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Syntheses of Colchicine and Isocolchicine Labelled with Carbon-11 or Carbon-13

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Summary

The syntheses of isotopically labelled (-)-10-[^{11}C/^{13}C]-colchicine and (-)-9-[^{11}C/^{13}C]-isocolchicine have been achieved from the reaction of (-)desmethylcolchicine with [^{11}C/^{13}C]-iodomethane. The radiolabelled compounds, (-)-10-[^{11}C]-colchicine (^{11}C-n-colchicine) and (-)-9-[^{11}C]-isocolchicine (^{11}C-i-colchicine), were isolated by reversed phase HPLC. The total synthesis time was approximately 60 minutes for both radiolabelled compounds with an average specific activity of 240 mCi/μmol calculated to EOB. Utilizing a similar synthetic strategy, we also report the synthesis of milligram quantities of the carbon-13 enriched compounds and the magnetic resonance signal assignment for (-)-9-[^{13}C] isocolchicine.

Key Words: [^{11}C/^{13}C]-n-colchicine, [^{11}C/^{13}C]-i-colchicine, ^{11}C/^{13}C-iodomethane, Positron Emission Tomography(PET), Magnetic Resonance Imaging(MRI).

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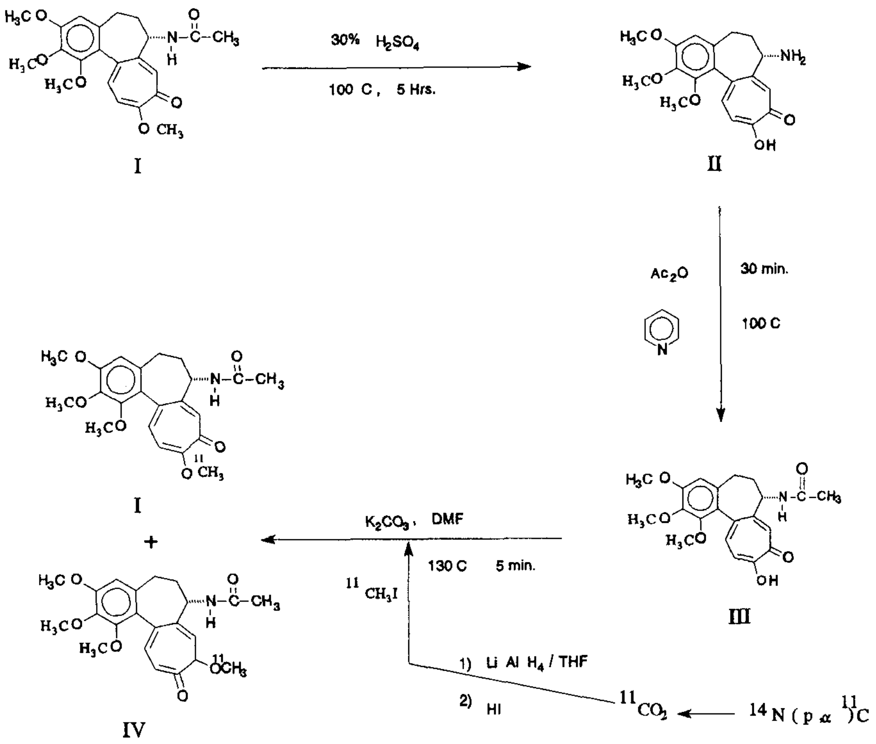
Introduction

Drug resistance, either intrinsic or acquired, is a major obstacle to the clinical management of cancer. Tumor cells that are resistant to natural-product anti-cancer agents such as vinca alkaloids or anthracyclines, often exhibit cross-resistance to other natural-product chemotherapeutic agents. Colchicine, a naturally occuring alkaloid and a potent inhibitor of cellular mitosis, is a member of the multi-drug resistance family of drugs(1,2). Although the specific mechanism of therapeutic and cytotoxic action is not precisely known, preliminary results with both carbon-14 and tritium labelled colchicine indicate that the carbon-11 radionuclide incorporated into the methoxy group attached to the 10-position should remain relatively non-metabolically labile for the short evaluation period required for the assessment of multi-drug resistance(3,4).

Isotopically labelled n-colchicine((-)-10-[^{11}C/^{13}C]-n-colchicine) and i-colchicine((-)-9-[^{11}C/^{13}C]-isocolchicine), were synthesized for pharmacokinetic and metabolic studies.

Results and Discussion

The synthesis of isomeric [^{11}C] n- and i-colchicine was accomplished by O-methylation of desmethylcolchicine (III) with ^{11}C-iodomethane (Scheme I). The requisite desmethylcolchicine (III) was prepared according to literature procedures(5,6,7). In agreement with the literature, care must be taken to dissolve the crude product (III) in 1 N sodium hydroxide with reprecipitation using concentrated hydrochloric acid and subsequent recrystallization from ethyl acetate to yield an analytically pure reagent. In the preparation of [^{11}C]-n-colchicine and [^{11}C]-i-colchicine, following the O-methylation of III in dimethyl formamide and solvent removal in vacuo, chromatographic purification of the products resulted in isolation of both isomers in average radiochemical yields of 20.9% and 24.7% respectively with specific activity of 240 mCi/μmole (EOB). The radiochemical purity of the respective compounds was in excess of 90% with the chemical impurity resulting from the close proximity of the HPLC separation of the two

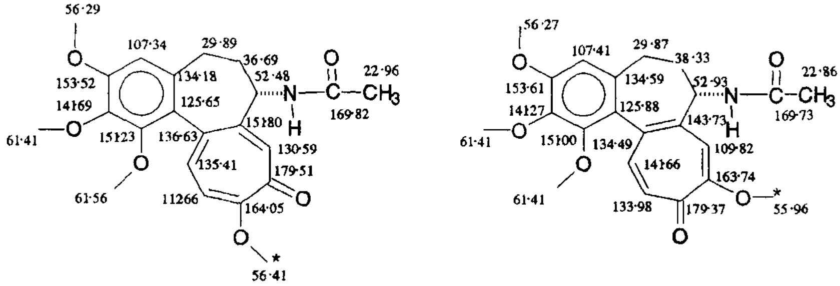


Scheme I

isomers and III. In our experiments, when the isolation of the n- and i-colchicine from the desmethylcolchicine was attempted without removal of DMF, the products had reduced chemical and radiochemical purities. With reference to the synopsis of reaction conditions (Table I), the apparent reduction in percentage yield for the two radiolabelled isomers was compensated by the shortest reaction time and the minimal amount of starting material.

For the preparation of the [^{13}C]-n-colchicine and [^{13}C]-i-colchicine, repetitive fractional injections and sharp collection of the HPLC peaks resulted in analytically pure carbon-13 labelled I and IV. The NMR spectra, TLC (R_f) and chromatographic behavior of labelled products were consistent with the authenticated standard. The [^{13}C]-i-colchicine magnetic resonance signal assignments, in ppm (δ) with reference to tetramethylsilane (δ = 0.0), along with those reported for [^{13}C]-n-colchicine(8) are shown in Figure 1. The ^{13}C-Nuclear Magnetic

Resonance (CMR) Spectra of I and IV exhibited the signal intensities for methoxy carbon at C-10 for I and C-9 for IV that were 2-3 orders of magnitude greater than corresponding signal intensities of the rest of the C-atoms, respectively.



[^{13}C]-n-colchicine **[^{13}C]-i-colchicine**

Nuclear Magnetic Resonance Signals(δ) for[^{13}C]-n-Colchicine and[^{13}C]-i-Colchicine

Figure 1

In summary, we have synthesized [^{11}C]-labelled n- and i-colchicine, compounds of potential value using PET for the evaluation of multi-drug resistance in patients undergoing chemotherapy. Following a similar synthetic scheme, but utilizing repetitive fractional separation technique, we have prepared [^{13}C] enriched n-colchicine and i-colchicine in milligram quantities for the evaluation of these compounds as an indication of MDR using MRI techniques.

Experimental

(-)Colchicine, potassium carbonate, ethyl acetate, pyridine, acetic anhydride, hydrochloric acid, triethylamine and [^{12}C/^{13}C]-iodomethane were purchased from Aldrich Chemical Company, Milwaukee, WI. Concentrated sulfuric acid, N,N-dimethyl formamide (DMF) and methanol were purchased from Fisher Scientific Company, Fairlawn, NJ. The silica gel plates (5cm x 20 cm, 250 μm thickness) used for autoradiography and thin layer chromatography (TLC) were purchased from Whatman International Limited, Clifton, NJ and ethanol (95%) was purchased from Aaper Alcohol and Chemical Company, Shelbyville, KY. Melting point determinations were done using Thomas Hoover Melting Point Apparatus model Unimelt and are reported uncorrected. The optical rotation was determined on a Preston Industries, Niles, IL model SR6 polarimeter. The ^{1}H and ^{13}C nuclear magnetic resonance spectra were recorded using 400MHz spectrometer model AMX 400 manufactured by Brooker Instruments, Billerica, MA. ATI Unicam Company, Madison, WI. model Automass 150 was used to record mass spectra. The radiochromatograms were scanned using BioScan Imaging Scanner model 400 equipped with Autochanger 1000 manufactured by BioScan Inc., Washington, D.C. For the purification/analyses of isomeric colchicines, their precursor and other intermediates a Waters High Performance Liquid Chromatographic (HPLC) system consisting of two Waters model 510 pumps coupled with a Waters Gradient Controller, a U6K Waters injector, LDC Analytical model SM 4000 variable wavelength ultraviolet detector, and Beckman radiation detector model 170 was utilized. ^{14}C-labelled colchicine (specific activity: 36.0 mCi/mmole) was purchased from DuPont NEN Research Products, Boston, MA and used as a reference compound. HPLC grade solvents were used whenever possible. Other solvents were purified by distillation prior to use.

(-)Trimethylcolchicinic Acid (II):

The title compound was prepared according to literature procedure(4). A mixture of 2 g (-)colchicine (5 mmol), 9.4 ml conc. sulfuric acid (170 mmole) and 37.7 ml water was heated for 5 hours at 100°C in an oil bath. The reaction mixture was neutralized with solid sodium carbonate while hot to pH 7-7.5. The resulting lemon yellow frothy mass was filtered, washed with ice-water and air dried while protected from light. The filtrate was extracted with chloroform (3 x 30 ml). The combined chloroform solution was washed with water, dried over anhyd. sodium sulfate. Upon removal of solvent in vacuo, the crude product was recrystallized from methanol to yellow colored fine needle shaped crystals (1.3 g, 76.2%): m.pt.: 153-157°C(lit. 152-158°C); $[\alpha]_D$:221.8°(c:0.586, chloroform (lit. -184.5°c:1, chloroform)); TLC: silica gel plate(250 μm thickness) , 60% 25 mM ammonium acetate:40% acetonitrile at pH 2 adjusted with conc. hydrochloric acid, Rf_{II}:0.64. The ^1H NMR spectrum of II is in agreement with that reported in the literature(9,10).

(-)Desmethylcolchicine(III):

As per the literature report(6), freshly distilled pyridine (6.0 ml, 74.2 mmole), acetic anhydride (2.0 ml, 21.2 mmole) and II (1.0 g, 2.9 mmole) were heated at 100°C for 30 minutes. Upon concentration in vacuo, a dark yellow colored glassy residue was obtained. The residue was dissolved in 20-30 ml 1 M sodium hydroxide. Following filtration, the filtrate was acidified to a pH=2 by dropwise addition of conc. hydrochloric acid. The resultant precipitate was filtered, washed with ice-water and air dried (crude product weight:1.0 g, 89%). The crude product was recrystallized from ethyl acetate to yield an off-white colored amorphous material: m.pt.: 177-178°C; $[\alpha]_D$: -249.8°(lit.(9,10) -252°) m/e: 385; TLC: silica gel plate and 95% acetonitrile:5% glacial acetic acid. Rf_{III}: 0.26. The ^1H NMR spectrum of III is in agreement with literature (10).

Table 1

Synopsis of Reaction Conditions for the Optimization
of
[^{11}C]-n-Colchicine and [^{11}C]-i-Colchicine Syntheses

Amount of Desmethyl-colchicine mg	Reaction Temperature 130°C Reaction time(min)	Starting No Carrier Added $^{11}CH_3I$ Act. mCi*	Yield of [^{11}C]-n-colchicine mCi* (%)	Yield of [^{11}C]-i-colchicine mCi* (%)
6.8	20	70.0	13.1(18.7)	15.9(22.7)
4.7	20	67.6	13.3(19.7)	15.8(23.9)
2.4	10	115.2	24.0(20.8)	28.0(24.3)
2.5	5	123.8	20.2(16.3)	23.8(19.3)
Syntheses of [^{13}C]-Labelled n- and -i-Colchicines				
20.1**	20	8.1mg $^{13}CH_3I$	7.1mg (34.2)	7.8mg (37.5)

* Corrected to the End of Bombardment (EOB)

** Following reaction and removal of the solvent, the product mixture containing [^{13}C]-n- and -i-colchicines was divided into aliquots and purified by HPLC. The individual fractions corresponding to [^{13}C]-n- and -i-colchicines were combined.

Preparation of ^{11}C-Labelled n-Colchicine(I) and i-Colchicine(IV): In order to deduce the optimal reaction conditions, ^{11}C-iodomethane was collected in a V-shaped reaction vial charged with 500μl DMF solution containing 2.5-10 mg desmethylcolchicine (III, 6.5-26.0 μmol) and 0.99-3.94 mg potassium carbonate (7.2-28.6 μmol) using a stream of nitrogen (Table I). The nitrogen carrier gas flow was stopped when the hot cell radiation detector reached a constant value and an additional 200μl DMF was added to the reaction vial. The reaction mixture was heated with stirring in an oil bath at 130°C for 5-20 minutes. The product mixture was transferred to a 10 ml round bottom flask and solvent was removed in vacuo. The residue was dissolved in 300-500 μl mobile phase and the mixture was loaded onto two serially connected 250mm x 10mm id Spherisorb 5μ ODS2 columns. Using 49.9% water: 49.9% methanol: 0.2% triethylamine as a mobile phase flowing at 2.0 ml/min, ^{11}C-labelled isomeric colchicines were separated from the product mixture. The retention times for I and IV were 23.7 minutes and 26.9 minutes respectively. Upon removal of the mobile phase in vacuo, [^{11}C]-n-colchicine and [^{11}C]-i-colchicine were each dissolved in 1-2 ml of 0.9% sodium chloride solution. Product sterilization was achieved by terminal filtration. The same methodology has been employed successfully to synthesize [^{13}C]-labelled n- and i-colchicine by reaction of commercially available [^{13}C]-iodomethane, III and potassium carbonate in DMF in a 1.1: 1.0: 1.1 molar ratio.

Determination of Radiochemical Purity and Specific Activity:

Radiochemical Purity: The radiochemical purity of the product was determined by TLC and analytical HPLC (see conditions below for assaying Specific Activity). Authenticated samples of [^{14}C]-n-colchicine and n-colchicine obtained from DuPont-NEN and Aldrich Chemical Company respectively, were used as chromatographic standards. The standards were either co-spotted or co-injected along with an aliquot of the radioactive product and the congruence of mass and radioactivity was ascertained. For TLC analyses, silica gel plates were used with visualization via short wavelength ultraviolet lamp or TLC scanner. The R_f values for n-colchicine and i-colchicine were 0.55 and 0.61 respectively in a solvent mixture of acetonitrile: aq.ammonia (95:5).

Specific Activity: An aliquot of the final product was assayed for specific activity by analytical HPLC (Spherisorb ODS, 5 μ, 250 x 10 mm column, water:methanol:triethylamine (49.9:49.9:0.2)). A LDC Analytical variable wavelength detector operated at 240 nm and Beckman radiation detector were used to detect mass and radioactivity. [^{11}C]-labelled n-colchicine and i-colchicine eluted at 8.05 minutes and 9.36 minutes respectively, with a flow rate of 3.0 mL/min.

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