on removal of the solvent was taken up in ether and freed of darkish impurities with the addition of petroleum ether. On removal of the solvent from the purified solution a colourless crystalline residue (VI) was obtained which on recrystallization from ethyl acetate-ether formed colourless plates melting at 115–16° (yield 50 %). It is soluble in methanol, ethyl acetate and chloroform, sparingly soluble in ether and insoluble in petroleum ether.

IR ($CHCl_3$) ν_{max} : 3400 (indolic N–H), 3330 and 3250 (–NH_2), 1630 (aromatic C = C).

Mass: M^+ = 245.1529 (Calcd. for $C_{14}H_{19}N_3O$ = 245.15279). Other prominent peak at *m/e* 230 (B.P.), 215, 201, 187.

–Picrate: yellow plates from methanol-benzene (1 : 1), m.p. 154–55° (decomp.), readily soluble in methanol, ethanol and acetone, sparingly soluble in ethyl acetate and insoluble in water.

pyN-Methyl-8-acetamidotetrahydroharmine (VII). pyN-Methyl-8-acetamidotetrahydroharmine was obtained on reaction of pyN-methyl-8-aminotetrahydroharmine with acetic anhydride/pyridine at room temperature for one hour, and working up the reaction mixture in the usual manner. It formed colourless bunches of needles from ethyl acetate-ether and melted at 120–2°. It is soluble in methanol, ethyl acetate and chloroform, sparingly soluble in ether and insoluble in petroleum ether.

IR (KBr) ν_{max} : 3300 – 3260 (amide NH and indolic NH), 1670 (amide C = O) and 1630 cm^{-1} (aromatic C = C).

Mass: M^+ = 287.16329 (Calcd. for $C_{16}H_{21}N_3O_2$ = 287. 163355). Other important fragments at *m/e* 272 (B.P.), 257, 244, 229, 201 and 187.

pyN-Methyl-8-benzenesulfonamidotetrahydroharmine (VIII). 250 mg of pyN-methyl-8-aminotetrahydroharmine were dissolved in 1 ml of pyridine and to it was added 0.5 ml of benzenesulfonyl chloride. After keeping at room temperature for about an hour the reaction mixture was poured into crushed ice, basified with ammonia and extracted out with ethyl acetate. Ethyl acetate layer on usual working afforded pyN-methyl-8-benzenesulfonamidotetrahydroharmine as colourless crystallizate which on repeated crystallization from methanol formed flowers of needles, m.p. 130–2° (yield 79 %). It is soluble in ethyl acetate, methanol, and chloroform, insoluble in ether and petroleum ether.

IR (KBr) ν_{max} : 3360 (indolic NH), 3240 (–$NHSO_2$–C_6H_5), 1630 (aromatic C = C), 1340 and 1160 cm^{-1} (O = S = O).

Mass: M^+ = 385.14647 (Calcd. for $C_{20}H_{23}N_3O_3S$ = 385.14698). Other diagnostic peaks at *m/e* 370, 229, 201, 186, 141 and 77 (B.P.).

Reduction of Harmidine to Tetrahydroharmine (IX). To a solution of 10 g harmidine in 10 % aqueous hydrochloric acid zinc dust was gradually added with occasional shaking. The reaction mixture was heated on water bath till the yellow colour disappeared. Unreacted zinc was filtered off and the colourless filtrate ammoniated with prior addition of ammonium chloride to prevent the precipitation of zinc hydroxide. The white crystalline mass which separated out immediately was shaken out with ethyl acetate. On working up the ethyl acetate layer tetrahydroharmine was obtained as colourless crystallizate in about 80 % yeild. On recrystallization from methanol-benzene it formed slender needles which melted at 199° (Lit. m.p. 199°) [3]. Mass, NMR, IR and UV spectra were found identical with those of an authentic sample of tetrahydroharmine.

pyN-Acetyltetrahydroharmine (X). To a solution of 2 g of tetrahydroharmine in 4 ml of dry pyridine was added 3 ml of freshly distilled acetic anhydride. After standing at room temperature for about an hour, the reaction mixture was diluted with water. The resulting crystalline precipitate was filtered and washed thoroughly with water. On recrystallization from methanol benzene (1 : 1) pyN-acetyltetra-

hydroharmine separated out as colourless fine needles, m.p. 242° (Lit. [6] m.p. 242°). (yield theroretical).

IR (KBr) ν_{max} : 1640 (amido C = 0), 3300 cm^{-1} (indolic NH).

Mass: 258 (M^+), 243, 215, 201, 186 and 43 (B.P.).

Tetrahydroharmine-6-sulfonic acid (XI). 2 g tetrahydroharmine with 20 ml concentrated sulfuric acid (d, 1.84) was heated at 80° for about 15 min. The reaction mixture was poured into crushed ice when tetrahydroharmine-6-sulfonic acid separated out in clusters of prismatic rods which did not melt upto 360° (yield 85 %). It is soluble in dilute alkali and concentrated ammonia, insoluble in water and other comm olvents. It analyzed for $C_{13}H_{16}N_2O_4S$, Found; C, 52.68; H, 5.5; N, 9.39; S, 10.79 % rquires: C, 52.7; H, 5.41; N, 9.46; S, 10.81 %,

IR (KBr) ν_{max} : 3400 – 2600 (indolic NH, SO_3H, py NH; It is likely that latter two exist in $>NH_2^+$ and $-SO_3^-$ form), 1165 and 1050, 1040 (S = O and C – O).

Trimethylsilyl Derivative of Tetrahydroharmine-6-sulfonic Acid. A solution of tetrahydroharmine-6-sulfonic acid (10 mg) in dry pyridine was kept with bis (trimethylsilyl) acetamide at room temperature for about an hour. On removal of the solvent under vacuum the reaction mixture gave cream coloured crystallizate of the di-trimethylsilyl derivative of tetrahydroharmine-6-sulfonic acid which showed M^+ at *m/e* 440 with other important fragments at *m/e* 425, 411, 368, 353, 339, 147, 75 and 73 (B.P.).

Tetrahydroharmine-6-sodiumsulfonate. Sodium salt of tetrahydroharmine-6-sulfonic acid came out as cream coloured shining plates on treating its methanolic suspension with dilute methanolic alkali, m.p. 270° (decomp.). It is soluble in methanol and water.

IR (KBr) ν_{max} : 3240 broad peak (indolic NH and pyNH), 1630 (aromatic C = C), 1620 (NH bending), 1180 and 1050 cm^{-1} (S = O stretching of the sulfonate salt).

UV (MeOH) λ_{max} : 232, 267, 302 and 350 nm. λ_{min} : 254, 283 and 330 nm.

^1H –NMR (D_2O –DSS as internal reference) δ :7.83 (1H, s,, C_5 – H), 6.95 (1H, s, C_8 – H), 3.9 (3H, s, OCH_3), 4.1 (1H, *q*, C_1 – H) and 1.27 (3H, d, C_1 –CH_3).

pyN-Acetyltetrahydroharmine-6-sulfonic acid (XII). A suspension of 250 mg of tetrahydroharmine-6-sulfonic acid in 2 ml of pyridine and 2 ml of acetic anhydride was kept at room temperature for 48 hours, and the reaction mixture was freed of the solvent under reduced pressure. The residue was taken up in little methanol and purified through ethyl acetate and ether. The purified solution yielded pyN-acetyltetrahydroharmine-6-sulfonic acid as colourless crystallizate, which formed short rods on rubbing with petroleum ether, m.p. 275–77° (decomp.). It is soluble in methanol, water and dilute alkali, sparingly soluble in acetone and insoluble in other common organic solvents.

It analyzed for $C_{15}H_{18}N_2O_5S$, (Found: C, 53.22; H, 5.29; N, 8.27; S, 9.49 % requires: C, 53.25; H, 5.33; N, 8.28; S, 9.47 %).

IR (KBr) ν_{max} : 3400 – 2600 (–SO_3H and indolic NH), 1640 (C = O), 1160 and 1350 cm^{-1} (S = 0).

UV (MeOH) λ_{max} : 230, 260, 302, 345 nm. λ_{min} : 250, 281 and 327 nm.

^1H –NMR (D_2O – DSS as internal reference) δ : 7.76 (1H, s , C_5 – H), 6.93 (1H, s, C_8 – H), 5.35 (1H, *q*, C_1 – H), 3.9 (3H, s, OCH_3), 2.2 (3H, s, $COCH_3$), 1.33 (3H, d, C_1 – CH_3).

Nitration of pyN-acetyltetrahydroharmine to 3-(2-acetamidoethyl)-2,7-dinitro-6-methoxyindole (XIII). 1 g of pyN-Acetyltetrahydroharmine was suspended in 10 ml of glacial acetic acid and to it was gradually added 2 ml of concentrated nitric acid (d, 1.4) with cooling (below 20°) and good shaking. A blood red solution was obtained within 1 min which suddenly turned to reddish brown. At this stage, it was poured into crushed ice and ammoniated with strong ammonia till pH 6. The amorphous yellow precipitate which separated out was filtered, washed with water and dried on porous plate. It was dissolved in a 1:1 mixture of methanol and benzene and kept overnight in cold, when 3-(2-acetamidoethyl)2,7-dinitro-6-methoxyindole came out as light yellow elongated rods melting at 208–10° (Yield 50 %). It is soluble in methanol, sparingly soluble in acetone, ethyl acetate and chloroform and insoluble in ether and petroleum ether.

IR (KBr) ν_{max} : 3350 – 3250 (amide NH and indolic NH), 1650 (amide C = 0), 1630 (aromatic C = C), 1320 and 1540 cm^{-1} (–NO_2).

UV (MeOH) λ_{max} : 205, 230 and 372 nm. λ_{min} : 222 and 282 nm. λ_{max} (MeOH – NaOH): 212, 247 and 400 nm. λ_{min} : 240 and 307 nm. λ_{max} (MeOH –HCl): 205, 230 and 372 nm. λ_{min} 222 and 282 nm.

Mass: M^+ = 322.09058 (Calcd. for $C_{13}H_{14}N_4O_6$, 322.09129). Other diagnostic peaks at *m/e* 305.08919 (Calcd. for $C_{13}H_{13}N_4O_5$, 305.08855) = $(M - OH)^+$, 288.0847 (Calcd. for $C_{13}H_{12}N_4O_4$, 288.08482) = $(M - OH - OH)^+$, 276.0985 (Calcd. for $C_{13}H_{14}N_4O_6$, 276.0984) = $(M - NO_2)^+$, 263.07735 (Calcd. for $C_{11}H_{11}N_4O_4$, 263.07799) = $(M - OH - COCH_2)^+$, 246.05114 (Calcd. for $C_{11}H_8N_3O_4$, 246.05145) = $(M - OH - NH_2COCH_3)^+$, 234.05081 (Calcd. for $C_{10}H_8N_3O_4$, 234.05145) = $(M - OH - C_3H_5NO)^+$, 46 = $(NO_2)^+$ and 43 = $(COCH_3)^+$. Peak at 305 was base peak.

Nitration of tetrahydroharmine-6-sulfonic acid to 3-(2-aminoethyl)-2-nitro-5-sulpho 6-methoxyindole (XIV). 200 mg of tetrahydroharmine-6-sulfonic acid in 1 ml of glacial acetic acid was cooled to 15° and 2 ml of 1:1 mixture of concentrated nitric acid (d, 1.4) and glacial acetic acid were added slowly with good shaking. The resulting yellow solution gradually turned reddish yellow and then reddish brown. At this stage, which was reached in

about 1 min, the reaction mixture was poured into crushed ice and strong ammonia was added on with vigorous stirring till pH 6.5. The yellowish fine needles of 3-(2-aminoethyl)-2-nitro-5-sulpho-6-methoxyindole were filtered, washed repeatedly with water and dried on porous plate, m.p. 330° (decomp.), (Yield 45 %). It is soluble in aqueous alkali, very sparingly soluble in methanol, methanol-benzene (1:1) and dimethylsulfoxide, insoluble in water and other usual solvents. It analyzed for $C_{11}H_{13}N_3O_6S$ (Found: C, 41.79; H, 4.17; N, 13.21; S, 10.18 %. Calcd. for $C_{11}H_{13}N_3O_6S$: C, 41.9; H, 4.13; N, 13.33; S, 10.16 %.

IR (KBr) ν_{max} : 3400 – 2200 broad (indolic NH, $-NH_2$ and $-SO_3H$; It is likely that latter two exist in $-NH_3^+$ and $-SO^-_3$ form), 1620 (aromatic C = C), 1150 (O = S = O), 1360 ($-NO_2$), 1560 ($-NO_2$), 1060 cm^{-1} (C – O/S = O).

Trimethylsilyl Derivative of (XIV). Tri-trimethylsilyl derivative of (XIV) was obtained as yellow crystalline solid in exactly the same manner as described for tetrahydroharmine-6-sulfonic acid. The mass spectrum of the TMS-derivative showed a peak at *m/e* 517 $(M - 15)^+$, and other diagnostic peaks at *m/e* 444, 430, 413, 311, 174, 147 (B.P.), 75 and 73.

3-(2-Aminoethyl)-2-nitro-5-sodiumsulfonate-6-methoxyindole. 250 mg of 3-(2-aminoethyl)-2-nitro-5-sulpho-6-methoxyindole was dissolved in dilute aqueous alkali and kept at room temperature with the addition of little methanol. The sodium salt came out in a few minutes as yellow short rods in about 85 % yeild. It recrystallized from dilute methanol and decomposed at 260–62°. It is readily soluble in water, soluble in methanol and insoluble in other usual solvents.

IR (KBr) ν_{max} : 3400 broad (indolic NH and NH_2), 1630 (aromatic C = C), 1564 ($-NO_2$), 1365 ($-NO_2$), 1180 (O = S = O) and 1065 cm^{-1} (O = S = O and C – O).

UV (MeOH) λ_{max}: 215, 240, 270 and 385 nm. λ_{min}: 233, 257 and 297 nm.

3-(2-Acetamidoethyl)-2-nitro-5-sulpho-6-methoxyindole (XV). 320 mg of 3-(2-aminoethyl)-2-nitro-5-sulpho-6-methoxyindole was suspended in 1 ml of dry pyridine and refluxed with 1 ml of freshly distilled acetic anhydride for about 10 hr. The residue left on removal of the solvent from the reaction mixture afforded 3-(2-acetamidoethyl)-2-nitro-5-sulpho-6-methoxyindole as yellow short rods on recrystallization from dilute methanol, m.p. 250° (decomp.). It is readily soluble in water, soluble in methanol and insoluble in other common solvents.

It analyzed for $C_{13}H_{15}N_3O_7S$ (Found: C, 43.68; H, 4.17; N, 11.80; S, 9.00 % requires: C, 43.70; H, 4.20; N, 11.76; S, 8.96 %).

IR (KBr) ν_{max}: 3340 (indolic N – H), 3300 – 2600 ($-SO_3H$ and amide NH), 1650 (amide C = O), 1620 (aromatic C = C), 1560 ($-NO_2$), 1380 ($-NO_2$ and S = O), 1155 (S = O).

UV (MeOH) λ_{max}: 215, 240, 270 and 385 nm; λ_{min} 232, 258 and 295 nm.

Mass: M^+ was not observed in the mass spectrum. Diagnostic peaks appeared at *m/e* 277.10598 (Calcd. for $C_{13}H_{15}N_3O_4$ = 277.106225) = $(M-SO_3)^+$, 260.10345 (Calcd. for $C_{13}H_{14}N_3O_3$ = 260.10349) = $(M-SO_3-OH)^+$, 243.09984 (Calcd. for $C_{13}H_{13}N_3O_2$ = 243.100755) = $(M - SO_3 - OH - OH)^+$, 46 = $(NO_2)^+$ and 43 (B.P.) = $COCH^+_3$. FI technique also failed to give M^+. It however showed a peak at *m/e* 277. ^1H –NMR (D_2O-DSS as internal reference) δ : 8.03 (1H, s, C_4 – H), 6.82 (1H, s, C_7 – H), 3.93 (3H, s, OCH_3) and 1.65 (3H, s, $NCOCH_3$).

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