Phytochemistry, Vol. 22, No. 10, pp. 2301–2304, 1983.
Printed in Great Britain.

0031–9422/83 $3.00 + 0.00
Pergamon Press Ltd.

ALKALOIDS FROM *MICROPLUMERIA ANOMALA**

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(*Revised received* 4 *March* 1983)

Key Word Index—*Microplumeria anomala*; Apocynaceae; indoline alkaloids; anomaline; demethoxyanomaline; 12-*O*-methylanomaline; NMR and mass spectra.

Abstract—Three indoline alkaloids have been isolated from the Amazonian plant *Microplumeria anomala* and their structures determined by spectral means.

INTRODUCTION

In continuation of a study of alkaloid constituents of the Apocynaceae an investigation of the tree *Microplumeria anomala*, indigenous to the Amazon region, was undertaken. The present communication reports the isolation and characterization of three bases from this plant.

RESULTS AND DISCUSSION

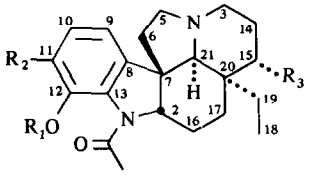
Extraction of the bark with ethanol and chromatography of the extract on alumina and silica gel yielded a colorless, crystalline compound $C_{22}H_{30}O_4N_2$, mp 216–218°. Its IR spectrum (KBr) exhibited a broad OH absorption band (3400 cm^{-1}) and strong amide carbonyl absorption (1630, 1600 and 1580 cm^{-1}) and its UV spectrum (methanol) showed maxima at 226 nm (log ε 4.27) and 259 nm (log ε 3.85). The ^1H NMR spectrum of a deuterochloroform solution of the alkaloid indicated the presence of a methoxyl singlet (δ 3.88), an acetyl methyl singlet (δ 2.33), a C-ethyl group bonded to a non-protonated carbon [0.66 (*t*, 3H, *J* = 7 Hz, Me), 1.37 (*dq*, 2H, *J* = 16, 7 Hz, CH_2)] and two vicinal aromatic hydrogens [6.62 (*d*, 1H, *J* = 8 Hz), 6.69 (*d*, 1H, *J* = 8 Hz)]. These data were reminiscent of the spectral characteristics of the *Aspidosperma* indoline alkaloid aspidocarpine (**1a**) [1, 2] and suggested the new natural base, named herewith anomaline, to be a hydroxyaspidocarpine. For this reason an exhaustive ^1H NMR spectral analysis of aspidocarpine (**1a**) was undertaken.

The ^1H NMR spectrum of a deuterochloroform solution of aspidocarpine (**1a**) showed signals at 0.64 (*t*, 3H, *J* = 7 Hz, ethyl Me), 1.38 (*q*, 2H, *J* = 7 Hz, ethyl CH_2), 2.23 (*s*, 1H, H-21), 2.32 (*s*, 3H, Ac Me), 3.05 (*ddd*, 1H, *J* = 10, 3, 2 Hz, H-3 or H-5), 3.12 (*dt*, 1H, *J* = 9, 3 Hz, H-3 or H-5), 3.88 (*s*, 3H, OMe), 4.06 (*dd*, 1H, *J* = 11, 6 Hz, H-2), 6.61, 6.70 (*d*, 1H each, *J* = 8 Hz, aromatic Hs) and 8.91 (*s*, 1H, OH), whose similarity with the hydrogen shifts of anomaline (**1b**) (see above) [2.49 (*m*, 1H, H-3 or H-5), 2.62 (*s*, 1H, H-21), 2.84 (*ddd*, 1H, *J* = 11, 3, 2 Hz, H-3 or H-5), 3.11 (*dt*, 1H, *J* = 9, 3 Hz, H-3 or H-5), 3.70 (*br s*, 1H, H-15), 4.10 (*dd*, 1H, *J* = 11, 6 Hz, H-2)] showed the latter to be indeed a hydroxyaspidocarpine. The addition of pyridine caused deshielding of the methylene unit of the quaternary ethyl group by 0.12 ppm [3] indicating the proximity of the latter to the non-aromatic hydroxyl group. Anomaline's aspidocarpine-like structure was confirmed by its mass spectrum. By analogy with the behavior of all *Aspidosperma* alkaloids the spectrum showed five characteristic fragments, the $[M]^+$ and cations **2**–**5** (R_1 = H, R_2 = OMe) at *m/z* 386 (97%), 358 (11), 190 (3), 176 (4) and 140 (100), respectively. These results corroborated the aspidocarpine-like structure and restricted the non-aromatic hydroxyl group to the piperidine ring in the vicinity of the angular ethyl group. The specific location of the alcohol function was derived from ^{13}C NMR data (see below).

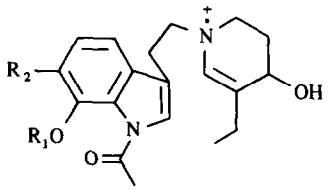
The anomaline-bearing, Amazonian plant yielded two, minor cogeners, demethoxyanomaline (**1c**) and 12-*O*-methylanomaline (**1d**) from the leaves, whose structural relationship with the major bark alkaloid was readily apparent from their spectral characteristics. Thus, the first base lacked a methoxyl signal in its ^1H NMR spectrum and showed an altered aromatic hydrogen region in the spectrum [6.70 (*d*, 1H, *J* = 8 Hz, H-9), 6.82 (*d*, 1H, *J* = 8 Hz, H-11) and 7.06 (*t*, 1H, *J* = 8 Hz, H-10)], while the second base revealed a two-methoxyl singlet (δ 3.88) and two vicinal, aromatic hydrogen doublets (δ 6.65 and 6.85, *J* = 8 Hz each). The IR spectra were anomaline-like. The UV spectrum (methanol) of **1c** exhibited maxima at 221, 260, 288 nm (log ε 4.13, 3.72, 3.47, respectively) and that of **1d** at 221, 253, 288 nm (log ε 4.29, 3.80, 3.49, respectively). These facts showed the substances to differ from anomaline in their aromatic substituents, as denoted in their names. The mass spectra of the minor bases confirmed these differences even more clearly. Thus although the compounds showed base peaks identical with anomaline (*m/z* 140; fragment **5**), the $[M]^+$ and fragments **2**–**4** of demethoxyanomaline [*m/z* 356 (34%), 328 (8), 160 (4), 146 (3)] differed from those of anomaline by −30 mu and the $[M]^+$ and ions **2**–**4** of 12-*O*-methylanomaline [*m/z* 400 (49%), 372 (17), 204 (5), 190 (4)] varied from those of the major alkaloid by +14 mu.

With the gross structures of the three alkaloids es-

*Part 78 in the series "Carbon-13 Nuclear Magnetic Resonance Spectroscopy of Naturally Occurring Substances". For part 77 see McPhail, A. T., Hagaman, E. W., Kunesch, N., Wenkert, E. and Poisson, J. (1983) *Tetrahedron* **39** (in press).



1a $R_1 = R_3 = H, R_2 = OMe$
1b $R_1 = H, R_2 = OMe, R_3 = OH$
1c $R_1 = R_2 = H, R_3 = OH$
1d $R_1 = Me, R_2 = OMe, R_3 = OH$



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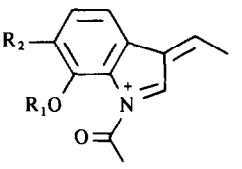

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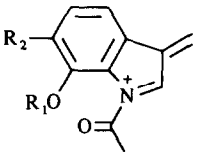

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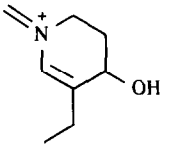

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tablished, it remained for ^{13}C NMR spectroscopy to remove any structural uncertainties. In order to assign the aromatic carbon shifts, it was necessary to rely on shift data for N_a-methyl-2β,16β-dihydrotabersonine (**6**) [4], a retuline derivative (**7**) [5] and a vobtusine derivative (**8**) [6], wherefrom it was possible to make complete shift assignments for the heretofore uninspected indoline alkaloids refractine (**9**) [7] and cylindrocarpine (**10**) [2, 8]. The non-aromatic carbon shifts of **9** were based on those of venalstonine [9] and those of **10** on the shift data of a variety of *Aspidosperma* bases [10].

The aromatic carbon shifts of 12-*O*-methylanomaline (**1d**) could be assigned with the use of **9** as a model and by the application of methoxyl substituent parameters [11] and these are listed on formula **11**. The δ values for the aromatic carbons of anomaline (**1b**) (methines: 109.9, 112.8; non-protonated carbons: 127.4, 132.8, 137.1, 149.2) and demethoxyanomaline (**1c**) (methines: 113.6, 117.5, 127.8; non-protonated carbons: 126.8, 140.6, 147.0) could not be assigned with certainty and will require a future model study for proper designation. The identity of the aromatic carbon shifts of anomaline (**1b**) with those of aspidocarpine (**1a**) revealed the nature of the aromatic substitution pattern of the former base.

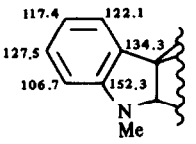
The non-aromatic carbon shifts of aspidocarpine (**1a**), anomaline (**1b**) and its two cogeners **1c** and **1d**, listed in Table 1, were based on shift data for various *Aspidosperma* alkaloids [10] and revealed the position of the hydroxyl group of the three new bases as C-15. Furthermore, a comparison of the δ values of the three new compounds (**1b–d**) with those of aspidocarpine (**1a**) showed the 15-hydroxyl group to be oriented axially

Table 1. Non-aromatic carbon shifts of alkaloids **1a–1d***

Carbon	1a	1b	1c	1d
2	70.0†	70.1	69.5	69.1
3	53.4	47.0	47.0	46.9
5	52.1	51.8	51.9	51.6
6	39.1	39.1	38.9	37.9
7	51.9	51.8	52.5	51.6
14	24.8	29.2	29.2	29.0
15	33.7	68.0	68.0	68.1
16	21.2	21.8	21.8	21.7
17	22.6	22.8	22.8	22.8
18	6.5	6.0	6.0	6.1
19	29.7	24.7	24.7	24.5
20	35.2	40.2	40.2	40.2
21	70.3†	65.8	65.9	66.2

*In $CDCl_3$ solutions; chemical shifts in ppm downfield from TMS; δ (TMS) = ($CDCl_3$) + 76.9 ppm. The acetyl C=O and Me shifts of **1a–c** are 169.1 and 22.5 ppm, respectively. The OMe shifts of **1a–b** are 56.1 ppm.

†The signals may be interchanged.



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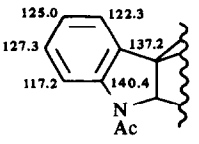

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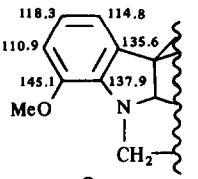

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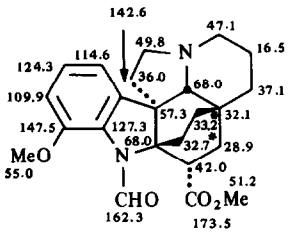

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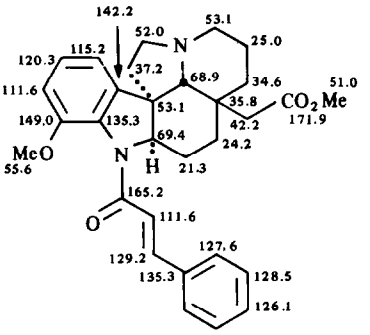

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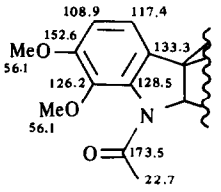

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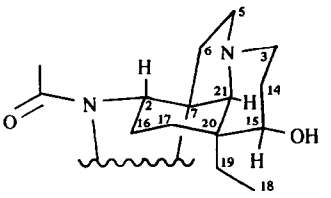

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within the conformational framework **12**. Such conformation accounted for the shielding (i.e. γ shifts) of C-3, C-19 and C-21 on introduction of the hydroxyl group into the piperidine ring. The combined data, and especially the ^{13}C NMR analyses, prove the three new *Aspidosperma* bases to possess the relative configurations depicted in formulas **1b–d**.

EXPERIMENTAL

MPs are uncorr. IR spectra were recorded in KBr pellets and UV spectra in MeOH solns. ^1H NMR spectra of $CDCl_3$ solns with TMS as int. standard ($\delta = 0$ ppm) were measured at 360 MHz. ^{13}C NMR spectra were obtained on a wide-bore, broad-band spectrometer, operating at 50.31 MHz in the Fourier transform mode. The carbon shifts denoted on formulas **6–11** are from $CDCl_3$ solns in ppm downfield from TMS; δ(TMS) $= \delta(CDCl_3) + 76.9$ ppm. The asterisked δ values of formula **9** may be interchanged.

Isolation of anomaline (**1b**). A *M. anomala* (M. Arg.) Mgf. plant (common name: cururu) (INPA-Manaus herbarium no. 78.959) was collected along the bank of the Rio Negro in the vicinity of Manaus. Its ground, dried bark (3.5 kg) was extracted twice for 8 days with 8 l. of EtOH. The extract was concd to one-third its vol. and filtered. Chromatography of the ppt (25 g) showed it to be a mixture of lupeol, lupenone and sitosterols. Vacuum evaporation of the filtrate yielded 165 g (5.5% dry bark) of residue. Chromatography of a portion (10 g) of the latter on neutral Al_2O_3 and elution with C_6H_6 gave more of the steroids and triterpenes, whereas elution with $CHCl_3$ yielded alkaloidal material. Prep. TLC of the latter on silica gel and development with $CHCl_3$–MeOH (9:1) yielded a solid, which on crystallization from EtOAc gave colorless needles of anomaline (**1b**), mp 216–218°. Found (exact mass): *m/z* 386.22053. $C_{22}H_{30}N_2O_4$ requires: *m/z* 386.22053.

Isolation of demethoxyanomaline (**1c**) *and* 12-O-*methylanomaline* (**1d**). Dried, powdered leaves (680 g) were sprayed with conc Na_2CO_3 soln (100 ml), left for 18 hr and then extracted twice for one week at room temp. with 10 l. of Et_2O. The extract was extracted exhaustively with 5% HCl soln. After adjustment of the aq. extract to pH 10 with NH_4OH it was extracted thoroughly with $CHCl_3$. The extract was evaporated and the residue (10.6 g, 1.6% of dry leaves) chromatographed on neutral Al_2O_3. Elution with C_6H_6 gave steroids and triterpenes, while elution with $CHCl_3$ yielded an alkaloid mixture. Prep. TLC of the latter on silica gel and development with $CHCl_3$–MeOH (9:1) led to an alkaloid-rich fraction, which was chromatographed on silica gel. Elution with C_6H_6 removed more non-polar, non-alkaloidal material, while elution with $CHCl_3$–C_6H_6 (4:1), $CHCl_3$ and $CHCl_3$–MeOH (9:1) yielded alkaloid fractions, whose constituents were monitored by ^1H NMR spectroscopy, especially the OMe and OH signals.

Crystallization of the constituents of the early eluates with EtOAc gave colorless needles of demethoxyanomaline (**1c**), mp 263–265°. IR ν(cm^{-1}) 3520 (OH), 1620, 1600, 1580 (C=O). ^1H NMR δ 0.70 (*t*, 3H, J = 7 Hz, Me), 2.40 (*s*, 3H, Ac Me), 2.52, 2.85, 3.14 (*m*, 1 each, CH_2NCH_2), 2.68 (*s*, 1H, H-21), 3.70 (*s*, 1H, OCH), 4.13 (*m*, 1H, H-2). Found (exact mass): *m/z* 356.2100.

$C_{21}H_{28}N_2O_3$ requires: *m/z* 356.2100. Crystallization of the constituents of the middle eluates with EtOAc yielded colorless needles of anomaline (**1b**), mp 216–218° (*see above*), while the later eluates with the same solvent led to the isolation of amorphous 12-*O*-methylanomaline (**1d**). IR ν (cm^{-1}) 3430 (OH), 1630 (*br*, C=O); ^1H NMR δ 0.66 (*t*, 3H, J = 7 Hz, Me), 2.25 (*s*, 3H, acetyl Me), 2.48, 2.82, 3.11 (*m*, 1H each, CH_2NCH_2), 2.59 (*s*, 1H, H-21), 3.67 (*s*, 1H, OCH), 3.80 (*m*, 1H, H-2). Found (exact mass): *m/z* 400.2383. $C_{23}H_{32}N_2O_4$ requires: *m/z* 400.2362.

Acknowledgements—We are indebted to Dr. B. Gilbert for samples of aspidocarpine, cylindrocarpine and refractine and to Dr. B. Monpon for the high-resolution MS. A. I. da R., on a leave of absence from the Universidade do Amazonas, expresses his gratitude to the John Simon Guggenheim Foundation for the award of a fellowship. A. I. da R., B. P. and E. W. are most grateful to the U.S. Public Health Service for partial support of this work.

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