Phytochemistry, 1975, Vol. 14, pp. 999–1003. Pergamon Press. Printed in England.

N-OXIDES OF HYOSCYAMINE AND HYOSCINE IN THE SOLANACEAE

J. DAVID PHILLIPSON and S. S. HANDA

Department of Pharmacognosy, The School of Pharmacy, University of London, 29–39, Brunswick Square, London, WC1N 1AX, England

(*Received* 21 *July* 1974)

Key Word Index—*Atropa*; *Datura*; *Hyoscyamus*; *Mandragora*; *Scopolia*; Solanaceae; tropane alkaloids; hyoscyamine *N*-oxides; hyoscine *N*-oxides.

Abstract—The *N*-oxides of (−)-hyoscyamine and (−)-hyoscine have been prepared and characterized. Two *N*-oxides of hyoscyamine have been isolated from species of *Atropa*, *Datura*, *Hyoscyamus*, *Scopolia* and *Mandragora*, and one *N*-oxide of hyoscine has been isolated from species of the first four genera.

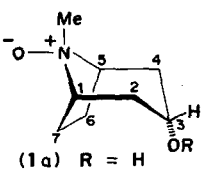
INTRODUCTION

Naturally-occurring *N*-oxides are well known for some of the pyrrolizidine alkaloids found in members of the Boraginaceae, Compositae and Papilionaceae [1]. Many of the plants in these families have been investigated in some detail because of their toxicity to livestock and as a result more than 100 alkaloids have been isolated, of which some 30 are known to occur as *N*-oxides [1, 2]. Other examples of naturally-occurring *N*-oxides are known and it has been suggested that they may be more widespread than the literature indicates since they can be missed by normal extraction procedures [2]. Tropane alkaloid *N*-oxides have not been reported previously as natural products* although they are readily prepared in the laboratory by oxidation of tertiary alkaloids.

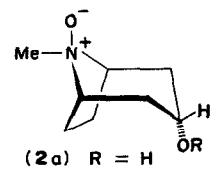
RESULTS AND DISCUSSION

The *N*-oxides of (−)-hyoscyamine and (−)-hyoscine have been prepared, separated and characterized. Each alkaloid forms two isomeric *N*-oxides which are termed isomer 1 (equatorial $N^+–O^-$) and isomer 2 (axial $N^+–O^-$). Both isomers of hyoscyamine *N*-oxide have been isolated from the roots, stems, leaves, flowers, pericarps and seeds of *Atropa belladonna* L., *Hyoscyamus niger* L., and *Datura stramonium* L. Isomer 1 of hyoscine *N*-oxide has been isolated from all parts of the latter two species and also from the leaves of *A. belladonna*. The roots, stems and leaves of *Scopolia lurida* Dun. and *S. carniolica* Jacq. were found to contain the two *N*-oxides of hyoscyamine and isomer 1 of hyoscine *N*-oxide, while the former two *N*-oxides were found in the roots, stems with leaves, and fruits of *Mandragora officinarum* L.

The amino–alcohol tropine forms two isomeric *N*-oxides in the ratio of 3:1; these isomers have been separated and the configurations at the *N* asymmetric centre of the major isomer (**1**a) and the minor isomer (**2**a) have been established [4–6]. The major isomer, which possesses the equatorial $N^+–O^-$ bond, can readily be distinguished from the minor isomer by means of the chemical shifts of the PMR signals attributed to the N^+–Me and



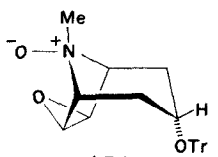
(1a) R = H
(1b) R = Tr



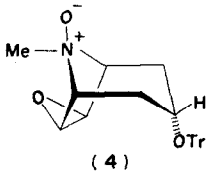
(2a) R = H
(2b) R = Tr

Tr = —CO.CH(CH_2OH).C_6H_5

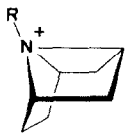
* A short communication reporting the natural occurrence of some tropane alkaloid *N*-oxides was presented at the British Pharmaceutical Conference held in September 1973 [3].



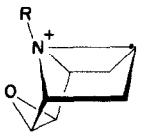
(3)



(4)



(5a) R = Me
(5b) R = H



(6a) R = Me
(6b) R = H

to H-2 and -4 and H-6 and -7 [5, 6]. These signals have been correlated with those found in the spectrum of the major isomer of (−)-hyoscine *N*-oxide (**3**), the structure of which has been established by X-ray crystallography. Although the isomers were not separated, the existence of a second *N*-oxide of hyoscine was revealed by the presence of two N^+–Me signals in the PMR spectrum. Two N^+–Me signals have been observed in the PMR spectrum of atropine [(±)-hyoscyamine] *N*-oxide but the mixture of *N*-oxides have not been separated [5].

The use of *m*-chloroperbenzoic acid [6] for the preparation of tropane alkaloid *N*-oxides has been criticized on the ground that the reaction was too slow and a preference was expressed for the use of H_2O_2 [5]. However, in our hands, H_2O_2 tended to give other reaction products whereas *m*-chloroperbenzoic acid yielded only the two *N*-oxides after 3 hr. Although (−)-hyoscyamine *N*-oxide gives one spot in many TLC systems, it can be resolved into two components and initial separation of the two isomers in the ratio of 3:2 was effected by preparative TLC. Subsequently, separation was accomplished by the differing solubilities in acetone, followed by crystallization of the hydrochlorides.

The major isomer (**1b**), which was acetone-soluble and had a lower R_f value, is referred to as isomer 1, while the acetone-insoluble isomer with higher R_f is referred to as isomer 2 (**2b**). Both isomers were readily reduced by H_2SO_3 to yield hyoscyamine. The important feature in the PMR spectra of the two *N*-oxides is the chemical shift of the N^+–Me signal, well downfield at δ 3·30 in the spectrum of isomer 1 and at δ 3·12 in the spectrum of isomer 2; the corresponding signals for the hydrochlorides appeared further downfield at δ 3·56 and δ 3·45 respectively. Since isomers of quaternised tropane alkaloids with axial N^+–Me substituents produce signals which resonate at lower field than the corresponding equatorial isomers [5, 8], it is concluded that isomer 1 has an axial N^+–Me and isomer 2 has an equatorial N^+–Me. Further support for these assignments comes from a study of the signals attributed to the axial H-2 and H-4 which appeared further downfield (δ 3·25) in the spectrum of isomer 2 than in the spectrum of isomer 1 (δ 2·40).

Treatment of (−)-hyoscine with *m*-chloroperbenzoic acid resulted in the formation of two isomers, obtained in the ratio of 3:1. The major isomer (**3**) was separated by crystallization of the hydrochloride and the minor isomer (**4**) was obtained by preparative TLC. The major isomer which had a lower R_f value is referred to as isomer 1, while the minor isomer is referred to as isomer 2. In contrast to the PMR spectra of (−)-hyoscyamine *N*-oxides, the hydrochloride of the major isomer 1 of (−)-hyoscine *N*-oxide has a signal for the axial N^+–Me (δ 3·45) which resonates at higher field than the signal for the equatorial N^+–Me of the hydrochloride of the minor isomer 2 (δ 3·62). These differences in chemical shift are due to the deshielding effect of the epoxide oxygen on the equatorial N^+–Me (**4**) [5]. Further differences between the two hyoscine *N*-oxide isomers were noted since in the PMR spectrum of the major isomer 1 (**3**), the signals for H-1 and H-5 (δ 4·43) and for H-6 and H-7 (δ 4·22) appeared at lower fields than the corresponding signals (δ 4·30 and δ 3·92 respectively) for the minor isomer 2 (**4**); these differences in chemical shift are due to the deshielding influence of the axial N^+–O^- bond in the minor isomer.

MS has proved useful in the structure determination of some tropane alkaloid esters (e.g. phyllalbin [9]) but generally such alkaloids give complicated spectra [10] and this was noted in the present work since the *N*-oxides undergo thermal decomposition. Each of the four *N*-oxides was heated to 200° and when the residues were examined by TLC, spots corresponding to the tertiary and nor-alkaloids were observed. This indicates that the MS obtained resulted from mixtures of *N*-oxide, tertiary and nor-alkaloids. When hyoscya-

mine *N*-oxide was heated, a portion of the *N*-oxides remained unchanged but no *N*-oxides were observed after heating hyoscine *N*-oxides. This is consistent with the absence of the M^+ in the spectra of the latter compounds. Despite thermal decomposition it was possible to distinguish the two *N*-oxides of hyoscyamine and of hyoscine. Both isomers of (−)-hyoscyamine *N*-oxide gave MS with peaks of low intensity at *m/e* 305 (M^+) and 289 (M^+-16) indicating the presence of the additional oxygen atom. The spectrum of isomer 1 had a base peak at *m/e* 124 (**5a**) which is the base peak of hyoscyamine. However, this ion was of lower intensity in the spectrum of isomer 2 and the peaks at *m/e* 139, 136 and 118 due to the tropic acid moiety were more pronounced. The hydrochlorides of both isomers gave spectra which differed from those of the bases and in which the first discernible fragment appeared at *m/e* 303. The presence of this ion, which cannot be explained by the normal mass fragmentation rules, and the further complexity of the spectra, indicated that thermal decomposition was taking place. The presence of peaks of low intensity at *m/e* 275 show that the *N*-oxides lose HCHO and form the nor-alkaloids. Both spectra also showed the presence of ions at *m/e* 124 (*ca* 30%) with a composition of $C_8H_{14}N^+$ (**5a**) and had base peaks at *m/e* 110 due to an ion of composition $C_7H_{12}N^+$ (**5b**). This latter ion cannot arise from an ion of structure **5a** and most probably is derived from nor-hyoscyamine. Peaks which appear at *m/e* 148 and 118 in the spectra of the two *N*-oxides are attributed to ions of composition O=C=C (CH_2OH) $C_6H_5^+$. ($C_9H_8O_2$) and O=C=CH. $C_6H_5^+$. (C_8H_6O) respectively.

The first discernible peak in the MS of the major isomer of (−)-hyoscine *N*-oxide appears at *m/e* 303 (M^+-16). The peak at *m/e* 138 has a relative abundance of 50% in the spectrum of the base but was the base peak in the MS of the hydrochloride. This peak can be assigned to an ion of structure **6a**, analogous to ion **5a** produced by hyoscyamine *N*-oxide. The MS of the hydrochloride showed peaks due to ions at *m/e* 303, 302 and 289 due to loss of O, OH and HCHO respectively. The ion which appeared at *m/e* 124 (90%), is attributed to an ion of structure **6b**. Hence the MS of the major isomers of hyoscyamine and hyoscine *N*-oxides show marked similarities. The MS of the hyoscine *N*-oxide major isomer was readily distinguished from that of the minor isomer since in the spectrum of the latter, peaks due to ions at *m/e* 138 and 124 were of much lower intensity.

Both isomers of hyoscyamine *N*-oxide and the major isomer of hyoscine *N*-oxide were obtained from fresh plant material and were characterized by their TLC behaviour, by reduction to the corresponding base and by their MS. The proportions of *N*-oxide to tertiary base varied between the different organs examined and also varied at different stages of development. Preliminary results indicate that this proportion is between 5 and 40% in *A. belladonna*, the highest *N*-oxide proportion being found in seeds of mature fruits. Considerable variations in the ratio of tertiary alkaloid to *N*-oxide have been noted in other plants and notably in *Senecio platyphyllus* [11]. Such variations in *N*-oxide to tertiary base ratio is an indication that the *N*-oxides are not artifacts and this was confirmed by control experiments in which hyoscyamine and hyoscine were subjected to the extraction methods; no *N*-oxides were produced. Despite the relatively high proportions present these *N*-oxides have not been detected previously in the Solanaceae and this is probably a direct result of the general method of isolation. Diethyl ether, in which the *N*-oxides are insoluble, is frequently used in extracting the alkaloids.

The function of the alkaloids and their associated *N*-oxides is not known. The MS of the *N*-oxides and their thermal decomposition show that they readily lose their oxygen to yield the tertiary alkaloid and that they are readily demethylated to the nor-alkaloids. It is possible that the alkaloids and their *N*-oxides are interconvertible in the plant or that the *N*-oxides may be involved in demethylation. Further studies of these possibilities are in progress.

EXPERIMENTAL

The 60 MHz and 100 MHz PMR spectra were determined in CD_3OD (hydrochlorides) or in $CDCl_3$ (bases), using TMS as internal reference; high resolution MS were determined at 70 eV. The TLC systems used were Si gel G with (A) EtOAc–*iso*PrOH–20%NH_4OH (45:35:15) for *N*-oxides and (B) Me_2CO–H_2O–conc NH_4OH (90:7:3) tertiary alkaloids. The alkaloids and their *N*-oxides were detected with Dragendorff's reagent.

Preparation of N-*oxides. General procedure.* Equal wts of alkaloid and *m*-chloroperbenzoic acid were stirred in $CHCl_3$ at 0° for 3 hr and then shaken with 10% aq. K_2CO_3 to remove any

excess acid. The $CHCl_3$ was washed with H_2O, dried and evaporated to dryness.

(−)-Hyoscyamine N-oxides. 200 mg Hyoscyamine yielded total *N*-oxide residue of 185 mg (88%). The residue was divided into Me_2CO soluble and insoluble portions and further purification was effected by preparative TLC (system A) or by recrystallization of hydrochlorides. The two isomeric *N*-oxides were obtained in a ratio of 3:2.

(−)-Hyoscine N-oxides. 380 mg Hyoscine yielded total *N*-oxide residue of 360 mg (82%). Recrystallization of the hydrochloride resulted in separation of the major isomer. Separation of the minor isomer was effected by preparative TLC (system A). The two isomeric *N*-oxides were obtained in a ratio of 3:1.

Characterization of prepared N-oxides. 1–2 mg of *N*-oxide was treated with 1 ml of 5% H_2SO_3 at 20° for 12 hr. TLC (systems A and B) indicated that each *N*-oxide resulted in only one alkaloid spot which corresponded to the tertiary base.

(−)-Hyoscyamine N-oxide, isomer 1, major isomer, hR_f 30, hydrochloride mp 165–170° (decomp.); PMR base (100 MHz) δ 7·27 (5H, *s*, aromatics), 5·02 (1H, *t*, H-3), 4·18 (1H, *t*, benzylic), 3·80 (2H, *m*, C$\underline{H}_2$OH), 3·30 (3H, *s*, N-Me, axial), 2·40 (2H, *m*, H-2 and 4, axial), 1·94 (2H, *m*, H-2 and 4 equatorial. PMR hydrochloride (60 MHz), δ 3·56 (3H, *s*, N-Me, axial). MS base (210°), *m/e* 305 (M^+, 1), 289 (M^+-16, 8), 288 (M^+-17, 0·7), 287 (1·4), 275 (1·4), 272 (8), 140 (35), 139 (15), 136 (21), 124 (100), 118 (20), 110 (28), 103 (49), 96 (56), 94 (63), 91 (42), 83 (63), 82 (77), 81 (21), 77 (56), 68 (35), 67 (49). MS hydrochloride (195°), *m/e* 305 (0·04), 303 (0·3), 289 (0·7), 275 (0·2), 148 (8), 136 (38), 124 (36), 118 (27), 110 (100), 103 (21), 91 (30), 82 (45), 77 (18), 68 (42). Accurate mass measurements, 303·1460, $C_{17}H_{21}NO_4$ requires 303·1470; 289·1681, $C_{17}H_{23}NO_3$ requires 289·1678; 124·1216, $C_8H_{14}N$ requires 124·1124; 110·0982, $C_7H_{12}N$ requires 110·0970; 148·0531, $C_9H_8O_2$ requires 148·0524; 136·0533, $C_8H_8O_2$ requires 136·0524; 118·0411, C_8H_6O requires 118·0419; 91·0541, C_7H_7 requires 91·0548.

(−)-Hyoscyamine N-oxide, isomer 2, minor isomer, hR_f 35, hydrochloride mp 175–180° (decomp.). PMR base (100 MHz) δ 7·26 (5H, *s*, aromatics), 5·08 (1H, *t*, H-3), 4·16 (1H, *t*, benzylic), 3·80 (2H, *m*, C$\underline{H}_2$OH), 3·12 (3H, *s*, N-Me, equatorial), 3·25 (2H, *m*, H-2 and 4 axial), 1·60 (2H, *m*, H-2 and 4 equatorial). PMR hydrochloride (60 MHz), δ 3·45 (3H, *s*, N-Me, equatorial). MS base (210°), *m/e* 305 (M^+, 5), 289 (M^+-16, 0·5), 287 (M^+-18, 0·3), 275 (M^+-HCHO, 0·24), 272 (2), 156 (4), 148 (4), 139 (55), 138 (16), 136 (55), 124 (16), 118 (50), 110 (38), 108 (28), 104 (38), 103 (28), 97 (28), 96 (55), 94 (28), 91 (72), 90 (33), 83 (38), 82 (100), 81 (38), 69 (38), 68 (100), 67 (44). MS hydrochloride (195°) *m/e* 305 (0·02), 303 (0·2), 289 (0·6), 275 (0·2), 148 (1), 136 (9), 124 (32), 118 (8), 110 (100), 103 (19), 91 (9), 82 (18), 68 (18). Accurate mass measurements, 303·1457, $C_{17}H_{21}NO_4$ requires 303·1470; 289·1690, $C_{17}H_{23}NO_3$ requires 289·1678; 275·1511, $C_{16}H_{21}NO_3$ requires 275·1521; 257·1420, $C_{16}H_{19}NO_2$ requires 257·1410; 110·0974, $C_7H_{12}N$ requires 110·0970.

(−)-Hyoscine N-oxide, isomer 1, major isomer, hR_f 20, hydrochloride mp 125–130° (decomp.). PMR hydrochloride (60 MHz), δ 7·32 (5H, *s*, aromatics), 5·07 (1H, *t*, H-3), 4·43 (2H, *m*, H-1 and 5), 4·22 (2H, *t*, H-6 and 7), 3·60–4·0 (3H, *m*, ar-C$\underline{H}$-C$\underline{H}_2$O), 3·45 (3H, *s*, N-Me axial). MS base (220°) *m/e* 303 (M^+-16, 5), 290 (1), 288 (2), 154 (10), 152 (10), 138 (50), 136 (45), 122 (60), 118 (35), 108 (35), 103 (65), 94 (100), 91 (65), 80 (65), 78 (65). MS hydrochloride (200°) *m/e* 317 (0·4), 303 (6·3), 289 (3·8), 286 (4·2), 152 (37), 148 (37), 138 (100), 136 (40), 124 (88), 122 (50), 118 (20), 108 (30), 103 (52), 94 (80), 91 (32), 80 (50), 78 (37).

(−)-Hyoscine N-oxide, isomer 2, minor, hR_f 45, hydrochloride-amorphous. PMR hydrochloride (60 MHz), δ 7·31 (5H, *s*, aromatics), 5·04 (1H, *t*, H-3), 4·30 (2H, *m*, H-1 and 5), 3·92 (2H, *t*, H-6 and 7), 3·30-3·90 (3H, *m*, ar-C$\underline{H}$-C$\underline{H}_2$O), 3·62 (3H, *s*, N-Me, equatorial). MS hydrochloride (190°), *m/e* 303 (1·6), 290 (1·5), 289 (1·3), 267 (29), 156 (16), 148 (43), 139 (33), 136 (66), 124 (59), 118 (43), 103 (100), 99 (36), 91 (66), 80 (35), 78 (57).

Thermal decomposition of N-oxides. A few crystals of base and of hydrochloride were heated in a capillary tube in a mp apparatus and on melting bubbles immediately formed (HCHO). Heating was continued until 200°, the residue was dissolved in $CHCl_3$ and examined by TLC (systems A and B). Spots corresponding to tertiary alkaloid and to nor-alkaloid were observed from the *N*-oxides of (−)-hyoscyamine (starting from base and from hydrochloride) and from the isomer 1 of (−)-hyoscine *N*-oxide. Some *N*-oxides remained when hyoscyamine *N*-oxides were heated but not when hyoscine *N*-oxide was heated.

Isolation of N-oxides from plant material. Fresh plant material was extracted in a blender with 5% NH_4OH in MeOH and maceration continued for 18 hr. The extract was filtered and concentrated under red. pres. to a semi-solid which was extracted into 2% H_2SO_4. The filtered acid extract was made alkaline with NH_4OH and extracted successively with $CHCl_3$ and $CHCl_3$–MeOH (9:1). All extracts were examined by TLC (system A) and the *N*-oxides were isolated by preparative TLC. Control extractions with tertiary alkaloids indicated that *N*-oxides were not formed by the isolation procedures.

Identification of natural N-oxides. The R_f's of the natural *N*-oxides were identical with those of the prepared compounds on TLC (system A) and on reduction with H_2SO_3 they gave spots with identical R_f's to the corresponding tertiary alkaloids (systems A and B). MS of natural hyoscyamine *N*-oxides 1 and 2 isolated from *A. belladonna* leaves and hyoscine *N*-oxide 1 isolated from *H. niger* leaves were identical with those of the prepared compounds.

Species and plant parts containing N-oxides. Atropa belladonna. Hyoscyamine *N*-oxides 1 and 2 were isolated from roots, stems, leaves, flowers, fruit pulp and seeds; hyoscine *N*-oxide 1 was isolated from the leaves. The ratio of *N*-oxide to tertiary base varies between each organ at different stages of growth and is between 5 and 40%, being highest in the seeds of mature fruits. *Hyoscyamus niger* and *Datura stramonium.* Hyoscyamine *N*-oxides 1 and 2 and hyoscine *N*-oxide isomer 1, were isolated from roots, stems, leaves, flowers, pericarp and seeds. *Mandragora officinarum.* Hyoscyamine *N*-oxides 1 and 2 were isolated from roots, stems and leaves, and fruits. *Scopolia lurida* and *S. carniolica.* Hyoscyamine *N*-oxides 1 and 2 and hyoscine *N*-oxide 1 were isolated from roots, leaves and stems.

Acknowledgements—We thank Mr D. Carter of The School of Pharmacy for determining the MS and we are grateful to Dr B. J. Millard for helpful discussions. The 100 MHz PMR spectra were determined by P.C.M.U. (Harwell) and the 60 MHz PMR spectra by Mr W. Baldeo of The School of Pharmacy. We thank Mr C. Smith of Myddelton House Gardens, Enfield, for growing the plant material, and Dr N. G. Bisset of Chelsea College, University of London, for helpful comments during the preparation of the manuscript.

REFERENCES

1. Bull, L. B., Culvenor, C. C. J. and Dick, A. T. (1968) *The pyrrolizidine alkaloids*, in *Frontiers of Biology*, Vol. 9. (Neuberger, A. and Tatum, E. L., eds.), North Holland, Amsterdam.
2. Phillipson, J. D. (1971) *Xenobiotica*, **1**, 419.
3. Phillipson, J. D. and Handa, S. S. (1973) *J. Pharm. Pharmac.* **25**, 117P.
4. Werner, G. and Schickfluss, R. (1971) *Ann. Chem.* **746**, 65.

5. Huber, C. S., Fodor, G. and Mandava, N. (1971) *Can. J. Chem.* **49,** 3258.
6. Bachmann, K. and von Phillipsborn, W. (1972) *Helv. Chim. Acta* **55,** 637.
7. Cymerman Craig, J. and Purushothaman, K. K. (1971) *J. Org. Chem.* **35,** 1721.
8. Fodor, G., Chastain, R. V., Frehel, D., Cooper, M. J., Mandava, N. and Gooden, E. L. (1971) *J. Am. Chem. Soc.* **93,** 403.
9. Parello, J., Longevialle, P., Vetter, W. and McCloskey, J. A. (1963) *Bull. Soc. Chim. France*, 2787.
10. Blossey, E. C., Budzikiewicz, H., Ohashi, M., Fodor, G. and Djerassi, C. (1964) *Tetrahedron* **20,** 585.
11. Areshkina, L. Ya. (1957) *Biokhimia* **22,** 487.