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Full Length Research Paper

Antimicrobial activities of a novel biflavonoid and other constituents from *Rhus natalensis*

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Phytochemical studies on *Rhus natalensis* root bark collected from Kenya led to the isolation and identification of a new biflavonoid (3-(1-(2,4-dihydroxyphenyl)-3,3-bis(4-hydroxyphenyl)-1-oxopropan-2-yl)-7-methoxy-4H-chromone-4-one (1), named rhuschromone, in addition to two other known compounds; 2',4'-dihydroxychalcone-(4-*O*-5''')-4'',2''',4'''-trihydroxychalcone (2) and 3-((*Z*)-heptadec-13-enyl) benzene-1,2-diol (3). The chemical structures of the isolated compounds were established using spectroscopic techniques including high field nuclear magnetic resonance (NMR). The total extracts and the isolated compounds were tested for their antimicrobial activities against different strains of bacteria.

Key words: *Rhus natalensis*, rhuschromone, anti-bacterial activity, isoflavones.

INTRODUCTION

The genus *Rhus* is made up of flowering plants in the family Anacardiaceae (US FDA, 2007) which are generally found in temperate and tropical regions. They may grow in non-agriculturally viable regions, and various species have been used by indigenous cultures for medicinal and other purposes worldwide (Van Wyk et al., 2004). The genus *Rhus* consists of over 200 species (Hutchings et al., 1996) and is known to be rich in biflavonoids and urushiols. Interest in biflavonoids has increased in recent times due to the realization of a variety of biological activities they manifest (Lin et al., 1999). Another class of compounds found in the genus *Rhus* are the bichalcones (Masesane et al., 2000).

Rhus natalensis (Anacardiaceae) is a tree with a smooth dark brown bark. The leaflets are elliptic or obvate with rounded apex. Their leaf margins do crenate at times. They produce cream or greenish yellow flowers that eventually bear green fruits, round in shape. The fruits are edible; a decoction of the fruit is employed against diarrhoea (Henk, 1991). The plant is common in wooded grassland thickets or grasslands with rocky sites. In the different regions of Kenya, the plant is widely recognized by the indigenous people for treating various microbial conditions. To the best of our knowledge, phytochemical studies on *R. natalensis* have not been previously reported. The present paper describes the isolation and characterization of three compounds (1-3) from the root bark of *R. natalensis,* where compound 1 is a new bi-isoflavonoid reported for the first time from this natural resource.

MATERIALS AND METHODS

Plant

Root bark from trees of *R. natalensis* was collected in June, 2008 from the banks of the Thika River in Gatanga division, Central Kenya. The plant was taxonomically identified at the Department of Botany, University of Nairobi, Kenya. The voucher specimen (HM 2008/01) was deposited at the Department of Botany Herbarium.

Extraction and isolation

Air-dried stem bark of *R. natalensis* was pulverized to give a fine powder (4.5 kg) which was extracted sequentially with solvents of

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increasing polarity, starting with *n*-hexane followed by dichloromethane (DCM), ethyl acetate (EtOAc) and finally methanol (MeOH). Each extraction involved maceration for a period of about 48 h, with three repetitions for each solvent except for methanol which was performed twice. The extracts were decanted, filtered using Whatman filter paper and concentrated under reduced pressure using a rotary evaporator at temperatures less than 50°C. Separation and purification of the plant constituents was carried out using one or the other of a combination of chromatographic techniques. The EtOAc extract (69.1 g) was adsorbed on silica gel and fractionated using increasing gradients of hexane-DCM, DCM-EtOAc in selected ratios, and finally, small portions of MeOH were introduced at 5% intervals in the mixture with EtOAc, up to 50% MeOH. Fractions collected were subjected to thin layer chromatography (TLC) analysis using DCM-MeOH (9.5:0.5) ratio. Fractions with similar TLC profiles were pooled together. The combined fraction from tubes 39 to 51 (800 mg) was chromatographed on Sephadex LH-20 using DCM-MeOH (50:50), followed by preparative TLC using DCM-MeOH (9:1) to give compound 2 (14.9 mg). Re-crystallization of fraction 23 to 32 (321 mg) and then preparative TLC using DCM-MeOH (8:2) ratio gave compound 1 (13.5 mg) as a yellow amorphous powder. The hexane extract (10.6 g) was also adsorbed on silica gel and eluted using similar solvent ratios as given earlier. The combined fraction from tubes 26 to 29 (400 mg) was chromatographed on a sephadex column using DCM-MeOH (1:1) leading to the isolation of compound 3 as a colorless oil (17.1 mg).

General experimental procedures

Nuclear magnetic resonance (NMR) spectra were measured on a Bruker DRX 300 instrument using CD_3OD as solvent. For TLC, pre-coated Si gel 60 F_{254} plates were used and spots were detected under ultraviolet (UV) light and further visualized by spraying with vanillin-sulfuric acid followed by heating at ±120°C until spots were revealed. Preparative TLC was performed on a 0.5 mm thick Si gel layer coated on 20×20 cm glass plates. Column chromatography was carried out on Si gel 60 (0.040 to 0.063 mm) (230-400 Mesh ASTM, Merck) as well as Sephadex® LH-20 (Pharmacia).

List of isolated compounds

(Rhuschromone)3-(1-(2,4-dihydroxyphenyl)-3,3-bis(4-hydroxyphenyl)-1-oxopropan-2-yl)-7-methoxy-4H-chromen-4-one (1)

An amorphous yellow powder, revealing a pseudo-molecular ion $[M+H]^+$ at m/z 524.1244, molecular formula $C_{31}H_{24}O_8$.^{1}H NMR (CD_3OD, 300 MHz) and ^{13}C NMR (300 MHz) (Table 1).

2', 4'-dihydroxychalcone-(4-O-5''')-4'',2''',4'''-trihydroxychalcone (2)

A yellow solid; MS indicated an ion at m/z 510.1212 (M^+, calculated 510.1314) consistent with a molecular formula of $C_{30}H_{22}O_8$.
^{1}H NMR (CD_3OD, 300 MHz) *δ* 8.01 (1H, *d*, *J* = 9.0 Hz, H-6'), 7.94 (1H, *s*, H-6'''), 7.85 (1H, *d*, *J* = 15.3 Hz, H-*β'*), 7.83 (1H, *d*, *J* = 15.3 Hz, H-*β*), 7.75 (2H, *d*, *J* = 8.4 Hz, H-2, H-6), 7.68 (1H, *m*, H-*α'*), 7.67 (2H, *d*, *J* = 8.4 Hz, H-2'', H-6''), 7.57 (1H, *m*, H-*α*), 6.96 (2H, d, *J* = 8.6 Hz, H-3, H-5), 6.87 (2H, d, *J* = 8.7 Hz, H-3'', H-5''), 6.61 (1H, s, H-3'''), 6.42 (1H, dd, *J* = 9.0, 2.4 Hz, H-5'), 6.31 (1H, d, *J* = 2.4 Hz, H-3'); ^{13}C NMR (CD_3OD, 300 MHz) *δ* 192.4 (s, C=O'), 192.0 (s, C=O), 166.2 (s, C-4'), 165.2 (s, C-2'), 160.4 (s, C-2'''), 160.3 (s, C-4''), 158.4 (s, C-4), 157.9 (s, C-4'''), 144.8 (d, C-*β'*), 143.6 (d, C-*β*), 132.1 (d, C-6'), 130.6 (d, C-6), 130.2 (d, C-2''), 129.9 (s, C-1), 129.1 (s, C-1''), 128.8 (d, C-2), 128.0 (d, C-6''), 127.3 (s, C-5'''), 126.4 (d, C-6'''), 118.3 (d, C-α, C-α'), 116.7 (d, C-3''), 115.3 (d, C-5''), 113.9 (s, C-1'), 113.3 (s, C-1'''), 108.1 (d, C-5'), 107.8 (d, C-3'''), 102.4 (d, C-3').

3-((Z)-heptadec-13-enyl) benzene-1, 2-diol (3)

A colorless oil, revealing a pseudo molecular ion $[M+H]^+$ at m/z 347.2945, (calculated 347.2945), molecular formula $C_{23}H_{38}O_2$.
^{1}H (CD_3OD, 600 MHz) *δ* 7.05 (*t*, H-5), 6.63 (*d, J* = 7.2 Hz, H-4), 6.57 (*d*, *J* = 7.8, H-6), 5.33 (2H, *dd*, CH=CH, *J* = 9.6, 5.4 Hz,), 2.52 (2H, *t*, CH_2Ar), 2.03 (4H, *m*, 2CH_2CH=), 1.29 (18H, $(CH_2)_9$) and 0.89 (3H, *t*, Me).
^{13}C (CD_3OD, 600 MHz) *δ* 158.3 (s, C-1), *δ* 145.6 (s, C-2), *δ* 130.9 (s, C-3), *δ* 130.8 (d, C-13'), *δ* 130.2 (d, C-14'), *δ* 116.2(d, C-4), *δ* 120 (d, C-5) and *δ* 113.5 (d, C-6), *δ* 36.9 (t, C-1'), *δ* 33.0(t, C-2'), *δ* 32.6 (t, C11'), *δ* 30.8 (t, C-15'), *δ* 30.7(t, C-6',C-7', C-8'',C-9',C-10'), *δ* 30.6(t, C-5'), *δ* 30.4(t, C-4'), *δ* 30.3(t, C-3'), *δ* 28.1(t, C-12'), *δ* 23.7(t, C-16') and *δ* 14.4(q, C-17').

In vitro antimicrobial screening

Antimicrobial screening bioassay of the crude extracts and the isolated compounds against micro-organisms was carried out using the agar diffusion technique (El-gayyar et al., 2000). Micro-organisms were obtained from the National Public Health Laboratories (NPHL). Gram-positive bacteria *Staphylococcus aureas* (ATCC 25923), *Escherichia coli* (ATCC 25922); Gram-negative bacteria *Pseudomonas aureginosa* (clinical isolate). Fungi included: *Cryptococcus neoformans* (clinical isolate), *Trichophyton mentagrophytes* (clinical isolate), *Penicillium* species (environmental isolate); yeast: *Candida albicans*. A colony of each bacterial strain was suspended in 1 ml of Mueller-Hinton broth and incubated for 18 h at 37°C. A subculture was diluted 1/50 in the same broth before use. Plant extracts and isolates were dissolved in appropriate solvents at a concentration of 100 mg/ml for extracts and 2 mg/ml for isolates. Aliquots of 20 µl were dispensed on 9 mm sterile paper discs. Chloramphenicol (2 mg/ml) was used as a positive control for bacteria and fluconazole (2 mg/ml) for fungi. The diluted cultures were spread on sterile Muller-Hinton agar plates. The antimicrobial activity was evaluated by measuring the diameter of inhibition zone. The experiments were carried out in triplicate and the mean of the diameter of the inhibition zones calculated. Antimicrobial inhibition activities were measured against those of the standards (Vlietinck, 1997).

RESULTS AND DISCUSSION

Sequential extraction of the dried root bark material of *R. natalensis* with solvents of increasing polarity, afforded, as one of the bioactive compounds in the ethyl acetate extract a new compound 1 and one known compound 2, whereas the hexane extract gave another known compound 3. The isolated compounds were identified using spectroscopy and then evaluated for their antimicrobial activity. Compound 1 (Figure 1) was obtained as an amorphous yellow powder, revealing a pseudo molecular ion $[M+H]^+$ at m/z 524 and 1244; calcd. 524.1212, corresponding to the molecular formula $C_{31}H_{24}O_8$. It gave a positive flavonoid test (Harbone, 1988). The ^{1}H NMR spectrum assigned with the aid of the COSY experiment

Table 1. ^{1}H NMR and ^{13}C NMR data of compound 1 in CD_3OD at 300 MHz.

Position	C (*δ*)	H (*δ* multiplicity, *J*)	HMBC
2	157.5	8.20 (*s*)	-
3	122.6	-	-
4	177.2	-	-
5	134.1	8.21 *d*, (*J* = 3.9)	C-4, C-7, C-9
6	108.6	6.44 *dd* (*J* = 2.1, 2.1)	C-8, C-10
7	166.9	-	
8	101.9	6.28 *d*, (*J* = 2.1)	C-6, C-7, C-10
9	159.5	-	-
10	115.2	-	-
11	45.1	6.02 *d*, (*J* = 12.0)	C-2,C-3,C-4,C-12,C-13
12	205.4	-	-
13	135.9	-	-
14	168.2	-	-
15	103.4	6.69 *d*, (*J* = 2.1)	C-14, C-16, C-17
16	164.9	-	-
17	116.7	6.83 *dd*, (*J* = 2.1,2.1)	C-13, C-15
18	128.3	7.87 *d*, (*J* = 8.7)	C-14, C-16
19	54.5	4.67 *d*, (*J* = 12)	C-11,C-12,C-21/C-25
20	134.9	-	-
21	129.9	7.15 *d*, (*J* = 9.0)	C-19, C-23, C-25
22	116.2	6.57 *d*, (*J* = 8.4)	C-20, C-23
23	156.8		-
24	116.3	6.57 *d*, (*J* = 8.4)	-
25	129.9	7.15 *d*, (*J* = 9.0)	-
26	134.9	-	-
27	130.6	7.15 *d*, (*J* = 9.0)	-
28	116.2	6.57 *d*, (*J*=8.4)	-
29	156.9	-	-
30	116.3	6.57 *d*, (*J* = 8.4)	-
31	130.6	7.15 *d* (*J* = 9.0)	-
-OCH_3	56.2	3.76 *s*	-

B and B' give signals with interchangeable values. HMBC: Heteronuclear multiple bond correlation.

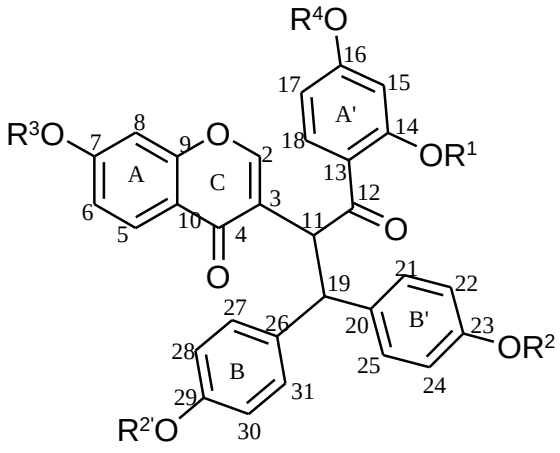

Figure 1. Compounds $R^1=R^2=R^{2'}=H$, $R^3=CH_3$, $R^4=H$ (1) and $R^1=R^2=R^{2'}=R^3=H$, $R^4=CH_3$ (4).

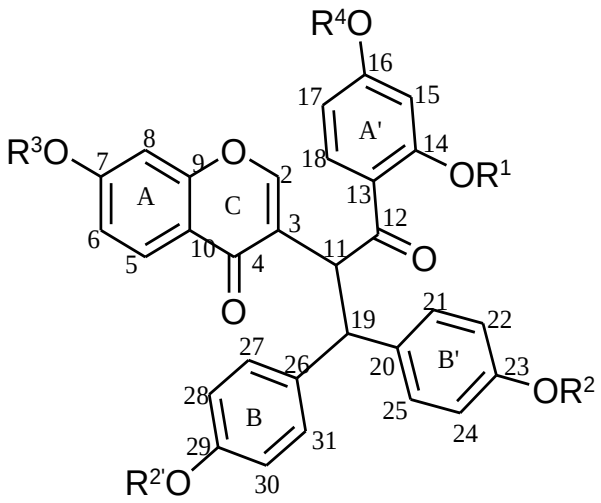

Figure 1. Compounds $R^1=R^2=R^{2'}=H$, $R^3=CH_3$, $R^4=H$ (1) and $R^1=R^2=R^{2'}=R^3=H$, $R^4=CH_3$ (4).

enabled the distinction of two 1, 4-disubstituted aromatic rings (B, B'), characterized by a symmetric set of peaks at H-21/H-25 (*δ* 7.15, s) and H-22/H-24 (*δ* 6.57, s), and which are interchangeable with H-27/H-31 and H-28/H30, respectively. Two 1, 2, 4-trisubstituted (A, A') aromatic rings were also discernible as suggested by the presence of the signals H-5 (*δ* 8.21), H-6 (*δ* 6.44), and H-8 (*δ* 6.28); H-18 (*δ* 7.87), H-17 (*δ* 6.83), and H-15 (*δ* 6.69), respectively. An AB system due to two protons at *δ* 6.02 and 4.67 could be associated with the mutually coupled H-11 and H-19 protons (J = 12). A typical singlet for proton H-2 of the isoflavone moiety was observed at *δ* 8.20 (s), whereas the signal which appeared at *δ* 3.76 was due to the methoxyl group.

The ^{13}C NMR spectrum showed the presence of 31 carbons (Table 1), which on the basis of the distortionless enhancement by polarization transfer (DEPT) experiment, could be grouped into 1 methyl, 17 methines and 13 non-protonated carbons, the latter including two carbonyl groups. All these features were in agreement with the presence of a bi-isoflavonoid skeleton carrying an extra methoxyl group. The spectral data of compound 1 were similar to those reported for calodenone (4) (Messanga et al., 1992), a compound isolated from the stem bark of *Ochna calodendron* (Ochnaceae), except for the location of the methoxyl group. The position of the methoxyl group in 1 was established through the detection of a nuclear overhauser effect spectroscopy (NOESY) interaction between the methoxyl protons, and H-6 (*δ* 6.44) and H-8 (*δ* 6.28) only (Figure 2). Unique features noted included modification of the chemical shifts of the A-ring protons, particularly H-6 (*δ* 6.44) and H-8 (*δ* 6.28) due to the presence of the methoxyl group. In 4 (Messanga et al., 1992), the methoxyl group is linked to A'-ring, where it affects the chemical shift of the corresponding protons, H-15 and H-17.

The other two compounds (2) and (3) were also identified using spectroscopic analysis, which showed results that were in agreement with the literature (Ladislaus et al., 2003; Valcic et al., 2002).

Iso-biflavonoids of this type are relatively rare in nature, and rhuschromone 1 represents the first hydroxyphenyl chromen-4-one to be reported from *R. natalensis* as well as the entire *Rhus* genus. Other phenoxychromones have been isolated from the roots of *Stellera chamaejasme* L. (Thymelaeaceae) (Masatake et al., 1984), and the stem barks of *Lophira lanceolata* (Ochnaceae) (Ghogomu et al., 1987) and *Ochna calodendron* (Ochnaceae) (Messanga et al., 1992). These isoflavonoids differ from known biflavonoids in having had an aryl shift of ring B from one flavone unit to the next, resulting in an isoflavone.

Following antimicrobial studies of the aforementioned isolates, rhuschromone was found to possess a relatively high activity against *S. aureus* ATCC 25923, which was comparable to that of the chloramphenicol standard (Table 2). Compound 3 displayed the least activity. No activity against the selected fungi was detectable. It was speculated that the activity observed with 1 may be associated with the unique positioning of the 7-OCH_3 group in ring A.

Conclusion

The investigation of this plant has led to the isolation of a novel compound called rhuschromone and which displays antimicrobial properties. However, further in depth studies would be required in order to establish whether rhuschromone would make a suitable candidate for novel

Table 2. Antimicrobial activity of isolated compounds.

Number of compound	Weight (mg/ml)	Amount/disc (µl)	Antibacterial assay			Antifungal assay		
			Sau	Eco	Pseudo	Can	TM	Mg
1	2	20	21	0	0	0	0	0
2	2	20	17	0	0	0	0	0
3	2	20	9	0	0	0	0	0
Chloramphenicol	2	20	20	0	0	0	0	0
Fluconazole	2	20	-	-	-	15	12	14

Sau: *Staphylococcus aureus* ATCC 25923; Eco: *Escherichia coli* ATCC 25922; Pseudo: *Pseudomonas aureginosa* clinical isolate; Can: *Candida albicans* ATCC 90028; Tm: *Trichophyton mentagrophytes* clinical isolate; Mg: *Microsporum gyseum* clinical isolate.

drug development.

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REFERENCES

Elgayyar M, Draughon FA, Golden DA, Mount JN (2000). Antimicrobial activity of essential oils from plants against selected pathogenic and saprophytic microorganisms. J. Food Prod. 64:1019-1024.

Ghogomu , Sondengam B, Martin M, Bodo B. Lophirone A (1987). A biflavonoid with unusual skeleton from *Lophira lanceolata.* J. Nat. Prod. 28:2967-2968.

Harbone J (1988). ‘‘The Flavonoids: Advances in Research since 1980.’’ Chapman and Hall, New York p.99.

Henk B(1991). Kenya trees shrubs and lianas, National Museums of Kenya Hutchings, A. Zulu Medicinal Plants*:* An Inventory; University of Natal Press: Pietermaritzburg p.180.

Ladislaus KM, Yeboah SO, Abegaz BM (2003). Rhuschalcones II-VI, five new bichalcones from the root bark of Rhus pyroides. J. Nat. Prod. 66:599-604.

Lin YM, Flavin MT, Schure R, Chen FC, Sidwell R, Barnard DL, Huffman JH, Kern ER (1999). Antiviral activities of biflavonoids. Planta Med. 65:120-125.

Masesane IB, Yeboah SO, Liebscher J, Mugge C, Abegaz BM (2000). A Bichalcone from the Twigs of Rhus pyroides. Phytochemistry 53:1005-1008.

Masatake N, Guo-Quan L, Hirosh, T, Yoshimasa H (1984). Chamaechromone, a novel rearranged biflavonoid from *Stellera chamaejasme L*. Tetrahedron Lett. 25:3735-3738.

Messanga B, Ghogomu R, Kimbu S, Sondengam B (1992). Calodenone, a new isobiflavonoid from *ochna calodendron*. J. Nat. Prod. 55:245-248.

US FDA (2007). Germplasm Resources Information Network. Beltsville, MD, USA: United States Department of Agriculture, Agricultural Research Service.

Van Wyk, E, Wink,M (2004). Medicinal Plants of the World. Portland, OR, USA: Timber Press.

Valcic S, Wachter GA, Eppler CM, Timmermann BN (2002). Nematicidal alkylene resorcinols from *Lithraea molleoides*. J. Nat. Prod. 65:1270-1273.

Vlietinck AJ (1997). Biologically active substances from traditional drugs. In: Hostettman K., Lea, PJ (Eds.), Proceeding of the Phytochemical Society of Europe on Biologically Active Natural Products. Oxford University Press, Oxford pp.33-47.