Part II

STUDIES ON THE DEBROMINATION OF 2- AND 4-BROMO TRITERPENOIDS AND BIOCIDAL ACTIVITY OF THE DEBROMINATED COMPOUNDS IN COMPARISON TO THE RESPECTIVE BROMO DERIVATIVES.

CHAPTER I

A SHORT REVIEW ON THE DEBROMINATION REACTIONS OF DIFFERENT BROMODERIVATIVES INCLUDING THAT OF TRITERPENOID SKELETON AND BIOCIDAL ACTIVITY OF THE RELATED COMPOUNDS

This chapter is divided into two sections, Section A and Section B.

SECTION A:

A SHORT REVIEW ON THE DEBROMINATION REACTIONS

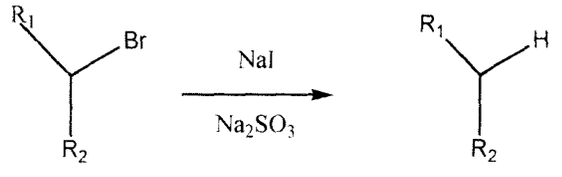
Triterpenoids represent a varied class of natural products. Thousands of structures have been reported with hundreds of new derivatives discovering each year. Among these are included the pentacyclic lupane and friedelin type triterpenes which are represented by a diverse assemblage of bioactive natural products. The author extracted several triterpenes from plants and studied their biological activities against different microorganisms and seeds and observed some interesting results.

So the author interested to introduce some groups or atoms to triterpenes through transformative debromination reactions. For this the author has undertaken these surveys to summarize the available literature. A number of computer based databases, journals and abstracts were utilized in literature search. Based on this search it was observed that more than 1000 publications were identified in which triterpenes were mentioned but very fewer publications mentioned the transformative debromination reactions of triterpenoids and biocidal activities of the derived compounds. A short review of the subject is presented here in this chapter.

The chemical methods of dehalogenation are important for degradation of halogenated compounds. Many reagents for reduction of α-bromoketones have been developed and could be divided in three categories: (1) reducing agents, (2) nucleophilic reducing agents, and (3) Pd-catalyzed hydrogenation. Among nucleophilic reducing agents, iodide ion has been used as a mild dehalogenating agent [1]. Excess amount of sodium iodide in the absence of any other additives or along with other carbonyl activating additive such as sulfuric acid, chlorotrimethylsilane have been employed for the reduction of α-haloketones. Recently, stoichiometric amount of HBr as bromide

source has been reported for regioselective bromination of α-haloketones in the presence of stoichiometric amount of sodium dithionite ($Na_2S_2O_4$) as a scavenger of bromine [2]. The limitations of these reaction conditions are the excess amount of reducing agent and additive, strong acidic condition or anhydrous conditions. During the study for the mild debromination condition, it occurred to us that catalytic amount of nucleophilic reducing agent could be a nice combination for the mild debromination [3]. The debromination using a catalytic amount of nucleophilic iodide anion in the presence of sulfur salts as a reducing agent was examined and it was found that α-bromoketones and *vic*-dibromides were debrominated efficiently (Scheme-I). The advantage of this method is that during the work-up, treatment of iodine monobromide with a large amount of sulphur reagent is not required.

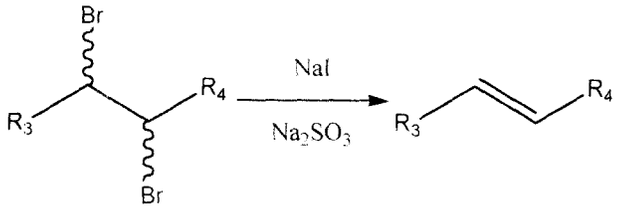
Scheme I



R_1 = ArCO and R_2 = H or Me

R_1 = BnOCO or PhNHCO and R_2 = H

R_1 = Ph and R_2 = CN



R_3 =Ar and R_4 = COMe or COPh or CO_2Me or Ph

Favorskii et al. [4] reported debromination using strong bases in a high boiling solvent but in practice those bases are corrosive to skin and clothing. They first reported *N,N*-diethylaniline as a dehydrobrominating agent. The viability of tertiary amines such as triethylamine, pyridine, quinoline and dimethylaniline as dehydrohalogenating agents have been demonstrated. In most reports these amines are used interchangeably without marked changes in reaction products or yields.

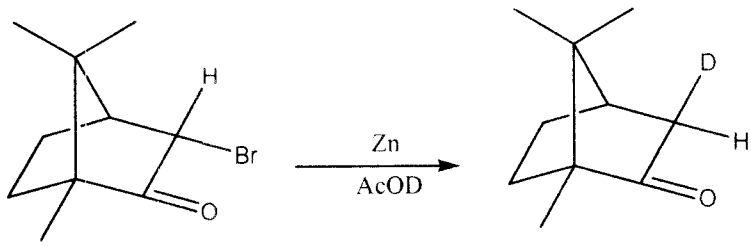
Stalick et al. [5] reported that in addition to the amine bases a few non-amine bases were also employed for debromination reactions; but in these cases the yields were unsatisfactory.

Curnow et al. [6] reported that debromination takes place by sodium hydride in THF followed by addition of aqueous hydrochloric acid; but the yield in each case was less than satisfactory and moreover, neither of them was attempted on triterpenoid skeleton.

Andy Hor et al. [7] reported Pd-catalysed reductive debromination of polybrominated benzens which also extended to polybrominated biphenyls (PBBs). A complete conversion of hexabromobenzene to benzene at room temperature had been achieved. Both of $PdCl_2[\ C_5H_4PPh_2)_2M]$ (M = Fe, Ru) showed excellent catalytic activities in the presence of $NaBH_4$ as a reducing agent and $Me_2NC_2H_4NMe_2$ as a base.

Thiemann et al. [8] found that zinc dust/sodium hydroxide/ammonium formate system is highly effective for the debromination of the ubiquitous pollutant tetrabromobisphenol A (TBPPA) to bisphenol A (BPA). Treatment of pure TBPPA with NaOH or with another alkali hydroxide in the presence of ammonium formate in an alcohol at elevated temperature (78-116^{0}C) leads to a successful debromination: an almost quantitative removal of TBBPA can be achieved.

Squers et al. [9] proved zinc-acetic acid to be a useful debrominating agent. A study of the zinc-acetic acid debromination of α-bromo camphor 1 was undertaken with the objective of evaluating the potential of the reaction to the stereo-specific synthesis of α-deuterio camphor 2.



1 2

Suzuki et al. [10] developed a low cost and high effective debromination of bromoform in a continuous flow system using zinc powder under mild conditions. The degradation efficiency with 3 gm zinc powder was namely 100% at temperature >40^{0}C. In the continuous flow system at 40°C, both the degradation efficiency and the bromide ion yield were relatively high with more than 4.5 gm of zinc metal. At 45^{0}C and 1 mL/minute treatment rate with 6 gm zinc metal powder, bromoform could be almost completely degradated for the long treatment (250 h). This research was promised to contribute to the treatment technology of bromoform contaminated ground water.

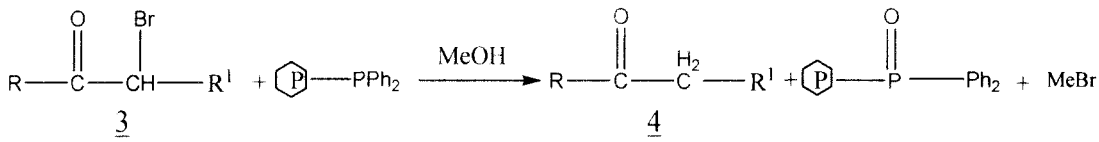
Jian Li et al. [11] found that the treatment of α-haloketones with 1.5 mole equilibrium of NH_4Cl in ethanol for 0.5 min gave the corresponding ketones with excellent yields under microwave irradiation. Applying their methodology, *vic*-dibromides and 2,2-dibromo acctophenone can be efficiently debrominated to alkenes and acetophenones respectively.

Majumdar et al. [12] reported that zinc with a trace of zinc chloride in methanol, ethanol, dioxane or diethyl ether is one of the most useful dehalogenating agents. However, the use of such low boiling solvents causes difficulties when the dehalogenated products have boiling points similar to those of solvents, when they form azeotropic mixtures with the solvents or when the products tend to polymerize in the reaction mixture and hence it is desirable to remove them as formed.

Sihai et al. [13] found that 1,4-dibromobenzene and 4,4′-dibromobiphenyl has been debrominated by a catalyst [$PdCl_2(PPh_3)_2$, $NiCl_2$(dppf) or $PdCl_2$ (dppf)] in a Schlenk tube.

Salunkhe et al. [14] reported the debromination of α-bromo ketones using polymer-supported triphenylphoshine as shown in Scheme-II. To a solution of the bromo ketone 3 in anhydrous benzene was added the insoluble phosphine reagent. This resulted in the formation of the phosphonium salt which was decomposed by alcohol to yield the corresponding ketone 4. The yield of the products and the rate of reaction, when carried out in toluene, acetonitrile, THF etc. were found to be very low. Polymeric phosphine oxide was obtained as a byproduct, which was then separated by filtration. Removal of the solvent under reduced pressure yielded the pure ketone 4.

Scheme-II



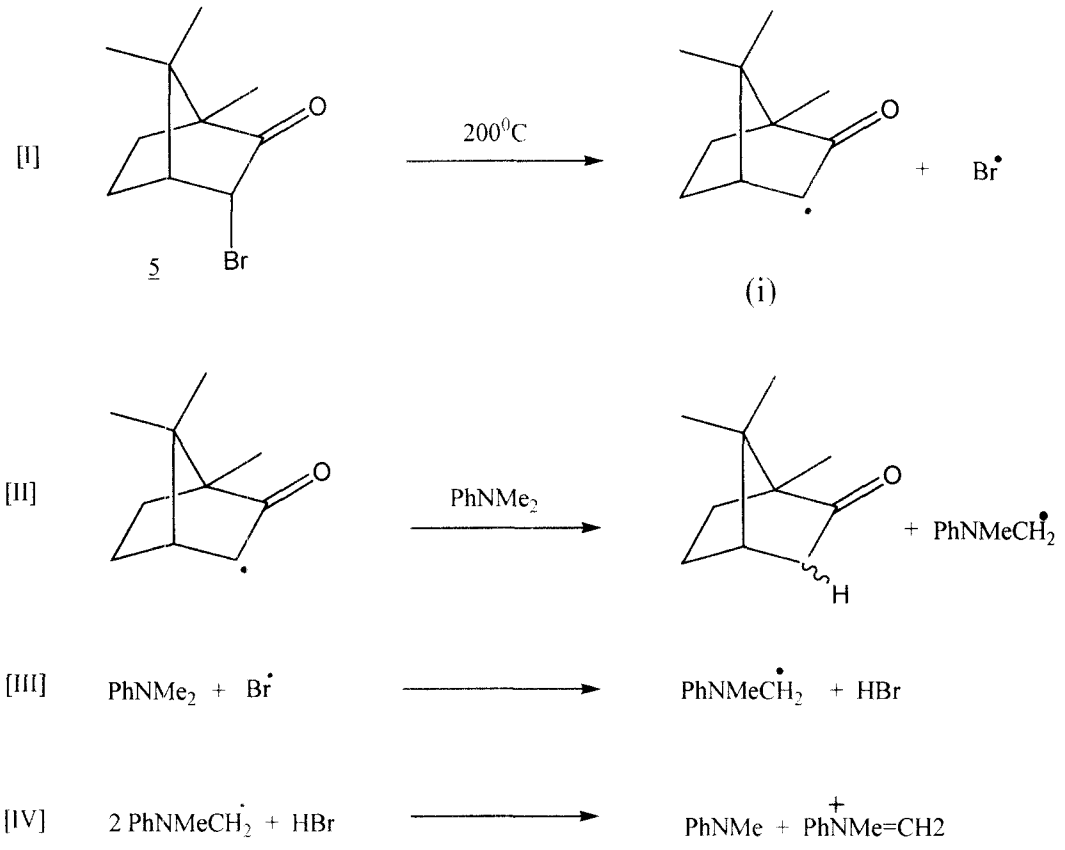
3 4

Chen et al. [15] reported that α-bromocamphor was reduced to yield camphor in the presence of *N,N*-dimethylaniline (DMA) at 200^{0}C. Scheme-III was suggested as the mechanism for the reductive debromination. The mechanism assumes that in a primary step the thermal cleavage of the carbon-bromine bond occurs readily at 200°C. Since the bond dissociation energy (BDE) for the carbon-bromine bond in 5 is expected to be approximately 64 kcal/mol (the carbon-bromine bond dissociation energy for α-bromoacetone) (III), it does not appear to be reasonable, even at high temperatures, that a non chain mechanism involving homolytic cleavage of the C-Br bond will lead to an efficient reductive debromination. In accord with this conclusion, the thermal decomposition of 5, itself, at 200^{0}C, yielded only a small amount of camphor and no other detectable products (II). The pathway leading to the oxidation product of DMA is likewise not clearly defined (II). Utilizing the thermolysis of 5 as an initiation step a chain sequence can be proposed that involves a halophilic abstraction of a bromine atom from 5. No direct evidence for this pathway was reported to be available, with the exception that the dehalogenation precedes by a chain reaction.

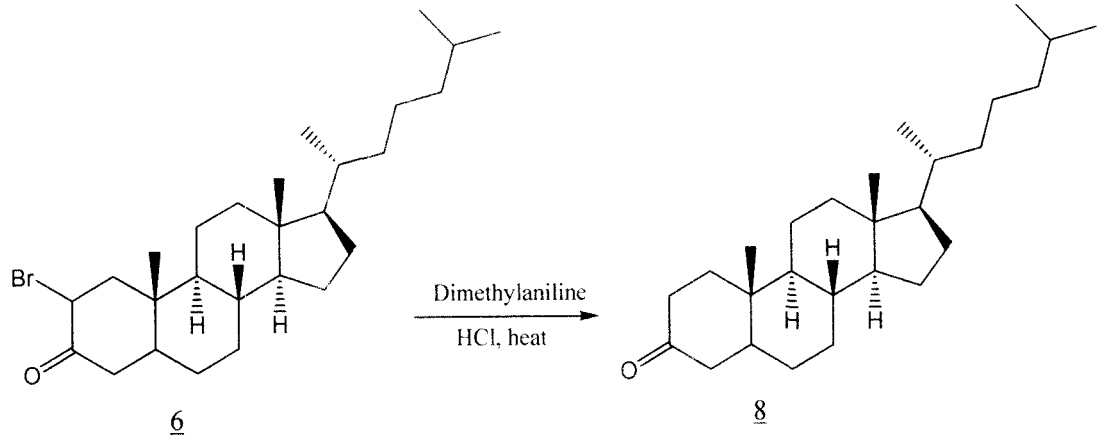
Since the dehalogenation of a number of α-haloketones by 1,3-dimethyl-2-phenylbenzimidazoline (DMBI) and *N*-benzyl-1,4-dihydronicotinamide (BNAH) proceeds via an electron transfer hydrogen abstraction chain sequence, a more likely

mechanistic rationalization for the reported debromination of 5, in the presence of an amine, is a similar chain sequence where ZH is *N,N*-dimethylaniline (DMA).

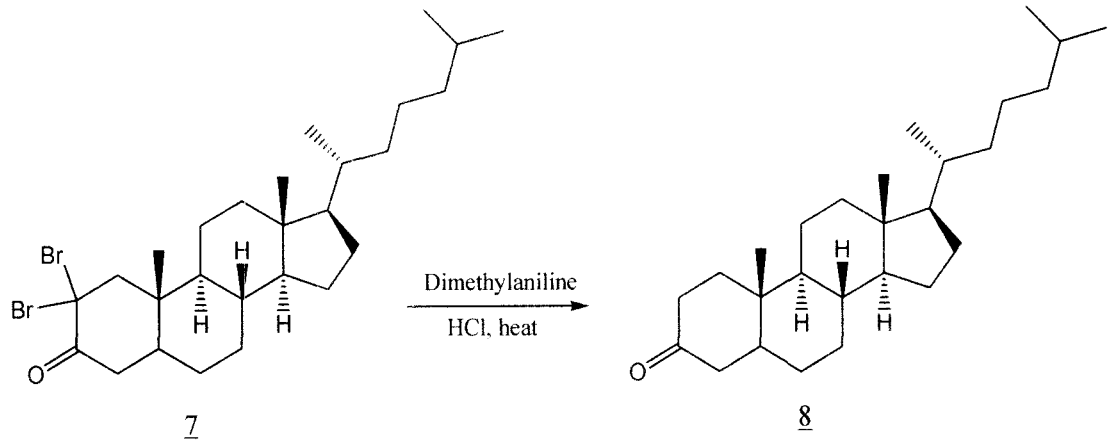
Scheme III



Schwenk et al. [16] found that the nature of the reagent used to remove hydrogen bromide from bromosterols has considerable influence on the course of reaction. Experiments with mono- and dibromocholestanone are illustrative of this point. From monobromocholestanone 6 and dibromocholestanone 7 by refluxing with dimethylaniline for eight hours, the product obtained was mainly cholestanone 8. In this case the bromine atom been replaced by hydrogen.



6 8



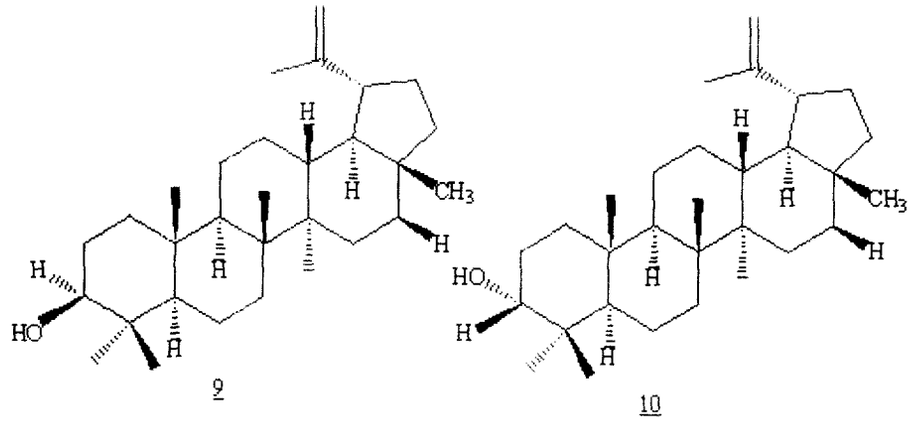
7 8

Thus from the above it is observed that debromination of a wide variety of bromo carbocyclic compounds including those of triterpenoid skeletons have been carried out by a number of useful reagents. However, debromination of 2-bromo- and 2,2-dibromo-3-ketotriterpenoids has not yet been performed. Thus a study was undertaken for the debromination of 2-bromo- and 2,2-dibromo-3-ketotriterpenoids by using *N,N*-dimethylaniline.

SECTION B:

A SHORT REVIEW ON THE BIOLOGICAL ACTIVITY OF TRITERPENOIDS AND THEIR DERIVATIVES

Akihisa et al. [17] isolated twenty eight 3-hydroxy triterpenoids from the non-saponifiable lipid fraction of the flower extract of *chrysanthemum* (*Chrysanthemum morifolium*) and one lupane-type 3-α-hydroxy triterpenoid 10 derived from 9 was tested for their antitubercular activity against Mycobacterium tuberculosis strain $H_{37}Rv$ using the Microplate Alamar Blue Assay (MABA). They observed that Cytotoxicity of compound 10 against Vero cells gave an IC_{50} value of over 62.5 microg/mL, suggesting some degree of selectivity for *M. tuberculosis*.



9 10

Ryu et al. [18] studied antiviral activity of triterpenoid derivatives and observed that 3-oxo- or/and 11-oxo-derivatives of natural 3-hydroxy triterpenes *i.e.*, 3-oxoursolic acid, 11-oxoursolic acid, 3,11-dioxoursolic acid, 3-oxobetulinic acid and 3-oxopomolic acid exhibited to show an increased anti-HSV-1 activity *in vitro*, four to ten times with respect to corresponding parent 3-hydroxy compounds.

Liby et al. [19] observed that synthetic oleanane triterpenoids have profound effects on inflammation and the redox state of cells and tissues, as well as being potent anti-proliferative and pro-apoptotic agents. Rexinoids are ligands for the nuclear receptor transcription factors known as retinoid X receptors. They found that both

classes of agents can prevent and treat cancer in experimental animals and these drugs have unique molecular and cellular mechanisms of action and might prove to be synergistic with standard anti-cancer treatments.

Tamura et al. [20] reported that the leaf beetle *Ophraella communa* infests almost exclusively *Ambrosia artemisiifolia* in the fields of Japan and a filter paper bioassay showed that the feeding of *O. communa* is strongly stimulated by methanolic extracts of *A. artemisiifolia*. They also reported that triterpenoid derivatives (α-amyrin acetate or β-amyrin acetate) and caffeic acid derivatives (3,5-dicaffoylquinic acid or 5-caffeoylquinic acid) showed feeding stimulant activity when mixed together.

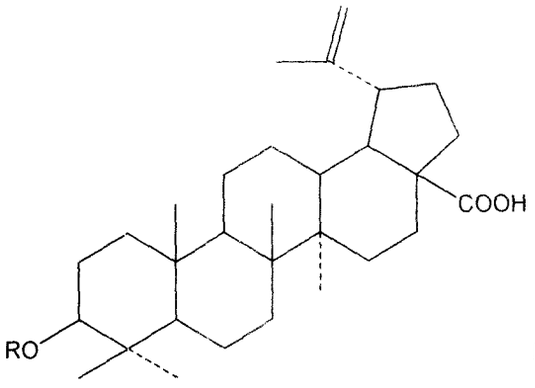
Reddy et al. [21] isolated lupeol from the leaves extract of *Aegle marmelos* and synthesized few novel derivatives from the naturally occurring lupeol and screened for their antihyperglycemic activity and antidyslipidemic activity. They found that lupeol derivative lowered the blood glucose levels by 18.2% and 25.0% at 5 h and 24 h, respectively, in sucrose challenged streptozotocin induced diabetic rats (STZ-S) model at the dose of 100 mg/kg body weight and the lupeol derivative also significantly lowered 40% (P <0.001) in triglycerides, 30% (P <0.05) in glycerol, 24% (P <0.05) in cholesterol quantity and also improved the HDL–cholesterol by 5% in dyslipidemic hamster.

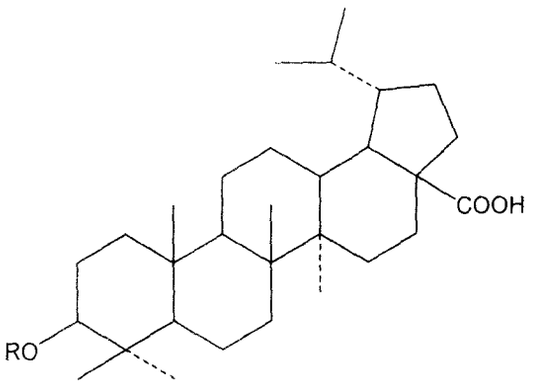
Meng et al. [22] synthesized and designed a series of boswellic acid derivatives in order to search for new potent anticancer agents and six of them were identified by IR, NMR and MS.

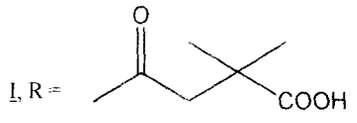
Woldmichael et al. [23] detected 16 saponins in the seeds of *Chenopodium quinoa*. They studied antifungal activity and hemolytic activity on erythrocytes of these compounds and derived monodesmosides against *Candida albicans*. They found that both bidesmosides and derived monodesmosides showed little antifungal activity whereas a comparatively higher degree of hemolytic activity could be determined for monodesmosides.

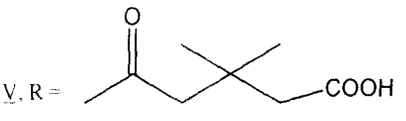
Tolstikov et al. [24] systematized the data on natural source of betulin and methods of its extraction, transformation and its available derivatives. They presented the data on the biological activity of betulin, its natural and synthetic analogs. They reported the promising character of the compounds based on betulin for creation of antiviral and antitumour agents.

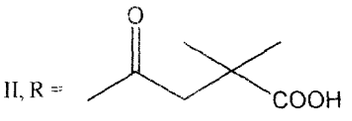
Lee et al. [25] reported betulinic acid, a triterpene isolated from *Syzigium claviflorum*, was active against HIV replication in H9 lymphocytes with an EC_{50} of 1.4 μM and a therapeutic index (TI) of 9.3. The related palatinic acid has an acetyl rather than an isopropenyl side chain and is less active with a slightly higher EC_{50} value (6.5 μM). Esterification of the C-3 hydroxyl of betulinic acid and its dihydro derivative led to 3-O-(3′,3′-dimethylsuccinyl)-betulinic acid (DSB) I and dihydrobetulinic acid II which were more potent (EC_{50}<$3.5x10^{-4}$ μM, TI = 12500). Other 3-acylated compounds including 3-O—(3,3′- -dimethylglutaryl)-betulinic acid III, dihydrobetulinic acid IV , 3-O-diglycolyi-betulinic acid V, dihydrobetulinic acid VI and 3-O-glutaryl betulinic acid VII were also potent inhibitors of HIV replication with EC_{50} values from 0.04 to $2.3x10^{-3}$ μM and TI values from 292 to 2344. Betulin VIII is less potent (≈ 16-fold) than betulinic acid, however, adding 3′,3′-dimethyl glutaryl esters at both the C-3 and C-28 hydroxy groups gave an extremely potent compound IX with EC_{50} and TI values of $6.6x10^{-4}$ μM and 21515 respectively.

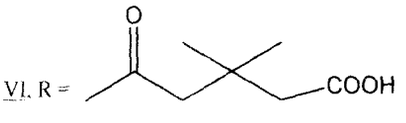


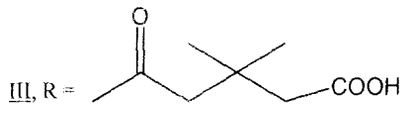


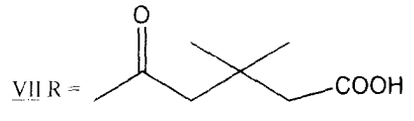


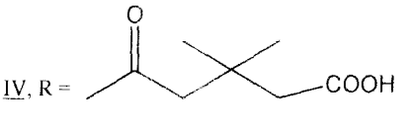


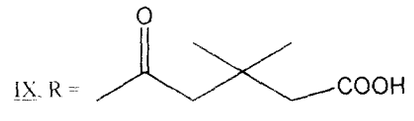










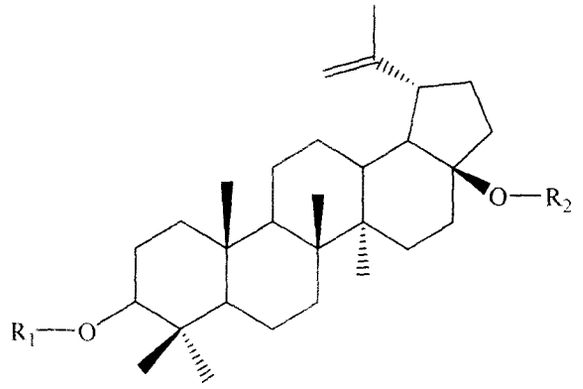


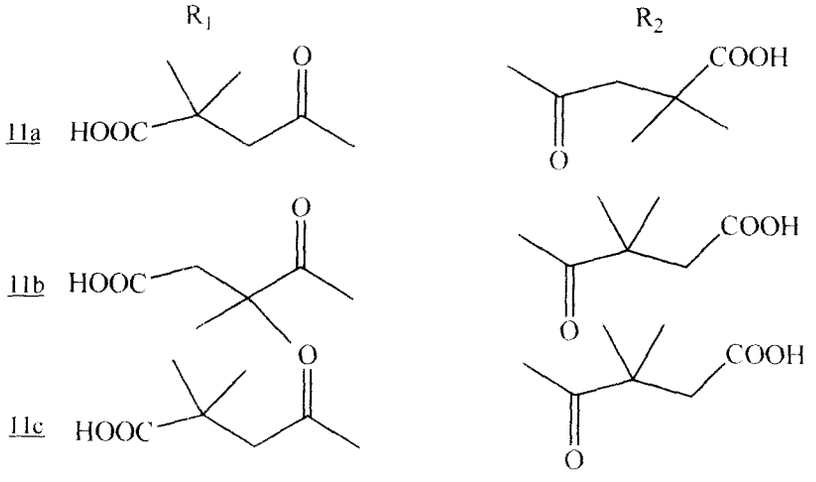
VIII, R = H

Schuhly et al. [26] isolated betulinic acid from the stem bark of Brazilian medicinal plant *Zizypus jaazerio* and its three new derivatives namely 7β-(4-hydroxybenzoyloxy) betulinic acid and 27-(4-hydroxy-3-methoxybenzoyloxy) betulinic

acid and 27-(4-hydroxy-3-methoxybenzoyloxy) showed considerable activity against gram-positive bacteria.

Kashiwada et al. [27] prepared four isomeric 3,28-di-O-(dimethylsuccinyl) betulin derivatives and evaluated their anti-HIV potency. Among these derivatives, 11c demonstrated the highest activity in acutely infected H_9 cells with an EC_{50} value of 0.87 μM and inhibited uninfected H9 cell growth with an IC_{50} value of 36.9 μM. Its calculated SI value (42,400) was comparable to that of zidovudine (41,622). Compound 11a was also extremely potent with an EC_{50} value of 0.02 μM and SI of 1680. Compound 11b displayed fair activity (EC_{50} =0.4 μM; SI=96.5) while 12b was toxic.



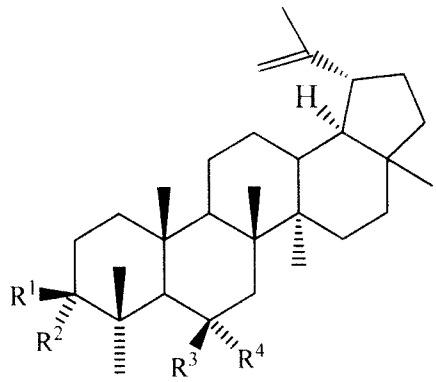


Su et al. [28] studied a series of triterpene derivatives for quantitative structure-activity relationship with multiple linear regression (MLR) and artificial neural networks (ANN). They observed that the linear model with MLR performed poorly while the nonlinear model with ANN preformed well. For the ANN model with architecture of 5-6-1, the root mean square error for the training set, validation set and the prediction set were 0.2019, 0.2214 and 0.2883, respectively. In this study they used different methods to select the most relevant descriptors for MLR and ANN and the result indicated that those descriptors werw playing an important role on the anti-HIV activity of triterpene derivatives.

Suh et al. [29] reported that the new synthetic oleanane triterpenoid 2-cyano-3, 12-dioxoolean-1,9-dien-28-oic acid (CDDO) is a potent, multifunctional molecule. It induces monocytic differentiation of human myeloid leukemia cells and adipogenic differentiation of mouse 3T3-L1 fibroblasts and enhances the neuronal differentiation of rat PC12 pheochromocytoma cells caused by nerve growth factor. They found that CDDO inhibited proliferation of many human tumor cell lines, including those derived from estrogen receptor positive and negative breast carcinomas, myeloid leukemias, and several carcinomas bearing a *Smad4* mutation and suppresses the abilities of various inflammatory cytokinase.

Baltina et al. [30] modified betulin and betulinic acid at the C-3 and C-28 positions and evaluated *in vitro* for antiviral activity. It was found that simple modifications of the parent structure of lupane triterpenes produced highly effective agents against influenza A and herpes simple type 1 viruses.

Mustafa et al. [31] reported that lupeol derivatives (III-V) containing functional groups in the ring B displayed a high inhibiting activity toward α-glucosidase and moderate antibacterial activity. Lupeol ester (VI) was found to display cytostatic activity against JB6 cells [32].



	R^1	R^2	R^3	R^4
III	OH	H	OH	H
IV	–	O	–	O
V	$COC_{17}H_{35}$	H	H	OH
VI	$OCOCHCHC_6H_{13}(OH)_2$	H	H	H

Cock [33] reported the antimicrobial activity of a methanolic extract of *Buckinghamia celsissima* leaves by disc diffusion assay against a panel of bacteria and fungi. *B. celsissima* leaf extract inhibited the growth of 5 of the 14 bacteria tested (36%). Gram-positive and gram-negative bacteria were both affected by *B. celsissima* extract although gram-positive bacteria were more susceptible. Out of 11 gram-negative 3, (27%) and out of the 3 gram-positive 2, (67%) bacteria tested by the group, had their growth inhibited by *B. celsissima* extract. *B. celsissima* leaf extract displayed antifungal activity towards *Candida albicans* when tested by disc diffusion assay and inhibited the growth of the yeast *Saccharomyces cerevisiae.*

Erturk et al. [34] reported that the antibacterial and antifungal activities of crude ethanolic extracts of 41 traditional medicinal plant species belonging to 26 families were tested against four bacteria and two fungi: *Bacillus subtilis, Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans* and *Aspergillus niger.* Of the 41 plants tested, 39 showed antimicrobial activity against one or more species of microorganisms. While the crude extracts from *Nigellea arvensis* did not show antimicrobial activity against the test microorganisms, *Pistasia lentiscus* showed only antifungal activity against *A. Niger.* The most active antimicrobial plants were

Cuminum cyminum, Jasminium officinale, Thymus capitatus, Viscum album, Tanecetum sorbifolium, Pimpinella anisum, Galega officinalis, Liguidamber orientalis, Rhus coriaria, Alnus glutinosa and *Cameli sinensis.*

Kucukboyaci et al. [35] investigated the aerial parts and seeds of *Sophora jaubertii* Spach (Leguminosae) growing in Turkey were investigated for their alkaloid compositions and antimicrobial activities. The alkaloid extracts were analyzed by capillary gas chromatography-mass spectrometry (GC-MS). The main components were identified as matrine (34.64%), sophoccarpine (15.32%), anagyrine (9.10%) and sophoridine (6.35%) in the aerial parts, while matrine (32.34%), sophocarpine (14.67%), cytosine (13.30%), sophoranol (10.98%) and sophoridine (8.57%) in the seeds of the plant. The alkaloid extract of the aerial parts of *S. jaubertii* presented significant activity against *Bacillus subtilis* with a minimum inhibitory concentration (MIC) of 31.25 µg/mL. The remaining MIC values were found in the range of 62.5-500 µg/mL.

Usha et al. [36] found that *Morinda Citrifolia* is one of the most important traditional Polynesian medicinal plants. This small evergreen tree is native from South Eastern Asia to Australia and now it has a pantropical distribution. It has antifungal, antibacterial, anti-inflammatory and antiviral activities. *Morinda citrifolia* (L.) was studied for its antimicrobial activity. The leaves of this plant were dried powdered and different extracts were prepared using different solvents like benzene, chloroform, ethyl acetate, ethanol and water. Four organisms, namely *E. coli, Staphylococcus aureus, Candida albicans and Aspergillus niger,* were used for investigation. The activity of each solvent extract was checked on each organism by disc diffusion method and then the zone size of each was measured. The results of our antimicrobial assay revealed that the plant extracts showed inhibitory activity against the tested organisms.

Bharathi et al. [37] reported the various extracts of leaves of *Barringtonia acutangula* (Lecythidaceae) *viz.*, n-hexane, chloroform, ethyl acetate and ethanol were subjected to preliminary phytochemical screening and screened for their antibacterial activity against gram-positive *(Staphyloccocus aureus, Entero cocci, Coagulase staphylococci)* and gram-negative bacteria *(Escherichia coli, Klebsiella, Citrobactor, Aceneto bacter, Pseudomonas, Salmonella paratyphi)* using Minimum Inhibitory

Concentration (MIC) and zone of inhibition by agar disc diffusion method. The results of the preliminary investigation revealed the presence of terpenoids, steroids, tannins, saponins, flavanoids and glycosides. Among the crude extracts. n-hexane extract showed good antibacterial activity against all tested organisms followed by chloroform (MIC = 100 µg/mL), ethyl acetate (MIC = 100 µg/mL), ethanol and aqueous extracts (MIC = 166.67 µg/mL). Results on the zone of inhibition (mm) revealed n-hexane extract as the maximum antibacterial potential followed by ethyl acetate, ethanol, aqueous and chloroform. The extracts were subjected to antifungal activity using MIC method against *Candida albicans, Aspergillus flavus, Aspergillus fumigates and Aspergillus Niger.* The n-hexane extract inhibited growth of pathogenic fungi at a lesser concentration followed by aqueous, ethanol, chloroform and ethyl acetate. The results revealed that the *Barringtonia acutangula* leaves possess potential antibacterial and antifungal activity.

Bari et al. [38] extracted the leaves stem, roots and inflorescence of *Solanum torvum* Sw. were extracted in two different organic solvents (chloroform and methanol). Antibacterial and antifungal effects of the extracts were tested on fifteen (six gram-positive and nine gram-negative) human pathogenic bacteria and on eight pathogenic fungi. Methanolic extracts of roots of *S. torvum* exhibited promising antibacterial and antifungal effects on all organisms tested in comparison with that observed in the leaves, stems and inflorescence extracts. The toxicity of the extracts was in the following order, root>stem>inflorescence>leaf. The minimum inhibitory concentration (MIC) values of methanolic extract of roots of *S. torvum* were in the range between 64-128 µg/mL. Chloroform extracts of roots were more toxic (LC_{50} 35.4629 ppm) than other extracts analyzed in Brine shrimp test. In conclusion, *S. torvum* appears to be an attractive material for the development of antimicrobial drugs and environment friendly biopesticides.

Hassan et al. [39] determine the antibacterial and antifungal activities of *Polygonum hydropiper* (L.) root extract on chloroform against both bacteria and fungi using the disc diffusion method. The extract showed significant antibacterial activities against four gram-positive (*Bacillus subtilis, Bacillus megaterium, Stapphylococcus aureus and Enterobacter aerogenes*) and four gram-negative (*Escherichia coli,*

Pseudomonus aeruginosa, Salmonella typhi and Shigella sonnri) bacteria. The minimum inhibitory concentration (MIC) values against these bacteria ranged from 16-64 μg/mL. The antifungal activities were found strong against six fungi *(Aspergillus fumigates, Aspergillus niger, Aspergillus flavus, Candida albicans, Rizopus oryzae and Tricophyton rubrum*). It can be used in the folk medicine at different parts of the world to treat many diseases including bacterial and fungal infections.

Nandini et al. [40] determined the antibacterial and antifungal activities of ethyanolic extract of *Luffa cylindrical* (Linn). The extract was prepared from fresh fruit of *Luffa cylindrical* by hot continuous percolation method in soxhlet apparatus. Ethanolic extract of *Luffa cylindrical* (Linn) were then tested for antibacterial and antifungal efficacy against gram positive and gram negative bacteria and fungi *viz. Aspergillus fumigates, Aspergillus niger and Candida albicans* organism. The ethanolic extract was found to be the most effective and showed antibacterial and antifungal activity against the entire organism tested. The zone of inhibition (mm) at various concentrations of ethanolic extract of *Luffa cylindrical* was found be in to the range 50 mg/mL to 150 mg/mL on all the tested all the test organisms. This study scientifically supports the usage of whole plant as a remedy for various superficial bacterial and fungal infections in traditional medicine.

Jayaraman et al. [41] reported that *Morinda citrifolia* (noni) was indigenous to tropical countries and also considered as an important traditional folk medicine. *M. citrifolia* fruits extracted with three solvents (methanol, ethyl acetate and hexane) were tested *in vitro* for their antibacterial, antifungal and antitumor activity. Among the three solvents tested, methanol extract was active against all the tested organisms with varied extents of antibacterial activity. Ethyl acetate extract was effective against most of the microorganisms tested except *Pseudomonas aeruginosa* and *Klebsiella pneumonia.* Hexane extract was ineffective against all tested microorganisms. Among the fungi tested, the maximum percentage of inhibition was observed against *Trichophyton mentagrophytes.* With the extracts of methanol (79.3%) and ethyl acetate (62.06%), nearly 50% inhibition was recorded against *Penicillium sp., Fusarium sp. and Rhizopus sp.* with methanol extract. None of the extracts were active against *Candida albicans* and *Aspergillus species.* The methanol extract showed maximum cytotoxicity on HEp2

cells followed by ethyl acetate extract. The overall results indicated promising baseline information for the potential uses of *M. citrifolia* fruit extracts in the treatment of infectious diseases and tumor.

Khan et al. [42] studied the antibacterial, antifungal and cytotoxic activities of the ethanolic extract of tuberous roots of *Amorphophallus campanulatus*. Disc diffusion technique was used to determine *in vitro* antibacterial and antifungal activities. Cytotoxicity was determined against Brine shrimp nauplii. In addition, minimum inhibitory concentration (MIC) was determined using serial dilution technique to determine antibacterial potency. The extract showed significant antibacterial activities against four gram-positive bacteria (*Bacillus subtilis, Bacillus megaterium, Staphylococcus aureus,* and *Streptococcus β-haemolyticus*) and six gram-negative bacteria (*Escherichia coli, Shigella dysenteriae, Shigella sonnei, Shigella flexneri, Pseudomonas aeruginosa, Salmonella typhi*). The MIC values against these bacteria ranged from 16 to 128 μg/mL. The antifugal activity was found weak against the tested fungi. In cytotoxicity determination, LC_{50} of the extract against Brine shrimp nauplii was 7.66 μg/mL.

From the above literature work the author has found some discrete work on phytochemical investigation of the plants particularly which are available in this region and no systematic study regarding their biocidal activity has so far been carried out. Thus it was felt necessary to undertake a thorough study towards the phytochemical investigation of medicinal plants available in this region of West Bengal and also to make a systematic study of the biocidal activity of the isolated plant materials in comparison to their prepared derivatives.