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(19)

(54) PTERIDINE COMPOUNDS

(71) We, RÖHM GMBH., a German Body Corporate, of Darmstadt, Germany, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement:—

The present invention relates to pteridine compounds having valuable diuretic activity.

2,4,7 - Triamino - 6 - phenyl - pteridine (which is hereinafter identified by its trivial name "triamterene") is employed in medicine as a diuretic. A disadvantage of this compound is its low water-solubility and the compound is thus limited in practice to use in pharmaceutical compositions for oral administration. Triamterene could not therefore be readily used in emergencies. Attempts to convert the above active compound into a water soluble (i.e. injectable) form have hitherto been unsuccessful.

Triamterene belongs to the group of potassium-retaining diuretics. Details of the mode of action of triamterene are known from model tests on the main excretory duct of the salivary glands of rats. This excretory duct was chosen as a model since its epithelium transports Na^+, K^+ and H^+/HCO_3^- in a similar manner to the distal kidney tubule (Knauf *et al.* Pflügers Arch., 333, 83—94 (1972); 361, 55 (1975)). Work done by Knauf *et al.* has shown that complete inhibition of Na^+ reabsorption is obtained using 10^{-4} M triamterene and the K^+ secretion is reduced to half its initial level. (Europ. J. clin Invest. 6, 43—50 (1976)).

These changes in the transport of electrolytes are caused by inhibition of the influx of Na^+ from the lumen into the cell, as can be inferred from permeability measurements. Since the influx of Na^+ is functionally coupled with the outflow of K^+, blocking this influx results in an inhibition of the K^+ secretion, so that the loss of potassium associated with many diuretics is substantially prevented in the case of treatment with triamterene. In the tests carried out by Knauf *et al.*, the technique of microperfusion described by Young *et al.*, (Pflügers Arch. 295, 157 (1967)) was used.

The activity of triamterene can be detected, in the test method mentioned above, by the reduction in electrical potential difference between lumen and interstice, which provides a measurement of Na^+ transport. The inhibiting effect can only be obtained from the lumen side, whereas even maximum doses of triamterene are inactive in the bath solution of the isolated salivary gland duct, e.g. from the blood side. The assumptions regarding the working mechanism of triamterene, supported by the results of the methods of measurement described above, are thus substantiated.

In order to solve the problem of converting triamterene into a soluble (injectable) form whilst keeping the same diuretic and potassium-retaining activity, it was possible to make use of the test methods worked out for testing triamterene.

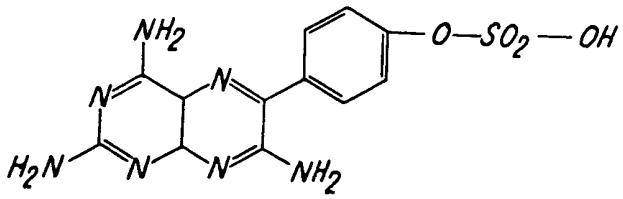
In 1965, Lehmann reported in "Arzneimittelforschung" 15 (7), pages 812—816 on the separation isolation and identification of the metabolites of triamterene produced by the kidneys. According to this report, in addition to unchanged triamterene, the hydrogen sulphate of 2,4,7 - triamino - 6 - p - hydroxyphenyl - pteridine and, in a smaller quantity, 2,4,7 - triamino - 6 - p - hydroxyphenyl - pteridine itself were produced as metabolites. In 1968, Weinstock *et al.*, reported on the diuretic effect of derivatives of 2,4,7 - triamino - 6 - phenyl - pteridine (J. Med. Chem. 11, 573—579 (1968)) and established that the p-hydroxy-analogue of

triamterene, one of the metabolites of this compound, does not show any diuretic effect. Those of the triamterene derivatives tested which have any diuretic effect at all are, according to Weinstock's investigations, compounds having apolar substituents, such as for example the p-toluyl-homologue of triamterene. On the other hand, derivatives with polar groups e.g. with an amino or nitro group, are diuretically inactive (*loc. cit.* Table VIII).

We have now surprisingly found that the hydrogen sulphate of the diuretically inactive p-hydroxy-triamterene i.e. a triamterene derivative with a polar group, has diuretic activity. This diuretic activity also extends to the water-soluble e.g. sodium and potassium, salts of the said hydrogen sulphate.

Thus, the hydrogen sulphate of 2,4,7 - triamino - 6 - p - hydroxyphenyl - pteridine has been found to have virtually the same activity as triamterene both with regard to the diuretic effect and also to potassium retention, but is clearly superior to triamterene owing to the solubility of the salts of the hydrogen sulphate. The solubility of the hydrogen sulphate of 2,4,7 - triamino - 6 - p - hydroxyphenyl - pteridine is sufficient for this active ingredient to be employed in injectible preparations.

According to one feature of the present invention we provide as new synthetic compounds 2,4,7 - triamino - 6 - *p* - hydroxyphenyl - pteridine hydrogen sulphate in the form of its water-soluble non-toxic salts, with the exclusion of the ammonium salt. It will be appreciated that the hydrogen sulphate can be represented as



or as the corresponding acid addition salt owing to the amphoteric nature of 2,4,7 - triamino - 6 - p - hydroxyphenyl - pteridine.

The term "non-toxic" is used herein in relation to the above salts to denote those salts, the cationic moiety of which is physiologically acceptable at dosages at which the salts are administered.

Examples of non-toxic water-soluble salts according to the invention include alkali metal e.g. sodium or potassium; substituted ammonium, e.g. tri-(C_{1-3}alkyl)amine, N—C_{1-3}alkyl-piperidine, procaine, alkanolamine or choline salts.

According to a further feature of the present invention we provide pharmaceutical compositions comprising as active ingredient 2,4,7 - triamino - 6 - p - hydroxyphenyl - pteridine hydrogen sulphate or a water-soluble non-toxic salt thereof in association with at least one pharmaceutical carrier or excipient.

The above compositions are advantageously formulated for parenteral administration, particularly intravenous administration, e.g. in ampoules, using for example a sterile aqueous injectable vehicle in which the active ingredient is dissolved. If desired, buffered solutions can be employed.

For parenteral, particularly intravenous administration, the amount of active ingredient administered is preferably such that the amount of the active ingredient in the plasma of the human adult is up to 20 mg, or up to 5 mg for special plasma concentrations.

According to a still further feature of the present invention we provide a process for the preparation of 2,4,7 - triamino - 6 - *p* - hydroxyphenyl - pteridine hydrogen sulphate which comprises reacting 2,4,7 - triamino - 6 - *p* - hydroxyphenyl - pteridine with sulphuric acid whereby the said hydrogen sulphate is produced. The reaction is generally effected in the presence of a condensation agent, preferably dicyclohexylcarbodiimide. The hydrogen sulphate thus produced can if desired be converted to a water-soluble non-toxic salt thereof.

The following Examples illustrate the present invention.

Example 1

124 mg of dicyclohexylcarbodiimide were dissolved in 0.2 ml of dimethylformamide dried over a molecular sieve (4A). To this solution was added a clear solution of 32 mg of 2,4,7 - triamino - 6 - p - hydroxyphenyl - pteridine in 0.7 ml of dry hexamethylphosphoric acid triamide (dried over a molecular sieve

10A), this clear solution having been prepared by dissolving the pteridine compound at the boiling point of the triamide and cooling to room temperature.

0.5 ml of 96% sulphuric acid were made up to 10 ml with ice-cold dry dimethylformamide, whilst cooling with ice. The dicyclohexylcarbodiimide/2,4,7 - triamino - 6 - p - hydroxyphenyl - pteridine solution was then mixed with 0.15 ml of the cold H_2SO_4/dimethylformamide solution, whilst cooling with ice. After a few minutes, the reaction mixture became cloudy; dicyclohexylurea was precipitated.

After 30 minutes, the mixture was diluted with 25 ml of dry dimethylformamide and neutralised with N NaOH solution whilst cooling with ice. The precipitated dicyclohexylurea was filtered off through a G3 frit and the filtrate was concentrated at a bath temperature of 30°C in the vacuum from an oil pump. Since the hexamethylphosphoric acid triamide did not pass over at this temperature, the residue was mixed with diethyl ether and the precipitate was removed by centrifuging. After the clear supernatant part had been decanted off, the mixture was dissolved in 0.5 ml of N-ammonia and 1 ml of dimethylsulphoxide and diluted with 20 ml of 0.01 M pyridinium acetate solution. The clear yellow solution was chromatographed on DEAE Sephadex (Registered Trade Mark) A25 (acetate form, column 3×23 cm), which was equilibrated with 0.01 M pyridinium acetate solution. Any unreacted hydroxy compound was immediately eluted, whilst the sulphate could not be separated until a gradient had been applied towards 1 M pyridinium acetate solution. The course of elution was monitored by measuring the absorbances of the individual fractions at 365 nm using a photometer. After identification by a thin layer chromatography, the sulphate-containing eluate was evaporated to dryness at 30°C in the vacuum from an oil pump. The residue was taken up in a little N ammonia and the filtered solution brought to pH 5—6 with a few drops of glacial acetic acid. When the solution was stored in the refrigerator, the hydrogen sulphate crystallised out.

Yield: 12 mg (29% of theory).

Purity check by thin layer chromatography (eluant: $CHCl_3$-methanol-water 65:35:4): uniform.

Example 2

500 mg of the hydrogen sulphate of 2,4,7 - triamino - 6 - hydroxyphenyl - pteridine is introduced with stirring into a solution of 1 g of triethylamine and 1 ml of water (suitable for injection). The mixture is diluted to 10 ml with water suitable for injection, and filtered through a fine-pored filter for germ removal and removal of any solid matter. The content of active material was then determined under aseptic conditions. Samples were adjusted with water for injection purposes to a content of about 5 mg/ml, then with HCl in water for injection purposes to a pH of 9, and finally diluted with water to the calculated volume. The solution was filled into ampoules under sterile conditions.

Experimental Study

Sufficient stability of the above-identified hydrogen sulphate is a pre-requisite for parenteral (intravenous) administration. The stability of the active ingredient against hydrolysis was determined by measuring the distribution between the lipophilic and aqueous phases. At the same time the measurement provides a reference point for the hydrophilic properties of the compound.

1) Determination of the Stability Under Hydrolytic Conditions

The stability of the hydrogen sulphate of 2,4,7 - triamino - p - hydroxyphenyl - pteridine under hydrolytic conditions can be determined by monitoring and measuring the distribution of the active ingredient between the aqueous phase and lipophilic phase, since the coefficient of distribution must change with the saponification of the ester.

Test Method

0.33 mg of the hydrogen sulphate of 2,4,7 - triamino - 6 - p - hydroxyphenyl - pteridine are dissolved in 10 mM Tris-HCl buffer (pH=7.4) to provide a 10^{-4}M solution. The aqueous phase is extracted with the same volume of n-octanol at constant temperature in defined dilution stages (equilibrated). After the phases have been separated by centrifuging, the particular ester concentration is determined photometrically. The coefficient of distribution of triamterene is measured in parallel, as a comparison value.

Result

Coefficient of distribution for triamterene in the n-octanol/water system is p=12.8 at room temperature; coefficient of distribution of the ester directly after being brought into solution in the same system at room temperature is p=0.029. After storage at room temperature for 72 hours, the quotient of distribution of the hydrogen sulphate remains unchanged (p=0.029 as the average from 10 measurements).

2) Diuretic Activity

During the luminal perfusion of the excretory ducts of the salivary glands of male rats using the method described by Knauf and Fromter (Pflüger's Arch. *316*, 238 (1970), the electrical potential difference was recorded continuously. A solution of 140 mEq. Na^+ buffered with 10 mM Tris-HCl, as a sulphate in water, was used as the perfusion solution. Under these conditions, the potential difference obtained between lumen and interstice is dependent only on the transport of Na^+. A reduction in the potential difference using triamterene also represents a reduction in the transport of Na^+ and thus a diuretic effect.

Result

Perfusion with the hydrogen sulphate of 2,4,7 - triamino - 6 - p - hydroxyphenyl - pteridine and 2,4,7 - triamino - 6 - phenyl - pteridine (triamterene) as a comparison in 10^{-4}M concentration results in a parallel reduction in the sodium potential in both cases. The effect is fully reversible. Determination of the 50% maximum inhibition I_{50}. The values given in the following table are determined in a 140 mEq. sodium sulphate solution. (Tris-buffered at pH 7.4), see above. The inhibition of sodium reabsorption is given as the 50% maximum effective dose I_{50}.

Active Ingredient	I_{50}
2,4,7 - Triamino - 6 - phenyl - pteridine (triamterene)	3×10^{-5}Mol
Hydrogen sulphate of 2,4,7 - triamino - 6 - p - hydroxyphenyl - pteridine	8×10^{-5}Mol

Summary of the Results

On a molar basis, the hydrogen sulphate has the same effect on the Na^+ potential as triamterene itself. The diuretic effect to be expected as a result of this finding is confirmed by transport studies.

3) Total Inhibition of Na^+ Transport-Investigation of the Secretion of K^+ According to Knauf loc. cit.

The transport of Na^+ is totally blocked with a 10^{-4} molar concentration of hydrogen sulphate in Tyrode solution (pH=7.4). Tyrode solution comprises, in parts by weight, 0.8 NaCl, 0.02 KCl, 0.02 $CaCl_2$, 0.01 $MgCl_2$, 0.005 NaH_2PO_4, 0.1 $NaHCO_3$, 0.1 of Glucose in 100 parts by weight of water. (Decline from 39±3 to 1.9±3.4 nEq/min, i.e. to a value not significantly different from zero). The measurement is carried out on the same size excretory duct. With triamterene, the flow is 1.5 nEq/min/excretory duct

The effect on the secretion of K^+ is best compared under conditions wherein the resorption of Na^+ is totally inhibited.

It has been found that the hydrogen sulphate of 2,4,7 - triamino - 6 - p - hydroxyphenyl - pteridine has a stronger inhibiting effect on the secretion of K^+ (i.e. it retains more K± ions) than triamterene. The secretion of K^+ falls from 27±3 nEq per min to 4±1 nEq per min. The measurement is carried out on the same size excretory ducts. With triamterene, the flow is 12 nEq per min per duct.

We make no claim herein to aqueous solutions comprising 2,4,7 - triamino - 6 - *p* - hydroxyphenyl - pteridine hydrogen sulphate and/or the ammonium salt of the said hydrogen sulphate as described in the above-mentioned article in "Arzneimittelforschung", 15 (7), pages 812—816.

Subject to the foregoing disclaimer.

WHAT WE CLAIM IS:—

1. 2,4,7 - Triamino - 6 - *p* - hydroxyphenyl - pteridine hydrogen sulphate in the form of its water-soluble non-toxic salts, with the exclusion of the ammonium salt.

2. 2,4,7 - Triamino - 6 - *p* - hydroxyphenyl - pteridine hydrogen sulphate in the form of its alkali metal or substituted ammonium salts.

3. 2,4,7 - Triamino - 6 - p - hydroxyphenyl - pteridine hydrogen sulphate in the form of its sodium or potassium salts.

4. Pharmaceutical compositions comprising as active ingredient, 2,4,7 - triamino - 6 - *p* - hydroxyphenyl - pteridine hydrogen sulphate or a water-soluble non-toxic salt thereof in association with at least one pharmaceutical carrier or excipient.

5. Compositions as claimed in Claim 4, wherein the said carrier or excipient comprises a sterile aqueous injectable vehicle.

6. Compositions as claimed in Claim 5 in the form of ampoules.

7. Compositions as claimed in any of Claims 4 to 6, wherein the active ingredient comprises a water-soluble non-toxic salt of the said hydrogen sulphate.

8. Compositions as claimed in Claim 7, wherein the active ingredient comprises the sodium, potassium or triethylamine salt of the said hydrogen sulphate.

9. Compositions as claimed in Claim 4 substantially as herein described in Example 2.

10. A process for the preparation of 2,4,7 - triamino - 6 - *p* - hydroxyphenyl - pteridine hydrogen sulphate which comprises reacting 2,4,7 - triamino - 6 - *p* - hydroxyphenyl - pteridine with sulphuric acid whereby the said hydrogen sulphate is produced.

11. A process as claimed in Claim 10, wherein the reaction is effected in the presence of dicyclohexylcarbodiimide.

12. A process as claimed in Claim 10 or Claim 11, wherein the hydrogen sulphate is converted to a water-soluble non-toxic salt thereof.

13. A process as claimed in Claim 12 in which the hydrogen sulphate is converted to an alkali metal salt thereof.

14. A process as claimed in Claim 10 substantially as herein described in Example 1.

15. 2,4,7 - Triamino - 6 - p - hydroxyphenyl - pteridine hydrogen sulphate and water-soluble non-toxic salts thereof, when prepared by a process as claimed in any of Claims 10—14.

16. Pharmaceutical compositions as claimed in any of Claims 4 to 8, wherein the active ingredient is as claimed in Claim 15.

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