BIOTRANSFORMATION OF CARYOPHYLLENE BY DIPLODIA GOSSYPINA

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Abstract—Fermentation of caryophyllene with *Diplodia gossypina* affords a number of products which are all derived from the 4,5-epoxide Twelve of these are described here for the first time. The main products are 12-hydroxy-4,5-epoxy-caryophyllene and the corresponding acid On side reactions additional hydroxylations occurred or the exomethylene group was also epoxidized and further degraded. Two rearranged compounds were found, one resulting from an epoxide rearrangement leading to an eight-membered ring, the other one being a cyclization product possessing the rare skeleton of the punctatin antibiotics.

INTRODUCTION

To date about 50 natural products are known which contain the bicyclic sesquiterpene skeleton of caryophyllane. Some of them are produced by fungi and are biologically active, such as naematoline, naematolone [1] or punctatin B [2]. The caryophyllane skeleton is also considered to be the precursor of some tricyclic sesquiterpenes, e.g. of the antibiotics punctatin A, D, E and F [3, 4] Total or partial syntheses of these compounds are difficult, even chemical derivatization of the natural products is often impossible. There is the challenge to carry out biotransformations of compounds which are available in larger quantities in order to obtain known or previously undescribed derivatives [5-7]. Biotransformation of caryophyllene (1) have been carried out by Devi [8] with Pseudomonas cruciviae resulting in 1-hydroxy-14-norcaryophyllene and by Ishida et al. [9] and Asakawa et al. [10], with rabbits yielding, 4,5-epoxy-12caryophyllenol (3)

RESULTS

Diplodia gossypina ATCC 10936 was selected after a screen for biotransformation of caryophyllene on a preparative scale. Fermentation of caryophyllene (1) with Diplodia gossypina ATCC 10936 leads to 14 different products all being epoxidation products of caryophyllene. The simplest product and probably the first one in this cascade is the epoxide 2 This compound is known from many essential oils and is also a component of marihuana essential oil [11] which exerts a strong attraction on the cocker spaniel. The observation that 3 is responsible for the recognition of Cannabis sativa by dogs has since been confirmed [12]. The next step seems to be the hydroxylation of C-12 to give 12-hydroxy-4,5-epoxycaryophyllene (3). This substance is known as a natural product which previously had been isolated from *Lactar-us camphoratus* [13]. The published ¹³C NMR data do not agree with our findings Even after adjustment of the chemical shifts by a constant amount (+2 ppm) one signal was missing and two others were wrongly assigned. Compound 3 is also the main metabolite of caryophyllene in rabbits [9, 10]. It is further oxidized to the corresponding acid 4 which is the main product of this biotransformation. On a side reaction the acid 4 is oxidatively decarboxylated to the *nor*-compound 5

Another group of compounds are hydroxylation products of 3. From the fermentation broth compounds 6-8 were isolated. Their constitution could easily be solved by comparison of their ¹³C NMR spectra and their configuration was elucidated by the aid of NOE difference experiments. The coupling between 5-H and 6-H in 6 is 9 Hz, requiring an anti-diaxial orientation which requires the α -orientation of the hydroxyl group In 7 a NOE was observed between H-7 and H-9 which is only possible for an α -oriented hydroxy group, while H-15 in 8 displayed a NOE to H-9. Therefore the hydroxymethyl group at C-4 must occupy the β -position The configuration at C-4 remains the same in all compounds isolated from the fermentation of caryophyllene with Diplodia gossypina and the observed relation between H-9 (the absolute configuration of C-9 being known) and H-15 also gave the absolute configuration of C-4 and C-5.

To a minor degree, subsequent epoxidation of the exomethylene group is observed. The epoxydiol 7 is further oxidized to give the diepoxydiol 9. This substance could be crystallized after the first crude chromatography on silica gel. Although we could not isolate the diepoxide of 3, some metabolites of it were found One of it seems to be the corresponding triol which reacted intramolecularly with the 4,5-epoxide forming the ethertriol 10. Its configuration was determined by comparison of its NMR spectra with related compounds [14] 12-Hydroxykobus-

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one (11) was also found in the extract, probably derived from the 8,14-diol by diol cleavage. An epimeric pair of diols (12/13) was isolated which showed no evidence for a double bond. The minor compound 12 was studied in detail Assignment of the protons using various 2D NMR methods could not solve the stereochemistry at C-8 unambigously. However, NOE difference experiments displayed a NOE between H-5 and H-1 and between H-15 and H-8. Such a configuration is only possible with an α -oriented hydroxymethyl group. Compound 13 with a β oriented hydroxymethyl group is more polar.

In addition, compounds with a new carbon skeleton were formed in minor amounts The first one (14) resulted from a contraction of the 9-membered ring A strong hydrogen bond between the hydroxy group at C-5 and the carbonyl at C-6 is observed in the IR and ¹H NMR spectra of 14 The reaction path is not very clear but 4,5epoxy-caryophyllene-6,12-diol 6 will probably be the precursor We assume a rearrangement similar to the one observed by treatment of epoxides with strong Lewis acids However, treatment of caryophyllene epoxide with boron trifluoride did not yield any aldehydes Interestingly, Bohlmann *et al.* [15] proposed the same mechanism for the rearrangement of humulene to lychnocolumnic acid, isolated from *Lychnophora columnaris* (Compositae)

The skeleton of compound 15 is even stranger Here a new bond is formed between C-4 and C-8, resulting in a tricyclo[$6.300^{2.5}$]undecane While the cyclopentane ring is *cis*-fused to the cyclohexane ring, the cyclobutane ring is still *trans*-fused as it is in the caryophyllene starting material Molecular models show that the *trans*-fusion of the cyclobutane ring induces substantial strain in the molecule To solve the structure of this compound various 2D NMR techniques were used including *J*-resolved ¹H NMR and homo- and heteronuclear shift correlation. A homonuclear shift correlation optimized for long-range spin couplings proved particularly helpful in establishing the position of the formyl group Although only 3 mg of substance were available, ¹H¹³C-shift correlation could still be obtained for the methyl-, methine-, and hydroxymethylene signals Compounds with this rare skeleton were recently described from *Poronia punctata* and originally named punctatins, now punctaporonins [16]

The metabolism of caryophyllene with *Diplodia gossypina* ATCC 10936 produced highly oxygenated compounds The main reaction path is the epoxidation at C-4/C-5 and hydroxylation at C-12 The same reactions had been described for the metabolism in mammals Compound **3** is then further hydroxylated or epoxidized Of particular interest are the rearranged products **14** and **15** The observation of a very rare carbon skeleton in **15** indicates the advantage of biotransformation in the formation of such strained skeletons. The compounds obtained from the biotransformation of caryophyllene are currently tested for some of their biological activities

Besides the production of previously unknown sesquiterpenes and the study of the selectivity of the strains used, the biotransformation described herein, produced valuable NMR reference data To date the prediction of ¹³C NMR shifts of such complex molecules is relatively uncertain. Now the effects of additional hydroxy groups in the caryophyllane molecule can be deduced from the data of the caryophyllanes described here This will facilitate predictions of the chemical shifts of related substances

EXPERIMENTAL

The microorganisms were precultivated at 27 ° and 100 r.p.m. in 100 ml Erlenmeyer flasks containing 20 ml of the following medium: 1% glucose, 1% universal peptone (Merck), 2% malt extract and 0.3% yeast extract. After 48 hr, 10 #1 of caryophyllene (1) dissolved in 10 #1 of DMF were added to the cultures. After 24, 48 and 72 hr samples were taken and analysed as follows: to 1 ml of culture broth 0.2 ml of EtOAc were added and the mixture was shaken for 2 rain prior to centrifugation. 10/~1 of the extract were developed on HPTLC plates with CH2CI2-Me2CO (9:1) [17]. The spots were made visible by spraying with anisaldehyde-H2SO4 in HOAc and heating to 110° for 1 rain. For biotransformations on a preparative scale the microorganisms were grown in 5×100 ml flasks, transferred, after 48 hr, into five 21 flasks containing 400 ml of the medium and incubated for another period of 24 hr. The substrate (0.3 g/flask dissolved in 0.4 ml of DMF) was then added aseptically.

Biotransformation of 20 g caryophyllene was carried out in a 50 1 draft tube fermentor. The vessel was filled with 451 of the above medium. After sterilization 1200 mt of a 48 hr preculture was used as inoculum. Applying 120 r.p.m, the aeration was satisfactory with 10 vols of air per 100 vols of culture, pH 5 was obtained using a 15° /, soln of H3PO 4 and 15% soln of KOH. Before adding substrate the culture was allowed to grow for a total of 16 hr. Then 10 g of the substrate was added aseptically and another 10g was administered 8 hr later. The curve of conversion was followed by HPTLC and was terminated after 72 hr of contact.

Extraction and purification. Culture medium and mycelia were separated by filtration and both extracted $\times 3$ with EtOAc. The solvent was evapd and the crude extract separated on Si-60 columns with a n-hexane-EtOAc gradient (changing from 19:1 to 1:1). When necessary the collected fractions were purified further by prep. TLC.

The ~HNMR spectra were obtained at 400 MHz and the 3CNMR spectra at 75.5 MHz, CDC13 was the solvent if not stated otherwise and TMS the int. standard [18]. IR spectra and [~]D were measured in CHCI3 unless otherwise stated. Mass spectra were recorded with 70 eV. Mps: uncorr. TLC solvents: A =CH2C12-Me2CO (4:1) or B=CH2C12-Me2CO (7:3). Biotransformation of 20 g caryophyllene (1) with *Diplodia 90ssypina* ATCC 10936 in a 501 fermenter yielded, after 72 hr, 2 (65 mg), 3 (300 mg), 4 (900 mg), 5 (220 mg), 6 (180 mg), 7 (150 rag), 8 (60 mg), 9 (45 rag), 10 (35 rag), 11 (120 rag), 12 (60 rag), 13 (95 rag), 14 (25 mg), and 15 (15 rag).

(1R,4R,5R,9S,11R) -4,5-*Epoxy-caryophyll-8*(14)-*en-12-oic acid* (4). Colourless viscous oil. *R*: 0.59 (A). 1H and ~3C NMR data are listed in Tables 1 and 2. MS (m/z): [M-Me] + 223.1691 (5%) (223.1698 calc. for C1,~H2302).

$$[\sim] \begin{array}{c} 589 \text{ nm} & 578 \text{ nm} & 546 \text{ nm} & 436 \text{ nm} & 365 \text{ nm} \\ \hline & -55.5^{\circ} & -.57.6^{\circ} & -.64.6^{\circ} & -102.8^{\circ} & -.145.1^{\circ} \end{array} (c \ 1.00).$$

(1R,4R,5R,9S,11R)-4,5-Epoxy- 12-nor-8(14)-caryophyllene-11ol (5). Colourless viscous oil. R: 0.53 (A). 1H NMR: 35.00 (IH, brs, 14-H), 4.89(1H, brs, 14'-H), 2.91 (1H, dd, J = 10 and 4 Hz, 5-H), 1.25 (3H, s, 15-H), 1.19 (3H, s, 13-H). 13C NMR data are listed in Table 2. MS (re~z):207.1385 (1%) [M -Me] + (207.1385 calc. for C13H1002), 43 (100).

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(1R,4R,5R,6R,9S,11R)-4,5-*Epoxy-8(14)-caryophyUene-6,12diol* (6). Colourless viscous oil. *R*: 0.31 (B). 1H and 13CNMR data are listed in Tables 1 and 2. MS ($re\sim z$): [M] \div 252.1724 (0.5%) (252A725 calc. for ClsH2403), 43 (100).

(c 1.00, MeOH).

(1R,4R,5R,7R,9S,11R)-4,5-Epoxy-8(14)-caryophyllene-7,12diol (7). Colourless oil. R: 0.47 (B). ~H and ~3CNMR data arelisted in Tables 1 and 2. MS (re~z): [M] + 252.1724 (0.5%)252.1725 calc. for C15H2aO3), 43 (100).

$$[\sim] 589nm 578nm 546nm 436nm 365nm (c 1.00).$$

(1R,4S,5R,9S,11R)-4,5-Epoxy-8(14)-caryophyUene-12,15-diol (8). Colourless viscous oil. R/0.42 (B). 1H and 13C NMR data are listed in Tables 1 and 2. MS (*re~z*): [M-Me] + 237.1494 (1%) (237.1491 calc. for C14H2103), 83 (82), 43 (100).

(1R,4R,5R,7R,9S,I1R)-4,5; 8, 14-Diepoxycaryophyllane-7,12diol (9). Colourless crystals, mp 209°. R: 0.34 (B). 1H and I3CNMR data are listed in Tables 1 and 2. MS (re~z): [M-Me] + 237.1492 (2%) (237.1491 calc. for C14H2103), 161 (4), 55 (78), 43 (100).

(1R,4S,5R,8R,9S,11 *R*)-4,8-*Epoxycaryophyllane-5,12,14-triol* (10). Colourless oil. *Rf* 0.11 (B). ~HNMR: 63.62 (1H, *dd*, J=8 and 3 Hz, 5-H), 3.39 (2H, s, t2-H), 3.28 (1H, d, J= 10 Hz, 14-H), 3.20 (1H, d, J=10 Hz, 10'-H), 1.22 (3H, s, 15-H), 1.08 (3H, s, 13-H).

[ct]
$$589$$
 nm 578 nm 546 nm 436 nm (c 0.46).
-9.4° -9.6~ -10.7° -16.3°

(1R,4R,5R,9S,11R)-4,5-Epoxy-8-oxo-*l4-norcaryophyllan-12-ol* (11). Colourless oil. *R*: 0.44 (A). XH and 13CNMR data are listed in Tables 1 and 2.

(1R,4R,5R,8R,9S,11R)-4,5-Epoxycaryophyllane-12,14-diol (12). Colourless oil. *R*: 0.29 (B). ~H and 13CNMR data are listed in Tables 1 and 2. MS (*m*/*z*): [M-CHzOH] + 223.1698 (6%) (223.1698 calc. for C14H2302), 43 (100).

(1R,4R,5R,8S,9S,11R)-4,5-Epoxycaryophyllane- 12,14-diol (13).Colourless oil. R: 0.22 (B). 1H NMR: c53.31 (2H, m, 14-H), 3.27 (2H, m, 12-H), 3.06 (1H, dd, J = 10 and 5 Hz, 5-H), 1.29 (3H, s, 15-H), 0.99 (3H, s, 13-H). 13CNMR data are listed in Table 2. MS (m/z): [M--CH2OH] + 223.1700 (3%) (223.1698 calc. for C14H2302), 43 (100).

(1R,9S,11R)-4,11-Bis(hydroxyrnethyl)-4,11-dimethyl-8-methylene-6-oxo-bicyclo[6.2.0]decane (14). Colourless oil. R: 0.68 (A). IR Vmaxcm-X: 3640, 1710. ~H and 13CNMR data are listed in Tables 1 and 2.

(1R,4R,8R,9S,11R)-4,11 -Dimethyl-8-formyl-5-hydroxymethyltricyclo[6.3.0.0]l"glundecan-5-ol (15). Colourless viscous oil. Rf

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	3	4	9	٢	œ	6	II	12	14	15
H-1	2 08 ddd	2,52 dd	1 88 dd	1 87 m	2 16 ddd	2 02 ddd	2 29 ddd	2 16 m	1 84 <i>dàd</i>	1 78 ddd
H-2	1,67 dddd	1,82 <i>dddd</i>	15 m	1 64 dddd	1 70 dddd	1 76 dddd	1 70 dddd	174 m	1 58 m	1 44 dddm
H-2′	1 49 <i>dddd</i>	1 56 dddd	1 76 dddd	1 53 dddd	1 41 m	1 62 <i>dddd</i>	1 60 <i>dddd</i>	1 44 m		1,4 m
H-3	1 00 ddd	1 04 ddd	1 08 ddd	2 13 ddd	2 50 ddd	2 10 ddd	2 15 ddd	2,06 m	2 32 <i>ddd</i>	1 25 m
H-3'	2 07 ddd	2 08 ddd	2 04 ddd	0 93 ddd	0 87 444	1 07 m	<i>ppp</i> 66 0	1 03 m	1 29 m	12 m
H-5	2,92 dd	2 88 dd	2 96 d	2 67 dd	3 08 dd	3 16 dd	2 73 dd	2 97 dd	4 40/4 29 <i>d</i>	3 65 dd
H-6	2 25 dddd	2 25 dddd	3 49 ddd	2 16 ddd	2 30 m	2 12 ddd	2 40 dddd	2 23 m	}	2 36 dddd
,9 - H	1 33 <i>dddd</i>	1 35 dddd		1 84 <i>ddd</i>	14 m	1 52 ddd	1 47 <i>dddd</i>	1 21 m	ł	1 77 dddd
Н-7	2 36 ddd	2,35 ddd	2 53 dd	4 11 dd	2 30 m	4 00 <i>dd</i>	2,58 m	1 59 m	2 65 ABq	2.10 ddd
H-7′	2 13 ddd	2 12 ddd	2 45 dd	ł	2 10 m			1 46 m		1 94 <i>ddd</i>
H-8	<u></u>	e. F	ł	1, 1	1		Ť	1 71 m		ł
H-9	2 67 ddd	2 70 ddd	2 79 ddd	2 48 <i>ddd</i>	2 69 ddd	2 5 ddd	3 09 ddd	2 37 dddd	2 15 m	2 39 dd
H-10	1 83 dd	2 34 dd	2 33 dd	1 70 ddd	1 80 <i>dd</i>	1 46 <i>dd</i>	2,25 dd	1 51 dd	1 73 m	1 53 dd
H-10′	1 57 dd	1 78 dd	1 55 dd	2 07 dd	1 56 dd	1,37 dd	1 58 dd	1 46 dd	1 73 m	1 43 <i>dd</i>
H-12	3 34 d	- I	3 32 d	3 37 s	3 34 s	3 22 ABq	3,40 d	3 31 s	3 35 s	3 42 s
H-12′	3 33 d		3 26 d			I	3 39 d			
H-13	1 06 s	135s	1 08 s	1 06 5	1 06 s	1 07 s	1,09 s	1 03 s	117s	1195
H-14	5 02 d	5 04 d	5 08 s	5 46 s	5 00 s	3 10 d		3 42 dd	482 s	9 68 s
H-14′	488 d	491 d	4,94 s	5 33 s	484s	2 68 d	÷	3 36 dd	4 58 s	ł
H-15	1,21 s	121 <i>s</i>	1 24 s	1 32 s	3 82 d	1 38 s	1 32 s	1 29 s	1 02 s	113 <i>s</i>
H-15′	ł	ł	ł		3 40 d	÷	ł	÷	ł	ł

Table 1 ¹H NMR data of compounds 3-9 (4001 MHz, CDCl₃, 6, 9 m CD₃OD, 15 m C₆D₆)

= 12, 2.3' = 4, 2', 3 = 4, 2', 3' = 3, 3.3' = 12, 5, 6 = 9, 6, 7 = 9, 6, 7' = 4, 7, 7' = 13, 9, 10 = 10, 9, 10' = 9, 10, 10' = 10, 12, 12' = 11, 7, 12 = 2, 1, 2' = 10, 2, 2' = 15, 2, 3 = 4, 2, 3' = 12, 2, 3' = 12, 3, 3' = 12, 5, 5' = 12, 5' = 1 $= 109 \ 12 \ 1,9 = 9 - 10, 5,6 = 36, 5,6' = 114, 8, 14 = 73, 8, 14' = 74, 8, 9 = 5, 9, 10 = 110, 9, 10' = 81, 10, 10' = 102, 14, 14' = 103 \ 14 \ 1,2 = 5, 1,2' = 10, 1,9 = 10, 2,3 = 4, 2,3' = 4, 3, 3' = 4, 3, 3' = 4, 3, 3' = 4, 3, 3' = 4, 3, 3' = 4, 3, 3' = 4, 3, 3' = 4, 3, 3' = 4, 3, 3' = 4, 3, 3' = 4, 3, 3' = 4, 3, 3' = 4,$ $\cdot 81, 6', 7' = 47, 7, 7' = 131, 9, 10 = 106, 9, 10' = 8.3, 10, 10' = [07, 12, 12' = 109, 14, 14' = 16, 41, 2 = 1, 1, 2' = 10, 1, 9 = 10, 2, 2' = 145, 2, 3 = 5, 2, 3' = 4, 2, 3 = 13, 2, 3' = 4, 2, 3 = 13, 2, 3' = 14, 2, 3 = 13, 2, 3' = 14, 3, 3' = 13, 3' = 14, 3'$ 3,3' = 13, 5,6 = 4, 5,6' = 10, 5,6,6' = 12, 5,6,7 = 8,6,7' = 4,5,6,7 = 4,5,6,7' = 8,7,7' = 12,5,9,10 = 10,9,10' = 9,10,10' = 10,5,14,14' = 1,5,6,1,9 = 10,2,2' = 13,2,3,10' = 10,10' = 10,5,10' = 10,10=4; $2^{2}3=3$, $2^{2}3=13$, $3^{2}3=13$, $5^{6}6=5$, $5^{6}6=125$, $6^{7}6=95$, $6^{7}7=65$, $9^{1}0=10$, $9^{1}0^{7}=10$, $10,10^{7}=11$, $1,2^{7}=10$, 1,9=10, $22^{7}=15$, 23=3, $23^{7}=12$, -55, 2', 3=4, 2', 3'=13, 3, 3'=13, 5, 6=11, 5, 6'=4, 9, 10=10, 9, 10'=9, 10, 10'=10, 15, 15'=11, 9, 1, 2'=10, 1, 9=10, 2, 2'=15, 2, 3=4, 2, 3=3, 2, 3'=3, 2, 3'=1, 2, 3'=1, 3, 3'=1, 3, 3'=1, 3, 3'=1, 3, 3'=1, 3, 3'=1, 3, 3'=1, 3, 3'=1, 3, 3'=1, 3, 3'=1, 3'== 13, 3, 3' = 13, 3, 6 = 55, 5, 6' = 95, 6, 6' = 12, 6, 7 = 105, 6', 7 = 5, 9, 10 = 10, 9, 10' = 9, 10, 10' = 10, 14, 14' = 55 11 1, 2 = 1 8, 1, 2' = 11 0, 1, 9 = 85, 2, 2' = 15, 2, 3 = 48, 2, 3' = 13, 3, 5' = 10, 10' = 10, =37, 2,3=13, 2,3'=37, 3,3'=131, 3,15>0, 3,15>0, 5,6=49, 5,6'=101, 5,15>0, 6,6'=132, 9,10'=82, 10,10'=112, 10,12>0, 10,13>0, 12,12'=12, 10,12'=112, 1=4, 3, 3'=14, 5, 5'=18 **15** 1, 2/1, 2'=4/11, 1, 9=125, 5, 5=55, 5, 6=12, 5, 15>0, 6, 6'=148, 6, 7=56, 6, 7'=117, 6, 7=102, 6, 7'=52, 7, 7'=143, 9, 10=71, 9, 10'= 110, 10, 100 = 98, 10, 12 > 0, 12, 13 > 0

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	e	4	Ś	9	L	æ	6	11	12	13	14	15
5	45,1 +*	477+*	53.8 +	43.4 +	44.5 +	43.7 +	45.4 +	45.4 +	39.9 +	45.2 + ª	447+	41.1 +
C-2	278 -	29.8 – ^b	26.5 -	28 2	28.0 -	27.1 -	287 -	272 -	278 -	$28.0 - ^{b}$	26.2 -	23.4 ^a
C-3	39,0 -	38.5	38.7	39.7	39.5	33.5 —	40.8 -	38.9 - *	38.3 -	40 3	38 9	36.6 ^a
0-4	5980	59.50	59.8 0	5930	59.00	62.7 0	6100	59.0 0	60.2 0	59.40	49.10	61.10 ^b
C-5	637+	63.5 +	638+	688+	59.5 +	65.0 +	59.2 +	617+	65.5 +	61.7 +	64.8 -	815+
C-6	30.0 -	29 7 - ^b	29.3 - a	730+	37.5 -	29.1 –	34.0 -	30.1 -	29.0 -	298 — ^b	21410	23.3 - ^a
C-1	30.0 -	$351 - ^{b}$	30 3 ª	39.7 -	73.4 +	342 -	723+	37.9*	218 -	28.3 – ⁶	45.2 -	32.9 - "
С-8	15170	150.40	150.60	14850	157.70	15140	6470	n.d	42.2 +	46.8 + ^a	15020	52 8 0 ^b
6-0	48,2 +	473+*	449+	48.7 +	50.1 +	48.5 +	44.6 +	51.7 +	42.3 +	458+ ^b	408+	352+
C-10	34,2 -	27.5 - b	42.6	35.1 -	34.0 –	30.1 -	30.5 –	24.7 -	28.7 -	$33.7 - ^{b}$	33.6	32.8 - *
C-11	3860	4180	7110	3990	37.50	39.0 0	38.90	39.0 0	39.7 0	3870	39.5.0	4400
C-12	- 70 7	182 8 0]	70.1 -	- 101	70.6 –	711 –	70 3 -	70.7 —	702 -	716 -	718 -
C-13	171+	$168 + ^{\circ}$	215+	174+	166+	16.9 +	166+	$16.3 + ^{b}$	167+	163+	17.2 +	16.2 +
C-14	113,1 -	1140 -	1138-	1151 -	111.7 -	113.6 -	510-	l	66.2 -	663 -	108 1 -	2076+
C-15	170+	16.4 +°	171 +	187+	179+	62.1 -	174+	174 + b	176+	173+	31.4 +	166 +

*Amplitude of signals in DEPT-135 spectrum (CH₃ or CH = +, CH₂ = -, quat C=0) ^{a - c}Assignments may be interchanged

Caryophyllene biotransformation

0 41 (A) ¹H and ¹³C NMR data are listed in Tables 1 and 2 MS (m/z) [M]⁺ 252 1724 (2%) (252 1725 calc for C₁₅H₂₄O₃), 221 [M-CH₂OH]⁺ (4), 134 (40), 107 (42), 43 (100)

$$[\alpha] = \frac{589 \text{ nm}}{-67} \frac{578 \text{ nm}}{-75^{\circ}} \frac{546 \text{ nm}}{-87^{\circ}} \frac{436 \text{ nm}}{-191} (c \ 100)$$

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