INTRODUCTION

The modified steroids are of considerable interest to medicinal chemists, since structural changes have led to the congeners possessing different biological activities. The organic chemists find the area fascinating as the preparative work itself is a challenge, and also for study of reaction mechanisms and stereochemistry the steroid nucleus provides a good base.

SYNTHETIC AZASTEROIDS

The heterosteroids constitute a class of modified steroids which has attracted a considerable attention. These may be divided into nucleo- and extranuclear categories. The former contain heteroatoms in the nucleus, and in the latter heteroatoms form part of a fused ring system, attached group or a side chain. The most prominent of the heterosteroids are azasteroids.

There are known hundreds of nucleo-heterosteroids which are purely of synthetic origin. The reviews by Morand and Lyall,¹ Gogte² and Huisman³ cover the relevant literature
on total synthesis of heterosteroids and analogues. For introduction of a heteroatom (or atoms) by total synthesis suitable mono- or bicyclic heterocyclic systems have been utilised to construct the heterosteroid skeleton, and the other approach is based on construction of heterocyclic component of the steroid nucleus through a sequence of special synthetic techniques.

The early selected references to the partial synthesis of heterosteroids were compiled by Toke's. For the synthesis of azasteroids the partial synthetic route employed most makes use of Beckmann rearrangement and the Schmidt reaction. The other procedures leading to azasteroids by partial synthesis have been listed.

Regarding heterosteroids in which the heteroatom(s) forms part of the ring attached to the steroid nucleus, a reference may be made to two reviews which cover literature in the area. Several synthetic heterosteroids are known in which heteroatom(s) is part of group or side chain attached to the steroid nucleus—the reviews by Martin-Smith and associates list selected cross references.

The literature on biological activity of heterosteroids, particularly azasteroids, has been the subject of some reviews. The research in certain instances in the synthesis of new heterosteroids has been the result of sound reasoning based on theoretical concepts of drug design.
There are known several synthetic heterosteroids which possess, for example, androgenic, anabolic, anti-inflammatory, ovulation inhibitory, antineoplastic, cholesterol biosynthesis inhibitory, CNS stimulant, anticonvulsant, neuromuscular blocking, ganglion blocking, analgetic, or antimicrobial activity.

Since the present thesis pertains mainly to the design of new azasteroid neuromuscular blocking agents, it is worthwhile to give a brief review of the work in the field done by other researchers.

AZASTEROIDAL NEUROMUSCULAR BLOCKING AGENTS

Two recent reviews on rational elements in the development of superior neuromuscular blocking agents and aspects of the pharmacology of aminosteroids include accounts of the azasteroid neuromuscular blocking agents. The following treatment traces the sequence of development of the agents and gives a brief description of the present state of our knowledge.

Though there are known many molecular features which effect the neuromuscular blocking activity, and there are pertinent alternative explanations, the potent neuromuscular blocking activity of certain bisquaternary salts is often explained in terms of a "two-point" attachment in which the cationic heads interact simultaneously with separate anionic sites of the acetylcholine receptors on the post-synaptic membrane. Earlier, the spatial separation of these anionic sites were considered in terms of the interonium distance of
the decamethonium molecule, and it was assumed that the decamethonium molecule interacted in the fully staggered conformation (interonium distance ca. 14 Å). However, conductimetric studies with polymethylene bisquaternary salts,\textsuperscript{23-27} indicated that extended conformation is not favoured in aqueous or ethanolic solution and the mean interonium distance is around 9.5 Å. These observations created doubts about the postulated optimal interonium distance of ca. 14 Å, and the latter assumption also lost validity from the observed high potencies in a number of bisquaternary salts not capable of an interonium distance that large.\textsuperscript{28-32}

In this context, the steroid nucleus as a supporting moiety for two cationic heads looked to be a proposition of good interest. The nucleus is relatively rigid and as such the spatial relationship between two onium centres would be more or less fixed and there will be a limited flexibility through conformational variations. That such compounds would possess appropriate hydrophilic to lipophilic ratio was apparent from discovery of neuromuscular blocking activity comparable to that of (+)-tubocurarine in the steroidal alkaloid malouetine (1)\textsuperscript{33,34} and its configurational isomers at C-3.
and C-20. In these a degree of variation in interonium distance, 11 to 12.5 Å, can be visualised due to free rotation of the side chain, and as such a study of the bisonium aza-steroids having both quaternary ammonium groups directly attached to the nucleus was considered worthwhile.

Martin-Smith and coworkers prepared a series of 3α,17α-bis(quaternary ammonium)-5α-androstanes (2), in which the interonium distance (9.2-10.6 Å) was near the favourable range, and steric hindrance to post-junctional binding by β-face angular methyl groups on C-10 and C-13 was excluded. These compounds showed activity, though less than tubocurarine. On the basis of studies on dipyramium chloride (3) and all the eight of its stereoisomers, tests being done in vivo on the cat or monkey sciatic nerve tibialis muscle preparation, it was seen that 3β isomers were in general more potent than the corresponding 3α compounds, and there was no general relationship between potency and interonium distances. On this basis, Bamford et al. tended to support the adumbration theory of Loewe and Harvey, who postulated a "one-point" attachment theory, where the bulk of the molecule, in this case presumably the steroid nucleus,
shields the receptor, rather than the suggestion of Cavallito and Gray and Waser that a two-point receptor complex could be formed. The relatively flat steroid nucleus in the 5α series may be more effective shield than the more folded nucleus in the 5β series, and therefore the 5α series should be more potent than the corresponding members of the 5β series, and that indeed was seen to be the case. By testing several monoquaternary androstane derivatives, Bamford et al. further support the "one-point" attachment theory, and again consider quaternary centre linked to position 3 to be more important than one attached to position 17 in determining potency in the series.

The activity of the steroidal quaternary compounds examined has been of non-depolarizing type. It was also so with the quaternary salts (4) derived from the alkaloid conessine. In the cat most of these compounds possessed short-acting muscle relaxant properties; block duration was shorter, and cumulation was less than with gallamine or (+)-tubocurarine. The bisquaternary compounds reported possess the interonium distance of 10.1 Å. Seven of the eight 3-mono
quaternary compounds tested were also potent neuromuscular blocking agents; this observation may not be taken as a convincing evidence for "one-point" attachment since the second nitrogen could get protonated in the system and thus provide the second cationic head.

Though there is lively discussion as to the mode of post-synaptic attachment, interest in the steroid quaternary compounds continues. Certain drugs thus evolved have shown promise on clinical testing. These include dipyridamol chloride (3),^47 N,N-dimethylconessine (4; R=H=-CH₃),^48 and pancuronium bromide (5).^49-52 Particularly pancuronium bromide (Pavulon, N.V. Organon) has become to be marketed and is advocated for use in clinical situations where a non-depolarizing muscle relaxant of medium duration of action is required, due to its high potency with minimal side effects. The details about pancuronium bromide and related compounds have been published recently;^53 a brief description may be given about its design and ancilliary aspects.

A programme on the synthesis and pharmacological study of 2β-amino-3α-hydroxy-5α-androstanes and derivatives^54 and
the corresponding 3α-amino-2β-hydroxy isomers, led to the observation that the corresponding monoguatemary salts possessed neuromuscular blocking activity; the most potent of the series, 3α-acetoxy-2β-piperidino-5α-androstan-17-one methobromide (6) has 1/16th the potency of (+)-tubocurarine. The 2β-piperidino and 3α-acetoxy groups are both considered to be pseudoequatorial due to the twisted boat conformation of ring A.\(^5^4\) In this preferred conformation which may be rigid due to steric compression,\(^5^6\) one may consider it to have ring A substituents in specific molecular conformation akin to the neurotransmitter acetylcholine (7), and thus (6) may be expected to occupy the transmitter’s site of action and neuromuscular transmission. As the monoquaternary analogue (6) had only a low activity, it was thought that a bisquaternary azasteroid may be potent and pancuronium bromide (5) got to be ultimately synthesised\(^5^3\) and tested.\(^5^7\) Here also the 16- and 17- substituents are pseudoequatorial.

Structure-activity studies\(^5^3\) on pancuronium bromide and other steroidal neuromuscular blocking agents containing acetylcholine fragments indicated that for high potency it is probably essential to have two nitrogen atoms in the molecule
and at least one of these nitrogen atoms should be quaternized. X-ray crystallographic studies with pancuronium bromide reveal the actual interonium distance in the solid state to be 11.08 Å as against 10.6 Å calculated from Dreiding models. The molecule has certain rigidity and unique hydrogen bonding systems, involving quasi-six-ring formation (C-C-N⁺-C-H...O-COCH₃), within each of the molecules acetylcholine-like fragments. The high potency and specificity of action of the agent at a neuromuscular receptor site may be associated with the particular molecular geometries and electronic structures of the acetylcholine-like moieties in the molecule.

A relative of pancuronium bromide, dacuronium bromide (2β,16β-dipiperidino-5α-androstane-3α,17β-diol 3α-acetate dimethobromide) on clinical studies showed that it possessed a rapid onset and shorter duration of action than that of pancuronium bromide but it lacked sufficient potency to be a clinically useful drug.

RESEARCH ENVISAGED

The above survey indicates the success achieved in the design of azasteroidal neuromuscular blocking agents. There is interest in the development of short-acting neuromuscular blocking agents and this need has been frequently stated, and studies in this direction continue. The azasteroid field holds promise for development of such agents.

At the Panjab University Department of Pharmaceutical Sciences there are in progress synthetic studies in the area
of heterosteroids for over a decade. It was considered of interest to develop a programme of work aiming at the design of neuromuscular blocking azasteroids. Preparation of bis-onium steroids with one or both of the cationic systems as part of the steroid ring skeleton at different interonium distances was envisaged, so that the cationic heads, one or both, may be in a more rigid setting.

This thesis embodies mostly the work carried out in the design of new neuromuscular blocking agents. Certain mono-quaternary azasteroids have also been prepared which are potent ganglionic blocking agents.

Reports on the studies carried out on the application of Vilsmeier-Haack reaction to steroid lactams and synthesis of a steroidal tetrazole system are also included.