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ALKALOIDS OF *ALSTONIA LENORMANDII*, A STRUCTURAL REVISION OF 10-METHOXYCOMPACTINERVINE*

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Key Word Index—*Alstonia lenormandii*; Apocynaceae; indole alkaloids; 12-methoxycompactinervine; ^{13}C NMR.

Abstract—Thirteen alkaloids have been isolated from the leaves and from the stem bark of two varieties of *Alstonia lenormandii*: variety *lenormandii* and variety *minutifolia* from New Caledonia. They are 10,11-dimethoxy-1-methyldeacetylpicraline, its 3′,4′,5′-trimethoxybenzoate and its benzoate, 10,11-dimethoxy-1-methylpicraline, akuammiline, picraline, lochnericine, 11-methoxyakuammicine, 11-methoxy- and 12-methoxycompactinervine, 12-methoxy-19α,20α-epoxyakuammicine, gentianine and 3′,4′,5′-trimethoxycinnamamide. The compactinervine which is methoxylated at C-12 was previously isolated from *A. lanceolata* and assigned the structure 10-methoxycompactinervine; this has been revised in the present work by the analysis of high field ^{1}H and ^{13}C NMR. The corresponding epoxide is a novel alkaloid isolated only in the *minutifolia* variety. Its structure has been established by spectral means and by chemical correlations.

INTRODUCTION

As part of a systematic investigation of the genus *Alstonia* (Apocynaceae) [1], we herein describe our results on the alkaloid content of two varieties of *A. lenormandii* (*A. l*), an endemic species of New Caledonia. They are *A. lenormandii* var. *lenormandii*, the most common variety, and *A. lenormandii* var. *minutifolia*, a novel variety proposed by P. Boiteau [2]. Extractions of the alkaloids were performed in the usual fashion [3] and yields were as follows: *A. l.* var. *minutifolia* leaves: 3.07 g/kg; trunk bark: 6.4 g/kg; *A. l.* var. *lenormandii* leaves: 4.25 g/kg; trunk bark: 8.45 g/kg.

RESULTS AND DISCUSSION

A unique feature of the alkaloid mixture (AM) from the leaves of var. *minutifolia* is its composition, since TLC reveals the presence of a single alkaloid in *ca* 50% yield. This alkaloid is obtained in pure form by crystallization of the crude AM from methanol; it is 10,11-dimethoxy-1-methyldeacetylpicraline-3′4′,5′-trimethoxybenzoate (**1**). Chromatography of the mother liquors of crystallization gives small amounts of the ubiquitous monoterpene pyridine alkaloid gentianine (**2**, 2% of AM) and of **3**, a methoxylated derivative of compactinervine (2% of AM), the structural assignment of which will be described later.

The alkaloid content of the bark of both varieties is similar (TLC) and only the bark of var. *lenormandii* has been investigated in detail. On TLC, one can distinguish two sets of alkaloids according to their colouration after Ce(IV) spraying: orange (corresponding to picraline-type structures) or blue, a characteristic of alkaloids which contain the β-anilinoacrylic moiety. From this mixture eight alkaloids are separable; they are in order of increasing polarity: (1) lochnericine (**4**, 0.2% of AM), (2) 10,11-dimethoxy-1-methyl picraline (**5**, 17% of AM), (3) 10,11-dimethoxy-1-methyl desacetyl picraline (**6**, 5% of AM), (4) akuammiline (**7**, 0.2% of AM), (5) picraline (**8**, 1% of AM), (6) 11-methoxyakuammicine (**9**, 1% of AM), (7) 11-methoxycompactinervine (**10**, 2% of AM) and (8) 12-methoxycompactinervine (**3**, 5% of AM).

Alkaloids **1**–**6** and **8** have previously been isolated from *A. lanceolata* [4] and alkaloids **7**, **9** and **10** from *A. lanceolifera* [5]. They have been identified by direct comparison on TLC and by comparison of their spectra (IR, UV, NMR, mass) with those of authentic samples. Alkaloid **10**, which is also known as alstovine has also been found in *A. vitiensis* [6] and in *A. plumosa* [7]. Previous structural assignments for **10** [5–7] are based on 60 MHz ^{1}H NMR spectra and on ^{13}C chemical shifts reported only for four aromatic carbons. After this novel isolation of **10**, 400 MHz ^{1}H NMR and 75 MHz ^{13}C NMR spectra have become available. Comparison of the high field parts of the NMR spectra of compactinervine and of **10** shows an almost perfect superimposition, which points to an identity of the skeletons including relative configurations (C-3, C-15, C-19 and C-20) of these two alkaloids. The ^{13}C spectrum of **10** (Table 1) is also in excellent agreement with Verpoorte's data on compactinervine [8] and on his proposed aromatic substituent induced chemical shifts [9]. All these data further support the original structure of **10**.

Alkaloid **3**, which is found in the two varieties of *A. lenormandii* is identical to an alkaloid isolated from *A. lanceolata* [4] and *A. lanceolifera* [5] which we have called 10-methoxy compactinervine. The ^{1}H NMR spec-

* Part IC in the series "Plants from New Caledonia". For Part IIC see Kan-Fan, C., Brillanceau, M. H., Pusset, J., Chauvière, G. and Husson, H.-P. (1985) *Phytochemistry* **24**, 2773.

Table 1. ^{13}C NMR of alkaloids **10**, **3** and **13**

C	10	3	13	C	10	3	13
2	168	169.2	167.8	14	25.6	25.8	29.1
3	60.5	60.8	60.9	15	35.3	35.2	31.2
5	51.5	51.9	54.3	16	98.8	99.1	99.3
6	43.1	43.3	44.4	18	14.8	14.8	14.4
7	56.8	58.3	58.8	19	69.9	70	61.3
8	127.7	137	137	20	72.6	72.6	62.1
9	120.5	112.5	112.7	21	53.8	54.2	54.6
10	106	122.4	122	22	172.5	172.4	170.7
11	160	110.2	110.5	$CO_2\underline{Me}$	52.1	51.9	50.7
12	97.4	144.5	144.5	Ar$O\underline{Me}$	55.6	55.5	55.5
13	144.7	132.3	132.8				

trum of **3** is characterized by an entangled aromatic area from which coupling constants were deduced after a solvent-induced shift study performed at 60 MHz. A now available 400 MHz ^{1}H NMR spectrum of **3** shows an AMX system with two 7 Hz coupling constants indicative of either 9- of 12-methoxy substitution; examination of the high field part of the ^{1}H NMR spectrum confirms that **3** belongs to the compactinervine series. Final location of the aromatic methoxyl can be deduced from comparison of the ^{13}C NMR spectra of **3**, of scholarine (four reported δ values) [10] and of vinervinine (12-methoxy-akuammicine) [11]. Compound **3** is thus 12-methoxy-compactinervine and not 10-methoxycompactinervine as previously stated. As a consequence, it is worth noting that, at the present time, there does not exist any alkaloid with the β-anilino acrylic substructure bearing a single oxygenated substituent on C-10. This fact might be of significance with regard to the mechanism of *in vivo* hydroxylations or rearrangements leading to the formation of this series of compounds.

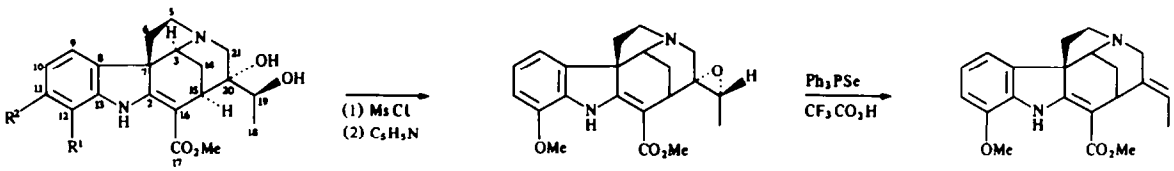
The alkaloid content of the leaves of var. *lenormandii* was more complex than that of var. *minutifolia*. It contained alkaloids **1** (17% of AM), **2** (1.5% of AM) and **3** (20% of AM), common to the two varieties but also 10,11-dimethoxy-1-methyl-desacetylpicraline benzoate (**11**, 2% of AM), 3',4',5'-trimethoxycinnamamide (**12**, 0.5% of AM) and 12-methoxy 19α,20α-epoxyakuammicine (**13**, 18% of AM).

Alkaloid **11** was also found in *A. lanceolifera* where it accompanied the corresponding trimethoxybenzoate **1**; these two alkaloids can only be separated by multiple migration TLC. The structure of alkaloid **12** was established after its spectral properties: UV (λ_{max}: 230, 300 nm), IR (1685 cm^{-1}), mass spectrum $[M]^+$ at *m/z* 237, $C_{12}H_{15}NO_4$) and ^{1}H NMR where all protons could unambiguously be assigned (see Experimental).

Compound **13** is a novel alkaloid classified in the β-anilino acrylic ester series after its blue colouration upon Ce(IV) spraying and its UV spectrum (λ_{max}: 213, 238, 290 and 335 nm). Its mass spectrum showed an $[M]^+$ at *m/z* 368 which analysed for $C_{21}H_{24}N_2O_4$ (calc. 368.174; found 368.167). The main fragment in the spectrum is found at *m/z* 231 corresponding to the main neutral loss in that of 11-methoxyakuammicine $[M-121]^+$. The most characteristic bands in the IR spectrum of **13** are a sharp NH or OH band at 3380 cm^{-1} and the typical double band of vinylic urethanes at 1600 and 1665 cm^{-1}. The ^{13}C NMR spectrum of **13** displayed signals for six quaternary carbons and two methines in the high frequency area; the chemical shifts of these signals are superimposable on those of the aromatic part of 12-methoxycompactinervine (**3**) (Table 1). The difference in M_r between compounds **3** and **13** (18 mu) corresponds to the loss of water, i.e. to the formation of an olefin or of a cyclic ether. Since no signals appear in the ^{13}C NMR spectrum for a supplementary double bond or for an alcohol bearing carbon, the second hypothesis is preferred. The presence of signals at δ62, 61 and 14.5 (C, CH, Me) suggests an epoxy ring on carbons 19 and 20 and we therefore propose for **13** the structure of 12-methoxy-19,20-epoxyakuammicine. This is further supported by analysis of the ^{1}H NMR spectrum of **13** obtained at 400 MHz (see Experimental). The enchainment of protons is found to be the same as that in compactinervine but minor differences appear for some chemical shifts, particularly for H-19 (shielded) and for the H-21 (singlet of two equivalent protons).

To complete the full structure establishment of **13**, it was necessary to determine the configuration of the two carbon atoms of the epoxide. Since few data existed on epoxides of the akuammicine series (apart from the parent compound prepared by Djerassi in the structural determination of compactinervine [12]), we decided to solve the problem by chemical means. Thus, 12-methoxy compactinervine was converted into its monomesylate which was not characterized but directly solvolysed into a single non-polar compound in all respects identical to **13**. Since the monomesylate is most probably formed on the secondary alcohol, which is inverted in the reaction sequence, compound **13** is the α-epoxide, i.e. the 19*S*,20*S*-isomer. The isolation of **13** was an opportunity to prepare the previously unknown 12-methoxyakuammicine by deoxygenation using Clive's reagent (triphenylphosphine selenide) [13]. The compound which was obtained strongly resembled akuammicine except for the effects of the aromatic substitution. It is worth nothing here that although Clive's reaction proceeds with double inversion, this does not constitute an unambiguous chemical correlation since two epoxides can yield the *E*-olefin (19*R*,20*R* or 19*S*,20*S*).

The division of *A. lenormandii* into two varieties is not totally justified, at least from a chemical standpoint. While the content in the barks of the two varieties is almost the same, the leaves mostly differ in the ratio of their alkaloids. The isolation of epoxide **13** in var. *lenormandii*, which is the principal difference between the two plants,



	R¹	R²
3	OMe	H
10	H	OMe

13

12 · OMe akuammicine

Scheme 1.

must not be considered as a strong separation argument because of the facile chemical interconversions between *vic*-diols and epoxides. The many isolations in our laboratory of compactinervines without any epoxides accompanying them, suggest that these compounds are true natural products and not isolation artefacts.

EXPERIMENTAL

General. Mps are uncorr. NMR were measured in $CDCl_3$ at 60 or 400 MHz on a prototype at the Institut d'Electronique Fondamentale (Orsay); ^{13}C NMR was obtained at 75 MHz. Colour reactions were obtained by spraying plates with a soln of Ce(IV) $(NH_4)_2SO_4$. *Alstonia lenormandii* var. *lenormandii* was collected at the Mouirange pass (March 12, 1981 and July 4, 1984); var. *minutifolia* was collected on February 28, 1984 at Foret Nord. Herbarium specimens are deposited at the ORSTOM Center in Noumea.

Extraction and isolation of alkaloids. Dried powdered leaves of *A. lenormandii* var. *minutifolia* (4.48 kg) were wetted with 50% NH_4OH and extracted with $CHCl_3$ and lixiviated with 40 l. of EtOAc. The lixiviate was extracted with 2% H_2SO_4 and the aq. phase 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 13.4 g of crude AM (yield 3 g/kg). The other extractions were performed in a similar fashion. Alkaloid **1** was separated by crystallization of the above mentioned AM from 150 ml of MeOH yield 8.2 g (59%). CC followed by prep. TLC gave pure **2** and **3**. AM from stem bark (10 g) was placed on a silica gel column (400 g) packed in $CHCl_3$ and eluted in 100 ml fractions with $CHCl_3$ (3 l.), $CHCl_3$–MeOH (49:1, 2 l.), $CHCl_3$–MeOH (19:1, 2 l.), $CHCl_3$–MeOH (9:1, 3 l.), $CHCl_3$–MeOH (17:3, 1 l.) and $CHCl_3$–MeOH (4:1, 1 l.). Alkaloid **4** was in fractions 30–40, alkaloids **5**–**7** in fr. 50–70, alkaloid **8** in fr. 70–80, alkaloid **9** in fr. 80–85 and the compactinervines **3** and **10** in fr. 100–120. The same type of purification was applied to the mixture extracted from leaves of var. *lenormandii*. Alkaloids **1** and **13** were eluted with $CHCl_3$–MeOH (49:1) and separated by crystallization from MeOH (**1**) and Et_2O (**13**); alkaloid **3** was eluted with $CHCl_3$–MeOH (24:1) and crystallized from MeOH.

New alkaloids. 12-*Methoxy compactinervine* (**3**): mp 155° (MeOH); $[\alpha]_D$ – 482° (*c* 1; $CHCl_3$); UV λ_{max} nm: 235, 288, 337; IR ν_{max} cm^{-1}: 3400, 1720, 1660, 1600; MS *m/z* 386 $[M]^+$, 352, 313, 298, 256, 255, 121; ^1H NMR (400 MHz): δ6.92 (*t*, *J* = 8 Hz, H-10), 6.89 (*d*, *J* = 8 Hz), 6.76 (*d*, *J* = 8 Hz), 3.89 (*br s*, H-3), 3.87 (*s*, 3H), 3.85 (*s*, 3H), 3.5 (*q*, *J* = 7 Hz), 2.93 (*br s*, H-15), 2.79 (*d*, *J* = 12 Hz, H-21), 2.03 (*d*, *J* = 12 Hz, H-21), 1.11 (*dt*, *J* = 12, 4 Hz, H-14), 1.05 (*d*, *J* = 7 Hz, Me-18). 12-*Methoxy*-19α,20α-*epoxy-akuammicine* (**13**): mp 188° (Et_2O); $[\alpha]_D$ – 590° (*c* 1; $CHCl_3$); UV λ_{max} nm (log ε): 213 (3.99), 238 (4.12), 290 (3.77), 335 (4.19); IR ν_{max} cm^{-1}: 3380, 1665, 1600, 1490, 1450, 1270, 1230; MS *m/z* (rel. int.): 368 $[M]^+$ (98), 353 (30), 350 (20), 337 (15), 323 (30), 312 (55), 281, 255, 246, 231 (100); ^1H NMR (400 MHz): δ8.98 (*s*, NH), 6.92 (*t*, *J* = 8 Hz, H-10), 6.85 (*d*, *J* = 8 Hz), 6.76 (*d*, *J* = 8 Hz), 4.0 (*br t*, *J* = 4 Hz), 3.87 (*s*, 3H), 3.73 (*s*, 3H), 3.12 (*dt*, *J* = 7, 14 Hz), 2.97 (*br t*, *J* = 4 Hz), 3.9 (*q*, *J* = 7 Hz, H-19), 2.73 (*s*, 3H, H-21), 2.53 (*dt*, *J* = 13, 4 Hz, H-14), 1.9 (*dd*, *J* = 7, 14 Hz), 1.41 (*d*, *J* = 7 Hz, Me-18), 1.37 (*dt*, *J* = 13, 4 Hz, H-14). 3′,4′,5′-*Trimethoxycinnamamide* (**12**): UV λ_{max} nm: 230, 300; IR ν_{max}^{nujol} cm^{-1}: 3420, 3180, 1690, 1610, 1585, 1500, 1330, 1125; MS *m/z* (rel. int.): 237 $[M]^{+\cdot}$ (100), 222 (40), 177 (25); ^1H NMR: δ7.56 (*d*, *J* = 16 Hz), 6.73 (*s*, 2H), 6.36 (*d*, *J* = 16 Hz), 5.6 (*br s*, 2H), 3.87 (*s*, 6H), 3.86 (*s*, 3H).

Preparation of **13** *from* **3**. The procedure described in ref. [12] was applied to **3** (50 mg). A compound in all respects identical to **13** was obtained in 68% yield (TLC, IR, UV, MS, ^1H NMR).

Deoxygenation of epoxide **13**. Compound **13** (35 mg) was dissolved in 1 ml CH_2Cl_2 and 65 mg of Ph_3PSe (2 eq.) were added. After dissolution, 50 μl of CF_3CO_2H were added and the mixture stirred at room temp. for 24 hr. The Se ppt was filtered off and the reaction mixture diluted with 10 ml of CH_2Cl_2 and 10 ml satd aq. Na_2CO_3. After separation of the phases, the organic layer was dried ($MgSO_4$) and evapd *in vacuo*. The residue (26 mg) was purified by prep. TLC and 14 mg of 12-methoxy-akuammicine was obtained. UV λ_{max} nm: 240, 290, 335; IR ν_{max} cm^{-1}: 3400, 1670, 1600; MS *m/z* (rel. int.): 352 $[M]^+$ (40), 321 (10), 293 (10), 246 (25), 211 (10), 121 (100); ^1H NMR (60 MHz): δ8.9 (*br s*, NH), 6.7 (*m*, 3H), 5.4 (*m*, 1H), 3.85 (*s*, 3H), 3.8 (*s*, 3H), 1.4 (*br d*, *J* = 7 Hz, Me-18).

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