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NITRATION STUDIES IN SOME β-CARBOLINE BASES

Part I. Mononitro Derivatives of Rescinnamine*

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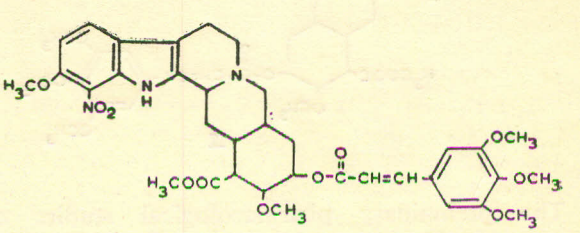
Abstract. Nitration studies in rescinnamine have yielded three mononitro position isomers. The chemical and spectral studies have revealed that electrophilic substitution has taken place at C-9, C-12 and N-1. Preliminary pharmacological studies have shown that the hypotensive action of 1- and 12-mononitrorescinnamine is lower in comparison to rescinnamine.

The establishment of the chemical constitution of the ajmaline series of bases communicated by Siddiqui and Siddiqui[1] in 1930 and some of the other Rauwolfia alkaloids carrying important therapeutic activities widely stimulated interest in the correlation of structural and physiological activity in this series of bases. Taking into account the fact that the mononitro derivative of ajmaline[2] was found to be more than twice as active in its antiarrhythmic cardiac activity than the mother base, it was considered of interest to study the substitution in benzene ring of the indole nucleus of reserpine and some of the other Rauwolfia bases with a β-carboline structure. As a result of a comprehensive nitration studies carried out by Siddiqui and Ataullah,[3] three mononitro position isomers of the base 1-, 12-and 9 nitroreserpine were obtained. Out of these, 1-nitro derivative which formed the major product of nitration was found to have the same hypotensive activity as reserpine without its undesirable side effects like tremor, ptosis and diarrhoea. On the other hand, 12-nitroreserpine required a higher dosage for the same order of hypotensive activity, but its action was noted to be more lasting in unanaesthetised dogs, continuing over 90 hr as against 20–24 hr in the case of reserpine.

Taking these findings into account it was considered of interest to extend these studies to rescinnamine. The nitration of this base was carried out in glacial acetic acid medium and, as in the case of reserpine, it was found that the critical conditions of nitration in respect of time, temperature and the proportion of the diluent in the reactants had to be carefully controlled, to avoid the resinification on the one hand and lack of reaction on the other. When rescinnamine dissolved in glacial acetic acid and the reaction mixture kept over a period of about 2 min with occasional shaking, the initial straw coloured solution turned to greenish yellow rapidly changing over to orange yellow, orange red and finally to deep red. At this stage the reaction mixture was worked up as described in the experimental. Following that procedure 12-nitrorescinnamine was obtained on repeated crystallisation from a mixture of methanol and acetone, as shining yellow needles, melting at 280–82°C and analysing for $C_{35}H_{41}O_{11}N_3$. When the reaction was extended over a longer period after the deep red colouration was attained, it failed to give any crystalline product due to resinification and formation of tarry materials. The hydrochloride, picrate, nitrate, hydroiodide and hydrobromide of 12-nitrorescinnamine were also prepared and analysed.

To elucidate the location of the nitro group, the alkaline hydrolysis of the mononitro derivative was carried out, whereby a nitrogen free carboxylic acid was obtained which could be identified with 3,4,5-trimethoxy cinnamic acid through mixed melting point with an authentic sample, and comparison of their IR spectra. While this excluded the presence of the nitro group in the cinnamoyl moiety of rescinnamine, it indicated the possibility of its location in the indole nucleus of the base. On the basis of NMR spectra, it has been concluded that the nitro group is situated at position 12 of the rescinnamine molecule (I). Its NMR spectrum in $CDCl_3$ indicated that two aromatic hydrogens are coupled so that they are *ortho* to each other, and the substitution has taken place at position 12 which is the point of highest electron density.



I

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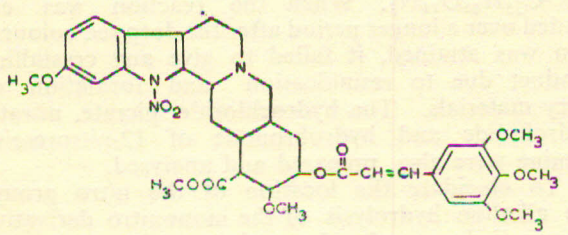
12-Nitrorescinnamine on its reduction yielded an amino base, m.p. 196°C which formed a crystalline hydrochloride.

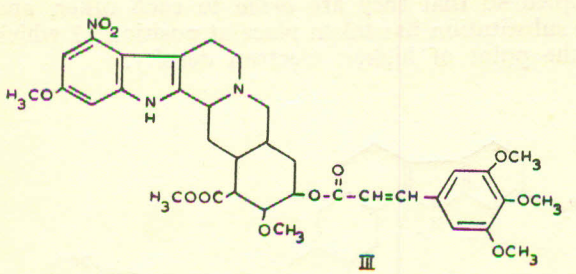
1-Nitrorescinnamine which, as in the case of reserpine, is the major product of nitration, was obtained from the mother liquors of 12-nitrorescinnamine according to the procedure described in the experimental. It formed a yellowish microcrystalline powder melting at 145–46°C with decomposition, and analysed for $C_{35}H_{41}O_{11}N_3$. Exhaustive attempts to get it in well-defined crystalline form did not succeed, but it gave a single spot in TLC, and its melting point could not be raised any higher through repeated crystallisations. Its alkaline hydrolysis and reduction failed to yield any crystalline product, in contrast to 12-nitrorescinnamine, due to the formation of resinified products.

It could be characterised as 1-nitrorescinnamine (II) mainly through spectral studies. There was no peak due to indolic NH in the IR and NMR spectra of the nitro base. The NMR spectra of the nitro compound showed signals for five and three protons respectively in the aromatic region. The H-12 signal is shifted downfield while H-3 is shifted upfield by 0.3 ppm compared with rescinnamine.



II

As in the case of reserpine, a third isomer (III) of its mononitro derivative was obtained in the form of carmine red needles in a small quantity. It analysed for $C_{35}H_{41}O_{11}N_3$ and charred without melting above 250°C. Due to lack of sufficient quantity, structural studies in it could not be carried out, but probably corresponds to 9-nitroreserpine which also has a carmine red colour and charrs without melting.



III

The preliminary pharmacological studies of 1-nitrorescinnamine revealed that its hypotensive action is slightly lower than that of reserpine, and that the action of 12-nitrorescinnamine is much lower in comparison. The results of further pharmacological work particularly in respect of its central nervous system section are still awaited.

Experimental

Rescinnamine (5 g) was dissolved in glacial acetic acid (100 ml), cooled to 18°C and 1:1 mixutre of concd HNO_3 acid (1.4 d) and glacial acid (4 ml) was added slowly in 20 sec with good shaking, whereby the solution acquired a greenish yellow colouration. The colour of the reaction mixture changed from greenish yellow to orange yellow going on to orange red and finally to deep red. At this stage, which was reached in about 2 min, the reaction mixture was poured into crushed ice and strong ammonia was added on with vigorous stirring till pH 6 was reached. The amorphous yellow precipitate was filtered, washed repeatedly with water, sucked, dried on a porous plate and dissolved in a 2 : 1 mixture of methanol and acetone. The solution was then kept overnight in the cold. The resulting crystallisate of 12-nitrorescinnamine was repeatedly recrystallised from methanol and acetone, when it finally melted at 280–82°C, and analysed for $C_{35}H_{41}O_{11}N_3$ (yield 20%).

The combined methanolic mother liquors and washings of 12-nitrorescinnamine were concentrated under reduced pressure. On the successive addition of small quantities of water to the solution in the hot, a darkish brown precipitate was thrown out which was cottoned. The resulting golden yellow solution was left overnight in a crystallising dish at room temperature, whereby a yellowish brown semi-crystalline mass settled down. On repeating this process of solvation in slightly diluted methanol and slow evaporation, 1-nitrorescinnamine was ultimately obtained as a yellowish microcrystalline product, which finally melted at 145–46°C with decomposition, and analysed for $C_{35}H_{41}O_{11}N_3$.

On freeing the mother liquors of the solvent under reduced pressure and working up the residue in the manner described above, a further quantity of 1-nitrorescinnamine was obtained (total yield 65%).

The darkish brown precipitates obtained in the foregoing operations on the addition of water to the concentrated methanolic mother liquors of 12-nitrorescinnamine, were taken together and subjected to purification through solvation in ethyl acetate and ether and precipitation with petroleum ether, whereby a small quantity of a reddish product was obtained on removal of the solvent. On repeated crystallisation of the residue from acetone, 9-nitrorescinnamine was finally obtained in the form of carmine red prismatic rods, which charred without melting above 260°C and analysed for $C_{35}H_{41}O_{11}N_3$.

Characterisation of the Bases 12-*Nitrorescinnamine.* 12-Nitrorescinnamine was crystallised from methanol and acetone in the form of brittle yellow needles which melted at 280–82°C (dec.) and analysed for $C_{35}H_{41}O_{11}N_3$. It is readily soluble in mixture of methanol–acetone, alcohol–benzene, sparingly soluble in these solvents individually and partly soluble in ether. (Found after drying over P_2O_5: C, 60.46; H, 6.58; N, 5.8%; Calcd for $C_{35}H_{41}O_{11}N_3$ C, 60.8; H, 6.3; and N, 6.1%.) The IR spectrum

in nujol indicated peaks at 1500 (NO_2), 1620 (aromatic ring), 1730 (ester carbonyl) and 3475 cm^{-1} (NH). Its UV spectrum in methanol indicated λ_{max} 224, 307 nm and λ_{min} 268 nm. Its NMR spectrum revealed that the nitro group has entered the position 12 of the rescinnamine molecule.

12-*Nitrorescinnamine Hydrochloride.* 12-Nitrorescinnamine was dissolved in alcoholic hydrochloric acid when the hydrochloride came out as yellow tapering needles. On recrystallisation from methanol it melted at 220–24°C. The hydrochloride is nearly insoluble in ether. (Found after drying at room temperature over P_2O_5 under reduced pressure: Cl, 4.7%. Calcd for $C_{35}H_{41}O_{11}N_3$: HCl: Cl, 4.96%).

12-*Nitrorescinnamine Hydroiodide.* To a solution of the base in 20% acetic acid was added KI till saturation and the resulting precipitate of the hydroiodide salt was filtered, washed with cold water and dried over porous plate. It was taken up in methanol and kept in the cold when 12-nitrorescinnamine hydroiodide crystallised out in the form of golden yellow rods. On recrystallisation from methanol it melted at 230–35°C and analysed for $C_{35}H_{41}O_{11}N_3$ HI. (Found after drying over P_2O_5 under reduced pressure I, 14.96%, Calcd for $C_{35}H_{41}O_{11}N_3$. HI: I, 15.73%).

12-*Nitrorescinnamine Hydrobromide.* The hydrobromide was prepared in the same manner as hydroiodide and crystallised from a small quantity of methanol, when it formed yellow needles, m.p. 245–48°C. (Found after drying over P_2O_5 under reduced pressure Br. 11.61% Calcd for $C_{35}H_{41}O_{11}N_3$. HBr: 10.52%).

12-*Nitrorescinnamine Picrate.* A solution of the base in alcohol alongwith a few drops of acetone was treated with a methanolic solution of picric acid and kept overnight in the cold, when the picrate crystallised out in the form of yellow needles, and analysed for $C_{35}H_{41}O_{11}N_3 . C_6H_3O_7N_3$. (Found after drying at room temperature under reduced pressure over P_2O_5: C, 54.3; H, 4.2 and N, 9.4%. Calcd for $C_{35}H_{41}O_{11}N_3 . C_6H_3O_7N_3$: C, 54.1; H, 4.8 and N, 9.25%).

12-*Nitrorescinnamine Nitrate.* To a solution of the nitro base in 20% acetic acid was added KNO_3 till saturation, and the resulting nitrate was filtered after cooling, washed with cold water and dried. On recrystallisation from methanol, it formed aggregates of short needles, m.p. 180–85°C and analysed for $C_{35}H_{41}O_{11}N_3 . HNO_3$. (Found after drying over P_2O_5 under reduced pressure. C, 55.2; H, 4.5; N, 11.3%. Calcd for $C_{35}H_{41}O_{11}N_3 . HNO_3$: C, 54.9; H, 4.3 and N, 11.6%).

Reduction of 12-*Nitrorescinnamine.* 12-Nitrorescinnamine (0.15 g) was dissolved in alcohol (2 ml) alongwith 30% HCl (4 ml) and zinc dust was gradually added to the solution with constant shaking till the yellow colour of the reaction mixture disappeared. Unreacted zinc was filtered off, and the clear solution ammoniated. The resulting precipitate which consisted of the reduced base and $Zn(OH)_2$ was sucked, washed, and extracted out with ethyl acetate. The ethyl acetate solution was washed with water, dried (Na_2SO_4), filtered and freed of the solvent under reduced pressure. On repeated recrystallisations of the residue from alcohol alongwith a few drops of acetone, 12-aminorescinnamine was finally obtained as colourless needles which melted at 195–98°C. It was soluble in alcohol, methanol and acetone. (Found after drying over P_2O_5 under reduced pressure: C, 61.9; H, 6.73; N, 6.32%. Calcd for $C_{35}H_{41}O_9N_2 . NH_2$: C, 61.31; H, 6.86; N, 6.13%).

Hydrolysis of 12-*Nitrorescinnamine.* 12-Nitrorescinnamine (0.15 g) was refluxed for 2 hr in a solution of methanol (20 ml), water (10 ml) to which 10 ml 5% aqueous NaOH had been added. At the end of this period, the deep golden yellow solution was concentrated under reduced pressure to remove the major portion of methanol. The concentrated solution was then diluted with an additional 20 ml water, cooled, adjusted to pH2 by the addition of concd HCl and extracted with chloroform. The combined chloroform extracts were concentrated under reduced pressure to a viscous mass which crystallised on standing in the form of white needles (30 mg). On recrystallisation it melted at 126–27°C and showed no depression on admixture with an authentic sample of 3,4,5-trimethoxy cinnamic acid. The IR and UV spectra were identical with those of the authentic sample. (Found: C, 66.62; H, 5.59; OCH_3, 38.49%. Calcd for $C_{12}H_{14}O_5$: C, 60.50; H, 5.92; OCH_3, 39.08%).

The aqueous layer after the chloroform extractions was concentrated under reduced pressure, placed in the refrigerator and allowed to stand overnight, when 12-nitroreserpic acid hydrochloride crystallised out (45 mg) in the form of orange prismatic rods, which melted at 292–94°C (dec). (Found: Cl, 7.25%. Calcd for $C_{22}H_{27}O_7N_3$. HCl; Cl, 7.58%).

1-*Nitrorescinnamine.* The major product (m.p. 145–46°C, yield 65%) could be characterised as 1-nitrorescinnamine by NMR spectrum in $CDCl_3$. It is readily soluble in alcohol, methanol, acetone, chloroform and ethyl acetate and sparingly soluble in ether. (Found after drying over P_2O_5: C, 60.41; H, 6.14; N, 6.6%. Calcd for $C_{35}H_{41}O_{11}N_3$: C, 60.8; H, 6.3; N, 6.1%). The IR spectrum in nujol showed absorption bands at 1504 (NO_2), 1704 (ester carbonyl), 1620 cm^{-1} (aromatic ring) Its UV spectrum in methanol shows λ_{max} 300 nm λ_{min} 263 nm.

1-Nitrorescinnamine on its hydrolysis under the procedure described for 12-nitrorescinnamine, yielded 3,4,5-trimethoxy cinnamic acid. (Found C, 60.62; H, 5.72; OCH_3, 38.93%. Calcd for $C_{12}H_{14}O_5$: C, 60.50; H, 5.92; OCH_3, 39.08%.) The aqueous fraction 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.

1-Nitrorescinnamine could not be reduced as it resinified to give tarry materials in acidic medium.

9-*Nitrorescinnamine.* The carmine coloured third position isomer of nitrorescinnamine charred above 250°C and analysed for $C_{35}H_{41}O_{11}N_3$. It is soluble in mixtures of methanol and acetone, methanol–benzene and sparingly soluble in these solvents individually. (Found after drying over P_2O_5 at

60°C under reduced pressure: C, 61.1; H, 6.5; N, 6.7%. Calcd for $C_{35}H_{41}O_{11}N_3$: C, 60.8; H, 6.3; N, 6.1%. The position of the nitro group is assigned to it at 9 on the fact that a corresponding base has been obtained in the case of reserpine.

References

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3. S. Siddiqui and M. A. Khan, Pakistan Patent 122708 (1972); British Patent 135 3667.