Abstract:
The invention discloses a novel process for quaternization of (2P,3A,5A,16P,17P)-2,16-bis(piperidino)-3,17-diacetoxy-5-androstan-3-one with methyl bromide in presence of a cyclic ether. The invention further discloses purification of quaternary salt to provide highly pure vecuronium bromide with unspecified impurity level not more than 0.1%.
"Novel process for quaternization of (2p,3a,5a,16p,17p)-2,16-bispiperidino-3,17-diacetoxy-5-androstane"

Technical Field:

The present invention relates to a novel process for quaternization of (2β,3α,5α,16β,17β)-2,16-bispiperidino-3,17-diacetoxy-5-androstane with methyl bromide in presence of a cyclic ether. The invention also relates to purification of quaternary salt to provide vecuronium bromide with unspecified impurity level less than 0.1%.

Background and Prior art:

Vecuronium bromide (1-[(2β,3α,5α,16β,17β)-3, 17-bis(acetyloxy)-2-(piperidin-1-yl)androstan-16-yl]-1-methylpiperidinium bromide) is a valuable drug having action as a muscle relaxant in the category of non-depolarizing blocking agents. It is an aminosteroidal competitive neuromuscular blocker. Vecuronium is used to promote skeletal muscle relaxation during surgery to aid controlled respiration by increasing pulmonary compliance, and to facilitate endotracheal intubation. Vecuronium has minimal histamine-release and is less likely to cause bronchospasm or cardiac adverse effects than neuromuscular blockers with significant histamine-releasing properties such as Atracurium, Mivacurium, Succinylcholine, and Tubocurarine.

Vecuronium bromide has following structure:

![Vecuronium Structure](image)

The structure of Vecuronium is derived from the same aminosteroid structure as pancuronium, but missing the methyl group on the piperidine nitrogen that is attached to the 'A' ring making it monoquaternary. It has the same configuration at all ten stereocentres as Pancuronium and is a single-isomer preparation.
Pancuronium bromide has following structure:

The final step in the manufacture of vecuronium bromide is quaternization of 
\[(2\beta,3\alpha,5\alpha,16\beta,17\beta)-2,16\text{-bispiperidino}\-3,17\text{-diacetoxy}-5\text{-androstan\-e} \] with methyl bromide. Till now the quaternization of androstan compounds has been carried out in various organic solvents such as diethyl ether, dichloromethane, acetonitrile, acetone or nitromethane. The preferred solvents for quaternization in the manufacture of vecuronium bromide were diethyl ether, acetone and acetonitrile, since it was considered to have desirable solubility and boiling point characteristics.

J. Med. Chem., vol. 16, issue 10, pages 1116-1124 (1973) discloses preparation of vecuronium bromide where the quaternization of \[(2p,3a,5a,16p,17P)-2,16\text{-bispiperidino}-3,17\text{-diacetoxy}-5\text{-androstan\-e} \] with methyl bromide was carried in diethyl ether.

The patent CN101684139 discloses a process for preparation of vecuronium bromide where the quaternization of \[(2p,3a,5a,16p,17P)-2,16\text{-bispiperidino}-3,17\text{-diacetoxy}-5\text{-androstan\-e} \] with methyl bromide was carried in diethyl ether and acetone. It was observed that use of diethyl ether results in more amount of impurity formation.

The process of quaternization of \[(2P,3a,5a,16p,17P)-2,16\text{-bispiperidino}-3,17\text{-diacetoxy}-5\text{-androstan\-e} \] with methyl bromide, was disclosed in Chinese J. Med. Chem., vol. 16, issue 4, pages 233 to 235 (2006). The reaction was carried out in acetonitile for 40 hours; followed by distillation, washing with propanol-ethyl acetate, and column chromatography to provide vecuronium bromide in 53.2% yield. Thus the reaction time
which was 40 hours, column chromatography and low yield were the main disadvantages of this process.

In the manufacture of drugs for human therapy high standards of purity have to be met and the manufacture to meet the required specification is often costly. Thus it is important in any of the steps of such manufacture to keep costs as low as possible without compromising on yield, purity of product, and ease on manipulation.

Like any synthetic compound vecuronium bromide can contain extraneous compounds or impurities that can come from any source. The impurities can be in the form of unreacted starting materials, by-products of the reaction, products of side reactions or degradation products. Impurities in vecuronium bromide are undesired and might even be harmful to a patient being treated with a dosage form containing the same.

The reported method for purification of vecuronium bromide is via column chromatography. Purification via column chromatography is not suitable for industrial application. Hence, presently there are no convenient and industrially viable methods for purification of vecuronium bromide.

Needless to say it is advantageous to develop cost effective and high yielding process for quatemization of \((2p,3a,5a,16p,17p)-2,16\)-bispiperidino-\(3,17\)-diacetoxy-5-androstane and purification of quaternary salt to provide highly pure vecuronium bromide with unspecified impurity level not more than 0.1%.

**Object of the invention:**

It is therefore an object of the invention is to overcome or ameliorate at least one disadvantage of the prior art or to provide a useful alternative.

Another object of the invention is to provide a novel process for quatemization of \((2p,3a,5a,16p,17P)-2,16\)-bispiperidino-\(3,17\)-diacetoxy-5-androstane in easily available
and inexpensive solvent in which the solubility of the androstane compound is much better than known solvents.

Yet another object of the invention is to provide a concise and industrially applicable process for purification of vecuronium bromide to provide highly pure vecuronium bromide with unknown impurity level not more than 0.1% and having desirable pharmacological activity, broad safety margins, without toxicity or unfavourable side effects.

Summary of the invention:

In accordance with the above objectives, the present invention provides an economic and industrially feasible process for quaternization of (2p,3a,5a,16p,17β)-2,16-bis(piperidino)-3,17-diacetoxy-5-androstane with methyl bromide in presence of cyclic ether to provide vecuronium bromide.

According to another aspect, the present invention provides a process for purification of crude vecuronium bromide to provide highly pure vecuronium bromide with unspecified impurity level not more than 0.1%.

Detailed description of the invention:

Unless specified otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art, to which this invention belongs. Although any methods and material or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods and materials are described. To describe the invention, certain terms are defined herein specified as follows:

Unless stated to the contrary, any of the words 'having', 'including', 'includes', 'comprising' and 'comprises' mean 'including without limitations' and shall not be construed to limit any general statement that it follows to the specific or similar items or matters immediately following it. Embodiments of the invention are not mutually exclusive, but may be implemented in various combinations. The described embodiments
of the invention are given for the purpose illustration rather than limitation of the invention as set forth the appended claims.

Accordingly, the present invention provides a process for quatemization of androstane compound of formula (I) with methyl bromide in presence of cyclic ether to provide vecuronium bromide of formula (II).

![Formula (I)](image1)

![Formula (II)](image2)

The process of the present invention is advantageously carried out by reacting the androstane compound of formula (I) with methyl bromide in presence of tetrahydrofuran (THF). The solvent THF shows excellent solubility for \((2\beta,3\alpha,5\alpha,16\beta,17\beta)-2,16\)-bispiperidino-3,17-diacetoxy-5-androstane, which is much better than any of the known solvents used for quatemization of above mentioned androstane derivative.

In a preferred embodiment the process of the present invention is carried out by passing gaseous methyl bromide into androstane compound of formula (I) in THF. The resulting reaction mixture was stirred to obtain a precipitation of vecuronium bromide.
Methyl bromide is conveniently purged in an amount, relative to the androstane compound of formula (I), preferably in a range between 1 to 10 moles, more preferably in a range between 3 to 7 moles. The most preferred quantity of methyl bromide is 5 to 6 moles.

The process of the present invention may be carried out at suitable temperature. To minimize the decomposition of products and impurity formation the reaction is carried out at 10°C to 50°C, more preferably at 15°C to 35°C. The most preferred reaction temperature is 25°C to 30°C.

The process of quatemization normally completes in a span of 10 to 20 hours, more preferably in a span of 12 to 13 hours.

On completion of the reaction vecuronium bromide can be recovered from the reaction mass in a convenient manner. The workup of the reaction involves washing the precipitate of vecuronium bromide with non polar solvent, medium polar solvent or mixture thereof. The solvent used for washing the precipitate of vecuronium bromide is selected from ether, hexane or benzene. The preferred solvent for washing the precipitate of vecuronium bromide is ether such as THF.

The process provides quantitative yield of vecuronium bromide in one lot which is not reported in the literature. The formation of bis-quaternary salt is not observed following this process.

In the second embodiment the present invention provides a process for purification of crude vecuronium bromide comprising the following steps:

a. dissolving vecuronium bromide in at least one water immiscible solvent; and
b. precipitating the resultant solution from at least one anti solvent.

The water immiscible solvent used for dissolving vecuronium bromide is selected from dichloromethane, dichloroethane, chloroform, ether, ethyl acetate or mixture thereof. The preferred solvent for dissolving crude vecuronium bromide is dichloromethane.
The anti solvent used for precipitation is THF.

The process of purification of vecuronium bromide further comprises washing the solution obtained in the step 'a' with water; followed by distilling the solvent.

The process of purification of vecuronium bromide further comprises adding at least one polar solvent to the solution obtained in the step 'a'. The polar solvent is selected from ethyl acetate, isopropyl acetate, acetonitrile or mixture thereof.

Thus the purification process of the present invention is highly cost effective and simple.

The other advantage of the purification processes of the present invention is that 1-[2β,3α,5α,16β,17β]-2,16-bispiperidino-3,17-bis(acetoxy)-2-(piperidin-1-yl)androstan-16-yl)methylpiperidinium hydrobromide, if formed as a by-product, is highly soluble in water.

The purified vecuronium bromide has HPLC purity of greater than 99% and contains not more than 0.1% of any unknown impurity.

The pure vecuronium bromide obtained by the process of the invention may be formulated into a dosage form by combining with one or more pharmaceutically acceptable excipients using known techniques. Further the dosage form may be immediate release or extended release.

Further details of the process of the present invention will be apparent from the examples presented below. Examples presented are purely illustrative and are not limited to the particular embodiments illustrated herein but include the permutations, which are obvious as set forth in the description.

**Examples:**

**Example 1**

A 100 ml round bottom flask was charged with (2p,3a,5a,16p,17β)-2,16-bispiperidino-3,17-diacetoxy-5-androstane (2.7 gm) and THF (32 ml) at room temperature. Methyl
bromide gas (5 moles) was purged at the same temperature and the reaction mixture was stirred for 12 hours. The solid was collected by filtration and dried under vacuum to provide vecuronium bromide (2.5 gm).

Example 2
Purification of vecuronium bromide

In a 100 ml round bottom flask crude vecuronium bromide (2 gm) was dissolved in dichloromethane (20 ml). The resulting solution was washed with distilled water. The organic layer evaporated under vacuum and oil was precipitated with THF (20 ml). The solid was collected by filtration and dried under vacuum to provide vecuronium bromide (yield 1.6 gm, HPLC purity 99.83%, any unspecified impurity not more than 0.1%).

Example 3
Purification of vecuronium bromide

A 100 ml round bottom flask was charged with dichloromethane (8 ml), vecuronium bromide (2 gm) and acetonitrile (4 ml). The resulting solution was charcolized, dichloromethane was evaporated under vacuum and the resulting oil was precipitated with THF. The solid was collected by filtration and dried under vacuum to provide vecuronium bromide (yield 1.8 gm, HPLC purity 99.89%, any unspecified impurity not more than 0.1%).
We claim,

1. A process for quatemization of (2p,3a,5a,16p,17P)-2,16-bispiperidino-3,17-diacetoxy-5-androstane of formula (I)

![Formula (I)](image)

comprising reacting the compound of formula (I) with methyl bromide in presence of cyclic ether.

2. The process as claimed in claim 1, wherein the cyclic ether is tetrahydrofuran.

3. The process as claimed in claim 1, wherein the process is carried out at 10°C to 50°C.

4. The process as claimed in claim 3, wherein the process is carried out at 25°C to 30°C.

5. The process as claimed in claim 1, wherein the compound of formula (I) is reacted with 1 to 10 moles of methyl bromide.

6. The process as claimed in claims 1 and 5, wherein the compound of formula (I) is reacted with 3 to 7 moles of methyl bromide.

7. The process as claimed in claim 6, wherein the compound of formula (I) is reacted with 5 to 6 moles of methyl bromide.

8. The process as claimed in claim 1, wherein the process is carried out for 10 to 20 hours.
9. The process as claimed in claim 8, wherein the process is carried out for 12 to 13 hours.

10. The process as claimed in claims 1 to 9, wherein the process provides vecuronium bromide.

11. A process for purification of vecuronium bromide comprising,
   a. dissolving vecuronium bromide in at least one water immiscible solvent to provide a solution; and
   b. precipitating the resultant solution from at least one anti solvent.

12. The process for purification of vecuronium bromide as claimed in claim 11, wherein the water immiscible solvent is selected from dichloromethane, dichloroethane, chloroform, ether, ethyl acetate or mixture thereof.

13. The process for purification of vecuronium bromide as claimed in claims 11 and 12, wherein the water immiscible solvent is dichloromethane.

14. The process for purification of vecuronium bromide as claimed in claim 11, wherein the anti solvent is selected from ethers.

15. The process for purification of vecuronium bromide as claimed in claims 11 and 14 wherein the anti solvent is tetrahydrofuran.

16. The process for purification of vecuronium bromide as claimed in claim 11, wherein the process further comprises washing the solution obtained in the step 'a' with water; followed by distilling the solvent.

17. The process for purification of vecuronium bromide as claimed in claim 11, wherein the process further comprises adding at least one polar solvent to the solution obtained in the step 'a'.
18. The process for purification of vecuronium bromide as claimed in claim 17, wherein
the polar solvent is selected from ethyl acetate, isopropyl acetate, acetonitrile or
mixture thereof.

19. The process for purification of vecuronium bromide as claimed in claims 17 and 18,
wherein the polar solvent is acetonitrile.
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

INV. C07J75/00

ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C07J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BEI LSTEIN Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

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Further documents are listed in the continuation of Box C.

See patent family annex.

Date of the actual completion of the international search: 29 February 2012

Date of mailing of the international search report: 06/03/2012

Name and mailing address of the ISA:
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Tabanel La, Stefania
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