# Synthesis of Deuterium Labeled Perillyl Alcohol and Dual C-13 and Deuterium Labeled Perillic Acid, Major Metabolites of d-Limonene

Haitao Chen and Kenneth K.Chan*

Colleges of Pharmacy and Medicine, and Comprehensive Cancer Center,

the Ohio State University, Columbus, OH 43210

## SUMMARY

Dual C-13 and deuterium labeled perillic acid, [(1,1-dideuterio-1-$^{13}$C-2-methyl)ethenyl]-1-cyclohexene-1-carboxylic acid (**6**) and deuterated perillyl alcohol, [(2,2-dideuterio-1-methyl)ethenyl]-1-deuteriohydroxymethyl-1-cyclo-hexene (**9**) were synthesized from commercially available (4S)-(-)-perillaldehyde (**1**). Compound **1** was first protected with ethylene glycol to yield the ethylene ketal followed by oxidation with $OsO_4/NaIO_4$ to cleave the terminal double bond to afford the key intermediate ketone, 4-acetyl-1-cyclohexene-1-carboxaldehyde ethylene ketal (**3**). **3** was then converted to the labeled perillyl aldehyde by Wittig reaction with prepared $Ph_3P^{13}CD_3I$ or $Ph_3PCD_3I$ followed by deprotection to give the labeled perillaldehydes, [(2,2-dideuterio-2-$^{13}$C-1-methyl)ethenyl]-1-cyclohexene-1-carboxaldehyde (**5**) or [(2,2-dideuterio-1-methyl)ethenyl]-1-cyclohexene-1-carboxaldehyde (**8**). **5** was further oxidized by freshly prepared $Ag_2O$ to give the desired compound **6**. **8** was reduced by $LiAlD_4$ to afford the desired compound **9**. The same synthetic procedure may be adopted to synthesize the radioactive isotope labeled perillic acid and perillyl alcohol.

**Key Word** : stable isotope; perillic acid; perilly alcohol; metabolites of d-limonene

---

*Author for correspondence

CCC 0362–4803/97/050369–09$17.50
©1997 by John Wiley & Sons, Ltd.

Received 1 October 1996
Revised 31 December 1996

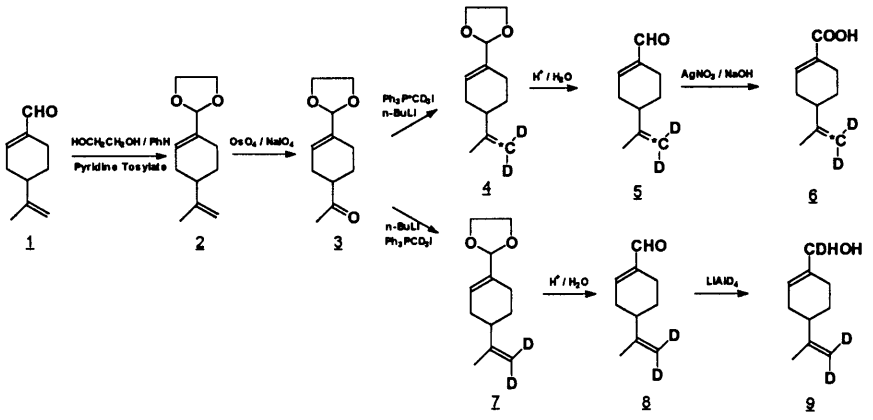
## INTRODUCTION

The naturally occurring compound d-limonene has recently been found to possess promising chemotherapeutic and chemopreventive properties which may be mainly attributed to its major metabolites perillyl alcohol and/or perillic acid based on the findings that both perillyl alcohol and perillic acid are more active than d-limonene itself *in vitro* (1,2). On this basis, perillyl alcohol has recently been selected for clinical evaluation by the National Cancer Institute (3). To support the future clinical development of this compound, we plan to develop a GC/MS method for the analysis of this compound and its metabolites perillic acid and dihydroperillic acid. It is generally agreed that stable isotopically labeled analogs of a compound are the best internal standards for a GC/MS assay and this is especially important for highly volatile molecules such as monoterpenoids. Additionally, stable isotopically labeled analogs, when used as the internal standards, have been shown to greatly improved the assay reproducibility of an ion-trap mass spectrometer which is a very sensitive instrument for quantitation of organic compounds (4). For this reason, we undertook the design and synthesis of stable isotope labeled perillyl alcohol and perillic acid.

## RESULTS AND DISCUSSION

The synthesis starting with the commercially available compound (S)-(-)-perillaldehyde (**1**) is shown in scheme 1.

Compound **1** was first protected by refluxing with ethylene glycol and the catalytic amount of pyridine tosylate in benzene with a water separator to afford an ethylene ketal **2**. There are two double bonds in **2**; one is a terminal double bond located on the side chain and the other is an endocyclic double bond adjacent to a bulky ethylene ketal group. The selective cleavage of the terminal double bond of **2** is the key step of the synthesis. Pappo, et.al.(5) reported that the osmium tetroxide-catalyzed periodate oxidation in two immiscible liquid phases, i.e. ethyl ether and




**Scheme 1**

water, could selectively cleave the double bond of cyclohexene but not that of 1-methylcyclohexene. The same method was first applied to **2**, but the yield of **3** was poor. The method was then modified to use acetonitrile and water as a one-phase solvent system, as expected the terminal double bond of compound **2** was cleaved selectively leaving the endocyclic double bond intact. The selective cleavage was confirmed by GC/MS which gave a mass spectrum consistent with the structure of **3**. The selectivity of the cleavage is probably driven by the steric factor where the bulky ethylene ketal prevents the oxidative cleavage of the endocyclic double bond. However, under such a condition, the ketals in substrate **2** and its product **3** were rapidly deprotected to give their corresponding aldehydes. The rapid deprotection was probably triggered by the acidic condition generated by osmium tetroxide; in fact, osmium tetroxide has frequently been used for cleavage of the ethylene ketal protection group(6). To prevent **2** from premature deprotection, a small amount of pyridine was added to the reaction mixture prior to the addition of osmium tetroxide. Under such a basic condition after stirring for 5 hrs, only very small amount of **1** (less than 8% of sum of **1**, **2**, and **3**) was detected by GC/MS. The yield of the desired compound **3** was about 83% in the reaction mixture. However, the reaction mixture still contained about 9% unreacted starting material **2**.

Fortunately, 1 was successfully removed by extraction with $NaHSO_3$ solution. After distillation, the product was a mixture consisting of a major compound 3 and a minor component 2. Attempts to separate these two compounds by repeated distillation was unsuccessful. Finally, they were completely separated by flash silica gel flash chromatography. The complete removal of 2 from 3 is extremely important, because as the synthesis progresses, compound 2 will be converted to unlabeled perillic acid or perillyl alcohol, which will reduce the isotopic purity of the desired products.

The introduction of the isotopically labeled moiety to 3 was accomplished by Wittig reaction. Treatment of 3 with the labeled triphenylphosphonium iodide in n-BuLi and DMF afforded the labeled compound 4 or 7. To minimize the potential interference due to contribution of the unlabeled entities from the labeled compound, when labeled and unlabeled compounds are used together during an assay procedure, the use of a minimum of 3 amu in the labeled compound is desirable. As the internal standard for perillic acid, the labeled analog with 3 amu higher will further avoid mass overlap with the metabolite dihydroperillic acid. The synthesis can be readily accomplished by the use of methyl-[$^{13}C$,$^{2}H_3$]triphenylphosphonium iodide which can be prepared from commercial $^{13}CD_3I$ and triphenylphosphine. Thus, compound 4 was prepared in 65.5% yield after distillation. Compound 4 was then deprotected in acidic condition to afford the labeled perillaldehyde 5 followed by oxidation to give 60% yield of the labeled perillic acid 6 using silver oxide freshly prepared by addition of NaOH to $AgNO_3$ solution. For perillyl alcohol, two deuterium atoms can be introduced by Wittig Reaction using deuterated methyl iodide and triphenylphosphine to yield labeled perillaldehyde 8 in 86% yield. Reduction of 8 by $LiAlD_4$ incorporated another deuterium to afford the 3 deuterium labeled perillyl alcohol 9. In this fashion, the use of more expensive $^{13}C$-labeled reagent as compared to deuterium labeled reagent such as that for 4 can be avoided.

Using the stable isotope labeled perillyl alcohol and perillic acid as the internal standard, a sensitive and reliable GC/MS assay has been developed. Description of the GC/MS assay and its application in metabolism and pharmacokinetic studies will be reported elsewhere.

## EXPERIMENTAL

$^1$H NMR spectra were recorded on a Bruker NR-250 spectrometer with tetramethylsilane as the internal standard. Mass spectra were obtained on a Finnigan MAT Ion Trap ITS40 GC/MS with ammonia as the chemical ionization reagent gas.

*4-(1-Methylethenyl)-1-cyclohexene-1-carboxaldehyde ethylene ketal* (2)

A mixture of 8.70 g (0.058mol) of (S)-(-)-perillaldehyde, 18.0 g (0.29 mol) of ethylene glycol and 1.00 g (4.0 mmol) of pyridinium tosylate in 150 ml of dry benzene was refluxed for 12 hrs while the water generated from the reaction was removed by a Dean-Stark trap. After cooling to room temperature, the organic solution was washed with 3x50 ml of water and dried over anhydrous $MgSO_4$. Evaporation of the organic solution gave an oily residue which was distilled to yield 9.10 g (81.3%) of colorless liquid, bp 104-107$^o$C/0.7 mm Hg . $^1$H NMR ($CDCl_3$) $\delta$ 1.4-2.4 (m, 7H, ring H), 1.7 (s, 3H, $-CH_3$), 3.8-4.1 (m, 4H, $-OCH_2CH_2O-$), 4.7 (br, 2H, $=CH_2$), 5.1 (s, 1H, -OCHO-), 5.9-6.0 (m, 1H, =CH-) ; MS (CI) m/z 195 (M+1), 151, 133.

*4-Acetyl-1-cyclohexene-1-carboxaldehyde ethylene ketal* (3)

To a solution of 3.67 g (18.92 mmol) of 2 in 2 ml of pyridine and 80 ml of acetonitrile immersed into an ice-bath was added 500 mg (1.97 mmol) of $OsO_4$. The resulting dark solution was stirred for 5 min and to it was added 16 ml of water. A total of 8.0 g (37.38 mmol) finely powdered $NaIO_4$ was then added to the mixture over a period of 2 hrs. The mixture was vigorously stirred for 5 hrs during which period the dark color of the reaction mixture changed to pale yellow. The resulting sodium iodate was

separated by filtration and the filtrate was extracted thoroughly with diethyl ether (4x100 ml). The combined ether extract was washed with cold water (2x50 ml), then with saturated $NaHSO_3$ solution (2x20 ml) and dried over anhydrous $MgSO_4$. After the solvent was removed on a rotary evaporator, the resulting oily residue was distilled to afford 2.63 g of slightly yellow liquid, bp 147-150°C/3.2 mm Hg. GC/MS showed that the liquid contained about 9% of the starting material **2** and 2% of perillaldehyde resulted from deprotection of **2** and 8% aldehyde from **3**. The liquid was redissolved in 10 ml of ether and washed with cold solution of saturated $NaHSO_3$ (3x5 ml) to remove the aldehydes. After the ether solution was dried over anhydrous $MgSO_4$ and then concentrated, the liquid residue was purified by flash silica gel chromatography (silica gel 60, EM Merck) with n-pentane: ether (4:1) as the eluant to afford 1.98 g (53.5%) of **3** . GC/MS (30 m DB5 capillary column, J&W, Folsom, CA) gave retention times of 7.5 and 8.4 min for **2** and **3**, respectively (60°C for 1 min than to 200°C at 10°C/min). $^1H$ NMR ($CDCl_3$) δ 1.4-2.7 (m, 7H, ring H), 2.2 (s, 3H, $-CH_3$), 3.9-4.1 (m, 4H, $-OCH_2CH_2O-$), 5.1 (s, 1H, -OCHO-), 5.9-6.0 (m, 1H, =CH-); MS (CI) *m/z* 197 (M+1), 153, 135.

*4-[(2,2-Dideuterio-2-$^{13}C$-1-methyl)ethenyl ]-1-cyclohexene-1-carboxaldehyde ethylene ketal* (**4**)

To a solution of 1.80 g (6.86 mmol) of triphenylphosphine in 5 ml of dry benzene immersed into a dry ace-acetone bath was added dropwise a solution of 1.00 g of methyl-[ $^{13}C,^2H_3$]iodide (Cambridge Isotopes, $^{13}C$, 99% and D 99%) in 2 ml of dry benzene. The resulting mixture was stirred overnight at room temperature. After that the mixture was filtered and the solid washed with dry benzene to give 2.50 g (89.3%) of methyl-[$^{13}C,^2H_3$]triphenylphosphonium iodide as a white solid. To a mixture of 1.23 g (3.0 mmol) methyl-[$^{13}C,^2H_3$]triphenylphosphonium iodide in 10 ml of anhydrous THF immersed into an ice bath was added 2.0 ml of 1.6 M of n-butyl lithium hexane solution. The solution was stirred for 30 min to give an orange colored solution to which was then added 0.29 g (1.47 mmol) of

1-ethylenedioxymethyl-4-acetyl-1-cyclohexene **3** over 1 hr. The mixture was refluxed for 2 hrs and then stirred at room temperature for overnight. The mixture was then filtered and the filtrate was concentrated *in vacuo* to give a residue which was then extracted with n-pentane. The combined n-pentane extracts was concentrated *in vacuo* to afford 0.28 g of residue which was distilled to yield 0.19 g (65.5%) of colorless liquid, bp 102-106°C/0.9 mm Hg. $^1$H NMR ($CDCl_3$) δ 1.2-2.4 (m, 7H, ring H), 1.7 (s, -$CH_3$), 3.9-4.1 (m, 4H, -$OCH_2CH_2O$-), 5.2 (s, 1H, -OCHO-), 5.9-6.0 (m, 1H, =CH); MS (CI) *m/z* 198 (M+1), 154, 136.

*[(2,2-Dideuterio-2-$^{13}$C-1-methyl)ethenyl]-1-cyclohexene-1-carboxaldehyde* (**5**)

A solution of 114 mg (0.58 mmol) of **4** in 10 ml of methanol and 1 ml of 1N $H_2SO_4$ was stirred at room temperature for 4 hrs. The solution was mixed with 10 ml of water and extracted with 3x30 ml of diethyl ether. Then the organic solution was washed with water (2x5 ml) and dried over anhydrous $MgSO_4$. After filtration and concentration, the mixture gave 81 mg (92.0%) of colorless liquid. $^1$H NMR ($CDCl_3$) δ 1.2-2.6 (m, 7H, ring H), 1.7 (s, 3H, -$CH_3$), 6.8-6.9 (m, 1H, -OCHO-), 9.5 (s, 1H, =CH-); MS (CI) *m/z* 154 (M+1), 136.

*[(1,1-Dideuterio-1-$^{13}$C-2-methyl)ethenyl]-1-cyclohexene-1-carboxylic acid* (**6**)

To a solution of 81 mg (0.52 mmol) of **5** and 135 mg (0.80 mmol) of $AgNO_3$ in 5 ml of ethanol and 1 ml of $H_2O$ was added 2 ml of 1N NaOH over a period of 30 min at room temperature. The resulting mixture was stirred at room temperature overnight and then filtered. The filtrate was acidified with 6N HCl and extracted with 3x10 ml of diethyl ether. The combined ether extract was then extracted with 3x5 ml of 1N NaOH. The combined NaOH extract was acidified with 6N HCl and then extracted with 3x10 ml of diethyl ether. The ether layer was dried over anhydrous $MgSO_4$ and then concentrated to give a residue. The residue was recrystallized

from petroleum ether to yield 53 mg (60.0 %) white solid, and the overall yield from 1 was 15.7%. $^1$H NMR ($CDCl_3$) δ 1.2-2.6 (m, 7H, ring H), 1.7 (s, 3H, $-CH_3$), 7.1-7.2 (m, 1H, =CH-), 11.8 (br 1H, -COOH); MS (CI) *m/z* 170 (M+1), 152, 124, 98, 81.

*4-[(2,2-Dideuterio-1-methyl)ethenyl ]-1-cyclohexene-1-carboxaldehyde ethylene ketal* (7)

The titled compound was prepared using the same procedure as that for compound 4 except that $CD_3I$ (Aldrich, D, 99.5%) was used. Yield was 63.8%, bp 114-117°C / 3.2 mmHg . $^1$H NMR ($CDCl_3$) δ 1.2-2.5 (m, 7H, ring H), 1.7 (s, 3H, $-CH_3$), 3.8-4.1 (m, 4H, $-OCH_2CH_2O-$), 5.1 (s, 1H, OCHO-), 5.9-6.0 (m, 1H, =CH-); MS (CI) *m/z* 197 (M+1), 153, 135.

*[(2,2-Dideuterio-1-methyl)ethenyl]-1-cyclohexene-1-carboxaldehyde* (8)

The titled compound was prepared using the same procedure as that for compound 5 except that 7 rather than 4 was used as the starting material. Yield was 86.0%, bp 64-67°C/3.0 mmHg;$^1$H NMR ($CDCl_3$) δ 1.2-2.6 (m, 7H, ring H), 1.7 (s, 3H, $-CH_3$), 6.8-6.9 (m, 1H, =CH-), 9.5 (s, 1H, -CHO) ; MS (CI) *m/z* 153 (M+1), 135.

*[(2,2-Dideuterio-1-methyl)ethenyl]-1-deuteriohydroxymethyl-1-cyclohexene* (9)

To a suspension of 0.15 g (3.58 mmol) of $LiAlD_4$ in 5 ml of diethyl ether was added dropwise a solution of 0.12 g (0.79 mmol) of 8 in 2 ml of dry diethyl ether over a period of 15 min. The mixture was refluxed for 3 hrs. After cooling to room temperature, the mixture was poured into ice-water and the mixture was extracted with 3x50 ml diethyl ether and the combined ether extract was dried over anhydrous $MgSO_4$. After filtration and concentration, it gave an oily residue which was distilled to yield 0.86 g (70.5%) of colorless liquid, bp 95-98°C/3.1 mm Hg. The overall yield of 9

from 1 was 16.8%. $^1$H NMR ($CDCl_3$) δ 1.2-2.4 (m, 7H, ring H), 1.7 (s, 3H, -$CH_3$), 4.0 (br,1H, -CHDO-), 4.6 (br, 1H, -OH), 5.7 (br, 1H, =CH-); MS (CI) *m/z* 155 (M), 138.

**Acknowledgement.** The support from the Contract NO1-CN-55083070 of the National Cancer Institute to the Cleveland Clinic Foundation subcontracted to the Ohio State University is gratefully acknowledged.

## REFERENCES

1. Crowell P.L., Ren Z., Lin S., Vedejs E., and Gould M.N.-Biochem. Pharmaco. 47:1405(1944).

2. Crowell P.L., Lin S., Vedejs E., and Gould M.N-Cancer Chemother.Pharmacol. 31:250 (1992).

3. Phillips L.R., Malspeis L., and Supko J.G.-Drug Metabolism Disposition. 23:676(1995) and references cited therein.

4. Wang J.-H. and Chan K.K.-J. Chromatogr. B. 674:205(1995).

5. Pappo R., Allen D.S., Lemieux R.U.Jr., and Johnson W.S.-J.Org.Chem. 21:478 (1956)

6. Arth G.E., Poos G.I., and Aareet L.H.-J.Amer.Chem.Soc. 77:3834 (1955)