TWENTY YEARS OF CHEMISTRY ON THE ALKALOIDS OF *ALSTONIA* SPECIES FROM NEW CALEDONIA

Georges MASSIOT

Laboratoire de Pharmacognosie, Faculté de Pharmacie, 51 rue Cognacq-Jay, 51096 Reims, France

Alstonia species have provided chemists with fascinating problems and important compounds for the past 60 years.To name just a few, we can mention echitamine, a quaternary ammonium salt isolated from more than fifteen *Alstonia* from South East Asia and which was used against malaria, tetrahydro alstonine and macralstonidine which retain part of the vocable *Alstonia* in their name.
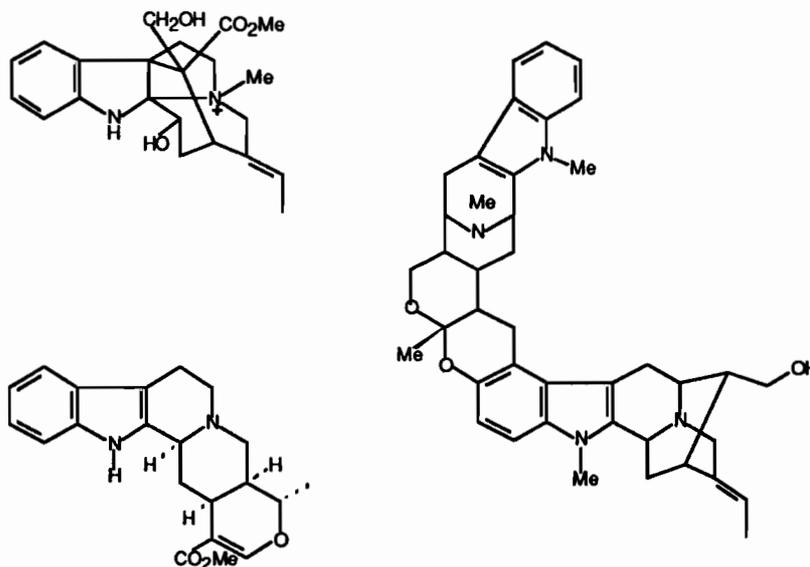
Some representative *Alstonia* alkaloids : echitamine, tetrahydroalstonine and macralstonidine

When it was realized that many species of *Alstonia* were growing in New Caledonia, chemists became almost immediately interested. Curiously, one of the first papers to be published on the subject was authored by Mr Paris from the Faculté de Pharmacie de Paris and dealt with the flavonoids from *Alstonia undulata* and *A. deplanchei* (1), two species whose name might become familiar to you at the end of this talk. Curiously also, this remains the only report on substances different from alkaloids in *Alstonia* from New-Caledonia. Plant material was provided by one of our hosts, ORSTOM but the CNRS, our other host and the Museum d'Histoire Naturelle de Paris were not inactive since in 1977, the late Dr Boiteau, Allorge and Sévenet published the botanical revision of the *Alstonia* from New-Caledonia, an authoritative article which remains a guide line for authors in the field (2). Boiteau, Allorge and Sévenet have retained 14 species and a few varieties, most of them being available to natural product chemists thanks to the efforts of several generations of collectors, the first one being Thierry Sevenet. The following two slides give the list of the official species and the name of the ones which have been investigated to-day on a chemical standpoint. A chemical study on a plant can never be claimed

to be completed but as far as alkaloids are concerned, there is little chance that totally unknown and original structures be discovered in the few varieties or parts of plant which have not yet been investigated. I therefore believe that it is time to try and summarize all of the work done on alkaloids from *Alstonia* from New-Caledonia. It will also be an opportunity to discuss the evolution of the techniques which have been used and to present tools for the future.

A. vieillardii *var vieillardii	A. boulindaensis *	
var glaberrima	A. sphaerocapitata *	
A. plumosa * var plumosa	A. lanceolifera*	
var communis	A. deplanchei var deplanchei *	
f. communis	var ndokoaensis*	
f. glabra	A.lenormandii var lenormandii *	
A. odontophora *	var comptonii	
A. undulata *	var minutifolia *	
A. balansae	A. saligna *	
A. lanceolata *	A. quaternata *	
A. coriacea *	A. legouixiae * var legouixiae	
	var linearis	

Alstonia **species from New Caledonia (stars refer to investigated material)**

This work has been carried out in three places in France : Gif, Chatenay and Reims . I am more familiar with the work from Reims but I shall do my best to give a fair credit to the other groups as well.

I believe that the first pieces of work on *Alstonia* species were done in Gif and Reims on a triad of plants named *Alstonia deplanchei*, *Alstonia balansae* and *Alstonia undulata*. The next slide gives you an impression on the complexity of the mixtures and on the colours of the alkaloids. People were really unlucky to have chosen these starting points because the mixtures are complex and the structures as well. *Alstonia undulata* has been almost continuously studied from 1975 up to now; not in a single assault but in several attacks each one yielding a little bit of information. Most of the structures of the complex alkaloids from *Alstonia undulata* were elucidated thanks to work done on other *Alstonia* except gentiacraline and the very recent ones which we will discuss at the end of this paper.

In the early seventies it was not easy to determine the structure of a simple new indole alkaloid and I am going to illustrate this by the story of two related alkaloids named cathafoline and cabucraline, the first one being known as alkaloid X from *Catharanthus longifolius* (3) and the second as an alkaloid from *Cabucala caudata* (4). Both were isolated on several occasions in *Alstonia* species, the work was carried out simultaneously in Gif and Reims but I think it is fair to say that the problem was solved thanks to the hard work of a student from Madagascar, Philippe Rasoanaivo in 1974. Alkaloid X had a molecular weight of 338 and the real problem was to tell the difference between a dihydroakuammicine skeleton as found in *Strychnos* alkaloids and a picraline skeleton. Numerous arguments were developed and many compounds in the two series were prepared containing or not an aromatic methoxy which is the difference between cathafoline and cabucraline. Although retro Diels Alder fragmentation led to the same ion in the two series, model compounds seem to indicate that a fragment containing indole and methyl acetate was only present in the picraline series. That was the first argument in favor of the picraline structure. Next came arguments based on Circular Dichroism, a technique which just proved to be of importance in the determination of the relative configuration of C-16 of the vinblastine type dimers. ^{13}C NMR was just becoming available in France thanks to the efforts of Lukacs who convinced people to prepare more than twenty reference samples on the millimole scale (several hundred milligrams were then required to record a ^{13}C spectrum !). The arguments retained to tell that cabucraline belonged to the picraline series was the chemical shifts

of C-6 and of C-3. The tryptamine carbon atom is more shielded by 15 ppm in this series and although modern methods had to interchange three pairs of assignments, the general idea still holds and it is remarkable that the assignments were so accurate, given the quality of the spectra at the time. Proton NMR could have been used to solve the problem and it should be easy to distinguish a system with 3 consecutive CH from a system with only 2. Unfortunately the geometry of this molecule is such that coupling constants with CH are small which make assignments difficult with the sole help of proton decoupling.

dihydro-akuammicine   **cathafoline**   **desacetyl desformo picraline**
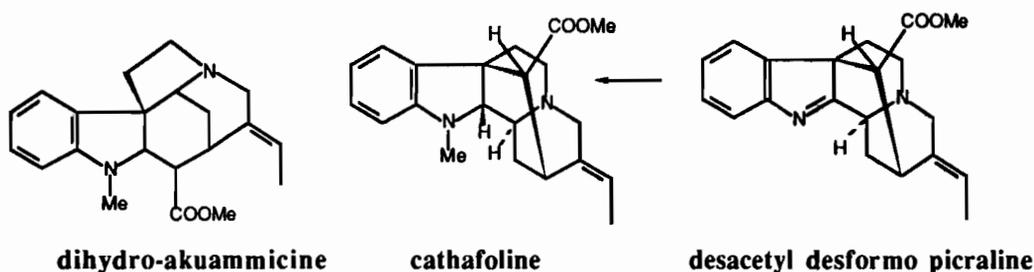
The definitive solution to the problem was brought by chemistry and cathafoline, alkaloid X, was made by reduction of desformoakuammiline. It was obtained as the minor reduction product and that gave an argument to assign configuration of C-2. I think that the CD studies were initiated to settle this point and they later A few derivatives of cathafoline and cabucraline were later isolated, one of them is 10-formyl cabucraline and illustrates the nucleophilicity of C-10 of cabucraline, a feature which is responsible for the isolation of a large number of dimeric alkaloids. A second example is this oxidation product of cathafoline, perhaps formed through the intermediacy of an endoperoxide. Both structural determinations were based on NMR and also on mass spectrometry (5).

Cabucraline,   **10-formyl cabucraline**   **and**   **Oxo cathafoline**
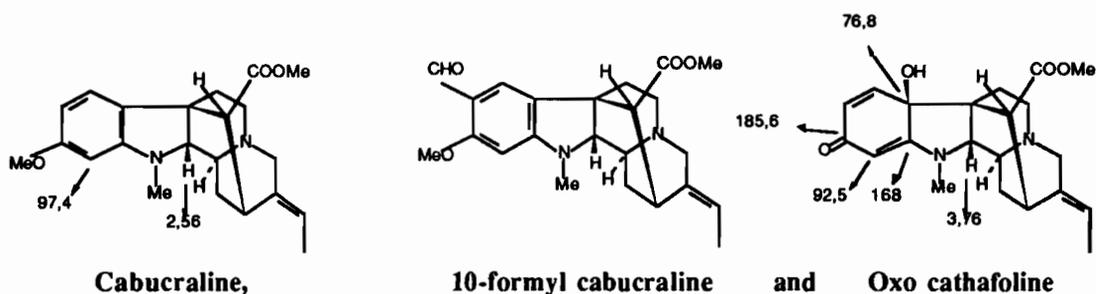
Cathafoline had been isolated from *Alstonia quaternata* by Mamatas and his work was published soon after Rasoanaivo's in 1975 (6). At the same time and one floor downstair, Cosson was fighting against the alkaloids of *Alstonia deplanchei*. That was a difficult plant to work with and in the alkaloids there were dimers of complex structures. The structure of pleiocorine was established first and it is one of the first successes of ^{13}C NMR in the field of dimeric indole alkaloids (7). Mass spectrometry brought very little, except an important information on the molecular ion and composition of the compound. $C_{41}H_{46}N_4O_5$ was the retained combination and no fragments were observed except loss of carbomethoxy group. The game was then to try to spot amongst the 41 carbon resonances, characteristic signals. The first clue was brought by a resonance at 97 ppm for a quaternary carbon which was indicative of a PhNCN arrangement like the one found in vincorine. Vincorine was also available from *Alstonia deplanchei* and its spectrum was also recorded. Two pairs of methylenes at 20 and 40 ppm were recognized as characteristic and were also present in the dimer. Then were assigned as C-6 and C-14 but it was recently demonstrated that this assignment had to be reversed. The second moiety was recognized as being a pleiocarpamine thanks to a typical doublet in the ^{1}H NMR spectrum at 4.66 ppm. Villalstonine, a dimer from Alstonia species also had this signal in a 2,7 dihydropleiocarpamine moiety and the recording of the ^{13}C NMR spectrum of villalstonine

confirmed this presence. Of course assignment of the ^{13}C NMR spectrum of villalstonine required idenfication of its macroline part and this was done thanks to the spectra of another dimer macralstonidine and of a monomer sarpagine. This determination illustrates a technique which was very fruitful at the time : comparison of ^{13}C NMR spectra of dimers and monomers. But that was not such an easy task because the only way to differentiate CH, CH_2 and CH_3 carbons was off resonance decoupling, an insensitive technique submitted to second order effects. Now we can use J modulated spin echo techniques developed in France by Lallemand and in Australia at the same time by Doddrell. The technique readily differentiates CH and CH_3 on one hand from C and CH_2 on the other. Its use makes the spectra of dimers much more legible. Soon after the structure of another dimer from *Alstonia deplanchei* was solved along the same principles. It was named pleiocraline (8) and contained an analogue of cabucraline instead of the vincorine unit. These two molecules are often met in the same plant as parts of similar dimers. They may be related by some chemical transformation which has not been mimicked by chemists so far. A few years later, the two dimers were isolated in Reims from Alstonia odontophora, and the family was enlarged to a third member nor pleiocorine, whose structure was demonstrated by a chemical correlation (9).

Pleiocorine R = Me
Nor pleiocorine R = H

Pleiocraline
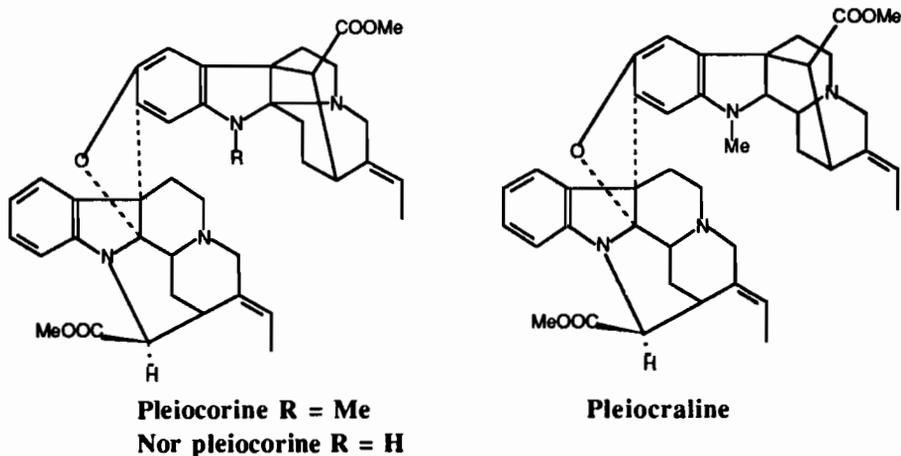
Related to these dimers is plumocraline from *Alstonia plumosa* and of course *Alstonia deplanchei* (10). This structure like the others, was established by ^{13}C NMR and also by a degradation into the two monomers and a partial synthesis from the monomers under acidic conditions. This was the first structure of an original dimer determined in Reims.

Plumocraline

Cabucraline + Pleiocarpamine
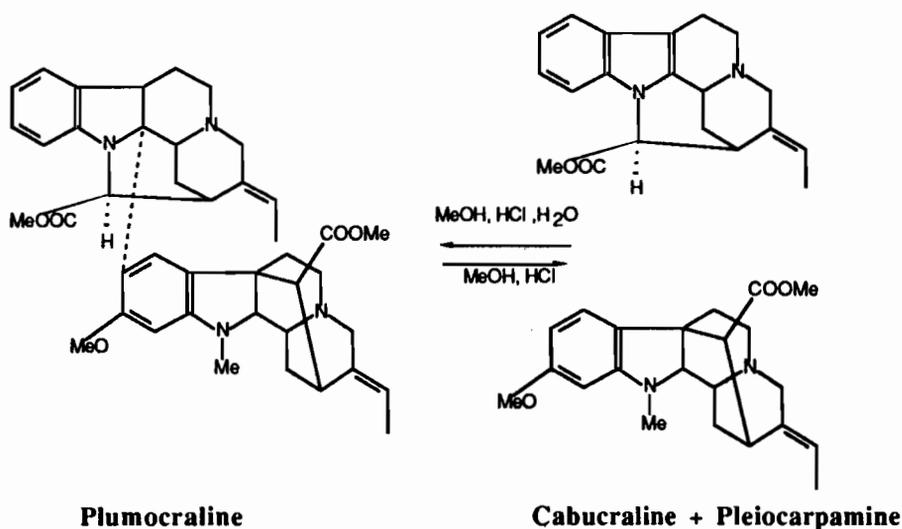
When speaking of these dimers we often qualify them as "dimers with two singlets", which means that in the aromatic part of their proton NMR spectrum, there are two singlets featuring substitutions at positions 10 and 11. When we examined the alkaloids from two other species *Alstonia sphaerocapitata* and *Alstonia plumosa* and later from *Alstonia deplanchei* and *Alstonia undulata,* we came across dimers with 4 singlets which meant that the aromatic parts were substituted on the 4 positions 10 and 11. The same alkaloids were found in *Cabucala caudata* and in *Tonduzia pittieri*, a genus from Guatemala closely related to *Alstonia*. Similar alkaloids were found in many other species, for example in *Petchya zeylanica* studied in Chatenay and our structures cabufiline and its deoxy derivatives were solved by superimposition of ^{13}C NMR spectra (11). This technique allows a rapid determination of the gross structure but if one looks carefully at the argumentation, there is no link given between the aromatic part of one moiety and its aliphatic partner. The two parts are separated on one side by a quaternary carbon atom C-7 and on the other by a Nitrogen atom. For cabufiline as an example, this leaves 4 possible structures,with different aromatic substitutions or locations of the epoxide. Location of the epoxide on the cabucraline or vincorine unit was made possible by the observation of a facile opening of the epoxide into a lactone under acidic conditions. This behaviour is typical of the cabucraline skeleton and to the best of my knowledge is not observed in the other series. Use of one bond correlated CH COSY allows assignments of proton and carbon resonances. When an oxygen substitutes position 11, C-12 is shielded well under 100 ppm. It is therefore possible to recognize H-12 and the neighbouring N-methyl thanks to a NOE. One of the N-methyl gives an NOE with H-2 and from there a proton analysis allows to link aromatic and aliphatic parts of the molecule. This is how these structures have been verified. Today,we are not able to prepare these molecules by partial synthesis and the only interesting reaction, published in the field, is a photochemical dimerization, which however does not give the correct regioisomer (12).
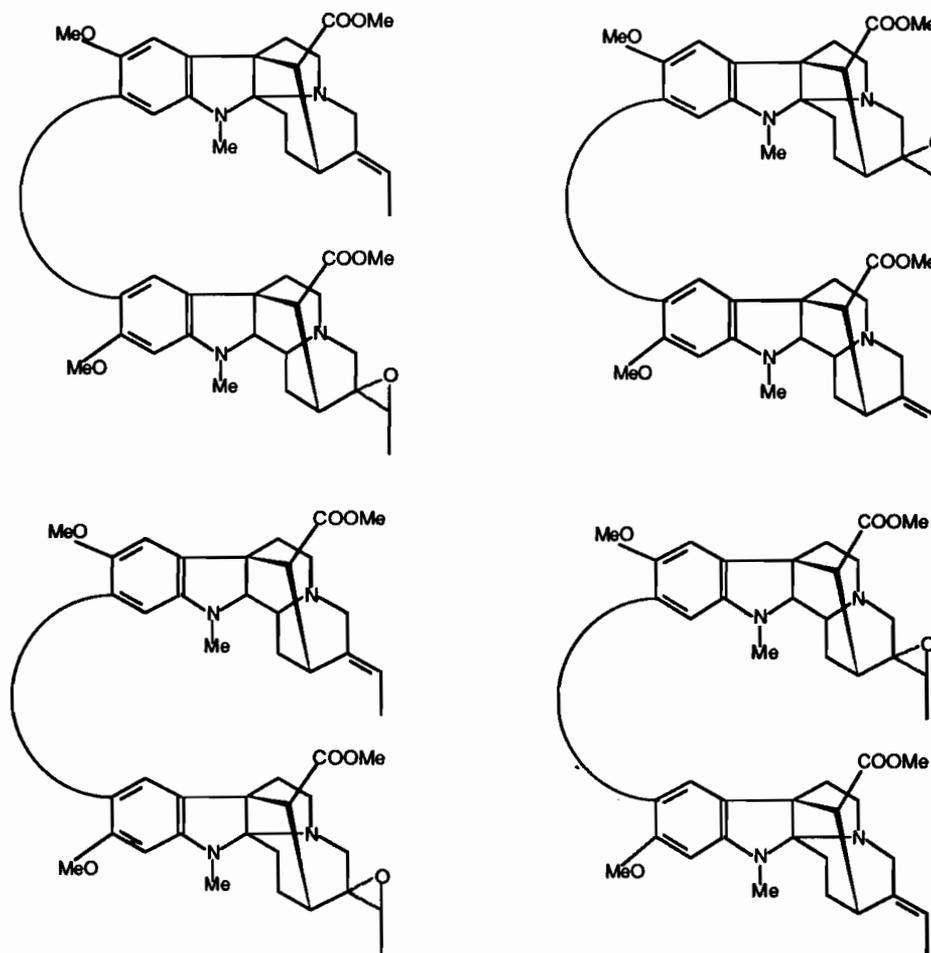
Cabufiline and position isomers

The structures of the monomers are not always easy to establish and we had in Reims for quite some time an alkaloid from *Alstonia lanceolifera* for which we could not decide whether it was a big molecule or not. It turned out to be methoxydeplancheine and the problem had been first solved in Gif by the infernal duet Das-Cosson (13). They were still working on *Alstonia deplanchei* and they had found this non polar molecule with a $C_{17}H_{20}N_2$ composition. They had an advantage upon us, they were mass spectrometry experts and it was not a big deal for them to come up with the correct structure. The compound was known as a synthetic compound made by Winterfeldt to illustrate his b aminoacid to lactam rearrangement. Another duet Besselievre and Husson rapidly succeeded in synthesizing deplancheine and this was followed by about ten other approaches. I would like to say one word about Joule's synthesis because it is beautiful and it uses a amino-nitrile chemistry which was soon to become Husson 's favourite weapon (14). We had to wait for a few years and Meyers to know the absolute configuration of deplancheine and it is not the most expected (15). Where does this alkaloid come from? Obviously from a type I indole alkaloid, but they are rare among *Alstonia* with a 3 b configuration.
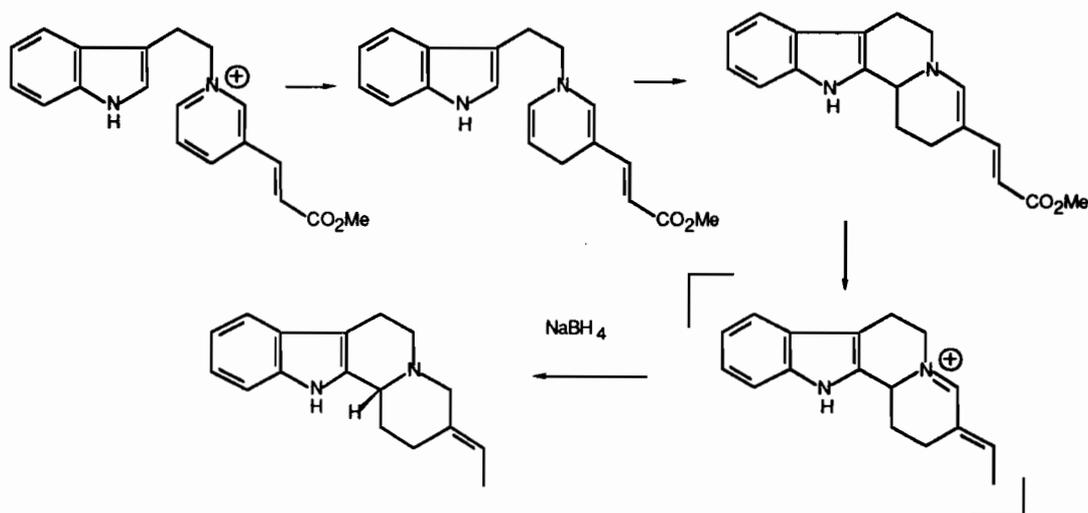
Deplancheine and Husson's synthesis of deplancheine

Tubotaiwine is an ubiquitous indole alkaloid, isolated from more than 40 sources. It was one of first alkaloids isolated by Mamatas from *Alstonia quaternata* (6). Until two years ago there was some confusion as regards configuration of tubotaiwine and even though Mamatas referred to one of the original publications, the configuration he gave was wrong. Proof came recently and three NMR articles gave the correct assignment with positive arguments in Verpoorte (16), negative arguments in Lounasmaa's (17) and confusing arguments in Atta-ur-Rahman's (18). Three total syntheses provided an alternative solution : one from Reims due to Vercauteren (19), one by Kuehne (20) and a third by Bosch (21).
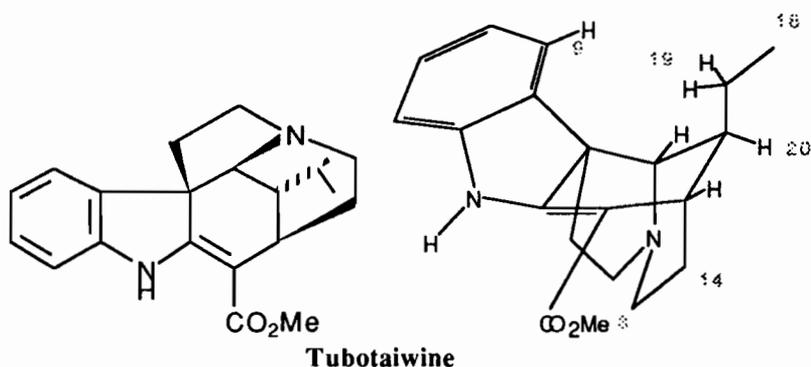
Tubotaiwine

The structural elucidation process is more difficult when the structures are totally unexpected and this is the case for two severely rearranged alkaloids lanceomigine (22) and corialstonine (23). Both are quinoline derivatives originating from indoles. Lanceomigine was first found by Vercauteren in Hunteria congolana and almost simultaneously in Alstonia lanceolata. Proton NMR revealed that this molecule contained a terpenic part similar to the one found in type I indole alkaloids. In the middle of the spectrum, in the vicinity of 5 ppm a one proton singlet was not easily accounted for. It was linked to a carbon atom resonating near 90 ppm therefore substituted by two heteroatoms. There were not that many ring systems that we could build and we soon realised that lanceomigine was not an indole alkaloid. Confirmation of this fact and establishment of the structure came through single crystal X-ray diffraction of a derivative. The structure of rhazinicine from Rhazya stricta was published after this (24) ; it is in fact nor lanceomigine and this was again established ,almost 10 years after our work, by X-ray diffraction (25).
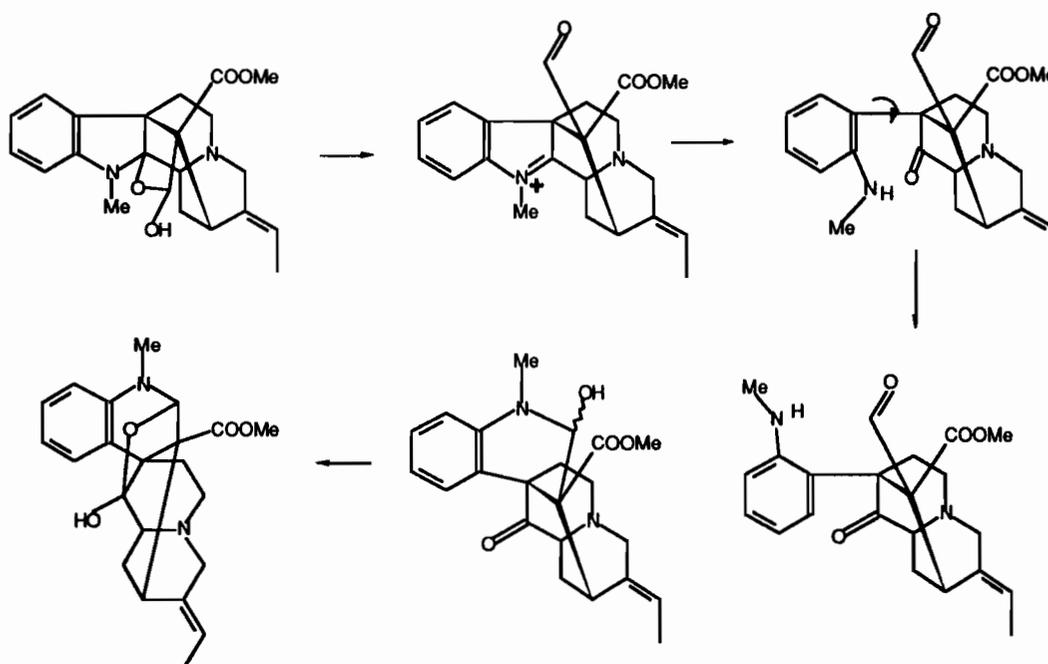
Lanceomigine and alledged biosynthesis from hydroxypseudoakuammigine

Corialstonine from *Alstonia coriacea* (23) is a genuine quinoleine and establishment of its structure rests upon observation of long range proton-carbon couplings thanks to the so called COLOC experiment. This experiment allows linking of small fragments derived from proton NMR even through quaternary carbon atoms or heteroatoms. Lanceomigine and corialstonine derive from indole alkaloids which we were lucky to isolate from the same plants and this gives clues for their alleged biosynthesis. In the case of lanceomigine the rearrangement from hydroxypseudoakuammigine was performed in a test tube.
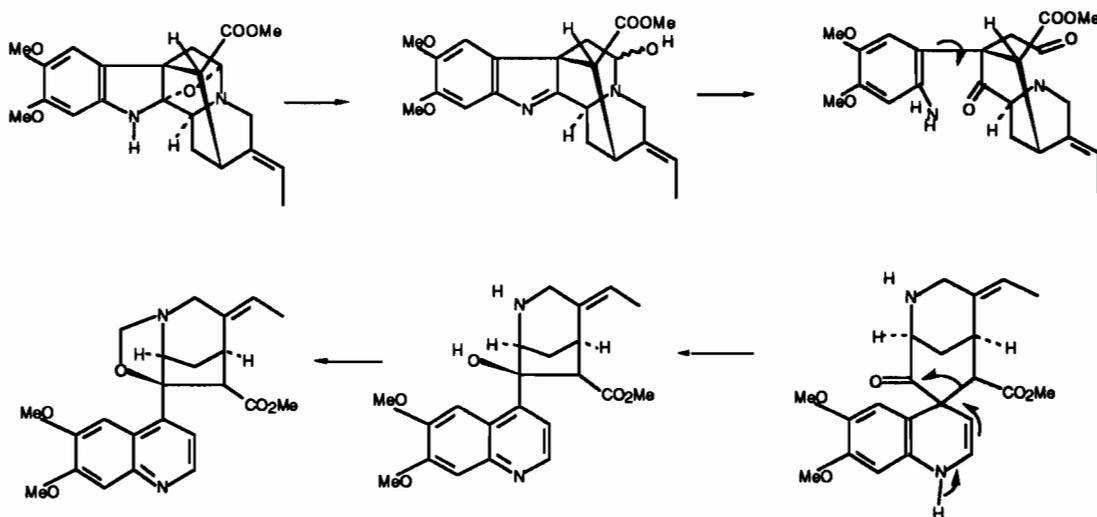
Corialstonine and alledged biosynthesis

To come back to the alkaloids of the dreaded *Alstonia undulata*, here is what we were able to find over the years. Plumocraline and some cabufiline were the first large structures to come. Then came gentiacraline an original combination of cabucraline and of gentianine (26). This brings me to a question I am often asked by Sevenet : is gentianine a natural alkaloid or an artefact ? The question deserves to be asked because precursors to gentianine, iridoids are very reactive and isolation work done in the presence of ammonia may well transform them into pyridines. In this case the formation of gentiacraline would request two reactions out of the plant : ammonia addition and reaction with cabucraline before dismutation to a pyridine. *Alstonia undulata* is not particularly rich in cabucraline and I still prefer to believe that most of the compounds that we isolate are natural compounds.
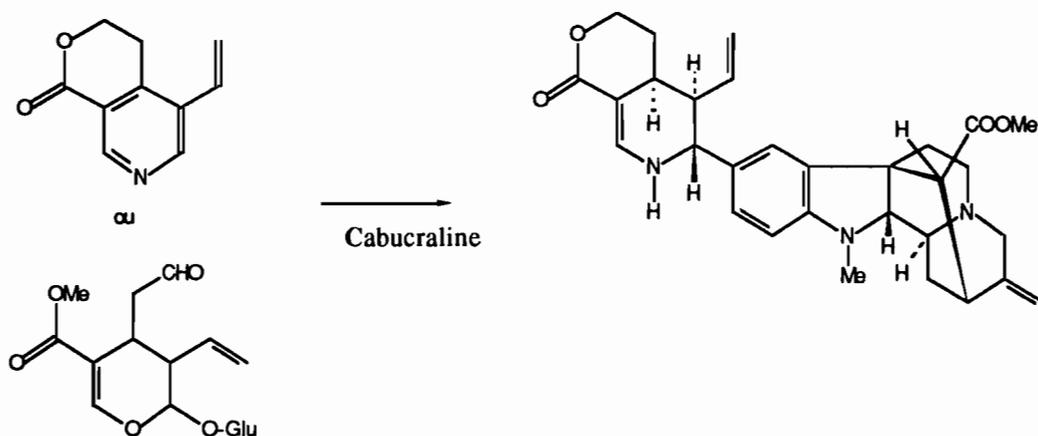
Gentiacraline and its possible precursors secologanin and gentianin

Very recently we started to understand what was going wrong with *Alstonia undulata* : it is able to oxidize unusual positions such as position 6 on the tryptamine chain and make reactive intermediate susceptible of duplication. Undulatine is the first such structure we were able to determine (27) and there is a second more oxidised and still tentative formula recently determined by Cherif. The first type of structure was chemically prepared using Yonemitsu oxidation of position 6 of indoles with DDQ (28). Such oxidised indoles are very reactive and upon acidic catalysis a cation is formed that might be trapped by passing-by nucleophiles. Dihydroxy 3 and 6 indole might be prepared and reacted to make the trimeric indole alkaloids. It

is not known yet if they will be chemically made first or if they exist here on one of the near-by islands.
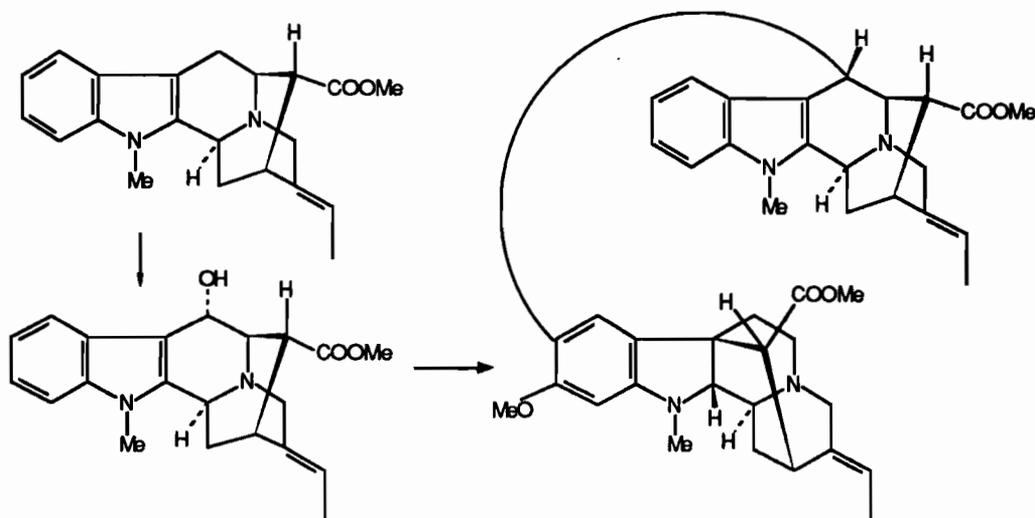
Partial synthesis of the new bisindoles from *Alstonia undulata*

Obviously work on *Alstonia* brought a lot of chemistry and helped many talented young people get acquainted with alkaloid chemistry. It also brought us a lot of intellectual work for the solving of complicated structures. In the last slides I would like to show you what is going to change in the years to come. Let us ask a naive question : what is a structure? It is a collection of atoms linked by chemical bonds. The number of atoms is determined by mass spectrometry, by proton and carbon NMR most of the time . The same techniques plus UV and IR allow to write fragments and partial structures. Our intelligence does the remaining and in the context of known biogenetical rules and plant knowledge, one is able to put structures forth. Now we have new wonderful tools which allow linking of carbon and proton atoms HMQC and HMBC, one bond and multiple bond correlations. Combination of these experiments provide fictitious carbon to carbon correlations. With those, a microcomputer and a pinch of artificial intelligence is able to provide solutions (21). Here is what we have for cabucraline, 22 separated carbons numbered 1-22 and 14 different proton signals numbered 1-14. Here are the correlation charts. We also know that there is a N-methyl, a O-methyl, a methyl ester, an ethylidene side chain. We these elements the computer provides about 200 solutions some of them completely unrealistic such as Dewar benzenes. If we give the computer a few more elements coming from COSY such as a 3-14 link or a 5-6 link the number of structures and computational time fall rapidly : one structure in a matter of minutes with a MacIntosh and Nuzillard who conceived and wrote the program is able to do much more (29).

All this could not have been thought of if we had not patiently done our work on the alkaloids of *Alstonia* species. The work could have been more motivating had some of these molecules shown new biological properties but that was not a priority when work was started and it is difficult to interest industry in these complex mixtures. But that nobody can tell before the extraction is done.
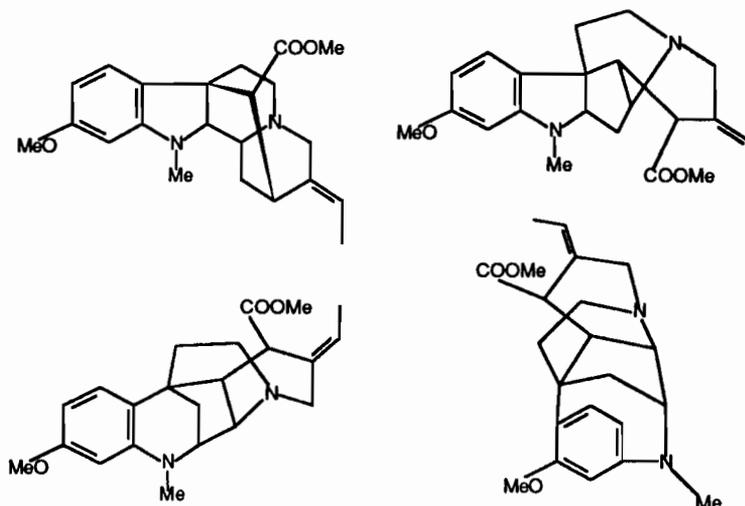
Four structures for cabucraline deduced from the LSD program (no indole substructure included)

Work on *Alstonia* from New Caledonia is the fruit of the efforts of more than 40 persons over 20 years. Here are the names of those whose left a trace in the literature. Limitation in time has not allowed reporting on everyone's contribution ; I had to choose and I apologize for those who feel ill treated.

References
1.   Duret S. and Paris R. R., *Plantes Méd. et Phytothér.* **6**, 56 (1972)
2.   Boiteau P., Allorge L.and Sévenet T., *Adamsonia*, 465 (1977)
3.   Rasoanaivo P., Langlois N., Potier P.and Bladon P., *Tetrahedron Letters*, 1425 (1973)
4.   Mansour M., *Thèse Reims, Structure of catbucraline*, (1974)
5.   Cherif A.,*Thèse Reims,Oxidised derivatives of cabucraline* (1989)
6.   Mamatas-Kalamaras S., Sévenet T., Thal C.and Potier P., *Phytochem.* **14**, 1849 (1975)
7.   Das B.C., Cosson J.P., Lukacs G.and Potier P., *Tetrahedron Letters*, 4294 (1974)
8.   Das B.C., Cosson J.P.and Lukacs G., *J. Org. Chem.* **42**, 2785 (1977)
9.   Vercauteren J., Massiot G., Sévenet T., Lévy J., Le Men-Olivier L.and Le Men J., *Phytochem.* **19**, 1729 (1979)
10.   Massiot G., Vercauteren J., Jacquier M.J., Lévy J.and Le Men-Olivier L., *C. R. Acad. Sc. Paris*, **292**,191 (1981)
11.   Massiot G., Vercauteren J., Richard B., Jacquier M.J.and Le Men-Olivier L., *C.R. Acad. Sc. Paris*, **294**,579 (1982)
12.   Erra-Balsells, *Phytochem.* **27**, 3395 (1988)
13.   Besselièvre R., Cosson J.P., Das B.C.and Husson H.P., *Tetrahedron Letters*, 63 (1980)
14.   Ashcroft W.R.and Joule J.A., *Tetrahedron Letters*, 2341 (1980)
15.   Meyers A.I ., Sohda T.and Loewe M.F., *J. Org. Chem.* **51**, 3108 (1986)
16.   Schripsema J., van Beek T.A., Verpoorte R., Erkelens P., Perera P.and Tibell C., *J. Nat. Prod.* **50**,89 (1987)
17.   Lounasmaa M., Koskinen A.and O'Connell J., *Helv. Chim. Acta* **69**, 1343(1986)
18.   Atta-ur-Rahman, Alvi K.A.and Muzaffar A., *Planta Medica*, 325 (1986)
19.   Vercauteren J., Legseir B., Henin J.and Massiot G. *submitted to publication*
20.   Kuehne M.E., Frasier D.A.and Spitzer T.D., *J. Org. Chem.***56**, 2696 (1991)
21.   Gracia J., Bonjoch J., Casamitjana N., Amat M.and Bosch J., *J. Chem. Soc. Chem.Comm.*, 614 (1991)
22.   Vercauteren J., Massiot G.,Le Men Olivier L., Lévy J., Prangé T.and Pascard C., *Tetrahedron Letters*, 2871(1981)
23.   Chérif A., Massiot G.and Le Men-Olivier L., *Heterocycles*, 3055 (1987)
24.   Atta-ur-Rahman and Khanum S., *Heterocycles*, 2183 (1984)

25. Hu Wen-Lan, Zhu Ji-Ping, Piantini A., Prewo R.and Hesse M., *Phytochem.* **26**, 2625 (1987)
26. Massiot G., Guillaume D., Vercauteren J., Le Men-Olivier L., *C. R. Acad. Sc. Paris*, 296, 977 (1983)
27. Nuzillard J.M., Pinchon T.M., Caron C., Massiot G.and Le Men-Olivier L., *C. R.Acad. Sc. Paris* 309,195 (1989)
28. Massiot G., Nuzillard J.M., Richard B.and Le Men-Olivier L., *Tetrahedron Letters*, 2883 (1990)
29. Nuzillard J.M.and Massiot G., *Tetrahedron* **47**, 3655(1991)

Further reading :
A. boulindaensis : Ind. J. Chem. 55, 1096 (1978)
A. lanceolata : Phytochemistry 20, 1411 (1981)
A. legouixiae : Ann. Pharm. Fr. 39, 273 (1981)
A. lanceolifera : Phytochemistry 20, 1987 (1981), 21, 2160 (1982)
A. plumosa : Phytochemistry 21, 2973 (1982)
A. Sphaerocapitata : Phytochemistry, 23, 1355 (1984)
A. lenormandii : Phytochemistry 25, 1735 (1988)

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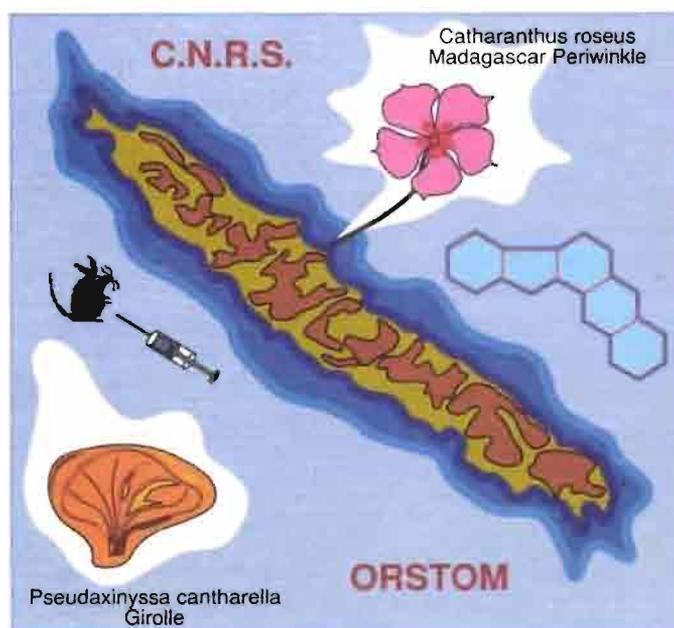
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