SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF AMIDE DERIVATIVES FROM ACRYLOPIMARIC ACID

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This paper reports on the synthesis of a series of amide derivatives from acrylopimaric acid (APA). The derivatives contained aromatic groups and were characterized by IR, ^{1}HNMR, MS, and elemental analysis. The antibacterial activity of the derivatives against Gram-negative bacteria and Gram-positive bacteria were also investigated. When compared with the other derivatives, compounds 3a and 3f showed much higher activity against *Escherichia coli* (Gram-negative bacteria) with inhibition zones of 5 mm and 5.5 mm, respectively. Structure-activity relationship analysis revealed that ortho-substituted phenyl derivatives and meta-substituted phenyl derivatives exhibited higher activity than the para-substituted derivatives. Meanwhile, the halogen-substituted compounds did not show visible antibacterial activity compared with other compounds, which may have been caused by the lower electron density of the halogen-substituted phenyl rings.

Keywords: Rosin; APA; Antibacterial activity; Structure activity relationship

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INTRODUCTION

As an important natural resource, rosin is widely available throughout China (Cheng 1996). Rosin, as well as its derivatives, has been developed as a starting material for synthesizing various chemicals and/or intermediates, which are renewable alternatives or substitutes of petrochemical-based chemicals (Lu et al. 2007; Wang et al. 2009). For example, rosin can be used as a paper sizing agent to resist penetration of aqueous liquids (Hubbe 2007). Jong and Sung (2002) reported that a negative photoresist made from copolymers containing rosin moiety showed favorable sensitivity and contrast.

Crystallization and oxidization are two factors that restrict the application of rosins. Because of this, chemical modification is required before the rosin can be utilized. Acrylopimaric acid (APA) is a Diels–Alder adduct of rosin and acrylic acid (Diels and Alder 1930) and has been isolated and characterized (Wang et al. 2009). It has been used in producing alkyd resins as well as used in paper sizing agents (Sabyasachi et al. 1990), coatings (Xie 1997), adhesives (Feng 2007), surfactants (Zhong et al. 2003), and photoresist (Jong and Sung 2002). In our earlier work, we have reported the synthesis and biological activity of diacylthiourea and dihydrazone derivatives from APA (Li et al. 2011a,b).

Amides have been used as fungicides for decades following the discovery of carboxin by Schmeling and Kulka (1966). Since then, more than 20 molecules with similar structures, such as benodani (Pommer et al. 1974), flutolanil (Yabutani et al. 1978), and trifluzamide (Alt et al. 1991) have been developed. The use of these toxic fungicides, however, may adversely impact the environment. As a feasible solution, products from natural resources can be chemically modified with similar structures.

In this work, a series of amide derivatives from APA were synthesized, and their structures were characterized by IR, ^{1}HNMR, MS, and elemental analysis. Meanwhile, their antimicrobial activities against *Staphylococcus aureus* (gram-positive bacteria) and *Escherichia coli* (Gram-negative bacteria) were also investigated. The goal of this exploration is to promote the application of rosin in antimicrobial activity aspects.

EXPERIMENTAL

Materials and Instruments

Gum rosin (Grade one) was obtained from a commercial source (Wu Zhou Pine Chemicals Ltd., Guang Xi, China) and used without further purification. All other chemicals were of reagent grade. The IR spectra were taken on a Nicolet IS10 FT-IR (Nicolet, Madison, USA) spectrophotometer (scan times, 16; resolution, 6.000). The ^{1}HNMR spectra were recorded using a Bruker AV-300 (Bruker, Karlsruhe, Germany) nuclear magnetic resonance spectrometer with dimethylsulfoxide (DMSO) as the solvent and tetramethylsilane (TMS) as the internal standard. The mass spectroscopy (MS) data were obtained using an Agilent-5973 (Agilent, Santa Clara, USA) spectrophotometer. The melting points were determined using an XT-5 (Saiao, Beijing, China) melting point apparatus. The elemental analysis (C, H, and N) was performed using a Vario EL-III (Elementar, Hanau, Germany) elemental analyzer; the results were in good agreement with the calculated values (within ±0.5%). All reactions were traced by thin layer chromatography (TLC). Fourteen new amide derivatives were synthesized from APA, and their synthetic routes are shown in Fig. 1.

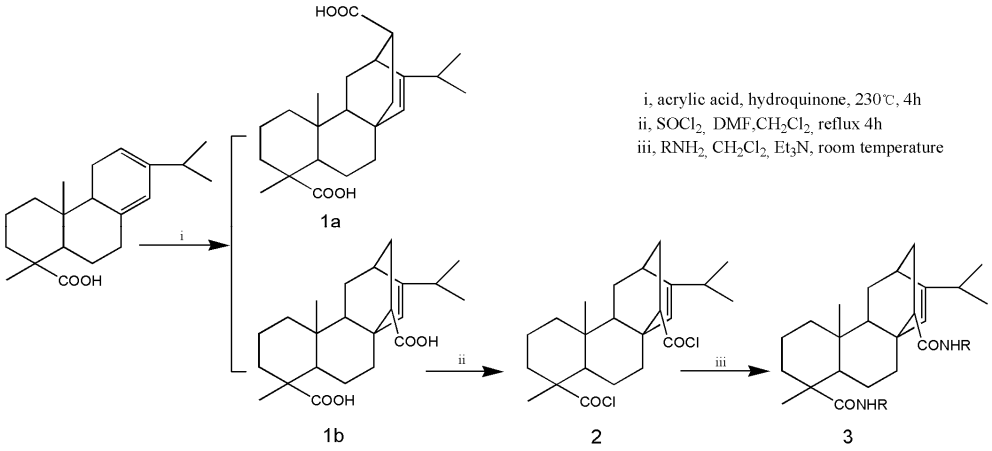


Fig. 1. Synthetic routes of the Acrylopimaryl amide derivatives

General Synthetic Procedure for Acrylopimaric acid (1b)

In accordance with the literature (Noah et al. 1961, 1972), the gum rosin (1000 g) and hydroquinone (5 g) were charged to a flask equipped with a stirrer, dropping funnel, N_2 inlet, thermometer, and water trap topped with a water-cooled condenser. The rosin was first heated using a slow stream of nitrogen and then stirred after melting had occurred. The temperature was adjusted to 220°C, and the acrylic acid was added dropwise within 0.3 h. The mixture was then heated to 230°C for a residence time of 4 h, and the products were collected after cooling to 170°C. Two isomers of the APA derivatives (1a and 1b in Table 1, with contents of 15% and 55%, respectively, in the products) were found within them, as determined by GC. The target chemical 1b (with content of 95%) was obtained by recrystallization with ethanol and sodium salts.

General Synthetic Procedure for Acrylopimaryl Chloride (2)

In a 250 mL flask with a water-cooled condenser, thermometer, drying tube, and dropping funnel, 20 mmol of APA and 80 mL of CH_2Cl_2 were stirred until the solid was dissolved. 50 mmol of thionyl chloride was then added dropwise through a dropping funnel within 1 h and refluxed for 4 h at 65 °C. Acrylopimaryl chloride was then obtained after removing the dichloromethane and excess thionyl chloride under reduced pressure.

General Synthetic Procedure for the Acrylopimaryl Amide Derivatives (3a-3n)

A solution of Acrylopimaryl chloride in 15 mL of CH_2Cl_2 was added dropwise to a solution of 60 mmol of amine and 60 mmol of triethylamine in 40 mL of CH_2Cl_2 within 30 minutes at room temperature. After reacting for 12 h, the mixture was washed using 50 mL of 0.1M hydrochloric acid three times and then deionized water. Purification of the residue by silica gel chromatography [V (ethyl acetate)/ V (petroleum ether) =1:10] gave the fourteen resulting derivatives, 3a to 3n (shown below).

Antibacterial Activity

The antibacterial activities of the derivatives were estimated by a disc paper method (Afroditi 1996; Bauer et al. 1959; Bauer et al. 1966). The test chemicals were dissolved in ethanol and sterile water to obtain a solution concentration of 256 μg/mL. As test species, *Staphylococcus aureus* and *Escherichia coli* were cultivated in beef extract-peptone for 1 week. Small amounts (1 to 2 scratches) of fresh bacteria from the culture medium were then added into the culture solution, and in turn diluted the solution 10 fold to a concentration of 5.0 to 10.0 × 10^6 (CFU) mL^{-1}. 1mL of the bacterial solution was then evenly coated on a 90 mm plate of the beef extract peptone medium. A 6 mm diameter sterile filter paper was dipped in the solution of chemicals for 10 minutes and then placed on the plate. The inhibition zones were measured by calipers (in units of mm) at the end of an incubation period of 24 h. All experiments were conducted in triplicate to be positive at a given concentration,

$$\text{Diameter of inhibition zone (mm)} = A_1 - A_0 \quad (1)$$

where A_0 is the diameter of the control (blank, ethanol and sterile water without

chemical), and A_1 is the diameter in the presence of test chemicals.

RESULTS AND DISCUSSION

As a dicarboxylic acid, APA was obtained by the Diels-Alder addition reaction between rosin and acrylic acid; the content of APA (Fig. 1) in the Diels-Alder adducts was 70%. Additional purifications were required to remove other reactants and separate the two isomers 1a and 1b (in Fig. 1). Following the approach given in the literature (Wang et al. 2009), the target isomer 1b was separated by recrystallization, and the content of 1b was 95%.

Fourteen amide derivatives from APA were prepared, and their structures are shown in Table 1. These compounds have two amide groups. At the same time, aromatic rings, as well as active atoms/groups such as fluorine, chlorine, bromine, and trifluoromethyl on the aromatic ring were introduced into the amide backbone, in order to carry out the preliminary analysis of structure-activity relationship. The derivative structures were characterized by IR, ^{1}HNMR, MS, and elemental analysis.

APA Amide Derivatives

N-benzyl-acrylopimaramide (3a)

White powder, m.p. 140.8-141.6°C, yield: 7.77 g (70.4%). IR (cm^{-1}): 3363, 3259 (N-H); 2926, 2854 (-CH_3, -CH_2); 1659 (N-C=O); 738, 697 (Ar-H). ^{1}HNMR (DMSO. δ/ppm. 300MHz): 9.61, 9.06 (m, 2H, CONH-); 7.61-6.96 (m, 10H, Ar-H); 5.29 (S, H, C=CH-); 4.71-4.30 (m, 4H, Ar-CH_2-); 2.57 (S, H, -CH-C=O-); 2.01-1.83 (m, 3H, -CH-); 1.82-1.24 (m, 14H, -CH_2-); 1.53 (m, H, -CH-$(Me)_2$); 1.14-0.61 (m, 12H, CH_3). ESI-MS m/z=553 $[M+H]^+$. Anal. Calcd for $C_{37}H_{48}N_2O_2$: C, 80.39; H, 8.75; N, 5.07. Found: C, 80.27; H, 9.00; N, 4.86.

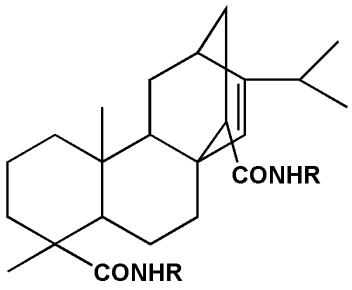
N-phenyl-acrylopimaramide (3b)

White powder, m.p. 135.6-137.4°C, yield: 7.55 g (72%). IR (cm^{-1}): 3354 (N-H); 2961, 2861 (-CH_3, -CH_2); 1663 (N-C=O); 754, 691 (Ar-H). ^{1}HNMR (DMSO. δ/ppm. 300MHz): 8.08, 7.95 (m, 2H, CONH-); 7.29-7.20 (m, 10H, Ar-H); 5.25 (S, H, C=CH-); 2.68 (S, H, -CH-C=O-); 2.50-1.28 (m, 14H, -CH_2-); 2.11-1.90 (m, 3H, -CH-); 1.57 (m, H, -CH-$(Me)_2$); 1.17-0.55 (m, 12H, CH_3). ESI-MS m/z=525 $[M+H]^+$. Anal. Calcd for $C_{35}H_{44}N_2O_2$: C, 80.11; H, 8.45; N, 5.34. Found: C, 79.90; H, 8.54; N, 5.08.

N-(o-methylphenyl)-acrylopimaramide (3c)

Yellow powder, m.p. 125.7-126.9°C, yield: 6.6 g (60%). IR (cm^{-1}): 3301 (N-H); 2924, 2864 (-CH_3, -CH_2); 1663 (N-C=O); 748 (Ar-H). ^{1}HNMR (DMSO. δ/ppm. 300MHz): 8.05, 7.99 (m, 2H, CONH-); 7.34-7.03 (m, 8H, Ar-H); 5.30 (S, H, C=CH-); 2.69 (S, H, -CH-C=O-); 2.20 (S, 6H, Ar-CH_3); 2.24-2.11 (m, 3H, -CH-); 2.19-2.04 (m, 14H, -CH_2-); 1.55 (m, H, -CH-$(Me)_2$); 1.21-0.62 (m, 12H, CH_3). ESI-MS m/z=553 $[M+H]^+$. Anal. Calcd for $C_{37}H_{48}N_2O_2$: C, 80.39; H, 8.75; N, 5.07. Found: C, 80.28; H, 8.80; N, 4.84.

Table 1. Structures of the Synthesized APA Amide Derivatives



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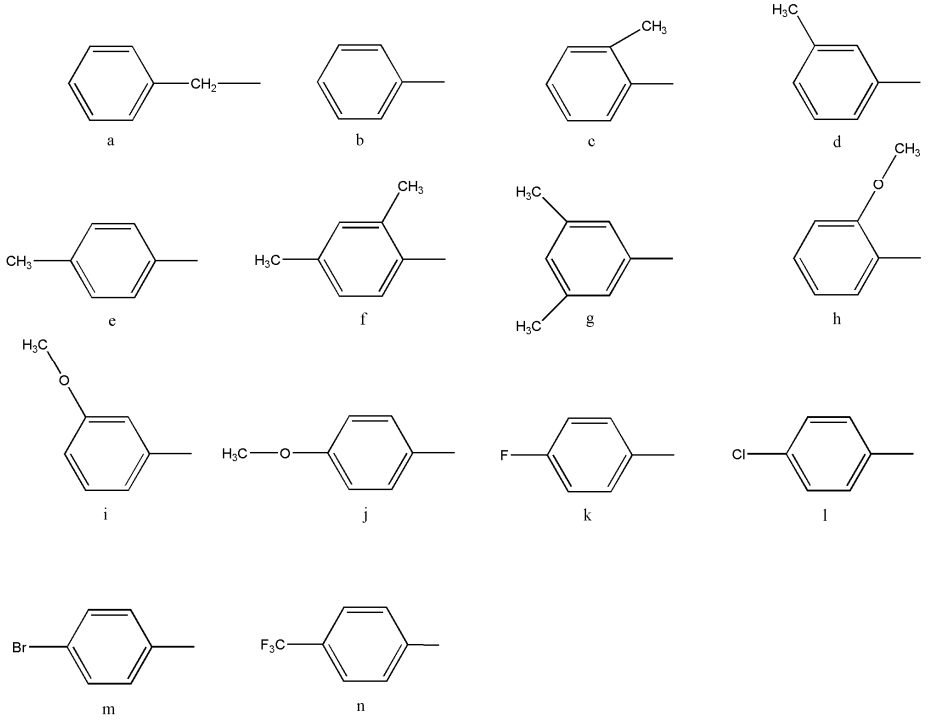

N-(m-methylphenyl)-acrylopimaramide (3d)

White powder, m.p. 126.9-127.8°C, yield: 4.97 g (45%). IR (cm^{-1}): 3338 (N-H); 2922, 2864 (-CH_3, -CH_2); 1663 (N-C=O); 778, 689 (Ar-H). ^{1}HNMR (DMSO. δ/ppm. 300MHz): 8.09, 7.98 (m, 2H, CONH-); 7.31-6.96 (m, 8H, Ar-H); 5.29 (S, H, C=CH-); 2.67 (S, H, -CH-C=O-); 2.31 (S, 6H, Ar-CH_3); 2.33-2.29 (m, 3H, -CH-); 2.17-1.27 (m, 14H, -CH_2-); 1.58 (m, H, -CH-$(Me)_2$); 1.17-0.59 (m, 12H, CH_3). ESI-MS m/z=553 $[M+H]^+$. Anal. Calcd for $C_{37}H_{48}N_2O_2$: C, 80.39; H, 8.75; N, 5.07. Found: C, 80.19; H, 8.97; N, 4.95.

N-(p-methylphenyl)-acrylopimaramide (3e)

White powder, m.p. 142.0-142.9°C, yield: 5.85 g (53%). IR (cm^{-1}): 3334 (N-H); 2922, 2864 (-CH_3, -CH_2); 1662 (N-C=O); 812 (Ar-H). ^{1}HNMR (DMSO. δ/ppm.

300MHz): 8.35, 8.06 (m, 2H, CONH-); 7.35-7.04 (m, 8H, Ar-H); 5.30 (S, H, C=CH-); 2.66 (S, H, -CH-C=O-); 2.28 (S, 6H, Ar-CH_3); 2.32-2.27 (m, 3H, -CH-); 2.17-1.26 (m, 14H, -CH_2-); 1.54 (m, H, -CH-$(Me)_2$); 1.16-0.70 (m, 12H, CH_3). ESI-MS m/z=553 $[M+H]^+$. Anal. Calcd for $C_{37}H_{48}N_2O_2$: C, 80.39; H, 8.75; N, 5.07. Found: C, 80.47; H, 8.85; N, 4.95.

N-(2, 4-dimethylphenyl)-acrylopimaramide (3f)

White powder, m.p. 207.7-208.5°C, yield: 5.45 g (47%). IR (cm^{-1}): 3265 (N-H); 2920, 2861 (-CH_3, -CH_2); 1670 (N-C=O); 804, 685 (Ar-H). ^{1}HNMR (DMSO. δ/ppm. 300MHz): 8.83, 8.71 (m, 2H, CONH-); 7.21-6.88 (m, 6H, Ar-H); 5.33 (S, H, C=CH-); 2.69 (S, H, -CH-C=O-); 2.49-1.58 (m, 14H, -CH_2-); 2.35-2.10 (S, 12H, Ar-CH_3); 2.29-2.17 (m, 3H, -CH-); 1.57 (m, H, -CH-$(Me)_2$); 1.47-0.61 (m, 12H, CH_3). ESI-MS m/z=581 $[M+H]^+$. Anal. Calcd for $C_{39}H_{52}N_2O_2$: C, 80.64; H, 9.02; N, 4.82. Found: C, 80.52; H, 9.50; N, 4.56.

N-(3, 5-dimethylphenyl)-acrylopimaramide (3g)

White powder, m.p. 140.8-142.9°C, yield: 7.50 g (68%). IR (cm^{-1}): 3348 (N-H); 2921, 2864 (-CH_3, -CH_2); 1664 (N-C=O); 836, 686 (Ar-H). ^{1}HNMR (DMSO. δ/ppm. 300MHz): 8.91, 8.68 (m, 2H, CONH-); 7.14-7.03 (m, 6H, Ar-H); 5.30 (S, H, C=CH-); 2.68 (S, H, -CH-C=O-); 2.54-1.26 (m, 14H, -CH_2-); 2.29-2.24 (m, 3H, -CH-); 2.28-1.42 (S, 12H, Ar-CH_3); 1.52 (m, H, -CH-$(Me)_2$); 1.14-0.61 (m, 12H, CH_3). ESI-MS m/z=553 $[M+H]^+$. Anal. Calcd for $C_{39}H_{52}N_2O_2$: C, 80.64; H, 9.02; N, 4.82. Found: C, 80.29; H, 9.43; N, 4.48.

N-(o-methoxyphenyl)-acrylpimaramide (3h)

Yellow powder, m.p. 129.8-130.5°C, yield: 8.53 g (73%). IR (cm^{-1}): 3343 (N-H); 2929, 2866 (-CH_3, -CH_2); 1672 (N-C=O); 745 (Ar-H). ^{1}HNMR (DMSO. δ/ppm. 300MHz): 8.52, 8.34 (m, 2H, CONH-); 7.80-7.43 (m, 8H, Ar-H); 5.29 (S, H, C=CH-); 3.87 (m, 6H, -OCH_3); 2.68 (S, H, -CH-C=O-); 2.55-1.02 (m, 14H, -CH_2-); 2.16-1.87 (m, 3H, -CH-); 1.54 (m, H, -CH-$(Me)_2$); 1.16-0.67 (m, 12H, CH_3). ESI-MS m/z=585 $[M+H]^+$. Anal. Calcd for $C_{37}H_{48}N_2O_4$: C, 75.99; H, 8.27; N, 4.79. Found: C, 76.01; H, 8.58; N, 4.45.

N-(m-methoxyphenyl)-acrylopimaramide (3i)

White powder, m.p. 120.0-120.5°C, yield: 8.76 g (75%). IR (cm^{-1}): 3349 (N-H); 2927, 2864 (-CH_3, -CH_2); 1664 (N-C=O); 770, 686 (Ar-H). ^{1}HNMR (DMSO. δ/ppm. 300MHz): 8.39, 8.22 (m, 2H, CONH-); 7.38-6.91 (m, 8H, Ar-H); 5.29 (S, H, C=CH-); 3.79 (m, 6H, -OCH_3); 2.68 (S, H, -CH-C=O-); 2.53-1.28 (m, 14H, -CH_2-); 2.19-1.87 (m, 3H, -CH-); 1.54 (m, H, -CH-$(Me)_2$); 1.21-0.65 (m, 12H, CH_3). ESI-MS m/z=585 $[M+H]^+$. Anal. Calcd for $C_{37}H_{48}N_2O_4$: C, 75.99; H, 8.27; N, 4.79. Found: C, 75.70; H, 8.53; N, 4.43.

N-(p-methoxyphenyl)-acrylopimaramide (3j)

White powder, m.p. 130.8-131.5°C, yield: 8.99 g (77%). IR (cm^{-1}): 3324 (N-H); 2925, 2864 (-CH_3, -CH_2); 1661 (N-C=O); 825 (Ar-H). ^{1}HNMR (DMSO. δ/ppm.

300MHz): 8.38, 8.29 (m, 2H, CONH-); 7.31-7.08 (m, 8H, Ar-H); 5.31 (S, H, C=CH-); 3.17 (m, 6H, -OCH_3); 2.67 (S, H, -CH-C=O-); 2.51-1.26 (m, 14H, -CH_2-); 2.16-1.83 (m, 3H, -CH-); 1.55 (m, H, -CH-$(Me)_2$); 1.19-0.61 (m, 12H, CH_3). ESI-MS m/z=585 $[M+H]^+$. Anal. Calcd for $C_{37}H_{48}N_2O_4$: C, 75.99; H, 8.27; N, 4.79. Found: C, 75.86; H, 8.31; N, 4.59.

N-(p-fluorophenyl)-acrylopimaramide (3k)

White powder, m.p. 129.3-130.4°C, yield: 7.62 g (68%). IR (cm^{-1}): 3298 (N-H); 2927, 2865 (-CH_3, -CH_2); 1647 (N-C=O); 827 (Ar-H). ^{1}HNMR (DMSO. δ/ppm. 300MHz): 8.42, 8.28 (m, 2H, CONH-); 7.37-7.19 (m, 8H, Ar-H); 5.29 (S, H, C=CH-); 2.68 (S, H, -CH-C=O-); 2.52-1.27 (m, 14H, -CH_2-); 2.08-1.93 (m, 3H, -CH-); 1.55 (m, H, -CH-$(Me)_2$); 1.16-0.65 (m, 12H, CH_3). ESI-MS m/z=561 $[M+H]^+$. Anal. Calcd for $C_{35}H_{42}F_2N_2O_2$: C, 74.97; H, 7.55; N, 5.00. Found: C, 74.86; H, 7.53; N, 4.91.

N-(p-chlorophenyl)-acrylopimaramide (3l)

White powder, m.p. 156.4-157.4°C, yield: 7.71 g (65%). IR (cm^{-1}): 3338 (N-H); 2925, 2865 (-CH_3, -CH_2); 1665 (N-C=O); 824 (Ar-H). ^{1}HNMR (DMSO. δ/ppm. 300MHz): 8.45, 8.39 (m, 2H, CONH-); 7.40-6.83 (m, 8H, Ar-H); 5.26 (S, H, C=CH-); 2.68 (S, H, -CH-C=O-); 2.51-1.27 (m, 14H, -CH_2-); 2.49-2.30 (m, 3H, -CH-); 1.56 (m, H, -CH-$(Me)_2$); 1.18-0.59 (m, 12H, CH_3). ESI-MS m/z=594 $[M+H]^+$. Anal. Calcd for $C_{35}H_{42}Cl_2N_2O_2$: C, 70.81; H, 7.13; N, 4.72. Found: C, 70.67; H, 7.07; N, 4.57.

N-(p-bromophenyl)-acrylopimaramide (3m)

White powder, m.p. 140.7-141.4°C, yield: 9.55 g (70%). IR (cm^{-1}): 3344 (N-H); 2924, 2864 (-CH_3, -CH_2); 1665 (N-C=O); 822 (Ar-H). ^{1}HNMR (DMSO. δ/ppm. 300MHz): 8.41, 8.35 (m, 2H, CONH-); 7.38-7.23 (m, 8H, Ar-H); 5.35 (S, H, C=CH-); 2.67 (S, H, -CH-C=O-); 2.54-1.18 (m, 14H, -CH_2-); 2.03-1.87 (m, 3H, -CH-); 1.54 (m, H, -CH-$(Me)_2$); 1.16-0.64 (m, 12H, CH_3). ESI-MS m/z=683 $[M+H]^+$. Anal. Calcd for $C_{35}H_{42}Br_2N_2O_2$: C, 61.59; H, 6.20; N, 4.10. Found: C, 61.76; H, 6.38; N, 3.98.

N-(p-trifluoromethylphenyl)-acrylopimaramide (3n)

White powder, m.p. 158.9-160.2°C, yield: 5.28 g (40%). IR (cm^{-1}): 3343 (N-H); 2928, 2868 (-CH_3, -CH_2); 1671 (N-C=O); 838 (Ar-H). ^{1}HNMR (DMSO. δ/ppm. 300MHz): 8.64, 8.33 (m, 2H, CONH-); 7.58-7.26 (m, 8H, Ar-H); 5.31 (S, H, C=CH-); 2.69 (S, H, -CH-C=O-); 2.54-1.21 (m, 14H, -CH_2-); 2.06-1.92 (m, 3H, -CH-); 1.56 (m, H, -CH-$(Me)_2$); 1.17-0.66 (m, 12H, CH_3). ESI-MS m/z=661 $[M+H]^+$. Anal. Calcd for $C_{37}H_{42}F_6N_2O_2$: C, 67.26; H, 6.41; N, 4.24. Found: C, 67.12; H, 6.50; N, 4.08.

The newly synthesized compounds were screened for their antibacterial activity against two kinds of bacteria, *Staphylococcus aureus* and *Escherichia coli*. Some of them were comparable to reference Bromogeramine. The antimicrobial activities of the amide derivatives from APA against tested micro-organisms are presented in Table 2.

Table 2. Inhibition Zone (mean diameter of inhibition/mm) as a Criterion of Antibacterial Activity of the Newly Synthesized Title Compounds (256 μg/mL solution in ethanol)

Compound	*Escherichia coli* (Gram-negative bacteria)[b]	*Staphyloccocus aureus* (Gram-positive bacteria)[b]
3a	5.00	0.50
3b	0.06	0.01
3c	0.73	0.01
3d	1.00	0.30
3e	0.07	0.01
3f	5.50	0.93
3g	0.02	0.01
3h	1.67	0.38
3i	0.50	0.30
3j	0.01	0.01
3k	0.02	0.03
3l	0.51	0.01
3m	0.01	0.33
3n	0.01	0.01
Bromogeramine[a]	7.90	17.9

[a] Reference compound
[b] Values are mean of three independent trials

It was shown that compounds 3a, 3c, 3d, 3f, 3h, 3i, 3l, and 3m possess anti-bacterial activity. Among them, compounds 3a and 3f exhibited excellent anti-bacterial activity against *Escherichia coli*, whereas compounds 3a, 3d, 3f, 3h, 3l, and 3m showed mild anti-bacterial activity against *Staphylococcus aureus*. The non-modified rosin showed some antibacterial activity over the Gram-positive bacteria only (Goodson et al. 1999; Rao 2007; Soderberg et al. 1990; Soderberg et al. 1991). The Acrylopimaryl amide derivatives displayed extensive anti-bacterial activity against *Escherichia coli*. Among them, the inhibition zones against *Escherichia coli* of compounds 3a and 3f were 5 mm and 5.5 mm, respectively. This may be due to two reasons. First, the APA contains two carboxyl groups. So the amide derivatives from APA will have two amide groups, while other amides from abietic type acids of rosin have only one. This may increase the chance of the antibacterial agent to penetrate the cell wall via suitable bindings, and improve its antibacterial activity. On the other hand, aromatic rings are introduced to the amide backbone, as well as active atoms/groups such as fluorine, chlorine, bromine, and trifluoromethyl are added on the aromatic ring. These changes may also enhance the biological activity of the compounds. These results were noticeable and showed promising applications.

According to certain structure activity relationship (SAR), it can be concluded that the ortho-substituted phenyl derivatives (3c and 3h) and the meta-substituted phenyl derivatives (3d and 3i) exhibited higher activity against selected microorganisms than the para-substituted derivatives (3e and 3j), which suggests that the ortho-substituted phenyl

and meta-substituted phenyl compounds can well penetrate the bacterial cells by suitable binding (Liu et al. 2008; Tanitame et al. 2004). It has been reported that the introduction of aromatic groups and halogen groups into the amide backbone can increase the antibacterial activity (Scott et al. 2007; Xu et al. 2010); however, in this work, the presence of halogen groups did not show a positive effect on the antibacterial activity of the compounds. The combination of the above facts reveals that the specific binding of the phenyl rings to the bacterial cells plays an important role in the amide's antibacterial process. An electron-rich group is beneficial for such binding. For those halogen-containing compounds, the electron density of the phenyl rings was lowered by the halogen's electron-withdrawing effect. As a result, the binding process to specific sites of the bacterial cells for these compounds became difficult, and their antibacterial activity was thus decreased. Certainly, the structural elements are also important in the antibacterial activity of amide groups. Further investigation on the structure antibacterial activity relationship is needed.

CONCLUSIONS

1. APA was synthesized by a Diels-Alder addition reaction between rosin and acrylic acid. Fourteen amide derivatives from the APA were prepared in this work. The structures of the derivatives were determined and characterized by IR, ^{1}HNMR, MS, and elemental analysis. This was the first time that such compounds have been reported based on synthesis from APA. This approach has the potential to give rise to added value development of this natural resource.

2. The antibacterial activity of amide derivatives from APA against *Staphylococcus aureus* and *Escherichia coli* were investigated. The results indicated that compounds 3a and 3f show much higher antibacterial activity than others against *Escherichia coli*. Structure-activity relationship analysis revealed that ortho-substituted phenyl derivatives and meta-substituted phenyl derivatives exhibited higher activity than the para-substituted derivatives. Meanwhile, the halogen-substituted compounds did not show visible antibacterial activity compared with other compounds, which may have been caused by the lower electron density of the halogen-substituted phenyl rings.

3. The promising results obtained from these new derivatives justify their consideration as potential candidates. This work can be expected to promote the application of rosin in antimicrobial activity aspects.

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