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β-Cyclodextrin and its derivatives directed axial attack of hydride ion in the reduction of (*R*)-(+)-pulegone and (2*S*,5*R*)-(−)-menthone

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Abstract

Sodium borohydride reduction of (*R*)-(+)-pulegone and (2*S*,5*R*)-(−)-menthone in aqueous alkali resulted in formation of *cis*-pulegol from pulegone and menthol from menthone, in larger proportions in the presence of derivatives of β-cyclodextrin (BCD), namely, heptakis-2,6-di-*O*-methyl-β-cyclodextrin (DM-BCD) and BCD–epichlorohydrin polymer (BCD-polymer), implying axial attack of hydride ion being preferred in inclusion complexes of (*R*)-(+)-pulegone and (2*S*,5*R*)-(−)-menthone. Higher conversion yields were registered in reactions where BCD and its derivatives were present. More of double bond migrated product (isopulegol) was found to be formed from pulegone in the presence of BCD. The results obtained from the reactions are explained in terms of the orientation of pulegone and menthone arrived at from UV–Vis, ^{1}H and ^{13}C-NMR spectroscopic measurements. Binding constant values for the 1:2 (BCD or DMBCD:pulegone) complexes of BCD and DMBCD with pulegone were evaluated to be $5.428 \pm 0.5 \times 10^{3}$ M^{-1} (BCD:pulegone) and 937 ± 50 M^{-1} (DMBCD:pulegone).

Keywords: Axial attack; Equatorial alcohols; β-Cyclodextrin; 1:2 complex; Double bond migration; Pulegone; Menthone

1. Introduction

(*R*)-(+)-Pulegone and (2*S*,5*R*)-(−)-menthone are present in many essential oils (80% pulegone in Pennyroyal oil [1], 51.7% pulegone and 46.8% menthone in *Minthostachys verticillata* oil [2], 35–74% menthone in the essential oils of peppermint, geranium and other mint oils [3]). Reduction by various reducing agents of both these ketones yields epimeric menthols [4]. Reduction by sodium dithionite of these ketones included in β-cyclodextrin and its methyl and polymer derivatives resulted in not only different relative proportions of epimeric alcohols with higher menthol/neomenthol ratios but the yields were also found to be enhanced [5,6]. In the present work sodium borohydride reduction of (*R*)-(+)-pulegone and (2*S*,5*R*)-(−)-menthone in aqueous alkaline solution carried out in the presence of β-cyclodextrin and its derivatives showed that the axial attack of the hydride ion resulted in products containing predominantly equatorial hydroxyl namely *cis*-pulegol, isopulegol and menthol.

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2. Results and discussion

2.1. (R)-(+)-Pulegone

The results of the reduction products of pulegone are summarised in Table 1. Although the products of reduction of pulegone with sodium dithionite and hydrogenation over Raney nickel were reported to be menthone and menthols [6], interestingly only the carbonyl function reduced products with intact olefinic bond yielding exclusively isopulegol and *cis*-pulegol were obtained on reduction with sodium borohydride in aqueous alkali (Scheme 1). Since pulegone was insoluble in water used as solvent in the present study, the control reaction gave only 71.6% of product alcohols. However, this problem of insolubility was overcome by use of BCD and its derivatives, which partially solubilized pulegone through formation of inclusion complex with the substrate. Thus presence of DM-BCD, BCD-polymer and BCD improved the yield with a maximum (99.0%) conversion in presence of BCD. In addition, the presence of BCD and its derivatives showed alteration in the relative proportion of the two alcohols formed. While the control reaction favoured the formation of *cis*-pulegol (77.1%), with a P/I (*cis*-pulegol/isopulegol) ratio of 3.0, that with BCD showed an increase in proportion of isopulegol (49.0% with a P/I ratio of 1.3). However, the presence of BCD-polymer and DMBCD showed increase in *cis*-pulegol proportion (70.7% and 82.6% with P/I ratios of 2.45 and 5.16, respectively). Except in the case of the BCD-mediated reaction where no unreacted pulegone could be detected, all the others showed 12.1–28.3% of unreacted pulegone, the highest being observed for the control.

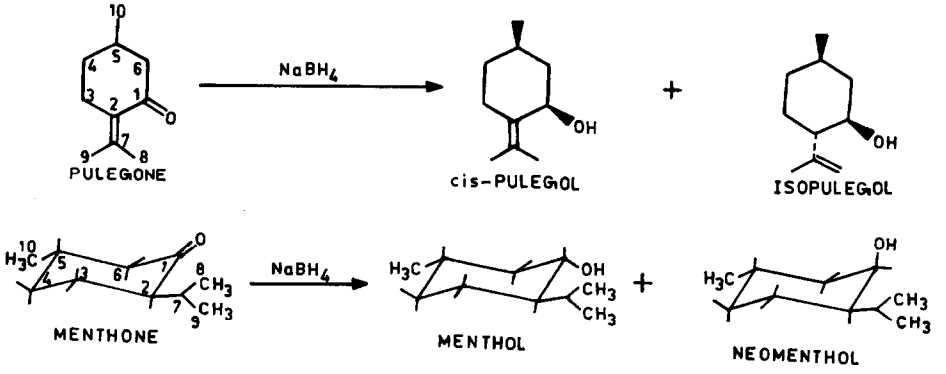
2.2. (2S,5R)-(−)-Menthone

The results obtained are shown in Table 2. In this case also, higher conversion yields resulting

Table 1
Sodium borohydride reduction products of (R)-(+)-pulegone

Catalyst	Unreacted pulegone [a] (%)	Relative percentage [b]		Yield of alcohols (%)	*cis*-pulegol/isopulegol [b]
		Isopulegol (%)	*cis*-pulegol (%)		
control	28.3	22.9/25.7	77.1/74.3	71.6	3.37/2.89
BCD	0	49.0/38.5	51.0/61.5	99.0	1.04/1.60
BCD-polymer	12.1	29.3/28.6	70.7/71.4	87.8	2.40/2.50
DMBCD	18.4	17.4/15.2	82.6/84.8	81.6	4.75/5.58

[a] GC analyses.
[b] GC/NMR analyses.



Scheme 1.

in alcohols were observed in case of BCD (> 99%) and BCD-polymer (> 99%) compared to control (87.9%) and DM-BCD (81.2%), consistent with facilitated partial solubilization of menthone in water by BCD and its derivatives. Also, the presence of BCD and its derivatives showed variation in the product proportion. Thus, while the control reaction gave a menthol/neomenthol ratio of 1.9, those with BCD, BCD-polymer and DM-BCD gave ratios of 4.6, 3.2 and 4.8, respectively. The highest ratio was observed with DM-BCD although the yield of alcohol was less than the rest. Thus, BCD and its derivatives clearly exhibited stereoselective reduction, by increasing the proportion of menthol among the eight optical isomers possible. Also, in the present reaction, only menthol and neomenthol could be detected and the other isomers were not traceable.

2.3. Structural studies

The inclusion complex formation of pulegone was studied by UV–Vis spectroscopy. Binding constant values were determined as described elsewhere [6,7]. A binding constant value of $5428 \pm 500\ M^{-1}$ was evaluated for the 1:2 (BCD:pulegone) complex in 40% aqueous ethanol [6]. Binding constant value of the 1:2 complex formed between DM-BCD and pulegone in chloroform (λ_{max} 258 nm, $\epsilon = 8386$) by Scatchard plot analysis was found to be $937 \pm 50\ M^{-1}$ (Fig. 1a and 1b).

UV–Vis spectroscopic studies did not give satisfactory results on complexation of BCD with menthone. Formation of a very weak complex between BCD and menthone was detected in fluorescence spectroscopy. An aqueous solution of *p*-toludinylnaphthalene sulphonic acid (TNS) (1.053×10^{-4} M) was found to show

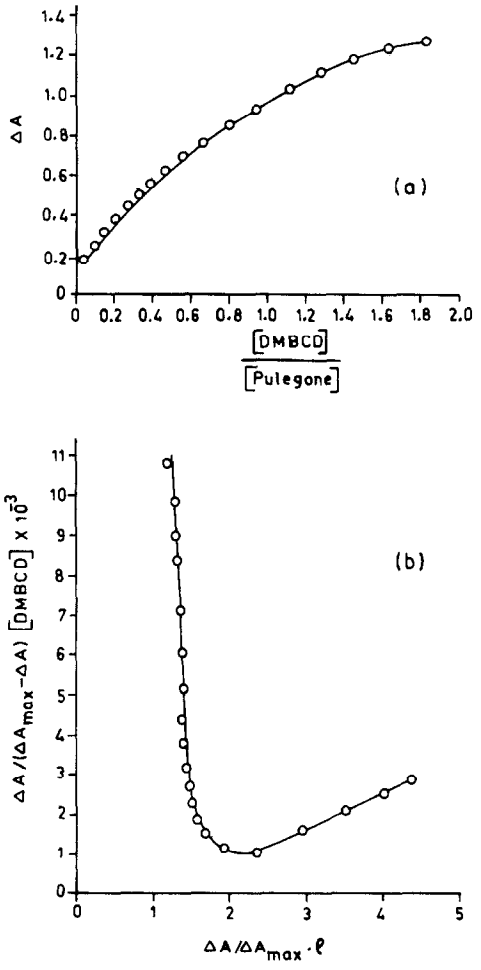


Fig. 1. Determination of (a) stoichiometry and (b) binding constant values for DMBCD–pulegone inclusion complex by UV–Vis spectroscopy, in chloroform. [pulegone] 1.908×10^{-4} M, [DMBCD] 1.890×10^{-3} M. The additions were monitored at 258 nm for evaluation of binding constant value by means of Scatchard analysis.

Table 2
Sodium borohydride reduction products of (−)-menthone [a]

Catalyst	Unreacted menthone (%)	Menthol (%)	Neomenthol (%)	Yield of alcohols (%)	Menthol/neomenthol
control	12.1	58.4	29.5	87.9	1.9
BCD	0	81.7	18.3	> 99.0	4.6
BCD-polymer	0	76.1	23.9	> 99.0	3.2
DMBCD	18.8	67.2	14.0	81.2	4.8

[a] Relative percentage by GC analyses.

fluorescence enhancement (excitation at 364 nm, emission at 464 nm) on adding increasing amounts of BCD. A maximum fluorescence emission (496 arbitrary units) was observed at about 5.8 equivalents of BCD. In the presence of 8.6 equivalents of menthone, fluorescence emission decreased to a value of 11 arbitrary units, indicating displacement of TNS by menthone. Since more equivalents of menthone were required to quench TNS fluorescence, a weak complex being formed between menthone and BCD was inferred.

Presence of two pulegone molecules inside the BCD cavity may lead to different orientations for pulegone: head-to-tail, head-to-head or tail-to-tail. The orientation of pulegone and menthone inside the BCD and DMBCD cavities was studied by ^{13}C and ^{1}H-NMR spectroscopy, and the data are given in Tables 3 and 4, respectively. In the case of the DMBCD–pulegone complex, large downfield shifts for C-1 (1.1 ppm), C-2 (0.31 ppm), C-5 (0.29 ppm) and C-7 (0.48 ppm) were observed. However, while all the pulegone signals showed downfield shifts, those of DMBCD showed upfield shifts. In the DMBCD signals, both 2-OCH_3 and 6-OCH_3 experienced slightly greater upfield shifts (0.07 ppm) compared to other glucose carbon signals (0.03–0.05 ppm).

The menthone–DMBCD complex exhibited large downfield shifts for C-1 (0.35 ppm), C-2 (0.19 ppm), C-8 (0.12 ppm) and C-9 (0.12 ppm). However, as observed in the pulegone complex, here also, all the signals of menthone invariably exhibited downfield shifts, while those of DMBCD showed upfield shifts. Again, large upfield shifts were observed for both 2-OCH_3 and 6-OCH_3 groups. Among the other glucose carbon signals, those of C-1 (0.1 ppm) and C-3 (0.11 ppm) showed slightly greater shifts than the others (0.07–0.09 ppm).

The ^{1}H-NMR spectra of pulegone and menthone with BCD and DMBCD were carried out and the data obtained are shown in Table 3. In the case of the BCD complexes with menthone and pulegone in D_2O, BCD signals could not be

Table 3
^{13}C-NMR chemical shift values of pulegone and menthone in free and complexed state [a]

Carbon signal	Free	Complex	Shift in ppm [b]	Carbon signal	Free	Complex	Shift in ppm
Pulegone ($CDCl_3$)				Menthone ($CDCl_3$)			
C-1	203.73	204.83	−1.10	C-1	212.22	212.57	−0.35
C-2	132.15	132.46	−0.31	C-2	56.19	56.38	−0.19
C-3	28.97	29.22	−0.25	C-3	28.34	28.43	−0.09
C-4	33.19	33.40	−0.21	C-4	34.39	34.49	−0.10
C-5	31.90	32.19	−0.29	C-5	35.91	35.99	−0.08
C-6	51.17	51.44	−0.27	C-6	51.26	51.37	−0.11
C-7	142.05	142.53	−0.48	C-7	26.36	26.46	−0.10
C-8	23.34	23.61	−0.27	C-8	21.59	21.71	−0.12
C-9	22.47	22.72	−0.25	C-9	19.12	19.24	−0.12
C-10	22.16	22.37	−0.21	C-10	22.71	22.81	−0.10
DMBCD ($CDCl_3$)				DMBCD ($CDCl_3$)			
C-1	102.01	101.97	+0.04	C-1	102.01	101.91	+0.10
C-2	82.76	82.73	+0.03	C-2	82.76	82.69	+0.07
C-3	71.02	70.97	+0.05	C-3	71.02	70.91	+0.11
C-4	84.25	84.21	+0.04	C-4	84.25	84.18	+0.07
C-5	73.90	73.86	+0.04	C-5	73.90	73.82	+0.08
C-6	71.57	71.53	+0.04	C-6	71.57	71.48	+0.09
2-OCH_3	61.00	60.93	+0.07	2-OCH_3	61.00	60.82	+0.18
6-OCH_3	59.68	59.61	+0.07	6-OCH_3	59.68	59.49	+0.19

[a] Assignments were based on Ref. [10].
[b] + indicates upfield shift, − indicates downfield shift.

Table 4
^{1}H-NMR chemical shift values of pulegone and menthone in free and complexed state [a]

Proton signal	Free	Complex	Shift in ppm [b]	Proton signal	Free	Complex	Shift in ppm
Pulegone ($CDCl_3$)				Menthone (Neat)			
H-3	2.26 (3.3 Hz)	2.48 (2.20 Hz)	−0.22	H-6a and b	2.08	2.06	+0.02
				H-3a and b	2.08	2.06	+0.02
H-4a and b	1.33	1.26	+0.07	H-4a and b	1.38	1.38	0.00
H-6a	2.71 (2.05 and 11.22 Hz)	2.72 (15.5 Hz)	−0.01	H-5	2.08	2.06	+0.02
				H-2	2.27 (11.58 Hz)	2.46	−0.19
H-6b	2.49 (2.2 and 10.8 Hz)	2.52 (2.14 Hz)	−0.03	H-7	2.08	2.06	+0.02
				9-CH_3	0.86 (4.95 Hz)	0.86	0
9-CH_3	1.98	1.97	+0.01	8-CH_3	0.93 (4.63 Hz)	1.06 (7.01 Hz)	−0.13
8-CH_3	1.78	1.77	+0.01				
10-CH_3	1.00 (6.15 Hz)	1.00 (6.15 Hz)	0	10-CH_3	1.03 (5.08 Hz)	1.18	−0.15
H-5	– [c]	–	–				
DMBCD ($CDCl_3$)				DMBCD ($CDCl_3$)			
H-1	4.96 (3.70 Hz)	4.97 (3.50 Hz)	−0.01	H-1	4.96 (3.70 Hz)	4.95 (9.60 Hz)	−0.01
H-2	3.25 (2.7 and 9.4 Hz)	3.28 (2.7 and 9.4 Hz)	−0.03	H-2	3.25 (2.70 and 9.40 Hz)	3.25 (2.70 and 9.40 Hz)	0
H-3	3.90 (9.4 Hz)	3.94 (9.1 Hz)	−0.04	H-3	3.90 (9.4 Hz)	3.89 (8.9 Hz)	−0.01
3-OH	5.05	5.09	−0.04	3-OH	5.05	5.05	0
H-4	3.44 (9.4 Hz)	3.48 (10.8 Hz)	−0.04	H-4	3.44 (9.4 Hz)	3.46 (11.0 Hz)	+0.02
H-5	3.68 (9.4 Hz)	3.71 (9.4 Hz)	−0.03	H-5	3.68 (9.4 Hz)	3.67 (6.0 Hz)	−0.01
H-6a and b and 2-OCH_3	3.60	3.64	−0.04	H6a and b, and 2-OCH_3	3.60	3.60	0
6-OCH_3	3.37	3.41	−0.04	6-OCH_3	3.37	3.37	0
Pulegone (neat)				Menthone (neat)			
H-3	3.01 (12.4 Hz)	2.14	+0.87	H1-6a and b	2.08	2.03	+0.05
				H-3a and b	2.08	2.03	+0.05
H-4a and b	2.22	2.21	+0.01	H-4a and b	1.38	1.35	+0.03
H-6a	3.48 (3.48 and 12.9 Hz)	2.59	+0.89	H-5	2.08	2.03	+0.05
				H-2	2.27 (11.5 Hz)	2.03	+0.24
H-6b	3.24 (3.6 and 10.8 Hz)	2.31	+0.93	H-7	2.08	2.03	+0.05
				9-CH_3	0.86 (4.9 Hz)	0.87	−0.01
9-CH_3	2.74	1.84	+0.90	8-CH_3	0.93 (4.6 Hz)	0.87	+0.06
8-CH_3	2.54	1.67	+0.87				
10-CH_3	1.76 (5.58 Hz)	0.88	+0.88	10-CH_3	1.03 (5.09 Hz)	0.87	+0.16
H-5	– [c]	–	–				
BCD (D_2O) [d]		–	–	BCD (D_2O) [d]		–	–

[a] ^{1}H NMR assignments except those of the three methyl groups [11] are tentative.
[b] + indicates upfield shift, − indicates downfield shift.
[c] Merged with CH_3 signals at 8, 9 and hence could not be detected.
[d] BCD signals were not detected.

detected due to precipitation. Also, since menthone was insoluble in water, comparison of chemical shift values of neat menthone and pulegone and their complexes with BCD gave unrealistic differences which could not be interpreted meaningfully. The very fact that menthone and pulegone signals in the spectra of their BCD complexes could be detected indicated that small amounts of menthone and pulegone became dissolved in water as the BCD complex.

However, pulegone and menthone complexes of DMBCD in $CDCl_3$ gave better indication of their complexation. In the case of pulegone, a maximum downfield shift was observed for H-3 (0.22 ppm) and H-4a and b (0.07 ppm) showed maximum upfield shift on complexation. Other signals showed both downfield as well as upfield shifts in the range 0.01–0.03 ppm. The signals from DMBCD showed downfield shifts in the order of 0.01–0.04 ppm.

However, in menthone, some of the signals were not resolved clearly like H-6a and b, H-3a and b, H-5 and H-7, as they were found merged in a huge envelope at 2.08 ppm. However, signals like H-4a and b, H-2 and methyl signals were clearly resolved. Maximum downfield shifts were observed for H-2 (0.19 ppm), 8-CH_3 (0.13 ppm) and 10-CH_3 (0.15 ppm). Other signals like H-6a and b, H-3a and b, H-5 and H-7 showed both upfield as well as downfield shifts of the order 0.02–0.05 ppm. DMBCD signals showed upfield and downfield shifts in the order of 0.01–0.02 ppm. Here also, the differences should be treated with caution since the comparison was between neat menthone and the complex in $CDCl_3$.

The studies described above have clearly shown that the reactivity of both the ketone function and the olefinic function are affected by inclusion of these groups inside the BCD cavity. While the disposition of the olefinic and keto group in pulegone is not clear, that of the ketone function in menthone may be envisaged to be projected into the mouth of the BCD cavity. The ^{13}C and ^{1}H-NMR studies of BCD and DMBCD complexes of pulegone show that the region around ketone and olefinic functions being affected more by complexation. In 1:2 complexes of pulegone with BCD or DMBCD, based on the observed product distribution on reduction, orientation of two pulegone molecules inside BCD cavity can be envisaged to be the one with both an olefinic and a keto group of one molecule at the wider end and a methyl group of another molecule projected inside at the narrower end. At the wider end, isopropylidene group of one pulegone molecule probably is projected into the cavity bringing the keto group close to secondary hydroxyl groups which also aid in stabilizing such a disposition through hydrogen bonding. Observed NMR and reactivity data support such disposition. The same data also exclude the disposition of both the pulegone molecules through projection of their olefinic and keto functions at both the ends of BCD cavity. This orientation is definitely not possible at the narrower end due to steric hindrance by the hydroxymethyl groups to the incoming isopropylidene group of pulegone. Similarly the orientation involving methyl group of two pulegone molecules projected into both the ends of BCD would leave the keto and olefinic functions exposed, affecting the observed selectivity. Hence, that orientation is also ruled out.

The products obtained from the two ketones, namely *cis*-pulegol and isopulegol from pulegone and menthol from menthone are due to attack of hydride ion (from $NaBH_4$) from the axial position. The attack from the equatorial position, namely the attack from the already more hindered side, is made further difficult by the defined orientation of the ketonic function inside the BCD cavity. The product of the equatorial attack namely, neomenthol from menthone showed decrease in its proportion with BCD and its derivatives. In pulegone the product of equatorial attack namely trans-pulegol was not detected. However, isopulegol, the product due to migration of double bond from 2 and 7 to 7 and 8 position was found to be

formed in larger proportion in reaction where BCD was present. In the presence of its methyl and polymer derivatives, more *cis*-pulegol, was detected in preference to the double bond migrated product, namely isopulegol. This may be due to complete ionisation of secondary hydroxyl groups on C-2 and C-3 atoms of each glucose unit in BCD (14 of them with a $pK =$ 12.0) [5,6] which probably accommodates pulegone in a slightly different orientation at both the ends compared to BCD-polymer and DMBCD where the number of secondary hydroxyl groups are reduced due to derivatisation. Although DMBCD contains seven hydroxyl groups on C-3 capable of undergoing ionisation, its orientation towards the centre of cavity in a slightly hydrophobic exterior (due to derivatisation of OH groups on C-2) may prevent the migration, mentioned above, to such an extent as to reduce the proportion of isopulegol formed.

3. Experimental

In a typical experiment, the substrate (13.0 mmol)/$NaBH_4$/NaOH in molar ratios 1:1:2 were stirred in 25 ml of water at room temperature with appropriate catalyst (Tables 1 and 2) for a day. The reaction mixture was then treated with ammonium chloride, extracted with ether, dried over anhydrous sodium sulphate, concentrated and analysed by GLC. Clear separation of isopulegol (RT 5.1 min), pulegone (8.3 min), pulegol (12.1 min), menthone (4.0 min), menthol (6.2 min) and neomenthol (6.9 min) were achieved. The products were separated by silica gel column chromatography with hexane as eluant and were characterised by comparison with authentic samples.

A Shimadzu GC-15A instrument fitted with 20% Carbowax 20 M, 3m column, with a 30 ml/min nitrogen flow rate was used. The injection and FID detection port temperatures were maintained at 200°C and 250°C respectively. The column was maintained at 130°C. For the structural studies, a Shimadzu UV-240 spectrophotometer was used at 20°C.

The BCD used was a gift from American Maize Products Company, USA. BCD-polymer and DMBCD were prepared by the procedures of Shaw and Buslig [8] and Szejtli et al. [9], respectively. Pulegone was purchased from Aldrich Chemical Company, USA. Menthone was prepared by the oxidation of (−)-menthol by chromic acid [5].

The reaction products so obtained were also analysed by ^{1}H-NMR in CCl_4 on a Varian EM-390 continuous wave 90 MHz NMR spectrometer, in order to determine the product distribution, from the areas of the H-1 protons at 5.7 ppm (*cis*-pulegol) and 3.4 ppm (isopulegol) and the relative proportions of both *cis*-pulegol as well as isopulegol so obtained were compared with the GC data.

^{1}H-NMR spectra for structural studies were recorded on a Bruker WH 270 instrument operating at 270 MHz, fitted with a Spectrospin magnet and an Aspect 2000 computer at 20 ± 1°C. Carbon-13 NMR spectra were also recorded on the same instrument operating at 67.5 MHz for carbon-13. Proton-noise decoupled spectra were obtained. Signals were referenced to within ±0.01 ppm from tetramethylsilane used as an internal reference. Typically 100–500 scans were accumulated to obtain good spectra.

^{1}H-NMR characteristics of the products of pulegone namely *cis*-pulegol and isopulegol are:

cis-Pulegol δ ppm ($CDCl_3$): 5.7 (m, 1H, H-1), 3.5 (m, 1H, OH), 1.6–1.7 (6H, CH_3), 1.2–2.2 (m, 7H), 1.0 (S, 3H, CH_3); isopulegol δ ppm ($CDCl_3$): 4.85 (m, 2H, $=CH_2$), 3.4 (d,d,t, 1H, 8.0 Hz, 4.0 Hz, H-1), 1.75 (S, 3H, CH_3), 1.2–2.2 (m, 9H), 1.0 (d, 3H, CH_3).

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