Chromone and Phenanthrene Alkaloids from *Dennettia tripetala*

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Dennettine, a new 2,6-dimethoxychromone and three known phenanthrene alkaloids (uvariopsine, stephenanthrine and argentinine) in addition to the phenolic and known compound vanillin were isolated from the roots of *Dennettia tripetala*. Their structures were determined by physical and spectroscopical one dimensional (1D) and 2D-NMR analysis, including heteronuclear multiple bond correlation and nuclear Overhauser enhancement spectroscopy.

Key words Annonaceae; *Dennettia tripetala*; 2,6-dimethoxychromone; phenanthrene alkaloid

Dennettia tripetala Baker F. (Annonaceae) is widely distributed in Africa. Its ethanolic extract has been used traditionally in Nigeria to combat the growth of *Ostrinia nubilaris* that affects significantly corn, cotton as well as other vegetable crops.[1)] Previous investigations have shown that the seed powder and the essential oil of *Dennettia tripetala* were effective to protect of cowpea and maize grains against infection by *Callosobruchus maculatus* and *Sitophilus zeamais*.[2)] *Dennettia* like other Annonaceae genus is an important source of isoquinoline alkaloids, such as benzylisoquinoline, aporphine which are widely distributed in the plant kingdom and more rarely phenanthrene alkaloids. The phenanthrene alkaloids are a small group of optically inactive bases, with pharmacological and chemotaxonomic relevance, derived from aporphine alkaloids by Hofmann reaction. Some benzylisoquinoline alkaloids isolated from plants have shown to behave as α-adrenoceptor blockers either in vascular or other smooth muscles. Inhibition of platelet aggregation by this alkaloids has been also observed. Interestingly, among the tested compounds the effect exerted by natural and synthetic phenanthrene alkaloids has been shown to be more powerful than that elicited by benzylisoquinoline and aporphine alkaloids.[3,4)] On the other hand, among the different phytochemical components present in the roots of *Dennettia tripetala* we have found a chromone, which is unusual in the Annonaceae family characterized mainly by the presence of alkaloids, terpenoids, styryl-lactones, acetogenins, essential oils and flavonoids, so the isolation of this phenolic compound has chemotaxonomic value.

Experimental

General Experimental Procedures Melting points were determined on a Fisher–John apparatus and are uncorrected. IR spectra (film) were run on a Perkin-Elmer 175 spectrometer. UV spectra were taken on a Shimadzu 2101UV/Vis spectrophotometer in MeOH solutions. MS measurement were performed using a VG Auto Spec Fisons spectrometer. ^{1}H- (300 or 400 MHz) and ^{13}C-NMR (100 MHz) spectra were recorded on a Bruker 300 or a Varian Unity-400 instrument, using the solvent signal as reference ($CDCl_3$ at δ 7.26 and δ 77.0). Multiplicities of ^{13}C-NMR resonances were determined by distortionless enhancement by polarization transfer (DEPT) experiments. Silica (Merck 7736) was used for column chromatography.

Plant Material The roots of *Dennettia tripetala* Baker F. (Annonaceae) were collected in 1998 in Calabar, Nigeria and identified by Dr. E. Anam of the University of Calabar, Calabar, Nigeria where a voucher specimen was deposited.

Extraction and Isolation Fresh roots of *D. tripetala* were extracted repeatedly with MeOH at room temperature. The MeOH extract was concentrated and precipitated with diethylether. The concentrated MeOH extract (5.2 g) was chromatographed over a Silica column (Merck 7736) (175 g) eluted with dichloromethane and methanol step gradient and collected 20 ml in each fraction as follows: fractions 1—3 (CH_2Cl_2/MeOH 9 : 1), 7—9 (CH_2Cl_2/MeOH 8 : 2). Fractions 7—9 were combined and rechromatographed on a Silica column eluted with dichloromethane–methanol mixtures. Fractions eluted with CH_2Cl_2/MeOH (9.9 : 0.1) gave compound **2** (12 mg), fractions eluted with CH_2Cl_2/MeOH (9.8 : 0.2) afforded compound **3** (20 mg) and fractions eluted with CH_2Cl_2/MeOH (9.7 : 0.3) gave compound **4** (3 mg). Fractions 1—3 were rechromatographed on a Silica columnn eluted with hexane, dichloromethane and methanol to afford compounds **5** (19 mg) eluted with CH_2Cl_2 and compound **1** (15 mg) eluted with CH_2Cl_2/MeOH (9.5 : 0.5).

Dennettine (**1**): $C_{11}H_{10}O_4$, white powder, UV (MeOH) λ_{max} (log ε) 285 (1.22), 262 (2.05), 216 (2.94) nm; IR (film) ν_{max} 2922, 2851, 1679 (γ-pyrone), 1597, 1523, 1469, 1335, 1206, 1032, 880 cm^{-1}; fast atom bombardment (FAB) *m/z* $[M+1]^+$ 207. NMR data in Table 1.

Uvariopsine (**2**): $C_{20}H_{21}O_3N$, white crystals, mp 71—72 °C (CH_2Cl_2/hexane); UV (MeOH) λ_{max} (log ε) 216 (3.09), 236 (5.00), 258 (5.00), 284 (2.25), 322 (1.44), 352 (0.43), 369 (0.38) nm; IR (film) ν_{max} 1951, 1537, 1367, 1356, 919, 890 cm^{-1}; electron impact (EI)-MS (70 eV) *m/z* (rel. int. %) 323 ([M$^+$], 2), 294 (2), 293 (10), 278 (1), 265 (2), 235 (3), 198 (9), 189 (3), 178 (2), 176 (4) 58 (100); high resolution (HR)-EI-MS *m/z* [M$^+$] 323.152119 (Cald for $C_{20}H_{21}O_3N$, 323.152144). NMR data in Table 2.

Stephenanthrine (**3**): $C_{19}H_{19}O_2N$, white needles, mp 92—93 °C (CH_2Cl_2/hexane); UV (MeOH) λ_{max} (log ε) 213 (0.81), 239 (1.07), 249 (1.31), 284 (0.43), 320 (0.25), 351 (0.04), 369 (0.03) nm; IR (film) ν_{max} 2923, 2853, 1597, 1390, 1282, 814 cm^{-1}; EI-MS (70 eV) *m/z* (rel. int. %) 293 ([M$^+$], 17), 235 (9), 189 (6), 176 (11), 58 (100); FAB-MS *m/z* $[M+1]^+$ 294. NMR data in Table 2.

Argentinine (**4**): $C_{19}H_{21}O_2N$, amorphous, IR (film) ν_{max} 3367, 2922, 2852, 2359, 2340, 1730, 1463, 1288, 1120 cm^{-1}; FAB-MS *m/z* $[M+1]^+$ 296. NMR data in Table 2.

Table 1. ^{1}H-, ^{13}C- and HMBC NMR Data for Compound **1** ($CDCl_3$, 400 MHz)

Position	**1**		
	δ_C	δ_H	HMBC
2	146.6		H-3
3	107.2	7.39, s	
4	171.2		H-3,5
4a	121.2		H-8
5	112.1	7.59, d (1.8)	H-7
6	146.2		H-8
7	125.2	7.73, dd (8.4, 1.8)	H-5
8	114.2	6.97, d (8.4)	
8a	150.8		H-5,7
2,6-OCH_3	56.4, 56.1	3.95, s (3H) 3.96, s (3H)	H-2,6

Multiplicities were obtained from DEPT experiments. δ ppm, *J* Hz.

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Table 2. ^{1}H- and ^{13}C-NMR Data for Compounds **2**, **3** and **4** ($CDCl_3$, 400 MHz)

Position	**2**		**3**		**4**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
1	131.9		131.9		131.9	
2	110.7	7.14 s	110.1	7.16 s	117.8	7.28 s
3	144.9		145.0		150.7	
4	142.6		142.3		147.8	
4a	117.1		117.1		124.1	
5	127.2	9.08 br d (9.2)	127.3	9.08 br d (9.6)	127.2	9.42 br d (9.2)
5a	128.8		128.6		128.9	
6	127.6	7.81 br d (9.2)	127.3	7.83 br d (9.6)	128.3	7.86 br d (9.2)
7	146.4		126.8	7.58—7.61 m	126.9	7.61 t d (8.4, 1.6)
8	106.5	7.44 s	126.3	7.58—7.61 m	126.2	7.53 br d (8.8)
8a	137.8		131.3		131.4	
9	125.5	7.58 d (9.2)	125.5	7.62 br d (9.6)	125.6	7.68 d (9.2)
10	122.3	7.85 d (9.2)	122.4	7.87 d (9.6)	121.8	7.91 d (9.2)
10a	125.9		125.9		125.2	
$CH_2\alpha$	59.5	2.92 ddd (2H) (16.8, 11.4, 5.4)	60.2	2.85 ddd (2H) (16.8, 11.2, 5,6)	58.7	3.21 ddd (2H) (16.8, 11.6, 5.2)
$CH_2\beta$	30.2	3.39 ddd (2H) (16.8, 11.4, 5.4)	30.9	3.38 ddd (2H) (16.8, 11.2, 5.6)	29.7	3.59 ddd (2H) (16.8, 11.6, 5.2)
$N(CH_3)_2$	43.8	2.61 s (6H)	44.5	2.56 s (6H)	43.3	2.84 s (6H)
OCH_3	56.2	3.93 s (3H)			60.1	3.84 s (3H)
OCH_2O	101.1	6.21 s (2H)	101.1	6.21 s (2H)		

Multiplicities were obtained from DEPT experiments. δ ppm, J Hz.

Vanillin (**5**): $C_8H_8O_3$, white crystals, mp 80—81 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 9.85 (1H, s, HCO), 7.35 (1H, dd, J=8.4, 2.0 Hz, H-6), 7.20 (1H, d, J=2.0 Hz, H-2),7.01 (1H, d, J=8.4 Hz, H-5), 3.90 (3H, s, OMe-3); ^{13}C-NMR ($CDCl_3$, 100 MHz) δ: 191.3 (HCO-1), 152.0 (C-4), 147.5 (C-3), 130.3 (C-1), 128.0 (C-6), 114.7 (C-5), 109.1 (C-2), 56.5 (OMe-3).

Results and Discussion

Separation of the compounds from the methanolic extract of the roots of *D. tripetala* by chromatographic techniques resulted in the isolation of the new chromone dennettine (**1**), three phenanthrene alkaloids identified as uvariopsine (**2**), stephenanthrine (**3**) and argentinine (**4**) and a simple phenolic compound vanillin (**5**). The structures were determined using one dimensional (1D) and 2D ^{1}H- and ^{13}C-NMR experiments in conjunction with the analysis of mass spectral and other spectroscopic data.

Compound **1** obtained as a white powder, showed UV absorption maxima at 285, 262, 216 nm in MeOH. Its IR spectrum gave absorption bands for aromatic ring (2922, 2851 cm^{-1}) and another band at 1679 cm^{-1} characteristic of a γ-pyrone ring.[5] The FAB-MS with a peak at *m/z* 207 [M^++1] showed the molecular weight.

The structure of **1** was mainly deduced from the ^{1}H-NMR spectrum which revealed four aromatic protons, three of them δ 7.59 (d, J=1.8 Hz), δ 7.73 (dd, J=8.4, 1.8 Hz) and δ 6.97 (d, J=8.4 Hz) indicating a trisubstituted benzene. The coupling constant pattern of the corresponding proton signals suggested that benzene substitution had to be of the 1, 2, 4 type. In addition, singlets at δ 3.95 and δ 3.96 due to two methoxy groups and the proton H-3 at δ 7.39 (s) of the pyrone ring, were observed.

The presence of a trisubstituted benzene ring was corroborated by which exhibited in the aromatic region three methine group (δ 112.1, 114.2, 125.2) and signals at δ 150.8, δ 146.2 and δ 121.2 corresponding to quaternary carbons (Table 1). Other characteristic signals up to a total of eleven were at δ 171.2 and δ 107.2 consistent respectively with the carbonyl and the methine attached to carbonyl group in the chromone skeleton. Two signals at δ 56.1 and δ 56.4 confirmed the existence of the two methoxy groups.

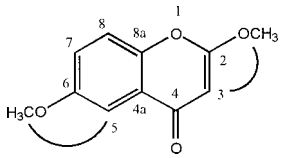


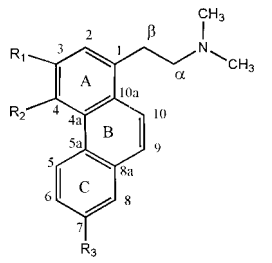
Fig. 1. The NOESY Correlations of **1**

Based on the analyses of ^{13}C-NMR (BB and DEPT), ^{1}H-detected heteronuclear multiple quantum coherence (HMQC) and heteronuclear multiple bond connectivity (HMBC) spectra, compound **1** was identified as a 2,6-dimethoxychromone, named dennettine. Nuclear Overhauser effect spectroscopy (NOESY) experiments (Fig. 1) corroborated the location of the two methoxyl groups since the irradiation of H-5 (δ 7.59) created a NOE of the methoxyl group at δ 3.96 and the irradiation of the two methoxyl groups resulted in a NOE of the signal at δ 7.59 (H-5) and δ 7.39 (H-3).

This is the first time that 2,6-dimethoxychromone has been isolated from a natural source. On the other hand it may be of chemotaxonomical value since *Dennettia* is to date the only Annonaceae genus in which 2,6-dimethoxychromone has been encountered.

Compound **2** was obtained as white crystals, positive to the Dragendorff's reactive. HR-EI-MS revealed a $[M]^+$ ion at *m/z* 323.152119, corresponding to the molecular formula $C_{20}H_{21}O_3N$. The fragments at *m/z* 293 $[M-(CH_3)_2]^+$, 278 $[M-CH_3NHCH_3]^+$, 265 $[M-CH_2-N(CH_3)_2]^+$ and the base peak at *m/z* 58 $[CH_2-N(CH_3)_2+H]^+$ demonstrated the characteristic cleavage of the (dimethylamino)-dimethylene side chain in the phenanthrene type alkaloids.[6]

The UV spectrum contained absorption bands typical of the phenanthrene skeleton,[7] and the IR spectrum of **2** exhibited absorption bands at ν_{max} 1367 and 919 cm^{-1} indicating



2 $R_1 + R_2 = OCH_2O$, $R_3 = OCH_3$
3 $R_1 + R_2 = OCH_2O$, $R_3 = H$
4 $R_1 = OH$, $R_2 = OCH_3$, $R_3 = H$

Fig. 2. Phenanthrene Alkaloids from *Dennettia tripetala* Root

methyl and methylenedioxy groups, respectively.

The ^{1}H-NMR spectrum of **2** indicated the presence of singlets at δ 2.61 (6H) and δ 3.93 (3H), which corresponded to two *N*-methyl groups and one methoxy group. In addition, a singlet at δ 6.21 (2H) was due to methylenedioxy protons. In the aromatic region, two singlets at δ 7.14 (1H, s) and δ 7.44 (1H, s), and two coupled protons at δ 7.58 (1H, d, J=9.2 Hz) and 7.85 (1H, d, J=9.2 Hz), characteristic of an AB system corresponding to H-9 and H-10 in the phenanthrene ring system, were detected (Fig. 2). The aromatic proton at δ 9.08 (1H, br d, J=9.2 Hz) corresponded to the H-5 in the phenanthrene alkaloid. This signal was consistent with the presence of a methylenedioxy group at C3—4 that resulted in an upfield shift of the H-5 proton signal to $\approx\delta$ 9.0, since phenanthrene alkaloids with methoxy and/or hydroxyl substituents at C3—4 show H-5 signals downfield between δ 9.3 and 9.9.[8)]

Finally, an AA′BB′ system at δ 3.39 and 2.92 was observed. This signal and not two quadruplets or multiplets that have been previously described for this compound[9,10)] was due to the 4 aliphatic protons. The AA′BB′ system resonated as a complex signal ought to the preferred anti conformation along the corresponding carbons bond.[11)]

In the HMQC spectrum, the protons resonanting at δ 2.92 showed a heteronuclear connectivity with the signal at δ 59.5 while the protons at δ 3.39 showed HMQC with the carbon at δ 30.2. The homonuclear correlation (COSY 45) beetwen δ 9.08 (H-5) and δ 7.81 (H-6) aided in establishing the location of the methoxy group in C-7 and the methylenedioxy group in C3—4 on the phenanthrene skeleton.

These results suggested that the compound **2** is a phenanthrene alkaloid named uvariopsine isolated from *Uvariopsis solheidii*,[9)] *U. guineensis*[10)] and *U. congolana*.[12)] No ^{13}C-NMR data have yet been published and not all the protons have been assigned up to now for this compound.

Comparison of the UV, IR, ^{1}H- and ^{13}C-NMR data of **3** and **4** with **2** suggested that the two alkaloids had the same phenanthrene skeleton, without methoxy group on C ring (compound **3**) and also with different functional group on A ring (compound **4**). Compound **3** is a known phenanthrene alkaloid named stephenanthrine previously isolated from *Stephania tetrandra* (Menispermaceae)[13)] and from *Monocyclanthus vignei* (Annonaceae)[14)] and the compound **4** is argentinine that has been isolated for the first time in *Aristolochia argentina*[15)] of the Aristolochiaceae family and more recently in the genus *Annona*,[16)] *Enantia*,[17)] *Guatteria*,[18,19)] *Monocyclanthus*,[14)] *Monodora*,[20)] *Oxymitra*,[21)] *Phaeanthus*[22)] and *Popowia*[23)] of the Annonaceae family, as well as in *Stephania*[13)] of the Menispermaceae family.

From a taxonomic point of view, it is interesting to note that this is the first report on the isolation of phenanthrene alkaloids from the genus *Dennettia*, which increase the number of genus belonging to the Annonaceae family with this class of compounds.

Despite phenanthrene alkaloids are closely related with the Annonaceae, their existence has also been reported in other families such as Aristolochiaceae, Fumariaceae, Lauraceae, Magnoliaceae, Menispermaceae, Monimiaceae and Ranunculaceae, suggesting that their distribution may be wider than previously thought.

Finally, the physical characteristics of the compound **5** allowed us its identification as vanillin. Its spectral data ^{1}H-, ^{13}C-NMR and DEPT was in concordance with authentical standard.

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