

## CHAPTER-III

### EXPERIMENTAL

Melting points are uncorrected. The petrol used throughout the investigation had b.p. 60-80°. All optical rotations were determined in chloroform solution. The I.R. spectra were recorded in Beckmann IR-20 spectrophotometer. The U.V. absorption spectra were taken in Beckmann DU-2 spectrophotometer in methanol solution. TLC was carried out on 12% silver nitrate impregnated silica gel G (E.Merck) plate and the spots were developed with sulfuric acid-acetic anhydride (1:9) mixture. Silica gel used for column chromatography was of 60-120 mesh (B.D.H) and alumina used for column chromatography was of active, basic grade (B.D.H).

#### Extraction of the Flowers of Eugenia Jambolana Lam and Separation of the Acid Part:

The dried and powdered flowers of Eugenia Jambolana Lam (2 kg) were extracted with benzene in a soxhlet apparatus for 24 hours. Benzene was distilled off and the gummy residue (85 g) was digested with ether. The ether solution was separated from ether-insoluble material by filtration. The ether solution was extracted with 10% aqueous potassium hydroxide solution (4 x 200 ml). The aqueous alkaline layer was acidified with cold dilute hydrochloric acid and the precipitated dark brown coloured acidic material (30g) was separated by filtration.

Chromatography of the Acid Part: Isolation of Crategolic

Acid (25):

A solution of the above acidic material (10 g) in benzene (100 ml) was placed over a column of silica gel (300 g) and the column was eluted with the following solvents (Table-I).

Table-I

Eluent	Fractions 250 ml each	Residue on evaporation
Benzene	1-4	Brown gum
Benzene: Ether (4:1)	5-18	Brown gum
Benzene: Ether (3:2)	19-27	Gummy solid (0.8 g), m.p. 232-272°
Benzene: Ether (2:3)	28-34	Gummy solid (0.3 g), m.p. 221-37°
Benzene: Ether (1:4)	35-41	Gummy solid (0.5 g), m.p. 259-266°
Ether	42-46	Trace gummy solid
Chloroform	47-51	Nil
Methanol	52-58	Gummy solid, m.p. 217-232°

The gummy solid (0.5 g) obtained from the fractions 35-41 (Table-I) was rechromatographed over a column of silica gel (30 g). The brownish solid (0.3 g) eluted with ether was dissolved in hot methanol, decolorised with active charcoal and filtered. Concentration of the methanol solution and dilution with water afforded crystalline crategolic acid (25, 0.2 g), m.p. 266-69° identical with an authentic specimen.

Found:	C, 76.09;	H, 10.16.
Calc. for $C_{30}H_{48}O_4$ :	C, 76.23;	H, 10.24%

IR  $\nu_{\text{max}}$  <sup>nujol</sup> 3350, 1680  $\text{cm}^{-1}$

Fig-1

Esterification of the Acidic Part: Isolation of Methyl Crategolate (26):

The ether solution of the total crude acid mixture (15' g) mentioned before was esterified with an ethereal solution of diazomethane prepared from nitrosomethylurea (15 g) in the usual way and the mixture kept overnight. The excess of diazomethane was decomposed with acetic acid (8 ml). The ether solution was washed with water, 10% aqueous potassium hydroxide solution and again with water till neutral and then dried ( $\text{Na}_2\text{SO}_4$ ). Evaporation

of ether gave a gummy residue (14.5 g). The crude ester mixture (14.5 g) dissolved in benzene (20 ml) was placed over a column of alumina (900 g; deactivated with 54 ml of 10% aqueous acetic acid) and the column was eluted with the following solvents (Table-II).

Table-II

Eluent	Fractions 250 ml each	Residue on evaporation
Petrol	1-12	Oil (2.4 g)
Petrol: Benzene (1:1)	13-20	Oil (1.1 g)
Benzene	21-33	Oily Solid (5.3 g), m.p. 104-54 <sup>o</sup>
Benzene: Ether (4:1)	34-39	Oil (0.4 g)
Benzene: Ether (3:2)	40-45	Oil (0.8 g)
Benzene: Ether (2:3)	46-51	Oil (0.6 g)
Benzene: Ether (1:4)	52-60	Oily Solid (1.2 g), m.p. 217-22 <sup>o</sup>
Ether	61-66	Gum (0.1 g)
Chloroform	67-75	Gum (0.2 g)

The solid (1.2 g) obtained from fractions 52-60 (Table-II) was rechromatographed over a column of alumina (54 g; deactivated with 3.2 ml of 10% aqueous acetic acid). The solid (0.9 g) eluted with ether on crystallisation from a mixture of benzene and petrol afforded crystals (0.6 g) of methyl crategolate (26), m.p. 224-27°, ( $\alpha$ )<sub>D</sub> 36° identical with an authentic specimen.

Found:	C, 76.32;	H, 10.18.
Calc. for C <sub>31</sub> H <sub>50</sub> O <sub>4</sub> :	C, 76.54;	H, 10.28%
IR $\nu$ <sub>max</sub> <sup>nujol</sup>	3200, 3100, 1710, 1230 cm <sup>-1</sup>	

Fig-2

Hydrolysis of Methyl Crategolate (26): Preparation of Crategolic Acid (25):

To a suspension of dry potassium tertiary butoxide (prepared from 2.8 g of metallic potassium and 35 ml of dry tertiary butanol) in distilled dimethyl sulfoxide (20 ml) was added a solution of methyl crategolate (25; 1 g) in distilled dimethyl sulfoxide (50 ml) and the reaction mixture was heated on an oil bath at 120° for 8 hours. The mixture was allowed to cool to room temperature and allowed to stand overnight. The reaction mixture was diluted with water (500 ml) and acidified

with cold dilute hydrochloric acid. The precipitated material was extracted with chloroform. The chloroform solution was washed with water till neutral and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the solvent gave a brown coloured gummy material (1.2 g) dissolved in benzene (12 ml) and was placed over a column of silica gel (75 g) which was eluted with the following solvents (Table-III).

Table-III

Eluent	Fractions 100 ml each	Residue on evaporation
Benzene	1-6	Oil
Benzene: Ether (4:1)	7-11	Oil
Benzene : Ether (3:2)	12-15	Trace oil
Benzene: Ether (2:3)	16-19	Trace oil
Benzene: Ether (1:4)	20-24	Trace oil
Ether	25-33	Partially crystalline solid (0.9 g), m.p. 262-67 <sup>o</sup>

Further elution with more polar solvents did not afford any solid material.

Fractions 25-33 (Table-III) were combined (0.9 g) dissolved in hot methanol, decolorised with active charcoal and filtered. Concentration of the methanol solution and dilution with water afforded crategolic acid (25, 0.6 g), m.p. 266-69° identical with the crategolic acid obtained earlier.

Acetylation of Crategolic Acid (25): Preparation of Crategolic Acid Diacetate (27):

To a solution of crategolic acid (25; 0.6 g) in pyridine (20 ml) was added acetic anhydride (20 ml) and the mixture was heated on a water bath for 12 hours. The reaction mixture was allowed to stand overnight at room temperature, then diluted with water (500 ml) and the precipitated material was extracted with ether. The ether layer was washed with water till neutral and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of solvent gave a slightly coloured residue (0.6 g) which was dissolved in benzene (6 ml) and placed on a column of silica gel (60 g) which was eluted with the following solvents (Table-IV).

Table-IV

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Eluent	Fractions 100 ml each	Residue on evaporation
Petrol	1-4	Oil
Petrol: Benzene (4:1)	5-8	Nil
Petrol: Benzene (3:2)	9-12	Nil
Petrol: Benzene (2:3)	13-16	Trace oil
Petrol: Benzene (1:4)	17-20	Trace oil
Benzene	21-25	Trace oil
Benzene: Ether (9:1)	26-36	Solid (0.5 g), m.p. 232-35 <sup>o</sup>
Benzene: Ether (4:1)	37-40	Trace oil

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Further elution with more polar solvents did not afford any solid material.

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The solid (0.5 g) from fractions 26-36 (Table-IV) was dissolved in hot methanol, boiled with active charcoal and filtered. Removal of the solvent gave a white solid (450 mg). The solid (450 mg) upon crystallisation from a mixture of ether and petrol afforded

needle shaped crystals (350 mg) of pure crategolic acid diacetate, (27), m.p. 234-37°, ( $\alpha$ )<sub>D</sub> 31°.

Found:	C, 73.26;	H, 9.35.
Calc. for C <sub>34</sub> H <sub>52</sub> O <sub>6</sub> :	C, 73.35;	H, 9.41%

IR  $\nu$ <sub>max</sub> <sup>nujol</sup> 1740, 1680, 1250 cm<sup>-1</sup>

Fig-3

Lead Tetraacetate Decarboxylation of Crategolic Acid Diacetate

(27):

To a solution of crategolic acid diacetate (27; 300 mg) in dry benzene (25 ml) was added pyridine (0.3 ml) followed by lead tetraacetate (0.5 g). The reaction mixture which immediately turned dark brown was stirred under nitrogen atmosphere for one hour at room temperature and then refluxed for 4 hours. The cooled mixture was filtered and the filtrate was concentrated under vacuum to yield a yellowish residue (300 mg) which was dissolved in benzene (3 ml) and placed on a column of alumina (30 g; deactivated with 1.8 ml of 10% aqueous acetic acid), which was eluted with the following solvents (Table-V).

Table-V

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Eluent	Fractions 50 ml each	Residue on evaporation
Petrol	1-12	Trace oil
Petrol: Benzene (4:1)	13-20	Foamy solid (63.3 mg), m.p. 114-170 <sup>o</sup>
Petrol: Benzene (3:2)	21-28	Trace oil
Petrol: Benzene (2:3)	29-32	Trace oil
Petrol: Benzene (1:4)	33-37	Trace oil
Benzene	38-42	Trace oil

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Further elution with more polar solvents did not afford any solid material.

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Examination of the Solid from Fractions 13-20 (Table-V):

Isolation of the Mixture of Diene Diacetates (30):

The foamy solid (63.3 mg) from fractions 13-20 (Table-V) showed a range of melting point from 114 to 170<sup>o</sup> and three spots on TLC.

Found: C, 77.73; H, 9.58.  
Calc. for  $C_{33}H_{50}O_4$ : C, 77.95; H, 9.44%

IR  $\nu_{\max}^{\text{nujol}}$  1740, 1240  $\text{cm}^{-1}$

Fig-4

UV  $\lambda_{\max}^{\text{MeOH}}$  237 ( $\epsilon$ , 9510), 244 ( $\epsilon$ , 10,050)  
and 252 nm ( $\epsilon$ , 7590).

The above physical data indicated that it was a mixture of the diene diacetates represented by structure (30).

Acid Isomerisation of the Mixture of Diene Diacetates (30):

Preparation of 2 $\alpha$ , 3 $\beta$ -Diacetoxy-28-nor oleana-12,17-diene (21):

To a solution of the above mixture of diene diacetates (30; 60 mg) in glacial acetic acid (3 ml) was added a 2(N) sulfuric acid solution (0.3 ml). The mixture was heated on a water bath for 2.5 hours, cooled to room temperature and poured into ice-cold water (100 ml), whereby a yellowish solid separated out. The latter solid was extracted with ether, and the ether layer was washed with water till neutral and dried ( $\text{Na}_2\text{SO}_4$ ). Removal of the solvent gave a yellow gum (66.3 mg) which was dissolved in benzene (3 ml) and placed on a column of alumina

(12 g; deactivated with 0.7 ml of 10% aqueous acetic acid). The column was eluted with the following solvents (Table-VI).

Table-VI

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Eluent	Fractions 25 ml each	Residue on evaporation
Petrol	1-10	Trace oil
Petrol: Benzene (9:1)	11-17	Trace oil
Petrol: Benzene (4:1)	18-35	Solid (40 mg), m.p. 184-86 <sup>o</sup>

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Further elution with more polar solvents did not afford any solid material.

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The solid (40 mg) from fractions 18-35 (Table-VI) on crystallisation from a mixture of chloroform and methanol afforded needle shaped crystals (25 mg) of 2 $\alpha$ , 3 $\beta$ -diacetoxy-28-nor oleana-12, 17-diene (21), m.p. 189-90<sup>o</sup>.

TLC : Single spot Rf = 0.34, Solvent- Benzene.

Found: C, 77.83; H, 9.53.

Calc. for C<sub>33</sub>H<sub>50</sub>O<sub>4</sub>: C, 77.95; H, 9.44%

U.V.  $\lambda_{\text{max}}^{\text{MeOH}}$  237 ( $\epsilon$ , 27,000), 244 ( $\epsilon$ , 28,300)  
and 252 nm ( $\epsilon$ , 20,200).

IR  $\nu_{\text{max}}^{\text{nujol}}$  1745, 1650 (w), 1255, 1220  $\text{cm}^{-1}$

The  $2\alpha$ ,  $3\beta$ -diacetoxy 28-nor oleana-12, 17-diene (21) synthesised in this manner was found to be identical (m.m.p, U.V. absorption, Co-TLC, superimposable IR spectra - Fig-5) with an authentic specimen of  $2\alpha$ ,  $3\beta$ -diacetoxy-28-nor oleana-12, 17-diene (21) previously obtained<sup>7,8</sup> from the degradation of baccatin (7a).