Biotransformation of Humulene by Fungi and Enantioselectivity of the Strains Used

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Z. Naturforsch. **42c**, 79–86 (1987); received September 2, 1986

Dedicated to Professor F. Bohlmann on the occasion of his 65th birthday

Biotransformation, Sesquiterpene, Humulene, Enantioselectivity, Kinetic Resolution

Humulene was transformed by strains of *Chaetomium cochlioides* and *Diplodia gossypina* ATCC 10936 affording 33 new humulanes. The first step is a non-enantioselective epoxidation of the 1,2-double bond, whereas the subsequent hydroxylations were performed preferably at the 1R,2R-epoxide. To proof these results the *anti*-1,2;8,9-diepoxide was synthesized and used as substrate. While the 1R,2R,8S,9S-antipode of this diepoxide was hydroxylated the other one was recovered unchanged. *Diplodia gossypina* ATCC 10936 gave the same products but differing in relative and absolute configurations.

Introduction

To date some thousands sesquiterpenes are known from a broad range of sources. Many of them are biologically active compounds. Syntheses of new sesquiterpenes for pharmaceutical screening are every time consuming and therefore uneconomical. Here biotransformations are advantageous because unexpensive and readily available starting materials can be converted to chiral products, enriched in one enantiomer or even being optically pure.

Previous attempts to convert sesquiterpene hydrocarbons were not very successful because of low yields. In 1962 Prema and Bhattacharyya [1] reported on the biotransformation of humulene with *Aspergillus niger*. They isolated an alcohol of unknown structure in very low yield, probably an artifact. In 1978 Devi [2] grew a strain of *Pseudomonas cruciviae,* isolated by enrichment cultures, on caryophyllene as sole carbon source. The structure of the isolated hydroxyketone of caryophyllene was elucidated mainly by its infrared spectrum. Recently we reported on the biotransformation of isolongifolene [3] and we now present the results of the transformation of humulene which gave products from moderate to good yields.

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Verlag der Zeitschrift für Naturforschung, D-7400 Tübingen
0341–0382/87/0100–0079 $ 01.30/0

Experimental

The fungi were precultivated at 27 °C and 100 r.p.m. in five 100 ml EM flasks containing 20 ml of the following medium: 1% of glucose, 1% of universalpeptone (Merck), 2% of malt extract and 0.3% of yeast extract. After 72 h the cultures were passed into five 2 liter flasks filled with 400 ml of the medium and incubated for another period of 48 h. Then the substrate (0.4 ml/flask) was added aseptically. After 16, 48, and 72 h samples were taken and analyzed as follows: To 1 ml of culture broth 0.2 ml ethylacetate was added and shaken for 2 min. prior to centrifugation. 10 µl of the extract were developed on HPTLC plates with dichloromethane–acetone 9:1 [4]. The spots were made visible by spraying with anisaldehyde-sulfuric acid in acetic acid and heating to 110 °C for 1 min.

Extraction and purification: Culture medium and mycelia were separated by filtration and both extracted three times with ethylacetate. The solvent was evaporated and the crude extract separated on Si-60 columns with a *n*-hexane/ethylacetate gradient (changing from 19:1 to 1:1). When necessary the collected fractions were purified further by preparative TLC.

Instruments used: NMR: The ^{1}H NMR spectra were obtained at 400 MHz on a Bruker WM 400 spectrometer and the ^{13}C NMR spectra at 75.5 MHz on a Bruker AM 300 spectrometer. If not stated otherwise $CDCl_3$ was the solvent and TMS the internal standard. IR: spectra were measured on a IR Spectral-Photometer 297, Perkin Elmer, in chloro-

Table I: ^{1}H NMR data of **2**–**4**, **6**, **8**–**11**, **13**–**15** (400.1 MHz, $CDCl_3$, coupling constants in Hz in parentheses).

	2	**3**	**4**	**6**	**8**	**9**	**10**	**11**	**13**	**14**	**15**
1-H	t 4.96 (7)	t 4.87 (7)	m 5.03	t 4.97 (7)	dd 2.58 (10.5, 2.5)	d 2.66 (8)	dd 2.41 (10, 5)	dd 2.92 (11, 1.5)	dd 2.52 (10, 4)	d 2.66 (9)	dd 2.38 (10, 5)
3-H	dd 2.60 (14, 7)	d 2.55	dd 2.79 (17, 8)	dd 2.74 (13, 4)	dd 2.60 (13, 5.5)	dd 2.61 (12, 5)	dd 2.64 (13, 5)	m 2.52	dd 2.64 (14,5)	ddd 2.55 (13.5, 5.5, 1.3)	dd 2.59 (11, 5)
3′-H	dd 2.42 (14, 8)	(7)	dd 2.38 (17, 7)	dd 1.50 (13, 10)	dd 1.77 (13, 9.5)	dd 1.74 (12, 10)	dd 1.56 (13, 12)	m 1.82	dd 1.66 (14, 12)	dd 1.86 (13.5, 9)	dd 1.54 (11, 10)
4-H	ddd 5.71 (15, 8, 7)	dt 5.70 (15, 7)	ddd 5.75 (16, 8, 7)	ddd 2.99 (10, 4, 2)	ddd 5.36 (16, 9.5, 5.5)	ddd 5.41 (15, 10, 5)	ddd 5.39 (15, 12, 5)	m 5.34	ddd 5.43 (16, 10, 6)	ddd 5.40 (15, 9, 5.5)	ddd 5.25 (16, 10, 5)
5-H	d 5.14 (15)	d 5.14 (15)	d 5.13 (16)	d 2.18 (2)	d 5.18 (16)	d 5.25 (15)	d 5.06 (15)		d 5.16 (16)	dd 5.24 (15, 1.3)	d 5.08 (16)
7-H	d 3.96 (10)	dd 2.25 (12, 11)	m 2.10	d 4.13 (10)	dd 2.13 (13.5, 9)	dd 2.16 (14, 9)	d 4.02 (10)	d 4.10 (10)	m 2.12	dd 2.18 (14, 10.5)	d(br) 1.83 (13)
7′-H	–	d(br) 1.80 (12)		–	dd 1.84 (13.5, 6)	dd 1.85 (14, 6)	–	–		d(br) 1.85 (14)	dd 2.12 (13, 11)
8-H	d 5.00 (10)	d 5.07 (11)	m 5.03	d 5.06 (10)	dd 5.04 (9, 6)	dd 5.00 (9, 6)	d 5.10 (10)	ddq 5.61 (10, 1, 1)	dd 5.00 (10, 3)	d(br) 5.13 (10, 5)	d(br) 5.18 (11)
10-H	m 2.05	dd 4.09 (10, 7)	dd 2.57 (14, 5)	m 2.0	m 2.22	m 2.39	m 2.2	t(br) 4.29 (3.5)	m 2.25	dd 2.53 (13, 4.5)	dd 4.16 (10, 6.5)
10′-H	to	–	m 2.06	to				–		dd 2.30 (13, 10)	–
11-H	m 2.15	dt 2.48 (13, 7)	dt 4.54 (11, 6)	m 2.3	dtd 2.03 (14, 5.5, 2.5)	dt 3.71 (8, 3)	m 2.0 (14.5, 11, 3.5)	ddd 1.63	m 1.80	ddd 3.66 (9.5, 9, 5)	ddd 2.26 (13.5, 10, 5)
11′-H		dt 2.09 (13, 10)	–	–	dddd 1.43 (14, 10.5, 9, 5.5)	–	m 1.4 (14.5, 3.5, 1.5)	ddd 2.15	m 1.32	–	ddd 1.67 (13.5, 10, 6.5)
12-H	s 1.56	s 1.50	s 1.72	s 1.64	s 1.29	s 1.32	s 1.31	s 1.23	s 1.32	s 1.34	s 1.32
13-H	s 1.13	d 3.48 (11)	d 3.47 (12)	s 1.18	d 3.64 (11)	d 3.58 (10)	s 1.16	s 1.18	d 3.52 (11)	d 3.58 (10.5)	s 1.12
13′-H	–	d 3.41 (11)	d 3.39 (12)	–	d 3.48 (11)	d 3.48 (10)	–	–	d 3.45 (11)	d 3.46 (10.5)	–
14-H	s 1.04	s 1.12	s 1.14	s 0.69	s 1.15	s 1.17	s 1.07	s 1.09	s 1.17	s 1.16	s 1.09
15-H	s 1.64	s 1.67	s 1.46	s 1.68	s 1.53	s 1.70	s 1.73	d 1.58 (1)	s 1.59	s 1.58	s 1.62

form. Mass spectra were recorded on a AEI 902S mass spectrometer with 70 eV. Melting points are uncorrected and were obtained at Büchi 510 melting point apparatus. Optical rotation: Perkin-Elmer Polarimeter 241.

Biotransformation of 1.7 g of humulene **1** with *Chaetomium cochlioides* DSM 63353 yielded within 96 h 85 mg **21**, 15 mg **16**, 14 mg **13**, 13 mg **23**, 7 mg **14**, 7 mg **29**, 5 mg **10**, 5 mg **27**, 4 mg **15**, 4 mg **26**, 4 mg **28** (8R, 9R; α_D +6.6°), 3 mg **3**, 3 mg **4**, 3 mg **18**, 3 mg **19**, 3 mg **20**, 3 mg **22**, 2 mg **7** + **12**, 2 mg **11**.

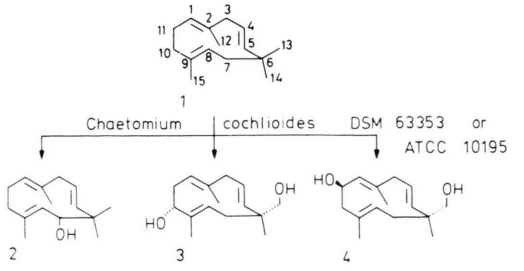

Fig. 1. Hydroxylation products of humulene.

Table I (continued): ^{1}H NMR data of **16–27** (400.1 MHz, $CDCl_3$ if not stated otherwise, coupling constants in Hz in parentheses).

	16	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**(C_6D_6)	**25**	**26**	**27**
1-H	dd 2.60 (10.5, 1.5)	m 2.2	d 2.54 (8)	dd 2.57 (10.5, 2.5)	dd 2.47 (11, 5)	dd 2.37 (10, 5)	d 2.64 (10)	d 2.59 (10)	d 2.32 (10)	dd 2.82 (8, 3)	dd 2.81 (11, 3)	dd 2.80 (11, 2.5)
3-H	dd 2.54 (13, 4.5)	dd 2.62 (12, 5)	dd 2.62 (12, 4)	dd 2.73 (12.5, 4)	dd 2.80 (12, 5)	dd 2.61 (12, 5)	dd 2.61 (12.5, 3)	dd 2.68 (14, 4)	dd 2.40 (13, 5)	m 2.2	dd 2.17 (14, 6)	dd 2.17 (14, 6)
3′-H	dd 1.83 (13, 6.5)	dd 1.53 (12, 11)	dd 1.62 (12, 11)	dd 1.77 (12.5, 10)	t 1.77 (12)	dd 1.58 (12, 10)	dd 1.80 (12.5, 7)	dd 1.87 (14, 9)	dd 1.66 (13, 9)	dd 1.54 (12, 9)	dd 1.54 (14, 8)	dd 1.49 (14, 9)
4-H	m 5.33	ddd 5.38 (16, 11, 5)	ddd 5.34 (15, 11, 4)	ddd 5.42 (16, 10, 4)	ddd 5.71 (16, 12, 5)	ddd 5.38 (16, 10, 5)	m 5.57	ddd 5.61 (15, 9, 4)	ddd 5.24 (16, 11, 5)	m 2.82	m 2.83	ddd 2.74 (9, 6, 2.5)
5-H		d 5.04 (16)	d 5.12 (15)	d 5.53 (16)	d 5.58 (16)	d 5.10 (16)		d 5.43 (15)	d 5.32 (16)	d 2.49 (3)	d 2.43 (3)	d 2.45 (2.5)
7-H	d 4.03 (10)	d 4.05 (10)	d 4.09 (10)	–	–	t 2.20 (12)	d 1.71 (14)	d 1.69 (14)	d 1.94 (14)	d(br) 1.92 (14)	d 4.19 (9)	dd 2.37 (15, 11)
7′-H	–	–	–	–	–	d(br) 1.79 (12)	dd 1.46 (14, 9.5)	dd 1.55 (14, 10)	m 1.17	m 2.2	–	d(br) 1.96 (15)
8-H	d 5.21 (10)	d(br) 5.26 (10)	d 5.03 (10)	s 6.07	s 6.13	d(br) 5.16 (12)	d 2.69 (10)	d 2.72 (10)	d 2.58 (10)	d 5.15 (11)	d 5.33 (9)	d 5.35 (11)
10-H	m 2.2	m 4.11	dd 2.49 (14, 1)	m 2.28	m 2.25	dd 4.13 (10, 6)	dt 2.23 (14, 4)	dt 2.25 (14, 4)	m 1.99	m 2.2	m 2.28	dd 4.35 (11.5, 4)
10′-H		–	dd 2.23 (14, 5)			–	td 1.47 (14, 3)	m 1.28	m 1.17			–
11-H	dtd 1.96 (14, 4.5, 1.5)	m 2.2	ddd 3.72 (8, 5, 1)	m 2.16	m 2.15	ddd 2.25 (12, 10, 5)	ddd 2.00 (14, 4, 3)	dt 2.00 (14, 4)	m 1.74	m 2.02	m 2.1	ddd 2.26 (13, 4, 2.5)
11′-H	dddd 1.46 (14, 12.5, 10.5, 5)	m 1.68	–	m 1.48	m 1.48	ddd 1.67 (12, 10, 6)	m 1.28	m 1.48	m 1.17	m 1.4	m 1.4 (13, 11.5, 11)	ddd 1.62
12-H	s 1.24	s 1.31	s 1.32	s 1.24	s 1.34	s 1.33	s 1.20	s 1.22	d 3.22 (10)	s 1.20	s 1.25	s 1.22
12′-H	–	–	–	–	–	–	–	–	d 3.18 (10)	–	–	–
13-H	s 1.19	s 1.16	s 1.18	s 1.24	ABq 3.70	ABq 3.45	s 1.18	d 3.47 (11)	s 0.93	s 1.13	s 1.18	s 1.14
13′-H	–	–	–	–			–	d 3.39 (11)	–	–	–	–
14-H	s 1.06	s 1.07	s 1.07	s 1.23	s 1.33	s 1.16	s 1.10	s 1.23	s 0.90	s 0.77	s 0.72	s 0.78
15-H	s 1.62	s 1.74	s 1.90	s 1.85	s 1.89	s 1.62	s 1.39	s 1.42	s 1.06	s 1.61	s 1.77	s 1.65

Biotransformation of 1.7 g of humulene **1** with *Chaetomium cochlioides* ATCC 10195 yielded within 72 h 166 mg **21**, 48 mg **13**, 46 mg **16**, 26 mg **10**, 20 mg **3**, 15 mg **25**, 15 mg **31**, 13 mg **19**, 11 mg **8**, 10 mg **14**, 10 mg **27**, 9 mg **28** (8R, 9R; α_D +14.8°), 8 mg **17**, 8 mg **24**, 7 mg **23**, 7 mg **26**, 6 mg **22**, 6 mg **33**, 5 mg **4**, 5 mg **9**, 5 mg **11**, 5 mg **20**, 4 mg **2**, 4 mg **15**, 4 mg **29**, 3 mg **5**, 3 mg **6**, 3 mg **7** + **12**, 2 mg **30**.

Preparation of *anti*-1,2-;8,9-diepoxy-humulene **28**: 1.5 g humulene were solved in 100 ml dried chloroform, cooled to 0 °C, and stirred. To this solution 2.5 g *m*-chloro-perbenzoic acid (90% peracid) solved in 50 ml chloroform was dropped during 1 h. Then the solution was stirred for another 23 h. After filtration the solution was washed twice with saturated sodium hydrogen carbonate and twice with water,

Table I (continued): ^{1}H NMR data of **28**–**31**, **33**–**38** (400.1 MHz, $CDCl_3$, coupling constants in Hz in parentheses).

	28	**29**	**30**	**31**	**33**	**34**	**35**	**36**	**37**	**38**
1-H	dd 2.74 (10, 4)	dd 2.75 (10, 4)	dd 2.48 (10, 4)	dd 2.36 (9.5, 5.5)	t 5.28 (7)	dd 2.73 (8, 4.7)	dd 2.73 (9.7, 5)	dd 2.63 (10, 5.2)	dd 2.67 (10, 4.5)	dd 2.50 (10, 3)
3-H	dd 2.65 (12, 5)	dd 2.68 (12, 5)	dd 2.68 (12, 2)	dd 2.68 (12, 3)	dd 2.68 (13, 3.7)	dd 2.70 (12, 4.7)	dd 2.74 (13, 2.7)	dd 2.63 (12.5, 5)	dd 3.06 (12.5, 5.2)	dd 2.60 (12, 5)
3′-H	dd 1.64 (12, 10)	t 1.71 (11)	dd 0.63 (12, 10)	dd 0.60 (12, 10.5)	dd 1.62 (13, 10)	dd 1.66 (12, 11)	dd 0.74 (13, 10.5)	dd 1.62 (12.5, 11)	dd 1.53 (12.5, 11)	dd 1.69 (12, 10)
4-H	ddd 5.49 (16, 10, 5)	ddd 5.65 (16, 11, 5)	dt 2.79 (10, 2)	ddd 2.77 (10.5, 3, 2.5)	ddd 3.01 (9.7, 3.7, 2.3)	ddd 5.53 (15, 11, 4.7)	ddd 2.94 (10.8, 2.7, 2.3)	ddd 5.46 (15, 11, 5)	ddd 5.46 (16, 11, 5)	ddd 5.43 (16, 10, 5)
5-H	d 5.31 (16)	d 5.38 (16)	d 2.23 (2)	d 2.19 (2.5)	d 2.36 (2.3)	d 5.26 (15)	d 2.34 (2.3)	d 5.28 (15)	d 5.20 (16)	d 5.24 (16)
7-H	d 1.62 (14)	d 1.60 (14)	dd 2.15 (14, 10)	dd 2.32 (15, 11.5)	d 1.68 (15)	d 1.84 (15)	dd 1.71 (15.5, 1.8)	d 1.63 (14)	dd 2.13 (13.5, 10.5)	dd 2.16 (14, 7)
7′-H	dd 1.38 (14, 10)	dd 1.44 (14, 10)	d(br) 1.94 (14)	ddq 2.00 (15, 2.7, 1.5)	dd 1.57 (15, 7)	dd 1.30 (15, 9.7)	dd 1.64 (15.5, 6.7)	dd 1.43 (14, 9.5)	dd(br) 1.78 (13.5, 3)	dd 1.99 (14, 7)
8-H	d 2.48 (10)	d 2.54 (10)	d(br) 5.18 (10)	d(br) 5.39 (11.5)	d 2.58 (7)	d 2.55 (9.7)	dd 2.64 (6.7, 1.8)	d 2.61 (9.5)	dd 4.96 (10, 3)	t 5.34 (7)
10-H	m 2.15	m 2.15		dd 4.23 (10.5, 7)	m 2.08	m 2.14	m 2.18	dd 3.13 (11.2, 6.2)	m 2.2	t 4.33 (6)
10′-H	m 1.08	td 1.10 (13, 5)	m2.3	–	m 1.15	m 1.08	ddd 1.13 (13, 6.5, 6)	–	m 2.0	–
11-H	m 2.25	m 2.24		ddd 2.29 (13, 10.5, 5.5)	m 2.32	m 2.24	m 2.28	ddd 2.21 (14, 11.2, 5.2)	m 2.2	m 2.34
11′-H	m 1.35	m 1.38	m 1.35	ddd 1.70 (13, 9.5, 7)	m 2.10	m 1.38	dddd 1.43 (12.3, 9.7, 6.5, 2.3)	ddd 1.68 (14, 10, 6.2)	m 1.5	m 1.56
12-H	s 1.31	s 1.32	s 1.38	s 1.40	s 1.71	s 1.31	s 1.38	s 1.31	d 3.93 (12)	s 1.31
12′-H	–	–	–	–	–	–	–	–	d 3.57 (12)	–
13-H	s 1.20	ABq 3.44	s 1.09	s 1.11	s 1.09	d 3.92 (10.5)	s 1.10	s 1.08	d 3.53 (10)	d 3.59 (10)
13′-H	–		–	–	–	d 3.72 (10.5)	–	–	d 3.46 (10)	d 3.49 (10)
14-H	s 1.08	s 1.23	s 0.78	s 0.80	s 0.84	s 1.14	s 0.88	s 1.19	s 1.16	s 1.18
15-H	s 1.31	s 1.32	s 1.68	t 1.72 (1.5)	s 1.25	s 1.31	s 1.36	s 1.31	s 1.58	s 1.56

dried over sodium sulfate, and evaporated. To separate the 1,2;8,9-diepoxide from the other epoxides formed in minor amounts was difficult and achieved by repeated chromatography (Si-60, 40–63 μm, *n*-hexane/ethyl acetate 97:3) yielding 480 mg **28**.

Biotransformation of 450 mg of *anti*-1,2;8,9-diepoxy-humulene **28** with *Chaetomium cochlioides* DSM 63353 yielded within 72 h 95 mg **29**, 84 mg **28** (8R, 9R; α_D + 77.3°), 15 mg **35**, 15 mg **36**, 4 mg **34**.

Biotransformation of 1.7 g of humulene **1** with *Diplodia gossypina* ATCC 10936 yielded within 72 h 11 mg **39**, 7 mg **4**, 5 mg **38**, 4 mg **24**, 3 mg **37**, 2 mg **7** + **12**.

*7-Hydroxy-humulene (**2**): MS (m/e):* M^+ 220.1824 (220.1827 calc. for $C_{15}H_{24}O$).

*10,13-Dihydroxy-humulene (**3**): MS (m/e):* M^+ 236.1776 (236.1776 calc. for $C_{15}H_{24}O_2$).

Table II: ^{13}C NMR data of **3**, **4**, **7**, **8**, **13**, **21**, **22**, **25**, **28**, **29**, **31**, **33**, **36**, **38**, (75.5 MHz, $CDCl_3$).

	3	**4**	**7**	**8**	**13**	**21**	**22**	**25**	**28**	**29**	**31**	**33**	**36**	**38**
1-C	d 131.3	d 131.7	d 62.0	d 62.6	d 61.8	d 59.2	d 63.1	d 61.5	d 64.7	d 64.2[a]	d 59.4	d 125.6	d 63.5	d 60.2
2-C	s 136.2	s 142.2	s 63.2	s 62.9	s 63.0	s 62.9	s 64.0	s 58.6	s 63.3	s 63.2[b]	s 57.5	s 132.7	s 63.2[a]	s 63.6
3-C	t 40.8	t 38.7	t 42.6	t 41.9	t 43.4	t 43.2	t 42.5	t 42.0	t 43.4	t 43.5	t 44.3	t 41.6	t 43.1	t 42.9
4-C	d 122.6	d 124.9	d 122.2	d 124.0	d 124.6[b]	d 126.3[a]	d 123.9	d 51.8	d 122.7	d 126.0	d 53.2	d 56.5	d 122.8	d 125.3
5-C	d 137.0	d 137.2	d 143.1	d 138.1	d 139.0	d 138.9	d 142.5	d 63.3	d 142.9	d 138.5	d 66.0	d 65.5[a]	d 142.7	d 138.9
6-C	s 43.0	s 42.9	s 36.5	s 41.9	s 42.5	s 42.6	s 34.6	s 34.5	s 35.7	s 41.5	s 34.3	s 33.3	s 35.7	s 41.8
7-C	t 36.6	t 36.5	t 40.6	t 36.0	t 34.9[a]	t 33.9[b]	t 40.8	t 38.9	t 38.5	t 33.5	t 38.5	t 38.4[b]	t 37.6	t 35.7[a]
8-C	d 124.6	d 131.1	d 125.8	d 135.7	d 125.4[b]	d 125.1[a]	d 63.1	d 123.0	d 60.3	d 60.3[a]	d 124.5	d 61.7[a]	d 57.7	d 123.3
9-C	s 139.9	s 132.6	s 132.0	s 133.2	s 132.7	s 135.0	s 60.6	s 133.8	s 60.1	s 60.2[b]	s 136.5	d 61.0	s 63.6[a]	s 135.3
10-C	d 78.9	t 49.7	t 36.7	t 37.2	t 36.5[a]	d 75.7	t 36.9	t 37.1	t 35.0	t 34.8	d 75.8	t 38.9[b]	d 74.9	d 74.9
11-C	t 31.9	d 66.4	t 24.8	t 24.3	t 24.9	t 33.4[b]	t 23.6	t 25.0	t 25.3	t 25.2	t 33.4	t 23.5	t 32.9	t 32.6[a]
12-C	q 17.9	q 16.5	q 15.1	q 15.3	q 15.0[c]	q 16.8	q 19.1[a]	q 18.0[a]	q 16.5	q 16.5[c]	q 18.9[a]	q 17.9	q 16.7	q 15.6
13-C	t 71.8	t 71.6	q 29.0	t 70.3	t 71.7	t 71.6	q 30.2	q 29.7	q 30.8	t 71.8	q 28.6	q 29.0	q 30.7	q 18.0
14-C	q 19.2	q 19.7	q 25.7	q 22.5	q 20.0	q 18.9	q 26.3	q 29.6	q 23.4	q 18.9	q 17.5[a]	q 18.3	q 23.4	t 70.7
15-C	q 10.3	q 19.6	q 17.3	q 18.3	q 16.9[c]	q 10.6	q 16.4[a]	q 20.4[a]	q 16.4	q 16.3[c]	q 10.5	q 16.4	q 11.5	q 10.8

[a], [b], [c] assignments may be interchanged.

*(4S,5S,7S)-4,5-Epoxy-7-hydroxy-humulene (**6**): MS (m/e):* M^+ 236.1770 (236.1776 calc. for $C_{15}H_{24}O_2$).
*(1S,2S,6R)-1,2-Epoxy-13-hydroxy-humulene (**8**): MS*
(m/e): M^+ 236.1766 (236.1776 calc. for $C_{15}H_{24}O_2$).
*(1S,2S,6R,11R)-11,13-Dihydroxy-1,2-epoxy-humulene (**9**): MS (m/e):* M^+ 252.1725 (252.1725 calc. for $C_{15}H_{24}O_3$).

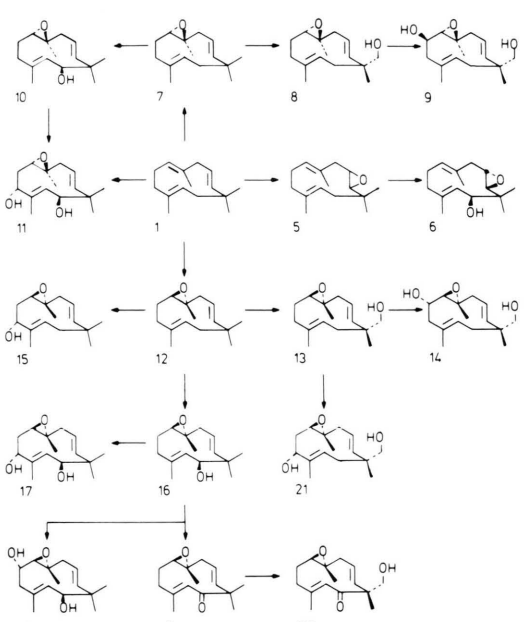

Fig. 2. Hydroxylation products formed *via* the humulene monoepoxides by *Chaetomium cochlioides*.

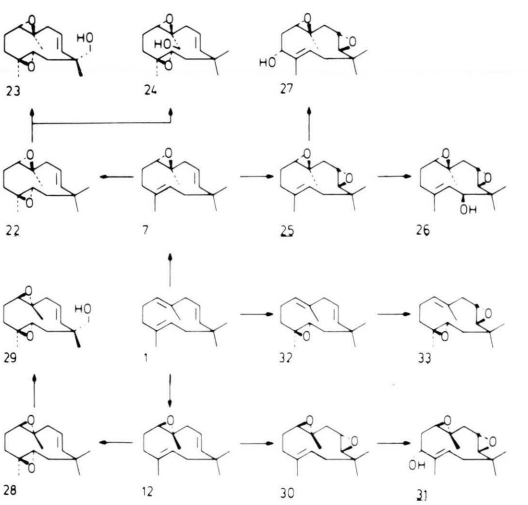

Fig. 3. Hydroxylation products formed *via* the humulene diepoxides by *Chaetomium cochlioides*.

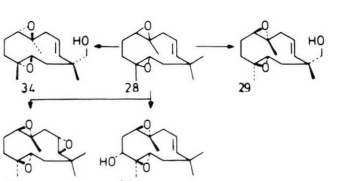

Fig. 4. Products of the biotransformation of rac. *anti*-1,2;8,9-humulene-diepoxide by *Chaetomium cochlioides*.

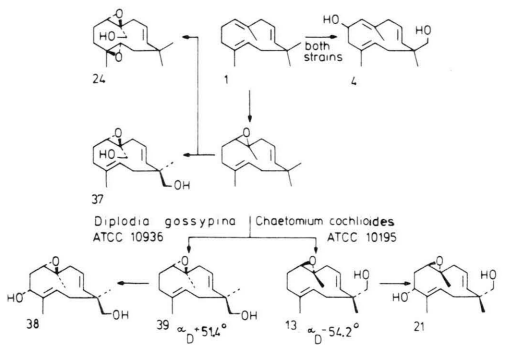

Fig. 5. Biotransformation products of humulene from *Diplodia gossypina* and *Chaetomium cochlioides*.

(1R,2R,6R)-1,2-Epoxy-13-hydroxy-humulene (**13**): *MS (m/e):* M^+ 236.1776 (236.1776 calc. for $C_{15}H_{24}O_2$).

(1R,2R,6R,11S)-11,13-Dihydroxy-1,2-epoxy-humulene (**14**): *MS (m/e):* M^+ 252.1717 (252.1725 calc. for $C_{15}H_{24}O_3$).

(1R,2R,10R)-1,2-Epoxy-10-hydroxy-humulene (**15**): *MS (m/e):* M^+ 236.1768 (236.1776 calc. for $C_{15}H_{24}O_2$).

(1R,2R,7S)-1,2-Epoxy-7-hydroxy-humulene (**16**): MS *(m/e):* M^+ 236.1776 (236.1776 calc. for $C_{15}H_{24}O_2$).

(1R,2R)-1,2-Epoxy-7-oxo-humulene (**19**): MS *(m/e):* M^+ 234.1610 (234.1620 calc. for $C_{15}H_{22}O_2$).

(1R,2R,6S)-1,2-Epoxy-13-hydroxy-7-oxo-humulene (**20**): IR: 3600–3500, 1680 cm^{-1}.

(1R,2R,6S,10R)-10,13-Dihydroxy-1,2-epoxy-humulene (**21**): Colourless cristals, m.p. 131 °C. MS *(m/e):* M^+ 252.1719 (252.1725 calc. for $C_{15}H_{24}O_3$).

anti-(1S,2S*,4S*,5S*)-1,2;4,5-Diepoxy-humulene* (**25**): Colourless cristals, m.p. 79 °C. MS *(m/e):* M^+ 236.1763 (236.1776 calc. for $C_{15}H_{24}O_2$).

anti-(1S,2S,4S,5S,7S)-1,2;4,5-Diepoxy-7-hydroxy-humulene (**26**): MS *(m/e):* M^+ 252.1725 (252.1725 calc. for $C_{15}H_{24}O_3$).

anti-(1S,2S,4S,5S,10R)-1,2;4,5-Diepoxy-10-hydroxy-humulene (**27**): MS *(m/e):* M^+ 252.1725 (252.1725 calc. for $C_{15}H_{24}O_3$).

anti-(1R,2R,6R,8S,9S)-1,2;8,9-Diepoxy-13-hydroxy-humulene (**29**): Colourless cristals, m.p. 127 °C. MS *(m/e):* M^+ 252.1725 (252.1725 calc. for $C_{15}H_{24}O_3$).

syn-(1R,2R,4S,5S,10R)-1,2;4,5-Diepoxy-10-hydroxy-humulene (**31**): MS *(m/e):* M^+ 252.1719 (252.1725 calc. for $C_{15}H_{24}O_3$).

anti-(4S,5S*,8S*,9S*)-4,5;8,9-Diepoxy-humulene* (**33**): MS *(m/e):* M^+ 236.1776 (236.1776 calc. for $C_{15}H_{24}O_2$).

anti-(1R,2R,8S,9S,10R)-1,2;8,9-Diepoxy-10-hydroxy-humulene (**36**): MS *(m/e):* M^+ 252.1725 (252.1725 calc. for $C_{15}H_{24}O_3$).

Optical rotations measured in chloroform.

	589 nm	578 nm	546 nm	436 nm	365 nm
2 (c = 0.52)	− 34.2°	− 35.5°	− 41.5°	− 83.7°	
3 (c = 1.00)	+ 72.4°	+ 75.9°	+ 87.1°	+156.0°	+294.4°
4 (c = 1.00)	+ 58.8°	+ 61.8°	+ 71.1°	+135.4°	
8 (c = 1.00)	+ 8.1°	+ 8.6°	+ 10.0°	+ 18.9°	+ 46.2°
10 (c = 0.59)	− 89.9°	− 93.7°	−107.5°	−192.5°	−320.6°
11 (c = 0.23)	− 75.8°	− 77.9°	− 89.1°	−159.8°	−264.9°
13 (c = 1.00)	− 54.2°	− 56.7°	− 64.4°	−109.9°	
14 (c = 0.74)	+103.7°	+109.3°	+119.0°	+209.6°	+358.6°
15 (c = 1.00)	− 37.2°	− 38.8°	− 44.6°	− 79.4°	
16 (c = 1.00)	− 34.8°	− 36.7°	− 41.9°	− 76.0°	−129.3°
18 (c = 0.28)	+ 26.5°	+ 27.9°	+ 32.6°	+ 61.0°	+145.1°
19 (c = 0.82)	− 88.5°	− 92.2°	−107.2°	−187.9°	
20 (c = 1.00)	+ 50.7°	+ 54.8°	+ 69.8°	+233.8°	
21 (c = 1.00)	− 19.4°	− 20.8°	− 23.3°	− 39.6°	− 61.7°
22 (c = 1.00)	− 48.3°	− 50.1°	− 57.1°	− 99.6°	
23 (c = 0.78)	−105.4°	−108.7°	−123.9°	−213.4°	−342.8°
25 (c = 1.00)	109.7°	−114.3°	−129.7°	−222.9°	
26 (c = 0.42)	− 97.8°	−103.0°	−117.7°	−205.0°	−329.2°
27 (c = 0.43)	− 68.3°	− 71.6°	− 80.1°	−137.6°	−218.6°
28 (c = 1.00)	+ 77.3°	+ 80.9°	+ 92.3°	+162.8°	+267.7°
29 (c = 0.50)	− 49.4°	− 50.7°	− 57.5°	−101.4°	−168.4°
33 (c = 0.58)	− 18.8°	− 20.7°	− 22.4°	− 36.9°	
38 (c = 1.00)	− 14.6°	− 15.3°	− 17.2°	− 30.0°	

Results

From a screen using about 300 strains *Diplodia gossypina* ATCC 10936 and *Chaetomium cochlioides* were selected for preparative fermentations. The biotransformation of humulene with *Chaetomium cochlioides* DSM 63353 or ATCC 10195 gave a multitude of products. Three compounds are simply hydroxylation products of humulene.

The main reaction path starts with the epoxidation of the 1,2-double bond in humulene. The monoepoxide is racemic because its optical rotation is α_D +2° compared with α_D −31° reported for the pure 1R,2R-product [5]. This epoxide is then further oxidized to afford diepoxides and hydroxy-epoxides. The corresponding 4,5- and 7,8-monoepoxides were also formed but in very low yield.

Very soon it became apparent that most products occurred in pairs. Using the nuclear Overhauser

enhancement difference technique it was possible to solve their relative configuration and to distinguish between the *syn*- and *anti*-compound in the pairs. The 3α-proton of the 1,2;4,5-diepoxy-humulen-10-ol (**31**) is shielded by the *syn*-diepoxide so its resonance occurred at δ = 0.60 in the ^{1}H NMR, while the *anti*-diepoxide **27** led to the resonance at δ = 1.49 for the same proton. This clear difference gave first hints for the relative configuration which was confirmed by NOE experiments.

But the question which center is epimeric was not solved by these findings. The answer came from the relative configurations of the higher oxidized humulenes. Here the relative configuration of compounds with the same constitution only changes with respect to the 1,2-epoxide, but remains constant to all other substituents.

The only exception from this rule is the hydroxy group at C-11. Here it depends on the epoxy group having always the *anti*-configuration relative to the methyl group at C-2. This result is plausible because of the proximity of the epoxide.

Both strains produced the 1,2-epoxy-humulene-10,13-diol (**21**). The relative configuration of this compound was derived by ^{1}H NMR using the NOE difference technique. The absolute configuration was then determined by Horeau's method [6]. To test the influence of the primary alcohol group on the result of the Horeau experiment, compound **13** was also subjected to this procedure and gave an optical yield of only 1.7% whereas **21** gave 22.5%, so the influence of the primary alcohol on the result is negligible. The absolute configuration determined by Horeau's method was adopted from the relative configurations determined by NOE experiments for all other compounds.

The isolated amounts of hydroxylated products reflect the enantioselectivity of the enzymes involved in the hydroxylation. Both strains form the enantiomers of the 1,2-epoxide in a 1R:1S-ratio of almost 1:1. If the DSM-strain is used in the oxidation the ratio of the two resulting 1,2-epoxy-humulen-7-ols **16** and **10** is 1.8:1 and that of the 1,2-epoxy-humulene-13-ols **13** and **8** is 4.4:1. In the biotransformation with the ATCC-strain the 1S-compound was not detected, so the 1R:1S-ratio here is better than 45:1. The situation is even more pronounced for the main product of the biotransformation of humulene, *viz.* for 1,2-epoxy-humulene-10,13-diol **21**. Both strains produce only the 1R,2R-compound and the ratio of the 1,2-epimers is better than 166:1. The formation of the diepoxides is not very stereoselective, but hydroxylation of these compounds displayed a good enantioselectivity.

To test our results we synthesized the *anti*-diepoxide of humulene. The biotransformation of the racemic *anti*-1,2;8,9-diepoxy-humulene (**28**) gave hydroxylation products in good yields. Better solubility of the substrate in water is the presumable cause that the fermentation proceeded much faster and gave better yields. We recovered unreacted *anti*-1,2;8,9-diepoxide which exhibited an optical rotation of α_D +77.3°. Using the work of Damodaran and Dev [5] we determined the absolute configuration of this compound to be 1S,2S,8R,9R. As expected only the 1R,2R,8S,9S-enantiomer was accepted by the enzymes while the antipode remained unchanged. This is a way to resolve the racemate especially because the optical rotation of the isolated product is about 50 times higher than reported by Damodaran and Dev. Another confirmation of this result is the fact that only small quantities of this antipode was hydroxylated at C-13 while hydroxylation of the other compound leads to **29** as the main product of this transformation.

These experiments solved another problem. We isolated some compounds with a hydroxyl vicinal to an epoxide group. It was not clear whether this hydroxylation occurred before or after epoxidation. Using the diepoxides we also obtained these vicinal hydroxylation products which in this case could be formed only after epoxidation.

To proof whether these results are limited to *Chaetomium cochlioides* only, we selected *Diplodia gossypina* ATCC 10936 from our screen. This strain also attacked humulene but in lower yields. Again the racemic 1,2-epoxide is formed which is further hydroxylated at C-13. The sign of the optical rotation of this compound is reversed relative to the product from *Chaetomium cochlioides,* so the enantiomer **39** is formed. As in the case of the former microorganism, this compound is further oxidized at C-10. Here the configuration is not reversed with respect to *Chaetomium cochlioides,* so the substance is (1S,2S,6S,10R)-1,2-epoxy-humulene-10,13-diol **38**.

Discussion

Our results reveal that *Chaetomium cochlioides* hydroxylates humulene to give 7S-, 10R-, 12-, and

13-alcohols. The 11-hydroxylation is controlled by the configuration of the 1,2-epoxide, so the 1S-epoxide led to the 11R- and the 1R-epoxide to the 11S-alcohol.

While the primary attack of humulene leads to a racemic epoxide the following reactions select one of the antipode for hydroxylation. These findings were further proofed by the transformation of the *anti*-1,2; 8,9-diepoxide. Here the unreacted starting material showed the expected absolute configuration. *Diplodia gossypina* gave products of different relative and absolute configuration. With these findings it is possible to use these strains for similar transformations of different substrates.

The biotransformation here gave 38 different products some in moderate yields which are in tests for their biological activities. 33 of them have never been described before. Whereever possible ^{13}C NMR data were measured giving references for further humulanes which will be isolated in the future.

Acknowledgements

We are grateful to Dr. G. Höhne, Inst. f. Org. Chemie, TU Berlin, for performing high resolution mass spectrometry, to Mrs. H. Schwab-Hanisch for her skilful technical assistance with the separation of the compounds, and Miss M. Mull for microbiological experiments.

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