FULL PAPER

A New Isoflavanol from the Fruits of *Kotschya strigosa* (Fabaceae)

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The phytochemical investigation of the MeOH extract from fruits of *Kotschya strigosa* using repeated normal and reversed-phase column chromatography and *Sephadex LH-20* chromatography led to the isolation and characterization of a new isoflavanol, named kotstrigoisoflavanol (**1**), together with three known compounds, diosmetin (**2**), *β*-sitosterol (**3**), and the 3-*O*-*β*-D-glucopyranoside of *β*-sitosterol (**4**). The antioxidant activity of crude extract, **1,** and **2** was determined using the 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\cdot}$) method. The crude extract (IC_{50} 61.7 ± 0.2 μg/ml) and **2** (IC_{50} 70.2 ± 0.1 μg/ml) showed moderate antioxidant activities, while **1** was weakly active (IC_{50} 153.1 ± 0.1 μg/ml), as compared with the standard reference L-ascorbic acid (IC_{50} 21.9 ± 0.0 μg/ml).

Keywords: *Kotschya strigosa*, Isoflavanol, Kotstrigoisoflavanol, Antioxidant activity.

Introduction

The genus *Kotschya* belongs to the Fabaceae family and consists of *ca.* 30 species, which are broadly distributed in tropical Africa and Madagascar [1 – 3]. Among them, *Kotschya strigosa* (Benth.) Dewit & P.A. Duvign grows in the highlands and is widespread in tropical east and west Africa, and Madagascar [3][4]. This plant is an erect or sprawling shrub (0.3 – 2.4 m tall), with bristly stems covered by tubercular-based hairs, fasciculate leaves, and 10 – 24 foliolates [3][4]. Its chestnut-colored seeds are bluntly trigonous, with dimensions of 2 – 2.5 mm and 1 mm [3].

Some species of the genus *Kotschya* have been used in folk medicine. In Tanzania, *Kotschya uguenensis* has been used in traditional medicine to repel the chicken mite *Dermanyssus gallinae* DeGeer (Acarina; Dermanyssidae) from infesting their hosts [1]. The ethnobotanical and ethnomedicinal surveys conducted in Baham (western region of Cameroon) reported that traditional healers used the fruits of *K. strigosa*, locally called 'tsoptsop', for the treatment of mycoses. In previous studies on species of the genus *Kotschya*, the larvicidal properties of extracts from *K. uguenensis* against *Anopheles gambiae s.s.* Giles larvae [1][5], and the isolation of terpenoids from *Kotschya africana* were reported [6]. Nevertheless, flavonoids are the major medicinal constituents of plants from the Fabaceae family [7 – 10]. To the best of our knowledge, no phytochemical study on *K. strigosa* has been performed.

In our continuing search for new bioactive constituents from medicinal plants collected in Cameroon [7][11][12], a new isoflavanol, kotstrigoisoflavanol (**1**), along with three known compounds, **2** – **4**, were isolated from fruits of *K. strigosa*. Herein, we report the isolation, structure elucidation, and antioxidant activity of the novel compound.

Results and Discussion

The MeOH extract from fruits of *K. strigosa* was subjected to repeated normal- and reversed-phase column chromatography, and *Sephadex LH-20* chromatography (Sigma-Aldrich, St. Louis, MO, USA), to afford the new isoflavanol, kotstrigoisoflavanol (**1**), along with three known compounds, diosmetin (**2**) [13], *β*-sitosterol (**3**) [14], and *β*-sitosterol 3-*O*-*β*-D-glucopyranoside (**4**; *Fig. 1*) [15]. The structures of the known compounds were identified by comparison of their spectroscopic data with those reported in the literature.

Kotstrigoisoflavanol (**1**) was obtained as yellowish powder. Its HR-ESI-MS exhibited a *quasi*-molecular-ion peak at *m*/*z* 301.0700 ($[M - H]^-$), corresponding to the molecular formula $C_{16}H_{14}O_6$ with 10 degrees of unsatu-

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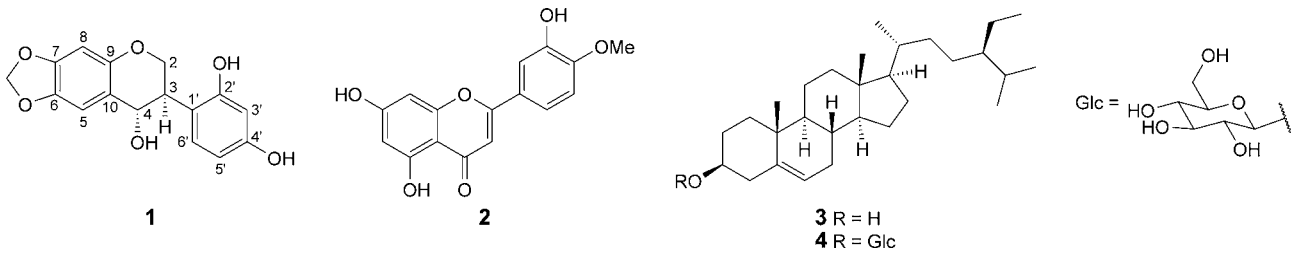

Fig. 1. Chemical structures of **1** – **4** isolated from *K. strigosa*.

ration. The IR spectrum of **1** displayed absorption bands at 3421 and 1621 cm^{-1}, assignable to OH and aromatic groups, respectively. The ^{1}H-NMR spectrum (*Table*) of **1** exhibited four signals, a *doublet* of *doublet*s at δ(H) 4.22 (J = 10.9, 4.6), a *triplet* at δ(H) 3.64 (J = 10.9), a *multiplet* at δ(H) 3.49 – 3.45, and a *doublet* at δ(H) 5.47 (J = 7.5), which could be assigned to H_a–C(2), H_b–C(2), H–C(3), and H–C(4) of 4-hydroxyisoflavan [16 – 18], respectively.

Moreover, the ^{1}H-NMR spectrum showed two sets of aromatic H-atom signals. One set was composed of two *singlet*s at δ(H) 6.72 and 6.44, assignable to H–C(5) and H–C(8) in the *A*-ring, respectively. The other set showed the presence of *ABX*-type aromatic H-atoms at δ(H) 6.41 (*d*, J = 2.9, H–C(3′)), 7.36 (*d*, J = 8.0, H–C(6′)), and 6.55 (*dd*, J = 8.0, 2.9, H–C(5′)), corresponding to the H-atoms in the *B*-ring. The ^{1}H-NMR spectrum also displayed two *doublet*s of one H-atom each at δ(H) 5.92 (*d*, J = 1.7) and 5.89 (*d*, J = 1.7), suggesting the presence of a OCH_2O group in **1**. The downfield CH_2 C-atom signal at δ(C) 101.3 indicated the presence of a OCH_2O group attached to the *A*-ring [16][17]. The 16 C-atoms of **1** were assigned by analyzing the ^{13}C-NMR, DEPT, and HMQC spectra. The ^{13}C-NMR spectrum indicated characteristic chemical shifts at δ(C) 66.4 (C(2)), 40.1 (C(3)), and 78.4 (C(4)) similar to those of reported isoflavans with a OH group at C(4) [16][17]. Furthermore, the ^{13}C-NMR spectrum exhibited five downfield signals assignable to O-bearing aromatic C-atoms, at δ(C) 157.1 (C(4′)), 156.6 (C(2′)), 154.2 (C(9)), 148.1 (C(7)), and 141.7 (C(6)), whereas five aromatic H-atom signals were observed at δ(C) 104.7 (C(5)), 93.8 (C(8)), 103.6 (C(3′)), 109.8 (C(5′)), and 132.1 (C(6′)). In the HMBC spectrum of **1** (*Fig. 2*), the correlations of the aromatic H-atoms at δ(H) 5.92 and 5.89 with the O-bearing aromatic C-atoms at δ(C) 141.7 (C(6)) and 148.1 (C(7)) allowed us to locate the OCH_2O group at C(6) and C(7) of the *A*-ring. The HMBC correlations of the H-atoms at δ(H) 4.22 (H_a–C(2)) and 3.64 (H_b–C(2)) with the C-atoms at δ(C) 40.1 (C(3)) and 78.4 (C(4)), of the H-atom at δ(H) 3.49 – 3.45 (H–C(3)) with the C-atoms at δ(C) 66.4 (C(2)), 117.9 (C(10)), and 156.6 (C(2′)), and of the H-atom at δ(H) 5.47 (H–C(4)) with the C-atoms at δ(C) 66.4 (C(2)), 40.1 (C(3)), 117.9 (C(10)), and 112.5 (C(1′)) indicated the further connectivity between the C-atoms C(2 – 4), as well as the interconnection between the *A*/*C* and *B*/*C* rings. Moreover, the HMBC correlations of the aromatic H-atom at δ(H) 6.72 (H–C(5)) with the O-bearing aromatic C-atoms at δ(C) 141.7 (C(6)), 148.1 (C(7)), and 154.2 (C(9)), and of the aromatic H-atom at δ(H) 6.44 (H–C(8)) with the aromatic C-atoms at δ(C) 141.7 (C(6)), 148.1 (C(7)), 154.2 (C(9)), and 117.9 (C(10)), confirmed that H–C(5) and H–C(8) were located at the *A*-ring, while the other aromatic H-atoms were located at the *B*-ring. The ^{1}H,^{1}H-COSY (*Fig. 2*) correlations between the H-atoms at δ(H) 6.41 (*d*, J = 2.9, H–C(3′)), 6.55 (*dd*, J = 8.0, 2.9, H–C(5′)), and 7.36 (*d*, J = 8.0, H–C(6′)), as well as the HMBC correlations of the H-atoms at δ(H) 3.49 – 3.45 (H–C(3)), 6.41 (H–C(3′)), and 7.36 (H–C(6′)) with the C-atom at δ(C) 156.6 (C(2′)), suggested the fixation of the first OH group on the *B*-ring at C(2′). The HMBC correlations of the H-atoms at δ(H) 6.41 (H–C(3′)), 6.55 (H–C(5′)), and 7.36 (H–C(6′)) with the C-atom at δ(C) 157.1 (C(4′)) indicated that the second OH group was located at C(4′).

The relative configurations at C(2) and C(3) were determined by analysis of the ^{1}H-NMR and NOESY experiments (*Fig. 2*), and comparison of our data with those of reported related structures. The coupling constant J = 7.5 at δ(H) 5.47 (H–C(4)) – *i.e.*, J(3, 4) – and the previous reports on the stereochemistry of 4-hydroxyi-

Table. ^{1}H- and ^{13}C-NMR data (500 and 125 MHz, resp.; in $CDCl_3$) for **1**. δ in ppm, J in Hz.

Position	δ(H)	δ(C)
2	4.22 (*dd*, J = 10.9, 4.6)	66.4
	3.64 (*t*, J = 10.9)	
3	3.49 – 3.45 (*m*)	40.1
4	5.47 (*d*, J = 7.5)	78.4
5	6.72 (*s*)	104.7
6		141.7
7		148.1
8	6.44 (*s*)	93.8
9		154.2
10		117.9
1′		112.5
2′		156.6
3′	6.41 (*d*, J = 2.9)	103.6
4′		157.1
5′	6.55 (*dd*, J = 8.0, 2.9)	109.8
6′	7.36 (*d*, J = 8.0)	132.1
–OCH_2O–	5.92 (*d*, J = 1.7)	101.3
	5.89 (*d*, J = 1.7)	

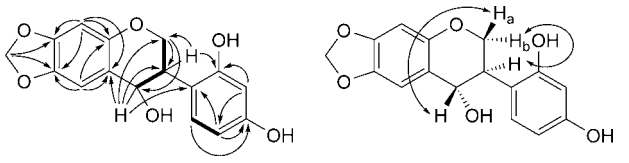

Fig. 2. Key HMBC (H → C), ^{1}H,^{1}H-COSY (▬), and NOESY (H ↔ H) correlations of **1**.

soflavan [19 – 21] indicated the 3,4-*trans* relationship and the α-orientation of the OH group at C(4). The NOESY correlations between the H-atom at δ(H) 4.22 (H_a–C(2)) and 5.47 (H–C(4)) and between 3.49 – 3.45 (H–C(3)) and 3.64 (H_b–C(2)) confirmed that H–C(4)) and H_a–C(2) were located at the same side of the molecule, as well as H–C(3) and H_b–C(2).

The absolute configuration of **1** was determined by CD experiment and comparison of the data with those of similar structures reported. The CD spectrum of **1** showed a positive *Cotton* effect between 230 and 260 nm and a negative *Cotton* effect between 290 and 325 nm corresponding to a (3*R*,4*S*)-configuration as compared to published CD data of 4-hydroxyisoflavans [22]. Based on the spectroscopic data and comparison with those of related published compounds [16][17][19], **1** was determined to be (3*R*,4*S*)-2′,4,4′-trihydroxy-6,7-(methylenedioxy)isoflavan, and was named kotstrigoisoflavanol.

The antioxidant properties of crude extract, **1** and **2**, were determined. Among them, the crude extract and **2** showed moderate activities (IC_{50} 61.7 ± 0.2 and 70.2 ± 0.1 μg/ml, resp.), as compared with the standard reference L-ascorbic acid (IC_{50} 21.9 ± 0.0 μg/ml). In contrast, **1** was weakly active (IC_{50} 153.1 ± 0.1 μg/ml).

To the best of our knowledge, no phytochemical and biological investigations have been reported for *K. strigosa*. Thus, our findings are useful for further investigations of the secondary metabolites of this plant.

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Experimental Part

General

M.p.: *Yanaco Micro* melting point apparatus (*Yanaco*, Kyoto, Japan); uncorrected. Thin-layer chromatography (TLC): pre-coated silica gel *60 F_{254}* plates (SiO_2; 0.25 or 0.50 mm thickness; *Merck*, Darmstadt, Germany), visualized by UV light at 254 and 365 nm, followed by spraying with 1% $Ce(SO_4)_2$ in 10% aq. H_2SO_4 and heating to 150 °C. Column chromatography (CC): *60N* spherical (40 – 50 μm) and neutral SiO_2 (*Kanto Chemical Co., Inc.*, Tokyo, Japan), *Cosmosil 75C_{18}-OPN* (*Nacalai Tesque, Inc.*, Kyoto, Japan), and *Sephadex LH-20* (*Dowex® 50WX2-100*; *Sigma–Aldrich*, St Louis, MO, USA). Optical rotations: *JASCO P-2100* polarimeter (Tokyo, Japan). UV Spectra: *Shimadzu UV-160 A* spectrophotometer (Kyoto, Japan); λ_{max} (log ε) in nm. CD Spectra: *JASCO J-805* spectropolarimeter; λ_{max} (Δε) in nm. IR Spectra: *JASCO FT/IR-460 Plus* spectrometer; $\tilde{\nu}$ in cm^{-1}. ^{1}H- and ^{13}C-NMR spectra: *Jeol-500* spectrometer (500 and 125 MHz, resp.); in $CDCl_3$; δ in ppm rel. to Me_4Si as internal standard, *J* in Hz. ESI-MS and HR-ESI-MS (neg.): *Shimadzu LCMS-IT-TOF* spectrometers (Kyoto, Japan); in *m*/*z*.

Plant Material

The fruits of *K. strigosa* (Benth.) Dewit & P.A. Duvign were collected in Baham in April 2015, and identified by Mr. *Nana*, a retired botanist, at the National Herbarium of Cameroon in Yaoundé, where our sample was compared to the available reference specimen, with voucher number 501240/HNC.

Extraction and Isolation

The dried and powdered fruits of *K. strigosa* (300 g) were macerated for 24 h in MeOH (3 × 5 l) to yield the crude extract (6.1 g, 2.1% yield) after filtration and removal of the solvent using a rotary evaporator. A part of the crude extract (5.8 g) was subjected to CC (*60N* spherical and neutral SiO_2 (1500 g); i.d., 8 cm; l, 90 cm; hexane (9 l)/AcOEt (8 l)/MeOH (4 l) in gradient polarity) to give 71 fractions of 300 ml each that were combined into six main fractions (1 – 9 = *Fr. 1*, 10 – 14 = *Fr. 2*, 15 – 26 = *Fr. 3*, 27 – 38 = *Fr. 4*, 39 – 56 = *Fr. 5*, and 57 – 71 = *Fr. 6*) using comparative TLC. *Fr. 1* (1.4 g) was constituted mainly of fatty acid derivatives while *Fr. 6* (1.7 g) was constituted of a complex mixture of polar compounds that were not further investigated. *Fr. 3* (300 mg) was subjected to CC (*60N* spherical and neutral SiO_2 (150 g); i.d., 3 cm; *l*, 40 cm; hexane/AcOEt 9:1 isocratic solvent system), and the subfractions *Frs. 3.35 – 3.42* (350 – 420 ml, 17.3 mg) were further separated by CC (*Sephadex LH-20* (40 g); i.d., 2.2 cm; *l*, 50 cm) to furnish **1** (14.7 mg; yellowish; R_f: 0.6 on normal-phase (NP) SiO_2 TLC (hexane/AcOEt 4:1)). *Fr. 5* (507.6 mg) was subjected to reversed-phase CC (*Cosmosil 75C_{18}-OPN*; *Nacalai Tesque, Inc*., Kyoto, Japan (300 g); i.d., 4 cm; *l*, 50 cm; MeOH/H_2O 2:1) to give **2** (*Fr. 5-3-7* (300 – 700 ml); 5.1 mg; yellowish; R_f: 0.9 on reversed-phase SiO_2 TLC (MeOH/H_2O 3:1)). *Frs. 2* (300 mg) and *4* (194.3 mg) were crystallized from acetone to give **3** (15 mg; white powder; R_f: 0.65 on NP SiO_2 TLC (hexane/AcOEt 85:15)) and **4** (10 mg; white powder; R_f: 0.4 on NP SiO_2 TLC ($CHCl_3$/MeOH 9:1)), resp.

Kotstrigoisoflavanol (= **(3*R*,4*S*)-2′,4,4′-Trihydroxy-6,7-(methylenedioxy)isoflavan; 4-[(7*R*,8*S*)-7,8-Dihydro-8-**

hydroxy-6*H*-1,3-dioxolo[4,5-*g*][1]benzopyran-7-yl]-1,3-benzenediol; **1**). Yellowish powder. M.p. 101 – 103 °C. $[\alpha]_D^{22}$ = +266 (*c* = 0.1, MeOH). UV (MeOH): 310 (4.0), 218 (4.2). CD (MeOH): 238 (+47.24), 308 (−9.46). IR (KBr): 3421, 2921, 1621, 1500, 1473, 1378, 1147, 1025, 986. ^{1}H- and ^{13}C-NMR: *Table*. HR-ESI-MS: 301.0700 ([*M* − H]$^-$, $C_{16}H_{13}O_6^-$; calc. 301.0718).

DPPH$^{\cdot}$-Radical-Scavenging Assay

The antioxidant activity was determined according to the previously reported method [23]. Samples were dissolved in HPLC-grade MeOH (*Wako*, Osaka, Japan) and were diluted twofold serially to concentration ranges of 1000 – 7.81 μg/ml for extract and 100 – 0.78 μg/ml for isolated compounds. Briefly, a volume of 40 μl of each concentration prepared was introduced into each well of a 99-well microtiter plate, followed by 160 μl of methanolic soln. of DPPH$^{\cdot}$; 3.7 mg/100 ml. After incubation in the dark for 30 min at room temperature, the absorbance was measured at 517 nm using a microplate reader (*Corona Electric SH-1200; Hitachi High-Tech Science*, Tokyo, Japan). L-Ascorbic acid (*Wako*, Japan) was used as standard antioxidant. The free-radical-scavenging activity of each sample and the reference standard was determined as percent of the inhibition obtained from the following formula: radical-scavenging capacity [%] = $[(100 - (A_{sample} - A_{blank})/A_{control}) \times 100]$, where A_{sample} is the absorbance of the sample with DPPH$^{\cdot}$, A_{blank} is the absorbance of the sample without DPPH$^{\cdot}$, and $A_{control}$ is the absorbance of DPPH$^{\cdot}$ in MeOH. The concentration of samples reducing 50% of free-radical DPPH$^{\cdot}$ (IC_{50}) was determined by plotting the percentage of inhibition against the sample concentrations. The assay was replicated three times and results are expressed as mean ± SD.

REFERENCES

[1] E. Innocent, C. C. Joseph, N. K. Gikonyo, M. H. H. Nkunya, A. Hassanali, *Int. J. Trop. Insect Sci.* **2008**, *28*, 220.

[2] J. B. Gillett, R. M. Polhill, B. Verdcourt, 'Leguminosae (Parts 3 and 4)', in 'Flora of Tropical East Africa', Eds. E. Milne-Redhead, R. M. Polhill, A. A. Balkema, Rotterdam, Netherlands, 1971.

[3] J. B. Gillett, E. Milne-Redhead, R. M. Polhill, in 'Flora of Tropical East Africa, Papilionoideae', Crown Agents for Oversea Governments and Administrations, London, 1971.

[4] H. M. Burkill, 'Families J – L', in 'Useful Plants of West Tropical Africa', 2nd edn., Royal Botanic Gardens, Kew, Richmond, United Kingdom, 1995, Vol. 3.

[5] E. Innocent, M. H. H. Nkunya, A. Hassanali, *J. Appl. Pharm. Sci.* **2013**, *3*, 122.

[6] L. M. Gitu, Ph.D. Thesis, Jomo Kenyatta University of Agriculture and Technology, **2009**.

[7] M. D. Awouafack, P. Tane, M. Spiteller, J. N. Eloff, *Nat. Prod. Commun.* **2015**, *10*, 1325.

[8] M. Parvaiz, K. Hussain, S. Khalid, N. Hussnain, N. Iram, Z.Hussain, M. A. Ali, *Global J. Pharmacol.* **2014**, *8*, 8.

[9] S. Touqeer, M. A. Saeed, M. Ajaib, *Phytopharmacology* **2013**, *4*, 598.

[10] P. M. Krishna, K. N. V. Rao, S. Sandhya, D. Banji, *Braz. J. Pharmacogn.* **2012**, *22*, 1145.

[11] M. D. Awouafack, L. J. McGaw, S. Gottfried, R. Mbouangouere, P. Tane, M. Spiteller, J. N. Eloff, B. M. C. Complement, *Altern. Med.* **2013**, *13*, 289.

[12] F. L. D. Mafodong, A. Tsopmo, M. D. Awouafack, R. T. Tchuenguem, J. P. Dzoyem, P. Tane, *Nat. Prod. Commun.* **2015**, *10*, 1709.

[13] Y. Park, B.-H. Moon, H. Yang, Y. Lee, E. Lee, Y. Lim, *Magn. Reson. Chem.* **2007**, *45*, 1072.

[14] A. Kamboj, A. K. Saluja, *Int. J. Pharm. Pharm. Sci.* **2011**, *3*, 94.

[15] M. Al-Oqail, W. H. B. Hassan, M. S. Ahmad, A. J. Al-Rehaily, *Saudi Pharm. J.* **2012**, *20*, 371.

[16] S. Ganapaty, S. T. Pannakal, G. V. K. Srilakshmi, P. Lakshmi, P. G. Waterman, R. Brun, *Phytochem. Lett.* **2008**, *1*, 175.

[17] G. Bojase, C. C. W. Wanjala, R. R. T. Majinda, *Phytochemistry* **2001**, *56*, 837.

[18] M. Ahmed, M. Khaleduzzaman, M. S. Islam, *Phytochemistry* **1990**, *29*, 2009.

[19] L. Bai, N. Masukawa, M. Yamaki, S. Takagi, *Phytochemistry* **1998**, *48*, 327.

[20] K. Wähälä, J. K. Koskimies, M. Mesilaakso, A. K. Salakka, T. K. Leino, H. Adlercreutz, *J. Org. Chem.* **1997**, *62*, 7690.

[21] S. Yamaguchi, S. Ito, A. Nakamura, N. Inoue, *Bull. Chem. Soc. Jpn.* **1965**, *38*, 2187.

[22] D. Won, B.-K. Shin, S. Kang, H.-G. Hur, M. Kim, J. Han, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1952.

[23] F. S. T. Ndjateu, R. B. N. Tsafack, B. K. Nganou, M. D. Awouafack, H. K. Wabo, M. Tene, P. Tane, J. N. Eloff, *S. Afr. J. Bot.* **2014**, *91*, 37.

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