Physical Sciences Section

Pakistan J. Sci. Ind. Res., Vol. 18, No. 5, October 1975

NITRATION STUDIES IN RESERPINE

SALIMUZZAMAN SIDDIQUI and MOHAMMAD ATAULLAH KHAN

H. E. J. Postgraduate Institute of Chemistry, University of Karachi, Karachi 32

(Received September 17, 1975)

Abstract. In the context of studies in the correlation of sturcture and activity three isomers of mononitroreserpine have been prepared and their structures established through chemical and spectral techniques. The results of pharmacological action of two of these isomers have been reported.

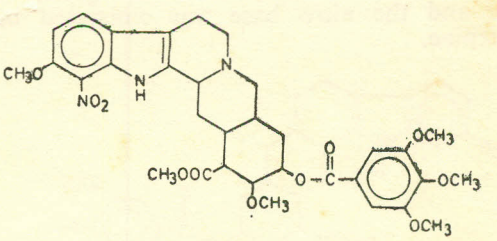
Reserpine[1] has found extensive therapeutic use in the treatment of hypertension on the one hand and a variety of mental ailments on the other. As a result of clinical experience over a long period, however, it has been noted that its extended use in the treatment of hypertension produces heavy depressions in about 50% of patients, leading on in many cases to suicidal tendencies and schizophrenia.[2] On account of this complication which is due to the dual activity of reserpine as a hypotensive and a sedative, a large number of derivatives of reserpine have been prepared with the object of eliminating or reducing either one or the other of these two actions, but none of those reported by different workers appears to have so far come into therapeutic use.

The pharmacological investigation of the antiarrythmic activity of nitroajmaline revealed that it is more than twice as active as ajmaline.[3] Profiting from this finding, it appeared of interest to extend the nitration studies to the reserpine molecule in order to note any changes in its dual hypotensive and sedative central depressant action.

Due to the extreme susceptibility of reserpine to resinification and formation of tarry material on reacting it with nitric acid, a great deal of difficulty was experienced in working out the optimum experimental conditions for this reaction. When the nitration of reserpine was carried out in glacial acetic acid at 18–20°C with a reaction period of about 4–6 min, three position isomers of mononitroreserpine could be obtained. Following patent coverage [4,5] of these isomers a preliminary account of nitration studies in reserpine was reported.[6] The present paper deals with full details of characterisation and structure elucidation of the three isomers. In the course of these studies it was noted that the experimental conditions in respect of the variables of concentration of reactants, temperature, duration, and the speed with which the reactants are brought together are highly critical. If these conditions are not meticulously observed, there is either failure of the reaction or formation of tarry material. For instance, if the optimum proportion of nitric acid (eight moles) in glacial acid (Merck) is added slowly drop by drop with mechanical stirring at 20°C to a solution of reserpine in glacial acetic acid, reserpine nitrate crystallises out and there is no formation of a nitro derivative of the base. On the other hand, when under the same conditions the reaction mixture is quickly run through a burette with slight manual shaking and allowed to stand for 4–5 min, the initial yellowish colour of the reaction mixture changes over to red and then deep red. On quenching the reaction at this stage by pouring into crushed ice, and working it up under the conditions described in detail in the experimental, 1-nitroreserpine, m.p. 146°C (dec) is obtained in a yield of 40–50%, 12-nitroreserpine, m.p. 231°C (dec) 10–12% and 9-nitroreserpine, m.p. 253°C (dec) only in minute quantities in the form of carmine red prismatic rods.

12-Nitroreserpine was isolated as hydrochloride from the mother liquors of 1-nitroreserpine and was further characterised through the formation of various salts described in the experimental portion.

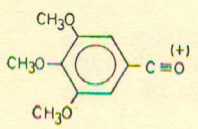
The location of the nitro group which was recognised at 1510 and 1330 cm^{-2} in the IR spectrum of the base was ascertained by the alkaline hydrolysis of the nitrobase, which afforded a nitrogen free acid identified as 3,4,5-trimethoxybenzoic acid through analysis, IR spectrum and mixed m.p. determination with an authentic sample, and a nitro amino acid isolated as hydrochloride. This led to



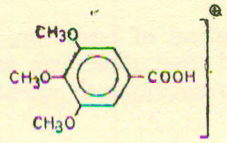
12-Nitroreserpine

the conclusion that the substitution has taken place in the reserpic acid moiety of the reserpine molecule. The NMR spectrum of the nitro base in $CDCl_3$ showed two signals at 2.35 τ and 3.10 τ due to H-9 and H-10 conforming to the characteristic *ortho* coupling of *J* 8.5 c/s, which indicated location of the nitro group at *C*-12.

The mass spectrum showed the molecular ion at *m/e* 653 conforming to the formula $C_{33}H_{39}O_{11}N_3$ for 12-nitroreserpine. The base peak at *m/e* 212 established the presence of 3,4,5-trimethoxy benzoyl and 3, 4, 5-trimethxybenzoic acid ions respectively, while the peak at *m/e* 636, which is M—OH ion

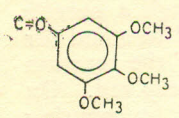


m/e 195



m/e 212

may be explained by the formation of an ion through the interaction of the nitro group and the indole nitrogen of the nitro base. Four further prominent peaks at *m/e* 458 (I), 441 (II), 426 (III) and 410 (IV) may be attributed to the formation of the following ions respectively.



M^+ *m/e* 458

I

1–OH

m/e 441

II

I–CH_3OH

m/e 426

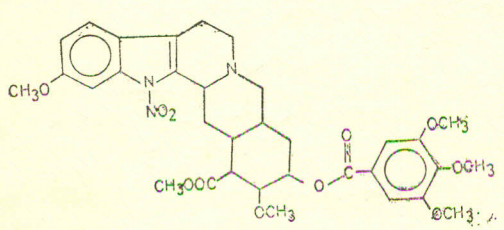
III

II–OCH_3

m/e 410

IV

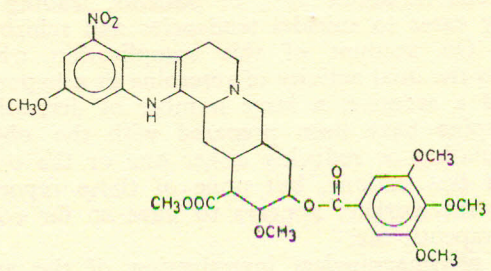
1-Nitroreserpine was isolated from the orange yellow initial product of nitration through direct fractional crystallisation in about 45% yield according to the procedure described in the experimental. On hydrolysis with alkaline aqueous alcohol it also afforded a nitrogen-free acid identified as 3,4,5–trimethoxybenzoic acid and an uncrystallisable amino acid, indicating the presence of the nitro group in the reserpic acid moiety. The typical —NH absorption was absent both in the IR and NMR spectra showing a substitution at the nitrogen atom. The NMR spectrum indicated signals for five protons in the aromatic region. The H-12 signal is shifted downfield, H-3 is shifted upfield by 0.3 ppm compared with reserpine. The position of the nitro group was further confirmed by the reduction of 1-nitroreserpine by zinc and dilute hydrochloric acid, whereby the nitro group was removed and the nitro base was converted back into reserpine.



1-Nitroreserpine

1-Nitroreserpine when heated for 5 min in 80% acetic acid isomerised to a slightly more basic red crystalline product, m.p. 180° (dec) which analysed for mononitroreserpine ($C_{33}H_{39}O_{11}N_3$) and was further characterised through the formation of various salts.

The final mother liquors from 1-nitroreserpine and 12-nitroreserpine hydrochloride were worked up according to the procedure described in experimental. Apart from small quantities of 12-nitroreserpine hydrochloride and 1-nitroreserpine, a carmine red crystalline substance was also thereby obtained, which melted at 253° (dec) and analysed for a mononitro derivative of reserpine ($C_{33}H_{39}O_{11}N_3$). Its NMR spectrum in $CDCl_3$ revealed the position of the nitro group at *C*-9. It showed signals at 3.10 τ due to H-12 proton, 3.0 τ due to H-9 proton and 3.60 τ due to the two protons in the trimethoxy benzoic acid moiety giving characteristic *meta* coupling of *J* 2.5 c/s only. The mass spectrum afforded the molecular ion peak at *m/e* 653 and a base peak at *m/e* 195 attributed to the presence of 3,4,5-trimethoxy benzoyl ion. It also showed major peaks at *m/e* 637, 636, 623, 558, 442, 441, 440, 424, 410, 382, 296, 259, 238, 226, 212, 211 and 197.



9-Nitroreserpine

Pharmacological tests of 12- and 1-nitroreserpine have shown that their hypotensive activity is of the same order as that of reserpine whereas their sedative action is less than one-eighth and one-third as compared with that of reserpine, and none of its side effects. 12-Nitroreserpine has much longer lasting hypotensive action than reserpine and showed better absorption through oral administration as compared with 1-nitroreserpine.

Experimental

A solution of reserpine (5 g) in glacial acetic acid (100 ml) cooled to 18°C and a 1 : 1 mixture of concentrated nitric acid (d 1.4) and glacial acetic acid (4 ml) was added dropwise in 20 sec with shaking. The initial yellow colour of the reaction mixture changed to orange yellow, going on to orange red and finally to deep red. At this stage, which was reached in about 4 min, the mixture was poured into crushed ice and basified by running in cold concentrated ammonia with vigorous stirring. The granular orange yellow precipitate was filtered, washed repeatedly with water, and dried over a porous plate. After considerable experimentation the following procedure was ultimately arrived at for

obtaining the various nitro derivatives from the total nitration product in optimum yields. The precipitate obtained was taken up in methanol with the addition of a little acetone and kept in the cold when 1-nitroreserpine crystallised out as nearly colourless prismatic rods.

The mother liquor of 1-nitroreserpine was freed of the solvent under reduced pressure and the residue digested with 10% acetic acid. The acetic acid solution was then treated with excess of NaCl and the resulting granular precipitate was filtered and crystallised from a mixture of methanol and acetone (yield, ca.12%), m.p. 207–12° (dec). It was treated with dilute ammonium hydroxide, and the liberated 12-nitroreserpine crystallised from a mixture of methanol and acetone, m.p. 231° (dec).

The combined final mother liquors of 1- and 12-nitroreserpines were taken together and freed of the solvent under reduced pressure. The residue was taken up in ethyl acetate and extracted out with 10% acetic acid with the addition of a small quantity of ether, whereby a negligible quantity of darkish resinous material remained insoluble in both the layers and was rejected. The organic layer was washed with 10% ammonium hydroxide and then repeatedly with water to neutral reaction, dried (Na_2SO_4) and freed of the solvent under reduced pressure. The residue was dissolved in the least quantity of acetone and kept in the cold, when 9-nitroreserpine crystallised out as carmine red prismatic rods, m.p. 253° (dec).

Characterisation of 1-*Nitroreserpine*

1-Nitroreserpine crystallises in colourless prismatic rods, m.p. 146°C (dec) and analysed for $C_{33}H_{39}O_{11}N_3$. Found after drying to constant weight at 50°C under 2 mm pressure over P_2O_5 : C, 61.04; H, 6.20; O, 26.36; N, 6.84%. Calcd. for $C_{33}H_{39}O_{11}N_3$: C, 60.64; H, 5.99; O, 26.90; N, 6.43%. The IR spectrum of the base in KBr indicated peaks at 3462, 2930, 2828, 1735, 1710 (ester carbonyl), 1620, 1590, 1540 (—NO_2), 1500, 1490, 1460, 1430, 1415, 1360, 1330, 1280, 1255, 1220, 1173, 1155, 1125, 1090, 1060, 1020, 1000, 980, 955, 920, 900, 860, 840, 825, 795, 760, 720 and 720 cm^{-1}. Its UV spectrum in methanol indicated λ_{max} 217.5, 233 and 266.5 nm and λ_{min} 253 nm. Its NMR spectrum in $CDCl_3$ indicated signal at 8.78 ppm with a quartet due to *ortho* coupling of the proton in the benzene nucleus of the reserpine molecule (J 8 c/s) and also signals at 4.08, 3.95, 3.90, 3.58, 3.20, 2.62 and 2.15 ppm (δ).

1-*Nitroreserpine Hydrochloride.* To a solution of 1-nitroreserpine (100 mg) in methanol was added a few drops of methanolic hydrochloric acid followed by a large quantity of ether and the resulting hydrochloride was filtered, washed thoroughly with ether and recrystallised from methanol, when it melted at 200–2°C (dec) with initial softening at 180°C. Found at room over P_2O_5 under reduced pressure: Cl, 4.83%. Calcd. for $C_{33}H_{39}O_{11}N_3.HCl$: Cl. 5.14%.

1-*Nitroreserpine Picrate.* A solution of the base (100 mg) in methanol was treated with a methanolic solution of picric acid and kept overnight in the cold. It was freed of the solvent over the water bath and the residue taken up in alcohol in the hot. On slow cooling, the picrate crystallised out in the form of yellow needles (120 mg), m.p. 228–30°C (dec) and analysed for $C_{33}H_{39}O_{11}N_3.C_6H_3O_7N_3.C_2H_5OH$. (Found at room temp under reduced pressure over P_2O_5 : C, 54.49; H, 5.46; O, 32.02; N, 8.75%. Calcd. for $C_{33}H_{39}O_{11}N_3.C_6H_3O_7N_3.C_2H_5OH$: C, 53.94; H, 5.26; O, 31.57; N, 9.21%.)

Reduction of 1-*Nitroreserpine*

1-Nitroreserpine (1 g) was treated with a warm suspension of iron powder in 50% acetic acid (10 ml) whereby the base went gradually into solution accompanied by brisk effervescence of hydrogen. The light yellow reaction mixture was filtered and washed with 10% acetic acid. On addition of potassium iodide to the combined filtrate and washings, the reduced base was completely precipitated out in the form of its crystalline hydroiodide which was filtered, washed and dried. The base was liberated from the hydroiodide by treating the aqueous suspension of the salt with ammonia, and extracted out with ethyl acetate. The ethyl acetate extract was washed with water, dried (Na_2SO_4) filtered and freed of the solvent under reduced pressure. The residue was dissolved in least quantity of methanol and kept in the cold when it crystallised out as prismatic rods. It analysed for reserpine and was identified with it through mixed m.p. determination, IR spectral data and TLC.

Hydrolysis of 1-*Nitroreserpine*

1-Nitroreserpine (1 g) was dissolved in 2.5% aqueous methanolic KOH solution (40 ml) and the reddish solution thus obtained was left overnight at room temperature. The reaction mixture was acidified with methanolic hydrochloric acid, then basified with methanolic ammonia, and acidified with methanolic acetic acid. A little ether was then added on and the inorganic salts precipitated during this process were filtered off. The filtrate was freed of the solvent under reduced pressure the residue taken up in about 5 ml water, and the aqueous solution was extracted three times with ethyl acetate The combined ethyl acetate extracts were washed with water, dried (Na_2SO_4), and freed of the solvent under reduced pressure when a crystalline residue (0.3 g) was obtained. On recrystallisation from methanol it formed colourless prismatic rods, m.p. 166°, and analysed for $C_{10}H_{12}O_5$. It was identified through IR spectrum and mixed m.p. determination as 3,4,5-trimethoxybenzoic acid obtained through the alkaline hydrolysis of reserpine. (Found after drying to constant weight at room temperature over P_2O_5 under reduced pressure: C, 56.46; H, 5.33; O, 37.92%. Calcd. for $C_{10}H_{12}O_5$: C, 56.60; H, 5.66; E, 37.73%).

The aqueous fraction from the above working was freed of the solvent under reduced pressure and

the residue taken up in small quantity of methanol. On the addition, of benzene to the methanolic solution some more inorganic matter precipitated out which was filtered off. The filtrate was freed of the solvent under reduced pressure. The brownish waxy residue thus obtained failed to yield the amino acid component of the hydrolysis in a crystalline form. It was also not possible to get the 1-nitroreserpic acid in the form of its salts due apparently to side reactions in the procedure of hydrolysis.

Isomerisation of 1-*Nitroreserpine-iso*-1-*Nitroreserpine*

1-Nitroreserpine (1 g) was dissolved in glacial acetic acid (20 ml) and heated at 90°C over a steam bath for 5 min, when a deep red persistent colouration appeared. The reaction mixture was diluted to a 10% acetic acid solution by adding 180 ml distilled water, and KI was then added to the solution till saturation. The resulting reddish crystalline precipitate was filtered, washed thoroughly with water and basified with 5% ammonium hydroxide in aqueous suspension. The liberated base was extracted out with ethyl acetate, which was repeatedly washed with water, dried (Na_2SO_4) and freed of the solvent under reduced pressure. The silky brownish residue was taken up in methanol and the solution treated with ethyl acetate followed by ether and a few drops of petroleum ether (b.p. 60–80°) which threw out a small quantity of dark brown sticky material which was neglected. The clear yellowish solution was freed of the solvent under reduced pressure, the residue was taken up in 70% methanol in the hot and the solution kept in the cold when iso-1-nitroreserpine (0.57 g) crystallised out as yellow needles, m.p. 180° (dec) and analysed for $C_{33}H_{39}O_{11}N_3$. (Found at 50°C over P_2O_5 under reduced pressure C, 60.98; 61.49; H, 6.32, 6.11; O, 26.14, 26.41; N, 6.38, 6.52%. Calcd. for $C_{33}H_{39}O_{11}N_3$: C, 60.64, H, 5.99; O, 26.90; N, 6.43%.

Isomeresation of 1-*Nitroreserpine-iso*-1-*Nitroreserpine Hydrochloride.* A methanolic solution of the base was treated with a few drops of alcoholic hydrochloric acid and an excess of ether added on to the solution, when the hydrochloride of the isonitro base came out in the form of reddish crystallisate which was filtered and washed with ether. On recrystallisation from aqueous methanol the hydrochloride formed deep red short needles which melted at 170°C (dec). (Found at room temperature over P_2O_5 under reduced pressure: Cl, 4.70. Calcd. for $C_{33}H_{39}O_{11}N_3$.HCl : Cl, 5.14%.

Isomeresation of 1-*Nitroreserpine-iso*-1-*Nitroreserpine Hydroiodide and Sulphate.* The hydroiodide and the sulphate of the iso base were also prepared by adding KI and sodium sulphate respectively to a 20%–acetic acid solution of the base and crystallising out the precipitated salts from dilute methanol. (Hydroiodide red prismatic rods, m.p. 180–2° (dec). Sulphate red prismatic rods, m.p. 184° (dec).

Isomerisation of 1-*Nitroreserpine-iso*-1-*Nitroreserpine Picrate.* The picric acid salt was prepared by bringing together the components in alcoholic solution and crystallising out the resulting precipitate from alcohol. (Fine short orange coloured needles, m.p. 205°C.)

Isomerisation of 1-*Nitroreserpine-iso*-1-*Nitroreserpine Methiodide.* To a solution of iso-1-nitroreserpine (100 mg) was added methyl iodide (1 ml) which gave an immediate deep red colouration, and the solution was kept overnight in the cold. It was freed of the solvent under reduced pressure and the deep red crystalline residue of iso-1-nitroreserpine methiodide was recrystallised from methanol, when it formed short red needles (60 mg), m.p. 184–85°C (dec.) (Found at room temp over P_2O_5 under reduced pressure. I, 15.92: 15.18%: Calcd. for $C_{33}H_{39}O_{11}N_3$. CH_3I : I, 15.97%.)

Characterisation of 12–*Nitroreserpine*

12–Nitroreserpine crystallises in cauliflower-like crystals, m.p. 231°C (dec), and analysed for $C_{33}H_{39}O_{11}N_3$. (Found at 50°C under 2 mm pressure over P^2O^5: C, 60.39; H, 6.18; O, 26.86; N, 6.48% and mol. wt. (Rast.), 628. Calcd. for $C_{33}H_{39}O_{11}N_3$: C, 60.64; H, 5.99; O, 26.90; N, 6.43% and mol wt. 653). It is readily soluble in $CHCl_3$, CCl_4 and ethyl acetate, sparingly soluble in methanol, acetone and ether, insoluble in petroleum ether (b.p. 60–80°C). Its IR spectrum in KBr indicated peaks at 3460 cm^{-1} (—NH), 2930, 2830, 1740, and 1710 (ester —CO), 1628, 1588, 1568, 1510 cm^{-1} (—NO_2), 1500, 1460, 1415, 1355, 1330 (—NO_2), 1305, 1295, 1270, 1220, 1170, 1150, 1125, 1090, 1040, 995, 980, 910, 870, 840, 795 and 760 cm^{-1}. Its UV absorption spectrum in methanol indicated λ_{max} 217.5, 264 nm and a flat maxima at 356 nm and λ_{min} 237.5, 320 nm. The NMR spectrum of 12-nitroreserpine in $CDCl_3$ indicated signals at 7.25, 3.86, 3.80, 3.50 and 2.14 p.p.m. (δ).

12-*Nitroreserpine Hydrochloride.* Hundred mg base was taken up in hot alcohol and treated with alcoholic hydrochloric acid at room temperature when the hydrochloride crystallised out as yellow needles (100 mg), m.p. 207–212°C (dec). (Found on analysis after recrystallisation from methanol for $C_{33}H_{39}O_{11}N_3$.HCl at room temp over P_2O_5 under reduced pressure: C, 56.87; H, 6.34, O, 25.08; N, 6.04; Cl. 5.20%. Calcd. for $C_{33}H_{39}O_{11}N_3$. HCl: C, 57.43; H, 5.80; O, 25.52; N, 6.09; Cl, 5.14%.)

12-*Nitroreserpine Picrate.* To a solution of the base (50 mg) in hot methanol (7 ml) was added a methanolic solution of picric acid, and the mixture warmed on the water bath for about 10 min, when the picrate of the base crystallised out as yellow flat needles (60 mg). On recrystallisation from a 1 : 1 mixture of benzene and methanol the picrate melted at 228–30°C (dec) with initial browning at 222°C, and analysed for $C_{33}H_{39}O_{11}N_3$. ($C_6H_3O_7N_3$) (Found at 50°C under 2 mm pressure over P2O5: C, 52.89; H, 4.87; O, 32.46; N, 9.55%. Calcd. for $C_{33}H_{39}O_{11}N_3(C_6H_3O_7N_3)$; C, 53.06; H, 4.87; O, 32.65; and N, 9.52%.)

12-*Nitrorespine Methiodide.* To a suspension of the base (50 mg) in hot methanol (2 ml) was added methyl iodide (1 ml) whereby a clear solution was obtained. It was kept overnight in the cold and

freed of the solvent over the water bath. The yellow residue was taken up in methanol and ether added to the solution till slight turbidity, when the methiodide crystallised out as short yellow needles (50 mg) on keeping in the cold. On recrystallisation from methanol it melted at 225°C (dec). (Found at 50°C under 2 mm pressure over P_2O_5: I, 16.60; *N*—CH_3, 3.80% Calcd. for $C_{33}H_{39}O_{11}N_3$. CH_3I: I, 15.97; *N*—CH_3, 3.64%.)

12-*Nitroreserpine Nitrate and Sulphate.* The nitrate and sulphate salts of the base were prepared by adding the corresponding alkali salts to a 20% solution of 12-nitroreserpine and crystallising the resulting salts of the base out of dilute methanol. (Nitrate, prismatic rods, m.p. 180°C (dec); sulphate, orange hexagonal plates, m.p. 198–200° (dec).

12–*Nitroreserpine Acetate.* The nitro base (100 mg) (100 mg) was dissolved in hot 20% acetic acid (5 ml) and rubbed vigorously with a glass rod when the acetate crystallises in the form of long yellow needles m.p. 220°C (dec). (Found at room temp under reduced pressure over P_2O_5: N, 5.93%. Calcd. for $C_{33}H_{39}O_{11}N_3$. $C_2H_4O_2$: N, 5.89%.)

12-*Nitroreserpine Oxalate and Citrate.* The oxalate and citrate were prepared by adding the corresponding alkali salts to a 35% acetic solution of the base and crystallising the resulting crystalline precipitate from alcohol. (Oxalte, yellow short needles, m.p. 180° (dec); citrate-yellow needles, m.p. 230–32° (dec).

12-*Nitroreserpine Ascorbate.* The nitro base (100 mg) was dissolved in 20% L(+)–ascorbic acid (10 ml) and rubbed with a glass rod, when the ascorbate crystallises out as yellow needles, m.p. 196–98° (dec).

Reduction of 12-*Nitroreserpine.* 12-*Aminoreserpine and the Acetylation of the Reduced Base*

The nitro base (100 mg) was dissolved in 50% acetic acid, and the solution was heated on the water bath for 15 min, after the addition of a pinch of iron dust. The original orange yellow colour of the solution started decolouring with brisk effervescence of hydrogen. The nearly colourless solution was filtered and basified with concentrated ammonia and the liberated base extracted out with a 1 : 1 mixture of ethyl acetate and ether. The ethereal extract was washed with water, dried (Na_2SO_4) and freed of the solvent, finally *in vacuo*. The greenish brown sticky residue (80 mg) failed to crystallise from the usual bench solvents. It is soluble in methanol, ethyl acetate, ether and insoluble in petroleum ether (b.p. 60–80°C). It formed an amorphous hydrochloride.

The uncrystallisable amino base (80 mg) was then dissolved in pyridine (1 ml), and freshly distilled acetic anhydride (1 ml) was added to the solution which was left overnight at room temperature. The reddish reaction mixture was freed of the solvent under reduced pressure, the last traces of pyridine being removed by repeatedly dissolving the residue in alcohol and evaporating it off under reduced pressure. The brownish residue was extracted out with ether and the light yellow crystalline residue (50 mg) obtained on removal of the solvent was recrystallised from a mixture of ethyl acetate and petroleum ether (b.p. 60–80°C), when it formed long colourless needles which metlted at 250–56° (dec). (Found at room temp over P_2O_5: under reduced pressure: N, 6.27%. Calcd. for $C_{33}H_{43}O_{10}N_3$: N, 6.31%.)

Hydrolysis of 12-*Nitroreserpine*

The nitro base (0.1 g) was dissolved in hot methanol and heated with 5% aqueous methanolic potassium hydroxide on water bath for about 20 min adding some more of dilute methanol to keep the base in solution. The initial orange colour of the reaction mixture gradually changed to orange red; it was then acidified with methanolic hydrochloric acid whereby the colour of the solution turned into light yellow. The methanolic solution was then basified with ammonia and acidified with methanolic acetic acid. The acidic solution, which now did not contain any free hydrochloric acid, was freed of the solvent under reduced pressure after filtering off the inorganic salts that had precipitated out in this process. The residue was taken up in ethyl acetate and ether with a touch of methanol and the solution was filtered off from the residual inorganic matter.

On keeping the ethyl acetate solution in the cold 12-nitroreserpic acid came out in the form of cauliflower like microcrystals. It cou'd not be characterised because of its extremely hygroscopic nature and was, therefore, converted into the hydrochloride by taking it up in 5% hydrochloric acid and allowing it to slowly concentrate on the water bath. The hydrochloride of the amino acid (40 mg) crystallised in the form of orange prisms which melted at 290°C (dec) with initial blackening at 220°C. (Found at room temp over P_2O_5 under reduced pressure. Cl, 7.49%. Calcd. for $C_{22}H_{27}O_7N_3$. HCl: Cl 7.58%.)

An excess of ether was added to the 12-nitroreserpic acid mother liquor, and a small quantity of coloured material thereby thrown out was filtered off. The filtrate was freed of the solvent under reduced pressure and the crystalline residue was recrystallised from methanol. The acid thus obtained in the form of colourless prismatic rods melted at 166° and could be identified through IR spectrum and mixed melting point determination as 3,4,5-trimethoxybenzoic acid. It analysed for $C_{10}H_{11}O_5$. (Found at room temp over P_2O_5 under reduced pressure. C, 56.48; H, 5.12; O, 3787%. Calcd. for $C_{10}H_{12}O_5$: C, 56.46; H, 5.66; O, 37.73%.)

Acknowledgements. The authors thank C. H. Boehringer Sohn, Chemische Fabrick, Ingelheim (Germany) for the generous supply of reserpine. The authors are also grateful to Dr. Zubeda Qureshi of the Jinnah Postgraduate Medical Centre, Karachi, and Dr. Kudszus and Dr. Stimming of C. H. Boehringer Sohn, Germany, for carrying out pharma-

cological tests of 1- and 12-nitroreserpine derivatives. Dr. Stimming also determined the NMR and mass spectra of various nitro derivatives.

References

1. J. M. Mueller, E. Schlittler and H. J. Bein, Experientia, **8,** 338 (1952); H. J. Bein,Experientia, **9,** 107 (1953).
2. R. V. Ford and J. H. Moyer, J. Am. Med. Assoc., **166,** 1793 (1958).
3. S. Siddiqui and V.U. Ahmad, Proc. Pakistan Acad. Sci., **5,** 1 (1968).
4. S. Siddiqui and Mohammad Ataullah Khan, Pakistan Patent 122708 (1972).
5. S. Siddiqui and Mohammad Ataullah Khan, Pakistan Patent 124142 (1974).
6. S. Siddiqui, V. U. Ahmad, Mohammad Ataullah Khan and Saira Ismail, Z. Naturforsch., **28b,** 486 (1973).