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Transesterification of a Natural Epoxythymol Is Favored under Alkaline Conditions, Preserving the Enantiomeric Purity †

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Abstract: Transesterification is a synthetic chemistry strategy promoted in acid or alkaline conditions, yielding a structural diversity of organic compounds. Epoxythymols comprise a class of chiral natural compounds with biological relevance, and the literature describes their chiral purity loss during acid transesterification reactions. This work reports the basic transesterification of the natural derivative (8*S*)-10-benzoylxy-8,9-epoxy-6-hydroxythymol under alkaline conditions. Herein, the formation of (8*S*)-10-benzoylxy-6-isobutyryloxy-8,9-epoxythymol isobutyrate is gained, avoiding the loss of optical purity. ^{1}H NMR-BINOL experiments revealed the enantiomeric purity of the product reaction. These results highlight that the implemented strategy promotes transesterification, preserving optical purity.

Keywords: epoxythymol; transesterification; chirality

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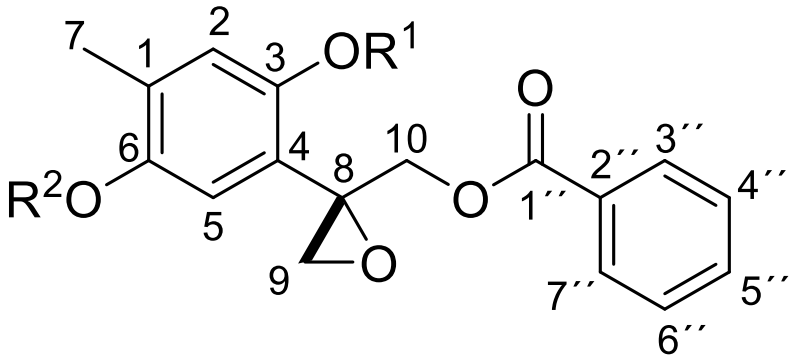
1. Introduction

Transesterification is a widely used chemical reaction to yield esterified derivatives. Several strategies can be used depending on the reaction medium and the starting material stability; however, acidic or basic conditions or catalysts with such properties are always considered. Sometimes, anhydrous conditions are used with sensitive substrates [1]. This type of reaction has been described as a result of spontaneous transformations of natural epoxythymol derivatives during or after their isolation [2–4]. This compound family originates from terpene biosynthesis and is found in 42 genera of the Asteraceae family, characterized by chirality due to the oxirane ring located at the C-8/C-9 position [5]. These compounds are important due to their biological potential as cytotoxic and antibacterial agents [6–11]. The literature suggested that these derivatives may be susceptible to the escalemization process [12] through acid transesterification reactions. Herein, escalemization proceeds via an intramolecular reaction mechanism involving the cleavage of the oxirane ring, thereby altering chirality [13]. Structural variation around the basic epoxythymol skeleton avoiding chiral alterations will enable the generation of novel derivatives with potential biological activity.

In the present work, the transesterification under basic conditions of (8*S*)-10-benzoylxy-8,9-epoxy-6-hydroxythymol isobutyrate (**1**) isolated from *Ageratina glabrata* is described, allowing the formation of (8S)-10-benzoylxy-6-isobutyryloxy-8,9-epoxythymol isobutyrate (**2**). After the reaction process, the optical purity of the reaction product was saved, which was validated through ^{1}H NMR-BINOL experiments. These results demonstrate that the implemented strategy promotes transesterification while preserving the chirality of

molecules containing asymmetric oxirane rings, such as those natural epoxythymol compounds (Figure 1).



1 R^1 = *i*-Bu R^2 = H

2 R^1, R^2 = H

Figure 1. Formulas of epoxythymol derivatives **1** and **2**.

2. Material and Methods

2.1. Experimental Section

All chemical reagents were acquired from Sigma-Aldrich and used as purchased. Solvents were distilled before use. Melting points were determined using a Fisher-Johns apparatus and were not corrected. Dichroic Circularly Polarized Light (CPL) and UV spectra were obtained using a Jasco CD-2095 circular dichroism detector, employing an ethanolic solution with 0.027 mmol of compound **1**. The results were processed using WinDaq software and plotted in Excel. The IR spectra were acquired using a Thermo Scientific Nicolet iS10 spectrophotometer employing the ATR (Attenuated Total Reflectance) technique. The ^{1}H and ^{13}C NMR spectra at 400 MHz and 100 MHz, respectively, were measured in a Varian Mercury 400 spectrometer, using solutions of $CDCl_3$ and tetramethylsilane (TMS) as the internal reference. Chemical shifts are reported in ppm, and coupling constants (*J*) are given in Hertz. The NMR spectra were processed using MestReNova software. The enantiomeric purity of thymol derivatives was determined by ^{1}H NMR using 6.0 and 3.0 mg of sample (**1** and **2**, respectively) and (S)-BINOL as the chiral solvating reagent, in quantities of 30.0 and 15.0 mg, respectively, and dissolving in all cases with 0.7 mL of $CDCl_3$. The enantiomeric ratio was established by analyzing the splitting of signals and their intensities. Purification of compounds was achieved by column chromatography using silica gel (230–400 mesh) as the stationary phase.

2.2. Plant Material

Specimens of *Ageratina glabrata* were collected during the flowering stage in February 2018, near km 4.5 of the federal road 200 from Pátzcuaro-Santa Clara del Cobre, Michoacán, Mexico, at N 19°29.516′ W 101°35.273′ and 2285 m above sea level. A voucher specimen (No. 226133) was deposited in the Herbarium of the Institute of Ecology, A. C., Regional Center of El Bajío, Pátzcuaro, Michoacán, Mexico.

2.3. Extraction and Isolation

A batch of dried leaves (1.5 kg) was macerated using hexanes (10 L) for 3 days, filtered, and concentrated under reduced pressure. This procedure was performed three times. Afterward, the same procedure was performed with dichloromethane (10 L). Macerates yielded 35 g (2.3%) of the hexanes extract and 147 g (9.8%) of the CH_2Cl_2 extract, respectively.

(+)-(8S)-10-benzoyloxy-6-hydroxy-8,9-epoxythymol isobutyrate (**1**) was isolated following the reported methodology [13]. Colorless crystals, m.p. 112–114 °C; UV (EtOH) λ_{max} (log ε) 230 (0.75), 275 (0.38) nm; ECD (EtOH) λ_{max} ($\Delta\varepsilon$): 226 (−185); ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.97 (2H, dd, J = 7.8, 1.5 Hz, H-3″, H-7″), 7.55 (1H, tt, J = 7.8, 1.5 Hz, H-5″), 7.41 (2H, t, J = 7.8 Hz, H-4″, H-6″), 6.93 (1H, s, H-5), 6.81 (1H, s, H-2), 4.76 (1H, d, J = 12.3 Hz, H-10a), 4.47 (1H, d, J = 12.3 Hz, H-10b), 3.10 (1H, d, J = 5.3 Hz, H-9a), 2.85 (1H, d, J = 5.3 Hz, H-9b), 2.82 (1H, hept, J = 6.8 Hz, H-2′), 2.21 (3H, s, H-7), 1.30 (3H, d, J = 6.8 Hz, H-3′), 1.29 (3H, d, J = 6.8 Hz, H-4′). ^{13}C NMR ($CDCl_3$, 100 MHz,) δ: 175.0 (C, C-1′), 166.0 (C, C-1″), 151.7 (C, C-6), 141.7 (C, C-3), 133.2 (CH, C-5″), 129.7 (CH, C-3″, C-7″), 129.5 (C, C-4), 128.4 (CH, C-4″, C-6″), 127.1 (C, C-2″), 125.8 (C, C-1), 124.7 (CH, C-2), 114.5 (CH, C-5), 65.5 (CH_2, C-10), 57.0 (C, C-8), 51.0 (CH_2, C-9), 34.1 (CH, C-2′), 19 (CH_3, C-3′, C-4′), 15.7 (CH_3, C-7).

2.4. Transesterification Reaction under Basic Conditions

A batch of 100 mg of **1** was dissolved in 60 mL of benzene, previously refluxed for 4 h using a Dean–Stark trap to remove moisture. Subsequently, 38 mg (0.95 mmol, 1.5 eq) of NaOH was added and stirred for 2 h under reflux. Afterward, the crude was tempered, poured over wet ice, and extracted with AcOEt (ethyl acetate) (3 × 50 mL). The organic phase was dried with Na_2SO_4 and evaporated under vacuum to obtain 45.6 mg of yellow oil, which was purified via column chromatography using silica gel as the stationary phase and hexanes-AcOEt (4:1) as the eluent, yielding 29.9 mg of a yellowish oily liquid.
(8*S*)-10-benzoyloxy-6-isobutyryloxy-8,9-epoxythymol isobutyrate (**2**). Yellow oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.97 (2H, dd, J = 7.4, 1.4 H-3″, H-7″), 7.54 (1H, tt, J = 7.4, 1.4 Hz H-5″), 7.42 (2H, t, J = 7.4 Hz, H-4″, H-6″), 7.21 (1H,s, H-5), 6.94 (1H, s, H-2), 4.75 (1H, d, J = 12.4 Hz, H-10a), 4.46 (1H, d, J = 12.4 Hz, H-10b), 3.10 (1H, d, J = 5.2 Hz, H-9a), 2.87 (1H, d, J = 5.2 Hz, H-9b), 2.82 (1H, hept, J = 7.0 Hz, H-2′), 2.82 (1H, sept, J = 7.0 Hz, H-2‴), 2.16 (1H, s, H-7), 1.34 (3H, d, J = 7.0 Hz, H-3′), 1.34 (3H, d, J = 7.0 Hz, H-3‴), 1.30 (3H, d, J = 2.8 Hz, H-4′), 1.30 (3H, d, J = 2.8 Hz, H-4‴).

2.5. Epimerization Reaction of Epoxythymol **1**

A suspension of 1 g of silica gel in 60 mL of benzene was refluxed for 4 h using a Dean–Stark trap to remove moisture. A sample (100 mg) of **1** (enantiomerically pure) was added, and the mixture was refluxed for 6 h. The crude reaction solution was filtered, evaporated under vacuum, and purified via column chromatography using hexanes-AcOEt (4:1) to yield 15.3 mg (15%) of **1** as a scalemic mixture [13].

3. Results and Discussion

Compound **1** was obtained as colorless crystals (m.p. 112–114 °C), whose spectroscopic data (see Supplementary Materials) were compared with an authentic sample and those reported values [14]. In the ^{1}H NMR spectrum, the doublet signals from CH_2-10 at δ 4.76 and 4.46 are highlighted since these protons confirmed the optical purity of **1** after the ^{1}H NMR-BINOL analysis (Figure 2a). It follows that compound **1** was subjected to transesterification using NaOH/benzene in a Dean–Stark trap. After the reaction, the crude product was purified via column chromatography, resulting in a yellowish oil. The ^{1}H NMR spectrum (see Supplementary Materials) revealed a pattern of signals similar to the starting material, where the singlet signals of the aromatic protons H-5 and H-2 appeared at δ 7.21 and 6.94, respectively. Additionally, a new set of signals from an isobutyrate moiety at δ 2.82 (1H, hept J = 7.0 Hz) and δ 1.34 (6-H, d, J = 7.0 Hz) appeared. This result suggested incorporating an isobutyrate group at position C-6 of the starting molecule, indicating the formation of 10-benzoylxy-6-isobutyryloxy-8,9-epoxythymol (**2**). This product suggests the concomitant generation of 10-benzoyloxy-6-hydroxy-8,9-epoxythymol (**3**), whose isolation and identification are currently under experimentation.

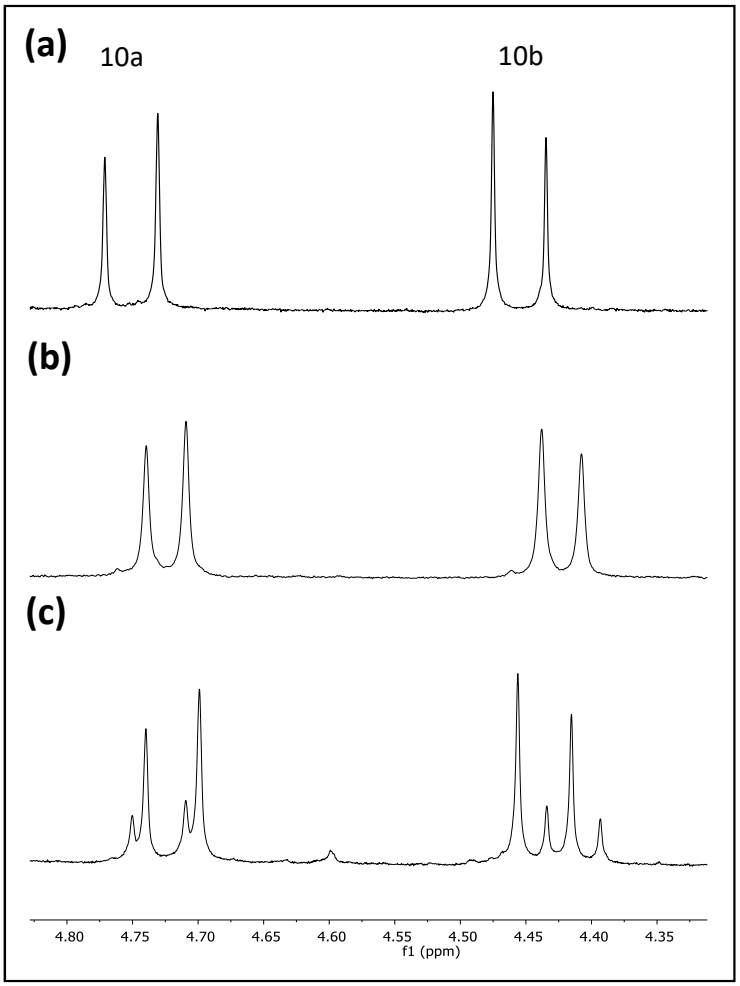
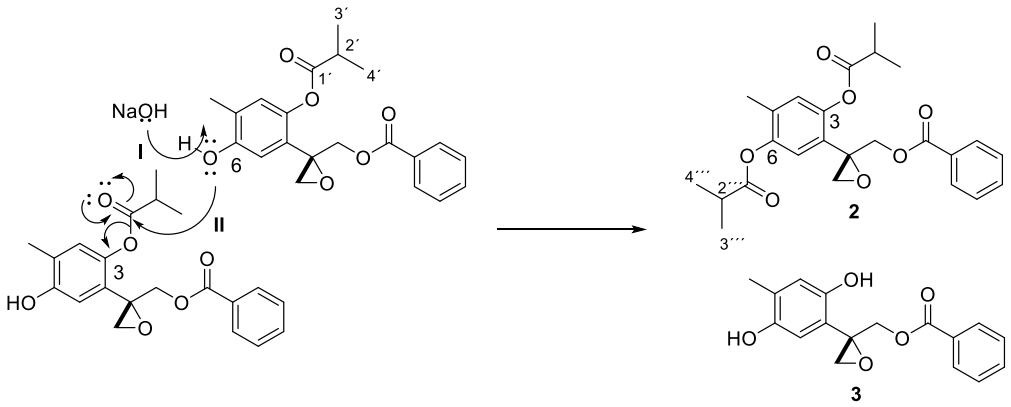


Figure 2. Optical purity analysis by ^{1}H NMR-BINOL methodology of (**a**) compound **1**, (**b**) transesterification product **2**, and (**c**) delivered escalimizated epoxythymol **1**, evidencing a 75:25 (8*S*:8*R*) ratio.

The literature mentions the transesterification of epoxythymol derivatives, where the reaction mechanism leads to racemization or escalemization. Therefore, it was decided to evaluate the enantiomeric purity of **2** using ^{1}H NMR-BINOL analysis, as reported in [13]. In this spectrum (Figure 2b), the enantiomeric purity of **2** was revealed when only one set of signals for CH_2-10 was observed.

A deliberate escalemization of **1** was achieved to validate the above enantiopurity analysis. Thus, compound **1** was subjected to reflux with benzene and silica gel for 6 h using a Dean–Stark trap, according to the methodology reported by Arreaga-González et al. [13]. In this spectrum (Figure 2c), two sets of signals for the AB system (δ 4.57) of methylene CH_2-10 were observed and assigned to each enantiomer of **1** in a 75:25 ratio (8*S*:8*R*). Based on this, it can be proposed that the transesterification reaction proceeds without affecting the optical purity of the stereogenic center C-(8S).

According to the experimental results, a mechanistic pathway is proposed to the obtention of **2**. It involves a concerted intermolecular transesterification process (Scheme 1), where NaOH facilitates the activation of O-6 through deprotonation (**I**). The produced phenoxide ion promotes a nucleophilic attack to the carbonyl of the isobutyrate group of a neighboring molecule (**II**) to create the new ester bond, concomitantly generating 10-benzoyloxy-6-hydroxy-8,9-epoxythymol (**3**).



Scheme 1. Proposed reaction mechanism for the transesterification of **1**.

4. Conclusions

The transesterification with NaOH/benzene using a Dean–Stark trap allowed for the optical pure epoxythymol derivative **2** to be obtained. Presumably, transesterification takes place intermolecularly, thus yielding the expected product, 10-benzoyloxy-6-hydroxy-8,9-epoxythymol; therefore, this method is suitable for generating various optically pure epoxythymol derivatives.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ecsoc-27-16140/s1, NMR, ^{1}H NMR-BINOL, ECD of compound **1.** NMR, ^{1}H NMR-BINOL of compound **2**.

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

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