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Studies on Natural Products†

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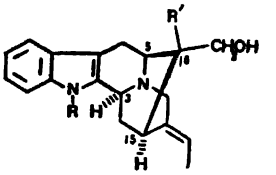
It is indeed a privilege to have been elected by the Indian Chemical Society to deliver the endowment lecture instituted in the memory of Dr. Basudev Banerjee. The scientific community had lost an organic chemist of great potential in the untimely death of Dr. Basudev Banerjee at the age of forty-two.

The present lecture reviews certain aspects of our work on Natural Products and fields related to it. India has a rich heritage of the use of medicinal plants in the traditional schools of medicine, viz. the Ayurveda system, the Unani system and the Siddha system. Many plant-based preparations were used in folk-medicine. Even now a very large number of drugs in current usage are either Natural Products or derived from Natural Products.

Natural Products Chemistry has been one of the most active fields of scientific endeavour in India. It is reported that Shaugnessy of the Calcutta Medical College, was the first to investigate the active principles of Indian Medicinal Plants as early as the 1840s[1]. The pioneering work of Siddiqui on alkaloids and of J. L. Simonsen on terpenoids in the early part of this century established the foundations of Natural Products research in India. In Calcutta, it was Acharya Prafulla Chandra Ray who initiated, albeit in a small way, work on the active principles of garlic and onions. Subsequently, a major school of Natural Products research was established at the Chemistry Department at Calcutta University through the dedicated efforts of Dr. P. K. Bose, Professor D. Chakraborty and Professor (Mrs.) A. Chatterjee. Natural Products research continues to be one of the major research activities of our Department at Calcutta.

Significant contributions have been made on the chemistry of alkaloids from our Department, and my initial research interests were in the field of indole alkaloids of *Tabernaemontana*, *Rhazya* and *Alstonia* species.

Two *Alstonia* species, *Alstonia scholaris* (Bengali : *Chhatim*) and *Alstonia macrophylla* grow abundantly in India. These plants have been extensively investigated for their alkaloidal content at our Department[2,3], the present author being involved in much of this work. Additionally other *Alstonia* species, such as *A. venenata* and *A. congensis*, have been also investigated. *A. scholaris* and *A. macrophylla* furnished a wealth of indole alkaloids of diverse structural patterns. It is perhaps chemotaxonomically significant that only bases belonging to those categories having an unrearranged Type I monoterpenoid part have been isolated from these plants. Rhazine (akuammidine)[4-6] (1) belonging to the sarpagine group has been obtained from *A. scholaris*[2] as well as *A. congensis*[7]. Affinisine (2), another sarpagine-type base, was obtained from *A. macrophylla*[11]. Among ajmaline-type bases, we obtained the known quebrachidine (3) from *A. macrophylla*[8], while the new alkaloid 17-*O*-benzoylvincamajine (4) was characterised by Chatterjee and Mukherjee[9]. Several



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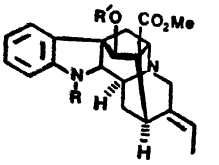
(2) R=Me, R^1=H



(3) R=Me, R^1=H
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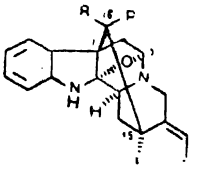

(5) R=H, R^1=CO_2Me
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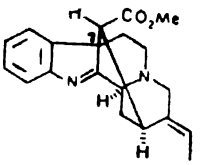

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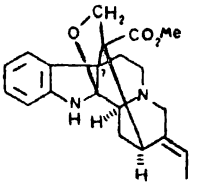

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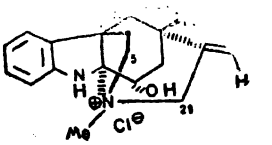

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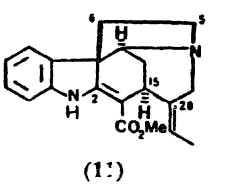
picraline type bases were also obtained. These included picrinine[10,11] (5), previously known as a transformation product[12], but first isolated from nature by Chatterjee and Mukherjee[10]; the

†Basudev Banerjee Memorial Award Lecture (1985) delivered at the 26th Annual Convention of Chemists, organised under the auspices of the Indian Chemical Society on 26th December, 1989 at Indore at the Devi Ahilyabai Viswavidyalaya.

corresponding C_{16}-epimer picralstonine (6)[11]—a new alkaloid; picralinal[13,30] (7), first isolated from *Picralima nitida* by Smith[12]; strictamine (8) isolated by Ray[14], which was obtained as a new base by Chatterjee *et al.* from *Rhazya stricta*[15]; and the known alkaloid pseudo-akuammigine[16] (9). Echitamine (10), an alkaloid whose structure-elucidation took about a century, was isolated from *Alstonia scholaris*[2], and recently from *Alstonia congensis*.

Picralstonine[11] (6) exhibited uv and mass spectra virtually identical with picrinine (5), with which it differed in physical properties (m.p., tlc and ir spectra). That picralstonine was epimeric with picrinine at C-16 was established by saponification experiments. Hydrolysis of the former with ethanolic KOH gave an amorphous acid, which on remethylation with diazomethane, furnished the original compound and another substance identified as picrinine (5). Further, when picrinine was refluxed with sodium methoxide in anhydrous methanol for 4 h, picralstonine was obtained in addition to unchanged picrinine. Picralstonine was thus characterised as 16-epi-picrinine (6). The ir spectra of both the compounds were very similar, with the exception that the position of the —NH— band in picralstonine shifted from 3 248 cm^{-1} observed in picrinine to 3 049 cm^{-1}. This was presumably due to the strong intramolecular hydrogen-bonding with the proximate carbomethoxyl group which would not be possible in picrinine.

Several Strychnos type bases, viz. akuammicine[18,19] (11), akuammicine-N_b-methosalt[19], akuammicine-N_b-oxide[19], N_a-methyl-2,16-dihydro-akuammicine[20] (12), tubotaiwine[19] (14), 18/19-hydroxy-19,20-dihydroakuammicine[19], echitamidine (15), (+)-lochneridine[21] (16), scholarine[22] (17) and scholaricine[23] (18) have been obtained from the two *Alstonia* species. The last two were new alkaloids. Scholarine[22] (17); $C_{21}H_{26}N_2O_4$ (M^+ 370, $[\alpha]_D^{25}$ 0° ($CHCl_3$), m.p. 205–06°d showed the uv (λ_{max}^{EtOH} 234, 291, 335 nm) and ir spectral characteristic of a β-anilinoacrylate derivative. Its uv and mass spectral fragmentation suggested a methoxy-echitamidine structure. This was confirmed by its reduction with zinc-methanolic sulphuric acid to a dihydro-derivative (19), $C_{21}H_{28}N_2O_4$ (M^+ 372), which gave very characteristic uv (λ_{max}^{EtOH} 230, 291 nm), ^1H nmr and mass spectra corresponding to 19 (Chart 1). The position of the methoxyl group at C-12 was settled by ^{13}C nmr studies. Scholarine was isolated as a racemate; to the present author's knowledge this is the second instance of the isolation of a racemic akuammicine-type alkaloid. The first instance was the isolation of pseudoakuammicine [(±)-akuammicine] by Henry from *Picralima nitida*. (—)-Scholarine has been obtained subsequently[21]. Scholaricine[23] (18) is the phenolic alkaloid corresponding to scholarine (17).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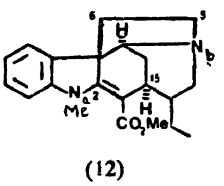
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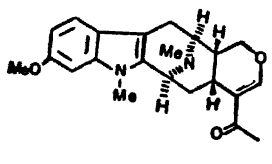
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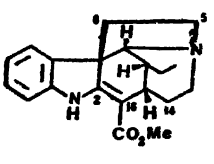
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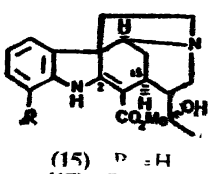
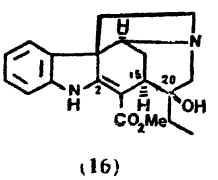

(15) R=H
(17) R=OMe
(18) R=OH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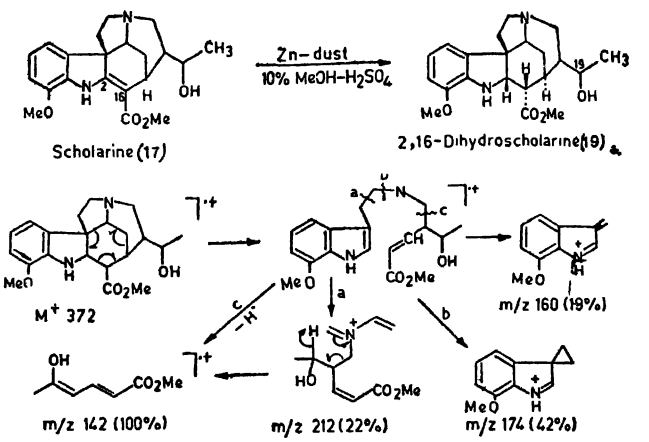


Chart 1. Scholarine.

Several interesting alkaloids have been isolated from *A. macrophylla* by research groups working at Calcutta and Zürich. Mention may be made of pleiocarpamine[24], and the three 'dimeric' indole alkaloids—villalstonine[25,26], macralstonidine[26], macrophylline[27] and macralstonine[26,27]. The macroline-type of base alstophylline (13)[28,29] and macrosalhine[29] have been obtained from *Alstonia macrophylla*.

The most interesting compounds obtained from *A. scholaris* were the novel alkaloids nareline[30] (20) and SA-I (5-epi-nareline) based on the unique indolo-aza-adamantane skeleton. Nareline (20); m.p. 275°, $[\alpha]_D^{28}$ —88° (pyridine) exhibited the typical uv characteristics of an indolenine derivative. Its

ir and ^1H nmr (100 MHz) spectra showed the presence of a carbomethoxyl, an ethylidene and an hydroxyl group. The involvement of a hydroxyl group in a hemiacetal moiety was shown by its conversion to a monomethyl ether with methanolic sulphuric acid, the ready formation of a monooxime and the appearance of a one-proton doublet at δ 3.91 in its ^1H nmr spectrum (d_6-DMSO), which changed to a singlet at δ 5.08 in its mono-acetate. The mass fragmentation of nareline and its derivatives were not characteristic of any previously known class of indole alkaloids. It became apparent at this stage that nareline did not belong to any of the known skeletal patterns. Its structure was settled after prolonged work which included a series of chemical transformations (Charts 2 and 3) often using deuterated reagents to introduce deuterium labelling at specific sites in the molecule, and detailed spectroscopical (^1H and ^{13}C nmr and MS) studies of nareline and its derivatives. The structure was confirmed finally by *X*-ray crystallographic analysis. This work was done in the 1970s when the modern two-dimensional nmr techniques had not yet come into vogue, nor were powerful computer-linked *X*-ray units developed. Hence its structure elucidation required about six years of intensive collaborative effort at Calcutta and Zürich, and substantial amounts of the alkaloid had to be painstakingly isolated by column chromatographic techniques, as the MPLC and HPLC instruments were not available in those days. In a biogenetic sense nareline is related to akuammiline and picraline from which it is derived by a sequence initiated by a 4,5-bond cleavage. An unusual oxidation of nareline with chromic acid in glacial acetic acid was observed to give oxo-nareline (22), a rearranged oxindole containing an acetal functionality (Chart 4)[30], via the seco-intermediate (21).

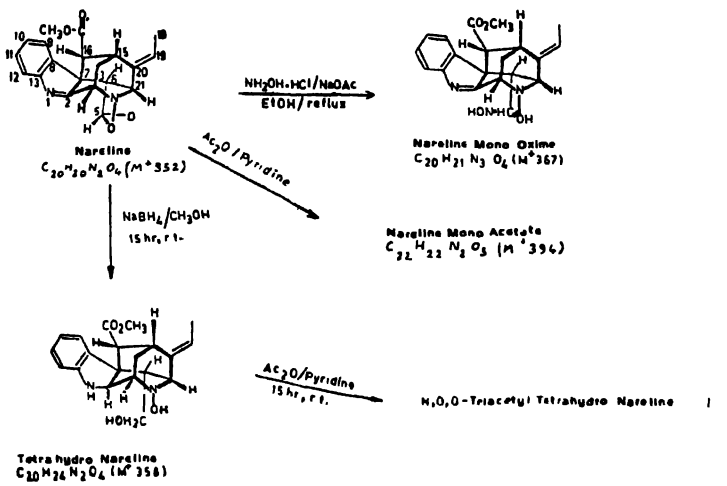


Chart 2. Reactions of nareline.

Our interest in the chemistry of indole alkaloids extend to the study of transformation reactions

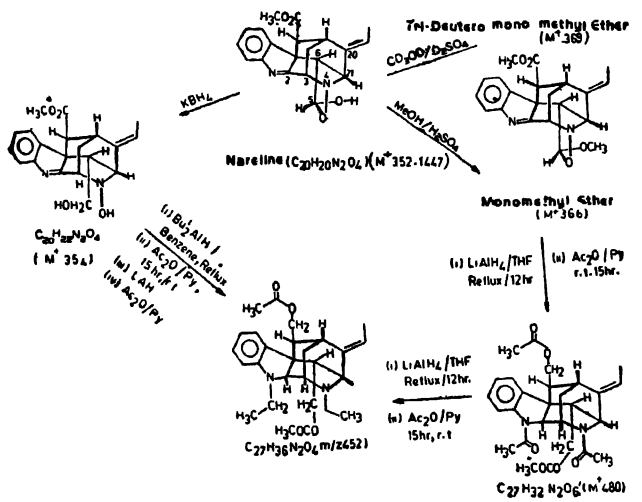


Chart 3. Reactions of nareline.

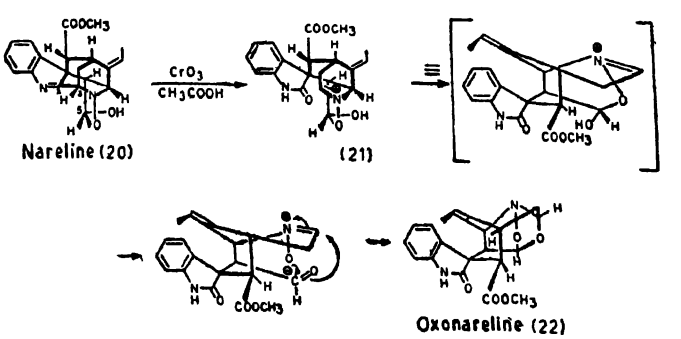


Chart 4. Formation of oxonareline.

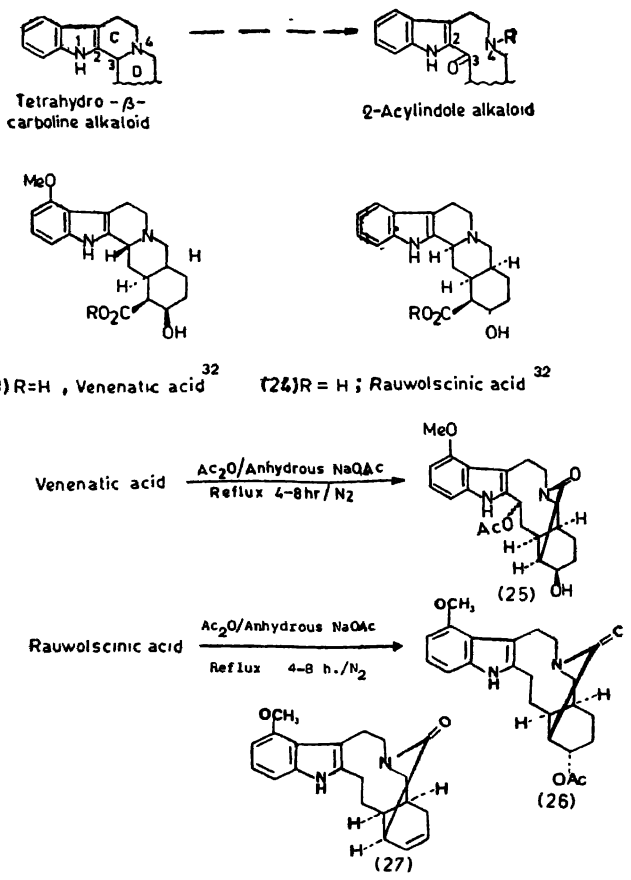


Chart 5. *C,D*-Ring cleavage of yohimbinoid alkaloids.

with special emphasis on the interconversions of different classes of alkaloids. Tetrahydro-β-carboline bases often co-occur with the corresponding 2-acylindole derivatives in the same plant. It is believed that the latter are biogenetically derived from the former by a reaction sequence which involves a 3,4-bond cleavage as the key step (Chart 5). This 3,4-bond cleavage reaction of tetrahydro-β-carboline alkaloids has been investigated *in vitro* by several research groups, including ourselves[32-40]. One strategy was to quaternise $N_{(b)}$ and carry out a nucleophilic attack at C_3. A carboxylic acid functionality suitably situated in the same molecule, can be utilised as the electrophilic reagent for quaternising $N_{(b)}$ (Chart 5). This strategy has been used by us with venenatinic acid (23) and rauwolscinic acid (24) which possess a *cis-D,E*-ring juncture and C_{16}-β-carboxyl group[32]. On refluxing these acids in acetic anhydride in presence of fused sodium acetate, a *C,D*-ring cleavage occurred to give 3,4-seco-lactams (25—27). The reaction was initiated by nucleophilic attack of $N_{(b)}$ on the carboxyl group, or rather the mixed anhydride formed under the reaction conditions, to give an unstable quaternary lactam (28). This was opened up on nucleophilic attack by acetate at C_3 to give the resultant seco-lactam (25). In the more flexible *cis-D,E* series a chair-to-boat transformation on ring-*D* would bring the N_b-lone pair close to the C_{16}-carboxyl group (29) which is the spatial requirement for the reaction to proceed (Chart 6)[32]. Alkaloids possessing a rigid *D,E-trans*-fused ring system, e.g. yohimbic acid, would be unable to undergo a similar change and hence did not

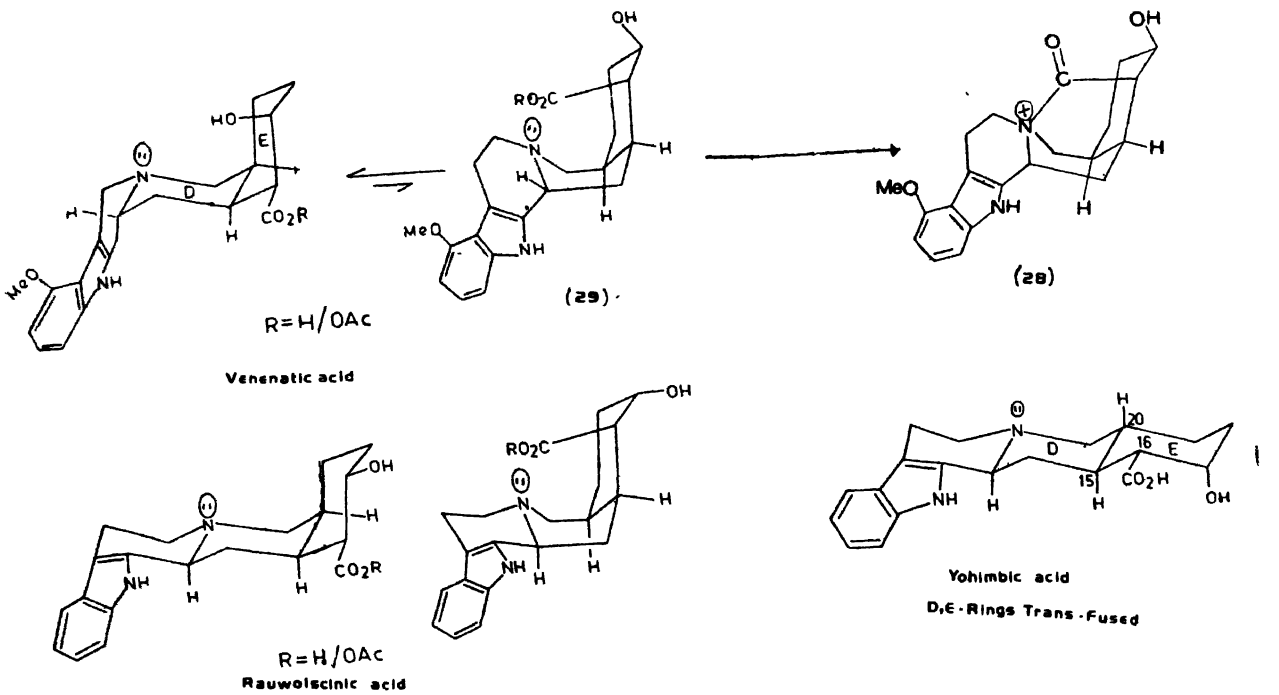


Chart 6. *C,D*-Ring cleavage of yohimbinoid alkaloids - conformational aspects.

undergo this type of reaction. The seco-lactam products (**26, 27**) derived from rauwolscinic acid were the results of further reactions where the intermediate 3,14-dehydro compound formed by loss of acetic acid was reduced, presumably by disproportionation reactions in the reaction medium.

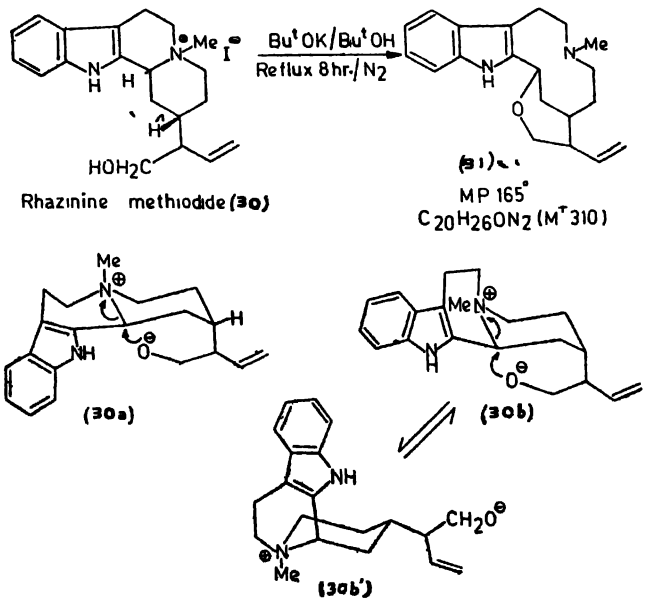


Chart 7. *C,D*-Ring cleavage of rhazinine methiodide.

The second example of a 3,4-bond cleavage was observed with rhazinine methiodide (**30**)[33]. An attempted Hofmann degradation of the methiodide with potassium tertiary butoxide yielded a single product (**31** ; Chart 7). This example is extraordinary in the sense that the attacking nucleophilic centre was situated elsewhere in the molecule.

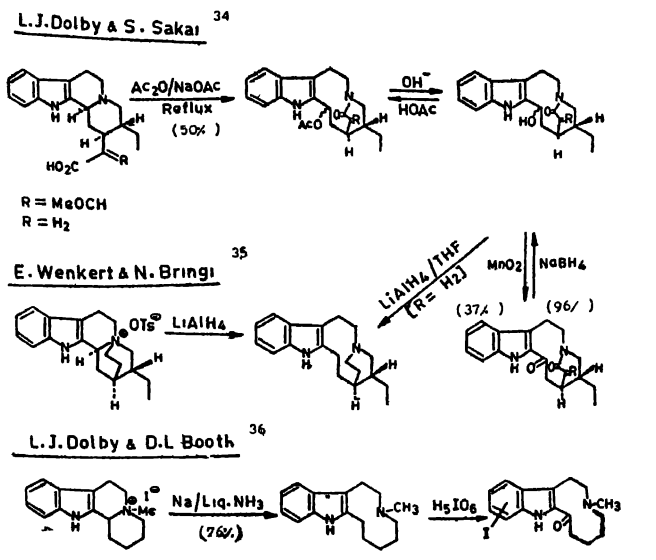


Chart 8. *C, D*-Ring cleavage of tetrahydro-β-carboline alkaloids.

The nucleophilic attack would occur in the appropriate conformations of the two alternative methiodides (**30a** and **30b**).

Several other research groups have done related work (Charts 8 and 9). Dolby and Sakai's investigations with the tetracyclic alkaloids utilised the intermediate formation of quaternary lactams[34]. Reductive cleavages of the 3,4-bond have beeu nsed by Wenkert[35] and Booth[36] with the use of lithium aluminium hydride and sodium/liquid ammonia

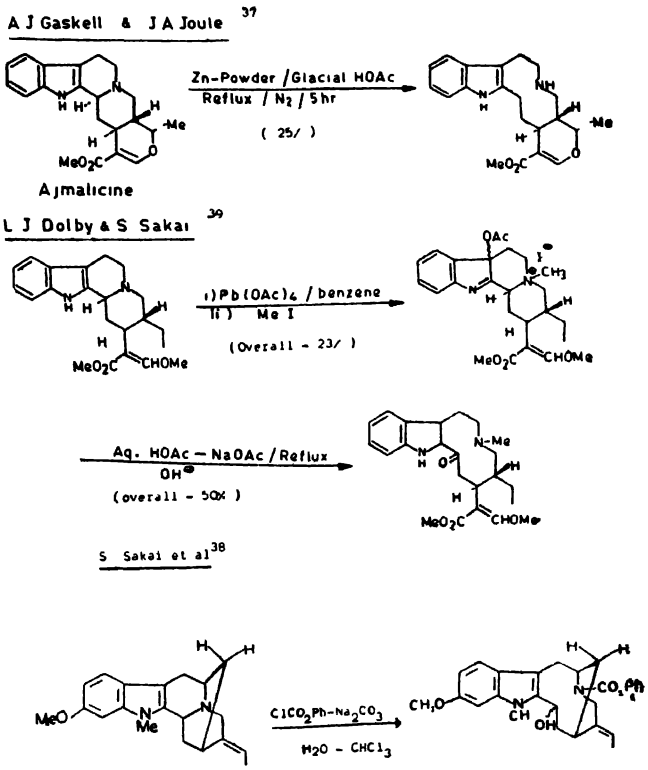


Chart 9. *C,D*-Ring cleavage of tetrahydro-β-carboline alkaloids.

respectively as reducing agents. Gaskell and Joule's method[37] was also reductive, with zinc and acetic acid being used as the reducing agents. Phenyl chloroformate has been used by Japanese chemists to convert sarpagine-type alkaloids to 3,4-seco-urethanes[38]. In another approach Dolby and Sakai utilised the 3-acetoxyindolenine intermediates[39]. Treatment of these with aqueous acetic acid-sodium acetate directly led to the 2-acylindole.

We have used a modified von Braun procedure using ethanol as solvent, to effect the 3,4-bond cleavage in venenatine (**32**) and rhazine (**1**)[40] (Charts 10—12). Diastereoismeric mixtures of 3-ethoxy-3,4-seco-cyanamides (**33** and **34** ; **35** and **36**) were obtained from both venenatine and rhazine. The use of ethanol as the nucleophilic solvent thus avoided the formation of unstable 3-bromo-secocyanamides, the handling of which is usually difficult. The C_3-configuration of the products could be determined on the basis of stereospecific ring closure

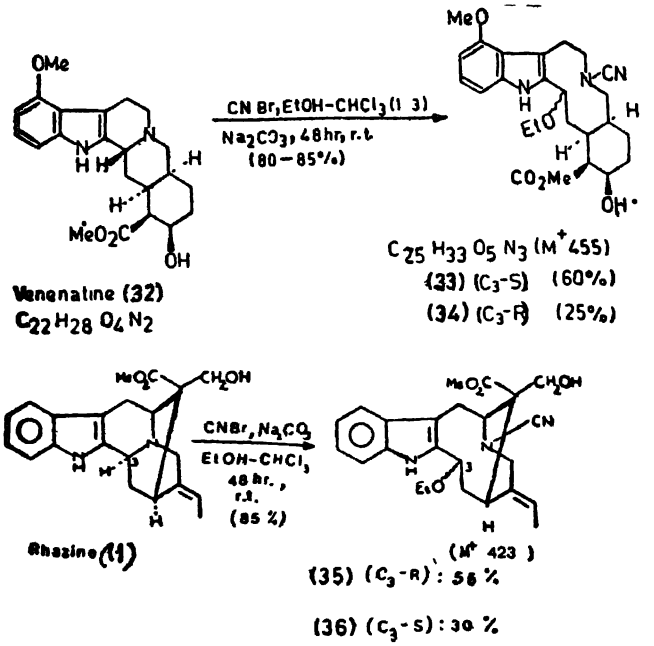


Chart 10. Modified von Braun cleavage of venenatine and rhazine.

achieved by treatment of the seco-cyanamides with glacial acetic acid at 70° (Chart 11). A S_N2 displacement at C_3 occurred to give venenatine exclusively from the major isomer (33), which therefore had the *S*-configuration at this centre. Alstovenine (37) resulted as the sole product from the C_3-*R* isomer (34). The diastereoisomeric mixture of seco-cyanamides was converted to the 3-chloroindolenines (38), which on subsequent warming with dilute methanolic acid gave the 2-acylindole (39). Hydrolysis of the cyanamide group could be achieved by warming with aqueous acetic acid-ammonium acetate or alternatively by hydrolysis of the imino-chloride obtained by the action of anhydrous methanolic HCl. Two-step methylation, first of the carboxylic acid and then of the N_b-methyl, furnished the desired 2-acylindole (40) (Chart 12).

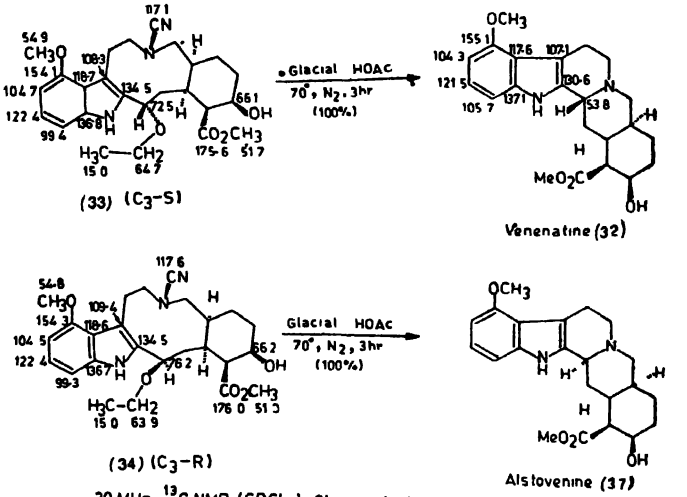


Chart 11. Determination of C_3-configuration of seco-cyanamides (33 and 34).

Our interest in indole alkaloids led us to another aspect of our work, viz. the reactions of simple indole derivatives with metallic reagents[41-44]. To alkaloid chemists it is a familiar fact that treatment of a tetrahydro-β-carboline base with mercuric acetate gives the $\Delta^{3,4}$-dehydro compound, only if the C-3 hydrogen is *syn*- and *anti*-periplanar to the nitrogen lone-pair. Lead tetraacetate, being a stronger oxidising agent converts tetrahydro-β-carboline bases to the π-dehydro compounds. As thallium occupies an intermediate

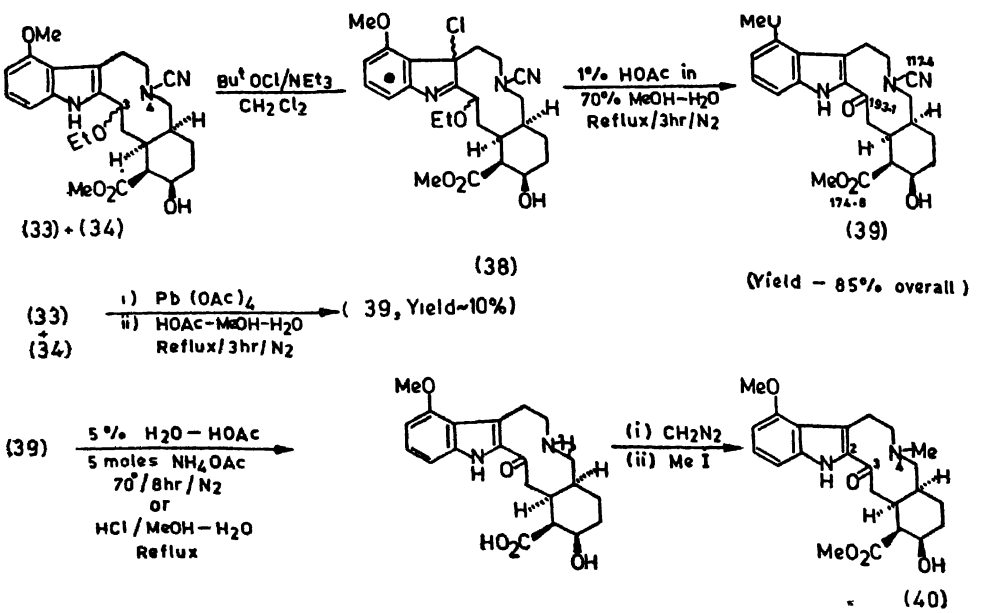


Chart 12. Conversion of seco-cyanamide to 2-acylindole.

position between mercury and lead, it was of interest to us to find out the action of thallium(III) salts on indole alkaloids as well as simple indole derivatives. Our interest was also raised by the fact that while the reactions of thallium(III) salts with various types of organic compounds had been investigated, reports on reactions with heterocycles were extremely rare.

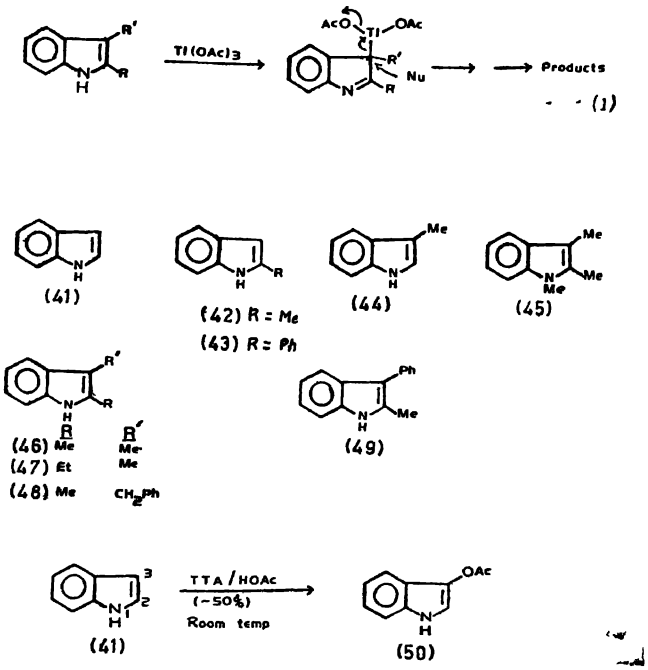
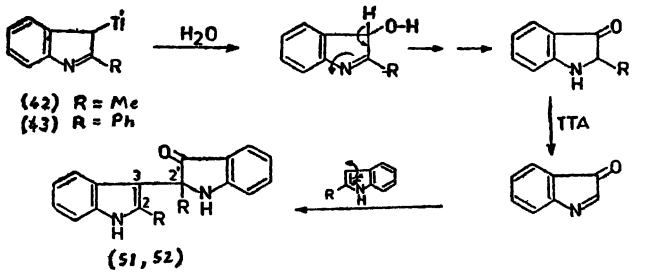


Chart 13.

Reactions of several indole derivatives (41—48; Chart 13), variously substituted in the 2- and 3-positions, with thallium triacetate (TTA) were investigated in acetic acid medium containing about 10% of water under nitrogen atmosphere[41-44]. Monomeric and dimeric oxidation products were obtained, the actual structure depending on the substituents in the 2- and/or 3-position. The formation of all the products could be explained



Substrate	*Reaction condition*	*Product (yield)*
2-Methylindole	TTA/HOAc, r.t., 20 h	2-Methyl-3-(2′-methyl-indoxyl-2′)indole (51) (15%)
2-Phenylindole	TTA/HOAc, r.t., 30°, 70 h ; 45 - 50°, 24 h	2-Phenyl-3-(2′-phenylindoxyl-2′)indole (52), (30%)

Chart 14. Reactions of 2-methyl- and 2-phenyl-indole.

by an initial electrophilic attack at the electron-rich 3-position of the indole to give a 3-thallated indolenine, which underwent further reactions *in situ* (equation 1). We were unable to isolate the intermediate organothallium derivatives. In most of the reactions thallium is displaced by a nucleophile to yield a product, which

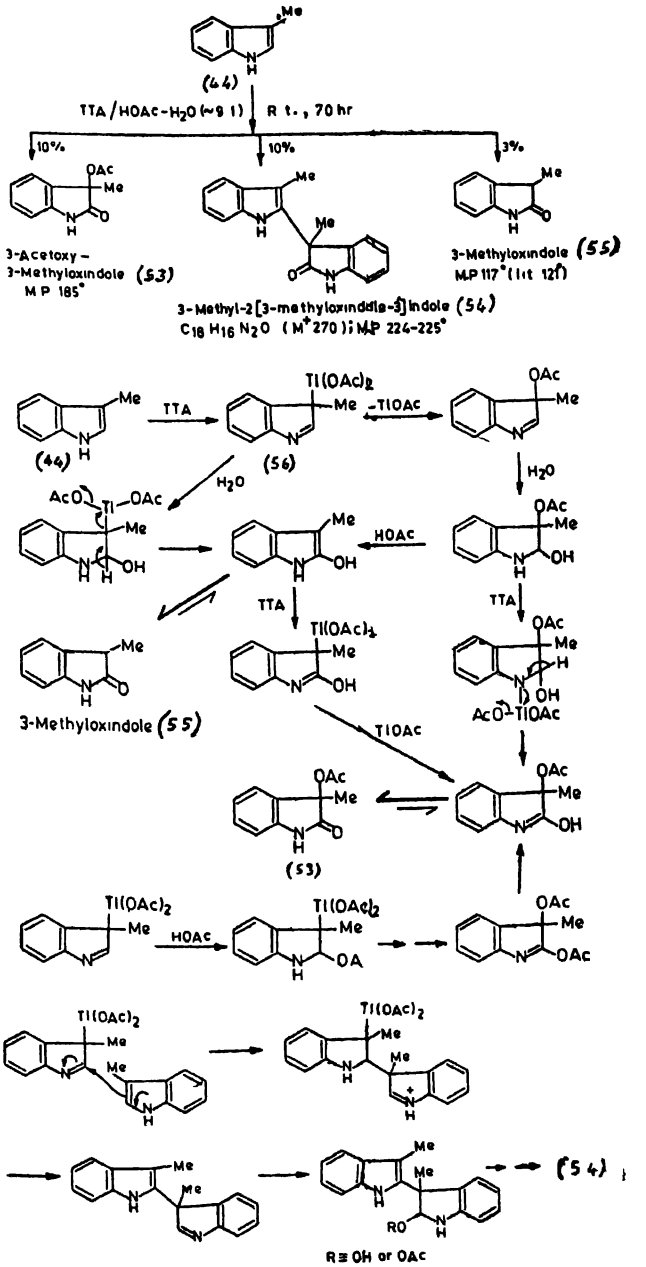


Chart 15. Reaction of 3-methylindole.

in many instances underwent further transformations itself. The overall result is thus the introduction of a nucleophile at C-3 of the indole nucleus, which is susceptible to electrophilic attack. This could be regarded as a sort of 'umpolung' of the indole nucleus via the intermediate formation of a thallated derivative. Another reaction

pathway involved nucleophilic attack on the indolenine double bond.

Indole furnished 3-acetoxyindole (50) in about 50% yield. This is a convenient method for the preparation of 3-oxygenated indoles, which can serve as building blocks for complex Natural Products. 2-Methylindole (42) and 2-phenylindole (43) both furnished the corresponding indoxylindole (51, 52 ; Chart 14). Presumably, an indoxyl intervenes as an intermediate ; this is then oxidised to the 1,2-dehydro species, which then suffers a nucleophilic attack on the 1,2-double bond by a second molecule of the substrate. Skatole (44) gave a complex mixture of products, from which three oxindole derivatives (53, 54, 55) were isolated and

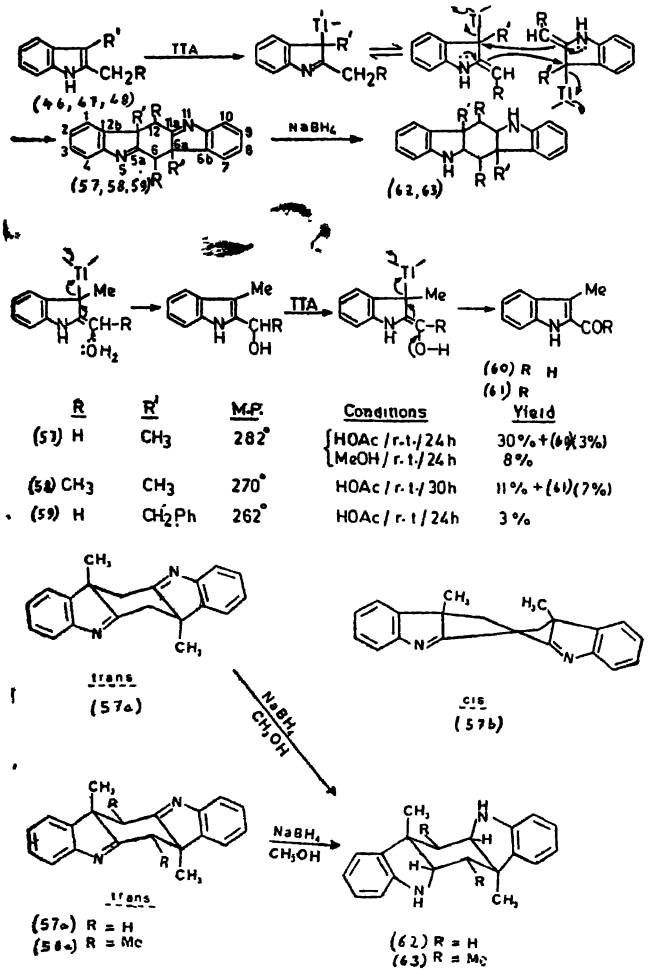


Chart 16. Reaction of 2,3-dimethyl-, 2-ethyl-3-methyl- and 3-benzyl-2-methyl-indole.

characterised (Chart 15). The reactions proceed by way of the 3-acetoxythallyl-3-methylindolenine (56) and involve nucleophilic attack at both 2- and 3-positions by solvent molecules as well as further oxidation steps. Nucleophilic attack by a second molecule of the substrate, rather than by a solvent molecule, led ultimately to the 'dimeric' 3-methyl-2-[3'-methyloxindole-3']indole (54).

The most interesting reactions were, however, observed with 2,3-dialkylindoles (46, 47, 48) which underwent a novel oxidative dimerisation with TTA to give 6,6*a*,12,12*a*-tetrahydroindolo[3,2-*b*]-carbazole (57, 58, 59 ; Chart 16). Small amounts of the 2-acetyl/formyl-3-alkylindoles (60, 61) were also obtained. The tetrahydroindolocarbazoles formed were new compounds with an unique substitution and double-bond pattern which had

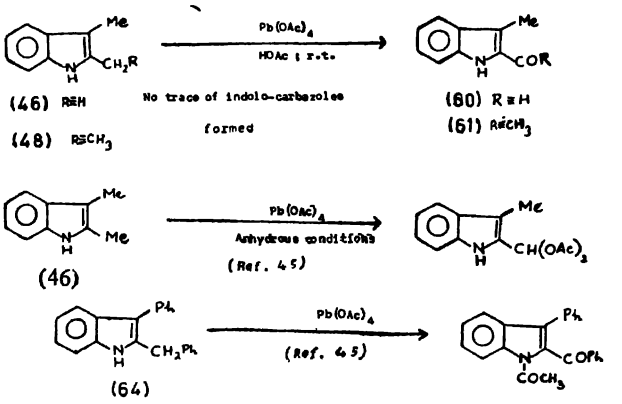


Chart 17. Reactions with lead tetra-acetate.

not been reported previously. The nmr and *X*-ray evidence decided in favour of the *trans*-(e.g. 57a, 58a) rather than the *cis*-structures (e.g. 57b). The thallium-induced oxidative dimerisation is the most direct method known to generate the indole[3,2-*b*]carbazole skeleton. Borohydride reduction of the tetrahydroindolocarbazoles furnished the corresponding octahydroindolocarbazoles (62, 63) which were also new compounds. Treatment of the same 2,3-disubstituted-indoles with lead tetra-acetete (LTA), failed to give the tetrahydroindolocarbazole products obtained by the TTA oxidation ; instead oxidation of the 2-alkyl group was the predominant process (Chart 17), in agreement with the observations made earlier by Chen and Leete[45]. There is thus a significant difference in the oxidation mechanisms of dialkylindoles by thallium(III) and lead(IV) species, thereby illustrating once again the enhanced selectivity of the thallium reagent over its lead(IV) counterpart. 1,2,3-Trimethylindole (45) remained unchanged even after prolonged contact with TTA, thus demonstrating that the lack of substitution at the nitrogen is necessary for TTA oxidations to occur. 2-Methyl-3-phenylindole (49) failed to give a tetrahydroindolocarbazole product, but instead gave a different unidentified dimeric compound.

The action of TTA on yohimbine in acetic acid solution was also investigated. A mixture of products were obtained with the Δ^3-product predominating. Thus TTA showed less selectivity than LTA or mercuric acetate in this reaction. This was in contrast to its greater selectivity for the simple indoles.

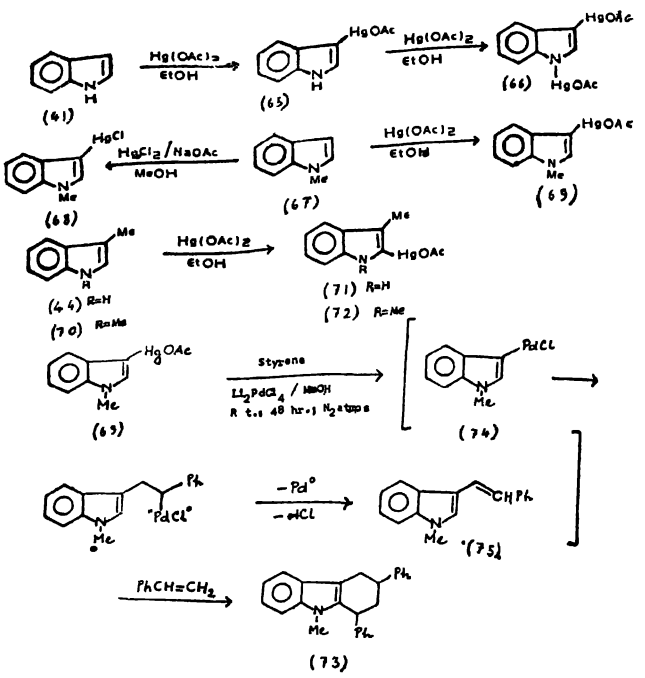


Chart 18. Mercuration of indoles.

The mercuration of indoles was also investigated by us (Chart 18)[46]. There were certain previous reports on this[47], some of which were contradictory, while at least one report was found to have given wrong information. In contrast to TTA, mercuric acetate and mercuric chloride reacted with various indoles (41, 44, 67, 70) to give fairly stable mercurated products (65, 66, 68, 69, 71, 72), which could be characterised spectroscopically. Mercuration occurred preferentially at the 3-position (65, 68, 69). In 3-substituted indoles (44, 70) mercuration occurred at the 2-position (71, 72). The mercurated indoles decomposed on heating for prolonged periods with acetic acid. 3-Acetoxymercuri indole (69) on treatment with styrene and Li_2PdCl_4 in methanolic medium furnished 73 as the final product[46]. Transmetalation, followed by the Heck reaction yielded 74. The latter yielded 73 by a Diels-Alder reaction with excess styrene followed by double-bond rearrangement.

First detection of azetinones :

In connection with my interest on heterocyclic systems present in Natural Products, I was involved for a brief while with some aspects of azetinone chemistry. The β-lactam unit is a part of many biologically active compounds, particularly antibiotics. Azetinones are the corresponding dehydro-compounds (Chart 19). Synthetic interest in azetinones derives from their attractiveness as intermediates in medicinally useful α,β-functionalised β-lactams. Theoretical interest in azetinone derives from its relationship to cyclobutadiene, and its projected antiaromaticity due to its violation of Hückel's $(4n+2)$ π-rule provided amide delocalisation occurs. Amide delocalisation can, however, be avoided by rehybridising the nitrogen from trigonal to tetrahedral and moving the substituent-R on nitrogen out of the ring-plane, so that the azetinone mimics a simple amino-ketone. This would increase the strain in the 4-membered ring. In either case, the azetinones are not expected to be particularly stable unless there is some stabilising feature in their structures such as fusion to an aromatic ring. Olofson *et al* [48] reported the first synthesis of stable benzoazetinones. *X*-Ray analysis of the *N*-adamantyl compound showed a planar 4-membered ring, which attested to the anti-aromaticity of the compound as well as some amount of bond-fixation in the benzenoid moiety. Treatment of an appropriate *N*-alkylbenzisoxazolium salt with a strong base results in deprotonation and then ring-opening followed by electrocyclisation to the azetinone system.

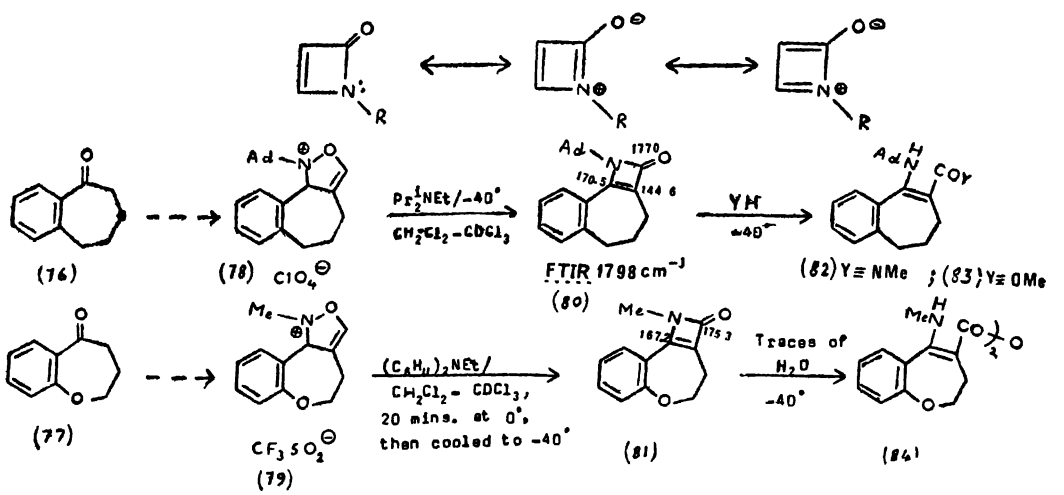


Chart 19. Azetinones revealed

Simple azetinones have been proposed as intermediates in some transformation reactions of biologically active β-lactams[49]. Organic chemists had been endeavouring since the early 1960s to isolate, or at least detect, the simple azetinones but there was absolutely no direct evidence. Occasionally, there have been reports of the synthesis and isolation of simple azetinones[50]. None of these claims, however, withstood the critical scrutiny of subsequent investigators who established that the products which were rather optimistically designated as azetinones actually had different structures[51]. While working at Pennsylvania State University on an UNESCO-UNDP project with Professor R. A. Olofson, I was involved in obtaining the first direct evidence of the existence of simple azetinones not fused to aromatic rings[52]. The necessary isoxazolium (78, 79) salts were obtained from appropriate ketonic precursors (76, 77) by Olofson's procedure. The isoxazolium salts were treated with strong, sterically hindered bases in rigorously dried solvent, to generate the azetinones (80, 81). The problems of thermal instability and extreme sensitivity to moisture had to be overcome in order to record spectroscopical (^{13}C NMR and FT-IR) evidence of the presence of azetinones. At low temperatures around −40°, low-field carbonyl signals at around δ170—180 corresponding to the azetinone carbonyl were observed. The chemical shift of these signals were indicative of the presence of amide delocalisation, and hence the anti-aromatic character of the azetinones. The azetinones were fairly stable at −40°, but on warming decomposed to give a mixture of products. Low temperature FT-IR data was also in agreement with the formation of a simple azetinone, with the relevant carbonyl band appearing at 1 798 cm^{-1}. Azetinone (80) could be trapped with methanol or diethylamine at low temperatures to give the expected iminoester (82) and iminoamide (83) respectively. In the presence of traces of moisture, the *N*-methylazetinone (81) furnished the rather stable anhydride (84). The evidence that simple azetinones are capable of existence, is expected to lead to the development of synthetic strategies based on the generation of these species as synthetic intermediates.

We have been interested in the investigations of the active constituents of indigenous *Piper* species. Plants belonging to this genus have been known since Ayurvedic times to possess important physiological properties. More recently it has been shown that extracts of some *Piper* species as well as some of the constituents, particularly the amides, possess significant pesticidal properties. Over the course of a few years, the plants *P. aurantiacum*[42,53-59], *P. sylvaticum*[42,43,55,60-68], *P. retrofractum*[52,73], *P. attenuatum*, *P. methysticum*[71,72], *P. argyrophyllum*[75] and *P. brachystachyum*[76] have been investigated by us. These included several new amieds and lignans in addition to known compounds. The chemistry of the amide derivatives will be dealt with briefly.

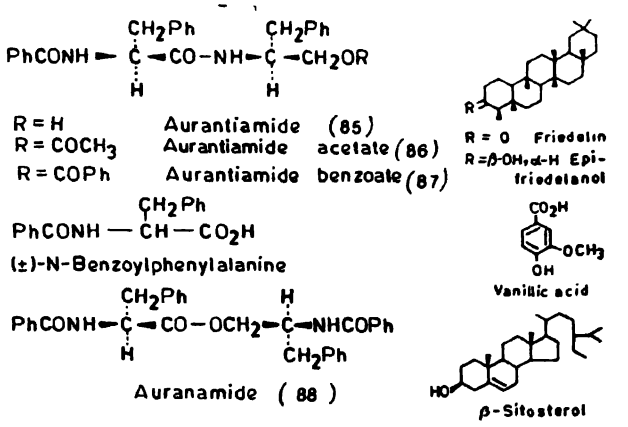


Chart 20. Constituents of *Piper aurantiacum* Wall.

Our earliest investigations were carried out with *P. aurantiacum*[42,43,54-59]. The compounds which were isolated and characterised from the seeds of this plant are shown in Chart 20. These included four new phenylalanine derivatives (85—88), three

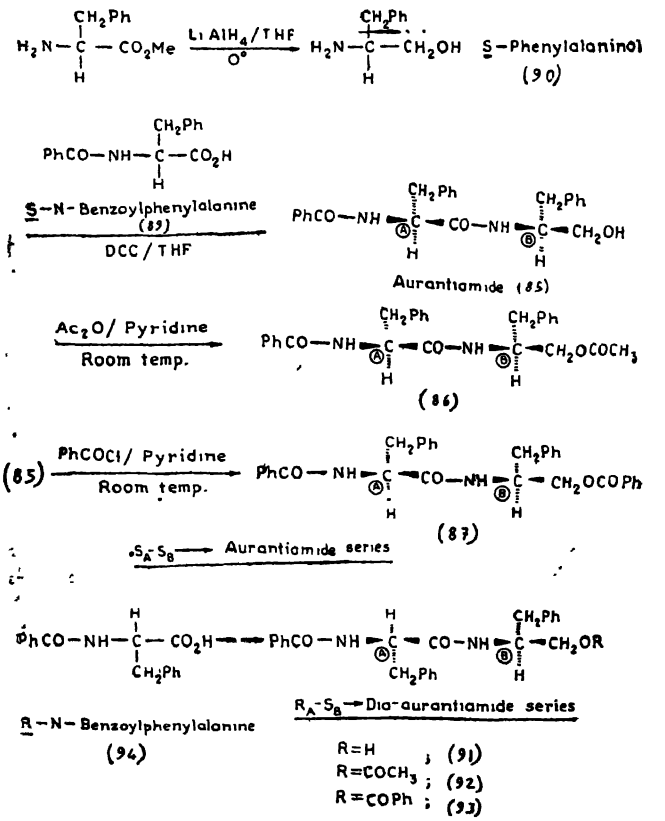


Chart 21. Synthesis of aurantiamide, dia-aurantiamide and their acetates.

belonging to a class of modified dipeptides, which we designated aurantiamides[42,56,57]. The fourth was a novel ester amide, auranamide (88)[58], in

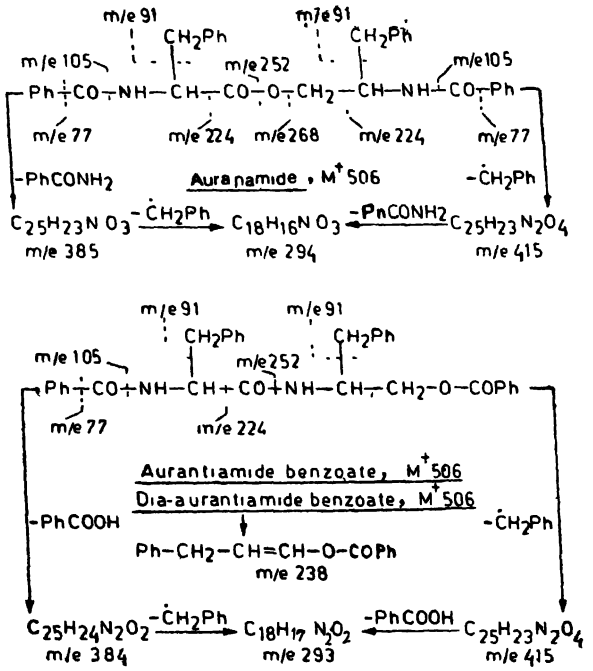


Chart 22. Mass spectral fragmentation patterns of aurantiamide benzoate (87), and dia-aurantiamide benzoate (92) and auranamide (88).

which the central amide linkage of the aurantiamides was replaced by an ester one. The structures of these compounds were settled from spectroscopic data including high-field ^{13}C and ^{1}H nmr

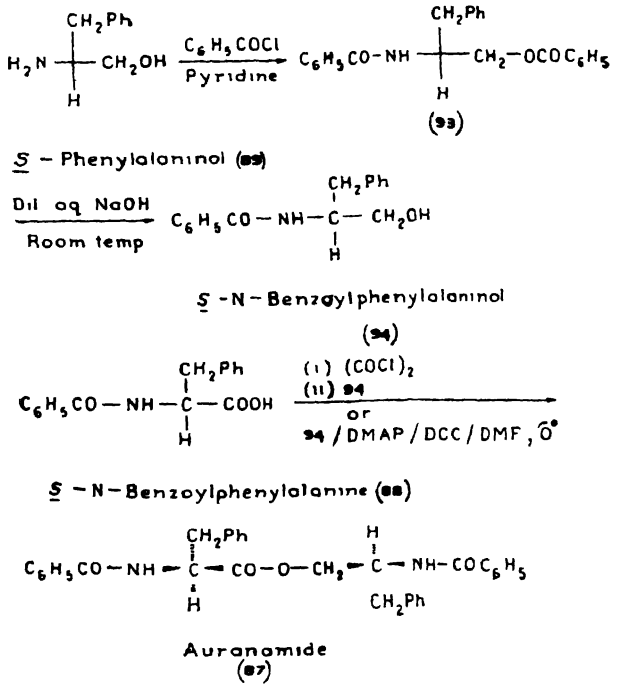


Chart 23. Synthesis of auranamide

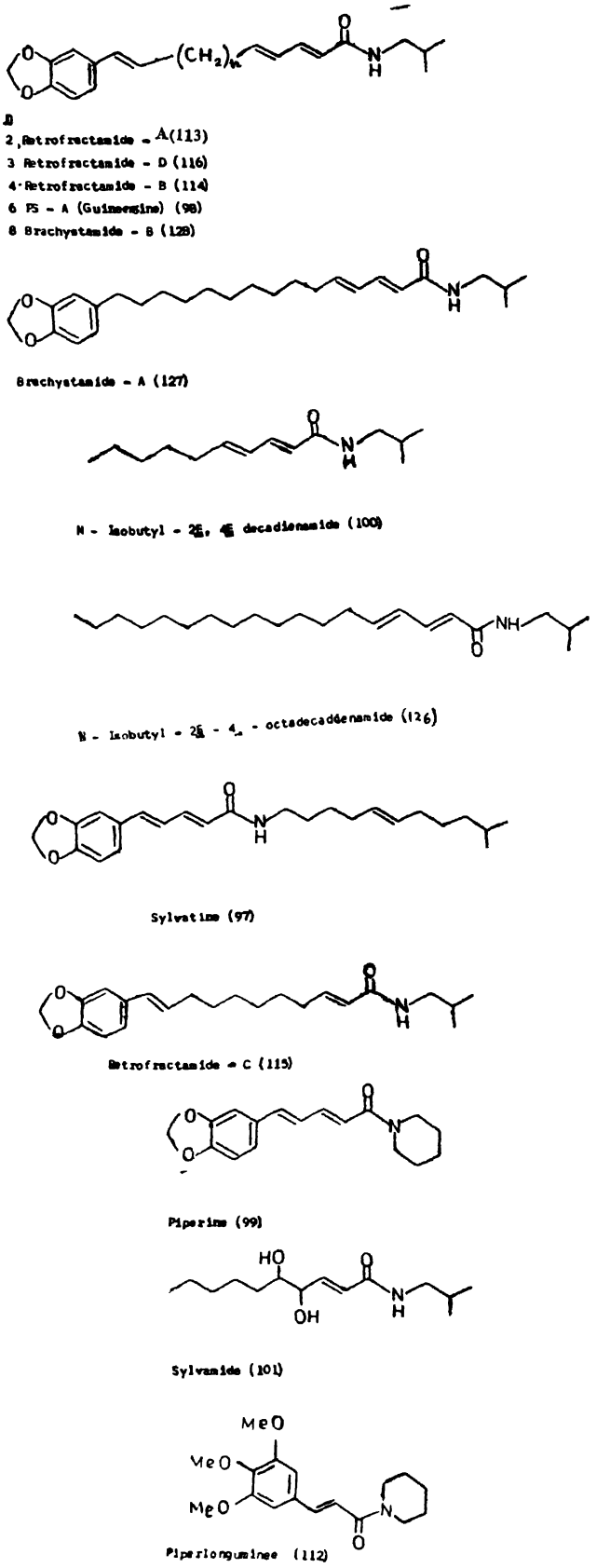


Chart 24. Amide constituents of *Piper*.

(with extensive decoupling experiments) and chemical reactions. Aurantiamide (85) was synthesised by the DCC-mediated condensation of *S*-*N*-benzoylphenylalanine (89) with *S*-phenylalaninol (90) (Chart 21)[42,56,57]. This established the S_A/S_B-configuration of the naturally occurring compounds. Aurantiamide could be readily acetylated with acetic anhydride-pyridine to 86 [42,56,57], and benzoylated with benzoyl chloride-sodium hydroxide to 87[53,54] (Chart 21). The diastereoisomeric series ($R_A S_B$)—the dia-aurantiamides (91—93) were prepared similarly from *R*-*N*-benzoylphenylalanine (94)[42,53,56,57,59]. The normal and diastereoisomeric series had different physical properties, and could be differentiated by their ^1H and ^{13}C nmr characteristics. The mass spectral fragmentation patterns of 85—88 also proved useful in structure elucidation (Chart 22). The aurantiamides showed characteristic peaks at *m/z* 384 ($C_{25}H_{24}N_2O_2$) and 293 ($C_{18}H_{17}N_2O_2$)[56-58]. Auranamide (88), isomeric with aurantiamide benzoate (87)[55,61] and dia-aurantiamide benzoate (93), could be readily distinguished as the corresponding peaks were shifted to *m/z* 385 ($C_{25}H_{23}NO_3$) and

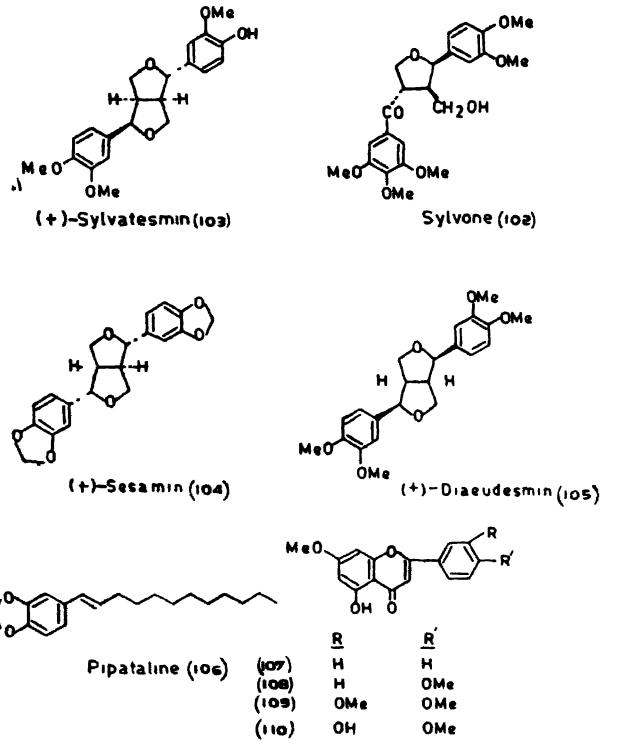


Chart 25. Non-amide constituents of *Piper* sylvaticum

294 ($C_{18}H_{16}NO_3$). This difference is diagnostic of the dipeptide and ester series of compounds. The structure and stereochemistry of auranamide was confirmed by synthesis from chiral precursors. Benzoylation of *S*-phenylalaninol (90) yielded the dibenzoyl derivative (95) which was selectively hydrolysed to 96. This was followed by the latters 4-DMAP-catalysed DCC-mediated condensation to 89 to give auranamide (Chart 23)[58].

The investigations on *P. sylvaticum*, *P. retrofractum*, *P. argyrophyllum* and *P. brachystachyum* led to the isolation of several alkamides, many of these

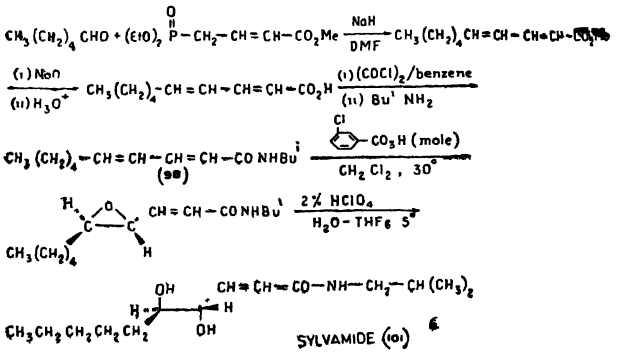


Chart 26. Synthesis of sylvamide.

being new compounds (Chart 24). Other constituents, mainly polypehnolics, were also obtained

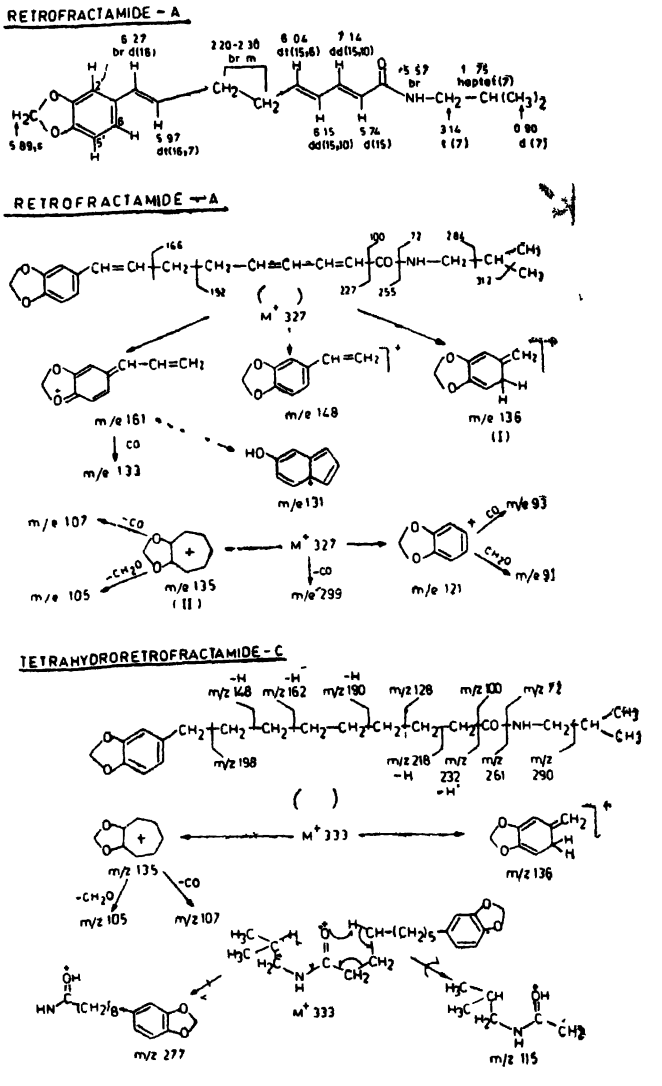


Chart 27. ^1H nmr and MS fragmentation patterns of the retrofractamides.

from these plants (Chart 25). The largest number of compounds obtained by us were from *Piper sylvaticum* (Charts 24, 25)[42,43,55,60–68]. The first new compound isolated from this plant was sylvatine (97). Other amide components included PS-A (guineensine) (98)[61], piperine (99)[68], *N*-isobutyl-2*E*, 4*E*-decadienamide (100)[67], the new dihydroxyamide-sylvamide (101)[62,63] and aurantiamide acetate (86)[55]. Detailed ^{13}C nmr and ^{1}H nmr (200 MHz) analysis of the compound PS-A showed the same gross structure given by earlier workers to guineensine, with the further information that all these double bonds had the *E*-configuration (98)[61]. Both sylvatine and PS-A guineesine have been synthesised by Vig's group[70]. Non-amide constituents isolated were the new lignans sylvone (102)[64] and sylvatesmin (103)[65] in addition to (+)-sesamin (104), (+)-diaeudesmin (105)[42,60], pipataline (106)[42,60], the flavones (107—110)[42,43,55,60] and β-sitosterol.

hydroxyl groups, one of which was attached to the allylic carbon. Structure (101) for sylvamide followed from a comparative ^{13}C nmr study with *N*-isobutyl-2*E*-decenamide (111) and the use of additivity parameters (Chart 28), as well as by detection of *n*-hexanal by GC among the lead tetra-acetate cleaved products[62]. The structure and relative stereochemistry of sylvamide as *N*-isobutyl-4*R**, 5*S**-dihydroxy-2*E*-decenamide (101) was finally confirmed by total synthesis (Chart 26). Chemoselective *trans*-hydroxylation of the 4,5-double bond in *N*-isobutyl-2*E*,4*E*-decadienamide (100) was achieved by epoxidation by an equimolar amount of *m*-chloroperbenzoic acid followed by cleavage of the epoxide under mild conditions by 2% aqueous perchloric acid in aqueous THF at 5°.

The petrol extract of the total above-ground parts of *Piper retrofractum*[53,78] led to the isolation of the known compounds sesamin (104), piperlongumine (112) and 3′,4′,5′-trimethoxyphenylpro-

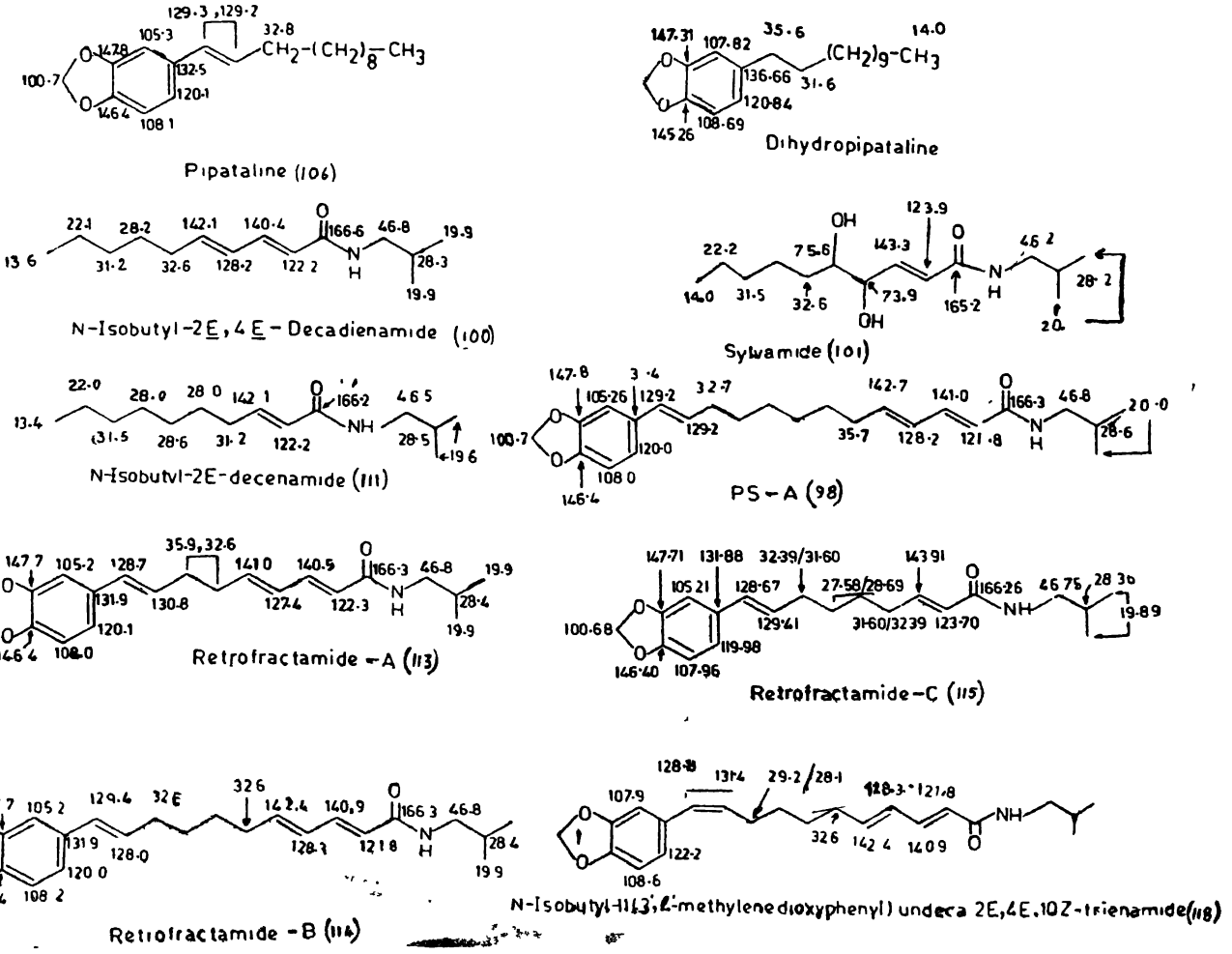


Chart 28. Comparison of ^{13}C nmr values.

Sylvamide (101)[62,63] showed the spectral characteristics of an α,β-unsaturated isobutylamide with the double bond having the *E*-configuration, with two

pionic acid along with a crude mixture of several alkamides, which were designated the retrofractamides (Chart 24). At least eight unsaturated iso-

butylamides were revealed by mass spectroscopical analysis : retrofractamide-A (113 ; M^+ 327), retrofractamide-B (114 ; M^+ 355), retrofractamide-C (115 ; M^+329): retrofractamide-D (116 ; M^+341) and others having M^+ 343, 345, 353 and 357. Careful chromatographic fractionation led to pure samples of some of these compounds. Retrofractamides-A and -C were new compounds, while retrofractamide-B (114) was indentical with pipericide, previously characterised by a Japanese group[77]. The structure and stereochemistry of retrofractamide-A (113) and retrofractamide-C (115) were deduced from spectroscopical and chemical data (Chart 27/28) and confirmed by total syntheses (Charts 29—31). Retrofractamide-A (113; $C_{20}H_{25}NO_3$, M^+ 327), showed the uv (λ_{max}^{EtOH} 262, 211 nm ; log ε 4.52, 4.44) and ir (ν_{max} 3 300, 1 657, 1 625, 998 cm^{-1}) characteristic of a dienamide moiety. It contained three double bonds as shown by ^1H nmr analysis (Chart 27) and hydrogenation experiments. Retrofractamide-C (115 ; $C_{20}H_{27}NO_3$, m.p. 120°) showed similar spectral properties, which however indicated the presence of one double bond less. Catalytic hydrogenation of both the compounds gave the same compound tetrahydroretrofractamide-C{≡hexahydroretrofractamide-A≡N-isobutyl-9-(3',4'-methylenedioxyphenyl)nonanamide} (117). The structure of the latter followed from its uv (λ_{max}^{EtOH} 287, 234, 216 nm ; log ε 3.53, 3.56, 3.48), ir, ^1H nmr data and particularly from its mass spectrum. The MS-fragmentation pattern (Chart 27) indicated the presence of a linear side-chain and a piperonyl moiety. Alternative McLafferty-type cleavages gave significant fragments at m/z 277 and 115. The position and stereochemistry of the double bonds in retrofractamide-A and retrofractamide C were established from their ^1H nmr (200 MHz) (Chart 27) and specially by ^{13}C nmr investigations (Chart 28)[53,73]. In connection with the structure elucidation of these compounds, detailed ^{13}C nmr investigations of naturally occurring alkamides and model compounds were undertaken (Chart 28)[74]. The ^{13}C nmr chemical shifts of the aromatic and olefinic carbons of retrofractamide-A (113) were very similar to those of pipataline (106) and to *N*-isobutyl-2*E*,4*E*-decadienamide (100) thus confirming the presence of an *E*,*E*-dienamide grouping as well as a *E*-(3',4'-methylenedioxy)styryl moiety. A *Z*-styryl double bond, as in the synthetic (118), causes downfield shifts of the aromatic and olefinic carbon signals. In a similar manner, the positions of the two double bonds in retrofractamide-C (115) could be settled, the close correspondence of its olefinic and aromatic ^{13}C nmr shifts now being with *N*-isobutyl-2*E*-decenamide (111) and pipataline (106).

The structure and stereochemistry of retrofractamide-A (113) and retrofractamide-C (115) were confirmed by total syntheses (Charts 29, 31). Retrofractamide-B/pipericide (114) was also synthesised for the first time (Chart 30). The most important feature of these synthesis is the stereoselective generation of the double bond. The aromatic unit (119) was derived from piperonal. The central 6-carbon unit of the retrofractamide-C side-chain was derived from cyclohexanone by the alternative reaction sequences outlined in Chart 29, the key step in both the routes being an initial

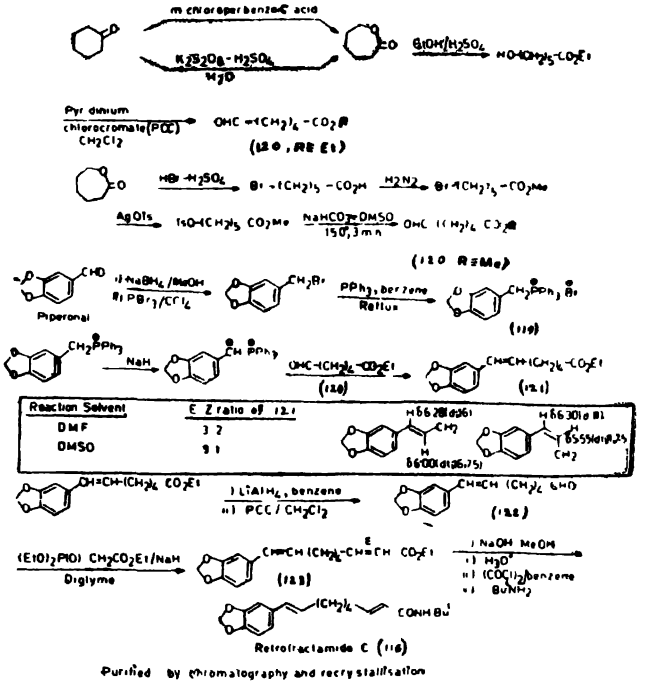


Chart 29. Synthesis of retrofractamide-C.

Baeyer-Villiger reaction. ε-Caprolactone on ethanolysis and PCC oxidation yielded the key ester-aldehyde (120, R=Et). Alternatively, HBr treatment of ε-caprolactone followed by diazomethane methylation and DMSO-$NaHCO_3$ oxidation also gave 120 (R=Me). The two units 119 and 120 are combined by the Wittig reaction with the stereoselective generation of the styryl double bond. The even more steroselective Horner-Emmons procedure proved impractical due to abysmally low overall yield. Reduction of the unsaturated ester (121) followed by PCC-oxidation gave the corresponding aldehyde (122) which underwent the Wittig-Horner-Emmons reaction with triethyl phosphonoacetate to stereospecifically generate the desired *E*-double bond in the product ester (122). Hydrolysis, conversion to acid chloride with oxalyl chloride, followed by the addition of excess isobutylamine afforded the synthetic amide identical in all respects with retrofractamide-C (115). The essential difference between retrofractamide-C (115) and retrofractamide-B (pipericide) (114) is the presence of an extra *E*-double bond in the latter. By utilising the same intermediate unsaturated aldehyde (a mixture of *E* and *Z* isomers) (122) and reacting it with phosphonocrotonate under Wittig-Horner-Emmons condi-

tions the 2*E*,4*E*-dienoate (124) was synthesised. This was converted by the usual reaction sequence to a mixture of 10*E* - and 10*Z*-isobutylamides (114 and 118). The 10*E*-isomer retrofractamide-B (114) was obtained from the mixture by ptlc over silver nitrate impregnated silica gel (Chart 30).

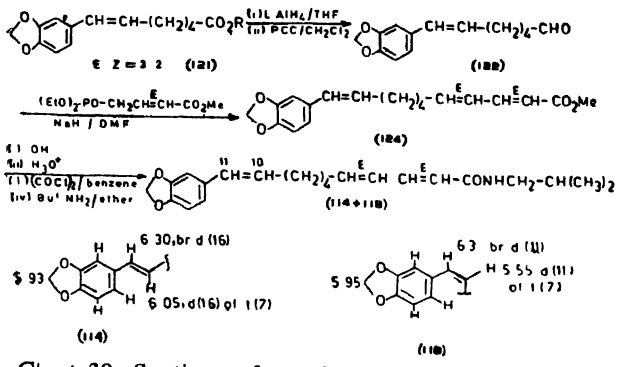


Chart 30. Synthesis of retrofractamide-B (Pipericide).

Retrofractamide-A (113) is a lower homologue of retrofractamide-B (114) with two methylene groups less. Essentially the same synthetic strategy was adopted, with the use of γ-butyrolactone instead of ε-caprolactone as the starting material (Chart 31). However, the ring-opening in the first step understandably proved to be a more difficult proposition, but by the use of Amberlyst ion-exchange resin as the catalyst and a large excess of ethanol a reasonable degree of conversion to the hydroxy-ester (125) was achieved.

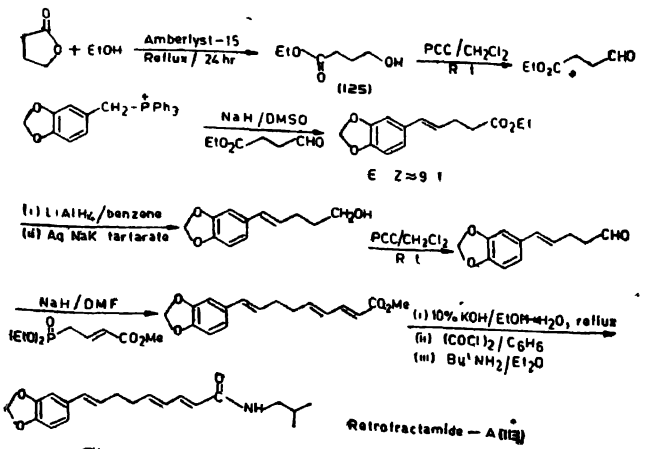


Chart 31. Synthesis of retrofractamide-A.

Subsequently, *Piper argyrophyllum*[75] and *Piper brachystachyum*[76] were also investigated. *P. argyrophyllum*[75] yielded sesamin (104), piperine (99) and *N*-isobutyl-2*E*,4*E*-octadecadienamide (126). The structure of the latter was suggested from spectroscopical data and confirmed by stereoselective synthesis. Investigation on *P. brachystachyum*[76] led to the isolation of the new amides brachystamide-A (120) and brachystamide-B (127) in addition to the known compounds sesamin (101), sitosterol, crotepoxide, caryophyllene oxide and triacontanol.

The uv and ir data of brachystamide-A (117; $C_{26}H_{39}NO_3$, M^+ 413) and brachystamide-B (118; $C_{26}H_{37}NO_3$, M^+ 411) suggested that the two compounds were α,β-unsaturated alkamides, the former containing one double bond less. The structures of the two compounds were settled by spectroscopic studies particularly by nmr. The latter involved (i) 75.5 MHz ^{13}C nmr investigations (^{13}C nmr assignments on structures) and comparison with model compounds (Chart 28), (ii) 300 MHz ^1H nmr investigations including decoupling experiments and (iii) a ^1H-^1H HOMCOR-COSY two-dimensional nmr spectrum to find out *J*-coupling information between protons (Fig. 1).

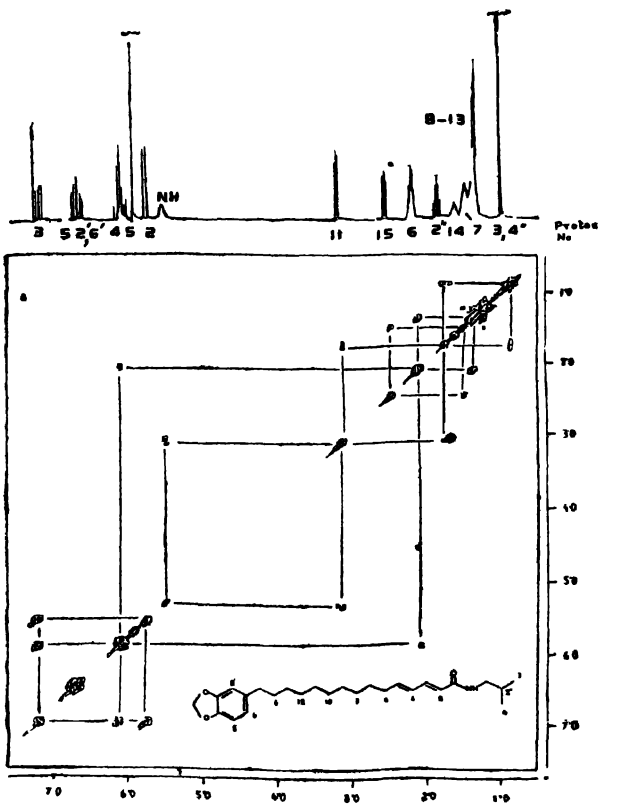
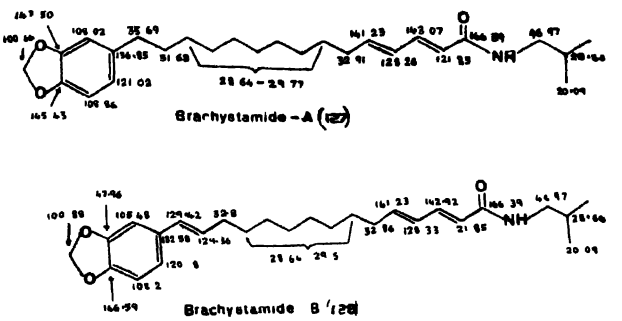


Fig. 1. 300 MHz HOMCOR-COSY of brachystamide-A (*Piper brachystachyum* Wall.).



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