Isolation and Identification of 7-Oxo-8,15-isopimaric Acid in UV-cured Products of Isopimaric Acid

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Abstract: A non-polymerized compound with strong UV absorption was detected during investigation on UV-cured product of isopimaric acid (iPA) by HPLC-UV. The compound was isolated through silica gel column chromatography and preparative HPLC in purity of 82.6 % analyzed with GC. It was identified as 7-oxo-8,15-isopimaric acid (7-O-8,15-iPA), a kind of oxidized derivative of iPA, by means of spectroscopic data of UV-vis, FT-IR, MS, ^{1}H NMR and ^{13}C NMR in accord with the data reported in references. The formation mechanism of 7-oxo-8,15-isopimaric acid from iPA was also predicted.

Key words: 7-oxo-8,15-isopimaric acid; isopimaric acid; UV-curing reaction; structure identification

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异海松酸光固化产物中 7-羰基-8,15-异海松酸的分离与结构鉴定

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摘 要: 利用 HPLC-UV 研究了异海松酸(iPA)UV 光固化反应产物,从中检测到了一种具有较强紫外吸收的非聚合物成分。通过硅胶柱色谱和制备型 HPLC 分离得到了该物质,经气相色谱分析检验,其纯度为 82.6 %。通过 UV-vis、FT-IR、MS、^{1}H NMR 及 ^{13}C NMR 等光谱分析研究,该物质的化学结构被鉴定为 7-羰基-8,15-异海松酸(7-O-8,15-iPA),这是异海松酸的一种氧化衍生物,与文献报道结构一致。还探讨了异海松酸发生氧化反应生成 7-羰基-8,15-异海松酸的反应机理。

关键词: 7-羰基-8,15-异海松酸; 异海松酸; UV 光固化反应; 结构鉴定

7-Oxo-8,15-isopimaric acid (7-O-8,15-iPA, **1**) was a kind of isopimaric acid (iPA, **2**) derivative. This compound was discovered as a component of extracts of some special plants, such as *Juniperus communis*[1], *Platycladus orientalis*[2], *Thuja occidentalis*[3]. There is little literature for compound **1**, but some for its epimer or analogues isolated or derived from the nature. Compound **1** was derived by Jones reagent oxidation of two analogous compounds isopimara-8,15-dien-7β,18-diol (**3**), and isopimara-8,15-dien-7β,18-diol (**4**),

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which was isolated from *Nepeta tuberosa* subsp. *reticulata*, and purified and characterized as a methyl ester dirivative (**5**)[4]. Herz et al[5] isolated an analogous compound methyl *ent*-7-oxopimaria-8,15-dien-19-oate (**6**, 16 mg) from 2 kg of above ground parts of *Helianthus strumosus*. Severiano et al[6] isolated 7-keto *ent*-pimara-8,15-dien-19-oic acid (**7**, epimer of 7-O-8,15-iPA, 8.5 mg) from 800 mg of microbial transformation product of *epi*-sandarapimaric acid (SA, **8**) by *M. rouxii*, and further purified it by reversed phase HPLC using an analytical column. Ulubelen et al[7] isolated 7-oxo-13-*epi*-pimara-8,15-dien-18-oic acid (**9**, an epimer of 7-O-8,15-iPA, 9 mg) from 1 kg of roots of *Salvia heldreichiana*. Chang et al[3] indicated that the biological activity of 7-O-8,15-iPA was more obvious in inhibiting transformation of JB6 mouse epidermal cells than PA.

In our previous studies[8-10], a technology has been discovered for preparation of iPA from the rosin of *Pinus elliottii*. Presently, while most researches focused on the UV-curing property of iPA, an UV responsible compound has been detected in the UV-cured product of iPA by HPLC-UV. It was identified to be 7-O-8,15-iPA. In order to recognize and utilize more valuably and efficiently 7-O-8,15-iPA in the cured products, it is necessary to investigate a practical preparative method on the basis of structural identification.

1 Experiment

1.1 Material and equipments

Isopimaric acid (**2**, purity 95.1%) was prepared according to literatures[8-10]. Initiator 2959 (2-hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone), hexane, ethyl acetate and tetrahydrofuran (THF) are analytic reagents. Methyl alcohol is chromatographic grade. Tinplate panels (120 mm × 50 mm × 2.8 mm) is produced by Kunshan Minglida Stainless Steel Co., Ltd.

Wet membrane preparation device is made by Shanghai Xiandai Environment Engineering Technique Co., Ltd. INTELLI-RAY 600 Shuttered UV Floodlight, MAGNA-IR 550 FT-IR Spectrometer (Nicolet), LC-20AT HPLC (Shimadzu), Waters Q-TOF Micro™ Mass Spectrometer (Micro), AV-300 Nuclear Magnetic Resonance Spectrometer (BRUKER), Lab Tech 9100D UV Spectrometer (Beijing Labtech Instrument Co., Ltd.) are used for analysis.

1.2 UV-curing reaction[11]

A varnish was prepared with iPA (0.45 g), initiator 2959 and THF in mass ratio of $m_{iPA}:m_{2959}:m_{THF}$ = 1:0.04:3, and coated on tinplates by wet membrane preparation device. The coated tinplates were exposed to ultraviolet light in INTELLI-RAY 600 Shuttered UV Floodlight. The irradiation conditions were as follows: the time 300 s, the space 4.5 cm between UV floodlight and tinplate. After the irradiation, the cured solid product (0.41 g) contained 7-O-8,15-iPA was rinsed from the tinplates with THF.

1.3 Isolation and purification of 7-O-8, 15-iPA

HPLC analysis was performed on a reversed phase C_{18} Hypersil ODS column (150 mm × 4.6 mm, 5 μm), detected at 254 nm by PDA. Sample injection was 10 μL. The column temperature was 40 ℃. The mobile phase was programmed with a liner gradient from 70 % A (purified water with 2 % acetic acid) and 30 % B (methanol with 2 % acetic acid), to 45 % B in 20 min, then to 73 % B in 5 min, at last, to 100 % B in 50 min, remained 5 min, with a flow rate of 0.3 mL/min.

The UV-cured product (0.41 g) was separated by a silica gel column chromatography eluted with hexane and ethyl acetate to give 60 mg of a fraction consisted of 7-O-8,15-iPA. The 32 mg of goal product 7-O-8,15-iPA was collected after the further purification by a preparative high performance liquid chromatography under

above conditions.

1.4 Instrumentals

1.4.1 GC The sample of 7-O-8,15-iPA pre-treated with NMe_4OH was analyzed by GC on a DB-5 quartz capillary chromatographic column under programmed column temperatures as 100 ℃ (remained 2 min) $\xrightarrow{5\ ℃/min}$200 ℃ (remained 2 min) $\xrightarrow{2\ ℃/min}$250 ℃ (remained 30 min). The temperatures of injection and FID were 270 ℃ and 280 ℃, respectively. The carrier gas was N_2.

1.4.2 FT-IR Several drops of 7-O-8,15-iPA gathered with HPLC, were coated on the infrared ray transmitting glass to analyze its particular functional groups.

1.4.3 UV-vis The UV-vis chart was scanned from 190 to 600 nm with sample dissolved in methyl alcohol.

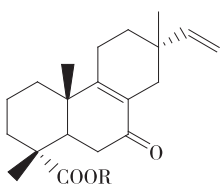
1.4.4 MS Mass spectrometric analysis was conducted with an electrospray interface operating in the negative ion mode. MS conditions were as follows: ion source temperature 100 ℃, desolvation temperature 200 ℃, cone voltage setting 30 V, capillary voltage setting 2 500 V, cone gas N_2 50 L/h, desolvation gas N_2 400 L/h. The scanned area of *m/z* was from 80 to 1 000.

1.4.5 ^1H NMR and ^{13}C NMR Both of ^1H NMR and ^{13}C NMR spectra were taken in $CDCl_3$ solution with a Bruker AV-300 spectrometer.

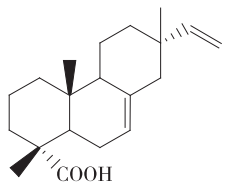
2 Results and Discussion

2.1 Detection and isolation of 7-O-8,15-iPA

Structures of compounds **1–9** were illustrated as the following:



1 R=H
5 R=CH_3



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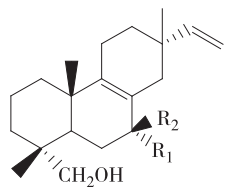
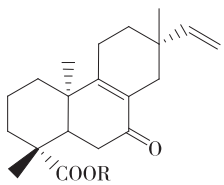
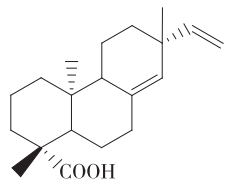


3 R_1=OH, R_2=H
4 R_1=H, R_2=OH



6 R=CH_3
7 R=H



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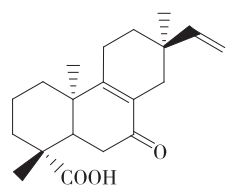


9

The HPLC analysis of the UV-cured products of iPA was shown in Fig. 1. There are a couple of conjugated double bonds and exocyclic vinyl double bond in the molecule of 7-O-8,15-iPA, which accorded with the structure of α,β-unsaturation carbonyl compounds. On basis of calculation, the longest UV absorption wavelength of 7-O-8,15-iPA would be 249 nm, but actually, the suitable UV absorption wavelength for HPLC detection is 254 nm for a stable base line. There are maximum absorptions at wavelength of 248.5 to 250 nm for abietic acid contained conjugated double bonds, and 203 nm for iPA with no conjugated double bond[11]. The HPLC chart (Fig. 1) for the UV-cured products of iPA showed that peak 1 belonged to iPA with little abietic acid, and peak 2 belonged to 7-O-8,15-iPA, which was eluted faster than iPA because of more polarity of 7-O-8,15-iPA on the reversed phase C_{18} Hypersil ODS stationary phase.

The isolated matter collected by preparative HPLC was analyzed with GC area normalization as shown in Fig. 2. The purity of 7-O-8,15-iPA was 82.6 %.

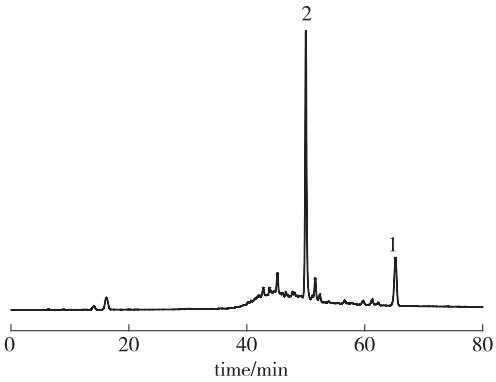
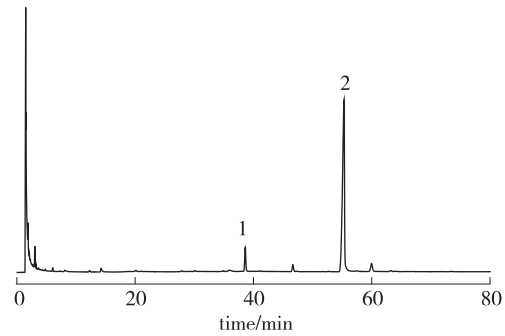


Fig. 1 HPLC chart of the UV-cured products of iPA



1. iPA; 2. 7-oxo-8,15-iPA

Fig. 2 GC chart of isolated 7-oxo-8,15-isopimaric acid

2.2 Structure characterization

2.2.1 Spectral data UV-vis(λ_{max}): 249 nm. FT-IR (υ_{max}, cm^{-1}): 3079, 2929, 1706, 1654, 1564, 1242, 913. MS(m/z): 315$[C_{20}H_{27}O_3]^+$ ($[M-1]^+$, 100), 316$[C_{20}H_{28}O_3]^+$ ($[M]^+$, 25). ^1H NMR (δ, 300 MHz, $CDCl_3$): 1.0058 (s, 3H, $C_{10}-CH_3$), 1.1252 (s, 3H, $C_{13}-CH_3$), 1.2759 (s, 3H, C_4-CH_3), 1.2302~1.2550 (m, 1H, C_5-H), 1.3252~1.3818 (m, 2H), 4.8651 (dd, 1H, J_1 = 17.49 Hz, J_2 = 1.25 Hz, CH), 4.9367 (dd, 1H, J_1 = 10.74 Hz, J_2 = 1.25 Hz, CH), 5.6809 (dd, 1H, J_1 = 17.50 Hz, J_2 = 10.75 Hz, CH). ^{13}C NMR (δ): The ^{13}C chemical shifts are listed in Table 1.

Table 1 ^{13}C NMR chemical shifts of 7-O-8,15-iPA

carbon number	δ	carbon number	δ
1	36.86	11	18.13
2	17.86	12	29.71
3	36.14	13	34.39
4	44.56	14	34.64
5	46.33	15	145.09
6	33.62	16	111.84
7	199.31	17	22.83
8	129.25	18	181.32
9	165.51	19	27.94
10	39.16	20	16.27

2.2.2 Structural resolutions According to experiment, the UV spectrum λ_{max} was 249 nm, which was in accord with the calculated value as well as being close to that in literature[1].

Under the selected negative ion electrospray conditions, the 7-O-8,15-iPA gave the base peak at m/z 315 $[M-H]^-$ and molecular ion at m/z 316$[M]^-$ with no other obvious fragment ions being observed.

Fig. 3 showed absorption bands at 1706 and 1242 cm^{-1} which confirmed the presence of a carbonyl group in carboxylic acid. 1654 and 1564 cm^{-1} belonged to α,β-unsaturated carbonyl group. 3079 and 913 cm^{-1} belonged to absorption $\upsilon_{C—H}$ in the exocyclic vinyl group[1-2,5]. Because of the hydrogen bond, the broad band at 3300 cm^{-1} was assigned to O—H in carboxylic acid. And the band at 2929 cm^{-1} belonged to the saturated absorption $\upsilon_{C—H}$ in 7-O-8,15-iPA.

In ^1H NMR spectrum (Fig. 4) of 7-O-8,15-iPA, there is not the signal of the carboxylic proton because

of $CDCl_3$ solvent. Apart from the one in carboxyl, the total of protons in 7-O-8, 15-iPA molecule was twenty seven. Compared with iPA, there was no difference for three exocyclic vinylic protons, but the proton at δ 5.2 observed in iPA disappeared. And three strong peaks of methylic protons were observed.

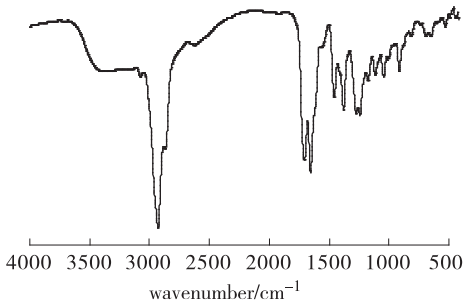


Fig. 3 FT-IR spectrum of 7-O-8,15-iPA

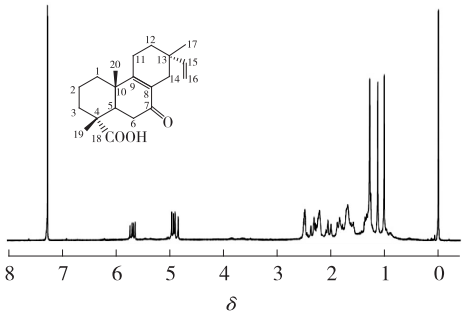


Fig. 4 ^{1}H NMR spectrum of 7-O-8, 15-iPA

According to literatures[4-5], the carbons in 7-O-8,15-iPA could be situated at their locations. It can be seen that there were six sp2 carbon (7, 8, 9, 15, 16, 19) in 7-O-8, 15-iPA. The chemical shifts of these six carbons were in upfield compared with the others′ (Fig. 5). The chemical shift of the carbonylic carbon conjugated with C═C bond in the ring moved to downfield than that of the carboxylic carbon.

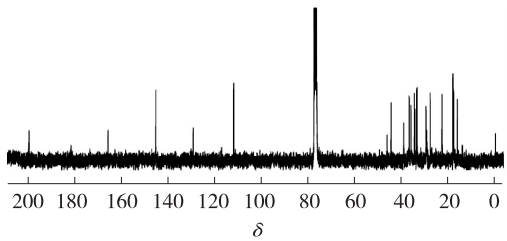
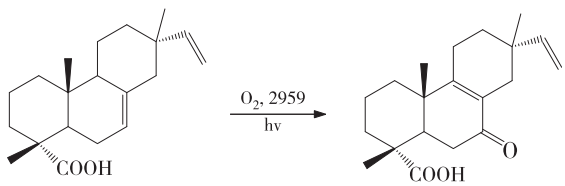


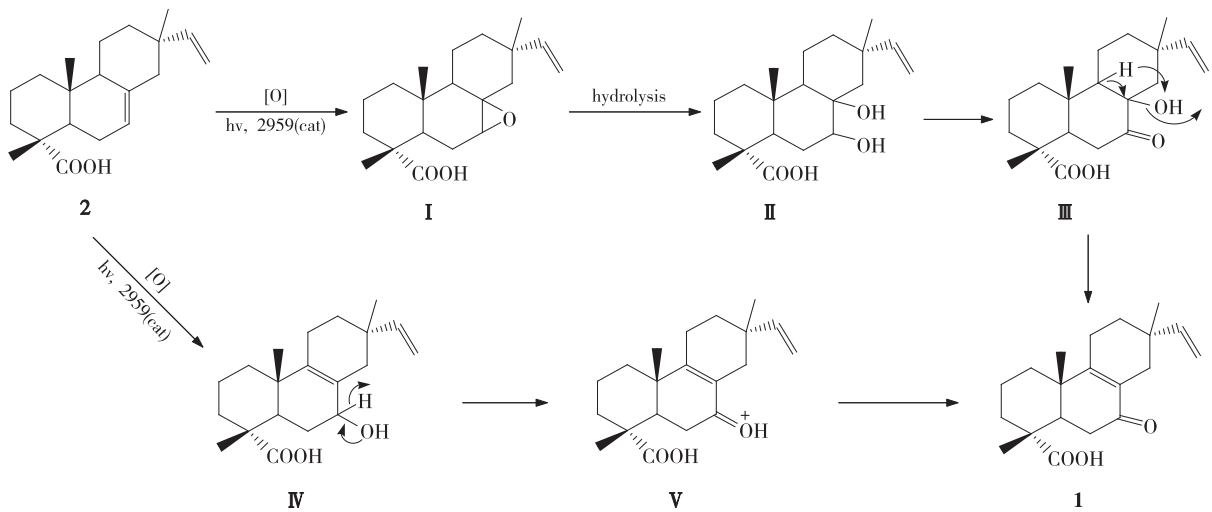
Fig. 5 ^{13}C NMR spectrum of 7-O-8, 15-iPA

2.3 Formation mechanism

As results described above, the oxidation equation about iPA to 7-O-8,15-iPA is deduced as follows.



The probable mechanism of the reaction may be predicted as follows.



The unsaturated double bond provided with electron-donating group could undergo epoxidation easily. Compared with the exocyclic vinyl bond in iPA (**2**), the cyclic double bond contained more electron-donating group may be more easily epoxidated with monomolecular oxygen activated by photo-initiator 2959 under UV

light[12]. Then, compound Ⅱ with two hydroxyl groups could yield from Ⅰ for the hydrolysis and the oxidation occurred on C-7 after the dehydration on C-8 and C-9 which was illustrated as Ⅲ. The other pathway is that the unsaturated bond between C-7 and C-8 transferred to the location between C-8 and C-9 at the same time of that the C-7 was attacked by the activated single molecular oxygen illustrated as Ⅳ. Then, compound **1** was formed from Ⅳ with the lost of two protons. At the end, the structural stable compound 7-oxo-8,15-isopimaric acid (**1**) formed from compound **2** by two pathways[13]. However, authenticity about the probable mechanism of the reaction was not tested.

3 Conclusions

3.1 The 7-oxo-8,15-isopimaric acid (7-O-8,15-iPA) was detected by a UV detector while analyzed with HPLC in the UV-cured product of isopimaric aci (iPA), and isolated from the product with a preparative HPLC in purity of 82.6 % (GC).

3.2 The structure of 7-O-8,15-iPA was characterized and identified by the spectral data of UV-vis, FT-IR, MS, ^{1}H NMR and ^{13}C NMR, in accordance with those reported in literatures. The oxidation equation and the probable mechanism from iPA to 7-O-8,15-iPA was deduced via a monomolecular epoxidation to the cyclic double bond.

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