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SHORT COMMUNICATION

CHEMICAL CONSTITUENTS OF THE ETHYL ACETATE EXTRACTS OF THE STEM BARK AND FRUITS OF *DICHROSTACHYS CINEREA* AND THE ROOTS OF *PARKIA BICOLOR*

J. Fotie[1*], A.E. Nkengfack[1], M.G. Peter[2], M. Heydenreich[2] and Z.T. Fomum[1]

[1]Department of Organic Chemistry, Faculty of Science, University of Yaounde I, P.O. Box 812, Yaounde, Cameroon
[2]Institut für Chemie, Universität Potsdam, D-14476 Golm, Germany

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ABSTRACT. The antibacterial activities of ethyl acetate, methanol and aqueous extracts of the stem bark of *Dichrostachys cinerea* and the roots of *Parkia bicolor* have been evaluated. Ethyl acetate extracts have been investigated, studies that led to a series of known compounds, amongst which many are reported here for the very first time from both the species.

KEY WORDS: *Dichrostachys cinerea, Parkia bicolor,* Chemical constituents, Antibacterial activity

INTRODUCTION

D. cinerea and *P. bicolor* both belong to the Leguminosae family (Mimosaceae). Taking previously as two species of the same genus [1, 2], *D. cinerea* (Linn) and *D. glomerata* (Forsk) had appeared to be the same species [3]. The other scientific names are *Mimosa cinerea* Linn, *Mimosa glomerata* Forsk, *Dichrostachys nutans* Benth, *Mimosa nutans* Benth [3]. Stem barks are used in local medicine in decoction against bad coughs in children, treatment of wounds and gynaecological troubles in adults [1-3]. The roots of *P. bicolor* are used against children measles, woman sterility and sexually transmitted diseases [4].

The antibacterial activities of fruits and leaves of *D. cinerea* have been described previously [5]. The antibacterial activities of ethyl acetate, methanol and aqueous extracts of the stem bark of *D. cinerea* and the roots of *P. bicolor* are investigated in this study.

Previously isolated constituents of *D. cinerea* are triterpenes [1, 2], sterols [2, 6], tannins [7], flavonoids [6, 7] and of *P. bicolor* are fatty acids [8]. This report describes the isolation of new constituents 7,4'-dihydroxyflavon, 7,3',4'-trihydroxyflavon, apigenin, luteonin, monoglycerides of tetracosanoic and 26-hydroxyhexacosanoic acids, 3-α-L-*O*-rhamnopyranosyl-(*2S,3R*)-5,7,4´-trihydroxyflavanon and 3-α-L-*O*-rhamnopyranosyl-(*2S,3S*)-5,7,4'-trihydroxy-flavanon from *D. cinerea* and lupeol, 1'-monoglyceride of octacosanoic acid, lichexanthone, gallic acid and methyl gallate from *P. bicolor*.

EXPERIMENTAL

General. The stem barks and fruits of *D. cinerea* and the roots of *P. bicolor* were collected at Nkolbisson (Yaounde suburban area) in January 2000. Both were identified by M. Kofani, botanist at the National Herbarium in Yaounde, where voucher specimens have been deposited.

*Corresponding author. Tel: (+237) 997 07 06. E-mail: fotie@uycdc.uninet.cm

Antibacterial activities

Inhibition zones were recorded by measuring the diameter of the area showing complete inhibition. The growth inhibition was calculated with reference to positive control, using standard microorganisms from Centre Pasteur of Yaounde. Tests were performed using the cup-plate agar diffusion protocol [9]. The results are reported in Table 1.

Table 1. Antibacterial activities of crude extracts from stem bark of *D. cinerea* and the roots of *P. bicolor.*

	Extract	Inhibition zone (mm)						
		Bc	Cd	Kp	Pm	Pa	St	Sp
Dichrostachys cinerea	Aqueous	9.5	10.0	9.0	10.5	12.0	12.0	9.5
(stem bark)	MeOH	10.5	11.0	10.5	10.0	10.0	10.0	11.5
	AcOEt	8.0	-	9.5	7.0	8.0	-	10.0
Parkia bicolor	Aqueous	10.5	10.0	8.0	13.5	12.0	11.0	12.0
(roots)	MeOH	12.0	11.0	10.5	11.0	13.5	10.5	12.5
	AcOEt	8.5	9.5	10.0	-	8.5	7.0	11.0
Amoxicillin.H_2O	(20 µg/mL)	15.0	16.0	11.0	13.0	12.0	15.0	13.0
Ampicillin.H_2O	(20 µg/mL)	15.0	18.0	10.0	15.0	15.0	15.0	10.0

Key. Bc: *Bacillus cereus*; Cd: *Corynebacterium diptheriae*; Kp: *Klebsiella pneumoniae*; Pm : *Proteus mirabilis*; Pa : *Pseudomonas aeroginosa*; St : *Salmonella typhi*; Sp : *Streptococcus pyogenes*.
Test concentration of extracts: 30 mg/mL; 0.1 mL/cup.

Examination of stem bark of D. cinerea

3 kg of air-dried bark powder was extracted at room temperature with a mixture of CH_2Cl_2/MeOH(1:1). The evaporation gave 250 g (8.33%) of a residue which was redissolved in 500 mL of 10% aqueous MeOH. The solution was extracted once with ethyl acetate. After evaporation, 96 g (3.2%) of a sticky brown oil was obtained. Chromatography over silica gel, eluted with mixtures of hexane and ethyl acetate in increasing polarities yielded **1** (200 mg, 0.007%), **2** (400 mg, 0.013%), **3** (1.62 g, 0.054%), **4** (30 mg, 0.001%), **5** (26 mg, 0.0009%), **6** (42 mg, 0.0014%), **7** (23 mg, 0.0008%), **8** (25 mg, 0.0008%), **9** (37 mg, 0.0012%), and **10** (28 mg, 0.0009%).

Compounds **1, 2, 3** and **10** were identified as friedelan-3-one, friedelan-3β-ol, betulinic acid and 3-*O*-β-*D*-glucopyranosyl-β-sitosterol, respectively, as revealed by NMR spectroscopy, mass spectrometry, melting point and specific rotations. **1** and **2** have previously been isolated from roots [6] and stem bark [2] of the same plant, while **3** and **10** was reported only from the stem bark [2, 6].

*1´-Monoglyceride of tetracosanoic acid **4***. m.p. 115-117 °C (lit. 112-114 °C [10]); IR (KBr): ν = 3450, 3560, 1770 cm^{-1}; MS (EI) *m/z* 308 (13.8%), 134 (64.4); HRMS (EI) *m/z* 442.6895 ($C_{27}H_{54}O_4$); cal. 442.6936. The structure was confirmed by a combination of spectroscopic data, ^{1}H NMR, ^{13}C NMR, HMBC and mass.

*1´-Monoglyceride of 26-hydroxyhexacosanoic acid **5***. m.p. 106-107 °C (lit.103-104 °C [11]); IR (KBr): ν = 3625, 3566, 1705 cm^{-1}; MS (EI) *m/z* 352 (17.8%), 134 (46.8); HRMS (EI) *m/z* 486.7510 ($C_{29}H_{58}O_5$); cal. 486.7678. The structure was confirmed by ^{1}H NMR, ^{13}C NMR spectra and HMBC.

Apigenin (4,7,4´-trihydroxyflavon) **6**. m.p. 347-348 °C; MS (EI) *m/z* 270 [M^+, 100%]; HRMS (EI) *m/z* 270.1828 ($C_{15}H_{10}O_5$); cal. 270.2369. ^{1}H NMR and ^{13}C NMR data correlate well with those from the literature [12].

7,4´-Dihydroxyflavon ***7***. m.p. 328-330 °C; MS (EI) *m/z* 254 [M+, 76.3%]; HRMS (EI) *m/z* 254.2579 ($C_{15}H_{10}O_4$); cal. 254.2375. ^{1}H NMR and the ^{13}C NMR spectra were identical to those reported in the literature [13, 14].

Luteonin (5,7,3´,4´-tetrahydroxyflavon) ***8***. m.p. 288-290 °C, MS (EI) *m/z* 286 [M^+, 100%]; HRMS (EI) *m/z* 286.2177 ($C_{15}H_{10}O_6$); cal. 286.2363. ^{1}H NMR and ^{13}C NMR spectra were identical to those reported in the literature [12, 15].

7,3´,4´-Trihydroxyflavon ***9***. m.p. 352-353 °C; MS (EI) *m/z* 270 [M^+, 84.5%]; HRMS(EI) *m/z* 270.2528 ($C_{15}H_{10}O_5$); cal. 270.2369. ^{1}H NMR and ^{13}C NMR spectra were identical to those reported in the literature [15].

Examination of the fruits of Dichrostachys cinerea

Powdered air-dried fruits (1 kg) were extracted at room temperature with 5 L of a mixture of CH_2Cl_2-MeOH (1:1). The evaporation of the solvent gave 200 g (20%) of a residue which was redissolved in 400 mL of 10% aqueous MeOH. The solution was extracted once with ethyl acetate. After evaporation, 56 g (5.6%) of a viscous sticky brown oil was obtained. Chromatography over silica gel eluted with mixtures of hexane and ethyl acetate in increasing polarities gave **7** (40 mg, 0.004%) and **9** (29 mg, 0.0029%) previously isolated from the stem barks, **11** (18 mg, 0.0018%), **12** (26 mg, 0.0026%) and **13** (26 mg, 0.0026%).

Isoastilbin (3-O-α-L-rhamnopyranosyl (2S3R)-5,7,4´-trihydroxyflavanon) ***11***. m.p. 285-286 °C, MS (EI) *m/z* 288 (25%), 165 (9.6), 153 (100); $[\alpha]_D^{26}$: –199 (c 0.27, EtOH) (lit.: –196°; c 0.27, EtOH [16]); CD (DMSO, c 1 g/L) $[\Theta]_{255}^{26}$: –25 x 10^3 $[\Theta]_{295}^{26}$: +50 x 10^3. The structure was determined using ^{1}H-NMR, ^{13}C-NMR, HMQC, HMBC, NOESY and mass spectra. The absolute configuration was obtained from the circular dichroism spectrum [17, 18].

Neoastilbin (3-O-α-L-rhamnopyranosyl (2S3S)-5,7,4´-trihydroxyflavon) ***12***. m.p. 178-180 °C, $[\alpha]_D^{26}$: –72.5° (c 0.56, EtOH) (lit.: –71.1°, c 0.55, EtOH [16]); CD (DMSO, c 1 g/L) $[\Theta]_{255}^{26}$: –25 x 10^3, $[\Theta]_{295}^{26}$: +12 x 10^3. The structure was determined by the use of ^{1}H NMR, ^{13}C NMR, HMQC, HMBC, NOESY and mass spectra. The absolute configuration was obtained from the circular dichroism spectrum [17, 18].

Catechine ***13***. m.p. 249-250 °C; MS (EI) *m/z* 290 [M+, 23%] 170 (100), 86 (47.8); HRMS (EI) *m/z* 290.2316 ($C_{15}H_{14}O_6$); cal. 290.2680. The ^{1}H NMR and ^{13}C NMR spectra were identical to those reported in the literature [19].

Examination of the roots of Parkia bicolor

Powdered air-dried roots (3 kg) were extracted at room temperature with 15 L of a mixture of CH_2Cl_2-MeOH (1:1). The evaporation of the solvent gave 300 g (10%) of a residue which was redissolved in 500 mL of 10% aqueous MeOH. The solution was extracted once with ethyl acetate. After evaporation, 87 g (2.9%) of a dark viscous and sticky mixture was obtained. Chromatography over silica gel eluted with mixtures of hexane and ethyl acetate in increasing

polarities yielded **14** (18 mg, 0.0006%), **15** (58 mg, 0.002%), **16** (65 mg, 0.002%), **17** (30 mg, 0.001%) and **18** (40 mg, 0.0013%).

Lichexanthone (1-hydroxy-8-methyl-3,6-dimethoxyxanthone) ***14***. m.p. 189-190 °C (lit. 189-191 °C [20]); MS (EI) *m/z* 286 [M+, 100%], 257(43); HRMS (EI) *m/z* 286.2794 ($C_{16}H_{14}O_5$); cal. 286.2793. The structure was determined using ^{1}H NMR, ^{13}C NMR, HMQC, HMBC and mass spectra [21].

Lupeol ***15***. m.p. 215-216 °C; MS (EI) *m/z* 426. The structure was determined by the use of ^{1}H NMR and ^{13}C NMR data and by comparison with authentic sample.

1'-Monoglyceride of octacosanoic acid ***16***. m.p. 107-108 °C (lit. 110-111 °C [22]); MS (MALDI-TOFF) *m/z* 520 [M^++ Na^+ - H]; MS (EI) *m/z* 134 (100%), 294 (12.3%), 378 (17.8%) and 498 (3.2%). The structure was determined by the use of ^{1}H NMR, ^{13}C NMR spectra and HMBC data that were similar to those of compound **4,** and in concordance with the mass spectrum.

Methyl gallate ***17***. m.p. 201-203 °C (lit. 200-202 °C [23]), MS (EI) *m/z* 184 [M^+, 100%]. The structure was determined using ^{1}H NMR, ^{13}C NMR and mass spectra.

Gallic acid ***18***. m.p. 249-250 °C (lit. 251-252 °C [23]); MS (EI) *m/z* 170 [M^+, 100%]. The structure was determined by the use of ^{1}H NMR, ^{13}C NMR and mass spectra.

CONCLUSION

Extensive NMR studies were performed in this work. The values for all the protons and the carbons were assigned by the use of ^{1}H NMR, ^{13}C NMR, HMBC, HMQC and NOESY spectra. The absolute configurations of stereogenic centres were determined using the circular dichroism spectra.

All tested extracts showed antibacterial activities. The results may provide a justification to the traditional usage of these plants in the treatment of some infectious diseases. At this level of study, we cannot recommend the intensification of their usage. Tests on toxicity of the extracts are in progress as well as antibacterial tests on isolated compounds.

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