Phytochemistry, Vol 23, No 10, pp 2355–2357, 1984
Printed in Great Britain

0031–9422/84 $3 00 + 0 00

ALKALOIDS OF *ALSTONIA SPHAEROCAPITATA**

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(*Received* 14 *February* 1984)

Key Word Index—*Alstonia sphaerocapitata*, Apocynaceae, indole alkaloids

Abstract—Twenty alkaloids have been isolated from the leaves, fruit and stem-bark of *Alstonia sphaerocapitata* from New Caledonia They were vincamedine, 10-methoxyvincamedine, *Z*-isositsirikine, akuammicine, quaternoline, 11-methoxyakuammicine, tubotaiwine, 10-methoxyvincamedine *N*(4)-oxide, cabucraline, cathafoline, caberoline, vincoridine, quebrachidine, quaternoxine, nor C-fluorocurarine, desoxycabufiline, nordesoxycabufiline and three alkaloids of unknown structure

INTRODUCTION

Alstonia sphaerocapitata Boit is an 8–15 m high tree of New Caledonia It is a new species, described by Boiteau in 1977 [1] As part of our chemotaxonomic work on the *Alstonia* from New Caledonia, we herein describe our results on its alkaloid content

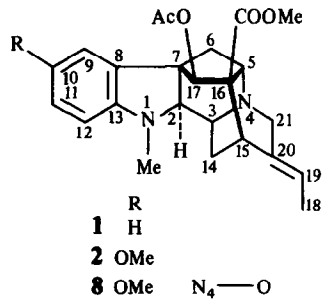
RESULTS AND DISCUSSION

Extractions were conducted in the usual fashion and the yield of alkaloids was 22 g/kg in the leaves, 2 g/kg in the fruit and 6 6 g/kg in the stem-bark The alkaloid mixtures (AM) were separated by a combination of medium pressure LC and prep TLC

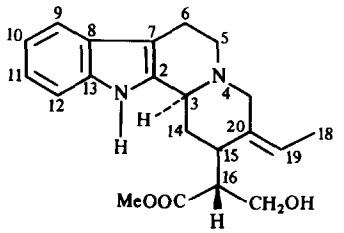
Twelve identified alkaloids were isolated from the leaves They were, in order of increasing polarity vincamedine **1** (1% of AM), 10-methoxyvincamedine **2** (2%), *Z*-isositsirikine **3** (0 02%), akuammicine **4** (3%), quaternoline **5** (0 5%), 11-methoxyakuammicine **6** (4%), tubotaiwine **7** (0 5%), 10-methoxy vincamedine *N*(4)-oxide **8** (2%), cabucraline **9** (8%), cathafoline **10** (8%), caberoline **11** (0 5%) and desoxycabufiline **12** (0 5%) Alkaloid **10** is also known as alkaloid X from *Catharanthus roseus* [2] and although not definitively proved, quaternoline **5** seems to be identical to raucubaine [3] Compounds **1**, **4–7**, **9–12** are known compounds identified by direct comparison with reference samples The major alkaloids of the leaves are the pairs cathafoline **10**/cabucraline **9**, quaternoline **5**/caberoline **11**, which only differ by a methoxyl substituent on the aromatic ring, separation of **9** and **10** is tedious and can only be achieved by multiple migration TLC

Vincamedine **1** (vincamajine acetate), also isolated from *A deplanchei* [4], is accompanied in *A sphaerocapitata* by compound **2**, which possesses very similar spectral properties Their ^{1}H NMR spectra are superimposable except for the aromatic area and for a three proton singlet at δ3 7, which is absent in the spectrum of **1** The mass spectrum of **2** shows an $[M]^+$ at *m/z* 438 which analysed for $C_{25}H_{30}N_2O_5$ Fragments pertaining to the indole part of the molecule are shifted 30 mu above the corresponding fragments of **1** These data led to the conclusion that **2** is a methoxylated vincamedine Location of the methoxyl group on the indole ring is secured by comparison of the ^{13}C NMR spectra of **1** and **2** (Table 1) As anticipated, these spectra are superimposable (±0 1 ppm) except for those carbons related to the aromatic ring We propose C-10 as the substitution position in **2** to account for the shifts of the aromatic carbons and especially that of C-12 (δ111 0) Ample precedent exists in the literature for such a use of ^{13}C shifts in the determination of a methoxyl substituent on a dihydroindole ring [5, 6]

Compound **8** displays spectral properties (UV, mass spectrum, IR) similar to those of **2**, slight differences are found in the ^{1}H NMR spectra, in the δ4 0–4 5 region,



1 R = H
2 R = OMe
8 R = OMe, N_4—O



3 *Z* - Isositsirikine

*Part 87 in the series "Plants from New Caledonia" For part 86 see Mehri, H, Sciamama, F, Plat, M and Sévenet, T, *Ann. Pharm* (in press)

Table 1 ^{13}C NMR spectral data of **1** and **2** ($CDCl_3$, δ, TMS, attributions according to ref [5])

Position	1	2	Position	1	2
C-2	75 6	75 6	C-16	59 0	59 1
C-3	53 2	53 3	C-17	75 0	75 1
C-5	61 7	61 7	C-18	12 7	12 7
C-6	36 5	36 6	C-19	116 7	116 7
C-7	56 2	56 2	C-20	136 9	136 9
C-8	129 1	130 6	C-21	55 6	55 7
C-9	123 3	109 7	N-Me	34 2	34 9
C-10	118 9	148 7	C-10-OMe	—	55 9
C-11	128 7	113 1	COOMe	172 3	172 3
C-12	109 4	111 0	COOMe	51 5	51 5
C-13	154 5	153 7	OCOMe	168 4	168 4
C-14	21 9	21 9	OCOMe	20 7	20 8
C-15	30 5	30 5			

which corresponds to the H–C–N protons of the molecule The hypothesis of **8** being the *N*-oxide of **2** stems from their behaviour on TLC (different mobilities but identical colours with spray reagents), this has been confirmed by the conversion of **2** into **8** using *p*-nitroperbenzoic acid

The non polar alkaloid **3** is an indole as shown by its UV absorption (227, 283 and 290 nm) Its mass spectrum is reminiscent of that of the sitsirikines In these compounds, the $[M]^+$ at *m/z* 354 is accompanied by an intense $[M-1]^+$ ion and by fragments corresponding to the loss of H_2O (*m/z* 336), CH_2OH (*m/z* 323), CO_2Me (*m/z* 295) and CH_2OH–CH–CO_2Me (*m/z* 251, 100%) Ions at *m/z* 156, 169, 170 and 184 derive from the β-carboline part of the molecule Compound **3** belongs to the isositsirikine series and not to the sitsirikine series, as demonstrated by the ^1H NMR spectrum of **3**, in which a quartet at δ 5 45 and a three proton doublet at 1 73 signify an ethylidene side chain Direct comparison of **3** and of 16*R* and of 16*S*-isositsirikines showed the three compounds to be different Recent publications by Husson *et al* [7] on the reduction products of 4,21-dehydrogeissoschizine and by Cordell *et al* [8] on the isolation of 16*S* *Z*-isositsirikine lead us to consider that **3** could be the missing 16*R* *Z*-isositsirikine That this was the case was demonstrated by comparison of our data and compounds with those of H P Husson Two unidentified monomers **18** and **19** have also been isolated, they are described in the Experimental

Similar extraction of the fruit gave a mixture in which 11 known alkaloids were separated In order of increasing polarity, they are vincoridine **13** (0 5% of AM), vincamedine **1** (5%), 10-methoxyvincamedine **2** (5%), quebrachidine **14** (2 5%), quaternoxine **15** (0 5%), akuammicine **4** (11%), 11-methoxyakuammicine **6** (9%), 10-methoxyvincamedine *N*-oxide **8** (1%), cabucraline **9** (17%), cathafoline **10** (11%) and desoxycabufiline **12** (8%) Among these alkaloids, compounds **1**, **2**, **4**, **6** and **8**–**12** are also found in the leaves, alkaloids **13**–**15** were identified by direct comparison with authentic samples

Analogous treatment of the stem-bark was also accomplished and 11 alkaloids were separated They are, in order of increasing polarity vincamedine **1** (2% of AM), 10-methoxyvincamedine **2** (3%), akuammicine **4** (7%), 11-methoxyakuammicine **6** (8 5%), nor C-fluorocurarine **16** (1%), 10-methoxyvincamedine *N*(4)-oxide **8** (1%), cabucraline **9** (estimated 26% of AM), cathafoline **10** (estimated 7%), desoxycabufiline **12** (5%), nor desoxycabufiline **17** (0 5%) and an unknown dimeric alkaloid **20** (1%) Compound **17** as well as compound **12** have also been found and identified in *A plumosa* [9, 10]

A sphaerocapitata, as in all other *Alstonia* from New Caledonia, contains mainly indole alkaloids of type I of the Le Men–Taylor classification However, it offers a rare combination of a tetracyclic alkaloid (16*R* *Z*-isositsirikine **3**) and of the corresponding alkaloids possessing a C-16/C-7 bond (**9**, **10**, **11**, **13** and **15**) or a C-16/C-5 bond (**1**, **2**, **8** and **14**), 'rearranged' alkaloids with a C-16/C-2 bond (**4**, **6** and **16**) are also present

As all the New Caledonian species of *Alstonia*, *A sphaerocapitata* belongs to the section *Dissuraspermum* Monachino, same considerations as mentioned in the work on *A plumosa* are of value here [9]

EXPERIMENTAL

General Mps are uncorr NMR were measured in $CDCl_3$ solns at 60 MHz or at 400 MHz on a prototype at the Institut d'Electronique Fondamentale (Orsay) Chemical shifts are given in δ-values with TMS as int standard, coupling constants are given in Hz Rotations are measured in $CHCl_3$ (10 cm cell) Medium pressure LC expts were run at 10 bar Colour reactions (CR) were obtained by spraying plates with a soln of Ce(IV) $(NH_4)_2SO_4$ Plant material was collected on the East Coast in a lowland rainforest near Poindimie (6 July, 1979) under reference Sevenet-Pusset 1722, an herbarium specimen is kept in the Herbarium of ORSTOM Center in Noumea

Extraction and isolation of alkaloids Finely ground leaves (1 04 kg) were wetted with 50% NH_4OH (624 ml—48 hr) and lixiviated by means of 26 l of EtOAc The lixiviate was extracted with 2% H_2SO_4 and the aq phase made alkaline with NH_4OH and thoroughly extracted with $CHCl_3$ The $CHCl_3$ layers were dried (Na_2SO_4) and evapd *in vacuo* to give 23 7 g of crude alkaloid mixture (yield 22 7 g/kg) The AM was fractionated on 1 kg silica gel H60 (elution pressure 10 bar, 30 ml fractions), the column was eluted with $CHCl_3$ (3 l), $CHCl_3$–MeOH (49 1, 1 2 l, 19 1, 6 l, 9 1, 2 l) The tubes were analysed by TLC and pooled according to their composition Vincamedine **1** and unknowns **18** and **19** were in fractions 101–118, 10-methoxy vincamedine **2** (430 mg) was in fractions 119–180 and *Z*-isositsirikine **3** in fractions 181–190 The content of tubes 225–265 was refractionated by CC to yield akuammicine **4**, quaternoline **5** and 11-methoxyakuammicine **6**, tubotaiwine **7** and the *N*(4)-oxide of 10-methoxyvincamedine **8** were found in fr 281–290 The major alkaloids cabucraline **9** (1 75 g), cathafoline **10** (1 78 g) and caberoline **11** (0 15 g) were in fractions 300–360 The dimer desoxy cabufiline **12** was eluted with $CHCl_3$–MeOH (9 1) in fr 361–427

In the same manner 1 08 g of crude AM was obtained from 540 g of dried fruit (yield 2 g/kg) The AM was fractionated on 250 g silica gel H60, elution solvents were CH_2Cl_2 (3 6 l), CH_2Cl_2–MeOH (99 1, 3 6 l, 49 1, 2 4 l, 19 1, 4 2 l, 9 1, 3 3 l, 4 1, 1 8 l) and MeOH (1 5 l) Vincoridine **13** (10 mg) was in fractions 81–120, **1** and **2** in fr 196–240, quebrachidine **14** (54 mg) in fr 280–340 quaternoxine **15** (14 mg) in fr 321–343, **4**, **6** and **8** in fr 344–434, **9** and **10** in fr 425–570 while **12** was in fr 588–680

Dried stem bark (3 5 kg) yielded 23 g of crude AM which was also separated on 1 kg silica gel Alkaloids **1** and **2** were eluted with CH_2Cl_2–MeOH (49 1) in fr 161–219, **4** in fr 271–470, fr 471–571 yielded **6**, **8** and nor C fluorocurarine **16** and **9** and **10** were in fr 572–913 The dimers **12** and **17** were in fr 914–1057 and the unknown **20** in fr 1057–1127

Alkaloids **1**, **4**–**7** and **9**–**17**, available from other studies in the Reims laboratory, were identified by direct comparison as well as by examination of their spectral properties

Description of new alkaloids 10-*methoxyvincamedine* **2** $C_{25}H_{30}N_2O_5$, (CR pink), $[\alpha]_D = -9°$ (*c* 1), UV λ_{max}^{MeOH} nm 247, 313, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1750, 1735, 1610, MS (rel int) *m/z* 438 (100), 423, 379, 264, 222, 200, 190, 187, 174, ^{1}H NMR (60 MHz) δ 5 68 (*s*, 1H), 5 25 (*q*, *J* = 7 Hz, 1H), 3 7 (*s*, 3H), 3 6 (*s*, 3H), 2 65 (*s*, 3H), 1 85 (*s*, 3H), 1 55 (*d*, *J* = 7 Hz, 3H) *Unknown* **18** (CR yellow), UV λ_{max}^{MeOH} nm 233, 280, $\lambda_{max}^{MeOH\text{-}H^+}$ nm 225, 258 (sh) 290, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3460, 1770, 1635, MS (rel int) *m/z* 366 (100), 336, 308, 265, 229 (85), 200 (98), 199, 182, 170, 158, ^{1}H NMR (400 MHz) δ 7 2 (*d*, *J* = 2 Hz, 1H), 7 15 (*d*, *J* = 8 Hz, 1H), 6 8 (*dd*, *J* = 2 Hz and 8 Hz, 1H), 4 6 (*m*, 2H), 3 85 (*s*, 3H), 3 1 (*s*, 3H) *Unknown* **19** (CR orange), UV λ_{max}^{MeOH} nm 233, 283, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1760, 1635, MS (rel int) *m/z* 336 (100), 306, 229 (22), 200 (40), 183, 170, 108, ^{1}H NMR (60 MHz) δ 7 2–6 7 (*m*, 3H), 3 8 (*s*, 3H), 1 1 (*t*, *J* = 7 Hz, 3H) 10-*Methoxyvincamedine N*(4)-*oxide* **8** $C_{25}H_{30}N_2O_6$ (CR red), $[\alpha]_D = -2°$ (*c* 1), UV λ_{max}^{MeOH} nm 212, 248, 305, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 1740, MS (rel int) *m/z* 454, 438 (100), 379, 264, 222, 212, 200, 190, 187, 174, ^{1}H NMR (60 MHz) δ 5 65 (*s*, 1H), 5 4 (*q*, *J* = 7 Hz, 1H), 3 7 (*s*, 3H), 3 65 (*s*, 3H), 2 6 (*s*, 3H), 1 85 (*s*, 3H), 1 55 (*d*, *J* = 7 Hz, 3H) *Z-Isositsirikine* **3** $C_{21}H_{26}N_2O_3$, mp 183°, CR pale yellow green, $[\alpha]_D = -32°$ (*c* 1), UV λ_{max}^{MeOH} nm 227, 283, 290, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3400, 1710, 1450, 1380, MS (rel int) *m/z* 354 (100), 353, 339, 337, 323, 295, 251, 249, 237, 184, 170, 169, 156, ^{1}H NMR (60 MHz) δ 8 6 (*s*, 1H), 7 5–6 9 (*m*, 4H), 5 45 (*q*, *J* = 6 Hz, 1H), 3 7 (*s*, 3H), 1 7 (*d*, *J* = 6 Hz, 3H) *Unknown dimer* **20** (CR purple), $[\alpha]_D = -32°$ (*c* 1), UV λ_{max}^{MeOH} nm 218, 231 (sh), 256 (sh), 288, 294, IR $\nu_{max}^{CHCl_3}$ cm^{-1} 3360, 1730, 1670, 1620, MS (rel int) *m/z* 732 (100), 702, 701, 656, 629, 381, 366, 365 (78), 350, 335, 194, ^{1}H NMR (400 MHz) δ 7 2 (*d*, *J* = 7 Hz, 1H), 7 1 (*d*, *J* = 7 Hz, 1H), 7 0 (*t*, *J* = 7 Hz, 1H), 6 85 (*t*, *J* = 7 Hz, 1H), 6 2 (*s*, 1H), 6 18 (*s*, 1H), 5 28 (*dq*, *J* = 7 Hz, 2H), 4 85 (*s*, 1H), 4 2 (*d*, *J* = 8 Hz, 2H), 3 9 (*s*, 3H), 3 6 (*s*, 3H), 3 0 (*s*, 3H), 2 8 (*s*, 3H), 2 63 (*s*, 3H), 1 6 (*d*, *J* = 7 Hz, 3H), 1 25 (*d*, *J* = 7 Hz, 3H) *Oxidation of* 10-*methoxyvincamedine* **2** 10-Methoxyvincamedine (20 mg) was dissolved in 2 ml CH_2Cl_2 to which 15 mg of *p*-nitroperbenzoic acid was added After 30 min to the soln was added 1 ml 2 N NaOH, usual treatment of the organic layer yielded 15 mg of 10-methoxyvincamedine *N*-oxide identical to **9** (TLC, UV, IR, MS)

Acknowledgements—We thank Dr S K Kan for 400 MHz ^{1}H NMR measurements and Drs H-P Husson and C Kan-Fan for a sample of *Z*-isositsirikine

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