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# Preparation of Two Stereochemically Defined Isomers of Deuterium Labeled δ-Cadinene

Robert D. Stipanovic,* Howard J. Williams,**,*** Isabel Sattler,** A. Ian Scott,** S. Bradleigh Vinson,*** and Jinggao Liu*****

*U.S. Department of Agriculture, Agricultural Research Service, Southern Crops Research Laboratory, College Station, TX 77845.

**Departments of Chemistry, ***Entomology, and ****Biochemistry and Biophysics, Texas A&M University, College Station, TX 77843.

## SUMMARY

Deuterated δ-cadinenes needed for biosynthetic feeding studies in cotton were prepared by acid catalyzed rearrangements of (-)-α-cubebene and (+,-)-α-copaene. In both cases, one deuterium was incorporated in the 7 position. Stereochemistry of the added deuterium was determined by $^1$H NMR spectroscopy to be exclusively *cis* to the isopropyl group in the α-copaene derived product and predominantly *trans* (20% cis) to the isopropyl group in the cubebene derived product.

**Key words:** cadinene, deuterium, enantioselective

## INTRODUCTION

Cotton produces a variety of volatile[1-7] and non-volatile[8,9] terpenes which in some cases have been shown to confer resistance to herbivory.[10] Many of the non-volatile components (i.e. gossypol

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and hemigossypol) are derived from a cadinene terpene skeleton. (+)-δ-Cadinene itself is present in small quantity in the volatile terpene fraction, and it has been proposed to be the biosynthetic precursor of these natural products.[11, 12] For our studies of this biosynthetic pathway we required labeled δ-cadinene, and a deuterium or $^{13}C$ label was preferred as an NMR probe of reaction mechanism.

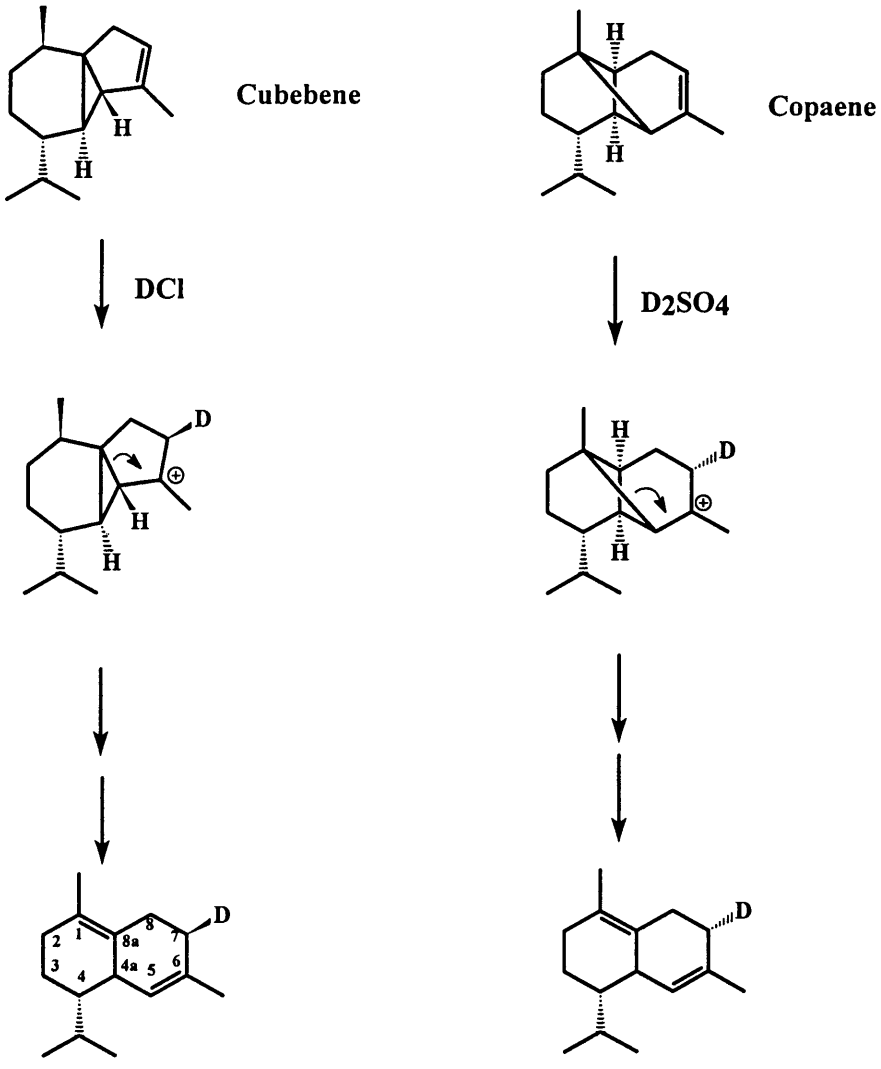
Several syntheses of the cadinene framework are found in the literature,[13-19] The two approaches which seemed most promising for simplicity and low cost were those based on rearrangements of α-cubebene and α-copaene.[20] Although the mechanisms of the two rearrangements are not known, it seemed reasonable that they involved protonation of a double bond followed by deprotonation at another position. If performed in a deuterated protic solvent these reactions should lead to a deuterated product. The position and stereochemistry of the added deuterium could then be determined by NMR spectroscopy.

## EXPERIMENTAL

Equipment. Capillary GC was performed on a Hewlett Packard 8390 instrument using a 25m X 0.32 mm I.D. Scientific Glass Engineering BP-1 column, He pressure 20 psig, temperature programmed 60° for 4 min, and then raised to 260° at 15° / min. Under these conditions, retention times were 10.90 min. for α-copaene, 10.61 min. for α-cubebene, and 12.19 min. for δ-cadinene. Preparative GC was performed using a 1.83m X 4 mm I.D. column packed with 3% OV-101 on Chromosorb 750, operated isothermally at 160°. The chromatograph was a Tracor 550 instrument with flame ionization detection and a Brownlee-Silverstein thermal gradient collection system[21] cooled with liquid nitrogen. NMR spectra were acquired in $CDCl_3$ solution using a Bruker ARX-500 500 MHz Fourier Transform instrument, utilizing a 45° pulse for both carbon and proton.

Preparation of (+)-(7R)-7-$^2$H)-δ-cadinene from (-)-α-cubebene. The method is based on that of Ohta *et al.*[20] Cubebene {Fluka, $[\alpha]_{546}^{20}$ -24.5 (in substance)(160.7 mg, 0.786 mmol)} dissolved in 10 ml of 0.012 M DCl in $D_2O$ : dioxane (2 : 8) was stirred at 50° for 2.5 h under nitrogen. The reaction mixture was poured into cold water (10 ml) and extracted with pentane (3 X 15 ml). The combined pentane extracts were washed with 1 M $NaHCO_3$ (6 ml) and distilled water (2 X 6 ml), and dried over sodium sulfate. Concentration gave 154.7 mg of crude product, shown by GC analysis to consist of δ-cadinene (51%), recovered cubebene (10%), and byproducts (39%). MS spectrum [e.i., m/z, (%)] 205 (46), 190 (16), 163 (25),162 (100), 135 (66), 134 (19), 121 (10), 120 (68), 119 (21), 94 (13), 93 (18), 92 (29), 91 (22), 82 (34), 81 (12), 79 (11), 78 (10), 77 (12), 69 (11), 55 (12). The product was purified by preparative GC for analysis and feeding studies.

Preparation of (+)-(7R)-7-$^2$H)-δ-cadinene from α-copaene. The method is based on that of Ohta *et al.* $^{20}$α-Copaene {Fluka, $[\alpha]_{546}^{20}$ -0.7 (in substance), 70:30 mixture of (-) and (+)-α-copaene) (210.5 mg, 1.03 mmol)} dissolved in 10 ml of 0.1 M $D_2SO_4$ in $D_2O$ : dioxane (2 : 8) was refluxed for 2 h under nitrogen. The mixture was poured into 10 ml cold water and then worked up as above to give 201.3 mg of crude product, shown by GC analysis to consist of δ-cadinene (46%), α-muurolene (38%), recovered copaene (6%), and other byproducts (10%). MS spectrum [e.i., m/z, (%)] 205 (34), 190 (14), 163 (33), 162 (100), 161 (21), 160 (47), 159 (23), 136 (13), 135 (61), 134 (22), 132 (12), 129 (12), 121 (13), 120 (71), 119 (27), 118 (11), 107 (12), 106 (52), 105 (45), 94 (12), 93 (18), 92 (28), 91 (23), 82 (32), 81 (13), 79 (11), 77 (13), 69 (10), 55 (12). The product was purified by preparative GC for analysis and feeding studies. The method is also based on that of Ohta *et al.*[20]




## RESULTS AND DISCUSSION

Both reaction pathways produced fair yields of labeled cadinene suitable for feeding studies. In both cases other products were also formed but the compound of interest could be separated by preparative gas chromatography. In the case of the cubebene based synthesis, the byproducts were chlorinated adducts which were easily separated while the copaene derived byproducts were isomeric to cadinene and the separation was more difficult.

Purification of sufficient material for NMR spectroscopy and feeding studies (10-100 mg) could be achieved in a single day for both products making a more complicated specific synthesis unnecessary. The difference in relative stereochemistry of the two deuterated reaction products will be useful in the biosynthetic studies. Thus, in the suggested biosynthetic conversion of δ-cadinene to hemigossypol pathway, one proton will be lost at C-7 as the hemigossypol product is aromatized. Performing the feeding experiment with the two different isomers will allow determination of the stereochemical nature of the oxidation process and may yield further clues concerning the enzymatic mechanism involved.

Proton and carbon-13 chemical shift assignments are given in Table 1. Unequivocal determination of the stereochemistry of the two products was straightforward from $^1$H NMR data. It depended on coupling constants determined for the isolated, well resolved peak of the neighboring C-8 equatorial proton. Apart from its geminal coupling of 12.9 Hz, this proton shows vicinal couplings of 4.7 and 2.5 Hz to its axial and equatorial C-7 neighbors, respectively. According to the Karplus curve, these coupling constants fit very well with dihedral angles of 47° and 71° determined by computer modeling. The corresponding C-8 equatorial proton peak for the deuterated product derived from α-cubebene is a simplified doublet of doublets with vicinol coupling of 4.7 Hz. This indicates 7-R stereochemistry with the equatorial C-7 proton replaced by deuterium. The coupling pattern of H-8 equatorial for the deuterated product derived from α-copaene shows a coupling of approximately 2 Hz, contaminated, however, with a small amount (approximately 23%) of the 7-R isomer produced in the cubebene experiment.

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**Table 1.** Proton and carbon-13 chemical shift assignments for δ-cadinene (non-deuterated).

| Position | Type | $^1H$ | $^{13}C$ |
|---|---|---|---|
| 1 | =C< | - | 124.5 |
| 2 | $CH_2$ | 1.93 m 2.0 m | 32.3 |
| 3 | $CH_2$ | 1.14 dddd 12.0, 11.9 11.9 5.6 | 21.2 |
| | | 1.59 ddd 12.4, 4.9 2.4 | |
| 4 | CH | 1.03 dddd 10.8, 10.8, 2.4, 2.4 | 45.3 |
| 4a | CH | 2.49 m | 39.4 |
| 5 | =CH | 5.46 br. s | 124.7 |
| 6 | =C< | - | 129.9 |
| 7 | $CH_2$ | 1.98 m | 31.9 |
| 8 | $CH_2$ | 1.92 m | 26.8 |
| | | 2.69 ddd 12.9, 4.7, 2.5 | |
| 9 | $CH_3$ | 1.63 s | 18.5 |
| 10 | $CH_3$ | 1.65 br. s | 23.6 |
| 11 | CH | 2.03 qqd 6.9, 6.8, 2.4 | 26.7 |
| 12* | $CH_3$ | 0.77 d 6.8 | 15.7 |
| 13* | $CH_3$ | 0.94 d 6.9 | 21.8 |

*Interchangeable

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