SYNTHESIS OF ^{3}H LABELED RIFAMYCIN L 105

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Summary
^{3}H labeled rifamycin L 105, a potential new intestinal disinfectant, was synthesized according to the Wilzbach method with the aim of performing radiopharmacological studies.

Keywords: ^{3}H-2-Amino-4-methyl-pyridine, ^{3}H-Rifamycin L 105,
Tritiation Wilzbach method, Radio HPLC

Introduction

Rifamycins are well-known antibiotics very active on Gram-positive bacteria and on mycobacteria through the

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inhibition of the bacterial enzyme DNA-dependent RNA polymerase[1-3]. Many hundreds of semisynthetic derivatives of the natural compounds have been prepared with the aim of increasing their intrinsic activity, of obtaining a wider spectrum of action, and of modifying their pharmacokinetic behavior[4,5]. Two of these compounds are nowadays used in therapy: they are rifamycin SV[6] and rifampicin[7] (Fig. 1).

The former, when administred per os, is rapidly excreted with bile, and its use is therefore limited to the therapy of biliary infections and to topical applications. The latter, instead, is well absorbed and represents a drug of choice in the therapy of tuberculosis.

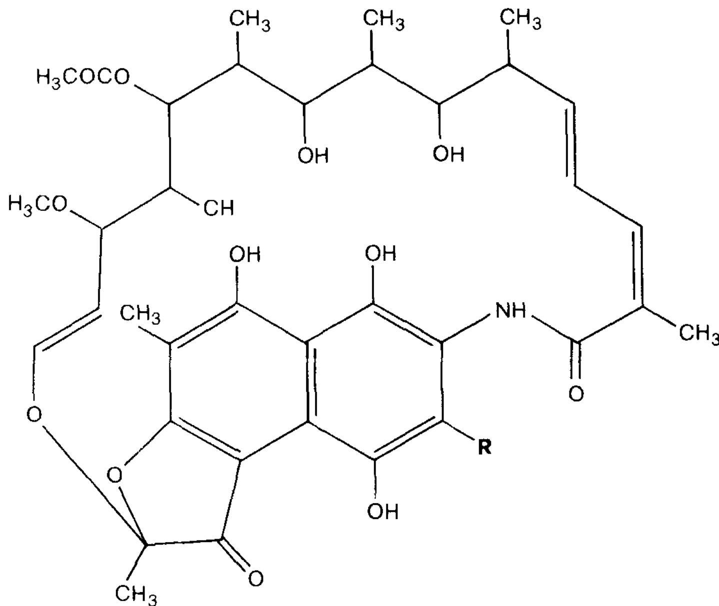
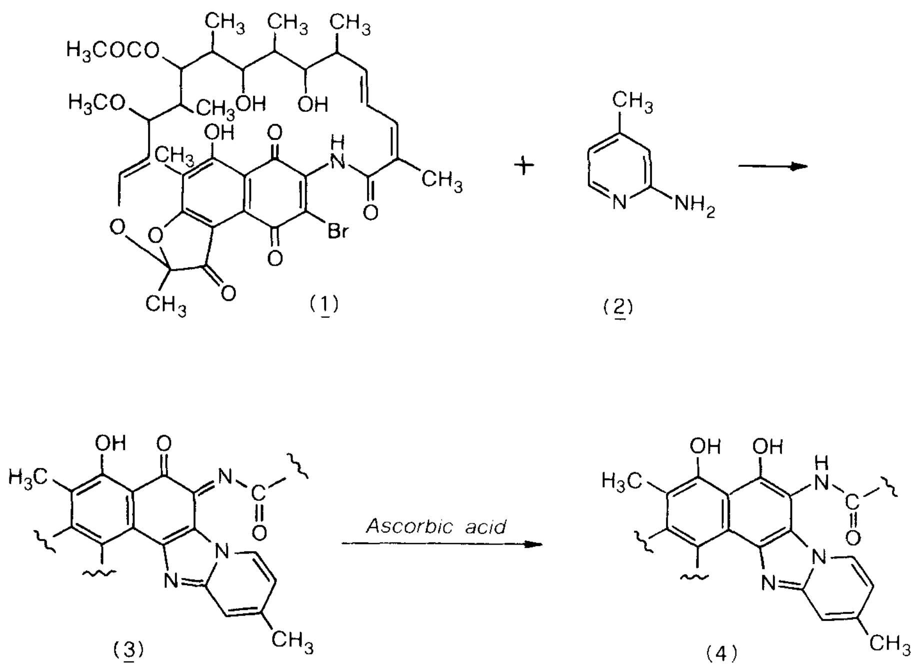


Fig. 1



Scheme 1

A new rifamycin has been recently prepared in the laboratories of Alfa Ricerche, Bologna, and beside being a potent antibacterial agent, displays the peculiar pharmacokinetic behavior of not undergoing biliary excretion and not being absorbed like the others. This new compound, named rifamycin L 105[8)] (4) (Scheme 1), appears as a promising potential intestinal disinfectant and has already provided very good results in pharmacological and clinical trials.

In order to enhance the sensitivity of the pharmacological tests carried out to determine the extent of absorption of the antibiotic in the body, its localization in the organs and fluids, and its recovery, radioactive rifamycin L 105 was required.

The choice of the labeling method

In planning the preparation of labeled (4) several considerations had to be taken into account. In fact, a compound with high specific activity had to be prepared in the light of the known structure-activity relationships[4,5], bringing about as little modifications as possible and without affecting its pharmacological properties. The balance of these rationals lead to the choice of labeling with tritium the precursor of the benzo-imidazo moiety of the antibiotic, namely the 2-amino-4-methyl-pyridine (2), prior to its condensation to 3-bromorifamycin S (1) (Scheme 1).
In fact, any interfering modification induced by the labeling procedure merely yields by-products from which tritiated (2) can be easily separated. On the contrary, the direct labeling of (4) would yield a mixture of closely related products much more difficult to separate. In addition, during the condensation reaction, no loss of activity from non-labile positions of the picoline occurs. Furthermore, first, the site of the rifamycin molecule at which the benzo-imidazo moiety is located, does not seem to be involved in the inhibitory activity. Second, the net replacement of hydrogen with tritium should not vary, in principle, the pharmacologycal properties of a molecule.

Therefore (2) was labeled with tritium according to the simple and rapid Wilzbach exposure method[9], largely experimented on aromatic compounds[10,11]. The labeled (2) was then reacted as outlined in Scheme 1 for the preparation of tritiated (4).

Synthesis and purification of ^{3}H-2-amino-4-methyl-pyridine (2)

Since our aim was to obtain a high radiochemical yield of an intermediate product and no specific labeling was required, we chose to follow the charcoal absorption method reported by Evans[11] as an efficient modification of Wilzbach procedure[9]

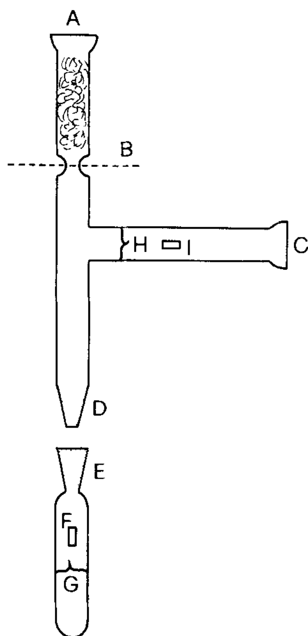


Fig. 2

Therefore, 135.29 mg (1.25 mmol) of (2)(Aldrich) were ground with 325.73 mg of charcoal. The resulting powder was introduced into a Pyrex reaction vessel (Fig. 2) to which an ampoulecontaining 2.60 Ci of tritium gas (Amersham) was previously sealed with seal-wax through the ground conical joints D and E. The region between the restriction B and the greaseless joint A was filled with glasswool. The apparatus was connected through A to a high vacuum line, evacuated, and sealed at B. The fragile point G was broken by the glass rod F by shaking and the vessel was stored for 21 days. Now and then during this period the vessel was shaken manually. Afterwards, the vessel was connected to a high vacuum line through the greaseless joint G, the fragile point H was broken by the iron rod I driven by an external magnet, and helium gas was admitted into the vessel. The unreacted tritium gas and helium were evacuated by a Toepler pump. Several washings with helium were carried out. At the end, at atmospheric pressure the vessel was disconnected from the vacuum line, broken inside a bea-

ker, and washed with 50 ml of methanol. The organic solution was separated from the charcoal powder by filtering on Celite, and eluting with methanol. The volume of the final solution was 200 ml.
Methyl alcohol was chosen as a solvent because of its hydroxyl hydrogens which induce hydrogen isotope exchange with tritium atoms incorporated in the labile positions of the products[12]. Therefore, it was excpected that the recovered radioactive products were labeled at the pyridine ring and at the methyl group and not at the amino group. Methyl alcohol was removed under vacuum with a rotatory evaporator, and the residue was washed several times with portions of methanol. The final solid was dissolved with acetone and purified by column chromatography on 20 g Silica gel 60 (Merck), eluted with acetone. The fractions were assayed by TLC on silica gel and by liquid scintillation counting. The fractions containing radioactive (2) were collected, and the solvent was evaporated yielding 113.27 mg of radioactive (2).

Synthesis of ^{3}H-rifamycin L 105(4)

390.0 mg of (1) (0.5 mmoles), prepared according to the literature, were dissolved in 10 ml of chloroform, and 108.0 mg of tritiated (2) (1.0 mmoles) were added.
The solution was stirred for two hours at room temperature, then washed with 0.1 N hydrochloric acid and finally with water. The organic phase was dried with anhydrous sodium sulphate, filtered and evaporated under vacuum. The remaining solid was purified by columm chromatography on 25 g of Silica gel 60 (Merck) eluted with chloroform-ethyl acetate (1:1). The fractions were controlled by TLC and collected accordingly; 300.0 mg of tritiated (4) in the quinonic form (3) (Scheme 1) were recovered. This compound was dissolved in 4 ml of methanol and 1 ml of 25% aqueous solution of

ascorbic acid was added. The mixture was stirred for fifteen minutes at room temperature, then added with 50 ml of chloroform and washed with brine. The organic phase was dryed on anhydrous sodium sulphate, filtered and evaporated under vacuum to give 258.21 mg of tritiated (4).

Chemical and radiochemical purity and yield of (4).

The chemical and radiochemical purity of tritiated (4) was controlled by radio HPLC.

A Perkin-Elmer 3B Chromatograph equipped with a columm 4 X 250 mm Lichrosorb RP8 10 μ (Merck) was used. The effluent from the column flowed through a u.v. Perkin-Elmer LC 75 recorder with Autocontrol, set at 289 nm for mass analysis, and, afterwards, through a Packard TRI-CARB RAM 7500 solid scintillation chamber for radiochemical analysis. Dead volumes and connecting tubes were reduced, so that, practically, simultaneous records of the u.v. and radiochemical traces occurred.

A typical chromatogram is presented in Fig. 3. The identification of rifamycin tritiated (4) was estabilished by the comparison of its retention volume with that of an authentic sample and by the addition method. Furthermore, u.v. spectra (Fig. 4) of the chromatographic peaks of labeled and unlabeled (4) were recordered by scanning on the peaks within the range 200-600 nm in the stop-flow method and compared.

Radio HPLC analyses were repeated several times under different conditions and with the detectors set at various levels of sensitivity. It was concluded that both the chemical and radiochemical purity of the ^3H rifamycin L 105 was better than 98%.

Specific activity determination

10, 50, and 100 μl of acetonitrile-water (6:4) solution of tritiated (4) whose concentration was determined by u.v. titration, ε_{450} = 12,650, were assayed by liquid scintilla-

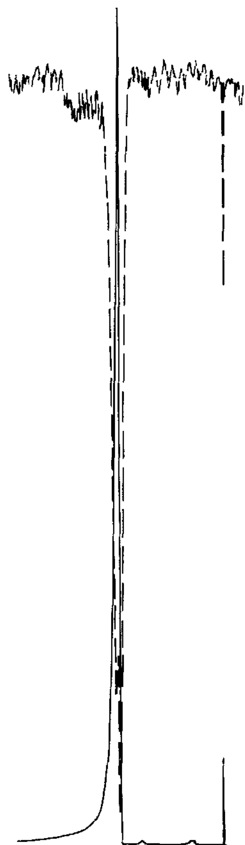


Fig. 3 Radio HPLC of ^{3}H rifamycin L 105; retention volume 5.4 ml; flow-rate 1 ml/min; chart speed 5 mm/min. Solid line: u.v. trace; broken line radio trace.

tion counting. A Packard instrument was employed and the counting parameters were set to give a standard deviation of $\sigma = 0.2$. A toluene-based scintillation cocktail was used, and the measures were repeated at different times to evaluate possible solubility effects on counting efficiency. Quenching curves were also drawn in the concentration and counting ranges of interest using acetonitrile-water solutions of inactive antibiotic and ^{3}H benzene of known specific activity.

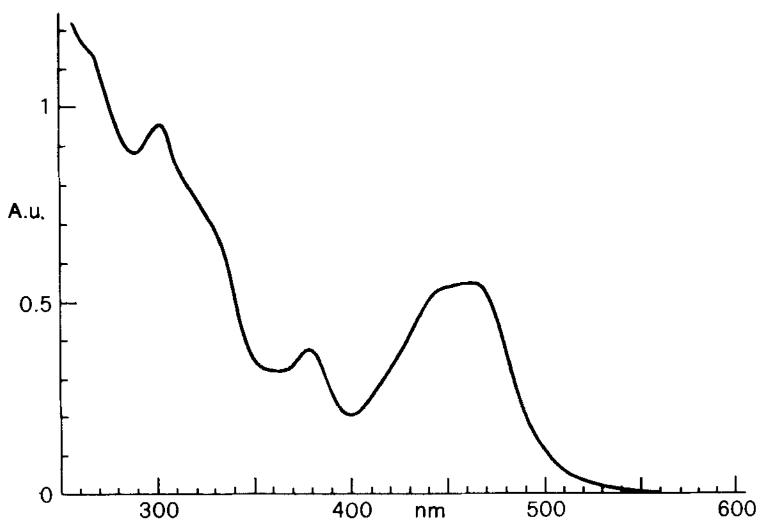


Fig. 4 Absorbance u.v. spectrum of rifamycin L 105 (4) in acetonitrile-water (6:4)

The resulting specific activity of labeled (4) was 33.79 Ci/mg. Since 258.21 mg of antibiotic was synthesized, the total activity recovered in the product was 8.72 ± 3% mCi. Therefore, 0.33% of the original tritium activity was found as incorporated in the final product. Considering the decay rate of tritium, the radioactivity incorporated accounts for more than 90% of the decay events occurred in the 21 expcsure days.

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