Research Journal of

Phytochemistry

ISSN 1819-3471

Academic
Journals Inc.

www.academicjournals.com

Research Journal of Phytochemistry 2 (1): 27-34, 2008
ISSN 1819-3471
© 2008 Academic Journals Inc.

Piptadenol A-C and α-Glucosidase Inhibitor From *Piptadenia africana*

[1,2]Roukayatou N. Mbouangouere, [1]Pierre Tane, [2]Mohamed I. Choudhary, [1]Pierre C. Djemgou, [3]Bonaventure T. Ngadjui and [1]Dieudonné Ngamga
[1]Department of Chemistry, Faculty of Science, University of Dschang, P.O. Box 67, Dschang, Cameroon
[2]H.E.J. Research Institute of Chemistry, University of Karachi, Karachi-75270, Pakistan
[3]Department of Organic Chemistry, Faculty of Science, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon

Abstract: Ten compounds were isolated from the CH_2Cl_2/MeOH (1/1) stem bark extract of *Piptadenia africana*, a western Cameroonian plant species. These compounds included three new lactone derivatives piptadenol A-C. The known compounds include 5,6-dimethoxy-7-hydroxyflavone, antiquol B, sistosterol, β-amyrine, betulinic acid, cholesterol and 24(S)-stigmat-5,22-dien-3β-O-glucopyranoside. The latter was found to be highly promising α-glucosidase inhibitor. These compounds were characterized using physical and spectroscopic methods. The plant extract and 24(S)-stigmat-5,22-dien-3 β-O glucopyranoside showed antibacterial activity.

Key words: *Piptadenia africana*, new lactones, α-glucosidase inhibitor

INTRODUCTION

The genus *Piptadenia* or *Piptadeniastrum* belongs to the family Leguminoseae. This genus possesses several species including: *P. peregrina*, *P. macrocarpa*, *P. excelsus*, *P. rigida*, *P. vidiflora*, *P. paraguayensi* and *P. Africana* (Hook.f). Brenan. (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 (Letouzey, 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). The leaves and fruits are used by traditional healers for aphrodisiac, tonic, enema urethritis and abortificient effect (Iwu, 1993); while the stems bark are use for gastric pain, fever and caught by local population of Noun division, Cameroon. Previous investigation on several species of this genus yielded mainly alkaloids. Phytochemical study of the seeds of *P. peregrina* yielded bufotenine (Stromberg, 1954), work on *P. macrocarpa* and *P. excelsa* revealed the presence of the base from tryptamine type (Iacobucci and Ruveda, 1964) which was absent in *P. rigida*, *P. vidiflora* and *P. paraguayensis* (Fellows and Bell, 1971). *P. africana* was suggested not to contain alkaloids (Paris *et al*., 1967). In the course of our phytochemical and pharmacological study on this species (Mbouangouere *et al*., 2007), we carried out the investigation of the stem bark of *Piptadenia africana* (Hook. f.) and we report herein the isolation and characterisation, of three new compounds from this natural source.

Corresponding Author: Mbouangouere Roukayatou Epouse Nsangou, Department of Chemistry, Faculty of Science, University of Dschang, Box 67, Dschang, Cameroon
Tel: 00237 75706965 Fax: 00237 3451735

Glucosidases have drawn the attention of the scientific community for its wide role in the living biological systems. Glucosidases are involved in several biological processes, intestinal digestion, the biosynthesis of glycoproteins and the lyposomal catabolism of the glycoconjugates (Asano *et al*., 1997). Glucosidase inhibitors are of considerable current interest in view of potential aspects in the treatment of AIDS (Acquired Immunodeficiency Syndrum), because of its anti-HIV (Human Immunodeficiency Virus) activity shown by the natural competitive inhibitors deoxynojirimycin (Josie *et al*., 1992). Intestinal α-glucosidases are involved in the final step of the carbohydrates digestion and convert them into monosaccharides, which are absorbed from the intestine, thus its inhibitors could therefore suppress the postprandial hyperglycemia and can be used for the treatment of the type II diabetes (Sou *et al*., 2000); α-glucosidase inhibitors have been also used as inhibitors of tumor metastasis, antiobesity drugs, fungistatic compounds, insects antifeedants, antiviral and immune modulators (El Ashry *et al*., 2000). We have focused to work in the discovery of effective inhibitors because of the multi dimensional scope of this enzyme. The acarbose is a very widely prescribed drug in the management of the type II diabetes.

MATERIALS AND METHODS

General

IR spectra were recorded on a Nicolet Magma 750 spectrophotometer with KBr disc and $CHCl_3$. ^{1}H-NMR spectra were recorded on advance 300 and 500 MHZ; while ^{13}C-NMR spectra were recorded on 100 MHZ. Chemical shifts are given in δ (ppm) with TMS as reference. NMR was taken in pyridine and $CDCl_3$. EIMS and HRMS were recorded on a JEOL MS Route. Silica gel (70-32, 230-400 mesh) (Merck) was used for column chromatography. Melting points were recorded in BUCHI 535 melting points apparatus. GC-MS (Masslab Trio 1000), column DB5-MS, 60 m, 0.25 mm internal diameter, 0.25 µm film thickness; injector, 325°C; initial oven temperature, 100°C (5 mn), 10°C/mn rate up to 240°C, 3°C/mn rate up to 300°C, 1°C/mn rate up to 325°C (10 mn).

Plant Material

The stem bark of *Piptadenia africana* was collected at Matachouom, Noun Division, Western province of Cameroon in February 2005. Plant identification was performed by Dr. Onana, National Herbarium, Yaoundé Cameroon where a voucher specimen (N°5000/12115/HNC) has been deposited. This work was conducted in the H.E.J. Research Institute of Chemistry, (International Center for Chemical Science), University of Karachi, Pakistan from April to September 2005.

Extraction and Isolation

The air-dried and powdered plant material (3 kg) was macerated in a mixture of dichloromethane and methanol (1:1) for 3 days. Removal of the solvent with rotary evaporator provided an organic extract (250 g). This extract was dissolved in methanol and re-extracted with petroleum ether to yield extract A (23 g). The residu was then concentrated and dissolved in water. This water part was partitioned with dichloromethane and ethyl acetate to yield extract B (22 g) and C (75 g). Extract A was concentrated and subjected to column chromatography, using a mixture of n-hexane-chloroform as eluent. Fractions of 200 mL each were collected and combined on the basis of their TLC profiles. The fraction eluted with n-hexane-chloroform (7:3) (450 mg) was further purified by column chromatography with silica gel (70-32 mesh) to yield compound 3 (4 mg) and antiquol (5 mg). Extract B was concentrated and subjected to column chromatography, using a mixture of n-hexane-acetone with increasing polarity as eluent. Fractions of 200 mL each were collected and combined on the basis of their TLC profiles. The fraction eluted with n-hexane-acetone (8:2) (410 mg) was further purified by

column chromatography with silica gel (70-32 mesh) to yield compound 2 (4 mg) and cholesterol (6 mg). Extract C was concentrated and subjected to column chromatography, using a mixture of n-hexane-acetone with increasing polarity. Fractions of 500 mL each were collected and combined on the basis of their TLC profiles. The main fraction eluted with n-hexane-acetone (8:2) (350 mg) was further purified by column chromatography on silica gel (70-32 mesh) to yield betulenic acid (6 mg), 5,6-dimethoxy-7-hydroxyflavone (4 mg), and β-amyrin (13 mg). The combined fractions (800 mg) eluted with n-hexane-acetone (1:1) revealed the presence of sitosterol. Further purification of this fraction by chromatography on silica gel column with dichloromethane-acetone as eluent afforded sitosterol (30 mg) and compound 1 (6 mg). Fraction eluted with *n*-hexane-acetone (7:3) (22 mg) yielded 24(S)-stigmat-5,22-dien-3β-O-glucopyranoside 4 (18 mg).

Piptadenol A 1 (27-Hydroxymethyl-25-Oxaheptacosan-27,1-Olide)

Amorphous solid, crystallized in methanol mp: 205°C (uncorrected). $[\alpha]^{27}_{D}$ +1.6°(c 0.06, $CHCl_3$/MeOH). IR (υ^{KBr}_{max} cm^{-1}): 3342, 1738 cm^{-1}; EIMS m/z 440, 382, 339 307, 146, 116, 101, 58; HREIMS found m/z 440.7014 (calc. 440.7038 for $C_{27}H_{52}O_4$). ^{13}C and ^{1}H NMR see Table 1 and 2, respectively.

Piptadenol B 2 (29-Hydroxymethyl-27-Oxanonacosan-29,1-Olide)

Amorphous solid, mp: 196°C (uncorrected), $[\alpha]^{27}_{D}$ +2.6°(c 0.01, $CHCl_3$/MeOH). IR (υ^{KBr}_{max} cm^{-1}): 3347, 1730 cm^{-1}; EIMS m/z 468, 440, 366, 348, 185, 98; HREIMS found m/z 468.7531, (calc. 468.7574 for $C_{29}H_{56}O_4$); ^{13}C and ^{1}H NMR see Table 1 and 2, respectively.

Table 1: ^{1}H-NMR data of compounds 1, 2 (in pyridine 300 MHZ) and 3 in $CDCl_3$ (500 MHZ)

1		2		3	
Position	^{1}H [δ, m, J (Hz)]	Position	^{1}H [δ, m, J (Hz)]	Position	^{1}H [δ, m, J (Hz)]
1	-	1	-	1	-
2	2.30, t (7.4)	2	2.30, t (7.0)	2	1.60, br(t)
3	1.60, m	3	1.60, m	3	2.30, t (7.0)
4	1.70, m	4	1.70, m	4	-
5-22	1.30-1.40, br(m)	5-24	1.10-1.30, br(m)	5	1.20, br(t)
23	1.50, m	25	1.50, m	6-45	1.22-1.30, br(m)
24	3.80, t (6.5)	26	3.80, t (6.3)	46	1.20, br(m)
1′	4.40, m	1′	4.38, m	47	0.80, t (6.3)
2′	4.10, d (5.4)	2′	4.10, d (5.2)	1′	4.15, br(t)
3′a	4.60, dd (6.3, 11.8)	3′a	4.60, dd (6.1, 11.4)	2′	4.10, br(t)
3′b	4.70, dd (6.2, 12.4)	3′b	4.70, dd (6.2, 12.4)		

δ: Chemical shift, m: Multiplet, t: Triplet, dd: Doublet of doublet, J: Coupling constant, br: Broad, Hz: Hertz

Table 2: ^{13}C-NMR data of compounds 1, 2 (in pyridine) and 3 (in $CDCl_3$) (100 MHZ)

1		2		3	
Position	^{13}C	Position	^{13}C	Position	^{13}C
1	173.7	1	173.8	1	173.9
2	34.4	2	34.4	2	24.9
3	25.5	3	25.3	3	34.1
4	33.9	4	33.7	4	177.4
5-22	26.5-29.9	5-24	26.5-29.9	5	29.0
23	26.5	25	26.5	6-45	22.6-33.5
24	62.1	26	62.2	46	22.4
1′	70.9	1′	70.9	47	14.1
2′	64.3	2′	64.3	1′	65.0
3′	66.7	3′	66.8	2′	68.4

Piptadenol C 3 (2'-Hydroxylethyl 4-Oxo-Tetracontanoate)

White solid, crystallized from acetone, mp: 73°C (uncorrected). $[\alpha]^{27}_{D}$ +0.24°(c 0.01, $CHCl_3$/MeOH). IR (υ^{KBr}_{max} cm^{-1}): 3324, 2917, 2849, 1730, 1463); EIMS m/z 748, 720, 157, 143, 113, 95, 71, 57; HREIMS found m/z 749.2921 (calc. 749.2934 for $C_{49}H_{96}O_4$). ^{13}C and ^{1}H NMR see Table 1 and 2, respectively.

Alkaline hydrolysis of 3: To 5 mL of 15 m mol L^{-1} $NaOCH_3$, prepared by dissolving metallic sodium in dry methanol, 2 mg of 3 was added. The mixture was kept under reflux for 1 h, before water was added and the organic compounds extracted with CH_2Cl_2. The mixture was analysed by GC-MS.

Microorganisms

The microorganisms used in this study included six clinical isolates of bacteria: *Bacillus subtilis*, *Escherichia coli*, *Shigella flexneri*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi* collected from the Dr Panjwani Center for Molecular Medecine and drug Research, University of Karachi in Pakistan. The bacterial isolates were grown at 37°C and maintained on nutrient agar Slant.

Antibacterial Activity

The preliminary screening test was performed by the agar well diffusion technique as per Atta-ur-Rahman *et al*. (2001) with slight modifications. Stock solution of the extracts (crude dichloromethane/methanol (1/1) extract and one pure compound) were prepared in 5% v/v aqueous Dimethyl Sulfoxyde (DMSO) at concentrations of 3 mg mL^{-1} (for crude extract and 1 mg mL^{-1} for pure compound) resulting in homogeneous solution of each one. The inocula of micro-organisms were prepared from 24 h old broth cultures. The absorbance was read at 530 um and adjusted with sterile distilled water to match that of a 0.5 Mc Farland Standard solution from the prepared stock solutions. Other dilutions with sterile distilled water were prepared to give a final concentration of 10^6 cfu mL^{-1}. Bottles containing 19.8 mL of sterile Mueller Hinton Agar (MHA) were maintained in a steam bath set at 40°C to prevent solidification and then inoculated aseptically with 200 μL of bacteria suspension followed by thorough mixing. Sterile Petri dishes (diameter, 90 mm) were filled to 20 mL final of each bottle to give a solid plate. Wells of 6 mm of diameter were bored aseptically on the solid plates and 10 μL of molten MHA were previously introduced into each well to ceal the bottom of the well. After this, 50 μL of stock solution of extracts (crude extract or pure compound) were finally introduced into each well and allowed for 2 h at + 4°C for extract to diffuse. The Petri dishes were then incubated at 37°C for 24 h. The final disc charges were 150 μg (for crude extract) and 50 μg (for pure compound) per well. The antibacterial activity was recorded by measuring the clear zone of growth inhibition on agar surface around the well. All the experiments were carried out in triplicate. Imipenum (reference drug) at 50 μg per well was used as positive control and 5% v/v aqueous DMSO as a negative control.

Enzyme Inhibition Assay

α-glucosidase (E.C.3.2.1.20) enzyme inhibition assay has been performed according to the slightly modified method of Matsui *et al*. (1996). α-Glucosidase (E.C.3.2.1.20) from *Saccharomyces* species, purchased from Wako Pure Chemical Industries Ltd. (Wako 076-02841). The enzyme inhibition was measured spectrophotometrically through continuous monitoring of the nitrophenyl produced by the hydrolysis of the substrate p-nitrophenyl α-D-glucopyranoside (PNP-G) (0.7 mM) and 500 milli units/mL of the enzyme used. Whole enzymatic reaction was performed at 37°C for 30 min. The increment in absorption at 400 um, due to the hydrolysis of PNP-G by α-glucosidase, was monitored continuously on microplate spectrophotometer (Spectra Max, Molecular Devices, USA). Phosphate

saline buffer at pH 6.9, which contains 50 mM sodium phosphate and 100 mM NaCl was used. 1-Deoxynojirimycin (0.425 mM) and Acarbose (0.78 mM) were used as positive controls.

RESULTS AND DISCUSSION

The CH_2Cl_2/MeOH (1/1) extract of the stem bark of *P. africana* was defatted with petroleum ether followed by extraction with dichloromethane and ethyl acetate. Purification of these fractions afforded ten compounds. Piptadenol A (1), piptadenol B (2), piptadenol C (3), 24(S)-stigmat-5,22-dien-3β-O-glucopyranoside (4) in addition to 5,6-dimethoxy-7-hydroxyflavone, antiquol B, sistosterol, β-amyrine, betulenic acid and cholesterol. Compound 1 was obtained as amorphous solid from methanol mp: 205°C. It molecular formula was deduced to be $C_{27}H_{52}O_4$ on the basis of a molecular ion peak [M^+] at m/z 440, from EIMS analysis. This was in agreement with the HREIMS analyses where the molecular ion was observed at m/z 440.7014. This accounts for two unsaturations including a carbonyl and a ring. Additionally, fragments could be observed at m/z 382[$M-H_2O]^+$, 339[382-$CH_3CO]^+$ and 58[$HOCH_2CHCH_2]^+$. The IR spectrum of compound 1 showed the presence of a carbonyl group at 1738 cm^{-1}, hydroxyl group at 3342 cm^{-1}. The ^{1}H-NMR spectrum (Table 1) revealed the presence of seven hydrogen atoms geminal to oxygen atom, at δ 3.80 (2H, t, J = 6.5, H-24), 4.10 (2H, d, J = 5.4, H-2'), 4.60 (1H, dd, J = 6.3, 11.8, H-3′a), 4.70 (1H, dd, J = 6.2, 12.4, H-3′b) and the multiplet at δ 4.40 (1H, m, H-1'). Moreover we could easily observe distinguish methylene proton at δ 2.30 (2H, t, J = 7.4, H-2), downfield because of the neighbouring carbonyl (Plasman *et al*., 1999). Furthermore other methylene protons were observed at δ 1.70 (2H, m, H-4), 1.60 (2H, m, H-3) and 1.50 (2H, m, H-23). A broad multiplet was observed between 1.30 and 1.40 ppm integrating for thirty six cyclic methylene protons. These data were close to those of macrolactone molecules (Plasman *et al*., 1999). The ^{13}C-NMR spectrum (Table2) confirmed this suggestion and showed no methyl carbon. Analysis of these data with the help of DEPT 90 and 135 lead to their classification into a carbonyl carbon at δ 173.7 (C-1), four carbon counected to oxygen atom at δ 70.9 (C-1'), 66.7 (C-3′), 64.3 (C-2') and 62.1 (C-24). In addition eighteen signals were found from δ 26.5 to δ 29.9 accounting for long chain methylene carbon. The carbon at δ 34.4 (C-2) and 33.8 (C-4) were deduced to be those of the shielded methylene proton at δ 2.30 and 1.70 respectively from HMQC experiments. COSY ^{1}H-^{1}H spectrum (Fig. 1) shows correlation between H-3 and H-2, in the other hand H-23 showed direct correlation with H-22, H-3′a/b and H-27. Analogously HMBC (Fig. 1) experiment showed correlation of H-2 with C-1and C-4, H-3 with C-1, C-2 and C-4, H-3' with C-1', C-2'. By comparing all these data with those of similar compound (Plasman *et al*., 1999), structure of 1 was assigned. The compound is a new natural product, trivially named Piptadenol A.

Compound 2 was obtained as amorphous solid from methanol mp: 196°C. The molecular formula of Compound 2 was deduced to be $C_{29}H_{56}O_4$ on the basis of a molecular ion peak at m/z 468 as shown by the EIMS. This was in agreement with the HREIMS analyses where the molecular ion was observed at m/z 468.7531. The mass spectra showed ion peaks at m/z 468[M]$^+$, 440[M-2$CH_2]^+$,

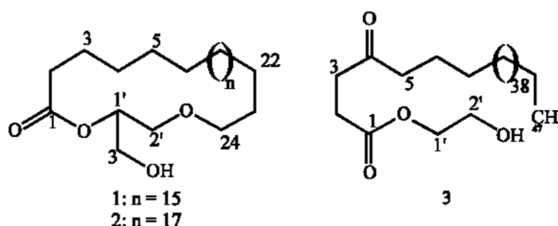

Fig. 1: Structures of the isolated

366[M-$HOCH_2CHCH_2O$]$^+$, 348[366-H_2O]$^+$. The IR showed the carbonyl group at μ 1730 cm^{-1}, one hydroxyl group at 3347 cm^{-1}. The ^{1}H-NMR spectrum (Table 1) revealed four groups of proton geminal to oxygen at 3.80, (2H, t, 6.5, H-26), 4.10 (2H, d, 5.4, H-2'), 4.38 (1H, m, H-1'), 4.60 (1H, dd, 6.2, 11.8, H-3′a) and 4.70 (1H, dd, 6.2, 12.4, H-3′b). A down field methylene proton was found at δ 2.30 (2H, t, 7.4, H-2). Three methylene protons could be observed at δ 1.70 (2H, m, H-4), 1.60 (2H, m, H-3), 1.50 (2H, m, H-25). A broad multiplet at δ 1.10-1.30 was attributed to fourthy cyclic methylene protons. No methyl signal was found in the spectra. The ^{13}C-NMR spectrum (Table 2) revealed the presence of a carbonyl at δ 173.8 (C-1). In addition, four carbons atoms connected to oxygen were found at δ 70.9 (C-1'), δ 66.8 (C-3′), δ 64.3 (C-2') and δ 62.2 (C-26). Twenty signals were found between 26.5-29.9 ppm for cyclic methylene carbons. No methyl carbon was observed. These data suggested that compound 2 has 2 more methylene groups than compound 1. From the COSY experiment, correlation between H-3 and H-2 were observed, furthermore H-25 showed direct correlation with H-24. Analogously HMBC experiment showed correlation of H-2 with C-1, C-3 and C-4, H-3 with C-1, C-2 and C-4, H-3′ showed correlation with C-1' and C-2'. Thus, compound 2 was assigned structure. Compound 2 is a new natural product and was trivially named Piptadenol B.

Compound 3 was isolated as white solid, mp: 73°C. Its cross formula was deduced to be $C_{49}H_{96}O_4$ as observed from a molecular ion peak at m/z 749[M+H]$^+$ on the EIMS and confirmed by the HRMS analyses. This molecular formula accounts for two unsaturations and equivalent to two carbonyl groups from the ^{13}C-NMR spectra. In the EIMS spectrum, fragments at m/z 720[M-2CH_2]$^+$ and 57[OCH_2CHOH]$^+$ were observed. In the other hand, a large number of fragment ions were observed exhibiting a uniform difference of 14 mass units, thus suggesting aliphatic long chain. More intense clusters of peaks corresponding to C_nH_{2n-1} (m/z 83, 97, 115, 125, 139 and 153), in comparison to those corresponding to C_nH_{2n+1} (m/z 85, 99, 113, 127, 141 and 155) also supported the acyclic olefinic nature of the compound (Silverstein and Basler, 1967). The ^{1}H-NMR of the compound (Table 1) revealed six groups of protons. Four methylene protons geminal to oxygen at δ 4.10 (2H, br (t), H-2′) and 4.15 (2H, br (t), H-1′). Two deshielded protons at δ 2.30 (2H, t, 7.0, H-3). Other distinguish methylene protons were found at δ 1.60 (2H, br(t), H-2) and 1.20 (2H, br(t), H-5). The broad multiplet at δ 1.22-1.30 (66 H) was attributed to a long chain methylene protons. The methyl proton appeared at δ 0.80 (3H, t, 6.3, H-47). The ^{13}C-NMR data of 3 (Table 2) determined from DEPT (45, 90 and 135) revealed the presence of two carbonyl carbons at δ 173.9 (C-1) and 177.4 (C-4), two carbons attached to oxygen at δ 68.4 (C-2′) and 65.0 (C-1′). A broad band at δ 29.6 was attributed to fourthy methylene carbons C-6-C-38. Moreover the COSY experiment showed correlations between H-1′and H-2′, H-2 and H-3. There was also correlation between H-5 and H-6. The HMBC spectrum exhibited correlation between H-2 and C-1, C-3 and C-4. H-3 showed correlation with C-2, C-4, C-5 and C-1. H-1′ showed correlation with C-1. By comparing these data with those previously published (Misra *et al*., 1991) compound 3 was assigned structure, has shown

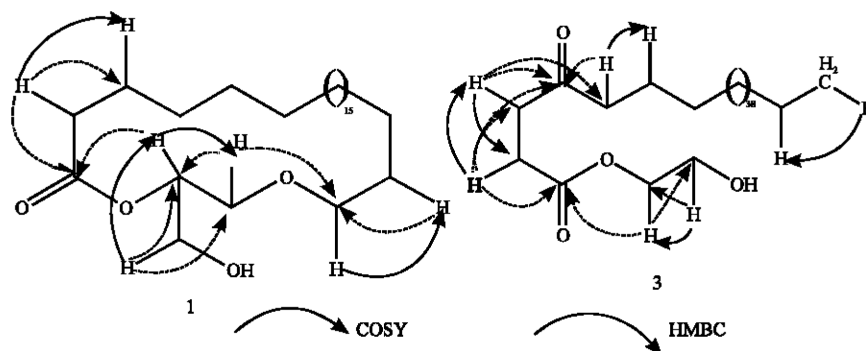

Fig. 2: Key HMBC and COSY correlations in compounds 1-3

in Fig. 2 which is a new natural product trivially named piptadenol C. The structure of 3 was further confirmed by the GC-MS analysis, which after alkaline hydrolysis yielded ethylene glycol and methylester heptatetracontanoic acid as the major products, based on the peaks at m/z 62 and 719, respectively

Compound 4 was obtained as white solid and was identified as 24(S)-stigmat-5,22-dien-3β-O-glucopyranoside by comparison with the authentic specimen available in our laboratory. The known compounds 5,6-dimethoxy-7-hydroxyflavone (Buschi *et al*., 1981), antiquol B (Mohan *et al*., 1990), sistosterol, β-amyrin, betulinic acid (Siddiqui *et al*., 1988) and cholesterol were identified by comparison of their (^1H and ^{13}C) NMR data with those reported in the literature and authentic specimen available in our laboratory.

The CH_2Cl_2-MeOH (1/1) extract of the stem bark of *P. africana* and 24(S)-stigmat-5,22-dien-3β-*O*-glucopyranoside were tested by Agar diffusion assay (Atta-ur-Rahman *et al*., 2001) against six species of bacteria comprises (*Bacillus subtilis*, *Escherichia coli*, *Shigella flexenari*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*). As result, good activity was observed with the extract against *E. coli*, *P. aeruginosa* and *S. typhi*; the compound 24(S)-stigmat-5,22-dien-3β-O-glucopyranoside showed potent activity against *S. flexnari*, *S. aureus* and *S. typhi*. This activity was low compared to that of reference drug on the same micro-organism. It was also low compared to other steroid such as 3-O-[beta-D-(6'-nonadeanoate)glucopyranosyl]-beta-sitosterol isolated from *Arctotis arctotoide*, while stigmaterol was not active (Sultana and Afavan, 2007). This indicated that the activity may be due to the aglycone instead of the basic squeleton.

The compound 24(S)-stigmat-5,22-dien-3β-O-glucopyranoside showed very potent and significant α-glucosidase inhibition compared with the standards drugs, while the other compounds were not tested due to their little amount.

CONCLUSIONS

The result from this work is of great interest: No alkaloid was found on the isolated as previously mentioned (Paris *et al*., 1967). The isolates included triterpenes, flovonoids ester derivatives and macrolactones. These isolated constituted the first report from this genus. This may constituted a chemotaxonomic marker for further investigations of plants species. The antibacterial activity showed by the extract as well as the α-glucosidase inhibition of the compound may confirmed that the plant could be use in this purpose.

ACKNOWLEDGMENTS

Mbouangouere R.N. is grateful to The H.E.J.R.I.C (International Center for Chemical Science) for the grant he provided to conduct this work and to COMSTECH (Organisation of Islamic Conference Standing Committee on Scientific and Technological Cooperation) for its financial support. Authors will like to thank Dr. Onana for plant identification and Mss. Samreen Hussein and Shamsun Khan for the biological activities.

REFERENCES

Asano, N., M. Nishida, H. Kizu, K. Matsui, A.A. Watson and R.J. Nash, 1997. Homonojirimycin isomers and glycosides from *Aglaonema treubii*. J. Nat. Prod., 60: 98-100.

Atta-ur-Rahman, M.I. Choudhary and W.J. Thomsen, 2001. Bioassay Technique for Drug Development. Amsterdam. The Netherlands Harwood Academy Publications, pp: 16-17.

Buschi, C.A., A.B. Pomilio and E.G. Gros, 1981. 5,6,7-Trisubstituted flavones from *Gomphrena martiana*. Phytochemistry, 20: 1178-1179.

El Ashry, E.S.H., N. Rashed and A.H.S. Shobier, 2000. Glycosidase inhibitors and their chemotherapeutic values. Part 1, Pharmazie, 55: 251-262.

Fellows, L.E. and E.A. Bell, 1971. Indole metabolism in *Piptadenia peregrine*. Phytochemistry, 10: 2083-2091.

Iacobucci, G.A. and E.A. Rúveda, 1964. Bases derived from tryptamine in argentine *Piptadenia species*. Phytochemistry, 3: 465-467.

Iwu, M.M., 1993. Handbook of African Medicinal Plants. CRC Press, Inc., pp: 41.

Josie, C.B., H.H. Alan and J.K.T. Richard, 1992. 2-Chloromethyl-4-nitrophenyl α-D-glucopyranoside: An enzyme-activated irreversible inhibition of yeast α-glucopyranoside. J. Chem. Soc. Chem. Commun., 15: 1039-1041.

Letouzey, R., 1969. Mannal of Forest Botanic of Tropical Africa, IIA, Imprimerie Jouve, Paris 6e, France.

Letouzey, R., 1985. Notice of Phytogeographic map of Cameroon, Toulouse, France.

Matsui, T., C. Yoshimoto, K. Osajima, T. Oki and Y. Osajima, 1996. *In vitro* survey of α-glucosidase Inhibitory food components. Biosci. Biotech. Biochem., 60: 2019-2022.

Mbouangouere, R.N., P. Tane, D. Ngamga, P. Djemgou, M.I. Choudhary and B.T. Ngadjui, 2007. Piptaderol from *Piptadenia africana*. Afr. J. Trad. CAM, 4: 294-298.

Misra, T.N., R.S. Singh and H.S. Pandey, 1991. Aliphatic compounds from *Adenocalymma alliaceum* leaves. Phytochemistry, 30: 541-543.

Mohan, B., M.H. Gewali, T. Yassnhiro, K. Tohru and N. Seneo, 1990. Constituents of the latex of *Euphorbia antiquorum*. Phytochemistry, 29: 1625-1628.

Paris, R., A. Saint-Firmin and S. Etchepare, 1967. On the alkaloids and flavonoids of a leguminous plant of Haiti: *Piptadenia peregrina* Benth. Absence of alkaloids in *Piptadenia africana* Hook. Ann. Pharm. Fr., 25: 509-513.

Plasman, V., D. Daloze, J.C. Braekman, S. Counétable, A. Robert and C. Bordereau, 1999. New macrolactones from the defensive salivary secretion of soldiers of the African termite *Pseudacanthotermes spiniger*. Tetrahedron Lett., 40: 9229-9232.

Siddiqui, S., F. Hafeez, S. Begum and B.S. Siddiqui, 1988. Oleanderol, a new pentacyclic triterpene from the leaves of *Nerium oleander*. J. Nat. Prod., 51: 229-233.

Silverstein, R.M. and G.C. Basler, 1967. Spectroscopic Identification of Organic Compounds. 2nd Edn., Wiley, New York, pp: 16.

Sou, S., S. Mayumi, H. Takahashi, R. Yamasaki, S. Kadoya, M. Sodeoka and Y. Hashimoto, 2000. Novel α-glucosidase inhibitors with a tetrachlorophthalimide skeleton. Bio. Med. Chem. Lett., 10: 1081-1084.

Stromberg, V.L., 1954. The isolation of bufotenine from *Piptadenia peregrine*. J. Am. Chem. Soc., 76: 1707.

Sultana, N. and A.J. Afavan, 2007. A novel daucosterol derivative and antibacterial activity of compounds from Arctotis arctotoides. Nat. Prod. Res., 21: 889-896.