Part IV

Oxidative Transformation of Cerin and Friedelin with 3D Molecular Docking and Antitopoisomerase Activity

Chapter I

Transformative reactions on friedelan triterpenoids

1. Introduction

The constituents of cork have been the subject of many researches for the past 150 years, and over this period a considerable number of pure substances have been isolated and characterized. The scientific literature on friedelin, 3-keto derivative of the hydrocarbon friedelan (Figure 1) is widely scattered. Kuloshreshtha *et al.*[1] reviewed the occurrence of triterpenoids for the period 1963-70. However in that review there were few listing of friedelan related compounds.

Friedel[2] according to Karrer[3] first isolated friedelin, however as much earlier reference is provided by Elsevier to Chevreul who obtained substances from cork which he called "**Cerine**".[3] In 1889 Istrati and Ostrogovich reported that cerine was actually composed of two different compounds, one they name friedelin in honour of their friend Friedel and the other as cerin.[3]

After the work of Karrer, almost 40 years later Drake and Jacobson[4] had repeated the isolation of friedelin and cerin and determined their empirical molecular formula. For the first time they claimed that both friedelin and cerin have the same skeleton (Figure 1) and friedelin has a keto group and cerin a hydroxyl ketone.

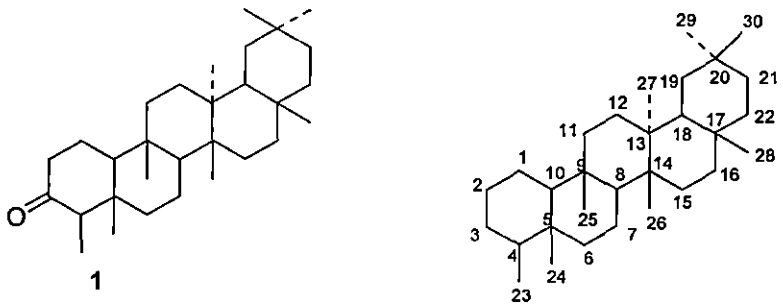
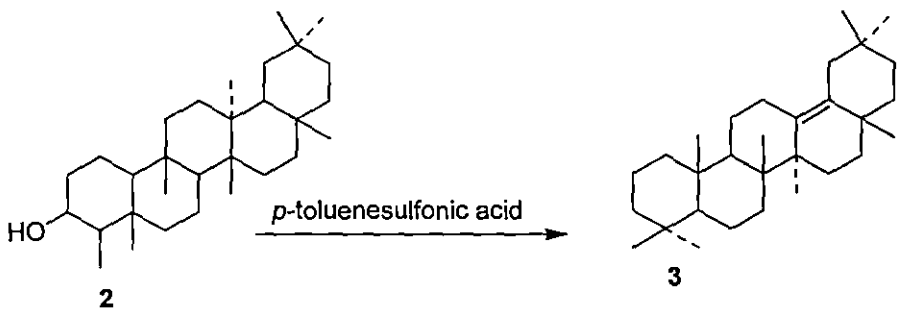


Figure 1 Structure of friedelan skeleton and friedelin, **1**

Efforts to elucidate the structure and confirmation were based initially on the preparation of derivatives and their comparison with the known compounds. Most modern physical

method was yet to be developed, but Drake and Wolfe[5] investigated the surface films formed by friedelin and cerin. Lander and his coworkers.[6] studied the structure of friedelin by using the dipole moment of cerin, friedelin and various other friedelan isomers. During the period of 1944-49 Ruzicka *et al.*[7,8] published the results of theit attempts to derive the structure of friedelin by some chemical means.[7,8] However it was 1955, two groups (Ruzicka *et al.* and Corey *et al.*) working independently, arrived at the accepted structure as a result of dehydrogenation studies and the isomerizaton[9,10] of friedel-3β-ol, **2** to olean-13(18)-ene, **3** (Scheme 1).

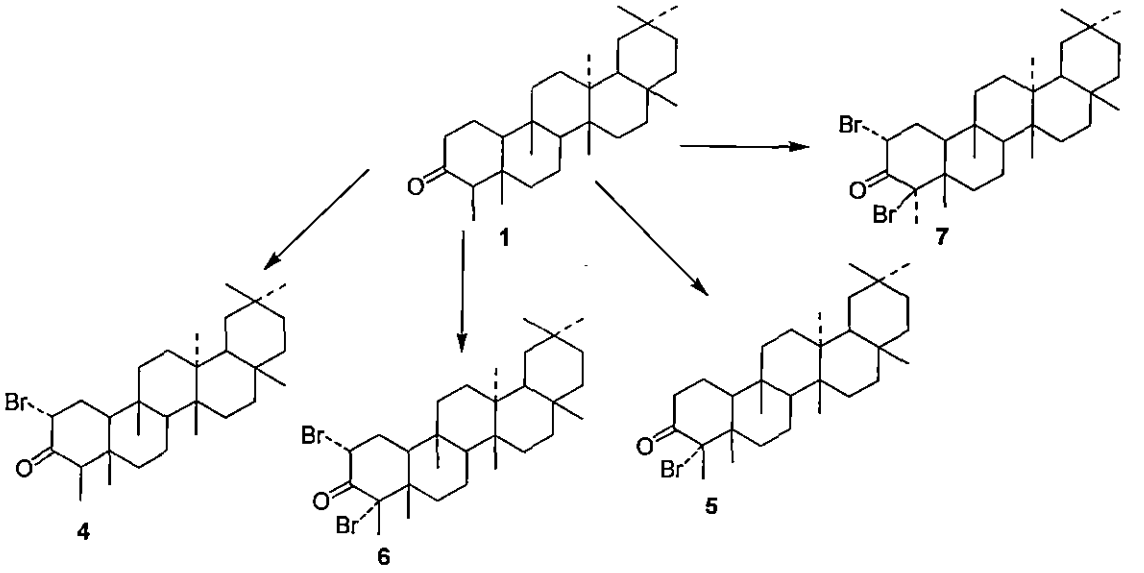


Scheme 1 Acid catalyzed conversion of friedelan to olean skleton

The rearrangement also proceeds well with Δ^3-friedelene and somewhat less readily with Δ^2-friedelene, the optimum results (55% yields) so far having been obtained from friedelan-3β-ol and hydrogen chloride in phenol at 110 °C. Further studies to friedelan-olean rearrangement continued with attempts to isolate the intermediated compounds.[11] Finally Carl Djerassi and his coworkers confirmed the confuguration of friedelin by optical rotatory dispersion studies.[12-13] After that several publications appeared dealing with the isolation and purification of friedelin and related triterpenoids. As the whole description of various methods of isolation and purification are beyond the scope of this review, the present author is more concerned to represent the various transformative reactions on friedelin and cerin.

Corey and Ursprung[9] had shown (Scheme 2) that friedelin on direct bromonation gave 2α-bromo friedelin (**4**) and bromination of appropriate enol benzoate gave the isomeric 4α-bromo friedelin (**5**). They have also prepared a dibromo friedelin (**6**) in presence of hydrobromic acid in chloroform. The dibromo friedelin has been assigned as 2α,4α-dibromo friedelin form the UV absorption at 332 nm. Carl Djerassi and his coworkers

have synthesized another dibromo friedelin (7) by bromination of 2α-bromofriedelin in glacial acetic acid. This prepared from dibromo friedelin was found different from that prepared by Corey. From UV absorption data (310.5 nm) they designated the compound as 2α,4β-dibromo friedelin (7).



Scheme 2 Direct bromination of friedelin

Takahashi *et al.* and Ourrison[14] also prepared a dibromo friedelin by the action of bromine in chloroform and acetic acid on friedelin. But they could not assign the structure of this compound although the compound showed UV absorption at 320 nm.

The reaction of friedelin and its derivatives with NBS was studied by Stevenson *et al.*[15] They found that friedelin on treatment with molar equivalent of NBS in carbon tetrachloride gave 4α-bromofriedelin in satisfactory yield. They also isolated 2α-bromofriedelin from the 4α-bromofriedelin by further treatment with bromine in glacial acetic acid. Hence in this reaction isomerization occurred rather than substitution. As was expected from the results, it was found that 4α-bromofriedelin, $[\alpha]_D = +92^\circ$ was unstable in chloroform-hydrobromic acid, the presumed equilibrium mixture $[\alpha]_D = -75^\circ$ being formed after 24 h. 2α-bromofriedelin, **4** also gave the same result on similar equilibration.

For obtaining dibromofriedelin, since this route was unsuccessful, an alternative route of treatment of 2α-bromofriedelin, **4** with NBS was attempted by Stevenson *et al.*[16] Treatment of 2α-bromofriedelin, **4** with NBS gave an unsaturated monoketone $C_{30}H_{47}OBr$ which showed positive TNM test, indicating thereby the presence of ethylinic linkage (Figure 2). UV and IR spectra of this compound showed that the double bond of this compound was not in conjugated to the carbonyl group and the α-bromine atom retained an axial orientation. Since it was known that acid isomerization of friedel-3-ene (**8**) afforded a mixture of olean-13(18)-ene (**3**) and 18α-olean-12-ene (**9**),[16,17] it was assumed that this nonconjugated bromoketone had probably arisen by molecular rearrangement of 2α,4α-dibromoketone intermediate with elimination of hydrobromic acid.

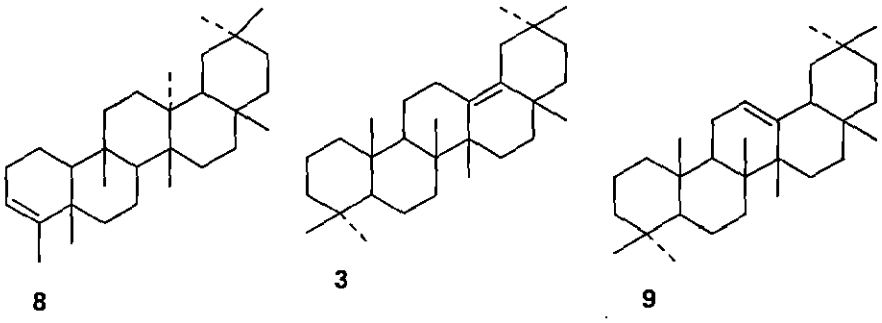
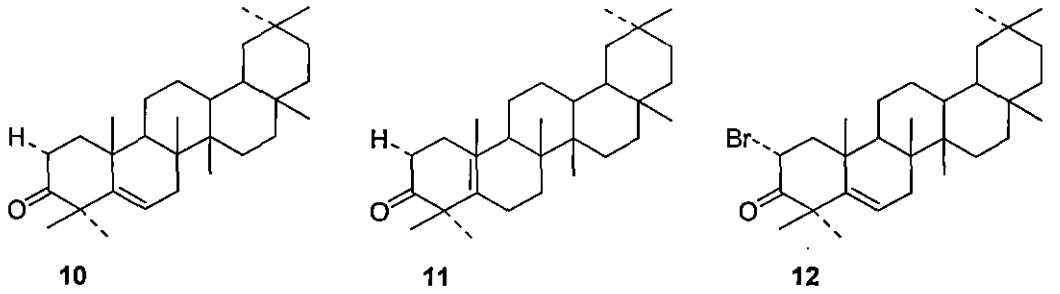


Figure 2 Structures of some different dehydrobrominated products from friedelin

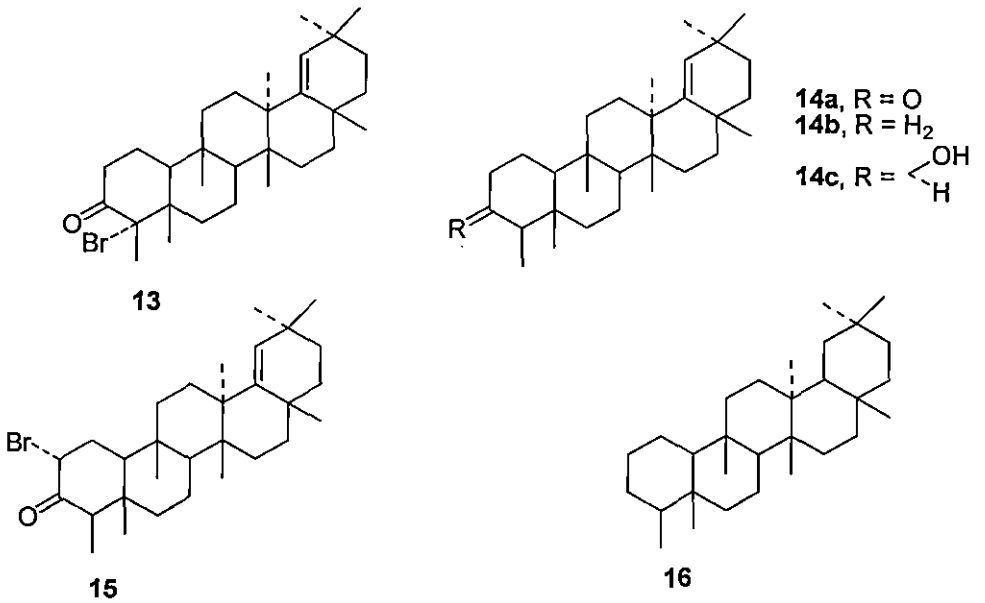
A precedent for such a rearrangement was provided by the action of silver acetate on 4α-bromofriedelin to yield a product **3** which was shown to be a mixture[18] of alnus-5-ene (**10**) and alnus-5-enone (**11**). The probability that the unsaturated bromoketone derived from 4α-bromofriedelin could be represented as 2-bromoalnus enone (**12**) was elucidated from the fact that the zinc debromination in neutral solution was different from either alnus enone **10** or **11** (Figure 3).



10 11 12

Figure 3 Structures of some alnus derivatives synthesized from friedelin

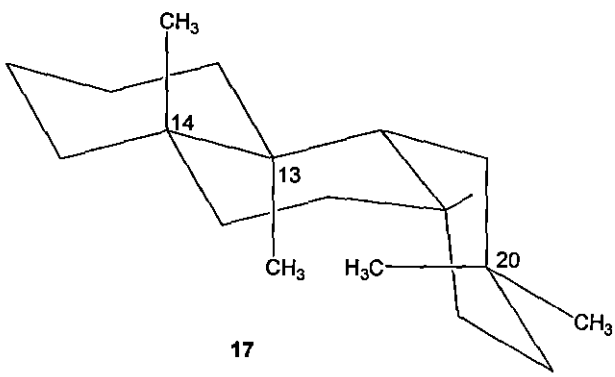
Treatment of 4α-bromofriedelin, **5** with NBS gave an isomeric non-conjugated axial bromo substituted ketone (**13**) having the molecular formula of $C_{30}H_{47}OBr$ which on dehydrobromination gave the identical ketone $C_{30}H_{48}O$ (**14**). LAH reduction of **14a** gave an alcohol **14c** and on Hung-Minlon reduction gave the hydrocarbon **14b**. From these observations the isomeric monobromoketones obtained from 2α-bromofriedelin and 4α-bromofriedelin was assigned structures 2α-bromofriedel-18-en-3-one, **15** and 2α-bromofriedel-18-en-3-one, **13** respectively. These assignments were also supported by specific rotation and ORD studies.



13 15 16

The experiments on friedelin and related triterpenoids as described by Kane and Stevenson established that in friedelin the tertiary axial α-hydrogen atom at position C-4 is more reactive towards NBS than the secondary hydrogen atoms at position C-2, but

that the presence of a 2α-bromine atom effectively prevents abstraction of the 4α-hydrogen atom by its 1:3-diaxial blocking effect to the approach of a succinimide radical. In absence of the activating C-3 carbonyl group or where there is deactivation due to the steric influence of the neighbouring axial halogen, the most reactive hydrogen is the tertiary C-18 atom. An examination of an all chair form (**17**) of friedelan shows that, as a consequence of the *cis*-junction of D and E rings, a severe steric interaction must exist between the 13α-and 20α-methyl groups. Whereas this interference is removed if the terminal ring E adopts a boat conformation (**18**), an unfavorable 1:4-diaxial "boat prow-and-stern" methyl interaction is a consequence. The steric strain inherent in both conformations of this *cis* D/E system is relieved by dissociation of the 18β-hydrogen atom and formation of the ethylenic trigonal system. The driving force for the backbone rearrangement of friedelene (**19**) to the oleanenes **3** and **20** can be attributed also to the same steric interactions.



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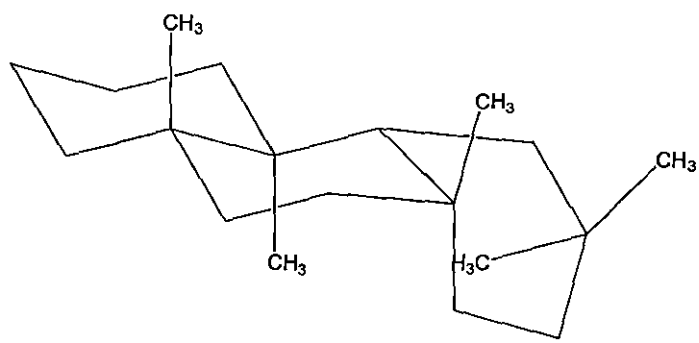

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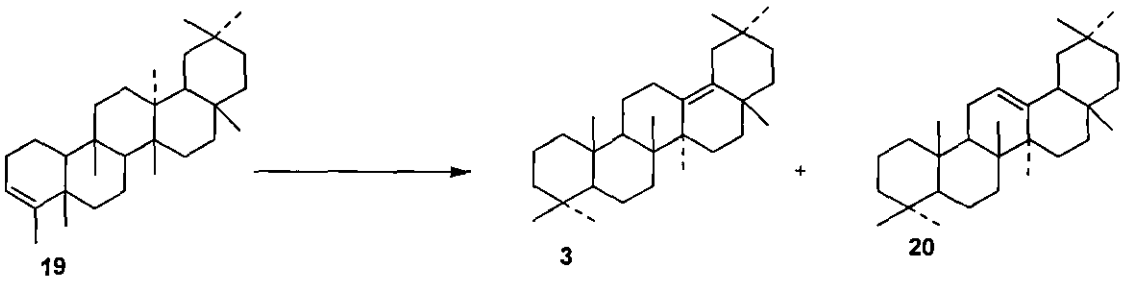
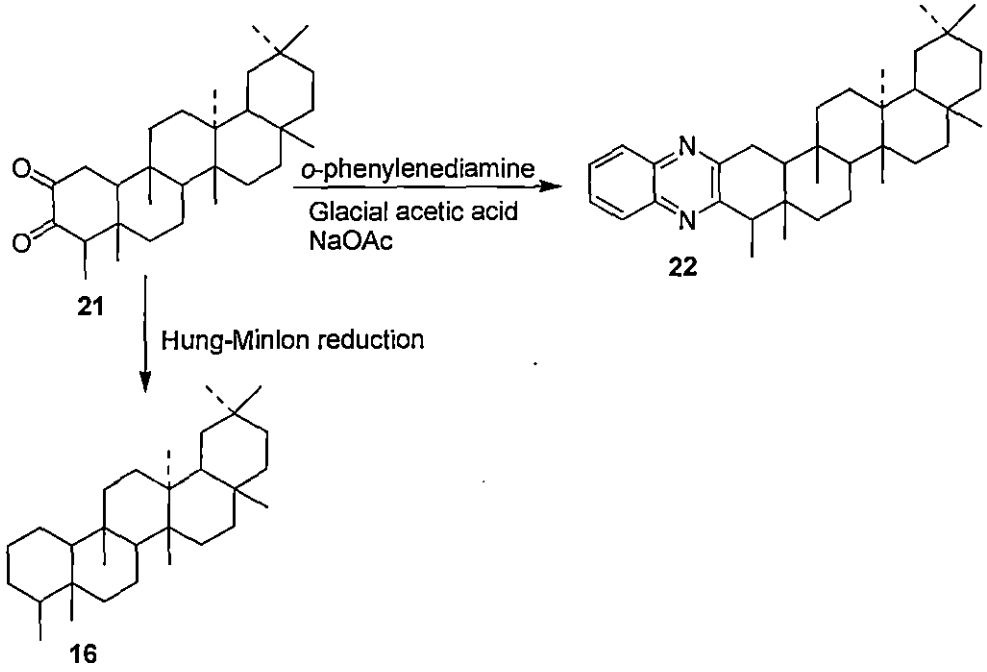


V. V Kane and R. Stivenson[19] in their continuous work on cork triterpenoid isolated friedelan-2,3-dione from cork smoker wash solid. The structure of this triterpenoid was established following the results of some chemical transformation. Hung-Minlon reduction of the isolated derivative gave exclusively the hydrocarbon friedelan readily identified by direct comparison with an authentic specimenand consequently restricted the possible formulations to friedelan-1,2-dione, friedelan-6,7-dione or friedelan-2,3-dione. A decision in favor of friedelan-2,3-dione, **21** was reached by conversion to the diketone to friedelan-3-one. For a final verification (Scheme 3) about their proposed structure the workers synthesized the quinoxaline derivative of friedelin under refluxing condition in glacial acetic acid in presence of sodium acetate. The ready formation of the quinoxaline derivative (**22**) (Scheme 3) firmly established the exact structure of the isolated triterpenoid that have two carbonyl groups at C-2 and C-3 positions of the pentacyclic ring structure.



Scheme 3 Transformation of 1,2-friedelan by Kane

Pradhan *et al.*[20] studied the reaction of NBS in DMSO on friedelin (Figure 4) and observed that it gave a mixture of five different products. From the spectral as well as chemical evidences they designated the compounds as 2α,23-dibromofriedelin (**23**), 2β,23-dibromofriedelin (**24**), 2α-bromofriedelin, 2,2-dibromofriedelin, **25** and 2,3-diketo friedelin, **21**.

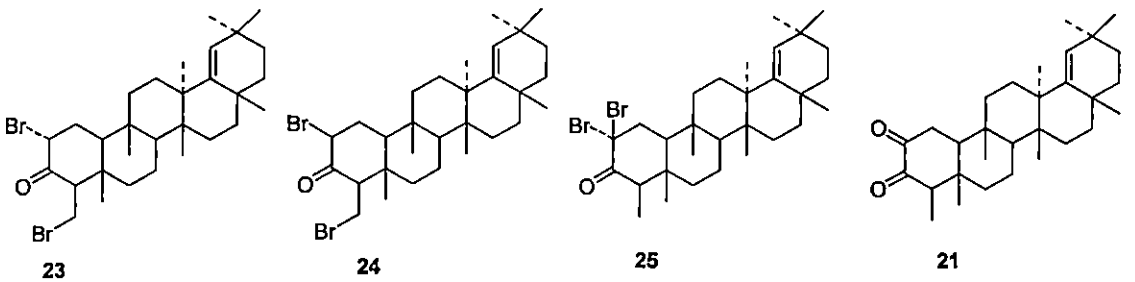
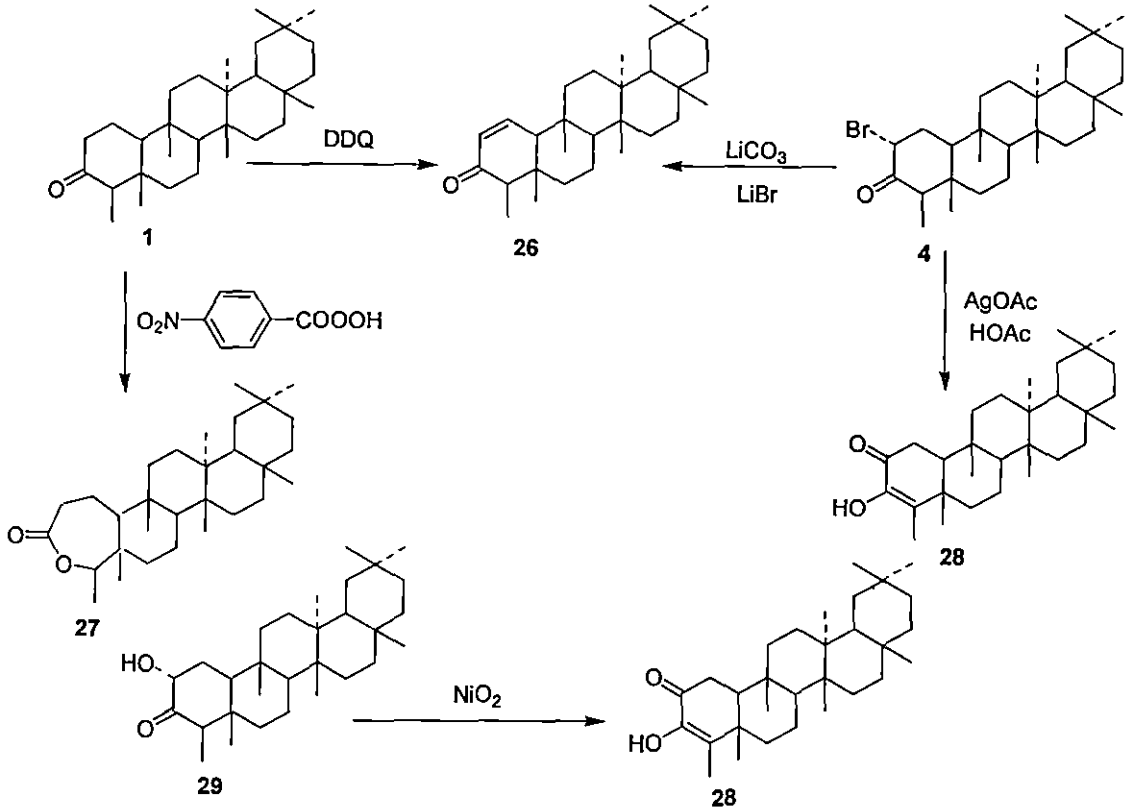
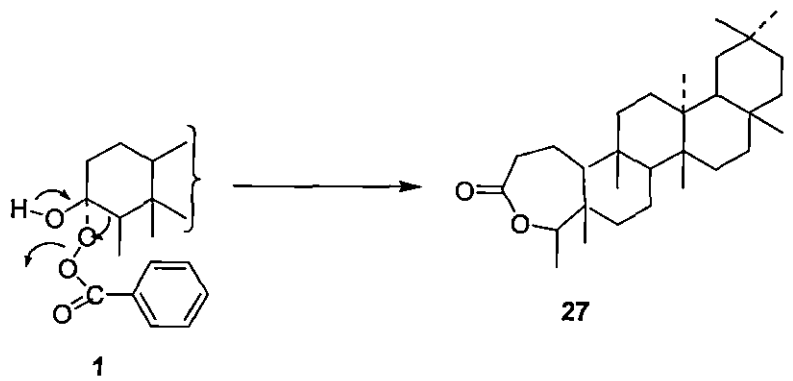


Figure 4 Products of NBS bromination of friedelin in DMSO by Pradhan *et al.*

Talapatra *et al.*[21] studied some oxidative transformation on friedelin (Scheme 4). DDQ dehydrogenation of friedelin afforded friedel-1-ene-3-one in a very low yield (9%). Treatment of 2α-bromofriedelin with LiBr and $LiCO_3$ furnished friedel-1-ene-3-one (20%) along with friedelin (15%). Reaction of 2α-bromofriedelin with AgOAc and glacial acetic acid leads to the formation of 3-hydroxyfriedel-3-ene-2-one (66%). Baeyer Villiger oxidation of friedelin with *p*-nitroperbenzoic acid produced the ε-lactone with 41% yield while NiO_2 oxidation of cerin afforded 3-hydroxyfriedel-3-ene-2-one and 3α-hydroxyfriedel-3-ene-2-one with 15%. They have also suggested a mechanism for their formation (Scheme 5).



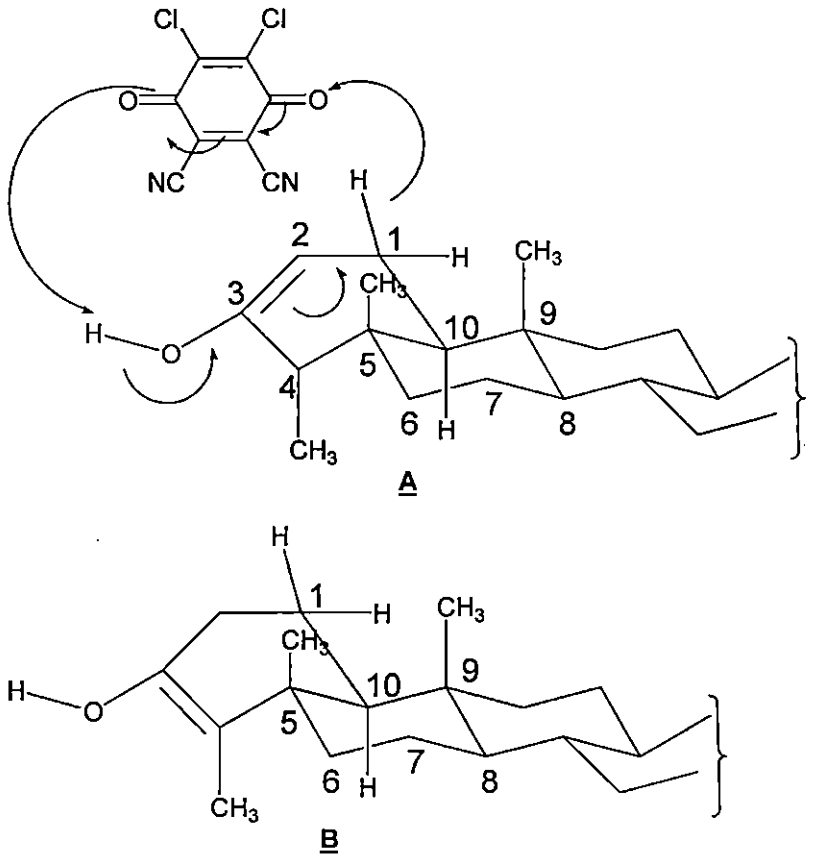
Scheme 4 Transformative reactions on friedelin and cerin by Talapatra *et al.*



Scheme 5 Proposed mechanism of formation of the ε-lactone by Talapatra *et al.*

Additionally, they have suggested an explanation (Scheme 6) to the observed low yield of friedel-1-ene-3-one by DDQ dehydrogenation of friedelin. According to them of the two enols A and B that friedelin can form, the population of A is much lower, B being more stabilized by the inductive effect of 4-Me and the pseudo-axial orientation of 5-Me,

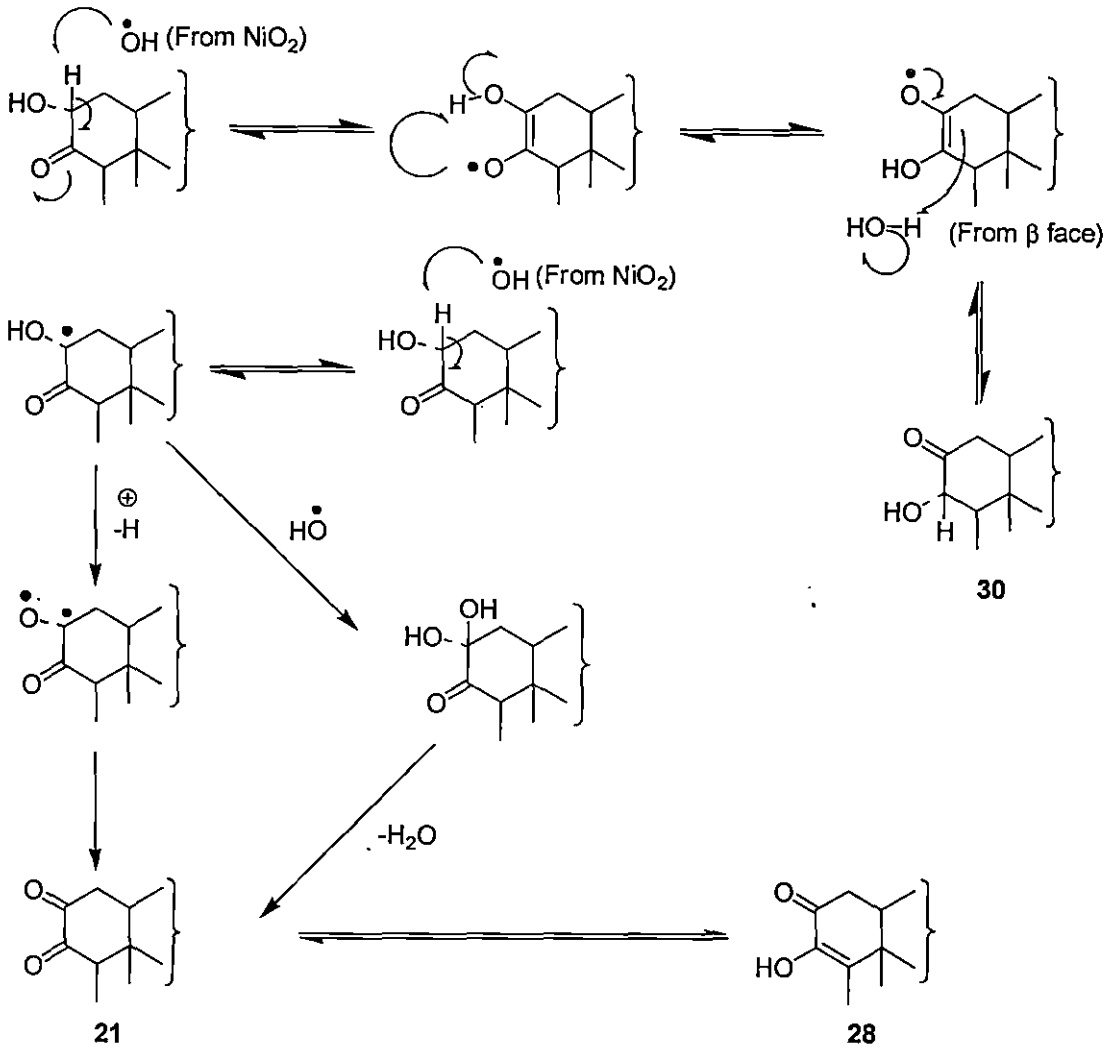
thereby alleviating the steric interaction with syn-axial 9-Me and 1β-H to some extent. Enolization is the first step for this dehydrogenation and the product is formed from the enol by the transfer of the pseudo-axial γ-H to the quinone maintaining continuous overlap of the π-orbitals of the double bond being formed with the remainder of the conjugated system. Thus the stable enol **B** is inert toward DDQ as it does not possess any pseudo-axial γ-H at C-5. On the contrary the less stable enol A possesses a pseudo-axial γ-H at C-1 sufficiently hindered due to the syn-axial 5-Me and 9-Me and hence its transfer to the quinone involves high activation energy resulting in a low yield of friedel-1-ene-3-one.



Scheme 6 Proposed mechanism for the dehydration reaction by DDQ by Talapatra *et al.*

The naturally occurring cerin on being refluxed with nickel peroxide in benzene (Scheme 7) afforded a solid residue which on subsequent chromatography over silica gel furnished 3-hydroxyfriedel-3-ene-2-one and 3α-hydroxyfriedel-3-ene-2-one. The mechanism as

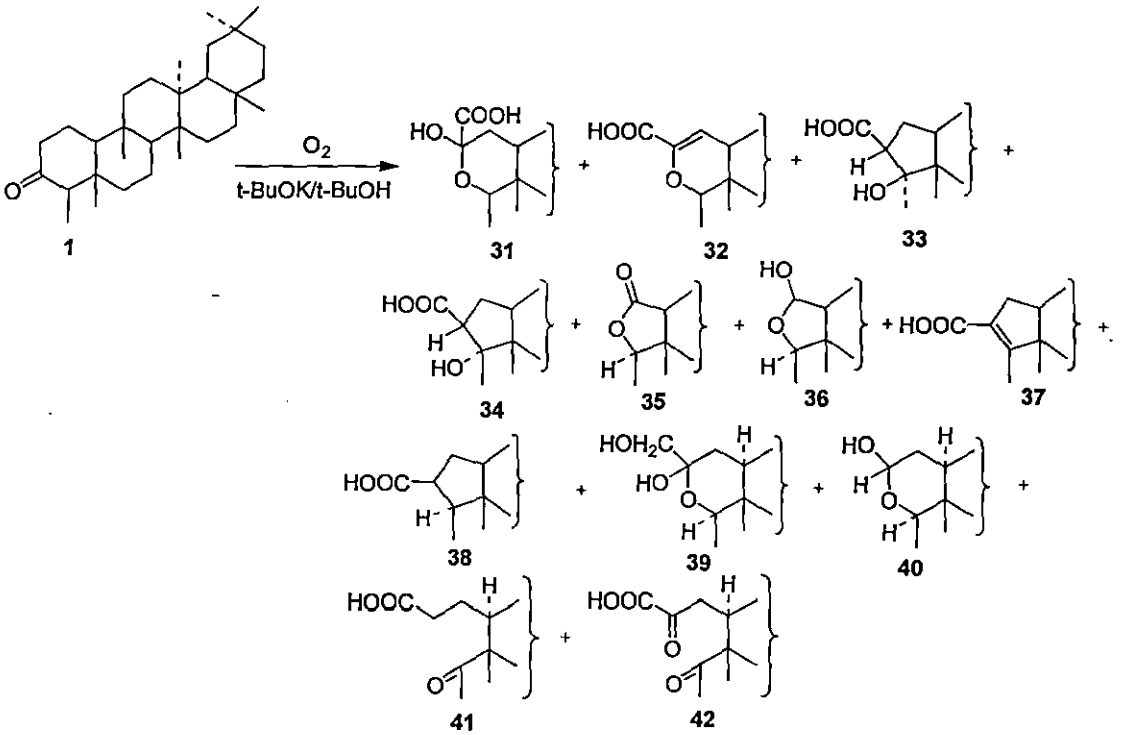
suggested for the formation of 3-hydroxyfriedel-3-ene-2-one from cerin was as follows.



Scheme 7 Proposed mechanism of formation of 3-hydroxyfriedel-3-ene-2-one by Talapatra *et al.*

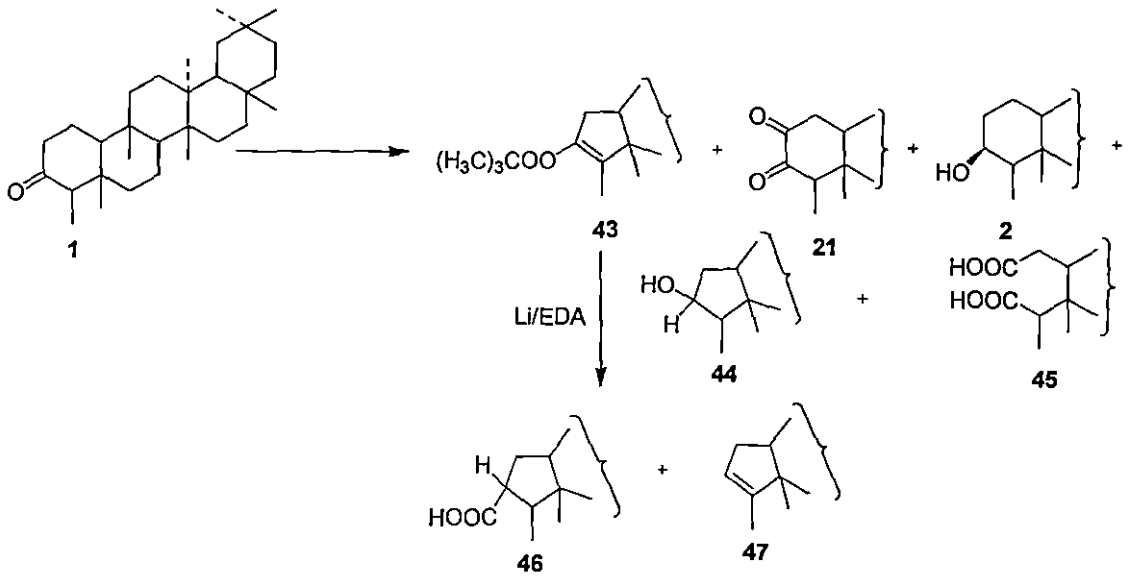
Auto oxidation of ketones with gaseous oxygen in the presence of strong bases is known as the convenient method for the preparation of α-diketones from the corresponding α-methylene ketones. The autooxidation of triterpene with a 4,4-dimethyl-3-keto structure was examined by many workers and in most of the cases they reported the formation of α-diketones in moderate yield. Nishihama *et al.*[22] for the first time reported the base catalyzed autooxidation of friedelin (Scheme 8) and also studied the mechanism of their

formation. Thay have synthesized a number of compounds, of which 2-hydroxy-3-oxafriedelane-2-carboxylic acid was the main product. 3-oxafriedel-1-ene-2-carboxylic acid was the dehydrated product of 2-hydroxy-3-oxafriedelane-2-carboxylic acid. The common intermediates to this compound was suggested to be 4-hydroperoxyfriedelan-3-one, not the friedelan-2,3-dione.



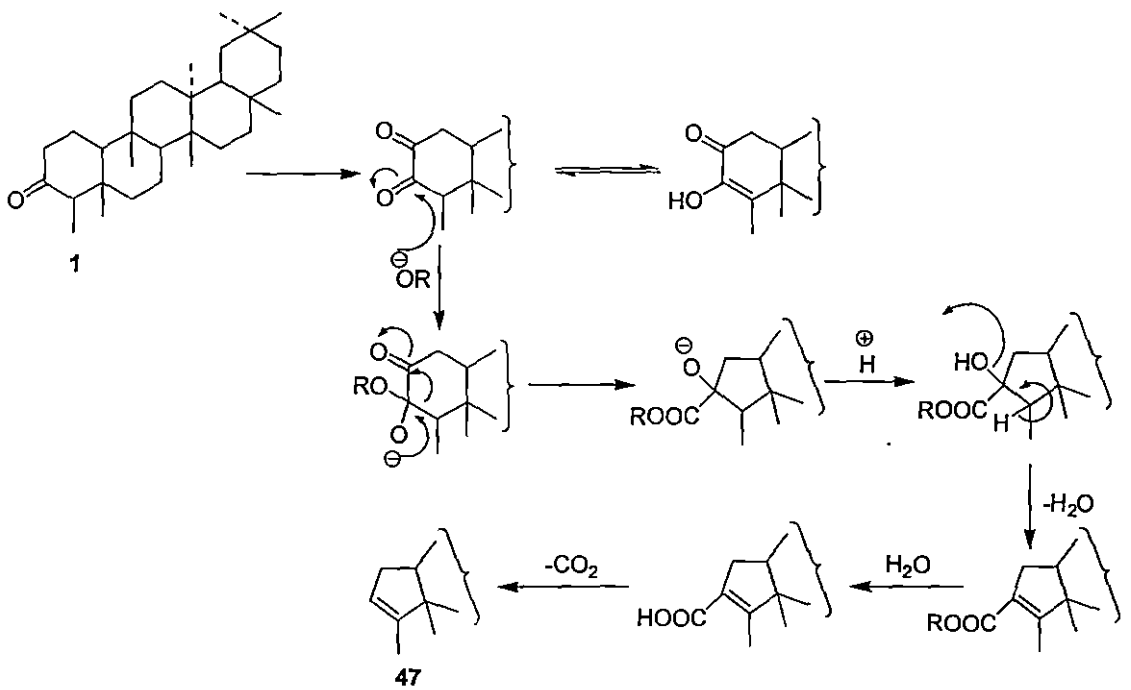
Scheme 8 Basic auto oxidation of friedelin by Nishihama *et al.*

In 1991 Pradhan *et al.*[23] once again studied the autooxidation of friedelin in t-BuOH in presence of t-BuOK as the catalyst (Scheme 9). Oxygenation of friedelin in presence of t-BuOK in t-BuOH furnished t-butyl A-nor-friedel-2(4)-en-2-carboxylate (**43**), friedel-2,3-dione (**21**), friedelan-3β-ol (**2**), 2β-hydroxy-A-nor-friedelan-2α-carboxylic acid (**44**) and friedelin dicarboxylic acid (**45**). Lithium-ethylenediamine reduction of t-butyl A-nor-friedel-2(4)-en-2-carboxylate gave A-nor-friedelan-2α-carboxylic acid (**46**) and A-nor-friedelene (**47**). Acetylation of 2β-hydroxy-A-nor-friedelan-2α-carboxylic acid gave friedel-2(4)-en-2-ol-acetate. They have characterized all the products with by spectral as well as chemical evidences.



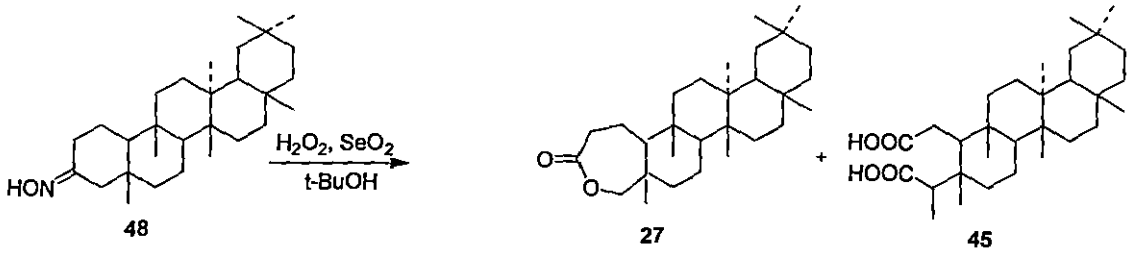
Scheme 9 Auto oxidation of friedelin by Pradhan *et al.*

Pradhan *et al.*[23] also proposed a mechanism (Scheme 10) for the formation of the above autooxidized compounds.



Scheme 10 Proposed mechanism for the auto oxidation of friedelin by Pradhan *et al.*

Pradhan *et al.* in a different attempt tried the oxidation of friedelin oxim with hydrogen peroxide and selenium dioxide in t-BuOH (Scheme 11). The reaction furnished a ε-lactone (**27**) and 2,3-seco-friedelonic acid (**45**).



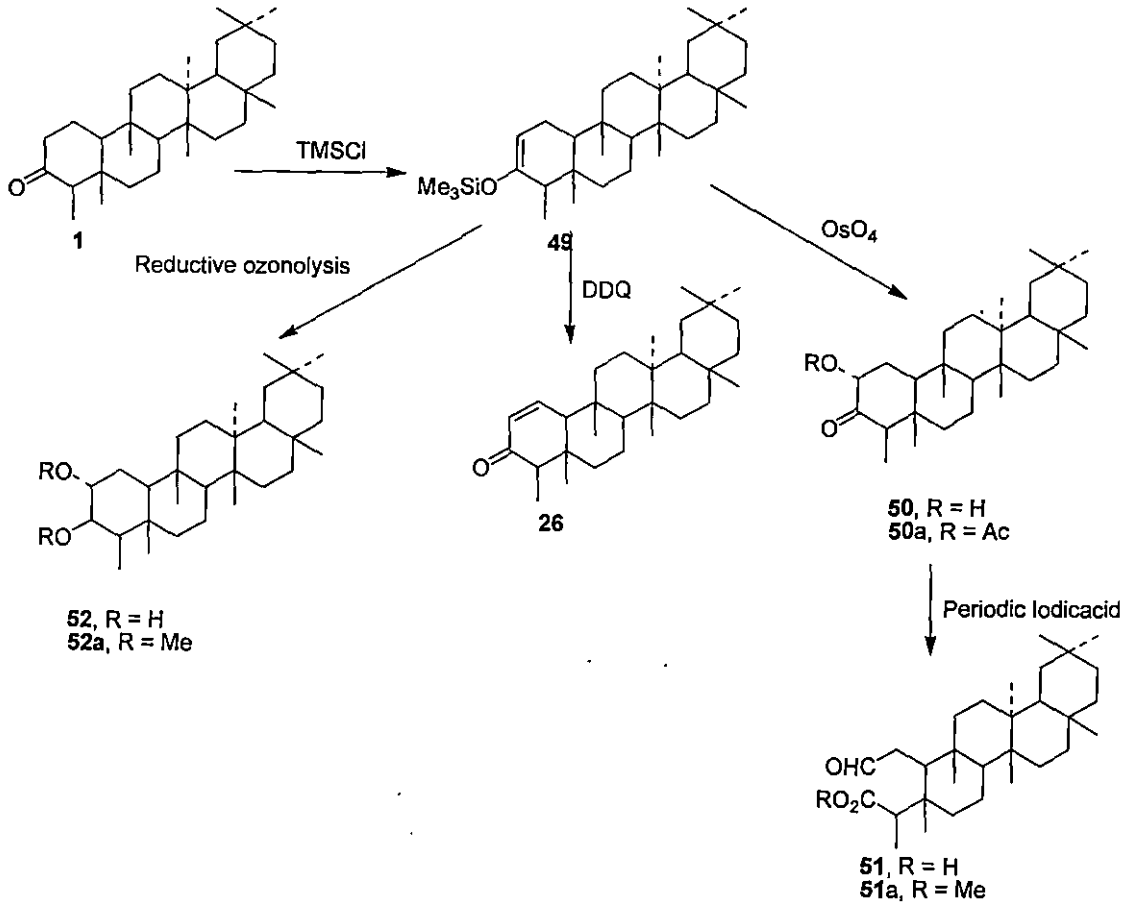
48 27 45

Scheme 11 SeO_2-H_2O_2 oxidation of friedelin oxim by Pradhan *et al.*

V.Anjaneyulu and G. Sambasiva Rao[24] also carried out the oxidation of friedelin under SeO_2-H_2O_2 in t-BuOH. They isolated the same compounds as found by Pradhan *et. al.* except friedel-1-ene-3-one.

It is interesting to note that these authors had not done any biological work on friedelin itself as well the synthesized derivatives from the various routs. Some biological works on friedelin and its derivatives had started late nineties of last century. The following part of the review will demonstrate some chemical transformative reactions as well as some reported biological activities of friedelin and its derivatives.

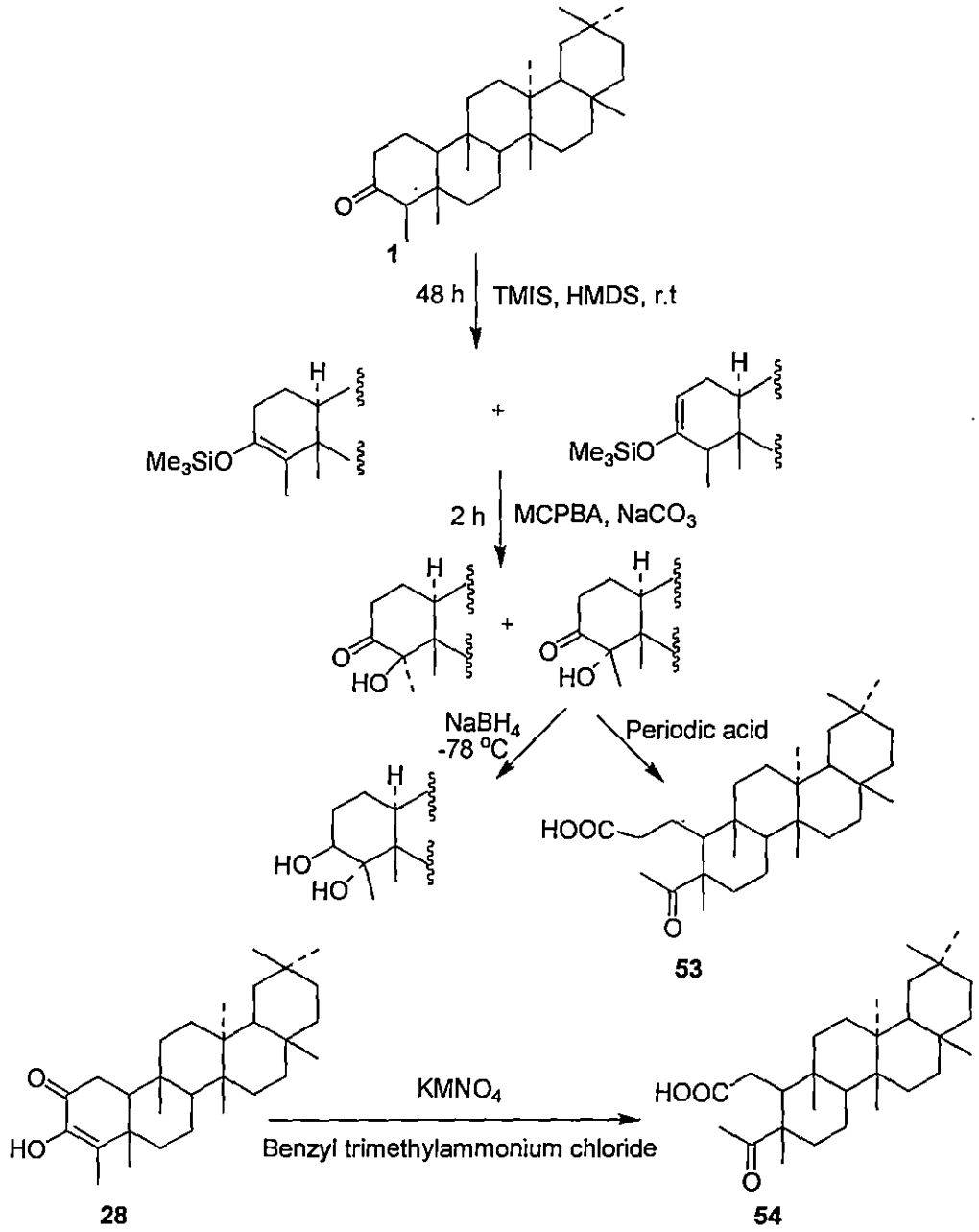
Moiteiro *et al.*[25] reported the formation of 3-trimethylsiloxyfriedel-2-ene (**49**) in high yields under the controlled silylation of friedelin (**1**) (Scheme 12) from cork smoker wash solids, a byproduct generated during processing of corkboard by steam baking. Oxidation of 3-trimethylsiloxyfriedel-2-ene with OsO_4/NMMO produced 2α-hydroxyfriedelan-3-one (cerin) (**50**) from which the new 2,3-secofriedelan-2-al-3-oic acid (**51**) as obtained quantitatively by periodic acid oxidation. Oxidation of **49** with DDQ afforded friedel-1-en-3-one (**26**). Reductive ozonolysis of **49** gave 2α,3β-dihydroxyfriedelane, pachysandiol A (**52**). Compound **51** proved to be a potent inhibitor of human lymphocyte proliferation (IC_{50} =10.7 μM) and of the growth of a human cancer cell line (GI_{50} = 5.4-17.2 μM).



Scheme 12 Transformative reactions on friedelin by Moiteiro *et al.*

Moiteiro *et al.*[26] in another attempt in 2004 reported the synthesis of two different seco-friedelinic acids **53** and **54** though silylation followed by oxidation (Scheme 13). **53** was isolated in a reaction between the trimethylsilyl ether of friedelin and periodic acid. On the other hand **54** was prepared by an effective reaction between friedel-2-keto-3-hydroxy-3-ene and potassium permanganate in benzymtrimethyl ammonium chloride. They have also synthesized three hydroxyl compounds by oxidation with MCPBA and via sodium borohydride reduction of the intermediate keto-hydroxy derivative. The total reaction sequence is shown below (Scheme 13). These hydroxyl and seco derivatives were evaluated fir their ability to inhibit in vitro the growth of three human tumor cell lines, MCF-7 (breast adenocarcinoma), NCI-H460 (nonsmall cell lung cancer), and SF-268 (CNS cancer). Only compounds 3,4-Secofriedelan-4-oxo-3-oic acid (**53**) and 3-Nor-

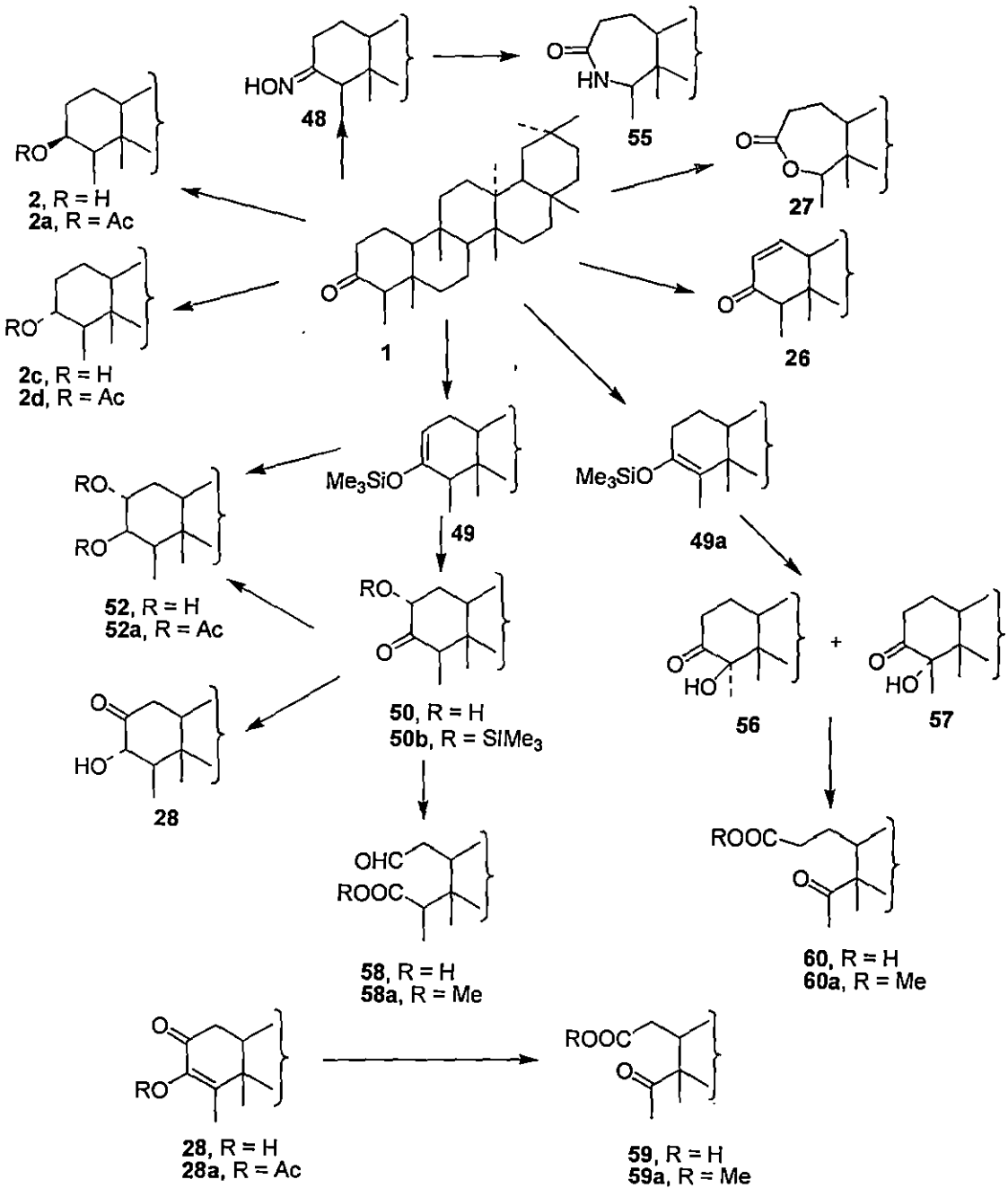
2,4-secofriedelan-4-oxo-2-oic acid (**54**) showed significant activities against all the tested cell lines exhibiting GI_{50} of 24.6 to 32.8 μM and 10.9 to 17.6 μM, respectively.



Scheme 13 Synthesis of some hydroxy and seco derivatives of friedelin by Moiteiro *et al.*

Gonzaalez-Coloma and his co-workers[27] described the synthesis, bioactivity screening, and structure-activity relationships of various synthetic triterpenoids prepared from the

cork processing byproducts friedelin (**1**) and 3-hydroxyfriedel-3-en-2-one (**28**) via oxidative procedures (Scheme 14). The synthesis of compounds 2R-trimethylsiloxyfriedelan-3-one (**50b**), friedelin-2,3-lactone (**27**), friedelin-3-oxime (**48**), and friedelin-3,4-lactam (**55**) was also described. They have studied the insecticidal and phytotoxic potential of these compounds, their selective cytotoxic effects on insect and mammalian cells, and their antiparasitic effects. Structural modifications of the A-ring of friedelin (**1**) improved its insecticidal activity with derivatives **50**, 2,3-secofriedelan-2-al-3-oic acid (**58**), its acetylated derivative **58a**, 3α- and 3R-hydroxyfriedelane (**2, 2a, 2c** and **2d**), 3R-hydroxyfriedel-2-one (**28**), 4α-hydroxyfriedel-3-one (**57**), the acetylated **2d**, 3,4-secofriedelan-4-oxo-3-oic-acid (**59**), lactone **27**, and the oxime **48** being stronger insecticides than the parent compound. Methyl-3-nor-2,4-secofriedelan-4-oxo-2-oic acid (**59**) and its acetylated derivative **59a** also showed insecticidal activity in contrast to their inactive parent compound **28** and **28a**. The postingestive effects and cytotoxicity of these compounds suggested a multifaceted insecticidal mode of action. According to them these structural modifications did not result in better phytotoxic agents than the parent compounds except for lactam **55** and yielded several moderately active antiparasite derivatives (seco acids **58**, **59**, **60** and 4α -hydroxyfriedel-3-one **57**) with cytotoxic effects on mammalian cells.



Scheme 14 Oxidative transformative reactions on friedelin by Gonzaalez *et al.*

Sheppaerd *et al.*[28] investigated the selective palladium mediated C–H functionalisation of appropriately functionalised derivatives of lanosterol, cholesterol, and friedelin. The desired equatorial aldehyde functionality was successfully introduced into the lanosterol skeleton as expected. Cyclopalladation of a cholesterol derivative proceeded as expected,

but during oxidation of the organopalladium intermediate, participation of the adjacent alkene functionality led to stereoselective formation of a cyclopropane and introduction of an acetate group into the steroid backbone at C-6. But the friedelin oxim failed to undergo a successful cyclopalladation reaction, presumably due to the unfavorable orientation of the single methyl group and/or the potential for β-hydride elimination from any resultant σ-organopalladium complex.