Part II

Synthesis of Pyrazine and Benzopyrazine Derivatives of Pentacyclic Triterpenoids with Antimicrobial and Antitopoisomerase Activity

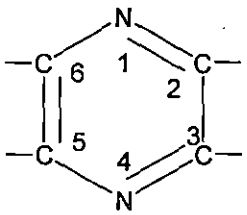
Chapter 1

1. A short review on pyrazine derivatives

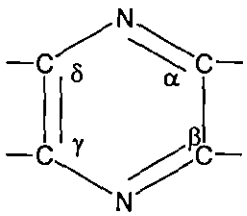
The flavor of foods has always been an interesting and complicated area worthy of investigation. Through the years many classes of compounds have been implicated as the key to the flavor properties of certain foods. During the past two decades, evidence has been accumulating that a class of heterocyclic nitrogen-containing compounds, namely pyrazines, directly contributes to the roasted or cooked flavor of foods.[1] A specific pyrazine compound that has been associated with potato flavor is 2,5-dimethylpyrazine.[2] At that time question arose how these pyrazine molecules were synthesized in foodstuffs. Many theories then came to explain the formation of pyrazines in foods. The role of carbohydrate degradation on pyrazine formation is well documented[3,4] and early reports exist of the isolation of substituted pyrazines from reaction mixtures of ammonia and hexose sugars.[5]

Theories exist for the formation of various types of pyrazines. In the case of simple alkylated pyrazines, Dawes and Edwards in 1966, using model systems containing fructose and amino acids, identified 2,5-dimethyl- and trimethyl pyrazine and concluded that **pyrazines in heated foods resulted from the condensation reactions between sugars and amino acids.**

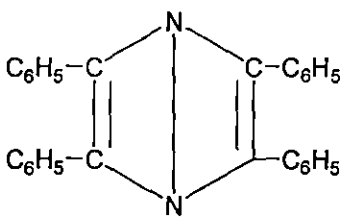
As will be indicated in the following historical section, the development of the nomenclature was beset with difficulties, owing to the confusion of the early workers with regard to the structure of pyrazine. In addition to understandable trivial names given to compounds discovered before their structure was elucidated, there arose such terms for pyrazine as aldine, paradiazine, and piazine. At one time, 2,5-dimethylpyrazine was thought to be the parent of the class, with the result that it was given the generic name "ketine." Following the Ring Index[6] the name pyrazine was established and the numbering system is as follows-



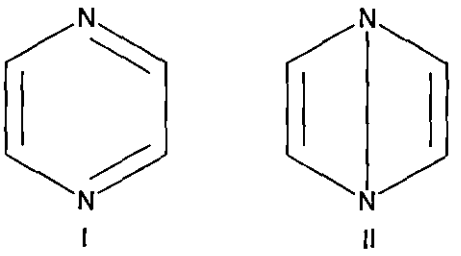
An alternative scheme is that used by T. Tictor and V. Richter in his *Lehrbuch der organischen Chemie:*



Historically the first structure elucidation of pyrazine derivative was made by Japp and Wilson and later by Pauling as well. Japp and Wilson[7] undertook the task of determining the true nature of Erdman's benzoinimide, which they renamed ditolanazotide, by repeating the original experiments. In the following year Japp and Burton[8] concluded that the compound was an azine, and assigned to it the structure, together with the name "tetraphenylazine."



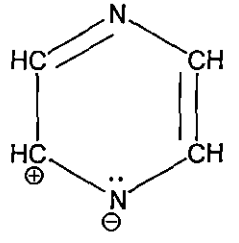
Snape and Brooke there upon repeated Laurent's original work and in 1897 published a paper[9,10] in which they revealed that amarone, benzoinimide, ditolanazotide and tetraphenylazine were all one and the same substance: tetraphenylpyrazine. There yet remained doubt as to the exact location of the double bonds in the pyrazine molecule. The Kekule type with its conjugated double bond system (I) and the Dewar type (II) with the long para bond, each had its adherents.[11]



I II

Bruhl finally established the validity of I after a study of the molecular refractions of a number of pyrazine derivatives.

Pauling and his collaborators[12] have made electron-diffraction studies on some cyclic systems, including pyrazine. Their results showed that pyrazine and benzene have almost identical structures. Further, although the C-C distances for the two molecules were almost identical, 1.39 Å, the value for the C-N distance in pyrazine is 1.36 Å, was greater than expected for the Kekule resonance, which would give this bond 50 per cent double-bond character. This is interpreted as being due to the large electronegativity of the nitrogen atom, which results in the introduction of additional resonating ionic structures, as:



Simple pyrazines is a heterocyclic aromatic compound with the chemical formula $C_4H_4N_2$. It is a symmetrical molecule with point group D_2h.

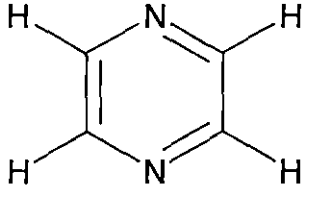


Figure 1 Finally accepted structure of pyrazine

Compounds containing *N*-heterocyclic moieties are a class of privileged compounds that have found numerous applications as pharmaceuticals as well as in medicines. Pyrazines are important component of aroma fragrances,[1] potential pharmacophore of a large

number of biologically active substances,[13-16] and widely used as agrochemicals.[17-19] For examples, methoxy pyrazines are relevant components of aromas of many fruits, vegetables and wines, methyl phenyl derivatives of dihydropyrazines inhibit the growth of *Echerichia coli* by generating hydroxyl and carbene-centered radicals that cause DNA strand breakage and alkylpyrazines have been recognized as flavor components in foods, as pheromones in various insect species [13-16] and as versatile synthetic intermediates. Pyrazine derivatives are known for use as relaxing cardiovascular and uterine smooth muscle, anti-thrombotic, anti-aggregation, COX-2 inhibiting and analgesic effects.[20] Tetra methyl pyrazine also known as ligustrazine is reported to scavenge superoxide anion and decrease nitric oxide production in human polymorphonuclear leukocytes.[21] It is also a component of some herbs in traditional Chinese medicine. Derivatives of pyrazine possess varieties of activities like antimicrobial, anti filarial, anti leukemia in mice against i.p. P388 and several pyrazines were more active than the corresponding oxazines or thiazines.[22-24] Also a series of pyrazine-carboxymides has been described as eukalemic agents possessing diuretic and natriuretic properties. Hence pyrazine is a lead compound for designing potential bioactive agents.

The importance of the pyrazine nucleus in life processes is indicated in its condensed derivative, riboflavin or vitamin B2.

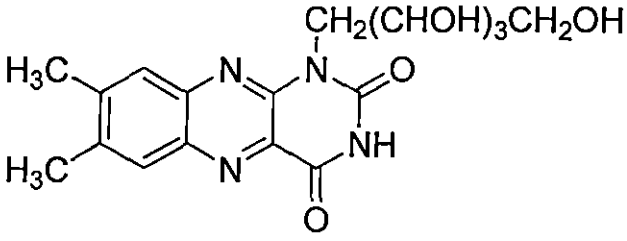
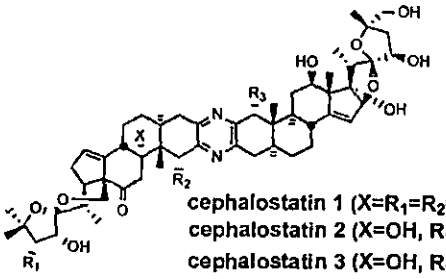
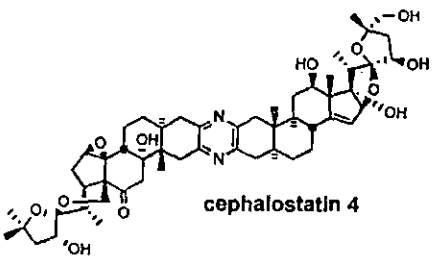


Figure 2 Structure of vitamin B2

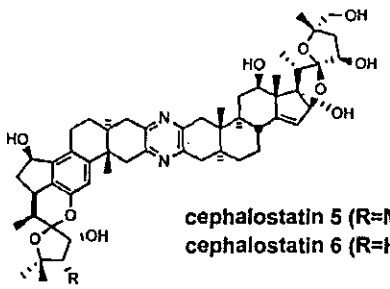
The cephalostatins along with the structurally related ritterazines form a unique class of trisdecacyclic compounds that consist of two steroidal units linked through a pyrazine ring.[25] The first cephalostatins were isolated by Pettit *et al.* from the Indian Ocean tube worm *Cephalodiscus gilchristi*.[26]



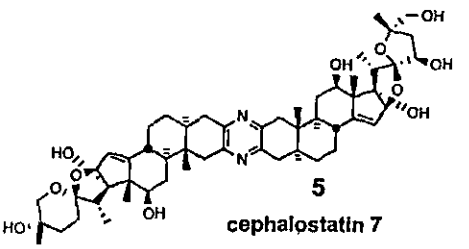
cephalostatin 1 (X=R_1=R_2=R_3=H)
cephalostatin 2 (X=OH, R_1=R_2=R_3=H)
cephalostatin 3 (X=OH, R_1=Me, R_2=R_3=H)
cephalostatin 10 (X=OH, R_1=R_2=H, R_3=OMe)
cephalostatin 11 (X=OH, R_1=R_3=H, R_2=OMe)
cephalostatin 18 (X=R_1=R_2=H, R_3=OMe)
cephalostatin 19 (X=R_1=R_3=H, R_2=OMe)



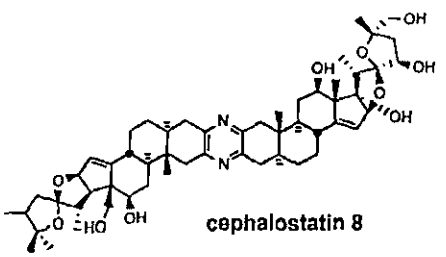
cephalostatin 4



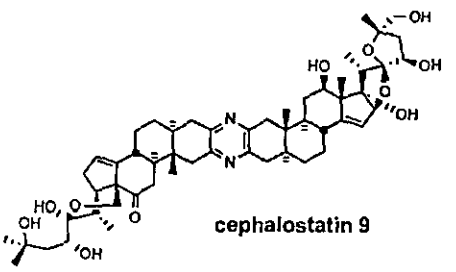
cephalostatin 5 (R=Me)
cephalostatin 6 (R=H)



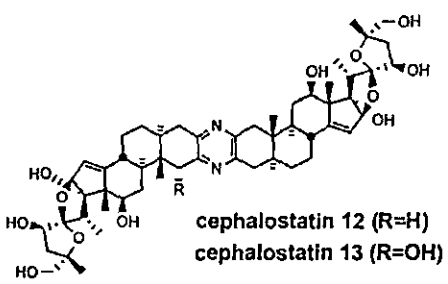
5
cephalostatin 7



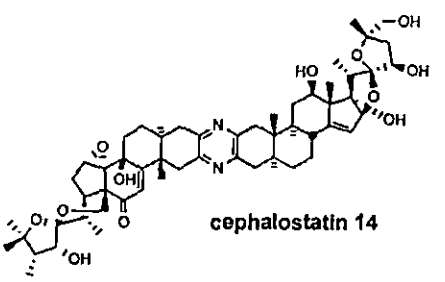
cephalostatin 8



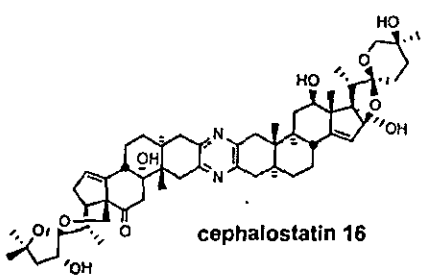
cephalostatin 9



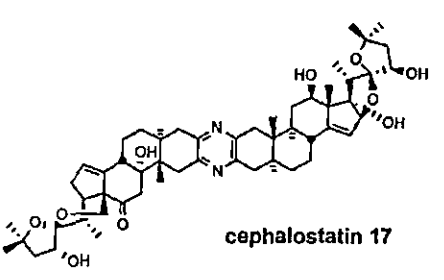
cephalostatin 12 (R=H)
cephalostatin 13 (R=OH)



cephalostatin 14



cephalostatin 16



cephalostatin 17

Figure 3 Structures of various naturally occurring cephalostatins

Cephalostatin 1 exhibits extraordinarily high cytostatic activity against a broad spectrum of cancer cell lines and proved to be one of the most powerful cell growth inhibitors ever tested in the NCI. It is considerably more active in vitro than paclitaxel and has an unprecedented mechanism of action.[27] More than 18 other cephalostatins were characterized and they showed the same unique cytotoxicity profile in the NCI-60 cell line panel.[28] Closely related ritterazines were isolated by Fusetani and co-workers from the Japanese marine tunicate *Ritterella tokioka* and they showed a similar pattern of cytotoxic activity.[29-30]

A great surprise was the isolation of ritterazines, compounds with structure analogous to cephalostatins, from the Japanese tunicates *Ritterela tokioka*.[31-32] They are not related to the hemichordate family, to which *Cephalodiscus* belongs. The presence of the latter was not observed in the samples collected. Both species *C. gilchristi* and *R. tokioka* live in environments inhabited by marine predators and perhaps the production of the alkaloids under discussion is a form of chemical defense. To date, 24 ritterazines were isolated (ritterazines A-Z).[33] Their chemical relationship to the cephalostatins is obvious (some have the identical structure as one of the steroid units), although the same alkaloid has not yet been isolated from both species.

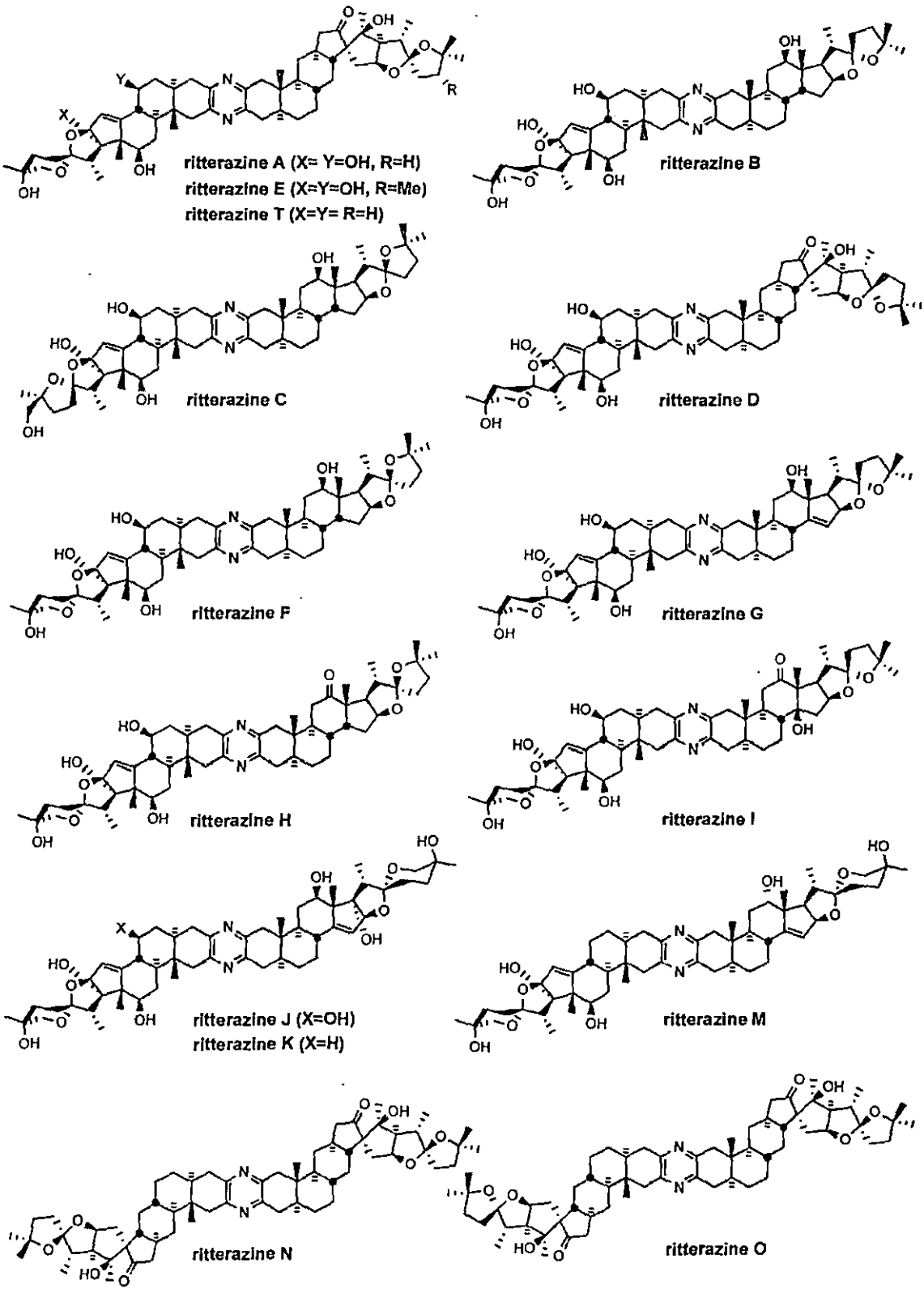
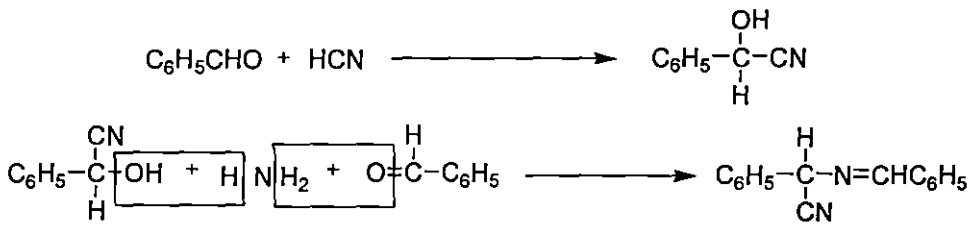


Figure 4 Structures of various naturally occurring ritterazines

Because of these associated wide variety of applications with the pyrazine moieties as well as their natural occurrence fused with steroidal skeleton, their synthesis has remained the goal of many research groups over the years.[34-40]

In recording the development of pyrazine chemistry, the author has attempted to preserve the original historical plan, presenting the facts in almost the same manner as they were exposed. This treatment may initially appear somewhat awkward, but it will be seen that the early and apparently unrelated observations form an important part of the entire pattern.

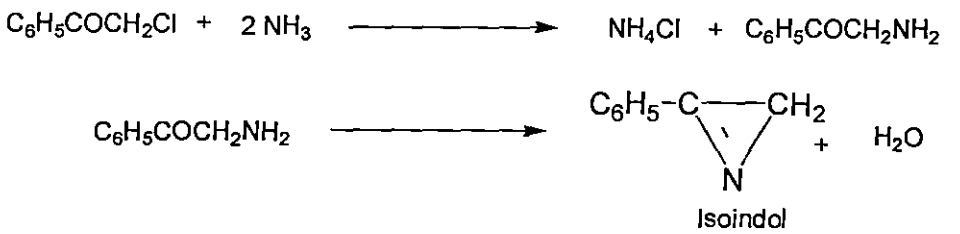
The first procedure for the synthesis of a pyrazine derivative was published by Laurent in 1844.[34] Starting with crude benzaldehyde, i.e., benzaldehyde containing some hydrogen cyanide, he treated it with ammonia to obtain what was then known as "benzoylazotid" actually α-benzalaminophenylacetonitrile:



Scheme 1 Synthesis of α-benzalaminophenylacetonitrile

Then, in a fashion typical of that period, he more or less destructively distilled "benzoylazotid," and was able to isolate amongst the products a substance which he called amarone.

Twenty one years later Erdmann reported[35] an apparently new substance, benzoinimide, which he obtained, along with some others, by the action of ammonia on benzoin. No further .progress was made until 1876, when Stadel and Rugheimer[36] published a paper describing the formation of a new compound, isoindol, by the action of ammonia on ω-chloroacetophenone. They postulated that isoindol was the inner anhydride of an amino ketone and was formed according to the following sequence:

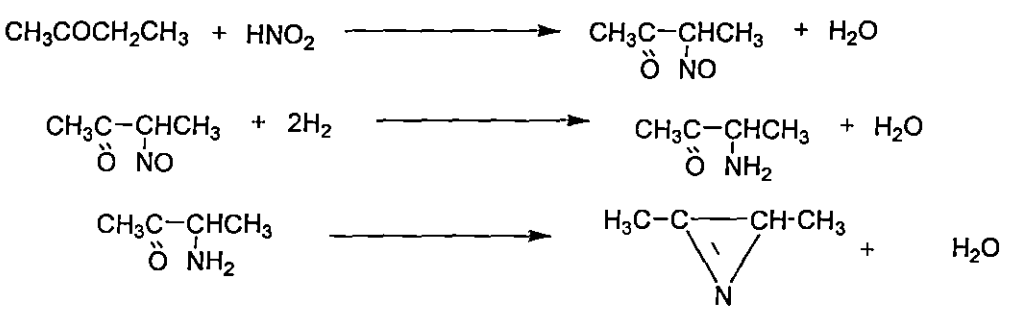


Scheme 2 Synthesis of isoindol

This is of interest since it represents the first recorded instance of a structural formula for a pyrazine.

In 1881 Etard[37] made what was seemingly an uncorrelated observation when he published the fact that a new substance, which he named glycolin, could be isolated from the distillate which resulted from heating a mixture of glycerol and an ammonium salt.

Two years prior to the appearance of Etard's article, work was progressing in Victor Meyer's laboratory at Zurich which was to be of great value in the elucidation of the nature of these compounds. At that time Meyer was interested in the study of nitroso derivatives. To H. Gutknecht he assigned the task of reducing the product of the action of nitrous acid on ethyl methyl ketone. We now know that this nitrous acid product was the oxime, but these investigators believed that they were dealing with a true nitroso compound. The results were piiblished[38] in 1879, with the conclusion that an inner anhydride of the amino ketone was obtained from the reduction, in exact analogy to the hypothesis of Stadel and Rugheimer:



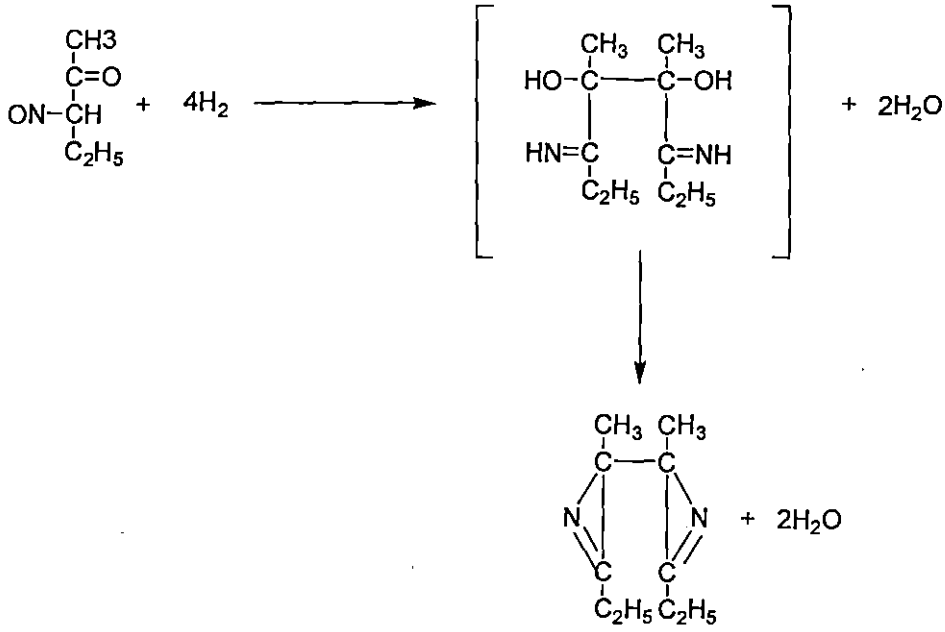
Scheme 3 Synthesis of substituted isoindol derivative

Analyses of the crystalline product were not too satisfactory because of difficulties in obtaining it in an absolutely anhydrous state. After consideration of the data, Gutknecht

advanced the possibility that the compound might contain one less hydrogen atom than was indicated by the formula.

This point was finally clarified by F. P. Treadwell[39] (of analytical fame) in the same laboratory in 1881. He had carried out a reduction of (iso) 'nitrosoethylacetone" and had isolated a crystalline hydrate. After placing these crystals in a desiccator over calcium chloride he noted that anhydrous oil had formed. Analysis of this oil proved that it possessed one hydrogen atom less than would be anticipated from the formula of the simple "inner anhydride" of an amino ketone. Vapor-density determinations further indicated that the molecular weight was about twice that which mould be expected for such a compound.

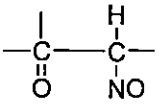
Using the reduction of acetone to pinacol as a model, Treadwell assumed that the reaction for (iso) "nitrosoethylacetone" proceeded as follows:



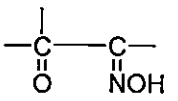
Scheme 3 Synthesis of nitrosoethylacetone

The name "ketine" vas applied to this new series of nitrogenous compounds to indicate their derivation from ketones. The simplest member, according to the Treadwell theory, would be that obtained from acetone; and this was specifically termed ketine. The other members were named as derivatives of ketine, so that Gutknecht's compound was called dimethylketine, and Treadwell's diethylketine.

The next year V. Meyer submitted a paper[40] in which he contributed two important suggestions. He first pointed out that although the products of the action of nitrous acid on the ketones were presumably nitroso compounds, they failed to respond to the Liebermann nitroso test. This led to the hypothesis that they were not true nitroso compounds but rather the isomeric oximes:



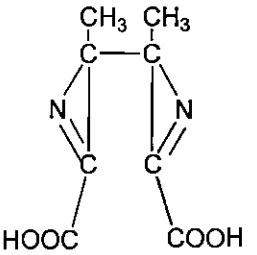
Nitroso ketone



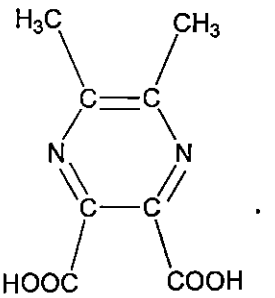
Oximino ketone

He then considered the reduction of these compounds. After making careful comparisons with the corresponding processes for nitro-amine and ketone-pinacol reductions, he concluded that the former was more analogous. This led to the abandonment of the Treadwell theory.

The first conception of the ketines as ring compounds was put forth, almost immediately, by Wleugel in an article[41] concerned with the reduction of (iso) "nitrosoacetoacetic" ester. Thus, instead of writing the structure for the "ketine" this was obtained as



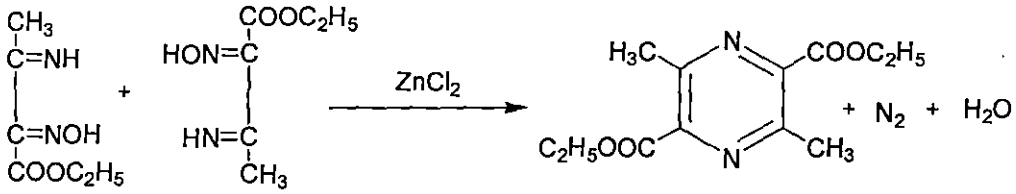
The six-membered ring was made closed by utilizing the C-C bonds involved in the two inner anhydride rings:



This resulted in the formulation of a heterocycle which, as Wleugel stated, could be conceived to be a pyridine in which the CH group para to the nitrogen atom was replaced

by another nitrogen atom. This is the modern concept of the pyrnzine nucleus; but there remained a flaw in its derivation, since Wleugel had still utilized the ketine (pinacol) mechanism.

It remained for L. Oeconomides[42] in 1886 to demonstrate experimentally that this mechanism was untenable. He attempted to dehydrate Wleugel's diacid to the acid anhydride, a reaction which should have been clearly possible if the two carboxyls were ortho to one another. This was unsuccessful, and the natural conclusion was that these functional groups had been assigned to incorrect positions on the ring. Verification came, together with a proof that the carboxyls were actually para, from the following experiment. Iminoisonitrosobutyric ester was heated with fused zinc chloride. An examination of the only plausible mechanism which could yield a "ketine" indicated that the carboxyl groups in such a compound would unambiguously be situated at the para positions:

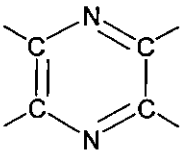


Scheme 4 Oeconomides's synthesis of pyrazine derivative

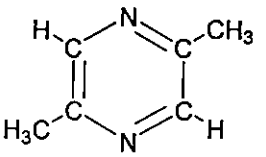
A small amount of free acid was isolated and compared with Wleugel's; the two were found to be identical. Oeconomides further called attention to the fact that Hinsberg[43] had synthesized quinoxaline, a condensed pyrazine, from *o*-phenylenediamine and glyoxal:

Scheme 5 Hinsberg's method of benzopyrazine synthesis

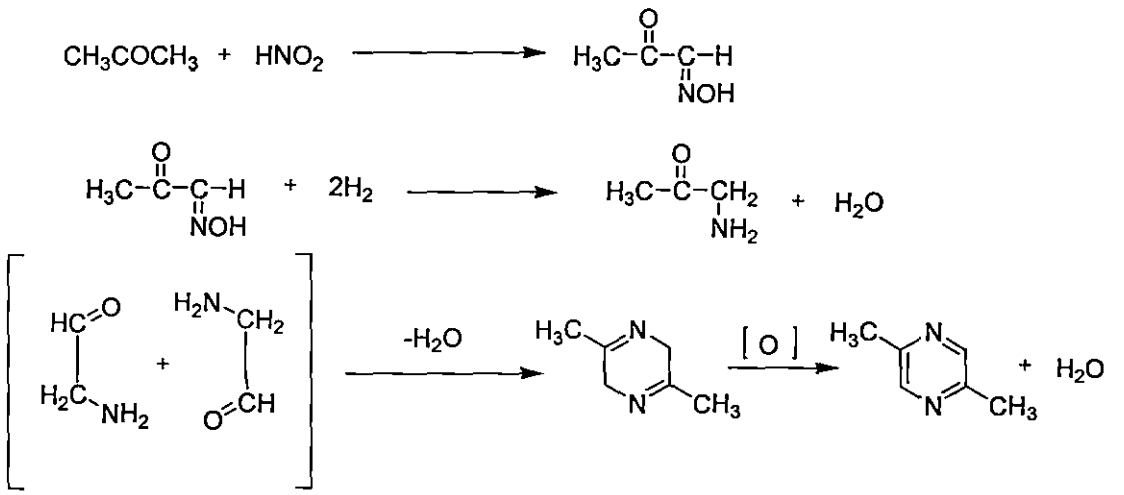
Thus, the ketine nucleus was firmly established as



and what was previously thought to be the simplest member, ketine, in fact was the dimethyl derivative.



The name "pyrazine" was independently suggested for the nucleus in the following year by Mason[44] and Wolff[45] in order to point up the correlation with pyridine. It is interesting to note that, in the same paper, Wolff acknowledged that the mechanism, fist inferred by Meyer, for the preparation of a pyrazine by the reduction of an isonitroso ketone involved an intermediate amino ketone which immediately condensed with it to yield a dihydropyrazine that was oxidized to the desired pyrazine. Thus, starting with acetone:

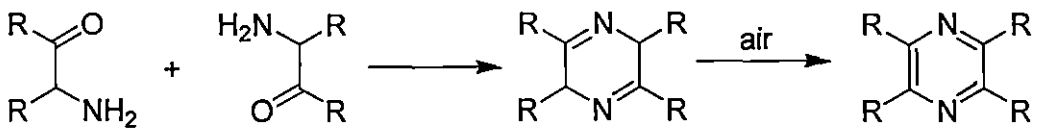


Scheme 6 Mason's synthesis of pyrazine synthesis

V. Meyer[46] objected to the term "pyrazine" on the grounds that Knorr[47] had already used it for pyrazole tetrahydride, and, in its stead, proposed the generic name "aldine," since the simplest member would result from the self-condensation of the hypothetical aminoacetaldehyde.

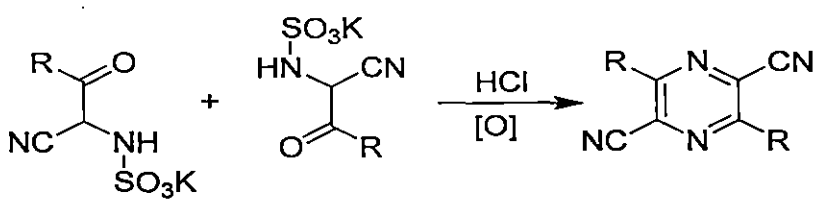
Widman[48] finally resolved the issues with a systematic nomenclature. He classified as azines those compounds which contained a six-membered ring consisting of nitrogen and carbon atoms. Hence, substances containing two nitrogen atoms in the ring were called diazines. These were further classified, according as to whether the nitrogens were ortho, meta or para, as o-diazines, m-diazines, or p-diazines. Mason condensed these names, respectively, into oiazines, miazines, and piazines; but it seems that he and his associates were the only ones to use this terminology consistently. In the light of the newly elucidated structures of the pyrazines, the results of the early workers were finally clarified.

Historically simple pyrazine was prepared as early as in 1876 by Stacdel-Rugheimer.[49] In the Stacdel-Rugheimer synthesis from 2-chloroacetophenone was reacted with ammonia to the amino ketone, then condensed and oxidized to pyrazine. In 1879 Gutknecth modified the process a little. The method was based on this self condensation but differing in the way the alpha-ketoamine is synthesised (the chlorine compound in the above method is a lachrymatory agent). [50]



Scheme 7 Gutknecth's method of pyrazine synthesis

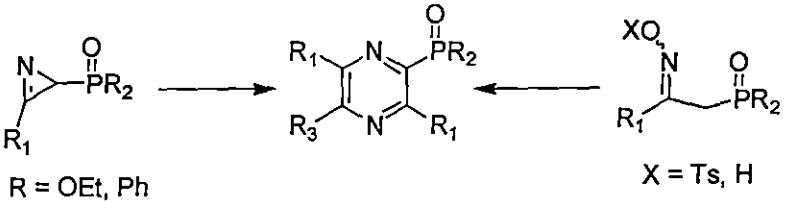
Fifty years later at the beginning of the twentieth century, Gastaldi synthesized pyrazine in a very convenient way.[51-52]



Scheme 8 Gastaldi's method of pyrazine synthesis

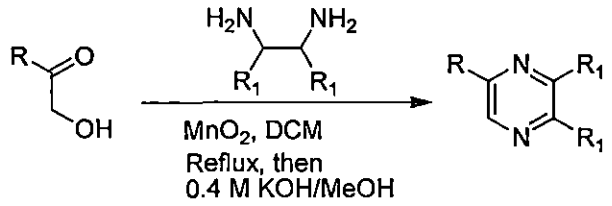
The above protocol served years to the synthetic organic chemistry. Moreover, it was a very good way for the incorporation of nitrile group into the heterocyclic moiety. Among the various methods developed, pyrazine compounds are synthesized by the reaction of diamines with diols in a vapour phase reaction in presence of granular alumina.[10] Catalytic systems such as copper-chromium,[11] copper-zinc-chromium,[12] zinc-phosphoric acid-manganese,[13] and silver[14] are also patented as catalysts for the preparation of 2-methylpyrazine from ethylenediamine and propylene glycol. Pyrazines are also obtained from condensation reaction of diamines and epoxides using copper-chromium catalyst,[15] condensation reaction between alkanolamines,[16] or cyclodehydrogenation of N-(-hydroxyalkyl) alkyldiamine[17] using the same catalysts. In the presence of palladium catalyst, dehydrogenation of piperazines yields corresponding pyrazines in high yield.[18]

Palacios *et. al.* synthesized tetrasubstituted pyrazines containing two phosphonate groups in positions 2 and 5 and trisubstituted pyrazines containing a phosphonate or a phosphine oxide group in position 2 by thermal treatment of 2*H*-azirine-2-phosphonates and -phosphine oxides.[53] This method involved simply heating the substrates at 80 °C without any added solvent, catalyst or any solid inorganic support.



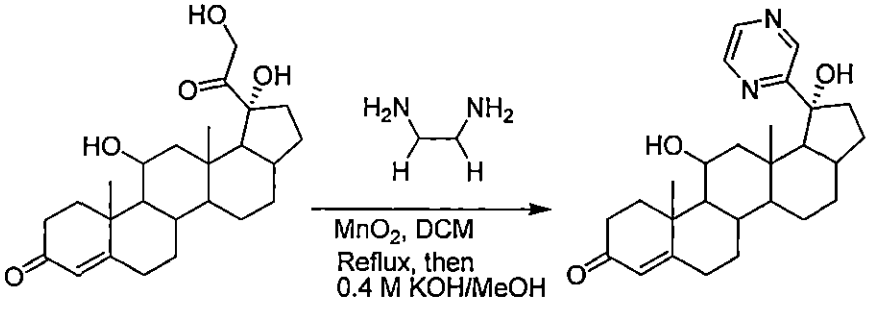
Scheme 9 Palacio's method of pyrazine synthesis

Recently, synthesis of pyrazines from α-hydroxy ketones and 1,2-diamines via MnO_2 catalyzed tandem oxidation process under refluxing conditions has been reported by Raw *et. al.* In this process α-hydroxy ketones underwent MnO_2 mediated oxidation followed by in situ trapping with aromatic and aliphatic 1,2-diamines to give quinoxalines or dihydropyrazines respectively in a one pot manner. Pyrazines were prepared in refluxing DCM with ten equivalents of MnO_2 and 0.4 M KOH solution.



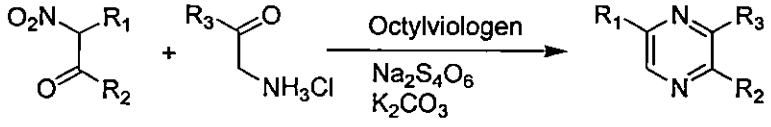
Scheme 10 Raw's method of pyrazine synthesis

In a dramatic move, they also reported the preparation of pyrazine derivatives of hydroxycortisone (a steroid) following their developed method in refluxing DCM with ten equivalents of MnO_2 and 0.4 M KOH solution. But the yield was very much poor, only 10 % isolated yield.[54]



Scheme 11 Raw's method of synthesis of pyrazine derivative of hydroxycortisone

In the communication the authors did not reported the synthesis of pyrazine derivatives of terpenoids and also the developed process required high loading of the corrosive catalyst. Elmaaty *et al.* described a new regiochemically controlled synthetic method and the optimal reaction conditions of pyrazine synthesis from α-nitro ketones. According to their report α-nitro ketones can be transformed selectively into trialkyl-substituted pyrazines via reaction with α-amino ketones using octylviologen as an electron-transfer reagent in basic condition.[55]

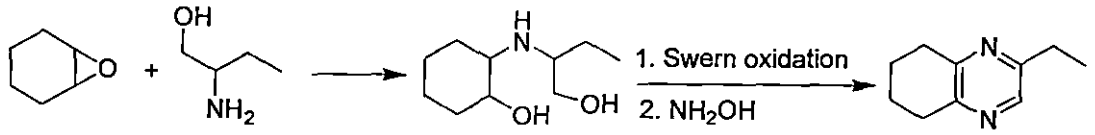


Scheme 12 Elmaaty's synthesis of pyrazine catalyzed by octylviologen

Although the process was very much utilizable for the synthesis of substituted pyrazines in good to excellent yields, but the syntheses of unsymmetrically substituted pyrazines

were a challenge to this developed method. Notably, it did not report the synthesis of pyrazine derivatives of naturally occurring steroids and terpenoids.

Taber *et al.*[56] in an interesting mode reported the preparation of pyrazine from epoxides under very mild way. Opening of the representative epoxide with 1,2-amino alcohols delivered the amino diols. The product diols were then oxidized under Swern conditions. The aminodiketones so prepared were not isolated, but were condensed directly with hydroxylamine to give the substituted pyrazines.



Scheme 13 Douglass's method of pyrazine synthesis from epoxides

In the experimental condition they[56] heated cyclohexene oxide and 2-amino-3-phenyl- 1-propanol under solvent-free conditions, but after 7 days only starting materials were visible by tlc. While $LiClO_4$ and $BF_3.OEt_2$ failed to activate the epoxide, the addition of a catalytic amount of $Yb(OTf)_3$ to the reaction facilitated an easy transformation to the amino diol. This is thought to be due to the oxophilicity of the early lanthanides. Further investigations later showed that identical loading of LiBr under solvent-free conditions effected an even faster transformation to the amino diol. When an activated epoxide was used, additions were carried out without catalyst. Indeed, if catalysts were added, an increased amount of the undesired regioisomer was observed.

The method of bubbling oxygen under refluxing condition[57] suffers from scientific drawbacks.

Synthetic transformations of natural compounds for the purpose of developing biologically active agents have become the basis of the actively advancing scientific direction of perfect organic synthesis and medical chemistry. The greatest attention of researchers is attracted by native compounds with reliably established biological activity. An attractive factor is the availability of natural metabolites due to frequent occurrence of the sources and technological reasonableness of the methods of isolation of natural substances. Widely known examples of medically successful transformations of steroids, antibiotics of penicillane and cephallosporane groups, alkaloids of morphinane series

have recently been supplemented with the modificants of cacrinostatic taxol, anti-glaucoma terpenoid forscoline, antiplasmodium medicine artemisinine and other preparations. Compounds combining availability with valuable biological activity are frequent in the class of triterpenoids. Speaking of purposeful synthetic transformations of triterpenoids for medical chemistry, perhaps the most advanced object is the glycoside of licorice, glycyrrhizinic acid and its aglycones.[58] The recent two decades gave us grounds to expect that the preparations based on triterpenoids of lupane series could be involved in therapy of a number of diseases. The expectations are undoubtedly connected with betulinic acid (3β-hydroxy-20(29)-lupene-28-oic acid) **1**, a triterpenoid surprisingly widespread in nature and easily available in almost any amount. The number of publications dealing with valuable biological activity of **1** and its natural and synthetic derivatives is increasingly growing. Numerous experimental and epidemiological studies have shown that several plant derived natural products may serve as effective anticancer drugs.[59] It was also documented in most of the literatures that suitable derivatives of naturally occurring triterpenois often showed greater biological activities in comparison to that of the naturally occurring triterpenoids. In this regard researchers around the globe had so far reported the preparation of pyrazine derivatives of naturally occurring triterpenoids.

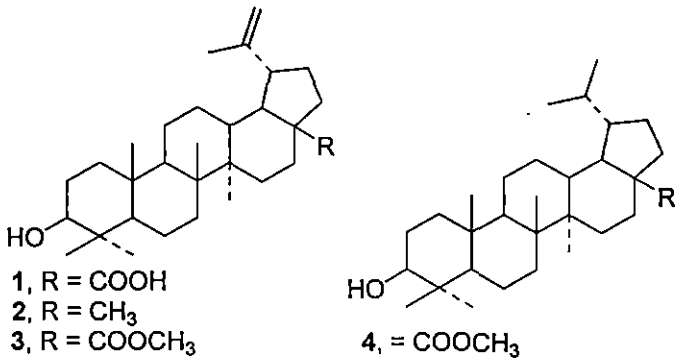
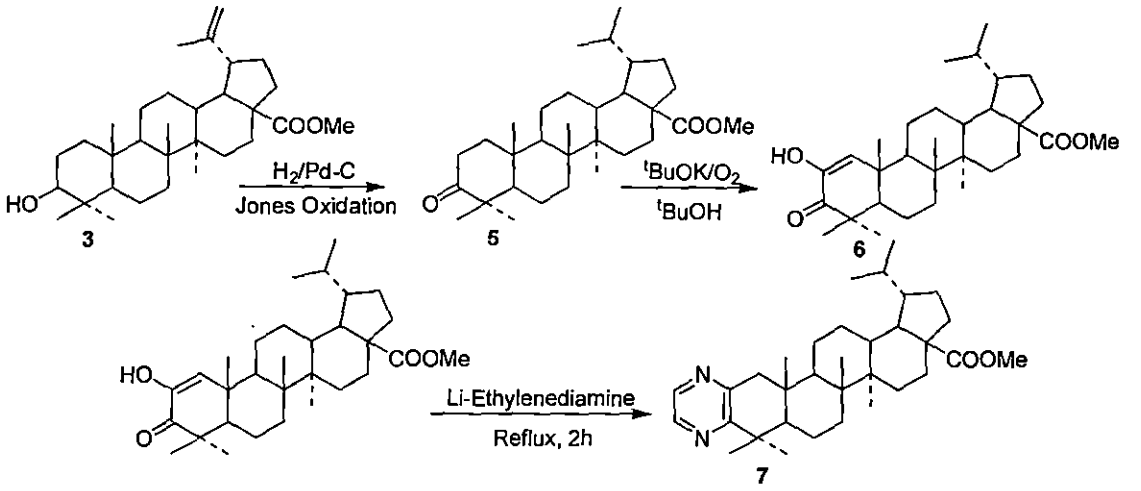


Figure 4 Lupane derived different triterpenoids

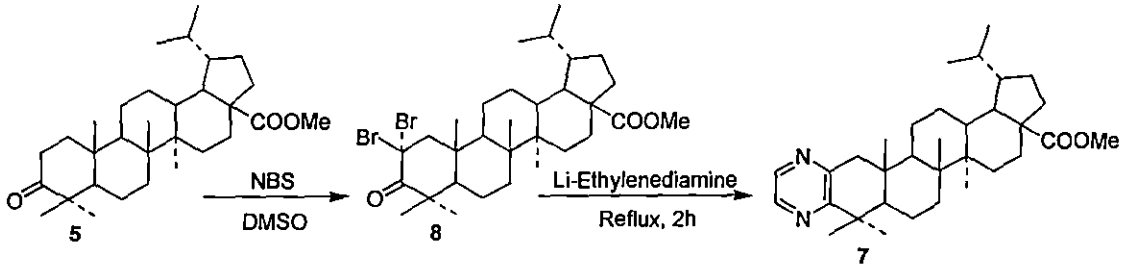
In an early attempt Pradhan *et al.* had reported the synthesis of pyrazine derivatives (scheme 7) of lupeol, **2** and methyl dihydrobetulinate, **4** in refluxing lithium-ethylenediamine in an expeditious manner.[60] Autooxidation of the respective triterpenoidal ketones (**5**) afforded the corresponding diosphenols, **6** that were then refluxed with excess ethylenediamine in presence of small pieces of lithium metals for 2

hours. After cooling to room temperature and usual work up followed by chromatographic purification afforded the corresponding pyrazine (7) derivatives with 52% yield. In this case, metallic lithium not only induced the required condensation of the diosphenol and ethylenediamine but also brought about the aromatization of the condensed compound in the same reaction media as well as in one pot.



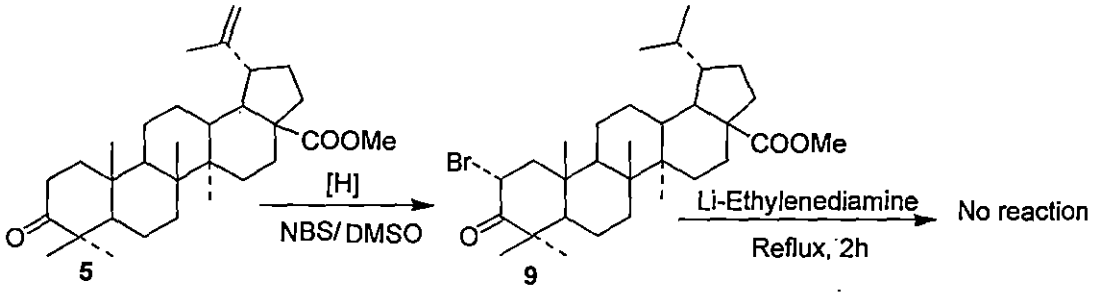
Scheme 14 Pradhan's synthesis of pyrazine derivatives of lupane triterpenoids using metallic lithium as the condensation agent

Later on the same authors also reported[61] another method of synthesis of 7 derivatives from 2,2-dibromo-28-carbomethoxyupane-3-one (8) in the same way. 2,2-dibromolupanes were prepared from the corresponding ketones in a reaction with NBS (*N*-bromosuccinimide) in DMSO (dimethyl sulfoxide) under dark condition.



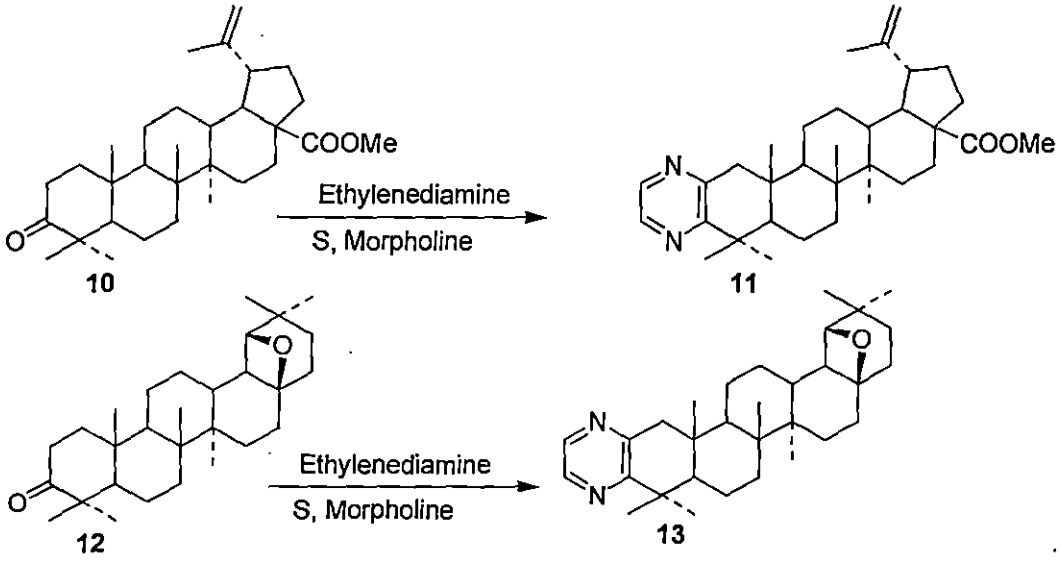
Scheme 15 Pradhan's synthesis of pyrazine derivatives of lupane triterpenoids from 2,2-dibromo-triterpenoid derivatives

Similar transformations to the corresponding pyrazine derivatives starting from monobromo triterpene derivatives *viz* 2α-bromo-methyldihydrobetulonate (**9**) derivative of lupanone were also attempted but were not successful.



Scheme 16 Failure of pyrazine synthesis from monobromo triterpenoid derivatives

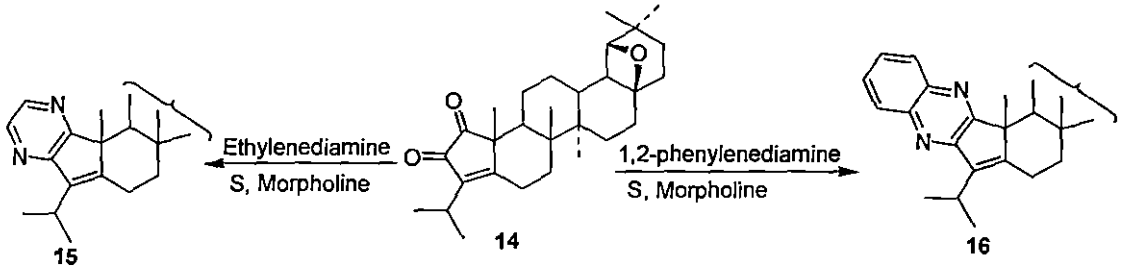
Very recently Urban *et al.*[62] has reported the synthesis of pyrazine derivatives of various triterpenoids from the respective ketones in one pot. In the experimental procedure they refluxed the ketones with amorphous sulfur using morpholine as the solvent efficiently for 4 hours. After the usual workup and purification over silica gel column they yielded the corresponding pyrazine derivatives.



Scheme 17 Urban's method of pyrazine synthesis at ring A of pentacyclic teriterpenoids

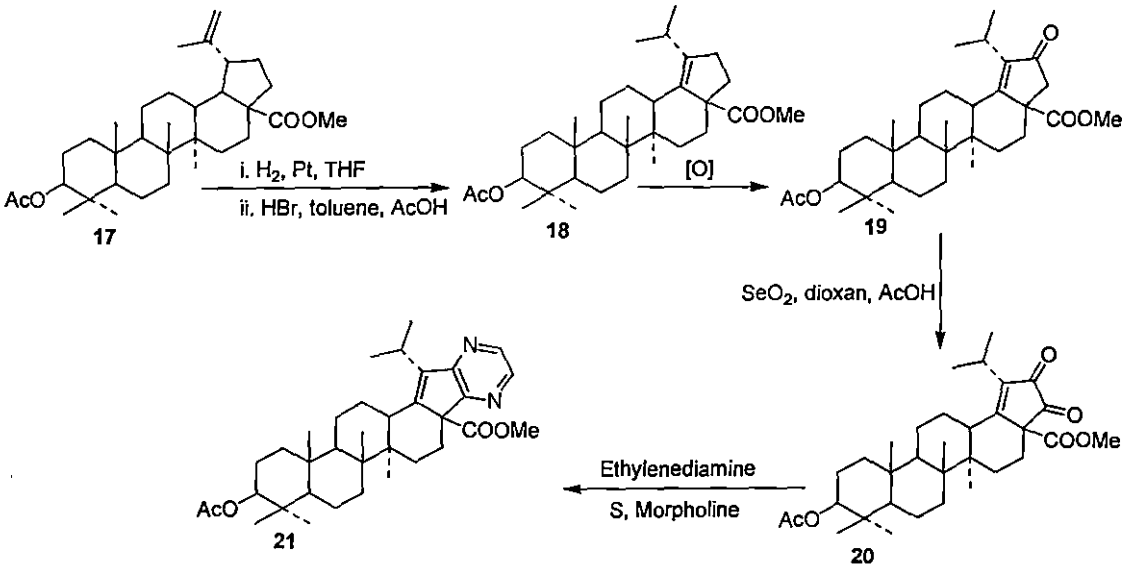
In an attempt in the same communication,[62] they suitably modified the ring A of allobetulene into a cyclopentane ring containing an isopropyl group by a series of transformative reactions. Bromination and subsequent debromination introduced a double bond in the cyclopentane ring. Afterward, a series of reactions were performed to

introduce a 1,2-diketone that then transformed chemically to the corresponding pyrazine derivative. Here also they used amorphous sulfur as the oxidant and morpholine as solvent under refluxing condition for the oxidative condensation followed by in situ aromatization. Although the yields were not good enough, the developed process was pretty good to introduce the pyrazine nucleus into the ring A of the pentacyclic triterpene skeleton.



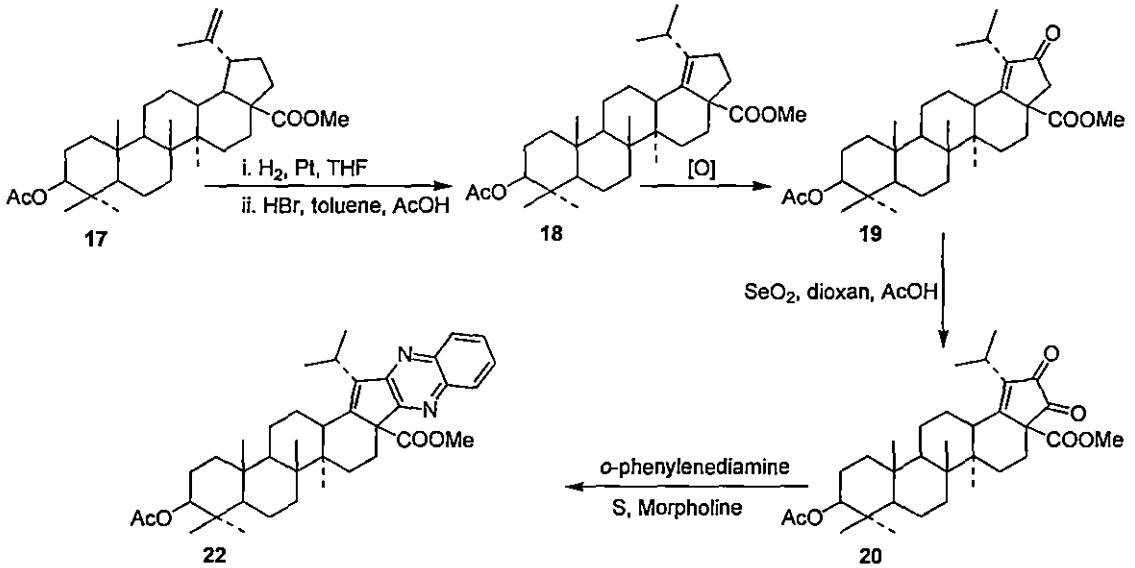
Scheme 18 Unban's method of pyrazine synthesis into transformed allobetulin derivatives

Following the procedure they[62] not only incorporated the pyrazine ring into the ring A of pentacyclic triterpenoids, but also able to incorporate the same into the ring E of the pentacyclic triterpenoids in the following series of transformative reactions.

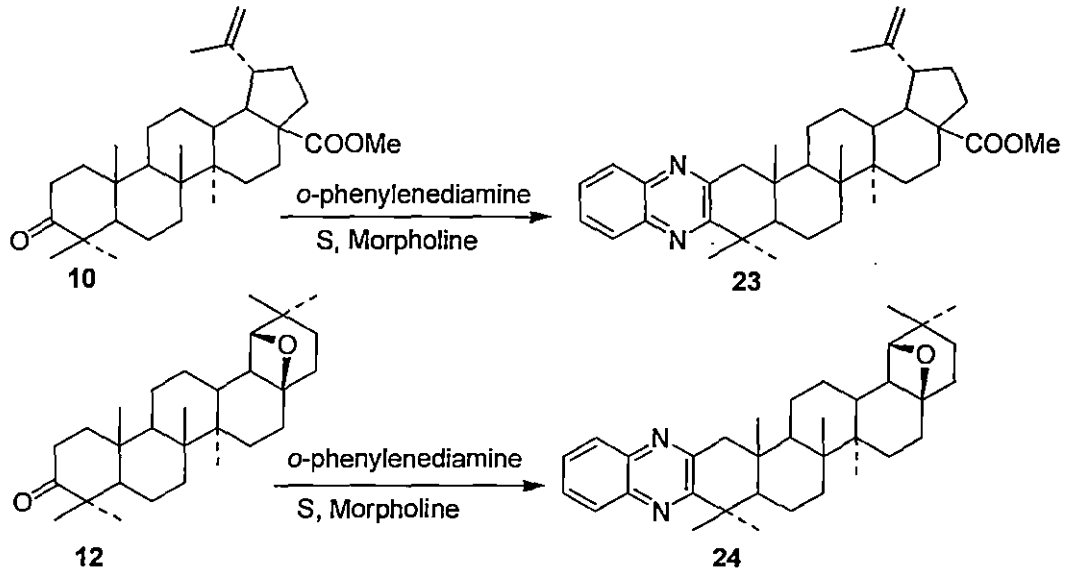


Scheme 19 Urban's method of pyrazine synthesis at ring E of pentacyclic teriterpenoids

In this same communication[62] they have reported also reported the formation of benzopyrazines or quinoxaline derivatives of triterpenoids following the same method developed by them.



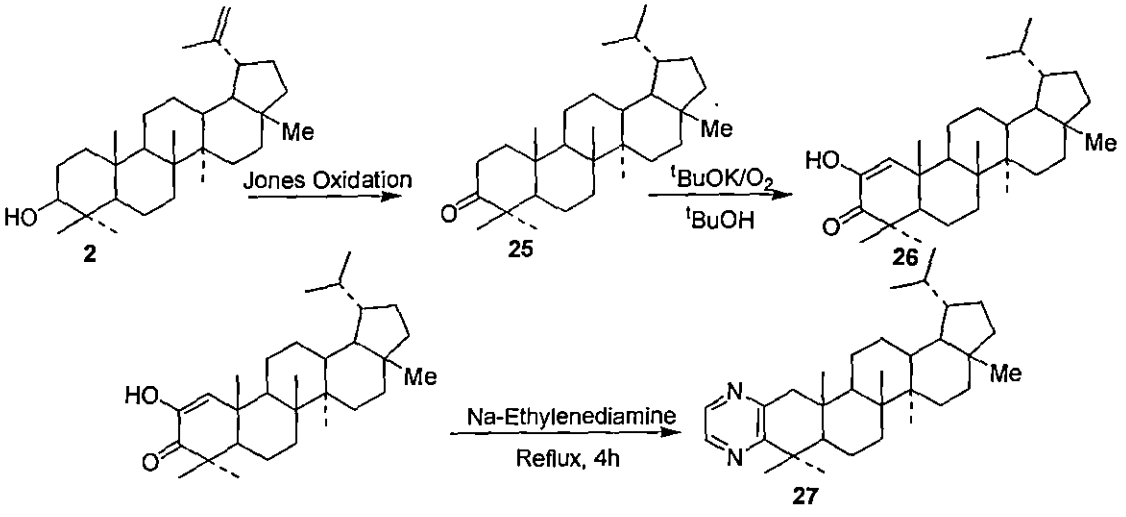
Scheme 20 Urban's method of benzopyrazine synthesis at ring E of pentacyclic teriterpenoids



Scheme 21 Urban's method of benzopyrazine synthesis at ring A of pentacyclic teriterpenoids

They method developed by Urban *et al.* is a slight modification to that developed by Sejbal *et al.*[63] as early as in 1986. They claimed that the purpose of their synthesis was to synthesize a series of pyrazine derivatives of pentacyclic triterpenes in a modified methodology. It although seemed that the method developed by Urban *et al.* was very efficient, the method used toxic amorphous sulfur and highly corrosive morpholine as the solvent which were against the principles of green chemistry as well as limits its widespread application as a modern green synthetic tool for the incorporation of pyrazine ring into ring A of triterpene skeleton.

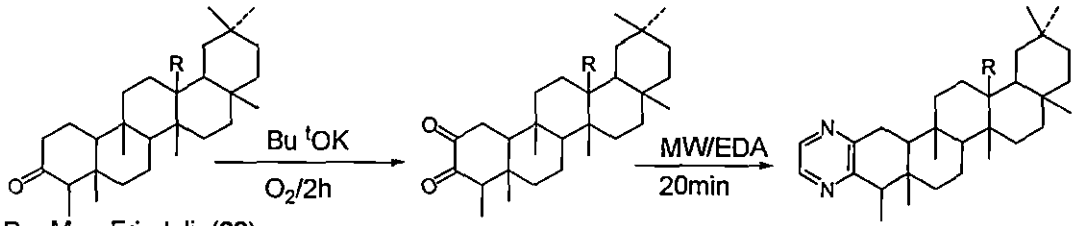
Ghosh *et al.*[64] in a good attempt to modify the work of Pradhan *et al.* carried out the same series of reactions (Scheme 8) to prepare the pyrazine derivatives of methyl dihydrobetulinate (**4**) and lupeol (**2**). In their modification they used metallic sodium instead of metallic lithium for the purpose of tandem condensation-aromatization. In their developed process they used a slight excess of ethylenediamine with the corresponding diketones or diosphenols in presence of metallic sodium without any other organic solvent and isolated the respective pyrazine derivatives in moderate to good yield. In that communication they used cheaper metallic sodium instead of lithium and therefore, the ease of toxicity associated with metallic lithium was eliminated and that their report is a forward footstep to the direction of "*Green Chemistry*".



Scheme 22 Synthesis of pyrazine derivatives of lupanol by Ghosh *et al.*

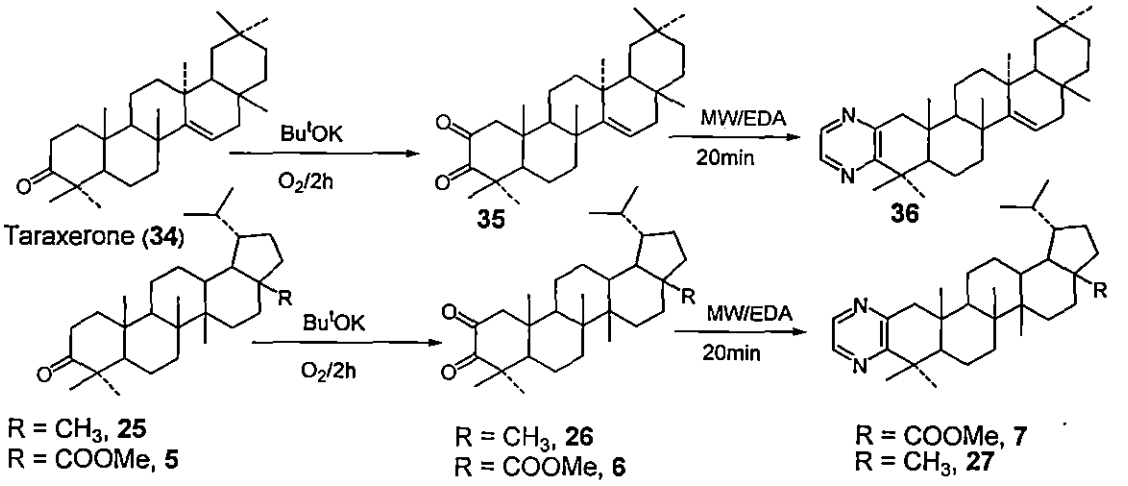
Although their developed protocol is a footstep towards green chemistry, the method suffers from certain drawbacks from the present perspective of green chemistry. It is not atom economic, not energetically safer technique and the time required for the process was high too. Too much ethylenediamine was used to carry out the transformation.

To overcome the above limitations Ghosh *et al.*[65] attempted another procedure (Scheme 13 and 14) in which they used microwave irradiation as the energy source remaining all other things as same as that reported by Pradhan *et al.*[62] The newly developed process is energetically efficient one and the duration of the reaction is very short too, only twenty minutes is sufficient to complete the reactions. In this method they were able to synthesize the pyrazine derivatives of five different pentacyclic triterpenoid molecules which include two examples from lupane skeleton, two from friedelan skeleton and one from taraxar skeleton. The potential application of microwave technology (MW) in organic synthesis is increasing[65] rapidly because of the reaction simplicity, less polluting and minimum reaction time providing rapid access to large libraries of diverse molecules. This technology has been implemented since the middle of 1980s in the field of organic chemistry.



R = Me - Friedelin(**28**)
R = COOMe- Methyltricadenate(**29**)

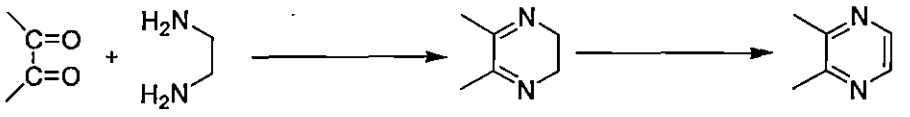
30 = Diketo friedelin
31 = Diketo derivative of the methyl ester of tricadenic acid
32 = Pyrazine derivative of friedelin
33 = Pyrazine derivative of methyltricadenate



Scheme 23 Synthesis of pyrazine derivatives of friedelin, lupanol and dihydro methylbetulonate by Ghosh *et al.* under microwave irradiation

Although the methods are useful for the incorporation of the pyrazine nucleus in the triterpenoid skeleton, but these methods do not satisfy completely the modern trends in "*Green Chemistry*". The use of excess corrosive ethylenediamine limits its varsatile application as a general tool for pyrazine synthesis. Although in modern days microwave irradiation has emerged as a safe source of energy to perform organic reactions, reactions performed at room temperature are always preferable. Additionally all the methods described above are not atom economic and in most of the cases the prepared side products are not environmentally safer.

Strategically, direct condensation reaction of 1,2-diketones with 1,2-diamine is the most straightforward as well as the classical route for the preparation of pyrazines via dihydropyrazine intermediates.



Scheme 24 Direct strategic approach to pyrazine

Although, a number of methods are reported in literature for the synthesis of pyrazine, none of them was found to be effective because of poor yield, harsh reaction condition and tedious work-up procedures.[21] Attempts to carry out dehydrogenation under a variety of milder and more convenient laboratory procedures were not successful. Although, some of them are apparently useful, most of them are limited by long reaction time, low yields, and use of toxic solvents or heavy metals as the catalyst.

"***Green chemistry***", also called sustainable chemistry, is a philosophy of chemical research and engineering that encourages the design of products and processes that minimize the use and generation of hazardous substances. Whereas environmental chemistry is the chemistry of the natural environment, and of pollutant chemicals in nature, green chemistry seeks to reduce and prevent pollution at its source. In 1990 the Pollution Prevention Act was passed in the United States. This act helped create a *modus operandi* for dealing with pollution in an original and innovative way. It aims to avoid problems before they happen. The main focus is on minimizing the hazard and maximizing the efficiency of any chemical choice. Paul Anastas, then of the United States Environmental Protection Agency, and John C. Warner developed 12 principles of green chemistry, which help to explain what the definition means in practice. The principles cover such concepts as:

- the design of processes to maximize the amount of raw material that ends up in the product;
- the use of safe, environment-benign substances, including solvents, whenever possible;
- the design of energy efficient processes;
- the best form of waste disposal: not to create it in the first place.

From green chemistry point of view a synthetic protocol should have the above criteria. All the existing methods for the synthesis of pyrazine derivatives although synthetically useful, but all of them suffer a number of limitations if we keep on looking from green chemistry point of view. Therefore, development of mild, efficient and environmentally

benign method for synthesizing pyrazine derivatives keeping the 12 points of green chemistry principles has been a major challenge in contemporary organic synthesis