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## STRUCTURE OF MICROBIOTOL, A NEW SESQUITERPENE ALCOHOL FROM NEEDLES OF *MICROBIOTA DECUSSATA*

A. V. TKACHEV,\* M. M. SHAKIROV, and V. A. RALDUGIN

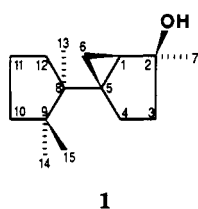
Novosibirsk Institute of Organic Chemistry, Siberian Division of the Academy of Sciences,  
USSR, 630090 Novosibirsk, USSR

**ABSTRACT.**—The sesquiterpene alcohol microbiotol, previously isolated from an Et<sub>2</sub>O extract of needles of *Microbiota decussata*, has been characterized as (–)-(1*R*,2*S*,5*R*)-2-methyl-5[(1*R*)-1,2,2-trimethylcyclopentyl]-bicyclo[3.1.0]hexan-2-ol [**1**] on the basis of chemical correlation and spectral data.

The monoecious evergreen bush *Microbiota decussata* Kom. is a representative of a monotypic genus of the Cupressaceae. *M. decussata* grows on the Sikhote-Alin mountain ridge (USSR) mainly in its southern part. An Et<sub>2</sub>O extract of *M. decussata* needles has earlier been found to contain the sesquiterpene alcohols hedycaryol, (+)- $\alpha$ -bisabolol, thujopsan-2 $\alpha$ -ol, (1*E*,5*S*,6*E*,8*S*)-Germacra-1,6-dien-5-ol,  $\beta$ -eudesmol, and a new sesquiterpene alcohol named microbiotol (**1**) whose structure remained undetermined. In this study, we show microbiotol [**1**] to be a tricyclic saturated alcohol with a new type of carbon skeleton.

Microbiotol is an easily sublimated substance crystallizing from various solvents in the form of very thin needles, and because of this we were unable to prepare crystals suitable for X-ray crystallographic analysis. Microbiotol is very unstable in acidic media and completely decomposes in several hours at room temperature in CHCl<sub>3</sub>. Attempts to obtain a crystalline derivative of the alcohol with the same carbon skeleton failed. From careful analysis of <sup>1</sup>H- and <sup>13</sup>C-nmr spectra of microbiotol and comparison of the calculated and experimental nmr data, as well as the chemical correlation with the aromatic hydrocarbon (+)-cuparene [**7**], we conclude that the structure and absolute configuration of the alcohol must be represented by formula **1**.

The chemical structure of microbiotol has been partially determined by analysis of carbon-carbon coupling constants <sup>1</sup>J(<sup>13</sup>C-<sup>13</sup>C) in the <sup>13</sup>C-nmr spectra [INADEQUATE experiment (2)]. How-



**1**

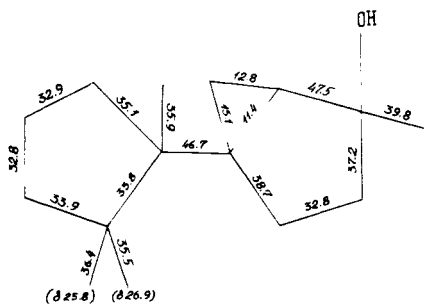


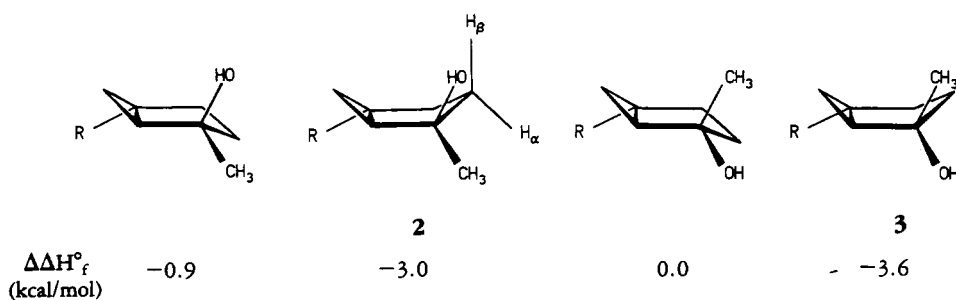
FIGURE 1. Carbon-carbon coupling constants ( $\pm 0.1$  Hz) in microbiotol [**1**] based on the <sup>13</sup>C-nmr INADEQUATE and 2D INADEQUATE experiments.

ever, the magnitudes of two coupling constants were identical ( $J_{3,4}$  and  $J_{10,11}$ ), and thus the final conclusion about the structure has been made on the basis of a 2D INADEQUATE experiment (2) (Figure 1).

The hydroxy group in the microbiotol molecule may be in the cis or trans position with respect to the cyclopropane unit. Molecular mechanics calculations (3) show the boat-like conformation to be more stable than the chair-like one for both the cis and trans isomers **2** and **3**

(Scheme 1).

These calculations agree with those of the parent hydrocarbon bicyclo[3.1.0]hexane, in which the boat-like conformation of the hydrocarbon is more stable than the chair-like form (4). The computed (2,5) proton-carbon coupling constants  ${}^3J_{C^7-H^1}$ ,  ${}^3J_{C^7-H^3}$ , and  ${}^3J_{C^7-H^4}$  are 3.3, 5.8, and 6.1 Hz, respectively, for conformation **2**, and 0.5, 0.1, and 5.2 Hz for conformation **3**. Comparison with the experimental values (3, 5, and 5 Hz, see also Table 1) shows a cis con-



SCHEME 1

TABLE 1.  ${}^{13}\text{C}$ - and  ${}^1\text{H}$ -nmr Data for Microbiotol [1].

Position	$\delta\text{C}^i$ (ppm) <sup>a</sup>	$J_{\text{C}^i\text{-H}^j}$ (Hz) <sup>b</sup>	$J_{\text{C}^i\text{-H}^j}$ (Hz) <sup>b</sup>	$\delta\text{H}^i$ (ppm) <sup>c</sup>
1 . . . . .	32.47	164.5	$\text{W}_{1/2} = 13$	0.877 ddd
2 . . . . .	79.28	—	$\text{W}_{1/2} = 17$	—
3 . . . . .	37.04	130.0	4,4,4,4,4,4	[1.473]
4 . . . . .	28.71	127.0	$\text{W}_{1/2} = 12$	$\alpha$ 1.972 dddd $\beta$ 1.732 ddd
5 . . . . .	33.80	—	$\text{W}_{1/2} = 20$	—
6 . . . . .	12.45	158.2	7,3,3	$\alpha$ 0.957 ddd $\beta$ 0.693 dd
7 . . . . .	28.94	124.5	5,5,3	1.317 d
8 . . . . .	47.56	—	$\text{W}_{1/2} = 14$	—
9 . . . . .	45.54	—	$\text{W}_{1/2} = 17$	—
10 . . . . .	42.17	127.4	$\text{W}_{1/2} = 20$	[1.710] [1.410]
11 . . . . .	20.27	130.6	7,7,4,3	[1.547]
12 . . . . .	35.58	127.0	$\text{W}_{1/2} = 13$	$\alpha$ [1.547] $\beta$ [0.917]
13 . . . . .	22.06	124.8	7,6, $\text{W}_{1/2} = 3$	1.043 d
14 . . . . .	25.81	124.6	5,5,5,5	1.020 s
15 . . . . .	26.87	124.6	6,6,6, $\text{W}_{1/2} = 5$	1.020 s

<sup>a</sup>Concn = 2.0 M in  $\text{CD}_3\text{OD}$ .

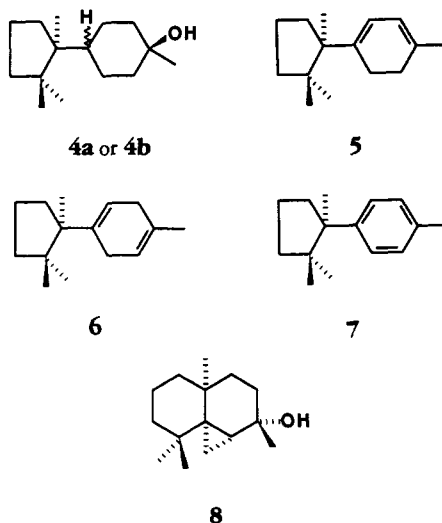
<sup>b</sup>From the proton-coupled  ${}^{13}\text{C}$ -nmr spectrum.

<sup>c</sup>Concn = 0.075 M in  $\text{CD}_3\text{OD}$ ; the  $\delta$ -values in square brackets are taken from the 2D carbon-proton shift correlation experiment; the proton-proton coupling constants  $\text{H}^i\text{-H}^j$  are as follows (Hz):  $J_{1,6\alpha} = 8.2$ ,  $J_{1,6\beta} = 3.4$ ,  $J_{1,3\alpha} = 0.8$ ,  $J_{3\alpha,3\beta} = 14.0$ ,  $J_{4\alpha,4\beta} = 12.5$ ,  $J_{3\alpha,4\alpha} = 8.3$ ,  $J_{3\alpha,4\beta} = 1.6$ ,  $J_{3\beta,4\alpha} = 11.1$ ,  $J_{3\beta,4\beta} = 7.8$ ,  $J_{4\alpha,6\alpha} = 1.4$ ,  $J_{6\alpha,6\beta} = 4.8$ ,  $J_{7,3\beta} = 0.9$ ,  $J_{10\alpha,10\beta} \approx 15$ ,  $J_{12\alpha,12\beta} \approx 15$ ,  $J_{13,12\beta} = 0.6$ .

figuration of the hydroxybicyclo[3.1.0]-hexane unit in microbiotol (as in formula 2).

Microbiotol [1] treated with aqueous  $\text{HCO}_2\text{H}$  in THF gave a mixture of epimeric alcohols 4a and 4b and hydrocarbons 5 and 6. Heating of the mixture of hydrocarbons with ethylenediamine lithium salt (6) leads to the aromatic hydrocarbon (+)-cuparene [7] of known absolute configuration (7).

The formation of (+)-cuparene [7] indicates the *R* configuration of the C-8 center, and thus microbiotol may be either the 1*R*,2*S*,5*R*,8*R* derivative or the diastereomeric 1*S*,2*R*,5*S*,8*R* derivative. Molecular mechanics calculations using the DRIVING procedure (8) show that a 1*R*,2*S*,5*R*,8*R* configuration should be practically conformationally homogeneous (see Figure 2), because the free energy of the conformation  $\phi(\text{C}^1-\text{C}^5-\text{C}^8-\text{C}^9) = -160^\circ$  is smaller by ca. 3.5 kcal/mol than that of other stable conformations. For the 1*S*,2*R*,5*S*,8*R* diastereomer,



calculations predict two conformers with  $\phi = 132^\circ$  and  $\phi = -87^\circ$  (ca. 25:75 ratio). In order to choose between these two diastereomers, we have recorded the  $^1\text{H}$ -nmr spectra of microbiotol [1] in the presence of  $\text{Eu}(\text{fod})_3$  and compared the experimentally obtained values of the

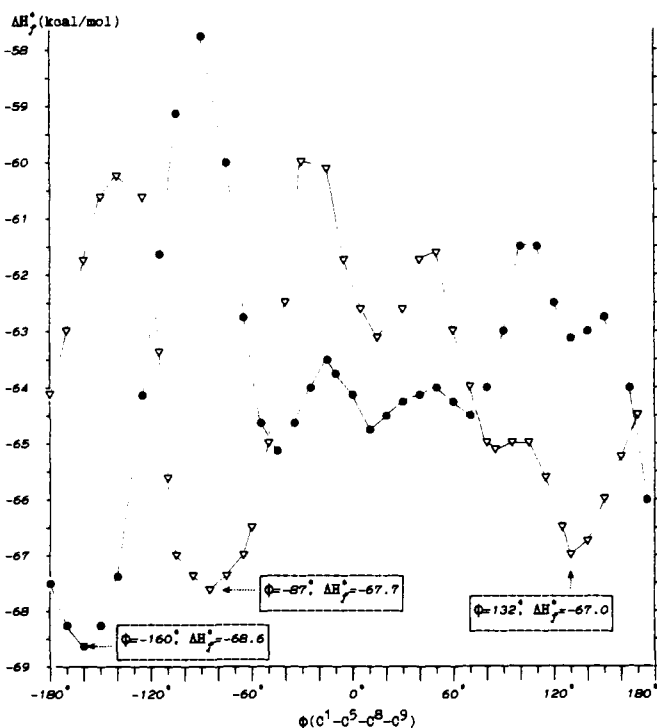


FIGURE 2. Heats of formation of different conformers of microbiotol [1] (●) and its 1*S*,2*R*,5*S*,8*R*-isomer (∇) obtained as a result of the molecular mechanics calculation.

lanthanide-induced shifts with the calculated values. The induced shifts were calculated using the qualified geometric parameters of the conformations shown in Figure 2. In our calculations we proceeded from the fact that the relative configuration of the molecular fragment containing the unit of bicyclo[3.1.0]hexane is already known (the *cis* boat-like form, structure 2); therefore the geometric optimization of the microbiotol-Eu(fod)<sub>3</sub> complex was carried out to provide the best agreement between the calculated and experimental values of the induced shifts exactly for the bicyclohexane unit. Using the lanthanide atom position found in this way, the induced shifts of control signals, namely the signals of the H<sub>β</sub>-12, Me-13, Me-14, and Me-15 atoms, were calculated. Comparison of the data listed in Table 2 shows the calculated data to be in good agreement with the experimental data for the 1*R*,2*S*,5*R*,8*R* derivative, but not for the 1*S*,2*R*,5*S*,8*R*-isomer.

These data allow us to suggest (–)-(1*R*,2*S*,5*R*)-2-methyl-5[(1*R*)-1,2,2-trimethylcyclopentyl]-bicyclo[3.1.0]hexan-2-ol [1] is the structure for microbiotol.

Finally, it should be stressed that the configuration of microbiotol [1] is very close to that of the sesquiterpene alcohol

thujopsan-2α-ol [8]. This alcohol is one of the main constituents of the Et<sub>2</sub>O extract of *M. decussata* (1). Thus the stereochemical similarity between these two components is an indirect confirmation of the configuration of microbiotol [1].

## EXPERIMENTAL

**GENERAL EXPERIMENTAL PROCEDURES.**—Nmr spectra were obtained on a Bruker WP 200 SY instrument (<sup>1</sup>H at 200.13 MHz, <sup>13</sup>C at 50.32 MHz). The INADEQUATE, 2D INADEQUATE, and heteronuclear shift correlation 2D nmr spectra were obtained using the standard Bruker nmr software system. Cc was performed on Chemapol L Si gel (40–70 μm). Analytical tlc was performed on ready-made plates Silufol coated with Silpearl Si gel. Visualization of the spots on tlc plates was achieved by spraying with H<sub>2</sub>SO<sub>4</sub> (95%).

**ISOLATION OF MICROBIOTOL [1].**—The fraction of terpene alcohols was obtained by cc of the Et<sub>2</sub>O extract of *M. decussata* needles (A voucher specimen for which is deposited in the Herbarium of Botanic Institute of the Academy of Sciences, Leningrad, USSR.) [SiO<sub>2</sub>, petroleum ether (bp 40–70°) Et<sub>2</sub>O (4:1)]. A portion of this fraction (20 g) after separation of hedycaryol (7 g) by vigorous shaking with a solution of AgNO<sub>3</sub> (15 g AgNO<sub>3</sub> + 50 ml H<sub>2</sub>O) (9) was charged on a Si gel (400 g) column. (1*E*,5*S*,6*E*,8*S*)-Germacrene-1,6-dien-5-ol (0.8 g), (+)-α-bisabolol (0.4 g), thujopsan-2α-ol + β-eudesmol (5 g), and crude microbiotol (3.0 g) were successively obtained by careful elution with petroleum ether-Et<sub>2</sub>O (4:1).

TABLE 2. Measured and Calculated Lanthanide-induced Shifts in the <sup>1</sup>H-nmr Spectra of Microbiotol [1].

Proton	δH <sup>i</sup> (ppm) <sup>a</sup>	ΔδH <sup>i</sup> (ppm) <sup>b</sup>	ΔδH <sup>i</sup> calculated (ppm)	
			1	1 <i>S</i> ,2 <i>R</i> ,5 <i>S</i> ,8 <i>R</i> diastereomer
H-1 . . . . .	(0.84)	4.82		
H-3 . . . . .	α (1.55)	4.17		
	β (1.40)	5.82		
H-4 . . . . .	α (1.84)	2.34		
	β (1.66)	2.06		
H-6 . . . . .	α (0.86)	2.16		
	β 0.60	4.17		
H-7 . . . . .	1.32	4.92		
H-12 . . . . .	β (1.10)	1.02	0.93	1.55
H-13 . . . . .	0.97	1.31	1.44	0.85
H-14 . . . . .	0.94	0.71	0.80	1.49
H-15 . . . . .	0.95	0.72	0.90	0.80

<sup>a</sup>Concn = 0.04 M in CDCl<sub>3</sub>, extrapolated shifts are given in parentheses.

<sup>b</sup>+0.5 mol/mol Eu(fod)<sub>3</sub>.

Crude microbiotol was purified by vacuum sublimation to yield 1.6 g of pure microbiotol (**1**): rose-colored spot on tlc plates;  $R_f$  0.2 {pentane-Et<sub>2</sub>O (3:2)}; mp 116–117° (MeCN);  $[\alpha]^{25}_D -13^\circ$  ( $c = 3.08$ , CHCl<sub>3</sub>); ir (1% in CCl<sub>4</sub>) 3620 cm<sup>-1</sup> ( $\nu_{O-H}$ ); eims  $m/z$  (rel. int.)  $[M]^+$  222 (3),  $[M - Me]^+$  207 (24),  $[M - H_2O]^+$  204 (15),  $[M - Me - H_2O]^+$  189 (21), 161 (13), 151 (19), 137 (20), 121 (31), 119 (28), 111 (100), 95 (45), 94 (83), 93 (56), 79 (36), 69 (60), 55 (43). Found C 81.3, H 11.6; C<sub>15</sub>H<sub>26</sub>O requires C 81.02, H 11.79.

REACTION OF MICROBIOTOL (**1**) WITH HCO<sub>2</sub>H.—A solution of **1** (100 mg) and 45% aqueous HCO<sub>2</sub>H (0.1 ml) in 2 ml of THF was heated to boiling and cooled to room temperature. After neutralizing with aqueous NaOH, saturation of the aqueous layer with NaCl, and extraction with Et<sub>2</sub>O, 90 mg of an oily product was obtained. Chromatography of the product on Si gel (5 g) with petroleum ether gave 40 mg of a mixture of **5** and **6** (3:4, according to <sup>1</sup>H nmr). Continued elution with petroleum ether-Et<sub>2</sub>O (3:1) furnished 45 mg of a mixture of **4a** and **4b** (45:55, <sup>1</sup>H nmr). <sup>1</sup>H-nmr data (0.1 M in CDCl<sub>3</sub>, CHCl<sub>3</sub> as internal standard at  $\delta$  7.24 ppm) for **4a** 0.76 (3H, s), 0.94 (3H, s), 1.04 (3H, s), 1.17 (3H, s), ca. 2.2 (1H, m), 5.48 (1H, ddd,  $J = 10.5, 2.5, 2.0$  Hz), 5.64 (1H, ddd,  $J = 10.5, 2.0, 2.0$ ); for **4b** 0.73 (3H, s), 0.94 (3H, s), 0.97 (3H, s), 1.17 (3H, s), ca. 2.2 (1H, m), 5.37 (1H, ddd,  $J = 11.0, 1.5, 1.5$  Hz), 5.50 (1H, br d,  $J = 11.0$  Hz,  $W_{1/2} = 5$  Hz); for **5** 0.78 (3H, s), 0.97 (3H, s), 0.99 (3H, s), 1.64 (3H, br s,  $W_{1/2} = 3$  Hz), ca. 1.8–2.2 (4H, m), 5.64 (2H, br s,  $W_{1/2} = 3$  Hz); and for **6** 0.80 (3H, s), 0.99 (3H, s), 1.01 (3H, s), 1.77 (3H, br s,  $W_{1/2} = 2.5$  Hz), ca. 2.6 (4H, m), 5.39 (1H, m,  $W_{1/2} = 8$  Hz), 5.49 (1H, m,  $W_{1/2} = 7$  Hz).

PREPARATION OF CUPARENE (**7**).—The reaction of the mixture of hydrocarbons **5** and **6** (100 mg) with a solution of lithium (30 mg) in 5 ml of NH<sub>2</sub>CH<sub>2</sub>CH<sub>2</sub>NH<sub>2</sub> (stirring under reflux, 6 h) gave 80 mg of an oil, which was treated with a solution of *p*-carbomethoxyperbenzoic acid (100 mg) of the peracid in 4 ml of CHCl<sub>3</sub>, 5 min at room temperature) followed by cc on Si gel (5 g) with petroleum ether to give 40 mg of (+)-cuparene (**7**),  $[\alpha]^{20}_{394} +64^\circ$  ( $c = 5.2$  CHCl<sub>3</sub>) [lit. (7)  $[\alpha]_D +65^\circ$  ( $c = 5.9$ , CHCl<sub>3</sub>)]. The <sup>1</sup>H-nmr

spectrum of cuparene obtained coincided with the one reported earlier (10).

The molecular mechanics calculations were carried out using the MM2S program with the MM2 force field (11).

The lanthanide-induced shifts calculations were conducted according to the McConnell-Robertson equation, using the atomic coordinates of stable conformations shown in Figure 2. The calculation procedure is described by Tkachev *et al.* (5).

#### ACKNOWLEDGMENTS

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