Phytochemistry, Vol. 20, No. 11, pp. 2575–2578, 1981.
Printed in Great Britain.

0031-9422/81/112575-04 $02.00/0

POLYAVOLINAMIDE, AN INDOLOSESQUITERPENE ALKALOID FROM *POLYATHIA SUAVEOLENS*

DOMINIC A. OKORIE

Department of Chemistry, University of Ibadan, Ibadan, Nigeria

(*Revised received* 12 *March* 1981)

Key Word Index—*Polyathia suaveolens*; Annonaceae; alkaloids; indolosesquiterpene; polyavolinamide; triterpene; aporphine alkaloid.

Abstract—A new indolosesquiterpene alkaloid, polyavolinamide, has been identified in the stem of *Polyathia suaveolens* accompanied by the three previously isolated indolosesquiterpene alkaloids, polyavolensin, polyavolensinol, polyavolensinone, as well as an unidentified triterpene and an aporhine alkaloids. Polyavolinamide was also the major component of the root bark. Its structure was assigned on the basis of ^{1}H and ^{13}C NMR, and mass spectrometry.

INTRODUCTION

Recently [1] we reported the isolation of three alkaloids belonging to a new class of indolosesquiterpenes, polyavolensin (**1**), polyavolensinol (**2**) and polyavolensinone (**3**), from the stem and stem bark of the Nigerian species of the medicinal plant *Polyathia suaveolens*. Other workers [2, 3] have isolated some aporhine alkaloids and an indolosesquiterpene alkaloid polyveoline (**4a**) from the trunk bark of this species. In continuation of our investigation of the medicinal plants of Nigeria [1, 4] we have now examined the stem of *P. suaveolens* and have isolated another new indolosesquiterpene alkaloid named polyavolinamide (**5**), unidentified triterpenes and an aporphine alkaloid, whose structure has not been fully elucidated, as well as the three alkaloids (**1**–**3**).

RESULTS AND DISCUSSION

Hot hexane extraction of the stem of *P. suaveolens* followed by chromatography afforded five compounds. The first compound (mp 210–212°, M^+ 379), the second compound (mp 185–187°, M^+ 335) and the third compound (mp 163–165°, M^+ 337) were identified [1] as the previously isolated indolosesquiterpenoids (**1**, **3**, **2**), respectively.

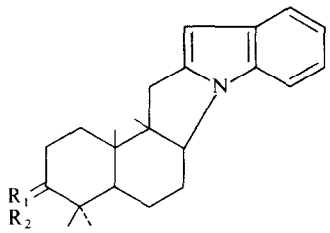
The fourth compound (mp 176–179°) did not contain N and was shown to be a triterpene from the spectroscopic data.

The fifth compound (mp 249–251°), which had an M^+ in the mass spectrum at *m/z* 381, analysed for $C_{25}H_{35}NO_2$ and was named polyavolinamide and assigned the structure **5**. Other significant peaks in the mass spectrum include *m/z* 363 ($M^+ - H_2O$), 348 ($M^+ - H_2O - Me$), 339 ($M^+ - CH_2CO$), 131, 130. The peak at *m/z* 130 was indicative of the presence of an indolic fragment [5]. This was confirmed by the UV spectrum which showed maxima at 217, 255, 285 and 293 nm ($\log\varepsilon = 4.10$, 4.02, 3.42 and 3.34), respectively. These UV absorptions are in fact more characteristic [6, 7] of an *N*-acyl dihydroindole moiety unsubstituted in the benzene ring. The IR spectrum showed bands at ν_{max} 3500 cm^{-1} (OH), 1640 cm^{-1} (CO) of an amide group [8], 1600 cm^{-1} (aromatic) and a strong band at 765 cm^{-1} confirming that the aromatic moiety carried four adjacent protons [8].

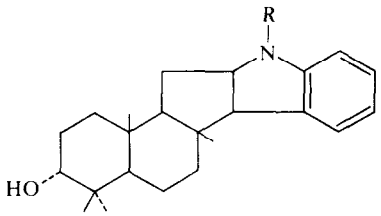
The ^{1}H NMR spectrum of **5** showed four aromatic protons absorbing as a one-proton doublet ($J = 8$ Hz) at δ 8.29 and a three-proton multiplet at δ 6.95–7.40; a one-proton complex quartet at δ 4.58 coupled to a one-proton doublet at δ 3.57 ($J = 11$ Hz); and a one-proton ABX multiplet at δ 3.40 with width at half-height of 8 Hz ($W_{h/2} = 8$ Hz). It also showed the N—COMe as a three proton singlet at δ 2.24 and the four tertiary C—Me groups as singlets at δ 1.40, 1.13, 0.81 and 0.80. With the exception of the absorption at δ 3.40 and those for the four tertiary C—Me groups, all the other absorptions so far mentioned were characteristic [7, 8] of the presence of an *N*-acetyl dihydroindole grouping such as are present in some aspidospermine alkaloids, e.g. deozyaspidospermine (**6**). The down-field absorption at δ 8.29 was therefore assigned to the aromatic proton at C-8[1]. The shift down-field was due to the proximity of the carbonyl group of *N*-acetyl [7–9]. The one-proton complex quartet at δ 4.58 was then assigned to the indoline C-2′ proton [7].

The splitting pattern of the C-2′ proton gave evidence that it must be flanked by CH_2 protons on one side and a proton at C-3′ on the other side. The proton at C-3′ must be responsible for the doublet absorption at δ 3.57. Because of the doublet nature, the C-3′ proton must be flanked by the proton at C-2′ and a tertiary carbon atom. These observations led to the partial structure **9**.

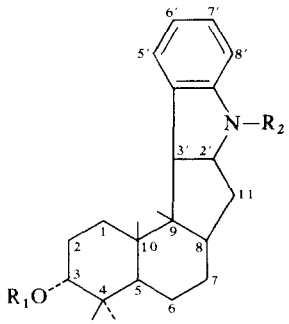
Considering the molecular formula of polyavolinamide $C_{25}H_{35}NO_2$, the rest of the molecule must be sesquiterpenoid. The ABX multiplet at δ 3.40 in the ^{1}H NMR was assigned to the base proton of the —OH group, since on acetylation of **5** to give the acetate **8**, it shifted to δ 4.63 and was still an ABX multiplet ($W_{h/2} = 8$ Hz). The low value of the width at half-height (less than 15 Hz) indicated [10] its equatorial nature, while the splitting pattern suggested it was next to a methylene. Combination of all these facts led to the formulation of **5** as the most probable structure for polyavolinamide.



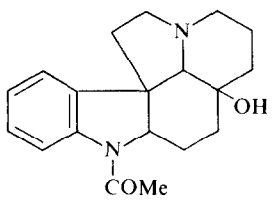
1 R_1 = OAc, R_2 = H
2 R_1 = OH, R_2 = H
3 R_1, R_2 = O



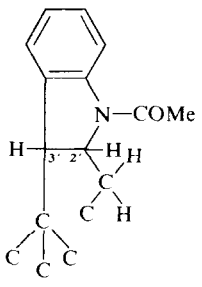
4a R = H
4b R = COMe



5 R_1 = H, R_2 = COMe
7 R_1 = H, R_2 = H
8 R_1 = COMe, R_2 = COMe



6



9

Structure **5** was confirmed by the ^{13}C NMR spectrum which showed six aromatic carbons, two as singlets at δ 144.2, 131.4 and four as doublets at δ 127.9, 124.3, 122.9 and 116.7. These were assigned to C-9′, C-4′, C-6′, C-7′, C-5′, and C-8′, respectively, by comparison with similar systems. The amide CO resonated at δ 169.3 as a singlet while the Me—CO appeared at δ 24.3 as a quartet. These values are similar to those recorded for acetanilide (169.5, *s*; 24.1, *q*). The peaks at δ 63.5 (*d*) and 60.8 (*d*) were assigned to N—CH at C-2′ and the indolinic–benzylic C—H at C-3′, respectively. The other signals in the ^{13}C NMR of **5** which could easily be assigned by comparison of the δ values with those of like sites in polyavolensinol [1] (**1**) and related systems [5, 11] included C-1 (37.3, *t*), C-2 (25.5, *t*), C-3 (76.4, *d*), C-4 (37.6, *s*), C-5 (59.0, *d*), C-6 (19.1, *t*), C-10 (36.7, *s*), 4α-Me (28.9, *q*), 4β-Me (23.3, *q*) 10β-Me (22.1, *q*).

An alternative structure to **5** would be **4b** which is the *N*-acetate of the known compound polyveoline [2, 3], whose structure was recently elucidated [3] by X-ray crystallography. Proof for the non-identity of the two compounds was shown by hydrolysis of **5** which afforded (see later) **7** whose mp, 150–152°, was quite different to that reported for **4a**, 172°. The spectral data for **4a** were, however, essentially similar to that of **7**. Also the hydrochloride of **7** had a mp of 210° while that of polyveoline was 297° [2]. Furthermore, the acetylation product **8** of **5** (see later) had a mp of 246–248° while that of the acetylation product of polyveoline (**4a**) was reported [2] to be 239°. There were no spectral data recorded in the report to enable comparison to be made.

Alkaline hydrolysis of **5** gave **7** whose IR spectrum, had a peak at 3300 cm^{-1} for an —OH and the new —NH groups but lacked any peaks for the CO group. The ^{1}H NMR spectrum of **7** lacked the one-proton doublet at δ 8.29 which was due to the deshielded aromatic proton at C-8′, and the NCOMe peak. Acid hydrolysis of **5** also gave the same *N*-deacetyl polyavolinamide (**7**). Acetylation of **7** gave the *O*,*N*-diacetate identical in all respects with the *O*-acetylpolyavolinamide (**8**) obtained earlier from the acetylation of **5**.

Examination of the stem bark of *P. suaveolens* gave four crystalline compounds, of which three were identified as the three alkaloids (**1**, **2**, **3**), while the fourth was found to be identical with the triterpene of unknown structure from the stem. Similar examination of the root bark of the plant yielded only polyavolensinol (**1**), the unidentified triterpene and the new alkaloid **5**.

EXPERIMENTAL

Mps were determined using a Kofler hot-stage microscope and are uncorr. IR spectra were recorded in Nujol and UV spectra were obtained in MeOH soln. NMR spectra were recorded in $CDCl_3$ as solvent with TMS as int. standard, chemical shifts are expressed in δ units. Si gel refers to Merck Kieselgel 60 (70–230 Mesh ASTM). Petrol refers to the fraction bp 60–80°.

P. suaveolens Engl and Diels was collected at Aponmu Forest Reserve, Ondo State, Nigeria, and identified by the Forestry Research Institute of Nigeria, Ibadan.

Extraction of stem [1]. Dried stems (4 kg) were milled and extracted with boiling hexane. The extract was concd to a gummy solid which was mostly dissolved in C_6H_6 and chromatographed on a column of Si gel eluting with increasing percentages of Et_2O–hexane. Hexane–Et_2O (9:1) eluted **1**, as white flaky crystals, mp 210–212°, $[\alpha]_D^{20}$ −3.8° ($CHCl_3$) (1.6 g). UV λ_{max} (log ε) nm: 238 (3.25), 278 (3.88), 285 (3.89), 295 (3.78). IR ν_{max} cm^{-1}: 1730, 1600, 1450, 1250, 1115, 765, 740, 730. ^1H NMR ($CDCl_3$): δ 6.94–7.63 (4 H, *m*, ArH), 6.18 (1 H, *m*, indole β-H), 4.55 (1 H, *t*, J = 7.5 Hz), 2.80 (1 H, *m*, NCH), 2.05 (3 H, *s*, OAc), 0.93 (6 H, *s*, 2Me), 1.07 (3 H, *s*, Me), 1.18 (3 H, *s*, Me); MS *m/z* 379 (M^+, base peak), 364, 319, 304, 182, 130. (Found: C, 79.16; H, 8.76; N, 3.76; $C_{25}H_{33}NO_2$ requires: C, 79.11; H, 8.76; N, 3.69%.)

Hexane–Et_2O (4:1) fractions furnished **3**, mp 185–187° as white, fluffy crystals (1.9 g). $[\alpha]_D^{20}$ +40.0° ($CHCl_3$). UV λ_{max} (log ε) nm: 237 (3.21), 279 (3.77), 285 (3.78), 294 (3.65); IR ν_{max} cm^{-1}: 1700, 1600, 1450, 1375, 768, 745. ^1H NMR ($CDCl_3$): δ 6.93–7.63 (4 H, *m*, ArH), 6.20 (1 H, *m*, indole β-H), 2.80 (1 H, *m*, NCH), 1.08 (6 H, *s*, 2Me), 1.12 (3 H, *s*, Me), 1.18 (3 H, *s*, Me). MS: *m/z* 335 (M^+), 320, 182, 168, 130 (base peak). (Found: C, 82.37; H, 8.80; N, 4.30; $C_{23}H_{29}NO$ requires: C, 82.34 H, 8.71; N, 4.18%.) Hexane–Et_2O (3:2) eluates afforded **2** as white crystals, mp 163–165°, $[\alpha]_D^{20}$ +6.2° ($CHCl_3$) (3.1 g). IR ν_{max} cm^{-1}: 3450, 1600, 1450, 1375, 1330, 1300, 1020, 768, 740, 730. ^1H NMR ($CDCl_3$): δ 6.93–7.60 (4 H, *m*, ArH), 6.17 (1 H, *m*, indole β-H), 3.2 (1 H, *t*, J = 7.5 Hz), 2.80 (1 H, *m*, NCH), 0.82 (3 H, *s*, Me), 1.0 (6 H, *s*, 2Me), 1.18 (3 H, *s*, Me). MS: *m/z* 337 (M^+), 322, 319, 304, 182, 168, 130 (base peak). (Found: C, 81.73; H, 9.23; N, 4.21; $C_{23}H_{31}NO$ requires: C, 81.85; H; 9.26; N, 4.15%.)

Hexane–Et_2O (1:4) eluted polyavolinamide (**5**) as white crystals (0.24 g), mp 249–251° (from $CHCl_3$–MeOH). UV λ_{max} (log ε) nm: 217 (4.10), 255 (4.02), 285 (3.42) and 293 (3.34). UV (MeOH + NaOH) λ_{max} (log ε) nm: 262 (3.89), 285 (3.48), 293 (3.40). IR ν_{max} cm^{-1}: 3500, 1640, 1600, 1300, 1250, 1175, 1125, 1055, 1030, 990, 910, 765: ^1H NMR δ 8.29 (1 H, *d*, J = 8 Hz, ArH), 6.95–7.4 (3 H, *m*, ArH), 4.58 (1 H, complex *q*, H-2′), 3.57 (1 H, *d*, J = 11 Hz, H-3′), 3.40 (1 H, ABX, $W_{h/2}$, J = 8 Hz, H-3), 2.24 (3 H, *s*, NCOMe), 1.40 (3 H, *s*, Me), 1.13 (3 H, *s*, Me), 0.87 (3 H, *s*, Me), 0.80 (3 H, *s*, Me): MS: *m/z* 381 (M^+), 363, 348, 339, 324, 282, 173, 159, 131, 130, 117 (base peak). (Found: C, 78.84; H, 9.31; N, 3.68; $C_{25}H_{35}NO_2$ requires: C, 78.68; H, 9.25, N, 3.67%.)

The hydrochloride was prepared from conc HCl and MeOH and recrystallized from Et_2O–MeOH to give white crystals, mp 210° (decomp).

Extraction of stem bark. Hot hexane extraction of the dried and milled stem bark (2 kg) afforded a gummy solid (24 g) which was dissolved in C_6H_6 and column chromatographed as described for the stem. Hexane–Et_2O (9:1) eluates gave polyavolensin (**1**) (0.74 g) identical in all respects with authentic sample. Hexane–Et_2O (4:1) afforded polyavolensinone (**3**) (2 g) identical with authentic sample from the stem. Hexane–Et_2O (3:2) yielded polyavolensinol (**2**) (1.3 g) identical with authentic sample. Hexane–Et_2O (1:1) furnished the same triterpene (0.9 g) as that isolated from the stem.

Extraction of root bark. The bark (324 g) was dried, milled and extrd with hot hexane to give a gummy solid (45 g). This was dissolved in C_6H_6 and chromatographed as described for the stem. Hexane–Et_2O (9:1) eluted **1** (0.3 g), identical with an authentic sample. Hexane–Et_2O (1:1) gave the triterpene (0.4 g) which was identical with samples from the stem and stem bark. Hexane–Et_2O (1:4) furnished **5** (1.1 g) identical in all respects with the sample from the stem.

Acetylation of polyavolinamide. **5** (0.054 g) was treated with Ac_2O (3 ml), pyridine (3 ml) and allowed to stand at room temp. for 18 hr. MeOH was added and when cool, the mixture was poured into cold H_2O when polyveolinamide-*O*-acetate (**8**) was precipitated as white crystals (0.05 g). Recrystallization from $CHCl_3$–MeOH gave mp 246–248°. UV λ_{max} (log ε) nm: 222 (3.66), 261 (3.94), 282 (3.76), 290 (3.68). IR ν_{max} cm^{-1}: 1725 (C=O, OAc), 1650 (C=O, N—COMe), 1600 (C_6H_6), 1380, 1280, 1250, 1175, 1130, 1025, 970, 760. ^1H NMR ($CDCl_3$): δ 8.29 (1 H, *d*, J = 8 Hz, ArH) 7.0–7.40 (3 H, *m*, ArH), 4.63. (1 H, ABX, *m*, $W_{h/2}$ = 8 Hz, CH—OAc), 4.58 (1 H, complex *q*), 3.58 (1 H, *d*, J = 11 Hz), 2.27 (3 H, *s*, N—COMe), 2.04 (3 H, *s*, OAc), 1.40 (3 H, *s*, Me), 1.17 (3 H, *s*, Me), 0.83 (3 H, *s*, Me), 0.77 (3 H, *s*, Me). MS: *m/z* 423 (M^+), 381, 366, 363, 348, 316, 299, 261, 248, 193, 176, 131, 130, 117. (Found: C, 76.43; H, 8.80; N, 3.33; $C_{27}H_{37}NO_3$ requires: C, 76:56; H, 8.81; N, 3.31%.)

Alkaline hydrolysis of **5**. **5** (0.15 g) was suspended in MeOH (50 ml) and 50% aq. KOH soln (20 ml) added. The mixture was refluxed at 100° for 6 hr. After cooling, the mixture was diluted with H_2O and extracted with Et_2O (2 × 100 ml). The combined Et_2O fractions were dried ($MgSO_4$) and evapd to give a gummy solid (0.13 g). TLC on Si gel using C_6H_6–EtOAc (3:1) showed that it contained some unhydrolysed starting material. The product was dissolved in C_6H_6 and chromatographed on a column of Si gel, eluting with increasing percentages as hexane–Et_2O.

Hexane–Et_2O (2:3) eluted the hydrolysis product (**7**) (0.04 g), mp 150–152° (from MeOH) UV λ_{max} (log ε) nm: 223 (3.60), 258 (3.75), 307 (3.35). IR ν_{max} cm^{-1}: 3300, 1600 (C_6H_6), 1250, 1060, 980, 970, 940, 740 cm^{-1}. ^1H NMR: ($CDCl_3$) δ 6.4–7.2 (4 H, *m*, ArH), 4.2 (1 H, complex *m*), 3.42 (2 H, *m*, H-3′ and C<u>H</u>—OH), 1.33 (3 H, *s*, Me), 1.13 (3 H, *s*, Me), 0.90 (3 H, *s*, Me), 0.83 (3 H, *s*, Me). (Found: C, 81.31; H, 9.86; N, 4.14; $C_{23}H_{33}NO$ requires: C, 81.36; H, 9.80. N, 4.13%.) Hexane–Et_2O (1:4) gave white crystals (0.07 g), mp 249–251° identical in all respects with the starting amide (**5**).

Acid hydrolysis of **5**. To **5** (0.13 g) suspended in MeOH (20 ml) were added conc. HCl (6 ml) and H_2O (5 ml). After refluxing for 4 hr at 100°, the mixture was cooled, diluted with H_2O and extracted with Et_2O (20 ml). This Et_2O extract was rejected. The aq. soln was then covered with a layer of Et_2O (20 ml) and neutralized with solid $NaHCO_3$. The Et_2O layer was separated and the aq. layer extracted with more Et_2O (2 × 20 ml). The combined Et_2O fractions were dried (Na_2SO_4) and evapd to give an orange-coloured gummy solid. This on purification by prep. TLC on Si gel (Merck Kieselgel $PF_{254+366}$ using C_6H_6–EtOAc (3:1) gave white crystals of **7**, mp 150–152° (from MeOH) (0.066 g). This was identical (mp IR, UV, ^1H NMR, MS) with the product from alkaline hydrolysis of **5**.

Acetylation of hydrolysis product **7**. Acetylation of **7** (0.042 g) with Ac_2O (2 ml) and pyridine (2 ml) at room temp. overnight and work up as was done above for **5**, gave white crystals (0.05 g), mp 246–248°. This was identical (mp, IR, UV, ^1H NMR, MS) with the acetylation product **8** of polyavolinamide.

Acknowledgements—The author thanks Professor David A. H. Taylor, Department of Chemistry, University of Durban, for ^{13}C NMR spectra and very useful discussions, and Mr. George A. Adesida, Department of Chemistry, University of Ibadan, for

plant collection and help in identification. The author is grateful to the International Foundation for Science, Sweden, for a research grant.

REFERENCES

1. Okorie, D. A. (1980) *Tetrahedron* **36,** 2005.
2. Cave, A., Guinaudean, H., Leboeuf, M., Rmahatra, A. and Razafundrazaka, J. (1978) *Planta Med.* **33,** 243.
3. Riche, C., Chiaromi, A., Dubois, G., Hocquimiller, R., Leboeuf, M. and Cave, A. (1980) *Planta Med.* **39,** 206.
4. Okorie, D. A. (1977) *Phytochemistry* **16,** 1591.
5. Leboeuf, M., Hammoniere, M., Cave, A., Gottlieb, H. E., Kunesch, N. and Wenkert, E. (1976) *Tetrahedron Letters* 3559.
6. Ferreira, J. M., Owellen, R. J., Swanholm, C. E., Budzikiewics, H., Durham, L. J. and Djerassi, C. (1962) *Tetrahedron Letters* 59.
7. Ikeda, M. and Djerassi, C. (1968) *Tetrahedron Letters* 5837.
8. Djerassi, C., Archer, A. A. P., George, T., Gilbert, B. Schoolery, J. N. and Johnson, L. F. (1960) *Experientia* **16,** 532.
9. Gilbert, B. (1965) in *The Alkaloids* (Manske, R. H. F., ed.) Vol. 8, Chap. 14. Academic Press, New York.
10. Hassner, A. and Heathcock, C. (1964) *J. Org. Chem.* **29,** 1350.
11. Buckwalter, B. L., Burfitt, I. R., Nagel, A. A., Wenkert, E. and Naf, E. (1975) *Helv. Chim. Acta* **58,** 1567.