ISOLATION OF (-)-MENTHOL-^{14}C AND (+)-NEOMENTHOL-^{14}C

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SUMMARY

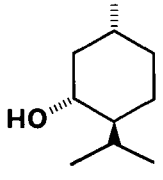
Uniformly labelled (-)-menthol-^{14}C and (+)-neomenthol-^{14}C were isolated and purified from the oil of peppermint plants grown in a $^{14}CO_2$ chamber by preparative high performance liquid chromatography. This procedure is much simplier than previous methods and allows for large amounts of labelled oil to be processed.

Key Words: (-)-Menthol-^{14}C, (+)-Neomenthol-^{14}C,

^{14}C-Peppermint oil

INTRODUCTION[1]

Several years ago a method for the biosynthesis and isolation of ^{14}C-labelled menthol (1) from peppermint oil was published.[2] We wish to report an improved isolation procedure using preparative high performance liquid chromatography (HPLC) that is both more rapid and applicable on a larger scale than previous methods. Neomenthol (2) can also be isolated from the peppermint oil.



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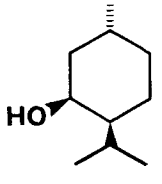

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0362-4803/82/010117-04$01.00

Received April 6, 1981
Revised April 24, 1981

INSTRUMENTATION

Analytical HPLC was accomplished with a Waters Associates Liquid Chromatograph, Model 201, equipped with a U6K loop injector, a Radial Compression Separation System (10 cm X 0.8 cm Radial-Pak B μPorasil cartridge, RCM-100 Module), and Model 401 Differential Refractometer.

A Waters Associates Prep-500 liquid chromatograph was used for the isolations, using one Silica Prep-Pak cartridge, and detection by differential refractive index.

Solvents and flow rates can be found in the appropriate figures.

BIOSYNTHESIS AND ISOLATION OF MINT OIL

Mint plants (Mentha arvensis L. var piperascens (Murray Hybrid)[3], were grown for thirty-five days with $^{14}CO_2$ and harvested as previously described[2]. Crushed leaves from the entire plant (890 g, 102 μCi/g) were exhaustively steam distilled in two batches. The combined distillate (ca. 1L) was extracted with pentane (6 x 200 mL), dried ($MgSO_4$) and the solvent carefully removed *in vacuo* on a rotary evaporator at 35°C to give crude labelled peppermint oil (ca. 7 g, 4.8 mCi). This oil contained about 60% menthol by analytical HPLC (Figure 1).[4]

PURIFICATION OF MENTHOLS 1 AND 2

All of the ^{14}C-peppermint oil was preparatively chromatographed (Figure 2). Fractions three and four were combined and carefully evaporated as before to give 3.8 g (2.52 mCi) of 1 (>99.5% by gas radiochromatography[2] and HPLC[4]). Fractions two and five were combined, evaporated (0.9g, 990 μCi) and rechromatographed (Figure 3). Fractions D and E gave an additional portion of pure 1 (0.4 g, 235 μCi). Fractions B and C were combined to give 0.1 g (111 μCi) of pure 2. A summary of all isolations are listed below.

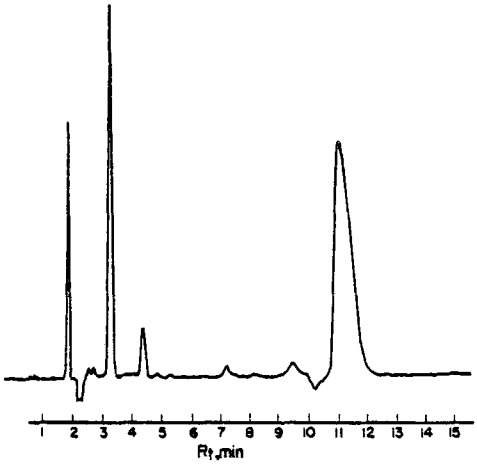

Figure 1. HPLC of ^{14}C-Peppermint Oil

Solvent-3% ethyl acetate/isooctane, Flow Rate = 3mL/min
a. Neomenthol b. Menthol

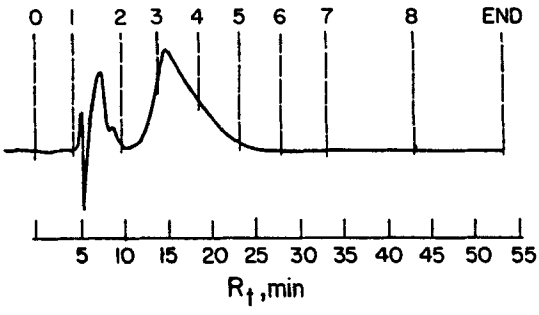

Figure 2. Prep-Chromatogram of ^{14}C-Peppermint Oil

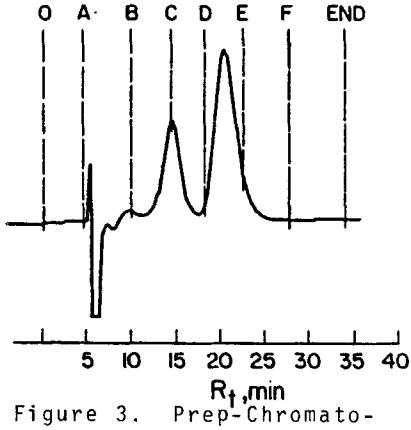

Figure 3. Prep-Chromatogram of first run fract #2&5

Solvent = 8% ethyl acetate/isooctane
Flow Rate = 100 mL/min

	Crushed Leaves	Crude Oil	(-)-Menthol-^{14}C	(+)-Neomenthol-^{14}C
Wt.	890 g	7 g	4.2 g	0.1 g
Activity (μCi)	-	4800	2860	111

CONCLUSION

This HPLC assisted isolation method is superior to previous gravity column[2] and low temperature crystallization methods[5] in that it is faster, allows for greater mass throughput and affords almost complete recovery of the labelled (-)-menthol from the peppermint oil.

ACKNOWLEDGMENTS

The authors wish to thank R. T. Bass and G. C. Newell for growing the mint plants and A. F. Frisch, R. A. Comes, B. W. Francis, M. D. Edmonds, K. R. Barlow and M. K. Chavis for assistance in harvesting the leaves. Additional thanks to Dr. R. W. Jenkins, Jr. under whose guidance and direction as Group Leader this work was developed.

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