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ALKALOIDS OF *ALSTONIA LANCEOLIFERA**

N. PETITFRERE-AUVRAY, J. VERCAUTEREN, G. MASSIOT, G. LUKACS, T. SEVENET, L. LE MEN-OLIVIER and the technical collaboration of B. RICHARD and M.-J. JACQUIER

Faculté de Pharmacie (E.R.A. au C.N.R.S., No. 319), 51, rue Cognacq-Jay, 51096 Reims Cédex, France

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Key Word Index—*Alstonia lanceolifera*; Apocynaceae; indole alkaloids.

Abstract—Four new indole alkaloids, 10-methoxydeplancheine, 10,11-dimethoxy-1-methyl-deacetyl picraline benzoate, 10,11-dimethoxy-1-methyl-deacetyl picraline 3′,4′,5′-trimethoxybenzoate and 10,11-dimethoxy-1-methyl-deacetyl picraline were isolated from the leaves of *Alstonia lanceolifera*.

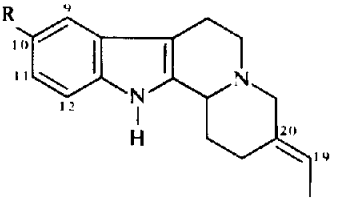
INTRODUCTION

As a continuation of our chemotaxonomic work on the New Caledonian *Alstonia*, we herein describe our results on the alkaloid content of *Alstonia lanceolifera* S. Moore [1]. Plant material was collected in the Tontouta Valley and was identified by T. Sevenet (Sevenet voucher No. 795). A previous report on the alkaloids of *A. lanceolifera* [2, 3] incorrectly identified the plant species and it has now been corrected to *A. boulindaensis* Boiteau [1, 4].

The present study was limited to the leaves, the alkaloid mixture (AM) of which was obtained by standard procedures (yield 3 g per kg of dried leaves). A combination of crystallization and column chromatography allowed isolation and identification of four components which, to the best of our knowledge, have never been previously encountered.

RESULTS AND DISCUSSION

10-Methoxydeplancheine (1) (0.3% of AM), M^+: 282 ($C_{18}H_{22}N_2O$), mp: 75° (from acetone), $[\alpha]_D$ +33° ($c = 0.9$, $CHCl_3$). Other than a strong peak at *m/z* 281 ($M^+ - 1$) the mass spectrum showed little fragmentation and was reminiscent of the spectrum of the recently described deplancheine (2) [5], the major peaks (*m/z* 186, 199, 200) being shifted by 30 amu indicating the presence of an extra OMe group. This was confirmed by the ^1H NMR spectrum showing a methyl at δ 3.80 and a three-proton multiplet between 6.6 and 7.3. Substitution at positions 9 or 12 was ruled out by examination of the 400 MHz ^1H NMR spectrum allowing analysis of the aromatic multiplet as an ABX with $J_{AB} = 7$ Hz, $J_{AX} = 0$, $J_{BX} = 2$ Hz. The location of the OMe was finally secured by an unambiguous total synthesis of 19,20-dihydro-10-methoxydeplancheine [6] whose UV spectrum and the aromatic part of the ^1H NMR spectrum were superimposable on those of 1.



1 R = OMe
2 R = H

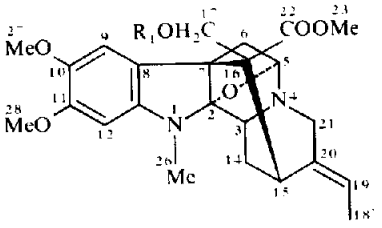
10,11-Dimethoxy-1-methyl-deacetyl picraline benzoate (3) (10% of AM), 10,11-dimethoxy-1-methyl-deacetyl picraline 3′,4′,5′-trimethoxybenzoate (4) (55% of AM) and 10,11-dimethoxy-1-methyl-deacetyl picraline (5) (5% of AM) were also isolated and their structures were established as follows.

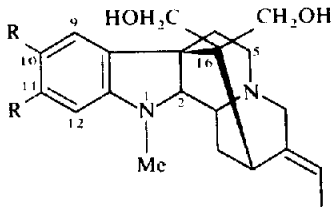
Mild alkaline hydrolysis of 3 and 4 yielded 5 and an acidic fraction shown to be benzoic acid (obtained from 3) and 3,4,5-trimethoxybenzoic acid (from 4). All three compounds were reduced ($LiAlH_4$, THF, reflux) to a single diol 6 whose spectral properties (UV, IR, MS) were very similar to those of pseudoakuammigol (7), the reduction product of pseudoakuammigine [7, 8]. Due to the poor solubilities of diols 6 and 7, these compounds were acetylated in order to compare their NMR spectra. These spectra were shown to be practically identical except for the effects of the double substitution on C(10) and C(11) (singlets at δ 3.75 (3 H), 3.85 (3 H), 6.30 (1 H) and 6.95 ppm (1 H)).

Assignment of stereochemistry at C(16) relied upon compound 8, which was isolated from *A. lanceolata* and hydrolysed to 5 [9]. In 8, an NMR signal at δ 1.60 was assigned to the acetyl methyl group (the upfield shift being due to the shielding by the aromatic ring current) [10]. Noteworthy is the fact that acetylation of 5 could never be performed without concomitant fragmentation of the carbinolamine bonds [11]. Finally, hydrolysis and deformylation of 4 (methanolic KOH treatment), yielded a compound in all respects identical to quaternine (9), an alkaloid previously isolated by Mamatas-Kalamaras *et al.* [12].

The ^1H NMR spectra of 3, 4, 5 and picraline (10) were strikingly similar, the most obvious differences resulting from varying degrees of shielding of the CH_2(17)

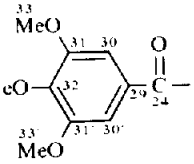
* Part LXX in the series "Plants from New Caledonia". For Part LXIX see Vercauteren, J., Massiot, G., Sevenet, T., Richard, B., Lobjois, V., Le Men-Olivier, L. and Levy, J. (1981) *Phytochemistry* **20**, 1411.



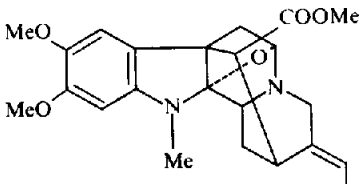


6 R = OMe
7 R = H

3 R_1 = $C_6H_5-\overset{\overset{24}{|}}{\underset{\underset{O}{||}}{C}}-$



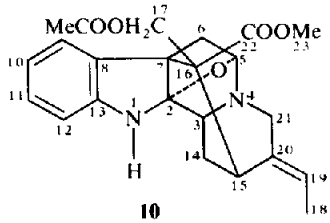
4 R_1 =



9

5 R_1 = H

8 R_1 = $Me-\overset{\overset{O}{||}}{\underset{25}{C}}-\underset{24}{}$



10

depending on substitution, from the singlets caused by the N-Me and the OMe and from the aromatic protons. The mass spectra of **3**, **4**, **5** and **10** displayed the same type of fragmentation characterized by a peak at *m/z* 411 (in **3**–**5**) or 337 in **10** (loss of CH_2(17)-ester) and by peaks at *m/z* 313 (239 in **10**) and 231 (157 in **10**) due to the fragmentation of rings A, B and C.

Structural resemblances between **4**, **5** and **10** were further reinforced by examination of the ^{13}C NMR spectra (Table 1). They all had in common the carbinolamine carbons at 108 ± 1 ppm (C(2)) and 87.0 ± 0.1 ppm (C(5)), as well as all the carbons belonging to the non-aromatic part of the molecule; substitutions at positions 10 and 11 caused the expected shifts of the aromatic carbons [13]. The shielding of C(14) and C(21) might be due to γ-interaction with the bridge oxygen (difference of 8 ppm with pseudoakuammigine) (Lanceomigine, unpublished work).

Among the 14 *Alstonia* species from New Caledonia, seven species have so far been investigated [2–5, 9, 11, 12,

Table 1. ^{13}C NMR spectral data of **10**, **5** and **4** ($CDCl_3$, δ, TMS = 0)

Position	**10**	**5**	**4**	Position	**10**	**5**	**4**
2	107.1	109.7	109.9	19	120.9	120.0	120.6
3	51.6	49.4	49.3	20	137.4	137.8	137.4
5	87.1	86.9	87.0	21	46.5	46.6	46.5
6	44.4	44.7	44.4	22	172.3	174.5	172.6
7	52.9	52.5	52.5	23	51.4	51.6	51.5
8	133.8	123.4	124.2	24	169.9	—	164.8
9	127.9	111.2	113.3	25	20.0	—	—
10	120.9	143.5	143.5	26	—	30.0	30.2
11	127.6	145.6	145.6	27	—	57.7*	56.8*
12	111.2	95.2	95.6	28	—	57.0*	56.2*
13	148.6	150.0	150.0	29	—	—	124.7
14	22.0	21.6	21.9	30–30′	—	—	106.8
15	35.6	33.2	35.1	31–31′			152.8
16	56.3	57.9	56.8	32			164.8
17	67.1	64.6	66.9	33′–33			56.2
18	13.2	13.1	13.2	34			60.8

* Values within the same column may be interchanged.

14–17]. All of the isolated alkaloids belong to the type I of the Le Men–Taylor classification [18], i.e. the most primitive one. The picraline type (with C(16) bound to C(7)) accounts for 70% of the alkaloid content of *A. lanceolifera* and is especially well represented in the Alstoniae.

EXPERIMENTAL

General. Mps were taken on a hot stage Reichert microscope and are uncorr. Rotations were determined in $CHCl_3$ and, unless otherwise stated, NMR were measured in $CDCl_3$ solns; chemical shifts are given in δ with TMS as the int. standard. ^{13}C NMR spectra were obtained at 15 or 22 MHz. 400 MHz ^1H NMR data were generously provided by Dr. S. K. Kan, Institut d'Electronique Fondamentale (Orsay). Chromatographic columns were packed with Al_2O_3, activity grade II–III. Prep. TLC plates were Merck 60F-254. Colour reactions (CR) were obtained by spraying plates with a soln of Ce (IV) ammonium sulfate.

Extraction and isolation of alkaloids. Dried ground leaves (8.7 kg) were wet with 50% NH_4OH and lixiviated by means of 160 l. of EtOAc. The lixiviate was extracted with 2% H_2SO_4, and the aq. phase was made alkaline with NH_4OH and extracted with $CHCl_3$. The $CHCl_3$ layers were dried (Na_2SO_4) and evapd *in vacuo* to give 28.8 g of crude alkaloid mixture (yield 3 g/kg). Direct crystallization of the alkaloid mixture (Me_2CO) yielded 11.3 g of alkaloid **3**. The C_6H_6-soluble fraction of the mother liquor (17.5 g) was chromatographed on an Al_2O_3 column (500 g) packed in C_6H_6, which was eluted in 400 ml fractions with: C_6H_6 (2.4 l.); C_6H_6–Et_2O, 99:1 (2.8 l.); C_6H_6–Et_2O, 49:1 (2 l.); C_6H_6–Et_2O, 19:1 (2.8 l.); C_6H_6–Et_2O, 9:1 (7.2 l.); C_6H_6–Et_2O, 4:1 (11.2 l.); C_6H_6–Et_2O, 1:1 (5.6 l.); Et_2O (6 l.); Et_2O–MeOH, 99:1 (2 l.); Et_2O–MeOH, 49:1 (2 l.); Et_2O–MeOH, 19:1 (2 l.); MeOH (2 l.).

No alkaloids were found in fractions 1–19 (1.8 g) from the column as well as in the C_6H_6-insoluble material left over from the mother liquor (2.3 g). Fractions 20–27 (272 mg) yielded 87 mg of methoxydeplancheine (**1**) (crystallization from Me_2CO; yield 0.3% of AM). Fractions 28–111 contained alkaloids **3**, **4** and **5** which were separated and purified by repeated crystallization; yield for compound **3** (*ca* 10% of AM). **4** (*ca* 55% of AM) including material from crystallization; **5** (*ca* 5% of AM).

10-Methoxydeplancheine (**1**). $C_{18}H_{22}N_2O$ (CR yellow); mp 75° (Me_2CO), $[\alpha]_D$ +33° (c = 0.9); UV λ_{max}^{MeOH} nm: 222, 277, 296 (s), 308 (s); IR ($CHCl_3$) cm^{-1}: 3420, 2280, 1590, 1480, 1450; MS *m/z* (rel. int.): 282 $[M]^+$ (100), 281 (98), 200 (18), 199 (40), 186 (35); ^1H NMR ($CDCl_3$): δ 7.9 (*s*, 1 H), 5.3 (*q*, J = 7 Hz, 1 H), 3.8 (*s*, 3 H), 1.65 (*d*, J = 7 Hz, 3 H).

10,11-Dimethoxy-1-methyl-deacetyl picraline benzoate (**3**). (CR orange) $C_{31}H_{34}N_2O_7$; mp 114° (MeOH), $[\alpha]_D$ −157° (c = 0.5); UV λ_{max}^{MeOH} nm: 220, 237, 282 (s), 305; IR (Nujol) cm^{-1}: 3600, 3400, 1730, 1610; MS *m/z* (rel. int.): 546 $[M]^+$ (100), 425 (15), 411 (65), 313 (75), 231 (60); ^1H NMR ($CDCl_3$): δ 7.05 (*s*, 1 H), 6.2 (*s*, 1 H), 5.4 (*q*, J = 7 Hz, 1 H), 4.75 (*d*, J = 2 Hz, 1 H), 4.3–4.6 (AB system, J_{AB} = 12 Hz), 3.7 (*s*, 6 H), 3.6 (*s*, 3 H), 2.9 (*s*, 3 H), 1.6 (*d*, J = 7 Hz, 3 H).

10,11-Dimethoxy-1-methyl-deactyl picraline 3′,4′,5′-trimethoxybenzoate (**4**). $C_{34}H_{40}N_2O_{10}$ (CR orange brown); mp 194° (MeOH), $[\alpha]_D$ −126° (c = 0.98); UV λ_{max}^{MeOH} (log ε): 212 (4.64), 255 (4.09), 302 (3.16); IR (Nujol cm^{-1}: 3600, 3400, 1730 (br), 1590, 1460; MS *m/z* (rel. int.) 636 $[M^+]$ (100), 425 (25), 411 (75), 313 (75), 231 (70); ^1H NMR ($CDCl_3$): δ 7.05 (*s*, 1 H), 7.0 (*s*, 2 H), 6.25 (*s*, 1 H), 5.4 (*q*, J = 7 Hz, 1 H), 4.75 (*d*, J = 2 Hz, 1 H), 3.9 (*s*, 6 H), 3.75 (*s*, 3 H), 3.7 (*s*, 3 H), 3.6 (*s*, 3 H), 2.95 (*s*, 3 H), 1.6 (*d*, J = 7 Hz, 3 H).

10,11-Dimethoxy-1-methyl-deacetyl picraline (**5**). (CR orange) $C_{24}H_{30}N_2O_6$; mp 204° (MeOH), $[\alpha]_D$ −90° (c = 0.7); UV λ_{max}^{MeOH} nm: 217, 248, 303; IR ($CHCl_3$) cm^{-1}: 3550, 3400, 1730, 1610; MS *m/z* (rel. int.): 442 $[M]^+$ (70), 411 (100), 313 (30), 231 (25); ^1H NMR ($CDCl_3$): δ 6.9 (*s*, 1 H), 6.3 (*s*, 1 H), 5.4 (*q*, J = 7 Hz, 1 H), 4.7 (*d*, J = 2 Hz, 1 H), 3.85 (*s*, 3 H), 3.75 (*s*, 3 H), 3.7 (*s*, 3 H), 2.9 (*s*, 3 H), 1.6 (*d*, J = 7 Hz, 3 H).

Hydrolysis of **4**: **4** → **5**. Alkaloid **4** (3.69 g) was dissolved in 120 ml 0.1 N methanolic KOH; the soln refluxed for 2 hr, then stirred at room temp. for 3 hr. The soln was then poured into H_2O and acidified to pH 4 (HOAc). After vacuum distillation to remove MeOH, the mixture was extracted with 3 × 120 ml of Et_2O. Usual treatment of the organic layers yielded 0.79 g of an acid identical to 3,4,5-trimethoxybenzoic acid. Alkalization of the mother liquor followed by extraction with Et_2O (6 × 150 ml) yielded 1.77 g of a homogeneous product identical to alkaloid **5**. Yield: 69%.

Hydrolysis of **3**: **3** → **5**. The same reaction performed on alkaloid **3** gave a 30% yield of alkaloid **5** and benzoic acid.

$LiAlH_4$ *reduction of* **5**: **5** → **6**. Alkaloid **5** (500 mg) was dissolved in 20 ml of dry THF to which 700 mg of $LiAlH_4$ was added. After refluxing for 6 hr, usual treatment and $CHCl_3$ extraction yielded 400 mg of crude diol. After purification by prep. TLC, 281 mg of pure diol **6** were obtained (yield 62%). (CR: pink); UV λ_{max}^{MeOH} nm: 212, 248, 306; IR ($CHCl_3$) cm^{-1}: 3400, 1615 (weak); MS *m/z*: 400 $[M]^+$, 383, 369, 218, 217, 204, 196, 178, 106.

Acetylation of **6**. Compound **6** (281 mg) was dissolved in a mixture of 0.2 ml of pyridine and 0.9 ml of Ac_2O. After 18 hr at room temp. the solvents were removed and the residue filtered on 3 g of Al_2O_3. This procedure yielded 197 mg of pure diacetate. UV λ_{max}^{MeOH} nm: 216, 250, 260, 313; IR ($CHCl_3$) cm^{-1}: 1740, 1610, 1490, 1220; MS *m/z* (rel. int.): 484 $[M]^+$, 425, 280, 218 (100), 204; ^1H NMR ($CDCl_3$): δ 6.85 (*s*, 1 H), 6.3 (*s*, 1 H), 5.52 (*q*, J = 7 Hz, 1 H), 4.6 (*s*, 2 H), 3.9 (*s*, 3 H), 3.85 (*s*, 3 H), 2.62 (*s*, 3 H), 2.1 (*s*, 3 H), 1.9 (*s*, 3 H), 1.67 (*d*, J = 7 Hz, 3 H). The ^1H NMR spectrum of this compound was superimposable with the NMR spectrum of pseudoakuammigol diacetate except for the aromatic region and the methoxy singlets.

Correlation between **4** *and quaternine*: **4** → **9**. Alkaloid **4** was refluxed for 10 hr in 2.5 N methanolic KOH. The mixture was worked up as previously described for the hydrolysis of **4** (see above). After TLC purification, 121 mg of quaternine was obtained: mp: 153° (Et_2O); $[\alpha]_D$ −26° ($CHCl_3$, c = 0.8). UV, IR, NMR and MS were identical to those of quaternine.

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REFERENCES

1. Boiteau, P., Allorge, L. and Sevenet, T. (1977) *Adansonia* **16**, 465.
2. Lewin, G., Kunesch, N. and Poisson, J. (1975) *C.R. Acad. Sci. Ser. C* **280**, 987.
3. Lewin, G., Kunesch, N., Cave, A., Sevenet, T. and Poisson, J. (1975) *Phytochemistry* **14**, 2067.
4. Lewin, G., Kunesch, N., Poisson, J. and Sevenet, T. (1978) *J. Indian Chem. Soc.* **55**, 1096.
5. Besselievre, R., Cosson, J.-P., Das, B. C. and Husson, H.-P. (1980) *Tetrahedron Letters*, 63.
6. Ferte, C., Sousa Olivera, F., Vercauteren, J., Massiot, G. and Le Men-Olivier, L. (1981) *Heterocycles* (in preparation).
7. Olivier, L., Lévy, J., Le Men, J., Janot, M.-M., Djerassi, C., Budzikiewicz, H., Wilson, J. M. and Durham, L. J., (1963) *Bull. Soc. Chim. Fr.* 646.

8. Olivier, L., Lévy, J., Le Men, J., Janot, M.-M., Budzikiewicz, H. and Djerassi, C. (1965) *Bull. Soc. Chim. Fr.* 868.
9. Vercauteren, J., Massiot, G., Sevenet, T., Richard, B., Lobjois, V., Le Men-Olivier, L. and Lévy, J. (1981) *Phytochemistry* **20**, 1411.
10. Pousset, J. L., Poisson, J., Olivier, L., Le Men, J. and Janot, M.-M. (1965) *C.R. Acad. Sci.* **261**, 5538.
11. Petitfrere-Auvray, N. (1978) Thèse de Doctorat de 3ème cycle en Pharmacie, Reims, No. 301.
12. Mamatas-Kalamaras, S., Sevenet, T., Thal, C. and Potier, P. (1975) *Phytochemistry* **14**, 1849.
13. Breitmaier, E. and Voelter, W. (1974) ^{13}C *NMR Spectroscopy*. Verlag Chemie, Weinheim.
14. Vercauteren, J., Massiot, G., Sevenet, T., Lévy, J., Le Men-Olivier, L. and Le Men, J. (1979) *Phytochemistry* **18**, 1729.
15. Das, B. C., Cosson, J. P., Lukacs, G. and Potier, P. (1974) *Tetrahedron Letters* 4299.
16. Das, B. C., Cosson, J. P. and Lukacs, G. (1977) *J. Org. Chem.* **42**, 2785.
17. Cosson, J. P. (1975) Thèse Doctorat d'Université, Université Paris-Sud.
18. Taylor, W. I. and Le Men, J. (1965) *Experientia* **21**, 508.