Chapter 4

EXPERIMENTAL & REFERENCES

Experimental – Related to Chemical work

All the melting points were determined by open capillary method and are uncorrected. All the microwave reactions were carried out in a 10ml sealed glass tubes in a focused mono-mode microwave oven,"Discover" by CEM Corporation, Matthews, NC at 100W(100°C). The NMR spectra were recorded in $CDCl_3$ solutions at ambient temperature on a Bruker Avance 300 MHz NMR spectrometer using 5mm BBO probe. The chemical shift δ are given in ppm related to tetra methyl silane (TMS) as internal standard. The coupling constant (*J*) are reported in Hz. The IR spectra were recorded in Shimadzu FT-IR spectrophotometer in KBr discs.

General procedure for the synthesis of 2, 3- diketo compounds

3-keto triterpenoids (1mol) suspended in potassium tertiary butoxide (prepared from 6g of potassium and 60ml of tertiary butanol) was shaken in a stream of oxygen for two hours. The reaction mixture was then diluted with water and then 6N HCl was added till the solution was acidic. It was then extracted with $CHCl_3$ (100ml) and the combined extract was dried (Na_2SO_4) and the solvent was removed under reduced pressure to yield a colourless solid which after crystallization from $CHCl_3$-MeOH mixture afforded crystals (0.5g). Some of the prepared diketones were compared with the authentic samples prepared earlier [75, 76].

General procedure for the synthesis of 1, 4-pyrazine derivatives

A solution of the substrate (0.001mol) in dry ethylene diamine was taken in a 10 ml sealed tube. Small pieces of Li (0.001mol) metal were then added to the solution of the sample. The reaction mixture was then irradiated under microwave. The reaction mixture was cooled, excess lithium was destroyed by solid ammonium chloride, diluted with cold water, extracted with ether and purified by column chromatography (over silica gel) followed by crystallization.

Extraction of Friedelin

2 kgs of finely powdered cork was extracted with petroleum ether in a soxhlet apparatus for 24 hours. After removal of the solvent, a white solid separated out. The solid was dissolved in minimum volume of benzene and chromatographed over silica gel column developed with petroleum ether. Elution of the column with petroleum ether gave shining crystals of friedelin [93], m.p. 261-263°C, $[\alpha]_D$ –48.7°.

IR: 1720 cm^{-1}

Auto oxidation of Friedelin

The oxidation was carried out following the general method as described above. The compound obtained was purified (column chromatography) and crystallised from a mixture of $CHCl_3$ – MeOH, m.p. 263-264°C, $[\alpha]_D$ + 16° [Lit 94, m.p. 265-67°C, $[\alpha]_D$ + 16.5°] characterized as 2,3-diketofriedelin by comparison with an authentic sample (m.m.p, CO TLC, CO IR)

	%C	%H
Analysis report found	81.8	10.90
Calculated for $C_{30}H_{48}O2$	81.80	10.90

TNM test: Positive

UV: 269 nm (ε=4.07)

IR: 3600, 3200(OH), 1685 (C=O), 1665, 1610, 840 cm^{-1}

Preparation of 1, 4-pyrazine derivative of friedelin (1b)

The reaction was carried out following the general method as described above. The compound obtained was purified by column chromatography followed by crystallization from a mixture of $CHCl_3$ - MeOH and characterized by spectral analysis.

Table 1. Chromatography of 1, 4 -pyrazine derivative of Friedelin

Eluent	Fractions 50 ml each	Residue	M.P.
Petroleum ether	1- 4	—	—
Pet. ether + ethyl acetate (96:04)	5-10	Solid	228°C

Examination of fraction 5-10 and isolation of compound 1b (1, 4-pyrazine derivative of friedelin)

Crystallization of the compound from $CHCl_3$-MeOH mixture furnished A, analyzed for $C_{32}H_{50}N_2$, m.p. 228°C, IR at 1650-70, 1430, 1120 cm^{-1} for pyrazine ring [69]. UV absorption maxima at 272 nm (ε = 5800) and 278 nm (ε = 5450). Anal. calc.: 83.12% C, 10.82% H; found 83.10% C, 10.81% H. It showed no depression in melting point when mixed with authentic sample of 1, 4-pyrazine derivative of friedelin and was found identical with the original sample of 1, 4-pyrazine derivative of friedelin (mmp, CO IR, CO TLC and spectral data)

UV: λ_{max}^{MeOH} 272 nm (ε= 5800)
278 nm (ε=5450)

IR: ν_{max}^{Nujol} 1650-70, 1430, 1120 cm^{-1}

^{1}H NMR ($CDCl_3$): 0.82-1.22 ppm (7s, 21H, 7t CH_3)
0.99 ppm (d, J = 6.5 Hz)
8.40 and 8.27 ppm (d, 2H, J = 3Hz)

Mass: m/z at 462.40[M^+], 463.40, 464.40.

Preparation of 1, 4 pyrazine derivative of taraxerone (2b)

The reaction was carried out following the general method as described above. The compound obtained was purified by column chromatography followed by crystallization from a mixture of $CHCl_3$ - MeOH and characterized by spectral analysis.

Table 2. Chromatography of 1,4-pyrazine derivative of taraxerone (2b)

Eluent	Fractions 50 ml each	Residue	M.P.
Petroleum ether	1-4	Nil	—
Pet. ether + ethyl acetate (96:04)	5-10	Nil	-
Pet. ether + ethyl acetate (94:06)	11-15	Solid	262°C

Examination of fraction 11-15 and isolation of compound 2b (1, 4-pyrazine derivative of taraxerone)

Crystallization from $CHCl_3$-MeOH mixture afforded B, analyzed for $C_{32}H_{48}N_2$, m.p. 262°C. IR peaks at 1600, 810, 1650, 1430 and 1120 cm^{-1}. The UV absorption maxima at 272nm (ε = 6150) and at 278 nm (ε = 5200). Anal. Calc.: 83.48% C, 10.43% H; found 83.40% C, 10.31% H. Mass spectrum showed molecular ion peak at m/z 460 as base peak. It showed no depression in melting point when mixed with authentic sample of 1, 4-pyrazine derivative of taraxerone and was found identical with the original sample of 1, 4-pyrazine derivative of taraxerone (mmp, CO IR, CO TLC and spectral data).

MeOH

UV: λ_{max} 272nm (ε = 6150)

278 nm (ε = 5200)

Nujol

IR: ν_{max} 1600, 810, 1650, 1430 and 1120 cm^{-1}

^{1}H NMR($CDCl_3$) : 0.81 to 1.25 ppm (8s, 24H, 8t CH_3), at 5.54 ppm (dd, 1H), 2.30 ppm (m, 1H), 8.40, 8.27 ppm (d, 2H, J = 3Hz).

Mass: 460.38, 461.39, 462.39.

Preparation of 1, 4 pyrazine derivative of methyl trichadenate (3b)

The reaction was carried out following the general method as described above. The compound obtained was purified by column chromatography followed by crystallization from a mixture of $CHCl_3$ - MeOH and characterized by spectral analysis.

Table 3. Chromatography of 1, 4-pyrazine derivative of methyl trichadenate (3b)

Eluent	Fractions 50 ml each	Residue	M.P.
Petroleum ether	1-4	Nil	—
Pet. ether + ethyl acetate (96:04)	5-10	Nil	-
Pet. ether + ethyl acetate (94:06)	11-15	Nil	-
Pet. ether + ethyl acetate (90: 10)	16-20	Solid	198°C

Examination of fraction 16-20 and isolation of compound 3b (1, 4-pyrazine derivative of methyl trichadenate)

Crystallization from $CHCl_3$-MeOH mixture afforded C, analyzed for $C_{33}H_{50}N_2O_2$, m.p.198°C. IR peaks at 1730, 1650–70, 1430, 1120 cm^{-1}. UV absorption at 272 nm (ε = 5785) and 278 nm (ε = 5600). Anal. Calc.: 78.26% C, 9.88% H, 5.53% N; found 78.25% C, 9.73% H, 5.50% N. Mass spectrum showed molecular ion peak at m/z 506 as base peak. ^1H NMR spectrum at 0.81, 0.85, 0.93, 0.99, 1.11 and 1.20 ppm (6s, 18H, 6t CH_3), at 0.74ppm (d, 3H, $CHCH_3$, J = 7Hz), at 3.67 ppm(s, 3H), at 2.30 ppm (m, 1H) and at 8.40 and 8.27 ppm (d, 2H, J = 3Hz). Thus from spectral analysis the structure for C has been assigned as 3b. It showed no depression in melting point when mixed with authentic sample of 1, 4-pyrazine derivative of methyl trichadenate and was found identical with the original sample of 1, 4-pyrazine derivative of methyl trichadenate (mmp, CO IR, CO TLC and spectral data)

Extraction of *Xanthoxylum budrunga*: Isolation of lupeol (1).

First collected barks of *Xanthoxylum budrunga* (3 Kg) plant from Darjeeling hilly region and dried it on sunlight and coarsely powdered. Then from these powdered materials the compounds were extracted using benzene as a solvent in a soxhlet apparatus for 30 hours. Benzene was distilled off and the gummy residue (15 g) was taken with ether solution (2 lit). The ether solution was washed by 10% aqueous sodium hydroxide (1.5 lit) solution. The aqueous alkaline layer was thoroughly shaken with ether to remove any neutral components that might be present on it. The portion was washed with water till neutral and dried by using sodium sulphate (Na_2SO_4). Ether was removed when a gummy residue of Lupeol was obtained. This residue dissolved in benzene (45 ml) and placed over a column of silica gel developed with petroleum ether and was eluted with the following solvents (Table 4).

Table 4. Chromatography of the *Xanthoxylum budrunga* extract

Eluent	Fractions each 100 ml	Residue on evaporation	Melting point
Petroleum ether	1—5	Oil	—
Petroleum ether + benzene (80:20)	6—8	Nil	—
Petroleum ether + benzene (70:30)	9—11	Nil	—
Petroleum ether + benzene (60:40)	12—19	Solid	212—213°C

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

Fractions 12—19 (Table 8) were mixed and crystallised by chloroform and methanol mixture. The white powdered compound (m.p. 212– 213°C) obtained was found to be identical[88] (m.m.p, CO IR, and CO TLC) with authentic specimen of lupeol (1).

IR: ν_{max}^{Nujol} 3610, 1020 cm^{-1}

3070, 1640, 887 cm^{-1}

^{1}H NMR($CDCl_3$,): δ_H 0.75, 0.77, 0.80, 0.92, 0.94 and 1.02, a vinyl methyl group at δ_H 1.66 (broad d J = 0.5 Hz)], a secondary carbinol group at δ_H 3.20 (dd, J = 9.6 and 6.2 Hz) and an exomethylene group at δ_H 4.58 (1H, triterpenoid[15-16] of lupeol) .

^{13}C NMR($CDCl_3$): δ_C 28.0 (C-23), 19.3 (C-30), 18.0 (C- 28), 16.1 (C-25), 15.9 (C-26), 15.4 (C-24), 14.5 (C-27), an exomethylene group at δ_C 150.8 (C-20), 109.3 (C-29) and a secondary hydroxyl bearing

carbon at δ_C 78.9 (C-3) in addition to ten methylene, five methine and five quarternary carbons.

Hydrogenation of lupeol: Preparation of lupanol (1a)

Lupeol (7 g) dissolved in a mixture of ethyl acetate and acetic acid (100 ml each) was shaken in an atmosphere of hydrogen in presence of PtO_2 catalyst for three hours until absorption of hydrogen ceased. Ethyl acetate was removed by distillation and the solution was diluted with water. A white solid (6.5 g) separated out which was collected by filtration. The solid obtained crystallised by using a mixture of chloroform and methanol which furnished colourless components 1a, m.p. 204°C, $[\alpha]_D$ +15°. The compound did not respond to the TNM test for unsaturation and Beilstein test for halogen indicating the absence of them in 1a. IR spectrum of the compound 1a, showed peak at 3330 cm^{-1} for hydroxyl functional group. The compound 1a was identified as lupanol by comparison (m.m.p., CO IR and CO TLC) with an authentic specimen of lupanol and by preparation of its acetate $C_{32}H_{54}O_2$, m.p. 243-244°C, $[\alpha]_D$ −1.6° [Lit 91 m.p. 245-246°C].

Jone's oxidation of lupanol: Preparation of lupanone (1b)

To a solution of lupanol (6 g) in pure acetone (600 ml) Jone's reagent was added drop wise with constant shaking until a faint orange colour persisted. The mixture was kept at room temperature for 1 h, diluted with water and extracted with ether. The ether layer was washed thoroughly with water, dried (Na_2SO_4) and evaporated. The residue (5.6 g) dissolved in benzene was chromatographed over a column of silica gel (150 g) developed with petroleum ether and then eluted with the following solvents (Table 5)

Table 5. Chromatography of oxidized lupanol residue

Eluent	Fraction 50 ml each	Residue on evaporation	Melting point
Petroleum ether	1–6	Nil	—
Petroleum ether +			

benzene (90:10)	7—11	Nil	—
Petroleum ether + benzene (80:20)	12—16	Nil	—
Petroleum ether + benzene (70:30)	17— 24	solid	209°—210°C

Further elution with more polar solvents did not yield any solid materials.

Fractions 17—24 were mixed and crystallised by using chloroform and methanol mixture. The crystallization furnished colourless solid 1c, m.p. 208°C, $[\alpha]_D$ + 15° [Lit m.p. 210°C, $[\alpha]_D$ +16.2°]

	%C	%H
Analysis report found	84.11	11.82
Calculated for $C_{30}H_{50}O$	84.52	11.74

TNM test: No yellow coloration.

IR: ν_{max}^{Nujol} 1712 cm^{-1} (CO)

Auto oxidation of lupanone

The oxidation was carried out following the general method as described above. The compound obtained was purified (column chromatography followed by crystallization from $CHCl_3$ – MeOH mixture) had m.p. 210-213°C, $[\alpha]_D$ 70.9°C and characterized as 2, 3 diketo lupanone by spectral data and by comparison with the data reported in literature 92[Lit m.p. 210-13°C]

	%C	%H
Analysis report found	81.79	10.90
Calculated for $C_{30}H_{50}O_2$	81.82	10.91

MeOH

UV: λ_{max} 270 nm (ε= 7932)

310 nm (in KOH)

Nujol

IR: ν_{max} 3640 (OH),

1670, 1650, 860 cm^{-1}

Neutral $FeCl_3$ colour test: Positive

Mass: m/z at 440 $[M^+]$, 425 $[M\text{-}CH_3]^+$, 397 $[M\text{-}CH(CH_3)_2]^+$, 312,231, 191, 154, 137, 123, 71, 57 (base peak).

Preparation of 1, 4 pyrazine derivative of lupanone (4b)

The reaction was carried out following the general method as described above. The compound obtained was purified by column chromatography followed by crystallization from a mixture of $CHCl_3$ - MeOH and characterized by spectral analysis.

Table 6. Chromatography of 1, 4 pyrazine derivative of lupanone

Eluent	Fractions 50 ml each	Residue	M.P.
Petroleum ether	1-4	Nil	—
Pet. ether + ethyl acetate (96:04)	5-10	Nil	—
Pet. ether + ethyl acetate (94:06)	11-16	Nil	—
Pet. ether + ethyl acetate (90: 10)	17-24	solid	220°C

Further elution with more polar solvents did not yield any solid materials.

Examination of fraction 17-24 and Isolation of compound 4b (1, 4-pyrazine derivative of lupanone)

Crystallization afforded fine needle shaped crystals of compound 4b, m.p. 220°C. It showed no depression in melting point when mixed with authentic sample of 1, 4-pyrazine derivative of lupanone and was found identical with the original sample of 1, 4-pyrazine derivative of lupanone [89] (mmp, CO IR, CO TLC and spectral data).

	%C	%H
Analysis report found	83.03	10.80
Calculated for $C_{30}H_{50}N_2$	83.12	10.91

UV: λ_{max}^{MeOH} 272 nm (ε= 5831)
278 nm (ε=5063)

IR: ν_{max}^{Nujol} 1650, 1430, 1120 (cm^{-1})

^{1}H NMR($CDCl_3$) : 0.78, 0.83, 0.98, 1.11, 1.29, 1.31, (6S, 18H,6t-CH_3)
0.77 and 0.86(2d, 6H, $CH(CH_3)$, 7 Hz)
2.47 and 3.04(2d, 2H, 2-CH_2, J=16Hz)
8.27 (d, 1H, J= 3 Hz)
8.41(dd, 1H, J=1 and 3 Hz) ppm

Mass: m/z at 462[M^+], 447, 419, 271, 258, 257, 256, 242, 241, 149, 123, 57.

Extraction of *Biscofia javanica* blume: Isolation of betulinic acid (6)

First collected the bark of Biscofia javanica blume from Darjeeling hilly region dried on sunlight and coarsely powdered (2.5 kg). These powdered materials were extracted with benzene in a soxhlet apparatus for 36 hours. Benzene was distilled off and

the gummy residue (12 g) was taken up in ether (1 lit). The ether solution was washed with 10% aqueous sodium hydroxide solution. The aqueous alkaline layer was thoroughly shaken with ether to remove neutral materials present in it. The aqueous layer was acidified (1 lit) when some insoluble solids separated out. The acidified portion was extracted with ether, washed with water until neutral and dried using sodium sulphate. Ether was removed when a gummy residue of betulinic acid (8 g) obtained and chromatographed. Elution by a mixture of benzene and ether (1:4) and crystallised from aqueous methanol afforded betulinic acid, m.p. 301—303°C.

Esterification of betulinic acid: Preparation of methylbetulinate.

To the crude acid (8 g) dissolved in ether was added to a solution of diazomethane in ether prepared from nitrosomethylurea (4 g) and was kept overnight. Next day excess of diazomethane was destroyed by acetic acid (CH_3COOH, 2 ml). The ether solution was washed with water, 10% sodium bicarbonate solution and again with water until neutral and dried by using sodium sulphate. Evaporation of the ether yielded a gummy residue (4g). This crude ester dissolved in benzene (20 ml) was placed over a column of silica gel (100 g) developed with petroleum ether and was eluted with the following solvents (Table 7)

Table 7. Chromatography of the esterified betulinic acid residue

Eluent	Fractions 50 ml each	Residue on evaporation	M. P
Petroleum ether	1—6	Oil	—
Petroleum ether + benzene (90:10)	7—10	Nil	—
Petroleum ether + benzene (85: 15)	11—14	Nil	—
Petroleum ether + benzene (80:20)	15—20	Solid	221°—223°C

Further elution with more polar solvent did not yield any solid materials.

Examination of fractions 15—20: Isolation of methylbetulinate (7).

The solid compound obtained from the fractions 15—20 (Table 11) were mixed (3.8 g) and crystallised from a mixture of chloroform and methanol to afford a colourless needle shaped methylbetulinate, m.p. 221—223°C, $[\alpha]_D$ +5.0°, identical with the original sample (m.m.p, CO IR and CO TLC) (Lit m.p 224—225°C, $[\alpha]_D$ +5.0°)

	% C	% H
Analysis report found	78.71	10.59
Calculated for $C_{31}H_{50}O_3$	79.10	10.71

IR: ν_{max}^{Nujol} 3540 cm^{-1} (—OH)
1730 cm^{-1} ($-COOCH_3$)
1660,
890 cm^{-1} ($=CH_2$)

Hydrogenation of methylbetulinate: Preparation of methyldihydrobetulinate (8).

Methylbetulinate (3.8 g) dissolved in ethyl acetate (200 ml) was shaken in an atmosphere of hydrogen in presence of palladium in charcoal catalyst (200 mg)for three hours until absorption of hydrogen ceased. Ethyl acetate was removed by distillation after filtering off the catalyst. The solution was diluted with water whereas a white solid (3.1 g) Separated out which was collected by filtration. Crystallization from a mixture of chloroform and methanol furnished colourless needle shaped of a compound m.p. 235—237°C, $[\alpha]_D$ +17.0°. This compound was found to be identical with an authentic sample of methyldihydrobetulinate (m.m.p, CO TLC, CO IR).

IR: ν_{max}^{Nujol} 3540 (—OH), 1705 cm^{-1} ($COOCH_3$)

Jone's oxidation of Methyldihydrobetulinate: Preparation of methyldihydrobetulonate (9)

To a solution of Methyldihydrobetulinate (2.95 g) in pure acetone added Jone's reagent drop wise with constant shaking until a faint orange colour persisted. The mixture was kept at room temperature for 1 h, diluted with water and extracted with ether. The ether layer was washed thoroughly with water, dried (Na_2SO_4) and the ether evaporated. The residue (2.5 g) dissolved in minimum volume of benzene was chromatographed over a column of silica gel (50 g). The chromatogram was developed with petroleum ether and eluted with the following solvents (Table 8)

Table 8. Chromatography of the oxidized methyldihydrobetulinate residue

Eluent	Fractions 50 ml each	Residue on evaporation	M.P.
Petroleum ether	1—5	Nil	—
Petroleum ether + benzene (90:10)	6—9	Nil	—
Petroleum ether + benzene (85:15)	10—13	Nil	—
Petroleum ether+ benzene (80:20)	14—23	Solid	196°—198°C

Further elution with more polar solvent did not afford any solid materials.

Fractions 14—23 (Table 8) were mixed and on crystallization from methanol furnished needle shaped crystals of methyldihydrobetulonate, m.p. 190—192°C, $[\alpha]_D$ +8.2°, identical with an authentic sample of methyldihydrobetulonate (m.m.p, CO TLC, CO IR) [90] [Lit m.p. 194°C, $[\alpha]_D$ + 8.4°].

	% C	% H
Analysis report found	79.22	10.56
Calculated for $C_{31}H_{50}O_3$	79.10	10.71

Nujol

IR: ν_{max} 1730 (—COOMe)
1708 cm^{-1} (CO)

Auto oxidation of methyldihydrobetulonate (9)

The oxidation was carried out following the general method as described above. The compound obtained was purified (column chromatography) followed by crystallization from $CHCl_3$ – MeOH mixture afforded colourless crystals, m.p. 131-132oC, $[\alpha]_D$ - 1.94° [95] [Lit m.p. 131-133°, $[\alpha]_D$ - 1.96°]. It gave a positive ferric chloride coloration for diosphenol and was identified as 2, 3-diketomethyldihydrobetulonate (5b).

Nujol

IR: ν_{max} 3460, 1730, 1670, 860 cm^{-1}

MeOH

UV: λ_{max} 271 nm (ε=7829), 310 nm (alkali shift)

Preparation of 1, 4-pyrazine derivative (5b) of methyldihydrobetulonate (9).

The reaction was carried out following the general method as described above. The compound obtained was purified by column chromatography followed by crystallization from a mixture of CHCl3 - MeOH and characterized by spectral analysis.

Table 9. Chromatography of 1,4-pyrazine derivative of methyl dihydrobetulonate (5)

Eluent	Fractions 50 ml each	Residue	M.P.
Petroleum ether	1-4	—	—
Pet. ether + ethyl acetate (96:04)	5-10	nil	
Pet. ether + ethyl acetate (94:06)	11-15	nil	-
Pet. ether + ethyl acetate (90: 10)	16-20	-	-
Pet. ether + ethyl acetate (86: 14)	21-25	-	220°C

Examination of fraction 21-25 and Isolation of compound 5b (1, 4-pyrazine derivative of methyldihydrobetulonate)

Crystallization afforded compound E, $C_{33}H_{50}O_2N_2$. m.p. 220°C. IR spectrum showed peaks at 1710 cm^{-1}(CO_2Me); 1665, 1430 and 1120 cm^{-1}. UV spectrum showed absorption maximum at 272nm (ε = 5712) and 278 nm (ε = 5603). Anal. Calc.: 78.26% C, 9.88% H, 5.53% N; found 78.25% C, 9.73% H, 5.50% N. It showed no depression in melting point when mixed with authentic sample of 1, 4-pyrazine derivative of methyldihydrobetulonate and was found identical with the original sample of 1, 4-pyrazine derivative [98] of methyldihydrobetulonate (mmp, CO IR, CO TLC and spectral data)

MeOH

UV: λ_{max} 272 nm (ε= 5712)

278 nm (ε=5603)

Nujol

IR: ν_{max} 1710 cm^{-1}(CO_2Me); 1665, 1430 and 1120 cm^{-1}

^1H NMR($CDCl_3$): 0.82, 0.985, 0.99, 1.28, 1.305, 0.76 and 0.88 ppm (2d, 6H, $CH(CH)_3$, J = 7 Hz); 2.48, 3.04 ppm (2d, j = 16 Hz); 8.27, 8.41 ppm (2d, J = 3 Hz) and at 3.66 ppm (1s, ester methyl)

Mass: 491$[M\text{-}CH_3]^+$, 463$[M\text{-}CH(CH_3)_3]^+$, 447 $[M\text{-}COOCH_3]^+$, 432,431, 258, 256, 241, 191, 187, 175, 159, 147, 133, 95, 55.

Treatment of lupanone 1c with N-bromosuccinimide: Formation of 2, 2-dibromolupanone 2 and 2α–bromolupanone (3)

A solution of 1c (4 g) was mixed with dimethylsulphoxide (100 ml). N-bromosuccinimide was then added to it in small lots in order to keep the temperature of the reaction mixture below 25°C and the mixture was kept in dark place for 12 days. The mixture was extracted with chloroform and it washed several times with water, dried (Na_2SO_4) and the solvent removed under reduced pressure. The residue (3.7 g) was chromatographed over a column of silica gel (100 g). The chromatogram was developed with petroleum ether and eluted with the following solvents (Table 10).

Table 10 .Chromatography of the lupanone and N-bromosuccinimide mixture

Eluent	Fraction 50 ml each	Residue on evaporation	Melting point
Petroleum ether	1—4	Nil	—
Petroleum ether	5—10	Solid	209°–210°C
Petroleum ether + Benzene (90:10)	11—14	Nil	—
Petroleum ether + Benzene (85:15)	15—18	Nil	—

Petroleum ether + Benzene (80:20)	19—25	Solid	220°—222°C

Examination of fractions 5—10: Isolation of 2, 2-dibromolupanone (2)

The fractions 5—10 (Table 10) showed homogeneity on TLC plate, hence these were mixed (1.7 g) and crystallised from a mixture of chloroform and methanol to afford a needle shaped crystals, m.p. 209—210°C and was identified as 2,2-Dibromolupanone (**2**).

	% C	**% H**
Analysis report found	61.53	13.87
Calculated for $C_{30}H_{48}OBr_2$	61.41	13.32

UV: λ_{max}^{MeOH} 221 nm (ε= 7925) 313 nm (ε=25)

IR: ν_{max}^{Nujol} 1725 cm^{-1} (CO)

CD: λ_{max}^{CHCl3} 239 nm (ϕ= +4590.18), 320 nm (ϕ= −8977.85)

^{1}H NMR ($CDCl_3$): 0.77, 0.94, 1.09, 1.24 (5S, 15H, 5t—CH_3), 0.78 and0.86 (2d, 6H, 2S—CH_3, J= 7 Hz), 3.13 and 3.64 (2d, 2H, CH_2, J=16 Hz) ppm

Mass: m/z at 586, 584, 582 (M+), 567, 569, 571,539,541, 543, 504, 506, 489, 491, 461,463, 426, 425, 424, 409, 285, 283, 274, 231, 206, 205, 191, 171, 163, 123

Examination of fractions 19—25 (Table 10): Isolation of 2α–bromolupanone (3)

The fractions 19—25 (Table 10) were mixed (2.0 g) and crystallised from chloroform and methanol mixture to afford amorphous white solid, m.p. 222—223°C. It showed positive Beilstein test for bromine.

	% C	% H
Analysis report found	71.58	9.33
Calculated for $C_{30}H_{49}OBr$	71.15	9.68

UV: λ_{max}^{MeOH} 226 nm (ε=7015 nm)
309 nm (ε=40 nm)

IR: ν_{max}^{Nujol} 1723 cm−1

CD: λ_{max}^{MeOH} 297 nm (ϕ= 2625.80)

^{1}H NMR ($CDCl_3$): 0.77 (S, 6H, 2—CH_3), 0.92, 1.10, 1.13, 1.2 (4S, 12H, 4t-CH_3), 0.76 and 0.85 (2d, 6H-2S-CH_3, J=7 Hz), 2.65 (dd, 1H, 1-C-H, J= 12 and 6 Hz), 5.06 (dd, 1H, 2—CH, J=12 and 6 Hz) ppm.

Mass: m/z at 506, 504 (M), 491, 489, 463, 461, 426, 425, 285, 283, 274, 206, 191, 163, 149, 123, (base peak)

Treatment of 2,2-dibromo lupanone with hydroxylamine hydrochloride: Preparation of 2, 3-dioximino lupane (4) and the subsequent cyclization of the dioximino derivative to lupan[2,3-C]-1′,2′, 5′-oxadiazole (5)

2,2-dibromo lupanone **2** (1.4 g) dissolved in pyridine was refluxed with hydroxyl amine hydrochloride in ethanol. The compound obtained from the reaction was purified by

repeated crystallization from chloroform-methanol mixture to obtain a white amorphous powder of compound A, analyzed for $C_{30}H_{50}O_2N_2$, m.p. 193°C, $[\alpha]_D$ +21.6°.

IR: ν_{max}^{Nujol} 3200-3400 cm^{-1} (C=N).

UV: λ_{max}^{MeOH} 220 nm (€=5100).

Mass: m/z 469$[M]^+$ 441, 439, 425, 424(base peak), 422, 380, 341, 340, 299, 231, 191, 163, 149, 136, 122, 121, 95, 81, 69

Cyclisation of the dioximino derivative (4) to lupan[2,3-C]-1',2', 5'-oxadiazole (5)

The dioximino derivative, 4 (1 g) and dry DMF (3 ml) was taken in a 10 ml sealed glass tubes in a focused mono-mode microwave oven ("Discover" by CEM Corporation, Matthews, NC) at 100W(100°C) in only 10 minutes reaction time. Small pieces of Li (0.001mol) metal were then added to the solution of the sample. The reaction mixture was then irradiated under microwave. The reaction mixture was cooled, excess lithium was destroyed by solid ammonium chloride and the residue obtained after usual work up was crystallised by chloroform-methanol mixture which afforded compound **5**, analyzed for $C_{30}H_{48}ON_2$ m.p.249-50°C.

Mass: m/z 452, 437 $[M-CH_3]^+$, 409 $[M-CH(CH_3)_2]^+$,(base peak), 367,271, 259, 245, 231, 206, 191, 163, 149, 123, 121, 109, 95, 81, 55

Extraction of *Biscofia javanica* blume: isolation of betulinic acid (6)

First collected the bark of Biscofia javanica blume from Darjeeling hilly region dried on sunlight and coarsely powdered (2.5 kg). These powders were extracted with benzene in a soxhlet apparatus for 36 hours. Benzene was distilled off and the gummy residue (12 g) was taken up in ether (1 lit). The ether solution was washed with 10% aqueous sodium hydroxide solution. The aqueous alkaline layer was thoroughly shaken with ether to remove neutral materials present in it. The aqueous layer was acidified (1 lit) when some insoluble solids separated out. The acidified portion was extracted with ether, washed with water until neutral and dried using sodium sulphate. Ether was removed when a gummy residue of betulinic acid (8 g) obtained and chromatographed. Elution by a mixture of benzene and ether (1:4) and crystallised from aqueous methanol afforded betulinic acid, m.p. 301—303°C.

Treatment of methyldihydrobetulonate with N-bromosuccinimide: Formation of 2, 2-dibromomethyldihydrobetulonate (10) and 2α-bromomethyldihydrobetulonate (11)

A solution of methyldihydrobetulonate (2.2 g) in chloroform (100ml) was mixed with dimethylsulphoxide (50 ml). N-bromosuccinimide (2.5 g) was then added to the solution with constant shaking in order to keep the temperature of the reaction mixture below 25°C and the mixture was kept in dark place for 10 days. The residue (2 g) obtained after usual workup two spots on chromatoplate. So the residue was chromatographed over silica gel column. The chromatogram was developed with petroleum ether and eluted with the following solvents (Table 11).

Table 11. Chromatography of the methyldihydrobetulinate and N-bromosuccinimide residue.

Eluent	Fractions 50 ml each	Residue on evaporation	M.P.
Petroleum ether	1—5	Nil	—
Petroleum ether +	6—12	White solid	157—158°C

benzene (90:10)			
Petroleum ether + benzene (85:15)	13—18	Nil	—
Petroleum ether + benzene (80:20)	19—22	Nil	—
Petroleum ether + benzene (75:25)	23--28	White solid	121—123°C

Further elution with more polar solvent did not yield any solid material.

Examination of fractions 6-12 (Table 15): Isolation of 2,2-dibromomethyldihydrobetulonate (10)

Fractions 6--12 (Table 11) showed homogeneity on TLC plate. They were mixed together (0.7 g) and crystallised by using chloroform and methanol mixture which afforded 2,2-Dibromomethyldihydrobetulonate m.p.161—163°C. It gave positive Beilstein test for halogen.

	% C	% H
Analysis report found	58.86	12.75
Calculated for $C_{31}H_{48}O_3Br_2$	59.05	12.90

IR: ν_{max}^{Nujol} 1725 cm⁻¹ (COOMe)
1705 cm⁻¹ (CO)

UV: λ_{max}^{MeOH} 219 nm (ε=7879)

^{1}HNMR ($CDCl_3$): 0.76 to 1.22 for seven methyl
3.11 and 3.63 (2d, 1H, 1-CH2, J= 16 Hz)
3.65 (s, 3H, COOCH3) ppm.

Mass: m/z at 628,626, 624(M^+), 571569, 567 $[M=COOCH3]^+$ 550,548.547 533, 531, 525, 523, 468, 470,471,453, 412, 411, 410, 409, 283,285, 274, 231, 205, 203, 177(base peak) .

Examination of fractions 23—28 (Table 10): Isolation of 2α-bromomethyl dihydrobetulonate (11).

Fractions 23—28 (Table 11) were mixed (0.9 g) and on crystallization from chloroform methanol mixture afforded crystals of 2α—Bromomethyldihydrobetulonate, m.p. 126-128°C. responded Beilstein test for halogen.

	% C	% H
Analysis report found	67.46	13.40
Calculated for $C_{31}H_{49}O_3Br$	67.64	13.77

IR: ν_{max}^{Nujol} 1725 (COOMe), 1705 cm^{-1} (CO)

^{1}HNMR ($CDCl_3$): 0.94, 0.97, 1.09, 1.13, 1.20 (4s, 12H, 4t-CH_3)
0.77 and 0.87 (2d, 6H, 2S-CH_3, 1-CH_{-e}, J=7 Hz)

2.65 (dd, 1H, 1-CH_{-a}, J=12 Hz)
5.06 (dd, 1H, 2-CH, J=12 and 6 Hz) ppm

Mass: m/z at 550, 548, (M^+) (1:1), 491, 489 $[M=COOCH_3]^+$, 471, 470, 469 [M—Br]; 412, 411 (100%) 410, 395, 275, 260, 250, 205, 191, 177, 174, 119.

Treatment of 2, 2-dibromomethyl dihydrobetulonate with hydroxylamine hydrochloride: Preparation of 28-carbomethoxy-2,3-dioximinolupane (12) and the subsequent cyclization of the dioximino derivative to 28-carbomethoxy lupan[2,3-C]-1',2', 5'-oxadiazole (13)

2,2-dibromomethyldihydrobetulonate (0.8 g) dissolved in pyridine was refluxed with hydroxyl amine hydrochloride in ethanol. The compound obtained from the reaction was purified by repeated crystallization from chloroform-methanol mixture to obtain a white amorphous powder (0.7 g), analyzed for $C_{32}H_{53}O_4N_2$.

UV: λ_{max}^{MeOH} 220 nm (€=5100).

IR: ν_{max}^{Nujol} 3200-3400 cm^{-1} (C=N) 1720 cm^{-1} (–COOMe)

Mass: m/z 529[M]+ m/z 530, 531, (base peak), 424, 380, 341 340, 299, 231, 191, 163, 149, 136, 122, 121, 95, 81, 69.

Cyclisation of the dioxime to 28-carbomethoxy lupan [2,3-C]-1',2', 5'-oxadiazole

The dioximino derivative (0.7 g) and dry DMF was taken in a 10 ml sealed glass tubes in a focused mono-mode microwave oven ("Discover" by CEM Corporation, Matthews, NC) at 100W(100°C) in only 10 minutes reaction time. Small pieces of Li (0.001mol) metal were then added to the solution of the sample. The reaction mixture was then irradiated under microwave. The reaction mixture was cooled, excess lithium was destroyed by solid ammonium chloride and the residue obtained after usual work up was crystallised by chloroform-methanol mixture which afforded compound D analyzed for $C_{31}H_{48}O_3N_2$.

UV: λ_{max}^{MeOH} 223 nm (€=5169)

Nujol

IR: ν_{max} 1620 cm^{-1} (—C=N-O)

890 cm^{-1}, 1720 cm^{-1} (–COOMe)

^{1}HNMR ($CDCl_3$): 77 (d, 3H, J=7 Hz) 0.78, 0.86 (d, 3H, J=7 Hz), 0.97, 1.10

Mass: 498,(base peak) 496 m/z 449.7 $[M-CH_3]^+$, 367,271, 259, 245, 231, 206, 191, 163, 149, 123, 121, 109, 95, 81, 55.

Biocidal work

Details of the experimental procedure have been described in the Experimental section of Part I, Chapter 4.

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