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# Syntheses of ( ± )-[$^{14}CO_2H$] Jasmonic Acid and its Pure Enantiomers

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## Summary

( ± )-Jasmonic acid (JA) was converted in 67% yield to the next lower bromomethyl derivative **2** by bromodecarboxylation following Barton's procedure. Protection of the 3-keto group by ketalization (ethanediol), condensation with K$^{14}$CN (DMSO) and deprotection gave the [$^{14}$CN] nitrile **6**. The diasteroisomeric semioxamazone derivatives **8a**, **8b** were resolved by flash chromatography. The hydrolysis of the nitriles **6a**, **6b** successively by NaOH, then 3N HCl gave (+)-[$^{14}CO_2H$] JA and (-)-[$^{14}CO_2H$] JA of high enantiomeric purity (ee≥ 98%).

**Key Words**: (3-oxo-2-pent-2Z-enyl-cyclopentyl)-[$^{14}CO_2H$] acetic acid, [$^{14}CO_2H$] jasmonic acid, diastereoisomer, enantiomer, chiral semioxamazone, Barton's bromodecarboxylation

## Introduction

Jasmonic acid (JA) is a phytohormone widely distributed within the plant kingdom. It contributes to many physiological and biochemical processes (1,2,3,4) such as tuberization, senescence, growth inhibition or chemical defense. Although many studies concerning the biosynthesis of jasmonic acid have been published (1,2,3), the knowledge of JA metabolism in plants is limited (5,6).

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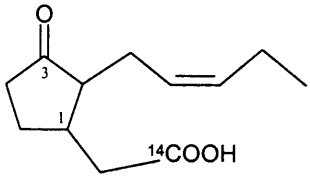
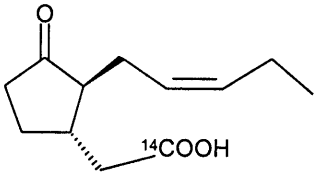
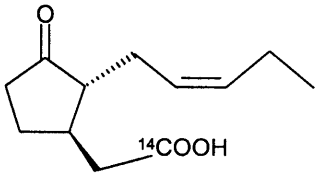
In order to study the in-vitro hydroxylation of jasmonic acid by plant cytochromes P450 (7) and the importance of its chiral centers, we synthesized ( ± )-[$^{14}CO_2H$] jasmonic acid **1** and its pure enantiomers (+)-[$^{14}CO_2H$] jasmonic acid **1a**, (-)-[$^{14}CO_2H$] jasmonic acid **1b**.




**1**




**1a**

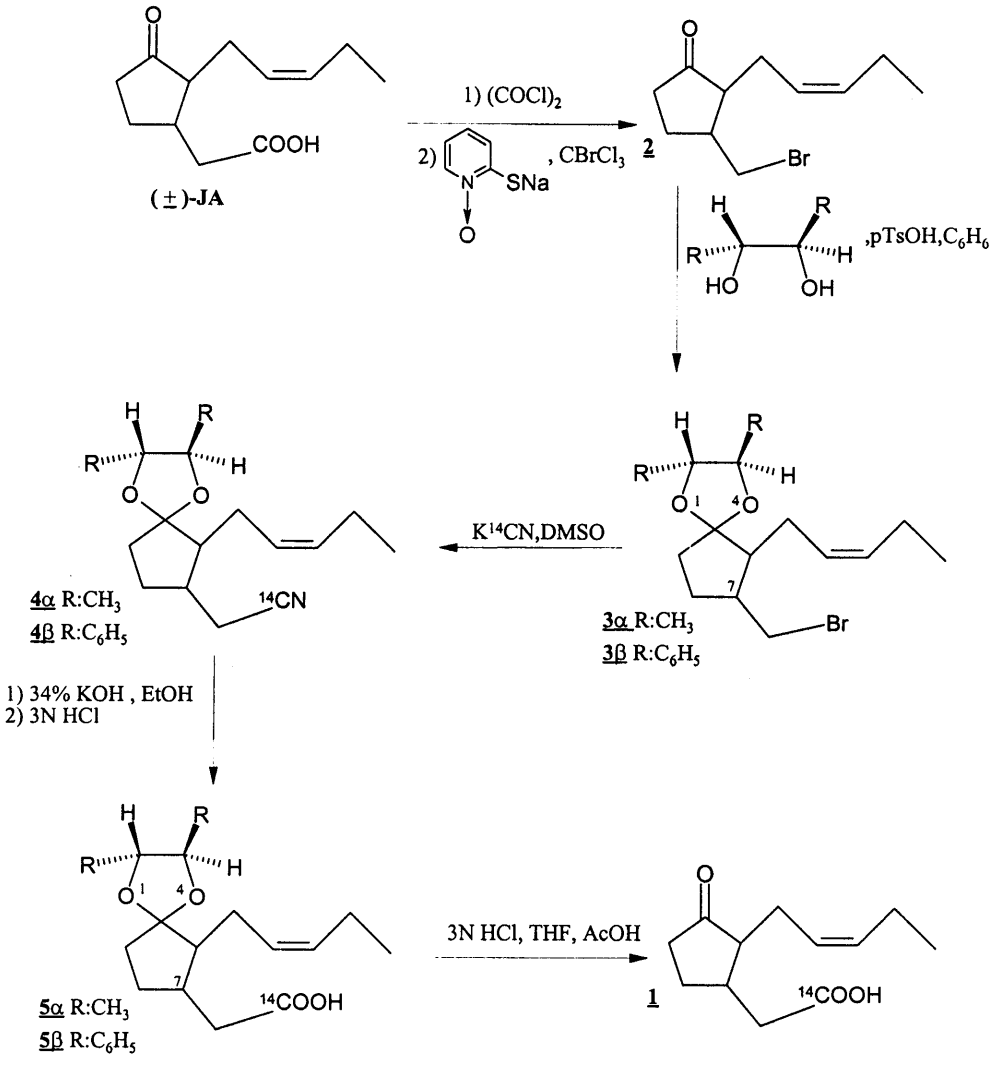



**1b**

Previous reports described the syntheses of ( ± )-[$^{14}C$] dihydrojasmonic acid (8) and ( ± )-[$^{14}C$] JA (9,10) using the Michael reaction as a basic reaction. This method required the syntheses of diethyl [2-$^{14}C$]-malonate and substituted cyclopentenones. We propose a new procedure for the preparation of [$^{14}CO_2H$] JA using commercially available ( ± )-JA as a convenient starting material and K$^{14}CN$ as labelling reagent.

## Results and Discussion

According to the procedure of Barton et al (11), ( ± )-JA was converted to the bromomethyl compound **2** (Scheme 1). Since some studies (12,13,14) had reported the separation of diastereomeric jasmonic acid derivatives by chromatographic methods, the oxo group of the bromo derivative **2** was protected by a chiral diol in an attempt to secure directly the enantiomeric forms of JA by such chromatographic procedures. So we prepared the racemates **3α** derived from (2R,3R)-(-)-2,3-butanediol and **3β** derived from (R,R)-(+)-hydroxybenzoin which gave respectively the nitriles **4α** and **4β** with K$^{14}CN$. Hydrolysis of **4α** and **4β** were carried out under aqueous alkaline conditions and afforded the acids **5α** and **5β**. Unfortunately, our attempts to resolve all these different diastereomeric compounds by flash chromatography over silica gel or by high performance liquid chromatography (HPLC) failed. The acids **5α** and **5β** were used without further purification and furnished ( ± )-[$^{14}CO_2H$] JA **1** by treatment with 3N HCl in THF / acetic acid. The overall chemical yield was 34 % from ( ± )-JA and the overall radioactive yield was 61 % based on K$^{14}CN$ when we used (2R,3R)-(-)-2,3-butanediol.

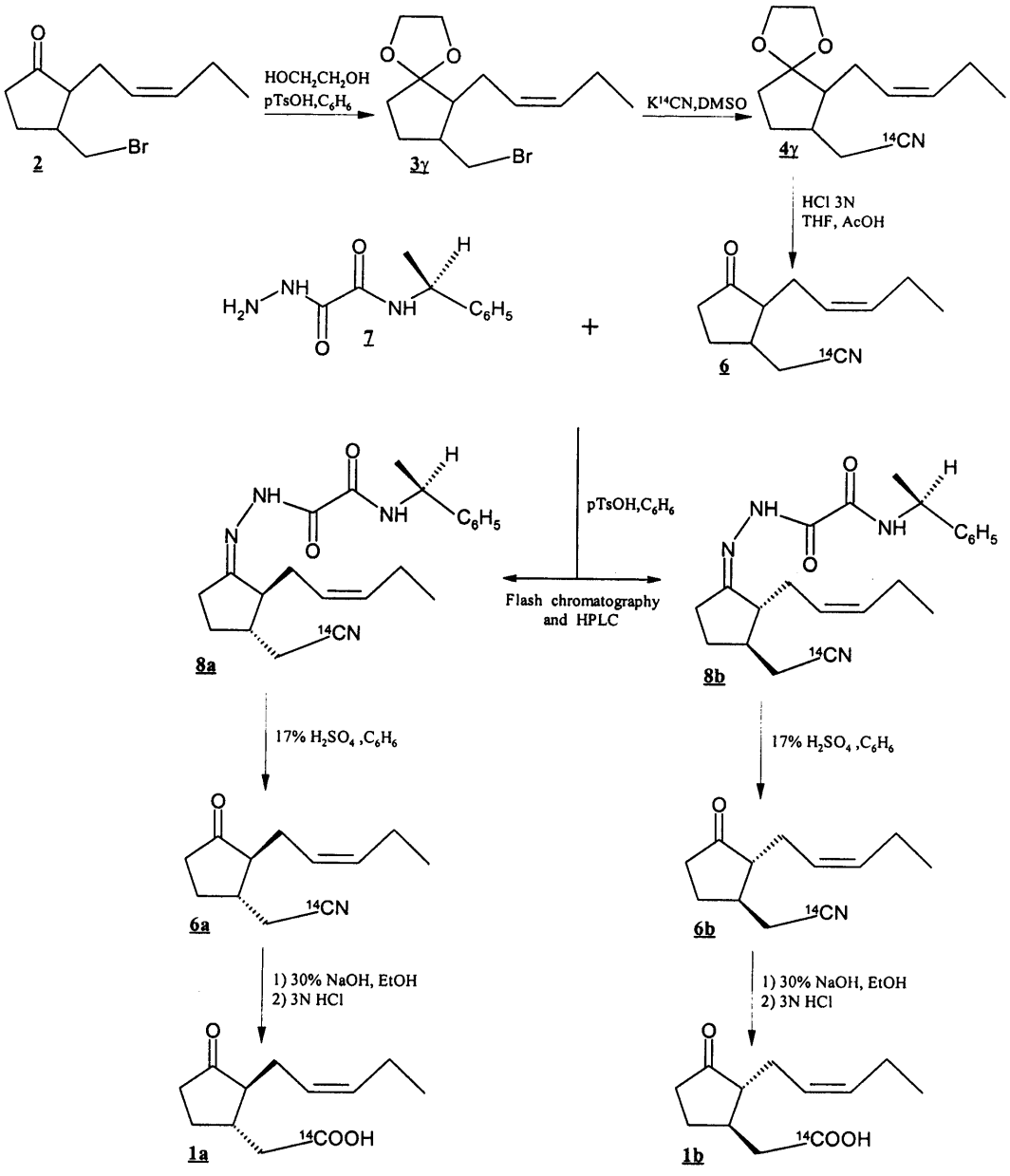



Scheme 1

Resolution of racemic derivatives of jasmonic acid was finally achieved using a semioxamazone (Scheme 2) prepared by reaction of the nitrile **6** with 2-hydrazino-2-oxo-N-((1S)-phenyl-ethyl)-acetamide **7** (15,16).

Attempts to synthesize the nitrile **6** directly from the bromomethyl derivative **2** with $K^{14}CN$ gave a yield of only 16%. So we decided to protect the keto function of **2** via the ketals **3γ** and **4γ** derived from ethylene glycol, which finally gave a 65% overall yield of **6**. After reaction of the nitrile **6** with the semioxamazide **7**, a mixture of the diastereoisomers **8a** and **8b** was obtained. Separation of these diastereoisomers was performed by flash chromatography over silica gel followed by preparative HPLC until complete resolution.




Scheme 2

The semioxamazones **8a** and **8b** were respectively converted to the nitriles **6a** and **6b** by acidic treatment. After hydrolysis of the nitriles **6a** and **6b** under aqueous alkaline conditions, the crude enantiomers of JA were purified by flash chromatography over silica gel. Then each enantiomer of JA was purified by

preparative HPLC until its radiochemical purity, determined by analytical HPLC, was better than 95%. The enantiomeric purities of **1a** (95.7%, ee=98%) and **1b** (97.6%, ee>98%) were determined by analytical HPLC with a Nucleodex β-PM column (17).

## Conclusion

( ± )-[$^{14}CO_2H$] JA and its enantiomers were obtained from commercially available ( ± ) JA using the Barton bromodecarboxylation method as the basic reaction. This procedure avoided the tedious and costly preparations of diethyl [2-$^{14}$C] malonate and 2-pent-2Z-enyl-cyclopent-2-enone.

## Acknowledgements

The authors thank Mrs Sophie Dezard (CEA) for preliminary studies and Mr Alain Valleix (CEA) for analyses.

## Experimental

All chemicals and reagents were used as received from the suppliers unless otherwise noted.

K$^{14}$CN (58 mCi/mmol) was purchassed from Amersham Life Science. Flash chromatography was performed using LiChroprep Si 60 (E Merck) and preparative HPLC by using a Gilson pump (Model 303) with a Berthold Nuclear Spectrometer Detector (Model LB 2040). Analytical TLC was performed with silica gel 60F-254 plates (E Merck). They were visualized with an anisaldehyde solution (anisaldehyde 12.5 mL, acetic acid 5 mL, sulfuric acid 17 mL, ethanol 95% 450 mL) by heating. Radioactivity measurements were carried out with Berthold Automatic TLC-Linear Analysers (Model LB 2820-1 or LB 2821). Analytical HPLC was conducted with a Merck pump (Model L-6200) and a Berthold HPLC Radioactivity Monitor (Model LB 503). NMR spectra were recorded on a Bruker AM 400 spectrometer (7.05 T; 300 MHz ($^1$H)) and the chemical shifts were reported in parts per million (ppm). Mass spectra were obtained on a Finnigan instrument (Model 4600). Specific activities were determined by mass spectrometry (DCI/$NH_3$). Radioactivity measurements were carried out with a Berthold Nuclear Spectrometer Detector (Model LB 2040) or a Liquid Scintillation Counter Wallac (Model 1409).

**3-Bromomethyl-2-pent-2Z-enyl cyclopentanone : 2**

In an oven dried 250 mL two-necked round-bottomed flask equipped with a magnetic stirring bar, we placed jasmonic acid **1** (2 g, 9.5 mmol) in 40 mL of dry toluene. Oxalyl chloride (8 mL, 91.7 mmol) was added dropwise under argon. After stirring at room temperature for 3 h, the reaction mixture was concentrated. Anhydrous toluene (2x20 mL) was added to the crude acyl chloride of JA and evaporated under vacuum to remove traces of oxalyl chloride. The crude acyl chloride was dissolved in 8 mL of bromotrichloromethane. A solution of 2-mercaptopyridine-1-oxide sodium salt (1.54 g, 10.3 mmol), 4-dimethylaminopyridine (0.11 g, 0.9 mmol) and 30 mL of bromotrichloromethane in a oven dried 100 mL two-necked round-bottomed flask was heated under reflux under argon. To this solution was added dropwise the solution of jasmonic acyl chloride. After stirring for 10 min under reflux, the reaction mixture was allowed to cool at room temperature, filtered, concentrated and purified on silica gel with hexane/ethyl acetate (95/5) as eluant. 1.57 g (6.4 mmol) of bromide **2** was obtained as a colorless oil. Yield: 67%. TLC: Rf=0.4 (silica gel; hexane/ethyl acetate (90/10)). $^1$H NMR ($CDCl_3$): 0.95 (t, J=7.5 Hz, 3H, $CH_2$-C<u>H</u>$_3$), 1.65 (m, 1H), 2-2.5 (m, 9H), 3.45 (dd, J=10.2, 6.5 Hz, 1H, CH-C<u>H</u>Br), 3.7 (dd, J=10.2, 3.2Hz, 1H, CH-C<u>H</u>Br), 5.25 (m, 1H, CH=C<u>H</u>), 5.5 (m, 1H, C<u>H</u>=CH)

**7-Bromomethyl-(2R,3R)-dimethyl-6-pent-2Z-enyl-1,4-dioxa [4.4] nonane : 3α**

In an oven dried 50 mL round-bottomed flask equipped with a magnetic stirring bar and a Dean-Stark apparatus were placed under nitrogen **2** (200 mg, 0.81 mmol), (2R,3R)-(-)-2,3-butanediol (290 mg, 3.2 mmol), p-toluenesulfonic acid (50 mg) and 25 mL of anhydrous benzene. The reaction mixture was heated under reflux under nitrogen for 4.5 h. The resulting mixture was washed with 30 mL of 5% aqueous sodium carbonate, then dried over magnesium sulfate, filtered and concentrated. The crude product was purified on silica gel with hexane/ethyl acetate (98/2) to give the expected compound **3α** (220 mg, 0.69 mmol). Yield: 85%. TLC: Rf=0.37 (silica gel; hexane/ethyl acetate (95/5)). $^1$H NMR ($CDCl_3$): 0.95 (t, J=7.5 Hz, 3H, $CH_2$-C<u>H</u>$_3$), 1.2 (d, J=6Hz, 3H, CH-C<u>H</u>$_3$), 1.25 (d, J=6Hz, 3H, CH-C<u>H</u>$_3$), 1.5 (m, 1H), 1.7-2.15 (m, 8H), 2.25 (m, 1H), 3.35 (m, 1H, CH-C<u>H</u>Br), 3.45-3.65 (m, 3H, CH-C<u>H</u>Br and 2 O-C<u>H</u>-CH$_3$), 5.3-5.45 (m, 2H, C<u>H</u>=C<u>H</u>)

**7-Bromomethyl-(2R,3R)-diphenyl-6-pent-2Z-enyl-1,4-dioxa [4.4] nonane : 3β**

3β was prepared by the same procedure described for 3α: **2** (150 mg, 0.61 mmol), (R,R)-(+)-hydroxybenzoin (360 mg, 1.7 mmol), p-toluenesulfonic acid (50 mg), anhydrous benzene (30 mL), reflux time: 7h. 3β: (170 mg, 0.38 mmol). Yield: 62%. TLC: Rf=0.38 (silica gel; hexane/ethyl acetate (95/5)). $^1$H NMR ($CDCl_3$): 0.9-1.1 (2t, J=7.5Hz, 3H, $CH_2$-C$\underline{H}_3$), 1.65 (m, 1H), 1.9-2.7 (m, 9H), 3.45 (m,;1H, CH-C$\underline{H}$Br), 3.65 (m, 1H, CH-C$\underline{H}$Br), 4.65 (d, J=15Hz, 2H, 2 O-C$\underline{H}$-$C_6H_5$), 5.4-5.65 (m, 2H, C$\underline{H}$=C$\underline{H}$), 7.1-7.45 (m, 10H, 2 $C_6\underline{H}_5$)

**7-Bromomethyl-6-pent-2Z-enyl-1,4-dioxa [4.4] nonane : 3γ**

3γ was prepared by the same procedure described for 3α: **2** (240 mg, 0.98 mmol), ethylene glycol (300 mg, 4.8 mmol), p-toluenesulfonic acid (50 mg), anhydrous benzene (25 mL). 3γ: (230 mg, 0.8 mmol). Yield: 81%. TLC: Rf=0.43 (silica gel; hexane/ethyl acetate (95/5)). $^1$H NMR ($CDCl_3$): 0.95 (t, J=7.5Hz, 3H, $CH_2$-C$\underline{H}_3$), 1.5 (m, 1H), 1.7-2.15 (m, 8H), 2.25 (m, 1H), 3.35 (dd, J=10.2, 6.5Hz, 1H, CH-C$\underline{H}$Br), 3.6 (dd, J=10.2, 3.2Hz, 1H, CH-C$\underline{H}$Br), 3.8-3.95 (m, 4H, O-($C\underline{H}_2$)$_2$-O), 5.25-5.5 (m, 2H, C$\underline{H}$=C$\underline{H}$)

**((2R,3R)-dimethyl-6-pent-2Z-enyl-1,4-dioxa-spiro [4.4] non-7-yl)-[$^{14}$CN] acetonitrile : 4α**

In a 100 mL round-bottomed flask were placed 3α (210 mg, 0.66 mmol), K$^{14}$CN (43 mg, 0.66 mmol, 58 mCi/mmol, 38.3 mCi) and 20 mL of freshly distilled DMSO stored on molecular sieve. The reaction mixture was heated at 80° C under nitrogen for 1.2 h. The mixture was cooled at room temperature and 150 mL of water were added. The mixture was extracted twice with 150 mL of diethylether. The combined organic layers were dried over magnesium sulfate, filtered and concentrated. The crude material was subjected to flash chromatography over silica gel with hexane/ethyl acetate (95/5) to give 4α (160 mg, 35 mCi, 0.60 mmol). Yield: 90%. TLC: Rf=0.27 (silica gel; hexane/ethyl acetate (95/5)).

**((2R,3R)-diphenyl-6-pent-2Z-enyl-1,4-dioxa-spiro [4.4] non-7-yl)-[$^{14}$CN] acetonitrile : 4β**

In a 100 mL round-bottomed flask were placed 3β (102 mg, 0.23 mmol), K$^{14}$CN (15.7 mg, 0.24 mmol, 58 mCi/mmol, 14 mCi) and 20 mL of freshly distilled DMSO stored on molecular sieve. The reaction mixture

was heated at 80° C under nitrogen for 1.7 h. The mixture was cooled at room temperature and 150 mL of water were added. The mixture was extracted twice with 200 mL of diethylether. The organic layer was dried over magnesium sulfate, filtered and concentrated. The crude material was purified on silica gel with hexane/ethyl acetate (95/5) to give 4β (9.4 mCi, 0.16 mmol) as a pale yellow oil. Yield from 3β: 70%. TLC: Rf=0.22 (silica gel; hexane/ethyl acetate (95/5)).

**(6-Pent-2Z-enyl-1,4-dioxa-spiro [4.4] non-7-yl)-[$^{14}$CN] acetonitrile : 4γ**

In a 100 mL round-bottomed flask were placed 3γ (210 mg, 0.73 mmol), K$^{14}$CN (47.1 mg, 0.72 mmol, 58 mCi/mmol, 42 mCi) and 30 mL of freshly distilled DMSO stored on molecular sieve. The reaction mixture was heated at 80° C under nitrogen for 5 h. The mixture was cooled at room temperature and 200 mL of water were added. The mixture was extracted twice with 200 mL of diethylether. The combined organic layers were dried over magnesium sulfate , filtered and concentrated. The crude material was subjected to flash chromatography over silica gel with hexane/ethyl acetate (90/10) to give 4γ (36 mCi, 0.62 mmol). Yield from 3γ: 85%. TLC: Rf=0.3 (silica gel; hexane/ethyl acetate (95/5)).

**((2R,3R)-dimethyl-6-pent-2Z-enyl-1,4-dioxa-spiro [4.4] non-7-yl)-[$^{14}CO_2H$] acetic acid : 5α**

In a 100 mL round-bottomed flask were placed 4α (19.8 mCi, 0.34 mmol), 25 mL of ethanol and 25 mL of aqueous potassium hydroxide (34%, d=1.33). The mixture was heated at 80° C overnight. 80 ML of water were added to the mixture and the aqueous solution was washed with 100 mL of diethylether, acidified with 3N HCl and then extracted with 100 mL of diethylether. The organic layer was dried over magnesium sulfate, filtered and concentrated for the next step. TLC: Rf of 5α=0.65 (silica gel; hexane/ethyl acetate/acetic acid (60/40/1)).

**((2R,3R)-diphenyl-6-pent-2Z-enyl-1,4-dioxa-spiro [4.4] non-7-yl)-[$^{14}CO_2H$] acetic acid : 5β**

5β was prepared from 4β by the same procedure described for the preparation of 5α: 4β (9.4 mCi, 0.16 mmol), 10 mL of ethanol, 10 mL of potassium hydroxide (34%, d=1.33). TLC: Rf of 5β =0.73 (silica gel; hexane/ethyl acetate/acetic acid (60/40/1))

**((1RS,2RS)-3-oxo-2-pent-2Z-enyl-cyclopentyl)-[$^{14}CO_2H$] acetic acid -( ( ± )-[$^{14}CO_2H$] JA) : 1**

In a 250 mL round-bottomed flask were placed 5α (14.8 mCi, 0.25 mmol), 50 mL of THF, 40 mL of acetic acid and 20 mL of 3N HCl. The mixture was stirred at room temperature for 3 h. 150 mL of diethylether were added and the mixture was washed with 5x100 mL of water. The organic layer was dried over magnesium sulfate, filtered and concentrated to give a brown oil which was purified on silica gel with hexane/ethyl acetate/acetic acid (60/40/0.1). 1 (13.5 mCi, 0.23 mmol) was obtained as a colorless oil. Yield from 4α: 68%.

-MS (DCI/$NH_3$): m/e (%) 213 (3.1), 228 (6), 229 (9), 230 (100), 231 (8.4). SA: 58 mCi/mmol

-TLC: Rf=0.31 (silica gel; hexane /ethyl acetate/acetic acid (60/40/0.1)) Radioactive purity: >99%

-HPLC: Column: Zorbax SB C18 (4.6x250 mm), Solvent system: acetonitrile/water/acetic acid (30/70/0.1) Flow rate: 1 mL/min. rt= 15 min. Radioactive purity: 99.5%

-$^1$H NMR ($CDCl_3$): 0.95 (t, J=7.5Hz, 3H, $CH_2$-C<u>H</u>$_3$), 1.55 (m, 1H), 1.9 (m, 1H), 2-2.45 (m, 10H), 2.75 (m, 1H), 5.25 (m, 1H, CH=C<u>H</u>), 5.45 (m, 1H, C<u>H</u>=CH). This NMR spectrum was indistinguishable from a NMR spectrum of authentic material.

1 (6.2 mCi, 0.10 mmol) was also prepared from 5β (7.2 mCi, 0.12 mmol). Overall yield from 4β: 66%

**((1RS,2RS)-3-oxo-2-pent-2Z-enyl-cyclopentyl)-[$^{14}$CN] acetonitrile : 6**

In a 250 mL round-bottomed flask were placed 4γ (36 mCi, 0.62 mmol), 30 mL of THF, 30 mL of acetic acid, 40 mL of 3N HCl. The mixture was stirred at room temperature for 2 h. 100 mL of diethylether were added and the mixture was washed with water. The organic layer was dried over magnesium sulfate, filtered and concentrated. The residue was chomatographed on silica gel with hexane/ethyl acetate (80/20). 6 (34.6 mCi, 0.59 mmol) was obtained as a colorless oil. Yield: 95%. TLC: Rf=0.4 (silica gel; hexane/ethyl acetate (70/30)). $^1$H NMR ($CDCl_3$): 0.95 (t, J=7.5 Hz, 3H, $CH_2$-C<u>H</u>$_3$), 1.7 (m, 1H), 1.9-2.55 (m, 10H), 2.7 (dd, J=16.8 Hz, 4.2 Hz, 1H), 5.2 (m, 1H, CH=C<u>H</u>), 5.5 (m, 1H, C<u>H</u>=CH)

**2-Hydrazino-2-oxo-N-((1S)-phenyl-ethyl)-acetamide : 7**

The compound 7 was prepared according to the procedure of Leonard et al (15). $^1$H NMR (DMSO): 1.45 (d, J=6.9 Hz, 3H, CH-C<u>H</u>$_3$), 4.5 (broad s, 2H, NH-N<u>H</u>$_2$), 4.95 (m, 1H, NH-C<u>H</u>-$CH_3$), 7.15-7.45 (m, 5H,

$C_6\underline{H}_5$), 9.1 (broad d, 1H, CH-N<u>H</u>), 10 (broad s, 1H, N<u>H</u>-$NH_2$).

mp: 169°C (lit (16): 168-169°C). $[\alpha]_D$= -99.4° ((589 nm), c 1, $CHCl_3$) (lit (16).: $[\alpha]_D$= -103°)

**2-[N'-(3-[$^{14}$CN] cyanomethyl-2-pent-2-enyl-cyclopentylidene)-hydrazino]-2-oxo-N-((1S)-phenyl-ethyl)-acetamide : <u>8a</u> and <u>8b</u>**

In an oven dried 100 mL two-necked round-bottomed flask were placed <u>6</u> (34.6 mCi, 0.59 mmol), <u>7</u> (183 mg, 0.88 mmol), catalytic p-toluenesulfonic acid and 50 mL of anhydrous benzene. The reaction mixture was heated at reflux under argon for 3.5 h. The mixture was concentrated and the resolution of 23.3 mCi of the diastereomeric crude mixture was achieved by flash chromatography using silica gel with toluene/ethyl acetate (80/20) followed by preparative HPLC on a ZORBAX SIL column (21.2x250 mm) with the same eluent until the other diastereomer could not be detected <u>8a</u> (10.1 mCi, 0.17 mmol) and <u>8b</u> (9.5 mCi, 0.16 mmol) were obtained.

<u>8a</u> : TLC: Rf=0.36 (silica gel; toluene/ethyl acetate (70/30)). $^1$H NMR ($C_6D_6$): 0.85 (m, 1H), 0.9 (t, J=7.5 Hz, 3H, $CH_2$-C<u>H</u>$_3$), 1.1 (d, J=7.3 Hz, 3H, CH-C<u>H</u>$_3$), 1.15-1.5 (m, 5H), 1.65 (dd, J=16.7, 4 Hz, 1H), 1.85-2 (m, 3H), 2.2-2.4 (m, 2H), 5.1 (m, 1H, NH-C<u>H</u>-$CH_3$), 5.25 (m, 1H, CH=C<u>H</u>), 5.4 (m, 1H, C<u>H</u>=CH), 6.95-7.15 (m, 5H, $C_6\underline{H}_5$), 7.9 (m, 1H, CH-N<u>H</u>-C=O), 9.65 (s, 1H, O=C-N<u>H</u>-N=C).

<u>8b</u> : TLC: Rf=0.23 (silica gel; toluene/ethyl acetate (70/30)). $^1$H NMR ($C_6D_6$): 0.9 (m, 1H), 0.9 (t, J=7.5 Hz, 3H, $CH_2$-C<u>H</u>$_3$), 1.15 (d, 7.3 Hz, 3H, CH-C<u>H</u>$_3$), 1.2-1.55 (m, 5H), 1.7 (dd, J=16.7, 4 Hz, 1H), 1.9-2.05 (m, 3H), 2.25-2.45 (m, 2H), 5.15 (m, 1H, NH-C<u>H</u>-$CH_3$), 5.3 (m, 1H, CH=C<u>H</u>), 5.4 (m,1H, CH=C<u>H</u>), 7-7.2 (m, 5H, $C_6\underline{H}_5$), 8.05 (broad d, 1H, CH-N<u>H</u>-C=O), 9.7 (s, 1H, O=C-N<u>H</u>-N=C).

**((1S,2S)-3-oxo-2-pent-2Z-enyl-cyclopentyl)-[$^{14}$CN] acetonitrile : <u>6a</u>**

In a 50 mL round-bottomed flask were placed <u>8a</u> (2 mCi, 0.034 mmol), 10 mL of benzene and 5 mL of 17% sulfuric acid. The reaction mixture was heated under reflux overnight. The organic layer was separated, washed with water, dried over magnesium sulfate and filtered. Benzene was removed and the crude product was purified on silica gel with hexane/ethyl acetate (80/20). <u>6a</u> (1.6 mCi, 0.027 mmol) was obtained as a colorless oil. Yield: 79%. Rf=0.4 (silica gel; hexane/ethyl acetate (70/30))

**((1R,2R)-3-oxo-2-pent-2Z-enyl-cyclopentyl)-[$^{14}$CN] acetonitrile: 6b**

**6b** (5.1 mCi, 0.088 mmol) was prepared from **8b** (5.8 mCi, 0.1 mmol) by the procedure described for **6a**. Yield: 88%.Rf=0.4 (silica gel; hexane/ ethyl acetate (70/30))

**((1S,2S)-3-oxo-2-pent-2Z-enyl-cyclopentyl)-[$^{14}CO_2H$] acetic acid- ( (+)-[$^{14}CO_2H$] JA): 1a**

In a 5 mL round-bottomed flask were placed **6a** (1.6 mCi, 0.027 mmol), 2 mL of ethanol, 1 mL of water, 0.05 mL of 30% sodium hydroxide. The reaction mixture was heated under reflux for 48 h. After acidification to pH=2 with 3N HCl, the mixture was concentrated. 30 mL of diethylether and 30 mL of water were added. The organic layer was separated and the aqueous layer was extracted with 30 mL of diethylether. The combined organic layers were dried over magnesium sulfate, filtered and diethylether was evaporated. The crude product was chomatographed on silica gel with hexane/ethyl acetate/acetic acid (60/40/0.1) and then submitted to preparative H.P.L.C. on a ZORBAX SIL column (21.2x250 mm) using hexane/ethyl acetate/acetic acid (80/20/0.1) as eluent until its radiochemical purity was better than 95%. **1a** (0.52 mCi, 0.009 mmol) was obtained as a colorless oil. Yied: 33%.

-MS (DCI/$NH_3$): m/e (%) 213 (4.1), 228 (7), 230 (100), 231 (17.3). SA: 58 mCi/mmol

-TLC: Rf=0.31 (silica gel; hexane /ethyl acetate/acetic acid (60/40/0.1)) Radioactive purity: 99.1%

-HPLC: Column: ZORBAX SB C18 (4.6x250 mm), Solvent system: acetonitrile/water/acetic acid (30/70/0.1). Flow rate: 1 mL/min. rt= 15 min. Radiochemical purity: 96.7%

Column: ZORBAX SIL 5µ (4.6x250 mm), Solvent system: hexane/ethyl acetate/acetic acid (60/40/0.2). Flow rate: 1mL/min. rt= 10.5 min. Radiochemical purity: 97.9%

Column: Nucleodex β PM (4x200 mm), Solvent system: methyl alcohol/TEA acetate 0.1% (pH=4) Flow rate: 0.8 mL/min. rt= 31 min. Radiochemical purity: **1a**: 95.7%. **1b**: 1%. [$^{14}CO_2H$] epijasmonic acid: 3.3%. Enantiomeric excess of **1a**: 98%

**((1R,2R)-3-oxo-2-pent-2-enyl-cyclopentyl)-[$^{14}CO_2H$] acetic acid -( (-)-[$^{14}CO_2H$] JA): 1b**

**1b** (0.74 mCi, 0.013 mmol) was obtained from **6b** (2.4 mCi, 0.041 mmol) by the procedure described above. Yield: 31%

-MS (DCI/$NH_3$): m/e (%) 213 (9.9), 228 (8.1), 229 (10.6), 230 (100), 231 (15). SA: 58 mCi/mmol

-TLC: Rf=0.31 (silica gel; hexane /ethyl acetate/acetic acid (60/40/0.1)) Radioactive purity: 99.6%

-HPLC: Column: ZORBAX SB C18 (4.6x250 mm), Solvent system: acetonitrile/water/acetic acid (30/70/0.1). Flow rate: 1 mL/ min. rt= 15 min. Radiochemical purity: 99.2%

Column: ZORBAX SIL 5μ (4.6x250 mm), Solvent system: hexane/ethyl acetate/acetic acid (60/40/0.2). Flow rate: 1 mL/min. rt= 10.5 min. Radiochemical purity: 96.5%

Column: Nucleodex β PM (4x200 mm), Solvent system: methyl alcohol/TEA acetate 0.1% (pH=4) Flow rate: 0.8 mL/min. rt= 21 min. Radiochemical purity: **1a**: <1%. **1b**: 97.6%. [$^{14}CO_2H$]-epijasmonic acid: 1%. Enantiomeric excess of **1b**: >98%

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