Preparation of ^{14}C-Labelled Vincristine and N-Desmethyl-N-formyl-leurosine

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SUMMARY

^{14}C-Labelled vincristine was prepared from N-desmethyl vinblastine by formylation with ^{14}C-formic acid. Excess radioactive formic acid was recovered as sodium formate. ^{14}C-Labelled N-desmethyl-N-formyl-leurosine was prepared analogously.

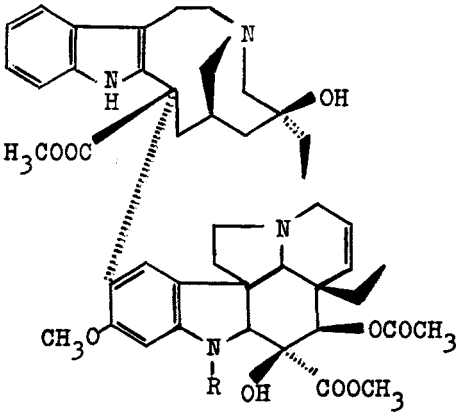
Key words: Vincristine, N-Desmethyl-formyl-leurosine, ^{14}C-Formic acid

INTRODUCTION

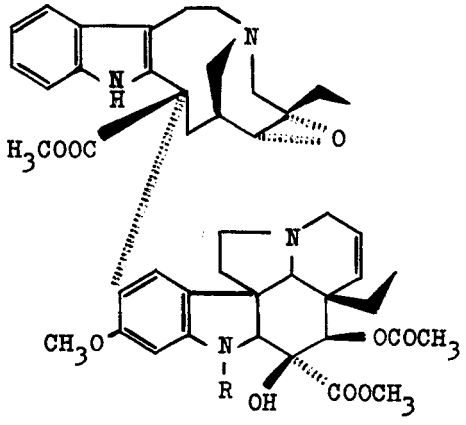
Owellen and Donigian[1] reported about the tritium-labelled vincristine (VCR) (3) which had been prepared by an exchange reaction with tritiated trifluoroacetic acid, in the presence of a special platinum catalyst. At the same time they announced that attempts had been made to desacetylate VCR and to reacetylate it with tritiated acetic anhydride. Although vinblastine (VLB) (2) had been labelled by Greenius and co-workers[2] by this latter method, in the case of VCR it proved to be unsuccessful.

The aim of the present work was to prepare ^{14}C-labelled VCR with high specific activity for the purpose of pharmacological testing. For this the following reaction sequence seemed to be appropriate: desacetylation and reacetylation of VLB with ^{14}C-

-acetic anhydride, by analogy of the tritium-labelling, and thereafter the preparation of VCR from the obtained labelled VLB by known methods, by an oxidative demethylation[3], followed by formylation[4]. Since N-desmethyl vinblastine (1) could be formylated with a relatively small excess of formic acid, further attempts were made to minimize the amount of formic acid. It was found that 1 can be transformed into VCR by reacting it with 12 moles of formic acid at room temperature for 48 hours. Excess formic acid could be recovered as sodium formate nearly quantitatively, and thus, using ^{14}C-formic acid, a rational method was found for the preparation of VCR positionally labelled with ^{14}C possessing a high specific activity, starting out from an easily available starting material, the N-desmethyl vinblastine[5]. Analogously, the ^{14}C-labelled N-desmethyl-N-formyl-leurosine (5) was prepared from N-desmethyl leurosine (4)[6] in a similar way.



1 R = H
2 R = CH_3
3 R = CHO



4 R = H
5 R = CHO

EXPERIMENTAL

VCR(formyl-^{14}C) sulfate

Onto 159 mg (0.2 mmoles) of 1 were distilled 110.5 mg (2.4

mmoles) of ^{14}C-formic acid, liberated from 163 mg (2.4 mmoles) of sodium formate-^{14}C (111.6 mCi, 46.5 mCi/mmole) with a stoichiometric amount of dry, gaseous hydrochloric acid. After shaking for a few minutes, a thick, syruplike solution was obtained, which was allowed to stand at room temperature in darkness for 48 hours. The mixture was then dissolved in 5 ml water, the solution rendered alkaline with 0.5 ml conc. NH_4OH, and extracted three times with 10 ml portions of dichloromethane. After drying over anhydrous $MgSO_4$ the solution was concentrated in vacuo. The glassy solid residue weighing 186 mg (theoretical yield: 164.6 mg) was chromatographed on a column (120 x 10 mm) filled with alumina (activity grade: III) by elution with the following solvent mixtures: 60 ml benzene/chloroform = 2:1 ; 120 ml benzene/chloroform = 1:1 and 60 ml benzene/chloroform = 1:2. The fractions containing pure VCR were collected and concentrated in vacuo. Yield: 153 mg (0.186 mmoles), 93 %.

The obtained base was dissolved in 2 ml absol. ethanol, and the solution was acidified with a freshly prepared solution of 0.3 ml conc. H_2SO_4 in 100 ml ethanol to pH = 4. Crystallization started upon scratching. After 1 hour standing at room temperature the crystals were collected by filtration, and washed twice with 1 ml portions of ethanol. The product is a white crystalline substance, weighing 141.2 mg (0.153 mmoles), yield: 76.5 %. This substance was dissolved in 1.5 methanol, 6 ml ethanol were added, whereupon crystallization started immediately. After standing for 1 hour at room temperature it was filtered off, and washed twice with 1 ml portions of ethanol. The obtained white crystalline substance weighed 131 mg (0.143 mmoles), yield: 71.5 %. Activity: 6.62 mCi (50.3 mCi/g, 46.3 mCi/mmole).

The chemical and radiochemical purity of the product was tested by tlc. (TLC plastic roll Silica Gel F_{254} Merck; chloroform/benzene/diethylamine = 20:40:30.) A contact autoradiogram was

prepared on Forte X-ray film. Radiochemical purity: > 98 %.

Recovery of the sodium formate-^{14}C

The aqueous solution after extraction with dichloromethane was treated with 2 ml 1 N NaOH solution, and evaporated to dryness in vacuo. The residue was dissolved in 1 ml water, and poured onto a DOWEX 50 $[H]^+$ column, which was eluted with 30 ml water. The eluate was neutralized with sodium hydroxide solution and evaporated to dryness. The recovered sodium formate weighed 147 mg (2.16 mmoles), activity: 100 mCi.

N-Desmethyl-N-formyl(^{14}C)-leurosine sulfate

N-Desmethyl-N-formyl(^{14}C)-leurosine sulfate was prepared as described for VCR, starting out from 198 mg (0.25 mmoles) of 4 and 204 mg (3 mmoles) of sodium formate-(^{14}C) (120.5 mCi, 43.5 mCi/mmole). Yield: 133 mg (1.45 mmoles), 58.1 %. Radiochemical purity: > 98 %, activity 6.2 mCi (46.6 mCi/g, 42.8 mCi/mmole). The recovered sodium formate weighed 178 mg (2.62 mmoles), 105 mCi.

LITERATURE

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