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A NEW CLASS OF INDOLOSESQUITERPENE ALKALOIDS FROM *POLYATHIA SUAVEOLENS*

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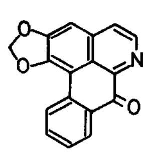
Abstract—Three new alkaloids, polyavolensin, polyavolensinol and polyavolensinone, isolated from the stem of *Polyathia suaveolens* are shown to belong to a new class of indolosesquiterpenes with structures **4**, **5**, **6** respectively by PMR, CMR and mass spectrometry. They have also been inter-related by chemical reactions.

Polyathia suaveolens Engl & Diels (Annonaceae) is a small tree which grows in the tropical forest zone of West Africa.[1] A decoction of the bark is drunk for blackwater fever and for stomach disorders.[2] Earlier work on the chemical examination of the genus *Polyathia* were on *P. nitidissma*[3] which agve an aporhine alkaloid liriodenine (**1**); *P. fragrans*,[4] from which a diterpene acid, polyathic acid (**2**) was isolated; on *P. canangiodes*,[5] in whose flower 'Diacetyl' was found to be present and on *P. oliveri*[6,7] which gave six aporphine alkaloids. Recently, it was reported that further examination of *P. oliveri* afforded a triterpene polycarpol[8] and a structurally novel indolosesquiterpene polyathenol (**3**).[9] In continuation of our investigation of the medicinal plants of Nigeria,[10] we have examined the stem and stembark of *P. suaveolens* which gave three new compounds belonging to a new class of indolosesquiterpene alkaloids. They were named polyavolensin (**4**), polyavolensinol (**5**) and polyavolensinone (**6**).

Freshly collected stem of *P. suaveolens* from Apomu Forest Reserve, Ondo State, Nigeria, were dried and extracted with hot hexane. The resultant gummy solid was chromatographed on a column of silica gel using increasing percentages of diethyl ether in hexane to give three white crystalline compounds all of which gave positive sodium fusion test for nitrogen.

The first compound m.p. 210–212°, M^+ 379, whose



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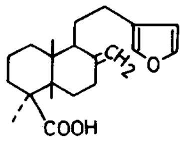

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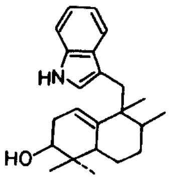

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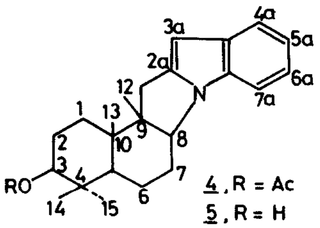
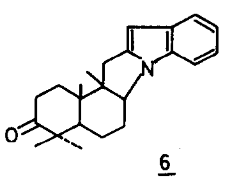


4, R = Ac
5, R = H



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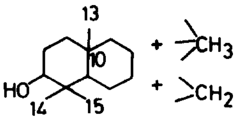

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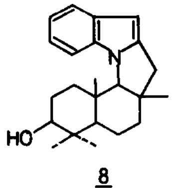

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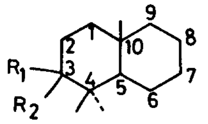
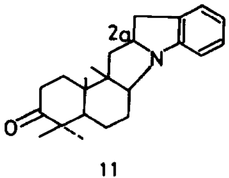


9 $R_1 R_2 = O$
10 $R_1 = \beta$-OH,
$R_2 = \alpha H$



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molecular composition was found to be $C_{25}H_{33}NO_2$ by combustion analysis and high resolution mass spectrometry was named polyavolensin and assigned structure 4. The UV spectrum showed maxima at 238 (log ε 3.25), 278 (log ε 3.88), 285 (log ε 3.89) and 295 nm (log ε, 3.78), which were characteristic of the presence of an indole nucleus. The MS had significant peaks at m/e 379 (M^+), 364 (M^+-CH_3), 319 (M^+-AcOH), 304 (M^+-CH_3-AcOH), 182, 130. The peak at m/e 130 which had almost the same intensity as the M^+ (base peak) confirmed the presence of indolic fragment.[9] The IR spectrum showed no absorptions for -OH or -NH groups but had CO absorption at 1730 cm^{-1} attributeable to an acetate and a peak at 1600 indicative of the presence of benzene (aromatic) ring. The IR spectrum in particular did not have any absorption signal at about 1660 cm^{-1}, which is the characteristic region for C=N bond, thus suggesting the absence of such a bond. The PMR spectrum was unchanged on the addition of D_2O and contained signals for four tertiary C-Me groups at δ 0.93 (6H, s), 1.07 (3H, s), 1.18 (3H, s); an acetate at 2.05 (3H, s), a base proton of an acetate at 4.55 (1H, t, J = 7.5 Hz) an indole *β-H* at 6.18 (1H, m) and four other indolic protons at 6.94–7.63 (4H, m).

The second compound m.p. 185–187°, polyavolensinone was assigned structure 6. The MS contained a molecular ion peak at m/e 335 corresponding to $C_{23}H_{29}NO$. Other strong peaks included m/e 320 (M^+-CH_3), 182 and the indolic fragment at m/e 130 (100%). The IR spectrum of 6 had bands at 1700, indicative of the presence of a saturated 6-membered ring ketone and at 1600 cm^{-1} for a benzene ring. The UV and PMR spectra of 6 were quite similar to those of polyavolensin 4 except that the PMR of the former lacked both the base proton of the acetate peak at δ 4.55 and the acetate peak at 2.05 which were present in the latter. Also the four tertiary C-Me groups showed up at δ 1.08 (6H, s), 1.12 (3H, s) and 1.18 (3H, s).

The third compound polyavolensinol 5 m.p. 163–165° had M^+ in MS spectrum at m/e 337, analysing for $C_{23}H_{31}NO$. Other significant peaks included m/e 322 (M^+-CH_3), 319 (M^+-H_2O), 304 (M^+-CH_3-H_2O), 182, 168, 130 (100%). The IR spectrum had bands for -OH group at 3450 and for benzene ring at 1600, but did not show any absorption due to CO group or C=N bond. The PMR spectrum of 5 was similar to that of 4, but it had a peak at δ 3.2 (1H, t, J = 7.5 Hz) corresponding to the base proton of the OH group instead of the triplet at δ 4.55 (1H) and the singlet at δ 2.05 (3H) belonging to the acetate group present in 4. The OH group in 5 must therefore be secondary. The four tertiary C-Me groups resonated at δ 0.82 (3H, s), 1.0 (6H, s) and 1.18 (3H, s).

The relationship between the three compounds was established as follows. Acetylation of the alcohol 5 with acetic anhydride in pyridine gave polyavolensin 4, identical with the natural product. Also alkaline hydrolysis of 4 with sodium hydroxide in methanol furnished 5, completely identical with natural product. Reduction of the ketone 6 with sodium borohydride in methanol gave 5 identical with the natural product. Polyavolensin (4) is therefore the acetate of polyavolensinol (5) while polyavolensinone (6) is the ketone analogue.

The N atom which had earlier been shown to be part of the indole ring, must be tertiary since no -NH proton could be detected in the IR and PMR spectra. Furthermore the acetylation of 5 gave only the O-acetate. As none of the three compounds 4, 5 and 6 contained any N-Me or N-Et group from PMR or C=N bond from IR spectra, the indolic N atom must also be involved in a second ring. From the PMR spectrum of say 5, only four aromatic proton signals were seen indicating that the benzene ring of the indole was unsubstituted. In addition only one other proton signal appeared at δ 6.17 (m) which was assigned to indolic β-H, for it is known[11,12] that indole β-H in N-Me, 2-Me, and 1,2-disubstituted indoles resonates at around δ 6.13 while the indole α-H in N-Me, 3-Me and 1,3-disubstituted indoles appears at around δ 6.8. Hence the indole moiety in 4, 5 and 6 is 1,2-disubstituted.

Subtraction of C_8H_5N, i.e. the 1,2-disubstituted indole fragment, from the molecular formula $C_{23}H_{31}NO$ of 5 leaves $C_{15}H_{26}O$, which shows 5 to be sesquiterpenoid. The base proton of the -OH group in 5 which appeared as a triplet with J = 7.5 Hz was highly indicative of an axial proton next to a methylene.[13] On comparing the chemical shift changes of the Me groups on going from the -OH to the acetate (5→4) and from the -OH to the ketone (5→6); it was apparent that only one Me group (δ 1.18 throughout) was far removed from the -OH group. Furthermore the chemical shift changes from -OH to acetate for two of the Me groups (0.82, 1.0→ 0.93, 0.93 ppm) were very similar to what was observed for gem-dimethyl groups in 3[9] suggesting identical location. The downfield shift by 0.07 ppm for the transformation -OH to acetate and by 0.12 ppm for -OH to ketone of the third Me group is comparable to that expected[13] if the third Me group were a C-13Me in a partial bicyclic sesquiterpene structure such as 7. The observation that sodium borohydride reduction of the keto group in 6 gave 5, which had been shown to be an equatorial alcohol (see above) was in accordance with the observation in tritepenoid-3-oxo-4, 4-dimethyl systems[13] further confirming the partial structure 7. Combination of the formular $C_{15}H_{26}O$ left after subtraction of 1,2-disubstituted indole moiety with the fact that the fourth Me group is tertiary coupled with biosynthetic considerations led to an expansion of the partial structure 7 to 5 or 8 for polyavolensinol. That 5 was the structure followed from the CMR spectrum of the acetate 4 which had signal at 65.1 ppm appearing as a doublet. In the ketone 6 the doublet resonated at 64.3. This must be the non aromatic N-C carbon and must carry only one H atom i.e. N-CH, because of its doublet nature. In the PMR spectrum this N-CH proton appeared as the X portion of an ABX multiplet at δ 2.8 (1H). The pattern of the splitting showed that this NCH proton must be located at position C-8 where it is flanked by CH_2 protons of C-7 and by no protons on C-9. If it was placed at C-9 where it would be flanked by two quartenary carbons at C-10 and C-8 as in structure 8, it would have resonated as a singlet. Hence structure 5 is the correct structure. The detailed CMR spectra (Experimental and below) of the acetate 4 and ketone 6 confirm the assigned structures.

In the case of 4, the CMR spectrum showed eight aromatic carbons, three as singlets at 142.9, 132.8, 131.9 ppm and five as doublets at 120.7, 120.3, 119.0, 109.6, 94.6 ppm. Six of the carbons are much the same as 1,2-dimethyl-indole,[14] the other two at 131.9(s) and 94.6(d) ppm are more in agreement with the figures recorded[14] for the C-2 and C-3 carbons of a 1,2-dimethylindole which are 138(s), 100.0(d), than for a 2,3-disubstituted.[15] The other signals in the PMR spectrum of 4 which could easily be assigned by comparison of the δ values with those of like carbon sites of **3**,[9] decalone (**9**), decalol (**10**)[16] and related triterpenes included C-1 (38.5, t) C-2 (22.8, t) C-3 (80.7 d), C-4 (37.9, s) C-5 (56.1, d), C-6 (19.9, t), C-10 (36.2, s) 4α-Me (28.1, q), 4β-Me (20.2, q), 10β-Me (16.2, q) and acetyl CO at (170.8, s) ppm. The remaining signals could not be assigned with certainty, but their splitting patterns were in conformity with the assigned structure **4**.

Hydrogenation of **6** gave a mixture of three products which were separated by column chromatography. The first compound was the unreacted ketone **6**. The second product, was found to possess an -OH group (3450 cm^{-1}) but no CO group in the IR spectrum. The MS spectrum showed the M^+ at *m/e* 337. More detailed analysis of the spectra (IR, NMR, mass spec) and the m.p. revealed that it was completely identical with an authentic sample of **5**. The third product from the catalytic hydrogenation was a dihydro product, m.p. 191–3°. MS gave M^+ 337 analysing for $C_{23}H_{31}ON$. The IR spectrum did not show any signals for -NH or -OH, the latter indicating the reduction did not involve mere reduction of the keto group to alcohol. The PMR spectrum showed the disappearance of the indolic β-H at δ 6.20 (1H, m), and the appearance of a new complex one proton multiplet at δ 4.22, which must be the C-2a (H) of an indoline ring. In the PMR spectrum, the four indole hydrogens now absorbed at 6.80–7.27 (4H, m) while the four tertiary Me's resonated separately at 1.13 (3H, s), 1.07 (3H, s), 1.02 (3H, s), 1.02 (3H, s) and 0.92 (3H, s). The only major changes in the CMR spectrum were the disappearance of the doublet at 94.6 which now appeared as a triplet at 41.5 ppm, and the disappearance of the aromatic singlet at 131.8 which now appeared as a doublet at 62.5. These changes were in accordance with the structure **11** for the dihydro compound. It is worth noting that there was no product in which both the keto group and the double bond of the pyrrole ring were reduced.

Treatment of **6** with tin and hydrochloric acid also gave three products. These were the unreacted ketone **6**, dihydropolyavolensinone (**11**), identical in all respects with that from catalytic hydrogenation of **6** and a third compound whose PMR spectrum resembled that of **11**, but the Me region was different. The structure of the product has not been fully elucidated.

It is important to point out that **5**, has the same molecular formular $C_{23}H_{31}NO$ as the previously reported **3**, but differed from it in having no olefinic proton, no–NH and no secondary C–Me group amongst others. The compounds **4**, **5**, **6** represent a new class of indolesesquiterpenes. All the compounds turn light-pink on standing in chloroform.

EXPERIMENTAL

M.ps were determined using a Kofler hot-stage microscope and are uncorrected. IR spectra were in Nujol and measured on a Perkin-Elmer 137 spectrometer. UV spectra were obtained in MeOH solution using a Perkin-Elmer 137 UV spectrometer. NMR spectra were recorded on Varian T-60 and on Varian CFT-20 instruments in $CDCl_3$ as solvent with TMS as internal standard, chemical shifts are expressed in δ units. Mass spectra were determined using a Perkin-Elmer RMU 6E instrument. High resolution mass spec were done at the University of Stracthclyde and microanalysis at the University of Ibadan. Silica gel refers to Merck Kieselgel 60 (70–230 mesh ASTM). Light petroleum refers to the fraction b.p. 60–80°.

Extraction of the stem

Dried stem of *Polyathia suaveolens* (3.75 kg) were milled and extracted with boiling hexane. The extract was concentrated to a gummy solid which was dissolved in C_6H_6 and chromatographed on a column of Silica gel eluting with increasing percentages of Et_2O–hexane. Hexane–Et_2O (9:1) eluted **4** as white flaky crystals m.p. 210–212° (1.2 g). UV: λ_{max} (log ε) 238 (3.25), 278 (3.88), 285 (3.89), 295 (3.78) nm; IR: ν_{max} 1730 (acetate ester), 1600 (Benzene ring), 1450, 1250, 1115, 765, 740 730 cm^{-1}. PMR ($CDCl_3$) δ 6.94–7.63 (4H, m, ArH), 6.18 (1H, m, indole β-H), 4.55 (1H, t, J = 7.5 Hz), 2.80 (1H, m, NCH), 2.05 (3H, s, OAc), 0.93 (6H, s, $2CH_3$) 1.07 (3H, s, CH_3), 1.18 (3H, s, CH_3). CMR: δ 170.8 (*s*), 142.9 (*s*), 132.8 (*s*), 131.9 (*s*), 120.7 (*d*), 120.3 (*d*), 119.0 (*d*), 109.6 (*d*), 94.6 (*d*), 80.7 (*d*), 65.1 (*d*), 63.1 (*s*), 56.1 (*d*), 38.5 (*t*), 37.9 (*s*), 37.7 (*t*), 36.5 (*t*) 36.2 (*s*), 28.1 (*q*), 23.5 (*q*), 22.8 (*t*), 21.5 (*q*), 20.2 (*q*), 19.9 (*t*), 16.2 (*q*); *MS*: *m/e* 379 (M^+, 100%), 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%). (Found *m/e* 379.2531. Required for $C_{25}H_{33}NO_2$: 379.2511).

Hexane–Et_2O (4:1) fractions furnished polyavolensinone **6** m.p. 185–187° as white fluffy crystals (1.7g). UV: λ_{max} (log ε) 237 (3.21), 279 (3.77), 285 (3.78), 294 (3.65) nm; IR: ν_{max} 1700 (C=O, ketone), 1600 (Benzene ring), 1450, 1375, 768, 745 cm^{-1}. PMR: ($CDCl_3$) δ 6.93–7.63 (4H, m, ArH), 6.20 (1H, m, indole β–H), 2.80 (1H, m, NCH), 1.08 (6H, s, $2CH_3$), 1.12 (3H, s, CH_3), 1.18 (3H, s, CH_3). CMR: δ 216.1 (s), 142.5 (s), 132.9 (s), 131.9 (s), 120.8 (d), 120.4 (d), 119.2 (d), 109.6 (d), 94.6 (d), 64.3 (d), 62.8 (s), 55.1 (d), 47.3 (s), 38.1 (t), 37.8 (t), 36.2 (s), 33.7 (t), 26.9 (q), 22.8 (t), 21.2 (t), 20.8 (q), 19.6 (q), 15.4 (q). MS: *m/e* 335 (M^+), 320, 182, 168, 130 (100%). [Found: C, 82.37; H, 8.80; H, 4.30; $C_{23}H_{29}NO$ requires: C, 82.34; H, 8.71; N, 4.18%]. (Found: *m/e* 335.2271. Required for $C_{23}H_{29}NO$: *m/e* 335.2249).

Hexane–Et_2O (3:2) eluates afforded polyavolensinol **5** as white crystals m.p. 163–165° (2.3g). IR: ν_{max} 3450 (–OH), 1600 (Benzene ring), 1450, 1375, 1330, 1300, 1020, 768, 740, 730 cm^{-1}. PMR: ($CDCl_3$) δ 6.93–7.60 (4H, m, ArH), 6.17 (1H m, indole β–H), 3.2 (1H, t, J = 7.5 Hz), 2.80 (1H, m, NCH), 0.82 (3H, s, CH_3), 1.0 (6H, s, $2CH_3$), 1.18 (3H, s, CH_3). MS: *m/e* 337 (M^+), 322, 319, 304, 182, 168, 130 (100%). (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%).

Alkaline hydrolysis of polyavolensin **4**

Polyavolensin (0.16g) was suspended in MeOH (60 ml) and refluxed with 2M NaOH for 4 hr. After cooling, the mixture was poured into water and extracted with Et_2O (2 × 100 ml). Combined Et_2O fractions were dried over Na_2SO_4 and evaporated down to give white crystalline compound (0.12g) which was identical (m.p. IR, UV, PMR, MS) with a sample of natural **5**.

Acetylation of polyavolensinol **5**

Polyavolensinol (0.11g) was treated with Ac_2O (3 ml), pyridine (3 ml) and allowed to stand at RT overnight (18 hr). Work up in the normal manner furnished white crystalline compound (0.09g) which was identical (m.p. IR, UV, PMR, MS) with a sample of natural **4**.

$NaBH_4$ *Reduction of Polyavolensinone* **6**

$NaBH_4$ (0.05g) dissolved in the minimum amount of MeOH was added to a soln of **6** (0.12g) in $CHCl_3$ (3 ml) and MeOH (20 ml). The mixture was stirred at RT for 6 hr, diluted with water and extracted with $CHCl_3$ (2 × 100 ml). Combined $CHCl_3$ fractions was dried with Na_2SO_4 and evaporated down to give an oil which crystallised from Et_2O–hexane (1:1) (0.07g). The product was identical (m.p, IR, UV, PMR and MS) with a sample of natural polyavolensinol.

Catalytic hydrogenation of polyavolensinone **6**

Polyavolensinone (0.50g) dissolved in $CHCl_3$ (5 ml) and MeOH (100 ml) was hydrogenated over Adams catalyst to give an oil. Tlc showed it to be a mixture of three compounds. This was dissolved in a little C_6H_6 and chromatographed on a column of silica gel eluting with mixtures of Light petroleum–Et_2O.

Light petroleum–Et_2O (17:3) gave white crystals (0.2g) m.p. 185–187° identical in all respects with the starting ketone **6**. Light petroleum–Et_2O (4:1) eluates furnished white crystals m.p. 164–165° (0.075g) which was completely identical (IR, UV, PMR, MS) with a sample of the natural **5**. Light petroleum–Et_2O (7:3) eluted the white crystalline **11**. (0.06g) m.p. 191–193°. IR: ν_{max} 1700 (C=O, ketone), 1600 (Bensene ring) 1375, 1260, 1215, 1120, 1060, 920, 840, 765, 735 cm^{-1}. PMR: ($CDCl_3$) δ 6.80–7.27 (4H, m, ArH), 4.22 (1H, m, indoline α-H), 1.13 (3H, s, CH_3). 1.07 (3H, s, CH_3), 1.02 (3H, s, CH_3), 0.92 (3 H, s, CH_3). MS: *m/e* 337 (M^+), 322 (100%), 220, 149. (Found: C, 81.80; H, 9.29; N, 4.30; $C_{23}H_{31}NO$ requires: C, 81.85; H, 9.26; N, 4.15%).

Reduction of polyavolensinone **6** *with* S_n *and* HCl

Polyavolensinone (0.40 g) was suspended in conc HCl (5 ml) and granulated Sn added. After about 14 hr, dissolution took place. Mixture was then heated on steam bath for 3 hr, cooled, neutralised with 2M NaOH, diluted with H_2O and extracted with Et_2O (2 × 100 ml). Combined Et_2O fractions was dried and evaporated down to give an oil. Tlc on EtOAc–C_6H_6 (1:3) showed three main products. Separation was effected by chromatography on a column of silica gel using mixtures of E t_2O–light petroleum. Light petroleum–Et_2O (17:3) gave the starting ketone (0.18g). Light petroleum–Et_2O (4;1) eluted white crystalline compound (0.025g) whose structure has not been fully elucidated. Light petroleum–Et_2O (7:3) eluates gave white crystalline compound (0.07g) m.p. 191–193° completely identical (m.p., IR, UV, PMR, MS), with an anthentic sample of **11** earlier prepared by catalytic hydrogenation.

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