Preparation of a Carbon-11 Labelled Antibiotic, Erythromycin A Lactobionate

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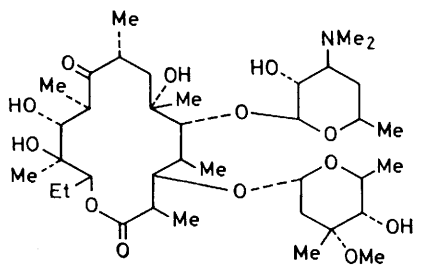
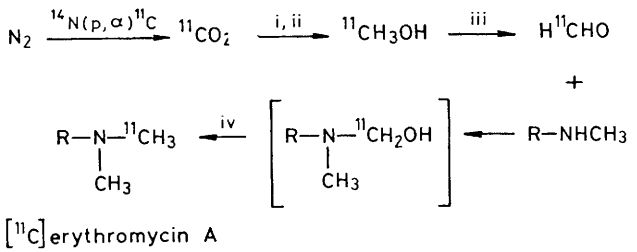
Erythromycin A is labelled with the positron-emitting radionuclide, carbon-11 ($t_{\frac{1}{2}}$ = 20.4 min), by the fast reductive methylation of *N*-demethylerythromycin A with [^{11}C]formaldehyde to provide an agent that permits the non-invasive study of the antibiotic *in vivo*.

Erythromycin A is produced by a strain of *Streptomyces erythreus* and is the best known of the medicinally important macrolide antibiotics.[1] We sought a fast method to label erythromycin A with the short-lived positron-emitting radionuclide, carbon-11 ($t_{\frac{1}{2}}$ = 20.4 min), in order to provide for the first time an agent that may be used with the quantitative technique of positron emission tomography[2] for the non-invasive study of an antibiotic *in vivo*. The choice of carbon-11 makes feasible the desired aim of labelling the antibiotic without alteration of its biological properties.

The erythromycin A molecule (**1**) contains the sugars, L-cladinose and D-desosamine, linked to the aglycone, erythronolide A.[3] A method for labelling the dimethylamino-



(1)



Scheme 1. R = erythromycin A residue, R–$NHCH_3$ = *N*-demethylerythromycin A. *Reagents and conditions:* i, $LiAlH_4$–tetrahydrofuran, ii, H_2O, iii, Ag, 550 °C, iv, H_2, Pd/C, 10 min.

group of the D-desosamine moiety with the long-lived radionuclide, carbon-14, has been reported.[4] However, because the half-life of carbon-11 is very short, a faster and more efficient method of labelling erythromycin A with carbon-11 had to be devised.

From kinetic studies we found that the reductive methylation of *N*-demethylerythromycin A[5] in methanol, using 1 equiv. of formaldehyde in the presence of hydrogen and palladium on charcoal at 18 °C, gives erythromycin A in 50% yield after only *ca.* 20 min of reaction. We therefore explored the possibility of labelling erythromycin A with carbon-11 by the fast reductive methylation of *N*-demethylerythromycin A with [^{11}C]formaldehyde (Scheme 1).

[^{11}C]Carbon dioxide (5.6—7.4 GBq) was produced in high radionuclidic and radiochemical purity by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction on nitrogen gas[6] and converted into [^{11}C]-methanol[7,8] by reaction with lithium aluminium hydride followed by hydrolysis. The [^{11}C]methanol was in turn partly oxidised to [^{11}C]formaldehyde over heated silver wool.[7,8] The resultant [^{11}C]formaldehyde/[^{11}C]methanol mixture was carried in nitrogen into cold (0—5 °C) ethanol (2.3 cm^3) containing *N*-demethylerythromycin A (10 mg) and palladium on charcoal catalyst (18 mg). The latter had been pre-activated by passing hydrogen through the suspension at 40 cm^3 min^{-1} at atmospheric pressure for 10 min. After 10 min the suspension was warmed to 40 °C and hydrogenated for 10 min as before. The suspension was then filtered and the radioactive filtrate injected onto a silica column eluted at 5.0 cm^3 min^{-1} with CH_2Cl_2–EtOH–NH_4OH (97.5/2.5/0.25 by volume). The column had been pre-conditioned by elution with CH_2Cl_2–EtOH–NH_4OH (50/50/5 by volume). [^{11}C]Erythromycin A, which was well separated from other radioactive components, eluted in 5—8 min and was collected. Unchanged *N*-demethylerythromycin A was retained on the column. Thin layer chromatography of the collected radioactive fraction followed by autoradiography verified the [^{11}C]erythromycin A to be radiochemically pure. Erythromycin A was the only stable compound detected in chromatographed samples.

The [^{11}C]erythromycin A was formulated for human injection as follows. Solvent was removed from the collected radioactive fraction by rotary evaporation and the radioactive residue dissolved in dextrose solution for injection (5% wt/v; 5 cm^3) containing lactobionic acid (6.7 mg). This solution was added to unlabelled erythromycin A (12.8 mg), shaken thoroughly and sterilised by filtration (0.22 μ pore size). Preparations of [^{11}C]erythromycin A lactobionate passed independent tests for apyrogenicity and sterility.

From the end of [^{11}C]carbon dioxide production the preparation requires only 42 min and provides an injectable solution of [^{11}C]erythromycin A lactobionate in 4—12% radiochemical yield (corrected for radioactive decay and based on the activity of the [^{11}C]carbon dioxide used at the end of proton irradiation). The short time and modest radiochemical yield of the synthesis we have described have enabled useful activities (56—185 MBq) of [^{11}C]erythromycin A lactobionate to be prepared for studies of the uptake of erythromycin A into normal and infected human lung by positron emission tomography. The results of these studies are the subject of a forthcoming publication.[9]

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