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Pimaric Acid from Manila Elemi derived from *Canarium luzonicum* as a resolving agent in the resolution of racemic compounds

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Manila elemi, an oleoresin comprising mostly of resenes, resin acids and volatile oils, is one of the well-known minor forest products of the Philippines. It is an exudate of the bark of *Canarium luzonicum*, locally known as pili tree. It is exported mostly as raw and unprocessed resin which it has both pharmaceutical and industrial uses. In order to enhance the economic potential of Manila elemi, this study was undertaken to isolate its major chiral resin acid component, pimaric acid which is then derivatized into its amine form. Pimaric acid and pimaryl amine were evaluated for its use as potential acid and base resolving agents in the resolution of racemic α-phenyl ethylamine and racemic ibuprofen, respectively. For both compounds favorable resolutions of racemic substances were realized.

Keywords: Manila elemi; pimaric acid; diastereomeric salt formation; resolution of racemic compounds; (±)-a-phenyl ethylamine, (±)-ibuprofen

INTRODUCTION

The rational synthesis of the growing number of chiral chemicals (drugs, agrochemicals and intermediates) either on a laboratory or industrial scale, calls for efficient methods for providing these compounds in enantiomerically pure form instead of a racemate. Although many strategies may be considered (asymmetric synthesis, synthesis from the chiral pool, chromatography, etc.), optical resolution of a racemate via diastereomeric salt formation remains the most widely used method for preparing single and pure enantiomers.

The general procedure for this resolution involves the formation of salts from racemic acids or bases by neutralization with optically pure bases or acids, respectively. The required optically pure reactants (resolving agents) are often available from natural sources since living organisms usually produce only one enantiomer of a pair. Resolving agents therefore are chiral chemicals, which can be used to separate racemate into the individual enantiomers.

Plant extracts provide a large reservoir of chiral compounds that can be used as resolving agents. For example, Manila elemi, a valuable oleoresin from the trunk of a mature pili tree (*Canarium luzonicum*), is a good source of chiral resin acids. These resin acids are primarily used in the manufacture of ester gums for use in lacquers and varnishes. They are also used in the manufacture of metal resinates, soaps, plastics and paper sizes. These compounds, however, are not listed in the literature under the category of resolving agents.

In this particular study, a chiral resin acid from Manila elemi will be isolated and then derivatized into its amine form. These compounds will then be evaluated for their potential use as resolving agents in the resolution of some selected racemic compounds.

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EXPERIMENTAL

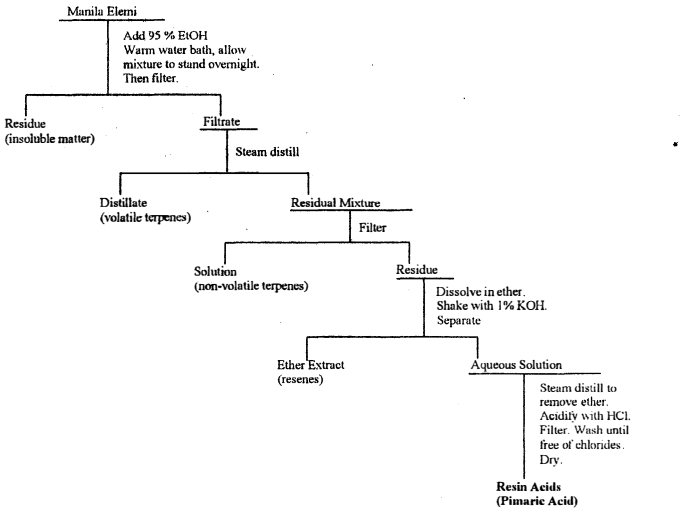
Sampling of Manila elemi. Manila elemi was purchased from a local dealer in Legazpi, Albay. It was collected from a mature pili tree, *C. luzonicum*. The tapping procedure involved making a series of cuts up the trunk of the tree, each cut resulting in the removal of bark and exudation of the oleoresin. The exuded sticky mass was collected at two-week intervals by scrapping it off the tree with a blunt-tipped bolo or stick.

General analytical procedure. Normal phase column chromatography with gradient elution was the technique employed in the purification of the products using silica gel 60 (63–200 mesh, Merck). Separation and reaction were monitored by thin layer chromatography using silica gel 60 F_{254} (Merck) pre-coated TLC plastic sheets. Dipping the plates in the eluent and then placing them in an iodine chamber or observing them under ultraviolet lamp enabled the visualization of the TLC spots.

Products were characterized by the conventional techniques. FTIR spectra were recorded using BIORAD FTS-40A spectrometer with KBr as reference. Mass spectral analyses were carried out using Finnigan MAT LCQ mass spectrometer. Optical activities of the resolved products were determined using Jasco CD-ORD spectrometer model J-715 and Atago polarimeter. HPLC analysis was done on Shimadzu SPD-10 AV chromatograph using UV-Vis (254 nm) as detector and LichroCart 250-4 (S, S)-Whelk-O-1) as chiral column. A Fisher-John melting apparatus was used to record the melting point of some of the products. The recorded melting points were all uncorrected.

Racemic ibuprofen used in the study was obtained from a commercial sample provided by United Laboratories, Inc. S-(+) ibuprofen (Aldrich), (+)-dehydroabietyl amine (Merck), (–) abietic acid (Fluka), racemic phenyl ethylamine (Fluka), S-(–)-phenyl ethylamine (Acros Organics) were used without further purification. All other solvents and reagents used in the experiment were of analytical grade. Solvents used in HPLC analysis were of HPLC grade. Solvents used for extraction, isolation, and purification were technical grade and were distilled before use.

Isolation of the resin acid components. To a 50-g sample of Manila elemi was added 600 mL of distilled 95% ethanol. The resulting mixture was warmed with occasional stirring until all the solids were dissolved. The light yellow solution was allowed to stand overnight and then filtered to remove insoluble matter consisting of foreign material. The filtrate was steam distilled to remove the volatile terpenes and the residual mixture left was filtered to remove the solid residue. The solid residue was dissolved in diethyl ether (200 mL) and extracted with 1% aqueous KOH solution (5 × 20 mL). The combined alkaline aqueous extracts were steam distilled to remove residual ether and then acidified (until pH 1) with concentrated HCl to precipitate the amorphous resin acids. The resin acids were filtered, washed with distilled water until filtrate is free with chlorides and then air-dried (see Scheme 1).



Scheme 1. Isolation of the resin acid components of Manila elemi

Separation and purification of pimaric acid. The crude resin acid extracts were chromatographed on a 2.0 cm column utilizing silica gel and employing gradient elution with hexane, hexane-ethyl acetate and ethyl acetate (10% increments). The major resin acid component was eluted with 7:3 hexane-ethyl acetate mixtures. After repeated recrystallizations from methanol, colorless crystals of the acid were obtained; $R_F = 0.64$ (hexane: ethyl acetate, 3:1) m.p. = 211–214°C; $[\alpha]$ 30/D = +90° (c = 0.5 in $CHCl_3$); λ_{max} = 220 nm; IR (KBr, cm^{-1}): 3168.65, 2937.08, 2643.76, 1694.32, 1466.61, 1277.5, 953.298; MS *m/z* (relative abundance): 302.2 (17) $[M^+]$, 286.2 (100) $[M-O]^+$, 240.2 (11) $[M\text{-}COOH\text{-}CH_3]^+$.

Synthesis of pimaryl amine. A solution of pimaric acid (3.78 g, 0.0125 mol) in $CHCl_3$ (20 mL) was placed in 1.5 mL of oleum (30% SO_3) and 4.5 mL of concentrated sulfuric acid in a 100 mL two-necked flask equipped with a reflux condenser and a dropping funnel. $CHCl_3$ (5 mL) was added and the temperature was raised to 45°C. The reaction mixture was stirred rapidly, after which 0.9 g of NaN_3 was added in small portions while maintaining the temperature at 35–45°C. The reaction was accompanied by foaming. After all the sodium azide has been added the temperature was raised so that the chloroform refluxes vigorously for 3 h. Then the reaction mixture was cooled and cautiously poured on to 25 g of crushed ice and then diluted with 150 mL of water. After 1 h, the solid was filtered at the pump, washed well with water and air-dried. The product amine is in the sulfate salt form. To convert it to free amine, 10% NaOH solution was added and then the free amine was extracted with diethyl ether. Repeated recrystallizations from ethanol afforded golden yellow viscous oil; $[\alpha]$ 27.5/D = +55° (c = 0.547 in CH_3OH); IR (film, cm^{-1}): 3400.23, 3319.18, 2927.56, 2867.61, 1612.73, 1498.53, 1463.19, 1382.78, 1062.78, 921.433, 883.629; MS *m/z* (relative abundance): 273.3 (100) $[M]^+$, 256.2 (51) $[M-NH_3]^+$, 258.1 (20) $[M-CH_3]^+$, 245.2 (19) $[M-CH_2=CH_2]^+$, 200.4 (18) $[M-CH_2=CH_2-3\,CH_3]^+$.

Diastereomeric salt resolution of racemic α-phenyl ethylamine using (+)-pimaric acid

(a.) To the solvent absolute methanol (25.00 mL) was added 1.19 g (9.82 mmol) of racemic α-phenyl ethylamine and 2.97 g (9.82 mmol) of pimaric acid. The resulting mixture was filtered and the residue transferred to a fresh 25 mL absolute methanol for another hour of stirring. The mixture was again filtered and the residue collected and air-dried. The residue was next treated with 10% NaOH to convert the amine salt to free amine which was then extracted with diethyl ether. The yield of the process was 27% of enriched α-phenyl ethylamine containing 60.17% R enantiomer or 20.34% enantiomeric excess.

(b.) The mother liquor obtained after salt formation was evaporated and the residue treated in the same manner as in 1 (a) above. The yield of the process was 23% of enriched α-phenyl ethylamine containing 76.56% S enantiomer or 53.12% enantiomeric excess.

Diastereomeric salt resolution of racemic ibuprofen using (+)-pimaryl amine

(a.) To the solvent absolute methanol (19.00 mL) was added 1.82 g (8.82 mmol) racemic ibuprofen and 2.52 g (8.82 mmol) of (+)-pimaryl amine. The resulting mixture was filtered and the residue transferred to a fresh absolute methanol (19.00 mL) for another hour of stirring. The mixture was filtered and the residue collected. The residue was next treated with 10% HCl to precipitate out the enriched ibuprofen which was then extracted with diethyl ether. The yield of the process was 22% of enriched ibuprofen containing 60% R enantiomer or 20% enantiomeric excess.

(b.) The mother liquor obtained after salt formation was evaporated and the residue treated in the same way as described above. The yield of the process was 25% of enriched ibuprofen containing 65% S enantiomer or 30% enantiomeric excess.

Analysis of the enantiomeric content of the resolved compounds. The enantiomeric content of the product obtained in the resolution of racemic α-phenyl ethylamine using (+)-pimaric acid was determined using CD-ORD methodology. The CD-ORD instrument was operated under the following measuring conditions.

Data mode: ORD	Response: 1 sec
Ch2- mode: HT	Speed: 200 nm/min
Range: 350–190 nm	Accumulation: 1
Bandwidth: 1.0 nm	Sensitivity: 1000 mdeg
Resolution: 0.5 nm	

A 1 cm quartz cell was used as sample holder. ORD values of sample solutions was measured and then converted to their corresponding molar rotations. Molar rotations were then related to % S-α-phenyl ethylamine using the prepared ORD standard curve.

Preparation of ORD standard curve. Standard solutions of racemic α-phenyl ethylamine and S-(–)-α-phenylethylamine were prepared (1.26×10^{-2} M) and combined at different proportions (Table 1). ORD values at 265–195 nm were determined, converted to molecular rotations and plotted against % S-(–)-α-phenylethylamine. From this standard curve, the % S-α-phenyl ethylamine and % R-α-phenyl ethylamine were determined for the resolved products.

Table 1. Volume ratio of S-α-phenyl ethylamine and racemic-α-phenyl ethylamine

Volume (mL) S-α-phenyl ethylamine[a]	*Volume (mL) Racemic-α-phenyl ethylamine*[a]	*% S*	*% R*
8	0	100	0
6	2	87.5	12.5
4	4	75	25
2	6	62.5	37.5
0	8	50	50

[a] 1.26×10^{-2} M

To analyze the enantiomeric content of the product obtained in the resolution of racemic ibuprofen using (+)-pimaryl amine, HPLC analysis was employed. A chiral column was used with the column specifications and operating conditions listed below.

Column: LichroCart™ 250-4 (S, S)-Whelk-O 1, 5 μm
Mobile phase: n-hexane/2-propanol/glacial acetic acid (99.5:0.5:0.5) (v/v)
Flow rate: 0.9 mL/min
Detection: UV (254 nm)
Injection vol.: 20 μL
Temperature: 30°C
Sample concentration: 1.00 mg in 10.00 mL solvent (mobile phase)

RESULTS AND DISCUSSION

Isolation, purification and characterization of the major resin acid of Manila elemi. The resin acid components of Manila elemi were found to have an average yield of 14.50% (s = 0.22, n = 3.

The resin acids were separated by means of flash column chromatography, from which the major resin acid component was eluted with 7:3 hexane-ethyl acetate mixtures. After three recrystallizations from methanol, colorless crystals of this resin acid were obtained. Yield was approximately 80%.

The IR spectrum of the major resin acid was taken to determine the functional groups present in the molecule. Table 2 lists the significant peaks in the IR spectrum of the major resin acid. The IR spectrum of the resin acid is shown in Fig. 1. The expected

Table 2. IR spectrum analysis of the major resin acid.

Wavenumber (cm^{-1})	*Peak or Band Assignment*
3168.65–2643.76	O-H stretch
2937.08, 2643.76	C-H (saturated) superimposed upon O-H stretch
1694.32	C=O stretch (carboxylic acid group)
1466.61	C-O-H bend
1277.5	C-O stretch
953.298	CHR = CH_2 stretch (vinyl group)

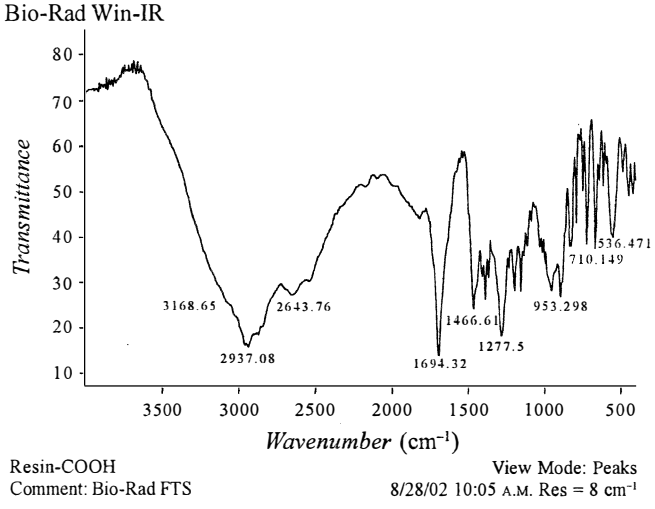


Fig. 1. FT-IR spectrum of pimaric acid

peaks in the IR spectrum of the resin acid include the O-H stretching of the carboxylic acid group in the region 3168.65–2643.76, the saturated C-H stretching of the alkyl group at 2937.08, 2643.76. One significant peak in the IR spectrum of the resin acid is the strong peak observed at 1694.32 which correspond to the C = O stretching of the carboxylic acid moiety. Another peak significant in the spectrum is at 953.298 that is due to vinyl group CHR = CH_2 present the resin acid.

Mass spectral analysis was used to verify the structure of the isolated resin acid by determining the molecular fragments corresponding to the mass to charge (m/z) peaks on the mass spectrum. Table 3 lists the significant peaks in the mass spectrum of the major resin acid. The mass spectrum of the major resin acid is shown in Fig. 2. Looking at the mass spectrum of the major resin acid, the parent peak is at 302.2 m/z that corresponds to its molecular mass. The m/z peak at 286.2 corresponds to the fragmentation of the molecule $[M - O]^+$, while that at 240.2 corresponds to the fragment where carboxylic acid and methyl groups were removed from the molecule, $[M - COOH - CH_3]^+$.

Table 3. Mass spectrum analysis of the major resin acid.

m/z	*Relative Abundance*	*Fragment*
302.2	17	[Molecular Ion]$^+$
286.2	100	$[M - O]^+$
240.2	11	$[M - COOH - CH_3]^+$

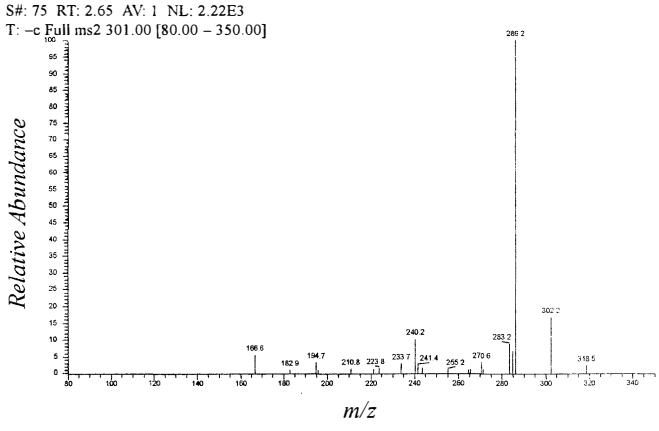
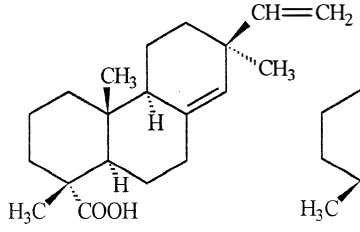


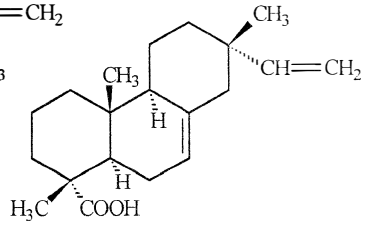
Fig. 2. Mass spectrum of pimaric acid.

The melting point and optical rotation were also determined to establish the identity of the major resin acid isolated.

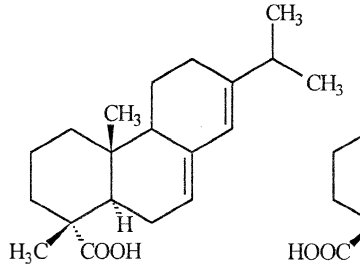
There are four possible resin acid isomers that may be present in Manila elemi. Their structures and some of their physical properties are shown below.



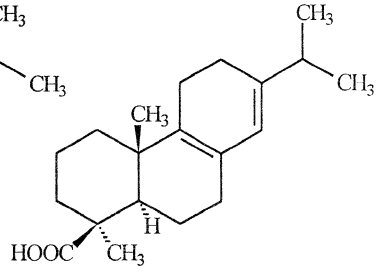
Pimaric Acid
m.p. = 217-219°C
$[a]_D^{20}$ = + 87.3° (c = 0.4 in $CHCl_3$)



Isopimaric Acid
m.p. = 160°C
$[a]_D^{16}$ = - 3.6 ° (c = 10.4 % solution in 1:1 alcohol: $CHCl_3$)



Abietic Acid
m.p. = 172-175°C
$[a]_D^{24}$ = -106° (c = 1 in abs. alcohol)



Palustric Acid
m.p. = 162-167 °C
$[a]_D$ = + 67.7°

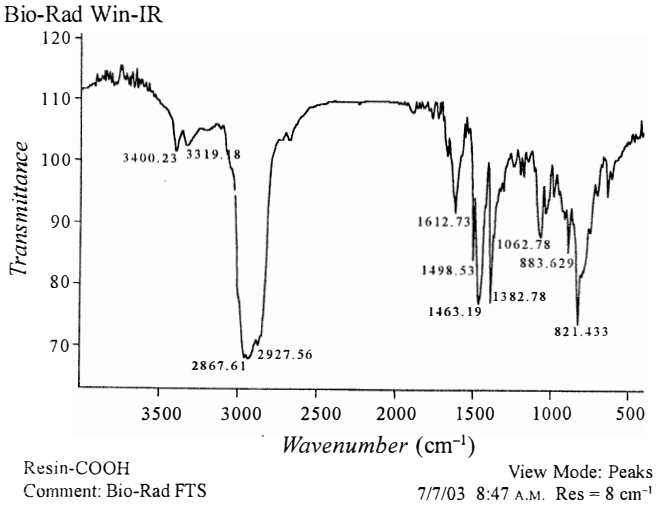


Fig. 3. FT-IR spectrum of pimaryl amine

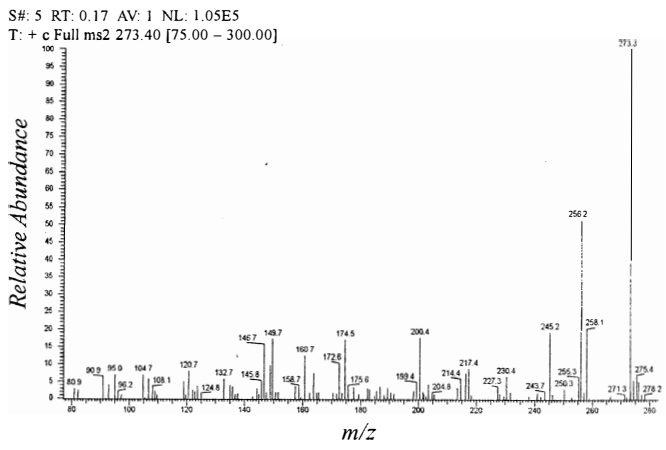


Fig. 4. Mass spectrum of pimaryl amine.

Table 4. IR spectrum analysis of pimaryl amine.

Wavenumber (cm^{-1})	*Peak or Band Assignment*
3400.23, 3319.18	N-H stretch, hydrogen bonded, primary amine
2927.56, 2867.61	C-H (saturated)
1612.73	N-H bend
1498.53, 1463.19	CH_2 (scissoring)
1062.78	C-N stretch
921–883.629	N-H wag

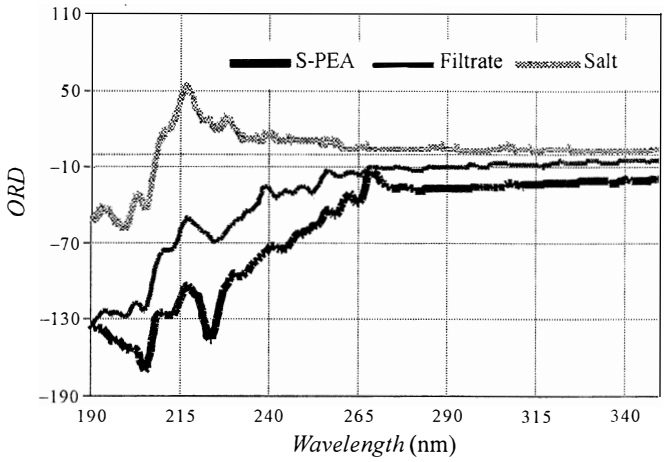


Fig. 5. Optical Rotatory Dispersion (ORD) spectrum of the resolved a-phenyl ethylamine with (+)-pimaric acid. From the graph shown, the resolution of a-phenylethyl amine gives a (+)-ORD spectrum for the salt or residue and a (–)-ORD spectrum for the filtrate as compared to the standard S-(–)-a-phenyl ethylamine.

Looking at the physical properties of these four isomeric resin acids, it can be established that the major resin acid isolated from Manila elemi is that of pimaric acid since the obtained melting point and optical rotation for the major resin acid isolate are similar to that of pimaric acid.

Synthesis of pimaryl amine. The Schmidt reaction was used to convert pimaric acid to pimaryl amine. This methodology involves the reaction of carboxylic acid with hydrazoic acid, HN_3, and H_2SO_4 where a primary amine is produced. The most important aspect of this reaction is that it preserves the chirality of the starting carboxylic acid. Thus this reaction was adapted for use in the preparation of pimaryl amine.

The IR spectrum of pimaryl amine was taken to determine the functional groups present in the molecule. Table 3 lists the significant peaks in its IR spectrum. The IR spectrum of pimaryl amine is shown in Fig. 3.

The expected peaks in the IR spectrum of pimaryl amine include the N-H stretching of the primary amine group in the 3400.23–3319.18 region, the saturated C-H stretching of the alkyl group at 2927.56, 2867.61, the N-H bend at 1612.73 and the C-N stretch at 1062.78.

Mass spectral analysis was used to verify the structure of the synthesized amine by determining the molecular fragments corresponding to the mass to charge (m/z) peaks on the mass spectrum.

Table 4 lists the significant peaks in the mass spectrum of the amine. The mass spectrum of pimaryl amine is shown in Fig. 4.

Looking at the mass spectrum of pimaryl amine, the parent peak is at 273.3 m/z that corresponds to its molecular mass. The m/z peak at 256.2 corresponds to the fragmentation $[M - NH_3]^+$, while that at 258.1 corresponds to the fragment $[M - CH_3]^+$.

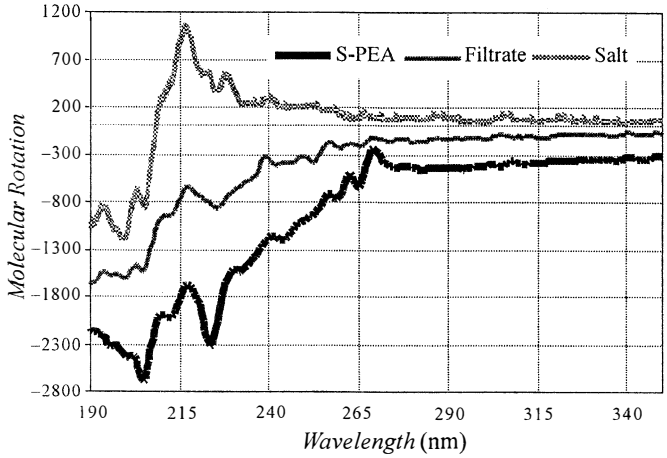


Fig. 6. Molecular rotation spectrum of the resolved a-phenyl ethylamine with (+)-pimaric acid. From the graph shown, the resolution of a-phenylethyl amine gives a (+)-rotation for the salt or residue and a (–)-rotation for the filtrate as compared to the (–)-rotation for the standard S-(–)-a-phenyl ethylamine.

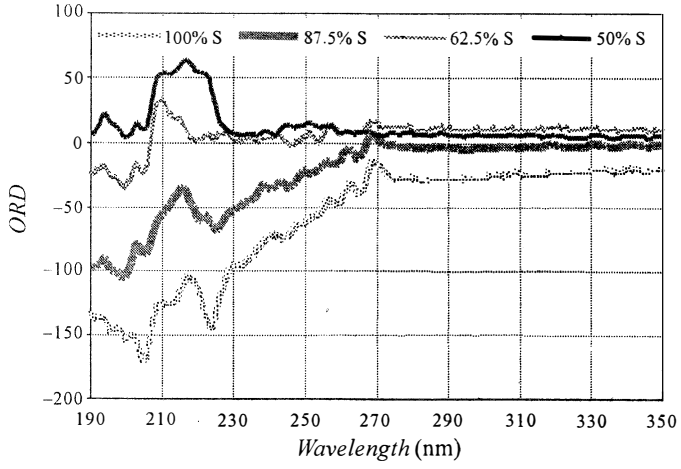


Fig. 7. Optical Rotatory Dispersion (ORD) Spectrum of Standard Solutions of S-(–)-a-phenyl ethylamine.

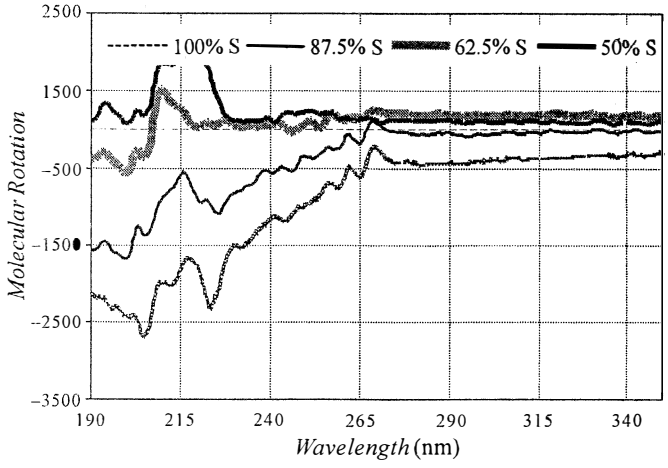


Fig. 8. Molecular Rotation spectrum of standard solutions. From this standard curve, the % S-a-phenyl ethylamine and % R-a-phenyl ethylamine were determined for the products of resolution of racemic a-phenyl ethylamine with (+)-pimaric acid.

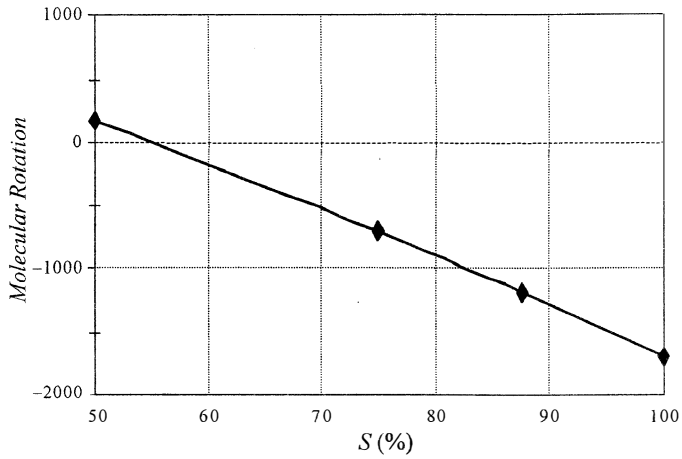


Fig. 9. Calibration plot at 224.5 nm of the standard solutions. From this plot, % S-a-phenyl ethylamine was determined for the products of resolution of racemic a-phenyl ethylamine with (+)-pimaric acid.

			Molar Rotation	% S
Slope	-34.01	Salt or Residue	401.116882	39.83
Intercept	1756.04	Filtrate	–848.074951	76.56
Reg. coeff.	0.9936004270			

The m/z peak at 245.2 corresponds to the fragmentation [M – $CH_2 = CH_2$]$^+$ and finally m/z peak at 200.4 for the fragment [M – $CH_2 = CH_2 - 3\ CH_3$]$^+$.

The optical rotation of the product amine was determined to have a value of [α] 27.5/D = +55° (c = 0.547 in CH_3OH), thus confirming the retention of chirality in the original compound.

Evaluation of pimaric acid and pimaryl amine as resolving agents. Diastereomeric salt resolution of racemic α-phenyl ethylamine using (+)-pimaric acid

(+)-Pimaric acid is an optically active acid and thus a very good candidate as acid resolving agent to racemic amines. In this study, racemic phenyl ethylamine was chosen as the model racemic amine for which (+)-pimaric acid could be used as resolving agent. Results were notably favorable. The enantiomeric content of the product obtained was determined using CD-ORD methodology. Figures 5 and 6 show the ORD and molecular rotation spectra of resolved α-phenyl ethylamine with (+)-pimaric acid. From the graph shown, the resolution of α-phenyl ethylamine gives a (+)-ORD curve or (+)-rotation for the salt or residue and a (–)-ORD curve or (–)-rotation for the filtrate as compared to the (–)-ORD or (–)-rotation for the standard S-(–)-α-phenyl ethylamine.

Figures 7 and 8 show the ORD and molar rotation plots of the standard solutions of S-PEA. From the standard calibration curve (Fig. 9), it was determined that the residue was enriched with 60.17% R enantiomer (20.34% ee) while the filtrate was enriched with 76.56% S enantiomer (53.12% ee).

Diastereomeric salt resolution of racemic ibuprofen using (+)-pimaryl amine. Racemic ibuprofen was used as model compound to be resolved by the synthesized (+)-pimaryl amine. The enantiomeric content of the resolved product was determined via HPLC analysis utilizing a Pirkle type chiral column. The first major peak on the HPLC chromatograms belong to R-ibuprofen while next to it is the peak for S-ibuprofen. S-ibuprofen was eluted out last because the chiral column used has more affinity for it than the R isomer. HPLC chromatograms revealed that the residue or salt was enriched with 60.1026% R-isomer while that of the filtrate was enriched with 65.3507 % S-isomer).

CONCLUSION

This study shows that (+)-pimaric acid is the major resin acid component of Manila elemi. Its potential use as acid resolving agent in the resolution of racemic a-phenyl ethylamine showed favorable separation where the R-enriched isomer is found in the residue and the S-enriched form in the filtrate. On the other hand, (+)-pimaryl amine, the synthesized amine form of (+)-pimaric acid, also showed promising results when used as basic resolving agent to racemic ibuprofen. Based on HPLC analysis, favorable separation of racemic ibuprofen was achieved where the R-enriched ibuprofen is found in the residue and the filtrate is enriched with S-ibuprofen. Although the % enantiomeric excesses for both compounds were low to moderate, the potential use of pimaric acid and pimaryl amine for affecting the resolution of racemic compounds can now be considered promising.

RECOMMENDATION

(+)-Pimaric acid has been isolated in moderate yield from Manila elemi. It is optically active acid and is a potential resolving agent for racemic amines. Therefore, other racemic organic amines must also be tried to be able to determine the extent of its potential use as acid resolving agent. Another chiral compound isolated in high yield from Manila elemi is (+)-amyrin. This compound has an –OH moiety and thus can be made to react with racemic carboxylic acids through an esterification reaction. A study is now being proposed to consider its potential use as a resolving agent in the resolution of racemic carboxylic acids through diastereomeric compound formation.

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