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(71) We, SOCIETA FARMACEUTICI ITALIA S.p.A., a body corporate organised and existing under the laws of Italy, of 1/2 Largo Guido Donegani -1 20121 Milan, Italy, 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 invention relates to anthracycline glycosides, and provides as new compounds the radiochemically labelled [14-^{14}C] daunorubicin and [14-^{14}C] doxorubidin and their hydrochlorides. These are important for the study of the distribution, pharmacokinetics and metabolism of these compounds which are antitumour medicines. The stability and specificity of the ^{14}C-label makes these compounds useful for both experimental and medical purposes.

The invention includes a process of preparing these compounds which comprises treating 9-deacetyl-9-formyl-N-trifluoroacetyl-daunorubicin with [^{14}C] diazomethane. The starting material can be obtained according to our British Patent Specification 1542920. The process is preferably carried out in a vacuum manifold as shown in the drawing accompanying the Provisional Specification. Three portions of the starting material, in the solid state, are put in the flasks 5, 6 and 7 and treated at ambient temperature under reduced pressurewith [^{14}C] diazomethane in a medium such as methylene dichloride and/or diethyl ether or another aprotic organic solvent.

More precisely, an ethereal solution of diazomethane is generated in flask 2, distilled into flask 3, and then transferred to flask 4 containing an equal volume of methylene dichloride or chloroform. The organic solution is then transferred by distillation into flask 5 containing the starting material. At the end of the reaction (60 minutes at ambient temperature) the excess diazomethane is distilled into flask 6 to react with a second portion of starting material. The consumption of the diazomethane is completed in flask 7. The solvents are recovered in flask 8. This allows the radioactivity to be bound completely, and therefore optimizes the yield of [14-^{14}C]N-trifluoroacetyl-daunorubicin.

The combined crude product is preferably purified by chromatographic separation using silica gel plates or a column of silicic acid and an eluent system consisting of a mixture of chloroform and acetone in a ratio of 4:1 (by volume). Mild alkaline treatment of the purified N-trifluoroacetyl-daunorubicin to hydrolyze the protective group is preferably carried out at from 0 to 5°C with aqueous 0.1 N sodium hydroxide or ammonium hydroxide for from 30 to 60 minutes. The glycoside can be recovered as the hydrochloride in the following manner: the alkaline solution is adjusted to pH 4.5 by addition of 0.5 N hydrochloric acid and extracted with chloroform to remove the aglycones. The solution is then brought to pH 8.5 and repeatedly extracted with chloroform. The combined extracts are evaporated to a small volume under vacuum and a stoichiometric amount of hydrogen chloride in methanol is added to give 14-^{14} daunorubicin hydrochloride. The radiochemical yield, based on [^{14}C] methylemine hydrochloride, the precursor of the [^{14}C] diazomethane via N-nitroso-N-[^{14}C]methyl-urea, is about 10%.

The preparation of [14-^{14}C]doxorubicin

can be performed in accordance with our British Patent Specification 1,217,133 for the chemical transformation of daunorubicin to doxorubicin. Treatment of [14-^{14}C] daunorubicin hydrochloride with bromine gives the corresponding [14-^{14}C]14-bromo-derivative which by hydrolysis is converted to [14-^{14}C]doxorubicin.

The invention is illustrated by the following Examples:

Example 1
[14-^{14}C]Daunorubicin

With reference to the drawing accompanying the Provisional Specification, flask 1 is charged with 50% aqueous potassium hydroxide. This is fed into flask 2 containing N-nitroso-[^{14}C]-N-methyl-urea in diethyl ether derived from 940 μCi of [^{14}C]methylamine hydrochloride ($NH_2{}^{14}CH_3$.HCl) (0.477 mmols, 1.97 MCi/mmols) for the production of [^{14}C]diazomethane. This diazomethane is distilled into flask 3 as an ethereal solution, and thence into flask 4 to give a solution in 1:1 by volume ether and methylene dichloride.

9-Deacetyl-9-formyl-N-trifluoroacetyldaunorubicin (0.118 g, 0.193 mmols) is put in three flasks 5, 6 and 7, 0.035 g, 0.055 g and 0.028 g respectively. The mixture of diethyl ether and methylene chloride (20 ml) containing [^{14}C] diazomethane is distilled into flask 5. After stirring for 60 minutes at room temperature, the excess of diazomethane is distilled into flask 6. The reaction is run as described above. Finally, residual radioactive reagent is transferred by distillation into flask 7. The solvents are recovered in flask 8.

The combined radioactive products (0.127 g) are purified by thin layer chromatography on silica gel plates (2 mm thick) using a chloroform-acetone (1:1 v/v) solvent system as eluent. The band containing N-trifluoroacetyl [14-^{14}C]daunorubicin is taken out, transferred into a beaker quantitatively, and washed with 5% aqueous methanol (10 ml), then with chloroform (20 ml). The organic phase is filtered through a porous glass filter. After six washings, the silica gel is completely colourless. The combined extracts are evaporated to dryness under vacuum. The residual solid, dissolved in 20 ml of chloroform and filtered through a paper filter, is evaporated to dryness yielding 0.047 g of [14-^{14}C]N-trifluoroacetyl-daunorubicin. This compound is identical with an authentic sample obtained by treatment of daunorubicin with trifluoroacetic anhydride [F. Arcamone et al., Chim. Ind. (Milan) 1969, *51*, 834].

A solution of this compound in 10 ml of 0.1 sodium hydroxide, after 60 minutes at 0°C, is adjusted to pH 4.5 by addition of 0.5 N hydrochloric acid and extracted with chloroform until the extracts are colourless. The aqueous solution is adjusted to pH 8.5 with 0.1 N sodium hydroxide and extracted with chloroform repeatedly (20 ml × 5) until the colour is completely transferred into the organic phase. A stoichiometric amount of 0.1 N methanolic hydrogen chloride is added to the combined organic extracts. The resulting red solution is evaporated to dryness at 35°C, giving 0.030 g of [14-^{14}C]daunorubicin hydrochloride, specific activity 3.25 μCi/mg (1.832 mCi/mmol).

Following the above outlined procedure and starting from 10 mCi of $NH_2{}^{14}CH_3$.HCl (specific activity 19.27 mCi/mmole) and 0.150 g of 9-deacetyl-9-formyl-N-trifluoroacetyl-daunorubicin, 44 mg of [14-^{14}C] daunorubicin hydrochloride with specific activity 19.18 mCi/mmol were obtained (15.0% radiochemical yield).

Example 2
[14-^{14}C]Doxorubicin hydrochloride

A solution of [14-^{14}C]daunorubicin hydrochloride in a mixture of methanol and dioxan is treated with bromine to give the corresponding 14-bromo derivative. Treatment with an aqueous solution of sodium formate at ambient temperature for 100 hours, followed by treatment with a stoichiometric amount of hydrogen chloride in methanol gives [14-^{14}C]doxorubicin as the hydrochloride.

Following this procedure [14-^{14}C]doxorubicin hydrochloride (29 mg, specific activity 14.04 mCi/mmol and 10 mg, specific activity 6.54 mCi/mmol) was obtained starting from [14-^{14}C]daunorubicin (54 mg, specific activity 14.18 mCi/mmole) with 60.4% overall radiochemical yield.

WHAT WE CLAIM IS:

1. [14-^{14}C]Daunorubicin or its hydrochloride.

2. [14-^{14}C]Doxorubicin or its hydrochloride.

3. A process for the preparation of [14-^{14}C] daunorubicin, the process comprising reacting 9-deacetyl-9-formy-N-trifluoroacetyl-daunorubicin with [^{14}C]diazomethane in an aprotic organic solvent and removing the N-trifluoroacetyl group from the resulting [14-^{14}C]-N-trifluoroacetyl-daunorubicin by mild alkaline hydrolysis.

4. A process according to claim 3 in which the aprotic organic solvent is methylene dichloride or diethyl ether.

5. A process according to claim 3 or claim 4 in which the reaction with [^{14}C]diazomethane is carried out at ambient temperature for 60 minutes under reduced pressure.

6. A process according to any of claims 3 to 5 in which the alkaline hydrolysis is effected with 0.1N aqueous sodium or

ammonium hydroxide at a temperature of from 0°C to 5°C for from 30 to 60 minutes.

7. A process according to any of claims 3 to 6 in which the [14-^{14}C]-N-trifluoroacetyl-daunorubicin is chromatographically purified.

8. A process according to claim 3 and substantially as described herein with reference to Example 1 and the drawing accompanying the Provisional Specification.

9. [14-^{14}C]Daunorubicin prepared by a process according to any of claims 3 to 8.

10. A process for the preparation of [14-^{14}C] daunorubicin hydrochloride, the process comprising treating [14-^{14}C]daunorubicin according to claim 9 with a stoichiometric amount of hydrogen chloride in methanol.

11. A process according to claim 10 and substantially as described herein with reference to Example 1 and the drawing accompanying the Provisional Specification.

12. [14-^{14}C]Daunorubicin hydrochloride prepared by a process according to claim 10 or claim 11.

13. A process for the preparation of [14-^{14}C]doxorubicin, the process comprising reacting [14-^{14}C]daunorubicin hydrochloride according to claim 12 with bromine and hydrolyzing the resultant 14-bromo derivative.

14. A process according to claim 13 in which the hydrolysis is effected with an aqueous solution of sodium formate.

15. A process according to claim 14 in which the hydrolysis is carried out at ambient temperature for 100 hours.

16. A process according to claim 13 and substantially as described herein with reference to Example 2.

17. [14-^{14}C]Doxorubicin prepared by a process according to any of claims 13 to 16.

18. A process for the preparation of [14-^{14}C]-doxorubicin hydrochloride, the process comprising treating [14-^{14}C]doxorubicin according to claim 17 with a stoichiometric amount of hydrogen chloride in methanol.

19. A process according to claim 18 and substantially as described herein with reference to Example 2.

20. [14-^{14}C]Doxorubicin hydrochloride prepared by a process according to claim 18 or claim 19.

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1566330 COMPLETE SPECIFICATION
1 SHEET *This drawing is a reproduction of the Original on a reduced scale*