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Research Article

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Fungal Biotransformation of Cedryl Formate

Sedril Formatın Fungal Biyodönüşümü

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ABSTRACT

Cedryl formate is a synthetic fragrance compound, which is an ester derivative of the sesquiterpenoid cedrol, found in different parts of the cedar tree/cedarwood with a characteristic fragrance. Cedryl formate is used in cosmetics and personal care products like fragrances, perfumes, as well as in the chemical and agriculture industries in various forms. In the present work, the microbial transformation of the formate was evaluated by 13 fungal cultures to produce new derivatives. Among the evaluated, the plant pathogenic fungus Aspergillus niger NRRL 326, biotransformed to the metabolite 8-cedren-3β-ol derivative with 6.2% yield. The structure of the chromatographically purified metabolite was elucidated by NMR and spectroscopic methods.

Key Words

Aspergillus niger, biotransformation, cedrol, fragrance compounds.

ÖZ

Sedril format sedrol molekülünün sentetik esteri olan bir koku bileşiğidir. Sedrol sedir ağacının uçucu yağı dahil farklı bulunan bir seskiterpen aromatik bileşiktir. Sedirin karakteristik kokusunu vermektedir. Sedril format, parfüm ve kozmetik gibi kişisel bakım ürünleri ile kimya, ve hatta tarım endüstrisi gibi alanlarda kullanılmaktadır. Bu çalışmada format substratı farklı 13 fungal kültür ile türevlendirilmesi incelenmiştir. Sonuçlar arasında, bitki patojeni olan Aspergillus niger NRRL 326, substratı %6.2 verimle 8-cedren-3β-ol metabolitine biyolojik olarak ilk defa dönüştürülmüştür. Kromatografik yöntemlerle saflaştırılan metabolitin yapısı NMR ve spektroskopik metotlar kullanılarak aydınlatılmıştır.

Anahtar Kelimeler

Aspergillus niger, biyotransformasyon, sedrol, koku bileşikleri.

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INTRODUCTION

Flavor and fragrance compounds, also known as aroma chemicals, are compounds that are used to create and enhance flavors and scents in a wide range of products, including food and beverages, perfumes and colognes, soaps and body washes, candles and air fresheners, and many other consumer products. Aroma compounds can also be used to mask unpleasant odors and tastes, making them a valuable tool in the food and beverage industries [1,2]. There are a wide variety of flavor and fragrance compounds available, each with their own unique properties and characteristics. Some of the most common types include essential oils, synthetic aroma chemicals, and natural extracts. Essential oils are derived from plants and are used to create natural, authentic flavors and scents. Synthetic aroma chemicals can be used to create a wide range of flavors and scents which serve many industries. Natural extracts, on the other hand, derived from natural sources such as fruits, vegetables, herbs and are still used for expensive authentic, natural flavors and scent demands [5-7].

Cedryl formate is a synthetic fragrance compound that mimics the aroma of cedarwood. It is synthesized by reacting cedrol with formic acid, resulting in an ester form. Cedryl formate is defined as a woody, spicy, and slightly sweet aroma, which is why it is prefered in perfumes and other specific woody fragrance products. It is also used as a fragrance ingredient in other personal care products such as soaps, lotions, and deodorants. In addition to its use in the fragrance industry, cedryl formate can also be found in other industries such as the chemical industry where it is used as a intermediate in the synthesis, and the agriculture industry as a repellent or attractant [8]. Additionally, it is documented that cedryl formate can also be used as an attractant to lure pests into traps, which makes it a versatile tool for pest management in both indoor and outdoor settings. The compound was also reported for its antimicrobial properties, which makes it a potential preservative for in personal care products. Furthermore, Cedryl formate is known to be biodegradable and non-toxic, making it an environmentally friendly alternative to traditional pesticides [9].

This present study aimed the production of new derivatives from the substrate cedryl formate, by microbial transformations. To the best of our knowledge, this is the first report on the microbial transformation from cedryl formate.

MATERIALS and METHODS

General experimental procedures

High purity cedryl formate was provided kindy by IFF Inc. Co., USA. Fourier Transform Infra Red (FTIR) spectra were obtained using KBr pellets on Bruker Tensor 27 Spectrometer. Optical rotations were recorded using the Krüss Optronic P8000-T polarimeter. Agilent 6890N GC system, GC/MS Agilent 5975 GC/MSD, Shimadzu QP2010 Plus systems mounted with an Innowax FSC column (60 m × 0.25 mm and 0.25 µm film thickness), and CPSil-5CB (25 m × 0.25 mm and 0.25 µm film thickness) were used for the separation and analysis of the mass spectra of the metabolites. High resolution mass spectra (HRMS) coupled an electrospray ionization (ESI) and with micro-TOF were used to record the specific mass of the purified metabolites. NMR spectra were acquired using a Varian Mercury Plus 400 system in deutero chloroform ($CDCl_3$). Silica gel (230–400 mesh, type 60) was used for column chromatography purifications. Thin Layer Chromatography (silica gel 60 GF_{254}) was performed using n-hexane/ethyl acetate in various proportions, where the metabolites were visualized by UV light and by acid staining followed by heating.

Microorganisms

Microbial strains were aquired from various resources such as the Agriculture Research Service Culture Collection (NRRL), American Type Culture Collection (ATCC), Leibniz-Institut DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSM) and the culture collection of the Faculty of Pharmacy at Anadolu University, Türkiye.

Fungal strains used for the biotransformation screens were; *Aspergillus parasiticus* NRRL 2999, *Aspergillus niger* ATCC 10549, *Aspergillus niger* NRRL 326, *Alternaria alternata* NRRL 20593, *Aspergillus alliaceus* NRRL 317, *Penicillium adametzii* NRRL 737, *Penicillium chrysogenum* NRRL 792, *Fusarium culmorum* isolate (Faculty of Sciences), *Hansenula anomala* ATCC 20170, *Sporobolomyces pararoseus* ATCC 11385, *Saccharomyces cerevisiae* ATCC 9763, *Trametes versicolor* ATCC 200801, and *Corynespora cassiicola* DSM 62475, respectively.

Conditions of cultivation and biotransformation

The biotransformation experiments were basically performed using the enrichted liquid α-medium as perviously described in detail [10,11].

Biotransformation by *A. niger*

The substrate cedryl formate was converted using the *A. niger* NRRL 326 the strain over 7 days at 25°C to the metabolite in 6,16% yield, as shown in Scheme 1. The metabolite was purified as an oily odorous compound [20% ethyl acetate (v/v) in hexane].

Cedryl formate: ^{1}H NMR ($CDCl_3$, 400 MHz): 0.85 (3H, d, *J* = 8 Hz, 12-CH_3), 1.01 (3H, s, 13-CH_3), 1.20 (3H, s, 14-CH_3), 1.36 (1H, m, 2-CH), 1.32 (1H, m, 3-CH_{ax}), 1.48 (2H, m, 10-CH_2), 1.55 (2H, m, 4-CH_2), 1.58 (3H, s, 15-CH_3), 1.68 (2H, m, 11-CH_3), 1.81 (1H, t, *J* = 4 Hz, 5-H), 1.90 (1H, m, 3-CH_{eq}), 2.05 (1H, m, 9-CH), 2.34 (1H, d, *J* = 4 Hz, 7-CH), 8.05 (H, s(br), 8-OCHO), ^{13}C NMR: Table 1.

8-Cedren-3β-ol: FT-IR ν_{max} cm^{-1}: 3431 (OH), 2958, 2931 (C-H), 1730 (C-H), 1664 (C-H), 1460 and 1373 (-C-O-H), 1245 (C-O), 1180 (C-CH_3), 1076 (C-O). ^{1}H NMR ($CDCl_3$, 400 MHz): 0.88 (3H, d, *J* = 8 Hz, 12-CH_3), 0.96 (3H, s, 13-CH_3), 1.04 (3H, s, 14-CH_3), 1.66 (3H, s, 15-CH_3), 4.30 (1H, m, 3-CHOH), 5.20 (H, s(br), 9-CH), ^{13}C NMR: is listed in Table 1.

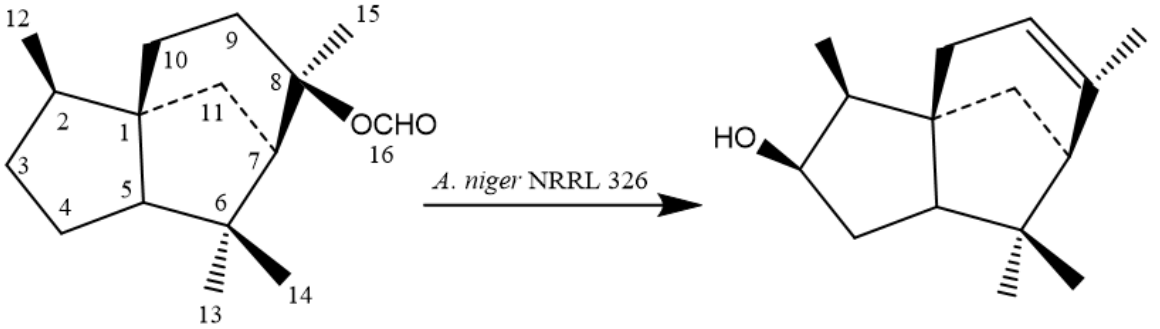
RESULTS and DISCUSSION

Among all the tested fungal cultures, the biotransformation by *A. niger* NRRL 326 was the richerst metabolite yielding microorganism reported in this present study. The molecular ion of the metabolite's MS data was *m/z* 220.18 representing $C_{14}H_{24}O$. The FTIR spectrum indicated a broad hydroxyl group absorption at 3431 cm^{-1}. The ^{1}H-NMR spectrum highlighted the proton signal at 4.30 ppm (1H, m, 3-CHOH) and the DEPT spectrum confirmed this finding by showing the presence of a signal at 79.07 ppm (-CHOH) indicating that a new hydroxyl group was introduced at the 8-cedren-3ß-ol structure. The position of the hydroxyl group was determined by the aid of HMBC experimental data, which indicated the key connectivity of proton signals at 4.30 (1H, m, 3-CHOH) to the signals at δC 56.86 (C-5) and δC 51.97 (C-1), respectively. It was confirmed that the hydroxylation was at the C-3 position. When comparing the ^{1}H NMR and ^{13}C NMR spectra of cedrenyl formate (*syn.* Cedryl formate) and 8-cedren-3β-ol, the singlet signal at δH 8.04 ppm in the substrate ^{1}H NMR spectrum was not observed in the proton spectrum of the metabolite (8-cedren-3β-ol). In the ^{13}C NMR spectrum, the C16 peak signals around 160 ppm indicated that the -OCHO functional group structure was not present in the metabolite. The metabolite also showed new peaks at C-8 (δC 140.16) and C-9 (δC 118.98), which confirmed that it was introduced at the 8 position (-C=C-) signals.

Table 1. Comparative ^{13}C NMR data for substrate and its metabolite.

	δC (ppm) for compounds	
C-position	Cedryl formate	Metabolite
C-1	53.85	51.97
C-2	40.99	45.82
C-3	36.93	79.07
C-4	26.39	33.23
C-5	56.73	56.86
C-6	43.56	47.37
C-7	57.36	55.05
C-8	87.48	140.16
C-9	33.16	118.99
C-10	31.13	39.91
C-11	41.26	40.90
C-12	15.47	9.40
C-13	27.17	27.39
C-14	28.48	25.96
C-15	25.26	24.67
C-16	160.72	-

As a result this metabolite was assinged as 8-cedren-3β-ol based on MS, FT-IR and NMR data.



Scheme 1. Biotransformation of Cedryl formate by *Aspergillus niger.*

The structure of the cedryl metabolite was determined by comparing its ^{1}H NMR and ^{13}C NMR spectral data with those of Luu and Ourisson [12], who reported *in vivo* biotransformation by rabbits. According to the literature, the cedrol metabolite(s)were reported by microbial transformation products in other conversion routes [13-16]. The structure was clearly supported and revealed through comparison with the results of the analysis.

Conclusion

This study revealed new findings for the biotransformation of the substrate cedryl formate, and its metabolite by different fungi. Today it is important to produce flavor and fragrance compounds by green technologies. Not only due to environmental concerns but also biotechnological production of aroma chemicals may have great impact on the industry. Still the operation yields of conventional industrial production of fragrances have a great impact, which can be improved by genetic engineering for microbial transformations.

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