Journal of Labelled Compounds and Radiopharmaceuticals—Vol. XXV, No. 4

[^{14}C] - Radiolabeling of {[trans-(8β)]-6-Methyl-1-(1-Methylethyl) Ergoline-8-Carboxylic Acid, 4-Methoxycyclohexyl Ester (Z)-2-Butenedioate (1:1)},

A Potent And Selective 5HT$_2$-Receptor Antagonist

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SUMMARY

[^{14}C]-Labeled {[trans-(8β)]-6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid, 4-methoxycyclohexyl ester (Z)-2-butenedioate (1:1)} (LY281067) (**10**) was synthesized from unlabeled 6-methyl-1-(1-methylethyl)ergoline-8-carboxylic acid (**2**). The [^{14}C] label was introduced into the carboxyl group attached to the 8 position of the ergoline nucleus. This site is stable to metabolism. The synthesis involves removal of the unlabeled carboxyl group from **2** and subsequent reinsertion of a [^{14}C]-labeled carboxyl group into the same position. The radiolabel is not introduced until near the end of the synthesis which allows for ease of handling and scale-up of intermediates.

Keywords: LY281067, ergoline, 5HT$_2$-receptor antagonist, [^{14}C]

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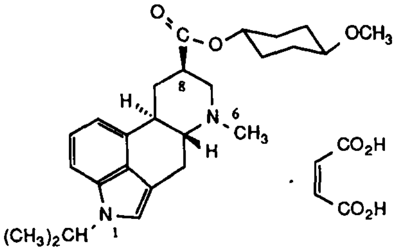
INTRODUCTION

LY281067 (**1**) has been shown to be a potent and selective 5HT$_2$-receptor antagonist.[1] Radiolabeling of **1** was required for metabolism studies in laboratory animals and man. It was desirable that a [^{14}C]-label be placed in a metabolically stable site in the



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0362–4803/88/040429–10$05.00

Received July 7, 1987
Revised August 6, 1987

ergoline nucleus. Previously, the ergoline nucleus has been radiolabeled biosynthetically by fermentation of [^{14}C]-D,L-tryptophan.[2] A [^{3}H]-label has also been introduced at the 8 position by tritium exchange[2,3], at the 9, 10 positions by tritium reduction of ergotamine[4] or 1-methyllysergamide[5], and at the 2 position by halogen-tritium exchange in 2-bromoergostine.[2] We have developed a synthetic route that would introduce a [^{14}C]-radiolabel into the ergoline nucleus on the C-8 carboxyl group.

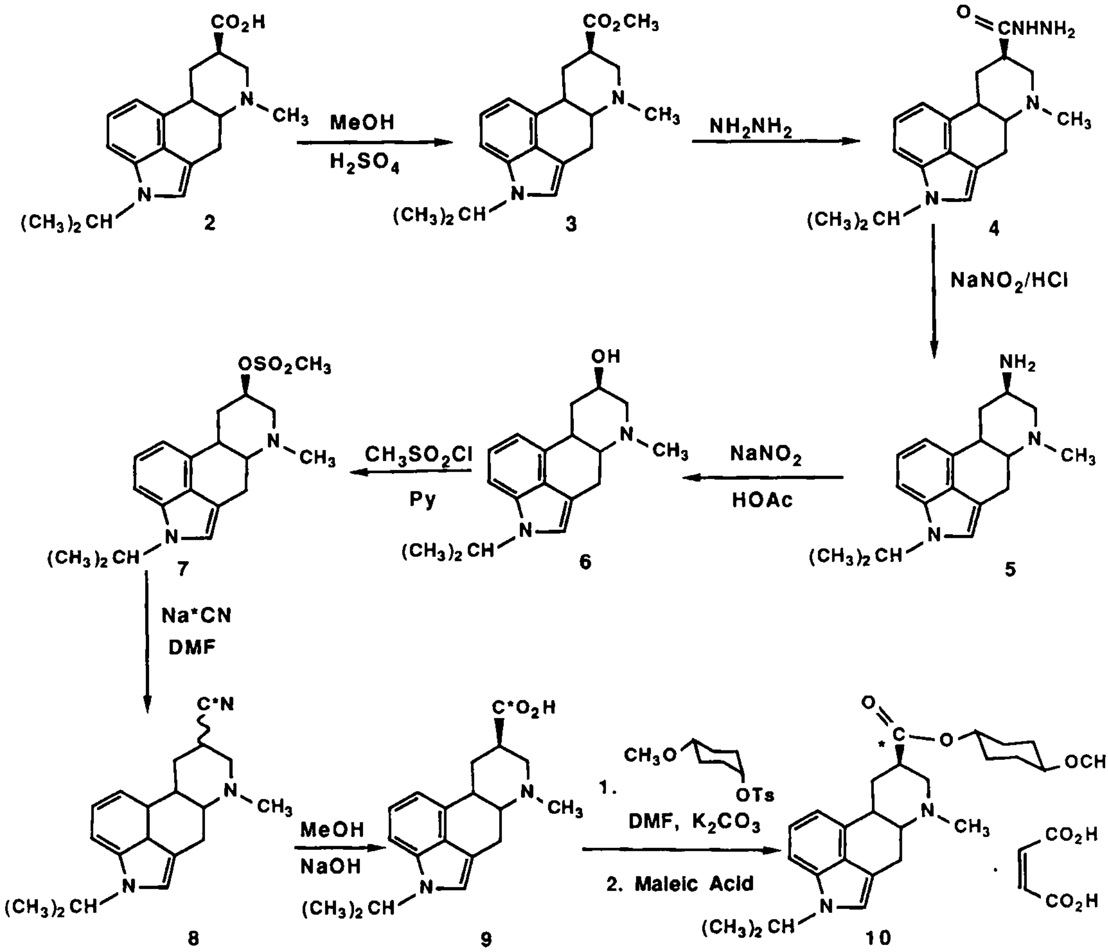
RESULTS AND DISCUSSION

Preliminary metabolism data indicated that the isopropyl group in the N^{1} position, the methyl group in the N^{6} position, and the ester were all subject to cleavage in vivo.[6] Placement of the radiolabel in any one of these positions, while relatively easy, would result in the loss of an unknown percentage of the radiolabel from the ergoline nucleus during metabolism. Accurate determination of the metabolic fate of the ergoline nucleus would therefore be impossible. The only remaining site available for modification without interfering with the integrity of the ring system was the carboxyl group attached to the 8 position.

Scheme I outlines the synthetic route used to incorporate the [^{14}C]-radiolabel. 6-Methyl-1-(1-methylethyl)ergoline-8-carboxylic acid (2), readily prepared from dihydrolysergic acid[7], was converted to the methyl ester 3 by stirring overnight in methanol containing sulfuric acid. Reaction of 3 with refluxing anhydrous hydrazine yielded hydrazide 4. Curtius rearrangement of the corresponding acylazide, prepared in situ by treatment of 4 with sodium nitrite in hydrochloric acid, gave primary amine 5. This reaction proceeded in a stereospecific manner yielding only the β-isomer. Conversion of 5 to alcohol 6 could be accomplished using diazotization chemistry.[8] Reaction of 5 with sodium nitrite in aqueous acetic acid yielded a mixture of 6 and the corresponding acetate. Isolation of this mixture followed by treatment with sodium hydroxide yielded impure 6. Crystallization of 6 as its maleate salt resulted in substantial purification. The salt was converted back to the free base for further reaction. While the yield of the reaction was lower than desired (~50%), only the β-isomer was isolated. Preparation of mesylate 7 from 6 by reaction with methanesulfonyl chloride in pyridine proceeded in high yield.

The [^{14}C]-radiolabel was introduced into the molecule at this point by reaction of 7 with sodium [^{14}C]-cyanide (specific activity of 49.5 mCi/mmol) in dimethylformamide at

Scheme 1



* INDICATES $[^{14}C]$ RADIOLABEL

60°C to yield 8. Somewhat surprisingly, the stereochemical integrity at the 8 position was lost during this reaction. The stereochemical scrambling is explainable if one assumes there is an equilibrium between free mesylate anion generated during the displacement reaction and unreacted 7, although this hypothesis was not tested. Base hydrolysis of 8 in aqueous methanolic sodium hydroxide yielded principally the desired β-isomer of 9. Examination of an unlabeled sample revealed that less than 3% of the α-isomer was present. This was fortuitous, but not surprising, in view of the earlier finding by Stoll et al., that the saponification of the methyl ester of dihydroisolysergic acid yielded the thermodynamically more stable dihydrolysergic acid.[9] Reaction of 9 with cis-4-methoxycyclohexyl tosylate[1], in dimethylformamide in the presence of potassium carbonate and subsequent conversion to the maleate salt, yielded 10 with a specific activity of 46.7 μCi/mg (26.8 mCi/mmol).

This synthetic route has a number of attractive features. Most importantly, the radiolabel is not introduced until near the end of the synthesis which allows for ease of handling and purification of the intermediates. Also, since the starting material contains all the chiral centers desired in the final product, no asymmetric synthesis is required. The stereochemistry of the ring system remains unaffected during the sequence of reactions. Finally, there is the potential of using this synthetic approach for the [^{14}C]-radiolabeling of other ergoline based drugs since many are also substituted with a carbon atom in the 8 position.

EXPERIMENTAL

Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Specific activity was determined in 1:10 methanol:PCS scintillation fluid (Amersham) and was measured on a Packard Model 1500 liquid scintillation spectrometer. Identities of all non-radioactive compounds were confirmed by ^{1}H-NMR, mass spectra, and combustion analysis. Radiolabeled compounds were compared to authentic samples of unlabeled compounds. Chemical and radiochemical purity was assessed by either TLC on Merck F254 silica gel plates or by HPLC using a 15 cm Altex ODS column (mobile phase: 75:25 MeOH/0.1MNH$_4$OAc, flow rate at 1.5 ml/min) using either an ultraviolet detector (254nm) or a Radiomatic Flo-One radiochemical detector. Microanalyses were provided by the Physical Chemistry department of the Lilly Research Laboratories. Physical data for all the unlabeled intermediates are given in Table I.

(8β)-6-Methyl-1-(1-methylethyl)ergoline-8-carboxylic acid, methyl ester (3)

A solution of 15.6 g (50.0 mmol) of 2 in 500 mL methanol and 9.8 g (100 mmol) of sulfuric acid was stirred at room temperature for 20 hr. The solution was partially evaporated until crystals formed, the pH of the mixture was adjusted to 8 by the addition of concentrated ammonium hydroxide, and then diluted by the dropwise addition of 400 mL water. After cooling, the product was collected and dried in vacuo to yield 12.4 g of 3 (76% theory yield) mp 109-110°C. TLC (chloroform/methanol/acetic acid 18:6:1) indicated that the material was pure (r_f=0.87).

TABLE I - PHYSICAL DATA FOR UNLABELED COMPOUNDS

Compound No.	MS(70ev)[a] M/e (%)	^{1}H-NMR[b](SOLVENT) ppm	M.P.[c] (Recrys. Sol.)	Analysis	Theory	Found
3	326 (M^+,100)	$CDCl_3$/TMS 1.5-1.8(M,7H), 2.2(dt,1H,J-3Hz) 2.35(t,1H,J=12Hz), 2.5(S,3H), 2.70(t,1H,J=12Hz), 3.0(M,3H), 3.25(d,1H,J=7Hz), 3.40(dd,1H,J=3Hz) 3.75(S,3H), 4.62(SEPT,1H,J=6Hz) 6.90(M,2H), 7.15(d,2H,J=3Hz)	109-110°C MeOH/H_2O	C H N	73.59 8.03 8.58	73.31 7.97 8.35
4	326 (M^+,100)	$CDCl_3$/TMS 1.52(dd,6H,J=2Hz), 2.75(QUAR,2H,J=12Hz) 2.15(dt,1H,J=6Hz), 2.50(S,3H), 2.7(M,3H) 2.45(dt,1H), 3.10(m,1H), 3.40(dd,1H,J=6Hz) 3.95(d,2H,J=6Hz), 4.60(QUINT,1H,J=6Hz) 6.90(m,3H), 7.15(M,2H)	216-219°C	C H N	69.91 8.03 17.16	69.84 8.14 16.93
5	283 (M^+,100)	$CDCl_3$/TMS 1.22(QUART,1H,J=12Hz), 1.5(dd,8H,J=3Hz) 1.98(t,1H,J=12Hz), 2.15(dt,1H,J=6Hz) 2.45(S,3H), 3.65(t,1H,J=12Hz), 2.8-3.2 (M,4H), 3.4(dd,1H,J=6Hz), 4.60(SEPT,1H,J=7Hz) 6.95(S,2H), 7.15(M,2H)	129.5-133.5°C MeOH/H_2O	C H N	76.28 8.89 14.83	75.99 8.60 14.58

a) Obtained on a CEC-21-110 Mass Spectrometer. b) Recorded on a GE QE-300 NMR Spectrometer. c) Measured on a Thomas-Hoover Capillary Melting Point Apparatus.

TABLE I - PHYSICAL DATA FOR UNLABELED COMPOUNDS

Compound No.	MS(70ev)[a] M/e (%)	^{1}H-NMR[b](SOLVENT) ppm	M.P.[c] (Recrys. Sol.)	Analysis	Theory	Found
6	284 (M^+,100)	CDCl$_3$/TMS 1.40(QUART,1H,J=12Hz), 1.50(dd,6H,J=3Hz) 2.15(M,2H), 2.45(S,3H), 2.65(t,1H,J=12Hz), 2.95(M,2H), 3.20(M,1H), 3.37(dd,1H,J=3Hz) 4.10(M,1H), 4.60(SEPT,1H,J=7Hz), 6.90 (M,2H), 7.15(M,2H)	114-117°C MeOH/H$_2$O	C H N	76.02 8.51 9.85	76.20 8.65 10.10
7	362 (M^+,50) 265 (M^+-97,100)	CDCl$_3$/TMS 1.52(dd,6H,J=3Hz), 1.70(QUART,2H,J=12Hz), 2.25(dt,1H,J=3Hz), 2.37(t,1H,J=12Hz), 2.50(S,3H), 2.6(t,1H,J=12Hz), 3.1(M,5H) 3.35(M,2H), 4.60(SEPT,1H,J=7Hz), 5.02 (M,1H), 6.90(M,2H), 7.15(M,2H)	206-211°C	C H N S	62.96 7.23 7.73 8.85	63.24 7.23 7.71 9.06
8	293 (M^+,100)	CDCl$_3$/TMS 1.50(dd,6H,J=3Hz), 1.70(QUART,1H,J=12Hz) 2.25(dt,1H,J=3Hz), 2.4-2.6(M,4H), 2.67 (t,1H,J=12Hz) 2.9-3.2(M,3H), 3.25(d,1H, J=12Hz), 3.35(dd,1H,J=3Hz), 4.60 (QUINT, 1H,J=7Hz), 6.90(M,2H), 7.15(M,2H)	117-121°C	C H N	77.78 7.90 14.32	77.98 7.86 14.25
2	312 (M^+,100)	D$_4$-HoAC/TMS 1.55(dd,6H,J=7Hz), 1.85(QUAR,1H,J=14Hz) 3.15(M,7H), 4.0(M,1H), 4.75(SEPT,1H,J=7Hz) 6.95(d,1H,5=7Hz), 2.05(S,1H), 7.18 (T,1H, J=7Hz), 7.25(d,1H,J=7Hz)	d			
1	424 (M^+,100) 312 (M^+-50,50)	D$_6$-DMSO 1.3-1.7(M,9H), 2.8-3.6(M,20H),3.75 (d,1H), 4.65(QUINT,1H,J=7Hz), 4.80(M,1H) 6.05(S,2H), 6.90(d,1H,J=7Hz), 7.15(t,1H,J=7Hz) 7.25(S,1H), 7.35(d,1H,J=7Hz)	172-173°C EtOH/Et$_2$O	C H N	66.65 7.46 5.18	66.42 7.26 5.12

d) Due to the hygroscopic nauture of this material, satisfactory analyses could not be obtained.

(8β)-6-Methyl-1-(1-methylethyl)ergoline-8-carboxylic acid, hydrazide (4)

A mixture of 50.0 g (0.153 mol) of 3 and 500 g of anhydrous hydrazine was heated to reflux. Reflux was maintained for 2.5 hrs and then the reaction was cooled to room temperature. The mixture was diluted with 1L water, cooled to 10°C, and the precipitate collected. On drying in vacuo, 47.8 g of 4 was recovered (96% theory yield) mp 216-219°C. TLC (chloroform/methanol/acetic acid 18:6:1) indicated that the material was homogeneous (r_f=0.31).

(8β)-6-Methyl-1-(1-methylethyl)ergoline-8-amine (5)

A solution of 51.0 g (0.156 mol) of 4, 406 mL 1N HCl, and 507 mL water was cooled in an ice bath and 1.093 L 0.2M $NaNO_2$ solution was added dropwise maintaining the reaction temperature at or below 10°C. After stirring for 30 min, 43 g (0.406 mol) of sodium carbonate was added and the mixture was filtered. The filter cake was washed with water, then returned to the reaction flask. The solid was slurried in 1872 mL water and 468 mL 1N HCl and heated to 75°C. After stirring at 70-75°C for 45 min, the reaction solution was cooled to room temperature. The pH of the solution was adjusted to 6.1 by the addition of 5N NaOH. The mixture was filtered through hyflo and the filter cake discarded. The pH of the filtrate was then adjusted to 12-13 with 5N NaOH. After cooling, the precipitate was collected and dried in vacuo to yield 36.8 g of crude 5 (HPLC assay = 91.3%). Several lots of crude 5 were combined for purification. A solution of 146.6 g of crude 5 in 3000 mL hot methanol was prepared and filtered through a hyflo pad. The filtrate was evaporated to a volume of 500 mL. This solution was then diluted by the dropwise addition of 1800 mL water. The mixture was cooled to 0°C and the purified product collected. After drying in vacuo, 115.3 g of pure 5 was obtained. (HPLC assay = 97%, r_t=2.01 min, 66% theory yield from 4) mp 129.5-133.5°C.

(8β)-6-Methyl-1-(1-methylethyl)ergoline-8-ol (6)

A solution of 13.83 g (48.80 mmol) of 5 and 277 mL glacial acetic acid was cooled in an ice bath to 10°C. A solution of 732 mL 0.2N $NaNO_2$ was then added over 20 minutes. The reaction solution was stirred at 10°C for 1 hr, then 500 mL methylene chloride was added and the reaction neutralized by the dropwise addition of 960 mL 5N NaOH. The layers were separated and the aqueous layer was washed a second time with 500 mL methylene chloride. The combined methylene chloride extracts were washed with water, saturated brine solution, and then dried over sodium sulfate. The dried solution was evaporated to yield 15.36 g of crude product. This residue was taken up in 150 mL

methanol and 39 mL 5N NaOH was added. After stirring for 30 min, the reaction was further diluted with 400 mL water. The precipitated product was collected and dried in vacuo to yield 10.67 g of crude 6 (HPLC assay = 67%). The crude product was taken up in 150 mL warm methanol and 5.04 g (43.4 mmol) of maleic acid dissolved in 60 mL methanol was added. The maleate salt of 6 was precipitated from solution by the addition of 300 mL diethyl ether. After cooling, the precipitated product was collected and dried in vacuo to yield 9.01 g of maleate salt of 6 (HPLC assay = 96%).
Several lots of the maleate salt were combined for conversion to 6. A total of 44.5 g of maleate salt was partitioned between 1500 mL methylene chloride and 400 mL aqueous sodium bicarbonate solution. The layers were separated and the aqueous layer washed with methylene chloride. The combined methylene chloride extracts were dried over sodium sulfate and evaporated. The residue was taken up in 180 mL methanol and diluted by the dropwise addition of 500 mL water. The precipitated product was collected and yielded on drying in vacuo 30.0 g of pure 6. (HPLC assay = 97%, r_t=2.37 min, 47% theory yield) mp 114-117°C.

(8β)-6-Methyl-1-(1-methylethyl)ergoline-8-ol, methane sulfonate (7)

A solution of 10.0 g (35.16 mmol) of 6 and 80 mL pyridine was prepared and cooled to 10°C. To this solution was added 11.76 g (105.47 mmol) of methanesulfonyl chloride, maintaining reaction temperature between 10°-20°C. The mixture was stirred for 2 hr at 10°-20°C and then the reaction was added to 300 mL water containing 10 mL concentrated ammonium hydroxide. After cooling, the precipitated product was collected and dried in vacuo to yield 11.55 g of 7 (91% theory yield) mp 206-211°C (with dec.). TLC (chloroform/methanol 9:1) aindicated a single product (r_f=0.71) with only traces of lower r_f impurities.

17-[^{14}C]-6-Methyl-1-(methylethyl)ergoline-8-carbonitrile (8)

A mixture of 0.956 g (2.64 mmol) of 7, 0.098 g (2.0 mmol) of sodium [^{14}C] cyanide (Pathfinder Laboratories, specific activity 49.5 mCi/mmol), 0.096 g (1.96 mmol) of sodium cyanide and 10 mL DMF was heated at 55-60°C for 4 hrs, then allowed to cool to room temperature. Stirring was continued overnight. The mixture was diluted by the dropwise addition of 25 mL water whereupon the material precipitated. The radiolabeled product was collected and dried in vacuo yielding 0.593 g of 8 (77% theory yield). This material was determined to be identical to unlabeled 8 by HPLC. HPLC analysis showed this material to be a mixture of the 8α- and 8β-isomers (r_t=3.26 and

4.38 min) in approximately a 75:25 ratio (it is unknown which is the predominant isomer).

17-[^{14}C]-(8β)-6-Methyl-1-(methylethyl)ergoline-8-carboxylic acid (9)

A solution of 0.593 g (2.02 mmol) of **8**, 25 mL methanol, and 13.3 mL 5N NaOH was stirred at reflux for 24 hr. The mixture was allowed to cool to room temperature and the methanol was evaporated. The resulting mixture was diluted with water (q.s. to dissolve solids) and the pH adjusted to 5.5 with glacial acetic acid. The product that precipitated was collected and dried in vacuo to yield 0.609 g of **9**. TLC (chloroform/methanol/acetic acid 18:6:1) indicated the product was not completely pure, so the dried product was dissolved in dilute ammonia solution and reprecipitated by the addition of glacial acetic acid. On drying, 0.557 g of **9** was recovered (88% theory yield). This material was identical to **2** by TLC using chloroform/methanol/acetic acid 18:6:1 as developing solvent (r_f=0.24).

17-[^{14}C]{[trans-(8β)]-6-Methyl-1-(1-methylethyl)ergoline-8-carboxylic acid, 4-methoxycyclohexyl ester (Z)-2-butenedioate (1:1)} (10) (LY281067-[^{14}C]).

A mixture of 0.557 g (1.79 mmol) of **9**, 2.72 g (19.7 mmol) of potassium carbonate, and 35 mL DMF was heated to 60°C, then 1.86 g (6.43 mmol) of cis-4-methoxycyclohexyl tosylate was added. The mixture was stirred at 65-70°C for 22 hr, then allowed to cool to ambient temperature. The cool reaction mixture was poured into 100 mL water and extracted with 150 mL ethyl acetate. The aqueous layer was extracted a second time with 50 mL ethyl acetate. The combined ethyl acetate layers were washed twice with water, then dried over magnesium sulfate. The mixture was filtered and treated with ethyl acetate saturated with HCl gas. On concentration, the desired material crystallized. After cooling, the hydrochloride salt was collected and dried in vacuo yielding 0.470 g solid. This material was suspended in methylene chloride and washed with saturated aqueous sodium bicarbonate solution. The aqueous layer was washed a second time with methylene chloride and the combined organic extracts were washed with water. The solution was dried over magnesium sulfate, decolorized with charcoal, then evaporated. The residue was taken up in 8 mL ethyl acetate and 0.148 g (1.28 mmol) of maleic acid was added. On stirring, **10** precipitated from solution. After cooling, the product was collected and dried. The crystals were redissolved in 50 mL ethanol and filtered through hyflo. The filtrate was concentrated, then diluted with diethyl ether. The mixture was cooled and the product collected yielding 0.327 g of **10** after vacuum drying. The product was reprecipitated from ethanol by the addition of diethyl ether, collected and dried yielding 0.29 g of **10**, specific activity 49.7 μCi/mg (26.8 mCi/mmol). This material was determined to be identical to an authentic sample of **1** by TLC using ethyl acetate/toluene/methanol 3:1:0.5 as developing solvent (r_f=0.55). The radiochemical purity was ≥ 98.8% by HPLC (r_t=11.3 min).

ACKNOWLEDGEMENTS

The authors thank Carmen McElravy for her help in preparing the manuscript.

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