

Article

Conformation Analysis and Stereodynamics of Symmetrically *ortho*-Disubstituted Carvacrol Derivatives

Miljana R. Đorđević Zlatković ¹, Niko S. Radulović ^{1,*}, Miroslav Dangelov ²  and Nikolay G. Vassilev ^{2,*} 

¹ Department of Chemistry, Faculty of Sciences and Mathematics, University of Niš, Višegradska 33, 18000 Niš, Serbia; miljana.djordjevic@pmf.edu.rs

² Institute of Organic Chemistry with Centre of Phytochemistry, Bulgarian Academy of Sciences, Acad. G. Bontchev Str. Bl. 9, 1113 Sofia, Bulgaria; miroslav.dangelov@orgchm.bas.bg

* Correspondence: niko.radulovic@pmf.edu.rs (N.S.R.); nikolay.vassilev@orgchm.bas.bg (N.G.V.)

Abstract: The design and synthesis of analogs of natural products can be a valuable source of medicinal preparations for the pharmaceutical industry. In the present study, the structural elucidation of eleven derivatives of 2,4-dihalogeno substituted synthetic analogues of the natural compound carvacrol was carried out by means of NMR experiments, and of another thirteen by DFT calculations. By selective NOE experiments and the irradiation of CH signals of the isopropyl group, individual conformers were assigned as *syn* and *anti*. By comparing GIAO/B3LYP/6-311++G(d,p)-calculated and experimentally measured vicinal $^3J_{CH}$ spin-spin constants, this assignment was confirmed. An unusual relationship is reported for proton-carbon vicinal couplings: $^3J_{CH}(180^\circ) < ^3J_{CH}(0^\circ)$. The conformational mobility of carvacrols was studied by 2D EXSY spectra. The application of homonuclear decoupling technique (HOBS) to these spectra simplifies the spectra, improves resolution without reducing the sensitivity, and allows a systematic examination of the rotational barrier of all compounds via their CH signals of the isopropyl group in a wider temperature interval. The rate constants of the isopropyl rotation between *syn* and *anti* conformers were determined and the corresponding energy barriers (14–17 kcal/mol) were calculated. DFT calculations of the energy barriers in carvacrol derivatives allowed the determination of the steric origin of the restricted isopropyl rotation. The barrier height depends on the size of the 2- and 4-position substituents, and is independent of the derivatization of the OH group.

Keywords: carvacrol; rotational barrier energy; dynamic NMR; HOBS EXSY experiment; DFT calculations



Citation: Đorđević Zlatković, M.R.; Radulović, N.S.; Dangelov, M.; Vassilev, N.G. Conformation Analysis and Stereodynamics of Symmetrically *ortho*-Disubstituted Carvacrol Derivatives. *Molecules* **2024**, *29*, 1962. <https://doi.org/10.3390/molecules29091962>

Academic Editor: Adel Nefzi

Received: 22 March 2024

Revised: 12 April 2024

Accepted: 19 April 2024

Published: 25 April 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Atropisomerism is a form of axial chirality whereby enantiomers or diastereomers, known as atropisomers and atropodiastereomers, emerge as a result of constrained or hindered rotation along a σ -bond axis [1–3]. Despite the increasing attention paid to atropisomerism in the pharmaceutical sciences over the past decade, the presence of configurationally stable, axially chiral stereochemical elements in commercially available drugs remains rare even a century after the initial mention of axial atropisomerism [4]. The racemization process may last from a few seconds to several months or even decades. LaPlante and co-workers categorized atropisomers into three groups based on the energy required for the chiral axis to undergo racemization through rotation [5,6]. Atropisomers in class 1 have rotation barriers of <84 kJ/mol (20 kcal/mol) and racemize rapidly, typically on a minute or faster timescale at room temperature. Class 2 atropisomers have rotation barriers ranging from 84 to 117 kJ/mol (20–28 kcal/mol) and racemize over the hour-to-month timescale at room temperature. On the other hand, class 3 atropisomers have rotation barriers exceeding 117 kJ/mol (28 kcal/mol) and undergo racemization on a year or longer timescale at room temperature. Class 1 atropisomers have gained prominence in the field of drug discovery. Around 30% of small-molecule drugs approved by the FDA

(Food and Drug Administration) since 2011 exhibit at least one chirality interconverting axis [7].

Ortho-substituted biaryl systems ($C(sp^2)-C(sp^2)$) are a class of widely distributed and extensively studied atropisomeric compounds. On the other hand, nonbiaryl atropisomers with conformationally stable $C(sp^2)-C(sp^3)$ single bonds have been left unexplored due to the rarity of restricted rotation around these bonds. Even though the first report suggesting the possibility of isolating sp^2-sp^3 atropisomers appeared in 1968 [8], only a few such compounds have been reported in the literature: carbinols [9–12], 9-arylfluorene derivatives and 9-aryl-9,10-dihydroanthracene [13,14], cannabidiols [15,16], 2-arylindoline derivatives [17] and hexahydro-1,2,4,5-tetrazines [18]. The existence of atropisomeric chirality has the potential to have a significant impact on the chemical, spectroscopic, and biological properties of natural products. Examples such as pegaharmols A–B [19], cordypyridones A–B [20,21], linderatin derivatives [22], and stilbene derivatives [23,24] illustrate stable natural atropisomers resulting from restricted rotation about the $C(sp^2)-C(sp^3)$ bond.

Carvacrol, a phenolic monoterpene, is a major component of the essential oils found in plants of the Lamiaceae family. It is classified as “Generally Recognized As Safe” and is permitted for use in food [25–27]. Known for its diverse properties, including antimicrobial, anti-inflammatory, antioxidant, antitumor, analgesic, anti-hepatotoxic, and insecticidal effects [27], carvacrol has been extensively studied for its antibacterial activity against a range of Gram-positive and Gram-negative bacteria in food [27,28]. Research on carvacrol derivatives aims to enhance its biological properties, with many derivatives showing improved features compared to the original compound [29–31].

Surprisingly, there are no data in the literature on iodinated derivatives of carvacrol, while information on chlorinated and brominated derivatives is very limited [32–35]. The aim of the present study was to synthesize new 2,4-substituted halogen derivatives of carvacrol as a starting scaffold for new biologically active compounds. One would expect that both halogen atoms (Cl, Br or I) would be positioned *ortho* to the isopropyl group, and *ortho*, i.e., *para* to the phenolic group. Such regiochemistry could potentially cause the rotation around the aromatic core–isopropyl group bond ($C(sp^2)-C(sp^3)$) to be difficult, and as a result there are diastereoisomers of the atropisomeric type [12]. Majetich and Grove reported on dibromo derivatives of carvacrol, and the excess of signals in NMR spectra were explained by “open-orbital-induced heavy-atom effects” rather than the presence of a mixture of diastereoisomers [35]. This further encouraged us to investigate (both experimentally with dynamical NMR and theoretically with DFT calculations) the conformational exchange due to restricted rotation of the isopropyl group in disubstituted carvacrols.

Herein, we reveal a new class of rare aryl- $C(sp^3)$ atropisomers obtained by the derivatization of the natural product carvacrol. We present the synthesis, combined dynamic nuclear magnetic resonance (DNMR) spectroscopy, and density functional theory (DFT) study of the restricted rotation of the isopropyl group in 2,4-disubstituted carvacrols (Figure 1). By applying the homonuclear decoupling technique (HOBS experiment) in 1D and 2D NOESY spectra, we determined the rate constants of the isopropyl rotation between *syn* and *anti* conformers.

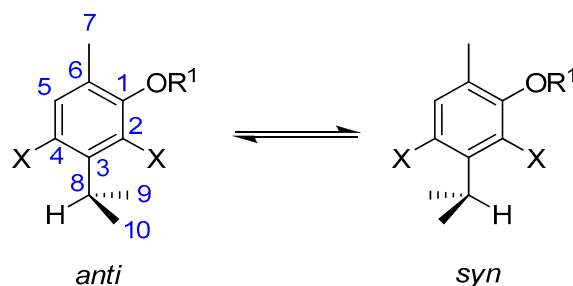


Figure 1. The studied atropisomers of 2,4-disubstituted carvacrols. For variation of substituents refer to Table 1.

Table 1. The studied 2,4-disubstituted carvacrols and NMR experimental condition.

Compound ^a	X	R ¹	H ^b	T Range, K	H ^c	T Range, K
1	Cl	H	H-5	233–258	H-8	233–268
2	Br	H	H-8	253–273	H-8	253–283
3	I	H	OH	253–293	H-8	263–293
4	Br	CH ₃	H-5	253–278	H-8	253–273
5	I	CH ₃	H-8	253–273	H-8	263–293
6	Br	Allyl	H-8	243–283	H-8	253–283
7	I	Allyl	H-8	253–273	H-8	263–293
8	Br	C(=O) <i>i</i> -Pr	H-5	253–273	H-8	263–288
9	I	C(=O) <i>i</i> -Pr	H-8	263–283	H-8	263–293
10	Br	C(=O)Et	H-5	253–293	H-8	263–293
11	I	C(=O)Et	H-8	273–313	H-8	273–313
12	F	H				
13	H	H				

^a Compounds **12** and **13** were studied only by DFT calculations. ^b Protons used in 2D EXSY experiments. ^c Protons used in 2D HOBS-NOESY.

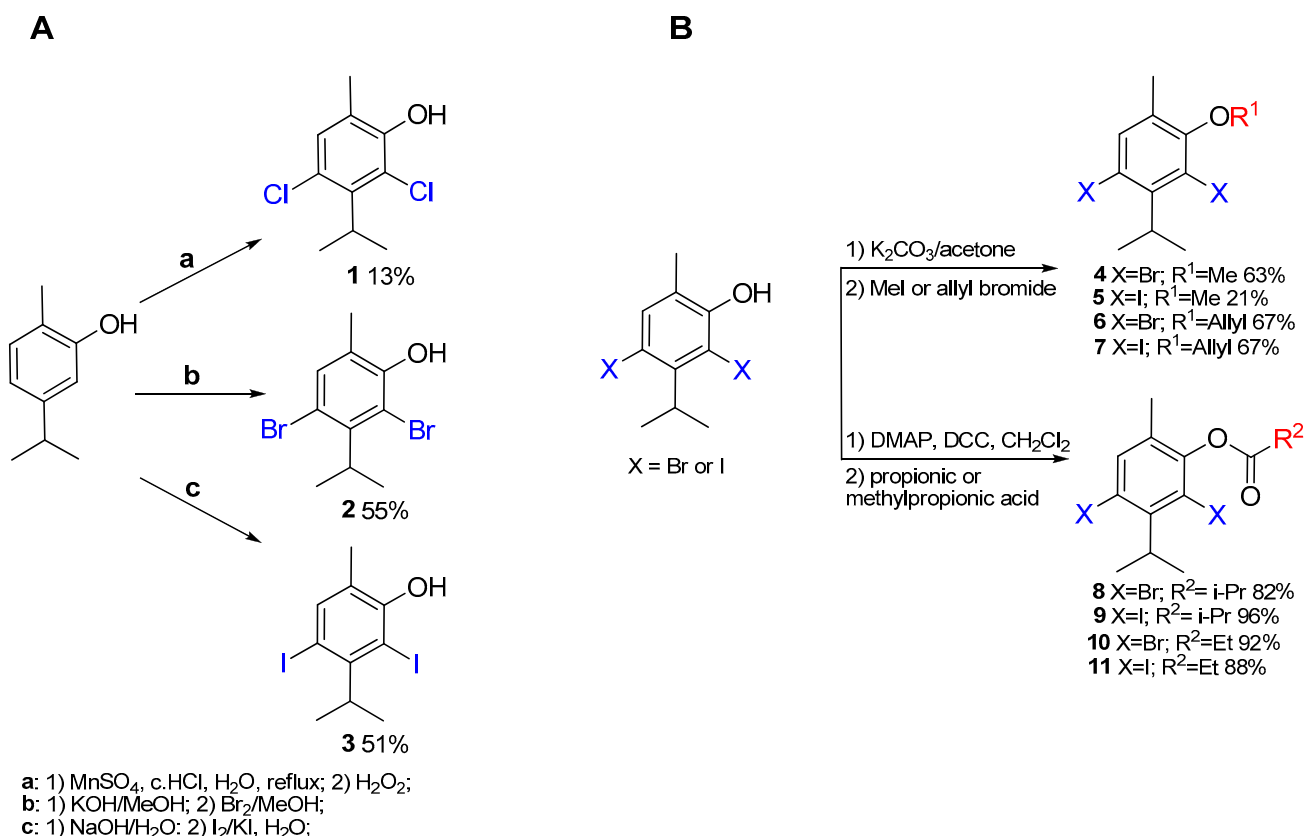
2. Results and Discussion

2.1. Synthesis of the Compounds

We were interested in synthesizing biologically active derivatives of natural products, and were prompted by the results obtained by studying the iodination reaction of thymol, including the isolation and identification of new, unexpected derivatives of thymol [36]. Here, carvacrol (a regioisomer of thymol) was subjected to iodination. The synthesis of 2,4-diiodo-substituted carvacrol was carried out in a similar manner to the iodation of thymol [36]. After repeated chromatographies on SiO₂ and Sephadex LH-20 columns, a sample of 2,4-diiodo-3-isopropyl-6-methylphenol (**3**), pure based on TLC and GC-MS analyses, was isolated. Two sets of signals with comparable integrals were observed in the recorded ¹H and ¹³C NMR spectra in deuterated chloroform at room temperature. A complete assignment of the observed resonances was accomplished by a careful analysis of the 1D and 2D NMR spectra (a series of selective homonuclear ¹H decoupling, and grHSQC, grHMBC, gr¹H–¹H-COSY, ROESY, and NOESY experiments), and this confirmed the presence of two stable atropodiastereomers. Attempts followed to preparatively separate these diastereomers (using chromatography on Sephadex LH-20 and silica gel), but without success. The interconversion of these diastereomers is likely fast at the (high) temperature at which they eluted from the GC column, resulting in only one peak being detected. To confirm the existence of analogous atropisomers, we subsequently synthesized both dichloro and dibromo derivatives of carvacrol, compounds **2** and **3** (see Scheme 1A). Furthermore, additional functionalizations of the halogenated derivatives **2** and **3** were performed; thereby, corresponding ethers and esters were obtained, compounds **4–11** (see Scheme 1B). All synthesized compounds represented mixtures of two rotamers, in approximately equal populations. These other halogenated derivatives (compounds **1** and **2**) demonstrated how the size of the *ortho* substituent affects the rotational barrier of the observed atropisomers, while the alkylation or acylation of the phenolic OH group of **2** and **3** revealed how changing the size of the *meta*-supporting group influences the stability of the rotamers.

Spectral data for most of the synthesized halogenated derivatives of carvacrol do not exist in the literature, except for those related to compounds **1**, **2**, and **4**. In the work of Miller and Haggerty (1986), the ¹H NMR spectra of compounds **1** and **2** are presented, without any mention of the presence of atropisomers, and they provide data only for one compound [33]. The ¹H and ¹³C NMR, IR, and HRMS spectra for compound **4** are listed in the work of Majetich and Grove [35], but the existence of atropisomers was not recognized in their study. An apparently unexpected larger number of signals was explained as being the consequence of the “open-orbital-induced heavy-atom effects”. In the proton NMR spectrum, the signals were described as doublets originating from a single compound split

by exceptionally high-value coupling constants. For example, in the ^1H NMR spectrum, the H-8 signal is described as a doublet of septets with an additional coupling constant $J = 46.8$ Hz, corresponding to a difference of 0.117 ppm (at 400 MHz), or the difference in the shift of the two atropisomers that we observed. By comparing our spectral data with those in the literature, all signals expected for both atropisomers of 1,3-dibromo-2-isopropyl-4-methoxy-5-methylbenzene (**4**) were accounted for, indicating an error in the existing literature. These authors also provided NMR data for 3,5-dibromo-4-isopropyl-2-methoxybenzyl acetate, from which it can also be concluded that atropisomers are present. However, their explanation of the doubling in number of signals in the NMR spectra remained the same as for compound **4**.



Scheme 1. Synthesis of compounds **1–11**. (A) Halogenation of carvacrol. (B) Etherification and esterification of compounds **2** and **3**.

2.2. Conformational Analysis and Molecular Geometry

The NMR spectra of the obtained and purified 2,4-disubstituted carvacrols **1–11** display signals indicating a mixture of two atropisomers, with their exchange occurring slowly on the NMR time scale. Our first task was to assign signals for the two isomers. Consequently, we measured the NOE under conditions of slow exchange, and the isomer is referred to as *anti* when the proximity of the methine proton of *i*-Pr (H-8) and H-5 was observed. In Figure 2, this approach is depicted for compound **1**. Although the NOE experiments reveal the *syn* and *anti* conformers, the observed NOEs were only in the order of 0.1%. Furthermore, in the case of compound **5**, the H-5 signals for the two atropisomers overlap. Consequently, we decided to employ a second approach: measuring the vicinal coupling constants between the CH proton from the *i*-Pr group and the carbon atoms C-2 and C-4. The usual relationship of the vicinal coupling constants should be $^3J_{\text{CH}}(180^\circ) > ^3J_{\text{CH}}(0^\circ)$. However, recently, an unusual relationship for proton vicinal couplings was reported, and we decided to calculate the vicinal couplings of the two rotamers of carvacrol (Figure 3) using GIAO/B3LYP/aug-cc-pVTZ-J [37,38] and GIAO/B3LYP/6-311++G*-J [39] methods.

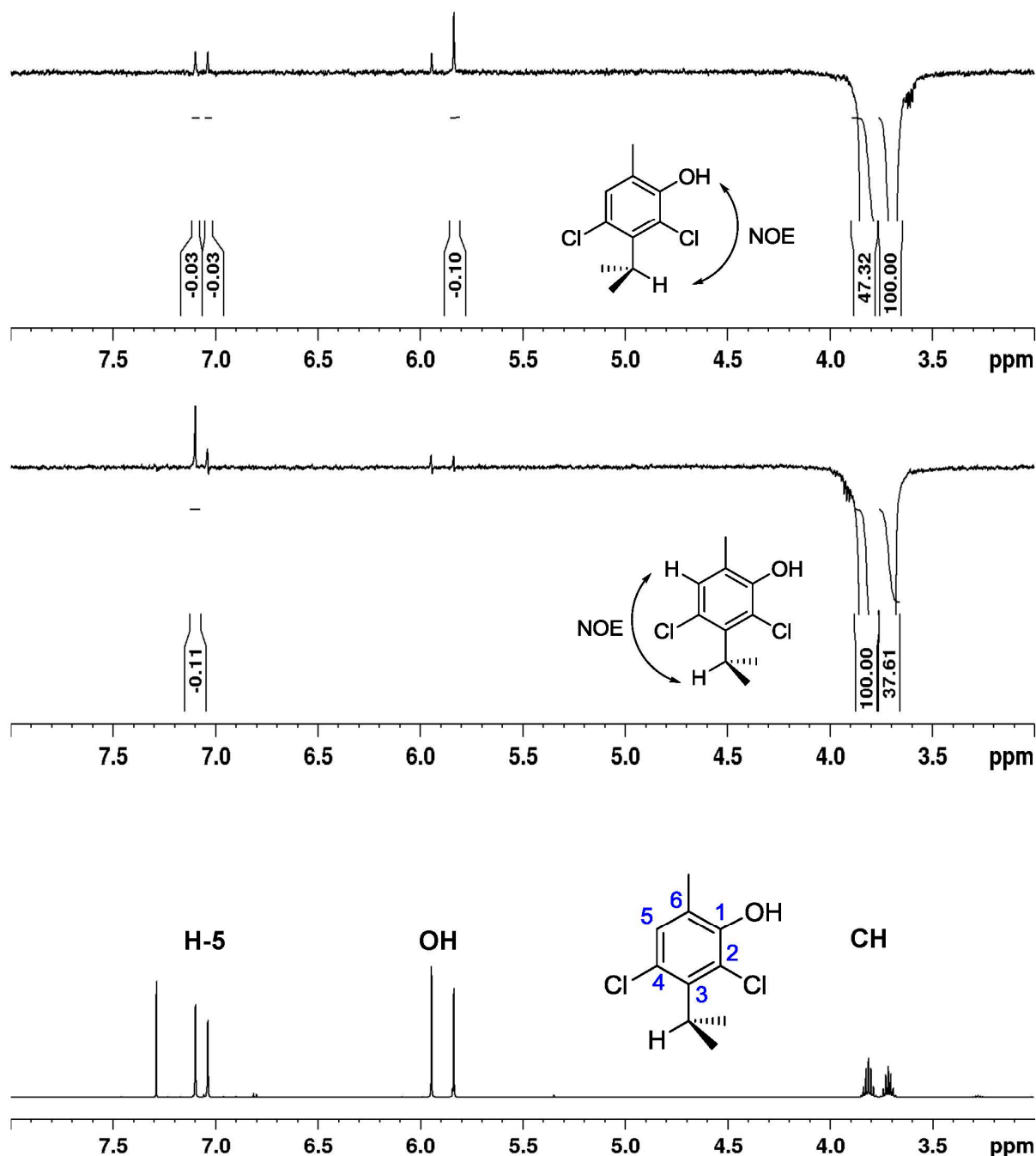


Figure 2. The NOE measurements and assignment of syn and anti atropisomers of **1**. The bottom ^1H spectrum represents a regular proton spectrum at 238 K. The middle spectrum is a 1D NOE spectrum obtained at 238 K by selective excitation of the signal at 3.78 ppm (0.11% NOE effect on H-5 signal indicate proximity of H-5 and CH protons). The upper spectrum is a 1D NOE spectrum at 238 K obtained by selective excitation of the signal at 3.69 ppm (0.10% NOE effect on OH signal indicates the proximity of OH and CH protons).

Lately, it has been shown on test compounds that these methods are very accurate in calculating coupling constants [39]. The results of the calculations show that, in the case of carvacrol and its derivatives, there is an unusual relationship of proton–carbon vicinal couplings: $^3J_{\text{CH}}(180^\circ) < ^3J_{\text{CH}}(0^\circ)$ (refer to the Supplementary Materials). The calculation of vicinal couplings for all compounds confirms the assignment from the selective NOE experiments with the exception of **1**, in which the two C-4 signals of two conformers overlap in the ^{13}C spectrum, and therefore the accurate determination of the coupling

constants is rather difficult. Consequently, in the case of **1**, our assignment relies on NOE measurements alone.

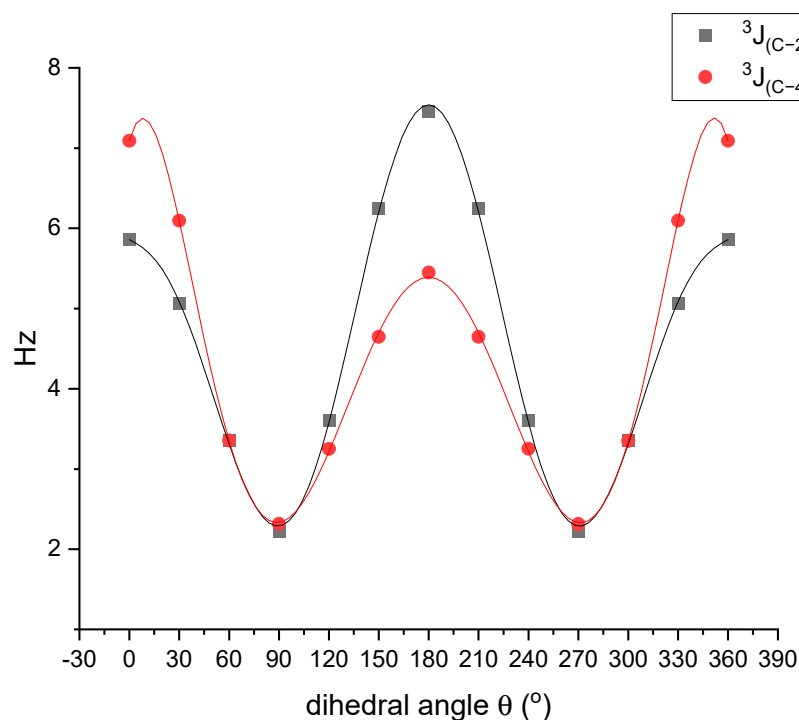


Figure 3. The relationship of vicinal coupling constants of carvacrol and the dihedral angle, based on GIAO/B3LYP/6-311++G*-J data.

2.3. Dynamic NMR Spectroscopy

Variable temperature ^1H NMR spectra of compounds **1–11** were measured in CDCl_3 in order to determine the effects of substituents on the rotational barrier. As the temperature decreases, the separation of signals for the two rotamers increases, allowing 2D EXSY spectra to be acquired in slow exchange over temperature intervals in order to obtain the rate constants using the volume integrals. The kinetic parameters for bond rotation were then extracted by Eyring analysis. In the case of compound **1**, the two isomers show well separated singlet signals for H-5 protons. The same signal of H-5 was used for dynamic NMR studies of compounds **4**, **8** and **10**. In most other studied compounds, with the exception of **3**, the signal for the CH protons from the isopropyl group was the only choice, since at a lower measured temperature the separation of other signals does not allow proper integration (Table 1). Thus, 2D EXSY spectra in the relevant region were measured in such a way as to extract the thermodynamic parameters of the exchange process. As a way to acquire more accurate data, by comparing the signals from the same group for all compounds, and to exclude the possibility of additional interactions (e.g., the phenolic proton of compound **3** also exchanges with the water present in the solvent), we chose to focus our efforts on “pure shift” techniques.

The broadband ^1H homodecoupled NMR [40,41], also known as a “pure shift” technique, relies on improving the resolution via refocusing the scalar coupling. This approach produces a spectrum displaying chemical shift resonances as collapsed singlets, avoiding the complications of overlapping multiplets. We implemented a homodecoupled band selective (HOBS) NMR [42–45] in 1D and 2D EXSY pulse programs to achieve homonuclear proton decoupling over a selected region of the whole spectrum, while the resonances outside this region are completely removed. Furthermore, HOBS, compared to other homonuclear decoupling approaches, is a quantitative method as it retains full sensitivity of the desired signals. Hence, we expect to obtain the same results for the rate constants as when using standard EXSY pulse programs (Figure 4; refer to Supplementary Mate-

rials for pulse program and more details). The rate constants of the isopropyl rotation between *syn* and *anti* conformers were determined and the corresponding energy barriers (14–17 kcal/mol) were calculated using HOBS EXSY spectra. The experimental activation parameters for both directions of the studied exchanging process are summarized in Tables 2 and S58–S61. In the case of rate constants, the HOBS EXSY results show a statistical deviation in some cases of up to 10% compared to the EXSY rate constants, which is absolutely within the range of the maximum relative error of the applied method. Accordingly, the comparison between the results derived from the HOBS EXSY and conventional EXSY spectra clearly demonstrates that the calculated energy barriers occasionally differ from each other. For example, the values for the *anti* to *syn* exchange process only in two cases exceed the limits of the statistical error (see Supplementary Materials, Tables S58–S61). By applying the HOBS EXSY experiment to determine the rate constants and activation parameters, we were able to study all compounds using the same signals for all of them (the methine proton of the isopropyl group), thus obtaining comparable accurate results for all compounds. This makes the application of HOBS EXSY quite valuable in cases of overlapping exchange signals to simplify proton spectra. As a result of the simplification of the spectrum, the temperature range of investigation is increased and reliable results can be obtained in an unambiguous way.

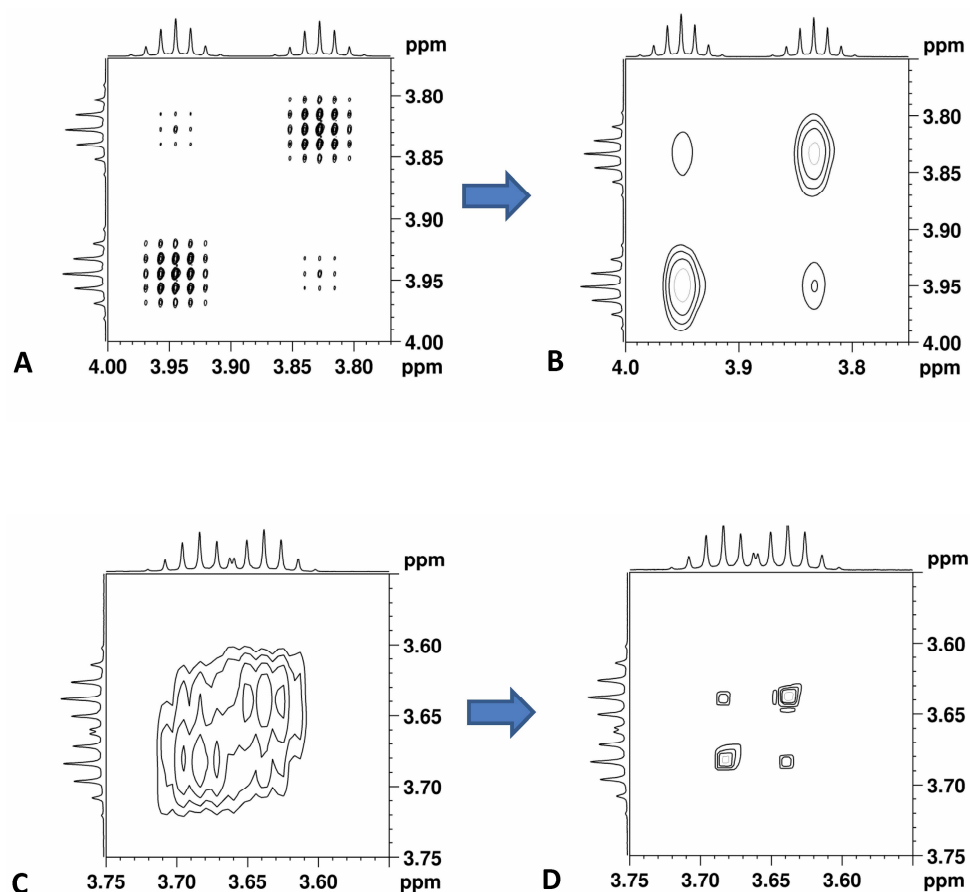
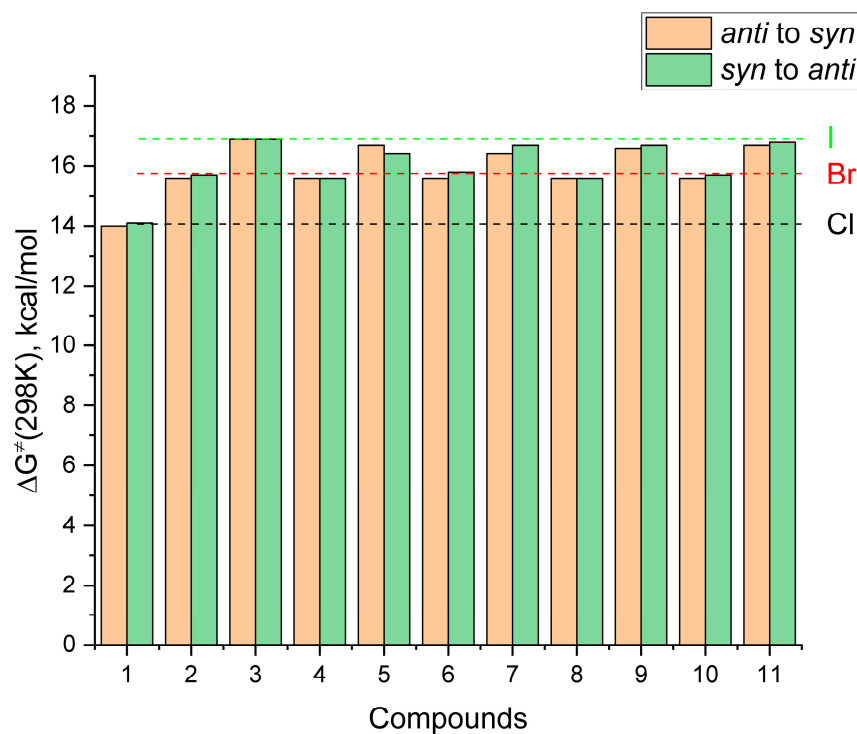


Figure 4. Comparison of the standard 2D ^1H – ^1H EXSY and 2D ^1H – ^1H HOBS EXSY experiments in the region of the methine proton of the isopropyl group. (A) ^1H EXSY spectrum of compound 6 in CDCl_3 at 253 K; (B) ^1H HOBS EXSY spectrum of compound 6 in CDCl_3 at 253 K; (C) ^1H EXSY spectrum of compound 3 in CDCl_3 at 253 K; (D) ^1H HOBS EXSY spectrum of compound 3 in CDCl_3 at 253 K.

Table 2. Experimental HOBS EXSY activation parameters for the *anti* to *syn* exchange process of the studied compounds in CDCl₃.

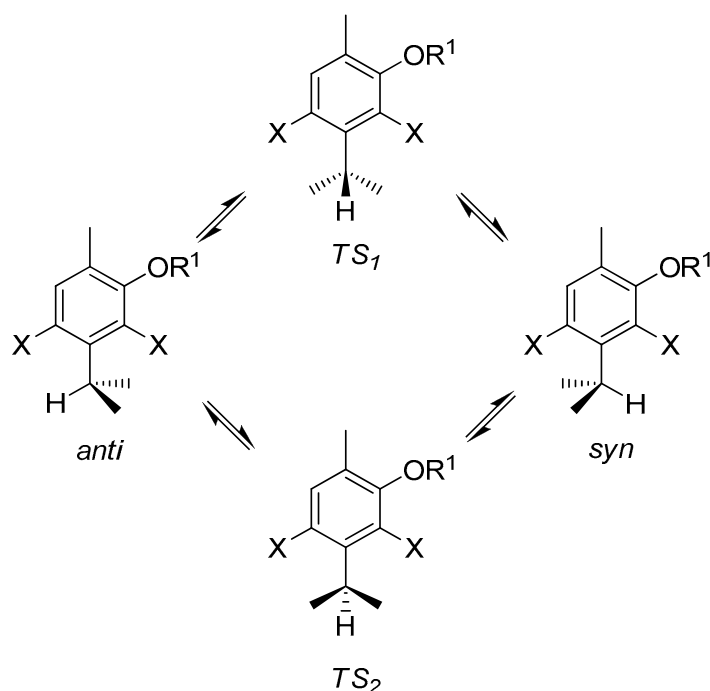
Compound	Process	X	R ¹	$\Delta H^\ddagger$ (298 K), kcal/mol	$\Delta S^\ddagger$ (298 K), e.u.	$\Delta G^\ddagger$ (298 K), kcal/mol	T Range, K
1	<i>anti</i> to <i>syn</i>	Cl	H	15.4 ± 1.0	4.2 ± 3.6	14.1 ± 0.1	233–268
	<i>syn</i> to <i>anti</i>			15.5 ± 0.7	5.0 ± 2.7	14.0 ± 0.1	
2	<i>anti</i> to <i>syn</i>	Br	H	16.2 ± 1.1	1.9 ± 3.2	15.6 ± 0.1	253–283
	<i>syn</i> to <i>anti</i>			17.5 ± 0.8	7.4 ± 3.0	15.3 ± 0.1	
3	<i>anti</i> to <i>syn</i>	I	H	12.3 ± 1.2	−15.2 ± 4.4	16.9 ± 0.1	263–293
	<i>syn</i> to <i>anti</i>			13.0 ± 1.0	−13.0 ± 3.5	16.9 ± 0.1	
4	<i>anti</i> to <i>syn</i>	Br	CH ₃	18.7 ± 1.2	10.9 ± 4.4	15.5 ± 0.1	253–273
	<i>syn</i> to <i>anti</i>			16.8 ± 1.6	3.9 ± 12.6	15.6 ± 0.1	
5	<i>anti</i> to <i>syn</i>	I	CH ₃	16.7 ± 0.9	0.0 ± 2.7	16.7 ± 0.1	263–293
	<i>syn</i> to <i>anti</i>			16.6 ± 0.9	0.0 ± 1.2	16.6 ± 0.1	
6	<i>anti</i> to <i>syn</i>	Br	Allyl	15.2 ± 0.8	−1.9 ± 2.4	15.8 ± 0.1	253–283
	<i>syn</i> to <i>anti</i>			16.3 ± 0.8	1.9 ± 2.5	15.7 ± 0.1	
7	<i>anti</i> to <i>syn</i>	I	Allyl	16.4 ± 0.9	−0.1 ± 1.4	16.4 ± 0.1	263–293
	<i>syn</i> to <i>anti</i>			16.3 ± 0.8	−1.1 ± 3.9	16.7 ± 0.1	
8	<i>anti</i> to <i>syn</i>	Br	C(=O) <i>i</i> -Pr	17.4 ± 1.1	7.2 ± 4.5	15.3 ± 0.1	263–288
	<i>syn</i> to <i>anti</i>			17.5 ± 1.4	7.6 ± 6.4	15.2 ± 0.1	
9	<i>anti</i> to <i>syn</i>	I	C(=O) <i>i</i> -Pr	18.1 ± 1.2	5.3 ± 3.7	16.6 ± 0.1	263–293
	<i>syn</i> to <i>anti</i>			17.0 ± 0.8	0.6 ± 3.2	16.8 ± 0.1	
10	<i>anti</i> to <i>syn</i>	Br	C(=O)Et	14.6 ± 0.9	−3.4 ± 2.9	15.6 ± 0.1	263–293
	<i>syn</i> to <i>anti</i>			14.8 ± 1.0	−2.9 ± 2.8	15.6 ± 0.1	
11	<i>anti</i> to <i>syn</i>	I	C(=O)Et	15.9 ± 0.8	−2.8 ± 3.1	16.7 ± 0.1	273–313
	<i>syn</i> to <i>anti</i>			15.4 ± 0.8	−4.8 ± 2.4	16.8 ± 0.1	

The results indicate that the energy barrier is strongly dependent on the size of the substituents at positions 2 and 4 and is almost independent of the derivatization of the OH group. The dichloro derivative **1** has an energy barrier of 14.0–14.1 kcal/mol, the dibromo derivatives **2**, **4**, **6**, **8** and **10** have energy barriers of 15.6–15.8 kcal/mol, and the diiodo derivatives **3**, **5**, **7**, **9** and **11** have energy barriers of 16.4–16.9 kcal/mol. The dependence of the rotational barrier of the isopropyl group on the substituents at positions 2 and 4 can be clearly seen in Figure 5.

**Figure 5.** Experimental rotational barriers of the studied compounds in CDCl₃.

2.4. DFT Studies

Further insights into the studied rotational isomerism come from DFT calculations, which were performed using SMD(CDCI₃)/B3LYP/6-311G(d,p) theory. In addition to the two ground states, two possible transition states were also located (Scheme 2). The two ground states (GSs) have almost the same energy, and the populations of the two rotamers are around 50 to 50. Therefore, the calculated GS stabilities and their respective populations cannot be used to assign the exchange signals in the NMR spectra to a particular rotamer, as we have applied this approach before [46–48]. Calculating chemical shifts is also not applicable because the difference in chemical shifts of rotamers is too small. We trusted the calculated coupling constants and the measured NOE effect (see the discussion about the conformational analysis and molecular geometry above).



Scheme 2. Ground state (*anti* and *syn*) and transition state (TS_1 and TS_2) structures of the studied carvacrols.

The localization of the two GSs and the two TSs enabled the calculation of the rotational energy barriers of the isopropyl group. The calculated thermodynamic parameters (Tables 3 and S62) effectively reproduce the experimental values.

In addition to the DFT calculations of 1–11, the same calculations were performed for carvacrol and 2,4-fluoro substituted carvacrol. The obtained results are in agreement with the expected energy barriers and with the dependence of this barrier on the steric hindrance of substituents. Steric hindrance is a consequence of steric effects. The steric properties of substituents have been evaluated in the literature via numerous methods: relative rates of chemical reactions [49], A-values (derived from equilibrium measurements of monosubstituted cyclohexanes) and others [50].

The van der Waals repulsion is related to steric hindrance. It is realized when two substituents in a molecule approach each other within a distance less than the sum of their van der Waals radii. The steric hindrance of substituents can be illustrated by the dependence of the energy barriers of compounds 1–11 measured (or calculated) from C-R bond lengths (Figure 6) or from van der Waals radii (Figure 7) of the substituents at position 2 and 4 of the studied carvacrols.

Table 3. SMD(CDCl₃)/B3LYP/6-311G(d,p) calculated activation parameters for the *anti* to *syn* exchange processes of the studied compounds (ZPE, thermal and entropy corrections are calculated using SMD(CDCl₃)/B3LYP/6-311G(d,p) theory for 298 K).

Compound	Barrier	$\Delta H^\ddagger$ (298 K)	$\Delta S^\ddagger$ (298 K)	$\Delta G^\ddagger$ (298 K)	$\Delta G^\ddagger$ (298 K) ^{eff}
1	TS1— <i>anti</i> -GS	14.4	−4.9	15.9	15.5
	TS2— <i>anti</i> -GS	14.4	−4.9	15.9	
2	TS1— <i>anti</i> -GS	16.0	−4.8	17.4	17.0
	TS2— <i>anti</i> -GS	16.0	−4.8	17.4	
3	TS1— <i>anti</i> -GS	17.1	−4.9	18.6	18.1
	TS2— <i>anti</i> -GS	17.1	−4.9	18.6	
4	TS1— <i>anti</i> -GS	15.6	−4.3	16.9	16.7
	TS2— <i>anti</i> -GS	16.2	−4.5	17.5	
5	TS1— <i>anti</i> -GS	16.3	−3.5	17.3	17.3
	TS2— <i>anti</i> -GS	17.1	−4.5	18.4	
6	TS1— <i>anti</i> -GS	16.1	−3.8	17.2	16.0
	TS2— <i>anti</i> -GS	15.5	−2.2	16.1	
7	TS1— <i>anti</i> -GS	17.2	−4.7	18.6	17.6
	TS2— <i>anti</i> -GS	16.4	−4.4	17.7	
8	TS1— <i>anti</i> -GS	15.5	−4.2	16.7	16.7
	TS2— <i>anti</i> -GS	16.3	−5.7	18.0	
9	TS1— <i>anti</i> -GS	16.0	−2.8	16.9	17.0
	TS2— <i>anti</i> -GS	17.4	−2.5	18.2	
10	TS1— <i>anti</i> -GS	15.6	−4.2	16.9	16.7
	TS2— <i>anti</i> -GS	16.2	−4.3	17.4	
11	TS1— <i>anti</i> -GS	16.5	−3.3	17.5	17.4
	TS2— <i>anti</i> -GS	17.3	−4.5	18.7	
12	TS1— <i>anti</i> -GS	6.2	−5.8	8.0	7.6
	TS2— <i>anti</i> -GS	6.2	−5.8	8.0	
13	TS1— <i>anti</i> -GS	1.9	−7.1	4.0	3.6
	TS2— <i>anti</i> -GS	1.9	−7.1	4.0	

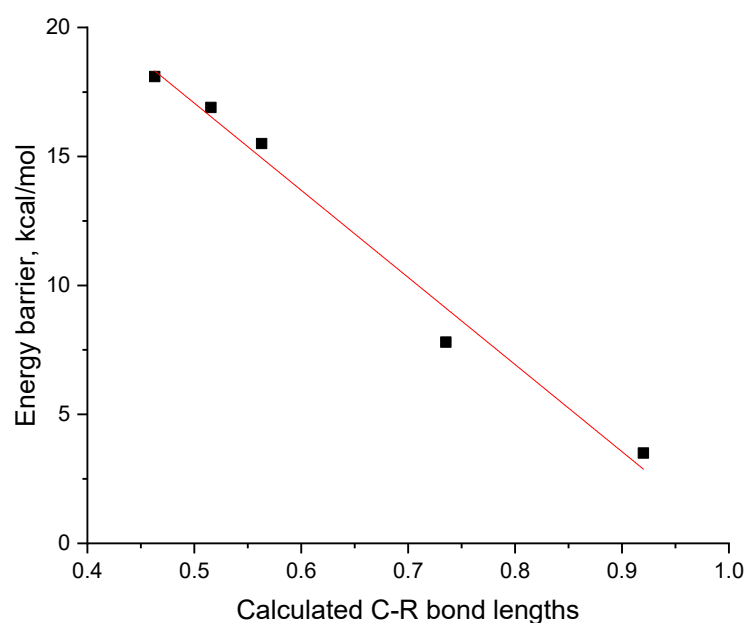


Figure 6. Dependence of the energy barriers of compounds 1–11 measured (or calculated) from the calculated bond lengths of substituents at positions 2 and 4 of the studied carvacrols.

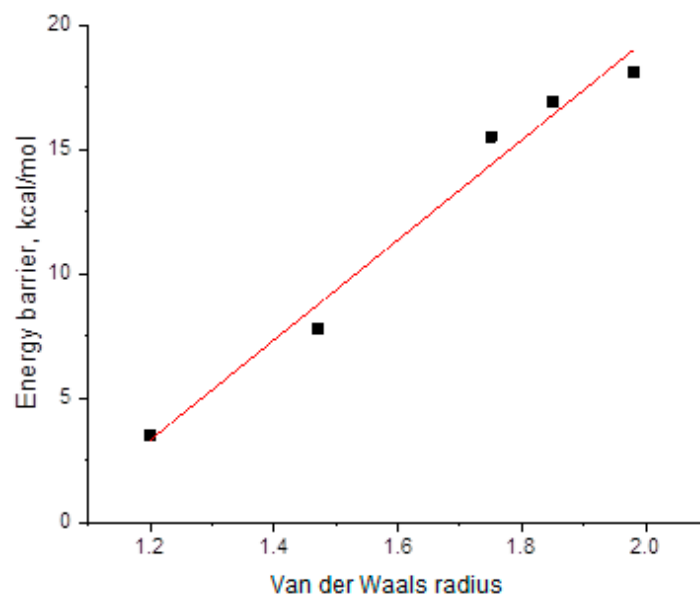


Figure 7. Dependence of the energy barriers of compounds **1–11** measured (or calculated) from the van der Waals radii of substituents at positions 2 and 4 of studied carvacrols.

During the rotation around the sp^3 - sp^2 bond of the isopropyl group, there is a change in the length of this bond. The *i*-Pr-C bond is elongated by 0.028 Å in the di-Cl compound, by 0.026 Å in di-Br compounds, by 0.022 Å in diiodo compounds, by 0.020 Å in the difluoro compound and by only 0.011 Å in carvacrol. This extension of the *i*-Pr-C bond lengths does not correlate with the steric properties of substituents, but results from non-bonding interactions with the substituents at positions 2 and 4.

3. Materials and Methods

3.1. General Information

All chemicals were commercially available and used as received (Merck, Darmstadt, Germany; Fluka, Deutschland, Germany), except for the solvents, which were purified via distillation. All reactions were carried out in oven-dried glassware under an inert atmosphere of nitrogen. Column chromatography was performed using silica gel 60 with a particle size distribution of 0.02–0.045 mm (Carl Roth, Karlsruhe, Germany). Also, preparative separation was carried out on a Sephadex LH-20 (Merck, Darmstadt, Germany) column with a length of 50 cm (diameter 1.5 cm) using a mixture of chloroform and methanol in a ratio of 1:1 (*v/v*) as the eluent. Thin-layer chromatography (TLC) was conducted on aluminum plates precoated with silica gel and fluorescence indicator F₂₅₄, with a layer thickness of 0.2 mm (Merck, Darmstadt, Germany). Visualization was achieved with UV light (254 nm) and by spraying the plates with a mixture of nitric and sulfuric acids (1:1), followed by brief heating at 110 °C. IR measurements (ATR-attenuated total reflectance) were performed using an FT-IR instrument, model 6700 (Thermo Nicolet, Waltham, MA, USA). UV spectra (in acetonitrile) were obtained using a UV-1800 spectrophotometer (Shimadzu, Tokyo, Japan). Microanalyses of carbon and hydrogen were carried out with a Carlo Erba Elemental Analyzer model 1106 (Carlo Erba Strumentazione, Inverio Italy). High-resolution mass spectrometry (HRMS) analyses were conducted using a JEOL MStation JMS-700 mass spectrometer (Akishima, Japan) with an ionization energy of 70 eV, an ionization trap current of 300 µA, and a source temperature of 230 °C. The error for each elemental composition data is given in units of atomic mass units (amu).

3.2. GC and GC-MS Analyses

GC-MS analyses were performed on a Hewlett-Packard 6890N gas chromatograph equipped with a fused silica capillary column DB-5MS (5% phenylmethylsiloxane,

30 m $\times$ 0.25 mm, film thickness 0.25 μ m, Agilent Technologies, Palo Alto, CA, USA) and coupled with a 5975B mass selective detector from the same company. The injector and interface were operated at 250 $^{\circ}$ C and 320 $^{\circ}$ C, respectively. The oven temperature was raised from 70 $^{\circ}$ C to 310 $^{\circ}$ C at a heating rate of 5 $^{\circ}$ C min $^{-1}$ and then isothermally held for 30 min. As a carrier gas, He at 1.0 mL min $^{-1}$ was used. Samples (1 μ L of the corresponding solutions in Et $_2$ O, 1 mg per 1 mL) were injected in split mode (the flow was 1.5 mL min $^{-1}$ for the first 0.5 min and then set to 1.0 mL min $^{-1}$ throughout the rest of the analysis: split ratio, 40:1). MS conditions included an ionization voltage of 70 eV, an acquisition mass range 35–650 amu, and a scan time 0.32 s. AMDIS software (version 2.73) was used for chromatogram deconvolution, and mass spectral libraries (Wiley 7, NIST 14, MassFinder 2.3, and Adams library [51]) were searched with NIST MS Search software (version 3.0).

3.3. Initial NMR Measurements at Room Temperature

Routine 1 H and 13 C NMR spectra were recorded on a Bruker Avance III spectrometer (Bruker, Ettlingen, Germany) operating at 400 and 100.6 MHz, respectively. Two-dimensional experiments (NOESY and gradient 1 H– 1 H COSY, HSQC, HMBC), as well as DEPT-90, DEPT-135, and selective 1 H homonuclear decoupling measurements, were run on same instrument with the built-in Bruker pulse sequences. All NMR spectra were measured at 25 $^{\circ}$ C in CDCl $_3$ with (CH $_3$) $_4$ Si as internal standard. Chemical shifts were reported as (δ) in parts per million (ppm) with respect to (CH $_3$) $_4$ Si, and coupling constants *J* values are given in Hertz. The following abbreviations were used to designate multiplicities: s, singlet; d, doublet; dd, doublet of doublets; ddd, doublet of doublets of doublets; dt, doublet of triplets; dq, doublet of quartets; sept, septet.

3.4. General Procedure for Preparation

3.4.1. Chlorination of Carvacrol

Carvacrol (5 g, 33 mmol), manganese sulphate (4.98 g, 33 mmol), and conc. hydrochloric acid (6.69 mL, 80 mmol) were added to water in a three-neck flask equipped with a reflux condenser (Merck, Darmstadt, Germany). The mixture was heated and stirred in an oil bath. Then, H $_2$ O $_2$ (10.47 mL, 92 mmol of a 30% aqueous solution) was added dropwise during the reaction. After the reaction was complete, the mixture was allowed to stir for 1.5 h at room temperature, resulting in the formation of a distinct organic phase separated from the aqueous solution at the bottom. After that, the reaction mixture was extracted three times with diethyl ether. The combined organic extracts were dried over anhydrous MgSO $_4$ and concentrated in vacuo. The crude chlorination products were purified via gradient flash dry column chromatography (eluent: *n*-hexane–diethyl ether (*v/v*)) to give the desired compound **1**.

3.4.2. Bromination of Carvacrol

Bromine solution (0.639 g, 4.0 mmol) in methanol (5 mL) was slowly added drop by drop to a stirred solution of carvacrol (0.50 g, 3.3 mmol) in methanol (10 mL) with added KOH (0.559 g, 9.9 mmol). The mixture was stirred for additional 30 min, then concentrated in vacuo. The resulting reaction mixture was poured into water, neutralized with aqueous HCl (1:1, *v/v*), and extracted with diethyl ether. The organic layer was washed with an aqueous Na $_2$ S $_2$ O $_3$ solution (10%) and dried over anhydrous MgSO $_4$, and the solvent was removed using a rotary evaporator. The resulting residue was purified via gradient dry flash column chromatography (*n*-hexane–diethyl ether mixtures of increasing polarity (*v/v*)). The first two fractions of the mixture, which contained 2,4-dibromo-3-isopropyl-6-methylphenol and 2-bromo-3-isopropyl-6-methylphenol, were combined and further subjected to chromatography on Sephadex LH-20 (Merck, Darmstadt, Germany) using a mixture of MeOH and CHCl $_3$ at a 1:1 (*v/v*) ratio.

3.4.3. Iodination of Carvacrol

Five grams (33 mmol) of carvacrol was dissolved in a solution of 1.2 g (30 mmol) of sodium hydroxide in 20 mL of water. While stirring on a magnetic stirrer, a solution containing 6.0 g of iodine (23.6 mmol) and 9.0 g (54 mmol) of potassium iodide dissolved in 10 mL of water was added dropwise. The decolorization of the reaction mixture was considered to be the end of the reaction. The reaction mixture was neutralized with hydrochloric acid (1:1, v/v) and extracted with diethyl ether, and the combined organic layers were washed with aqueous sodium thiosulfate solution (10%). The organic layer was dried and concentrated in vacuo.

The obtained mixture (6.67 g) was initially fractionated via gradient dry-flash column chromatography (*n*-hexane–diethyl ether mixtures of increasing polarity (*v/v*)) on silica gel. The fractions were pooled based on thin-layer chromatography (1% diethyl ether in *n*-hexane (*v/v*)). Based on GC–MS analysis, fraction I (pure *n*-hexane was used as the eluent) contained a mixture of 3-isopropyl-2,4-diiodo-6-methylphenol and 3-isopropyl-2-iodo-6-methylphenol (*o*-iodocarcavacrol), so it was further separated on Sephadex LH-20. After several re-chromatographies on Sephadex LH-20 with a mixture of MeOH and CHCl₃ at a 1:1 (*v/v*) ratio, pure compounds were obtained, which were further used for the synthesis of diiodocarcavacrol derivatives.

3.4.4. Etherification of Compounds 2 and 3

Either phenol 2 or 3 (0.174 mmol) was added to a suspension of anhydrous potassium carbonate (48 mg, 0.350 mmol) in acetone (5 mL). Methyl iodide (25 mg, 0.178 mmol) or allyl bromide (22 mg, 0.178 mmol) was then added, and the mixture was stirred for 24 h at room temperature. After that, acetone was evaporated under reduced pressure, and water was added. The resulting reaction mixture was extracted three times with diethyl ether. The combined extracts were dried and evaporated. The crude methylation product was purified via gradient silica gel column chromatography (eluent: *n*-hexane–diethyl ether (*v/v*)) to give the desired compounds 4, 5, 6, and 7.

3.4.5. Esterification of Compounds 2 and 3

A solution containing phenols 2 or 3 (0.075 mmol), 4-(dimethylamino)pyridine (DMAP, 1.2 mg, 0.01 mmol), *N,N'*-dicyclohexylcarbodiimide (DCC, 16.9 mg, 0.082 mmol), and either propanoic acid (6 mg, 0.082 mmol) or 2-methylpropanoic acid (7.2 mg, 0.082 mmol) in dry CH₂Cl₂ (10 mL) was stirred overnight at room temperature. For the workup, silica gel (5 g) was added to the resulting suspension. Dichloromethane was then removed under vacuum, and the resulting residue was subjected to purification using gradient silica gel column chromatography (eluent: *n*-hexane–diethyl ether (*v/v*)), yielding the desired esters 8, 9, 10, and 11.

3.5. Dynamic NMR Studies

¹H spectra (0.1 M in 0.6 mL CDCl₃) were recorded on a Bruker II+ 600 instrument (BBO probe) at 600.13 MHz in steps of 5 K (for T ranges refer to Table 1). Temperature calibration was carried out with a B-VT 3000 unit (it was checked and calibrated with methanol and ethylene glycol reference samples). ¹H NMR spectra were acquired using a spectral width of 10 kHz, an acquisition time of 1.7 s, and 32 scans, zero-filled to 64 k datapoints (0.15 Hz per point) and processed without apodization. Peaks were fitted to a Lorentzian lineshape. ¹H EXSY spectra were recorded on a BBO probe in steps of 5 K (for T ranges refer to Table 1). The spectra were acquired using a spectral width of 1.2 kHz, 2048 × 256 complex time domain data points, mixing times in the range of 0.03 to 0.3 s, and 2 scans in about 45 min. Linear prediction (32 coefficients and 256 points) in F1 was applied. The spectra were zero-filled to 4096 × 4096 data points and processed with a shifted square sine bell apodization in both dimensions. The populations were obtained via the integration of 1D ¹H signals, and the exchange rates were calculated using the program

EXSYCalc (MestreLab Research S.L., Santiago de Compostela, Spain)) from diagonal- and crosspeak integrals.

Errors analysis: Usually, the presented errors in the activation parameters are the statistical errors based on scattering of the data points around the Eyring straight line only. The errors in this analysis are due to inaccuracies in both the calculated rate constants, k , and the measured temperatures, T , and are computed according to the error propagation equations of Binsch [52] and Heinzer and Oth [53], in which errors due to both the calculated rate constants and the measured temperature are taken into account. The absolute temperature errors were assumed to be $\sigma T = \pm 0.5$ K, and the maximum relative error in rate constants was taken to be $\sigma k/k = \pm 10\%$.

3.6. HOmodecoupled Band-Selective NMR Experiments (HOBS)

^1H HOBS EXSY spectra were recorded using hobs_noesy pulse program (refer to Section III. Pulse program codes for the HOBS-EXSY experiments) on a BBO probe in steps of 5 K typically between 233 and 313 K. The spectra were acquired using a spectral width of 2.4 kHz, 1024×128 complex time domain data points, mixing times in the range of 0.03 to 1.0 s, and 8 scans in about 45 min. Linear prediction (32 coefficients and 256 points) in F1 was applied. The spectra were zerofilled to 4096×4096 data points and processed with a shifted square sine bell apodization in both dimensions. The populations were obtained via the integration of 1D ^1H signals, and the exchange rates were calculated using the program EXSYCalc (MestreLab Research S.L.) from diagonal- and crosspeak integrals.

3.7. NOE Experiments

Selective NOE ^1H spectra were recorded on a Bruker NEO 600 instrument (prodigy BBO probe) at 600.18 MHz at the lower measured temperature (0.1 M in 0.6 mL CDCl_3). ^1H NMR spectra were acquired using the *selnpg* pulse program, an 80 ms selective Gaussian pulse, a mixing time of 1 s, a spectral width of 10 kHz, an acquisition time of 4.6 s, and 256 scans, zerofilled to 64 k datapoints (0.15 Hz per point) and processed with EM (LB=1) apodization.

3.8. DFT Calculations

All calculations were performed by means of quantum chemical calculations at the density functional theory (DFT) level using the Gaussian09 program package [54]. The geometries of all compounds were fully optimized, and the corresponding transition states were localized using B3LYP [55] functional with a 6-311++G(d,p) basis set [56]. Solvent effect was included implicitly in the optimizations via the SMD [57] model with the built-in parameters for the solvent (CDCl_3). The nature of all critical points was confirmed by means of the vibrational analysis. The ΔH , ΔS , and ΔG values were calculated at $T = 298.15$ K at the same level of theory including zero-point energy in the particular solvent environment (represented by relative permittivity) and vibrational, rotational, and translational thermal energy corrections.

The NMR coupling constants of two rotamers of the studied compounds were calculated using the GIAO approximation [58,59] at the GIAO/B3LYP/6-311++G**-J computational level. The test calculations of the coupling constants [60–63] of two rotamers of carvacrol were performed using the GIAO/B3LYP/aug-cc-pVTZ-J [37,38] and GIAO/B3LYP/6-311++G**-J [39] methods. Recently, a 6-311++G**-J basis was introduced and shown on test compounds to be very accurate for calculating coupling constants [39].

4. Conclusions

The eleven (eight new) derivatives of 2,4-dihalogeno-substituted synthetic analogues of the natural compound carvacrol were synthesized and studied by dynamic NMR spectroscopy and DFT calculations. By selective NOE experiments and irradiating the CH signals of the isopropyl group, the individual conformers were assigned as *syn* and *anti*. By comparing GIAO/B3LYP/6-311++G(d,p)-calculated and experimentally measured vicinal $^3J_{\text{CH}}$ spin-spin constants, this assignment was confirmed.

The conformational mobility of carvacrols was studied by 1D and 2D NOESY and ROESY spectra. The application of the homonuclear decoupling technique (HOBS) to these spectra simplifies the spectra, improves resolution without reducing the sensitivity, and allows a systematic examination of the rotational barrier of all compounds by their CH signals of the isopropyl group in a wider temperature interval. The rate constants of the isopropyl rotation between *syn* and *anti* conformers were determined, and the corresponding energy barriers (14–17 kcal/mol) were calculated. The DFT calculations of the energy barriers in carvacrol derivatives allowed the determination of the steric origin of the restricted isopropyl rotation. The barrier values mainly depend on the size of the 2- and 4-position substituents, and are almost independent of the derivatization of the OH group.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules29091962/s1>, Synthesis and characterization data and NMR for compounds 1–11. Variable-temperature NMR spectra of compounds 1–11 with assignment of *anti* and *syn* rotamers using $^3J_{(C-2,CH)}$ and $^3J_{(C-4,CH)}$ coupling constants and NOE effects, analysis of 1H EXSY and HOBS EXSY spectra, pulse program codes for the HOBS-EXSY experiments, DFT optimized structures of the two ground state conformations of 1–13, optimized TS1 and TS2 transition state structures for compounds 1–13, and theoretical predictions of coupling constants in carvacrol. References [37–39,51–63] are cited in the Supplementary Materials.

Author Contributions: Conceptualization, N.S.R. and N.G.V.; methodology, N.S.R. and N.G.V.; formal analysis, M.R.Đ.Z. and M.D.; investigation, M.R.Đ.Z. and M.D.; resources, N.S.R. and N.G.V.; writing—original draft preparation, M.R.Đ.Z. and M.D.; writing—review and editing, N.S.R. and N.G.V.; supervision, N.S.R. and N.G.V.; funding acquisition, N.S.R. and N.G.V. All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Ministry of Science, Technological Development and Innovations of the Republic of Serbia (Contract Numbers 451-03-65/2024-03/200124 and 451-03-66/2024-03/200124), and by the Bulgarian Ministry of Education and Science under the National Research Programme “Young scientists and postdoctoral students” approved by DCM No 577/17.08.2018.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data are available within the article and Supplementary Materials.

Acknowledgments: Research equipment of Distributed Research Infrastructure INFRAMAT, part of the Bulgarian National Roadmap for Research Infrastructures, supported by the Bulgarian Ministry of Education and Science, was used in this investigation. We acknowledge the provided access to the e-infrastructure of the NCHDC—part of the Bulgarian National Roadmap on RIs, with financial support given by the Grant No. D01-168/28.07.2022.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Cheng, J.K.; Xiang, S.-H.; Li, S.; Ye, L.; Tan, B. Recent Advances in Catalytic Asymmetric Construction of Atropisomers. *Chem. Rev.* **2021**, *121*, 4805–4902. [[CrossRef](#)] [[PubMed](#)]
2. Kumarasamy, E.; Raghunathan, R.; Sibi, M.P.; Sivaguru, J. Nonbiaryl and Heterobiaryl Atropisomers: Molecular Templates with Promise for Atropselective Chemical Transformations. *Chem. Rev.* **2015**, *115*, 11239–11300. [[CrossRef](#)] [[PubMed](#)]
3. Smyth, J.E.; Butler, N.M.; Keller, P.A. A Twist of Nature—The Significance of Atropisomers in Biological Systems. *Nat. Prod. Rep.* **2015**, *32*, 1562–1583. [[CrossRef](#)] [[PubMed](#)]
4. Lanman, B.A.; Parsons, A.T.; Zech, S.G. Addressing Atropisomerism in the Development of Sotorasib, a Covalent Inhibitor of KRAS G12C: Structural, Analytical, and Synthetic Considerations. *Acc. Chem. Res.* **2022**, *55*, 2892–2903. [[CrossRef](#)]
5. LaPlante, S.R.; Fader, L.D.; Fandrick, K.R.; Fandrick, D.R.; Huckle, O.; Kemper, R.; Miller, S.P.F.; Edwards, P.J. Assessing Atropisomer Axial Chirality in Drug Discovery and Development. *J. Med. Chem.* **2011**, *54*, 7005–7022. [[CrossRef](#)]
6. LaPlante, S.R.; Edwards, P.J.; Fader, L.D.; Jakalian, A.; Huckle, O. Revealing atropisomer axial chirality in drug discovery. *ChemMedChem* **2011**, *6*, 505–513. [[CrossRef](#)] [[PubMed](#)]
7. Toenjes, S.T.; Gustafson, J.L. Atropisomerism in medicinal chemistry: Challenges and opportunities. *Future Med. Chem.* **2018**, *10*, 409–422. [[CrossRef](#)]

8. Chandross, E.A.; Sheley, C.F., Jr. Some 9-aryl fluorenes. Ring-current effects on nuclear magnetic resonance spectra, carbonium ions, and the 9-mesitylfluorenyl radical. *J. Am. Chem. Soc.* **1968**, *90*, 4345–4354. [\[CrossRef\]](#)
9. Lomas, J.S.; Dubois, J.-E. Conformational isomerism in *o*-tolyl-di-*tert*-butylcarbinol. *J. Org. Chem.* **1976**, *41*, 3033–3034. [\[CrossRef\]](#)
10. Lomas, J.S.; Luong, P.K.; Dubois, J.-E. Nucleophilic addition of *o*-tolyl-lithium compounds to di-*tert*-butyl ketone. Thermal and organolithium-catalyzed isomerization of *o*-tolyl-di-*tert*-butylcarbinol rotamers. *J. Org. Chem.* **1977**, *42*, 3394–3399. [\[CrossRef\]](#)
11. Casarini, D.; Lunazzi, L.; Mazzanti, A. Conformational Studies by Dynamic Nuclear Magnetic Resonance. 59. (1) Stereodynamics of Conformational Enantiomers in the Atropisomers of Hindered Naphthylcarbinols. *J. Org. Chem.* **1997**, *62*, 3315–3323. [\[CrossRef\]](#) [\[PubMed\]](#)
12. Casarini, D.; Lunazzi, L.; Mancinelli, M.; Mazzanti, A. Conformation and Stereodynamics of Symmetrically *Ortho*-Disubstituted Aryl Carbinols and Aryl Ethers. *J. Org. Chem.* **2007**, *72*, 998–1004. [\[CrossRef\]](#) [\[PubMed\]](#)
13. Mori, T.; Ōki, M. Restricted Rotation Involving the Tetrahedral Carbon. XXXVIII. Barriers to Rotation and Population Distributions of 9-(8-Methyl-1-naphthyl)fluorene and Its 1-Methyl Derivative. *Bull. Chem. Soc. Jpn.* **1981**, *54*, 1199–1202. [\[CrossRef\]](#)
14. Nakamura, M.; Ōki, M. Restricted Rotation Involving the Tetrahedral Carbon. XXXIII. Restricted Rotation about a Csp^3 – Csp^2 Bond in 10,10-Disubstituted 9-(2,6-Xylyl)-9,10-dihydroanthracene Derivatives. *Bull. Chem. Soc. Jpn.* **1980**, *53*, 2977–2980. [\[CrossRef\]](#)
15. Berber, H.; Lameiras, P.; Denhez, C.; Antheaume, C.; Clayden, J. Atropisomerism about Aryl– Csp^3 Bonds: The Electronic and Steric Influence of *ortho*-Substituents on Conformational Exchange in Cannabidiol and Linderatin Derivatives. *J. Org. Chem.* **2014**, *79*, 6015–6027. [\[CrossRef\]](#)
16. Flos, M.; Lameiras, P.; Denhez, C.; Mirand, C.; Berber, H. Atropisomerism about Aryl– $C(sp^3)$ Bonds: Conformational Behavior of Substituted Phenylcyclohexanes in Solution. *J. Org. Chem.* **2016**, *81*, 2372–2382. [\[CrossRef\]](#) [\[PubMed\]](#)
17. Eto, M.; Yamaguchi, K.; Shinohara, I.; Ito, F.; Yoshitake, Y. Conformation of aromatic rings in isolable atropisomers of 2-arylindoline derivatives and kinetic evidences for π – π interaction. *Tetrahedron* **2010**, *66*, 898–903. [\[CrossRef\]](#)
18. Toda, Y.; Kooguchi, A.; Sukegawa, K.; Kikuchi, A.; Suga, H. Ring-fused hexahydro-1,2,4,5-tetrazines: Synthesis, structure, and mechanistic studies on isolable rotational isomers. *Chem. Commun.* **2023**, *59*, 700–703. [\[CrossRef\]](#) [\[PubMed\]](#)
19. Li, S.-G.; Wang, Y.-T.; Zhang, Q.; Wang, K.-B.; Xue, J.-J.; Li, D.-H.; Jing, Y.-K.; Lin, B.; Hua, H.-M. Pegaharmols A–B, Axially Chiral β -Carboline-quinazoline Dimers from the Roots of *Peganum harmala*. *Org. Lett.* **2020**, *22*, 7522–7525. [\[CrossRef\]](#)
20. Isaka, M.; Tanticharoen, M.; Kongsaree, P.; Thebtaranonth, Y. Structures of cordypyridones A–D, antimalarial *N*-hydroxy- and *N*-methoxy-2-pyridones from the insect pathogenic fungus *Cordyceps nipponica*. *J. Org. Chem.* **2001**, *66*, 4803–4808. [\[CrossRef\]](#)
21. Jones, I.L.; Moore, F.K.; Chai, C.L.L. Total Synthesis of ($\pm$)-Cordypyridones A and B and Related Epimers. *Org. Lett.* **2009**, *11*, 5526–5529. [\[CrossRef\]](#) [\[PubMed\]](#)
22. Delaye, P.-O.; Lameiras, P.; Kervarec, N.; Mirand, C.; Berber, H. Improved Enantioselective Synthesis of (–)-Linderol A: Hindered Rotation about Aryl– Csp^3 Bond. *J. Org. Chem.* **2010**, *75*, 2501–2509. [\[CrossRef\]](#) [\[PubMed\]](#)
23. He, Q.-F.; Wu, Z.-L.; Huang, X.-J.; Xia, T.-Q.; Tang, G.; Tang, W.; Shi, L.; Ye, W.-C.; Wang, Y. Cajanusoids A–D, Unusual Atropisomeric Stilbene Dimers with PTP1B Inhibitory Activities from the Leaves of *Cajanus cajan*. *J. Org. Chem.* **2021**, *86*, 5870–5882. [\[CrossRef\]](#)
24. He, Q.-F.; Wu, Z.-L.; Huang, X.-J.; Zhong, Y.-L.; Li, M.-M.; Jiang, R.-W.; Li, Y.-L.; Ye, W.-C.; Wang, Y. Cajanusflavanols A–C, Three Pairs of Flavonostilbene Enantiomers from *Cajanus cajan*. *Org. Lett.* **2018**, *20*, 876–879. [\[CrossRef\]](#) [\[PubMed\]](#)
25. EAFUS. A Food Additive Database Centre for Food Safety Applied Nutrition; Food, and Drug Administration: Washington, DC, USA, 2006.
26. European Parliament and Council. Regulation (EC) No 2232/96 the European Parliament, and of the Council on 28 October 1996, Commission Decision of 23 February 1999 adopting a register of flavouring substances used in or on foodstuffs. *Off. J. Eur. Commun.* **1996**, *39*, 1–37.
27. Hyldgaard, M.; Mygind, T.; Meyer, R.L. Essential Oils in Food Preservation: Mode of Action, Synergies, and Interactions with Food Matrix Components. *Front. Microbiol.* **2012**, *3*, 12. [\[CrossRef\]](#) [\[PubMed\]](#)
28. Langeveld, W.T.; Veldhuizen, E.J.A.; Burt, S.A. Synergy between essential oil components and antibiotics: A review. *Crit. Rev. Microbiol.* **2014**, *40*, 76–94. [\[CrossRef\]](#) [\[PubMed\]](#)
29. De Oliveira, A.S.; Llanes, L.C.; Brighente, I.M.C.; Nunes, R.J.; Yunes, R.A.; Máximo, N., Jr.; Baumgart, A.M.K.; Aust, A.N.; Cruz, A.B. New Sulfonamides Derived from Carvacrol: Compounds with High Antibacterial Activity against Resistant *Staphylococcus aureus* Strains. *J. Biosci. Med.* **2016**, *4*, 105–114. [\[CrossRef\]](#)
30. Bansal, A.; Saleh-E-In, M.; Kar, P.; Roy, A.; Sharma, N.R. Synthesis of Carvacrol Derivatives as Potential New Anticancer Agent against Lung Cancer. *Molecules* **2022**, *27*, 4597. [\[CrossRef\]](#)
31. Cocolas, A.H.; Parks, E.L.; Ressler, A.J.; Havasi, M.H.; Seeram, N.P.; Henry, G.E. Heterocyclic β -keto sulfide derivatives of carvacrol: Synthesis and copper (II) ion reducing capacity. *Bioorg. Med. Chem. Lett.* **2019**, *29*, 126636. [\[CrossRef\]](#)
32. Cocker, W.; Shannon, P.V.R.; Dowsett, M. The chemistry of terpenes. Part 27. The halogenation of (+)-thujone and of (–)-carvotanacetone, and the stereochemistry and mechanism of formation of ‘tribromothujone’. *J. Chem. Soc. Perkin Trans.* **1988**, *1*, 1527–1535. [\[CrossRef\]](#)
33. Miller, B.; Haggerty, J.G. Effects of halogen substitution on reactions of *o*-quinol acetates with isopropylmagnesium bromide and diisopropylmagnesium. Competition between unimolecular decomposition and bimolecular reactions of radical anions. *J. Org. Chem.* **1986**, *51*, 174–179. [\[CrossRef\]](#)
34. Soderberg, B.C.; Fields, S.L. Expedient syntheses of espintanol, *p*-methoxycarvacrol and thymoquinone dimethyl ether. *Org. Prep. Proced. Int.* **1996**, *28*, 221–225. [\[CrossRef\]](#)

35. Majetich, G.; Grove, J.L. Total Synthesis of (+)-19-Deoxyicetexone, (–)-Icetexone, and (+)-5-Epi-icetexone. *Org. Lett.* **2009**, *11*, 2904–2907. [[CrossRef](#)] [[PubMed](#)]
36. Radulović, N.S.; Đorđević, M.R.; Blagojević, P.D. Structural revision of aristol: A fresh look at the oxidative coupling of thymol under iodination conditions. *RSC Adv.* **2016**, *6*, 69067–69082. [[CrossRef](#)]
37. Enevoldsen, T.; Oddershede, J.; Sauer, S.P.A. Correlated calculations of indirect nuclear spin-spin coupling constants using second-order polarization propagator approximations: SOPPA and SOPPA (CCSD). *Theor. Chem. Acc.* **1998**, *100*, 275–284. [[CrossRef](#)]
38. Provasi, P.F.; Aucar, G.A.; Sauer, S.P.A. The effect of lone pairs and electronegativity on the indirect nuclear spin-spin coupling constants in CH_2X ($\text{X}=\text{CH}_2$, NH , O , S): *Ab initio* calculations using optimized contracted basis sets. *J. Chem. Phys.* **2001**, *115*, 1324–1334. [[CrossRef](#)]
39. Kjær, H.; Sauer, S.P.A. Pople Style Basis Sets for the Calculation of NMR Spin-Spin Coupling Constants: The 6-31G-J and 6-311G-J Basis Sets. *J. Chem. Theory Comput.* **2011**, *7*, 4070–4076. [[CrossRef](#)] [[PubMed](#)]
40. Castañar, L.; Parella, T. Broadband ^1H homodecoupled NMR experiments: Recent developments, methods and applications. *Magn. Reson. Chem.* **2015**, *53*, 399–426. [[CrossRef](#)]
41. Zangger, K. Pure shift NMR. *Prog. Nucl. Magn. Reson. Spectrosc.* **2015**, *86–87*, 1–20. [[CrossRef](#)]
42. McKenna, J.M.; Parkinson, J.A. HOBBS methods for enhancing resolution and sensitivity in small DNA oligonucleotide NMR studies. *Magn. Reson. Chem.* **2015**, *53*, 249–255. [[CrossRef](#)] [[PubMed](#)]
43. Ying, J.; Roche, J.; Bax, A. Homonuclear decoupling for enhancing resolution and sensitivity in NOE and RDC measurements of peptides and proteins. *J. Magn. Reson.* **2014**, *241*, 97–102. [[CrossRef](#)] [[PubMed](#)]
44. Ilgen, J.; Nowag, J.; Kaltschnee, L.; Schmidts, V.; Thiele, C.M. Gradient selected pure shift EASY-ROESY techniques facilitate the quantitative measurement of ^1H , ^1H -distance restraints in congested spectral regions. *J. Magn. Reson.* **2021**, *324*, 106900. [[CrossRef](#)] [[PubMed](#)]
45. Castañar, L.; Nolis, P.; Virgili, A.; Parella, T. Full Sensitivity and Enhanced Resolution in Homodecoupled Band-Selective NMR Experiments. *Chem.—Eur. J.* **2013**, *19*, 17283–17286. [[CrossRef](#)] [[PubMed](#)]
46. Dangalov, M.; Stoyanova, M.; Petrov, P.; Putala, M.; Vassilev, N.G. Fluxional Pd (II) NHC complexes—Synthesis, structure elucidation and catalytic studies. *J. Organomet. Chem.* **2016**, *817*, 1–14. [[CrossRef](#)]
47. Philipova, I.; Stavrakov, G.; Vassilev, N.; Nikolova, R.; Shivachev, B.; Dimitrov, V. Cytisine as a scaffold for *ortho*-diphenylphosphinobenzenecarboxamide ligands for Pd-catalyzed asymmetric allylic alkylation. *J. Organomet. Chem.* **2015**, *778*, 10–20. [[CrossRef](#)]
48. Philipova, I.; Stavrakov, G.; Dimitrov, V.; Vassilev, N. Galantamine derivatives: Synthesis, NMR study, DFT calculations and application in asymmetric catalysis. *J. Mol. Struct.* **2020**, *1219*, 128568. [[CrossRef](#)]
49. Smith, M.B.; March, J. *March's Advanced Organic Chemistry: Reactions, Mechanisms, and Structure*; Wiley: Hoboken, NJ, USA, 2007.
50. Eliel, E.L.; Wilen, S.H.; Mander, L.N. *Stereochemistry of Organic Compounds*; Wiley: Hoboken, NJ, USA, 1994.
51. Adams, R.P. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*; Allured Publishing Corporation: Carol Stream, IL, USA, 2007.
52. Binsch, G. *Dynamic NMR Spectroscopy*; Jackman, L.M., Cotton, F.A., Eds.; Academic Press: New York, NY, USA, 1975; pp. 45–82.
53. Heinzer, J.; Oth, J. Iterative Least-Squares Lineshape Fitting of ^1H -decoupled ^{13}C -DNMR. *Spectra. Helv. Chim. Acta* **1981**, *64*, 258. [[CrossRef](#)]
54. Frisch, M.J.; Trucks, G.W.; Schlegel, H.B.; Scuseria, G.E.; Robb, M.A.; Cheeseman, J.R.; Scalmani, G.; Barone, V.; Petersson, G.A.; Nakatsuji, H.; et al. *Gaussian*; Gaussian, Inc.: Wallingford, CT, USA, 2013.
55. Becke, A.D. Density-functional thermochemistry. III. The role of exact exchange. *J. Chem. Phys.* **1993**, *98*, 5648–5652. [[CrossRef](#)]
56. Hariharan, P.C.; Pople, J.A. The influence of polarization functions on molecular orbital hydrogenation energies. *Theoret. Chim. Acta* **1973**, *28*, 213–222. [[CrossRef](#)]
57. Marenich, A.V.; Cramer, C.J.; Truhlar, D.G. Universal solvation model based on solute electron density and on a continuum model of the solvent defined by the bulk dielectric constant and atomic surface tensions. *J. Phys. Chem. B* **2009**, *113*, 6378–6396. [[CrossRef](#)] [[PubMed](#)]
58. Wolinski, K.; Hinton, J.F.; Pulay, P. Efficient implementation of the gauge-independent atomic orbital method for NMR chemical shift calculations. *J. Am. Chem. Soc.* **1990**, *112*, 8251–8260. [[CrossRef](#)]
59. Cheeseman, J.R.; Trucks, G.W.; Keith, T.A.; Frisch, M.J. A comparison of models for calculating nuclear magnetic resonance shielding tensors. *J. Chem. Phys.* **1996**, *104*, 5497–5509. [[CrossRef](#)]
60. Helgaker, T.; Watson, M.; Handy, N.C. Analytical calculation of nuclear magnetic resonance indirect spin-spin coupling constants at the generalized gradient approximation and hybrid levels of density-functional theory. *J. Chem. Phys.* **2000**, *113*, 9402–9409. [[CrossRef](#)]
61. Sychrovský, V.; Gräfenstein, J.; Cremer, D. Nuclear magnetic resonance spin-spin coupling constants from coupled perturbed density functional theory. *J. Chem. Phys.* **2000**, *113*, 3530–3547. [[CrossRef](#)]

62. Peralta, J.E.; Scuseria, G.E.; Cheeseman, J.R.; Frisch, M.J. Basis set dependence of NMR spin–spin couplings in density functional theory calculations: First row and hydrogen atoms. *Chem. Phys. Lett.* **2003**, *375*, 452–458. [[CrossRef](#)]
63. Deng, W.; Cheeseman, J.R.; Frisch, M.J. Calculation of nuclear spin–spin coupling constants of molecules with first and second row atoms in study of basis set dependence. *J. Chem. Theory Comput.* **2006**, *2*, 1028–1037. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.