Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXVII, No. 8

TOTAL SYNTHESIS OF [7-^{14}C]-(±)-COLCHICINE*

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SUMMARY

The synthesis of (±)-colchicine 1 labelled with carbon-14 at the 7-position of the B ring was achieved by a sixteen step sequence with an overall radiochemical yield of 2.5% from [$Ba^{14}CO_3$] (Specific activity : 55 mCi·mmol^{-1}).

Keywords : Colchicine, (3,4,5-trimethoxyphenyl)butyric acid, carbon-14

INTRODUCTION

Colchicine 1 is an alkaloid occurring in members of the Liliacea, especially in meadow saffron (Colchicum autumnale) and in the African climbing lily (Glorosia superba). Compound 1 is used, probably for several centuries[1], in the treatment of acute gouty arthritis and more recently against the familial mediterranean fever[2] , the Behçet's syndrome[3]... Colchicine is a potent mitotic inhibitor[4] but is too toxic to be of value as an antitumor drug[5]. Although its specific mechanism of therapeutic and cytotoxic action is still imperfectly known the pharmacological effects of colchicine may be related to its binding to tubulin which prevents microtubule polymerization and consequently cell division[6]. Investigations on the biotransformation of colchicine have been limited

* Presented at the Third International Symposium on the Synthesis and Applications of Isotopically Labelled Compounds, Innsbruck, Austria, July 17-21, 1988.
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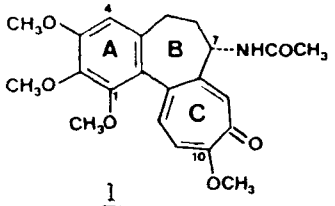
0362–4803/89/080927–11$05.50

Received December 20, 1988
Revised February 14, 1989

to those employing molecules labelled on a methoxy group. Thus Schönharting et al.[7], then Hunter and Klaassen[8] reported that this drug undergoes oxidative O-monodemethylation at the 2, 3, or 10-positions. In an attempt to study the hepatic metabolism of colchicine in the rat[9] (biotransformation, metabolic activation) we have first prepared[10] the (±)-colchicine tagged in the 1-methoxy group described as a non metabolically labile group[7]. With a view for in vivo assays, a synthesis of (±)-colchicine labelled with carbon-14 in the ring has been undertaken.



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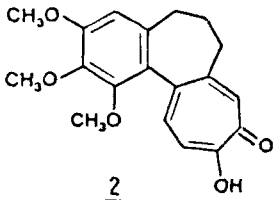

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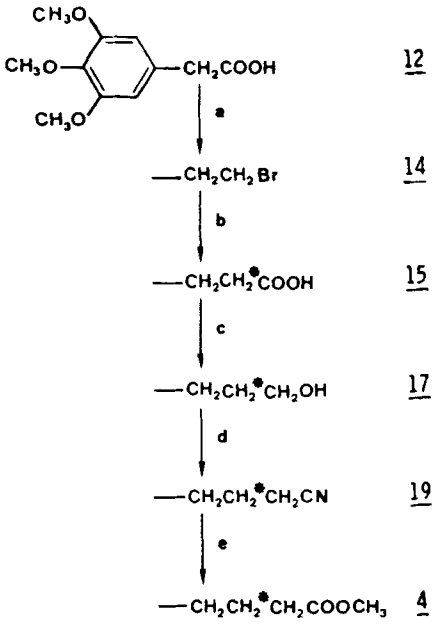
Since 1961 some twelve total syntheses of (±) colchicine 1 have been reported[11]. In all but three instances[11c,e,k] desacetamidocolchiceine 2 was the key intermediate. The final introduction of the acetamido group, required in the conversion of 2 to (±)-colchicine 1, was detailed in the syntheses devised by Schreiber et al.[11a] and van Tamelen et al.[11b], but with a poor yield, below 1%.

Among the three syntheses giving rise directly to (±)-colchicine 1 or to its amino derivative, desacetylcolchiceine 3, the notable work of Evans et al.[11k] was the most suitable process for a radiolabelled preparation. Herein we have worked out the design of a total synthesis of colchicine including the sequence of reactions described by these authors (scheme II). By this strategy the sixteen carbons, as constituent part of the colchicine tricyclic skeleton, are provided in the first step by the two components : methyl 4-(3,4,5-trimethoxyphenyl)butyrate 4 and 4,5,5-trimethoxy-bicyclo[4.1.0]hept-3-en-2-one 5. We have opted for radiolabelling of the ester 4 as outlined in scheme I, by two successive homologation reactions of 3,4,5-trimethoxyphenylacetic acid 12. By this way the total synthesis of colchicine labelled with carbon-14 at the 7- position was attempted.

RESULTS AND DISCUSSION

Acid 12 was converted into the bromide 14 (71 % overall yield) on lithium aluminium hydride (LAH) reduction[12] to give the alcohol 13, and subsequent treatment with the N-bromosuccinimide/triphenyl-phosphine reagent[13]. Carbonation with $^{14}CO_2$ (1700 mCi, SA : 55 mCi or 2035 MBq·mmol^{-1}) of the Grignard reagent of 14 afforded [carboxyl-^{14}C] 3-(3,4,5-

trimethoxyphenyl)propionic acid 15 in 85% yield. Experimental conditions were defined by "low activity" carbonation assays on Grignard reagent (excess 50%)[9]. Acid 15 was successively treated in diethyl ether solution with diazomethane and lithium aluminium hydride giving rise to the γ-phenylpropanol 17[11h] which was converted to the chloride 18[14] using thionyl chloride and N,N-dimethylaniline (DMA). 18 condensed with sodium cyanide in dimethylformamide (DMF) provided the nitrile 19[15] which was hydrolyzed with potassium hydroxide into the butyric acid 20, isolated in a 54% overall yield from $Ba^{14}CO_3$. Upon treatment with diazomethane, 20 provided [2-^{14}C] methyl 4-(3,4,5-trimethoxylphenyl)butyrate 4 which displayed spectroscopic properties identical with those disclosed for the same compound previously prepared by Evans et al.[11k] from 3,4,5-trimethoxycinnamic acid.

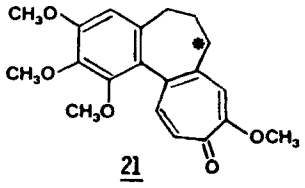


a) 1- LAH, ether, 34 °C, 2h, 82%
2- NBS-Ph_3P, 20 °C, 16h, 87%
b) 1- Mg, THF, 50 °C, 4h.
2- $^{14}CO_2$, -20 °C, 3h, 85% from $^{14}CO_2$
c) 1- CH_2N_2, ether, 20 °C, 3h.
2- LAH, ether, 20 °C, 3h, 92%
d) 1- $SOCl_2$, DMA, $CHCl_3$,. 61 °C, 1.5h, 85%
2- NaCN, DMF, 40 °C, 5h, 99%
e) 1- EtOH, KOH, 100 °C, 4h, 83%
2- CH_2N_2, ether, 20 °C, 3h
* denotes position of ^{14}C label.

Scheme I : Synthesis of [2-^{14}C]-methyl 4-(3,4,5-trimethoxyphenyl)butyrate 4

Conversion of the methyl δ-phenylbutyrate ester 4 to (±)-desacetylcolchiceine 3 was realized according to the procedure described by Evans et al.[11k] as outlined in scheme II. Ester 4 deprotonated with lithium diisopropyl amide (LDA) in tetrahydrofuran at -78°C was condensed with the cyclopropyl ketone 5 to afford alcohol 6. Without purification, 6 was hydrolyzed with aqueous oxalic acid in tetrahydrofuran to provide the γ-hydroxyenone 7, in 60% yield after chromatogaphy

as a mixture of two diastereomers. Compound 7 showing a pronounced instability, mainly due to radiolysis, must be immediately used after purification. Treatment of 7 with refluxing trifluoroacetic acid (TFA) for 40 min afforded diastereomeric dihydrotropolones 8 in 70% yield after chromatography. As reported by Evans et al.[11k] this cyclization process requires the intervention of a spiran intermediate which then rearranges, with aryl migration, to afford the tricyclic compound 8. Esters 8 were oxidized with 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) to tropolone ether 9 then hydrolyzed (NaOH-aqueous methanol for 40 h) at room temperature to afford tropolonic acid 10 in 98% yield. When the hydrolysis was carried out at reflux[11k] a by-product was formed, proportionally to the reaction time, probably the corresponding tropolone[16]. Acid 10 was converted to amine through a modified Curtius reaction. Treated with diphenylphosphoryl azide (DPPA) and triethylamine in tert-butyl alcohol, 10 gave rise to the carbamate 11 in 49% yield after chromatography. 8% of a decarboxylated product, [7-^{14}C]-desacetamidoisocolchicine 21, was also isolated. By acid hydrolysis at reflux for 2 h, carbamate group and ether linkage were cleaved to provide [7-^{14}C]-desacetylcolchiceine 3 (92%), which displayed spectroscopic (uv, ^{1}H nmr, ms) and chromatographic properties identical with those of an authentic sample of (-) 3 prepared from (-)-colchicine via standard procedures[17]. The [7-^{14}C]-(±)-desacetylcolchiceine 3 was obtained in an overall yield of 6.5% from $Ba^{14}CO_3$.



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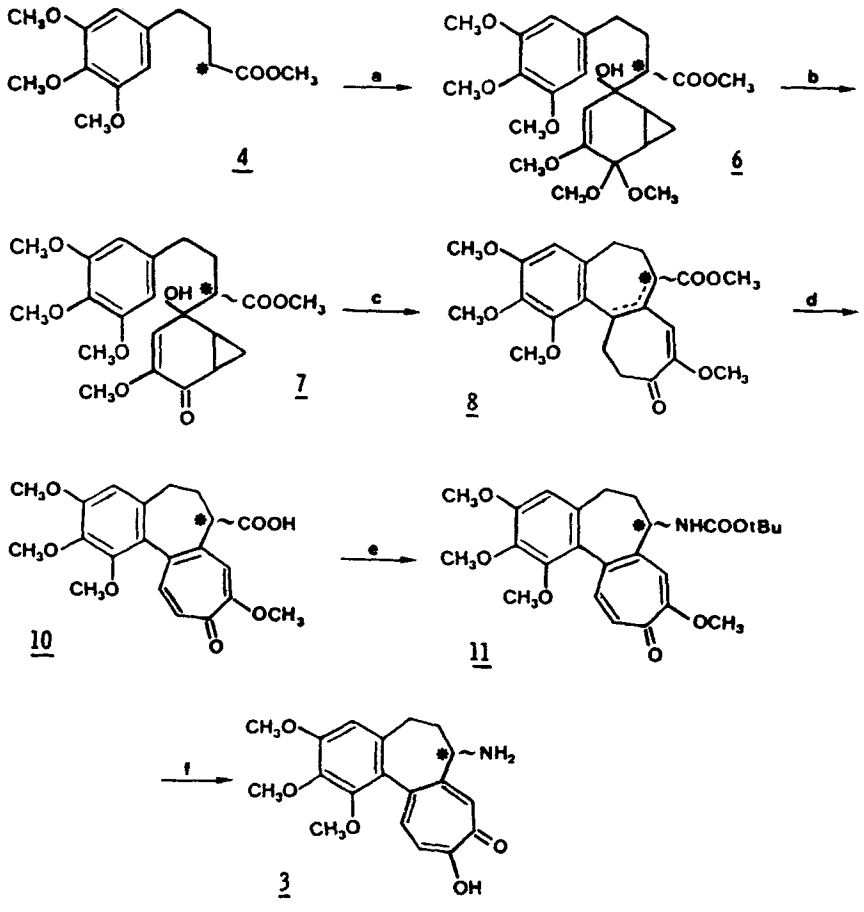
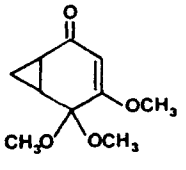
Completion of the synthesis was accomplished by means of N-acetylation and O-methylation reactions[17,18]. The yield of colchicine depends on the order chosen to carry out these two steps. Since tropolone methylation is not regioselective two isomeric ethers are produced, normal and iso colchicinic derivatives. Methylation of desacetylcolchiceine 3 promotes formation of the iso derivative (3:1)[17,19], however methylation of the acetylated product, colchiceine 22, provides a mixture of isocolchicine 23 and colchicine 1, the latter being favoured[20]. Accordingly we have opted for methylation of colchiceine 22 (Scheme III).



a) 1- LDA, THF, -78 °C, 0.5h
2- 5, -78 °C, 35 min then -5 °C, 35 min
b) THF-H_2O, HOOC-COOH, 20 °C, 4.5h, 60%
c) TFA, 72 °C, 40 min, 70%
d) 1-DDQ, Bz, 80 °C, 18h, 66%
2-MeOH, NaOH, 20 °C, 40h, 98%
e) tBuOH, DPPA, Et_3N, 82 °C, 18h, 49%
f) HCl 2N, 100 °C, 2h, 92%



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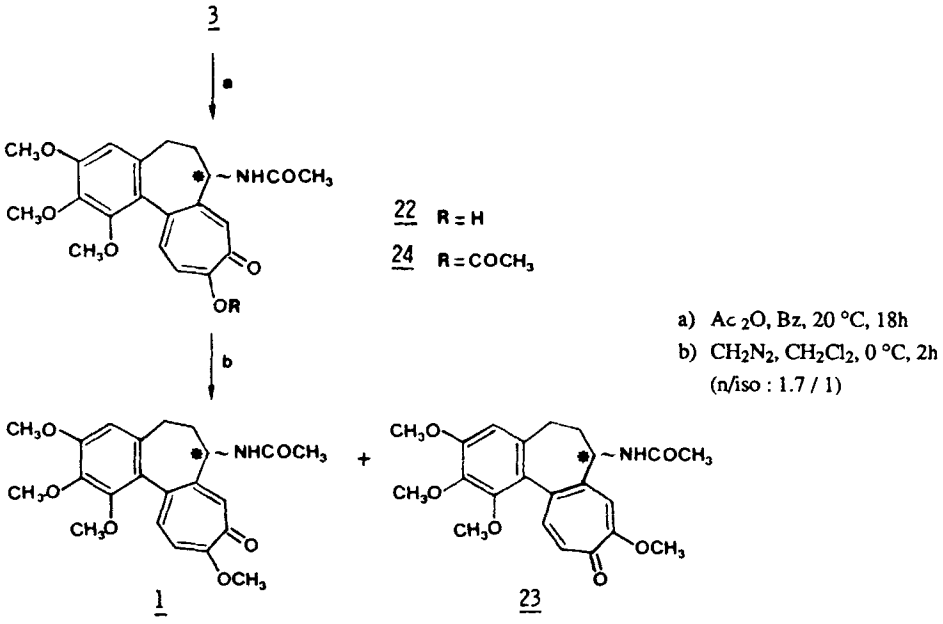
Scheme II : Synthesis of [7-^{14}C]-(±)-Desacetylcolchiceine 3[11k] :

Scheme III



Upon treatment with acetic anhydride in benzene[21] at room temperature compound 3 afforded the required colchiceine 22 (80%) contaminated with 2% of the diacetylated product, O-acetylcolchiceine 24[9]. O-methylation of crude 22, using diazomethane, provided regioisomers 1 and 23 as a 1.7:1 mixture determined by radiochromatography. After chromatographic purification on silica gel column [7-^{14}C]-(±)-colchicine 1 was obtained in an overall yield of 2.5% from $Ba^{14}CO_3$. The chemical and radiochemical purity were determined by HPLC and TLC, and were better than 98%. Specific activity measured by mass spectrometry on molecular ion (m/z:401) was 55 mCi or 2035 MBq · mmol^{-1}, in agreement with that calculated from ultraviolet spectrometry analysis and liquid scintillation counting. Comparison by means of spectroscopic (^{1}H nmr, ms, uv) and chromatographic data showed that the synthetic radiolabelled (±)- colchicine was indistinguishable from the natural product, except for its optical rotation.

EXPERIMENTAL

When necessary, solvents and reagents were dried prior to use. Tetrahydrofuran was distilled from sodium and benzophenone, dimethyl formamide from phosphorus pentoxide. All other solvents were of reagent grade and stored on molecular sieves. All anhydrous reactions were run under argon with rigorous exclusion of moisture. $Ba^{14}CO_3$ was a gift of Service des Molécules Marquées (CEN-

Saclay, France). Analytical thin-layer chromatography (TLC) was carried out on silica gel (60F-254 Merck) or reverse phase (KC18F Whatman) plates. Radiochemical purity was determined by radiochromatogram scanning of TLC on a Berthold scanner, model LB-282/511. Preparative liquid chromatography was carried out on a Jobin-Yvon Miniprep LC system by using TLC Kieselgel 60H Merck (5-40 μm) silica gel powder. Radioactivity was measured with a Bremsstrahlung counter (Berthold LB 2040) or a liquid scintillation counter (LKB 1211 Rackbetta). Melting points were taken on a Reichert apparatus and are uncorrected. Infrared spectra (ir) were recorded on a Beckman spectrometer, ultraviolet spectra (uv) on a Beckman 5230 instrument and mass spectra (ms) on a Varian CH 7A spectrometer. ^{1}H nuclear magnetic resonance spectra (nmr) were determined on a Perkin Elmer R12 (60 MHz) or a Bruker WP100 (100 MHz) spectrometers in $CDCl_3$ with tetramethylsilane (TMS) as the internal reference. Chemical shifts are expressed in ppm downfield from TMS. When the chemical shifts and the coupling constants are in agreement with the literature values, only appropriate references are given. ^{14}C-intermediates have spectral and chromatographic properties identical to the authentic samples. Except for (±)-colchicine 1 cited values for physical data are those of unlabelled compounds.

[Carboxyl-^{14}C]-3-(3,4,5-trimethoxyphenyl)propionic acid (15)

A mixture of 4.68 g (17 mmol) of 2-(3,4,5-trimethoxyphenyl)ethylbromide 14[12,13] and 0.468 g (17 mmol) of dry magnesium turnings in 57 ml of anhydrous THF was warmed at 50° C for 4 h. 56 ml of the resulting Grignard solution (50% excess) were carbonated, at -20 °C for 3h, by dry $^{14}CO_2$ (10.9 mmol) generated from $Ba^{14}CO_3$ (610 mCi, 11 mmol, 55 mCi·mmol^{-1}) and concentrated sulfuric acid. The reaction mixture was acidified with 20 ml of 6N H_2SO_4 and continuously extracted with ether. The organic phase was concentrated in vacuo to a small volume, the residue taken up in 30 ml of 10% Na_2CO_3 and the impurities extracted with ether. The aqueous phase was acidified and extracted thoroughly with ether. The combined ether layers were dried ($MgSO_4$) to afford 512 mCi of 15. On the whole three successive carbonations from 12.9 g (47.1 mmol) of bromide 14 and 1700 mCi of $Ba^{14}CO_3$ gave 1462 mCi (26.5 mmol) of acid 15 in 86 % yield from $Ba^{14}CO_3$ and with a radiochemical purities of 90-98%. ms : m/z : 240 (M^+), 225, 181.^{1}H-nmr : lit.[22].

[1-^{14}C]-3-(3,4,5-trimethoxyphenyl)propanol (17) :

A solution of 410 mCi (7.4 mmol) of acid 15 in 200 ml of dry diethylether was treated at 0°C with 23 ml of an ethereal solution of CH_2N_2 (0.48 M). After stirring 3h at room temperature radiochromatography showed that methylation is achieved (purity > 98%). The solvents were

evaporated to dryness. To the residue redissolved in 150 ml of dry diethyl ether and cooled in an ice bath was added 80 ml of lithium aluminium hydride solution in the same solvent (0.2 M). After stirring at room temperature for 3 h, the excess reagent was destroyed by adding moist ether and then water. The resultant mixture was treated with 6N H_2SO_4, the ether layer separated and the aqueous phase extracted well. The combined ether extracts were washed with water and dried ($MgSO_4$). Three similar reductions from 1462 mCi of acid 15 afforded 1345 mCi (92%) of alcohol 17, purity >95%. ms m/z : 226 (M^+), 181. ^1H nmr : lit.[11h].

[1-^{14}C]-1-chloro-3-(3,4,5-trimethoxyphenyl)propane (18) :

Over a period of 70 min a solution of 15.8 mmol of thionyl chloride in 15 ml of dry chloroform was added to a solution of 580 mCi (10.5 mmol) of alcohol 17 and 2 ml (15.7 mmol) of dimethylaniline in 60 ml of dry chloroform cooled in an ice bath. The reaction mixture was stirred at 0°C for an additional 30 min and then was heated at reflux for 1.5 h. After cooling 60 ml of ice-water were added and the aqueous phase extracted with chloroform. The combined organic phase was dried ($MgSO_4$) and concentrated in vacuo. The residual brown oil was chromatographed on a column of silica gel eluted with dichloromethane-methanol (99.5 : 0.5) to afford 440 mCi of chloride 18. By two successive preparations from 1345 mCi of 17, 1143 mCi of chloride 18 were obtained (85%, purity >97%). ms m/z : 246 and 244 (M^+), 231 and 229, 181. ^1H nmr (60 MHz, $CDCl_3$) : δ 2.00 (m, 2H, C-2-CH_2), 2.67 (m, 2H, C-3-CH_2), 3.50 (m, 2H, C-1-CH_2), 3.78 (s, 3H, OCH_3), 3.80 (s, 6H, two OCH_3), 6.38 (s, 2H, ArH).

[2-^{14}C] 4-(3,4,5-trimethoxyphenyl)butyronitrile (19) :

To a solution of 383 mCi of 18 in 50 ml of anhydrous DMF was added in glove box NaCN (452 mg, 9.2 mmol). The mixture was heated at 40°C for 5 h, concentrated in vacuo to about 2 ml, taken up with ice water (50 ml), and extracted with dichloromethane (4 x 50 ml) to yield 379 mCi of the nitrile 19. Three successive preparations from 1143 mCi of 18 provided 1131 mCi of nitrile 19 (99%, purity>96%). The unlabelled compound was obtained as colourless crystals (ether-petroleum ether); mp 36°C (lit.[15] bp 150-160°C at 0.4 mm). ms m/z : 235 (M^+), 220, 181.^1H nmr (60 MHz, $CDCl_3$) : δ 1.99 (m, 2H, C-2-CH_2), 2.38 (m, 2H, C-1-CH_2), 2.77 (m, 2H, C-3-CH_2), 3.87 (s, 3H, OCH_3), 3.90 (s, 6H, two OCH_3), 6.47 (s, 2H, ArH).

[2-^{14}C] 4-(3,4,5-trimethoxyphenyl)butyric acid (20) and methyl ester (4) :

To a solution of 440 mCi (8mmol) of nitrile 19 in 20 ml of ethanol was added 20 ml of 4M KOH.The mixture was heated at 100°C for 4h, concentrated in vacuo and diluted with 150 ml of water. Impurities (21 mCi) were eliminated by continuous extraction with ether. The aqueous phase

was acidified with 17 ml of 6N H_2SO_4 and extracted thoroughly with ether. The organic layer was washed with water and dried ($MgSO_4$) to give 360 mCi of 20. Thus, by three hydrolyses from 1120 mCi of the nitrile 19, 930 mCi of acid 20 were prepared (83%, purity >98%) .ms m/z : 254 (M^+), 239, 195, 181. ^{1}H-nmr (60 MHz, $CDCl_3$): 2.02 (m, 2H, C-2-CH_2), 2.47 (m, 2H, C-1-CH_2), 2.67 (m, 2H, C-3-CH_2), 3.90 (s, 3H, OCH_3), 3.93 (s, 6H, two OCH_3), 6.48 (s, 2H, ArH). Esterification was achieved according to the procedure used in the synthesis of 16. So, 930 mCi of the methyl butyrate 4 were obtained with a purity of 98%. ^{1}H-nmr : lit[11k].

[7-^{14}C]-(±)-Desacetylcolchiceine (3) :

Ester 4 (867 mCi) was converted into 3 (72 mCi) in seven steps according to the procedure previously described[11k] as outlined in scheme II. This material was identical (^{1}H nmr, ms , TLC : reverse phase : methanol - 0.1M K_2HPO_4, 7:3) with an authentic sample of 3 prepared by acidic hydrolysis of colchicine[17]. ms m/z : 343 (M^+), 328, 312, 207.

[7-^{14}C]-(±)-Colchiceine (22) :

Desacetylcolchiceine 3 (56 mCi, 1.1 mmol) in 22 ml of benzene and 1.2 ml of acetic anhydride were stirred at room temperature for 18 h. The mixture was hydrolysed by ice water and extracted with dichloromethane (2 x 40 ml). The combined organic layers were dried ($MgSO_4$) and provided, on evaporation, 45 mCi of (±)-colchiceine 22 which contained 2% of O-acetylcolchiceine 24 (purity of 80% determined by radiochromatography on TLC : reverse phase, methanol - 0.1M K_2HPO_4, 7:3), ^{1}H nmr : lit.[23].

[7-^{14}C]-(±)-Colchicine (1) and [7-^{14}C]-(±)-Isocolchicine (23) :

A solution of 43 mCi (0.8 mmol) of colchiceine 22 in 20 ml of dry dichloromethane was treated with 8 ml of ethereal diazomethane (0.15M) at 0°C for 2h. Radiochromatography (TLC, silica gel, chloroform-acetone-diethylamine, 7:2:1) of an aliquot of the mixture, showed that 90% of colchiceine was converted into two methylated isomers, with a 1/0.6 ratio of colchicine (Rf : 0.65) and isocolchicine (Rf : 0.40) . The solvents were evaporated in vacuo and the residue chromatographed on a column of silica gel (4 x 45 cm) eluted with chloroform-isopropanol, 9:1 (12 ml / min / fraction). Fractions 43-58 afforded 21 mCi (49%) of labelled (±)-colchicine 1 (purity > 98%). ms m/z : 401 (M^++2), 373, 314. ^{1}H-nmr : lit.[23]. [α] = 0.00° (c 0.29, $CHCl_3$). Fractions 102-130 gave 14 mCi of labelled (±)-isocolchicine 23. HPLC : column Sup-RS (Prolabo); 25 cm x 4.5 mm i.d; mobile phase : methanol-H_2O-triethylamine, 50:50:0.05; flow rate 0.5 ml / min; uv detector at 254 nm; R_T 1: 20.2 min, R_T 23: 22.4 min.

ACKNOWLEDGEMENTS

We are grateful to Drs. A. Vanhove and J. P. Beaucourt for their kind hospitality at the Service des Molécules Marquées, Centre d'études Nucléaires de Saclay, where this synthesis has been carried out. One of us (RP) acknowledges Prof. L. Pichat for stimulating discussions and his constant interest to this work and L. Sergent for mass and nmr spectral measurements.

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