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An alkaloid, two conjugate sesquiterpenes and a phenylpropanoid from *Pachypodanthium confine* Engl. and Diels

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Abstract

Two sesquiterpene-trimethoxystyrene conjugates (*E*)-1-[3′-(4″,8″-dimethylnona-3″,7″-dienyl)cyclohex-3′-enyl]-2,4,5-trimethoxybenzene (**1**) and (*Z*)-1-[3′-(4″,8″-dimethylnona-3″,7″-dienyl)cyclohex-3′-enyl]-2,4,5-trimethoxybenzene (**2**), a phenylpropanoid 1,2,4-trimethoxy-5-(1-methoxy-ethyl)-benzene (**3**), and an aporphine alkaloid *N*-acetylpachypodanthine (**4**), were isolated in addition to several known compounds from cyclohexane, dichloromethane and alkaloid extracts from bark of *Pachypodanthium confine*. The structures of these compounds were established based on the interpretation of their high resolution NMR (HSQC, HMBC, COSY and NOESY) spectral data.

Keywords: *Pachypodanthium confine*; Annonaceae; Phenylpropanoid; Conjugate sesquiterpenes; *N*-Acetylpachypodanthine

1. Introduction

The genus *Pachypodanthium* belongs to the family of Annonaceae, which consists of 120 genera subdivided in 1100 species. According to Le Thomas (1969), *Pachypodanthium confine* belongs to the family of Annonaceae, subfamily of Annonoideae, tribe of Uvarieae. In 1998, Chatrou (IPNI, 2006) assigned this plant to the genus *Duguetia* and was named *Duguetia confinis* (Engl. and Diels) Chatrou. It is a tree reaching 30 m high, growing in wet forests of Cameroon, Gabon, Congo and Angola (Le Thomas, 1969) and is characterized by its leaves presenting many yellow hairs growing on the inferior face. It is used in traditional medicine as an antitussive or analgesic. For these reasons, several papers regarding its phytochemistry have been reported (Cavé et al., 1973; Bévalot et al., 1976b, 1977b). Recently Fekam Boyom et al. published a study of the essential oil composition of *P. confine* and its anti-plasmodial activities (Fekam Boyom et al., 2003). Furthermore, the species *P. staudtii* has been the subject of numerous studies (Bévalot et al., 1976a, 1978; Waterman, 1976; Cavé et al., 1980; Ngadjui et al., 1987, 1989; Agnaniet et al., 2004).

Within the scope of our ongoing program aimed at the systematic chemical studies of African plants with a medicinally interesting profile, we reported our findings on cyclohexane, dichloromethane and alkaloid extracts of the bark of *P. confine*. Three new natural compounds numbered **1**, **3** and **4** were isolated and compound **2** was characterized as a new compound and is an isomer of compound **1** (Fig. 1). Compound **4** was described for the first time in this paper as natural product; Bévalot et al. described it as hemisynthetic compound from pachypodanthine (Bévalot et al.,

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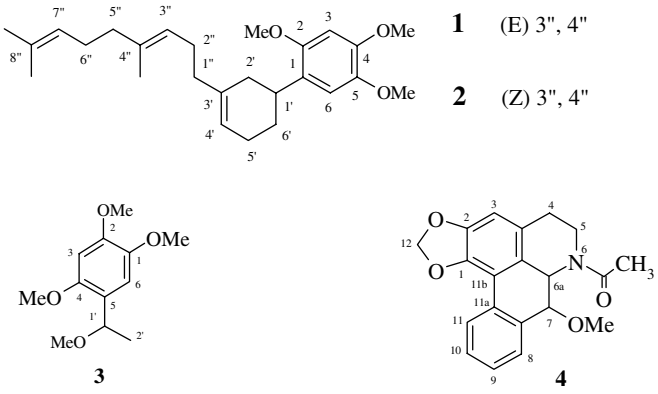

Fig. 1. Structural formulae of compounds **1**–**4**.

1976a). In addition this study led us to isolate three known alkaloids govanine (**8**) (Mehra et al., 1976), discretine (**9**) (Bévalot et al., 1977a) and pachypodanthine (**10**) (Bévalot et al., 1976a; Hamonnière et al., 1977; Jackman et al., 1979), one flavonoid pachypodol (**6**) (Cavé et al., 1973), two lignans, pachypostaudine A (**5**) and pachypostaudine B (**7**) (Ngadjui et al., 1987, 1989) (Fig. 2). In this paper, we report the structural elucidation of four new natural compounds.

2. Results and discussion

Dried and powdered barks of *P. confine* were extracted successively with cyclohexane and dichloromethane. The repeated column chromatography of each extract was followed by GC–MS analyses.

The study of cyclohexane extract led us to isolate compounds **1** and **2** with known pachypostaudine A (**5**) and pachypodol (**6**).

Compound **1**, obtained as yellowish powder, displayed in its EIMS the molecular ion peak [$M^{+\cdot}$] at *m*/*z* 398. The IR spectrum showed strong vibrations at 2850, 1216, 1039 cm^{-1} indicative of the presence of methoxy groups and 1710, 1506 cm^{-1} that confirm the existence of the aromatic benzene ring. The UV spectrum of **1** showed maxima at 227 and 289 nm, characteristic of a benzene chromophore substituted by methoxy groups. More interestingly, the ^{13}C NMR and DEPT experiments of **1** showed 26 resonance lines consisting of six methyl carbons including three methoxy signals at δ 56.20, 56.64 and 56.73 ppm, six methines, three of them were olefinic and appeared at 120.44, 124.30 and 124.35 ppm, seven methylene carbons and seven quaternary carbons, which inverted and disappeared, respectively in the DEPT program (Table 1). The ^1H NMR spectrum of compound **1** displayed resonance of two *para*-coupled aromatic protons at 6.54 and 6.77 ppm. These information permitted us to deduce the molecular formula of compound **1** as $C_{26}H_{38}O_3$. The analysis of these data suggests a terpenic structure containing a chain, three double bonds not in conjugation positions, a cyclohexene ring coupled with a substituted aromatic cycle. Location of these substitutions was finally carried out using an array of 2D NMR experiments.

In particular, the NOESY offers an interesting connection between H_3C-4″ and H_2-1″ at 1.61 and 2.01/2.11 ppm, respectively, as well as between H-3″ and H_a-5″ at 5.14 and 2.06 ppm, respectively. Three sets of consecutive protons, the first set H-2′, H-1′, H-6′, H-5′ and H-4′, the second set H-1″, H-2″ and H-3″ and the third set of consecutive protons, H-5″, H-6″ and H-7″ are also revealed from COSY spectrum.

These assignments were checked by correlations observed in the HSQC, HMBC spectrum. The configuration of all substituents in the structure of isomer (*E*)-1-[3′-(4″,8″-dimethylnona-3″,7″-dienyl)cyclohex-3′-enyl]-2,4,5-trimethoxybenzene (**1**) was based on correlations in the NOESY spectrum.

The analysis by GC–MS of another cyclohexanic fraction showed the presence in an equal proportion of compound **1** (R_t: 29.12 min) and compound **2** (R_t: 30.07 min) with identical mass spectrum. The ^1H and ^{13}C NMR anal-

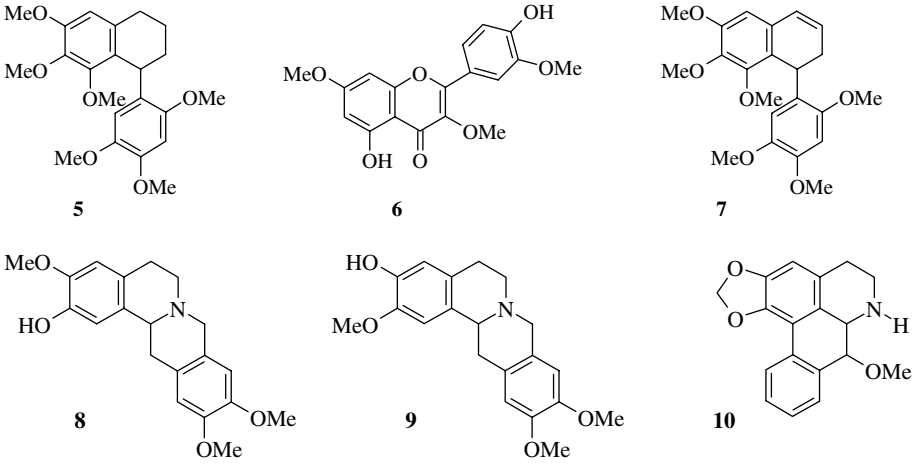

Fig. 2. Structural formulae of compounds **5**–**10**.

Table 1
NMR spectral data of compound **1** ($CDCl_3$, 600 MHz)[a]

Position	^1H	^{13}C
1		127.42
2		151.06
2-OCH_3	3.81 (3H, *s*)	56.64
3	6.54 (1H, *s*)	98.09
4		147.44
4-OCH_3	3.89 (3H, *s*)	56.20
5		143.17
5-OCH_3	3.85 (3H, *s*)	56.73
6	6.77 (1H, *s*)	111.43
1′	3.19 (1H, *m*)	32.80
2′	2.03 (1H, *m*) 2.16 (1H, *m*)	35.65
3′		137.78
4′	5.47 (1H, *m*)	120.44
5′	2.16 (2H, *m*)	25.87
6′	1.69 (1H, *m*)/1.79 (1H, *m*)	28.67
1″	2.01 (1H, *m*)/2.11 (1H, *m*)	37.81
2″	2.01 (1H, *m*)/2.11 (1H, *m*)	26.38
3″	5.14 (1H, *m*)	124.30
4″		134.95
4″-CH_3	1.61 (3H, *s*)	15.97
5″	1.99 (1H, *m*)/2.06 (1H, *m*)	39.71
6″	1.99 (1H, *m*)/2.07 (1H, *m*)	26.74
7″	5.11 (1H, *m*)	124.35
8″		131.25
8″-CH_3	1.61 (3H, *s*)	17.66
9″	1.69 (3H, *s*)	25.66

[a] Chemical shifts (δ) are in ppm.

ysis of this sample also shows a great similarity with the spectra of compound **1** described above. Furthermore, a great difference was observed in NOESY spectrum at the connection between H–C5″ and H-1″, suggesting that compound **2** corresponds to form *Z* of compound **1**.

The study of dichloromethanic extract led us to isolate compounds **3** and **4** with known pachypostaudine B (**7**).

Compound **3** displayed in its EIMS a molecular ion [$M^{+\cdot}$] at *m*/*z* 226, consistent with a molecular formula $C_{12}H_{18}O_4$ (4 degrees of unsaturation). The IR spectrum showed absorption bands centered at 2931 cm^{-1} (methoxy groups) and an aromatic ring at 1610, 1508 cm^{-1}, whereas the UV spectrum of **3** exhibited absorption maxima at 230 and 290 nm characteristic of a benzene chromophore substituted by methoxy groups. The ^1H NMR spectrum revealed the presence of four methoxy groups, two *para*-coupled aromatic protons, one doublet methyl at 1.38 ppm and one quaternary proton at 4.72 ppm. A combination of ^{13}C DEPT, ^{13}C–^1H COSY and HMBC spectroscopic data led to the assignment of ^1H and ^{13}C NMR signals of **3** (Table 2). All available evidence suggested that **3** is an aromatic component presenting an original structure 1,2,4-trimethoxy-5-(1-methoxy-ethyl)-benzene (Fig. 1).

Compound **4** was isolated from the dichloromethane extract of *P. confine* as yellowish powder. IR spectrum of **4** showed bands at 1641 (carbonyl), 1435, 1414 (aromatic nucleus) 1041 and 942 cm^{-1} (methylenedioxy fragment). The EIMS of compound **4** displayed a molecular ion [$M^{+\cdot}$] at *m*/*z* 337 which was confirmed by CIMS. Its ^{13}C

Table 2
NMR spectral data of compound **3** ($CDCl_3$, 600 MHz)[a]

Position	^1H	^{13}C
1		143.54
1-OCH_3	3.86 (3H, *s*)	56.49
2		148.45
2-OCH_3	3.89 (3H, *s*)	56.13
3	6.52 (3H, *s*)	97.52
4		150.86
4-OCH_3	3.81 (3H, *s*)	56.46
5		123.38
6	6.93 (1H, *s*)	109.61
1′	4.72 (1H, *q*, 6.5)	72.71
1′-OCH_3	3.25 (3H, *s*)	56.41
2′	1.38 (3H, *d*, 6.5)	22.67

[a] Chemical shifts (δ) are in ppm, and coupling constants (*J* in Hz) are given in parentheses.

NMR combined with DEPT experiments showed 20 resonance lines consisting of three methylene carbons at 29.72, 36.52 and 101.14 ppm characteristic of methylendioxy group, two methyl carbons including a methoxy signal at δ 61.17 ppm and *N*-acetyl at 22.43 ppm, seven methines carbons, five of them were aromatic and two were deshielded at 59.05 and 80.63 ppm and eight quaternary carbons, one of them at 171.61 ppm was characteristic of a carbonyl group. These data of **4** were consistent with the molecular formula $C_{20}H_{19}NO_4$. The ^1H NMR data revealed the presence of two AB coupled protons at δ 4.77 (1H, *d*, $J = 12$ Hz, H–C6a) and 4.42 (1H, *d*, $J = 12$ Hz, H–C7) and also showed a methylenedioxy group at δ 6.00 (1H, *d*, $J = 1.4$ Hz) and 6.11 (1H, *d*, $J = 1.4$ Hz). In addition, the ^1H NMR spectrum contained four aromatic coupled protons, one singlet aromatic proton at 6.61 ppm, a methoxy group at δ 3.64 (3H, *s*), and a methyl group at δ 2.23 (3H, *s*) suggesting an *N*-acetyl group. One set of consecutive aromatic protons, H-8, H-9, H-10 and H-11, the other sets of consecutive protons, on the one hand H-4 and H-5, and on the other hand H-6a and H-7 were also revealed by using COSY spectrum. In particular, HSQC shows the following connections (i) Ha-12 and Hb-12 at 6.00 and 6.11 ppm and C-12 at 101.14 ppm, (ii) H-6a at 4.77 and C-6a at 59.05 and H-7 at 4.42 and C-7 at 80.63 ppm. The HMBC spectrum confirmed the correlations between H-5 protons and the ketone (δ 171.6), suggesting the location of the carbonyl on *N*-6. Further, the H-4, H-5, H-6a protons showed correlations with C-3a (δ 129.14). Based on the above analysis, the structure of compound **4** was elucidated as *N*-acetylpachypodanthine (**4**) (Fig. 1). The proposed structure is in agreement with the fragmentations observed in EIMS: the loss of CH_3OH rising to the *m*/*z* 305 [$M-32$]$^{+\cdot}$, followed by the loss of CH_2CO leading to *m*/*z* 263 [$M-32-42$]$^{+\cdot}$. This structure was also confirmed by comparison with spectral data of the product stemming from pachypodanthine acetylation with acetic anhydride in pyridine, and also with spectral data of hemisynthetic product previously described by Bévalot et al. (1976a). Additional NMR data are presented in Table 3.

3. Conclusion

The isolation of these new compounds offers an explanation of the biogenesis of metabolites already described. The compound **3** could be biogenetically derived from the oxidation of the 2,4,5-trimethoxystyrene previously described and isolated from different *Pachypodanthium* (Bévalot et al., 1976b; Waterman, 1976). Biogenetically, the new conjugates sesquiterpenes **1** and **2** seems to arise from the condensation of 2,4,5 trimethoxystyrene with a farnesyl cation followed by a cyclization. The skeleton of 2,4,5-trimethoxystyrene is present in the structure of compounds **5** and **7** and may be a chemical marker of the *Pachypodanthium* genus, moreover this compound is the major constituent (70%) of the essential oil of *P. staudtii* (Agnaniet et al., 2004).

In more the discovery of these four new natural compounds, this work led us to show for the first time to our knowledge the presence in *P. confine* of pachypostaudine A, pachypostaudine B, govanine, discretine and pachypodanthine.

4. Experimental

4.1. General experimental procedures

NMR spectra were recorded on a Bruker Avance DMX 600 spectrometer. The assignment of ^{1}H and ^{13}C signals was supported by one- and two-dimensional ^{1}H–^{1}H COSY, DEPT, NOESY, ^{1}H–^{13}C HMBC, and HSQC experiments.

Table 3
NMR spectral data of compound **4** ($CDCl_3$, 600 MHz)[a]

Position	^{1}H	^{13}C	HMBC
1		142.75	
2		147.49	
3	6.61 (1H, *s*)	107.85	C1, C2, C4, C11c
3a		129.14	
4	2.60 (1H, *m*)/2.80 (1H, *m*)	29.72	C3, C3a, C11c, C5
5	2.79 (1H, *m*)/5.03 (1H, *m*)	36.52	C3a, C4, C6a, C7 C=O
N-$\underline{C}OCH_3$		171.61	
N-CO$\underline{CH_3}$	2.23 (3H, *s*)	22.43	
6a	4.77 (1H, *d*, 12.0)	59.05	C3a, C5, C7, C11b, C11c
7	4.42 (1H, *d*, 12.0)	80.63	
7-OCH_3	3.64 (3H, *s*)	61.17	
7a		138.17	
8	7.63 (1H, *m*)	124.38	C11a
9	7.38 (1H, *m*)	128.26	C7a, C11
10	7.40 (1H, *m*)	127.93	C11, C11a
11	8.06 (1H, *m*)	127.15	C11b, C7a, C10
11a		129.15	
11b		116.53	
11c		123.25	
12	6.00 (1H, *d*, 1.4) 6.11 (1H, *d*, 1.4)	101.14	C1, C2

[a] Chemical shifts (δ) are in ppm, and coupling constants (J in Hz) are given in parentheses.

All the experiments were recorded using $CDCl_3$ as solvent. UV spectra (λ_{max} in nm) were recorded in EtOH spectroscopic grade on a Beckman Model DU-600 spectrometer.

For IR spectra a chloroformic solution of each compound was deposited on a KBr plate leaving a slight film, spectra were obtained with Perkin–Elmer FT-IR Spectrometer Paragon 500.

Optical rotation was measured on a Perkin–Elmer 341 polarimeter.

Analytical TLC was realized on aluminium sheets (Merck, silica gel 60 F_{254}). Column chromatographies were carried out with silica gel 20–45 μm. Flash column chromatographies were conducted using silica gel 60 Merck (35–70 μm) with an overpressure of 300 mbar.

Mass spectra were recorded with a Hewlett Packard 5973 spectrometer, using electron impact (EIMS) and chemical ionization (CIMS; reagent gas: NH_3 or CH_4) techniques.

GC–MS analyses were performed with an Agilent 6890 gas chromatograph directly interfaced with the mass spectrometer described below. GC analyses were performed using a (5% phenyl 95% methyl polysiloxane) Optima 5 column (30 m × 0.25 mm internal diameter × 0.25 μm film thickness) from Macherey Nagel. Oven chromatographic conditions were the following: the temperature was maintained at 60 °C during 2 min after injection, and increased at a rate of 10 °C/min–280 °C. The final temperature was maintained during 25 min. Split ratio was 1:12. Injector and interface were maintained at 280 °C. Mass spectrometric conditions were the following: ion source 230 °C, quadrupole 150 °C, scan: 35–700 unit mass atomic.

4.2. Plant material

Specimen was collected in Libreville Estuary (Gabon), in August 2003. A voucher specimen (#1894) was deposited in the National Herbarium of Gabon (Libreville).

4.3. Extraction and isolation

4.3.1. Classical extraction

The dried barks (968 g) of *P. confine* were extracted successively with cyclohexane (3 × 3 l) and dichloromethane (4 × 3 l). The extracts of same solvent were pooled and concentrated under vacuum, to give dark residues of cyclohexane (5.87 g) and dichloromethane (17.14 g).

The 5.87 g of cyclohexanic extracts were chromatographied on column silica gel using a gradient of cyclohexane/CH_2Cl_2 followed by a gradient of CH_2Cl_2/MeOH as mobile phase. This separation led to 25 fractions (**A–Y**). Fraction **K** was purified on silica gel with an eluant phase consisting of a mixture of cyclohexane/EtOAc (80/20) and led to the isolation of compounds **1** and **2**. The fraction **P** was purified on silica gel using cyclohexane/EtOAc (65/35) as mobile phase and led to the isolation of 28.5 mg of compound **5** and 12.4 mg of compound **6**.

The 17.14 g of dichloromethane extract were fractionated successively on silica gel using cyclohexane/EtOAc

mixtures with increasing polarity: (50/50) for 51 fractions and (25/75) for 24 fractions. The end of the elution was realized with a mixture of EtOAc/MeOH (99/1). Based on these procedures, 108 fractions were obtained. The fraction 12 was purified by chromatography with a mixture of cyclohexane/EtOAc (50/50) as eluent and yielded 40.8 mg of compound **7**.

Fraction 25 was chromatographied on column silica gel using a mixture of cyclohexane/acetone/EtOAc (60/30/10) and yielded 13 mg of compound **3**.

Finally, fractions 35 and 36 were pooled, and then chromatographied with a mixture of CH_2Cl_2/EtOAc (80/20) and yielded 3.2 mg of compound **4**.

*4.3.1.1. (E)-1-[3′-(4″,8″-Dimethylnona-3″,7″-dienyl)cyclohex-3′-enyl]-2,4,5-trimethoxybenzene (**1**) and (Z)-1-[3′-(4″,8″-dimethylnona-3″,7″-dienyl)cyclohex-3′-enyl]-2,4,5-trimethoxybenzene (**2**).* Product **1** was isolated as yellowish powder; TLC: R_f 0.54 (EtOAc/cyclohexane (20/80)) reagent: sulfuric vanillin; $[\alpha]_D^{20} + 5°$ (EtOH, *c* 0.6); UV λ_{max}^{MeOH} nm (log ε): 227 (3.94), 289 (3.68); IR ν_{max}^{KBr} (cm^{-1}): 2917, 2850, 1710, 1506, 1216 and 1039; EIMS 70 eV, *m/z* (rel. int.): 399(13), 398[$M^{+\cdot}$](47), 195(14), 194(100), 179(17), 151(9), 69(10); CIMS (CH_4), 235 eV, *m/z* (rel. int.): 378[$M+C_3H_5$]$^+$(6), 366[$M+C_2H_5$]$^+$(22), 338[M+H]$^+$(88), 306(100); (Found: C, 78.37; H, 9.68. $C_{26}H_{38}O_3$ requires: C, 78.46; H, 9.62%). Retention time in GC–MS (chromatographic conditions described in Section 4.1): 29.12 min.

Compound **2**. TLC: R_f 0.54 (EtOAc/cyclohexane (20/80)) reagent: sulfuric vanillin; EIMS 70 eV, *m/z* (rel. int.): 399(13), 398[$M^{+\cdot}$](47), 195(14), 194(100), 179(17), 151(9), 69(10); CIMS (CH_4), 235 eV, *m/z* (rel. int.): 378[$M+C_3H_5$]$^+$(6), 366[$M+C_2H_5$]$^+$(22), 338[M+H]$^+$(88), 306(100). Retention time in GC–MS (chromatographic conditions described in Section 4.1): 30.07 min.

*4.3.1.2. 1,2,4-Trimethoxy-5-(1-methoxy-ethyl)-benzene (**3**).* Yellowish powder; TLC: R_f 0.71 (cyclohexane/acetone/EtOAc (60/30/10)) reagent: sulfuric vanillin; $[\alpha]_D^{20} - 17°$ (EtOH, *c* 1.12); UV λ_{max}^{MeOH} nm (log ε): 230(3.92), 290(3.626); IR ν_{max}^{KBr} (cm^{-1}): 2932, 1610, 1508, 1398 and 1204; EIMS 70 eV, *m/z* (rel. int.): 226[$M^{+\cdot}$](38), 212(14), 211(100), 196(12), 195(48), 181(14), 180(10), 151(10). (Found: C, 63.83; H, 8.11. $C_{12}H_{18}O_4$ requires: C, 63.77; H, 8.03%.)

*4.3.1.3. N-Acetylpachypodanthine (**4**). N-Acetylpachypodanthine isolated from bark of P. confine.* Yellowish powder; TLC: R_f 0.35 (EtOAc/CH_2Cl_2 (20/80)), reagent: sulfuric vanillin; $[\alpha]_D^{20} - 64.3°$ (EtOH, *c* 0.28); UV λ_{max}^{MeOH} nm (log ε): 277 (3.92); IR ν_{max}^{KBr} (cm^{-1}): 2918, 2849, 1734, 1641, 1435, 1414, 1230, 1210, 1127, 1041 and 942; EIMS 70 eV, *m/z* (rel. int.): 337[$M^{+\cdot}$](1), 306(21), 305(100), 280(22), 265(17), 264(21), 263(87), 262(11) 251(24), 176(8), 165(15); CIMS (CH_4), 235 eV, *m/z* (rel. int.): 378[$M+C_3H_5$]$^+$(6), 366[$M+C_2H_5$]$^+$(22), 338[M+H]$^+$(88), 306(100). (Found: C, 71.49; H, 5.72; N, 4.26. $C_{20}H_{19}NO_4$ requires: C, 71.28; H, 5.68; N, 4.15%.)

Hemisynthetic N-acetylpachypodanthine. The acetylation of 10 mg (0.034 mmol) of pachypodanthine with acetic anhydride 5.32 M in pyridine resulted in *N*-acetylpachypodanthine. Spectral data are similar to those observed for the compound **4** extracted from bark of *P. confine*. (Found: C, 71.37; H, 5.63; N, 4.11. $C_{20}H_{19}NO_4$ requires: C, 71.28; H, 5.68; N, 4.15%.)

*4.3.1.4. Pachypostaudine A (**5**).* 28.5 mg; TLC: R_f 0.33 (EtOAc/cyclohexane (35/65)), reagent: sulfuric vanillin; ^1H NMR, ^{13}C NMR and MS are in agreement with published data (Ngadjui et al., 1987, 1989).

*4.3.1.5. Pachypodol (**6**).* 12.4 mg; TLC: R_f 0.55 (EtOAc/cyclohexane (50/50)), reagent: sulfuric vanillin; ^1H NMR and ^{13}C NMR are in agreement with published data (Cavé et al., 1973; Sy and Brown, 1998).

*4.3.1.6. Pachypostaudine B (**7**).* 40.8 mg; TLC: R_f 0.67 (EtOAc/cyclohexane (50/50)), reagent: sulfuric vanillin; ^1H NMR, ^{13}C NMR and MS are in agreement with published data (Ngadjui et al., 1987, 1989).

4.3.2. Alkaloidic extraction

One kilogram of powdered bark of *P. confine* was extracted at room temperature with CH_2Cl_2/NH_4OH. The extract was subjected to an alkaloidic extraction procedure and produced a total of 2.02 g (yield 0.2% dry wt.). The extract was chromatographied over silica gel and eluted with a mixture of CH_2Cl_2/MeOH/NH_4OH (97/3/10). Two hundred fractions were obtained and analyzed by TLC. Similar fractions were pooled. Fractions (55–64) was purified by CC (mobile phase: EtOAc/MeOH (85/15)) and yielded 26 mg of compound **8**. Fraction (39–45) was purified on silica gel (mobile phase: EtOAc/MeOH (85/15)) and led us to isolate 29 mg of compound **9**. Fractions (24–29) was chromatographied and eluted with EtOAc/MeOH (85/15) and led to the isolation of 52 mg of compound **10**.

*4.3.2.1. Govanine (**8**).* 26 mg; TLC: R_f 0.57 (EtOAc/MeOH (85/15)), reagent: sulfuric vanillin, spectral data agree with those reported in the literature (Mehra et al., 1976).

*4.3.2.2. Discretine (**9**).* 29 mg; TLC: R_f 0.67 (EtOAc/MeOH (85/15)), reagent: sulfuric vanillin; spectral data agree with those reported in the literature (Bévalot et al., 1977a).

*4.3.2.3. Pachypodanthine (**10**).* 52 mg; TLC: R_f 0.32 (EtOAc/MeOH (85/15)), reagent: sulfuric vanillin; ^1H NMR, ^{13}C NMR, MS are in agreement with published data (Bévalot et al., 1976a; Hamonnière et al., 1977; Jackman et al., 1979).

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