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NITRATION STUDIES IN THE β– CARBOLINE ALKALOID, YOHIMBINE

SALIMUZZAMAN SIDDIQUI, SABIRA BEGUM AND ABDUL MALIK

H.E.J. Research Institute of Chemistry, University of Karachi, Karachi, Pakistan

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Nitration studies in the β carboline alkaloid, yohimbine (1), afforded two isomeric mononitro derivatives II and III, which have been characterized as 12-nitroyohimbine and 9-nitroyohimbine respectively through chemical and spectral studies. A series of new potentially significant derivatives (IV-IX) of II have also been prepared.

Key words: Yohimbine, β–Carboline, Nitration, 12-Nitroyohimbine, 9-Nitroyohimbine.

Introduction

In view of the earlier observations that introduction of a nitro group plays an important role in the physiological activity of ajmaline [1,2] and reserpine [3,5] series of β carboline Rauwolfia bases, it was considered of interest to carry out nitration studies on another Rauwolfia base yohimbine [6] possessing the basic reserpine skeleton. As a result, a number of new derivatives *(II-IX)* have been prepared and characterized through chemical and spectral studies.

Experimental

All melting points were recorded in glass capillary tubes and are uncorrected. IR spectra were recorded on Unicam SP. 200 G and JASCO IRA-1 Diffraction Grating Infrared Spectrophotometers. Mass spectra were recorded on Finnigan MAT - 112 and Finnigan MAT - 312 double facussing mass spectrometers connected to PDP 11/34 computer system. High resolution mass measurements were carried out by peak matchings and by measurements on Spectrosystem 188 computer linked to Finnigan MAT 312 mass spectrometer ^{1}H-NMR spectra were recorded on Bruker WP-100-SY FT-NMR spectrometer operating at 100 MHz, with TMS as internal reference. The purity of the samples was checked on tlc (silica gel 60 PF 254).

Nitration of I. A solution of I (5g) in glacial acetic acid (25 ml) was cooled to 20° and a mixture of (1:1) conc.nitric acid (d 1.4) and glacial acetic acid (8ml) was added in 30 sec. with manual shaking. The initial yellow colour of the mixture first changed to deep yellow going on to orange yellow and finally to reddish orange. At this stage, which was reached in about 2 min., the reaction mixture was poured in crushed ice and brought to pH 5 by running in cold concentrated ammonia with vigorous stirring. On treating with aqueous solution of potassium nitrate till faint turbidity and keeping overnight in the cold, semicrystalline nitrate of II separated out, which formed lemon yellow needles from methanol-water (1:3), (yield 2.85g). The mother liquor was treated with cold conc. ammonia and the liberated base divided into benzene soluble and benzene insoluble fractions.

The former on purification with petroleum ether afforded a second crop of the nitrate of II (yield 1.75g). On treating the nitrate with conc. ammonia, II was ultimately obtained, which formed bright orange silky needles from methanol-benzene (1:1), m.p. 248-50° (decomp.) yield 63%.

The benzene insoluble fraction was partitioned between ethyl acetate and 5% acetic acid. The ethyl acetate layer was neutralized with dilute ammonia, washed, dried and purified through ether and petroleum ether. The slightly yellowish solution was freed of the solvent under reduced pressure. The residue was subjected to column chromatography (neutral alumina) and elution was carried out in the order: benzene, benzene-ethyl acetate (1:1) ethyl acetate and benzene containing increasing proportions of methanol. The residue from benzene-ethyl acetate (1:1) and ethyl acetate fractions were taken together in dilute acetic acid and treated with NaCl. The darkish yellow insoluble material that settled down was dissolved in (1:1) methanol-acetone and kept in the cold, when hydrochloride of III came out as yellow rods. m.p. 257-58° (decomp). The base liberated from the hydrochloride formed pale yellow microcrystalline powder which showed single spot on TLC with Rf value slightly lower than that of II (yield 150 mg).

Characterization of II. M.p. 264-65° (Found: C,63; H, 6.41; N, 10.55; O, 20.07% and *m/z* 339 (M^+). Calcd. for $C_{21}H_{25}N_3O_5$, C, 63.15; H, 6.31; N, 10.52; O, 20.05%); IR (KBr) υ cm^{-1} : 3500-3300 (indolic NH and OH), 1730 (ester C=O), 1600 (aromatic C=C), 1510 and 1320 (-NO_2), 1070-1080 (C-O); EIMS *m/z* (%): 399.1792 (100%) (Calcd. for $C_{21}H_{25}N_3O_5$ = 399.1793), 398 (76.58, M^+ -1) 382 (10 M^+ - OH), 281 (45.65, M^+ -H_2O), 369 (80.62, M^+ - NO), 368 (78.25, M^+ -OCH_3), 353 (50.56, M^+ -NO_2) 340 (12, M^+ - $COOCH_3$), 229 (30.65), 215 (20), 214 (18.65) and 201 (30.65) [7]; ^{1}H-NMR ($CDCl_3$) δ:8.70 (1H, br s, indolic NH), 7.10 (1H, t,J = 8.0 Hz, H-10), 7.51 (1H, dd, $J_{9,10}$ = 8.0 Hz, $J_{9,11}$ = 2.5 Hz, H-9), 7.9 (1H, dd $J_{11,10}$ = 8.0 Hz, $J_{11,9}$ = 2.5 Hz, H-11), 4.23 (1H, s, OH) and 3.76 (3H, s, $OCOCH_3$).

Hydrochloride and hydroiodide. On bringing the components together in acetone and crystallizing the salts in

the same solvent, the hydrochloride formed lemon yellow needles, m.p 288° (decomp) (Found: Cl, 8.11 Calcd. for $C_{21}H_{25}N_3O_5$-HCl, Cl 8.04%) and the hydroiodide formed deep yellow prismatic rods m.p. 291° (decomp).

Hydrobromide. Prepred through the procedure described above, lemon yellow prismatic plates, (1:1 methanol-benzene), m.p. 282-83° (decomp.) (Found Br. 16.54% Calcd. for $C_{21}H_{25}N_3O_5$ HBr, Br, 16.47%).

Picrate. On treating the solution of II in dilute acetic acid with aqueous solution of picric acid, the picrate was obtained as pale orange plates (1;1 benzene-ether), m.p. 182-83° (decomp.) (Found: C, 51.60; H, 4.51; N, 13.29%, Calcd. for $C_{21}H_{25}N_3O_5$. ($C_6H_3O_7N_3$), C, 51.58; H, 4.49; N, 13.37%).

Chloroplatinate and nitrite. These were formed on reacting II with 1.5% chloroplatinic acid and sodium nitrite respectively in the manner described for picrate. Chloroplatinate: yellow powder. (Found: Pt, 16.11%, Calcd. for $(C_{21}H_{25}N_3O_5)_2$. H_2PtCl_6 Pt, 16.14%; nitrite: deep yellow prismatic rods (methanol), m.p. 185-86°. (decomp).

12-Aminoyohimbine (IV). To a solution of II (1g) in 30% aqueous hydrochloric acid was added zinc dust with heating on the water bath till the reddish orange colour of the solution became pale yellow . It was filtered, treated with an excess of ammonium chloride and basified with ammonia in cold. The liberated base was extracted out with 1:1 benzene-ethyl acetate, which on usual work up afforded IV, m.p. 253-54° (colourless slender needles, methanol), yield 50% (Found, C, 68.30; H, 7.40; N, 11.32% Calcd. for $C_{21}H_{27}N_3O_3$ C, 68.26; H, 7.37; N, 11.38%); EIMS *m/z* (%): 369.2051 (100 M^+ Calcd. for $C_{21}H_{27}N_3O_3$, 369.2052, 368 (56.57, M^+ –1), 351(30.65), M ± H_2O), 310 (27.53, M^+ –$COOCH_3$), 338 (12.86, M^+- OCH_3); IR (KBr) υ cm^{-1} ; 3400 (indolic NH and OH), 3350, 3300 (-NH_2) 1730 (ester C=O) and 1580 (aromatic C=C).

12-Nitroyohimbine-17-O-acetate (V). A mixture of II (250mg), pyridine (3ml) and acetic anhydride (1.8 ml) was kept over night at room temperature. The residue obtained after usual work up was taken in acetone and treated with acetonic solution of hydrobromic acid. On concentration and cooling, the hydrobromide of V came out as greenish yellow spindle shaped needles, m.p. 322-23°. V was obtained by rubbing the hydrobromide with dilute solution of alcoholic ammonia as bright orange silky needles m.p. 170-71° yield 84.6% (Found: C, 62.5; H, 6.19; N, 9.59% Calcd. for $C_{23}H_{27}N_3O_6$, C, 62.56; H 6.16; N, 9.52%); EIMS *m/z* (%) 441.1898 (10.25, M^+, Calcd. for C_{23} $H_{27}N_3O_6$ 441.1899), 440 (3.25, M^+ -1), 399 (88.36, M^+-$COCH_2$), 398 (100, M^+ -$COCH_3$), 382 (37.31, M^+ -$COOCH_3$), 368 (16.52, M^+ -$COCH_2$-NO) and 353 (42.09, M^+ -$COCH_2$-NO_2); IR (KBr) υ cm^{-1}: 3400 (indolic NH), 1730 and 1715 (ester C=O), 1610 (aromatic C=C) and 1520, 1340 (NO_2).

12-Nitroyohimbine-17-O-benzoate (VI). To a solution of II (250 mg) in pyridine (3ml) was added benzoyl chloride (2 ml) and the solution was kept at room temperature over night. VI, obtained after usual work up, was purified through its hydrochloride salt (m.p. 286.87°) which yielded VI as lemon yellow needles m.p. 244-45° (decomp.) yield 69% (Found: C, 66.79; H, 5.68; N, 8.35%, Calcd. for $C_{28}H_{29}N_3O_6$: C, 66.77; H, 5.61; N, 8.35%(; EIMS *m/z* (%): 503.2054 (26.52, M^+. Calcd. for $C_{28}H_{29}N_3O_6$: 503.2055), 502 (21.65, M^+ -1), 457 (10.25, M^+ -NO_2), 105 (100, $COC_6H_5^+$) and 77 (46.56, $C_6H_5^+$); IR (KBr) υ cm^{-1}: 3400 (indolic NH), 1730, 1710 (ester C=O), 1520 and 1340 (-NO_2).

12-Nitroyohimbine-17-O-benzenesulfonate (VII). 250 mg of II were kept at room temperature overnight with 1 ml pyridine and 1 ml benzenesulfonyl chloride. The reaction mixture was diluted with water and treated with ammonia. The resulting yellow precipitate, formed pale yellow flowers of fine needles from methanol, m.p. 270-71° (decomp.) yield 84%. (Found: C, 60.07; H, 5.34; N, 7.69; S, 5.95%. Calcd. for $C_{27}H_{29}N_3O_7S$: C, 60.10; H, 5.38; N,. 7.79; S, 5.93%) ; EIMS *m/z* (%): M^+ (not observed), 336.1838 (100, M^+ -NO_2 -SO_3-C_6H_5, Calcd. for $C_{21}H_{24}N_2O_2$, 336.1837), 335 (95, M^+ -NO_2-C_6H_5 -SO_3H), 141 (8, $SO_2C_6H_5^+$)and 77 (50, $C_6H_5^+$); IR (KBr) , υ cm^{-1} 1180 and 1360 (O=S=O).

12-Nitroyohimbyl alcohol (VIII). Method 1. LiAIII$_4$ reduction of II. To an ethereal solution of $LiAlH_4$ (0.5g) was gradually added an ethereal solution of II (100 mg) with stirring at room temperature under anhydrous conditions. After 5 hr stirring and usual work up the reaction mixture afforded VIII as a yellow residue which formed yellow flowers of needles from methanol-acetone (2:1), m.p. 190 -92°, yield (55%). EIMS *m/z* (%): 371.1843 (100, Calcd. for $C_{20}H_{25}N_3O_4$, 371.1844), 370 (73.90, M^+ -1), 354 (16.91, M^+ -OH), 341 (8.82,M^+ -NO), 340 (10.66, M^+ -CH_2OH), 325 (20.59, M^+ -NO_2), 229 (20.59), 215 (11.40), 214 (29.41) and 201 (6.25); IR (KBr) υ cm^{-1} 3400-3250 br (indolic HN/OH), 1565 (aromatic C=C), 1520, 1320 (NO_2), 1120 and 1065 (C-O).

Method 2: Borohydride reduction of II: To a refluxing solution of II (250 mg) and sodium borohydride (0.5 mg) in tertiary butyl alcohol 5 ml of methanol were added and refluxing was continued for 1 hr. The reaction mixture was treated with water and extracted out with ethyl acetate. On usual working the organic phase afforded VIII as a yellow crystallizate in nearly theoretical yield m.p., m.m.p tlc and spectroscopic data of the product are comparcable with the product obtained through $LiA1H_4$ reduction of II.

12-Nitroyohimbic acid (IX): IX was obtained in nearly

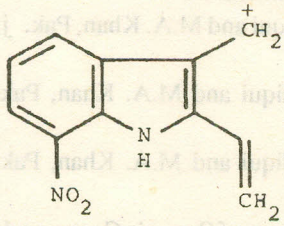
theoretical yield on refluxing II with 2% methanolic potassium hydroxide for about 1/2 hr and working up the reaction mixture in the usual manner. m.p. 225- 27° (decomp.) (yellow plates, (1:1) methanol-benzene). (Found: C, 62.26; H, 6.01; N, 10.89; O, 20.68%; Calcd. for $C_{20}H_{23}N_3O_5$ C, 62.31; H, 6.02; N, 10.9 l; O, 20.76%); EIMS *m/z* (%): 385.1636 (58.95, M^+, Calcd. for $C_{20}H_{23}N_3O_5$, 385.1637), 384 (46.52, M^+ -1), 368 (16.56, M^+ OH), 355 (4.81, M^+ -NO), 340 (7.32, M^+ -COOH), 339 (15.78, M^+-NO_2), 229 (16.67), 215 (14.62), 214 (10.25) and 201 (7.95) [7]; IR (KBr) υ cm^{-1} :3400-2600 br (indolic NH, -OH, -COOH), 1530 and 1350 (-NO_2).

Methylation of IX. On reaction of IX (methanolic solution) with diazomethane (ethereal solution) II was obtained in nearly theoretical yield which could be identified through m.p., mm.p and comparison of spectral data with those of an authentic sample.

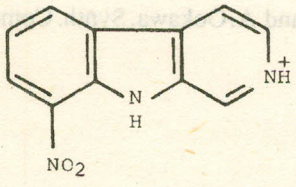
Characterization of III. (Found: C, 63.05; H, 6.36; N, 10.55 ; O, 20.02% and *m/z* 339 (M^+). Calcd. for $C_{21}H_{25}N_3O_5$, C, 63.15; H, 6.31; N, 10.52; O, 20.05%); IR (KBr) υ cm^{-1}: 3500-3300 br (indolic NH, and -OH), 1730 (ester C=O), 1630 (aromatic C=C), 1515, 1330 (-NO_2) and 1070 (C-O): EIMS *m/z* (%): 399.1791 (100, M^+, Calcd. for $C_{21}H_{25}N_3O_5$, 399.1793), 398 (67.85, M^+ -1), 381 (40.67, M^+ -H_2O), 369 (60.56, M^+ -NO), 368 (59.00, M^+ -OCH_3), 353 (60.75, M^+ -NO_2), 340 (16.56, M^+ $COOCH_3$), 229 (36.16), 215 (22.72), 214 (16.85) and 201 (28.27) [7]; ^{1}H-NMR ($CDCL_3$) δ : 8.60 (1H, br, indolic NH), 7.7 (1H, t, J=8.5 Hz, H-11), 7.3 (1H, dd, $J_{10,11}$ = 8.5 Hz, $J_{10,12}$ =2.5Hz, H-10), 6.9 (1H, dd $J_{12,11}$=8.5 Hz, $J_{12,10}$ = 2.5 Hz, H-12), 4.12 (1H, s, OH) and 3.80 (3H, s, $OCOCH_3$).

Results and Discussion

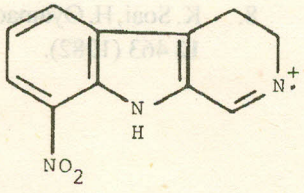
Due to extreme susceptibility of yohimbine to resinification and formation of tarry material on reaction with nitric acid, a great deal of difficulty was experienced in working out the optimum experimental conditions for the reaction. After considerable experimentation it was ultimately found that the two mononitro position isomers (II and III) were obtained in optimum yield when the reaction was carried out in glacial acetic acid medium at 20° with a reaction period of about 2 min.



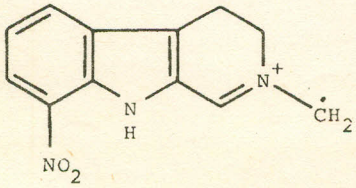
A, m/z 201



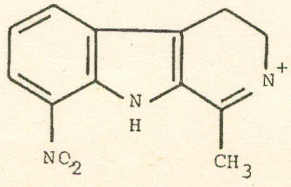
B, m/z 214



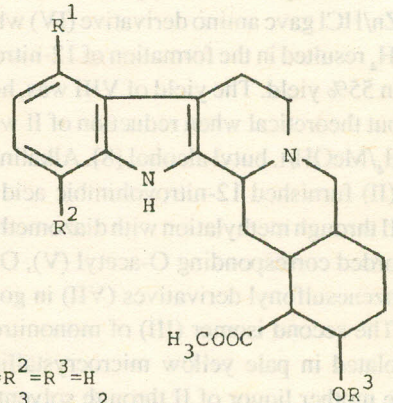
C, m/z 215



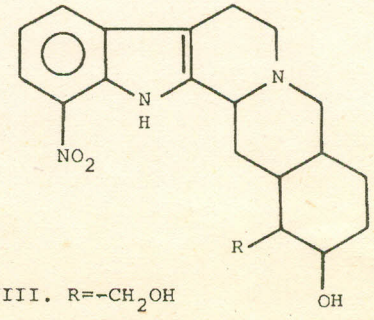
D, m/z 229



E, m/z 229



I. $R^1=R^2=R^3=H$
II. $R^1=R^3=H$, $R^2=-NO_2$
III. $R^2=R^3=H$, $R^1=-NO_2$
IV. $R^1=R^3=H$, $R^2=-NH_2$
V. $R^1=H$, $R^2=-NO_2$, $R^3=-COCH_3$
VI. $R^1=H$, $R^2=-NO_2$, $R^3=-COC_6H_5$
VII. $R^1=H$, $R^2=-NO_2$, $R^3=-SO_2C_6H_5$



VIII. R=-CH_2OH
IX. R=-COOH

Following the procedure described in the Experimental, the major nitration product (II) could be isolated from the reaction mixture throug its crystalline nitrate. Its molecular formula, $C_{21}H_{25}N_3O_5$, was confirmed through exact mass measurement of the molecular ion peak. The IR spectrum indicated the presence of nitro group (1510 and 1320 cm^{-1}) which was located at C-12 through ^{1}H-NMR studies. The signal at δ 7.1 was assigned to H-10 showing ortho couplings with both H-9 and H-11 (J=8.0 Hz) while H-9 and H-11 appeared as doublets of doublet at δ 7.51 ($J_{9,10}$ = 8.0 Hz, $J_{9,11}$ = 2.5 Hz) and δ 7.9 ($J_{11,10}$ =8.0 Hz, $J_{11,9}$ =2.5 Hz). Slightly down field appearance of H-11 signal is due to electron withdrawing nitro group at C-12. In the mass spectrum significant peaks appeared at *m/z* 398 (M^+ -1, due to loss of H from C-3), 381 $(M-H_2O)^+$, 369 $(M-NO)^+$, 353 $(M-NO_2)^+$, and 340 $(M-COOCH_3)^+$. Other prominent peaks at *m/z* 201, 214, 215 and 229 have been assigned to the ions (A-E) in correlation with the published mass spectral fragmentation of yohimbine [7].

II, formed crystalline hydrochloride, hydroiodide, hydrobromide, picrate, chloroplatinate and nitrite. Its reduction with Zn/HCl gave amino derivative (IV) while reduction with $LiAlH_4$ resulted in the formation of 12-nitroyohimbyl alcohol VIII in 55% yield. The yield of VIII was, however, improved to about theoretical when reduction of II was performed with $NaBH_4$/MeOH/t. butyl alcohol [8]. Alkaline hydrolysis of the base (II) furnished 12-nitroyohimbic acid (IX), which gave back II through methylation with diazomethane. Furthermore, II afforded corresponding O-acetyl (V), O-benzoyl (VI) and O-benzenesulfonyl derivatives (VII) in good yields.

The second isomer (III) of mononitroyohimbine could be isolated in pale yellow microcrystalline form from the nitrate mother liquor of II through solvent fractionation and subsequent chromatographic procedures (Vide Experimental). Its IR spectrum indicated the presence of nitro group and the molecular ion corresponded to the formula $C_{21}H_{25}N_3O_5$. Fragmentation pattern in the mass spectrum was found similar to II showing that the nitro group has been introduced in the indole nucleus which was located at C-9 on the basis of ^{1}H-NMR spectrum in which H-12 and H-10 appeared at δ 6.9($J_{12,11}$ =8.5 Hz, $J_{12,10}$ =2.5 Hz) and δ 7.3 ($J_{10,11}$ =8.5 Hz, $J_{10,12}$ =2.5 Hz) respectively as doublets of doublet, while signal of H-11 was observed at δ 7.7 showing ortho coupling with both H-10 and H-12 (J=8.5 Hz).

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