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STABLE WATER SOLUBLE SALTS OF OESTRONE SULFATE AND PROCESS OF MAKING SAME

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This invention relates to novel stable water soluble salts of oestrone sulfate. More particularly, the invention concerns new oestrone sulfate salts derived from organic bases, and to the method of their preparation.

It is known that the alkali metal salts of oestrone sulfate, which as water soluble derivatives of oestrone possess considerable industrial importance in the manufacture of pharmaceutical and cosmetic preparations, cannot be preserved as such for prolonged periods of time, because these salts, even when stored in the anhydrous state and with careful exclusion of moisture, spontaneously decompose with liberation of acid, after a brief period of time.

Attempts have been made previously to increase the storability of oestrone sulfate by transforming it into other salts, such as salts of organic bases. All efforts in this direction, even after seemingly promising beginnings have ultimately proved to be failures.

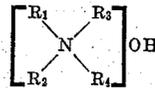
Only certain salts of oestrone sulfate with quaternary ammonium bases did in fact exhibit good stability on storage. Unfortunately, the solubility in water of these stable salts was insufficient for industrial applications of the type previously mentioned; the solubility was, in fact, so limited, that these same salts served admirably as a means of precipitating the oestrone present in urine in very low concentrations. On the basis of this previous work the view was widely held in the art that quaternary salts of oestrone sulfate were to be considered as having a general characteristic of low solubility.

In accordance with the present invention, it was found unexpectedly, and in contradiction of the foregoing assumption concerning solubility, that certain new and previously undescribed salts of oestrone sulfate with quaternary ammonium bases possessed both the properties of excellent stability and surprisingly high water solubility. The new quaternary ammonium bases which possess these advantages are those which contain alkyl groups attached to the quaternary nitrogen atom, instead of aryl or aralkyl groups as in the case of the previously used bases.

It has been found in accordance with this invention that the presence of a bivalent saturated alkyl radical attached to the nitrogen atom imparts markedly increased solubility. The second bond of such alkyl radical may also be attached to the quaternary nitrogen atom to form a heterocyclic ring. The bivalent alkyl radical may impart even greater increase in water solubility when it is interrupted by the presence of other heteroatoms, such as, for example, oxygen or nitrogen atoms. An additional increase in solubility is obtained through the presence of a hydroxy-group in one of the other alkyl-groups attached to the quaternary nitrogen atom.

Thus, the quaternary ammonium base which is used is one having the general formula

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5 wherein R_1 , R_3 and R_4 are alkyl radicals, R_2 is a member of the group consisting of alkyl radicals and hydroxy alkyl radicals; and R_1 and R_2 are each alkyl, R_3 and R_4 may be bivalent alkylene radicals joined to the quaternary N atom by one valence, and joined together by 10 other valences to form a member of the group consisting of a heterocyclic ring, and an oxygen containing heterocyclic ring.

The improvement in solubility resulting from the formation of the novel quaternary ammonium base derivatives of oestrone sulfate in accordance with the present invention will be readily apparent from the solubility properties of several of these new compounds as set forth in the following table:

TABLE 1

	Quaternary Ammonium Base	Water solubility of the corresponding Oestrone Sulfate salt in percent by weight at 23° C.
1	$[(CH_3)_4N]OH$	1.7
2	$[CH_3-N \langle \text{benzene ring} \rangle]OH$	2.5
3	$[HO.C_2H_4-N(CH_3)_3]OH$	6
4	$[CH_3-N \langle \text{pyrrolidine ring} \rangle]OH$	6.7
5	$[CH_3-N \langle \text{piperidine ring} \rangle]OH$	8.0
6	$[CH_3-N \langle \text{piperazine ring} \rangle]OH$	20
7	$[HO.C_2H_4-N \langle \text{piperazine ring} \rangle]OH$	36

By way of comparison, there are given the solubilities of two typical examples of salts of arylated or aralkylated ammonium bases:

8	$[\langle \text{benzene ring} \rangle N(CH_3)_3]OH$	0.35
9	$[\langle \text{benzene ring} \rangle CH_2N(CH_3)_3]OH$	0.25

Among the quaternary ammonium bases which may be successfully used in preparing the novel oestrone sulfate derivatives of this invention there may be mentioned: N-(β -hydroxyethyl)-N-methyl-morpholinium hydroxide, N-methylpyridinium hydroxide, N- β -hydroxyethyl-N-methylpiperidinium hydroxide, tetramethylammonium hydroxide, and N- β -hydroxyethyl-N-methylpyrrolidinium hydroxide, but it will be understood that any base of this type which has the previously indicated characteristics may be used.

The oestrone sulfate starting material may be prepared by any suitable method, but it has been found preferable to proceed in either of two ways:

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Method A.—1.0 g. of oestrone is converted by heating under reflux for 1 hour with 1.18 g. of pyridine-SO₂ adduct (prepared from freshly distilled chlorosulfonic acid or stabilized SO₂) and dry pyridine in 12 ml. of chloroform. Finally the chloroform and the pyridine are removed by distillation and the oily residue is worked up with absolute ether. The solidified precipitate is separated from the ether by decantation, dissolved in 100 ml. of methanol, filtered, and the solution used for the preparation of the salts described in Examples 1, 2, and 3, below.

Method B.—10 g. of oestrone are converted into the sulfate by heating with stirring with 11 g. aminosulfonic acid dissolved in 50 cc. of pyridine for approximately 20 minutes at about 100° C. under an atmosphere of nitrogen. After distilling off the pyridine in vacuo, the residue is dried in vacuo at 70° C. There is obtained 24 g. of material (instead of the calculated 21 g.). The 24 g. are ground in a mortar with 150 cc. of methanol for ½ hour, filtered by suction and washed in portions with 60 cc. of methanol. The total methanol solution amounted to 210 cc. and was divided into 3 equal portions for further treatment, in accordance with Examples 4, 5, and 6, below.

The method of preparing the novel quaternary ammonium salts of oestrone may be illustrated by the following examples, but the invention is not to be considered as limited thereto.

Example 1.—*N*-(β -hydroxyethyl)-*N*-methyl-morpholinium salt of oestrone sulfate

To 50 ml. of the methanol solution of oestrone sulfate, prepared as described under method A, and corresponding to 500 mg. of oestrone, there is added about 0.5–2 cc. of a methanol solution of *N*- β -hydroxyethyl-*N*-methyl-morpholinium hydroxide until a pH of 11.0 is reached. A precipitate which formed is filtered off. The filtrate is adjusted to about 20 ml. and a little ether is carefully added thereto. An oil is precipitated, which after settling, may be separated from the supernatant liquid by decantation, whereupon the solution is treated again with ether. There occurs a turbidity, which soon is transformed into crystals of the *N*-(β -hydroxyethyl)-*N*-methyl-morpholinium salt, having the following properties:

Yield 480 mg., M. Pt. 135–142° C., $[\alpha]_D^{+84}$ C. in H₂O. Solubility in water, 20%.

From the precipitated oil and the filtrate, by renewed solution in methanol after separating a precipitate by means of ether, further significant amounts of the oestrone sulfate salt may be recovered.

Example 2.—*N,N*-dimethyl-morpholinium salt of oestrone sulfate

To 50 ml. of oestrone sulfate solution (method A), corresponding to 500 mg. of oestrone, there is added, as previously described, a methanol solution of *N,N*-dimethyl-morpholinium hydroxide until a pH of 10.2 is reached. The solution is then concentrated in vacuo to 20 ml. and treated with ether. There results a turbidity, from which crystals are formed of the *N,N*-dimethylmorpholinium salt of oestrone sulfate, which have the following properties:

Yield 445 mg., M. Pt. 173–176° C., $[\alpha]_D^{+91}$ C. in water. Solubility in water at 25° C. 8%.

Example 3.—Tetramethylammonium salt of oestrone sulfate

A solution of 2 g. of oestrone sulfate prepared as described under method A is treated with a solution of tetramethylammonium hydroxide in methanol, until a pH value of 9.2 is reached. After standing for several hours (no precipitation) the solution is treated with an equal amount of ether, the resulting precipitate is filtered by suction, and washed with a 1:1 ether-methanol mixture. There is obtained as a primary crystallization

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product 1.96 g. of the tetramethylammonium salt, having a M. Pt. of 229–230° C. (decomposition), yield 62.7% of theory. The mother liquor is again treated with the same volume of ether and yields as a second crystallization product 0.86 g. having a melting point of 232–237° C., corresponding to 27.5% of theory, total yield 90.2% of theory. The mother liquor is evaporated to dryness, taken up with 8 cc. of methanol, and precipitated with ether, whereby there is obtained as a third crystallization product 0.1 g. of material having a M. Pt. 232–234° C., corresponding to 3.19% of theory. The total yield is thus 93.4% of theory.

By hydrolysis of the mother liquors with dilute sulfuric acid there is recovered 0.96 g. of oestrone having a M. Pt. of 250–254° C., corresponding to 4.8% of theory.

The solubility of the oestrone salt in water is 1.6–1.7% at room temperature; the $[\alpha]_D^{20}$ is 96.9° C. in water.

Example 4.—*N*-methyl-pyridinium salt of oestrone sulfate

To 70 cc. of methanolic oestrone sulfate solution prepared in accordance with method B above, and corresponding to 3.3 g. of oestrone, there is added 63 cc. of a methanol solution of *N*-methyl-pyridinium hydroxide, which contains 0.074 g. of base in 5 cc., until a pH of 10.2 is reached. The solution is then treated with 130 cc. of absolute ether. The amorphous by-product thereby precipitated (80 mg.) is filtered off by suction. Further addition to the filtrate of 130 cc. of ether yield after two hours standing at 0° C. a precipitate of 2.27 g. of a colored crude product, which is then recrystallized from ethanol, and finally from methanol-ether.

The yield of *N*-methylpyridinium salt is 41.5% of theory, M. Pt. 152–155°; solubility in water is 2.5% by weight at 23° C., $[\alpha]_D^{24}$ is 93.3° C. in water.

Example 5.—*N*- β -hydroxyethyl-*N*-methyl-piperidinium salt of oestrone sulfate

Similarly to Example 4, to 70 cc. of oestrone sulfate solution (method B), containing an amount of oestrone sulfate corresponding to 3.3 g. of oestrone, is added a methanol solution of *N*- β -hydroxyethyl-*N*-methylpiperidinium hydroxide until a pH of 10.2 is reached. The processing is conducted in such manner that the entire methanol solution is evaporated to dryness under vacuo. The residual syrup is rubbed with isopropyl alcohol, whereupon extensive crystallization takes place. The crude product of M. Pt. 140–145° after being recrystallized from methanol-ether mixture furnishes well-formed colorless crystals having a M. Pt. of 147–149°, in good yield (about 80% of theory). The solubility of the resulting salt in water is extraordinarily high, namely 36% by weight at 23° C. The $[\alpha]_D^{24}$ is +85.0° C. in water

Example 6.—*N*- β -hydroxyethyl-*N*-methyl-pyrrolidinium salt of oestrone sulfate

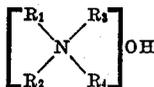
To 70 cc. (the last third of the solution of oestrone sulfate prepared by method B, above) there is added a methanol solution of the pyrrolidine base until a pH of 9 is reached, as described in the foregoing examples. The turbid solution is evaporated completely in vacuo. The residue (4 g.) is rubbed with 10 cc. of isopropyl alcohol. The crystallized portion is washed twice on the suction filter with 2 cc. of isopropyl alcohol. The weight of dried substance amounts to 2.1 g. (36% of theory) which has a M. Pt. 141–148° C. This product is then precipitated from alcohol-ether, and finally recrystallized from methanol-ether. There are obtained fine needles having a M. Pt. of 148–150°. The working up of the mother liquors yields additional salt, so that a yield of 60% of theory can be attained without difficulty. The solubility of the salt in water is 6.7% at 23° C., the $[\alpha]_D^{24}$ is +82° C.

We claim:

1. Quaternary ammonium salts of oestrone sulfate,

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which are derived from quaternary ammonium bases having the general formula



wherein R_1 , R_3 , and R_4 are alkyl radicals, R_2 is a member of the group consisting of alkyl radicals and hydroxyalkyl radicals; when R_1 and R_2 are alkyl, R_3 and R_4 are bivalent alkylene radicals joined to the quaternary N atom by one valence, and joined together by the other valence to form a member of the group consisting of a heterocyclic ring, and an oxygen containing heterocyclic ring, said salts being stable and possessing good water solubility.

2. Quaternary ammonium salts of oestrone sulfate which are derived from quaternary ammonium bases having 4 alkyl radicals attached to the nitrogen atom, said salts being stable and possessing good water solubility.

3. Quaternary ammonium salts of oestrone sulfate which are derived from quaternary ammonium bases having 3 alkyl radicals and 1 hydroxyalkyl radical attached to the nitrogen atom, said salts being stable and possessing good water solubility.

4. Quaternary ammonium salts of oestrone sulfate which are derived from quaternary ammonium bases which contain at least one alkyl radical attached to the nitrogen atom, and in which said nitrogen atom forms part of a six-membered heterocyclic ring, said salts being stable and possessing good water solubility.

5. Quaternary ammonium salts of oestrone sulfate which are derived from quaternary ammonium bases which contain at least one alkyl radical attached to the nitrogen atom, and in which said nitrogen atom forms part of a 5-membered heterocyclic ring, said salts being stable and possessing good water solubility.

6. Quaternary ammonium salts of oestrone sulfate which are derived from quaternary ammonium bases which contain at least one alkyl radical attached to the nitrogen atom, and in which said nitrogen atom forms part of a 6-membered oxygen containing heterocyclic

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ring, said salts being stable and possessing good water solubility.

7. N-(β -hydroxyethyl)-N-methyl-morpholinium salt of oestrone sulfate.

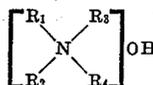
8. N-(β -hydroxyethyl)-N-methyl-pyrrolidinium salt of oestrone sulfate.

9. N-(β -hydroxyethyl)-N-methyl-piperidinium salt of oestrone sulfate.

10. N.N-dimethyl-morpholinium salt of oestrone sulfate.

11. Tetramethylammonium salt of oestrone sulfate.

12. The method of preparing stable, water soluble quaternary ammonium salts of oestrone sulfate which comprises reacting the oestrone sulfate with a quaternary ammonium bases having the general formula



wherein R_1 , R_3 , and R_4 are alkyl radicals, R_2 is a member of the group consisting of alkyl radicals and hydroxyalkyl radicals; when R_1 and R_2 are alkyl, R_3 and R_4 are bivalent alkylene radicals joined to the quaternary N atom by one valence, and joined together by the other valence to form a member of the group consisting of a heterocyclic ring, and an oxygen containing heterocyclic ring, said salts being stable and possessing good water solubility.

13. The method of claim 12 in which the salt forming reaction takes place in methanol solution.

14. The method of claim 13 in which the salt forming reaction is continued until a pH value of 9 to 11 is reached.

15. The method of claim 12 in which the quaternary ammonium base contains at least one alkyl radical.

16. The method of claim 12 in which the quaternary ammonium base contains a hydroxyalkyl group attached to the nitrogen atom.

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Notice of Adverse Decision in Interference

In Interference No. 89,538 involving Patent No. 2,828,306, E. Griebisch, G. Zuehlsdorff, and K. Pirner, Stable water soluble salts of oestrone sulfate and process of making same, final judgment adverse to the patentees was rendered February 4, 1959, as to claim 3.

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