

Synthesis of 3-Oxo-4-aza-5 β -System from (25R)-3-Oxo-A-Norspirostane

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Azasteroids possess a wide range of biological activities. Neuromuscular blocking agents belonging to the class of azasteroids are bisquaternary ammonium compounds. On account of the importance of azasteroids, it was planned to synthesize a compound (4) having one nitrogen at position-3 in ring-A and the other nitrogen attached to the C-16 position. Diosgenin (12), a cheap precursor of basic steroid skeleton was used as the starting material, Diosgenin (12) was converted to (25R)-4-hydroxy-4-spirosten-3-one (13) by the known method. Benzilic acid rearrangement of the enol (13) offered the *t*-alcohol (14) which was oxidized to the ketone (15), thus reducing the size of ring-A. Oximation and Beckmann rearrangement of the oxime (16) offered lactam (17). Although, nitrogen at position-3 in ring-A could not be introduced by the designed scheme, it provided an efficient method for the preparation of 3-oxo-4-aza-5 β -steroids from a cheap starting material like diosgenin (12).

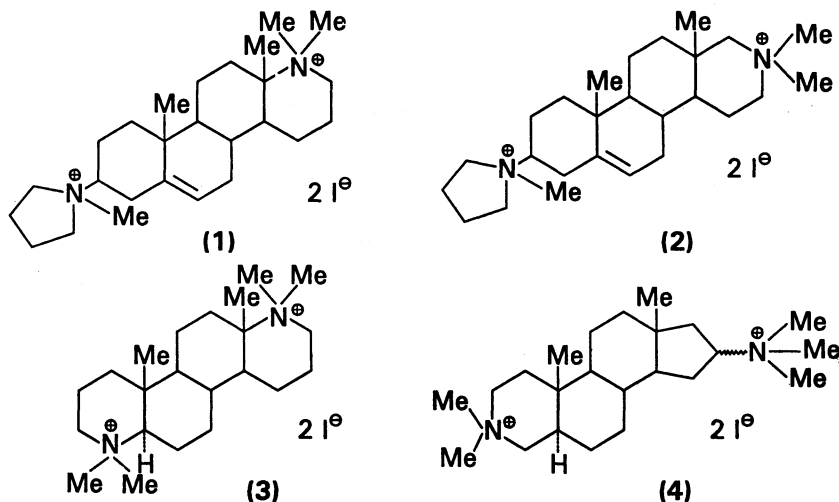
Key Words: Synthesis, Azasteroids, Norspirostane.

INTRODUCTION

Azasteroids have been reported to possess wide range of biological activities¹ like anabolic/androgenic, progestational, anti-progestational, aromatase and 5 α -reductase inhibiting, corticoidal, and neuromuscular blocking. We were interested in developing azasteroids possessing bisquaternary ammonium centres as potential neuromuscular blocking agents. Chandonium² (1) (renamed as candocurionium³) is an azasteroidal compound possessing one quaternary nitrogen attached to 3 β -position and the other at 17 α -position. The interonium distance has been reported⁴ to be 10.29 Å for chandonium. A chandonium analog (2) which possesses nitrogen at 17-position in place of 17 α -position has been reported⁵. This compound (2) has slightly higher interonium distance of 10.7 Å (Chem 3D Ultra 6.0 software⁶). Compound (2) has been found to be less active than chandonium (1). Another bisquaternary ammonium compound⁷ (3) with an interonium distance⁴ of 8.17 Å is not only less active but possesses undesirable

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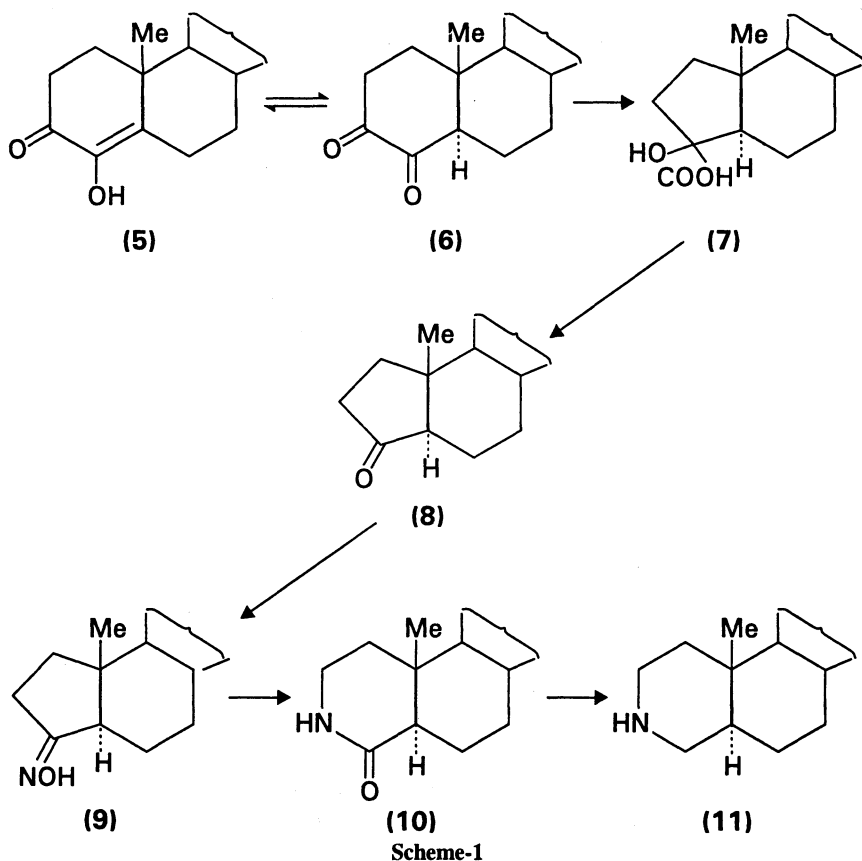
ganglion blocking activity^{8,9}. Optimal neuromuscular blocking activity is obtained in compounds having bisquaternary centres separated by a distance in the range of 10–11 Å, and the cationic heads should have large hydrocarbon environment to effectively deny acetylcholine access to the post-synaptic receptors¹⁰.



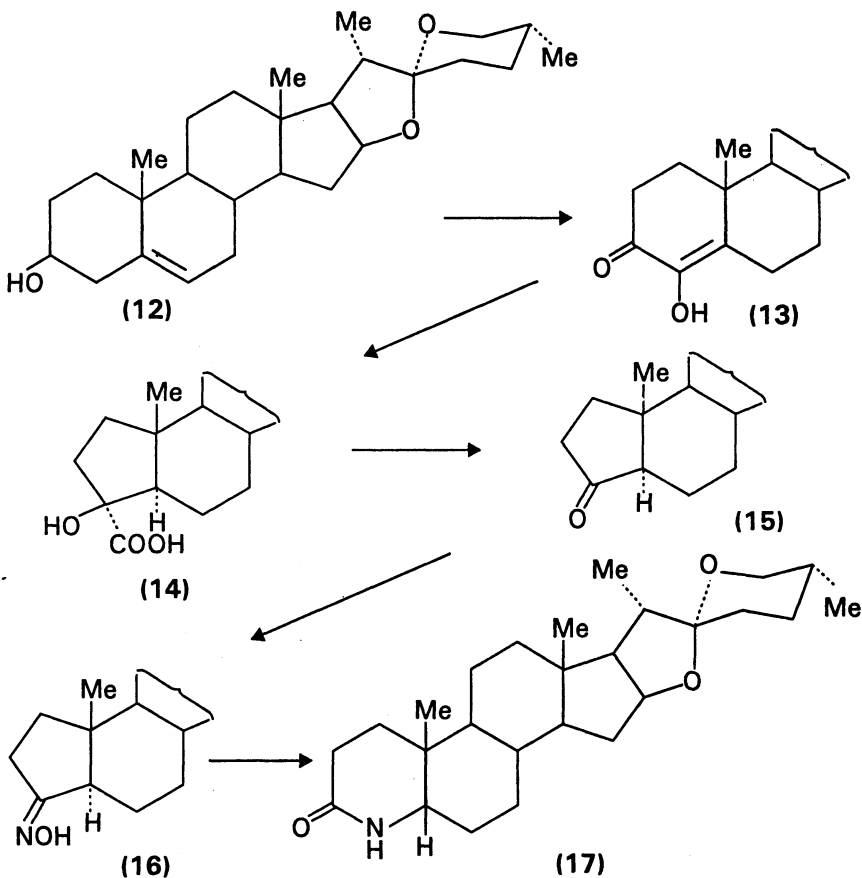
The interonium distance for compound (4) was determined to be 9.9 Å (for 16 α -) and 10.27 Å (for 16 β -) by Chem 3D Ultra 6.0 software⁶, which are very close to the interonium distance present in chandonium (1). In order to synthesize the target compound (4), nitrogens were to be introduced at 3-position in the steroid skeleton and the other linked to 16-position. A method for the attachment of nitrogen functionality at C-16 has already been reported⁵. But survey of literature revealed no simple or straightforward method for the preparation of 3-azasteroids. In this scheme of synthesis it was planned to carry out benzilic rearrangement of 4-hydroxy-4-en-3-one system (5) and tautomerize to the α -diketone (6) in basic medium. Oxidation of the benzilic acid rearranged tertiary alcohol (7) to the ketone (8) followed by oximation and Beckmann's rearrangement of the oxime (9) should theoretically provide compound (10). The lactam (10) could be reduced to the amine (11) to complete the job of introducing nitrogen at position-3 of ring-A (Scheme-1).

RESULTS AND DISCUSSION

In order to see the synthetic viability of the designed route, a cheaper starting material like diosgenin (12) was utilized. Diosgenin (12) was converted into (25R)-4-hydroxy-4-spirosten-3-one (13) by the reported procedure¹¹. Benzilic acid rearrangement of the keto enol (13) was performed using potassium hydroxide in *n*-pentanol to afford compound (14). It showed a band at 1710 cm^{-1} for (C=O stretching) and 3445 cm^{-1} (for O—H stretching) in IR spectrum. Signals appeared at δ 0.77 (s, 3H; 18- CH_3), 0.97 (s, 3H; 19- CH_3), 3.40 (m, 2H;



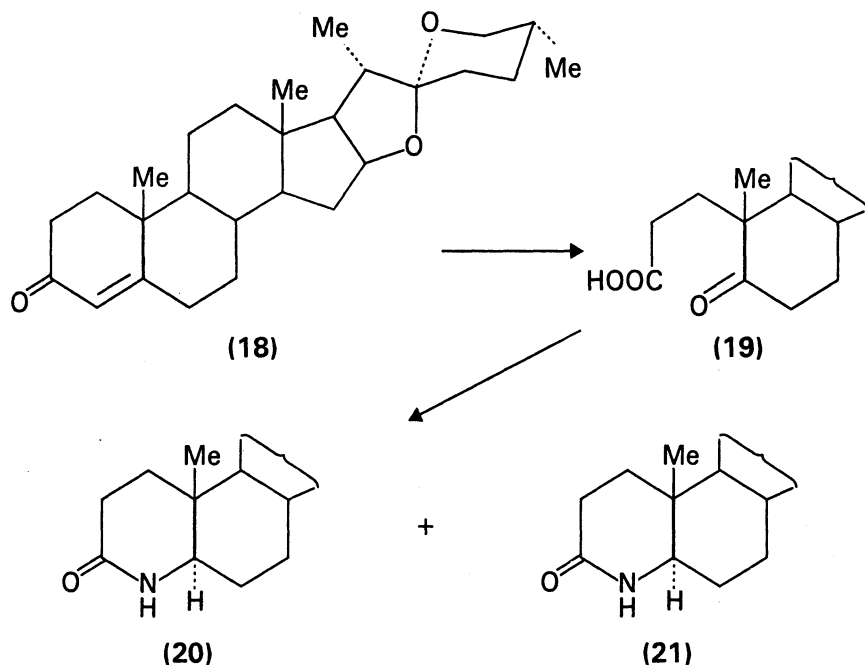
26-CH₃-O) and 4.30 (m, 1H, 16-CH-O) in PMR spectrum. Singlet due to the 4-OH in the compound (13) became absent in compound (14). It was tried to perform benzilic acid rearrangement in milder conditions like using potassium hydroxide and various alcohols like ethanol, *n*-propanol, *n*-butanol, *t*-butanol and ethylene glycol; and bases like sodium ethoxide/potassium *t*-butoxide in benzene/toluene systems. Unfortunately, starting material (13) was obtained as the major product under all these conditions. A very high concentration of potassium hydroxide in *n*-pentanol only under nitrogen atmosphere offered the best yields of the product (14). Oxidation of compound (14) with Jones's reagent¹² was performed to yield the five-membered ketone (15). It showed a strong band at 1744 cm⁻¹ in the IR spectrum. PMR spectrum gave signals at δ 0.77 (s, 3H; 18-CH₃), 0.78 (s, 3H; 19-CH₃), 3.40 (m, 2H; 26-CH₂-O) and 4.40 (m, 1H; 16-CH-O). The 5 α -configuration in compounds (14 and 15) and the configuration at 3-position in (14) are given on the basis of earlier observations^{13, 14}. Molecular ion peak at *m/z* 400 was obtained in the MS for compound (15). The ketone (15) was converted into the oxime (16) using hydroxylamine hydrochloride-sodium acetate in ethanol. The keto peak of compound (15) simply became extinct in the oxime (16). In order to obtain the desired system (10), Beckmann's rearrange-



Scheme-2

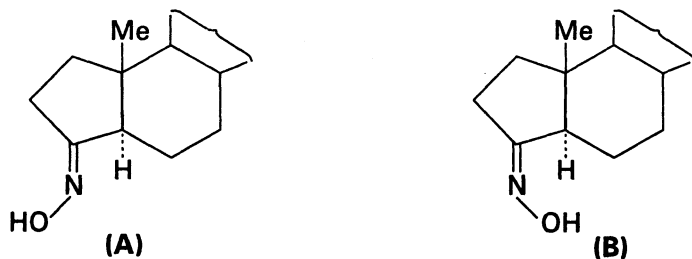
ment of the oxime was performed using thionyl chloride. Lactam yielded during the reaction was not the desired lactam (17). The structure of lactam (17) could be established on the basis of spectral data. Characteristic signals appeared at δ 2.42 (dd, 2H for 2- CH_2 -), 3.98 (t, 1H for 5- CH -) and 5.87 (b, 1H; exchangeable with deuterium for 4- NH) in the PMR spectrum. Other signals were at δ 0.80 (s, 3H; 18- CH_3), 0.98 (s, 3H; 19- CH_3), 3.47 (m, 2H; 26- CH_2 -O) and 4.43 (m, 1H; 16- CHO). IR spectrum gave a strong band at 1670 cm^{-1} for the lactam keto stretching vibrations. Although NMR data of the compound is in complete conformity with the 4-aza-3-keto-system as present in compound (17) and not with 3-aza-4-keto system as present in compound (10), the structure (17) was further confirmed beyond doubt by performing mixed melting point and Co-IR studies with the compound (20) obtained by the reported method¹⁵ as shown in Scheme-3.

Since the designed Scheme-1 did not lead to the formation of the desired system of 2-aza-4-keto in ring A (10), it could be assumed that the oxime (9) existed in the undesired anti-configuration (A). So, it was planned either to



SCHEME-3

prepare the oxime in *syn*-configuration (**B**) or to convert the anti-configuration (**A**) into the *syn*-configuration (**B**) by chemical means. Oximation of the keto (**15**) was performed under different conditions like heating it in presence of hydroxylamine hydrochloride with pyridine/collidine/ethylene glycol-potassium hydroxide. But, the products so isolated in all these reactions showed same physical characteristics as the oxime (**16**) and also they gave the same lactam (**17**) on Beckmann's rearrangements. Failure in obtaining this desired isomer of oxime (**B**) prompted us to try for conversion of the oxime (**A**) into its geometric isomer (**B**) by subjecting it to different acidic and basic conditions. The oxime (**16**) was heated separately in ethanolic solution containing conc. HCl and basic solution (10% KOH) and in basic ethylene glycol for a period of 24 h, without success in the desired conversion.



Although, the target of introducing nitrogen at C-3 position in ring-A to provide 3-azasteroids could not be achieved by the designed route, the method reported herein provides an efficient route for the preparation of 3-oxo-4-Aza-5 β -steroids.

EXPERIMENTAL

All melting points reported were taken on Veego make silicone oil bath-type melting point apparatus and are uncorrected. NMR spectra (on Varian 90 MHz) were recorded in CDCl_3 (unless specified) with TMS as internal reference (chemical shift in δ , ppm), IR spectra (Shimadzu FT-IR, 8300) in KBr (ν_{max} in cm^{-1}) and UV spectra (Shimadzu, UV-240) in methanol (λ_{max} in nm; figures within parentheses refer to $\log \epsilon$ values). Elemental analysis has been performed on Carlo-Erba elemental analyzer. Silica gel-G (E. Merck) was used for TLC and iodine vapours used for exposure of TLC plates. Anhydrous sodium sulphate (S.D. Fine Chemicals) was used as drying agent.

(25R)-3 β -Hydroxy-A-nor-5 α -spirostan-3-carboxylic acid (14): (25R)-4-Hydroxy-4-spirosten-3-one¹¹ (2 g) was dissolved in *n*-pentanol (100 mL). Potassium hydroxide (10 g) was added to the above solution and the reaction mixture refluxed for 36 h under an atmosphere of nitrogen. *n*-Pentanol was removed by steam-distillation, the reaction mixture cooled to 15°C, acidified with conc. hydrochloric acid and extracted with chloroform (3 \times 50 mL). The organic extract was dried and chloroform removed to obtain a semi-solid mass which was crystallized from methanol to afford the product (14) as a white solid (1 g; 48%); m.p. 248–50°C; IR (cm^{-1}) (ν_{max}): 3445, 1718, 1456, 1975, 1050 and 983; PMR (DMSO-d_6): 0.77 (s, 3H), 3.40 (m, 2H) and 4.30 (m, 1H). (Calcd. for $\text{C}_{27}\text{H}_{42}\text{O}_5$: C, 72.60; H, 9.48; found: C, 71.95; H, 9.73%).

(25R)-A-Nor-5 α -spirostan-3-one (15): (25R)-3 β -Hydroxy-A-nor-5 α -spirosten-3-carboxylic acid (14) (2 g) was dissolved in aldehyde-free acetone (30 mL). Jones reagent¹² was added dropwise to this solution below 20°C to maintain the colour of the reagent with constant stirring. The mixture was poured into water (150 mL) and extracted with chloroform (3 \times 50 mL). The chloroform extract was washed with water, dried and the solvent removed. The residue so obtained was crystallized from methanol to obtain the compound (15) (1.4 g; 78%), m.p. 194–96°C; IR (cm^{-1}) (ν_{max}): 1735, 1445, 1072 and 980; PMR: 0.77 (s, 3H), 0.78 (s, 3H), 3.40 (m, 2H) and 4.40 (m, 1H); MS: M^+ , m/z 400; Base peak at m/z 139 (other peaks at m/z 341, 328, 286, 271, 257, 115, 83, 69, 55) (Calcd. for $\text{C}_{26}\text{H}_{40}\text{O}_3$: C, 77.95; H, 10.07; Found: C, 77.57; H, 10.31%).

(25R)-3-Oximino-A-nor-5 ξ -spirostane (16): (25R)-A-Nor-5 α -spirostan-3-one (15) (1 g) was dissolved in aldehyde-free ethanol (40 mL). A solution of sodium acetate trihydrate (2 g) and hydroxylamine hydrochloride (1 g) in water (10 mL) was added to the above solution. After 2 h of refluxing the reaction mixture was gradually diluted with water and left to stand overnight. The crystals were separated by filtration to afford the oxime (16) (0.9 g, 87%); m.p. 220–222°C (d); IR (cm^{-1}) (ν_{max}): 3300, 1506, 1074, 1053, 981 and 886.

(25R)-4-Aza-5 β -spirostan-3-one (17): A solution of thionyl chloride (0.20 mL) in dioxan (7 mL) was added to a solution of (25R)-3-oximino-A-nor-5 ξ -spirostane (16) (0.5 g) in dry benzene (20 mL) and dioxan (5 mL) with stirring, maintaining the temperature below 15°C. The reaction mixture was stirred for 15 min. at 20–25°C, neutralized by addition of cold water (100 mL) and ammonia solution to the basic pH. Both the layers were separated, aqueous layer extracted with chloroform (3 \times 25 mL) and the combined organic layer washed with water

and dried. After removal of the solvent, the residue was crystallized from methanol to afford the lactam (**17**) (0.3 g, 60%); m.p. 301–302°C (d) [lit¹⁴ 299–302°C (d)]; IR (cm⁻¹) (ν_{\max}): 3185, 3020, 1669, 1313, 1240, 1072, 1051, 980 and 898; PMR: 0.80 (s, 3H), 0.98 (s, 3H), 2.42 (s, 2H), 3.03 (t, 1H), 4.43 (m, 1H) and 5.87 (b, 1H; exchangeable with D₂O) (Calcd. for C₂₆H₄₁NO₃: C, 75.13; H, 9.94; N, 3.37; Found: C, 74.50; H, 10.22; N, 3.36%).

Attempted conversion of Anti-form (A) of oxime (16) to syn-form (B)

Method A: The oxime (**16**) (0.5g) was dissolved in ethanol (25 mL). Conc. HCl (2 mL) was added to the reaction mixture and it was refluxed for 24 h, concentrated under vacuum and diluted with water (50 mL) to give a precipitate that corresponded with the oxime (**16**) in TLC.

Method B: 20% KOH (5 mL) was added to a solution of oxime (**16**) (0.5 g) in ethanol and the reaction mixture refluxed for 24 h on a water bath. The reaction mixture was concentrated under vacuum, diluted with water (50 mL), acidified with conc. HCl and filtered to obtain a product that was similar in TLC and melting point to the starting material (**16**).

Method C: The reaction was carried out as above except that ethanol was replaced with ethylene glycol and the reaction mixture was refluxed on oil bath for 24 h and processed as above to obtain a product which compared with the starting material (**16**) in TLC and melting point.

Products obtained from all the three methods (A, B and C) were subjected to Beckmann rearrangement as described above separately, to afford the same lactam (**17**).

REFERENCES

1. H. Singh, D.P. Jindal, M.R. Yadav and M. Kumar, in: G.P. Ellis and G.B. West (Eds.), *Heterosteroids and Drug Research in Progress in Medicinal Chemistry*, Vol. 28, Elsevier-North Holland-Biomedical Press, Amsterdam, p. 233 (1991).
2. H. Singh and D. Paul, *J. Chem. Soc., Perkin I*, 1475 (1974).
3. D.P. Jindal, *Indian J. Pharm. Edu.*, **30**, 45 (1996).
4. R.A. Palmer, M.A. Kalam, H. Singh and D. Paul, *J. Cryst. Mol. Struct.*, **10**, 31 (1980).
5. M.R. Yadav, *Indian J. Chem.*, **32B**, 746 (1993).
6. C.S. Chem. 3D 6.0 Molecular Modeling and Analysis, Cambridge Soft Corporation, Cambridge, USA, Reg. Code 7X62-L3FF-7767-2VG.
7. H. Singh, D. Paul and V.V. Parashar, *J. Chem. Soc. Perkin I*, 1204 (1976).
8. I.G. Marshall, D. Paul and H. Singh, *J. Pharm. Pharmacol.*, **25**, 441 (1973).
9. ———, *Eur. J. Pharmacol.*, **22**, 129 (1973).
10. A. Gringauz, *Introduction to Medicinal Chemistry: How Drugs Act and Why*, Wiley-VCH, New York, p. 377 (1997).
11. H. Singh and K.L. Bedi, *Indian J. Chem.*, **4**, 377 (1966).
12. K. Bowden, I.M. Heilbron, E.R.H. Jones and B.C.L. Weeden, *J. Chem. Soc.*, 39 (1946).
13. B. Camerino and V. Valeavi, *Gazz. Chim Italy*, **93**, 723 (1963); British Patent, 892, 170 (1962).
14. ———, *Gazz. Chim. Italy*, **93**, 735 (1963).
15. H. Singh, P.P. Sharma and R.B. Mathur, *Indian J. Chem.*, **11A**, 1254 (1973).