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PIPTADEROL FROM *PIPTADENIA AFRICANA*

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Abstract

A new glyceryl derivative (Glyceryl-1-hexacosanoate) and a flavone derivative (methyletherapigenin) were isolated from the stem bark extract of *Piptadenia africana*, a western Cameroonian plant species. Common terpenes like sitosterol, β-amyrin and eicosane were also isolated. These compounds were identified using physical and spectroscopic methods including mp, IR, ^{1}H and ^{13}C-NMR, DEPT, COSY, HMQC, HMBC, EI MS, HREI MS as well as some chemical transformations. The antibacterial activity of the extract, the fractions and the pure compounds is also discussed.

Key words: *Piptadenia africana*, Leguminoseae, Glyceryl-1-hexacosanoate, Methyletherapigenin, Chemotaxonomy, Antibacterial activity.

Introduction

The genus *Piptadenia* or *piptadeniastrum* belongs to the family Leguminoseae. This genus possesses several species including: *P. peregrina, P. macrocarpa, P. rigida, P. vidiflora P. paraguayensi* and *P. africana* (Letouzey, 1969). These species are distributed in tropical Africa from Senegal to Angola and across the Congo region to Uganda (Letouzey, 1969). Only the last species is found in Cameroon (Letourzey, 1985). *P. africana* is a tree, the leaves are very small and numerous, contiguous, about 3-8 mm long and 0.8-1.5 mm broad, fruits are broadly linear, elongated, 17-36 cm long and 2-3 cm broad. They are found in mixed deciduous and evergreen forest, it often stands as a single tree on farmlands (Letouzey, 1969). Previous investigation on the seeds of *P. peregrina* yielded bufotenine (Stromberg, 1954), work on *P. rigida* revealed the presence of tryptamine (Iacabucci *et al*., 1964) which was absent in *P. rigida, P. vidiflora* and *P. paraguayensis* (Linda *et al*., 1971), in the other hand *Piptadenia africana* was suggested not to contain alkaloids (Paris et al., 1967) . As part of our contribution to phytochemical, chemotaxonomic and biological survey of the genus *Piptadenia*, we carried out the investigation of the stem bark of *Piptadenia africana* (Hook. f.) previously not investigated and we report herein the isolation and characterisation, of a new compound in addition to the full NMR assignment of methyletherapigenin. and sitosterol, β-amyrin and eicosane. At low concentration the activity of the crude extract for both gram positive and negative organisms was non existent however at highest concentration the crude extract showed a weak inhibition of gram negative microrganisms. The fractions and pure compounds did not show any activity.

Experimental

General

IR spectra were recorded on a Nicolet Magma 750 spectrophotometer with KBr disc. ^{1}H-NMR spectra

were recorded on Avance 400 MHz Bruker NMR while ^{13}C-NMR spectra recorded at 100 MHz. Chemical shifts are given in δ (ppm) taking tetramethylsilane (TMS) as reference and relative to the solvent used. NMR was taken in pyridine and $CDCl_3$. EI MS were recorded on a JEOL MS Route. Silica gel (60-230, 230-400 mesh) (Merck) was used for column chromatography. Melting points were recorded on BUCHI 535 melting point apparatus.

Plant Material

The stem bark *of Piptadenia africana* was collected at Matachouom, Noun Division, and Western province of Cameroon in February 2005. Plant identification was performed by Dr. Onana, Botanist at the National Herbarium, Yaoundé, Cameroon, where a voucher specimen (N° 5000/12115/HNC) has been deposited.

Extraction and isolation

The air dried and pulverized plant material (500 g) was macerated in a mixture of dichloromethane and methanol (1:1) for 3 days. Removal of the solvent in vacuo provided an organic extract (45 g). The extract (45 g) was dissolved in methanol and re-extracted with petroleum ether to yield fraction A (3 g). The resulting organic phase was then concentrated and dissolved in water. This aqueous phase was extracted with dichloromethane, ethyl acetate and butanol to yield fractions B (10 g), C (7 g) and D (11 g). Fraction B was then concentrated and subjected to column chromatography, using a mixture of *n*-hexane-acetone with increasing polarity as eluent. Fractions of 200 ml were collected and combined on the basic of their TLC profiles. The fraction eluted with *n*-hexane-acetone (8:2, 350 mg) was further purified by column chromatography on silica gel (60-230 mesh) to yield eicosane (4 mg) and 4′, 7-dihydroxy-5-methoxyflavone (**2**, 6 mg). The combined fractions (800 mg) eluted with *n*-hexane-acetone (1:1) revealed the presence of sitosterol. Further purification of this fraction by column chromatography on silica gel with dichloromethane-acetone afforded β-amyrin (23 mg), sitosterol (30 mg) and glyceryl-1- hexacosanoate (**1**, 12 mg).

Hydrolyses of glyceryl-1-hexacosanoate (1)

5 mg of this compound was dissolved in 2 ml of pyridine in a 50 ml round flask, to which 10 ml of HCl 5$^{o}/_{o}$ was added and refluxed at 60° C for 14 hours required for complete hydrolysis. The solution was kept for 24 hours to evaporate pyridine. The remaining mixture was concentrated and further purification yielded two main compounds: Glycerol was obtained as oil and hexacosanoic acid as a white solid (mp 88-89° C). Both products were identified by analysis of their mass spectrum and comparison with an authentic specimen available in our laboratory.

Glyceryl-1-hexacosanoate (12 mg, mp 91-93° C). White solid, (CH_2Cl_2/acetone), EI MS *m/z* (re lint.): 470, M^+, 439 (2), 396 (7), 379 (5), 351 (3), 176 (3),134 (47), 98 (91) and 57 (100); IR (KBr) 2918, 2850, 1732 cm^{-1}, ^{1}H-NMR, ^{13}C-NMR and HMBC, see Table 1

4′, 7, Dihydroxy-5-methoxyflavone (6 mg, mp 279-283° C). Colourless powder (*n*-hexane/acetone). EI MS: *m/z* (re lint.) 270 (100), 242 (11), 153 (31), 121 (24), 69 (23). ^{1}H-NMR and ^{13}C-NMR, see Table 2

Sitosterol. $C_{29}H_{50}O$, cristallised from acetone as white powder, 50 mg, mp 134-136 °C. The physical and NMR data (^{13}C and ^{1}H) are in agreement with those reported in the literature (Goad L. J. 1991).

β-Amyrin. $C_{30}H_{50}O$, cristallised from methanol as colourless needles, 30 mg, mp 197-199 °C. The physical and NMR (^{1}H and ^{13}C) data are in agreement with those reported in the literature(veterberg *et al*.1925).

Eicosane. $C_{20}H_{42}$, crystallised in Ethanol as white solid 4 mg, mp 38 °C. The physical and NMR data (^{13}C and ^{1}H) data are in agreement with those reported in the literature.

Results and Discussion

A dried powder sample of the stem bark of *P. africana* was extracted by maceration in CH_2Cl_2/MeOH (1/1). Concentration yielded an extract, which was defatted with petroleum ether followed by extraction with dichloromethane, ethyl acetate and butanol. Fractionation of the CH_2Cl_2 extract was achieved and purified by column chromatography over silica gel to afford five compounds. Glyceryl-1-hexacosanoate **1**, 5-methoxy-4′, 7-dihydroxyflavone (methyletherapigenin) **2**, sitosterol, β-amyrin and eicosane . Compound **1** was isolated as a white solid (12 mg, mp 91-93° C). Its cross formula was deduced to be $C_{29}H_{58}O_4$ on the basis of a molecular ion peak at *m/z* 470 from EIMS and was confirmed by HREI MS analyses. The peak at 469 (M-1) suggested the presence of hydroxyl group in the molecule. On the other hand, fragments could be observed at m/z 439 (M-

Table 1: ^{1}H and ^{13}C-NMR data for compound **1** (400 and 100 MHz, δ values in C_5D_5N)

Position	^{1}H [m, *J* (Hz)]	^{13}C
1a	3.97 (dd, 6.3,11.8)	66.7
1b	3.95 (dd, 4.8,11.8)	
2	3.70 (q, 5.8)	70.9
3a	3.46 (dd, 4.2,12.4)	64.3
3b	3.39 (dd, 6.1,12.4)	
1′		173.7
2′	2.19 (t, 15)	34.4
3′	1.46 (q, 14)	25.3
4′-24′	1.03-1.11 (br s)	29.4-32.1
25′	1.13 (br s)	22.9
26′	0.72 (t, 13)	14.2

Table 2: ^{1}H and ^{13}C-NMR data for compound **2** (400 and 100 MHz, δ values in $CDCl_3$)

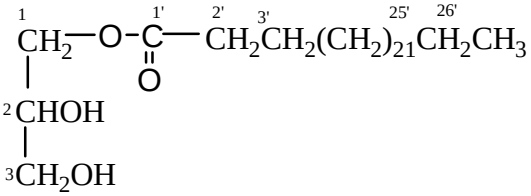
Position	^{1}H [m, *J* (Hz)]	^{13}C
2	-	163.8
3	6.34 (s)	115.8
4		182.4
5		161.4
6	7.61 (d, 2.8)	115.6
7		160.6
8	6.76 (d, 2.8)	115.8
9	-	164.5
10	-	128.1
1′	-	122.3
2′	6.26 (dd, 2.1, 8.8)	99.1
3′	6.09 (dd, 2.1, 8.8)	103.2
4′	-	160.6
5′	6.09 (dd, 2.1, 8.8)	104.4
6′	6.26 (dd, 2.1, 8.8)	99.1
OMe	3.70 (s)	53.2

Table 3: In vitro Antibacterial bioassay of *P. africana*.

Name of Bacteria	Zone of Inhibition of Sample (mm)	Zone of inhibition of Std. Drug (mm)[a]
Escherina coli	16	30
Bacillus subtilis	13	33
Shigella flexenari	9	27
Staphylococcus aureus	10	33
Pseudomonas aeruginosa	15	24
Salmonella typhi	14	25

Key: Concentration of Sample 3mg/ml of DMSO Size of well 6mm (diameter)
[a] Std. Drug Imipenum

CH_2OH), 410 ($-C_3H_7$), 351 (-CO), 154, 134, 98. An interesting fragment was observed at *m/z* 379 [M-glycerol]$^{+}$, which suggested the fatty acid as hexacosanoic acid. The identity of the fatty acid was proved from HREI MS which gave a molecular ion peak at *m/z* 470.4342, consistence with our suggested molecular formula ($C_{29}H_{58}O_4$). The IR spectrum showed the presence of a carbonyl group at 1732 cm^{-1}, hydroxyl group at 2918 and 2850 cm^{-1}. The ^{1}H-NMR spectrum of **1** (Table 1) revealed the presence of five hydrogen germinal to hydroxyl group at δ 3.97 (1H, dd, *J* = 6.3, 11.8 Hz, H-1a), 3.95 (1H, dd, *J* = 4.8, 11.8 Hz, H-1b), 3.70 (1H, q, *J* = 5.8 Hz, H-2), 3.46 (1H, dd, *J* = 4.2, 12.4 Hz, H-3a) and 3.39 (1H, dd, *J* = 6.1, 12.4 Hz, H-3b), three methylene



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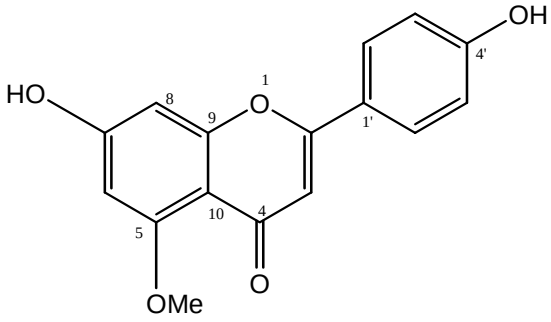

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Figure 1: Structure of Compound **1** and **2**

protons at δ 2.19 (2H, t, *J* = 15.0 Hz, H-2′), 1.46 (2H, q, *J* = 14.0 Hz, H-3′) and 1.13 (2H, br s, H-25′), a methyl at δ 0.72 (3H, t, *J* = 13.0 Hz, H-26′), in addition the signal of a large number of cyclic methylene proton between δ 1.03 and 1.11 was observed. These chemical shifts are characteristic of oxomethylene proton signal for an -O-CH_2-CH(O)-CH_2-O- spin system attributable to glycerol (Nasim *et al*., 1999). The ^{13}C-NMR spectrum (Table 1) confirmed these suggestions and showed an ester carbonyl signal at δ 173.7 (C-1′), three carbon atoms connected to oxygen at δ 66.7 (C-1), 70.9 (C-2) and 64.3 (C-3), signals of aliphatic methylene carbon at δ 34.4 (C-2′), 25.3 (C-3′) and 22.9 (C-25′), a methyl carbon was found at δ 14.2 (C-26′), from δ 29.4 to 32.1 we had and a peak integrating for a large number of CH_2. In HMBC experiment (Table 1), the methylene protons at δ 3.97/3.95 ppm (H-1) showed a ^{3}J correlation with the carbonyl. From these shifts the idea of a monoglyceride was evident. The results described and by comparison with those of similar compound (Nasim *et al*., 1999), as the NMR revealed a single linear alkyl chain this must be C-26 and accordingly, **1** is glyceryl -1- hexacosanoate which is a new natural product named as piptaderol.

Compound **2** was obtained as colorless needles mp 279-283°C and gave positive Mg-HCl test characteristic of flavonoids (Mabry *et al*., 1970). It was assigned the molecular formula $C_{16}H_{12}O_5$ as deduced from EI MS and HREI MS where a molecular ion peak was observed at *m/z* 282 and 282.1231 respectively. In addition fragments were observed at *m/z* 270, 242, 153, 149, 121, 83 and 69. The ^{1}H-NMR spectrum of **2** (Table 2) showed one singlet at δ 6.34 (1H, H-3), two doublets at δ 7.61 (1H, *J* = 2.8 Hz, H-6) and 6.76 (1H, *J* = 2.8 Hz, H-8), characteristic of meta-related proton (Dey and Harbone, 1989) along with two doublets of doublets at δ 6.26 (2H, *J* = 2.1, 8.8 Hz, H-2′, H-6′) and δ 6.09 (2H, *J* = 2.1, 8.8 Hz, H-3′, H-5′) revealing ortho coupled proton. A singlet at δ 3.70 integrating for the methoxyl proton was observed. It was clear that The compound has a flavone basic skeleton. This was confirmed by the ^{13}C-NMR spectrum from which signals for sixteen carbon atoms were observed. Analysis of ^{13}C-NMR, DEPT (90 and 135) and HMQC lead to their classification into a carbonyl at δ 182.4 (C-4), two unsaturated quaternary carbon link to hydroxyl group at δ 160.6 (C-7, C-4′), a carbon link to a methoxyl at δ 161.4 (C-5). Four aromatic quaternary carbon at δ 163.8 (C-2), 164.5 (C-9), 128.1 (C-10) and 122.3 (C-1′), in addition we could also found seven aromatic methine carbon atom at δ 115.8 (C-3, C-8), 115.6 (C-6), 99.1 (C-2′, C-6′), 103.2 (C-3′) and 104.4 (C-5′), a methoxyl at δ 53.2. As the chemical shifts of C-2′ and C-6′ were similar, like C-3′ and C-5′ were very closed, deduced the hydroxyl group to be at C-4′. The assignment of the hydroxyl group was also based on the chemical shift of the proton, their form on the spectrum, their coupling constant and according to the HMQC. Comparing our data to those of similar compounds 4′,5-dihydroxy-7-methoxyflavone (Bosabalidis, 1998), as there was no signal between 10-14 ppm for OH-5 (Dey and Harborne, 1989), this flavone must be 5-methoxy-4′,7-dihydroxyflavone. This compound was previously isolated from the flowers of *Saccharum officinarum* (Misra *et al*., 1979) and appears to be common in plant kingdom, but to best of our knowledge, the complete assignment of its NMR data is described for the first time.The other compounds were isolated and characterized from comparison of their physical and NMR data (^{1}H and ^{13}C) to those reported in the literature.

Conclusion

Previous investigation of members of this genus yielded alkaloids, but we found no traces of this class of compounds. Further more, flavonoids have also been previously reported from other species of the genus. These may constitute a possible chemotaxonomic marker for the investigation of other species of the genus.

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