JOURNAL OF NATURAL PRODUCTS

pubs.acs.org/jnp

Article

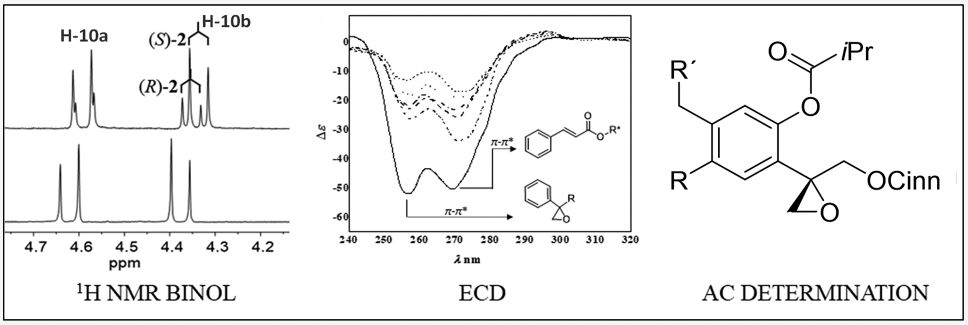
Methodology for the Absolute Configuration Determination of Epoxythymols Using the Constituents of *Piptothrix areolare*

Héctor M. Arreaga-González, Antonio J. Oliveros-Ortiz, Rosa E. del Río, Gabriela Rodríguez-García, J. Martín Torres-Valencia, Carlos M. Cerda-García-Rojas,* Pedro Joseph-Nathan, and Mario A. Gómez-Hurtado*

Cite This: *J. Nat. Prod.* 2021, 84, 707−712

Read Online

ACCESS | Metrics & More | Article Recommendations | Supporting Information

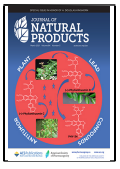


ABSTRACT: Since epoxythymols occur in Nature either as scalemic mixtures or as pure enantiomers, the knowledge of their chiral composition and of the absolute configuration (AC) of the dominant enantiomer turns out to be mandatory. This task has already been faced using 1,1-bis-2-naphthol (BINOL), as a chiral solvating agent in accurate ^{1}H NMR quantifications to determine the enantiomeric ratio, and vibrational circular dichroism (VCD) to evidence the AC of the dominant enantiomer. We now explore the use of electronic circular dichroism (ECD) to determine the AC of an epoxythymol for which time-expensive DFT calculations would be required unless the AC of a related molecule is already known, from either VCD studies or single-crystal X-ray diffraction analysis, since one could correlate the ECD Cotton effect with the AC because in ECD only chromophores and their neighborhoods are evidenced. This method is now applied by using the epoxythymols from *Piptothrix areolare*. Known areolal (**1**) and 10-cinnamoyloxy-8,9-epoxythymol isobutyrate (**2**) were isolated from the roots, while known 7-acetoxy-10-cinnamoyloxy-8,9-epoxythymol isobutyrate (**3**) and 10-cinnamoyloxy-7-hydroxy-8,9-epoxythymol isobutyrate (**4**), as well as the new enantiopure 7-acetoxy-10-cinnamoyloxy-6-hydroxy-8,9-epoxythymol isobutyrate (**5**) and 10-cinnamoyloxy-8,9-epoxy-6-hydroxy-7-*nor*thymol isobutyrate (**6**), were obtained from the extract of the flowers. Chemical correlation of epoxythymols **1** and **3** was achieved. Compounds **1**−**4** were obtained as scalemic mixtures, and **5** and **6** as the pure (8*S*) enantiomers. In addition, the new 10-cinnamoyloxy-7-oxo-8,9-dehydrothymol isobutyrate (**7**) was isolated from the roots. The structures of **5**−**7** followed from NMR and HRMS data, while enantiomeric compositions of **1**−**6** were determined by ^{1}H NMR-BINOL measurements. The AC determination for **2**−**6** was done by ECD using a sample of **1** to reference the ECD Cotton effect. In turn, the AC of **1** was determined by VCD and extensive DFT calculations. The ECD-BINOL methodology turned out to be some 500 times more sensitive than that combining VCD and ^{1}H NMR-BINOL.

Electronic circular dichroism (ECD) and vibrational circular dichroism (VCD) are optical methodologies that require circularly polarized radiation for the absolute configuration (AC) determination of organic molecules.[1] ECD uses circularly polarized light in the UV region and is highly sensitive, thus demanding small quantities of sample, but requires the presence of appropriate chromophores and only provides a limited number of broad absorption bands.[2] In contrast, VCD is based on the absorption difference of circularly polarized light by a chiral molecule in the infrared (IR) region, and therefore any chiral organic compound will provide narrow absorption bands attributed to the chirality of the molecule. However, the lower sensitivity of VCD, as compared to ECD, and the need to perform density functional theory (DFT) calculations, in typical AC determinations, limits the methodology.[3,4]

Special Issue: Special Issue in Honor of A. Douglas Kinghorn

Received: October 15, 2020
Published: March 8, 2021



Downloaded via UNIV OF CALIFORNIA SANTA BARBARA on May 15, 2021 at 17:43:42 (UTC).
See https://pubs.acs.org/sharingguidelines for options on how to legitimately share published articles.

ACS Publications

© 2021 American Chemical Society and American Society of Pharmacognosy

The occurrence of enantiomerically pure, scalemic, or racemic mixtures of epoxythymols is prevalent in Asteraceae species,[5] and these chiral variations have been related to isolation processes, degradation in plant materials,[6−8] or metabolic processes.[9,10] Thus, an easy methodology to establish the enantiomeric purity of natural epoxythymols by using 1,1-bis-2-naphtol (BINOL) as a chiral solvating agent for precise ^{1}H NMR quantification was recently described,[9] while the AC determination was done using VCD. This stereochemical aspect becomes relevant since antiproliferative,[11] antibacterial, anti-inflammatory, antioxidant, antiprotozoal, cytotoxic, piscicidal, and allelopathic[5] activities have been described for epoxythymols.

Application of an ECD-based method could be an alternative strategy for the AC determination of epoxythymols due to the presence of an aromatic chromophore. Its feasibility is herein reported using the constituents of *Piptothrix areolare*. Thus, known areolal (**1**) and 10-cinnamoyloxy-8,9-epoxythymol isobutyrate (**2**) from roots and known 7-acetoxy-10-cinnamoyloxy-8,9-epoxythymol isobutyrate (**3**) and 10-cinnamoyloxy-7-hydroxy-8,9-epoxythymol isobutyrate (**4**) as well as the new epoxthymols (8*S*)-7-acetoxy-10-cinnamoyloxy-6-hydroxy-8,9-epoxythymol isobutyrate (**5**) and (8*S*)-10-cinnamoyloxy-8,9-epoxy-6-hydroxy-7-*nor*thymol isobutyrate (**6**) from flowers (Figure 1) were studied by ECD. Also, the new 10-cinnamoyloxy-7-oxo-8,9-dehydrothymol isobutyrate (**7**) was isolated from the roots. The enantiomeric purities of **1**−**6** were determined by ^{1}H NMR-BINOL experiments as shown in Figure 2 for **1**−**4**, while epoxythymols **5** and **6** were enantiomerically pure compounds. UV and ECD data of **1**−**6** (Figure 3) revealed similar spectra for all compounds. Since the AC of (−)-(8*S*)-areolal (**1**) is known,[10] the AC of the dominant enantiomer in the **2**−**4** scalemic mixtures and of enantiopure **5** and **6** also followed to be (8*S*). Chemical correlation between **1** and **3** revealed structural similarity of epoxythymols from the roots and flowers of *P. areolare*.

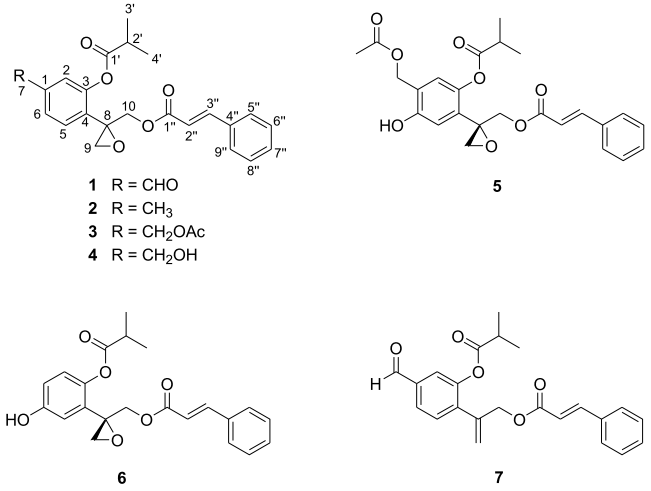


Figure 1. Formulas of epoxythymols **1**−**7** from *Piptothrix areolare*.

RESULTS AND DISCUSSION

Chromatography of the root extract of *P. areolare* afforded thymol derivatives **1**, **2**, and **7**, while the flower extract yielded epoxythymols **3**−**6** (Figure 1). Areolal (**1**) was originally described as racemic crystals,[12] and, recently, this natural product (**1**) was isolated as scalemic mixtures.[10] These variations encouraged us to study the AC of the dominant enantiomer, (−)-(8*S*)-areolal, using VCD measurements in combination with DFT calculations.[10] The NMR data of **1**−**4** were similar to the reported values.[12−14]

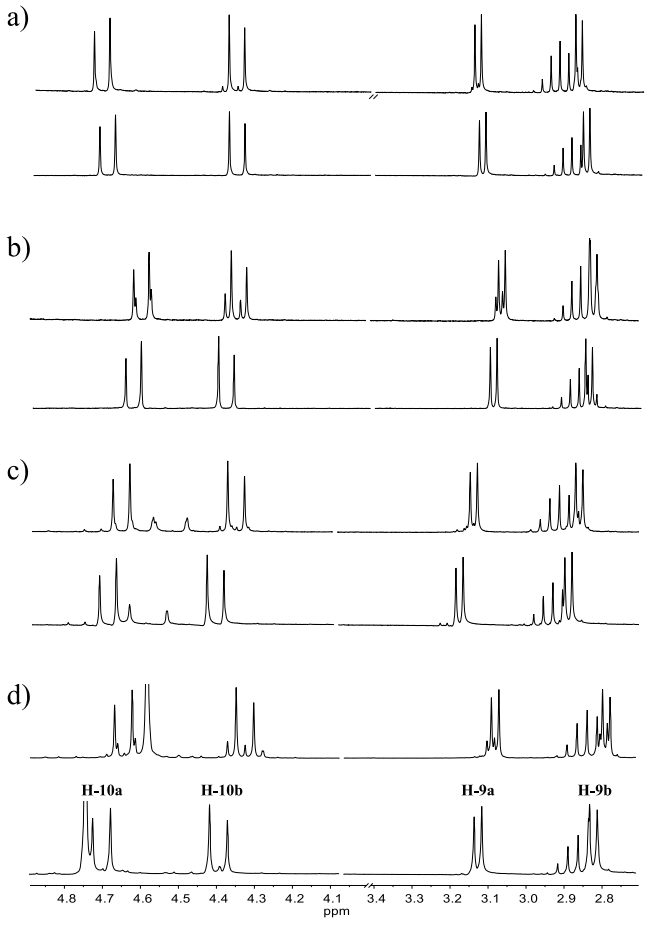


Figure 2. Comparison of the ^{1}H NMR spectra of epoxythymols **1**−**4** before (lower traces) and after (upper traces) (*S*)-BINOL addition. Enantiomeric proportions are (a) **1**, 91:9 *S*/*R*, (b) **2**, 62:38 *S*/*R*, (c) **3**, 92:8 *S*/*R*, and (d) **4**, 81:19 *S*/*R*.

The HRESIMS data of the epoxythymol analogue **5** showed an $[M + H]^+$ ion at m/z 455.1703 (calcd as 455.1700 for $C_{25}H_{26}O_8 + H^+$). Its ^{1}H NMR spectrum displayed two singlets at δ 7.03 and 6.92 for H-2 and H-5, respectively, indicative of a 1,2,4,5-tetrasubstituted aromatic ring. The typical resonances of a *trans*-cinnamate moiety were observed at δ 7.59 and 6.33 as doublets (J = 16.0 Hz) and the aromatic protons in the δ 7.44−7.31 range. An isobutyrate moiety was also revealed by the δ 2.78 (1H, sept, J = 7.0) and 1.25 (6H, d, J = 7.0 Hz) resonances. The signals of these two ester moieties are in close analogy with those of **1**−**4**, as are two pairs of methylene protons at δ 4.56 and 4.30 (J = 12.2 Hz) and at δ 3.01 and 2.77 (J = 5.2 Hz) assigned to CH_2-10 and CH_2-9, respectively. These and other data are summarized in Table 1. The ^{13}C NMR spectrum showed 23 signals including three carbonyl carbon signals at δ 175.6 (C-1′), 173.6 (Ac), and 166.3 (C-1″) and 10 aromatic carbon signals in the δ 153.2−118.1 range, of which the C-6′/C8′ (δ 128.9) and C-5″/C-9″ (δ 128.1) pairs of signals are more intense. The isobutyrate signals appeared at δ 34.1 (CH-2′), 19.0 (CH_3-3′), and 18.9 (CH_3-4′), while a signal at δ 20.9 was attributed to the acetoxy methyl group

https://dx.doi.org/10.1021/acs.jnatprod.0c01113
J. Nat. Prod. 2021, 84, 707−712

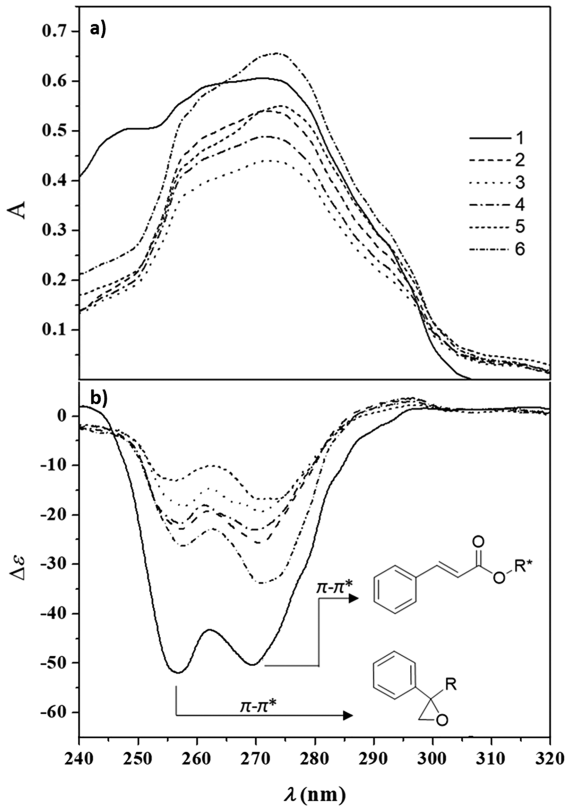


Figure 3. Comparation of (a) UV and (b) ECD spectra of epoxythymols **1**−**6** and transition assignments according to published data for analogue moieties.[21,24]

(Table 1). There is again a significant similarity of signals between **5** and **1**−**4**. Carbons attached to hydrogen atoms were verified by a HETCOR experiment, while quaternary carbons and positional assignments were done by an HMBC experiment, where the correlation between H_2-7 and the acetoxy carbonyl carbon is evident. The data of **5** particularly resembled those of **3**,[12] but are indicative of an additional hydroxy group at C-6.

The HRESIMS data of **6** showed an $[M + H]^+$ ion at m/z 383.1492 (calcd as 383.1489 for $C_{22}H_{22}O_6 + H^+$). Its ^{1}H NMR spectrum showed doublets at δ 6.90 (J = 3.0 Hz) and 6.84 (J = 8.7 Hz) and a doublet of doublets at δ 6.72 (J = 8.7, 3.0 Hz), assigned to H-5, H-2, and H-1, respectively, evidencing a trisubstituted aromatic ring. Methylene protons at δ 4.59 and 4.32 (J = 12.2 Hz) as well as at δ 3.02 and 2.78 (J = 5.2 Hz) were assigned to CH_2-10 and CH_2-9, respectively. In addition, cinnamate and isobutyrate moieties were evidenced as in the previous case (Table 1). The ^{13}C NMR spectrum showed 20 signals, of which two carbonyl carbon signals appeared at δ 175.8 (C-1′) and 166.4 (C-1″). The aromatic carbon signals appeared in the δ 153.5−115.4 range, and the cinnamate and isobutyrate signals resemble those of **1**−**5** (Table 1). The HMBC plot showed correlations of H-1 with C-3 (δ 141.9), C-5 (δ 115.4), and C-6 (δ 153.5), while H-2 correlated with C-3, C-4 (δ 130.0), and C-6, demonstrating the structure of the *nor*-epoxythymol derivative **6**.

The HRESIMS data of **7** showed an $[M + Na]^+$ ion at m/z 401.1360 (calcd as 401.1359 for $C_{23}H_{22}O_5 + Na^+$). Its ^{1}H NMR spectrum was similar to that of **1**,[12] but showed doublets at δ 5.50 (J = 1.0, H-9a) and 5.26 (J = 1.0, H-9b), indicating a vinylic methylene instead of the epoxide AB spin system. The ^{13}C NMR spectrum showed 20 signals (Table 1), with the

Table 1. NMR Data of 5−7 in $CDCl_3$ (δ in ppm, J in Hz)

	5			6			7		
position	δ_C	δ_H (J in Hz)	HMBC	δ_C	δ_H (J in Hz)	HMBC	δ_C	δ_H (J in Hz)	HMBC
1	122.6			116.4	6.72 dd (8.7, 3.0)	3, 5	137.0		
2	125.7	7.03 s	3, 6, 7	123.5	6.84 d (8.7)	3, 4, 6	130.8	7.43 m	1, 4, 6, 7
3	141.6			141.9			148.8		
4	131.8			130.0			138.9		
5	118.1	6.92 s	3, 6, 8	115.4	6.90 d (3.0)	3	123.6	7.52 d (1.5)	
6	153.2			153.5			127.2	7.69 dd (7.8, 1.5)	1, 2, 7
7	62.4	4.99 s	1, 2, 6, Ac (C)				190.8	9.92 s	
8	56.8			57.0			139.8		
9a	50.9	3.01 d (5.2)	8, 9, 10	51.0	3.02 d (5.2)	4, 8	118.9	5.50 d (1.0)	8, 10
9b		2.77 d (5.2)	4, 8, 10		2.78 d (5.2)	4, 8		5.26 d (1.0)	8, 10
10a	65.2	4.56 d (12.2)	8, 9, 1″	65.0	4.59 d (12.2)	4, 8, 9, 1″	65.8	4.9 s	8, 9, 1″
10b		4.30 d (12.2)	4, 8, 9, 1″		4.32 d (12.2)	4, 8, 9, 1″			
1′	175.6			175.8			175.1		
2′	34.1	2.78 sept (7.0)	1′, 3′, 4′	34.1	2.78 sept (7.0)	1′, 3′, 4′	34.1	2.75 sept (7.0)	1′, 3′, 4′
3′	19.0	1.25 d (7.0)	1′, 2′, 4′	19.0	1.25 d (7.0)	1′, 2′,4′	18.8	1.24 d (7.0)	1′, 2′, 4′
4′	18.9	1.25 d (7.0)	1′, 2′, 3′	18.9	1.25 d (7.0)	1′, 2′, 3′	18.8	1.24 d (7.0)	1′, 2′, 3′
1″	166.3			166.4			166.3		
2″	145.6	7.59 d (16.0)	1″, 5″, 8″	145.7	7.58 d (16.0)		145.5	7.60 d (16.0)	1″
3″	117.1	6.33 d (16.0)	1″, 4″	117.1	6.32 d (16.0)	1″, 4″	117.4	6.34 d (16.0)	4″
4″	134.1			134.1			134.2		
5″, 9″	128.1	7.31 m	4″	128.1	7.31 m		128.1	7.31 m	4″, 7″
6″, 8″	128.9	7.44 m	7″	128.9	7.43 m		128.9	7.43 m	4″, 5″, 9″
7″	130.4	7.31 m		130.5	7.31 m		130.5	7.31 m	
Ac (C)	173.6								
Ac CH_3)	20.9	2.03 s	Ac (C)						

https://dx.doi.org/10.1021/acs.jnatprod.0c01113
J. Nat. Prod. 2021, 84, 707−712

vinylic carbons resonating at δ 139.8 (C-8) and 118.9 (C-9). Comparison of the structures of **1** and **7** readily suggested that **7** could be the biosynthetic precursor of **1**.

Once the identification of the known compounds and the 2D structure elucidation of the new metabolites was completed, attention was turned to the determination of the AC of **2**–**6**. It should be kept in mind that areolal (**1**) was originally isolated as achiral crystals. To confirm its racemic nature, two ^{1}H NMR doublets of equal intensity were observed[12] for H-10b in the presence of a chiral europium shift reagent. In contrast, for the quantification of enantiomers significantly milder conditions were used since (*S*)-BINOL acts as a chiral solvating agent, which is equivalent to using a chiral solvent. The (*S*)-BINOL methodology was used for recent[9,10] epoxythymol studies; thus, its validity to define enantiomeric compositions of this type of molecule is well established. In addition, the enantiomeric mixture of epoxythymols can be recovered by column chromatography separation after NMR-BINOL quantifications.

The technique provides enantioresolution of some proton signals close to the epoxythymol stereocenter, producing sufficient ^{1}H NMR signal splitting for the determination of enantiomeric excess.[15,16] The enantiomeric excess quantification is also done by CH_2-10 signal intensity measurements from spectra determined under good magnetic homogeneity conditions, since the peak separations are too close for routine integration[9,10] (Figure 2), where **1** showed a 91:9 proportion, 82% ee,[9] as seen in traces (a). Its chemical shift splitting was similar to that reported.[10] Compound **2** exhibited a 62:38 (24% ee) proportion (traces b), **3** showed a 92:8 (84% ee) ratio (traces c), and **4** gave an 81:19 (62% ee) proportion (traces d).

For all ^{1}H NMR-BINOL measurements, the dominant enantiomer showed the H-9a and H-10b proton signals significantly shielded, in contrast to the respective H-9b and H-10a resonances. The signal splitting magnitudes of H-9a were $\Delta\delta$ = 0.008, 0.017, 0.009, and 0.010, while those for H-10b were $\Delta\delta$ = 0.017, 0.041, 0.019, and 0.020 for **1**–**4**, respectively. Quantification of the scalemic mixtures was feasible in spite of the fact that the signal of H-10a in **4** appeared close to the OH signal at δ 4.58 (Figure 2d, upper trace), or the H-9b signals of **1**–**4** overlap with the methine proton of the isobutyrate moiety. This is because an advantage of this methodology is that the scalemic proportion can be determined from at least one visible signal, H-10b for instance. In the enantiopure analyses of **5** and **6**, no splitting was observed after addition of BINOL, revealing only one set of slightly shielded signals.

After enantiopurity determination of **1**–**6**, UV and ECD data were recorded. These analyses were not explored previously for the AC determination of epoxythymols despite the higher sensitivity of the method, faster measurement times, and the presence of suitable chromophores close to the stereogenic center. ECD has classically been used in organic chemistry and in natural product studies for the AC determination by data comparison using model compounds,[17] although with the advent of contemporary computational resources, the required time-dependent DFT calculations for new groups of molecules are currently feasible,[18,19] although quite expensive in terms of computation time.

A sample of enantiomerically enriched (−)-(8*S*)-areolal (**1**) enabled ECD data comparison for the AC determination of **2**–**6**. The UV spectrum of **1** (Figure 3a) showed λ_{max} at 248 and 271 nm, which were assigned to π–π^* transitions of the benzaldehyde[20] and cinnamate moieties,[21−23] respectively. In the ECD spectrum, negative Cotton effects at 258, 263, 272, and 298 nm were observed (Table 2). The first two negative Cotton effects were associated with the π–π^* transition of the epoxystyrene moiety,[24] while the negative Cotton effect at 272 nm was assigned to the π–π^* transition of the cinnamate functionality. Finally, the weak positive Cotton effect at 298 nm was related to the n–π^* cinnamate transition.[21]

Table 2. Maximum UV Absorptions and Main ECD Transitions of Epoxythymols 1–6

compound	λ/nm (log ε)[a]	λ/nm ($\Delta\varepsilon$)[a]
1	248 (4.91)	258 (−52.2)
	271 (4.98)	263 (−42.4)
		272 (−49.9)
		298 (1.3)
2	273 (4.92)	259 (−21.5)
		265 (−17.7)
		272 (−22.8)
		299 (3.2)
3	275 (4.87)	259 (−20.3)
		263 (−17.5)
		272 (−22.3)
		298 (2.3)
4	275 (4.88)	260 (−45.4)
		264 (−41.7)
		273 (−57.0)
		298 (3.1)
5	274 (4.99)	257 (−16.4)
		263 (−12.7)
		271 (−19.4)
		298 (3.1)
6	274 (4.99)	258 (−24.6)
		263 (−20.3)
		272 (−30.5)
		298 (2.5)

[a]Data for ε are given in L mol^{-1} cm^{-1}.

Once the UV and ECD spectra of (8*S*)-**1** were assigned, the AC of epoxythymols **2**–**6** became plausible, since, as seen in Figure 3b, the ECD spectra of **1**–**6** are quite similar and further reveal a qualitative absorption dependence associated with the enantiomeric excess of the studied samples. All the UV spectra showed λ_{max} in the 273–275 nm range associated with π–π^* transitions of the cinnamate moiety, as in **1** (Figure 3a). All ECD spectra showed negative Cotton effects in the 257–260 and 263–265 nm regions associated with π–π^* transitions of the epoxystyrene unit, while the π–π^* transitions of the cinnamate moieties were related to the negative Cotton effects in the 272–273 nm region. All spectra also showed weak positive Cotton effects at 298–299 nm for the n–π^* transition of the cinnamate moieties (Table 2). On the basis of these results, the AC of the major enantiomers in **2**–**4** and those for the enantiomeric pure epoxythymols **5** and **6** are (8*S*), thus completing the structure and AC determination of the new epoxythymols **5** and **6**.

Compound **3** was previously isolated as a natural product from *P. areolare*, but its chiroptical properties and absolute configuration were not examined at that time.[25] The 92:8 *S*/*R* scalemic mixture of **3** isolated herein showed a levorotatory specific rotation $[\alpha]_{589}^{22\,^\circ C}$ −5.7 (*c* 1.1, $CHCl_3$), while the 91:9

https://dx.doi.org/10.1021/acs.jnatprod.0c01113
J. Nat. Prod. 2021, 84, 707−712

S/*R* mixture of areolal (**1**) showed $[\alpha]_{589}^{22\,^{\circ}C}$ −5.4 (*c* 1.0, $CHCl_3$). In order to collect independent evidence for the absolute configuration of **3**, a sample of areolal (**1**), enriched in the dextrorotatory (8*R*) enantiomer of $[\alpha]_{589}^{22\,^{\circ}C}$ + 0.5, $[\alpha]_{578}$ +0.5, $[\alpha]_{546}$ +0.6, and $[\alpha]_{436}$ +2.6 (*c* 2.17, $CHCl_3$), was converted into **3** by using a described sequence.[12] Areolal (**1**) was treated with $NaBH_4$ in MeOH at room temperature, and the reduction product was immediately acetylated with Ac_2O in pyridine. The hemisynthetic sample of **3** showed identical ^1H and ^{13}C NMR spectra to those of natural **3**, but dextrorotatory values of $[\alpha]_{589}^{22\,^{\circ}C}$ +0.7, $[\alpha]_{578}$ +0.7, $[\alpha]_{546}$ +0.8, $[\alpha]_{436}$ +2.1, and $[\alpha]_{365}$ +6.2 (*c* 3.9, $CHCl_3$), thus reinforcing the specific rotation sign/absolute configuration relationship for (−)-(8*S*)-**3** and (+)-(8*R*)-**3**. It also follows that the chemical changes at C-7 in **1**−**4** or the presence of a phenolic hydroxy group at C-6 in **5** and **6** is not influencing the ECD spectra. As more epoxythymols become available for this type of study, there will be more data to compare and assign the AC of this kind of compound, many of which presumably occur as scalemic mixtures.

In conclusion, the AC determination of epoxythymols using ECD spectroscopy requires a fraction of a milligram compared to several milligrams for VCD data acquisition. The sample amount ratio for enantiomerically pure epoxythymols seems to be around 1:500 in favor of ECD. Furthermore, inspection of the ECD spectra of **1**−**6** showed that relatively small enantiomeric excesses would be sufficient to define the chirality of the dominant enantiomer in scalemic mixtures. This is an additional advantage over VCD determinations, in which, due to the inherent lower sensitivity, samples having some 50% ee seem to be a practical limit.[9] In addition, since VCD provided the AC of a sample of **1**, its ECD spectrum can now be used as a reference for the AC determination of related epoxythymols, thereby precluding the need to perform expensive computer TDDFT calculations.

EXPERIMENTAL SECTION

General Experimental Procedures. Optical rotations were recorded on a PerkinElmer 341 polarimeter. UV and ECD spectra were obtained on a stand-alone JASCO CD-2095 circular dichroism detector. 1D and 2D NMR spectra were measured at 300 MHz for ^1H and 75.4 for ^{13}C on a Varian Mercury 300 NMR spectrometer in $CDCl_3$ using TMS as the internal reference. Chemical shift values are reported in ppm, and coupling constants (*J*) are given in Hz. HRESIMS data were acquired on a Waters Synapt G2 spectrometer at the Department of Biochemistry, University of Colorado, Boulder, CO, USA. Silica gel 230−400 mesh (Merck) was used for column chromatography.

Plant Material. *Piptothrix areolare* (DC.) R.M. King & H. Rob. specimens were collected during the flowering stage, in September and October 2019, near km 35 of the Tiripetío-Eréndira federal road (N 19°20.645′, W 101°21.348′) at 2509 m above sea level. A voucher specimen (246342) is deposited at the Herbarium of Instituto de Ecología, A. C., Centro Regional del Bajío, Pátzcuaro, Michoacán, México.

Extraction and Isolation of Epoxythymols. Fresh roots (2 kg) of *P. areolare* were macerated in hexanes (3 × 3 L) at room temperature for 3 days. Filtration and solvent evaporation gave 5 g (0.3%) of a yellowish oil. Column chromatography of the extract, using hexanes−EtOAc mixtures in ascending polarity, afforded **7** (20 mg 0.001%) with 9:1 to 7:3 mixtures, **1** (22 mg, 0.001%) with a 7:3 mixture, and **2** (60 mg, 0.003%) with a 3:2 mixture.

Dried flowers (200 g) were macerated in CH_2Cl_2 (3 × 1 L) at room temperature for 3 days. Filtration and solvent evaporation gave 10 g (5%) of a yellowish oil. Column chromatography of a 5 g aliquot using a hexanes−EtOAc mixture in ascending polarity afforded **3** (73 mg, 0.04%) and **6** (20 mg, 0.01%) with a 7:3 mixture and **5** (8 mg, 0.004%) and **4** (12 mg, 0.006%) with a 3:2 mixture.

Determination of Scalemic Ratios Using (*S*)-BINOL. The enantiomeric ratio for each epoxythymol was determined from the height of the signals from ^1H NMR measurements obtained under good magnet homogeneity (better than 0.3 Hz), considering that the width at half-height of the evaluated signals in both enantiomers is the same, by using 6 mg of **1** and **2**, 3 mg of **3**−**6**, and in each case 30 mg of (−)-(*S*)-BINOL in 0.7 mL of $CDCl_3$.

ECD Spectroscopic Measurements. The measurements were performed in the 220 to 420 nm region using solutions containing 10 μg of individual epoxythymols **1**−**6** in 1 mL of EtOH at 25 °C. The ECD spectra were baseline corrected by subtracting the spectrum of the solvent acquired under identical instrument conditions.

*(8S)-7-Acetoxy-10-cinnamoyloxy-6-hydroxy-8,9-epoxythymol isobutyrate (**5**):* pale yellow oil; $[\alpha]_{589}^{22\,^{\circ}C}$ +2.4, $[\alpha]_{578}$ +2.7, $[\alpha]_{546}$ +2.4, $[\alpha]_{436}$ −2.7, $[\alpha]_{365}$ −27.2 (*c* 0.29, $CHCl_3$); ^1H and ^{13}C NMR ($CDCl_3$) see Table 1; HRESIMS *m*/*z* 455.1703 $[M + H]^+$ (calcd for $C_{25}H_{26}O_8 + H^+$, 455.1700).

*(8S)-10-Cinnamoyloxy-8,9-epoxy-6-hydroxy-7-northymol isobutyrate (**6**):* pale yellow oil; $[\alpha]_{589}^{22\,^{\circ}C}$ −3.8, $[\alpha]_{578}$ −4.2, $[\alpha]_{546}$ −5.4, $[\alpha]_{436}$ −17.6 (*c* 5.34, $CHCl_3$); ^1H and ^{13}C NMR ($CDCl_3$) see Table 1; HRESIMS *m*/*z* 383.1492 $[M]^+$ (calcd for $C_{22}H_{22}O_6 + H^+$, 383.1489).

*10-Cinnamoyloxy-7-oxo-8,9-dehydrothymol isobutyrate (**7**):* pale yellow oil; ^1H and ^{13}C NMR ($CDCl_3$) see Table 1; HRESIMS *m*/*z* 401.1360 $[M]^+$ (calcd for $C_{23}H_{22}O_5 + Na^+$, 401.1359).

Chemical Correlation of Epoxythymols 1 and 3. A solution of 360 mg of areolal (**1**) of $[\alpha]_{589}^{22\,^{\circ}C}$ +0.5, $[\alpha]_{578}$ +0.5, $[\alpha]_{546}$ +0.6, and $[\alpha]_{436}$ +2.6 (*c* 2.17, $CHCl_3$) in 15 mL of MeOH was stirred in the presence of 70 mg of $NaBH_4$ at room temperature for 30 min. The reaction mixture was extracted with CH_2Cl_2 (2 × 20 mL), and the combined extracts were washed with 50 mL of H_2O, dried over anhydrous Na_2SO_4, filtered, and evaporated to yield 70 mg of **4**. A portion of the residue (49 mg) was dissolved in 5 mL of CH_2Cl_2 and 0.5 mL of pyridine and treated with 0.5 mL of Ac_2O at room temperature for 1 h. After extraction and isolation, the oily residue (50 mg) showed $[\alpha]_{589}^{22\,^{\circ}C}$ +0.7, $[\alpha]_{578}$ +0.7, $[\alpha]_{546}$ +0.8, $[\alpha]_{436}$ +2.1, and $[\alpha]_{365}$ + 6.2 (*c* 3.9, $CHCl_3$). EIMS *m*/*z* (rel int) 389 (0.1), 290 (33), 232 (35), 220 (100), 204 (94). The ^1H and ^{13}C NMR spectra were identical with those of natural **3**.[12]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.0c01113.

^1H and ^{13}C NMR spectra of **5**−**7** (PDF)

AUTHOR INFORMATION

Corresponding Authors

Carlos M. Cerda-García-Rojas − *Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City 07000, Mexico;* orcid.org/0000-0002-5590-7908; Phone: +52 55 5747 4035; Email: ccerda@cinvestav.mx; Fax: +52 55 5747 7137

Mario A. Gómez-Hurtado − *Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Michoacán 58030, Mexico;* orcid.org/0000-0002-2386-4819; Phone: +52 443 326 5788; Email: magomez@umich.mx; Fax: +52 443 326 5790

https://dx.doi.org/10.1021/acs.jnatprod.0c01113
J. Nat. Prod. 2021, 84, 707−712

Authors

Héctor M. Arreaga-González − *Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City 07000, Mexico;* orcid.org/0000-0002-3746-0137

Antonio J. Oliveros-Ortiz − *Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Michoacán 58030, Mexico*

Rosa E. del Río − *Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Michoacán 58030, Mexico;* orcid.org/0000-0001-8932-552X

Gabriela Rodríguez-García − *Instituto de Investigaciones Químico Biológicas, Universidad Michoacana de San Nicolás de Hidalgo, Michoacán 58030, Mexico;* orcid.org/0000-0002-9840-9968

J. Martín Torres-Valencia − *Área Académica de Química, Universidad Autónoma del Estado de Hidalgo, Hidalgo 42184, Mexico;* orcid.org/0000-0001-6426-7562

Pedro Joseph-Nathan − *Departamento de Química, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional, Mexico City 07000, Mexico;* orcid.org/0000-0003-3347-3990

Complete contact information is available at: https://pubs.acs.org/10.1021/acs.jnatprod.0c01113

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank CIC-UMSNH and CONACYT-Mexico (Grant No. 284194) for partial financial support. H.M.A.G. and A.J.O.O. are grateful to CONACYT-Mexico for scholarships 295638 and 956977, respectively.

■ DEDICATION

Dedicated to Professor Dr. A. Douglas Kinghorn, The Ohio State University, for his pioneering work on bioactive natural products.

■ REFERENCES

(1) Mándi, A.; Kurtán, T. *Nat. Prod. Rep.* **2019**, *36*, 889−918.
(2) Berova, N.; Di Bari, L.; Pescitelli, G. *Chem. Soc. Rev.* **2007**, *36*, 914−931.
(3) Joseph-Nathan, P.; Gordillo-Román, B. Vibrational Circular Dichroism Absolute Configuration Determination of Natural Products. In *Progress in the Chemistry of Organic Natural Products*; Kinghorn, A. D., Falk, H., Kobayashi, J., Eds.; Springer International Publishing: Cham, Switzerland, 2015; Vol. *100*, pp 311−451.
(4) Burgeño-Tapia, E.; Joseph-Nathan, P. *Nat. Prod. Commun.* **2015**, *10*, 1785−1795.
(5) Talavera-Alemán, A.; Rodríguez-García, G.; López, Y.; García-Gutiérrez, H. A.; Torres-Valencia, J. M.; del Río, R. E.; Cerda-García-Rojas, C. M.; Joseph-Nathan, P.; Gómez-Hurtado, M. A. *Phytochem. Rev.* **2016**, *15*, 251−277.
(6) Bohlmann, F.; Niedballa, U.; Schulz, J. *Chem. Ber.* **1969**, *102*, 864−871.
(7) Tori, M.; Ohara, Y.; Nakashima, K.; Sono, M. *J. Nat. Prod.* **2001**, *64*, 1048−1051.
(8) Jiang, H. X.; Li, Y.; Pan, J.; Gao, K. *Helv. Chim. Acta* **2006**, *89*, 558−566.
(9) Arreaga-González, H. M.; Pardo-Novoa, J. C.; del Río, R. E.; Rodríguez-García, G.; Torres-Valencia, J. M.; Manríquez-Torres, J. J.; Cerda-García-Rojas, C. M.; Joseph-Nathan, P.; Gómez-Hurtado, M. A. *J. Nat. Prod.* **2018**, *81*, 63−71.
(10) Arreaga-González, H. M.; Rodríguez-García, G.; del Río, R. E.; Ferreira-Sereno, J. A.; García-Gutiérrez, H. A.; Cerda-García-Rojas, C. M.; Joseph-Nathan, P.; Gómez-Hurtado, M. A. *J. Nat. Prod.* **2019**, *82*, 3394−3400.
(11) Adorisio, S.; Giamperi, L.; Bucchini, A. E. A.; Delfino, D. V.; Marcotullio, M. C. *Molecules* **2020**, *25*, 1893.
(12) Hernández, J. D.; Román, L. U.; Rodríguez, M. J.; Espiñeira, J.; Joseph-Nathan, P. *Phytochemistry* **1986**, *25*, 1743−1744.
(13) Delle Monache, G.; Delle Monache, F.; Becerra, J.; Silva, M.; Menichini, F. *Phytochemistry* **1984**, *23*, 1947−1950.
(14) Tamayo-Castillo, G.; Jakupovic, J.; Bohlmann, F.; Rojas, A.; Castro, V.; King, R. M. *Phytochemistry* **1988**, *27*, 2893−2897.
(15) Wenzel, T. J.; Wilcox, J. D. *Chirality* **2003**, *15*, 256−270.
(16) Redondo, J.; Capdevila, A.; Latorre, I. *Chirality* **2009**, *22*, 472−478.
(17) Slade, D.; Ferreira, D.; Marais, J. P. J. *Phytochemistry* **2005**, *66*, 2177−2215.
(18) Li, X. C.; Ferreira, D. *Curr. Org. Chem.* **2010**, *14*, 1678−1697.
(19) Ding, Y.; Li, X. C.; Ferreira, D. *J. Org. Chem.* **2007**, *72*, 9010−9017.
(20) Kawamura, K.; Yasuda, T.; Hatanaka, T.; Hamahiga, K.; Matsuda, N.; Ueshima, M.; Nakai, K. *Chem. Eng. J.* **2016**, *285*, 49−56.
(21) Abu-Eittah, R. H.; Khedr, M. K.; Goma, M.; Zordok, W. *Int. J. Quantum Chem.* **2012**, *112*, 1256−1272.
(22) Ravindram, B.; Madhurambal, G.; Mariappan, M.; Ramamurthi, K.; Mojumdar, S. C. *J. Therm. Anal. Calorim.* **2011**, *104*, 909−914.
(23) Madhurambal, G.; Ravindran, B.; Mariappan, M.; Mojumdar, S. C. *J. Therm. Anal. Calorim.* **2010**, *100*, 811−815.
(24) Kland-English, M.; Summerbell, R. K.; Klotz, I. M. *J. Am. Chem. Soc.* **1953**, *75*, 3709−3712.
(25) Fang, N.; Gage, D. A.; Mabry, T. M. *Phytochemistry* **1988**, *27*, 203−205.